A Simple Device for Culturing Marine Calanoid Copepods And Notes on the Biology of Eurytemora hirundoides Nordquist

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A Simple Device for Culturing Marine Calanoid Copepods and Notes on the Biology of *Eurytemora hirundoides* NORDQUIST

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Contents

I. ABSTRACT

A simple device for culturing marine calanoid copepods is described. Calanus helgolandicus Pacificus, Acartia clausi GIESBRECHT Temora longicornis MULLER, and Eurytemora hirundoides NORDQUIST, were reared in this device. The latter has been bred in the laboratory for several generations. Antibiotics were used only once when

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the experiment was started. Filtered sea-water was used, with a salinity of about 29.5 ± 0.5 ‰. Skeletonema costatum, Isochrysis galbana, Chlamydomonas sp., Phaeodactylum tricornutum, Monochrysis sp., and Dunaliella sp., were used as food (none of the media used was bacteria-free).

The effect of temperature on the life cycle of Eurytemora hirundoides has also been studied.

II. INTRODUCTION

Early in this century it was realized that copepods appear to be a rather important link in the marine food web. Particularly, calanoid copepods are the principal link between primary (photosynthetic) producers and carnivores in the sea (ZILLIOUX & WILSON, 1966).

Usually it is impracticable to study the effect of ecological factors on copepods in natural conditions. Therefore scientists try to rear and breed them in the laboratory.

ALLEN & NELSON (1910) kept Calanus finmarchicus for a few weeks. RAYMONT & GROOS (1942) kept Calanus finmarchicus for 3 to 9 weeks healthy and active. CONOVER (1962, 1967) has reared some individuals of Calanus hyperboreus from egg to adult, as referred to by VILELA (1972). CORKETT (1967) reared Temora longicornis MULLER and Pseudocalanus minutus KROYER.

Some species of calanoid copepods have successfully been cultured in the laboratory for several generations: Pseudodiaptomus coranatus WILLIAMS (JACOBS, 1966), Acartia tonsa DANA (ZILLIOUX & WILSON, 1966) Calanus helgolandicus PACIFICUS and Rhincalanus nasutus (MULLIN & BROOKS, 1967), Pseudocalanus elongatus BOECK (KATONA & MOODIE, 1969), Eurytemora affinis POPPE and Acartia tonsa DANA

(HEINLE, 1969), Acartia clausi GIESBRECHT and Acartia tonsa DANA (ZILLIOUX, 1969), Calanus helgolandicus (PAFFENHÖFER, 1970), Gladioferens imparipes THOMSON (TAKANO, 1971), and Acartia grani SARS (VILELA, 1972).

Also there has been success in rearing and breeding harpacticoid copepods: Tigriopus fulvus (FRASER, 1936); PROVASOLI, SHIRAISHI, & LANCE (1959), as referred to by MULLIN & BROOKS (1967). Tisbe furcata BAIRD (JOHNSON & OLSON, 1948), Euterpina acutifrons DANA (NEUNES & PONGOLINI, 1965), Tisbe sp. (VILELA, 1969), Tigriopus japonicus MORI (TAKANO, 1971), and Tisbe pori (BETOUHIM-EL & KAHAN, 1972).

The cyclopoid copepod Oithona nana was successfully reared by MURPHY (1923).

This paper describes the rearing of several calanoid copepod species, using a new simple device. Only Eurytemora hirundiodes NORDQUIST could be reared for several generations in succession. Therefore this species was chosen to study its life cycle and the influence of temperature on its development.

The genus Eurytemora includes about 15 species, all of Northern distribution and characteristically found in coastal and brackish marine environments (GURNEY, 1931, as referred to by KATONA, 1970). Eurytemora hirundoides is sometimes an important constituent of the zooplankton and the food of herring (WIMPENNY, 1966).

III. MATERIAL AND METHODS

a. Collection of Copepods

On February 9, 1973, copepods were obtained from offshore localities in the Dutch Wadden Sea, near the NIOZ harbor at Texel. They were collected by towing a net for a few minutes, using a 100 µ meshes net (30 cm. diameter), the cod end provided with a 300 ml. conical

flask. Copepods were collected one meter beneath the water surface. The organisms were carried to the laboratory in a 10 liter plastic container. The container stayed outside the laboratory for a few hours, and then inside for another few hours to acclimatize with respect to temperature. Copepods were taken from the collection at random. Most of them were Eurytemora hirundoides. Apart from these there were four individuals of Calanus helgolandicus, two of Acartia clausi, and a few of Temora longicornis. They were washed in filtered sea-water.

The copepods selected were transferred to the working device about six hours afte the time of capture.

b. Description of the new device

Many different kinds of containers have been used for rearing and breeding copepods. At the onset of the culturing of calanoid copepods many problems occurred with respect to the survival, reproduction, feeding of the copepods, and the ability to float of the animals and their food organisms.

The main problems encountered were:

1 - Attachment of copepods to the walls of the containers on which bacteria and protozoan ciliates accumulated.

2 - Precipitation of algae and diatoms on the bottom, and mixing of these foods organisms with the fecal pellets of the copepods.

3 - Accmulation of algae and diatoms producing large clusters, which settle on the bottom of the experimental vessels; moreover these clusters attached to the distal parts of the urosome and antennae, hindering movement and facilitating infection by parasites.

4 - The choice of the right type of food which can support the

development of the copepods from egg to mature adult.

Most of these problems were solved with the appearance of new devices for culturing copepods.

