Reproductive dynamics of the male Norway lobster,

Nephrops norvegicus (L.).

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Presented in Candidature for the degree of Doctor of Philosophy, to the Institute of Biomedical and Life Sciences, University of Glasgow.

Summary

The aim of this research was to examine various aspects of the reproductive biology of male *Nephrops norvegicus*. Due to differences in the reproductive behaviour between the sexes males suffer greater fishing mortality. A reduction in the numbers and size of males within a fished population may have effects on the reproductive output of the population.

The structure and functioning of the testes of *N. norvegicus* were examined histologically. The testis appeared to be made up of many acini, which drain into convoluted collecting tubules that carry the developing spermatozoa to the vas deferens. Each acinus appears to have an independent cycle of production by which secondary spermatophores are produced.

There is a pronounced seasonal development of the ovaries in female *N. norvegicus*, however, no such seasonality has been observed in the testes. This study used histological and biochemical analyses to determine any changes in the level of reproductive output of the testes over the course of the year. There were no observed changes in the structure of the testes. There was no thickening of the tubule walls which has been observed during the breeding season in other decapods. There was no change in the amount of sperm produced over the course of the year. There were no seasonal changes in the levels of protein and RNA in the testes indicating that there was no period of increased reproductive activity in

this tissue. Spermatophores were present in the vasa deferentia throughout the year. These results concur with previous research that there is no cyclical activity in the testes of *N. norvegicus*.

Positive allometric growth of secondary sexual characters has been used to determine the size at maturity of a wide range of decapod crustaceans. Several different techniques were used in order to determine male maturity in *N. norvegicus* from areas of differing sedimentology (indicative of populations with different biological characteristics) and also between different fishing grounds from around Scotland. It was found that those methods which used morphometric measurements from males alone provided estimates of maturity much greater than that of the observed physiological maturity. In contrast, those methods that compared the differences between the allometric growth of males and females provided estimates nearer to physiological maturity. These methods may prove to be more reliable in the calculation of size at onset of maturity. There was a great deal of variability in the maturity estimates of animals from different grounds and also from different sediment types, although no clear pattern was observed. It may be more appropriate, therefore, to assign a size range at maturity for different grounds.

The reproductive behaviour of *N. norvegicus* was examined through observations of laboratory populations and also interactions between individuals. Laboratory populations displayed daily activity patterns similar to those that would occur in the wild. Over the duration of the experiment a reduction in the levels of

agonistic activity was seen which may indicate the formation of dominance hierarchies within the populations. There was a high degree of movement by both sexes between the artificial burrows provided and this resulted in the frequent observation of eviction behaviours. In observations of pairs of males relatively little agonistic behaviour was observed, although this may be due to the holding conditions prior to observation. The duration of encounters between animals of similar size was shorter than between those differing in size, which was unexpected. This was not reflected in encounters between an intact animal and an animal missing a claw. Evidence that pheromones in *N. norvegicus* are not related to the moulting hormones is also presented.

The incidence of insemination of female *N. norvegicus* was examined in two separate months during the breeding season. It was found that a small percentage of reproductively active females (i.e. with developing ovaries) were unmated following the moult. Although this value was not statistically significant, it may be an important factor in the reproductive output of a fished population.

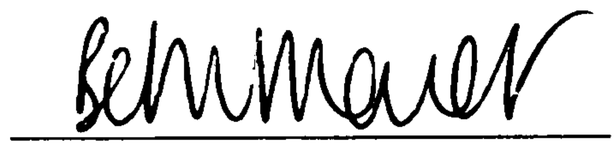
N. norvegicus in the Firth of Clyde are subject to seasonal infection by parasitic dinoflagellates of the genus *Hematodinium*. The effects of *Hematodinium* sp. infection on the reproductive organs of both male and female *N. norvegicus* were examined. Histological, immunological and molecular techniques were used to identify the parasite in the blood and tissue of the individuals sampled. It was found that *Hematodinium* was present in the haemal spaces of infected individuals, but was not seen invading the tissue. The ovaries of infected females

did not show any development even in mature moulted individuals. There was substantial disruption of the tissue and evidence that ovarian development had been terminated. There was no such evidence of disruption in the testes and sperm were present in the tubules of heavily infected males. Multinucleate parasite cells were seen attached to the basal laminae of the tissues and this may explain the disparity between tissue cell counts and ELISA results from the haemolymph of infected individuals.

The results of these investigations are discussed in relation to the possible effects on the reproductive output of fished populations.

Candidates Declaration

I declare that the work recorded in this thesis is entirely my own, unless otherwise stated and that it is of my own composition. No part of this work has been submitted for any other degree.



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Date 12-12-02

Acknowledgements

I would very much like to thank my supervisors; Professor Alan Taylor, Professor Jim Atkinson and Nick Bailey for their patient help throughout my PhD. Special thanks must also go to June Freel for her help with all kinds of practical work. Others who provided valuable technical expertise were; John Laurie for his help in the aquarium, Dr Ian Montgomery, Dr Kath Sloman and Kate Orr for assisting me with histology. Dr Ian Tuck for help with volumetric analyses and Dr Sue Marrs and Barry Ward for providing me with their extensive effort mapping data and also to Dr Ian Morgan for his help with the biochemical analyses. I would also like to thank Dr. Grant Stentiford from CEFAS, Weymouth for providing images and advice for the *Hematodinium* sp. work and Dr. Graeme Ruxton and Dr. Stuart Humphries helping me with some statistics. Thanks!

A special thanks goes to Mr Mick Parker and the crew of RV Aora and Mr Howard McKrindle and the crew of RV Aplysia for their help with the collection of animals in all sorts of weather and at all times of the day and night! I would also like to thank the Captain, crew and scientific staff aboard RV Scotia for many a pleasant week watching prawns and some late night trivial pursuit competitions!

Many thanks to Dave Bova and Adrian Wheetman from my CASE partner, FRS Marine Laboratory in Aberdeen, for their help with setting up the behavioural

study, and also the aquarium staff Ben Williamson and Rory Long. Thanks to Kenny, Magnus, and Dickie Livingstone of the Fram III and Jim Kinnear (FRS) for their help in collection of animals in Loch Torridon.

I would like to thank my office mates for putting up with me over the years! Dr. Jason Hall-Spencer, Kath Sloman, Kevin Butterworth, Dom Lewis, Sarah Brown, Tina Schmitt, Andrew Jackson, and especially Hamish Small and Iain Ridgway. Also thanks goes to all those people who kept me sane - Jo Smith, Jen Ward, Jen Andrew, Dave Stevens, Lorna Kennedy, Jo Mould, and Gill Taylor.

Most of all I would like to thank my parents and my brother for their constant support and encouragement.

Contents

Chapter 1

General Introduction

The Norway lobster *Nephrops norvegicus* (L.) is a burrowing decapod crustacean belonging to the infraorder Astacidea and family Nephropidae. This species forms the basis of important commercial fisheries on the continental shelf and the marginal seas of the eastern North Atlantic (Hill and White, 1990) and has been the subject of several reviews (Figueiredo & Thomas 1967a; Farmer, 1975; Chapman 1980; Sardà, 1995). *N. norvegicus* was a bycatch species in the Firth of Clyde before being commercially targeted in the 1950s. It has since become the most valuable species in the area (Bailey *et al.*, 1986; Scottish Fisheries Statistics, 2001). The Scottish catch of this species makes up about one third of the world total and the British catch is currently worth around £68.5 million per annum at first sale (Source: Sea Fish Industry Authority, 2001). *N. norvegicus* has a fairly wide distribution and can be found at depths of 10 - 800m (Farmer, 1975); it is, however, spatially constrained by its requirement for soft substrata in which to burrow. Although local movements take place, *N. norvegicus* do not carry out any large-scale migrations (Chapman, 1980, Chapman & Bailey, 1987). Due to its largely sedentary, benthic lifestyle, and the patchy distribution of populations, *N. norvegicus* is vulnerable to local scale depletion (Tully and Hillis, 1995).

N. norvegicus is dioecious and mating is promiscuous (Farmer, 1975). Mating occurs just after moulting in the female which occurs from May through to August

(Farmer, 1974a). Once the spermatophore has been transferred the male departs and the female stores the sperm packet until egg laying which occurs in August and September (Farmer, 1974a). Prior to egg laying there is a period of ovarian development which has been variously classified into five main stages (Thomas, 1964; Bailey, 1984). Following egg laying there is a period of incubation, where the eggs are carried on the pleopods of females. Incubation will last until the following April/May or sometimes as late as June (Farmer 1974a) when the planktonic larvae are released. During the incubation period the ovigerous females remain largely in their burrows and are less attracted to food (Dunthorn, 1967).

There have been several studies of the reproductive biology of *N. norvegicus* throughout its range (e.g. Thomas, 1964; Bailey, 1984; Nicolajsen & Eiriksson, 1990; Sarda, 1991; Orsi Relini *et al.*, 1998). It has been shown that the duration of the reproductive cycle varies geographically and this may well be related to environmental factors such as temperature. In the Adriatic, the period of hatching occurs earlier in the year than in Scottish waters (Karlovac, 1953), whereas females in Icelandic populations carry their eggs for up to 13 months (Eiriksson, 1970). The tendency for ovigerous females to remain within their burrows causes a bias in the apparent sex ratio of the population. Catches from Adriatic populations show a more even sex ratio (Karlovac, 1953) than that seen in Scotland, where females are largely absent from catches during the winter months. In Iceland, however, where the incubation period is much longer, males make up the majority of the catch throughout the year (Eiriksson, 1970). There are also

geographical variations in the female size at maturity (see Anon., 1982, Sardà, 1995).

The reproductive cycle of *N. norvegicus* in temperate waters is an annual one (e.g. Thomas, 1964; Figueiredo & Thomas, 1967b), unlike the American and European lobsters which reproduce every two years and the spiny lobster *Panulirus homarus* which can produce several broods in one year (Aiken & Waddy, 1980). There are some suggestions, however, that the length of the reproductive cycle of females may depend on their size and that larger females may take longer to recover from breeding and may therefore only reproduce every second year. This has been reported, for example, in the Firth of Clyde (Bailey, 1984; Bailey *et al.*, 1986) and the central North Sea (Sterk & Redant, 1989).

It has been noted that male *N. norvegicus* from waters around the British Isles are able to produce spermatophores throughout the year (Storrow, 1912; Farmer, 1974a). However, it has been reported for Portuguese stocks that, although spermatogenesis occurs all year round, spermatophore production occurs only from July onwards (Figueiredo and Barraca, 1963). In an investigation of males from the Irish Sea, histological investigations revealed no annual pattern in spermic activity (Farmer, 1974a). Similarly, a study on males from the West Coast of Scotland also reported no annual change in dry weight measurements of the testes (Bailey, 1984). It is suggested that there may well be seasonal changes in the testis that are not associated with weight change (Bailey, 1984).

It has been reported that it is only the largest males that carry out most of the mating in polygamous mating systems of nephropid and palinurid lobsters (Sastry, 1983). It is thought that the initiation of male mating behaviour in *N. norvegicus* is caused by the release of pheromones by the newly moulted female (Farmer, 1975). The existence of pheromones has been demonstrated in *Homarus americanus* and it would appear that *N. norvegicus* courtship behaviour is very similar to that of the American lobster as described by Atema (1986). The presence of a 'soft' female results in the male walking on extended periopods. The male will straddle the female, after stroking her with his antennae and he will then flip her over onto her back before placing the first pair of pleopods into the female thelycum. During copulation the spermatophore is passed to the female via grooves in the first pair of pleopods by the action of the appendices masculina on the second pair.

Observations carried out on experimental animals have shown that only one spermatophore was transferred during each copulation and penetration lasted around 2 seconds (Farmer, 1974a). Unlike the American lobster there is no mate guarding or cohabitation and, following copulation, the male and female separate (Farmer, 1974a). Following transfer, the spermatophores are stored at the point furthest from the opening of the thelycum. The exact mechanism for fertilisation of the eggs has yet to be demonstrated. Figueiredo & Thomas (1967a) suggested that the eggs must be fertilised during laying when they pass over the surface of the thelycum. This is, however, unlikely as the thelycum will harden with the rest of the exoskeleton following the moult. Farmer (1974a) suggested that fertilisation

may occur through the temporary formation of tubules from the thelycum to the oviduct as in the spiny lobster *Jasus lalandii* (Silberbauer, 1971).

Male and female *N. norvegicus* differ in their secondary sexual characteristics (Farmer, 1974b, c). In males there is positive allometric growth of the chelipeds, while there is widening of the abdomen in females. In addition to this there is differentiation of the pleopods in both sexes (Farmer, 1974c). The change in body dimensions that takes place can be used to determine the size at onset of maturity (SOM). There is considerable variation in the size at maturity between different populations of *N. norvegicus* (e.g. Thomas, 1965; Figueiredo & Thomas, 1967b; Anon., 1982; Bailey, 1984). It was originally thought that this variability in the size of *N. norvegicus* was mainly due to differences in fishing effort (Thomas 1965). However, it has since been related to the differences in density and growth rate between populations (Bailey & Chapman, 1983; Bailey *et al.*, 1986; Chapman & Bailey, 1987; Chapman & Howard, 1988; Tuck *et al.*, 1997a, 2000).

The behaviour of *N. norvegicus* affects its fishery, as populations from different depths show different endogenous emergence patterns in relation to light intensity (Chapman & Rice, 1971; Chapman & Priestley, 1972; Farmer, 1974d; Aréchiga & Atkinson, 1975; Chapman *et al.*, 1975; Atkinson & Naylor, 1976; Chapman & Howard, 1979; Aréchiga *et al.*, 1980; Moller & Naylor, 1980). As previously described, the breeding behaviour of *N. norvegicus* causes changes in the sex ratio of the fishery. Ovigerous females remain in their burrows while incubating their eggs and during this time they are largely unavailable to the fishery. The

percentage of males in the catch may vary from 33% to 96% (Farmer, 1975). It is interesting to note that in Mediterranean fisheries where the incubation period is shorter, there appears to be a female bias in the catch (Sardà, 1998). In the fisheries in Scottish waters the fluctuation in apparent sex ratio means that males are subject to greater fishing mortality and will also be exposed to a greater risk of natural mortality than females. The proportion of females emerging from their burrows, and subsequently present in the catch, increases in the summer months in the period between egg hatching and laying (Farmer, 1975). It can be seen that in larger size groups within fished populations the majority of individuals are female (Bailey, 1984) and if the sex ratios are calculated on the basis of age groups then females are the only sex present at older ages. This would be expected as a result of the reduced fishing pressure experienced by females. Large males have been shown to be less inclined to burrow in laboratory experiments (Farmer, 1974e). This could increase fishing mortality in the larger size classes; however, there is no evidence to suggest that this occurs in their natural environment.

The problem of selective capture is further complicated by the fact that mature male *N. norvegicus* display a greater rate of growth than females and thus reach a catchable size at a younger age (Briggs, 1997). The calculation of sex ratio by size group also masks an additional problem caused by differences in growth rate (Bailey, 1984). Since males exhibit faster growth rates than females they will be younger than females of a comparable size. Selective fishing of males from the population will, therefore, result in males that are younger and much smaller than the females in the population. This fishing pressure could even be selecting for

(physiological) maturity at smaller sizes (Bailey, 1984). It has been noted that there has been a reduction in the size of individuals present in fished populations (Karlovac, 1955; Anon, 1984; Hillis, 1988a; Hillis & Tully, 1993). Fishing could therefore lead to sperm limitation in terms of the amount of sperm transferred during copulation between a small male and a large female. Sperm limitation could take the form of reduced mating opportunities through reduced numbers of males. A further problem could be that large females might be unable to find a mate of a suitable size. This problem could be further exacerbated if the larger, and therefore more fecund females, breed biennially.

Sperm limitation as a result of a male biased fishery has already been reported in the Dungeness crab fisheries (Smith & Jamieson, 1991). Here the fishery concentrates mainly on males because few females reach the legal landing size and the male crabs have a better meat yield. The fishermen are reluctant to land females as they see it as a poor management strategy. Female Dungeness crabs require a mate larger than themselves to reproduce successfully (Butler, 1960; Snow & Neilsen, 1966). In areas of heavy fishing pressure it is thought that many males will be removed from the population before they have a chance to reproduce (Smith and Jamieson, 1991). If this is the case then, even with male polygamy (Butler, 1960, Snow & Neilsen, 1966), many females will go unmated and the population will be reduced. It is therefore important to understand the importance of functional maturity in the breeding system of *N. norvegicus*, if the fishery is to be regulated appropriately.

Another major influence on both populations of *N. norvegicus* and the fishery, especially in the Clyde Sea area, is the seasonal occurrence of infection by dinoflagellate parasites of the genus *Hematodinium*. This parasite was first reported in the 1980s (Field *et al.*, 1992) and has since received much study. Although the effects of infection on the reproductive abilities of individuals is not clear, overall prevalence has reached levels of up to 25% in the catch in recent years (Stentiford *et al.*, 2001a), a reduction on the very high levels (70%) of prevalence that were reported on the early 1990s (Field *et al.*, 1992).

The aim of the research reported herein was to investigate the reproductive dynamics of male *N. norvegicus*, to determine the possible effects of continued intensive fishing on populations. The research areas were that of the structure and function of the male testes, specifically in relation to any seasonal pattern in the production of gametes that might have been related to the female reproductive cycle. The determination of size at first maturity (SOM) was an extremely important parameter for investigation in exploited populations due to the likely changes in the size structure of fished populations. Variation in SOM both geographically (see Figure 1.1 for sampling areas) and in relation to different sedimentology was also investigated to determine the possible implications for future management of the fisheries. The mating behaviour and strategies of male *N. norvegicus* were studied in order to determine the possible impacts of fishing on population structure and the ability of males to mate. The reduction in the size and numbers of males within a fished population could result in sperm limitation particularly in larger females. In combining behavioural studies with an

investigation into the levels of insemination of reproductively active females, the importance of sperm limitation to the reproductive output of the population was examined. The Clyde Sea area is particularly affected by *Hematodinium* sp. infection, and the effects of infection on the gonads of both male and female individuals were examined to determine the possible implications for reproduction. By using this multifaceted approach it was hoped to develop a better understanding of male reproductive biology and the possible impacts of sex biased fishing on the reproductive output of exploited populations.

