TEMPORAL VARIATION IN DIGESTIVE ENZYME ACTIVITY OF *Euphausia Pacifica*

by

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ABSTRACT

Field investigations into the temporal variation of digestive enzyme activity in the planktonic crustacean, *Euphausia pacifica* Hansen, 1911 (Crustacea; Eucarida) were carried out in Saanich Inlet, British Columbia, Canada over a period of nine months from March through November 1989. The results obtained from analysis of the activity of laminarinase, a dominant polysaccharidase in zooplankton, suggest that the animal feeds preferentially on phytoplankton which are high in the carbohydrate storage product, laminarin (ß-1, 3-glucan). Other food types in the Inlet, such as phytoplankton with lower amounts of laminarin, detritus and zooplankton larvae do not appear to be a major part of the animal's diet. A second enzyme, a trypsin-like protease was also analyzed for activity and showed little temporal variation over the study period. Mean levels of protease activity observed in field animals were always higher than mean levels seen in starved laboratory animals. After a period of starvation, specimens of *E. pacifica* maintained in the laboratory were fed high carbohydrate diatoms and *Artemia* nauplii in order to induce laminarinase and protease activity respectively. Animals were fed the diatom, *Skeletonema costatum* and laminarinase activity was observed to more than double within six hours

of this feeding to approximately 1300 μg glucose produced per minute/g wet weight. Peak laminarinase activity of approximately 1700 μg glucose produced per minute/g wet weight was observed thirty hours after the initial feeding. Animals fed on *Artemia* and analyzed for laminarinase activity showed no increase in enzyme activity over pre-feeding values. Variations in trypsin-like protease activity were not inducible in the laboratory regardless of proffered food type.

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Chapter 1

INTRODUCTION

The particulate environment in which zooplankton live and feed displays a great deal of temporo-spatial heterogeneity in terms of the food types available for ingestion (Mackas et al., 1985). Animals that can adapt to this patchiness of nutritional resources have an advantage over others that cannot. Spawning cycles timed to coincide with the appearance of food, vertical and horizontal migration patterns and larval over-wintering are all strategies used by zooplankton to increase their chances of obtaining sufficient nutrition to grow and reproduce (Mayzaud et al., 1985). Apart from behavioral and reproductive strategies, animals that display physiological adaptations that allow them to take the greatest advantage of ephemeral food sources would likely have an energetic and ultimately an evolutionary advantage over those that lack such capabilities. Regulation of the production and secretion of digestive enzymes, as a response to the biochemical composition of the particulate matter in the animal's diet would be such an advantage.

Grossman et al. (1943) found that in both rats and dogs the average enzyme composition of the pancreatic tissue was proportionally related to the biochemical composition of the diet of the animals. In the case of invertebrates, numerous recent laboratory studies on assorted species of zooplankton have shown that the activity of several classes of digestive enzymes varies proportionally with their specific substrate concentrations in the diet of the animal (Cox, 1981; Cox et al., 1982; Mayzaud et al., 1985; Willason and Cox, 1987) yet few field studies have been done on long term temporal variation of major digestive enzymes. Because of the temporal patchiness (in terms of both quantity and quality) of food particles available to zooplankton in coastal waters, these animals must display some type of regulation of the production and secretion of specific digestive enzymes, in order to obtain the maximum nutritional benefit for the lowest energetic cost. Mayzaud et al, (1985) state that "digestive enzyme activity of zooplankton is the functional link between ingestion and assimilation. It has been shown to be representative of the feeding adaptability to changes in quantity or quality of the food supply...". Such a tight linkage between an environmental parameter such as food type, and a specific physiological response, in this case production and secretion of digestive enzymes, has energetic implications for zooplankton.

Enright (1977) and Enright and Honegger (1977) proposed that energetic economy is the driving force behind vertical migration: digestion at depth of material ingested at the surface provides a migrator with an energetic advantage over non-migrators in that the cooler temperatures at depth lower the basal metabolic rate (BMR) of the animal. A lower BMR allows the animal to use a larger part of the energy obtained from a recent meal for reproduction or growth rather than for maintenance (McLaren, 1963). Similarly, in the case of production and secretion of digestive enzymes, an animal that is able to regulate the production of its enzymes in concert with the types of food available would have an energetic advantage over those animals without this control. The ability of an animal to compete and survive, in terms of energetic economy and competition for resources in a patchy food environment, would then depend upon the degree of control it had over its enzyme production and secretion.

In this thesis, the temporal regulation of two digestive enzymes of a common North Pacific euphausiid, *Euphausia pacifica* was studied on two time scales: long-term variation over a nine-month period and daily variation under laboratory conditions. The purpose of these experiments was to estimate the degree of control that *E. pacifica* has in regulating its digestive enzyme activity by observing its ability to quickly to changes in available food types under both field and laboratory conditions.

The animal of interest in this study, *Euphausia pacifica*, is a common and usually dominant member of the zooplankton community of the eastern North Pacific Ocean (Kathman et al., 1986). This animal has been found in prolific swarms in Saanich Inlet on Vancouver Island, up to densities of $10,000/m^3$ (Mackie and Mills, 1983). It can be easily differentiated from the confamilial *Thysanoessa raschii*, the other present but less common euphausiid in the study area, by the obvious lack of a rostral spine. While found as deep as 300 meters in some locations in the North Pacific, the animal's maximum depth range is limited in Saanich Inlet by the presence of an oxycline in the inlet that is usually found around 150 meters (Kathman et al., 1986). *E. pacifica* is a major part of the food web in northeast Pacific waters both ecologically and economically. It is an important food source for seabirds, baleen whales, squid and a vital part of the diet of salmonids (Mauchline, 1980; Kathman, 1986). Recently, interest has centered around the establishment of commercial fisheries for *E. pacifica* and commercial cultivation of the animal for the manufacture of feeds for farm fish.

The trophic classification of *E. pacifica* appears to be the subject of some controversy in the literature. Ponomareva (in Lasker, 1966) stated in an early work (1955) that while phytoplankton is generally not an important food source for *E. pacifica*, some feed upon it in the spring. Lasker (1966) classified the animal as an omnivore with a preference for the cultured branchiopod crustacean, *Artemia* over phytoplankton and Willason et al. (1986) classified *E. pacifica* as primarily herbivorous. Mauchline and Fisher (1969) classified the animal as an omnivore that eats everything from algae and echinoderm larvae to other crustacea (presumably larvae) and detritus. Each of these studies took place in a different geographical location ranging from the Bering Sea to southern California and while the same organism was observed, the type of particulate matter available to the animal may have been completely different. *Euphausia pacifica* is likely best classified as an opportunistic omnivore that takes advantage of the most prevalent food type available at a particular time. Mayzaud and Mayzaud (1981) found omnivorous copepods to be equipped with a wide range of digestive enzymes capable of digesting almost all possible food types in their environment and this would appear to be a reasonable assumption for *E. pacifica* in Saanich Inlet.

The two major enzymes of interest are the dominant polysaccharidase unique to herbivorous zooplankton (Cox, 1981), laminarinase (ß-1,3-glucanohydrolase) and a trypsin-like alkaline protease. These enzymes meet several important criteria for the study: both appear to be membrane-bound within the digestive tissues of *E. pacifica* (Cox, 1981; Gates and Travis, 1969) thus rendering them fairly stable to the rigors of net capture and ultralow temperature freezing. Together they represent opposite ends of the nutritional spectrum in terms of the food types on which they act, and the analysis of activity of the enzymes is simple enough so as to reduce the possibilities of compound errors that may result from complex biochemical protocols.

Laminarinase is extremely substrate-specific, catalyzing the hydrolysis of sub-units of laminarin (ß-1,3 glucan), a short chain carbohydrate storage product common to many species of phytoplankton (Handa and Tominaga, 1969). This substance, also known as chrysolaminarin, exists in soluble and insoluble forms; the soluble form is the primary carbohydrate storage product in a wide variety of phytoplankton groups. Handa and Tominaga (1969) isolated it from several brown algae and from *Euglena* sp., as well as from the diatom species *Skeletonema costatum* and *Phaeodactylum tricornutum*. It has also been isolated from a number of species of the genus *Chaetoceros* which are common in the waters of Saanich Inlet (Hobson, 1983). Cox (1981) reported that "laminarinase" activity was due to the combined actions of two forms of the enzyme: an endo-ß-1, 3-glucanase which cleaves oligosaccharide fragments from within the chain, and an exo-ß-1, 3-glucanase which produces free glucose by cleaving single subunits from the end of the chain. Of interest in this study is the exo-ß-1, 3-

glucanase: the end-product of the reaction of this form of the enzyme with laminarin is free D-glucose which is detectable using a simple colormetric assay. Mayzaud and Mayzaud (1981) investigated the kinetic properties of laminarinase and reported an optimal pH of 5.0 and an optimal temperature of 50°C. While the optimal pH value falls within the natural range of pH for digestive juices of Crustacea as a class (5.0 to 7.5; Mayzaud and Mayzaud, 1981), the enzyme operates at far from optimal temperature in the natural environment. The enzyme has a reported Michaelis constant (Stryer, 1981; K_m) of 0.37±0.07 mg/mL±SE for the substrate laminarin (sic, Mayzaud and Mayzaud, 1981).

While the presence of acidic and alkaline trypsin-like proteases has been confirmed in various species of crustacea (Gates and Travis, 1969; Mayzaud, 1979; Mayzaud and Mayzaud, 1981) very little work has been done on the activity of these enzyme classes. Alkaline proteases belong to a family of enzymes with molecular weights between 20,000 and 25,000 and catalyse the hydrolysis of bonds in peptides involving the carboxyl group of either arginine or lysine (Keil, 1971). Alkaline proteases isolated from copepods have similar properties to mammalian trypsin (Mayzaud and Mayzaud, 1981) and while trypsinogen, the inactive precursor form of trypsin, has not been found in invertebrates (Keil, 1971), it is likely that invertebrate trypsin-like proteases require the presence of a divalent cation for activation. In an experiment where p-toluene-sulfonyl-L-arginine methyl ester hydrochloride (TAME) was used as a substrate, Mayzaud and Mayzaud (1981) found the optimal pH for alkaline proteases as a group to be 9.0. An optimal temperature and Michaelis constant (K_m) were not reported for the enzyme in the same paper. In a paper describing a method for the determination of mammalian trypsin, Hummel (1959) suggested a temperature of 25°C at a pH of 8.1 as conditions for maximum activity of this enzyme.

Investigations into seasonal cycles of nutritional physiology provide necessary data from which a better understanding of euphausiid feeding ecology and physiology may be obtained. Use of this information may subsequently lead to the development of a euphausiid aquaculture industry such as that already in existance for the brine shrimp, *Artemia*. This industry, in turn would benefit both the salmon aquaculture industry and the wild stocks of euphausiids by preventing overfishing of the natural populations.

Chapter 2

MATERIALS AND METHODS

Sampling

(a)Field Station

All field samples were taken from Station "E" (Lat. 48°37'N, Long. 123°30'W) in Saanich Inlet, a fjord located on the southeast tip of Vancouver Island, British Columbia, Canada (Figure 1). Samples were taken at approximately 1000 hours local time throughout the study. (See section (c).ii in Field Experiments)

(b)Water Sampling

Seawater samples were obtained with hydrocasts at depths of 0, 3, 6, 9, 12, 15, 20 and 30 meters using a 30L Niskin bottle. An aliquot (100 mL) of seawater from each depth was removed directly from the Niskin bottle. This seawater any organisms therein were immediately preserved in standard Lugol's Solution (Parsons et al., 1984). The remaining water was filtered through a 500-μm Nytex mesh to remove detritus and large zooplankton and then stored in 20L Nalgene carboys. These carboys were stored in the dark at 9°C upon return to the laboratory.

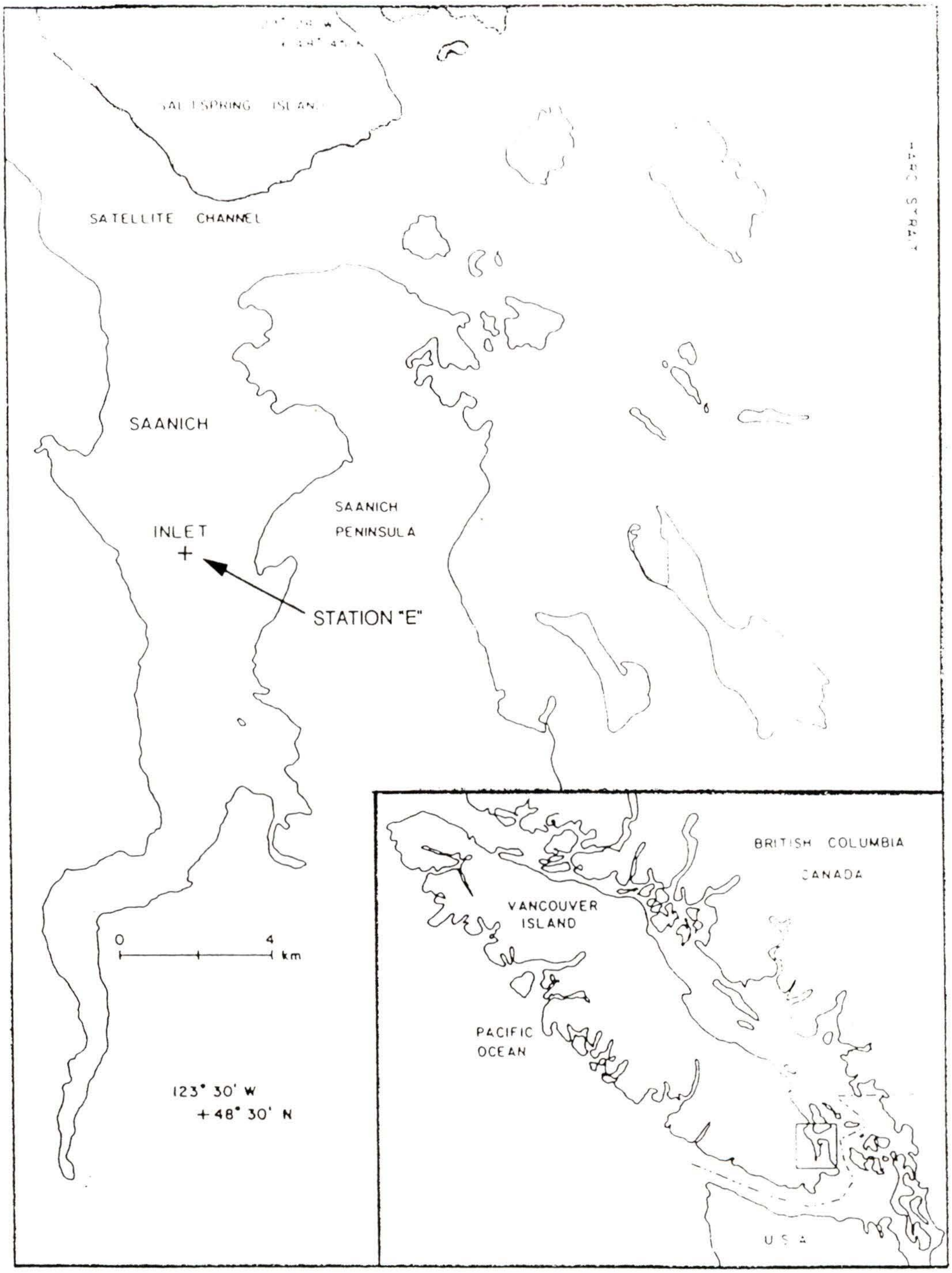

Figure 1: Map of Saanich Inlet, British Columbia and location of Station "E". (after Stucchi and Giovando, 1984)

(c) Collection of Animals

(i) Field animals

Specimens of *Euphausia pacifica* Hansen 1911 (Crustacea; Eucarida) were collected using a Reeve plankton net (Reeve, 1981; mesh size 333 μm) equipped with a large vented cod-end to prevent the animals from being crushed. The net was lowered on a hydrographic wire from the rear deck of the M.S.S.V. John Strickland and oblique tows, from below the scattering layer to the surface, were carried out at a speed of 2 knots. Depth of the scattering layer was determined using the shipboard Furuno echo depth sounder operating at 200 KHz. Upon completion of the tow, the cod end was removed and the animals contained therein were poured gently onto a coarse mesh filter. *E. pacifica* were identified and placed in Whirl-pak bags containing seawater collected from 50m. The bags were labeled with the date and location of the sample site and the contents quick-frozen by immersion in 95% ethanol cooled with dry ice. The temperature obtained with this solution was approximately -80°C (Ashwood-Smith, pers. com.), which was cold enough to stop the enzymatic processes of interest in the euphausiids (Willason and Cox, 1987).

