Biology of deep-sea calanoid copepod genus *Pleuromamma* with particular references to phylogeny, pore signatures, moulting and life history

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by

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Abstract

The pore signature of calanoid copepods is of increasing interest in phylogenetic and biogeographical studies. Some recent studies have been restricted to the urosome on the assumption that most of the species-specific information resides there. The present study tests that assumption in eight *Pleuromamma* species by assessing the signatures of the cephalosome, metasome and urosome separately in each species. Most of the species-specific information is in the urosome, but a significant proportion also resides in the cephalosome and a lesser component in the metasome. Changes in the pore signatures between the species parallel changes in other morphological characters. The pore signatures shed further light on the phylogeny of the *Pleuromamma* species which had been diffuse in studies of conventional morphology of this genus. Interspecific differences in the pore signatures occur as early as copepodid III. Sexual differences in the pore signature are found in copepodid IV and are primarily evident in the urosome of the adult. The species-specific components of the urosomal signature are greater in the female than in the male. In general, the degree of the intraspecific variation in the pore signature increases with increasing number of the total integumental pores and is limited to 10% or less of the total. Inter- and intraspecific variation are an expression of the phylogenetic grouping of species, or generic similarities between the species within the genus.

In calanoid copepods, external and internal morphological changes associated with the intermoult cycle in *Pleuromamma robusta* conform with those in other crustaceans. Early stages of the intermoult cycle are the completion of the integumental structure and somatic tissue after the previous ecdysis and late stages are in preparation for the next ecdysis. Adult copepods do not moult, but progress through part of the moult cycle exhibited by earlier copepodid stages. Changes in the integumental structure were evident as increases in the thickness of the cuticle throughout the period of the intermoult cycle, which also affect the body appearance of copepodids such as soft and hard bodies. The different body appearance is of great use for studies of population dynamics, e.g. detection of the timing of the population reconstruction, overlapping generations, the identification of the newly recruited stock and the seasonal changes of the recruitment rate. The Rockall Trough population of *P. robusta* produces three generations per year. The peak of reproduction of this species was in September and was followed by a hiatus of reproduction in November. In addition, significant relationships between day and night vertical distribution of individuals of copepodid V and adults at different stages of the intermoult cycle in *P. robusta* were found in relation to their mating behaviour.

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Contents

CHAPTER 1:

General Introduction

1.1. General background

This study is a part of the SAMS project on deep-sea biology of the Rockall Trough, northeastern Atlantic. The continental slope and rise of the Rockall Trough extend through a bathymetric transect ranging from approximately 400 to 2,900 m depth. The taxonomic composition of the pelagic and benthic fauna was mostly described during the period of 1880 to 1910, as reviewed by Currie (1986), Mauchline (1986) and Mauchline *et al.* (1986). In the present project, time series of samples were collected in the area of 55°N, 12°W during the years 1973 to 1978. A vertical series of samples were collected between the surface and 1900 m depth at Station 10105 of the Institute of Oceanographic Sciences; this station was located at 54°30'N, 13°W near the station at which all the time series samples were collected. Earlier studies of the project concentrated on examining seasonality of pelagic, benthic and fish fauna and examined trophic structures within the assemblages of fish in the Rockall Trough (e.g. Mauchline and Gordon 1980, 1983, 1984).

New aspects of the project have since been developed and the work is still ongoing. One such aspect is the structure and dynamics of the pelagic biota of the Rockall Trough aimed at examine the comparative rates of growth and evidence of seasonality in a deep oceanic water column; to define the importance of the different processes controlling population balance within species; and to understand how different bathymetric regimes affect rates of growth and production at the species and population level. The results of this project, dealing mainly with the congeneric species of the calanoid genus *Euchaeta*, have been published (Mauchline 1992a, b, 1994 a, b).

Another genus with congeneric species occurring in the water column of the Rockall Trough is *Pleuromamma*. The genus has 9 species, 6 of which occur in the Trough. The dominant species is *P. robusta* which occurs in large numbers in the mesopelagic. *Pleuromamma* species are important in the economic's of the Rockall Trough, contributing

to the diets of the common deep-sea species of fish such as *Benthosema glaciale*, *Coryphaenoides rupestris* and *C. guentheri* (Kawaguchi and Mauchline 1982, Mauchline and Gordon 1984).

1.2. The calanoid genus *Pleuromamma*

Species of the calanoid copepod genus *Pleuromamma* are easily distinguished from all other copepods by the presence of a rounded, black cuticular structure known as a pigment knob that occurs on the left or right side of the second metasomal segment depending on the species. Claus (1863) first used the name of this genus '*Pleuromma*' derived from Greek meaning "side" and "eye" because the pigment knob was thought to be a lateral eye. Richard (1892) showed that the gross morphology of the pigment knob is different from that of the copepod nauplius eyes. The name of *Pleuromamma* was given by Giesbrecht (1898), derived from the Greek words "side" and "teat". Dahl (1893) suggested later that the pigment knob might be a luminescent organ. Blades-Eckelbarger and Youngbluth (1988), however, showed that the pigment knob does not secrete a luminescent material nor does it resemble the structure of any known luminescent gland. The central part of the pigment knob is occupied by a mass of pigment granules and its end connected with a cuticular pore opening to the outside. They suggested that the pigment knob has a secretory function, but the nature and function of this unique morphological structure remains undetermined.

Almost sixty years ago Steuer (1932) found that the position of the pigment knob is related to morphologically asymmetrical appendages, namely the antennules, fifth pairs of swimming legs, and the genital opening in males (see Table 3.1). On the basis of these morphological and asymmetrical characters, he divided the species of *Pleuromamma* into four groups: 1) *P. xiphias* and *P. abdominalis*; 2) *P. indica*; 3) *P. robusta* and *P.*

quadrungulata; 4) *P. gracilis* and *P. borealis*. In his study, *P. scutullata* was not examined and *P. piseki* was regarded as a subspecies of *P. gracilis*. Ferrari (1984) termed the relationship between the position of the pigment knob and asymmetrical characters as a "unique concordance" and found other asymmetrical characters in adult males and females. He divided the species into three groups based on the frequent position of the asymmetrical characters, the latter two groups of Steuer being regarded as one. Steuer (1932) and Ferrari (1984) made some suggestions about the phylogeny of this genus, but there remains disagreement in phylogenetic relationship between individual species (see Fig. 3.1).

The *Pleuromamma* species are common from boreal to subtropical latitudes of most oceanic regions and are often numerically dominant in some zooplankton communities (Steuer 1932, Roe, 1972b, Deevey and Brooks 1977, Hayward and McGowan 1979, Hopkins 1982). They play a significant role in the oceanic food web as an important prey for the decapods and the fishes (Foxton and Roe 1974, Merrett and Roe 1974, Hopkins and Baird 1977, 1985, Mauchline and Gordon 1980, 1983, 1984, Baird and Hopkins 1981, Heffernan and Hopkins 1981, Scotto di Carlo *et al.* 1982, Roe and Badcock 1984). The biology of the genus *Pleuromamma* is best known from studies of day and night vertical distribution because of their marked diel migratory behaviour (e.g. Moore and O'Berry 1957, Roe 1972b, 1984, Ambler and Miller 1987, Haury 1988, Bennett and Hopkins 1989, Hattori 1989). Despite their ecological importance, there have been no published studies of the population dynamics of this genus.

This thesis is divided into two main parts. The first part concentrates on the taxonomic and phylogenetic significance of the integumental pore signatures of the metridinid genus *Pleuromamma*. The second part of this thesis deals with the ecological aspects of this genus, and attempts to apply aspects of the intermoult cycle to marine ecological studies of calanoid copepods as a tool for the interpretation of population parameters.

1.2.1. The integumental pore signatures

As in all arthropods, the integument of calanoid copepods and other crustaceans has integumental organs, consisting of sensilla and openings of underlying glands. With (1915) first noted the presence of these organs in calanoid copepods. Sewell (1929, 1932, 1947) showed considerable interest in the general distribution of integumental organs on the body, as well as appendages. Clarke *et al.* (1962) and Elofsson (1971) demonstrated that the neural innervation of the sensilla and the gland ducts pass through the integument to the subcuticular layer.

The first major study to examine the pore signatures in calanoid copepods was conducted by Fleminger (1973). He showed that the presence of integumental organs is most easily determined by digestion of the soft tissue associated with the integument. Digestion by hot potassium hydroxide leaves pores at the sites of the subcuticular integumental organs. These pores are the holes in the integument through which the internal structures of sensilla and glands are connected with the outside. In adults, the spatial distribution of integumental organs is species-specific and is known as an integumental perforation pattern by Fleminger (1973) and pore signature by Mauchline and Nemoto (1977).

Fleminger (1973) examined the overall species pore signatures in 17 *Eucalanus* species and divided the species into four groups based on their signatures. His division agrees with that derived from other morphological characters of the species. Mauchline (1988) constructed 7 superfamilial pore signatures of calanoid copepods derived from a study of 289 species representing 89 genera ascribed to 28 families. His study showed that changes in the superfamilial pore signature corresponded with current concepts of the phylogeny of the calanoid superfamilies presented by Andronov (1974) and Park (1986) based on general morphological characteristics of the animals. The taxonomic and

phylogenetic usefulness of the species pore signature has been shown at several taxonomic levels.

The species pore signature has been described in a number of calanoid copepods during the last two decades. The species pore signatures have been derived in three different ways, using either the whole integument (e.g. Fleminger 1973, Mauchline 1977, Campaner 1984, Koomen 1992); or the metasome or the prosome (e.g. Bradford 1974, Mauchline 1988); or the urosome or the genital somite of adult females (e.g. Fleminger and Hulsemann 1977, 1987, Hulsemann and Fleminger 1990) because it is still unknown where the species-specific information primarily resides in the integumental tagmata. In addition, most studies on the species pore signature tend to consider only adult females and very little is known of its ontogenetic development and sexual differences.

Not only is there interspecific variation in the pore signature, but intraspecific variation. This intraspecific variation is evident between individuals within a single geographical area (within-sample variation) and between different geographical areas (geographical variation). Intraspecific variation has been studied on a geographical scale under the assumption that the species-specific signature is most clearly seen in the urosome of adult females (Fleminger 1973, Fleminger and Hulsemann 1977, 1987, Hulsemann and Fleminger 1990). In these studies, the extent of the within-sample variation is neglected and a "single" geographical area includes up to 24 different regional locations, some separated by over 200 miles, on different dates. There remains some question as to how contributory intraspecific variation in the pore signature is to the determination of geographical variation. In fact, the extent of the within-sample variation at a single geographical location has not yet been adequately assessed.

The integumental organs of the genus *Pleuromamma* were first noted by Sewell (1929). Mauchline (1988) described part of the pore signature in a number of genera of calanoid copepods including that of *Pleuromamma* derived from five species: *P. gracilis*, *P.*

piseki, *P. robusta*, *P. scutullata* and *P. xiphias*. He described only the generic pore signature on the metasomal segments and did not describe the overall species pore signature.

1.2.2. The intermoult cycle

The modern concept of the intermoult cycle was advanced by Drach (1939). He found that the calcification of the old-exuvial exoskeleton, increase in the amount of the somatic tissue, developing new setae, the decalcification of the old-exuvial exoskeleton and the formation of the pre-exuvial exoskeleton sequentially occur throughout the period of the intermoult cycle. On the basis of the degree of these sequential changes, the intermoult cycle is divided into the postmoult, intermoult and premoult stage (see also Table 6.4). Drach (1944) and many others have modified Drach's original criteria for various stages of the intermoult cycle so that it is now applicable to a variety of crustaceans (e.g. Hiatt 1948, Carlisle 1960, Passano 1960, Sheer 1960, Drach and Tchernigovtzeff 1967, Stevenson *et al.* 1968).

In calanoid copepods, Currie (1918) noted that during the process of moulting in *Calanus finmarchicus* new setae are developed in the somatic tissue of appendages in preparation for the next ecdysis (called here setogenesis). Dexter (1981) described the intermoult cycle of *Calanus marshallae* as a well-defined sequence of stages and divided the intermoult cycle into the postmoult, intermoult and premoult stage based on the degree of setogenesis. Her descriptions correspond with other crustacean criteria. The formation of new setae, however, is only a part of the moult process and a detailed intermoult cycle has not yet been studied in calanoid copepods.

As in most calanoid copepods, there are six naupliar stages followed by six copepodid stages, the last of which is the adult in *Pleuromamma* species. In the traditional concept of copepod growth, body size of adults is a comparable entity between species

because they do not moult, and size variation is derived from continuous development of copepodid cohorts (broods or generations) with different growth rates (Marshall 1949, Miller *et al.* 1977, McLaren 1978). Variation in body size, or different growth rates, has been studied relative to the influenced of temperature and food in field and laboratory observations. Growth rate is proportional to food concentration and is strongly influenced by food quality (e.g. Paffenhöffer 1976, Huntley *et al.* 1987, Klein Breteler *et al.* 1990). Changes in temperature do not always affect copepod size (e.g. Deevey 1960, McLaren 1963, 1965, Klein Breteler and Gonzalez 1982, 1988, Escribo and McLaren 1992).

Ultimately, moulting is the means of crustacean growth. The intermoult cycle is not only in preparation for the next ecdysis, but also the completion of the integumental structure and somatic tissue after the previous ecdysis. The most notable changes related to the intermoult cycle are the formation of the old- and pre-exuvial exoskeleton (see Fig. 6.4). Morphological changes in the integumental structure are also evident as an increase in thickness of the cuticle throughout the intermoult cycle in *Balanus amphitrite* (David *et al.* 1973). Subsequent somatic tissue growth has been noted but has not been adequately quantified. Lasker (1964, 1966) showed that, in juvenile *Euphausia pacifica*, dry weight of exuviae is approximately 6 to 11% of total body dry weight and that the growth rate is 0.048 mm day^{-1} in laboratory culture and 0.02 mm day^{-1} in oceanic population where the moult interval is between 3 and 8 days. Different growth rates may be due to different growth factors between field and laboratory experiments, but their growth continues throughout an intermoult interval.

The hypothesis of this study is that copepod body size and weight, including those of adults, increase continuously throughout the period of the intermoult cycle and the stage-specific size is determined by the maximum size limit of each copepodid stage. In nature, copepodid stages develop continuously and within the copepodid stage various stages of the intermoult cycle co-exist. The degree of morphological and physiological development

related to the intermoult cycle may be evident as a variation in body size.

Since copepod age is determined by the number of moults, individuals of each life stage can be further divided based on the intermoult cycle. Copepod recruitment from one stage to the next, therefore, can be predicted from the proportion of different stages of the intermoult cycle within the copepodid stage. For example, the high proportion of early stages of the intermoult cycle indicates active recruitment from the previous life stage and vice versa.

The aims of this thesis are:

1) to describe the overall species pore signature in eight of the nine *Pleuromamma* species and then to assess the species-specific information content of the cephalosomal, metasomal and urosomal signature separately (chapter 2)

2) to compare species pore signatures with conventional morphological characters of the *Pleuromamma* species and to clarify current disagreement of the phylogenetic relationships between individual species of this genus presented by Steuer (1932) and Ferrari (1984) (chapter 3).

3) to examine the stage at which interspecific and sexual differences in the pore signature develop and the species-specific signature is completed (chapter 4).

4) to assess the extent of the within-sample variation in the species pore signature at a single geographical location (chapter 5). This chapter also re-examines published data on intraspecific variation on a geographical scale.

5) to examine changes in the integumental structure in association with the intermoult cycle using the Transmission Electron Microscope and to quantify them by measuring the increase in the thickness of the integument (chapter 6).

6) to test the potential usefulness of the intermoult cycle as an indicator of the recruitment rate in a study of the population dynamics of *Pleuromamma robusta* (chapter 7). In addition, seasonal fluctuations of the abundance, body size, sex ratio and gonad

maturity are examined to understand the life history of this species.

7) to study whether the extent of the vertical distribution of individuals of copepodid stage II to adult is related to the intermoult cycle and to see whether the individual vertical distribution is related to gonad maturity (chapter 8).

CHAPTER 2:

Interspecific differences

in integumental pore signatures

2.1. Introduction

The integument of calanoid copepods, and other crustaceans, has integumental organs, consisting of sensilla and subcuticular gland openings. The internal structures of sensilla and glands are connected to the epidermis and pass through the endo- and exocuticle to the epicuticle (Clark *et al.* 1962, Eloffson 1971, Briggs 1978, Gharagozlou van Ginneken 1979, Nishida 1989, Bannister 1993). The presence of integumental organs is most easily determined by digestion of the integument in potassium hydroxide which removes all soft tissues. Subsequent staining of the integument reveals patterns of pores in the integument that are the sites of integumental sensilla and gland openings. The spatial arrangement of these pores in adults is peculiar to the species and is therefore called a pore signature (Fleminger and Hulsemann 1977, Mauchline and Nemoto 1977). The species-specific pore signatures, often in restricted areas of the integument, have been described in a variety of calanoid copepods (Fleminger 1973, Bradford 1974, Fleminger and Hulsemann 1977, 1987, Mauchline 1977, 1988, Mauchline and Nemoto 1977, von Vaupel Klein 1982, Campaner 1984, Hulsemann and Fleminger 1990, Ohtsuka and Mitsuzumi 1990, Hulsemann 1991, 1994, Koomen 1992).

The first major study of the species pore signature in calanoid copepods was made by Fleminger (1973). He described the overall species pore signatures in 17 species of the genus *Eucalanus* and divided the species into four groups according to their signatures. His division conformed with that derived from other morphological characteristics of the species. Since then, the pore signature has been of increasing interest in taxonomic, phylogenetic and biogeographical studies (Fleminger and Hulsemann 1977, 1987, Mauchline 1988, Hulsemann 1991, 1994, Hulsemann and Fleminger 1990).

Fleminger and Hulsemann (1977, 1987), Hulsemann and Fleminger (1990) and Hulsemann (1994) examined the urosomal signatures of the adult female in studies of

taxonomic divergence within a species. The use of the urosome in isolation presupposes that enough species-specific information resides in it. This derives from the assumption that the primary component of the species pore signature is developed in the urosome of the adult female. However, this assumption has not been proven and species-specific characters in the signatures are known to exist elsewhere in the integument.

Mauchline (1988) described the generic, familial and superfamilial signatures derived from a study of 289 species representing 89 genera ascribed to 28 families of calanoid copepods. The generic signatures contribute to the familiar signatures and finally to the superfamilial signatures. His study showed that changes in the superfamilial pore signatures corresponded with current phylogenetic relationships of the calanoid superfamilies, based on the morphological characteristics, presented by Andronov (1974) and Park (1986). Hulsemann (1991) described the metasomal signature of the family Clausocalanidae to compare four generic signatures of this family, *Pseudocalanus*, *Clausocalanus*, *Farrania* and *Drepanopus*. The taxonomic and phylogenetic usefulness of the pore signature has been shown at the specific, generic, familial and superfamilial level (Fleminger 1973, Mauchline 1988, Hulsemann 1991).

The integument organs of the genus *Pleuromamma* Giesbrecht 1898 were first described by Sewell (1932, 1947). All species of *Pleuromamma* have additional luminescent glands on their body and appendages so most of the studies on integumental organs of this genus have been made with respect to copepod luminescence (Dahl 1893, 1894, Giesbrecht 1895, Clarke *et al.* 1962, Rudjakov and Voronina 1967, Evstigneev 1982, 1983a, b, Buskey *et al.* 1987, 1989, Herring 1985, Bannister and Herring 1989, Latz *et al.* 1990). Mauchline (1988) described the pore signatures of a number of calanoid genera including that of *Pleuromamma*. He described only the generic pore signature on the metasomal segments and did not describe the overall species pore signature. His generic signature was derived from *P. gracilis*, *P. piseki*, *P. robusta*, *P. scutullata* and *P. xiphias*. Therefore,

a study on the overall species pore signature of this genus has not been published.

In the present study, adult females of eight of the nine species of the metridinid genus *Pleuromamma* were examined. The aims of this study are to describe overall species pore signatures and to present interspecific differences in the pore signatures. In addition, the information content of the pore signature of the cephalosome, metasome and urosome is assessed separately.

2.2. Materials and methods

Specimens of eight *Pleuromamma* species were collected in the north Atlantic, western Pacific and western Indian Ocean (Table 2.1).

Samples were preserved in 5% hexamine buffered formalin in sea water prior to potassium hydroxide treatment. They were washed in distilled water and transferred to a test tube containing 20 ml of 20% aqueous potassium hydroxide (KOH). Each test tube contained 10 individuals. The test tubes were placed in a sand bath on a thermostatically controlled hot-plate at 90-100°C for approximately 8 to 12 hours.

When the integuments were completely free from soft tissues, they were decanted into a glass Petri dish, washed in distilled water and placed in 70% ethanol for a few minutes. Then specimens were stained in a 1% solution of Chlorazol Black E in 70% ethanol. The stained specimens were washed again in distilled water, placed on a slide in polyvinyl lactophenol and dissected.

The ventral regions of the cephalosome and metasome with all attached appendages were removed using dissecting needles. The cephalosome was cut with a pair of iridectomy scissors (John Weiss B 1053 RL) or a scalpel and spread flat on the slide. The metasomal segments were separated from each other and displayed individually. Displaying the urosomal segments proved to be the most difficult part of the treatment. The

urosome had to be cut in order to be laid flat because pores often remain concealed if the segments are viewed as a tube. A pair of iridectomy scissors was of great use in cutting these segments. Some species were too small to use these scissors. In this case, the urosome was cut by a pair of dissecting needles. Duplicate urosomal segments were cut in different orientations, both along the dorsal and ventral sides, in order to accurately examine the positions of the pores. This dissection and spreading procedure allowed easy and accurate mapping of the pore distribution. The flattened integuments were mounted in polyvinyl lactophenol with some Lignin Pink (Gurr, London, England) added.

After the digestion in hot potassium hydroxide most of the integumental organs are destroyed so that only holes or pores are left at their sites in the integument. When the dissected and stained integuments are flattened on a glass slide and viewed under the light microscope these pores show as spots of light on the surface of the black integument. To identify the site of each integumental organ accurately several individuals of each species were placed in lactic acid for approximately 10 hours in a small petri dish and then examined without dissection.

Observations on slide preparations were made both under the dissecting and compound microscopes and magnification ranged from 50 to 400x. Drawings were made with the aid of a drawing tube. The site at which a pore occurs does not vary relative to adjacent sites and so the same site is easily recognized in different integuments.

Mauchline (1988) defined the extent of pore complexes within areas of the metasomal segments and his terminology, as far as possible, is followed here (Fig. 2.1). In addition to the rostrum and rostral base, the cephalosome was divided into five cephalic regions (Fig. 2.1). The species pore signatures described represent the pores that occur on more than 50% of the individuals examined. Within each defined area, the pores that occur in all species are termed a complex. The large and small pores are represented as large and small circles respectively in Figures 2.2-2.9. The terms 'pore' and 'site' are used in

different contexts here: a pore is the hole in the integument left after KOH treatment; a site is the position in the integument where a pore is expected to occur.

Jaccard's (1908) index of similarity is used to examine the similarity of the pore signatures between the eight species examined. The index of similarity (IS) is defined as

$$IS = \frac{c}{a+b+c}$$

where *a* and *b* are the number of pores peculiar to the respective species, and *c* is the number of pores common to the two species. The pores are either present or absent in a pore signature and this index is a reflection of this property. The pore signatures of the cephalosome, metasome and urosome are examined separately (Tables 2.3-2.5).

2.3. Results

The pore signatures of the eight species of *Pleuromamma* are shown in Figs. 2.2-2.9. The number of pores on the whole body is more than 320 in *P. xiphias*, *P. abdominalis*, *P. scutullata* and *P. robusta*, and is less than 300 in *P. indica*, *P. gracilis*, *P. piseki* and *P. borealis* (Table 2.2). A more detailed description and comparison of the eight species follows.

The rostrum

All species of *Pleuromamma* have two pores sited on the rostrum. The number of pores on the rostral base differs between species: *P. xiphias* possess six pores (Fig. 2.2); *P. scutullata* has seven pores (Fig. 2.4); *P. abdominalis* and *P. robusta* have nine pores (Figs. 2.3, 2.5); *P. indica*, *P. gracilis*, *P. piseki* and *P. borealis* have five pores (Figs. 2.6-2.9).

The first cephalic region

Differences between species are confined to the median area of the region. At this site, an extra pair of pores is peculiar to *Pleuromamma xiphias* (Fig. 2.2). In *P. scutullata* and *P. robusta* (Figs. 2.4, 2.5), a pair of pores between the rostral base and the median complex is unique. The pore signatures are identical in *P. abdominalis*, *P. indica*, *P. gracilis*, *P. piseki* and *P. borealis* (Figs. 2.3 and 2.6-2.9), these species having three large pores and four small ones.

The second cephalic region

On the median complex all species have two large and four small pores. One more pore is present in *Pleuromamma xiphias*, *P. abdominalis*, *P. scutullata*, *P. robusta* and *P. indica* (Figs. 2.2-2.6). An extra pair of pores is present in the lateral area of *P. scutullata* and *P. robusta* (Figs. 2.4, 2.5). *P. scutullata* also have an extra pair in the dorsal area (Fig. 2.4).

The third cephalic region

The pore signatures of *Pleuromamma xiphias*, *P. abdominalis*, *P. scutullata*, *P. robusta* and *P. indica* are identical (Figs. 2.2-2.6). In *P. gracilis*, *P. piseki* and *P. borealis* one pair of pores on the dorsal area is absent (Figs. 2.7-2.9). One pair of pores on the lateral area is peculiar to *P. gracilis*, *P. piseki* and *P. borealis*, while one pair is missing in *P. piseki*. One large pore on the median is unique to *P. gracilis* (Fig. 2.7).

The fourth cephalic region

Four small pores are present on the median complex in all species. At this site, *Pleuromamma xiphias*, *P. abdominalis*, *P. robusta* and *P. indica* have one extra pair of large pores (Figs, 2.2, 2.3, 2.5, 2.6), *P. scutullata* has two extra pairs of large pores (Fig. 2.4), and *P. indica* and *P. borealis* have an unique median large pore (Figs. 2.6, 2.9). The pore signatures on the dorsal and lateral areas vary between species.

The fifth cephalic region

There are considerable variations between species in all three areas of this region (Figs. 2.2-2.9).

The first metasome

The pore signatures on the median, the posterior dorsal and the posterior lateral area are identical in all species. Variation is primarily restricted to the anterior dorsal and lateral areas of the segment where the numbers and positions of the large pores vary between species. The overall pore pattern in this segment is identical in *Pleuromamma scutullata* and *P. robusta* (Figs, 2.4, 2.5) and a different pattern is shown by *P. gracilis*, *P. piseki* and *P. borealis* (Figs. 2.7-2.9).

The second metasome

Pleuromamma xiphias has three small pores on the lateral margins of the second to fourth metasome (Fig. 2.2). These pores distinguish this species from the others which

have two small pores.

On the anterior dorsal area, *P. scutullata* has three pores (Fig. 2.4), *P. robusta* has two (Fig. 2.5) and the other species have only one. On the anterior lateral ares, *P. xiphias*, *P. abdominalis*, *P. scutullata* and *P. robusta* have six large pores (Figs. 2.2-2.5), *P. indica* has five (Fig. 2.6), *P. gracilis*, *P. piseki* and *P. borealis* have four (Figs. 2.7-2.9). In *P. xiphias*, the number of pores on the median area is 12 (Fig. 2.2) which is different from all others which have eight pores. In *P. scutullata* one pair of large pores is absent from the posterior lateral area (Fig. 2.4).

The third metasome

Pleuromamma xiphias has three small pores on the lateral margin and 18 pores on the median area (Fig. 2.2). All other species have 12 pores on the median. All species have the same number of pores on the posterior dorsal and lateral areas. The overall pore pattern in this segment is closely similar in *P. indica*, *P. gracilis*, *P. piseki* and *P. borealis* (Figs. 2.6-2.9). The other species all show minor differences.

The fourth and fifth metasome

Pleuromamma xiphias has three small pores on the lateral margin of the fourth metasome similar to the preceding two metasomal segments (Fig. 2.2). *P. scutullata* has two more pores on the dorsal area of the fourth segment (Fig. 2.4). All other species share the same pattern in the fourth segment. The pattern in the fifth segment is basically the same in *P. abdominalis*, *P. scutullata*, *P. robusta*, *P. indica* and *P. gracilis* (Figs. 2.3-2.7). Minor differences are apparent in the other three species.

The first urosome

The greatest variation in pore patterns occurs in the first segment of the urosome, i.e. the genital somite (Figs. 2.2-2.9). The simplest pattern occurs in *Pleuromamma piseki* (Fig. 2.8), the most complex in *P. xiphias*, *P. scutullata* and *P. robusta* (Figs. 2.2, 2.4, 2.5). No two species show the same pattern.

The second urosome

The pore pattern is much simpler than on the first urosome. The same pattern is shown in *Pleuromamma scutullata*, *P. gracilis*, *P. piseki* and *P. borealis* (Figs. 2.4, 2.7-2.9). However, *P. robusta* and *P. indica* have one more large pore (Figs. 2.5, 2.6), and *P. xiphias* and *P. abdominalis* have three more large pores (Figs. 2.2, 2.3).

The third urosome or anal segment

More pores occur in this segment than the last and different groups of species share the same patterns. The same pattern is present in *Pleuromamma xiphias*, *P. abdominalis* and *P. scutullata* (Figs. 2.2-2.4). A second pattern is present in *P. robusta*, *P. indica* and *P. borealis* (Figs. 2.5, 2.6, 2.9), while a third pattern is present in *P. gracilis* and *P. piseki* (Figs. 2.7, 2.8).

Grouping the species pore signatures

In *Pleuromamma xiphias*, *P. abdominalis*, *P. scutullata* and *P. robusta*, the overall pore pattern differs from that in *P. indica*, *P. gracilis*, *P. piseki* and *P. borealis* (Figs. 2.2-

2.9). In the latter four species one or two large pores, present in the former four species, are absent on the median area of the cephalic II, IV, and V, the dorsal area of the cephalics III and IV, the lateral area of the cephalic V, the anterior lateral area of the first three metasomal segments, and the first urosome.

The pore signature of *Pleuromamma indica* (Fig. 2.6), however, is intermediate between the two groups. The pore pattern on the cephalic II, III, IV and V is close to the first group, whilst the pattern on the rostral base and the first urosome is close to the second group. The number of pores on the anterior lateral area of the second metasome is five in *P. indica*; six in *P. xiphias*, *P. abdominalis*, *P. scutullata* and *P. robusta*; four in *P. gracilis*, *P. piseki* and *P. borealis* (Figs. 2.2-2.9). In addition, the first metasomal signature of *P. indica* is distinct from all other species. Thus, *P. indica* is separated from *P. gracilis*, *P. piseki* and *P. borealis* (Figs. 2.6-2.9).

The pore signatures are similar in *Pleuromamma xiphias*, *P. abdominalis*, *P. scutullata* and *P. robusta* (Figs. 2.2-2.5). The signatures of *P. xiphias* and *P.abdominalis*, however, are different from those of *P. scutullata* and *P. robusta*. On the dorsal area of the cephalic V, two pores are peculiar to *P. xiphias* and *P. abdominalis* (Figs. 2.2, 2.3). The pores on the lateral area of the cephalic II and the anterior dorsal area of the first and second metasomal segments are particular to *P. scutullata* and *P. robusta* (Figs. 2.4, 2.5). The first urosomal signature of *P. xiphias* and *P. abdominalis* differs from *P. scutullata* and *P. robusta*. *Pleuromamma xiphias* and *P. abdominalis* are, therefore, separated from *P. scutullata* and *P. robusta*.

From the species pore signatures, *Pleuromamma* species are divided into four groups: 1) *P. xiphias* and *P. abdominalis*; 2) *P. scutullata* and *P. robusta*; 3) *P. indica*; 4) *P. gracilis*, *P. piseki* and *P. borealis*.

Analysis of the pore signature

The similarities in pore signatures between species were tested using the index of similarity (Jaccard 1908). The similarity values for the signatures of the cephalosome are greatest between *Pleuromamma xiphias* and *P. abdominalis*. *Pleuromamma scutullata*, *P. robusta* and *P. indica* are closely similar to *P. xiphias* and *P. abdominalis* (Table 2.3). *Pleuromamma gracilis*, *P. piseki* and *P. borealis* have high similarity values, but are dissimilar to the previous five species. The metasomal signatures of *P. abdominalis*, *P. scutullata* and *P. robusta* have high similarity values (Table 2.4). *Pleuromamma gracilis* and *P. piseki* show the greatest similarity and are also similar to *P. borealis* and *P. indica*. *Pleuromamma xiphias* is distinctive, its signature being closest to that of *P. abdominalis*. The similarity values for the urosome are most similar in *P. scutullata* and *P. robusta* and *P. gracilis* on the one hand, and *P. piseki* and *P. borealis* on the other (Table 2.5). The mean index of similarity between the eight species is 82±7 for the cephalosome signatures, 83±5 for the metasome signatures and only 73±1 for the urosome signatures. This means that the bulk of the species component, as opposed to the generic component, of the signature resides in the urosome.

Pore signature in relation to certain morphological structures

Pleuromamma xiphias and *P. scutullata* each have an unique morphological structure. *Pleuromamma xiphias* has a process directed forward at the midline of the rostral base. At this area the three median pores, present in *P. abdominalis*, *P. scutullata* and *P. robusta* (Figs. 2.3-2.5), are absent in *P. xiphias* (Fig. 2.2). *Pleuromamma scutullata* has a carina passing between the cephalosome and the first metasome, and the signature at each area around the carina is peculiar to this species, with one or two extra pores present (Fig.

2.4). These diagnostic signatures correspond with the presence of these unique structures.

In female *Pleuromamma xiphias* and *P. abdominalis*, the pigment knob occurs on the left or right side of the first metasome segment. There is no difference in pore signatures between females having the pigment knob on the left or right side. Therefore, the position of the pigment knob does not affect the individual's pore signature. Only *P. indica* with the pigment knob on the left side were available for the present study.

2.4. Discussion

The species pore signatures have been derived using either the whole integument (Fleminger 1973, Strickler 1975, Mauchline 1977, Mauchline and Nemoto 1977, von Vaupel Klein 1982, Malt 1983, Campaner 1984, Ohtsuka and Mitsuzumi 1990, Koomen 1992); or the metasome or the prosome (Bradford 1974, Mauchline 1988, Hulsemann 1991); or the urosome (Fleminger and Hulsemann 1977, 1987, Hulsemann 1994, Hulsemann and Fleminger 1990). In the previous studies, only interspecific differences in the pore signatures have been described at several taxonomic levels. There has been no objective assessment of the species pore signature, where the species-specific information primarily resides in the integumental tagmata.

The present study shows that most of species-specific information of the signature resides in the urosome as had previously been assumed (Fleminger 1973, Fleminger and Hulsemann 1977, 1987, Hulsemann 1994, Hulsemann and Fleminger 1990) (Tables 2.3-2.5). The cephalosome and metasome, however, have additional species-specific components. In addition, the *Pleuromamma* species are divided into four groups on the basis of their pore signatures: 1) *P. xiphias* and *P. abdominalis*; 2) *P. scutullata* and *P. robusta*; 3) *P. indica*; 4) *P. gracilis*, *P. piseki* and *P. borealis*. A simple index of similarity is of great use to confirm this division. Although the primary component of the pore signature

is in the urosome, the exclusive use of the urosome in phylogenetic and geographical studies may not provide enough information in some genera because the urosomal signature involves a small number of pores. In eight *Pleuromamma* species, about 16% of the total number of integumental pores reside in the urosome.

A relevant study of the pore signatures of congeners using the whole integument was made by Fleminger (1973). On re-examination of his data on adult females of 17 *Eucalanus* species, the mean index of similarity is 56±2 for the cephalosome signatures, 64±1 for the metasome signatures and only 53±2 for the urosome signatures (Tables 2.6-2.8). Only 10% of the total number of integumental pores occur in the urosome. These values disagree with Fleminger's conclusion that the species-specific pattern is most clearly seen in the adult female urosome. In his study, the species signatures do not include all pores occurring on the whole integument. Only pores that occur in more than 80% of individuals are described; the pores that occurred on the paired lateral area (ca. 16 to 34% of the total number of sites) are omitted because he did not examine them thoroughly. It is, therefore, difficult to quantify the extent of the species-specific proportion in *Eucalanus* species signatures. Fleminger's values are unlikely to indicate that a minority of the species-specific component resides in the urosomal signature.

Fleminger (1973) found that the species pore signatures of 15 of the 17 *Eucalanus* species are peculiar, while the overall species pore signature is identical between *E. subtenuis* and *E. bungii*. Hulsemann (1994) also noted the same prosomal signatures in 15 *Calanus* species. In the present study, the prosomal signatures of *Calanus finmarchicus*, *C. helgolandicus*, *C. hyperboreus* and *C. minor* were available (data from Mauchline, personal communication). According to Mauchline's data, interspecific differences are evident in the cephalosomal and/or metasomal signatures of four *Calanus* species including *C. finmarchicus* and *C. helgolandicus* which were described as sharing the same prosomal signature by Hulsemann (1994).

