EVALUATION OF LIVE PHYTOPLANKTON AND LIVE ALGAE SUBSTITUTE DIETS FOR FEEDING JUVENILE OYSTERS (CRASSOSTREA GIGAS) IN NURSERY CULTURE

JEFFEREY W. BABUIN

EVALUATION OF LIVE PHYTOPLANKTON AND LIVE ALGAE SUBSTITUTE DIETS FOR FEEDING JUVENILE OYSTERS (*CRASSOSTREA GIGAS*) IN NURSERY CULTURE

by

© Jefferey W. Babuin

A thesis submitted to the

School of Graduate Studies

in partial fulfillment of the

requirements for the degree of

Master of Science (Aquaculture)

School of Fisheries, Marine Institute

Memorial University of Newfoundland

July, 2009

St. John's Newfoundland and Labrador

Abstract

The aim of this study was to mitigate summer mortality of juvenile Pacific oysters (*Crassostrea gigas*) by developing a low-cost, low-intensity system of supplemental feeding of juvenile oysters that could be used during times of low natural phytoplankton production. Three experiments tested the effectiveness of supplemental feeding of juvenile oysters in nursery culture using live algae and live algae substitute diets. Experiment 1 tested five dietary formulations consisting of two *Schizochytrium*-based live algae substitute diets (Algamac 3050™ and Rotimac™) and *Isochrysis galbana* (clone T-Iso) in 1:1 (dw:dw) and 100% formulations. Oysters were fed for 60 days and the Algamac 3050™/T-Iso diet produced the highest percentage increase in dry weight over 60 days (+106%) followed by the reference T-Iso diet (+65%). Experiment 2 tested the effectiveness of using protease absence/presence as an indication of dietary suitability. The oysters that were fed the diets that produced poor growth (Algamac 3050™, Rotimac™ and the starved control) also failed to produce proteases. Experiment 3 tested three dietary formulations consisting of two mixed *Schizochytrium*-based live algae substitute (Algamac 2050™ and Rotimac™) and live algae diets (using phytoplankton grown in outdoor bloom tanks dominated by *Thalassiosira nordenskioeldii*; 1:1 (dw:dw)) and a reference 100% *T. nordenskioeldii* diet. Both mixed diets produced a significant increase in mean oyster dry weight and overall energy storage levels compared to the *T. nordenskioeldii* diet following 30 days of feeding. The results of the three experiments suggest that juvenile oyster energy storage levels and overall health could be improved through a supplemental feeding regime.

Acknowledgements

This project was funded through AquaNet, a Network Centres of Excellence in Aquaculture grant (339857). In-kind support was provided by Pearl Sea Products, Aquafauna Biomarine and Unique Seafarms. I would like to thank my committee members: Dr. J.N.C. (Ian) Whyte, Dr. G.J. (Jay) Parsons, Cyr Couturier and Dr. Neil Ross for their assistance, guidance and most of all patience. I would also like to thank Norma Ginther, Laurie Keddy, Kathleen Sherry, Nicole Brun for their assistance and advice. Lastly, I would like to thank my wife, Tatiana, for her never ending support.

Table of Contents

List of Tables

List of Figures

1.0 Introduction

1.1 Oyster aquaculture in British Columbia

Pacific oyster aquaculture in British Columbia currently consists of the purchase or rearing of seed oysters (~1–2 mm) from hatchery facilities for ongrowing in ocean-based systems using floating upweller systems (FLUPSY), lantern nets, suspended trays, cages or by seeding beach tenures. In all cases, oyster growth is constrained by the natural production of phytoplankton. Phytoplankton abundance fluctuates throughout the annual production cycle as environmental conditions are continually changing. British Columbia's coastline is situated in the Northern Temperate climate zone and seasonality of phytoplankton production occurs due to shifting environmental conditions such as day length, temperature, rainfall, and nutrient availability (Harrison *et al.* 1983). A critical period in the annual production cycle occurs between the February – May diatom bloom and the September – October diatom bloom (Harrison *et al.* 1983; Haigh *et al.* 1992) when vulnerable juvenile Pacific oysters must survive on energy stored during the February – May diatom bloom event. Although the winter period of low natural food availability is longer (late October – early February), the lower water temperature during the winter period causes the dormant Pacific oysters to enter a hibernation-like state that results in a lower rate of energy utilisation (J.N.C. Whyte, pers. comm.). Therefore, the elevated temperature and lack of food make the mid-summer (May – September) period a potential source of mortality for vulnerable seed oysters with compromised stored energy levels.

The usefulness of supplementing the natural diet of the juvenile Pacific oysters during this critical time would be demonstrated in maintenance of optimum energy levels between bloom events and by promoting growth and increased survival. The advantage to the grower would be seen in increased production and efficiency through decreased seed purchases and a larger percentage of original seed growing to a marketable size. Brown *et al.* (1998) noted similar problems and potential advantages of supplemental feeding in the temperate climate zone of Tasmania. Brown and McCausland (1999) found that supplemental feeding of juvenile oysters in hatchery culture had the potential to increase the cost-effectiveness of rearing because it is less expensive to feed oysters prepared diets when compared to mass reared phytoplankton systems. Costs for maintaining live algae culture systems range from US $160 to US $400·kg^{-1} and algal production is estimated to account for approximately 30% of a hatchery's operating costs (Coutteau and Sorgeloos 1992).

1.2 Summer mortality of juvenile oysters and probable causes

Summer mortality of juvenile oysters in nursery culture is a challenge faced by British Columbia Pacific oyster (*Crassostrea gigas*) aquaculturists. This problem is not a new phenomenon, nor is it a problem that is only experienced in the Northeast Pacific. Summer mortalities have been documented in the Puget Sound region of the Northeast Pacific, France, New Zealand, and Japan (Cheney *et al.* 2000). Several hypotheses have been proposed as to the cause of the mortality events, including high levels of toxic phytoplankton species resulting in low dissolved oxygen levels (Cheney *et al.* 2000),

elevated summer water temperatures resulting in resorption of the gonad (Perdue 1983), accelerated gonadal development also due to elevated water temperatures and high nutrient levels (Imai *et al.* 1965; Tamate *et al.* 1965), and a bacterial disease, nocardiosis, was also proposed as a possible cause (Elston *et al.* 1987; Friedman 1991a; Friedman 1991b).

The two hypotheses regarding resorption of the gonad and rapid gonadal development both highlight a fundamental problem, a lack of sufficient nutrition. If insufficient food is available, the oyster will begin to resorb the glycogen and lipid stores in the gonad in an effort to fuel catabolic and anabolic processes. Also, rapid gonadal development requires sufficient energy from food sources to first fuel metabolic and anabolic processes, then to allow excess energy to fuel gonadal development. Both of these situations require that excess energy be available and, without sufficient energy, both processes were theorised to have caused the observed mortalities (Imai *et al.* 1965; Tamate *et al.* 1965; Perdue 1983).

1.3 Summer mortality of juvenile oysters at Sykes Island farm site

The aim of my study was to gain a better understanding of the causes of summer mortalities of juvenile Pacific oysters (*Crassostrea gigas*) experienced by the original industrial partner Pearl Sea Products. Pearl Sea Products (now Mac's Oysters) is a large-scale Pacific oyster (*Crassostrea gigas*) aquaculture company based in Sechelt, British

Columbia, Canada with its main farm site located in the vicinity of Sykes Island in Jervis Inlet, also in British Columbia (49° 49.516' N, 124° 00.139' W).

The project was designed based on observations by Pearl Sea Products' staff over successive growing seasons during which large mortalities of seed oysters <6 mm in size seemingly coincided with the mid-summer drop in naturally occurring phytoplankton (J. Manders, pers. comm.) common to the temperate marine regions of the Pacific Ocean (Harrison *et al.* 1983).

Two hypotheses for this phenomenon were:

a) An insufficient food supply of wild phytoplankton for juvenile oysters <6 mm in shell length (based on anecdotal information and farm records indicating juvenile *C. gigas* larger than 6 mm experienced markedly higher survival) resulted in critically low energy reserves.

b) The occurrence of potentially harmful phytoplankton species possibly coinciding with low food abundance resulted in the observed mortalities.

The mortalities were thought to occur as a result of insufficient natural feed in the water to sustain and promote growth and to allow the juvenile *C. gigas* to accumulate sufficient energy stores to survive the extended winter period of low phytoplankton abundance. Therefore, the original goal for the first experiment was to test the effectiveness of supplemental feeding of juvenile oysters susceptible to summer mortality with local

phytoplankton species grown in bloom tanks and commercially available live algae substitute diets as partial and whole substitutions for live micro-algae.

Previously studied mortality events were concerned with adult oysters; however, Pearl Sea Products' farm records indicated that the mortalities were isolated to juvenile oysters mainly <6 mm in size (J. Manders, pers. comm.). Scientists from the Shellfish Pathology Unit at the Canada Federal Department of Fisheries and Oceans (DFO) Pacific Biological Station facility examined moribund oysters from Pearl Sea Products' Sykes Island site and no primary pathogen was detected. Hinge ligament disease, *Cytophaga*-like bacteria (CLB), was present in some samples; however, it was not present during all of the mortality events and only affects weakened oysters (G. Meyers, pers. comm.).

1.4 Previously studied effects of toxic algae on *Crassostrea gigas* larvae and adults

Crassostrea gigas larvae were shown to be susceptible to high levels (10^7 cell·L^{-1}) of some toxic phytoplankton species, *Alexandrium tamarense*, *A. taylorii*, *Gymnodinium mikimotoi*, and *Heterocapsa circularisquama* in a past study (Matsuyama *et al.* 2001). The same study demonstrated that at lower cell densities ($\leq 10^6$ cells·L^{-1}) only *A. taylori* and *H. circularisquama* caused mortality in *C. gigas* larvae. *Alexandrium minutum* was fed to adult *C. gigas* and it was demonstrated that there is an initial period of approximately 8 to 15 days where oyster feeding activity was reduced. However, following introduction of a diatom species, markedly higher feeding activity resumed (Lassus *et al.* 1999). The reduced feeding effect caused by toxic phytoplankton exposure

coupled with elevated temperatures could cause a rapid loss in energy reserves as the oysters utilise stored energy in feeding but lack quality food species to acquire additional energy. The oyster mortalities during the winter period were observed to be lower and this could be attributed to the oysters' lower metabolic activity and lack of food species allowing the organism to enter a state of aestivation. A parallel research project focussing on phytoplankton community structure and dynamics was directed towards evaluating the effects of two potentially toxic species as the cause of the mortalities (D. Cassis, pers. comm.).

It was hypothesised that the summer mortality experienced at Pearl Sea Products' Sykes Island site was not caused by disease, low dissolved oxygen levels, resorption of the gonad, or accelerated gonadal development. Mortality caused by the presence of toxic phytoplankton species also seems unlikely as the species present are larger than juvenile Pacific oysters can ingest (Jørgensen 1996; Ropert and Goulletquer 2000). It was hypothesised that the mortality events were caused by the lack of nutritious phytoplankton species for a prolonged period, coupled with elevated water temperatures leading to the rapid dissipation of energy reserves. This weakened state could have caused death by starvation alone or by an unknown stressor or factor not sufficiently pathogenic to kill healthy oysters. In Tasmania, Brown and McCausland (1999) found that food availability was the main constraint to growth at a commercial *C. gigas* hatchery utilising natural algal production by pumping unfiltered seawater through larval and juvenile rearing tanks.

1.5 *Schizochytrium*-based diets

Schizochytrium-based diets were chosen because they are available commercially at a much lower cost than cultured algae (~US $28 per kg), they are easy to use and store, and have sufficient levels of nutritional components in comparison to commercially utilised live algae species (Coutteau and Sorgeloos 1992). *Schizochytrium* diets upon re-suspension in seawater yield particles of an appropriate size (3–10 μm) for oyster consumption (Sornin *et al.* 1988; Jørgensen 1996) although a previous study by Baldwin and Newell (1995) suggested that *Crassostrea virginica* larvae ingestion rates in nature are regulated by food quantity and nutritional quality and are less dependent on particle size.

Previous studies have examined the Algamac 2000™ *Schizochytrium*-based diet as a partial supplement for live algae in hatchery-reared juvenile *Crassostrea gigas* (Boeing 1999; Brown and McCausland 2000). Boeing (1999) demonstrated a significantly higher growth rate using a 40% *Schizochytrium* substitution versus a 100% *Tetraselmis suecica* diet. Brown and McCausland (2000) tested Algamac 2000™ as a supplement to a natural phytoplankton diet in a flow-through nursery culture system and found it to be approximately 50% effective when compared with the *Isochrysis galbana* (clone T-Iso) control diet. The mussel species, *Mytilus galloprovincialis*, was shown to be able to subsist on a 100% *Schizochytrium* diet (Onal and Langdon 1999; Whyte 2003).

In Experiment 1, the effectiveness of the live algae substitute diets were evaluated as whole substitutes and partial substitutes and further experiments were designed. The goal of the additional experiments was to test a method of supplementing juvenile *C. gigas* in a commercial nursery culture system in an effective and efficient manner.

1.6 Evaluation of alternative tests for determination of diet efficacy

Experiment 2 was intended to aid in future feeding studies by developing alternative methods of assessing the effectiveness of a test diet. Therefore, it was not intended to be directly relevant to the overall project objectives. Experiment 2 was originally designed to test the use of biochemical markers (*e.g.*, HSPs, enzymes) as indicators of dietary stress in juvenile *C. gigas*. It was hypothesised that oysters that were presented with an ineffective diet would display signs of stress such as production of HSP70 stress proteins or an altered profile of digestive enzymes. It was further hypothesised that if the tests showed that an ineffective diet could be demonstrated through the production of a stress response, future feeding studies could possibly be shortened as lengthy growth and biochemical measurements determinations would be unnecessary.

Experiment 2 focussed on assessing protease activity profiles. A growing oyster that is receiving an effective diet will stimulate protease activity for tissue production. An ineffective diet may cause the oyster to lower or alter protease production as protein assimilation and growth ceases. Protease activity was correlated with growth to determine if this qualitative method is appropriate for the assessment of diet

effectiveness. Other methods of assessing metabolic activity were used in past studies with other species (*e.g.*, *Mytilus californicus*) such as calorimetry, oxygen consumption, RNA:DNA ratios and the activity of key metabolic enzymes (*e.g.*, citrate synthase and malate dehydrogenase) (Wright and Hetzel 1985; Dahlhoff *et al.* 2002); however, protease activity was not measured in these studies.

1.7 *Schizochytrium*-based diets and bloom tank algae

The objective of Experiment 3 was to develop and test methods and diet formulations for supplementing a natural phytoplankton diet that could be used in remote locations similar to that of Pearl Sea Products' Sykes Island FLUPSY (Floating Upweller System) operation. Experiment 1 (E1) demonstrated the effectiveness of a 1:1 (dw:dw; dry weight/dry weight) substitution of Algamac 3050™ and Rotimac™ with a natural phytoplankton diet (T-Iso). Algamac 3050™ was determined to have unnecessarily high lipid levels and Algamac 2050™was tested instead. As original plans specifying the use of live phytoplankton cultured in bloom tanks on site and field trials were not possible at the appropriate time (June - September 2002), the experiment was moved to Unique Seafarms' hatchery location where operating outdoor bloom tanks existed. Therefore, live phytoplankton cultured in outdoor bloom tanks was used as feed for nutritionally compromised juvenile *C. gigas* supplemented with Algamac 2050™and Rotimac™.

Using the results from Experiment 3, a determination was made on the effectiveness of the theoretical bloom tank live algae and substitute diet formulations for application in remote oyster culture locations.

1.8 Objectives

The main objective of this research project was to design and test a system of supplementary feeding of juvenile *C. gigas* in nursery culture that could be used in remote locations. The goal of the development of such a system would be to enhance juvenile oyster storage energy levels prior to the summer growing season, limiting summer mortality of nutritionally-depressed oysters. This was achieved through the completion of three objectives:

1. Determination of the effectiveness of two live algae substitute diets as whole and partial replacements for a live algae diet.
2. Evaluation of qualitative protease activity analysis as a tool for assessing dietary effectiveness.
3. Testing of bloom tank algae and two live algae substitute diets as mixed diets for juvenile *C. gigas*.

2.0 Materials and Methods

2.1 Experiment 1

2.1.1 Experimental setup

This experiment tested two *Schizochytrium*-based dietary formulations: Algamac 3050™ and Rotimac™ that were previously untested for feeding *Crassostrea gigas*. Unlike the previous studies by Boeing (1999) and Brown and McCausland (2000), this experiment used 50% substitutions for the live algae *Isochrysis galbana* (clone T-Iso) and assessed growth and biochemical parameters in determining the diet's effectiveness. *Isochrysis galbana* (clone T-Iso) was used as a reference diet due to its excellent nutritional profile, ease of culture and wide use by industry and in previous research studies (Helm and Laing 1987; Whyte 1987; Coutteau and Sorgeloos 1992; Brown *et al.* 1998).

Table 1 summarises the nutritional information for each of the test diets. The pure *Schizochytrium* sp. diet, Algamac 3050™, is currently used for enriching *Artemia* and rotifers in larval finfish culture as it is very rich in lipid, specifically, the long chain polyunsaturated fatty acids docosahexaenoic acid (DHA; 22:6ω3) and eicosapentaenoic acid (EPA; 20:5ω3) (Aquafauna Biomarine 2005; Table 1). Both fatty acids were implicated in a previous study to be of key importance for *C. gigas* (Langdon and Waldock 1981). Rotimac™ was designed for rotifer enrichment for larval finfish culture and is not a pure *Schizochytrium* diet; it has a higher carbohydrate component and lower lipid fraction (Table 1).

For this experiment six discrete upwelling systems were used each consisting of a semi-circular 75-L reservoir tank (65 cm x 45 cm x 28 cm L x W x D) with a submerged aquarium pump (Hagen 402 Powerhead) pumping seawater into a 135-L upper tank (57 cm x 39.5 cm x 61 cm) fitted with three 55 cm x 12 cm ID round upwellers with 100-µm nitex screen. Three hundred millilitres of juvenile *C. gigas* were introduced into each upweller for a total of 900 mL of Pacific oyster seed per treatment. Due to the need of Pearl Sea Products to improve the growth of poor performing oysters, nutritionally-depressed oysters were provided for use in this experiment.

All incoming seawater was filtered to 1-µm using 25-µm and 1-µm cartridge filters plumbed in series and aeration was continually supplied to each reservoir tank. When the oysters were not being fed, fresh seawater was pumped into the upwellers and the tanks acted as a flow-through system.

Ambient seawater was used and this resulted in water temperatures similar to local winter marine conditions (Harrison *et al.* 1983). Temperature was measured using automatic temperature loggers (Onset Computer Corporation Stowaway Tidbit -5°C to +37°C). Temperature was measured in the 100% T-Iso tank, the 1:1 (dw:dw) Algamac 3050™/T-Iso tank and the starved tank to assess the range of possible temperatures (precision ± 0.2°C). Temperature range varied in each of the three measured tanks due to the different initial temperatures of the introduced seawater containing the feeds and as a

result of the surrounding air temperature gradually warming the water during the six-hour static feeding cycle.

The test oysters were provided by Pearl Sea Products from their Sykes Island FLUPSY for use in this experiment. It was indicated by Pearl Sea Products' staff that the oysters were those remaining in the 4.8 – 6.4 mm size range after several size grading events during the 2001 summer growing season. Therefore, the oysters used for this experiment were determined to be slow-growing and unresponsive to the natural diet present at the Sykes Island farm site. In order to ensure that the oysters' enzymatic systems were given sufficient time to be able to efficiently digest the test diets, the oysters were fed daily with cultured *Isochrysis galbana* (T-Iso) for a period of three weeks prior to the start of the experiment.

For the experiment, the oysters were fed to satiation for a period of six hours per day for 60 days. The experimental period lasted from December 11th, 2002 – February 14th, 2003. The time period of 60 days was chosen because the prevailing time period for juvenile oyster feeding studies is 21 – 33 days (Knauer and Southgate 1996; Brown *et al.* 1998; Soudant *et al.* 2000; Knuckey *et al.* 2002). Brown and McCausland (1999) determined that a period of 28 days was sufficient to statistically detect a significant difference among diets and to observe a 20% increase in growth from a control diet, *Isochrysis galbana* (clone T-Iso). In the study of Brown and McCausland (1999) the experimental temperature was higher and the oysters were not identified as slow-

growing. Therefore, for the purposes of this experiment, a longer feeding period was used to allow a sufficient period of growth in order to enable firmer conclusions to be drawn as to the effectiveness of the test diets.

A six-hour static feeding cycle was used because the total volume of water in the tank systems was not large enough in comparison to the amount of oysters in each tank to ensure that the feed would be sufficient to satiate the oysters for longer than six hours. In order to assume that the oysters were fed to satiation, cell particle levels were measured over a 6 hour feeding period using a Coulter Counter (Model T). The 6-hour feeding cycle was also used to simulate a feeding cycle that could be used in a remote location by commercial oyster farmers during an 8-hour work shift. Diet feeding efficiency (D_{fe}) or the percentage difference of suspended food particles at time 0 (C_{ti}) and 6 hours post-introduction (C_{tf}) was computed:

$$\mathbf{D_{fe}\,(\%) = (1 - (C_{tf}/C_{ti}))*100}$$

Diets were introduced into the experimental tank systems and evenly distributed by the aeration of the reservoir tank and the incoming seawater was shut off to create a re-circulating upweller system. Following addition of the live algae to each of the mixed diet and live algae control diet tanks, the water level was brought to maximum re-circulated volume for all of the tanks by addition of fresh 1 μm-filtered seawater. Live algae substitute diets were assumed to remain suspended in the water column for the 6 hour static feeding cycle. Gouthro *et al.* (1998) found that there was no settlement of

Schizochytrium cells out of the water column after 5 hours in a tank system with constant aeration. Dissolved oxygen and ammonia levels were monitored for the first week of feeding to assess the range of values during the six-hour static feeding cycle. Prior to the start of the experiment, 0.2 g of both *Schizochytrium*-based diets (Algamac 3050™ and Rotimac™) were added to 1 L of 1 μm-filtered seawater and blended for 3 minutes at high speed in a Waring blender. Number of cells·mL^{-1} and range of cell sizes were measured using a Coulter Counter (Multisizer II). Algamac 3050™ yielded approximately 72 million cells·mL^{-1} and cells ranged in size from 3.1 – 11.6 μm. Rotimac™ yielded approximately 120 million cells·mL^{-1} and cells ranged in size from 2.3 – 20.1 μm.

2.1.2 Summary of diets tested

For this experiment six treatments were used. A control diet of *Isochrysis galbana* (clone T-Iso) was used because it was shown to be an effective diet for juvenile *C. gigas* in several past studies (Wilson 1987; Brown *et al.* 1998; Onal and Langdon 1998; McCausland *et al.* 1999; Brown and McCausland 2000) and it is also in wide use in commercial rearing facilities due to its ease of growing and favourable nutritional profile (Coutteau and Sorgeloos 1992). A starved control treatment was used to assess the ability of the juvenile *C. gigas* to withstand a prolonged period of starvation by maintaining the oysters in 1 μm filtered seawater. The starvation treatment was also used to measure the survival of the nutritionally depressed oyster seed provided by Pearl Sea Products for this experiment and compare it to the other treatments.

The two *Schizochytrium*-based diets that were used as live algae substitute diets were Algamac 3050™ and Rotimac™ supplied by Aquafauna Biomarine (Hawthorne, CA, USA). Algamac 3050™ is a pure spray-dried, heterotrophically-grown *Schizochytrium* diet. *Schizochytrium* is a thraustochytrid protist species that is naturally occurring in tropical mangrove marine habitats (Raghukumar 1992). Rotimac™ is a blended *Schizochytrium* diet that was originally developed for rotifer enhancement due to its high carbohydrate content and much lower lipid content when compared to the Algamac 3050™ pure *Schizochytrium* diet. Table 1 summarises the biochemical properties of the five test diets. Diets were prepared by mixing a pre-weighed amount in ambient, 1 µm-filtered seawater for approximately 3 minutes.

A total of six treatments were used in this experiment: a 100% Algamac 3050™ diet; a 100% Rotimac™ diet; a 1:1 (dw:dw) mixed T-Iso/Algamac 3050™ diet; a 1:1 (dw:dw) mixed T-Iso/Rotimac™ diet; a 100% T-Iso control diet; and a starved treatment. All diets were measured on a dry weight basis with moisture content of the live algae substitute diets determined prior to the commencement of the experiment. The feeding ration was determined as 1% of the initial dry weight of the oysters. The dry weight of the oysters was calculated using the average moisture content value of three sub-samples taken prior to the start of the experiment. The initial volume of oysters in each upweller was converted to a dry weight equivalent and the dry weight of the ration was determined. For determination of the live algae ration size, a known volume of algae

was vacuum-filtered with 8 mm diameter glass-fibre filters (pre-combusted at 500°C for 48 hours) and the average dry weight per cell was determined by dividing the dry weight per mL by the mean number of cells per mL in the original sample determined using a Coulter Counter (Model T). Therefore, the live algae ration was determined by volume using daily cell counts.

2.1.3 Algal culture

Isochrysis galbana (T-Iso, clone CCMP 1324, Bigelow Laboratories) was maintained as a stock culture by transferring 50 mL of actively growing 48-hour stock culture to 600 mL 20°C pasteurised seawater (1-µm and charcoal filtered prior to 85°C pasteurisation) enriched with modified Harrison nutrient mix (Harrison *et al.* 1980). The Harrison media was modified by substitution of 25 g $NaH_2PO_4 \cdot 6H_2O$ for 25 g $Na_2 \cdot glycero \cdot PO_4 \cdot 6H_2O$. The remaining culture was transferred to a 20-L carboy containing pasteurised seawater enriched with nutrient mix and was gently aerated. When the cell density reached mid-exponential level, the content of the carboy was transferred to 250-L of pasteurised seawater in a 350-L fibreglass column with nutrient mix added. The culture was gently aerated and pH was kept in the 7.4 – 7.8 range by bubbling CO_2 into the culture using solenoids to control the flow when the pH fell below 7.4. A pH meter was installed in the column to regulate CO_2. All cultures received 24 hour illumination at 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ provided by full spectrum fluorescent lights. The temperature in the culture laboratory was kept constant at 18 ± 1°C (L. Keddy, pers. comm.).

All phytoplankton used in this experiment were harvested during the late exponential phase of development. Cell counts were performed daily using a Coulter Counter (Model T).

2.1.4 Biochemical analysis of oysters and test diets

Two oyster samples of approximately 1.5 g wet weight were taken every 10 days from each upweller (yielding triplicate samples per diet) immediately before feeding. The oysters were starved for a period of at least 16 hours prior to sampling in order to allow for clearing of the gut contents prior to proximate analysis. Whole oyster samples were rinsed with cold 0.5 N ammonium formate to remove excess salt, patted dry with a paper towel to absorb excess water and then weighed to determine wet weight. Next, the samples were stored in sealed glass vials and placed at -80°C for 24 hours, lyophilised in a freeze-drying unit for 48 hours and re-weighed to determine dry weight and to calculate original moisture content. All samples were stored as lyophilised samples, sealed in glass vials, vacuum-sealed in a plastic bag, stored at -80°C and were analysed within 8 months of sampling. It was previously shown by Whyte (1987) that samples stored in this manner do not show measurable degradation of biochemical components, notably lipid and protein components following a period of one year.

