# ベーリング海におけるCalanus cristatusの呼吸と摂食についての予備船上実験


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# Preliminary Shipboard Culture Experiments on the Feeding and Respiration of an Oceanic Copepod, *Calanus cristatus*, in the Bering Sea*

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**Abstract**

*Calanus cristatus* stage V was maintained on board the ship in the Bering Sea for 16 days in an apparatus through which fresh sea water continuously flows. Food uptake, respiration rate and assimilation efficiency of ingested food were measured using this apparatus.

Sea water used in the experiments contains 132-432 $\mu$g particulate organic matter per liter (carbohydrate, 20-36%; lipid, 3-10%; protein, 56-77%). Dominant micro-organisms in the water were diatoms and dinoflagellates amounting to 10-1000 $\times 10^6$ cells per cubic meter altogether.

Food uptake by *C. cristatus* varies from 0.22 to 10.90 $\mu$g organic matter/animal/hour with positive correlation to the flow rate of water through the system. Respiration rate observed is as high as two times (1.26-4.25 $\mu l$ $O_2$/animal/hour) that measured using closed bottle without food (1-2$\mu l$ $O_2$/animal/hour; Ikeda, 1970). The proportion of organic carbon to dry weight of seston in the sea water is less than in faecal pellets produced by the animals, suggesting that there is selection of food by the animals. The maximum assimilation efficiency calculated from that assumption in percent of organic carbon to dry weight of food is 50% to about 68%.

Based on the results obtained in the present experiments the daily uptake of food by *C. cristatus* stage V is estimated as 7% of body weight.

## Introduction

Nutrition and metabolism of marine zooplankton are important problems in the studies of marine ecosystems. The knowledges on these problems, however, are limited in few species, mostly neritic copepods (ref. to the reviews of Anraku, 1963; Raymont, 1963; Conover, 1964 Corner and Cowey, 1968). The main reason for the lack of knowledge on oceanic species is in the difficulty of maintaining such species in laboratory conditions similar to. Since 1968, the author has tried shipboard culture of various oceanic zooplankton for the purpose of experimental studies on nutrition and metabolism of oceanic plankton animals and some results have already been published (Ikeda, 1970 ; 1971).

*Calanus cristatus* is one of the representative oceanic herbivorous copepods which occur; abundantly in the shallow water of the northern North Pacific and the Bering Sea in spring through summer.

In the present experiments, *C. cristatus* was maintained in a continuous flow system of fresh sea water on board the "Oshoro Maru" during her cruise 32 to the northern North Pacific and

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Bering Sea. Food uptake, assimilation and respiration rates were measured.

## Material and Methods

*Animals*: *Calanus cristatus* stage V were caught with the same manner as previously reported (Ikeda, 1970) from the sea surface at 52°24′N 171°52′W on June 13, 1969. The animals were sorted in the ship's laboratory into culture glass bottles, 250m*l* in capacity, soon after sampling, and maintained under the condition of a continuous flow of fresh sea water. The following experiments were conducted while the ship was sailing on the course shown in Fig. 1.

