# Lipid Changes during Life Cycle of Marine Copepod, *Euchaeta japonica* Marukawa

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## ABSTRACT

All stages from egg to adult of the North Pacific copepod, *Euchaeta japonica* contained wax esters in their lipid stores, while triglycerides were important only in the eggs, early naupliar stages, and adults. The large lipid reserves of the eggs were wax esters and triglycerides (58% and 19% of the lipid, respectively), both of which were used rapidly during the early stages of development. Wax esters continued to decrease after triglycerides had been utilized completely for energy. The slow metabolism of lipid during starvation indicated that lipid stores in adult females may be conserved for egg production. The dominant alcohols of the wax esters of all stages were tetradecanol (24-42% of the total) and hexadecanol (25-65%). Only minor amounts of polyunsaturated alcohols were observed. There was, however, a high proportion of polyunsaturation in the wax ester fatty acids, even though octadecenoic was generally predominant (16-46% of the total wax ester fatty acids). The polyunsaturation of the wax esters fatty acids and the presence of 21:6 hydrocarbon suggest phytoplankton in the diet of adults and in the younger stages. Cholesterol was the main sterol, but there were minor amounts of desmosterol (1-12% of the total sterols) present. The latter sterol has not been found previously in copepods, although reported from Cirripedia and Decapoda.

## INTRODUCTION

The calanoid copepods are an important part of the marine food web because of their predominance in the zooplankton. In previous work, dealing mainly with adult forms, we have noted that wax esters are an important reserve lipid in many species of copepods (1-3). The type of lipids in the early developmental stages of copepods and the possible presence of wax esters in these stages are largely unknown. *Euchaeta japonica* is a common copepod in the North Pacific (4), and adults of the genus *Euchaeta* are known to contain large stores of wax esters (3). The life history and techniques for maintaining the early life history stages are well known from the work by Campbell (5) and Lewis and Ramnarine (6,7). The life history consists of an egg stage, followed by six naupliar and six copepodid stages (the copepodid VI is the adult).

We present, in this article, the results of lipid analyses of the various stages of *Euchaeta japonica*. Both field collected and laboratory reared stages were used. In addition, the rate of utilization of lipids by the adults is given.

## METHODS

*E. japonica* adults (both male and female), copepodid V, copepodid II, and copepodid I stages were collected for analysis by vertical net hauls (0-200 m) at Indian Arm, an inlet of the Strait of Georgia near Vancouver, British Columbia, Canada. The bright blue egg clusters were detached from females and some of the eggs were kept for analysis while the remainder were placed in large glass bowls and raised in the laboratory at 10 C. The colorless first naupliar stage is spent in the egg. So called late eggs were eggs which showed less of the blue color, thus indicating the presence of a high proportion of first naupliar stage individuals; these eggs were analyzed separately. Naupliar stages 2-4 were kept in filtered sea water without feeding. Nauplius stage 5-copepodid II were fed a mixture of algae, *Dunaliella tertiolecta* and *Phaeodactylum tricornutum*. Immature adults (200) were picked from plankton samples and transferred to filtered sea water for starvation experiments. At selected intervals, females were removed for analysis as described below.

The lipids of all stages were extracted by covering live animals with chloroform:methanol (2:1 v/v) and grinding gently with a glass rod. Contact at room temperature (30 min) was sufficient to extract all the lipid. The carcass left after lipid extraction was weighed to allow determination of lipid as a percent of dry wt. All subsequent work was carried out under nitrogen. The lipid was weighed and, for those stages where more than 6 mg was available, fraction-

