CHANGES IN RESPIRATION RATE AND IN COMPOSITION OF ORGANIC MATTER IN *CALANUS CRISTATUS* (CRUSTACEA COPEPODA) UNDER STARVATION

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Introduction

Planktonic herbivorous copepods, which are widely distributed in the seas at high latitudes, largely consume phytoplankton when it is abundant in those seas during certain seasons. However, they survive when phytoplankton is scarce, probably depending on energy sources other than phytoplankton.

It has been suggested that the animals survive during unfavourable periods by using fat reserves in their bodies. In general, animals inhabiting high latitudes contain a large amount of body fat (cf. Wimpenny, 1941; Sheard, 1953; Littlepage, 1964). Conover (1962, 1964) showed that *Calanus hyperboreus* could survive for several months without food by using their stored fat. In the experiments of Cowey & Corner (1963) and Linford (1965), *Calanus helgolandicus* lost mainly protein during starvation. The loss of body protein is too great for *C. helgolandicus* to survive for very long; hence, it would have to feed on whatever is available (Cowey & Corner, 1963).

The present experiments were carried out on board the "Oshoro Maru" during Cruise 28 to the North Pacific and the Bering Sea in June through August 1968. The experiments are concerned with the changes in respiration rate and in the amount of protein, chitin, lipid and carbohydrate of *C. cristatus* stage V during the process of starvation.

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Methods and material

Animals: Samples were taken by towing a specially designed tow net (0.35× 0.35 mm mesh size, 56 cm in dia., 100 cm in length, with a large polyethylene tail

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bucket, 15 cm in dia., 25 cm in length) through the surface water (0–10 m). Samples were obtained in the area from 49° to 60°N on 179°W (June 10–16). *C. cristatus* (stage V) were separated from the samples with a large bore pipette (0.8 cm in dia.) and transferred to glass bottles, 1 l in capacity, filled with non-filtered surface sea water. The bottles were placed in a light-tight wooden tank in which surface sea water was circulating continually. After 24 hours *C. cristatus* were placed in other bottles (1 l in capacity) filled with HA millipore (0.45 μ) filtered, aerated sea water, and kept without food. Fifteen bottles each containing 50 *C. cristatus* were prepared. During the starvation period, the filtered sea water in the bottles was renewed evecy 2 days. At the same time, dead *C. cristatus*, if present, were removed. The faecal pellets produced by the animals which sank to the bottom were collected in the same way and their size and content were examined under the microscope. The number of dead specimens was recorded on the two bottles over the period of starvation for estimating the survival rate. As a control, another bottle filled with natural sea water (without filtration) containing 50 *C. cristatus* was prepared, and the survival rate of these animals was recorded. At a certain intervals, several animals were picked up for measurement of respiration rate and for determination of chemical constituents. The temperature in the tank containing the experimental bottles ranged from 6.9° to 9.4°C over the first 6 days, but increased from 9.9° to 11.9°C between the 15th and 36th days.

Respiration rate: The water bottle method described by Marshall *et al.* (1935) and Conover (1956) was adopted to measure respiration. In a series of experiments, three oxygen bottles, 250 ml in capacity, were prepared. Five *C. cristatus* were transferred from the rearing bottles into each two experimental oxygen bottles filled with filtered sea water, and a remaining oxygen bottle with similar water, but without *C. cristatus*, were used as a control. Then, the water in the bottle was again renewed by siphoning HA millipore filtered and re-aerated water, repeatedly seven to eight times. The bottles were immersed in a light-tight wooden tank for about 24 hours through which surface sea water was circulating. At the end of the experiments, the water was withdrawn with a siphon from the experimental and control bottles, transferring each into two small oxygen bottles, 100ml in capacity. Dissolved oxygen was determined by the Winkler method (Strickland & Parsons, 1965). In every case thiosulfate factor was standardized at the beginning and at the end of one series of experiments. The respiration rate was calculated from the difference in dissolved oxygen between experimental and control bottles at the end of the experiments. After finishing the experiments on respiration, the animals were rinsed quickly with distilled water and stored in a freezer at -20°C. Later the dry weight of the animals was determined in the land laboratory.

Biochemical constituents in the body: *C. cristatus* were sacrificed from the

rearing bottles at appropriate intervals (0, 2, 6, 15, 24 and 36 days) during starvation. They were rinsed quickly with distilled water and the excess of water was removed by rolling the animals on the blotting paper. Then, *C. cristatus* were placed on a piece of aluminium foil and stored in a air-tight plastic ointment pot (2.5 cm in dia.) in the dark at -20°C. Twenty *C. cristatus* were used for one determination of dry weight and ash weight, and a single *C. cristatus* (3.5–10.6 mg wet weight) was used for one determination of each organic constituent. Protein and chitin were measured following the method of Strickland & Parsons (1965), and carbohydrate following the method of Raymont *et al.* (1964). Lipid was not analyzed directly, but was calculated from the difference between dry weight and the total weight of protein, chitin, carbohydrate and ash. Carbohydrate was analyzed immediately on board the ship but the other components were analyzed in the laboratory after the completion of the cruise. Dry weight was determined by drying *C. cristatus* in a reduced pressure dessicator until constant weight was obtained at room temperature; ash weight, by burning *C. cristatus* on a glass fiber filter in an electric muffle furnace at 450°C until constant weight was obtained. An additional analysis of crude fiber on the freshly caught *C. cristatus* was made by Strickland & Parsons (1965) method. The Mettler balance was used for weighing and the Hitachi 139 Spectrophotometer was used for colorimetric analyses.

