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A GENERAL METHOD FOR THE MONOXENIC CULTIVATION OF THE DAPHNIDAE

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This laboratory has attempted to obtain culture conditions which should satisfy the members of the family Daphnidae that are commonly found in the lakes of the Northeastern United States. We have succeeded with all the commonly available species and have fourteen under monoxenic continuous cultivation, using *Chlamydomonas reinhardii* as the sole food organism.

The classical method for cultivating Cladocera is Banta's stable tea, a pond water extract of horse manure and garden soil (Needham, Gatz and Lutz, 1937). Other successful methods have been developed using mixtures in which either bacteria, protozoa, yeast, or algae are the principal food supply (Mortimer, 1936; Beerstecher, 1952; Murachi and Imai, 1954; Watanabe, Ito and Sasa, 1955; Frank, Bolland and Kelly, 1957; Sasa, Kunieda and Tamiya, 1960; Dewey and Parker, 1964). A monoxenic system was developed for *D. magna* by Treillard (1924) using rabbit erythrocytes and for *Moina macrocopa* by Stuart, McPherson and Cooper (1931) using sterile pond water with suspensions of living bacteria. The individual specimens were freed of microorganisms by repeated washings prior to inoculating the food organism. Fritch (1953) showed that *Chlamydomonas* sp. would support the growth of *D. pulex* if pantothenic acid was added to a system in which bacteria were present. Recently Taub and Dollar (1968) studied the inadequacies of *Chlamydomonas reinhardii* and *Chlorella pyrenoidosa* as food for *D. pulex*.

None of these systems has proved completely satisfactory because of uncontrollable variation in results. Banta (1939) reviewed the problem of "depression" or periods when cultures die out or show a reduced reproduction rate and pointed out that no method was known to prevent the phenomenon. Dewey and Parker (1964) describe the difficulties in obtaining natural water of constant composition free from insecticides and other toxic substances. Anthony D'Agostino and Luigi Provasoli, St. Johns University and Haskins Laboratories (personal communication) have succeeded in devising a dixenic system with *Chlamydomonas reinhardii* and *Scenedesmus obliquus* which will support *D. magna* in continuous culture. The medium contains several salts and vitamins B_{12}, pantothenic acid and thiamin. The method should prevent the aforementioned problems as the cultures can be isolated from variations in food organisms and changes in the composition of the medium.

Materials and Methods

Algae. *Chlamydomonas reinhardii* Indiana U. strain #90 and *Scenedesmus obliquus* Indiana U. strain #393 obtained from Dr. Luigi Provasoli were used throughout these experiments as the principal food supply of the *Daphnia.* They

TABLE I

*Composition of the medium (mg/liter)**

	Basic medium	Enriched medium	
		Early formula	Later (improved) formula
Calcium acetate · X H_2O	177.0	59.0	59.0
Potassium penicillin (U.S.P.)	645.0 (10^6 units)	215.0	215.0
Streptomycin sulfate (U.S.P.)	20.0	7.0	7.0
Bovine albumin, fraction V (Armour)	200.0	67.0	67.0
Calcium pantothenate	20.0	7.0	7.0
B_{12}	0.001	0.0003	0.0003
Thiamin	1.0†	0.6	0.6
Riboflavin		0.03	0.4
Nicotinamide		0.3	1.3
Folic acid		3.3	3.3
Biotin		0.3	0.3
Putrescine		0.3	0.3
Choline		0.3	5.0
Inositol		0.6	11.0
Hutner's Trace Elements‡		0.3 ml	0.3 ml

* The medium is made up at three times concentration and frozen in 250 ml amounts. When needed it may be thawed, diluted and filtered through a 2 micron porcelain filter cylinder. Concentrated stock solutions of all the vitamins except biotin, which precipitates, may be stored frozen.

† Only included for a brief time.