The two separate samples were used for measurements and determination of biochemical components. Survival was estimated by counting the amount of empty bottom valves

present in the measurement sample (dead oysters were determined by gently pushing on the umbo and those shells where the valves separated easily and no organic tissue remained inside were counted as dead) versus live oysters. A sub-sample of thirty live oysters was used to measure shell height, width and depth using digital calipers to ± 0.02 mm (Mitutoyo Model #CD-8"CS). The biochemical component sample, following the removal of dead oysters, was manually ground to a fine powder using a mortar and pestle. This powdered sample was then used in analyses of ash content, crude protein, lipid, mono- and oligosaccharide, polysaccharide and fatty acid content.

To determine ash content and ash free dry weight (AFDW), triplicate samples of approximately 100 mg were weighed into pre-combusted (500°C for 24 hours and stored in a desiccator) porcelain crucibles with aluminium foil covering the opening to prevent loss of sample from explosive combustion. Samples were combusted at 500°C for 48 hours and placed in a desiccator after cooling to 100°C. Samples were weighed to within 0.01 mg after cooling to room temperature on a microbalance (Mettler AT201).

Test diets were lyophilised to determine ash free dry weight and stored at -80°C for a maximum of 8 months prior to analysis. T-Iso samples were taken at Days 0, 30, and 60 by centrifugation with a Sharples centrifuge at 12,000 rpm that provided a paste that was transferred to a plastic bag, frozen to -80°C, lyophilised, and stored at -80°C until analysed.

Crude protein, lipid, mono- and oligosaccharide, and polysaccharide concentrations were determined using the methodologies described by Whyte (1987). The following energy equivalents were used in calculating the energy potential of lipid, protein and carbohydrate: 9.8, 4.8 and 4.2 $kcal \cdot g^{-1}$, respectively (Whyte 1987).

2.1.5 Statistical analysis

Data from the feeding experiment were compared using analysis of variance (ANOVA) followed by Tukey's post hoc test for pair wise comparisons. Prior to analysis by ANOVA, the homogeneity of variance among the upwellers in each tank was confirmed using the Levine test. Data from the upwellers were grouped to yield a single mean value per tank for comparison among tanks. Tamhane's test was used to analyse data that failed the Levine test for homogeneity of variance.

The instantaneous growth rate (k) was calculated as outlined by McCausland and Brown (1999) for comparison to past feeding studies and to allow comparison of the relative effectiveness of the reference diet, *I. galbana* (clone T-Iso) at suboptimal experimental temperatures.

$k = (\ln M_t - \ln M_0)/t$

where M = measured weight (mg) and t = experiment length (days)

All results were reported to ± one standard error (± SE).

2.2 Experiment 2

2.2.1 Experimental setup

Please refer to section 2.3.1 – 2.3.2 for details on the treatments used for Experiment 2. Samples were taken at Days 0, 20 and 60 for comparison of protease activity. The Day 20 oysters were used to indicate if zymography could be used for early detection of a poor response to a test diet.

The original goal of the starvation trial was to measure the loss in nutritional components of the starved oysters to determine which components were preferentially used during starvation. This would allow one to hypothesize about the relative importance of different nutritional components to juvenile *C. gigas* and aid in determining the structure of an optimum diet. Due to time constraints and logistical problems, the starvation samples were not processed.

During sampling, 15 live oysters were randomly chosen from one upweller in each tank to yield 5 samples per upweller and the oysters were rinsed with cold distilled water to remove faeces and other organic matter that could contaminate the preserved sample. The oysters were then rinsed with cold 0.5 N ammonium formate to remove salt deposits and then dissected to remove all tissue for storage. Throughout sampling, the oysters were kept on ice unless otherwise noted. A total of three individual oysters was grouped to yield a sufficient sample size (>1 mg wet weight of tissue) for analysis and also to

limit potentially confounding genetic variability. Plastic 5 mL genetic storage cryovials were used for storage and all samples were frozen at -80°C for 24 hours, then lyophilised for a minimum of 48 hours, vacuum-sealed in a plastic bag and stored at -80°C until analysed. All samples were analysed within 6 months of sampling.

2.2.2 Analysis of protease activity

Qualitative protease analysis was performed at the Laboratory of Dr. Neil Ross at the National Research Council of Canada Institute for Marine Biosciences in Halifax, Nova Scotia, Canada.

Prior to qualitative protease analysis, protein concentration was determined using the Bradford protein assay (Bradford 1976) using a commercial reagent (BioRad). A 250 µL aliquot of Bradford Reagent diluted with double distilled water (1:4) was added to 5 µL of unknown sample dissolved in 40 mM NaH_2PO_4 buffer solution (pH 6.2). Samples were analysed in 5 mL wells (64 well plate) and absorbance at 590 nm read using a Thermomax microplate spectrophotometer (Molecular Devices, Sunnyvale, CA, USA). Bovine gamma globulin was used as a standard.

Qualitative protease analysis was performed by separating the protease enzymes using gel electrophoresis (Ross *et al.* 2000). Separating gels were formulated by mixing 1.175 mL double distilled water (ddH_2O, 0.625 mL 1.5M TrisHCl buffer (pH 8.8), 50 µL SDS (10% w/v) and 2.0 mL 30% Acrylamide/Bis. After degassing for 15 minutes, 0.5 mL

gelatine (50 mg gelatine dissolved in 5 mL boiling ddH_2O), 25 µL 10% ammonium persulfate (APS) and 2.5 µL TEMED were added. Stacking gels were formulated by mixing 3.05 mL ddH_2O, 1.25 mL 0.5M TrisHCl buffer (pH 6.8), 50 µL 10% SDS (w/v) and 0.65 mL 30% Acrylamide/Bis. After degassing for 15 minutes, 25 µL 10% APS and 5 µL TEMED were added.

For each tank, a total of five replicates were run for Days 0 and 60. Five µg of sample protein was added to each well and feed samples were also run but in triplicate. Gels were run at 150V until the dye front had reached approximately 25 mm from the bottom of the plate. Gels were then rinsed in wash buffer for 10 minutes three times on ice and then incubated at 30°C for approximately 19 hours in a temperature controlled agitator (~70 rpm). Following incubation, the gels were stained with 0.1% amido black in $MeOH/H_2O/AcOH$ (45:45:10) for 1 h and destained with $MeOH/H_2O/AcOH$ (50:48:2) until desired contrast was obtained.

2.2.3 Analysis of zymography gels

Zymography gels were saved as digitised images and the resultant data were a qualitative assessment of the absence/presence of protease enzymes in the oysters and no quantitative measure of protease activity was attempted. Digitised images of zymography gels were used to identify protease protein bands and low molecular weight protein markers were used to estimate the size of the proteases.

2.3 Experiment 3

2.3.1 Experimental setup

This experiment was conducted at Unique Seafarms Inc., a commercial scale multi-species bivalve hatchery located in Cedar, British Columbia (49° 06.184' N, 123° 48.201' W). For this experiment, three discrete recirculating feeding systems were used, each consisting of a modified circular 165-L reservoir tank (106 cm x 58 cm ID; Barr Plastics, Abbotsford, British Columbia, Canada) connected to an upper 135-L tank (57 cm x 39.5 cm x 61 cm) fitted with three round upwellers (55 cm x 12 cm ID) with 100 μm nitex screen to retain the oysters. A water pump (Iwaki PM20) was plumbed in series to pump water from the bottom of the reservoir tank to the upper tank. All incoming seawater was filtered through two sand filters, then a UV-filtration system and was assumed to be free of particles larger than 1 μm in size. Bloom tank water was introduced into the tanks without filtration. Aeration was provided by the water returning from the upper tank into the reservoir tank. When the oysters were not being fed, fresh seawater was pumped into the upwellers and the tanks acted as a flow-through system. Both the bloom tank seawater and the incoming fresh seawater were at ambient temperature levels and were unheated prior to introduction into the feeding systems.

Ambient seawater was used and this resulted in water temperatures similar to local winter marine conditions (Harrison *et al.* 1983). Temperature (±0.3°C) was measured using automatic temperature loggers (Vemco Incorporated Minilog TX 8-Bit -5°C +35°C). Temperature was measured in all of the tanks to assess any difference in

temperature between the mixed diets and the 100% bloom tank algae. Temperature range varied in each of the three measured tanks due to the different initial temperatures of the introduced seawater containing the feeds and as a result of the surrounding air temperature gradually warming the water during the 24-hour static feeding cycle.

The test oysters were provided by Unique Seafarms from their nursery surplus for use in this experiment. The test oysters were oysters from the 2002 growing season that were unsold and had been unfed for most of the winter period. They were of a poor overall quality in comparison to other faster growing seed from the same year class. Therefore, the oysters used for this experiment were determined to be of a poor overall quality and unresponsive to the bloom tank diet. Unlike Experiment 1, the oysters were not fed prior to the beginning of the experiment and they were placed in the experimental tanks for 48 hours in ambient running seawater to acclimatise before commencement of the feeding trial.

As in Experiment 1, 300 mL of oysters were added to each upweller to give a total of 900 mL of oysters per tank. Diets were fed as 1% of the initial dry weight per tank of oysters calculated prior to the start of the experiment by lyophilising three subsamples (~30 individual oysters) of juvenile oysters and using the moisture content to determine the initial oyster dry weight per tank.

The oysters were batch fed to satiation levels (1% initial dry weight of oysters) once per day and the tanks were left to recirculate for approximately 24 hours for 30 days. The oysters were assumed to be satiated following the feeding period because food particles were still present. Therefore, the tanks were completely drained and rinsed with cold filtered seawater before addition of the diets. The experiment lasted from January 25th – February 23rd, 2003. The 30 day time period was chosen strictly for logistical reasons as a longer experiment would not enable completion by August 2003. The 24-hour feeding cycle was also chosen for logistical reasons as the after hours access required for a shorter feeding time was not possible at Unique Seafarms Inc.'s facility.

2.3.2 Summary of diets tested

The two *Schizochytrium*-based diets that were used as live algae substitute diets were Algamac 2050™ and Rotimac™ supplied by Aquafauna Biomarine (Hawthorne, CA, USA). Diets were prepared by mixing a pre-weighed amount in ambient sand- and UV-filtered seawater for approximately 3 minutes.

Three diet formulations were tested: a 1:1 (dw:dw) formulation of Rotimac™ and *Thalassiosira nordenskioeldii*, a 1:1 (dw:dw) formulation of Algamac 2050™ and *T. nordenskioeldii* and a 100% *T. nordenskioeldii* formulation. Table 2 shows the proximate component values for each of the pure diets as well as theoretical compositions of the mixed diets based on equal contributions. Table 3 outlines the fatty acid values for each of the pure diets as well as the two mixed diets. Myristic acid

(14:0), palmitic acid (16:0) and docosahexaenoic acid (DHA; 22:6ω3) fatty acid values in the Algamac 2050™ and Rotimac™ diets were much higher than the *T. nordenskioeldii* diet; however, eicosapentaenoic acid (EPA; 20:5ω3) was higher in the *T. nordenskioeldii* diet (Table 3).

All diets were measured on a dry weight basis with moisture content of the live algae substitute diets determined prior to the commencement of the experiment. The feeding ration was determined as 1% of the initial dry weight of the oysters. The dry weight of the oysters was calculated using the average moisture content value of three sub-samples taken prior to the start of the experiment. The initial volume of oysters in each upweller was converted to a dry weight equivalent and the dry weight of the ration was determined. For determination of the live algae ration size, 50 mL of live algae was filtered through a pre-weighed, glass-fibre filter (combusted at 500°C for 48 hours) that was lyophilised and reweighed to determine dry weight per mL. This estimate was used to calculate the ration size in litres added to the tanks. In the mixed diet tanks, after addition of the bloom tank algae ration, fresh seawater was added to make the final tank volume double that in the 100% *T. nordenskioeldii* tank.

Dissolved oxygen was measured daily and ammonia was measured once per day for the first week of the experiment and ammonia was assumed to be below detectable levels for the remainder of the experiment.

2.3.3 Algal culture

Bloom tank algae was grown in outdoor 2000-L concrete tanks supplied with inorganic fertilisers and organic waste by-products provided by recirculating seawater from bivalve rearing tanks. A series of four 2000-L concrete tanks with 2.7 cm ($^3/_4$") diameter PVC outlets were plumbed in parallel to a junction with an electric pump that was hooked up to a sterilised rubber hose to deliver bloom tank algae for use in the experiment (rinsed with ~80°C freshwater prior to use). Natural sunlight and ambient outside temperature were used for promoting phytoplankton growth in the bloom tanks.

Daily phytoplankton samples were taken to assess the dry weight per mL of bloom tank algae. Samples for determination of chlorophyll a were also taken every 3 days. Every 7 days from the commencement of the experiment, 40 L of bloom tank algae was concentrated by centrifugation with a Sharples centrifuge at 12,000 rpm, providing a paste that was transferred to a plastic bag, frozen to -80°C, lyophilised, and stored at -80°C until analysed.

2.3.4 Biochemical analysis of oysters and test diets

Two oyster samples of approximately 1.5 g wet weight were taken every 6 days from each upweller (yielding triplicate samples per diet) immediately before feeding. The oysters were then placed in filtered flowing seawater for 24 hours to allow for clearing of the gut contents prior to analysis of the biochemical components. Samples were rinsed with cold 0.5 N ammonium formate to remove excess salt, patted dry with a paper towel

to absorb excess water and then weighed to determine wet weight. Next, the samples were stored in sealed plastic vials and placed at -80°C for 24 hours, lyophilised in a freeze-drying unit for 48 hours and re-weighed to determine dry weight and to calculate original moisture content. All samples were sealed in plastic vials, vacuum-sealed in a plastic bag, stored at -80°C and were analysed within 6 months of sampling.

The two separate samples were used for measurements and determination of biochemical components. Survival was estimated by counting the amount of empty bottom valves present in the measurement sample (dead oysters were determined by gently pushing on the umbo and those shells where the valves separated easily and no organic tissue remained inside were counted as dead) versus live oysters. A sub-sample of 30 live oysters was used to measure shell height, width and depth (±0.02 mm) using digital calipers to (Mitutoyo Model #CD-8"CS). The biochemical component sample, following the removal of dead oysters, was manually ground to a fine powder using a mortar and pestle. This powdered sample was then used in analyses of ash content, crude protein, lipid, mono- and oligosaccharide, polysaccharide and fatty acids.

To determine ash content and ash free dry weight (AFDW), triplicate samples of approximately 100 mg were weighed into pre-combusted (500°C for 24 hours and stored in a desiccator) porcelain crucibles with aluminium foil covering the opening to prevent loss of sample from explosive combustion. Samples were combusted at 500°C for 48

hours and placed in a desiccator after cooling to 100°C. Samples were weighed to within 0.01 mg after cooling to room temperature on a microbalance (model Mettler AT201).

Test diets were lyophilised to determine ash free dry weight and stored at -80°C for a maximum of 8 months prior to analysis. Bloom tank samples were taken every 7 days by centrifugation with a Sharples centrifuge at 12,000 rpm. The centrifugation provided a paste that was transferred to a plastic bag, frozen to -80°C, lyophilised, and stored at -80°C until analysed.

Crude protein, lipid, mono- and oligosaccharide, and polysaccharide concentrations were determined using the methodologies described by Whyte (1987). Fatty acid concentrations were determined using the methodology described by Whyte (1988).

2.3.5 Statistical analysis

See section 2.1.4 for a description of the statistical methods used for analysis. All results were reported to ± one standard error (± SE).

3.0 Results

3.1 Experiment 1

3.1.1 Efficiency of diet uptake over a 6 hour feeding cycle

The diet feeding efficiency ranged in value from 25.14% (T-Iso) – 88.58% (Rotimac™/T-Iso) (Figure 2). The two mixed diets (Algamac 3050™/T-Iso and Rotimac™/T-Iso) yielded the highest percentage of ingested cells (assuming insignificant settlement of cells) at 81.05% for the Algamac 3050™/T-Iso diet and 88.58% for the Rotimac™/T-Iso diet (Figure 2). Statistical analysis was not performed on the data as there were only single samples analysed for each diet and time period.

3.1.2 Experimental temperature

The mean temperature in the Algamac 3050™/T-Iso tank was 9.49 ± 0.01°C (range 7.90 – 12.25°C). The mean temperature in the T-Iso tank was 9.59 ± 0.01°C (range 7.87 – 12.66°C). The mean temperature in the starved control tank was 9.07 ± 0.01°C (range 7.82 – 11.83°C).

3.1.3 Shell height, dry weight and ash-free dry weight

The initial shell height for test oysters (*Crassostrea gigas*) was 9.59 ± 0.11 mm (± SE). There were no significant differences in shell height between Day 60 oysters and Day 0 oysters for any of the test diets and the starved control. The range in shell height at Day 60 was 9.31 ± 0.26 mm (Algamac 3050™ diet) to 10.35 ± 0.31 mm (Rotimac™/T-Iso diet).

The initial dry weight per individual test oyster was 60.67 ± 0.42 mg (± SE). Table 4 shows the mean initial and Day 60 individual oyster dry weight per tank and the percentage change in dry weight. The range in individual oyster dry weight at Day 60 was 52.46 ± 0.35 mg (starved control) to 125.24 ± 1.54 mg (Algamac 3050™/T-Iso) (Table 4). Both mixed diets (Algamac 3050™/T-Iso and Rotimac™/T-Iso) and the T-Iso diet Day 60 dry weight per oyster were significantly higher than the mono-*Schizochytrium* diets and the starved control (Table 4). Individual dry weight for oysters fed the Algamac 3050™/T-Iso diet was significantly higher than the Rotimac™/T-Iso and T-Iso diets at Day 60 and there was no significant difference between the Rotimac™/T-Iso and T-Iso diets at Day 60 (Table 4). The instantaneous growth rates (k) for each diet tested were as follows: 0.012 (AM3050™/T-Iso), 0.001 (AM3050™), 0.008 (Rotimac™/T-Iso), 0.001 (Rotimac™), 0.008 (T-Iso), -0.002 (Starved).

The ash-free dry weight (AFDW) per oyster at Day 0 was 4.63 ± 0.26% (Table 5). The AFDW values at Day 60 ranged from 9.39 ± 0.58% (T-Iso) to 5.50 ± 0.32% (Algamac 3050™) (Table 5). AFDW was significantly higher in the Algamac 3050™/T-Iso, T-Iso and starved control diet compared to the *Schizochytrium* mono-diets (Algamac 3050™ and Rotimac™) (Table 5). AFDW in the Rotimac™/T-Iso diet was not significantly different from the Algamac 3050™ diet (Table 5).

3.1.4 Crude protein content

Crude protein content (%) at Day 60 for oysters fed both mixed diets (Algamac 3050™/T-Iso and Rotimac™/T-Iso), as well as the T-Iso-fed oysters, were significantly higher ($p<0.05$) than the oysters at Day 0 (Figure 4). At Day 60 oysters fed both mixed diets (Algamac 3050™/T-Iso and Rotimac™/T-Iso) had significantly higher crude protein content (%) than oysters fed the *Schizochytrium*-based diets (Algamac 3050™ and Rotimac™) and the starved control (Figure 4). The crude protein content (%) for oysters fed the T-Iso diet was significantly higher than oysters fed the Algamac 3050™ and the starved control; however there was no significant difference between oysters fed the T-Iso and Rotimac™ diets (Figure 4).

Crude protein content (dw) per oyster at Day 60 ranged from 4.87 ± 0.13 mg (Algamac 3050™/T-Iso) to 1.20 ± 0.02 mg (starved control) (Figure 5). The Algamac 3050™/T-Iso-fed oysters had crude protein content (dw) that was significantly higher than all other diets (Figure 5). Oysters fed the Rotimac™/T-Iso and T-Iso crude protein content (dw) were not significantly different but they were significantly higher than oysters fed both mono-diets (Algamac 3050™ and Rotimac™) and the starved control oysters (Figure 5).

3.1.5 Crude lipid content

Crude lipid content (%) at Day 60 for oysters fed all diets was significantly higher than the oysters at Day 0 (Figure 6). At Day 60 oysters fed the AM3050™/T-Iso diet had significantly higher crude lipid content (%) than all other diets (Figure 6).

Crude lipid content (dw) at Day 60 ranged from 0.12 ± 0.01 mg (starved control) to 1.40 ± 0.09 mg (Algamac 3050™/T-Iso) (Figure 7). Oysters fed the Algamac 3050™/T-Iso diet had crude lipid content (dw) that was significantly higher than oysters fed all other diets (Figure 7). Oysters fed all remaining diets were significantly higher than the starved control oysters at Day 60 (Figure 7).

3.1.6 Crude mono/oligosaccharide content

Oyster crude mono/oligosaccharide content (% and dw) at Day 60 for all diets was not significantly higher than the oysters at Day 0 (Figures 8, 9). Tamhane's test was used to analyse significant differences between tanks at Day 0 and Day 60 as equal variances could not be assumed (Levine's test of homogeneity of variances $p < 0.001$).

Oyster crude mono/oligosaccharide content (dw) at Day 60 ranged from 0.06 ± 0.01 mg (starved control) to 0.43 ± 0.08 mg (Algamac 3050™/T-Iso) (Figure 9).

3.1.7 Crude polysaccharide content

Oysters fed the Rotimac™/T-Iso and T-Iso diets showed a significantly higher crude polysaccharide content at Day 60 compared to Day 0 (Figure 10). Oysters fed the mixed Rotimac™/T-Iso crude polysaccharide content were not significantly different from oysters fed the Algamac 3050™/T-Iso diet at Day 60. Oysters fed the T-Iso diet had

significantly higher crude polysaccharide content than all diets with the exception of oysters fed the Rotimac™/T-Iso diet at Day 60 (Figure 10).

Oyster crude polysaccharide content (dw) at Day 60 ranged from 0.07 ± 0.01 mg (starved control) to 2.25 ± 0.20 mg (T-Iso) (Figure 11). The Rotimac™/T-Iso fed oysters' crude polysaccharide content (dw) was significantly higher than all other diets except for the T-Iso and Algamac™/T-Iso diets (Figure 11). Oysters fed the T-Iso diet were significantly higher than all diets except for oysters fed the Rotimac™/T-Iso (Figure 11). Oysters fed all remaining diets were not significantly higher than the starved control oysters at Day 60 (Figure 11).

3.2 Experiment 2

3.2.1 Protease activity

The oysters at Day 0 showed a single intense band of proteases between 53 and 91 kilo Daltons (kDa; Figure 12). Oysters fed both mixed diets (Algamac3050™/T-Iso, Rotimac™/T-Iso) and the T-Iso reference diet showed higher protease activity as well as a higher diversity of protease molecular weights after 20 and 60 days of feeding (Figures 13 - 16). There were no protease bands present <53 kDa in oysters fed the 100% *Schizochytrium*-based diets and the starved control diet following 20 and 60 days of feeding (Figure 13 - 16). At Day 20, oysters fed the two mixed *Schizochytrium* diets and the T-Iso diet showed two distinct protease bands of approximately 35 and 29 kilo Daltons (Figures 13 and 14). These protease bands were also present in oysters fed the

mixed and T-Iso diets after 60 days of feeding. The lower molecular weight protease bands (29 – 35 kDa) were only present in oysters fed the diets that produced an effective growth response (Figures 15 and 16).

3.3 Experiment 3

3.3.1 Experimental temperature

The mean temperature in the Rotimac™/*Thalassiosira nordenskioeldii* tank was 10.32 ± 0.01°C (range 10.02 – 11.13°C). The mean temperature in the Algamac 2050™/*T. nordenskioeldii* tank was 10.22 ± 0.01°C (range 9.78 – 11.02°C). The mean temperature in the *T. nordenskioeldii* control tank was 10.18 ± 0.01°C (range 10.00 – 10.87°C).

3.3.2 Survival

Oyster survival ranged from 86.5 ± 0.6 % (Rotimac™/*T. nordenskioeldii*) to 81.3 ± 1.9 % (*T. nordenskioeldii*) at Day 30. Survival at Day 30 for oysters fed the Algamac 2050™/*T. nordenskioeldii* diet was 86.2 ± 1.9 % (±SE). There were no significant differences in survival among oysters fed the test diets at Day 30.

3.3.3 Dry weight and ash-free dry weight

The initial (Day 0) dry weight per individual test oyster was 15.92 ± 0.20 mg(Figure 17). The range in individual oyster dry weight at Day 30 was 17.99 ± 0.97 mg (*T. nordenskioeldii*) to 31.28 ± 0.34 mg (Algamac 2050™/*T. nordenskioeldii*) (Figure 17). The individual dry weight for oysters fed both mixed diets (Algamac 2050™/*T.*

nordenskioeldii and Rotimac™/*T. nordenskioeldii*) was significantly higher than oysters at Day 0 and oysters fed the Day 30 *T. nordenskioeldii* diet (Figure 17). There was no significant difference among the mixed diets at Day 30 and between the *T. nordenskioeldii* diet at Day 0 and Day 30 (Figure 17). The instantaneous growth rate (k) was 0.022 day^{-1} for both mixed diets and 0.003 day^{-1} for the *T. nordenskioeldii* diet.

The oyster ash-free dry weight (AFDW) at Day 0 was 4.86 ± 0.17% (Figure 18). The oyster AFDW values at Day 30 ranged from 11.11 ± 0.63% (Rotimac™/*T. nordenskioeldii*) to 9.23 ± 0.22% (*T. nordenskioeldii*) (Figure 18). AFDW among oysters fed all test diets at Day 30 was significantly higher than the Day 0 oysters (Figure 18). There were no significant differences in AFDW among the test diets at Day 30 (Figure 18).

3.3.4 Crude protein content

Oyster crude protein content (%) was 3.10 ± 0.34 at Day 0 and ranged from 6.21 ± 0.18 (Algamac 2050™/*T. nordenskioeldii*) to 6.54 ± 0.39 (Rotimac™/*T. nordenskioeldii*) at Day 30 (Figure 19). Oyster crude protein content (%) at Day 30 was significantly higher than at Day 0 for all test diets (Figure 19). There was no significant difference among oysters fed each of the diets at Day 30 (Figure 19).

Oyster crude protein content (dw) at Day 30 ranged from 2.03 ± 0.12 mg (Rotimac™/*T. nordenskioeldii*) to 1.16 ± 0.06 mg (*T. nordenskioeldii*) (Figure 20). All test oysters (all

diets) at Day 30 had a significantly higher protein content (dw) than at Day 0 (Figure 20). Oyster crude protein content (dw) for both mixed diets (Rotimac™/*T. nordenskioeldii* and Algamac 2050™/*T. nordenskioeldii*) were significantly higher than oysters fed the *T. nordenskioeldii* diet at Day 30 (Figure 20). There was no significant difference in oyster crude protein content (dw) between the mixed diets at Day 30 (Figure 20).

3.3.5 Crude mono/oligosaccharide content

Oyster crude mono/oligosaccharide content (%) at Day 0 was 0.107 ± 0.007 (Figure 21). Oyster crude mono/oligosaccharide content (%) ranged from 0.197 ± 0.013 (Rotimac™/*T. nordenskioeldii*) to 0.150 ± 0.016 (*T. nordenskioeldii*) at Day 30 (Figure 21). Oysters fed the Rotimac™/*T. nordenskioeldii* diet had crude mono/oligosaccharide content (%) at Day 30 that was significantly higher than oysters at Day 0 (Figure 21). There were no significant differences among oysters fed each of the diets at Day 30 and no difference between the Day 30 and Day 0 oyster crude mono/oligosaccharide contents (%) for the Algamac 2050™/*T. nordenskioeldii* and the *T. nordenskioeldii* diets (Figure 21).

Oyster crude mono/oligosaccharide content (dw) at Day 0 was 0.017 ± 0.001 mg (Figure 22). Oyster crude mono/oligosaccharide content (dw) ranged from 0.061 ± 0.004 mg (Rotimac™/*T. nordenskioeldii*) to 0.028 ± 0.003 mg (*T. nordenskioeldii*) at Day 30

(Figure 22). There were no significant differences among oysters fed the test diets at Day 30 and Day 0 (Figure 22).

