Fatty acids
Lipids
Paracalanus parvus
Copepods
Zooplankton
Acides gras
Lipides
Paracalanus parvus
Copépodes
Zooplancton

Lipid composition of *Paracalanus parvus*

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ABSTRACT

The lipid composition of the Copepod *P. parvus* collected in July and October on the Atlantic coast of Argentina was studied. Lipid content, which was moderate in July, increased in October, as did the ratio of simple to complex lipids. Triacylglycerols predominated in the simple lipids, while phosphatidyl-choline and -ethanolamine predominated in the complex lipid fractions. Palmitic, 16 : 1 ω 7, 18 : 0, 18 : 1 ω 9, 20 : 5 ω 3 and 22 : 6 ω 3 were the main fatty acids. The lipid distribution and fatty acid composition suggest that some of these fatty acids are very probably dependent on the amount and composition of the diet. The amount of 20 : 5 ω 3 and 22 : 6 ω 3 acids in this Copepod would appear to depend to a great extent on the abundance of the particular species of phytoplankton which provide these acids or corresponding precursor fatty acids.

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RÉSUMÉ

Composition lipidique du Copépode *Paracalanus parvus* recueilli dans l'Atlantique du Sud

Nous avons étudié la composition lipidique du Copépode *Paracalanus parvus* récolté en juillet et en octobre dans les eaux de la côte argentine de l'Atlantique Sud. Le contenu des lipides, modéré en juillet, augmente en octobre en même temps que le rapport lipides simples lipides complexes. Les triacylglycéroles prédominent dans les lipides simples, tandis que la phosphatidylcholine et la phosphatidyléthanolamine prédominent dans les lipides complexes. Les principaux acides gras sont les suivants : 16 : 1 ω 7, 18 : 0, 18 : 1 ω 9, 20 : 5 ω 3 et 22 : 6 ω 3. La distribution des lipides et la composition des acides gras suggèrent que quelques-uns de ces acides gras dépendent probablement de la quantité et de la composition de leur nourriture. La quantité des acides 20 : 5 ω 3 et 20 : 6 ω 3 dans le Copépode est apparemment en rapport avec l'abondance d'espèces déterminées du phytoplancton qui contiennent ces acides gras ou les acides gras précurseurs correspondants.

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INTRODUCTION

The lipid composition of marine organisms is to a great extent conditioned by ecological and physiological factors. Their fatty acid composition may therefore be modified by the type and amount of food available throughout the year, and by the internal biosynthetic activity of the organism. Since calanoid Copepods are components of the plankton and occupy a key position in the marine food chain, it is important to study the composition of their lipids and fatty acids in their natural environment.

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A certain amount of information is available concerning the lipid composition of Northern Hemisphere marine Copepods harvested in their natural environment during specific periods of the year (Morris, 1971 *b*), (Ackman *et al.*, 1974), (Lee *et al.*, 1971 *b*) or grown in the laboratory with special diets (Lee *et al.*, 1971 *a*).

The present paper reports the lipid class distribution and fatty acid composition of the calanoid Copepod *Paracalanus parvus*, taken at two different periods of the year from inshore waters on the coast of Argentina.

MATERIALS AND METHODS

Paracalanus parvus Copepods were caught on the surface by a plankton net (160-180 µm mesh) in the inshore water of Mar del Plata (38°00S; 57°20'W), Buenos Aires Province, Argentina. The temperature of the surface water was 9.8°C in July and 13.1°C in October. Total salinity at the point of collection was 33.98‰ (July) and 33.77‰ (October), while nitrate concentration was 1.94 µg-at $N-NO_3^-$/lt (July) and 1.06 µg-at $N-NO_3$/lt (October). Phosphate concentration was 1.29 µg-at $-P-PO_4$/lt (July) and 1.06 µg-at $-P-PO_4$/lt (October) (Carreto *et al.*, 1973, Roa *et al.*, 1974). The collected Copepods were filtered slowly in the laboratory through a coarser sieve to eliminate organisms larger than *P. parvus*. They were then concentrated at the surface of the water, taking advantage of their positive phototropism. This procedure made it possible to obtain a sample of *P. parvus* which was more than 90% pure. The Copepods were then filtered through a sieve of 160-180 µm mesh and dried between filter paper, and the lipids were immediately extracted.