(ii)Laboratory animals

Animals to be used for laboratory experiments were captured in a similar manner to those to be analyzed for field enzyme activity levels. The contents of the cod-end were gently poured through a coarse mesh filter and the animals caught on the filter were resuspended in a shallow pan filled with seawater from 50m. *E. pacifica* were sorted with a fine paintbrush and transferred to jars containing 50m seawater. The jars were filled to a density of approximately 20 animals/L and placed in dark, ice-filled coolers until their return to the laboratory. There, the animals were transferred to 30L glass aquaria, filled with filter-sterilized (0.45-μm, Millipore) seawater collected from 50m. (See Laboratory Animal Maintenance for further details).

Seawater Analysis

(a)Direct Observation of Preserved Samples via Inverted Microscope

Preserved subsamples (100 mL) of seawater from each sample depth were examined to determine the type of particulate matter available to *E. pacifica*. Samples were allowed to settle for 24 hours in 25 mL Utermöhl chambers and then examined using a Wild-Leitz inverted microscope. Organisms in five replicate areas of 0.235 cm^2 were counted and were separated into five classes of particles:

Bacillariophyceae (diatoms), Dinophyceae (dinoflagellates), Chrysophycean-like flagellates, zooplankton (usually larvae) and detritus (items that were identifiable as organic non-living matter). Replicate counts were averaged and the number of counts per mL was calculated for each of the five classes. Average cell volumes of the three phytoplankton classes were calculated using length, height, and diameter measurements of cells taken using an ocular micrometer. These numbers were entered into the appropriate formula for the geometric shape best representing the cell shape (e.g. cylinder = some diatoms, pyramidal = armoured dinoflagellates etc...) and volumes calculated (Jiménez et al., 1987). This procedure converted the raw number counts to a measure of biovolume as a measure of biomass.

(b) Chlorophyll a Analysis

Water in the carboys was analyzed within twenty-four hours for the plant pigment, chlorophyll a[1] (and asssociated

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[1] Chlorophyll a shares an almost identical absorbtion spectrum with phaeophytin, the by-product of its acid hydrolysis. If present in the water samples, phaeophytins would lead to an overestimate of the amount of chlorophyll a present. Although chlorophylls b and c may also have been present in the sample, the selection of excitation and emission filters used in the fluorometer ensured that it was chlorophyll a primarily being measured. A Corning 2-64 emmision filter was used which, because of its narrow bandwidth did not allow the passage of emission fluoresence from chlorophyll c and phaeophytin c (Yentsch and Menzel, 1963; Holm-Hansen et al., 1965; Lorenzen, 1966; Turner Designs, 1981; Turner Designs, 1983).

phaeopigments[2]), by means of a modified method of the flow-through fluorometry technique first reported by Lorenzen (1966) utilizing filters as specified in Turner, 1983. The method involved pumping seawater through a flow-through cuvette in a Turner Designs Model 10 fluorometer and directly measuring the fluorescence of the phytoplankton in the water sample. The fluorometer was directly connected to a computer, and combined chlorophyll a and phaeopigment a values were calculated and printed out in mg/m^3. Fluorometer readings were calibrated using pure cultures of the diatom, *Thalassiosira weissfloggii* Hustedt, the chlorophyll a content of which were obtained through trichromic pigment analysis. The sensitivity of the TD Model 10 allowed for a range of chlorophyll values from 0.1-31,600 mg/m^3 with precision and accuracy suitably comparable for this study to discrete sample methods (Flemer, 1969 in Turner Designs, 1981). This precision and accuracy was checked periodically by performing a trichromic chlorophyll analysis (Jeffrey and Humphrey, 1975 in Parsons et al, 1984) on the water samples and comparing the results to those obtained with the flow-through method (see Appendix Table 14 for a comparison of the two methods).

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[2] Chlorophyll breakdown pigments, phaeophytin, phaeophorbide and other phaeopigments are also reported as pheophytin, pheophorbide and pheopigments in many journals. The spelling used in this paper is consistent with that used in Parsons et al, 1984.

(c) Total Particulate Protein (TPP) Analysis

Following chlorophyll analysis, aliquots of the same seawater samples were analyzed in triplicate for total particulate protein, standardized against known concentrations of bovine serum albumin. Samples were filtered through nitrocellulose membrane filters (47-mm, 1.0-μm pore size) that had been coated with a thin layer of an $MgCO_3$ suspension prior to filtration of the sample, and the precipitate on each filter was rinsed into acid-cleaned beakers. The suspension was then centrifuged and the precipitate analyzed using the modified micro-Lowry method of Peterson (1977), and reagents as supplied by Sigma Chemical Company (Lowry Protein Assay Kit P5656). Following reagent addition to unknowns, blanks and Bovine Serum Albumin (Sigma # P7656) standards, all samples were incubated at room temperature for thirty minutes and after further centrifugation to remove particles of $MgCO_3$ from suspension, the absorbances of the supernatants were read on an LKB Biochrom Ultrospec II, Model 4050, at 750 nm. A BSA standard curve was plotted and a regression line fitted to the straight part of the curve ($m=0.27 \times 10^{-3} \pm 4.1 \times 10^{-4}$, 95% c.i.). The protein concentrations of the unknown samples were obtained in bovine serum albumin equivalent units from this regression.

(d)Total Particulate Matter (TPM) Analysis

Using aliquots of the same seawater samples, the weight of the total particulate matter in seawater at each depth was determined in triplicate using the gravimetric method of Banse et al. (1963) as outlined by Strickland and Parsons (1972). Samples of 500 mL were filtered onto 47-mm diameter, 1.2-μm glass fiber filters that had been previously washed, dried and weighed. After all sample water had passed through the filters, the filter flasks were rinsed twice with small amounts of distilled water and these rinses were filtered. With vacuum still applied, the filter flasks were removed and the edges of the filters carefully rinsed with distilled water to remove any salts that may have accumulated during filtration. The filters and precipitate were dried and weighed again, and by subtraction, the dry weight of the "micro-seston" from each depth was calculated in g/m^3. Similarly treated filters, through which no sample had been filtered, were used as controls to correct for any changes in weight that may have occurred over time. Replicate weights were averaged and integrated over depth.

Experimental Protocols

(a)Laboratory Animal Maintenance

Animals to be used in laboratory experiments were maintained in seawater in 30L glass aquaria. The water was taken from a depth of 50m in Saanich Inlet and filtered using 0.45-μm Millipore filters. The aquaria were kept in the dark at a temperature of 9.0°C and any observations of the animals were made under red light conditions. To prevent bacterial growth from stressing the animals, the aquaria water was filtered (0.45-μm Millipore filters) every two days and dead animals removed daily. Initial percentage mortality upon transfer to the aquaria were observed to be approximately 5% and percentage survivorship was approximately 85% after three weeks.

(i)Laboratory Food Types

Animals maintained in the laboratory were fed on one of two food types: a high protein cultured branchipod larva, *Artemia* sp., or the diatom, *Skeletonema costatum* (Greville) Cleve known to have a high concentration of stored carbohydrate (Handa and Tominaga, 1969). *Artemia* nauplii were hatched from desiccated cysts in 50 ppt seawater under strong illumination at room temperature (20°C). Immediately after hatching, live nauplii were separated from unhatched cysts and transferred to fresh brine. The nauplii were allowed to develop for 24 hours before being used as food for experimental euphausiids. *Skeletonema costatum* cultures were grown using the "f/20" nutrient media of Guillard and Ryther (1962) and used as food four to seven days after inoculation. This delay was to ensure that the cultures had reached "stationary phase" growth, when levels of the carbohydrate storage product, soluble ß-1,3-glucan were highest (Cox, 1981).

(b) Field Experiments

(i) Field Experiment #1 - Seasonal Variation of Digestive Enzymes

Specimens of *Euphausia pacifica* were taken from Station "E" in Saanich Inlet (see Sampling for details) every two weeks from March 23, 1989 to November 27, 1989 (see Appendix Table 2). For each sample date, triplicate samples of three or more animals were analyzed for both laminarinase activity and trypsin-like protease activity. The results of these analyses were correlated with chlorophyll measurements, TPP, TPM and available food types in an attempt to determine proximal causes for the patterns of temporal variation that were observed.

(ii) Field Experiment #2 - Day/Night Comparison of Laminarinase Activity

In order to ensure that there was no significant difference in levels of laminarinase activity in animals collected during the day from below the scattering layer, and those collected during the night from within the area of maximum chlorophyll a in the water column, a day/night sampling regime was executed. Samples of water and animals were taken at approximately 1000h PDT and similar samples were taken at 2200h PDT at the same sample site. At both sampling times, chlorophyll a profiles of the water column from 0 to 30 meters were measured, using the method described earlier. During the morning sample time, animals were taken from below the scattering layer, sorted and frozen in the usual manner. During the evening sample, the animals were taken from that depth in the water column showing the maximum chlorophyll a value as indicated by the recently measured profile. These animals were observed for degree of gut fullness and color to ensure that they were feeding animals and not non-feeding vertical migrators. These observations were recorded. The animals were frozen in the previously-described manner. Several replicates of both groups of animals were later analyzed for laminarinase activity and the Student's *t* test was performed to determine if there were any significant differences in the levels of activity.

(c)Laboratory Experiments

(i)Laboratory Experiment #1 - Background-Baseline Enzyme Activity

Because whole animal homogenates were used for both laminarinase and protease enzyme analyses, it was desirable to identify levels of enzyme activity in tissues other than those involved in digestion (background activity) and levels of activity in the digestive tissues of starved animals (baseline activity). This was achieved by analyzing whole animal homogenates of specimens that had been starved for at least two weeks and attributing the measured activity to a combination of both baseline and background sources. Willason and Cox (1987) found that after four days starvation, activity levels stabilized at or near to zero. Two weeks starvation was chosen to be completely sure that levels of activity had sufficient time to stabilize at their "starvation" level of activity. For each analysis (laminarinase or protease), three or more specimens were sampled three times a day (0900, 1500 and 2100h) for two days, the results for each sample time averaged and 95% confidence intervals calculated. Using the six datapoints obtained over the two days, average values and 95% confidence intervals were calculated for both laminarinase and trypsin-like protease activity in starved animals.

(ii)Laboratory Experiment #2 - Laminarinase Induction

Two groups of *E. pacifica* were maintained under different feeding conditions and individuals from each treatment were removed periodically and analyzed for laminarinase activity. Initially, both groups were starved for one week at the end of which triplicate samples were taken at three times during the day. One group was then fed stationary phase cells of the diatom *Skeletonema costatum* and the other group fed *Artemia* nauplii once a day for four successive days. Triplicate samples were removed three times a day from each treatment tank and analyzed for laminarinase activity. Chlorophyll a and phaeopigment values were measured in the algal treatment tank at the same times that animals were taken, in an attempt to correlate the amount of food available with the level of enzyme activity observed.

(iii)Laboratory Experiment #3 - Sustained Laminarinase Activity

To observe the effects of "bloom condition"[3] amounts of food upon laminarinase activity levels, a group of *E. pacifica* were maintained for four days on a high concentration diet of stationary phase *Skeletonema costatum* cells by feeding them three times a day as opposed to just once a day as in other experiments. Feedings consisting of 250 mL of *S. costatum* culture were administered immediately before each sample time, and as with the previous experiment, triplicate samples of animals were removed three times a day for laminarinase analysis; chlorophyll a and phaeopigment values were also measured according to the method of Jeffrey and Humphrey, 1975 in Parsons et al, 1984.

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[3] Chlorophyll values throughout the year in Saanich Inlet are usually below 3 mg/m^3 except in the spring and the late fall when increases up to 20 mg/m^3 and occasionally 30 mg/m^3 have been observed (Finnigan, pers. obs.;Hobson, 1983)

(iv)Laboratory Experiment #4 - Trypsin-like Protease Induction

The procedure for this experiment was the same as for the laminarinase induction experiment (Exp. #2) except that samples were analyzed for a trypsin-like protease enzyme.

Enzyme Activity Analysis

(a)Laminarinase Activity Analysis

Activity of the digestive enzyme laminarinase (ß-1,3-glucanohydrolase) was measured using the method of Cox (1981). Animals collected and frozen in the field were thawed at room temperature and triplicate samples, each containing 3 or more animals, from each sample date were blotted lightly and weighed to the nearest 0.001 g. The animals were homogenized with a motorized mortar and pestle tissue grinder in standard phosphate buffer (0.15M, pH 5.0) at 4°C using 1 mL buffer per animal; the whole animal homogenate was kept on ice at all times during the procedure. An aliquot of homogenate (100 μL) was mixed with laminarin substrate (extracted from *Laminaria digitata* Sigma # L9634) at a concentration of 2.5 mg/mL, shown by Cox (1981) and Mayzaud and Mayzaud (1981) to be sufficient to saturate enzyme activity. Samples were incubated at 37°C for one hour and the reaction stopped by the addition of 1°C distilled water. Following a five minute centrifugation at 12,000 g to precipitate proteins (Willason and Cox, 1987), supernatants were analyzed for free glucose using the Sigma Glucostat method (Sigma # 115.). This procedure involves a hexokinase-mediated conversion of glucose to glucose-6-phosphate followed by the reduction of nicotinamide adenine dinucleotide phosphate (NADP) to NADPH by means of glucose-6-phosphate dehydrogenase (G-6-PDH). Phenazine methosulphate (PMS) in the presence of NADPH is reduced to PMSH and in turn reduces iodonitrotetrazolium (INTCl) chloride to INTH, which is then measured colormetrically at 520 nm (Carroll et al., 1970 in Sigma Procedure No. 115) The homogenates and the substrates were also tested for free glucose as a form of reagent blank. Because excess substrate was used, all values for the activity of the enzyme represented V_{max} and were expressed as μg glucose produced per min/ g wet weight.

(b) Trypsin-like Protease Activity Analysis

Activity of the trypsin-like protease digestive enzyme was measured using a modified version of the method of Hummel (1959). Replicate samples of three or more *E. pacifica* were thawed, blotted lightly and weighed to the nearest 0.001 g. The animals were then homogenized in 0.001M HCl at a ratio of 1 mL acid per 2 animals. The homogenates were placed in Eppendorf micro-centrifuge tubes and either frozen at -80°C for analysis at a later date or centrifuged immediately and the supernatants drawn off for enzyme analysis. Aliquots (100 μL) of the homogenate supernatant were added to 300 μL of a 0.01M solution[4] of p-toluenesulfonyl-L-arginine methyl ester hydrochloride (TAME, Sigma # T4626) and 2.6 mL of a TRIS buffer (0.046M, pH 8.1 containing 0.0115M $CaCl_2$) in 1-cm quartz glass spectrophotometer cuvettes. The absorbances of these solutions were measured at 247 nm against a similarly prepared blank containing 100 μL of 0.001M HCl instead of the enzyme solution. Absorbance measurements were taken every twenty seconds for five minutes and absorbancy versus time was plotted. From the straight line portion of the curves, ΔA/minute was calculated and the following formula (Worthington, 1972) was used to calculate the activity of amount of trypsin-like protease present in the cuvettes.

(see over for formula)

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[4] Hummel (1959) showed that this concentration of TAME was sufficient to saturate the enzyme thus allowing the relationship $V_{max} = k_3[E_T]$ to hold true where V_{max} = maximum velocity of the reaction, k_3 = the turnover rate of the enzyme and $[E_T]$ = the concentration of the enzyme. It is upon this relationship that the formula used to calculate enzyme activity (Worthington, 1972) relies.

activity units/g wet weight =

$$\frac{\Delta_{247}/\text{min x 1000 x 3 mL}}{\text{540 x g homog. in cuv.}}$$

where one activity unit = the hydrolysis of one micromole of TAME per minute per g homogenate at 25°c and pH 8.1 in the presence of 0.01M calcium ion,

ΔA_{247}/min = maximum velocity of the reaction in terms of absorbancy units @ 247 nm,

540 = molar absorbancy index of p-toluenesulfonyl-L-arginine at 247 nm,

and g homogenate in cuvette = the amount of homogenate in grams in 100 μL.

Statistical Analysis

As no model of expected results was created for this study, statistical analysis was restricted to descriptive statistics only. All calculations and analyses were performed on an IBM AT personal computer using SAS« statistical analysis software package Version 6.0. Data were analyzed in several ways: multiple linear regression analysis was performed on data where one of the parameters displayed little or no error; Pearson product-moment correlation analysis was performed on data where error existed in all parameters. Means were compared using Student's unpaired *t*-test for unequal sample sizes. When more than two means were compared Tukey's HSD (honestly significantly different) Multiple Comparison Test was used (SAS Institute Inc., 1985). For comparison of regression coefficients the SAS-General Linear Models procedure was used. This analyzes data sets for differences over time within a group, between groups and for differences in regression coefficients between groups. All tests were performed at the 95% confidence level.