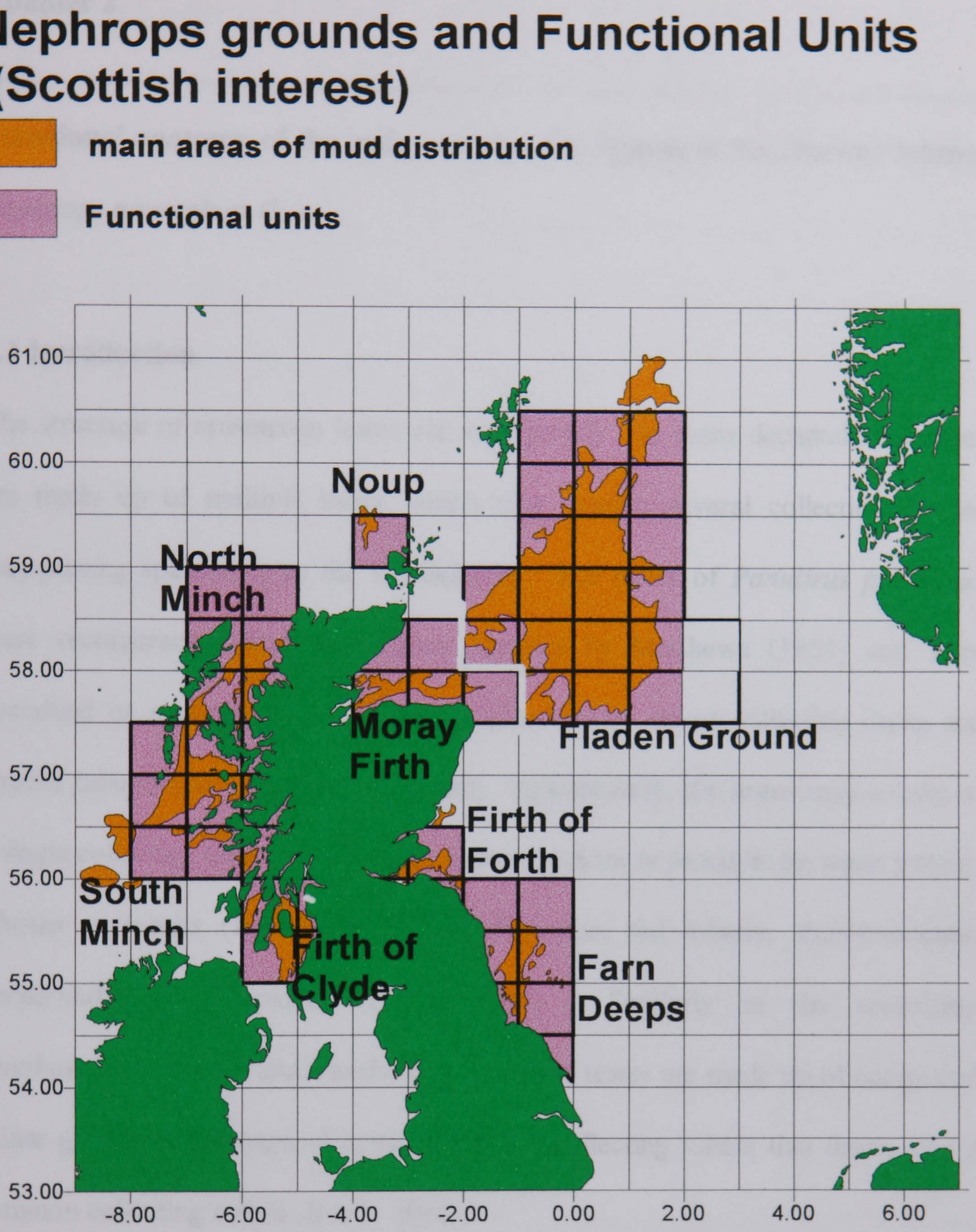


Figure 1.1: A map of the functional units for *Nephrops norvegicus* population assessment around the Scottish coast and the main areas of mud distribution. The areas sampled in this study included: the Firth of Clyde and the North and South Minches on the west coast, and the Fladen Ground in the Northern North Sea. (Map provided by Dr I. D. Tuck, FRS Marine Laboratory, Aberdeen.).

Chapter 2

Functional anatomy of the male reproductive system of the Norway lobster, *Nephrops norvegicus* (L.).

2.1 Introduction

The structure of crustacean testes can vary greatly. In many decapods the testes are made up of multiple lobes, which may contain several collecting tubules transporting spermatids to the vas deferens. The testes of *Panulirus pencilatus* were reconstructed from histological samples by Matthews (1951) and were described as a compound or racemose gland made up of collecting ducts and smaller tubules ending in acini or follicles. Alternatively, the testes may consist of a single collecting tube surrounded by numerous acini as found in the squat lobster, *Thenus orientalis* (Burton, 1995) and Hawaiian red lobster, *Enoplometopus occidentalis* (Nephropidae) (Haley, 1984). Similarly in the crawfishes *Cambarellus shufeldti* and *Cambarellus puer* the testes are made up of compound acinar glands with groups of acini joined to a collecting tubule that drains into a common collecting tubule (Black, 1966).

The testes of *Nephrops norvegicus* make up an H-shaped organ situated dorso-laterally within the thoracic cavity (Figure 2.1). It has two anterior arms which extend forward almost to the cephalic ganglion. The posterior arms extend back under the abdominal segments and pass over the dorso-lateral abdominal muscles alongside the midgut. Previously, the bridging point of the organ, which lies just

under the heart, has defined the anterior and posterior testes. In *N. norvegicus* this bridge occurs anterior to the vas deferens. For this study, the testis was divided into anterior and posterior with respect to the vas deferens under the assumption that they are functionally separate. In support of this, there is evidence from previous histological studies that the collecting tubules from each part of the testis both join at the proximal vas deferens (O'Neill, 1992).

Spermatogenesis occurs rapidly in most invertebrates (Barnes *et al.*, 1993). Germ cells develop into gonial cells, which multiply rapidly by mitotic division before meiosis (Sastry, 1983). The first stage in the process is the transformation of spermatogonia to primary spermatocytes, which then give rise to four spermatids. These spermatids differentiate to produce spermatozoa (Sastry, 1983). Marine invertebrates typically have a simple round-headed spermatozoan, which is generally associated with pelagic development from external fertilisation. Some species, however, have more advanced spermatozoa which have been associated with sperm storage or internal fertilisation or where eggs are highly protected (Barnes *et al.*, 1993). The spermatozoa of *N. norvegicus*, which have been described as early as the beginning of the 20th century, are non-motile. The sperm of reptantian decapods become embedded in a gelatinous matrix on their passage from the testes to the anterior vas deferens (Hinsch, 1991). They take the form of a highly convoluted ribbon or cord, or can be seen in individual bundles (Hinsch, 1991). The matrix is then surrounded by further components that make up the wall of the spermatophore, which is produced in the vas deferens.

The vasa deferentia of *N. norvegicus* can be divided into three sections each with a separate function (Farmer, 1974a). The proximal region of the vas deferens has a secretory function (Hinsch, 1991); this is followed by the intermediate sphincter region and a distal ejaculatory region (Farmer, 1974a). The vas deferens is lined with columnar epithelium, of which the cells decrease in height towards the posterior section. These cells contain cytoplasmic organelles that are associated with synthetic activity. As the sperm chord moves along the vas deferens, successive layers of acellular mucopolysaccharide are added to its surface (Hinsch, 1991; Subramoniam, 1991). The spermatophore is then held in storage by the sphincter muscle and ejaculatory regions prior to transfer to the female thelycum (Farmer, 1974a). The process of sperm transfer via a spermatophore occurs through the genital openings by rupture of the thin membranes that normally cover these apertures (Farmer, 1974a).

The function of the layers of acellular mucopolysaccharides laid down in the spermatophore walls and contained in the seminal fluid are as yet unclear (Hinsch, 1991), although it is suggested that they may have roles in preventing dehydration or providing nourishment to spermatozoa during storage (Subramoniam, 1991). In some species, for example the tanner crab (*Chionoecetes bairdi*), viable sperm have been found in storage for as long as two years (Paul, 1984). Anilkumar *et al.* (1996) report that the spermatophore of the brachyuran crab, *Metopograpsus messor*, consisted mainly of fat and protein. They go on to suggest that the fat and lipid may be used as an energy store by sperm during aerobic respiration following the breakdown of the spermatophore wall (as described by Hinsch, 1991). This is

in contrast to the findings of Jeyalectumie & Subramonium (1991), who reported a decrease in carbohydrates during storage in the spermatheca. They postulate that this is due to anaerobic respiration.

It has been shown that, like vertebrate semen, the seminal plasma of some crustaceans has antibacterial functions, for example the mud crab *Scylla serrata* (Jayasankar & Subramoniam, 1999). Such antibacterial activity could be important should any microbes enter the reproductive tract of the female during the mating process. Thus males could be protecting their investment during storage by the female. It has been shown that there are fewer bacteria associated with the spermathecae of multiparous female snow crabs, *Chionoecetes opilio*, than in primiparous or old barren females (Benhalima & Moriyasu, 2001).

The aim of this study was to describe the structure of the testes of *N. norvegicus*. Through this it was hoped to develop a better understanding of the functioning of the testes in relation to the reproductive biology of this species.

2.2 Methods

Nephrops norvegicus were collected from south of Little Cumbrae in the Clyde Sea area (55.41°N, 4.56°W) throughout the year. The testes were dissected intact from animals of around 30 mm carapace length and larger, to ensure that they were physiologically mature, since Farmer (1974a) reported physiologically mature animals of 18mm in the Irish Sea. Once removed, the testes were preserved in 10% formalsaline prior to dehydration through graded alcohols (one hour in each of 30%, 50%, 70%, 90%, and repeated in absolute alcohol) and embedding in paraffin wax. Sections of 7μm thickness were stained using Haematoxylin and Eosin (H&E). The sections produced were examined using a digital camera attached to a compound microscope with an image capture programme (PCImage).

In order to determine any differences between the anterior and posterior testis, the gonad was divided at the point of joining with the vas deferens and each part was processed, sectioned, and observed separately so that a comparison could be made. The vas deferens was also processed for examination.

2.3 Results

Observations of the stained testicular material showed that there were several different tissue types within the testes. The testes of *Nephrops norvegicus* appear to be made up of many individual acini (Figure 2.2a), which drain into what appears to be a single, highly convoluted collecting tubule. Part of the connecting tubule can be seen in Figure 2.2b. Each acinus contains a layer of spermatogonial cells that are located on the basal membrane of the acini (Figure 2.3a & b). These are relatively large cells that stain darkly under H&E (Figure 2.3a & b). It can be seen that the spermatogonial cells vary in size (Figure 2.3b). The spermatogonia undergo mitotic division to produce primary spermatophores. These cells usually fill the acinus and can often be seen at various stages of meiotic division (Figure 2.3). They are smaller than the spermatogonia and stain lighter. They have a very large nucleus that can fill up to three-quarters of the cell. These cells divide by meiosis to become secondary spermatocytes. Accessory cells can also be seen in the follicles (Figure 2.3b) in association with spermatocytes. The accessory cells can also be seen in association with more developed spermatozoa, prior to leaving the acinus (Figure 2.4). In this case, bundles of what appear to be spermatids or developing spermatozoa can be seen in association with the accessory cells. Accessory cells have also been observed outwith the acinus and may enter the collecting tubule with a mixture of unidentified cellular matter, associated with the completion of spermatogenesis in the acinus (Figure 2.5). It is not completely clear at which point the secondary spermatocytes transform into spermatids, whether it is before leaving the acinus or upon entry into the collecting tubules.

The collecting tubule appears to be very convoluted and in many cases it is not possible to get a clear cross section of the tubule (Figure 2.6). The walls of the tubule appear to be quite thick in most cases (Figure 2.7) but do not consist of spermatogonia and therefore do not have any germinative function (Figure 2.8). They act purely as a conduit to transport the spermatids/spermatozoa to the proximal vas deferens. The structure of the collecting tubule can be seen in Figure 2.7. It appears that the lumens of the collecting tubules are not completely filled with spermatozoa. This could be an artifact of preservation, dehydration and staining of the tissues, or it could be that there is a fluid surrounding the spermatozoa, which is lost during the processing of the tissue.

No musculature is seen in association with either the acini or the collecting tubules, although there is connective tissue associated with the structures of the testes (Figure 2.9). Observations were made on both the anterior and posterior testis and no discernible differences were observed (Figure 2.10). There were also no differences observed between the right and left testis within an individual male (Figure 2.10).

The vas deferens of *N. norvegicus* proved to be very difficult to process and section, particularly the broader storage region of the mid-vas deferens. However, some good images of the proximal vas deferens were obtained. It can be seen that in the vas deferens, unlike the testes, movement of the spermatozoa is no longer passive and there is a musculature associated with the proximal vas deferens (Figure 2.11). It can also be seen that there is a columnar epithelium associated

with the proximal vas deferens (Figure 2.12). It was also noted from the vas deferens of one animal, that there might have been packets of material that are passed along the vas deferens separately by muscular contraction called a 'string of pearls' (Figure 2.13).

2.4 Discussion

It was often difficult to determine the nature of the tubules, collecting ducts and acini from the sections of *Nephrops norvegicus* testis, as spermatids/spermatozoa appeared to be present in all of these structures. The acini can be distinguished from the collecting tubules by the presence of spermatogonia in their walls and the spermatocytes, which are quite distinct from the cells that make up the tubule walls. It is more difficult to determine any difference between the ducts, which connect the acini to the collecting tubule and the collecting tubule itself. There appear to be many acini connected to convoluted collecting tubules. The number of collecting tubules is not obvious from the sections; however, observations made on the testes using illumination from below seemed to show that there was only one main collecting tubule. Unfortunately, it was not possible to reconstruct this from the histology and attempts at repeating the observation were unsuccessful. The external structure of the testis could suggest a single collecting tubule, as the testis is not lobular. One convoluted tubule running the length of each of the anterior and posterior testis would therefore be able to connect to all the acini along the length of the testis. The convoluted nature of the tubules of the testes was apparent through histological investigation, although it is possible that the presence of connecting tubules from the acini somewhat confused these observations.

Within the acini of the testes several different cell types were seen. The spermatogonia that were observed appeared to vary in size. Fasten (1926) described two different types of spermatgonial cell in the black-clawed crab

(*Lophopanpeus bellus*) and suggested that the larger cells were primary spermatogonia and the smaller cells were secondary spermatogonia. No difference in the function of these two cell types was ascribed by Fasten (1926), however, and in both types division was observed to proceed in the same manner. It is likely that the different sized spermatogonial cells seen in the testes of *N. norvegicus* represent these primary and secondary types.

It was not possible in this study to determine the different stages of spermatogenesis that would be expected in the acini. Although it was noted that within each acinus all of the developing cells appeared to be at a similar stage of development as noted by O'Neill (1992) (Figures 2.2 – 2.4). Cells of differing size were observed in the acini and it could be hypothesised that these represented both primary and secondary spermatocytes, the primary type being the larger cells. Spermatids are produced from secondary spermatocytes through the second meiotic division, and it was not possible to discriminate between these cell types. It is likely that the spermatids in *N. norvegicus* mature rapidly into spermatozoa. Where cells were observed in the lumen of the collecting tubules a single cellular projection was always seen. It is unlikely that this projection was one of the several non-motile processes that are common in decapod crustaceans, and of which *N. norvegicus* has three (see Afzelius, 1995). It is likely that the projection observed on these cells was the acrosomal projection (see Chevaillier, 1966), which is more substantial than the flagellae that also radiate from the spermatozoa. This would indicate that the maturation process from spermatid to spermatozoon is occurring either in the acinus, or very shortly thereafter.

The accessory cells that were seen to be associated with the spermatogonia and spermatids have been described previously for a number of decapod crustaceans (e.g. Fasten, 1926; Cronin, 1947; Matthews, 1954). Cronin (1947) noted that they were most often seen close to, or in association with, the lobule walls in the testis of *Callinectes sapidus*. This did not appear to be the case, however, for *N. norvegicus* where the accessory cells were observed throughout the lumen of the acinus. The accessory cells observed by Cronin (1947) were described as forming a wall of columnar/cuboidal epithelium on the completion of spermatogenic activity. There was no evidence for this in *N. norvegicus*; indeed accessory cells were often seen in the lumen of the collecting tubule presumably having been expelled from the acinus. On examination of the testis of *Enoplometopus occidentalis*, Matthews (1954) referred to these cells as sustentacular cells, which were found dispersed throughout the spermatocytes although there is no reference to their function. Haley (1984), in a study on the same species, suggests that the accessory cells arise from the same basal cells as the spermatogonia, although their exact origin and function is unknown.

There were no discernible differences in the structure of the testis bilaterally, nor was there any difference between the anterior and posterior regions of the testis. From these observations it would seem appropriate to use the connection with the vas deferens as the point of division of the testis. Either side of this point should lie a separate functional 'lobe' of the testis, each with its own system of collecting tubules and acini. The previous use of the bridging point of the testes to divide

each testis into anterior and posterior units relied on gross morphology and had no functional basis.

The difficulties experienced in obtaining sections from the vas deferens were probably caused by the nature of the spermatophores found in the median and distal portions. It was found that these areas did not appear to be well preserved and were not readily embedded with paraffin wax for sectioning. The sections obtained of the proximal vas deferens showed that there was associated musculature. It is reported that the function of this musculature is not to move the contents of the vas deferens along, but rather to mould them into shape (Greenwood, 1972). The presence of columnar epithelial cells is also expected in the proximal region, as the epithelium decreases in height with cuboidal epithelial cells located in the distal region as previously stated.

The nature of the testes of *Nephrops norvegicus* collected in this study suggests that males would be capable of producing sperm all year round, although there was no indication of what proportion of the testes is contributing to the output of spermatozoa at any one time. This agrees with data from animals in the Irish Sea and observations on animals from Scottish populations, which are able to produce spermatophores all year round. Males from other populations of *N. norvegicus*, for example in the Adriatic (Karlovac, 1955), and in Portuguese waters (Figueiredo & Barraca, 1963), have also been shown to carry out spermatogenesis throughout the year, although they only produce spermatophores during the breeding season. Having individual acini at different stages of production will mean that there will be

a constant supply of maturing spermatozoa. This subject is discussed in greater detail in Chapter 3.

2.5 Conclusions

- The testes of *N. norvegicus* are made up of a number of individual acini attached to one or more collecting tubules. It was not possible to determine the number of collecting tubules within the testis.
- Each acinus contains spermatocytes at the same stage of development, which are produced through a cycle of development apparently independent of the other acini. It was not possible, however, to determine what proportion of acini were contributing to sperm production at any one time.
- There appear to be both primary and secondary spermatogonial cells lining each acinus and accessory cells can be seen in association with the spermatocytes within the lumen of the testes.
- There were no differences between the left and right testis, nor was there any difference between the anterior and posterior testis. The division of the testes into anterior and posterior from the vas deferens rather than the bridging point of the organ appears to be an appropriate functional definition.

