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Journal

Marine Ecology Progress Series, 42(2)

ISSN

0171-8630

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Publication Date

1988

DOI

10.3354/meps042143

Peer reviewed

Vol. 42: 143–153, 1988 | MARINE ECOLOGY – PROGRESS SERIES Mar. Ecol. Prog. Ser. | Published February 18

Sources of variability in measurements of copepod lipids and gut fluorescence in the California Current coastal zone

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ABSTRACT: The spatial association of zooplankton nutritional state with particulate food abundance was tested along the central California coast in March 1986. Suspension-feeding copepods *(Metridia pacifica, Calanus pacificus californicus, Rhincalanus nasutus, Eucalanus californicus)* and suspended particulate matter were sampled at stations with median nearest neighbor distance of 19.6 km. In this field condition of relatively small horizontal gradients in food supply, copepod gut fluorescence, triacylglycerols and wax esters showed little or no association with spatial variations in particulate nitrogen, particulate carbon or chlorophyll *a*. In contrast, strong diel variation in gut fluorescence was observed including post-sunset increases in ingestion. For the conditions observed during this study, diel variations in feeding behavior explained more of the variance in copepod ingestion than did horizontal variations in food supply. Efficiency of extraction of copepod lipids by homogenization and by standing in chloroform : methanol was also compared. A new double development, single scan method for separation and analysis of zooplankton lipids by Thin Layer Chromatography/Flame Ionization Detection is presented.

INTRODUCTION

Responses of marine zooplankton to spatial variations in food supply are of broad interest because of the effects of resource limitation on recruitment rates of natural populations (e.g. Checkley 1980, Frost 1985, Lampert 1985, Runge 1985, Razouls et al. 1986, Hirche & Bohrer 1987). This article reports on the spatial association of zooplankton nutritional state with suspended particulate concentrations in a nearshore region of the California Current. The study was conducted in late winter conditions of relatively small lateral gradients, as determined on a scale of 9 to 30 km. The magnitude of spatial variations in zooplankton nutrition is also contrasted with that of diel variations.

Zooplankton nutritional state was inferred from 3 measures that differ in characteristic response time to altered concentrations of food substrates. Gut fluorescence, an index of recent ingestion of phytoplankton containing chlorophyll *a* (Nemoto 1968), responds within minutes to altered food concentrations (e.g. Dagg 1983, Mackas & Burns 1986). Triacylglycerols, a class of storage lipids common among both aquatic and terrestrial organisms (Hadley 1985), change within several hours to a day or more of exposure to altered food abundance (Lee et al. 1971, Sargent et al. 1977a, Hakanson 1984). Wax esters, another class of depot lipids that is widely distributed among marine metazoans (Benson & Lee 1975, Sargent et al. 1977b), are usually metabolized more slowly than triacylglycerols. Biosynthesis of copepod wax esters occurs within 1 to 4 d of exposure to high substrate concentrations (Sargent et al. 1977a, Hakanson 1984) and depletion begins within 2 to 7 d after the onset of starvation (Lee et al. 1971, Sargent et al. 1977a, Hakanson 1984). For zooplankton in a state of reduced metabolic activity, however, wax ester catabolism may extend to several weeks to months (Lee 1974, Ohman 1987), in which case lipid content will reflect not recent, but rather previous ingestion.

Four species of calanoid copepod commonly found in the California Current are contrasted in their nutritional status: *Metridia pacifica* Brodskii, *Calanus pacificus californicus* Brodskii (hereafter *C. pacificus*), *Rhincalanus nasutus* Giesbrecht, and *Eucalanus californicus* Johnson. In addition, the efficiency of extraction of copepod lipids by 2 methods is compared,

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and a new solvent system is proposed for separating copepod lipids for analysis by Thin Layer Chromatography/Flame Ionization Detection.

METHODS

Sampling and suspended particulate analysis. Sampling was conducted along the Central California coast between 16 and 20 March 1986. The stations where zooplankton and suspended particulate matter were collected were embedded in an array of stations for conductivity-temperature-depth (CTD) profiling and deployment of satellite-tracked drifters (Fig. 1). The median nearest neighbor distance between zooplankton stations was 19.6 km (range 8.8 to 29.7 km).

Suspended particulate matter (SPM) was collected in Niskin or Nansen bottles and aliquots filtered onto Whatman GF/C glass fiber filters for phytoplankton pigment analysis or Whatman GF/F filters for analysis of particulate nitrogen (PN) and particulate carbon (PC). Aliquots filtered for PN and PC analysis were prescreened through a 183 μm mesh Nitex screen to exclude larger organisms. The maximum sampling depth for PN and PC was usually 80 m while pigments were usually sampled to 240 m. Vertical integrations of all 3 SPM constituents were made to 80 m depth, using the trapezoidal rule. Chlorophyll *a* (chl *a*) and pheopigments were extracted in refrigerated 90 % aqueous acetone in the dark for 26 to 57 h and analyzed on a Turner Designs model 10 fluorometer. Filters for PN and PC analysis were precombusted at 500 °C, aliquots filtered, filters dried at 55 °C then analyzed on a Hewlett-Packard model 185B CHN analyzer according to Sharp (1974). Chl *a* was analyzed at one station more than PN and PC.

