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WAYNE STATE UNIVERSITY

Department of Biology

FINAL REPORT

ON

DRIFT STATION BIOLOGY:

ZOOPLANKTON TAXONOMY

AND

BIOCHEMICAL AND PHYSIOLOGICAL ADAPTATIONS OF ARCTIC MARINE ANIMALS

NOVEMBER, 1972-SEPTEMBER, 1977

Prepared under contract with the Office of Naval Research N00014-77-C-0013 NR 307-270/9-2-76 461

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Principal Investigator: Dr. H.R.C. Fernandez Associate Professor Department of Biology Wayne State University Detroit, Michigan 48202



INTRODUCTION

The Arctic is one of the last frontiers left to mankind which remain relatively unknown. It is a region of great importance for a number of reasons. First of all because it is an important factor which the determines the weather pattern on a global scale. It is a potential source of natural resources in the form of minerals and possibly food. Thirdly is geographic location makes it an important geopolitical factor. For these reasons it behooves man to gain as much information as possible about this particualr environment.

Prior to the present contract, this investigator was involved in another ONR supported project whose principal objective was to establish the taxonomic identity of arctic marine animals that are found in the water column. The taxonomic and general biological information garnered in that effort formed the basis for the present work. The main objectives of this work were as follows:

- 1- Description of the time and depth distribution of some of the most important zooplankton species. A particular effort was made to also determine the nature of those factors which determine the distribution of a particular species in the water column.
- 2- Ellucidation of the freezing resistance mechanism in an invertebrate species.
- 3- Description of the anatomical, biochemical and physiological adaptations of the visual receptors of selected animal species. Particular emphasis was given to vertical migrators and to a teleost species which reputedly is a component of arctic scattering laeyrs.
- 4- Adaptation of the energy storage mechanisms, particularly the storage of lipids.

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1-Time and Depth Distribution Studies.

During the contract period the following studies were carried out. Each study is preceded by a short description after which resulting manuscripts are inserted into the text of the report.

The Vertical Distribution and Rerpoductive Biology of <u>Pelogobia</u> <u>longicirrata</u> (Annelida) in the Central Arctic Ocean. by Douglas Yingst.

In this investigation Dr. Yingst made the first time and depth distribution analysis of a cosmopolitan worm not only in the Arctic Ocean but of any other water mass in the world. The work describes the distribution of the species in terms of time and depth over a two year period and gives information on the life cycle and pattern of reproduction.

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THE VERTICAL DISTRIBUTION AND REPRODUCTIVE BIOLOGY OF PELOGOBIA LONGICIRRATA (ANNELIDA) IN THE CENTRAL ARCTIC OCEAN

DOUGLAS YINGST

Reprinted from BIOLOGICAL BULLETIN, Vol. 147, No. 2, October, 1974 Printed in U. S. A.

Reference: Biol. Bull., 147: 457-465. (October, 1974)

THE VERTICAL DISTRIBUTION AND REPRODUCTIVE BIOLOGY OF PELOGOBIA LONGICIRRATA (ANNELIDA) IN THE CENTRAL ARCTIC OCEAN

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Although holopelagic polychaetes are widely distributed throughout the oceans, very little quantitative information exists on their vertical distribution and even less is known of their reproductive biology and life histories. Most of our knowledge on these subjects comes from the many expeditions and surveys of the last one hundred years which, although of great importance in many respects, are of limited value for analytical evaluations and comparisons. This is due to differences in the type of collecting gear and sampling methods employed and in the depths sampled, and the fact that most cruises covered too wide a geographic range to intensively study any one area over an extended period of time. As a result the information which can be obtained on any particular species from a review of such data is necessarily incomplete and sometimes even contradictory. More recent quantitative approaches to plankton analysis have not increased our knowledge either, since they have generally not included pelagic polychaetes. Of the few studies on holopelagic polychaetes in which closing nets have extensively been used, Mileikovsky (1969 and 1962), Tebble (1960), Friedrich (1950), and Hardy and Gunther (1935) deserve special mention. Of the polychaetes studied by them, one of the most abundant was Pelogobia longicirrata Greeff 1879, a cosmopolitan species which reaches a maximum length of approximately 12mm in 26 to 30 segments and has been collected from below 4000m to the surface and throughout the world: Støp-Bowitz, 1948 (north Atlantic); Friedrich, 1950 (Atlantic); Tebble, 1960 (south Atlantic); Hardy and Gunther, 1935 (South Georgia); Augener, 1929 (Weddell sea); Berkeley and Berkeley, 1964 (Peru); Dales, 1957 (central Pacific); Tebble, 1962 (north Pacific); Mileikovsky, 1969 (northwest Pacific); and Okuda, 1937 (Japan). For a more complete listing of the literature concerning this species the reader should see Dales (1972).

P. longicirrata is also present in the central Arctic ocean (Uschakov, 1957; Knox, 1959; and Yingst, 1972) and was the most abundant holopelagic polychaete collected in a two year program of systematic plankton sampling conducted from Fletcher's Ice Island T-3. This paper presents data from that two year study on the reproductive biology and vertical distribution of the larvae, juveniles, and adults of *P. longicirrata* and proposes a life history for this species. Fletcher's Ice Island T-3 served as a stable working platform which permitted long term quantitative sampling with closing nets in a restricted oceanic region and thereby allowed researchers to amass the type of data so difficult to obtain by more conventional means in the open sea. Likewise, the direct development of the larvae of *P. longicirrata* into a form clearly recognizable and comparable with the adults (Nolte, 1938) has facilitated this study. Since *P. longicirrata* is an abundant cosmopolitan species, it is hoped that this Arctic study will also be of general

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interest to those concerned with the distribution and natural history of holopelagic polychaetes in other seas.

MATERIALS AND METHODS

A one meter closing net with a number 6 mesh (215 μ opening) was used to sample discrete vertical intervals from 0 to 1500 m in the central Arctic basin. All sampling was done by oceanographic teams from the University of Southern California and the University of Washington from Fletcher's Ice Island T-3, while the island drifted from 84° 24'N, 112° 30'W (March, 1970) to 84° 20'N, 86° 17'W (December, 1971). Complete series of tows from 0 to 1500 m were taken an average of 2 or 3 times a month from March, 1970 to November, 1971 with the exception of September, October and November, 1970. During 1970 the upper 300 m (0-300 m) was sampled in 50 m increments; successive tows of 200 m were taken from 300 m to 900 m followed by 300 m tows from 900 m to 1500 m. In 1971 the sampling program from 0 to 500 m was modified, such that 25 m tows were taken from 0 to 400 m, and 100 m tows from 400 m to 500 m. For purposes of this report, data in the upper 500 m from both years was pooled to give three intervals: 0-100 m, 100-300 m, 300-500 m. Thus, a complete series of tows from 0 to 1500 m includes the following: 0-100 m, 100-300 m, 300-500 m, 500-700 m, 700-900 m, 900-1200 m, 1200-1500 m. All data presented here are from complete series of tows with the exception of July/August, 1970 when there were no tows from 1200-1500 m and December, 1970 when the results from one complete series from 0-1500 m were extrapolated to a partial second series extending from 0 to 700 m. In this case, the same number of specimens found in each size class and in each depth interval from 700 m to 1500 m in the complete series was added to the data from the partial series from 0 to 700 m. The net result is the adjusted equivalent of two complete series for December, 1970.

Each tow was fixed and preserved in 10% formalin in seawater. Later, the USC Arctic project personnel sorted the animals to phylum and gave the annelids to the author who identified them to species. The total number of complete specimens and fragments bearing a prostomium of *P. longicirrata* were then counted and separately recorded for each tow. Likewise, the number of true segments (the entire worm excluding the prostomium and the pygidium) was determined for each complete specimen in a given tow. On the basis of its number of true segments, each animal was placed in one of 8 size classes. Each class contained specimens in a three segment range, so that the first group included specimens with 1 to 3 segments, the second those with 4 to 6 segments, and the eighth those with 22 to 24 segments.

In order to compare the vertical distribution of the size classes throughout the year, the above data for each vertical tow were grouped into the depth intervals previously noted and pooled for a two month period. Due to the differences in the lengths of the depth intervals comprising the series of tows from 0–1500 m, the volume of water filtered by the net varied considerably from interval to interval. To remove this volume bias in comparing the data in the different intervals, the number of animals in each size class in the 100 m intervals was multiplied by 3 and those in the 200 m intervals by 1.5, so that the volume of water equalled the amount filtered in the largest intervals of 300 m. After this correction, the num-

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ber of animals in each size class at each depth was determined as a percentage of the total number of complete specimens in a given two month period.

The above manipulation of the volume filtered was possible because the filtering efficiency of the net was not found to vary considerably between tows. The filtering efficiency was calculated by dividing the amount of water actually filtered by the net (as measured by a flow meter) by the maximum theoretical filtering capacity of the net. The ratio averaged approximately 0.5.

The average density of animals from 0-1500 m was calculated by dividing the total number of complete and incomplete specimens in a two month period by the total volume of water filtered by the net, as calculated from the filtering efficiency and the length of the tow.

Oocytes were counted through the body wall under a compound microscope and measured with an ocular micrometer. If an animal had a minimum of 4 oocytes in each of at least 3 segments, it was recorded as being ovigerous. The total number of ovigerous animals taken in a given two month period was then figured as a percentage of all the specimens with more than 12 segments in that time period. Larvae are defined here for convenience as specimens with 6 or less segments, juveniles those with 7 to 12 segments, and adults those with 13 or more segments.

RESULTS

Vertical distribution and relative age

The vertical distribution of *P. longicirrata* changed seasonally as a function of the relative age of the specimens (Fig. 1). Animals with 13 or more segments were primarily found above 500 m where at least 60% of the total population was collected throughout the year in any given two month period. During the summer (and early fall) the percentage of animals under 500 m increased, reaching a maximum between July and October (Fig. 1). In contrast to those above, most of the individuals below 500 m had less than 13 segments. In fact, those with the fewest number of segments were found the deepest. For example, specimens with 1 to 3 segments were primarily found below 900 m and made up most of the population from 900 m to 1200 m (Fig. 1).

Density

The average density of animals from 0–1500 m fluctuated somewhat during the two year sampling period, but did so without demonstrating any apparent seasonal trends (Fig. 1). The mean density for 1970 and 1971 was 46/1000 m³ and 37/1000 m³, respectively.

The following is a list of the number of complete series of tows from 0-1500 m, the numbers of whole specimens (ws), and fragments bearing a prostomium (f) collected in a given two month period: March/April, 1970 (ws = 158, f = 30, 13 series from 0-1500 m); May/June, 1970 (ws = 71, f = 21, 5 series from 0-1500m); July/August, 1970 (ws = 73, f = 53, 4 series from 0-1200 m); December, 1970, (ws = 54, f = 8, 1 series from 0-1500 m, and 1 series from 0-700 m); March/April, 1971 (ws = 90, f = 14, 3 series from 0-1500 m); May/June, 1971 (ws = 106, f = 28, 4 series from 0-1500 m); July/August, 1971 (ws = 38, f = 52,



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5 series from 0-1500 m); September/October, 1971 (ws = 47, f = 6, 4 series from 0-1500 m); and November, 1971 (ws = 17, f = 3, 2 series from 0-1500 m).

Occurrence of oocytes

With the hope of defining the breeding season and the development of sexual maturity in *P. longicirrata*, the occurrence of oocytes throughout the sample population was investigated. Oocytes as viewed through the body wall with a compound microscope were opaque, either spherical or ovoid, and contained a large amount of yolk. In general they were similar to the oocytes of this species shown by Reibisch (1895).

Oocyte diameters ranged from 8 μ to 120 μ , but most were between 20 μ and 40 μ . The ratio of the yolk diameter to the total oocyte diameter decreased from 0.7 in oocytes with a diameter of 20 μ to 40 μ to about 0.4 in the 60 μ to 120 μ range. These data, however, do not suggest at what size the oocytes are sexually mature or even what diameter constitutes a maximum size for this species. Occasionally, the coeloms of the middle segments of specimens were densely packed with 30 to 40 oocytes per segment. In most specimens, however, the oocyte density was 6 to 10 per segment in each of 5 to 10 of the middle segments. Of the 104 specimens which contain a minimum of 4 oocytes in each of at least 3 segments, 82% had 15 to 20 segments. No oocytes were visible in specimens with less than 13 segments. Thus, on this basis, the breeding population is probably comprised of animals wth 15 or more segments.

As to seasonal variations in numbers, the peak occurrence of oocytes in both years was in the two month period of May/June when 30% of those with more than 12 segments bore oocytes (Fig. 2). The rapid decline in percentages from May/June to July/August suggests that spawning took place in early summer.

Developmental features concerned with food procurement

Specimens with more than 5 segments were found to have a mouth, anus, a continuous digestive tract, and a muscled pharynx which was often everted. Although these eversions were probably evoked during fixation of the specimens, they do indicate a capacity for eversion, a necessary prerequisite for feeding. On the other hand, the digestive tracts of animals with two or three segments were incomplete and appeared to be restricted to the anterior half of the body. Well preserved specimens of this size, very similar to the figure shown by Mileikovsky (1970), had a dense opaque area in the anterior half. Such an area could indicate the presence of yolk, which may play an important role in the survival of the larvae below 900 m. As an adult, *P. longicirrata* does not develop jaws on its proboscis which implies that it is not carnivorous.

FIGURE 1. The corrected annual vertical distribution of P. longicirrata. The specimens were placed into 8 size classes based on the number of segments. Each size class is coded by a different type of shading. The horizontal length of the bars is proportional to the relative number of specimens from each depth interval and in each size class in a two month period. The solid black line near the top of the figure is equivalent to a 10% contribution. The numbers on the lower left side of each two month distribution represent the average density from 0 to 1500 m for the two month period.

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FIGURE 2. The percentage of specimens with 13 or more segments in a given two month period possessing a minimum of 4 oocytes in each of at least 3 segments. There are no data for the period of September-October, 1970.

DISCUSSION

Although it appears that P. longicirrata spawns during the late spring and early summer, it is doubtful that spawning is restricted to this period, as evidenced by the presence of some specimens with oocytes as early as March/April and as late as October (Fig. 2) and the occurrence of at least a few larvae for as much as 6 months of the year (Fig. 1). Since the breeding population is chiefly composed of animals living from 100 m to 500 m and very few ovigerous specimens were found below 500 m, spawning probably occurs above 500 m. At present nothing is known about fertilization or the subsequent fate of the spawners. It is possible, however, that females could either spawn more than once or shed the eggs or embryos over an extended period of time. According to Goodrich (1945) the Phyllodocidae, which at that time included the genus Pelogobia, possess a protonephromixium which generally provides a duct through which genital products can be shed. If P. longicirrata has such nephromixia, it is unlikely that the body wall would have to be ruptured and the female sacrificed to release the ova or embryos. However they are released, these ova or embryos evidently descend from the surface because most larvae are first found between 900 m and 1200 m. It is doubtful that the larvae feed at these depths; instead, they probably live on stored yolk retained from the egg. As they mature and slowly develop the morphology necessary for feeding, the juveniles move up in the water column. This upward movement occurs during and just after the yearly phytoplankton bloom in July and early August (English, 1961).

Although it was not appreciated at the time, the occurrence of the larvae below the adults may have been indirectly confirmed for P. longicirrata by two former studies. In the first, no larvae or juveniles were found during the summer in the upper 500 m of the Norwegian and Barents sea, although adults were numerous in this range (Mileikovsky, 1962). To explain this absence of larvae, Mileikovsky proposed that the adults reproduce at greater depths, implying that the adults and larvae should be found together at depths below 500 m. To test this hypothesis,

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two series of vertical tows were taken during August of one year in the Kurile-Kamchatka trench (Mileikovsky, 1969). Here adults were most numerous from 200 m to 1000 m and larvae from 300 m to 2000 m (Mileikovsky, 1969). These data were then interpreted as substantiating the above hypothesis that adults and larvae are found in the same depth range below 500 m (Mileikovsky, 1969). However, in light of the information now available from the Arctic ocean, the sampling program in the Kurile-Kamchatka trench may not have been sufficient to unequivocally delimit the vertical distribution of the larvae and adults. For instance, only two sets of tows in one month of one year were taken. Secondly, the lengths of the tows in the first set of samples covered too great a vertical distance. Thirdly, the number of animals listed for each depth interval in both series is uncorrected for differences in the lengths of the tows. Fourthly, it is not clear that the same number of tows was taken at each depth. Thus, the data from the Kurile-Kamchatka trench may not be inconsistent with the pattern present in the Arctic ocean, in which the larvae are found deeper than the adults.

On the other hand, it is possible that the vertical distribution of the larvae and juveniles relative to the adults may in part vary with the oceanic region in question in accordance with factors not yet appreciated, for there are some observations in the literature which are difficult to reconcile with the view put forth in this paper. For instance, young approximately 2 mm long were found in the same sample with mature adults in a 11 m tow taken near Hut Point, Antarctica (Ehlers, 1912). Friedrich (1950) also notes that samples with many individuals collected on deep tows in the Atlantic were either of just young animals or a mixture of young and mature, whereas no samples contained only adults. From these data Friedrich concluded that a small number of adults produce many offspring and that due to the negligible amount of turbulence they tend to remain together in the deep sea, and only slowly and passively disperse. Thus, although Friedrich confirms the presence of young in deep water, he apparently would disagree with the idea that the adults spawn in shallower water than that subsequently occupied by the larvae. In this case, however, any apparent contradiction cannot be resolved, because we do not know the exact size of the immature specimens and too few such samples were found by Friedrich to be certain that he presents a general case. On the other hand and in apparent support of deep living ovigerous individuals, three females with ova were collected between 750 m and 1250 m in the north Atlantic at two separate stations (Støp-Bowitz, 1948). However, only 5 specimens of this species in total were collected on the entire cruise and none of these were reported as immature. Another somewhat ambiguous picture of the vertical distribution of the adults versus the juveniles emerges from the work of Hardy and Gunther (1935) from South Georgia, Antarctica. From November to May the vertical distribution of the adults of P. longicirrata from 50 m to 1000 m tended to overlap the vertical distribution of the juveniles of all species of polychaetes collected. Although most of the juveniles were thought by the authors to be P. longicirrata, the fact that all species were lumped together in their data and that we again do not know the relative sizes of the juveniles precludes a critical evaluation of their results.

Finally, Tebble (1960) has found independent support for the contention made here that *P. longicirrata* is capable of seasonal changes in its vertical position.

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Examining data collected by a vertical closing net on a transect following the Greenwich meridian from 70° S to 35° S, he determined that during the summer and early fall from November to April *P. longicirrata* was most abundant in the upper 100 m, but that during the winter it migrated into deeper water where it was fairly abundant throughout the year. Tebble speculates that its presence in the surface waters may be for purposes of breeding and feeding, although he presents no data to support these possibilities.

It is, of course, tempting to directly compare and discuss the vertical distribution of the adults of P. longicirrata with that found by others such as Tebble (1960), Gunther and Hardy (1935), and Mileikovsky (1962 and 1969) in different parts of the world. That temptation, however, is resisted for many of the reasons stated earlier in this paper. More productive comparisons can hopefully be made when the results on other groups of planktonic organisms collected in the same program from Fletcher's Ice Island T-3 become available.

In conclusion, the occurrence of the larvae below the adults is an interesting and even a bit unusual, because as Marshall (1954) notes, the larvae of most species are either found above or in the same range as the adults. With *P. longicirrata* one can presently only speculate on an explanation for this phenomenon. One possibility, however, is that living deeper may reduce predator pressure when the larvae are too young to feed and thus unable to exploit the surface waters.

I especially thank Drs. Kristian Fauchald and Hector R. Fernandez for their helpful suggestions and for reviewing the manuscript, Robert Smith for his computer programing, and Josephine Yingst for her help in counting oocytes and segments in many specimens. Special appreciation is also extended to Dr. Paul Schroeder who reviewed the manuscript and made important suggestions on gametogenesis in polychaetes. I also thank Dr. Thomas English of the University of Washington for providing some of the samples on which this study is based. This work was supported by ONR contract N 00014–67-A-0269–0013 to Dr. Hector R. Fernandez.

SUMMARY

During a two year period the vertical distribution of *Pelogobia longicirrata* (Polychaeta, Annelida) in the central Arctic ocean changed seasonally in conjunction with the relative age of the organisms. Adults with thirteen or more segments were primarily found throughout the year from 100-500 m. The peak occurrence of oocytes in the two month period of May/June was followed by a rapid decline in July/August, suggesting a spawning season in early summer or late spring. Larvae subsequently appeared below 500 m where specimens with one to three segments accounted for most of the animals collected between 900 m and 1200 m. Specimens with six or more segments were the youngest observed to have a mouth, anus, a continuous digestive tract, and a muscled pharynx, indicating that larvae smaller than these are probably incapable of feeding. They may instead live off of stored yolk retained from the egg. It is suggested that as the larvae mature they move up the water column and eventually join the adult population. The number of animals collected throughout the two years varied somewhat without demonstrating any apparent seasonal trends. The mean density from 0-1500 m was 46/1000 m⁸ in 1970 and 37/1000 m⁸ in 1971.

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Growth Cycle and Related Vertical Distribution of the Thecostomatous Pteropod <u>Spiratella</u> (<u>Limacina</u>) <u>helicina</u> in the Central Arctic Ocean. by Dr. Hester A. Kobayashi

The growth cycle and vertical distribution of <u>Spiratella helicina</u> was investigated because evidence has been presented which implicates this organism with the sonar scattering layer found at certain times of the year in the 75-100 meters below the surface. The vertical distribution of the organism is cyclical and appears to be influenced by the availability of suspended particulate organic matter which accumulates near the picnocline and by the photic quality of the water column.