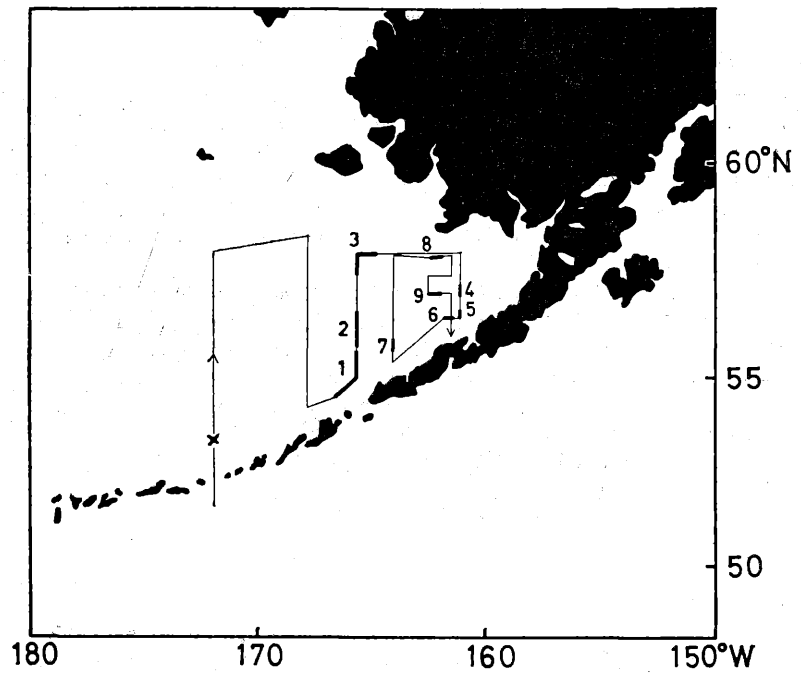


Fig. 1. Track of the "Oshoro Maru" (thin line) and approximate locations (heavy line) where experiments were carried out during her sailing. A cross on the thin line shows the station where *Calanus cristatus* were caught.

*Continuous flow system*: Shematic diagram of this system is shown in Fig. 2. The surface sea water was first pumped up with a rotary pump (1) into a water tank (20 *l* in capacity) (2). Animals in the water were removed with a net (0.35 mm mesh openings) (3) covering on an inlet tube inserted into the tank. This net was renewed daily. Excess water introduced into the tank flowed continuously through an over flow tube, so as the level of water in the tank was kept constant. Then, the water in the tank was poured into two vinyl tubes (4mm in dia.) (4), then to two series of culture glass bottles. The water poured into each culture bottle was withdrawn through an outlet tube inserted into the bottle. The open end of the tube was covered with a net (0.35mm mesh openings). Subsequently, the water which has flown

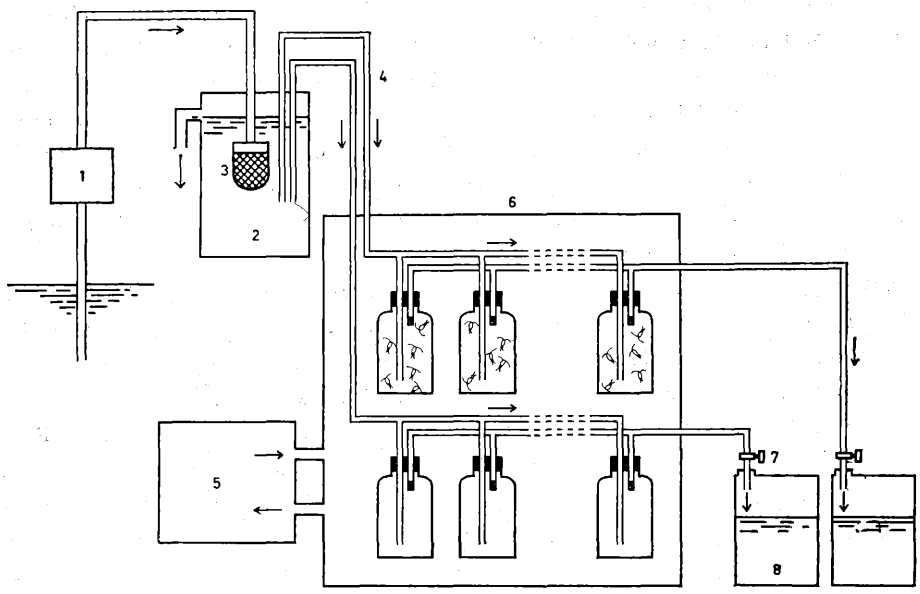


Fig. 2. Shematic diagram of continuous flow system for the maintenance of *Calanus cristatus*.
1 Rotary pump 2 Water tank 3 Net 4 Vinyl tube
5 Coolnics 6 Water bath 7 Pinch cock 8 Sample collector