‡ Levine and Ebersold's (1958) modification of a trace element mixture designed by Hutner, Provasoli, Schatz and Haskins (1950). We heated the mixture to 100° C and brought it to pH 6 with KOH while hot. The solution is deep green but turns to deep purple on standing. The mixture is stable.

were grown on a yeast extract-acetate medium described by Levine and Ebersold (1958) enriched with one gram per liter of N-Z-Case peptone (Sheffield Chemical Co.) and with agar omitted. The cells were harvested sterilely by low speed centrifugation after 3 or 4 days culture, washed twice in deionized water and added to the *Daphnia* medium (Table I). A delay of 1–2 hours is allowed before the animals are added to allow time for a rise in pH to occur.

Daphnia. The species that are currently under monoxenic cultivation have been obtained from the following sources. *Daphnia magna* from the laboratory of Dr. Luigi Provasoli in bacteria-free continuous dixenic cultivation. *D. pulex* (four strains), 1. Connecticut Valley Biological Supply Co., Southampton, Massachusetts, 2. General Biological Supply House, Chicago, Illinois, 3. Pine Swamp Pond, Connecticut (Lat. 41°53′63N., Long. 73°24′04W.), 4. Candlewood Lake, Connecticut (Lat. 41°28′93N., Long. 73°27′63W.), *D. catawba,* Croton Reservoir, New York (Lat. 41°15′55N., Long. 73°50′54W.), *D. parvula,* Pocantico Lake, New York (Lat. 41°07′06N., Long. 73°50′02W.), *D. retrocurva,* Bantam Lake, Connecticut (Lat. 41°42′66N., Long. 73°13′63W.), *D. ambigua* (two strains), 1. Croton Reservoir, New York, 2. Lake Giles, Pennsylvania (Lat. 41°22′54N., Long. 75°05′90W.), *D. laevis,* Pine Swamp Pond, Connecticut, *D.*

dubia, Candlewood Lake, Connecticut, *D. galeata mendotae* (four strains), 1. Swan Lake, New York (Lat. 41°06′62N., Long. 73°49′94W.), (two strains), 2. Candlewood Lake, Connecticut, 3. Croton Reservoir, New York, *Simocephalus serrulatus,* Upper Hadlock Pond, Maine (Lat. 44°19′19N., Long. 68°17′28W.), *Scapholeberis mucronata,* Swan Lake, New York, *Ceriodaphnia reticulata,* Dark Entry Forest Pond, Connecticut (Lat. 41°47′70N., Long. 73°21′82W.), *C. quadrangula,* Swan Lake, New York, *Moina macrocopa americana,* ephippeal eggs obtained commercially from John and Ruth Fenneberg, P.O. Box 1043, Victorville, California, *M. macrocopa americana* variant, same as above.

Individuals to be isolated are rinsed once or twice and transferred to Falcon 60 × 15 mm "Tissue Culture" petri dishes containing 10 ml of medium with 2×10^6 cells/ml *Chlamydomonas.* Tentative identification is made at this time by stranding the animal on a clean microscope slide. Identifications have been made using Brooks' monograph (1957) for the genus *Daphnia* and Goulden's monograph (1968) for the Moinidae.

TABLE II

The effect of antibiotics and protein on egg development of D. pulex *and survival of young*

	Deionized distilled water	Water with* antibiotics	Water with* protein	Water with* antibiotics and protein
Egg tested	19	9	24	32
Number developing into young	18	4	24	26
Number stuck at interface	9	1	0	0
Survival of young for one day	0	0	16	24
Per cent survival for one day	0%	12%	67%	75%

* Concentration same as in Basic Medium (Table I).

Bacteria-free animals were obtained by the following modifications of the methods of Stuart, McPherson and Cooper (1931) and of Obreshkove and Fraser (1940). As soon as the progenitrix of the strain to be isolated shows well-developed, eyed embryos, it is transferred six or more times through large droplets of sterile medium (Table I) in the hydrophobic top of the plastic petri dish and left for one hour in a dish with 10 ml of medium. It is then rinsed six more times and the brood pouch of the carapace is opened with sterile needles. Care is taken not to express the gut contents during this procedure but doing so does not necessarily mean failure in obtaining bacteria-free young. The embryos in groups of 1–3 are rinsed twelve or more times in droplets of sterile medium on petri dish tops and placed in sterile medium. The important part of this final washing is to use a new sterile capillary pipette for each transfer step and to carry over with the animals as little medium as possible. This procedure usually yields bacteria-free young. If an original culture is especially heavily contaminated the number of rinses may be doubled. Eggs a few hours old may be used as they will develop and hatch but they can be easily damaged by the washing step.