Undoubtedly, species-specific characters in the pore signature reside on the cephalosome, metasome and urosome to a different degree. In the present study, the dissected and stained integuments were separated from each other and then spread flat on slides. Displaying the urosomal segments, although there are only small numbers of pores involved, is the most difficult procedure in the KOH treatment, while the easiest is preparing the metasomal segments. Without the dissection and spreading flat of each segment, the parts of the integument are viewed as a tube. At certain areas, pores remain overlapped and it is difficult to detect them thoroughly. This method was used by Fleminger (1973), Fleminger and Hulsemann (1977, 1987) and Hulsemann (1994) so in these studies the species signature drawn did not describe all pores throughout the whole integument. The exceptional cases of species sharing the same signature within the genus could be partly explained by their method of KOH treatment, as mentioned by Fleminger (1973).

Studies on bioluminescence of marine calanoid copepods show that all species of the family Metridinidae, Augaptilidae, Lucicutidae and Heterorhabdidae have luminescent organs on their body and appendages (Giesbrecht 1895, Clarke *et al.* 1962, Mauchline 1977, 1988, Herring 1985). Species of the metridinid genera, *Metridia*, *Gaussia* and *Pleuromamma* have been the most intensively studied (Dahl 1893, 1894, Giesbrecht 1895, David and Conover 1961, Clarke *et al.* 1962, Rudjakov and Voronina 1967, Barnes and Case 1972, Mauchline 1977, Evstigneev 1982, 1983a, b, Herring 1985, Buskey *et al.* 1987, 1989, Bannister and Herring 1989, Latz *et al.* 1990). In the genus *Pleuromamma*, close observation of the location of bioluminescent organs were made in *P. robusta* by Clarke *et al.* (1962) and in *P. abdominalis*, *P. gracilis* and *P. piseki* by Evstigneev (1982).

In this investigation, attempts were made to identify the accurate position of luminescent glands in the pore signatures of *Pleuromamma robusta*. Specimens were obtained during a cruise on the RRS Challenger (May and September, 1993). On the ship, observation was carried out on freshly caught specimens under the epifluroscent

microscope, but it was difficult to examine bioluminescence. Buskey and Stearns (1991) reported that freshly caught copepods have a reduced bioluminescence capacity because they are mechanically stimulated to luminescence during net capture. The recovery time for bioluminescence potential was 10 to 16 h in *Pleuromamma xiphias*. The main reason for the failure to examine bioluminescence organs may be the use of freshly caught animals whose luminescence has been exhausted.

This study examined only pore signatures of *Pleuromamma* species. The various structural types of the integumental organs in calanoid copepods are generally classified as either a hair, peg and pit sensilla or gland opening (e.g. Fleminger 1973, von Vaupel Klein 1982, Koomen 1992). Further histological and ultrastructural studies on the integumental organs are required to show the various structures and functions of these organs. The KOH-treatment will provide useful information on the accurate detection of pore sites for further detailed SEM and TEM studies.

Table 2.1. *Pleuromamma* species. Specimens examined, sample position and date of collection.

Species	No. of specimens	Sample position	Date of collection
P. xiphias	22	37°27'N 17°10'W	15 Oct 75
P. abdominalis	20	37°27'N 17°10'W	15 Oct 75
P. scutullata	13	42°05'N 144°29'E	29 Jun 83
P. robusta	27	55°16'N 11°32'W	4 Sep 75
P. indica	22	25°N 63°E	21 Mar 93
P. gracilis	18	54°54'N 12°20'W	17 Nov.75
P. piseki	17	32°27'N 17°10'W	15 Oct 75
P. borealis	18	54°54'N 12°20'W	17 Nov 75

Table 2.2. *Pleuromamma* species. The number of integumental pores, large (L) and small (S), that occurred on the different areas and segments of the integuments of adult females of the different species. Only pores that occurred in more than 50% of the females examined are included. The values in parentheses are total numbers of pores per area of the integuments.

		xiphias		*abdominalis*		*scutullata*		*robusta*		*indica*		*gracilis*		*piselo*		*borealis*	
		L	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S
Rostrum		8	0	11	0	9	0	11	0	7	0	7	0	7	0	7	0
cephalic	I	9	8	7	8	9	8	9	8	7	8	7	8	7	8	7	8
	II	9	6	9	6	13	6	11	6	9	8	10	6	6	6	6	6
	III	20	8	20	8	20	8	20	8	20	8	21	8	18	8	20	8
	IV	10	6	10	6	16	6	10	6	11	6	8	6	8	6	9	6
	V	16	14	16	14	19	16	12	16	10	16	8	16	8	16	8	16
Subtotal		72	42	73	42	86	44	73	44	64	44	61	44	56	44	59	44
		(114)		(115)		(130)		(117)		(108)		(105)		(100)		(103)	
Metasome	I	23	20	22	20	25	20	23	20	15	20	19	20	19	20	19	20
	II	22	22	18	20	20	20	20	20	16	20	14	20	14	20	14	20
	III	14	26	12	22	16	22	14	22	10	22	10	22	10	22	10	22
	IV & V	16	26	16	26	18	26	16	26	16	26	16	24	20	24	16	26
Subtotal		77	96	68	88	79	88	73	88	57	86	59	86	63	86	59	86
		(173)		(156)		(167)		(161)		(145)		(145)		(149)		(145)	
Urosome	I	29	12	11	12	25	12	28	12	9	12	12	12	6	12	7	12
	II	7	4	7	4	4	4	5	4	5	4	4	4	4	4	4	4
	III	11	4	11	4	11	4	11	2	11	2	9	2	9	2	11	2
Subtotal		47	20	29	20	40	20	44	18	25	18	25	18	19	18	22	18
Total		196	158	170	150	205	152	190	150	146	150	143	148	138	148	140	148
		(354)		(320)		(367)		(340)		(298)		(293)		(286)		(288)	

Table 2.3. *Pleuromamma* spp. The index of similarity of the cephalosomal pore signatures.

	xiphias	*abdominalis*	*scutullata*	*robusta*	*indica*	*gracilis*	*piseki*	*borealis*
xiphias		0.95	0.76	0.87	0.88	0.77	0.73	0.75
abdominalis			0.80	0.91	0.88	0.78	0.74	0.75
scutullata				0.87	0.81	0.74	0.71	0.72
robusta					0.90	0.79	0.76	0.77
indica						0.85	0.82	0.83
gracilis							0.92	0.93
piseki								0.93
borealis								

Table 2.4. *Pleuromamma* spp. The index of similarity of the metasomal pore signatures.

	xiphias	*abdominalis*	*scutullata*	*robusta*	*indica*	*gracilis*	*piseki*	*borealis*
xiphias		0.90	0.81	0.86	0.83	0.82	0.84	0.83
abdominalis			0.90	0.95	0.92	0.91	0.90	0.92
scutullata				0.94	0.84	0.83	0.82	0.84
robusta					0.90	0.88	0.87	0.90
indica						0.93	0.92	0.94
gracilis							0.98	0.98
piseki								0.97
borealis								

Table 2.5. *Pleuromamma* spp. The index of similarity of the urosomal pore signatures.

	xiphias	*abdominalis*	*scutullata*	*robusta*	*indica*	*gracilis*	*piseki*	*borealis*
xiphias		0.73	0.73	0.76	0.64	0.64	0.55	0.57
abdominalis			0.73	0.68	0.87	0.84	0.75	0.79
scutullata				0.90	0.68	0.71	0.61	0.63
robusta					0.69	0.69	0.59	0.61
indica						0.86	0.86	0.88
gracilis							0.86	0.80
piseki								0.92
borealis								

Table 2.6. *Eucalanus* spp. The index of similarity of the cephalosomal signatures. Data from Fleminger's (1973) figures.

	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	E13	E14	E15	E16
E1		0.32	0.31	0.30	0.30	0.35	0.37	0.41	0.33	0.31	0.41	0.31	0.40	0.38	0.35	0.41
E2			0.62	0.68	0.73	0.67	0.57	0.62	0.52	0.88	0.61	0.41	0.66	0.57	0.70	0.59
E3				0.84	0.84	0.75	0.53	0.59	0.51	0.41	0.44	0.28	0.47	0.52	0.51	0.51
E4					0.96	0.76	0.62	0.68	0.48	0.47	0.45	0.29	0.52	0.53	0.56	0.48
E5						0.89	0.62	0.69	0.48	0.39	0.45	0.31	0.56	0.53	0.63	0.48
E6							0.65	0.68	0.45	0.40	0.43	0.30	0.52	0.53	0.56	0.45
E7								0.81	0.46	0.41	0.46	0.36	0.54	0.49	0.58	0.50
E8									0.42	0.46	0.54	0.35	0.56	0.47	0.60	0.49
E9										0.63	0.76	0.54	0.63	0.61	0.60	0.71
E10											0.73	0.72	0.59	0.55	0.57	0.60
E11												0.66	0.75	0.70	0.72	0.76
E12													0.54	0.50	0.51	0.54
E13														0.90	0.92	0.87
E14															0.82	0.86
E15																0.84
E16																

E1, *crassus*; E2, *longiceps*; E3, *monachus*; E4, *mucronatus*; E5, *subtenuis*; E6, *pileatus*; E7, *dentatus*; E8, *subcrassus*; E9, *inermis*; E10, *elongatus*; E11, *hyalinus*; E12, *californicus*; E13, *parki*; E14, *sewelli*; E15, *langae*; E16, *attenuatus*.

Table. 2.7. *Eucalanus* spp. The index of similarity of the metasomal signatures. Data from Fleminger's (1973) figures.

	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	E13	E14	E15	E16
E1		0.61	0.63	0.58	0.52	0.55	0.54	0.63	0.47	0.56	0.48	0.63	0.59	0.84	0.54	0.56
E2			0.81	0.89	0.71	0.75	0.44	0.66	0.61	0.60	0.66	0.59	0.59	0.57	0.65	0.55
E3				0.89	0.70	0.75	0.65	0.55	0.63	0.67	0.69	0.58	0.68	0.63	0.59	0.67
E4					0.76	0.65	0.68	0.71	0.57	0.71	0.61	0.66	0.70	0.58	0.59	0.66
E5						0.75	0.67	0.73	0.55	0.79	0.50	0.65	0.73	0.70	0.70	0.64
E6							0.63	0.70	0.70	0.68	0.60	0.57	0.68	0.55	0.56	0.53
E7								0.92	0.50	0.66	0.45	0.57	0.56	0.55	0.53	0.47
E8									0.49	0.60	0.43	0.53	0.55	0.52	0.55	0.49
E9										0.65	0.83	0.54	0.56	0.73	0.73	0.59
E10											0.55	0.84	0.67	0.65	0.70	0.63
E11												0.45	0.46	0.60	0.51	0.58
E12													0.75	0.65	0.76	0.68
E13														0.77	0.92	0.80
E14															0.85	0.84
E15																0.88
E16																

E1, *crassus*; E2, *longiceps*; E3, *monachus*; E4, *mucronatus*; E5, *subtenuis*; E6, *pileatus*; E7, *dentatus*; E8, *subcrassus*; E9, *inermis*; E10, *elongatus*; E11, *hyalinus*; E12, *californicus*; E13, *parki*; E14, *sewelli*; E15, *langae*; E16, *attenuatus*.

Table. 2.8. *Eucalanus* spp. The index of similarity of the urosomal signatures. Data from Fleminger's (1973) figures.

	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	E13	E14	E15	E16
E1		1.00	0.43	0.25	0.67	0.57	0.62	0.41	0.43	0.56	0.62	0.53	0.38	0.44	0.45	0.36
E2			0.70	0.80	0.67	0.69	0.69	0.48	0.77	0.56	0.75	0.53	0.47	0.44	0.45	0.83
E3				0.88	0.70	0.58	0.58	0.33	0.30	0.39	0.64	0.37	0.37	0.54	0.37	0.29
E4					0.80	0.69	0.67	0.38	0.35	0.44	0.73	0.42	0.42	0.50	0.42	0.33
E5						0.83	0.57	0.48	0.32	0.40	0.91	0.38	0.53	0.77	0.53	0.42
E6							0.71	0.57	0.40	0.50	0.92	0.48	0.55	0.56	0.48	0.44
E7								0.57	0.52	0.67	0.64	0.63	0.41	0.39	0.41	0.38
E8									0.42	0.34	0.48	0.43	0.60	0.48	0.60	0.55
E9										0.78	0.36	0.83	0.35	0.43	0.33	0.34
E10											0.45	0.95	0.37	0.41	0.42	0.40
E11												0.43	0.58	0.60	0.43	0.58
E12													0.41	0.39	0.41	0.43
E13														0.68	0.81	0.79
E14															0.52	0.54
E15																0.79
E16																

E1, *crassus*; E2, *longiceps*; E3, *monachus*; E4, *mucronatus*; E5, *subtenuis*; E6, *pileatus*; E7, *dentatus*; E8, *subcrassus*; E9, *inermis*; E10, *elongatus*; E11, *hyalinus*; E12, *californicus*; E13, *parki*; E14, *sewelli*; E15, *langae*; E16, *attenuatus*.

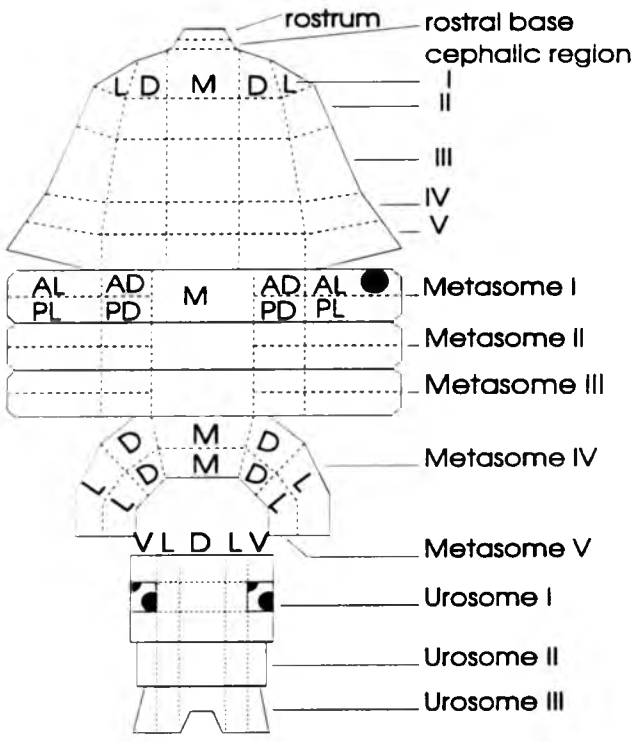


Fig. 2.1. Terminology refers to pores on the integument. The cephalosome is considered as having a median area (M) and paired dorsal (D) and lateral areas (L); the whole cephalosome is divided into five regions, as indicated by the horizontally dashed lines. The segments of the metasome have a median area (M), paired dorsal and lateral areas, these latter being divided into anterior and posterior regions (AD, PD, AL, PL). The fourth and fifth metasome segments are fused in *Pleuromamma* species but are recognized by their pore signatures; they are divided into paired dorsal (D) and lateral (L) areas. The genital somite of the urosome of the adult female is considered as having an anterior (UI-1), median (UI-2) and posterior (UI-3) area; the median area is defined as containing the genital opening. The urosomal segments have a dorsal (D), ventral (V), and paired lateral (L) area. The pigment knob is shown as a black circle in metasome I. The genital opening is shown as a square in urosome I, cut medially.

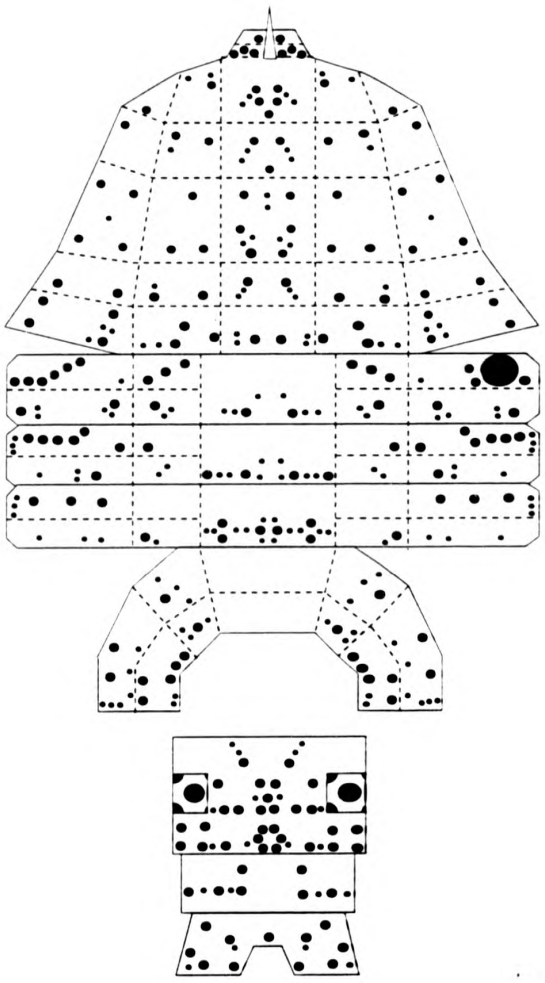


Fig. 2.2. Pore signature of adult female *Pleuromamma xiphias*.

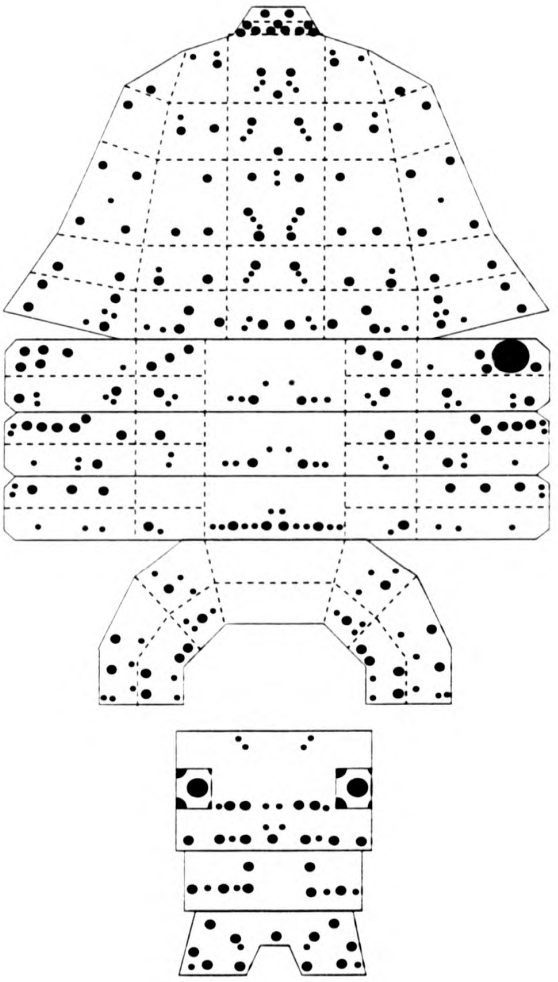


Fig. 2.3. Pore signature of adult female *Pleuromamma abdominalis*.

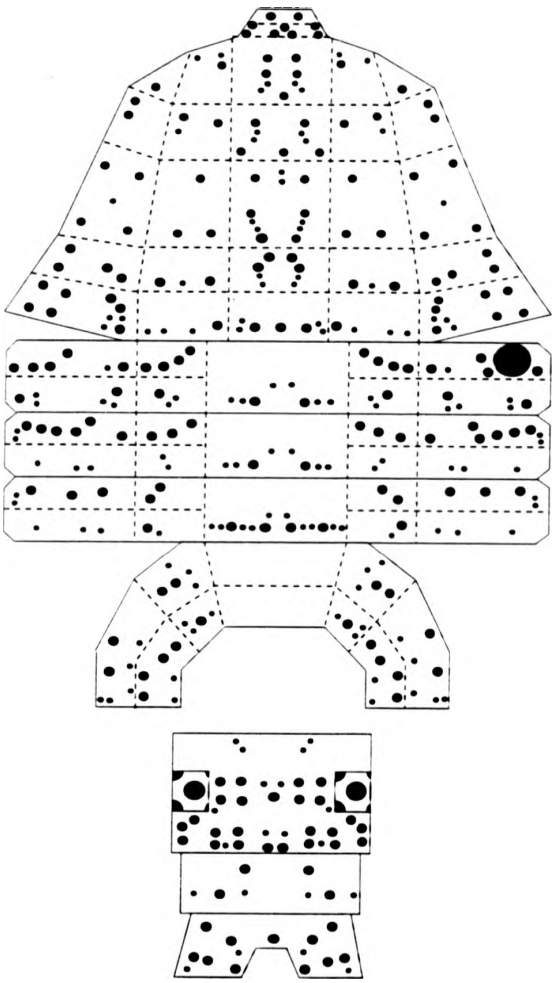


Fig. 2.4. Pore signature of adult female *Pleuromamma scutullata*.

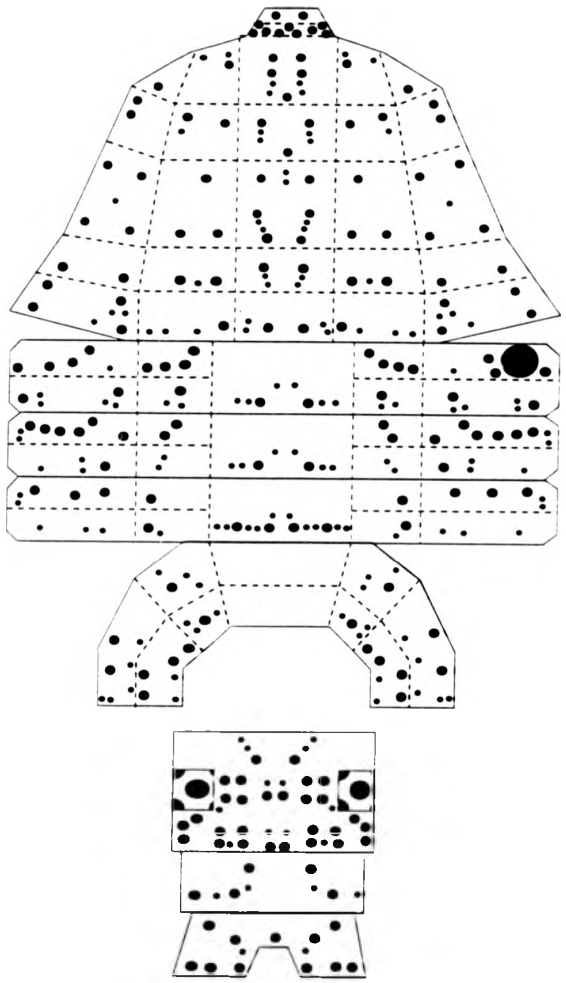


Fig. 2.5. Pore signature of adult female *Pleuromamma robusta*.

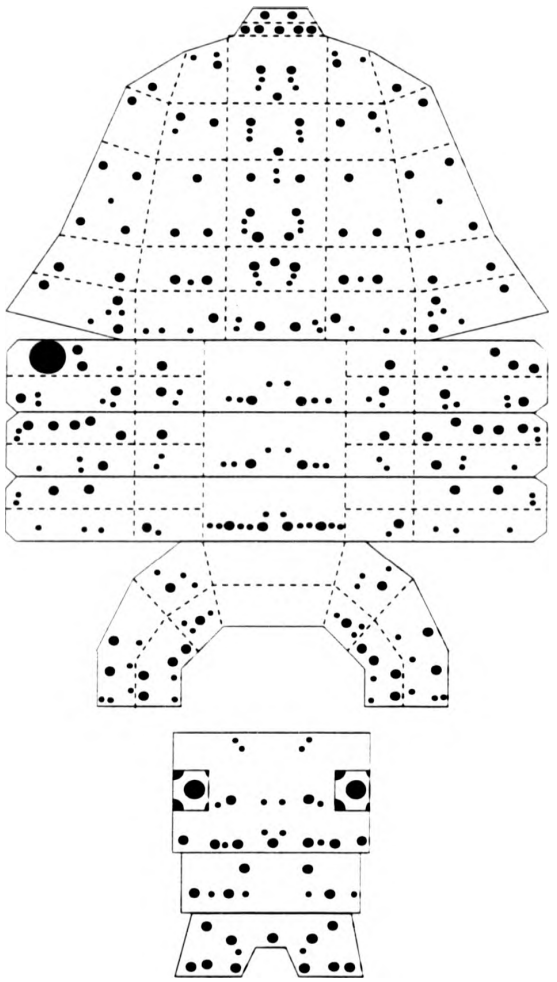


Fig. 2.6. Pore signature of adult female *Pleuromamma indica*.

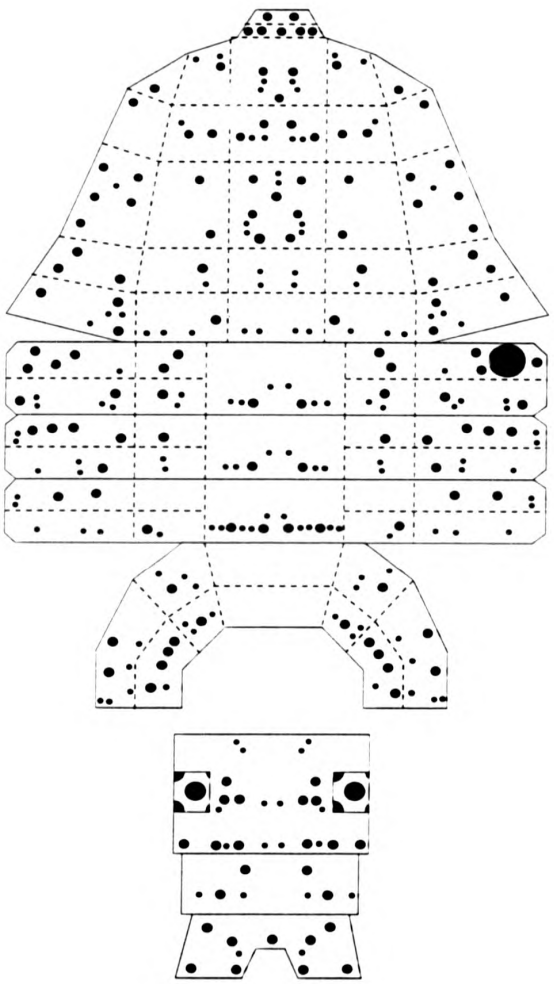


Fig. 2.7. Pore signature of adult female *Pleuromamma gracilis*.

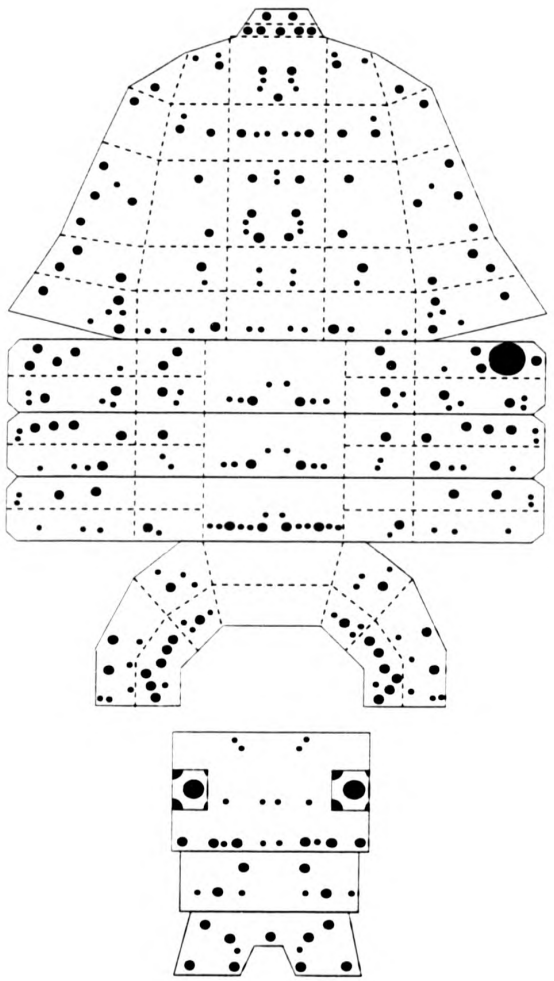


Fig. 2.8. Pore signature of adult female *Pleuromamma piseki*.

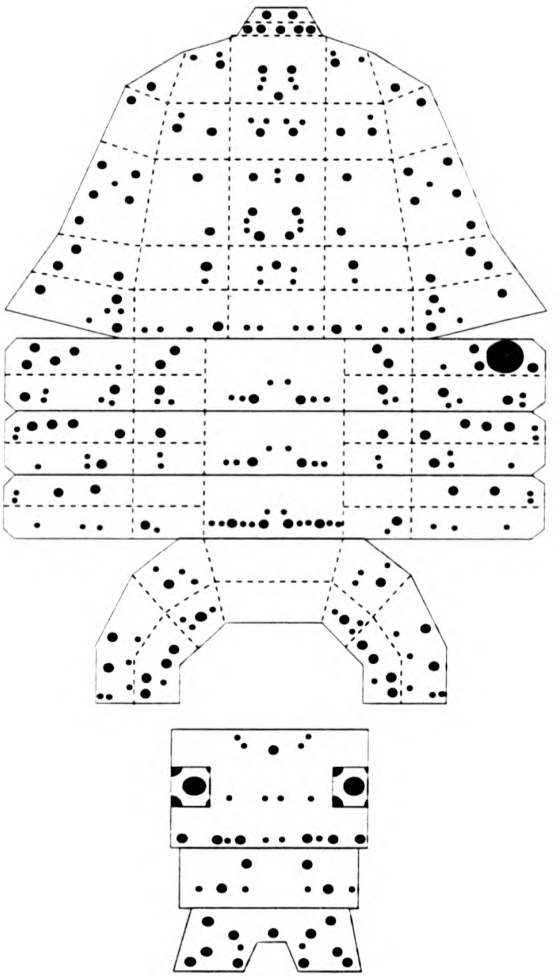


Fig. 2.9. Pore signature of adult female *Pleuromamma borealis*.

CHAPTER 3:

Phylogenetic relationships within the genus *Pleuromamma*

3.1. Introduction

The calanoid copepod genus *Pleuromamma* Giesbrecht 1898 has an unique organ consisting of a rounded, black or dark-red cuticular structure known as a pigment knob. The pigment knob occurs on the left or right side of the second metasomal segment, also termed the first pedigerous segment, depending on the species. The position of the pigment knob is related to morphologically asymmetrical appendages, namely the antennules, fifth pair of swimming legs, and the genital opening in males (Steuer 1932) (Table 3.1). The pigment knob, the non-geniculate antennule, the spermatophore-holder of the fifth legs and the genital opening always occur together on the same side of the body. The geniculate antennule and the female holder of the fifth legs always occur on the opposite side of the body. Ferrari (1984) termed this relationship of asymmetrical characters "unique concordance" and found other asymmetrical characters in adult males and females.

Steuer (1932) divided the species of *Pleuromamma* into four groups based on their morphological and asymmetrical characters: 1) *P. xiphias* and *P. abdominalis*; 2) *P. indica*; 3) *P. robusta* and *P. quadrungulata*; 4) *P. gracilis* and *P. borealis*. In his study, *P. scutullata* was not examined and *P. piseki* was regarded as a subspecies of *P. gracilis*. Ferrari (1984) divided the species into three groups based on the frequent position of the asymmetrical characters, the latter two groups of Steuer being regarded as one. The concepts of the phylogeny of the genus have been advanced by Steuer (1932) and Ferrari (1984), but there remains disagreement in phylogenetic relationships between individual species (Fig. 3.1).

In the present study, the morphological and asymmetrical characters are reexamined and the pore signatures of eight of the nine species of *Pleuromamma* were examined to clarify the phylogenetic relationships of the species. The results are then compared with those derived from interspecific comparisons of conventional morphological characteristics.

3.2. Materials and methods

Specimens of eight of nine *Pleuromamma* species were collected in the north Atlantic, western Pacific and western Indian Ocean. Specimens were treated with 20% aqueous potassium hydroxide (KOH) to examine the integumental organs as described in chapter 2. The terminology for describing the sites of the pores on the whole body follows Fig. 2.1.

3.3. Result

Species pore signatures

The species pore signatures and interspecific differences in species signatures were described in detail in the previous chapter (Figs. 2.2-2.9). The number of pores on the whole body is more than 320 in *Pleuromamma xiphias*, *P. abdominalis*, *P. scutullata* and *P. robusta*, and is less than 300 in *P. indica*, *P. gracilis*, *P. piskei* and *P. borealis* (Table 2.2). In the latter four species, one or two large pores, present in the first four species, are absent on the median area of the cephalic II, IV, and V, the dorsal area of the cephalics III and IV, the lateral area of the cephalic V, the anterior lateral area of the first three metasomal segments, and the first urosome. The overall pore signatures of *P. xiphias*, *P. abdominalis*, *P. scutullata* and *P. robusta*, therefore, are different from those of the remaining four species.

The pore signatures are similar in *Pleuromamma xiphias*, *P. abdominalis*, *P. scutullata* and *P. robusta* (Figs. 2.2-2.5). The signatures of *P. xiphias* and *P.abdominalis*, however, are different from those of *P. scutullata* and *P. robusta*. On the dorsal area of the cephalic V, two pores are peculiar to *P. xiphias* and *P. abdominalis* (Figs. 2.2, 2.3). The

pores on the lateral area of the cephalic II and the anterior dorsal area of the first and second metasomal segments are particular to *P. scutullata* and *P. robusta* (Figs. 2.4, 2.5). The first urosomal signature of *P. xiphias* and *P. abdominalis* differs from *P. scutullata* and *P. robusta*. *Pleuromamma xiphias* and *P. abdominalis*, therefore, separated from *P. scutullata* and *P. robusta*.

In *Pleuromamma indica*, the pore pattern on the cephalic II, III, IV and V is close to *P. xiphias*, *P. abdominalis*, *P. scutullata* and *P. robusta*, whilst the pattern on the rostral base and the first urosome is close to *P. gracilis*, *P. piseki* and *P. borealis* (Figs. 2.2-2.9). The number of pores on the anterior lateral area of the second metasome is five in *P. indica*; six in *P. xiphias*, *P. abdominalis*, *P. scutullata* and *P. robusta*; four in *P. gracilis*, *P. piseki* and *P. borealis*. In addition, the first metasomal signature of *P. indica* is distinct from all other species. Thus, *P. indica* is separated from *P. gracilis*, *P. piseki* and *P. borealis* (Figs. 2.6-2.9).

From the species pore signatures, *Pleuromamma* species are divided into four groups: 1) *P. xiphias* and *P. abdominalis*; 2) *P. scutullata* and *P. robusta*; 3) *P. indica*; 4) *P. gracilis*, *P. piseki* and *P. borealis*. The distinction between the species grouping is based on the overall species pore signatures, and it has not been possible to identify a suitable statistical test to confirm these grouping of species.

The morphological and asymmetrical characteristics

The morphological and asymmetrical characteristics of the nine *Pleuromamma* species are summarized in Table 3.1, as had previously been documented by Steuer (1932) and Ferrari (1984). Specimens of *P. quadrungulata* were not available for the species pore signatures, but their morphology follows Steuer's (1932) description.

3.4. Discussion

From the species pore signatures, *Pleuromamma* species are divided into four groups: 1) *P. xiphias* and *P. abdominalis*; 2) *P. scutullata* and *P. robusta*; 3) *P. indica*; 4) *P. gracilis*, *P. piseki* and *P. borealis*. How does the grouping of species derived from pore signatures compare with that derived from conventional morphological characters in the genus *Pleuromamma* species.

The antennules and the fifth pair of swimming legs are morphologically asymmetrical in the male of many calanoid copepods. When one of the male antennules is geniculate, it occurs consistently on one side of the body in a given group of copepods (Andronov 1974, Park 1986). However, in the genus *Pleuromamma* the geniculate antennule occurs either on the left or right side (Table 3.1): in *P. xiphias* and *P. abdominalis* on the right side; in *P. scutullata*, *P. quadrungulata*, *P. robusta*, *P. gracilis*, *P. piseki* and *P. borealis* on the left; in *P. indica* on the left or right. The pigment knob is a morphological structure unique to this genus. The pigment knob, the non-geniculate antennule, the spermatophore-holder of the fifth legs and the genital opening occur together on the same side of the body of the male; the geniculate antennule and female-holder of the fifth legs occur on the opposite side to the first four characters (Steuer 1932, Ferrari 1984).

