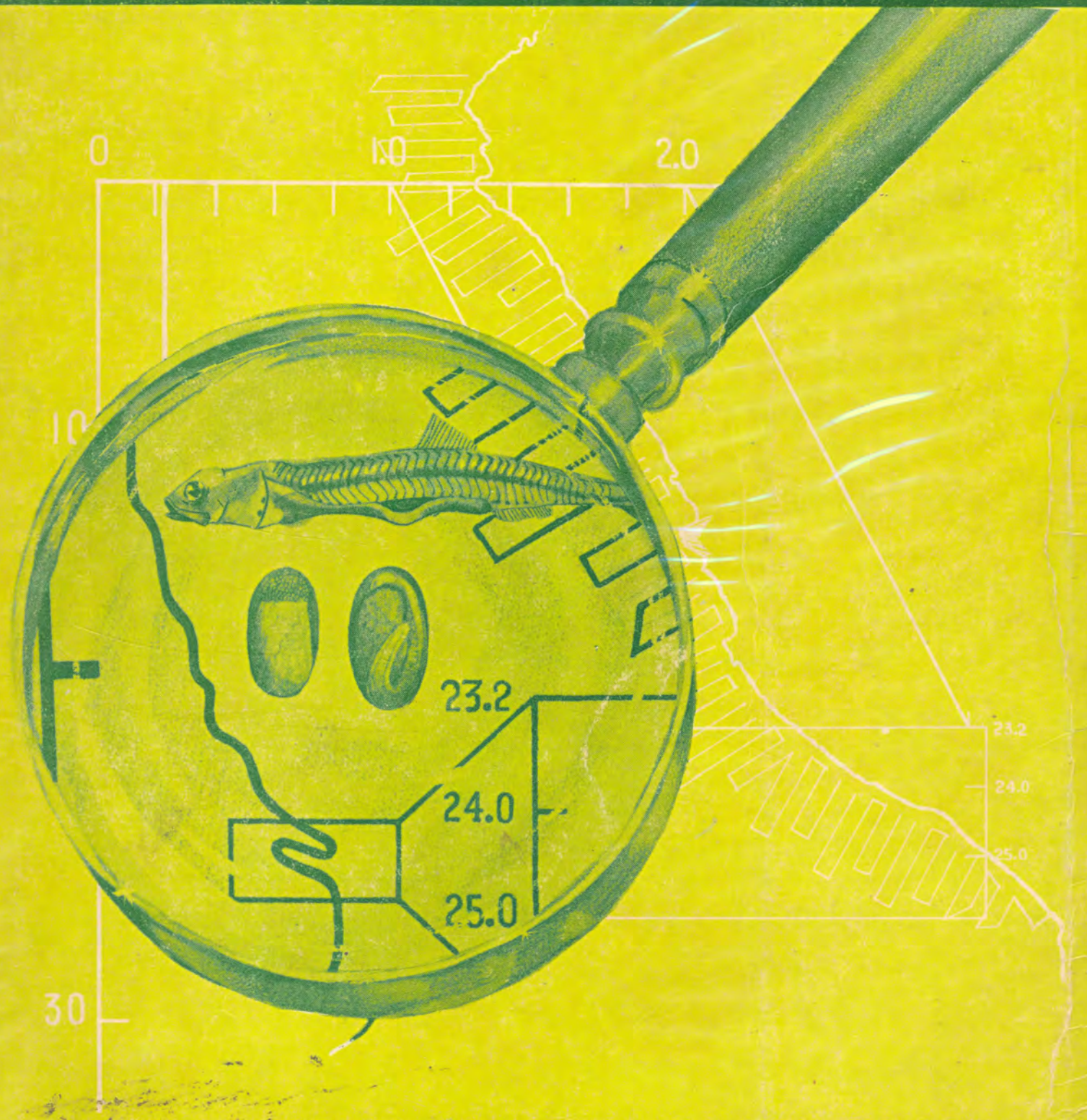
INSTITUTO DEL MAR DEL PERU

Boletín

ISSN - Q 378 - 7699
VOLUMEN EXTRAORDINARIO



INVESTIGACION COOPERATIVA DE LA ANCHOVETA Y SU ECOSISTEMA - ICANE - ENTRE PERU Y CANADA CALLAO 1981 PERU

GRAZING PATTERNS OF COPEPODS IN THE UPWELLING SYSTEM OFF PERU

Reprinted from: Limnology and Oceanography, Vol 25

Carl M. Boyd,
Sharon L. Smith[1],
Department of Oceanography
Dalhousie University
Halifax, Nova Scotia

and

Timothy J. Cowles
Woods Hole Oceanographic Institution
Woods Hole, Massachusetts 02543

ABSTRACT

The amount of food eaten by copepods of three genera (estimated from chlorophyll and pheophytin in the guts of the animals) was measured to determine the depth and also the time of day at which the maximun and minimum intensity of feeding occurred.

Copepods were obtained with a large volume (800 liters min^{-1}) pumpig system at five depths (0 to 85 m) and twelve samplig intervals (every 4 hours) at three stations in the Peruvian upwelling system. Results indicate that **Eucalanus** could withstand periods of 12 hours in anoxic layers, but **Calanus** and **Centropages** tended to be found in waters having more than 0.8 and 0.2 ml $liter^{-1}$ respectively, that in situ filtration rates could be derived which were in accord with filtration rates obtained from laboratory studies, that individuals of the three genera migrated in and out of the 5 m surface layer when food was abundant but did not show a coherent diurnal vertical migration when food was scarce, and that the three genera maintained different feeding strategies that were amplified when food was scarce. The results are consistent with the concepts of resource allocation and separation of niches by species.

RESUMEN

Se midió la cantidad de alimento ingerido (mediante la estimación de la clorofila y la feofitina encontrada en el tracto intestinal) para tres géneros de copépodos con el fin de determinar la profundidad y la hora del día en que se dan el máximo y el mínimo de actividad alimentaria.

Los copépodos se obtuvieron de cinco profundidades (entre 0 y 85 m) y a intervalos de muestreo de 4 horas (12 intervalos) mediante un sistema de bombeo de gran volumen (800 litros por minuto) en tres estaciones en la zona de afloramientos del Perú. Los resultados indican que **Eucalanus** podría resistir períodos de 12 horas en ambiente anóxico, que **Calanus** y **Centropages** tienden a encontrarse en ambientes con mas de 0.8 y 0.2 ml O_2 por litro respectivamente, que puede derivarse tasas de filtración in situ muy de acuerdo con las tasas obtenidas en el laboratorio, que individuos de los tres géneros salieron y entraron a la capa superior de los 5 m cuando el alimento fue abundante pero que no mostraron coherentemente ninguna migración vertical cuando el alimento fue escaso, y que los tres géneros mantuvieron diferentes estrategias de alimentación que se acentuaron cuando el alimento fue escaso. Los resultados son consistentes con los conceptos de asignación de recursos y separación de nichos por especies.