GREVE (1968) developed a device for culturing zooplankton, resolving the problems of the attachment of organisms to the walls of the experimental vessel, sedimentation of food particles, and oxygen supply. ZILLIOUX (1969) developed a continuously recirculating culture system for planktonic copepods, to provide the laboratory with sufficient numbers of calanoid copepods for research work. He succeeded in culturing Acartia clausi and Acartia tonsa for 14 and 10 months, respectively. Then ZILLIOUX & LACKIE (1970) developed another recirculating culture system, which reduces the dissolved organic carbon by facilitating the removal of foam, in which Acartia clausi and Acartia tonsa were successfully cultured for more than a year.

In this paper a new device for rearing and breeding calanoid copepods is described. Its main characteristic is, that it is easy to prepare, clean, use, and examine the copepods reared, and it is possible to use it to study the effect of ecological factors such as temperature and salinity on the developmental stages of calanoid copepods. The device consists of a main cylinder (30.2 cm. total hight, 17 cm. diameter), which is separated into four parts, from the bottom upwards:

1 - The perspex base (4.7 cm. high) provided with " n and o shape openings".

2 - The bottom sieve (a 44 or 150 µ mesh-size stainless steel screen), mounted in a polythene ring, which fits around the base and the next part.

3 - The perspex dish ring (5.5 cm. high).

4 - A perspex cylinder (20 cm. high) provided with four windows, lined with stainless steel screens (44 or 150 /u mesh-size).

It is connected to the dish ring with an outer polythene ring. A hollow basal disk (15.5 cm. diameter, 4.5 cm. high) is placed beneath the bottom sieve, within the base (Fig. 1). It consists of a ring, which is closed by a bottom plate and a top plate. The latter is provided with 7 pores (2 mm. diameter) in a circle 3 cm. from the margin (one of them in the center). Two instruments were used, one for gravid females and nauplii with screens of 44 /u mesh-size, and one for copepodites and adults with 150 /u mesh-size.

Before using the device it is submerged in tapwater for two days, in sea-water for two days, and in distilled water for one day, in order to make sure that the screens are without poisonous ionic materials. Then it is placed in a glass aquarium (47 x 29 x 28 cm.), which is filled with filtered sea-water in such a way, that the water level is below the top of the device.

A waterpump sucks the water into a siliconrubber tube of 2.5 meter length, which is closed at the end and is provided with about 20 pores (1.5 mm. diameter) distributed at regular intervals. This tube is coiled around the apparatus in the aquarium. The outlet of the pump is connected with the basal disk by a siliconrubber tube. The water is pushed through the pores in the dick and the bottom sieve into the main cylinder, which contains the copepods. From this it flows back into the aquarium through the screened windows.

The water in the aquarium is bubbled with air (Fig. 2) and the cultures of algae are added. In this way the algae remain in suspension and the copepods are continuously supplied with water rich in oxygen and food particles.

In case of examination or cleaning, the water in the aquarium

is symphoned out. At the same time the screen of the cylinder is rinsed with a weak stream of water, to prevent the attachment of copepods to the screen, until the water inside the cylinder appears beneath the dish ring. Then the base in removed, and the intermediate dish is closed from below by a perspex closing plate by means of a rubber ring. Then the main cylinder id removed. The three parts (dish ring, bottom sieve, and closing plate) are carried to a dish container (perspex 19.2 cm. diameter, 9 cm. high Fig. 3) and the copepods can be examined with a magnifying glass, or with a binocular microscope.

c. Algal Cultures

Food plays an important role in the culturing of copepods, because not all algal cultures give positive results. NASSOGNE (1970) concluded that some points must still receive further attention:

1 - Availability and/or preference of copepods for certain species of phytoplankton.

2 - Suitability of a given algae species to support the development of the copepods from egg to mature adult.

3 - Efficiency of food conversion.

PAFFENHÖFER (1971) noticed that not only the food quantity, but condition and age of the food organisms as well, influenced the rate of mortality of Calanus helgolandicus during its early lifetime, when feeding on 2-8 days old algal cultures.

Many workers used algal cultures as a food source only (CORKETT, 1967; 1970; HEINLE, 1969; ZILLIOUX, 1969; & VILELA, 1972).

Others used diatoms, (ALLEN, 1910; RAYMONT & GROSS, 1942). Mixtures of algae and diatoms were used also (ZILLIOUX & WILSON, 1966; KATONA & MOODIE, 1969; PAFFENHÖFER, 1970). Others used algae

and bacteria (JACOBS, 1961), a mixture of diatoms and Artemia salina "for older copepodites" (MULLIN & BROOKS, 1967), or a mixture of diatoms, wheat flour and soya flour (TAKANO, 1971).

Several authors have reported that mixture of algae are a better diet than unialgal cultures (DAVIS & GUILLARD, 1958; PROVASOLI et al., 1959; referred to by BETOUHIM-EL & KAHAN, 1972).

NASSOGNE (1970) advised to provide copepods with a wide range of algal species in the nutrient solution. He concluded that not only the size of the algae (which varies only between 6 and 15 µ) is important, but also the presence of certain factors such as micronutrients and vitamins.

In the present work six algal and diatom cultures were used, none of them bacteria-free: Skeletonema costatum, Isochrysis galbana, Chlamydomonas sp., Monochrysis sp., Phaeodactylum tricornutum, and Dunaliella sp.. All of them were provided by the Central Laboratory "TNO", Delft, The Netherlands.