through two series of bottles was collected in two sample collectors (8) respectively. One flow series consisting of ten culture glass bottles containing 5 animals each was prepared as experimental; 50 animals were kept in a total of 10 bottles. The another flow series consisting of similar 10 bottles but without animals was also prepared as a control. Both experimental and control flow series were immersed in a dark water bath (6) which was connected to Coolnics (5) to keep the temperature similar to the sea temperature at which the animals had lived (7.5°C). The desired flow rate was obtained by regulating the height between outlet tube in the first water tank and subsequent inlet tube in the sample collector, and screw pinch cocks (7) attached to the tubes were handled to adjust the space through which the water passed. The water flow of experimental and control series was kept at the same rate. Such parallel arrangement of culture bottles advantages the use of several number of bottles keeping the same flow rate of water in every bottle. The animals were maintained for 6 days prior to the beginning of experiments for adaptation. The experiments were carried out under various flow rates of water through the system. During the time when no experiment was made and according to circumstances, the water was continualy passed through the whole systems as much as 5 liters per hour until the next experiments started.

*Food uptake*: In one series of experiment approximately 20 liters of sample water which flowed through both control and experimental series were collected in the two sample collectors (Fig. 2 (8)). On the control water seston (particulate materials) were separated by means of Millipore filters (HA type) and the amount of carbohydrate, lipid and protein were measured immediatly on board following the manual of Strickland and Parsons (1965). Usually 3 liters

of water were used in each analysis. On the experimental water only carbohydrate was measured and food uptake was calculated on the assumption that the percent removal of lipid and protein was the same as that of carbohydrate. To test the change in the rate of food uptake with the flow rate of water in the system the latter was changed to several grades. For colorimetric analyses of carbohydrate, lipid and protein the Hitachi 101 spectrophotometer was used. At the same time 500 milliliters of control water were taken from 20 liters sample water and preserved by adding a small amount of formalin for later microscopic examination and cell count of micro-organisms in the water.

*Assimilation efficiency* : The efficiency was estimated from the difference in the proportion of organic carbon to dry weight between food and faecal pellets produced by the animals using the ratio method described by Conover (1966a). Data on dry weight of seston was referred to the measurement by Mr. T. Ichikawa who was aboard with the author. For the measurement of organic carbon in seston, 4 to 6 liters of water were filtered through Whatman GF/C glass fiber filter which had been pre-ignited in muffle furnace at 450°C for 1 hour. A small quantity of 3% sodium chloride solution was then passed through the filter to replace the soluble carbonate materials trapped in the filter. The filters were stored in a desiccator at −20°C in a deep freezer. After returning to the land laboratory, the organic carbon was analyzed with the Hitachi 026 CHN analyzer.

Faecal pellets produced by the animals always sank to the bottom of culture bottles and were never disturbed by the water flow. Collection of the pellets was not carried out at each series of experiment, but all pellets were collected after completing experiments. The pellets were picked up with a pipette and stored on a glass fiber filter in a desiccator placed in the deep freezer. Just before the analysis of organic carbon of the pellets their dry weight was obtained with the Mettler UM-7 Ultra-Micro Balance.

*Respiration rate*: Oxygen bottles (300m*l* in capacity) were placed under the outlet tubes replacing the sample collectors. Dissolved oxygen was measured on both experimental and control waters by the Winkler method (Strickland and Parsons, 1965) three to four times during one experiment. The rate was calculated from the difference in the amount of dissolved oxygen between experimental and control waters. The rate was measured in the experiment series nos. 4, 6, 7 and 9.