Lipid extraction and fractionation

The Copepods were homogeneized with a Potter-Elvehjem homogeneizer and the lipids extracted by the procedure of Folch *et al.* (1957). Total lipids were expressed as percentage of wet weight. Simple and complex lipids were separated by absorption on silicic acid (Wren, 1960) to facilitate the subsequent fractionation, identification and estimation of the lipid classes by thin layer chromatography (TLC) on silica gel G. The following solvent systems were used to analyse the simple lipids: petroleum ether (60-90°C) -ethyl ether-acetic acid (90: 10: 1 v/v) for general separation; and petroleum ether (60-90°C) -ethyl ether-acetic acid-methanol (90: 50: 2: 5 v/v) to separate the sterols from diacylglycerols. Identification of the components was done by comparison of the R_f with standards run on the same plate and by means of specific reactions (Lowry, 1968; Reitsema, 1954). The amount of the components was measured by extraction of the lipids from the silica gel with chloroform, evaporation of the solvent and weighing. The relative percentage of the total weight of the lipids was calculated.

The chloroform-methanol-water (65: 25: 4 v/v) solvent system was used to analyse complex lipids. Identification of the components was also effected by comparison of their R_f with standards run on the same plate and with specific reactions (Wagner *et al.*, 1961; Siakotos *et al.*, 1965). Once the complex lipids were chromatographed and identified, their phosphorus content was determined by the method of Bartlett (1959) modified by Doizaki and Zieve (1963).

Fatty acid composition

The total lipids were saponified with alcoholic KOH for a period of 45 minutes at 80°C, and unsaponifiable material was extracted with petroleum ether. Free fatty acids were recovered from the acidified medium, and esterified with methanol-HCl acid, purified by sublimation (Stoffel *et al.*, 1959) and finally analysed in a Hewlett-Packard 700 gas liquid chromatograph with a flame ionization detector. The columns were packed with 15% diethylene glycol succinate on Chromosorb W (80-100 mesh).

Fatty acids in the chromatogram were identified by comparison of their retention times with standards, using the graphic procedure of the retention times vs. chain length (Ackman, 1962), Ackman's separation factors I, II and III (Ackman, 1963; Ackman *et al.*, 1963 *a* and 1963 *b*) and Haken's factor (Haken, 1966). The percentage of each fatty acid was calculated by measuring the area of peaks. To confirm the chain length of the unsaturated fatty acids, the samples

Table 1
Composition of simple lipids of Paracalanus parvus (October).

Lipid classes	R_f [a]	Reactions: Iodine	Reactions: Dinitrophenyl-hydrazine	Reactions: Cl_3Fe	Distribution (%)
Sterol esters	0,89	+	−	+	17,6
Neutral plasmalogens + alkyldiacylglycerols	0,69	+	+	−	4,7
Triacylglycerols	0,58	+	−	−	45,5
Free fatty acids	0,32	+	−	−	11,8
Diacylglycerols + sterols	0,12	+	−	+	8,9
Monoacylglycerols, pigments and phospholipids	Origin	±	−	−	11,5

[a] Thin layer chromatography in petroleum ether (60-90°C) -ethyl ether-acetic acid (90: 10: 1 v/v).

were rechromatographed after hydrogenation (Farquhar *et al.*, 1959).

RESULTS

The amount of total lipids found in natural *P. parvus* samples collected in July (winter) was 2.0% (wet weight), and was higher in October (spring), when it reached 6.8%. These lipids were mainly stored in dorsal sacs extended along the body and easily visible because of their orange colour through the transparent chitinous exoskeleton.

In July, simple lipids accounted for 46,3% of the total; the remaining 53,7% were complex. From July to October, simple lipids increased to 76,3% and correspondingly complex lipids decreased to 23,7%.