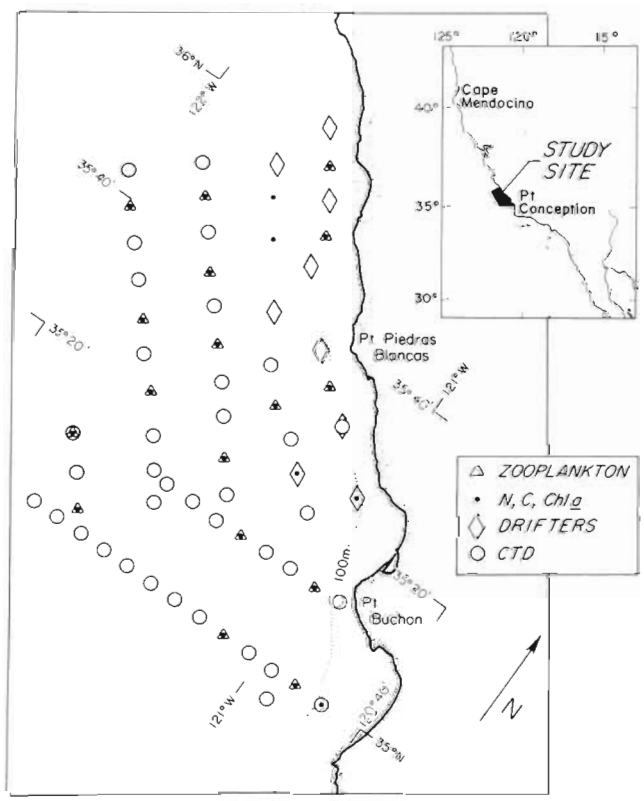

Fig. 1 Sampling locations along the central California coast, 16 to 20 Mar 1986. Legend identifies stations sampled for macrozooplankton (> 202 μm), particulate nitrogen, particulate carbon, chlorophyll *a*, CTD profiling and the deployment locations for satellite-tracked drifters

Zooplankton collections were made, on average, 35 min after bottle casts for SPM. Animals were collected in a 202 μm mesh, 71 cm diameter bongo net towed obliquely between 210 m and the surface, or from shallower depths if restricted by bathymetry. Zooplankton was promptly frozen in liquid nitrogen aboard ship and maintained in liquid nitrogen prior to analysis of lipids, gut fluorescence and dry mass. Copepods for dry mass analysis were rinsed briefly in distilled water then added to precombusted, tared aluminum boats in lots of 2 to 5 individuals. After desiccation at 55 °C animals were weighed on a Cahn 29 electrobalance.

Gut fluorescence. Fluorometric analysis of copepod gut contents was performed by rinsing animals briefly in distilled water then macerating whole copepods in 90 % aqueous acetone. Between 2 and 4 individuals selected without bias were pooled per analysis; a single animal was sometimes analyzed in the case of the less abundant copepodid Stage V. A Turner Designs model 10 fluorometer was used to assay for chlorophyll *a* and pheopigments. Usually 10 replicate extractions (range: 1 to 10) were analyzed per station, the number dictated by the availability of animals in each collection. Total pigment content per copepod was obtained by summing the mass of chl *a* and pheopigments derived from the Strickland & Parsons (1972) equations. No correction was made for possible degradation of pigments by copepods as reported by Conover et al. (1986), as these losses appear to be variable (Shuman & Lorenzen 1975, Baars & Helling 1985, Wang & Conover 1986) and apparently of lower magnitude than determined by Conover and co-workers (see Dagg & Walser 1987, Kiørbe & Tiselius 1987). Background fluorescence of each species/stage in the absence of ingested pigments was determined by holding live animals in filtered seawater in the dark for 2 to 3 d at 15 °C, then extracting and analyzing pigments as above.

Lipid extraction and analysis. Although standard procedures for extracting lipids from metazoans call for homogenizing (Kates 1986) or sonicating (Parrish 1987) tissues, some analysts of zooplankton lipids omit this

step. An extraction experiment was therefore conducted to compare the efficiency of extraction of different copepod lipid classes by standing in a solvent mixture versus mechanical homogenization according to the method of Bligh & Dyer (1959). The temperature-dependence of extraction rate was also evaluated. Replicate lots of adult females of *Calanus pacificus* (n = 40 ind. per extraction) and *Rhincalanus nasutus* (n = 20) collected from a single net haul in the study site were sorted into vials. Chloroform:methanol (2:1, v/v) was added to the vials which were then purged with nitrogen gas and placed in the dark at −20°C, +1°C or +23°C. At intervals (12, 24, 48 and 72 h) the extracting solvent was removed, copepods were washed 2× with CH_3Cl:MeOH and the total solution analyzed for lipid content by Thin Layer Chromatography/Flame Ionization Detection (TLC/FID). After solvent removal, fresh CH_3Cl:MeOH was added to each vial and extraction continued under nitrogen. After 72 h, the CH_3Cl:MeOH mixture was removed, animals macerated in CH_3Cl:MeOH:H_2O (1:2:0.8, v/v/v) with a glass homogenizer, then taken through the remaining extraction procedure of Bligh & Dyer (1959) with the addition of a benzene washing step (Kates 1986). Bligh & Dyer extraction was conducted twice then all lipids analyzed by TLC/FID. Extracted copepod lipids were maintained at −20°C in a N_2 atmosphere for less than 2 wk prior to analysis.

Lipids were analyzed by Thin Layer Chromatography/Flame Ionization Detection (TLC/FID) using an Iatroscan TH-10 (see Ackman 1981, Parrish & Ackman 1985). Lipids were spotted onto SII Chromarods then separations performed using a new double development, single scan procedure that was suggested by the results of Parrish & Ackman (1983). No single solvent system was found adequate for consistent baseline separation of copepod lipid classes. Chromarods were humidified over saturated $CaCl_2$ and developed first in hexane:diethyl ether (95:5, v/v) for 30 min to separate wax esters. They were then dried at 110°C, rehumidified and developed in hexane:diethyl ether:formic acid (82:18:1.0, v/v/v) for 20 min to separate triacylglycerols, free fatty acids, free alcohols, sterols and phospholipids. Rods were scanned in one uninterrupted pass at 40 s scan^{-1} with hydrogen flow at 160 ml min^{-1} and air flow at 2000 ml min^{-1}. Peak area was recorded on a Shimadzu CR3A integrator. The above solvent systems do not separate sterol esters from wax esters, but no sterol esters were detected in these samples using plate thin-layer chromatography and the reaction of Jatzkewitz & Mehl (1960).