Moina macrocopa must be handled differently. The brood sac is closed in this species and the eggs rupture on release. However, satisfactory results are obtained by washing the young immediately after they are released by the mother.

Bacteriologically sterile animals are transferred twice weekly into new petri dishes with sterile medium and algae and maintained in constant temperature boxes at either 15° C or 20° C constantly illuminated with two 15 watt cool white fluorescent bulbs. They may also be kept in screwtop Falcon 30 ml "Tissue Culture Flasks." Sterility was routinely checked using thioglycollate medium. Anaerobes were ruled out both by darkfield and phase microscopic examination and by the culture method of Schaedler, Dubos and Costello, (1965).

Results

Requirement for embryonic development

Bacteriologically sterile eggs from *D. pulex,* obtained from Connecticut Valley Biological Supply Co., completed embryonic development as well in deionized distilled water as in salt solution. When the young became motile they developed

Table III

The effect of various salts tested singly and in pairs on survival of D. pulex *for more than three days (four animals per test)*

	NaCl	KCl	$CaCl_2$	$MgSO_4$	PO_4 buffer
NaCl	0	0	4	0	0
KCl	0	0	4	0	0
$CaCl_2$	4	4	4	4	4
$MgSO_4$	0	0	4	3 (weak)	0
PO_4 buffer	0	0	4	0	0

The salts were dissolved in deionized distilled water with bovine albumin, penicillin and streptomycin as in Basic Medium (Table I) with *Chlamydomonas* and *Scenedesmus* and NaCl 0.004 *M*, $CaCl_2$ 0.001 *M*, $MgSO_4$ 0.0005 *M*, KCl 0.0001 *M*, Na phosphate buffer (pH 6.9) 0.002 *M*.

a tendency to stick to the air-water interface. This difficulty was solved by the addition of bovine albumin, fraction V. Table II shows that 0.2 grams per liter accomplished the purpose and that penicillin and streptomycin may be added to the medium to reduce the probability of bacterial contamination.

It had previously been observed that penicillin at 1000 units/ml and that streptomycin at 20 mg/liter were not toxic.

Requirement for juvenile development

The above medium did not support the young even though they were fed a mixture of washed *Chlamydomonas reinhardii* and *Scenedesmus obliquus* whereas a salt mixture allowed continued development. Table III shows that calcium was the necessary factor, for without it, the *D. pulex* died in the next ecdysis. While several calcium salts were tried, calcium acetate was chosen because it is neutral and improves the appearance of the *Chlamydomonas.* Dilutions lower than 10^{-4} molar did not support young of *D. pulex* as can be seen from Table IV. Other salts are brought into the medium with the antibiotics and the algae protoplasm.

TABLE IV

Development from eggs and survival for one week of young of D. pulex *in various concentrations of calcium acetate*

	None added	Concentration of calcium acetate			
		10^{-6} *M*	10^{-5} *M*	10^{-4} *M*	10^{-3} *M*
Number tested	11	13	14	13	14
Number developing	3	10	7	10	10
Number surviving one week	0	0	0	1	8

Medium consists of bovine albumin, penicillin, and streptomycin as in Basic Medium (Table I) with *Chlamydomonas* and *Scenedesmus* added.