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TABLE I

Lipid Changes during Development of *Euchaeta japonica*[a]

| Stage | Lipid/individual (mg) | Lipid (% dry wt) | Wax ester (% of lipid) | Triglyceride (% of lipid) |
|---|---|---|---|---|
| Eggs | 0.59[b,c] | 64.4 | 58 | 19 |
| Eggs (late stage) | 0.39[b] | 58.1 | 50 | 17 |
| Nauplius 2 | 0.02 | 43.8 | 61 | 17 |
| Nauplius 3 | 0.02 | 30.8 | 56 | 5 |
| Nauplius 4 | 0.02 | 25.0 | 20 | 3 |
| Nauplius 5 | 0.04 | 21.2 | 15 | 1 |
| Nauplius 6 | 0.04 | 17.0 | 12 | absent |
| Copepodid I | 0.03 | 14.2 | 9 | absent |
| Copepodid I (field collected) | 0.05 | 23.6 | 29 | absent |
| Copepodid II | 0.03 | 11.6 | 12 | absent |
| Copepodid IV | 0.20 | 31.2 | 40 | 3 |
| Copepodid V | 0.52 | 50.1 | 81 | 2 |
| Adult ♀ (immature) | 0.44 | 41.3 | 54 | 18 |
| Adult ♀ (mature) | 0.60 | 52.2 | 60 | 17 |
| Adult ♂ (mature) | 0.58 | 49.2 | 78 | 9 |

[a]Egg sacs were removed from females and placed in filtered sea water. Naupliar stages 2-4 were reared in filtered sea water at 10 C without feeding. Naupliar stages 5-copepodid II were raised in the laboratory by feeding them a mixture of phytoplankton. The remaining stages were field collected.

[b]Per egg cluster.

[c]Average 0.04 mg lipid/egg.

ated on a silicic acid column. Different lipid classes were eluted with solvents of increasing polarity, as described by Nevenzel, et al., (8) and weighed. The procedures for analyzing the five different lipid fractions (hydrocarbons, wax esters, triglycerides, sterols, and phospholipids) or their component acids and alcohols by gas liquid chromatography (GLC) are given in a previous paper (9). A Varian Aerograph (series 1800) gas chromatograph equipped with a digital print-out for peak areas was used for the work. The two columns used were a 2.4 m x 3.2 mm (outside diameter) column of 10% diethylene glycol succinate polyester (DEGS) and a 1.8 m x 3.2 mm column of 3% OV-1 on 60-80 mesh Gas-Chrom P (both supplied by Applied Science Laboratories, State College, Pa). Several different temperatures were used, depending upon the compounds being analyzed. Hydrocarbons were run at 150 and 170 C on OV-1 and DEGS, methyl esters of fatty acids at 160 and 190 C on DEGS, and at 170 and 200 C on OV-1, trifluoroacetate derivatives of long chain alcohols at 150 and 180 C on DEGS and 160 and 180 C on OV-1, and the sterol trifluoroacetates were run at 250 C on OV-1. Hydrogenation of fatty acid and alcohol mixtures was carried out to aid in identifications. The structures of some fatty acids were verified using an LKB gas chromatography-mass spectrometer (GC-MS) (model 9000).

When less than 6 mg total lipid was available, the lipids were separated by silicic acid thin layer chromatography (TLC), and the amounts of wax esters and triglycerides were determined spectrophotometrically by the procedure of Armenta (10) using dichromate digestion and measurement at 350 nm.

The procedures used to separate, identify, and quantitate the phospholipids were essentially those described by Parsons and Patton (11) using TLC and phosphorus analysis. The identities of the predominant phospholipids were confirmed by IR spectrum (Perkin-Elmer Infracord spectrometer, model 137) of the lipids after deposition as thin films on KBr pellets.