Results

Through the whole starvation period, *C. cristatus* were quite motionless, floating near the surface, sometimes suspended in the middle layer or resting on the bottom. A hopping movement was observed everytime when the rearing bottle was taken out from the dark tank and exposed to room light. This movement in response to light was not reduced during the whole starvation period. As progress of starvation, red body color of *C. cristatus* faded.

Survival rates of starved and fed animals: Survival rates in starved and fed *C. cristatus* are shown in Figure 1. Mortality per day was 1.69% for starved *C. cristatus* and 0.87% for fed ones. In a similar starvation experiment by Cowey & Corner (1963), the mortality rate of *C. helgolandicus* was 5.0–6.5% per day for starved animals and below 1% for fed ones (calculated from Cowey & Corner, 1963). Hence, *C. cristatus* seems to be more tolerant of fasting than *C. helgolandicus*.

Ivlev (1955) obtained a characteristic survival curve for the fish during starvation. The mortality of fish was high at the beginning, followed by a moderate rate, and then became high again. In the present experiment on *C. cristatus*, however, the mortality was rather constant over the period of starvation.

Faecal pellets: Just after the starvation had begun *C. cristatus* fed on their own feces. Conover (1966) observed that *C. hyperboreus* rejected their feces

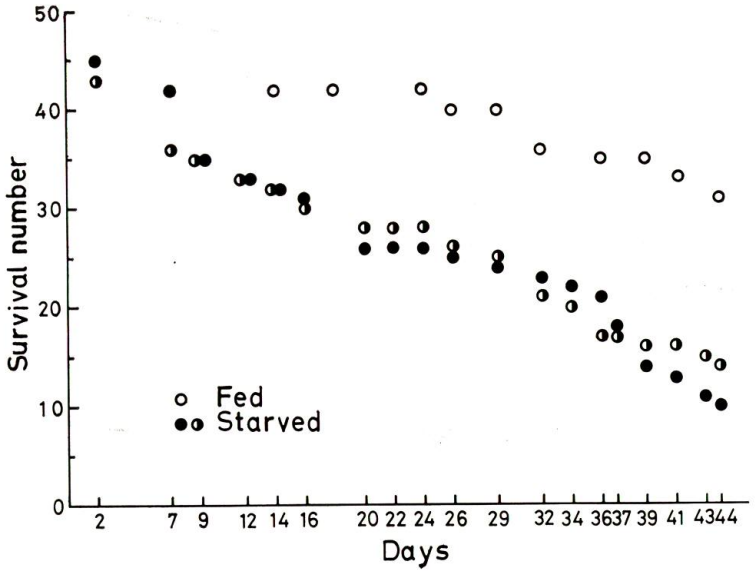

Fig. 1. Survival rate of *Calanus cristatus* under fed and starved conditions

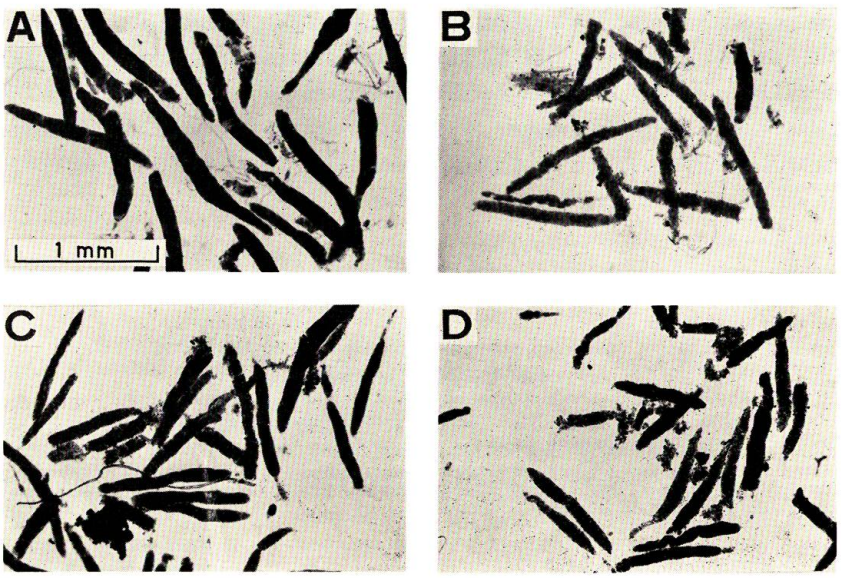

Fig. 2. Faecal pellets of *Calanus cristatus* produced after 1(A), 15(B), 25(C) and 34(D) days of starvation

selectively from other suitable food. Such selective feeding might occur only when plenty of normal food is present.

