POLYTENY AND SIZE VARIATION IN ARCTIC FJORD *PSEUDOCALANUS*

by

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POLYTENY AND SIZE VARIATION IN THE COPEPOD, PSEUDOCALANUS, FROM TWO SEMI-LANDLOCKED FIORDS IN BAFFIN ISLAND.

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INTRODUCTION

Seasonal size variation within local populations of the copepod genus Pseudocalanus has recently been studied in relation to temperature and food differences (Deevey, 1960; McLaren, 1963, 1965b). Collections taken from June 1 to October 4 during 1957 by I.A. McLaren from Ogac Lake, a warm semi-landlocked fjord in Baffin Island, however, revealed size variation within a population of Pseudocalanus undergoing similar environmental influences. This situation was found later, in 1964, to occur clearly in Pseudocalanus samples collected from the semi-landlocked head of Winton Bay on the east coast of Baffin Island. The positions of Ogac Lake at 62°52'N and 67°21'W and Winton Bay at 63°24'N and 64°39'W are shown in Figure 1.

Previous collections taken from Ogac Lake in 1952 by an expedition arranged by M.J. Dunbar under the auspices of the Arctic Institute of North America and later samples collected there in 1962 and 1965 by I.A. McLaren have been examined for the appearance of these size forms.

The larger forms bear eggs of a significantly larger size than those of the smaller form. No mention of such a large-egged form occurring elsewhere has been found in the literature. It was considered possible (McLaren, 1965b) that these large animals were polyploid, but this hypothesis was not examined further at that time. Therefore a comparative study of the size forms, their chromosomes, and their nuclear DNA contents has been carried out and is presented here.

The morphological features of both forms, excluding size, coincide with the detailed description of Pseudocalanus gracilis by Sars (1903). However, it is uncertain whether the genus Pseudocalanus is comprised of three distinct species, P. elongatus Boeck,

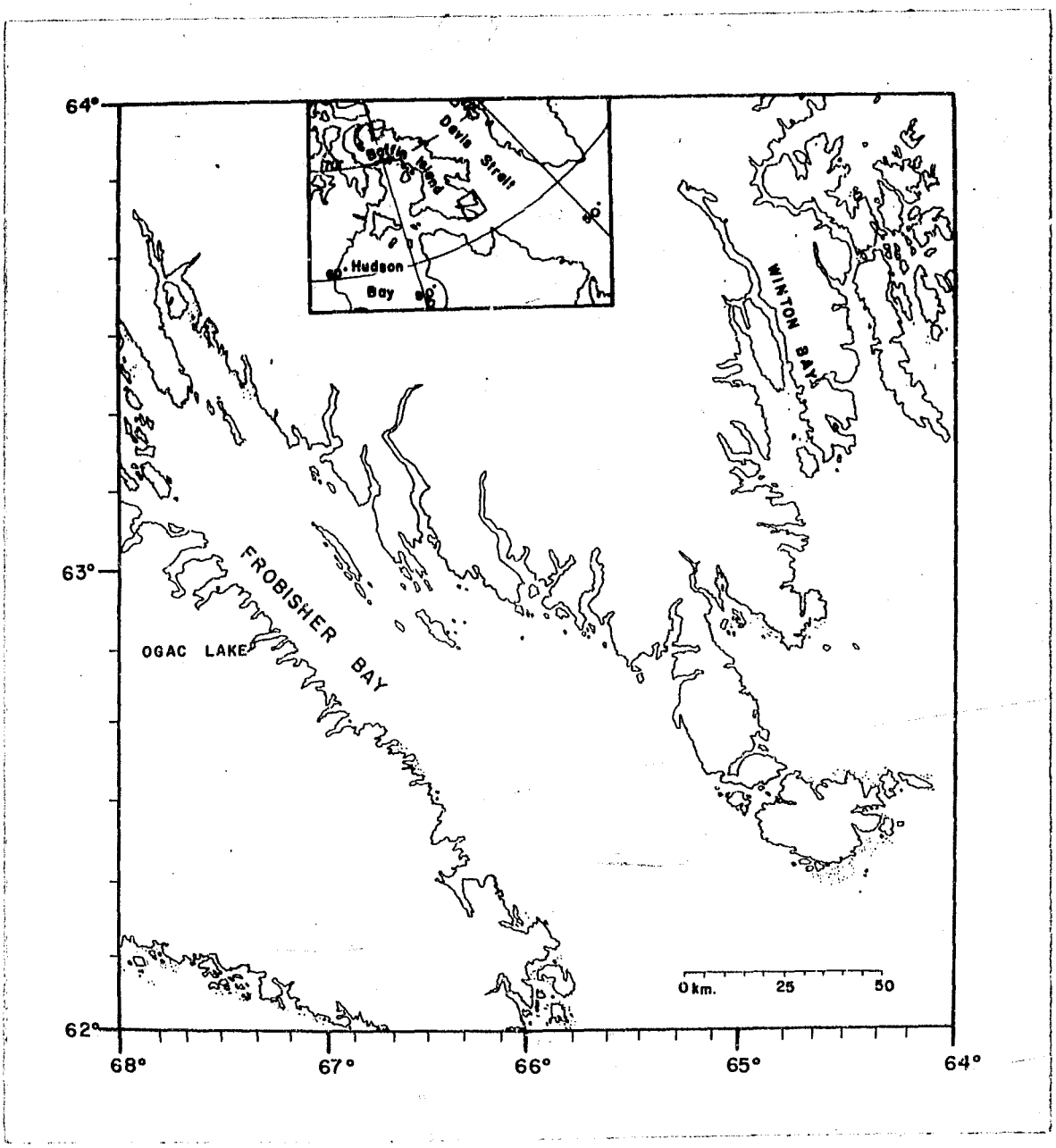

Figure 1 Map showing location of Ogac Lake and Winton Bay.

P. gracilis Sars, and P. major Sars (see Brodsky, 1950; Wiborg, 1954, 1955; Sars, 1903) or whether the species described are actually synonymous with P. minutus (Krøyer) as assumed by some authors (Jespersen, 1923; With, 1915; Størmer, 1929; Marshall, 1949; Farran, 1951; Fontaine, 1955; Østvedt, 1955). For this reason, and also due to the appearance of the hitherto unknown large-egged form, the animals discussed here will be referred to only by the generic name.

MATERIALS AND METHODS

Sampling methods. The methods used in the collection of Pseudocalanus were based essentially on those of McLaren (MS 1961). The schedule of the hauls from which the various values were determined is tabled in Appendix I. Some other samples referred to in the text, for example from Frobisher Bay and elsewhere, were not collected quantitatively and are not listed in Appendix I. The material from 1957 and 1962 was analysed by I.A. McLaren and made available for investigation here. It can be seen that a variety of net sizes and meshes was used from year to year. All the vertical hauls with the no. 6 nets were made from the anoxic depths (below 30 m.) to the surface and thus can be assumed to have sampled the whole water column. In all years, except 1952, these nets were hauled at a rate of approximately 0.5 m. per sec., which allows estimates for numbers per $m.^2$ to be made (see below).

Depending upon the abundance of the copepodite stages in the complete haul, the counts of the stages were assessed from either the whole sample or a random subsample. Egg-bearing females were removed from the whole sample to avoid detaching the eggs in the act of sub-sampling. The subsamples were taken from thoroughly mixed samples, from which large obstructing organisms had been removed, by a large bore suction pipette and the volumes measured in a graduated cylinder. The sizes of the subsamples varied but they were usually 10% or greater. Counts of the naupliar stages were made from random subsamples of 10 - 20% which were measured in the above manner.

In order to estimate the abundance of copepodites of the small and large forms, the total numbers of older copepodites (stages IV to

VI, or stage VI alone where these were sufficiently common) were calculated for the no. 6 nets, since these are assumed to be 100% efficient in retaining animals of this size. The estimates could then easily be converted to numbers per $m.^2$ from the known net diameters (Appendix I). In order to estimate the abundance of younger stages (copepodites I - III), their numbers were calculated relative to those of older stages in samples from the no. 20 nets, which are assumed to retain all stages with equal, but less than 100% efficiency. The one exception was the determination of the abundance on August 3, 1965 in Winton Bay. The estimates for all stages were calculated from a no. 20 net as only the one collection was taken that day. Unfortunately, not all the no. 20 nets were hauled from full depth, and some stages may be disproportionately represented. Unfortunately also, only samples from fine-mesh, no. 18 net hauls are available from Ogac Lake in 1952. Numbers are, therefore, calculated assuming this net to be 100% efficient for all stages. It is possible that this efficiency was, in fact, almost achieved in the phytoplankton-poor waters of August when the samples used here were collected.

The cephalothorax lengths of the counted copepodites were measured under a dissecting microscope while the cephalosome lengths of the naupliar stages and the egg diameters were measured under a compound microscope. The naupliar stages were identified from the diagrams by Ogilvie (1953). The egg diameters were determined from the average of the maximum and minimum diameters of each egg if it was not perfectly spherical. In order to compare the adult female cephalothorax lengths and the egg diameters, only those eggs still attached to the females were measured.

Determination of volume ratios. The volume ratios of adult females and stage I copepodites were calculated by cubing mean cephalothorax lengths, assuming that the large and small forms were the same shape. The volumes of the small and large eggs were calculated from the mean diameters, assuming the diameters to be those of two spheres.

To estimate the volume ratio of the nuclei of the small and large forms, ten adult females of each form were stained in haematoxylin and squashed in permount on separate slides. Like other organisms, copepods exhibit somatic ploidy (Stich, 1962) and therefore only a single common type of nucleus - spherical and densely staining - was measured. The diameters of ten such nuclei in each female were measured and the mean diameter of the two forms calculated. Again, assuming the diameters to be those of two spheres, the relative volumes were calculated from the mean diameters.

Chromosome staining. Incidentally to studies by I.A. McLaren on development rate of copepod eggs (McLaren, 1966), fresh eggs were secured for chromosome study. The eggs or early embryos were squashed in aceto-orcein stain. The squashes were then covered and the slides sealed. Only the chromosomes of eggs at the metaphase stage of division were used for comparison.