Steuer (1932) divided the species of *Pleuromamma* into three groups (Fig. 3.1). The first group comprised *P. xiphias* and *P. abdominalis* with the pigment knob occurring on the left side in males and on the left and right side in females (Table 3.1). The second group consisted of only *P. indica* with the pigment knob on the left or right side in the males and female. The third group included all other species in which the pigment knob is fixed on the right side in both sexes. In his study, *P. scutullata* was not examined and *P. piseki* was regarded as a subspecies of *P. gracilis*.

Steuer (1932) also described other morphological characters. In *Pleuromamma*

xiphias, *P. abdominalis*, *P. quadrungulata* and *P. robusta*, the proximal segments of the antennules have denticles, in females the fifth pair of legs consists of four segments and the genital pore is of a similar structure. In *Pleuromamma xiphias* and *P. abdominalis*, however, the pigment knob is not fixed on one side of females but it is fixed on the left side of males, opposite to all other species (Table 3.1). The male urosome is morphologically asymmetrical in *P. xiphias* and *P. abdominalis*, whilst it is symmetrical in the other species (Table 3.1).

In *Pleuromamma indica*, *P. gracilis*, *P. piseki* and *P. borealis*, the antennules have no denticles and the body size is smaller than in the former four species (Table 3.1). In female *P. gracilis*, *P. piseki* and *P. borealis* the fifth legs have only two or three segments and the genital pore is specialized. However, these structures of female *P. indica*, the sole species in Steuer's second group, are similar to those of *P. xiphias*, *P. abdominalis*, *P. quadrungulata* and *P. robusta*.

All species have a hook on one or both of the inner edges of the first endopodite of the second pair of legs. This is a diagnostic character of the family Metridinidae. Females of the genus *Pleuromamma* have a hook either on the left or right side. In males the position of the hook in *P. xiphias* and *P. abdominalis* is on the left side, in *P. quadrungulata*, *P. robusta* and *P. indica* on both left and right sides, and in *P. gracilis*, *P. piseki* and *P. borealis* on the right side (Table 3.1). Therefore, the third group of species of Steuer (1932) is subdivided into two groups: one consisting of *P. robusta* and *P. quadrungulata* and the other of *P. gracilis* and *P. borealis* (Fig. 3.1). In spite of Steuer's (1932) detailed investigations of the morphological and asymmetrical characters, his original form ("stem form") of the genus is confused. He stated that in members of genus *Pleuromamma* the pigment knob is not fixed on one side, the antennules are without denticles and the body size is small. He, therefore, classified *P. indica* close to the stem form of the genus and placed it between the two subgroups of the third group (Fig. 3.1). *P. xiphias* and *P.*

abdominalis were considered distant from the original form.

Ferrari (1984), however, suggested that *Pleuromamma xiphias* and *P. abdominalis* are the oldest species, *P. indica* is next oldest and all the others are younger (Fig. 3.1). He noted that males of both *Metridia* and *Gaussia* of the family Metridinidae have the geniculate antennule on the right side. The oldest species was defined by the right geniculate antennule, the left genital opening and the spermatophore-holder of the fifth legs, as in the two other metridinid genera. However, at least seven species of *Metridia* males, namely *M. asymmetrica*, *M. brevicauda*, *M. longa*, *M. lucens*, *M. macrura*, *M. ornata* and *M. princeps*, have the geniculate antennule on the left side (Sars 1903, Sewell 1932, Rose 1933, Brodsky 1950, van Breemen 1964). In the species of the family Metridinidae, the geniculate antennule is more commonly on the right side than the left. A reduction in the number of component segments, setae and spines of the fifth pair of legs is believed to be an evolutionary trend (Andronov 1974, Park 1986). Therefore, in Ferrari's younger group species, the number of segments of the fifth legs and the specialized genital opening should be considered in female *P. gracilis*, *P. piseki* and *P. borealis*. The species of Ferrari's younger group should be divided into two according to these two characters.

In the present study, the *Pleuromamma* species are divided by their pore signatures into four groups (Fig. 3.1): 1) *P. xiphias* and *P. abdominalis*; 2) *P. scutullata* and *P. robusta*; 3) *P. indica*; 4) *P. gracilis*, *P. piseki* and *P. borealis*. Specimens of *P. quadrungulata* were not available to this study, but might belong to the second group of species, *P. scutullata* and *P. robusta*. This division is in agreement with Steuer's derived from morphological and asymmetrical characters. The possible phylogenetic relationships, however, of the individual species is different from both that of Steuer and Ferrari (Fig. 3.1).

The evolutionary trends in the species of *Pleuromamma* might be apparent in body length, the male urosome, the antennules, and the fifth pair of legs and genital opening of females (Table 3.1). Body length in the genus *Pleuromamma* seems to be progressively

decreasing. It is noteworthy that the pore signature of the first urosomal segment of *P. abdominalis* is more close to that of *P. indica* and *P. gracilis* than to that of *P. xiphias*, even though the morphological and asymmetrical characters are different. The asymmetrical urosome of male *P. xiphias* and *P. abdominalis* might evolve into the symmetrical urosome. The presence or absence of denticles on the antennules corresponds to the pore signature on the rostral base (Figs. 2.2-2.9 in chapter 2). Finally, the fifth pair of legs and the structure of the genital complex seem to be specialized in female *P. gracilis*, *P. piseki* and *P. borealis* (see Figs, Steuer 1932, Ferrari 1984).

The pore signatures shed further light on the phylogeny of the species which is not evident in studies of the morphology and asymmetry. The pore signatures provide presence/absence data that are discrete in contrast to other morphological data that are often diffuse. Changes in the pore signatures between species parallel changes in other morphological characters as has previously been shown at the generic, familial and superfamilial levels (Mauchline 1988). The correspondence between changes in the pore signatures and conventional morphological characters in two different genera of calanoid copepods, namely *Eucalanus* studied by Fleminger (1973) and *Pleuromamma* examined here, infers the general usefulness of this character in speciation studies.

Table 3.1. *Pleuromamma* species. Comparison of morphological and asymmetrical characters described by Steuer (1932). The position of the male geniculate (G) antennule, the pigment knob of both sexes, and hook of the male second pair of swimming legs (P2) that occur on the left (L) and/or right (R). Male urosome is asymmetrical (asym) and symmetrical (sym). The antennules have denticles (yes) and no denticles (no) in males and females. The female fifth pair of swimming legs (P5) have four (4), two (2) or three (3) segments.

	Antennules		Pigment knob		Hook of P2	Female P5	Male Urosome	Body length (mm)
	Denticles	Male (G)	Female	Male	Male			
xiphias	Yes	R	L or R	L	L	4	Asym	3.0-6.42
abdominalis	Yes	R	L or R	L	L	4	Asym	2.4-5.0
scutullata	Yes	L	R	R	L & R	4	Sym	3.6-4.0
quadrungulata	Yes	L	R	R	L & R	4	Sym	3.0-5.0
robusta	Yes	L	R	R	L & R	4	Sym	3.0-4.89
indica	No	L or R	L or R	L or R	L & R	4	Sym	1.7-2.5
gracilis	No	L	R	R	R	2 or 3	Sym	1.2-2.55
piseki	No	L	R	R	R	2 or 3	Sym	1.2-2.0
borealis	No	L	R	R	R	2 or 3	Sym	1.6-2.46

* Records of total body lengths from Sewell (1932, 1947), Rose (1933), Brodsky (1950), Vervoort (1957, 1965), van Breemen (1964), Bradford (1972), Hayward (1980), Ferrari (1985), Hopkins (1985), Bennett and Hopkins (1989).

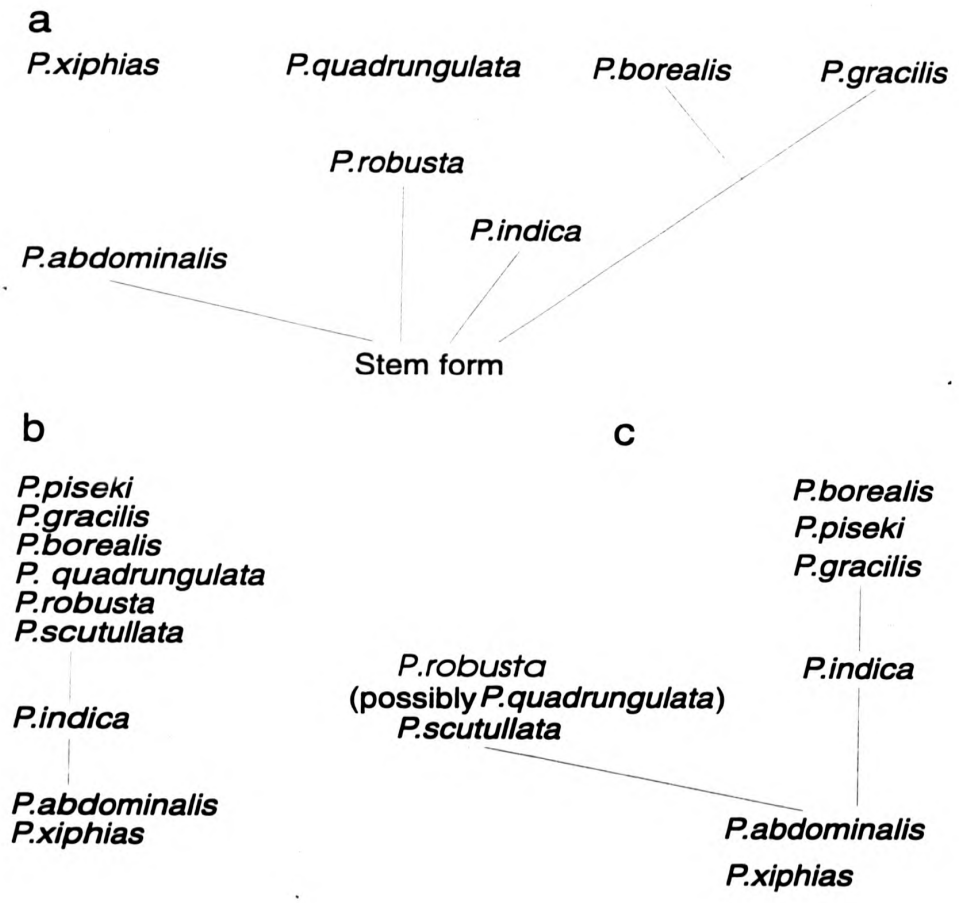


Fig. 3.1. *Pleuromamma* species. Phylogenetic relationships.
(A) Steuer (1932), (B) Ferrari (1984), (C) Present study

CHAPTER 4:

Ontogenetic development of species pore signatures

4.1. Introduction

Since most studies on the pore signature of calanoid copepods have been made on adult females, very little is known of the ontogenetic development of the pore signatures and their sexual differences. Fleminger (1973) noted that in *Eucalanus* species the prosomal signature is complete in the copepodid III, and the urosomal signature in the copepodid VI, the adult. Mauchline and Nemoto (1977), however, found that the species pore signatures in both the prosome and the urosome are only completed in the copepodid VI, adult stage, in *Paraeuchaeta norvegica*, *Neocalanus plumchrus* and *N. cristatus*. Sexual differences in the pore signature have been described in two species of *Arietellus* by Bradford (1974) and in *Paraeuchaeta norvegica* by Mauchline and Nemoto (1977). The previous studies do not show differences in the pore signatures during the copepodid ontogeny between the species of a genus, nor do they describe sexual differences with respect to the species-specific components.

The aim of this study is to identify the stages at which interspecific and sexual differences in the pore signature are developed and the species-specific signature is completed. The sequential development of the pore signature is described in male and female copepodid stages of five species in the metridinid genus *Pleuromamma*: *P. robusta*, *P. xiphias*, *P. abdominalis*, *P. pseki* and *P. gracilis*. In addition, the sexual differences in the pore signature are assessed.

4.2. Materials and methods

Copepodid stages were collected in the Rockall Trough, northeastern Atlantic Ocean at approximately 54°N 12°W. The number of specimens examined in male and female copepodid stages of five *Pleuromamma* species is shown in Table 4.1. To examine the

integumental organs, specimens were treated with 20% aqueous potassium hydroxide (KOH). The terminology for describing the pore sites on the whole integument follows Fig. 2.1 in chapter 2.

Jaccard's (1908) index of similarity is used to examine the similarity of the urosomal signature between the adult male and female of the five *Pleuromamma* species examined. The index of similarity (IS) is defined as

$$IS = \frac{c}{a+b+c}$$

where *a* and *b* are the number of pores peculiar to the respective species, and *c* is the number of pores common to the two species. The pores are either present or absent in a pore signature; the index is a reflection of this property.

4.3. Results

The pore signatures from copepodid II to VI in *Pleuromamma robusta*, copepodid III to VI in *P. xiphias*, copepodid IV to VI in *P. abdominalis* and *P. piseki* and copepodid V to VI in *P. gracilis* are shown Figs. 4.1-4.5. The quantitative development of integumental pores is shown in Table 4.1.

Copepodid I

No representatives of this stage were found in the samples.

Copepodid II

Of five species, only copepodid II of *Pleuromamma robusta* was present in the

samples (Fig. 4.1, Table 4.1). The number of metasomal segments is four, and the number of urosomal segments is two. About 17% of the integumental organs of the adult are present on the cephalosome and the first three metasomal segments (Table 4.1). The fourth metasomal and two urosomal segments have no pores.

Copepodid III

This stage was available only in *Pleuromamma robusta* and *P. xiphias* (Figs. 4.1, 4.2, Table 4.1). The number of metasomal segments is five. The segmentation between the fourth and fifth metasomal segments is clearly recognizable in the KOH-treated integument. These two segments are fused in the subsequent stage. In *P. robusta*, more pores than in the previous stage are developed on the cephalosome and the first three metasomal segments. A few pores occur on the fourth and fifth metasomal and two urosomal segments. The cephalosomal and urosomal signatures are identical in *P. robusta* and *P. xiphias*. However, the signatures of the first and second metasomal segments differ between the two species (Figs. 4.1, 4.2); *P. robusta* has additional pores on the paired anterior dorsal area of the first and second metasome.

Copepodid IV

There are four free metasomal segments as the fourth and fifth segments are fused. The overall pore pattern is similar in *Pleuromamma robusta*, *P. xiphias*, and *P. abdominalis*. The pore patterns of these three species are different from that of *P. piseki* (Figs. 4.1-4.4). Interspecific differences occurred in the previous stage between *P. robusta* and *P. xiphias*, but they now have the same pattern in this stage. Minor interspecific differences in the prosomal signature are found between all the species. About 50% of the integumental

organs of the adult are present (Table 4.1).

There are three urosomal segments. The first segment in each species shows two kinds of pore patterns; two large and two small pores or four small pores (Figs. 4.1-4.4). The urosomal signature of *P. piseki* was not examined.

Copepodid V

The adult cephalosomal signature is complete, except in *Pleuromamma robusta* where two pores on the median and the paired lateral area of the first and the second cephalic region are not developed (Fig. 4.1). The metasomal and urosomal signatures are not complete (Figs. 4.1-4.5). Interspecific differences occur on the whole integument and the species have peculiar pore patterns. The number of integumental organs in *P. robusta*, *P. xiphias*, and *P. abdominalis* is greater than that in *P. piseki* and *P. gracilis* (Table 4.1).

The number of urosomal segments is four. Sexual differences occur in the first and second urosomal signatures.

Copepodid VI or adult

The development of integumental organs is completed (Figs. 4.1-4.5, Table 4.1). A pair of small pores, present in the previous stage of all species examined, is not found on the paired dorsal and lateral area of the first and second cephalic region in the adult.

There are no sexual differences between the cephalosomal signatures of the five species. In *Pleuromamma robusta*, *P. piseki* and *P. gracilis* the metasomal signature is identical between sexes, whilst sexual differences are found in *P. xiphias* and *P. abdominalis* (Figs. 4.1-4.5).

In *Pleuromamma xiphias*, one large pore on each of the paired posterior lateral

margins of the first metasomal segment is present in the female but absent in the male (Fig. 4.2). Females have three small pores on each of the paired anterior lateral margins of the second to fourth metasomal segments, whilst males have only two small pores at these sites. In addition, the patterns in the median area of the second and third metasomal segments differ between sexes.

In *Pleuromamma abdominalis*, a large pore on each of the paired posterior lateral margins of the first metasomal segment, present in the female, is absent in the male (Fig. 4.3). Both sexes have a row of six and three large pores respectively on each of the paired anterior lateral areas of the second and third metasomal segments; in the male the outermost pore in the row of the opposite side to the pigment knob is replaced by a group of pores in the form of a rosette.

The adult male has five urosomal segments, while in the female the first three segments are fused, so that the apparent number of segments is three. Thus, the first urosomal segment of the female is divided into three regions: the anterior, medial and posterior region (Fig. 2.1 in chapter 2). The medial region is defined as containing the genital opening. The pore signatures of these three regions are comparable to those of the first three urosomal segments of the male. Sexual differences in the urosomal segments are found in all *Pleuromamma* species examined.

The urosomal signatures of males and females at this stage are assessed separately using the index of similarity (Jaccard 1908). The mean index of similarity between the five species is 0.78±0.07 for males and 0.71±0.10 for females (Tables 4.2, 4.3). These values suggest that the interspecific differences in the urosomal signatures are greater in females than in males.

4.4. Discussion

Mauchline and Nemoto (1977) found that the copepodid I of *Pareuchaeta norvegica* has only one pore and a few thin integumental sites on the metasome. The thin integumental sites are represented by pores in the subsequent stage. No pores are present on the cephalosome and the urosome. Evstigneev (1982) observed bioluminescence of *Pleuromamma abdominalis*, *P. piseki* and *P. gracilis*. The number of bioluminescent glands is the same in the copepodid stages I to VI; 15 in *P. abdominalis* and 18 in *P. piseki* and *P. gracilis*. The naupliar stages V and VI of *P. piseki* have three bioluminescent organs. These bioluminescent glands open externally through integumental pores (Clarke *et al.* 1962).

In the present study, the copepodid I of *Pleuromamma* species was not present in the samples. Evstigneev's (1982) study, however, indicates the presence of at least bioluminescent glands and their integumental opening in the copepodid I stage. The copepodid II of *P. robusta* has 59 pores (Table 4.1). Evstigneev (1982) noted that four or eight bioluminescent organs are present on the anal segment and the furcae. In this study, the copepodid II of *P. robusta* has not pores on the fourth metasomal segment and two urosomal segments; the furcae were not examined (Fig. 4.1).

Fleminger (1973) found that the copepodid III of *Eucalanus* species has the adult pattern of the integument organs on the prosome, but that that of the urosome is not completed until the copepodid VI, adult stage. Mauchline and Nemoto (1977), however, showed that these organs develop sequentially throughout the successive copepodid stages and that the final adult pattern on the whole integument is completed in the copepodid VI stage in *Pareuchaeta norvegica*, *Neocalanus cristatus* and *N. plumchrus*. In the present study, the copepodid III stage has about 30% of the adult organs (Table 4.1). At this stage, interspecific differences in the pore signature occur. The species pore signature is complete

in the copepodid VI stage. The cephalosomal signature, however, is complete in the copepodid V stage, except in *Pleuromamma robusta*.

In *Pleuromamma* species, interspecific differences in the pore signatures of copepodid stages arise from three sources. The first is where the development of the adult pore signature takes place at different rates and corresponding copepodids are not at the same stage of development. An example of this is the first and second metasomal signatures of the copepodid III of *P. robusta* and *P. xiphias* (Figs. 4.1, 4.2) where the pattern is different in the copepodid III but becomes identical in the copepodid IV. The second source of differences in the pore patterns of copepodids is the development of the species-specific components of the pore signatures. These differences occur at sites where the adult pore patterns are different between species, for example the interspecific differences in the copepodid V of all five species (Figs. 4.1-4.5). The third source of differences in the copepodid patterns arise from expressions of the genetic or phylogenetic grouping of species within the genus. Thus, the overall developmental pattern of the pore signature is similar in *P. robusta*, *P. xiphias* and *P. abdominalis*, and different from that in *P. piseki* and *P. gracilis* (Figs. 4.1-4.5, Table 4.1). In the genus, interspecific relationships have already been derived from the species pore signatures of adult females, and morphological and asymmetrical characteristics (see chapters 2, 3). The ontogenetic developmental pattern of the species signature reflects not only the final species pore signature but the grouping of the species within the genus.

Sexual differences in the pore signature were first noted on the prosome in adult *Arietellus aculeatus* and an unidentified *Arietellus* sp. by Bradford (1974). Female *Arietellus* species have a few more pores than the corresponding males. Mauchline and Nemoto (1977) found sexual differences in the pore patterns of male and female *Pareuchaeta norvegica*; the differences are described as slight and consist of presence or absence of certain pores over the whole integument. Mauchline and Nemoto (1977) also noted that

sexual differences do not develop until the copepodid VI stage. In this study, sexual differences are found in the metasomal and urosomal signatures in copepodids and adults. In the metasome, sexual differences occur in the copepodid VI stage. Differences in the urosomal signature, however, occurred in the copepodid V stage. In the copepodid IV stage, the first urosomal signature show two patterns, but the sex can not be identified at this stage. These differences may be sexual characteristics.

Boxshall (1985), Huys and Boxshall (1991) and Hulsemann (1991) illustrate the ontogenetic pattern of urosomal segments based on the general anatomy and morphology of the copepodids of the genus *Euaugaptilus* and *Drepanopus*. The number of urosomal segments is two until the copepodid III stage. A new segment is added at each successive copepodid stage between IV and VI. The fusion of female urosomal segments occurs in the copepodid VI stage. This segmentation pattern of the urosome is also evident in the sequential development of the urosomal signature of *Pleuromamma* species (Fig. 4.6) and confirms the developmental sequence of these previous authors.

In the present study, only the pore signature was examined. There remains further histological and ultrastructural studies on integumental organs, especially to show the various types and the function of the pores peculiar to the male or female of the species.

Table 4.1. The number of pores on the cephalosome (C), metasome (M) and urosome (U) in the copepodid and adult stages of *Pleuromamma* species. Only pores that occurred in more than 50% of the specimens examined are included. The numbers of each copepodid examined are shown in parenthesis.

Species	CII	CIII	CIV		CV		CVI	
			F	M	F	M	F	M
P.robusta								
C	36	61	94	94	119	119	117	117
M	23	45	73	73	117	117	161	161
U	0	5	19	19	33	35	62	68
Total	59	111	186	186	269	271	340	346
Number	(3)	(4)	(9)		(6)	(8)	(20)	(20)
P.xiphias								
C	-	61	94	94	118	118	114	114
M	-	41	71	71	114	114	173	156
U	-	5	19	19	33	36	67	65
Total	-	107	184	184	265	268	354	334
Number	-	(5)	(11)		(10)	(6)	(20)	(20)
P.abdominalis								
C	-	-	91	91	119	119	115	115
M	-	-	69	69	114	114	156	159
U	-	-	19	19	30	36	49	59
Total	-	-	179	179	263	269	320	333
Number	-	-	(7)		(7)	(9)	(20)	(20)
P.piseki								
C	-	-	82	82	104	104	100	100
M	-	-	67	67	109	109	149	149
U	-	-	?	?	21	29	37	51
Total	-	-	-	-	234	242	286	300
Number	-	-	(5)		(5)	(2)	(20)	(8)
P.gracilis								
C	-	-	-	-	109	109	105	105
M	-	-	-	-	109	109	145	145
U	-	-	-	-	27	29	43	45
Total	-	-	-	-	245	247	293	295
Number	-	-	-	-	(3)	(2)	(20)	(12)

Table 4.2. The index of similarity of the urosomal pore signature in female *Pleuromamma* species.

	robusta	*xiphias*	*abdominalis*	*piseki*	*gracilis*
robusta		0.77	0.68	0.60	0.69
xiphias			0.73	0.55	0.64
abdominalis				0.76	0.80
piseki					0.86
gracilis					

Table 4.3. The index of similarity of the urosomal pore signature in male *Pleuromamma* species.

	robusta	*xiphias*	*abdominalis*	*piseki*	*gracilis*
robusta		0.77	0.81	0.77	0.68
xiphias			0.88	0.73	0.69
abdominalis				0.80	0.76
piseki					0.88
gracilis					

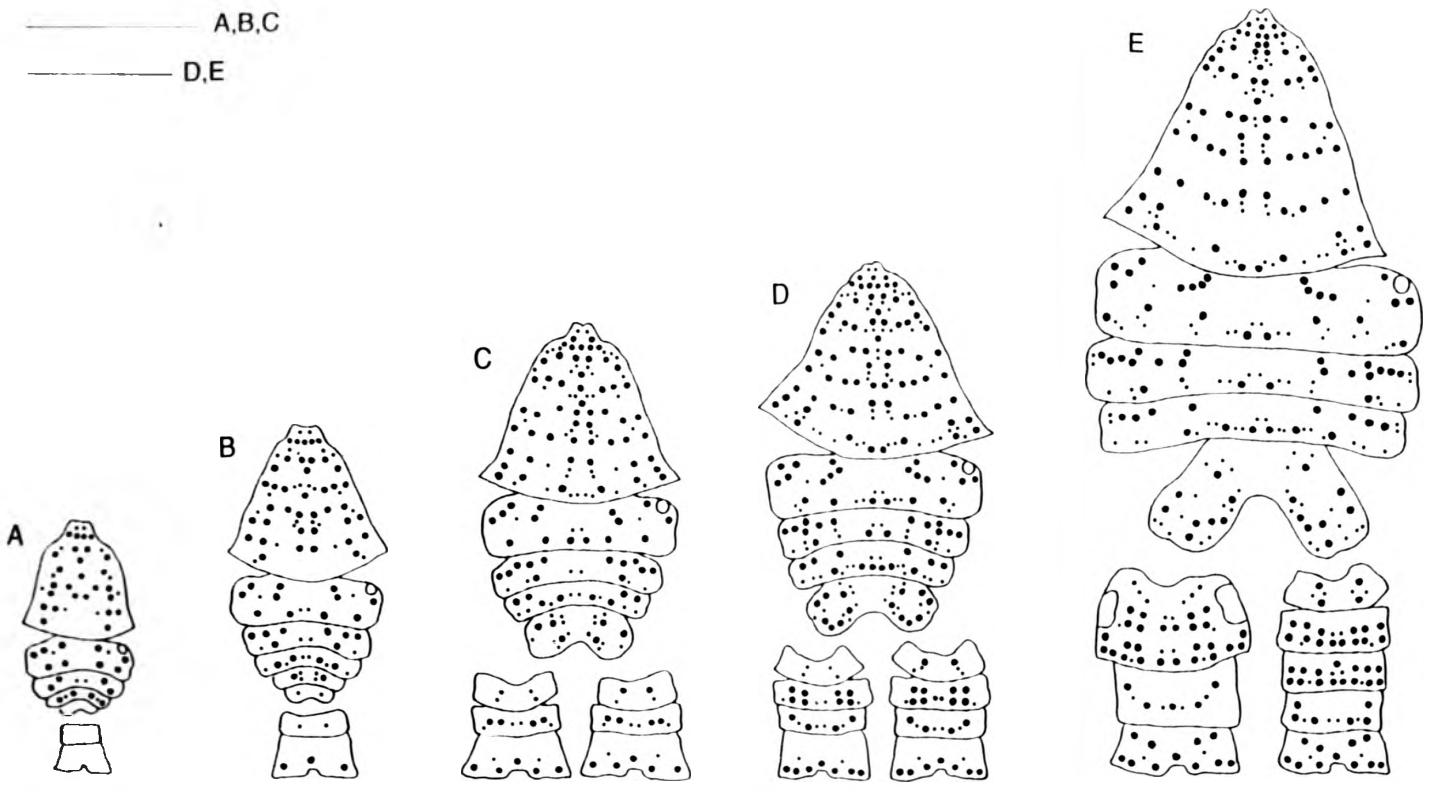


Fig. 4.1. Pore signature in the KOH-treated integument of *Pleuromamma robusta*. The pigment knob is shown as an open circle in metasome I of all copepodids; the urosomal signature of the female on the left and that of the male on the right in the copepodid IV to VI. The genital opening of the female in copepodid VI is shown as a square in urosome I. Scale: 1 mm. (**A**) copepodid II, (**B**) copepodid III, (**C**) copepodid IV, (**D**) copepodid V, (**E**) copepodid VI.

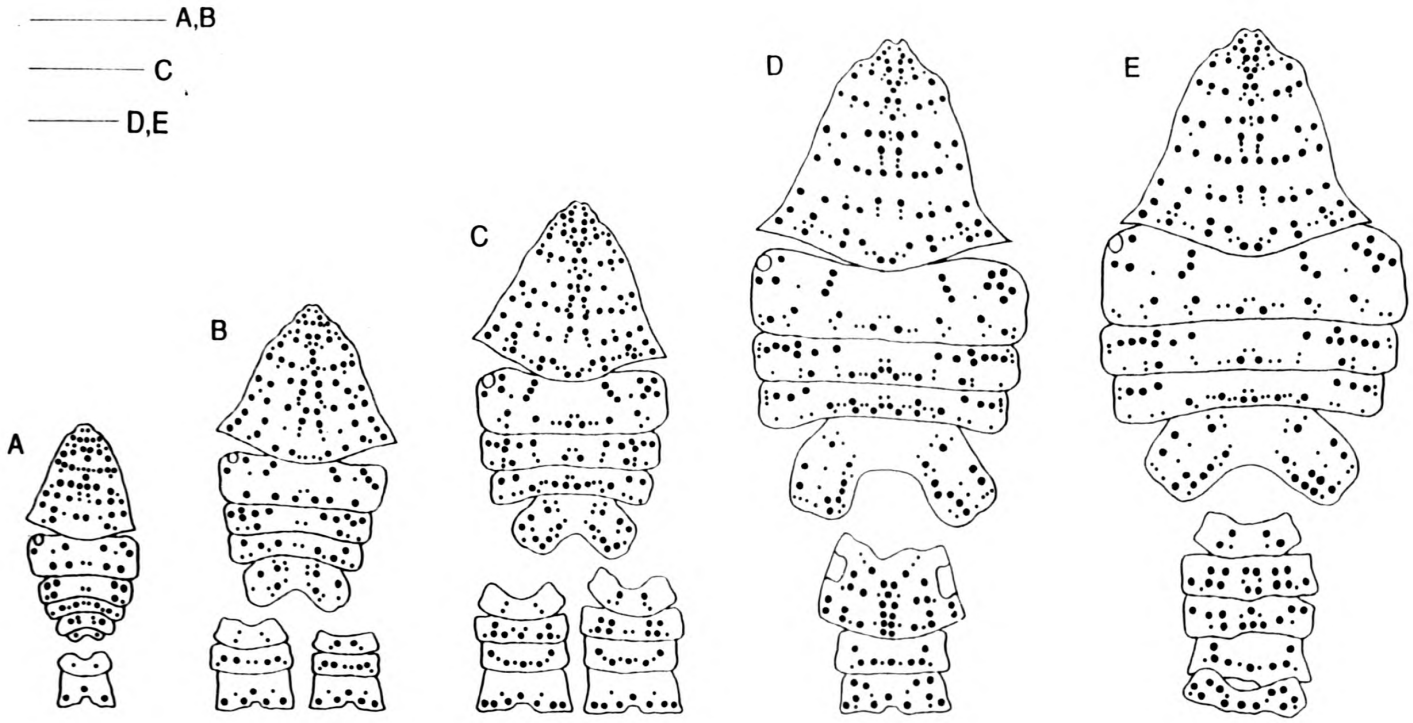


Fig. 4.2. Pore signature in the KOH-treated integument of *Pleuromamma xiphias*. The format of the figures is explained in legend to Figure 1. **(A)** copepodid III, **(B)** copepodid IV, **(C)** copepodid V, **(D)** female of copepodid VI, **(E)** male of copepodid VI.

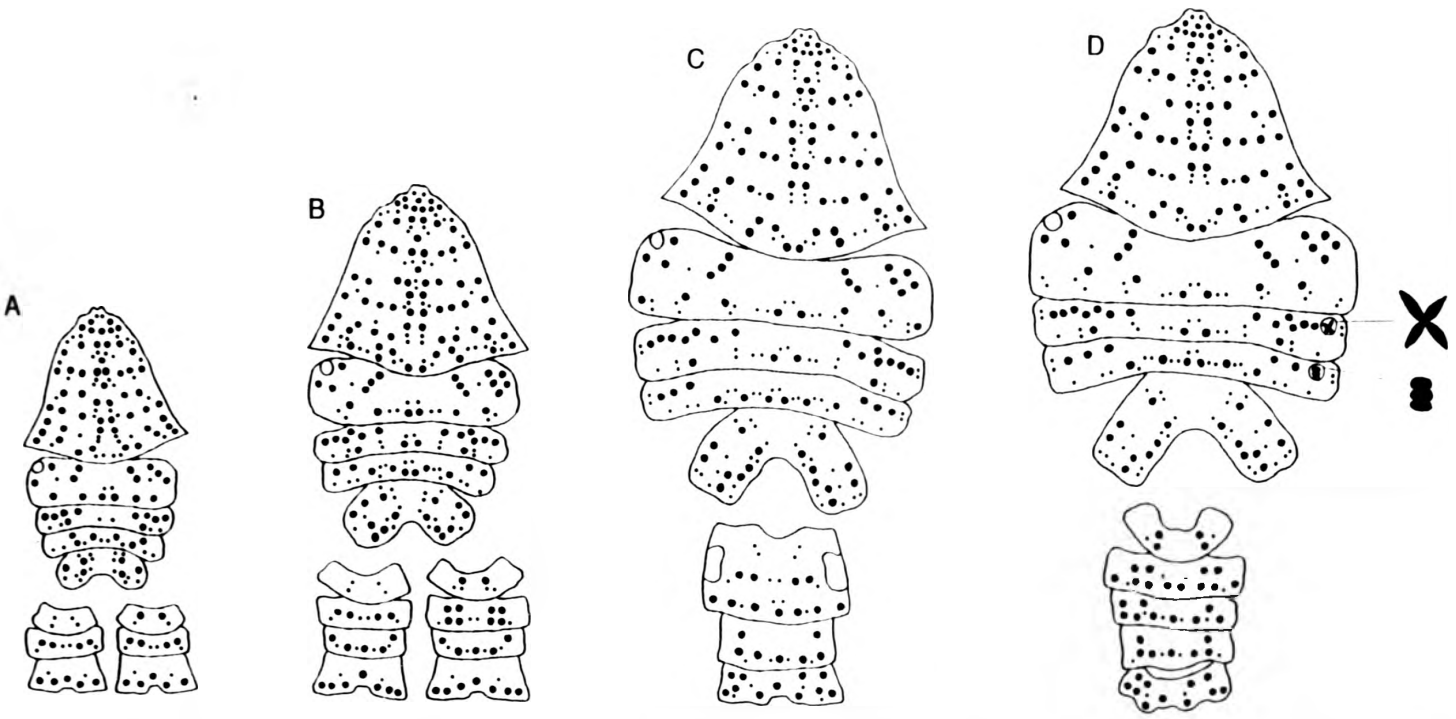


Fig. 4.3: Pore signature in the KOH-treated integument of *Pleuromamma abdominalis*. The format of the figures is explained in legend to Figure 1. **(A)** copepodid IV, **(B)** copepodid V, **(C)** female of copepodid VI, **(D)** male of copepodid VI.

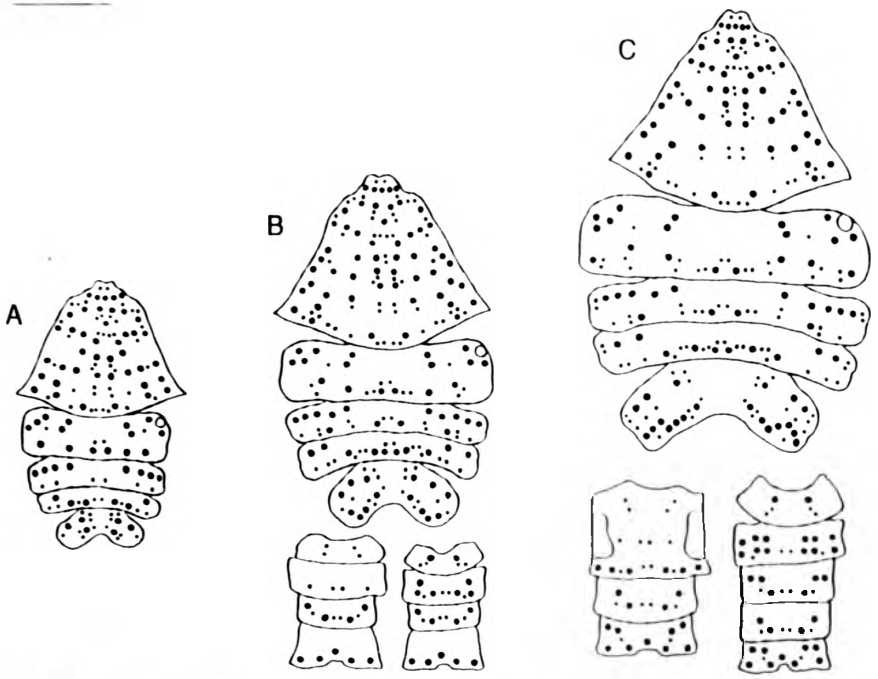


Fig. 4.4: Pore signature in the KOH-treated integument of *Pleuromamma piseki*. The format of the figures is explained in legend to Figure 1. **(A)** copepodid IV, **(B)** copepodid V, **(C)** copepodid VI.