In the present experiments, the pellets produced on the 15th day of starvation were more transparent than those seen on the lst day (Fig. 2). These pellets resembled somewhat the "ghost pellet" described by Marshall & Orr (1955). However, the pellets became slightly more dense on the 25th and 34th days than those on the 15th day. With progressive starvation the size of pellets became gradually smaller.

During the first half of the starvation period, the mouth of some dead specimens was choked with faecal pellets. On the 15th day or thereabouts, dismembered corpses and carcasses with the dorsal body wall torn open appeared in some rearing bottles and afterwards this became common in all rearing bottles. This period coincided with the time when the faecal pellets became slightly more dense again. Whereas the mortality was almost constant through the whole starvation period, living *C. cristatus* began feeding on dead specimens only in the later period. The term necrophagy is better suited than cannibalism to describe this phenomenon. Feeding on dead specimens was not observed when the bottles were taken from the dark tank into the lighted room for 12 hours. The experiments suggest that if this sort of feeding occurs in nature, it would probably occur only in deep water in continuous darkness.

Respiration rate: Beklemishev (1954) referring to Vinogradov (1952) reported that the respiration rate of *C. cristatus* was 0.84 μl O_2/*Calanus*/hour at 6.8°C. In an earlier experiment (unpublished), the author obtained a value of 1.73 at 7.0°–7.5°C for this species collected off Kushiro, Hokkaido, on the "Tansei Maru" Cruise 68–9 (May, 1968). In the present experiments on the "Oshoro Maru" Cruise 28, the rate of respiration at the start (0 day) was 1.24 at 7.4°–8.2°C (Table 1). Up to the end of the first 6 day's starvation the respiration rate tended to increase becoming 1.37 at 6.9°–9.4°C.

Temperature changes might cause the increasing rate ot respiration. However,

Table 1. Change in respiration rate in *Calanus cristatus* under starved condition

Days	Temperature (°C)	Respiration rate	
		μl O_2/*Calanus*/hour	μl O_2/mg dry weight of *Calanus*/hour
0	7.4- 8.2	1.24	0.69
2	7.1- 8.8	1.22	0.82
6	6.9- 9.4	1.37	0.92
15	10.3-11.4	0.76	0.55
24	9.9-11.6	0.91	0.51
36	10.4-11.9	0.86	0.51

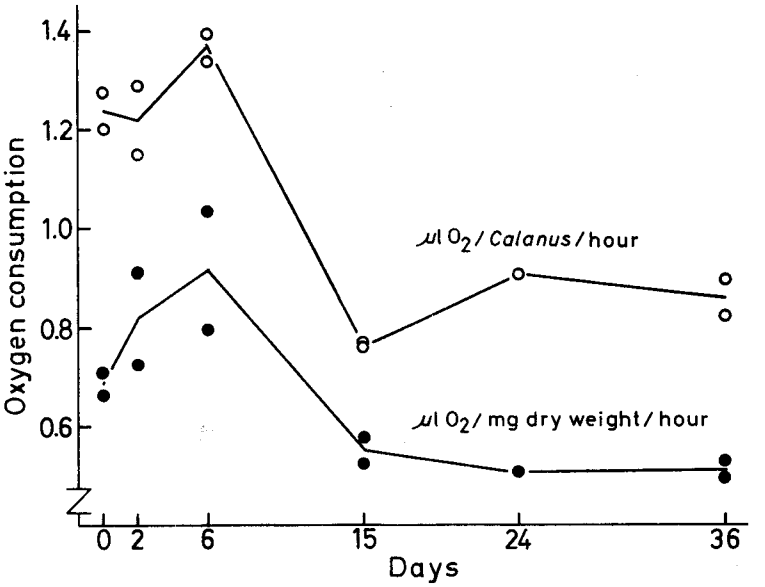

Fig. 3. Change in respiration rate of *Calanus cristatus* under starved condition

Conover (1962) also observed an increase in respiration rate in *C. hyperboreus* after some time in laboratory culture. A sudden decrease in the rate occurred on the 15th day after which the rate remained at constant level. The pattern of variation of the rate when expressed by μl O_2 per *Calanus*-hour was almost the same as expressed by μl O_2 per mg dry weight-hour (Fig. 3), so that the variation of the rate was nearly independent of changes in body weight.