Feeding by herbivorous copepods has been extensively studied in the last decade in an attempt to understand energy exchanges in the aquatic environment. A great deal has been learned about feeding behavior by capturing copepods of interest, bringing them into the laboratory, and allowing them to feed under controlled conditions. The studies of Mullin (1963), Frost (1972), Paffenhofer (1971), and others have established the transfer funtions that relate rates of food ingestion to con-

1 Present Adress: Brookhaven National Laboratory, Upton, New York 11973.

centrations of food available to the animals, and have stimulated interest in studies of diurnal feeding rhythms, mechanisms of food retention, and strategies of feeding of marine copepods.

While these laboratory studies have elucidated many components of feeding by copepods, they are limited by the artificial constraints of the laboratory experiment wherein animals that have been brusquely captured by a plankton net are kept in an abnormal light regime, in containers that restrict excursions, and are fed phytoplankton species that are often unknown to them. Other workers have attempted to remove the element of unfamiliar food by allowing animals to feed in the laboratory on particles that occur coincidently with the copepods in nature (Poulet 1974, Cowles 1979). The criticism and virtues that derive from bringing animals into the laboratory still pertain to this type of study.

It is difficult or impossible to answer several very interesting and important questions with existing laboratory techniques: Do animals eat more at the surface than at depth in keeping with some current ideas concerning vertical migration? Do animals eat all the time or intermittently? Do animal synchronize their feeding activity according to diurnal rhythms? Are the vertical excursions of copepods associated with the availability of food and/or other environmental characteristics? Do animals partition their environment? These questions have important consequences in interpreting the biology of copepods, and served as focal-points in this study.

Attempts to study feeding in situ have been made by Haney (1971) who measured ingestion of prepared food by zooplankters trapped for a short period in a large water bottle, and by Mackas and Bohrer (1976) who measured the quantity of chlorophyll and pheophytin in the guts of selected species of copepods taken from plankton samples.

The procedure proposed by Mackas and Bohrer has the advantage that all elements that might regulate feeding of the individual are uninfluenced by man or his experimental design up to the instant the animal is captured. With some qualifications, the quantity of chlorophyll and its degradation products in the gut of a copepod is an indication of how much phytoplankton the animal has recently ingested and should be correlated with its grazing rate. Chlorophyll serves as a naturally occurring label, and it can be used conceptually as if it were a radio-isotopic label. Among the limitations of the technique are that only feeding on particles containing chlorophyll or its degradation products will be measured; if the animal had been feeding on detritus devoid of these compounds, no feeding would be measured, and if the animal were feeding carnivorously, only the chlorophyll products that might have been in the gut of the prey species would be detected. It is therefore necessary to limit the use of this technique to those species whose feeding characteristics are to some extent known.

We used the technique of Mackas and Bohrer (1976) in a study of grazing by four species of calanoid copepods in the region of upwelling off the coast of Peru at about 15°S as part of the Coastal Upwelling Ecosystems Analysis, expedition JOINT II during April, 1977. The four species chosen, **Calanus chilensis** Brodsky, **Eucalanus inermis** Giesbrecht, **Eucalanus subtenuis** Giesbrecht and **Centropages brachiatus** Dana were abundant copepods in the system (Heinrich 1973); **Calanus** and **Eucalanus** are believed to be predominantly herbivorous genera, and **Centropages** an omnivorous genus.

Samples were taken from three locations at five depths; sampling was repeated at 4 hour intervals for 44 hours at the following stations:

Midshelf: water depth = 118 m; 15° 5.0' S, 75° 30.5' W; 12, 13, 14 April 1977; surface water temperature = 16.3°C; at 85 m = 15.0°C.

Shelfbreak: water depth = 480 m; 15° 10.0'S, 75° 35.0'W; 15, 16, 17 April 1977; surface water temperature = 16.3°C; at 85 m = 14.5°C.

Offshore: depth = 3100 m; 15° 51.5'S, 76° 25.O'W; 21, 22, 23 April 1977; surface water temperature = 21.0°C; at 85 m = 14.0°C.

The tree areas were separated by 8 and 64 nautical miles (15 and 119 km) respectively; the fundamental difference was proximity to the coastline and a gradation in effects of coastal upwelling.

This project was carried out as part of the Coastal Upwelling Ecosystems Analysis study Joint II in March and April of 1977 off the coast of Peru. The cooperative aspects of CUEA gave us access to data, equipment, and samples that would rarely be available in a smaller study. Dr. David Judkins, of the Brookhaven National Laboratory, provided us the opportunity to collect plankton samples from the large pumping system and Mr. Warren Keith of Scripps Institution of Oceanography (R.V. Melville) helped us in sample collection and analysis. We are especially indebted to Ms. Olga Gomez of the Instituto del Mar de Peru for assitance in sorting the copepods. The CUEA study and the research of two of the authors (S.L.S. and T.J.C.) were funded through the U.S. National Sciences Foundation; S.L.S. was also supported by a Killam Postdoctoral Fellowship. The research expenses of C.M. Boyd were funded by grants from the National Research Council of Canada and the North Atlantic Treaty Organization.

Animals were collected with a pump-system having a 12.6 cm i.d. hose which was lowered vertically to depth. Water was discharged by a centrifugal pump on the ship through a fine mesh conical plankton net (202 μm) at volumes of about 800 liters min^{-1}. Samples were collected as the inlet of the hose was lowered from one depth to another over an interval of about five minutes, yielding a sample from about 4 m^3 of water for each stratum; 1-5 m, 10 -15 m, 15-20 m, 25-35 m, and 65-84 m. Sampling intervals chosen represent a compromise between a desire for total vertical coverage and the

practical constraints imposed by frequent sampling during a 44 hour time series. We left a large interval between 35 m and 65 m since very few copepods were found within this oxygen-poor zone. Plankton samples were examined immediately under a dissecting microscope, and twenty specimens of each of four species (when available) were pipetted into vials containing a small amount of 0.22 μm filtered seawater. These specimens included adults and late copepodites. The contents of the vials were filtered onto 25 mm Gelman type A-E glass-fiber filters and ground with 10 ml 90o/o aqueous acetone. The operation, from collection of the samples to extraction, required only a few minutes. The slurry containing filter fibers, acetone and extracted pigments was filtered through a Gelman A-E filter, and the fluorescence of the filtrate was measured before and after acidification with 5o/o vol/vol HC1 with a Turner Designs 10-050 fluorometer. Chlorophyll and pheophytin concentrations in the guts of the four species of copepods were calculated from fluorescence of pigments extracted from whole bodies of the copepods. The following formulae give concentrations of pheophytin a and chlorophyll a in ng·animal^{-1}.

$$\text{pheophytin a (ng animal}^{-1}) = \frac{K\,[(RFa)-Fo]v}{n}$$

$$\text{chlorophyll a (ng animal}^{-1}) = \frac{K\,(Fo - Fa)\,v}{n}$$

The constant K (75.4 during this study) was derived from instrument calibration and is normally used to express concentration of pigments in μg per liter of sea water. The value v is the volume in ml of 90o/o acetone in which the pigments were extracted,n is the number of animals in the sample (usually 20), Fo and Fa are the fluorescence readings before and after acidification with two drops of 5o/o vol/vol HC1. The acidification ratio (R) was 2.08. These formulae depart from those used by Mackas and Bohrer (1976) and express pheophytin a and chlorophyll a in absolute units (ng pigment per animal). Jeffrey (1974) points out that digestion of clorophyll by copepods results in pheophorbide, the double-decomposition product of chlorophyll, and that very little pheophytin occurs in fecal pellets. We were not able to carry out chromatographic separation required to distinguish between pheophorbide and pheophytin, and consequently group the two compounds as "pheophytin".