All figures were created using Sigmaplot 4.02 (Jandel Scientific Inc.) and best-fit and regression lines were created with the same program.

Chapter 3

RESULTS

Seawater Analysis

(a) Direct Observation of Preserved Samples

Of the five categories of planktonic cells identified and counted in Utermöhl chambers, diatoms made up the largest biovolume, attaining peaks of 11.7 x 10^{13} $\mu m^3/m^2$ on May 15 and 9.7 x 10^{13} $\mu m^3/m^2$ on October 2. Dinoflagellates were the next most prevalent cell type and peaked in midsummer with a value of 3.7 x 10^{13} $\mu m^3/m^2$. Flagellates, while numerically dominant, made little contribution to total phytoplankton biomass (Figure 2a). Plot comparisons over time of total phytoplankton with individual species (Figure 2b) revealed that diatoms alone were responsible for spring and fall peaks while the combined biovolume of diatoms and dinoflagellates caused the midsummer peak. The two other categories measured were zooplankton (usually larvae) and detritus. Neither of these "cell" types had sufficient biovolume to be worthy of comment.

(b) Chlorophyll a and Phaeopigments

Integration of chlorophyll a and phaeopigment values, measured at eight depths of the water column, from surface to 30 meters, helped to eliminate interference from ephemeral surface blooms caused by localized, short term atmospheric conditions, and provided an overall view of

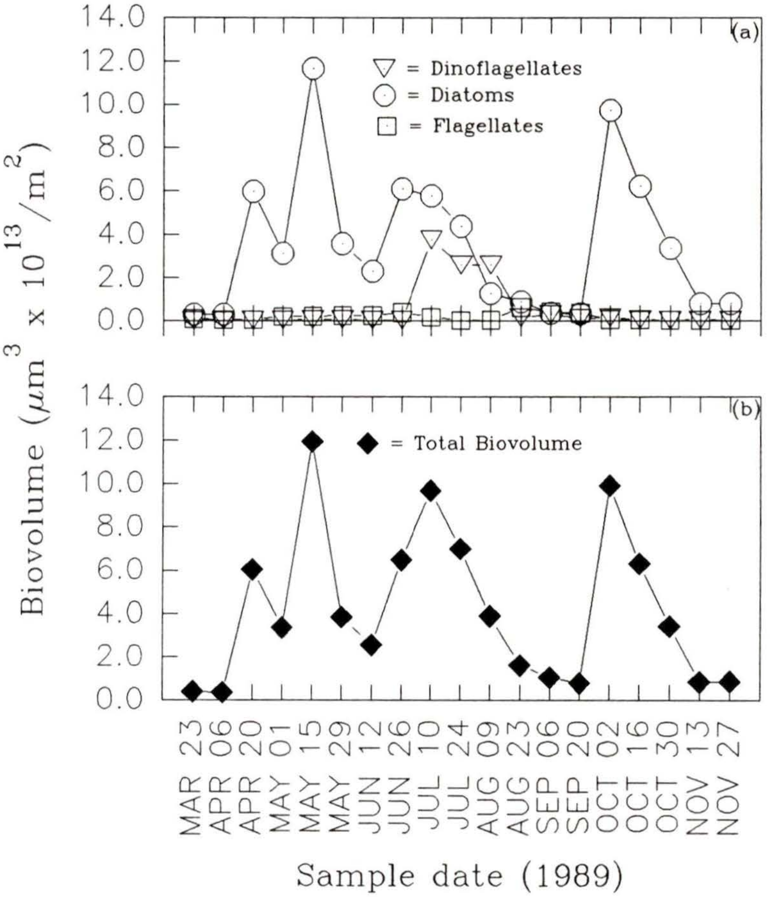

Figure 2a, b: Integrated biovolume of (a) diatoms, dinoflagellates and flagellates, and (b) all phytoplankton groups combined, for top 30m of Stn. "E", Saanich Inlet, from March through November 1989.

phytoplankton biomass in the euphotic zone of Saanich Inlet. Values for integrated chlorophyll a and phaeopigments fluctuated in a seemingly random manner over the nine-month study period (Figure 3a), ranging from less than 50 mg/m^2 in the early spring to a mid-summer high of 260 mg/m^2. There were however three distinct pigment peaks during the time period: the first occurring on April 20, the second on July 10 and the third on October 16. Using phytoplankton biovolume data (Section (a) of Results), the species of phytoplankton primarily responsible for the various pigment peaks were identified. There was a significant correlation between diatom biovolume and the April 20th and October 16th pigment peaks (Pearson Correlation Coefficient $R=0.55729$, $P<0.05$, $DF=5$; Figure 3b) and similarly the mid-summer pigment peak (July 10) correlated with the cumulative biovolumes of diatoms and dinoflagellates ($R=0.62349$, $P<0.05$, $DF=5$; Figure 3c). There were no other significant correlations between total integrated chlorophyll and phytoplankton biovolume. While these correlations may be statistically significant, neither one accounts for the majority of the observed variance and this is important to note when attempting to explain causation from correlation.

(c) Total Particulate Protein (TPP)

Total particulate protein values (Figure 4a), integrated over the surface 30 meters of the water column,

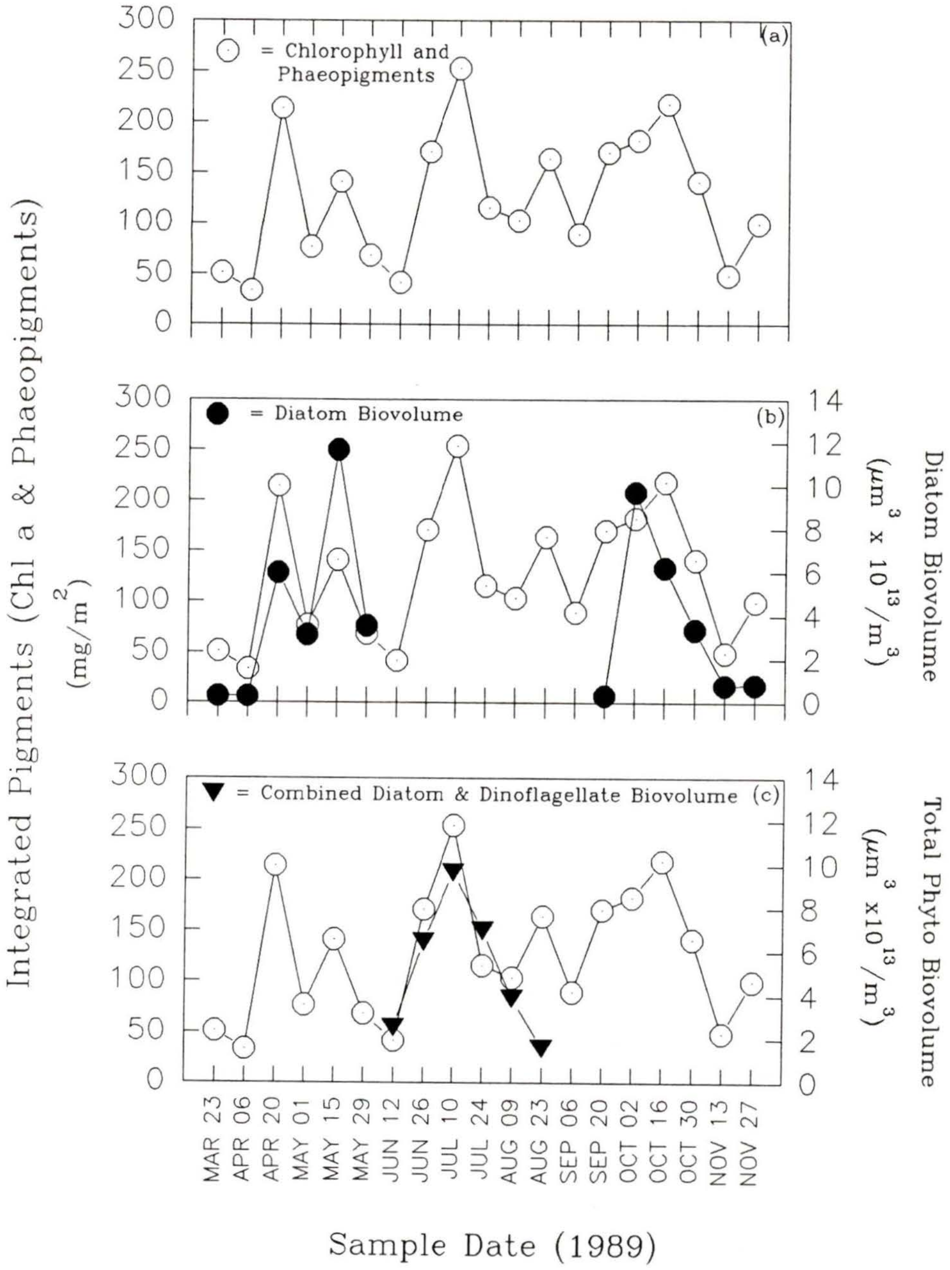

Figure 3a-c: (a) Integrated plant pigments (chl a and phaeopigments), for top 30m of Stn. "E", Saanich Inlet (b) Integrated pigments correlated with diatom biovolume (c) Integrated pigments correlated with combined diatom and dinoflagellate biovolume.

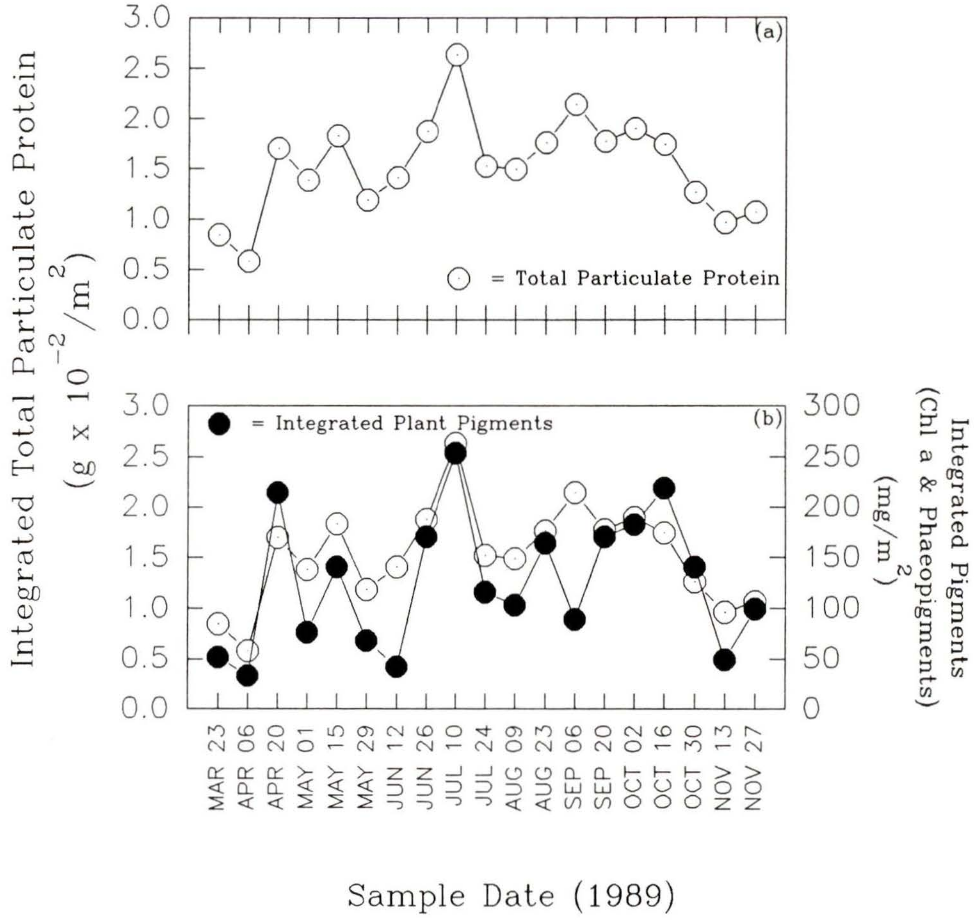

Figure 4a, b: (a) Integrated total particulate protein (TPP), for top 30m of Stn. "E", Saanich Inlet (b) TPP correlated with integrated plant pigments, from March through November 1989.

showed significant correlation with integrated plant pigment values (Pearson Correlation Coefficient R=0.771, P<0.05, DF=18, Figure 4b). As with integrated plant pigments, there were three distinct peaks; the highest, having a maximum value of approximately 2.6 x 10^{-2} g TPP/m^2, occurring on July 10, and the two lesser peaks occurring on May 15 and September 6. TPP, however did not show the same degree of fluctuation over time as seen in the chlorophyll values. Instead, TPP rose from 0.8 x 10^{-2} g/m^2 in late March to a mean level of 1.5 x 10^{-2} g/m^2 by the end of April; TPP remained near this level for the whole summer, only decreasing to 1 x 10^{-2} g/m^2 by November.

(d) Total Particulate Matter (TPM)

Figure 5 shows that levels of total particulate matter in the surface 30 meters of the water column remained constant over time at a level of approximately 0.5 g/m^2. Three unaccountable peaks in TPM were observed and likely represent sampling error where surface water samples were taken at the air-water interface, rather than from just under the water surface. This may have resulted in sampling of the neuston and/or of any debris present on the suspect sample days.

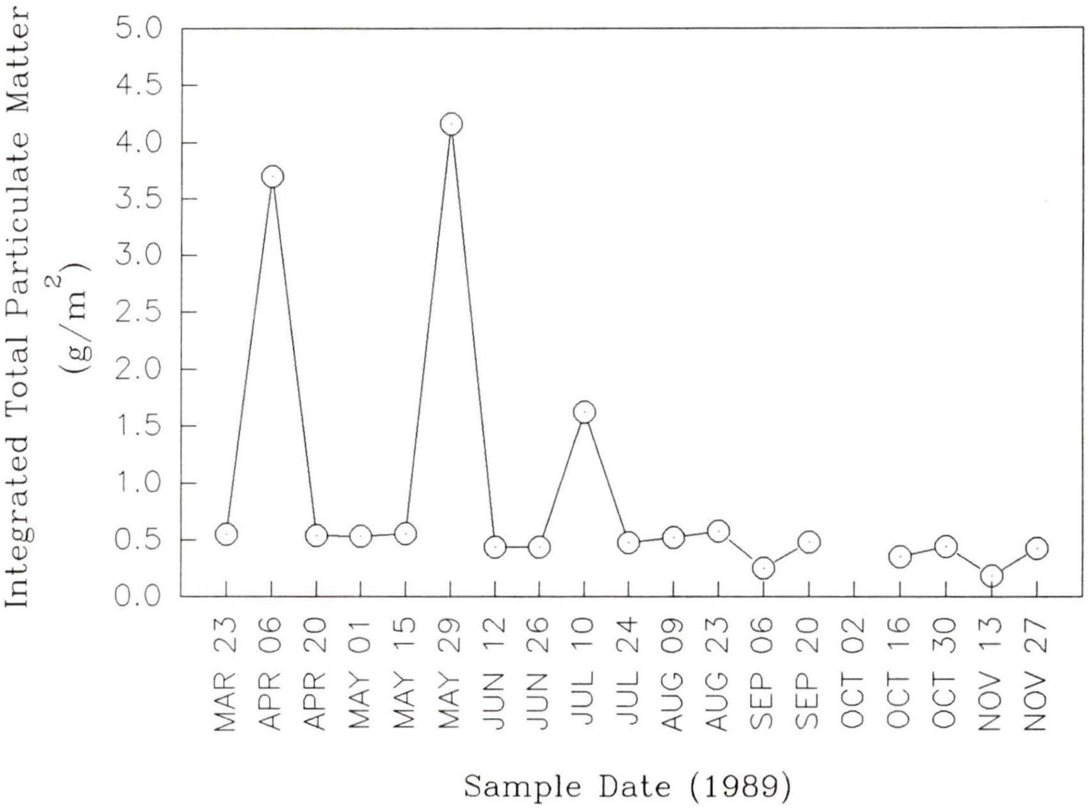

Figure 5: Integrated total particulate matter (TPM), for top 30m of Stn. "E", Saanich Inlet. Data not available for October 2nd.

Experimental Analyses

(a) Field Experiments

(i) Experiment 1 - Seasonal Variation of Digestive Enzymes

Specimens of *E. pacifica* analyzed for the digestive enzyme laminarinase (ß-1,3-glucanohydrolase) showed a significant pattern of temporal variation in enzyme activity.(ANOVA, $P<0.01$, DF=18,). Having established that differences existed between the sample means, a Tukey HSD multiple comparison analysis was performed on the individual data points to see where the differences actually existed (Table 1).

