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Hopkins, CCE

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THE BREEDING BIOLOGY OF EUCHAETA NORVEGICA (COPEPODA: CALANOIDA)

Thesis submitted for the degree of Doctor of Philosophy in the
University of Stirling

by

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February, 1976

Ph.D. awarded ~~March~~ May 1976
Conferred March 1977

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ACKNOWLEDGEMENTS

I wish to sincerely thank my supervisors, Dr John Mauchline (S.M.B.A., Oban) and Dr Donald McLusky (Department of Biology, University of Stirling) who have given me constant help and encouragement.

I also acknowledge with gratitude the help of Mr T Forrest (Department of Biology, University of Stirling) and Mr R I Banks (Department of Medical Pathology, University of Edinburgh) who supervised my entry into the field of transmission electron microscopy. Acknowledgement is also due to Mr David Machin (Department of Mathematics, University of Stirling) for his assistance in selecting the appropriate statistical techniques and in running the computer programs for me. Dr C M Lee and Mr D H Jones, both former colleagues at the Oceanographic Laboratory, Edinburgh (I.M.E.R.), also provided helpful discussion and encouragement when the author was at that establishment. Mr A W G John (I.M.E.R.) provided help in scanning electron microscope techniques. Mr A Edwards (S.M.B.A.) gave advice on the hydrography of Loch Etive as well as kindly allowing me to use information prior to its publication, and Dr J Gordon (S.M.B.A.) provided information on the feeding habits of fish in Loch Etive.

I thank Professor F G T Holliday, C.B.E., Professor H Meidner (Department of Biology, University of Stirling), and Mr R I Currie (S.M.B.A.) for use of facilities within their establishments.

The financial assistance which enabled this work to be carried out was provided by my parents, Mr & Mrs H E Hopkins, and by my uncle, Major F L Hopkins, to all of whom I am most grateful.

Finally, I would like to thank my wife, Margaret, for her help and encouragement.

CHAPTER 1

GENERAL INTRODUCTIONOBJECTIVES:

Euchaeta norvegica (Boeck, 1872) is one of the larger species of calanoid copepod (adult females = 7-11mm total length, adult males 5.5-7mm total length) and has a widespread distribution in northern waters. Details of its distribution provided from the records of the Continuous Plankton Recorder Survey (Edinburgh Oceanographic Laboratory, 1973) show that it is present throughout the northern temperate waters of the north Atlantic Ocean, with its centre of abundance situated in the boreal waters of the Greenland-Labrador Current Complex.

Hedgpeth (1957), in his classification of marine environments, showed the boundary between oceanic and neritic as approximating to the position of the 100-fathom (ca.182 metre) depth contour. Colebrook (1964, 1972) has since found that further divisions in the classification of geographic areas and water types are possible and has classified E. norvegica as being a typical component of the "north-west oceanic" plankton community. Relatively few specimens of E. norvegica are found in neritic seas although it is often found in abundance in Norwegian fjords and Scottish west coast sea lochs, and the deep waters associated with them. In its typical oceanic environment the adults occupy an essentially mesopelagic depth distribution whilst the younger copepodite stages are found in the more surface waters.

A large body size and its often high abundance makes E. norvegica one of the dominant biomass copepods of the northern temperate and boreal plankton communities. This, coupled with the fact that it appears to be wholly carnivorous (see Harding, 1974), emphasises the importance of

this copepod in marine ecosystems. It is thus surprising that little published information is available about the biology and ecology of this species.

The necessity to mate and breed is a dominant factor in an animal's life. The research presented in this thesis was carried out in order to examine these important processes in E. norvegica with particular reference to those aspects which are likely to determine the breeding intensity of a population. This study has, therefore, concentrated on an examination of sperm and spermatophore production in relation to fertilization and subsequent egg-laying, rather than the development of generation cycles after the eggs have hatched.

The female reproductive system has been described, with an emphasis on its function during mating and egg-laying. The male reproductive system has been described and its histology and ultrastructure have been investigated with regard to secretion of the various components of the spermatophore. The mating process and the inseminatory position adopted by the male has been predicted. Analyses have been made of the frequency distributions of spermatophores found on adult females as well as the patterns of distribution of these spermatophores on the different areas of the female genital segment. The significance of such distributions have been discussed in terms of mating behaviour. The relationship between maternal body size and the size of the egg-clutch produced has been quantified. Seasonal variations in the sex-ratio of adults, as well as stage IV and stage V copepodites, have been followed and possible factors responsible for these changes have been discussed. Finally, the proportion of males present in the adult population has been examined as a factor determining the numbers of spermatophores attached to adult females and the quantity of egg-sacs subsequently produced by the population.

The concept of examining "reproductive potential" adopted for this study requires assessments to be made of the numbers of spermatophores found on adult females, the number of egg sacs these females are carrying, as well as the sex ratio of the participants. However, an accurate delineation of breeding and its intensity requires the study to be made of a natural population in reproductive isolation. In oceanic areas the effects of current systems in causing immigration and emigration of individuals from different populations, which may be out of phase in terms of a reproductive "reaction time lag" (see Krebs, 1972), would significantly reduce the degree of information received from this type of study. In accordance with these considerations, Loch Etive, Argyll, was selected as a most suitable area for sampling as its structure and hydrography act to maintain the integrity of the large population of E. norvegica found there.

In Loch Etive E. norvegica is one of the dominant members of the plankton community and is associated there with populations of the mysid Schistomysis ornata (G.O. Sars), the chaetognath Sagitta elegans Verrill and the copepod Calanus finmarchicus (Gunnerus).

LOCH ETIVE

Loch Etive (Fig. 1) opens into the Firth of Lorne at its junction with Loch Linnhe. It is situated in the western part of the Grampian Highlands, and historically is best regarded as a submerged land valley over-deepened by ice-erosion during the glacial period. The loch is 28km long, varying in width from 0.5km to 1.2km with two main sills, one at the entrance (Connel Bridge) and the other in the region of the Bonawe narrows (Fig. 2). A third, smaller sill is present about 4km

from the entrance at Connel Bridge. The sill at Connel ensures that the entrance channel to the loch is very shallow (8m) with exposed low water rocks under the Bridge. The sill at Bonawe divides the loch into two major basins. The upper basin has a maximum depth of 150m and runs north-east to south-west between mountainous terrain. The lower basin has a maximum depth of 60m and runs east to west in fairly low lying country.

Loch Etive has a large catchment area of 1400km^2 over which the average rainfall is about 2400mm per year peaked so that 75% of it occurs between September and February. The freshwater run-off from the surrounding area determines much of the hydrography of the loch, with the drainage from the river Awe being particularly important as this river drains 60% of the catchment and flows from Loch Awe to enter Loch Etive at Bonawe.

In the loch precipitation and run-off exceed evaporation. In such a situation a surface layer of low salinity and corresponding low density is developed. This layer has a mean seawards motion as shown by Wood et al (1973). At the base of this layer is the primary halocline, below which a return layer of intermediate salinity moves landwards to replace salt entrained into the brackish layer. The base of the return layer is marked by a secondary halocline, below which "stagnating" water of slowly varying salinity and temperature normally shows slowly decreasing dissolved oxygen levels and currents of the order of a few cm/sec.

The salinity of the brackish and return layers is inversely related to run-off; the salinity of the bottom water is usually near $28^{\circ}/\text{oo}$ falling by $1^{\circ}/\text{oo}$ per year and apparently unrelated to run off. The temperature of

the return flow varies between 7°C and 14°C , following local coastal sea conditions. In the brackish layer this cycle is modified towards an earlier lower winter minimum and earlier summer maximum. Cooling is never sufficient to drive a convective overturning to erode the primary halocline, which in winter usually shows about 10% change in salinity. In the deep water of the upper basin the temperature is nearly constant during stagnation, showing no seasonal progression.

The slow variation of deep water salinity and temperature is occasionally interrupted by rapid change. At such time bottom currents increase considerably, oxygen levels rise to saturation, phosphate and nitrate levels fall to coastal levels, the secondary halocline disappears and bottom density rises. The temperature and salinity of the water which has apparently flushed the upper basin are close to those of the return water at the Bonawe sill and suggests a density driven flow of new water from the sill into the deep basin. The cause of this dense water is a period of low run-off; its effects are important in determining bottom conditions for months or years afterwards. If the temperatures of the new and old waters are very different then the rapid changes which accompany mixing may disturb pelagic or planktonic stages of plants and animals found in the bottom water. However, the mean time between renewals is 1.3 years (Edwards & Edelsten, in press) so conditions are normally very stable.

The majority of *E. norvegica* in Loch Etive are found in the upper basin and are thus maintained as an isolated population separated from extraneous populations by the barriers created by the Connel sill and the prominent sill at Bonawe. Any mixing of individuals from within the loch and those outside would be likely to occur outside the entrance channel as a result of movement of the more surface dwelling juvenile

stages outwards on the ebb tide. Movement of immigrants into the upper basin of the loch during the sampling period (27th September 1971-24th August 1972) was most unlikely as renewal of water in the upper basin was absent throughout this period of study (Edwards, pers.comm; Edwards & Edelsten, in press).

SAMPLING PROGRAMME

Plankton samples were collected from the upper basin of Loch Etive at approximately 10 day intervals between 27th September 1971 and 24th August 1972, using a one metre diameter stramin net of 1mm^2 mesh size towed for 30 minutes duration at a ship's speed of about 2 knots. The net was shot and hauled in such a manner that the whole of the water column was sampled with no obvious bias being placed on any particular depth horizon. The plankton was preserved in neutral 4% formaldehyde in sea water using hexamine as the buffering agent.

The 21 plankton samples so collected contained such large quantities of E. norvegica that each sample was first poured into a large beaker and the volume made up to 1 litre with preservative. The contents were then thoroughly stirred with a spatula or other flat blade-shaped paddle in an irregular manner to achieve a random distribution of organisms. The shape of the stirring vessel is not important as long as the worker learns how to stir in such a way as to avoid vortices that serve to concentrate the organisms in one part of the vessel (Edmondson, 1971). A stainless steel ladle of 50ml volume was used to remove each aliquot. The aliquots were then poured, one at a time, into a perspex rafter cell until 500 adult females had been encountered. All other specimens of E. norvegica regardless of copepodite stage, which were found whilst counting the standard 500 adult females, were removed and tubed together with them for further examination.

EXTERNAL ANATOMY

The body is divisible into three primary tagmata, the cephalosome, the metasome, and the urosome. The cephalosome consists of the cephalic somites bearing the antennules, the antennae, the mandibles, the maxillules and the maxillae, and the first true thoracic somite bearing the maxillipeds. The metasome contains the five thoracic somites, each of which bears a pair of swimming limbs. The left and right legs of the 1st-4th pairs are symmetrical. The cephalosome and the metasome together make up the prosome.

The first four pairs of swimming limbs have the following typical structure: each swimming limb consists of two basal segments, the proximal and distal basipodites. The distal basipodite bears two branches or rami, the lateral exopodite and the medial endopodite. The segments of the rami are numbered in sequence from proximal to distal joint. Both rami are typically three-jointed, the endopodite being shorter than the exopodite. Fusion of joints results in one or two-jointed branches; this occurs in the 1st pair of legs and in the endopodite of the second pair.

The fifth pair of limbs of the male do not resemble the preceding pairs. In the male it is transformed into a copulatory organ which holds the female and attaches the spermatophore to her genital aperture. The male fifth limbs are asymmetrical whilst in the female the fifth pair are absent altogether.

The urosome is composed of five segments in the male but in the female it is four-segmented as the first two have fused. The stage of

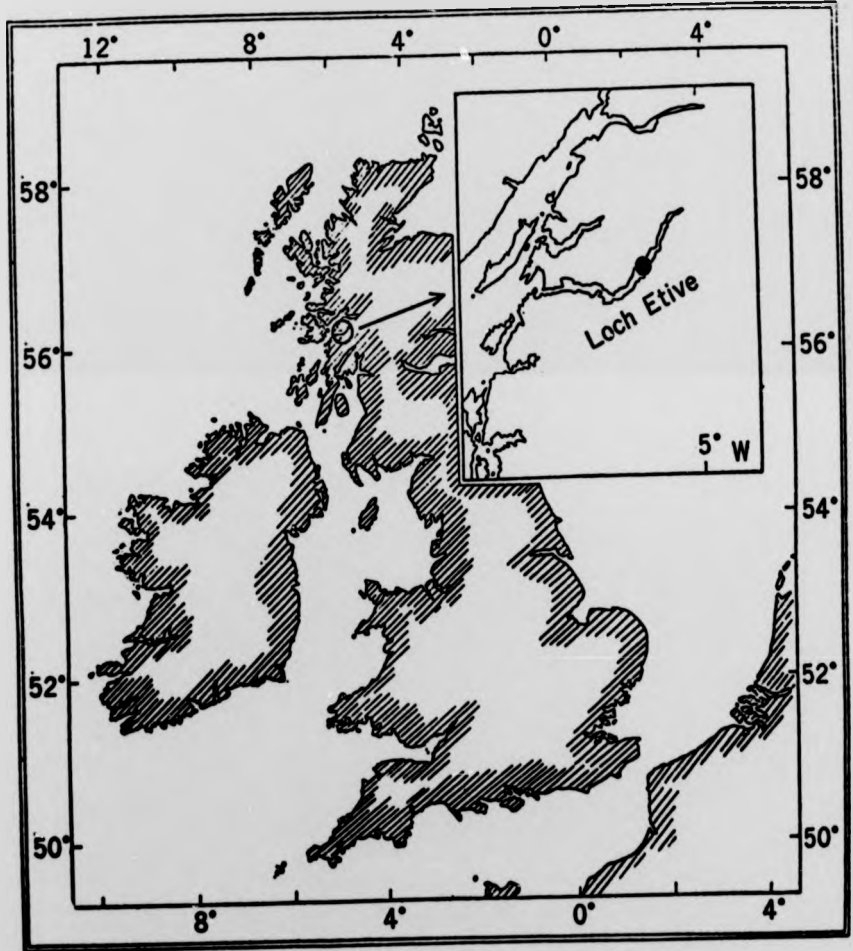
development of the immature stages may be determined from the number of urosome segments together with the number of swimming limbs (see Nicholls, 1934).

The 1st urosome segment is known as the genital segment, and in the female is larger than any of the others. In the female it is ventrally developed in the form of a massive protuberance which bears a number of processes as well as the genital aperture. The genital segment of the male (likewise the most anterior segment of the urosome) is shorter than each of the rest and is slightly asymmetrical since the genital opening lies asymmetrically to the left of the mid-line. The last segment of the urosome is the anal (perianal) segment ending in furcae or caudal rami bearing long setae and feather-like bristles.

Further details of the segmentation of the body and legs are given by Sars (1903), With (1915), and Nicholls (1934). Specimens were originally identified as E. norvegica using the criteria given in Sars (1903), Rose (1933) and Brodskii (1950).

Fig. 1 Sampling locality of Loch Etive in Scotland

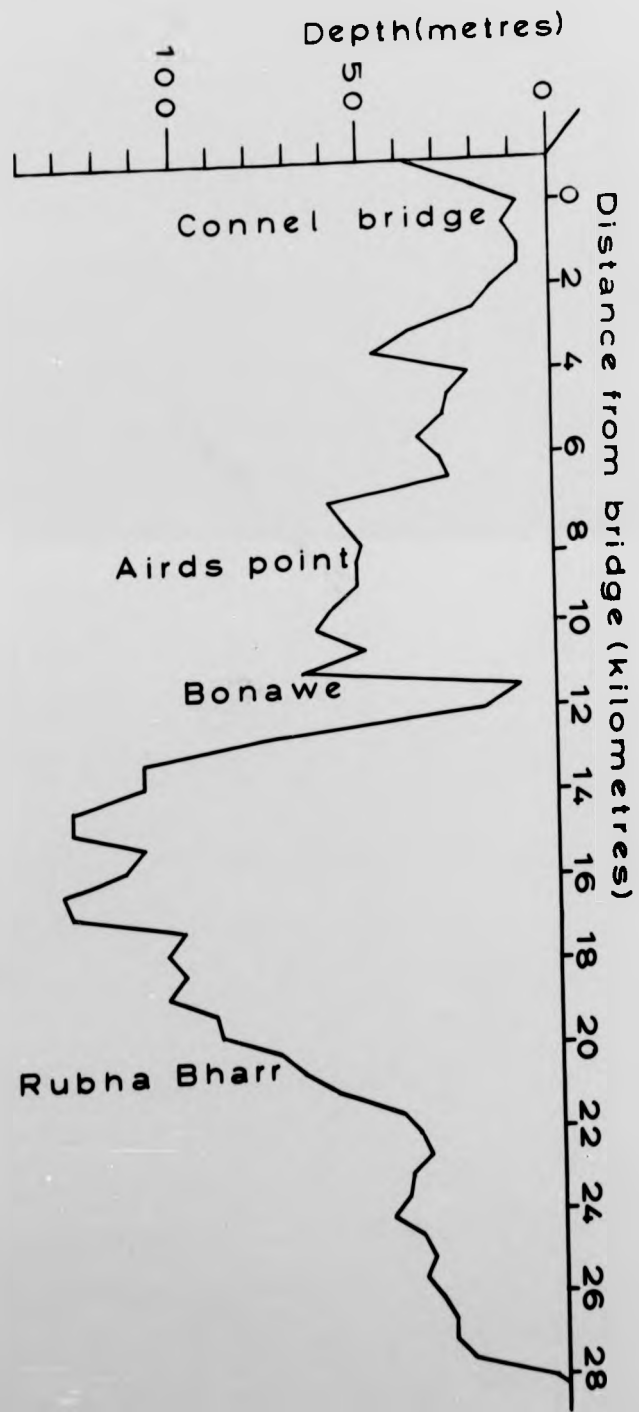
and



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Fig. 2 Bathymetry of Loch Etive (drawn from Admiralty charts).
The lower basin stretches from Connel bridge to the
prominant sill at Bonawe. The upper basin stretches
from Bonawe to the head of the loch.

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CHAPTER 2

THE FEMALE REPRODUCTIVE SYSTEMINTRODUCTION

A number of aspects of the female reproductive system have been described in various species of copepod. Among the more notable studies describing the morphology and histology of the entire genital system are those of Lowe (1935) for Calanus finmarchicus, Fahrenbach (1962) for Diarthrodes cystoecus, and Park (1966) for Epilabidocera amphitrites. Heberer (1930) has studied the genital system of Eucalanus elongatus with the major emphasis on oogenesis, and Raymont et al (1974) have provided an account of an electron microscope investigation of the body of C. finmarchicus which includes brief details of the fine structure of parts of the female reproductive system. In addition, C. finmarchicus has been the subject of further attention with detailed studies having been made by Hilton (1931) on oogenesis, and Grobben (1881) and Harding (1963) having concentrated on the chromosomes of the maturation divisions.

The female reproductive system of Euchaeta norvegica has not been described before and details of the female's reproductive biology have previously been lacking.

The female reproductive system of E. norvegica (Figs. 3 & 4) is basically the same as that described for other calanoid copepods in that it consists of a single ovary, two oviducts passing to the paired openings entering the genital cavity, and a pair of sperm storing receptacles. However, there are a number of substantial differences in the arrangement and functioning of the female reproductive system of E. norvegica when compared with the published descriptions for other copepods, especially C. finmarchicus, which show that the reproductive system of the former species is distinctive and highly specialized.

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A number of aspects of the female reproductive system have been described in various species of copepod. Among the more notable studies describing the morphology and histology of the entire genital system are those of Lowe (1935) for Calanus finmarchicus, Fahrenbach (1962) for Diarthrodes cystoecus, and Park (1966) for Epilabidocera amphitrites. Heberer (1930) has studied the genital system of Eucalanus elongatus with the major emphasis on oogenesis, and Raymond et al (1974) have provided an account of an electron microscope investigation of the body of C. finmarchicus which includes brief details of the fine structure of parts of the female reproductive system. In addition, C. finmarchicus has been the subject of further attention with detailed studies having been made by Hilton (1931) on oogenesis, and Grobden (1881) and Harding (1963) having concentrated on the chromosomes of the maturation divisions.

The female reproductive system of Euchaeta norvegica has not been described before and details of the female's reproductive biology have previously been lacking.

The female reproductive system of E. norvegica (Figs. 3 & 4) is basically the same as that described for other calanoid copepods in that it consists of a single ovary, two oviducts passing to the paired openings entering the genital cavity, and a pair of sperm storing receptacles. However, there are a number of substantial differences in the arrangement and functioning of the female reproductive system of E. norvegica when compared with the published descriptions for other copepods, especially C. finmarchicus, which show that the reproductive system of the former species is distinctive and highly specialized.

MATERIALS AND METHODS

Narcotised, live specimens were used for in situ examination of the female reproductive system. Histological studies were made using the methods described in Chapter 3. Examination of the genital segment of adult females was carried out using a scanning electron microscope (Cambridge Instruments Series IIA).

In the scanning electron microscope a beam of electrons is passed by a condenser lens to a fine point on the specimen and made, by scanning coils, to scan or sweep over the specimen's surface in a "raster" as in a television set. The electrons which hit or slightly penetrate the surface cause secondary electron emission. These secondary electrons, their number and energy governed by the nature of the surface from which they emerge and the energy which is applied to them, are collected by a scintillator/photomultiplier system. This collected signal is used to produce a scanned image, displayed, after amplification, on a long-persistent cathode-ray tube screen (Oatley & Smith, 1955; Oatley, Nixon & Pease, 1965). The scanning electron microscope gives a detailed view of the surface of the specimen, with a pronounced three dimensional effect due to the great depth of focus possible with the electron beam; the resolution lies conveniently mid-way between that of the light and transmission electron microscopes and varies, depending on the accelerating voltage of the beam, from about $0.2\mu\text{m}$ at 3kV to 20nm at 20kV.

All specimens in the scanning electron microscope build up a charge as the electrons strike them. Unless the surface is conducting this distorts the image. Non-conducting surfaces, such as copepod cuticle, must be coated with conducting material to drain away the charge. Different types of specimen need different treatments and further details are provided by Echlin (1968).

The technique used here for preparing specimens of E. norvegica for examination in the microscope was adapted from Lee (1972). The formalin preserved specimens were washed in distilled water, and then dehydrated by passing them through a graded series of tertiary butyl alcohol, from 30% to absolute in 5% steps. The specimens were kept for 10 minutes in each alcohol concentration (c.f. 5 minutes for Lee's specimens) because of the large size of Euchaeta, and then dried in a desiccator containing silica gel. The tertiary butyl alcohol hardens the body musculature which provides a skeletal support for the cuticle when the fluid has been removed. After 48 hours the dried specimens were taken out of the desiccator and mounted in the required positions on the specimen stubs using Durofix glue. The mounted specimens were then coated with a layer of gold-palladium ca. 30nm thick whilst under a vacuum of 10^{-5} to 10^{-6} torr.

RESULTS AND DISCUSSION

THE OVARY:

The ovary is single and is situated close to the median line of the body within the cephalosome, in a position directly above the point of attachment of the maxillipeds. It is about $500\mu\text{m}$ long by $250\mu\text{m}$ wide in its fully mature state and is subcylindrical in shape being slightly compressed in a dorso-ventral plane. The organ is slightly rounded at its anterior end and tapers in width at its posterior end. The ovary is connected to the oviducts at its left and right antero-lateral surfaces.

The ovary varies in size according to the physiological condition of the female but it is always small in comparison to the body of the copepod, no matter what the stage of ripeness. It therefore differs

significantly from the ovary of C. finmarchicus which Marshall & Orr (1952) have described as undergoing large variations in size depending on the state of maturity of the individual. The ovary of E. norvegica appears to be an organ primarily for the production of eggs which migrate into the oviducts for maturation, growth and storage, whereas in C. finmarchicus (Fig. 5) the ovary, when fully ripe, occupies a significant proportion of the body as it becomes the primary area for maturation, growth and storage.

Oogenesis has not been studied in detail in E. norvegica during the present investigation but it is evident that the ovary exhibits three distinct zones. The posterior end of the ovary contains oogonia undergoing multiplication by mitosis; the second zone, anterior to the first, contains oocytes in early stages of maturation divisions; the third zone, which occupies the most anterior position of the ovary, is the growth zone where initial growth and maturation of the oocytes occur. The oocytes continue to develop in the oviducts where they reach the fully grown condition prior to fertilization and egg laying.

THE OVIDUCTS:

The oviducts (Figs. 3 & 4) are paired ducts which arise from the antero-lateral sides of the ovary but the actual point of demarcation between the ovary and oviducts is usually not very clear. In the mature state the position of the oviducts anterior to the ovary proceed forward and slightly inward in a horizontal plane for about 400 μ m before turning and running backward and upward at an angle of about 45 $^{\circ}$ C. The two oviducts eventually meet with their inner walls being closely apposed, at a point directly dorsal to the ovary. These anteriorly and dorsally

directed limbs of the oviduct are known as the anterior or head diverticula.

The section of the oviducts posterior to the ovary pass backwards and inwards until at a distance of about 500 μ m behind the ovary the walls of the two ducts are only separated by about 60 μ m. They then continue to pass backwards and carry on through the metasome segments and give off three diverticula on their outer sides. The first, second, and third diverticula occur intersegmentally at about the junctions of the cephalosome and metasome, first and second metasome segments, and second and third metasome segments respectively. The diverticula project outwards in the direction of the ventro-lateral exoskeleton, occupying the space between the segmental muscles. The oviducts narrow and approach one another as they near the posterior of the metasome. At this point the lumen of the ducts decrease in diameter owing to the decrease in the external diameter of the ducts combined with an increase in the thickness of their walls. Once within the urosome the oviducts begin to widen and having reached nearly to the middle of the 1st urosome (genital) segment the ducts become thick walled and lined with cuticle. These cuticular lined portions of the oviducts are called the receptacula seminis.

Lowe (1935) has stated that in C. finmarchicus the anterior diverticula of the oviducts are each divided into an upper and a lower canal by a thin membranous partition which she believed represented the fused adjacent walls of a U-shaped loop. The oocytes from the ovary pass forward into the upper channels and then back along the ventral channels and into the oviducts proper. Park (1966) could not detect a similar

partition in the calanoid Epilabidocera amphitrites and such an arrangement is also absent in E. norvegica.

In females which are in an immature or spent condition the head diverticula and posteriorly directed oviducts are difficult to see and may appear to be absent. In the semi-ripe condition the living female is transparent enough for the ovary to just become visible and the oviducts can be seen as two dark, thin cords containing immature eggs. In the mature condition the oviducts and anterior diverticula are much expanded, so that the ovary is partly masked from view, and are filled with a row of large, ripe eggs. The three lateral diverticula are prominent. The largest and most fully developed eggs are found at the distal end of the ducts whereas the smallest eggs are present at the narrower, more anterior ends of the ducts. Marshall & Orr (1952) state that the oviducts of C. finmarchicus in medium or fully mature conditions can contain more than one row of eggs, whilst Park (1966) has described female E. amphitrites as having a single row of eggs anteriorly and several rows posteriorly. The condition of both these copepods differ appreciably from that of E. norvegica which has never been observed with more than one row of eggs present in each oviduct.

The walls of the oviducts consist of a very thin, often indistinct, layer of epithelial cells. The nuclei of the cells are clearly seen as positively staining, elongated structures. The lumen of the ducts in ripe females are greatly distended with enlarging eggs and the walls are so stretched that they are often so tenuous as to appear invisible in light microscope preparations stained with haematoxylin and eosin. The extreme dilation of the oviducts around the maturing eggs, as well as

the constriction of the same ducts between individual eggs suggests that the walls possess elastic-like properties. The fully mature oocytes that are found in the oviducts are elongated in shape as a result of the pressure in the duct.

THE GENITAL SEGMENT:

The genital segment is the name given to the 1st urosome segment and follows on immediately posterior to the last segment of the metasome. The structure of the genital segment is complex, and its shape and arrangement are key features used in the taxonomic separation of females of the family Euchaetidae (see Rose, 1933; Brodskii, 1950; Geptner, 1968). The present description is an attempt to analyse the structure of the genital segment and the genital region from the standpoint of functional morphology as well as to relate the female genital segment to the later description of spermatophore placement in this region by the male.

The genital segment (plates 1 & 2) can be divided into an elongated, more or less cylindrical main part and the genital prominence. The genital prominence is the name given to the large, readily visible part of the genital segment which is directed ventrally away, at approximately 90° , from the cylindrical portion of the segment and upon which the genital field is situated. The proximal part of the segment is articulated with the distal part of the last metasome segment. The genital segment, like the urosome in general, can be moved in a vertical plane. Upward movement is effected by three pairs of short muscles, whilst downwards movements are made by two, long powerful muscles which pass through the prosome and are attached to the exoskeleton of the cephalosome.

The central cavity of the cylindrical part of the genital segment is occupied by the gut, the nerve cord, various muscle bands as well as the

genital organs. The genital organs are the receptacula seminis and the paired accessory glands. Externally the cylindrical part of the segment is devoid of any prominent structures except for a well defined ridge which is mainly situated on the left ventral aspect anterior to the genital prominence (Plates 1 & 2).

The receptaculum seminis is essentially a tube, bent at right angles, with thick, semi-rigid walls. The proximal straight horizontal part lies along the articulation of the urosome with the metasome, at which point the tube becomes constricted and merges with the main thin walled portion of the oviduct. The distal end is narrowed and proceeds ventrally to terminate in the region of the genital field.

The accessory glands (Figs. 3 & 4) lie along the lateral wall of the segment at the same level as the proximal halves of the receptacula. The inner aspects lie close to the alimentary canal. Each gland gives off a duct which opens in the genital region. The function of the glands are not known but it seems reasonable to envisage that they are connected with the reproductive system. They undergo cyclical changes in size and activity which appear to be associated with mature oocytes and egg laying; they may be involved in some way with secretion of the egg sac.

THE GENITAL FIELD:

The distal parts of the genital system are contained in the genital prominence, where they have the advantage of being removed from the proximity of the urosome musculature. The genital prominence and associated genital field have developed in E. norvegica as structures more or less separated from the rest of the segment.

The genital field is a complex part of the integument in the form of a system of folds, bulges and thickenings of the cuticle which surrounds the openings of the ducts of the reproductive organs (Plates 2 & 3). The male attaches its spermatophore to the genital field, and later the egg sac is formed and attached to the field.

Geptner (1968) in a general description of the morphological features of the genital segment of members of the family Euchaetidae has divided the genital field into three major parts. These are the valve, the pads and the posterior wall of the field; the latter bounding a deep recess in the integuments, the half-closed genital cavity into which the ducts of the receptacula seminis and accessory glands open. The genital field accessory glands are situated under the integument of the field. Their function, like that of the main accessory glands, is unknown but their connection with the reproductive system is obvious.

The genital valve is a flap of integument from the anterior wall of the prominence, curving over on its apex where it forms a "roof". The valve appears to possess some degree of mobility and probably acts to close the entrance to the genital cavity in the manner of a door. Morphologically the genital valve is seen as a complex, doubly curved plate. The outer visible part occupies a large area of the field. The lamella of the valve eventually merges with the lining of the genital cavity, where the ducts of the receptacula and accessory glands have their openings. The lining of the posterior wall of the genital cavity surfaces to form the posterior wall or posterior border of the genital field, which in turn merges into the posterior wall of the prominence. The posterior wall of the prominence has a large outgrowth on the right side and is therefore asymmetrical. Viewed from above the valve flap is

seen to be heart-shaped in outline with a V-shaped notch in the centre of its posterior border. The upper external part of the valve flap has an intricate relief and structure due to bulges and convexities in the valve flap as well as local thickenings of the integument. By far the most pronounced structures situated in the flap are the paired elongated lateral folds. In addition the left and right outer portions of the valve flap each bear a distinct tooth which curves outwards and ends in close proximity to the inner edge of the respective genital pads. Paired muscles are attached to the under surface of the genital valve while the other ends are attached to the anterior and lateral walls of the genital prominence; when they contract they probably function to enlarge the genital opening during egg laying and possibly during copulation.

The genital pads are symmetrical paired structures, lying along the sides of the genital valve. The body of the pad is formed by a layer of integument from the lateral wall of the genital prominence. The axis of the pad runs parallel to the long axis of the genital segment and urosome.

FERTILIZATION AND EGG MEMBRANES:

The eggs of E. norvegica are of such a size when mature that the egg number can often be discerned with relative ease, and enable egg counts to be made within the oviduct before egg laying has occurred. This feature coupled with counts of eggs in the egg sacs after extrusion has taken place demonstrates that all the eggs seen in the oviducts are usually shed together in one "spawning".

The extruded eggs within the egg sac are surrounded by three membranes (Plate 4):

1. The primary egg membrane or perivitelline membrane
2. A second membrane which is external and concentric to the perivitelline membrane, and
3. The egg sac membrane which surrounds the extruded egg mass and helps to bind the eggs together as well as attach them to the genital orifice.

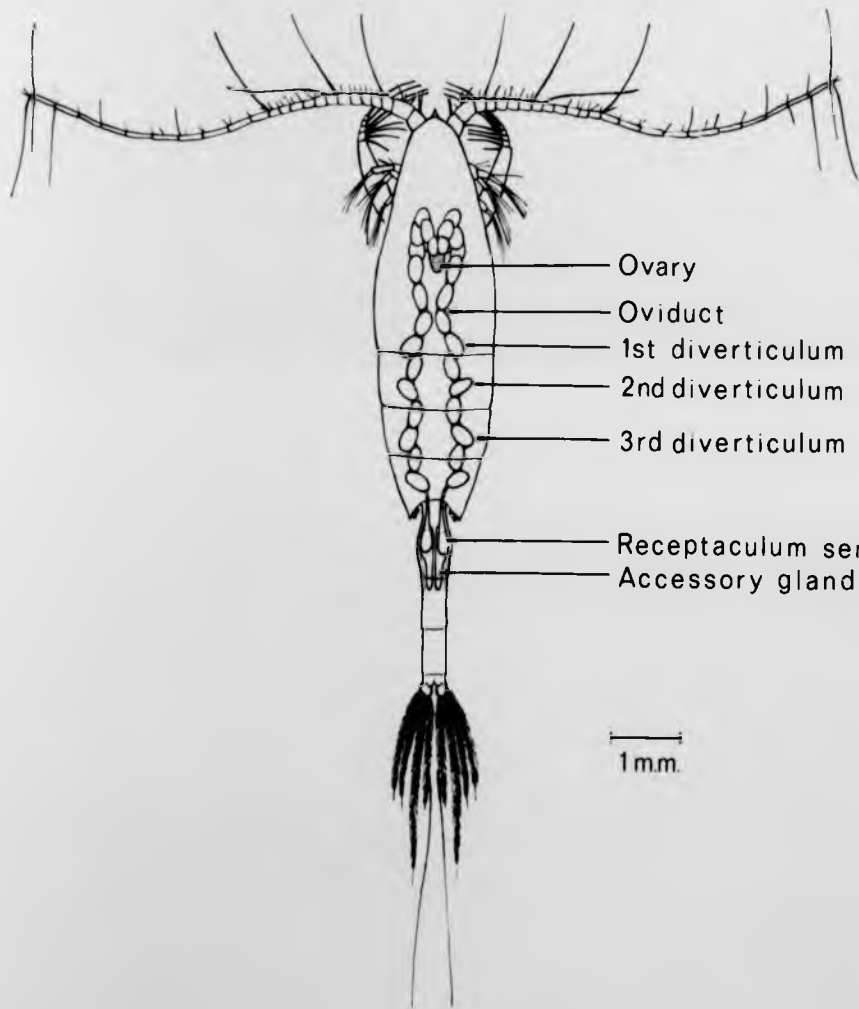
Light microscope preparations show the perivitelline membrane to be present around the egg in the ovary. The second egg membrane appears after the egg has reached the distal end of the oviduct which suggests that it is added by the relatively more developed, secretory walls of this region of the duct. The egg sac membrane is added last around the egg mass and is probably associated with increased secretory activity of the accessory glands.

The presence of two non-living membranes around the developing eggs has been clearly demonstrated in various species of Decapod crustaceans (Yonge, 1935, 1937). An inner chitinous membrane secreted by the oviducal epithelium is closely applied to the protoplasmic membrane which already surrounds the eggs in the ovary. An outer cuticular membrane is later added by a secretion flowing round the eggs when they pass back through the genital openings to the underside of the abdomen during the process of egg laying. This flowing round is possible owing to the low surface tension of the cuticular material (Yonge, 1932). Two secreted, non-living membranes exist in the branchiopod crustacean Chirocephalus diaphanus (Mawson & Yonge, 1938) and in Copepoda (Hacker, 1901; Ziegelmeier, 1926). Raymont et al (1974) have indicated the presence of a primary egg membrane

in the copepod Calanus finmarchicus using transmission electron microscope observations and have made histochemical studies on its composition which indicate an abundance of both basic proteins, mucoproteins and lipids.

Fertilization of the eggs of E. norvegica probably takes place when the eggs pass through the seminal receptacles and genital cavity when egg laying takes place, as the sperm are non-motile and are only found in the genital cavity and receptacula seminis. Fusion of the sperm and egg probably occurs some time between the addition of the second egg membrane and the egg sac membrane. The eggs are not shed free into the water column but are firmly attached to the parent copepod in the form of a single egg mass which is carried around until after the nauplii have hatched out.

Fig. 3. Diagram of an adult female Euchaeta norvegica viewed from the dorsal aspect to show the arrangement of the component parts of the reproductive system in the fully developed condition, just prior to egg laying. The oviducts are shown packed with mature eggs.



Ovary

Oviduct

1st diverticulum

2nd diverticulum

3rd diverticulum

Receptaculum seminis

Accessory gland

1 mm.

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ve

Fig. 4. Diagram of an adult female Euchaeta norvegica viewed from the left side to show the arrangement of the component parts of the reproductive system in the fully developed condition, just prior to egg laying. The oviducts are shown packed with mature eggs.

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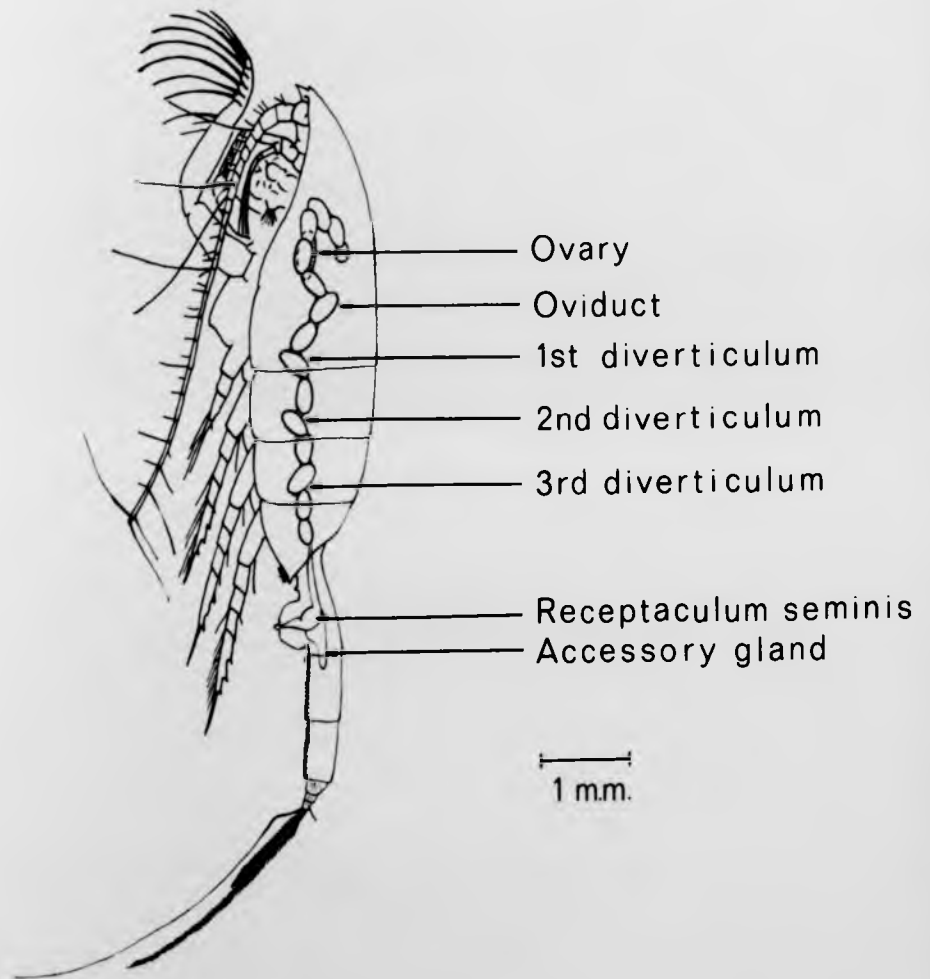


Fig. 5. Diagram of an adult female Calanus viewed from the left side (after Marshall & Orr) showing the arrangement of the component parts of the reproductive system.

a: aorta; an: anus; br: brain; fo: frontal organ;
g: gut; h: heart; me: median eye; mo: mouth; mxg:
maxillary gland; o: ovary; o di: oviducal diverticula;
od: oviduct; oes: oesophagus; os: oil sac; rf: rostral
filament; sp: spermathecal sac; vnc: ventral nerve
cord

the

gan;

mxg:

articula;

: rostral

nerve

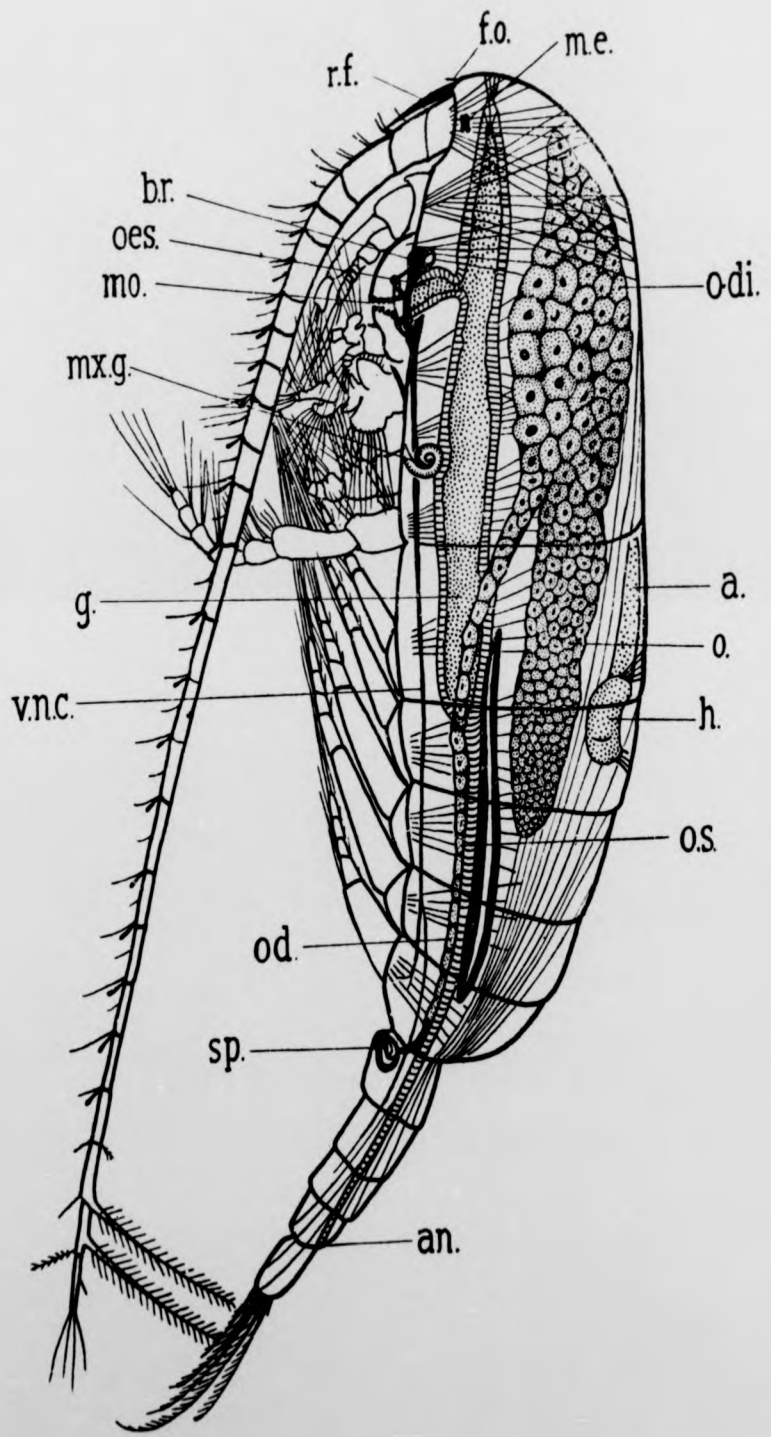


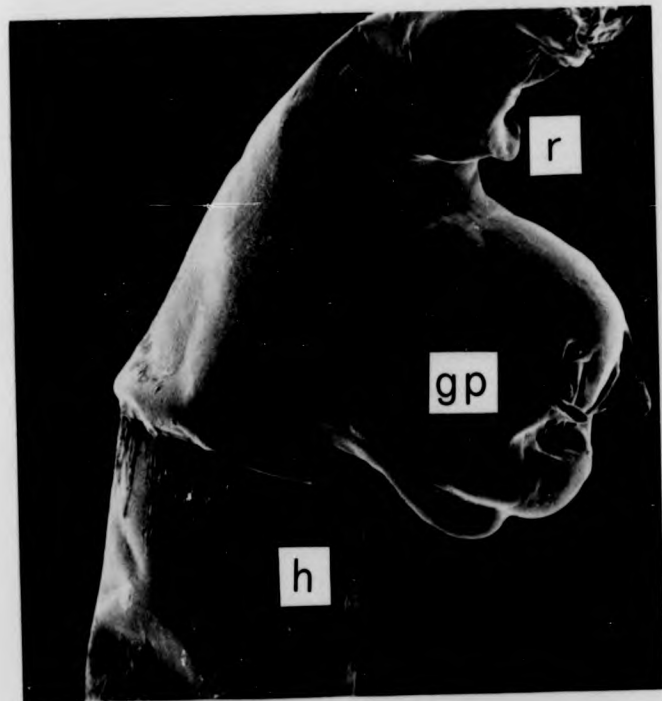
Plate 1. Scanning electron micrograph showing the adult female genital segment of Euchaeta norvegica viewed from the right side.

gp: genital prominence; r: ventral ridge; h: hairs found mainly on the 2nd urosome segment but also, to a lesser extent, on the posterior border of the genital segment.