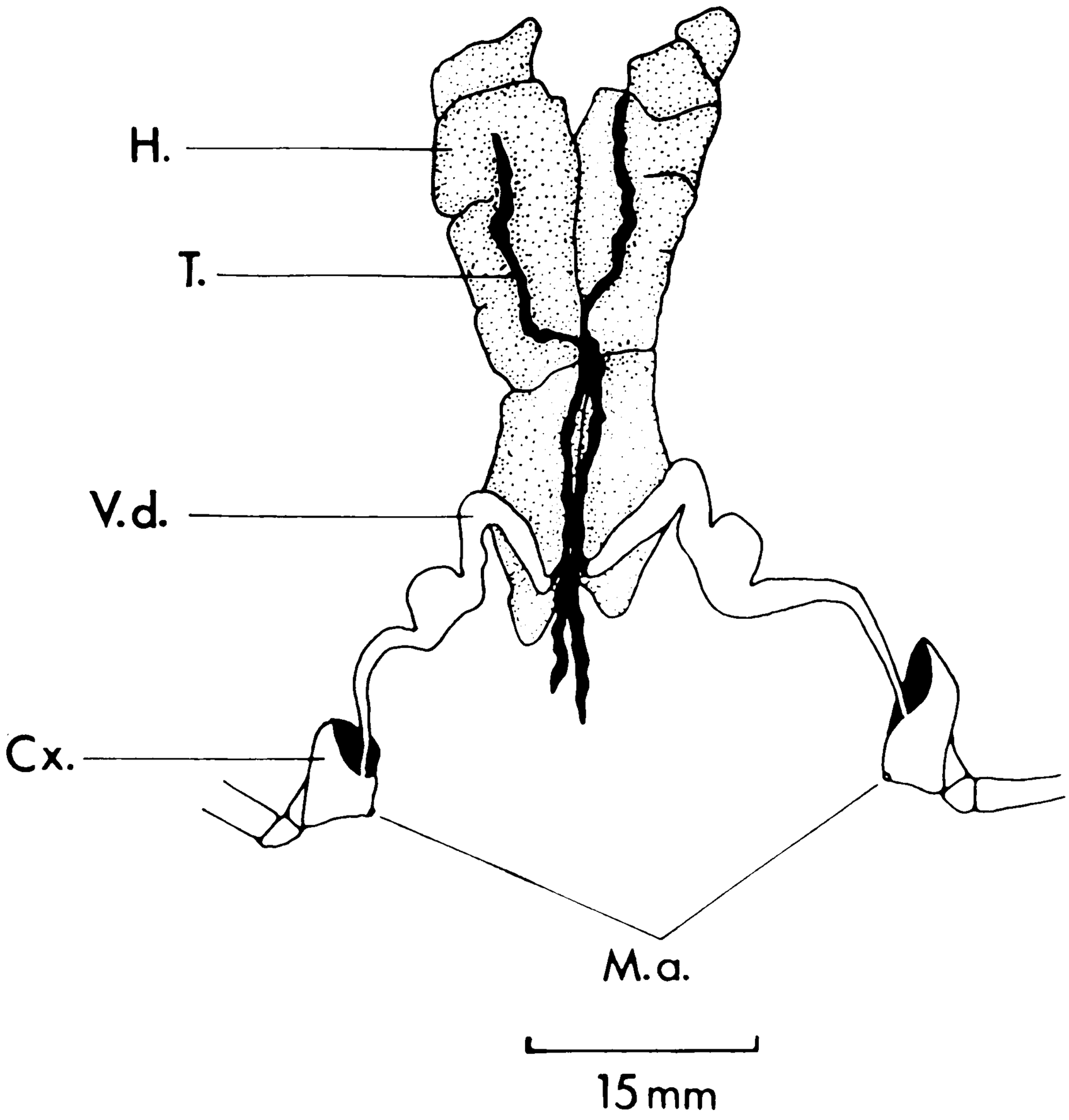


Figure 2.1: Anatomy of the male Norway lobster *Nephrops norvegicus* (from Farmer 1974a). The diagram is a dorsal view showing the hepatopancreas (H) and the overlying testes (T), which join with the vas deferens (V.d.). The male reproductive system ends at membrane covered apertures (M.a.) on the coxopodites of the fifth pereiopods. In this study it was noted that the posterior testes (shown here at the bottom of the diagram) were greater in length than represented in this diagram.

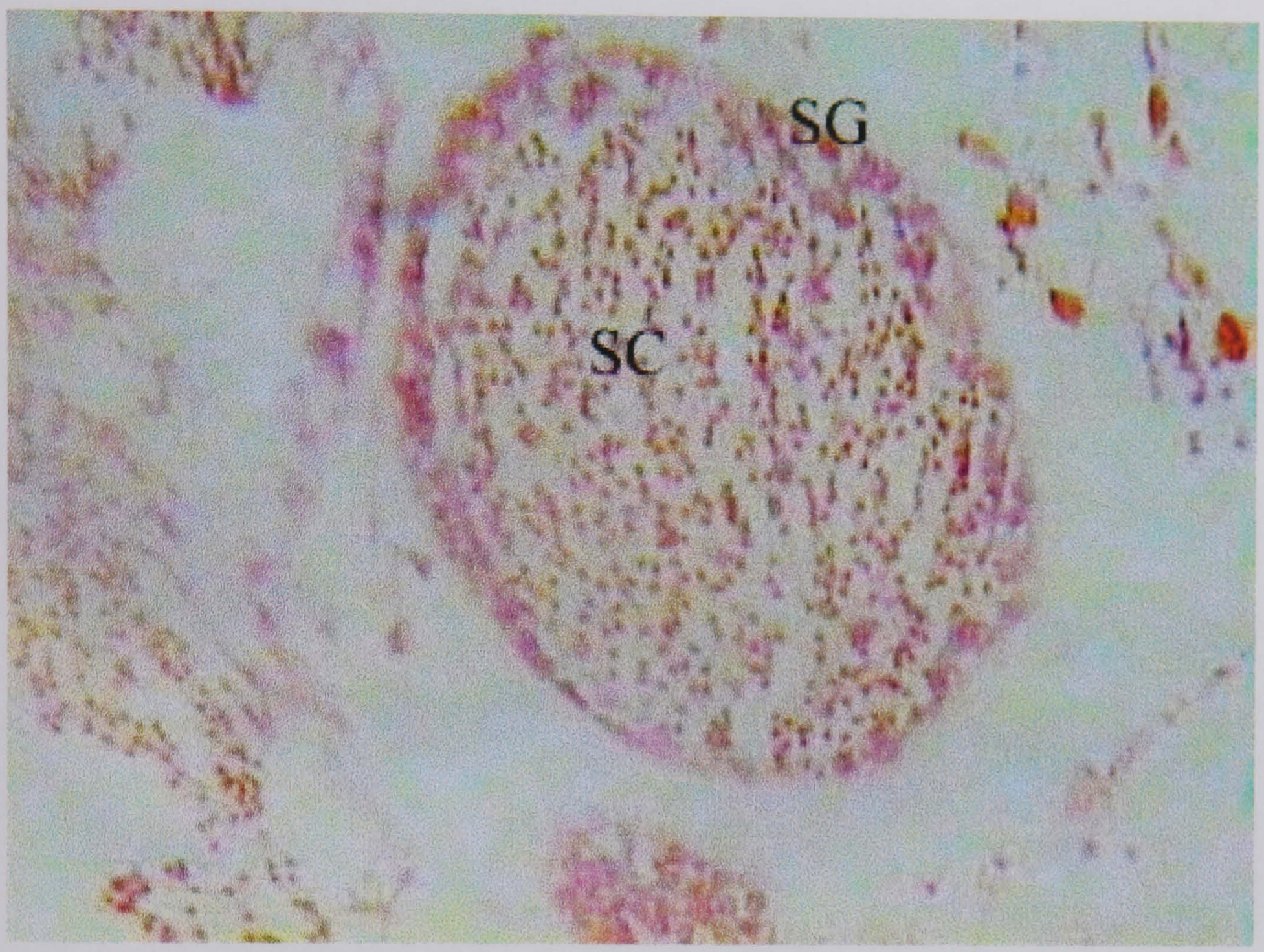


Figure 2.2a: An acinus from the testes of *Nephrops norvegicus* (H & E x10) showing the darker staining layer of spermatogonia (SG) around the edge of the acinus and the lighter staining spermatocytes (SC) in the centre.

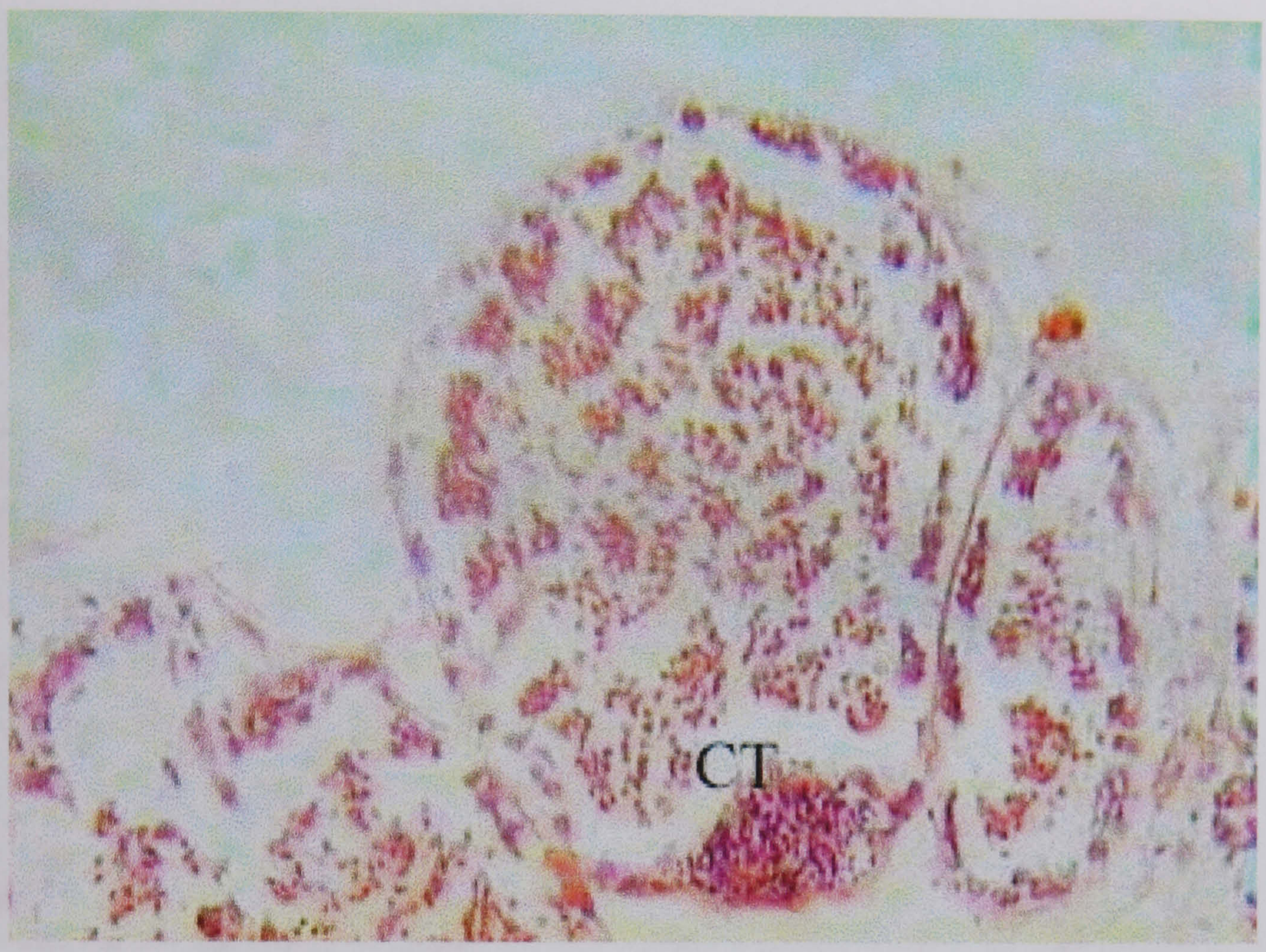


Figure 2.2b: The connecting tubule (CT) of this acinus can also be seen in this section, filled with spermatozoa. (H & E x 10).

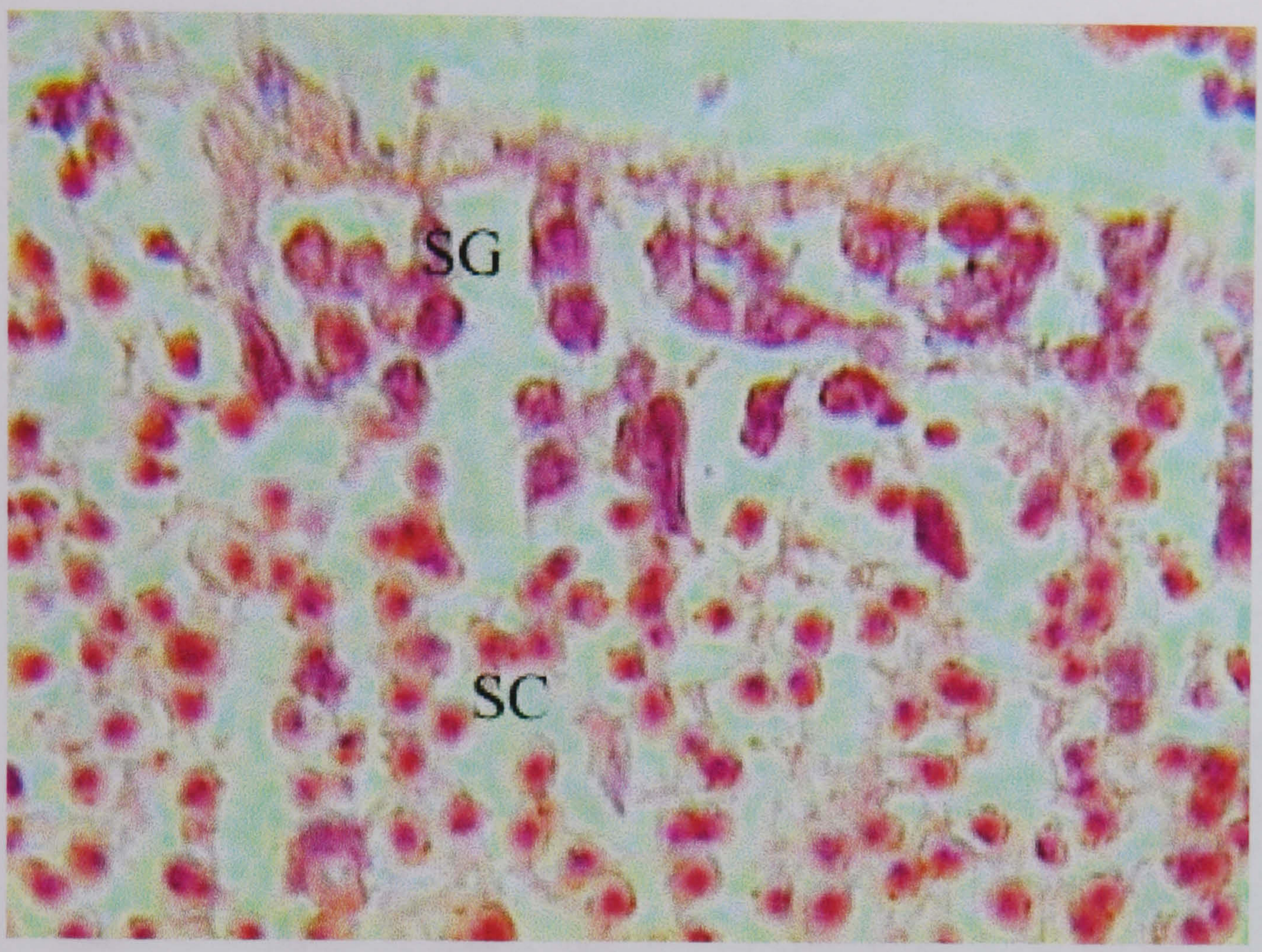


Figure 2.3a: The wall and some of the contents of an acinus from the testis of *Nephrops norvegicus*. The previously described darkly staining spermatogonia (SG) and lighter spermatocytes (SC) can be seen in greater detail at the edge of the acinus. (H & E, x 40).

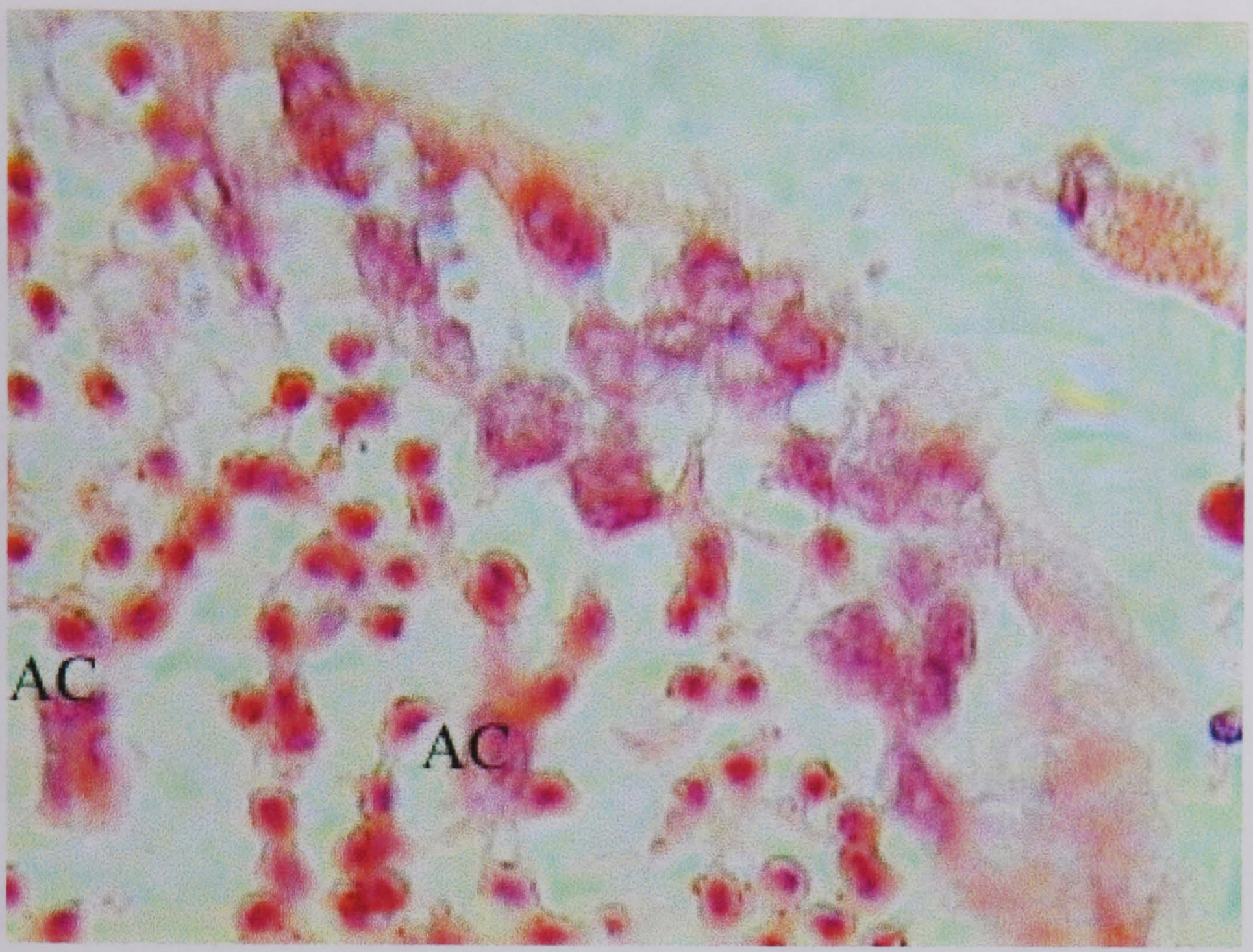


Figure 2.3b: The wall of an acinus from the testis of *Nephrops norvegicus* along with the previously described spermatogonia and spermatocytes, some larger darker staining cells can also be seen inside the acinus, these are accessory cells (AC). (H & E, x 40).