Table 1: Tukey HSD Multiple Comparisons of Means of Field Laminarinase Activity: Matrix of Pairwise Comparison Probabilities. Comparisons were made at 95% confidence level.

Group	Mar 23-Apr 20	May 01-Aug 23	Sep 06-Nov 27
Mar 23 to Apr 20	--		
May 01 to Aug 23	<0.05	--	
Sep 06 to Nov 27	>0.05	<0.05	--

Samples taken from March 23 to April 20 showed levels of activity between 500-1000 μg glucose produced per minute/g wet weight (Figure 6). This level of activity

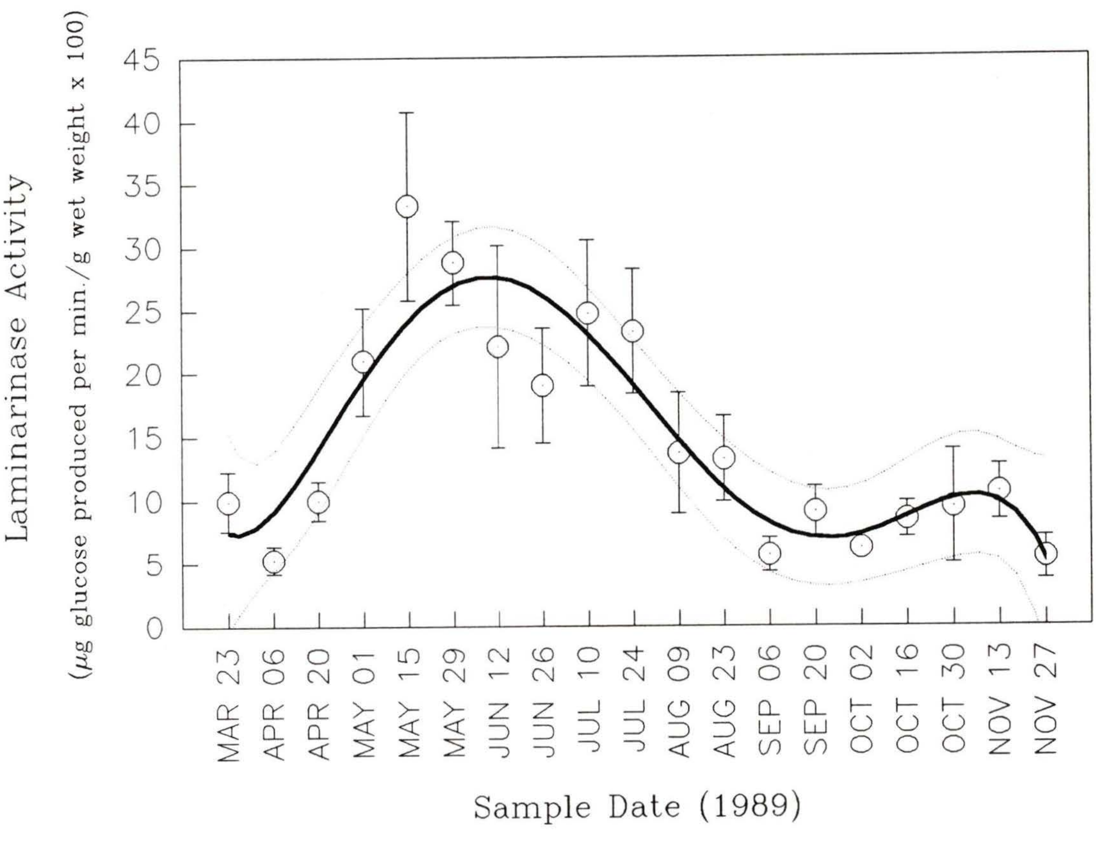

Figure 6: Laminarinase activity of *E. pacifica* collected at Stn. "E", Saanich Inlet from March through November 1989. Solid line is a computer generated best fit line. N=9 for each data point and error bars and dotted lines represent 95% confidence limits of the data and the best-fit line. Error bars fall within sample variance.

increased with time, reaching a peak of just under 3500 μg glucose produced per minute/g wet weight on May 15. Activity decreased slowly over the summer and by September 6 had reached a level similar to that observed in early spring. A second smaller peak was observed at the end of October, reaching a maximum level of activity on November 13.

To determine proximal causes for this pattern of activity, the three dominant phytoplankton food types available to *E. pacifica* in Saanich Inlet were regressed with laminarinase enzyme activity as the dependent variable using multiple linear regression. Only diatom biovolume showed a significant relationship with the observed pattern of laminarinase activity (Table 2 and Figure 7a).

Table 2: Multiple Linear Regression Analysis for Field Laminarinase Activity of *Euphausia pacifica*.

Food Type in Model	R^2	F statistic	Prob>F
Diatoms	0.210	4.54	0.048
Dinoflagellates	0.097	2.75	0.118
Flagellates	0.031	2.26	0.153
All three food types	0.384	3.12	0.057

While the correlation coefficient, R^2, was higher when all three food types (diatoms, dinoflagellates and flagellates) were entered into the regression model (R^2=0.384), the

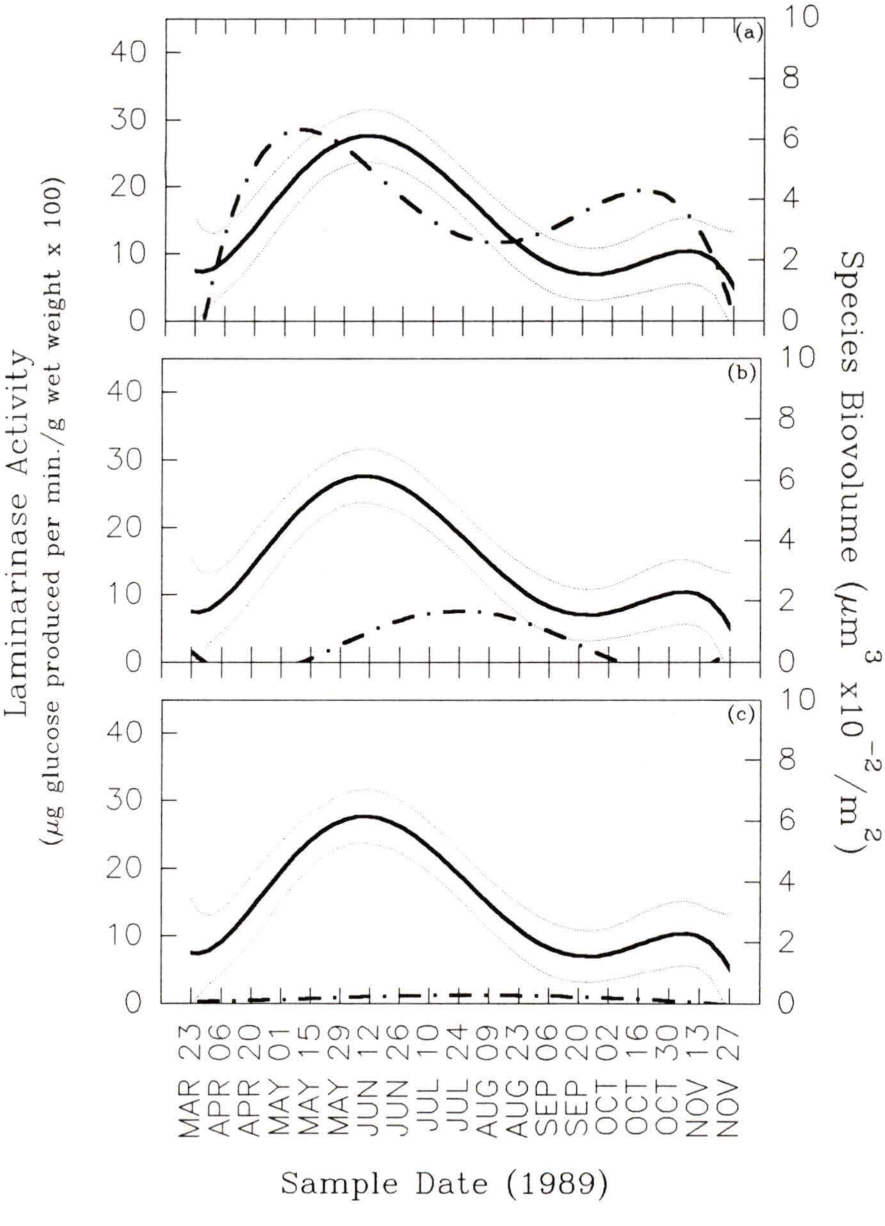

Figure 7a-c: Field laminarinase activity of *E. pacifica* (solid line) compared with best fit lines (dash-dot lines) of (a) diatom biovolume (b) dinoflagellate biovolume and (c) flagellate biovolume.

correlation was not significant at the 95% confidence level. Dinoflagellates and flagellates did not appear to influence laminarinase activity in the presence of diatoms (Figures 7b & 7c).

Analysis of variance of specimens of *E. pacifica* analyzed for a trypsin-like protease digestive enzyme indicated a significant pattern of temporal variation (Figure 8; ANOVA, $P<0.01$, DF=18). A subsequent Tukey HSD Multiple Comparison test however showed that only one data point was responsible for the significant result from the ANOVA test: activity on May 1st was significantly higher than levels of activity on the dates immediately preceding and following at the 95% confidence level (Tukey HSD Multiple Comparison Analysis, $P<0.05$, DF=18,). In light of the other Tukey multiple comparisons within the same data set, it is likely that this represents an anomalous point and that no significant difference exists in activity levels over the study period. The mean level of activity over the study period was 9.2 ± 5.6 (95% c.i.) μmol p-tosylsulfonyl-L-arginine produced per minute/g wet weight. Multiple linear regression of the activity values with phytoplankton food types and total particulate protein was performed. There were no significant relationships between trypsin-like protease activity and any food type (Table 3).

Table 3: Multiple Linear Regression Analysis for Field Trypsin-like Protease Activity of *Euphausia pacifica*.

Food Type in Model	R^2	F statistic	Prob>F
Diatoms	0.01	0.01	0.95
Dinoflagellates	0.01	0.01	0.89
Flagellates	0.01	0.05	0.81
All three food types	0.01	0.01	0.95
Total Particulate Protein	0.03	0.53	0.47

(ii) Experiment 2 - Day/Night Comparison of Laminarinase Activity

Mean laminarinase activity levels for specimens of *E. pacifica* taken during the day (1100h) and during the night (2300h) were compared using Student's *t*-test (Figure 9). There was no significant difference between day and night samples at the 95% confidence level (Student's unpaired *t*-test, $t=-1.545$, $P<0.05$, $DF=3$).

(b) Laboratory Experiments

(i) Laboratory Experiment 1 - Background-Baseline Enzyme Activity

Animals maintained in the laboratory under starvation conditions for two weeks were analyzed for levels of laminarinase activity and trypsin-like protease activity (Figure 10a, b). Mean laminarinase activity over two days was 410±170 (95% c.i.) μg glucose produced per minute/g wet weight (n=18); mean trypsin-like protease activity over a day and a half was 2.8±3.5 (95% c.i.) μmol p-tosylsulfony-L-arginine produced per minute/g wet weight.

(ii) Laboratory Experiment 2 - Laminarinase Induction

Animals fed on the marine diatom *Skeletonema costatum* (treatment #1) showed a significant increase in laminarinase activity over time (Figure 11a) compared with groups of animals that were either starved (figure 10a) or fed high protein food such as *Artemia* nauplii (experimental treatment #2; Heterogeneity of Slope Test, F=20.93, P<0.01, DF=1, ;Figure 11b). Animals were fed first at 0900h of Day 2 and the reaction to feeding in terms of increased enzyme activity was very quick with significantly higher levels of activity observed six hours later (Student's *t*-test, P<0.01,). Remaining animals were fed a second time at 0900h of Day 3, and again laminarinase activity increased after feeding. Peak enzyme activity occurred approximately 12 hours after the second feeding and 36 hours after the initial feeding. Third and fourth feedings were given at 0900h of Days 4 and 5, but unlike the earlier responses to feeding, enzyme activity dropped to a mean value of 580±230 (95% c.l.) μg glucose produced per minute/g wet weight. This level of activity was significantly different however from the pre-feeding mean level of 200±110 μg glucose produced per minute/g wet weight (Student's *t*

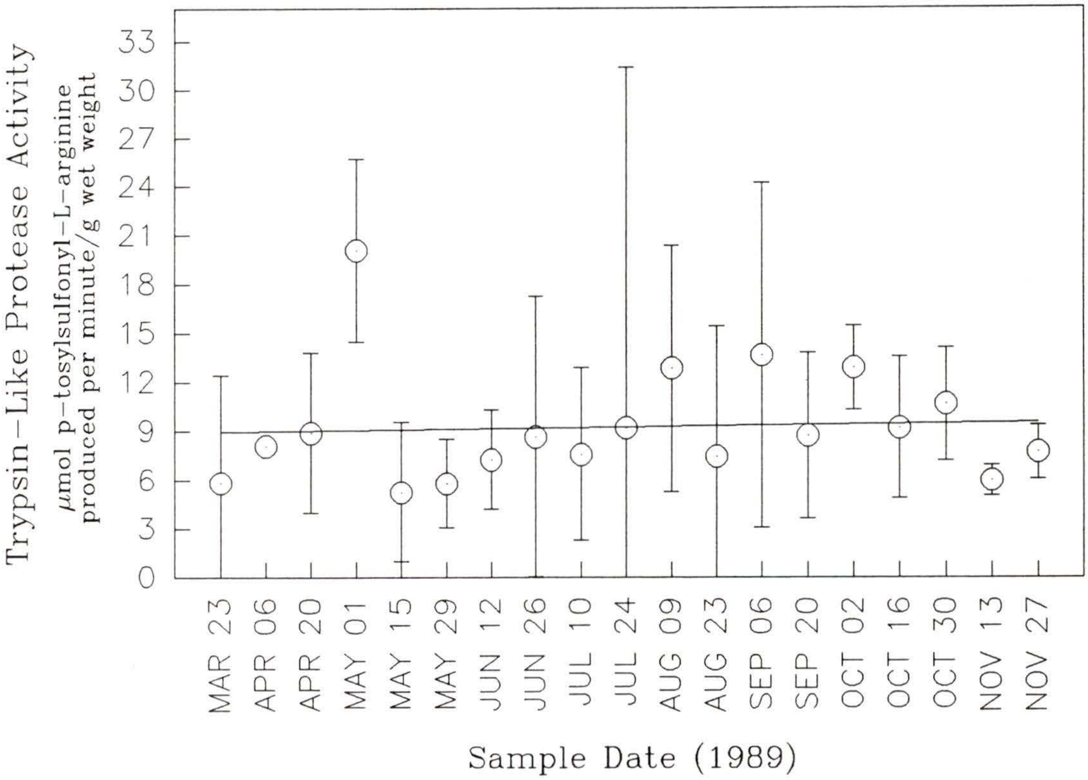

Figure 8: Trypsin-like protease activity of *E. pacifica* collected at Stn. "E", Saanich Inlet from March through November 1989. N=3 for each data point and error bars represent 95% confidence limits. Solid line is a computer generated best-fit line.

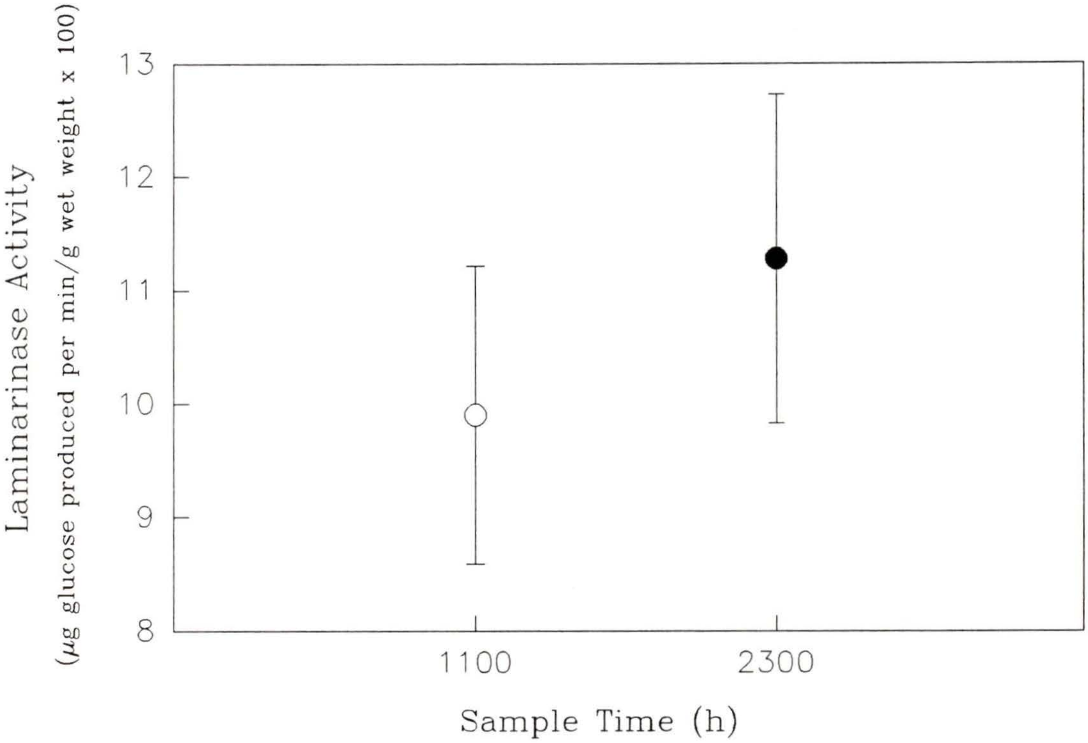

Figure 9: Day/night comparison of laminarinase activity in *E. pacifica*. N=4 for each data point and error bars represent 95% confidence limits.

