Seasonal Variation in the Carotenoid Pigment Content and Composition of *Euphausia pacifica*

by

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We accept this thesis as conforming to the required standard

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ABSTRACT

The carotenoid pigment content and composition of *Euphausia pacifica*, a preferential herbivore, was determined at monthly intervals from March, 1988 to May, 1989 to determine whether or not season and/or sexual maturity affected the carotenoid content/composition. The total weight specific carotenoid content varied from 306-536 μg/g dry weight over the 15 month period. The maximum occurred in Feburary and March, just prior to sexual maturity and spawning, and the minimum occurred in October, 1988. The weight specific carotenoid content remained relatively constant throughout the summer and early fall (June-Nov., 1988) and then increased over the winter as the average dry weight of the euphausiid population decreased. The data suggested that *E. pacifica* utilized energy stores such as lipid and protein preferentially to carotenoid pigments during times when the food supply was limited. *E. pacifica* also appeared to accumulate carotenoid pigments on an individual basis during early spring (Feb.-Apr.,1989) in preparation for spawning. Results from two-dimensional silica TLC and RP-HPLC suggest that 90% of the carotenoid pigments present in *E. pacifica* consist of astaxanthin, its mono- and diesters each contributing 5, 49, and 40%, respectively. The astaxanthin molecules appear to be esterified to only two different fatty acids; the combinations producing two monoesters and three diesters. The relative contribution of all the carotenoid fractions isolated from *E. pacifica* remained remarkably constant throughout the study period suggesting functional importance.

Examiners:

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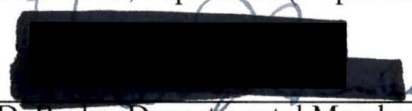

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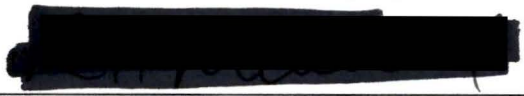

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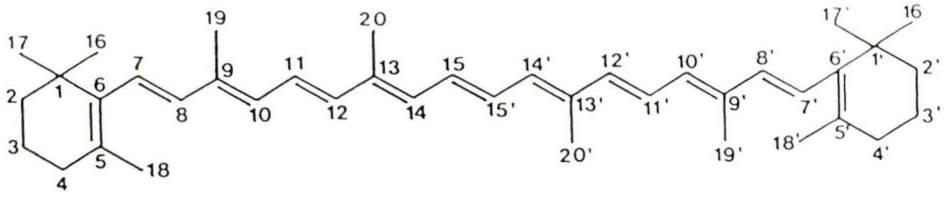
INTRODUCTION

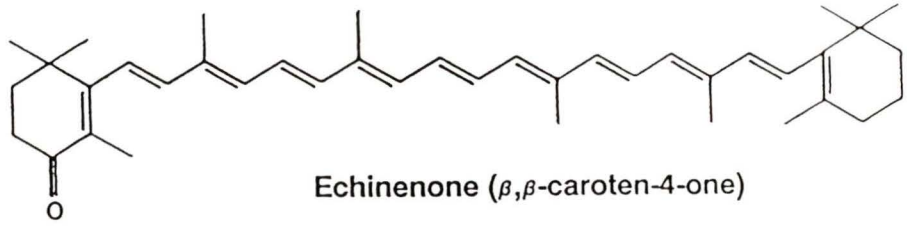
Carotenoid pigments, by definition, are isoprenoid polyenes which typically possess a carbon skeleton consisting of forty carbon atoms (figure 1). The polyene chain, consisting of seven to thirteen conjugated double bonds, is the chromophore, absorbing light in the 400-500 nm range depending on the length of the conjugated system. Carotenoid pigments may be divided into two categories: carotenes and xanthophylls. Carotenes are those which only contain hydrogen and carbon atoms (β-carotene in figure 1), while xanthophylls also contain atoms of oxygen which may be present in alcohol, aldehyde, ketone, epoxy, and/or carboxyl functional groups (figure 1 depicts a few derivatives).

In recent years, considerable attention has been given to carotenoid metabolism in salmonids. Salmonids are quite unique among fishes in their ability to accumulate carotenoid pigments in their flesh. The unique bright orange-red colour of the flesh is aesthetically pleasing to the consumer and has lead to a large increase in the market value of salmon. As a result, salmon aquaculturists have sought to maximize the carotenoid content in the flesh of various farmed salmon species .

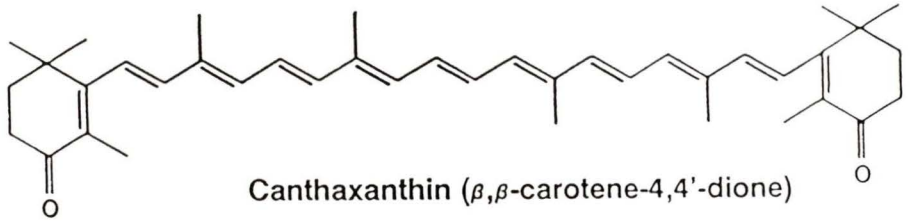
There are inherent differences among salmonids in their ability to accumulate carotenoid pigments (Foss et al., 1987; Schiedt et al., 1981, 1985; Storebakken et al., 1986). Several biological factors have also been shown to affect the degree of flesh pigmentation within a given species. Some important factors are fish size and growth rate (Spinelli et al., 1978; Torrissen, 1986; Torrissen et al., 1984), sexual maturation (Kitahara, 1983; Torrissen et al., 1985, 1988), age (Sivtseva et al., 1981; Torrissen et al., 1988) and genetic background (McCullum et al., 1987; Torrissen et al., 1984, 1988).



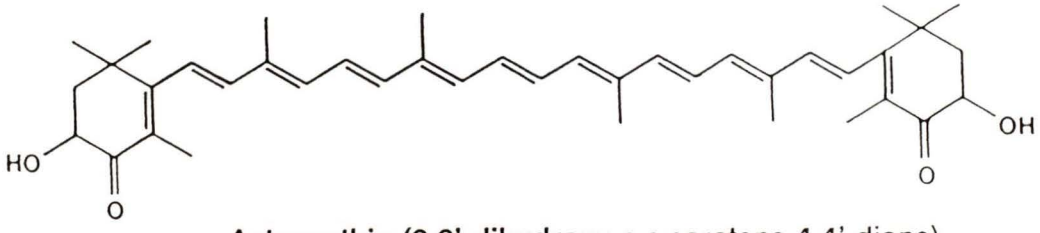
β-carotene (β,β-carotene)



Echinenone (β,β-caroten-4-one)



Canthaxanthin (β,β-carotene-4,4'-dione)



Astaxanthin (3,3'-dihydroxy-β,β-carotene-4,4'-dione)

Figure 1: Some examples of C_{40} carotenoid molecules. The common names and systematic names are given. The carbon atoms of β-carotene are numbered to illustrate the systematic naming procedure.

Canthaxanthin (β,β-carotene-4,4'-dione) and unesterified astaxanthin (3,3'-dihydroxy-β,β-carotene-4,4'-dione) (figure 1) are the only two xanthophylls reported to be present in the flesh of salmonids. Studies have shown that these two dietary pigments are transported directly across the intestinal wall and to the muscle without any structural modifications (Foss et al., 1984; Schiedt et al., 1985). Dietary astaxanthin is transported across the intestinal wall and deposited in the flesh preferentially compared to canthaxanthin (Storebakken et al., 1987; Torrissen, 1986). The optical isomers of astaxanthin (3R-3R', 3S-3S', and the meso form) are absorbed equally (Foss et al., 1984; Schiedt et al., 1981, 1985). Fish fed a diet containing esters of astaxanthin will accumulate free astaxanthin but at a slower rate because hydrolysis of the ester linkage is required before transport can occur and this step is rate limiting (Foss et al., 1987; Schiedt et al., 1985; Storebakken et al., 1987). The ester hydrolase responsible for hydrolysis of the ester linkages appears to be stereospecific as there is an accumulation of the 3R, 3R' isomer in fish when they are fed a mixture of astaxanthin diester enantiomers (Schiedt et al., 1985).

Presently, astaxanthin and canthaxanthin are purchased from Hoffmann-La Roche Inc., the only company presently synthesizing these pigments on a commercial scale. The incorporation of these pigments into fish feed is expensive for both the feed manufacturer and the farmer and consequently, alternative sources are being sought.

Euphausiids, commonly called krill, have been investigated as a feed and pigment source for farmed salmon (reviewed by Storebakken, 1988). Krill are marine, pelagic crustaceans belonging to the order Euphausiacea. The biochemical composition of the Antarctic krill, *Euphausia superba*, and a few other euphausiid species are reasonably well documented (Bottino, 1974, 1975; Clarke, 1980; Ellingsen et al., 1981; Falk-Petersen, 1981; Falk-Petersen et al., 1981; Littlepage, 1964; Raymont et al., 1964, 1969, 1971a, 1971b; Reinhardt et al., 1986; Saether et al., 1985, 1986a, 1986b; Sargent et al., 1981; Suyama et al.,

1965). Owing to an extremely high fluoride content (Soevik & Braekkan, 1979), euphausiids are not suitable for human consumption and therefore, those species that are fished commercially are used in animal feeds.

Krill appear to be suitable as a fish feed ingredient for several reasons: the fluoride from krill is not accumulated in the flesh of salmonids (Grave, 1981; Tiews et al., 1982), the protein content of krill is of high nutritional value for fish and krill are a good source of essential fatty acids--including omega-3 fatty acids (reviewed by Storebakken, 1988). There is also limited evidence that fish fed a diet supplemented with either frozen krill or krill meal have an increased growth rate and feed conversion efficiency (Allahpichay et al., 1984; Grave et al., 1979; Koops et al., 1979).

One other important reason for euphausiids being sought as an ingredient for salmon feed is due to their relatively high levels of carotenoid pigments (Clarke, 1980; Czeczuga & Klyszejko, 1978; Fisher et al., 1952, 1954, 1955; Lambertsen & Braekkan, 1971; Storebakken, 1988; Yamaguchi et al., 1983). However, the suitability of krill as a pigment source for salmonids has not been thoroughly investigated. There have been no studies monitoring the deposition of pigments from frozen krill or krill meal in salmon, and there are conflicting results concerning the types of carotenoids present in euphausiids. Lambertsen & Braekkan (1971) reported that a mixed sample of euphausiid species contained free astaxanthin (3%) as well as its mono- (49%) and diesters (48%). Results reported by Yamaguchi et al. (1983) for *E. superba* were similar. However, Czeczuga et al. (1978) and Jackowska et al. (1980) reported the presence of seven different carotenoids in *E. superba* and *E. crystallorophias*.

In British Columbia a limited commercial fishery has developed for the local euphausiid, *Euphausia pacifica*, to be used primarily as a source of carotenoid pigments for farmed salmon. The actual usefulness of *E. pacifica* as a pigment source is not known as

there have been no detailed biochemical studies on this species. The present research was undertaken to investigate the content and composition of carotenoids in *E. pacifica*.

Primary producers are the only organisms capable of synthesizing carotenoid pigments (Goodwin, 1984). The role of carotenoid pigments in photosynthetic systems has been the subject of considerable attention and a summary of these roles is necessary for an understanding and an appreciation of the multi-functional nature of carotenoid molecules. The two primary functions of carotenoid pigments in photosynthetic organisms are believed to be a light-harvesting function and a protective function against photosensitized damage (recent reviews by Cogdell, 1985; Cogdell & Frank, 1987; Mathis & Schenck, 1982).

The light-harvesting function of carotenoid pigments is perhaps intuitively the more obvious. Chlorophyll (CHL) and bacteriochlorophyll (BCHL) pigments absorb light most efficiently at wavelengths centered around 440 and 670nm. Carotenoid pigments absorb in the 420 to 500nm range where chlorophylls are less efficient. The light energy absorbed by the carotenoid pigment causes the carotenoid to rise to its excited singlet energy state. This energy is then transferred to the chlorophyll molecule resulting in the formation of its excited singlet energy state (Cogdell, 1985; Cogdell & Frank, 1987; Mathis & Schenck, 1982). The efficiency and mechanism of this energy transfer varies due to the different reaction centres (light-harvesting centres). The reaction centres, located in photosynthetic membranes, may be described as caroteno-chlorophyll pigment-protein complexes. Reaction centres vary tremendously in terms of the CHL to carotenoid ratio, as well as in the types of carotenoid pigments and proteins present in the centres (Cogdell & Frank, 1987; Pardo et al., 1980; Ridley & Ridley, 1979; Song et al., 1976). The key, therefore, to understanding the *in vivo* mechanism and efficiency of a singlet-singlet energy transfer lies in understanding the detailed structure of the reaction centres. Razi-Naqvi (1980) discusses energy transfer mechanisms.

In contrast to the antenna function of carotenoid pigments, they also serve a protective function. Photosynthetic systems are prone to photosensitized damage due to the presence of either CHL or BCHL. There are two paths leading to photosensitized damage in biological systems (Foote, 1968b; Krinsky, 1979). Mandatory in both paths is the excitation of a sensitizer, usually a fluorescent substance, to its singlet energy state. Then, typically through intersystem crossing, the short lived singlet state molecule changes to a new excited species called the triplet sensitizer (Gollnick & Schenck, 1964). The triplet sensitizer is longer lived than the singlet state (Gollnick & Schenck, 1964) and thus can react directly with a substrate to produce free-radicals (Type I reaction) or with molecular oxygen to produce singlet oxygen ($^1O_2$) (Type II reaction) (Gollnick & Schenck, 1964; reviewed by Krinsky, 1979). Both free-radicals (reviewed by Pryor, 1976) and singlet oxygen (reviewed by Berson et al., 1966; Foote, 1968a, 1968b) are extremely harmful to biological systems. Production of the latter is more common and is referred to as photodynamic action. Photodynamic action is defined as an oxidation by molecular oxygen brought about in a biological system exposed to radiation through the agency of a fluorescent substance (the photodynamic agent, i.e. CHL) (Clare, 1956).

The role of carotenoid pigments in prevention of photodynamic action first became recognized by Stanier and his colleagues (Griffiths et al., 1955) who studied the carotenoidless mutant of the non-sulphur purple bacterium *Rhodopseudomonas spheroides*. They found extremely low cell viability when mutants were exposed to light and oxygen. The photosensitizer responsible for the photodynamic action resulting in cell death was the intracellular BCHL. Carotenoid pigments were also found capable of preventing photodynamic action in non-photosynthetic bacteria; cells lacking BCHL. Mathews & Sistrom (1959) found that only the carotenoidless mutants of *Sarcina lutea* died when

exposed to high levels of sun light and oxygen. Krinsky (1968) provides a comprehensive review on photodamage due to visible light in organisms which lack carotenoid pigments.

Carotenoids were proven capable of interacting with or quenching triplet sensitizers, free radicals produced in type I reactions and $^1O_2$ molecules produced in type II reactions. The triplet sensitizer is quenched equally well by either β-carotene or molecular oxygen (Chessin et al., 1966; Fujimori & Livingston, 1957). Therefore, provided the quenching reaction is diffusion controlled, production of $^1O_2$ would necessarily occur unless the concentration of carotenoid molecules greatly exceeded that of molecular oxygen (Foote & Denny, 1968).

The quenching of $^1O_2$ by carotenoid molecules is dependent on the length of the conjugated system (Foote et al., 1970a, 1970b). Foote et al. (1970a) found that carotenoid molecules having fewer than nine conjugated double bonds were very inefficient at quenching $^1O_2$. As proposed by Foote et al. (1970a) and proven by Mathis (1970), the energy level of the triplet state carotenoid increases as the length of the conjugated system decreases. The triplet state energy levels of carotenoid molecules having fewer than nine conjugated double bonds exceeds that of $^1O_2$, thus preventing the quenching reaction (Foote et al., 1970a).

There is also evidence that the quenching of free radicals (type I reactions) by carotenoids may be operative *in vivo*. Anderson & Krinsky (1973a, 1973b) demonstrated that free-radical induced lipid peroxidation could be prevented if carotenoid pigments were introduced into the system. However, it appears that carotenoids can inhibit only certain radical-induced reactions from proceeding and that the carotenoids may be oxidized during this process (for review see Krinsky, 1979).

The importance of the quenching pathway utilized by the carotenoid pigments is dependent not only on the energy levels of both the triplet sensitizer and the triplet

carotenoid, but also on the biological system. For example, in photosynthetic systems, the quenching of the triplet state BCHL by carotenoid pigments can be so efficient due to the position of carotenoid pigments in the light-harvesting complex that production of $^1O_2$ does not occur (Cogdell & Frank, 1987). Therefore, as first pointed out by Boucher et al. (1977), the relative effectiveness of carotenoids as protective agents must be a function both of the structure of the carotenoid and the manner in which it binds to the reaction centre.

From the preceding discussion, the complexity of carotenoid structure and function in photosynthetic systems should be apparent. Although the exact mechanisms by which carotenoid pigments function are not clearly understood, their importance as antenna molecules and as protective agents in photosynthetic systems cannot be disputed.

Carotenoid pigments are also widely distributed in the animal Kingdom (Goodwin, 1984) but their functional importance is less well understood. Apart from speculations, there have been few experiments relating carotenoid pigments to photosensitivity in animals. Mathews (1964) was one of the first investigators to establish β-carotene as a photoprotector in animals. She showed that the lethal photosensitization of albino mice, injected with the photosensitizer, haematoporphyrin, was markedly reduced by administration of β-carotene. Further studies directed towards the efficacy of β-carotene as a treatment for photosensitivity diseases (reviewed by Mathews-Roth, 1982, 1987) have revealed that the mechanism of symptom prevention may be effected through the quenching of singlet oxygen molecules and free radicals (Anderson & Krinsky, 1973b; Krinsky & Deneke, 1982; Mathews-Roth, 1984; Nilsson et al., 1975).

Carotenoids have also been found to prevent or slow down the development of skin tumors induced by UV-B light (290-320nm) (Epstein,1973; Mathews-Roth, 1982; Mathews-Roth & Krinsky, 1987). Although it is felt that $^1O_2$ and free radicals may be involved in the development of UV-B induced skin tumors (Black & Chan, 1977) and that carotenoids can

quench such excited species in epidermis exposed to UV-B (Mathews-Roth, 1986), it has not yet been possible to establish any causal role between anti-cancer activity and excited species quenching, nor between cancer development and the production of $^1O_2$ and free radicals (Mathews-Roth, 1986).

The protective role of carotenoid pigments against photodynamic action as well as the harmful reactions caused by free radicals has been extrapolated to animal systems to explain the ubiquitous occurrence of carotenoids. Limiting this discussion to crustaceans, certain observations have supported the photoprotective function of carotenoids in animal systems. For example, the cladoceran *Daphnia magna* was reported to increase its carotenoid content when grown under high light intensities (Green, 1957; Herring 1968b). Hairston (1976) found that unpigmented copepods died more quickly at high light intensities than pigmented ones. Any changes in the carotenoid concentration or distribution such as the deposition of carotenoids into eggs has thus been explained as a photoprotective phenomenon (Gilchrist & Lee, 1972; Green, 1965, 1966; Hairston, 1979b). The validity of such explanations remains to be proven but in most cases it seems reasonable.

As mentioned previously, animals cannot synthesize their own carotenoid pigments; the carbon skeletons must be derived from plants, via the food chain (Buchecker, 1982; Goodwin, 1984). However, the accumulation of carotenoids by crustaceans is not necessarily passive by nature. That is, the ability of animals to deposit and structurally manipulate dietary carotenoids is a highly variable, and often an energy requiring process. For example, the brine shrimp, *Artemia salina*, converts β-carotene to echinenone (β,β-caroten-4-one) and then to canthaxanthin (figure 1) (Davies et al., 1965, 1970; Hsu et al., 1970; Krinsky, 1965). The introduction of a carbonyl oxygen at carbons 4 and 4' appears to occur in an enzyme complex which forms the secondary alcohol and then converts it to a

ketone. The enzyme complex is specific to the unsubstituted ring of β-carotene (Davies, 1985; Davies et al., 1970; Soejima et al., 1980).

Other crustaceans convert dietary carotenoids to astaxanthin (figure 1). The formation of astaxanthin is more complex than that of canthaxanthin as there are several potential routes, each with its own set of intermediate molecules which may require specific enzymes. Also, astaxanthin has two chiral centers at C-3 and 3' resulting in three possible optical isomers: the 3S, 3S' and 3R, 3R' being enantiomers and 3S, 3R' being the meso form. The presence of stereospecific enzymes may limit the manipulation and deposition of dietary molecules.