## Results

In the present experiments the nature of water taken into the culture bottles varied with each experimental series because the ship was sailing. *C. cristatus* did not inhabit the water areas where the experiment series nos. 3, 4, 5, 6, 8 and 9 were carried out. The salinity of these areas was slightly low (31.5-32.0‰) compared with that of oceanic water from which *C. cristatus* was collected (32.5-34.5‰). Notwithstanding this changed condition mortality of *C. cristatus* was unexpectedly low throughout the whole experiments. Only 2 specimens died during the 16 day's experiments. The mortality rate per day was 0.25%. In the previous experiments on the same species (Ikeda, 1971) in which the animals were maintained in closed bottles, renewing non-filtered natural sea water every two days, the mortality rate of animals was 0.87% per day. The results suggest that the condition of culture in the present apparatus was fairly satisfactory. The average body weight of animals when caught from the sea was 1.99mg and it

Table 1. Organic fraction of seston (particulate materials) and micro-organisms in it, which were offered for the feeding experiments of *Calanus cristatus*.

| Expt. | Date and Time | Organic fraction ($\mu g/l$) Carbohydrate | Lipid | Protein | Total | Dominant micro-organisms ($\times 10^6$ cells/m$^3$) | |
|---|---|---|---|---|---|---|---|
| 1 | June 18 2240<br>19 0730 | 87.24 | 12.57 | 332 | 432 | *Chaetoceros* spp.<br>Dinoflagellates | (20.0) |
| 2 | 19 0730<br>1745 | 70.01 | 21.71 | 150 | 242 | *Chaetoceros* spp.<br>Dinoflagellates | (32.6) |
| 3 | 20 0910<br>21 0520 | 70.58 | 21.90 | 162 | 254 | *Chaetoceros concavicornis*<br>*Rhizosolenia hebetata* f. *semispina*<br>*Thalassiothrix* sp. | (79.4) |
| 4 | 22 0434<br>0629 | 63.53 | 15.62 | 140 | 219 | *Chaetoceros concavicornis*<br>Dinoflagellates<br>Tintinnids | (15.5) |
| 5 | 23 0015<br>0530 | 51.57 | 18.67 | 123 | 193 | Dinoflagellates<br>*Chaetoceros* spp. | ( 9.4) |
| 6 | 24 0018<br>1130 | 98.81 | 22.63 | 155 | 276 | *Rhizosolenia delicatula* (?)<br>Dinoflagellates | (959.5) |
| 7 | 25 0905<br>1055 | 41.10 | 8.00 | 83 | 132 | *Chaetoceros* spp.<br>Dinoflagellates<br>*Rhizosolenia delicatula* (?) | (78.5) |
| 8 | 27 0900<br>28 0100 | 72.84 | 20.57 | 150 | 243 | Dinoflagellates | ( 7.4) |
| 9 | 28 0210<br>29 0500 | 56.01 | 9.60 | 132 | 198 | Dinoflagellates<br>Tintinnids | (11.6) |

became 2.36mg at the end of the experiments after 16 days.

The chemical composition of seston, cell number of micro-organisms and dominant species in the control water at each experiment series are shown in Table 1. Except for two extreme values of experiment no. 1 and no. 7 the total amount of carbohydrate, lipid and protein remained on a fairly constant level (200-250$\mu g/l$). Average composition of this organic fraction showed that carbohydrate accounted 20-36%, lipid, 3-10% and protein 56-77%. Dominant species of micro-organisms in seston varied with cases, but diatoms and dinoflagellates were usually the main components. Their cell numbers attained about 10-1000 $\times$ $10^6$ cells/$l$.

Table 2 shows the results of food uptake and respiration experiments. Flow rate of water through the system was changed from 0.62 to 11.42 liters per hour. There was a tendency that food uptake of *C. cristatus* was accelerated with the increasing flow rate of water (Fig. 3).