## RESULTS

### Changes in Lipid during Development

Wax esters were present in all stages of the life history of *E. japonica*, but triglycerides were important constituents only in the eggs, the early naupliar stages (to N2), and the adults (Table I). The eggs when first removed from the female had 0.59 mg lipid/egg cluster (0.04 mg/egg), but, for eggs in the late stage of development, this value had dropped to 0.39 mg of lipid/egg cluster (0.03 mg/egg). Lipid made up a decreasing proportion of the dry wt in the stages from nauplius 2 to copepodid II, although the absolute amount of lipid/animal was at a minimum until nauplius 4 (in nature, feed-

ing commences with the third naupliar stage but is not appreciable until nauplius 4) and then increased to a somewhat higher plateau from nauplius 5 until copepodid II. The lipids then increased rapidly to high contents (31-52%) in copepodid IV to adults. Triglycerides were used preferentially as energy source until, by nauplius stage 3, they had been largely depleted, and the major utilization of wax esters began. By nauplius stage 6, triglycerides had been used up completely, and even the wax ester content was approaching the minimum seen in copepodid stages I and II (ca. 0.4 μg wax esters/individual).

So called immature and mature adults (females) were analyzed separately. Mature adults showed internal evidence of developing eggs, whereas immature adults had no evidence of eggs. Mature adults were higher in lipid and wax ester content than immature adults, yet still did not have sufficient lipid to account for the total in the eggs. Thus, our mature adults may have been in the process of accumulating lipid stores for later transfer to the eggs.

#### Lipid Utilization by Starved Adults

Since the eggs are so high in lipid, the female must use most of her lipid stores for the production of eggs. One female apparently can produce several clutches of eggs, since animals with attached egg sacs were observed to be forming additional eggs. Although *E. japonica* reproduces throughout the year (5, 6), we assume that, during the winter, the availability of food is quite low so that utilization of lipid by adults must be carefully controlled during this period to produce eggs with sufficient fat stores. In starved immature females, the amount of total lipid went down slowly, confirming the hypothesis of careful control over wax ester mobilization. For the first seven days of starvation, they utilized triglycerides, with little or no depletion of wax ester stores (Table II). After the seventh day, when most of the triglycerides had been used, the wax esters began to be metabolized, and, by the end of the experiment, this lipid type was reduced to ca. a third of its original content.

Starvation experiments with mature females also showed rapid utilization of triglycerides but much slower utilization of wax esters, so that only after starvation for 37 days had the wax esters dropped to a third of their original content.

TABLE II

Starvation Experiments with *Euchaeta japonica*[a]

| Starvation time (days) | Lipid (% dry wt) | Wax ester (% of lipid) | Triglyceride (% of lipid) |
|---|---|---|---|
| 0 | 41 | 54 | 18 |
| 7 | 39 | 68 | 2 |
| 10 | 32 | 46 | absent |
| 12 | 33 | 38 | absent |
| 15 | 27 | 26 | absent |

[a]Immature adult females were collected in the field and transferred to filtered sea water (at 10 C).

#### Fatty Acids and Long Chain Alcohols

Table III presents the fatty acids and alcohols of the wax esters, triglycerides, and phospholipids in several stages of *E. japonica*, including eggs, copepodid V, and adults. Immature, mature, and seven day starved immature adults were analyzed. Only the data for adult females is reported, but the fatty acid and alcohol analyses for mature males were similar.

The 14:0 and 16:0 alcohols accounted for 21-24% and 52-65%, respectively, of the total alcohols in eggs and mature adults (copepodid Vs also showed a predominance of these alcohols). Beside major amounts of 14:0 and 16:0 alcohols, immature adults had 15% of 20:1 and 9% of 22:1 homologues; however, in the eggs, the latter two constituents accounted for less than 1% of the alcohols. There was no correlation between the fatty acids and alcohols of the wax esters in any stage of *E. japonica*.

There were a broad spectra of fatty acids in the different stages, and several interesting observations can be made. In most stages, the 18:1 fatty acid predominated in the wax esters, constituting 41-46% of the total in eggs and copepodid V and 16-27% in adults. The wax esters of immature adults were characterized by 40% of 16:2 fatty acid, while in other stages this fatty acid never accounted for more than 4% of the total. In a second collection of immature adults the amount of 16:2 acid in the wax esters was 12% and subsequent collections of immature adults has revealed that the amount of this acid is highly variable. The identification of the 16:2 acid from *Euchaeta* was based upon the fact that it had an identical retention time on the polar column with authentic 16:2 acid prepared from spinach chloroplasts. The one peak seen for 16:1, 16:2, and 16:3 acids on the nonpolar column was equal in amount to the sum of these individual peaks seen on the polar column. Also, hydrogenation gave the expected amount of 16:0 acid. The 14:1 fatty acid of the wax esters of immature adults was identified tentatively on similar grounds. The predominant fatty acids of triglycerides were 16:0, 18:1, and 20:5. The fatty acids of wax esters and triglycerides of different stages were similar, with the exception that in