Fluorescence of organic compounds and pigments other than chlorophyll and pheophytin would add an error to these measurements. To assess this potential error, we measured fluorescence of copepods that had been starved in filtered seawater for 24 h, and found low concentrations indicating that background levels of fluorescence where generally not a significant component of the fluorescence of animals taken recently from the ocean. The background levels are compared to fluorescence levels from fed animals in Table 1.

Table 1. Mean background levels of gut contents of starved copepods (ng. animal^{-1}) relative to midrange levels of gut contents of animals captured at the shelfbreak station.

	Starved ng. animal^{-1}		Natural levels ng animal^{-1}	
	pheophytin	chlorophyll	pheophytin	chlorophyll
Calanus chilensis	0.098	0.226	3.935	0.445
Centropages brachiatus	0.203	0.143	3.610	1.005
Eucalanus inermis	0.276	0.132	9.340	0.980

Water samples drawn from the discharge of the pump were analyzed for chlorophyll a and pheophytin a (Lorenzen 1966) and nutrients (Dugdale, et al. 1977); oxygen samples were drawn fron Niskin bottles lowered to depth and were analyzed by the Winkler technique according to Strickland and Parsons (1972).

The technique of extraction of fluorescent pigments adapted itself well to the analysis of 20 pooled copepods, and we used that number as an index of abundance of each species. Generally this quantity was readily available in the sample of four m^3, but at some times or depth we were unable to find sufficient numbers for analysis. We have considered 20 or more animals in a sample to be the indicator of "presence" at a given depth; when fewer than 20 but more than four were found we regard that species as "rare", and when fewer than four (1 animal m^{-3}) were present we regard that species as "absent". While quantitative aspects are lost by this procedure, it is useful for generalization concerning the influence of environmental parameters such as depth, oxygen, and food concentrations on distribution.

Even though the study was made in the Peruvian coastal upwelling system where chlorophyll concentrations occasionally exceed 40 μg liter^{-1}, the highest concentrations of chlorophyll measured during the collection of these data was 2.1 μg liter^{-1}. Physical and chemical measurements made along the cost as part of this study indicated that our mid-shelf and shelf-break stations were located in a plume of upwelling water and suggest that this water had not been at the surface long enough for algal populations to proliferate. The plume supplied nutrients to the band of water along the coast that supported the high concentrations of phytoplankton well-known in Peruvian waters. The upwelling water, although sparse in phytoplankton, had high concentrations of pheophytin (3.5 μg liter^{-1}) which we believe originated from fecal material and senescent algal cells. These cells had precipitated from the surface waters into the upwelling source waters as they moved underneath regions of high phytoplankton biomass. Consequently, animals living in the near-surface waters in the upwelling

plume were frequently exposed to higher concentrations of non-living organic matter than of living phytoplankton.

Because the midshelf and shelfbreak stations were located in the plume of upwelling water, they are similar in many respects and the pair of them can be contrasted with the offshore station, which was typical of a "blue water" station. Figure 1 indicates that inshore stations had (relatively) high chlorophyll concentrations, low oxygen concentrations, and high concentrations of pheophytin. In contrast, the offshore station had very little chlorophyll (< 0.5 μg liter^{-1}), virtually no pheophytin (< 0.1 μg liter^{-1}), and was well-oxygenated down to our lowest sampling point (85 m). The two inshore stations could not be distinguished from each other ($p > 0.5$) on the basis of plant material in the guts of copepods, but those animals living at the offshore station had significantly less food than their congeners at the two inshore stations. We have accordingly assessed the responses of the copepods in the analysis that follows within the context of the "two inshore stations" relative to the "offshore station".

Oxygen concentrations, food concentration and distribution of copepods – One of several notable features of the Peruvian upwelling system is the occurrence of water with very low concentrations of dissolved oxygen within layers normally occupied by surface-living species of copepods. Figure la and b indicates that water on the shelf had less than 2 ml O_2 liter^{-1} (~38% saturation) even at the surface and was often virtually oxygen-free at depths below 35 m. Based on our criterion of at least 20 adult animals in 4 m^3 of water, **Calanaus chilensis** was limited to the upper 35 m at the two inshore stations (Fig. 2a and b), suggesting that its vertical distribution was limited by the low O_2 concentrations at greater depths. At the offshore station, where oxygen concentratios were higher at all sampling depths, **Calanus** ranged from the surface to our maximum samplig depth of 85 m (Fig. 2c).

Centropages brachiatus ranged slightly deeper at the two inshore stations than did **Calanus**, and occasionally was found in waters having as little as 0.2 ml O_2 liter^{-1} (Figs. 3 and 5). **Centropages**

Figure 1. Concentrations of chlorophyll (μg.liter^{-1}), pheophytin (μg . liter^{-1}) and oxygen (ml . liter^{-1}) from surface to 85 m at three stations in the Peruvian upwelling zone, 15°S, a, the midshelf region; b, the shelfbreak; c, offshore.