Staining and cytophotometric analysis of nuclear DNA. Cytophotometric analysis has been employed to study the relative amount of nuclear DNA in eggs of the small and large forms. These were from formalin-preserved plankton samples taken at Ogac Lake in 1965.

Eggs of each form were treated concurrently to avoid introduction of variants due to the staining procedure. The eggs were deformalinized by washing in distilled water for 15 min. and soaking in two 24 hr. changes

of 20% aqueous chloral hydrate. They were then washed for 15 min. in distilled water before staining (Lotho and Vaz Ferriera, cited by Davenport, 1960).

The Feulgen reaction (de Tomasi, 1936) was employed as a measure of relative amount of nuclear DNA. The specificity and stoichiometry of this reaction have been validated by Novikoff (1955), Stowell (1946), Dodson (1946), Swift (1953, 1955) and Leuchtenberger (1958). Following deformalinization, the eggs were hydrated in 70% alcohol for 2 hr., 50% alcohol for 1 hr. and 30% alcohol for 1 hr., then kept in distilled water for 12 hr. They were rinsed in cold 1N HCl for 2 min. and then hydrolyzed in 1N HCl for 9 min. at 60°C. After hydrolysis, the eggs were rinsed in cold 1N HCl for 2 min. and washed in three 10 min. changes of distilled water. The eggs were next treated with Schiff's reagent for 45 min. Several non-hydrolyzed eggs served as a control. The eggs were removed from the reagent and were transferred through three 10 min. changes of sulphurous acid rinse. They were then washed in three 10 min. changes of distilled water, dehydrated in absolute alcohol for 10 min. and cleared in alcohol-xylene (1:1). Squash preparations of these eggs were made and mounted in oil of refractive index (R.P. Cargille Inc.) to match the nuclear refractive index (small form, 1.580; large form, 1.592).

Measurements were made on squash preparations of the small and large eggs each mounted on separate slides in oil of refractive index by a cytophotometer constructed by Otto C. Watzka Co. Ltd. (Montreal). For the theoretical background and details regarding equipment, the reader is referred to the original papers (Caspersson, 1950; Swift, 1950; Pollister, 1952; Swift and Rasch, 1956; Pollister and Ornstein, 1955, 1959; Mendelsohn, 1958a). The cytophotometric technique used was

the aperture method, and all measurements were by the two wavelength method (Ornstein, 1952; Patau, 1953).

Absorption spectrum measurements were taken 10 mμ apart; the maximum absorption at $\lambda_{570m\mu}$ was selected as one wavelength. The second wavelength was estimated by linear regression (Garcia, 1962) and ultimately established by trial and error at $\lambda_{500m\mu}$. Subsequently, transmissions at $\lambda_{570m\mu}$ and $\lambda_{500m\mu}$ were used as a measure of amount of nuclear DNA per cell.

Duplicate readings were taken of 10 nuclei in five eggs each of the small and large forms at the 32 cell stage. The average extinction for each field was obtained from Mendelsohn's Tables (Mendelsohn, 1958b). Since the entire nucleus was included in the photometric field, the total amount of chromatophore per nucleus was estimated by multiplying the average extinction by the area of the photometric field (small, 523.0 μ^2; large, 2084.6 μ^2; see Table I).

ENVIRONMENT

General description of the environment. Ogac Lake, its hydrography and its zooplankton have been described in detail by McLaren (MS 1961, 1967). The lake lies at the head of Ney Harbour, an inlet on the southwest shore of Frobisher Bay. It is divided into three basins by shallow sills with oxygen absent and H_2S present below 25 m. in the lower basin, 30 m. in the middle basin, and 32.5 m. in the upper basin. The surface waters are fresh and the salinity increases to 27°/oo at depth. The threshold of the lake is so high that only the highest tides from Frobisher Bay are able to flow into the lake replenishing its salinity. Throughout the year Ogac Lake is much warmer than the sea outside due to its small size, its landlocked nature, and the conservation of radiant heating by its highly stable waters. Radiation causes considerable warming at depths to about 30 m. During the winter it is insulated from heat loss by its ice and snow cover.

The head of Winton Bay, which is situated on the southwest shore of Robinson Sound, is similar to Ogac Lake in many of its physical aspects (McLaren, 1967). It is divided into two basins by a shallow sill. The inner basin is devoid of oxygen at 31 m. as is the outer basin at 32 m. and the salinity increases to 32°/oo at depth. The waters are warmer than those of the bay outside due to the same causes which prevail in Ogac Lake but are cooler than those of Ogac Lake.

Both Ogac Lake and Winton Bay are extreme examples of the fjord condition as described by Dunbar (1958).

Environmental factors affecting size. Deevey (1960) showed

that size of copepods is strongly correlated with temperature of the environment and estimated amounts of food, but that the degree of dependence upon these factors varies with the geographical area, and especially the temperature range of the species being considered. McLaren (1963) has demonstrated that for some groups of poikilotherms, including calanoid copepods, size at any stage of development is a function of temperature alone although lack of food may thwart growth and development. The size-temperature curve given by McLaren (1965b) for Pseudocalanus in the Eastern Canadian Arctic shows a rather steep, inverse size-temperature relationship.

In the six geographical areas studied by McLaren (1965b) only in the one locality of Loch Striven was egg size found to be negatively correlated with temperature and positively correlated with the mean female length. Even then, although the female length varied about 35%, egg size varied only 7%. Correlations in other locations were not significant.

THE LARGE AND SMALL FORMS OF PSEUDOCALANUS

Morphology. As aforementioned, two forms of Pseudocalanus which vary only in size are found coexisting in both Ogac Lake (McLaren, 1965b) and Winton Bay (Figure 2). The morphological features are similar to those attributed to P. gracilis by Sars (1903). The head of the adult copepodite is slightly tapered. The first legs of the adult female are smaller than the other legs and bear one long seta on the inner side of each basipod. The basal segments of the asymmetrical fifth pair of legs of the adult male are approximately equal in length. The pointed terminal segment of the right leg, which consists of three segments, is about equal in length to the basipod. The left leg is longer than the right and, including the basipod, consists of five segments. The terminal segment of the left leg is tipped with a slender spinule. The authors (Brodsky, 1950; Sars, 1903) who have differentiated between the species, employ the fifth pair of appendages in the adult male and the first pair in the adult female as two of the principal distinguishing features of each species. The respective appendages showing the similarity of the large and small forms are presented in Figures 3 and 4.

Body size. All copepodites and nauplii contained in complete samples or subsamples collected from Winton Bay in 1964 and Ogac Lake in 1965 were measured either for cephalothorax length in the case of the copepodites or for cephalosome length in the naupliar stages. The frequencies and size distributions recorded from one collection taken from Ogac Lake on August 6, 1965 and one from Winton Bay on August 15, 1964 are presented in Figures 5 - 7. The frequencies shown for the

Figure 2 Forms of Pseudocalanus -
upper: from western Hudson Strait
middle: large-egged form from Ogac Lake
lower: dominant small form from Ogac Lake

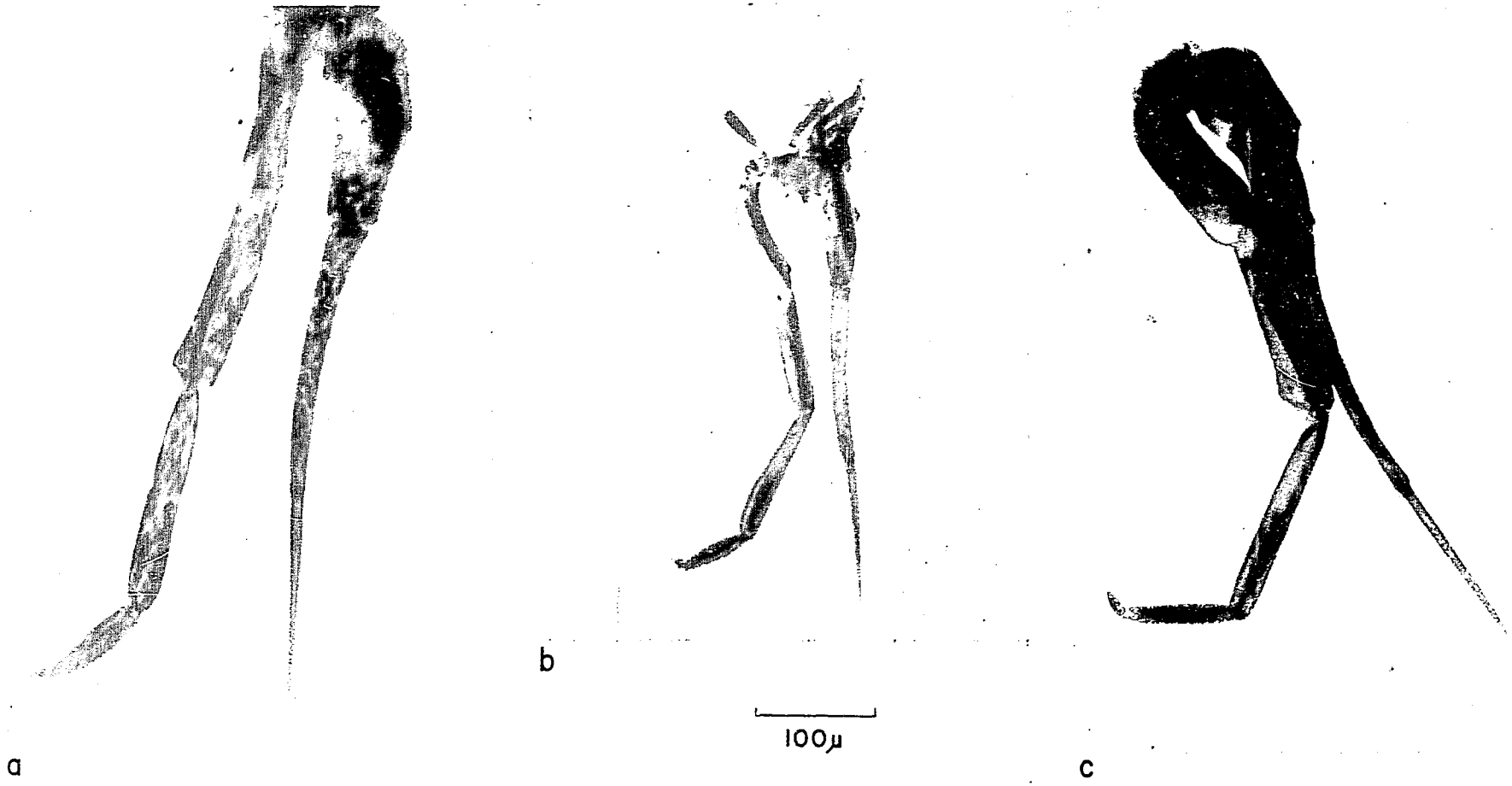

Figure 3 Fifth leg of adult males (with cephalothorax lengths) of Pseudocalanus from a) Winton Bay (large form = 1090.3µ), b) Winton Bay (small form = 687.5µ), and c) western Hudson Strait (1097.2µ).
All are to the same scale (above).