3.3.6 Crude polysaccharide content

Oyster crude polysaccharide content (%) at Day 0 was 0.88 ± 0.02 % (Figure 23). Oyster crude polysaccharide content (%) ranged from 2.78 ± 0.21 % (Rotimac™/*T. nordenskioeldii*) to 2.22 ± 0.07 % (Algamac 2050™/*T. nordenskioeldii*) at Day 30 (Figure 23). The *T. nordenskioeldii* fed oysters at Day 30 had a significantly higher crude polysaccharide content than at Day 0 (Figure 23). There were no significant differences among oysters fed each of the diets at Day 30 and the crude polysaccharide content (%) of oysters fed the mixed diets (Rotimac™/*T. nordenskioeldii* and Algamac 2050™/*T. nordenskioeldii*) was not significantly different from the Day 0 value (Figure 23).

Oyster crude polysaccharide content (mg) at Day 0 was 0.14 ± 0.01 (Figure 24). Oyster crude polysaccharide content (mg) ranged from 0.86 ± 0.06 mg (Rotimac™/*T. nordenskioeldii*) to 0.47 ± 0.02 (*T. nordenskioeldii*) at Day 30 (Figure 24). There were no significant differences in crude polysaccharide content (mg) among oysters fed the test diets at Day 30 and among oysters fed the test diets at Day 30 and the Day 0 value (Figure 24).

3.3.7 Crude lipid content

Crude lipid content (%) at Day 0 was 0.31 ± 0.01 % (Figure 25). Crude lipid content (%) ranged from 1.27 ± 0.11 % (Algamac 2050™/*T. nordenskioeldii*) to 0.73 ± 0.04 % (*T. nordenskioeldii*) at Day 30 (Figure 25). There were no significant differences among oysters fed each of the diets at Day 30 or Day 0 (Figure 25).

Oyster crude lipid content (mg) at Day 0 was 0.06 ± 0.01 (Figure 26). Oyster crude lipid content (mg) ranged from 0.94 ± 0.09 (Algamac 2050™/*T. nordenskioeldii*) to 0.13 ± 0.01 (Rotimac™/*T. nordenskioeldii*) at Day 30 (Figure 26). There were no significant differences in crude lipid content (mg) of oysters fed each of the diets at Day 30 and Day 0; however, the crude lipid content of oysters fed the *T. nordenskioeldii* diet was significantly higher at Day 30 than oysters fed the Rotimac™/*T. nordenskioeldii* diet at Day 30 (Figure 26).

3.3.8 Fatty acid content

Table 6 shows the fatty acid content ($mg \cdot g^{-1}$ dry weight and % total fatty acid) for juvenile oysters at Day 0 and at Day 30. Myristic acid (14:0) content (% TFA) increased approximately 200% in the oysters fed the Algamac™ 2050 mixed diet and there was little increase in myristic acid (14:0) in the oysters fed the Rotimac™ mixed diet and the bloom tank algae diet (Table 6). Palmitic acid (16:0) content (% TFA) in the oysters following 30 days of feeding showed a similar increase (~100%) in the oysters fed the lipid-rich Algamac 2050™ mixed diet, as compared to the Rotimac™ mixed diet and the

bloom tank algae diet (Table 6). Eicosapentaenoic acid (20:5n3) content (% TFA) increased ~100% in the oysters fed the bloom tank algae (comprised mainly of the diatom species *Thalassiosira nordenskioeldii*). Docosahexaenoic acid (22:6n3) content (% TFA) showed the largest increase in the oysters fed the Algamac 2050™ mixed diet (~ 140%).

4.0 Discussion

The mixed diets tested as partial replacements for a live phytoplankton diet produced identical growth characteristics to the *I. galbana* (clone T-Iso) reference diet in the case of the Rotimac™/T-Iso diet ($k = 0.008\ day^{-1}$) and superior growth with respect to the Algamac 3050™/T-Iso diet ($k = 0.012\ day^{-1}$). This indicated that both Algamac 3050™ (AM3050™) and Rotimac™ (RM™) were effective as lower-cost partial replacements for a live phytoplankton diet in a hatchery or nursery setting. Complete replacement of live microalgae by AM3050™ and RM™ were also tested and both diets failed to produce growth. Growth characteristics for the 100% *Schizochytrium*-based diets were not significantly different than the starved control diet following 60 days of feeding. The pre-Experiment 1 measurement of suspended particles during a six hour feeding cycle demonstrated that cells were being actively removed from the recirculating seawater. There was active filter feeding of the suspended AM3050™ and RM™ particles but it is likely that they were rejected as pseudofaeces. Both Boeing (1997) and Brown and McCausland (2000) concluded that a similar *Schizochytrium*-based diet (AM2000™) was ineffective as a single diet for feeding juvenile *Crassostrea gigas*. Experiment 1 showed that AM3050™ and RM™ are also ineffective as single diets. *Schizochytrium* was shown to be effective as single diet for feeding *Mytilus galloprovincialis* so it is likely that *C. gigas* lacks the necessary enzymes to derive sufficient nutrition from *Schizochytrium* cells (Whyte *et al.* 2003).

Comparison to past studies is difficult given the lower experimental temperature used in Experiments 1 and 3. Both feeding experiments were undertaken during the northern temperate winter season (between November and April). This resulted in experimental seawater temperatures that were not optimal for achieving maximum growth potential.

The low experimental seawater temperatures (9 - 10°C) used for both feeding experiments caused a lack of a comparable growth response to previous studies that used higher experimental temperatures. There are no prior feeding studies involving *C. gigas* that used temperatures lower than 14°C (Langdon and Waldcock 1981; Waldcock and Holland 1984; Parker and Selivonchick 1986; Knauer and Southgate 1996; Thompson *et al.* 1996; Knauer and Southgate 1997; Brown *et al.* 1998; Caers *et al.* 2000; Robert *et al.* 2001; Flores-Vergara *et al.* 2004). Enzymatic systems function more efficiently with rising temperature until excessively high temperatures cause damage to the enzyme's protein structure and limit the effectiveness (Dahlhoff *et al.* 2002). For molluscs, there is a linear relationship between the rate of enzymatic activity and temperature with oxygen consumption increasing with increased enzymatic activity (Dahlhoff *et al.* 2002). Therefore, the low experimental temperatures limited the amount of growth possible during the experimental period because metabolic activity was not operating efficiently. Due to the effect of low temperature on metabolic rates of ectothermic bivalves it is assumed that the growth response would be higher in seawater >14°C. McCausland *et al.* (1999) fed juvenile oysters *I. galbana* (clone T-Iso) and achieved an instantaneous growth rate of 0.059 day^{-1} at temperatures ranging from 15.4 – 17.8°C, 637% higher than

the *I. galbana* (clone T-Iso) fed oysters in the present study ($k = 0.008\ day^{-1}$; Experiment 1). In laboratory studies, the use of higher experimental temperatures is beneficial as it takes advantage of higher metabolic activity and contributes to larger differences in growth parameters such as shell height and dry weight (Flores-Vergara *et al.* 2004). It also mimics the optimal growing conditions that occur during summer months (May – September) when oyster growth is accelerated by high natural phytoplankton abundance and elevated oceanic temperatures. Although this practice will accentuate differences in dietary outcome, it fails to elucidate the effect that differing diets would have on *Crassostrea gigas* under non-ideal conditions likely to occur outside of the relatively small summer window of maximum growth.

The winter period represents a time of the year where food availability is constrained by low seawater temperatures and natural light. It is a period that could greatly enhance growth and survival of existing farm stock if the oysters were able to postpone the winter hibernation-like state and continue feeding. Filter-feeding is accelerated and digestion is faster and more efficient as enzymatic systems function at peak capacity. Faster digestion of incoming nutrients allows more excess energy to be available as base metabolic demands are met relatively quickly allowing surplus energy to be used for anabolic processes or stored for later use.

Although the maximum growth rate for Experiment 1 was lower than in past studies using higher temperatures (McCausland *et al.* 1999; Brown and McCausland 2000),

when the lower metabolic rate and poor initial physiological condition of the test oysters are considered, the results look promising. The overall condition of the oysters improved during the 60 day feeding cycle and there were increases in all proximate components for both mixed diets and the reference diet. Lower seawater temperature was a factor in the lower growth rate; however, the oysters were previously identified as poor performing seed and grew slower than other oysters in the same cohort under identical growing conditions. Therefore, it is possible that poor genetic characteristics were partially responsible for the lower growth rate.

The low initial condition index[1] (4.63 ± 0.26%) of the test oysters indicated that they were nutritionally depressed with low energy stores. A survey of incoming seed lots for Pearl Sea Products from 2000 – 2002 showed a range in initial condition indices for 2 – 6 mm juveniles of 6 – 13 % (Babuin, unpublished data). Although more research is needed to correlate initial condition index with growth and survival, it is assumed that oysters with condition indices <6 % represent individuals with depleted energy stores. The combination of low experimental temperature and poor genetic traits contributed to a minimal change in shell height following 60 days of feeding. There are no previous oyster feeding studies that used slow-growing oysters for dietary testing (Langdon and Waldcock 1981; Waldcock and Holland 1984; Parker and Selivonchick 1986; Knauer and Southgate 1996; Thompson *et al.* 1996; Knauer and Southgate 1997; Brown *et al.* 1998; McCausland *et al.* 1999; Caers *et al.* 2000; Robert *et al.* 2001). The use of slow-

[1] Condition index was calculated as CI = ((O/C)*100) where O = organic content and C = ash content

growing or poor performing oysters and winter conditions for Experiments 1 and 3 allowed for a rigorous assessment of the effectiveness of supplemental feeding for increasing energy stores prior to the limited *C. gigas* growing season.

The low experimental temperature resulted in a minimal change in shell height among all diets tested, a measure of dietary effectiveness in some past studies (Holland and Spencer 1973; Holland and Hannant 1974; Wilson 1978; Brown 1988). If only changes in shell height were used to assess dietary effectiveness, the results of both feeding experiments would suggest that all diets tested were ineffective. Change in shell height is a popular measure of dietary effectiveness as it reflects the rate at which the experimental oysters will reach a marketable size as well as being a cost-effective means to assess differences between diets. A prior study (Holland and Spencer 1973) showed that shell height increased while juvenile *Ostrea edulis* were being starved and it cannot be a useful indicator of dietary effectiveness for shorter duration studies (<30 days). This is further supported by the resulting 106% increase in mean weight for the Algamac 3050™/T-Iso diet during Experiment 1 coupled with a 7% increase in shell height following 60 days of feeding.

Most nutritional studies measure shell height and dry weight as indicators of the dietary performance. Complete proximate analysis represents a more thorough approach to understanding the effect that different diets have on the physical makeup of the oysters and how dietary energy is utilised and stored. Proximate analysis provides a powerful

tool for assessing dietary effectiveness and determining nutritional requirements for achieving superior growth. The low experimental temperature, poor prior growth characteristics of the test oysters and the limited duration of feeding (60 days) necessitated the addition of proximate analysis for determining differences among test diets.

There were no significant differences in shell height among all diets tested at Day 60 and Day 0 as was discussed previously. The AM3050™/T-Iso diet produced a significant increase in dry weight (mg) over all other diets tested and the RM™/T-Iso and T-Iso diets produced significantly higher average dry weights (mg) than the AM3050™, RM™ and starved control diets at Day 60. The data suggest that live phytoplankton were required to encourage a feeding response or that the AM3050™ and RM™ diets were missing one or more key dietary components necessary to result in growth. The large increase in mean weight compared to the change in shell height indicated that the 60 day feeding cycle represented a period of replenishment in depleted energy stores. Ash free dry weight (AFDW) was significantly higher in the AM3050™/T-Iso and T-Iso diets compared to the AM3050™, RM™ and starved control diets. The subsequent increase in AFDW and condition index supported the conclusion that depleted energy stores were first replenished prior to having excess energy for resuming growth. This lag period in the resumption of growth was also influenced by the low temperature. The oysters' metabolic rate was negatively affected by the low experimental temperature and it is likely that the oysters were not able to assimilate all available dietary energy despite the

satiation levels of food particles present. Further research is required to assess the effect of low temperature on growth rates and dietary utilisation, specifically, the efficiency of enzymatic function in *C. gigas* and digestibility of all available dietary components at suboptimal temperatures.

Oyster protein content (mg dw) was significantly higher in the AM3050™/T-Iso diet than oysters fed all other diets and this is not surprising given the >100% increase in oyster dry weight (mg) during feeding (Figure 5). The higher oyster protein content of the AM3050™/T-Iso diet represented a 290% increase over the initial Day 0 oyster protein content (Figure 5). The RM™/T-Iso and the T-Iso-fed oysters' protein contents (mg dw) were significantly higher than oysters fed the AM3050™, RM™ and starved control diets. The significantly higher protein content of oysters fed the AM3050™/T-Iso diet indicated that the oysters were utilising dietary protein from the *Schizochytrium* cells. If the oysters were not able to use the protein present in the *Schizochytrium* cells, there would be a comparable increase in protein content to the T-Iso diet or a smaller amount as the mixed AM3050™/T-Iso diet presented a lower density of T-Iso cells. The RM™/T-Iso and T-Iso diets both had higher crude protein content than the AM3050™ diet yet produced a smaller increase in protein content (mg dw). There were also no significant differences in protein content (%) among oysters fed these three diets (Figure 4). This indicated that there were other factors that contributed to the better performance of the AM3050™/T-Iso diet. It is likely that all of the protein assimilated by the oysters

fed the mixed diets was derived from the T-Iso portion of the diet because the oysters were fed to satiation level.

An interesting result was the increase (insignificant) in protein content of the oysters fed the RM™ diet. The higher carbohydrate content of the RM™ diet provided more polysaccharide and monosaccharide for utilisation as energy thus allowing the oysters to conserve protein rather than utilise it as an energy source. This was evident in the decrease in protein content of oysters fed both the AM3050™ diet and the starved control diet (Figure 5). *C. gigas* adults were shown in a past study to utilise protein as a source of energy during extended periods of starvation (Whyte 1994) and the results of Experiment 1 indicate that protein was used during 60 days of starvation by the AM3050™ and starved oysters. An organism cannot grow if protein is not being assimilated from dietary sources and the data from Experiment 2 confirmed this assumption by measuring protease activity in the starved and fed oysters.

Proteases are enzymes that aid in digestion of dietary protein and the presence of proteases may be used to assess the metabolic activity of an organism similar to the use of DNA:RNA ratios as an indicator of metabolic activity (Wright and Hetzel 1985). If protease enzyme molecules are not present, it can be assumed that the test oysters were not assimilating dietary protein and building tissue. Since the structural tissues in oysters are mainly comprised of protein, protease activity was used to determine growth activity. In addition to the evidence of a lack of assimilation of nutritional components from the

Schizochytrium-based diets by the test *C. gigas* juveniles, the results from the analysis of protease activity (Experiment 2) also demonstrate a lack of measureable protease levels in starved oysters and oysters fed mono-*Schizochytrium*-based diets at Day 20 and Day 60 (Figures 13 - 16). Proteases in the molecular weight range of 29 – 35 kDa were only present in oysters that were fed superior diets (Figures 13 – 16). Therefore, the data suggest that proteases were secreted by the test oysters when presented with a suitable diet indicating that digestion and assimilation of dietary protein was taking place. The lack of protease activity in oysters fed both the starved control and the *Schizochytrium*-based diets suggests an indirect correlation between protease levels and weight gain (Figures 3 and 13 – 16).

The use of protease measurements was shown in Experiment 2 to be an effective means of assessing dietary suitability and performance and may serve as an indicator of growth potential of oysters on a particular diet. Further experimentation is needed to assess whether this technique could be used for testing a diet's acceptance in a time period shorter than 30 days. If an effective and replicable protocol was developed, it would have the potential to make diet evaluation much more efficient by eliminating the need for time-consuming proximate analysis and could also save money.

Due to the qualitative nature of the zymography used to assess protease activity, it was not possible to assess the overall rate of protease activity for each of the test diets. Other protease assays could be used to quantify protease levels as a function of diets. It was

clear from the statistically insignificant differences in mean dry weight (mg) among both 100% *Schizochytrium*-based diets and the starved control that the *C. gigas* juveniles were able to enter a hibernation-like state of low metabolic activity and stored energy was mostly conserved for 60 days (Figure 3, Table 4). Figure 3 shows an increase in mean weight for oysters fed both mixed diets (AM3050™/T-Iso and RM™/T-Iso) and the 100% T-Iso diet following 40 days of feeding. This suggests the oysters exited a period of hibernation and low enzymatic activity and were able to more efficiently assimilate available dietary components. When *C. gigas* are presented with nutritionally inferior or foreign food particles, they enter a state of aestivation to conserve energy stores until a more suitable diet is available (J.N.C. Whyte, pers. comm.). The ability to enter an energy conserving state would allow oysters grown in southern BC waters to withstand the extended periods between major algal blooms when natural food abundance is low.

Lipid content (% and dw) was significantly higher in oysters fed the AM3050™/T-Iso diet than all other diets (Figures 6 and 7). The lipid content (%) of oysters fed the AM3050™ diet was 239% higher than the oysters fed T-Iso and it resulted in much higher crude lipid levels (% and mg dw) (Figures 6, 7). All of the remaining diets produced lipid contents (% and mg dw) that were higher than the starved control oysters at Day 60 (Figures 6 and 7). Therefore, it can also be assumed that the *Schizochytrium*-derived dietary lipid was being metabolised by the juvenile *C. gigas* because lipid content was not significantly different among all single diet treatments (T-Iso, AM3050™, RM™). The high content of total lipid present in the AM3050™ diet

appeared to be effectively assimilated when presented with a high quality phytoplankton diet.

There are several past studies that have focussed on total lipid and key fatty acids as being the most important dietary components when evaluating test diets (Langdon and Waldock 1981; Thompson *et al.* 1996; Knauer and Southgate 1997; Caers *et al.* 2000; Pernet 2005; Milke *et al.* 2006, 2008; Garcia *et al.* 2008). The increase in lipid content in oysters fed the AM3050™ diet indicated that there was ingestion and assimilation of the dietary lipid present in the *Schizochytrium* cells (Figures 6 and 7). The increase in percent lipid content and dry weight of lipid in the *Schizochytrium*-based single treatment diets failed to promote growth and the subsequent loss of protein over 60 days indicated that lipid stores were not used as an energy source by the oysters. Dietary lipid present in AM3050™ was assimilated and contributed to the higher growth rate seen in oysters fed the mixed AM3050™/T-Iso diet. The higher lipid content of the AM3050™/T-Iso diet provided the oysters with 31% more available energy per unit measure assuming 1:1 inputs of each diet and total oxidation (Table 1). The RM™/T-Iso diet and the T-Iso provided similar available energy per unit measure and there were no significant differences among lipid content (% and mg dw) for oysters fed those diets. Fatty acid analysis was not completed for Experiment 1 due to time constraints. Fatty acid data were analysed for Experiment 3 and will be discussed along with the results from that experiment.

There were no significant differences among oysters fed each of the diets with respect to monosaccharide content (% and mg dw). This was not an unexpected result considering monosaccharide would be utilised for short term energy requirements or converted to glycogen, a polysaccharide for storage. Therefore, major differences in carbohydrate content among oysters fed the differing diets would be expressed in the change in polysaccharide content over time.

The Rotimac™ diet had the highest carbohydrate content of the test diets (35.0%) yet the T-Iso polysaccharide content was not significantly different from the RM™/T-Iso diet following 60 days of feeding (Figures 10 and 11). This indicated that the dietary carbohydrate present in the RM™ and AM3050™ diets was not being digested. Since T-Iso was shown to support oyster growth, it is likely that the dietary carbohydrate present in the T-Iso in the mixed diets was sufficient to meet both short and long term energy requirements. It is possible that the form of the carbohydrate present in the *Schizochytrium*-based diets was non-digestible by *C. gigas* and that this caused the lack of a growth response in the oysters fed the AM3050™ and RM™ diets. Further research is required to analyse the carbohydrate present in the *Schizochytrium* cells and determine if *C. gigas* is capable of digesting the saccharides present in *Schizochytrium*.

The results of Experiment 1 were consistent with past research involving partial replacement of live phytoplankton by *Schizochytrium*-based diets (Boeing 1997; Brown and McCausland 2000). Both of those research projects tested an earlier *Schizochytrium*

formulation Algamac 2000™. This experiment represents the first time that Algamac 3050™ and Rotimac™ were tested for feeding *C. gigas*. It is clear that *Schizochytrium* did not stimulate feeding and the results suggest that the carbohydrate present in the *Schizochytrium* cells is non-digestible for juvenile *C. gigas*. Oysters fed the AM3050™ and RM™ diets were indistinguishable through proximate analysis from the starved control oysters. The addition of the analysis of protease activity confirmed that there was little digestion of dietary protein taking place (thus indicating a lack of growth activity) and the oysters had entered a hibernation-like state of energy conservation. The low experimental temperature aided in the conservation of energy and it is hypothesised that if the experimental temperature approximated mid-summer conditions (15 - 20°C) the increase in metabolic rate would result in an energy deficit producing higher mortalities. Further research is needed to confirmed this hypothesis.

Both mixed diets (1:1 dw:dw) provided sufficient nutrients to sustain maintenance energy. The lack of a significant increase in shell height following 60 days of feeding, combined with the poor initial condition of the oysters, indicated that excess energy was being used for rebuilding depleted energy reserves and emaciated tissues. The increase in dry weight (mg) for the mixed diets and the T-Iso diet indicated that the diets provided adequate nutrition with the AM3050™/T-Iso diet providing the highest amount of energy ($J \cdot mg^{-1}$) and also producing the highest increase in dry weight (mg) (Table 4). The mixed RM™/T-Iso diet produced results that were very similar to the T-Iso diet; however, RM™ is less expensive (US$ $20 \cdot kg^{-1}$ versus US$ $28 \cdot kg^{-1}$) than AM3050™ and

it is much less expensive than cultured live algae (estimated US$ 200·kg^{-1}: Coutteau and Sorgeloos 1992). AM 3050™ was the superior supplemental diet but both *Schizochytrium*-based diets would result in cost savings for hatcheries, nurseries and grow out operations by increasing storage energy and providing more surplus energy for anabolic processes. A more detailed cost/benefit analysis is required to properly determine the efficiencies of using AM3050™ versus RM™ for supplemental feeding.

Experiment 3 tested a different *Schizochytrium*-based dietary formulation, Algamac 2050™ and Rotimac™, as partial replacements for bloom tank algae (>95% *T. nordenskioeldii*). Survival (%) was highest in the mixed diets (~86) versus the bloom tank algae diet (~81) but was not significantly different. Both mixed diets (AM2050™/*T. nordenskioeldii* and RM™/*T. nordenskioeldii*) resulted in a ~100% increase in both dry weight (mg) (Figure 17) and AFDW (mg) (Figure 18) compared to the control diet (100% *T. nordenskioeldii*) following 30 days of feeding. AFDW was highest in the RM™/*T. nordenskioeldii* (11.1%) and was the highest value of any oysters tested (Experiments 1 and 3). The instantaneous growth rate (k) for both mixed diets (0.022 day^{-1}) was comparable to the growth rate achieved by Brown and McCausland (2000) with juvenile *C. gigas* fed Algamac 2000 (0.024 day^{-1}) as a supplemental diet for naturally occurring phytoplankton. This result is encouraging when one factors in the higher experimental ocean temperatures (14 - 19°C) used by Brown and McCausland (2000).

Oyster protein content (mg) was significantly higher for both mixed diets at Day 30 compared to the control diet and protein content was not a limiting factor in the diets as the *T. nordenskioeldii* diet had the highest crude protein content (35.5%) yet produced the smallest increase in protein content (135% increase in mg dry wt.: Figure 20). There was no significant difference between protein content (% or mg) for oysters fed the mixed diets despite the protein content of the RM™/*T. nordenskioeldii* being ~39% greater than the AM2050™/*T. nordenskioeldii* diet (Figures 19 and 20). Most of the observed difference in protein content (mg dw) between oysters fed the control diet and the mixed diets occurred between day 24 and 30 as the protein content, a measure of overall metabolic activity and growth, of oysters fed the mixed diets increased substantially (Figure 20). Webb and Chu (1983) found that most phytoplankton species that were tested for feeding oysters were able to provide adequate protein and amino acids. The lack of significant difference between the protein content (%) for all diets at day 30 demonstrated that the protein available for all three diets was adequate to promote growth (Figure 19). The dietary protein utilised by the oysters could have been provided exclusively by the *T. nordenskioeldii* portion of the diet because food levels provided were to satiation level. The results of Experiment 1 agreed with the conclusion that the oysters were preferentially utilising the dietary protein available in the phytoplankton portion of the diet.

The RM™/*T. nordenskioeldii* diet resulted in significantly higher monosaccharide content (%) following 30 days of feeding (Figure 21). This result is not surprising

considering the monosaccharide level of the RM™/*T. nordenskioeldii* diet was 46% higher than the AM2050™/*T. nordenskioeldii* diet and 12% higher than the *T. nordenskioeldii* diet (Table 2). The higher level of monosaccharide following 30 days of feeding indicated that the oysters had more simple sugars available for energy conversion. This represented an improvement in the condition of the oysters following 30 days of feeding. The overall level of monosaccharide as a component of the total energy stores of the oysters was small and adequate to meet short term energy requirements.

It is assumed that excess monosaccharide present in the RM™/*T. nordenskioeldii* diet was converted to glycogen for storage. This was evident in the higher percentage of polysaccharide content in the oysters fed the RM™/*T. nordenskioeldii* diet (Figure 23). Polysaccharide content (mg dw) was higher in both mixed diets compared to the *T. nordenskioeldii* diet but there were no significant differences among the oysters following 30 days of feeding (Figure 24).

The polysaccharide glycogen is the major energy storage component in the oysters and its assimilation through carbohydrates in food organisms is paramount. Diatoms have higher carbohydrate content than flagellate species and diatoms comprise the majority of phytoplankton species present during the two major annual microalgae blooms in BC (Harrison *et al.* 1983). The polysaccharide content (%) was significantly higher after 30 days of feeding in the oysters fed the *T. nordenskioeldii* diet despite there being no

significant differences among oysters fed the three diets at Day 30 (Figure 23). The increase in polysaccharide content for the *T. nordenskioeldii* diet indicated that the carbohydrate supplied by the diatom *T. nordenskioeldii* was the main source of digestible carbohydrate for the test oysters. This result agrees with the previous finding (Experiment 1) that the chemical structure of dominant carbohydrates in *Schizochytrium* cells is unaffected by saccharide enzyme activity in *C. gigas* and limits carbohydrate assimilation.

The AM2050™/*T. nordenskioeldii* diet produced oysters with the highest lipid content (% and mg) but there were no significant differences among oysters fed the diets at Day 0 or Day 30 (Figures 25 and 26) despite the >600% difference in lipid content (mg) between the mixed diets (Figure 26). There were no clear trends with regards to the differences between diets as the total energy available in the mixed AM2050™ diet was 15% higher than the *T. nordenskioeldii* diet yet produced an approximate 80% increase in growth (mg).