Plate 1. Scanning electron micrograph showing the adult female genital segment of Euchaeta norvegica viewed from the right side.

gp: genital prominence; r: ventral ridge; h: hairs found mainly on the 2nd urosome segment but also, to a lesser extent, on the posterior border of the genital segment.

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500 μ m

Plate 2. Scanning electron micrograph showing the ventral aspect of the genital segment of an adult female Euchaeta norvegica. The broken line delineates the approximate boundary of the genital field.

r: ventral ridge; h: hairs on 2nd urosome segment.

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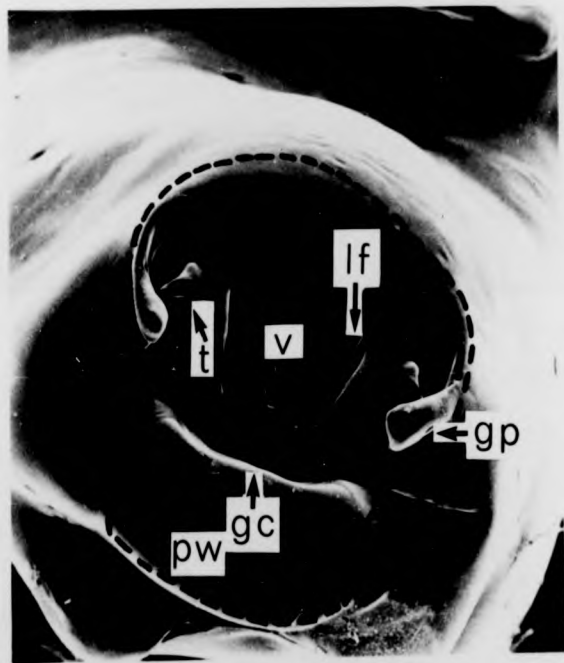


500 μ m

Plate 3. Scanning electron micrograph of the genital field of an adult female Euchaeta norvegica. The broken line delineates the approximate boundary of the genital field.

v: valve flap; lf: lateral fold; gc: genital cavity;
pw: posterior wall of field; gp: genital pad; t: lateral
tooth

al field
he broken
of the
tal cavity;
pad; t: lateral



200 μ m

Plate 4. Section through the egg sac of an adult female
Euchaeta norvegica to show the arrangement of the
egg membranes.

1: perivitelline membrane; 2: secondary egg
membrane; 3: egg sac membrane

female
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egg



500 μ m

CHAPTER 3

THE MALE REPRODUCTIVE SYSTEMINTRODUCTION

The reproductive system of male animals is primarily orientated towards the production and transference of sperm. In the Copepoda the male system is responsible for the packaging of the sperm and associated secretions within a flask-shaped spermatophore. The ripe spermatophore is eventually extruded from the male's body and attached to the female genital orifice in such a manner that discharge of the contents causes internal fertilization of the female's eggs. The spermatophore may thus be thought of as acting in place of a male intromittent organ.

Heberer, in a series of papers (1924, 1926, 1932a, b, 1937, 1955a, b), has made notable studies of the male reproductive system and spermatophore in a number of calanoid, cyclopoid and harpacticoid copepods. These studies have been supplemented by Lowe's (1935) short account of the male reproductive system of Calanus finmarchicus and more recently by the comprehensive studies of Fahrenbach (1962) and Park (1966) on Diarthrodes cystoecus and Epilabidocera amphitrites respectively. Raymont et al (1974) have provided, for the first time in a copepod, brief details of the fine structure of aspects of the male reproductive system and spermatophore of Calanus finmarchicus. The last named investigators have also indicated the remarkable degree of discord which exists over the exact methods of production and entry of the various components of the spermatophore.

The present study is an attempt to provide details of the morphology, histology and ultrastructure of the male reproductive system of Euchaeta norvegica, as well as to elucidate the manner of production and function

of the calanoid spermatophore. Investigations were initially made using light microscope techniques which, although adequate for determining the more gross histology, soon revealed that the vital, dynamic aspects of the system were beyond the limit of resolution of the light microscope. Accordingly the relationship between structure and function was pursued with the superior potential of the transmission electron microscope.

MATERIALS AND METHODS:

Standard techniques were exploited for examination of histology and ultrastructure. However, certain problems arose with the material which required modification and development of these techniques. Further details have been provided in the form of appendices, where it has been considered necessary.

A. Whole Mounts

Copepods required for viewing of the intact reproductive system in situ within the body were treated in the following manner:

1. Formalin-fixed material was placed directly into Anthracene blue staining solution but material stored in 70% alcohol was washed in 50% alcohol before being placed directly into the staining solution.

The staining solution was prepared by diluting an Anthracene blue solution (0.5gm of Anthracene blue W.R. "Becker" in 100ml of 5% aluminium sulphate solution) with 5% aluminium sulphate solution until a pale purple-pink colour is obtained (approx 1 part stain to 20 parts of aluminium sulphate solution).

The copepods were left in the stain for approx 36 hours. Over-staining rarely occurred but when this happened excess stain was removed by immersing in 1% aqueous aluminium sulphate solution for several hours.

2. Stained specimens were washed for 1 hour in several changes of distilled water.
3. Dehydrated through a graded alcohol series (i.e. 30%, 50%, 70%, 90%, absolute alcohol, allowing 10 minutes per grade and giving 2 changes of absolute alcohol).
4. Cleared in cedarwood oil (12 hours) followed by xylol (2 changes of 5 minutes each).
5. Finally mounted in DPX synthetic resin under a supported cover slip.

Permanent whole mounts prepared in this way enable the internal anatomy of the specimens to be easily viewed through the now transparent body.

B. Specimen Preparation for Light Microscope Histology

The specimens were fixed in Bouin's fluid which was made up according to the recipe given by Bradbury (1969).

The reasons for using Bouin's fluid were two-fold:

- i. The fixative contains acid and is a mild decalcifying agent, thus helping to soften the tissue making it easier to cut at a later stage.
- ii. The fixative is yellow and stains the tissue. As the specimens are fairly small this fixative makes the tissue easier to see and thus easier to orientate when embedding.

A fixation time of 48 hours was used. This was probably excessive for the size of specimen but did not appear to be detrimental to the end result.

After fixation the specimens were then processed by the "double embedding" method. This method is so called because it combines the celloidin method of processing with the paraffin wax method. It is carried out by firstly impregnating with celloidin and subsequently embedding in paraffin wax. The advantage of this method is that it helps the tissue layers to maintain their architecture and lessens the possibility of sections falling to pieces.

The double embedding method employed is given in Appendix 1.

Sections were cut at a thickness of $5\mu\text{m}$ using a heavy rotary microtome. The sections were floated on top of 30% spirit on glass microscope slides coated with "tissue tek" (a commercially made adhesive) and then lowered gently into water. The resulting change in surface tension causes the sections to stretch and flatten.

The wax was removed by soaking the slides bearing the sections in xylol for 5 minutes and the xylol was in turn removed by rinsing in absolute alcohol for 1 minute. The sections were then taken down through descending strengths of alcohol before being stained by the haematoxylin and eosin method (see Bradbury, 1969), dehydrated by passing through ascending strengths of alcohol, cleaned in xylol and mounted in DPX synthetic resin.

C. Specimen Preparation for the Transmission Electron Microscope

Correct preparation of material for E/M is important as cells deteriorate rapidly after the death of the organism. The primary rule to be followed is that material should be kept relatively small in size as fixatives do not penetrate very far into the tissue. The copepods were killed by

dropping them into buffered 3.1% gluteraldehyde solution. After death had occurred (usually less than 1-2 minutes) they had the last segments of their urosomes and their heads anterior to the testis cut off to facilitate speedy entry of fixative. Other specimens were killed in a similar manner and then removed to filtered sea water before quickly having their entire reproductive system carefully dissected out and immersed in fixative. Damage of the reproductive system should be kept to a minimum.

Fixation:

The cell structure of the tissue to be examined in the E/M must be stabilised by suitable fixatives. These fixatives stabilise cell structure by creating cross-linkages between some of the constituent molecules, thereby providing a framework so that fine structure does not change through stresses caused by dehydration and embedding. Although it is known that this cross linkage chiefly involves proteins and lipids, the full details of the chemistry of fixation are not well understood. Cell constituents which do not react with fixatives may be washed out; on the whole it is only the macromolecular skeleton of the cell which is preserved.

Double fixation was found to be the most satisfactory method of fixation available. This involved the use of gluteraldehyde ($\text{CHO}(\text{CH}_2)_3\text{CHO}$) and osmium tetroxide (OsO_4), often mutually incompatible, in sequence to obtain better fixation than would be obtained with either alone. The image resulting from double fixation is the most complete yet obtainable. The less toxic gluteraldehyde used first enables the tissue to be handled during fixation. Since gluteraldehyde penetrates faster than osmium, cellular activity stops more quickly which results in better preservation

of fine structure. All traces of gluteraldehyde should be removed from the tissues before post-fixation in osmium, as the two chemicals react to produce a fine electron-dense precipitate which pervades the preparation. The material being fixed was thus washed in several changes of buffer to remove all gluteraldehyde before being placed in osmium. Only cacodylate buffer is suitable for both fixatives. It was customary to use chilled solutions to avoid as far as possible unwanted chemical extraction of components of the cells. After fixation the specimens were washed thoroughly in buffer as any free osmium remaining in the tissue will be reduced during dehydration in ethanol and leave an electron-opaque precipitate throughout the tissue.

The recipes and processing schedule used during fixation and dehydration are given in Appendix 2.

Embedding:

After fixation and dehydration the specimens were placed in a clearing agent such as propylene oxide, miscible with with alcohol and the embedding medium, which was used to provide a transition to the final stage of processing. The pure propylene oxide was gradually replaced by the embedding epoxy resin Epon over a period of 12 days. This abnormally long period of transition was necessary to cause full infiltration of the specimen by the medium, as shorter periods were found to introduce extreme compression during sectioning or even failure to satisfactorily cut the spermatophore within its sac. Full replacement of propylene oxide by pure Epon was particularly difficult to achieve when shorter infiltration times were employed on whole male copepods.

Epon embedding modified from Luft (1961) was found to be the most suitable embedding medium for use with E. norvegica as it has the advantage of being able to produce embedded specimens free from polymerization damage, the plastic does not sublime in the microscope and so helps provide continual support and preservation of fine detail. In addition Luft's medium allows very hard blocks to be prepared by varying the quantity of the constituents employed, thus helping support the copepod body components which range in toughness from the strong chitinous exoskeleton to the soft internal organs.

After the required infiltration period had taken place the specimens were embedded in No. 3 beam capsules prior to polymerization. The specimens should be placed in the top of capsules filled with fresh Epon; any further infiltration necessary will occur as the specimens sink to the capsule bottom in the oven. Very small specimens, such as the dissected reproductive system or parts of it, were prevented from dropping to the capsule bottom by polymerizing a little resin in the capsule first. If misorientation of the specimens occurred during polymerization they were cut out with a jewellers saw and re-embedded.

The recipe used to make the Epon embedding medium and the processing schedule used during embedding are given in Appendix 3.

Section Cutting:

Ultrathin sectioning of crustacean tissue presents problems not generally encountered with other animal material. The most important of these is the vast range of variation in toughness found in a single block of a whole male Euchaeta. The microtome knife must cut the exoskeleton which

is relatively hard. At the same time it must cut the internal organs which are much softer. This conflict sets up considerable strain at the cutting face and can easily result in imperfect sectioning of one or more of the components, or in distortion of the section. A further problem is the presence in the body cells of hard inclusions and secretion granules, as well as the resistant wall of the spermatophore. These features are difficult to section and may be roughly fractured, knocked out completely or pushed through the tissue by the knife edge, tearing the section.

These problems had to be overcome, in part, at the embedding stage. The resin had to be uniformly hard throughout, and the hardness had to be correct to support the tissues concerned. Without this, attempts at sectioning were futile. In addition, trimming of the block to expose the specimen in the required orientation and with a correctly shaped face became vitally important.

Thick sections were cut at $2\mu\text{m}$ thickness for light microscopy and stained with methylene or toluidine blue or were examined unstained by phase contrast microscopy in order to localise areas of interest for subsequent electron microscopy. The area of the block face was then trimmed down to include only those structures of interest in the electron microscope investigation.

Sections for E/M were cut on LKBIII and Reichert OmU3 ultramicrotomes at silver (60-90nm thickness) or grey (<60nm thickness) interference colours. Glass microtome knives of 45° angle were generally employed but the hardness of the Epon resin quickly damaged the cutting edges. Accordingly

a diamond knife was used when serial sections were required. The sections were floated and collected on the surface of a water bath and mounted on copper grids ("MICRON" grids; type 200 & 400) without a supporting film. The absence of a supporting film allowed opportunities for higher resolution and better contrast.

Staining:

Staining for E/M means increasing specimen contrast by depositing in certain areas atoms of high atomic number which have greater electron scattering power than the atoms (C,O,N,H) of the tissue. In addition the fixative osmium tetroxide adds some contrast by selective deposition of its heavy atoms by increasing the contrast of phospholipid membranes, proteins and unsaturated lipids.

A variety of heavy metal stains (lead hydroxide, lead acetate, lead citrate, uranyl acetate and phosphotungstic acid) were assessed for staining performance but the most successful results were produced by staining the grids initially in a saturated alcoholic solution of uranyl acetate, followed by an aqueous solution of lead citrate.

The grids were submerged with the aid of forceps in drops of stain in covered watch-glasses. When using lead preparations a few beads of potassium hydroxide or similar carbon dioxide removing agent were placed in the dish along with the drops of stain to remove carbon dioxide contamination. Care was taken not to breathe over the drops. The grids were not allowed to dry between staining and washing as drying causes deposition of a contaminated layer. After a final rinse the grids were placed on a filter paper, sections upwards, to dry.

The recipes and staining schedule used during staining are given in Appendix 4.

D. The Transmission Electron Microscope

The great advantage of the transmission electron microscope lies in its far greater resolving power as compared with the light microscope. The resolving power of the latter, that is its ability to differentiate between two small objects close together so that they appear separate and not as one, is a function both of the numerical aperture of the objective and the wavelength of light; in practice, the minimum resolvable distance between two objects is about 200nm. The wavelength of the electron beam in the E/M, however, is very much less, so that its resolving power, and hence its ability to reveal detail, is greatly increased. A consequence of this is that the photographic negatives obtained can be greatly enlarged to reveal an enormous amount of detail.

The transmission electron microscope used throughout the present study was an AEI Corinth 275. This microscope has a resolving power of 0.9nm, and a theoretical resolution of 0.35nm. The magnification provides a working range of 600x-100000x. An accelerating voltage of 60kV was used throughout.

Further details concerning the functioning of the transmission electron microscope and the preparation of tissues for use with it are provided by Pease (1964), Kay (1965), Wischnitzer (1967), Sjostrand (1967) and Meek (1970).

RESULTS

The male reproductive system of E. norvegica (Fig. 6) consists of a single testis and a single genital duct which passes posteriorly in the left of the perivisceral cavity and opens to the exterior in the left side of the first urosome segment. There is no sign of the right genital

duct in E. norvegica but Lowe (1935) describes it as being present in a rudimentary condition as a cord of cells lying in the right side of the pericardial floor in Calanus finmarchicus.

Four distinct regions can be differentiated in the structure of the wall of the male genital duct in E. norvegica. Following Heberer's (1924) terminology for the duct of Heterocope these regions have been termed the vas deferens, the seminal vesicle, the spermatophore sac and the ductus ejaculatorius (Fig. 7).

A. Testis:

The single pear-shaped testis lies entirely within the anterior region of the cephalosome. In its fully developed condition it extends from the level of the maxillipeds forward to the maxillae, and measures about 600 μ m in length by 390 μ m in width at its widest point, which is close to the rounded anterior end. The posterior, narrow end of the testis is bifurcated, with each bifurcation terminating in a small rounded bulb.

The main body of the testis is displaced slightly to the left of centre in the cephalosome and is suspended in the main perivisceral cavity by connective tissue mesenteries; the dorsal surface of the testis is attached by mesenteries to the ventral wall of the main aorta, whilst the ventral aspect of the organ is often closely applied to the dorsal surface of the gut.

Spermatogenic stages are arranged in sequential order, starting with spermatogonia at the posterior end and ending at the anterior with mature spermatozoa. The posterior region or multiplication zone is occupied by spermatogonia, ca. 25-30 μ m in size. The next, more anterior layer is

the zone of spermatogenesis consisting of the spermatocytes which are ca. 18-22 μ m in size and are thus smaller than the spermatogonia. The anterior of the testis is occupied by spermatids and maturing spermatozoa (Plate 6A).

Each sperm is ca. 16-20 μ m long by 8-10 μ m in diameter and are thus slightly spindle-shaped. The wall of the sperm (Plates 6B, 6C) is ca. 270nm thick and is covered on the outside by a double layered cell membrane of ca. 50nm thickness. Just interior to the membrane is a layer of granules of ca. 30nm diameter which are spaced 30-100nm apart. These granules closely resemble glycogen particles in structure. The internal cytoplasm of the sperm consists of a uniformly granular ground substance which may, in part, be ribosomal. Situated at the periphery of the internal cytoplasm is a zone of vesicles. Each vesicle is ca. 1.8 μ m in diameter and can be seen to be made up of a system of extremely thin concentric membranes which produces an overall whorl-like appearance. The sperm, as is usual in crustaceans, do not have a "tail".

The anterior of the testis contains a central cavity (Plate 5A) ca. 40 μ m in diameter into which the mature sperm are released. The central cavity courses forwards to the left anterior end of the testis where it grades into the vas deferens.

B. Vas Deferens:

The vas deferens is a thick-walled, glandular tube arising from the left anterior end of the testis, which proceeds ventrally and posteriorly at an angle of about 15 $^{\circ}$ to the main longitudinal axis of the prosome for a distance of 390 μ m before generally travelling dorsally backwards for 400 μ m.

This last mentioned region is, however, characterised by the lumen of the duct forming a number of loops, the limbs of which have fused to such an extent that the walls are no longer distinct from each other, and thus when viewed externally make this region thicker than any of the rest of the vas deferens. The duct then passes straight backwards as a single, unlooped tube for about 300 μ m before passing in a ventral direction at an angle of nearly 45^o for 290 μ m. The vas deferens then continues to travel backwards at an angle of about 15^o for nearly 600 μ m. The thickness of this last mentioned part of the vas deferens is about 100 μ m. The duct then turns inwards at right angles to the longitudinal axis of the body for 300 μ m before passing upwards and forwards for 160 μ m. At this point the external diameter of the duct becomes constricted to only 50 μ m before entering the seminal vesicle.

Anterior vas deferens

The anterior portion of the vas deferens is vascular and consists of elongate columnar cells. The lumen of the proximal region is relatively narrow and sperm passing into it from the testis are often packed in tightly so that some of the sperm assume semi-hexagonal profiles where their walls have become distorted in shape owing to compaction (Plates 7A, 7B). The walls of the sperm are thus fairly soft, non-rigid structures.

Interspersed between the tightly massed sperm are numbers of small electron-dense granules, ca. 800nm in size, which vary in shape from sub-spherical to elongate (Plates 7A-F). These granules have been termed alpha granules and consist of a homogeneous, fairly granular material surrounded by a delicate limiting membrane. Both the sperm and alpha particles are encompassed in a matrix secreted by the proximal region of the vas deferens. The matrix can sometimes be seen to include a feint, filamentous component (Plate 7C).

The alpha granules are secreted by the whole length of the anterior region of the vas deferens, and they first appear in the lumen of the duct soon after the junction point with the testis. Large quantities of these granules are liberated into the duct and the numbers found increase as the vas deferens proceeds backwards. The cells of the anterior vas deferens are devoted towards producing alpha granules within numerous secretory vacuoles which are scattered throughout the cytoplasm but are generally crowded towards the lumen (Plates 7B, 7D).

The formation and secretion of alpha granules has been extensively studied during this investigation, and it is evident that their manufacture is closely connected with the presence of numerous prominent Golgi apparatus. The Golgi apparatus in the vas deferens of E. norvegica viewed under the electron microscope has the typical structure described by Favard (1969) for a wide range of organisms, and consists of membrane lamellae forming stacks of flattened saccules, dilated membrane bound vesicles and smaller transition vesicles (Plate 8A). Each stack of *saccules* is termed a dictyosome.

Also associated with the well defined Golgi apparatus is an elaborate system of granular endoplasmic reticulum (GER) which fills most of the cytoplasm (Plate 7D). The cisternae of the GER are long, extensively interconnected and at times so closely packed that the cytoplasmic space containing the ribosomes between adjacent cisternae is narrower than the width of the individual cisternae. The contents of the cisternae are usually of low density in comparison with the adjacent cytoplasmic ground substance with its numerous ribosomes, however, at times the cisternae may be dilated and the material contained in the cavities may be flocculent and suggestive of a proteinaceous precipitate.

Morphological observations at the ultrastructural level have established the presence of numerous minute vesicles, each about 40nm in size, in the space between the most external Golgi saccules and the GER. Intensive investigation of the GER in this zone has revealed that the cisternae nearest the dictyosomes are agranular and appear to bud off small vesicles. This suggests that the transfer of proteins occurs within the vesicles which arise from the GER and then open into the most internal Golgi saccule. These vesicles which form what have been called the transition elements (Ziegel & Dalton, 1962) can be seen in many types of cell (Palade, 1961; Friend, 1965; Kessel, 1966) and transfer by similar vesicles has been demonstrated by Jamieson & Palade (1965; 1967a).

It is now generally accepted that proteins are carried from the GER toward the Golgi apparatus inside transition vesicles whose membrane isolates them from the hyaloplasm. The saccules of the dictyosomes seem to form the final secretion granule by the accumulation of material of appreciable density. These secretion granules are released in the form of vesicles budded off from the saccules which may fuse together to form larger secretion granules (Plates 8A-D). Sometimes the secretory vesicles can be seen to aggregate into small clumps each of 3-12 granules. The mature alpha granules are transported to the cell surface where they are released by fusion of the limiting membrane of the vesicle with the apical cell membrane by the process of merocrine secretion.

The alpha granules, when present in the lumen of the duct in large numbers, give the impression that they are repellent to the sperm in some way, as the latter are often surrounded by a "halo" from which the

granules are absent (Plate 9B). The granules appear very electron dense in the proximal region of the vas deferens but gradually become less dense as they pass posteriorly along the duct. The reason for this change is not known but may possibly be due to a change in the pH or nature of other secretion products within the vas deferens.

Posterior vas deferens

The cells of this region of the vas deferens are intensely secretory and the wall of the duct becomes thicker than that of the anterior region. Morphologically and functionally the posterior vas deferens is considered to begin at the position where the secretory activity of the alpha granule producing cells decrease considerably to the point of complete absence and is replaced by a new type of cell which produces secretion responsible for the formation of the wall of the presumptive spermatophore.

The GER of the posterior vas deferens is very conspicuous and the cytoplasm contains larger quantities of the rosette types of ribosome than the anterior vas deferens. A number of ribosomes concerned in the manufacture of a single large protein molecule may be arranged together in a functional group known as a polyribosome or polysome, probably linked by the thread of messenger RNA (Toner & Carr, 1968) and it is likely that the rosettes and spirals of ribosomes commonly seen in electron micrographs of this portion of the duct may represent these functional units of protein synthesis.

The highly basophilic cytoplasm of this region, contains large numbers of vesicles, each between 600nm and 1.8 μ m in diameter when fully developed which enclose globules of fibrillar material (Plate 9C) and occasionally small very electron dense granules (Plates 9D, 9E) which have been termed beta granules. Each beta granule is nearly spherical with a diameter,

after liberation into the lumen of the duct, of ca. 600nm. The vesicles containing globules of spermatophore wall secretion are membrane limited, and besides containing beta granules, often enclose a uniformly dense amorphous component (Plate 9C) which suggests that formation of the fibrillar globules may involve a condensing process within the vesicle. Mitochondria (Plate 9E) are more evident in this region than in the anterior vas deferens.

The globules of fibrillar secretion are released into the lumen and merge together, taking up a position closely applied to the lining of the duct where they form the presumptive spermatophore wall (Plates 9A, 9B). The beta granules are liberated along with the globules of fibrillar secretion and become arranged interior to the forming wall (Plate 9F).

The lumen of the distal region of the posterior vas deferens can now be seen to contain a central core of alpha granules set in a moderately dense matrix around which are arranged a single, sparsely distributed layer of sperm. Outside the sperm zone, the beta granules are found closely applied to the inner surface of the presumptive spermatophore wall, which, in turn, encapsulates the whole assemblage. Both the sperm and the beta granules are set in a matrix which is less dense than that of the alpha granules. The recently applied spermatophore wall has a semi-fluid, viscous consistency and frequently contains vesicles (Plate 9F) which decrease in number towards the distal end of the vas deferens. These vesicles, visible within the newly produced spermatophore wall often contain beta granules which appear to be in the process of migrating through to the other side. The presumptive spermatophore wall lines the whole of the posterior vas deferens and reaches its maximum thickness of ca. 3 μ m at the distal end, just prior to entering the seminal vesicle.

The end of the vas deferens is marked by a well defined thinning of the walls of the reproductive tract and a decrease in the diameter of the lumen to about 25 μ m.

C. Seminal Vesicle

The beginning of the section of the reproductive tract known as the seminal vesicle is indicated by a noticeable widening of the external diameter of the tube after the point of constriction marking the termination of the vas deferens.

The seminal vesicle commences by proceeding upwards and forwards at an angle of about 45^o to the longitudinal axis of the male's body for 130 μ m before bending backwards and upwards for about 350 μ m. The seminal vesicle has now reached an external diameter of nearly 100 μ m and bends round forwards and downwards for about 500 μ m so that a loop is formed in which the ascending and descending limbs of the duct are separated from each other by an average of 20 μ m. The external diameter of the duct has by this point increased to 180 μ m. The descending limb now curves round posteriorly and passes backwards for about 400 μ m at which point it reaches its maximum diameter of 160 μ m. The reproductive tract now rapidly narrows to about 20 μ m in external diameter before entering the right side of the spermatophore sac at a distance of about 50 μ m from the latter's anterior end.

Freshly dissected whole mounts of the seminal vesicle can be seen to have an extremely thin hyaline wall through which the contents are easily visible. In all dissections (over 120 specimens) the seminal vesicle was always seen to contain a presumptive spermatophore in the process of manufacture.

The wall of the seminal vesicle varies in thickness from place to place depending upon the size of the developing spermatophore assemblage but it is usually only 2-3 μ m in diameter (Plate 10A). The cells of the duct form a single layer in which are interspersed a small number of highly elongated nuclei, however, in the places where the two adjacent loops of the duct meet the junctional nuclei tend to assume a more ovoid shape (Plates 10B, 10C, 10D).

The cytoplasm of the cells contain an abundant, convoluted network of GER as well as many vesicles containing globules of fibrillar spermatophore wall material. These vesicles range in size from 100nm to 2 μ m and are similar in structure to those produced in the posterior vas deferens except that beta granules are absent from the cells of the seminal vesicle. E/M preparations frequently show globules of fibrillar secretion merging with and adding to the already present spermatophore wall (Plate 10C). The seminal vesicle thus acts in a supplementary capacity to the posterior vas deferens in secreting the spermatophore wall material. The fibrillar structure of the spermatophore wall is particularly visible in higher power electron micrographs taken within this region of the reproductive tract (Plate 10E).

Preparations of the distal, widened portion of the seminal vesicle often reveal the presence of foamy, vesicular-like areas within the outer matrix of the presumptive spermatophore (Plate 10F). These foamy areas are sometimes visible in the posterior vas deferens but generally become more apparant as the various secretions pass posteriorly along the duct.

The spermatophore assemblage in the form of the cylindrical contents of the seminal vesicle is continuous anteriorly throughout the vas deferens.

The various components of the formative spermatophore, which have been secreted into the vas deferens in different regions along its length pass down, assuming an organized radial symmetry, into the cavity of the seminal vesicle. The contents are collected in the lumen whilst maintaining the same order of arrangement as that in which they were originally secreted. The components of the spermatophore accumulate and become compacted to varying degrees prior to the final shaping in the spermatophore sac. The seminal vesicle thus mainly acts as a compartment for the collection and storage of the constituents of a definitive spermatophore, and it is here that they take up their final positions.

The short section of the reproductive tract following the seminal vesicle proper, and which continues into the anterior of the spermatophore sac, is believed to be equivalent to the "Former" (= shaper, moulder) described by Heberer (1932b, 1937) in other copepods. The highly constricted lumen of the Former appears to act to cut off the cylinder of secretion contained within the seminal vesicle by fusing the distal end of the spermatophore wall in the manner of a round-bottomed flask. The rounded end of the spermatophore flask often displays an irregular, wavy pattern in the wall which may indicate that the viscous material has been closed by fusing after the neck of the preceding spermatophore was pinched off by the Former. Park (1966) has recorded a similar occurrence in the calanoid copepod Epilabidocera amphitrites and has also noted that this closure of the spermatophore flask results in the normally ordered arrangement of the contents of the presumptive spermatophore often being disrupted at the distal end of the seminal vesicle. An analogous situation has been observed in the harpacticoid copepod Diarthrodes cystoecus by Fahrenbach (1962), who has further supported

the above hypothesis by noting that the distal end of the spermatophore flask has a terminal, bulbous portion in the wall; probably as a result of fusion.

The natural passage of the spermatophore assemblage from the seminal vesicle into the spermatophore sac has not been observed in E. norvegica, however, the artificial passage of the assemblage has been followed after careful application of pressure to the anterior metasome of the live male copepod. This increase in internal pressure resulted in the extrusion of the ripe spermatophore already contained within the spermatophore sac and its replacement by the passage of the crude spermatophore into the vacant cavity of the sac. The former was seen to clamp down onto the narrowing spermatophore cylinder after the wider flask had passed through it. This had the effect of stretching and compressing the narrow strand of material until the proximal end of the newly formed spermatophore had become moulded into a short, narrow neck. Further observations were made impossible owing to the copepod becoming opaque prior to its death, however, it appears very likely that the narrow cylindrical strand of secretion is severed, probably by the action of the wall between the seminal vesicle and the spermatophore sac. Under natural conditions it is probable that the crude spermatophore assemblage, partly preformed within the seminal vesicle, passes down into the spermatophore sac in a single, smooth continuous motion, similar to that observed in other calanoid copepods by Heberer (1932b).

D. Spermatophore Sac

The spermatophore sac is an extensive spindle-shaped tube which occupies a position that is slightly displaced to the left side of the body, about

three-fifths of the distance between the ventral and dorsal surfaces of the metasome. The proximal end of the sac usually extends forwards into the middle of the first free metasome segment. The size of the sac can be very variable and in its most developed condition the proximal end of the organ will extend into the cephalosome as far as the position of the first pair of thoracic limbs. Distally the spermatophore sac extends as far back as half-way into the last metasome segment.

The spermatophore sac is usually about 1100-1300 μ m long, and reaches its greatest width of 200-250 μ m two-thirds of its way back. The walls of the duct are thick and glandular with the cytoplasm of the cells being packed with numerous globules of secretion (Plate 5C). The cytoplasm is highly vacuolated and the cisternae of the GER are characteristically wide and distended (Plate 11B). The ellipsoid shaped nuclei are abundant and vary from 7-11 μ m in length and are about 5 μ m wide. The secretory cells are often multinucleate and tend to have their nuclei distributed towards the external surfaces of the sac.

The wall of the sac displays irregular, wavy outer contours and is of varying thickness. The greatest thickness of the wall is found in the proximal third of the tract and gradually becomes thinner towards the distal end where it finally passes into the ductus ejaculatorius. The anterior 210-230 μ m of the sac is narrower than the rest and is bulb-shaped. The thin constricted duct leading from the seminal vesicle enters the anterior end of the spermatophore in this region (Plates 13A, 13B). This part of the sac is equivalent to Heberer's Former and has thick walls which form the main secretory sites in the sac. The cytoplasm of the cells in this area is crowded with secretory vesicles, which congregate toward the lumen.

The external cell membrane of the sac's surface is bounded by a thin diffuse basal lamina, beneath which are circular and longitudinal muscle fibres (Plate 11A). These muscles consist of spindle-shaped cells in which most of the cytoplasm is occupied by myofilaments with a predominantly longitudinal orientation without evidence of a pattern of repeating units. On these criteria the fibres can be diagnosed as being smooth muscle.

The circular muscles are situated at the periphery of the spermatophore sac close to the external cell membrane, whilst the longitudinal muscles are arranged in distinct bundles underneath the enclosing circular muscle layer. Smooth muscle is characterised by a slow and sustained mode of contraction which is independent of voluntary control. Such fibres have a great capacity for deformation, much greater than that for striated muscle. Smooth muscle generally may contract by as much as 400-500% whereas a corresponding figure for striated muscle would be 20% or less (Barrington, 1967). Therefore, the arrangement found in the spermatophore sac of E. norvegica is ideally suited for lengthy maintenance of the tonus required to cause extrusion of the mature spermatophore from within the sac.

The secretory activities of the spermatophore sac vary considerably from region to region and the vesicles found in the wall of the sac vary in size and staining properties. These vesicles have been classified into three main types on the basis of their appearance under the E/M.

The first type of secretion consists of electron dense, homogeneous globules which range in size from 1-4 μ m (Plates 11C, 11D). These

globules are secreted throughout most of the length of the spermatophore sac but become reduced in number towards the neck of the spermatophore. The globules which lie closer to the external periphery of the cell are often paler and more mottled in texture than those which have passed to their storage area in the cell apex. Pale inclusions of this type near the cell periphery appear to be condensing vesicles which apparently mature to form the final globule during the course of their passage towards the lumen of the duct.

The second type of secretion consists mainly of electron dense globules, similar to those first described, but also contain small very electron dense granules (Plate 11E). These granules are ca. 300-700nm in size and are usually situated centrally within the globule of secretion. This type of secretion is mainly liberated towards the proximal half of the spermatophore sac, although smaller quantities of it can also be encountered within the distal portion of the lumen.

The first and second types of secretion can be found distributed along most of the inner surface of the spermatophore sac and are responsible for the addition of a new layer to the outside of the spermatophore flask (Plates 11D, 11E; Plates 12A-D). The layer covering the distal region of the spermatophore flask (Plate 12A) incorporates only small numbers of the dense granules which, however, become more prevalent towards the proximal end of the spermatophore flask (Plate 12D). This recently added layer formed by the first and second secretions is 600-1000nm thick over most of the area of the flask but increases to a thickness of over 3 μ m at the proximal end where the flask starts to narrow (Plate 12D). In the region of the spermatophore neck the added secretion becomes noticeably thicker and the electron dense granules

in it increase in size to a maximum of ca. 500nm (Plate 12E). However, it is unlikely that these secretions modify the properties of any components of the spermatophore in the sense of a "differentiation secretion" such as described by Heberer (1932b). Instead it is more likely that the layer constitutes a spermatophore lubricant ("Gleitsekret" of Heberer) which aids ejaculation of the spermatophore.

The third type of secretion found within the cells of the spermatophore sac consists of birefringent globules, each one of which is made up of a centrally located pale spherule surrounded by a slightly more dense corona (Plate 11F). This secretion fills the cavity of the spermatophore sac in the region of the former and forms an opaque jelly-like layer (Plate 12F) which proximately becomes compacted into an ovoid body, 140 x 80 μ m in size, around the terminal portion of the spermatophore neck (Plate 13C). The secretion causes the neck of the spermatophore to be sticky to the touch and thus has been called the adhesive secretion ("Klebssekret" of Heberer). In calanoid copepods of the families Pontellidae and Centropagidae the spermatophore neck is developed into a large and highly-specialised coupling device ("Koppler" of Heberer, 1937) which aids the accurate transfer and location of the opening of the spermatophore on the females genital orifice (see Lee, 1972). In view of the fact that the relatively unmodified mass of secretion completing the spermatophore neck in E. norvegica cannot be construed to form part of a true coupler in the original sense of Heberer, it has been considered more appropriate to follow the terminology of Fahrenbach (1962) and call it the "adhesive body".

In the region of the spermatophore neck encompassed by the adhesive body, the added secretions become thicker than the spermatophore wall material (Plate 12F), as extra electron-opaque material is added. This electron-opaque secretion also forms the adhesive body and is composed of electron dense granules and particles each of which is surrounded by moderately dense homogeneous material set in an electron opaque matrix. The birefringent secretory globules probably account for the majority of the less dense material of the matrix of the adhesive body, whilst the granules and other particles can be traced back to the electron dense granules of the second type secretion which coats the spermatophore flask.

The manner of release of all the secretory products in the spermatophore sac appears to be typically merocrine as the products, after being synthesised and stored in the cells, are released by fusion of their limiting membranes with the cell membrane thus allowing the contents to be released without breakdown of the structural integrity of the cell.

The lumen of the spermatophore sac is lined by a brush border of abundant microvilli (Plates 11C-E). Each microvillus is round in cross section and cylindrical in overall shape except for a domed distal extremity. The microvilli are of uniform size being ca. 980nm long and 100nm in diameter. They are invested by a membrane continuous with the plasma membrane of the cell, and comprising part of it. Axial longitudinal filaments within the microvilli are not evident and a glycocalyx, common in mammalian organization, appears to be absent. These microvilli are of uncertain function since they have no obvious association with absorptive activity, however, they may aid the distribution of secretion by increasing the area of the duct constantly in contact with the spermatophore.

The spermatophore sac of E. norvegica nearly always contains a spermatophore (Plate 13A). The proximal end of the spermatophore tapers into a slender neck, about 270 μ m long by 40 μ m wide, which has been shaped by the action of the Former (Plates 13B, 13C). The lumen of the spermatophore neck is about 15 μ m in diameter. The distal end of the spermatophore is flask-shaped and has a maximum diameter of about 160 μ m. The total length of the ripe spermatophore within the spermatophore sac on average is about 885 μ m long.

The neck of the spermatophore is usually found a short distance posteriorly to the constricted anterior end of the sac (ie in the posterior region of the Former) whilst its body occupies all except the posterior 25% of the sac. This last part of the spermatophore sac is characterised by having thinner walls than the anterior of the sac. Behind the distal end of the spermatophore flask the sac and rest of the tract are empty throughout the ductus ejaculatorius.

The constituents of the definitive spermatophore, within the spermatophore sac, maintain essentially the same relative symmetry as was present in the seminal vesicle. The beta granules do, however, decrease in number towards the distal end of the spermatophore flask whilst they increase in number and appear to merge into larger bodies (Plate 12D) towards the spermatophore neck. Sections of the proximal region of the spermatophore flask thus show the presence of a thick outer layer of beta granules (Plate 5B) whereas sections of the distal region demonstrate the near absence of this layer (Plate 5C). Coupled with the decrease in the number of distally situated beta granules is an increase in the quantity of foamy, vesicle-like bodies found in the pale outer matrix of this layer. This arrangement results in the distal

end of the spermatophore, when viewed through the semi-transparent body of the live copepod, appearing to be filled with a clear, foamy mass; the core of alpha granules maintains its central position into the distal region but the beta granules and sperm are displaced proximally.

The wall of the completed spermatophore is about 8-10 μ m thick on average, and consists of the fibrillar wall proper with its newly applied outer coating. There is a tendency for the wall of the distal half of the spermatophore flask to be thinner than the wall of the narrower proximal third of the flask. The fibrillar material of the spermatophore neck is quite thin, 3-5 μ m, although the addition there of the thick outer coat of secretion gives it more substance.

The consistency of the spermatophore wall, within the sac, becomes more firm than it previously was in the seminal vesicle although it is still moderately pliable. There is an absence of definite layering or banding within the spermatophore wall (Plates 12C, 12D) although Park (1966) believes the spermatophore wall of Epilabidocera amphitrites to be composed of four distinct layers and Raymont et al (1974) have described it in Calanus finmarchicus to consist of about seven layers.

E. Ductus Ejaculatorius

The ductus ejaculatorius is that section of the reproductive tract which leaves the spermatophore sac terminally and passes posteriorly to the external genital aperture situated on the left lateral surface of the first urosome segment. The total length of this section is about 350 μ m.

The walls of the duct are only 1.5-3.0 μ m thick (Plate 5D) and are composed of elongated epithelial cells in which are distributed a few

scattered ellipsoidal nuclei. The ductus ejaculatorius is distinct in structure from the end of the spermatophore sac.