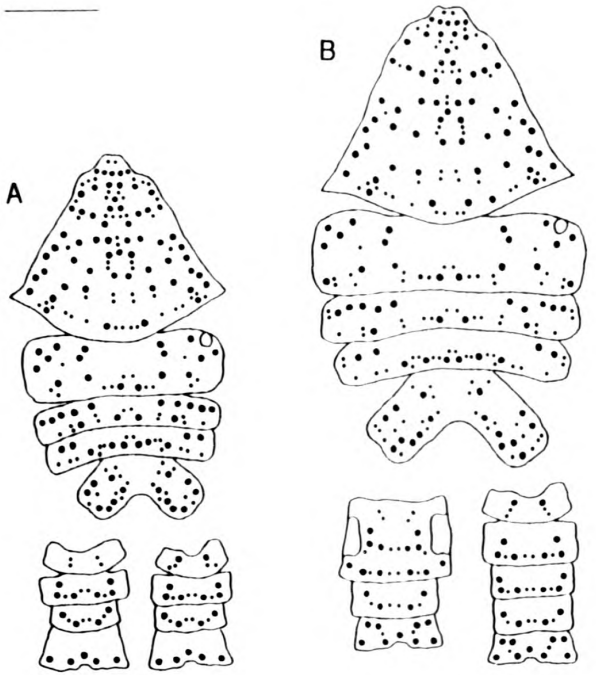


Fig. 4.5. Pore signature in the KOH-treated integument of *Pleuromamma gracilis*. The format of the figures is explained in legend to Figure 1. **(A)** copepodid V, **(B)** copepodid VI.

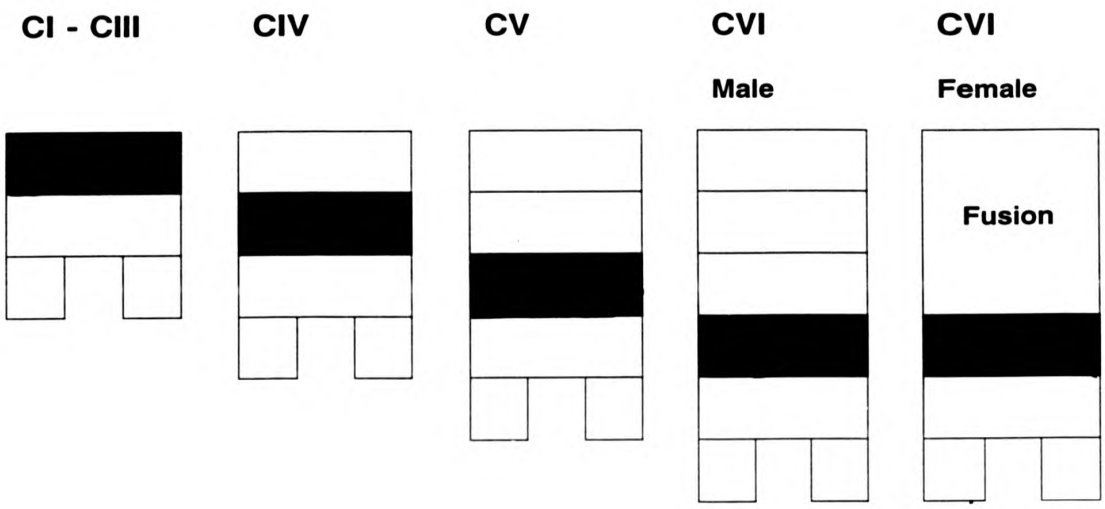


Fig. 4.6. The ontogenetic development of urosomal segments in the genus *Pleuromamma*. Dashed segments represent a new segment which is added at each stage.

CHAPTER 5:

Intraspecific variation in the pore signature

5.1. Introduction

The species pore signatures of calanoid copepods show intra- and interspecific variation. The taxonomic and phylogenetic value of interspecific variation in the pore signature has already been shown at the specific, generic, familial and superfamilial level (Fleminger 1973, Mauchline 1988, Hulsemann 1991). Intraspecific variation has been noted in a number of calanoid copepod species, but there has been no adequate assessment of it.

Intraspecific variation in the pore signatures is evident between individuals of a single geographical area, and between different geographical areas (Fleminger 1973, Fleminger and Hulsemann 1977, 1987, Malt 1983, Mauchline 1988, Hulsemann and Fleminger 1990). The former is the within-sample variation, and the latter is the geographical variation. Intraspecific variation has been studied on a geographical scale and it is mainly evident in the prosomal signature of *Eucalanus subtenuis* (Fleminger 1973); in the urosomal signature of *Calanus helgolandicus* (Fleminger and Hulsemann 1977, 1987); in the genital somite of *Pontellina plumata* and *P. morii* (Hulsemann and Fleminger 1990). In several of these studies, however, samples assumed to be representative of a single geographical area were often not confined to single locations. Such single geographical areas include up to 24 different regional locations, and locations were separated in time and space. Thus, it is probable that different populations of a species were represented and an element of geographical variation inadvertently included. The extent of the within-sample variation in the species pore signature at a single geographical location, therefore, has not yet been adequately assessed.

The present study assesses the proportion of pores that vary within species of the genus *Pleuromamma* in adult females. The specimens of each species were collected at the same location, while different species were collected from different locations. These

results are used to assess the results presented by Fleminger (1973), Fleminger and Hulsemann (1977, 1987), and Hulsemann and Fleminger (1990).

5.2. Materials and methods

Specimens of eight *Pleuromamma* species were collected in the north Atlantic, western Pacific and western Indian Ocean (Table 2.1 in chapter 2). Specimens were treated with 20% aqueous potassium hydroxide (KOH) to examine the integumental organs. The terminology for describing the sites follows Fig. 2.1.

5.3. Results

The integument of the whole body is divided into 89 areas (Fig. 5.1, Table 5.1). The pores in 21 areas of the integument are consistent in the eight *Pleuromamma* species. The number of pores, common to all species, is 269 and is the pore signature of the genus. The remaining 63 areas of the integument show intra- and interspecific variation, dependent on the species.

Intraspecific variation occurs in the cephalosomal, metasomal and urosomal pores in all species, except in the cephalosomal pores of *Pleuromamma xiphias* and in the urosomal pores of *P. piseki* (Table 5.1). In general, the pores are bilaterally symmetrical in their distribution over the integument, except those occurring in the median dorsal line. Intraspecific variation is bilaterally symmetrical at about half the variable sites while the other half has a pore either present or absent on one side of the integument only (Table 5.1).

The total number of integumental sites at which pores are recorded in each species are listed in Table 5.2, along with the numbers that do and do not vary. The percentage of

sites that are variable ranges from 4.0% in *Pleuromamma borealis* to 11.7% in *P. robusta*. The mean intraspecific variation is 7.7±3.2% of total sites in the eight *Pleuromamma* species.

About 50% of the total number of integumental pores in the eight species (Table 5.3) reside in the metasome, while 34% reside in the cephalosome, and 16% in the urosome. The extent, however, of intraspecific variation is greater in the urosomal pores (11.6±9.2%) than in the metasomal (8.7±2.9%) or the cephalosomal (3.7±4.1%) pores. Intraspecific variation occurs in 0 to 28.2% of urosomal pores, 0 to 12.1% of cephalosomal pores, and 5.0 to 13.4% of metasomal pores. About half the sites on the urosome occur in the genital somite (U1) (Table 5.4). Variation of sites in the second (U2) and third (U3) urosomal segment is restricted, being absent in three of the species and confined to one to three sites in the others. Variation in the sites of the genital somite ranges from nil to that in *P. robusta* where 17 of the 47 sites are variable (Table 5.4).

From the data in Table 5.3, it is calculated that some 88 to 96% of total integumental sites are represented by a pore in every individual, 92 to 97% occur in more than 80% of individuals, 94 to 98% occur in more than 50% of individuals. At least 91% of cephalosomal and metasomal pores occur in more than 80% of individuals, and 93% occur in more than 50% of individuals. Intraspecific variation in the urosomal pores is very variable; 91 to 100% occur in all individuals of *P. abdominalis*, *P. indica*, *P. gracilis*, *P. piseki* and *P. borealis*; 87 to 90% occur in more than 50% of individuals of *P. xiphias*, *P. scutullata* and *P. robusta* (Tables 5.3, 5.4).

5.4. Discussion

In eight *Pleuromamma* species, intraspecific variation occurs in about 10% (7.7±3.2%) of total integumental sites (Table 5.2). The percentage of intraspecific variation

ranges from 4.0 to 11.7%, depending on the species; it is greater in *P. xiphias*, *P. scutullata*, *P. robusta* and *P. indica* than in the remaining species. The species of *Pleuromamma* were divided into four groups on the basis of their pore signatures and other morphological characters (see chapters 2, 3). These groups are 1) *P. xiphias* and *P. abdominalis*; 2) *P. scutullata* and *P. robusta*; 3) *P. indica*; 4) *P. gracilis*, *P. piseki* and *P. borealis*. These groups are not reflected in Table 5.3 in terms of the proportion of pores occurring in all individuals. There is, however, some correspondence between these groups and the areas of the integument having variable sites (Table 5.1). Most of intraspecific variation occurs at the sites which also show interspecific variation within the genus.

Only 16% of the total number of integumental pores of the species reside in the urosome. The extent of intraspecific variation is, however, greater in the urosomal pores (Table 5.3). Up to 47% (23.8±15.0) of total variable sites over the integument reside in the urosome. Within the urosome, 84.1±12.4% of variable sites are present in the genital somite (U1) of the adult female (Table 5.4).

Most of the species-specific information of the pore signature resides in the adult female urosome, although a significant proportion resides in the cephalosome (see Tables 2.3-2.5). Sexual differences in the species pore signatures are primarily evident in the adult urosome (see chapter 4). The present study shows that the urosome also contains the greatest amount of the intraspecific variation.

In other genera, the species pore signatures have been derived from the pores that occurred in more than 80% of individuals in 17 *Eucalanus* species by Fleminger (1973); in more than 50% in three *Calanus* species by Fleminger and Hulsemann (1977); in 100% in *Calanus helgolandicus* by Fleminger and Hulsemann (1987); in 40% in four *Pontellina* species by Hulsemann and Fleminger (1990); in 50% in eight *Pleuromamma* species by the present study. The present investigation confirms that setting the level of occurrence of pores at 40, 50 or 60% does not cause significant changes in the species pore signature,

even in the urosome (Tables 5.3, 5.4).

Intraspecific variation in the pore signature is present between individuals collected at one and the same station, and between those from different geographical areas. The former is the within-sample variation, and the latter is the geographical variation. In the previous studies, some samples said to represent a single geographical area of the species were in fact obtained from several regional oceanic locations, some separated by over 200 miles, on different dates. Variation found within these groups of samples was assumed to be on the intraspecific rather than the geographical scale. In the present investigation, representatives of each *Pleuromamma* species were collected at only one station (Table 2.1, chapter 2), thus intraspecific variation is here synonymous with within-sample variation in the species pore signature.

The first study of intraspecific variation in the pore signature of calanoid copepods was made by Fleminger (1973). After examination of the pore signatures of 17 *Eucalanus* species, he described 16 different species signatures whereby those of two species, *E. californicus* and *E. bungii*, were indistinguishable (Table 5.5). He also compared the pore signatures of adult female *E. subtenuis* on a worldwide geographical scale in units of 60° longitude, bounded by 34°N and 34°S (Table 5.6). The ranges of the total number of pores differ between geographical locations. He concluded that roughly 80% of the sites were represented by a pore in every individual of each species; a further 10% of sites had a pore in 80-99% of individuals. The final 10% of the sites were occupied by a pore in less than 80% of individuals, but he pointed out that these include sites subject to variation on a geographical scale. The geographical variation was most clearly seen in the urosomal signatures of adult females. Since then, the pore signature of the adult female urosome, or of the genital somite, alone has been studied as evidence of geographical variation, or genetic variability within a species (Fleminger and Hulsemann 1977, 1987, Hulsemann 1994, Hulsemann and Fleminger 1990).

On re-examination of Fleminger's (1973) data in adult females of 17 *Eucalanus* species, 83 to 100% of integumental pores occur in more than 80% of individuals. Intraspecific variation occurs in 15% (14.2±9.8%) of integumental sites and ranges from 0% in *E. longiceps* to 38% in *E. subtenuis* (Table 5.5). About 49% of total integumental pores reside in the cephalosome, 40% in the metasome, and 10% in the urosome. Intraspecific variation occurs in 12.4±12.8% of the cephalosomal pores, 11.8±11.1% of the metasomal pores, and 23.0±19.8% of the urosomal pores. The values of standard deviation suggest that the degree of variation is very variable, depending on the species. In Fleminger's study, the range of variation is expected to be greater because the pores on the paired dorsal areas (ca. 16 to 34% of the total sites) (Fig. 2.1) were not counted (Table 5.5). The specimens of 17 *Eucalanus* species were collected at from 5 to 24 different regional locations so that intraspecific variation of the species can not be represented by the within-sample variation because it may contain a geographical component.

In *Eucalanus subtenuis*, 38% of the sites on the whole integument are variable sites, the greatest in this genus (Table 5.5). The percentage of the variable sites is 41% in the Atlantic, and 29% in both the Indian and Pacific Ocean (Table 5.6). The total number of pores in the urosome of *E. subtenuis* is seven. Only one pore is variable and occurs in 72% of individuals in the Atlantic, 50% in the Indian, and 75% in the Pacific Ocean. The pore signature and the variable site on the urosome of *E.subtenuis* are identical to those of *E. mucronatus*. Contrary to Fleminger's (1973) suggestion, there is no evidence that the geographical variation in the pore signatures is primarily evident in the adult female urosome; geographical variation is clear in the prosomal signature.

Fleminger and Hulsemann (1977) examined the urosomal signatures of four geographical populations of adult female *Calanus helgolandicus* from the North Atlantic Ocean (Table 5.7). In their study, geographical variation was assessed using four pairs of pores on the ventral areas of the second and third urosomal segments, and these pores

occurred in 5 to 30% of individuals examined. The percentage occurrence of these pores differed between the populations.

In *Calanus helgolandicus* the urosomal signature was re-examined for further evidence of geographical divergence in eight regional populations of the North Atlantic Ocean including the Mediterranean and the Black Sea by Fleminger and Hulsemann (1987) (Table 5.7). Four pairs of pores on the ventral areas of the second and third urosomal segments, which were assessed again for evidence of geographical variation, occurred in 5 to 36% of individuals. Using the percentage occurrence of pores on the second and third urosomal segments, eight populations were divided into three phenetic populations: Atlantic and western Mediterranean; eastern Mediterranean; Black Sea.

In Fleminger and Hulsemann's (1977, 1987) studies, the same sample of specimens of adult female *Calanus helgolandicus* from the North Atlantic location was described, although a single geographical area included several regional populations (Table 5.7). The results of both studies, however, on the percentage occurrence of intraspecific variation are different. Intraspecific variation in the pore signatures, therefore, is very variable depending on the sample size.

There is one instance of the intraspecific variation between individuals at one station. This is found in *Calanus helgolandicus* from the Aegean Sea (Fleminger and Hulsemann 1987) (Table 5.7). The percentage occurrence of intraspecific variation of the Aegean Sea is not significantly different from that of the other geographical areas containing several different regional locations.

In *Calanus helgolandicus*, the pores which provide evidence of geographical variation occur in less than 35% of specimens examined, but these pores occur in more than 90% of the other species of the *C. helgolandicus* group, i.e., *C. finmarchicus* and *C. glacialis* (Fleminger and Hulsemann 1977) and *C. sinicus*, *C. orientalis*, *C. chilensis* and *C. australis* (Fleminger and Hulsemann, unpublished data).

Hulsemann and Fleminger (1990) described the pore signatures of the genital somite of *Pontellina plumata*, *P. platychela*, *P. morii* and *P. sobrina*. In *P. plumata* and *P. morii*, variation was compared with geographical areas (Table 5.8); within the genus the latter species has the most complex signature and the former the simplest. In *P. plumata*, one pore is absent in the Atlantic Ocean, but occurs in between 60 and 79% of individuals from the Pacific Ocean. In *P. morii*, five pores are absent in the East Pacific Ocean, but occur in from 0 to 100% of individuals of the Indian and West Pacific. In Hulsemann and Fleminger's study, only pores that occurred in more than 40% of individuals are described. It is not clear whether geographically variable pores occur at a level of less than 40% of individuals from different areas. However, the signature of *P. plumata* is closely similar to that of *P. platychela*, and *P. morii* to *P. sobrina*. In *Pontellina plumata* and *P. morii* the pores showing geographical variation are closely related to those in the pore signatures of *P. platychela* and *P. sobrina* respectively.

The present study confirms that intraspecific variation is evident in urosomal pores, and the genital somite in particular. The degree of intraspecific variation, however, increases with the number of urosomal pores found in the species being compared (Tables 5.4, 5.5). Geographical variation possibly occurs in less than 40% of individuals, as had previously been assessed by Fleminger and Hulsemann (1977, 1987). Urosomal pores that occur in more than 80%, and possibly 50%, of individuals are unlikely to provide evidence of geographical variation.

In the previous studies, specimens of each species were examined using pooled samples of between at least 10 and 110 individuals whereas the current samples are 13 to 27 individuals. Corresponding supernumerary pores in *Pleuromamma* species are probably severely underestimated in Table 5.2 as many could only be expected in one out of 20 individuals examined. However, variation in the pore signature within a species is severely restricted. Samples as small as 5 individuals will identify more than 95% of

potential sites for the pore signature of the species. Detection of geographical variation will require large samples as this component resides in the rarer supernumerary pores occurring in less than 5% of individuals.

Fleminger and Hulsemann (1987) suggested that intraspecific variation in the pore signature is due to (1) environmental factors; (2) hybridization or past introgressions; (3) genetic differences. In the previous studies of intraspecific variation in the pore signature, however, only the genetic variability within a species has been emphasized. It should be noted that the pore signatures, and their variations, reflect the genetic similarities between the species within a genus. Intraspecific variation might be an expression of the phylogenetic grouping of species, which is also evident in the development of the pore signatures (see chapter 4). Intra- and interspecific variation might be restricted to certain limits at the genus level.

In this study only variation in the number of pores was examined. The various structural types of the integumental organs in calanoid copepods are generally classified as either a hair, peg and pit sensilla or gland opening (Fleminger 1973, Mauchline 1977, Gill 1986, Hulsemann and Fleminger 1990, Othsuka and Mitsuzumi 1990, Kurbjeweit and Buchholz 1991, Koomen 1992, Bannister 1993, Weatherby *et al.* 1994). Variation also occurs in the location and type of some integumental organs (Fleminger 1973, Von Vaupel Klein 1982, Hulsemann and Fleminger 1990, Koomen 1992). Further histological and ultrastructural studies on the integumental organs are required to show the function of these organs. The KOH-treatment will provide information on the accurate detection of pore sites for further detailed SEM and TEM studies, and the number of specimens required.

Table 5.1. *Pleuromamma* species. Variation areas (+) in the pore signature. Variation can occur bilaterally in the corresponding paired areas of the integument (Fig. 1) and is designated by (++).

		xiphias	*abdominalis*	*scutullata*	*robusta*	*indica*	*gracilis*	*piseki*	*borealis*
RB					+	+			
CI	M			+		+			
CII	L					+			
	M							+	
CIII	L					++	++		++
CIV	L			++		++			
	D					++	++	++	
	M			+		+			
CV	L			++		++	++	++	++
	D		++			++			
	M			+					
MI	AL	+	++	++	++		+		
	AD	++	++	++	++			+	+
	PL	++	++	++		++	++	+	+
MII	AL				++	++	++	++	
	AD			++	++			++	
	M	+	+	+	+	+	+	+	
MIII	AL	++		+	++	++	++		
	AD				+		+		
	M	+	+	+	+	+		+	
MIV	L	++				++		+	
	D			++	++		++		
MV	L	++		++	++		++	++	
	M				+				
UI-2	L	++	++	++			++		
	D	+		+	+				
UI-3	L		++	++	++		++		+
	D	+	+	+	+	+			
	V	+		+	+				
UII	I		+	+	+				
No. of variable areas		19	16	30	25	25	23	16	7
No. of non-variable areas		71	74	60	65	65	67	74	83

Table 5.2. *Pleuromamma* species. Total number of integumental pores in each species and the proportion of non-variable and variable pores. Numbers in parentheses are percentages of pores that do and do not vary, respectively.

Species	Total No. of sites	No. of sites that do not vary	No. of sites that vary
P. xiphias	369	331(89.7)	38(10.3)
P. abdominalis	327	313(95.7)	14(4.3)
P. scutullata	381	341(89.5)	40(10.5)
P. robusta	360	318(88.3)	42(11.7)
P. indica	309	279(90.3)	30(9.7)
P. gracilis	301	282(93.7)	19(6.3)
P. piseki	296	282(95.3)	14(4.7)
P. borealis	298	286(96.0)	12(4.0)

Table 5.3. *Pleuromamma* species. The number of sites in different regions of the integument occupied by pores in all individuals (100%) is compared with those that occur in decreasing percentages of individuals of the different species. Numbers in parentheses are percentages of pores that occur in different percentages of individuals.

Species	100%	80-99%	50-79%	20-49%	<20%	Total
P. xiphias						
Cephalosome	114(100.0)	0	0	0	0	114
Metasome	155(86.6)	17(9.5)	1(0.6)	4(2.2)	2(1.1)	179
Urosome	62(81.6)	3(3.0)	2(2.6)	4(5.3)	5(6.6)	76
Grand Total	331(89.7)	20(5.4)	3(0.8)	8(2.2)	7(1.9)	369
P. abdominalis						
Cephalosome	113(98.3)	2(1.7)	0	0	0	115
Metasome	153(95.0)	3(1.9)	0	2(1.2)	3(1.9)	161
Urosome	47(92.2)	0	2(3.9)	2(3.9)	0	51
Grand Total	313(95.7)	5(1.5)	2(0.6)	4(1.2)	3(0.9)	327
P. scutullata						
Cephalosome	123(91.8)	4(3.0)	1(0.7)	0	6(4.5)	134
Metasome	163(90.6)	1(0.6)	5(2.8)	5(2.8)	6(3.3)	180
Urosome	55(82.1)	3(4.5)	2(3.0)	2(3.0)	5(7.5)	67
Grand Total	341(89.5)	8(2.1)	8(2.1)	7(1.8)	17(4.5)	381
P. robusta						
Cephalosome	116(98.3)	0	1(0.9)	1(0.9)	0	118
Metasome	151(88.3)	6(3.5)	4(2.3)	6(3.5)	4(2.3)	171
Urosome	51(71.8)	7(9.9)	4(5.6)	4(5.6)	5(7.0)	71
Grand Total	318(88.3)	13(3.6)	9(2.5)	11(3.1)	9(2.5)	360
P. indica						
Cephalosome	102(87.9)	4(3.5)	2(1.7)	4(3.5)	4(3.5)	116
Metasome	136(91.3)	7(4.7)	2(1.3)	2(1.3)	2(1.3)	149
Urosome	41(93.2)	0	2(4.5)	1(2.3)	0	44
Grand Total	279(90.3)	11(3.6)	6(1.9)	7(2.3)	6(1.9)	309
P. gracilis						
Cephalosome	103(98.1)	0	0	0	2(1.9)	105
Metasome	138(91.4)	7(4.6)	2(1.3)	2(1.3)	2(1.3)	151
Urosome	41(91.1)	0	2(4.4)	2(4.4)	0	45
Grand Total	282(93.7)	7(2.3)	4(1.3)	4(1.3)	4(1.3)	301
P. piseki						
Cephalosome	100(98.0)	0	0	0	2(2.0)	102
Metasome	145(92.4)	2(1.3)	2(1.3)	0	8(5.1)	157
Urosome	37(100.0)	0	0	0	0	37
Grand Total	282(95.3)	2(0.7)	2(0.7)	0	10(3.4)	296
P. borealis						
Cephalosome	103(98.1)	0	0	0	2(1.9)	105
Metasome	143(94.7)	2(1.3)	0	2(1.3)	4(2.7)	151
Urosome	40(95.2)	0	0	0	2(4.8)	42
Grand Total	286(96.0)	2(0.7)	0	2(0.7)	8(2.7)	298

Table 5.4. *Pleuromamma* species. The number of sites in the first (U-1), second (U-2) and third (U-3) urosomal segments represented by pores occurring in all individuals (100%) is compared with those occurring in decreasing percentages of individuals of the different species. Numbers in parentheses are percentages of pores that occur in different percentages of individuals.

Species	100%	80-99%	50-79%	20-49%	<20%	Total
P. xiphias						
U-1	37(77.1)	2(4.2)	2(4.2)	2(4.2)	5(10.4)	48
U-2	10(76.9)	1(7.7)	0	2(15.4)	0	13
U-3	15(100.0)	0	0	0	0	15
Grand Total	62(81.6)	3(3.9)	2(2.6)	4(5.3)	5(6.6)	76
P. abdominalis						
U-1	22(88.0)	0	1(4.0)	2(8.0)	0	25
U-2	10(90.9)	0	1(9.1)	0	0	11
U-3	15(100.0)	0	0	0	0	15
Grand Total	47(92.2)	0	2(3.9)	2(3.9)	0	51
P. scutullata						
U-1	32(76.2)	3(7.1)	2(4.8)	2(4.8)	3(7.1)	42
U-2	8(100.0)	0	0	0	0	8
U-3	15(88.2)	0	0	0	2(11.8)	17
Grand Total	55 (82.1)	3(4.5)	2(3.0)	2(3.0)	5(7.5)	67
P. robusta						
U-1	30(63.8)	6(12.8)	4(8.5)	4(8.5)	3(6.4)	47
U-2	8(72.7)	1(9.1)	0	0	2(18.2)	11
U-3	13(100.0)	0	0	0	0	13
Grand Total	51(71.8)	7(9.9)	4(5.6)	4(5.6)	5(7.0)	71
P. indica						
U-1	19(90.5)	0	2(9.5)	0	0	21
U-2	9(90.0)	0	0	1(10.0)	0	10
U-3	13(100.0)	0	0	0	0	13
Grand Total	41(93.2)	0	2(4.5)	1(2.3)	0	44
P. gracilis						
U-1	22(84.6)	0	2(7.7)	2(7.7)	0	26
U-2	8(100.0)	0	0	0	0	8
U-3	11(100.0)	0	0	0	0	11
Grand Total	41(91.1)	0	2(4.4)	2(4.4)	0	45
P. piseki						
U-1	18(100.0)	0	0	0	0	18
U-2	8(100.0)	0	0	0	0	8
U-3	11(100.0)	0	0	0	0	11
Grand Total	37(100.0)	0	0	0	0	37
P. borealis						
U-1	19(90.5)	0	0	0	2(9.5)	21
U-2	8(100.0)	0	0	0	0	8
U-3	13(100.0)	0	0	0	0	13
Grand Total	40(95.2)	0	0	0	2(4.8)	42

Table 5.5. *Eucalanus* species. The number of integumental pores that occur in all individuals (100%) is compared with those that occur in decreasing percentages of individuals of the different species. Numbers in parentheses are percentages of pores that occur in different percentages of individuals. Data drawn from Fleminger's (1973) figures.

Species	100%	80-99%	10-79%	Total	Tergal[1)]	Total[2)]
E. subtenuis	91(61.90)	31(21.09)	25(17.00)	147	32	179
E. mucronatus	94(89.52)	3(2.86)	8(7.62)	105	22	127
E. crassus	81(96.43)	3(3.57)	0(0.00)	84	16	100
E. longiceps	84(100.00)	0(0.00)	0(0.00)	84	28	112
E. monachus	87(94.57)	3(3.26)	2(2.17)	92	34	126
E. pileatus	82(89.13)	7(6.60)	3(3.26)	92	30	122
E. subcrassus	94(84.68)	15(24.59)	2(1.80)	111	54	165
E. dentatus	100(84.75)	9(7.63)	9(7.63)	118	50	168
E. elongatus	84(79.25)	7(6.60)	15(14.15)	106	28	134
E. hyalinus	90(73.77)	30(24.24)	2(1.63)	122	56	178
E. inermis	83(81.37)	14(13.73)	5(4.90)	102	30	132
E. californicus	130(95.50)	3(2.27)	3(2.27)	136	72	208
E. bungii	130(95.50)	3(2.27)	3(2.27)	136	72	208
E. attenuatus	96(72.73)	32(24.24)	4(3.03)	132	40	172
E. sewelli	96(83.48)	19(16.52)	0(0.00)	115	26	141
E. parki	110(94.02)	1(0.85)	6(5.13)	117	38	155
E. langae	109(89.34)	8(6.56)	5(4.10)	122	30	152

[1)] In Fleminger's (1973) data, pores that occur in the paired lateral areas (see Fig. 2.1), termed "tergal sites", were not counted in terms of total number of pores over the integument.

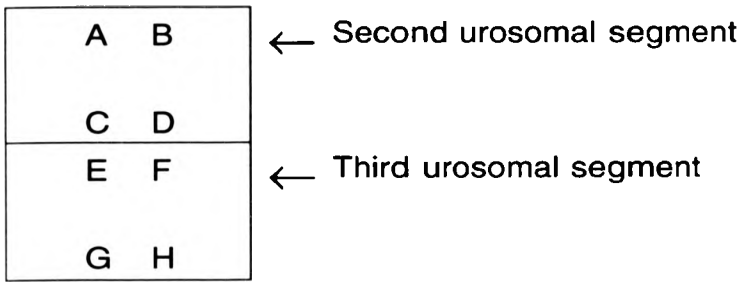
[2)] Total number of integumental pores including pores that occur in the paired lateral areas.

Table 5.6. *Eucalanus subtenuis*. Range, mean number, and standard deviation of total integumental pores and the percentage of intraspecific variation in three geographical areas; N=number of specimens examined, n=number of locations where specimens were collected. Data from Fleminger's (1973) results.

	N	n	range	Mean±Standard Deviation	% of intraspecific variation
Atlantic Ocean	50	15	125-141	134.8±3.25	41%
Indian Ocean	24	10	119-139	130.0±4.9	29%
Pacific Ocean	36	10	114-135	123.6±4.98	29%

Table 5.7. *Calanus helgolandicus*. The percentage occurrence of intraspecific variation between different geographical areas. (A) Schematic of the ventral area of the second and third urosomal segments of the adult female; alphabetic capitals indicated the sites occupied by pores. At these sites, numbers B-D, show the percentage occurrence of each of the eight pores; N=number of specimens examined, n=number of locations where specimens were collected. Data from Fleminger and Hulsemann (1977), (B) and Fleminger and Hulsemann (1987), (C) and (D).

A



B

Western North Atlantic (36-39oN, 72-74°W) N=19, n=4		Mid-North Atlantic (40-47°N, 35-36°W) N=20, n=3		Eastern North Atlantic Europe (45-49°N, 4-16°W) N=20, n=4		Eastern North Atlantic Africa (19-28°N, 15-18°W) N=20, n=4	
11	0	30	15	10	10	10	25
15	0	25	20	10	10	25	25
5	0	5	0	25	20	10	10
5	11	5	0	25	20	15	20

C

Western North Atlantic (36-39°N, 69-75°W) N=100, n=7		Mid-North Atlantic (40-47°N, 35-36°W) N=50, n=3		Eastern North Atlantic Europe (42-50°N, 4-16°W) N=50, n=5		Eastern North Atlantic Africa (19-28°N, 15-18°W) N=100, n=11	
24	15	28	18	20	18	23	22
23	16	22	16	18	16	19	17
19	18	14	18	16	14	18	13
19	17	10	10	14	12	15	8

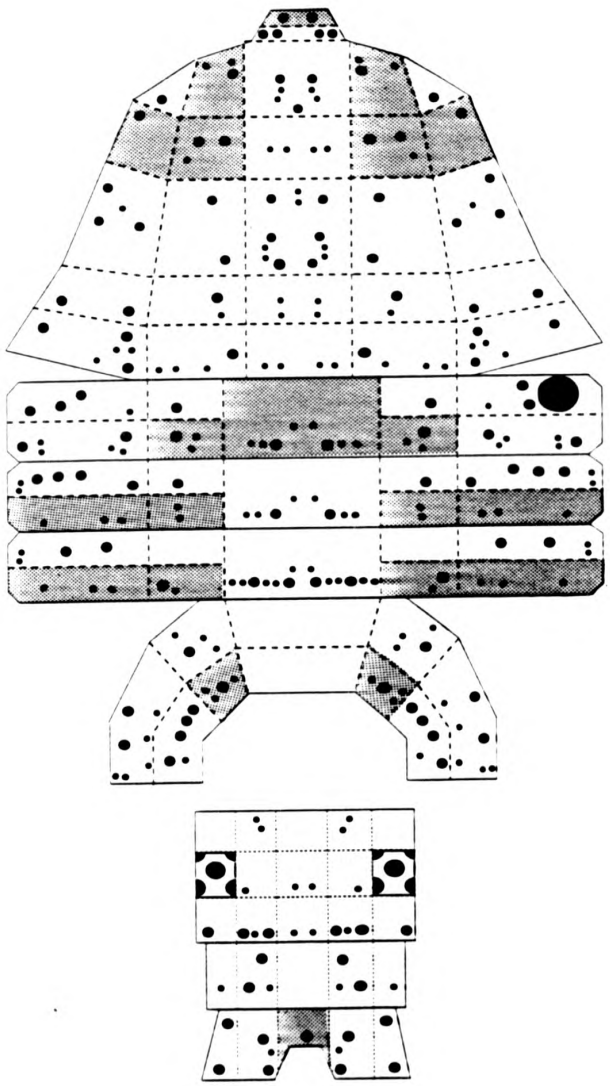
D

Western Mediterranean (43-43°4'N, 5-7°E) N=100, n=2		Adriatic (40-44°N, 14-19°E) N=100, n=7		Aegean (39°N, 20°E) N=48, n=1		Black Sea (42-44°N, 29-33°E) N=100, n=4	
15	17	8	8	10.4	10.4	34	25
13	15	7	8	8.3	8.3	31	24
10	11	6	6	12.5	12.5	36	34
6	10	6	5	10.4	12.5	36	32

Table 5.8. *Pontellina* species. The number of sites in the genital somite of the adult female represented by pores in all individuals (100%) is compared with those that occur in decreasing percentages of individuals of the different species.
Data from Hulsemann and Fleminger (1990).

Species	100%	80-99%	60-79%	40-50%	Total[1)]
P. platychela	?	?	?	?	21
P. plumata					
Pacific	5	9	2	1	17
Atlantic	6	6	2	2	16
P. sobrina	?	?	?	?	44
P. morii					
Indian	37	9	1	2	49
West Pacific	26	18	4	1	49
East Pacific	29	11	4	1	45

[1)] In Hulsemann and Fleminger's (1990) data, only pores that occurred in more than 40% of individuals were described.



5.1. The generic pore signature obtained from the eight *Pleuromamma* species. Shaded areas of the integument designate the areas that are consistent in all species.

CHAPTER 6:

Aspects of the intermoult cycle in calanoid copepods

6.1. Introduction

The majority of metazoan secondary production is represented by crustaceans in aquatic environments. Crustacean growth is accompanied by a periodic moulting of their exoskeleton. Currie (1918) first noted the process of moulting in small planktonic copepods in terms of setogenesis and showed new setae are formed in the somatic tissue of appendages to replace existing or old setae lost with the old exoskeleton at ecdysis. The concept of an intermoult cycle in crustaceans as a well-defined sequence of stages was advanced by Drach (1939). He found that the calcification of the old-exuvial exoskeleton, increase in the amount of the somatic tissue, developing new setae, the decalcification of the old-exuvial exoskeleton, and the formation of the pre-exuvial exoskeleton sequentially occur throughout a period of the intermoult cycle. Drach's original criteria for various stages of the intermoult cycle have been modified so that it is now applicable to a variety of crustaceans (Drach 1944, Scheer 1960, Kurup 1964, Davis *et al.* 1973, Stevenson *et al.* 1968, Jennings and Halverson 1971, Aiken 1973, Reaka 1975). The intermoult cycle comprises more than 15 stages and is mainly divided into the postmoult (stage A to B), intermoult (stage C), premoult (stage D) and ecdysis (stage E) (see Table 6.4).

