Vol. 64: 69–79, 1990 | MARINE ECOLOGY PROGRESS SERIES
Mar. Ecol. Prog. Ser. | Published June 28

Variation of vitamin C in some common species of marine plankton

A. M. Hapette, S. A. Poulet

Centre d'Etude d'Océanographie et de Biologie Marine, CNRS et Université P. et M. Curie, Place Georges Teissier, F-29211 Roscoff, France

ABSTRACT: Occurrence of vitamin C, observed in 26 species of the major zooplankton taxa, confirms the ubiquity of this essential micronutrient in eukaryotes. Short- and long-term variations of vitamin C in copepods reflected the dependence of vitamin incorporation upon phytoplankton. The link existing between the source of vitamin C, or its precursor, and the rank of the organisms in the marine food-chain is illustrated by the significant difference found between carnivorous and omnivorous/herbivorous species. Copepods and their fecal pellets are substantial carriers of vitamin C, constituting a potential pathway from phytoplankton to consumers in higher trophic levels and probably unable to synthesize this molecule. Information about properties and functions of vitamin C, known for plants and vertebrates, is scant in zooplankton, although it could be relevant to plankton ecology involving feeding, reproduction and growth.

INTRODUCTION

L(+)-ascorbic acid (vitamin C:L(+)-threo-2-hexenono-1,4-lactone) is a ubiquitous oligonutrient whose biochimestry, biosynthesis and physiological functions is well documented (Englard & Seifter 1986, Loewus & Loewus 1987). It is a product of hexose metabolism (Loewus 1971), the main precursor of which is D-glucose produced by photosynthetic organisms. The 2 known biosynthetic conversions of D-glucose into vitamin C reported for eukaryotic organisms (Grün & Loewus 1984) involve either a direct process or an inversion pathway, depending on species. The direct process, characterized by the oxidation of D-glucose and conservation of the hydroxymethyl group at C_6, has been studied in higher terrestrial plants (Loewus & Helsper 1982) and in some algae such as *Chlorella pyrenoisa* (Renstrøm et al. 1982/83) and *Cyclotella cryptica* (Grün & Loewus 1984). The second pathway is characterized by the conservation of the C_6-carbon chain of the sugar and by the inversion of the sequence of carbons (Renstrøm et al. 1982/83). This pathway seems to be typical for animals synthesizing ascorbic acid, such as rats (Loewus et al. 1960), although it has been described in a few species of algae (Shigeoka et al. 1979, Helsper et al. 1982).

Chatterjee et al. (1975) listed species not capable of synthesizing vitamin C, in relation to their phylogeny. Organisms that lack L-gulonolactone oxidase or D-glucurono-reductase, 2 enzymes required for the biosynthesis of ascorbic acid (Wilson 1973, Yamamoto et al. 1978), have to find this molecule in their food. Vitamin C is known as a micronutrient essential to life, but the capability of most marine organisms to synthesize this molecule has not been established. In crustaceans and fishes, the ability to synthesize vitamin C is either absent or weak (Guary & Guary 1975, Kean et al. 1985, Dabrowski et al. 1988). Recent application of high-performance liquid chromatography (HPLC; Poulet et al. 1989, Hapette & Poulet 1990) to the measurement of vitamin C in zooplankton should allow a re-examination of these questions, which have remained unanswered since Fisher's (1960) review.

It is tempting to extend to zooplankton the inability of invertebrates and fishes to synthesize ascorbic acid (Chatterjee 1973), as well as their physiological requirement for this molecule (Guary & Guary 1975, Halver et al. 1975, Barnes & Barnes 1982). If this is the case, it becomes important to study the flux of ascorbic acid between the primary and secondary planktonic trophic levels, as well as to investigate the physiological role of this molecule in the plankton. Before such an investigation can take place, 2 fundamental questions must be answered: (1) Does vitamin C occur and vary in plankton? (2) Does vitamin C incorporation in zooplankton reflect food conditions?

0171-8630/90/0064/0069/$ 03.00

In this report, short- and long-term variations of vitamin C in copepods have been investigated in relation to the standing stock of phytoplankton. The occurrence and variations of vitamin C have been compared among various species of plankton in relation to their position in the marine food chain.

MATERIAL AND METHODS

Field observations. Comparison of the vitamin C content in micro-, meso- and macrozooplankton was made with species belonging to different taxa, sampled in the Celtic and Irish Seas in 1987 and 1988 ('Rosimer' cruises) and in the Mediterranean sea in 1989 ('Tomofront-89 b' cruise). Bongo, WP-2 and Isaacs Kidd nets (200 to >500 µm mesh size) were used, depending on depth of capture (0 to 1000 m) and size of the plankton. Samples were also collected in the English Channel, in coastal waters (surface to 40 m) off Plymouth (UK) and off Roscoff (France), and in the Baie du Morbihan (Kerguelen Island, South Indian Ocean), using conical plankton nets (75, 200 and 710 µm mesh size). Samples were stored in liquid nitrogen (−196 °C), except those from Kerguelen which were kept at −80 °C.

Seasonal variations of vitamin C in 2 representative species of copepods, *Acartia clausi* and *Calanus helgolandicus*, were evaluated from samples collected weekly or monthly in coastal waters off Roscoff and Plymouth from 1987 to 1989. Live specimens picked from each plankton haul were identified, accurately weighed (<250 mg wet weight [ww]) and stored in liquid nitrogen until analysis.

Shipboard incubations of natural assemblages of copepod populations were conducted overnight at different sites located in the Celtic, Irish and Mediterranean seas (Claustre et al. unpubl.). Containers filled with 20 or 400 l of pre-sieved seawater (100 µm mesh size) were used on deck for the incubations. Fecal pellets were collected and concentrated <10 to 12 h later, according to a method described by Poulet et al. (1986). Feces were separated from other particles, organisms and eggs by sequential filtering, followed by microscopic sorting. Sub-samples were preserved in formaldehyde (4 %) for counting and stored in liquid nitrogen until chemical analysis. Precise composition of the copepod populations (dominant species were *Calanus helgolandicus, Temora longicornis, Oithona* sp., *Acartia* sp., and *Centropages typicus*, depending on site), as well as the chemical analyses of fecal pellets, will be reported elsewhere (Claustre et al. unpubl., Williams et al. unpubl.).

Salinity, temperature and chlorophyll *a* concentration were evaluated on board, using the Guildline CTD (General Oceanics) or the Seabird model SBE 19, (Seabird Co., equiped with a fluorometer Seatech), or from frozen (−20 °C) phytoplankton samples concentrated on GF/C filters, from which chlorophyll *a* and phaeophytin contents were determined, using a Turner Designs fluorometer following Yentsch & Menzel (1963) and Lorenzen (1967).