Given that animals are specific in the types of carotenoids they deposit and that structural manipulation of dietary pigments occurs, it seems reasonable to argue that the deposition of carotenoid pigments is not a passive process. It follows that the expenditure of energy in the deposition of carotenoids would not occur if the pigments served no function.

The objectives of this research are three-fold. The carotenoid pigments present in *Euphausia pacifica* will be characterized and quantified. The carotenoid content/composition of *E. pacifica* will be monitored for a one year period to observe any variations which may be a function of season and/or sexual maturity. Finally, laboratory experiments will be carried out to determine whether or not the dietary pigment composition affects either the quantity, and/or composition of carotenoids present in *E. pacifica*.

MATERIALS AND METHODS

COLLECTION

Euphausiids were collected aboard the MSSV John Strickland from Saanich Inlet, British Columbia (48° 37'N: 123° 30'W). Many zooplankters are diel vertical migrators--swimming to the surface at dusk to feed and swimming down again to a substantial depth at dawn. The zooplanktonic organisms tend to exist at the same depth during the day forming a concentrated layer of organic matter known as the scattering layer. The scattering layer was located using a depth sounder and was usually present between 90 and 110m. Horizontal tows of this layer were carried out using a one meter plankton net. Samples were collected at monthly intervals starting March 17, 1988 and ending May 1, 1989 (table 1). Euphausiids were separated from the other zooplankters and quick frozen in seawater using a dry ice-ethanol bath. They were stored at -80° C until analyzed.

PIGMENT EXTRACTION

Animals were thawed and sorted under a dissecting microscope to ensure that only undamaged individuals belonging to the species *E. pacifica* were used in the analyses. Euphausiids were lyophilized and a pooled sample having a dry weight of approximately 300mg provided sufficient material for quantification. Three samples were analyzed for each collection date with a few exceptions (table 1).

Table 1: Dates corresponding to the times *E. pacifica* was collected from Saanich Inlet. The number of replicate samples, the average dry weight and the approximate growth rate for each sample date are also listed. The growth rates are expressed as the percent increase in dry weight per day with respect to the initial dry weight.

Date	No. of smpl. replicates	Avg. dry wt. (mg)	Growth rate (%incr./day)
Mar. 7	3	4.4	
			2.27
Apr. 18	3	7.6	
			0.74
May 4	3	8.5	
			1.04
June 6	2	11.4	
			0.25
July 11	2	12.4	
			[1]N/D
May 4	2	0.17	
			4.82
June 6	2	0.44	
			11.4
July 11	2	2.2	
			1.32
Aug. 11	3	3.1	
			2.53
Aug. 25	3	4.2	
			0.45
Oct. 6	3	5.0	
			1.22
Nov. 3	3	6.7	
			-0.02
Nov. 28	2	6.3	
			-0.07
Dec. 16	2	5.6	
			-0.06
Jan. 12	2	4.7	
			-1.15
Feb. 9	2	3.2	
			0.47
Mar. 8	3	3.6	
			0.64
Apr. 3	2	4.2	
			3.07
May 1	3	7.8	
			[1]N/D

[1]N/D-no data

Following sorting, the euphausiids were lyophilized and the average dry weight determined. All subsequent analyses were carried out under minimal light, at temperatures no greater than room temperature (20 ° C), and under nitrogen. The dried euphausiids were ground in 100% acetone using a motorized teflon pestle and a glass mortar. During homogenization, the mortar and its contents were cooled in an ice bath. The extract was filtered through a glass fibre filter and the debris re-extracted until the acetone remained clear. An equal volume of diethyl ether (ether) was added to the acetone extract followed by a sufficient volume of 0.8% KCl to form a biphasic system. The ether hyperphase which contained the carotenoid pigments was removed and placed in a separatory funnel. The hypophase was washed several times with ether to ensure complete recovery of the pigments. The combined ethereal washings were then washed with distilled water to remove all the salt and most of the acetone/water. The ether was then evaporated under a stream of nitrogen and the remaining acetone/water removed in the freeze drier. The dried pigment was then dissolved in petroleum ether (b.p. 40-60 ° C).

QUANTIFICATION

Aliquots were removed from the total sample and diluted to 1.0 mL with pet. ether. Dilutions were carried out to yield absorbance values ranging from 0.35 - 1.20 (Append. I). The samples were scanned in the 400-500 nm range using a Beckman DU-8 spectrophotometer. The absorbance maximum was picked by the spectrophotometer. The absorbance value was converted to micrograms of astaxanthin equivalents using the specific extinction coefficient for canthaxanthin in pet. ether ($E^{1\%}_{1cm}=2200$) (Isler & Schudel, 1963) which was first corrected by the molecular weight ratio of canthaxanthin to astaxanthin

(0.9464) as described by Lambertsen & Braekkan (1971). The resulting specific extinction coefficient value of 2082 was used to quantify all carotenoid fractions.

The quantity of carotenoids present in *E. pacifica* was determined on both an individual (μg astaxanthin equivalents/individual) and a weight specific (μg astaxanthin equivalents/gram dry tissue) basis using the equation described by McBeth (1972):

$$\frac{\mu g \text{ carotenoid}}{g \text{ dry tissue}} = \frac{(abs)(dil.\ factor)(Total\ vol)10000}{E^{1\%}_{1cm}\ (Dry\ wt.\ of\ sample)}$$

FRACTIONATION OF PIGMENT EXTRACT

The pigments were separated on silica thin-layer plates. The plates were 20 x 20cm with a silica coating 500μm in thickness. The pigments were loaded onto the plates using a plate-streaker and then developed in a saturated chamber with dichloromethane and ethyl acetate (80:20 by volume) (Bolliger, 1965). While adsorbed to silica, pigments are extremely prone to cis/trans isomerization by light and are more readily oxidized. Therefore, the chambers were covered with aluminum foil to prevent exposure to light during development and the anti-oxidant butylated hydroxy toluene was added to the solvent system at a concentration of 50-100 mg/L (Wren, 1960).

The resulting fractions were scraped off the plate and eluted from the silica with ether or 10% methanol in ether for the more polar carotenoids. Each fraction was taken to dryness, dissolved in 1.0 mL of pet. ether, and appropriate dilutions carried out to yield accurate absorbance values. The quantity of astaxanthin equivalents was then determined as described above.

The quantities of all the fractions were summed and the percent contribution of each determined. The percent value was multiplied by the total carotenoid content to determine the quantity of each fraction. This was necessary as there was some unavoidable loss of pigments during loading of the plates and recovery from the plates. This method of quantification assumes equal loss of all carotenoid fractions.

After quantification, the fractions were loaded onto silica TLC plates and developed in a second solvent system consisting of 30% acetone in hexane (Renstrom et al., 1981c). Two of the fractions showed further separation. These were eluted from the silica and quantified on a percent basis as described above.

STATISTICAL ANALYSES

All of the error bars in the figures represent 95% confidence limits (C.L.). A one-way analysis of variance was used to test for significant differences (alpha level 0.05) among the carotenoid concentrations at different sample dates.

IDENTIFICATION OF CAROTENOID PIGMENTS

Carotenoid standards of free astaxanthin, astaxanthin monopalmitate and astaxanthin dipalmitate were received as a gift from Hoffmann-La Roche Inc. The relative mobilities of the standards on TLC as compared to the solvent front (R_f values) and the retention times on reverse-phase high-performance liquid chromatography (RP-HPLC) were compared to those values obtained for the major carotenoid pigments isolated from *E. pacifica*. The

HPLC system was also used to purify the unknowns so that mass spectrometric data of the unknowns and the standards could also be compared.

The HPLC used was a Varian, model 5000 liquid chromatograph. A C_{18} column, 30cm x 4mm, with a particle size of 10μm, was used for the separation. The mobile phase was methanol and water (Taylor & Ikawa, 1980). During elution, the flow rate was maintained at 1.0ml per minute and the carotenoids were detected at 315nm (a detector for the visible range was not available). The elution profile for the purification of free astaxanthin consisted of a 0.5% per minute gradient from 80% methanol in water to 100% methanol. This profile was modified for the purification of the euphausiid pigment fraction corresponding to the astaxanthin monoester standard to provide better resolution. A 1.0% per minute gradient was employed from 80% to 90% methanol followed by a 0.5% per minute gradient to 95% methanol. The system was isocratic at 95% methanol for 10min. and was then taken to 100% methanol with a 1.0% per minute gradient. The pigments were loaded onto the column in 100% methanol as they were insoluble in 80% methanol. Pigments which were more hydrophobic than the monoester standard could not be run using this system and therefore, no HPLC data is available.

The mass spectrometer used was a Finnegan 3300 GSMS system. The carotenoids were analyzed through positive and negative chemical ionization at 200-300 ° C.

DIETARY EFFECTS ON PIGMENT CONTENT--Experimental design

Euphausiids were collected on November 3, 1988. Sufficient individuals were frozen to provide three replicate samples at time zero. Approximately 300 live euphausiids were collected and placed into three separate containers. Each container was filled with

approximately 30L of 0.7 μm filtered sea water. The euphausiids were maintained at 9 ± 2 °C throughout the experiment.

Two days after collection the dead animals were removed and feeding was initiated. One group, consisting of 88 individuals, were fed *Artemia salina* nauplii which were less than 5 days old. Another group (69 individuals) was fed the diatom *Skeletonema costatum* and the third group (83 individuals) was fed the naked dinoflagellate *Amphidinium carterae*. The concentration of food was maintained at 900-1200 μg carbon per litre as recommended by Ross (1981) (Append. II).

The euphausiids were transferred to fresh sea water every 10 days and dead animals were removed daily. Half the animals were removed and frozen (-80 °C) after 31 days and the remaining euphausiids were frozen after an additional 12 days (T=43 days). Sufficient animals could not be maintained in the laboratory to provide replicate samples at the two sampling times and therefore, a statistical analysis of the carotenoid content over the test period was not possible.

The carotenoid pigments present in each of the diets were also characterized. The brine shrimp nauplii were analyzed using the same procedures described for the euphausiids.

The two species of phytoplankton were grown at 15 °C with a 12:12hr light-dark photo-period and analyzed during the logarithmic phase of their growth curve. Three litres of each culture were concentrated using a flow-through centrifuge. The pellets were combined and washed with distilled water. The pellet was then ground in 100% acetone using a motorized teflon pestle and glass mortar. The pigments were transferred to ether and taken to dryness as previously described. The pigments were dissolved in pet. ether, loaded onto a silica plate and developed in pet. ether, ethyl acetate and diethyl amine (58:30:12 by volume) (Riley & Wilson, 1965).

RESULTS

LIFE CYCLE

The majority of the population was spawning by April. The females collected at this time were what Ross, et al. (1982) described as stage IV females: the thorax was swollen due to the ovary filling the thoracic cavity and pushing aside the other organs. Two weeks later, May 4, 1988, the adult population appeared very much reduced in numbers and larvae were present. Although the adult population (1987 cohort) became scarce by July, there were still some adults present throughout the summer and larvae were noted again on October 6, 1988.

The larvae, first noted and collected on May 4, 1988, had already reached the furcilia stage. When the larvae molt from calyptopis 3 to furcilia I, the relatively large compound eyes become free from the carapace (Ross, 1981) making the larvae much more visible. These larvae (1988 cohort) had an average individual dry weight of 0.17mg (table 1). The larvae increased in size throughout the summer reaching a maximum dry weight of 6.8mg in November (figure 2A). The average individual dry weight of the cohort began decreasing starting the end of November and became significantly reduced at the 0.05 significance level in February when the average dry weight was 3.2mg. From February 9, 1989 to April 3, 1989 the euphausiids showed a small increase in average dry weight (3.2 to 4.2mg). During the next month (April-May) the average individual dry weight increased dramatically as the euphausiids became reproductive. The same rapid increase in dry weight was observed the previous spring (March-April, 1988) for the 1987 cohort (figure 2A).

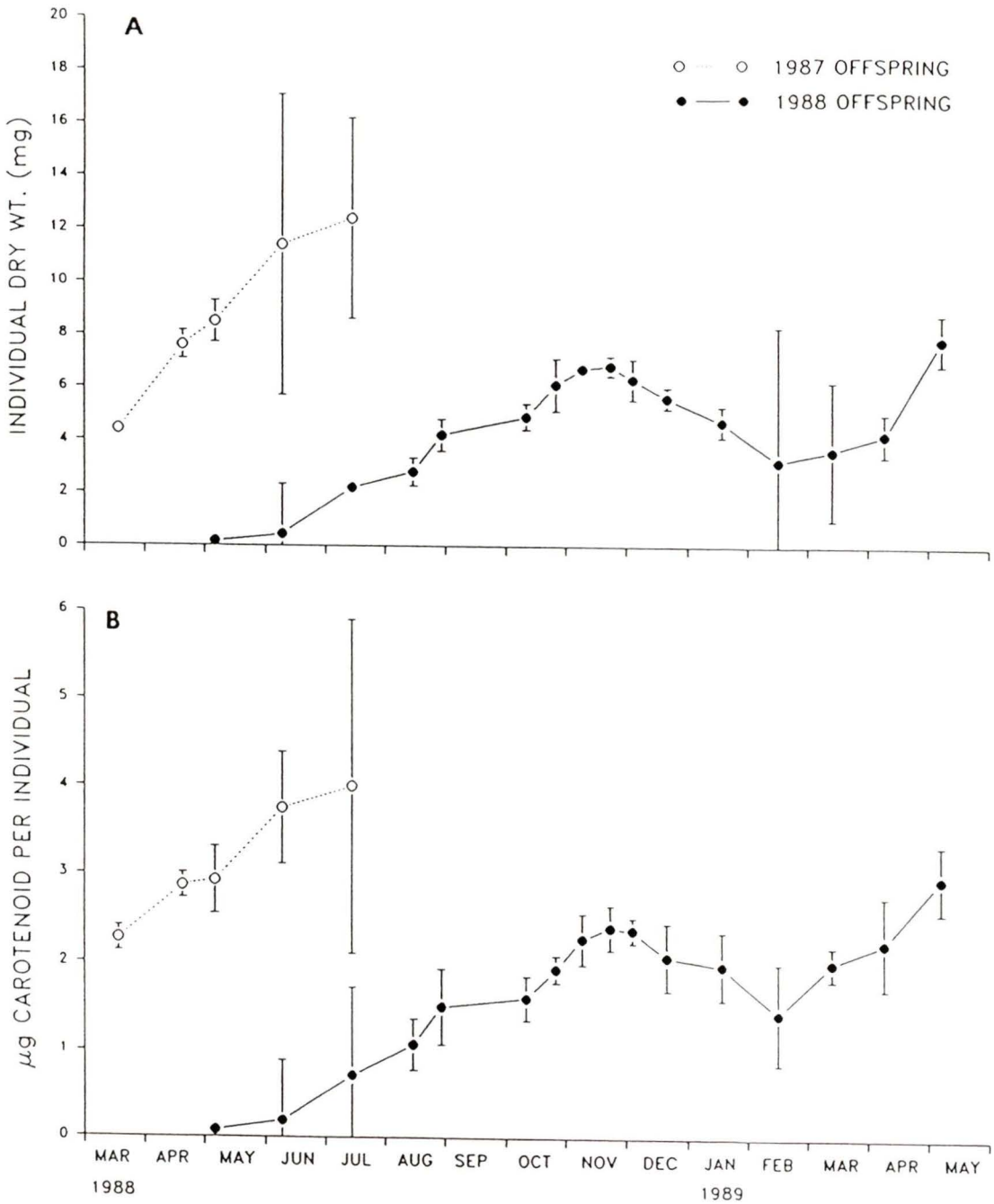


Figure 2: **A.** Average individual dry weight of *E. pacifica* determined at monthly intervals over a 15 month period. **B.** Average individual carotenoid content of *E. pacifica*. All error bars represent 95% C.L.

The average dry weight of sexually immature and mature males and females were compared on March 8 and May 1, 1989, respectively. The dry weights of males and females collected in March were not significantly different at the 0.05 significance level. However, the average dry weight of the females (7.9 ± 0.8mg) collected in May was significantly greater than that of the males (6.1 ± 0.3mg). The data point representing the dry weight of euphausiids collected in May, 1989 (figure 2A) represents only the females. The sexually maturing males and females were not separated during the spring of 1988 and this may partially account for the large 95% C.L. for this population during June and July, 1988.

SEASONAL VARIATION IN THE TOTAL CAROTENOID CONTENT

The carotenoid pigments were unevenly distributed throughout the euphausiid body. To gain some knowledge of the carotenoid distribution the eyes were separated from the body and the carotenoid content of the two fractions were determined for a discrete sample date (Dec. 16). Two pooled samples revealed the eyes to contain 53% and 60% of the carotenoid pigments. Also, all the carotenoid pigments observed on TLC when the euphausiids were analyzed as whole organisms were present in both the eyes and body fractions and in the same relative proportions.

The amount of carotenoid per individual generally varied in parallel with the dry weight (figure 2). The amount of carotenoid pigments in the 1987 euphausiid cohort increased throughout the spring and early summer on an individual basis as did their average dry weight (figure 2). The individual carotenoid content of the offspring (1988 cohort) increased throughout the summer, reaching a maximum in November. The individual carotenoid content decreased over the winter reaching a minimum in February as was

observed for the dry weight measurement (figure 2). The average individual carotenoid content of the population then increased throughout the spring of 1989 as the euphausiids became sexually mature; a pattern also observed during the previous spring (figure 2).

The weight specific carotenoid content of *E. pacifica* varied from an average of 306 to 536 μg carotenoid/g dry weight over the 15 month period. The water content of *E. pacifica* was 86%. Using the conversion factor of 7.14g wet weight per g dry weight, the weight specific carotenoid content can be expressed in terms of the wet weight for comparison purposes (42.0-75.6 μg carotenoid/g wet wt.).

The weight specific carotenoid content also showed a seasonal variation but it was quite distinct from that observed for both the dry weight and the individual carotenoid content (figure 3). The large increase in dry weight observed between March 17 and April 18, 1988 (figure 2A) corresponded to a dramatic decrease in the weight specific carotenoid content (figure 3). Therefore, although the euphausiids were still accumulating carotenoids on an individual basis, the accumulation of carotenoids did not keep pace with the overall increase in dry weight. The weight specific carotenoid content of the sexually maturing 1987 cohort continued to decrease throughout the spring (figure 3).

The growth rate of *E. pacifica* was estimated over the sample intervals to see how the growth rate affected the weight specific carotenoid content. The growth rate was expressed as the percent increase in dry weight per day, relative to the average dry weight of the euphausiids at the beginning of each sample interval (table 1). During the above mentioned increase in dry weight, the weight specific carotenoid content decreased most dramatically when the growth rate was greatest (table 1; figure 3).

The weight specific carotenoid content of the furcilia larvae collected on May 4 and June 6, 1988 showed large variability. Due to the large number of individuals required for one sample, only two replicates were analyzed for each of these sample dates. A sample

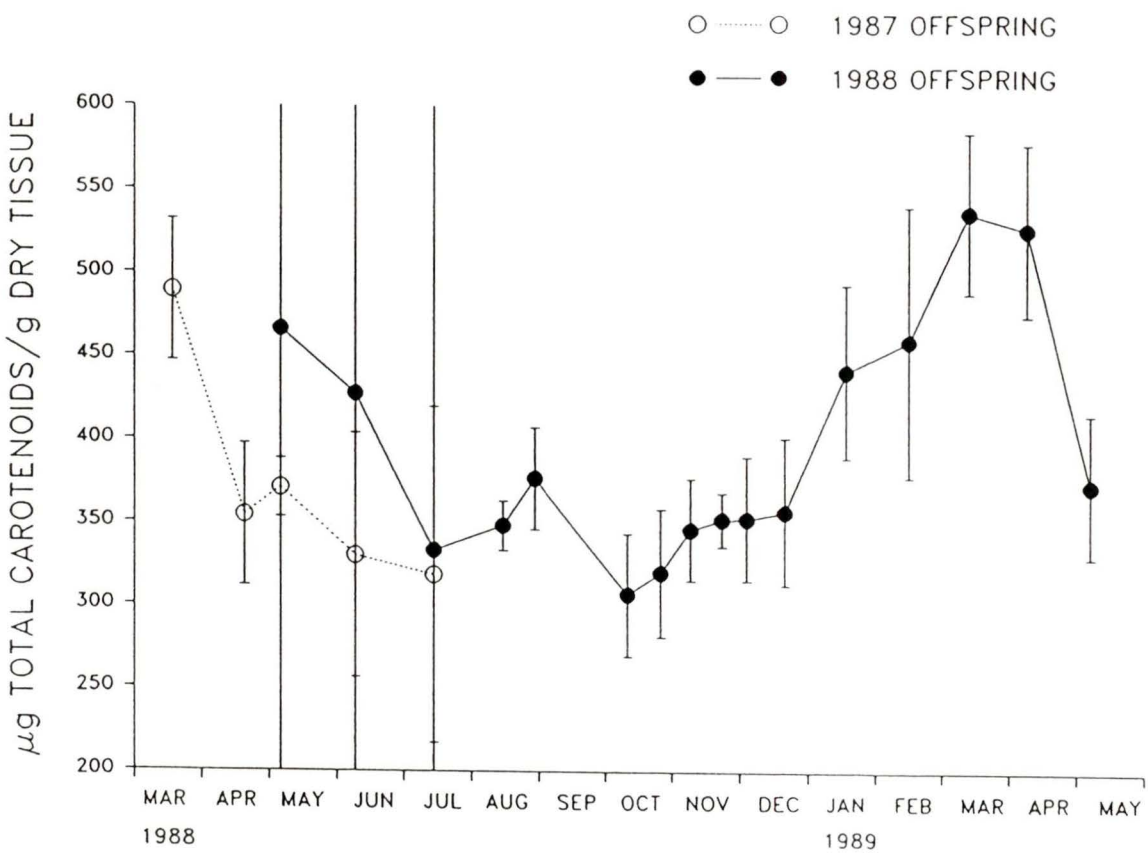


Figure 3: Seasonal fluctuations in the average weight specific carotenoid content of *E. pacifica*.

size of two, combined with isolated laboratory problems, resulted in very large 95% C.L. (figure 3). On a statistical basis therefore, these data are not very illuminating. However, the sample means do suggest a weight specific decrease in carotenoids from May to July. This decrease also occurred during a time when the euphausiid larvae had a high growth rate (table 1). After July, the growth rate decreased and remained relatively constant through to December (table 1) as did the weight specific carotenoid content (figure 3). The weight specific carotenoid concentration increased from December 16, 1988 through to February 9, 1989 while the dry weight and individual carotenoid content were decreasing (figures 2&3).