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Marine Biology 26, 295-301 (1974) © by Springer-Verlag 1974

Growth Cycle and Related Vertical Distribution of the Thecosomatous Pteropod Spiratella ("Limacina") helicina in the Central Arctic Ocean

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Abstract

The growth cycle and related vertical distribution of the thecosomatous pteropod Spiratella ("Limacina") helicina (Phipps) were studied. S. helicina has a life cycle of approximately 1.5 to 2 years in the central Arctic Ocean (Canada Basin). It spawns mainly during the spring to summer period, and on a small scale during the winter. The young double their sizes during the winter months of October to May, slow down in growth until late summer, and attain maximum size in early winter. The oldest disappear by late March. Gonadal tissue was first seen in young pteropods of 0.7 mm diameter, the predominant size from February to April. S. helicina 0.8 mm in diameter, the size predominant from May through July, are mature and hermaphroditic. Growth during the winter months suggests that particulate organic matter is available during this period to these obligate ciliary feeders. Vertical distribution is size and seasondependent. The youngest specimens collected (0.2 to 0.4 mm) were found concentrated in the first 50 m. The larger sizes dispersed during the summer months, and tended to concentrate in the top 150 m during the rest of the year. They aggregated in the top 50 m from late winter through early spring, and fall through early winter; then concentrated in the 100 to 50 m level until the end of winter. Numerous environmental factors seem to be involved in determining the vertical distribution of the species in the central Arctic Ocean.

Introduction

Spiratella ("Limacina") helicina (Phipps), a prominent member of marine zooplankton in polar and boreal waters, is the only the cosomatous pteropod recorded in the central Arctic Ocean (Leung, 1971). The role of S. helicina as a member of the Arctic fauna has become of particular interest as a result of Hansen and Dunbar's (1970) report of the species in the acoustical scattering layer associated with the 50 m pycnocline in the central Arctic Ocean (Canada Basin). It has been found concentrated at depths corresponding to locations of the scattering layers, and has been shown to be physically capable of causing accoustical reverberations. Earlier studies by Brodskii and Nikitin (1955), and Harding (1966) have established the distribution of *S. helicina* to be mainly within the Arctic layer of the water column (0 to 250 m). Aside from these observations, however, very little has been known of the biology of the species in the central Arctic Ocean.

This paper deals with the growth cycle, related vertical distribution and environmental factors which may be of influence in determining vertical distributional patterns of the species.

Materials and Methods

Samples used in this study were collected from the drifting ice stations: "Arlis I" at the periphery of the central Arctic Basin $(74^{\circ}-75^{\circ}N; 143^{\circ}-166^{\circ}W)$, October, 1960-March, 1961, 29 stations; "Arlis II" in the central Arctic $(83^{\circ}-86^{\circ}N; 169^{\circ}-174^{\circ}W)$, October, 1962-March, 1963, 16 stations; "T-3" in the central Arctic $(84^{\circ}-83^{\circ}N; 112^{\circ}-85^{\circ}W)$, March, 1970-October, 1972, 85 stations.

The "T-3" series was collected by field workers from the University of Southern California (USC) and the University of Washington, in vertical tows made consistently at the following increments: 50 to 0 m, 100 to 50 m, 150 to 100 m, 200 to 150 m, 250 to 200 m, 300 to 250 m, 500 to 300 m, 700 to 500 m, 900 to 700 m, 1200 to 900 m. 1 m diameter, closing nets of 215 µm mesh were used. Samples from "Arlis I" and "Arlis II" were obtained by vertical and horizontal tows with 0.5 m nets varying in mesh from 73 to 215 µm. USC field workers did the collecting.

Shell diameters were determined by measuring across the shell from the end of the outer whorl with an ocular micrometer.

Vertical distribution was investigated among samples collected from "T-3" between March, 1970 and December, 1971, the period of most intensive sampling.

Gonadal maturation was determined from histological slides of *Spiratella helicina* of different sizes, at the period of their peak occurrence. Slides were prepared (method of Hsiao, 1939a) from specimens preserved in 7 to 10% buffered formalin, decalcified in 1% acetic acid, and whole-stained with methylene blue for ease in handling. 5 μ m thick paraffin sections were prepared and stained with hematoxylin and eosin.

Results and Discussion

Growth-Cycle

Shell Diameters. Shell-diameter measurements of Spiratella helicina are presented in Table 1 as average monthly values. Two values are provided for months during which the array of sample diameters showed bimodal populations, one of large individuals (parent generation) and the other of smaller specimens (offspring generation). Individual measurements were pooled to derive the schematic growth cycle shown in Fig. 1.



Fig. 1. Spiratella helicina. Schematic growth cycle based on pooled data from samples collected from the drifting ice stations "Arlis I", "Arlis II" and "T-3". G: parent generation of G_1 ; G_1 : parent generation; G_2 : offspring generation

During the growth cycle, young individuals of 0.3 mm diameter begin to appear in August and are prominent until November. The average diameter of the population of young specimens increases throughout the winter. The sizes double from 0.4 mm in October to 0.8 mm in May. External growth ceases between May and July, and continues again to an average maximum diameter of 2.8 mm. During the period of final growth, however, the population declines very noticeably in the proportion of individuals of 2 mm and larger found. Memberss of the adult population seem to disappear completely by the end of March; large individuals are not collected until the following winter (October).

Data in Table 1 show that veligers of 0.3 mm diameter appear again in March. The numbers, however, are so small that their presence is not reflected in Fig. 1, based upon average sizes for each month. This appearance of young suggests that spawning occurs during the winter months on a limited scale.

Gonadal Development. The adult Spiratella is hermaphroditic. Its gonad, or ovotestis, is a spiral structure posterior to the digestive gland, occupying the posterior whorl of the spiral shell. Detailed description of the structure and organization is available in the work of Meisenheimer

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(1905) and Hsiao (1939a), who worked on S. helicina and S. retroversa, respectively. Hsiao found that mature ova in S. retroversa ranged from 50 to 70 µm , had dark staining granules in their cytoplasm, and a poorly staining nucleus. Two prominent, mature accessory reproductive organs he described were the shell gland and the seminal vesicularis. The shell gland, according to the description, is histologically characterized by the presence of large secretory cells and tall, ciliated columnar cells. The seminal vesicularis is lined with cuboidal epithelial cells and is an elongated, pear-shaped organ connected to the hermaphroditic duct. These prominent characteristics of the mature hermaphroditic system were used to determine the maturity of the pteropods examined. Table 2 summarizes the observations on S. helicina of different sizes.

The data show that maturation of gonads does not occur on the average until the pteropods reach about 0.7 mm diameter, the size found predominantly from February to April. Accessory organs were difficult to identify until they reached about 0.8 mm diameter, the size predominant in May. Histologically, the hermaphroditic system of Spiratella helicina of 0.8 mm and larger were very similar.

Discussion

Derivation of Growth Cycle. The size of specimens taken at corresponding months from the different ice stations overlap within I standard deviation (Table 1). The periods in the growth cycle when the smallest and largest individuals occur, and when the largest forms disappear also overlap. The appearance of a small number of veligers, 0.3 mm diameter, in March is also consistent.

Growth and reproduction are influenced by fluctuations in a number of recognized environmental features. Among the most important seem to be light, temperature, and availability of food (Kinne, 1970). Throughout the central Arctic Ocean (Canada Basin) these features appear to be similar during any one period of the year. Because, hydrographically, the central Arctic water column is composed of 3 major water masses which vary very slightly in depth from one location to another (Coachman, 1963), temperatures experienced by animals at corresponding depths in different locations are very similar. Temperatures in the most fluctuating part of the column, the top 50 m, vary by a maximum of only $2C^{\circ}$ throughout the year. Winter temperatures range between -1.65° and -1.85° C, and summer temperatures between -1.7° and 0° C. Light penetration, energy for primary production, is sharply controlled by the light cycle and the occurrence of snow cover during similar periods throughout the area. The availability of food is, thus, similarly limited until the spring-summer period occurring roughly from June-August (English, 1963, 1965).

On the basis of the shell-diameter measurements and the consistency of environmental features, I

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Table	

Month	"Arli	"I 8		"Arli	"II s		"T-3"	(0261)		"T-3"	(1261)		"T-3"	(1972)	
	Diame (mm)	ter Range (mm)	sb**	Diame (mm)	ter Range (mm)	(f)	Diamet (mm)	ter Range (mm)	(Ŧ)	Diamet((mm)	er Range (mm)	(†) SD	Diamet (mm)	er Range (mm)	(Ŧ)
January	0.6	0.4-0.9	0.10	0.5	0.5-0.6	0.02							0.5	0.4-0.6	0.09
February	0.7 2.2	0.5-0.8	0.09	0.5 2.8											
March	0.6 2.0	0.3-0.8	0.11	0.5			0.7 2.8	0.3-0.9 2.5-3.0	0.10	2.0	1.9-2.0				
April							0.7	0.5-0.9	0.08				0.7		
May							0.8	0.7-0.8	0.06				6.0	0.7-1.0	0.10
June							0.8	0.6-0.8	0.06						
July							0.8	0.7-1.0	0.10						
August										0.3	0.3-0.4	0.06			
							1.2	1.0-1.4	0.30	0.8					
September							0.3	1.0-1.4		0.3	0.3-0.5	0.02			
October	0.3 2.1	0.2-0.3	0.02	0.5	0.3-0.6	0.07				0.3	0.3-0.4	0.06	2.4	1.7-2.9	0.10
November	0.3	0.3-0.5	0.08	0.4	0.3-0.5	0.08				0.5	0.4-0.6	60.0			
	2.3	1.7-2.4	0.30	2.9	1.8-3.7	1.34									
December	0.5	0.3-0.7	0.10	2.3	1.3-2.9	0.36	2.1	1.6-2.4	0.22						

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Size (mm)	Gonadal state	Accessory organs	Comments	Month
0.4	No differentiation	Undeveloped	Primordial cells through- out body, in clumps	September
0.5	No differentiation	Undeveloped	Primordial cells through- out body, in clumps	November
0.6	No differentiation	Undeveloped	Primordial cells through- out body, in clumps	November
0.7	Some differentia- tion, but details indeterminate	Present, but not completely distinguishable	Differentiation through- out body	March, April
0.8	Mature ova, 37-46µ seen, male tissue present	Hermaphroditic organs present	Little histological difference from this size on	May, July
0.9	Mature ova, 43-71µ seen, male tissue present	Similar to that in 0.8 mm specimen		July
1.4	Mature ova, 43-57µ seen, male tissue present	Similar to that in 0.8 mm specimen		August

Table 2. Spiratella helicina. Shell size and gonadal development

have interpreted the growth cycle of *Spiratella helicina* to be generally a uniform one within the central Arctic Ocean, and have therefore combined the data to derive the growth cycle shown in Fig. 1.

The life cycle of Spiratella helicina in the central Arctic Ocean appears to extend over 1.5 to 2 years. Supporting this estimate are the observations that the major spawning period starts in May when the population averages 0.8 mm, that veligers grow to 0.8 mm by May of the following year, and that an average of 2.8 mm (maximum 3.7 mm) is reached by November, after which the large specimens decline and disappear by March of the following year. If larger S. helicina such as the 8 mm individuals reported by van der Spoel (1967) in the sub-Arctic occur, then the life cycle of the species in the central Arctic Ocean may be longer. Our records of many years of sampling in the region, however, do not show such large specimens. Despite the obvious handicap of being unable to make fast tows routinely from the ice stations except when wind conditions cause fast movement of the islands, we have been able to collect adult specimens of such fast-swimming zooplankton as Hymenodora glacialis (decapod), Clione limacina, various medusae, an occasional octopus, and large amphipods; even fish have been caught. If such large S. helicina occur it is reasonable to assume that a few would have been collected over the long period of collections. It may be that larger S. helicina are unable to sustain themselves on the

quality and quantity of food available to them during the long winter, and hence die out upon reaching a maximum sustainable size.

Spawning. Histological data show that Spiratella helicina has mature ova and fully developed accessory organs by about 0.8 mm diameter, the average size attained by the population in May (end of winter). This suggests that they can spawn from that size. Hansen and Dunbar (1970) have reported specimens of this size carrying egg masses. Since the major population of veligers appear in late summer and early winter (August-November) it appears that the major spawning period occurs during the spring and summer, starting in the late winter and ending before the following winter. The peak probably occurs during the May-July period, when cessation of external growth in the population was noted.

The cessation of growth in overall body size in Spiratella helicina during the reproductive period is common among animals. Energy demands require that only one of these processes predominate (Kinne, 1970). Growth after the major reproductive period is not as common; however, this feature does not appear to be an Arctic adaption since it is also displayed by the very closely related species found in the Gulf of Maine, S. retroversa (Hsiao, 1939b; Redfield, 1939).

Spawning does not seem to end entirely with the onset of winter. There appears to be some winter spawning on a small scale, for individuals of 0.3 mm diameter occasionally have been found in March (Table 1). Protracted reproduction has been found to exist among *Spiratella helicina* by Paranjape (1968).

The growth cycle of *Spiratella helicina* in the central Arctic is not unique to this region, but merely adapted to the occurrence of the spring-summer period of the area. It parallels that of *S. retroversa* (Redfield, 1939), which has its major spawning earlier in the year than *S. helicina*, during the spring period of the temperate region.

Major Growth Period and Food Supply. The fact that growth continues during the winter (October-May), with major size increase and gonadal development occurring during the dark months (October-March) suggests that food is available even during the winter months to these animals. Spiratella spp. are entirely ciliary feeders (Morton, 1954) dependent, therefore, upon the presence of particulate organic material (POM) of suitable size (phytoplankton, nannoplankton, detritus) for nutrition. Unlike a number of other Arctic and deepwater plankton from temperate areas which store reserves in the form of wax esters, pteropods do not appear to have this ability (Lee, personal communication), and hence are dependent throughout the year upon available food.

The presence of POM is dependent upon primary productivity, and the subsequent events in the food chain which lead to nannoplankton and detrital production. In the central Arctic, primary productivity occurs in an annual mono-cyclical manner in close association with local seasons (English, 1963, 1965), peaking during the biological summer (roughly mid-July through August), declining through September (autumn), pausing during the dark months (October-March) and resuming again from late winter as the sun appears. POM estimates, based upon amounts of particulate organic carbon for summer 1970 and 1971 (English, unpublished data for chlorophyll $a \ge 75$, where 75=C/Chl a: commonly used ratio for phytoplankton) and April 1971 (Holm-Hansen, unpublished data), shortly after the end of the dark period, at 25 m depth, were 32, 28, and 3 μg C/1, respectively. Because extensive annual surveys of organic particulates have not been conducted in the central Arctic Ocean, the major available data being limited to the end of winter (Kinney et al., 1971; Holm-Hansen, unpublished), little can be said of the actual quantities of particulate material available to support POM-feeding zooplankton throughout the year. It can be said, however, that at a minimum, concentrations are as high as that of other deep ocean basins which support POM-feeding populations (Hobson and Menzel, 1969; Holm-Hansen, 1969). In the central Arctic, only a small proportion of the POM-feeding zooplankton is dependent throughout the year on external energy sources. Hopkins (1969) has shown that roughly 86% of the biomass is composed of copepods, and recent lipid studies (Lee et al., 1972) have shown that the most prominent of these are capable of wax-ester production for

energy reserves. Numerous other zooplankton are also capable of this activity, hence only a very small portion of the total biomass remains which requires POM throughout the year, *Spiratella* spp. being included.

Vertical Distribution

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The data show seasonal vertical migration (Fig. 2). Spiratella helicina concentrated in the 50 to 100 m interval during the late winter months of March and April, migrated to the top 50 m during late winter to early spring (May-June), dispersed during the summer months, and reconcentrated at the end of summer. During September, the major concentration was in the top 50 m; however, in December there was a trend toward reconcentration at the 50 to 100 m depth interval.



Fig. 2. Spiratella helicina. Vertical distribution of specimens collected from "T-3" during March-December, 1970. Values of 5% and less are indicated by lightly shaded, open bars

Vertical distribution limits varied with season. From late winter to the end of spring 90% of the population remained in the top 150 m of the water column, but dispersed down as far as 900 to 1200 m depth during the summer, continuing to be diffuse as late as December.

Vertical distribution as a function of size is shown in Fig. 3. Spiratella helicina larvae of 0.2 to 0.4 mm diameter (prominent from August to November) concentrated primarily in the top 50 m, while individuals of 0.5 to 0.7 mm (prominent from December to April) tended to concentrate at the 50 to 100 m interval. The persistence of small sizes less than 0.5 mm in the top 50 m accounts for the top-heavy distribution in September, and the shift to the 50 to 100 m interval by December as the pteropods on the average become 0.5 mm and larger. Sizes 0.8 to 0.9 mm (prominent from May to early July) were found mainly in the first 100 m. Specimens 1.0 to 1.4 mm, common during the summer, were dispersed - as would be expected



Fig. 3. Spiratella helicina. Vertical distribution, as a function of size, of specimens collected from "T-3" during March-December, 1970. Values of 5% and less are indicated by lightly shaded, open bars

from the general population trend in Fig. 2. S. *helicina* of 1.9 to 2.5 mm, found during fall and early winter, displayed the reconcentrating tendency described for the total population during this season.

Discussion

Seasonal migratory behavior as seen here is a phenomenon common to central Arctic zooplankton. Hughes (1968) reported the seasonal vertical migration of several copepods. Dawson (personal communication) has shown that the copepodites of *Calanus hyperboreus* concentrate in the top 50 m during the summer, and gradually migrate to deeper water by winter. Such migratory and aggregative behavior often is interpreted as response to a number of factors in the biological and physical environment. These include the availability of food, light, pressure, salinity, and avoidance of predators.

The availability of food in the water column throughout the year is of great importance to Arctic Spiratella helicina since, as mentioned earlier in the discussion of the growth cycle, these pteropods require a constant source of nutritive POM. Studies of POM in the Arctic water column made in April, shortly after the end of the completely dark period, have shown that it concentrates in the vicinity of 50 m (Holm-Hansen, unpublished data). At this time, S. helicina concentrates in the 50 to 100 m depth range (Fig. 2). Veligers of 0.2 to 0.3 mm are concentrated in the top 50 m (Fig. 3) - the euphotic zone of the Arctic water column (English, 1965) and hence the depth range of highest POM levels during productive months. Larvae of this size lack the adult swimming appendages, or "wings" (Paranjape, 1968), and therefore may find advantage in being present in the layers of highest food concentration.

Among other environmental factors influencing vertical migrations, l i g h t appears to be of some importance in the orientation and maintenance of the young in the top 50 m, since larvae are positively phototactic (Paranjape, 1968). The migration of adults to the top 50 m in May and June may also be influenced by light, as a trigger and in directional orientation.

The influence of t e m p e r a t u r e on the migration of central Arctic Spiratella helicina seems to be minimal, in view of the very small temperature change (about $0.5C^{\circ}$) that these pteropods normally experience in their vertical migration from the top 50 m to the 100 to 50 m interval. This species spawns, hatches, and spends its most formative period at temperatures ranging from -1.6° to $-1.0^{\circ}C$. The maximum change it may experience would occur during summer dispersal, and may be roughly about $2C^{\circ}$.

Hydrostatic pressure does not appear to impose limits on the population, at least within 100 m depth. The downward movement is, on the whole, seasonal (Fig. 2). It is possible that tolerance varies with size, for only large, mature individuals have been found in depths below 200 m.

Salinity is often considered to pose physiological limits on vertical migration of zooplankton. It appears that in the Arctic this factor is influential in setting an upper distribution limit. Spiratella helicina concentrates at the 50 m level in preference to other parts of the top 50 m (Hansen and Dunbar, 1970). They thus concentrate in the salinity range of 29.8 to 32.00/00 (data collected by the University of Washington at 50 m: Stations W1-W2, 1965; Stations 012-013, 1970), instead of spreading throughout the top 50 m of the water column which range in salinity from 28.5 to 33.5 %/oo (Coachman, 1963). In their dispersal down the water column during the summer, S. helicina live at salinities as high as 35°/00, however this migration appears to be limited to adults.

A voidance of predators by migration does not appear to be an advantage of the vertical movement of *Spiratella helicina*. *Clione limacina*, its well-defined predator (Lalli, 1970), has a parallel pattern of distribution (Mileikovsky, 1970).

Acknowledgements. This work was supported by the U.S. Office of Naval Research Contract NOOO14-67-A-0269-0013, NR 307-270 with the University of Southern California. My deepest appreciation goes to project principal investigator, H. R. Fernandez, for reviewing the manuscript, and to O. Holm-Hansen and T.S. English for the use of unpublished data.

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Date of final manuscript acceptance: April 29, 1974. Communicated by J. Bunt, Miami

On the Ostracod Fauna of the Arctic Ocean by Yuk M. Leung. Ostracods are among the most prominent zooplankton constituents of the Arctic Ocean. In this investigation Mr. Leung the geographic and seasonal vertical distribution of five species .