Laboratory experiments. Incorporation and decrease of ascorbic acid in the body pool of *Acartia clausi* and *Calanus helgolandicus* were measured in the laboratory by starving and feeding experiments. Copepods were collected in coastal waters off Roscoff and Plymouth, using conical plankton nets (200 and 710 µm mesh size) towed oblically from 40 to 0 m at <1 knot. Stocks of copepods were pipetted gently into 1 l beakers containing filtered seawater (0.45 µm) and sorted according to species and sex using a binocular microscope. These selected sub-samples were formed of 100 % adult male and female *C. helgolandicus*, and of 70 to 100 % *A. clausi* including mixed developmental stages (C IV to adult); the remaining 30 % was formed of various species of copepods, decapod, annelid and cirriped larvae. Sub-samples from these initial stocks were accurately weighed prior to vitamin C analysis to estimate initial content in copepods before incubations started. Copepods were then starved for 10 h or 3 d in filtered seawater under temperature and light conditions similar to those measured in situ at time of capture. At the end of this phase, the remaining population of live copepods was transferred into new containers filled with filtered seawater containing a known concentration of cultivated phytoplankton cells. The duration of the feeding period varied from 12 h to 3 d. Visual observation of the variation of the fullness and color of copepod gut contents gave a rough evaluation of the feeding by copepods. Counts of suspended particles in seawater before, during and after both the starving and feeding periods, using a Coulter counter (Model ZBC 1000), in conjunction with the measurement of total gut pigments (Dagg 1983, Stearns et al. 1989), was used to estimate the feeding activity. Variation of vitamin C in copepods was estimated in samples (20 to 60 µg ww) collected at regular time intervals during the 2 phases of the experiment.

Calanus helgolandicus were incubated in 1 or 2 l of filtered seawater (0.45 µm) at 15 to 20 ind. l^{-1}, kept gently suspended with a plankton wheel (ca 1 rpm; Robins & Bellan 1986) in a room at constant temperature (12 °C). Containers of 1 m^3, containing 200 to 300 l of filtered seawater (0.6 µm), were utilized for *Acartia clausi* at a concentration of 6.6 to 13.3 µg ww l^{-1} in order to obtain enough sub-samples despite the high mortality rate (50 to 70 %) during starvation. Air bubbling facilitated resuspension of copepods and particles during the incubation.

Phytoplankton cultures. *Thalassiosira weissflogii*

(Bacillariophyceae) and *Cryptomonas maculata* (Cryptophyceae) were obtained from Plymouth Marine Laboratory (UK). Phytoplankton was grown in batch cultures at 15 °C in f/2 medium (at Roscoff: Guillard & Ryther 1962; at Plymouth: Eppley 1977). Cells at a concentration of 10^4 to 10^6 l^{-1} depending on species and experiment, were fed to copepods at the end of the starvation periods. Other species of phytoplankton (*Dunaliella primolecta, Chaetoceros* sp. and *Phaeocystis pouchetii*) were also cultivated at Roscoff to compare their intracellular pool of vitamin C.

Chemical analyses. In addition to the chlorophyll *a* and phaeopigment analyses reported above, the total protein content in copepods was measured according to Bradford's method (1976). Vitamin C in plankton was analysed using HPLC following a method recently described by Hapette & Poulet (1990). A polystyrene divinyl benzene column (PLRP-S: 25×4.6 mm I. D.; 100 Å; 5 µm – Polymer Laboratories) was used for all the analyses reported here. Results were expressed in pg per cell or µg per g wet wt (ww). Wet weight was used instead of a dry weight unit, to avoid the potential degradation of vitamin C by heat.

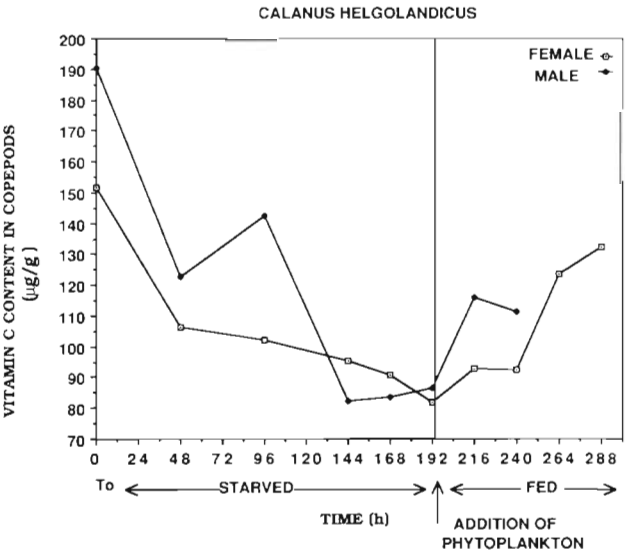

Fig. 1 *Calanus helgolandicus*. Variation of vitamin C in copepods collected in July 1988 in the English Channel off Plymouth (UK), kept in filtered seawater (starved) and then supplied every day with 10^5 cells l^{-1} of *Thalassiosira weissflogii* from 193 to 288 h. T_o: initial content in freshly collected copepods prior to experiment

RESULTS

Vitamin C was analyzed among 26 common species of zooplankton found in European coastal and shelf waters and in the Indian ocean. Values ranged from 10 to 800 µg g^{-1} ww (Table 1). Vitamin C in zooplankton was not related to any environmental conditions such as temperature or salinity at the site of capture. Rather, interspecific variations of vitamin C were related to the mode of feeding of the organisms (Table 1). The mean vitamin C content in carnivorous species (36.89± 21.60 µg g^{-1} ww) was 6 times less per unit weight than in omnivorous/herbivorous species (183.37± 134.87 µg g^{-1} ww).

Short-term variation of ascorbic acid in relation to food and feeding was studied in copepods. Initial content of vitamin C in naturally occurring adult male and female *Calanus helgolandicus* was 190 and 150 µg g^{-1}, respectively. They were starved for 192 h in filtered seawater, then fed 66 h on cultures of *Thalassiosira weissflogii* renewed daily, at constant concentration ($<10^6$ cells l^{-1}). The decrease of vitamin C in the body pool of the females was 54 % of the initial content, corresponding to 0.28 % h^{-1} during the starving period. The rate of increase was 1.54 % h^{-1} during the following feeding phase, corresponding to an uptake of 62.12 % of the content measured at 192 h (Fig. 1). Uptake rate was 5 times higher than rate of decrease. Similar results were obtained for males. Neither males nor females had recovered their initial pool of vitamin C by the end of these incubations. Vitamin C in *C. helgolandicus* also exhibited variability with time (Table 2).