Palmitic acid (16:0) was 189% and 99% higher in the oysters fed AM2050™/*T. nordenskioeldii* diet than the *T. nordenskioeldii* and RM™/*T. nordenskioeldii* diets, respectively (Table 6). Eicosapentaenoic acid (EPA; 20:5ω3) was highest in oysters fed the *T. nordenskioeldii* diet, reflective of the approximately 100% higher levels in the *T. nordenskioeldii* (Tables 3 and 6). Docosahexaenoic acid (DHA; 22:6ω3) was 87% and 288% higher in the RM™/*T. nordenskioeldii* and AM2050™/*T. nordenskioeldii* diets

compared to the *T. nordenskioeldii* diet (Table 3). Previous research has shown that EPA and DHA are essential fatty acids for *C. gigas* (Langdon and Waldock 1981; Waldock and Holland 1984; Thompson *et al.* 1996; Knauer and Southgate 1997; Caers *et al.* 2000; Knuckey *et al.* 2002). The results of this experiment support those findings with respect to DHA but EPA was highest in the *T. nordenskioeldii* diet that produced the least amount of growth (Table 6). Garcia *et al.* (2008) noted that EPA is a structural component of membranes and can also be oxidised as an energy source. Thompson *et al.* (1996) found that an abundance of dietary EPA had a negative effect on growth rates of larval *C. gigas*. The high retention of DHA in the mixed diets that had the highest increase in dry weight (mg) suggests a major role for this fatty acid in supporting growth. The PUFA docosapentaenoic acid (DPA 22:5n6) was identified in previous feeding studies involving larval sea scallops (*Placopecten magellanicus* and *Argopecten irradians*) as a possible essential fatty acid (Pernet *et al.* 2005; Milke *et al.* 2006, 2008). The juvenile oysters fed the two mixed diets (RM™/*T. nordenskioeldii* and AM2050™/*T. nordenskioeldii*) that demonstrated superior growth to the single *T. nordenskioeldii* diet had DPA levels that increased from trace amount to 4.17% and 6.40% of the total fatty acids respectively (Table 6). Although it is difficult to correlate growth performance to DPA given the similiarity in growth outcome and the varied levels of DPA in AM2050™ and RM™, the accumulation of this fatty acid in the test oysters demonstrating superior growth warrants further research.

There was no clear relationship between oyster growth and the proximate composition of each diet, but the structural nature of components would affect this correlation. Brown *et al.* (1998) tested several phytoplankton diets and also concluded that the growth rates of test *C. gigas* were correlated with available assimilatable energy and not the gross composition of the individual diets. The energy composition of the single phytoplankton diets was a better indicator of dietary outcome (Brown *et al.* 1998) because they contained components assimilatable by the oyster and the results of this experiment are in agreement with those findings with respect to the mixed AM2050™ diet but not the mixed RM™ diet. The total energy ($J \cdot mg^{-1}$) assuming total oxidation was 31% higher in the AM2050™ diet and 8% higher in the RM™ diet when compared to the *T. nordenskioeldii* diet (Table 2). Assuming the oysters ingested food particles in the same ratio (1:1), the total energy ($J \cdot mg^{-1}$) assuming total oxidation was 15% and 4% higher in the mixed AM2050™ and RM™ diets, respectively (Table 2). The energy content of the oysters following 30 days of feeding was approximately 100% higher in both mixed diets compared to the *T. nordenskioeldii* diet (Figure 27).

The use of both AM2050™ and RM™ as 50% replacements for live phytoplankton was more effective than feeding with bloom tank algae (*T. nordenskioeldii*). It is unclear why the mixed *Schizochytrium*-based diets were superior to the bloom tank algae diet. It is generally accepted that mixed diets are better at providing a balanced diet; species of differing proximate composition can provide higher levels of specific nutrients (Webb and Chu 1983). *Schizochytrium*-based diets were insufficient as single diets for *C. gigas*

juveniles and the results of this experiment suggested that the diatom *T. nordenskioeldii* is also lacking one or more key dietary components, possibly lipid or more specifically the fatty acid DHA (22:6ω3). The success of the mixed diets was evident in the higher instantaneous growth rate (0.022 day^{-1}) than the mixed AM3050™/T-Iso diet (0.012 day^{-1}) in Experiment 1. The average experimental temperature was ~1°C higher in Experiment 3 and the oysters were obtained from different origins and, therefore, genetic differences could have also played a role in the difference in peak growth rate between the two experiments.

5.0 Conclusions and Recommendations

Prior studies used bloom tank algae to enhance oyster production and it resulted in comparable growth to feeding with intensively cultured phytoplankton (Spencer *et al.* 1986; Laing and Chang 1998). Bloom tank algae can be produced at remote oyster growing locations using inexpensive culture systems and small amounts of commercially available fertilisers. Supplementation of enhanced natural phytoplankton with *Schizochytrium*-based feeds that are commercially available and can be stored at 4°C for up to one year should enable oyster producers to enhance production in a cost-effective manner. Rotimac™ was the least expensive *Schizochytrium*-based product used in both feeding experiments and it produced enhanced growth under suboptimal experimental temperature conditions using previously identified slow-growing *C. gigas* juveniles. The final proximate composition of the test oysters represented a marked improvement in physical condition and storage energy. The rehabilitation of poorly-performing oysters has the potential to improve time to market, survival and growth efficiency by building up storage energy levels and providing excess energy for fuelling anabolic processes.

The original aim of this research was to test the effectiveness of supplemental feeding as a means of mitigating summer mortality of *C. gigas* juveniles. The lack of a significant difference in survival between test oysters for both feeding experiments seemingly contradicted the hypothesis that the summer mortalities were caused by a lack of sufficient nutrition. There are two factors to consider. First, the experimental temperature was lower than the average oceanic temperature during the Northeast Pacific

summer season (June – September). Secondly, the oysters were only presented with a single component diet consisting mainly of a *Schizochytrium* strain that is naturally occurring in tropical mangrove habitat, or seawater screened to bacterial level (1 μm). *C. gigas* are unable to effectively select particulates <2.5 μm in size (Sornin *et al.* 1988). Therefore, both alternatives presented foreign particulates or no particulates and the oysters were not stimulated to continue to utilise stored energy for feeding activity.

It is hypothesised that low levels of nutritious microalgal species present in natural *C. gigas* growing waters will continue to stimulate feeding activity. Past studies that used chlorophyll content as a measure of overall microalgal abundance showed that low levels of microalgae are consistently present in natural temperate oceanic habitats (Brown and McCausland 2000). Therefore, feeding activity coupled with higher metabolic activity and low natural microalgal availability could result in an energy imbalance that would deplete stored energy. In a juvenile *C. gigas* individual with low stored energy, this situation could result in critically low maintenance energy levels and possible mortality. A contributing factor would also be higher seasonal oceanic temperatures resulting in increased metabolic activity. Flores-Vergara *et al.* (2004) experimented with feeding juvenile *C. gigas* (initial shell height ~3 mm) a superior diet (*I. galbana* (clone T-Iso)/*Pavlova lutheri*) and an inferior diet (*Dunaliella tertiolecta*) at temperatures ranging from 23 - 32°C. They found that at temperatures above 30°C growth was negatively affected regardless of food quality. Oceanic temperatures do not reach 30°C in British Columbian waters and the effect of acclimation to the cooler temperatures prevailing in

northern temperate oceans must be considered. It is probable that growth could be negatively affected at a lower temperature in British Columbian waters. The extended period of low natural phytoplankton availability between the spring and autumn diatom blooms also coincides with peak oceanic temperatures (~24°C). Further research is required to determine if high experimental temperatures and low food availability of diatom of flagellate species will cause an energy imbalance and mortalities in juvenile *C. gigas*.

6.0 References

Albentosa, M., A. Pérez-Camacho, U. Labarta, M.J. Fernández-Reirez. 1997. Evaluation of freeze-dried microalgal diets for the seed culture of *Ruditapes decussatus* using physiological and biochemical parameters. Aquaculture 154: 305 – 321.

Almeida, M.J., J. Machado, J. Coimbra. 1999. Growth and biochemical composition of *Crassostrea gigas* (Thunberg) and *Ostrea edulis* (Linné) in two estuaries from the north of Portugal. J. Shellfish Res. 18(1): 139 – 146.

Alpine, A.E., J.E. Cloern. 1992. Trophic interactions and direct physical effects control phytoplankton biomass and production in an estuary. Limnol. Oceanogr. 37(5): 946 – 955.

Baldwin, B.S., R.I.E. Newell. 1995. Feeding rate responses of oyster larvae (*Crassostrea virginica*) to seston quality and composition. J. Exp. Mar. Biol. Ecol. 189: 77 – 91.

Battaglene, S.C., J.E. Seymour, C. Ramofafia, I. Lane. 2002. Spawning induction of three tropical sea cucumbers, *Holothuria scabra*, *H. fuscogilva* and *Actinopyga mauritiana*. Aquaculture 207: 29 – 47.

Bayne, B.L., D. Hedgecock, D. McGoldrick, R. Rees. 1999a. Feeding behaviour and metabolic efficiency contribute to growth heterosis in Pacific oysters [*Crassostrea gigas* (Thunberg)]. J. Exp. Mar. Biol. Ecol. 233(1): 115 – 130.

Bayne, B.L., S. Svennson, J.A. Nell. 1999b. The physiological basis for faster growth in the Sydney rock oyster, *Saccostrea commercialis*. Biol. Bull. 197: 377 – 387.

Beiras, R., A. Pérez-Camacho, M. Albentosa. 1993. Influence of food concentration on energy balance and growth performance of *Venerupis pullastra* seed reared in an open-flow system. Aquaculture 116: 353 – 365.

Beiras, R., A. Pérez-Camacho, M. Albentosa. 1994. Comparison of the scope for growth with the growth performance of *Ostrea edulis* seed reared at different food concentrations in an open-flow system. Mar. Biol. 119: 227 – 233.

Berthelin, C., K. Kellner, M. Mathieu. 2000. Storage metabolism in the Pacific oyster (*Crassostrea gigas*) in relation to summer mortalities and reproductive cycle (West Coast of France). Comp. Biochem. Phys. B 125: 359 – 369.

Boeing, P. 1999. Use of spray-dried *Schizochytrium* sp. as a partial algal replacement for juvenile bivalves. Pacific Seafarms International S.A. de C.V., Mazatlan, Sinaloa, Mexico (unpublished).

Bougrier, S., A.J.S. Hawkins, M. Héral. 1997. Preingestive selection of different microalgal mixtures in *Crassostrea gigas* and *Mytilus edulis*, analysed by flow cytometry. Aquaculture 150: 123 – 134.

Bradford, M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of proteins using the principle of protein-dye binding. Anal. Biochem. 72: 248 – 254.

Brown, J.R., 1988. Multivariate analyses of the role of environmental factors in seasonal and site-related growth variation in the Pacific oyster *Crassostrea gigas*. Mar. Ecol. Prog. Ser. 45: 225 – 236.

Brown, M.R., 1991. The amino acid and sugar composition of 16 species of microalgae used in mariculture. J. Exp. Mar. Biol. Ecol. 145: 79 – 99.

Brown, M.R., G.A. Dunstan, S.W. Jeffrey, J.K. Volkman, S.M. Barrett, J-M. LeRoi. 1993. The influence of irradiance on the biochemical composition of the prymnesiophyte *Isochrysis* sp. (Clone T-Iso). J. Phycol. 29: 601 – 612.

Brown, M.R., S.M. Barrett, J.K. Volkman, S. P. Nearhos, J.A. Nell, G.L. Allan. 1996. Biochemical composition of new yeasts and bacteria evaluated as food for bivalve aquaculture. Aquaculture 143: 341 – 360.

Brown, M.R., M.A. McCausland, K. Kowalski. 1998. The nutritional value of four Australian microalgal strains fed to Pacific oyster *Crassostrea gigas* spat. Aquaculture 165: 281 – 293.

Brown, M.R., M.A. McCausland. 1999. Evaluation of live microalgae and microalgal pastes as supplementary food for juvenile Pacific oysters (*Crassostrea gigas*). Aquaculture 174(3 – 4): 323 – 342.

Brown, M.R., McCausland M.A. 2000. Increasing the growth of juvenile Pacific oysters *Crassostrea gigas* by supplementary feeding with microalgal and dried diets. Aquaculture Res. 31(8 – 9): 671 – 682.

Brown, M.R., R. Robert. 2002. Preparation and assessment of microalgal concentrates as feeds for larval and juvenile Pacific oysters (*Crassostrea gigas*). Aquaculture 207: 289 – 309.

Caers, M., P. Coutteau, P. Sorgeloos. 2000. Incorporation of different fatty acids, supplied as emulsions or liposomes, in the polar and neutral lipids of the *Crassostrea gigas* spat. Aquaculture 186: 157 – 171.

Cigarría, J., 1999. Effects of age, size, and season on growth of soft tissue in the oyster *Crassostrea gigas* (Thunberg, 1793). J. Shellfish Res. 18(1): 127 – 131.

Cheney, D.P., B.F. MacDonald, R.A. Elston. 2000. Summer mortality of Pacific oysters, *Crassostrea gigas* (Thunberg): Initial findings on multiple environmental stressors in Puget Sound, Washington. J. Shellfish Res. 19(1): 353 – 359.

Coutteau, P., P. Sorgeloos. 1992. The use of algal substitutes and the requirement for live algae in the hatchery and nursery rearing of bivalve molluscs: An international survey. J. Shellfish Res. 11(2): 467 – 476.

Coutteau, P., K. Curé, P. Sorgeloos. 1994. Effect of algal ration on feeding and growth of juvenile manila clam *Tapes phillippinarum* (Adams and Reeve). J. Shellfish Res. 13(1): 47 – 55.

Dahlhoff, E.P., J.H. Stillman, B.A. Menge. 2002. Physiological community ecology: Variation in metabolic activity of ecologically important rocky intertidal invertebrates along environmental gradients. Integr. Comp. Biol. 42(4): 862 – 871.

Enright, C.T., G.F. Newkirk, J.S. Craigie, J.D. Castell. 1986. Evaluation of phytoplankton as diets for juvenile *Ostrea edulis* L. J. Exp. Mar. Biol. Ecol. 96: 1 – 13.

Elston, R.A., J.H. Beattie, C. Friedman, R. Hedrick, M.L. Kent. 1987. Pathology and significance of fatal inflammatory bacteraemia in the Pacific oyster, *Crassostrea gigas* Thuenberg. J. Fish Dis. 10(2): 121 – 132.

Flores-Vergara, C., B. Cordero-Esquivel, A.N. Ceron-Ortiz, A. Nallely, B.O. Arredondo-Vega. 2004. Combined effects of temperature and diet on growth and biochemical composition of the Pacific oyster *Crassostrea gigas* (Thunberg) spat. Aquaculture Res. 35(12): 1131 – 1140.

Friedman, C.S., J.H. Beattie, R.A. Elston, R.P. Hedrick. 1991a. Investigation of the relationship between the presence of a Gram-positive bacterial infection and summer mortality of the Pacific oyster, *Crassostrea gigas* Thunberg. Aquaculture 94(1): 1- 15.

Friedman, C.S., R.P. Hedrick. 1991b. Pacific oyster nocardiosis: Isolation of the bacterium and induction of laboratory infections. J. Inv. Path. 57(1): 109 – 120.

Garcia, A.S., C.C. Parrish, J.A. Brown. 2008. Growth and lipid composition of Atlantic cod (*Gadus morhua*) larvae in response to differently enriched *Artemia franciscana*. Fish. Physiol. Biochem. 34: 77 – 94.

Gouthro, M.A., T.P. Odabasi, E.L. Martin, J.R. Rosowski, J.P. Davis, V.T. Sato. 1998. Properties of a *Schizochytrium* (Chromophyta)-based HUFA enrichment and larval feed (Docosa Gold™) suspended in saltwater. J. Shellfish Res. 17(1): 326 – 327.

Haigh, R., F.J.R. Taylor, T.F. Sutherland. 1992. Phytoplankton ecology of Sechelt Inlet, a fjord system on the British Columbia coast. I. General features of the nano- and microplankton. Mar. Ecol. Prog. Ser. 89(2 – 3): 117 – 134.

Harrison, P.J, J.D. Fulton, F.J.R. Taylor, T.R. Parsons. 1983. Review of the biological oceanography of the Strait of Georgia: Pelagic environment. Can. J. Fish. Aquatic Sci. 40(7): 1064 – 1094.

Harrison, P.J., R.E. Waters, F.J.R. Taylor. 1980. A broad spectrum artificial seawater medium for coastal and open ocean phytoplankton. J. Phycol. 16: 28 – 35.

Helm, M.M., I. Laing. 1987. Preliminary observations on the nutritional value of 'Tahiti *Isochrysis*' to bivalve larvae. Aquaculture 62: 281 – 288.

Holland, D.L., P.A. Gabbott. 1971. A micro-analytical scheme for the determination of protein, carbohydrate, lipid and RNA levels in marine invertebrate larvae. J. Mar. Biol. Ass. U.K. 51: 659 – 668.

Holland, D.L., P.J. Hannant. 1974. Biochemical changes during growth of the spat of the oyster, *Ostrea edulis* L. J. Mar. Biol. Ass. U.K. 54: 1007 – 1016.

Holland, D.L., B.E. Spencer. 1973. Biochemical changes in fed and starved oysters, *Ostrea edulis* L. during larval development, metamorphosis and early spat growth. J. Mar. Biol. Ass. U.K. 53: 287 – 298.

Imai, T., K. Numachi, J. Oizumi, S. Sato. 1965. Studies on the mass mortality of the oyster in Matsushima Bay II. Search for the cause of mass mortality and the possibility to prevent it by transplantation experiment. Bull. Tohoku Reg. Fish. Res. Lab. 25: 27 – 28.

Jørgenson, C.B. 1996. Bivalve filter feeding revisited. Mar. Ecol. Prog. Ser. 142: 287 – 302.

Knauer, J., P.C. Southgate. 1996. Nutritional value of a spray-dried freshwater alga, *Spongiococcum excentricum*, for Pacific oyster (*Crassostrea gigas*) spat. Aquaculture 146: 135 – 146.

Knauer, J., P.C. Southgate. 1997. Growth and fatty acid composition of Pacific oyster (*Crassostrea gigas*) spat fed microalga and microcapsules containing varying amounts of Eicosapentaenoic acid and Docosahexaenoic acid. J. Shellfish Res. 16(2): 447 – 453.

Knauer, J., P.C. Southgate. 1999. A review of the nutritional requirements of bivalves and the development of alternative and artificial diets for bivalve aquaculture. Rev. Fish. Sci. 7(3&4): 241 – 280.

Knuckey, R.M., M. R. Brown, S.M. Barrett, G.M. Hallegraeff. 2002. Isolation of new nanoplanktonic diatom strains and their evaluation as diets for juvenile Pacific oysters (*Crassostrea gigas*). Aquaculture 211: 253 – 274.

Laing, I., R-M. Chang. 1998. Hatchery cultivation of Pacific oyster juveniles using algae produced in outdoor bloom tanks. Aquaculture International 6: 303 – 315.

Langdon, C.J., M.J. Waldcock. 1981. The effect of algal and artificial diets on the growth and fatty acid composition of *Crassostrea gigas* spat. J. Mar. Biol. Ass. U.K. 61: 431 – 448.

Lassus, P., M. Bardouil, B. Beliaeff, P. Masselin, M. Naviner, P. Truquet. 1999. Effect of a continuous supply of the toxic dinoflagellate *Alexandrium minutum* Halim on the feeding behaviour of the Pacific oyster (*Crassostrea gigas* Thunberg). J. Shellfish Res. 18(1): 211 – 216.

Lefebvre, S., Barillé, L., Clerc, M. 2000. Pacific oyster (*Crassostrea gigas*) feeding responses to a fish-farm effluent. Aquaculture 187: 185 – 198.

Maguire, J.A., P.G. Fleury, G.M. Burnell. 1999. Some methods for quantifying quality in the scallop *Pecten maximus* (L.). J. Shellfish Res. 18(1): 59 – 66.

Manahan, D.T. 1983. The uptake and metabolism of dissolved amino acids by bivalve larvae. Biol. Bull. 164: 236 – 250.

Manahan, D.T., W.B. Jaeckle, S.D. Nourizadeh. 1989. Ontogenic changes in the rates of amino acid transport from seawater by marine invertebrate larvae (Echinodermata, Echiura, Mollusca). Biol. Bull. 176: 161 – 168.

Manzi, J.J., N.H. Hadley, M.B. Maddox. 1986. Seed clam, *Mercenaria mercenaria*, culture in an experimental-scale upflow nursery system. Aquaculture 54: 301 – 311.

Matsuyama, Y., H. Usuki, T. Uchida, Y. Kotani. 2001. Effects of harmful algae on the early planktonic larvae of the oyster, *Crassostrea gigas*. Harmful Algal Blooms 2000: 411 – 414.

McCausland, M.A., M.R. Brown, S.M. Barrett, J.A. Diemar, M.P. Heasman. 1999. Evaluation of live microalgae and microalgal pastes as supplementary food for juvenile Pacific oysters (*Crassostrea gigas*). Aquaculture 174: 323 – 342.

Milke, L.M., J.E. Ward. 2003. Influence of diet on pre-ingestive particle processing in bivalves II: Residence time in the pallial cavity and handling time on the labial palps. J. Exp. Mar. Biol. Ecol. 293: 151 – 172.

Milke, L.M., V.M. Bricelj, C.C. Parrish. 2006. Comparison of early life history stages of the bay scallop, *Argopecten irradians*: Effects of microalgal diets on growth and biochemical composition. Aquaculture 260: 272 – 289.

Milke, L.M., V.M. Bricelj, C.C. Parrish. 2008. Biochemical characterization and nutritional value of three *Pavlova* spp. In unialgal and mixed diets with *Chaetoceros muelleri* for postlarval sea scallops, *Placopecten magellanicus*. Aquaculture 276: 130 – 142.

Murata, M., M. Sakaguchi. 1986. Changes in contents of free amino acids, trimethylamine, and nonprotein nitrogen of oyster during ice storage. Bull. Japan. Soc. Sci. Fish. 52(11): 1975 – 1980.

Nell, J.A., J.A. Diemar, M.P. Heasman. 1996. Food value of live yeasts and dry yeast-based diets fed to Sydney rock oyster *Saccostrea commercialis* spat. Aquaculture 145: 235 – 243.

O'Connor, W.A., J.A. Nell, J.A. Diemar. 1992. The evaluation of twelve algal species as food for juvenile Sydney rock oysters *Saccostrea commercialis* (Iredale & Roughley). Aquaculture 108: 277 – 283.

Onal, E., C. Langdon. 1999. Replacement of living microalgae with spray-dried diets for the marine mussel *Mytilus galloprovincialis*. Aquaculture 180(3-4): 283 – 294.

Park, M.S., H.J. Lim, Q. Jo, J.S. Yoo, M. Jeon. 1999. Assessment of reproductive health in the wild seed oysters, *Crassostrea gigas*, from two locations in Korea. J. Shellfish Res. 18(2): 445 – 450.

Parker, R.S., D.P. Selivonchick. 1986. Uptake and metabolism of lipid vesicles from seawater by juvenile Pacific oysters (*Crassostrea gigas*). Aquaculture 53: 215 – 228.

Perdue, J.A. 1983. The relationship between the gametogenic cycle of the Pacific oyster, *C. gigas*, and the summer mortality phenomenon in strains of selectively bred oysters. Washington Univ. Sea Grant Program, Seattle, WA.

Pernet, F., V.M. Bricelj, C.C. Parrish. 2005. Effect of varying dietary levels of ω6 polyunsaturated fatty acids during the early ontogeny of the sea scallop, *Placopecten magellanicus*. J. Exp. Mar. Biol. Ecol. 327: 115 – 133.

Raghukumar, S. 1992. Bacterivory: a novel dual role for thraustochytrids in the sea. Mar. Biol. 113: 165 – 169.

Rice, M.A., G.C. Stephens. 1987. Uptake and internal distribution of exogenously supplied amino acids in the Pacific oyster, *Crassostrea gigas* (Thunberg). Aquaculture 66: 19 – 31.

Robert, R., G. Parisi, L. Rodolfi, B.M. Poli, M.R. Tredici. 2001. Use of fresh and preserved *Tetraselmis suecica* for feeding *Crassostrea gigas* larvae. Aquaculture 192: 333 – 346.

Robinson, A. 1992. Dietary supplements for the reproductive conditioning of *Crassostrea gigas kumamoto* (Thunberg): II. Effects on glycogen, lipid and fatty acid content of broodstock oysters and eggs. J. Shellfish Res. 11(2): 443 – 447.

Rodhouse, P.G., M. O'Kelly. 1981. Flow requirements of the oysters *Ostrea edulis* L. and *Crassostrea gigas* Thunb. in an upwelling column system of culture. Aquaculture 22: 1 – 10.

Ropert, M., P. Goulletquer. 2000. Comparative physiological energetics of two suspension feeders: polychaete annelid *Lanice conchilega* (Pallas 1766) and Pacific cupped oyster *Crassostrea gigas* (Thunberg 1795). Aquaculture 181: 171 – 189.

Ross, N.W., K.J. Firth, A. Wang, J.F. Burka, S.C. Johnson. 2000. Changes in hydrolytic enzyme activities of naïve Atlantic salmon (*Salmo salar*) skin mucus due to infection with the salmon louse (*Lepeophtheirus salmonis*) and cortisol implantation. Dis. Aquat. Org. 41: 43 – 51.

Sakaguchi, M., M. Murata. 1989. Seasonal variations of free amino acids in oyster whole body and adductor muscle. Nippon Suisan Gakkaishi 55(11): 2037 – 2041.

Shumway, S.E., T.L. Cucci, R.C. Newell, C.M. Yentsch. 1985. Particle selection, ingestion, and absorption in filter-feeding bivalves. J. Exp. Mar. Biol. Ecol. 91: 77 – 92.

Sornin, J.M., J.M. Deslous-Paoli, O. Hesse. 1988. Experimental study of the filtration of clays by the oyster *Crassostrea gigas* (Thunberg): Adjustment of particle size for best retention. Aquaculture 69(3 – 4): 355 – 366.

Soudant, P., M.V. Sanles, C. Quere, J.R. LeCoz, Y. Marty, J. Moal, J.F. Samain, P. Sorgeloos. 2000. The use of lipid emulsions for sterol supplementation of spat of the Pacific oyster, *Crassostrea gigas*. Aquaculture 184(3 – 4): 315 – 326.

Spencer, B.E., M.J. Akester, I. Mayer. 1986. Growth and survival of seed oysters in outdoor pumped upwelling systems supplied with fertilized sea water. Aquaculture 55: 173 – 189.

Spencer, B.E., C.J. Gough. 1978. The growth and survival of experimental batches of hatchery-reared spat of *Ostrea edulis* L. and *Crassostrea gigas* Thunberg, using different mehods of tray cultivation. Aquaculture 13: 293 – 312.

Tamate, H., K. Numachi, K. Mori, O. Itikawa, T. Imai. 1965. Studies on the mass mortality of the oyster in Matsushima Bay. VI. Pathological studies. Bull. Tohoku Reg. Fish. Res. Lab. 25: 89 – 104.

Thompson, P.A., M. Guo, P.J. Harrison. 1996. Nutritional value of diets that vary in fatty acid composition for larval Pacific oysters (*Crassostrea gigas*). Aquaculture 143: 379 – 391.

Waldcock, M.J., D.L. Holland. 1984. Fatty acid metabolism in young oysters, *Crassostrea gigas*: Polyunsaturated fatty acids. Lipids 19(5): 332 – 336.

Ward, J.E., J.S. Levinton, S.E. Shumway. 2003. Influence of diet on pre-ingestive particle processing in bivalves I: Transport velocities on the ctenidium. J. Exp. Mar. Biol. Ecol. 293: 129 – 149.

Webb, K.L., F.L. Chu. 1983. Phytoplankton as a food source for bivalve larvae. In: G.D. Pruder, C. Langdon and D. Conklin (Editors), Biochemical and physiological approaches to shellfish nutrition. Proc. 2nd Int. Conf. Aquaculture Nutr. World Maricult. Soc. Spec. Publ. No. 2, Lousiana State University, LA, pp. 272 – 291.