TABLE III

Compositions (wt %) of Fatty Acids and Long Chain Alcohols of Different Stages of *Euchaeta japonica*[a]

| Homologue | Eggs | | | | Copepodid V | | | | Immature adults | | | | Mature adults | | | | Immature adults[c] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | WE | WE | TG | PL | WE | WE | TG | PL | WE | WE | TG | PL | WE | WE | TG | PL | WE | WE | TG | PL |
| | AL | FA | FA | FA | AL | FA | FA | FA | AL | FA[b] | FA | FA | AL | FA | FA | FA | AL | FA | FA | FA |
| 14:0 | 21 | 2 | 9 | 9 | 42 | 1 | 7 | 12 | 36 | 3 | 7 | 3 | 29 | 8 | 9 | 9 | 26 | 2 | 7 | 5 |
| 16:0 | 65 | 3 | 20 | 21 | 53 | 1 | 31 | 29 | 29 | 5 | 17 | 14 | 52 | 4 | 18 | 14 | 50 | 1 | 14 | 14 |
| 16:1 | ND | 14 | 10 | 3 | ND | 16 | 7 | 2 | 1 | 6 | 10 | 1 | ND | 10 | 11 | 7 | ND | 10 | 9 | 5 |
| 16:2 | ND | 2 | ND | ND | ND | 2 | 1 | Tr | ND | 39 | 1 | ND | ND | 4 | 1 | Tr | ND | 1 | 2 | Tr |
| 16:3 | ND | ND | ND | ND | ND | ND | 1 | ND | ND | 4 | Tr | ND | ND | 4 | 11 | ND | ND | 1 | 14 | ND |
| 18:0 | 3 | Tr | Tr | 10 | 2 | Tr | 2 | 11 | 2 | 1 | Tr | 5 | 4 | Tr | 1 | 5 | 8 | Tr | 2 | 8 |
| 18:1 | 5 | 47 | 30 | 19 | 1 | 41 | 16 | 14 | 3 | 16 | 29 | 10 | 4 | 27 | 25 | 16 | 9 | 53 | 23 | 12 |
| 18:2 | ND | 3 | 3 | 1 | Tr | 3 | 4 | 1 | Tr | Tr | 4 | 1 | Tr | 5 | 5 | 2 | Tr | 4 | 2 | 3 |
| 18:3 | ND | 1 | 3 | 3 | ND | 2 | 3 | 1 | ND | 3 | 4 | Tr | ND | 6 | 3 | Tr | ND | 2 | 5 | Tr |
| 18:4 | ND | Tr | 3 | ND | ND | 2 | 3 | ND | ND | 3 | 3 | ND | ND | 2 | 2 | ND | ND | 3 | 2 | ND |
| 20:1 | 1 | 1 | Tr | 1 | Tr | Tr | 2 | 5 | 15 | 3 | 3 | 1 | 3 | 3 | 1 | 11 | 3 | 2 | 1 | 2 |
| 20:4 | ND | Tr | Tr | 2 | ND | Tr | 3 | 1 | ND | Tr | 2 | 2 | ND | Tr | 1 | 8 | ND | 1 | 1 | Tr |
| 20:5 | ND | 10 | 17 | 8 | ND | 17 | 10 | 5 | ND | 3 | 14 | 11 | ND | 14 | 7 | 9 | ND | 10 | 10 | 11 |
| 22:1 | 1 | ND | ND | ND | Tr | Tr | ND | ND | 9 | ND | ND | Tr | 2 | ND | ND | ND | 1 | ND | ND | ND |
| 22:5 | ND | ND | Tr | ND | ND | Tr | Tr | Tr | ND | ND | ND | 4 | ND | Tr | ND | Tr | ND | 1 | ND | 2 |
| 22:6 | ND | 15 | 3 | 15 | ND | 10 | 6 | 9 | ND | 1 | 4 | 40 | ND | 8 | 2 | 17 | ND | 6 | 4 | 30 |
| 24:1 | 1 | ND | ND | ND | Tr | ND | ND | ND | ND | ND | ND | 1 | ND | ND | ND | ND | 1 | ND | ND | ND |