The first three cultures were used in the breeding experiments with Eurytemora hirundoides, but all six cultures were used in the rearing of Calanus helgolandicus, Acartia clausi, and Temora longicornis.

The medium used in culturing algae and diatomes is provided by the Marine Microbiology department of NIOZ:

1	Liter	Distilled water
30	gm	Artificial Sea salt "HW-MEERESSALZ"
50	mg	Na_2EDTA "Ethylenediaminetetraacetic Acid, Disodium salt"
10	mg	Yeast Extract
5	ml	Soil Extract
20	mg	K_2HPO_4
100	mg	$NaNO_3$

200 mg $NaHCO_3$

with pH of 8 ± 0.2, HCl used as control. The medium was filtered, and sterilized in an autoclave (120 °C, 15 Lb., for 15 minutes). The cultures were incubated at 18 °C under fluorescent tubes, with light intensity of 0.002 gm Cal/cm^3/minute, in cotton-stoppered conical flasks of one liter, containing 500 ml. of the filtered medium.

d. Medium for Copepods

Most of the culturing experiments reported have been carried out by using natural sea-water (sterile or filtered). Few authors used artificial sea-water (ALLEN, 1910; RAYMONT, 1942; HEINLE, 1969).

Till now there is no clear idea about using antibiotics. Many workers used it continuously during the experiments (MULLIN & BROOKS, 1967; CORKETT & URRY, 1968; ZILLIOUX, 1969; CORKETT, 1970). NEUNES & PONGOLINI (1965) used antibiotics only for the initiation of a laboratory culture, because it is possible that the resistance of copepods against excessive bacterial growth is extremely low during the initial period in which they are probably in a weakened condition.

JACOBS (1961), KATONA & MOODIE (1969) considered bacteria as a source of food. VILELA (1969), used unfiltered sea-water.

The experiments with copepods described in this paper were carried out with fresh, filtered sea-water with a salinity of 29.5 ± 0.5 promille. Antibiotics were used only once when the experiment was started: 50 I.U. Penicilline + 50 µg streptomycine sulphate per milliliter sea-water (OPPENHEIMER, 1955), as recommended by FONDS (1970) for rearing fish eggs. The sea-water was changed weekly. Then fresh algal cultures were added, 1-2 weeks old.

Skeletonema costatum, Isochrysis galbana, Chlamydomonas sp., Phaeodactylum tricornutum, Monochrysis sp., and Dunaliella sp., were used in rearing Calanus helgolandicus, Acartia clausi, Temora longicornis, and Eurytemora hirundoides.

The first three algal cultures were used in rearing and breeding Eurytemora hirundoides. Using this medium the copepods collected could be kept alive for about one month. Table I gives for the different species the time, after which all copepods have died.

Table I

Maximal length of time the copepods stayed alive.

Species	Time in days
Calanus helgolandicus	45
Acartia clausi	34
Temora longicornis	21
Eurytemora hirundoides	36

IV. LIFE CYCLE AT DIFFERENT TEMPERATURES

To study the effect of the temperature on the life cycle of Eurytemora hirundoides, flat-bottomed tubes (3.2 x 10.5 cm.) were filled with 50 ml. of equal volumes from the Skeletonema costatum, Isochrysis galbana, and Chlamydomonas sp. media, and 30 ml. of filtered sea-water.

One or two gravid females were transferred to these tubes, which were incubated at different temperatures in small aquaria (20 x 12 x 12 cm.), which were placed in large concrete tanks, in which the following temperatures were maintained: 10, 15, 20, and 25 °C.

a. Copulation

JACOBS (1961) described the mating of Pseudodiaptomus coronatus. Pairs may remain copulated for hours, and sometimes for days. Mating in Acartia tonsa was never observed to last longer than a few seconds. In Pseudodiaptomus coronatus the female is the more active and leading partner.

The duration of copulation in Tisbe sp. varies from 3 hours to 3 days (VILELA, 1969). TAKANO (1971) described the mating of the calanoid copepod Gladioferens imparipes, and the harpacticoid copepod Tigriopus japonicus.

KOGA (1970) observed that the mature male of Tigriopus japonicus frequently copulates with the immature female, even with the female as young as in the first or second period of the copepodite stage. HANOOKA (1940) concluded that the length of time of mating is flexible from a few minutes to several days, as referred to by TAKANO (1971).

In Acartia grani the mating period is very short (VILELA, 1972).

Copulation of Eurytemora hirundoides was observed at room temperature ($16^{\circ} \pm 0.5\ ^{\circ}C$), and at 10 $^{\circ}C$. As soon as females matured, males approached them by very quick movements, attaching themselves to the right ramus of the female urosome by means of the right junculate antenna, turned upside down in the same direction as the female, which moved very quickly and appeared to try to escape from the male. The movements take about 30 minutes at room temperature, but about 2 hours at 10 $^{\circ}C$.

At the end of the movements of the female slow down maybe because she becomes exhausted. Then the male jumps on her back, facing the opposite direction to her, pushing the cylindrical spermatophore through the genital opening of the female.

Another mode of copulation was observed when the male lost the distal part of his right antenna (from the junction) before copulation. Then he attached himself to the genital segment of the female instead of the ramus, but the rest took place as described above.

b. Duration of embryonal development

After copulation has taken place the egg sack appears. The development of the egg sack is affected by temperature. At 15 °C it appears after not more than of an hour, whereas at 10 °C it appears the next day.

There are six naupliar stages, and six copepodite stages in the life cycle of Eurytemora hirundoides, the last copepodite stage is the adult stage (DAVIS, 1943).