Whyte, J.N.C. 1987. Biochemical composition and energy content of six species of phytoplankton used in mariculture of bivalves. Aquaculture 60: 231 – 241.

Whyte, J.N.C. 1988. Fatty acid profiles from direct methanolysis of lipids in tissue of cultured species. Aquaculture 75: 193 – 203.

Whyte, J.N.C., J.R. Englar. 1982. Seasonal variation in the chemical composition and condition indices of Pacific oyster, *Crassostrea gigas*, grown in trays or on the sea bed. Can. J. Fish. Aquat. Sci. 39: 1084 – 1094.

Whyte, J.N.C., K. Sherry, N. Ginther. 2003. Growth and nutritional condition of *Mytilus galloprovincialis* fed *Schizochytrium*. Pacific Biological Station, Fisheries and Oceans Canada, Nanaimo, BC (unpublished).

Wilson, J.H. 1978. The food value of *Phaeodactylum tricornutum* Bohlin to the larvae of *Ostrea edulis* L. and *Crassostrea gigas* Thunberg. Aquaculture 13: 313 – 323.

Wolfe, G.V. 2000. The chemical defense ecology of marine unicellular plankton: constraints, mechanisms, and impacts. Biol. Bull. 198: 225 – 244.

Wright, D.A., E.W. Hetzel. 1985. Use of RNA: DNA ratios as an indicator of nutritional stress in the American oyster *Crassostrea virginica*. Mar. Ecol. Prog. Ser. 25(2): 199 – 206.

7.0 Appendix 1

Table 1. Proximate composition of diets used for feeding *Crassostrea gigas* juveniles for 60 days at 1% dry weight day^{-1}. Mixed diets were fed at 1:1 (dw:dw) and total oxidation was assumed for calculating total energy. Algamac 3050™ and Rotimac™ are *Schizochytrium*-based dry feeds (Aquafauna Biomarine, Hawthorne, CA, USA). T-Iso was the flagellate species *Isochrysis galbana* (clone T-Iso).

Dietary Component	T-Iso	Algamac 3050	Rotimac	Algamac/T-Iso	Rotimac/T-Iso
Protein (%)	33.4	17.6	37.4	25.5	35.4
Carbohydrate (%)	9.2	15.9	35.0	12.6	22.1
Lipid (%)	23.5	56.2	11.7	39.9	17.6
Total Energy (J/mg)	1595.5	2590.0	1690.6	2095.4	1643.0

Table 2. Proximate composition of diets used for feeding *Crassostrea gigas* juveniles for 30 days at 1% dry weight day^{-1}. Mixed diets were fed at 1:1 (dw:dw) and total oxidation was assumed for calculating total energy. Algamac 2050™ and Rotimac™ are *Schizochytrium*-based dry feeds (Aquafauna Biomarine, Hawthorne, CA, USA). *Thalassiosira nordenskioeldii* (*T.n.*) was the dominant species (>90%) in bloom tank algae used for feeding.

Nutritional Component/Diet	*T. nordenskioeldii*	Algamac 2050	Rotimac	AM2050/*T.n.*	Rotimac/*T.n.*
Ash content	**37.93**	**20.23**	**7.86**	**29.08**	**22.90**
Crude protein (%)	**35.50**	**20.45**	**42.63**	**27.98**	**39.07**
Mono/oligosaccharide (%)	**9.57**	**5.09**	**11.83**	**7.33**	**10.70**
Polysaccharide (%)	**14.08**	**15.97**	**32.54**	**15.03**	**23.31**
Crude lipid (%)	**16.22**	**39.20**	**6.48**	**27.71**	**11.35**
Total energy (kJ/g)	**1622.34**	**2124.83**	**1759.80**	**1873.58**	**1691.07**

Table 3. The fatty acid composition of test diets (n = 3; ± S.E.) fed to juvenile Pacific oysters (*Crassostrea gigas*). The dominant phytoplankton species (>90%) in outdoor grown bloom tank algae cultures was *Thalassiosira nordenskioeldii*. Rotimac™ and Algamac 2050™ are spray-dried *Schizochytrium*-based diets (Aquafauna Biomarine, Hawthorne, California, USA).

	Algamac 2050		Rotimac		T-Iso	
Fatty acid	**Conc. (mg*g^{-1} DW)**	**%TFA**	**Conc. (mg*g^{-1} DW)**	**%TFA**	**Conc. (mg*g^{-1} DW)**	**%TFA**
Saturates						
14:0	43.20 ± 0.55	8.85	9.78 ± 0.15	6.46	18.27 ± 0.29	27.95
iso 15:0	-	-	-	-	0.20 ± 0.00	0.30
15:0	2.09 ± 0.03	0.43	0.80 ± 0.02	0.53	-	-
16:0	122.19 ± 1.27	25.02	37.37 ± 0.89	24.68	8.04 ± 0.07	12.29
iso 17:0	-	-	-	-	-	-
17:0	-	-	0.54 ± 0.11	0.35	-	-
18:0	-	-	6.36 ± 0.19	4.20	11.73 ± 0.11	17.93
Total	167.48	34.30	54.85	36.22	38.24	58.47
Monounsaturates						
16:1n9	-	-	-	-	0.58 ± 0.05	0.88
16:1n7	-	-	-	-	0.11 ± 0.06	0.18
16:1n6					0.25 ± 0.01	0.38
16:1n5					1.28 ± 0.02	1.96
18:1n9	-	-	-	-	-	-
18:1n7	2.74 ± 0.02	0.56	19.02 ± 0.52	12.56	0.94 ± 0.01	1.44
18:1n5	-	-	3.29 ± 0.11	2.17	-	-
20:1n11					5.09 ± 2.84	7.16
20:1n7	-	-	-	-	-	-
Total	2.74	0.56	22.31	14.73	8.25	12.00

Table 3 continued.

Fatty acid	Algamac 3050 Conc. (mg*g^{-1} DW)	%TFA	Rotimac Conc. (mg*g^{-1} DW)	%TFA	T-Iso Conc. (mg*g^{-1} DW)	%TFA
Polyunsaturates						
16:2n6	1.18 ± 0.01	0.24	8.29 ± 0.10	5.48	-	-
16:2n4					0.44 ± 0.01	0.67
16:3n4	1.28 ± 0.02	0.26	0.65 ± 0.02	0.43	-	-
16:4n1	-	-	-	-	-	-
18:2n6	-	-	-	-	2.46 ± 1.19	4.01
18:2n4	-	-	4.93 ± 0.19	3.25	1.34 ± 1.14	1.80
18:3n4	1.45 ± 0.01	0.30	1.03 ± 0.03	0.68	-	-
18:3n3	-	-	0.86 ± 0.02	0.56	2.26 ± 1.04	3.66
18:4n1	1.62 ± 0.03	0.33	-	-	-	-
18:4n3	-	-	-	-	8.59 ± 2.71	13.66
20:2n6	8.59 ± 3.87	1.74	2.33 ± 0.06	1.54	0.83 ± 0.62	1.14
20:3n6					0.18 ± 0.01	0.28
20:4n6					0.17 ± 0.00	0.26
20:3n3					0.58 ± 0.01	0.89
20:5n3	9.39 ± 0.15	1.92	8.12 ± 0.22	5.36	-	-
22:4n6					0.19 ± 0.00	0.28
22:4n3	-	-	10.09 ± 0.14	6.67	-	-
22:5n3	-	-	2.40 ± 0.08	1.58	0.50 ± 0.01	0.76
22:5n6	86.82 ± 0.95	17.78	-	-	1.38 ± 0.02	2.11
22:6n3	194.68 ± 2.56	39.87	31.45 ± 0.61	20.78	11.20 ± 0.25	17.09
Total	305.01	62.44	70.15	46.33	30.77	46.61
Unidentified						
Total	5.72	0.84	4.12	2.72	0.74	1.13

Table 4. Individual Pacific oyster (*Crassostrea gigas*) dry weight after 60 days of feeding. Diets were batch fed for six hours at 1% of the initial dry weight of oysters per tank per day. The reference diet used was *Isochrysis galbana* (clone T-Iso) and Algamac 3050™ and Rotimac™ are spray-dried *Schizochytrium*-based diets (Aquafauna Biomarine, Hawthorne, California, USA).

Diet	Initial weight (mg)	Day 60 weight (mg)	Change (%)
Algamac 3050™/T-Iso	60.674 ± 0.418	125.235 ± 1.536	106
Algamac 3050™	60.674 ± 0.418	62.333 ± 1.238	3
Rotimac™/T-Iso	60.674 ± 0.418	95.226 ± 0.765	57
Rotimac™	60.674 ± 0.418	63.227 ± 1.536	4
T-Iso	60.674 ± 0.418	100.363 ± 1.155	65
Starved (control)	60.674 ± 0.418	52.459 ± 0.351	-14

Table 5. Individual Pacific oyster (*Crassostrea gigas*) AFDW after 60 days of feeding. Diets were batch fed for six hours at 1% of the initial dry weight of oysters per tank per day. The reference diet used was *Isochrysis galbana* (clone T-Iso) and Algamac 3050™ and Rotimac™ are spray-dried *Schizochytrium*-based diets (Aquafauna Biomarine, Hawthorne, California, USA).

Diet	Initial AFDW (%)	Day 60 AFDW (%)	Change (%)
Algamac 3050™/T-Iso	4.63 ± 0.26	8.94 ± 0.42	93
Algamac 3050™	4.63 ± 0.26	5.50 ± 0.32	19
Rotimac™/T-Iso	4.63 ± 0.26	7.62 ± 0.28	65
Rotimac™	4.63 ± 0.26	6.86 ± 0.72	48
T-Iso	4.63 ± 0.26	9.39 ± 0.58	103
Starved (control)	4.63 ± 0.26	6.34 ± 0.56	37

Table 6. The fatty acid composition of juvenile Pacific oysters (*Crassostrea gigas*) fed three test diets (n = 3; ± SE). The dominant phytoplankton species (>90%) in outdoor grown bloom tank algae cultures was *Thalassiosira nordenskioeldii* (Bloom). Rotimac™ and Algamac 2050™ (AM 2050™) are spray-dried *Shizochytrium*-based diets (Aquafauna Biomarine, Hawthorne, California, USA).

Fatty acid	Day 0 Conc. ($mg^{*}g^{-1}$ DW)	%TFA	Day 30 Rotimac/Bloom Conc. ($mg^{*}g^{-1}$ DW)	%TFA	Day 30 AM 2050/Bloom Conc. ($mg^{*}g^{-1}$ DW)	%TFA	Day 30 Bloom Conc. ($mg^{*}g^{-1}$ DW)	%TFA
Saturates								
14:0	0.019 ± 0.001	1.48	0.110 ± 0.005	1.93	0.354 ± 0.016	4.55	0.073 ± 0.003	1.51
iso 15:0	trace	-	0.017 ± 0.000	0.30	-	-	0.013 ± 0.000	0.27
15:0	trace	-	0.016 ± 0.001	0.28	0.023 ± 0.001	0.30	0.013 ± 0.000	0.27
16:0	0.126 ± 0.007	9.82	0.733 ± 0.030	12.83	1.465 ± 0.055	18.82	0.506 ± 0.021	10.48
iso 17:0	0.018 ± 0.001	1.40	0.039 ± 0.001	0.68	0.028 ± 0.001	0.36	0.045 ± 0.003	0.93
17:0	0.016 ± 0.001	1.25	0.039 ± 0.002	0.68	0.043 ± 0.002	0.55	0.037 ± 0.005	0.77
18:0	0.083 ± 0.003	6.47	0.261 ± 0.008	4.57	0.313 ± 0.012	4.02	0.216 ± 0.006	4.47
20:0	0.085 ± 0.003	6.63	0.219 ± 0.006	3.83	0.209 ± 0.008	2.68	0.277 ± 0.011	5.73
Total	0.347	27.05	1.434	25.11	2.435	31.27	1.180	24.43
Monounsaturates								
16:1n9	-	-	-		-	-	-	-
16:1n7	0.060 ± 0.003	4.68	0.216 ± 0.005	3.78	0.312 ± 0.013	4.01	0.181 ± 0.009	3.75
18:1n9	0.027 ± 0.002	2.10	0.203 ± 0.009	3.55	0.055 ± 0.009	0.71	0.039 ± 0.000	0.81
18:1n7	0.044 ± 0.001	3.43	0.299 ± 0.010	5.24	0.387 ± 0.015	4.97	0.299 ± 0.010	6.19
18:1n5	-	-	trace	-	-	-	-	-
20:1n7	-	-	-	-	-	-	-	-
Total	0.131	10.21	0.718	12.57	0.754	9.68	0.519	10.75

Table 6 continued.

Fatty acid	Day 0 Conc. (mg*g^{-1} DW)	%TFA	Day 30 Rotimac/Bloom Conc. (mg*g^{-1} DW)	%TFA	Day 30 AM 2050/Bloom Conc. (mg*g^{-1} DW)	%TFA	Day 30 Bloom Conc. (mg*g^{-1} DW)	%TFA
Polyunsaturates								
16:2n6	-	-	0.028 ± 0.001	0.49	0.029 ± 0.004	0.37	0.029 ± 0.002	0.60
16:3n4	trace	-	0.059 ± 0.004	1.03	0.219 ± 0.160	2.81	0.069 ± 0.004	1.43
16:3n3	0.033 ± 0.008	2.57	0.025 ± 0.007	0.44	-	-	-	-
16:4n1	-	-	0.048 ± 0.004	0.84	0.056 ± 0.003	0.72	0.046 ± 0.003	0.95
18:1n9	-	-	-	-	-	-	-	-
18:1n7	-	-	-	-	-	-	-	-
18:1n5	-	-	-	-	-	-	-	-
18:2n6	-	-	0.133 ± 0.007	2.33	0.025 ± 0.003	0.32	0.033 ± 0.002	0.68
18:2n4	-	-	-	-	-	-	-	-
18:3n4	-	-	-	-	-	-	-	-
18:3n3	-	-	-	-	-	-	-	-
18:4n3	-	-	0.085 ± 0.005	1.49	0.093 ± 0.004	1.19	0.095 ± 0.005	1.97
18:4n1	-	-	-	-	-	-	-	-
20:2n6	-	-	0.018 ± 0.001	0.32	0.029 ± 0.001	0.37	-	-
20:5n3	0.201 ± 0.004	15.67	1.100 ± 0.056	19.26	1.161 ± 0.042	14.91	1.304 ± 0.067	27.00
21:5n3	-	-	0.055 ± 0.003	0.96	0.041 ± 0.002	0.53	0.083 ± 0.004	1.72
22:4n3	-	-	-	-	-	-	-	-
22:5n6	-	-	0.238 ± 0.008	4.17	0.498 ± 0.017	6.40	0.014 ± 0.001	0.29
22:5n3	0.015 ± 0.001	1.17	0.029 ± 0.001	0.51	0.026 ± 0.001	0.33	0.044 ± 0.003	0.91
22:6n3	0.173 ± 0.001	13.48	0.727 ± 0.031	12.73	1.502 ± 0.049	19.29	0.387 ± 0.021	8.01
Total	0.422	32.89	2.545	44.56	3.679	47.25	2.104	43.56
Unidentified								
Total	0.383	29.85	1.014	17.76	0.918	11.79	1.027	21.26

8.0 Appendix 2

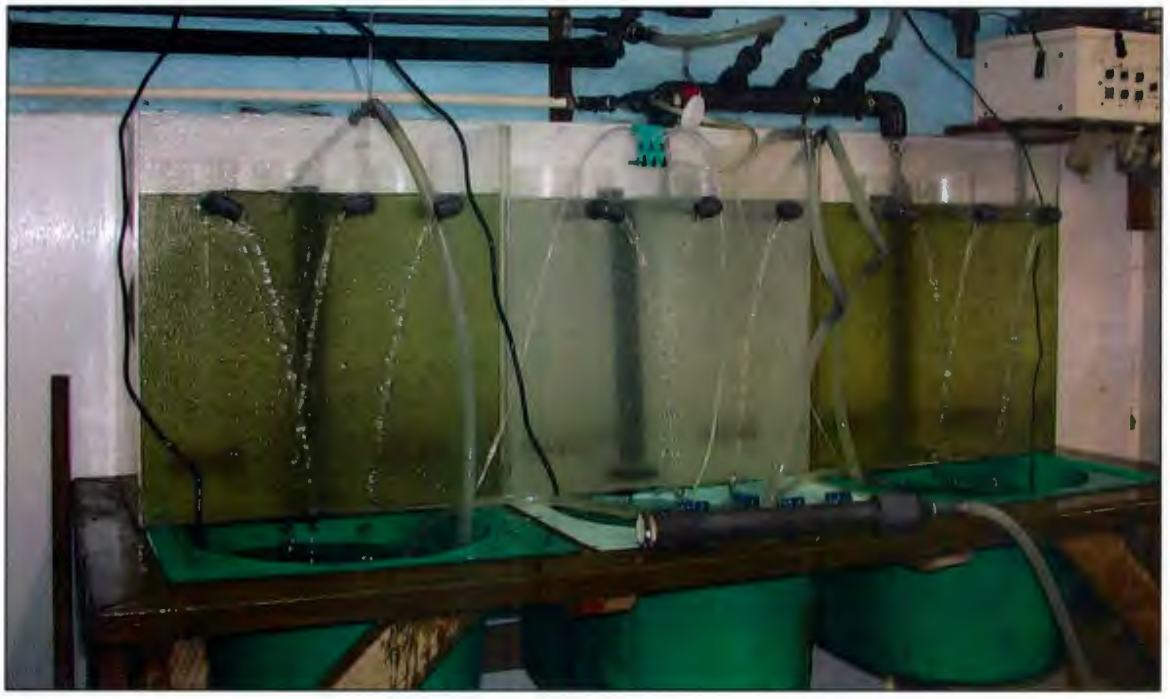

Figure 1. Photograph of the tank setup for Experiment 1. Each plumbed circuit consisted of a top tank with three upwellers holding juvenile oysters (*Crassostrea gigas*) above a reservoir tank using a mechanical pump to recirculate seawater during feeding. Following feeding, ambient 1 μm-filtered seawater was allowed to flow through the top tank.

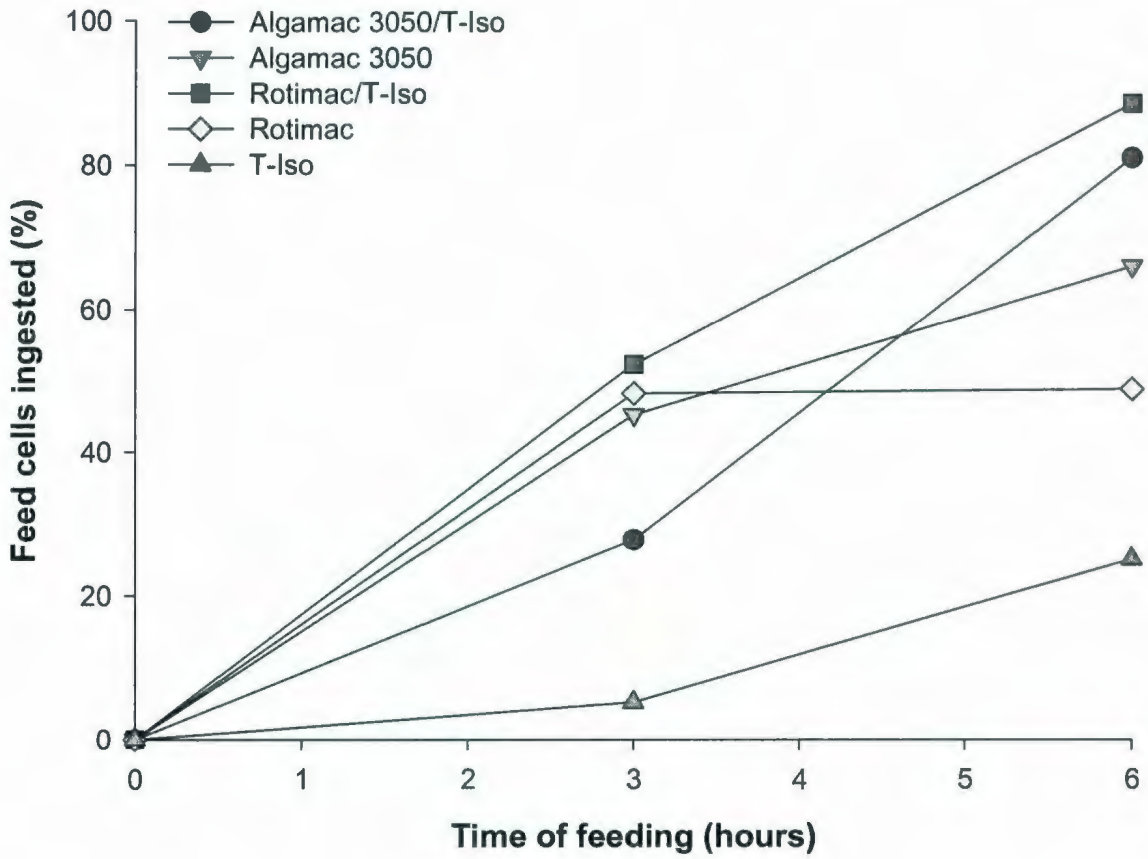

Figure 2. Relative feeding rate of juvenile Pacific oysters (*Crassostrea gigas*) as measured by the percentage reduction in cell counts of five diets. Diets were fed at 1% the initial dry weight (mg) of oysters per tank for a period of 6 hours. Algamac 3050™ and Rotimac™ are both *Schizochytrium*-based live algae substitute diets and the reference diet used was *Isochrysis galbana* (clone T-Iso).

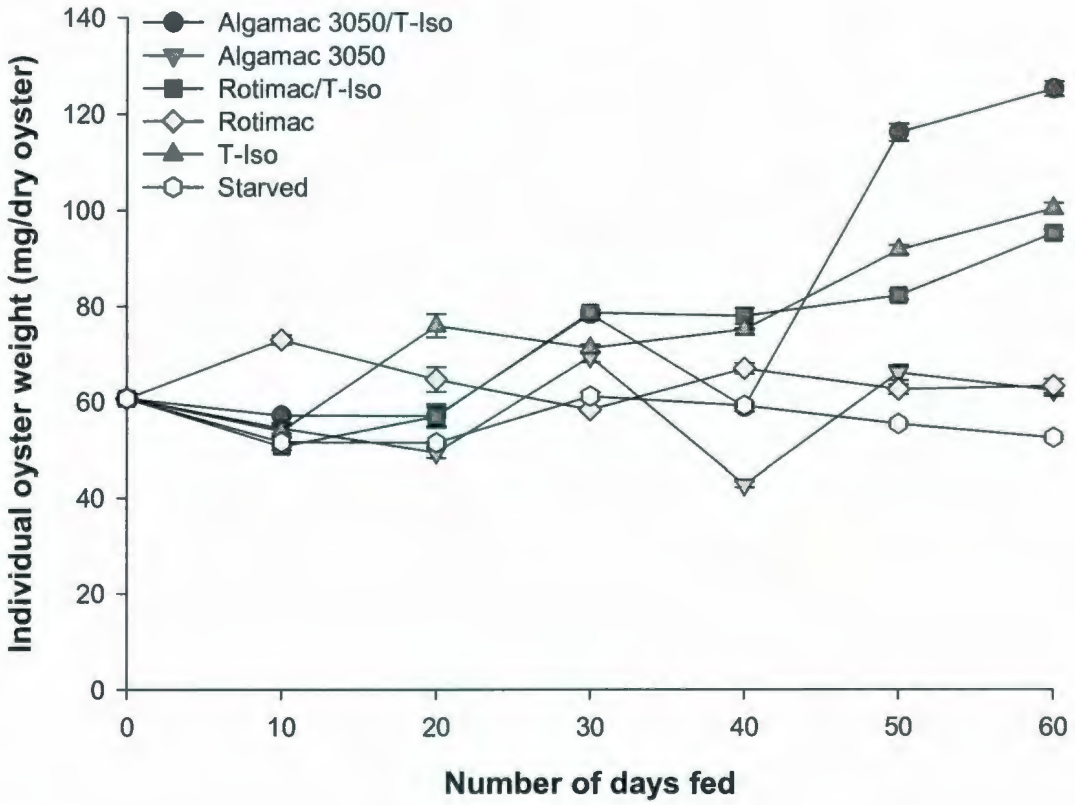

Figure 3. Mean dry weight (mg ± SE) of individual Pacific oysters (*Crassostrea gigas*) following 60 days of feeding. Oysters were fed 1% (dw) of the initial dry weight of oysters per tank. The reference diet used was *Isochrysis galbana* (Clone T-Iso) and Algamac 3050™ and Rotimac™ are spray-dried *Schizochytrium*-based diets (Aquafauna Biomarine, Hawthorne, California, USA).

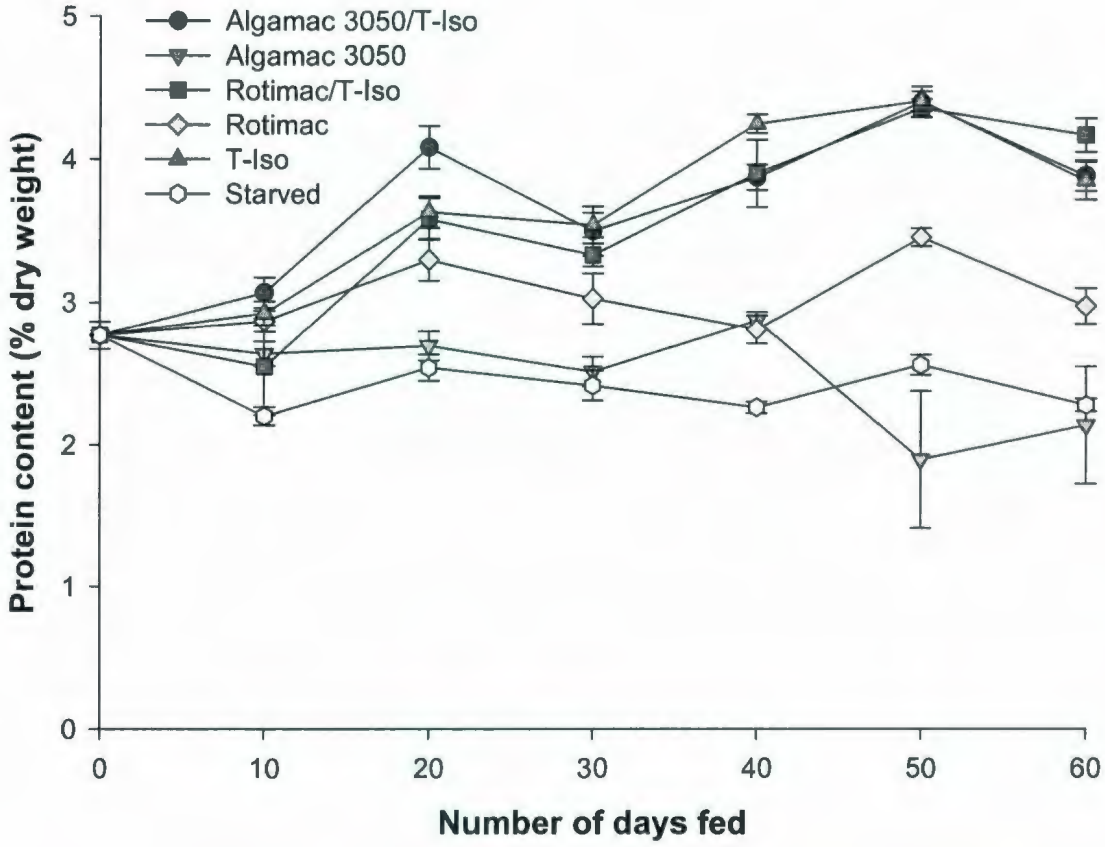

Figure 4. Percentage (± SE) protein content of juvenile oysters (*Crassostrea gigas*) fed microalgal and live algae substitute diets. Juvenile oysters were batch fed 1% of the initial dry weight of oysters per tank for 60 days. The live algae reference diet was *Isochrysis galbana* (clone T-Iso) and Algamac 3050™ and Rotimac™ are spray-dried *Schizochytrium*-based live algae substitute diets (Aquafauna Biomarine, Hawthorne, California, USA).