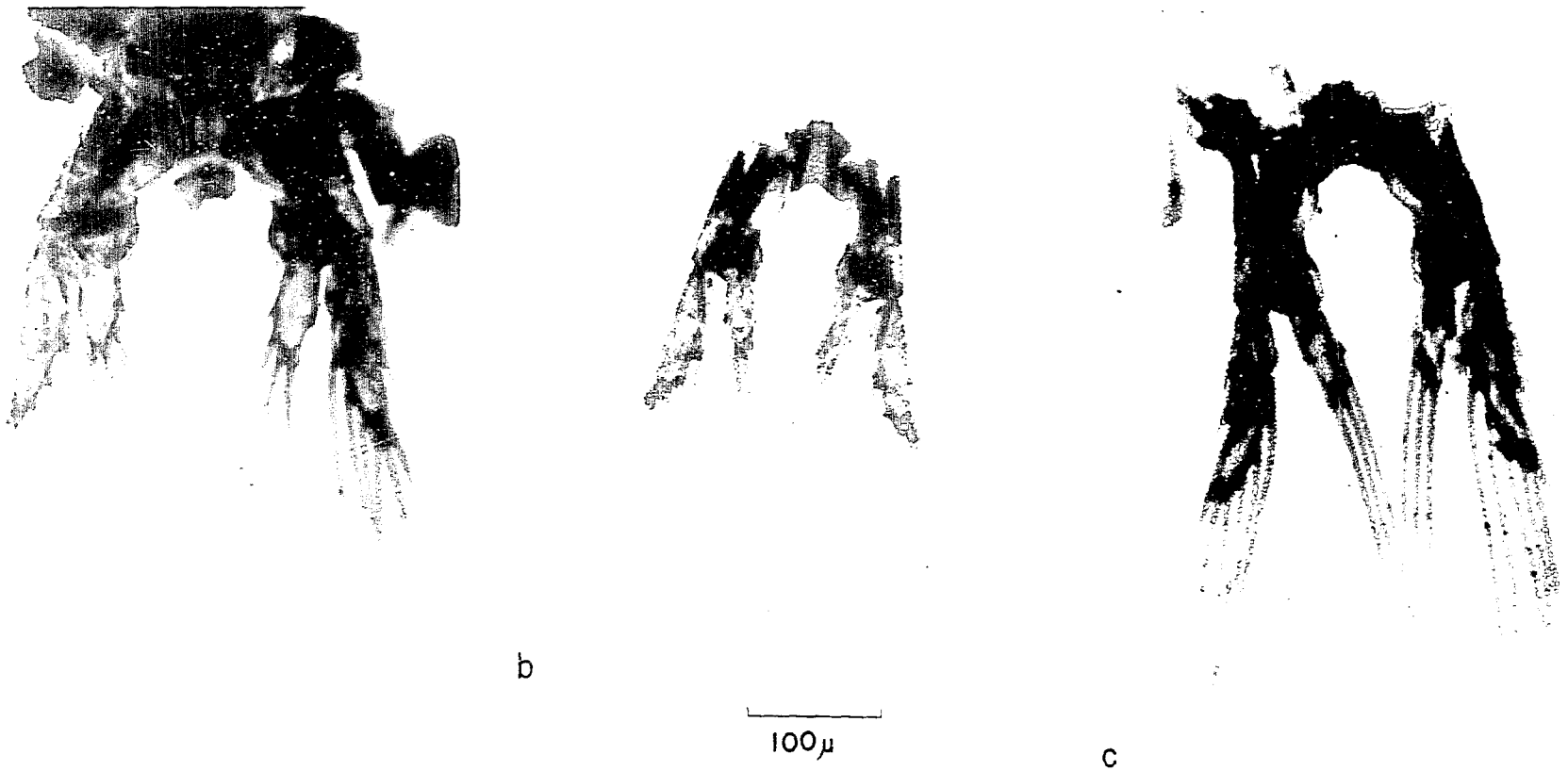

Figure 1 First leg of adult females (with cephalothorax lengths) of Pseudocalanus from a) Winton Bay (large form = 1215.3μ), b) Ogac Lake (small form = 886.9μ), and c) western Hudson Strait (1263.9μ).
All are to the same scale (above).

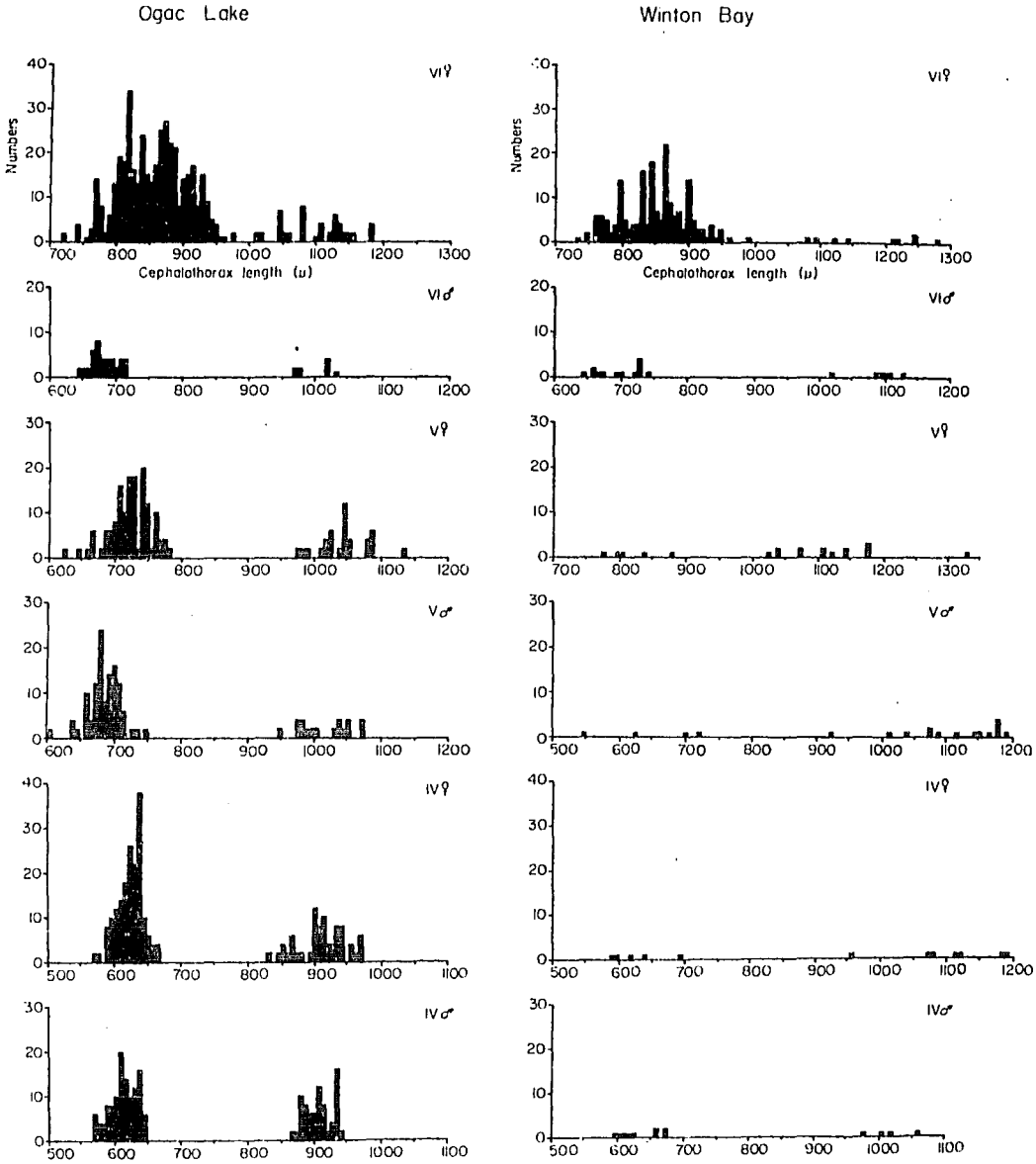

Figure 5 Size distribution of copepodite stages ♂ and ♀ IV - VI inclusive from Ogac Lake (August 6, 1965) and Winton Bay (August 15, 1964). Results are determined from full volume of sample.