As described previously, the euphausiid population reached a minimum dry weight in February and showed only a small increase in dry weight from February to April. During this time, the euphausiid population actually accumulated carotenoid pigments as evidenced by a dramatic increase in the weight specific carotenoid content (figure 3) and more importantly, by the significant increase ($p = 0.05$) in the individual carotenoid content (figure 2B).

Starting April 3 to May 1, 1989 the weight specific carotenoid content of the euphausiid population decreased at the same time their specific growth rate increased (figure 3 and table 1). The same phenomenon occurred during the spring of 1988.

The carotenoid content of sexually immature and mature male and female euphausiids were compared as was the dry weight described above. When sexually immature, the weight specific and individual carotenoid contents of the two sexes were not significantly different. When spawning males and females were compared (collected May 1, 1989) no significant difference was observed in the individual carotenoid content, but the weight specific content of females (375 μg carotenoid/g dry wt.) was significantly reduced compared to males (440 μg carotenoid/g dry wt.). It should be noted that the weight specific carotenoid value for

the May, 1989 sample date (plotted in figure 3) represents only the females. The data points plotted on figure 3 for the 1987 cohort during the spring of 1988 was an average of males and females which may account for the large 95% confidence limits during June and July, 1988 (data for figures in appendix III).

The average individual dry weight and the individual of carotenoid content were found to be related by a power function. A regression analysis of the log of the individual carotenoid content verses the log of the individual dry weight resulted in a slope of 1.04 and an r value of 0.99 (figure 4).

CHARACTERIZATION/IDENTIFICATION OF CAROTENOID PIGMENTS

RESULTS FROM SILICA TLC

The R_f values of the various carotenoid pigments present in *E. pacifica* are listed in table 2. Three main carotenoid fractions were produced following development in the first solvent system (dichloromethane, ethyl acetate 80:20 v/v). Each of these fractions corresponded exactly with the free astaxanthin, the astaxanthin monopalmitate and the astaxanthin dipalmitate standards (table 2). The euphausiid carotenoid fraction corresponding to the astaxanthin standard was again inseparable from the standard in the second dimension (hexane:acetone 70:30 v/v) (plate I; table 2).

The euphausiid carotenoid band corresponding to the mono-ester was separated into two distinct bands in the second dimension (plate I; table 2). The most hydrophobic of these two bands co-migrated with astaxanthin monopalmitate (plate 1; table 2). Lastly, the band corresponding to the diester standard in the first dimension, separated into three

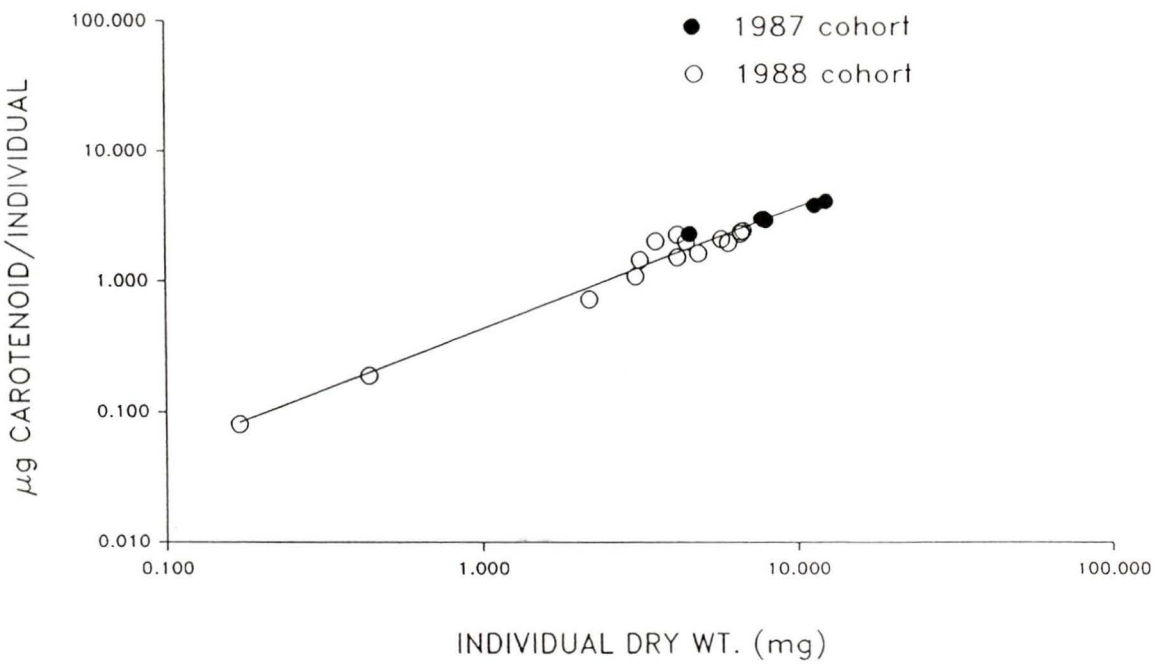


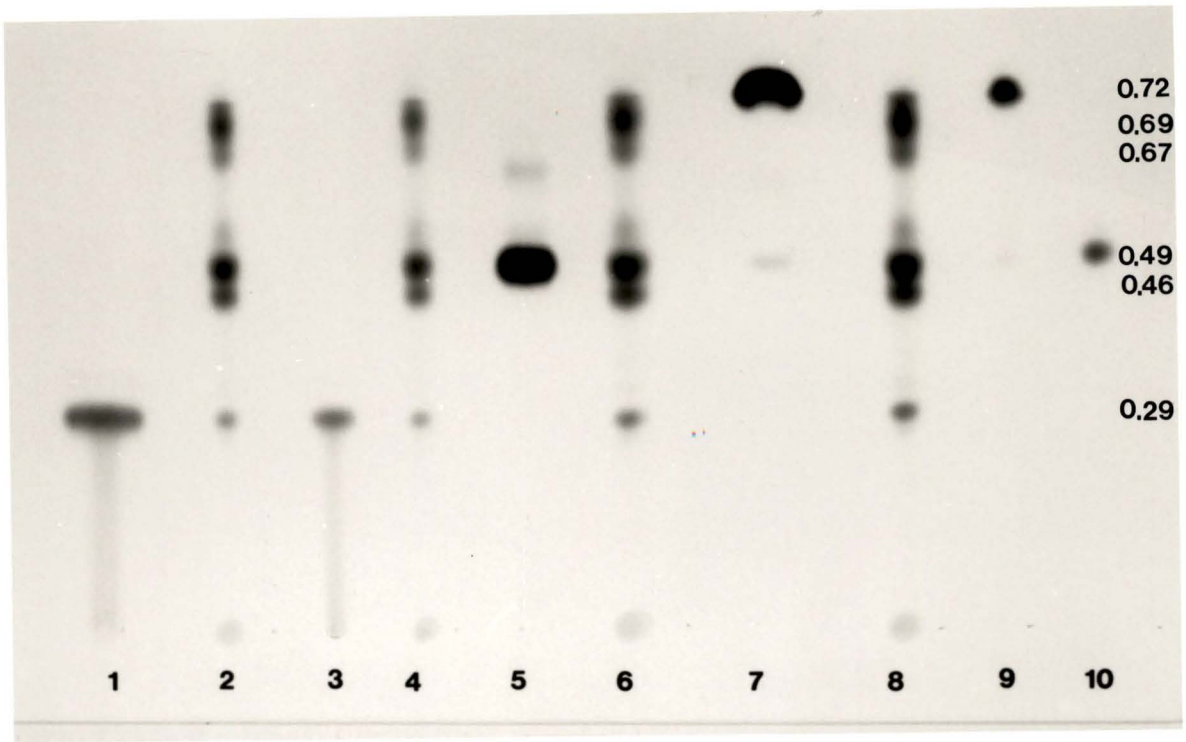
Figure 4: The log relationship of the individual carotenoid content against the individual dry weight. A regression analysis found the slope to equal 1.04 and the r value to be 0.99.

Table 2: The retention values of carotenoid pigments isolated from *E. pacifica* and *A. salina* as well as the carotenoid standards on silica TLC and reverse-phase HPLC.

Source	Pigment	R_f values 1st Dimen.	R_f values 2nd dimen.	Retention time on RP-HPLC (min)
Hoffmann-La Roche	Astaxanthin	0.33	0.29	34.4
	Asta. Mono-palmitate	0.61	0.49	47.0
	Asta. Di-palmitate	0.89	0.72	N/D[1]
E. pacifica	Astaxanthin	0.33	0.29	34.4
	Monoester	0.61		
	Monoester #1		0.46	39.8-43.6
	Monoester #2		0.49	46.2-47.0
	Diester	0.89		
	Diester #1		0.65	N/D
	Diester #2		0.69	N/D
	Diester #3		0.72	N/D
A. salina	Canthaxanthin	0.62	0.47	36.2

[1]N/D-no data

Plate I: Relative mobilities of carotenoid standards and carotenoid fractions isolated from *E. pacifica* and separated in the 2nd dimension on silica TLC (30% acetone in hexane). Lanes 1 and 2 are astaxanthin standard (R_f=0.29). Lanes 5 and 10 represent the monopalmitate standard (R_f=0.49) and lanes 7 and 9 represent the astaxanthin dipalmitate standard (R_f=0.72). Lanes 2, 4, 6 and 8 represent all the carotenoid fractions from *E. pacifica,* separated on TLC . The R_f values of each carotenoid fraction is reported along the right margin.


0.72
0.69
0.67
0.49
0.46
0.29
1
2
3
4
5
6
7
8
9
10

bands of similar but distinct R_f values in the second dimension (plate 1; table 2). The most hydrophobic of these three bands had the same mobility as the dipalmitate standard.

RESULTS FROM REVERSE-PHASE HPLC

The elution times of the standards and the unknowns from RP-HPLC are listed in table 2. The astaxanthin standard was eluted after 34.4 min., identical to the elution time of the most polar carotenoid pigment isolated from *E. pacifica* and presumed to be astaxanthin from the TLC results (figures 5A&B).

The elution profile of the monoester band from *E. pacifica*, separated during the first dimension on TLC was quite complex (figure 5C). The two groups of peaks presumably corresponded to the two bands obtained after the second dimension on TLC. Monoester #2 from *E. pacifica* (the most hydrophobic) had the same retention time on RP-HPLC as the monopalmitate standard (figure 5C&D).

MASS SPECTROSCOPIC DATA

The free astaxanthin standard was the only standard which gave a molecular ion. The peak was obtained only when the carotenoid was loaded in its crystallized form (as opposed to being dissolved in methylene chloride). It is interesting that a substantial amount of the standard was required to get the molecular ion and that no other peaks were noted in the upper portion of the spectrum except at M-91 and M-105 (table 3). There was also an unidentified peak at 286 mass units. The astaxanthin monoester and diester standards were too large for the machine to detect a molecular ion and only two peaks which could not be identified were observed at 286 and 258 mass units. The pigments isolated from *E. pacifica*

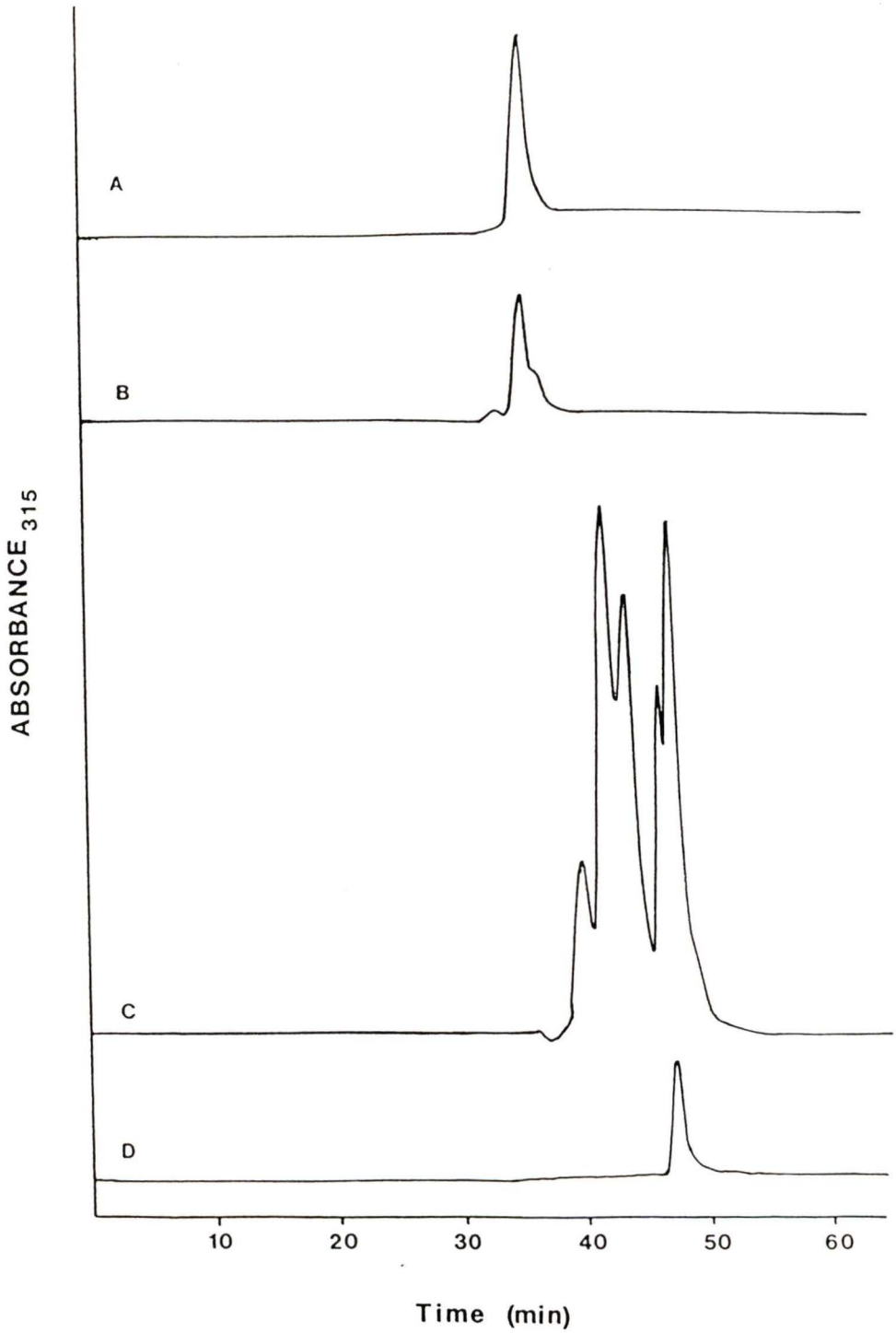


Figure 5: A trace of the elution profile of various carotenoid pigments from RP-HPLC using methanol and water as the mobile phase. **A**. The most polar carotenoid isolated from *E. pacifica* obtained after the first dimension on TLC. **B**. Astaxanthin standard obtained from Hoffmann-La Roche Inc. **C**. The carotenoid fraction isolated from *E. pacifica* which co-migrated with the astaxanthin monopalmitate in the first dimension on TLC. **D**. Astaxanthin monopalmitate standard obtained from Hoffmann-La Roche Inc.

Table 3: Mass spectrometric data of carotenoid standards, and carotenoid fractions isolated and purified from *E. pacifica* and *A. salina* nauplii. The astaxanthin standard gave a molecular ion (M + 1) and peaks at M-91 and M-105. A molecular ion was also observed for canthaxanthin (565), isolated from *A. salina* nauplii. Canthaxanthin showed a loss of 92 and 108 mass units corresponding to peaks at 472 and 456, respectively. None of the peaks observed for the pigments isolated from *E. pacifica* could be identified. However, the peaks at 547 and 456 as well as those at 578 and 487, observed only in the monoester fraction, represent the loss of 91 mass units.

Source	Pigment	Molecular weight of the main fragments
Hoffmann-La Roche	Astaxanthin	597 506 492 286
	Monopalmitate	286 258
E. pacifica	Astaxanthin	547 456 286 258
	Monoester	682 597 578 547 487 456 258
A. salina	Canthaxanthin	565 564 472 456

and purified on RP-HPLC did not give any diagnostic data when run on the mass spectrophotometer. None of the peaks listed in table 3 for the free astaxanthin and the astaxanthin monoester fractions isolated and purified from *E. pacifica* could be identified. It was, however, interesting that the peaks observed at 286 and 258 for the standards were also present in those pigments isolated from *E. pacifica*. Also, many of the peaks observed for astaxanthin and its monoesters from *E. pacifica* were the same (table 3). Three of the monoesters separated on RP-HPLC were analyzed on the mass spectrometer and gave very similar fragment patterns and, therefore, only one monoester fragment pattern was listed in table 3. The monoester fraction gave peaks at 682 and 597, the latter being the molecular weight of astaxanthin. Strong peaks at 547 and 456, corresponding to the loss of 91 mass units, were observed for both astaxanthin and its monoester (table 3). The monoester fraction showed an additional loss of 91 mass units with peaks at 578 and 487 (table 3).

The carotenoid pigment isolated and purified from *A. salina* nauplii gave a strong molecular ion at 564 which is the molecuar weight of canthaxanthin. There were however, no other peaks of diagnostic value--only the loss of 92 and 108 mass units (table 3).

TLC and RP-HPLC results suggest that the three main carotenoid fractions present in *E. pacifica* listed in order of increasing hydrophobicity, are astaxanthin, its mono- and diesters. To facilitate a discussion of the data, the two fractions originating from the proposed monoester band will be referred to as monoester #1 and #2 corresponding to the carotenoids in order of increasing hydrophobicity (increasing mobility on silica TLC) (table 2). Likewise, the three bands originating from the diester band, will be referred to as diester #1, #2 and #3 in order of increasing hydrophobicity.

CAROTENOID COMPOSITION OF *E. PACIFICA*

The three main carotenoid pigments in *E. pacifica*, identified as free astaxanthin, its mono- and diesters, comprised 85-90% of the total pigment content. The relative contribution of each of the three main carotenoid fractions remained remarkably constant throughout the sampling period. Free astaxanthin contributed only 5% of the total carotenoid content (figure 6A). Statistical analysis showed the sexually maturing euphausiids and the furcilia larvae to contain more free astaxanthin than animals collected at other times of the year (figure 6A).

The relative contribution of the monoester fraction in *E. pacifica* was 49% and did not vary significantly throughout the sample period (figure 6B). The diester fraction averaged 40% of the total carotenoid content. Only the youngest furcilia larvae appeared to have less diester than animals collected at all other times of the year (figure 6C). The three or four pigments which were combined and collectively called the unknown fraction contributed between 5% and 10% of the total (figure 6D).