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On the Ostracod fauna of the Arctic basin

YUK M. LEUNG

Leung, Y. M. 1975 On the Ostracod fauna of the Arctic basin. Astarte 8, 41-47. This study is based on some 7,000 individuals collected at the American basin of the Arctic Ocean. Five species have been recorded: four of Halocyprididae and one of Paradoxostomatidae. Conchoecia borealis maxima is the predominant species inhabiting depths ranging from 1500 m to the surface. One of the species, Bathy-conchoecia arctica, is new to science, and is described elsewhere. Conchoecia skogsbergi is a new record for the Arctic basin. Acetabulastoma arcticum, a parasitic form, lives attached to the gills and gnathopods of certain amphipods. The size-range of the specimens of C. elegans and C. skogsbergi exceed that of typical specimens and their systematic status merits a thorough study.

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Though the ostracods are among the prominent constituents of the high Arctic fauna, they have hitherto been but little investigated. A number of species occur at various depths ranging from 1500 meters to the surface, and some also occur in abundance on the continental shelves. In earlier literature, Skogsberg (1920) mentioned two marine species (Philomedes globosus and Conchoecia obtusata) found in the Arctic Ocean. Sars (1928) revealed that nine Norwegian species were distributed in the Arctic Sea: one Cypridinidae, three Conchoechiidae, and five Cytheridae. In this report five species are recorded: one belongs to the Paradoxostomatidae (Cytheridae) and the others to the Halocyprididae (Conchoechiidae). One of the species of Bathyconchoecia turned out to be new to science, and the other, a 'long form' of the rotundata group, is a new record in the Arctic Basin.

This paper is based on some 7,000 samples taken from the drifting stations ARLIS-I, ARLIS-II, and T-3 (Fletcher's Island) by many field biologists of the University of Southern California and the University of Washington, collected from 1952 to 1971. Most of the individuals were caught in plankton net tows, with non-closing nets and half-meter closing nets of 73 μ m and 215 μ m mesh opening, and the host gammarids were taken with a wire mesh trap and from the surface of the hydrohole. The depth of collecting was recorded from each station.

SYSTEMATIC ACCOUNT

Family HALOCYPRIDIDAE Genus Conchoecia Dana, 1849 mollis group Conchoecia borealis maxima Brady & Norman, 1896

Conchoecia maxima, Brady & Norman 1896, p. 686, Pl. LXI, figs. 1-8; Sars 1900, pp. 127-137, Pls. 35, 36.

Remarks. Conchoecia borealis maxima is the predominant species in the samples, distributed from the surface to 1500 meters in great

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abundance. Collectively, the features agree with material from the north Atlantic described by Brady & Norman (1896) and Sars (1900), although some appendages display some trifling variations. In females the frontal organ is club-shaped, but the apex is not so acutely pointed as mentioned by Brady & Norman; in the first antenna, the first two segments of the stem are not of equal size, and the basal one is longer than the second; the mandibles are visibly chitinized, the cutting edge is armed with a row of teeth, the basal segment of the endopodite is much longer than the other three, and its lower edge also bears a row of teeth; the second pair of legs is the longest, and the last segment is armed with two slender setae of subequal size; the arrangement of furcal claws corresponds with Sars' description, including the two small bristles behind the last pair which is slender and some distance apart. In the male, the apex of the frontal organ is not so dilated as in the female; the first antenna is strongly built and the proximal long seta is armed with a series of short recurved marginal spines in the middle; the mandibles resemble that of the female, but the basal segment of the endopodite is almost as long as the two following segments combined; the furca is relatively larger than that of the female, and the copulatory organ is well developed, located adjacent to the furca extending to the ventral edge of the shell.

Conchoecia borealis maxima is very closely allied to Conchoecia borealis borealis, but positively separated by larger size; the males measure 3.2 mm and the females 3.5 mm in length (compared with 2.3 mm and 2.6 mm, respectively, in the typical form). The Arctic form also resembles the Antarctic species, C. antipoda Muller, particularly in the armature of the male first antenna. It deviates, however, in that the shoulder vaults of the former are rounded, whereas in the C. antipoda they are raised to a sharp edge, while also the carapace length is smaller.

Distribution. The type species was described as occurring off Greenland and extending southward to the Faroe Channel. Sars (1900) recorded it as far north as $84^{\circ}47'N$. In the present collection the habitat was recorded at T-3 Ice Island from $74^{\circ}27'N$ to $76^{\circ}08'N$, at depths of 1500 m to the surface; at ARLIS–I from 74°18'N to 74°54'N at depths from 850 m to the surface; and at ARLIS–II from 83°31'N to 84°55'N at depths of 150 m to the surface. 1.

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elegans group

Conchoecia elegans G. O. Sars, 1865

Conchoecia elegans, Sars 1865 p. 117; Skogsberg 1920, pp. 624-636; Sars 1928, pp. 22-24, Pls. 11, 12.

Remarks. Conchoecia elegans made up approximately 0.74 % of the collection (totalling 52 specimens); they were associated with C. borealis maxima at depths from 750 m to the surface. It occurred at T-3 and ARLIS-I. but no record has yet been reported from ARLIS-II. The present form is quite similar to the type specimens (Sars 1865) from the Norwegian Sea with the exception of its larger size. Since C. elegans has a cosmopolitan distribution from the Arctic to the Antarctic, it varies considerably in size: Claus (1891) recorded it as 1.20-1.30 mm from the Atlantic; Muller (1906) as 1.00-2.00 mm from the German deep sea expedition; Sars (1928) as 2.30 mm from the Norwegian coast; the male specimens investigated by Skogsberg (1920) were 2.10-2.25 mm from Lofoten, 1.20 mm from the Atlantic, 2.05-2.20 mm from the Arctic, and 1.45-2.00 from the Antarctic. The present form has a range from 2.35-2.40 mm in both sexes, larger than all the previous records. According to the reports of the earlier workers, several size groups exist with distinct geographical and vertical distributions which are in a state of taxonomic confusion. Angel (pers. comm.) disclosed that wherever size groups overlap geographically, they are separated vertically, and the large form appears to extend far south in the northern Atlantic, but the median depth of its population descends, and its population density decreases by about two orders of magnitude. At least three species may be included within the present concept of the species.

Distribution. Sars (1865) described the species from the Norwegian coast, and it is distributed in the Atlantic Ocean (Claus 1891), Indian Ocean, and Antarctic (Skogsberg 1920); the most northerly record was 79°58'N (Aurivillius 1898). The present record has

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Table I. A yearly record of vertical distribution of Conchoecia borealis maxima in the Arctic Basin

Depth	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
50-0	2	0	0	1	3	0	0	0	0	1	1	0	8
100-50	7	0	33	7	2	2	3	2	4	1	6	10	77
150-100	11	0	29	34	6	6	8	8	2	6	6	5	121
200-150	4	7	12	21	14	13	17	34	0	2	10	10	144
250-200	3	2	12	12	14	39	20	25	6	7	2	21	163
300-250	2	7	18	13	22	14	14	29	3	9	3	17	151
500-300	4	17	76	58	55	21	65	65	3	10	9	72	455
700-500	0	18	27	51	13	8	30	16	15	5	6	13	207
900-700	3	2	14	22	8	10	14	11	8	2	1	11	106
1200-900	8	3	18	14	13	10	13	21	8	8	5	14	135
1500-1200	2	0	16	49	15	18	6	0	3	13	11	21	154
2000-1500	0	0	7	0	0	0	0	0	11	20	0	0	38
	46	56	262	282	170	141	190	211	63	84	60	194	1759

extended this to $85^{\circ}29'N$ at T-3 Ice Island and $74^{\circ}54'N$ at ARLIS-I. The depth is ranging from 750 m to the surface.

rotundata group

Conchoecia skogsbergi E. J. Iles, 1953

Conchoecia rotundata, Muller 1890, p. 83; Muller 1906, pp. 29–54; Skogsberg 1920, pp. 648–658. Conchoecia skogsbergi, Iles 1953, pp. 259–280; Angel 1968.

Remarks. Along with several juvenile Conchoecia borealis maxima, an adult female Conchoecia skogbergi of the rotundata group was caught in a plankton ret tow at $85^{\circ}58'N$, $91^{\circ}10'W$. It measured 1.5 mm. Although this species is commonly found in the Antarctic as far as $65^{\circ}S$ and in the north Atlantic to $66^{\circ}N$ (Angel 1968), this recent finding is a new faunal record in the Arctic Basin.

The systematic status of C. rotundata has long been confused since Muller (1906) separated the species into two groups: the 'long form' and the 'short form'. Iles (1953) referred to both forms as genuine species: he named the 'long form', as described by Skogsberg (1920), C. skogsbergi, a cold water species; and the 'short form' C. teretivalvata, a warm water species. No description of C. skogsbergi had been given for northern latitudes, however, until Angel (1968) recorded and described a female from the Norwegian Sea. Recent investigation has shown that this group demonstrates considerable variation with latitude. Angel (1972) revealed that at least 11 forms fall within the original concept. The individual collected from the

Arctic Basin has several features which show variation from Atlantic specimens. Although the Arctic form is similar to the specimen from the Norwegian Sea (Angel 1968), the size exceeds the Norwegian specimen which measured only 1.33 mm. The whole group needs drastic revision.

Genus Bathyconchoecia Deevey, 1968

Bathyconchoecia arctica Angel (Crustaceana, in press)

Remarks. A single female Bathyconchoecia arctica was sampled at the Station 49-IH-45 of T-3 Ice Island. Angel (in press) remarked the Arctic form is distinguished from known species by the vertical carapace striations, the broad rostrum, the notched gland openings, and the prominent frontal organ. Since Deevey (1968a) described six new species in the Gulf of Mexico, Poulsen (1969a, 1969b, 1972) added more from the Central Atlantic, Malayan Archipelago, and the Azores, respectively, and Kornicker (1969) described a further species from the Peru-Chile Trench System. Thus the genus is reported as occurring widely in temperate and tropical waters. However, the finding of an Arctic form suggests that the genus inhabits an even wider range of habitat.

Family PARADOXOSTOMATIDAE Genus Acetabulastoma Schornikov, 1970 Acetabulastoma arcticum E. I. Schornikov, 1970

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Paradoxostoma rostratum, Baker & Wong 1968, pp. 307-311; non Sars 1865, pp. 97-98, 256, Pl. 119, nec Elofson 1940, pp. 1-8, figs. 1-11. Acetabulastoma arcticum Schornikov 1970, pp. 1132-1143.

Remarks. Acetabulastoma arcticum is a parasitic form frequently found on Gammarus wilkitzkii and Gammaracanthus loricatus, and was referred to as Paradoxostoma rostratum by Baker & Wong (1968). The species measures 0.74-0.90 mm in length. It fastens habitually to the gills and the base of the gnathopods of the hosts by means of the sucking disc of the oral cone. Its systematic status was in a state of confusion before it was finally assigned to its appropriate species and genus. Sars (1865) originally described Paradoxostoma rostratum from two detached valves found in shell sand at Øxfjord, Norway, but details were not available until Elofson (1940) gave a complementary description of the carapace and appendages from a collection caught at Mortensas, Norway. Baker & Wong (1968) described it as a new species solely on the basis of its commensalism with certain Arctic gammarids which populated the icewater interface of the drifting ice stations. Eventually, Schornikov (1970) erected a new genus Acetabulastoma, in which he included five new species and one sub-species, all probably parasitic on amphipods: four are ectoparasitic on Gammarus and Amphitoe, but the hosts of the other two have not been determined. One of his new species, A. arcticum, was taken from the brood pouch of Gammarus wilkitzkii in the Arctic Basin, and Schornikov considered Paradoxostoma rostratum Baker & Wong to be synonymous with his new species, as he regarded it not to be the same as P. rostratum Sars, Baker & Wong (1968) stated that their Arctic species agreed with Elofson's description, except the proximal side projection of the distal segment of the copulatory organ which was more prominent, while the apparent spine-like process on the outer margin at the proximal end of the distal segment was not evident. Schornikov (pers. comm.) has confirmed by examination of their specimens that P. rostratum Baker & Wong was a synonym of A. arcticum.

Baker & Wong (unpublished report) examined metazoans and parasitic ostracods from some 960 amphipods, including Gammaracanthus loricatus, Gammarus wilkitzkii, Anonyx nugax, Hyperia galba, and Pseudalibrotus nanseni. It is of interest that Gammaracanthus loricatus and particularly Gammarus wilkitzkii were the only two species which were parasitized by A. arcticum. Hence, it is likely that the parasitic ostracods show host specificity. Since the ostracod is not freeswimming, infestation probably spreads by bodily contact. As the hosts are members of the under-ice population which occurs in the restricted habitat of the ice-water interface, the chance of bodily contact is high. Schornikov (1970) also showed host specificity by demonstrating that Acetabulastoma littorale and A. robustum parasitized two closely related species, Gammarus oceanicus and G. setosa, respectively, which were found to occur together in the littoral zone of the east Murman coast.

Distribution. The genus is distributed from the Arctic Basin along the east coast of the Bering Sea to the Sea of Japan. Hart (1971), however, described a new species, A. kozloffi, from San Juan Island, Washington; thus the known distribution has been extended to the eastern Pacific. The type locality of A. arcticum is in the Arctic Basin, but no exact position was given (Schornikov 1970). In the present collection, it ranged from 74°54'N at ARLIS-I to 82°04'N at ARLIS-II and 86°53'N at T-3 Ice Island.

SEASONAL VERTICAL DISTRIBUTION

Vertical diurnal migration is a natural phenomenon in many planktonic species. In pelagic Halocypriformes, Fowler (1909), Mackintosh (1937), Iles (1953), McHardy & Bary (1965), and Leveau (1969) reported that planktonic ostracods show vertical migration toward the surface at night, and return into deeper waters during the daylight hours. Deevey (1968b), on the other hand, found no significant evidence of vertical migration in the common Conchoecia species off Bermuda. Poulsen (1969b) also pointed out that a number of common species collected in the tropical eastern Atlantic did not demonstrate diurnal vertical migration. Angel (1972) observed that some species showed nocturnal migrations, while others showed little if any vertical movement, and two species even had a reverse pattern of migration off the Canaries.

There are no reports of diurnal migration by pelagic ostracods in the Arctic Basin, although some early workers (Walther 1893, Russell 1927, Bogorov 1946) gave different views on nocturnal migration of Arctic planktonic organisms in general. It is not clear whether the polar seasons affect vertical movements: there is continuous daylight in summer when the sun is above the horizon at midnight from mid-March to early October, and there is continuous darkness during the winter months. Table I shows a yearly record of Conchoecia borealis maxima samples at various depths at T-3 Ice Island between 82°50'N to 85°29'N. Although the collecting hours were between 01.03 and 23.50, they have not been considered separately as day samples or night samples. However, the number of individuals caught each month varied. The catches were abundant in March, April, and August when there was a cycle of light and dark, but the yields were comparably low in January, February, September, October, and November in conditions of total or near total darkness. Thus there is evidence of a seasonal migration into shallower depths during the Arctic spring and early summer.

Indication shows that each species of planktonic animals may have its own vertical zone. Brady & Norman (1896) recorded C. borealis maxima in abundance at the depth of 350 fathoms (about 830 m) off Greenland at 74°49'N, 11°30'W, and of 200 fathoms (about 475 m) at 60°20'N, 00°07'W; Sars' (1900) animals were collected at depths of 300 m in the Arctic Ocean (from 78°N, 136°E to 85°13'N, 79°E); Zenkevitch (1963) remarked that Conchoecia species were abundant at depths of 200-500 m in the Sea of Japan. Table I shows that the largest number of ostracods were caught at 500-300 m and 700-500 m (455 and 207 individuals taken respectively) off T-3 Ice Island, and that the number of individuals sharply declined below 1500 m and above 50 m. In general, the depth-zone of C. borealis maxima is from 830-300 m at high latitudes.

DISCUSSION AND CONCLUSION

The five species described in this report are based on some 7,000 individuals collected from 1952–1971. Except for *Conchoecia borealis maxima* and *C. elegans*, the other

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species do not match any of the Norwegian species (Philomedes globosus, Conchoecia borealis. Hemicythere emarginata, Cytherura similis, C. clathrata, Schlerochilus contortus and Paradoxostoma variabile) which were presumed to occur in the Arctic as suggested by Sars (1928, p. 14, 24, 184, 204, 216, 248 and 257), who probably used records from other less reliable sources. It is questionable if these species exist in the Arctic Ocean as neither the present collections nor those in the seas of USSR (Zenkevitch 1963) have included any of these species. Moreover, Sars (1900) found but one species, C. borealis maxima, in the Norwegian North Polar Expedition, and no other species was encountered

C. borealis maxima is the predominant species of the planktonic ostracod fauna of the Arctic Ocean. C. elegans and C. skogsbergi, found in abundance in the N. Atlantic, also inhabit the Arctic Basin. However, it is uncertain whether these two species are endemic or migrants from the Atlantic current, but their large sizes do appear to distinguish them from the type description. It is generally accepted that the size of marine animals is correlated with temperature, as plankton of northern and cold-water forms attain a larger size than the same or closely related species in warmer waters. The larger size of the Arctic forms reported here compared with those collected from temperate waters as recorded by earlier workers is consistent with this generalization. Angel (1972) showed that C. elegans is composed of various sizes ranging from 1.1 mm in tropical waters to 2.1 in the Arctic, but is made up of at least three consistently sized populations within this size-range. The status of the'rotundata' group is even more confused because it includes at least 11 forms additional to the original two species, C. skogsbergi and C. teretivalvata. The Arctic specimen of C. skogsbergi may represent yet another form, since its size is larger than any of the previously known species. Angel (pers. comm.) remarked that the Arctic form is clearly different from specimens that have been collected from various positions in the N. Atlantic (11°-60°N). However, there is some likelihood that low temperature phenotypically influences the development of animal structure. It supports Hesse's hypothesis (1924)

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that delayed sexual maturity favors growth for different species, but also for individuals of different stages of development of a species, sometimes even for the different sexes. It is well known that there are several factors controlling vertical movement. Light is not the only factor regulating vertical migratory behaviour, but other physical factors and chemical properties such as temperature, salinity, currents and water mass also play an important role in influencing vertical distribution.

This study is a preliminary one. There are many facets of problems which require further investigation: the chaotic systematic status of both C. elegans and C. skogsbergi needs clarification; the study of breeding cycles of C. borealis maxima is essential for understanding the production of standing crop; vertical migration and vertical distribution need further investigation; the role of ostracods in the food chain and their metabolism needs to be explored. The study of all these aspects would provide important contributions to our understanding of the Arctic ecosystem.

ACKNOWLEDGEMENTS

I am grateful to the many field biologists of the University of Southern California and the University of Washington whose collections made this study possible. I am indebted to Dr. G. B. Deevey of the Florida State Museum, Dr. C. W. Hart, Jr. of the Academy of National Sciences of Philadelphia, and Dr. E. I. Schornikov of the Academy of Sciences of USSR for identification, and particularly to Dr. M. V. Angel of the National Institution of Oceanography of England for his helpful criticism of the manuscript. The use of the library and laboratory of Allan Hancock Foundation is gratefully acknowledged. This work was supported by a contract between the University of Southern California, Dr. Hector R. Fernandez, chief investigator, and the Arctic Program, ONR, Dept. of the Navy, under Contract N00014-67-A-0269-0013.

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Distribution of Calanus hyperboreus in the Central Arctic Ocean by John K. Dawson. (submitted for publication to Limnology and Oceanography) <u>Calanus hyperboreus</u> is one of the four most important copepod species in the Arctic Ocean. Mr. Dawson describes the annual vertical distribution of adults and of copepodite stages II to V.

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Distribution of Calanus hyperboreus

in the Central Arctic Ocean¹

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Running Head: Distribution of Arctic Ocean Calanus

¹This work was supported by an Office of Naval Research contract, N00014-67-A-0269-0013NR307-270.

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Abstract

A study was undertaken to determine the vertical distribution of Calanus hyperboreus in the central Arctic Ocean.

The immature stages of <u>C</u>. <u>hyperboreus</u> show a sequence of molting from stage II copepodite to the adults, with immature stage distribuions similar to each other, but dissimilar to the adults. The concentration centers of copepodites range from 300 to 500 m during most of the year, with the exception of summer when they rise to the surface.

The adult female population center showed a gradual increase in depth from 100 to 300 m in spring. During the summer, females are found at the surface and by the fall, the population is centered about 150 m. Females remain in the upper 150 m throughout winter and by early spring the population is centered about 100 m, after which it again starts its slow sinking during the spring.

Adult males are centered deeper (400-700 m) than the females and are present only during the spring and early summer.

As far as can be determined, the population of \underline{C} . <u>hyperboreus</u> has a generation length of at least three years.

DISTRIBUTION OF CALANUS HYPERBOREUS IN THE CENTRAL ARCTIC OCEAN

INTRODUCTION

The first plankton collection from the high polar basin was by Fridtjof Nansen in 1893 when the <u>Fram</u> drifted for three years with the ice pack from the New Siberian Islands to north of Spitzbergen. The Crustacea collected during this drift were later described by Sars (1900). In 1937 a similar drift was followed by the <u>G. Sedov</u> but at higher latitudes (Bogorov, 1946). The most fruitful research in the central Arctic Ocean has been by Russians and Americans working from flows and ice islands (e.g. North Pole Series, ARLIS II, and T-3) drifting with the polar ice pack.

In 1952 a station on T-3 Ice Island was established. Since then this slab of glacial ice has drifted through two cycles in the anticyclonic drift system of the Beaufort Sea. This study is based on nearly two years of collecting while T-3 drifted about one-third the way between Ellesmere Island and the North Pole.