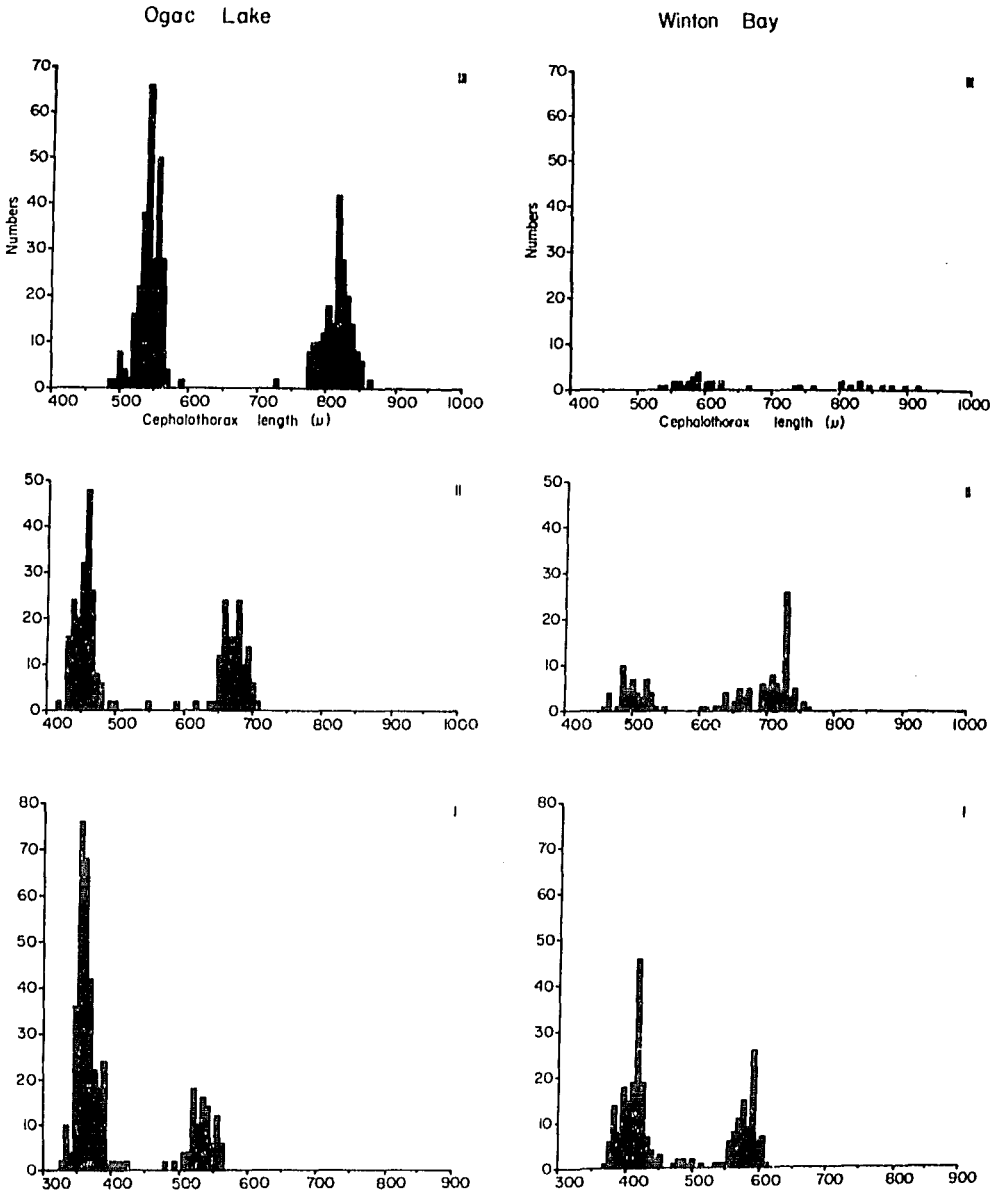

Figure 6 Size distribution of copepodite stages I - III inclusive from Ogac Lake (August 6, 1965) and Winton Bay (August 15, 1964). Results are determined from full volume of sample.

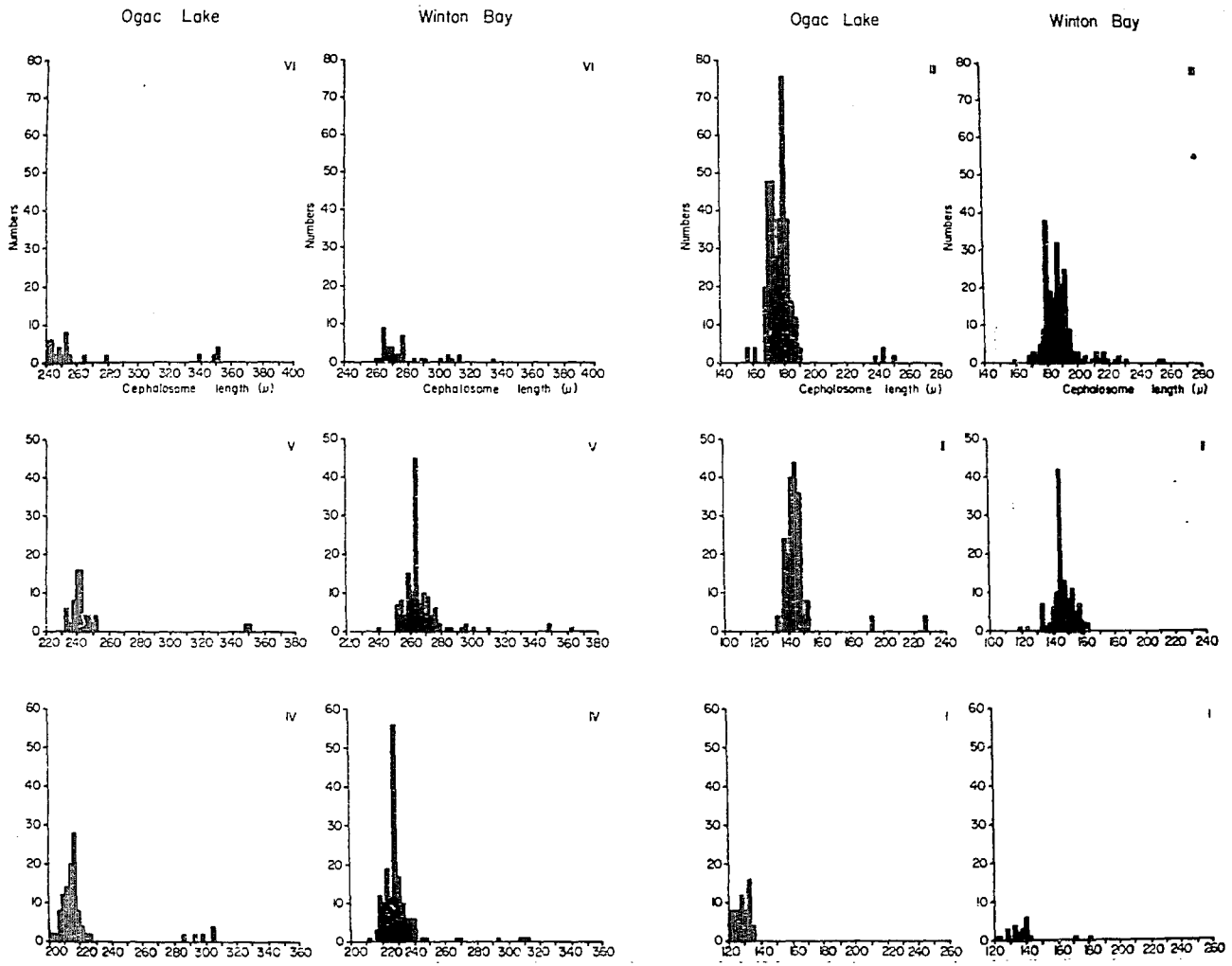

Figure 7 Size distribution of nauplius stages I - VI inclusive from Ogac Lake (August 6, 1965) and Winton Bay (August 15, 1964). Results are determined from subsamples of 1/10 the volume of the sample.

copepodite stages represent the complete sample while the frequencies of naupliar stages were determined from a subsample of 1/10 volume of the full haul.

The size ranges (Fig. 5 - 7) of all stages from first nauplius to adults for both Ogac Lake and Winton Bay appear to be bimodal. The distribution of sizes obtained in two samples was tested for the significance of the difference between the two means (Stanley, 1963) and the difference was found to be significant for each stage (Appendix II).

The size distributions and frequencies obtained from the other samples collected during 1964 and 1965 are recorded as the mean and two standard errors for each size mode for each stage in Appendix III. The animals collected in hauls taken in Ogac Lake during 1952, 1957 and 1962 also exhibit equally clear bimodality in their size distributions for some of the stages examined. They are not recorded here as either the measurements were only taken for the copepodite stages or in some cases the measurements were recorded only as the total number of animals occurring in each size group.

Collections of living material were made at Ogac Lake in 1965 by I.A. McLaren, for the purpose of studying embryonic development rate of the large and small forms (McLaren, 1966). Nauplii of the large and small form, hatched at the same temperature (ca. 5°C), are shown in Figure 8. Clearly, then, the size differences are not environmentally induced.

It is interesting to note that both size groups appearing in the Winton Bay collections average larger than those in Ogac Lake. The significance of the difference between the means was tested (Appendix IV) and in a slight majority of the stages was found to be significant. Since body size in Pseudocalanus is negatively correlated with temperature

Figure 8 Nauplius stage I hatched from adult ♀♀ from Ogac Lake with nauplius cephalosome length and female cephalothorax length.
left: large form (nauplius = 168μ; ♀ = 1069.4μ)
right: small form (nauplius = 129.6μ); ♀ = 902.8μ)
All are to the same scale (above).

(McLaren, 1965b) the cooler waters of Winton Bay (McLaren, 1967) are effecting the increase in body size there.

Egg size. In addition to the variation in body size of the two forms, the eggs born by the large form are conspicuously larger than those of the small form. The mean diameters of eggs are plotted (Figure 9) against cephalothorax length of the females bearing the eggs in hauls from Winton Bay in 1964, Ogac Lake in 1965, and from Frobisher Bay in 1965.

Two distinct size groups appear in the lakes except for a few large forms bearing eggs of intermediate size (Fig. 9 a,b). McLaren (1961, 1965b) has found that small numbers of large Pseudocalanus enter Ogac Lake at times of the highest tides but do not appear to survive long and are presumably ill-adapted to the sudden environmental change. The larger body size of these outside animals, which average one-third longer than the small fjord form (McLaren 1965b; see also Fig. 2), is due to the colder temperatures prevalent in Frobisher Bay. It is probable, therefore, that the large specimens bearing small eggs had recently come into the lakes from outside. Interestingly, their eggs are comparable in size with those from Frobisher Bay (c.f. Figure 9 a,b, with Figure 9c). If the eggs were, in fact, produced in the warmer waters of the fjords, this implies that egg size is a function of female size as such, and not temperatures at the time of spawning.

Chromosomes. It seems clear that neither food nor temperature could cause the size bimodalism of the indigenous populations of Pseudocalanus in the two landlocked fjords as these animals are experiencing the same environmental influences.