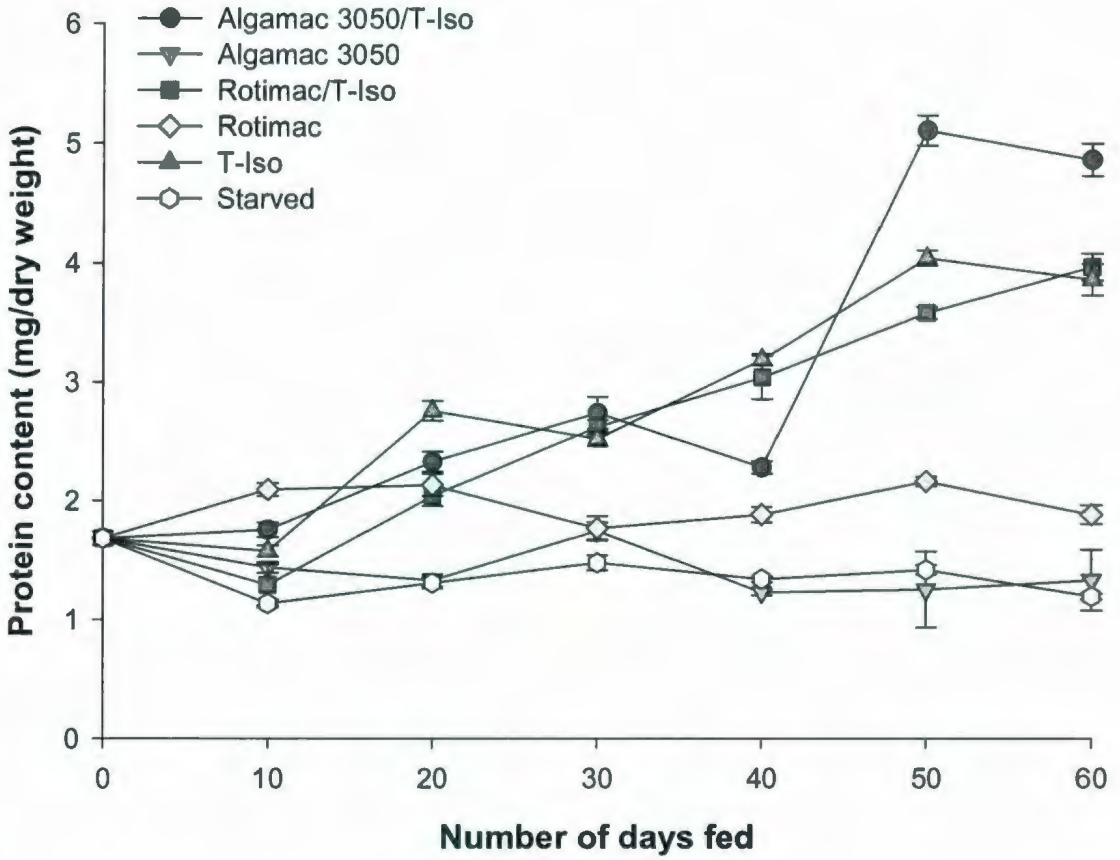

Figure 5. Protein content expressed as mg per individual dry oyster (± SE) following 60 days of feeding test diets. Diets were fed at 1% of the initial total oyster dry weight per tank and the reference diet used was *Isochrysis galbana* (clone T-Iso). Algamac 3050™ and Rotimac™ are spray-dried *Schizochytrium*-based diets (Aquafauna Biomarine, Hawthorne, California, USA).

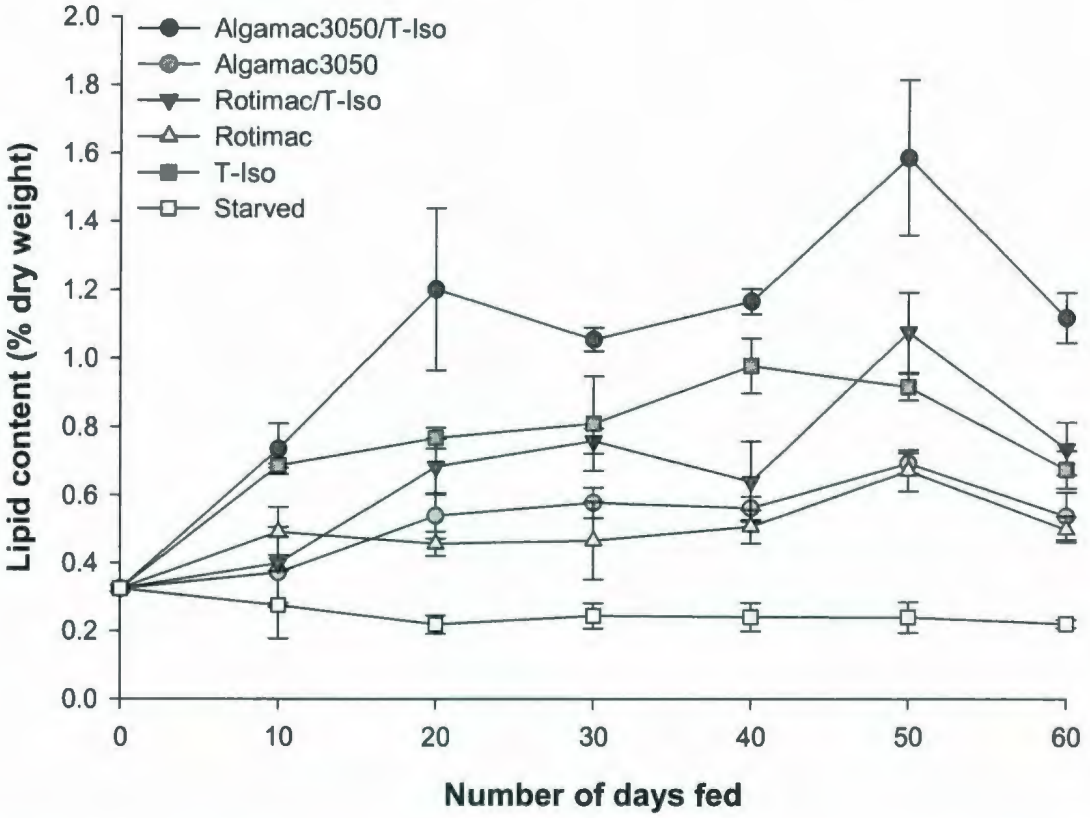

Figure 6. Percentage (± SE) lipid content of juvenile oysters (*Crassostrea gigas*) fed microalgal and live algae substitute diets. Juvenile oysters were batch fed 1% of the initial dry weight of oysters per tank for 60 days. The live algae reference diet was *Isochrysis galbana* (clone T-Iso) and Algamac 3050™ and Rotimac™ are spray-dried *Schizochytrium*-based live algae substitute diets (Aquafauna Biomarine, Hawthorne, California, USA).

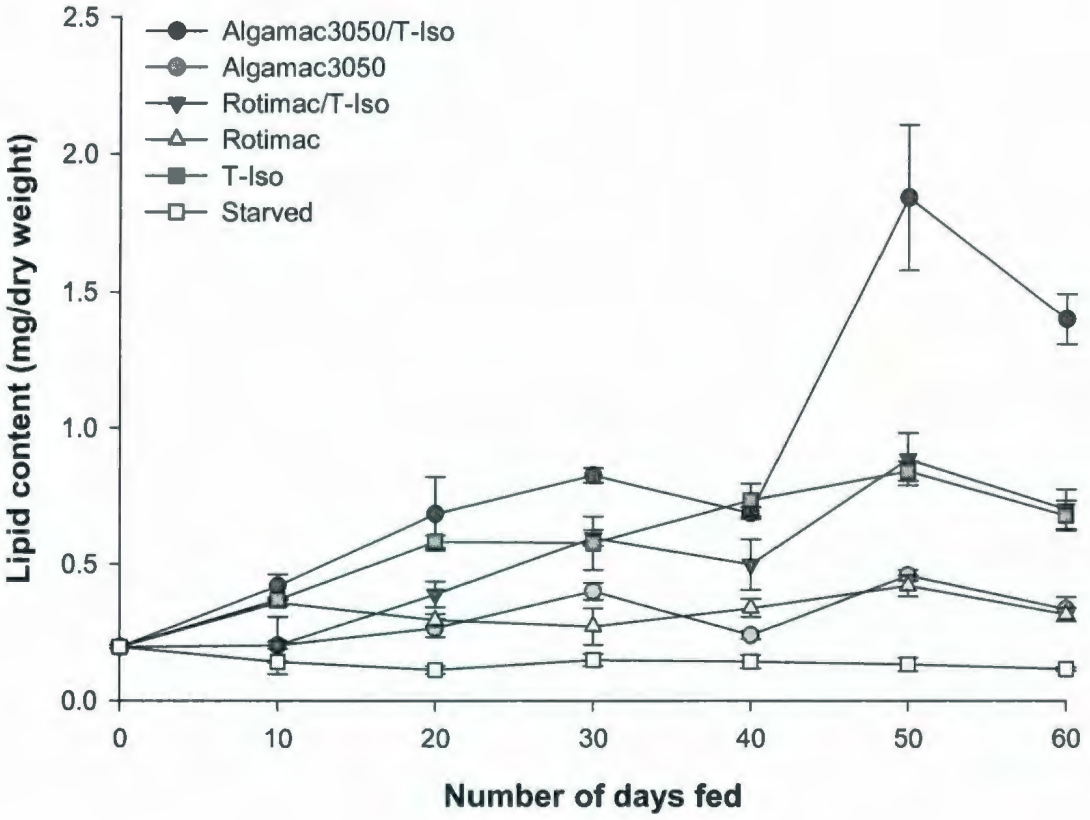

Figure 7. Lipid content expressed as mg per individual dry oyster (± SE) following 60 days of feeding test diets. Juvenile oysters were batch fed 1% of the initial dry weight of oysters per tank for 60 days. The live algae reference diet was *Isochrysis galbana* (clone T-Iso) and Algamac 3050™ and Rotimac™ are spray-dried *Schizochytrium*-based live algae substitute diets (Aquafauna Biomarine, Hawthorne, California, USA).

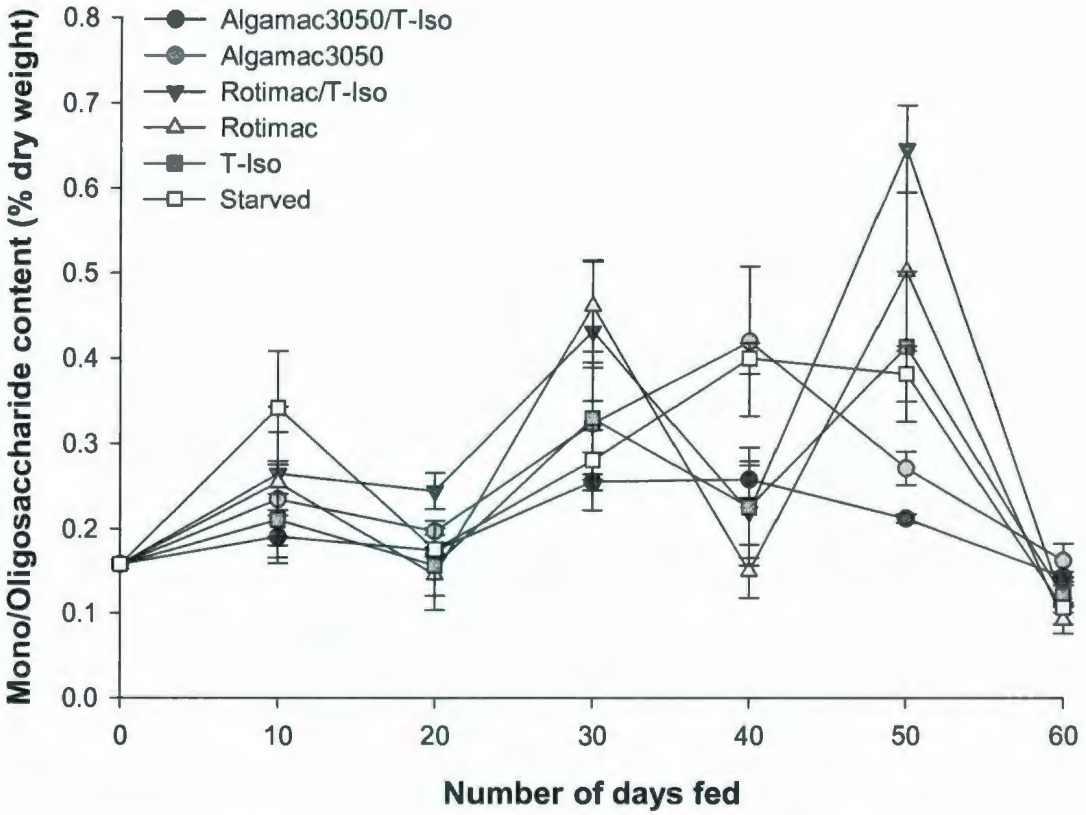

Figure 8. Percent (± SE) mono/oligosaccharide content of juvenile oysters (*Crassostrea gigas*) fed microalgal and live algae substitute diets. Juvenile oysters were batch fed 1% of the initial dry weight of oysters per tank for 60 days. The live algae reference diet was *Isochrysis galbana* (clone T-Iso) and Algamac 3050™ and Rotimac™ are spray-dried *Schizochytrium*-based live algae substitute diets (Aquafauna Biomarine, Hawthorne, California, USA).

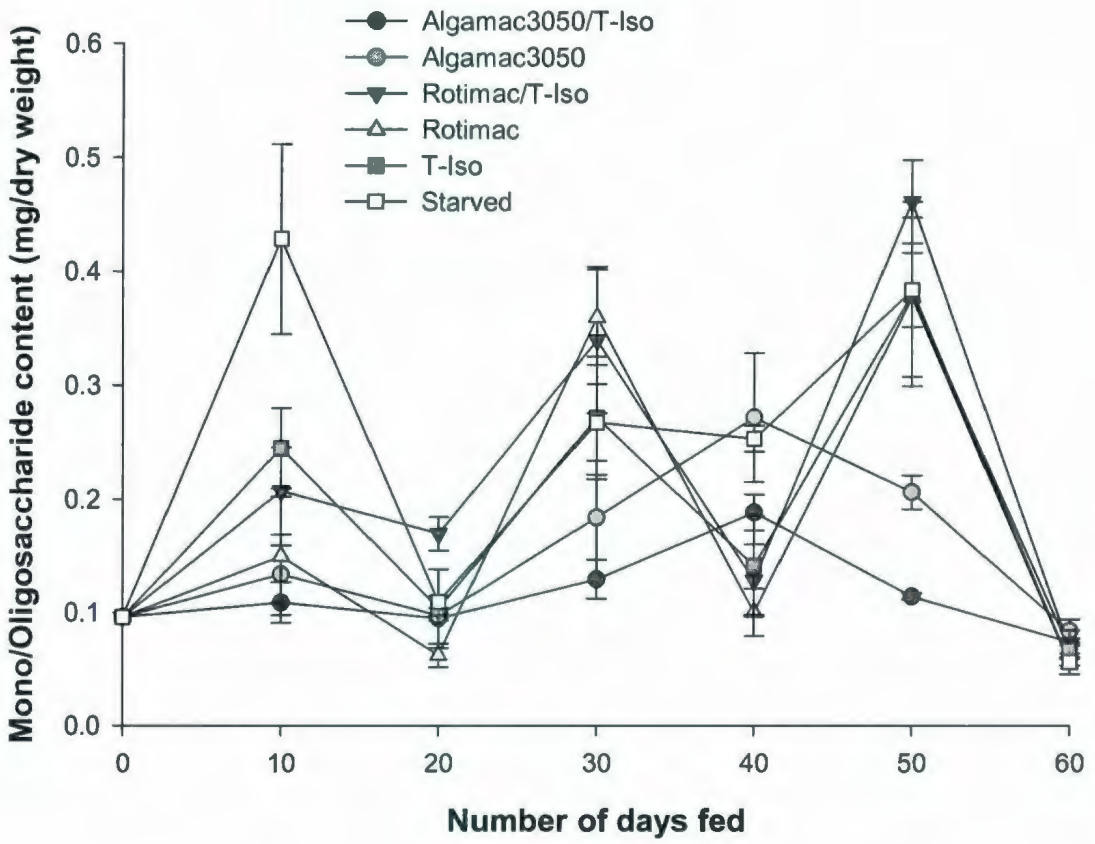

Figure 9. Mono/oligosaccharide content expressed as mg per individual dry oyster (± SE) following 60 days of feeding test diets. Juvenile oysters were batch fed 1% of the initial dry weight of oysters per tank for 60 days. The live algae reference diet was *Isochrysis galbana* (clone T-Iso) and Algamac 3050™ and Rotimac™ are spray-dried *Schizochytrium*-based live algae substitute diets (Aquafauna Biomarine, Hawthorne, California, USA).

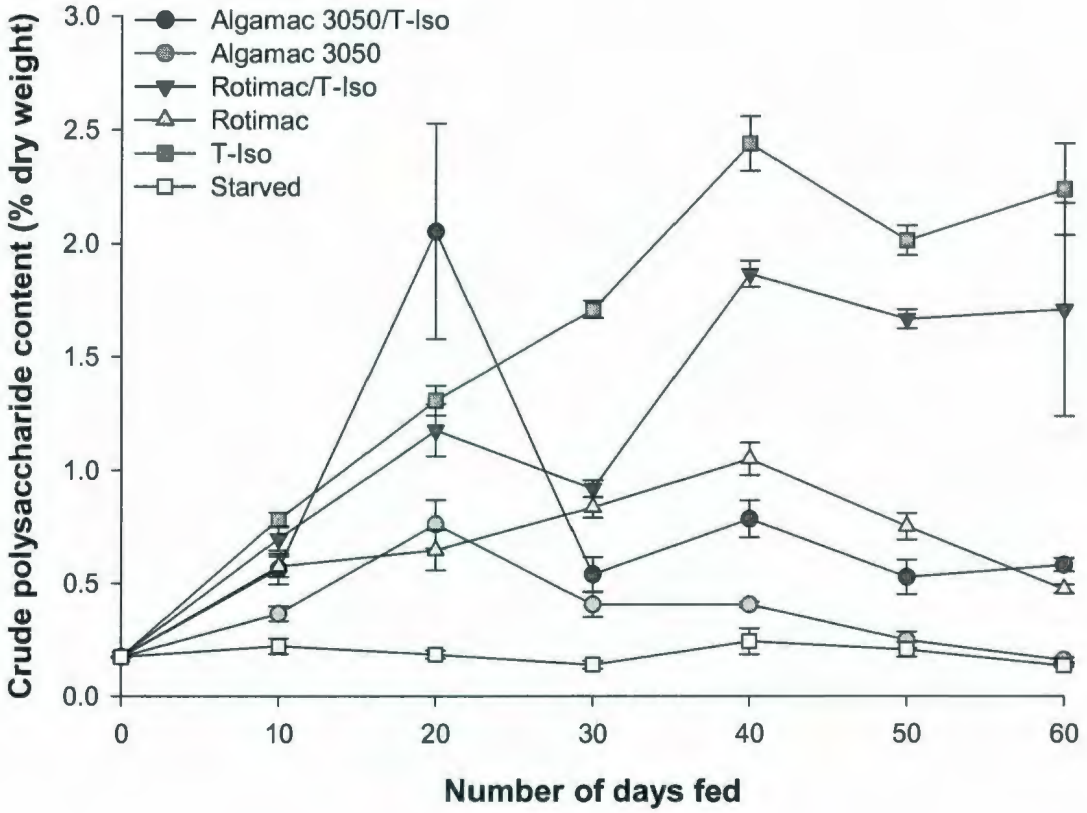

Figure 10. Percentage (± SE) polysaccharide content of juvenile oysters (*Crassostrea gigas*) fed microalgal and live algae substitute diets. Juvenile oysters were batch fed 1% of the initial dry weight of oysters per tank for 60 days. The live algae reference diet was *Isochrysis galbana* (clone T-Iso) and Algamac 3050™ and Rotimac™ are spray-dried *Schizochytrium*-based live algae substitute diets (Aquafauna Biomarine, Hawthorne, California, USA).

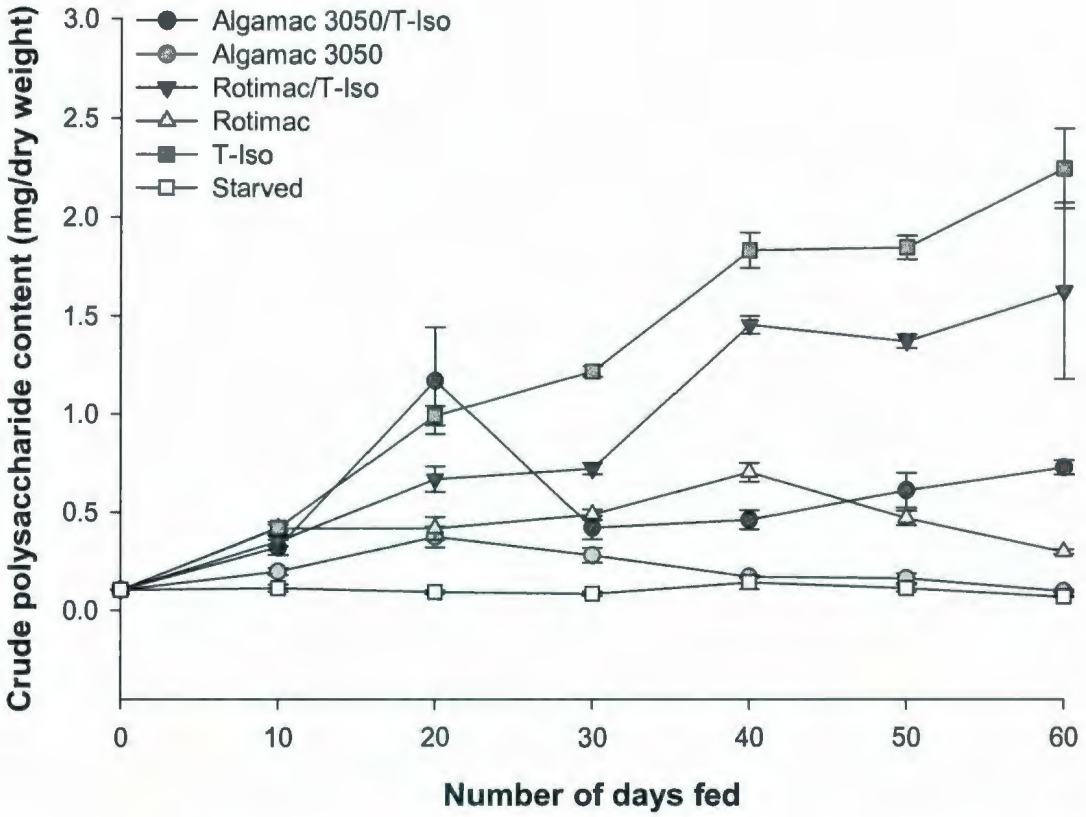

Figure 11. Polysaccharide content expressed as mg per individual dry oyster (± SE) following 60 days of feeding test diets. Juvenile oysters were batch fed 1% of the initial dry weight of oysters per tank for 60 days. The live algae reference diet was *Isochrysis galbana* (clone T-Iso) and Algamac 3050™ and Rotimac™ are spray-dried *Schizochytrium*-based live algae substitute diets (Aquafauna Biomarine, Hawthorne, California, USA).

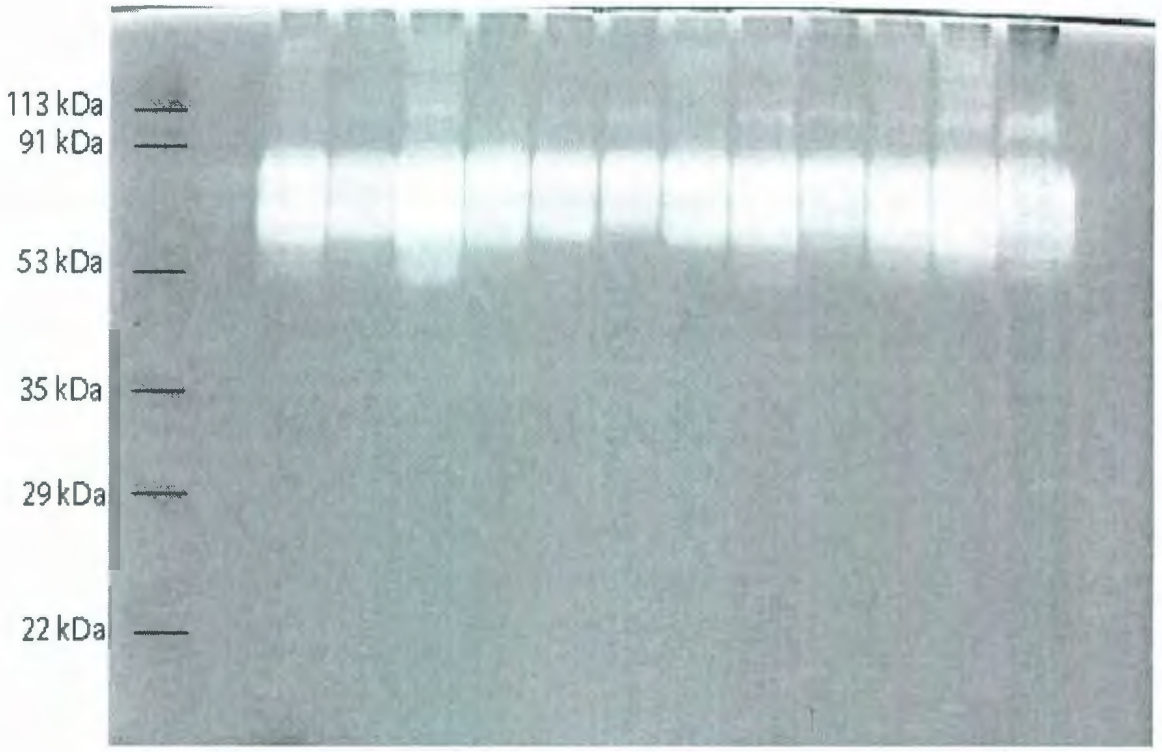

Figure 12. Polyacrylamide gel zymography of homogenised tissue from juvenile Pacific oysters (*Crassostrea gigas*) at Day 0 of a 60 day feeding experiment. Each lane represents tissue from three oysters (n = 3). Low molecular weight marker proteins were used to estimate protease size (kDa).