Morphological, physiological and histochemical studies of the moult cycle have been carried out in a variety of crustaceans since the pioneer work of Drach (1939) (Drach 1944, Passano 1960, Scheer 1960, Kurup 1964, Lasker 1964, 1966, Stevenson *et al.* 1968, Jennings and Halverson 1971, Aiken 1973, David *et al.* 1973, Reaka 1975, Skinner and Kumari 1992, Moss 1994, Roff *et al.* 1994). In calanoid copepods, the moult cycle has not yet been studied, except the morphology of setogenesis (Currie 1918, Dexter 1981). Dexter (1981) divided the intermoult cycle in *Calanus marshallae* into the postmoult, intermoult and premoult stage and her descriptions correspond with other crustacean criteria for the different stages of the moult cycle. The formation of new setae, however, is a part of the

moult process.

The most notable external changes are development of new setae and the formation of the old- and pre-exuvial exoskeleton throughout the intermoult cycle. The integument of crustaceans, including copepods, consists of the epicuticle, exocuticle, endocuticle, epidermis and basal lamina (see Fig. 6.4). It is generally accepted that the epicuticle of arthropods contains no chitin and chitin is present in the exocuticle and endocuticle. The exocuticle is not fully calcified in the early postmoult stage and its calcification and the formation of the endocuticle is complete in the late postmoult stage by subsequent enzyme secretion (Drach 1939, 1944, Carlisle 1960, Carlisle and Dohrn 1953, Knowles and Carlisle 1956, Passano 1960, Scheer 1960, Kurup 1964, Stevenson *et al.* 1968, Aiken 1973). The intermoult stage is a period of stability with no distinctive morphological and physiological changes. This stability is due to the absence of the moulting hormone or the moulting hormone is suppressed by the moult inhibiting hormone. When the moulting hormone is released from the inhibitory influence of the moulting inhibiting hormone, striking changes in the cuticle begin in preparation for the next ecdysis; this is the premoult stage. The endocuticle of the old-exuvial exoskeleton is reabsorbed and the epidermis and exocuticle of the pre-exuvial exoskeleton are produced by enzyme secretion. These sequential changes are also evident as an increase in thickness of the cuticle throughout the intermoult cycle in *Balanus amphitrite* (David *et al.* 1973).

The integument has been studied in a number of calanoid copepods with respect to its structure and integumental organs (Bouligand 1966, 1972, Fleminger 1973, Raymont *et al.* 1974, von Vaupel Klein 1982, Bresciani 1986, Brunet 1991, Koomen 1992, Bannister 1993). These studies show that the structure and thickness of the integument varies not only between species, but also between individuals of a species, and different parts of the body of a species, even though the intermoult cycle was not concerned. Bresciani (1986) noted that some cuticular layers can be absent in certain individuals of the species.

The typical calanoid copepod life cycle comprises six naupliar and copepodid stages. The sixth copepodid stage is the adult stage with no moult, unlike other crustaceans whereby the moult cycle continues throughout their life span or until they reach an upper limit of body size (Hiatt 1948, Carlisle 1960, Lasker 1966). Extensive studies of the intermoult cycle in other crustaceans suggest that in adult copepods, just after moulting from the copepodid V stage, the exocuticle and endocuticle are not fully developed. Independently of the next moulting these become complete as time goes by. In copepodids, the formation of the exo- and endocuticle of the old-exuvial exoskeleton should occur in the early moult cycle and that of ecdysial cuticular layers of the pre-exuvial exoskeleton in the late moult cycle. Simultaneously, the amount of somatic tissue increases throughout the intermoult cycle (Drach 1939, 1944, Dexter 1981). Changes in the cuticle and the amount of somatic tissue which is produced during periods of the intermoult cycle possibly affect copepod body size and weight.

In the present study, copepodids and adults of the calanoid copepod *Pleuromamma robusta* were examined. Different stages of the intermoult cycle were identified by morphological characteristics of setogenesis. Changes in cuticular structures in association with the intermoult cycle were examined using Transmission Electron Microscopy. Sequential structural changes in the cuticle were quantified by measuring the increase in the thickness of the cuticle. In addition, copepodids and adults were divided into "soft" and "hard" bodies based on texture and hardness of the outer integument, and/or on the body appearance. The relationship between body appearance and different stages of the intermoult cycle was assessed. Body length was measured to compare length variation between individuals at different stages of the intermoult cycle.

6.2. Materials and methods

Specimens of the calanoid copepod *Pleuromamma robusta* were collected in the Rockall Trough in September, 1975 and November, 1994 using a Rectangular Mid-water Trawl (RMT 1) with 295 µm mesh size of its cod end. Specimens obtained from 1975 were fixed in 5% hexamine buffered formaldehyde and then transferred to Steedman's (1976) preservative: 25 ml of 40% formaldehyde, 10 ml propylene phenoxetol, 100 ml propylene glycol and 865 ml sea water. Specimens obtained in 1994 were preserved in 0.1 M sodium cacodylate buffered with 0.35 M sucrose (pH 7.9).

Light microscopy for staging the intermoult cycle

Different stages of the intermoult cycle were identified by the morphology of setogenesis in copepodid II to VI, adult stage (Drach 1939, 1944, Scheer 1960, Kurup 1964, Stevenson 1969, Stevenson *et al.* 1968, Jennings and Halverson 1971, Aiken 1973, Reaka 1975, Dexter 1981). Live specimens were not available for this study so specimens preserved as described above were used. A pair of swimming legs and caudal rami were examined and dissected using a scalpel and dissecting needles. Dissected appendages were mounted in polyvinyl lactophenol on microscope slides. Observations on slide preparations were made with a compound light microscope at 400 power magnification. Drawings were made with the aid of a drawing tube.

Transmission Electron Microscopy

Specimens, obtained in 1975, were washed and placed in distilled water overnight. They were then washed in 4% glutaraldehyde in 0.1 M sodium cacodylate buffered with

0.35 M sucrose (pH 7.9) for 2 h. Live specimens obtained from 1994 were preserved in 4% glutaraldehyde in 0.1 M sodium cacodylate buffered with 0.35 M sucrose (pH 7.9). Both groups of specimens were postfixed in buffered 1% osmium tetroxide for approximately 24 h, dehydrated in a graded ethanol series and then embedded in L. R. White resin. Ultrathin (90 nm, Reichert Jung Ultracut OM CL3 microtome) sections were stained with uranyl acetate and lead citrate and viewed in an AEI Corinth 275 TEM at 80 kV. In addition, 1 µm thick sections (Glass microtome) were made to compare the thickness of the integument between different stages of the intermoult cycle. These sections were stained with methylene blue and then examined under the compound microscope. The structure and thickness of the integument varied with different parts of the body. All sections were, therefore, made around the middle area of paired dorsal longitudinal muscles of the second metasomal segment; on the right side of this area the pigment knob also occurs which is an unique morphological structure of the genus *Pleuromamma*.

Body lengths

Copepodids and adults were divided into "soft" and "hard" bodies based on texture and hardness of the integument, or the body appearance. Different stages of the intermoult cycle were determined by the morphology of setogenesis. Prosome lengths by ocular micrometer with a dissection microscope were measured to compare variation in body lengths between different stages of the intermoult cycle. In lateral view, prosome length was defined as from the anterior end of the rostrum to the segmentation, or boundary between the prosome and the urosome.

6.3. Results

The intermoult cycle as determined by the morphology of setogenesis

Different stages of the intermoult cycle were identified by the morphological characteristics of developing new setae in the somatic tissue in copepodids and adults of *Pleuromamma robusta*; the texture and hardness of the integument, or the body appearance were compared. The intermoult cycle is divided into the postmoult, intermoult and premoult stage.

The postmoult stage (stage A to B): The integument is very soft and the surrounding tissue in body appendages is unevenly distributed. The appearance of the whole body is, therefore, transparent. The distal shafts of some setae have not completely emerged into the exuvial setae and still extend beneath the cuticular nodes (Figs. 6.1, 6.2). These characteristics are similar in the swimming legs and caudal rami throughout all copepodid stages.

The intermoult stage (stage C): The integument is firm or hard with an even distribution of surrounding tissue. Granular patches appear in surrounding tissue of body appendages. The body appearance is neither transparent nor opaque. All distal shafts have now completely emerged into the inside of the exuvial setae. No shafts of the developing new setae are visible in the somatic tissue (Figs. 6.1, 6.2).

The premoult stage (stage D): The integument is very hard. Surrounding tissue is condensed and the body is opaque in appearance. At this stage, the most distinctive features are developing new setae. The proximal and distal shafts of new setae appear throughout somatic tissue of body appendages in copepodid II to V stages, but not the adult stage which does not moult (Figs. 6.1, 6.2). In adult *P. robusta*, however, changes in texture and hardness of the integument and the amount of somatic tissue are similar to those in

other copepodids.

Ultrastructure and thickness of the integument

Transmission Electron Microscopy was used to examine integumental structures in association with the intermoult cycle. Examinations were made in females of the copepodid V and adult stage. In adult females different stages of the intermoult cycle were determined by texture and hardness of the integument because they do not moult, but the same terms for the moult cycle are used. Two specimens of the postmoult, intermoult and premoult stage were examined from the 1975 sample; one specimen of each stage was examined from the 1994 sample.

TEM micrography of the integument is shown in Fig. 3. The integument consists of the epicuticle, exocuticle (p^1 or external procuticle), endocuticle (p^2 or internal procuticle), epidermis and basal lamina. Structural changes related to the intermoult cycle are found in the cuticle, or in the first three layers (Fig. 6.3). The epicuticle is the outermost thin and electron-dense layer. The exocuticle and endocuticle show stratification all the way across the cuticle.

In the postmoult stage the exocuticle and endocuticle are not distinguishable (Fig. 6.3). The exocuticle and endocuticle are discernable in the intermoult stage and the exocuticle is more electron opaque than the endocuticle. These changes are found in both copepodid V and adult females. In the pretmoult stage one more cuticular layer is recognizable between the exo- and endocuticle in copepodid V females only, and is not present in adult females. The lamellar structure is seen in the exocuticle in both copepodid V and adult females.

The thickness of the total cuticle varies depending on different stages of the intermoult cycle in the same copepodid stage (Table 6.1). The epicuticle is about 0.2 µm

thick in the copepodid V stage and 0.7 μm thick in the adult throughout all stages of the intermoult cycle. The exo- and endocuticle comprise up to 95% of the total cuticle. The thickness of the exo- and endocuticle gradually increases throughout the intermoult cycle, even in adult females where no moult is scheduled. The cuticle in the premoult stage is about twice as thick as in the postmoult stage. Increase in the thickness of the cuticle corresponds with changes in the cuticular structure (Table 6.1, Fig. 6.3). The thickness of the cuticle ranges between 2.3 and 8.2 μm in copepodid V females, and between 2.3 and 11.8 μm in adult females. The cuticle of the adult is greater in thickness than that in copepodid V stage.

Applying the intermoult cycle to field samples

On the basis of texture and hardness of the integument or the appearance of the body, copepodids and adults of *Pleuromamma robusta* were divided into "soft" and "hard" bodies; females of copepodid V and adult stages into "very soft", "soft", "hard" and "very hard" bodies. Copepodids and adults were then examined to identify different stages of the intermoult cycle by the morphology of setogenesis. All copepods with soft bodies were the postmoult stage, and those with hard bodies were the intermoult or premoult stage (Table 6.2). It was difficult to distinguish between the intermoult and premoult stages by the overall body appearance alone. The terms soft and hard bodies correspond with the postmoult, and the intermoult and premoult stage respectively and are used together throughout this study.

Prosome lengths of soft bodies (postmoult stage) and hard bodies (intermoult and premoult stage) were measured separately in all copepodid stages. Only females were examined in the copepodid V and adult stage. The mean, standard deviation, and range of prosome lengths are tabulated in Table 6.3. The mean prosome length of hard bodies is greater than that of soft bodies throughout all copepodid stages (Table 6.3). The standard

deviation gradually increases from the copepodid II to adult stages. In copepodid IV to adult stages the range of prosome lengths of hard bodies is greater than that of soft bodies.

6.4. Discussion

Ecdysis is important in the physiology of copepods and all other crustaceans because they grow through a periodic moulting of their exoskeleton. Internal physiological and biochemical changes affect changes in external morphology and behaviour (Drach 1939, 1944, Passano 1960, Scheer 1960, Kurup 1964, Lasker 1964, 1966, Stevenson 1969, Stevenson *et al.* 1968, Jennings and Halverson 1971, Aiken 1973, David *et al.* 1973, Reaka 1975, Skinner and Kumari 1992, Moss 1994, Roff *et al.* 1994). External and internal changes, therefore, continue throughout a period of the intermoult cycle. The most distinctive external changes are the formation of the old-exuvial exoskeleton, developing new setae and the formation of the new-exuvial exoskeleton. In calanoid copepods, the moult cycle has only been studied by an examination of the morphology of setogenesis in *Calanus finmarchicus* by Currie (1918) and *Calanus marshallae* by Dexter (1981).

In the present study, developing new setae and changes in cuticular structure related to the intermoult cycle are examined in *Pleuromamma robusta*. The intermoult cycle is divided into the postmoult, intermoult and premoult stage. Morphological characteristics of specific stages of the intermoult cycle found here are summarized in Table 6.4. The exocuticle and endocuticle of the old exuvial exoskeleton are formed in the postmoult stage in both copepodids and adults (Figs. 6.3, 6.4). New setae are formed throughout somatic tissue of appendages (Fig. 6.2) and ecdysial layers of the pre-exuvial exoskeleton are formed between the exocuticle and endocuticle of the old-exuvial exoskeleton (Fig. 6.3). These two occur in the premoult stage of copepodids because adult copepods do not moult. Various structures of the integument have been studied in a number of calanoid copepods,

even though the intermoult cycle was not examined (Bouligand 1966, 1972, Raymont *et al.* 1974, Bresciani 1986). Bresciani (1986) pointed out that some cuticular layers can be absent in certain individuals of a species. This may be due to individuals being at different stages of the intermoult cycle.

Structural changes in the cuticle are quantified by increases in the thickness of the cuticle throughout the period of the intermoult cycle (Table 6.1). The thickness of the cuticle varies according to different stages of the intermoult cycle in females of copepodid V and adult *Pleuromamma robusta*. The thickness of the cuticle in adult females is greater than in the copepodid V. Increase in thickness of the cuticle of adult females can be explained by the formation of the exocuticle and endocuticle of the exoskeleton in the postmoult stage (Fig. 6.3). In adult females, however, the thickness of the cuticle continuously increases throughout the intermoult and premoult stage as in copepodid V females where it forms the new-exuvial exoskeleton. This might be explained in the adult by two possibilities: 1) Increase in the thickness of the cuticle is with times of secretion and is independent of the moult; 2) Variation in the thickness may occur between individuals or different parts of the body. In the present study, all sections of cuticle were made using the well defined dorsal median area of the second metasomal segment; three specimens of each of the postmoult, intermoult and premoult stage were examined in the copepodid V and adult female, respectively; at least three serial sections (1 µm) were made in every single specimen. Variation in the thickness of the cuticle presented in this study is, therefore, unlikely to be due to the second possibility.

Body length increases with successive copepodid stage. Within the copepodid stage, the mean prosome length of the intermoult and premoult stage (hard bodies) is greater than those in the postmoult stage (soft bodies). Differences in body length between soft and hard bodies of the same copepodid stage is partially related to the intermoult cycle because the amount of the exoskeleton and somatic tissue increases throughout this period. The extent

of variation in body length may be related to the relative duration of different stages of the intermoult cycle. In the present study, duration of the intermoult cycle was not examined and no relevant data were found in calanoid copepods. Table 6.5 shows relative durations of different stages of the intermoult cycle, as a percentage of the total, in a variety of crustacean taxa. The postmoult stage (soft bodies) is about 10% (3 to 20%) of the total and the rest is the intermoult and premoult stage. In general, the intermoult stage is regarded as a period of stability with no distinctive morphological and physiological changes and this stability can be permanent (Drach 1939, 1944, Carlisle and Dohrn 1953, Knowles and Carlisle 1956, Passano 1960, Scheer 1960, Kurup 1964, Stevenson *et al.* 1968, Aiken 1973).

In the traditional concept of copepod growth, body size of adults is constant because they do not moult and size variation is due to continuous development of copepodid cohorts (broods and generation) with different growth rates (Marshall 1949, Marshall *et al.* 1934, Miller *et al.* 1977, McLaren 1978). Variation in body size, or different growth rates has been studied in relation to the influence of temperature and food in field and laboratory observations. The growth rate is proportional to food concentration and is strongly influenced by food quality (Mullin and Brooks 1970, Paffenhöffer 1976, Diel and Klein Breteler 1986, Huntley *et al.* 1987, Klein Breteler *et al.* 1990). Changes in temperature do not always affect copepod size (Deevey 1960, McLaren 1963,1965, Mullin and Brooks 1970, Corkett and McLaren 1978, Klein Breteler and Gonzalez 1982, 1988, Escribano and McLaren 1992). It is suggested that copepod body size and weight, including those of adults, increases continuously throughout a period of the intermoult cycle and the stage-specific size is determined by the maximum size of each copepodid stage. Different stages of the intermoult cycle are important in copepod size, in addition to growth factors from the ambient environment. In nature, copepodid stages develop continuously and within the copepodid stage various stages of the intermoult cycle coexist. Morphological and

physiological changes in association with the moult cycle may be evident as variation in body size.

The morphology of setogenesis and cuticular structures related to the moult cycle examined in the present study have already been documented in other crustaceans (Drach 1939, 1944, Scheer 1960, Passano 1960, Kurup 1965, Stevenson *et al.* 1968, Aiken 1973). This study confirms the usefulness of general information on the moult cycle in calanoid copepods. This study attempts to introduce the intermoult cycle as a source of variation in body size. It is, however, difficult to quantify the relationship between the intermoult cycle and body size variation.

Table 6.1. Copepodid V and adult females of *Pleuromamma robusta*. Comparison of the cuticular thickness in association with different stages of the intermoult cycle. Values in parenthesis are ranges of the cuticular thickness.

	Thickness of epicuticle* (μm)	Thickness of exo- and endocuticle* (μm)	Mean thickness of cuticle and standard deviation (μm)
Copepodid V females			
postmoult I	0.16	2.12	2.49±0.42 (2.3-4.7)
postmoult II	0.17	2.84	3.39±1.17 (2.3-4.7)
intermoult	0.17	3.35	4.32±0.83 (2.3-7.1)
premoult	0.19	4.45	5.05±1.63 (4.7-8.2)
Adult females			
postmoult I	0.65	4.75	5.39±1.78 (2.3-7.1)
postmoult II	0.79	6.40	7.19±1.17 (2.3-7.1)
intermoult	0.71	7.18	7.89±1.12 (4.7-8.2)
premoult	0.68	8.89	9.66±1.45 (7.6-11.8)

*These values were calculated from the TEM sections because the epicuticle and exo-and endocuticle were not identified under the compound microscope.

Table 6.2. Copepodids and adults females of *Pleuromamma robusta*. Relationship between body appearance and the intermoult cycle.

	Postmoult	intermoult	premoult	Total
Copepodid II				
soft	12	4	0	16
hard	2	17	16	35
Copepodid III				
soft	36	6	0	42
hard	4	21	17	42
Copepodid IV				
soft	35	3	0	38
hard	0	19	25	44
Copepodid V females				
soft	45	0	0	45
hard	0	12	33	45
Adult females				
soft	50	0	0	50
hard	0	18	32	50

Table 6.3. Copepodids and adults of *Pleuromamma robusta*. Comparison of prosome length of "soft" (postmoult stage) and "hard" (intermoult and premoult stage) bodies. Values in parenthesis are range of prosome length.

Copepodid stages	Soft bodies (mm)	Hard bodies (mm)
CII	0.61±0.02 (0.56-0.63)	0.63±0.11 (0.61-0.66)
CIII	0.83±0.03 (0.76-0.87)	0.85±0.02 (0.80-0.92)
CIV	1.08±0.04 (0.97-1.14)	1.10±0.32 (0.83-1.17)
CV females	1.47±0.08 (1.39-1.54)	1.52±0.08 (1.37-1.62)
Adult females	1.97±0.07 (1.88-2.11)	2.10±0.09 (1.71-2.24)

Table 6.4. Morphological characteristics of different stages of the intermoult cycle in *Pleuromamma robusta*.

Moult cycle	Characteristics
Postmoult	
(stage A to B)	1. Integument is soft.
	2. Body appearance is transparent.
	3. Exocuticle calcification; secretion begins to form endocuticle (Fig. 6.3).
	4. Surrounding tissue growth begins
	5. Distal shafts do not completely emerge into exuvial setae (Fig. 6.2).
Intermoult	
(stage C)	1. Integument is firm to hard.
	2. Formation of endocuticle is complete (Fig. 6. 3)..
	3. Main tissue growth continue; granular patches appear in surrounding tissue.
	4. Distal shafts of existing setae fully emerge (Fig. 6.2).
Premoult	
(stage D_1	1. Integument is hard.
	2. Tissue is condensed and body appearance is opaque.
	3. New setae grow out from somatic tissue of appendages and all morphological details of new setae invagination are not visible, except in adult stage (Fig. 6.2).
	4. Epicuticle and exocuticle of pre-exuvial exoskeleton may be formed, old-exuvial endocuticle reabsorbed.*
	5. Finally, ecdysial sutures may open.*
Ecdysis	
(stage E)	

*These characteristics were not examined in the present study (see Drach 1939, 1944, Scheer 1960, Passano 1960, Kurup 1964, Stevenson *et al.* 1968, Aiken 1973).

Table 6.5. Various durations (days) of different stages of the intermoult cycle in other crustacean taxa. Data for cirripeds (David *et al.* 1973), stomatopods (Reaka 1975), natantians (Drach 1944), macrurans (Stevenson *et al.* 1968), anomurans (Kurup 1964), and brachyurans (Drach 1939).

Moult stages	Cirripeds *Balanus*	Stomatopods *Gonodactylus*	Natantians *Leander*	Macrurans *Orconectes*	Anomurans *Petrolistes*	Brachyurans *Cancer*
Postmoult	15.0	2.6	19.0	5.1	6.5	10.0
Intermoult	45.0	26.6	21.5	59.6	49.2	76.0
Premoult	30.0	71.2	53.7	34.7	44.5	23.0

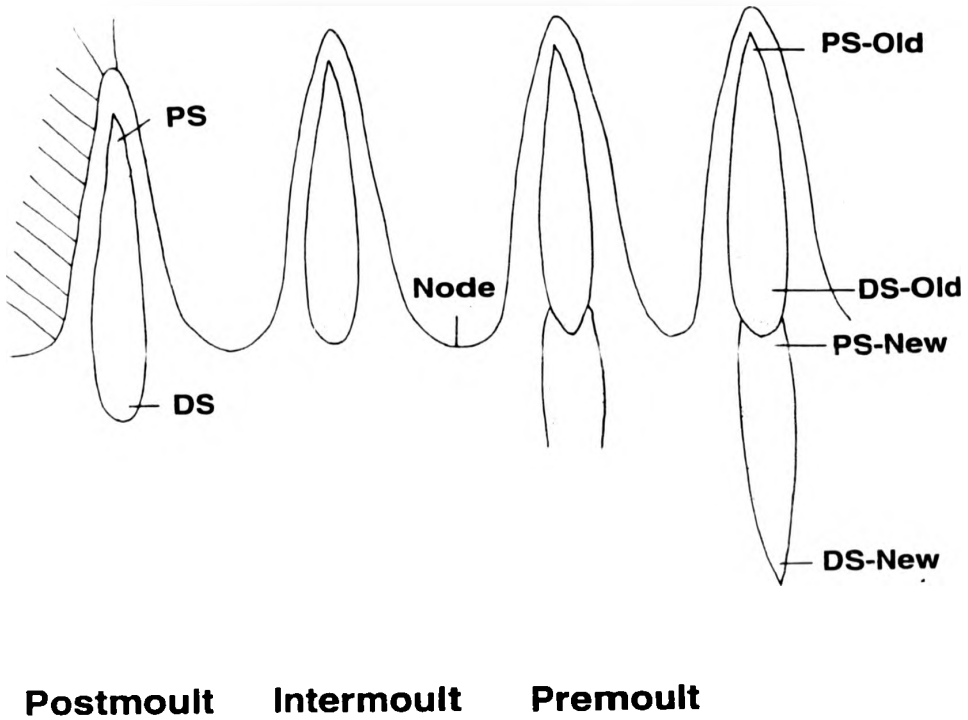


Fig. 6.1. Terminology used for developing setae in *Pleuromamma robusta*. PS, proximal shaft; DS, distal shaft; old, exuvial setae; new, new setae.

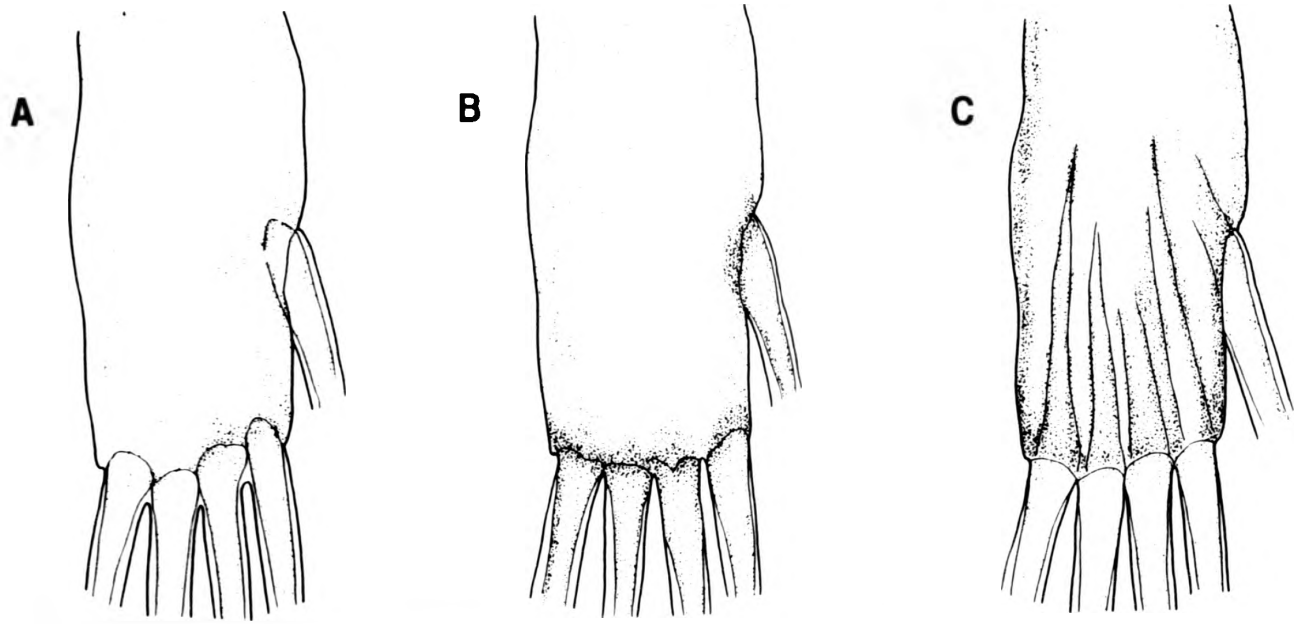


Fig. 6.2. Development of new setae in copepodid V female *Pleuromamma robusta*. (A) Postmoult stages of the caudal rami. (B) Intermoult stage. (C) Premoult stage.

Fig. 6.3. TEM micrographs. Transverse sections of the integument of *Pleuromamma robusta*. EP, epicuticle; EX, exocuticle; EN, endocuticle; ED, epidermis; MS, moulting space. (A) Late postmoult stage of the adult female (x25,000). (B) Intermoult stage of the copepodid V female (x25,000). (C) Premoult stage of the copepodid V female (x25,000). (D) Premoult stage of the copepodid V female (x10, 000) with the moulting space. (E) Premoult stage of the adult female (X10, 000). Scale bar= 2 μm.

Pleuromamma

moulting space.

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nale (x25,000).

ing space. (E)

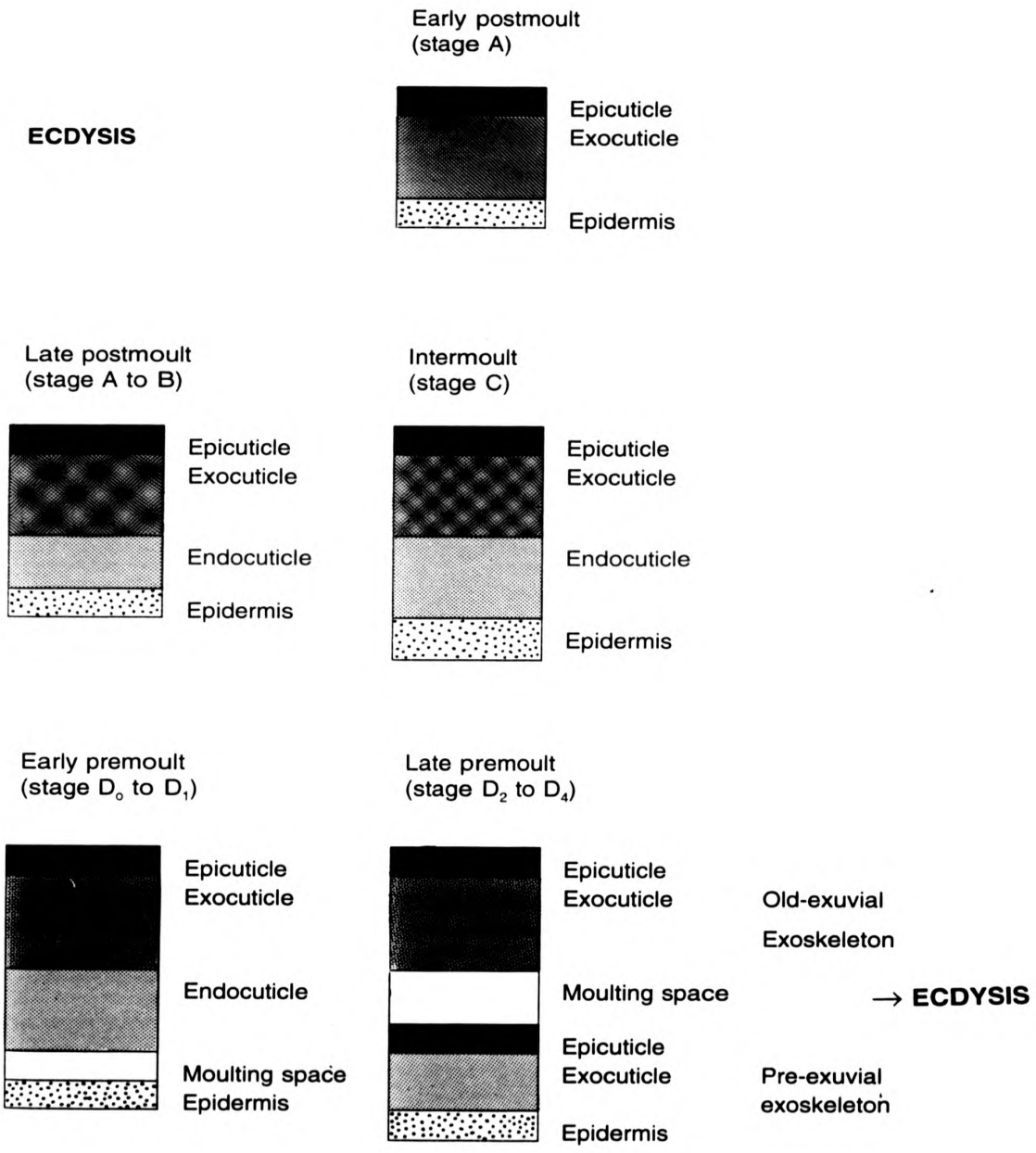

Fig. 6.4. Diagram of cross-section of successive changes in the integumental structure throughout a period of the intermoult cycle in *Pleuromamma robusta*.

CHAPTER 7:

The population dynamics of *Pleuromamma robusta*

7.1. Introduction

The oceanic calanoid copepod *Pleuromamma robusta* is an important component of the zooplankton community of the northeastern Atlantic Ocean and plays a significant role in the oceanic food wed as prey for foraging species for midwater micronekton (Mauchline and Gordon 1980, 1983, 1984, Scotto di Carlo *et al.* 1982, Kawaguchi and Mauchline 1982, Roe 1984, Roe and Badcock 1984). The biology of this species is best known from studies of its vertical distributions in various limited locations over short periods of time (Roe 1972b, Longhurt and Williams 1979, Roe 1984). There have been no published studies of the population dynamics of *P. robusta* or the other congeners, despite their ecological importance.

Ferrari and Hayek (1990) reported that in *Pleuromamma xiphias* some of adult males and females have a soft exoskeleton and undeveloped internal tissue, and others have a hard exoskeleton and well-developed internal tissue. They also noted that males without a spermatophore in the spermatophore sac, and females without a dark mass in the genital opening, usually have a soft exoskeleton, while males with a spermatophore and females with a dark mass usually possess a hard exoskeleton. In their study, the anatomical nature of the dark mass in the genital opening was not clear, nor was a relationship shown between the body appearance and status of the gonad structure, although copepods with a soft exoskeleton were assumed to be recently moulted. Ferrari and Hayek (1990) suggested the potential usefulness of the moult cycle as an indicator of the recruitment rate in a study of population dynamics.

As in all crustaceans, copepod growth, or recruitment from one stage to the next is accompanied by moulting. The life span is a time series of the intermoult cycle. The intermoult cycle is mainly divided into the postmoult, intermoult, and premoult stage. The calcification of the old-exuvial exoskeleton, increase in the amount of the somatic tissue,

developing new setae, decalcification of the old-exuvial exoskeleton, and the formation of the pre-exuvial exoskeleton sequentially occur through a period of the intermoult cycle. In particular, the first two changes affect the body appearance of copepods (see chapter 6). Since the development of the old-exuvial exoskeleton and the somatic tissue is not complete in the postmoult stage, the exoskeleton is soft and the body is transparent in appearance. The exoskeleton later becomes hard and the body acquires an opaque appearance; these are the intermoult and premoult stages.

The major events in the intermoult cycle of copepods are the completion of external and internal structures after moulting and preparation for the successive moulting. Most of the notable changes occur in the cuticular structure. In the previous chapter, these sequential changes of the cuticular structure of *Pleuromamma robusta* were quantified by measuring the increase in the thickness of the cuticle throughout the intermoult cycle. In addition, adult copepods do not moult but progress through part of the intermoult cycle exhibited by earlier copepodid stages, except the formation of new setae and the pre-exuvial exoskeleton. The previous study suggested that copepod body size and weight increase continuously throughout a period of the intermoult cycle. Although further study is needed in order to prove this relationship, it is worth applying the intermoult cycle to marine ecological studies of calanoid copepods as a tool for interpretation of the population parameters.

In the present study, different stages of the intermoult cycle were identified throughout copepodid stages II to VI of *Pleuromamma robusta*. Seasonal fluctuations in abundance and body size were examined for all copepodid stages. In addition, the gonad structure of CVI males and females was examined to describe the seasonal variation of gonad maturity. Abundance and body size were examined separately between individuals of each copepodid stage at different stages of the intermoult cycle and/or different status of gonad maturity.

7.2. Materials and methods

Field sampling

Samples of *Pleuromamma robusta* were taken at approximately two month intervals from March 1975 to February 1976 in the Rockall Trough near 55°N, 12°W, in the northeastern Atlantic Ocean. The samples were collected using a rectangular midwater trawl of 1 m^2 mouth area with 2 m codend constructed of 295 µm mesh, as described by Baker *et al.* (1973) and Roe *et al.* (1980). With a ship speed of about 30 m/min, the net was lowered slowly at about 6 m/min. The net was towed open with a ship speed of about 62 m/min, while wires were hauled in at about 10 m/min with the same ship speed as with the wire out. A time series of samples were done in a constant manner on each cruise at about 2000 m depth, except for July, when the depth was 800 m. Samples were fixed in 5% hexamine buffered formaldehyde and then transferred to Steedman's (1976) preservative: 25 ml of 40% formaldehyde, 10 ml porphylene phenoxetol, 100 ml propylene glycol and 865 ml sea water. The hydrogrphic data were not available for the present study.

Identification of copepodid stages and sexes

Copepodid stages of *P. robusta* were identified by the number of urosomal segments and swimming legs (Table 7.1). Sexes of copepodid V were identified by the shape and setal number on the fifth legs (see Ferrari 1985). The number of urosomal segments is five in adult males and three in adult females because of the fusion of the first two urosomal segments. Sexes of adults were readily distinguished.

Different stages of the intermoult cycle

In copepodid stages II to VI of *P. robusta*, different stages of the intermoult cycle were identified by the hardness of the integument, or the body appearance, and were divided into "soft" and "hard" bodies (see chapter 6). Here, the terms soft and hard bodies correspond with the postmoult, and intermoult and premoult stage respectively and are used together throughout this study.