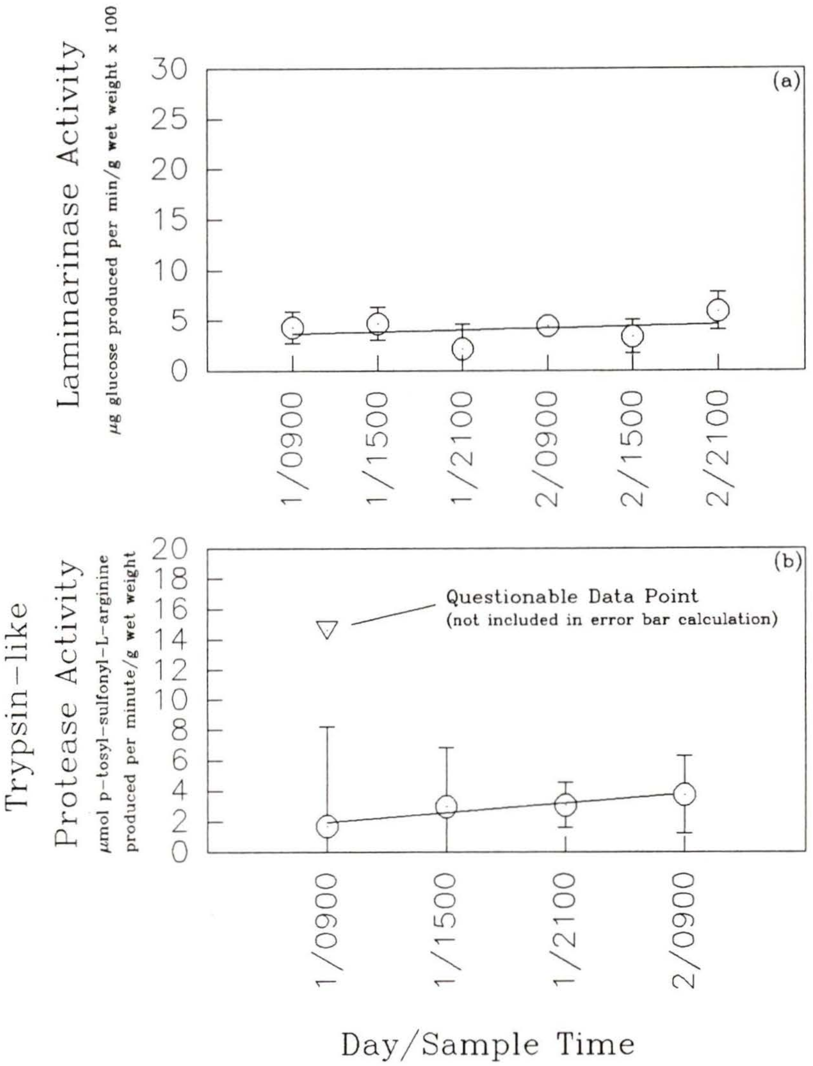

Figure 10a, b: Enzyme activity of starved specimens of *E. pacifica*. (a) laminarinase activity, n=9 for each data point (b) trypsin-like protease activity, n=3 for each data point. Error bars represent 95% confidence limits and solid lines represent computer-generated best-fit lines.

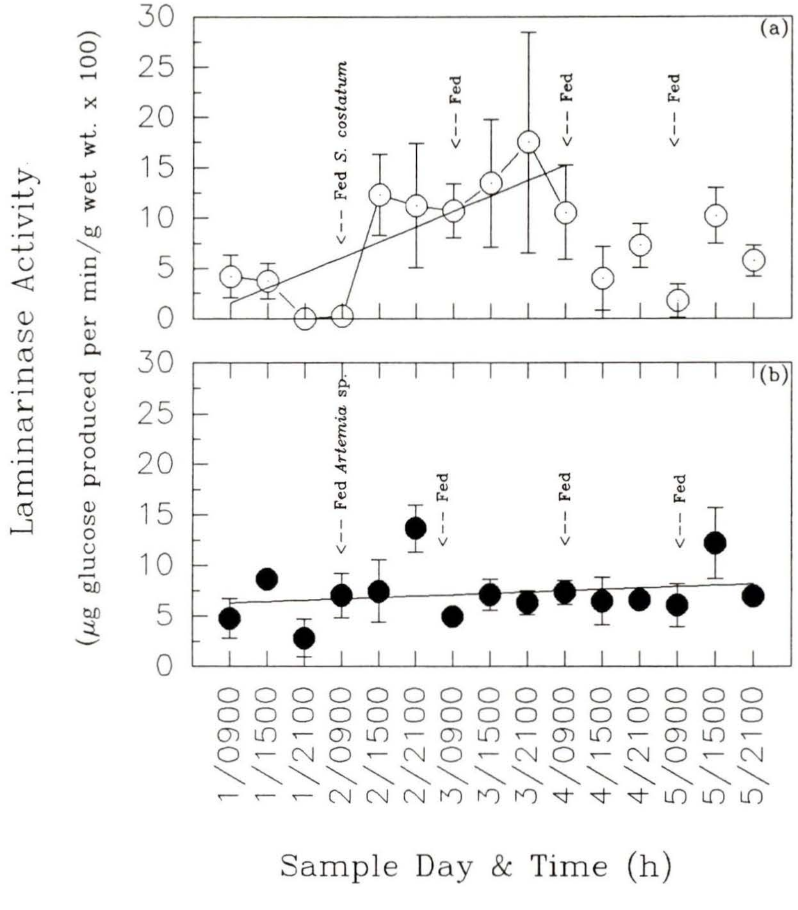

Figure 11a, b: Laminarinase activity of *E. pacifica* fed on (a) *S. costatum* and (b) *Artemia* nauplii. N=3 for each data point and error bars represent 95% confidence limits. Solid lines represent first order regression of enzyme activity with time.

test, t=7.826, $P<0.01$, DF=77,), indicating that the animals had not returned to a "starvation" state of enzyme activity but were maintaining activity at an intermediate level between low starvation values and peak activity values. Chlorophyll levels in the aquarium reached approximately 3 mg/m^3 by Day 4 and there was no change in the chlorophyll/phaeopigment ratio in the aquarium (≈3) to indicate that the animals had stopped feeding (Figures 12a & 12b).

(iii) Laboratory Experiment 3 - Sustained Laminarinase Activity

The results of Experiment 2 prompted further investigation into the sudden drop in laminarinase activity after a certain level of peak activity was reached. Working on the premise that higher food concentration led to higher enzyme activity levels, an experiment was carried out in which specimens of *E. pacifica* were provided with food three times a day as opposed to once a day as in the previous experiment. Increasing the food available to the animals by increasing frequency of feeding did not increase or prolong levels of enzyme activity seen in Experiment 2 (Figure 13). In fact, the pattern of activity over time displayed by this group of animals was not significantly different from those patterns shown by the groups either fed on *Artemia* nauplii or starved for two weeks (Heterogeneity of Slope Test, F=0.34, $P>0.05$, DF=1,).

As in Experiment 2, a relatively stable ratio of chlorophyll to phaeopigment (≈3, Figure 14a) indicated that the animals were feeding and frequent observation of specimens' hepatopancreases confirmed this. Unlike Experiment 2 however, the chlorophyll level in the Experiment 3 aquarium reached a level of 3 mg/m^3 by 0900h of Day 2 (Figure 14b). It was at a chlorophyll level of 3 mg/m^3 that animals in Experiment 2, which had earlier shown significant increased enzyme activity, began to show decreased levels of activity (See Laboratory Experiment 2 for details). Also as in Experiment 2, activity levels after the initial feeding were significantly different from pre-feeding levels (Student's *t*-test, t=3.01, P<0.05, DF=17,) but then dropped after 1500h on Day 2 and remained around a mean level of 470±50 (95% c.l.) for the duration of the experiment. A Student's *t*-test showed there to be no significant difference between this mean activity level and the level of 580±230 (95% c.l.) μg glucose produced per minute/g wet weight shown on and after Day 4 in Experiment 2 (t=-1.84, P>0.05, DF=179,). Also like Experiment 2, the mean level of activity observed after Day 2 was significantly different from the pre-feeding level of activity.

(iv) Laboratory Experiment 4 - Trypsin-like Protease Induction

Animals fed on *Artemia* nauplii showed no significant differences in trypsin-like protease activity over time

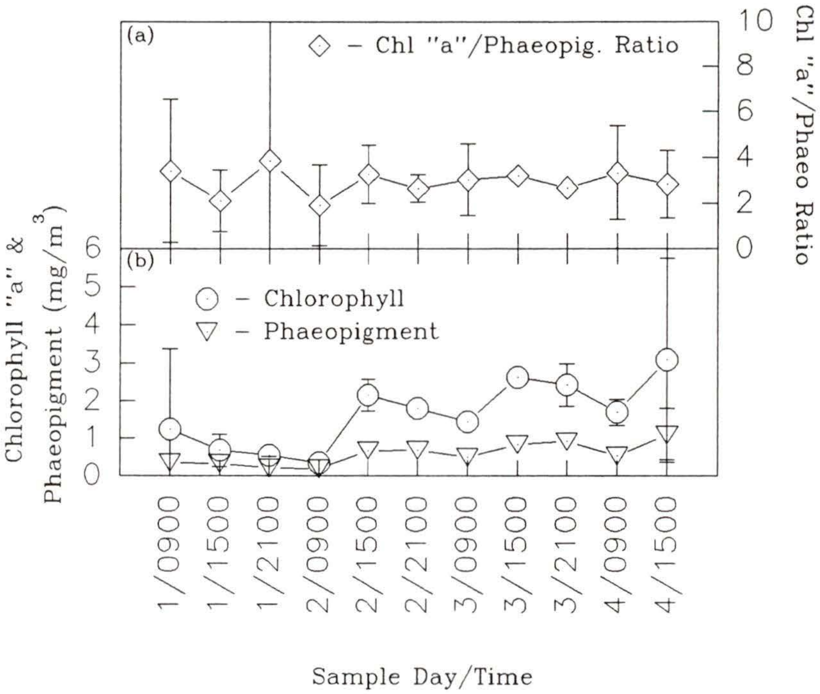

Figure 12a, b: (a) Changes in chlorophyll a/phaeopigment ratio over time in treatment #1 (*S. costatum*) of Experiment 2 (b) changes in chl a and phaeopigment values over time in same tank. N=3 for all data points and error bars represent 95% confidence limits.

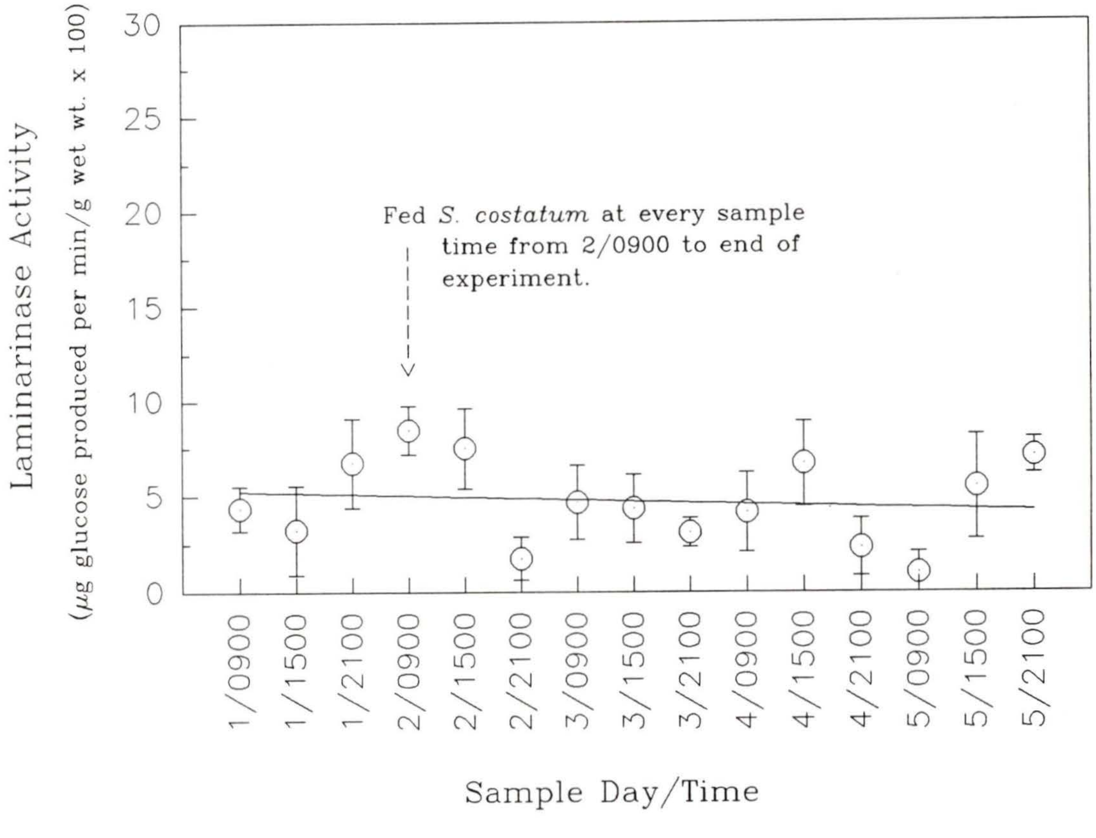

Figure 13: Laminarinase activity of *E. pacifica* fed three times a day on *S. costatum*. N=3 for each data point and error bars represent 95% confidence limits. Solid line represents a first order regression of enzyme activity with time.

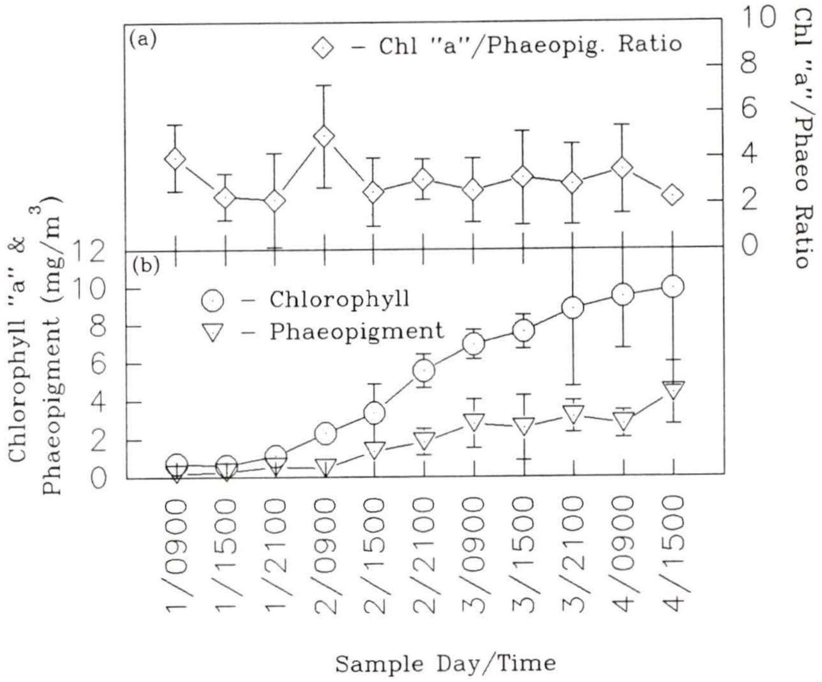

Figure 14a, b: (a) Changes in chlorophyll a/phaeopigment ratio over time in treatment #1 (*S. costatum*) of Experiment 3 (b) changes in chl a and phaeopigment values over time in same tank. N=3 for all data points and error bars represent 95% confidence limits.