Plankton collection from T-3 affords a unique opportunity to sample continually over a long period of time; furthermore, T-3 provides a stable platform from which to accurately sample discrete depths. Investigations dealing with the distribution of <u>Calanus</u> <u>hyperboreus</u> Kroyer in the central Arctic Ocean (Bogorov, 1946; Brodsky and Nikitin, 1955; Johnson, 1963; Minoda, 1967; Hansen, Bulleid, and Dunbar, 1971) have been based on either relatively short sampling periods, limited depths, or both. This study, however,

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incorporates over 700 plankton samples taken throughout the water column over a two year period.

I wish to express my gratitude to T. S. English, Department of Oceanography, University of Washington, who kindly furnished many of the samples upon which this study is based.

METHODS

Zooplankton were collected by vertical tows with a one meter, number 6 mesh (215 μ opening) closing net while T-3 drifted from 84° 24' N, 112° 30' W in February 1970 to 84° 20' N, 86° 17' W in January 1972. From February to December 1970, the upper 300 m were samples in 50 m increments. From December 1970 through the end of the sampling period, the upper 400 m were sampled in 25 m increments, and the deeper waters in 100, 200, 300, and 500 m increments to the bottom. The filtration efficiency of the net was about 50% according to flow meter tests.

Plankton samples were collected via a hole cut through the sea ice, preserved in 7% buffered Formalin and sent to the University of Southern California for identification and analysis.

RESULTS

Three distinctly different types of distribution are evident for copepodite stages II through V, adult females and adult males.

Distribution of copepodites

The copepodites have a vertically undulating seasonal distribution descending into deep water in winter and returning to shallow depths

in summer. Fig. 1 shows the beginning of this type of distribution when stage II copepodites (CII) are found in deep water in the latter part of winter and spring. Before the upward summer migration it appears, assuming no catastrophic mortality, that most CII molt to CIII. The surface concentration during the summer is probably the result of the concentration effect of remaining CII moving to the surface for summer.

CIII (Fig. 2) show a similar distribution to CII during the winter to spring period. However, in May, they start their ascent and by June, July, and August they are at or near the surface presumably feeding on phytoplankton present at that time (English 1961). Following the summer growth of CIII, the population has moved into deeper water and waned, suggesting a molting of CIII to CIV.

Prior to the upward movement of CIII to the surface, CIV (Fig. 3) were not present in the water column in significant numbers. Only with the growth and molting of CIII did CIV come into being. Following summer, the population starts its return to deeper water and by December the population is centered between 300 and 400 m. The CIV population has dwindled by early spring of 1971 suggesting that by this time most of CIV have molted to CV, however, sufficient numbers remain to indicate a return migration to the surface for the summer of 1971.

The distribution of CV is characterized by relatively low numbers collected in comparison with other copepodite stages (Fig. 4), resulting perhaps from rapid development through stage V. Sufficient numbers of CV were collected to show that the vertical

distribution of this stage is similar to those of stages II, III and IV. During the spring of 1971, CV return to the surface for the summer months and in the fall and winter of 1971-1972, the population begins to wane suggesting that following the 1971 summer, most of the copepodites have reached the adult stage.

Distribution of adult females

Adult females show the second type of distribution (Fig. 5). During the spring of 1970, females descend from between 50 and 100 m in March to between 250 and 300 m in June at about 50 m per month. The concentration at the surface in July represents an upward movement of existing females, molting CV to adults, or both. By early fall, the female population is centered around 150 m and it is believed that the adult females stay in the upper 150 m through the remaining fall and winter. The deeper females shown in Fig. 5 during the winter of 1970-1971 are probably the result of CV at those depths molting to adult females, rather than an actual downward movement of existing females.

By early spring 1971, the population center has returned to about 100 m and again the females start their spring descent. The summer of 1971 distribution is similar to the summer of 1970, and by early fall the population is centered at about 150 m. Throughout the remaining fall and winter, the female population remains in the upper 150 m. Since at this time almost all of the CV have matured to adults, there is no large female population found in deeper water as there was the previous winter.

During the spring of both years, from February to June,

gravid females were encountered. During April 1970 and March 1971, gravid female percentages peaked at 30 and 20% respectively.

Distribution of adult males

The third type of distribution is that of the adult males (Fig. 6). The bulk of the males are clearly deeper than the females, with the males being found primarily between 400 and 800 m. The males are also time limited, being present only during the spring and early summer months. When males are present, the females far outnumber the males in the water column by a ratio of roughly 30 to 1.

DISCUSSION

Distribution

The undulating seasonal distribution pattern of immature <u>C</u>. <u>hyperboreus</u> copepodites is supported by earlier findings (Ussing, 1938; Brodsky and Nikitin, 1955; Ostvedt, 1955; Hughes, 1968). Ussing (1938) proposed that their annual ascent to the surface in early summer is in response to light. Recently Fernandez and Yingst (pers. comm.) have measured the spectral sensitivity of the adult female <u>C</u>. <u>hyperboreus</u> eye and found it to be maximally sensitive near 540 nm, the same wave length peak of light found by Maykut and Greenfell (1975) to occur beneath sea ice with algae present. This suggests that <u>C</u>. <u>hyperboreus</u> is especially adapted to wave lengths penetrating the Arctic Ocean beneath the ice cover. English (1961) reported maximal intensity of light from May through June, preceding the highest chlorophyll a concentration by about 4 to 6 weeks. This late July-early August phytoplankton maximum is strongly supported by Ussing (1938) and Halldal (1953), but Shirshov (1938) found the phytoplankton bloom restricted to August.

Although previous investigators found adult females and immature copepodites with similar distributions (Ussing, 1938; Ostvedt, 1955; Hughest, 1968), my study shows their distributions and vertical migrations are dissimilar. The short increments in which these samples were taken in the upper 300 or 400 m clearly demonstrate a slow descent of females from about 100 to 300 m. This distribution differs considerably from that of the immature copepodites.

The large number of females present between 200 and 300 m during the summer months suggests that they do not migrate to the surface with the copepodites. Those females found near the surface are probably the result of molting CV to new adults.

Previous workers (Ostvedt, 1955; Hughes, 1968) have indicated that after summer the adult females, along with the immature copepodites, descend into deeper water for the winter. My study, however, shows that although large numbers of adult females are found with the immature copepodites as they descend into deep water, many adult females remain in the upper 150 m. It appears that the females already present in the fall remain in the upper 150 m during winter while those females found deeper are recently molted from CV already in deeper water. Supporting this view is the fact that during the fall and winter of 1971-1972, when very few immature copepodites remained in the water, the adult female population was found primarily in the upper 150 m with no large population of females in deeper water. Brodsky and Nikitin (1955) have also indicated that part of the population of adult females remains relatively close to the surface during winter.

The presence of adult males have been reported to occur only during the winter and spring months. Brodsky and Nikitin (1955) and Ostvedt (1955) noted their presence from November to May and December to June, respectively. Others (Wiborg, 1940; Grainger, 1959), however, have reported the males limited to January and February. While my study found males to be present primarily during the spring and early summer months (March through July), two individuals were found in September and January.

The data of Brodsky and Nikitin (1955) support vertical segregation of males and females, with the females being abundant primarily in the upper 400 m and the bulk of the males being found between 400 and 800 m. This raises the question of sufficient contact between the two sexes which is further compounded by the relatively low numbers of males as indicated by my study and others (Somme, 1934; Conover, 1965). This phenomenon of a high ratio of females to males is not unusual according to Mednikov (1961), particularly in deep water copepods. In areas of restricted food supply as is found in the central Arctic Ocean and in deep water of other oceans, a low male to female ratio may be a definite selective advantage by resulting in a greater fecundity for the species.

Spawning

Unlike many herbivorous species of copepod which spawn during or after the phytoplankton bloom, <u>C</u>. <u>hyperboreus</u> belong to a group

which reproduce during the winter or spring (Heinrich, 1961). Since the phytoplankton bloom is thus anticipated, there is essentially no delay in grazing activity. The presence of gravid females during spring of both years support this notion, and is further supported by other investigators (Somme, 1934; Wiborg, 1940, 1954).

Generation length

Conover (1965) reports generation length of <u>C</u>. <u>hyperboreus</u> in the Gulf of Maine to be one year. However, it is well known that as a species' distribution extends into higher latitudes its development rate becomes slower. In the Canadian Arctic some <u>C</u>. <u>hyperboreus</u> developed to adults within a single year, while others required two or more years (Grainger, 1959). Cairns (1967) described the development rate of <u>C</u>. <u>hyperboreus</u> as extremely slow and concluded that because of this, they probably breed only during occasional years.

To estimate the generation length of <u>C</u>. <u>hyperboreus</u> during this study, it is necessary to establish when CII and CIII present in March of 1970 were spawned. Since these stages occurred concurrently with gravid females, one might expect that CII resulted from the gravid females already present. However, considering this species' very slow development rate, and the fact that gravid females were still present following the CII and CIII molt, it is reasonable to assume that these copepodites were spawned the previous spring. Harding (1966) supports this belief by collecting large numbers of CII in September, which remained as CII or CIII through the winter. He concluded that these individuals resulted

from spawning in the previous spring. Thus it appears that the copepodites present before this study began were spawned in the spring of 1969, reached CII and CIII during the winter of 1969-1970, and were collected as one year olds in March of 1970. Since no CII were collected following the spring of 1970, it must be assumed that this potential brood was, for some unknown reason, unsuccessful.

By the spring of 1971, all CII and most CIII have developed to CIV, CV, and adults, all being two years old at this time. Again, a percentage of the females was gravid during this spring, but as in the previous year, apparently no progeny developed from them as evidenced by the lack of collected CII following the period of gravid females. By fall 1971, almost all of the copepodites present in the water have molted to adults and it can be speculated that by the spring of 1972 the gravid females, now three years old, finally produced a successful brood and the generation cycle would be complete. Thus, it appears that if reproduction occurs at all in the central Arctic Ocean, the generation length, i.e., the period from successful brood to successful brood in <u>C</u>. <u>hyperboreus</u>, is a minimum of three years.

The reason for the lack of young following the two periods of gravid females remains unresolved. Some possibilities, however, can be considered. It is conceivable that reproduction is never achieved in the central Arctic Ocean and copepodites are recruited from more productive continental shelf waters. On the other hand, if <u>C</u>. <u>hyperboreus</u> does reproduce in the central Arctic Ocean and the generation length is three years, the distribution of males may be such that two out of three years the females do not mate. Perhaps only in the third year does the male distribution overlap with the female distribution such that copulation may occur. If this is the case, the delay in production of young until all of the copepodites already in the water have matured to adults may be advantageous to <u>C</u>. <u>hyperboreus</u> considering the very limited food supply available to them in the central Arctic Ocean.

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FIGURE DESCRIPTIONS

Fig. 1.

Vertical distribution of <u>C</u>. <u>hyperboreus</u> copepodite II. Contours are drawn at 10, 20, 40, 60, 80, etc. copepodites/20 m^3 .

Fig. 2.

Vertical distribution of <u>C</u>. <u>hyperboreus</u> copepodite III. Contours are drawn at 100, 200, 300, 400, 500, etc. copepodites/20 m^3 .

Fig. 3.

Vertical distribution of <u>C</u>. <u>hyperboreus</u> copepodite IV. Contours are drawn at 50, 100, 200, 300, 400, etc. copepodites/20 m^3 .

Fig. 4.

Vertical distribution of <u>C</u>. <u>hyperboreus</u> copepodite V. Contours are drawn at 5, 10, 20, 30, 40, etc. copepodites/20 m³.

Fig. 5.

Vertical distribution of <u>C</u>. <u>hyperboreus</u> adult females. Contours are drawn at 10, 20, 30, 40, 50, etc. females/20 m^3 .

Fig. 6.

Vertical distribution of <u>C</u>. <u>hyperboreus</u> adult males. Contours are drawn at 1, 2, 3, 4, 5, etc. males/20 m³.









des.





2- Ellucidation of the Freezing Resistance Mechanism in an Arctic Isopod. The Freezing Resistance of and Arctic Isopod, <u>Mesidotea entomon</u> by Dr. Douglas Yingst. in press Journal of Comparative Physiology.

This investigation by Dr. Yingst has revealed that in <u>Mesidotea</u> freezing resistance occurs in the following manner: At the surrounding sea water begins to freeze and the ionic concentration of sea water increases, the concentration of ions in the hemolymph also increases. This depresses the freezing point of the hemolymph. The animal does not freeze as ions are taken up from the surrounding sea water, even when the animal is surrounded by ice because the cuticle <u>prevents</u> the external ice from inoculating the hemolymph. Evidence that the cuticle is an effective barrier to ice propagation is presented.

THE FREEZING RESISTANCE OF AN ARCTIC ISOPOD, MESIDOTEA ENTOMON (LINNAEUS, 1758)

by

Douglas Roy Yingst

A Dissertation Presented to the FACULTY OF THE GRADUATE SCHOOL UNIVERSITY OF SOUTHERN CALIFORNIA In Partial Fulfillment of the Requirements for the Degree DOCTOR OF PHILOSOPHY (Biological Sciences)

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August 1975

ACKNOWLEDGMENTS

I thank Dr. Hector R. Fernandez for providing the laboratory space, equipment, financial support, and encouragement which enabled me to undertake and complete this dissertation. I also thank the administration and staff of the Naval Arctic Research Laboratory(NARL) at Barrow, Alaska for providing laboratory space and logistic support during the springs of 1973 and 1974. While at NARL I was the recipient of advice and assistance of many, but I want particularly to thank Dr. Rita A. Horner and Dr. Robert I. Lewellen for introducing me to field work in the Arctic and Dr. Horner for assisting me with aspects of the field work.

I am indebted to Dr. William T. O'Day for his long hours helping me with the ultrastructure of the cuticle and to D. Wesley Corson for his computer programing. For their help with instrumentation, I thank D. Wesley Corson, Dr. Alvin A. Sieger, and Jonathan J. Shoukimas. I thank Dr. Samuel E. Allerton for the use of his gel electrophoesis apparatus and both Dr. Allerton and Mrs. Connie Chen for their technical help in preparing, running, staining, and interpreting the electrophoretic results. I am grateful to Dr. Robert M. Chew for providing a cold room, to Dr. Dorothy F. Soule for permitting me to use her atomic absorption spectrophotometer, to Dr. Donald J. Marsh for the use of his cryostat, and to Dr. Robert F. Bils for permission to use the facilities of the dark room and the electron microscope laboratory.

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Dr. Fernandez, Dr. Josephine Y. Yingst, Mr. Corson, Dr. O'Day, and Mr. Shoukimas all provided fruitful discussion and helpful suggestions at many times during this study.

My appreciation is also extended to Dr. Fernandez, Dr. Allerton, Dr. Kristian Fauchald, Dr. Leonard Muscatine, and Dr. Robert G. Douglas for reviewing this dissertation and serving on my committee.

Finally, a special thank you is due to my wife Josephine who packed boxes, made collecting traps, helped with laboratory analyses, fed animals, hauled me to and from the airport, typed, reviewed this manuscript, cheered, fed, and generally encouraged a fellow graduate student.

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INTRODUCTION

The blood of most teleosts freezes approximately 1°C above the freezing point of normal seawater (Black, 1951 and 1957). Hence, without some form of protection, these fish could freeze in polar seas where ice is present. Physiologists now know of at least three basic mechanisms by which ice crystal formation in the blood is either prevented or reduced. These include: the manufacture of antifreeze agents (DeVries and Wohlschlag, 1969; DeVries, 1971a), the depression of plasma freezing points through colligative action (Scholander, et al., 1957; Gordon, et al., 1962; Umminger, 1967 and 1969a; Smith, 1972), and the ability to supercool (Scholander, et al., 1957; Gordon, et al., 1962; Umminger, 1967 and 1969b; Smith, 1972). The blood of most marine invertebrates, on the other hand, is isoosmotic with seawater and would therefore freeze at the same temperature as seawater. For this reason, most investigators have assumed that freezing is not a danger for invertebrates unless they become entrapped in the ice (Scholander, et al., 1957; DeVries, 1971b). This notion has been so pervasive that to date no one has carefully investigated the freezing resistance of a marine invertebrate. Yet, if the blood of invertebrates freezes at the same temperature as seawater, why would it not freeze along with the seawater? This is especially pertinent for animals which live near the surface of the ocean, in

shallow areas, or in lagoons where they intimately contact growing and established ice. In Antarctica, for instance, Dayton, <u>et al</u>. (1969) reports numerous examples of live mobile epibenthic invertebrates entrapped in or firmly frozen into the sea ice. Do animals living in such close association with the sea ice live with ice crystals in their blood or do they somehow prevent the formation of such crystals and remain unfrozen?

Personal observations at Point Barrow, Alaska suggested that under some circumstances they do remain unfrozen. For instance, specimens of <u>Mesidotea entomon</u> (Linnaeus, 1758), a large benthic isopod, were often frozen into sea ice in the laboratory where they were maintained at -1.8 to -2.2°C in seawater with a salinity of approximately 33°/oo. If these animals were chipped free of the ice, they immediately crawled away. Those that were only partially encased often moved their free appendages. Also, rather than evading ice in the environment, this species actually demonstrated a predilection for it. For instance, specimens on the ice-free bottom of a twenty gallon aquarium filled with seawater with a salinity of 33°/oo at -1.9°C would swim to the surface to crawl, perch, and evidently to feed in the midst of growing ice spears and crystals on the underside of floating ice.

Since the above observations suggested that <u>M. entomon</u> did not freeze with the seawater or even when the animals became entrapped in the ice, previous explanations for the freezing resistance of marine invertebrates appeared inadequate (Scholander, <u>et al.</u>, 1957). In terms of M. entomon, some of the outstanding problems were: What is the

freezing point of the hemolymph? If it is the same as the seawater, does <u>M</u>. <u>entomon</u> freeze along with the seawater? Does <u>M</u>. <u>entomon</u> freeze when it becomes entrapped in ice? If <u>M</u>. <u>entomon</u> does not freeze, how is freezing prevented? If it does freeze, at what temperature does freezing begin? These questions were the impetus for my study and their investigation is the subject of this dissertation.

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MATERIALS AND METHODS

Field Work

During the late spring of 1973 and 1974 specimens of <u>Mesidotea</u> <u>entomon</u> were collected in baited traps lowered through holes cut in the sea ice of Elson Lagoon at Point Barrow, Alaska. Within the Lagoon the primary collecting holes were near Eluitkak Pass. Collected specimens were taken to the Naval Arctic Research Laboratory (NARL), maintained there in seawater and some were then shipped by air to Los Angeles in refrigerated styrofoam containers. In Los Angeles these isopods were maintained at -1.0° C to -2.0° C in twenty gallon aquaria filled with seawater with a salinity of approximately $34^{\circ}/00$.

The depth of the Lagoon at the collecting sites was sounded by a weighted rope marked in intervals of 0.5 m. The thickness of the ice was estimated by measuring the length of a sipre core drilled through the ice. Bottom water temperatures were measured by a partial immersion thermometer (marked in intervals of 0.2°C) immersed in a two liter water sample collected with a snatch bottle. The snatch bottle consisted of a weighted plastic bottle stoppered with a cork to which a light weight cord was afixed. When the bottle was on the bottom, the cork was pulled out, the bottle was allowed to fill, and then it was hauled to the surface. Water samples for chemical analysis were collected in the same manner in 120 ml plastic bottles. These samples were stored at 4°C up to two weeks until water samples were removed, filtered through a

0.8 μ Millipore filter, placed in 2 dram vials of borosilicate glass, and frozen at -20°C for subsequent chemical analysis. The salinity of the remaining water was determined on a standard Beckman salinometer.

Adaptation to Combinations of Temperature and Salinity

At NARL in 1973 animals were adapted for 6 days to 20 different combinations of salinity (4, 8, 16, 32, and $44^{\circ}/\circ\circ$) and temperature (2, 0, -1, -2°C). Seawater samples with salinities of $32^{\circ}/\circ\circ$ and $44^{\circ}/\circ\circ$ were collected from Elson Lagoon, whereas salinities of 4, 8, and $16^{\circ}/\circ\circ$ were made by diluting seawater of $32^{\circ}/\circ\circ$ with distilled water. The freezing points of hemolymph collected from animals at each combination of temperature and salinity were measured as described below and the results were both graphed and analyzed by a two-way analysis of variance. Although many more samples were collected, the data graphed and analyzed here are based on two separate hemolymph samples from each combination of temperature and salinity. The freezing point of each sample was measured once.

Extraction of Hemolymph

Hemolymph samples from animals in the above experiment were extracted by heart puncture with a 1 ml syringe fitted with a 24 gauge hypodermic needle. After extraction hemolymph samples were placed in 1 ml plastic centrifuge tubes which were covered with parafilm, and immediately frozen at -20°C. Sample processing was performed in the laboratory at 22 to 26°C.

At NARL in 1974 hemolymph was extracted within 30 hours of specimen collection and all samples were processed in a cold room between 2 and 8°C. After extraction the hemolymph was centrifuged 15 minutes at 500 g; the supernatant was removed, placed in capped vials of borosilicate glass, and frozen at -20°C. After collection and prior to extraction, specimens were maintained at -1 to -2°C in water from the collection sites.

In Vitro Melting and Freezing Points

The melting points of the hemolymph samples collected in the two different years were later determined in Los Angeles on two different instruments using the basic method of Ramsay and Brown (1955). The 1973 samples were measured using a cryostat with thermoelectric temperature control. Drops of hemolymph under parafin oil were placed in the holes of a small brass plate otherwise filled with parafin oil. This plate was sealed in a chamber filled with nitrogen, frozen to -5°C, and then automatically warmed at a rate of 0.01°C per minute. The hemolymph samples were observed using a dissecting microscope and the melting point was determined as the temperature at which the last crystal disappeared. The temperature of this melting point was determined in reference to the theoretical melting point temperatures of 3 salt solutions of known concentrations.