The composition of the simple lipid fraction of copepods caught in October is shown in Table 1. The amount of copepods caught in July was unfortunately small, and it was impossible to analyse the lipids by thin layer chromatography. Triacylglycerols were found to be the principal components. Free and esterified sterols were identified by the positive reaction given with $FeCl_3$. In this zone, small amounts of waxes and hydrocarbons might be present. The zone of R_f 0,69 was tentatively identified as a mixture of neutral plasmalogens and alcohoxydiglycerides, since they showed positive reactions with iodine and 2,4-dinitrophenylhydrazine reagent (Christie, 1973).

The composition of complex lipids was established by phosphorus determination in the TLC plate. The results are shown in Table 2. Phosphatidyl choline was the principal component. Other phospholipids found were phosphatidyl ethanolamine, sphingomyelin, cardiolipin and lysophospholipids. Some spots which gave a positive phosphorus reaction were not identified.

The fatty acid composition of the lipids is illustrated in Table 3. The composition of simple, complex and total lipids was determined independently. The addition of simple and complex lipid composition reconstituted quite satisfactorily the total composition. The predominant fatty acids in the periods studied were palmitic, palmitoleic, eicosa-5, 8, 11, 14, 17-pentaenoic, oleic, stearic and docosa-4, 7, 10, 13, 16, 19-hexaenoic acids. Polyunsaturated fatty acids of ω 3 series predominate largely over acids of ω 6 family, as may be expected in the case of marine organisms.

In July, saturated acids accounted for 48.0% of the total acid, which was the highest value found. This was due to an increased accumulation in both simple and complex lipids. In October, the figure decreased to 35,4%, palmitic acid being primarly responsible for the change.

Monoenoic and for the most part 16:1 and 18:1 acids also showed the highest percentages in July. Palmitoleic acid was mainly found in complex lipids, and decreased in October.

A different change was shown in polyunsaturated acids. Both 20:5 ω 3 and 22:6 ω 3, which were major components of both simple and complex lipids, increased from July to October.

DISCUSSION AND CONCLUSION

Lipids

The lipids of *P. parvus* are largely stored in dorsal oil sacs and increased from July (winter) to October (spring), probably in response to the increased availability of food, since the phytoplankton concentration at the point of sampling increased from 290000 cells/lt (July) to 420000 cells/lt (October) (Carreto *et al.*, 1973), (Roa *et al.*, 1974). The principal change found was in the neutral lipids, which increased during this period, and could represent an enhanced energy store.

The samples of *Paracalanus parvus* caught in the water of Mar del Plata showed a surprisingly low wax ester content, although there are many references to high wax ester levels in calanoid Copepods (Benson *et al.*, 1972, Sargent *et al.*, 1976). Notwithstanding the predominance of triacylglycerol and the apparent absence or reduced amount of wax esters, the results found in our *P. parvus*

Table 2
Phospholipid composition of complex lipids of Paracalanus parvus (October).

Phospholipid classes	R_f *	Reactions: Dragendorff	Ninhidrine	I_2	Phosphorus (%)
Cardiolipin	0,73	−	−	+	8,3
Phosphatidyl ethanolamine	0,62	−	+	+	16,3
Phosphatidyl choline	0,37	+	−	+	36,2
Sphingomyelin	0,29	+	−	+	10,0
Lysophosphatidyl ethanolamine	0,24	−	+	+	7,8
Lysophosphatidyl choline	0,16	+	−	+	3,3
Unidentified Components	Origin	−	+	+	10,0
	0,62	−	−	+	2,1
	0,81	−	−	+	2,5
	1,00	−	−	+	3,5

* Thin layer chromatography in chloroform: methanol: water (65:25:4 v/v).

samples are similar to those obtained by others for crustaceans likely to live in superficial or temperate waters. Culkin and Morris (1969) found no waxes in one euphausiid and six zooplanktonic Decapods, while Morris (1971 *a*) found wax esters in only two of 12 euphausiids studied. Of 10 epipelagic Copepods studied by Lee *et al.* (1971 *b*), four were absolutely free of waxes, two contained 1%, two 21% and only one contained 69% in total lipids. Our results would therefore appear to agree with the statement that the wax ester content of oceanic crustaceans may be correlated with the depth at which the crustaceans are found (Lee *et al.*, 1971 *b*, Morris, 1972), and would be also related to the temperature and availability of food (Sargent, 1976). In species found in deep and cold oceanic waters (Lee *et al.*, 1971 *b*), triglycerides are replaced by wax esters as the source of metabolic fuel (Lee *et al.*, 1970).