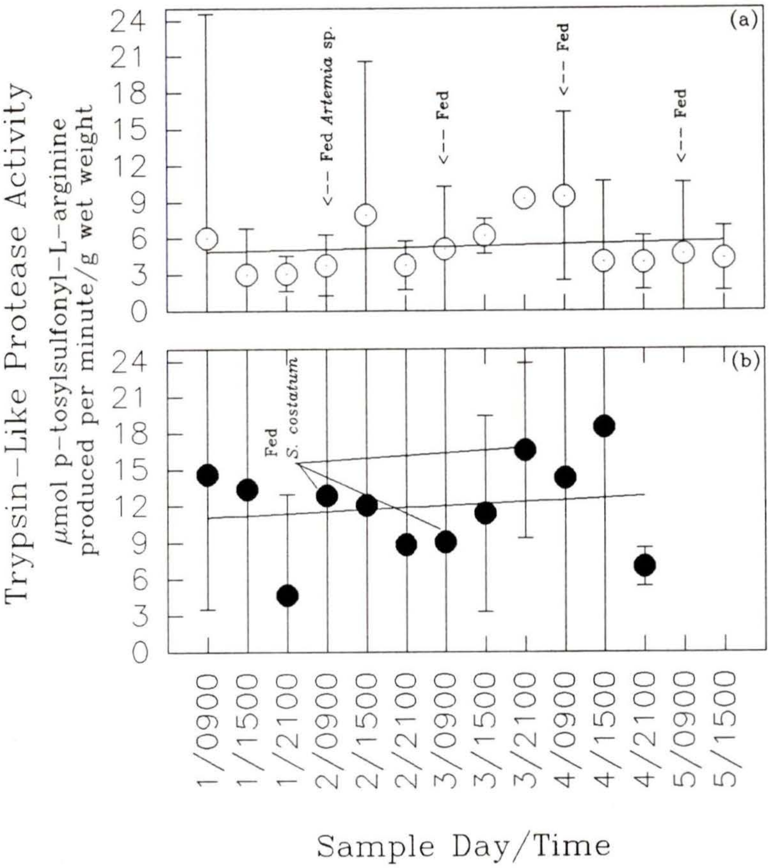

Figure 15a, b: Trypsin-like protease activity of *E. pacifica* fed on (a) *Artemia* nauplii and (b) *S. costatum*. N=3 for all data points and error bars represent 95% confidence limits. Solid lines represent first order regression of enzyme activity with time.

(SAS-General Linear Models, F=1.76, P>0.05, DF=13, Figure 15a). Variation among replicates was very high, making it difficult to detect any type of pattern in terms of activity. Similarly, animals fed on the marine diatom *S. costatum* (Figure 15b) showed no significant differences in trypsin-like protease activity over time (SAS-GLM, F=1.76, P>0.05, DF=13). A Heterogeneity of Slope Test indicated that the only significant difference between the two treatment groups was the overall mean level of activity. The group fed on *Artemia* nauplii had a mean activity level of 5.3±0.9 (95% c.l.) μmol p-tosylsulfonyl-L-arginine produced per minute/g wet weight while the group fed on *S. costatum* had a mean activity level of 11.9±2.1 (95% c.l.) μmol p-tosylsulfonyl-L-arginine produced per minute/g wet weight (SAS-GLM, F=39.84, P=<0.01, DF=1).

Chapter 4

DISCUSSION

Field Experiments

Seasonal Variation of Digestive Enzyme Activity

In order to take advantage of the natural variation in planktonic food types that occurs seasonally in Saanich Inlet, field studies were conducted from early spring (March) through to late autumn (end of November). Data presented in Figure 2a shows that taxonomic groups of phytoplankton in Saanich Inlet followed a typical pattern of growth (Raymont, 1980). Diatoms were responsible for two "blooms", one in the late spring and another smaller one in late fall. Phytoplankton biomass in midsummer was lower than in either spring or fall and no one group appeared to dominate the species assemblage. Dinoflagellate contribution to total biomass increased in the summer for two months and then decreased to earlier low levels. Flagellates as a group, because of their small cell size, never dominated the biomass, yet were always present in relatively large numbers. These findings are concordant with past works on phytoplankton growth and succession in coastal waters (Raymont, 1980; Smayda, 1980; Hobson, 1983)

Amounts of both total particulate matter and total particulate protein showed variation over the nine-month period. Total particulate matter (Figure 5) varied little from a level of approximately 0.5 g/m^2 except for three very high peaks on April 6th, May 29th and July 10th. These peaks did not correlate with any of the other environmental parameters measured, such as chlorophyll, total particulate protein or phytoplankton biomass and appear to be the result of either sampling or analysis error. Inadvertent sampling of the neuston may have occurred during collection of surface water samples if on occasion the Niskin bottle had not been lowered to at least 1 m below the surface. Strickland and Parsons (1972) advise that water samples taken for total particulate analysis should not be allowed to stand for extended periods of time after collection as an increase in particulate matter often occurs due to bacterial action. This also may be the cause of the anomalous peaks as samples were left overnight on several occasions before analysis.

Total particulate protein showed a close correlation with integrated plant pigments (figure 4b). It is hypothesized from these data that the proteins detected and measured by the Lowry method were for the large part plant proteins of living phytoplankton cells and not proteinaceous detritus from either plant or animal sources; detrital phaeopigments however cannot be eliminated as a possible cause for this correlation. While this relationship did not provide any information about the amount of non-phytoplanktonic proteinaceous food in the water column for *E. pacifica*, it suggested a possibly useful relationship for indirect fluorometric determination of phytoplankton metabolites by measuring chlorophyll fluorescence. Parsons et al. (1961) in an examination of the chemical content of asssorted marine phytoplankton groups found that within families, there was a fairly constant ratio of protein to chl a. This relationship did not hold between families however. Harwood et al. (1988) found a strong positive correlation between cholorphyll fluorescence and major molecular species of phosphatidylglycerol in the chloroplasts of some marine chlorophytes.

Laminarinase Activity

Activity of the digestive enzyme laminarinase in *Euphausia pacifica* showed statistically significant variation over the nine-month period (Figure 6). While correlation does not imply causation, from the data collected it appears that this variation was caused by changes in the particulate food environment in Saanich Inlet and many papers have been written that support this statement (Mayzaud and Poulet, 1978;Cox, 1981; Mayzaud et al., 1985; Willason and Cox, 1987). All of the potential food types observed in Saanich Inlet fell within the size range acceptable to *E. pacifica* for ingestion (8-70 µm in longest dimension, Parsons et al., 1967; ≤375 μm in longest dimension, Lasker, 1966); however, only diatom biovolume regressed significantly with laminarinase activity over the study period (Table 2). This does not mean that *E. pacifica* was not ingesting all three phytoplanktonic foods or any detrital matter in the water column, but that only diatoms were of sufficient quantity or quality to induce laminarinase production and secretion. When all three phytoplankton food types were entered into the stepwise linear regression model the coefficient of regression, R^2, was higher than it was for diatoms alone, but it was not significant at the 95% confidence level. This indicates that either the other two phytoplanktonic foods had little or no influence on the enzymatic activity or that a more complicated relationship between these food types and *E. pacifica* exists and is not detectable through simple correlation analysis. Both Parsons et al. (1967) and Lasker (1966) have shown that *E. pacifica* is a selective feeder in terms of particle size and Lasker (1966) showed that the animal displayed preferences for one type of food over another when given two palatable choices. Thus, it is possible that the animal either selectively feeds upon diatoms as a preferred food type, having already produced suitable enzyme (constitutive enzyme production) to digest that particular type of phytoplankton, or it ingests all particles in a certain size range and the chemical composition of the food stimulates production of the appropriate enzyme (inductive enzyme production).

Analysis of the chemical composition of the three phytoplanktonic food types supports the proposal that enzyme production and secretion is stimulated by food quality. The preferred substrate of the enzyme laminarinase is the short-chain carbohydrate, ß-1,3-glucan, a polymer of D-glucose. Table 4 (after Cox, 1981) shows the percentages of total carbohydrate stored as both soluble and insoluble ß-1,3 glucans in cultures of three diatom species and in naturally occurring assemblages of material. The mixed diatom sample contained on average 50% more soluble ß-1,3 glucans than did naked flagellates and four times more than dinoflagellates. Given the substrate-specific nature of laminarinase, it is not surprising that the food type containing the largest amount of laminarin (diatoms) showed a significant correlation with enzyme activity.

It can be seen from Figure 7a that there is a considerable lag time between increases in diatom biovolume and corresponding increases in laminarinase activity. On first glance it would appear that *E. pacifica* is physiologically insensitive to changes in its food environment, resulting in a lag of approximately two weeks. However, laboratory results from this study have shown response times to changes in food type and/or amount to be

Table 4: Average Carbohydrate Composition of Selected Groups of Phytoplankton Found in Saanich Inlet, British Columbia. (after Cox, 1981).

	% of total carbohydrate		
Phytoplankton	soluble ß-1,3 glucan	insoluble. ß-1,3 glucan	other polysaccharides
Phytoplankton cultures (stationary phase)			
Chaetoceros debilis	89	0	11
Chaetoceros socialis	93	0	7
Skeletonema costatum	90	7	3
Net tow material			
mixed diatoms	58	16	26
naked flagellates (*Phaeocystis* dominant)	39	11	50
dinoflagellates	16	71	13

considerably less than two weeks. These results will be discussed in more detail in a later section. The apparent asynchrony between diatom biovolume and laminarinase activity in field animals then is not the result of physiological limitations but is likely due to the expected daily biologically patchy nature of the animals' feeding environment (Mackas et al, 1985).

Mayzaud et al. (1985) best describe the effects of patchiness on such a study, stating that "the field definition of food supply is made difficult by spatial and temporal differences between the location of the animals at the time of capture and the particulate environment in which they were feeding". Phytoplankton patchiness can occur on a scale of meters (Mackas et al., 1985), but in this study the capture of animals and collection of water samples was spatially removed on a vertical scale of tens of meters.

The degree of resolution of this study must be considered. Over a nine-month period, only nineteen observations were made. While for each sample time a minimum of two replicates of each analysis were performed (a minimum total of three samples analysed per sample date), this procedure provided no measure of variance on a finer temporal scale. However, the animals had on average six to eight hours in the surface waters in which to feed on the phytoplankton present before sampling. Because of the ability of the animal to swim short horizontal distances (on a scale of meters) the animal would likely have overcome small-scale phytoplankton patchiness and any physiological responses to feeding would be to the food environment as a whole (Mullin and Brooks, 1976; Cox et al., 1982). Thus, because of integration over time during feeding before each sample date, interpretation of the dynamic nature of the physiological digestive processes of the euphausiids remains unaffected by the coarse degree of resolution.

A comparison of laminarinase activity in field animals taken from surface waters at night to animals taken from below the scattering layer during the day (Figure 9) revealed that both groups of animals experienced the same feeding environment despite a temporal separation of the two feeding groups of twelve hours. A *t*-test suggested that there was no difference in the mean activity between the groups. This is contradictory to the findings of Willason and Cox (1987), where they reported significant differences between average day activity levels and average night activity levels, but is in agreement with Cox et al. (1982) who found that activity levels in laboratory animals did not decrease significantly for at least eighteen to twenty hours after removal from the feeding environment. Based on literature data and the data presented in Figure 9, it appears that the spatial separation between the collection of animals and sampling of the particulate feeding environment had no impact on the validity of the results obtained.

Trypsin-like Protease Activity

Apart from data obtained on May 1st, the trypsin-like protease enzyme did not show a significant pattern of activity over time (Figure 8). Given the linear relationship between an enzyme's activity and the concentration of its potential substrate (Mayzaud and Poulet, 1978), the lack of any significant variation in trypsin-like protease activity indicates that either there must have been no changes over time in the amount or quality of proteinaceous food available to *E. pacifica* or that the type of proteinaceous food available did not provide a suitable substrate for the enzyme in question. However, the level of activity observed in field animals was significantly higher than that observed in starved animals in the laboratory, indicating that some proteinaceous source in the diet of field animals was stimulating production and secretion of the protease or that the trypsin-like protease is a constitutive enzyme in *Euphausia pacifica*.

Sources of proteinaceous food were not measured directly, but the observations that were taken on food types available in the inlet give a good approximation of the amount of protein present in the water column over the study period. The largest source of protein appeared to be in phytoplankton cells. The amount of protein stored in an algal cell varies depending upon the species and its nutritional state: on average, diatoms and dinoflagellates contain 30% protein and Chrysophycean-like flagellates as a group contain 50% protein (Parsons et al., 1961). Of the three phytoplanktonic foods measured, flagellates were present throughout the study period with little variation in the number of cells and the amount of protein present. Working on the assumption that *E. pacifica* filters all particles in a given size range and selectively digests material of sufficient nutritional quality, the unvarying presence of high protein flagellates in the diet of the animal may explain the steady level of trypsin-like protease activity observed over nine months. Although the measure of TPP is dominated by phytoplankton proteins (as can be seen in the close correlation between total phytoplankton biomass and total particulate protein in Figure 4b), the relatively consistent pattern displayed by TPP over time (Figure 4a) indicates that protein is always present in the particulate environment, whether it be in the form of plant cells or detritus from one of many sources.

Laboratory Experiments

Background-Baseline Enzyme Activity

The decision to use whole animal homogenates for enzyme analysis involved several compromises. In past works (Gates and Travis, 1969; Mayzaud et al., 1985) the digestive organs of the animal (mouthparts, hepatopancreas, and gut) were dissected out for analysis, eliminating much "noise" originating in tissues containing enzymes not involved with digestion. This procedure was not used in this study however for two reasons. Cox (1981) and Gates and Travis (1969) had shown both enzymes to be restricted to digestive tissues and not present in somatic tissue: thus it was felt that any possible activity from non-digestive tissue would be minimal and not affect measures of activity from digestive tissues. Secondly, Willason and Cox (1987) reported that non-digestive tissues in whole-animal homogenates did not interfere with, in any way, the laminarinase activity of whole-animal homogenates.

Changes in wet weight of individual animals due to either increases or decreases in non-digestive tissues might have an effect on the apparent amount of enzyme activity observed; however, this did not appear to be the case in this study. Appendix Figure 1 shows the mean wet weight of individual animals over the nine-month period and when compared with Figure 6, it is clear that the observed slight increases in weight do not decrease activity values. Mean wet weight over the study period was 0.036±0.016g (±95% c.i.) and while no ANOVA test was performed on these data, the overlapping 95% c.i. error bars indicate that none of the changes in weight are significant.

However, for both field and laboratory experiments a baseline measurement of enzyme activity was necessary to determine if changes due to different feeding treatments were statistically significant. Results obtained from analyzing animals that had been starved for two weeks were representative of both background activity and baseline activity of the enzymes in digestive tissues. Cox (1981) reported values of 0 for laminarinase activity in starved *E. pacifica* obtained from the Santa Barbara Channel off California, yet activity values for laminarinase in this study of starved animals were somewhat higher than zero (Figures 10a) and this was in agreement with Willason and Cox (1987). Similarly, the starved activity values for the trypsin-like protease were above zero (Figure 10b) The reasons for this variation in activity in supposedly starved animals are not fully understood, as no food of any sort was provided for two weeks and the water was filter-sterilized every three days. Furthermore, in laboratory feeding experiments, activity values of zero and very close to zero were observed at two times preceeding initial feedings (Figure 11a). Animals used for both feeding and starvation experiments were captured at the same time from the same location in Saanich Inlet. Without further work on this point, the variation seen in starved animal activity can only be attributed to natural variation within the sample population.

Laminarinase Induction

Production and secretion of laminarinase was induced in laboratory specimens of *E. pacifica* by feeding them stationary phase cultures of the marine diatom *Skeletonema costatum* (Figure 11a). This particular species of phytoplankton was chosen for its high concentration of ß-1,3 glucans in stationary phase (see Table 4). A response to the change in the animals' food environment was observed six hours after feeding, in the form of a significantly higher level of laminarinase activity. Yet production and/or secretion of the enzyme continued for another 30 hours, at which point a peak activity level was observed. Following this peak, levels dropped rapidly and stabilized around a mean activity that was significantly higher than pre-feeding levels.

Whether or not the drop in activity represented a drop in production or a drop in secretion of already produced enzyme is unclear, but these data would appear to indicate that the animals produce and secrete enzyme in excess when stimulated by a sudden change in food quantity or quality. Upon acclimation to the amount or quality of the new food type, enzyme production levels drop to a lower, more energetically economic level and remain there until further change stimulates a new response (Hassett and Landry, 1983). Food concentrations in the aquarium were monitored throughout the experiment by measuring chlorophyll a and phaeopigments in the water. The sudden drop in enzyme activity correlated with chlorophyll a values of approximately 3 mg/m^3. This relationship is discussed in more detail in the following section.