The duration of the embryonal development (incubation period) was estimated by noting the first appearance of the egg-sack and the subsequent hatching of the nauplius larvae (JOHNSON & OLSON, 1948). It seems to depend upon the temperature, being shorter at the highest temperatures (MARSHALL & ORR, 1953; VILELA, 1969; BETOUHIM-EL & KAHAN, 1972).

CORKETT (1972) shows that the development time increases with decreasing temperature in Calanus helgolandicus.

The experiments carried out at room temperature clearly proved that the incubation period, and the development of early naupliar stages are more sensitive to temperature than the copepodite stages, which always have an almost equal development time at different temperatures (Table IV).

c. Number of Broods produced by a Female

JOHNSON and OLSON (1948) observed that the females of Tisbe furcata produced 5, 9 and 12 broods before dying. VILELA (1969) found that the maximum number of egg-sacks formed by the same female of Tisbe sp. was 10. (HANAOKA, 1940, observed that a female of Tigriopus japonicus formed 14 egg batches during her life) as referred to by TAKANO (1971). TAKANO (1971) noticed that the same species produced nauplii 11 times.

For Eurytemora hirundoides the production of egg batches was observed at different temperatures, as shown in Table II.

Table II

The average egg batches produced by one female at different temperatures.

	$16^{\circ} \pm 0.5\ ^{\circ}C$ (Room temp.)	10 °C	15 °C	20 °C	25 °C
Number of batches	4-5	2-3	4	4-5	no reproduction

The results indicate, that the number of batches produced decreased when temperature decreased.

d. Number of Nauplii in one Brood

Until now no one observed 100% hatching of copepod eggs to nauplii. JOHNSON and OLSON (1948) mention about 80% survival in Tisbe furcata. MULLIN and BROOKS (1967) found survival of Rhincalanus nasutus from eggs to seven-weeks-old adults of no more than 30%. CORKETT (1972) concluded that the accumulation of detritus on the bottom of the experimental vessel decreased the hatching success. KATONA (1970) observed a high mortality rate at the lower temperatures

The number of eggs in one egg-sack of a wild female of Eurytemora hirundoides was about 35-45. Not all of them hatched successfully. The hatching of eggs was affected by temperature as shown in Table III.

Table III

The maximum numbers of nauplii per egg sack.

	16° ± 0.5 °C (Room temp.)	10 °C	15 °C	20 °C	25 °C
number of nauplii	29	13	21	32	8

The number of eggs hatching increases with increasing temperature up to 20 °C. At this temperature we find the maximum number of nauplii per egg sack.
At 25 °C the copepods seem to come under thermal stress the females do not produce other egg sacks.

KATONA (1970) observed the same phenomenon, that Eurytemora herdmani does not reproduce at 18 °C, Eurytemora affinis at 21.5 °C.

e. Duration of the Generation Cycle

In Calanus finmarchicus, MARSHALL and ORR (1953) observed that the developmental time for the eggs increased with decreasing temperature from 5° to 0 °C. KATONA (1970) concluded that Eurytemora herdmani developed faster and more successfully at their lowest temperature (2 °C), but Eurytemora affinis developed well at 5 °C.

The developmental rates in Eurytemora hirundoides increased with increasing temperature until 20 °C, at which the shortest time of one generation occurred.

When temperature rises to 25 °C, this induces thermal stress

in the organism, the copepods cannot reproduce.

The duration of one generation of Eurytemora hirundoides was studied at different temperatures, Table IV gives the results.

Table IV

Number of days required for each stage, and the mean time of one generation, at different temperatures, for Eurytemora hirundoides.

Temperature	Incubation period	Naupliar stage	Copepodite stage, until the appearance of the egg sacks	Total
Room temp. 16° ± 0.5 °C	1-2	7	10	18-19
Room temp. 15° ± 0.5 °C	2	8	10	20
10 °C	8	After 12 days all nauplii were dead		
15 °C	3	After 9 days all nauplii were dead		
20 °C	1	5	10	16
25 °C	less than one day	After 5 days all nauplii were dead. The parents did not produce other offspring.		

In Eurytemora hirundoides, gravid females appeared within no more than one day after the last moult. Therefore we can conclude that the period from moult to adult to the appearance of the egg sack (the preoviposition period) is very short. MULLIN and BROOKS (1967) concluded that the copulation occurs shortly after the females moult to adult, in both Calanus helgolandicus and Rhincalanus nasutus.

f. Size of Copepods

The size of copepods can be affected by ecological factors such as Temperature, Salinity, Oxygen, amount of phytoplankton, and light (CONOVER, 1956). DEEVEY (1960) observed that temperature is one of the most important variables governing copepod size, as referred to by KATONA (1970). MCLAREN (1963) believed, that the development of copepods is retarded by low temperature. MARSHALL (1963) gave evidence that small adults can be found at the end of winter, as published by MCLAREN (1963). KATONA and MOODIE (1969) observed, that Pseudocalanus elongatus cultured in the laboratory appear to be longer than wild animals. KATONA (1970) added salinity as a factor affecting the size of E. herdmani at moderate temperatures, and he observed that the size of E. herdmani raised in the laboratory has always been smaller than those of specimens collected in the field. For E. affinis he concluded that the length decreased with increasing temperature.