The complex lipid composition of *P. parvus* is similar to that of other marine organisms, and of crustaceans in particular (De Koning *et al.*, 1966; Shieh, 1969).

Fatty acids

The lipids of naturally growing *P. parvus* are particularly rich in 16:0, 16:1, 20:5 ω 3 and 22:6 ω 3 acids, as they are in other marine Copepods (Lee *et al.*, 1971 *b*; Ackman *et al.*, 1974; Morris 1971 *b*; Ackman, Hooper, 1970). Polyunsaturated acids of ω 3 series are found in either simple or complex lipids, but predominate in the former (Table 3). The storage of 20:5 ω 3 acid in triacylglycerols and phospholipids but not in the wax esters of zooplanktonic organisms has been already recognized by Lee *et al.* (1971 *b*). These results would therefore agree with the low levels of found wax esters.

P. parvus is a herbivore (diatoms, dinoflagellates) and feeds on detritus (Ramírez *et al.*, 1973). Diatoms largely contain 14:0, 16:0 and 20:5 ω 3 acids, whereas dinoflagellates mainly provide 16:0, 18:0, 20:5 ω 3 and 22:6 ω 3 acids (Ackman *et al.*, 1968; Chuecas *et al.*, 1969). Detritus may provide mainly saturated and monoenoic fatty acids of 14, 16 and 18 carbons (Williams, 1965). It may therefore be considered that one of the main factors which determine the different fatty acid composition of *P. parvus* caught in July and October is the composition of the diet. In July, the availability of food at the point of sampling was lower than in October. It contained more detritus, while 25% of the phytoplankton was composed of diatoms (mainly *Thalassionema nitzchioides, Thalassiosira rotula* and *Chaetoceros spp.*) (Carreto *et al.*, 1973). Roa *et al.* (1974) have shown that in October, not only was the availability of food higher, 420000 cells/lt, but also that 90% were dinoflagellates, preferentially *Gymnodinium, Peridinium* and *Gyrodinium* but also including diatoms, silicoflagellates and cyanophyceae.

Table 3
Percentage fatty acid composition of the lipids of Paracalanus parvus.

	July			October		
Fatty acids[a]	Total lipids	Simple lipids	Complex lipids	Total lipids	Simple lipids	Complex lipids
12:0	0,8	0,6	1,5	0,8	0,9	1,0
13:0	–	–	0,7	0,3	–	0,5
14:0	7,6	11,1	7,6	5,9	6,2	6,2
14:1	0,5	–	–	0,6	–	1,0
15:0	0,9	0,1	0,9	0,8	2,6	0,4
15:1	0,7	0,9	–	0,6	–	–
16:0	29,4	28,1	25,7	17,0	17,1	17,8
16:1 ω 7	13,7	8,1	28,0	9,8	9,0	10,4
17:0	0,8	0,8	0,6	2,4	3,9	2,3
16:2 ω 4	1,6	0,6	2,1	1,9	1,9	2,4
18:0	8,4	11,5	10,8	7,9	10,6	7,8
18:1 ω 9	9,9	10,1	7,6	9,1	9,3	9,4
18:2 ω 9	1,0	0,9	–	2,2	1,8	2,7
18:2 ω 6	0,8	1,4	0,4	1,5	1,1	1,8
18:3 ω 6 20:0	0,4	0,6	–	0,6	0,6	0,8
18:3 ω 3	0,1	–	–	0,8	–	–
20:1 ω 9	0,5	–	2,0	0,5	–	–
20:2 ω 9	1,9	1,8	0,2	3,9	2,0	4,9
20:3 ω 6	0,3	0,4	0,2	0,8	–	–
22:0	0,1	0,3	–	0,3	–	–
20:3 ω 3	0,6	2,0	–	0,9	0,4	–
20:4 ω 3	0,4	1,0	–	0,2	–	–
20:5 ω 3	12,5	10,1	6,9	17,0	17,0	18,3
22:3 ω 6	0,5	–	–	0,4	–	–
22:3 ω 3	0,8	2,0	2,4	–	–	–
22:4 ω 3	–	–	–	1,2	–	–
22:6 ω 3	5,8	7,6	2,4	12,6	15,6	12,3