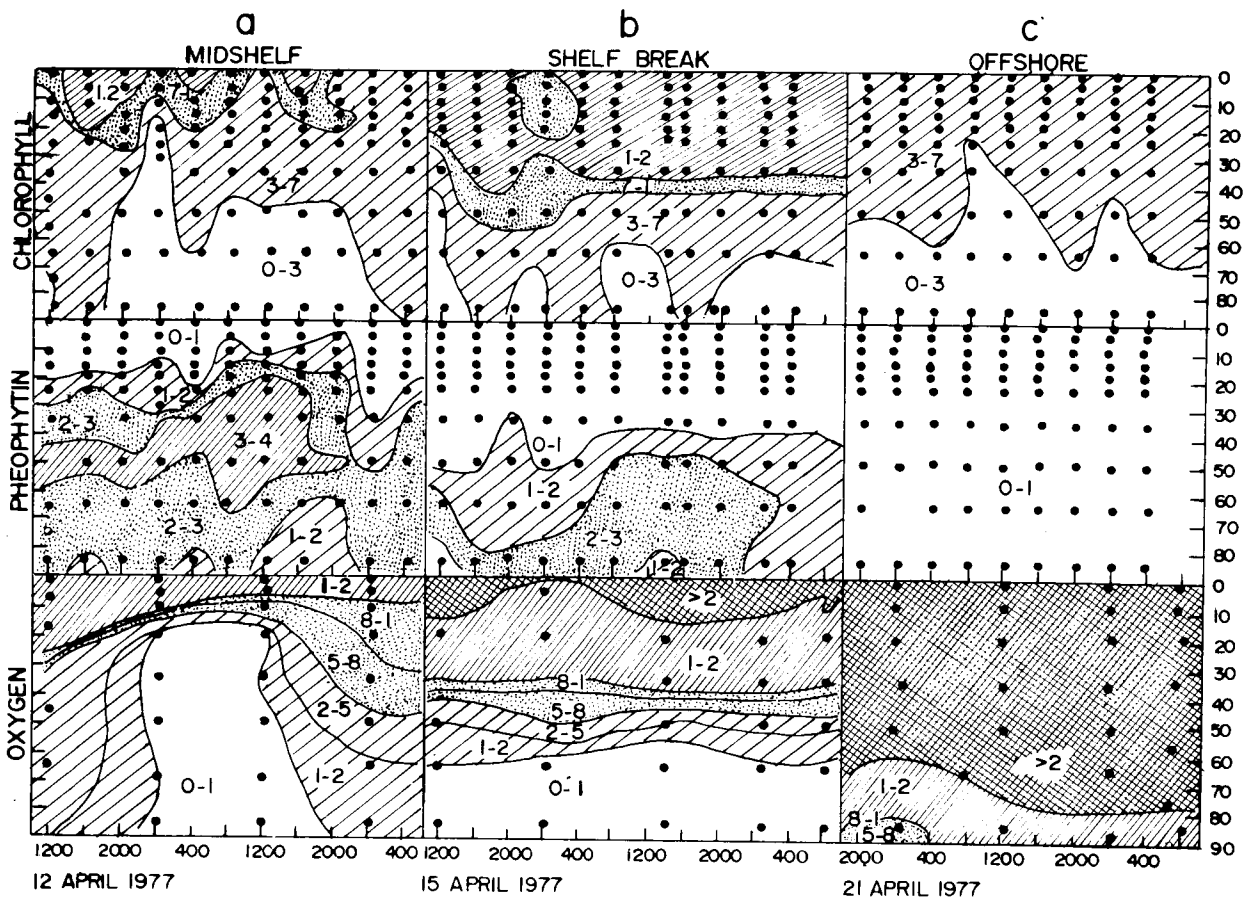
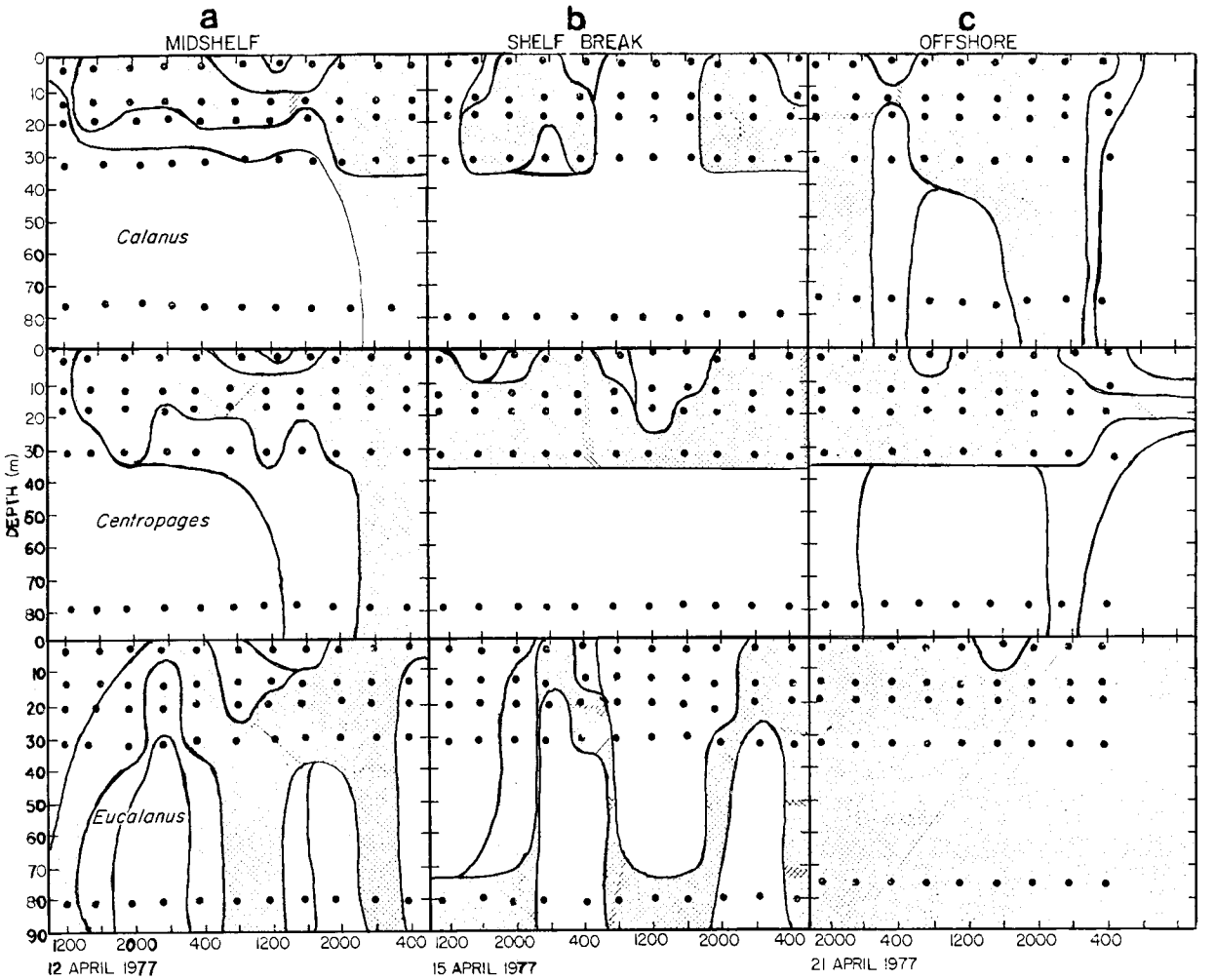


Figure 2. Occurrence with depth and time of **Calanus, Centropages** and **Eucalanus** at (a) the midshelf station, (b) the shelfbreak station, and (c) the offshore station. Darker crosshatching indicates locations where the animals were found abundantly (classed as "present" in the text; > 4 animals m^{-3}), the lighter stippling indicates where animals were "rare" (1-4 animals m^{-3}); unshaded indicates animals were "absent" (<1 animal m^{-3}).



occurred at depth of 65-84 m only when the surface layer with higher concentrations of chlorophyll and oxygen warped down from 35 to 85 m (Figs. 2a and b). In the offshore area where oxygen concentrations were higher at all depths sampled, **Centropages** was found primarily in the surface layer (0-35 m) where chlorophyll was most abundant (Figs. 2c).