SARS (1903) found that the size of the adult female of E. hirundoides can be up to 1.15 mm., whereas DAVIS (1943) observed that the length of the female was 1.482-1.708 mm., and of the male 1.403-1.647 mm. DEEVEY (1948) reported, that in Tisbury great pond the size of the male was 0.8-1.25 mm and the female 0.9-1.4 mm. The length of the wild copepods of this species collected from the Dutch Wadden Sea is 1.280-1.440 mm for the female, and 1.168-1.387 mm for the male.

The mean length of E. hirundoides bred in the laboratory is given by Table V.

Table V

Mean size of E. hirundoides bred in the laboratory in mm.

	Temperature	Male	Female
Parents	Dutch Wadden Sea 4 °C	1.182	1.397
1st. generation	Room temperature 16° ± 0.5 °C	1.121	1.261
1st. generation	20 °C	1.039	1.211
2nd. generation	20 °C	1.000	1.159

From all data mentioned we can conclude, that the temperature is the main factor which affects the size of the copepods at early developmental stages.

Copepods of the 2nd. generation at 20 °C are smaller than those of the 1st. generation. Presumably this difference follows from a higher vitality of the 1st. generation, as wel as from the new environmental factors.

g. Survival of Copepods

Survival may be defined as the period of time in days from maturation untill death.

For Calanus firmarchicus, CLARKE and BONNET (1939) concluded, that at low temperatures survival was high. The same was indicated by CORKETT and URRY (1968) for Pseudocalanus elongatus. KATONA (1970) observed, that the survival time of E. affinis and E. herdmani increased when temperature decreased. In nature DAVIS (1943) observed that E. hirundoides can reproduce at 2.5° to 24 °C. DEEVEY (1948) reported, that the temperature range of E. hirundoides in Tisbury

Great Pond was 0° to 24 °C.

From the results of NASSOGNE (1970) for Euterpina acutifrons, food constituents may be considered as a factor affecting the survival.

E. hirundoides differs from other copepods with respect to survival. It was found that the survival time increases with temperature until an optimum temperature at 20 °C; beyond 20 °C the survival time decreases again as is clear from the results obtained at 25 °C.

The results of Table VI shows the maximal time of survival

Table VI

The maximum survival time in days for E. hirundoides.

	Room temp. 16° ± 0.5 °C	10 °C	15 °C	20 °C	25 °C
Survival time (maximum in days)	26	25	29	32	16

V. DISCUSSION

Collection of the copepods

Copepods are very sensitive organisms. Once collected they must be treated very carefully.

Most copepods collected by simple netting and exposed to the atmosphere were dead. It is necessary to use a conical flask at the cod end of the net used, and it is important to wash the net until all copepods settle on the bottom.

The new device

In the discussion of a paper by CONOVER (1970) Gold advised to design culturing systems for the cultivation of plankton more in conformity with the natural environment. The new device described in the present report is mainly based on this idea of natural design approaching the natural marine environment, as to currents, nutrients, and organic and biotic substances.

In the device there are three main currents working together to keep the copepods and food materials in suspension. One current is produced by air bubbles in the exterior container. Another is directed upwards inside the cylinder as a result of the inlet of water through the bottom sieve into the cylinder. The third one turned out as a reaction on the pressure of water pushed by the basal disk against the bottom sieve. This current passes through the (n and o shape) openings of the base, washing the bottom of the aquarium and preventing precipitation of the food.

It is possible to use one instrument in culturing all stages to provide the laboratory with a sufficient quantity of healthy copepods, when the apparatus is provided with screens of 74 /u mesh-size, as those used by ZILLIOUX (1969). This mesh-size let the food organisms pass through it freely, in the same time preventing all stages of E. hirundoides from passing through, even the eggs (95 /u).

The movement of water reduces the mortality of nauplii, by preventing them from attaching to the surface, a mortality factor mentioned by CORKETT (1967).

Algal cultures

The algal cultures used in the rearing experiments are not bacteria-free. This may have increased the mortality rate of all stages of E. hirundoides. It is better when bacteria-free algal cultures are used.

Sometimes Skeletonema costatum showed a poor growth, decreasing the food concentration in the medium of the copepods. Many authors consider Skeletonemaas a main source of food for calanoid copepods. One cause of the poor growth was the rather low light intensity. Using an autoclave in the sterilization of the media may cause damage to the biotic substances of it. For this reason I suggest sterilization by filtration.

Medium for copepods

According to CORKETT (1967) the best medium for copepods is fresh filtered sea-water, which is taken from the same locality where the copepods were collected.

The medium used in the present work was suitable because of the following reasons:

1 - As mentioned before, bacteria were given as a constituent of the food. CONOVER (1964) concluded that herbivorous copepods may feed well on protozoa. MARSHALL and ORR (1956) observed that nauplii stage I and II of Calanus finmarchicus do not feed on algae and diatoms.

From these observations we can conclude that the early naupliar stages of copepods may feed on bacteria and protozoan ciliates.

2 - The micronutrients and biotic substances of the natural sea-water will be carried to the experimental vessels, providing the cultured copepods with the same natural medium, especially

when this medium is refreshed weekly.

3 - According to observations of NEUNES & PONGOLINI (1965), the presence of bacteria increases the resistance of copepods against bacterial infections. Although bacteria in the algal cultures which are alien to the natural system may be dangerous for copepods, the presence in the natural sea-water may be advantageous because they are presumably part of the natural food source.