The anterior region of the duct, lying within the metasome, is dorso-ventrally compressed so that it is about 130 μ m wide and 40 μ m high. However, once within the urosome the duct becomes dorso-ventrally elongated and measures 140 x 60 μ m as it approaches the slit-like genital aperture. The terminal section of the duct has a thin lining of cuticle and is kept firmly closed except during ejaculation of a spermatophore.

The ductus ejaculatorius appears to be non-secretory and functions solely as a tube along which the spermatophore passes to the outside of the body. No dilator muscles are connected with it nor are any myofibrils visible in its wall. Both Fahrenbach (1962) and Park (1966) have noted an absence of associated muscles in their studies of the ductus ejaculatorius in Diarthrodes cystoecus and Epilabidocera amphitrites respectively, although Marshall & Orr (1955) describe this region of Calanus finmarchicus as being short and muscular.

F. Spermatophore Discharge

Adult male E. norvegica can often be found in plankton hauls with spermatophores gripped in the terminal processes of their left fifth limbs (see Chapter 4). These spermatophores are nearly always identical in size and structure to the pre-ejaculation spermatophore found within the spermatophore sac. Occasionally, however, the spermatophore gripped by the male may show signs of some secretion having emerged from the neck at, or just before the adhesive body. The quantity of secretion seen to have escaped in these circumstances is very small.

Spermatophores are frequently found attached to the genital segment of adult female E. norvegica (see Chapter 5). These attached spermatophores differ from the spermatophore held in the male's fifth limbs in a number of respects. The main point of difference is that after attachment the spermatophore gains a long tube (Plates 13E-G) that is continuous with its short original neck and which has approximately the same external diameter as the originally present adhesive body. In some instances this newly added "extended neck" may be more than five times the length of the original, short spermatophore neck (Plate 13G).

It is highly unlikely that the extended neck of the spermatophore is formed before attachment to the female as the necks of spermatophores carefully removed from the adult male fifth limbs (Plates 13C, 13D) have necks which closely resemble that of the ripe spermatophore present in the spermatophore sac. If the spermatophore did increase in length during the period between ejaculation and attachment it is probable that examples in this condition would have been found considering the large number of specimens examined.

The first section of the tube leaving the flask of the attached spermatophore is the original short neck of the spermatophore as seen in the male's fifth limbs. Beyond this region the colour and refractive index of the tube increase to a dark brown shade and indicate the beginning of the extended neck. With the aid of high power magnification and frequent re-focussing it is possible to discern the widening at the end of the true spermatophore neck which marks the previous position of the adhesive body (Plate 13F). However, the bulk of the secretion forming the adhesive body itself has disappeared.

Spermatophores found attached on the genital segment can on the basis of their position of attachment and appearance be grouped into two main types; direct and non-direct placement spermatophores.

Direct placement spermatophores are those which have been attached immediately over the female's genital pores. These spermatophores are nearly always found empty as they have discharged their contents into the female's genital cavity. Their flasks are about the same size as those of spermatophores found gripped in the male's fifth limbs and their extended necks are usually about 454 μ m long.

Non-direct placement spermatophores are those which are found outside the region of the female's genital field and thus have not been attached over the genital pores. They can be found attached in nearly every part of the genital segment (see Chapter 5) and often may be some distance from the genital pores. Such spermatophores are usually found half-full and use of Duncan's Multiple range test (Duncan, 1955) has shown that they have significantly larger flasks than those of direct placement spermatophores or those from the male's fifth limb or spermatophore sac (Table 1). Their extended necks are appreciably longer than those of direct-placement spermatophores, usually being about 870 μ m or more, and end in a large circular mass of secretion on the female's cuticle (Plate 13G). This circular mass of secretion has been named the "attachment disc" and represents the original point of attachment of non-direct placement spermatophores to the genital segment.

The majority of non-direct placement spermatophores (69.7%) had no form of connection between the point of attachment and the female's

TABLE 1: The results of comparing the mean size (μm) of samples of spermatophores of Euchaeta norvegica obtained from different sources using Duncan's Multiple Range Test.

	Spermatophore Sac	Male Fifth Limbs	Direct Placement	Non-Direct Placement	S.E.
Length	885	876	826	1207	26
	a	a	a	c	
Width	142	138	133	163	30
	a	ab	b	c	

Samples with mean values which are not significantly different ($P = 0.05$) share similar identificatory letters. The length of the spermatophore was measured from the distal end of the flask to the start of the adhesive body on the short neck, whilst the spermatophore width was measured as the greatest diameter of the flask ($n = 20$ from each source).

genital orifice. The flask of these spermatophores are never found fully empty and thus are unlikely to be viable for fertilization of the female. The remaining non-direct placement spermatophores (30.3%) had, however, a connection with the female's genital orifice in the form of a very thin tube traversing the cuticle from their attachment discs (Plate 13H). These thin tubes, which have been called "fertilization tubes", enable their spermatophores to fully empty by passing their contents into the female's seminal receptacles. Spermatophores with fertilization tubes are thus likely to be viable for fertilization purposes.

Examination of over 6000 spermatophores on females failed to reveal any attached spermatophores which had not developed their extended necks. This strongly suggests that the extended spermatophore neck is produced immediately after attachment and that the time taken from the start of extrusion of this structure to the point where it is fully developed is very short indeed.

Although adult males with spermatophores in their fifth limbs can be brought into the laboratory and kept alive for a week or more, it is extremely rare for spermatophore attachment to take place under such conditions. Only one mating took place in the laboratory during the period of this study but it occurred, however, unobserved between midnight and six o'clock in the morning. The directly attached spermatophore flask had fully emptied through the already formed extended neck by the time observations were made. The male responsible had previously been carrying the spermatophore in his fifth limb for over 24 hours without the contents having started to extrude.

Adult males which were taken into the laboratory with spermatophores already gripped in their fifth limbs were frequently discovered to have released their spermatophores after about 2-3 days. The released spermatophores were collected from the bottom of the confining container and, in the majority of cases, were found to have produced long extended necks. These observations indicate that the discharge of spermatophore contents does not happen automatically within a few hours of ejaculation and further suggest that there is a critical, limiting period of time within which attachment has to occur otherwise the contents will spontaneously start to extrude.

In the absence of observations of mating and spermatophore attachment, spermatophores were squeezed out of live males in seawater and used for experimental work to determine the mechanism of discharge of the spermatophore contents. Once in seawater the distal foam-like bodies of the flask expand to produce a polygonal pattern on the inside of the spermatophore wall in the manner of fusing soap bubbles. It is, however, most uncommon for any of the spermatophore contents to extrude naturally in such recently extruded spermatophores. Nevertheless discharge of the contents can be brought about experimentally by cutting the short neck of the spermatophore in the region of the adhesive body. This results in the core secretion flowing out through the short neck of the spermatophore and quickly solidifying on contact with the seawater to form an elongated tube, since the liquid interior continues to push forward. The tube so formed is identical in every respect with the extended neck of those spermatophores found attached to the female genital segment.

Electron microscope preparations of the extended neck confirm that it is

constituted from solidified alpha granules and matrix from the core secretion of the spermatophore flask (Plates 14A, 14B). The alpha granules in the outer wall of the extended neck appear to increase in size towards the outside where they have been in contact with seawater; the process of solidification of the newly extended neck may well be associated with enlargement of the alpha granules.

In artificially activated spermatophores the process of extrusion of the spermatophore contents usually stops, probably because of a pressure balance between the swollen material at the distal end of the flask and the blocking effect due to the solidification of the central secretion at the end of the extended neck. It is noticeable, even with the formation of a fairly long extended neck, that the central core secretion is still present in quantity within the flask (Plate 13E). This is probably because the formation of a fairly long narrow neck causes relatively little drain on the material of the spermatophore core as well as the fact that enlargement of the alpha granules by imbibition of water makes the contents go further. Repeated cutting of the extended neck along its length causes further discharge of the spermatophore until, finally, the whole of the flask is empty except for the foamy material which eventually begins to lose its identity.

During extrusion of its contents the flask of the long-necked spermatophore often increases in length and width. Any increase in size is maintained even after the contents have been extruded and even puncturing of the empty flask does not cause it to shrink. These events indicate that the spermatophore probably stretches to a certain degree owing to increased pressure within the flask, but if it stretches beyond a critical threshold level it appears to be unable to contract back to its former dimensions.

The consistency of the spermatophore wall also changes noticeably after the spermatophore has been extruded into seawater. When gripped in the male fifth limbs it is more difficult to section than when in the spermatophore sac, although it is still reasonably pliant. After the spermatophore has been attached to the female and the extruded neck has formed the wall hardens to such an extent that sectioning of the specimen is impracticable without very lengthy impregnation. Dehydration and embedding causes the surface of the spermatophore flask to reflect light and exhibit a shiny appearance. Similar changes in consistency and appearance have been noted in the post-ejaculation spermatophore of Epilabidocera amphitrites by Park (1966), although he further records that the spermatophore of this species shrinks after discharge of the contents.

The ejaculated spermatophore, consisting of the flask and short neck, is fixed to the female by the left fifth limb of the adult male (see Chapter 4). Initial attachment of the spermatophore is probably helped by the stickiness of the adhesive body situated at the end of the short spermatophore neck. Artificial attachment of spermatophores removed from the fifth limbs of males has demonstrated that fixation to the female by the adhesive body alone is insecure as spermatophores so applied are often easily dislodged by natural swimming movements. Puncture of the spermatophore neck at or close to the adhesive body quickly causes the extrusion of material from the central core onto the surrounding cuticle. At the start of extrusion of the contents the tip of the short spermatophore neck remains in contact with the female's cuticle, but as more of the material comes out the spermatophore is slowly forced away on the end of the lengthening tube of the extended neck. A circular disc of secretion, larger in diameter than the width

of the extended neck, forms at the place where the spermatophore contents meet the female's cuticle thereby demonstrating the origin and mode of formation of the attachment disc. The central core secretion of the spermatophore is sticky and helps, when it solidifies on contact with seawater, to glue the spermatophore securely to the female.

Park (1966) has postulated that the canal through which the sperm can pass into the female orifice in Epilabidocera is possibly opened by an action of the female, such as secretion of an enzyme or lysin, after the spermatophore has been glued down. Discharge of the contents of the spermatophore placed over the genital opening of a female Euchaeta may possibly be caused by the same means as has just been outlined, however, similar activation of a spermatophore attached elsewhere is improbable as the glands necessary to secrete such chemicals are absent outside the genital field.

Occasionally spermatophores have been observed with developed extended necks within the spermatophore sac of a dead male. This rare occurrence is probably brought about by passage of seawater into the body tissues and spermatophore resulting in triggering of the extrusion mechanism. It must be pointed out, however, that production of the extended spermatophore neck has only been observed within the spermatophore sac of obviously decomposing individuals.

Under natural conditions a lack of delay in effecting extrusion of the spermatophore contents after attachment would be beneficial if not indispensable in cementing it to the female. This requirement, coupled with an absence of attached spermatophores without extended necks in plankton hauls, is good reason to believe that the male, either intention-

ally or inadvertently, punctures and opens the spermatophore neck to enable the contents to discharge.

Although there is a significant difference in size between direct and non-direct placement spermatophores, removal and measurement of spermatophores from within the spermatophore sac has failed to reveal the presence there of more than one morphological type or modal size of spermatophore produced by the male. Ejaculated spermatophores found within the male fifth limb also exhibit a general uniformity in size. Thus it appears to be the position of attachment (ie direct or non-direct placement) on the female's genital segment which influences the size of the spermatophore flask found there, as direct-placement spermatophores have smaller flasks than non-direct-placement spermatophores. This dimorphism of size present in post-attachment spermatophores may be associated with the markedly different length of the extended neck in the two main types of attachment.

The extended neck of the direct-placement spermatophore is short because, being placed over the female genital orifice, most of its contents flow straight into the female's seminal receptacles and only a small quantity of spermatophore core material solidifies outside her body on contact with seawater. There is thus unlikely to be much resultant back pressure even though the contents of the flask obviously swell. This probably results in the pressure within the flask being speedily relieved and the flask of the direct-placement spermatophore not being significantly different in size to that of the pre-attachment structure.

The non-direct placement spermatophore, being attached some distance away from the female's genital pores, is not able to extrude its core

material directly into the seminal receptacles and so must form a characteristically long extended neck due to solidification of this material with seawater. The pressure set up within the flask is unlikely to be relieved speedily as continued solidification of the core material at the end of the lengthening neck is likely to cause a persistent build up of pressure within the tube and finally result in the visibly expanded flask. The previously mentioned fact that many non-direct-placement spermatophores never succeed in fully discharging their contents is probably because they may not be able to overcome the back pressure within the narrow bore of the long neck or else as a consequence of material solidifying and blocking its end.

DISCUSSION

Examination of the ultrastructure of the male reproductive system of E. norvegica has demonstrated a noteworthy intensity of secretion of these tissues as revealed by the presence of abundant ribosomes, secretory vacuoles and Golgi apparatus. The vas deferens in particular, and to a slightly lesser extent, the spermatophore sac, exhibit many of the intrinsic features of a characteristic protein-secreting cell as typified by Jamieson & Palade's descriptions (1967a, b; 1968a, b) of the zymogenic cell of the mammalian exocrine pancreas. The presence of well defined and abundant Golgi apparatus in animal tissue is generally associated with the packaging of secretory products to form secretory granules which may be either polysaccharides (it should be noted that in general these polysaccharides are associated with proteins to form mucoprotein secretory granules), lipoprotein or protein in nature (see Favard, 1969). It is most likely, therefore, that many of the components of the spermatophore in E. norvegica are proteinaceous. Raymond et al

(1974) summarize results of their histochemical tests carried out on the genital system of Calanus which indicate that the spermatophore and its contents contain appreciable quantities of basic proteins and mucoproteins.

Heberer (1932b) has proposed that the proximal part of the vas deferens secretes the core substance of the spermatophore and has emphasised the highly secretory nature of the epithelium of the vas deferens in Calanus. Lowe (1935) has, on the other hand, claimed that the core substance of the spermatophore is formed in the testis and then passes down the vas deferens. Park (1966) describes the anterior part of the vas deferens in Epilabidocera as producing the secretion which forms the central core of the spermatophore but states that the anterior secretory cap of the testis, which he acknowledges as being the beginning of the vas deferens in this species, also produces small quantities of the same material. This smooth grading of the vas deferens into the testis may possibly account for Lowe's statement concerning the origin of the core secretion of the spermatophore. The present E/M studies of the genital tract of E. norvegica appear to affirm Heberer's view.

Heberer (1932b) has further claimed that the distal part of the vas deferens secretes the spermatophore wall and that the seminal vesicle merely acts as a storage space. Lowe (1935) accepts that the seminal vesicle is apparently non-glandular but has postulated that the spermatophore wall secretion is produced by the thick epithelium of the spermatophore sac and is passed forwards and moulded in the seminal vesicle. Raymont et al (1974) have produced electron micrographs which show that the spermatophore sac of Calanus produces abundant vacuoles whose contents are discharged from the cell to form a mass of unchanged

secretory material outside the laminated wall of the fully formed spermatophore, and on these grounds have tended to favour Lowe's suggestion for the origin of the spermatophore wall. In E. norvegica, however, electron micrographs provide irrefutable evidence that the spermatophore wall secretion is produced by the cells of the posterior vas deferens as well as by the seminal vesicle, but fail to show the secretion of this material by the spermatophore sac. The spermatophore sac of E. norvegica, as in Calanus, is intensely secretory but the globules which are released there form a distinct layer outside the true wall of the spermatophore and this layer is likely to constitute a spermatophore lubricant. A similar external layer has been noted as covering the spermatophore wall in Diarthrodes cystoecus (Fahrenbach, 1962) and Epilabidocera amphitrites (Park, 1966). In addition, both Fahrenbach (1962) and Park (1966) have described the spermatophore wall as already being present in the posterior region of the vas deferens.

The discovery that the thin walled seminal vesicle of E. norvegica is strongly secretory and is, together with the posterior vas deferens, responsible for addition of the presumptive spermatophore wall is contrary to the account of Lowe (1935) and Marshall & Orr (1955) who state that this region of the genital duct in Calanus has no secretory function. It is very likely that the still pliable wall of the presumptive spermatophore needs to have more material added to it after the assemblage has filled up to occupy the space left in the seminal vesicle by the passage of the previous assemblage into the spermatophore sac. The seminal vesicle thus probably acts to strengthen the presumptive spermatophore wall as well as acting as a storage space for the various constituents to take up their positions.

The muscular sphincter described by Heberer (1932b; 1937) as separating the seminal vesicle from the spermatophore sac in other calanoid copepods is absent from E. norvegica. Park (1966) has assumed that the wall between the seminal vesicle and the spermatophore sac must be ruptured by pressure in Epilabidocera amphitrites but substantial tearing of this region has not been observed in specimens of E. norvegica which already have a spermatophore in the sac. Separation of the newly formed short neck of the spermatophore from the proximal assemblage may simply be achieved by the slight tension of the wall tissue. However, specimens have occasionally been found in which the spermatophore assemblage is continuous between the seminal vesicle and the spermatophore sac; probably indicating that severance of the narrow interconnecting portion is not necessarily instantaneous.

The spermatophore of E. norvegica corresponds fairly closely in its manner of function with that described for Diarthrodes cystoecus by Fahrenbach (1962), primarily because it lacks the dualism of spermatozoa originally recorded by Heberer (1932b) which has been found to be present in all other copepods investigated. This functional dualism is manifested by Heberer's Q (Quell = Swell) spermatozoa, which provide by swelling the propulsive force inside the spermatophore to expel the B (Befruchtungs = fertilization) spermatozoa. He states that the spermatophore sac secretes two different kinds of material, the proximal and distal "differentiation secretions". The former increases the swelling resistance of the B-sperm, while the later eliminates the swelling resistance of the Q-sperm which swell up completely with the admission of water; this develops a strong pressure and thus the Q-sperm are mainly instrumental in emptying the spermatophore. However, no evidence as to this supposition has been found in E. norvegica in the present study.

Three different spermatophore structural types have been declared by Heberer (1937) to exist within the calanoid copepods. These were differentiated as Calanus-, Pleuromamma-, and Acartia-types on the basis of the pattern of arrangement of their contents. In the Calanus-type all the sperm lie parietally, in one or several layers, all the spermatophore secretions being situated in the interior. In the Pleuromamma-type there is a layer of secretion between the sperm and the spermatophore wall; this according to Heberer is the differentiation secretion which gives rise to the functional dualism of sperm. The centre of the spermatophore is occupied by the core secretion. In the Acartia-type there is no longer any core secretion as the sperm occupy the centre of the spermatophore. Although there is no functional dualism of sperm in E. norvegica the general arrangement of the spermatophore contents in this species appears to closely resemble the Pleuromamma-type. The beta granules in E. norvegica occupy the position of Heberer's differentiation secretion.

The absence of such dualism of spermatozoa in E. norvegica indicates that the propulsive force generated to empty the spermatophore must originate from one or more of the other spermatophore components other than the sperm. A likely source of this motive force is the vesicular foam bodies within the distal portion of the spermatophore flask which may swell by water imbibition. This swollen mass can be seen to have the effect of pushing the outer layer of the spermatophore, which contains the sperm, down into the proximal end of it. Fahrenbach (1962) has referred to the vesicular bodies which are responsible for the ejection of the spermatophore contents as "Q-bodies" by analogy to Heberer's Q-spermatozoa. The central core of alpha granules in E. norvegica may also help generate more force as they too have been shown to expand

and gel on contact with seawater before hardening to form the extended neck of the spermatophore. The swelling of the spermatophore contents strongly suggests that their extrusion is dependent on the penetration of water through the spermatophore wall. Park (1966) has postulated that the distal surface of the spermatophore, perhaps by receiving a particular secretion while it is in the sac or by the nature of the jelly coat on it, may pass water more readily than other areas.

The manner of formation of the spermatophore's lengthy neck has not always been fully understood before by various workers. Lowe (1935), for example, believed, because the substance forming the spermatophore is extensible, that "the long tube by which the spermatophore is attached to the genital opening of the female is formed when the body is being extended", and Marshall & Orr (1955) have reiterated this claim. The present study, however, has demonstrated in E. norvegica that the extrusion of the central core is responsible for the extended neck after the spermatophore has been attached to the female. Marshall & Orr (1955) have drawn attention to the fact that in Calanus finmarchicus the neck of the abnormally positioned spermatophore may be much longer than usual and may appear to be still full of sperm. Such observations are explained by reference to the fact that in E. norvegica the secretion passing out to form the extended neck merely flows directly into the female's genital cavity in accurately positioned spermatophore placements, whilst abnormally positioned examples (ie non-direct-placements) have long extended necks caused by continued hardening of the secretion on contact with seawater. Abnormally positioned spermatophores with long extended necks are much more liable to suffer blockage of the lumen of the tube and are thus likely to remain full. However, the tendency of the spermatophore contents

to act as a hydrophilic gel may also partially account for these spermatophores still being reasonably full even though they have obviously already extruded appreciable quantities of material.

The presence in and around the spermatophore of different secretions of various origins from within the genital tract raises the question of the function of these materials. Besides the obvious functions of effecting contact with the female, causing adhesion of the spermatophore and forming a channel through which sperm is pushed into the female's seminal receptacles, the additional possibility that part of the core material is nutritive and is transferred with the sperm has been put forward by Raymont et al (1974). Indeed, the passage of core material with the sperm into the female is a standard occurrence in E. norvegica. The sperm tend to have a long life in the spermathecae of Calanus finmarchicus after their transference to the female (see Marshall & Orr, 1952) and there is a strong suggestion that in Calanus hyperboreus a considerable delay occurs between spermatophore transference and fertilization (see Conover, 1962). Evidence is also presented in Chapter 6 which indicates that the sperm of E. norvegica are also long lived after transference. In all these cases the nutrition and homeostasis of the sperm must be critically important. Katona (1970) has suggested that the females of Eurytemora affinis and E. herdmani in nature can mate, survive long unfavourable periods, and then produce offspring when conditions become favourable without a further mating. Avoiding the necessity for an additional mating would be especially advantageous during times of low population density when males and females might have problems finding each other for mating.

The ductus ejaculatorius is only slightly smaller in width than the spermatophore and its walls probably have elastic properties as no signs

of rupture have been observed in preparations of this section of the genital tract in males which have an already ejaculated spermatophore gripped in their left fifth limbs. This apparent lack of self-imposed damage caused during spermatophore ejaculation, coupled with the discovery that numerous males have been found with a spermatophore in their fifth limbs in addition to another fully formed spermatophore within the spermatophore sac provides strong evidence to support the belief that males are able to safely ejaculate two spermatophores during their life. The existence of another, albeit presumptive, spermatophore within the seminal vesicle raises the potential production to at least three spermatophores.

In E. norvegica microforce balance weighings (Table 2) show that on average a male only transfers 0.2% of its wet body weight and 0.9% of its dry body weight into each ripe spermatophore. This is much less than the levels of 5.8% of wet body weight and 25.3% of dry body weight found to be transferred by the female in producing a single egg-clutch (see Chapter 6). There is evidence which indicates that male E. norvegica do not feed whilst in the adult stage (see Chapter 7) and thus spermatophore synthesis and ejaculation must place a substantial drain on the body reserves, even though the spermatophore is quite small.

Lee (1972) has commented that ejaculation of the large spermatophore coupling device of the Centropagidae must cause an immense distortion of the genital segment, even allowing for the fact that the coupling plates would be flexible, possibly to the extent of killing the male. It is therefore reasonable to postulate that the production of multiple spermatophores may be associated with the absence of a large and well developed coupling device.

TABLE 2: Output, in terms of Wet Weight and Dry Weight, when an Adult Male Euchaeta norvegica of average size produces a spermatophore (n = 50 in both cases).

<u>Wet Weight</u>				
Total Length of male	Prosome Length of male	Body Weight of male	Spermatophore weight	<u>Spermatophore wt</u> body weight %
6.21mm	4.37mm	4796 μ g	11 μ g	0.2
<u>Dry Weight</u>				
6.21mm	4.37mm	992 μ g	9 μ g	0.9

Fig. 6. Diagram of an adult male Euchaeta norvegica
viewed from the dorsal aspect to show the
position of the component parts of the
reproductive system.

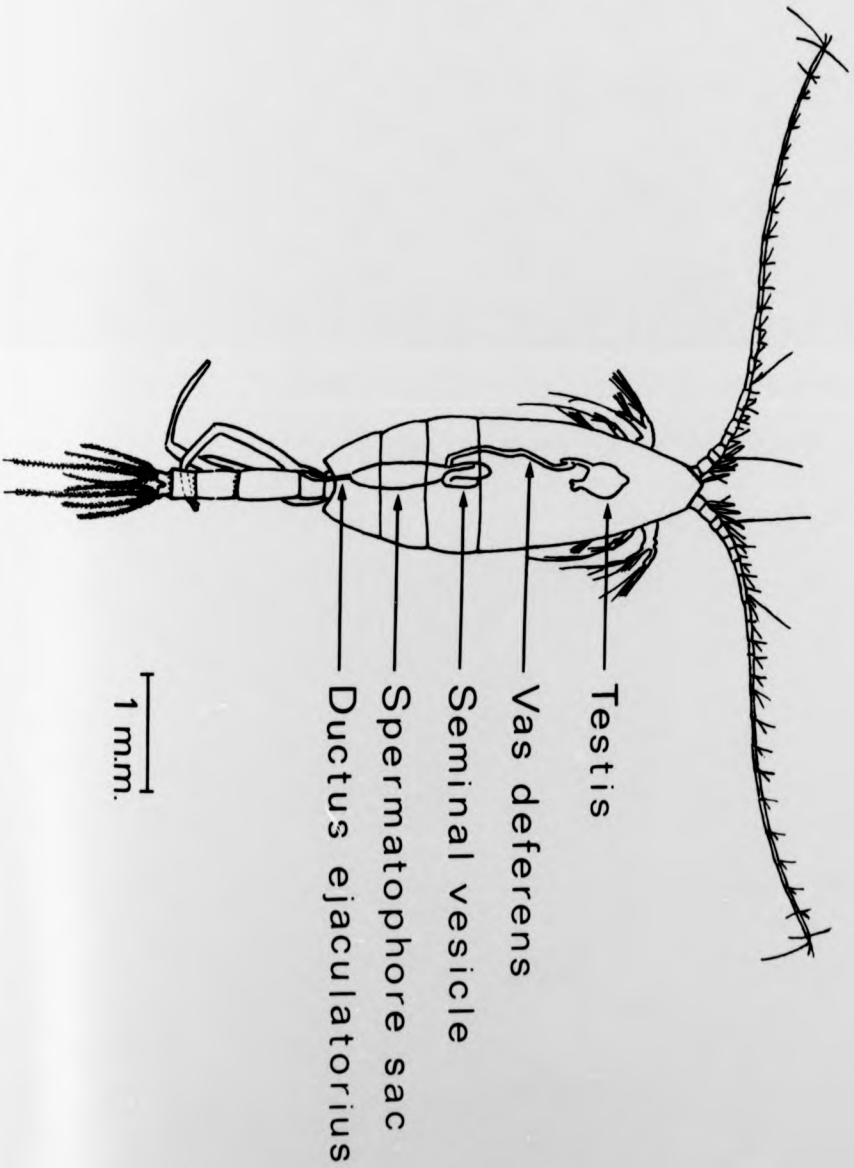
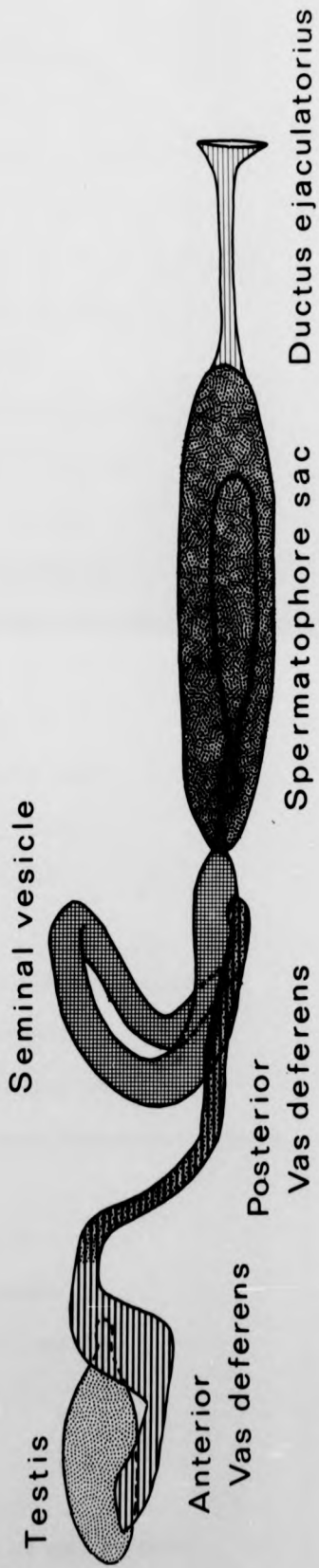


Fig. 7. Diagram of the reproductive system of an adult male Euchaeta norvegica viewed from the left side.



KEY TO LETTERING ON PLATES 5-14

α	=	alpha granule
β	=	beta granule
ab	=	adhesive body of spermatophore
ad	=	attachment disc of spermatophore
bg	=	birefringent globule
bl	=	basal lamina
c	=	core secretion of spermatophore
cm	=	cell membrane of sperm
cw	=	cell wall of sperm
de	=	<u>ductus ejaculatorius</u>
dm	=	dorsal longitudinal muscle of metasome
F	=	Former
fb	=	foam-bodies
ft	=	fertilization tube
fg	=	fibrillar globule
G	=	Golgi apparatus
g	=	gut
ger	=	granular endoplasmic reticulum
gg	=	granular globule
gs	=	ground substance of sperm
hg	=	electron dense homogeneous globule
lu	=	lumen
m	=	mitochondrion
mc	=	circular muscle
ml	=	longitudinal muscle
mv	=	microvilli
mx	=	matrix
n	=	nucleus
ns	=	short neck of spermatophore
ne	=	extended neck of spermatophore

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mx	=	matrix
n	=	nucleus
ns	=	short neck of spermatophore
ne	=	extended neck of spermatophore

ol = outer layer of spermatophore wall
os = electron opaque secretion
psw = presumptive spermatophore wall
s = sperm
sf = spermatophore flask
sps = spermatophore sac
sv = seminal vesicle
sw = spermatophore wall
t = testis
v = vesicle
vdp = posterior vas deferens
vt = transition vesicle
w = whorl-like vesicle of sperm
wne = wall of extended neck of spermatophore
wsps = wall of spermatophore sac
wsv = wall of seminal vesicle
wvda = wall of anterior vas deferens

Plate 5.

- a. Transverse section of the testis (t) showing sperm in the central cavity (arrowed) which leads into the lumen of the anterior vas deferens. A number of dark staining nuclei are visible in the wall of the anterior vas deferens (wvda)
- b. Transverse section of the anterior region of the spermatophore flask within the spermatophore sac. The central core secretion (C) of the spermatophore is surrounded by a thick layer of beta granules (β). Two dark staining sperm can be seen between the core secretion and the beta granules. Many dark staining nuclei are evident in the wall of the spermatophore sac (wsps)
- c. Transverse section of the spermatophore sac half-way along its length showing the prominent central core secretion (C) of the spermatophore contained within it. Eight darkly staining sperm are visible between the central core and the spermatophore wall (sw). The thin layer of beta granules characteristic of sections of this region is visible as a dark layer applied to the inside of the spermatophore wall. Note the numerous moderately staining secretion globules present in the wall of the spermatophore sac near the lumen. The larger, darker bodies towards the outside of the sac are nuclei
- d. Transverse section of the ductus ejaculatorius (de) showing the thin wall (arrowed). Part of the gut (g) is applied to the dorsal surface of the duct.

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Plate 6.

- a. Maturing sperm (s) within the testis showing the whorl-like vesicles (w) situated at the periphery of the internal cytoplasm
- b. The edge of a sperm showing its cell wall (cw), the whorl-like vesicles (w) and ground substance (gs) of the internal cytoplasm
- c. The cell wall (cw) of a sperm showing the double layered cell membrane (cm)

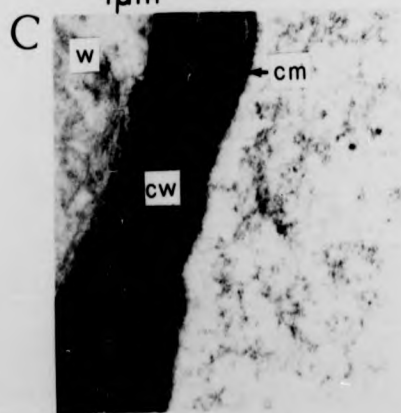
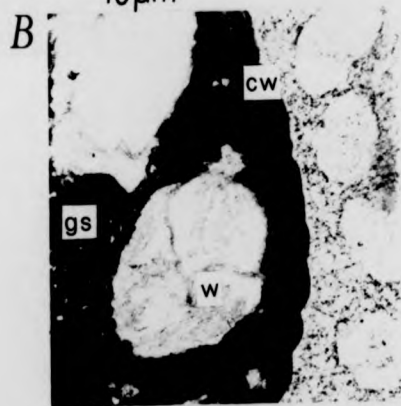
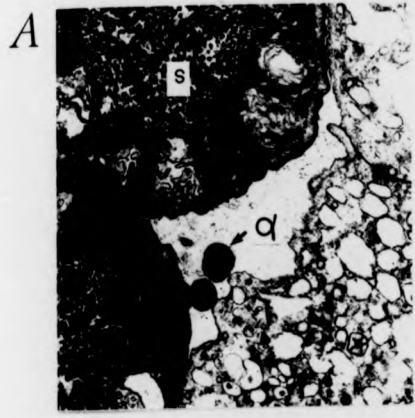


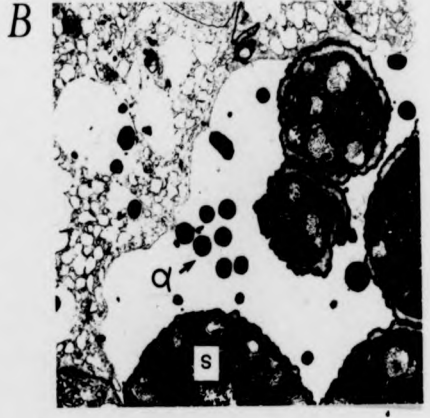
Plate 7

- a. Anterior vas deferens showing sperm (s) and alpha granules (α) within the lumen of the duct. The wall of the duct is seen on the right of the micrograph
- b. Anterior vas deferens showing alpha granules (α) recently liberated into the lumen of the duct. Other alpha granules are evident within the wall of the duct
- c. Three alpha granules (α) close to the wall of the anterior vas deferens (at left of micrograph). Note the filamentous component in the matrix (mx)
- d. Anterior vas deferens showing an alpha granule (α) within the lumen of the duct. A developing alpha granule is visible within a vesicle at the top right of the micrograph. The granular endoplasmic reticulum (ger) of the wall of the duct is very prominent
- e. A group of alpha granules in the lumen of the anterior vas deferens
- f. Alpha granules surrounded by matrix in the lumen of the anterior vas deferens

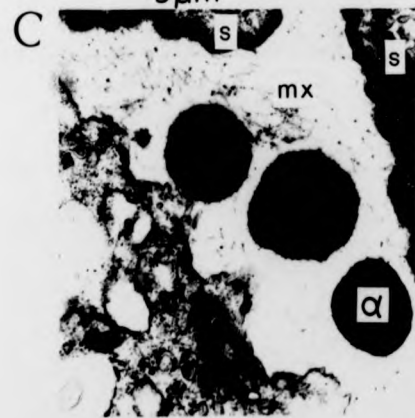
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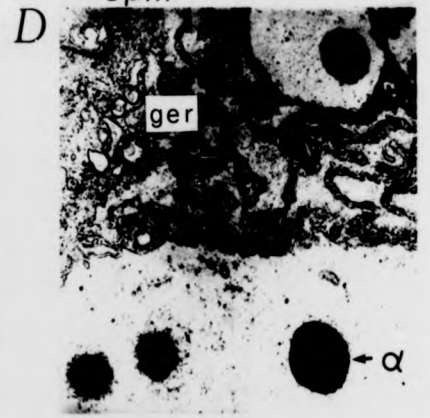
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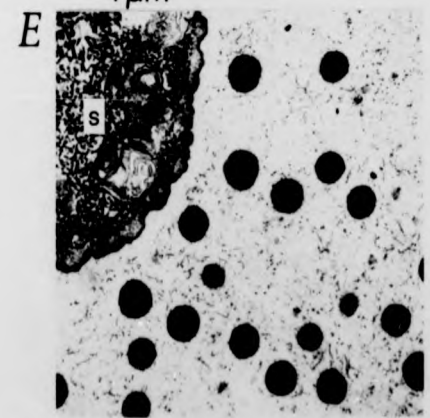
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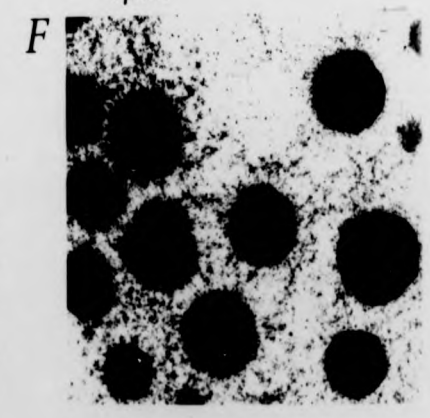
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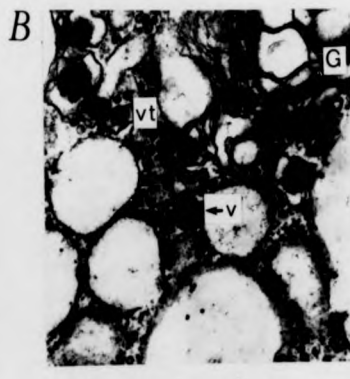
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Plate 8

- a. An alpha granule starting to develop within its vesicle (v) in the wall of the anterior vas deferens. Transition vesicles (vt) and Golgi apparatus are also visible
- b. An association of abundant transition vesicles (vt), vesicles containing alpha granules (v) and Golgi apparatus (G) in the anterior vas deferens
- c. A developing alpha granule in its vesicle (v) showing the well defined limiting membrane (arrowed) within the wall of the anterior vas deferens
- d. Aggregation of alpha granules to form a large vesicle (v) in the wall of the anterior vas deferens. Numerous Golgi apparatus (G) are dispersed throughout the cytoplasm

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- a. An early stage in the accumulation of the presumptive spermatophore wall (psw) within the posterior vas deferens. Four beta granules (β) are evident close to the periphery of the duct, whilst the alpha granules (α) are congregated towards the centre of the duct
- b. A later stage in the formation of the presumptive spermatophore wall (psw) within the posterior vas deferens. Vesicles of spermatophore wall secretion can be seen fusing with the presumptive spermatophore wall at the lower left corner of the micrograph
- c. A fibrillar globule (fg) of spermatophore wall secretion forming in a vesicle within the wall of the posterior vas deferens. Note the presence of flocculent material of moderate electron density in the left side of the vesicle. The cytoplasm of the cell is packed with abundant granular endoplasmic reticulum (ger)
- d. Posterior vas deferens showing a group of three vesicles containing spermatophore wall material. The two lower vesicles each contain a single electron dense beta granule (arrowed)
- e. Posterior vas deferens showing four fibrillar globules (fg) of spermatophore wall material. The top right globule has a developing beta granule associated with it. Mitochondria (m) are often found close to fibrillar globules
- f. Distal region of the posterior vas deferens showing the well developed presumptive spermatophore wall (psw). Six vesicles are present within the wall of the presumptive spermatophore, the lower one of which contains a mature beta granule. A group of globules of spermatophore wall secretion are present in the wall of the genital duct at the mid-right of the micrograph

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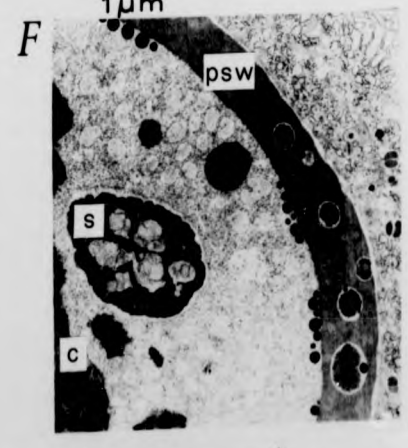
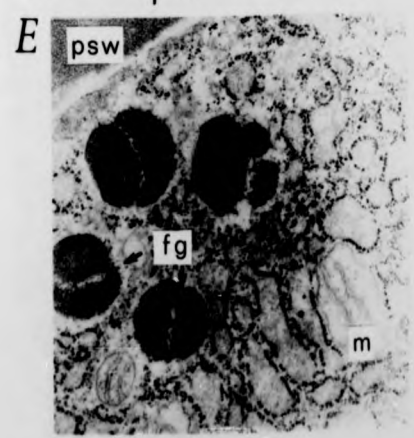
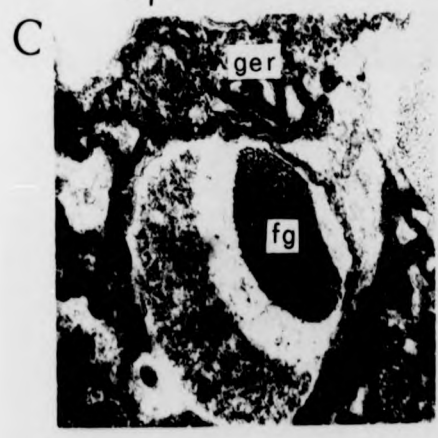
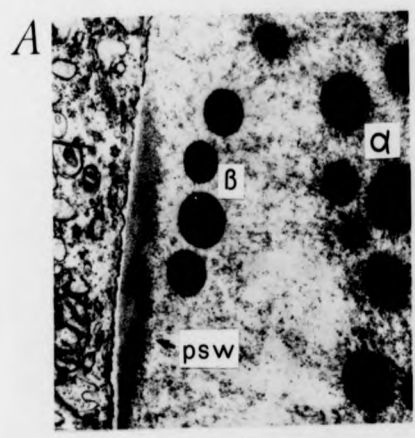
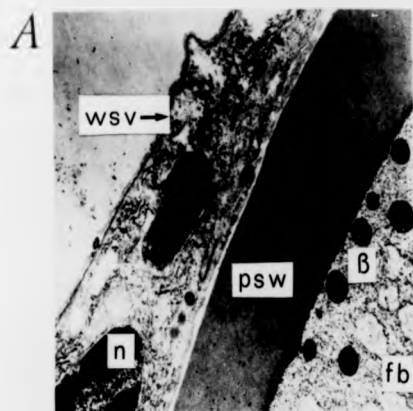
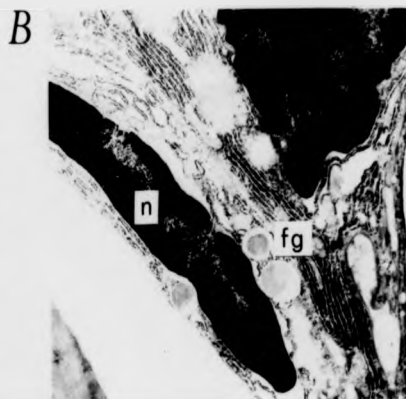