It was considered possible (McLaren, 1965b) that the large forms could be polyploids as had been discovered in Calanus finmarchicus

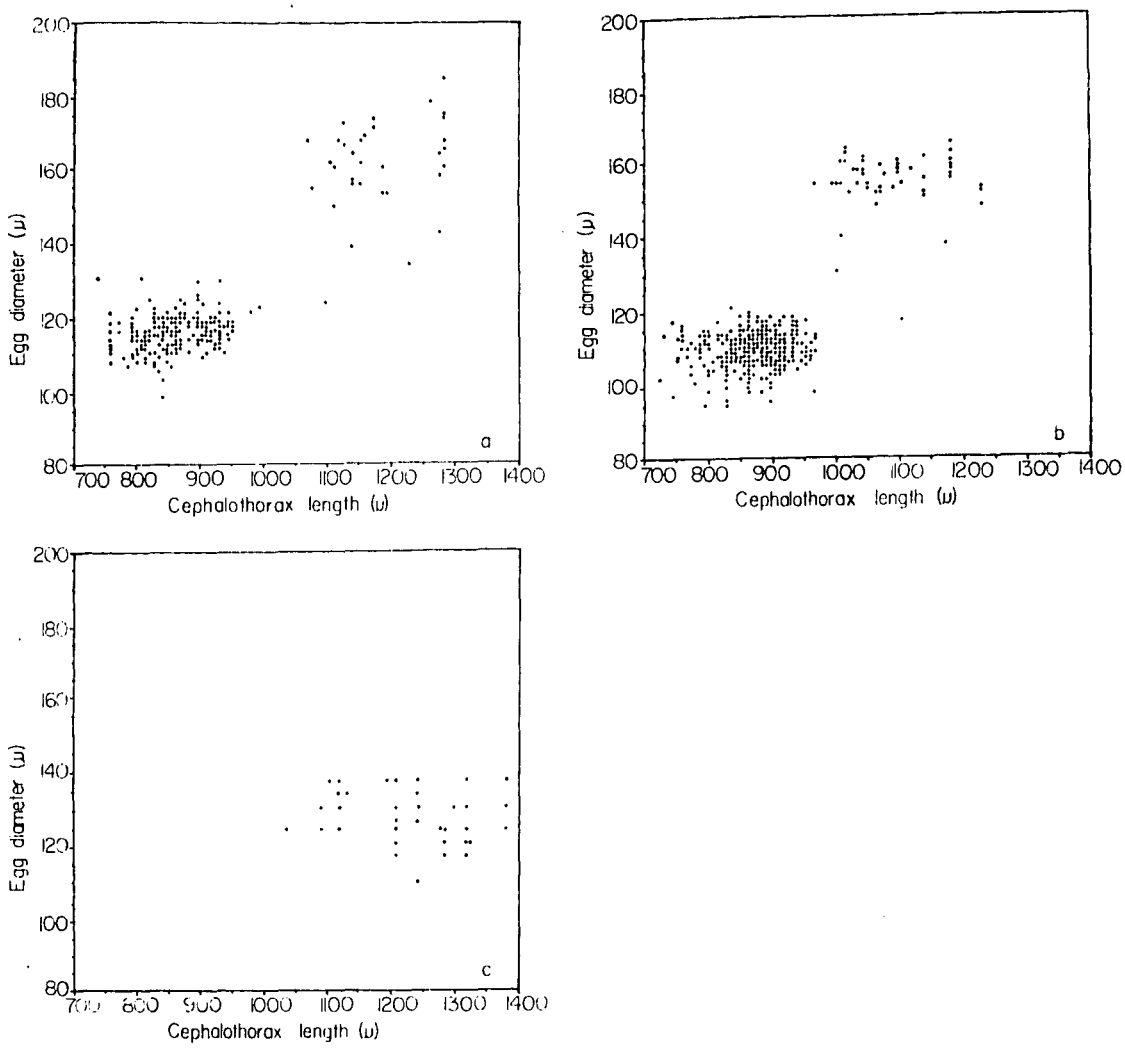

Figure 9 Graphs comparing adult female cephalothorax lengths and diameters of the attached eggs of Pseudocalanus from a) Winton Bay - 1964, b) Ogac Lake - 1965, and c) Frobisher - 1965.

(Harding and Marshall, 1955; Marshall and Orr, 1955). Aceto-orcein squash preparations of fresh eggs revealed that the large forms in Ogac Lake possess the normal calanoid chromosome complement of n=16 (Marshall and Orr, 1955) as do the small forms (Figure 10). Two eggs only of one of the large females were discovered to have a chromosome complement of n=17 (Figure 10b). According to Dawson (1962) this is caused by the phenomenon referred to as non-disjunction where pairs of chromosomes fail to separate at meiosis.

The chromosomes of the large form, however, were noticeably larger than those of the small form and also larger than those found in fresh material collected in Frobisher Bay, N.W.T., Halifax, N.S., and Millport, Scotland. Formalin preserved material was available for staining from Winton Bay but the development of the embryos was too advanced to allow a detailed picture of the chromosomes.

Polyteny and nuclear DNA content. It is an acceptable theory (Hughes-Schrader and Schrader, 1956) that an important factor in the determination of chromosome size is the occurrence of polyteny, in which a chromosome endoreplicates simultaneously a number of times and, instead of separating, the daughter strands remain together forming a many-threaded polytenic cable (Herskowitz, 1962; Altenburg, 1957; Mazia, 1961).

Darlington (1958) has stated that "the chromosome is a nucleo-protein . . . . . probably based on units consisting of poly-peptide chains to which are attached double columns of polymerised nucleotides forming desoxyribose nucleic acid" and that "it seems safe to assume that DNA is ordinarily restricted to the chromosomes" (Darlington, 1955) . Therefore, as chromosome size increases with ascending levels of polyteny, the nuclear DNA content increases proportionally (see Hughes-Schrader and Schrader, 1956; Kurnick and Herskowitz, 1952;

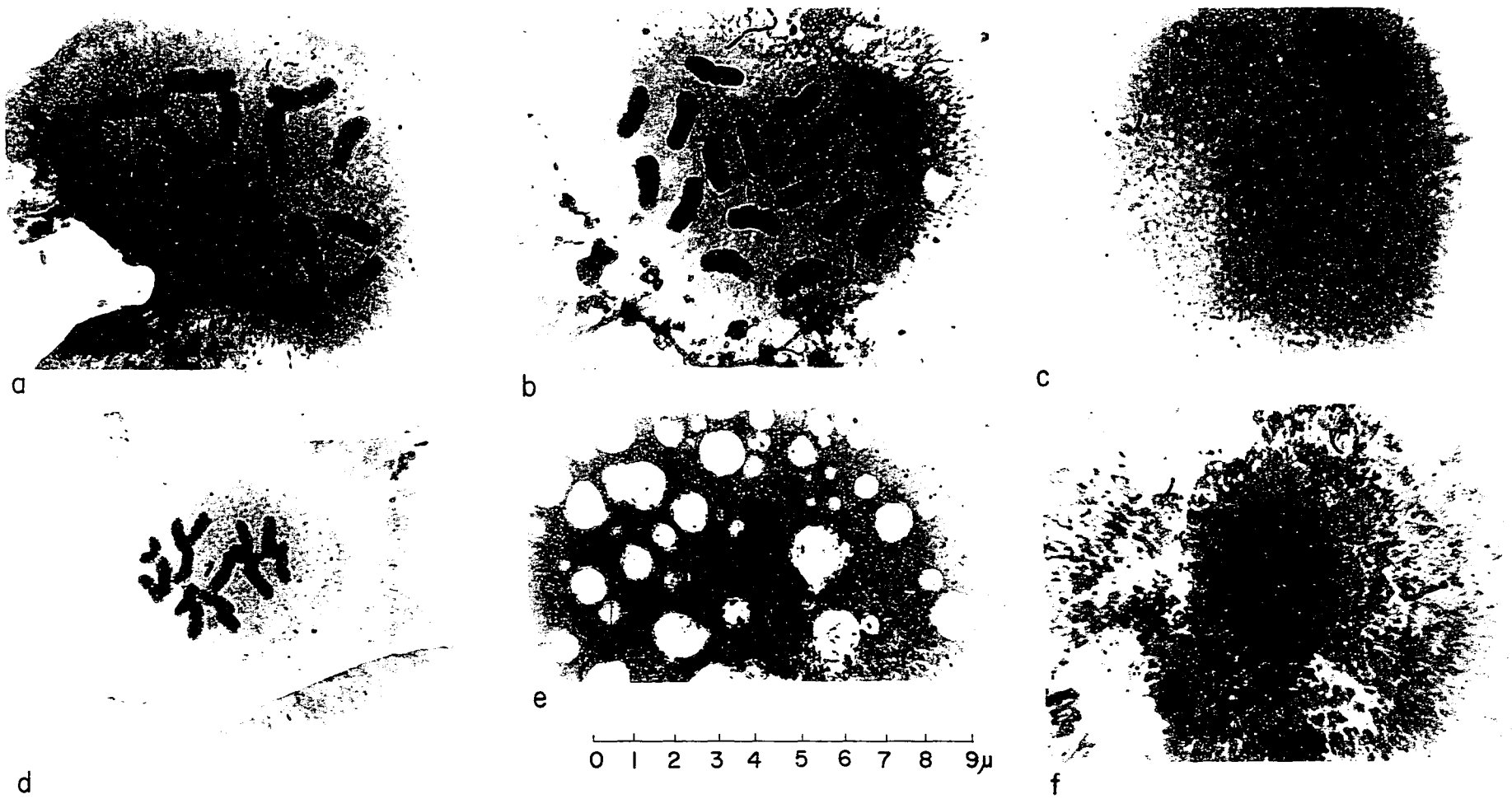

Figure 10 Aceto-orcein-stained haploid sets of metaphase chromosomes from fresh eggs of a) the large form of *Pseudocalanus* from Drac Lake, b) the large form from Drac Lake with n=17, and of *P. minutus* from c) Drac Lake, d) Frobisher, N.W.T., e) Halifax, N.S., and f) Millport, Scotland.
All are to the same scale (above).