[a] The number before the colon represents the number of carbons in the chain. The number after the colon indicates the number of double bonds. The number after ω indicates the number of carbons between the last double bond and the methyl end.

This type of food composition would therefore preferentially evoke a higher proportion of 20:5 ω 3 and 22:6 ω 3 acids in October (Table 3), an interpretation which is supported by experiments carried out with other zooplanktonic organisms. Jezyk and Penicnak (1966) and Hinchcliffe and Riley (1972), for example showed that *Artemia* fed on algae cultures reflected the composition of the diet. Similar results were also found by Ackman *et al.* (1970); Culkin and Morris (1970), Bottino (1974) and Jeffries (1970), working with wild zooplankton in their natural environment.

Notwithstanding these results, the importance of the participation of fatty acid biosynthesis in the building of the final composition of *P. parvus* lipids should not be overlooked. Although *P. parvus* is able to synthesize its own 20:5 ω 3 and 22:6 ω 3 acids from 18:3 ω 3 (Moreno *et al.*, 1979 *b*), the presence of this precursor in diatoms and dinoflagellates is not very pronounced, whereas 20:5 ω 3 and 22:6 ω 3 acids are relatively abundant in the same food sources. Moreno *et al.* (1979 *a* and *b*) have shown that *P. parvus* actively synthesizes *de novo* saturated and monounsaturated fatty acids, preferentially 16:0, 16:1 and 18:1 acids. On the other hand, *P. parvus* is unable to synthesize linoleic or α-linolenic acids. The biosynthesis of 20:5 ω 3 and 22:6 ω 3 requires the precursors α-18:3 or other fatty acids of the α-linolenic family. Biosynthesis takes place according to the "animal" route (Moreno *et al.*, 1979 *a*). Therefore, since the concentration of 18:3 ω 3 in diatoms, dinoflagellates and other elements of the phytoplankton which constitutes *P. parvus* food is not high, while 20:5 ω 3 and 22:6 ω 3 acids are generally abundant in the same organisms (Sargent, 1976), it is possible to speculate that they are preferentially provided by the food. These results consequently agree with the conclusions of the preceding paragraph. However, the saturated and monounsaturated acids would be easily synthesized by the Copepod when required.

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REFERENCES

Ackman R. G., 1962. Structure and retention time in the gas-liquid chromatography of unsaturated fatty acids on polyester substrates, *Nature*, **194**, 970-971.

Ackman R. G., 1963. An analysis of separation factors applicable in the gas-liquid chromatography of unsaturated fatty acid methyl esters on a polyester substrate, *J. Am. Oil Chem. Soc.*, **40**, 564-567.

Ackman R. G., Hooper S. N., 1970. Analysis of fatty acids from Newfoundland Copepods and sea water with remarks on the occurrence of arachidic acid, *Lipids*, **5**, 417-421.

Ackman R. G., Burgher R. D., 1963 *a*. A proposed basis for the systematic identification of unsaturated fatty acid esters through gas-liquid chromatography on polyester substrates, *J. Chromatogr.*, **11**, 185-194.

Ackman R. G., Burgher R. D., Jangaard P. M., 1963 *b*. Systematic identification of fatty acids in the gas-liquid chromatography of fatty acid methyl esters: a preliminary survey of seal oil, *Can. J. Biochem. Physiol.*, **41**, 1627-1641.