It is known that animals reduce their metabolism in starvation. This was demonstrated in copepods by Conover (1956, 1962, 1964), Comita (1968) and Conover & Corner (1968). In the natural habitat a decrease in respiration rate

Table 2. Relative amount of biochemical fractions in the body of *Calanus cristatus*

Fraction	Mean % wet weight	Mean % dry weight
Water	76	
Solid material	24	
*Protein		62.2
Lipid		20.6
**Chitin		5.2
***Carbohydrate		0.7
***Crude fiber		Trace
Ash		11.3

* Casein equivalent ** Glucosamine equivalent *** Glucose equivalent

Table 3. Organic contents of planktonic copepods (% of dry

Author	Species
Brandt, 1898	Copepods
Brandt & Raben, 1919–1922	Copepods
Klem, 1932	*Calanus finmarchicus*
Orr, 1934 a	*Euchaeta norvegica*
Orr, 1934 b	*Calanus finmarchicus*
Krey, 1950	Copepods
*Kizevetter, 1954	Copepods
Lafon, *et al.*, 1955	Copepods (mainly *Acartia* sp.)
Nakai, 1955	*Calanus cristatus*
	Calanus helgolandicus?
	Calanus plumchrus
	Paracalanus parvus
	Pseudocalanus elongatus
	Euchaeta japonica
	Acartia clausi
Saiki & Mori, 1956	*Calanus cristatus*
Raymont & Krishnaswamy, 1960	*Calanus finmarchicus*
Raymont & Conover, 1961	*Calanus finmarchicus*
	Calanus hyperboreus
Fisher, 1962	*Calanus finmarchicus*
	Pareuchaeta norvegica
Cowey & Corner, 1963	*Calanus helgolandicus*
**Littlepage, 1964	*Euchaeta antarctica*
Vinogradova, 1964	*Calanus helgolandicus*
	Limnocalanus grimaldii
Yamada, 1964	*Calanus plumchrus*
	Calanus cristatus
	Metridia longa
Linford, 1965	*Calanus helgolandicus*
Beers, 1966	Copepods
Conover & Corner, 1968	*Calanus hyperboreus*
	Calanus finmarchicus
	Metridia longa
	Calanus glacialis
	Pareuchaeta norvegica
	Centropages typicus
	Euchirella rostrata
	Pleuromamma robusta
	Rhincalanus nasutus

* Cited by Zenkevitch (1963)

** Cited by Raymont *et al.* (1969)

in copepods takes place during the food-scarced seasons (Pütter, 1924–1925; Marshall & Orr, 1958; Cowey & Corner, 1963; Marshall & Orr, 1966; Haq, 1967; Conover & Corner, 1968).

Biochemical constituents in fresh C. cristatus: Table 2 shows the results of analyses of major biochemical constituents of freshly caught *C. cristatus*. Data from previous workers on the biochemical composition of copepods are tabulated in Table 3. The analytical methods employed differ with workers and also the composition will change in a given species with seasons and geographical

weight) (Supplemental to Raymont *et al.*, 1964)

Carbo-hydrate	Lipid	Protein	Chitin	Ash
20	7	59	4.7	9.3
—	—	—	4.0	14.8
—	31.3-46.2	—	—	—
—	18-36	31-44	3.1-5.0	3.6-4.4
—	10.5-47.0	30-77	3.0	4.0
0-4.4	4.6-19.2	70.9-77.0	—	4.2-6.4
8.9-16.1	17.1-38.6	29.6-45.3	—	14.6-28.7
—	9.5	—	12.6	—
—	36.1	53.4	—	1.7
—	5.2-11.0	75.2	—	2.8
—	31.4-53.3	34.8-53.4	—	0.96-2.4
—	19.1	70.1	—	2.9
—	17.3	71.5	—	2.3
—	33.8	51.8	—	2.1
—	5.8	82.6	—	3.3
—	58.63	—	—	—
0.4	—	—	—	—
***0.5-2.0	—	—	—	—
***0.7-1.5	—	—	—	—
—	1.5-9.5	—	—	—
—	2.7-11.0	—	—	—
—	—	****45.7-50.3	—	—
—	?-46	—	—	—
9.1	46.2	42.7	—	2
12.9	31.9	51.5	—	3
—	***12.9-31.0	—	—	—
—	***23.1-33.6	—	—	—
—	***20.4	—	—	—
—	*** 7.1-23.1	—	—	—
0.30-0.67	—	51.0-69.8	—	—
—	21.8-53.7	31.1-47.8	—	—
—	20-47	40.6-56.3	—	—
—	12-31	50.0-68.8	—	—
—	33	36.3-50.6	—	—
—	25-50	41.3-51.9	—	—
—	14.0	82.4	—	—
—	21.5	53.5	—	—
—	12.1	56.2	—	—
—	44.5	32.5	—	—

*** Dry weight assumed to be one fifth the wet weight **** Total of amino acids

locations (e.g. Marshall *et al.*, 1934; Orr, 1934a, b; Cowey & Corner, 1962; Fisher, 1962; Conover & Corner, 1968).

The present data on lipid of *C. cristatus* is low compared with those of Nakai (1955), Saiki & Mori (1956) and Yamada (1964). One might suppose that this low lipid content is due to nutritionally unfavourable condition in the present case. However, the surface water (0–10 m in depth) from which *C. cristatus* was collected contained about 2 mg chlorophyll a/m^3 (Anonymous, 1969). This level

Table 4. Changes in dry and ash weight, total lipid, total protein, total chitin and total carbohydrate in *Calanus cristatus* during starvation (μg/*Calanus*)

Days	Dry weight	Ash weight	Lipid	*Protein	*Chitin	*Carbohydrate
0	1800	204	371	1119±58	92.8±6.4	12.6±0.7
2	1740	227	360	1059±69	82.1±8.0	11.7±0.8
6	1500	266	284	873±38	66.4±6.4	10.6±0.8
15	1380	326	150	831±60	59.2±7.1	14.1±1.0
24	1580	334	215	939±64	79.5±6.0	12.4±0.7
36	1520	308	154	940±40	103.5±8.5	13.6±0.7

* Mean of eight determinations±Probable error

of phytoplankton standing crops seem to be sufficient for the nutritional requirement of *C. cristatus*.