Because standing in solvents without homogenization failed to give complete extraction of all lipid classes, extractions of field samples were performed using the Bligh & Dyer (1959) procedure. Thawed copepods were sorted by developmental stage and species, rinsed briefly in distilled water, lipids extracted and analyzed by TLC/FID. Between 4 and 8 (mode = 5) replicate analyses were done per extraction. The coefficient of variation of replicate analyses varied with mass of lipid applied, declining from an average of 15.5 % at the 1.0 μg lipid application level to 6.2 % at the 10.0 μg level.

Iatroscan calibration was performed with authentic lipid standards purchased from Sigma: phospholipids (PL) – L-α-phosphatidylcholine; sterols (ST) – cholesterol; free fatty acids (FFA) – palmitic acid; triacylglycerols (TAG) – tripalmitin; wax esters (WE) – palmitic acid palmityl ester. The calibration relationship between peak area and lipid concentration was described by power functions which provided better statistical fits than rectilinear functions, based on minimizing the error term and randomizing residuals.

Lipid content and dry mass were determined on separate lots of animals drawn from the same sample. Because the t-distribution is inappropriate for the ratio of these 2 quantities, the standard error of the ratio of lipid/dry mass was estimated as indicated in Enright (1967) and the confidence limit of this ratio approximated as twice the standard error.

RESULTS

Extraction method

Standing in 2:1 chloroform:methanol, in the absence of mechanical disruption, resulted in incomplete extraction of copepod phospholipids (Fig. 2d, h). After 12 h, only 51 to 89 % of phospholipids were extracted from copepods, with the lowest efficiency at −20°C. After 24 h, 94 to 96 % of phospholipids were in solution, but complete extraction of this lipid class required homogenization. In contrast, extraction of wax esters, triacylglycerols and sterols was 99 to 100 % complete after 24 h, and subsequent homogenization and phase separation by the Bligh & Dyer (1959) procedure did not result in the detection of additional lipid (Fig. 2). *Calanus pacificus* lipids extracted more slowly at −20°C than did *Rhincalanus nasutus* lipids. *C. pacificus* storage lipids appear to be localized in a lipid sac while those of *R. nasutus* are more dispersed throughout the body cavity.

Based on these experiments it is recommended that copepods be homogenized if complete extraction of all lipid classes is desired. If only wax esters, triacylglycerols and sterols are to be analyzed, animals should stand in 2:1 chloroform:methanol, under nitrogen, for at least 24 h. Subsequent lipid results reported below were obtained from Bligh & Dyer extractions.

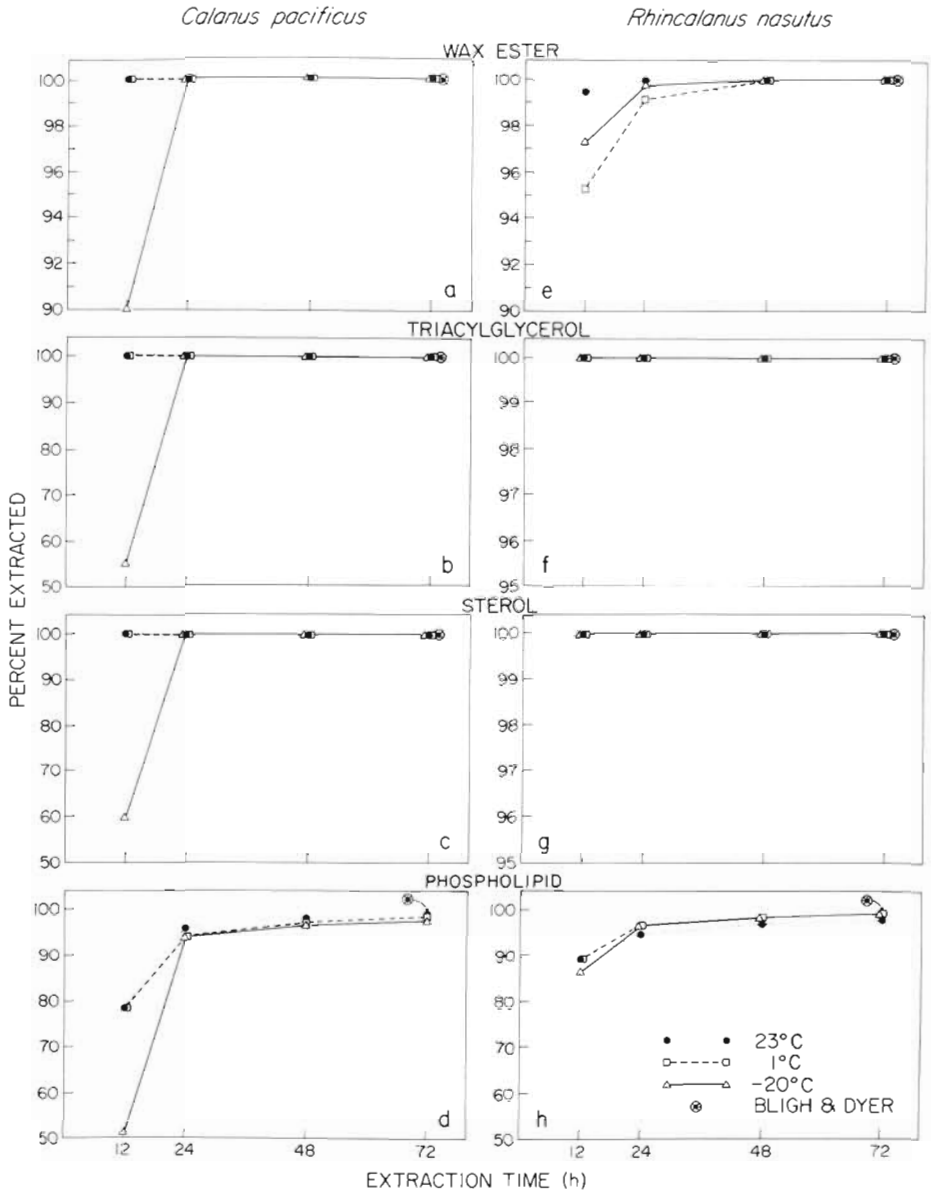

Fig. 2. *Calanus pacificus* and *Rhincalanus nasutus*. Time course of extraction of different lipid classes from adult females. After standing for 72 h in chloroform:methanol, copepods were homogenized according to the method of Bligh & Dyer (1959). Coefficient of variation of replicate analyses averaged 3.8%