The melting points of hemolymph and seawater samples from 1974 were also determined by the direct observation of crystals, but in a different apparatus. Samples with a volume of approximately 10^{-6} 1

were taken up under parafin oil into capillary tubes of borosilicate glass. These capillaries were mounted under an observation window in a rectangular container 10 cm x 10 cm x 18 cm through which a 1:1 mixture of methanol and water was circulated. A thermistor (Yellow Springs Instruments (YSI) #427; t.c.= 300 ms) was mounted next to these tubes and with accompanying instrumentation, was used to read the bath temperature just outside the tubes. The fluid was circulated and the temperature maintained at any desired level within 0.02°C by a Lauda super K-2 circulating bath. To freeze the samples the capillary tubes were sprayed with freon outside the cooling box. Then the tubes were placed in the box where the circulating fluid had been precooled. This fluid was warmed at a rate of 0.01°C per minute and the melting point was determined by the forementioned criteria. In this case, however, temperature was determined directly, instead of in reference to standards. The melting points of the seawater and hemolymph were compared using a one-way analysis of variance.

The freezing behavior of the hemolymph was also studied using the above apparatus. Frozen samples were warmed to within a few hundreths of a degree of their melting points and then the circulating fluid was cooled down again while the behavior of the crystals was observed.

Chemical Analysis

Sodium concentrations were measured in triplicate by flame photometry. Samples were diluted in double glass distilled water and run
against standards prepared from a 1000 ppm stock solution manufactured by Harleco (Philadelphia, Pa.).

Chloride concentrations were determined using a silver nitrate titration with a potentiometric endpoint as described in the first micro method of Ramsay, <u>et al</u>. (1955). All samples were run in triplicate against a set of stock solutions made from reagent grade NaCl in double glass distilled water.

The concentrations of sodium and chloride in the seawater and hemolymph were compared with a one-way analysis of variance.

Macromolecular constituents of the hemolymph were separated by gel electrophoresis. For comparison, full strength hemolymph was run against controls of normal human serum (NHS) diluted in water and sucrose. Gels were stained with Coomassie Dlue for protein and with periodic acid-Schiff (PAS) for carbohydrates and glycoproteins. All gels were prerun for 30 minutes at 4mA/gel, 20 minutes at 2mA/gel, and 97 minutes at 4mA/gel.

In Vivo Freezing Point

To detect the freezing point of the hemolymph <u>in vivo</u>, a thermistor was inserted in the hemocoel of <u>M</u>. <u>entomon</u>. Then the animal was cooled at a steady rate in seawater seeded with ice, and its internal temperature was plotted versus time. On such a graph the temperature decreases steadily until it either levels off or begins to rise to a temperature at which it does plateau. After reaching a plateau the temperature again decreases. The length of time the temperature remains at the plateau is inversely related to the cooling

rate and in the case of fast cooling rates the plateau appears more like a peak. In either case, the temperature at the plateau is the <u>in vivo</u> freezing point of the hemolymph.

The chamber built for these experiments is shown in Plate 1. Throughout the experiments animals were immobilized and fastened to a plexiglass stand with rubber bands (Plate 2). The thermistor which was cemented in the tip of a 26 gauge hypodermic probe (YSI #524; t.c.=100 ms) was inserted approximately 3 mm under the cuticle and cemented in place with a cyanoacrylic glue which was coated with silicon grease when dry. All specimens were between molts and were the same size (approximately 57 mm long). With one implanted thermistor, the probe was always inserted through the dorsum in the middle of the thoracic hemocoel. With two probes one was inserted into the thoracic hemocoel off the midline just behind the head. The second was approximately 15 mm more posterior in the most posterior region of the thoracic hemocoel. After the insertion of the probes, the preparation was placed in the center of the cooling chamber (Plate 3), submerged in approximately 100 ml of seawater, and cooled at a chosen rate by circulating cooling fluid in the exterior chamber. When the temperature of the seawater was about 0.2°C below its freezing point, it was artificially seeded by adding ice, and then further cooled.

Most animals were frozen in segwater with a salinity of $34^{\circ}/00$ in which they had been living at -1 to $-2^{\circ}C$ for several months. In one control experiment 0.4 ml of hemolymph from each of two animals was removed with a syringe and an equal amount of seawater with a salinity of $34^{\circ}/00$ was injected in its place. The thermistor probe was inserted

into the hole made by the hypodermic needle and the experiment was carried out as described above. In another set of experiments animals from the aquaria were frozen in instant ocean with salinities of $15^{\circ}/\circ\circ$ and $42^{\circ}/\circ\circ$ to which they had been adapted for at least 48 hours.

Cuticle Permeability

To experimentally study the ability of the cuticle to retard the propagation of ice, the permeability of the cuticle was measured <u>in</u> <u>vitro</u>. Fresh pieces of cuticle, minus the epidermis and underlying tissue, were cemented with cyanoacrylic glue over a 5 mm diameter hole between two chambers each with a 50 ml capacity. The chambers were filled with seawater, cooled, and the chamber on the exterior side of the cuticle was seeded with ice. After seeding the temperature in both chambers was either progressively lowered or set at a predetermined level while the unseeded chamber (equivalent to the inside of the animal) was visually observed with a dissecting microscope to determine when and how it would become nucleated. The temperature in each chamber was measured by a thermistor (YSI #427; t.c.= 300 ms) placed at the level of the hole on either side of the partition separating the two chambers.

Thermistor Calibration

Thermistors were connected to a Wheatstone bridge and a chart recorder through a preamplifier. Each thermistor was individually calibrated against a total immersion thermometer (Brooklyn thermometer

#22620) graduated in hundreths of a degree centigrade. This thermometer without calibration is accurate to \pm 0.03°C and is precise to \pm 0.005°C. To increase its accuracy, this thermometer was calibrated in an ice bath made from double glass distilled water following the methods and procedures outlined for such calibrations in monograph #90 of the National Bureau of Standards (Swindells, 1965) and in Hall (1953). Corrections for use of the thermometer at partial immersion were also made following the procedure outlined in monograph #90.

After calibration, temperatures determined with the thermistors were precise to \pm 0.01°C and accurate to \pm 0.04°C. During the course of the various experiments using thermistors, the thermometer was calibrated on two separate occasions and the thermistors four different times, equivalent to once every three weeks of use. From one calibration to the next there was very little variation in the thermistor readings.

Structure of the Cuticle

Cuticle to be examined in the transmission electron microscope was dissected from fresh specimens. Pieces of cuticle were removed from a ventral stergite, the last pair of pleopods, and the arthrodial membrane between two ventral stergites. Fixation was 2 hours in 2.5% gluteraldehyde in Millonig phosphate buffer (pH 7.4), and then rinsed in Millonig buffer. Pieces of ventral cuticle and arthrodial membrane were decalcified for 48 hours in a 10% solution of ethylenediamine tetraacetate (the disodium salt) in Millonig buffer (pH 7.4), whereas

cuticle from the last pair of pleopods was not decalcified. Post fixation was in osmium tetroxide for two hours and embedding was in hard epon. Thin sections were cut on a Sorvall M-2 microtome, stained with uranyl acetate and lead, and examined and photographed on an electron microscope.

Pieces of cuticle to be examined in the scanning electron microscope (SEM) were dissected from freshly sacrificed specimens and fixed in 5% gluteraldehyde for 30 days. After fixation the epidermis and underlying tissue were removed from the cuticle. The pieces were then critically point dried and subsequently shadowed with a mixture of platinum and gold. After this preparation the cuticle was observed and photographed on a scanning electron microscope.

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RESULTS

Physical Description of the Collecting Area

The environmental conditions at the collection sites in Elson Lagoon during 1974 are summerized in Table 1 and 2.

Chemical Analyses of Seawater and Hemolymph

Ionic concentrations and freezing point. The mean freezing point of the hemolymph from freshly collected animals (Table 3) was not significantly different (P> 0.05) from that of the seawater (Table 2) in which they were living (Table 4). Similarly, the concentration of sodium and chloride in the hemolymph (Table 3) was not significantly different (P> 0.05) (Tables 5 and 6) from the concentration of these ions in seawater (Table 2). If concentrations of the other cations in the hemolymph are assumed to be equal to their concentrations in seawater, and the concentrations of all ions in the hemolymph are summed, the theoretical freezing point based on their total concentration accounts for most of the measured freezing point (Table 3).

Organic constituents of the hemolymph. To determine the relative amounts of protein, glycoprotein, and carbohydrate present in the

Physical Description of the Collecting Area in Elson Lagoon, Alaska

Sample	Date	Depth	Thickness	Temperature	Salinity
		(ш)	07 1CC (m)	of water (°C)	(00/0)
1	5/30/74	4.5	1.90	-1.8	32.0131
2	5/30/74	2.8	1.90	-1.8	31.5688
3	6/15/74	4.5	1.78	-1.8	33.5984
4	6/21/74	4.5	÷.	-1.8	35.5645
ı×	•	•	•	-1.8	33.1862

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Chemical Description of the Seawater from the Collecting Area in Elson Lagoon (The number in () is the number of replicates)

ing Point (°C)	37 (7)	32 (3))2 (7)	18 (5)	L L
Freez	-1.8	-1.8	-2.(-2.(0 [-
C1 ⁻ (mM/Kg H ₂ 0)	534 (3)	508 (3)	561 (3)	559 (3)	
Na+ (mM/Kg 1120)	422 (3)	449 (3)	459 (3)	430 (3)	440
Salinity (0/00)	32.0131 (1)	31.5688 (1)	33.5934 (1)	35.5645 (1)	33.1862
Date	5/30/74	5/30/74	6/15/74	6/21/74	
Sample	-	2	3	4	×

The Ionic Composition and Freezing Point of Hemolymph withdrawn from Mesidotea entomon soon after capture (F.P. is an abbreviation for freezing point) (The number in () is the number of replicates)

Sample	Date	Na+	-C1-	Estimated conc.	Experimental	Calculated
	I	(mM/Kg 1120)	(mi/Kg H ₂ 0)	or other cations (mM/Kg H ₂ 0)	F.P. (°C)	F.P. due to ions (°C)
-	5/30/74	397	508		-1.97 (10)	
2	6/6/74	445	524		-1.82 (8)	
5	6/11/74	442	519		-2.01 (8)	
4	6/18/74	449	517	· ·	-1.95 (10)	
23	6/18/74	459	529		-1.99 (15)	
				68		-1.94
×		438	519		-1.95	

Comparison of the Freezing Points of Seawater and Hemolymph, using One-Way Analysis of Variance

Hypotheses:

 $H_0: \mu_1 = \mu_2$ H1: 11 # 12

One-Way Analysis of Variance Table:

Source	SS	df	MS	F	
between	5.56 x 10^{-7}	1	5.56 x 10^{-7}	5.76 x 10^{-5}	
error	6.76×10^{-2}	7	9.65 x 10^{-3}	-	
total	6.76×10^{-2}	8	9.82×10^{-2}	-	

Critical value: F 0.05 (1,7) = 5.59

Conclusion: Since 5.76 x 10^{-5} < 5.59, H_o is accepted. (There is no significant difference (P > 0.05) between the freezing points of the seawater and hemolymph.)

Comparison of the Concentration of Sodium in the Seawater and in the Hemolymph, using Gne-Way Analysis of Variance

Hypotheses:

$$H_0: \mu_1 = \mu_2$$

 $H_1: \mu_1 \neq \mu_2$

One-Way Analysis of Variance Table:

Source	SS	df	MS	F
between	5.69	1	5.69	1.25×10^{-2}
error	3.17×10^3	7	4.53×10^2	-
total	3.18×10^3	8	2.13 x 10 ¹	-

Critical value: F 0.05 (1,7) = 5.59 Conclusion: Since 1.25 x 10^{-2} < 5.59, H₀ is accepted. (There is no significant difference (P > 0.05) between the concentration of sodium in the seawater and in the hemolymph.)

Comparison of the Concentration of Chloride in the Seawater and in the Hemolymph, using One-Way Analysis of Variance

Hypotheses: $H_0: \mu_1 = \mu_2$

$$H_1: \mu_1 \neq \mu_2$$

One-Way Analysis of Variance Table:

Source	SS	df	MS	F	
between	9.89 x 10^2	1	9.89 x 10 ²	3.28	
error	2.11 x 10^3	7	3.01×10^2	-	
total	3.10×10^3	8	1.70×10^{1}	-	

Critical value: F 0.05 (1,7) = 5.59

Conclusion: Since 3.28 < 5.59, H_0 is accepted. (There is no significant difference (P > 0.05) between the concentration of chloride in the seawater and in the hemolymph.)

hemolymph, the position of protein bands separated on an acrylomide gel (stained with Coomassie Blue) are compared to the positions of bands of glycoprotein and/or carbohydrate on another gel (stained with PAS). This comparison of the positions of the bands (Plate 4) demonstrates that out of the approximately 6 bands of protein, one (band A), and perhaps four (bands A, B, C, and D), are glycoprotein. By quantity, however, the preponderance of the protein (bands E and F, and perhaps also B, C, and D) is not glycoprotein. The comparison shown in Plate 4 also demonstrates that little if any high molecular weight carbohydrate is present in the hemolymph.

To demonstrate the reliability of the separation and staining techniques which form the basis of the above comparisons and to provide a qualitative measure of the mobility of the macromolecular species represented by the bands, each of the gels shown in Plate 4 are individually compared to centrols of normal human serum (NHS) run and stained along with the samples of hemolymph. The bands in the control of NHS stained for protein (Plate 5) have the following identities: $1 = \gamma$ globulin, $3 = \beta - 2$ globulin, $4 = \beta - 1$ globulin, transferrin, $5 = \alpha - 2$ globulin, $6 = \alpha - 1$ globulin, and 7 = albumin. In Plate 6 the bands in the control of NHS stained for carbohydrates and glycoproteins are: 1 to 6 = same as in Plate 4 and 7 = prealbumin.

The Influence of Temperature and Salinity on the Freezing Point of the Hemolymph

The <u>in vitro</u> freezing points of hemolymph extracted from animals adapted to twenty different combinations of temperature and salinity

were affected by salinity (P< 0.05), but not by temperature (P> 0.05), nor by the interaction of temperature and salinity (P> 0.05), as measured by a two-way analysis of variance (Table 7) and as shown graphically (Fig. 1).

Data from these same series of experiments also demonstrated that specimens adapted to salinities below $23^{\circ}/oo$ maintain their hemolymph hyperosmotic to the ambient seawater. This phenomenon is shown in Fig. 1 by the position of the mean freezing points of the hemolymph above the unit slope line from which the freezing point of seawater with a given salinity can be determined. On the other hand, animals adapted to seawater with salinities from $28^{\circ}/oo$ to $32^{\circ}/oo$ may maintain their hemolymph slightly hypoosmotic to the seawater. Finally, animals adapted to seawater with salinities of $34^{\circ}/oo$ and above have hemolymph concentrations which tend to be isoosmotic with the seawater (Fig. 1).

A Comparison of the Melting and Freezing Points of Hemolymph

The melting and freezing points of hemolymph from five different specimens were equal. Single rounded crystals in capillary tubes a couple hundreths of a degree below their melting points would become rectangular and immediately begin to grow without hysteresis when the temperature of the bath was reduced as little as 0.05°C. In this respect the crystals behaved identically to crystals in solutions of sodium chloride.

The rate at which the crystals grew, however, was very sensitive to the cooling rate. Also, the manner in which the crystals in the

The Effect of Temperature (Factor A) and Salinity (Factor B) on the Freezing Point of the Hemolymph, using Two-Way Analysis of Variance

Null Hypotheses:

Two-Way Analysis of Variance Table:

Source	SS	df	MS
Factor A	0.16	3	0.0560
Factor B	8.73	4	2.1800
Interaction AB	2.25	12	0.1870
Error (E)	2.52	19	0.1330
Total	13.70	39	-

Null Hypotheses Examined:

 $H_{o}: all (αβ)_{ij} = 0$ $F = MS_{AB} / MS_{E} = 1.41$ Critical value: F 0.05 (12,19) = 2.31Conclusion: Since 1.41 < 2.31, H_o is accepted. (There is no interaction between temperature and salinity.)

$$H_0$$
: all $\alpha_i = 0$
 $F = MS_A / MS_E = 0.40$
Critical value: F 0.05 (3,19) = 3.13

Table 7 - Continued

Conclusion: Since 0.40 < 3.13, H_o is accepted. (There is no temperature effect.)

$$H_0: all \beta_j = 0$$

 $F = MS_B / MS_E = 16.39$

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Critical value: F 0.05 (4,19) = 2.90

Conclusion: Since 16.39 >2.90, H_0 is rejected. (There is a salinity effect.)

Fig. 1 The <u>in vitro</u> freezing point of hemolymph from <u>Mesidotea</u> <u>entomon</u> versus the salinity to which specimens were adapted at different temperatures. Each open symbol is the mean for two separate hemolymph samples, each measured once. The closed symbol is the mean for the four values at each salinity. The expected freezing point of seawater of any given salinity can be determined from the unit slope line.

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hemolymph enlarged was qualitatively different from crystals in solutions of sodium chloride with similar freezing points. Such differences prevented a quantitative comparison of the rates of crystal growth in the two types of solutions.

Measurement of the In Vivo Freezing Point

Animals adapted to seawater with a salinity of $34^{\circ}/\circ o$ at approximately -1.5°C, which represents conditions very similar to those during the winter in Elson Lagoon, had <u>in vivo</u> freezing points approximately 0.3°C below the temperature one would expect of an animal which was isoosmotic with the seawater (Fig. 2).

When two probes were placed in the hemocoel of animals adapted to seawater of $34^{\circ}/_{\circ\circ}$, the anterior probe recorded freezing a few minutes before the posterior probe (Fig. 3). Nevertheless, in a given experiment the freezing point as given by the plateau temperatures were approximately the same for the two probes.

If the hemolymph was removed from specimens adapted to seawater with a salinity of $34^{\circ}/\circ\circ$ and seawater with a salinity of $34^{\circ}/\circ\circ$ was injected in its place, the freezing point of the seawater <u>in vivo</u> was also 0.3° C below its in <u>vitro</u> freezing point (Fig. 4).

Specimens adapted to seawater with a salinity of $42^{\circ}/co$ also froze approximately 0.3°C below the expected <u>in vitro</u> freezing point (Fig. 5). On the other hand, animals adapted to seawater of $15^{\circ}/co$ froze <u>in vivo</u> (Fig. 5) at approximately the temperature one would expect based on <u>in vitro</u> freezing point measurements (Fig. 1). Fig. 2 The temperature and <u>in vivo</u> freezing point of <u>Mesidotea</u> <u>entomon</u> adapted to seawater with a salinity of approximately <u>34⁰/oo</u>, temperature recorded from the center of the thoracic hemocoel. A continuous line through a series of symbols of a given design represents an idealization of the data from one experiment. Each experiment is indicated by a symbol of a different design.

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Fig. 3 The temperature and in vivo freezing point of <u>Mesidotea</u> entomon adapted to seawater with a salinity of approximately 34°/00, temperature recorded simultaneously from the anterior and posterior regions of the thoracic hemocoel.

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Fig. 4 The temperature and <u>in vivo</u> freezing point of <u>Mesidotea</u> <u>entomon</u> with the hemolymph removed and seawater with a salinity of 34⁰/oo injected in its place, temperature recorded from the center of the thoracic hemocoel.

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Fig. 5 The internal temperature and in vivo freezing point of <u>Mesidotea entomon</u> adapted to different salinities, recorded from the center of the thoracic hemocoel. Two separate experiments were run at each salinity; triangles represent animals adapted to seawater with a salinity of 420/00, squares those adapted to 340/00, and circles those adapted to 150/00. The data in [] is the expected in vitro freezing point for animals adapted to seawater with the same salinity.

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Cuticular Permeability

Even after considerable ice formation (artificially induced) in the chamber representing the outside of the animal, no seeding of the supercooled chamber representing the inside of the animal was seen to occur across the cuticle mounted over a hole between the two chambers (Table 8). In experiments during which the chamber representing the inside of the animal did freeze (whether or not it was caused by inoculation through the cuticle), it did so most often at approximately -5.0°C when the supercooled water was unstable and freezing could be induced by the slightest vibration of the chamber.