Swift and Rasch, 1954).

Since the DNA content in the resting nucleus remains constant (Darlington, 1955) a cytophotometric analysis, as described in Materials and Methods, was carried out on the Feulgen-stained interphase nuclei of the small and large forms. Although the Feulgen test cannot by itself establish the absolute amount of nuclear DNA, if the differences in DNA content are sufficiently large the test does supply accurate estimates of the relative amounts (Leuchtenberger, 1954; Swift, 1950).

The representative absorption spectrum for the Feulgen-DNA complex is presented in Appendix V, plotted as per cent of maximum extinction (from the average absorption spectrum for each form - see Appendix VI) versus wavelength to facilitate comparison. The average absorption spectra were calculated from the absorption spectrum measurements taken for each of the eggs of both forms.

The average extinction values for each series of photometric measurements were analysed by the Chi^2 test (Snedecor, 1956; Duncan, 1959) and were found to have a normal distribution (Appendix VII). The results of the photometric analysis show that the ratio of the relative amount of DNA per nucleus in the large form to the small form is approximately 7:1 (Table I), and this seems safely attributable to polyteny.

Effects of DNA. Commoner (1964) has demonstrated that in bacteria the cellular DNA content is directly related to the cell volume and in various vertebrate erythrocytes the relationship is directly proportional. Mirsky and Ris (1950) also state that "in general . . . . the greater the DNA content, the larger the cell". Furthermore, since over-all cell size is generally inversely related to the metabolic rate, a species' characteristic rate of oxidative metabolism is inversely related to the

TABLE I

Results of photometric measurements of DNA-Feulgen complex on nuclei of five small and five large eggs corresponding to the 32 cell stage. Amount of chromatophore is determined as an average of duplicate readings of ten nuclei each per egg.

	Average Extinction ($\bar{\mu} \pm 6$)	Photometric Area (μ^2)	Total Amount Chromatophore
Small	.122 ± .025	523.0	63.8
	.138 ± .021		72.2
	.141 ± .036		73.7
	.115 ± .012		60.2
	.146 ± .016		76.4
Amount DNA/ Nucleus	.132 ± .022		69.0
Large	.241 ± .030	2084.6	502.4
	.233 ± .029		485.7
	.279 ± .030		581.6
	.202 ± .026		421.1
	.238 ± .012		496.1
Amount DNA/ Nucleus	.239 ± .035		498.2

DNA content. These relationships have also been discussed by McLaren (1965a), Yčas, Sugita, and Bensam (1965), and Stebbins (1966). In addition, it was found that nuclear volume is positively related to the length of the mitotic cycle (Van't Hoff and Sparrow, cited by Stebbins, 1966). Therefore, an increase in the amount of DNA results in an increase in the cell volume but a decrease in the metabolic and division rates. Since copepods show determinate growth and probably determinate cell number (McLaren, 1965b) an increase in the amount of DNA would be expected to increase the size and to reduce the development rate of the entire animal.

The relative volumes of the adult females of the two fjord forms were compared along with the volumes of their eggs, nuclei, and the stage I copepodites (Table II). The ratios of these volumes were approximately 3:1 as shown also by McLaren (1965b). Therefore, since the relative nuclear DNA contents of the two forms of Pseudocalanus are in the proportion of 7:1, the DNA content is directly related to the cell volume but the relationship is not proportional.

McLaren (1965b) investigated the development rates from field samples of the small and large forms in Ogac Lake and showed that the mean rate in the small form was significantly greater at 0.42 stages per day than in the large form at 0.28 stages per day. Later (McLaren, 1966) he was able to show experimentally that eggs of the small form develop about 1.7 times as fast at any temperature.

Thus, as a consequence of polyteny, the nuclear DNA contents of the large form have increased, causing an increase in cell volume and therefore body size, and a decrease in development rate.

Adaptive value of polyteny. It is a reasonable assumption that the greater the amount of DNA, the more complex the organism, and

TABLE II

Comparisons of sizes of the large and small forms of Pseudocalanus from Ogac Lake, 1965 and Winton Bay, 1964. Values are means and two standard errors.

	Large form (μ)	Small form (μ)	Volume ratio
Ogac Lake -			
Egg diameter	158.71 ± 0.70	110.23 ± 0.19	2.98
Nucleus diameter	5.19 ± 0.07	3.53 ± 0.03	3.16
♀ cephalothorax length	1089.37 ± 3.97	851.12 ± 0.89	2.10
Stage I copepodite length	532.40 ± 1.33	362.04 ± 0.94	3.18
Winton Bay -			
Egg diameter	162.46 ± 0.60	116.04 ± 0.32	2.74
Nucleus diameter	5.04 ± 0.04	3.54 ± 0.04	2.77
♀ cephalothorax length	1159.27 ± 4.39	840.77 ± 0.69	2.62
Stage I copepodite length	558.00 ± 0.83	398.41 ± 0.35	2.75

consequently the greater the variation possible in its adaptability (Herskowitz, 1962; Stebbins, 1966). However, this can only be a long-term value, and the polytenic Pseudocalanus does not appear to differ in ways unrelated to size.

In the inner basin of Ogac Lake three broods of the small form of Pseudocalanus are produced during the year (McLaren, MS 1961). However, only the first brood survives as it is spawned during the phytoplankton bloom; the numbers of the second brood decrease sharply and the third brood (actually the first of a second generation) completely disappears due largely to the depleted food conditions. The reproductive cycle in the middle basin is similar to that in the upper basin with the slow growth of the first brood, the attentuation of the second brood and the obliteration of the third. The pattern in the outer basin differs from that of the other basins as only two broods are spawned and both of these may survive due to the maintenance of the Chaetoceros supply.

Therefore, in Ogac Lake two generations of the small form are produced in a year in the outer basin while the reproductive cycles in the middle and inner basins are essentially annual but with two abortive broods being produced. The reproductive cycle of Pseudocalanus minutus in ordinary arctic waters is usually annual (Digby, 1954; Ussing, 1938; Fontaine, 1955; Østvedt, 1955) and, as aforementioned, the normal arctic Pseudocalanus are of a much greater size than the small form in the two landlocked fjords. Thus, as a result of the unusually warm waters in the fjords the size of P. minutus is greatly reduced and the development rate increased.

The larger size and the reduced development rate of the polytene animals may, therefore, represent "an evolutionary attempt to restore

normal size and development rates for these high latitudes" (McLaren, Woods, and Shea, 1966), perhaps with less "wastage" of reproductive products on abortive broods.

Abundance of the polytene Pseudocalanus. Estimates of the numbers per $m.^2$ of copepodites of the large form of Pseudocalanus appearing in all available samples collected from the middle basin of Ogac Lake in 1965 and from Winton Bay in 1964 are presented in Table III. Collections from one day only, August 6, were available from Ogac Lake. The small form in these samples appears to be approximately three times as numerous as the large form. On August 7, 1964 in Winton Bay the large form was one-fifth as numerous as the small form. It might thus be considered that the large form is relatively more abundant in Ogac Lake than it is in Winton Bay. However, on August 3, 1964 in Winton Bay the large form outnumbered the small form by about 3:1 while on August 15 the small form was only twice as abundant as the large form. The small form also outnumbered the large form approximately 5:1 and 4:1 on August 20 and August 28 respectively. If the estimates for the Winton Bay collections are totalled the ratio of the number of large forms to the number of small forms is 1:3 as it is in the August 6, 1965 collection from Ogac Lake. Therefore, since there is such a variation in the ratios of the relative abundance of the small and large forms it is felt that further investigation is necessary before a conclusion can be reached as to whether the large form is consistently more abundant in one fjord than in the other.

The variation in the abundance of the large form during a thirteen year period was calculated from collections taken from the middle basin of Ogac Lake in 1952, 1957, 1962, and 1965 (Table IV). In 1952 the large

TABLE III

Estimated numbers per m^2 of copepodites of the large form of Pseudocalanus in all available samples from the middle basin of Ogac Lake in 1965 and from the outer basin of Winton Bay (except the collection on August 20 which was taken from the inner basin) in 1964. Calculated as discussed in text.

Locality and date	Numbers per m^2 of copepodites of large form						Total copepodites per m^2	
	I	II	III	IV	V	adult ♀	large form	small form
Ogac Lake -								
August 6	1330	1870	2780	2350	1130	710	10,170	27,840
Winton Bay -								
August 3	3150	30	10	310	1750	240	5,490	1,620
August 7	1240	110	60	10	100	450	1,970	9,650
August 15	500	460	70	60	150	50	1,290	2,310
August 20	240	110	40	110	420	100	1,020	5,370
August 28	90	2500	2040	180	890	500	6,200	26,880

TABLE IV

Estimated numbers per m^2 of copepodites of the large form of Pseudocalanus in the middle basin of Ogac Lake in all available samples from August of four years. Calculated as discussed in text.

Date		Numbers per m^2 of copepodites of large form						Total copepodites per m^2	
		I	II	III	IV	V	adult ♀	large form	small form
1952	*August 7	10	70	50	80	60	20	290	10,120
	August 12	10	30	50	10			100	6,380
1957	August 3	630				400	1400	2,430	98,600
	August 17		180	1550	260	200		2,090	50,800
	August 31	150					120	270	28,000
1962	August 10							0	251,600
	August 22	220	220	880				1,320	129,200
1965	August 6	1330	1870	2780	2350	1130	710	10,170	27,840

* estimates probably too low since only less efficient fine-mesh net used

form was outnumbered by the small form on August 7 by about 35:1 and on August 12 by 64:1. The ratios varied in 1957 from 24:1 (small to large) on August 17 to 104:1 on August 31. No large forms were collected in hauls on August 10 in 1962 but on August 22 the ratio was approximately ninety-eight small to one large form. As mentioned above, the large forms on August 6, 1965 were one-third as numerous as the small forms. Therefore, the marked variations from year to year in relative abundance of the small and large forms may be taken as evidence that they are differentially adapted to the environment, but whether or not the large form is better adapted (i.e., increasing at the expense of the small form) cannot be said on present evidence.