The weight specific quantity of each of these fractions (figure 7A-D) displayed the same seasonal variation as did the total weight specific carotenoid content (figure 4). All the fractions were at a maximum in early spring, prior to the rapid growth phase (table 1). A significant decrease in all carotenoid fractions was observed during the spring of 1988 which corresponded to the time of rapid growth (table 1) and spawning.

The data obtained from the early larval samples showed considerable variation as indicated by the 95% C.L. (figure 7A-D) due to the variation observed in the total carotenoid content (figure 4). Therefore, on a statistical basis, there were no differences to note. However, the sample means indicate that the weight specific concentration of all of

Figure 6: The relative contribution of the four carotenoid fractions separated during the first dimension on silica TLC. **A.** Free astaxanthin **B**. Astaxanthin monoester **C**. Astaxanthin diester **D**. A pooled sample of approximately four unidentified pigments.

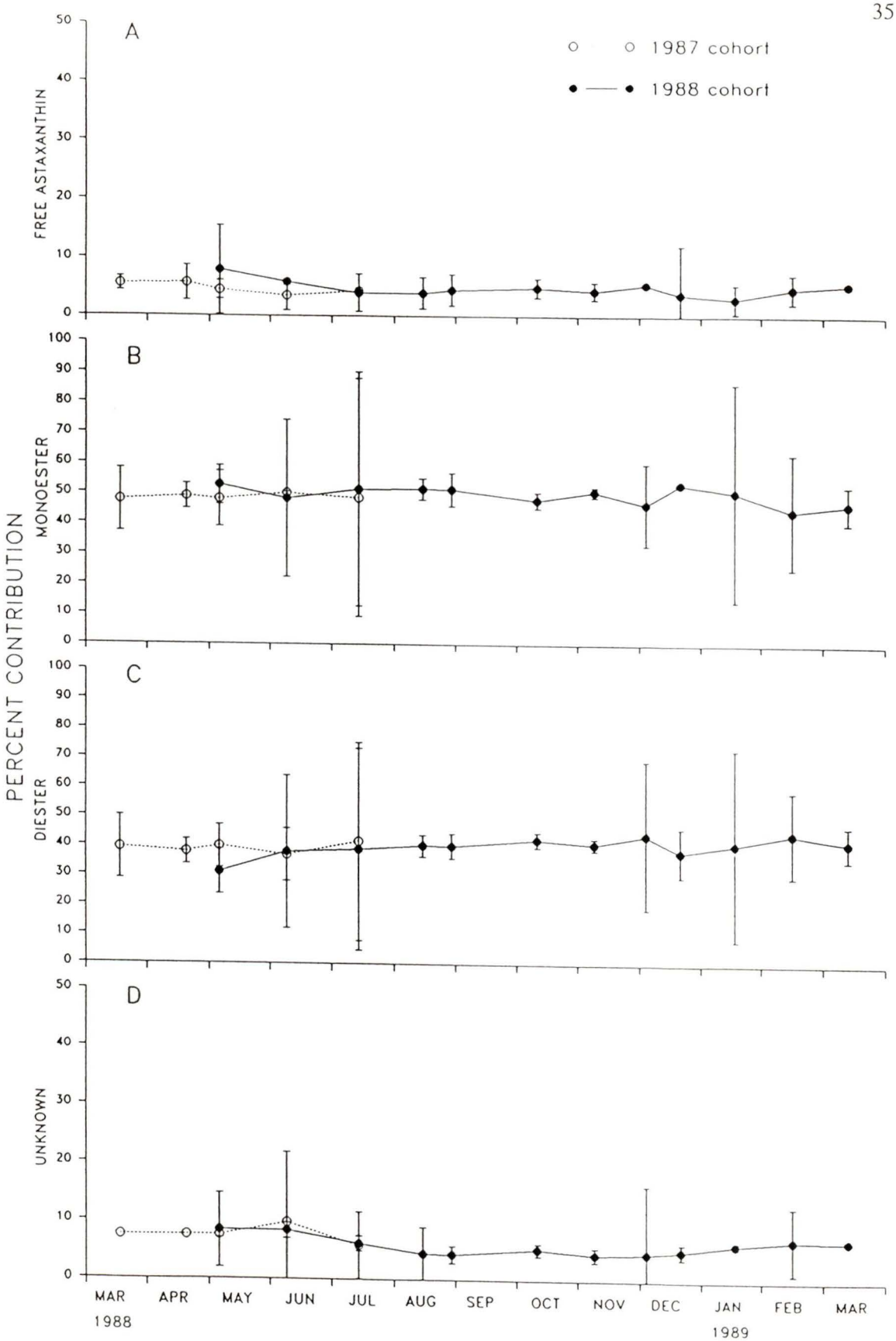
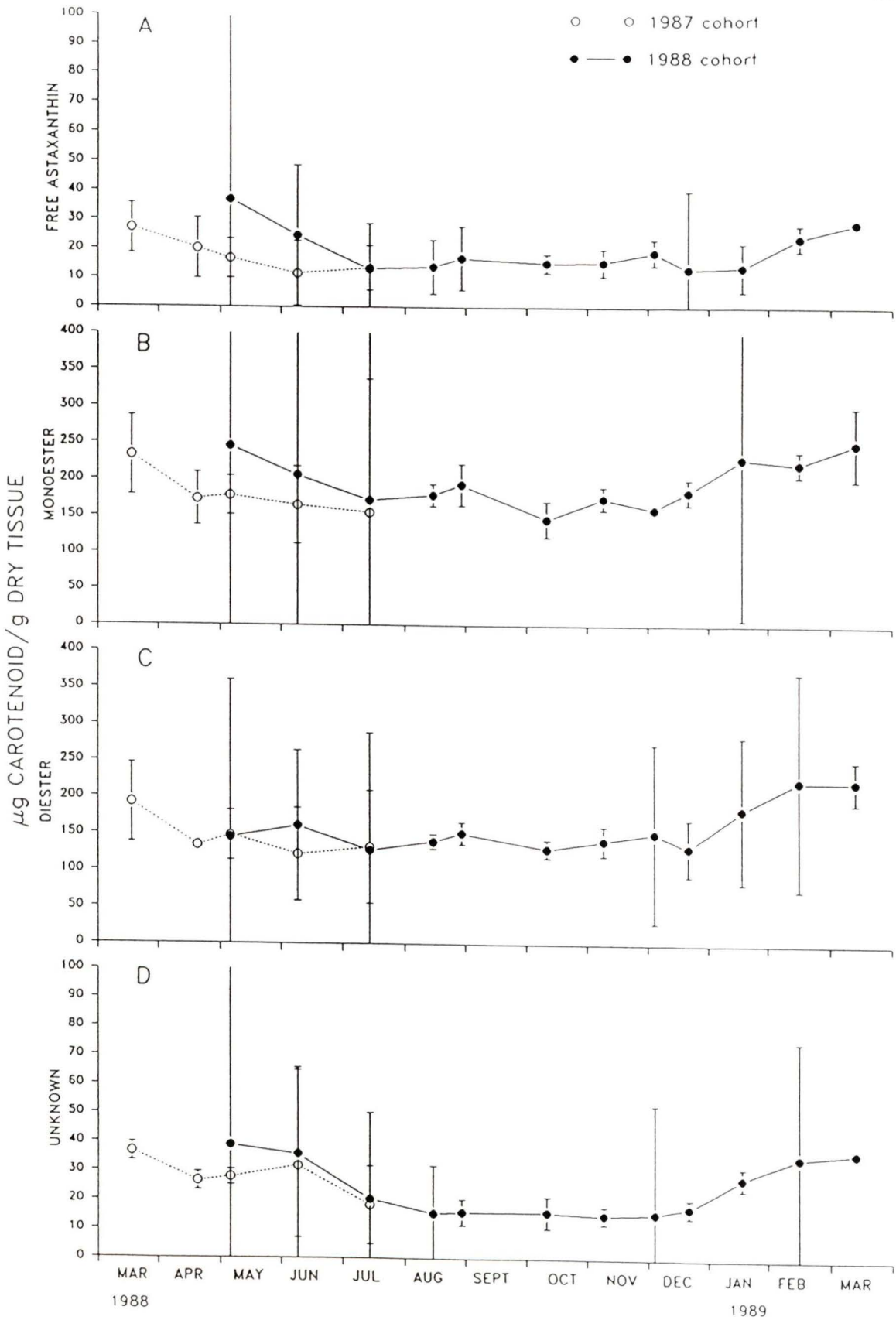

A
1987 cohort
1988 cohort
FREE ASTAXANTHIN
B
MONOESTER
C
DIESTER
D
UNKNOWN
PERCENT CONTRIBUTION
MAR
1988
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
JAN
1989
FEB
MAR

Figure 7: The weight specific concentration of the four carotenoid fractions present in *E. pacifica* separated during the first dimension on TLC. **A**. Free astaxanthin **B**. Astaxanthin monoester **C**. Astaxanthin diester **D**. The unidentified pigments.


A
B
C
D
1987 cohort
1988 cohort
FREE ASTAXANTHIN
MONOESTER
DIESTER
UNKNOWN
μg CAROTENOID/g DRY TISSUE
MAR
APR
MAY
JUN
JUL
AUG
SEPT
OCT
NOV
DEC
JAN
FEB
MAR
1988
1989

the carotenoid fractions decreased from May to July when the growth rate of the larvae was greatest (table 1), with the exception of the diester fraction (figure 7A-D).

MONOESTERS

Mono-ester #1, the more polar of the two, contributed between 13% and 21% to the total carotenoid content while mono-ester #2 varied from 28% to 37% of the total (figure 8). The percent contribution of mono-ester #1 was greater in the euphausiids caught during the summer and late fall (June to November, 1988) than during the spring of 1988 and 1989 (figure 8A). Mono-ester #2, although showing some small fluctuations, remained relatively constant in terms of its percent contribution throughout the sample period (figure 8B).

The weight specific quantity of mono-ester #1 did not vary over the sample period (figure 9A). The quantity of mono-ester #2 displayed the same seasonal pattern observed for the weight specific carotenoid content. Mono-ester #2 must, therefore, be solely responsible for the seasonal variations observed in the weight specific quantity of the total mono-ester fraction (figure 7B).

DIESTERS

The most polar of the three di-esters, #1, contributed between 5% and 8% to the total carotenoid content and this contribution did not fluctuate significantly throughout the sample period (figure 10A). Diesters #2 and #3 contributed equally to the carotenoid content, making up between 14% and 20% of the total pigment content (figure 10B&C).

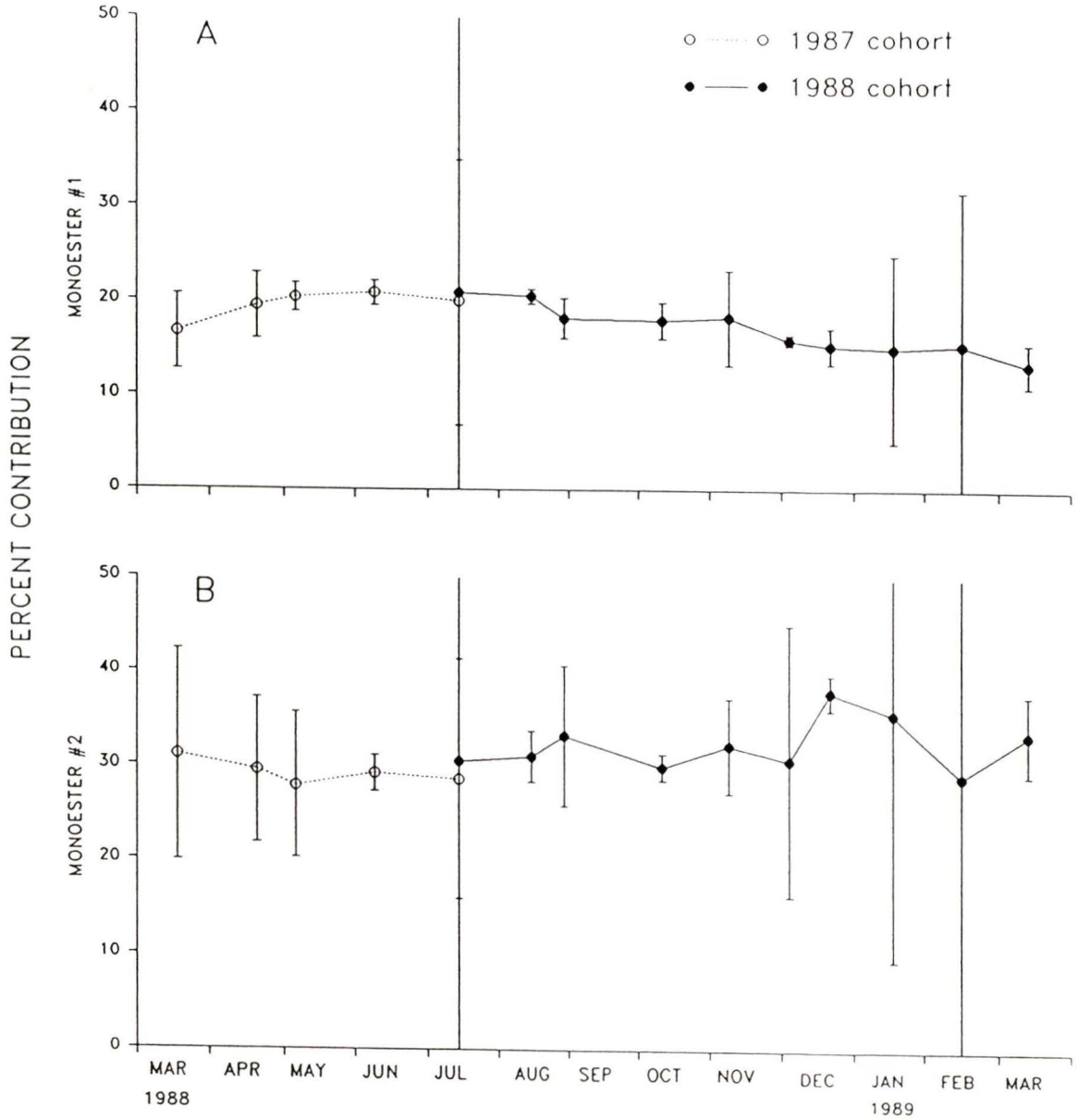


Figure 8: The percent contribution, relative to the total carotenoid content, of the two monoesters separated during the second dimension on TLC . A. The more polar monoester (R_f=0.46) called monoester #1. B. The more hydrophobic monoester (R_f=0.49) called monoester #2.

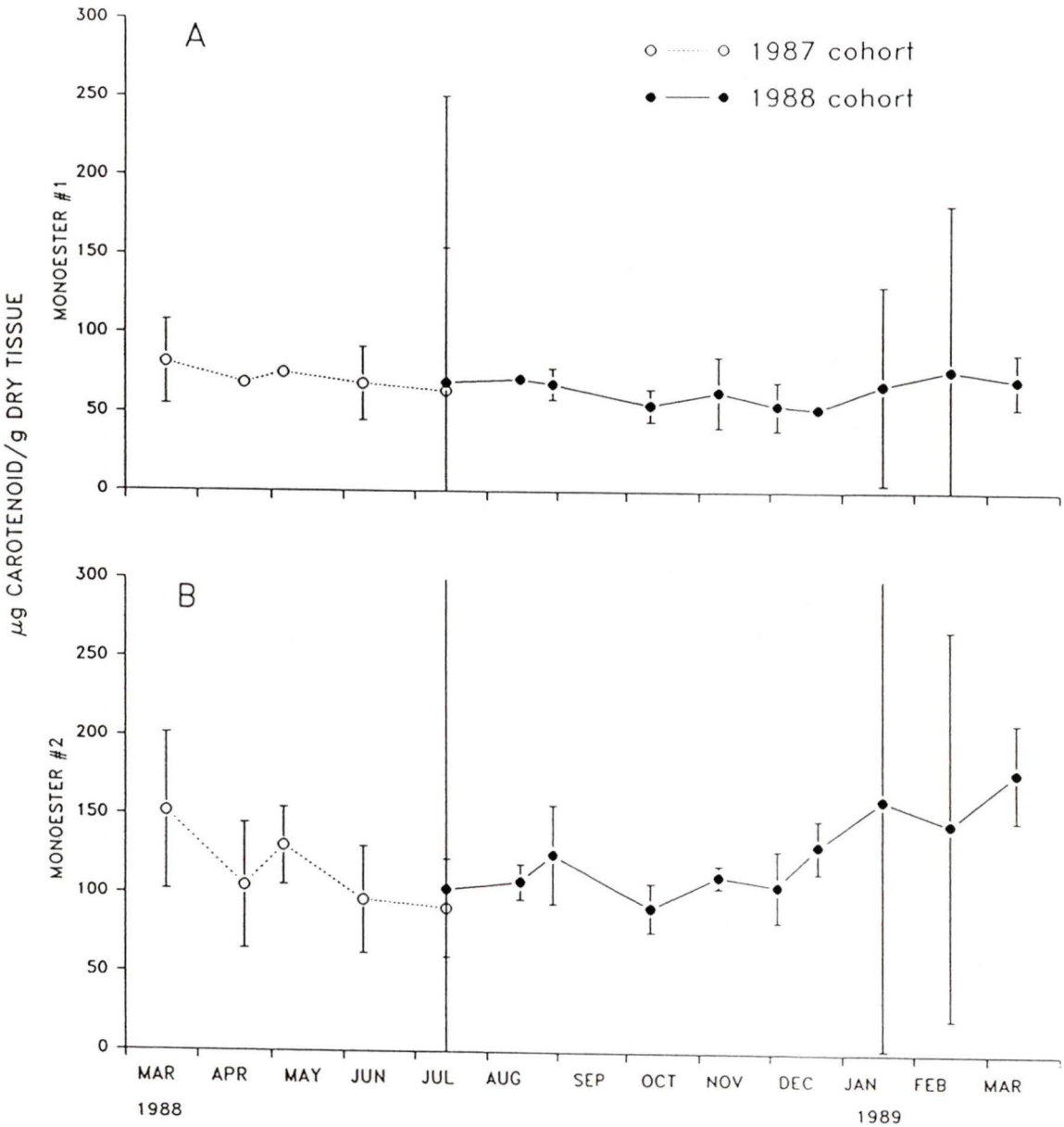


Figure 9: The weight specific concentration of the two monoesters separated during the second dimension on TLC. **A**. Monoester #1 **B**. Monoester #2.

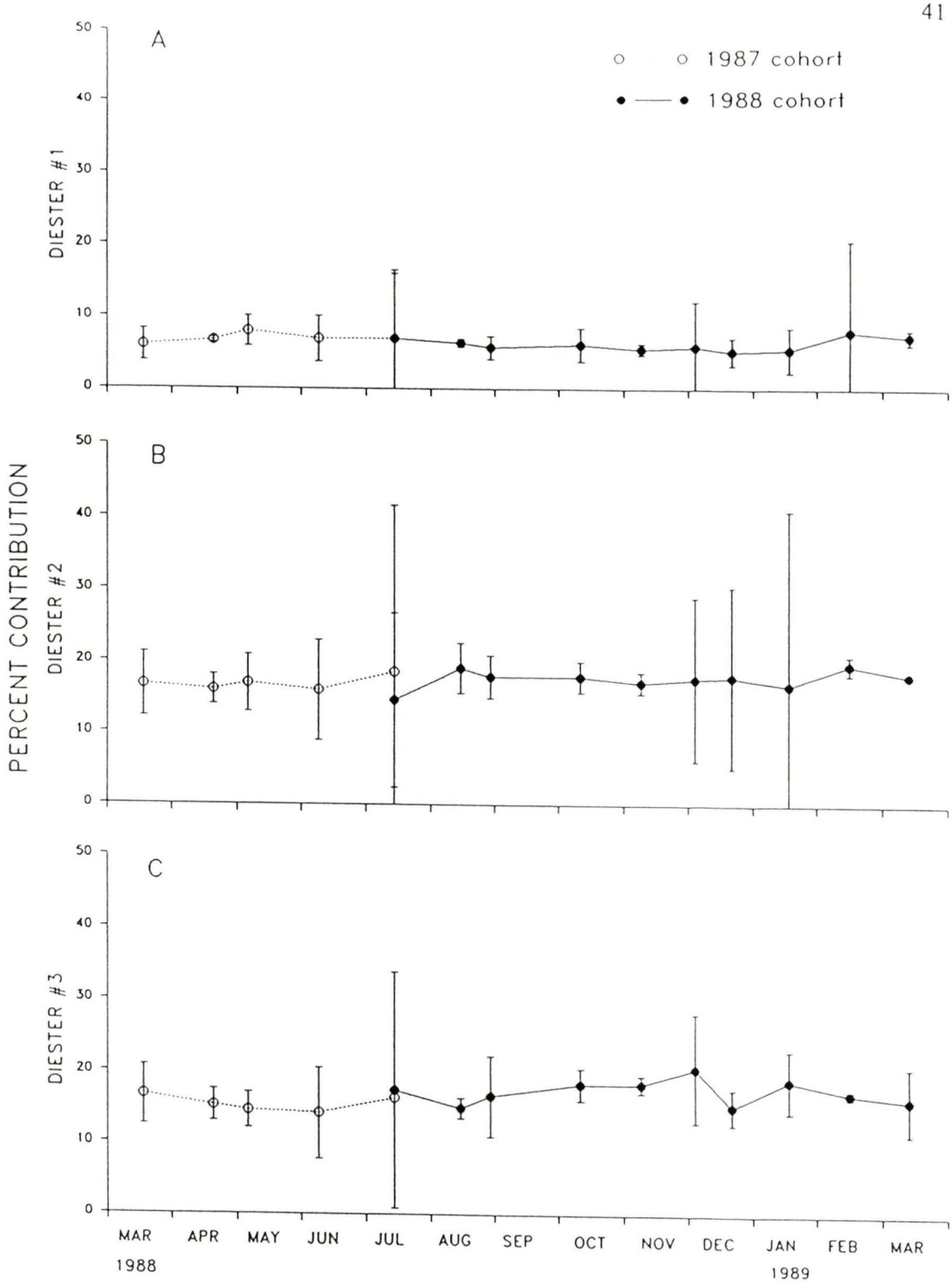


Figure 10: The percent contribution, relative to the total carotenoid content, of the three diesters separated in the second dimension on TLC. **A-C** represent diesters numbered in order of increasing hydrophobicity.