Antibiotics are necessary to provide early hatched nauplii with resistance against bacterial infections, and I commend to add them once in each generation period (i.e. once each 16 days at 20 °C, or once each 18-19 days at room temperature). MARSHALL & ORR (1958) observed that antibiotics increased the duration of the experiment when carried out in a small volume of water with small animals, in which the oxygen remained constant for 11 to 12 days.

Sterile sea-water was given once in the tubes used in the experiments carried out to investigate the influence of temperature on the life cycle. A high mortality rate in the late copepodite stage was observed. Needle-like crystals appeared, which mixed with the food and caused death to this stage.

The reason why Calanus helgolandicus, Acartia clausi and Temora longicornis did not reproduce is not known, because there were not enough animals. The survival period was sufficiently long for the production of egg sacks.

Hatching of eggs

MCLAREN, WALKER, & CORKETT (1968) noticed a high mortality of developing eggs in all experiments carried out to study the effect of temperature-salinity combinations on mortality and development

rate of eggs of Pseudocalanus minutus.

In the present work not all of the egg batches of E. hirundoides hatched, the highest percentage at 20 °C. For all other conditions no more than 2 sacks hatched per female. This low percentage of hatching may be due to the fact that the eggs were not fertilized. There may have been physiological factors affecting fertility in both males and females, due to the change in habitat and environmental components.

Size of copepods

KATONA (1970) observed that salinity and temperature affected the size of E. herdmani. PAFFENHÖFER (1970) remarked, that the size of Calanus helgolandicus females is affected by the type of food. MCLAREN (1970) found, that there is a relation between the size of the cephalothorax and the size of the eggs. He reported the length of the cephalothorax of E. hirundoides to be 0.70 mm., and the mean diameter of eggs 82 µ (MACLAREN, 1966; MCLAREN et al. 1969). These dimensions differ from those mensioned in the present work. The cephalothorax of wild females from the Dutch Wadden Sea measured 0.84 mm. on the average, and the mean diameter of the eggs was 95 µ.

MARSHALL (1949), DAVID (1961) assumed, that larger females carried more eggs, as published by MCLAREN (1963). Also there is a relation between the size and respiration, and between temperature and respiration as noticed by GAULD & RAYMONT (1953) for Acartia clausi.

From the dimensions of E. hirundoides given by SARS (1903), DAVIS (1943), DEEVEY (1948), the sizes of the wild copepods of the Dutch Wadden Sea, and for those of the laboratory, we can conclude that environmental factors such as temperature, salinity, food

constituents, micronutrients, and biotic substances, influence size in this copepod species.

Survival, Fecundity, and Reproduction

Survival in E. hirundoides differs from all other copepod species, in which survival increases when temperature decreases.

During the time the flat-bottomed tubes were used to study the effect of temperature on the life cycle of E. hirundoides, Chlamydomonas accumulated in large clusters attached to the distal parts of the antennae and urosome, impeding the movements of the copepods, and giving bacteria and protozoan ciliates a good chance to settle on the animals. This increased the mortality rate and decreased the time of survival. Accumulation of algae as clusters attaching to the urosome minimized the fertility of the eggs. It was noticed, that one egg sack remained attached to the female until her death.

KATONA (1970) considered raising of the temperature to 23.5° ± 1.5 °C as a lethal factor for algae. BETOUHIM-EL & KAHAN (1970) however remarked that the shortest developmental time of Tisbe pori was 26° to 28 °C. Combined results of KATONA, BETOUHIM-EL & KAHAN display, that algae can survive at 28 °C. It is possible that E. hirundoides does not reproduce at 25 °C, but likely this was not caused by the death of the food organisms, but must have been an effect of temperature on the reproductive system. At this temperature copepods remained alive for 16 days without egg production.

From all factors mentioned we can conclude, that all factors cooperate to affect survival, fecundity, and egg production of the copepods. E. hirundoides was more or less affected by combinations

of the factors when it was cultured in the laboratory.

VI. SUMMARY

1 - A new device for rearing and breeding calanoid copepods is described. The apparatus is easy to use and to assemble and is, moreover, cheap. Copepods can be readily examined in this device.

2 - Calanus helgolandicus, Acartia clausi, and Temora longicornis were reared for about one month. E. hirundoides was bred for several generations.

3 - The effect of temperature on the life cycle of E. hirundoides was studied. The shortest life cycle was found at 20 °C, which seems to be the optimum temperature for E. hirundoides collected from the Dutch Wadden Sea.

4 - The length of the incubation period and developmental period of early naupliar stages is very sensitive to small differences in temperature.

5 - The number of broods produced by a female decreases with temperature. At 10 °C a female produced 2 egg batches, whereas a female at 20 °C produced 5 egg batches.

6 - The percentage of eggs hatched increases with temperature, until an optimum at 20 °C. No more than 13 nauplii hatched per egg sack at 10 °C, but 32 nauplii hatched per egg sack at 20 °C.

7 - At 25 °C the copepods become under thermal stress and do not reproduce.

8 - The size of copepods is large at a low temperature. At room temperature (16° ± 0.5 °C) the size of the male E. hirundoides was 1.121 mm., of the female 1.261 mm., whereas at 20 °C the male measured 1.039 mm., the female 1.211 mm.

9 - The size of copepods changed in the course of the generations.

In the first generation at 20 °C the size of the male was 1.039 mm. and of the female 1.211 mm. In the second generation this size was 1.000 mm. for the male, and 1.159 mm. for the female.

10 - The survival time increased with temperature until an optimum at 20 °C. At 10 °C the adults stayed alive for 25 days, at 20 °C for 32 days, but at 25 °C survival time was only 16 days.