Lipid composition

Table 1 presents the body dimensions and lipid content of copepods collected in the study site. Total lipid as a percentage of dry mass varied by developmental stage within a species. In 3 of the 4 species the percentage of dry mass as lipid was nearly twice as high for the copepodid Stage V (CV) as for the copepodid Stage VI female. In *Rhincalanus nasutus*, females had higher lipid content. For all species but *R. nasutus* the depot lipid ratio, i.e. the sum of wax esters and triacylglycerols in CV's divided by that in adult females, was greater than 1. The quantity of depot lipid of CV's was significantly different from that of females for all 4 species ($p < 0.05$, Mann-Whitney U test, 2-tailed). The phospholipid ratio, by contrast, was less than 1 for all 4 species (Table 1). Copepodid Stage V and females differed significantly in quantity of phospholipid in *Metridia pacifica* and *Calanus pacificus* ($p < 0.01$, Mann-Whitney U, 2-tailed) but not in *R. nasutus* and *Eucalanus californicus* ($p > 0.05$).

Differences between developmental stages and among species are further reflected in the distribution of lipid classes (Fig. 3). These data (Fig. 3 & Table 1) illustrate the magnitude of lipid variations observed across the study site. Sterol, free fatty acid and triacylglycerol content did not differ significantly between CV and female for any of the 4 species ($p > 0.05$). Wax ester content tended to be higher in the CV stage than in females for all species except *Rhincalanus nasutus*.

Table 1. Body dimensions and lipid content of copepods collected in the study site during March 1986. N in Total lipid/Dry mass column refers to the number of independent lipid extractions; standard error calculated as indicated under 'Methods'

	Prosome length (μm)	Dry mass (μg)	Total lipid/Dry mass (%)	Depot lipid ratio	Phospholipid ratio
	$\bar{x}$ ± 95 % (n)	$\bar{x}$ ± 95 % (n)	$\bar{x}$ ± 2 SE (N)	$\frac{\text{CV (WE + TAG)}}{\text{♀ (WE + TAG)}}$	$\frac{\text{CV (PL)}}{\text{♀ (PL)}}$
Metridia pacifica CV	1219 ± 26 (32)	64.0 ± 16.4 (4)	14.3 ± 3.2 % (7)	3.79	0.69
Metridia pacifica ♀	1661 ± 17 (45)	101.0 ± 11.0 (23)	8.2 ± 1.1 % (12)		
Calanus pacificus CV	1977 ± 40 (15)	130.6 ± 25.1 (7)	15.8 ± 5.2 % (12)	3.04	0.72
Calanus pacificus ♀	2527 ± 33 (45)	206.7 ± 10.7 (30)	8.7 ± 1.1 % (16)		
Rhincalanus nasutus CV	2772 ± 99 (18)	142.5 ± 12.7 (20)	25.8 ± 4.1 % (15)	0.37	0.77
Rhincalanus nasutus ♀	3670 ± 61 (45)	246.8 ± 20.2 (30)	33.8 ± 5.2 % (16)		
Eucalanus californicus CV	4498 ± 192 (6)	220.3 ± 17.1 (32)	20.8 ± 4.8 % (6)	1.57	0.81
Eucalanus californicus ♀	5512 ± 106 (45)	363.1 ± 23.5 (33)	11.5 ± 2.1 % (14)		

Spatial distributions of suspended particulate matter

Vertical distributions of PN, PC and chl *a* as indicators of particulate food substrates for suspension-feeding zooplankton are illustrated in Fig. 4. The 3 indices of particulate food covaried in a similar manner ($W = 0.810$, $p < 0.001$, Kendall's Coefficient of Concordance). For each variable, the maximum concentration in vertical profile was significantly correlated with the vertically integrated value (PN: $r_s = 0.840$; PC: $r_s = 0.796$; chl *a*: $r_s = 0.935$; $p < 0.001$, Spearman's rho; $n = 21$ or 22 vertical profiles). No significant depth variation of the C:N ratio was observed (Fig. 4d). Temperature differences with depth were also rela-