Biochemical constituents in starved C. cristatus (Table 4 and Figure 4)

Dry weight and ash weight: After starvation began, the dry weight of *C. cristatus* decreased until the 15th day (Fig. 4. A). The rate of decrease from the 6th to the 15th day was small. This period corresponded to the time of sudden decrease in respiration mentioned above. After the 15th day the dry weight increased. The time of recovery in the dry weight coincided with the apparent increase in content of the faecal pellets produced and the appearance of dismembered corpses in the experimental bottles. There seems to exist reciprocal relation of the changing pattern between dry weight and ash weight of *C. cristatus* (Fig. 4. A and B). The ash weight of starved *C. cristatus* increased to more than one and half times that of initial value for 15 days and after the 15th day it remained fairly constant level.

Lipid: The variation of lipid during the starvation process was parallel to that of dry weight (Fig. 4. C). It decreased until the 15th day, recovered slightly by necrophagy on the 24th day, and then, decreased on the 36th day. In the first 2 days, the rate of loss in lipid was slower than that from the 2nd to the 15th day.

Utilization of lipid by *C. hyperboreus* during starvation was reported by Conover (1962, 1964). On the other hand, in the Linford's experiment (1965) lipid levels in the experimental *C. helgolandicus* did not differ significantly from the control animals, leading to the conclusion that protein might be catabolized in *C. helgolandicus* during starvation.

In the starvation experiment with *C. cristatus* in this study, the decrease in lipid paralleled fairly well with the reduction in the size of oil-sac in the abdomen of *C. cristatus*. Petipa (1964) observed a diurnal rhythm of the consumption and accumulation of the fat droplets lying along the intestine of *C. helgolandicus* decreasing during the day, when the animals do not feed.

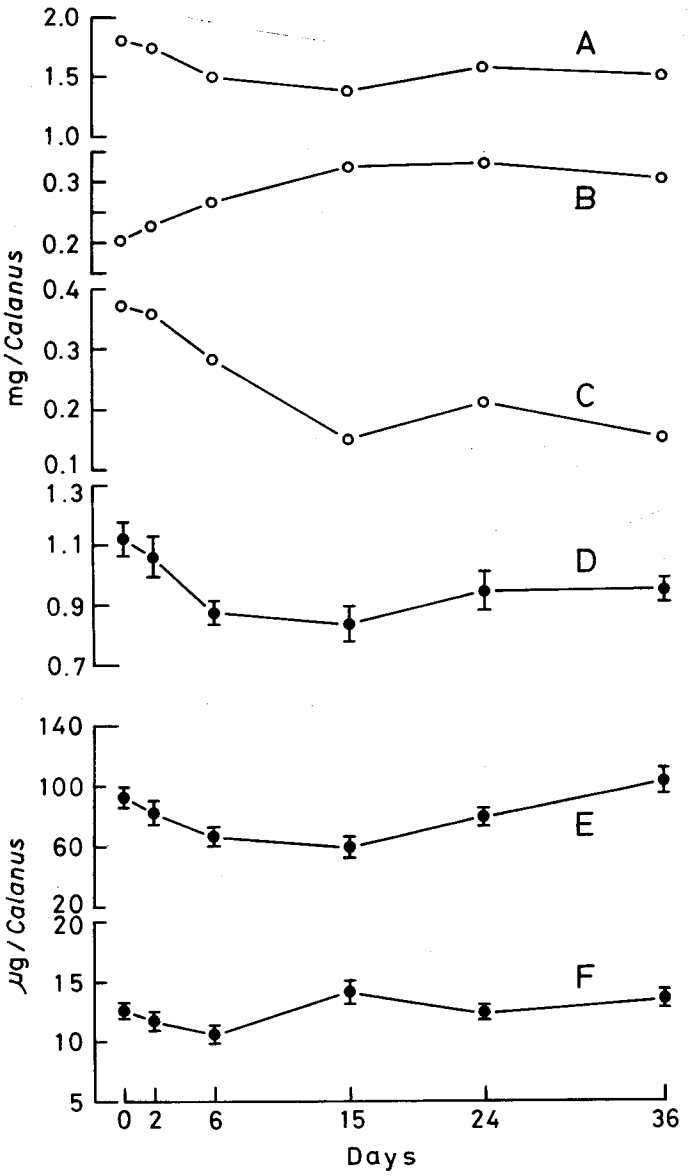

Fig. 4. Changes in dry weight (A), ash weight (B), total lipid (C), total protein (D), total chitin (E) and total carbohydrate (F) in *Calanus cristatus* during starvation. Vertical lines through points represent the range of a probable error of mean

Protein: Protein changed in the same manner as dry weight and lipid (Fig. 4. D). In the first 6 days during which necrophagy did not yet occur, *C. cristatus* lost 3.7% of total body protein per day. At the end of the first 15 days when necrophagy began, it was reduced to 1.7%, which agrees well with the results of Cowey & Corner (1963) who found that *C. helgolandicus* lost 1.8–2.1% of body protein per day during 10–14 day's starvation. The increase in dry weight in *C. cristatus* after the 15th day was mainly contributed by the increase in protein.