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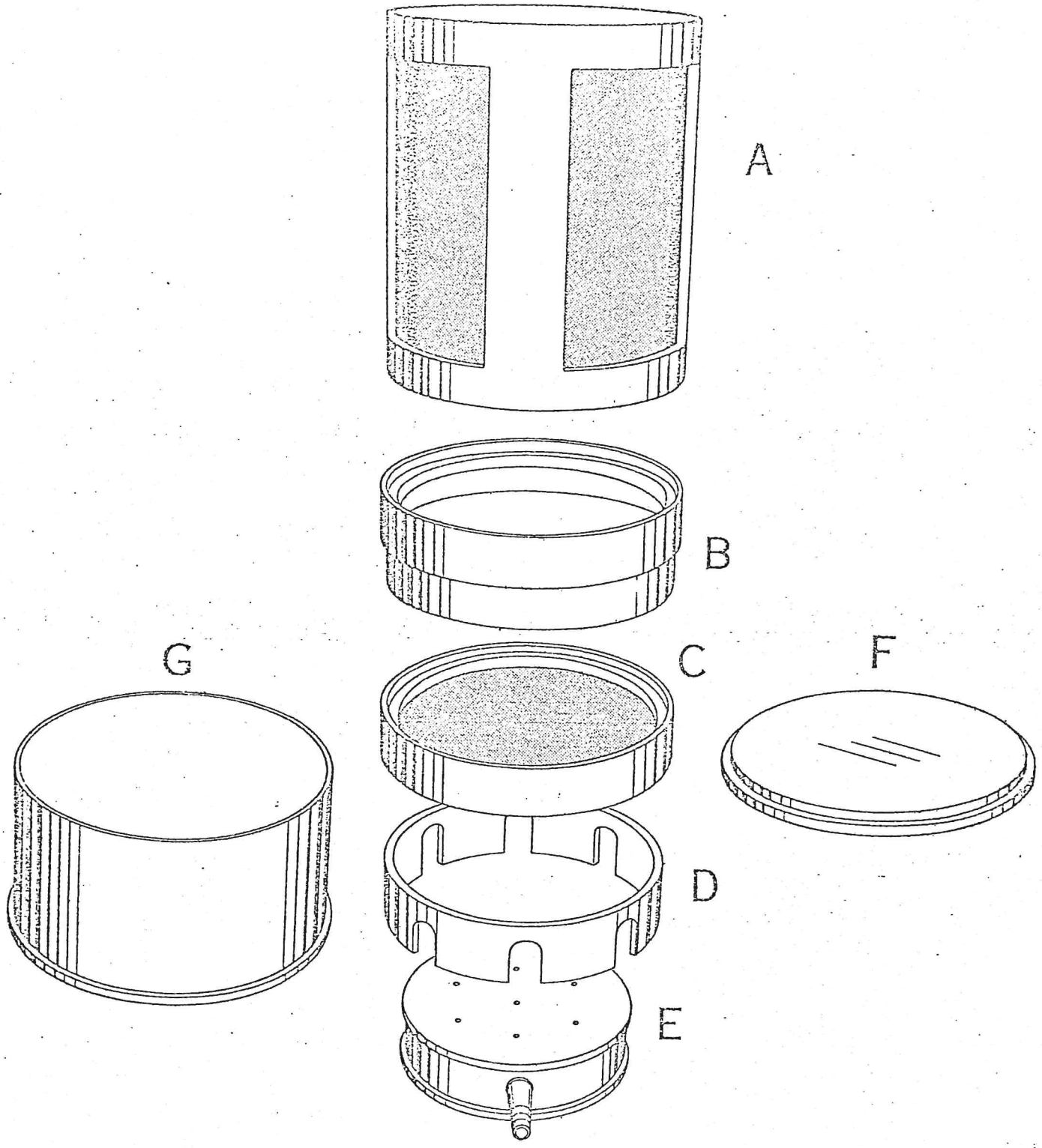
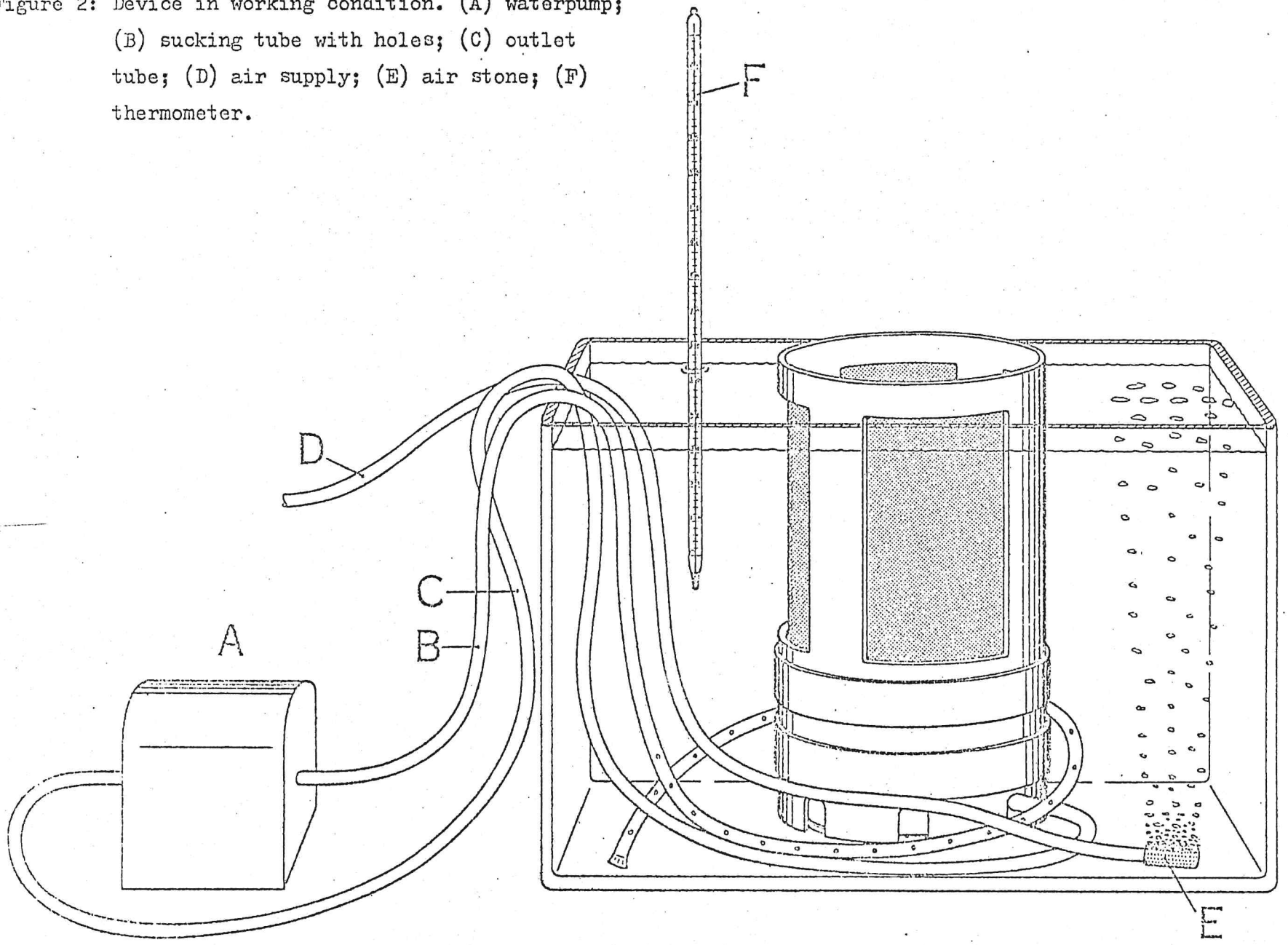