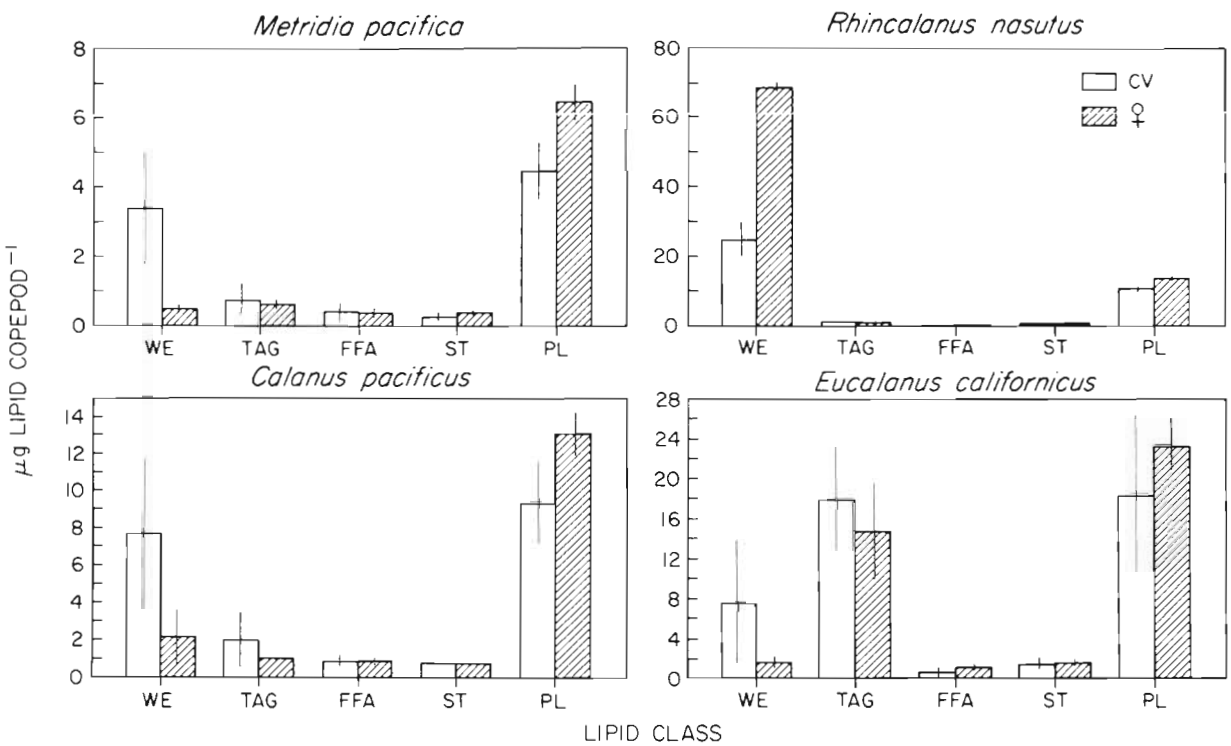

Fig. 3. Comparative lipid class composition of copepodid Stage V (open bars) and adult females (shaded bars) for 4 species of calanoid copepods collected in the study site ($\bar{x}$ ± 95 %). Lipid classes are wax esters (WE), triacylglycerols (TAG), free fatty acids (FFA), sterols (ST) and phospholipids (PL)

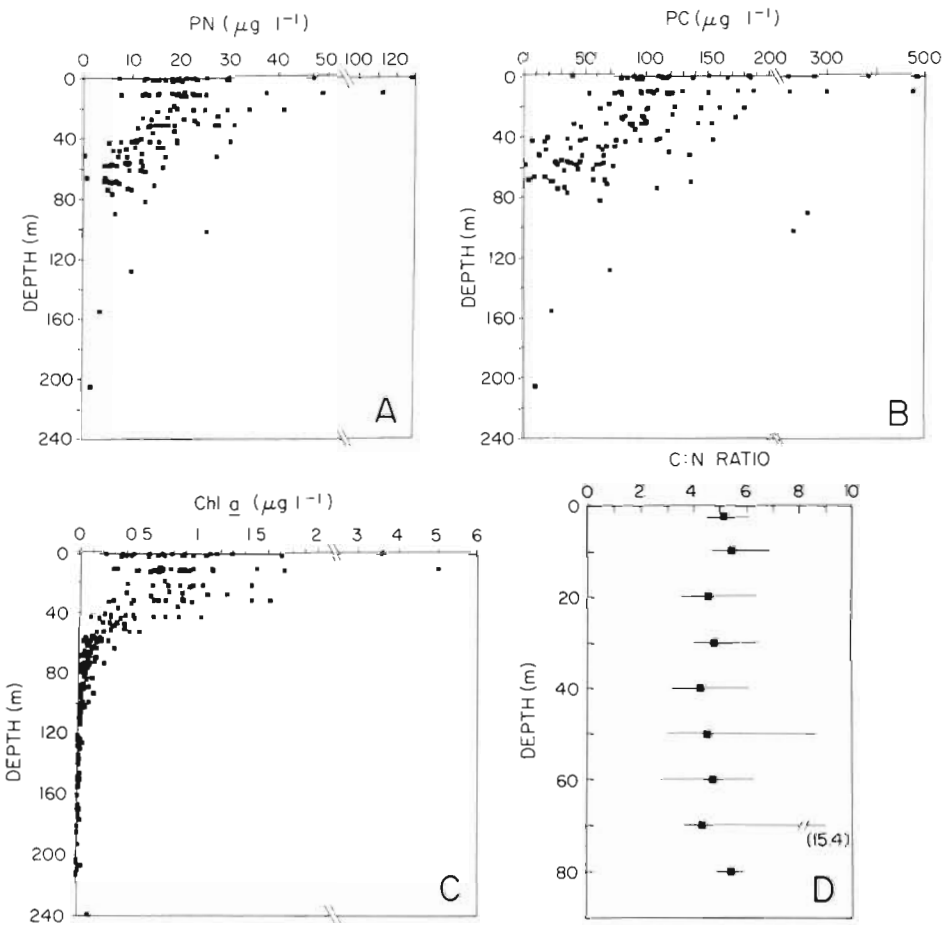

Fig. 4. Vertical distribution of (A) particulate nitrogen, (B) particulate carbon, (C) chlorophyll *a*, (D) C:N mass ratio of particulate matter in the study site. (D) illustrates the median ± 95% CL for data pooled in 10 m depth intervals excepting near-surface values which were pooled between 0 and 5 m

tively small, the difference between 0 and 50 m ranging between 1.3 and 1.9 C° (median 1.5 C°). At no station were surface waters undersaturated with respect to oxygen concentration (T. L. Hayward pers. comm.). Sharp frontal features were not observed, although a region of horizontal shear was detected between the drifters deployed inshore and those slightly further offshore (P. P. Niiler pers. comm.).

The horizontal distribution of particulate nitrogen is illustrated in Fig. 5 as the maximum concentration determined in each vertical profile. Toward the southern end of the study region one value ranged to 128 μg N l^{-1} but in all other profiles PN maxima ranged between 15 and 49 μg N l^{-1}.