Chitin: Chitin varied in the same manner as dry weight, lipid and protein (Fig. 4. E). The variation in chitin observed in the experiments suggest that this substance was also used as an energy substrate during the starvation period.

In an experiment with barnacles, Barnes *et al.* (1963) observed the fall in the proportion of insoluble glycogen to the total glycogen in the body toward the end of the period of starvation, and suggested that glyco-protein would be broken down.

In the cuticles of decapods the amount of glucosamine (the principal ingredient of chitin) changes in relation to molting (refer to Nicol, 1960; Passano, 1960). However, in the present experiments, change in chitin was seen in *C. cristatus* which did not molt during the starvation period.

Carbohydrate: In the first 6 days the carbohydrate level dropped, but then recovered when necrophagy began on the 15th day. After the 15th day, carbohydrate decreased slightly and increased again (Fig. 4. F).

Even if the carbohydrate was metabolized completely, it could not meet more than a half-day metabolic requirement for *C. cristatus* as calculated from respiration rate. It will not be an important metabolite during starvation.

Raymont & Krisnaswamy (1960) observed that carbohydrate in *C. finmarchicus* decreased under artificial fasting. However, Raymont & Conover (1961) did not obtain similar results with other zooplankton crustaceans such as *C. hyperboreus*, *Neomysis americana*, *Meganyctiphanes norvegica* and *Thysanoessa* sp.

The maintenance of carbohydrate levels (largely as glucose in blood and glycogen in muscle and liver) during starvation is also generally seen in mammals (Suzuki & Nikuni, 1954). In this case carbohydrate is derived from the body protein and lipid. In crustaceans, however, the conversion of protein and lipid to carbohydrate has not been verified.

Discussion

Knowledge of life history of *C. cristatus* in the Bering Sea and North Pacific is increasing (Beklemishev, 1954; Heinrich, 1962). The reproduction of *C. cristatus* occurs in winter and there is one brood a year. The new brood reaches the I-II copepodite stages by the beginning of the period of phytoplankton

growth. These stages feed on phytoplankton and rapidly grow to stage V copepodites. They store large reserves of fat. Then, the stage V copepodites begin to sink to the deep layers in late summer. After they molt to adults, the masticatory edges of mandibles degenerate and they no longer take any food. They live at depths greater than 200 m and subsist on the fat stored in their bodies (Heinrich, 1962).

It is unknown how much loss in body weight can occur before the death of *C. cristatus*. Omori & Tanaka (1967) found thin and transparent specimens of *C. cristatus* in waters deeper than 400 m off east-central Honshu, Japan. The dry body weight of these specimens was about one fourth that of specimens collected in the North Pacific. They considered that these specimens of *C. cristatus* were transported with submerged Oyashio water and that the low body weight resulted from the lack of food (phytoplankton) during the long journey in the undercurrent.

Figure 5 shows the variation in relative amount of protein, lipid, chitin and carbohydrate to the total organic matter in starved *C. cristatus*. Apparent decrease in the relative proportion of lipid occurred after the 15th day of starvation. Due to this decrease in lipid, the relative amount of protein, chitin and carbohydrate increased gradually toward the end of starvation. It seemed that the relative amount of utilization of lipid as energy substrate for *C. cristatus* in food scarced condition is larger than the other three organic body constituents, and

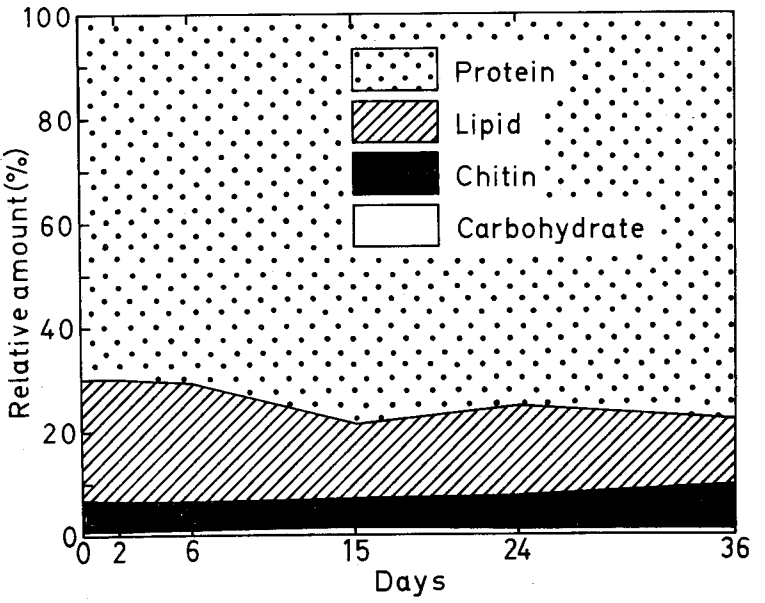

Fig. 5. Change in relative amount of each organic body component in *Calanus cristatus* during starvation

when some foods (dead individuals in this experiments) are gained during starvation its energy is used to repair the loss in carbohydrate, protein and chitin in preference to lipid.