Plate 10

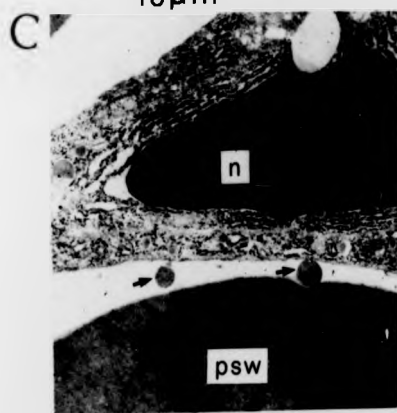
- a. Seminal vesicle (wsv) showing the typically thin wall of this part of the genital duct. Beta granules (β) and vesicular foam-bodies (fb) are evident within the presumptive spermatophore
- b. Seminal vesicle showing fibrillar globules (fg) of spermatophore wall secretion within the thick-walled junction connecting the ascending and descending limbs of the duct. The presumptive spermatophore wall is visible within the lumen of the duct at the lower left corner of the micrograph
- c. Seminal vesicle showing two fibrillar globules (arrowed) about to merge with the presumptive spermatophore wall (psw). The wall of the seminal vesicle appears thick in this micrograph as the section was taken across a junction connecting the ascending and descending limbs of the duct
- d. Seminal vesicle showing the thick presumptive spermatophore wall (psw) of a well developed spermatophore assemblage. Another portion of presumptive spermatophore wall is visible at the top right of the micrograph where a loop in the duct is present
- e. The fibrillar structure of the presumptive spermatophore wall (psw) found within the seminal vesicle
- f. The final arrangement of the contents of the spermatophore assemblage found at the distal end of the seminal vesicle. The prominent core secretion (C) occupies most of the area within the spermatophore. The sperm (S) lie outside the core secretion and are partly surrounded by foam-bodies (fb). A number of beta granules (β) are visible close to the presumptive



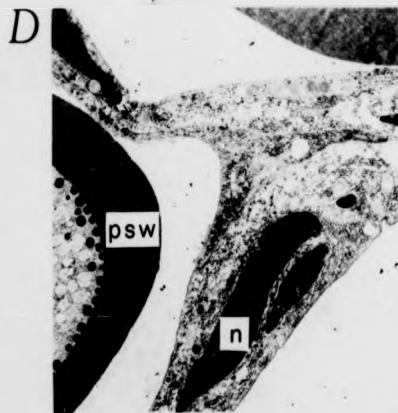
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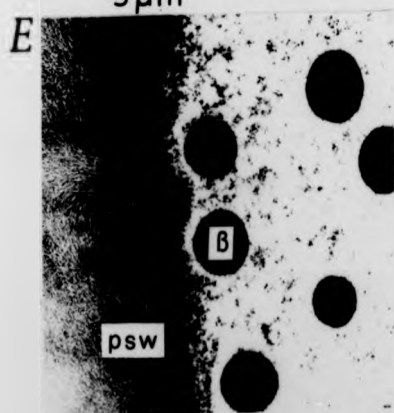
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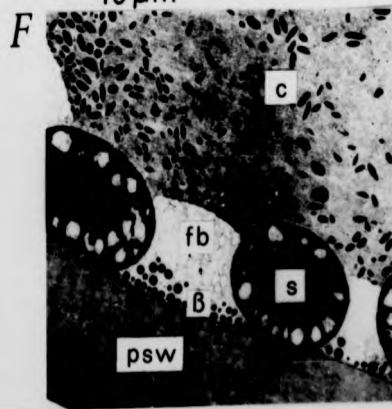
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Plate 11

- a. Outer region of the spermatophore sac showing the basal lamina (bl), circular muscle (mc) and longitudinal muscle (ml).
- b. Spermatophore sac showing a typical nucleus (n) from this region and the characteristic vacuolar cytoplasm surrounding it. The cytoplasm appears vacuolar as a result of the numerous distended cisternae of the endoplasmic reticulum
- c. Spermatophore sac showing electron dense homogeneous globules (hg) within the cytoplasm. A layer of microvilli (mv) project into the lumen of the duct. Pale globules (arrowed) are also visible
- d. Spermatophore sac showing the developing outer layer (ol) of the spermatophore. Electron dense homogeneous globules (hg) are evident within the cytoplasm close to the border of microvilli (mv) which lines the duct
- e. Spermatophore sac showing granular globules (gg) near the outer layer (ol) of the spermatophore
- f. A birefringent globule (bg) within the cytoplasm of the spermatophore sac

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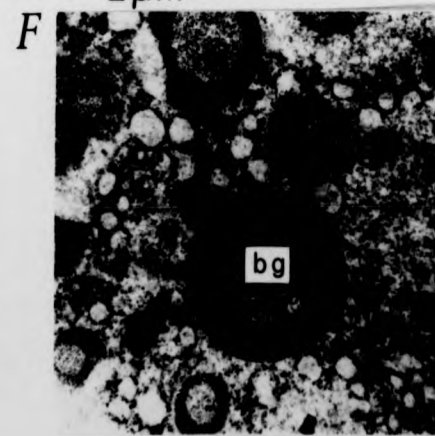
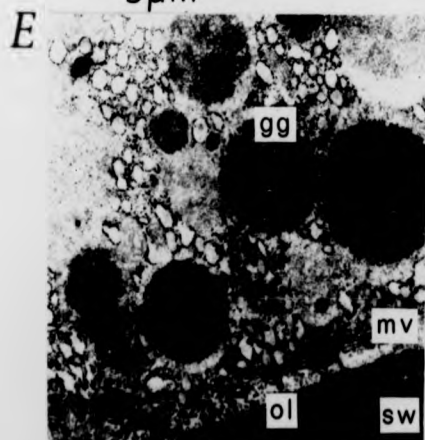
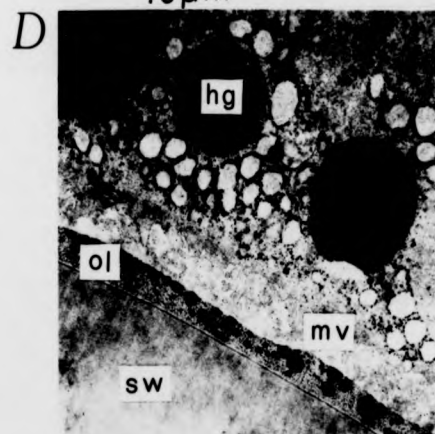
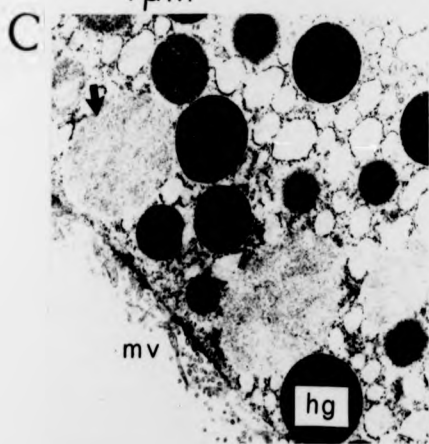
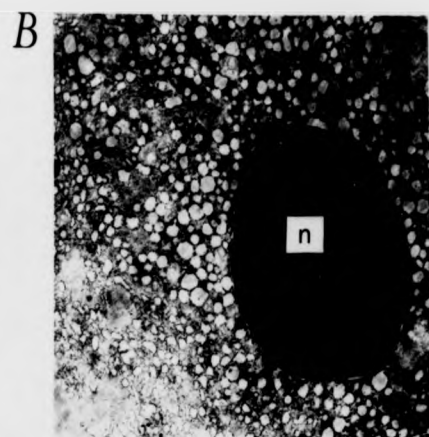
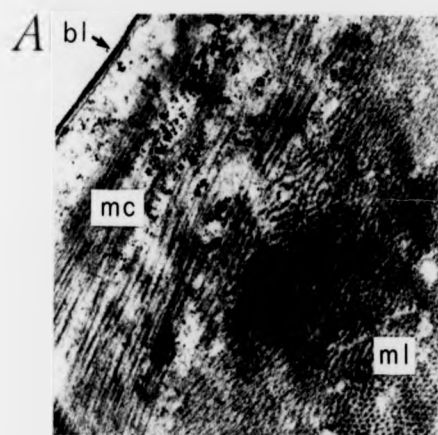
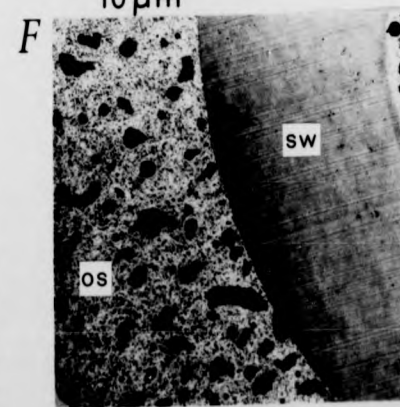
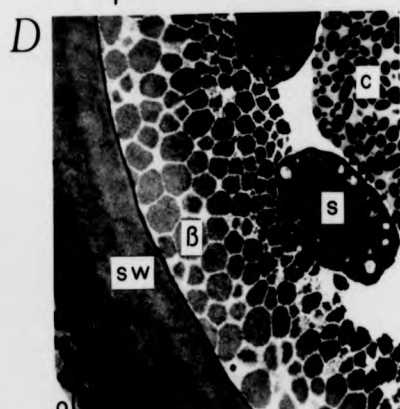
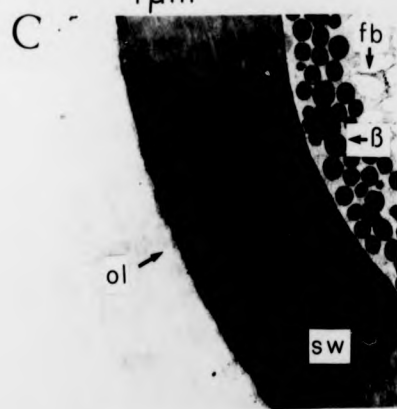
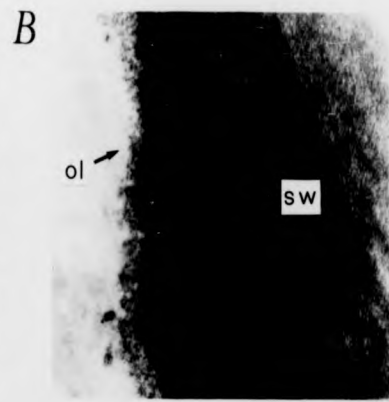
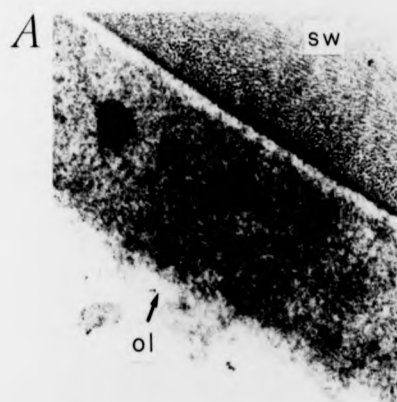
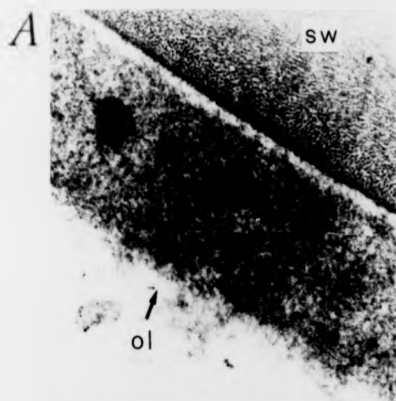


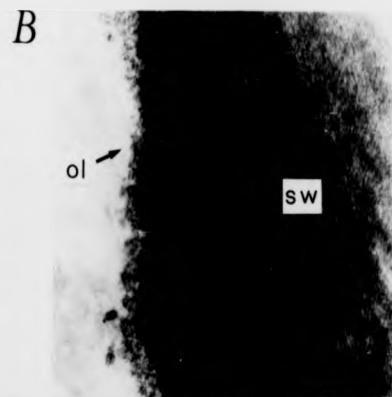
Plate 12

- a. The outer layer (ol) added to the spermatophore wall (sw) in the distal region of the spermatophore sac. Note the lack of electron dense granules
- b. The outer layer (ol) added to the spermatophore wall (sw) in the mid-region of the spermatophore sac. Note the abundance of electron dense granules
- c. The spermatophore wall (sw) and its outer layer (ol) from the mid-region of the spermatophore sac. A number of beta granules (β) and foam-bodies (fb) are evident within the spermatophore
- d. The spermatophore wall (sw) and its outer layer (ol) from the anterior region of the spermatophore sac. The section is taken from the proximal part of the spermatophore flask. Note the well developed outer layer. The beta granules have merged to form larger masses (β)
- e. The spermatophore wall (sw) close to the spermatophore neck showing the thick outer layer (ol)
- f. The spermatophore wall (sw) in the region of the short neck of the spermatophore. The opaque secretion (os) forms an outer layer which is also found around the adhesive body at the tip of the short neck

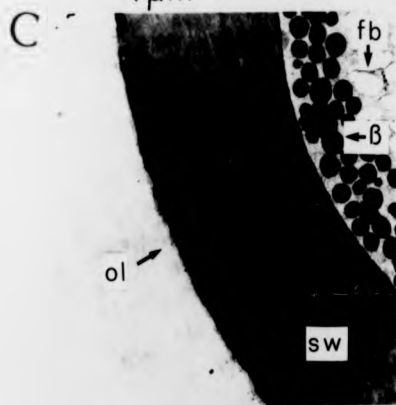




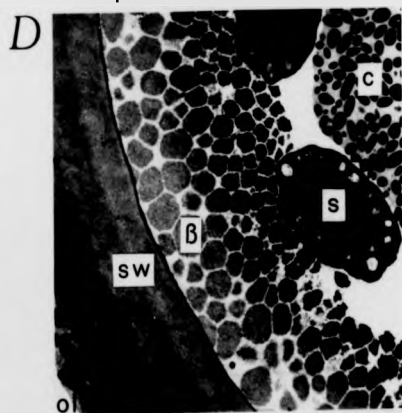
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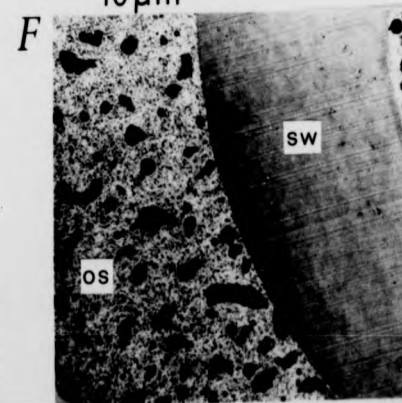
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Plate 13

- a. Seminal vesicle (sv) and spermatophore sac (sps) showing the demarcation point (arrowed) between these two regions of the genital tract. Note the spermatophore present within the spermatophore sac
- b. The proximal end of the spermatophore sac showing the region of the Former (F). The dark-coloured adhesive body of the ripe spermatophore is visible within the lumen
- c. A spermatophore removed from the left fifth limb of an adult male
- d. The short neck (ns) and adhesive body (ab) of a spermatophore that has been removed from the left fifth limb of an adult male. Note the opaque secretion (os) around the short neck and adhesive body
- e. A partially emptied spermatophore removed from a non-direct placement position on the genital segment of an adult female showing part of the extended neck. Note the core secretion (C) is still present and that the foam-bodies (fb) occupy most of the periphery of the spermatophore flask. The sperm and other lateral secretions have been displaced to the proximal end of the flask (arrowed)
- f. An empty spermatophore showing the point of extrusion of the extended neck (ne) from the short neck (ns). The remains of the adhesive body (arrowed) is visible at the end of the short neck
- g. The attachment disc (ad) at the end of the extended neck (ne) of a non-direct placement spermatophore
- h. The attachment disc (ad) and part of the fertilization tube (ft) of an empty, non-direct placement spermatophore

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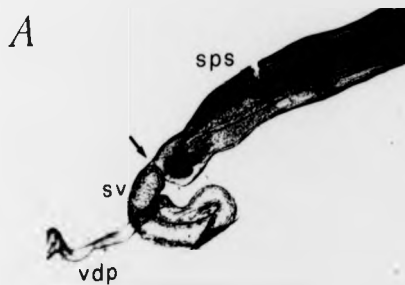
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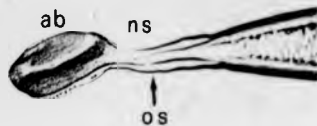
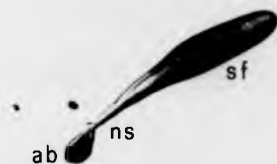


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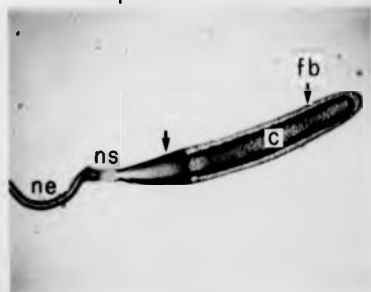


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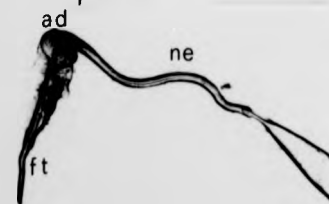
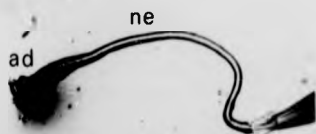


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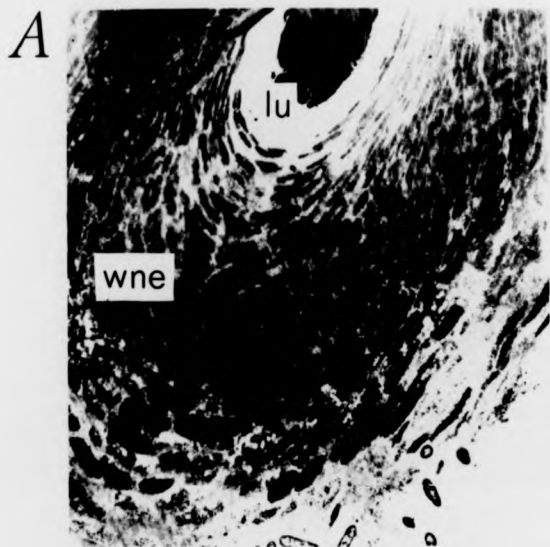
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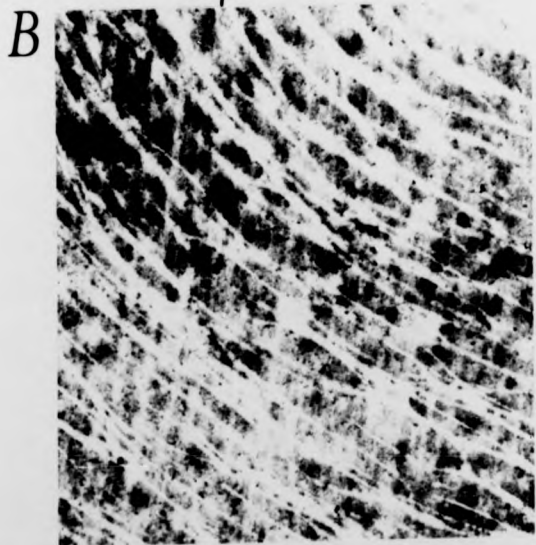
Plate 14

- a. Transverse section of the extended neck of a spermatophore showing its wall (wne) and central lumen (lu). Note how the component alpha granules which form the wall of the extended neck are appreciably smaller near the lumen than at the outer periphery
- b. Magnified view of alpha granules from the wall of the extended neck

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in describing copulatory positions; Hill & Coker (1930), in particular, have drawn attention to instances of initial clasping positions having been incorrectly interpreted by various observers as examples of copulatory positions, and point out that these positions are frequently not final, copulatory holds as transfer of the spermatophore from such positions is often seemingly impossible.

The terminology employed here to describe the structural components of the male fifth limbs is based upon that of Vervoort (1957, 1963), with modifications and additions where considered necessary.

MATERIALS AND METHODS

The specimens used in this study were selected from net haul material collected during the standard sampling programme in Loch Etive. Details of the sampling programme have been given in Chapter 1.

At the laboratory a number of specimens of stage IV and V copepodites as well as stage VI adults were removed for examination under a low power stereomicroscope. After examination the fifth limbs of male specimens were removed by hand using tungsten wire needles sharpened in molten sodium nitrite. The removed limbs were then mounted in a lignin pink and polyvinyl lactophenol mixture, which allowed the limbs to be mounted directly from an aqueous medium. Line drawings were made from permanent mounts using a Gillet and Sibert projection microscope.

Detailed examination of the fifth limbs of adult males was made using a scanning electron microscope (SEM). Information on the techniques used in preparing specimens for examination under the SEM has already been provided in Chapter 2.

CHAPTER 4

COPULATION AND SPERMATOPHORE TRANSFERINTRODUCTION

Copulation is the general rule in reproduction in crustaceans, the male usually having certain appendages modified for clasping the female (Barnes, 1968). In copepods, clasping of the female by the male and the movement of the partners into a copulatory position has been well documented on a number of occasions. Among the more detailed records of copepod copulation are those of Wolf (1905), Johnson (1948), Gauld (1957) and Fleminger (1967) for calanoids, Hill & Coker (1930) for cyclopoids, and Fahrenbach (1961) for a harpacticoid. These descriptions exhibit examples of the main phases of mating which Lee (1972) has described as consisting of "first the approach of the male to the female with a certain amount of ritual followed by the first contact and, secondly, the movement to the copulatory position with the subsequent transfer of the spermatophore to the female, often with another specially modified appendage".

The fifth pair of swimming limbs in males of the genus Euchaeta are highly modified appendages which play a primary part in transferring and attaching the spermatophore to the adult female's genital segment. A number of workers (Vervoort, 1957, 1963; Tanaka, 1958; Fontaine, 1967) have described in detail the adult male fifth limbs of various species of Euchaeta, however, the sparse descriptions of the adult male fifth limbs of E. norvegica that are available (Sars, 1903; With, 1915; Brodskii, 1950) provide an abbreviated and insubstantial account of the structure of the fifth limbs for this species. Wilson (1932) uses an illustration by Rathbun which is incorrect in a number of details, the most important being in their segmentation. Boeck (1872), in his original description

of the copepod, fails to mention the fifth limbs. With (1915) briefly describes and illustrates the stage IV male and stage V male fifth limbs, and Nicholls (1934) acknowledges that they are only present in the male and first appear on moulting to the stage IV copepodite.

The male fifth limbs may be regarded, with reference to functional morphology, as being part of the male's reproductive apparatus. As such, their accepted taxonomic application as the fundamental character used in identifying males of the species in the genus Euchaeta has an important biological significance in being based upon features which probably operate to ensure reproductive isolation of the species.

There have been no published accounts of copulation having been observed in E. norvegica, nor was copulation seen during the present study. Lacking direct observations, mating ethology, morphology and morphometry in this species can be related only by inference. However, the descriptions involving clasping and copulation in a large variety of other copepods, when analysed in detail, shed some light on mating behaviour and orientation of copepod partners in general. These considerations, when assessed together with functional aspects of the anatomy and morphology of male and female E. norvegica, have been used here to reasonably postulate the broader aspects of mating behaviour as well as to deduce and discuss the likely positions of the mating partners.

Clasping is defined as any gripping or grasping carried out by the partners, especially by the male, during the mating process. The term 'copulation' has been restricted to the eventual clasping and other manoeuvres performed, including transfer of the spermatophore, whilst the mating partners are actually in the inseminatory position. The distinction has been made in order to attempt to reduce instances of possible ambiguity

in describing copulatory positions; Hill & Coker (1930), in particular, have drawn attention to instances of initial clasping positions having been incorrectly interpreted by various observers as examples of copulatory positions, and point out that these positions are frequently not final, copulatory holds as transfer of the spermatophore from such positions is often seemingly impossible.

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Detailed examination of the fifth limbs of adult males was made using a scanning electron microscope (SEM). Information on the techniques used in preparing specimens for examination under the SEM has already been provided in Chapter 2.

THE MALE FIFTH LIMBSDevelopment and Structure

The male fifth limbs first become apparent in the stage IV male copepodite (Fig. 8). The limb is biramous and is approximately 390 μ m in length. Left and right rami consist of a 1st and 2nd basipodite, and a single endopodite and exopodite attached to the second basipodite. The left and right sides of the limb are equal in length, although the right endopodite is slightly larger than the left one (1.3:1.0).

On moulting to the stage V copepodite, the fifth limbs increase in size to approximately 980 μ m in length, but still consist of the same number of segments as the previous stage copepodite (Fig. 9). The moult to stage V has, however, increased the exopodites greatly in length with respect to the total length of the limb. The right endopodite has also enlarged greatly being now twice as long as the left one, whilst the right exopodite is now 20% longer than the left one.

The fifth limbs attain the adult condition on moulting to the stage VI copepodite (Fig. 10). The limbs have increased again in size from the stage V condition, the left and right rami now being approximately 2.80 and 3.27mm long respectively when fully extended, and have the final number of developed segments.

The adult left leg has two basipodites; the first being short, with the second over 3.5 times as long as the first. The second basipodite has a raised lateral denticle two-thirds of the way along its inner margin. The left endopodite arises from the distal end of the second basipodite, and is indistinctly two-segmented with its apex curved internally. The left first exopodite is 0.67 times as long as the

second basipodite segment, and has a flange along part of its outer surface, with a small semi-rounded peg at its proximal end and a small delicate bristle at the distal end of the flange. The second exopodal segment of this leg has its apex terminating in a partly toothed, internally curved dagger-shaped spine or lamella. A cylindrical, digitiform process arises from the second exopodal segment. The third exopodal segment is slender and slightly longer (1.15:1.0) than the digitiform process just mentioned. At the base of the third exopodal segment is a small haired tubercle or pad. The structure of the distal region of the left limb will be described in detail later in this chapter.

The right leg also consists of two basipodite segments. The first segment is of a similar size to its counterpart on the left leg, whereas the second segment is only half the length of its adjacent counterpart. The right endopodite is styliform and is slightly longer than the first segment of the exopodite. The proximal 44% of this segment is wider than the distal part, forming a rounded carina at their junction. The first exopodite is devoid of any distinct diagnostic characters. The right leg terminates at the second exopodite which is slightly longer than the first exopodal segment. The second exopodite is slightly curved internally and ends in a thickened and rounded apex.

Handling of the Spermatophore

The adult fifth limbs have lost their locomotory capacity, and have become totally adapted to a reproductive function. During normal relaxed swimming movements the fifth limbs are generally carried pointing towards the head of the copepod, whilst forward movement of the copepod is brought about by a rotatory action of the second antennae. Sometimes during swimming

the fifth limbs will be seen to take up a position pointing backwards parallel to the urosome; however, this is mostly taken up after a quick escape flexure has been made, by the first to fourth pairs of swimming limbs. Their long and slender shape, together with the absence of setae and other similar structures for aiding swimming movements, emphasise the loss of locomotory function. The male fifth limbs have become ideally modified for copulation, and the transference of the male's spermatozoa in the form of the spermatophore.

The spermatophore is extruded through the male pore on the left side of the first urosome segment, flask end first and neck last. Males with spermatophores gripped in their fifth limbs can be common, especially during February and March. Inspection of these spermatophores found gripped by males reveals that they are always held in the same position in the terminal processes of the left limb, and at the same point on the spermatophore neck. The left fifth leg grips the spermatophore, as it is parting company with the opening of the ductus ejaculatorius, before it is free in the surrounding water. A proposal, from examination of the structure and arrangements of the joints of the stage VI male left fifth limb, has been put forward as to the course of events at extrusion of the spermatophore.

The concept was promoted after observing the final position that the left leg assumed on death due to sudden immersion in 4% buffered formaldehyde in seawater. It was noted that the left fifth limb, on death, was often contracted to its fullest extent at the joints, and that in this position the terminal processes assume a position touching or close to the external opening of the ductus ejaculatorius. The use of buffered formaldehyde in seawater helps to nullify the effects upon the cuticle and tissues caused by changes in body osmotic pressure and pH.

In some crustacean appendages, such as *phyllopodia*, the cuticle is everywhere flexible. In most cases, including *Euchaeta*, the appendage consists of more or less rigid exoskeletal segments, flexibly connected at the joints by the cuticular arthrodistal membrane. Mechanically the appendage comprises a jointed system of hollow levers moved by internal muscles. The direction and freedom of movement at each joint is determined by the extent and physical properties of the arthrodistal membrane, and by the shapes of the opposing skeletal parts (Lochhead, 1961). In *E. norvegica* simple hinge joints, often with two pairs of bearing surfaces, occur frequently in the fifth limbs, but in some cases these joints also permit a slight amount of rotation. More complex arrangements, analogous to the vertebrate ball and socket joint are absent but some degree of freedom of movement for the fifth limbs is provided by a number of the hinge joints having their axes orientated at different angles.

It is known that crustacean joints without a ball and socket type of arrangement can only perform limited movements within a set path. The left fifth limb of the adult male *E. norvegica* is bound by the same considerations; it too is limited to a certain number of relatively fixed movements and positions. Due to these limited patterns of movements, the articulation of the terminal processes is such that when the exopodite of the left leg is brought close to the male genital segment there is only one position it can take up in relation to that segment. Every left fifth limb examined with a spermatophore grasped in its pincer-like terminal processes has the spermatophore orientated in the same position in space with regard to the genital segment. In each instance the spermatophore neck has been orientated towards the anterior of the animal, and the flask end towards the posterior of the animal. It is most likely that the left leg is bent up to grasp the neck of the sperm-

atophore during the actual process of extrusion, thus accounting for the spermatophore being gripped in the same position in the male fifth limbs.

The terminal portion of the left leg, as already stated, ends in a pincer-like arrangement (Plate 15) which consists of three processes and a pad or tubercle. The first process is the third exopodal segment (Fig. 11) which is about 260 μ m long. It has two closely associated groups of hairs at its tip which grow at right angles to the longitudinal axis of the process (Plate 16). Dissection of the third exopodal segment from the rest of the "pincer" reveals that the inner face is strongly concave, especially towards the base. The interior of this scooped area is covered with fine hair (Plate 17).

Close to the base of the first process arises a small chitinous tubercle, (approximately 120 μ m long) which is covered with numerous short, strong bristles. The area of this process facing the inner surface of the third exopodal segment has a dense coating of bristles, whereas the outer surface of the tubercle is devoid of bristles and is covered by smooth cuticle (Plate 17). The third exopodite and its associated tubercle are freely moveable and are attached to a powerful adductor muscle which passes backwards through the second exopodite.

The second process is the digitiform process which is approximately 225 μ m long, and articulates at its base with the terminal portion of the second exopodite. The surface of this thumb-like structure is covered with numerous ridges which run around the process at right angles to its longitudinal axis. The external pattern of the end of the process closely resembles a fingerprint (Plate 18).

The third process is a toothed lamella which ends in a curved and strongly developed cultriform spine. The teeth of the lamella run from the internal margin of the second exopodal segment and terminate at some distance under the apex of the lamella. The orientation of the teeth is such that they generally point towards the apex of the process. In the Loch Etive population the number of teeth on the internal margin of the lamella is variable with 9, 10, 11 and 12 toothed forms being found. The 10 toothed form is however the most common. The bottom tooth of the row is set apart from the others, some distance back along the second basipodite. A single, sharp tooth occurs on the external surface of the lamella at a point opposite the most distal tooth of the inner margin, although occasionally one or even two small, additional teeth can be discerned. The internal multiple-toothed ridge and the external ridge form the boundary to a concave area between them.

The third exopodal segment and its adjacent pad-like tubercle play an important role in gripping the spermatophore after it is extruded, and during its subsequent attachment to the genital segment of the adult female copepod. Specimens of stage VI males with spermatophores gripped in their left fifth limbs were used to describe the position of the segments of the fifth limbs during ejaculation, as well as the manner in which the left limb holds the spermatophore.

In order to grip the spermatophore as it is extruded from the external opening of the ductus ejaculatorius the left limb has to assume a position similar to that shown in Fig. 10. This involves the long axes of both fifth limbs lying back essentially parallel to the male urosome. The left second basipodite is bent slightly upwards and backwards at an angle which may, to some extent, pass across the posterior surface

of the right limb. In this position the left first exopodite, with its adductor muscles fully contracted, will be bent at about 70° to the long axis of the left second basipodite and will pass ventrally to the urosome, in a slightly anterior direction. The left second exopodite, with its adductor muscles fully contracted, is flexed at right angles to the first exopodite and thus faces upwards and forwards, coming to rest just behind the male genital slit on the left ventro-lateral side of the first urosome segment. The right fifth limb, however, probably plays no part in gripping the extruding spermatophore.

The spermatophore is grasped by the terminal portion of the left leg. The swelling at the end of the freshly extruded spermatophore neck is held between the third exopodal segment and the pad so that the latter keeps the swelling in close contact with the concave face of the former process. The swelling thus fits into the interior face of the third exopodite; the hairs on the inner faces of both factors make intimate contact with the surface of the swelling. Contact of the third exopodite and tubercle with the spermatophore is further aided by the surface of the swelling at the end of the short spermatophore neck being covered with an adhesive secretion. This arrangement ensures that the spermatophore is firmly held by the left fifth limb and is not lost before it has been attached to the adult female's genital segment.

MATING

Location of the female

The manner of approach of the male copepod to the female copepod, prior to copulation, has been observed on relatively few occasions, and the mechanisms involved in effecting contact of the partners has similarly been the subject of few observations and little experimental work.

Holmes (1909), in a limited study of the question of sex recognition in cyclopoids of the genus Cyclops found no evidence that an odour or scent emitted by the female played any part in initiating a mating reaction and concluded that the association of the sexes in mating was entirely due to chance collisions. However, Holmes thought that "olfactory" stimuli may play an important role in causing males to remain with females longer than they otherwise would and that they may render males more prone to seize females than other males. This initially non-selective impetus in Cyclops has been corroborated by the observations of Hill & Coker (1930) who found that a male Cyclops swimming in a culture dish tends to seize any individual of the same species with which it may collide, regardless of sex. This readiness to collide and mate has not been seen in E. norvegica and lengthy observations under laboratory conditions reveal that remarkably few collisions occur, either at random or otherwise, between individuals of opposite or similar sex. However, the mating drive in male copepods can be intense as witnessed in Pseudodiaptomus coronatus when males will occasionally attempt to copulate with other males and even females carrying egg sacs (Jacobs, 1961).

Parker (1902) working on Labidocera aestiva performed a number of simple experiments which support the idea that some form of chemical substance leads the male to the female. The fact that the 1st antennae of male copepods, particularly those of E. norvegica, possess characteristically a greater number of sense organs leads one to believe that these organs have a sexual function, although it remains to be proven that these supernumerary sense organs of males respond to chemical rather than to tactile stimuli.

Jacobs (1961) observed in Pseudodiaptomus coronatus that the males were more likely to clasp females "when they chanced to become situated some 5mm obliquely behind a female" and formed the impression that the stimulus to start the clasping attack might be triggered by the female feeding current. In addition, sudden changes in the swimming direction of males regularly occurred but similar behaviour was never observed in females. Jacobs postulated that this type of activity was a searching movement which may serve to increase the chance of finding females.

Triggering of the copulatory attacks in such circumstances as described for P. coronatus could either be as a result of changes in mechanical force brought about by fluid movement of feeding and swimming currents, or as a result of these currents acting to disperse chemical messenger substances such as pheromones. The widespread sensitivity among aquatic crustaceans to external changes in mechanical force as a result of contact with fluid movement has been well documented by Cohen & Dijkgraaf (1961), and the perception of high amplitude, strongly damped pressure waves, such as those caused by tapping against an aquarium wall, is evident in E. norvegica, although man-made vibrational stimuli of this kind, in general, only evokes flight reactions or reflex-like jumps.

The observations outlined here imply that males and females come into close proximity as a result of various behavioural interactions, called "mating behaviours". Such mating behaviours, of necessity, involve co-ordination of seasonal development so that both sexes are ripe and active at the same appropriate season. It is also of primary importance that the sexually mature animal must be capable not only of recognizing the species and sex, but also the exact status of sexual readiness of a prospective mate. In the light of such considerations it seems reasonable to postulate that precopulatory location and interrogation

of a prospective mating partner is to a great extent controlled by a pheromone system such as that described for Portunus sp by Ryan (1966). Such behaviour would serve a clear cut, useful function in reproduction in that it enables males to inseminate those females which are physiologically ready for fertilization, and therefore can be readily understood in terms of adaptive behaviour. Successful reproduction requires as a minimum the co-ordination of male and female activity with respect of insemination, and natural selection against interactions that waste gametes can be expected to act upon the ethology of mating as well as on the actual copulatory mechanism.

Evidence provided, in Chapter 5, from statistical analysis of the incidence and frequency of spermatophores placed on adult female E norvegica strongly indicates that mating is non-random in this species.

Clasping

Clasping of the female by the male and the movement of the partners into the copulatory position has been relatively well documented in copepods. The marine calanoid copepod Labidocera jollae has been briefly observed during copulation on two occasions (Fleminger, 1967). The two mating individuals were at first side by side but then quickly changed their positions "pivoting about the genital segment like the hands of a clock" so that the prosomes pointed in opposite directions and the urosomes crossed at about the level of the genital segments. The male was seen to hold the female around the urosome with the chela of its left fifth swimming limb.

Gauld (1957) made a number of incidental observations on the manner of pairing in the estuarine-coastal planktonic copepods Centropages hamatus, Temora longicornis, Eurytemora velox and Acartia clausi.

The first event in the pairing which was observed was the seizure of the female by the male with the latter sex holding the female copepod around the terminal segment of the urosome or the caudal rami by means of the right geniculate first antenna. After this had been accomplished the male attempted to exchange this grip for a second in which the female was gripped either immediately in front of, or immediately behind, the genital segment by using the fifth limb or limbs. In most pairs the animals were lying "head to tail" with their ventral sides opposed.

Wolf (1905) provides one of the most detailed accounts of copulation in a calanoid copepod in his description of Diaptomus gracilis. In this species the male's right first antenna is geniculate and is used to grip the furca of the female. In this first clasping stage the spermatophore emerges from the male's body and ejaculation of the spermatophore continues even if copulation is interrupted. The spermatophore comes out of the male's genital opening with the rounded flask end first, and is grasped by the male's left fifth swimming limb. In the next stage the male twists his urosome around that of the female and clasps her urosome with his right fifth limb which is developed in the form of a prehensile chela. The grip by the male's right 1st antenna on the female's furca weakens as soon as the embrace of the right fifth limb has been established and the male assumes a position dorsal to the female, facing posteriorly with respect to her long axis. The left fifth limb has been seen to make movements of an exploratory nature on the female genital segment before attaching the spermatophore to the genital pore. This exploratory activity and movement of the fifth limb on the genital segment is likely to be of importance in locating the female genital pore,

but in addition it may also possibly be a sign of action designed to sexually excite and arouse the female. Similar, possibly stimulatory activity has been observed in Cyclops americanus and C. bicuspidatus where the swimming feet of the clasping male occasionally brush the genital segment of the female, sometimes rather violently (Hill & Coker, 1930).

Lee (1972) in a study of the structure and function of the spermatophore and its coupling device in four species of Centropagidae described a number of structures which he interpreted as locking devices of the "lock and key" type. He suggested that these structures, together with a strong correlation between chela capacity and the reach of the left exopodite of the fifth limb should make it possible to describe a method by which copulation was achieved in the species examined, and deduced the relative positions of the male and female during mating. In Centropages the chela is used to grip the female urosome so that transfer of the spermatophore and its coupling device is carried out by the left fifth limb. In the case of Centropages typicus, C. chierciae and C. brachiatus, the form and location of the couplers on the female urosome indicates that, in order to release the female following transfer and attachment, the male must grip the female urosome at the genital segment. In the case of C. typicus and C. brachiatus, if the male was dorsal to the female facing posteriorly with respect to the long axis of the female, the male genital opening and the point of attachment of the spermatophore stalk would be adjacent.

The male copepod often has to overcome the initial resistance of the female and evidence has been provided of violent movements and attempts to escape in females of Diaptomus gracilis (Wolf, 1905), various species of Cyclops (Hill & Coker, 1930), Centropages hamatus, Temora longicornis,

Eurytemora velox and Acartia clausi (Gauld, 1957), as well as for the algal dwelling harpacticoid Diarthrodes cystoecus (Fahrenbach, 1961). During this stage of the procedure the male copepod allows itself to be passively carried around by the female, sometimes for hours in the case of Cyclops (Hill & Coker, 1930) or even several days in the case of Eurytemora (Gauld, 1957). The urge to clasp probably reaches its zenith in Pseudodiaptomus euryhalinus, a species in which the male has a tendency to continue grasping the female even though spermatophores have been transferred and full egg sacs developed (Johnson, 1948).

Most of the time consumed in the copepod mating process is taken by the male in overcoming the resistance of the female, in effecting his final hold, and perhaps, in getting himself in readiness. Once the final inseminatory position has been adopted the actual process of attachment of the spermatophore usually takes only a matter of seconds (Hill & Coker, 1930; Fahrenbach, 1961).

An analysis of these published studies reveals a number of important features which appear to be common, if not characteristic, in mating in the class Copepoda. These can be summarised as follows:

1. The active role of the male during location and copulation, and
2. The adoption of an initial seizing position followed, after a period of time, by
3. The final inseminatory and full copulatory position.

In those calanoids in which the males have modified fifth swimming limbs, the right fifth limb is used as a clasping appendage for gripping the female urosome in order to maintain the position of the male static

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relative to the female, whilst the left fifth limb is used to transfer and attach the spermatophore. In members of the calanoid sub-order Heterarthandria (eg Centropages, Temora, Eurytemora, Acartia, Labidocera and Diaptomus the male 1st antenna is geniculate and is used in a prehensile manner during the initial seizure of the female. However, in the sub-order Amphiscandria (eg Euchaeta) the male 1st antenna is unmodified and subsequently is unlikely to be used in a prehensile manner during initial clasping activity. In the final, copulatory position the male calanoid faces posteriorly with respect to the long axis of the female and the ventral surface of the former may be opposed either to the dorsal or to the ventral surface of the female.

Deduced Inseminatory Position in E. norvegica

Mating requires the male Euchaeta to firstly eject, position and attach a spermatophore while limited in movement because it must hold the female with clasping appendages and maintain a position from which accurate spermatophore placement is feasible. Males and females of E. norvegica are able to perceive variations in light intensity but are essentially non-visual animals in that they do not possess advanced light sensitive organs which discern shape or form. It therefore appears reasonable to believe that the act of orientation into a copulatory position depends to a great extent on tactile and mechanical responses. Insemination of the female is dependent on the tip of the spermatophore neck being brought to bear on the female's genital aperture situated in the middle of the genital field on the ventral side of the genital prominence. This requirement for precise attachment of the spermatophore indicates a need for accurate transfer and location, and this, in turn, implies that structure and dimension play a vital role in their accomplishment.

Males of E. norvegica, as mentioned previously, do not possess a prehensile 1st antenna as is found in the Heterarthandria. It therefore seems unlikely that the 1st antennae carry out a major clasping function in this species, and whatever clasping that needs to be performed is likely to be made by the modified fifth limbs of the male. The distal region of the left fifth limb, as already shown, is used to grip the spermatophore and it is probably also the limb which plays the important role in locating the position of the female genital aperture and in attaching the spermatophore. Wolf (1905) mentions that the left fifth limbs of Diaptomus gracilis, which enclosed the spermatophore neck, moves to and fro on the female genital segment, palpating with its long searching bristle prior to attaching the spermatophore to the genital orifice. The pronounced tuft of hairs at the end of the 3rd exopodal segment of E. norvegica, in a position just above the tip of the enclosed adhesive spermatophore neck, could similarly play an important part in any tactile response involved in positioning the spermatophore. The remarkable structure of the thumb-like process arising from the 2nd exopodite also suggests a possible tactile function. The right fifth limb of the male E. norvegica, although it does not closely resemble in structure the highly specialised chelate right limbs of some copepods (eg Labidocera and Centropages), is likely to play an important part in helping to hold the female whilst the left fifth limb is attaching the spermatophore.