Spatial and diel variations of nutrition

A test of the spatial association of wax esters, triacylglycerols and gut fluorescence with the particulate food available in the water column was made using Spearman's rank correlation statistic. Table 2 reveals that spatial variations in wax ester content of copepodid Stage V of all 4 species and of adult females of 3 of 4 species were uncorrelated with spatial variations in suspended particulate matter. One correlation was observed between *Eucalanus californicus* females and chl *a*, though this was not reflected in the relationship with PN and PC. If the α-level for significance is corrected for multiple testing (Miller 1977), allowing for chance correlations, then no significant association was detected between wax ester content and particulate matter for any of the 4 species.

Triacylglycerol content similarly showed no significant relationship in 23 out of 24 tests of correlation with particulate food (Table 2). In the case of gut fluorescence assays enough specimens were available in the samples for determinations with only *Calanus pacificus* and *Rhincalanus nasutus*. Two weak, but significant, correlations were detected between gut fluorescence and chl *a* (Table 2). These correlations were not confirmed by correlation analysis with PN and PC, and correcting for multiple testing would render these non-significant as well. Results of correlation of WE, TAG and GF with pheopigments were very similar to results with chl *a*, with 19 out of 20 correlations non-significant.

Using vertically integrated particulate matter as a

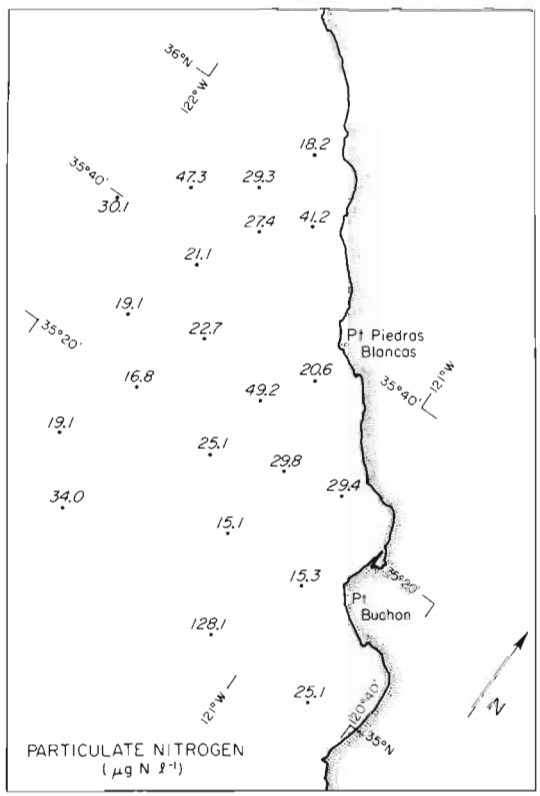

Fig. 5. Spatial distribution of maximum concentration of particulate nitrogen (μg N l^{-1}) determined in vertical profiles at each station

measure of available food, rather than the maximum concentration, very little difference occurred in correlation patterns. Differences included the complete lack of significant correlations between gut pigments and integrated PN, PC or chl *a* ($p > 0.05$), and a significant correlation between *Eucalanus californicus* female wax ester content and integrated PC ($r_s = 0.569$, $p < 0.05$). Again, weak or no association between indices of nutritional status and particulate food was the consistent result.

Turning from spatial variations in gut fluorescence and depot lipids to temporal variations as measured on a diel time scale, a different pattern was observed. Gut pigment content as a function of time of day is illustrated in Fig. 6 with all samples expressed on a common axis irrespective of the collection date. For each of the 4 stage/species combinations, significantly higher gut fluorescence was observed at night than by day ($p < 0.001$, Mann-Whitney U test, 2-tailed). A post-sunset increase in pigment content was observed in all 4 cases. Further, some evidence for a secondary pre-sunrise peak in pigment content was observed for *Calanus pacificus* CV and both stages of *Rhincalanus nasutus*, suggesting possible crepuscular feeding.

In contrast to diel variations in gut fluorescence, no significant day-night difference was observed in triacylglycerol content in any of the 8 species/stages analyzed ($p > 0.10$, Mann-Whitney U). Wax ester content of only *Eucalanus californicus* females was significantly higher in night samples than in day samples ($p < 0.01$). This result is not robust to correction for multiple testing, unlike the result for gut fluorescence.

Two-way analysis of variance tested for interactions between time of day and food concentration in affecting gut fluorescence level. The data were transformed [ln(x + 1)] then pooled by time of day (day or night) and by food concentration (<0.80, 0.80–1.60, >1.60 μg chl *a* l^{-1}). As in the above analysis, a highly significant effect of time of day was observed for all 4 species/stages ($p < 0.01$ or $p < 0.001$) and a barely significant

Table 2. Spearman's rank correlation between vertical maxima in particulate nitrogen (PN), particulate carbon (PC), chlorophyll *a* (chl *a*) and 3 indices of copepod nutrition: copepod wax ester content (WE), triacylglycerol content (TAG), gut fluorescence (GF). Gut fluorescence analyses were performed only on *Calanus pacificus* and *Rhincalanus nasutus*. Reported correlation coefficients are significant at $p < 0.05$; ns = $p > 0.05$

	PN			PC			Chl *a*		
	WE	TAG	GF	WE	TAG	GF	WE	TAG	GF
Copepodid V									
Metridia pacifica	ns	ns		ns	ns		ns	ns	
Calanus pacificus	ns	ns	ns	ns	ns	ns	ns	ns	ns
Rhincalanus nasutus	ns	ns	ns	ns	ns	ns	ns	ns	0.54
Eucalanus californicus	ns	ns		ns	ns		ns	ns	
Adult ♀									
Metridia pacifica	ns	0.62		ns	ns		ns	ns	
Calanus pacificus	ns	ns	ns	ns	ns	ns	ns	ns	ns
Rhincalanus nasutus	ns	ns	ns	ns	ns	ns	ns	ns	0.49
Eucalanus californicus	ns	ns		ns	ns		0.65	ns	