The theoretical respiration rate of animal is calculated from food uptake in two ways. In one way it is assumed that the total amount of carbohydrate and lipid ingested by the animal had been completely oxidized in the body (Table 2, column (1)). In the other way the total amount of protein ingested is also taken into the calculation, in addition to those of carbohydrate and lipid (Table 2, column (2)). Calculated values in the former way are less than one half those in the latter one. Comparing the results with those directly observed, those calculated in the former way were close to those of observed values of the experiment series no. 4 and no. 7 in which the flow rate of water was larger than 10 $l$/hour. However, the rate was considerably lower than those of the experiment series no. 6 and no. 9 in which the flow rate was 1.39 and 0.62 $l$/hour. It is thought that the supply of food in the culture bottles by a flow of water at

Table 2. Data on food uptake and respiration of *Calanus cristatus* measured in the continuous flow system.

| Expt. | Flowrate (*l*/hr) | Uptake of organic matter (μg/animal/hr) | | | | Respiration rate (μl $O_2$/animal/hr) | | |
|---|---|---|---|---|---|---|---|---|
| | | Carbohydrate | Lipid | Protein | Total | *Calculated (1) | *Calculated (2) | Observed |
| 1 | 2.20 | 0.11 | 0.02 | 0.43 | 0.56 | 0.13 | 0.58 | – |
| 2 | 2.05 | 0.19 | 0.06 | 0.41 | 0.66 | 0.28 | 0.71 | – |
| 3 | 1.07 | 0.06 | 0.02 | 0.14 | 0.22 | 0.09 | 0.24 | – |
| 4 | 10.96 | 3.16 | 0.78 | 6.96 | 10.90 | 4.20 | 11.44 | 4.25 |
| 5 | 3.79 | 0.26 | 0.10 | 0.63 | 0.99 | 0.42 | 1.08 | – |
| 6 | 1.39 | 0.49 | 0.11 | 0.78 | 1.38 | 0.63 | 1.44 | 1.94 |
| 7 | 11.42 | 1.14 | 0.22 | 2.30 | 3.66 | 1.39 | 3.78 | 1.26 |
| 8 | 1.32 | 0.27 | 0.08 | 0.56 | 0.91 | 0.39 | 0.97 | – |
| 9 | 0.62 | 0.07 | 0.01 | 0.16 | 0.24 | 0.08 | 0.25 | 2.88 |

*It is assumed that total amount of (1) carbohydrate and lipid (2) carbohydrate, lipid and protein ingested were completely oxidized in the animal body. The volume of oxygen required to metabolize 1 g of carbohydrate, lipid and protein is 0.83, 2.02 and 1.04 *l*, respectively.