Seasonal variation in *Acartia clausi* was monitored from 1987 to 1989 (Fig. 2). Vitamin C in *A. clausi* varied seasonally, reaching minimum values in fall and winter and maxima in spring and summer. Values could changed by ±50 to 100 µg g^{-1} within a week; during the year the maximum amplitude was 330 µg g^{-1} During the same length of time, maximum variation of the concentration of chlorophyll *a* in seawater was ±1 µg l^{-1}. In 1987, the monthly mean values of vitamin C co-varied with the standing stock of chlorophyll *a* measured in the same area (coefficient of correlation: $R = 0.89$; variance: $F = 23.2$; $n = 8$; $\alpha < 0.01$). In 1988–89, these 2 variables were not significantly correlated ($R = 0.24$; $n = 18$). Because in situ chlorophyll *a* and vitamin C samples were not always collected each month in the same week, only 8 and 18 paired data collected on the same day in 1987 and in 1988–89 respectively were statistically compared.

The relationship between food concentration and vitamin C content was further tested with *Acartia clausi* kept for 156 h in microcosms (Fig. 3). The mean concentration of suspended particles in the incubators varied from 200 to 850 ml^{-1} after addition of copepods, then decreased to 300 ml^{-1} (Fig. 3B). From 0 to 95 h, particles were mainly detritus and broken fecal material having negligible food value for copepods; this phase was defined as a 'starvation' period. At time

Table 1. Occurrence and variation of vitamin C in some common species of zooplankton collected in European coastal and shelf waters and the South Indian Ocean

Type of plankton	Taxon	Species	Vitamin C content ($\mu g\ g^{-1}$ ww)	No. of samples	Sampling area[a]	Feeding mode[b]
Meroplankton	Decapoda	*Palinurus elephas*[c]	41.82	1	D	OMN
		Nephrops norvegicus[c]	94.69–123.33	2	B	OMN
		Pasiphaea sp.	90.90–139.71	2	B	OMN
		Sergestes corniculum	28.60	1	A	CAR
Zooplankton	Copepoda	*Calanus helgolandicus*	85.59–436.33	27	B, C, D, E	OMN
		Temora longicornis	125.54–368.10	4	B	OMN
		Acartia clausi	30–260	48	D	OMN
		Anomalocera patersoni	11.66	1	C	CAR
		Centropages typicus	52.78–110.02	7	A	OMN
		Drepanopus pectinatus	9.29–13.83	2	F	OMN
		Nauplii (*A. clausi*, *T. longicornis*)	201.52–234.51	2	D	HER
	Euphausiacea	*Meganyctiphanes norvegica*	25.47–66.07	8	A, B, C	OMN
	Pteropoda	*Cavolinia inflexa*	147.04	1	A	HER
		Limacina inflata	31.73–41.70	2	C	HER
	Thaliacea	*Thalia democratica* (oozoid)[d]	2.42	1	A	HER
	Chaetognatha	*Sagitta elegans*	15.50	3	C	CAR
	Siphonophora	*Velella velella*	59.36	1	A	CAR
	Ctenophora	*Pleurobrachia pileus*	24.22	3	B	CAR
	Isopoda	*Idotea metallica*	272.58	1	A	OMN
	Amphipoda	*Phrosina semilunata*	52.60	1	A	CAR
		Platyscelus serratulus	77.82	1	A	CAR
		Phronima sedentaria	55.87	1	A	CAR
Ichthyoplankton	Fish eggs	*Scomber scombrus*	106.72–155.52	4	C	
		Sprattus sprattus	207.88	1	B	
	Fish larvae	*Scomber scombrus*	204.21–439.25	13	C	OMN
		Sprattus sprattus	151.23–831.42	112	B	OMN
		Solea solea	223.10–565.61	15	B	OMN
Necton	Teleostean	*Argyropelecus hemigymnus*	44.30	1	A	CAR
		Cyclothone braueri	20.87	1	A	CAR

[a] A = Mediterranean Sea; B = Irish Sea; C = Celtic Sea; D = English Channel, Roscoff; E = English Channel, Plymouth; F = Kerguelen Island
[b] OMN = omnivorous; HER = herbivorous; CAR = carnivorous
[c] Larvae
[d] Probably underestimated due to high water concentration, or non-feeding

Table 2. *Calanus helgolandicus*. Seasonal variation of vitamin C in copepods collected in the English Channel off Plymouth (UK). n: number of replicate samples. nd: below detection limit. Unit: $\mu g\ g^{-1}$ wet weight

Date	Female	Male
25 Aug 1988	106.22 n=1	122.79 n=1
30 Sep 1988	187.37 n=5	122.31 n=3
4 Oct 1988	229.42 n=8	nd n=3
29 Nov 1988	193.39 n=8	102.37 n=9

t = 95 h, addition of phytoplankton (*Cryptomonas maculata* or *Thalassiosira weissflogii*: 50×10^3 to 10^5 cells l^{-1}) increased the background level of particles. The feeding activity of copepods was demonstrated by the variations of the gut pigment concentration (Σ chlorophyll *a* + phaeopigments: Fig. 3C). During the starvation period, the decrease both of the gut pigments from 42 to 18 $\mu g\ g^{-1}$ ww and of the proteins from 65 to 37 $mg\ g^{-1}$ ww confirmed that these particles were not suitable food and that copepods did starve. Addition of phytoplankton was followed by an increase in the gut pigment concentration. Then it decreased again in conjunction with the clearance of particles. Total protein concentration in copepods (Fig. 3D) also varied significantly during the 2 phases of the experiment. Maximum values were observed 40 to 50 h after the food was added to the incubators. Copepods could lose 83 % of their initial content of vitamin C in 24 h when starved (Fig. 3A). The content of vitamin C remained below 35 $\mu g\ g^{-1}$ during most of the starvation phase. Food uptake by *A. clausi* (Fig. 3C) induced an increase of vitamin C in the body between 101 and 106 h into the experiment (Fig. 3A). During this short period