Observed values for laminarinase activity in laboratory experiments were not as high as the values observed in field specimens. This discrepancy has been noted by other workers doing similar experiments (Cox, 1981) and has been attributed in the past to capture and laboratory-induced stress.

Sustained Laminarinase Activity

Experiment 3, in which animals were fed three times a day, provided further support for the idea that the levels of activity of digestive enzymes change in response to ambient concentrations of food. As a consequence of feeding the animals three times a day, food concentrations in the aquarium rose quickly and continued to rise for the duration of the experiment (Figure 14b). The steady chlorophyll a/phaeopigment ratio (Figure 14a) indicated that the animals were actively feeding but not able to clear from the water the total amount of food provided them. While it appears from data in Figure 13 that there may have been a slight increase in laminarinase activity after the initial feeding, levels of activity remain low after the second feeding at 1500h on Day 2. Data in Figure 14b shows that at this time, chlorophyll a values had reached 3 mg/m^3, the same value at which enzyme activity leveled off in the previous experiment. A Student's *t*-test was used to compare mean activity levels after chlorophyll a values had reached 3 mg/m^3, and there was no significant difference.

The rate of digestive enzyme production has been shown to be related to feeding rates and, by extension, to concentrations of food in the particulate environment (Mayzaud and Poulet, 1978; Cox, 1981). Frost (1972) and others found that certain concentrations of food resulted in maximal feeding rates in copepods and accompanying maximal rates of enzyme production. This model of feeding has been referred to as the saturation model and appears to be a true representation of physiological events in the short term (i.e. half a day to two days). However, given a certain amount of time to acclimate to a new quantity or quality of food (>two days), an animal will no longer display saturation-model feeding and the high peaks of enzyme activity associated with it, but will display a more linear relationship between food concentration, feeding rate and digestive enzyme activity (Mayzaud and Poulet, 1978).

The environmental stimulus that controls the feeding rate of an animal appears to be the frequency of feeding (Hassett and Landry, 1983): not surprisingly, animals that have not fed for an extended period of time will display a much higher feeding rate upon exposure to a suitable food source than will animals that have access to a continuous supply of food. Control of the production of enzymes may be a combination of frequency of feeding and the degree of gut filling that takes place during the feedings. Animals that feed at such a rate as to fill their guts to capacity will produce more enzyme(s) than those animals that do not feed to the same extent (Mayzaud and Poulet, 1978).

The observed differences in laminarinase activity of groups fed on *Skeletonema costatum* once a day and three times a day (Figures 11a and 13 respectively) can be explained by the physiological responses discussed above. The animals fed once a day after a two week starvation period initially displayed a maximal feeding rate as represented by high laminarinase activity. After a period of two days, the drop in activity to an intermediate position between pre-feeding and maximal levels indicated that the animals had become acclimated to the new food and that a threshold concentration of food had been reached, stimulating a continuous but lower rate of production of laminarinase. This threshold concentration appeared to be a chlorophyll value of approximately 3 mg/m^3 for the animals used in these experiments. The animals that were fed three times a day acclimated to the high concentrations of food provided them very quickly. As in the group fed once a day, enzyme activity leveled off when food concentrations reached approximately 3 mg/m^3 of chlorophyll a. These results imply that acclimation to new food sources is very fast and within the order of half a day.

A physiological model of the relationship between ingestion, feeding rate and digestive enzyme activity (after Mayzaud and Poulet, 1978) is presented in Figure 16 and summarizes the two possible pathways that an animal's nutritional physiology may follow under different conditions. Animals that experience a sudden favorable change in their feeding environment follow the path from step 1 to step 4, a maximum feeding rate. Step 4 leads to step 5 where digestive enzymes are produced and secreted in excess. Positive feedback between step 4 and 5 occurs with enzyme production and secretion stimulating a maximum

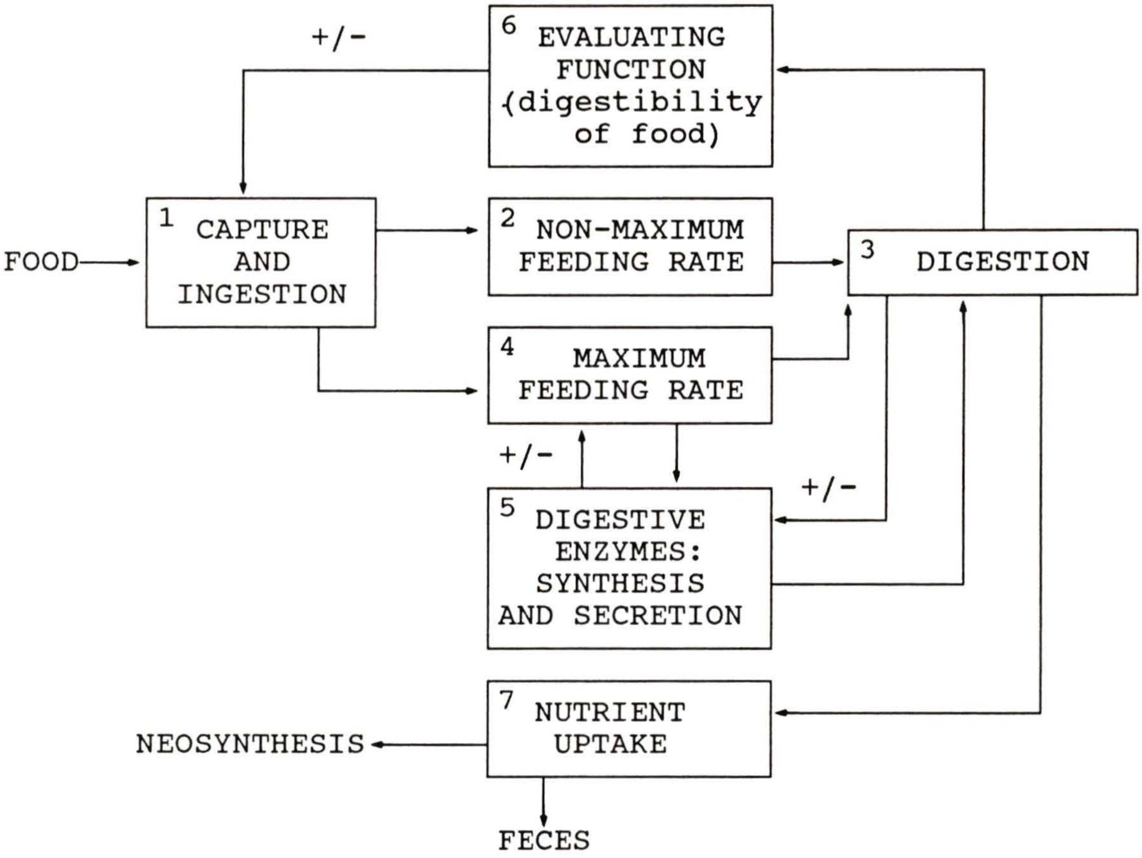

Figure 16: Physiological model of relationship between ingestion, feeding rate and digestive enzyme activity in *Euphausia pacifica* (after Mayzaud and Poulet, 1978) +/- indicates the type of feedback between each step.

feeding rate and vice versa for a period of 6 to 30 hours. During this period of cyclical stimulation, digestion and nutrient uptake (steps 3 and 7) are occurring. After this period of acclimation the feeding rate of the animal drops to below maximum (step 2) and digestion and nutrient uptake follow as before. The animal is still being supplied with digestive enzymes, but this time along a path from step 5 to 3 where the rate of production is controlled by the rate of digestion and not the rate of feeding (gut filling). Step 6 acts to control the system by evaluating the palatability and digestibility of the ingested food and sending positive or negative feedback to step 1.

The group of animals fed on *Artemia* nauplii and analyzed for laminarinase activity provided a form of control for the experiments on laminarinase induction (Figure 11b). This group showed no significant increase in laminarinase activity, supporting the idea that the enzyme is substrate-inducible (Willason et al., 1986;Mayzaud et al., 1985;Cox, 1981). While Willason (1983, in Willason et al., 1986) also reported that *E. pacifica* showed slightly increased levels of laminarinase activity when it ingested small particles of unreactive charcoal, and Head and Conover (1983 in Willason et al., 1986) reported laminarinase induction in copepods fed on algae that did not contain laminarin, no evidence of this response was observed in *E. pacifica* fed on *Artemia* nauplii.

Trypsin-like Protease Induction

It was difficult to determine if production and secretion of the trypsin-like protease digestive enzyme was stimulated in *E. pacifica* by feeding either *Artemia* nauplii or *Skeletonema costatum* (Figures 15a, b). Because there was no significant change in enzyme activity over time in either group, and in both treatments pre-feeding activity levels were higher than post-feeding levels, it appears that neither of the two food types used was effective in inducing activity above and beyond levels already present. The only significant difference between the groups was that the mean level of activity of the group fed on *S. costatum* was higher than the mean level of activity of the group fed on *Artemia*. That there was such a large difference between the two groups before the first feeding is also somewhat unusual as both groups were collected at the same time and experienced the same conditions before the experiments were started.

Conclusions

The data obtained from laboratory experiments indicates that *E. pacifica* has tight control over the production and secretion of the digestive enzyme, laminarinase. Initial response time for the production of new enzyme given a sudden favorable change in the particulate food environment appears to be less than six hours and acclimation to a new quantity or quality of food takes approximately thirty to thirty-six hours. Enzyme induction also appears to be substrate-specific as no activity could be induced by feeding the animals food that did not contain laminarin. The animals appear to acclimate to relatively constant levels of food by reducing the amount of enzyme produced; this is in contrast to the large amount of enzyme produced when favorable food is encountered after a period of starvation. This high degree of control is masked in the field results however because of temporo-spatial heterogeneity in the natural environment. Because of patchiness, it appears that the animals are in a constant state of acclimation to new quantities and qualities of food and the pattern observed over the nine-month study period represents the average response to the average environment.

The only conclusion to be made from the analysis of the trypsin-like protease is that it is always present in *Euphausia pacifica* and is induced by some component of the natural diet of the animal. Laboratory induction of the enzyme was unsuccessful as was identification of the particulate matter that induced activity in field animals. Given the degree of substrate specificity displayed by this class of protease, the results of this analysis were not entirely unexpected.

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APPENDIX

Table A1: List of Abbreviations used in Text

Abbreviation	Meaning
BSA	Bovine Serum Albumin
Chl	Chlorophyll
h	hours (24 hour clock)
LA	Laminarinase Activity
PDT	Pacific Daylight Time
ppt	parts per thousand
TPM	Total Particulate Matter
TPP	Total Particulate Protein

Table A2: Sample Date Weather Data and Scattering Layer Depth.

Date mm/dd/yy	Temperature (°C)	Wind Speed (kn.)	Sky Cond.	Scat. Layer (m)
03/23/89	12	10-15	part. cldy	110-150
04/06/89	8	20	fog, rain	90-110
04/20/89	15	0	fog, rain	50-80
05/01/89	15	20	overcast	90-120
05/15/89	26	0	sunny, clr.	150
05/29/89	12	0	overcast	110
06/12/89	18	0	overcast	100-120
06/26/89	14	25	overcast	70-130
07/10/89	21	0	sunny, clr.	90-120
07/24/89	26	10	sunny, clr.	90-110
08/09/89	17	15	sunny, clr.	---*
08/23/89	18	0	sunny, clr.	---*
09/06/89	12	5	sunny, clr.	---*
09/20/89	16	0	sunny, clr.	---*
10/02/89	15	0	sunny, clr.	110
10/16/89	10	0	sunny, clr.	90-120
10/26/89 (AM)	12	0	sunny, clr.	90-110
10/26/89 (PM)	4	18	drizzle	12
10/30/89	8	5	sunny, clr.	90-110
11/13/89	7	20	overcast	110-120
11/27/89	6	0	overcast	90-110

* M.S.S.V. John Strickland out of commission for this period and no depth sounder was available on the replacement vessel, the Raymont.

Table A3: Average Chlorophyll a Values in mg/m^3 from 0-30m for Sample Period.

Date (mm/dd/yy)

Z (m)	03/23/89	04/06/89	04/20/89	05/01/89	05/15/89
0	2.31	1.51	5.34	1.46	6.43
3	2.19	1.42	7.45	1.44	6.74
6	1.76	1.48	9.63	1.67	8.32
9	1.74	1.33	9.18	1.93	7.93
12	2.43	1.52	8.80	4.00	6.37
15	1.77	1.14	6.78	4.43	4.98
20	1.44	0.82	5.44	3.95	2.30
30	1.15	0.62	6.66	---	1.19

	05/29/89	06/12/89	06/26/89	07/10/89	07/24/89
0	1.89	0.71	8.45	13.44	3.94
3	2.37	0.75	7.88	12.89	4.03
6	2.74	0.97	6.74	12.96	5.08
9	3.51	0.74	5.94	11.16	3.73
12	3.36	1.35	5.82	11.01	3.56
15	2.68	2.13	6.07	8.40	8.17
20	1.85	1.74	5.04	5.64	2.45
30	1.03	1.58	2.61	2.74	1.88

	08/09/89	08/23/89	09/06/89	09/20/89	10/02/89
0	5.24	11.23	3.95	9.41	11.59
3	5.77	12.94	4.03	10.65	11.10
6	5.39	6.12	3.07	8.81	10.29
9	4.23	7.84	3.31	7.10	7.29
12	3.38	5.01	2.88	4.66	7.97
15	3.86	5.88	3.52	9.63	5.72
20	2.57	2.95	2.41	2.31	3.33
30	0.85	1.16	2.25	1.38	1.42

	10/16/89	10/30/89	11/13/89	11/27/89
0	14.62	9.18	2.98	8.84
3	15.24	7.22	3.54	8.08
6	15.15	9.26	3.80	6.80
9	11.01	6.98	1.89	4.12
12	8.96	5.27	1.43	2.90
15	6.01	4.07	1.16	2.15
20	2.23	2.36	0.79	1.54
30	1.00	1.36	0.40	0.09

N = 3 for each datapoint.

Table A4: Average Particulate Protein Values in µg/ml from 0-30m for Sample Period.

	Date (mm/dd/yy)				
Z (m)	03/23/89	04/06/89	04/20/89	05/01/89	05/15/89
0	0.193	0.132	0.291	0.187	0.360
3	0.196	0.128	0.317	0.191	0.386
6	0.147	0.130	0.322	0.226	0.410
9	0.140	0.118	0.328	0.231	0.408
12	0.156	0.097	0.315	0.253	0.382
15	0.150	0.107	0.298	0.285	0.341
20	0.122	0.064	0.247	0.249	0.244
30	0.102	0.076	0.234	0.184	0.132

	05/29/89	06/12/89	06/26/89	07/10/89	07/24/89
0	0.203	0.208	0.403	0.556	0.337
3	0.193	0.212	0.378	0.536	0.315
6	0.222	0.280	0.372	0.610	0.375
9	0.216	0.237	0.336	0.574	0.277
12	0.242	0.222	0.321	0.529	0.273
15	0.231	0.275	0.336	0.460	0.344
20	0.182	0.236	0.296	0.356	0.194
30	0.145	0.201	0.198	0.218	0.112

	08/09/89	08/23/89	09/06/89	09/20/89	10/02/89
0	0.468	0.430	0.468	0.431	0.483
3	0.444	0.473	0.426	0.495	0.462
6	0.443	0.321	0.398	0.412	0.446
9	0.329	0.386	0.404	0.340	0.341
12	0.312	0.299	0.365	0.294	0.368
15	0.371	0.413	0.427	0.436	0.326
20	0.067	0.194	0.289	0.165	0.238
30	0.035	0.122	0.270	0.123	0.165

	10/16/89	10/30/89	11/13/89	11/27/89	
0	0.492	0.336	0.261	0.323	N = 3 for each datapoint.
3	0.499	0.295	0.253	0.298	
6	0.459	0.332	0.251	0.273	
9	0.399	0.285	0.178	0.214	
12	0.365	0.243	0.159	0.169	
15	0.278	0.222	0.146	0.149	
20	0.162	0.130	0.118	0.123	
30	0.098	0.095	0.085	0.098	

Table A5: Average Total Particulate Matter Values in g/m^3 from 0-30m for Sample Period.