Cuticle Morphology

<u>Stergite</u>. The general organization of the ventral cuticle of <u>M. entomon</u> follows the general arthropod pattern (Hackman, 1971), as may be partially seen in Plate 7. The proximal epidermis (EP) gives rise to the more distal endocuticle (EN) which is in turn overlain by the exocuticle (EX) (Plate 7). The epicuticle (EPC), the outermost subdivision of the cuticle, (Plate 11) is torn from the section in Plate 7, but if present it would be attached to the distal border of the exocuticle located in the upper right hand corner of Plate 7. Pore canals which might serve as paths through which external ice could grow into the hemocoel arise at the inner boundary of the endocuticle (Plate 7, EN, arrow; and Plate 8, arrow) and slowly enlarge until they reach their largest diameter in the exocuticle (Plate 7, EX, arrow; and

The Permeability of Various Cuticle Pieces to Ice Propagation Measured In Vitro

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Type of cuticle preparation	Conditions i when "outside artificia "inside" temp./time (°C) (min.)	<pre>n the chambers " chamber was]]y seeded "outside" temp./time (°C) (min.)</pre>	Conditions at experiment and/ "inside" ch "inside" temp./time (°C) (min.)	the end of the or at the moment amber froze "outside" temp./time (°C) (min)	How "inside" chamber froze
Tergite Run l.	-3.5 0 (unfrozen)	-1.8 0 (frozen)	-4.5 34 (frozen)	-2.5 34 (frozen)	not known
Run 2.	-2.4 0 (unfrozen)	-1.8 0 (frozen)	-5.5 90 (frozen)	-3.8 90 (frozen)	not known
Stergite with arthrodial membrane Run l.	-2.7 0 (unfrozen)	-1.9 ((frozen)	-5.0 50 (frozen)	-2.7 50 (frozen)	chamber jarred
Run 2. Bun 3.	-2.4 0 (unfrozen) -3.0 0	-2.0 0 (frozen) -1 9 0	-2.7 180 (unfrozen) -2.6.440	-2.8 180 (frozen)	•
Tergite with arthrodial membrane	(unfrozen)	(frozen)	(unfrozen)	(frozen)	
Run 1. 36	-2.9 0 (unfrozen)	-1.9 0 (frozen)	-5.0 240 (frozen)	-5.0 240 (frozen)	not known

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Plate 9, arrow). Accordingly, the diameter of pores in the center of the endocuticle (Plate 8, arrow) is approximately 710 Å whereas those in the distal exocuticle are 1,700 Å (Plate 9, arrow). Some of the pores in the exocuticle contain filaments which may be cytoplasmic extensions of the epidermis (Plate 10, arrow). Pores approximately 50 Å in diameter do enter the epicuticle (Plate 11, EPC, arrow), but they do not seem to traverse the outermost layer of the epicuticle which, incidentally, supports many bacteria.

<u>Arthrodial membrane</u>. The general structure of the arthrodial membrane, including the location of pores, is similar to the stergites. The diameter of the pores in the epicuticle is 210 Å (Plate 12). In Plate 12 note that the pores do continue from the exocuticle into the epicuticle (arrow), but that these pores do not seem to traverse the outermost layer of the epicuticle.

<u>Pleopods</u>. Although the cuticle of the pleopods (Plate 13) is much thinner than that of the stergites or arthrodial membrane, its general structure is similar to that of the stergite and arthrodial membrane. One important difference, however, is that pores of the size found in the other types of cuticle are not visible. Attempts to photograph the cuticle at higher magnification, in order to look for smaller pores, failed due to poor resolution at those magnifications.

<u>Surface structure of a tergite</u>. The exterior surface of the epicuticle of a tergite is shown in Plates 14 and 15. No pore-like openings are present as can be seen in Plate 15 which shows an area representative of those examined. If pores 100 Å in diameter were present in Plate 15, they would appear as structures 0.35 mm in

diameter and would be clearly visible. The homogeneous surface of the epicuticle (Plates 14 and 15) should be compared with the interior surface of the endocuticle of the same tergite (Plates 16 and 17) which is both fibrous and porous and therefore could give rise to numerous pores. This fibrillar surface of the endocuticle interfaces with the epidermis which has been removed.

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DISCUSSION

The Difference between the <u>In Vitro</u> and <u>In Vivo</u> Freezing Points

Using the criteria of earlier investigators such as Scholander, et al. (1957), Mesidotea entomon should freeze along with the seawater under the winter conditions existing in Elson Lagoon, because the in vitro freezing point of the hemolymph is the same as the freezing point of the seawater. Yet, M. entomon actually freezes 0.3°C below the freezing point of the seawater as shown by the in vivo freezing points of the hemolymph in animals adapted to seawater with a salinity of 34⁰/00. This difference in freezing points is evidently responsible for the ability of M. entomon to remain active and unfrozen when in contact with established ice and growing crystals, and its ability to move its free appendages while partially frozen into the ice, as observed in the laboratory. Due to the shallow habitat of this species, similar conditions undoubtedly arise in the field and this difference between the in vitro and in vivo freezing points may represent the margin of safety which permits M. entomon to live in such close association with the ice. This difference in freezing points also clearly suggests the naivete of assuming that in vitro measurements are reliable estimates of physiological freezing points, as others have often inferred about invertebrates (Scholander, et al.,

1957; DeVries, 1971b).

Possible Explanation for the Difference in Freezing Points

Antifreeze. The difference between in vitro and in vivo freezing points could be explained by a chemical antifreeze similar to a glycoprotein present in the blood of some Antarctic fishes of the genus Trematomus (DeVries and Wohlschlag, 1969; DeVries, 1971a). This antifreeze is effective by somehow creating a hysteresis between the melting and freezing points of the blood of the fish. For example, the blood of these fish containing the antifreeze might have a melting point of -0.8°C, determined by the temperature at which the last crystal melts in a small sample in a capillary tube. On the other hand, when a crystal is present in the sample at a temperature just below the melting point, and the temperature of the sample is then lowered, the crystal will not enlarge until the temperature is lowered more than a degree centigrade. The physical-chemical mechanism responsible for this hysteresis is not yet understood, but it is not primarily due to the colligative action of the glycoprotein or to supercooling.

An antifreeze, however, is probably not present in the hemolymph of <u>M</u>. <u>entomon</u> for the following reasons. First, the <u>in vitro</u> melting and freezing points of the hemolymph are the same, i.e., an ice crystal in a hemolymph sample just below its melting point will enlarge as soon as the temperature is lowered. Second, there are no large quantities of glycoproteins present in the hemolymph. Third,

specimens with seawater injected in place of the hemolymph freeze at the same temperature as those with their hemolymph intact.

<u>Artifacts</u>. Two possible experimental artifacts might also explain the difference between the <u>in vivo</u> and <u>in vitro</u> freezing points. The first possibility is that freezing <u>in vivo</u> may have begun undetected at a point distant from the thermistor. Then as water was removed from the solution as ice, the freezing point of the remaining solution was progressively depressed until the hemolymph which froze in range of the probe actually had a lower freezing point than the original solution. The results of the dual probe experiment (Fig. 3) do in fact show that all of the body fluids do not freeze simultaneously, because the temperature at the anterior probe does rise a few minutes before the temperature at the posterior probe. On the other hand, the freezing point (as given by the plateau temperature) is almost the same for the two probes in a given experiment, demonstrating that the freezing point of the hemolymph is not significantly depressed by prior freezing.

Another potential artifact is that the temperature of the hemolymph did not rise to its true freezing point owing to the loss of too much heat through the cuticle and into the bath of ice and water. Indeed, it is difficult to entirely eliminate this factor as a potential source of error. On the other hand, if the freezing point of the hemolymph had been sensitive to heat loss, one would expect the freezing point to vary with the cooling rate, i.e., the rate at which heat is removed from the hemolymph. The data in Fig. 2, however, show that freezing points did not vary with the cooling rates employed,
although the faster cooling rates did markedly decrease the time at which the temperature remained at the freezing point. Therefore, the loss of latent heat is not sufficient to explain the existence of a difference between the <u>in vitro</u> and <u>in vivo</u> freezing points, although it may have slightly affected the magnitude of the differences.

Hypothesized Mechanism by which the In Vivo Freezing Point is Lowered

<u>Mechanism</u>. As the seawater freezes, <u>M. entomon</u> may take up ions attempting to remain isotonic with the seawater which is becoming progressively more concentrated as it freezes. During the uptake of ions, inoculation of the hemolymph is prevented by the cuticle which effectively isolates the hemolymph from the external ice. Without this cuticular barrier, ice would penetrate into the animal and the hemolymph would freeze along with the seawater, because at no point during the process is the freezing point of the hemolymph lower than that of the seawater. Thus, according to this explanation, the freezing resistance of <u>M. entomon</u> is based on a dual mechanism: prevention of inoculation by the cuticle and an ability to depress the freezing point of the hemolymph as the concentration of salts in the seawater increases due to freezing.

<u>Initiation of ice formation in the hemolymph</u>. The emphasis on inoculation of the hemolymph by the external ice as the mechanism by which freezing is initiated in the hemolymph, versus seeding by some other component of the hemolymph or spontaneous nucleation, is founded on the following considerations. First, pure water in the absence of

nucleating agents can be cooled many degrees past its thermodynamic equilibrium freezing point, i.e., supercooled before it spontaneously freezes. For instance, a drop of pure water 1 cm in diameter will not spontaneously freeze until the temperature is below -30°C (Fletcher, 1962 in Mazur, 1966). The temperature at which supercooled water freezes may be raised considerably by nucleating agents. Effective agents, however, are crystalline substances similar to ice which are not readily soluble in water (Mazur, 1966) and are therefore probably not present in the hemolymph. Solute mclecules in solution apparently are not nucleating agents either. They have in fact been shown to lower supercooling points approximately in proportion to the amount they reduce equilibrium freezing points (Lusena, 1955; Pruppacher, 1963). Organic molecules in solution and structural proteins are also ineffective nucleating agents (Mazur, 1966).

The data of Salt (1965) which shows that many insects supercool 20°C in dry air, and most of them at least 10°C, substantiate the contention that the hemolymph of arthropods does not contain effective nucleating agents. Sømme (1964) also found that most insects could be supercooled in dry air to at least -20°C, and some to as much as -44°C, although in the latter case the extent of supercooling may have been increased by the presence of glycerol which is thought to stabilize supercooled fluids.

Considering these facts and the experimental procedure used to freeze <u>M</u>. <u>entomon</u>, it is safe to assume that freezing of the hemolymph occurred through inoculation of the hemolymph by the external ice. For

this inoculation to occur, the external ice would have to pass through various barriers which might include: the cuticle, the basement membrane, membranes of the alimentary canal or any other barrier between the ice and the hemolymph.

<u>Permeability of the cuticle to ice</u>. <u>In vitro</u> studies of seeding across the cuticle substantiate the view that the cuticle is capable of thwarting the growth of ice (Table 8). In this respect the cuticle is quite different from the integument of some fishes through which ice easily grows (Scholander, 1957).

The structure of the cuticie in turn supports the view that the cuticle is capable of being a barrier to ice propagation. Pore canals with diameters comparable with those in the endocuticle and exocuticle of the fiddler crab (Green and Neff, 1972) are present in the endocuticle and exocuticle of M. entomon. Such pores could be channels for ice propagation, but in M. entomon these pores, as examined in the transmission electron microscope and SEM, teminate before they traverse the outermost layer of the epicuticle. This result stands in contrast to the cuticle of the crayfish in which much larger pores traverse the entire cuticle (Travis, 1965), but is similar to the cuticle of the fiddler crab in which pore canals do not enter the epicuticle. If the presence of this outermost layer of the epicuticle in M. entomon is primarily responsible for preventing ice from growing across the cuticle, then this layer would be serving a function similar to the outermost layer of the epicuticle in many insects, which determines the permeability of the cuticle to the transpiration of water (Ebeling, 1964; Locke, 1965).

On the other hand, the impermeability of the cuticle to the growth of ice may primarily, or at least in part, be due to the small diameters of the pores in the epicuticle (Salt, 1963). Determining the temperature at which ice will grow through a pore of submicron dimensions is equivalent to finding the freezing point of the solution in the pore. Other things being equal, that freezing point depression is inversely related to the diameter of the pore (Mazur, 1966).

Mazur (1966) derived a quantitative expression describing the growth of ice through pores in cellular membranes which is helpful in determining if the diameter of the pore canals of <u>M. entomon</u> are small enough to constitute a barrier to the propagation of ice. His expression is equivalent to the Kelvin equation and is based on changes in free energy associated with the transfer of ice in the environment to ice in the pore and in the free energy changes associated with the transfer of bulk water to water in the pore (see Fig. 1 in Mazur, 1966). In this expression the depression of the freezing point of pure water in the pore (Δ T) is:

$$\Delta T = \frac{2V_1 T_f \sigma_{s1} \cos \Theta}{r L_f}$$

where V₁ is the molar volume of water, T_f is the freezing point of bulk water, σ_{s1} is the interfacial energy between the ice and water, 0 is the angle of contact between the ice and the wall of the pore, r is the radius of the pore in angstroms, and L_f is the molar heat of fusion. By estimating σ_{s1} to be 20 erg/cm² for the ice-water interface (Fletcher, 1962 in Mazur, 1966), and assuming that 0 is 0° (complete wetting of the

pore wall by water), and substituting numerical values for V_1 , T_f , and L_f , this equation becomes

$$\Delta T = \frac{300}{r}$$

Hence, a pore with a radius of 25 Å, as measured in the epicuticle of <u>M. entomon</u>, would depress the freezing point of any solution it contained by 12°C. A pore with a radius of 300 Å would depress the freezing point 1°C.

There is evidence, however, that the Kelvin equation in the final form above may overestimate the depression caused by pores with small diameters. The pores in cellular membranes are a case in point. Estimates for the radii of pores in the cellular membrane range from 3.5 Å to 8 Å (Solomon, 1960; Whittembury, 1962, Villegas, 1963). Thus, using the Kelvin equation one would expect a cellular membrane to be a barrier to ice propagation somewhere between -38°C and -86°C. Yet, there is a variety of experimental evidence (Mazur, 1965) which suggests that the cellular membrane ceases to be a barrier below -10°C. Various numerical values in the Kelvin equation may be changed to increase the agreement between the experimental data and the theoretical predictions. For instance, if the angle of contact between the ice and the pore wall is greater than 0°C, as one might expect if the interior of the pores is hydrophobic, then $\cos \Theta$ is less than 1 and the freezing point depression is less than the previous estimate. Since one might expect that the walls of the pore canals in M. entomon to be hydrophobic, it is plausible that 12°C is an overestimate for the freezing point depression of fluid in a pore with a radius of 25 Å. Also, as Mazur (1965) himself notes and Viaud (1972) demonstrates, the

Kelvin equation must be cautiously applied to microscopic systems where concepts such as interfacial energies, contact angles, and radii of curvature begin to lose their meaning. However, even with these shortcomings, the Kelvin equation is sufficiently accurate in its predictions for one to assume that the diameter of pores in the epicuticle of <u>M</u>. <u>entomon</u> could depress the freezing point of fluid they contained an amount sufficient to constitute an effective barrier to the penetration of ice, because even a depression as small as 1° C would be effective in preventing ice propagation at the temperatures relevant to <u>M</u>. <u>entomon</u>.

This conclusion is supported by the experimental data of Viaud (1972) who demonstrated that Millipore filters having pores with a radius of 125 Å will lower the freezing point of water 2.05°C and that those with pores with radii of 500 Å will depress the freezing point 1.65°C. These results further substantiate the contention that the impermeability of the cuticle to the growth of ice across it, as directly measured in the <u>in vitro</u> studies of ice propagation and as required by the <u>in vivo</u> freezing experiments, could be explained in terms of pore size alone, since the pores in the epicuticle have radii in the range of 25 to 100 Å.

All of the above discussion of the effect of pore sizes on the freezing point of water assumed, among other things, that the pores had a uniform radius and were filled only with pure water. Of course, the diameter of pore canals in the arthropod cuticle are irregular and there is evidence that they contain filaments (Plate 10) (Neville and Luke, 1969; Neville, et al., 1969) which may be cytoplasmic extensions

of the epidermis (Neville and Luke, 1969), not to mention inorganic solutes and organic molecules. Salt (1963) qualitatively discussed some of these factors affecting the growth of ice through the cuticle of insects. He also experimentally demonstrated that the cuticles of several insects do retard the propagation of ice and prevent inoculation while the cuticle is in contact with ice and the blood is supercooled several degrees centrigrade.

Wherever the actual site of inoculation, the mechanism of inoculation appears to be sensitive to the concentration of salts in the hemolymph, as shown by the set of adaptation experiments in Fig. 5. In these experiments animals adapted to seawater with a salinity of 150/00 froze in vivo at the temperature expected on the basis of in vitro measurements, whereas those adapted to salinities of 34°/oo and 420/oo froze approximately 0.3°C below the temperature expected on the basis of in vitro measurements. Although one would expect increased salt concentrations in the cuticular pores to decrease the freezing point of the hemolymph contained therein (Lusena and Cook, 1953), the small diameter of the pores in the cuticle of M. entomon would alone depress the freezing point a number of degrees centigrade below the temperature at which inoculation occurs. Therefore, in the temperature range of interest, freezing of the pore fluid would not be sensitive to changes in salt concentrations. This consideration plus the probable termination of the pores in the epicutic's, make it unlikely that inoculation occurs across the cuticle proper.

Possible Site of Inoculation

In addition to a sensitivity to the concentration of salts in the medium and/or hemolymph, another clue to the location of inoculation is given in the dual probe experiment (Fig. 3). In both experiments freezing first began in the anterior of the animal and moved posteriorly (Fig. 3). The one site in M. entomon which would be sensitive to varying salt concentrations in the hemolymph and is also located in the anterior of the animal, is the excretory organ, the maxillary gland. If at low salinities this gland produced a urine hypotonic to the hemolymph as part of the mechanism by which the hemolymph was maintained hyperosmotic to the seawater, the freezing point of the urine would be higher than that of the hemolymph. Since the maxillary gland opens to the exterior by an aperture, freezing could begin in this gland before the temperature in the animal dropped to the freezing point of the hemolymph. Also, if the fluid channels through any barriers between the lumen of the gland and the hemolymph were larger than the pores through the cuticle, the freezing point of the fluid in them might be sensitive to changes in salt concentrations in the temperature range at which inoculation occurs. In the case of a hypotonic urine, the freezing point of the fluid in the channel between the gland lumen and the hemolymph might be sufficiently high to allow the passage of ice which could in turn inoculate the hemolymph. If the urine were isoosmotic with the hemolymph at salinities of 320/00 and above, the freezing point of the fluid between the gland and the hemolymph might be high enough to prevent inoculation until the temperature dropped to a critical point, i.e., 0.3°C below

the in vitro freezing point of the hemolymph.

Whether or not the above is a plausible description of how inoculation might occur in the maxillary gland, depends both on the morphology of the maxillary gland and its physiological capabilities. The latter has never been studied in the maxillary gland of any crustacean and the former is only very generally known for isopods (Goodrich, 1945). In the brine shrimp <u>Artemia salina</u>, however, the ultrastructure of the maxillary gland is at least consistent with the ultrafiltration of a primary urine (Tyson, 1968), which could be hypoosmotic to the hemolymph. Thus, the impermeability of the cuticle, the probably location of the inoculation site in the anterior of the animal and the apparent sensitivity of the inoculation temperature to the salt concentration of the hemolymph are at best circumstantial evidence in favor of the maxillary gland as the site of inoculation. Actual identification of the inoculation site would require additional investigation.

Concluding Remarks

A number of questions and problems raised in this study remain unresolved. For instance, is it the small diameter of the pores or the integrity of the outermost layer of the epicuticle which is primarily responsible for the impermeability of the cuticle? Experimentally this problem could be approached by removing this layer and looking for permeability changes <u>in vitro</u>. If the layer is important, its chemical composition would be of real interest. Also in terms of inoculation mechanisms, the site of inoculation should be identified.

Another problem which received little attention in this study, but warrants investigation, is the complicated relationship between freezing, physiological impairment, and death. In this study slight freezing did not seem to harm most (but not all) organisms, whereas prolonged freezing (more than 12 hours) at temperatures as little as 2°C below the <u>in vivo</u> freezing point caused paralysis of some appendages and often death.

There are also a number of intriguing evolutionary questions. For instance, do the mechanisms used by <u>M</u>. <u>entomon</u> to resist freezing represent special adaptations to the Arctic environment or are they preadaptations? Cuticular impermeability and the ability to take up ions from solution are, of course, characteristics shared by many arthropods. In terms of the general cuticle structure which is responsible for at least part of the mechanism, there do not appear to be any large differences between the cuticle of <u>M</u>. <u>entomon</u> and that of other crustaceans, although there are too few studies on other species to warrant a searching comparison. Finally, it would be enlightening to learn if other polar invertebrates, especially other crustaceans living in similar habitats utilize the same mechanisms as <u>M</u>. <u>entomon</u> to resist freezing. Both of these questions might be best answered through comparative studies on other polar invertebrates and closely related temperate species.

SUMMARY

1. Specimens of <u>Mesidotea entomon</u> were collected through 1.9 m of sea ice from shallow areas of Elson Lagoon in seawater with a salinity of $33^{\circ}/\circ \circ$ and a temperature of -1.8° C. The hemolymph from these animals was isoosmotic with the seawater and had concentrations of sodium and chloride equal to that in the seawater. By adapting specimens to four different salinities (4, 8, 16, 32, and $44^{\circ}/\circ \circ$) at four different temperatures (2, 0, -1, -2° C) it was determined that salinity, but not temperature, affects the <u>in vitro</u> freezing point of the hemolymph. At salinities of 4, 8 and $16^{\circ}/\circ \circ$ the hemolymph was hyperosmotic to the seawater, whereas it was isoosmotic at $44^{\circ}/\circ \circ$.

2. The hemolymph of specimens of <u>M. entomon</u> adapted to salinities of either 34 or $42^{\circ}/\circ\circ$ froze <u>in vivo</u> approximately 0.3°C below the expected <u>in vitro</u> freezing point of the hemolymph. In contrast, the hemolymph of specimens adapted to seawater with a salinity of $15^{\circ}/\circ\circ$ froze in vivo at the same temperature as it did <u>in vitro</u>.