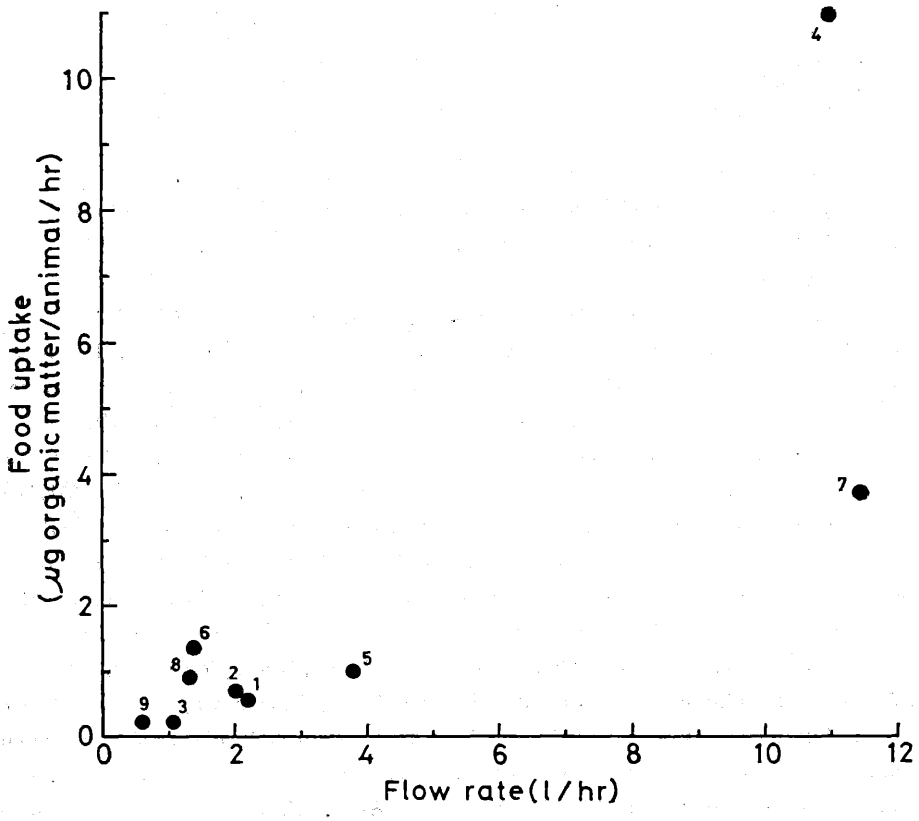


Fig. 3. Relationship between flow rate of water through the continuous flow system and food uptake of *Calanus cristatus*.

least as much as 10 *l*/hour is necessary to meet respiration requirement of animals if only carbohydrate and lipid are oxidized. If this were true, one might expected that the animals of present experiments had fallen into undernutrition resulting in loss of body weight and in high mortality as indicated in the previous starvation experiments on the same species (Ikeda, 1971), because the flow rate of water through the system was less than 5 liters per hour for almost all the experimental duration in the present experiments. However, the results disprove this idea because the animals showed no decrease in body weight and low mortality. A possible explanation will be that a certain proportion of protein ingested is utilized to meet respiration requirement of *C. cristatus*. The values in the column (2) of Table 2 are calculated as the maximum rate ignoring the utilization of protein for growth of animals. The results of more recent observation that the representative herbivorous copepod, *C. cristatus*, ingests *Artemia* nauplii (protein rich animal food) and produce small faecal pellets support this assumption to a certain extent. Feeding on *Artemia* nauplii or other animal food by primarily herbivorous calanoid copepods has been reported by several workers (e.g. Marshall, 1924; Conover, 1966b; Mullin, 1966).

Data on assimilation efficiency in *C. cristatus* are shown in Table 3. The value of dry weight of seston in the water was about 1000 to 4000 $\mu$g per liter in the sea where the present whole experiments were conducted. As the organic carbon concentration was 100 to 200 $\mu$g per liter, maximum percent of organic carbon to dry weight of seston was assumed to be 20%. On the other hand, the amount of organic carbon in the faecal pellets was measured as high as 24%

Table 3. Data on organic carbon and dry weight of food and faecal pellets of *Calanus cristatus*.

| | (1) Organic carbon | (2) Dry weight | (1)/(2) × 100 |
|---|---|---|---|
| Food (particulate materials) | 100-200 ($\mu$g/*l*) | *1000-4000 ($\mu$g/*l*) | <20 |
| Faecal pellets | 115.40 ($\mu$g) | 472.59 ($\mu$g) | 24.4 |
| | 71.87 | 293.10 | 24.5 |

*After Ichikawa (personal communication)

The results suggest that the animals do not feed on any seston by mechanical filtering, but choose selectively foods of high content of organic matter such as cells of phytoplankton and dinoflagellates in the seston. Selective feeding of copepods has indicated by various workers (Harvey, 1937; Corner, 1961; Mullin, 1963; Conover, 1966b). If the maximum percentage of organic carbon of food fed by *C. cristatus* in the present experiments is assumed to be 50% of dry weight of food, this value (50%) coincides with the maximum value on phytoplankton and dinoflagellates studied by Parsons *et al.* (1961). On this assumption the maximum assimilation efficiency is calculated from the following equation :

$$(0.5-0.245)/[(1-0.245)\times 0.5]\times 100=68\ (\%)$$

Assimilation efficiency of some zooplankton have been reported to vary greatly with species and with workers (Conover, 1964). The value obtained by the above calculation closes to that

of *C. hyperboreus*, which is an allied species to *C. cristatus*, studied by Conover (1966a).