Eucalanus is the most remarkable of the genera examined in this study. These animals existed in water virtually lacking in dissolved oxygen, (Figs. 2a and b) and underwent obvious diurnal vertical migrations. This genus was represented by two species in our study area: **Eucalanus inermis** was the more abundant member of the genus at the two inshore stations, while **E. subtenuis** was the more abundant member of the genus at the offshore station.

Comparison of Figs. 1a and 1b with Figs. 2a and b shows that **Eucalanus** generally migrated to the surface each night and descended to anoxic waters during daytime at the two inshore stations. We assume the animal existed via anaerobic metabolic processes while in the anoxic zone, and perhaps used its residence in the oxygenated surface waters at night to discharge any accumulated oxygen debt.

Diurnal Vertical Migration – Migrational patterns for the three genera at the inshore stations differed from patterns at the offshore area. The presence or absence of the animals in the top 5 m during the day versus the night was tested by the 2x2 constingency method (model II, Sokal and Rohlf 1969); the results indicate that all three genera tended to leave the surface 5 m during the day at the inshore stations ($p < 0.05$; all genera) but all forms occurred at the surface indiscriminately during the day and night at the offshore station (no significant day: night differences). When the obser-

vations from the two inshore stations are pooled, the analysis indicates that **Calanus** and **Centropages** left the upper 5 m during the day but were present in the 10-15 m stratum, hence the vertical range of the migration was not great. The amount of suspended material at these stations, however, was such that the intensity of light at 10-15 m was generally less than 5o/o of the surface intensity. **Eucalanus** ranged to greater depths than **Calanus** or **Centropages** at the inshore stations and often was present only at depths greater than 65 m (Figs. 2a and b).

Migrational patterns were greatly modified at the offshore station. No trends were apparent for **Centropages** or **Eucalanus**, and only **Calanus** exhibited a day: night pattern; individuals were concentrated in the upper 35 m during the day but were dispersed through the water column during the night, showing what might be called "midnight sinking" in absence of any daylight cues.

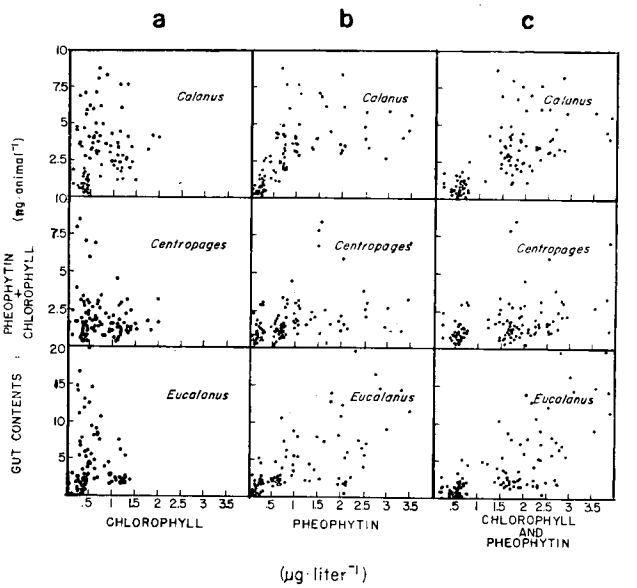
Our criterion of presence or absence would indicate only migrational patterns that were synchronous between individuals. It is quite possible that individual animals at the offshore station migrated in and out of the upper 5 m stratum independently of the time of day and of each other. We cannot assess this, and can only say this migrational pattern existed in the inshore areas (where food was abundant) and did not exist in the offshore area (where food was scarce).

Food and feeding responses – the amount of food available to an animal from the water surrounding it is known to relate to the quantity of ingested food (Mullin 1963, Frost 1972), and one would secondarily expect a correlation between available food and the degree of fullness of the gut. In Figure 3 the concentration of plant material available to the animal as food (expressed as pheophytin, chlorophyll, and a summation of the two) at the point where the animal was captured, is plotted against the quantity of pheophytin and chlorophyll in the gut of the animal. Correlation coefficients and F statistics from analysis of variances of the data shown in Figure 3 are presented in Table 2.

Most of the chlorophyll-like pigment in the gut existed as degradation products of chlorophyll, here called pheophytin. Chlorophyll was always less abundant than pheophytin in the guts of these species, averaging 13.0o/o for **Calanus**, 23.6o/o for **Centropages**, and 11.4o/o for **Eucalanus** of the combined concentrations of chlorophyll and pheophytin. This reflects the abundance of particles containing pheophytin and also indicates that the decomposition of chlorophyll is rapid relative to the time required for the copepod to clear its gut. We have used the sum of chlorophyll and pheophytin in the guts of the animals as our index of fullness of guts in Figure 3.

Data collected concurrently in Peruvian waters as part of the CUEA exercise indicated significant relationships between chlorophyll and carbon [carbon μg liter^{-1} = 41.86 + (43.10 μg chl liter^{-1})

Figure 3. Gut contents of **Calanus, Centropages,** and **Eucalanus** from the three stations expressed as ng chlorophyll plus pheophytin animal^{-1}. These concentrations are plotted relative to the amount of plant material available to the animals at the point where the animals were captured, expressed as (a) chlorophyll, (b) pheophytin, and (c) chlorophyll plus pheophytin, all expressed in μg liter^{-1}.



r^2 = .85] pheophytin and carbon [carbon = 68.46 + (70.34 μp pheophytin liter^{-1}); r^2 = .43], and summed chlorophyll and pheophytin [carbon = 37.80 + (29.8 μg chlorophyll + pheophytin liter^{-1}); r^2 = .77]. The samples taken for these analyses were all from the upper 50 m over the Peruvian shelf. The chlorophyll/carbon regression is in keeping with that of Lorenzen (1968) calculated from samples from the same region. The regressions imply that the animals we were studying generally had access to less than 150 μg carbon liter^{-1} at all three stations.

DISCUSSION

In figure 3 the food ingested by copepods is shown relative to the amount of food available to them, and the figures are in a format similar to curves of ingestion presented by Mullin (1963) and Frost (1972). Our data, however, represent plant food ingested and **retained**, and as such are not identical to the feeding curves described by other workers. With this caveat in mind, one notes that gut contents are generally higher at high food concentrations and that the figures give some insight into the feeding behavior of these copepods. In order to compare the relationships shown in Figure 3 we have carried out simple statistical analyses (Table 2) which express correlations between gut contents and concentrations of pheophytin and chlorophyll in the water. The poor correlations between gut contents and available chlorophyll are out of keeping with known responses

Table 2. Regression equations, correlation coefficients, coefficients of determination (r^2), and F statistics of regressions. The equations predict the amount of plant material (pheophytin plus chlorophyll; ng. $animal^{-1}$) in the guts of the copepods relative to the amount of plant material (X; chlorophyll and/or pheophytin; μg . $liter^{-1}$) at the point the animal was found. Significant regression ($p < 0.01$) are marked with an asterisk (*).