Requirement for fertility

Animals raised to maturity from eggs in the medium as developed to this point, if maintained bacteriologically sterile, survived but produced infertile eggs. These "unproductive" animals constituted the test subjects for the following series of experiments. Table V shows that vitamin B_{12} increased their fertility. However,

TABLE V

Number of young produced with and without vitamin B_{12}

	Number females tested	Viable young before B_{12} 1st clutch	Viable young after B_{12} 2nd clutch
Control without B_{12}	20	0	10
With B_{12}	19	0	53

Medium consists of calcium acetate, bovine albumin, penicillin, streptomycin as in Basic Medium (Table I) with *Chlamydomonas* and *Scenedesmus*. B_{12} was added immediately after the first clutch.

the effect was temporary and the animals became "unproductive" again. In another experiment shown in Table VI, calcium pantothenate (Fritsch, 1953) was used and

TABLE VI

Number of viable young and undeveloped eggs produced with and without calcium pantothenate (20 mg/liter)

Medium*	Number females tested	Clutch before pantothenate		Clutch after pantothenate	
		Viable young	Undeveloped eggs	Viable young	Undeveloped eggs
Control without Ca pantothenate	11	2	69	5	40+
With Ca pantothenate	6	1	42	36	0

* Medium consists of calcium acetate, bovine albumin, penicillin, streptomycin, B_{12}, as in Basic Medium (Table I), with *Chlamydomonas* and *Scenedesmus*. Calcium pantothenate was added immediately after the third clutch.

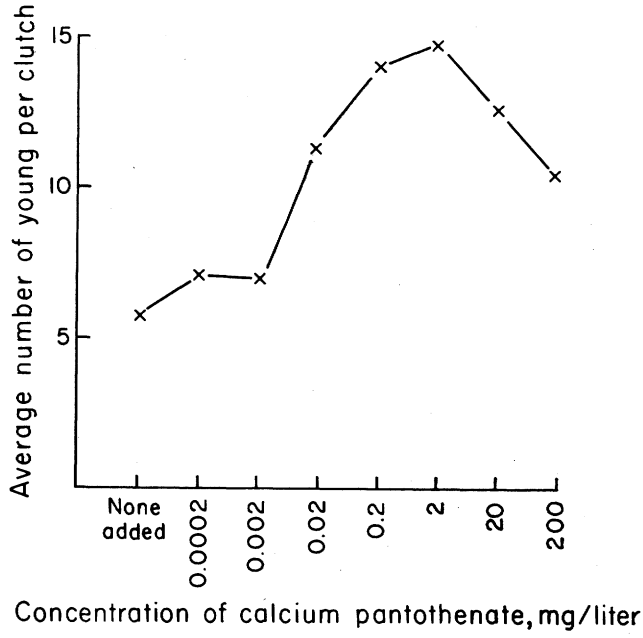

FIGURE 1. Average number of young per clutch in various concentrations of calcium pantothenate (summary of over 400 clutches).

caused a very striking increase in the number of viable young. Raising the amount of vitamin B_{12} 10 or 100 times did not alter the results but leaving it out of the medium produced a sharp reduction in lifespan and in the number of young produced. Calcium pantothenate on the other hand had a definite optimal concentration for production of young (see Fig. 1), but its presence or absence had no effect on the lifespan which averaged 41 days from the day embryological development began. The animals averaged twelve clutches of eggs apiece regardless of the presence or absence of added pantothenate.

Algae requirement

A final experiment in this series was done to find out if it was necessary to add both algae to these cultures. Washed eggs of *D. pulex* were started in basic

TABLE VII

Effect of Chlamydomonas *and* Scenedesmus *on* D. pulex *in basic medium*

	No. females	Average number of young		
		Clutch 1	Clutch 2	Clutch 3
Chlamydomonas alone	15	6.4	9.9	14
Scenedesmus alone	15	0	<1	1.9
Both algae	15	8.4	11.1	18.5

medium (Table I) and fed *Chlamydomonas* alone, *Scenedesmus* alone, or a combination of the two. Table VII shows that cultures with both algae were superior to either alone.