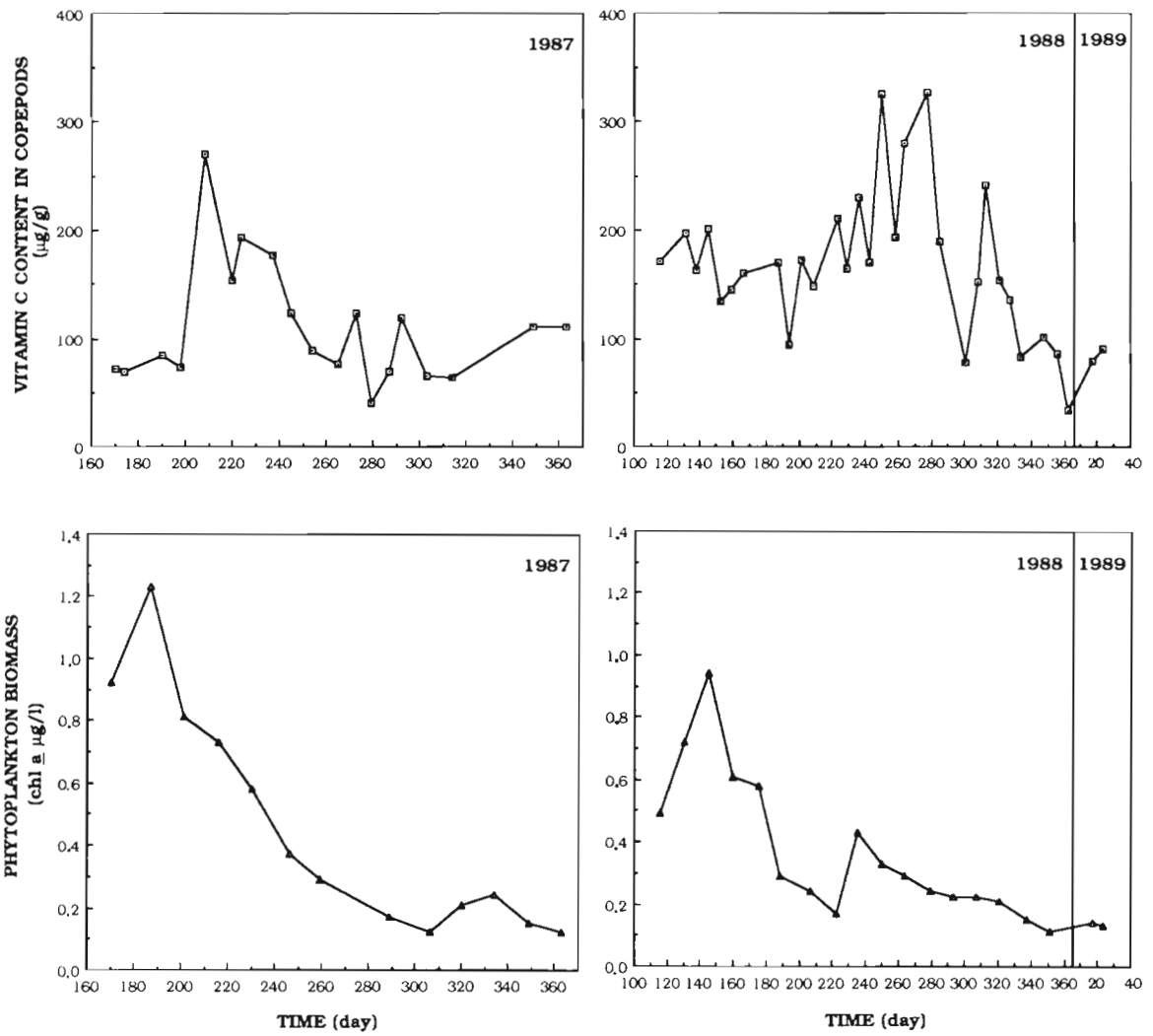

Fig. 2. *Acartia clausi*. Seasonal variation of vitamin C in copepods collected in coastal waters off Roscoff (France) and of the phytoplankton biomass measured in seawater. For comparison, the 1987 data were taken from Poulet et al. (1989)

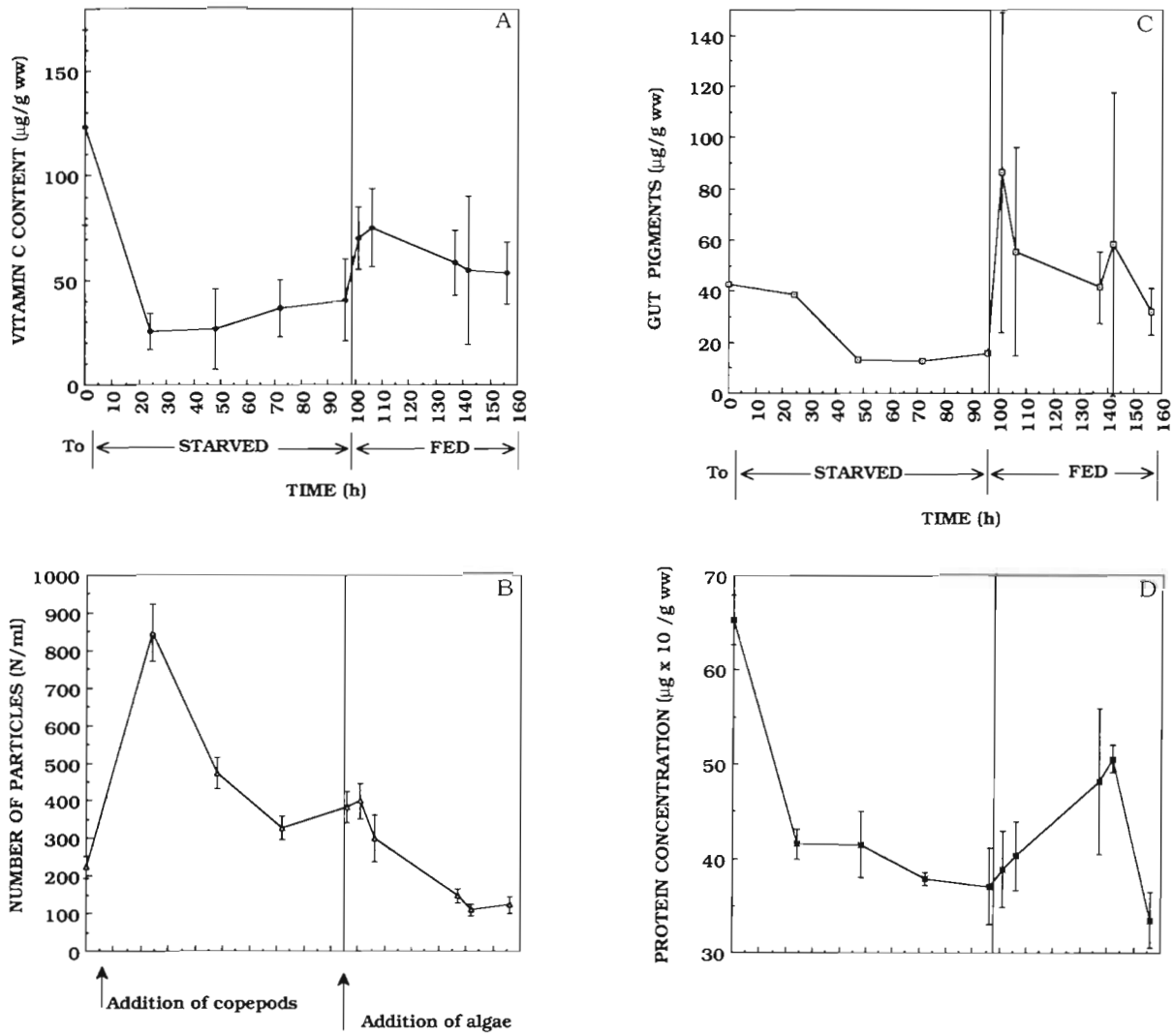

Fig. 3. *Acartia clausi*. (A) Variation of vitamin C in the body pool in relation to (B) food level, (C) gut pigments characterizing the feeding of copepods, and (D) total protein concentration in copepods. T_o: initial value in freshly collected samples prior to experiment. Values are means ± standard deviations (bars), corresponding to A: n = 2 to 6; B: n = 8; C: n = 1 or 2 and D: n = 2 replicate samples. Algae provided only once to copepods, at time T = 95 h, were either *Cryptomonas maculata* or *Thalassiosira weissflogii*. Gut pigments correspond to total pigment concentrations (chlorophyll *a* + phaeophytin) measured in acetonic extracts of copepods

(<10 h) vitamin C increased at a rate of <4 % h^{-1}. As food decreased in quantity (after 110 h), both gut pigment and vitamin C followed similar fluctuations with time. Although gut pigments of fed copepods were equal or higher than nonstarved specimens, as shown at the start of the experiment, neither vitamin C nor proteins ever returned to their initial levels.