Ackman R. G., Tocher C. S., McLachlan J., 1968. Marine phytoplankter fatty acids, *J. Fish. Res. Board Can.*, **25**, 1603-1620.

Ackman R. G., Eaton C. A., Sipos J. C., Hooper S. N., Castell J. D., 1970. Lipids and fatty acids of two species of North Atlantic krill (*Meganyctiphanes norvegica* and *Thysanoëssa inermis*) and their role in the aquatic food web, *J. Fish. Res. Board Can.*, **27**, 513-533.

Ackman R. G., Linke B. A., Hingley J., 1974. Some details of fatty acids and alcohols in the lipids of North Atlantic Copepods, *J. Fish. Res. Board Can.*, **31**, 1812-1818.

Bartlett G. R., 1959. Phosphorus assay in column chromatography, *J. Biol. Chem.*, **234**, 466-470.

Benson R. A., Lee R. F., Nevenzel, 1972. Wax-esters: major marine metabolic energy sources, *Biochem. Soc. Symp.*, **35**, 175-187.

Bottino N. R., 1974. The fatty acids of Antartic phytoplankton and euphausiids. Fatty acid exchange among trophic levels of the Ross Sea, *Mar. Biol.*, **27**, 197-204.

Carreto J. I., Ramírez F., Roa B. H., Verona C. A., 1973. Plancton y condiciones ecológicas en las aguas de la plataforma bonaerense, frente a Mar del Plata. II. Campaña "Transección II" (Julio 1972) Proy. Desar. Pesq. FAO Doc. Téc. Prel. No. 30, Mar del Plata, 1-13.

Christie W. N., 1973. *Lipid analysis*, Pergamon Press, Oxford, p. 167.

Chuecas L., Riley J. P., 1969. Component fatty acids of the total lipids of some marine phytoplankton, *J. Mar. Biol. Ass. UK*, **49**, 97-116.

Culkin F., Morris R. J., 1969. The fatty acids of some marine crustaceans, *Deep-Sea Res.*, **16**, 109-116.

Culkin F., Morris R. J., 1970. The fatty acid composition of two marine filter-feeders in relation to a phytoplankton diet, *Deep-Sea Res.*, **17**, 861-865.

De Koning A. J., McMullan K. B., 1966. Phospholipids of marine origin. II. The rock lobster (*Jasus lalandii*), *J. Sci. Food Agri.*, **17**, 117-120.

Doizaki W. M., Zieve L., 1963. Quantitative estimation of some phosphatides and their hydrolisis products by thin-layer chromatography, *Proc. Soc. Exp. Biol. Med.*, **113**, 91-94.

Farquhar J. W., Insull W. Jr., Rosen P., Stoffel W., Ahrens E. H. Jr., 1959. The analysis of fatty acid mixture by gas-liquid chromatography: construction and operation of an ionization chamber instrument, *Nutr. Rev.*, **17**, Suppl. 1-30.

Folch J., Lees M., Sloane-Stanley G. H., 1957. A simple method for the isolation and purification of total lipids from animal tissues, *J. Biol. Chem.*, **226**, 497-509.

Haken J. K., 1966. Retention time relationship in the gas chromatography of the methyl esters of fatty acids, *J. Chromatogr.*, **23**, 375-381.

Hinchcliffe P. R., Riley J. P., 1972. The effect of diet on the component fatty acid composition of *Artemia salina*, *J. Mar. Biol. Ass. UK*, **52**, 203-211.

Jeffries H. P., 1970. Seasonal composition of temperate plankton communities: fatty acids, *Limnol. Oceanog.*, **15**, 419-426.

Jezyk P. F., Penicnak A. J., 1966. Fatty acid relationship in an aquatic food chain, *Lipids*, **1**, 427-429.

Lee R. F., Nevenzel J. C., Paffenhöfer G. A., Benson A. A., 1970. The metabolism of wax esters and other lipids by the marine copepod, *Calanus helgolandicus*, *J. Lipid Res.*, **11**, 237-240.