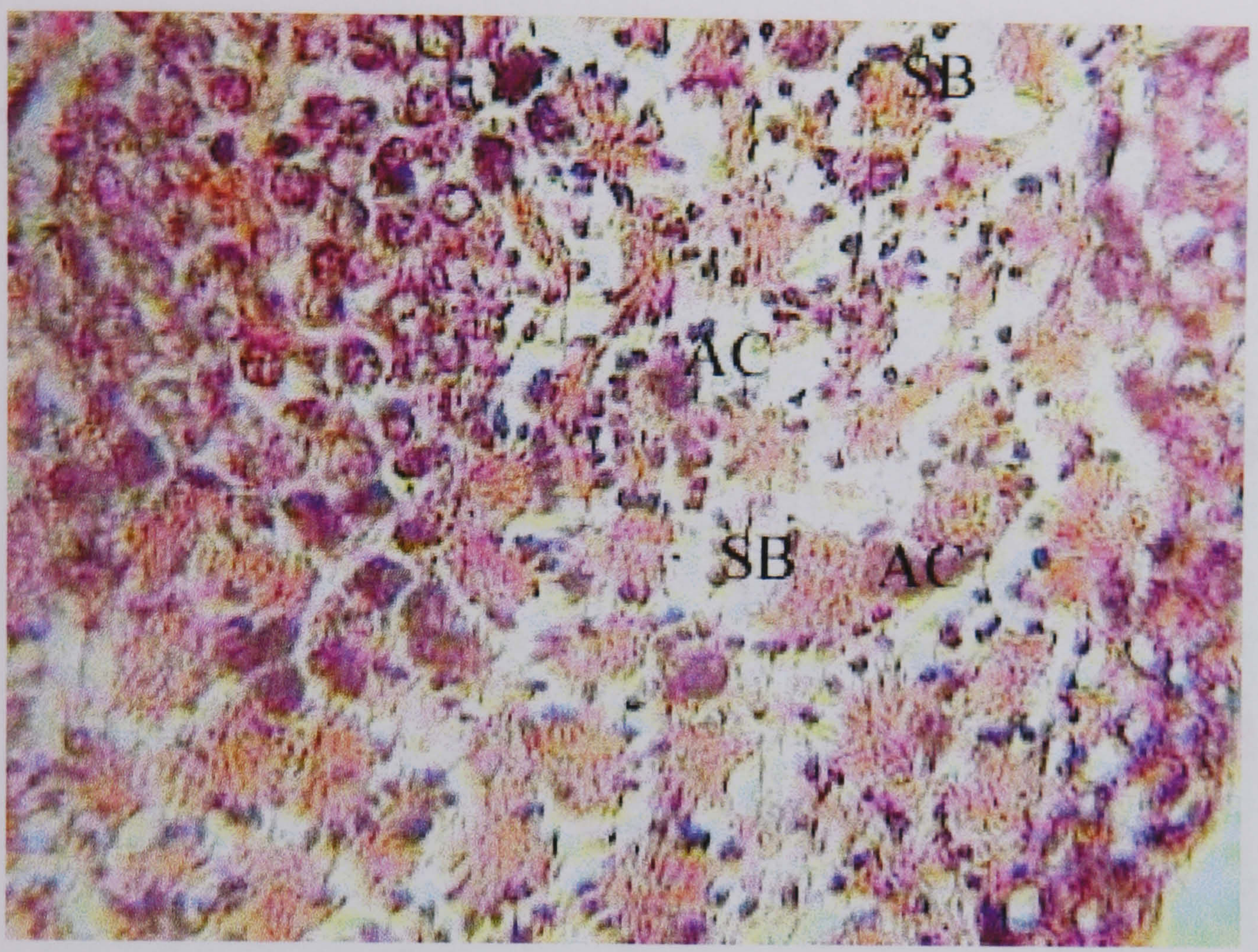


Figure 2.4: A transverse section showing apparent association between spermatozoa and accessory cells. There appear to be bundles of spermatozoa (SB) attached to accessory cells (AC) within an acinus. This could represent the stage of development prior to the expulsion of the spermatozoa into the lumen of the tubules. (H & E, x 40).

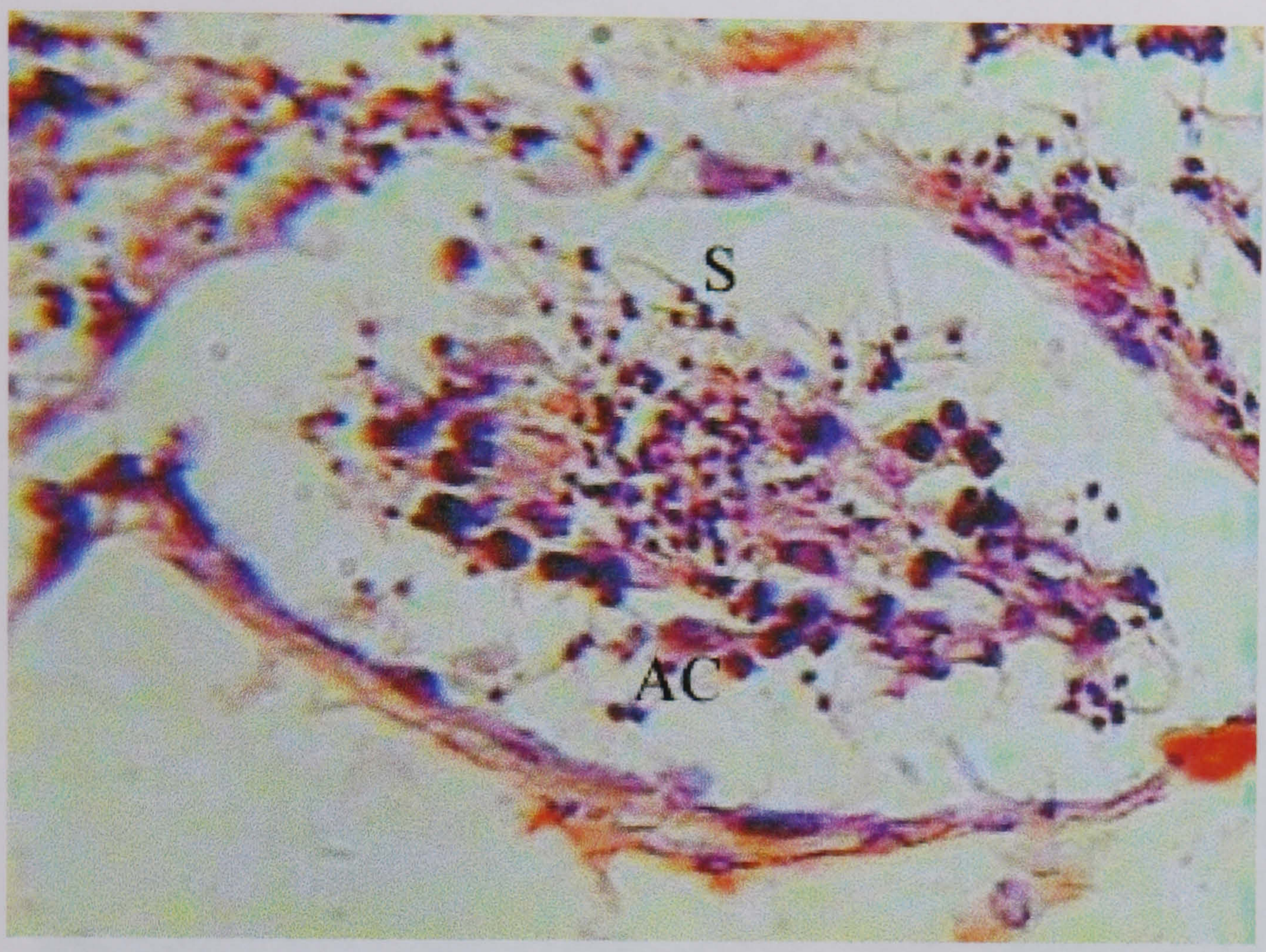


Figure 2.5: Spermatozoa (S) and unidentified cellular matter, which has been ejected from an acinus in the testes of *Nephrops norvegicus*. The accessory cells (AC) can still be seen in association with the spermatozoa. (H & E, x 40)

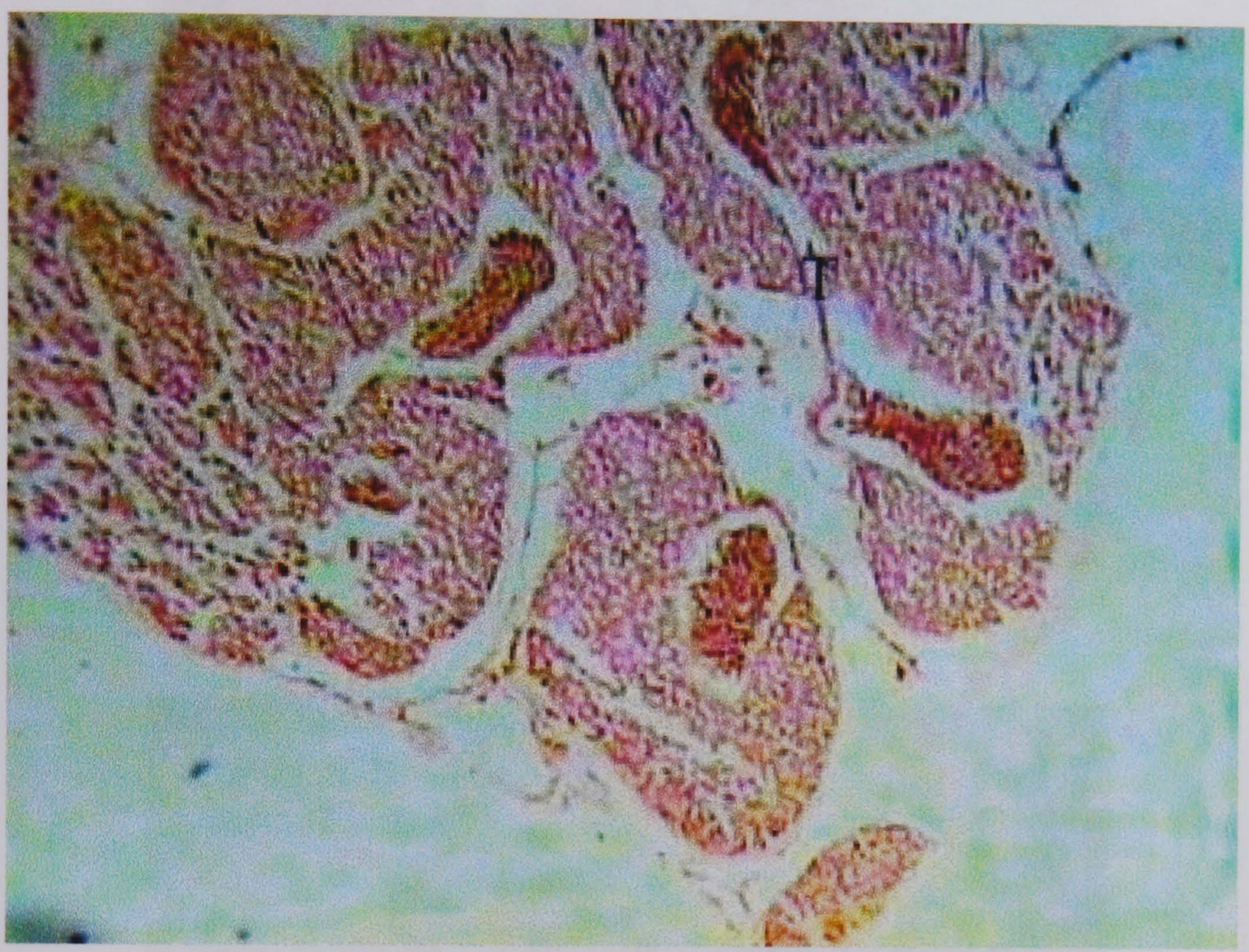
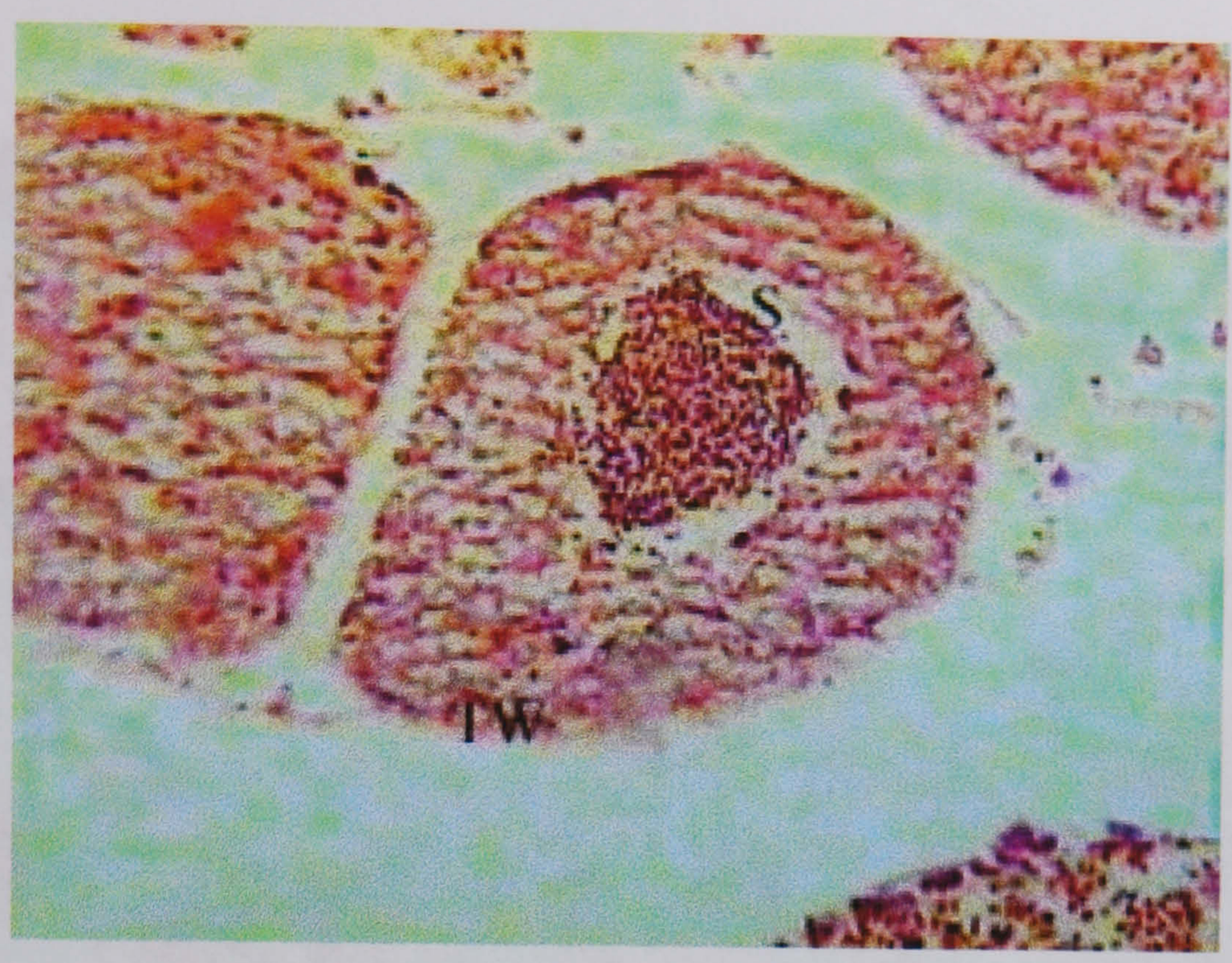


Figure 2.6: The convoluted collecting tubules in the testis of *Nephrops norvegicus*. A bend in the tubule (T) is seen in cross section, these features were common in all of the testis sections examined. (H & E, x 4)



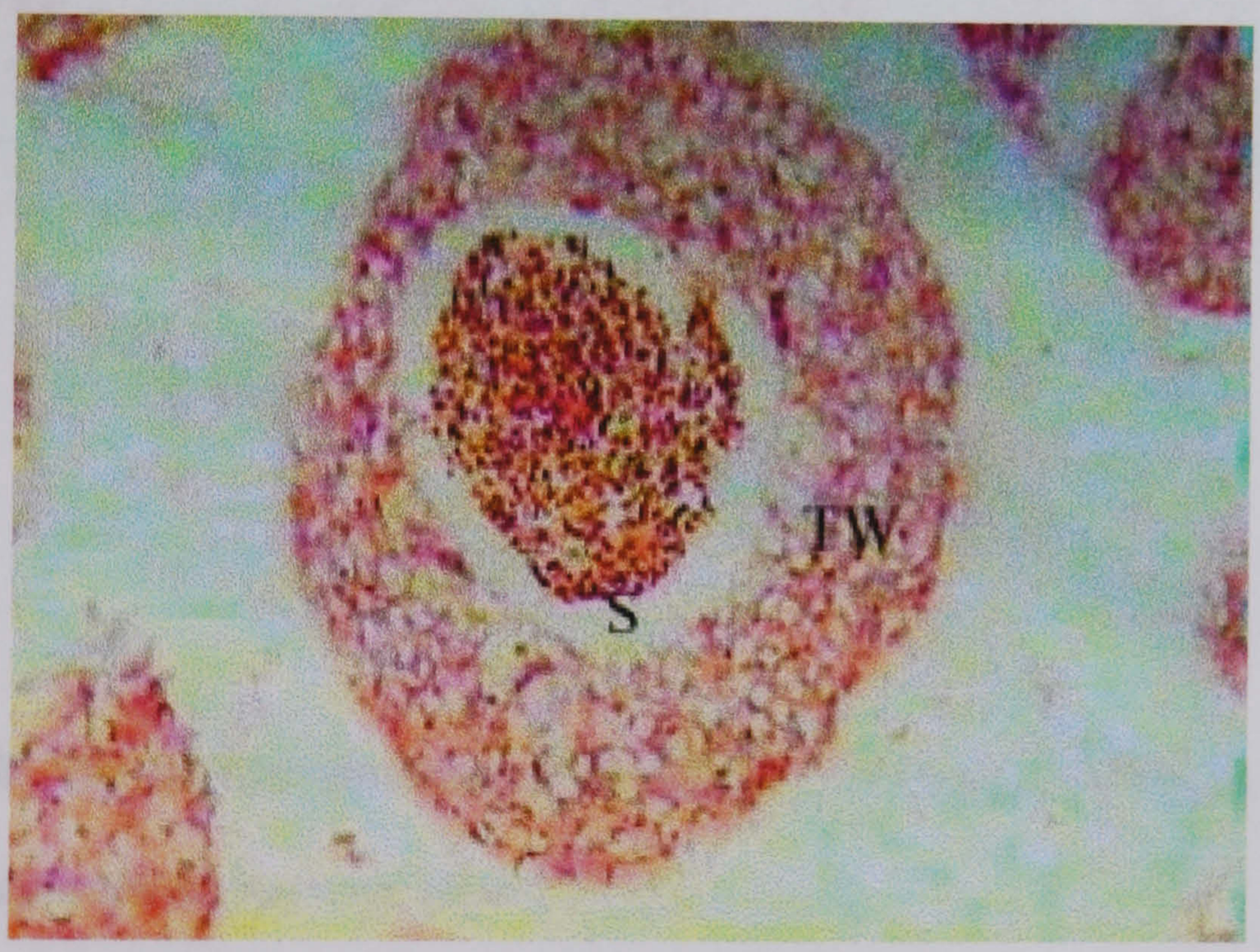


Figure 2.7: Two cross sections of collecting tubules taken from the testis of different male *Nephrops norvegicus*. The tubule wall (TW) is quite thick, although does not consist of spermatogonia. Spermatozoa (S) can be seen in the lumen of the tubules, the cells are quite densely packed. (H & E, x 10)