Content of vitamin C in copepod fecal pellets was evaluated. Natural populations of copepods, primarily *Calanus helgolandicus, Temora longicornis*, or *Centropages typicus*, were incubated on deck on 3 occasions (April to June), to sample fresh fecal material. Vitamin C incorporation into copepod feces occurred but was less than 0.7 pg per fecal pellet (Table 3). Furthermore, vitamin C in feces was detectable only when chlorophyll *a* was >1.5 µg l^{-1}.

Preliminary measurements of vitamin C in 5 species of phytoplankton are shown in Table 4. The intracellular content of vitamin C varied with species by about 20-fold. The mean ratios (n = 10 replicate samples) between vitamin C and pigment content, evaluated in *Dunalliela primolecta*, were 7.8×10^{-5} for vitamin C/chlorophyll *a* and 6.9×10^{-5} for vitamin C/phaeophytin.

DISCUSSION

Our results have emphasized the ubiquity and unevenness of vitamin C in plankton. The occurrence

Table 3. Vitamin C in copepod fecal pellets in relation to in situ chlorophyll *a* concentration. nd: below detection limit

Site	Food level chlorophyll *a* ($\mu g\ l^{-1}$)	Copepod species	Feces vitamin C content (pg $pellet^{-1}$)	Range of samples	No. of samples
Celtic Sea	1.5–2.5	*Calanus helgolandicus*	0.61	nd–0.61	2
Irish Sea	>2	*Temora longicornis*	0.59	nd–0.59	4
Mediterranean Sea	<1.2	*Centropages typicus*	nd	nd	5

Table 4. Vitamin C in some species of phytoplankton

Class	Species of phytoplankton	Vitamin C content (pg $cell^{-1}$)	No. of samples
Chlorophyceae	*Dunaliella primolecta*	0.35	3
Bacillariophyceae	*Thalassiosira weissflogii*	4.23	3
	Chaetoceros sp.	0.96	3
Cryptophyceae	*Cryptomonas maculata*	6.45	3
Haptophyceae	*Phaeocystis pouchetii*	3.05×10^{-2}	3

and variation of vitamin C in other vertebrate and invertebrate species are reviewed in Table 5 to compare with results in Table 1. Although methods used by most authors were different, at this stage of generalization it appears that vitamin C is highly variable. Values reported for non-planktonic species and for zooplankton fall generally into the same range. Contents in some plankton species (e.g. omnivorous-herbivorous copepods; fish larvae) are higher than whole body, or as high as in specific tissue, of vertebrates known for their ability to store or synthesize vitamin C (e.g. liver, kidney). In humans, the average total vitamin C content is 20 to 50 $\mu g\ g^{-1}$, but it can be as high as 500 $\mu g\ g^{-1}$ in specific tissues (Courtand & Sagaut 1987). These data confirm the universal presence of ascorbic acid in eukaryotes.

One may wonder about the relationship between vitamin C and size of zooplankton. This question was addressed recently for copepods by Poulet et al. (1989). Intraspecific variations reported for this group were of the same order of magnitude as the interspecific variations detected among taxa (Table 1). These results underline some interaction between vitamin C and physiological conditions, plankton life, growth rate, stage of growth and mode of feeding, rather than species, as shown in Table 1, when comparing meroplankton against holoplankton, fish larvae against eggs and carnivorous against omnivorous species.

The pool of vitamin C in the body of marine carnivorous species seems to be 10 times less than in omnivorous-herbivorous species (Table 1). An order of magnitude difference between these 2 groups probably reflects some relationship between their trophic position in the marine food chain and their specific capability to incorporate vitamin C. Comparison of the seasonal variations between free-swimming zooplankters (e.g. copepods: Table 2; Figs. 2 and 3) and benthic organisms (e.g. *Balanus balanoides, Chthamalus* spp.: Barnes & Barnes 1982) show that they differ by a factor of 10, despite both species being crustaceans and herbivorous. The lack of information on the biochemistry of vitamin C in most invertebrates is still a major problem in interpreting such biochemical differences.

The true relationship between vitamin C and food could not be investigated in nature, due to various interfering and uncontrolled factors, such as the adequacy and concentration of food, the hunger and selectivity of copepods, and match-mismatch between prey and predator in time and space. Nevertheless, contradicting results in Fig. 2 did not rule out the relationship found with *Calanus helgolandicus* between food and vitamin C. The response of *Acartia clausi* to the ingestion of food was illustrated by a slow incorporation of proteins (Fig. 3D), reflecting hte transformation of amino acids during proteogenesis. Time lag between ingestion of food and the biochemical response of these copepods was 40 to 50 h for proteins and <10 h for vitamin C or gut fullness. Considering that copepods do not synthesize chlorophyll *a* and phaeophytin and knowing that both vitamin C and gut pigments varied quickly following food uptake, results in Fig. 3 thus suggest that biosynthesis of vitamin C does not occur in copepods. This hypothesis is confirmed when chlorophyll *a* (0.75 to 1.5 $\mu g\ l^{-1}$) or number of cells (between 50×10^3 and 10^5 cells l^{-1}) corresponding to the addition of phytoplankton into the incubators is converted into vitamin C. Knowing that *Dunalliela primolecta* has a chlorophyll *a* concentration per cell similar to *Thalassiosira weissflogii* or *Cryptomonas maculata* (e.g. Moal et al. 1987), conversion (pg vit. C

Table 5. Summary of the values and ranges of vitamin C measured in non-planktonic organisms