## Discussion

The respiration rates of *C. cristatus* measured in the closed bottles without food on the same cruise as that of present experiments ranged from about 1 to 2 $\mu l$ $O_2$/animal/hour (Ikeda, 1970). The rates observed in the present experiments of water flow system were as high as about two times that of those in closed bottles. The high values in the present case would be the effect of specific dynamic action for feeding and the motion of animals created by a water stream.

In the earliest work on the nutrition and metabolism of zooplankton, Corner (1961) measured food uptake, selectivity and assimilation of food by *C. helgolandicus* using a continuous flow system in which the culture bottles were arranged in cascade, while in the present experiments a parallel arrangement of culture bottles were employed. Corner (1961) calculated the respiration rate assuming that total quantities of assimilated carbohydrate and lipid were completely oxidized. His calculated value of respiration rate was higher than those reported by previous workers. He proposed three possibilities to explain this difference. The results of the present experiments suggest that his third possibility that animals which are continuously feeding have a metabolic rate higher than that of animals kept in filtered sea water is correct.

As shown in Fig. 3 food uptake increases with the increase of flow rate of water through the system. This is not the case in the respiration rate. The present author thinks that this phenomenon is the result of feeding on cells of diatoms and dinoflagellates by the animals selectively from other non-living particulate organic matter. Practically, the difference in the concentration of particulate organic matter between experimental and control water is approximately 10% throughout almost all the experiments irrespective of flow rate of water in the system. Accordingly, particulate organic matter making available as nutriments for animals is in limited matter and normally occupies only small portion in total particulate organic matter present in the water. The result of assimilation efficiency supports this conception. In the present experiments food uptake is estimated from direct analysis of only carbohydrate between experimental and control waters assuming that the percent removal of lipid and protein is equal to that of carbohydrate. Usually, micro-organisms which might be fed preferably by the animals contain a larger amount of organic matter than non-living seston, so that the results on food uptake in the present experiments may be underestimated. For the precise estimation of food uptake simultaneous analyses of protein and lipid on both experimental and control waters are needed.

It is difficult to determine realistic values of food uptake and respiration rate of *C. cristatus* stage V in the present experiments because of large scatter of both values. If one accepts the assumption that assimilation efficiency is 68%, hourly growth is 0.05% $[(2.36-1.99)/(1.99\times16\times24)\times100]$ and respiration rate is 3 $\mu l$ $O_2$/animal/hour, one *C. cristatus* weighing 1.99 mg will need about 6 $\mu g$ of organic matter as food hourly. In this calculation, composition of organic matter of food analyzed in the present experiments was used. Daily food requirement of *C. cristatus* is calculated as 7% of body weight. The value is fairly low compared with 25.3% for *C. helgolandicus* reported by Corner (1961). This difference is reasonably explained from the difference in body size of animals, that is, generally weight specific metabolic rate of animals decreases with increase of body weight (Ikeda, 1970). Body dry weight of *C. helgolandicus* is 0.098–0.117mg com-

pared with 1.99mg of *C. cristatus*.

Finally, accurate simultaneous measurements of food uptake and the respiration rate of zooplankton under simulated condition to nature are almost impossible so far as closed bottles are used. The continuous flow system used here will be one step for this purpose. However, as was already shown, the determination of flow rate of water appropriate for normal life of the animals is a difficult problem, because the animals may fall into undernutrition in a slow stream, and, on the other hand, they may be forced to excess movement in a fast stream. At any rate the condition of continuous flow system is far close to the natural condition in the sea compared with closed bottles experiments. In the present experiments, the ship sailed to the areas where the experimental animals do not inhabit so that replicate experiments are limited.

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