Figure 1: Parts of the main cylinder. (A) cylinder; (B) dish ring; (C) bottom sieve; and (D) base. Basal disk (E), closing plate (F), dish container (G).

Figure 2: Device in working condition. (A) waterpump; (B) sucking tube with holes; (C) outlet tube; (D) air supply; (E) air stone; (F) thermometer.



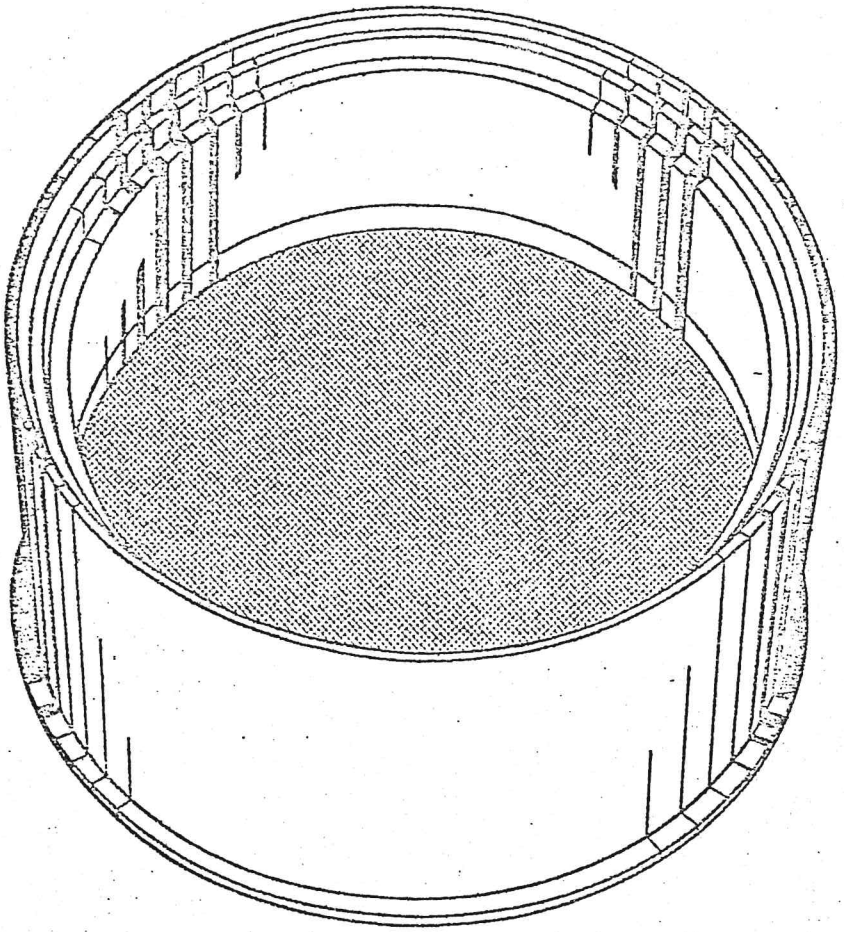


Figure 3: Examination parts (dish ring, bottom sieve, and closing plate) in the dish container.