[a]Only the principal components are given and they are rounded off to the nearest whole percent. AL = long chain alcohols, FA = fatty acids, WE = wax esters, TG = triglycerides, PL = phospholipids, ND = not detected, and Tr = trace.

[b]A principal fatty acid of this wax ester was 14:1 (9%).

[c]Starved seven days.

all cases the 16:0 fatty acid was a major component of triglycerides (14-30%), but only minor amounts of this fatty acid were in the wax esters (1-5%).

The phospholipid fatty acids were highly unsaturated in the adults, but a predominance of saturated fatty acids was evident in the phospholipids of eggs and copepodid V. As expected, the phospholipid fatty acid pattern in eggs was closer to that of mature adults than to that of immature adults, since mature adults contain developing eggs. During starvation, there were no changes in the phospholipid fatty acids, but the fatty acid composition of wax esters was altered dramatically, especially with respect to the relative amounts of 14:1, 16:2, 18:1, 18:2, and 20:5 fatty acids.

Our first analyses of the copepodid V phospholipid fatty acids were questioned because of low values 20:5 and 22:6. Because of this criticism and to verify the other data, we made a second collection of copepods from the same area and at the same time of the year. Analysis of all phospholipids was completed within two days of the end of the cruise, and the relative amount of 20:5 and 22:6 in copepodid V, as reported here, was higher than we previously found, although still much lower than the levels of these fatty acids in the adults. The fatty acids and alcohols of the other lipid classes were not found to be significantly different, with the exception of the 16:2 acid in immature adults noted above, for copepod stages collected during the two cruises.

#### Lipid Composition Data

Wax esters were the dominant lipid type in egg, copepodid V, and adult stages, and neutral lipids (wax esters plus triglycerides) accounted for ca. 80% of the total lipid; the structural lipids (phospholipids and sterols) amounted to less than 20%. The chain length (alcohol plus fatty acid) composition of wax esters showed no major differences in the different stages with a range of carbon numbers from $C_{26}$-$C_{40}$ (Table IV). In all stages, $C_{32}$ and $C_{34}$ were the major components, totaling 47-93% of the wax esters and $C_{36}$, $C_{38}$, and $C_{30}$ (generally in that sequence) accounting for most of the remainder. The predominance of $C_{32}$ and $C_{34}$ wax esters would be expected, since 18:1 was the main fatty acid and 16:0 and 14:0 were the dominant alcohols.

Hydrocarbons were less than 2% of the lipid, with pristane as the major hydrocarbon in all stages (30-50% of the total hydrocarbon). Of special interest was the presence in all stages of 21:6 hydrocarbon which was previously reported in phytoplankton (12-14). Between 30-40% of the hydrocarbons of adults and copepodid V and 7% of the egg hydrocarbons were composed of this polyunsaturated hydrocarbon. In addition, we noted minor amounts of a series of straight chain saturated and monounsaturated hydrocarbons ranging in length from $C_{17}$-$C_{26}$.