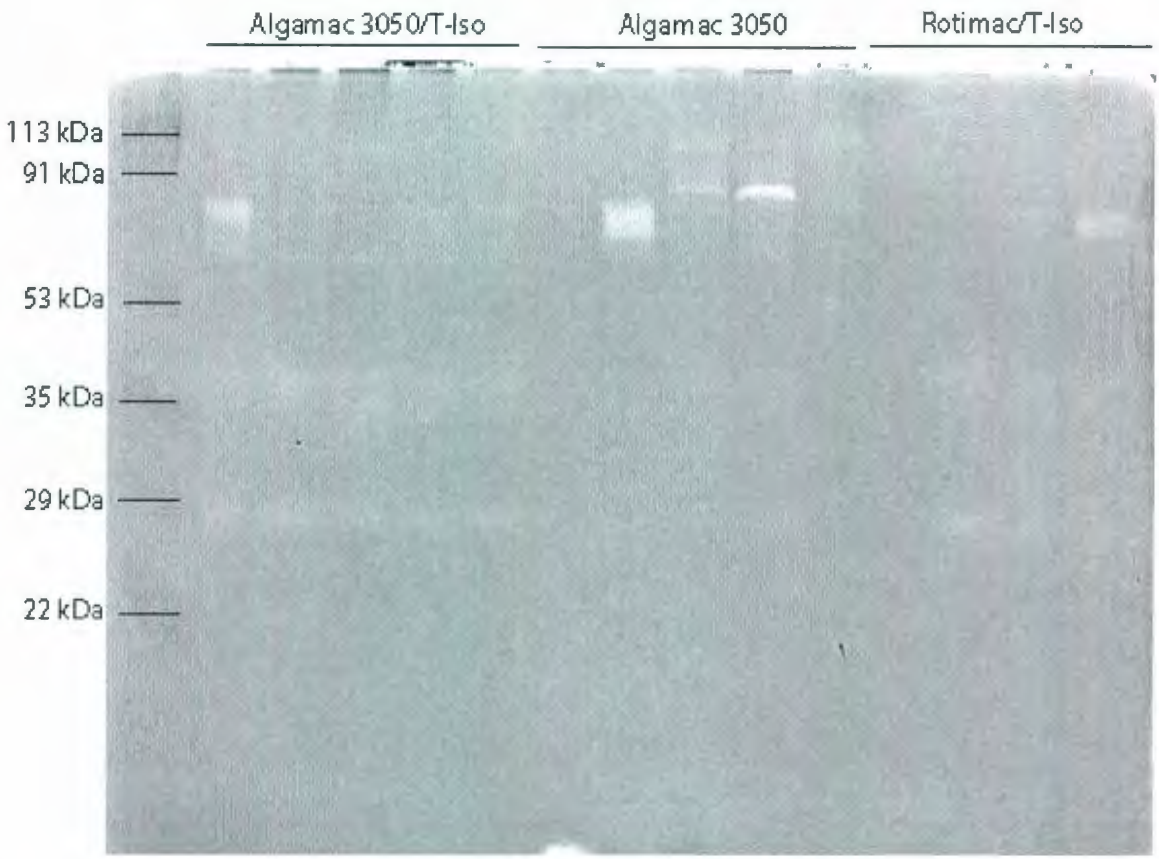

Figure 13. Polyacrylamide gel zymography of homogenised tissue from juvenile Pacific oysters (*Crassostrea gigas*) at Day 20 of a 60 day feeding experiment. Low molecular weight marker proteins were used to estimate protease size (kDa). Each lane represents tissue from three oysters (n = 3). Algamac 3050™ and Rotimac™ are *Schizochytrium*-based live algae substitute diets (Aquafauna Biomarine, Hawthorne, California, USA). *Isochrysis galbana* (clone T-Iso) was used as a control diet of known quality for feeding juvenile oysters (*C. gigas*). Juvenile oysters were batch fed 1% of the initial dry weight of oysters per tank for 60 days and mixed diets were presented as 1:1 (dw:dw) formulations.

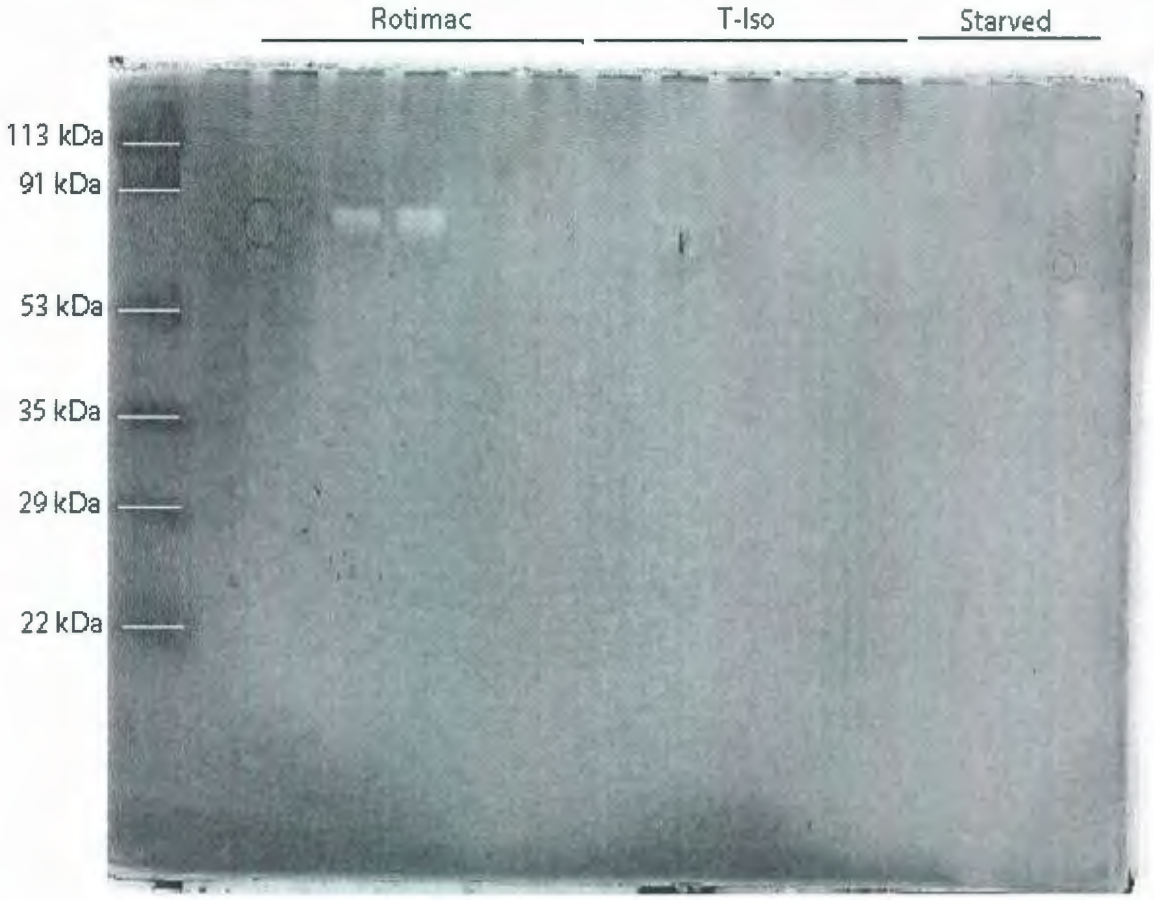

Figure 14. Polyacrylamide gel zymography of homogenised tissue from juvenile Pacific oysters (*Crassostrea gigas*) at Day 20 of a 60 day feeding experiment. Low molecular weight marker proteins were used to estimate protease size (kDa). Each lane represents tissue from three oysters (n = 3). Rotimac™ is a *Schizochytrium*-based live algae substitute diet (Aquafauna Biomarine, Hawthorne, California, USA). *Isochrysis galbana* (clone T-Iso) was used as a reference microalgal diet of known quality for feeding juvenile oysters (*C. gigas*). Juvenile oysters were batch fed 1% of the initial dry weight of oysters per tank for 60 days and mixed diets were presented as 1:1 (dw:dw)

formulations. Control oysters were starved to bacterial level (1 μm filtration of incoming seawater).

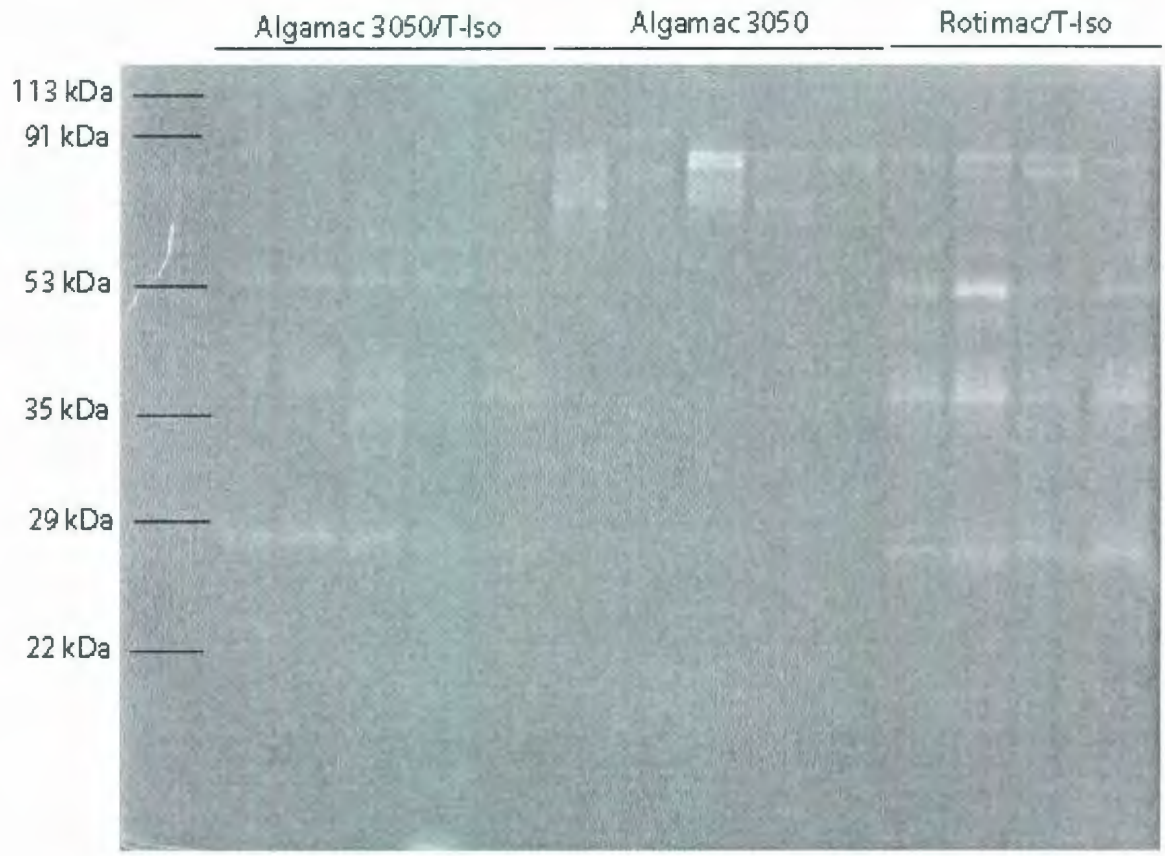

Figure 15. Polyacrylamide gel zymography of homogenised tissue from juvenile Pacific oysters (*Crassostrea gigas*) at Day 60 of a 60 day feeding experiment. Low molecular weight marker proteins were used to estimate protease size (kDa). Each lane represents tissue from three oysters (n = 3). Algamac 3050™ and Rotimac™ are *Schizochytrium*-based live algae substitute diets (Aquafauna Biomarine, Hawthorne, California, USA). *Isochrysis galbana* (clone T-Iso) was used as a control diet of known quality for feeding juvenile oysters (*C. gigas*). Juvenile oysters were batch fed 1% of the initial dry weight of oysters per tank for 60 days and mixed diets were presented as 1:1 (dw:dw) formulations.

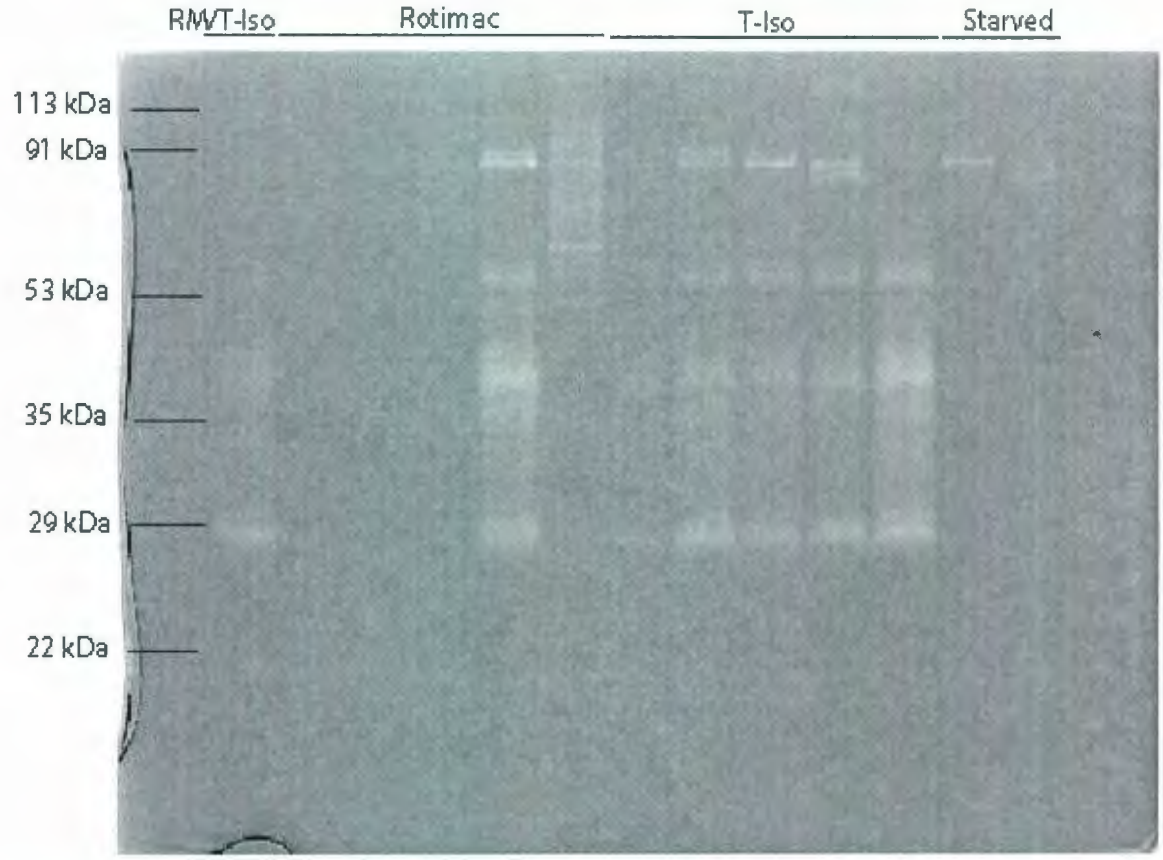

Figure 16. Polyacrylamide gel zymography of homogenised tissue from juvenile Pacific oysters (*Crassostrea gigas*) at Day 60 of a 60 day feeding experiment. Low molecular weight marker proteins were used to estimate protease size (kDa). Each lane represents tissue from three oysters (n = 3). Algamac 3050™ and Rotimac™ are *Schizochytrium*-based live algae substitute diets (Aquafauna Biomarine, Hawthorne, California, USA). *Isochrysis galbana* (clone T-Iso) was used as a reference microalgal diet of known quality for feeding juvenile oysters (*C. gigas*). Juvenile oysters were batch fed 1% of the initial dry weight of oysters per tank for 60 days and mixed diets were presented as 1:1 (dw:dw) formulations. Control oysters were starved to bacterial level (1 μm filtration of incoming seawater).

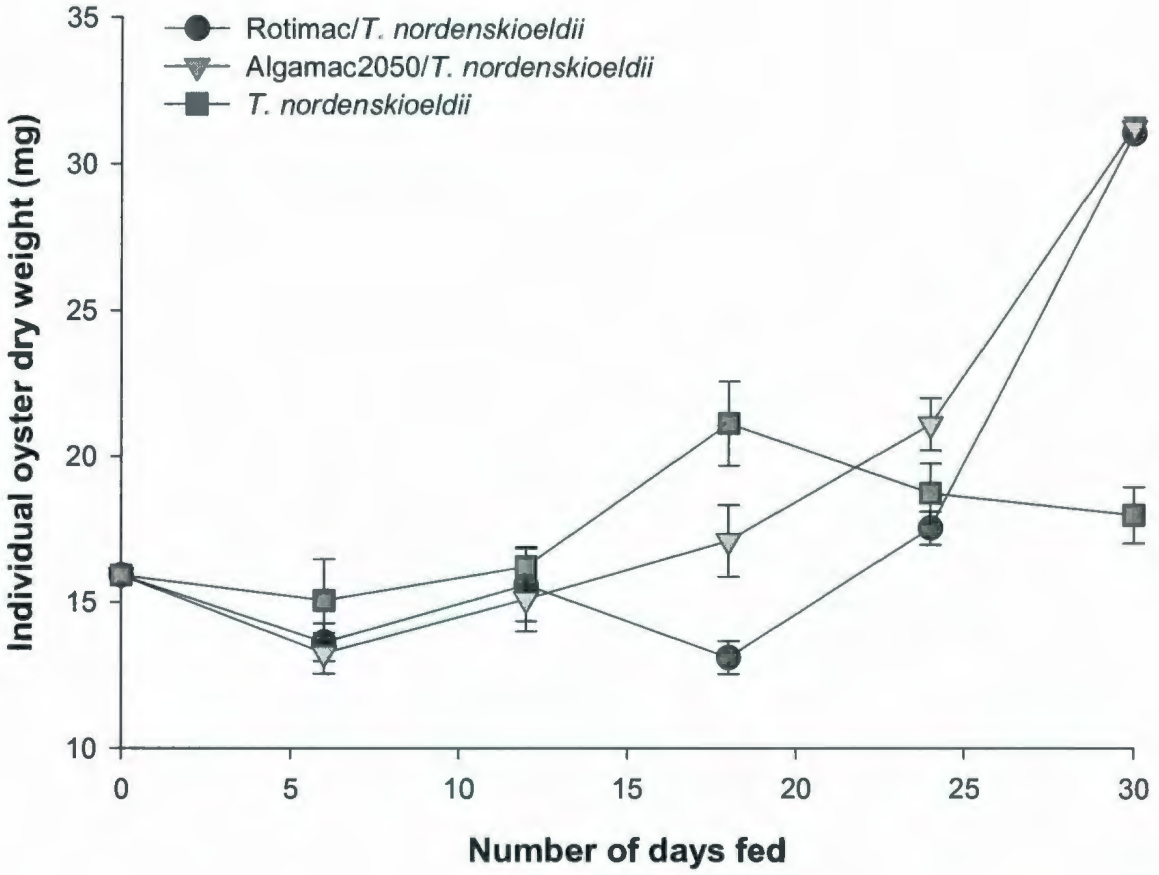

Figure 17. Dry weight (mg ± SE) per individual Pacific oyster (*Crassostrea gigas*; n = 30) batch fed once per 24 hours test diets at 1% of the initial dry weight of oysters per tank for 30 days. *Thalassiosira nordenskioeldii* was the dominant phytoplankton species (>90%) in outdoor grown bloom tank cultures. Rotimac™ and Algamac 2050™ are spray-dried *Schizochytrium*-based live algae substitute diets (Aquafauna Biomarine, Hawthorne, California, USA).

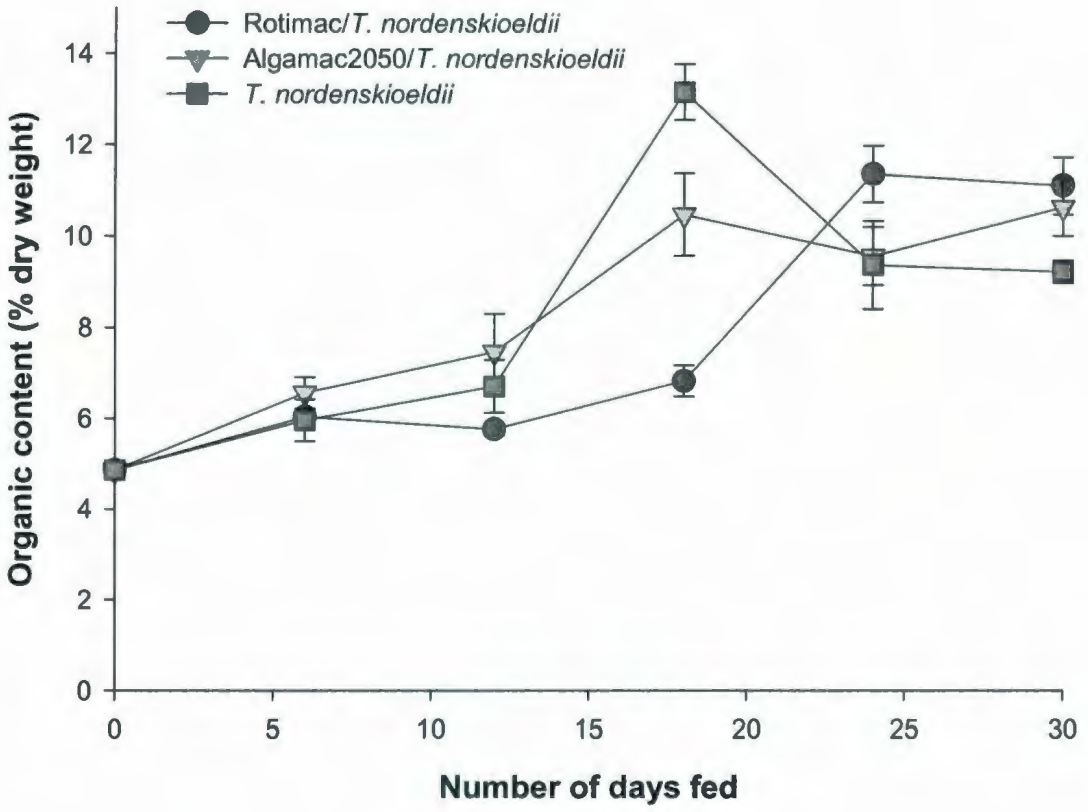

Figure 18. Percentage organic content or ash-free dry weight (± SE) of juvenile Pacific oysters (*Crassostrea gigas*) batch fed test diets at 1% of the initial dry weight of oysters per tank for 30 days. *Thalassiosira nordenskioeldii* was the dominant phytoplankton species (>90%) in outdoor grown bloom tank cultures. Rotimac™ and Algamac 2050™ are spray-dried *Schizochytrium*-based live algae substitute diets (Aquafauna Biomarine, Hawthorne, California, USA).

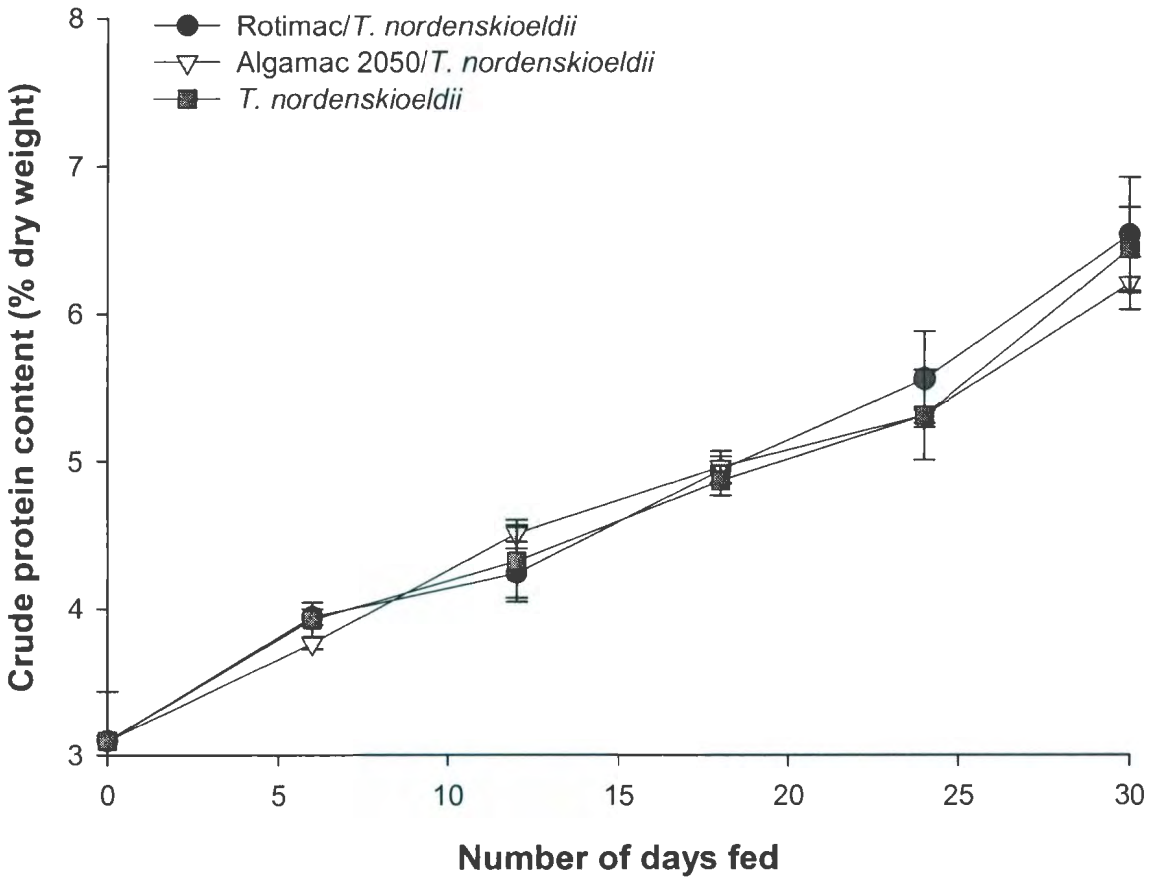

Figure 19. Percentage (± SE) crude protein content per individual dry Pacific oyster (*Crassostrea gigas*) batch fed once per 24 hours test diets at 1% of the initial dry weight of oysters per tank for 30 days. *Thalassiosira nordenskioeldii* was the dominant phytoplankton species (>90%) in outdoor grown bloom tank cultures. Rotimac™ and Algamac 2050™ are spray-dried *Schizochytrium*-based live algae substitute diets (Aquafauna Biomarine, Hawthorne, California, USA).

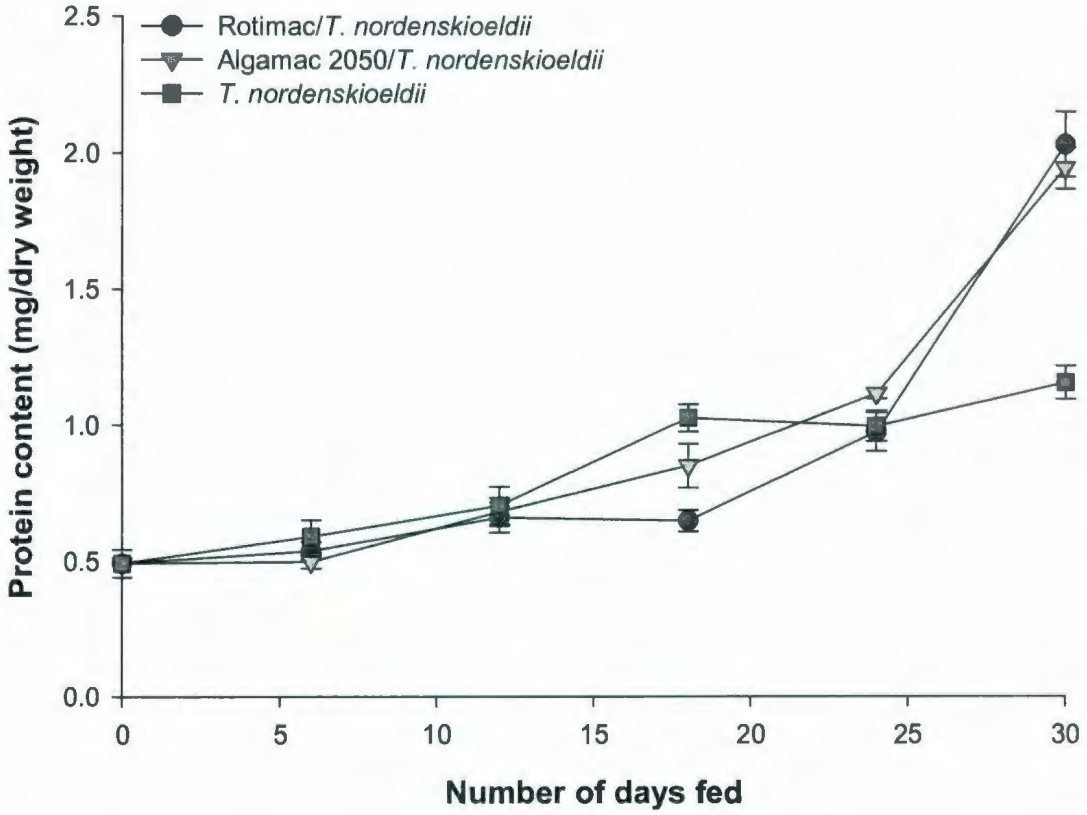

Figure 20. Crude protein content (mg ± SE) per individual dry Pacific oyster (*Crassostrea gigas*) batch fed once per 24 hours test diets at 1% of the initial dry weight of oysters per tank for 30 days. *Thalassiosira nordenskioeldii* was the dominant phytoplankton species (>90%) in outdoor grown bloom tank cultures. Rotimac™ and Algamac 2050™ are spray-dried *Schizochytrium*-based live algae substitute diets (Aquafauna Biomarine, Hawthorne, California, USA).