Date (mm/dd/yy)

Z (m)	03/23/89	04/06/89	04/20/89	05/01/89	05/15/89
0	0.02216	0.12905	0.01738	0.01744	0.02330
3	0.02434	0.12853	0.02186	0.01930	0.02093
6	0.02015	0.12367	0.01763	0.01567	0.01982
9	0.01890	0.12164	0.01673	0.01548	0.01916
12	0.01721	0.12401	0.01528	0.01573	0.02016
15	0.01615	0.12319	0.01799	0.01650	0.01926
20	0.01903	0.12066	0.01818	0.01997	0.01606
30	0.01372	0.12223	0.01847	0.01852	0.01636

	05/29/89	06/12/89	06/26/89	07/10/89	07/24/89
0	0.14435	0.01707	0.01697	0.05482	0.01835
3	0.14278	0.01379	0.01416	0.05411	0.01566
6	0.13885	0.01517	0.01514	0.05472	0.01751
9	0.13759	0.01291	0.01381	0.05281	0.01495
12	0.13973	0.01593	0.01529	0.05752	0.01871
15	0.13653	0.01313	0.01405	0.05588	0.01401
20	0.13957	0.01607	0.01557	0.05391	0.01728
30	0.13569	0.01332	0.01295	0.05192	0.01324

	08/09/89	08/23/89	09/06/89	09/20/89	10/02/89
0	0.02097	0.02154	0.01310	0.01673	--*
3	0.01790	0.01967	0.00710	0.01593	---
6	0.01956	0.01908	0.00890	0.01687	---
9	0.01608	0.01900	0.00843	0.01540	---
12	0.01778	0.02166	0.01057	0.01733	---
15	0.01622	0.01835	0.01103	0.01587	---
20	0.01839	0.02006	0.00703	0.01640	---
30	0.01503	0.01659	0.00643	0.01467	---

	10/16/89	10/30/89	11/13/89	11/27/89
0	0.01647	0.01685	0.00736	0.01558
3	0.01180	0.01399	0.00674	0.01437
6	0.01580	0.01637	0.00965	0.01697
9	0.01127	0.01423	0.00623	0.01173
12	0.01440	0.01596	0.00807	0.01503
15	0.01020	0.01403	0.00509	0.01177
20	0.01147	0.01565	0.00559	0.01565
30	0.00873	0.01295	0.00329	0.01339

* Data not available for this date. N = 3 for each datapoint.

Table A6: Integrated Total Biovolume (m^3/m^2) of Diatoms, Dinoflagellates and Flagellates for Sample Period.

Date	Diatoms	Dinos	Flags	Total
03/23/89	2.96×10^{-7}	7.76×10^{-9}	8.85×10^{-8}	3.92×10^{-7}
04/06/89	3.01×10^{-7}	5.98×10^{-9}	3.61×10^{-8}	3.43×10^{-7}
04/20/89	5.96×10^{-6}	2.71×10^{-8}	3.53×10^{-8}	6.03×10^{-6}
05/01/89	3.14×10^{-6}	3.38×10^{-8}	1.85×10^{-7}	3.36×10^{-6}
05/15/89	1.16×10^{-5}	4.97×10^{-8}	2.04×10^{-7}	1.19×10^{-5}
05/29/89	3.56×10^{-6}	5.23×10^{-8}	2.28×10^{-7}	3.84×10^{-6}
06/12/89	2.31×10^{-6}	3.01×10^{-8}	2.22×10^{-7}	2.56×10^{-6}
06/26/89	6.08×10^{-6}	2.60×10^{-8}	3.67×10^{-7}	6.47×10^{-6}
07/10/89	5.77×10^{-6}	3.74×10^{-6}	1.54×10^{-7}	9.67×10^{-6}
07/24/89	4.38×10^{-6}	2.60×10^{-6}	8.62×10^{-9}	6.99×10^{-6}
08/09/89	1.27×10^{-6}	2.61×10^{-6}	2.03×10^{-8}	3.89×10^{-6}
08/23/89	8.74×10^{-7}	1.37×10^{-7}	6.06×10^{-7}	1.61×10^{-6}
09/06/89	3.57×10^{-7}	2.88×10^{-7}	4.05×10^{-7}	1.05×10^{-6}
09/20/89	3.26×10^{-7}	1.09×10^{-7}	3.47×10^{-7}	7.83×10^{-7}
10/02/89	9.72×10^{-6}	1.39×10^{-7}	4.67×10^{-8}	9.91×10^{-6}
10/16/89	6.22×10^{-6}	4.39×10^{-8}	3.81×10^{-8}	6.31×10^{-6}
10/30/89	3.38×10^{-6}	2.19×10^{-8}	2.24×10^{-8}	3.42×10^{-6}
11/13/89	7.90×10^{-7}	3.96×10^{-8}	1.56×10^{-8}	8.45×10^{-7}
11/27/89	8.09×10^{-7}	3.32×10^{-8}	1.89×10^{-8}	8.61×10^{-7}

Table A7: Laminarinase Activity (μg glucose produced per minute/g wet weight) of Field Samples of *Euphausia pacifica*.

Date (mm/dd/yy)

Rep.	03/23/89	04/06/89	04/20/89	05/01/89	05/15/89
1	1000	540	1100	2400	3990
	890	460	770	1520	3510
	550	540	940	1160	3810
2	1200	410	720	1720	3720
	1560	490	870	1910	3990
	1200	810	930	2200	4630
3	700	480	1280	2840	2100
	810	340	1160	2610	2020
	1040	680	1220	2500	2180

	05/29/89	06/12/89	06/26/89	07/10/89	07/24/89
1	2960	3440	1820	2700	1900
	2520	3790	1460	2450	1490
	2890	3510	1010	2870	1490
2	3270	1460	1900	1730	2080
	3340	1660	2570	1280	2430
	3550	1810	3040	1640	2500
3	2460	1360	1790	3310	3180
	2460	1300	1790	2990	2710
	2460	1570	1740	3310	3180

	08/09/89	08/23/89	09/06/89	09/20/89	10/02/89
1	1310	1580	430	790	620
	1470	1060	270	750	620
	650	530	540	460	660
2	2060	1640	560	920	560
	1880	1930	560	980	600
	2250	1450	660	1110	600
3	1340	1480	620	880	690
	670	850	920	940	530
	670	1380	530	1380	690

cont'd....

Table A7: cont'd.

	Date (mm/dd/yy)			
Rep.	10/16/89	10/30/89	11/13/89	11/27/89
1	980	500	1150	370
	570	470	770	350
	680	400	610	280
2	920	1680	950	490
	800	1730	900	360
	610	1660	1030	730
3	1050	800	1400	630
	1030	400	1310	730
	980	870	1400	910

Table A8: Trypsin-like Protease Activity (μmol p-tosylsulfonyl-L-arginine produced per minute/g wet weight) of Field Samples of *Euphausia pacifica*.

	Date (mm/dd/yy)				
Rep.	03/23/89	04/06/89	04/20/89	05/01/89	05/15/89
1	3.23	7.99	6.64	18.19	3.93
2	5.76	8.09	9.88	19.48	4.65
3	8.55	8.24	10.23	22.61	7.22
	05/29/89	06/12/89	06/26/89	07/10/89	07/24/89
1	4.79	5.86	5.03	5.75	3.89
2	5.70	7.92	8.99	7.12	4.19
3	6.98	8.06	11.98	9.94	19.54
	08/09/89	08/23/89	09/06/89	09/20/89	10/02/89
1	10.12	5.49	8.86	6.57	11.80
2	12.26	5.81	15.10	8.94	12.99
3	16.13	11.19	17.00	10.65	13.87
	10/16/89	10/30/89	11/13/89	11/27/89	
1	7.83	9.07	5.75	6.99	
2	8.68	11.37	5.85	7.94	
3	11.20	11.57	6.44	8.27	

Table A9: Laminarinase Activity (μg glucose produced per minute/g wet weight) of Laboratory Specimens of *Euphausia pacifica* fed on *Skeletonema costatum*. (Laboratory Experiment #2)

	Day/Time				
Rep.	1/0900	1/1500	1/2100	2/0900	2/1500
1	240	500	0	0	1720
	240	0	0	230	1890
	120	230	0	0	1890
2	260	220	0	0	600
	350	260	0	0	760
	350	260	0	0	850
3	480	640	0	0	800
	730	540	0	0	1040
	990	700	0	0	1520

	2/2100	3/0900	3/1500	3/2100	4/0900
1	660	1110	2770	0	1530
	180	1290	0	430	100
	60	860	1660	290	0
2	800	520	1700	1820	1390
	1070	890	1770	1220	1770
	1070	820	1770	1560	1230
3	2320	1490	840	3510	1180
	1970	1030	530	3560	1110
	1970	1610	1050	3370	1180

	4/1500	4/2100	5/0900	5/1500	5/2100
1	0	1070	0	1910	410
	0	760	0	700	600
	0	310	0	1000	450
2	550	320	0	810	330
	550	1080	420	740	660
	70	950	490	1080	990
3	450	700	300	940	410
	1160	620	380	990	710
	800	740	0	1040	610

Table A10: Laminarinase Activity (μg glucose produced per minute/g wet weight) of Laboratory Specimens of *Euphausia pacifica* fed on *Artemia* sp. nauplii.
(Laboratory Experiment #2)

	Day/Time				
Rep.	1/0900	1/1500	1/2100	2/0900	2/1500
1	780	990	30	680	830
	870	760	0	430	860
	780	580	0	490	950
2	250	970	590	1120	210
	250	870	570	960	240
	290	870	550	1120	260
	340	910	220	410	1080
	310	910	320	570	1080
	430	910	270	540	1220

	2/2100	3/0900	3/1500	3/2100	4/0900
1	1690	470	870	650	710
	1440	440	690	630	490
	1550	660	610	700	490
2	1570	630	840	410	850
	1600	560	960	410	870
	1530	600	960	550	870
3	900	390	450	800	730
	960	350	530	830	850
	1080	390	510	740	770

	4/1500	4/2100	5/0900	5/1500	5/2100
1	1090	700	330	1400	750
	990	600	380	1400	750
	1020	550	410	1400	820
2	470	640	450	630	610
	440	710	490	630	610
	670	710	520	690	670
3	380	650	980	1680	--
	350	730	890	1800	--
	440	680	1030	1360	--

Table A11: Laminarinase Activity (μg glucose produced per minute/g wet weight) of Laboratory Specimens of *Euphausia pacifica* fed three times a day on *Skeletonema costatum*. (Laboratory Experiment #3)

	Day/Time				
Rep.	1/0900	1/1500	1/2100	2/0900	2/1500
1	780	0	1130	660	620
	500	0	880	690	390
	460	0	790	770	350
2	350	400	270	990	730
	430	180	270	990	680
	470	310	360	1170	830
3	270	550	780	740	1100
	330	770	820	790	1030
	360	700	750	840	1030

	2/2100	3/0900	3/1500	3/2100	4/0900
1	0	1100	130	510	0
	60	490	130	340	70
	60	430	260	170	130
2	240	360	500	230	470
	0	470	500	320	600
	340	470	360	270	600
3	220	320	590	310	600
	370	340	680	370	680
	300	230	770	260	600

	4/1500	4/2100	5/0900	5/1500	5/2100
1	640	0	40	50	690
	220	0	0	170	590
	320	80	0	320	490
2	940	150	170	520	650
	1000	230	0	520	900
	1060	230	0	580	730
3	570	450	200	620	720
	570	500	400	1070	800
	700	450	100	1070	800

Table A12: Trypsin-like Protease Activity (μmol p-tosylsulfonyl-L-arginine produced per minute/g wet weight) of Laboratory Samples of *Euphausia pacifica* fed on *Artemia* sp. nauplii. (Laboratory Experiment #4)

	Day/Time				
Rep.	1/0900	1/1500	1/2100	2/0900	2/1500
1	1.18	1.58	2.42	2.63	4.41
2	2.26	2.72	3.25	4.01	5.57
3	14.64	4.65	3.57	4.62	13.79
	2/2100	3/0900	3/1500	3/2100	4/0900
1	2.88	3.52	5.59	9.01	6.00
2	3.91	4.32	6.11	9.14	9.44
3	4.49	7.45	6.78	9.60	12.20
	4/1500	4/2100	5/0900	5/1500	5/2100
1	1.11	3.07	2.94	3.20	--
2	4.51	3.95	3.58	4.32	--
3	6.44	4.87	7.36	5.36	--

Table A13: Trypsin-like Protease Activity (μmol p-tosylsulfonyl-L-arginine produced per minute/g wet weight) of Laboratory Samples of *Euphausia pacifica* fed on *Skeletonema costatum*. (Laboratory Experiment #4)

	Day/Time				
Rep.	1/0900	1/1500	1/2100	2/0900	2/1500
1	18.89	9.69	2.79	10.78	10.50
2	9.97	21.08	8.58	22.70	19.21
3	14.98	9.47	2.79	5.06	6.47
	2/2100	3/0900	3/1500	3/2100	4/0900
1	4.31	8.67	13.49	18.35	19.33
2	18.97	15.94	7.64	13.22	7.74
3	3.25	2.54	13.03	18.18	15.80
	4/1500	4/2100	5/0900	5/1500	5/2100
1	26.47	6.96	--	--	--
2	10.17	7.66	--	--	--
3	18.76	6.41	--	--	--

Table A14: A comparison of trichromic pigment analysis and fluorometric pigment analysis for the determination of chlorophyll a at three pigment concentrations. Chlorophyll concentrations in mg/m^3.

Method		
Fluorometric	Trichromic	% difference
0	0	0
0	0.0001	0
1.90	2.20	15
1.89	2.21	16
1.89	2.20	16
1.88	2.23	18
1.90	2.19	15
1.88	2.19	16
4.85	5.00	3
4.89	4.92	1
4.91	4.91	0
4.88	4.94	1
4.89	4.89	0
4.90	4.90	0
	Average % Difference =	7.2

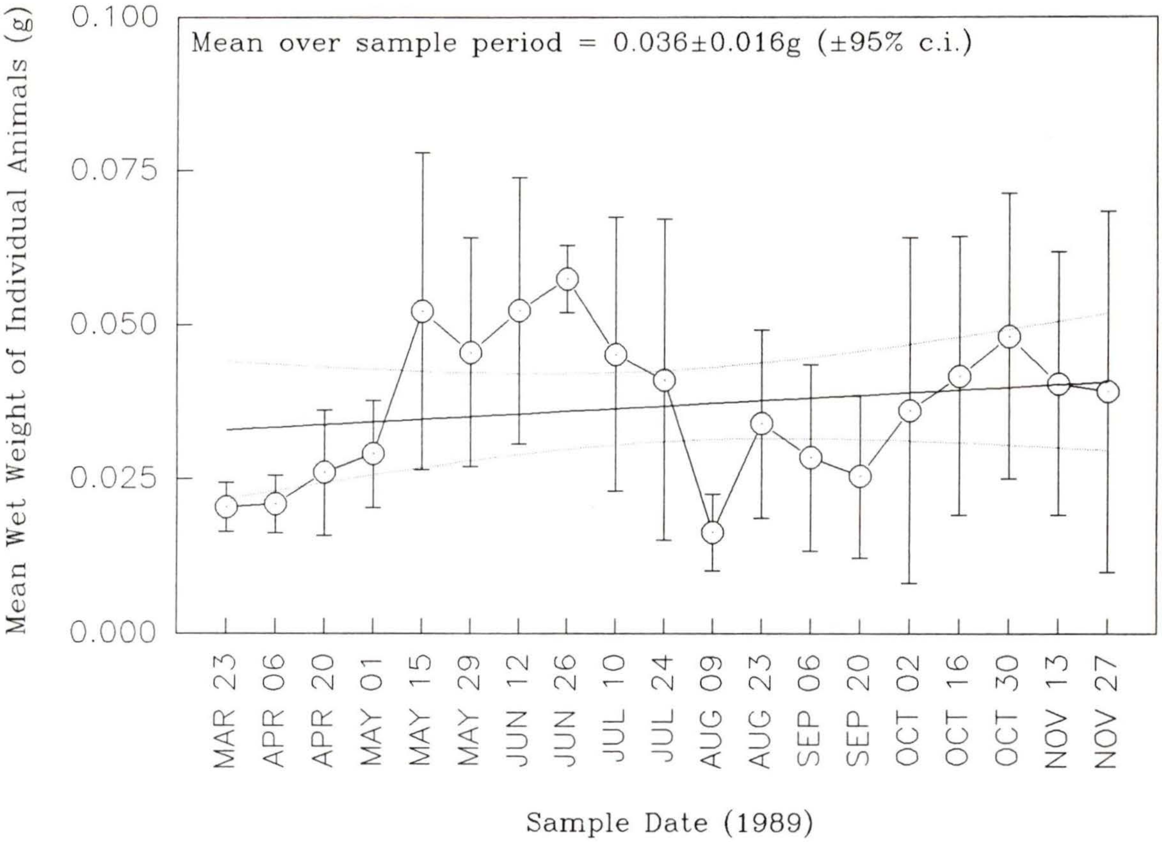

Figure A1: Mean wet weight of individual specimens of *Euphausia pacifica* taken from Stn. "E", Saanich Inlet, from March through November, 1989.

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October 1, 1990