In order to attach a spermatophore correctly the male Euchaeta must take up a position in close proximity to the female's urosome, otherwise the "pincers" of the left fifth limb will not be within reach of the genital prominence. The most satisfactory way to do this is for the male to grasp the female with his fifth limbs at or around the area of her genital segment, with the long axis of his body orientated parallel to

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the long axis of the female urosome. This would allow the male to maintain his body in a static position relative to the female genital aperture; a condition which is likely to be advantageous should the female make any swimming or retaliatory movements. Furthermore, the arrangement of the male fifth limbs is such that they are able to grip the female's urosome best when the latter passes between the left and right rami. An alternative copulatory orientation in which the male's body traverses the female's urosome at or about 90° renders insemination virtually impossible, as flexing of the left fifth limb with its spermatophore would not bring the "pincers" into contact with the female genital aperture, and either of the male's fifth limbs would be unable to encircle the female urosome without a high chance of self-imposed breakage.

Copulation with the male's ventral surface facing the ventral surface of the female urosome is unlikely because clasping of the female would be made difficult by the close proximity of the female's swimming legs, which even if conveniently directed towards the head of the female could still be flexed and thus effectively dislodge the male. Secondly, if male and female were facing in the same direction the swimming legs of both partners, even if slung forward, would probably increase the distance between their bodies and so add to the problems of clasping.

Another possible choice is for the partners to face in opposite directions with their ventral surfaces opposed. In such a position clasping would be dependent upon the swimming legs of each partner being directed forwards towards their own respective heads, thereby not obstructing access of the male to the genital prominence. However, even with clasping of the female urosome now being possible in this attitude, placement of the spermatophore is essentially ruled out as the left fifth limb

of the male is adapted in its movement patterns to encircle the female urosome and is not able to reach all the way round the dorsal surface of the female urosome and then down and backwards to the genital aperture.

A spermatophore could theoretically be accurately attached by a male which has its ventral surface facing the female's ventral surface and its anterior directed towards the caudal furcae of the female, provided that the male fully extended its left fifth limb straight across to the female's genital aperture; however, clasping of the female urosome would be impossible, and any clasping from this position would probably have to rely solely on the male's right fifth limb gripping the female's fourth pair of swimming legs. A possible disadvantage in gripping the female's fourth swimming legs is that they are moveable in relation to the female urosome and any reliance on them for clasping is unlikely to accentuate problems of accurate location of the female genital aperture. Grasping of the female's fourth swimming legs by the male, although possibly disadvantageous, of itself is unlikely to act as a major barrier in insemination of the female. However, when assessed in conjunction with the limited movement available to the male's left fifth limb it is unlikely that this position is commonly adopted in E. norvegica but it is pertinent to record that Hill & Coker (1930) observed in a number of freshwater cyclopoids that regardless of which female structure was initially grasped, the male would later move its position until it had secured itself to the female's fourth pair of swimming legs.

The position most likely to be adopted by the copulatory partners to ensure insemination is one in which the ventral surface of the male's body is apposed to the dorsal aspect of the female urosome, whilst his

head is directed posteriorly with respect to the long axis of the female's urosome (Fig. 12). In such a position the male's right and left fifth limbs would be on opposing sides of the female's urosome, and by contraction of their main adductor muscles would exert a scissor-like grip on the female's urosome in the region of the genital segment. A firm grip on the female would be aided by the effect of contraction of the adductor muscles in the right 1st exopodite, which being attached at one end of the proximal part of the right 2nd exopodite would raise the latter segment upwards under the female urosome. This would enable the left fifth limb to be free to search for the genital aperture with its terminal segments. Once the spermatophore is against the genital aperture, further contraction of the adductor muscles of the male fifth limb would have the effect of pushing the distally directed teeth on the terminal lamella of the "pincer" against the female's genital field whilst adhesion of the spermatophore takes place. The male right fifth limb is able to grip the female urosome either anteriorly or posteriorly to the genital prominence, no matter whether the male is facing backwards or forwards with respect to the female. The major benefit of the right limb gripping the female urosome anterior to the genital aperture is that in this position the end of the female prosome and the genital prominence are likely to fulfil a requirement for suitably placed anterior and posterior "stops" limiting the extent of any slippage of the encircling limb.

Copulation with the male facing towards the anterior of the female whilst in the dorsal position is also possible, but the position is likely to be relatively unstable owing to the necessity of the male's prosome being raised upwards at an angle of nearly 20° in order to clear the large, rounded, dorsal surface of the female prosome. A hydro-

dynamic force acts on a body when it moves through a fluid (Tietjens, 1957) and similar forces will act on the attached male copepod to effect its stability during forward swimming movements by the female. In such circumstances a slight movement of the male's raised prosome off the direct course of motion could produce turning movements tending to increase the deflection still further. Any unstable movements of the male body, so caused, would have to be borne by the fifth limbs, with possible harmful effects.

Fig. 8. The fifth pair of swimming limbs of a male stage IV copepodite of Euchaeta norvegica, viewed from the posterior aspect.

B1: 1st basipodite; B2: 2nd basipodite; Ri:
internal ramus; Re: external ramus

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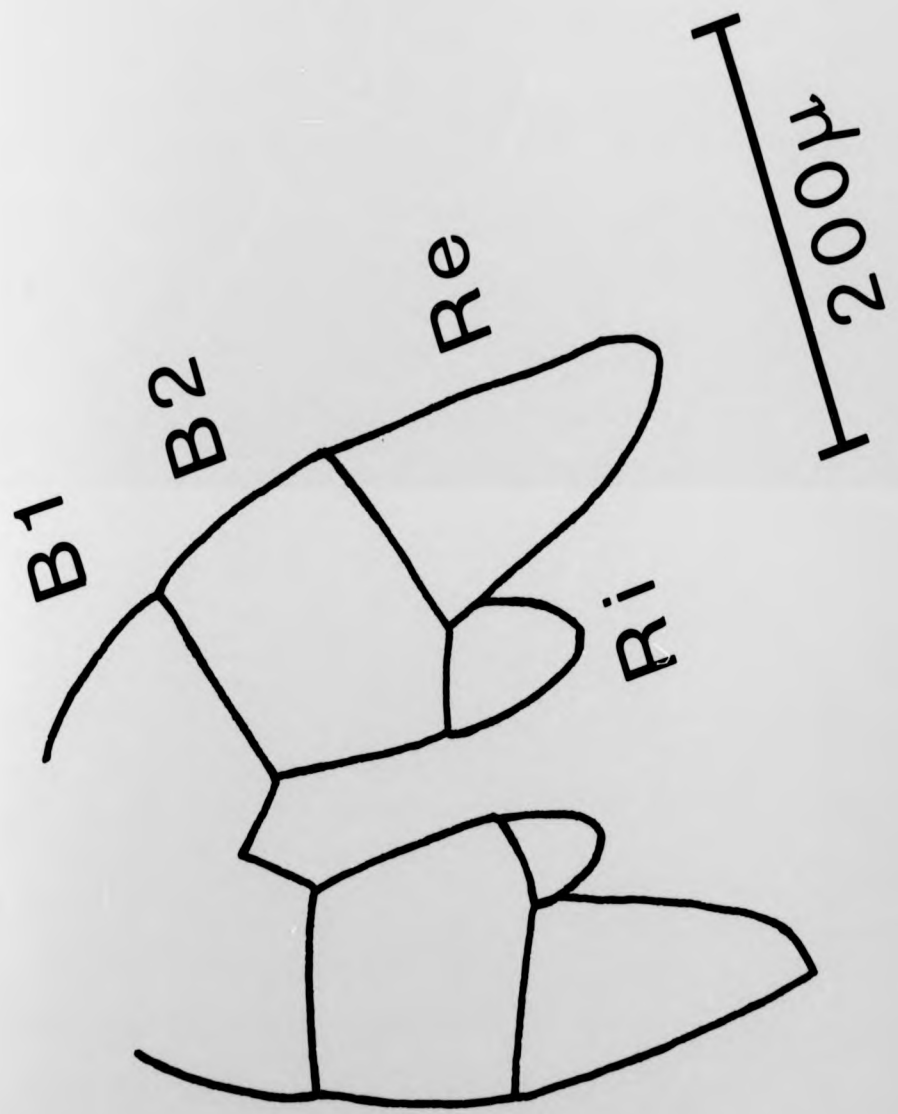


Fig. 9. The fifth pair of swimming limbs of a male stage V copepodite of Euchaeta norvegica, viewed from the posterior aspect.

B1: 1st basipodite; B2: 2nd basipodite; R1: internal ramus; Re: external ramus

Stage V

in the

internal

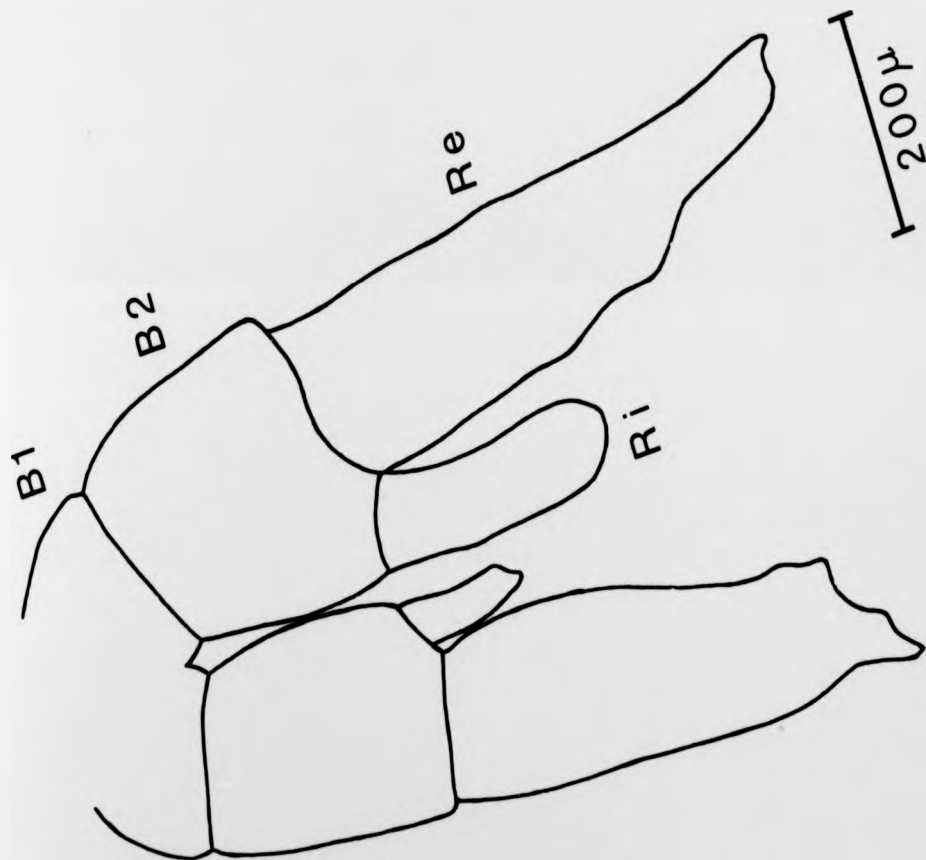
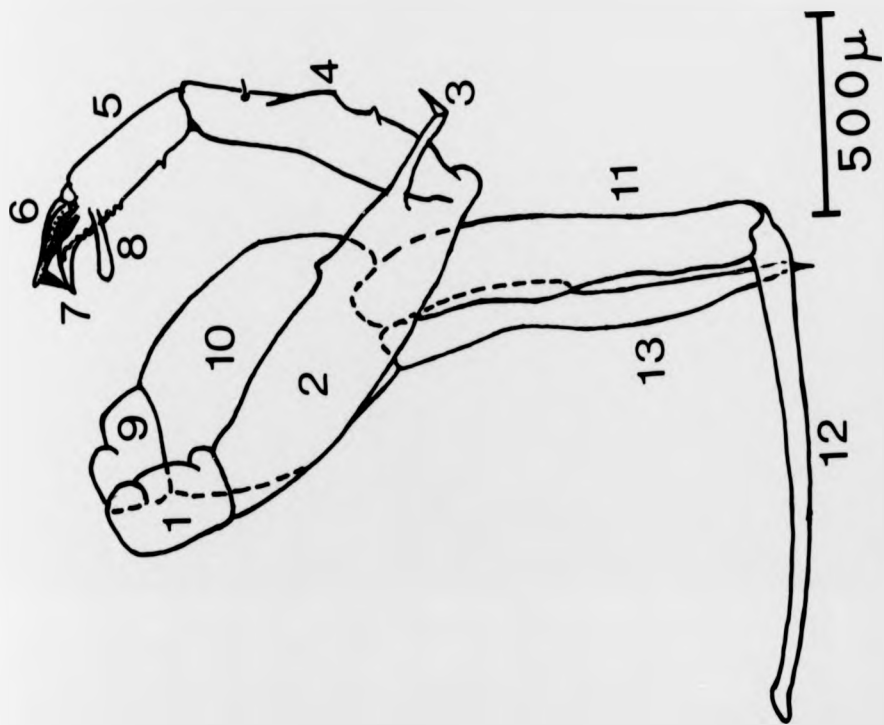


Fig. 10. The fifth pair of swimming limbs of an adult
(stage VI) male Euchaeta norvegica, viewed from
the posterior aspect.



1. Left 1st. Basipodite
2. Left 2nd. Basipodite
3. Left Endopodite
4. Left 1st. Exopodite
5. Left 2nd. Exopodite
6. Left 3rd. Exopodite
7. Lamella
8. Digitiform Process
9. Right 1st. Basipodite
10. Right 2nd. Basipodite
11. Right 1st. Exopodite
12. Right 2nd. Exopodite
13. Right Endopodite

Fig. 11. The third exopodal segment and associated tubercle
from the left fifth swimming limb of an adult male
Euchaeta norvegica.

tubercle
ult male

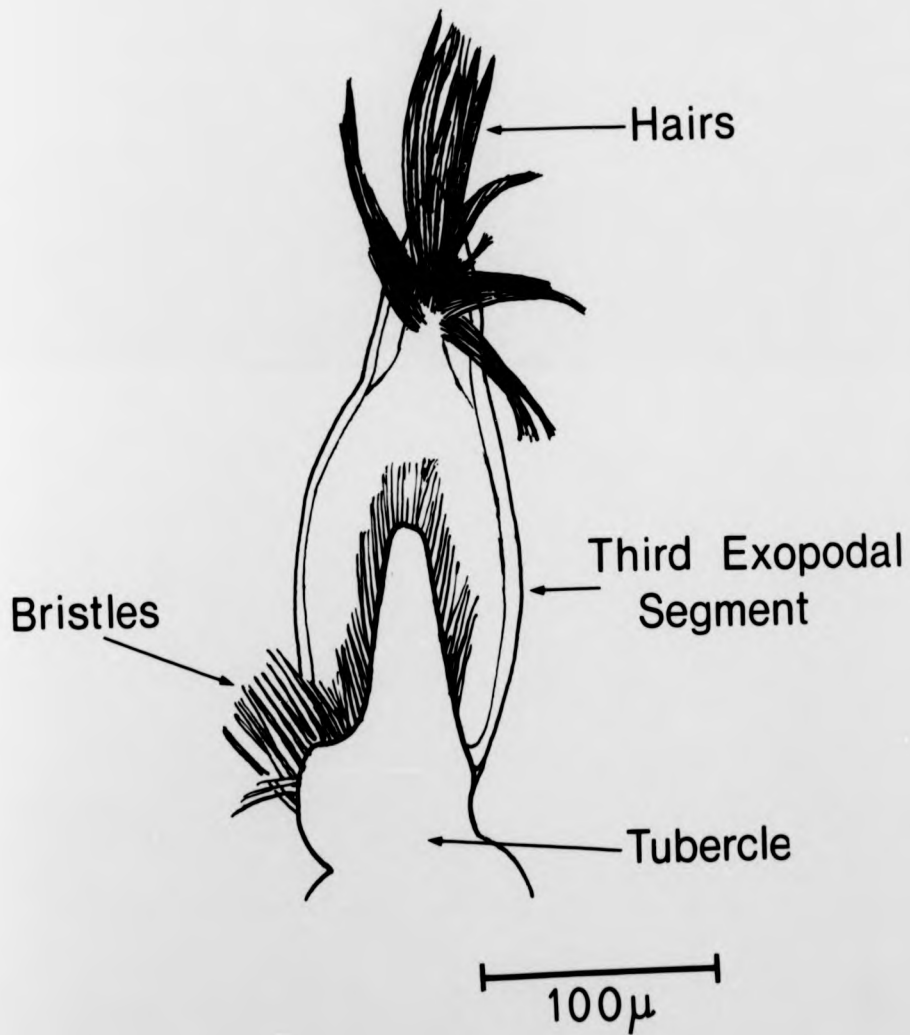


Fig. 12. A diagram showing the deduced relative positions
of the male (above, right) and female (below, left)
Euchaeta norvegica during spermatophore attachment

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ttachment

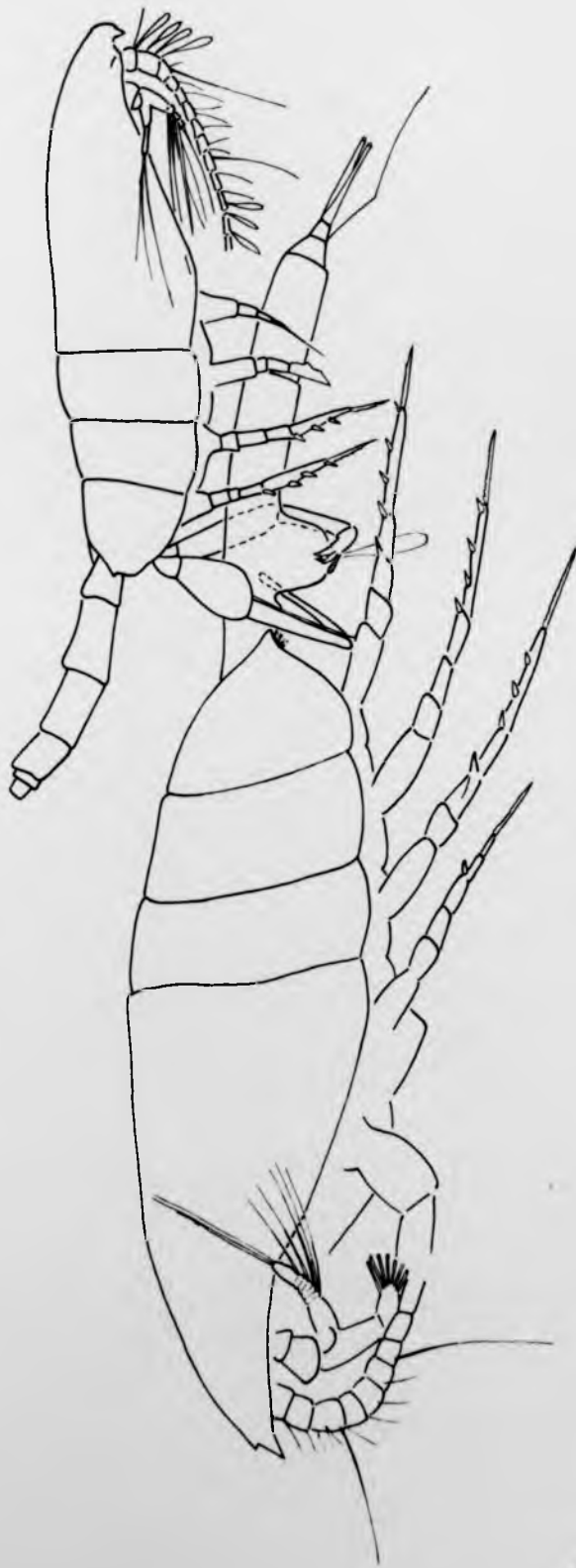


Plate 15. Scanning electron micrograph showing the arrangement of the terminal processes of the left fifth swimming limb of an adult male Euchaeta norvegica.

3 exp: 3rd exopodite; 2 exp: 2nd exopodite;

1 exp: 1st exopodite; l: lamella; dp: digitiform process; ur: urosome

Plate 16. Scanning electron micrograph of the hairs at the tip of the third exopodal segment of the left fifth swimming limb of an adult male Euchaeta norvegica.

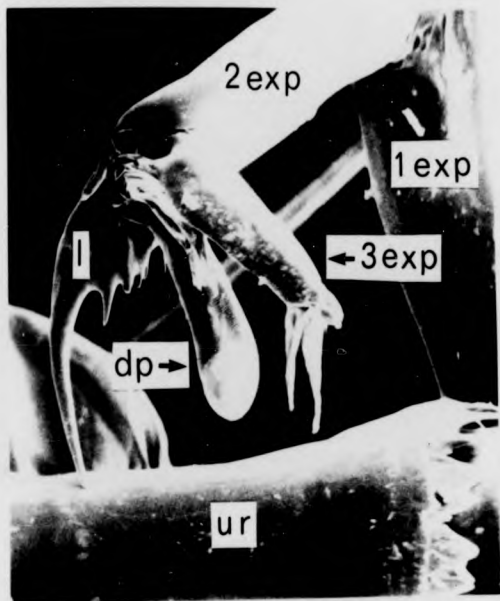
h: hairs

arrangement

fth swimming

te;

gitiform



200 μ m



50 μ m

at the

left fifth

orvegica.

Plate 17. Scanning electron micrograph of the end of the left fifth swimming limb of an adult male Euchaeta norvegica showing the fine hairs lining the inner surface of the third exopodite, the bristles of the associated tubercle, and the long digitiform process. A number of teeth of the lamella can be seen, three of which have been broken.

3 exp: 3rd exopodite; l: lamella; dp: digitiform process; t: tubercle

Plate 18. Scanning electron micrograph showing the "thumbprint" and well defined ridges at the distal end of the digitiform process of Euchaeta norvegica.

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20 μm

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CHAPTER 5

PATTERNS OF SPERMATOPHORE DISTRIBUTION AND PLACEMENTINTRODUCTION

The spermatophore in the Copepoda is an elongated flask-shaped object produced by the male copepod in which are contained the sperm and associated secretions. The male must attach the spermatophore to the female genital segment in such a position that the opening to the neck of the spermatophore, through which the sperm come out, is in contact with the opening of the female cavity. Placement of the spermatophore elsewhere, in such a manner that connection with the female genital cavity is not achieved, may not cause fertilization and will waste sperm.

In some families of calanoid copepod (for example Centropagidae and Pontellidae) the opening of the spermatophore neck is situated amidst a coupling device composed of one or more plates. Lee (1972) has shown that a strong relationship exists between the micromorphology of the female urosome and the coupling devices of the spermatophore in members of the Centropagidae which ensures that the spermatophore neck is brought to bear on its precise attachment point by the automatic location of the coupling device. It is thus not surprising that the presence of adult females with multiple spermatophore placements are rare in species which have couplers.

In the majority of calanoids, including Euchaeta norvegica, a specialised coupling device is absent and the unmodified spermatophore neck is attached to the female solely by a cement-like secretion extruded from the spermatophore itself. However it is clear from work on both calanoids with couplers and those without such devices (for example Fleming, 1967, in Labidocera jollae; Frost & Fleming, 1968, in Clausocalanus spp; Lee,

1972, in Centropages spp) that the site and mechanism of attachment is precise, species specific and, in the case of Labidocera jollae (Fleminger, 1967), racially specific.

Multiple placements of spermatophores have been described as occurring in various copepod species (Wolf, 1905; Rose, 1933; Marshall, Nicholls & Orr, 1934; Gibbons, 1936; Marshall & Orr, 1955; Fleminger, 1967) but most individuals carry a single, normally positioned spermatophore.

It was noticed during the course of the present study of the breeding biology of Euchaeta norvegica in Loch Etive, that examples of females carrying multiple spermatophore placements could be found regularly throughout the year. It was therefore decided that a special investigation of the frequency distribution of spermatophores as well as their positional placement on females would probably provide fundamental information about the breeding biology and ethology of this species, in the absence of observations on live material.

MATERIALS AND METHODS

The specimens used for this study were collected during the standard sampling programme (see Chapter 1).

The number of adult males encountered whilst counting 500 adult females was noted, and varied in proportion to their relative abundance in the population at the particular time of sampling. The proportion of males, p , present at any one time in the population was estimated from this by use of the unbiased estimator:

$$\hat{p} = \frac{m}{f + m - 1} \quad (1)$$

Where f , the number of females present is 500, and m , the number of males present varies from sample to sample. The estimated variance of \hat{p} is:

$$\text{var } \hat{p} = \frac{p(1-\hat{p})}{(f+m-2)} \quad (2)$$

see Kendall & Stuart (1961, p.593) for further details.

Each of the 500 adult females selected from each of the 21 plankton samples was carefully examined for attachment of spermatophores to the genital (1st urosome) segment. The number of spermatophores attached to each female as well as the position of attachment on the genital segment was recorded. In order to aid recording of the position of placement of the spermatophore the surface of the adult female genital segment was divided up into a system of grids, and the point of attachment noted on a diagram.

Fig. 13 shows the diagram of the genital segment of a typical adult female E. norvegica, viewed from left and right sides, which was used to record spermatophore placement positions. The boundaries of the grids were chosen arbitrarily for ease and speed of use rather than for similarity of surface texture, relief or area. Spermatophores were found to have been attached in 19 of the possible grids and these grids have been given the identificatory letters A to S, in order of decreasing frequency of spermatophore attachment.

The surface of the cuticle of all the grids, except A and B, are smooth and devoid of prominent features. Grid A encompasses the genital field, which is strongly folded and uneven around the genital pores, whilst grid B, on the left antero-ventral side of the segment is characterised by the well defined ventral ridge which is most highly developed in that grid.

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The grids which have been drawn to meet along the imaginary join-line of left and right sides of the genital segment in Fig. 13 should be visualised as curving round and assuming a 3-D aspect and that they meet in the centre of the dorsal or ventral regions of the segment. This holds true in all cases except for those of grids D and H which do not meet in the ventral mid-line along the whole of their border but are considered to form two halves of a circular band around the region of the A placement grid. However (E), (F), (R), and (S) represent positions along the mid join-lines where spermatophores were found to have been attached but could not be safely judged to have occurred in grids either to the left or to the right of the mid-line. The grids identified by stars are grids in which no spermatophores were found to have occurred. Spermatophores were found to be absent from the starred grids for the very good reason that these grids are overshadowed closely by the sweep of the metasome "wings", making it practically impossible for the male copepod to reach these areas, and thus any spermatophores that were successfully placed there are likely to be dislodged during any articulatory activity of the urosome with the metasome, such as happens close to this point during swimming or escape movements.

RESULTS

During the course of this study spermatophores were not found attached to any copepodite stages other than the adult female or to any region of the female's body except the genital segment.

The mean number of spermatophores per female

The mean number of spermatophores per female and its sample variance were calculated for each of the 21 samples collected. The mean number of spermatophores per female varied throughout the year and is graphed in Fig. 14

which also includes a graph of the changing proportion of males. It is clear that peaks of spermatophore attachment, and therefore probably also of fertilization, occurred during February and March, and from June through to early August. Troughs occurred in late October and mid-April. The proportion of males present was at its lowest during October and November and reached its highest in February and March, with the appearance of a secondary trough in mid-April to mid-June and a secondary peak in July.

Fig. 15 illustrates the strong positive correlation ($r = 0.83$, $p = < 0.001$) between the mean number of spermatophores per female and the proportion of adult males present. There is some evidence of a tailing-off of the relationship when high proportions of males are present in the adult population; perhaps as the result of a saturation effect. The mean number of spermatophores per female varied from a minimum of 0.316 ± 0.022 to a maximum to 0.998 ± 0.039 , a threefold change, whilst the proportion of males underwent an almost twelvefold change, from a minimum of 0.027 ± 0.007 to a maximum of 0.315 ± 0.017 .

Distribution of the number of spermatophores per female

Adult females were found carrying from 0-6 spermatophores attached to their genital segments. Fig. 16 shows the frequency distribution of adult females carrying different numbers of spermatophores based upon all information gained over the entire sampling period. Females without spermatophores accounted for 50.5% of all females examined. Individuals found carrying a single spermatophore accounted for 42.3% of all females but their spermatophores formed 71.6% of all attached spermatophores. Females found with double spermatophore placements (ie two attached spermatophores) made up 5.8% of all adult females and these double

placements accounted for 19.8% of all attached spermatophores. Females found carrying three spermatophores made up 1.3% of all adult females and triple spermatophore placements accounted for 6.5% of all attached spermatophores. Females found with four, five, and six spermatophores were rare and together accounted for less than 1.0% of all adult females examined and the spermatophores attached to these females made up less than 2.1% of all attached spermatophores. Thus it is only the single and double placements of spermatophores which appear with any degree of frequency.

The seasonal distribution of females found carrying different numbers of spermatophores was found to vary and are presented in Table 3. It is evident that significant proportions of the female population were found without attached spermatophores in every sample, even though mating continued throughout the year. It is also apparent that females with three or more spermatophores are mainly found at two peak times of the year, February-March and July-August.

Even though the proportion of multiple spermatophore placements can increase appreciably at certain times of the year the mean number of spermatophores per female did not exceed unity in any of the 21 samples examined. Fig. 17 shows a plot of the mean number of spermatophores per female per sample with the corresponding variances, and indicates that the two are strongly associated. In every case the sample variance is less than the mean and the coefficient of dispersion (C)

$$C = \frac{\text{Variance}}{\text{Mean}} \quad (3)$$

averages out at 0.74 for the 21 samples.

TABLE 3: Euchaeta norvegica. Seasonal variation in the number of adult females carrying different numbers of spermatophores per standard sample of 500 adult females examined.

Date	Number of Attached Spermatophores							Total Spermatophores
	0	1	2	3	4	5	6	
27. 9.71	244	232	21	2	1			284
13.10.71	273	222	5					232
25.10.71	342	154	4					162
1.11.71	318	176	6					188
12.11.71	299	189	11	1				214
30.11.71	276	215	8	1				234
14.12.71	275	199	24		2			255
4. 1.72	236	227	36	1				302
17. 1.72	216	217	50	17				368
6. 2.72	175	233	67	22	2	1		446
24. 2.72	137	264	72	22	1	3	1	499
7. 3.72	164	246	66	14	7	3		463
20.3. 72	175	254	52	18	1			416
17. 4.72	348	146	6					158
1. 5.72	316	157	25	2				213
16. 5.72	276	210	14					238
7. 6.72	222	246	27	4		1		317
17. 6.72	273	210	14	2	1			248
4. 7.72	224	231	32	10	3			337
1. 8.72	185	248	52	13	2			399
24. 8.72	310	162	21	6	1			226

The samples were examined in order to ascertain whether the distribution of spermatophores on females followed a clumped, random, or regular pattern. Counts were made of the number of females out of each sample of 500 females which had no spermatophore, one spermatophore, two spermatophores, three spermatophores and so on. The total number of spermatophores per sample were recorded and the average number of spermatophores per female per sample calculated. This was required to construct the expected proportion of females which had various numbers of spermatophores. The collected data were then tested for randomness by application of the Poisson distribution. This tells us what to expect if the attachment of spermatophores on females were isolated occurrences happening as a result of chance, randomly and independently of one another.

One characteristic of the Poisson distributed is that variance and mean are equal. Thus departure from a random distribution may be in one or other of two directions:

$$\text{Variance } (\sigma^2) < \text{Mean } (\mu) = \text{Underdispersion}$$

$$\text{Variance } (\sigma^2) > \text{Mean } (\mu) = \text{Overdispersion}$$

Underdispersion implies that organisms or structures are more evenly spaced than would be predicted from the Poisson model. Extreme overdispersion, on the other hand, will produce a clumping or clustering of organisms or structures. Further details of the theory behind the application of the Poisson distribution and the measurement of "aggregation" in ecological studies are provided by McArthur & Connell (1966), Andrewartha (1961), and Cassie (1971).

If the males of E. norvegica were mating with the females purely at random, ie if a male placed a spermatophore on the first female he came across irrespective of whether she was already carrying a spermatophore

or not, then one would expect a value of C close to 1 and the underlying distribution to be Poisson of the form:

$$P(X = x) = e^{-\lambda} \frac{\lambda^x}{x!}, \quad \lambda > 0, \quad x = 0, 1, 2, \dots \quad (4)$$

Where for a particular sample we estimate λ by the corresponding sample mean \bar{x} . In fact the Poisson distribution gives a poor fit to the data, as one might have expected, since $C < 1$ for every sample. It thus seems that once a female has a spermatophore the males tend to avoid attaching another spermatophore to her and instead search rather for a "virgin" female; as already mentioned, he is not always successful since multiple placement of spermatophores can occur. Fig. 18 shows the Poisson fit to the data for samples 7, 10, and 14 and they are clearly inadequate. Various mathematical models based upon negative binomial distributions have been developed to describe overdispersion (eg Bliss, 1953; Cassie, 1961) but they are neither applicable nor satisfactory in describing underdispersion. Ecological models describing underdispersion are not so familiar.

One model due to Greenwood & Yule (1920), see Appendix 5 for details, has two parameters α and β the first α corresponding to the λ of equation (4) above, the second β , for these data always less than α , corresponds to λ again but now referring only to those females who have spermatophores attached to them - effectively at any one instant of time the population of females is divided into "virgin" females and females with attached spermatophores with the probability of placement of a spermatophore for the two groups being different. A second alternative model is due to Consul & Jain (1973) and which again has two parameters ξ and η , their model is:

$$P(X = x) = \xi (\xi + x\eta)^{x-1} \exp\{-\xi - x\eta\} / x! \quad (5)$$

$$= 0 \quad \text{if} \quad \xi + x\eta \leq 0$$

where $\xi > 0$ and $x = 0, 1, 2, \dots$

and which reduces to the Poisson model if $\eta = 0$. In this model the probability of attachment of a further spermatophore on the female decreases (for these data) linearly with the number already on the female. Consul & Jain give simple moment estimators of ξ and η as:

$$\hat{\eta} = 1 - (\bar{x}/s^2)^{\frac{1}{2}} \quad \xi = \bar{x}(1 - \hat{\eta})$$

The disadvantages of this model for the present data is that $x > -\xi/\eta$ then $P(X = x) = 0$ whereas it is required that it should be small.

Table 4 shows the values of $\lambda, \alpha, \beta, \xi$ and $(\xi + \eta)$ estimated from the 21 samples. The last column of this table indicates the model that gives closest agreement to the data. The fits of the Greenwood & Yule and Consul & Jain models are typically good and are illustrated in Fig. 18. The least satisfactory fit was obtained from sample 10 and this is included in Fig. 18. The Greenwood & Yule model gives the closest fit in the majority of cases.

The positional placement of the spermatophore on the female

Spermatophores were found to have been placed in 19 of the grids on the female genital segment. The frequency distribution of spermatophores within the various grids based upon processing the information recorded for all spermatophores found on all females during the study are shown in Fig. 19. Spermatophores placed in the A grid of the genital segment are by far the most common (70.1% of all attached spermatophores), with the B grid being the second most common (14.6% of spermatophores) and the C position being the third most common (5.3% of spermatophores). Other placement positions together accounted for less than 10% of all spermatophore placements.

The data for spermatophore placement positions have been split up into information on females carrying one, two or three spermatophores.

TABLE 4: Estimates of the parameters of the alternative models
describing spermatophore distribution in Euchaeta
norvegica

Sample Number	Poisson λ	Greenwood and Yule α	Consul and Jain β	ξ	$(\xi + \eta)$	Best Fit
1	0.568	0.7066	0.2168	0.6962	0.4705	GY
2	0.464	0.6054	0.0395	0.6092	0.2963	GY
3	0.324	0.3521	0.1699	0.3800	0.2071	CJ
4	0.376	0.4529	0.0600	0.4529	0.2483	GY
5	0.428	0.5127	0.1234	0.5100	0.3183	GY
6	0.468	0.5918	0.0871	0.5909	0.3284	CJ
7	0.510	0.5969	0.2704	0.5797	0.4430	GY
8	0.604	0.7779	0.2077	0.7459	0.5109	CJ
9	0.736	0.8341	0.5288	0.8155	0.7075	GY
10	0.892	1.0269	0.6669	0.9944	0.8796	GY
11	0.998	1.1981	0.7184	1.1440	0.9977	GY
12	0.926	1.0291	0.7543	1.0025	0.9199	GY
13	0.832	1.0283	0.5051	0.9888	0.8003	GY
14	0.316	0.3628	0.0726	0.3621	0.2161	CJ
15	0.416	0.4384	0.3185	0.4415	0.3802	CJ
16	0.476	0.5961	0.1093	0.5937	0.3465	GY
17	0.634	0.7876	0.2888	0.7708	0.5550	GY
18	0.496	0.5930	0.1980	0.5865	0.4040	GY
19	0.674	0.7680	0.4563	0.7524	0.6360	GY
20	0.798	0.9742	0.4860	0.9407	0.7619	GY
21	0.452	0.4663	0.3946	0.4642	0.4373	GY

Females with more than three spermatophores were so rare that data gathered from these females were considered insufficiently numerous for significant analysis of results to be made. The results for placement positions of spermatophores on females with one, two and three placements are graphed in Fig. 20. It can be seen that the A placement position is the most frequent position used for single, double and treble spermatophore placements. However, it is evident that the grid selected for attachment of the spermatophore in adult females with a single placement was not always the A grid. There is a distinct trend for a greater percentage of spermatophores to go into other grids besides the A grid with the addition of second and third spermatophores to the female genital segments.

Table 5 shows the data from which Fig. 20 has been constructed and these data have been used to produce Table 6 which displays the probabilities of the first, second and third spermatophores being placed in each of the grids (A-S) of the genital segment.

The method of determining the probability of the first spermatophore being placed in a stated grid of the genital segment is straightforward in that it is a standard conversion of percentage occurrence to probability. However it is not possible to determine with certainty which of the spermatophores on a female carrying two or three spermatophores were placed in what order. Nevertheless it has been considered reasonable to provide an estimate of the likelihood of any trends in probability and order of placement of subsequently attached spermatophores in the various grids by using information obtained from females with single spermatophores. For example, in the case of A placements we know from data processed from females with single spermatophores that 78.5%

TABLE 5: Euchaeta norvegica. Frequency of occurrence of attached spermatophores in each of the regions of the genital segment, on adult females carrying one, two and three spermatophores.

Genital Segment grid	One Spermatophore		Two Spermatophores		Three Spermatophores	
	Number in grid	Percentage in grid	Number in grid	Percentage in grid	Number in grid	Percentage in grid
A	3484	78.5	622	50.7	180	44.4
B	465	10.5	300	24.5	113	27.9
C	165	3.7	101	8.2	45	11.1
D	76	1.7	48	3.9	20	4.9
E	56	1.3	37	3.0	7	1.7
F	53	1.2	30	2.4	12	3.0
G	44	*	29	2.4	8	2.0
H	41	*	16	1.3	5	1.2
I	26	*	20	1.6	6	1.5
J	13	*	11	*	6	1.5
K	7	*	3	*	0	*
L	2	*	3	*	1	*
M	1	*	2	*	0	*
N	2	*	2	*	0	*
O	0	*	0	*	2	*
P	2	*	0	*	0	*
Q	0	*	1	*	0	*
R	0	*	1	*	0	*
S	1	*	0	*	0	*
	4438 spermatophores on 4438 females		1226 spermatophores on 613 females		405 spermatophores on 135 females	

* = < 1.0% of spermatophores
in the stated grid

TABLE 6: Euchaeta norvegica. Probabilities of the first, second and third spermatophores being placed in each of the regions of the female genital segment.

Genital Segment grid	Probability of the Spermatophore being placed in the stated grid		
	First Spermatophore	Second Spermatophore	Third Spermatophore
A	0.78	0.23	0.32
B	0.11	0.38	0.35
C	0.04	0.12	0.17
D	0.02	0.06	0.07
E	0.01	0.05	*
F	0.01	0.04	0.04
G	*	0.04	0.01
H	*	0.02	0.01
I	*	0.03	0.01
J	*	0.02	0.03
K-S	*	*	*

* = < 0.01 probability

of first spermatophores were attached in the A grid. It is logical to assume that females carrying two spermatophores will have a similar percentage of their first placed spermatophores going into the A position. The total number of spermatophores found on females which carried double spermatophore placements was 1226 of which half (613) would obviously have been attached first as single placements. Therefore it has been assumed that 78.5% of these 613 spermatophores would have gone into the A position ie 481.2 spermatophores. But from analysis of both spermatophores on females carrying double spermatophores it was found that 622 out of the 1226 spermatophores were A placements. This 481.2 out of the 622 spermatophores placed in the A grid would have occurred as first placements; the remaining 140.8 would have been second placements. Therefore the probability of the second attached spermatophore occurring in an A grid would be 0.23. The probability for the other placements of second attached spermatophores has been calculated in a similar manner.

In the case of the third placement spermatophore we know from the processed data from females with two attached spermatophores that 50.7% of the first and second (double) placements occurred in the A grid. The total number of spermatophores on females which carried three spermatophores was 405, of which 135 would have been placed first, 135 would have been placed second, and 135 would have been placed third. Thus it has been assumed that 50.7% out of the 270 first and second spermatophores would have gone into the A position ie 136.9. Analysis of data for females carrying three spermatophores revealed that 180 out of the 405 spermatophores were A placements. Therefore 136.9 out of the 180 spermatophores which were found on the A grid would have been made up of first and second placements; the remaining 43.11 A placed spermatophores would be third placements. Thus 43.11 out of the 135 third placed spermatophores were estimated as A placements ie 31.9%. The probability

of the third attached spermatophore occurring in the A grid would be 0.32. The probability for the third placed spermatophore being placed in other grids has been calculated in a similar manner.

The data presented in Table 6 reveals that the A grid is by far the most likely grid for the first spermatophore to be placed in. The second spermatophore is more likely to be placed in the B grid ($P = 0.38$) but A spermatophores are nearly as likely ($P = 0.23$) as second placements. The third attached spermatophore is again most likely to be a B placement ($P = 0.35$) with A placements nearly as frequent ($P = 0.32$). However it is evident that it is only the first, A placement spermatophore which can be said to have a distinct probability of placement; the positions used for the addition of further spermatophores after the first are relatively indiscriminate.