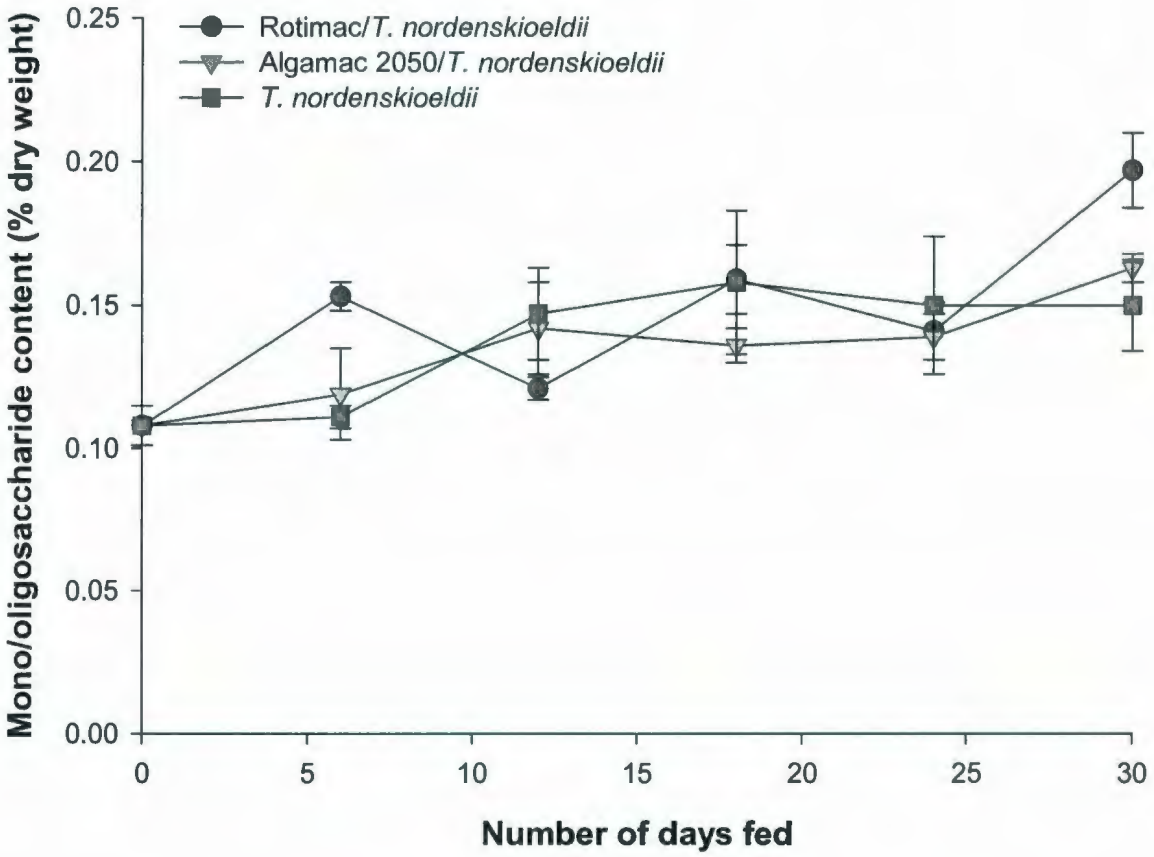

Figure 21. Percentage (± SE) crude mono/oligosaccharide content per individual dry Pacific oyster (*Crassostrea gigas*) batch fed once per 24 hours test diets at 1% of the initial dry weight of oysters per tank for 30 days. *Thalassiosira nordenskioeldii* was the dominant phytoplankton species (>90%) in outdoor grown bloom tank cultures. Rotimac™ and Algamac 2050™ are spray-dried *Schizochytrium*-based live algae substitute diets (Aquafauna Biomarine, Hawthorne, California, USA).

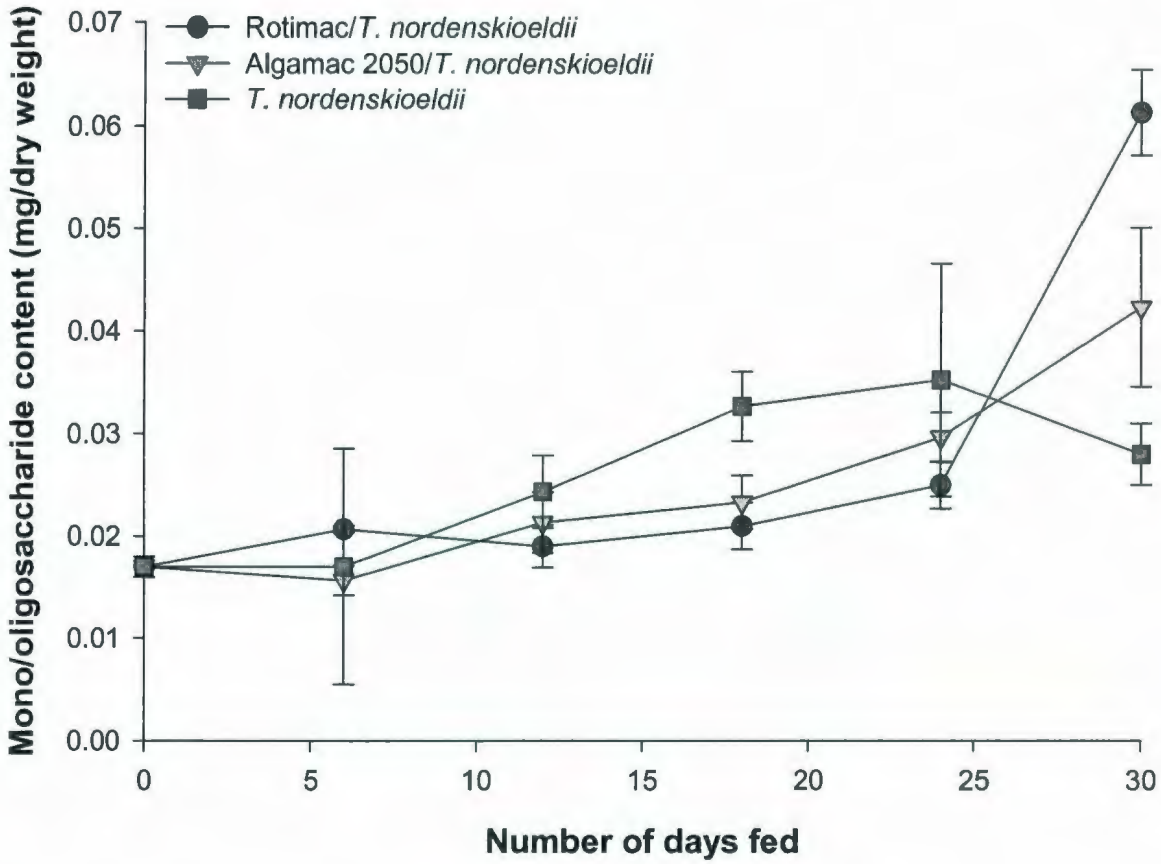

Figure 22. Crude mono/oligosaccharide content (mg ± SE) per individual Pacific oyster (*Crassostrea gigas*) batch fed once per 24 hours test diets at 1% of the initial dry weight of oysters per tank for 30 days. *Thalassiosira nordenskioeldii* was the dominant phytoplankton species (>90%) in outdoor grown bloom tank cultures. Rotimac™ and Algamac 2050™ are spray-dried *Schizochytrium*-based live algae substitute diets (Aquafauna Biomarine, Hawthorne, California, USA).

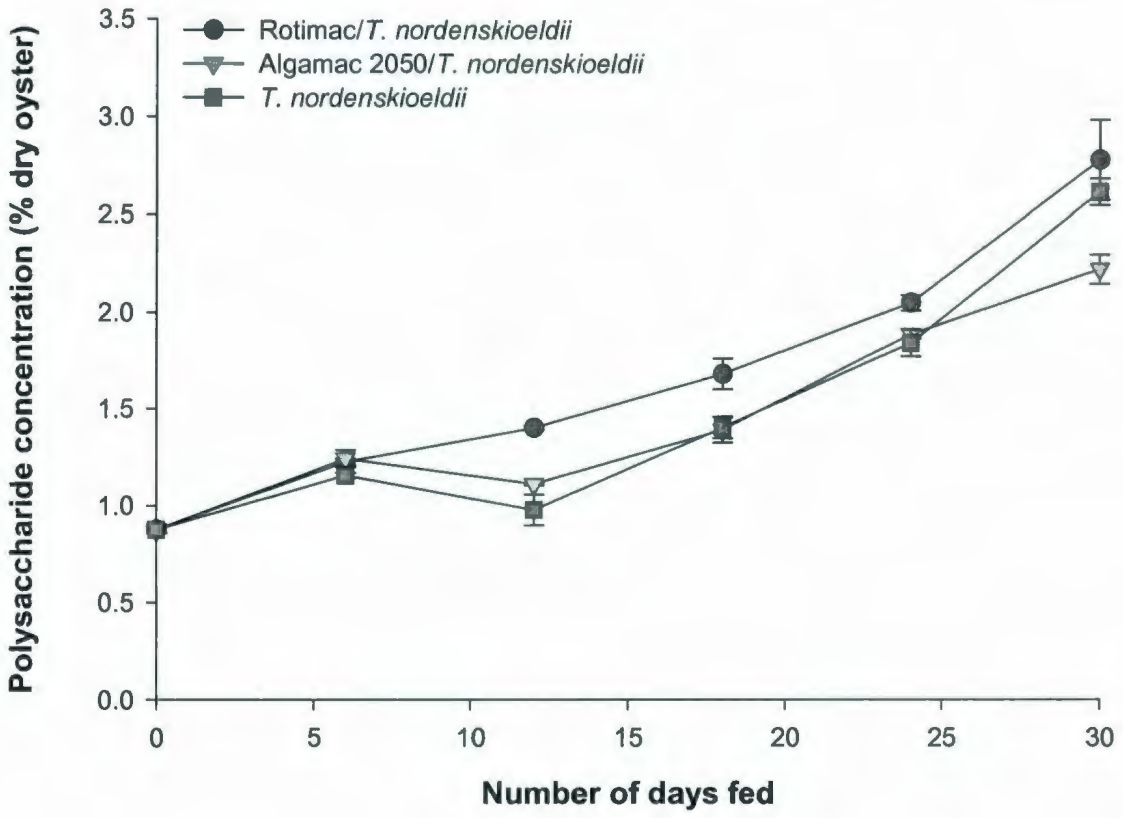

Figure 23. Percentage (± SE) crude polysaccharide content per individual Pacific oyster (*Crassostrea gigas*) batch fed once per 24 hours test diets at 1% of the initial dry weight of oysters per tank for 30 days. *Thalassiosira nordenskioeldii* was the dominant phytoplankton species (>90%) in outdoor grown bloom tank cultures. Rotimac™ and Algamac 2050™ are spray-dried *Schizochytrium*-based live algae substitute diets (Aquafauna Biomarine, Hawthorne, California, USA).

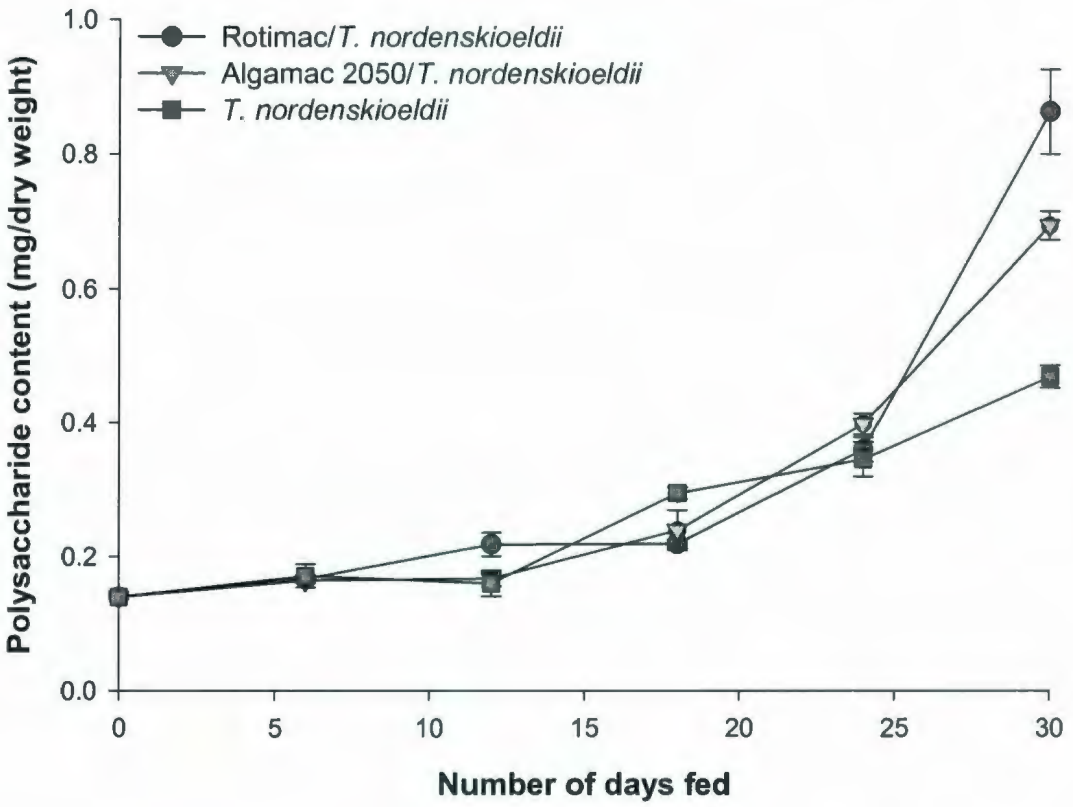

Figure 24. Crude polysaccharide content (mg ± SE) per individual Pacific oyster (*Crassostrea gigas*) batch fed once per 24 hours test diets at 1% of the initial dry weight of oysters per tank for 30 days. *Thalassiosira nordenskioeldii* was the dominant phytoplankton species (>90%) in outdoor grown bloom tank cultures. Rotimac™ and Algamac 2050™ are spray-dried *Schizochytrium*-based live algae substitute diets (Aquafauna Biomarine, Hawthorne, California, USA).

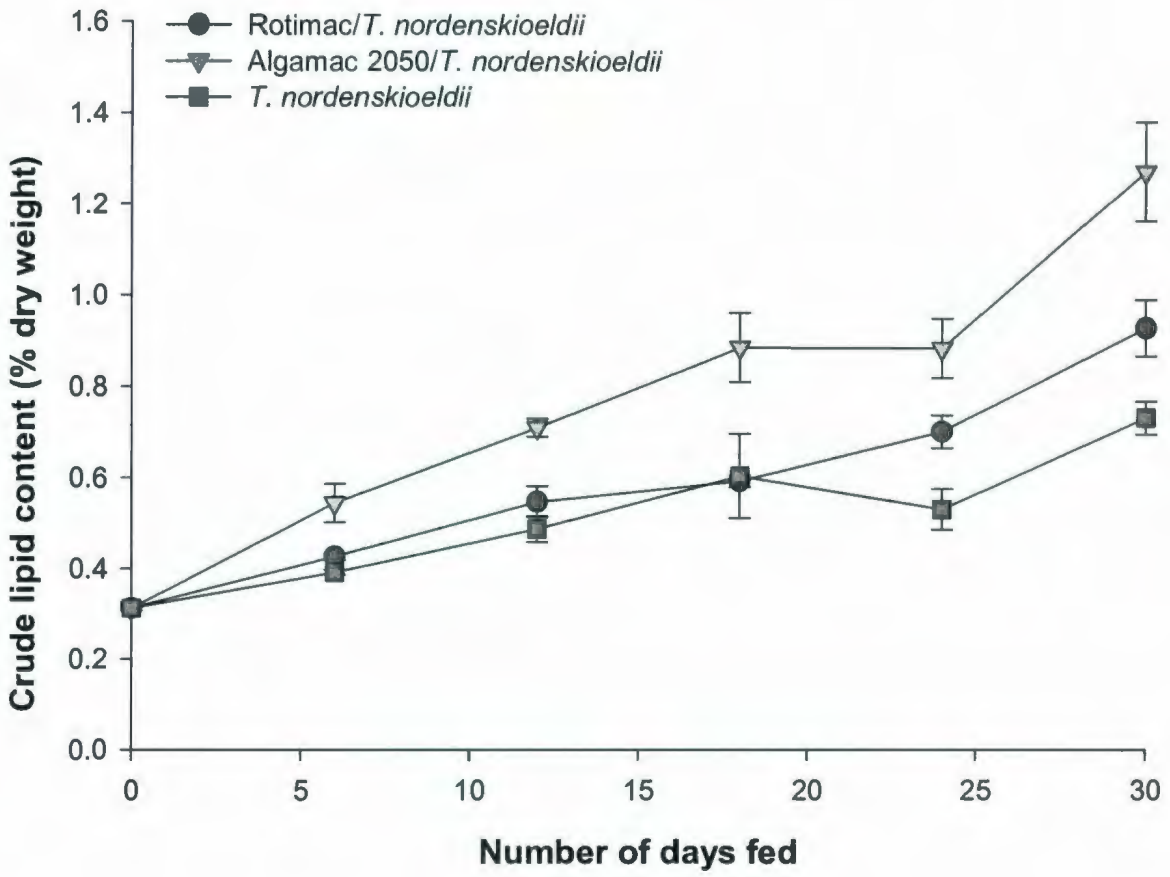

Figure 25. Percentage (± SE) crude lipid content per individual Pacific oyster (*Crassostrea gigas*) batch fed once per 24 hours test diets at 1% of the initial dry weight of oysters per tank for 30 days. *Thalassiosira nordenskioeldii* was the dominant phytoplankton species (>90%) in outdoor grown bloom tank cultures. Rotimac™ and Algamac 2050™ are spray-dried *Schizochytrium*-based live algae substitute diets (Aquafauna Biomarine, Hawthorne, California, USA).

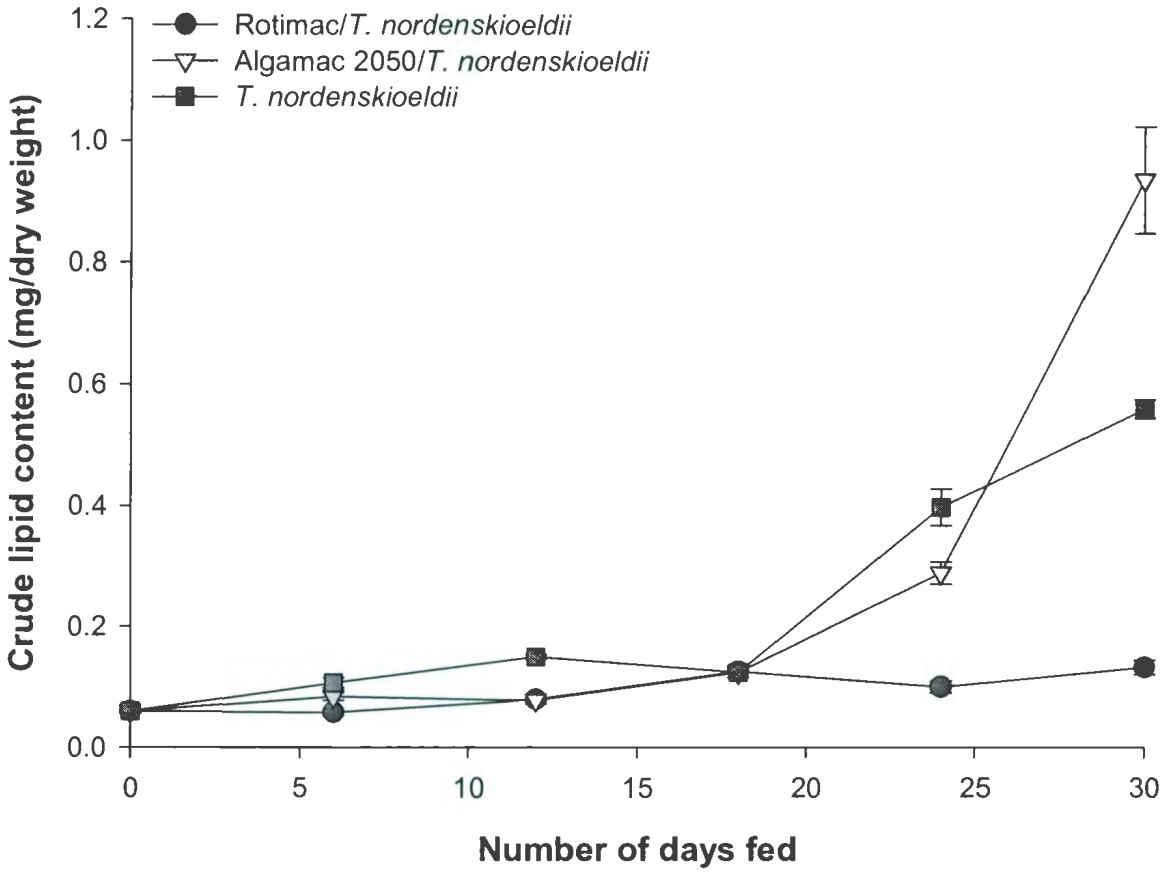

Figure 26. Crude lipid content (mg ± SE) per individual Pacific oyster (*Crassostrea gigas*) batch fed once per 24 hours test diets at 1% of the initial dry weight of oysters per tank for 30 days. *Thalassiosira nordenskioeldii* was the dominant phytoplankton species (>90%) in outdoor grown bloom tank cultures. Rotimac™ and Algamac 2050™ are spray-dried *Schizochytrium*-based live algae substitute diets (Aquafauna Biomarine, Hawthorne, California, USA).

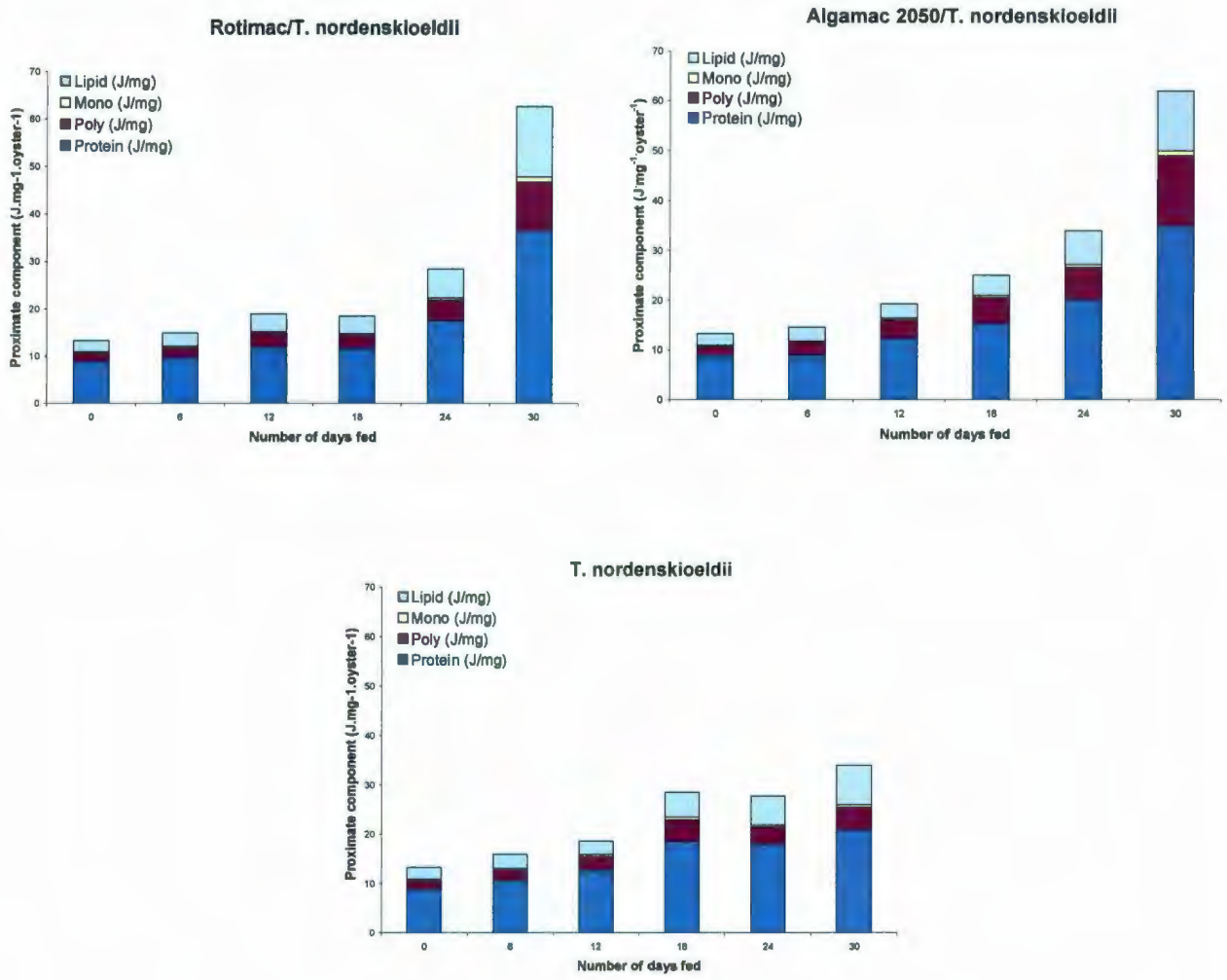

Figure 27. Energy equivalent of the proximate composition of the organic fraction of juvenile *Crassostrea gigas* fed test diets for 30 days. Total components is the sum of tested components (protein, lipid, polysaccharide and mono/oligosaccharides; Joules·mg^{-1}·oyster^{-1}). Oysters were batch fed once per 24 hours test diets at 1% of the initial dry weight of oysters per tank for 30 days. *Thalassiosira nordenskioeldii* was the dominant phytoplankton species (>90%) in outdoor grown bloom tank cultures. Rotimac™ and Algamac 2050™ are spray-dried *Schizochytrium*-based live algae substitute diets (Aquafauna Biomarine, Hawthorne, California, USA).