Results with other species

The above basic medium (Table I) permitted us to establish four species of *Daphnia* in bacteria-free continuous cultivation with two algae and another species with one algae. By the time this medium was improved (Table I), we had carried *D. pulex* (Connecticut Valley) 84 generations, *D. pulex* (General Biological) 10 generations, *Scapholeberis mucronata* 63 generations, *Simocephalus serrulatus* 40 generations, *D. magna* 17 generations, and with *Chlamydomonas* alone, *Moina macrocopa* 135 generations. On several occasions these cultures

TABLE VIII

Average number of young on the first brood in three media

	D. pulex Connecticut Valley	*D. pulex* General Biological	*Moina macrocopa**	*Scapholeberis mucronata*	*D. magna*	*Simocephalus serrulatus*	*D. galeata mendotae*
(1.) Basic medium with two algae	4.0 (21)	4.4 (38)	13.3 (80)	2.9 (40)	5.8 (17)	3.2 (20)	0
(2.) Enriched medium with two algae early formula	4.6 (33)	5.6 (52)	15.9 (116)	2.7 (84)	8.8 (18)	4.0 (52)	2.7 (25)
(3.) Enriched medium with *Chlamydomonas* alone, later formula	5.2 (69)	6.0 (76)	17.7 (175)	2.6 (46)	10.1 (51)	3.7 (24)	2.2 (16)
(4.) Repeat of number 3 above	5.8 (28)	6.4 (44)	19.6 (112)	2.8 (41)	10.0 (20)	4.4 (30)	3.1 (38)

* *Moina macrocopa* was always cultured with *Chlamydomonas* alone.

Rows 1, 2 and 3 were run simultaneously. The numbers in parenthesis are the total broods considered in the adjacent average.

became contaminated and were immediately reestablished in bacteria-free culture by isolating and washing embryos or newborn young. When yeast or fungus occurred as contaminants, daily transfer for a few days was usually all that was necessary to leave it behind.

Many other species of *Daphnia* were tried in this medium both with and without bacteria with little success. A clone of *D. galeata mendotae* (Swan Lake) became established in a $\frac{1}{3}$ dilution but only when bacteria and a protozoan (*Anisonema*) were present. Passages had to include sufficient old medium to quickly re-establish the contaminants. This indicated that it was likely that the medium was deficient rather than toxic. However, no single factor could be found which would improve the medium enough to support the species *D. galeata mendotae* so we resorted to "shot gun" methods.

Enriched medium, early formula

Immediate success in cultivating *D. galeata mendotae* on algae alone followed adding to the medium a mixture of eight vitamins and Hutner's Trace Elements

(Table I). Table VIII, under the heading "Enriched medium, early formula," demonstrates the effect this medium had on six species of *Daphnia*. It can be seen that *Scapholeberis* may not have done as well but all the others improved as measured by the number of young in their first clutch. Other parameters also showed improvement. They matured slightly sooner, and *D. magna* particularly increased markedly in size and vigor. When tried on other species of *Daphnia* the medium was unsatisfactory. It would support *D. ambigua* and *D. retrocurva* but only when the medium was contaminated with bacteria. It did not support *Ceriodaphnia reticulata, C. quadrangula, D. parvula, D. catawba,* and *D. dubia* even when bacteria were present.

Enriched medium, later formula

At this point a large number of media modifications were tested on the production of young by *D. retrocurva, D. galeata mendotae* and *Simocephalus serrulatus* under unsterile conditions using *Chlamydomonas* without *Scenedesmus*. These preliminary experiments indicated that the medium might be improved by increasing the amounts of choline, pyridoxal, inositol, nicotinamide and riboflavin and leaving out *Scenedesmus*. The formulation (Table I) was tested on our seven strains already in bacteria-free serial passage with the type of results shown under "Enriched medium, with *Chlamydomonas* alone, later formula" (Table VIII). The final row shows the results six months later when we had less difficulty with insecticide. It can be seen that each medium modification causes a definite improvement in most strains.

When this final formulation was tried on a wide variety of species of *Daphnia* it was found to support continuous monoxenic cultivation of eight more. No species of the Daphnidae yet tested has failed to reproduce and become established in the presence or absence of bacteria. All the species develop from isolated eggs and are readily obtained bacteria-free. Fourteen species are now under continuous monoxenic cultivation and doing well. They are listed below in order of increasing difficulty found in establishing and maintaining them in the laboratory. The last two have not been tested on anything but the later formula medium with *Chlamydomonas* alone.