On a weight specific basis, all three diesters contributed to the summer minimum and early spring increase in astaxanthin diester (figure 11A,B&C)(data for figures are in appendix III).

DIETARY EFFECTS ON THE PIGMENT CONTENT OF *E. PACIFICA*

CHARACTERIZATION OF DIETARY PIGMENTS

It is well established that the primary carotenoid pigment in *Artemia salina* is canthaxanthin (Davies et al., 1965, 1970; Hsu et al., 1970; Krinsky, 1965; Soejima et al., 1980). The R_f values of canthaxanthin isolated from *A. salina* in the two solvent systems on TLC are listed in table 2. Canthaxanthin had the same approximate mobility on silica as the astaxanthin mono-ester. Its retention time on RP-HPLC, using the same elution profile as was used for the astaxanthin mono-ester fraction from *E. pacifica*, was approximately 10min less than the retention time of the most polar monoester (table 2). Canthaxanthin, isolated and purified from *A. salina*, was the only pigment which gave a molecular ion on the mass spectrometer and thus the only pigment which could be positively identified.

The R_f values of the xanthophylls and carotenes present in the two algal species are listed in table 4. Although the overall pigment compositions of the two species were quite similar, the R_f values of the xanthophylls suggested differences. Published data reports *S. costatum* as containing fucoxanthin and neofucoxanthin (Jeffrey, 1961; Stauber & Jeffrey, 1988) and *Amphidinium sp.* as containing peridinin and neoperidinin as their primary xanthophylls (Bunt, 1964; Jeffrey, 1968). The presence of astaxanthin in either species has not been reported .

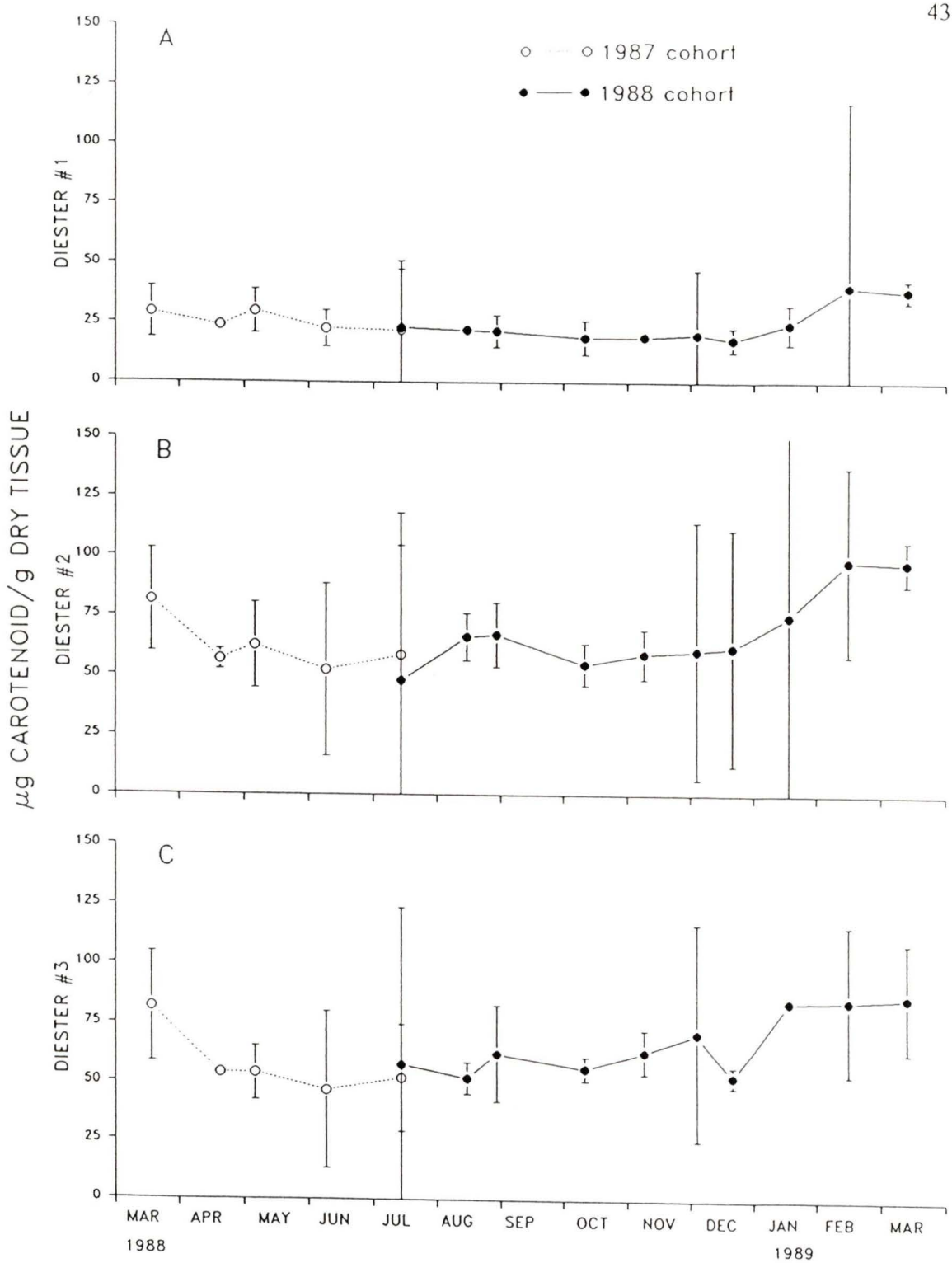


Figure 11: The weight specific concentration of the three diesters present in *E. pacifica*. Plots **A-C** represent diesters numbered in order of increasing hydrophobicity.

Table 4: Retention values of pigments from *A. carterae* and *S. costatum* on silica TLC developed in petroleum ether, ethyl acetate, and diethyl amine (58:30:12 by volume).

Pigments	*S. costatum* R_f values	*A. carterae* R_f values
Xanthophylls	0.33	0.26
	0.36	0.28
	0.44	0.36
	--	0.40
Carotenes	0.80	0.80

FEEDING EXPERIMENTS

The weight specific carotenoid content of *E. pacifica* collected on November 3, 1988 was 346 ± 31 μg carotenoid/g dry tissue. After 31 days of being fed different diets, there were no large differences among the three groups of euphausiids (table 5; figure 12). All three groups of euphausiids analayzed at this time had a weight specific carotenoid content of approximately 400 μg carotenoid/g dry wt (table 5); approximately 50 μg/g dry wt. more than at the initiation of the experiment. However, the amount of carotenoid per individual remained relatively constant, indicating the the increase in the weight specific carotenoid content was due to a decrease in the average dry weight as opposed to an accumulation of carotenoids (table 5).

The average dry weight and the weight specific carotenoid content remained relatively constant during the last 12 days of the experiment (table 5; figure 12). There were, however, a few small differences. For example, the individual and weight specific carotenoid content of the euphausiids maintained on *S. costatum* increased compared to their content at 31 days and compared to the other groups at 43 days of being fed specific diets (table 5; figure 12).

The relative contribution of the four main carotenoid fractions also remained constant throughout the experimental period (figure 13). One small variation to this was the apparent increase in the percent contribution of monoester and the decrease in diester in the group of euphausiids maintained on brine shrimp (figure 13B&C). However, with a sample size of one it is difficult to substantiate such an observation(all data for the feeding experiments are shown in appendix IV).

Table 5: The average dry weight and carotenoid content of *E. pacifica* on a weight specific and an individual basis after being fed specific diets consisting of *A. salina* nauplii, *A. carterae* or *S. costatum* for a period of 43 days.

Time	Diet	Avg. individ. dry wt. (mg)	Wt. specific pig. content (μg/g dry wt.)	Individ. pigment content (μg/ind.)
T=0		6.7	346 ± 31	2.32
T=31	*A. salina*	5.6	401	2.24
	S. costatum	4.9	404	1.98
	A. carterae	4.9	428	2.10
T=43	*A. salina*	5.4	417	2.25
	S. costatum	5.0	470	2.35
	A. carterae	5.5	436	2.40

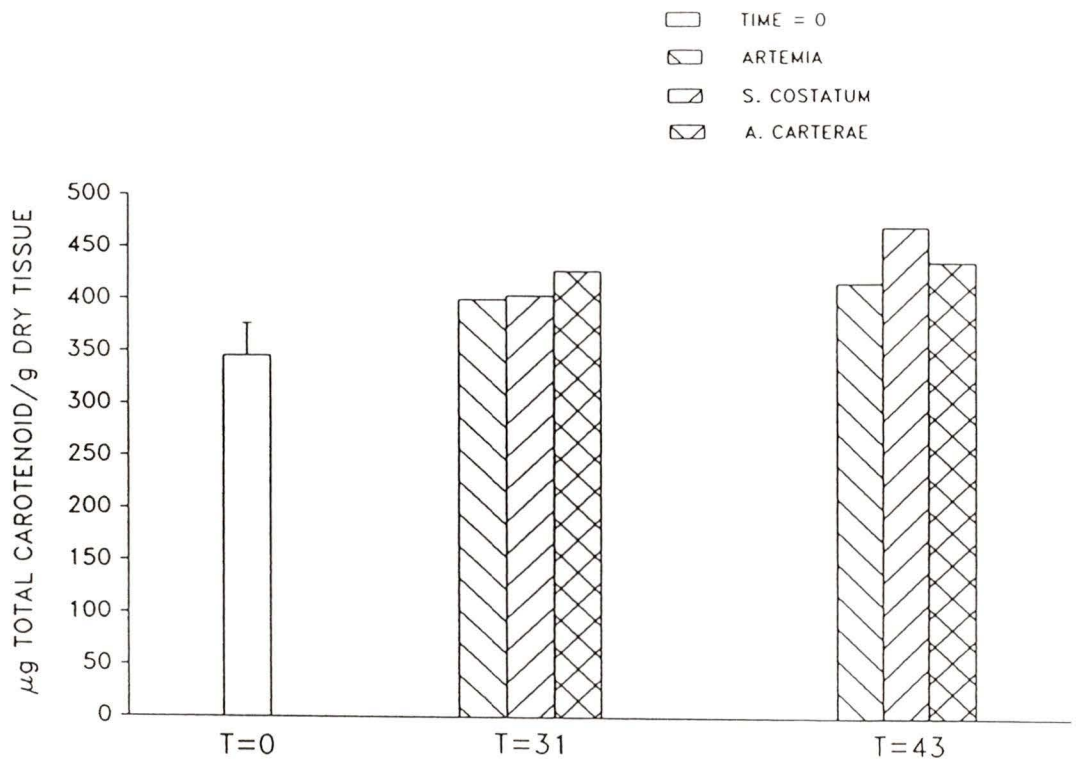
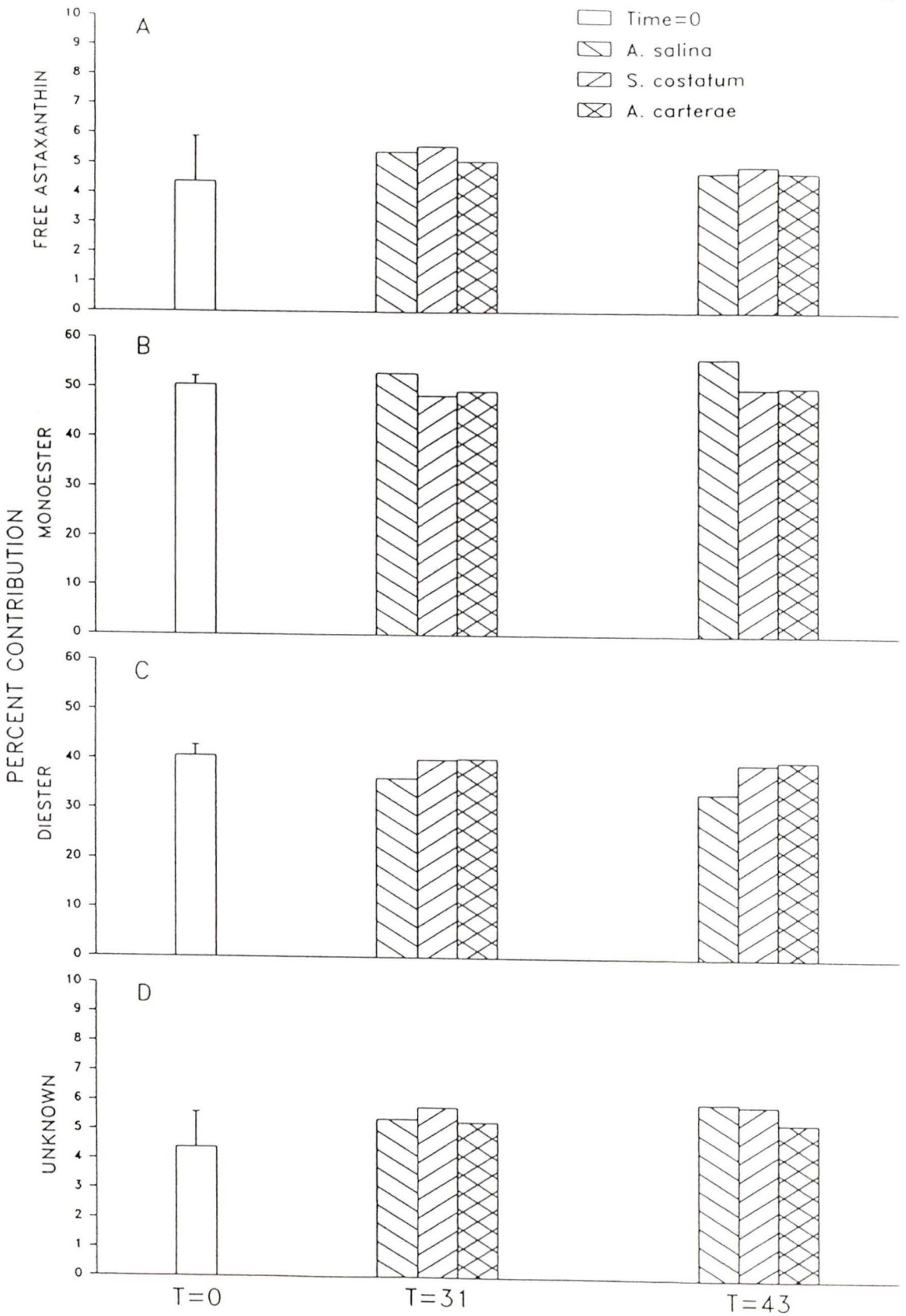


Figure 12: The total weight specific carotenoid content of *E. pacifica* after 0, 31 and 43 days of being fed specific diets.

Figure 13: The relative contribution of the four carotenoid fractions separated during the first dimension on TLC after 0, 31 and 43 days of the euphausiids being fed specific diets.


A
B
C
D
Time=0
A. salina
S. costatum
A. carterae
FREE ASTAXANTHIN
MONOESTER
DIESTER
UNKNOWN
PERCENT CONTRIBUTION
T=0
T=31
T=43

DISCUSSION

Before a discussion of the results can occur, one point must be acknowledged. *Euphausia pacifica* was collected from the same location in Saanich Inlet throughout the study period. However, there is no way to establish with absolute certainty that the euphausiids collected at different times were from the same population. Euphausiids originating outside the inlet may have been carried into the inlet by currents/tidal movements at any point in time. Therefore, it must be acknowledged that any of the observed changes in the dry weight, the individual or the weight specific carotenoid content over time may be due to differences among populations. The data, however, are discussed under the assumption that the same population was sampled throughout time.

LIFE CYCLE

The generation time of *E. pacifica* is approximately one year and was found to be relatively synchronous, giving rise to yearly cohorts. The majority of the 1987 cohort spawned starting in April and continued through to mid May, 1988 as estimated by the presence of stage IV females and furcilia larvae. Furcilia larva were first observed on May 5, 1988. According to Ross (1981), from hatching to the first furcilia stage takes approximately three weeks, thus indicating that spawning must have started in early April.

This, however, was not the only spawning period as larvae were again noted on October 6, 1988. Ross et al. (1982) also reported a second spawning period by *E. pacifica* in northern Puget Sound. Ross et al. attributed the second spawning to individuals hatching early in the spring of the same year and becoming reproductive by late July or early August. Based on my observations, this does not seem probable. The larvae were noted on October 6 making

the approximate time of spawning the beginning of September. Based on the average dry wt of the 1988 cohort there were no individuals of sufficient size at this point in time that could have been reproductive (figure 2A). Therefore, it appears most probable that the larvae noted in early October were from slower developing individuals hatched the previous year, 1987.

The average individual dry weight, as shown by the 95% C.L. in figure 2A, was relatively uniform at any given sample date. The seasonal fluctuations observed in the average dry weight showed a decrease in the average dry weight over the winter starting November, 1988 becoming significant by February, 1989 (figure 2A). The question is whether or not this decrease is real? It is well established that planktonic crustaceans which are primarily herbivorous typically decrease in weight during the winter months due to the utilization of lipid stores when phytoplankton crops are low (Bamstedt, 1976; Falk-Petersen, 1981; Falk-Petersen et al., 1981; Littlepage, 1964; Saether et al., 1986). Therefore, the observed decrease in dry weight of *E. pacifica*, a preferential herbivore, seems quite reasonable. However, it is also possible that the decrease in dry weight was due to a gradual incorporation of the October offspring into the samples. Based on the 95% C.L. it can be argued that the euphausiids collected up to the February sample date were primarily from the spring spawning as the size variation was small (figure 2A). The decrease in dry weight, however, did not become significantly different from the November maximum until February when the variation in the average dry weight was large. The increased variation may have been due to the October spawned euphausiids reaching a size where they were no longer excluded from the samples. These individuals would have been smaller than those hatching in the spring of 1988 and perhaps owing to their smaller size they would have been able to increase in weight despite the low food availability due to their smaller food

requirements. The inclusion of these smaller individuals in the samples may account for the significant decrease in dry weight and the increased sample variation.

Alternatively, the population as a whole may have been decreasing in size due to the utilization of energy stores. The individuals in the population may not lose weight at the same rate and thus large variations in the average dry weight would be observed by the end of the winter.

SEASONAL VARIATIONS IN THE TOTAL CAROTENOID CONTENT

The weight specific carotenoid content of *E. pacifica*, on a wet weight basis (42-76 μg/g wet wt.), was similar to values reported for other euphausiid species (Fisher et al., 1952, 1954, 1955; Lambertsen & Braekkan, 1971; Yamaguchi et al., 1983). The high percentage of carotenoids in the eyes of *E. pacifica* also appears to be standard for many euphausiid species (Fisher et al., 1955). The fluctuations observed in the weight specific carotenoid content appear to be influenced by both season and sexual maturity. The 1988 spring cohort had a relatively constant weight specific carotenoid content throughout the summer and early fall. During this time, dietary carotenoid pigments would have been abundant due to relatively high phytoplankton concentrations, allowing the carotenoid concentration of the euphausiids to increase at the same rate as their dry weight. Therefore, the season was responsible for the carotenoid concentration.