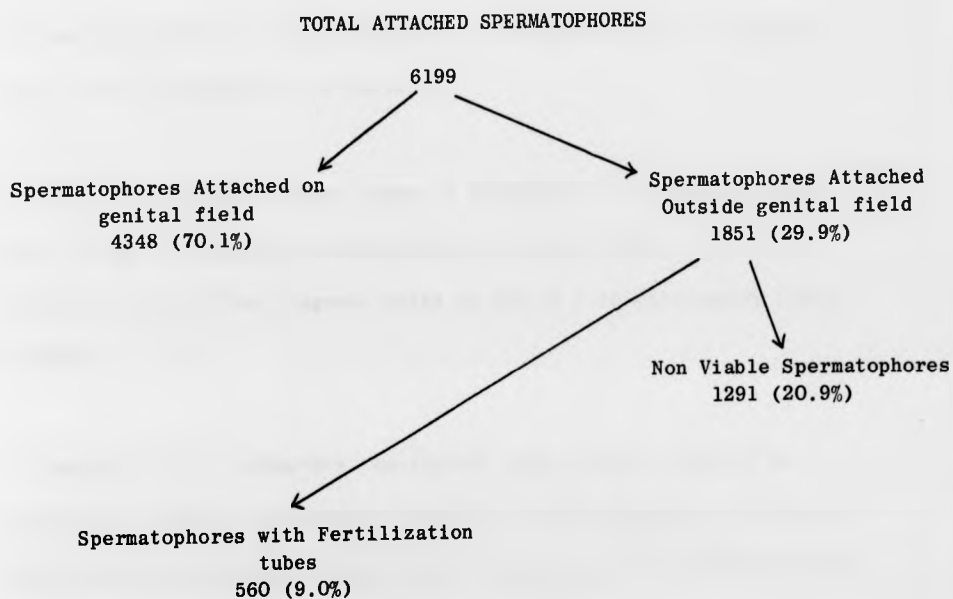
In E. norvegica the spermatophore prior to attachment on the adult female consists of an elongated flask and a short spermatophore neck. However, after attachment the spermatophore is found to have gained a long tube that is continuous with the short original neck, through which sperm and other secretions are transmitted. This "extended neck" is formed by extrusion of some of the non-spermatozoan contents of the spermatophore flask. If the spermatophore has been attached directly onto the female genital cavity (ie A placement region) the extension tube merely disappears within the female cavity, but if the attachment is made away from the genital field the end of the extension tube flows onto the surrounding cuticle to form a circular mass of cement-like material. This circular mass represents the original point of attachment to the genital segment in non-direct placement spermatophores, and is here referred to as the "attachment disc".

The majority of the imperfectly positioned spermatophores (see Table 7) have no connection between the attachment disc and the females seminal receptacles and cannot be thought of as viable for fertilization. However, the remainder have a thin tube (here termed the "fertilization tube") traversing the cuticle between attachment disc and seminal receptacle, and thus spermatophores with fertilization tubes must be considered as fully viable. The manner of formation of the fertilization tube and the reasons why it is not found in all indirectly placed spermatophores are not known and must await further research.

Only spermatophores which have a direct connection with the females genital cavity will result in fertilization. A placement spermatophores meet this requirement and their frequency (70.1% of all attached spermatophores) makes them the most important spermatophore attachments. A placement spermatophores with fertilization tubes present account for 9.0% of all attached spermatophores and so when they are added to the number of A placements they increase the total proportion of viable spermatophores to 79.1%. The B grid of the female genital segment accounts for over half of the fertilization tubes and emphasises the value of this area as a site of attachment additional to the A placement grid.

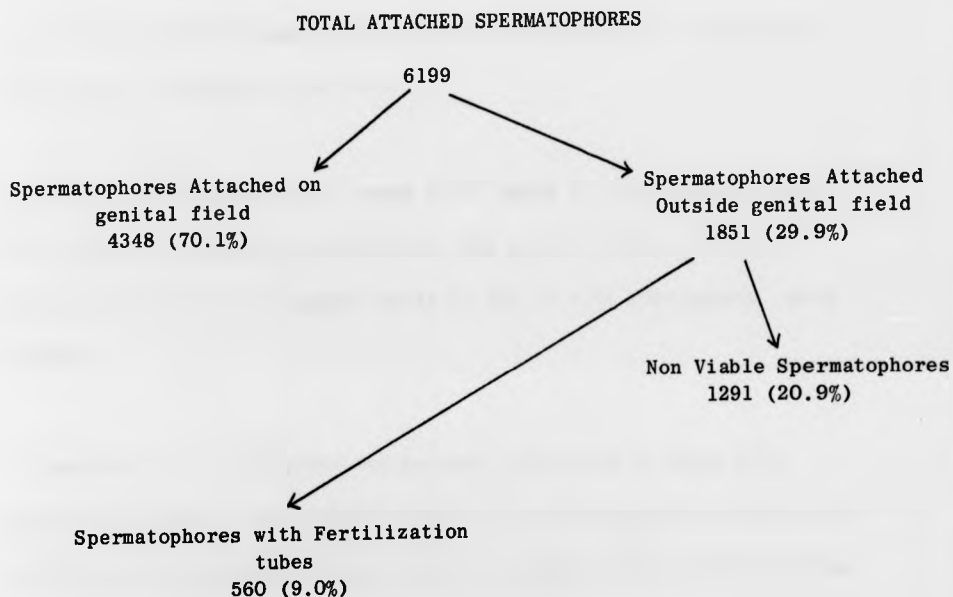
The analysis of positional placement of spermatophores has so far examined the attachment of spermatophores purely on a simple frequency basis and provides no information about whether the position of an attached spermatophore exerts any kind of influence, attractive or otherwise, on the placement of other spermatophores. In order to aid analysis of the degree of association between spermatophore positions the 19 regions have been reduced to 5 regions in the following way:

TABLE 7: *Euchaeta norvegica*. Distribution of spermatophores on and off the genital field, indicating the frequency of occurrence of fertilization tubes in the placement grids of the female genital segment.



Placement grid	No. of Spermatophores
B =	346
C =	82
D =	22
E =	32
F =	21
G =	17
H =	9
I =	18
J =	6
K =	5
L =	0
M =	1
N =	1
O-S =	0

TABLE 7: *Euchaeta norvegica*. Distribution of spermatophores on and off the genital field, indicating the frequency of occurrence of fertilization tubes in the placement grids of the female genital segment.



Placement grid	No. of Spermatophores
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G =	17
H =	9
I =	18
J =	6
K =	5
L =	0
M =	1
N =	1
O-S =	0

(A) —————→ A
(B,E,G) —————→ W
(C,F,J) —————→ X
(D,H) —————→ Y
(I,K,L,M,N,O,P,Q,R,S) —————→ Z

If one now considers those females with two spermatophore attachments they can be tabulated as in Table 8.

It should be remembered that there is no means of telling (for these data) which of the two spermatophores was placed first so that one cannot divide off the diagonal cells in the (5 x 5) contingency table further.

An analysis of an incomplete contingency table such as Table 8 is described by Bishop and Fienberg (1969). On the assumption of the null hypothesis that there is independence of placement the expected values corresponding to those of Table 8 are given in Table 9.

Comparisons of Tables 8 and 9 shows good agreement between observed and expected, the calculated value of χ^2 is 5.61 whereas from tables the 5% point with 6 degrees of freedom (see Bishop and Fienberg) is 12.65, indicating no association between the position of spermatophore placements.

The sample intra class correlation (see Fisher, 1967) for these data is -0.048 and clearly confirms the above analysis.

A similar analysis restricted to those females with no A placements gave the same result.

TABLE 8: Euchaeta norvegica. Association between positional placements of those females with two spermatophores.

	A	W	X	Y	Z	Total
A	139	208	87	32	18	484
W		57	23	14	7	101
X			9	9	4	22
Y				4	2	6
Z					0	0
Total	139	265	119	59	31	613

TABLE 9: Euchaeta norvegica. Expected values for the Table 8 data following the Bishop & Fienburg analysis.

	A	W	X	Y	Z	Total
A	139	204.99	82.07	37.97	19.97	484
W		60.01	24.03	11.13	5.83	101
X			12.90	5.97	3.13	22
Y				3.93	2.07	6
Z					0	0
Total	139	265	119	59	31	613

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Z					0	0
Total	139	265	119	59	31	613

For those females with more than two spermatophores attached no appropriate analysis appears to be available, and even if implemented would be unlikely to indicate a different behavioural response involved in attaching additional spermatophores.

DISCUSSION

In E. norvegica the strong positive correlation found between the mean number of spermatophores per female and the proportion of adult males present suggests that the intensity of mating and breeding is determined by the adult sex ratio; the more males present the greater the quantity of spermatophores that are produced and attached. An increase in the proportion of adult males in February-March and July-August has been seen to result in large increases in the mean number of spermatophores carried per female. Such an increased production of spermatophores ensures a "blanket" fertilization of all needy females. However, the relationship between males and attached spermatophores appears to tail off when relatively high proportions of males are present (see Fig. 15). This is compatible with the fact that underdispersion of spermatophores has been shown to occur and that males are likely to avoid placing a spermatophore on a female which already carries one. This may result in a saturation effect as at higher proportions of males in the adult population the proportion of already fertilized females will be high also and so males will have to spend relatively more time searching for the few remaining unfertilized females and will attach proportionately fewer spermatophores in a given time.

Mating ethology which results in underdispersion of spermatophores on females is necessary and advantageous in terms of natural selection as it tends to result in an even distribution of spermatophores throughout

the adult female population and fertilization of a greater quantity of available females. Nevertheless it has been shown that multiple placements of spermatophores on females become more frequent at times of high male:female sex ratios indicating that the underdispersion mechanism becomes less stable. Obviously at high adult male:unmated female ratios there is less chance that multiple placements will have adverse effects upon the survival of the population by reducing the quantity of females fertilized. With many males searching for dwindling numbers of unfertilized females it becomes more likely that a number of males will simultaneously attempt to mate with a single female. Cases of a number of males attempting to copulate at the same time with one female have been reported for various copepods (eg Hill & Coker, 1930; for Cyclops Americanus; and Jacobs, 1961, for Pseudodiaptomus Coronatus) and it seems likely that this can happen, under certain circumstances, in E. norvegica. This type of intense copulatory competition would certainly help account for the greater frequency of multiple placements which occur at the height of the breeding peaks in February-March and July-August when high proportions of males and low proportions of females with unattached spermatophores are present. Thus in E. norvegica multiple spermatophore placements are probably a measure of the reproductive competition between males for available females. This supposition is strengthened by the work of Fleminger (1967) who has proposed that in Labidocera jollae the presence of high percentages of females carrying one or more spermatophores in a sample may be caused by the time of sampling having coincided with a possible local mating swarm, and further postulated that sexual swarming coupled with a high male:female sex ratio could be the basis for large numbers of misplaced spermatophores.

The fact that single spermatophore placements were by far the most frequent number of spermatophores found attached (71.6% of all spermatophores) provides strong evidence that the contents of a single correctly placed spermatophore is enough to provide the quantity of sperm necessary for fertilization of a single egg clutch for a female. As is to be expected the majority (78.5%) of first placement spermatophores are attached directly onto the entrance to the genital cavity in the A grid thus causing the female to be successfully fertilized. However, there are a small quantity (21.5%) of single placement spermatophores which are not attached in the A grid. This may suggest that a degree of "misplacement" occasionally occurs but it can also be accounted for by the fact that A placement spermatophores are not as firmly attached to the female as spermatophores in other placement regions, and as they are relatively easily dislodged when empty they do not remain on the female for as long as other placements. Thus a number of the females recorded with their single spermatophore present in a non-A placement grid could possibly be carrying a spermatophore which was originally one of two; the A placement spermatophore having fallen off or subsequently been dislodged. The quantity of A placement spermatophores recorded is thus likely to be an underestimate.

Spermatophores were able to occur in practically any combination of placement positions such that a number of similarly placed spermatophores sometimes occurred together on one female. Instances of two or more spermatophores of one placement type superimposed on one another could be the product of a single male that fails to release the female after attaching the first spermatophore, or, the outcome of matings by other males. Adult males can often be found in plankton samples with a spermatophore gripped in the left fifth limb ready for attachment, as well as

having a fully formed spermatophore in the spermatophore sac ready for future ejaculation. Histological studies have further revealed the presence of a third spermatophore in the process of being formed further up the reproductive tract in the seminal vesicle (see Chapter 3). Adult males are thus potentially capable of attaching at least two, and possibly three spermatophores within a short space of time. There are thus no hard and fast rules of exclusion governing placement of spermatophores, so that the presence of an A placement spermatophore, for example, does not automatically prevent the next spermatophore from being attached there also.

It has been predicted that the adult male must position himself in a specific manner relative to the orientation of the female in order to correctly position a spermatophore to her genital orifice (see Chapter 4). The presence of a number of males simultaneously attempting to attach their spermatophores is likely to increase the quantity of misplaced spermatophores and this agrees well with a distinct trend for a greater percentage of spermatophores to go into other areas of the genital segment besides the A grid in those females carrying single, double, and treble spermatophore placements. The occupation of the genital field by a spermatophore thus tends to cause more of the later attached spermatophores to be "misplaced". However the establishment that there is no association between spermatophore positions indicates that the position of a spermatophore does not influence the choice of grid in which the next spermatophore is placed. Of the other areas of the genital segment the B grid is more favoured than the others; perhaps the presence there of the pronounced ventral ridge exerts some kind of attractive tactile response from the adult males left fifth limb which carries out the attachment of the spermatophore.

The adaptation of the mathematical models of Greenwood & Yule and Consul & Jain have been most successful in describing the frequency distribution of spermatophores on females. However it is essential to bear in mind that no matter how good the fit between observed and predicted results the models chosen must also be compatible with a sound knowledge of the biology of the species under study. The essential difference between the two models, as applied to this study, is that the model of Greenwood & Yule implies that the male copepod is only able to distinguish between females with spermatophores and females without spermatophores, whereas the model of Consul & Jain suggests that the male is, in addition, able to assess the quantity of spermatophores already attached to the female. Greenwood & Yule's model is more acceptable in biological terms, whereas the ability of the male copepod to enumerate the quantity of spermatophores present is less plausible.

It is extremely important that the adult male be capable of recognizing the exact state of readiness of his prospective mate, and for under-dispersion of spermatophores to take place it is obviously necessary for the male partner to determine the presence or absence of an attached spermatophore on a female. This "interrogation" of an adult female suggests the involvement of both chemical and physical factors before selection and copulation take place. The structure of the left fifth limb of the adult male, suggests that it is well adapted to a tactile role in locating the female genital field and in positioning the spermatophore; in such circumstances it is reasonable to postulate that it can also determine the presence of a previously attached spermatophore. However, for adult males to have to rely solely on tactile examination of every female encountered would be extremely time consuming and in terms of natural selection would be unsatisfactory.

The presence in a crustacean of a pheromone system has been verified by a number of workers (eg Ryan, 1966 for the decapod Portunus; Dahl et al 1970 for the amphipod Gammarus, and Katona, 1973 for some planktonic copepods) and the state of sexual receptivity of female E. norvegica may well be advertised with production of analogous pheromones in a similar manner to the silk moth Bombyx (see Karlson, 1960; Wright, 1964). A plausible mode of operation of this system in E. norvegica is one in which a receptive female without a spermatophore on her genital field would advertise her state of readiness until the presence of a spermatophore placed correctly over the genital orifice would cause the production of sexual pheromones to be "shut off" until after egg laying had occurred. The attachment of incorrectly placed spermatophores would not influence the production of such display pheromones as only fertilization of the female would merit cessation of the advertisement of receptivity. This method of publicity would be advantageous in providing the male with valuable information about his prospective mate in a speedy and efficient manner, without him entering into the copulatory position which is necessary for tactile examination of the female genital segment. The advantage of such a system would also lie in its benefit both to the individual as well as the population. It firstly benefits the individual female in increasing her chances of fertilization by advertising her possible availability, but it additionally aids the population as deactivation of the advisary system by a female after successful fertilization helps to withdraw her from competition with unfertilized females for the males attentions.

At times of high proportions of adult males and low proportions of receptive unmated females it would probably result in a number of males attempting to either mate simultaneously with a female or in consecutive males attempting to mate with her before the display pheromone system

has been fully closed down, thereby helping to explain the occurrence of multiple spermatophore placements.

Rose (1933) has stated that the male of Calanus is not very efficient in transferring the spermatophore and that it is quite frequently found attached elsewhere than to the genital segment. Marshall & Orr (1955) have questioned Rose's statement by reporting that occasionally a female Calanus finmarchicus is found carrying more than one, but rarely more than two or three spermatophores. Obviously gross inefficiency in attaching the spermatophore correctly would endanger the survival of the species. The frequency of occurrence of multiple spermatophore placements in copepods are generally low even though occasionally large numbers of spermatophores are found on individual females (for example Gibbons, 1936 has reported 15 on a single female Calanus, and Wolf, 1905 has stated that in Diaptomus gracilis 5-10 can be found on one female). In the present study only 17.5% of female E. norvegica with spermatophores carried more than one, and so multiple placements can be thought of as being relatively infrequent.

All the evidence presented for E. norvegica leads to the conclusion that placement of the first spermatophore on a female is precise and efficient. The attachment of additional spermatophores are not functionally important and so the increase in misplacement with extra attachment of spermatophores is inconsequential, provided of course that the majority of females are fertilized. The potential of every adult male to be able to produce more than one spermatophore in his life-time is likely to increase the capacity for multiple placements under conditions of high reproductive competition. The prediction of the functioning of a sexual information system, possibly of the pheromone type, which provides details of the state of receptivity of a female emphasises the need for biochemical research associated with mating and breeding in copepods.

Fig. 13. Euchaeta norvegica. Diagramatic representation of the genital segment of an adult female to show the regions of spermatophore placement. See text for further details.

Fig. 14. Euchaeta norvegica. Seasonal variations in the mean number of spermatophores per female and the changing proportions of males in the adult population

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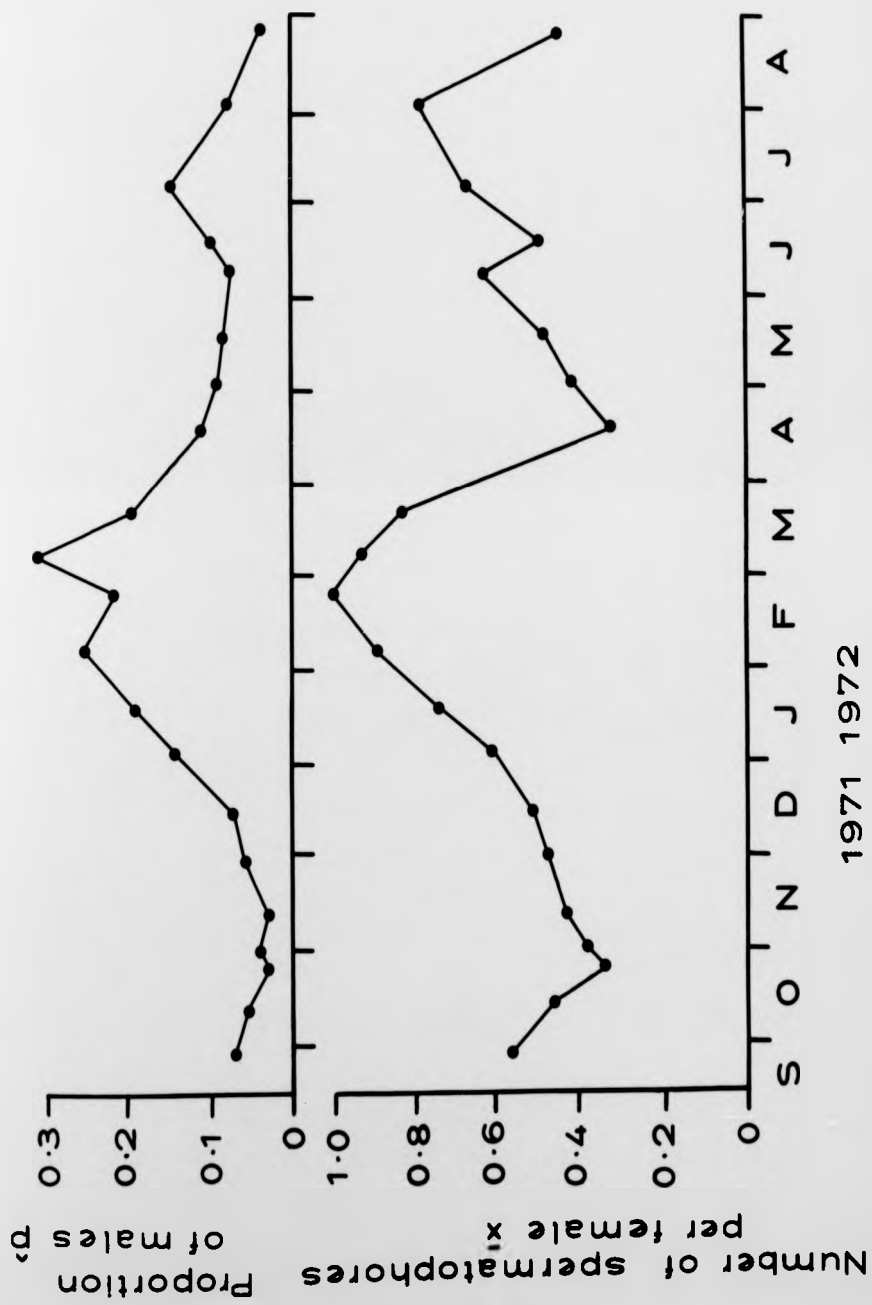


Fig. 15. Euchaeta norvegica. The relationship between
the mean number of spermatophores per female
and the proportion of adult males present

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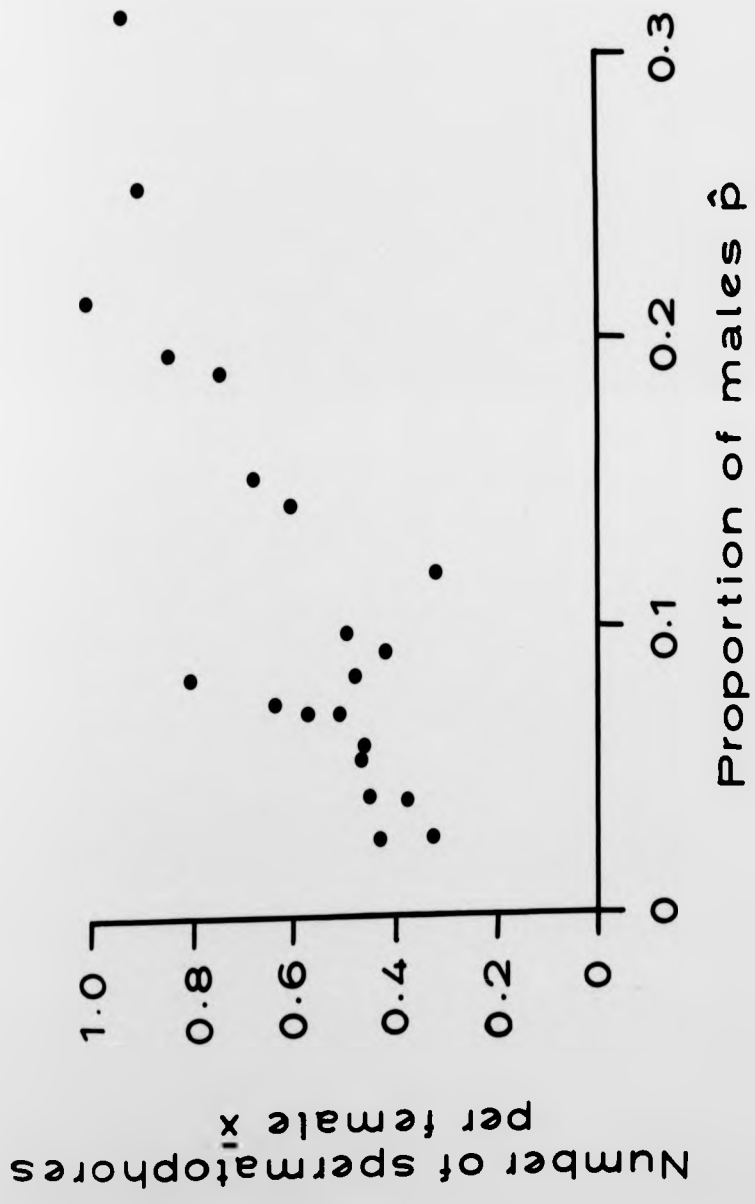


Fig. 16. Luchania norvegica. Percentage frequency
distribution of adult females carrying
different numbers of spermatophores

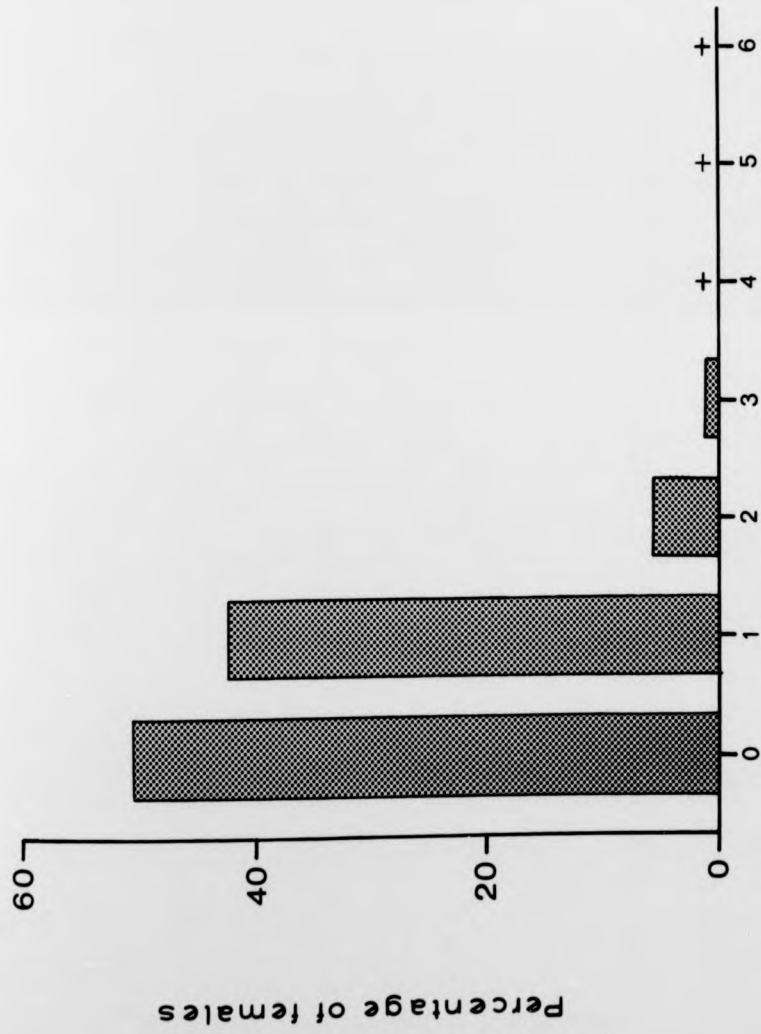
100% = 10,500 females

* = occurrence at < 1.0%

Fig. 16. Euchaeta norvegica. Percentage frequency
distribution of adult females carrying
different numbers of spermatophores

100% = 10,500 females

+ = occurrence at < 1.0%



Number of spermatophores

Fig. 17. Euchaeta norvegica. The mean variance relationship
for the number of spermatophores per female

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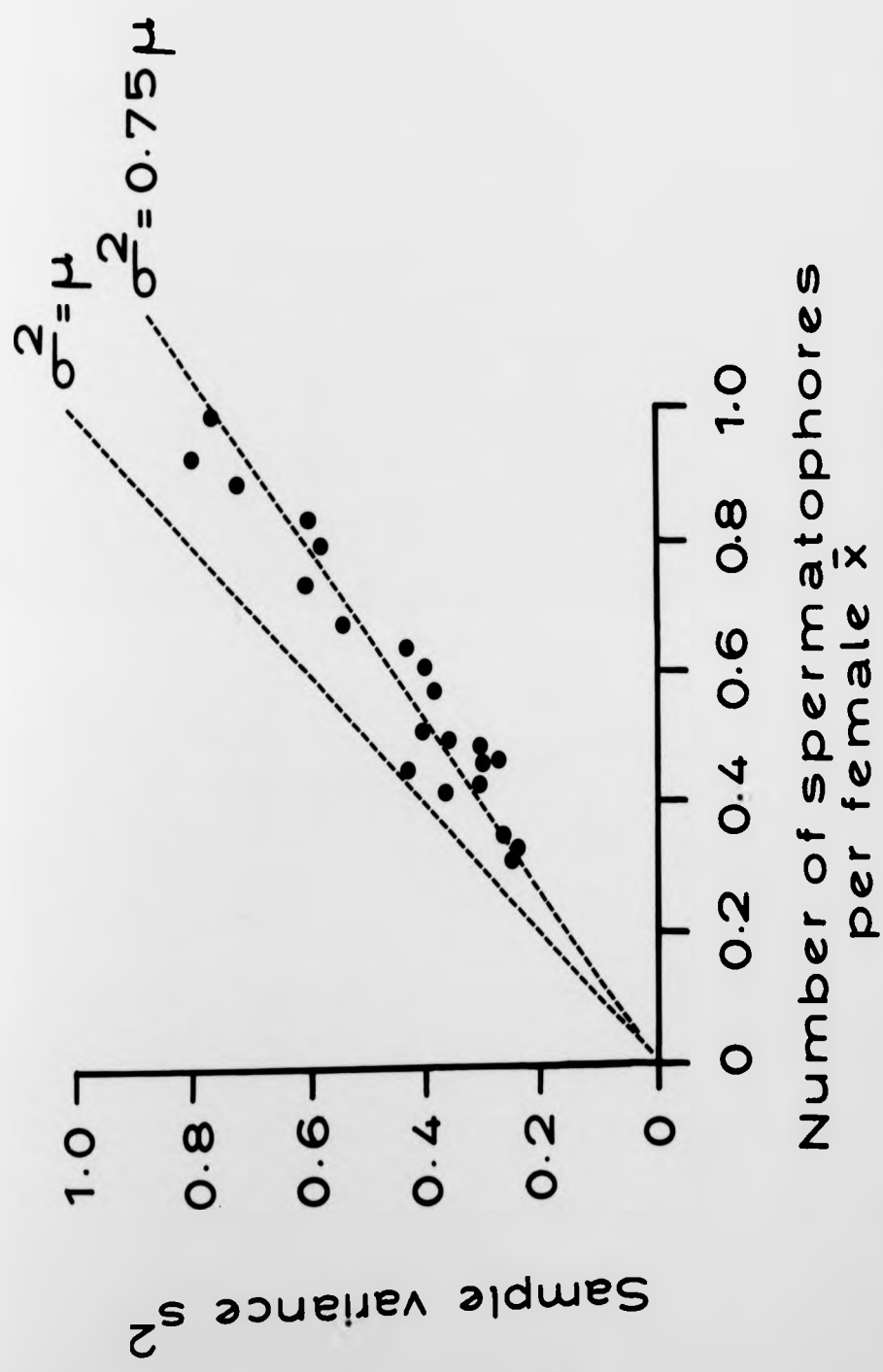


Fig. 18. Euchaeta norvegica. Comparison of three alternative models used to describe the frequency distribution of spermatophores on adult females. The probabilities quoted are those obtained from a chi-squared goodness of fit test. The hypothesis of a Poisson model is rejected in each case

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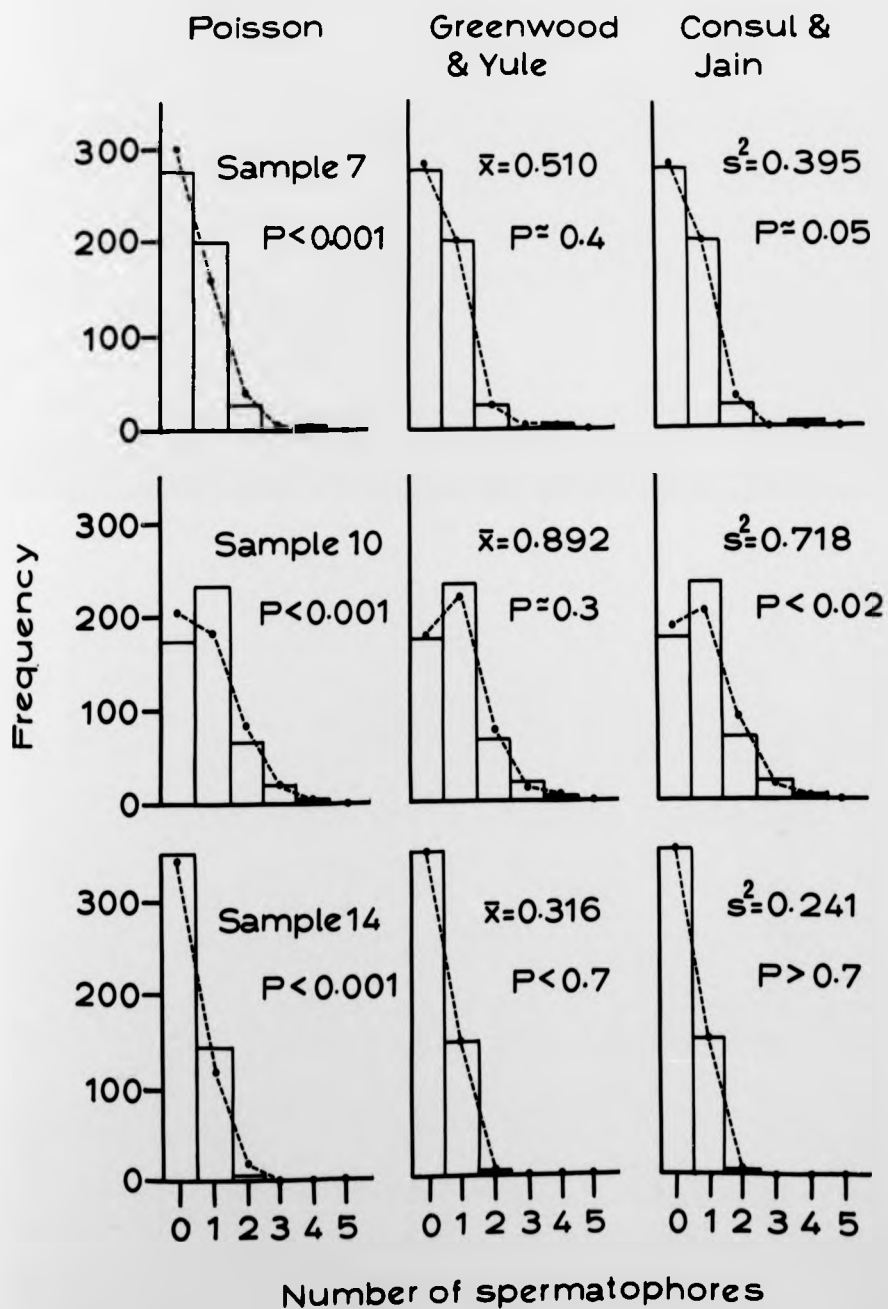


Fig. 19. Euchaeta norvegica. Percentage frequency distribution of spermatophores found in the different regions of the female genital segment. Based on data from all females examined from all samples

100% = 6,199 spermatophores

+ = occurrence at < 1.0%

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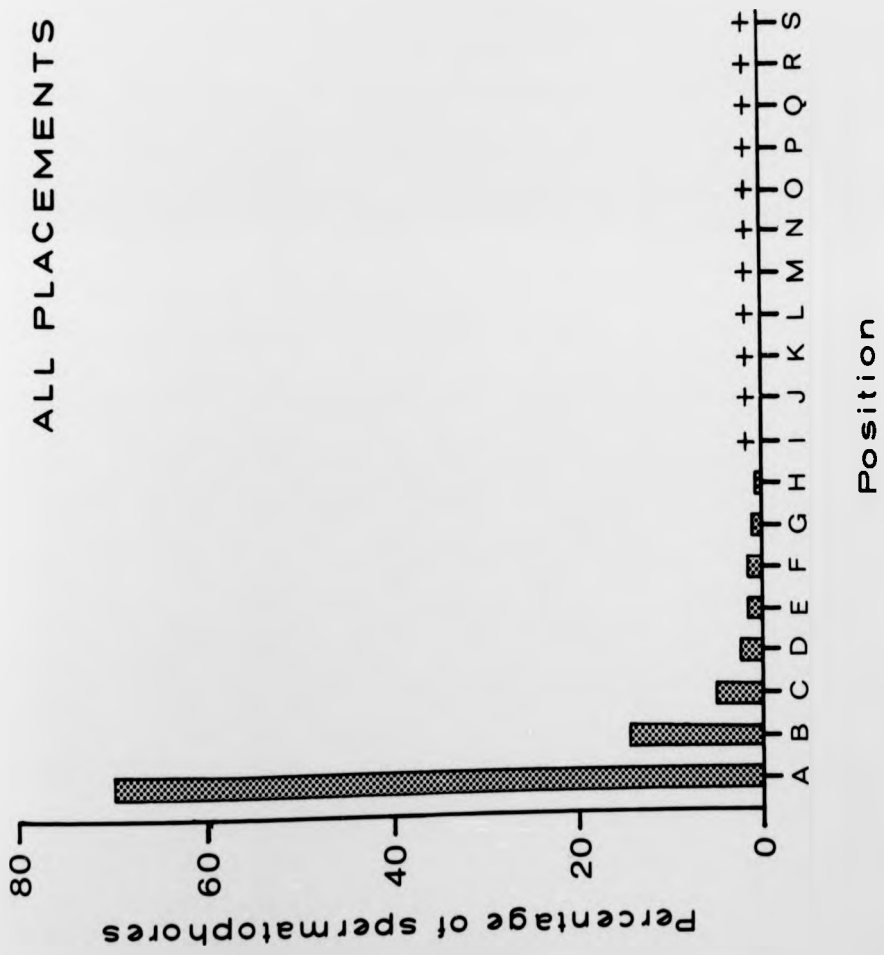


Fig. 20. Euchaeta norvegica. Percentage frequency distribution of spermatophores found in the different regions of the genital segment of females carrying (a) single (b) double and (c) treble spermatophore placements

100% = 4,438 spermatophores for single placements

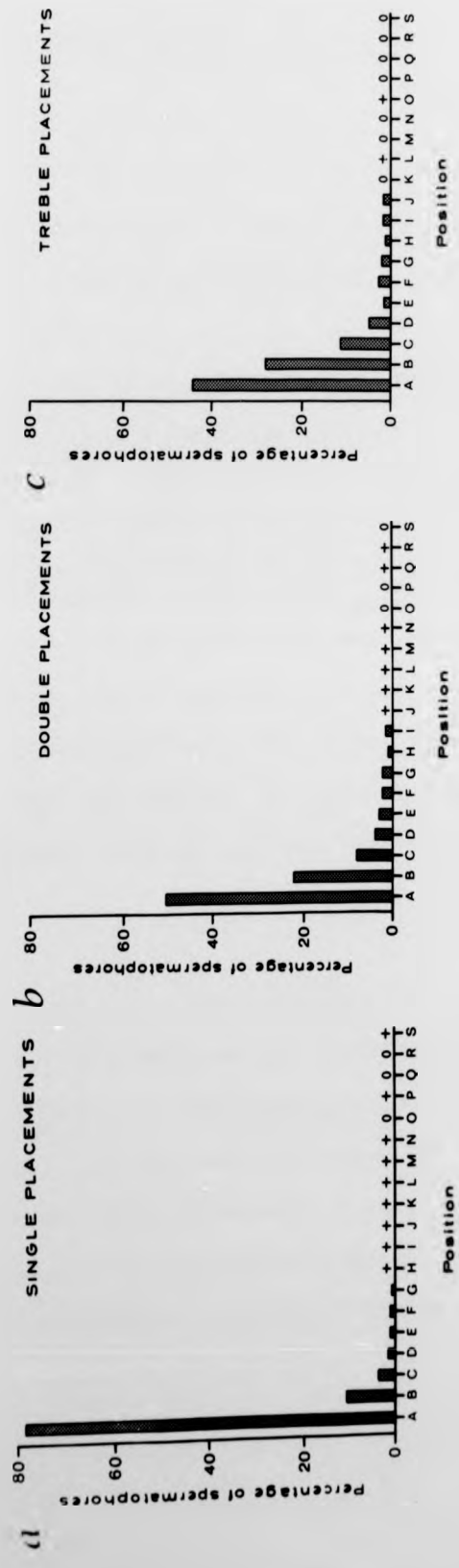
100% = 1,226 spermatophores for double placements

100% = 405 spermatophores for treble placements

+ = occurrence at < 1.0%

0 = absence of spermatophores from the grid

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CHAPTER 6

THE RELATIONSHIP BETWEEN MATERNAL BODY SIZE AND CLUTCH SIZEINTRODUCTION

Jensen (1958) on the basis of collected material and statements in the available literature made a number of analyses of the relationship between the size and the number of eggs per female in various decapods, isopods, cumaceans and mysids. The examination was made by means of regression analyses, the basis being a hypothesis to the effect that the number of eggs per female is a linear function of the length of the female raised to the power of 3, and he found that it held true for the majority of cases.

In copepods, small adult females carry fewer eggs than large females and correlations between female size and egg number have been shown for freshwater diaptomids (Ravera and Tonolli, 1956; Davis, 1959) and cyclopoids (Margalef, 1953, 1955; Elgmork, 1959; Elbourn, 1966), as well as for marine species of calanoids (Marshall, 1949; McLaren, 1963, 1965).

This chapter presents the results of an investigation into the relationship between maternal body size and clutch size as well as providing some information on egg development time, in Euchaeta norvegica. The relationship between females and egg clutches were investigated using "pooled" data collected from plankton hauls over a period of nearly a year, in order to produce general equations which quantify the mean relationship over the normal range of environmental conditions likely to be encountered in Loch Etive.

An egg sac is defined here as a group of eggs plus their surrounding egg sac membrane. The term 'egg clutch' is used for the eggs contained within an egg sac. The eggs do not account for all the available space within the egg sac and therefore clutch volume, although being synonymous with total egg volume, is not necessarily synonymous with egg sac volume.

MATERIALS AND METHODS

Sampling and selection of specimens

Approximately 10 adult female *E. norvegica* with undamaged egg sacs were removed from each of the 21 plankton hauls collected during the standard sampling programme in Loch Etive. These specimens were selected in the order in which they were encountered in the rafter cell sorting tray. Only undamaged specimens in which the body was relatively straight and not flexed excessively from the longitudinal axis of the prosome were chosen for measurements of body tagmata and length.

Measurements were made of total length, prosome length, maximum height and width of the prosome, together with the maximum width of the urosome as measured across the dorsal aspect of the genital segment. The length of the urosome was also recorded. The egg sacs were then detached from the genital segments and the length and width of the sac noted. The eggs were carefully dissected out of the protective egg sac material, and the number of eggs per female recorded. Ten eggs were then taken at random from each female and their diameters measured.

Prosome length was measured from the tip of the rostrum to the small spines on the posterior 'wings' of the fifth thoracic somite. Total length was measured in the standard manner from the tip of the rostrum to the end of the caudal furcae, excluding setae. Egg diameter was

measured as the greatest length of the egg because the egg becomes slightly subspherical after development has reached the early gastrula stage.

The basic data were distributed into 13 size-classes based upon the prosome length of the parent copepod and regression analyses were made of number of eggs on prosome length, number of eggs on prosome length³, and brood volume on prosome length³ using the class mid-points. All computations were based upon measurements of prosome length rather than total length because it is a more accurate evaluation of parental size. The urosome is more often affected by flexure and by post-mortem telescoping of segments than is the prosome, and thus if incorporated into measurements of length can introduce sources of biometric error.

Clutch volume was calculated for each female by multiplying the number of eggs in the sac by its mean volume; the latter having been obtained by assuming the eggs to be spherical, and applying the formula $\frac{4}{3}\pi r^3$, using the mean egg diameter recorded for that female.