	Available plant material ($\mu g.liter^{-1}$)	Correlation coefficient	Coefficient of determination r^2	F
Calanus	Chlorophyll: $y = 1.86 + 1.32X$	.256	6.5%	7.49*
	Pheophytin: $y = 1.41 + 1.61X$	.619	38%	66.3 *
	Chlorophyll + Pheophytin: $y = 0.50 + 1.49X$	.660	44%	82.8 *
Centropages	Chlorophyll: $y = 2.05 + (-.415X)$	-.127	1.6%	1.91N.S.
	Pheophytin: $y = 0.971 + 0.908X$	.493	24%	37.6 *
	Chlorophyll + Pheophytin: $y = 0.796 + 0.595X$	.376	14%	19.3 *
Eucalanus	Chlorophyll: $y = 4.09 + (-.055X)$	-.003	.001%	0.0 N.S.
	Pheophytin: $y = 0.69 + 3.53X$	.715	51%	111.74*
	Chlorophyll + Pheophytin: $y = -0.38 + 2.89X$	.648	42%	77.3 *

of copepods to increasing food concentrations, but the correlations more nearly follow the anticipated positive slope when pheophytin is included as an indicator of food, at least for **Calanus** and **Eucalanus**. These results indicate that in field studies it is essential to consider concentrations of both chlorophyll and pheophytin as indicators of food availability.

The best correlation as indicated by ther r^2 and the F test was associated with the gut fluorescense of **Eucalanus** and available pheophytin, and the weakest was between gut fluorescence of **Eucalanus** and the concentration of chlorophyll (presumably indicating live phytoplankton cells) in the water. These statistics suggest that **Eucalanus** was feeding to a large extent on particles of decomposed algal cells and fecal material, and to a lesser extent on cells containing chlorophyll. Since **Eucalanus** had access to both living and nonliving algal material, it is tempting to postulate an acceptance: rejeciton of individual food particles. The analysis of feeding periodicities discussed below, however, shows that such a pattern is not required: **Eucalanus** did most of its feeding during the day when it had access to high concentrations of detrital plant material at depth. When it migrated to the surface at night, leaving behind high detrital concentrations and entering the layers containing a greater proportion of live cells, it was found to have less material in its gut, suggesting that **Eucalanus** did not discriminate against what it ate, but only against when and where it ate.

The relationships shown in Figure 3 indicate that **Calanus** also fed to a large extent on particles containig decomposed algal material and that particles containing chlorophyll were only a small component of its diet. The best fit of a regression occurred when gut contents of **Calanus** were related to the summed plant material available to the animal; this relationship also accounted for the highest amount of variance in the series ($r^2 = .44$), and is in accord with the view that **Calanus** is a non-selective filter-feeder.

Centropages is known to eat both plants and animals, and we believe that this omnivorous feeding behaviour is reflected in our data. There was no indication that **Centropages** was feeding on live cells in proportion to their abundance (r^2 =

0.02) and the relationship between the quantity of dead plant cells available to the animal and the material in the gut is weak ($r^2 = 0.24$). **Centropages** certainly did ingest plant material but not to a degree related to the quantity available; we believe the animal was coincidently eating other zooplankton in the water, and that its predacious feeding habits obscured a relationship to associated plant material.

The best feeding relationship found in this study (**Eucalanus** feeding on particles containig pheophytin) accounted for only 51% of the variance of gut contents data, indicating that factors other than food concentrations acted to regulate the amount of plant material in guts of these animals. We believe that, aside from omnivory as it influenced the feeding of **Centropages**, much of the scatter of data points in Figure 3 results from intermittent feeding. If the animals did not feed at a constant rate (proportional to the amount of food available to them) but ate and egested intermittently and remained empty, we would expect results similar to those obtained. In all species, data points close to the abscissa of Figure 3 indicate that many animals were exposed to food concentrations in excess of accepted minimum thresholds (Front 1975), and the conclusion must be that many animals had not eaten for at least one hour prior to their capture. Animals with little or no food in their guts were captured during both day and night, suggesting intermittent rather than continuous feeding and egestion. The possibility exists that these "empty" animals had been feeding carnivorouusly and were, in fact, full though not with plant pigments. We believe that **Centropages** was feeding in part carnivorously, and that the lack of correlation between gut contents and available plant food can be attributed to this feeding behavior, but that the scatter in the data for the more strictly herbivorous **Calanus** and **Eucalanus** is indicative of intermittent herbivory. Recent experiments in which females of **Calanus finmarchicus** were fed pure cultures of **Thalassiosira fluviatilis** and were subsequently analyzed individually, indicate that most animals were empty most of the time.These preliminary experiments support field observations by S. Schanck (1975) and imply that intermittent feeding at high rates for short periods of time followed by egestion and periods of no feeding is commonplace.

By knowing the amount of plankton pigment in the guts of these species in ng.animal^{-1} and by knowing the amount of plant pigment in the water in μg liter^{-1}, we can calculate the volume of water that the average animal must have filtered in order to obtain the plant material found in its guts:

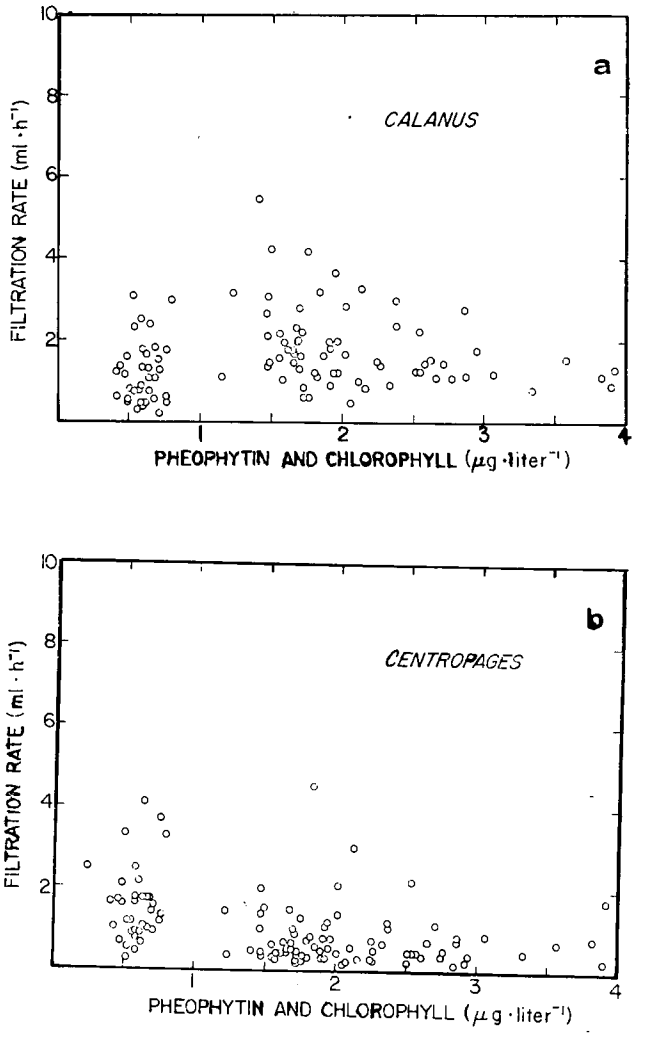
$$\text{water filtered (ml)} = \frac{\text{quantity of Chl + pheophytin in gut (ng. animal}^{-1})}{\text{concentration of Chl + pheophytin (μg .liter}^{-1})}$$