The increase in the weight specific carotenoid content that occurred from December, 1988 to February, 1989 was also probably governed by the season. This increase in carotenoid concentration is not indicative of an accumulation of carotenoids by the individuals. The average dry weight of the euphausiid population was decreasing during this

time and the carotenoid content per individual was also decreasing (figure 2). However, the quantity of carotenoids per individual did not decrease as quickly as the dry weight and thus the weight specific carotenoid content increased (figure 3). It seems reasonable to suggest that the decrease in average dry weight was real and that the euphausiids utilized material such as lipid and perhaps protein preferentially to carotenoids as a source of energy when the food supply was limiting.

Variations observed in the weight specific carotenoid concentration of sexually maturing euphausiids during the spring of 1988 and 1989 is less easily attributed to a single factor. The increase in the weight specific carotenoid content occurring from February 9 through to April 3, 1989 was actually an accumulation of carotenoids on an individual basis (figure 2A). During this time the food availability was probably increasing due to early spring blooms of flagellates (Hobson, 1988) and thus a source of carotenoids was available making the accumulation of pigments possible. During the next month (April to May) carotenoids were also accumulated per individual but the weight specific carotenoid content showed a dramatic decrease (figures 2&3). There are two factors which could be affecting the weight specific carotenoid content during this time period: growth rate and/or the stage of sexual maturity. The rapid increase in the weight specific carotenoid concentration which started in February and lasted through to April, occurred when the growth rate was minimal (table 1). The subsequent decrease in the weight specific carotenoid content occurred when the growth rate was markedly increased (table 1). The above changes could, therefore, simply be a function of the growth rate. Provided a source of carotenoid pigments is available, the euphausiids may accumulate these at a fixed and perhaps passive rate so that the weight specific carotenoid content is ultimately dependent on the growth rate.

Alternatively, the second factor, sexual maturity and/or spawning, may also have caused the observed changes in the carotenoid concentration. Crustaceans as well as many

other animals usually transfer carotenoids to the eggs (Gilchrist & Green, 1960; Green, 1957; Goodwin, 1951; Hairston, 1979b; Krinsky, 1965; Soejima et al., 1980), and subsequently, to the developing embryos (Goodwin, 1951; Hairston, 1979b; Herring, 1968b). Assuming euphausiids also deposit carotenoids in their eggs, it seems reasonable to suggest that the increase in the weight specific carotenoid content which began in February and lasted through to April was in preparation for spawning and the pronounced decrease in the weight specific carotenoid content was due to an actual loss of pigments through the eggs and spermatophores as spawning commenced. Unfortunately, this suggestion cannot be substantiated as the euphausiids collected in April were still quite small (avg. dry wt. of 4.2mg) and showed minimal gonadal development. By the next sample date, May 1, 1989 the majority of the population were ripe and had probably already started spawning. The large gap in stage of sexual maturity between these two sample dates makes it impossible to say whether the euphausiids reached a maximum carotenoid concentration just prior to spawning and were then unable to maintain that level due to losses during spawning.

One final point of interest is that the sexually mature, stage IV females (collected on May 1, 1989), had a lower weight specific carotenoid content than males of a comparable stage. Assuming *E. pacifica* deposit carotenoids in their eggs and sperm, and given that female *E. pacifica* may release as many as 31 batches of eggs during one season (Ross et al., 1982), it is probable that females would experience a larger drain on their carotenoid content than males. However, the fact remains that there are no data directly demonstrating the deposition of carotenoids in the eggs or sperm and certainly no quantitative data on the amount of carotenoids which may be transferred.

E. pacifica larvae begin feeding at the calyptopis I stage and it then takes them approximately one week to reach the first stage which was collected in this study: furcilia I (Ross, 1981). Had pre-feeding larval stages been collected, inferences concerning the

quantity of carotenoid pigments transferred to the eggs could have been made based on the carotenoid content of the pre-feeding larvae. Given that this is not possible, it can still be argued that the larvae receive a substantial amount of their carotenoids from the egg as the carotenoid concentration of the larvae was high after only one week of feeding (466 μg carotenoid/g dry tissue).

E. pacifica larvae were not collected until their growth rate was relatively high; a phenomenon which does not occur until the larvae start feeding. Prior to the onset of feeding the larvae must utilize energy reserves from the egg resulting in a decrease in the individual dry weight as the larvae proceed through their pre-feeding stages (Hairston, 1979b). Carotenoid pigments do not appear to have a metabolic function in the crustacean embryos and thus the individual content tends to remain constant throughout embryological development (Goodwin, 1951; Herring, 1968b; Hairston, 1979b). The individual carotenoid content has also been shown to remain constant in pre-feeding larval stages which results in an increase in the weight specific carotenoid content of pre-feeding larval stages (Hairston, 1979b). It would have been interesting to see how the individual carotenoid content of pre-feeding *E. pacifica* larvae fluctuated. The apparent decrease in the weight specific carotenoid concentration observed for the furcilia larvae during the period of rapid growth was a phenomenom also observed by Hairston (1979b) when studying diaptomus copepods. So although the 95% C.L. reported for the larval samples were large, the overall trend seems reasonable.

PIGMENT CHARACTERIZATION AND IDENTIFICATION

The identification of carotenoid molecules by biologists has, in the past, been based solely on the absorption spectra and the chromatographic behavior of the molecules. Unfortunately, the information available through these techniques is insufficient to warrant identification, and therefore, as pointed out by Davies (1985), "there is a jungle of mis-identifications in animal carotenoid literature". Advancements in physico-organic techniques such as circular dichroism (CD) (Sturzenegger et al., 1980), nuclear magnetic resonance (NMR) (Englert, 1985) and resonance raman spectroscopy (Merlin, 1985), now enable chemists to describe newly-isolated carotenoids in remarkable structural detail. However, many of these techniques require large quantities (i.e. mg) of highly purified material and therefore, are of limited utility for field biologists.

Mass spectroscopic data has proven to be most useful to the biologist as only 5-10 μg of purified material is typically required. Mass spectroscopic data of many carotenoid pigments have been published (Budzikiewicz et al., 1967; Enzell & Francis, 1969). However, the fragment pattern is not always enlightening. For example, it is characteristic of the polyene chain of carotenoid molecules to cyclize resulting in the loss of toluene (M-92) or xylene (M-106) (Budzikiewicz, 1982). The loss of 91 and 105/108 mass units observed for many of the samples analyzed, probably corresponds to such losses and although it can be stated that the such losses are characteristic of carotenoids, it says nothing about the structure of the end groups.

Crucial to the identification of a carotenoid is the identification of the molecular ion (the molecular weight + 1 mass unit in the case of positive chemical ionization). Also of diagnostic value is the loss of an end group (Enzell & Francis, 1969). The chances of detecting a molecular ion is based on the stability of the molecule and, therefore, this peak

may or may not be observed. Because it was difficult to observe a molecular ion for the astaxanthin standard, it is not surprising that molecular ions were not observed for those pigments isolated from *E. pacifica*. The presence of identical peaks among the standards and unknowns from *E. pacifica* may suggest molecular homology but because the peaks have not been identified, molecular structure cannot be inferred. The peak at 682, observed for the monoester fraction from *E. pacifica*, indicates that it is larger than astaxanthin and may, therefore, be an ester of astaxanthin, but this is all only speculative.

As mentioned, the diagnostic peaks commonly referred to in the literature usually refer to the loss of an end group. However, the loss of an end group is a rare occurrence, producing a peak that has an intensity of less than 2% relative to the strongest peak (Budzidiewicz et al., 1967; Enzell & Francis, 1969). So again, it is not surprising that these peaks were not observed for any of the samples run on the mass spectrometer.

The end result of the above discussion is that only the chromatographic behavior of the various carotenoids may be used in the determination of their molecular identities. Due to the improved resolving power of HPLC systems compared to TLC, the chromatographic behavior of carotenoids on HPLC allows identifications to be made with more confidence. For example, the R_f values of canthaxanthin and astaxanthin monoester on silica TLC were very similar (table 2) but when run on RP-HPLC, canthaxanthin was eluted almost 10 min before the monoester.

Therefore, based on the HPLC results, it seems reasonable to say that the most polar carotenoid present in *E. pacifica* is free astaxanthin. The carotenoids originating from the band which co-migrated with the monoester standard in the first dimension on TLC are not readily identified. The HPLC results suggest that there are three different molecules contributing to monoester #1 and two different molecules contributing to monoester #2. It is quite possible that the two bands obtained after the second dimension on TLC do

represent astaxanthin esterified to two fatty acids which differ in length and the separation observed on HPLC is a reflection of a mixture of optical isomers (the enantiomeric and meso forms). The presence of these isomers is quite probable as such mixtures have been reported in the shrimp *Pandalus borealis* (Renstrom et al., 1981a). If this is the case, it is curious that only one of the monoesters consists of all three isomers while the monoester which appears to be esterified to a fatty acid similar in length to palmitate only displayed two peaks. It is also unexpected that the free astaxanthin isolated from *E. pacifica* did not indicate the presence of optical isomers when run on RP-HPLC (figure 5A). It is possible that the C_{18} column was better able to separate the optical isomers when the hydroxyl group was esterified. Esterification would reduce the polarity and thus increase the interaction time between the carotenoid and the column, perhaps leading to increased resolution. The elution profile used for the purification of the monoester fraction also would have increased the interactions between the carotenoid and the column compared to the elution profile used in the purification of free astaxanthin.

The carotenoids which co-migrated with the diester standard were not analyzed by HPLC and, therefore, there is little evidence to validate the theory that the three bands originating in the second dimension are due to two fatty acids which differ in length. If this were the case, the band migrating furthest on TLC (diester #3) would theoretically be esterified to two fatty acids similar in length to palmitate. Diester #1 would be esterified to two fatty acids shorter in length than palmitate and diester #2 could be esterified to one of each.

The accuracy of this theory remains to be proven but it must be pointed out that the shrimp *P. borealis* showed no preference in the fatty acids it attached to its astaxanthin molecules (Renstrom et al., 1981b). However, Renstrom et al. (1981b) also did not report distinct bands separating when developed in 30% acetone in hexane indicating that the two

animals may be quite different..

EFFECT OF DIET ON THE CAROTENOID CONTENT/COMPOSITION OF *E. PACIFICA*

As outlined in the materials and methods section, only one sample was analyzed for each diet at the two sample dates and therefore, the information gained by these experiments is somewhat limited. According to the dry weight data, the euphausiids decreased in size considerably during this first month (table 5). However, such a large drop in dry weight seems unlikely as the euphausiids had more food than normally available to them and the fulness of their stomachs indicated that they were eating. Perhaps the apparent decrease was at least in part due to the small sample size at 31 days (n = 1). Alternatively, the euphausiids may have actually lost weight as a result of a longer than expected acclimation period. Due to the uncertainty in the dry weight measurement between the time zero euphausiids and those sampled after 31 days, the significance of the relatively large increase in the weight specific carotenoid content is also suspect as this value is based on the dry weight.

Most useful in this circumstance is the carotenoid content per individual which essentially remained constant from zero to thirty days and may have increased during the last 12 days of the experiment in all three test groups of euphausiids (table 5).

It is not possible to say with any confidence whether any of the subtle changes observed in average dry weight or carotenoid content over the experimental period were significant, but three conclusions can be made. Firstly, examination of the discarded exoskeletons revealed that euphausiids were losing carotenoid pigments in their molts. Therefore, in

order to maintain a constant individual carotenoid content, the euphausiids must have incorporated dietary carotenoid pigments throughout the experiment. Secondly, the differences in the individual carotenoid content among the groups were small suggesting carotenoids from each of the diets were incorporated with the same efficiency. Thirdly, the carotenoid composition of *E. pacifica* in terms of the types of pigments present and the relative contribution of each, did not vary either over the experimental period nor among the three groups. Firstly, this indicates that *E. pacifica* will only deposit specific molecular species. Secondly, since the carotenoid content of the three diets was markedly different and astaxanthin was not present in any of the diets, *E. pacifica* may have the metabolic capacity to structurally manipulate a wide variety of carotenoid pigments. It follows that since the dietary pigments were changed to astaxanthin and then esterified, the accumulation of carotenoid pigments by *E. pacifica* is a non-passive process.

FUNCTION(S) OF CAROTENOID PIGMENTS IN *E. PACIFICA*

Further support for the non-passive nature of carotenoid accumulation comes from the seasonal carotenoid composition. The relative contribution of astaxanthin, its mono- and di-esters remained remarkably constant throughout the entire sample period. The relative contribution of the two mono-esters and the three diesters also remained constant throughout the sample period. Further, the carotenoid composition of the body was identical to that of the eyes. It seems evolutionarily significant that specific carotenoids are accumulated and that their relative ratios are maintained. Selective pressures may have been responsible for the evolution of carotenoid metabolism in *E. pacifica* but the pressures and thus the functions of carotenoids remain unknown.

As outlined in the introduction, carotenoid pigments are capable of protecting biological systems from photosensitized damage (induced by visible light) as well as from damage caused by UV-B, though the latter process is less well understood than the former. Due to these well documented functions, the presence of carotenoids in crustaceans has led researchers to make the assumption that these pigments must be affording the organism with protection (Gilchrist & Lee, 1972; Green, 1965, 1966; Hairston, 1979a, 1979b). Clarke (1980) found that the pigment content and the wet weight of *E. superba* were related by a power function where the exponent, 0.76, was not significantly different than 0.67, the implication being that the pigment content increases approximately with the surface area. If the carotenoids were serving a photoprotective function, it is quite probable that they would be present primarily on the surface of the animal and thus increase with the surface area. However, such a relationship in *E. pacifica* was not found (figure 4) which is not suprising considering 50-60% of the pigments are located in the eyes.

Intuitively, *E. pacifica* would not seem to require protection against photosensitized damage as they show a vertical diel migration pattern and therefore, are exposed to minimal light levels. During the day, in Saanich Inlet, euphausiids are found from 90m to 120m in depth. The animals swim to the surface during dusk and feed on phytoplankton during the night. At dawn they begin their descent.

If the pigments in *E. pacifica* do not serve a photoprotective function, then what function do they serve? I propose that in *E. pacifica*, carotenoid pigments serve three functions. As just stated, *E. pacifica* displays a vertical diel migration pattern. The point of interest is what acts as a cue for their ascent and descent each day?

It is believed that euphausiids follow isolumes during their ascent and descent (Mauchline and Fisher, 1969). The compound eyes are required to detect the low light levels. At their daytime depth (>100m) blue light (450-500nm) is most abundant provided the water is relatively free of debris and it is in this range that both the visual pigments and the carotenoid pigments of euphausiids absorb most strongly (Denys and Brown, 1982). The visual pigments are responsible for detecting light and relaying the message to the brain as an electrical impulse (Wald, 1968). The role of carotenoids is less clear. In stomatopod crustaceans carotenoids have been found to function as light filters: regulating the wavelengths of light reaching the visual pigments (Cronin and Marshall, 1989). In euphausiids such a function seems unlikely. Euphausiids must detect low levels of light in the blue to green range and, therfore, a filter which absorbs light in this range, preventing its detection by the visual pigments does not seem useful. In euphausiid eyes, carotenoids are primarily located below the basement membrane of the compound eye which is below the level of light absorption by the visual pigments (Mauchline and Fisher, 1969). The carotenoids may function to absorb any light which is not absorbed by the retinular cells. The prevention of light scattering in the eye may in some way increase the eyes' sensitivity to light and thus aid the euphausiid in detecting and following isolumes.

The second function of carotenoid pigments in *E. pacifica* may be one of camouflage. In water the red wavelengths are absorbed first so that at depth there is effectively no red light and thus, red appears black. The large compound eyes of *E. pacifica* would therefore, not be seen by visual predators. The importance of the patchy distribution of carotenoid pigments on the body surface also becomes apparent. The carotenoids would function in breaking up the outline of the euphausiid so that a visual predator looking upwards, towards the light, would not see a distinct outline of the euphausiid. Also, if a predator were looking down, away from the light source, the predator would only see black.

The third function of carotenoid pigments that I propose is actually a photoprotective function. I have made a case, based on my results, that sexually maturing euphausiids accumulate carotenoid pigments in early spring and deposit them in their eggs. Euphausiids lay their eggs during the night when they are at the surface. The eggs, therefore, unlike the swimming individuals, are subjected to high light intensities. Carotenoid pigments may, therefore, be deposited in the eggs to prevent damage induced by visible and/or UV-B light.

The only variations observed in the contribution of astaxanthin and its esters occurred either in the spawning individuals or the early larval stages. For example, the spawning euphausiids and the earliest larval stages had a greater proportion of free astaxanthin than euphausiids collected at all other times of the year. The larvae also had a smaller proportion of diester. It is possible that free astaxanthin is a more efficient photoprotective agent and thus a greater proportion of this carotenoid is transported to the eggs. The adults, therefore, in preparation for spawning, hydrolyse the ester linkages of the mono- and diesters and hence have a greater proportion of free astaxanthin. Presumably, the adults take equally from the monoester and diester carotenoid pools as the relative contribution of these fractions did not change. The larvae of course have a greater proportion of free astaxanthin, and the lower than normal levels of diester appears to suggest the diesters are less important as photoprotective agents.

One last aspect of carotenoid pigments which deserves discussion is their role as membrane moderators. Membrane moderators lower the temperature at which the lipid membrane changes from a liquid to a solid state; referred to as its phase transition. Moderators also make this transition less abrupt so that there is a larger range of temperatures at which the membrane will be in between the solid and liquid phases. Moderators typically fit between the fatty acyl chains of the phospholipids making close association and crystallization more difficult. Moderators also stabilize membranes by

sterically blocking large motions of fatty acyl chains, thus making the membrane less fluid. The orientation of single carotenoids in membranes is perpendicular to the membrane interfaces and parallel to the fatty acyl chains. In this orientation C_{40} carotenoids extend through both layers of the membrane and may therefore, act in a rivet-like fashion to stabilize membranes (Rohmer et al., 1979). Dipolar carotenoids have been shown to depress and broaden the transition temperature of membranes (Yamamoto & Bangham, 1978). Milon et al. (1986) and Lazrak et al. (1987) also demonstrated that dipolar carotenoids do exert a mechanical reinforcing effect in some membranes but both the nature of the phospholipid and the carotenoid molecule are important. These factors are important as both affect the stability of the system. That is, the system would be unstable if the polar groups on a carotenoid molecule were located in the hydrophobic portion of the membrane, or if a hydrophobic end group of a particular carotenoid molecule were forced to associate with the polar head groups of the phospholipid bilayer. The nature of the phosopholipid membrane dictates the thickness of the membrane and thus determines the positioning of the carotenoid molecule in the membrane. The carotenoid pigments of *E. pacifica* could conceivably function as membrane moderators. The carbonyl groups would be primarily responsible for anchoring the carotenoid in the membrane and for bracing the two lipid layers together as most of the hydroxyl groups are esterified to fatty acids. Because over 80% of the carotenoid pigments are the mono- or diesterified form of astaxanthin, one would have to assume that the esterified fatty acids penetrate back into the hydrophobic centre of the membrane. However, cholesterol is the primary membrane moderator in eukaryotic systems and since euphausiids do contain sterols (Bottino, 1975; Clarke, 1980; Falk-Petersen et al., 1981; Saether et al., 1986a; Sargent et al., 1981) the role of carotenoid pigments in *E. pacifica* as membrane moderators may be of little importance.

If one assumes that the carotenoid pigments do provide a variety of functions as I have just suggested, this still does not explain the constancy of the relative contribution of each carotenoid fraction. In fact, one may expect the carotenoid pigments in the eyes, which function primarily to absorb all available light, to differ from those in the body, which function primarily to camouflage the animals. It may be that the observed ratio of carotenoid pigments was capable of performing all of the necessary functions, and thus it was energetically favourable for the euphausiids to evolve a single metabolic pathway leading to the deposition of all carotenoids. Also, the constancy of the carotenoid composition may be a function of the method of transportation. Movement of hydrophobic molecules through an aqueous environment typically occurs through association with proteins as is observed in the transport of lipid in animals. Perhaps the apoproteins responsible for the movement of carotenoid molecules in *E. pacifica*, require a fixed proportion of each carotenoid fraction and thus maintains the relative amounts of each carotenoid pigment in the euphausiid body.