Polytene Pseudocalanus as a new species. Hughes-Schrader (1951, 1953) demonstrated that in the morphologically similar species of the mantid genus Liturgousa the amount of nuclear DNA could be employed as a species constant in considering interspecific relationships. Evidence presented by Mirsky and Ris (1949, 1950), Ris and Mirsky (1949), and Swift (1950) also supports the hypothesis that the nuclear DNA content is a constant characteristic of a species. On this basis the polytene form of Pseudocalanus could be considered as a new species. However, as the taxonomy of the genus Pseudocalanus is still indefinite, it is felt that further examination of the taxonomy is necessary before the status of the polytene form can be decided. Further, it is quite possible that the polytene form has arisen independently from the ordinary form in Ogac Lake and Winton Bay. This obviously raises conceptual difficulties for systematic analysis.

SUMMARY

1. A brief description of Ogac Lake and Winton Bay is given in order to show the similarity in the physical features of the two landlocked fjords. The waters in Winton Bay are cooler than the waters in Ogac Lake but the temperatures in both fjords are much warmer than those present in the waters outside.

2. The morphological features of the adults of the two size forms of Pseudocalanus appearing in the fjords are discussed and are found to be close to those of P. gracilis Sars. They do not appear to differ morphologically from the ordinary arctic forms outside the fjords.

3. Collections taken from Ogac Lake in 1965 and Winton Bay in 1964 are examined for the length distributions of the naupliar and copepodite stages. Bimodality is significantly apparent in all stages. Both forms in Winton Bay are larger as a result of colder temperatures there.

4. The larger form is shown to bear eggs which are conspicuously larger than those of the small form. A few large individuals in the two fjords are observed to bear small eggs. These are attributed to the larger outside animals which enter during the highest tides, but which are not able to survive in the fjord conditions.

5. Chromosome stains reveal that both size forms possess the same number of chromosomes (n=16) thus eliminating polyploidy as the causative factor in the size variation. However, the chromosomes

of the large form are notably larger than those of the small form.

6. The relative nuclear DNA contents of the two forms were determined by a cytophotometric analysis and the large form is shown to contain about seven times as much DNA as the small form. Therefore, it is concluded that polyteny is effecting the bimodality of size distributions.

7. A brief account is given of the effects of variation in the amount of DNA. The sevenfold increase in DNA has been shown to decrease the development rate of the large form in addition to being the source of its increase in cell size and therefore body size.

8. Reasons for this adaptation are discussed and it is suggested that the increased cell size and decreased development rate are an attempt to restore the "normal" arctic size and development rate to a high latitude location containing extraordinarily warm waters.

9. The relative abundance of the large form in the two fjords is estimated but it is concluded that further investigation is necessary in order to determine whether the large form is consistently more abundant in one fjord than in the other. From collections taken from Ogac Lake over a thirteen year period it appears that the large form has varied considerably in abundance in relation to the small form from year to year.

10. The classification of the polytene form as a new species is considered but it is concluded that further examination of the taxonomy of Pseudocalanus is necessary before its definite status can be decided.

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Appendix I

Table of routine vertical plankton hauls from Ogac Lake and Winton Bay used in this study.

Location	Date Yr.	Time	Day	Month	Depth m.	Mesh size	Net diameter
Winton Bay	1964	-	7	8	35-0	6	1/2 m.
		-	15	8	32-0	6	1/2 m.
		-	15	8	32-0	6	1 ft.
		-	20	8	35-0	6	1/2 m.
		-	28	8	33-0	6	1/2 m.
		-	3	8	30-0	20	1 ft.
		-	7	8	35-0	20	1 ft.
		-	15	8	32-0	20	1/2 m.
		-	20	8	35-0	20	1 ft.
		-	28	8	33-0	20	1 ft.
Ogac Lake (all from the middle basin)	1952	1420	7	8	40-0	18	1/3 m.
		0950	12	8	30-0	18	1/3 m.
	1957	-	3	8	35-0	6	1 ft.
		-	17	8	35-0	6	1 ft.
		-	31	8	35-0	6	1 ft.
		-	3	8	35-0	20	1 ft.
		-	17	8	20-0	20	1 ft.
		-	31	8	20-0	20	1 ft.
	1962	-	10	8	35-0	26	1/2 m.
		-	22	8	35-0	6	1/2 m.
		-	10	8	35-0	20	1 ft.
		-	22	8	35-0	20	1 ft.
	1965	-	6	8	35-0	6	1 ft.
		-	6	8	35-0	20	1 ft.

Appendix II

Table of mean cephalosome lengths of the naupliar stages and of mean cephalothorax lengths of the copepodite stages of Pseudocalanus and T test values to verify the bimodality of the length distribution. Values are expressed as mean and two standard errors.

	Date	Stage	Small (μ)	Large (μ)	T test value	Difference between means
Winton Bay	3/8/64	Naup.I	131.52± 1.51	_____	_____	-
		Naup.II	145.92± 0.69	_____	_____	-
		Naup.III	185.04± 0.82	239.52±12.50	4.347	s*
		Naup.IV	227.76± 1.25	291.12±18.84	3.355	s
		Naup.V	260.88± 2.35	346.32± 6.82	11.845	s
		Naup.VI	259.92± 6.79	356.64± 2.64	13.276	s
		Cop.I	421.59± 4.83	556.83± 1.52	26.706	s
		Cop.II	505.08±15.80	638.25±10.35	7.049	s
		Cop.III	585.12± 6.56	_____	_____	-
		Cop.IV ♂	715.53±49.82	976.35±12.35	5.081	s
		Cop.IV ♀	629.97±17.94	927.36± 7.94	15.157	s
		Cop.V ♂	703.80± 0.00	1074.33± 6.97	53.160	s
		Cop.V ♀	793.50±55.34	1082.61± 6.28	5.190	s
		Cop.VI ♂	663.78±15.25	1035.00± 0.00	24.341	s
		Cop.VI ♀	818.34± 8.35	1089.51±13.66	16.937	s

	Date	Stage	Small (μ)	Large (μ)	T test value	Difference between means
Winton Bay	7/8/64	Naup.I	134.16± 0.82			-
		Naup.II	146.16± 0.79	174.00± 6.00	4.600	s
		Naup.III	183.84± 0.86	226.08± 1.27	27.535	s
		Naup.IV	224.64± 1.27	264.00± 0.00	30.992	s
		Naup.V	261.12± 1.73			-
		Naup.VI	275.28± 1.34	374.40± 8.42	11.625	s
		Cop.I	414.69± 3.38	563.73± 2.83	33.811	s
		Cop.II	485.07± 6.49	669.99±15.80	10.826	s
		Cop.III	576.84±12.49	812.82±28.08	7.679	s
		Cop.IV♂	621.00±12.28			-
		Cop.IV♀	658.95±31.05	979.80± 0.00	10.333	s
		Cop.V ♂	718.98±18.42	1101.93±17.94	14.894	s
		Cop.V ♀	731.40±41.40	1109.52±10.28	8.867	s
		Cop.VI♂	675.51± 6.49			-
		Cop.VI♀	850.08± 2.69	1158.51± 2.50	83.972	s

*s=significant

Appendix III

Table of mean cephalosome lengths of the naupliar stages and of mean cephalothorax lengths of the copepodite stages of Pseudocalanus as determined from vertical plankton hauls from Winton Bay and Ogac Lake in 1964 and 1965 respectively. The values are expressed as mean and two standard errors.