The degree of the gonad development

On the basis of the structure of the gonad system, adult males and females of *P. robusta* were classified into "immature" or "mature" condition. Adult males without a spermatophore in the spermatophore sac were recorded as immature, whilst those with a spermatophore were recorded as mature. Reproductively immature adult females were defined by one of two criteria; either by the diverticula not extending very far forward from the ovary, or, if the diverticula extended to the posterior body, they did not carry large egg masses. Copepodid V females in mature condition were defined as those containing multi-layered, large egg masses in the diverticula. Adult males and females in a post-reproductive condition were not identified and were included with those in an immature condition.

Although the genital system of copepodid V females of *P. robusta* was not anatomically examined, they can be classified by the presence and absence of a rounded structure over the ventral areas of the first and second urosomal segments, presumably corresponding with the spermathecae on the first urosomal somite of adult females. The ventral areas of the first two urosomal segments of copepodid V females were ventrally swollen, but the rounded structure was not formed, individuals were termed "immature". Copepodid V females with a well-formed, rounded structure were termed "mature".

Measurement of body size

Prosome lengths were measured by ocular micrometer under a dissection microscope. In lateral view, prosome length was defined as from the anterior end of the rostrum to the posterior area of the lateral end of the prosome. Measurements were made separately between individuals of each copepodid stage at different stages of the intermoult cycle and/or different status of gonad maturity.

7.3. Results

Abundances

Naupliar stages and Copepodid I of *P. robusta* were not available for this study. Copepodid stages II to VI were present throughout the year, except in February 1976 when copepodid II and III were absent (Tables 7.2, 7.3, Fig. 7.1). The monthly abundance of copepodid stages varied within an order of magnitude for each stage (0-83 for copepodid II, 0-520 for copepodid III, 1-1259 for copepodid IV, 16-689 for copepodid V males, 18-1078 for copepodid V females, 68-1327 for adult males, and 564-2472 for adult females).

The abundance of copepodid II and III was seasonally lowest in March and, thereafter, increased progressively (Table 7.2, Fig. 7.1). The peak of abundance was in September for copepodid II, and in November for copepodid III. Copepodid II decreased in abundance in November, but still remained abundant. No copepodid II or III were found in the following February.

Copepodid IV and copepodid V males and females exhibited two peaks of abundance: the first, small peak occurred in May and the second, large peak occurred in September (Tables 7.2, 7.3, Fig. 7.1). The September peak of copepodid IV and copepodid

V males was about twice as high as the May peak, but this was not true for the copepodid V females. Following each peak, there was a rapid decrease in abundance in July and November respectively. The abundance of these stage appeared to decrease throughout the winter, reaching its minimum in the following February.

The overall seasonal cycle of the abundance of adult males was close to that of copepodid V females (Table 7.3, Fig. 7.1). Adult females exhibited a distinct pattern of abundance throughout the year. The abundance of adult females increased from March to May and declined in July. Adult females showed a strong peak of abundance in September and was followed by a rapid decrease in abundance in November. The abundance of adult females increased slightly from November to the following February.

Different stages of the intermoult cycle

Seasonal fluctuations in the abundance of soft (postmoult stage) and hard (intermoult and premoult stage) bodies for each stage are given in Tables 7.2 and 7.3, along with fluctuations in monthly stage abundance. Within individual copepodid stages, the seasonal pattern of the abundance of soft bodies agrees with that of stage abundance, except that the peak of the abundance of copepodid II, copepodid V females, and adult females was in September, while the peak of the abundance of soft bodies was in July for copepodid II and in May for copepodid V females and adult females.

The degree of the gonad development

The degree of gonad development was examined in copepodid V females and adult males and females of *P. robusta*, in combination with the intermoult cycle. None of these stages with soft bodies (postmoult stage) were in a reproductively mature condition, while

significant proportions of hard bodies (intermoult and premoult stage) were in immature condition (Table 7.2, Fig. 7.1). This means that the development of the gonad system from an immature into a mature condition depends on the time elapsed after moulting.

The yearly average percentage of copepods in a mature condition is 46.6% for adult males, and 17.7% for adult females (Table 7.3). The percentage of adult males in mature condition varied among months from 13.5% in March to 67.7% in July. The percentage of reproductively mature condition of adult females ranged from 0% in November to 24.7% in July. These values suggest that adult females take longer to reach gonad maturity. Adult females, therefore, live longer than adult males, in terms of sex-limited, or specific longevity.

This study has inferred the seasonal timing and intensity of reproduction of *P. robusta* from the condition of the oocytes of the oviducts of adult females. Reproductively mature adult females occurred from March to September with increasing numbers, were absent in November, and reappeared at lower levels in following February (Table 7.3). The peak of the abundance of mature adult females was in September, when 52% of total mature females occurred. The percentage of mature adult females was seasonally highest in July (24.2%) and September (22.2%).

In the present study, 5973 adult females were examined, and among them, two females (one in May and the other in November) had a spermatophore attached to the genital opening. They had soft bodies. Adult females always contained the spermatophoric contents in the spermatophore sac. Only one female with an empty spermatophore sac was found in July; it had a soft body.

Sex ratio

The yearly average sex ratio, as the percentage of males per females, was similar for copepodid V (55.7%) and adults (55.3%) (Table 7.3). Copepodid V and adults, however,

exhibited different seasonal patterns of the sex ratio (Fig. 7.2). The pattern of seasonal fluctuations in the sex ratio of copepodid V was closely similar between soft and hard bodies, while there were significant differences in the seasonal pattern of the sex ratio between adults with soft and hard bodies.

Body size

Tables 7.4-7.10 show the mean and standard deviation of prosome length for copepodid stages II to adult, and for each copepodid stage at different stages of the intermoult cycle and/or different status of gonad maturity. The mean prosome length seasonally oscillated for all copepodid stages (Tables 7.11, 7.12), while the standard deviation of prosome length of all stages was relatively constant throughout the year.

Within each copepodid stage of a given month, the mean prosome length of hard bodied individuals was nearly always greater than that for soft bodied one (Tables 7.4-7.10). There are, however, some exceptional cases. The mean prosome length of soft bodies was larger than that of hard bodies in the following February for copepodid V females and in May for adult females. Females of copepodid V and adult were considerably larger than the males. The general pattern of seasonal fluctuations in prosome length was similar for all stages in that there was a pronounced increase in prosome length in May and thereafter prosome length decreased. This seasonal pattern of prosome length of each stage is well illustrated in the length-frequency distributions (Figs. 7.3-7.7).

7.4. Discussion

Seasonal dynamics

It is most convenient to begin to evaluate the life cycles of *Pleuromamma robusta* by examining the data on the adult females first. In the length-frequency distributions, adult females can be divided into the small (< 2mm in prosome length) and the large (> 2mm) size groups (Fig. 7.7). The smaller females dominate the March, November and February samples, while the large females dominate the May, July and September samples. There was extensive mixing between these two size groups in May and September, when two peaks of abundance occurred (Table 7.4, Fig. 7.7). In the remaining months, when abundance was relatively constant, one size group was exclusively dominant. The two peaks of the abundance of adult females, therefore, result from overlapping generations. The population of adult females is evidently reconstructed in May and September by recruitment from copepodid V stage.

Although no adult females were in a reproductively mature condition in November, the magnitude of the abundance of early copepodid stages, derived from the active breeding in September, indicates a future period of active recruitment to the adult population (Tables 7.3, 7.4). In the present study, however, major recruitment to November stocks of adults was not observed. It had, however, taken place by February (Fig. 7.7).

During the hiatus of reproduction of adult females, it is clear that no further production of early life stages will take place and the existing stocks of earlier copepodid stages in November will develop to the subsequent stages. The abundance of copepodid stages II to V appeared to decrease continuously from November to the following February, whilst the abundance of adult females held nearly steady, or rather increased slightly (Tables 7.2, 7.3, Fig. 7.1). During this period, a continuous decrease in body size of adult

females must be caused by continuous recruitment from the earlier copepodid stages (Fig. 7.7). This suggests that most of November stocks of early life stages recruit to CVI females throughout the winter.

The peak of the abundance and reproduction of adult females was in September and was followed by a hiatus of reproduction in November (Table 7.3). This can be explained by the fact that adult females which were reproductively active must then die off, while the gonads of the newly recruited small females have not had time to develop the gonad system into a mature condition. In the following February, however, no early copepodid stages were found. It is possible that some adult females had released their eggs, but these had not yet had time to develop into various copepodid stages. A considerable proportion of the new generation of adult females is reproductively mature (Fig. 7.7).

Since there are several sources of recruitment of CVI females throughout the winter, it is difficult to estimate the main source of adult females in the March population. There are no stocks of copepodid II and III present in the population in February (Table 7.2) that could develop to copepodid IV and the adult stage by March. Nauplii were too small to be retained by the nets but if recruitment was from them, then some indication of their presence would be expected in the copepodid II to CIII stages by February. The lack of breeding in November suggests that such a stock of nauplii would not be present in February. The principal difference between the stock of adult females in February and March is an increase in the number of a single size class. The one at 2.00 mm which doubles in size, while numbers of the others remain relatively constant. They could originate from the new breeding period which had started by February as indicated by the presence of mature females in the population (Fig. 7.7) and the appearance of early copepodid stages in the March samples (Table 7.2). They are in the smallest size class of the 'large' summer females.

The March (late winter) population of adult females was overwhelmed by the new generation in May (Fig. 7.7). Since a significant proportion of adult females of the new generation in May was in reproductively mature condition, the recruitment of the generation takes place some time before May, possibly as early as March because there are significant differences in body size of adult females with soft bodies between March and May, and a significant proportion of the new generation in May was in mature condition (Table 7.3). The May (spring) population of adult females was again overwhelmed by the autumn population. The population of adult females of *P. robusta*, therefore, comprise of three generations over the period of a year: the winter/early spring, the spring/summer, and the later summer/autumn.

All copepodid stages occurred in March, but the abundance of earlier copepodid stages was seasonally low (Tables 7.2, 7.3, Fig. 7.1). A very rapid increase in the abundance of all copepodid stages followed in May, due to the appearance of the new generation. There was also a pronounced increase in body size of all copepodid stages, but after May their body size decreased again. The spring population of all copepodid stages can, therefore, readily be distinguished from the other (Tables 7.4-7.10, Figs. 7.3-7.7). This spring generation could be produced from reproductive activity of the winter population of adult females. After May, the abundances of copepodid II and III increased progressively and reached their maximum in September and November respectively, while the abundances of copepodid stages IV to the adult oscillated.

Copepodid stages IV to adult exhibited two peaks of abundance: the first, smaller peak occurred in May and the second, major peak occurred in September (Tables 7.2, 7.3). As discussed above, one more peak is expected from the magnitude of the abundance of early copepodid stages in November. Three cohorts per year, therefore, can be passed through by *P. robusta* in the Rockall Trough.

In the present study, early (nauplii and copepodid I) life stages of *P. robusta*, which

are necessary for a thorough evaluation of the life history of copepods, were not available. This data set consists of samples collected at approximately two month intervals. In retrospect, this time interval is too long to result in population histograms that are coherent from one sample to the next. Significant recruitment to the adult population from populations of early copepodid stages appears to take place within overall periods of 4 to 6 weeks. Monthly samples would probably be close enough in time to document these changes, although samples at 2-weekly intervals would be nearer the optimum. The overall seasonal cycle of the abundance of early copepodid stages, therefore, disagrees with that of late copepodid stages (Tables 7.2, 7.3). However, seasonal fluctuations in the abundance of CVI females in reproductively mature condition agrees with the amplitude of the fluctuations in the abundance of early copepodid stages.

Sex-limited longevity

In *Pleuromamma robusta*, reproductively mature copepodid V females and adult males and females always had hard bodies (intermoult and premoult stage), and none of those with soft bodies (postmoult stage) were in a mature condition (Table 7.3). However, a significant proportion of copepodids with hard bodies was in immature condition. Since the classification of the intermoult cycle depends entirely upon the time elapsed after moulting, those mature copepodids with hard bodies must be older than immature ones. Differences in the percentage of gonad maturity between adult males and females suggests that adult females require more time to reach gonad maturity than adult males (Table 7.3).

There are several difference between the life cycles of adult males and females. First, the abundance of adult males and females increased from March to May and decreased in July (Table 7.3, Fig. 7.1). This event is due to the appearance of the new generation with large body size. In adult males with soft and hard bodies, and adult females

with soft bodies, an increase in body size occurred in May (Tables 7.9, 7.10, Fig. 7.7). In adult females with hard bodies, however, obvious size changes delayed and occurred in July. In May, the majority of adult males (56.7%) had already reached gonad maturity, while only 15.2% of adult females were in mature condition.

Second, the numbers of adult males and females with soft bodies were very similar between July and November (Table 7.3). Reproductively mature adult females occurred throughout this period, whilst no adult females were found in a mature condition in November.

Third, a proportion of the November stocks of earlier copepodid stages finally develop in November not only into adult females, but also into adult males. The abundance of adult males decreased continuously throughout the winter, but the abundance of adult females remained nearly steady (Table 7.3).

Adult females were long-lived relative to the males. Differences in the life cycles between adult males and females may be caused by sex-specific longevity. The sex-limited duration of the life cycle might be differentiated as early as copepodid IV because females of copepodid V and adult were considerably larger than the males of the same stage (Tables, 7.7-7.10, Figs. 7.6, 7.7). At the copepodid IV stage, secondary sexual characteristics also develop on the fifth pair of swimming legs and in the pore signature of the first urosome segment (see chapter 4).

In the present study, out of 5973 adult females of *P. robusta*, two specimens had a spermatophore attached to the genital opening and one specimen had empty spermathecae. All of these had soft bodies. Adult females always contained the spermatophoric contents in the spermathecae. It is probable that adult males transfer their spermatophore to the females just after moulting of copepodid V females and that adult females retain the spermatophoric content in the spermathecae until fertilization. If so, the timing of transfer of the spermatophore from the male to the females is entirely independent

of the stage of gonad maturity of adult females, but, instead, related to the moulting cycle of copepodid V females. In fact, the amplitude of the abundance fluctuations of adult males was close to that of copepodid V females throughout the year (Table 7.3). There was no significant relationship between seasonal variations in gonad maturity between adult males and females, nor between seasonal fluctuations in the sex ratio and gonad maturity of adult males and females (Table 7.3, Fig. 7.2).

Intermoult cycle

In calanoid copepods, Dexter (1981) first described the intermoult cycle of *Calanus marshallae* as a well-defined sequence of stages in copepodid stages II to V. Different stages of the intermoult cycle were characterised by the degree of setogenesis which had taken place in the somatic tissue of appendages in preparation for the loss of existing setae with the old exoskeleton at ecdysis. The formation of new setae, however, is a part of the moult process.

Detailed studies of the intermoult cycle in other crustaceans, have shown that the calcification of the old-exuvial exoskeleton, increase in the amount of the somatic tissue, developing new setae, the decalcification of the old-exuvial exoskeleton, and the formation of the pre-exuvial exoskeleton occur sequentially throughout a period of the intermoult cycle (e.g., Drach 1939, 1944, Passano 1960, Ailken 1973). The previous chapter confirmed the usefulness of general information on the moult cycle in calanoid copepods, and showed that adult copepods do not moult but progress through the intermoult cycle, with the exception of developing new setae and the pre-exuvial exoskeleton. These sequential changes are also evident as an increase in thickness of the cuticle throughout the intermoult cycle. The previous chapter suggested that copepod body size and weight, including those of adults, increase continuously throughout the period of the intermoult cycle and that the stage-

specific size is determined by the maximum size of each copepodid stage. In the present study, body size of soft bodies (postmoult stage) is always greater than that of hard bodies (intermoult and premoult stage) throughout the year in all copepodid stages of *P. robusta* (Tables 7.4-7.10), except that generations with different body size occur together. It is also suggested that differences in the body size between males and females of the same stage are due to sex-specific longevity. However, it is still difficult to quantify the relationship between the intermoult cycle and body size variation. Additional detailed physiological and morphological studies are needed in order to prove this relationship.

In the present study, the seasonal dynamics of *P. robusta* are evidenced by changes in abundance of each stage. The length-frequency distributions and the intermoult cycle provide further information, e.g., the timing of the population reconstruction or overlapping generations, detection of the property of the newly recruited stock, and the seasonal intensity of the recruitment rate.

Table 7.1. The number of urosomal segments and of pairs of swimming legs in the copepodid stages.

Copepodid stage	I	II	III	IV	V	VI	
						Male	Female
No. of urosomal segments	2	2	2	3	4	5	3
No. of swimming legs	3	4	5	5	5	5	5

Table 7.2. *Pleuromamma robusta*. Seasonal fluctuations of abundance of copepodid stages II to CV males, showing the numbers of individuals with soft (postmoult stage) and hard (intermoult and premoult stages) bodies.

	1975 Mar	May	July	Sept	Nov	1976 Feb	Grand Total
CII							
Total	3	20	53	83	54	0	213
Soft	0	1	17	11	10	0	39
Hard	3	19	36	72	44	0	174
CIII							
Total	35	135	175	410	520	0	1275
Soft	11	11	74	94	146	0	336
Hard	24	124	101	316	374	0	939
CIV							
Total	23	618	213	1259	359	1	2473
Soft	13	128	94	555	191	0	981
Hard	10	490	119	704	168	1	1492
CV Males							
Total	26	274	145	686	189	16	1336
Soft	3	58	52	147	76	8	344
Hard	23	216	93	539	113	8	992

Table 7.3. *Pleuromamma robusta*. Seasonal fluctuations in the abundance of CV females and CVI males and females, along with the corresponding numbers of individuals with soft (postmoult stage) and hard (intermoult and premoult stages) bodies and at different status of gonad development.

	1975 Mar	May	July	Sept	Nov	1976 Feb	Grand Total
CV Females							
Total	47	865	150	1078	240	18	2398
Soft Immature	9	335	57	199	120	12	732
Hard Immature	17	407	34	557	58	4	1077
Hard Mature	21	123	59	322	62	2	589
CVI Males							
Total	215	1080	387	1327	240	68	3317
Soft Immature	21	104	70	168	59	7	429
Hard immature	165	364	57	629	90	37	1342
Hard Mature	29	612	260	530	91	24	1546
CVI Females							
Total	578	1049	848	2472	462	564	5973
Soft Immature	91	182	63	160	49	25	570
Hard Immature	417	708	576	1764	413	473	4351
Hard Mature	70	159	209	548	0	66	1052

Table 7.4. Copepodid II of *Pleuromamma robusta*. Seasonal variations of mean and standard deviation of prosome length (mm) with number of specimens examined in parentheses.

	Soft bodies	Hard bodies	Total
Mar 1975	-	0.62±0.00 (2)	0.62±0.00 (2)
May	-	0.65±0.12 (18)	0.65±0.12 (18)
July	0.61±0.02 (16)	0.63±0.01 (36)	0.62±0.02 (52)
Sept	0.61±0.01 (11)	0.62±0.01 (64)	0.62±0.01 (75)
Nov	0.59±0.01 (9)	0.61±0.01 (38)	0.61±0.02 (47)
Feb 1976	-	-	-

Table 7.5. Copepodid III of *Pleuromamma robusta*. Seasonal variations of mean and standard deviation of prosome length (mm) with number of specimens examined in parentheses.

	Soft bodies	Hard bodies	Total
Mar 1975	0.83±0.02 (6)	0.83±0.01 (21)	0.83±0.02 (27)
May	0.86±0.02 (11)	0.88±0.02 (107)	0.88±0.02 (118)
July	0.83±0.03 (57)	0.85±0.02 (85)	0.84±0.03 (142)
Sep	0.81±0.02 (68)	0.83±0.02 (169)	0.83±0.02 (237)
Nov	0.81±0.02 (55)	0.82±0.02 (217)	0.82±0.02 (272)
Feb 1976	-	-	-

Table 7.6. Copepodid IV of *Pleuromamma robusta*. Seasonal variations of mean and standard deviation of prosome length (mm) with number of specimens examined in parentheses.

	Soft bodies	Hard bodies	Total
Mar 1975	1.07±0.03 (11)	1.09±0.04 (10)	1.08±0.04 (21)
May	1.12±0.02 (98)	1.17±0.03 (273)	1.16±0.04 (371)
July	1.08±0.04 (65)	1.10±0.03 (109)	1.09±0.04 (174)
Sep	1.06±0.03 (180)	1.12±0.03 (229)	1.09±0.04 (409)
Nov	1.06±0.04 (65)	1.10±0.03 (114)	1.08±0.04 (179)
Feb 1976	-	1.11±0.00 (1)	1.11±0.00 (1)

Table 7.7. Copepodid V male of *Pleuromamma robusta*. Seasonal variations of mean and standard deviation of prosome length (mm) with number of specimens examined in parentheses.

	Soft bodies	Hard bodies	Total
Mar 1975	-	1.38±0.03 (26)	1.38±0.03 (26)
May	1.40±0.04 (49)	1.46±0.02 (120)	1.44±0.05 (169)
July	1.37±0.03 (40)	1.41±0.04 (87)	1.39±0.04 (127)
Sept	1.35±0.04 (106)	1.40±0.04 (240)	1.38±0.05 (346)
Nov	1.33±0.03 (36)	1.39±0.04 (80)	1.37±0.05 (116)
Feb 1976	1.40±0.03 (7)	1.37±0.04 (6)	1.39±0.04 (13)

Table 7.8. Copepodid V female of *Pleuromamma robusta*. Seasonal variations of mean and standard deviation of prosome length (mm) with number of specimens examined in parentheses.

	Soft bodies	Hard bodies I	Hard bodies II	Total
Mar 1975	-	1.48±1.03 (12)	1.49±0.07 (15)	1.49±0.05 (27)
May	1.55±0.05 (148)	1.59±0.04 (170)	1.60±0.05 (105)	1.58±0.05 (423)
July	1.47±0.04 (51)	1.51±0.05 (59)	1.52±0.06 (31)	1.50±0.05 (141)
Sept	1.44±0.05 (104)	1.49±0.05 (211)	1.49±0.05 (86)	1.48±0.06 (401)
Nov	1.40±0.05 (50)	1.47±0.05 (54)	1.47±0.05 (30)	1.45±0.06 (134)
Feb 1976	1.40±0.03 (7)	1.36±0.04 (4)	1.39±0.01 (2)	1.39±0.04 (13)

Table 7.9. Copepodid VI male of *Pleuromamma robusta*. Seasonal variations of mean and standard deviation of prosome length (mm) with number of specimens examined in parentheses.

	Soft bodies	Hard bodies I	Hard bodies II	Total
Mar 1975	1.68±0.04 (9)	1.69±0.05 (74)	1.65±0.05 (29)	1.68±0.05 (111)
May	1.73±0.05 (26)	1.76±0.04 (118)	1.76±0.04 (282)	1.76±0.04 (426)
July	1.69±0.04 (53)	1.72±0.06 (57)	1.76±0.04 (232)	1.74±0.05 (342)
Sept	1.67±0.04 (101)	1.69±0.05 (180)	1.70±0.05 (281)	1.69±0.05 (562)
Nov	1.62±0.03 (30)	1.68±0.04 (53)	1.68±0.05 (113)	1.67±0.05 (195)
Feb 1976	1.59±0.00 (2)	1.62±0.03 (12)	1.61±0.03 (22)	1.61±0.03 (35)

Table 7.10. Copepodid VI female of *Pleuromamma robusta*. Seasonal variations of mean and standard deviation of prosome length in mm with number of specimens examined in parentheses.

	Soft bodies	Hard bodies I	Hard bodies II	Total
Mar 1975	1.89±0.09 (67)	1.89±0.09 (224)	1.89±0.10 (70)	1.89±0.10 (361)
May	2.05±0.08 (123)	2.02±0.13 (350)	2.02±0.11 (134)	2.03±0.12 (607)
July	1.97±0.07 (52)	2.11±0.09 (552)	2.11±0.09 (206)	2.10±0.09 (810)
Sept	1.89±0.08 (156)	2.01±0.12 (420)	2.04±0.11 (300)	2.00±0.12 (876)
Nov	1.77±0.08 (25)	1.94±0.09 (296)	-	1.93±0.10 (321)
Feb 1976	1.80±0.09 (17)	1.87±0.10 (203)	1.85±0.09 (66)	1.86±0.10 (286)

Table 7. 11. Copepodid stages II to IV of *Pleuromamma robusta*. The value for student's t-test for prosome length beweeen months, *ns* indicates a probability value greater than 0.05. * indicates a probability value less than 0.05 and greater than 0.01. ** indicates a probability value less than 0.01 and greater than 0.001.

	CII	CIII	CIV
Mar vs May	0.862*ns*	11.627**	8.919**
May vs July	1.056*ns*	12.903**	19.048**
July vs Sept	0.000*ns*	3.571**	0.000*ns*
Sept vs Nov	0.007*ns*	5.649**	2.793*
Nov vs Feb	-	-	10.036**

Table 7. 12. Copepodid stages V and VI of *Pleuromamma robusta*. The value for student's t-test values for prosome length between months, *ns* indicates a probability value greater than 0.05, * indicates a probability value less than 0.05 and greater than 0.01. ** indicates a probability value less than 0.01 and greater than 0.001.

	CV males	CV females	CVI males	CVI females
Mar vs May	8.571**	9.090**	16.000**	19.529**
May vs July	10.000**	16.461**	6.060**	12.069**
July vs Sept	2.247*	3.870**	14.586**	19.607**
Sept vs Nov	1.866*ns*	5.011**	4.812**	10.152**
Nov vs Feb	1.666*ns*	4.899**	9.677**	8.750**

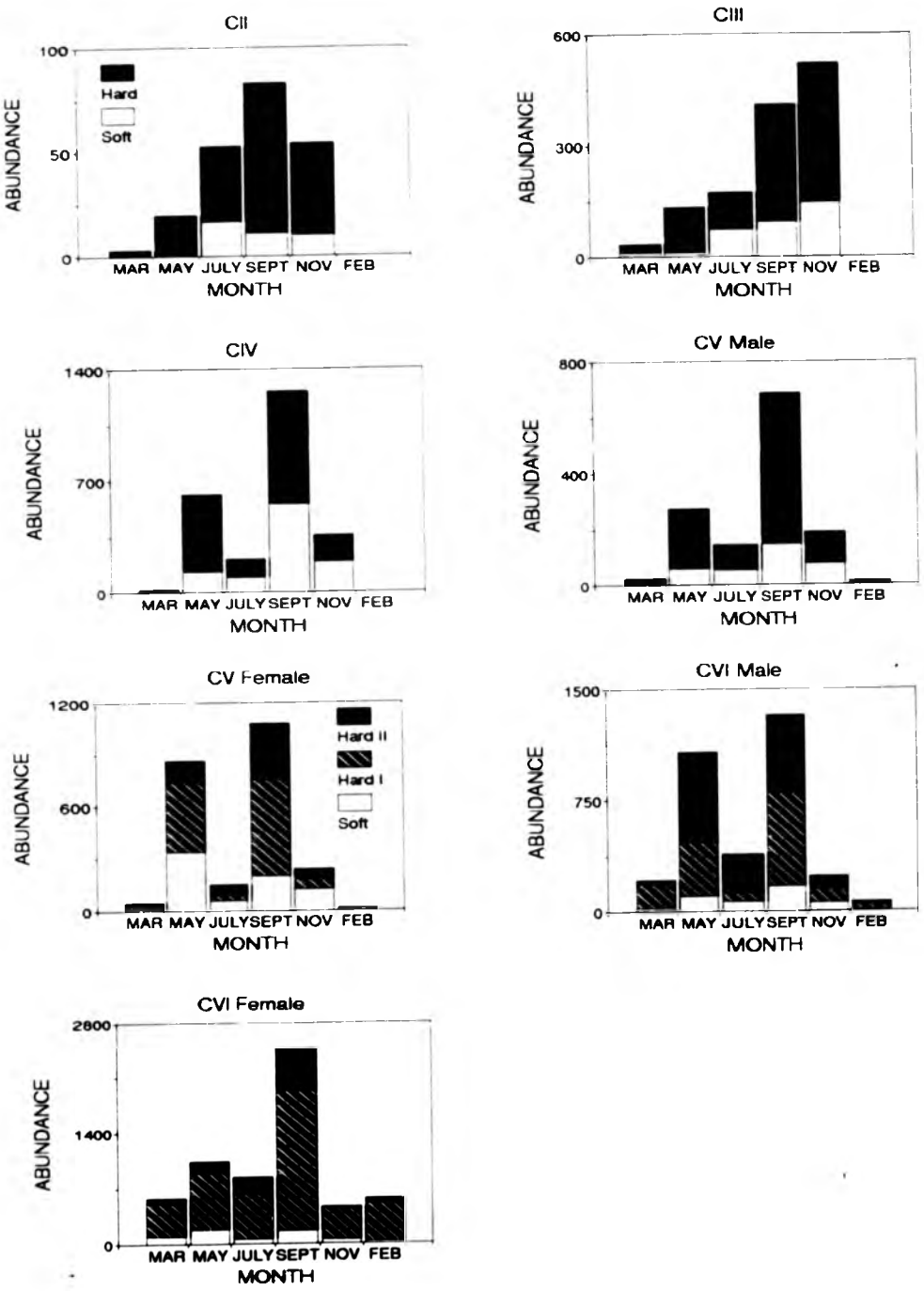

Fig. 7.1. *Pleuromamma robusta*. Seasonal changes in abundance of copepodid stage II to VI.

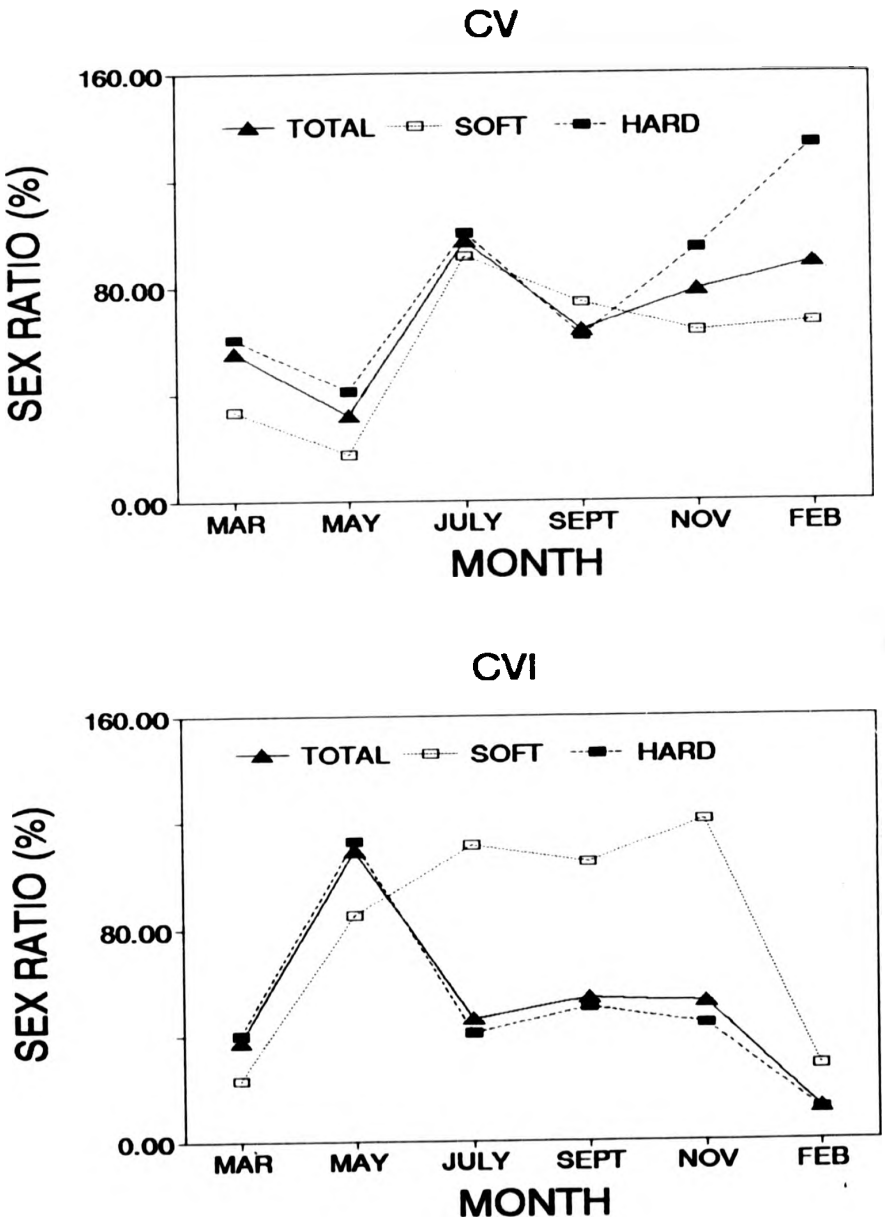

Fig. 7.2. *Pleuromamma robusta*. Seasonal fluctuations in sex ratios of copepodid stages V and VI.

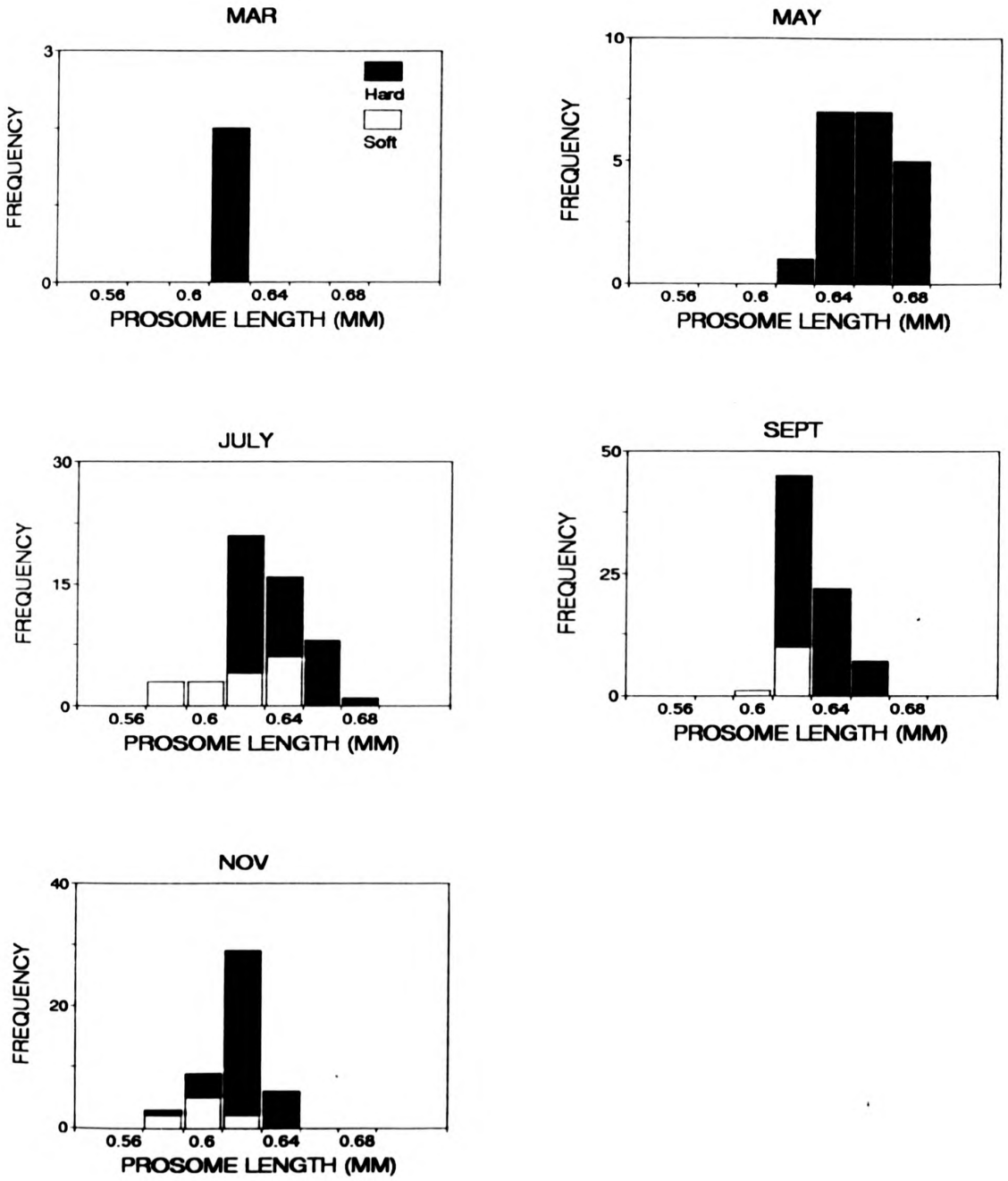

Fig. 7.3. Changes in the length-frequency distributions of CII of *Pleuromamma robusta*.