3. The difference between the <u>in vitro</u> and <u>in vivo</u> freezing points of the hemolymph was not due to an antifreeze similar to the glycoprotein found in fish of the genus <u>Trematomas</u> for the following reasons. First, there was no hysteresis between the <u>in vitro</u> melting

and freezing points of the hemolymph. Second, the amount of glycoprotein in the hemolymph was small; and third, the <u>in vivo</u> freezing point of the animal did not change when the hemolymph was replaced with seawater.

4. Instead, the difference appears to be due to the following mechanism: As the surrounding water began to freeze and the ionic concentration of seawater increased, the concentration of ions in the hemolymph also increased, which in turn depressed the freezing point of the hemolymph. The animal did not freeze as ions were taken up from solution, even though it was surrounded by ice, because the cuticle prevented the external ice from inoculating the hemolymph. Without this barrier the hemolymph would have frozen along with the seawater, because at no point was the freezing point of the hemolymph lower than that of the seawater.

5. Measurements of the permeability of the cuticle <u>in vitro</u> demonstrated that the cuticle was sufficiently impermeable to act as a barrier to ice propagation in the temperature range of interest.

6. The structure of the cuticle was also consistent with its functional impermeability. Pore canals arising from the epidermis and extending through the endocuticle, exocuticle, and the proximal epicuticle did not appear to traverse the outermost layer of the epicuticle. Even if they had, their small diameters in the epicuticle (50 Å to 200 Å) would have depressed the freezing point of any fluid they contained more than enough to create an effective barrier to the propagation of ice.

7. Due to the impermeability of the cuticle, the sensitivity of the inoculation temperature to the concentration of salts in the hemolymph, and evidence that freezing began in the anterior of the animal, it was suggested that the hemolymph may not have been inoculated through the cuticle, but rather through some specialized anterior region such as the maxillary gland.

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PLATES

PLATES CAN NOT BE REPRODUCED BY CONVENTIONAL XEROX METHODS

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3- Description of the anatomical, biochemical and physiological adaptations of selected species of animal species.

The main objective in these studies has been to establish the adaptive properties of visual organs, particularly the photoreceptors, of a vertical migrating copepod and of the arctic cod (<u>Arctogadus borisovi</u>) which is thought to be a component of arctic scattering layers.

The Spectral Sensitivity of the Naupliar Eye of a Copepod: <u>Calanus hyperboreus</u>. by Hector R.C. Fernandez and Douglas Yingst. to be submitted to the Journal of Comparative Physiology.

In this investigation the spectral sensitivity of the naupliar eye of <u>C</u>. <u>hyperboreus</u> was measured. The sensitivity exibits two maxima. The most prominent of the two is near 540nm and matches very closely the maximum intensity of down-welling light in the arctic water column.

Structural and Functional Proeprties of Rods and Twin Cones in the Retina of the Arctic Cod <u>Arctogadus borisovi</u>. by Hector R.C. Fernandez, David E. Menter and David. W. Corson, Jr. submitted to Journal of Ultrastructural Research.

This investigation represents the most complete description of a retina which contains twin cones. The anatomical and functional properties of the photoreceptors suggest that it is admirably suited for operation in relatively bright photoenvironments as found in the vicinity of iceleads and for a predatory mode of life.

The Spectral Sensitivity of the Naupliar Eye of a Copepod:

Calanus hyperboreus

Hector R. Fernandez and Douglas Yingst Department of Biological Sciences and Allan Hancock Foundation University of Southern California Los Angeles, California 90007 U. S. A.

by

Spectral Sensitivity of a Naupliar Eye.

Abstract.

The spectral sensitivity of the dark adapted naupliar eye of <u>C</u>. <u>hyperboreus</u> has been obtained from the responselog intensity curve and the spectral efficiency. The ERG has similar polarity, shape and time characteristics to the ERG of the compound eye of other crustaceans. The spectral sensitivity of the dark adapted eye is maximal near 543 nm and declines rapidly on either side of the maximum. There is a shoulder between 440 nm and 480 nm. It is proposed that the bimodal character of the spectral sensitivity is an adaptation to the photic environment which <u>C</u>. <u>hyperboreus</u> encounters during its vertical migration in the Arctic Ocean.

2

Introduction

Light is one of several environmental factors which seem to play an important role in the vertical migrations of some marine organisms. So far, the physiological aspects of this important question have not received the degree of attention which they deserve.

Recently in our laboratory, Dawson (1972) described the annual vertical distribution of the copepod <u>Calanus hyper-</u> <u>boreus</u> Kröyer in the Central Arctic Ocean and he found that the distribution pattern appears to be very closely associated with the annual light cycle. The eye of <u>C</u>. <u>hyperboreus</u> is of the naupliar type. In adult females, it is situated ventrally, just posterior to the large pair of antennas and is composed of three clusters of receptor cells. One of the clusters is central to the other two and is composed of ten receptors. The remaining two clusters are dorsolateral to the central one and each has nine receptors. It seems that each receptor gives rise to an axon.

In this paper we describe the spectral sensitivity of the dark adapted eye of adult females of <u>C</u>. <u>hyperboreus</u>. As far as we know, this is the first naupliar eye ever studied in this manner.

Materials and Methods

Adult females of <u>C</u>. <u>hyperboreus</u> were collected in the Central Arctic Ocean from Ice Island T-3 by means of vertical tows. The specimens were then sent in refrigerated containers to our laboratory in Los Angeles, where they were kept in aquaria at 4°C. In this manner, the animals could be maintained for periods of six months or longer in good physiological condition.

For recording purposes, the animal was mounted on a small aluminum block and held immobile with the aid of rubber bands and cotton moistened in sea water. The mounted specimen was then placed in a dish filled with sea water whose temperature was maintained at 3°C.

The recording electrode was a glass pipette filled with 3M KCl and with a tip diameter of 35µ. By means of a chlorided silver wire, the pipette was connected to an AC coupled preamplifier with a time constant of one second. Another chlorided silver wire, in contact with the sea water bathing the animal, served as a reference electrode. The recording electrode was placed near the eye through a small opening in the exoskeleton. Responses to light stimuli were displayed on an oscilloscope screen from which they were photographed with a movie camera.

The light source was a 150 watt xenon arc lamp. Flashes of 0.5 second duration were obtained with an electronically controlled camera shutter. A set of narrow band interference filters (Baltzer B-40, half band width 9 nanometers) was used to isolate spectral bands from 407 nanometers (nm) to 701 nm in approximately 20 nm intervals. The intensity was controlled with a circular neutral density wedge with an attenuation range of 4 log units. The stimulus was focused to a spot of 0.4 millimeter in diameter. The optical system was calibrated with an Eppley thermopile. Furthermore, in order to obtain the spectral efficiency of the eye, appropriate settings of the neutral density wedge were calculated for each wave length so that one could stimulate with flashes of equal quantum content. By stimulating the eye in this manner, one obtains the spectral efficiency curve of the eye. Before each experiment, the animal was dark adapted for one hour.

Results

Electroretinogram

Fig. 1

The electroretinogram (ERG) of <u>C</u>. <u>hyperboreus</u> is a negative slow wave. It is similar in many respects to the ERG of decapod crustaceans. A typical ERG is shown in Fig. 1. At low light intensities, near threshold, the response is sustained while the stimulus is present. At high intensities,

the response reaches its maximum amplitude shortly after the onset of the stimulus and then declines slowly during the remaining time of the stimulus. The maximum amplitude of the response varied from one experiment to another but in general it ranged from 40 microvolts to 200 microvolts. The latency near threshold was 85 milliseconds, and as the amplitude of the response increased, it became shorter so that a response of 110 microvolts had a latency of 20 milliseconds.

Spectral Sensitivity

The spectral sensitivity of the dark adapted eye was calculated from the spectral efficiency and the response-log intensity curve at the wave length of maximum sensitivity. The spectral sensitivity is defined as the logarithm of the reciprocal number of quanta required to ellicit a response of constant amplitude. It was assumed that the response-log intensity curves for all wave lengths tested were essentially parallel to each other. The response-log intensity curve for 540 nm is shown in Fig. 2. It can be seen that the amplitude of the response is linearly related to the stimulus intensity over 2.5 log units. The spectral efficiency curve for the same eye from which the reponse-log intensity curve of Fig. 2 was obtained, is shown in Fig. 3. The response is maximal when the

Fig. 2

Fig. 3

14.10

eye was stimulated with a light of 540 nm. On either side of the maximum, the responses decline fairly rapidly and there is a prominent shoulder in the region of 440 nm to 480 nm. The spectral sensitivity was calculated from these data and it is shown in Fig. 4. The maximum sensitivity lies near 543 nm and it is about one log unit higher than the sensitivity at the shoulder between 440 nm and 480 nm. Fig. 5 shows the average spectral efficiency from three different animals. There is a clear maximum near 540 nm and a secondary maximum near 460 nm.

Discussion

The ERG of the naupliar eye of <u>C</u>. hyperboreus has several characteristics which are similar to the ERG's from the large compound eyes of other crustaceans (Goldsmith & Fernandez, 1968). It is a negative, slow potential change whose amplitude is linearly related to the logarithm of the stimulus intensity over several log units. The latency is inversely related to the amplitude. In addition, the shape of the response changes with the amplitude. At low amplitudes the response is sustained while the stimulus is on. At larger amplitudes, it reaches a maximum very quickly, shortly after the onset of the stimulus, and then declines

Fig. 4

Fig. 5

gradually.

The fact that the spectral sensitivity of the dark adapted eye is bimodal with a maximum near 543 nm and a shoulder between 440 nm and 480 nm raises some interesting points, when one considers the migratory habits and the type of photic environment in which C. hyperboreus lives. Dawson (1972) has shown that in the Central Arctic Ocean C. hyperboreus undergoes an annual vertical migration which follows very closely the annual light cycle. He has also shown that the migratory depth range for adult females is from the surface to 400 meters. This means that C. hyperboreus must deal with a complex photic environment with great variations in intensity and spectral quality. At present there is no adequate information on the spectral properties of the ambient light in the waters of the Central Arctic Ocean and one can only speculate on the spectral characteristics of the photic environment. However, measurements of waters from northern latitudes have been made by a number of investigators. Utterback (1936) found that in the upper five meters of North Pacific waters the wave lengths of maximum transmission were 515 nm, 520 nm, and 490 nm. Jerlov and Kullenberg (1946) reported that the wave lengths of maximum transmission in Gullmar Fjord ranged from 530 nm to 590 nm. Jerlov (1970) has also found that in the

Baltic Sea the wave length of maximum transmission is around 550 nm in the upper 20 meters. Greenbank (1945) has shown that certain ice conditions have a strong filtering effect on the wave lengths of transmitted light which tend to favor the transmission of the reds and greens and to a smaller extent the blues. In addition, the presence of phytoplankton in the upper layers of the water and in association with the undersurface of the ice must have similar wave length selective effects on the light. Recently Fernandez (1971) reported that the spectral sensitivity of a pelagic amphipod from the surface waters of the Central Arctic Ocean had two maxima: one at 560 nm and another at 500 nm. On the other hand as the light penetrates deeper, the usual selective filtering effect of the water will tend to eliminate primarily the long wave length components so that the relative contribution of the blue components will increase. Thus one can say that in all probability C. hyperboreus must encounter a changing spectral background during its vertical migration. One way of coping with the changing spectral background is to have a broad spectral sensitivity range, which in fact is the case in C. hyperboreus. Furthermore, the fact that the spectral sensitivity is bimodal suggests the possibility that

the eye of <u>C</u>. <u>hyperboreus</u> has two receptor types with maximum sensitivity near 543 nm and around 460 nm respectively. Preliminary selective adaptation experiments suggest that this is the case.

Acknowledgements

This work was supported by the Office of Naval Research Contract NR 307-270 and by U. S. Public Health Service Grant No. EY00706. Live specimens were provided by Dr. T. S. English, Department of Oceanography, University of Washington, Seattle, Washington.

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Figure Legends

Fig. 1.	The ERG of the dark adapted eye (upper trace).
	The response is preceded by a calibration pulse
	of 200 microvolts amplitude and 100 milliseconds
	duration. The lower trace is the response of a
	photocell to the stimulus flash.

Fig. 2 Response-log intensity curve to stimuli of 540 nm.

Fig. 3 The spectral efficiency curve of the dark adapted eye.

- Fig. 4 The spectral sensitivity of the dark adapted eye obtained from the spectral efficiency curve of Fig. 3 and the response-log intensity curve of Fig. 2.
- Fig. 5

The mean of three spectral efficiency measurements obtained from three different animals. The vertical bars are the standard error of the mean: $s.E. = \pm \sqrt{\frac{\sum(x-x)^2}{n-1}}$





.:



"

Fig. 3



:

Fig. 4


Structural and Functional Properties of Rods and Twin Cones in the Retina of the Arctic cod <u>Arctogadus borisovi</u>.

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Abstract

The retina of the arctic cod Arctogadus borisovi has been examined by means of light and electron microscopic methods, retinal visual pigment extracts have been analyzed and the spectral sensitivity of eye cup preparations in dark and red light adapted states have been measured. The structural analysis reveals a retina with many characteristics commonly found in other gadids, in particular those species which live in bright photoenvironments and which are active predators. The retina has also many ultrastructural features which are found in other nongadid species. Of special importance are the large dimensions of the twin cones in the Arctogadus retina and the well defined cone mosaic. The cones show many indications of being in a very active metabolic state. Visual pigment extracts contain one predominant component which absorbs maximally at 503nm. The extract also contains a second component which is mainly sensitive to blue lights and which on the basis of spectral sensitivity measurements it is thought to be the visual pigment from a blue sensitive cone. Spectral sensitivity measurements indicate that in the scotopic condition the cones may be responsible for part of the sensitivity at wavelengths longer than 540nm. Eye cup preparations adapted to 601nm light have a spectral sensitivity with two distinct maxima located near 460nm and 540nm and which probably represent two different types of cones.

Introduction

The gadid retina has been a subject of considerable interest to investigators of retinal anatomy and evolution for several reasons. First of all, due to the phyllogeny of the family Gadidae, Walls (1942) considered the study of these retinas essential to the ellucidation of the origin of twin cones. The family is composed of a large number of species which are found in many different kinds of habitats and which have many different modes of life (Walls, 1942; Engstrom, 1961, 1963; Ali and Hanyu, 1963; Ali et al, 1968). Thus gadids are a convenient group in which to study anatomical and functional adaptations of the retina. In addition to the above reports, there have been many others, some dating back to the last century, which are also concerned with the anatomy of gadid retinas (Nunnely, 1858; Cobbold, 1862; Friis, 1879; Krause, 1925, 1929; Menner, 1929; Lyal1,1957a,b). Despite these numerous reports, however, there are no previously published descriptions of gadid photoreceptor ultrastructure and with the exception of a recent description by Borwein and Hollenberg (1973) of the twin cones of Anableps anableps (a nongadid species) there are no other reports on the ultrastructure of twin cones. Dartnall and Lythgoe (1965) and Beatty (1969) have analyzed visual pigment extracts from nine species of gadids but there are no previous studies of the physiological porperties of gadid photoreceptors.

This paper presents recent observations made on the retina of a predatory arctic cod <u>Arctogadus borisovi</u>. According to Walls (unpublished observations) arctic cod retinas have the largest cone photoreceptors that he ever observed during his extensive anatomical study of vertebrate retinas. <u>Arctogadus</u> is one of several species of cods found in the Arctic Ocean. It is usually found in the vicinity of sea ice, particularly where breaks in the ice occur (ice leads), exposing the underlying sea to the atmosphere. The cod feeds primarily on large arctic amphipods which are found at these locations. In the Arctic Ocean where

even in the summer months most of its surface is covered by an ice sheet, the ice lead is an important ecological factor. It is here that the surface waters are exposed directly to solar radiation, thus making this location a relatively bright photoenvironment. This report includes light and electron microscopic observations, visual pigment analysis and measurements of the spectral sensitivity of dark adapted and red light adapted retinas.

Materials and Methods

Specimens of the arctic cod <u>Arctogadus borisovi</u> (Drjagin) were collected by hook and line from ice leads, at the time of ice break-up in late June, in Barrow, Alaska. In the laboratory they were kept up to several months in refrigerated aquaria near 0° C and fed regularly small pieces of shrimp meat. All observations were performed on specimens which ranged in standard length from 12cm to 14cm.

Anatomical Studies

For structural studies only light adapted specimens were used. After enucleation the eyes were fixed in 2 percent glutaraldehyde in either Millonig phosphate or sodium cacodylate buffer (pH 7.45 and 7.2, respectively). Then the retinal tissues were postfixed in 1 percent osmium tetroxide in Millonig phosphate buffer and imbedded in Epon 812. Thick and thin sections were obtained with a Sorvall MT-2 ultramicrotome. Photomicrographs were taken with a Zeiss photomicroscope and a Leitz orthoplan orthomat compound microscope. Thin sections were examined with a Philips 201 electron microscope operated at 80 kv. The dimensions of the photoreceptors were obtained directly from light microscopic observation of thick sections and from electron micrographs of thin sections.

Visual Pigment Analysis

A visual pigment extract was prepared using 2 percent aqueous digitonin (w/v), Fisher Scientific Co.) from the retinas of six dark adapted fish. Details

of the extraction procedure are described elsewhere (O'Day and Fernander, 1974). All steps in the extraction procedure were carried out either in darkness or in dim red light (Kodak filter no. 1) and near 0° C.

Spectral analysis of the extract was performed with a Zeiss PM QII spectrophotometer in which the cuvette compartment was maintained at $5 \pm 1^{\circ}$ C. The visual pigment was characterized by the difference spectrum and according to the partial bleaching methods of Dartnall (1952) in the presence of 0.04M hydroxylamine neutralized with sodium hydroxyde. A 150 watt quartz-iodine lamp (Ealing) was used to bleach the extract in conjunction with interference filters (Balzers B-40, half band width 10nm), and heat filters (Corning no. 3391 and 1cm water). Further information on the color filters is given in the text and appropriate figure legends.

Physiological Studies

Spectral sensitivity measurements were made in eye cup preparations. After enucleation in dim red light, the frontal half of the eye was removed along with the lens. Some of the vitreous humor was also removed with filter paper. The eye cup was then placed on a piece of filter paper in contact with a chlorided silver wire which served as reference electrode inside a chamber which was maintained at 5° C. A second chlorided silver wire was lowered into the eye cup with a micromanipulator until it made contact with the vitreal surface of the retina and served as recording electrode. The electrodes were connected to an AC-coupled preamplifier (Tektronix 122) which was operated at one second time constant and 1 kHerz. Further details on the recording procedure as well as on the characteristics of the photostimulator are given elsewhere (Fernandez, 1973). In these experiments the entire retina was stimulated with flashes of 250 msec duration that were administered every three minutes to avoid light adaptation. The stability of the preparation was monitored at regular time intervals by measuring the sensitivity of the retina to a standard flash of

501nm light. Eyes prepared in this manner remained in good physiological condition for at least six hours.

The relative spectral sensitivity (the logarithm of the reciprocal of the relative number of quanta required to ellicit a response of constant amplitude) was obtained in the following manner. The eye cup was stimulated with at least three different intensities at ten different wavelengths from 403nm to 601nm. A plot of the response amplitude in microvolts versus the logarithm of the stimulus intensity at each wavelength was used to find the relative energy required to produce a criterion response of 15 microvolts amplitude in the height of the b-wave of the electroretinogram. These values were then expressed in terms of the relative number of quanta. A total of nine experiments were performed. In seven of them the spectral sensitivity was measured after the retina was allowed to dark adapt for one hour. In the remaining two experiments the spectral sensitivity was measured while the retina was exposed to an adapting light of 601nm. All calculations of spectral sensitivity were performed with a computer program designed by one of us (D.W.C.) using an IBM 360 computer.

Results

Anatomy and Ultrastructure

The retina of <u>Arctogadus</u> is characterized by well defined layers (Fig 1,A and B). One of its most prominent features is the pallisade of very large cones in the receptor layer. The cones appear to be exclusively twins and the base of their myoids is located at the outer limiting membrane (Fig. 1 A). In contrast, the rods are considerably smaller and are located at various levels with respect to the twin cones (Fig. 1 A). Tangential sections at the level of the cone ellipsoids reveal that twin cones are the only type of cone present (Fig. 2). The twin cones are arranged in rows which are staggered with respect to each other. This arrangement produces a cone mosaic in which each twin cone is surrounded by six of its neighbors in a hexagonal pattern (Fig. 2). Rods are

TABLE I - PHOTORECEPTOR DIMENSIONS^a OF <u>ARCTOGADUS BORISOVI</u>

		RODS			CONES		
		x(µm) Range		ge	x (µm)	Range	e
Length from synapse to tip of outer segment	rods between ellipsoids rods between outer seg.	60.8	58	63	101.4	94	105
		87.9	83	93			
Length from synapse to outer segment base					72.8	67	77
Outer segment length	rods between ellipsoids rods between outer seg.	9.7	7	15	28.1	19	30
		12.5	10	15			
Diameter of outer segment base		2.9	1.8	4.0	5.2	4.5	5.5
	5μ	3.0	1.8	4.5	5.2	4.5	5.5
Diameter (χμ) from outer segment base	10µ	2.5	1.0	3.3	4.6	3.7	5.1
	15µ	2.0	0.5	3.0	4.2	3.5	4.5
Ellipsoid length		6.0	4.5	8.2	23.8	22	27
Maximum ellipsoid diameter	÷	3.0	2.2	across single width 3.5 across double width	11.4	10.3	12.7
					14.5	13.6	15.3
Mitochondria diameter		1.2	0.5	outer 1.6 edges near center	0.6	0.5	0.7
					1.3	1.1	1.5
Nucleus length		6.3	4.9	9.1	10.6	9.1	12.7
Nucleus width		2.3	1.5	3.3	4.2	3.6	4.5
Synapse length		4.3	2.4	7.3	8.3	7.8	9.5
Synapse width		2.2	1.1	2.5	8.8	7.4	13.1

a) each mean value is based on ten measurements

located in the interstices between the cones and are not arranged in any special pattern (Fig. 2). Table I summarizes the main dimensional characteristics of rods and twin cones. Receptor density was estimated from receptor counts in tangential sections at various levels of the receptor layer. There are about 8,400 cones/mm² and 14,600 rods/mm² of retinal surface. Thus there is a ratio of cones to rods of 0.57.