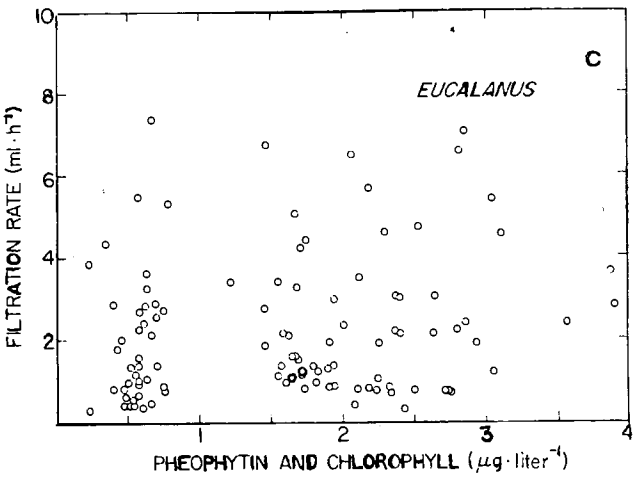
This measurement may be converted into an estimate of filtering rate if the rate at which food is removed from the gut is known. Appropriate values have been documented for copepods as being between 0.5 and 2 h (Mackas and Bohrer 1976, Marshal and Orr 1955), and we have taken an intermediate value of 1 h for our calculations.

While we have suggested above that the copepods examined may have fed intermittently, it is likely that our samples (containing at least 20 individuals) smoothed out a large portion of the inter-animal variability in gut contents. We feel it is useful to use calculated filtration rates to compare our "instantaneous" rates within and between species and locations. Use of these calculations does not imply that the animals are necessarily filtering at this rate all the time.

Estimates of filtration rates for the three genera indicate that the animals were typically filtering between 1 and 8 ml of water per hour (Fig. 4). If the extremes of gut clearance times,

Figure 4. Filtration rates for **Calanus** (4a), **Centropages** (4b), and **Eucalanus** (4c) calculated from the amount of water (ml) that each animal would have had to filter in 1 hour to obtain the material found in its gut relative to the quantity of plant material (pheophytin plus chlorophyll) available to it.





0.5 and 2 hours, are chosen, the rates would correspondingly be doubled or halved. These values are similat to those obtained for these species in shipboard experiments (Cowles 1977) and in laboratory experiments on **Calanus helgolandicus** by Mullin (1963) and on **C. pacificus** by Frost (1972).

Diurnal Feeding Patterns – The manner in which the plankton samples were taken (5 stratified depths, 12 series over 44 hours, 3 stations) permits us to analyze the gut-contents data for the three genera to ask if feeding was more intense at depth or at a certain period of 24h cycle. We have accordingly analyzed the data to detect trends of feeding intensity of animals caught: **a** at the surface (0-15m) vs. depth (15-85 m) and, **b** during the day vs. night. Chi^2 values and associated probabilities were calculated from 2 x 2 contingency tables, testing the frequency of samples with more or less than the median value of gut contents in the relevant series of samples. The median was chosen as an expression of central tendency rather than the mean because values of gut contents were not normally distributed. As earlier noted, concentrations of food in the guts of animals from the two inshore stations were indistiguishable, and samples from these stations were pooled to be contrasted with samples from the offshore station. The results of these analyses are tabulated in Table 3; we have also summarized these data in Figure 5 to illustrate schematically the trends indicated by the statistical analysis. All of the patterns shown on Figure 5 are statiscally significant, and describe the behavior of most (i.e. > 50%) of the animals; the patterns illustrate trends in the data that are superimposed on scatter introduced by various factors which we could not evaluate or were unknown to us. Surprisingly, the overall picture of feeding intensity versus time and depth was virtually identical whether one tested gut fullness or filtration rate (Fig. 6). The only differences were that **Calanus** showed higher filtration rates in the upper 15 m relative to the 15-85 m stratum at the inshore stations, and at the offshore stations, **Calanus** showed uniform filtration rates from surface to depth during the day (Figure 6).

Figure 5. Feeding patterns based on quantity of food in guts of copepods and on their vertical distribution. Diagrams are schematic and represent statistically significant trends of behaviour of animals as indicated by the statistical analysis of Table 3.

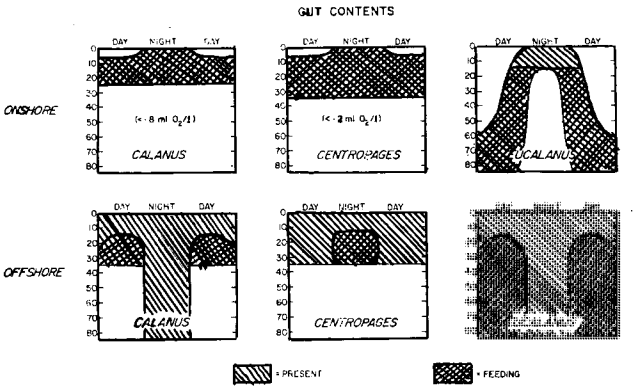
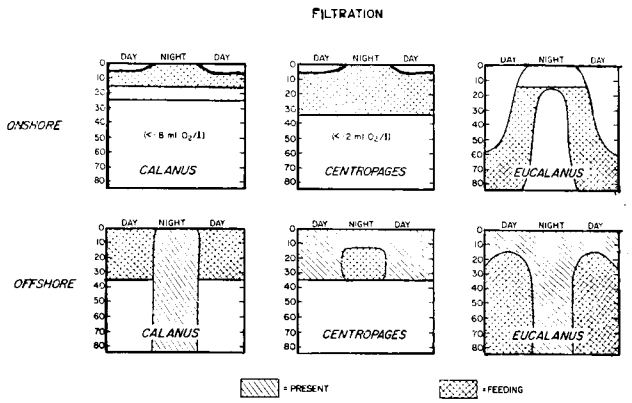


Table 3. Result of 2 x 2 contingency table analysis of feeding patterns.