Organism	Range of vit. C ($\mu g\ g^{-1}$)	Location	Species	Source
Vertebrate				
Fish	1.5–450	Liver, kidney, gill, brain, muscle, ovaries, egg	10 species: including *Mugil cephalus, Gadus morrhua*	Giroud & Rakoto-Ratsimamanga (1936), Gordon & Martin (1980), Sandnes & Braekkan (1981), Carr et al. (1983), Thomas et al. (1985)
Rat	12–151	Liver, kidney, lung, testis, brain, muscle		Carr et al. (1983)
Primate	0.7–500	Suprarenal, hypophysis, liver, lung, kidney, testicle, thyroid, heart, muscle, brain, pancreas, eye, plasma, saliva	*Homo sapiens*	Courtand & Sagaut (1987)
Invertebrate				
Insect	60–470	Gonad, gut, muscle, egg	*Dytissus marginalis Anthomomus grandis*	Giroud & Rakoto-Ratsimamanga (1936), Vanderzant et al. (1962)
Crustacean	10–320	Hepatopancreas, gonads, gill, muscle, egg, cephalotorax, whole body	9 species including: *Cragon vulgaris, Palaemonetes pugio, Balanus balanoides*	In Fisher (1960): reported by Nespor & Wenig (1939), Giroud & Rakoto-Ratsimamanga (1936), von Euler & von Euler (1933), Barnes & Barnes (1982), Carr et al. (1983)
Mollusc	20–1300	Hepatopancreas, gut, gill, gonads, muscle	*Octopus vulgaris, Mytilus galloprovincialis*	Giroud & Rakoto-Ratsimamanga (1936)
Annelid	4–25	Gut, muscle, posterior segments	*Neanthes virens*	Carr & Neff (1980)
Echinoderm	30–400	Hepatopancreas, gonads, gut	6 species	Giroud & Rakoto-Ratsimamanga (1936)
Coelenterae	50–180	Whole body	*Anemonia sulcata*, Jellyfish	Giroud & Rakoto-Ratsimamanga (1936)

per cell × number of cells added in 200 l, taking 5 pg $cell^{-1}$ as the average vitamin C $cell^{-1}$; Table 4) gives a maximum yield of vitamin C of the order of 50 to 100 µg 200 l^{-1}, a value of the same order of magnitude as the quantity incorporated by copepods (i.e. 37.8 to 79.8 µg vit. C for the entire population of copepods in the incubators, knowing that the range of copepod wet weight was 1260 to 2660 µg in 200 l) 10 h after they had started to feed (Fig. 3A). The ratio between gut pigments and vitamin C content was close to 1 during this period (Fig. 3A, C:t = 100 to 110 h), demonstrating further a direct link between food uptake and incorporation of vitamin C by copepods. These conclusions, based on only a few observations, should be ultimately tested through enzymatic assays, similar to those performed on rat and fish (Loewus et al. 1960, Chatterjee 1973, Thomas et al. 1985, Koshizaka et al. 1988).

Minimum concentrations of vitamin C in both *Calanus helgolandicus* and *Acartia clausi* starved in the laboratory were close to values recorded in naturally occurring copepods during winter, or on stations where the concentration of chlorophyll *a* in seawater was <0.5 µg l^{-1}. The effects of food concentration upon feeding (e.g. Paffenhöfer 1971), growth (e.g. Huntley et al. 1987) and production (e.g. Stearns et al. 1989) rates are well documented for copepods. However the importance of food quantity against food quality is still controversial (e.g. Kiørboe 1989). The concept of food quality, based on the induction of some specific biological function, could be defined better by vitamin C. For example, one known function of vitamin C among crustaceans is to stimulate reproduction. Collier et al. (1956) reported that *Balanus* sp. exposed to 14 µg l^{-1} vitamin C immediately started reproducing. In nature, input of dissolved vitamin C in relation to extracellular excretion by phytoplankton, which could be as high as 70 % of the quantity synthesized in some alga (Helsper et al. 1982), might induce such a response. In the decapod *Palaemon serratus*, Guary & Guary (1975), and Guary et al. (1975) have shown that vitamin C

increases both in the female ovaries before hatching and in eggs during embryogenesis. These authors suggested that vitamin C is required during egg division and should favour survival of larvae. Furthermore, a relationship between vitamin C and molting has been reported in *Palaemon serratus* (Guary et al. 1975) and in *Homarus americanus* (Desjardins et al. 1985). The dependence of reproduction and growth upon vitamin C or its precursor might have also great ecological importance in zooplanktonic crustaceans. Earlier studies on population dynamics, reported for *Acartia clausi* in the coastal waters off Roscoff (Le Fèvre-Lehoërff 1972), showed some coincidence in time (e.g. spring-summer) between maximum values of vitamin C and population density. Considering these results with those in Fig. 2, it would be speculated that vitamin C might also induce reproduction in copepods.

Three other functions might be relevant to plankton. Vitamin C is a factor in detoxification mechanisms against heavy metals (Kanner et al. 1977, Thomas et al. 1982) and is an essential micronutrient for fish (e.g. growth and reproduction: Millikin 1982). These functions would be worth exploring in zooplankton. Furthermore, vitamin C could be used as a new biochemical marker, characterizing phytoplankton-zooplankton interactions as shown in Figs. 1 to 3. The gut pigment/vitamin C ratio varied from 1 to 3 among fed copepods, while it was <1 during starvation (Fig. 3). Its nutritional and physiological function could offer this marker a subsidiary advantage over the classical index of zooplankton feeding using measurements of gut pigments (Mackas & Bohrer 1976, Dagg 1983, Conover et al. 1986), which have no such function.

Transfer of vitamin C between trophic levels relating phytoplankton, zooplankton and fish has some importance, mainly for species unable to synthesize this vitamin. Droop & Scott (1978) observed that as much as 60% of labeled vitamin B_{12} contained in the alga *Bachiomonas submarina* was incorporated into the rotifer *Brachionus plicatilis* through direct feeding. The role of copepods as a source of food for fish is well established (Turner 1984). As demonstrated for polyunsaturated fatty acids (Watanabe et al. 1983), well-fed copepods could be a source of vitamin C for fish. The importance of such a transfer would be quantified when requirements of planktivorous fish are known. This information is scant for most fish. Turnover times for ascorbic acid in primates are 20 d, and 2 to 3 d in guinea-pigs and rats (Tolbert 1979). If vitamin C and size of organism are related, then turnover in fish might be on the order of days to hours, depending on the stage of growth. According to Millikin (1982), Dabrowski (1986) and Soliman et al. (1986), the daily requirement of fish (juvenile to adult) is of the order of 1.25 $\mu g\ g^{-1}$ of dry diet. Such a quantity could be supplied by 10 to 50 copepods of the size of *Calanus helgolandicus*. Copepod fecal pellets could transfer low amounts of vitamin C to the next trophic level, except under oligotrophic conditions (e.g. Mediterranean Sea: Table 3). Copepods exposed to a high quantity of food would produced pellets enriched with vitamin C, as a result of unabsorbed organic matter (e.g. Clarke et al. 1988). Vitamin C found in fecal pellets (Table 3) is more likely an excretory pathway when the amount supplied by food exceeds copepod needs, as mentioned by Tolbert (1979) for urine of rats or primates.