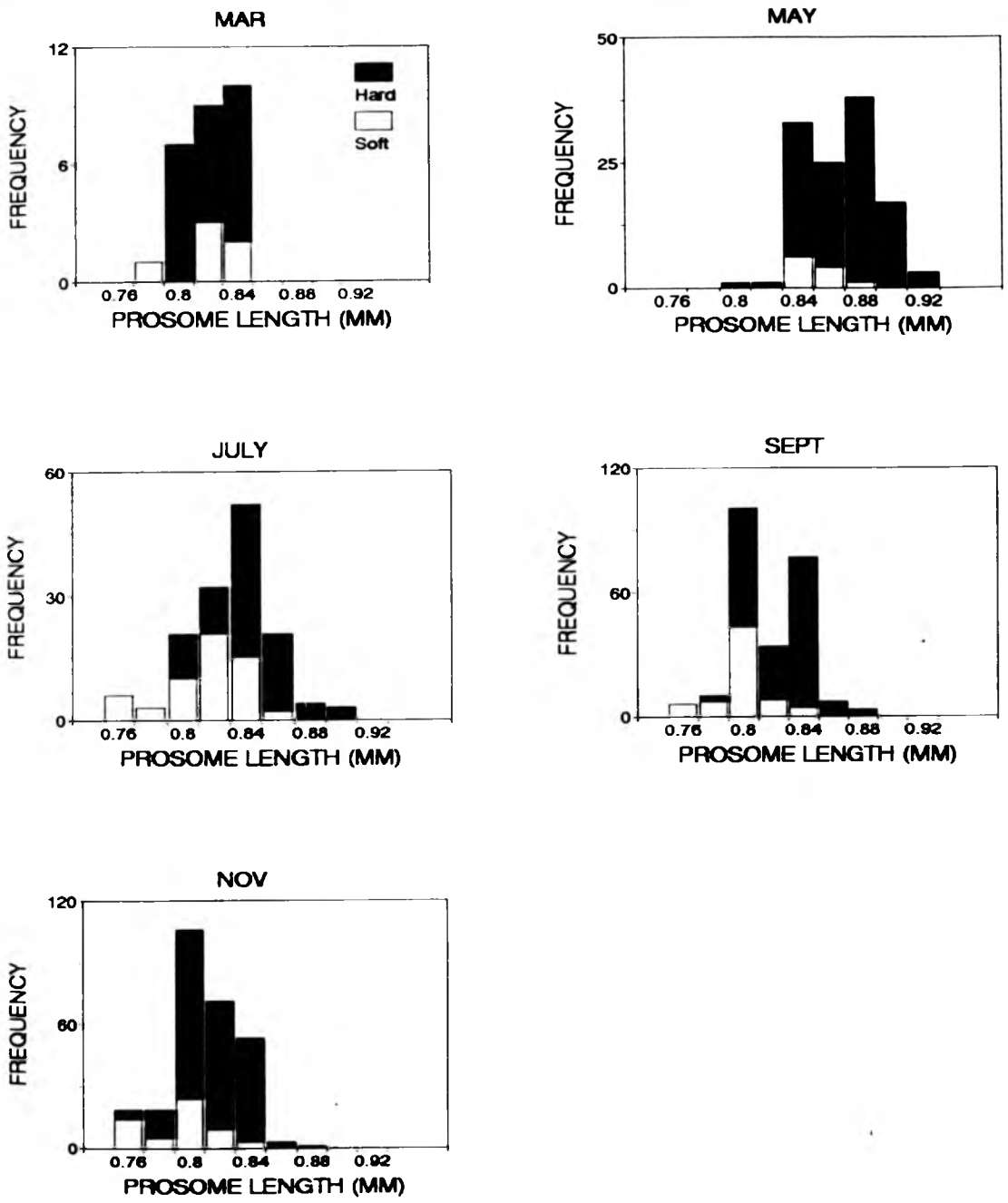

Fig. 7.4. Changes in the length-frequency distributions of CIII of *Pleuromamma robusta*.

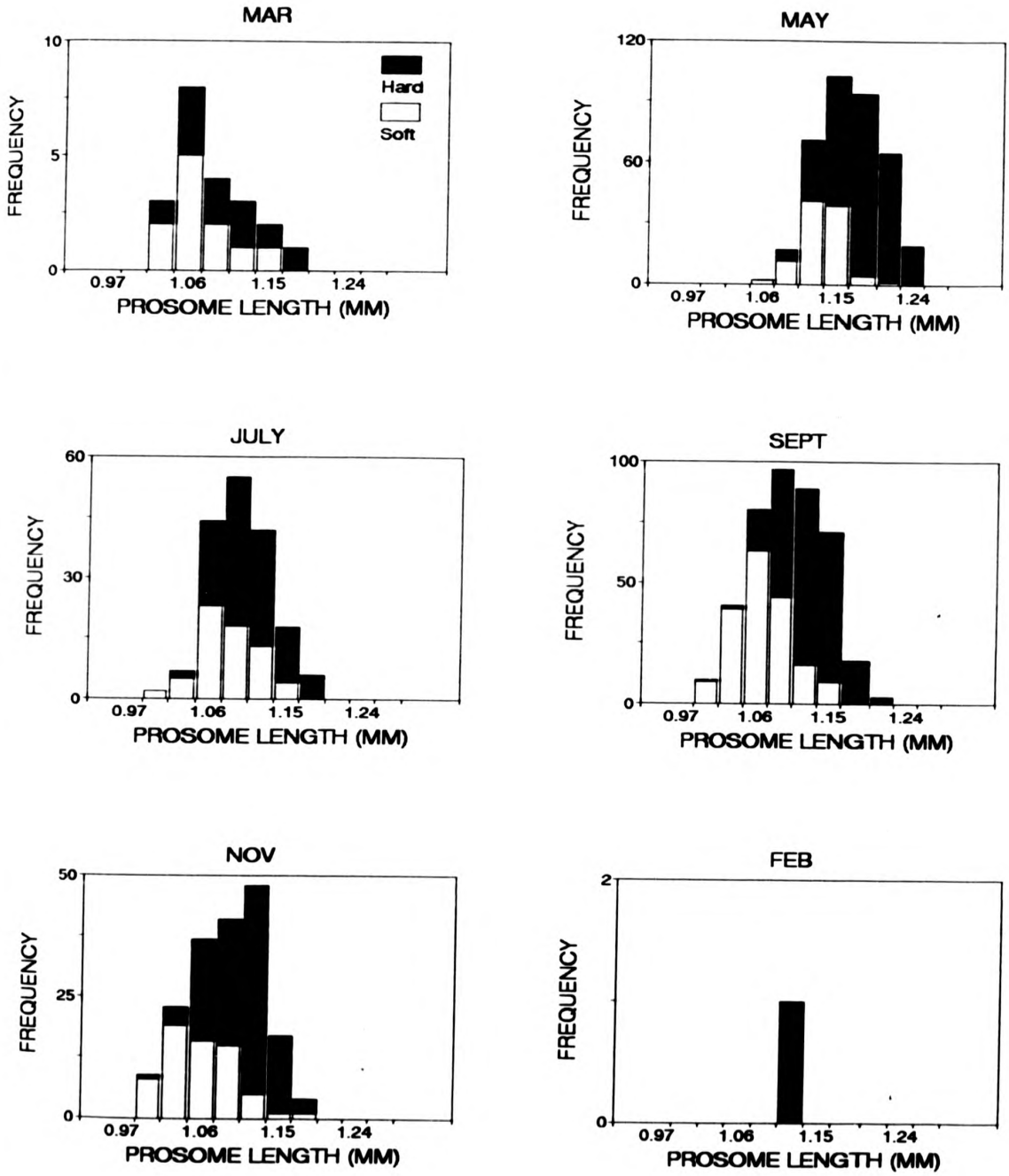

Fig. 7.5. Changes in the length-frequency distributions of CIV of *Pleuromamma robusta*.

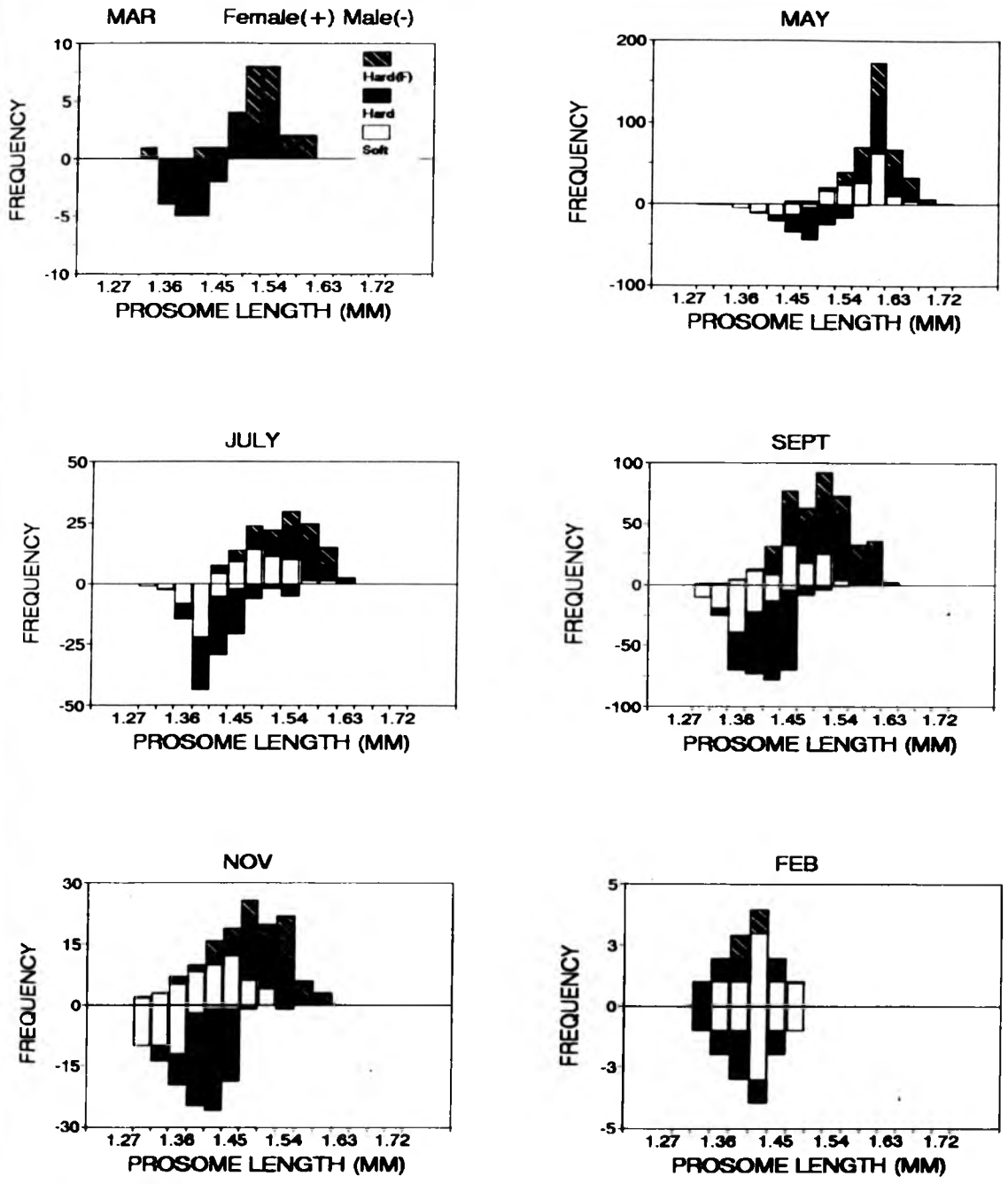

Fig. 7.6. Changes in the length-frequency distributions of CV males and females of *Pleuromamma robusta*.

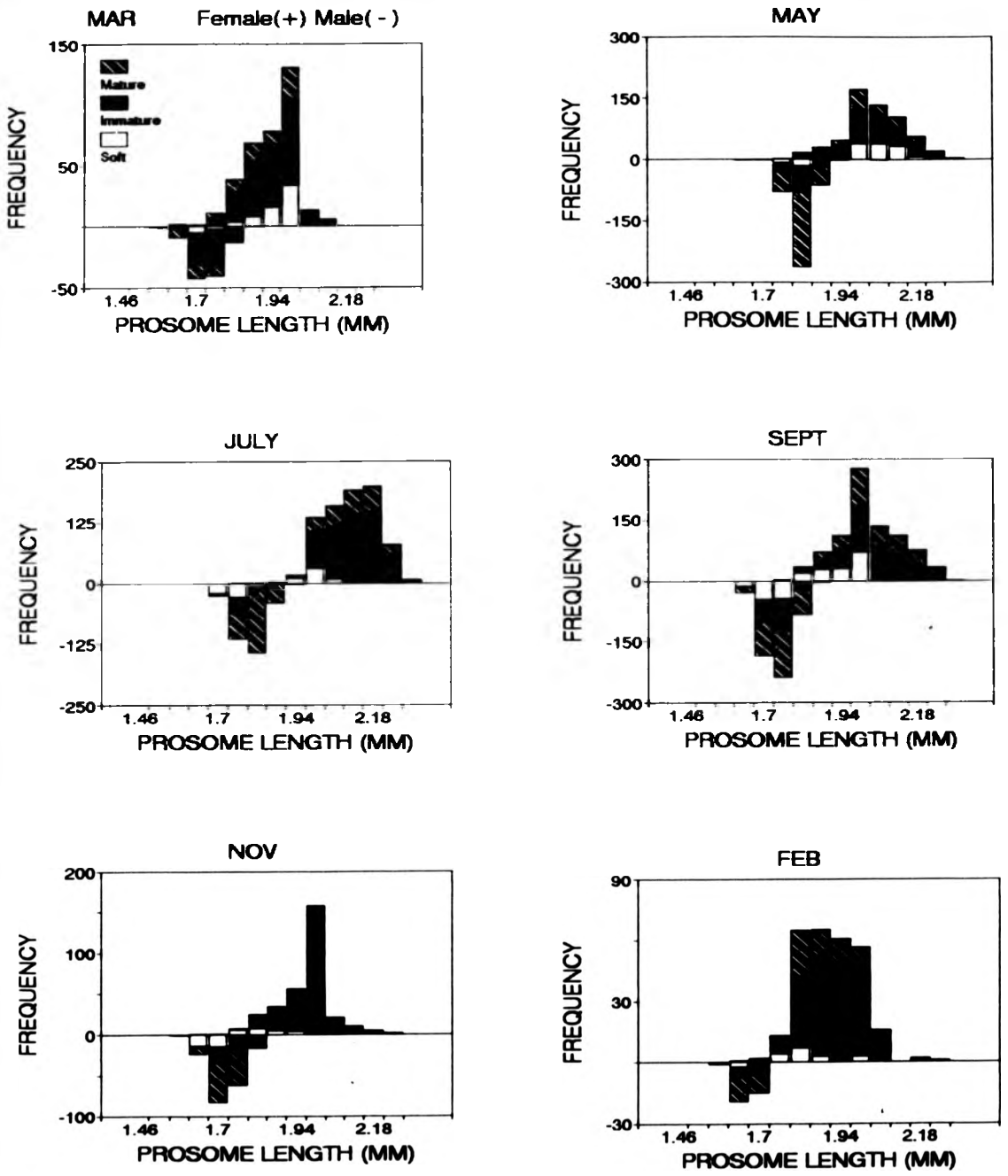

Fig. 7.7. Changes in the length-frequency distributions of CVI males and females of *Pleuromamma robusta*.

CHAPTER 8:

Vertical distribution of calanoid genus *Pleuromamma*

8.1. Introduction

Species of the calanoid copepod genus *Pleuromamma* are common from boreal to tropical latitudes of most oceanic regions and are often numerically dominant in some zooplankton communities (Steuer 1932, Roe 1972b, Deevey and Brooks 1977, Hayward and McGowan 1979, Hopkins 1982). Since all species are marked diel migrators, the day and night vertical distributions of the species, except for *P. indica*, have been described in various locations of the world's oceans (Moore and O'Berry 1957, Roe 1972a, b, 1984, Hure and Scotto di Carlo 1974, Hayward 1980, Sameoto 1986, Wisher and Allison 1986, Ambler and Miller 1987, Haury 1988, Bennett and Hopkins 1989, Hattori 1989, Weikert and Trinkaus 1990).

Daytime vertical distributions of the *Pleuromamma* species show a size-related pattern. The small species (< 2mm) such as *P. gracilis*, *P. piseki* and *P. borealis* are mainly found between 200 and 400 m. The large species (> 2.5 mm), *P. xiphias*, *P. abdominalis*, *P. scutullata*, *P. quadrungulata* and *P. robusta*, are distributed below 300 m. All species migrate vertically into near the surface layers at night and nighttime distributions are overlapped between co-occurring congeners at the same locations. The size-related pattern of vertical distribution is also seen within the species in that successively older stages occur increasingly deeper in the water column (Longhurst and Williams 1979, Ambler and Miller 1987, Bennett and Hopkins 1989).

As in the majority of calanoid copepods, there are six naupliar stages followed by six copepodid stages, the last of which is the adult. Copepodid stage is determined by the number of moults. Similarly, individuals of each life stage can be subdivided based on the intermoult cycle. The intermoult cycle of calanoid copepods is mainly divided into the postmoult, intermoult and premoult stage (see chapter 6). It is possible that individuals at different stages of the intermoult cycle have different patterns of vertical distribution and

migratory behaviour, as reflected in an ontogenetic vertical distribution.

The intermoult cycle is related to the degree of gonad development of adult copepods and gonad maturity is obtained in the intermoult and premoult stage (Ferrari and Hayek 1990, the previous chapter). The previous chapter showed that, in *Pleuromamma robusta*, adult females with a spermatophore attached to the genital opening always have a soft exoskeleton and undeveloped internal tissue; this is the postmoult stage. The previous chapter also showed that there was no significant relationship between seasonal variations in gonad maturity between adult males and females, nor between seasonal fluctuations in the sex ratio and gonad maturity in the populations of adults. The magnitude of the fluctuations in abundance of adult males was closely related to those of the copepodid V females throughout the year. It was, therefore, suggested that the timing of transference of the spermatophores from the male to the female is just after moulting of the copepodid V females.

Hayward (1981) found that reproductively mature adult males of *Pleuromamma piseki* migrated to slightly shallower depths at night than those in immature condition. Although the degree of gonad maturity of adult females was not examined, he suggested that the diel vertical distribution and migration pattern is somehow related to mating behaviour. Vertical aggregation of mature adult males could increase their chances of finding a mate.

In the present study, different stages of the intermoult cycle were identified throughout copepodid stages II to adult of *Pleuromamma robusta* to see whether the extent of the vertical distribution of individuals of each copepodid stage is related to the intermoult cycle. In addition, the degree of the gonad development was examined in copepodid V females and adult males and females to see whether the individual's vertical distribution is related to its stage of gonad development.

8.2. Materials and methods

Field sampling

Vertical stratified zooplankton samples were collected from 31 August to 3 September centred on 54°30'N, 13 °W in the Rockall Trough, northeastern Atlantic Ocean as detailed by Hargreaves *et al.* (1984), at the Institute of Oceanographic Sciences' Stn 10105 using a Multiple Rectangular Midwater Trawl of mouth area 1 m^2 and a mesh size of 0.33 mm (Roe and Shale 1979, Roe *et al.* 1980, Hargreaves 1984, 1985). The samples were collected from 100 m depth strata between the surface and 900 m depth by both day and night to examine the diel vertical migrations of species. Angel *et al.* (1982) concluded that there would be no influence of down-welling daylight at depth greater than 900 m and so samples were collected below 900 m to depths of 1900 m irrespective of time day or night. The net was opened and closed automatically, and the flow of water through the net were measured. More detailed sampling techniques were described by Baker *et al.* (1973) and Roe *et al.* (1984). Samples were fixed in a 5% formaldehyde-seawater solution buffered with hexamine and then transferred to Steedman's preserving fluid (Steedman 1976).

Identification of copepodid stages and sexes

Copepodid stages of the *Pleuromamma* species were identified by the number of urosomal segments and swimming legs (see Table 7.1). Sexes of copepodid V stage were identified by the shape and setal number on the fifth legs (see Ferrari 1985). The number of urosomal segments is five in adult males and three in adult females because of the fusion of the first two urosomal segments in females (see Fig. 4.6). Sexes of the adult stage were readily distinguished.

Copepodids of *P. xiphias*, *P. abdominalis*, and *P. robusta* were larger in body size than *P. gracilis*, *P. piseki* and *P. borealis* (see Table 3.1). Copepodids of *P. xiphias* were distinct from co-occurring congeners through possession of a process at the middle of the rostral base (see Ferrari 1985). Copepodids of *P. abdominalis* has more robust denticles on the proximal segments of the first antennules than those of *P. robusta*. In the small species of *P. gracilis*, *P. piseki* and *P. borealis*, adult males and copepodid stages V and IV were combined in single categories respectively because of difficulties in distinguishing them. Sexes of copepodid V stage of these three species were not identified.

The intermoult cycle and gonad maturity

In copepodid stages II to adult of *Pleuromamma robusta*, different stages of the intermoult cycle were identified by the hardness of the integument, or the body appearance, and were divided into "soft" and "hard" bodies (see Chapter 6). Here, the terms soft and hard bodies correspond with the postmoult and with the intermoult and premoult stage respectively and are used together throughout this study.

On the basis of the structure of the gonad system, adult males and females of *P. robusta* were classified into "immature" and "mature" condition (see chapter 7). Adult males and females in a post-reproductive condition were not identified and were included with those in an immature condition. Although the genital system of copepodid V females was not anatomically examined, they can be classified by the presence and absence of a round structure over the ventral areas of the first and second urosomal segments, presumably corresponding with the spermathecae on the first urosomal somite of adult females. The ventral areas of the first two urosomal segments of copepodid V females were ventrally swollen, but the rounded structure was not formed; individuals were termed "immature". Copepodid V females with a well-formed, round structure were termed "mature".

8.3. Results

Pleuromamma robusta

Both adult females and males of *P. robusta* were most abundant at 400 to 500 m and were found, in reduced numbers, as deep as 1900 m during the day (Tables 8.1, 8.2). Adult females mainly occurred between 300 and 600 m (87%) by day; only three females were caught in the upper 300 m. At night, 66% of adult females migrated into the upper 200 m and the remaining portions were reasonably evenly distributed between 300 and 900 m at about 4% levels. From the work of Angel *et al.* (1982), it is assumed that the animals caught below 900 m during the day are still there at night because they do not perform a diel migration since there are no diel changes in light intensity at these depths. During the day, adult males (62%) were concentrated between 300 and 600 m, but a substantial fraction (36%) was also found between 600 and 1100 m. Although 17% of nighttime distributions of adult males performed a diel vertical migration at night, the majority (79%) were found between 600 and 900 m. To these must be added the non-migrating components of the male population below 900 m.

More than 90% of both copepodid V females and males of *P. robusta* occurred between 300 and 500 m and the "deep tail" down to 1300 m during the day (Tables 8.3, 8.4). At night, roughly 30% of the stocks of both sexes were found in each of the 10 to 200 m, 400 to 500 m, and 600 to 700 m layers respectively.

Copepodid stages IV, III, and II of *P. robusta* were distributed unimodally in the daytime (Tables 8.5-8.7). The night centre of distribution for the migrating population of these stages was at 100 to 200 m, shallower than that of copepodid V and adults. Copepodid IV was most abundant at 300 to 400 m (84%) during the day, but occurring as deep as 1300 m (Table 8.5). At night, 32% or more of copepodid IV were in 100 to 200 m,

400 to 500 m and 600 to 700 m respectively. About 80% of nighttime distributions of copepodid IV were found at 100 to 200 m, with some 20% reaching near the surface depths (10 to 100 m).

Almost 95% of copepodid III of *P. robusta* occurred between 200 and 400 m and with a "deep tail" down to 700 m by day (Table 8.6). At night, copepodid III mainly occurred at 200 to 300 m (33%) and 400 to 500 m (43%), similar to the previous stage. During the day, copepodid II was centred at 200 to 300 m (79%), occurring between 200 and 500 m (Table 8.7). At night, 88% of copepodid II was found between 300 and 500 m, with some 11% occurring at 100 to 200 m; none of them were found close to the surface (10 to 100 m).

The intermoult cycle and gonad maturity

Different stages of the intermoult cycle were identified as "soft (postmoult stage)" and "hard (intermoult and premoult stage)" bodies in copepodid stages II to adult of *P. robusta*. In addition, the degree of the gonad development was examined in copepodid V females and adult females and males and was divided into "immature" and "mature". More advanced stages of gonad maturity of these stages occurred in later stage of the intermoult cycle, i.e. the intermoult and premoult stage (hard bodies). None of soft bodies were in reproductively mature condition, while a considerable number of hard bodies was in immature condition (see also the previous chapter). Day-night comparisons of depth distribution of individuals at different stages of the intermoult cycle and/or different degree of gonad maturity for each copepodid stage are shown in Tables 8.1-8.7.

Soft and hard bodies of adult females of *P. robusta* showed a similar pattern in daytime distributions (Table 8.1). At night, more than 72% of adult females with hard bodies in both immature and mature condition migrated into the upper 200 m, while only 22% of

females with soft bodies moved into this zone. The majority of adult females with soft bodies (63%) were found between 700 and 900 m at night. In addition, out of 7967 adult females of *P. robusta* caught in both the day and night samples, three specimens with a spermatophore attached to the genital opening were found, one specimen in each of the following depth layers: at 600 to 700 m and 900 to 1100 m layer by day, and 800 to 900 m layer at night. Each of these females with a spermatophore had soft bodies. Adult females always contained the spermatophoric contents in the spermathecae. Only three females had empty spermathecae, one specimen each at 600 and 700 m and 1100 and 1300 m in the daytime and one specimen at 700 to 800 m at night respectively. Each of these females had soft bodies.

In adult males of *P. robusta*, 77% of soft bodies, 61% of immature hard bodies, 44% of mature hard bodies occurred at 400 and 500 m during the day (Table 8.2). More than half (54%) of adult males in mature condition were found between 500 and 1100 m. The migrating portions of nighttime distributions, occurring in the upper 200 m, were 34% for soft bodies, 23% for immature hard bodies, and 14% for mature hard bodies; 58% of soft bodies, 67% of immature hard bodies, and 85% of mature hard bodies were found below 600 m. There was no clear relationship between the day and night depth distributions of adult females and males in reproductively mature condition. The nighttime distributions of adult females with soft bodies were close to those of mature adult males.

About 90% of immature copepodid V females of *P. robusta* occurred between 300 and 500 m during the day, while nearly all of those in reproductively mature condition were found below 500 m (Table 8.3). At night, 68% of soft bodies and 38% of immature hard bodies migrated into the upper 200 m. None of mature copepodid V females were found in the upper 400 m and 99% occurred between 600 and 900 m. Mature copepodid V females, therefore, were different from those in immature condition in both day and night distributions, close to those of mature adult males and the nighttime distributions of adult

females with soft bodies (Tables 8.1-8.3). The degree of gonad development of copepodid V males was not examined in this study. The daytime distributions of copepodid V males with soft and hard bodies were similar (Table 8.4). At night, however, copepodid V males with soft bodies showed more active migration (63%) than hard bodies (24%).

The daytime distribution peak of copepodid IV with soft (98%) and hard bodies (74%) was at 300 to 400 m and about 40% of soft and hard bodies of nighttime distributions migrated into the upper 200 m (Table 8.5). There is no clear difference in day and night distributions between soft and hard bodies of copepodid II and III of *P. robusta* (Tables 8.6, 8.7).

Species difference

Six *Pleuromamma* species occur in the north Atlantic ocean; *P. robusta*, *P. xiphias*, *P. abdominalis*, *P. gracilis*, *P. piseki*, and *P. borealis* (Steuer 1932). In the present study area of 54°N, 12°W, *P. robusta* was the dominant species and the other congeners were rare. Contrary to present results, Roe (1972a, b) found that *P. robusta* was the scarce species at 30°N, 15°W and the other congeners were common. Among the six species, *P. robusta* seems to be the most northern species in its geographical distribution.

In the present study, adult females of *P. xiphias* were common between 400 and 600 m, adult males at 500 to 600 m, copepodid V females at 300 to 400 m, copepodid V males at 400 to 500 m, and copepodid stages IV and III at 200 to 300 m during the day (Tables 8.8-8.10). Adult females of *P. abdominalis* were common at 300 to 400 m, adult males between 300 and 500 m, copepodid V females and males and copepodid IV at 300 to 400 m in the daytime (Tables 8.11, 8.12). Adult females of *P. gracilis*, *P. piseki* and *P. borealis* were centred at 200 to 300 m by day (Table 8.13). Combined adult males of these three species mainly occurred between 200 and 400 m and copepodid stages V and IV

were common at 200 to 300 m in the daytime (Tables 8.13, 8.14). Daytime distributions of *P. xiphias* and *P. abdominalis* were deeper than those of *P. gracilis*, *P. piseki* and *P. borealis* (Tables 8.1-8.14), as had previously been studied (e.g. Moore and O'Berry 1957, Roe 1972b, 1984, Wisher and Allison 1986, Ambler and Miller 1987, Haury 1988, Bennett and Hopkins 1989, Weikert and Trinkaus 1990).

8.4. Discussion

This study is primarily concerned with the day and night vertical distribution of *Pleuromamma robusta* in relation to its intermoult cycle. The vertical distribution of this species presented here is not new, having been previously studied by Roe (1972a, b, 1984), Longhurst and Williams (1979) and Pipe and Coombs (1980), although previous studies tended either to consider only the adult, often regardless of sex, or to combine all copepodid stages together.

The vertical distribution of *P. robusta* was size-related (Tables 8.1-8.7). The peak and range of daytime distributions of individual copepodid stages increased with increasing age of copepodids. Nighttime distributions of the migrating population of all copepodid stages heavily overlapped in the upper 200 m. The night centre of distribution for the migrating population of copepodid stages II to IV was shallower (10 to 100 m) than that of copepodid V and adults (100 to 200 m). Present results of the ontogenetic distribution pattern of *P. robusta* agree with previous studies: in copepodid stages I to adult of this species by Longhurst and Williams (1979); in copepodid stages III or IV to adult of *P. gracilis*, *P. piseki*, *P. abdominalis* and *P. xiphias* by Ambler and Miller (1987) and Bennett and Hopkins (1989).

In *P. robusta*, no significant difference was found between day and night vertical distributions of individuals of each of copepodid stages II to IV at different stages of the

intermoult cycle, but differences exist in copepodid V and adults (Tables 8.1-8.7). The intermoult cycle is related to the degree of the gonad development of copepodid V females and adult females and males. Gonad maturity of copepodid V males was not examined. Since the classification of the intermoult cycle depends on the time elapsed after moulting, mature copepodids with hard bodies are deemed to be at an older stage than immature ones.

In copepodid V females and males and adult males of *P. robusta*, the older stage of the intermoult cycle lived deeper than earlier stages in both day and night distributions and the migrating portions of nighttime distributions decreased with successively older stages of the intermoult cycle (Table 8.2-8.4). None of mature copepodid V females were found in the upper 300 m in both day and night. Adult females with soft and hard bodies showed a similar pattern of daytime distributions, but differed in nighttime distributions (Table 8.1). Night distributions of adult females with soft bodies were close to those of adult males in reproductively mature condition. Day and night distributions of adult males in mature condition were similar to those of mature copepodid V females.

In the previous chapter, adult females of *P. robusta* always contained the spermatophoric content in the spermathecae. Among 5973 adult females, two specimens had an attached spermatophore and one specimen had empty spermathecae. All of these had soft bodies. It was, therefore, suggested that adult males transfer their spermatophore to females just after moulting of copepodid V females and that adult females retain the spermatophoric content until fertilization. This means that the timing of transfer of the spermatophore from the male to the female is related to the moulting cycle of copepodid V females. In the previous study, in fact, the magnitude of the abundance fluctuations of adult males was close to that of copepodid V females throughout the year (see Table 7.3). There was no significant relationship between seasonal variations in gonad maturity between adult males and females, nor between seasonal fluctuations in the sex ratio and

gonad maturity of adult males and females (see Table 7.3, Fig. 7.1).

Similarly, in the present study, out of 7967 adult females of *P. robusta*, three specimens had a spermatophore attached to the genital opening and empty spermathecae respectively. All of these six had soft bodies and were found below 600 m in both day and night. Notably, mature copepodid V females, adult females with soft bodies, and mature adult males showed similar vertical distributions (Tables 8.1-8.3). These stages mainly occurred below 600 m in both day and night, but adult females by day only. This correspondence could be related to mating behaviour. Hayward (1981) found that in the sibling species, *P. piseki*, adult males in reproductively mature condition migrated to slightly shallower nighttime levels than immature ones and were aggregated in the 0 to 25 m layer. Different migration patterns of mature adult males between *P. robusta* and *P. piseki* may be related to different mating behaviour.

Paffenhöfer (1983) suggested that the main factor controlling vertical distribution of copepods is to find enough food. Hattori (1989) showed that in *Pleuromamma scutullata* adult females take food during the night but feeding activity of females was similar between surface migrators and non-migrators at deeper levels. In fact, the *Pleuromamma* species are omnivores, so their diel migration does not entirely depend on food. The present study shows that mating behaviour of *P. robusta* is not limited to adults, but extends to copepodid V females. Their vertical separation, or aggregation could decrease their effort and cost required to find a mate. Within the same stage, the migration population which is mostly not in immediate copulatory condition could be sufficient to prevent competitive exclusion and to optimize its use of resources.

Since all copepodid stages of *P. robusta* were distributed below 200 m during the day and migrated into the upper 200 m at night, upward migration was easily recognized (Tables 8.1-8.7). The migrating distance of each copepodid stage can be roughly estimated by comparisons between daytime distribution peaks and night centres of distribution for the

migrating population; 300 or 400 m for adults, 200 to 300 m for copepodid V and IV, and 100 to 200 m for copepodid III and II. Throughout the water column, changes in day and night depths of the major portions of the population do not deviate from this estimated migration distance. This distance could reflect the swimming ability of each copepodid stage.

Diel vertical migrations of the *Pleuromamma* species performs between sunset (Hure and Scotto di Carlo 1974, Roe 1984, Hattori 1989). In adults *P. scutullata*, upward swimming speeds were 15.9 to 46.5 m h^{-1} for females and 12.2 to 48.9 m h^{-1} for males, and downward swimming speeds were 48.6 m h^{-1} for females and 40.5 n h^{-1} (Hattori 1989). The average swimming speeds of *P. gracilis* and *P. xiphias* was 48.2 m h^{-1} and 52.9 m h^{-1} (Buskey and Swift, unpublished data, presumably adults but sexes are not clear). The distance between the mean daytime distribution peak and the night centre of distribution of the upward migrating population of adults was 250 to 400 m for *P. scutullata* (Hattori 1989), 250 to 350 m for *P. gracilis* and 400 to 500 for *P. xiphias* (Ambler and Miller 1987, Haury 1988, Bennett and Hopkins 1989). These swimming speeds would allow them to cover the distance between mean daytime and nighttime distribution peaks in less than 8 h, which agrees with the migration distance. If so, 50 m depth is enough distance to make niche separation between different life stages. Current sampling intervals of 50 or 100 m are too wide to detect small-scale partitioning of species or within species. In the present study, day estimates of abundance exceed those for night which could be influenced by the sampling time of day because of diel vertical migratory behaviour, in addition to sampling variation and patchness.

Table 8.1. *Pleuromamma robusta*. Vertical distribution of adult females, showing the numbers of individuals with soft (postmoult stage) and hard (intermoult and premoult stage) bodies and at different status of gonad development. Numbers per 1000 m^3 water filtered in parentheses. At night, deeper samples were not available and are designated by (-).

Depth (m)	Day				Night			
	Immature soft bodies	Immature hard bodies	Mature hard bodies	Total	Immature soft bodies	Immature hard bodies	Mature hard bodies	Total
10-100	0	1	0	1 (0.4)	28	617	242	887 (343.8)
100-200	0	0	1	1 (0.4)	46	497	139	682 (262.3)
200-300	0	1	0	1 (0.4)	7	61	27	95 (44.8)
300-400	36	212	165	413 (191.2)	6	64	18	88 (34.1)
400-500	526	1935	1410	3871 (1500.4)	0	104	22	136 (53.3)
500-600	51	331	207	589 (227.4)	3	67	11	81 (31.3)
600-700	18	75	32	125 (49.0)	23	59	11	93 (36.2)
700-800	16	72	48	136 (52.9)	129	38	15	182 (45.2)
800-900	4	43	18	65 (25.2)	83	40	7	130 (50.8)
900-1100	34	128	38	200 (93.0)	-	-	-	-
1100-1300	7	60	22	89 (17.3)	-	-	-	-
1300-1500	3	31	17	51 (9.9)	-	-	-	-
1500-1700	3	20	6	29 (5.6)	-	-	-	-
1700-1900	2	18	2	22 (4.3)	-	-	-	-
Grand Total	700	2927	1966	5593	335	1547	492	2374

Table 8.2. *Pleuromamma robusta*. Vertical distribution of adult males, showing the numbers of individuals with soft (postmoult stage) and hard (intermoult and premoult stage) bodies and at different status of gonad development. Numbers per 1000 m^3 water filtered in parentheses. At night, deeper samples were not available and are designated by (-).