Here, it must note that; relative loss in lipid is the largest among four organic constituents, however, absolute quantity of loss is the largest in protein over the first 6 days of starvation during which necrophagy does not yet occur (Table 4).

It is suggested from the results of the present experiments that for *C. cristatus* under starvation all organic constituents are utilized simultaneously. The relative amounts of chitin and carbohydrate in the total body constituents are small. Therefore, protein and lipid must be the most important metabolites quantitatively.

Protein or lipid as the most important energy sources in starvation is related to those original amount in the animal body; that is, if the lipid content is initially low, the major metabolite keeping the animals alive is apparently protein. On the other hand, if the lipid is originally abundant, the apparent major metabolite used during starvation is probably lipid. The heat production per unit weight of lipid in oxidation in the body is more than two times that of protein. Hence, very fat animals could tolerate a longer period of starvation than fat poor animals.

Conover & Corner (1968) studied the changes in respiration rate, nitrogen excretion (ninhydrin method) and chemical composition over a year on freshly caught copepods. In general their results agree with the above assumption. In the case of *C. hyperboreus*, which contain more than 50% fat per unit dry weight, not only dry weight and fat content but also total Kjeldahl nitrogen ($\times 6.25$= protein) are greatly reduced by the end of winter. During this period, the relative amount of fat per unit dry weight is reduced also, but a relative amount of total Kjeldahl nitrogen per unit dry weight remains remarkably constant indicating that relatively more fat is used than protein.

In their experiments, Conover & Corner (1968) found that the ratio of oxygen used for respiration to nitrogen excreted by copepods (O:N ratio, by atoms) is a good index of the major substrate which is oxidized in the animal's body. When fat and protein are oxidized in equivalent weight the O:N ratio would be about 24 (assuming that oxygen demand for complete combustion is 2.02 l/g for lipid, *1.04 l/g for protein, and protein is 16% nitrogen; from Suzuki & Nikuni, 1954). When food is scarce in nature, the O:N ratio of *C. hyperboreus* is usually higher than 24, indicating that the major metabolite is fat. In the cases of *C. finmarchicus* and *Metridia longa* the O:N ratio is usually lower than 24, especially in *M. longa* for which the lowest ratio is obtained among three species, indicating that the more important metabolite is protein. Of the three species, *C. hyperboreus* is the highest in fat (21.8–53.7% of dry weight), *C. finmarchicus* is the mediate

* Calculated as nitrogen excretion is entirely in the form of ammonia.

(20–47%) and *M. longa* the lowest (12–31%).

C. helgolandicus mainly utilizes protein when starved in the experiments of Cowey & Corner (1963) and Linford (1965), while *C. hyperboreus* utilizes fat in Conover's experiments (1962, 1964). Here also, the difference in substrates oxidized is probably related to the original amount of fat in the animal's body. *C. hyperboreus* contains 55.6% fat per unit dry weight (calculated from Conover, 1964), whereas *C. helgolandicus* contains only 7.1–23.1% (calculated from Linford, 1965).

Tentative calculation was carried out on the energy balance of *C. cristatus* when they sank into the deep water. It is assumed that starved *C. cristatus* respire at 0.84 μl O_2/*Calanus*/hour, which is an average rate over the later half of the present starvation experiments. The animal can survive until it loses 75% of body weight (refer to Omori & Tanaka, 1967). Accordingly, the survival period of this species is calculated to be about 2 months. The energy required for reproduction and molting to adult is not considered in this case. However, this survival period is insufficient for *C. cristatus* to complete the life span in the deep water (at least 4 months) where plant food is almost absent.

In nature, when stage V copepodites of *C. cristatus* descend to the deep layers they may encounter temperatures of 2°–3°C. The present experiments were done at temperatures from 6.9°–11.9°C, differing from the temperature in natural deep water. However, it seems reasonable to think that the regulation of respiration may occur in *C. cristatus* over its normal temperature range (1°–12°C, in the Bering Sea) as was shown in *C. hyperboreus* by Conover (1962) and in *Euphausia superba* by McWinnie (1964). It is generally considered that the metabolism of many poikilotherms is relatively independent of temperature within certain limits in nature (Bullock, 1955). Even if the law of Q_{10} can be applied to the respiration rate in *C. cristatus*, the energy source required for reproduction and molting is still insufficient.