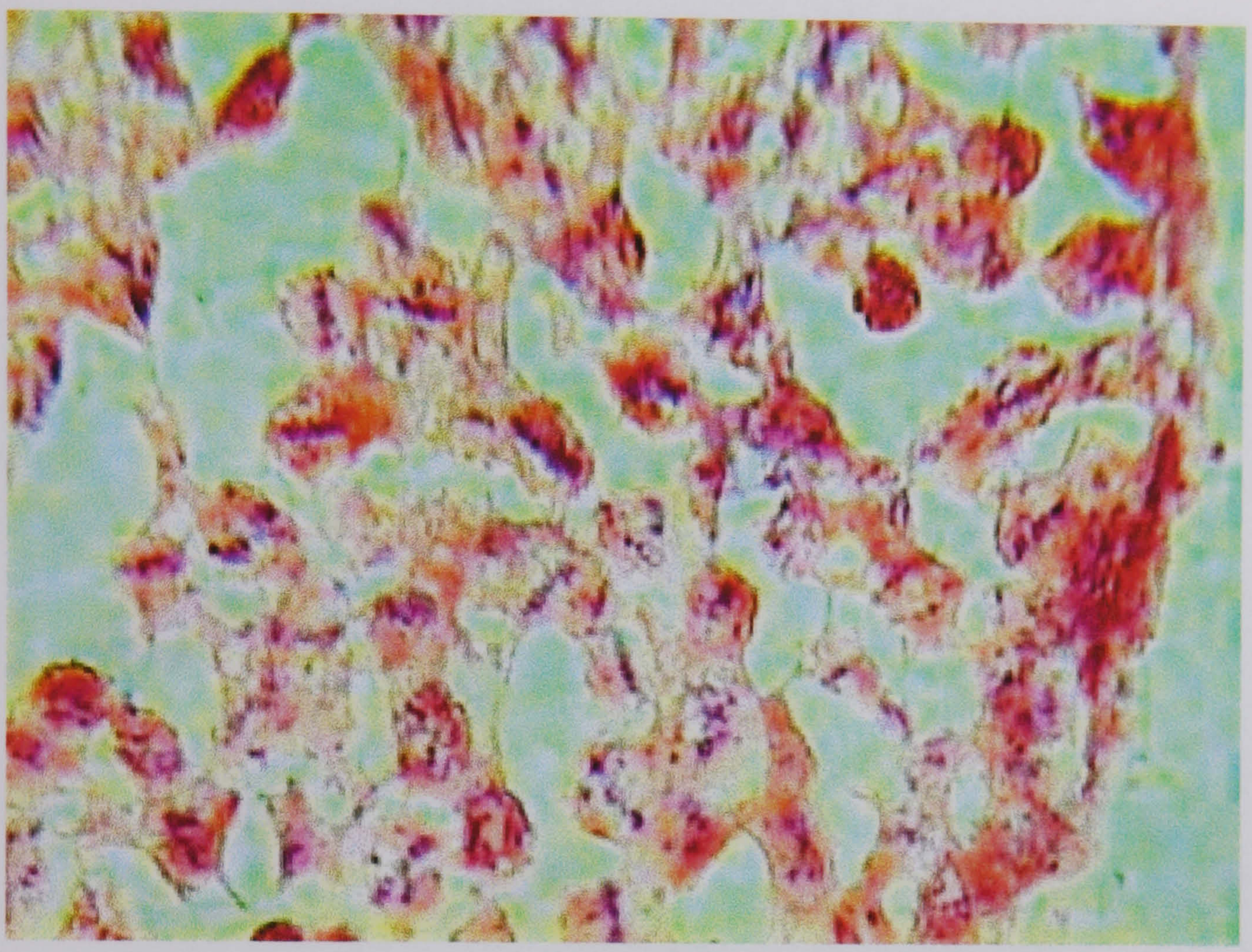


Figure 2.8: The cellular structure of an area of collecting tubule wall, from the testis of *Nephrops norvegicus*. (H & E, x 40)

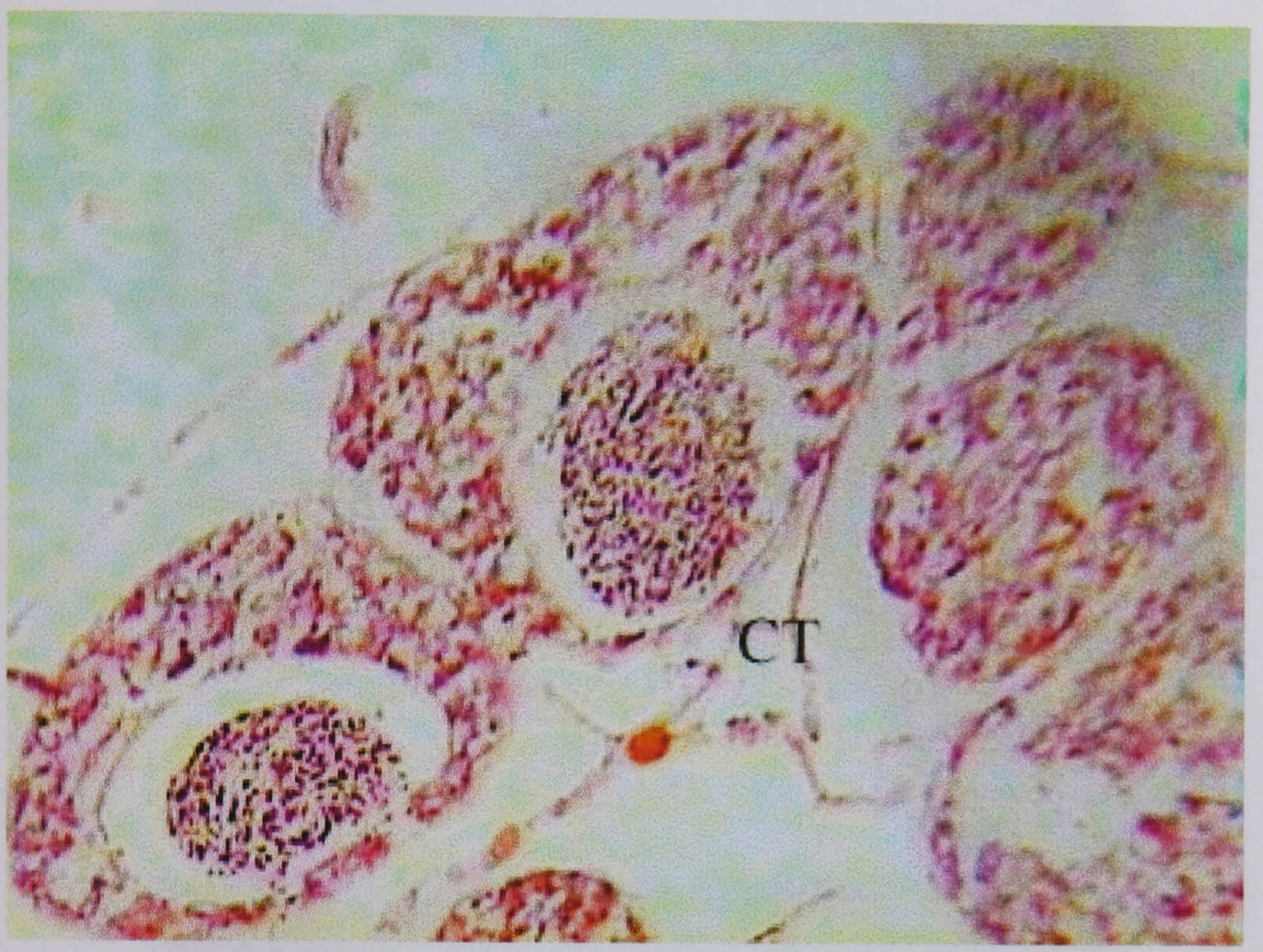
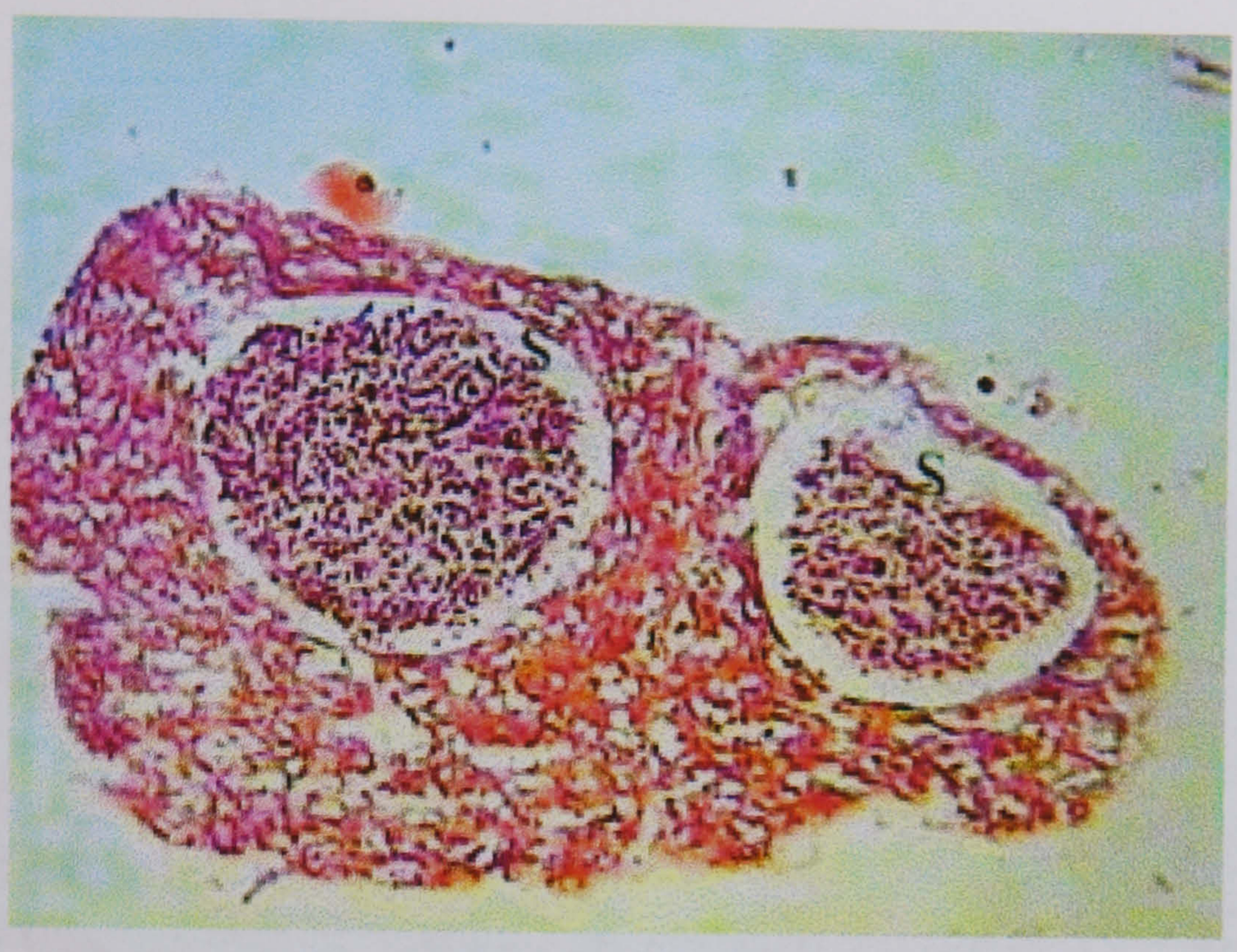


Figure 2.9: Connective tissue (CT) can be seen associated with the collecting tubule in the testis of *Nephrops norvegicus*, although processing and staining the testes may have caused it to dissociate from the organ. (H & E, x 10).

a)



b)

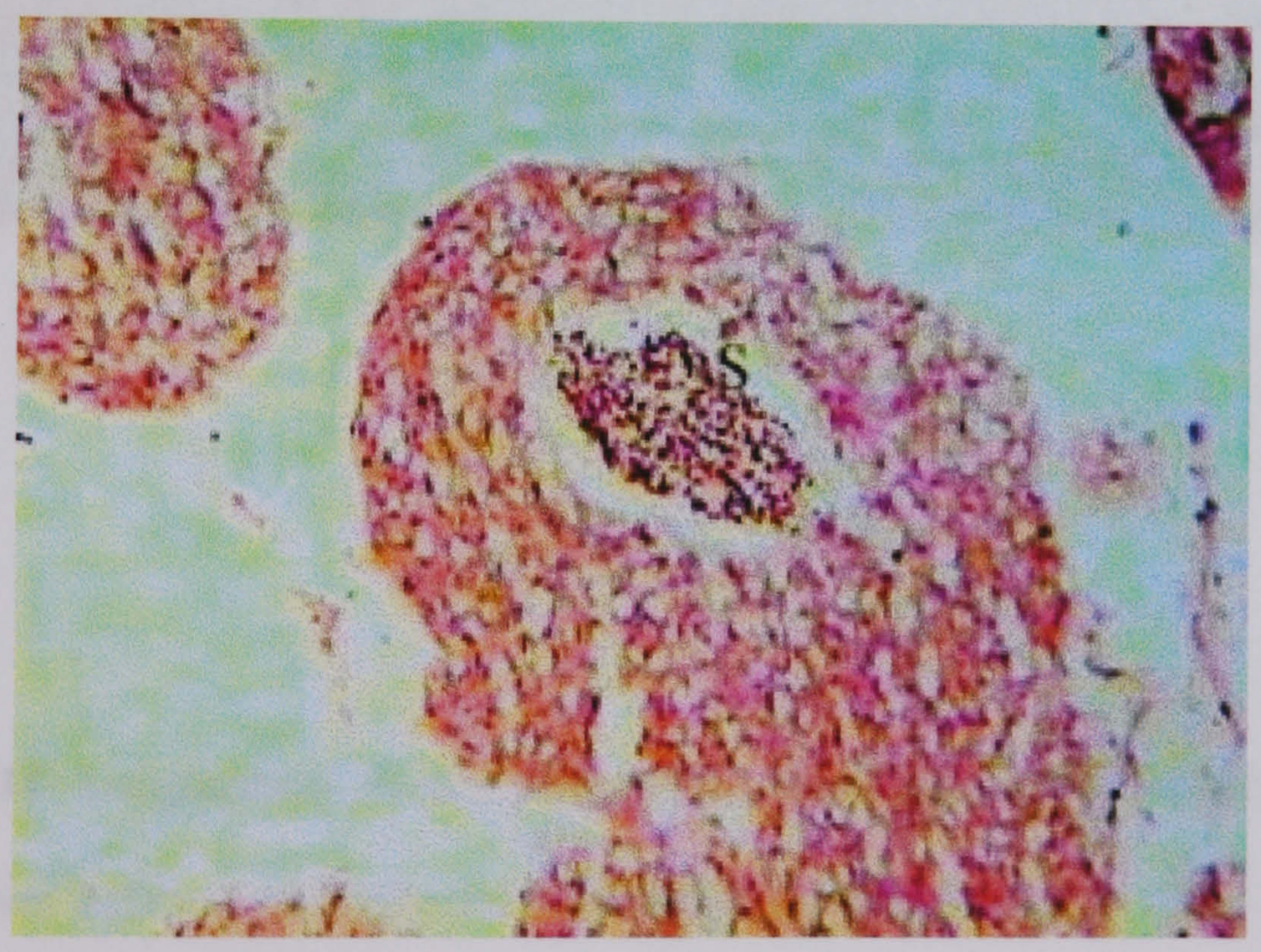


Figure 2.10: Tubules from a) the anterior right testis and b) the posterior left testis of *Nephrops norvegicus*. There are no discernible differences between the right and left testis, or between the anterior and posterior portions of the testis (as divided from the vas deferens). (H & E, x 10).

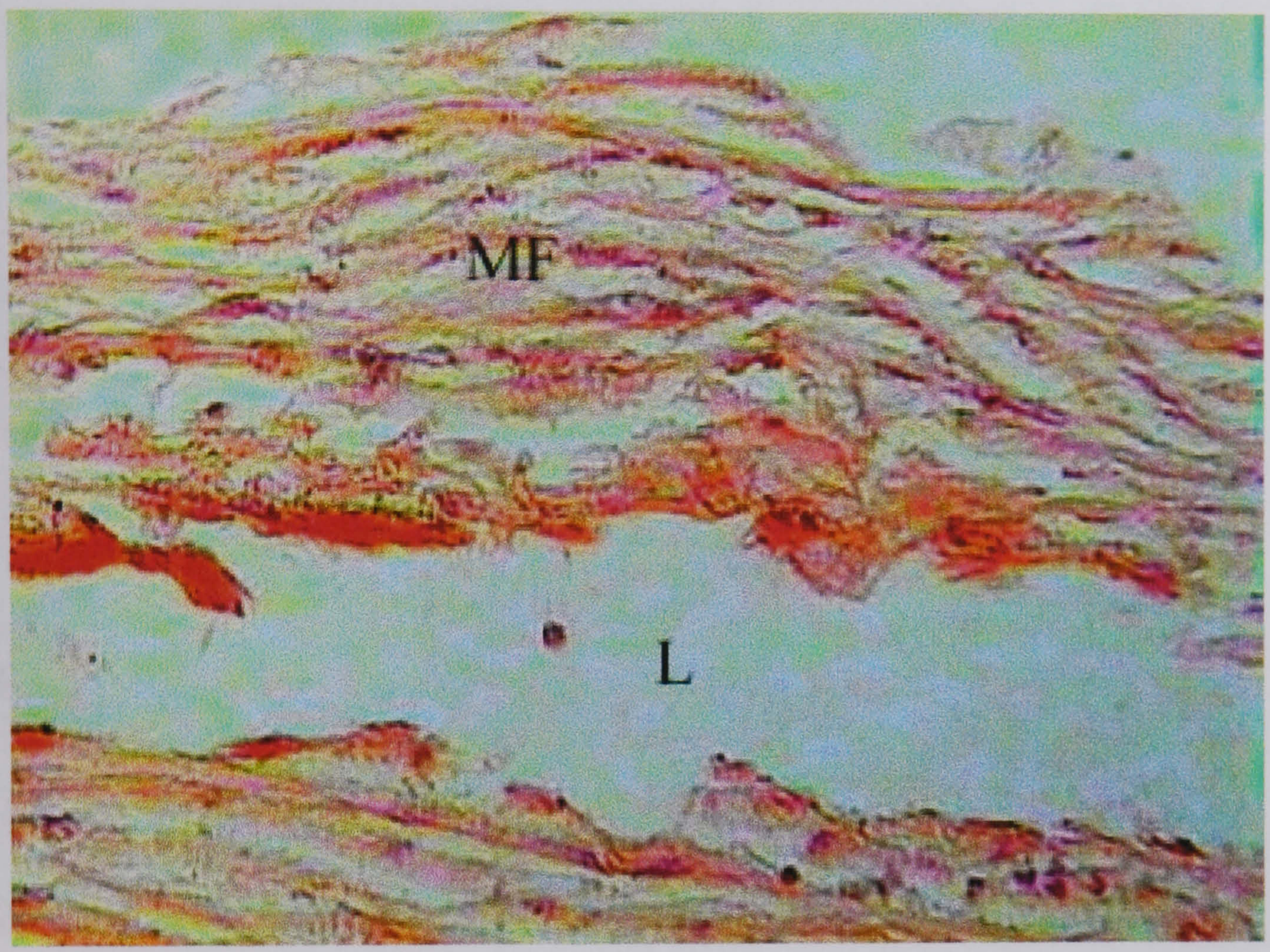


Figure 2.11: Longitudinal muscle fibres (MF) either side of the lumen (L) of the proximal vas deferens of *Nephrops norvegicus*. (H & E, x 40)