Rods

Rod outer segments vary markedly in ultrastructural appearance and in overall dimensions (Table I, Figs. 3 and 5). Outer segment disc membranes appear compact in some and loosely organized in others (Fig. 3), while in tangential sections, outer segments usually appear irregularly shaped (Figs. 2 and 4). The inner and outer segments are much smaller than those of the twin cones (Table 1, Figs. 4, 6 and 7). In many sections a basal body and lateral sacs can be readily seen (Figs. 5 and 6). The ellipsoid contains few but large mitochondria which fill completely the cytoplasmic space in this region of the cell (Figs. 3 5, 6 and 7). Mitochondria vary considerably in shape and the membrane profiles are thin (Figs. 5, 6 and 7). At their vitreal end the ellipsoids taper rapidly into a myoid which assumes various lengths. Many microtubules are present in the cytoplasm in this region (Fig. 7). Rod nuclei are also smaller than those of cones (Table I, Fig. 8) and their nuclear material appears very dense, mainly as heterochromatin. The perinuclear space between outer and inner membranes of the nuclear envelope is greatly enlarged in these cells; Slender cytoplasmic bridges traverse this gap and are associated with nuclear pores (Figs. 8 and 9). In radial sections through the outer nuclear layer, the rod nuclei appear arranged in columns bounded on the sides by twin cones (Figs. 8 and 11). Cones

Random sampling of different regions of the retina revealed that only large twin cones are present. The members of each twin cone appear similar in every

respect and are in close apposition to each other along the vitreal end of their outer segments, ellipsoids and nuclear regions. In tangential section, the outer segments appear almost circular and near the junction with the inner segment they are surrounded by calical processes (Fig. 3, insert). Cone ellipsoids are very large (Table I, Figs. 4 and 7) and exhibit many interesting ultrastructural characteristics. For example, highly concentrated and irregularly shaped mitochondria fill most of the cytoplasmic space with the exception of a narrow band of cytoplasm which separates them from the plasma membrane (Fig. 4). With the exception of those mitochondria which are located in the periphery, the more centrally located have very dense membrane profiles (Figs. 4 and 6). Each mitochondrion appears to be contained within a double membrane network (Fig. 6, insert). Furthermore, close examination of the membrane profiles where the two ellipsoids are juxtaposed also reveals the presence of subsurface membranes which form a flattened membrane sac in each cone cell. The subsurface membranes are found adjacent to and parallel to the plasma membrane along the entire length of the ellipsoids (Fig. 4, insert). Tangential and radial sections through the myoid also show finlike extensions of the plasma membrane (Fig. 10). The extracellular space between the fins is mostly occupied by slender processes from Müller cells

Most cone nuclei lie just below the outer limiting membrane. They are large and elongated in shape (Table I, Fig. 8). The nuclear material appears dispersed mainly as in the euchrochromatin state. In addition, the perinuclear space is much reduced and many cytoplasmic bridges traverse it (Figs. 8 and 9). The surrounding cytoplasm contains an extensive endoplasmic reticulum network with many cisternae which appear to be preferentially alligned with the longitudinal axis of the cell. Also alligned in similar manner are numerous microtubules and microfilaments (Fig. 9). In contrast to the ellipsoid region, the plasma membranes where the two cells are juxtaposed are very close to each other and there

are no subsurface membranes associated with them (Fig. 9).

At their scleral ends, both rods and twin cones are in close contact with a well developed pigment epithelium. In these light adapted preparations, the pigment epithelium cells contain rod shaped melanosomes which are concentrated sclerally to the cone outer segment tips and portions of phagocytosed outer segments within large phagosomes (Fig. 7, insert).

Photoreceptor Terminals

Cone pedicles are readily distinguished from rod spherules on the basis of several characteristics (Table I, Fig. 11 and 12). Pedicles usually terminate more vitreally and in some sections appear paired. They are further distinguished from spherules by their greater cytoplasmic density, higher concentration of presynaptic vesicles, greater number of presynaptic ribbons and more complex postsynaptic ultrastructure (Figs. 11 and 12).

Visual Pigment Analysis

Seven bleaching experiments were carried out in 0.04M hydroxylamine. In three experiments, samples of the extract were bleached completely with a single light exposure of wavelengths longer than 560nm (Corning filter 3480). Three other samples were bleached in successive stages with lights of 664nm, 639nm, 618nm, and 506nm, in that order. The first three exposures yielded difference spectra with maximum absorbance loss near 503nm. The last exposure, however, yielded a difference spectrum that was shifted towards shorter wavelengths and with a maximum near 500nm. A seventh experiment was performed which is shown in Fig. 13 and illustrates the observed phenomenon. In this experiment, after an initial measurement of the absorbance spectrum, the extract was exposed for 3.5 hours to a light of 639nm. The spectrum was measured once again and the extract was bleached completely with a light of 506nm, The resulting difference spectra (Fig. 13, circles and triangles respectively) are compared with the spectrum for VP 503₁ (Fig. 13, solid line) from the nomogram of Darnall

(1953). Inspection of the figure indicates that the difference spectrum produced by the 639nm light agrees well with the nomogram spectrum while that produced by the 506nm light is displaced towards shorter wavelengths. In this experiment the exposure with 506nm represented 17 percent of the total absorbance change at 503nm. Figure 14 (circles) shows the averaged normalized difference spectrum for the seven experiments. The solid line is the normalized spectrum for V.P. 503_1 from the nomogram of Dartnall (1953). The agreement is quite good. Finally, the endproduct in every bleaching experiment absorbed maximally near 367nm and identifies the oxime of retinal.

Spectral Sensitivity

Spectral sensitivity determinations were made in seven dark adapted eye cups; figure 15 (circles) illustrates the averaged results. The sensitivity maximum is rather broad extending from 480nm to 540nm. Beyond this range the sensitivity falls rapidly. The dark adapted spectral sensitivity is compared with the spectrum for VP 503₁ assuming a visual pigment absorbance in the retina of 0.39 (Fig. 15, solid line). There is good agreement between the two sets of data at wavelengths shorter than 520nm.

In two additional experiments, the spectral sensitivity was measured while the eye cup preparation was illuminated with a constant background of 601nm. The intensity of the adapting light was adjusted so that the sensitivity at 501nm was depressed an average of 1.35 log units; the results are shown in Fig. 16. The spectral sensitivity curve now consists of two well defined maxima, one near 460nm and another near 540nm.

Discussion

The present anatomical studies indicate that the retina of <u>Arctogadus</u> bears a strong resemblance to other gadid retinas, particularly those species which are predatory and which live in bright photoenvironments (Walls, 1942; Engstrom, 1961, 1963; Ali and Hanuy, 1963; Ali et al., 1968; Ali and Wagner, 1975). In other gadid species the retinal layers are well developed and even though both

rods and cones are present, the cones appear to play a dominant role. Furthermore, the cones form a well defined mosaic and in addition to single cones, these retinas contain double and twin cones. In the case of <u>Arctogadus</u> all these features are present with the peculiarities that the cones are exclusively twins and the receptor mosaic has a hexagonal pattern.

There are no previous reports on the ultrastructural characteristics of gadid retinas and there is only one earlier report which discusses twin cone ultrastructure (Borwein and Hollenberg, 1973) in a nongadid teleost. The present findings indicate that there are strong similarities in ultrastructure between the photoreceptors of Arctogadus and those of nongadid species which have been previously studied. For example the mitochondria in the twin cone ellipsoids have membrane profiles which resemble those of Lebistes reticulatus (Berger, 1967, Borwein and Hollenberg, 1973). Esox lucius (Braekvelt, 1975), Anableps anableps, Stizostedion vitreum and S. canadense (Zysnar and Ali, 1975) and Fundulus heteroclitus (Anctil and Ali, 1976). These mitochondrial specializations enable the cone ellipsoids to funnel incoming light rays onto the photoreceptor outer segments. In some fishes (Berger, 1966; Borwein and Hollenberg, 1973; Arnott and Best, 1973; Ali and Anctil, 1973) the mitochondria further specialize into oil droplets, however, this does not occur in Arctogadus. The membrane network which surrounds each individual mitochondrion is similar to that in Esox lucius (Braekvelt, 1975) and subsurface membranes in the cone ellipsoids have also been reported in double cones of Lebistes reticulatus (Berger, 1967). Calical processes in cones and lateral sacs in both rods and cones have been described in many other species of teleosts (Engstrom, 1961, 1963; Stell, 1965; Dathe, 1969; Locket, 1971a, 1971b; Borwein and Hollenberg, 1973; Fineran and Nicol, 1974; Fredericksen, 1976; Munk, 1977). Finally, finlike extensions of the plasma membrane in the myoid region have been found in some labrid fishes (Fineran and Nicol, 1974), Anableps anableps (Borwein and Hollenberg, 1973) and Scho-

pelarchus guntheri (Locket, 1971b).

There are marked differences in ultrastructure between rods and twin cones of <u>Arctogadus</u>. The twin cone inner segment, nuclear region and synaptic terminals have a general appearance which is usually indicative of cells with high metabolic activity. This is supported by the preponderance of euchromatin in the nuclear material, the presence of numerous cytoplasmic bridges which connect the nucleus with surrounding cytoplasm, an elaborate endoplasmic reticulum network and many microtubules and microfilaments. The cytoplasm in the cone pedicles is also more dense and contains a greater number of presynaptic vesicles and ribbons.

Identity of the Visual Pigments

Dartnall and Lythgoe (1965) and Beatty (1969) have extracted and identified ten visual pigments from nine gadid species. In eight species only one photopigment was found; their absorption maxima ranged between 485nm and 500nm. In the lake dwelling species Lota lota Beatty (1969) found two photopigments with respective maxima at 523nm and 527nm. All these visual pigments have retinal as chromophore with the exception of the 527nm photopigment of Lota lota which has 3-dehydroretinal; all are presumably of rod origin.

In <u>Arctogadus</u> extracts there appears to be a mixture in which the main component absorbs maximally at 503nm. When the absorption spectrum of this photopigment was compared with the spectral sensitivity in the dark adapted state (Fig. 15) and assuming a retinal absorbance of the photopigment of 0.39 (Witkovsky et al., 1973), there is good agreement between them at wavelengths shorter than 520nm. This suggests that the 503nm pigment is the main rod pigment in the retina of <u>Arctogadus</u>. In addition to this pigment however, there seems to be at least one more photosensitive component in the extract but it is present in very small concentration. Even though its absorbance spectrum

could not be determined, it must lie at shorter wavelengths than the 503nm pigment because only lights of relatively short wavelengths caused difference spectra with maxima different from 503nm. At present, one can not exclude the possibility that this is a second rod pigment, however, the spectral sensitivity of retinas adapted with 601nm light clearly shows a distinct maximum near 460nm. This maximum most probably represents a blue sensitive cone and thus the blue sensitive photopigment might represent the visual pigment of this type of cone. Although generally cone pigments are not amenable to conventional methods of extraction, Munz and McFarland (1975) have recently presented evidence which indicates that some extracts from teleost retinas contain cone visual pigments. Spectral Sensitivity

The dark adapted retina of Arctogadus has a very broad spectral sensitivity maximum (Fig. 15). When the sensitivity spectrum is compared with the spectrum of V.P. 503, assuming a retinal density of 0.39 (Witkovsky et al., 1973), there is good agreement between them at wavelengths shorter than 520nm. At longer wavelengths they differ markedly from each other. This discrepancy could be due to a contribution by the cones to the spectral sensitivity in the dark adapted retina. There have been earlier reports (Burkhardt, 1966; Witkovsky, 1968) which indicate that in some teleosts the spectral sensitivity in the dark adapted state is much more sensitive to long wavelengths than would be predicted by the spectrum of the rod pigment present in them. The difference has been attributed to a contribution by the cones. In contrast to these observations, in other teleost species there appears to be a good match between the rod visual pigment spectrum and the scotopic spectral sensitivity (Easter and Hamasaki, 1973). In Arctogadus the anatomical evidence indicates that the retina is not only relatively rich in cones (cone/rod ration of 0.57) but that the cones by virtue of their greater dimensions occupy a larger proportion of the retinal surface. Thus it is conceivable that even in the dark adapted state they may be

involved in determining sensitivity. In addition, selective adaptation experiments indicated that by only depressing the sensitivity 1.35 log units from the dark adapted state, the spectral sensitivity curve underwent a marked change in shape, which can only be attributed to a shift from a rod dominated to a cone dominated condition. In the latter condition the spectral sensitivity has two distinct maxima near 460nm and 540nm and they probably represent the maximum sensitivity of two different cone types. The present results cannot exclude the possibility that there might be other cone types although that seems unlikely, nor can they determine the spectral characteristics of each cone cell. However, Harosi and McNichol (1974) and Stell and Harosi (1976) have reported that in goldfish double cones one member is green sensitive while the other is red sensitive. There is no previous evidence for spectrally pure double or twin cones and thus it is possible that in <u>Arctogadus</u> twin cones the two members are also spectrally different.

Finally, there remains the question of the relation between anatomical and functional properties of this retina to the photic environment and mode of life of <u>Arctogadus</u>. Recent measurement of the spectral distribution of light beneath sea ice in Barrow, Alaska (Maykut and Grenfell, 1975) indicates that the preence of ice and in particular such factors as ice thickness and age, snow cover, and presence of an algal layer in the ice displace the wavelength of maximally transmitted light to the green region of the spectrum. In <u>Arctogadus</u> the scotopic spectral sensitivity is rather broad and its maximum fits fairly closely the wavelenth range of maximally transmitted light below the ice. Furthermore, the anatomical characteristics of the retina are in accord with features found in other predatory species, namely well defined retinal layers, a strong representation of cones (in this case twin cones), and a well developed retinal mosaic.

Acknowledgements

The authors wish to thank Dr. William T. O'Day for fixation and imbedding of the cod retinas. This investigation was supported by O.N.R. Contract N00014-77-E-0013 and N.S.F. Grant BNS 76-80501.

Figure Legends

- Fig. 1. Radial section of the Arctogadus retina
 - A Receptor layer, i rods and cones, ii outer limiting membrane, iii - outer nuclear layer. (500X)
 - B Continuation of the same section showing central regions of the retina. iii outer nuclear layer, iv outer plexiform layer, v bipolar and horizontal cell bodies, inner nuclear layer, vi inner plexiform layer, vii ganglion cell layer. (500X)
- Fig. 2. Tangential section at the level of twin cone ellipsoids. Twin cones form hexagonal cellular arrays of adjacent rows. The ellipsoids (cytoplasmic) membranes of twin cone pairs show an increase in complexity at the twin juncture. Rod outer segments (arrows) occupy the interstices between twin cones. (500X)
- Fig. 3. Radial section through the outer segments of the <u>Arctogadus</u> retina. Rods appear at various levels in relation to the twin cones (T). Note the loose appearance of the rod outer segment disc membranes. In comparison, twin cone outer segment membranes appear more compact. Lateral sacs course alonside rod and cone outer segments, (arrows). (4000X). Insert - Twin cone outer segments in tangential section near their base. Note the calical filaments in cross section which partically surround each outer segment (arrows). (3000X)
- Fig. 4. Tangential section through a twin cone ellipsoid. Mitochondria increase in size from the cell periphery inwards. The largest mitochondria lie next to the subsurface membrane complex (sm). A peripheral band of cytoplasm surrounds the ellipsoid. Section passes through rod outer (ro) and inner (ri) segments. In some rod inner segments the basal body is clearly visible. (6,650X) Insert - shows the subsurface membrane complex of a twin cone ellipsoid. (35,000X)
- Fig. 5. Radial section showing rod outer segments and inner segment ellipsoids (re) as well as myoids (rm). Note the irregular size of the mitochondria and the presence of basal bodies (b) in rod ellipsoids. Rod ellipsoids taper rapidly into elongated myoids which are filled with microtubules. (19,000X)
- Fig. 6. Radial section through rods and cones illustrates the difference in their ultrastructure. Basal bodies (b) commonly seen in rod inner segments were never observed in twin cones. Twin cone mitochondria have dense membrane profiles and appear surrounded by a membrane matrix (insert, 43,000X). Mitochondria of rods are more polymorphic and lack comparable matrices. Both rods and cones exhibit cytoplasmic extensions of inner segments (arrows) in the form of calical processes (cones) and lateral sacs (rods and cones). (14,000X)
- Fig. 7. Twin cone ellipsoid in radial section. The subsurface membrane complex extends along the entire length of ellipsoid region, (3450X) Insert - Portion of the pigment epithelium cell which contains rod shaped melanosomes and phagosomes, (p). (5950X)

- Fig. 8. Radial section through the scleral end of the outer nuclear layer. Rod (rn) and Müller cell (M) nuclei are arranged in columns between cones. All receptor nuclei lie ritread to the outer limiting membrane (ol). (3275X)
- Fig. 9. Radial section through the outer nuclear layer at higher magnification. Chromatin material is mainly in the euchromatic state in twin cone nuclei (cn) and in the heterochromatic state in rod nuclei (rn). The perinuclear space appears enlarged in rods when compared to cones and cytoplasmic bridges connect the nucleus with surrounding cytoplasm at the area of the nuclear pores (arrows). In twin cones the cytoplasm is filled with smooth and rough endoplasmic reticulum as well as microfilaments and microtubules. Note the distinct appearance of a Müller cell nucleus (M). (8000X)
- Fig. 10. Radial section showing the juction between the ellipsoid and myoid regions of a twin cone. The section shows cytoplasmic extensions of a twin cone at the level of the ellipsoid/myoid junction. The extensions are separated by spaces filled with Müller cell processes (arrows). (4700X)
- Fig. 11. Radial section illustrating the relative position of photoreceptor nuclei. Cone pedicles (ct) are located vitread to rod spherules (rt). Note the complexity of horizontal and bipolar cell processes which interdigitate with cone terminals. In contrast rod spherules contain fewer invaginations. Variations in cytoplasmic density between them are also clearly visible. (3800X)
- Fig. 12. Radial section of a cone pedicle (ct) and adjacent rod spherules (rt). Note the greater complexity, number of presynaptic ribbons and density of presynaptic vessicles in the cone pedicles. (7375X)
- Fig. 13. Partial bleaching of an alliquot of digitonin extract from <u>Arctogadus</u> retinas. Filled circles represent the difference spectrum which resulted when the extract was bleached with a light of 639nm for 3.5 hours, while the triangles represent the resulting difference spectrum when the extract was subsequently bleached with a light of 501nm for 15 min. Solid line is the spectrum for VP 503, (Dartnall, 1953).
- Fig. 14. Comparison of the averaged difference spectra with the nomogram spectrum of VP 503. The filled circles represent the average of seven experiments, while the solid line is the nomogram spectrum (Dartnall, 1953).
- Fig. 15. Averaged scotopic spectral sensitivity determined from seven experiments (filled circles), vertical bars indiacte the 95% confidence limits. Solid line represents the logarithm of relative absorption of VP 503 (Dartnall, 1953) computed for a maximum optical density of 0.39 in the retina. The expression in the right hand y-axis is the formula for the logarithm of relative absorption.
- Fig. 16. Averaged spectral sensitivity determined from two experiments in which the eye cup preparation was continuously exposed to an adapting light of 601nm. The adapting light depressed the sensitivity 1.35 log units at 501nm. Vertical bars indicate the 95% confidence limits.

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4- Adaptations of the energy storage mechanisms, particularly the storage of lipids.

Lipid composition of three species of arctic amphipods. by Mr. Philip Oshel. The present investigation by Mr. Oshel, during the time of the contract, has focused on the analysis of the fatty acid composition of phospholipids in three species of arctic amphipods.

In this investigation specimens of three speceis of amphipods (<u>Pseudalibro-tus littoralis</u>, <u>Gammarus locusta</u> and <u>Anonix nugax</u>) were collected over the period of one year. Total lipid extracts were prepared by the Folch method for lipid extraction. The first phase of the work consisted in the separation of the various lipid classes by two stage thin layer chromatography. From this separation, the phospholipid fraction was taken an after hydrolysis and subsequent methylation, the fatty acids composition was analysed by liquid gas chromatography. Sample records of the chromatograms are shown as Figs. 1,2,and 3.

Inspection of the chromatogram reveals a rather unusual fatty acid pattern. In all three species there is a high proportions of very low chain and very long chain fatty acids. Middle range fatty acids, those with carbon chain lengths of 14 to 18 are in very small concentration. The fatty acids were identified through the use of standards and by the retention time. A high degree of unsaturation in the carbon chains was found, suggesting an adaptation of the molecular composition of the membranes to ambient low temperatures. Eventhough there was variation from one species to another in tha actual identity of the fatty acids, there is a general pattern which the three share. That is that the chains tend to be mostly unsaturated and of great length when compared to species of lower latitudes. Further investigation of these lipids is being carried out presently.