Question	Onshore			Offshore		
	Calanus	Centropages	Eucalanus	Calanus	Centropages	Eucalanus
a. More gut contents in 0-15 m layer in day or night?	N.S.	N.S.	absent from surface layer	p=0.0019; more feeding during day	N.S.	N.S.
b. More gut contents in 15-85 m layer in day or night?	N.S.	N.S.	p=0.012; more feeding at day	N.S.	p=0.027; more feeding at night	p=0.043; more feeding during day
c. More gut contents at day or night regardless of depth?	N.S.	N.S.	p=0.0016; more feeding during the day	p=0.013; more feeding during day	p=0.044; more feeding at night	p=0.055; more feeding during day
d. More gut contents in upper layer (0-15 m) or lower layer (15-85 m)?	N.S.	N.S.	p=0.168; more feeding at depth	p=0.034; more feeding at depth	p=0.0023; more feeding at depth	p=0.0015; more feeding at depth
e. Were animals present or absent in surface layer (0- 5 m) in day or night?	p=0.001; avoided surface during day	p=0.0007 avoided surface during day	p=0.0012; avoided surface during the day	N.S.	N.S.	N.S.

Figure 6. Feeding patterns as in Figure 5 but based on calculated filtration rates.



One of the interesting trends suggests that patterns of vertical migration were modified according to the quantity of food available to the animals. At the two inshore stations all the species examined in this study avoided the surface 5 m during the day but swam up into this layer after sunset. This pattern disappeared at the offshore station where no coherent vertical migration in and out of the upper 5 m was noted in any of the species. This change in behavior is in keeping with observations of several authors who found that either the phototrophic response (Singarajah et al. 1967; Pearre 1973; Forward 1976), or the tendency to migrate vertically (Mackas and Bohrer 1976; Dann and Ringleberg, 1969) was associated with feeding satiety, and that satiated animals would migrate while unsatiated animals would not migrate.

We believe the most important variable in determining both the pattern of diurnal feeding of these copepods and their migrational pattern was the concentration of food available to them, and that each of these species responded in a manner that would amplify the differences between species when food became less abundant. At the inshore stations, **Calanus** avoided the surface during the day and fed evenly day and night; at the offshore station it became more of a daytime grazer, and in both regions fed on both live and dead particles with patterns expected of a non-selective filter-feeder. We believe that **Centropages** was feeding (Figure 5) carnivorously on other zooplankters and also fed on plant material; at the onshore stations it fed on plant material uniformly day and night, but it became a night-time feeder at the offshore station. **Eucalanus** also possesed behavioural patterns that isolated it from the other two genera; at the onshore stations it alone entered the anoxic zone where it fed during the day on detrital particles, and at night it swam to the surface but reduced its intensity of feeding. It kept its pattern of day-time feeding at the offshore station, but minimized interactions with **Calanus** by living at greater depth than **Calanus.**

The differing behavioral patterns of these copepods fit into the concepts of niche separation and resource allocation as advocated by Schoener (1974a) and others. Schoener (1974b) argues that when food is scarce animals will broaden their habitat (in this case the copepod species will range through greater depths of water) and will exhibit a decreased specialization on food types; Cowles (1979) has documented this latter modification of feeding behavior for **Calanus chilensis, Centropages brachiatus** and **Eucalanus inermis.** Schoener also argues that temporal specialization of feeding is unlikely to occur when food is relatively abundant (inshore), but becomes increasingly likely as food abundance decreases (offshore). Temporal specialization can occur when it becomes less expenssive in terms of energy for an animal to wait out periods of low food abundance. The uniqueness of these behavioral patterns is perhaps a device by which three kinds of apparently similar animals - **Calanus, Centropages,** and **Eucalanus** – can co-exist during periods of low food availability in an environment that is superficially homogeneous.

REFERENCES

COWLES, T. 1977. Copepod feeding in the Peru upwelling system. Ph. D. thesis, Duke University. 173 pp.

——— 1979. The feeding responses of copepods from the Peru upwelling system: food size selection. **J. Mar Res.** 37:601-622.

DANN, N. and J. RINGLEBERG. 1969. Further studies on the positive and negative phototactic reaction of **Daphnia magna** Straus. **Netherlands J. Zool.** 19:525-540.

DUGDALE, R., J. GOERING, R. BARBER, R. SMITH and T. PACKARD. 1977. Denitrification and hydrogen sulfide in the Peru upwelling region during 1976. **Deep-Sea Res.** 24:601-608.

FORWARD, R., Jr. 1976. Light and diurnal vertical migration: photobehavior and photophysiology of plankton. **in** Photochemical and Photobiological Reviews; **edited** by Kendric C. Smith. p. 157-209. Vol. 1, Plenum.

FROST, B. 1972. Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod **Calanus pacificus. Limnol. Oceanogr.** 17:805-815.

——— 1975. A threshold feeding behaviour in **Calanus pacificus. Limnol.Oceanogr.** 20:263-266.

HANEY, J. 1971. An **in situ** method for the measurement of zooplankton grazing rates. **Limnol. Oceanogr.** 16:970-976.

HEINRICH, A. 1973. Horizontal distribution of copepods in the Peru current region. **Oceanology.** 13: 97-103.

JEFFREY, S. 1974. Profiles of photosynthetic pigments in the ocean using thin-layer chromatography. **Mar. Biol.** 26:101-110.

LORENZEN, C. 1966. A method for the continuous measurement of **in vivo** chlorophyll concentration. **Deep-Sea Res.** 13:223-227.

MACKAS, D. 1968. Carbon/chlorophyll relationships in an upwelling area. **Limnol. Oceanogr.** 13:202-204.

——— and R. BOHRER. 1976. Fluorescence analysis of zooplankton gut contents and an investigation of diel feeding patterns. **J. Exp. Mar. Biol. Ecol.** 25:77-85.

MARSHALL, S. and A. ORR. 1972. The biology of a marine copepod. Springer.

MULLIN, M. 1963. Some factors affecting the feeding of marine copepods of the genus **Calanus. Limnol. Oceanogr.** 8:239-250.

PAFFENHOFER, G. 1971. Grazing and ingestion rates of nauplii, copepodids and adults of the marine planktonic copepod **Calanus helgolandicus. Mar. Biol.** 11:286-298.

PEARRE, S., Jr. 1973. Vertical migration and feeding in **Sagitta elegans** Verrill. **Ecology.** 54:303-314.

POULET, S. 1974. Seasonal grazing of **Pseudocalanus minutus** on particles. **Mar. Biol.** 25:109-123.

SCHNACK, S. 1975. Untersuchungen zur Nahrungsbiologie der Copopeden (Crustacea) in der Kieler Bucht. Ph. D. thesis. Inst. Meereskunde, Christian-Albrechts Univ., Kiel. 141 p.

SCHOENER, T. 1974. Resource partitioning in ecological communities. **Science.** 185:27-39.

——— 1974. The compression hypothesis and temporal resource partitioning. Proc. Nat. Acad. Sci. USA. 71:4169-4172.

SINGARAJAH, K., J. MOYSE and E. KNIGHT-JONES. 1967. The effect of feeding upon the phototactic behaviour of cirriped nauplii. **J. Exp. Mar. Biol. Ecol.** 1:144-153.

SOKAL, R. and F. ROHLF. 1969. Biometry. Freeman.

STRICKLAND, J. and T. PARSONS. 1972. A practical handbook of seawater analysis. 2nd. ed. **Bull. Fish. Res.. Bd. Canada.** 167.