The so called polar lipid fraction, including free fatty acids, pigment, and sterols, accounted for 8-12% of the lipid. The main pigment was astaxanthin in all stages. As collected, the eggs were a bright blue, but, when extracted with chloroform, a red-orange solution was obtained. In benzene solution, the egg pigment had an absorption spectrum identical to that of authentic astaxanthin. The eggs of the barnacle *Lepas fasicularis* also contain a blue chromoprotein in which the main pigment is astaxanthin (15). Recently, Zagalsky and Herring (16) purified a blue carotenoprotein containing astaxanthin from the pontellid copepod, *Labidocera acutifrons*. The astaxanthin of the eggs is metabolized in the early naupliar stages of *E. japonica* so that late naupliar stages are colorless. The copepodid stages apparently have the ability to synthesize astaxanthin.

The major sterol of all stages was cholesterol (0.1-0.8 wt percent of the total lipids). Desmosterol, generally a minor constituent (less than 1%), amounted to 12 and 26% of the total sterols in, respectively, the seven day starved adults and the eggs. The source of this desmosterol is most probably dietary, since it generally is accepted that crustaceans, like other arthropoda, cannot biosynthesize cholesterol de novo but can convert dietary phytosterols to cholesterol via desmosterol (17). Why desmosterol should seemingly accumulate during starvation is not clear.

The phospholipids of eggs, adult male, and adult female were analyed by TLC. The major phospholipids of the adults were phosphatidyl choline (51-62%), phosphatidyl ethanolamine (32-47%), sphingomyelin (3-5%), phosphatidyl inositol (5-7%), and lysophosphatidyl choline (1-2%), while the egg phospholipids were phosphatidyl choline (67%), phosphatidyl ethanolamine, and phosphatidyl inositol (1%). No sphingomyelin was detected in the egg phospholipids.

## DISCUSSION

The data presented here demonstrate the important role of wax esters in providing sustenance for the young naupliar stages of *E. japonica*. The wax esters and triglycerides of the eggs must provide all the energy for the nauplius 1 and nauplius 2 stages since these are nonfeeding stages, and, in fact, *Euchaeta* can be

TABLE IV

Wax Ester Compositions of *Euchaeta japonica* Stages (wt %)

| Stage | Eggs | Late eggs | Naupliar 2 | Naupliar 5 | Naupliar 6 | Copepodid I | Copepodid II | Copepodid IV | Adult females | | | Adult males |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Mature + eggs | Mature − eggs | Starved[a] | |
| Chain lengths of wax esters | | | | | | | | | | | | |
| $C_{28}$ | | 0.06 | | | | 0.05 | | | 0.29 | 0.06 | 0.37 | 1.2 |
| 29 | | 0.3 | | | | | | | | 0.3 | | |
| 30 | 3.8 | 4.9 | 4.4 | 4.4 | 9.1 | 4.9 | 3.9 | 7.3 | 10.6 | 10.2 | 9.1 | 8.1 |
| 31 | 0.6 | | | 0.8 | 1.4 | | 0.2 | 0.9 | | | | |
| 32 | 19.0 | 28.1 | 27.7 | 28.7 | 40.1 | 31.0 | 35.9 | 34.3 | 35.7 | 37.2 | 35.1 | 36.6 |
| 33 | 0.8 | 1.4 | | 2.2 | | | | 1.4 | | | | |
| 34 | 28.4 | 42.6 | 42.1 | 46.3 | 38.8 | 50.2 | 57.8 | 26.6 | 34.0 | 37.2 | 34.7 | 37.8 |
| 36 | 14.1 | 18.4 | 14.3 | 12.8 | 10.6 | 12.0 | 2.2 | 18.1 | 12.1 | 11.9 | 12.0 | 10.3 |
| 38 | 16.3 | 4.1 | 11.4 | 4.7 | ND[b] | 1.8 | ND | 11.4 | 7.2 | 2.9 | 7.8 | 4.5 |
| 40 | 6.7 | 0.07 | ND | Tr[c] | | ND | | Tr | ND | 0.2 | 0.7 | 0.5 |
| 42 | 6.6 | ND | | ND | | | | ND | | ND | ND | 0.3 |
| 44 | 2.3 | | | | | | | | | | | |

[a]Immature, starved 7 days.