A survey of the vitamin C literature (e.g. Cowey & Sargent 1979, Englard & Seifter 1986, Loewus & Loewus 1987) reveals little information regarding marine plankton compared with other plants and vertebrates; the existing plankton information is generally dated (e.g. Fisher 1960, Swift 1980, Barnes & Barnes 1982, Poulet et al. 1989, and results therein). Biochemical aspects, such as biosynthesis ability, metabolism pathways and requirements have been investigated in the past for non-planktonic organisms (e.g. Guary & Guary 1975, Tolbert 1979, Soliman et al. 1986, Koshizaka et al. 1988). Hopefully, this information should make the study of vitamin C more popular among marine plankton biologists and will encourage new research regarding vitamin C in marine plankton.

Acknowledgements. We thank the officers and crews of NO 'Thalassa', 'RRV' Challenger and NO 'Georges Petit'; IFREMER, CNRS and NERC for ship time; J. L. Birrien for the pigment data, A. Kueff for the analyses of proteins and V. Martin-Jézéquel for providing the phytoplankton cultures. We are grateful to Dr R. J. Conover for his comments during the preparation of the manuscript. Anonymous referees are greatly acknowledged for their comments and suggestions. This research was supported by CNRS (ROSIMER and TOMOFRONT-89 b cruises), TAAF (PEGASE programme) and EEC (ROSIMER programme: Action de Stimulation, contract no. ST2J-0369). We gratefully acknowledge the use of facilities at the Plymouth Marine Laboratory by one of us (A.M.H.) during this work.

LITERATURE CITED

Barnes, M., Barnes, H. (1982). The total ascorbic acid content and its variation with seasonal and nutritional factors in some common cirripedes. J. exp. mar. Biol. Ecol. 60: 261–273

Bradford, M. M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analyt. Biochem. 72: 248–254

Carr, R. S., Bally, M. B., Thomas, P., Neff, J. M. (1983). Comparison of methods for determination of ascorbic acid in animal tissues. Analyt. Chem. 55: 1229–1232

Carr, R. S., Neff, J. M. (1980). Determination of ascorbic acid in tissues of animals by liquid chromatography with electrochemical detection. Analyt. Chem. 52: 2428–2430

Chatterjee, I. B. (1973). Evolution and biosynthesis of ascorbic acid. Science 182: 1271–1272

Chatterjee, I. B., Majumber, A. K., Nandi, B. K., Subramanian, N. (1975). Synthesis and some major functions of vitamin C in animals. Ann. N.Y. Acad. Sci. 258: 24–47

Clarke, A., Quetin, L. B., Ross, R. M. (1988). Laboratory and field estimates of the rate of fecal pellet production by Antarctic krill, *Euphausia superba*. Mar. Biol. 98: 557–563

Collier, A., Ray, S., Wilson, W. B. (1956). Some effects of specific organic compounds on marine organisms. Science 124: 220

Conover, R. J., Durvasula, R., Roy, S., Wang, R. (1986). Probable loss of chlorophyll-derived pigments during passage through the gut of zooplankton, and some of the consequences. Limnol. Oceanogr. 31: 878–887

Courtand, M., Sagaut, P. (1987). Acide ascorbic (Vitamine C). In: Munnich, A., Ogier, H., Saudubray, J.-M. (eds). Les vitamines, aspects métaboliques, génétiques, nutritionnels et thérapeutiques. Masson, Paris, p. 326–346

Cowey, C. B., Sargent, J. R. (1979). Nutrition: In: Hoar, W. S., Randell, D. Y., Brett, J. (eds) Fish physiology, Vol. VIII, Bioenergetics and growth. Academic Press, New York, p. 1–69

Dabrowski, K. R. (1986). Ontogenetical aspects of nutritional requirements in fish. Comp. Biochem. Physiol. 85A, (4): 639–655

Dabrowski, K. R., Hinterleitner, S., Sturmbauer, C., El-Fiky, N., Wieser, W (1988). Do carp larvae require vitamin C? Aquaculture 72: 295–306

Dagg, M. J. (1983). A method for the determination of copepod feeding rates during short intervals. Mar. Biol. 75: 63–67

Desjardins, L. M., Castell, J. D., Kean, J. C. (1985). Synthesis of dehydroascorbic acid by subadult lobsters (*Homarus americanus*). Can. J. Fish. Aquat. Sci. 42: 370–373

Droop, M. R., Scott, J. M. (1978). Steady state energetics of a planktonic herbivore. J. mar biol. Ass. U. K. 58: 749–772

Englard, S., Seifter, S. (1986). The biochemical functions of ascorbic acid. Ann. Rev. Nutr. 6: 365–406

Eppley, R. W (1977). The growth and culture of diatoms. The biology of diatoms. Bot. Monogr. 13, 24–64

Fisher, L. R. (1960). Vitamins. In: Waterman, T. H. (ed.) The physiology of Crustacea. Vol. 1. Metabolism and growth. Academic Press, New York, p. 259–289

Giroud, M. M. A., Rakoto-Ratsimamanga, A. (1936). Distribution de la vitamine C chez les invertébrés. Bull. Soc. Chim. biol. 18: 375–383

Gordon, D. T., Martin, R. E. (1980). Vitamins and minerals in sea foods of the Pacific Northwest. In: Martin, R. E., Flick, G. J., Hebard, C. E., Ward, D. R. (eds.). Chemistry and biochemistry of marine food products. Avi Publishing Company, Westport, p. 429–445

Grün, M. Loewus, F. A. (1984). L-Ascorbic acid biosynthesis in the euryhaline diatom *Cyclotella cryptica*. Planta 160: 6–11

Guary, M. M., Ceccaldi, H. J. A., Kanazawa, A. (1975). Variations du taux d'acide ascorbique au cours de développement de l'ovaire et du cycle d'intermue chez *Palaemon serratus* (Crustacea: Decapoda). Mar. Biol. 32: 349–355

Guary, M. M., Guary, J.-C. (1975). Variations de la teneur en acide ascorbique dans les oeufs de *Cancer pagurus* et *Palaemon serratus* (Crustacea: Decapoda) au cours de l'embryongenèse. Mar Biol. 32: 357–363

Guillard, R. R. L., Ryther, J. H. (1962). Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. Can. J. Microbiol. 8: 229–239

Hapette, A. M., Poulet, S. A. (1990). Application of high-performance liquid chromatography to the determination of ascorbic acid in marine plankton. J. Liquid Chromatogr. 13: in press

Halver, J. E., Smith, R. R., Tolbert, B. M., Baker, E. M. (1975). Utilization of ascorbic acid in fish. Ann. N. Y. Acad. Sci. 258: 81–102