Current status of strains under cultivation

Moina macrocopa (two strains) is by far the easiest species to cultivate. It will withstand 0.05 molar salt concentrations, heavy bacterial contamination, and is more resistant to insecticides. It has been maintained bacteria-free for over 200 generations.

Scapholeberis mucronata is also very resistant to insecticides and extremely hardy. The species is small, and while the adults are easy to see because they are deeply pigmented, the newborn young are nearly invisible to the naked eye. It has been maintained for over 200 generations.

D. pulex (four strains) is easy to culture in the laboratory and one strain has been maintained bacteria-free for over 100 generations.

D. magna, although widely used in research, is not by any means as easy to culture as the above. It is also hard to obtain. We have never found it in nature,

and although the biological supply houses advertise it, they actually supply *D. pulex*. It is sensitive to insecticide. We have maintained it through 35 and 30 generations. The species was lost once due to insecticide and replenished from a sealed manure-water and algae culture that had supported a small population for one year.

Simocephalus serrulatus is very common in local ponds but compares with *D. magna* in difficulty of maintenance. As soon as the species is obtained bacteria-free it develops the remarkable open-carapace deformity described by Agar (1913). We have not succeeded in preventing this abnormality, but it does not seem to affect their survival and reproduction. It has been maintained for over 80 generations.

D. galeata mendotae is common and easy to maintain in complex medium. Under our conditions either helmeted or unhelmeted animals isolated from nature develop moderately helmeted progeny. It has been maintained for over 30 generations and several strains are under cultivation.

TABLE IX

Comparison of number of young and lifespan of Moina macrocopa *in two media*

	No. animals	No. young		Lifespan	
		Average	Range	Average (days)	Range
Basic medium	37	48.7	7–97	10.6	7–15
	63	34.3	4–88	10.4	3–14
Enriched medium later formula	111	88.4	17–181	11.8	3–20

D. retrocurva is fairly easy to obtain but more difficult to maintain. All the newborn young of some clones immediately become stuck on the air-water interface and must be sunk daily. They remain moderately helmeted and are most sensitive to insecticide. We have maintained it for 18 generations bacteria free.

D. ambigua is a small species, common and easy to cultivate in the more complex medium. It is sensitive to insecticides. It has been maintained for over 40 generations and repeatedly isolated.

D. parvula is similar to *D. ambigua* but requires the later formula medium. It also has been maintained for over 40 generations.

D. dubia is not very common in our experience and has the same characteristics as *D. retrocurva*. We currently have a strain that has gone well for 15 generations and does not get stuck at the interface.

D. catawba is fairly common. We have maintained it for 13 generations but it did not adapt easily to our culture conditions. It is very sensitive to insecticides.

Ceriodaphnia quadrangula compares with *D. ambigua* and *parvula*. It is harder to remove eggs from *Ceriodaphnia* females because of their almost spherical body shape. *Ceriodaphnia* from Swan Lake have large single-spiked fornices in nature and resemble *C. lacustris*, but these spikes are lost on cultivation. The spikes are similar to those described by Rzoska (1956) and Zaret (1969). After 8 generations this species was lost due to insecticide and a new isolate has gone 19 more.

D. laevis was only obtained by us recently, having hatched from ephippeal eggs and has gone through 13 generations. It is a large vigorous animal and probably is among the easier to maintain. Brooks (1957) has identified this as "Banta's *D. longispina*" (page 118) and it is therefore a well known experimental species.

C. reticulata was obtained only recently but has been easy to maintain for 14 generations. As with *C. quadrangula,* the eggs are hard to remove from the female. Older females are preferable because they are much larger than primigravida and have many more eggs.

Effect of medium enrichment on Moina

The effect of the change in medium on the lifespan and total number of young produced by *Moina macrocopa* is shown in Table IX. The later formula medium produces a marked improvement in both parameters particularly if the extremes are considered. Of the 111 animals in the experiment in enriched medium, twenty five or 22% had lifespans of over 15 days. The three experiments reported were done at different times but smaller numbers tested simultaneously show the same general result. We have seen no tendency for the clone of Moina to improve with time alone when the medium is unchanged.