Microforce balance weighings:

Mean wet and dry body weights for 10 egg sac bearing females of the most common modal length (prosome length 5.40-5.50mm) of the population were obtained by individual weighings of fresh specimens on a microforce balance after their egg sacs had been carefully removed. These individuals were gently rolled with a soft artist's brush on blotting paper to remove any extraneous fluid from their body surfaces before wet weighing. Care was taken not to break any appendages or rupture any membranes, nor to allow removal of internal body fluids through excess exposure

to the air or blotting paper. After wet weighing the specimens were placed in a desiccator with self-indicating silica-gel at room temperature for 24 hours before being weighed dry. After removal from the desiccator the specimens were speedily weighed, as lengthy exposure of desiccated material to the air can result in appreciable weight increases caused by uptake of moisture from the atmosphere (Lovegrove, 1966). Mean wet and dry egg weights were obtained by weighing ten batches of ten eggs; each egg having been previously dissected undamaged out of the egg sac. The eggs were touched briefly on blotting paper before being wet weighed and were then dry weighed after having been desiccator dried. The remaining 10 empty egg sacs were then dry weighed in one batch.

RESULTS

Egg development and mortality

Observations were made twice daily on the state of development of the ovaries of thirty adult females brought into the laboratory in early March 1974, and maintained at a constant temperature of 10°C, which closely approximated the prevailing water temperature in the loch. Artemia nauplii were provided as food.

Each of the females was selected for study on two criteria. Firstly each individual had to have a spermatophore attached to the genital field indicating that mating had taken place and that since mating no egg sac had been produced. The other criterion used was that the ovaries should only just be visible under a low power stereoscopic microscope as a small pigmented structure less than 0.5mm long, and the oviducts, if discernible, should be present only as thin strands, thereby showing that the ovaries were still in an immature state of development.

The ovaries proceeded to develop fully in every instance and mature eggs filled the oviducts, with the most developed eggs being found at the very end of the ducts in the last metasome segment. However, only six adult females successfully produced egg sacs. The average time taken to produce the egg sacs at 10°C was (mean and one S.D.) 22.3 ± 4.1 days. The range of time taken was 15-26 days. These experiments suggest that approximately three weeks are needed, after mating, for maturation of the ovary and eggs to fully take place before the clutch is laid.

The same six egg sac bearing females were then used to determine the time taken for the eggs to hatch out as recorded by the appearance of the first observed free swimming nauplius. The maximum and minimum times taken by an individual egg sac from the time of first observation of egg laying to time of first observation of a free swimming nauplius were 21 days and 17 days respectively. The average time taken at 10°C was 18.7 ± 1.2 days. All the nauplii emerged from the sac within 2 days of the first nauplius having escaped.

The females with gravid ovaries and oviducts but which never laid eggs lived for a further two months before dying. Their eggs, within the body, appeared to be in prime condition for a further two weeks after the other females had produced sacs, however, they then began to resorb their eggs which disintegrated in the oviducts, mainly into oil globules, before finally disappearing.

Further observations were made on another 25 females with attached egg sacs and revealed that an average of 4% of eggs in the sac failed to hatch out, but results varied from a minimum of 0% to a maximum of 17%. Analysis of the number of eggs per sac and mortality within the sac based

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on such low numbers failed to disclose any increased mortality in females with large numbers of eggs per clutch. No examples were found of mortality of the nauplii having occurred within the egg sac membrane.

The eggs, when first passed into the egg sac are bluish-green in colour and generally spherical. As development proceeds the egg becomes subspherical, being elongated along the anterior-posterior axis, with the late stage egg becoming more yellow owing to the presence of many large orange oil globules. The mean egg diameter within the sac is $421\mu\text{m}$ (2300 eggs) but sizes may vary from $351\text{--}507\mu\text{m}$ depending upon whether the egg has recently been extruded or whether it is at the point of developing into the 1st nauplius stage. The latter, when still within the sac can vary between $468\mu\text{m}$ and $546\mu\text{m}$ in length.

Relative Growth of Prosome and Urosome:

Measurements of the dimensions of the prosome and urosome of adult female E. norvegica over the whole of their size range in Loch Etive show that the proportions of the prosome and urosome are maintained constant. The following proportions were recorded for the population:

Prosome length	:	Urosome length	:	Prosome width	:	Urosome width
1.00	:	0.43	:	0.34	:	0.08

The ratio of Total length : Prosome length was found to be 1.43:1.00

In animals which maintain their body proportions as they grow the relationship of body volume to body length is constant, and as the mature ovary is likely to have a similar shape and extent within the body of nearly all individuals of the same species, it is safe to assume a relatively constant relationship between the volume of the ovary and

the body. The potential number of eggs produced by a female at each egg laying is therefore related to the volume of each egg and to the volume of the prosome, which contains the bulk of the ovary and oviducts.

Clutch Size and Body Size:

The mean number of eggs per sac have been plotted against the respective mean prosome lengths of the maternal copepods (Fig. 21) and show that there is a highly significant relationship ($r = 0.94$, $P < 0.001$). The regression equation is $N = 6.716 L - 6.11$

where N = mean number of eggs per sac, and L = prosome length (mm). The mean numbers of eggs per sac have been compared with the respective mean prosome lengths of the parent copepods raised to the third power (Fig. 22); this assumes that no interference from allometric growth takes place and that L^3 is thus an expression of volume. The correlation coefficient is highly significant ($r = 0.93$, $P < 0.001$). The regression equation is:

$$N = 0.080 L^3 + 17.464$$

where N = mean number of eggs per sac, and L = prosome length³.

Mean clutch volumes have been plotted against the cube of the respective mean prosome lengths (Fig. 23). The correlation coefficient is highly significant ($r = 0.93$, $P < 0.001$). The regression equation is $V = 0.004 L^3 + 0.596$

where V = clutch volume, and L = prosome length³.

On the other hand the size of the mother does not significantly influence the size of the eggs produced ($r = 0.39$, $P > 0.1$).

It is therefore evident that larger females, in general, produce more eggs and a greater volume of eggs per clutch than smaller females. However, there does appear to be a slight 'tail-off' from the fitted regression lines for the smallest size-class of females.

The mean, range and standard deviation of the observations of the dependent variables used to investigate the relationship between parental size and clutch size are given in Table 10. The wide range in both egg number and clutch volume within individual size-classes, and the large degree of overlap between the ranges indicate that the egg producing performance of females can be highly variable. This variability suggests that the opportunity exists for smaller females in good reproductive condition to be able to produce as many if not more eggs than the largest females if the latter have declined below their mean egg potential.

Mean wet and dry body weights of 10 adult female E. norvegica (prosome length 5.40-5.50mm) which had their egg sacs removed before weighing were found to be 12630 μ g and 1804 μ g respectively, having a wet weight:dry weight body ratio of 7.0:1.0. Mean wet and dry weights for an egg dissected out of the egg sac were 25 μ g and 15 μ g respectively, giving a wet weight:dry weight ratio of 1.6:1.0. The mean dry weight of the empty egg sacs was 21 μ g.

A reasonably accurate assessment of the body volume of adult female E. norvegica has been made by considering the copepod body to be comprised of two cylinders; the prosome and the urosome.

Therefore, Body Volume = Prosome Volume + Urosome Volume

$$" \quad " \quad = \pi r_p^2 l_p \quad + \quad \pi r_u^2 l_u$$

TABLE 10: Observed variations in egg numbers and clutch volume within, and between, different maternal body size groups of Euchaeta norvegica

Maternal Body Size PR.L(mm)	Number of Observations	Number of Eggs/sac			Clutch Volume (mlx10 ⁻³)		
		Mean	Range	S.D.	Mean	Range	S.D.
4.65	12	23.17	17-31	3.99	0.88	0.60-1.21	0.17
4.75	2	25.50	25-26	0.71	0.94	0.77-1.10	0.23
4.85	10	26.60	19-40	6.31	1.08	0.68-1.46	0.25
4.95	9	27.55	19-37	6.40	1.02	0.71-1.35	0.22
5.05	23	29.17	20-37	3.94	1.11	0.80-1.50	0.19
5.15	3	29.00	25-33	4.00	1.03	0.98-1.08	0.05
5.25	7	30.43	23-37	4.61	1.17	0.89-1.46	0.20
5.35	13	29.15	17-36	5.10	1.18	0.83-1.70	0.25
5.45	54	30.43	17-39	4.96	1.18	0.62-1.88	0.25
5.55	26	30.46	18-40	5.76	1.17	0.82-1.59	0.21
5.65	27	32.48	15-41	6.50	1.30	0.72-2.17	0.33
5.75	10	32.30	22-37	4.97	1.24	0.80-1.64	0.25
5.85	9	32.22	25-40	5.93	1.30	0.87-1.64	0.29

where r_p and r_u are the radius of the prosome and urosome, and l_p and l_u are the length of the prosome and urosome. These dimensions have been estimated for each of the body size-classes being investigated by application of the ratios governing relative growth of the prosome and urosome.

A summary of the results of the microforce balance weighings and of the estimates of body and clutch volumes for the most common body size (prosome length class mid-point of 5.45mm) of adult females is provided in Table 11. It shows that the output of a female in producing a clutch in terms of loss of wet weight biomass is small (5.8%), but when expressed as a drain in dry weight biomass, and therefore in organic material which is not easily replaced, it accounts for a quarter of the body weight. It is also interesting to note that the urosome volume accounts for less than 3% of the combined volume of the prosome and urosome.

A comparison of mean total body volume and mean clutch volume for the various sizes of adult female has been made in Table 12. The data indicate that the smaller sizes of female have, on average, produced a greater volume of eggs per unit body volume than larger females when producing a clutch. This, however, implies that smaller females undergo proportionately more dry weight loss, and consequently energy loss, in producing a clutch of eggs than larger females, given that volume for volume the calorific values of the body and eggs do not change between various size groups.

DISCUSSION

It is generally agreed that the number of eggs produced by the female

TABLE 11: Estimation of output in terms of wet weight, dry weight and volume when an adult female

E. norvegica of 5.45mm prosome length (7.79mm total length) produces of clutch of eggs.

Body Weight	Egg No/Sac	<u>Wet Weight</u>		Clutch Weight	$\frac{\text{Clutch Weight}}{\text{body weight}} \%$
		Mean Egg Weight	Clutch Weight		
12630 μG	30.43	24 μG	730 μG		5.8
1804 μG	30.43		456 μG		25.3
		<u>Dry Weight</u>			
			15 μG		
		<u>Volume</u>			
Total Body Volume ml x 10 ⁻³	Prosome Volume ml x 10 ⁻³	Prosome Volume Total Body Volume %	Clutch Volume ml x 10 ⁻³	$\frac{\text{Clutch Volume}}{\text{Total Body Volume}} \%$	
15.15	14.80	97.7	1.18	7.8	

TABLE 12: Comparison of calculated mean total body volume and
observed clutch volume for various sizes of adult female
Euchaeta norvegica.

Prosome length (mm)	Total Body Volume (ml x 10 ⁻³)	Clutch Volume (ml x 10 ⁻³)	$\frac{\text{Clutch Volume}}{\text{Total Body Volume}}$ %
4.65	9.34	0.88	9.4
4.75	10.01	0.94	9.4
4.85	10.47	1.08	10.3
4.95	11.24	1.02	9.1
5.05	12.00	1.11	9.3
5.15	12.83	1.03	8.1
5.25	13.37	1.17	8.7
5.35	14.23	1.18	8.3
5.45	15.15	1.18	7.8
5.55	15.76	1.17	7.4
5.65	16.75	1.30	7.8
5.75	17.75	1.24	7.0
5.85	18.42	1.30	7.0

of any species is associated with the degree of parental care or other protection accorded to the eggs and larvae following fertilization. The greater care the fewer eggs produced. E. norvegica produces relatively few, but large yolky eggs which being carried within an egg sac are provided with a degree of parental care. Egg protection within the sac permanently attached to the adult female copepod would confer upon the developing embryos comparative safety from predators. Natural selection, however, will only favour the evolution of a small egg number if there is a compensatory advantage such that those adults which lay fewer eggs at one time produce more, not fewer, surviving offspring. Lack (1954) has suggested that the chief advantage of this lies in the production of a larger egg.

An account of the naupliar stages of E. norvegica is given by Nicholls (1934) who indicated that they do not feed, and have none of the setae which Gauld (1959) found to be principally concerned with feeding in other species of crustacea examined. The nauplii therefore have to subsist on internal food stores. The accommodation of adequate food stores will also result in the eggs being large, which in turn means that the parents produce fewer eggs. The provision of an internal food supply transferred with the nauplius ensures that the young are independent of external food, and this rather than protection from possible enemies may be the greatest advantage of this copepod having large eggs. The fat filled, non-feeding nauplii can thus essentially be regarded as motile eggs.

A survey of the published literature on copepod egg sizes and development rates shows that the mean egg diameter of $421\mu\text{m}$ recorded here for E. norvegica easily surpasses that of Calanus hyperboreus which at $190\mu\text{m}$ (Conover, 1967) appears to have been, previous to this study, the largest

copepod egg about which development times were available. McLaren et al (1969) observed a development time to hatching of 2.98 days at 6.89°C for C. hyperboreus, and fitted Bělehrádek's (1935, 1957) temperature function equation to data on temperature related development times which predict a hatching time at 10°C of ca. 2.5 days. Both these hatching times are over six times shorter in duration than the mean of 18.7 days found in E. norvegica for the eggs to hatch after the egg sac has been produced. The maximum development time to hatching previously recorded at or around 10°C was that of 6.0 days for the small copepod Acartia tonsa (McLaren et al, 1969). The egg of E. norvegica is therefore notable both for its large size and long developmental time.

There is a well defined linear relationship in E. norvegica between the mean size of the clutch produced and the mean size of the female producing the clutch, with larger sizes of female generally laying more eggs and greater volumes of eggs than smaller females. Similar relationships have been found to exist in many crustaceans (Jensen, 1958; Kinne, 1960, 1961; Sheader and Fu-Shiang Chia, 1970; Mauchline 1973). Green (1956) in cladocerans, and McLaren (1963, 1965) in the copepod Pseudocalanus, have treated the relationship between length and egg number as an example of relative growth of the power curve type using the equation $E = mL^n$, where E is egg number, L is parental length and m and n are constants. It is apparent, however, that the power curve relationship does not fit the data for E. norvegica.

There was nonetheless much variability in the performance of females within the same size group. This large range in the number of eggs per

sac between individuals of the same size-class is probably partly caused by seasonal effects emphasised by combining data from throughout the whole sampling period, but it is also evident that variations in the number of eggs per sac occurred between females of the same size-class collected from the same plankton sample. Consequently it appears unlikely that these changes of clutch size within similar prosome size classes can be accounted for solely by environmental conditions at or close to the time of egg laying. The issue is further complicated by evidence of experimental work on factors affecting egg production in various copepods which often indicate that different species of copepod have different responses to similar ecological factors. For instance, Marshall and Orr (1952) have shown that the quality and quantity of food have a direct effect upon the average number of eggs produced by Calanus finmarchicus, but a more recent study by Corkett and McLaren (1969) on Pseudocalanus spp suggests that food concentration has little effect on the immediate or realized size of the clutches produced once the adult stage has been attained, but acts mainly to lengthen the period between hatching of eggs from one egg sac and the production of the next. The last named authors also found some evidence to indicate that after a period of starvation females lost the reproductive potential of that period.

In E. norvegica, which has a long adult life span of at least three months, the ovigerous population is comprised of females of different ages and even of different generations. Amongst these females there will be individuals which have developed under different conditions of temperature, food abundance and quality, and competition, which are likely to determine the eventual size of the copepod (Bogorov, 1933;

Marshall, 1933; Marshall, Nicholls and Orr, 1934; Ussing, 1938; Wiborg, 1955; Grainger, 1959). It would thus appear that a number of cumulative factors act on individuals to determine the body size of the adult female, which in turn controls the mean size of the clutch produced. The size of egg clutches of Pseudocalanus has been shown to be predictable from size of females, which is in turn determined by environmental temperature (McLaren, 1963). However, the range in clutch size found within individuals of the same body size in E. norvegica suggests that there is some more remote restriction on clutch performance, such as number of broods previously produced and the physiological condition of the individual. Jensen (1958) has suggested that the absolute number of eggs is dependent on external factors but that the relative number depends on the volume of the female. This belief has been substantiated by work on the freshwater egg sac bearing copepod Cyclops strenuus abyssorum (Smyly, 1973) which has demonstrated that seasonal variation in clutch size is not simply a function of seasonal variation in body size but is also influenced by food supply. If this line of reasoning applies to E. norvegica it would imply that at any point in the female's adult life cycle there is an optimum percentage of available energy which can be channeled into egg production to maximise the copepods contribution to future generations. The energy available for reproduction would therefore be limited, by various factors to a finite amount at any time.

Smyly (1973) has found in C. strenuus abyssorum that the upper limit to clutch volume set by thoracic size is of the order of 20% of the thorax. The upper limit to clutch volume set by total body volume in E. norvegica has been found in the present study to be about 14% of the body, with a mean value of about 8%, which is appreciably less than in C. strenuus

abyssorum even if the latter clutch volume was expressed in terms of total body volume and not thoracic volume. It is however comparable with the levels of about 10% of the body volume of the parent found in the euphausiids Meganyctiphanes norvegica and Thysanoessa raschi (Mauchline, 1968) and in many British mysids (Mauchline, 1973).

Wilson and Parrish (1971) have found in Acartia tonsa kept under controlled laboratory conditions that females eventually produce 100% infertile eggs if they are not remated, as well as strong suggestions that remating also caused an increase in the egg production rate which existed prior^{ly} to mating. The subsequent remating also resulted in all eggs hatching. Such considerations may partly account for those adult female E. norvegica found carrying low numbers of eggs within their sacs with respect to body size, however, it is doubtful whether shortage of sperm for egg fertilization is a limiting factor, at the population level, during synchronized mating cycles under natural conditions. Examination of females carrying low numbers of eggs for their body size, collected from the main breeding period of February/March in Loch Etive, does not indicate any unusual features in the percentage of infertile eggs, and Marshall and Orr (1955) remark *that* the fact that very few Calanus finmarchicus remain unfertilized is obvious from examination of spermathecal sacs which are almost always dark and full.

One of the few published estimates of levels of copepod egg mortality under situations where females have had free access to males is that of Paffenhoffer (1970) for Calanus helgolandicus, who discovered an egg mortality rate of 16% under laboratory conditions, which was close to values obtained from spermatophore carrying females from the ocean. The 4% egg mortality level found here for E. norvegica is lower than the values found for Calanus, a situation probably to be expected in a

copepod which produces fewer eggs and carries them within an egg sac.

Large amounts of lipid and protein, normally synthesised from the food intake, are needed during development of the crustacean ovaries (Pillay and McNair, 1973). The low wet weight:dry weight ratio of 1.6:1.0 found for the eggs of E. norvegica demonstrates that the water content of the eggs is very low, which further suggests that the quantity of organic material within the eggs is very high. This organic material is presumably mainly accounted for by lipid and yolk protein, the lipid being easily seen in histological preparations in the form of numerous globules. Some indication of the drain on the organic reserves of the female is shown by the fact that the amount of dry weight transferred to the eggs may exceed 25% of that remaining in the female's body. Given that the mean development time of the eggs to maturity in the ovary and oviducts has been found to be about 22 days this would then represent an increase in the weight of the eggs in the ovary and oviducts of approximately 1.1% of maternal dry body weight per day.

In the majority of marine copepods the eggs are shed directly into the surrounding water thereby ridding the mother of the weight of the eggs. However, egg sac bearing copepods transport their eggs for a further period of time until hatching occurs. In the case of E. norvegica the effort involved in movement whilst carrying the egg sac, which is equivalent to 6% of its live body weight and 8% of its body volume, for 19 days must be of consequence in terms of the drag likely to be exerted. Thus energy expended in egg production in Euchaeta is not restricted solely to the maturation of the ova within the body, but encompasses additional energy metabolised whilst transporting the egg sac.

On average larger sizes of female E. norvegica produced a greater volume and number of eggs than smaller females but there was evidence to suggest that smaller sizes of females generally produced a greater number and volume of eggs per unit body volume than larger females. As there is no significant difference between the size of eggs produced by large and small females it may imply that the smaller females are more efficient in producing egg material, but as yolk is generally synthesised from food intake it requires additional information on ingestion and assimilation values before efficiency can be discussed meaningfully in terms of gross and net egg production.

The observed potential of females to delay the calculated time of egg laying by an estimated two weeks, under what could be construed as abnormal environmental conditions, and then to eventually resorb the egg material back into the body when these conditions are prolonged even further, suggests a number of possible advantages to the individual and the population. Hatching of eggs under adverse environmental conditions is likely to increase the stress and mortality exerted upon the younger developmental stages, and so affect eventual recruitment to the adult stock. However, if conditions beneficial to the young appear within a short period of time egg laying may be able to proceed unhindered. If adverse conditions persist for long periods then any eggs within the female can be resorbed with possible benefit to the mother. An ability to delay liberation of ova has been postulated in Calanus, which though ready to lay eggs, do not necessarily do so, but may hold them up until conditions are favourable (Marshall and Orr, 1955).

Fig. 21. The relationship between the prosome length (mm)
of adult female Euchaeta norvegica and mean numbers
of eggs in their egg sacs.

(mm)
numbers

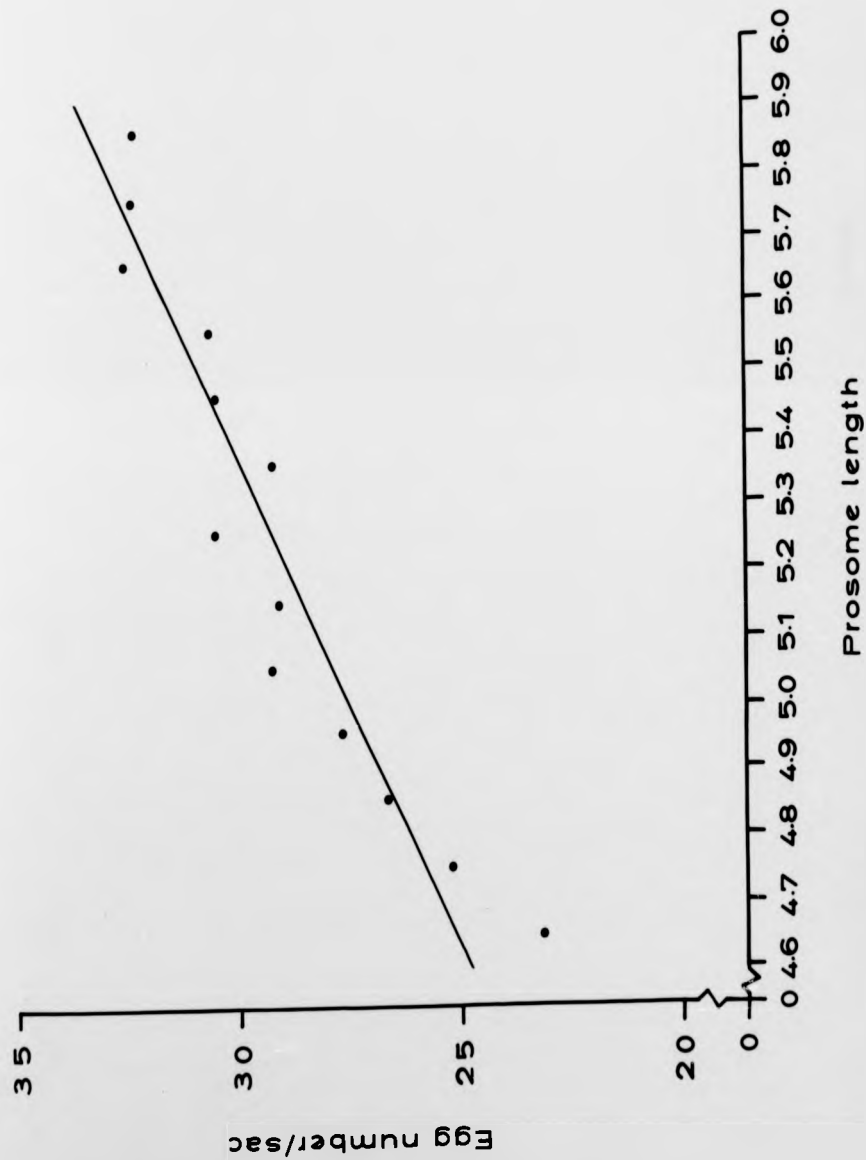


Fig. 22. The relationship between the prosome length³
of adult female Euchaeta norvegica and mean
number of eggs in their egg sacs.

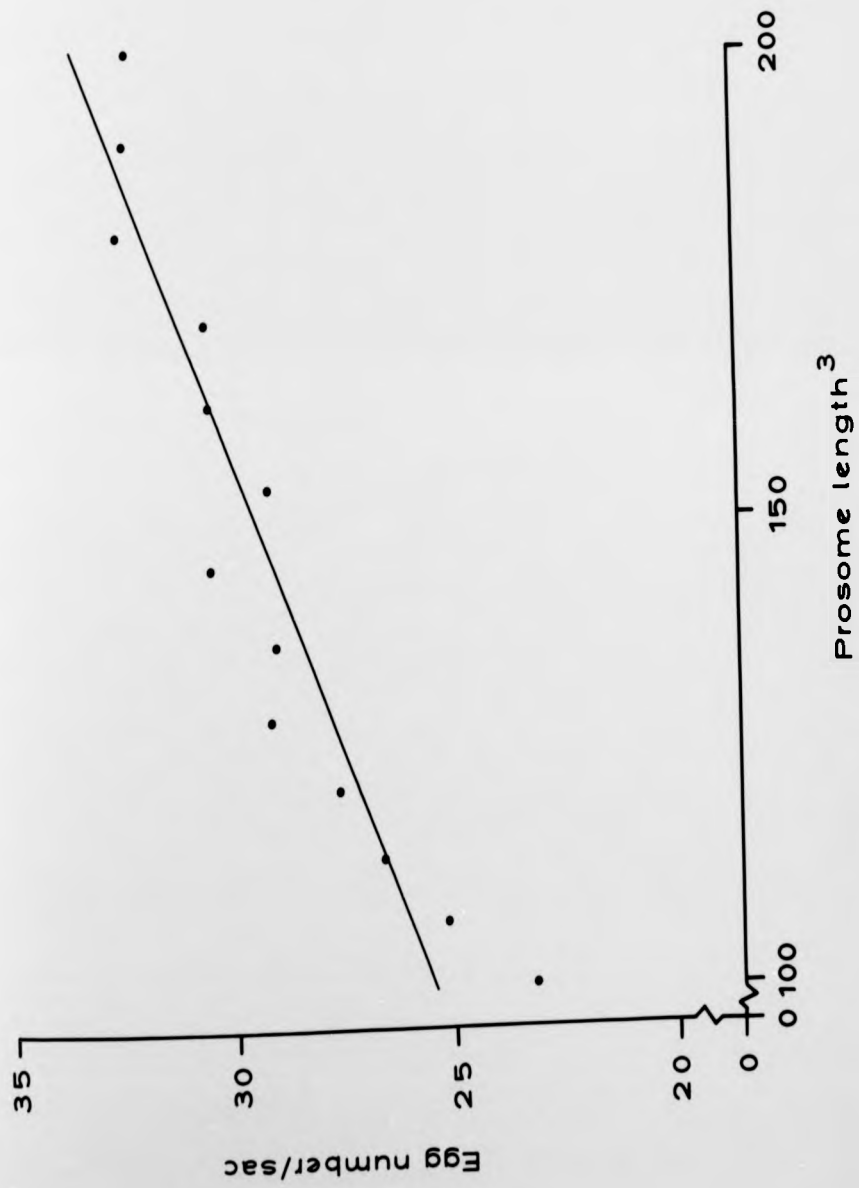
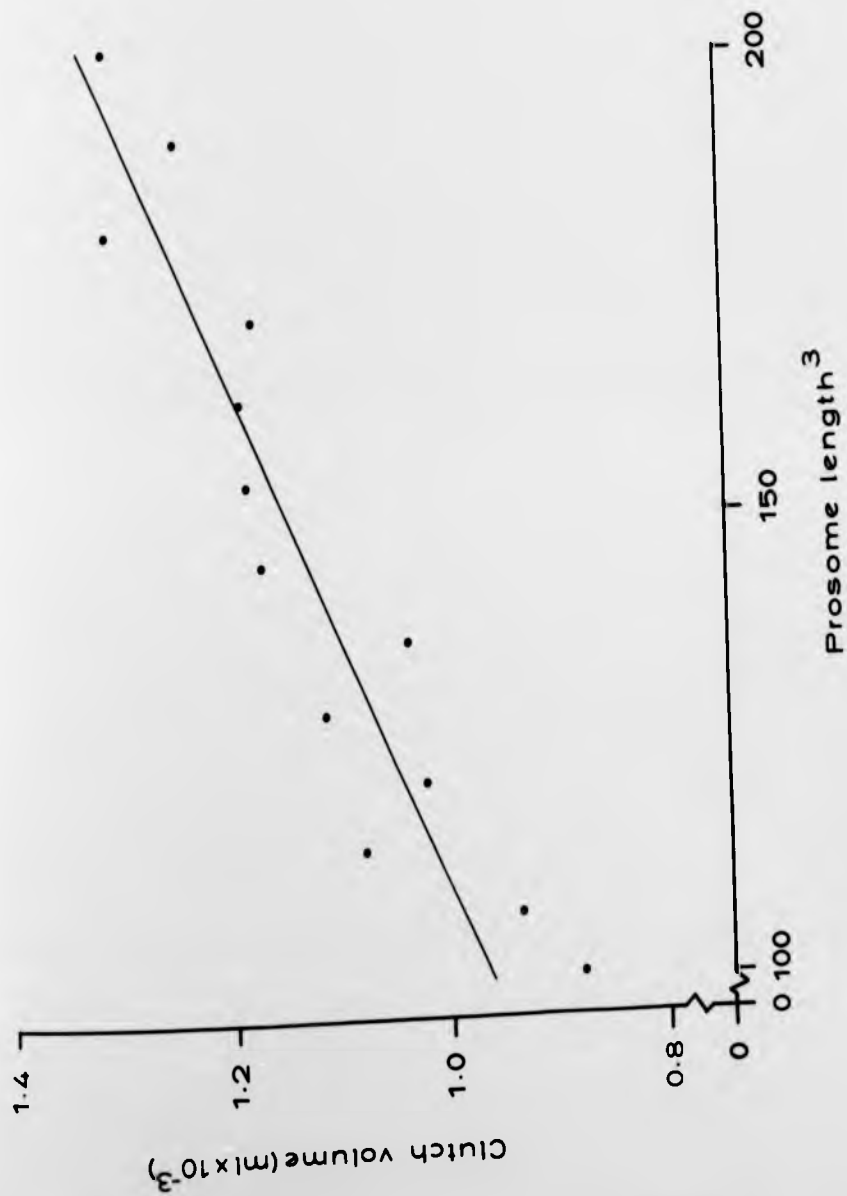


Fig. 23. The relationship between the prosome length³
of adult female Euchaeta norvegica and the mean
volumes of the egg clutch.

3
mean



CHAPTER 7

SEX RATIOS AND BREEDING CYCLESINTRODUCTION

The study of sex ratios in animals has begun to receive an increasing amount of attention in recent years. A number of investigators have stressed the selective advantage in most populations of having an equal frequency of males and females at birth or at the time of gaining independence from the parents (eg McArthur, 1961; Leigh, 1970). However, many others have emphasised deviations from an expected 1:1 sex ratio (eg Mednikov, 1961; Conover, 1965; Darnell, 1962; Maly, 1970).

Fisher's theory of sex ratio (Fisher, 1930; Kolman, 1960) favours a 1:1 expenditure by the parents on male and female offspring. It has been pointed out by Willson & Pianka (1963) that an equal sex ratio is expected with Fisher's theory provided that it is equally expensive to produce individuals of both sexes. They have also noted that differential mortality between the sexes and other factors which create a differential in the costs of producing offspring of each sex, such as differential growth rates or size differences between the sexes during periods of dependence upon the parents, can produce skewed sex ratios.

In temperate northern waters the breeding cycle in calanoid copepods usually starts in spring and proceeds through the summer with the production of one or more generations, with mating and egg laying having ceased by late autumn (Fish, 1936a, b, c; Marshall, 1949; Digby, 1950, 1954; Deevey, 1952a, b; Marshall & Orr, 1955). Even in calanoids with polycyclical generations there is a relatively well defined breeding

season resulting in the production of an overwintering population of adults and late stage copepodites which, as implied, are able to pass through the winter months and initiate breeding the following spring. The different generations are usually incompletely separated, with breeding becoming more or less continuous during the summer and the early autumn. Raymont (1963) has provided an extensive summary of work carried out on the breeding cycles of the various species of planktonic copepods which supports this generally accepted breeding pattern.

Our present knowledge of the breeding cycles of Euchaeta norvegica is generally sparse and is mainly dependent on the published works of Bigelow (1926), Ruud (1929), Runnstrom (1932), Wiborg (1940, 1953) and Østvedt (1955). More recently, brief additions to our knowledge have been made by Williams & Hopkins (1975, 1976), whilst more substantial contributions have been made by Båmstedt (1975) and Båmstedt & Matthews (1975).

This chapter describes the results of investigations into the juvenile and adult sex ratios as well as the breeding cycle of E. norvegica in Loch Etive.

It has previously been standard marine ecological practice to study breeding cycles of copepods using the generation cycle method which utilizes counts of the abundance of the various developmental stages. This method has the advantage of allowing the researcher to follow successive broods (cohorts) as they pass through the various stages and, ideally, to determine rates of production. However, most workers have tended to avoid counting back further than the 1st stage copepodite or naupliar stages and thus the time of mating and fertilization can be

defined only with difficulty. The present study has been designed to trace the precise time of mating and breeding back as closely as possible, by making counts of egg sacs and attached spermatophores associated with standard samples of adult females. Analysis of this kind has in its favour the fact that it involves determinations of the rate and intensity of mating and breeding using the raw materials of a generation ie sperm and eggs. These features, and especially their rate of "turn-over", are to a great extent responsible in determining the increment that is likely to be made to the population as well as the initial potential strength of a generation.

MATERIALS AND METHODS

The material used to determine sex ratios, spermatophore abundance and egg sac production was collected during the course of the standard plankton sampling programme in the upper basin of Loch Etive (see Chapter 1).

500 adult females were always examined to determine the frequency of spermatophores and egg sacs. The number of adult males examined varied in proportion to their relative abundance in the adult population at the time of sampling. Stage V copepodites of E. norvegica are efficiently sampled by the 1 metre diameter stramin net and sex ratios for this copepodite stage were based on counts of at least 200 individuals. The majority of stage IV copepodites, however, are lost through the meshes of the net during towing and thus, even though 100 individuals were always counted to determine the sex ratio, the relative abundance of this copepodite stage and the larger developmental stages cannot be directly compared without applying a correction factor which would take into account loss of individuals through the meshes.

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For the analysis of egg production both egg sacs still attached to the females urosomes as well as detached egg sacs were counted and the number of each were separately recorded. Orr (1934) found that analysis of ovigerous E. norvegica was to some extent a risky proceeding, as it is not always possible to tell if a so-called non-ovigerous female was one in which the egg sac had become detached. He also discovered that comparatively slight disturbance was often enough to cause the egg sac to be dropped. The loose egg sacs in the samples were therefore counted to attempt to ascertain to what extent the results might be affected by ignoring detached egg sacs.

The information obtained has been used to define seasonal cycles in sex ratio, spermatophore attachment and egg production with the subsequent determination of breeding cycles.

RESULTS

Sex Ratios in copepodite stages IV and V

When sampling started on 27 September 1971, 93.8% of the stage IV copepodites (Fig. 24) and 97.8% of the stage V copepodites (Fig. 25) were males. Males remained numerically dominant in both stage IV and stage V copepodites until late December and early January, when a sharp change took place in the sex ratio of these two stages which resulted in a rapid increase in the percentage of females present. The percentage of males in the stage IV copepodite population reached its lowest value of 14.7% on 24 February 1972 whilst the lowest value of 21.5% for stage V males occurred approximately one month later on 20 March. The male sex ratio then began to rise steadily once more and by the end of sampling on 24 August the male sex ratio of stage IV and stage V copepodites was close to reaching its maximum level once more. The stage IV and stage V sex ratio therefore varies widely during the year with males being significantly dominant from June to January, and females being especially

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abundant during March, April and May. However, taken over the whole sampling period males and females in copepodite stages IV and V were equally numerous even though there were large seasonal variations in the sex ratio.

Sex ratio in the adults:

The adult (stage VI) sex ratio strongly favoured females throughout the year (Fig. 26) but there were two peak periods when the percentage of males in the adult population noticeably increased. The major peak in abundance of adult males (31.4% of the adult population) occurred in early March 1971 whilst the second, smaller peak occurred in early July. The percentage of adult males fell to its lowest value of 2.7% on 25 October and again on 12 November. The mean adult sex ratio per haul taken over the whole sampling period was 11.3% males and 88.7% females (0.13:1.0 in favour of females).

Breeding cycles:

Females with attached spermatophores and carrying egg sacs were found in all the plankton hauls taken. At the start of sampling on 27 September 1971 the number of attached spermatophores and the total number of egg sacs were low (284 spermatophores and 60 egg sacs per 500 adult females) and declined towards a combined low in late October (Fig. 27a). At this time egg laying was at a minimum with only 2-3 per cent of the adult female population carrying egg sacs. After this, spermatophore attachment and egg production increased steadily throughout November, December and January to reach maximum numbers of attached spermatophores and total egg sacs in late February and early March (499 spermatophores and 242 egg sacs per 500 adult females). Once this peak had been reached the strength and intensity of breeding declined rapidly

so that by the middle of April a basal level, similar to that of the previous October, was again reached. Spermatophore attachment and egg production began to pick up very quickly and soon reached a summer peak of egg laying in June (total of 184 egg sacs per 500 adult females) and a maximum number of attached spermatophores (399 spermatophores per 500 adult females) in late July and early August. The intensity of breeding then began to decrease steadily once more towards the low October level.

The number of detached egg sacs in the samples varied from 5.7% to 66.1% of the total egg sacs. The greatest percentages of detached egg sacs generally occurred in February, March and June. The lowest proportion of detached egg sacs occurred in late October/early November and from April to mid-May.

The number of adult males found per standard sample of 500 adult females is graphed below the numbers of spermatophores and egg sacs in Fig. 27b, and generally shows a close agreement with the trends in spermatophore attachment and egg sac production. The main discrepancy between the two sets of graphs is visible in the occurrence of the highest proportion of males in early March whilst the maximum number of spermatophores is found in late February. Fig. 30 illustrates the strong positive correlations which exist between the proportion of adult males present and:

- a) the total number of spermatophores attached ($r = 0.81$, $P < 0.001$)
 - b) the number of females found with attached spermatophores ($r = 0.71$, $P < 0.001$)
 - c) the total number of egg sacs ($r = 0.76$, $P < 0.001$)
- in each standard sample of 500 adult females in the 21 hauls.

Water Temperature:

Surface water temperatures found in Loch Etive between 9 September 1971 and 1 September 1972 are graphed in Fig. 28. These temperatures followed the general pattern found in most Scottish west-coast, fjordic sea lochs in the fact that the water reaches its highest temperatures between July and September and becomes coldest in February and March. The winter water temperature range in the loch is usually 5.6-8.5°C and the summer range is in the order of 15.0-13.4°C (both surface to bottom) followed by stratification in the autumn (Milne, 1972). The surface water temperature records are shown starting on 9 September 1971 at 14.7°C and thereafter show a continued fall in temperature to a minimum temperature of 5.0°C on 4 February 1972. Temperatures then proceeded to increase steadily reaching a maximum of 18.7°C on 20 July 1972. However, this pronounced peak in late July is somewhat unusual and was the result of a short period of combined high air surface temperatures and lack of wind. The surface water temperature of 15.0°C recorded on 1 September is more characteristic of the normal annual maximum temperature.

DISCUSSION

Sex ratios:

Østvedt (1955) working at Weather Station "Mike" (66°N 2°E) recorded mean sex ratios that are very similar to those found during the present study in Loch Etive. He found in stage IV and stage V that there were about 50% of each sex, while in stage VI the males were much fewer, being only 17.5% of the adult population. Wiborg (1954) working in Norwegian coastal waters found relatively higher percentages of adult males taken over the whole sampling year in 1949 and 1950 with 26.0% and 27.0% respectively but found in the copepodites of stage V that the two sexes were represented in about equal numbers. The majority of authors working on copepods have not discussed the sex ratios of immature copepodites

but a similar situation to that found for E. norvegica in Loch Etive has been described by Marshall (1949) who found that even while females predominated in the adult stage, the sex ratios were nearly even among the copepodites.

The dramatic seasonal variation in the sex ratio of copepodite stage IV and copepodite stage V in Loch Etive suggests that the sex ratio may well be influenced by environmental factors acting on the female copepod, at, or close to the time of egg laying, or else upon the early developmental stages before the moult to stage IV copepodites when sexual differentiation first becomes externally evident in the presence or absence of the fifth pair of thoracic limbs.

It is known that there exists a genetic difference between male and female organisms which is responsible for sex determination, and Heberer (1924) and Harding (in Marshall & Orr, 1955) have favoured the view that sex determination and sexual differentiation in copepods are the result of a balance of male and female genes. However, this classic concept of "genetic balance of sex" (see Johnson, 1961; Tinturier-Hamelin, 1963) confuses the existence in all individuals of genes for all sex characters with the genetic system controlling the activity of these genes.

Battaglia (1958a, b, 1960) has argued that environmental factors, especially temperature, seem to determine sex ratio in certain crustaceans. He observed in the benthic species Porcellidium ^mfibriatum that the number of males decreased as the conditions become colder and correlated fluctuations in the sex ratio with the water temperature. Predominance of males during warm periods and of females during cold periods have been reported for different copepods by a number of authors (Ruttner, 1929; Takeda, 1950; Egami, 1951).

Takeda (1939, 1941a, b) found that various chemical substances could act as sex controlling factors in the marine copepod Tigriopus japonicus, and later showed that temperature had the same effect (1948, 1950). Egami (1951), again working with T. japonicus, found that lowering of pH and decrease of temperature induced a condition "favourable for feminization", and stated that feminization of the copepod took place, as a rule, under conditions which caused a decrease of developmental velocity. His experiments indicated that the sex of T. japonicus was determined between the last naupliar stage and copepodite stage I.

Kinne (1959) working with the amphipod Gammarus duebeni observed that variations in the sex ratio were the result of a temperature controlled sex determination but concluded, however, that low temperature increased the ratio of males. Under experimental conditions he was able to vary the sex ratio by altering the temperature conditions. Parents kept in temperatures below 5°C produced only males, parents kept in temperatures above 8°C produced only females, whilst in those kept between 5° and 8°C broods consisted of males and females. He also discovered that there was a sensitive phase during which temperature influenced the sex of the eggs in the ovarium and that this was restricted to a short period before oviposition.

Battaglia (1958a) conducted various inbreeding experiments on the marine copepod Tisbe gracilis which pointed to the existence of various sex genotypes, and put forward the hypothesis that development into one or other sexual phenotype is conditioned by the degree of heterozygosity of a number of genes closely concerned with sex determination. The manifestation of the female sex would require a high level of heterozygosity whereas homozygosity would lead to a development of the male phenotype.

More recently, however, the hormonal control of sexual differentiation in invertebrates has been established by means of experimental sex reversal in such diverse groups as the higher crustaceans, coleopterans and hydroids (Charmaux-Cotton 1959, 1960, 1965). The results of these experiments have shown in a number of crustaceans that females may be completely masculinized by grafting the androgenic tissue which secretes the male hormone. Removal of the androgenic tissue converts a male crustacean into a female; its testis becomes an ovary and secretes hormones responsible for the female's secondary sex characters.