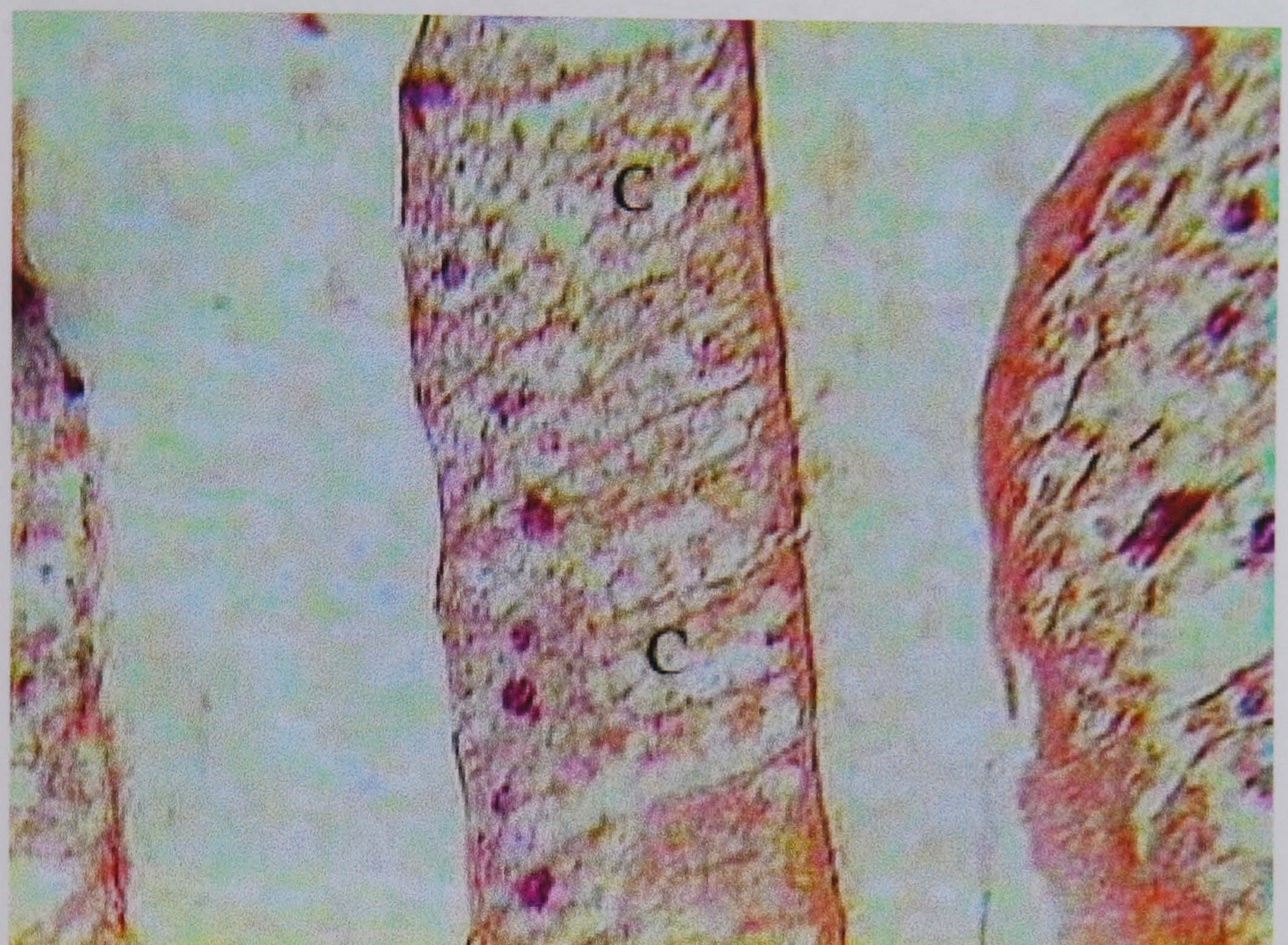


Figure 2.12a: Longitudinal section of columnar epithelium from the proximal vas deferens of *Nephrops norvegicus*. Individual tall epithelial cells (C) can be seen. (H & E, x 40).

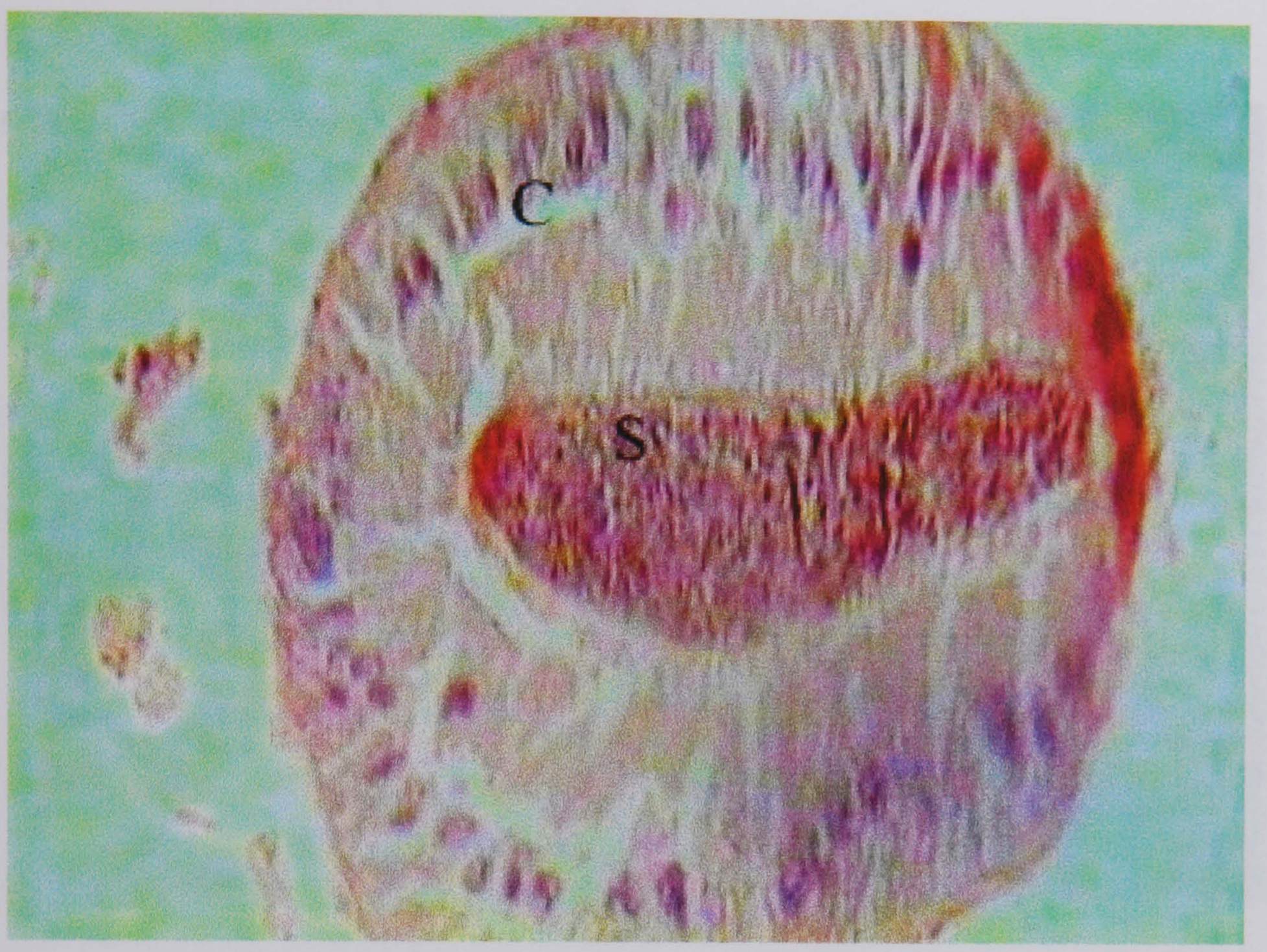


Figure 2.12b: Transverse section of the proximal vas deferens of *Nephrops norvegicus*. Columnar epithelial cells (C) can be seen surrounding the lumen of the vas deferens which contains spermatozoa (S). H & E x 10.

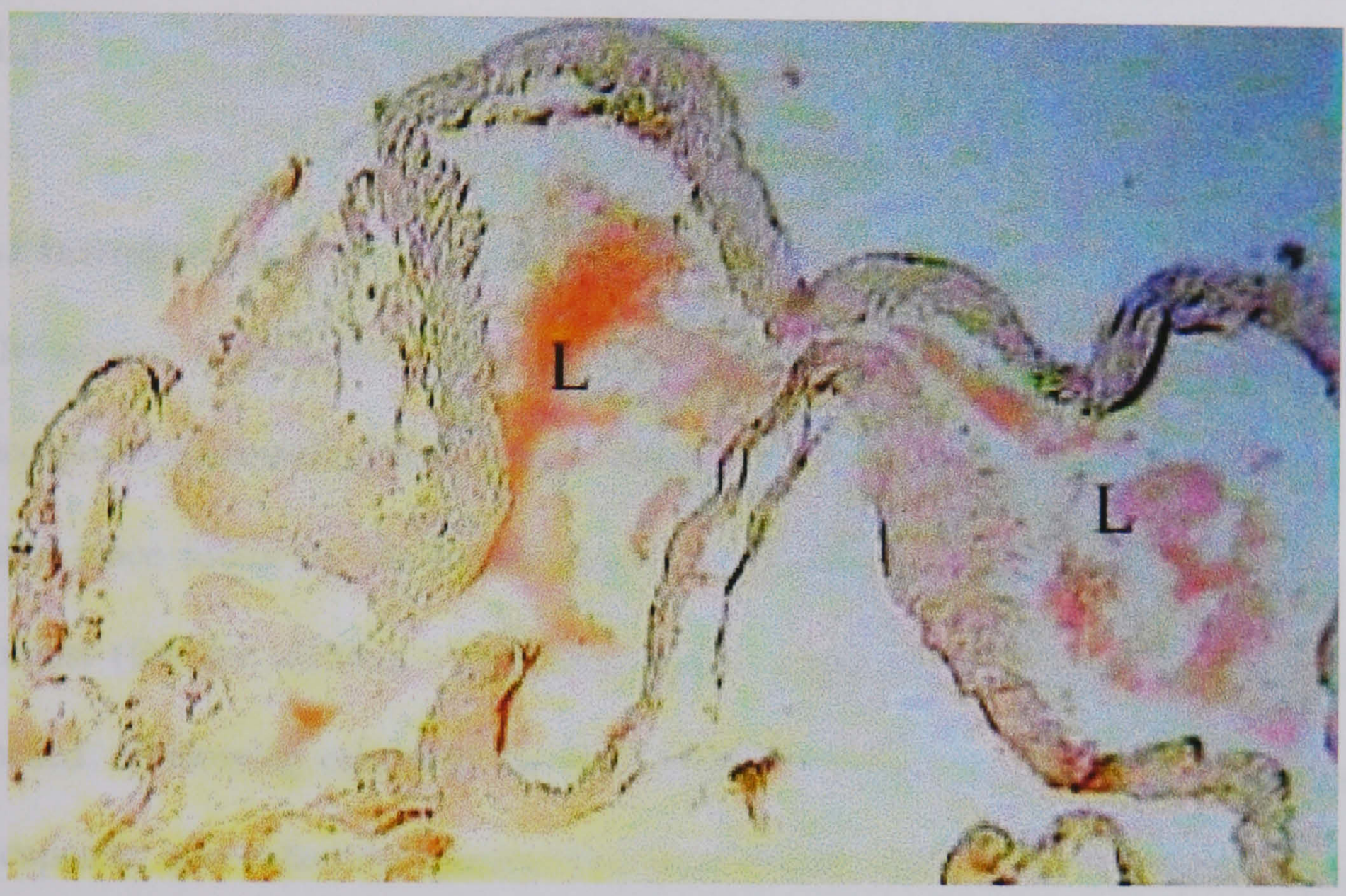


Figure 2.13: A 'string of pearls' seen in the proximal vas deferens of *Nephrops norvegicus*. This represents packets of material that are being passed along the lumen (L) of the vas deferens separately by musculature contraction to give this effect. (H & E, x10).

Chapter 3

Determination of cyclical production in the testes of *Nephrops norvegicus* (L.).

3.1 Introduction

The annual reproductive cycle of *Nephrops norvegicus* in the Clyde Sea area incorporates a breeding season from May to September. Mating in *N. norvegicus* takes place following the female moult, which may occur from May through to August (Farmer, 1974a). Copulation takes place while the female is still soft shelled. Following courtship the male transfers a spermatophore to the seminal receptacle, also called the spermatheca or thelycum. The inseminated females then carry the spermatophore through ovarian maturation until egg laying, which occurs in August and September (Farmer, 1974a). The method of fertilisation is as yet unknown in *N. norvegicus*. Farmer (1974a) suggests that fertilisation in *N. norvegicus* must occur internally because the spermatheca is completely closed to the external environment, and the spermatophore itself is stored at the point furthest from the opening of the thelycum. Silberbauer (1971) discovered two very fine paired ducts in mature hard shelled female spiny lobsters (*Jasus lalandii*), but not in newly moulted females suggesting that these structures were seasonally developed and resorbed during the period of incubation. Farmer (1974a) also discovered such ducts in a female lobster (*Homarus gammarus*) and suggests that internal fertlisiation may occur in the Nephropidae, Astacidea and Parastacidae. Following laying, the eggs are carried on the pleopods of the females until

April/May when hatching commences. This hatching period may last into June (Farmer, 1974a). During the incubation period ovigerous females remain largely in their burrows.

The ovary undergoes a pronounced and well-described development prior to egg laying, with an increase in the proportion of the body weight represented by the ovary, of almost a factor of three (Tuck *et al.*, 1997b). There does not, however, appear to be a concurrent development of the testes. Production of spermatophores occurs throughout the year in Irish Sea populations (Farmer, 1974a). However, it has been reported for Portuguese stocks that, although spermatogenesis occurs all year round, spermatophore production occurs only from July onwards (Figueiredo & Barraca, 1963). In Irish Sea populations, histological analysis revealed no pattern of increased spermic activity over the course of the year (Farmer, 1974a). Studies on *N. norvegicus* from the West Coast of Scotland have also reported no cyclical development of the testis, where dry weight measurements remained constant throughout the year (Bailey 1984). It is suggested, however, that there may well be seasonal changes in the testis that are not associated with weight change (Bailey, 1984). This also appears to be the case for male *Metanephrops* (as *Nephrops*) *andamanicus* in the southern hemisphere where investigations found no evidence for changes in the spermatogenic activity over the course of the year (Berry, 1969).

Male crustaceans show a number of reproductive strategies. In many species with a discontinuous breeding season, for example in the spiny lobster *Panulirus*

japonicus (Minagawa, 1999), the freshwater crabs *Barytelphusa cunicularis* (Diwan & Nagabhushnam, 1974), and *Potamon koolooense* (Joshi & Khanna, 1982), and the prawn *Penaeus indicus* (Subrahmanyam, 1963), there is a distinct seasonal peak in the productive activity of the testicular tissue. There are also examples of continuous breeders, for example the subterranean shrimp *Palaemonetes antrorum* (Strenth & Longley, 1990), and the red crab, *Geryon maritae* (Melville-Smith, 1987), in which the testes do not show cyclical activity. Other species have a distinct breeding season, yet show continuous levels of spermatogenesis in the testes, for example; the American lobster, *Homarus americanus* (Aiken & Waddy, 1980) and also *Metanephrops andamanicus* in South African waters (Berry, 1969).

A number of methods have been used to investigate seasonal development of the testes. A gonadosomatic index can be applied to determine any changes in the weight of the reproductive organs relative to the weight of the animal, as used by Bailey (1984) to investigate changes in the testes of *N. norvegicus*. Other techniques include histological methods, which have been used to observe cyclical activity in the spiny lobster *Panulirus japonicus* (Minagawa, 1999). This methodology allows observation of any changes at the cellular level within the testes. It relies on the assumption that an increase in production will be accompanied by an increase in the number of germinative cells within the seminiferous tubules. Biochemical analyses have also been used to determine the maturation cycle of the ovary of *N. norvegicus* (Tuck *et al.*, 1997b) and of the testes of the freshwater crab, *Barytelphusa cunicularis* (Diwan & Nagabhushnam,

1974). In both of these studies it was shown that the development of the gonad was accompanied by an increase in the protein content of the tissue. Castille & Lawrence (1989) also showed that the testes of developing male *Penaeus setiferus* contained significantly less protein than the testes of mature individuals. Increases in protein synthesis have also been recorded in crustacean muscle tissue associated with the moult (El Haj & Houlihan, 1987). It might be expected that a concurrent increase in RNA levels would be seen in tissue that was going through a phase of increased cellular production.

The aim of this study was to investigate any seasonal change in the reproductive activity of the testes to determine if males exhibit increased sperm production during the breeding season. Within the context of the fishery for *N. norvegicus* this is important as males are subject to greater fishing pressure than females throughout most of the year because of behavioural differences.

3.2 Methods

Male *Nephrops norvegicus* were collected from south of Little Cumbrae in the Clyde Sea area (55.41°N, 4.56°W) from March to October 2000 and November 2001 to February 2002. To allow comparisons with other fishing grounds, sampling was also carried out at sites around the Scottish coast (the North and South Minch on the West Coast and the Fladen ground in the North Sea, see Figure 1.1, Chapter 1) during the months of June and September 2000 and 2002. Within each fishing ground samples were taken from areas with different sediment types (Table 3.1). All animals were trawl caught, thus avoiding any physiological differences between samples (see Harris *et al.*, 1997). Carapace length (the distance from the posterior margin of the orbit to the midline of the posterior carapace edge) was measured using dial calipers for each male sampled. Animals of ~ 30mm carapace length (CL) and greater were selected to ensure that individuals were mature. Farmer (1974b,c) estimated that animals in the Irish Sea were physiologically mature at less than 18mm CL and morphometrically mature at 26mm CL. More recently, physiological maturity based on the presence of spermatophores in the vas deferens has been recorded at 15.1 mm CL and morphometric maturity at 25.9 – 31.0 mm CL (N. McQuaid pers. comm.). These definitions of maturity are described in greater detail in Chapter 4.

On each sampling day, the testes of 15 individuals were dissected as soon after capture as was practicable, for both histological and biochemical analysis. Samples of both testes and abdominal muscle were taken for biochemical analysis. Muscle tissues were taken to provide baseline data for each animal on the assumption that

there would be no biochemical changes in the abdominal muscle as a result of testicular maturation. Care was taken to select samples only from the deep abdominal flexor muscles to ensure that the sampling methodology was uniform. Each individual was also moult-staged using the pleopod method of Aiken (1980), and any animals in late premoult were rejected from the analysis. In order to examine the relationship between testes size and animal size, wet and dry weights of both the testes and the whole animal were taken. The testes were frozen at -20°C and then dried in a freeze drier for 48 hours. Whole animal dry weight was determined by oven drying at 80°C for 48 hours.

To establish the presence of any seasonal pattern in spermatophore production the vasa deferentia were also examined for the presence of spermatophores by a squash method. One of the vasa deferentia was removed from each male sampled and compressed between two glass slides and the contents examined under a compound microscope for the presence of spermatozoa.