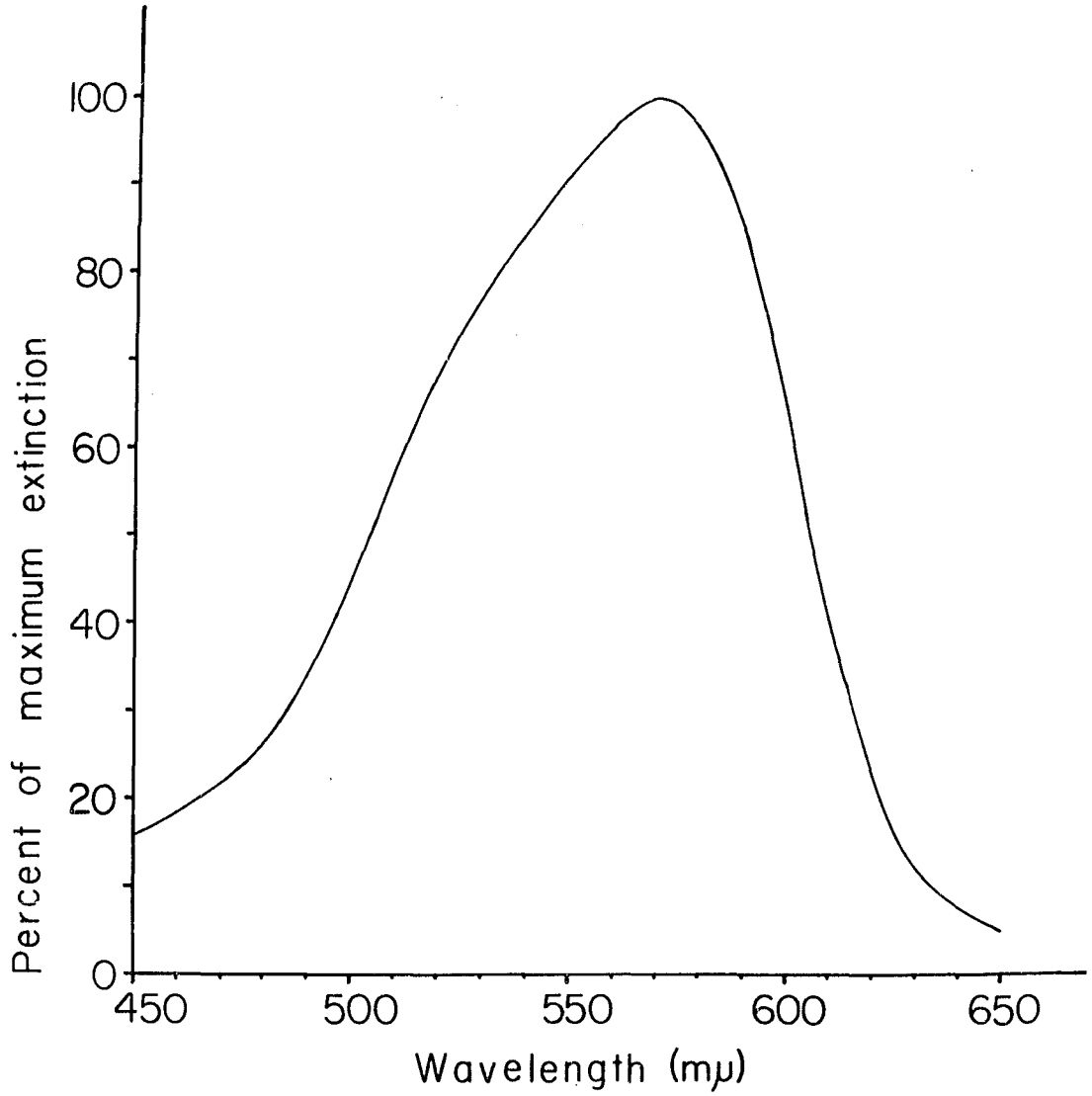
Date and location	Winton 15/8/64		Winton 20/8/64		Winton 28/8/64		Ogac 6/8/65	
Stage	Small (μ)	Large (μ)	Small (μ)	Large (μ)	Small (μ)	Large (μ)	Small (μ)	Large (μ)
Naup.I	133.68± 1.29		131.04± 1.80	187.20± 0.00	137.52± 1.80	170.40±14.40	127.44±1.10	
Naup.II	144.96± 0.65		148.80± 0.89		148.32± 0.86		142.56±0.60	208.80±16.80
Naup.III	185.28± 0.46	220.08± 3.41	183.36± 0.69	214.80± 3.60	185.76± 0.69	204.00± 0.00	176.64±0.46	243.12± 2.47
Naup.IV	227.52± 0.46	292.80± 8.42	226.80± 1.01	270.72± 8.26	226.80± 0.69	264.00± 0.00	217.92±2.47	304.80± 0.00
Naup.V	264.72± 0.69	333.60±12.14	264.48± 1.22	302.40± 0.00	262.32± 0.94	324.72±18.07	241.20±2.74	349.20± 1.20
Naup.VI	275.76± 2.38	333.60± 0.00	266.88± 2.09		267.36± 3.52	348.00± 0.00	249.36±2.30	346.80± 2.86
Cop.I	407.79± 1.45	571.32± 2.55	389.85± 1.73	522.33±11.79	396.75± 0.62	527.85±14.35	365.70±2.42	534.75± 2.07
Cop.II	497.49± 2.89	698.97± 3.73	472.65± 4.89	623.76±13.52	479.55± 1.31	709.32± 2.35	451.26±1.79	667.23± 2.42
Cop.III	583.05± 6.07	822.48±15.39	538.20± 4.07	763.83±32.43	552.69± 2.00	844.56± 2.69	538.20±1.45	812.13± 2.14
Cop.IV ♂	632.04±10.14	1010.85±15.53	616.17± 4.97	1009.47±16.49	611.34± 3.04	952.89±16.84	609.96±2.69	903.21± 3.38
Cop.IV ♀	623.76±18.56	1099.17±29.95	594.78±11.45	993.60±19.94	618.24± 4.76	1014.30±21.67	621.00±2.00	907.35± 5.24
Cop.V ♂	645.15±39.26	1107.45±19.25	703.80± 9.59	1105.38±10.28	678.27± 3.52	1113.66±11.59	682.41±3.11	1012.92± 9.32
Cop.V ♀	815.58±18.15	1119.87±20.29	665.85± 3.45	1139.19±21.87	721.74± 7.04	1128.15± 6.90	718.98±3.52	1038.45± 7.04
Cop.VI ♂	705.18± 6.69	1083.99±26.77	688.62± 7.52	1146.09±12.69	693.45± 7.66	1101.93±28.91	676.20±3.86	986.01±10.97
Cop.VI ♀	845.59± 2.17	1178.38± 3.55	839.[illegible]± 3.31	1144.71±17.80	848.01± 3.66	1188.87±16.84	850.77±2.54	1092.72± 5.80

Appendix IV

Table of mean cephalosome lengths of the naupliar stages and of mean cephalothorax lengths of the copepodite stages of both the small and large forms from hauls from Winton Bay (August 7, 1964) and Ogac Lake (August 6, 1965) and the T test values and significance for the difference between these means. The values for the mean lengths are mean and two standard errors with the $\sum f$ value in parentheses.

Stage	Winton Small (μ)	Ogac small (μ)	T test value	Difference between means	Winton large (μ)	Ogac large (μ)	T test value	Difference between means
Naup.I	134.16± 0.82(7)	127.44±1.10(16)	4.897	s[1]				-
Naup.II	146.16± 0.79(27)	142.56±0.60(45)	3.629	s	174.00± 6.00(1)	208.80±16.80(2)	1.950	ns
Naup.III	183.84± 0.86(92)	176.64±0.46(168)	7.382	s	226.08± 1.27(5)	243.12± 2.47(4)	6.133	s
Naup.IV	224.64± 1.27(47)	217.92±2.47(54)	2.419	ns[2]	264.00± 0.00(1)	304.80± 0.00(2)		-
Naup.V	261.12± 1.73(18)	241.20±2.74(31)	6.148	s		349.20± 1.20(2)		-
Naup.VI	275.28± 1.34(6)	249.36±2.30(17)	9.737	s	374.40± 8.42(3)	346.80± 2.86(4)	3.103	s
Cop.I	414.69± 3.38(29)	365.70±2.42(162)	11.784	s	563.73± 2.83(88)	534.75± 2.07(41)	8.265	s
Cop.II	485.07± 6.49(13)	451.26±1.79(94)	5.022	s	669.99±15.80(8)	667.23± 2.42(66)	0.172	ns
Cop.III	576.84±12.49(5)	538.20±1.45(136)	3.073	s	812.82±28.08(4)	812.13± 2.14(97)	0.024	ns
Cop.IV ♂	621.00±12.28(4)	609.96±2.69(59)	0.878	ns		903.21± 3.38(39)		-
Cop.IV ♀	658.95±31.05(2)	621.00±2.00(88)	1.219	ns	979.80± 0.00(1)	907.35± 5.24(44)	13.826	s
Cop.V ♂	718.98±18.42(5)	682.41±3.11(60)	1.958	ns	1101.93±17.94(3)	1012.92± 9.32(16)	4.402	s
Cop.V ♀	731.40±41.40(2)	718.98±3.52(76)	0.298	ns	1109.52±10.28(4)	1038.45± 7.04(24)	5.703	s
Cop.VI ♂	675.51± 6.49(20)	676.20±3.86(28)	0.091	ns		986.01±10.97(9)		-
Cop.VI ♀	850.08± 2.69(360)	850.77±2.54(416)	0.186	ns	1158.51± 2.50(20)	1092.72± 5.80(50)	10.409	s

[1] s = significant difference. [2] ns = non-significant difference.



Appendix V

Representative absorption spectrum for DNA-Feulgen complex displaying maximum absorption at 570 mμ expressed as percent of maximum extinction versus wavelength.

Appendix VI

Values for representative absorption spectrum determined by expressing the mean extinction values at the wavelengths as percentage of the mean extinction value at λ 570mμ.

Wavelength	Per cent of maximum extinction		
	E450-650/Eb Av L	E450-650/Eb Av S	Mean
450	14.82	16.76	15.79
460	17.58	19.92	18.75
470	22.41	21.47	21.94
480	27.93	24.49	26.21
490	35.86	32.77	34.31
495	40.00	38.61	39.30
500	46.55	42.88	44.71
505	51.37	49.91	50.64
510	57.93	56.24	57.08
520	68.96	67.49	68.22
530	79.31	75.82	77.56
540	85.86	83.18	84.52
550	92.41	90.69	91.55
560	97.58	96.32	96.95
565	98.96	98.86	98.91
570	100.00	100.00	100.00
575	99.31	98.70	99.00
580	97.58	94.37	95.97
590	87.24	80.85	84.04

Wavelength	Per cent of maximum extinction		
	E450-650/Eb Av L	E450-650/Eb Av S	Mean
600	68.62	59.81	64.21
610	44.13	36.93	40.53
620	25.51	19.63	22.57
630	13.44	9.62	11.53
640	8.27	6.38	7.32
650	5.51	4.21	4.86

Appendix VII

Chi^2 test for the extinction values of the nuclear DNA both for the small eggs and the large eggs to test the hypothesis of normal distribution.

	Upper class X	Limit of interval $x=X-\bar{X}$	Limits in standardized units $Z\ (X-\bar{X})/\sigma$	Relative frequency from $-\infty$ to Z	Relative frequency of interval	Absolute theoretical frequency (f)	Actual frequency (F)	F - f	$(F-f)^2$	$\frac{(F-f)^2}{f}$
Large	.19	-.049	-1.40	.0808	.0808	4.0	4	0	0	0.000
	.22	-.019	-0.54	.2946	.2138	10.7	7	3.7	13.69	1.279
	.25	.011	0.31	.6217	.3271	16.4	16	.4	.16	0.009
	.28	.041	1.17	.8790	.2573	12.9	16	3.1	9.61	0.744
	∞	∞		1.0000	.1210	6.1	7	.9	.81	0.132
						50.1	50			2.164
Small	.10	-.032	-1.45	.0735	.0735	3.7	5	1.3	1.69	0.456
	.12	-.012	-0.54	.2946	.2211	11.1	11	.1	.01	0.000
	.14	.008	0.36	.6406	.3460	17.3	13	4.3	18.49	1.068
	.16	.028	1.27	.8980	.2574	12.9	15	2.1	4.41	0.341
				1.0000	.0102	5.1	6	.9	.81	0.158
						50.1	50			2.023

	$\bar{X}$	σ	$\sum \frac{(F-f)^2}{f}$	$Chi^2_{0.05(n=2)}$	Decision
Large	.239	.035	2.164	5.99	Accept (normal distrib.)
Small	.132	.021	2.023	5.99	Accept (normal distrib.)