The sex of the individual crustacean thus can depend on the presence or absence of the male hormone. A female hormone also exists in some crustaceans which, in the absence of the male hormone, controls the formation of the secondary sex characters but does not control the sex of the organism. Each individual therefore possesses all the genes necessary for the differentiation of the primary and secondary characters of both sexes, however, the activity of this hermaphrodite genome is regulated by the male hormone. In its presence genes controlling male characters are active, in its absence female genes are active. Since sex is under genetic control it is logical to assume that secretion of androgenic hormone in the male, and its absence in the female are due to genetic differences between the sexes. Genetic determination of sex thus consists of the genetic control of secretion of an androgenic hormone. These genes are, however, different from those genes which are responsible for the formation of male and female sex characters, and which are possessed by all individuals. While the androgenic gland has not been demonstrated so far in the Copepoda, neurosecretory cells have been (Carlisle & Pitman, 1961) and it is a distinct possibility that sexual differentiation in copepods is under the control of a simple endocrine system, the function of which could be affected by environmental factors.

The close similarity in pattern seen in the seasonal change in surface water temperature and the seasonal variation in sex ratio within copepodite stage IV suggests that these two features may well be related. There is a greater percentage of stage IV males present in the loch during the warmer months and a high proportion of stage IV females present during the colder months of the year; a situation which is comparable with the findings of Battaglia (1958a, b, 1960). It is well known that juvenile copepods tend to inhabit the more surface waters of the sea (Nicholls, 1933; Marshall & Orr, 1955; Raymont, 1963), and it appears to be plausible to expect the juvenile stages of E. norvegica which contain large proportions of oil, to congregate in the more surface waters of Loch Etive. Grøn (1902), Wolfenden (1904), With (1915) and Østvedt (1955) found that the younger copepodite stages of E. norvegica were more frequently collected in the surface water layers, whilst the older stages were mostly found in the deeper layers. It is therefore in the more surface waters that the effects of temperature would be most likely to act upon the younger copepods if it were to have any influence on their sexual differentiation.

The mean sex ratio of copepodite stage IV in each of the 12 months sampled has been plotted against the respective mean monthly sea surface temperatures in Fig. 29 and reveals that a strong positive correlation ($r = 0.89$, $P < 0.001$) exists between the two variables. If water temperature and sex determination are related in E. norvegica it may well be as a result of temperature acting directly upon morphogenesis in the manner described for Carausius morosus by Bergerard (1961), or it may act indirectly through the functioning of a hormonal system comparable to that described for Orchestia gamarella by Charniaux-Cotton (1959). Heinle (1970) has suggested in Acartia tonsa that a portion of the genetic males in fact become phenotypic females during the course of their growth.

Obvious intersex forms of E. norvegica were absent from Loch Etive but stage IV females with small cuticular protuberances where a developing fifth limb would appear in the male, were often common and may possibly represent a similar state of affairs to that suggested for A. tonsa. Correlation of water temperature and juvenile sex ratio in E. norvegica may also come about as a result of temperature acting on the seasonal succession of phytoplankton and zooplankton which are incorporated in the diet of younger copepodites. Paffenhoffer (1970) discovered that different food species can cause markedly different sex ratios in Calanus helgolandicus under controlled laboratory conditions and came to the general assumption that food quality may be one of the major factors influencing sex ratio.

The adult sex ratio of E. norvegica in Loch Etive differs greatly from that of the stage IV and stage V copepodites, and would imply that the change in pattern of the sex ratio from stage V to stage VI comes about as a result of a significant alteration in differential mortality between the sexes in the adult stage. Differential mortality of the sexes may arise as a result of a number of factors. Maly (1970) investigated the effects of differential mortality produced by one sex having a different life habit (eg behaviour, colouration) which may result in that sex either escaping predation or actually being selectively preyed upon. He concluded from laboratory studies that predators can alter the sex ratio in prey species if there is a sexual dimorphism or behavioural difference between the two sexes. The main predatory organisms large enough to tackle adult E. norvegica in Loch Etive are fish, chaetognaths and medusae. Various species of fish are present in the loch (eg Whiting Merlangus merlangus and Herring Clupea harengus) but the presence of either male or female E. norvegica in their guts is

rare (J D Gordon, Pers.comm.). Chaetognaths found in the loch (Sagitta elegans) are able to feed on the younger copepodite stages but are not large enough to prey on the adult stages. Medusae (eg Aurelia aurita) can often be found in large numbers and although theoretically large enough to capture adult E. norvegica have not provided any evidence of actually having done so. Adult E. norvegica therefore appear to be practically free from predation, and selective predation of adult males is highly unlikely to be a cause of the well defined departure from an expected 1:1 sex ratio.

A shorter life span in the adult male which may be caused by a restricted nutrition is a likely factor influencing the adult sex ratio in E. norvegica. The scarcity of adult males coupled with the fact that they are, on average, only slightly larger and heavier than stage V males in Loch Etive suggests that little or no increase in weight takes place after a stage V male moults into an adult male (Table 13). Sars (1903) showed that the feeding appendages of adult male E. norvegica are greatly reduced when compared with the female condition, and With (1915) stated that the maxillules are useless for mastication. An examination made during the course of the present study of male and female mouth parts of E. norvegica in Loch Etive has revealed that they are very similar in both sexes in stage IV and stage V copepodites; the mandibles in particular being practically identical in both size and dentition. On moulting into adults the male mouth parts are appreciably smaller than those of the female copepod. In the adult stage the male mandible is much smaller than the mandible of the stage V male and lacks developed denticles along the mandibular blade, being reduced to a small rounded peg by that stage of development. Examination of histological preparations of adult male and female alimentary tracts reveal that the male gut is

TABLE 13: Length/weight characteristics of stage V and stage VI Euchaeta norvegica from Loch Etive showing the increase in growth achieved by the stage VI male and female from the stage V copepodite.

Stage	Total Length (mm)	% Increase	Wet Weight (μ g)	% Increase	Dry Weight (μ g)	% Increase
VI ♀♀	7.79	36.7	12630	166.4	1804	162.2
V (♂♂+♀♀)	5.70		4740		688	
VI ♂♂	6.19	8.6	4796	1.2	992	44.0
V (♂♂+♀♀)	5.70		4740		688	

greatly atrophied, having appreciably thinner walls and fewer and shorter villi than that of the female sex. These details support the suggestion made by Orr (1934) that after moulting takes place the adult male does not feed, and would likely account for the drastic reduction in the proportion of males present when compared with the stage V copepodite. Mednikov (1961) suggested the possibility of an ecological cause for the scarcity of adult males in some species of copepod and concluded that a "trophic" factor was involved and that a low proportion of males is an adaptation for increasing fecundity when food is scarce. Non-feeding adult males in E. norvegica ideally fulfill Mednikov's hypothesis in that they offer little if any competition for food resources with adult females, and therefore have the advantage of not placing a toll upon the energy budget of the population.

Bogorov (1939), Campbell (1934) and Chislenko (1964) have reported that specimens of male copepods were short lived and died soon after copulation, and have postulated that this leads to the inequality of the sex ratio in marine collections. Observations made during the present study on adult male E. norvegica have shown that males of this species are able to survive for at least 2-3 days after they have ejaculated their spermatophores and have gripped them in their left fifth thoracic limbs.

Bamstedt (1975) has pointed out that as adult males do not feed, the period an individual can spend in the population is ultimately limited by the food resources and their rate of utilization. Knowledge of the rate of lipid utilization and the individual lipid content of adult males have allowed him to define a life expectancy in excess of 20 days

for 80% of the stock. He has also been able to calculate a mortality rate of 0.8% per day for the first 20 days and 3.6% during the following period; after 29 days 50% of the starting stock would have died, and after 41 days the whole stock would be replaced by new individuals.

It has been estimated that an adult male Euchaeta in Loch Etive is likely to produce about three spermatophores during its life and that each spermatophore represents approximately 0.9% of the total dry body weight (see Chapter 3). This knowledge used in conjunction with Bámstedt's figure of a 50% mortality level after 29 days for adult males allows the total spermatophore production during the adult developmental stage to be estimated at about 0.1% of total dry body weight per day. However, such an apparently small drain on body reserves probably assumes a greater significance when one considers that this and other losses are not being replaced by feeding. Nevertheless, the actual process of spermatophore production and extrusion is unlikely to be the cause of early death and uneven sex ratios, although the act of clasping the female may possibly impose some degree of additional strain on the male.

Breeding cycles:

It has been revealed that there are two main periods of breeding activity in E. norvegica in Loch Etive. The first, main peak of activity involving the attachment of spermatophores and subsequent production of egg sacs occurs in late February and early March, whilst the second less highly developed peak occurs from June-August. Provided that each adult female copepod requires a standard number of spermatophores and hence sperm to fertilize a given number of eggs, and that the offspring produced from both peaks of breeding are able to develop into

adults without any unusual set-backs, then it seems reasonable to infer that this copepod produces two major generations per year in Loch Etive.

These results are in general agreement with previously published details of the reproductive cycles of E. norvegica, most of which have been based on sampling conducted in waters between Iceland and Norway.

Runnstrom (1932) reported that propagation takes place from January to April and from June to September in the fjords near Bergen in Norway. Wiborg (1954) in studies made in Norwegian coastal waters found females carrying egg sacs in nearly every month of the year and postulated that reproduction took place all the year round, as stated by earlier investigators (Bigelow, 1926; Ruud, 1929; Wiborg, 1940), but maxima in reproduction seemed to occur in two periods, the first from December to January, decreasing to May, and the second somewhat more extended, in June-August. Østvedt (1955) recorded nauplii, males with spermatophores and females bearing spermatophores and egg sacs at all times of the year and postulated that "spawning" took place throughout the year although it was at its most intense in winter and early spring.

Båmstedt & Matthews (1975) working on a deep-water population of E. norvegica in Korsfjorden, Western Norway, have drawn attention to the production of two major generations but have emphasised the fact that they had quite distinctive features; the winter generation had a brief, intensive fertilization period followed by concentrated spawning, while the summer generation was more protracted and less intense. There was also a difference in recruitment success; the winter stock of adult females gave rise to twice as many adult females the following summer.

Recent work in the north-east Atlantic at Ocean Weather Station "INDIA" (59°00 N 19°00 W) reported by Williams & Hopkins (1975, 1976) suggests that there is one major peak of breeding with occasional signs of a weak, second peak of breeding in late summer. It may be a characteristic of E. norvegica that populations living in sea lochs, fjords and coastal waters produce a well defined 2nd generation in summer whilst deep sea, oceanic populations have more of a tendency towards producing a single spring-early summer generation, with occasional signs of a poorly developed later breeding period.

Orr (1934) has stated that comparatively slight disturbance of adult female E. norvegica is sufficient to cause their egg sacs to become detached. However, an intensive series of experiments and observations made on live, egg sac bearing adult females during the course of the present study has revealed that a high degree of disturbance, coupled with physical contact, was required to dislodge the egg sac from the female's urosome. Struggling females carrying egg sacs were often removed from the water using forceps without their sacs becoming dislodged. The more recently produced egg sacs were firmly attached whereas there was a tendency for the more mature egg sacs, with their contents in a relatively well advanced state of development, to be the ones more likely to drop off. These observations, made on living material, imply that most egg sacs become detached after the samples have been preserved in formalin.

It can be seen from Fig. 27 that there is a well defined difference between the graph line showing the number of egg sacs attached to females and the graph line which includes detached egg sacs. It is evident that there are substantial numbers of detached egg sacs at the height of each of the breeding peaks. Calculation of the "standing stock"

of attached and total egg sacs over the period sampled (330 days) reveals that the detached egg sacs accounted for 41.0% of the total number of egg sacs produced. Future assessments of production involving calculations based only on the attached egg sacs found in plankton samples could well introduce unacceptably high levels of error.

The close correlation between the proportion of adult males present in the population and

- i the total number of spermatophores attached to adult females
- ii the number of adult females found with attached spermatophores, and
- iii the total number of egg sacs present

per standard sample of 500 adult females is shown in Figs. 30a, b and c respectively and, as is to be expected, demonstrates that these features are closely related in influencing the breeding cycle. There are, however, signs in these graphs that there is some "tail-off" in the relationships at high male:female ratios, and this is reflected in Fig. 27 where it is evident that the highest proportion of males in the adult population occurs in early March, some two weeks after the peak of attached spermatophores has occurred. This discrepancy may have occurred as a result of sampling "error" but may well be the result of a "saturation" effect in which the presence of attached spermatophores on females causes a slowing down in the rate of further attachments. Additional evidence of this "saturation" effect has been provided in a statistical analysis of spermatophore placement patterns in Chapter 5.

The interdependence of adult sex ratio, spermatophores and egg sacs is logical, as it is reasonable to believe that the number of spermatophores produced and attached is determined by the proportion of males present in the adult population. The number of available spermatophores, up to the

point where multiple attachments of spermatophores on females may become fairly frequent, is likely to influence the proportion of females which are mated, fertilized and subsequently produce egg sacs. Thus the adult sex ratio appears to be a major factor controlling the rate and intensity of breeding in E. norvegica in this loch.

Fig. 24. Seasonal variation in the sex ratio of stage IV
copepodites of Euchaeta norvegica in Loch Etive

stage IV
beh Etive

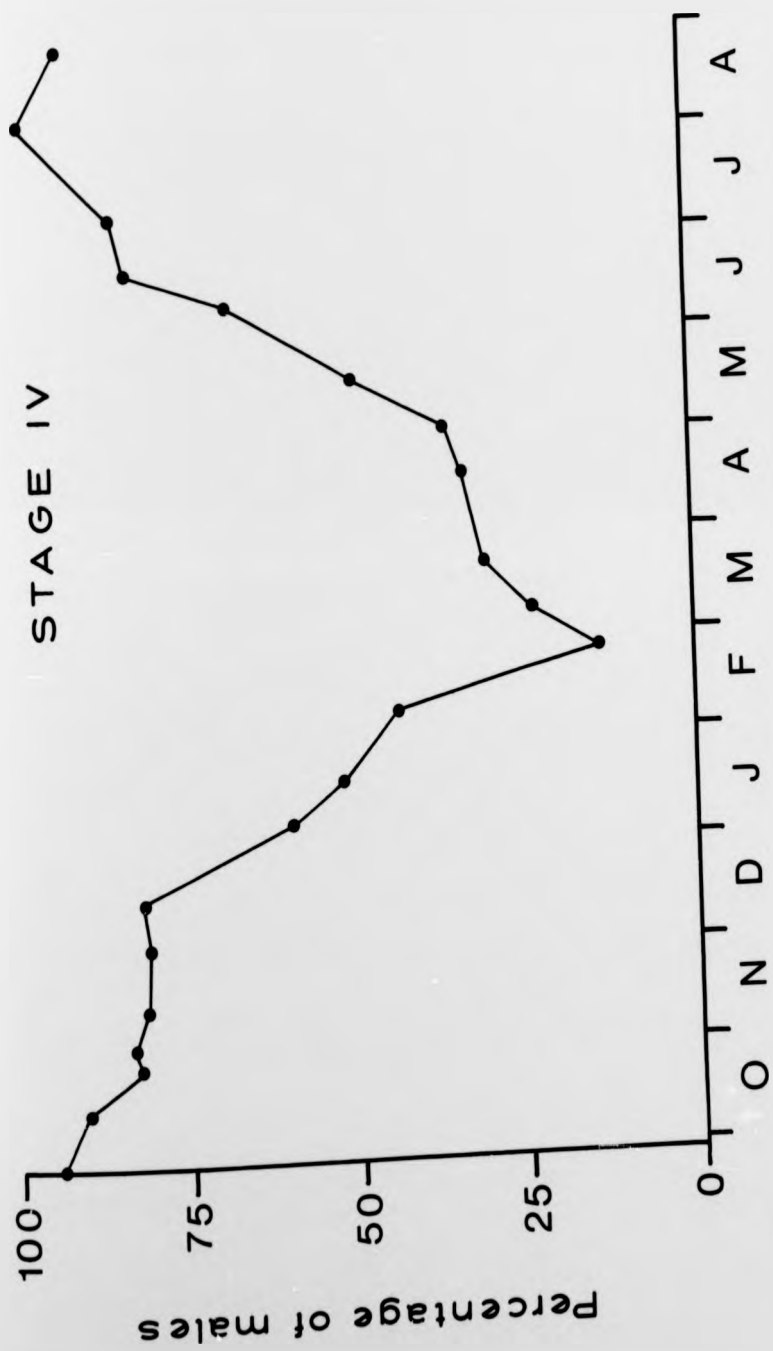


Fig. 25. Seasonal variation in the sex ratio of stage V
copepodites of Euchaeta norvegica in Loch Etive

stage V
och Etive



Fig. 26. Seasonal variation in the sex ratio of stage VI
copepodites of Euchaeta norvegica in Loch Etive

f stage VI

Loch Etive

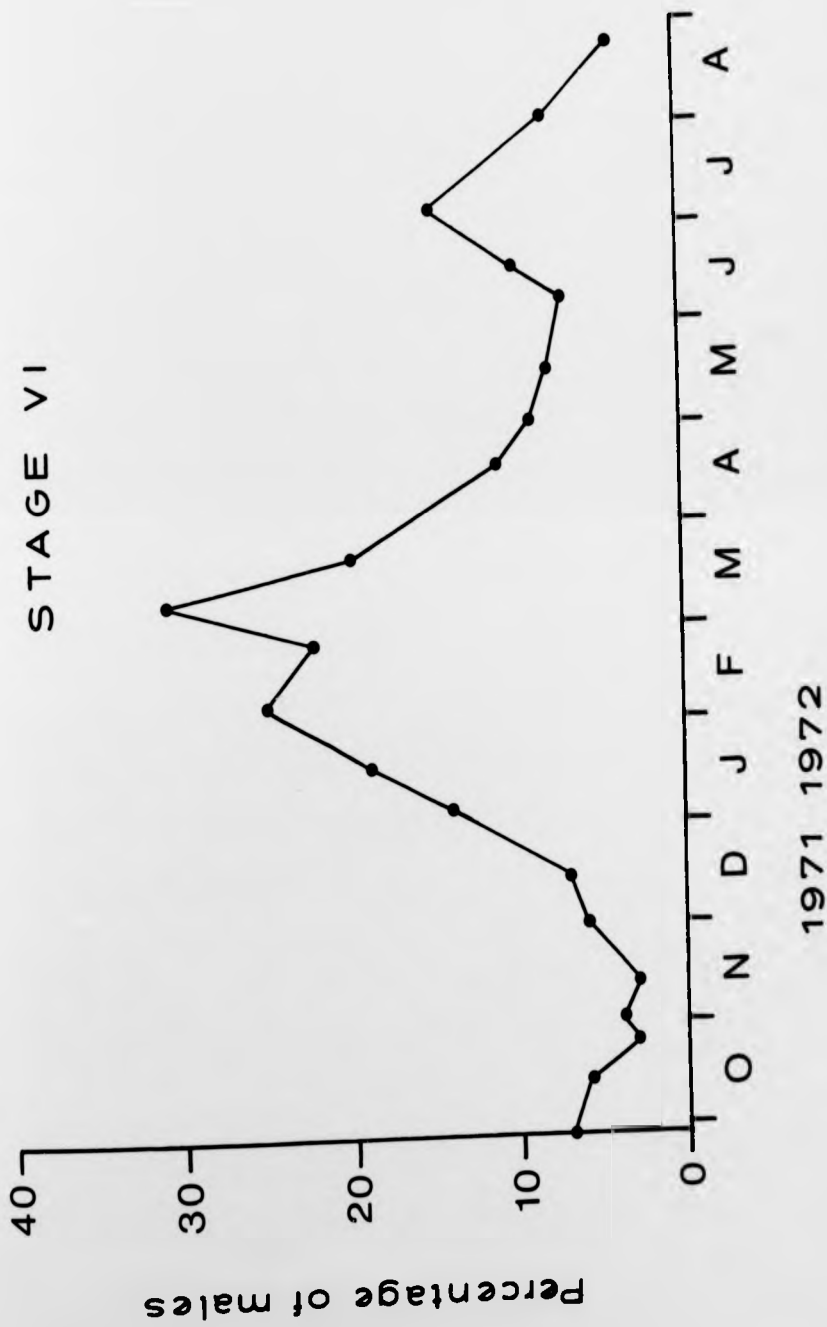


Fig. 27

- a. Seasonal variation in the number of attached spermatophores (solid line with stars) and egg sacs (total egg sacs = solid line with filled-in circles; attached egg sacs = broken line with open circles) for Euchaeta norvegica in Loch Etive
- b. Seasonal variation in the number of adult males of Euchaeta norvegica in Loch Etive. All numbers in (a) and (b) are expressed as numbers found per standard sample of 500 adult females

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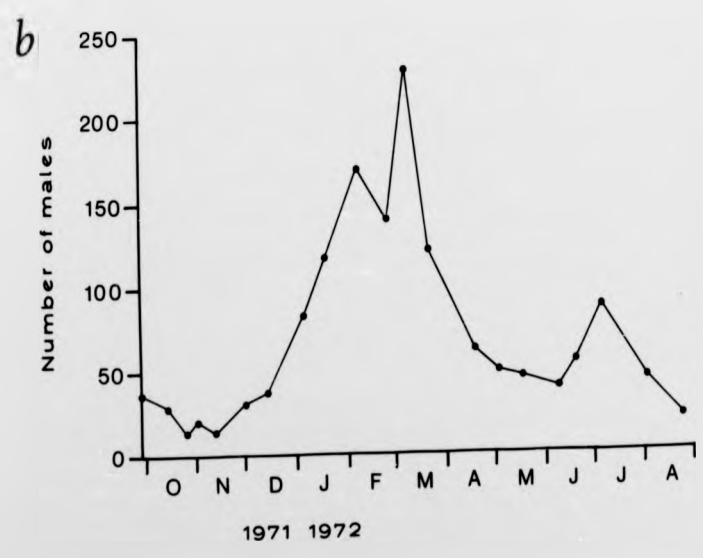
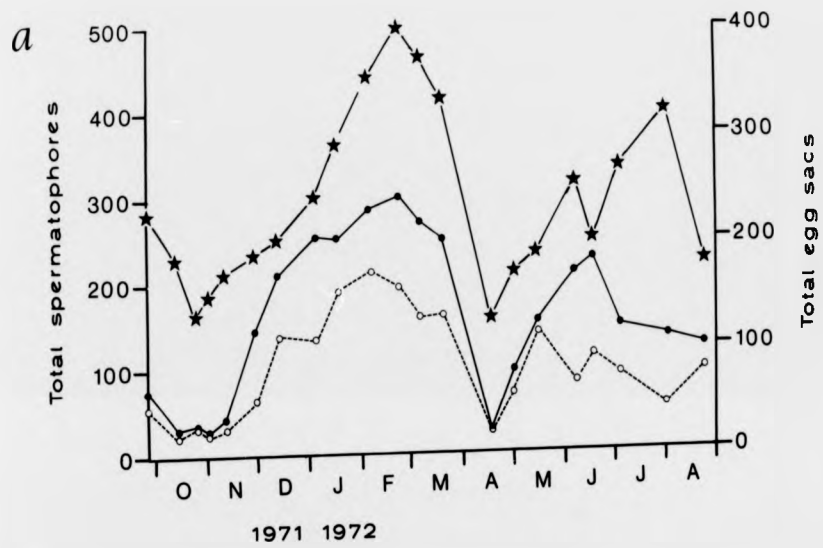


Fig. 28. Seasonal variation in the sea surface temperature
of the upper basin of Loch Etive (from Edwards,
unpublished information)

temperature

m Edwards,



1971 1972

Fig. 29. The relationship between the mean sex ratio of copepodite stage IV and the mean monthly sea surface temperature in each of the 12 months sampled ($r = 0.89$, $P = < 0.001$)

ratio of
ly sea
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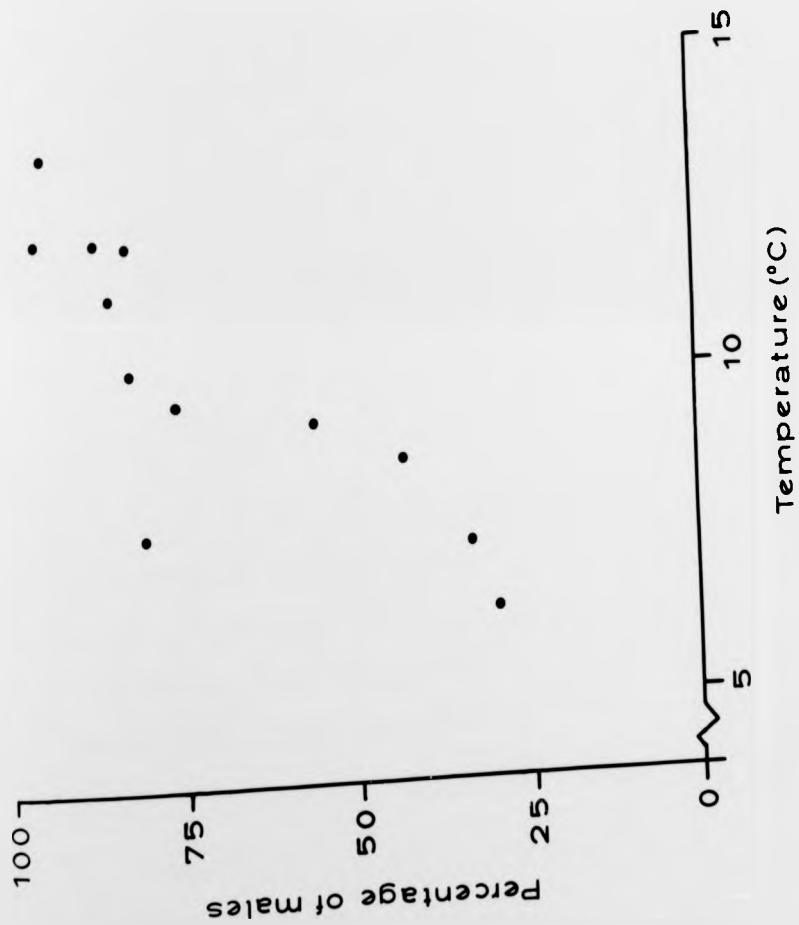


Fig. 30

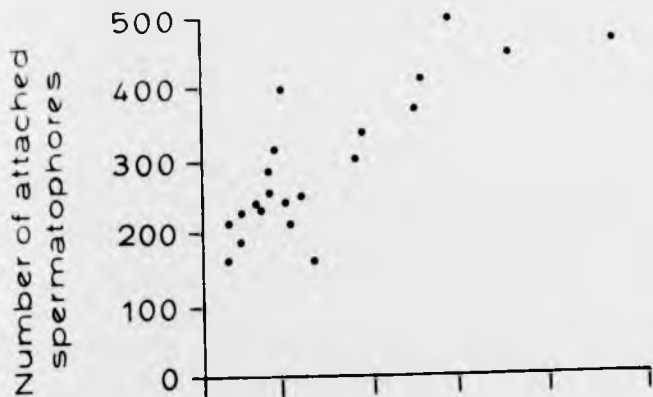
- a. The relationship between the number of males in the adult population and the number of spermatophores found attached to adult females ($\gamma = 0.81$, $P < 0.001$)

- b. The relationship between the number of males in the adult population and the number of adult females found with attached spermatophores ($\gamma = 0.71$, $P < 0.001$)

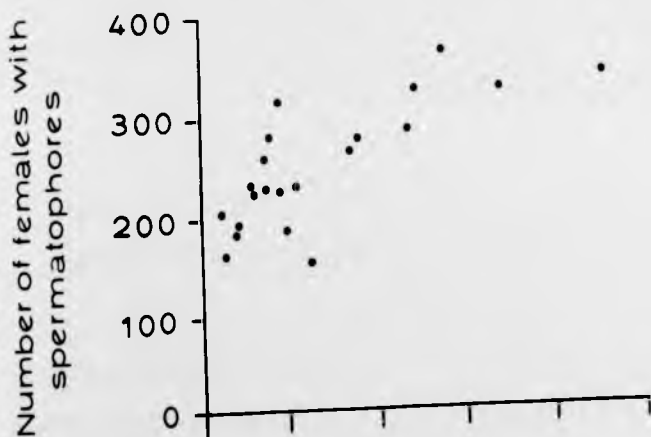
- c. The relationship between the number of males in the adult population and the total number of egg sacs present ($\gamma = 0.76$, $P < 0.001$).
All numbers in a, b and c are expressed as numbers found per standard sample of 500 adult females

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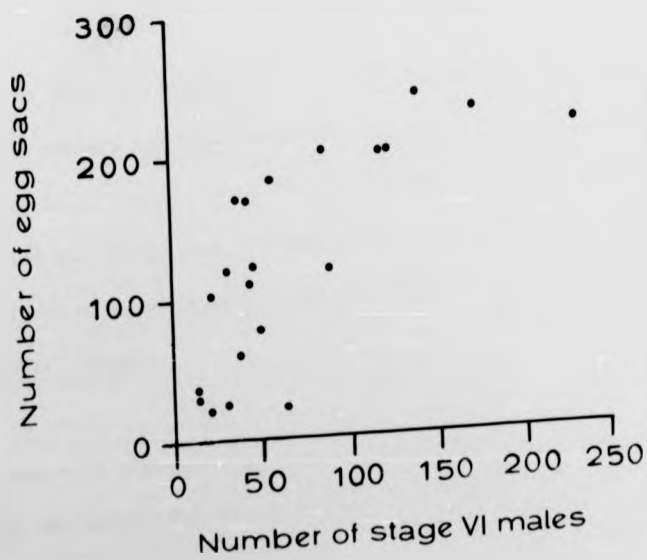
a



b



c



GENERAL SUMMARY

1) The female reproductive system and genital segment of E. norvegica have been described.

The ovary appears to be equivalent to the germinal zone of the ovary of most other crustaceans, as little growth of the eggs occurs there. The oviducts in the gravid female are large and act as a storage area for the maturing eggs. This situation is quite different from that found in other copepods where the ovary undergoes large variations in size owing to its role as a germinal site as well as a structure for storage of the developing eggs.

True spermathecal sacs, distinct from the oviducts, are absent from E. norvegica. Instead, the terminal cuticular portion of the oviducts, the receptacula seminalis, serve as sperm storage receptacles.

The genital field is complex and, because of its importance as a site for both spermatophore and egg sac attachment, its structure has been described in detail.

The presence of three membranes around the extruded egg have been revealed. The primary egg (perivitelline) membrane is already present around the egg in the ovary; the second egg membrane appears after the egg has reached the distal end of the main oviduct; the third membrane forms the egg sac membrane which surrounds the whole egg mass and attaches it to the genital field.

2) The structure of the male reproductive system has been described and its histology and ultrastructure have been examined in detail.

The secretion and development of the spermatophore have been followed. The function of the various regions of the genital duct in secreting the components of the spermatophore have been discussed with regard to other copepods.

Evidence has been provided which indicates that a male's potential output of spermatophores during his life time is three or more.

The manner of discharge of the spermatophore contents has been described and the role of the various components within the spermatophore during this process have been outlined. Swelling of the spermatophore contents, by infiltration of seawater, appears to generate the propulsive force necessary to eject the sperm. "Dualism of sperm", characteristic of many copepods, is absent from E. norvegica.

Spermatophores found attached to the female genital field have been shown to have smaller flasks than those attached elsewhere on the genital segment. This disparity in flask size has been related to differences involved in the dynamics of content extrusion in spermatophores that have been attached on and off the genital field.

3) The development and structure of the male fifth limbs have been followed in stage IV and stage V copepodites as well as in the adult stage.

Scanning electron microscope studies have revealed that the structure of the terminal portion of the adult male fifth limb is ideally suited for handling and transferring the spermatophore to the female genital segment.

Examination has been made, from published information, of the features which appear to be common, if not characteristic, in the mating of copepods. The relative inseminatory positions adopted by the copulatory partners has been deduced.

4) Analysis of the frequency distribution of spermatophores found on adult females has indicated that underdispersion of these structures takes place. This suggests that once a female has a spermatophore the males tend to avoid attaching another spermatophore to her. The data describing frequency distribution of spermatophores has been modelled and the merits of the models have been reviewed.

The functioning of a pheromone system, ensuring underdispersion of spermatophores has been postulated. A hypothetical method of functioning for such a system has been proposed and discussed with reference to the fact that multiple spermatophore placements become more frequent when relatively high proportions of adult males are present.

The underdispersion of spermatophores has been considered to provide strong evidence to support the view that a single well placed spermatophore is sufficient to meet a female's requirements to fertilize a single egg-clutch.

Analysis of the pattern of placement of spermatophores on different areas of the genital segment has indicated that the genital field is actively sought for spermatophore attachment, but as additional spermatophores are added to the female the percentage of placements occurring outside the genital field increases. The majority of spermatophore placements occurring outside the genital field have been considered to be misplacements but a few have connections with the genital cavity and so must be considered viable for fertilization.

5) Studies have been made of egg development, egg mortality, and the relationship between maternal body size and clutch size.

Experimental work has indicated that at 10°C about 22 days are needed, after mating, for maturation of the ovary and eggs to fully take place before the egg-clutch is laid. Such findings indicate that sperm are able to survive in a viable state for long periods of time. The eggs have been shown to require a further 19 days to hatch into nauplii. Egg mortality within the sac was found to be low at about 4%.

There is a well defined linear relationship between the mean size of the egg-clutch produced and the mean size of the female producing the clutch, with larger sizes of female generally laying more eggs and greater volumes of eggs than smaller females. Equations describing the various relationships have been generated. There is a suggestion, however, that small females are able to produce a greater volume of eggs per unit body volume than larger females.

The loss in weight of an adult female caused by egg laying is substantial. The amount of dry weight transferred to the eggs may exceed 25% of that remaining in the female's body.

There are indications that adult females have a potential to delay the calculated time of egg laying by about two weeks.

6) Studies have been made of sex ratios and breeding cycles.

The sex ratio of stage IV and stage V copepodites showed seasonal variations which appear to be related to changes in the temperature of

the more surface waters. Males were dominant when conditions were warm, whilst females were dominant when the water was cold. Taken over the whole sampling period, however, the sex ratios of males and females of stage IV and stage V copepodites were approximately equal. The mechanisms by which temperature may influence sex determination and the balance of the sex ratio are discussed.

The mean adult sex ratio per haul, taken over the whole sampling period, was 11.3% males and 88.7% females. The maximum sex ratio attained by males was 31.4% at the height of the main breeding peak in February whilst the other peak of males coincided with the secondary breeding peak in July. This drastic reduction in the proportion of males present in the adult stage when compared with the stage V copepodite has been accounted for by the much shorter life span of the adult male, probably caused by its non-feeding habit. On moulting from stage V copepodite to the adult, males only gain about 44% in dry weight whilst females have been found to increase their dry weight by some 162%.

Spermatophore production has been calculated as representing about 0.1% dry male body weight per day, knowing that a male produces about three spermatophores each equivalent to 0.9% of his dry body weight during an average adult life span of 29 days.

A close correlation has been shown to exist between the proportion of adult males in the population and:

- a) the number of spermatophores attached to adult females
- b) the number of females found with attached spermatophores
- c) the number of egg sacs present

The intensity of breeding in E. norvegica is apparently controlled by the proportion of adult males present in the population. They determine the proportion of females which are mated and subsequently produce egg sacs.

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APPENDIX 1

DOUBLE EMBEDDING METHOD

- | | |
|--|-----------|
| 1. 5% phenol in 70% alcohol (the phenol acts as a softening agent) | 2 hours |
| 2. 95% alcohol | 2 hours |
| 3. 99% alcohol | 2 hours |
| 4. Absolute alcohol | 2 hours |
| 5. Absolute alcohol/amy l acetate 50/50 | Overnight |
| 6. 100% amy l acetate | 4 hours |
| 7. 100% amy l acetate | 4 hours |
| 8. 1% celloidin in methyl benzoate | 24 hours |
| 9. 1% celloidin in methyl benzoate | 24 hours |
| 10. 1% celloidin in methyl benzoate | 24 hours |
| 11. Benzene | 2 hours |
| 12. Benzene | 2 hours |
| 13. Benzene | 2 hours |
| 14. Immerse in paraffin wax of melting point 54°C | Overnight |
| 15. Vacuum specimens in 54°C paraffin wax | 1 hour |
| 16. Finally embed in paraffin wax with a melting point of 56°C. | |

The reason for using the two different paraffin waxes is that, as the specimens are tough, the harder the wax used for embedding the easier the block will be to cut because it gives the tissue more support. The lower melting point wax is used for impregnation as it penetrates the tissues more easily than a harder wax of higher melting point.

APPENDIX 2

RECIPES USED FOR PREPARATION OF FIXATIVES AND BUFFER

Gluteraldehyde: 3.1% 0.075M

25% gluteraldehyde	5 mls
0.2M cacodylate buffer	15 mls
distilled water	20 mls

Sodium cacodylate buffer: 0.2M pH 7.4

sodium cacodylate	42.8gm
N.HCl	6.9ml
distilled water	to 1000ml

Osmium tetroxide: 1.0%

osmium tetroxide	0.5gm
0.2M cacodylate buffer	50ml

NB. The colour of the freshly prepared solution should be a very pale straw, and if any other colour (eg red, black, or purple) is observed the sample should be discarded.

The processing schedule used during fixation and dehydration is as follows:-

- | | |
|--|----------|
| 1. 3.1% gluteraldehyde (cacodylate buffered) @ 4°C | 24 hours |
| 2. Sodium cacodylate buffer @ 4°C | 2 hours |
| 3. 1% osmium tetroxide (cacodylate buffered) @ 4°C | 2 hours |
| 4. Sodium cacodylate buffer | 2 hours |
| 5. 10% ethanol | ½ hour |
| 6. 10% ethanol | ½ hour |

7. 10% ethanol	½ hour
8. 30% ethanol	1 hour
9. 50% ethanol	1 hour
10. 70% ethanol	1 hour
11. 90% ethanol	1 hour
12. Absolute alcohol	2 hours
13. Absolute alcohol	2 hours
14. Absolute alcohol	2 hours
15. Absolute alcohol	2 hours

APPENDIX 3

The recipe used to make the Epon embedding medium is given below:

a) Epikote 812 (Epon 812)	16ml
b) DDSA, dodecenyl succinic anhydride	10ml (softener)
c) MNA, methyl nadic anhydride	9ml (hardener)
d) DMP-30, 2,4,6-dimethylaminomethyl-phenol	0.15ml/ 10ml mixture

Mix constituents a, b and c together very thoroughly but care must be taken not to aerate the mixture excessively. The mixture can safely be kept in a refrigerator for 2-3 days. When needed 10ml of the mixture can be removed with a pipette and 0.15ml of d (DMP-30) carefully stirred into it.

The processing schedule used during embedding is as follows:

- | | |
|---|--------|
| 1. Propylene oxide (100%) | 1 hour |
| 2. Epon/propylene oxide 50/50 | 3 days |
| 3. Epon/propylene oxide 75/25 | 3 days |
| 4. Epon/propylene oxide 95/5 | 3 days |
| 5. Epon (100%) | 1 day |
| 6. Epon (100%) | 1 day |
| 7. Epon (100%) | 1 day |
| 8. Epon (100%)....embed in beam capsules and allow to
polymerize at 60°C for 3 days. | |

APPENDIX 4

The recipe used to make the staining solutions are given below:

Uranyl acetate

Use as a saturated solution in 50% ethanol. Adjust to pH 4-5 with NaOH. Store in the dark.

Lead acetate (Reynolds, 1963)

1. Mix 1.33gm of lead nitrate ($\text{Pb}(\text{NO}_3)_2$), 1.76gm of sodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$) and 30ml of distilled water in a 50ml volumetric flask.
2. Shake the resultant suspension vigorously for 1 minute and then allow to stand for 30 minutes with intermittent shaking to ensure complete conversion of lead nitrate to lead citrate.
3. Add 8.0ml of N-NaOH and dilute to 50ml with distilled water. Mix by inversion. The lead citrate dissolves and the staining solution should be centrifuged immediately before use. Store in the dark. The pH is 12.0 ± 0.1 .

The staining schedule used is as follows:-

- | | |
|---|---------|
| 1. Stain in uranyl acetate | 15 mins |
| 2. Rinse in 50% ethanol | 1 min |
| 3. Rinse in 50% ethanol | 1 min |
| 4. Rinse in distilled water | 1 min |
| 5. Stain in lead citrate | 5 mins |
| 6. Rinse in 0.02 N-NaOH | 1 min |
| 7. Rinse in 0.02 N-NaOH | 1 min |
| 8. Rinse in distilled water | 1 min |
| 9. Dry on filter paper and store in a desiccator before | |

viewing

APPENDIX 5

The Greenwood-Yule distribution

Greenwood and Yule (1920)* describe a generalised Poisson distribution in which, in our terminology, the chances of successive matings vary with the number of spermatophores present on the female. They give, page 281, the first six terms of such a series. For the particular case in which the probability of the first mating is different from the remainder, all of which then are the same, they give, page 283, in our notation:

$$P(X = 0) = e^{-\alpha}$$

$$P(X = 1) = e^{-\alpha} \alpha (1 - e^{-\delta}) / \delta$$

$$P(X = 2) = e^{-\alpha} \alpha \beta \{ (1 - e^{-\delta}) / \delta^2 - e^{-\delta} / \delta \}$$

when $\delta = \beta - \alpha$. For $x = 3$ the corresponding term is:

$$P(X = 3) = e^{-\alpha} \alpha \beta^2 \{ (1 - e^{-\delta}) / \delta^3 - e^{-\delta} / \delta^2 - e^{-\delta} / (2\delta) \} .$$

No simple expression is available for the general term. Greenwood and Yule give a method of moments solution for the estimates $\hat{\alpha}$ and $\hat{\beta}$ of α and β by means of the following non linear equations:

$$\bar{x} - (1 - \frac{\hat{\beta}}{\hat{\alpha}}) (1 - e^{-\hat{\alpha}}) - \hat{\beta} = 0$$

$$\frac{\sum x^2}{n} - \bar{x} - \hat{\beta}(\hat{\beta} + 2) + 2 \frac{\hat{\beta}}{\hat{\alpha}} \bar{x} = 0 .$$

A computer program has been written to solve the above equations using the Newton-Rapheson technique, this program gave the estimates for α and β listed in Table 4.

*Corrigenda to this paper are listed at the end of this appendix.

Corrigenda to Greenwood & Yule (1920)

page 283, line 19: $q_0^t = (1 - \frac{\lambda_0}{t})^t = e^{-\lambda_0}$

line 21: the last expression should read $q_0^{t-1} \lambda_0 \frac{1 - e^{-\delta}}{\delta}$

line 28: should read $p_0 p A_2 = q_0^{t-2} \lambda_0 \lambda_1 \left(\frac{1 - e^{-\delta}}{\delta^2} - \frac{e^{-\delta}}{\delta} \right)$

page 285, equation (39) should be

$$M = \left(1 - \frac{\lambda_1}{\lambda_0}\right) (1 - e^{-\lambda_0}) + \lambda_1$$

The solution to (39) and (40) for the 60 lb shrapnel data should be

$$\lambda_0 = 0.5984, \quad \lambda_1 = 0.5079.$$

Corrigenda to Greenwood & Yule (1920)

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