Helsper, J. P., Kagan, L., Hilby, C. L., Maynard, T M., Loewus, F. A. (1982). L-ascorbic acid biosynthesis in *Ochromonas danica*. Plant Physiol. 69: 465–468

Huntley, M. E., Ciminiello, P., Lopez, M. D. G. (1987). Importance of food quality in determining development and survival of *Calanus pacificus* (Copepoda: Calanoida). Mar. Biol. 95: 103–113

Kanner, J., Mendel, H., Budowski, P. (1977). Prooxidation and antioxidant effects of ascorbic acid and metal salts in a β-carotene-linoleate model system. J. Food Sci. 42: 60–64

Kean, J. C., Castell, J. D., Trider, D. J. (1985). Juvenile lobster (*Homarus americanus*) do not require dietary ascorbic acid. Can. J. Fish. Aquat. Sci. 42: 368–370

Kiørboe, T. (1989). Phytoplankton growth rate and nitrogen content: implication for feeding and fecundity in a herbivorous copepod. Mar. Ecol. Prog. Ser. 55: 229–234

Koshizaka, T., Nishikimi, M., Ozawa, T., Yagi, K. (1988). Isolation and sequence analysis of a complementary DNA encoding rat liver L-guluno-γ-lactone oxidase, a key enzyme for L-ascorbic acid biosynthesis. J. biol. Chem. 263: 1619–1621

Le Fèvre-Lehoërff, G. (1972). Populations planctoniques d'un estuaire à marée: la rivière de Morlaix. Leurs relations avec les conditions hydrologiques. Thèse 3ème cycle. Université Paris VI

Loewus, F. A. (1971). Carbohydrate interconversions. Ann. Rev. Plant Physiol. 22: 337–364

Loewus, F. A., Helsper, J. P. F. G. (1982). Metabolism of L-ascorbic acid in plants. Adv. Chem. Ser 200: 249–262

Loewus, F. A., Kelly, S., Hiatt, H. H. (1960). Ascorbic acid synthesis from D-glucose-2-^{14}C in the liver of the intact rat. J. biol. Chem. 235: 937–939

Loewus, F. A., Loewus, M. A. (1987). Biosynthesis and metabolism of ascorbic acid in plants. CRC Crit. Rev. Plant Sci. 5: 101–119

Lorenzen, C. F. (1967). Determination of chlorophyll and pheo-pigments: spectrophotometric equations. Limnol. Oceanogr 12: 343–346

Mackas, D., Bohrer, R. (1976). Fluorescence analysis of zooplankton gut contents and an investigation of diel feeding patterns. J. exp. mar. Biol. Ecol. 25: 77–85

Millikin, M. R. (1982). Qualitative and quantitative nutrient requirements of fishes: a review. Fish. Bull. U.S. 80: 655–686

Moal, J., Martin-Jézéquel, V., Harris, R. P., Samain, J.-F., Poulet, S. A. (1987). Interspecific and intraspecific variability of the chemical composition of marine phytoplankton. Oceanologica Acta 10: 339–346

Paffenhöffer, G. A. (1971). Grazing and ingestion rates of nauplii, copepodids and adults of the marine planktonic copepod *Calanus helgolandicus*. Mar. Biol. 11: 286–298

Poulet, S. A., Hapette, A. M., Cole, R. B., Tabet, J. C. (1989). Vitamin C in marine copepods. Limnol. Oceanogr 34: 1325–1331

Poulet, S. A., Harris. R. P., Martin-Jézéquel, V., Moal, J., Samain, J.-F. (1986). Free amino acids in copepod faecal pellets. Oceanologica Acta. 9: 191–197

Renstrøm, B., Grün, M., Loewus, F. A. (1982/83). Biosynthesis of L-ascorbic acid in *Chlorella pyrenoidosa*. Plant Sci. Lett. 28: 299–305

Robins, D. B., Bellan, I. E. (1986). A controlled-temperature plankton wheel. Mar. Biol. 92: 587–593

Sandnes, K., Braekman, O. R. (1981). Ascorbic acid and the reproductive cycle of ovaries in cod (*Gadus morrhua*). Comp. Biochem. Physiol. 70A: 545–546

Shigeoka, S., Nakano, Y., Kitaoka, S. (1979). The biosynthetic pathway of L-ascorbic acid in *Euglena gracilis*. J. Nutr. Sci. Vitaminol. 25: 299–307

Soliman, A. K., Jauncey, K., Roberts, R. J. (1986). The effect of varying forms of dietary ascorbic acid on the nutrition of juvenile tilapias (*Oreochromis niloticus*). Aquaculture 52: 1–10

Stearns, D. A., Tester, P. A., Walker, R. L. (1989). Diel changes in the egg production rate of *Acartia tonsa* (Copepoda, Calanoida) and related environmental factors in two estuaries. Mar. Ecol. Prog. Ser. 52: 7–16

Swift, D. G. (1980). Vitamins and phytoplankton growth. In: Morris, I. (ed.), The physiological ecology of phytoplankton. Stud. Ecol. 7: 329–368

Thomas, P., Bally, M., Neff, J. M. (1982). Ascorbic acid status of mullet, *Mugil cephalus* Linn., exposed to cadmium. J. Fish. Biol. 20: 183–196

Thomas, P., Bally, M., Neff, J. M. (1985). Influence of some environmental variables on the ascorbic acid status of mullet, *Mugil cephalus* L., tissues. II. Seasonal fluctuations and biosynthetic ability. J. Fish. Biol. 27: 47–57

Tolbert, B. M. (1979). Ascorbic acid metabolism and physiological function. Int. J. Vitam. Nutr Res. 19: 127–142

Turner, J. T. (1984). The feeding ecology of some zooplankters that are important prey items of larval fish. NOAA Tech. Rep. NMFS 7: 1–28

Vanderzant, E. S., Pool, M. C., Richardson, C. D. (1962). The role of ascorbic acid in the nutrition of three cotton insects. J. Insect Physiol. 8: 287–297

Watanabe, T., Tomiya, T., Oka, A., Hirata, M., Kitajima, C., Fujita, S. (1983). Improvement of dietary value of live foods for fish larvae by feeding them on ω_3 highly unsaturated fatty acids and fat-soluble vitamins. Bull. Jap. Soc. Scient. Fish. 49: 471–479

Wilson, R. P. (1973). Absence of ascorbic acid synthesis in channel catfish, *Ictalurus punctatus* and blue catfish, *Ictalurus frucatus*. Comp. Biochem. Physiol. 46 B: 635–638

Yamamoto, Y., Sato, M., Ikeda, S. (1978). Existence of L-gulanolactone oxidase in some teleosts. Bull. Jap. Soc. Scient. Fish 44: 775–779

Yentsch, C. S., Menzel, D. W. (1963). A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. Deep Sea Res. 10: 221–231

This article was submitted to the editor

Manuscript first received: October 19, 1989
Revised version accepted: March 29, 1990