[b]ND = not detected.

[c]Tr = trace.

raised to copepodid I without feeding (18 and A.G. Lewis, unpublished data). Since *E. japonica* reproduces throughout the year (6), the ability of young stages to use lipid stores would be important in the winter when food supply is low.

The egg clusters from females averaged 0.59 mg lipid, while mature adults with developing eggs had a total of only 0.60 mg lipid. Our mature copepods still may have been feeding so that their lipid stores had not yet reached maximum values at the time of analysis. Another explanation may be that the mature adults which we collected in September would not be producing their egg clusters until October or later; at this later time, there are fewer eggs/cluster (6) and possibly less lipid/egg. Maximum egg production takes place during the spring when lipid stores in the adult are probably at their maximum. Littlepage (19) has noted a large increase in lipid in *Euchaeta antarctica* during the summer when egg production occurs in this copepod. Corkett and McLaren (20) suggested that *Pseudocalanus* lengthens its period between egg production during times of low food. The slow utilization of lipid by starving females (Table II) indicates that lipid stores may be saved for egg production.

The eggs of *Euchaeta media*, collected off California, and *Euchaeta marina*, collected near Tahiti, were also rich in lipid with wax esters accounting for 72 and 58% of their lipid, respectively (21). Thus, most species of *Euchaeta*, regardless of environment, are assumed to have wax esters in their egg lipid stores. Lipid stores are present in the eggs of many marine invertebrates (22-24), and the thorough study of lipids in the young stages of the barnacles, *Balanus balanoides* and *Balanus balanus*, by Dawson and Barnes (25) demonstrated that the eggs of the barnacles were rich in triglycerides, which were consumed rapidly during development.

The wax esters of the filter feeding copepods, such as *Calanus* and *Rhincalanus* resemble their phytoplankton diet in having high proportions of polyunsaturated fatty acids (3, 9, 26). However, the wax ester fatty acids of deep water predatory copepods are mainly saturated and monounsaturated, with 18:1 accounting for over 50% of the total (3). Our data indicate that *E. japonica* is omnivorous, since the wax ester fatty acids show both high polyunsaturation and high 18:1 content (16-50%). This agrees with Pandyan's (27) observation that adult *E. japonica* would feed on both large phytoplankton and zooplankton. The immature adults seem to be exceptions with their high content of 16:2, a characteristic fatty acid (together with the 16:3, 18:3, and 18:4 homologues) of phytoplankton (28, 29). Immature adults collected at different times had quite variable contents of 16:2 acid. This could result if they graze extensively on phytoplankton for brief periods, since marine phytoplankton fatty acids may contain up to 14% of the 16:2 homologue (29). Similarly, in marine fish, the presence of small amounts of such acids, variable with season, has been interpreted as temporary survival from dietary phytoplankton (30). In both *Euchaeta* and fish, the characteristic phytoplankton acids are significant constituents only in the neutral storage lipids. The dominance of hexadecanol and absence of appreciable polyunsaturation in the wax ester alcohols suggest that the carbon chains of phytoplankton fatty acids are not converted directly into alcohols in *E. japonica*. Hexadecanol previously has been noted as the dominant alcohol of the wax esters of deep water copepods and fish (3, 31).

The presence in *E. japonica* of 21:6 hydrocarbon, which is synthesized by phytoplankton (12), also indicates the presence of phytoplankton in the diet of the various stages. The presence of 21:6 hydrocarbon in the eggs shows that the adult is able to transfer this hydrocarbon to the eggs. Because of the sizeable amounts of 21:6 hydrocarbon in the adults and copepodid V, it would appear that phytoplankton is important in the diet of these stages. Among all the other copepod species examined, including herbivorous species of *Calanus* (12), only *Rhincalanus nasutus* contained this hydrocarbon. The reason for its retention by *Euchaeta japonica* and *Rhincalanus nasutus* is not clear.

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