3.2.1 Histology

Histological samples were fixed in 10% formalsaline for at least 12 hours prior to dehydration through graded alcohol (one hour in each of 30%, 50%, 70%, 90%, and twice in 100%). The samples were then embedded in paraffin wax and cut into 7 micron sections. Sections were stained using Haematoxylin and Eosin before analysis. Analysis was carried out using a digital camera attached to a compound microscope with an image capture programme (PCImage) to capture images of the seminiferous tubules. These images were then analysed using a computer package

(Scion Image) to measure the diameter of seminiferous tubules and also of the lumen (Figure 3.1). The methodology of Minegawa (1999) was used to calculate the ratio of the cross sectional area of the lumen of the seminiferous tubules to that of tubule itself (lumen area/tubule area x 100 = RAL). For each of the 15 animals dissected on each date 10 or more images were obtained (where possible) and the RAL was calculated. An average value of RAL was then calculated for each individual to determine any relationship between carapace length and RAL within each month. This methodology was also used to observe the relationship between the area occupied by spermatozoa and both tubule area (sperm area/tubule area x 100 = RAS) and lumen area (sperm area/lumen area x 100 = RLS).

3.2.2 Biochemistry

Samples were stored at -20°C (some at -70°C) prior to analysis. Biochemical analysis was carried out to determine the protein and RNA content within the testes throughout the year. Protein analysis was carried out using a colorimetric method based on the Lowry method (Pierce) and RNA analysis using the Orcinol method (Munro & Fleck, 1966). Samples of testes and abdominal muscle (25µg fresh weight) were placed in 4ml 0.5M NaOH and incubated in a water bath at 50°C for 90 minutes, or until all the tissue had dissolved. The samples were then centrifuged at 3000g and 0°C for 15 minutes. For protein analysis 0.02ml samples of the supernatant were added to 200µl of the colorimetric reagents (50 parts A to 1 part B, Pierce) in a microwell plate. The plate was then agitated on a plate shaker for 30 seconds before incubating on a hotplate at 37°C for 35 minutes. The absorbance of each of the samples was then read on a Titertec Multiscan©

MCC/340 plate reader at 680nm. Standards were prepared using stock bovine serum albumen (BSA) diluted in 0.5M NaOH. The mean standard curve is shown in Figure 3.2.

For RNA analysis, 1ml of sample was added to 1 ml 0.5M HCl and 2ml orcinol reagent (120mg orcinol dissolved in 20ml of 20mg $FeCl_3$, in 100ml conc. HCl) was added. A standard stock solution was prepared by dissolving 5mg RNA in 5ml 0.5M NaOH, this was then made up to 10ml with 1M HCl. Standards were prepared from a dilution of the stock to 10ml in 0.5m HCl. The samples and standards were then placed in closed tubes in a heating block at 90°C for 30 minutes before cooling to room temperature. The absorbance of standards and samples was then read on a Shimadzu UV1201 spectrophotometer at 665nm. The mean standard curve is shown in Figure 3.3. In addition to this curve an internal standard was also assessed using measured amounts of pure RNA, which confirmed the methodology. Selected testes and muscle samples were also freeze dried to determine their water content for calculation of the percentages of total protein and total RNA.

3.3 Results

Analysis of the data on testes weight and whole animal weight produced a linear relationship for both wet ($r^2 = 0.89$) and dry weight ($r^2 = 0.90$) (Figure 3.4). There were significant positive correlations between the weight of the whole animal in relation to the weight of the testes for both the wet and dry weights (Spearman rank correlation wet weight $r_s = 0.93$; dry weight $r_s = 0.941$; $p<0.001$ in both cases). In each case the slope was not statistically different from unity (wet weight 95% confidence intervals = 0.87 – 1.15; Dry weight 95% confidence intervals – 0.83 – 1.07). A significant correlation was determined between carapace length and tubule area ($p<0.001$; $r = 0.289$) (Figure 3.5). There were no significant relationships between the calculated ratio of tubule area to lumen (RAL, $p = 0.372$) and sperm area (RAS, $p = 0.221$), and the carapace length of any animal studied.

It was not possible to carry out all the histological sampling from the Little Cumbrae site during the same year; however, for ease of observation and comparison the data are displayed as though all were taken from the same 12 month period. Samples from March through to November were taken in the year 2000 while the December sample was collected in 2001 and January and February sampled in 2002.

Analysis of the vasa deferentia showed that spermatophores were present throughout the year. The results of monthly histological analyses showed that within each month there was a high degree of variability in RAL, but there was no significant correlation between RAL and carapace length (ANOVA $p = 0.372$, $r^2 =$

0.238) (Figure 3.6 & Table 3.2). No clear seasonal pattern in RAL was observed (Figure 3.7). Nor was there any significant relationship between carapace length and any of the calculated ratios for each month, apart from RLS in March where there was a highly significant negative correlation ($p = 0.003$, $r = -0.718$) (Table 3.2). There was less variability seen in the relationship between lumen area and sperm area, than that between tubule area and sperm area. Fifth order non-linear regressions (See Appendix 2) fitted to the data to detect any seasonal pattern produced very low R^2 values in all cases (RAL R^2=0.17; RAS R^2=0.07; RLS R^2=0.15) indicating that only a small proportion of the variance was described by the regression. This indicates that there is no seasonal relationship in the data (see Figures 3.7, 3.8 & 3.9). The two RLS data points available for December (Figure 3.9) were removed from the regression analysis, as they appeared to be outliers. This is probably due to difficulties that were experienced in the histological processing of the tissue samples from this month rather than any actual trend.

The water content of the abdominal muscles and testes was measured prior to biochemical analysis. The testes contained a higher water content (82.07% ± 1.37) than the muscle tissue (77.89% ± 0.89). The protein content of the testes and muscle averaged 0.29 $mg.mg^{-1}$ and 0.32 $mg.mg^{-1}$ dry weight, respectively, and generally did not deviate greatly from these values from month to month (Figure 3.10). The RNA content in both tissues was lower than the protein content and was relatively stable at 0.097 $mg.mg^{-1}$ in the muscle and 0.099 $mg.mg^{-1}$ in the testes (Figure 3.11). The pattern seen in both RNA and protein content of the muscle and testes over time was the same; there was no difference between either

of the variables nor were the slopes significantly different from zero. RNA concentrations appeared to be more variable than protein concentrations. Fifth order non-linear regressions (Appendix 2) were carried out on the data and again the R^2 values were low indicating that a small proportion of the variance was described by the regression (protein: muscle R=0.35, testes R=0.26, RNA: muscle R=0.26, testes R=0.26). This indicates that there was no cyclical pattern in the levels of protein and RNA in the muscle or testes

Samples of testis and abdominal muscle were also analysed from sites with varying sedimentology (Table 3.1) around the Scottish coast (Figures 3.12 & 3.13). The RNA and protein content of the testes and muscle were similar to those seen in the main study site in the Clyde. Protein contents (Figure 3.12) from the sites with fine sediments (mud, see Table 3.1) appeared to be higher than other sediment types (except the testes samples taken in the Clyde). Samples from sites with an intermediate sedimentology followed a similar pattern to the mud sites, although values from the Fladen were relatively low (Figure 3.12). Sites with coarse sediments (muddy sand, see Table 3) had the lowest protein content (Figure 3.12). The relationship between RNA content and sedimentology (Figure 3.13) appears to be more variable between sites and samples from coarse sediments are relatively higher than for protein (Figures 3.12 & 3.13). The RNA content of abdominal muscle particularly does not appear to display any relationship with sediment type (Figure 3.13). It would appear that there is a relationship between sediment type and protein content of the tissue; however this relationship is not as apparent in the RNA content of the tissue.

3.4 Discussion

The good correlation between testes weight and whole animal weight for both wet and dry tissue indicates that there was a high degree of accuracy in the measurement of wet weight and that the proportion of water is similar in animals of all sizes. These correlations indicate that testicular growth is isometric with that of the whole body growth. Initial analysis of the relationship between tubule area and carapace length within each month showed a slightly negative relationship, which suggested that the testes showed negative allometric growth. However, the isometric relationship between tubule area and animal size (Figure 3.5) negates the need for any transformation of tubule area relative to carapace length, as it is likely that tubule size is relative to testis size.

The results of histological analysis using the method of Minegawa (1999) showed that there was no seasonal pattern in the development of testicular tissue of *Nephrops norvegicus*. Although there appeared to be a pattern in the RAL data there was no biological reason to suspect seasonal change, and this was proven using non-linear regression (Appendix 2). Histological investigation of the testes of *N. norvegicus* showed that they were made up of numerous acini attached to collecting tubules which carry the spermatozoa at various stages of development to the vasa deferentia (see Chapter 2). The acini of *N. norvegicus* testes have also been shown to go through independent maturation cycles (O'Neill, 1992). Haley (1984) studied the testes of another nephropid lobster, *Enoplometopus occidentalis*, and reported a similar acinar structure to the testes. It was noted, however, that the acini showed a 'wave of development' along the length of the

testes similar to the mammalian seminiferous tubule. It is not yet known if this is the case in *N. norvegicus*, but the structure of the testes also indicates that constant production of spermatozoa occurs through the independent and non-synchronous development of the spermatogonia within each acinus. This theory concurs with the analysis of the vasa deferentia, which showed that spermatophores are present throughout the year.

The methodology of Minegawa (1999) using RAL to calculate testicular productivity, may not be well suited to the assessment of seasonality in *N. norvegicus*, due to the lack of germinative seminiferous tubules. The technique also proved to be problematic due to the convoluted nature of the collecting tubules. Few sections provided a true cross section of the tubule and in some cases no cross sections were seen and the sample was omitted from the analysis. In addition, the processing technique used prior to sectioning caused a certain amount of shrinkage of the tissue (Figure 3.14), which meant that in some cases the exact area of the tubules was difficult to determine accurately. The variation in RAL measurements between individuals in each month could indicate that this method is inaccurate or that the testes of *N. norvegicus* are made up of tubules with varying RAL.

As there was no evidence to show seasonal changes in RAL, the area of sperm within the tubules was examined. It would be expected that the area of sperm would increase relative to the area of the tubule and the area of the lumen if there were an increase in the productivity of the acini of the testes. There was no

relationship between the ratio of tubule area to sperm area (RAS) and carapace length, on any of the sampling dates. There was also no seasonal change in RAS, which could indicate that there is no change in acinar output. There was often a large size difference between the tubule size and the sperm area, however, so the area of sperm was investigated in relation to the area of the lumen. No relationship was observed between RLS and carapace length on any of the sampling dates apart from the sample taken in March where there was a significant negative correlation. It is unlikely that this correlation reflects any underlying biological function, however, as it is just an individual case. There was no cyclical seasonal relationship between RLS and time of year (Figure 3.9 & Appendix 2). The results could be affected by the relationship between tubule area and lumen area, which are unlikely to be completely independent, as the sperm area is restricted by lumen area. It is also possible that the density of sperm within the lumen of individual tubules could vary thus affecting the results. In this way observations of sperm area may prove to be unreliable.

It would appear from all these results that the tubules of *N. norvegicus* do not undergo any germinative changes during the year, which agrees with observations made on the gross morphology. The lack of seasonality also indicates that there is no increase in the output of individual acini to compensate for reproduction. This would indicate that individual males could be subject to sperm limitation in terms of the number of spermatophores they can produce, should they mate with many females over a short period of time.

Biochemical analyses confirmed previous reports that there was no seasonal change in the productivity of the testes. There was no overall change in RNA and protein in the testes throughout the year, nor were there changes in the abdominal muscle, also reported by Rosa & Nunes (2002), indicating that the analysis was not affected by other factors such as moult stage. The protein content of the testes and abdominal muscle was comparable with values reported for abdominal muscle and other tissues by Parslow-Williams (1998) and Parslow-Williams *et al.* (2002) for this species. The levels of RNA seen in the tissue are not comparable, however, being higher than were reported for the same stock by Parslow-Williams (1998). Thorough examination of the methodology of the RNA assay and repeated analyses of the results could not find any reason for this discrepancy. Re-calibration of the standards was carried out with an internal standard using Torula yeast RNA to determine that the assay was functioning in an accurate manner and the results provided were consistent with the prepared standards which showed a good standard curve (Figure 3.3).

It is likely that the greater degree of variability seen in the RNA data is due to differences in the accuracy of the techniques used. In some cases the samples of testis tissue had been contaminated with hepatopancreas tissue. This appeared to increase the levels of RNA recorded for such samples, it was not possible to quantify or qualify the underlying causes and therefore the samples were included in the analysis. Parslow-Williams *et al.* (2001) showed that levels of RNA were around three times higher in hepatopancreas tissue than in abdominal muscle tissue. As the RNA content of the testes is similar to that in the abdominal muscle (Figure

3.11) it is likely that contamination was the cause of the high levels of RNA in the testes recorded at sites in the South Minch (Figure 3.13).

The relationship between sedimentology and protein content of the tissues (Figure 3.12) indicated that there was greater productivity in the tissues of *N. norvegicus* found on fine mud sediments. It would be expected, however, that sites with higher growth rates would have higher protein contents, and these are usually associated with intermediate sediment types (Afonso-Dias, 1997), with the lowest productivity observed in areas with coarse sedimentology. There is not such a clear relationship between sedimentology and RNA content, however (Figure 3.13), and this may be due to differences between the assays as previously mentioned. It has been shown that there are differences in growth rates associated with the different densities of animals supported by different sediment types (Bailey & Chapman 1983).

A possible reason for the apparent lack of seasonality seen in this study is that there may be mating opportunities for males outwith the main breeding season. Bailey (1984) found that in the Clyde, small females showed a peak of moulting during the early summer with larger females moulting somewhat later in the year during the autumn. It was also reported, however, that females in the size class 21–30 mm CL showed peaks of moulting in both spring and winter as juvenile females approached maturity. Examination of soft juvenile females has revealed the presence of spermatophores in the thelycum (Bailey, 1984) indicating that males will mate with soft females throughout the year. Sardà (1991) has also

shown that mature moulting females are found, albeit in small numbers, all year round in the Catalan Sea.

The main breeding season of *N. norvegicus* is timed to allow ovary maturation following the moult. Egg laying is timed so that the main hatching period occurs when there is sufficient planktonic food for larvae. Should moulting occur outside this period, spermatophores would have to be stored by females prior to egg laying. The storage of spermatophores for long periods of time has been recorded for many decapod species, for example the giant crab *Pseudocarcinus gigas* (Gardner & Williams, 2002). However, juvenile females that are reaching maturity are likely to be moulting annually and therefore any stored spermatophores would be lost prior to the main breeding season. Alternatively, females maturing in the winter delay their next moult until after egg laying and hatching. Large females appear to be able to spawn two years in succession without moulting (C. Chapman, pers. comm), which could indicate longer term sperm storage, or possibly intermoult mating. For *Metanephrops andamanicus*, the breeding period in South Africa coincides with that of *N. norvegicus* in the Northern Hemisphere (Berry, 1969) at a time when (off the coast of South Africa) there would be reduced quantities of planktonic food for larvae. There is increasing evidence, however, that the larvae of *M. andamanicus* settle almost immediately after hatching (Berry, 1969). Immediate settlement would negate the requirement for a good supply of planktonic food and thus the time of hatching would not need to coincide with the spring bloom. The driving forces behind these different reproductive approaches in nephropids are as yet unclear.

There are many possible factors that influence the various reproductive strategies displayed by male crustaceans. Environmental influences are likely to be fundamental in the regulation of reproduction. In the spiny lobster *Panulirus argus*, photoperiod and temperature were found to affect the development of the female reproductive system, yet they had no effect on the testes (Lipcius & Herrnkind, 1987). The relationship between timing of breeding and latitude is related to cyclical maturation of the ovaries of *N. norvegicus*, as summarised by Sardà (1995).

As previously stated, seasonality of reproduction is likely to be related to seasonal change in the environment. Crustaceans found in environments with little seasonal influence, for example the deep sea or the tropics would be expected to show continuous reproduction. This is true for the deep sea red crab *Geryon maritae* (Melville-Smith, 1987), although, *G. fenneri* does show seasonal testicular development in deep water (Hinsch, 1988). In tropical waters, the male blue crab (*Portunus pelagicus*) does not show synchronous cyclical development (Batoy *et al.*, 1987) although individual males go through a distinct testicular cycle. The subterranean shrimp *Palaemonetes antrorum* is a continuous breeder; however, this is not the rule in such environments (Strenth & Longley, 1990). Although the testes of *N. norvegicus* from temperate Scottish waters would be expected to show a strong seasonal affinity, it can be seen that different reproductive strategies occur within apparently similar environments and there are no clear driving forces.

It would appear that male *N. norvegicus* are not able to increase production of sperm in the testes and therefore do not show a peak in reproductive output to coincide with the main period of female moulting. In an unexploited population this could be due to the relatively high density in which the Norway lobster is found in comparison with other lobsters. Under these conditions, it is possible that the chances of finding a partner of a suitable size in close proximity are good, but even so most males would be expected to have limited opportunities to mate due to intense competition. In heavily exploited populations, however, where there is a reduction in numbers of males, sperm limitation could occur in females due to a lack of suitable mates. The presence of reproductively active males throughout the year, however, may allow mating to occur outwith the main reproductive period.

3.5 Conclusions

- Histological studies have shown that there is no seasonal change in the germinative output of the testes of *Nephrops norvegicus* through the year.
- Biochemical analyses of the testes have shown that there is no seasonal change in the levels of protein and RNA in the testes of *N. norvegicus*.
- There is an apparent site-related relationship (e.g. differences in sedimentology) between levels of protein seen in the testes and abdominal muscle of *N. norvegicus*, although this does not follow the expected pattern as the highest productivity was observed on fine sediments. There is no concurrent pattern in the RNA analyses from different areas around the Scottish coast; however, this may be due to differences between the techniques used.

Site	Sediment Type	Silt Clay Content
Cumbrae	Mud	90 – 100%
Ailsa Craig	Sandy Mud	50 – 90%
Clyde 3	Muddy Sand	10 – 50%
Fladen 4	Mud	80 – 100%
Fladen 2	Muddy Fine	55 – 79%
Fladen 1	Muddy Coarse	40 – 54%
Fladen 3	Muddy Sand	10 – 39%
North Minch1	Mud	90 – 100%
North Minch 2	Sandy Mud	50 – 90%
North Minch 3	Muddy Sand	10 – 50%
South Minch 1	Mud	90 – 100%
South Minch 2	Sandy Mud	50 – 90%
South Minch 3	Muddy Sand	10 – 50%

Table 3.1: Sediment types at sampling sites from around the Scottish coast from BGS (1987) data and Fisheries Research Services data for the Fladen ground (Afonso-Dias, 1997).

	RAL		RAS		RLS	
Month	r	p=	r	p=	r	p=
January	-0.368	0.216	-0.231	0.448	-0.093	0.762
February	-0.036	0.899	-0.275	0.321	-0.211	0.451
March	-0.220	0.431	-0.273	0.324	-0.718	0.003*
April	-0.185	0.546	0.088	0.775	0.223	0.223
May	-0.281	0.311	-0.102	0.718	0.443	0.098
July	-0.067	0.837	-0.119	0.712	-0.161	0.617
Sept 1st	-0.009	0.975	-0.265	0.341	-0.120	0.671
Sept 13th	-0.244	0.250	-0.487	0.487	-0.127	0.680
Sept 20th	0.500	0.117	0.300	0.320	0.415	0.158
October	-0.195	0.487	-0.207	0.485	0.002	0.995
November	-0.279	0.315	-0.007	0.980	0.168	0.550

Table 3.2: Level of significance and r values for the Spearman Rank Correlation analyses on the relationship between carapace length and the ratio of the tubule to the lumen (RAL), the ratio of the tubule to the area of sperm (RAS) and the area of sperm in relation to the lumen (RLS). * Denotes a statistically significant result.