Depth (m)	Day				Night			
	Immature soft bodies	Immature hard bodies	Mature hard bodies	Total	Immature soft bodies	Immature hard bodies	Mature hard bodies	Total
10-100	0	0	0	0	20	30	88	138 (53.5)
100-200	0	0	0	0	16	43	67	126 (48.7)
200-300	0	0	0	0	5	3	3	11(5.2)
300-400	36	51	31	118 (54.6)	0	3	2	5 (1.9)
400-500	458	465	1013	1936 (750.4)	5	12	10	27 (10 6)
500-600	11	40	183	234 (90.4)	0	16	2	18 (7.0)
600-700	15	68	249	332 (130.2)	36	81	124	241 (93.8)
700-800	21	19	196	236 (91.8)	17	25	531	557 (142.2)
800-900	42	67	348	457 (177.1)	6	110	314	430 (168.0)
900-1100	11	40	263	314 (146.1)	-	-	-	-
1100-1300	1	2	6	9 (1.8)	-	-	-	-
1300-1500	1	5	6	12 (2.3)	-	-	-	-
1500-1700	0	0	2	2 (0.4)	-	-	-	-
1700-1900	1	2	2	5 (1.0)	-	-	-	-
Grand Total	597	759	2299	3655	105	323	1141	1553

Table 8.3. *Pleuromamma robusta*. Vertical distribution of CV females, showing the numbers of individuals with soft (postmoult stage) and hard (intermoult and premoult stage) bodies and at different status of gonad development. Numbers per 1000 m^3 water filtered in parentheses. At night, deeper samples were not available and are designated by (-).

Depth (m)	Day				Night			
	Immature soft bodies	Immature hard bodies	Mature hard bodies	Total	Immature soft bodies	Immature hard bodies	Mature hard bodies	Total
10-100	0	0	0	0	26	66	0	92 (35.7)
100-200	0	0	0	0	45	60	0	105 (40.5)
200-300	0	0	0	0	1	0	0	1 (0.5)
300-400	1084	1188	2	2274 (1052.8)	0	22	0	22 (8.5)
400-500	220	979	4	1203 (466.3)	8	133	1	142 (55.7)
500-600	4	35	61	100 (38.6)	2	14	2	18 (7.0)
600-700	12	50	60	122 (47.8)	19	21	65	105 (40.9)
700-800	0	3	59	62 (24.1)	1	6	106	113 (28.0)
800-900	0	2	32	34 (13.2)	2	6	20	28 (10.9)
900-1100	1	7	27	35 (16.3)	-	-	-	-
1100-1300	4	0	0	4 (0.8)	-	-	-	-
1300-1500	0	0	0	0	-	-	-	-
1500-1700	0	0	0	0	-	-	-	-
1700-1900	0	0	0	0	-	-	-	-
Grand Total	1325	2264	245	3834	104	328	194	626

Table 8.4. *Pleuromamma robusta*. Vertical distribution of CV males, showing the numbers of individuals with soft (postmoult stage) and hard (intermoult and premoult stage) bodies. Numbers per 1000 m^3 water filtered in parentheses. At night, deeper samples were not available and are designated by (-).

	Day			Night		
Depth (M)	Soft bodies	Hard bodies	Total	Soft bodies	Hard bodies	Total
10-100	0	0	0	13	39	52 (20.2)
100-200	0	0	0	34	33	67 (25.9)
200-300	1	1	2 (0.8)	3	1	4 (1.9)
300-400	475	769	1244 (575.9)	5	24	29 (11.2)
400-500	43	307	350 (135.7)	4	90	94 (36.9)
500-600	2	37	39 (15.1)	1	8	9 (3.5)
600-700	3	78	81 (31.8)	15	97	112 (43.6)
700-800	0	13	13 (5.1)	0	6	6 (1.5)
800-900	0	0	0	0	2	2 (0.8)
900-1100	0	4	4 (1.9)	-	-	-
1100-1300	0	0	0	-	-	-
1300-1500	0	0	0	-	-	-
1500-1700	0	0	0	-	-	-
1700-1900	0	0	0	-	-	-
Grand Total	**524**	**1209**	**1733**	**75**	**300**	**375**

Table 8.5. *Pleuromamma robusta*. Vertical distribution of CIV, showing the numbers of individuals with soft (postmoult stage) and hard (intermoult and premoult stage) bodies. Numbers per 1000 m^3 water filtered in parentheses. At night, deeper samples were not available and are designated by (-).

	Day			Night		
Depth (M)	Soft bodies	Hard bodies	Total	Soft bodies	Hard bodies	Total
0-100	0	0	0	10	54	64 (24.8)
100-200	0	0	0	62	162	224 (86.5)
200-300	1	1	2 (0.77)	0	4	4 (1.9)
300-400	602	672	1274 (589.8)	1	43	44 (17.1)
400-500	2	117	119 (46.1)	82	151	233 (91.4)
500-600	1	10	11 (4.3)	3	9	12 (4.6)
600-700	5	103	108 (42.4)	2	100	102 (39.7)
700-800	0	4	4 (1.6)	0	4	4 (1.0)
800-900	0	0	0	0	0	0
900-1100	0	2	2 (0.9)	-	-	-
1100-1300	4	1	5 (1.0)	-	-	-
1300-1500	0	0	0	-	-	-
1500-1700	1	0	1 (0.2)	-	-	-
1700-1900	0	0	0	-	-	-
Grand Total	616	910	1526	160	527	687

Table 8.6. *Pleuromamma robusta*. Vertical distribution of CIII, showing the numbers of individuals with soft (postmoult stage) and hard (intermoult and premoult stage) bodies. Numbers per 1000 m^3 water filtered in parentheses. At night, deeper samples were not available and are designated by (-).

	Day			Night		
Depth (m)	Soft bodies	Hard bodies	Total	Soft bodies	Hard bodies	Total
10-100	0	0	0	3	8	11 (4.3)
100-200	0	0	0	25	115	140 (54.1)
200-300	76	400	476 (183.1)	0	5	5 (2.4)
300-400	57	202	259 (119.9)	4	65	69 (26.7)
400-500	0	34	34 (13.2)	48	138	186 (74.4)
500-600	0	3	3 (1.6)	1	9	10 (3.9)
600-700	3	7	10 (3.9)	0	10	10 (3.9)
700-800	0	0	0	0	0	0
800-900	0	0	0	0	0	0
900-1100	0	0	0	-	-	-
1100-1300	0	0	0	-	-	-
1300-1500	0	0	0	-	-	-
1500-1700	0	0	0	-	-	-
1700-1900	0	0	0	-	-	-
Grand Total	136	646	782	81	350	431

Table 8.7. *Pleuromamma robusta*. Vertical distribution of CII, showing the numbers of individuals with soft (postmoult stage) and hard (intermoult and premoult stage) bodies. Numbers per 1000 m^3 water filtered in parentheses. At night, deeper samples were not available and are designated by (-).

	Day			Night		
Depth (m)	Soft bodies	Hard bodies	Total	Soft bodies	Hard bodies	Total
10-100	0	0	0	0	0	0
100-200	0	0	0	3	11	14 (5.4)
200-300	21	81	102 (39.2)	0	0	0
300-400	2	16	18 (8.3)	0	58	58 (22.5)
400-500	0	9	9 (3.5)	5	54	59 (23.1)
500-600	0	0	0	2	0	2 (0.8)
600-700	0	0	0	0	0	0
700-800	0	0	0	0	0	0
800-900	0	0	0	0	0	0
900-1100	0	0	0	-	-	-
1100-1300	0	0	0	-	-	-
1300-1500	0	0	0	-	-	-
1500-1700	0	0	0	-	-	-
1700-1900	0	0	0	-	-	-
Grand Total	23	106	129	10	123	133

Table 8.8. *Pleuromamma xiphias*. Vertical distribution of adult females and males. Numbers per 1000 m^3 water filtered in parentheses. At night, deeper samples were not available and are designated by (-).

	Females		Males	
Depth (m)	Day	Night	Day	Night
10-100	0	7 (2.7)	0	5 (1.9)
100-200	0	4 (1.5)	0	4 (1.5)
200-300	0	0	0	0
300-400	0	1 (0.4)	1 (0.5)	1 (0.4)
400-500	15 (5.8)	0	4 (1.6)	0
500-600	16 (6.2)	2 (0.8)	12 (4.6)	1 (0.4)
600-700	3 (1.2)	4 (1.6)	2 (0.8)	3 (1.2)
700-800	0	12 (3.0)	1 (0.4)	7 (1.7)
800-900	1 (0.4)	0	0	2 (0.8)
900-1100	1 (0.5)	-	0	-
1100-1300	0	-	0	-
1300-1500	0	-	0	-
1500-1700	2 (0.4)	-	0	-
1700-1900	1 (0.2)	-	0	-
Total	39	30	20	23

Table 8.9. *Pleuromamma xiphias*. Vertical distribution of CV females and males. Numbers per 1000 m^3 water filtered in parentheses. At night, deeper samples were not available and are designated by (-).

	Females		Males	
Depth (m)	Day	Night	Day	Night
10-100	0	0	0	1 (0.4)
100-200	0	2 (0.8)	0	0
200-300	0	0	0	0
300-400	40 (18.5)	0	7 (3.2)	2 (0.8)
400-500	12 (4.7)	2 (0.8)	24 (9.3)	2 (0.8)
500-600	3 (1.2)	0	2 (0.8)	1 (0.4)
600-700	3 (1.2)	0	0	3 (1.2)
700-800	3 (1.2)	1 (0.3)	5 (2.0)	5 (1.2)
800-900	0	0	0	1 (0.4)
900-1100	0	-	1 (0.5)	-
1100-1300	1 (0.2)	-	0	-
1300-1500	0	-	0	-
1500-1700	1 (0.2)	-	0	-
1700-1900	1 (0.2)	-	1 (0.2)	-
Total	64	5	40	15

Table 8.10. *Pleuromamma xiphias*. Vertical distribution of CIV and CIII. Numbers per 1000 m^3 water filtered in parentheses. At night, deeper samples were not available and are designated by (-).

	CIV		CV	
Depth (m)	Day	Night	Day	Night
10-100	0	8 (3.1)	0	0
100-200	0	21 (8.1)	0	0
200-300	70 (26.9)	0	34 (13.1)	0
300-400	22 (10.2)	8 (3.1)	9 (4.2)	11 (4.3)
400-500	17 (6.6)	10 (3.9)	0	0
500-600	0	0	0	0
600-700	2 (0.8)	3 (1.2)	0	0
700-800	0	0	0	0
800-900	0	0	0	0
900-1100	0	-	0	-
1100-1300	0	-	0	-
1300-1500	0	-	0	-
1500-1700	0	-	0	-
1700-1900	0	-	0	-
Total	111	50	43	11

Table 8.11. *Pleuromamma abdominalis*. Vertical distribution of adult females and males. Numbers per 1000 m^3 water filtered in parentheses. At night, deeper samples were not available and are designated by (-).

	Females		Males	
Depth (m)	Day	Night	Day	Night
10-100	0	2 (0.8)	0	4 (1.6)
100-200	0	0	0	0
200-300	0	0	0	2 (0.8)
300-400	56 (25.9)	1 (0.4)	31 (14.4)	1 (0.4)
400-500	11 (4.3)	0	46 (17.8)	0
500-600	2 (0.8)	0	2 (0.8)	0
600-700	1 (0.4)	0	0	0
700-800	0	0	2 (0.8)	0
800-900	0	2 (0.8)	1 (0.4)	0
900-1100	2 (0.9)	-	0	-
1100-1300	2 (0.4)	-	2 (0.4)	-
1300-1500	0	-	0	-
1500-1700	2 (0.4)	-	0	-
1700-1900	1 (0.2)	-	0	-
Total	77	5	84	7

Table 8.12. *Pleuromamma abdominalis*. Vertical distribution of CV females and males and CIV. Numbers per 1000 m^3 water filtered in parentheses. At night, deeper samples were not available and are designated by (-).

	CV females		CV males		CIV	
Depth (m)	Day	Night	Day	Night	Day	Night
10-100	0	1 (0.4)	0	0	0	0
100-200	0	0	0	0	0	0
200-300	0	6 (2.8)	0	5 (2.4)	7 (2.7)	6 (2.8)
300-400	39 (18.1)	9 (3.5)	17 (7.9)	6 (2.3)	14 (6.5)	0
400-500	18 (7.0)	1 (0.4)	2 (0.8)	1 (0.4)	2 (0.8)	3 (1.2)
500-600	0	0	0	0	0	2 (0.8)
600-700	0	0	0	0	0	2 (0.8)
700-800	1 (0.4)	0	1 (0.4)	0	0	2 (0.5)
800-900	0	0	0	0	0	0
900-1100	1 (0.5)	-	0	-	0	-
1100-1300	0	-	2 (0.4)	-	0	-
1300-1500	0	-	0	-	0	-
1500-1700	0	-	0	-	0	-
1700-1900	1 (0.2)	-	1 (0.2)	-	0	-
Total	60	17	23	12	23	15

Table 8.13. *Pleuromamma gracilis*, *P. piseki* and *P. borealis*. Vertical distribution of adult females. Numbers per 1000 m^3 water filtered in parentheses. At night, deeper samples were not available and are designated by (-).

	P. gracilis		*P. piseki*		*P. borealis*	
Depth (m)	Day	Night	Day	Night	Day	Night
10-100	0	5 (1.9)	1 (0.4)	6 (2.3)	0	1 (0.4)
100-200	0	0	0	2 (0.8)	0	0
200-300	18 (6.9)	0	206 (79.2)	0	8 (3.1)	0
300-400	2 (0.9)	0	11 (5.1)	0	3 (1.4)	0
400-500	0	0	0	5 (2.0)	0	0
500-600	0	0	0	0	0	0
600-700	0	0	4 (1.6)	2 (0.8)	0	0
700-800	1 (0.4)	0	0	3 (0.7)	0	0
800-900	0	0	0	2 (0.8)	0	0
900-1100	0	-	2 (0.9)	-	0	-
1100-1300	0	-	2 (0.4)	-	0	-
1300-1500	0	-	0	-	0	-
1500-1700	0	-	0	-	0	-
1700-1900	0	-	0	-	0	-
Total	21	5	226	20	11	1

Table 8.14. *Pleuromamma gracilis*, *P. piseki* and *P. borealis*. Vertical distribution of adult males, CV and CIV. Numbers per 1000 m^3 water filtered in parentheses. At night, deeper samples were not available and are designated by (-).

	adult males		CV		CIV	
Depth (m)	Day	Night	Day	Night	Day	Night
10-100	0	7 (2.7)	0	4 (1.6)	0	0
100-200	0	0	0	2 (0.8)	0	0
200-300	50 (19.2)	0	250 (96.2)	1 (0.5)	59 (22.7)	0
300-400	56 (25.9)	21 (8.1)	12 (5.6)	21 (8.1)	0	0
400-500	22 (8.5)	21 (8.2)	0	7 (2.8)	0	0
500-600	0	0	0	0	0	0
600-700	0	0	0	0	0	0
700-800	0	2 (0.5)	0	0	0	0
800-900	0	0	0	0	0	0
900-1100	0	-	0	-	0	0
1100-1300	0	-	0	-	0	0
1300-1500	0	-	0	-	0	0
1500-1700	0	-	0	-	0	0
1700-1900	0	-	0	-	0	0
Total	128	51	262	35	59	0

CHAPTER 9:

General Discussion

9.1. The integumental pore signature

The species pore signature of calanoid copepods has been used for around 20 years since the pioneer work of Fleminger (1973). Despite repeated mention of the taxonomic and phylogenetic usefulness of the species pore signature, there remained two conceptually important questions. First, where the species-specific information primarily resides in the integumental tagmata. Second, how significant is the within-sample variation and how many specimens are required to describe quantitatively the species pore signature, with respect to intraspecific variation. Different authors define the pore signature by considering pores that occur in 40, 50, 80, or 90% of individuals examined. The choice of the significant level of percentage occurrence has been relatively arbitrary. Likewise, the numbers of individuals used to determine the pore signature of a species has varied from about 2 to 100. Consequently, it has been important to assess the intraspecific variation properly in order to assess the value of the earlier work realistically.

The present study shows that most of the species-specific information is in the urosome of adult females, but a significant proportion also resides in the cephalosome and a lesser component in the metasome in eight *Pleuromamma* species (Tables 2.3-2.5). This study, however, points out that the exclusive use of the urosome in phylogenetic and geographical studies may not provide enough information in some genera because the urosomal signature contains a small number of pores and the greatest amount of the intraspecific variation. In eight *Pleuromamma* species, about 16% of the total number of pores of the species are in the urosome, 34% in the cephalosome and 50% in the metasome (Tables 2.2, 5.3). The extent of intraspecific variation is greater in the urosomal pores (11.6±9.2%) than in the cephalosomal (3.7±4.1%) and the metasomal (8.7±2.9%) pores. On the re-examination of Fleminger's (1973) data in 17 *Eucalanus* species, about 10% of total integumental pores reside in the urosome, 50% in the cephalosome and 40%

in the metasome. Intraspecific variation occurs in 23.0±19.8% of the urosomal pores, 12.4±12.8% of the cephalosomal pores, and 11.8±11.1% of the metasomal pores.

The present study shows that setting the level of occurrence of pores at 40, 50 or 60% does not cause significant changes in the species pore signature, even in the urosome (Tables 5.3, 5.4). In general, the degree of intraspecific variation increases with increasing number of the total integumental pores and is limited to 10% or less of the total (Table 5.2). The re-examination of published data on intraspecific variation in pore signatures, namely Fleminger (1973), Fleminger and Hulsemann (1977, 1987), and Hulsemann and Fleminger (1990), showed that intraspecific variation depends on the sample size (see chapter 5). Intraspecific variation in the pore signature within a species is, however, severely restricted. Samples as small as five individuals will identify more than 95% of potential sites for the pore signature of a species. Detection of geographical variation will require large samples as this component resides in the rare supernumerary pores occurring in less than 5% of individuals.

The species of *Pleuromamma* were divided into the four groups based on their pore signatures: 1) *P. xiphias* and *P. abdominalis*; 2) *P. scutullata* and *P. robusta*; 3) *P. indica*; 4) *P. gracilis*, *P. piseki* and *P. borealis* (see chapter 2). Specimens of *P. quadrungulata* were not available for pore signature studies, but might belong to the second group of species, *P. scutullata* and *P. robusta*, considering their morphology and asymmetry (see chapter 3). This division agrees with that derived from other morphological characters of the species (Table 3.1), as had previously been shown in 17 *Eucalanus* species by Fleminger (1973). This correspondence between changes in the pore signatures and conventional morphological characters in two different genera of calanoid copepods proves the general taxonomic usefulness of the pore signature in speciation studies. Changes in general morphological characters are often diffuse and their detection requires subjective decisions. The data from pore signatures are of the presence/absence type and avoid subjective

decisions. Care has to be taken in the preparation of the integuments for observations of the pores. They have to be cleaned and stained properly so that none of the pores present are overlooked. This requires practice and generally is not a source of errors in the data.

The pore signatures are casting a new light on the phylogeny of the *Pleuromamma* species which had been diffuse in studies of conventional morphology of this genus (Steuer 1932, Ferrari 1984) (Fig. 3.1). There is some correspondence between phylogenetic grouping of species and the areas of the integument having variable sites (Figs. 2.2-2.9, Table 5.2). Most of the intraspecific variation in the pore signatures occurs at the sites which show interspecific variation within the genus. Inter- and intraspecific variation, therefore, are an expression of the phylogenetic grouping of species, or generic similarities between the species within a genus, which is also evident in the ontogenetic development of the pore signature of the species (see chapter 4).

Interspecific variation in the pore signatures occurs as early as copepodid III in *Pleuromamma robusta* and *P. xiphias* (Figs. 4.1, 4.2). Interspecific difference in the pore signatures of the copepodids arise from two sources: 1) species-specific rates of development of the adult signature; 2) development of the species-specific components of the adult signature. Sexual differences in the pore signature are found in copepodid IV and are primarily evident in the urosomal signatures of adults in *P. robusta*, *P. xiphias*, *P. abdominalis*, and *P. gracilis* (Figs. 4.1-4.4). The species-specific components of the urosomal signature are greater in the female than in the male (Tables 4.2, 4.3).

When this project started it was planned to examine the pore signatures of the species of the other two metridinid genera, *Metridia* and *Gaussia*, for describing the generic and familial signature on the entire integument. It was expected that the pore signature of early copepodid stage would reflect the generic and familial signature. The familial signature would precede the generic pore signature, the species pore signature would appear later than the generic signature. The generic signature, however, is basically derived from the

adult species pore signature and the adult signature has peculiar pores that occur only in the adult stage. Although in the species of the same genus the pore signature of early copepodid stages shows some similarity, this similarity does not correspond with the generic or familial signature (Figs. 4.1-4.5). It was also difficult to describe, accurately, the generic signature of the genus *Metridia* because this genus includes at least 23 species, many not occurring in the Rockall Trough.

This study only examined the pore signature of *Pleuromamma* species. Various structural types of the integumental organs have been described in a number of calanoid copepods (Fleminger 1973, Mauchline 1977, Gill 1986, Othuka and Mitsuzumi 1990, Kurbjeweit and Buchholz 1991, Koomen 1992, Bannister 1993). These integumental organs are generally classified as either a hair, peg, and pit sensilla or gland opening. The three types of sensilla are composed of one to several sensory neurons and are generally accessory cells. The latter have the external features which may be in the form of an outgrowth such as the hair and the peg or an ingrowth such as the pit with sensory cell bodies located underneath (Laverack 1969, Schneider 1969, Struckow 1970). Some gland openings may constitute excretory ducts, others may be associated with various sense organs. These integumental organs are functionally chemoreceptors, mechanoreceptors and secretory glands, but their functions are for the greater part unknown. The development of the species pore signature is progressively increasing the number of integumental organs with increasing age, or developmental stages of the life history (Table 4.1, Fig. 4.1-4.5). This should be related to the increasing biological activity of copepods and to the requirement for increased sensory information, i.e. that involved in seeking mates.

Some calanoid copepods have luminescent organs on their body and appendages that produce bright bioluminescent displays. The most extensive study has been done using the species of the family Metridinidae, *Metridia*, *Gaussia* and *Pleuromamma* (David and Conover 1961, Clarke *et al.* 1962, Artiomkin *et al.* 1966, Rudjakov and Voronina 1967,

Barnes and Case 1972, Evstigneev 1983a, b). These genera have luminous glands variously distributed on the cephalosome, metasome, caudal furcae and some limbs. Evstigneev (1983b) examined 18 luminescent organs in *Pleuromamma gracilis* and *P. piseki* respectively and 15 in *P. abdominalis*. The location of these organs in the first two species is alike, but different from that of these organs in *P. abdominalis*. This difference also reflects the phylogenetic grouping of these species (see Fig. 3.1). In the present study, attempts were made to identify the accurate position of luminescent glands in the pore signatures of *P. robusta*. It was difficult to examine bioluminescence. Live individuals were sampled on two occasions in the Rockall Trough, some being transported to the Dunstaffnage Marine Laboratory. Luminescence of the copepods was observed but the accurate identification of its origins relative to the pore signature was not possible (see discussion section of chapter 2).

Barnes and Case (1972) showed that there is no indication of sexual dimorphism in the glandular sites. Experiments by David and Conover (1961) showed that the glands were employed when individual *Metridia* were captured by euphausiids. Luminescence develops in the early stages, for nauplii and copepodids of several genera, as seen in their response to stimulation, although the luminescent potential increases with maturity (Rudjakov and Voronina 1967, Evstigneev 1983a). The function of these organs is presumably as a deterrent or distraction to a predator. They may be also function as recognition signals between males and females at mating.

Intraspecific variation also occurs in the location and types of some integumental organs (e.g. Fleminger 1973, von Vaupel Klein 1982, Koomen 1992). Some integumental organs appear to be spatially linked and such classes are referred to as "cohort" by Fleminger (1973), "pore complex" by Mauchline (1988) and "associated pairs and triplets" by von Vaupel Klein (1982) and Koomen (1992). Von Vaupel Klein (1982) stated that this spatial linkage may be due to the ontogenetic linkage or the functional relationship or both.

Further histological and ultrastructural studies on the integumental organs are required to show the various structures and functions of these organs.

9.2. Ecological aspects of the *Pleuromamma* species

Life histories of calanoid copepods are best known for small coastal and estuarine species (Marshall 1949, Marshall *et al.* 1934, Landry 1975, 1978, McLaren 1978, McLaren and Corkett 1981, Uye 1982). One of the common features of these studies is to trace cohorts (broods or generations) by the abundance survey of each stage in time series samples from small, enclose bodies of water. These studies were, in fact, facilitated by frequent sampling of the population relative to the generation time of the copepods and supplementary laboratory experiments. Study of the population dynamics of oceanic copepods is more difficult because of difficulties in obtaining sequential time-series samples of the same population. There are no published studies comparable to those of the coastal species.

Chapter 7 concentrated on the population dynamics of *Pleuromamma robusta*. However, there were two limits to the analysis of results. First, the fine-mesh sampling for early (naupliar) life stages, which are necessary for a thorough evaluation of the life history of copepods, was not available. Samples were collected with a mesh size of 295 μm which quantitatively retained the adult and older copepodid stages, but not the abundant early copepodid stages. Second, samples were collected at approximately two month intervals, which is too long to result in population histograms that are coherent from one sample to the next. The abundance survey alone, therefore, is not enough to provide the data for interpreting the life history of this species.

Reproduction is the most important parameter in population growth. This study inferred the seasonal timing and intensity of reproduction of *Pleuromamma robusta* from the

condition of the oocytes in the oviducts of adult females. Reproductively mature adult females occurred from March to September in increasing numbers, were absent in November, and reappeared at lower levels in the following February. Seasonal fluctuations in the abundance of adult females in reproductively mature condition agree with the magnitude of the fluctuations in the abundance of early copepodid stages (Tables 7.2, 7.3). The population of *P. robusta* in the Rockall Trough was seasonally highest in May and September and lowest in March and the following February.

Few aspects of the population dynamics of calanoid copepods are as important as moulting. Moulting is a means of copepod growth as well as recruitment from one stage to the next. Most of the copepod's life is affected by periodically recurring moults, in terms of the completion of the internal structure after ecdysis and preparations for the following ecdysis. According to the time elapsed after ecdysis, the intermoult cycle is mainly divided into the postmoult, intermoult, and premoult stages. Ideally, within each copepodid stage, the proportion of different stages of the intermoult cycle can indicate the timing and intensity of the recruitment rate. For studies of the population dynamics of copepods, however, a suitably rapid method for staging of the intermoult cycle had not been developed.

Different stages of the intermoult cycle are most easily recognized by the degree of setogenesis which has taken place in the somatic tissue of appendages in preparation for the loss of existing setae with the old-exuvial exoskeleton at ecdysis. This universal method for staging the intermoult cycle, however, is in practice difficult to apply to ecological studies because it requires detailed examination of each individual, dealing with dissected appendages.

The main events in the period of the intermoult cycle are the calcification of the old-exuvial exoskeleton, increase in the amount of the somatic tissue, developing new setae, decalcification of the old-exoskeleton, and the formation of the pre-exuvial exoskeleton (Table 6.4). The first two changes affect the body appearance of copepods. Since the

development of the old-exuvial exoskeleton and the somatic tissue is not complete in the postmoult stage, the exoskeleton is soft and the body is transparent in appearance. The exoskeleton then becomes hard and the body acquires an opaque appearance: these are the intermoult and premoult stages. This method is now applicable to field studies, although it is difficult to distinguish between the intermoult and premoult stage by the body appearance.

In the present study, different stages of the intermoult cycle were simply identified by the body appearance, or the hardness of the integument, and were divided into soft (postmoult stage) and hard (intermoult and premoult stage) bodies in copepodid stages II to the adult of *Pleuromamma robusta* (chapters 7 and 8). This relationship between the intermoult cycle and the body appearance was assessed throughout copepodid stages II to the adult of *Pleuromamma robusta* (Table 6.2). It was useful in detecting the timing of the population reconstruction, overlapping generations, the identification of the newly recruited stock and the seasonal changes of the recruitment rate (chapter 7).

The different body appearance, soft and hard bodies, has previously been noted in calanoid copepods. Oh *et al.* (1991), pointed out that in copepodid V *Neocalanus cristatus* specimens from the North Pacific area had robust and fat bodies, while specimens from Sagami Bay had weak and transparent bodies. Prosome length and wet weight of specimens in the North Pacific were greater than those in Sagami Bay. They suggested that different body appearance is related to the nutritional condition: transparent bodies are due to the poor nutritional condition and vice versa.

Similarly, Ferrari and Hayek (1990) examined adult *Pleuromamma xiphias* and found that some have a soft exoskeleton and undeveloped internal tissue and others have a hard exoskeleton and well-developed internal tissue. In addition, males without a spermatophore in the spermatophore sac, and females without a dark mass in the genital opening, usually have a soft exoskeleton, while males with a spermatophore and females with a dark mass

usually possess a hard exoskeleton. They assumed that copepods with a soft exoskeleton had recently moulted and pointed out the potential usefulness of the intermoult cycle as an indicator of the recruitment rate in a study of population dynamics, although the anatomical nature of the dark mass in the genital opening was not clear, nor was a relationship shown between the body appearance and status of the gonad structure.

In chapter 7, the degree of gonad development of adult males and females of *Pleuromamma robusta* was examined based on the structure of gonad system. Although the genital system of copepodid V females was not anatomically examined, they were classified by the presence and absence of a rounded structure over the ventral areas of the first and second urosomal segments, presumably corresponding with the spermathecae on the first urosomal somite of adult females. None of copepodid V females and adult males and females with soft bodies were in reproductively mature condition, while a significant proportion of hard bodies were in immature condition (Table 7.2). This means that the development of the gonad from an immature to a mature condition depends on the time elapsed after ecdysis. In other words, reproductively mature copepodids with hard bodies are older than immature ones.

In the present study of 11,566 adult females of *Pleuromamma robusta*, five specimens had a spermatophore attached to the genital opening and four specimens had empty spermathecae. All of these had soft bodies (see chapter 7 and 8). Adult females always contain the spermatophoric contents in the spermathecae. It is suggested that adult males transfer their spermatophore to the females just after moulting of copepodid V female and that adult females retain the spermatophoric content in the spermathecae until fertilization. This implies that the timing of transfer of the spermatophore from the male to the female is entirely independent of gonad maturity of adult females, but, instead, related to the moulting cycle of the copepodid V females. Chapter 7 shows that there was no significant relationship between seasonal variations in gonad maturity between adult males

and females, nor between seasonal fluctuations in the sex ratio and gonad maturity of adult males and females (Table 7.3, Fig. 7.1). The amplitude of the abundance fluctuations of adult males was close to that of copepodid V females throughout the year.

In *Pleuromamma robusta*, day and night distributions of mature copepodid V females, adult females with soft bodies and mature adult males were separated from the others of the same copepodid stages at different stages of the intermoult cycle and gonad maturity (Tables 8.1-8.3). These stages mainly occurred below 600 m in both day and night, but adult females by day only, having similar day and night depth distributions. Adult females with a spermatophore attached to the genital opening and with empty spermathecae were also found below 600 m in both day and night. This correspondence may be related to mating behaviour. Mating behaviour of *P. robusta* is not restricted to adults, but extends to copepodid V females.

No significant difference was found between day and night vertical distributions of individuals of copepodid stages II to IV at different stages of the intermoult cycle in *Pleuromamma robusta* (Tables 8.5-8.7). The present sampling intervals of 100 m may be too wide to detect small-scale niche separation between individuals of each copepodid stage at different stages of the intermoult cycle. It is still possible that individuals at different stages of the intermoult cycle have different patterns of vertical distribution and migratory behaviour, as reflected in an ontogenetic vertical distribution.

The vertical distribution of *Pleuromamma robusta* is size-related (Tables 8.1-8.7). The peak and ranges of daytime distributions of individual copepodid stages increase with increasing developmental stage of copepodids. Nighttime distributions of the migrating population of all copepodid stages heavily overlapped in the upper 200 m. The night centre of distribution for the migrating population of copepodid stages II to IV was shallower (10 to 100 m) than that of copepodid V and adults (100 to 200 m). These results agree well with studies of the ontogenetic distribution of the other congeners (Longhurst and Williams 1979,

Ambler and Miller 1989, Bennett and Hopkins 1989).

In *Pleuromamma robusta*, the migrating distance of each copepodid stage was roughly estimated by comparisons between daytime distribution depths and night centres of distribution for the migrating population; the distance migrated is 100 to 200 m for copepodid II and III, 200 to 300 m for copepodid IV and V, and 300 or 400 m for adults. Throughout the water column, changes in day and night depths of the major portions of the population do not deviate from this estimated migration distance. This distance is size-related and could reflect the swimming ability of each copepodid stage.

The most notable changes associated with the intermoult cycle are the formation of old- and pre-exuvial exoskeleton (Table 6.4). These structural changes are also evident as increases in the thickness of the cuticle throughout the period of the intermoult cycle in females of copepodid V and adult *P. robusta* (Table 6.1). In adult females, increase in the cuticular thickness can be explained by the completion of the integumental structure after ecdysis. Adult females, however, do not moult but the thickness of the cuticle continuously increases throughout the intermoult and premoult stage as in copepodid V females where it forms the new-exuvial exoskeleton. It is possible that the thickness of the cuticle increases with times of secretion.

In calanoid copepods, one of the essential tools to trace cohorts is the abundance survey (e.g. Marshall 1949, Marshall *et al.* 1934, Carter 1965, McLaren 1969, Rigler and Cooley 1974). This method is severely affected by different sampling efficiencies such as the mesh-size used, or different durations of the life stages. McLaren (1978) used variation in body size to distinguish cohorts, mainly in adult copepods. His study was made under the assumption that body size of adult copepods is constant because they do not moult and that the main source of size variation is environmental temperature change. It was hypothesized that if copepodid stages develop continuously throughout a period when temperatures also change continuously, then changes in body size are also continuous.

Changes in body size of one copepodid stage are followed by the next and are finally evident in changes in body size of adults. The discontinuities of changes in body size of successively older stages of the life cycle are due to long sampling intervals, or relatively scarce number of new generations. The present study agrees with McLaren's hypothesis, but points out that copepod body size and weight, including those of adults, increases continuously throughout a period of the intermoult cycle.

Size variation is partially subject to different stages of the intermoult cycle within the same life stage because body size of hard bodies is always greater than that of soft bodies throughout all copepodid stages of *Pleuromamma robusta*, except that generations with different body size overlaps (Tables 6.3, 7.4-7.10). The amount of the exoskeleton and somatic tissue increases with the time elapsed after ecdysis, which could be evident in differences in body size between soft and hard bodies. This also contributes to bimodal size distributions of copepodid stages, in addition to overlapping generations.

It is believed that durations of various life stages of *Pleuromamma robusta* increase progressively with the increasing age of copepodids, unlike *Acartia* species whereby all stages have about the same duration at given temperatures and good nutritional conditions (Miller *et al.* 1977). In *P. robusta*, the thickness of the cuticle in adult females is greater than in the copepodid V females. This may be related to longer duration of adult females, in terms of longevity. Progressively longer durations of copepodid stages may be related to the increasing body mass. In addition, body size of adult females is considerably larger than the males throughout the year and adult females take longer to reach gonad maturity (Tables 7.2, 7.9, 7.10). Adult females, therefore, live longer than adult males, in terms of sex-limited longevity. This sex-limited longevity can explain the difference in life cycles between adult males and females.

Throughout chapter 6 to 8, this study introduces the intermoult cycle as a tool of interpretation of population parameters in ecological studies of copepods, e.g. the timing

of the population reconstruction, the identification of the newly recruited stock and seasonal intensity of the recruitment rate. This study also suggests that the intermoult cycle is a source of variation in body size. However, it was difficult to quantify the relationship between the intermoult cycle and body size variation. Further study is needed in order to properly quantify this aspect.

Neville (1963) used daily growth layers in solid endocuticle for determining the age of a grasshopper population. Yano and Kobayashi (1969) found the helicoidal structure of lamellae in the crab cuticle and estimated approximately the age of crabs by counting the number of lamellae. This lamellar structure is also seen in the cuticle of calanoid copepods: 16 or 17 layers in *Calanus finmarchicus* (Raymont *et al.* 1974); about 38 in adult male *Pleuromamma abdominalis* and more than 30 in adult male *P. piseki* (Blades-Eckelbarger and Youngbluth 1988); more than 60 in adult *P. robusta* in the present study. Although it is not clear whether the lamellar structure might represent daily growth layers, it is worth questioning whether there are daily growth layers in crustacean cuticle. Is it true?

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