The evolution of carotenoid pigments in animals appears to be an unbroached subject. With respect to photosynthetic systems, the presence or absence of carotenoids in plants is dependent on the pigments of the endosymbiont which gave rise to the plastids (Whately, 1981). The molecular evolution of membranes with respect to carotenoid pigments may have occurred through two different processes. Carotenoids may have arisen in prokaryotic systems as membrane moderators (Rohmer et al.,1979) or as photoprotective agents in photosynthetic bacteria to protect against photosensitized oxidation when the atmosphere changed from one that was primarily reducing to the present, oxidizing environment (Mathews & Sistrom, 1959). Based on the distribution of carotenoids in photosynthetic systems and the evidence supporting their role as photoprotecting agents, the latter theory seems more probable. The question of carotenoid origin lies in the function of the

carotenoids in the prokaryotes (and eukaryotes) prior to their endosymbiosis: membrane moderators or photoprotectors? Whatever the answer, it is probably of little import to a discussion on the evolution of carotenoid pigments in animal systems as the presence of carotenoid pigments in animals is dependent on the dietary source of carotenoid moleules and the metabolic capacity of the particular species. The diversity of carotenoid pigments in animals (Goodwin, 1984) may be a result of these molecules serving different functions and thus, a common evolutionary theme will not be found. The function of carotenoid molecules in a species must be established prior to the formation of a theory of their evolution.

LITERATURE CITED

Allahpichay, I. and Shimizu, C. 1984. Extraction of growth promoting fractions from non-muscle krill meal of *Euphausia superba* and its effects on fish growth. Bulletin of the Japanese Society of Scientific Fisheries 50: 821-826.

Anderson, S.M. and Krinsky, N.I. 1973a. Protective action of carotenoid pigments against photodynamic damage to liposomes. Photochemistry and Photobiology 18: 403-408.

Anderson, S.M. and Krinsky, N.I. 1973b. The inhibition of photosensitized oxidations in liposomes by carotenoid pigments and free radical quenchers. Federation Proceedings 32: 562.

Bamstedt, U. 1976. Studies on the deep-water pelagic community of Korsfjorden, western Norway: changes in the size and biochemical composition of *Meganyctiphanes norvegica* (Euphausiacea) in relation to its lifecycle. Sarsia 61: 15-30.

Berson, J.A., Wall. R.G. and Perlmutter, H.D. 1966. A preference for enoid addition in the ene synthesis. Journal of the American Chemistry Society 88: 187-188.

Black, H.S. and Chan, J.T. 1977. Experimental ultraviolet light carcinogenesis. Photochemistry and Photobiology 26: 183-199.

Bolliger, H.R. 1965. Vitamins, *In, Thin-layer chromatography: a laboratory handbook,* Stahl, E. (ed.). Academic press Inc., New Yord, USA, pp. 212-219.

Bottino, N.R. 1974. The fatty acids of Antarctic phytoplankton and euphausiids. Fatty acid exchange among trophic levels of the Ross Sea. Marine Biology 27: 197-204.

Bottino, N.R. 1975. Lipid composition of two species of Antarctic krill: *Eupausia superba* and *E. crystallorophias*. Comparative Biochemistry and Physiology 50B: 479-484.

Boucher, F., van der Rest, M. and Gingras, G. 1977. Structure and function of carotenoids in the photoreaction centre from *Rhodospirillum rubrum*. Biochimica et Biophysica Acta 461: 339-357.

Budzikiewicz, H. 1982. Mass spectra of carotenoids -- labelling studies. *In, Carotenoid chemistry and biochemistry*, Britton, G. and Goodwin, T.W. (eds.). Pergamon Press, New York, USA, pp. 155-165.

Budzikiewicz. H., Djerassi, C. and Williams, D.H. 1967. *Mass spectrometry of organic compounds*. Holden-Day, San Francisco, USA, pp. 5-49.

Bunt, J.S. 1964. Analysis of algal pigments by thin-layer chromatography. Nature 203: 1261-1263.

Chessin, M., Livingston, R. and Truscott, T.G. 1966. Direct evidence for the sensitized fromation of a metastable state of β-carotene. Transactions of the Faraday Society 62: 1519-1524.

Clare, N.T. 1956. Photodynamic action and its pathological effects. *In. Radiation biology, Vol. III: Visible and near-visible light*, Hollaender, A. (ed.). McGraw-Hill Book Company, Inc., New York, pp. 693-723.

Clarke, A. 1980. The biochemical composition of krill, *Euphausia superba* Dana, from South Georgia. Journal of experimental marine Biology and Ecology 43: 221-236.

Cogdell, R.J. 1985. Carotenoids in photosynthesis. Pure & Applied Chemistry 57: 723-728.

Cogdell, R.J. and Frank, H.A. 1987. How carotenoids function in photosynthetic bacteria. Biochimica et Biophysica Acta 895: 63-79.

Cronin, T.W. and Marshall, N.J. 1989. A retina with at least ten spectral types of photoreceptors in a mantis shrimp. Nature 339: 137-140.

Czeczuga, B. and Klyszejko, B. 1978. Carotenoid contents in *E. superba* (Dana 1850), (Euphausiacea), representative of krill from Antarctic waters. Polish Archives of Hydrobiology 25: 657-662.

Davies, B.H. 1976. Carotenoids. *In Chemistry and biochemistry of plant pigments*, vol. 2, 2nd edn., Goodwin, T.W. (ed.). Academic press, London, pp. 38-155.

Davies, B.H. 1985. Carotenoid metabolism in animals: a biochemist's view. Pure and Applied Chemistry. 57: 679-684.

Davies, B.H., Hsu, W.-J. and Chichester, C.O. 1970. The mechanism of the conversion of β-carotene into canthaxanthin by the brine shrimp, *Artemia salina* L. (Crustacea: Branchiopoda). Comparative Biochemistry and Physiology 33: 601-615.

Davies, B.H., Hsu, W.-J. and Chichester, C.O. 1965. The metabolism of carotenoids in the brine shrimp *Artemia salina*. Biochemistry Journal 94: 26.

Denys, C.J. and Brown, P.K. 1982. Euphausiid visual pigments: The rhodopsins of *Euphausia superba* and *Meganyctiphanes norvegica*. Journal of General Physiology 80: 451-472.

Ellingsen, T.E. and Mohr, V. 1981. Lipids in Antarctic krill. Composition and post mortem changes. Proc. 11th Scandinavian Symposium on Lipids. Marscuse, R. (ed.). LIK, Gothenburg, Sweden, pp. 110-116.

Englert, G. 1985. NMR of carotenoids -- new experimental techniques. Pure and Applied Chemistry 57: 801-821.

Enzell, C.R. and Francis, G.W. 1969. Mass spectrometric studies of carotenoids 2. A survey of fragmentation reactions. Acta Chemica Scandinavica 23: 727-750.

Epstein, J.H. 1973. Effects of β-carotene on ultraviolet induced cancer formation in the hairless mouse skin Photochemistry and Photobiology 25: 211-213.

Falk-Petersen, S. 1981. Ecological investigations on the zooplankton community of Balsfjorden, northern Norway: seasonal changes in body weight and the main biochemical composition of *Thysanoessa inermis* (Kroyer), *T. raschii* (M. Sars), and *Meganyctiphanes norvegica* (M. Sars) in relation to environmental factors. Journal experimental marine Biology and Ecology 49: 103-120.

Falk-Petersen, S., Gatten, R.R., Sargent, J.R. and Hopkins, C.C.E. 1981. Ecological investigations on the zooplankton community in Balsfjorden, northern Norway: seasonal changes in the lipid class composition of *Meganyctiphanes norvegica* (M. Sars), *Thysanoessa raschii* (M. Sars), and *T. inermis* (Kroyer). Journal expermental marine Biology and Ecology 54: 209-224.

Fisher, L.R., Kon, S.K. and Thompson, S.Y. 1952. Vitamin A and carotenoids in certain invertebrates I. Marine crustacea. Journal marine biology Association U.K. 31: 229-258.

Fisher, L.R., Kon, S.K. and Thompson, S.Y. 1954. Vitamin A and carotenoids in certain invertebrates II. Studies of seasonal variations in some marine crustacea. Journal marine biology Association U.K. 33: 589-612.

Fisher, L.R., Kon, S.K. and Thompson, S.Y. 1955. Vitamin A and carotenoids in certain invertebrates III. Euphausiacea. Journal marine biology Association U.K. 34: 81-100.

Foote, C.S. 1968a. Photosensitized oxygenations and the role of singlet oxygen. Accounts of Chemical Research 1: 104-110.

Foote, C.S. 1968b. Mechanisms of photosensitized oxidation. Science 162: 963-970.

Foote, C.S. and Denny, R.W. 1968. Chemistry of singlet oxygen VII. Quenching by β-carotene. Journal of the American Chemistry Society 90: 6233-6235.

Foote, C.S., Chang, Y.C. and Denny, R.W. 1970a. Chemistry of singlet oxygen. X. Carotenoid quenching parallels biological protection. Journal of the American Chemistry Society 92: 5216-5218.

Foote, C.S., Chang, Y.C. and Denny, R.W. 1970b. Chemistry of singlet oxygen. XI. Cis - trans isomerization of carotenoids by singlet oxygen and a probable quenching mechanism. Journal of the American Chemistry Society 92: 5218-5219.

Foss, P., Storebakken, T., Austreng, E. and Liaaen-Jensen, S. 1987. Carotenoids in diets for salmonids V. Pigmentation of rainbow trout and sea trout with astaxanthin and astaxanthin dipalmitate in comparison with canthaxanthin. Aquaculture 65: 293-305.

Foss, P., Storebakken, T., Schiedt, K., Liaaen-Jensen, S., Austreng, E. and Streiff, K. 1984. Carotenoids in diets for salmonids I. Pigmentation of rainbow trout with the individual optical isomers of astaxanthin in comparison with canthaxanthin. Aquaculture 41: 213-226.

Fujimori, E. and Livingston, R. 1957. Interactions of chlorophyll in its triplet state with oxygen, carotene, etc. Nature 180: 1036-1038.

Gilchrist, B.M. and Green, J. 1960. The pigments of *Artemia*. Proceedings of the Royal Society of London, Series B 152: 118-136.

Gilchrist, B.M. and Lee, W.L. 1972. Carotenoid pigments and their possible role in reproduction in the sand crab, *Emerita analoga* (Stimpson, 1857). Comparative Biochemistry and Physiology 42B: 263-294.

Gollnick, K. and Schenck, G.O. 1964. Mechanism and stereoselectivity of photosensitized oxygen transfer reactions. Pure and Applied Chemistry 9: 507-525.

Goodwin, T.W. 1951. Carotenoid metabolism during development of lobster eggs. Nature 167: 559.

Goodwin, T.W. 1984. *The biochemistry of the carotenoids, 2nd edn., vol. 2 (Animals)*. Chapman & Hall, London.

Grave, H. 1981. Fluoride content of salmonids fed on Antarctic krill. Aquaculture 24: 191-196.

Grave, H., Schultz, A. and Van Thielen, R. 1979. The infuence of the blue mussel (*Mytilus edulis*) and krill (*Euphausia superba*) on growth and proximate composition of rainbow trout, *Salmo gairdneri*. Proc. World symp. Finfish Nutrition and Fishfeed Technology. Hamburg, 20-23 June, 1978. Vol. I. Heenemann, Berlin, pp. 575-586.

Green, J. 1957. Carotenoids in *Daphnia*. Proceedings of the Royal Society of London, Series B 147: 392-401.

Green, J. 1965. Chemical embryology of the Crustacea. Biology Review 40: 580-600.

Green, J. 1966. Variation in the pigmentation of *Simocephalus vetulus* (Crustacea: cladocera). Journal of Zoology, London 149: 174-187.

Griffiths, M., Sistrom, W.R. Cohen-Bazire, G. and Stanier, R.Y. 1955. Function of carotenoids in photosynthesis. Nature 176: 1211-1215.

Hairston, N.G. Jr. 1976. Photoprotection by carotenoid pigments in the copepod *Diaptomus nevadensis*. Proceedings of the National Academy of Science, USA 73: 971-974.

Hairston, N.G. Jr. 1979a. The adaptive significance of colour polymorphism in two species of *Diaptomus* (Copepoda). Limnology and Oceannography 24: 15-37.

Hairston, N.G. Jr. 1979b. The relationship between pigmentation and reproduction in two species of *Diaptomus* (Copepoda). Limnology and Oceanography 24: 38-44.

Herring, P.J. 1968b. The carotenoid pigments of *Daphnia magna* Straus--II. Aspects of pigmentary metabolism. Comparative Biochemistry and Physiology 24: 205-221.

Hobson, L.A. 1988. Volume-frequency distributions of marine phytoplankton in a temperate neritic environment. Marine Ecology Progress Series 48: 47-55

Hsu, W.-J., Chichester, C.O. and Davies, B.H. 1970. The metabolism of β-carotene and other carotenoids in th brine shrimp, *Artemia salina* L. (Crustacea: Branchiopoda). Comparative Biochemistry and Physiology 32: 69-79.

Isler, O. and Schudel, P. 1963. *In, Carotine und carotinoide* (Wiss. Veroff. dt. Ges. Ernahr., Col. 9), Steinkopff, Darmstadt, pp. 54-104. (c.f. Davies, 1976).

Jackowska, H., Czerpak, R. and Mical, A. 1980. Carotenoids of Antarctic krill (*Euphausia superba* Dana, *Euphausia crystallorophias* Holt et Tattersall) independence on age structure and sex. Polish Archives of Hydrobiology 27: 291-303.

Jeffrey, S.W. 1961. Paper-chromatographic separation of chlorophylls and carotenoids from marine algae. Biochemistry Journal 80: 336-342.

Jeffrey, S.W. 1968. Quantitative thin-layer chromatography of chlorophylls and carotenoids from marine algae. Biochimica et Biophysica Acta 162: 271-285.

Kitahara, T. 1983. Behavior of carotenoids in the chum salmon (*Oncorhynchus keta*) during anadromous migration. Comparative Biochemistry and Physiology 76B: 97-101.

Koops, H., Tiews, K., Gropp, J. and Beck, H. 1979. Krill in trout diets. Proc. World symp. Finfish Nutrition and Fishfeed Technology. Hamburg, 20-23 June, 1978. Vol. II. Heenemann, Berlin, pp. 281-292.

Krinsky, N.I. 1965. The carotenoids of the brine shrimp, *Artemia salina*. Comparative Biochemistry and Physiology 16: 181-187.

Krinsky, N.I. 1968. The protective function of carotenoid pigments. *In*, *Photophysiology*, Vol. III, Giese, A.C. (ed.). Academic Press, New York, USA, pp. 123-196.

Krinsky, N.I. 1979. Carotenoid protection against oxidation. Pure and Applied Chemistry 51: 649-660.

Krinsky, N.I. and Deneke, S.M. 1982. Interaction of oxygen and oxy-radicals with carotenoids. Journal of the National Cancer Institute 69: 205-209.

Lambertsen, G. and Braekkan, O.R. 1971. Method of analysis of astaxanthin and its occurrence in some maring products. Journal of the Science of Food and Agriculture 22: 99-101.

Lazrak, T., Milon, A., Wolff, G., Albrecht, A.-M., Mieke, M., Ourisson, G. and Nakatani, Y. 1987. Comparison of the effects of inserted C_{40}- and C_{50}-terminally dihydroxylated carotenoids on the mechanical properties of various phospholipid vesicles. Biochimica et Biophysica Acta 903: 132-141.

Littlepage, J.L. 1964. Seasonal variation in lipid content of two Antarctic marine Crustacea. *In, Actualites scientifiques industrielles: Biologie Antarctique*. Hermann, Paris, pp. 463-470.

Mathews, M.M. 1964. Protective effect of β-carotene against lethal photosensitization by haematoporphyrin. Nature 203: 1092.

Mathews, M.M. and Sistrom, W.R. 1959. Function of carotenoid pigments in non-photosynthetic bacteria. Nature 184: 1892-1893.

Mathews-Roth, M.M. 1982. Beta-carotene therapy for erythropoietic protoporphyria and other photosensitivity diseases. *In, The science of photomedicine*, Regan, J. and Parrish, J. (eds.). Plenum Press, New York, USA, pp. 409-440.

Mathews-Roth, M.M. 1984. Porphyrin photosensitization and carotenoid protection in mice; *in vitro* and *in vivo* studies. Photochemistry and Photobiology 40: 63-67.

Mathews-Roth, M.M. 1986. Carotenoids quench evolution of excited species in epidermis exposed to UV-B (290-320nm) light. Photochemistry and Photobiology 43: 91-93.

Mathews-Roth, M.M. 1987. Photoprotection by carotenoids. Federation Proceedings 46: 1890-1893.

Mathews-Roth, M.M. and Krinsky, N.I. 1987. Carotenoids affect development of UV-B induced skin cancer. Photochemistry and Photobiology 46: 507-509.

Mathis, P. 1970. Thesis, Orsay, France. c.f. Mathis & Kleo (1973).

Mathis, P. and Kleo, J. 1973. The triplet state of β-carotene and of analog polyenes of different length. Photochemistry and Photobiology 18: 343-346.

Mathis, P. and Schenck, C.C. 1982. The functions of carotenoids in photosynthesis. *In, Carotenoid chemistry and biochemistry*, Britton, G. and Goodwin, T.W. (eds.). Proceedings of the 6th international symposium on carotenoids, Liverpool, July 1981. Pergamon Press, New York, USA, pp. 339-351.

Mauchline, J. and Fisher, L.R. 1969. The biology of euphausiids. Advances in Marine Biology 7: 464pp.

McBeth, J.W. 1972. Carotenoids from nudibranchs. Comparative Biochemistry and Physiology 41B: 55-68.

McCullum, I.M., Cheng, K.M. and March, B.E. 1987. Carotenoid pigmentation in two strains of Chinook salmon (*Oncorhynchus tshawytscha*) and their crosses. Aquaculture 67: 291-300.

Merlin, J.C. 1985. Resonance Raman spectroscopy of carotenoids and carotenoid containing systems. Pure and Applied Chemistry 57: 785-792.

Milon, A., Lazrak, T., Albrecht, A.-M., Wolff, G., Weill, G., Ourisson G. and Nakatani, Y. 1986. Osmotic swelling of unilamellar vesicles by the stopped-flow light scattering method. Influence of vesicle size, solute, temperature, cholesterol and three α,x-dihydroxy carotenoids. Biochimica et Biophysica Acta 859: 1-9.

Nilsson, R., Swanbeck, G. and Wennersten, G. 1975. Primary mechanisms of erythrocyte photolysis induced by biological sensitizers and phototoxic drugs. Photochemistry Photobiology 22: 183-196.

Pardo. A.D. and Schiff, J.A. 1980. Plastid and seedling development in SAN-9789 (4-chloro-5-(methylamine)-2-(α,α,α-trifluoro-m-tolyl)-3-(2H)-pyridasinone)-treated etiolated bean seedlings. Canadian Journal of Botany 58: 25-35.

Pryor, W.A. 1976. The role of free radical reactions in biological systems. *In, Free radicals in biology,* Vol. I., Pryor, W.A. (ed.). Academic Press, New York, USA, pp. 1-49.

Raymont, J.E.G., Austin, J. and Linford, E. 1964. Biochemical studies on marine zooplankton V. The composition of major biochemical fraction in *Neomysis integer*. Journal of the marine biology Association U.K. 48: 735-760.

Raymont, J.E.G., Srinivasagam, R.T. and Raymont, J.K.B. 1969. Biochemical studies on marine zooplankton--IV. Investigations on *Meganyctiphanes norvegica*. Deep-Sea Research 16: 141-156.

Raymont, J.E.G., Srinivasagam, R.T. and Raymont, J.K.B. 1971a. Biochemical studies in marine zooplankton IX. The biochemical composition of *Euphausia superba*. Journal of the marine biology Association U.K. 51: 581-588.

Raymont, J.E.G., Srinivasagam, R.T. and Raymont, J.K.B. 1971b. Biochemical studies on marine zooplankton--VII. Further investigation on *Meganyctiphanes norvegica* (M. Sars). Deep-Sea Research 18: 1167-1178.

Razi-Naqui, K. 1980. The mechanism of singlet-singlet excitation energy transfer from carotenoids to chlorophyll. Photochemistry and Photobiology 31: 523-524.

Reinhardt, S.B. and Van Vleet, E.S. 1986. Lipid composition of twenty-two species of Antarctic midwater zooplankton and fish. Marine Biology 91: 149-159.

Renstrom, B. and Liaaen-Jensen, S. 1981b. Fatty acid composition of some esterified carotenols. Comparative Biochemistry and Physiology 69B: 625-627.

Renstrom, B., Borch, G., Skulberg, O.M. and Liaaen-Jensen, S. 1981c. Optical purity of (3S, 3'S)-astaxanthin from *Haematococcus pluvialis*. Phytochemistry 20: 2561-2564.