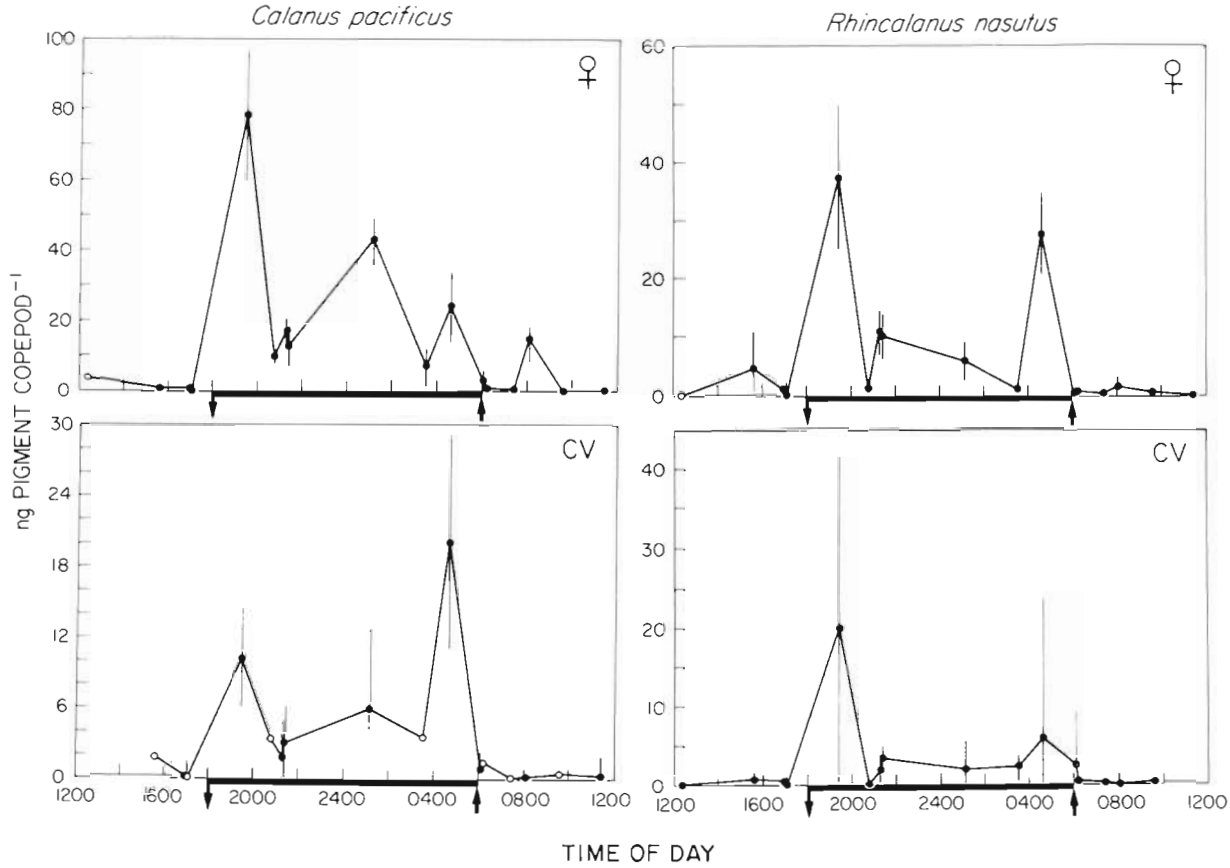

Fig. 6. *Calanus pacificus* and *Rhincalanus nasutus*. Diel variations in gut pigment levels for copepodid Stage V and adult females ($\bar{x} \pm 95\%$). Confidence limits reflect variability among subsamples from a single net haul at each station. Dark horizontal bar: period of darkness, with sunset (↓) and sunrise (↑) also illustrated. Open circles: unreplicated analyses

effect of food concentration was detected for *Rhincalanus nasutus* CV ($p = 0.05$). The interaction of time with food concentration was significant in the case of *Calanus pacificus* CV and *R. nasutus* female ($p < 0.05$). Hence, higher food concentrations at night were associated with higher gut fluorescence in half the cases but in all cases most of the variance in gut fluorescence levels was explained by time of day alone.

DISCUSSION

Stage/species-specific lipid storage

Elevated depot lipid content of copepodid Stage V by comparison with adult females is consistent with laboratory observations that CV storage lipids are metabolized and used in egg production by the female (Lee et al. 1972, Gatten et al. 1980, Ohman 1987). Departure of *Rhincalanus nasutus* from this pattern may reflect a different timing of life history events; developing oocytes were less apparent in female *R. nasutus* than in the other 3 species. Higher lipid content of *R. nasutus* than *Calanus pacificus* is in accord with the results of Lee & Hirota (1973) and with the higher C:N ratio of *R. nasutus* documented by Mullin & Brooks (1970). *Eucalanus californicus* departed from the other 3 species in that triacylglycerols rather than wax esters were the principal storage lipid. This difference among species merits further investigation.

Spatial and diel patterns

The absence of strong spatial correlations between particulate food and copepod nutrition in this study site contrasts with previous studies in this region of the California Current. Cox et al. (1982) observed significant correlations between digestive enzyme (laminarinase) activity of a mixed plankton assemblage and chlorophyll *a* concentration. Willason et al. (1986) found that laminarinase activity of *Calanus pacificus*

collected in the upper 210 m was correlated with surface chl *a*. It should be noted, however, that digestive enzyme activity is not related in a simple manner to substrate concentration (Mayzaud 1986) or to ingestion rate (Hassett & Landry 1983). In the study of Willason et al. (1986), total lipid content of *C. pacificus* showed little spatial association with surface chl *a* concentration, although the relationship was not analyzed statistically and depot lipids were not distinguished from other lipid classes. Hakanson (1985) reported an association between ambient chl *a* and the wax ester and triacylglycerol content of copepodid Stage V of *C. pacificus*. He also suggested that lipid content was better correlated with primary production rate than with chl *a*, although analysis of only those stations where both primary production rate and chl *a* were measured suggests that the strength of the 2 correlations was similar. The apparent association of lipid content with primary production rates may be influenced by a cross correlation between integrated primary production rates and integrated chl *a* ($r_s = 0.93$, $p < 0.01$, $n = 12$).