Discussion

The general approach used in this study has been to alternate between modifying the medium to improve the growth of one species and testing the modification on as large a number of species as were currently available. This two-pronged method has the advantage of improving the medium for the species under study and finding new species of *Daphnia* that will grow in it. Working on numerous types has also allowed us to find better species for a given experiment. The best animal to use for testing a modification seems to be one that will survive in unsterile culture, but which, when monoxenic, will not produce more than an occasional fertile egg. If the correct modification is made, this animal will produce viable young almost immediately. In contrast, a species that dies out yields little information and presents problems of obtaining enough animals, while one that produces a few young may, in a better medium, merely produce slightly more young, a less critical endpoint. The assumption underlying this approach is that what is good for one animal is good for another. So far, this has been true in all cases except a clone of *Scapholeberis* which became slightly less productive in the more complex medium. However, it was not necessary to make up a special medium to support this species.

The problem of laboratory contamination with insecticide is most troublesome. If a species is brought into the laboratory and will not survive, the question always arises whether the culture medium is deficient or whether the medium is toxic. Any species that is more sensitive to insecticide than usual will simply seem harder to maintain if the environment is slightly contaminated. The presence of chronic or low level insecticide poisoning is almost impossible to evaluate until sensitivity studies have been made with all the species under cultivation. It is safe to say that all the species reported here have been repeatedly subjected to

insecticide and *Moina* and *Scapholeberis* in particular have repeatedly survived levels that have killed off most of the others.

The increase in lifespan and production of young of *Moina* in the enriched medium is remarkable since the strain already was highly productive relative to other species. Even further improvement seems possible since the spread of values for individual animals is very large and the medium has never been modified by direct experimentation with *Moina*. That a relative deficiency in vitamins should have an effect on the production of eggs and young is not surprising but it is not easy to understand why lifespan should be so markedly affected. Vitamin deficiency in higher animals is known to produce specific disease syndromes and may produce death, but an effect on lifespan has not been recognized. There is suggestive evidence that aged humans are "less resistant than the young to the ill effects of restriction of B complex vitamins" (Horwitt, Liebert, Kreisler and Wittman, 1948, page 106) but that is all.

On the other hand, variation in lifespan with diet is a common occurrence in experiments with various species of the Daphnidae. It may be that the biosynthetic mechanism leading toward egg production is capable of drawing so heavily on the reserves of the animal that upon completion of a clutch of eggs, the animal is thrown into pathological deficiency. It is possible that the nutritional state of the parent will also influence the longevity of the young.

The powerful effect vitamins have on the rate of reproduction of Cladocera and other crustacea (Provasoli and Shiraishi, 1959; Shiraishi and Provasoli, 1959; Provasoli and D'Agostino, 1969) may be important in the understanding and control of lake ecology. The evidence of the varying requirements of different species of *Daphnia* may help explain their distribution in nature.

I wish to thank Dr. Luigi Provasoli for his most valuable advice and for providing some of the organisms used in this study. I am much indebted to Mrs. Nancy Michael, Mrs. Margot Butler, and Miss Marjorie Offinger for technical assistance and to Mrs. Victoria Murphy, Miss Wendy Murphy and Miss Carol Murphy for help in the field.

Summary

Fourteen species of the family Daphnidae have been established under continuous monoxenic cultivation utilizing *Chlamydomonas reinhardii* as sole food organism in a medium consisting of calcium acetate, antibiotics, albumin, trace elements and the water soluble vitamins, folic acid, B_{12}, calcium pantothenate, choline, pyridoxal, inositol, thiamin, nicotinamide, riboflavin, biotin and putrescine. The Daphnidae under cultivation include *Daphnia magna, D. pulex, D. galeata mendotae, D. laevis, D. dubia, D. retrocurva, D. parvula, D. ambigua, D. catawba, Moina macrocopa, Scapholeberis mucronata, Simocephalus serrulatus, Ceriodaphnia reticulata,* and *C. quadrangula.* The requirements for vitamins for some species are more complex than for others. The complete medium is superior for all but *Scapholeberis mucronata* and markedly increases the lifespan and fertility of *Moina macrocopa.*

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