Renstrom, B., Gunner, B. and Liaaen-Jensen, S. 1981a. Natural occurrence of enantiomeric and meso-astaxanthin 4. Ex Shrimps (*Pandalus borealis*). Comparative Biochemistry and Physiology 69B: 621-624.

Ridley, S.M. and Ridley, J. 1979. Interaction of chloroplasts with inhibitors. Plant Physiology 63: 392-398.

Riley, J.P. and Wilson, T.R.S. 1965. The use of thin-layer chromatography for the separation and identification of phytoplankton pigments. Journal of the marine biology Association U.K. 45: 583-591.

Rohmer, M., Bouvier, P. and Ourisson, G. 1979. Molecular evolution of biomembranes: structural equivalents and phylogenetic precursors of sterols. Proceedings of the National Academy of Science, USA. 76: 847-851.

Ross, R.M. 1981. Laboratory culture and development of *Euphausia pacifica*. Limnology and Oceanograpgy 26: 235-246.

Ross, R.M., Daly, K.L. and English, T.S. 1982. Reproductive cycle and fecundity of *Euphausia pacifica* in Puget Sound, Washington. Limnology and Oceanography 27: 304-314.

Saether, O., Ellingsen, T.E. and Mohr, V. 1985. The distribution of lipid in the tissues of Antarctic krill, *Eupausia superba*. Comparative Biochemistry and Physiology 81B: 609-614.

Saether, O., Ellingsen, T.E. and Mohr, V. 1986a. Lipolysis post mortem in north Atlantic krill. Comparative Biochemistry and Physiology 83B: 51-55.

Sargent, J.R. and Falk-Petersen, S. 1981. Ecological investigations on the zooplankton community in Balsfjorden, northern Norway: lipids and fatty acids in *Meganyctiphanes norvegica*, *Thysanoessa raschii* and *T. inermis*. Marine Biology 62: 131-137.

Schiedt, K., Leuenberger, F.J. and Vecchi, M. 1981. Natural occurrence of enantiomeric and meso-astaxantin 5. Ex Wild salmon (*Salmo salar* and *Oncorhynchus*). Helvetica Chimica Acta 64: 449-457.

Schiedt, K., Leuenberger, F.J., Vecchi, M. and Glinz, E. 1985. Absorption, retention and metabolic transformations of carotenoids in rainbow trout, salmon and chicken. Pure and Applied Chemistry 57: 685-692.

Sivtseva, L.V. and Dubrovin, V.D. 1981. Some patterns in the quantitative distribution of carotenoid pigments in the body of rainbow trout, *Salmo gairdneri*. Journal of Ichthyology 221: 142-148.

Soejima, T., Katayama, T. and Simpson, K.L. 1980. International study on *Artemia* XII. The carotenoid composition of eight geographical strains of *Artemia* and the effect of diet on the carotenoid composition of *Artemia*. *In, The brine shrimp* Artemia, vol. 2, Persoone, G., Sorgeloos, P., Roels, O. and Jaspers, E. (eds.). Universa Press, Wetteren, Belgium, pp.613-622.

Soevik, T. and Braekkan, O.R. 1979. Fluoride in Antarctic krill (*Euphausia superba*) and Atlantic krill (*Meganyctiphanes norvegica*). Journal of the Fisheries Research Board of Canada 36: 1414-1416.

Song, P.-S., Koka, P., Prezelin, B.B. and Haxo, F.T. 1976. Molecular topology of the photosynthetic light-harvesting pigment complex, peridinin-chlorophyll a-protein, from marine dinoflagellates. Biochemistry 15: 4422-4427.

Spinelli, J. and Mahnken, C. 1978. Carotenoid deposition in pen-reared salmonids fed diets containing oil extracts of red crab (*Pleuroncodes planipes*). Aquaculture 13: 213-223.

Stauber, J.L. and Jeffrey, S.W. 1988. Photosynthetic pigments in fifty-one species of marine diatoms. Journal of Phycology 24: 158-172.

Storebakken, T. 1988. Krill as a potential feed source for salmonids. Aquaculture 70: 193-205.

Storebakken, T., Foss, P., Huse, I., Wandsvik, A. and Berg Lea, T. 1986. Carotenoids in diets for salmon III. Utilization of canthaxanthin from dry and wet diets by Atlantic salmon, rainbow trout and sea trout. Aquaculture 51: 245-255.

Storebakken, T., Foss, P., Schiedt, K., Austreng, E., Liaaen-Jensen, S. and Manz, U. 1987. Carotenoids in diets for salmonids IV. Pigmentation of Atlantic salmon with astaxanthin, astaxanthin dipalmitate and canthaxanthin. Aquaculture 65: 279-292.

Sturzenegger, V., Buchecker, R. and Wagniere, G. 1980. Classification of the CD. spectra of carotenoids. Helvetica Chimica Acta 63: 1074-1092.

Suyama, M., Nakajima, K. and Nonaka, J. 1965. Studies on the protein and non-protein nitrogenous constituents of *Euphausia*. Bulletin of the Japanese Scientific Fisheries 31: 302-306.

Taylor, R.F. and Ikawa, M. 1980. Gas chromatography, gas chromatography-mass spectrometry, and high-pressure liquid chromatography of carotenoids and retinoids. Methods in Enzymology 67: 233-261.

Tiews, K., Manthey, M. and Koops, H. 1982. The carry-over of fluoride from krill meal pellets into rainbow trout (*Salmo gairdneri*). Archiv fuer Fischereiwissenschaft 32: 39-42.

Torrissen, K.R. and Torrissen, O.J. 1985. Sexual maturation: effect on protease activities and carotenoid levels in Atlantic salmon (*Salmo salar*). Aquaculture 50: 113-122.

Torrissen, O.J. 1986. Pigmentation of salmonids -- a comparison of astaxanthin and canthaxanthin as pigment sources for rainbow trout. Aquaculture 53: 271-278.

Torrissen, O.J. and Naevdal, G. 1984. Pigmentation of salmonids -- genetic variation in carotenoid deposition in rainbow trout. Aquaculture 38: 59-66.

Torrissen, O.J. and Naevdal, G. 1988. Pigmentation of salmonids -- variation in flesh carotenoids of Atlantic salmon. Aquaculture 68: 305-310.

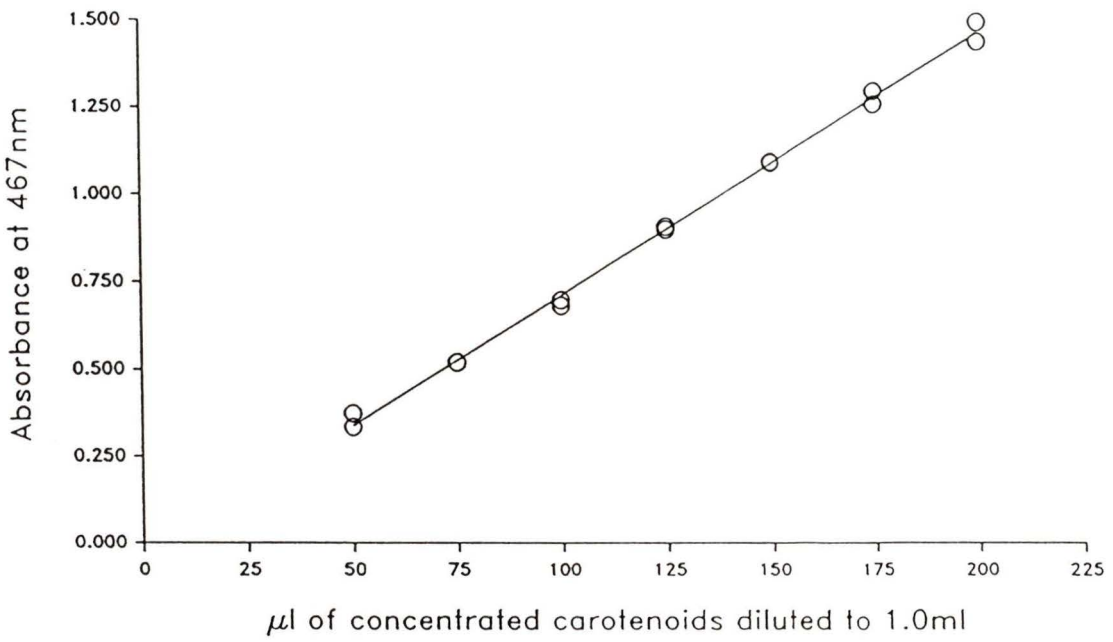
Wald, G. 1968. The molecular basis of visual excitation. Nature 219: 800-807.

Whately, J.M. 1981. Chloroplast evolution -- ancient and modern. *In, Origins and evolution of eukaryotic intracellular organelles*, Fredrick, J.F. (ed.). New York Academy of Sciences, New York, USA, pp. 154-165.

Wren, J.J. 1960. Chromatography of lipids on silicic acid. Journal of Chromatography 4: 173-195.

Yamaguchi, K., Miki, W., Toriu, N., Kondo, Y., Murakami, M., Konosu, S., Satake, M. and Fujita, T. 1983. The composition of carotenoid pigments in the Antarctic krill *Euphausia superba*. Bulletin of the Japanese Society of Scientific Fisheries 49: 1411-1415.

Yamamoto, H.Y. and Bangham, A.D. 1978. Carotenoid organization in membranes -- thermal transition and spectral properties of cartenoid-containing liposomes. Biochimica et Biophysica Acta 507: 119-127.



Appendix I: A determination of the applicability of Beers law for carotenoid pigments. This demonstrates the range of absorbance values that fall within the linear portion of the curve.

Appendix II: The carbon content of the diets fed to *E. pacifica* during the feeding experiments, expressed as a percent of the dry weight. The dried samples were analyzed by Canadian Microanalytical service, Ltd.

Species	Carbon content
A. carterae	41%
S. costatum	35%
A. salina	41%

An aliquot of the stock culture was filtered onto a pre-weighed glass fibre filter, washed with distilled water and dried. The dry weight per volume of culture was determined and using the above values, the carbon content per volume was determined. The same was determined for the seawater containing the euphausiids, and thus the amount of carbon required in each experimental chamber to maintain the desired carbon concentration, could also be determined.

APPENDIX III: Data obtained from euphausiids collected from Saanich Inlet at monthly intervals. Data are plotted in figures in the text.

date	dry wt.	C.I.	car/ind	C.I.	car/wt.	C.I.	n	%1	C.I.	%2	C.I.	%3	C.I.	%rest	C.I.
Mar 7	4.4	0.22	2.2	0.22	489	43	3	5.5	1.2	47.7	10.4	39.3	10.7	7.5	0
Apr 18	7.6	0.54	2.7	0.38	355	43	3	5.7	3	48.9	4.2	37.9	4.2	7.5	0
May 4	8.5	0.8	3.2	0.34	371	17.4	3	4.5	1.6	48.1	9.2	39.8	7.2	7.5	0.5
larvae	0.17		0.079		466	540	2	7.9	7.6	52.7	6.4	31.1	7.6	8.3	6.4
Jun 6	11.4	5.7	3.8	2.08	330	73.4	2	3.5	2.5	50.0	0.6	36.9	8.9	9.7	12.1
larvae	0.44	1.9	0.19	0.83	427	271	2	5.8	0.6	48.0	26	38	26	8.3	1.3
Jul 11	12.4	3.8	3.9	1.73	318	101	2	4.3	0.6	49.0	39	42	34	5.8	5.7
larvae	2.2	0.25	0.73	0.84	333	380	2	4	3.2	51.0	39	39	34	6.1	1.3
Aug 11	2.8	0.52	0.97	0.19	348	15	3	4	2.7	51.4	3.5	40.1	3.7	4.4	4.5
Aug 25	4.2	0.6	1.58	0.26	376	31	3	4.5	2.7	51.2	5.5	40	4.2	4.3	1.5
Oct 6	5.0	0.3	1.5	0.24	306	37	3	4.9	1.6	47.7	2.7	42.2	2.5	5.2	1.1
Nov 3	6.7	0.2	2.32	0.22	346	31	3	4.4	1.5	50.5	1.7	40.7	2.1	4.4	1.2
Nov 28	6.3	0.77	2.2	0.36	353	37	3	5.4	0	46.4	13.5	43.8	25.1	4.6	11.7
Dec 16	5.6	0.4	2.0	0.29	357	44	3	3.8	8.3	53.1	0.6	38.1	8.3	5.1	1.3
Jan 12	4.7	0.58	2.07	0.35	441	52	3	3	2.5	51.0	36	41	32	6.2	0.6
Feb 9	3.2	5.1	1.47	2.35	459	81	4	4.7	2.5	44.2	18.9	44.3	14.4	7.0	5.7
Mar 8	3.6	2.6	1.93	1.4	536	48	3	5.4	0.5	46.4	6.2	41.3	5.7	6.9	0.4
Apr 3	4.2	0.8	2.2	0.47	526	52	3								
May 1															
females	7.8	0.96			373	43	4								
males	6.2	1.12			430	126	3								

Footnotes:

dry wt.: Average individual dry weight (mg)
C.I.: 95% confidence limits for the preceding column
Car/ind: µg carotenoids per individual
car/wt: µg carotenoids per gram dry tissue
n: number of pooled samples analyzed
%1: % contribution of astaxanthin
%2: % contribution of all monoesters
%3: % contribution of all diesters
%rest: % contribution of unidentified fractions

APPENDIX III (continued): Data points and 95% confidence limits (C.I.) used in construction the figures for the yearly study.

Date	%2-1	C.I.	%2-2	C.I.	%3-1	C.I.	%3-2	C.I.	%3-3	C.I.
Mar 7	16.6	4	31.1	11.2	6	2.2	16.7	4.5	16.7	4.2
Apr 18	19.4	3.5	29.5	7.7	6.7	0.4	16	2.1	15.2	2.2
May 4	20.3	1.5	27.9	7.7	8	2.1	16.9	4	14.5	2.5
larvae										
Jun 6	20.8	1.3	29.2	1.9	6.9	3.2	15.9	7	14.1	6.4
larvae										
Jul 11	19.9	52	28.6	12.7	6.9	9.5	18.5	23	16.2	0
larvae	20.8	14	30.5	53	6.9	9	14.5	12	17.3	16.5
Aug 11	20.4	0.8	31	2.7	6.3	0.6	19	3.5	14.8	1.4
Aug 25	18.1	2.1	33.2	7.4	5.7	1.6	17.8	3	16.5	5.7
Oct 6	17.9	1.9	29.9	1.4	6.1	2.3	17.8	2.2	18.2	2.3
Nov 3	18.2	5	32.2	5	5.5	0.8	17	1.5	18.2	1.2
Nov 28	15.8	0.6	30.6	14.4	5.8	6.3	17.5	11.4	20.5	7.6
Dec 16	15.2	1.9	37.8	1.9	5.2	1.9	17.8	12.6	15.1	2.5
Jan 12	14.9	9.9	35.5	26	5.4	3.1	16.6	24.2	18.7	4.4
Feb 9	15.3	16.2	28.9	34.1	7.9	12.6	19.5	1.3	16.9	0.6
Mar 8	13.2	2.3	33.3	4.2	7.2	1	18	0.1	16	4.7

Footnotes:

%2-1: % contribution of monoester #1
%2-2: % contribution of monoester #2
%3-1: % contribution of diester #1
%3-2: % contribution of diester #2
%3-3: % contribution of diester #3

APPENDIX III (continued): Data points and 95% confidence limits (C.I.) used in constructing the figures for the yearly study.

Date	wt.1	C.I.	wt.2	C.I.	wt.3	C.I.	wt.rest	C.I.	wt.2-1	C.I.	wt.2-2	C.I.	wt.3-1	C.I.	wt.3-2	C.I.	wt.3-3	C.I.
Mar 7	27.1	8.7	233	54	192	54	36.7	3.2	81.4	26.2	152	50	29.3	10.8	81.5	21.6	81.7	23.1
Apr 18	20.2	10.4	173	36	134	4.5	26.6	3.2	68.6	4.2	104.9	40	23.9	3	56.8	4.2	53.8	1.8
May 4	16.6	6.7	178	26	148	34	27.9	2.7	75.1	2.1	103.2	24.6	29.8	9.2	62.6	17.9	53.9	11.7
larvae	37	89	245	383	145	215	39	87										
Jun 6	11.6	11	165	53	122	63	32	34	68.1	23.3	96.2	33.8	22.6	7.6	52.3	36.2	46.5	33.4
larvae	24.6	24.2	206	274	161	103	36	29										
Jul 11	13.5	7.7	155	182	132	77	18.3	13.4	63.7	187	90.9	31.1	21.8	25.5	58.6	59.5	51.6	22.8
larvae	13.1	15.6	172	378	128	160	20.3	30	69	85	103	316	22.9	27.9	47.9	56.5	57.3	66.2
Aug 11	13.9	9.2	179	15	139.6	10.2	15.5	16.4	71	4	107.8	11.2	21.9	1.1	66.2	9.9	51.6	6.7
Aug 25	16.9	0.9	193	28	150.7	15.1	16	4.5	68	9.9	125.1	31.3	21.4	6.7	67.1	13.4	62.2	20.4
Oct 6	15.1	3.2	146	24	129.9	11.9	15.9	5.5	54.8	10.4	91.6	15.4	18.7	7.2	54.7	8.7	55.8	5.2
Nov 3	15.3	4.7	175	16	140.8	19.9	15.1	3	63.2	22.6	111.3	7.2	18.9	2.5	59	10.4	63.1	9.4
Nov 28	18.7	4.4	160	8	150.9	122	15.6	37.7	54.3	15.3	105.4	22.5	20	30	60.4	53.9	70.9	45.8
Dec 16	13	27	184	17	131.9	38.6	17.7	3.1	52.5	2.5	130.9	17.1	17.8	5.1	61.7	49.4	52.4	4.4
Jan 12	13.6	8.3	228	221	183	100	27.8	3.8	67.4	62.9	160.4	158	24.2	8.3	74.8	90	84.2	1.3
Feb 9	23.6	4.4	222	17	222	148	35	40	76.7	105	145	123	39.8	77	97.9	39.5	84.6	31.4
Mar 8	28.7	1.2	249	50	221.5	29.3	36.8	1.9	70.7	17.4	178.4	31	38.2	4.7	97.2	9.2	85.9	22.8

Footnotes:

wt.1: concentration (conc.) of astaxanthin (μg carotenoids per gram dry tissue)
wt.2: conc. all monoesters
wt.3: conc. all diesters
wt.rest: conc. unidentified fractions
wt.2-1: conc. monoester #1
wt.2-2: conc. monoester #2
wt.3-1: conc. diester #1
wt.3-2: conc. diester #2
wt.3-3: conc. diester #3

APPENDIX IV: The data used in constructing the figures for the feeding experiments.

		ind wt.	car/ind	car/wt.	%1	%2	%3	%rest	wt.1	wt.2	wt.3	wt.rest
T=0		6.8	2.4	359.0	4.5	50.0	41.6	3.9	16.1	179.2	149.1	14.0
T=0		6.7	2.2	333.7	5.0	50.2	39.9	4.9	16.7	167.5	133.1	16.4
T=0		6.5	2.2	345.1	3.8	51.3	40.6	4.3	13.1	177.0	140.1	14.8
T=31	Artemia	5.6	2.24	400.6	5.4	53.0	36.2	5.4	21.6	212.3	145.0	21.6
	S.costatum	4.9	1.98	404.0	5.6	48.4	40.1	5.8	22.6	195.5	162.0	23.4
	A.carterae	4.9	2.10	427.9	5.1	49.4	40.3	5.3	21.8	211.4	172.4	22.7
T=43	Artemia	5.4	2.25	416.6	4.7	55.9	33.5	6.0	19.6	232.9	139.6	25.0
	S.costatum	5.0	2.35	470.2	4.9	49.9	39.3	5.9	23.0	234.6	184.8	27.7
	A.carterae	5.5	2.4	436.1	4.7	50.2	39.9	5.3	20.5	218.9	174.0	23.1

Footnotes:

ind wt: average individual dry wt. (mg)
car/ind: µg of carotenoids per individual
car/wt.: µg of carotenoids per gram of dry tissue
%1: percent contribution of astaxanthin
%2: percent contribution of all monoesters
%3: percent contribution of all diesters
%rest: percent contribution of unidentified fractions
wt.1: µg astaxanthin per gram dry tissue
wt.2: µg monoester per gram dry tissue
wt.3: µg diester per gram dry tissue
wt.rest: µg unidentified fractions per gram dry tissue

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Title of Thesis: Seasonal variation in the carotenoid pigment content and composition of *Euphausia pacifica*

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