The range of phytoplankton concentrations observed in the preceeding studies was considerably greater than in the present study. Previous investigations were conducted somewhat later in the year than the present March study: April (Willason et al. 1986); April-May (Hakanson 1985), or July (Cox et al. 1982). In each case spatial gradients were well-defined and surface chlorophyll concentrations ranged by over a factor of 100 (L. R. Haury pers. comm., based on discrete calibration samples for underway data in Cox et al. 1982; SIO Reference 84-25 for the study of Hakanson 1985; Willason et al. 1986). In the present study, the range of surface values was an order of magnitude lower (Fig. 4). In the presence of a broader range of prey concentrations a stronger spatial signal is present and correlation with nutritional status may be more readily detected against a background of small scale variability. Additionally, all 3 studies sampled over a larger spatial scale than the present study. Sampling over a larger scale, during conditions of a stronger spatial signal, may increase the likelihood that animals are captured in a water parcel with food concentrations that reflect their recent nutrition.

The weak spatial correlations observed in this study could also be influenced by the small sample sizes for some correlation analyses, by population mixing or by the lack of assessment of food 'quality', including the lipid composition of particulate matter (Sargent & Henderson 1986). An additional factor of importance is the uncertainty of the true depth or range of depths where copepod feeding occurred. The vertical distribution of zooplankton feeding activity remains controversial but the assumption that animals fed at the depth of the plant biomass maximum is consistent with the results of Napp (1986).

The relative importance of horizontal and vertical variations in food supply in the study site is approximated in Fig. 7 as the magnitude of horizontal versus vertical gradients in particulate nitrogen concentration. The median gradient in the vertical plane is 3 orders of magnitude greater than the median gradient in the horizontal plane ($p < 0.0001$, Kolmogorov-Smirnof 2-sample test), suggesting that zooplankters seeking suitable feeding grounds would more profitably allocate effort by vertical searching activity. Furthermore, the vertical changes, over a scale of tens of meters, are

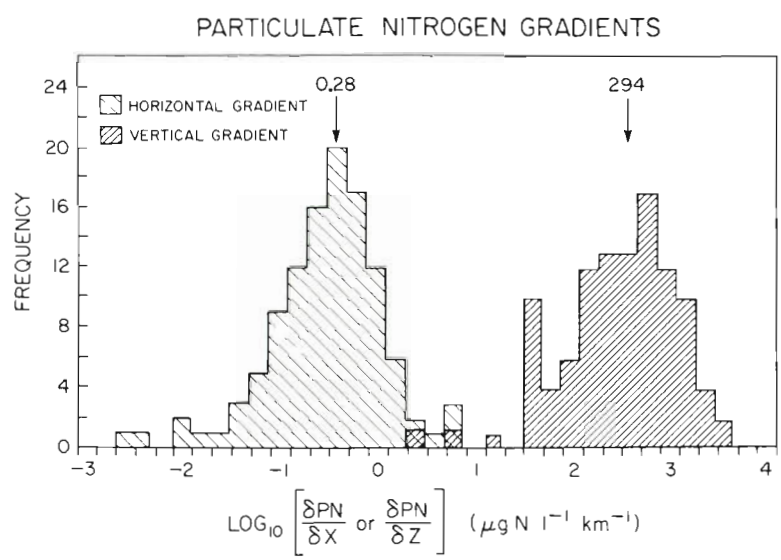

Fig. 7. Frequency distribution of gradients in particulate nitrogen in horizontal $\left(\frac{\delta PN}{\delta x}\right)$ and vertical $\left(\frac{\delta PN}{\delta Z}\right)$ dimensions in the study site. Arrows: median gradients

within the daily ambit of the organisms. This analysis of the importance of vertical gradients further supports the results summarized in Mullin (1986).

Diel variation in zooplankton feeding activity is commonly observed (e.g. Duval & Geen 1976, Mackas & Bohrer 1976, Hayward 1980, Head et al. 1985, Kleppel et al. 1985, Willason & Cox 1987). In the present study nocturnal increases in gut pigment levels could be accounted for either by endogenous feeding rhythms (e.g. Boyd et al. 1980, Dagg 1985, Stearns 1986), by nocturnal vertical migration into the euphotic zone or by elements of both behaviors.

In summary, diel variation in feeding behavior appeared more important than horizontal variation in food supply in controlling copepod ingestion in the study site. This conclusion appears applicable during the present study conditions of relatively little horizontal spatial change, though it probably does not apply in regions of defined mesoscale features with steeper lateral gradients (cf. Hirche & Bohrer 1987), or in other seasons. Nonetheless, suspension-feeding zooplankton can experience low magnitude horizontal gradients for considerable periods of time. In such conditions the reward to the zooplankter, as well as to the investigator, will be greater for searches conducted in the vertical plane.

Acknowledgements. J. Wilkinson provided valuable technical assistance during this study. I thank T L. Hayward for the invitation to participate in this cruise and for providing an early version of Fig. 1. R. W. Eppley provided access to a CHN analyzer and A. A. Yayanos to an Iatroscan. A. Fleminger confirmed identifications. E. L. Venrick and J. T Enright discussed methods for estimating the standard error of a continuous function. Anonymous referees furnished criticisms of the manuscript. Supported by the Academic Senate of the University of California at San Diego and by the Marine Life Research Group of Scripps Institution of Oceanography.

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This article was presented by Professor T. R. Parsons; it was accepted for printing on October 23, 1987