C. cristatus is a primarily herbivorous species, but coprophagy and necrophagy have been found in the present experiments, suggesting that this species, when sinking into deep layers at stage V, can get some energy by feeding on an alternative food source. Cannibalism seemingly does not occur in the present experiments, so that active carnivorous feeding in the deep water may not be important. There is a possibility that suspended detritus may play a role as available food to this species in deep layers. McAllister *et al.* (1960) and Parsons & Strickland (1962) suggested that detritus constitutes the bulk of potential food stuffs for zooplankton living in deep layers.

Table 5 shows the respiration rate of *C. cristatus* observed in the experiments and the theoretical oxygen demand calculated from the assumption that all organic materials lost during the first six days of starvation were oxidized completely. The oxygen consumption observed is about half to one third of the theoretical oxygen

Table 5. Observed oxygen consumption and theoretical oxygen demand of *Calanus cristatus* during starvation. The figures in parentheses are oxygen amounts in μl O_2 necessary to oxidize completely the total amount in μg of that component

	Duration of starvation (in days)	
	0–2	2–6
Calculated		
Protein (1.04)	62.40	193.44
Lipid (2.02)	22.22	153.52
Chitin (*0.87)	9.31	13.66
Carbohydrate (0.82)	0.74	0.90
Total	94.67	361.52
Observed		
Oxygen consumption	59.04	124.32

* The value was calculated from following equation: $C_{30}H_{50}N_4O_{19}+30O_2 = 30CO_2+19H_2O+4NH_3$

demand calculated. A possible explanation of this result may be that *C. cristatus* does not metabolize all the organic matter by pure oxidation, but excretes some high energy organic compounds under certain conditions.

Satomi & Pomeroy (1965) observed that O:P ratio (by atoms) calculated from oxygen consumption and phosphorus excretion is low in planktonic animals compared with benthic ones. They stated that "Zooplankton may be engaged in some aerobic fermentation, excreting lactic acid or other organic acids and thereby consuming less oxygen." There are other papers describing the excretion of soluble organic phosphorus and nitrogen compounds by zooplankton (Pomeroy & Bush, 1959; Pomeroy, Mathews & Min, 1963; Johannes & Webb, 1965; Webb & Johannes, 1967; Hargrave & Geen, 1968). However, organic phosphorus and nitrogen compounds excreted into the water by zooplankton might include some compounds which are not assimilated, but rather simply pass through their gut (Corner & Cowey, 1968).

Dr. Conover (personal communication) does not agree with the author's above interpretation of the experiments. Dr. Conover's major criticism concerns the rather crowded conditions of *C. cristatus* and higher temperatures in the present experiments.

On the former point, Corner & Newell (1967) reported that the forms of nitrogen excreted by *C. helgolandicus* is largely in the form of ammonia; however, at an abnormally high experimental density of animals the levels of ninhydrin-positive nitrogen (e.g. amino acids, amines, amino sugars, etc.) are greatly increased. The density of 50 *C. cristatus*/l in the present experimental condition is fairly high compared with that occurring naturally. This crowded condition may bring the result showed in Table 5. However, it has been confirmed that the

density of 50 *C. cristatus*/l does not affect seriously on respiration rate so far as the metabolism of this species are concerned in the author's experiments (unpublished) of different densities of *C. cristatus*.

Regarding high experimental temperature (6.9°–11.9°C), *C. cristatus* stage V are actually distributed at a temperature about 1°–12°C in the Bering Sea and the author found healthy specimens of this species even in waters at 15°C off Hokkaido. The experimental culture of this species proved that they can tolerate up to 20°C.

At any rate, it is certain that the conditions of the present experiments are fairly differ from the living condition in nature for *C. cristatus*. This may cause arguments against the tentative interpretations presented by the author.

Summary

1. *Calanus cristatus* (stage V) were successfully maintained in a closed tank on board the ship without food for 36 days at a temperature of 7°–12°C.

2. Changes in respiration rate were measured, and changes in body constituents, such as protein, chitin, lipid, carbohydrate and ash were followed on the specimens sacrificed at appropriate intervals. At the same time, the faecal pellets produced were collected and examined.

3. Mortality rate of starved *C. cristatus* was twice that of the control (fed).

4. *C. cristatus* fed on their own feces when starved and after about 15 days of starvation fed on dead bodies of individuals of the same species.

5. Faecal pellets produced by *C. cristatus* became gradually smaller and more transparent with progressive starvation period, but its content became dense again after necrophagy had begun.

6. All organic constituents in *C. cristatus* decreased and ash increased in starved condition.

7. The relative amount of lipid to total organic body constituents in starved *C. cristatus* decreased and that of protein, chitin and carbohydrate slightly increased toward the end of starvation period, indicating high utilization of lipid in starvation. However, absolute quantity of loss in protein was larger than that in lipid for the first 6 days of starvation.

8. It is suggested from the calculation of the energy requirement for survival of starved *C. cristatus* that the animal in nature, when it sinks down to deep water where no phytoplankton exist, perhaps utilize suspended organic detritus as a source of energy.

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