Lee R. F., Nevenzel J. C., Paffenhöfer G. A., 1971 *a*. Importance of wax esters and other lipids in the marine food chain: phytoplankton and copepods, *Mar. Biol.*, **9**, 99-108.

Lee R. F., Hirota J., Barnett A. M., 1971 *b*. Distribution and importance of wax esters in marine copepods and other zooplankton, *Deep-Sea Res.*, **18**, 1147-1165.

Lowry R. R., 1968. Ferric chloride spray detector for cholesterol and cholesteryl esters on thin-layer chromatogram, *J. Lipid Res.*, **9**, 397.

Moreno V. J., Moreno J. E. A. de, Brenner R. R., 1979 *a*. Fatty acid metabolism in the calanoid Copepod *Paracalanus parvus*. 1. Polyunsaturated fatty acids, *Lipids*, **14**, 313-317.

Moreno V. J., Moreno E. J. A. de, Brenner R. R., 1979 *b.* Fatty acid metabolism in the calanoid Copepod *Paracalanus parvus.* 2. Palmitate, stearate, oleate and acetate, *Lipids,* **14,** 318-322.

Morris R. J., 1971 *a.* Variations in the fatty acid composition of oceanic euphausiids, *Deep-Sea Res.,* **18,** 525-529.

Morris R. J., 1971 *b.* Comparison of the composition of oceanic copepods from different depths, *Comp. Biochem. Physiol.,* **40** *B,* 275-281.

Morris R. J., 1972. The occurrence of wax esters in crustaceans from the north-east Atlantic ocean, *Mar. Biol.,* **16,** 102-107.

Ramírez F., Roa B. H., Verona C. A., Carreto J. I., 1973. Plancton y condiciones ecológicas en las aguas de la plataforma bonaerense, frente a Mar del Plata. III. Campaña "Transección III" (agosto 1972) Proy. Desear. Pesq. FAO Doc. Téc. Prel. No. 33, Mar del Plata, 1-14.

Roa B. H., Verona C. A., Carreto J. I., Ramírez F., 1974. Plancton y condiciones ecológicas en las aguas de la plataforma bonaerense, frente a Mar del Plata. IV. Campaña "Transección IV" (Octubre de 1972) Proy. Desar. Pesq. FAO Doc. Téc. Prel. No. 40, Mar del Plata, 1-38.

Reitsema R. H., 1954. Characterization of essential oils by chromatography, *Annal. Chem.,* **26,** 960-963.

Sargent J. R., 1976. The structure, metabolism and function of lipids in marine organisms, in *Biochemical and Biophysical Perspectives in Marine Biology,* Vol. 3, edited by D. C. Malin and J. R. Sargent, Academic Press, London, 149-212.

Sargent J. R., Lee R. F., Nevenzel J. C., 1976. Marine waxes, in *Chemistry and biochemistry of natural waxes,* edited by P. E. Kolattukudy, Elsevier, Amsterdam, 50-91.

Shieh H. S., 1969. The biosynthesis of Phospholipids in the lobster, *Homarus americanus, Comp. Biochem. Physiol.,* **30,** 679-684.

Siakotos A. N., Rouser G., 1965. Analytical separation of nonlipid water soluble substances and gangliosides from other lipids by detran gel column chromatography, *J. Am. Oil Chem. Soc.,* **42,** 913-919.

Stoffel W., Chu F., Ahrens Jr., 1959. Analysis of long-chain fatty acids by gas-liquid chromatography. Micromethod for preparation of methyl esters, *Anal. Chem.,* **31,** 307-308.

Wagner H., Hörhammer L., Wolff P., 1961. Dünnschichtchromatographic von phosphatiden und Glykolipiden, *Biochem. Z.,* **334,** 175-184.

Williams P. M., 1965. Fatty acids derived from lipids of marine origin, *J. Fish. Res. Board Can.,* **22,** 1107-1122.

Wren J. J., 1960. Chromatography of lipids on silicic acid, *J. Chromatogr.,* **4,** 173-195.