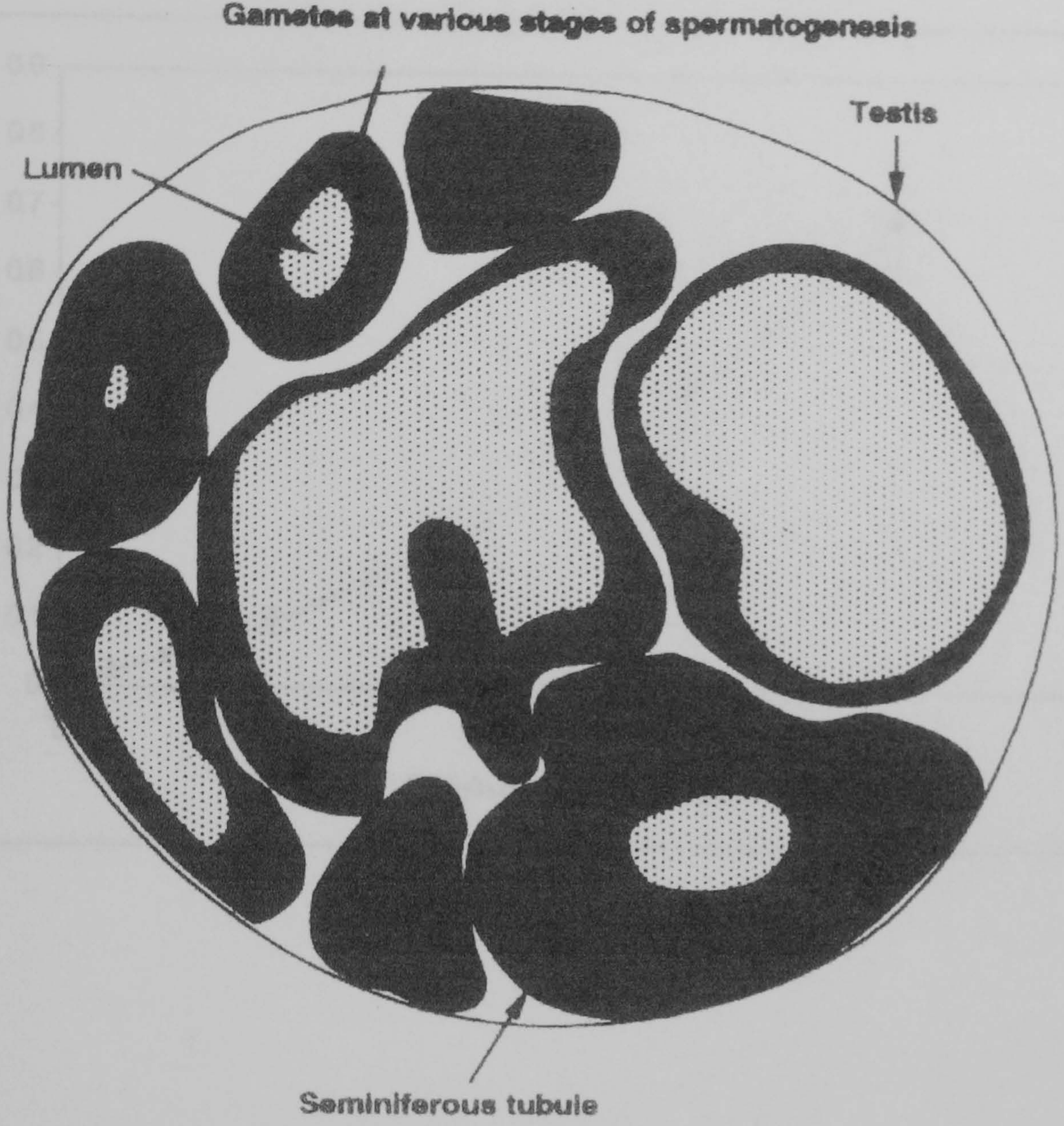


Figure 3.1: Diagram of the testis showing the tubules made up of gametes at different developmental stages (darkly shaded), the lumens are lightly shaded (from Minegawa 1999).

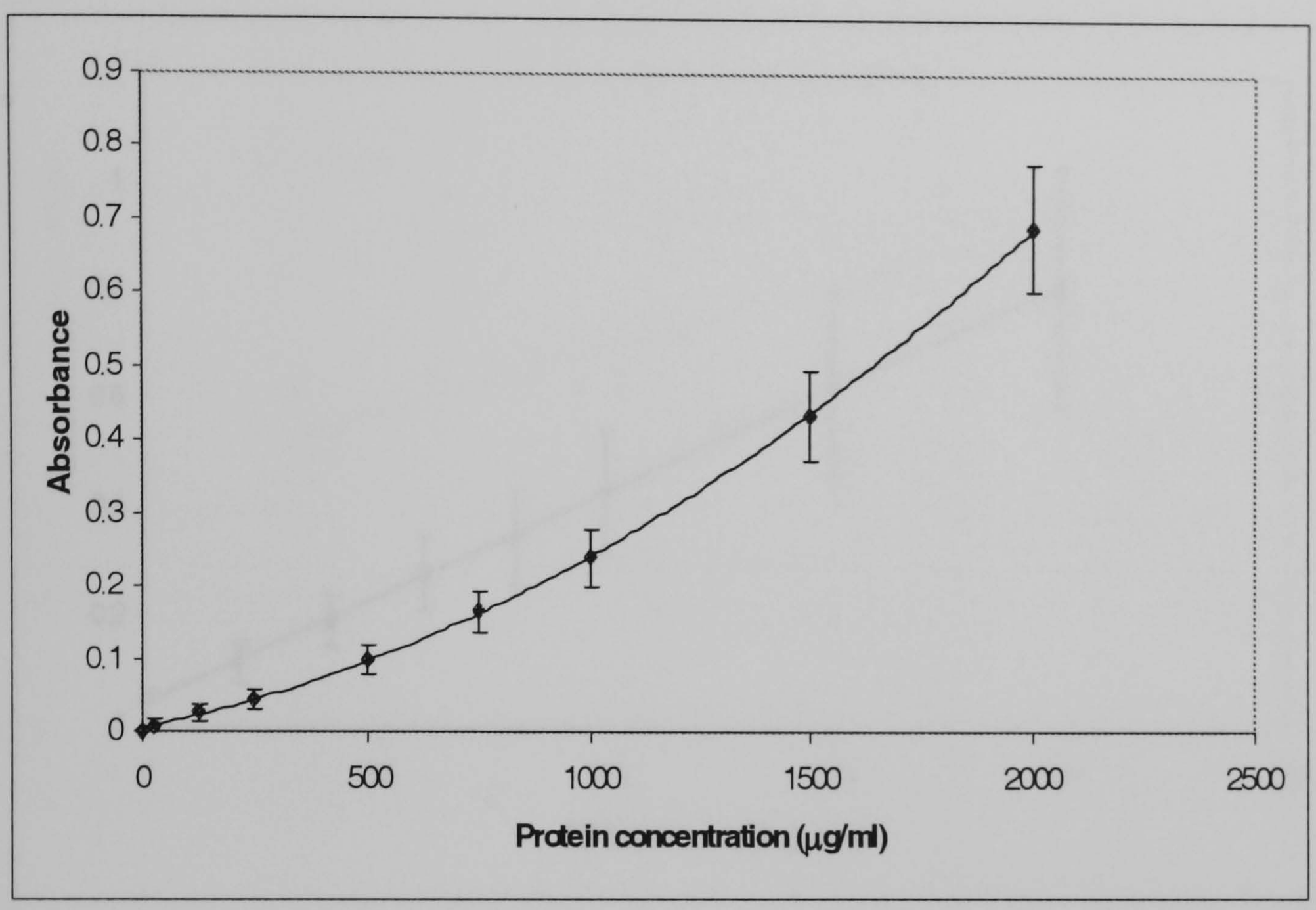


Figure 3.2: Protein analysis standard curve calculated using a Pierce assay kit. The standard curve is a mean (± S.D.) of the standards analysed concurrently with each sample analysis.

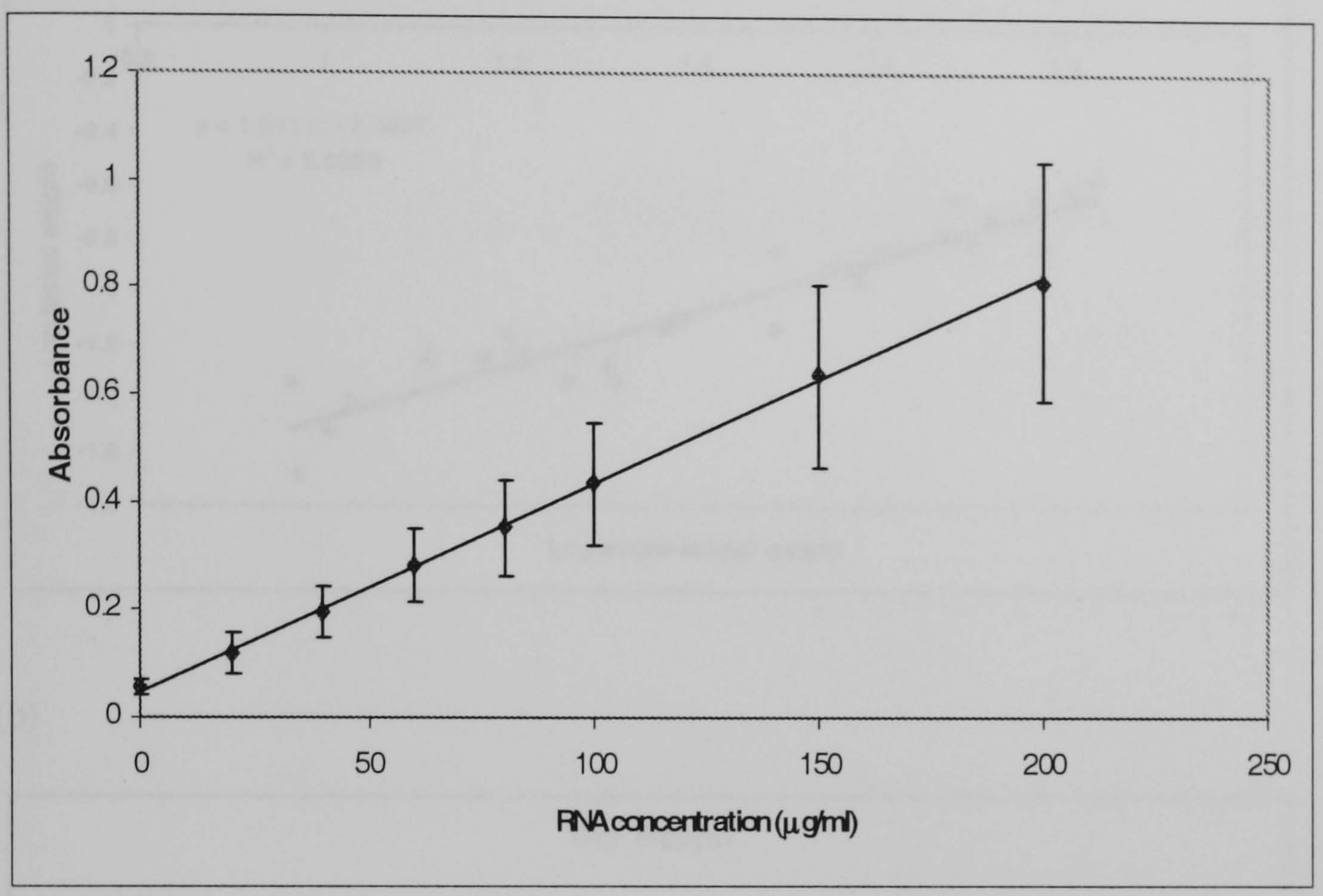
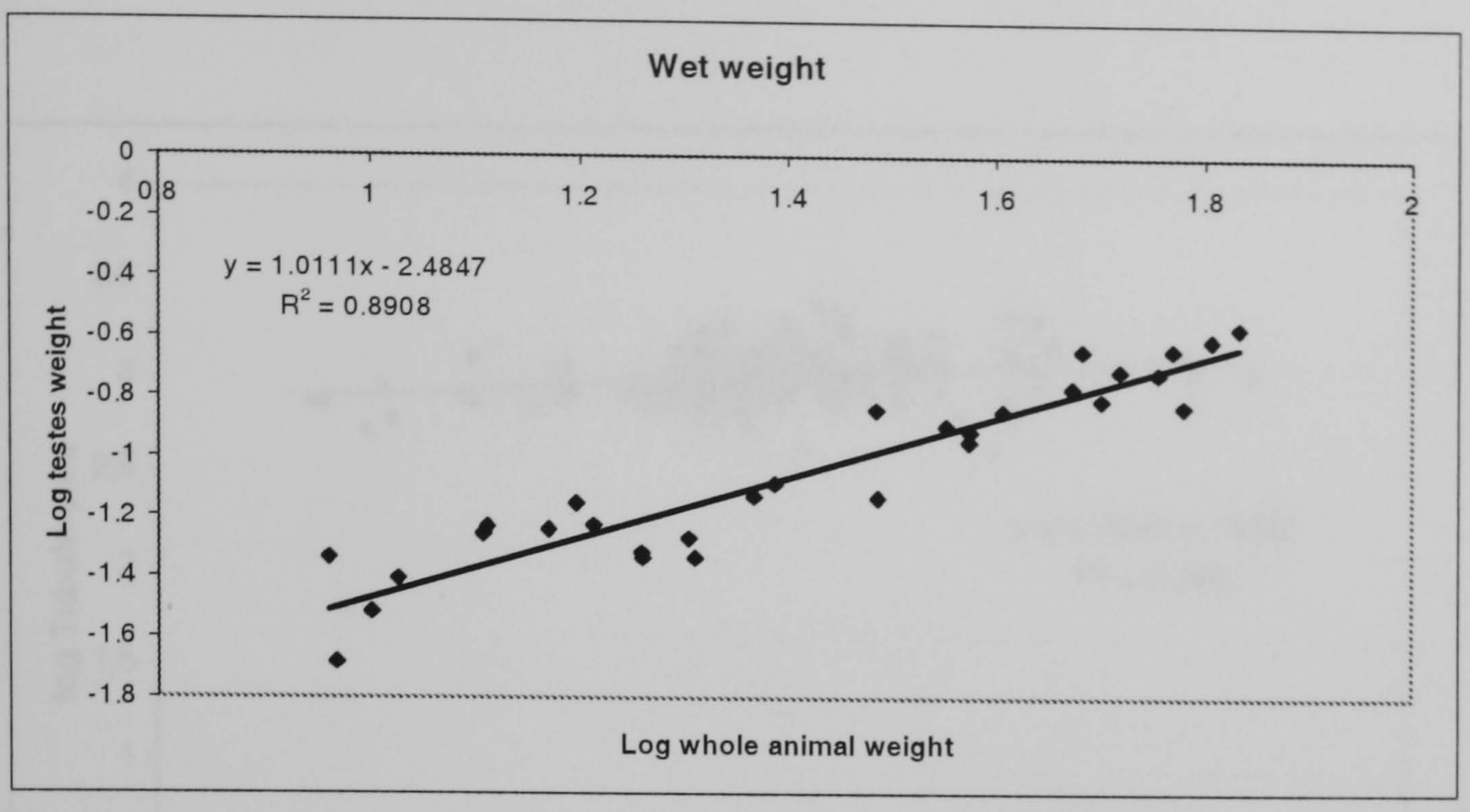


Figure 3.3: Calibration curve for Orcinol RNA assay, prepared using Torula yeast RNA (Sigma). The standard curve is a mean (± S.D.) of the standards analysed concurrently with each sample analysis.

(a)



(b)

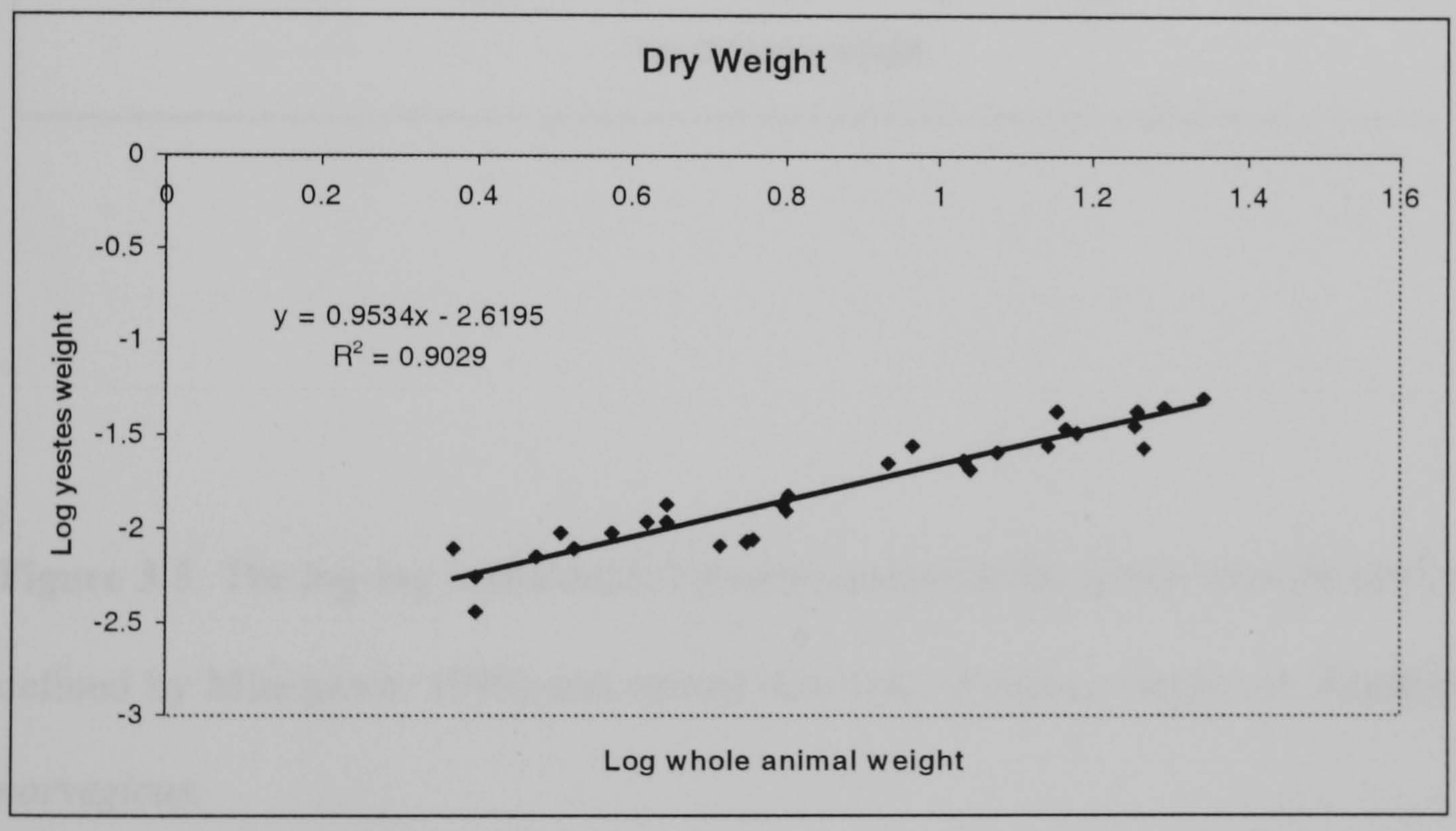


Figure 3.4: The log-log relationships between testes weight and whole animal fresh weight (a) and dry weight (b) of male *Nephrops norvegicus* from the Clyde sea area. In both cases the slope was not statistically different from unity.

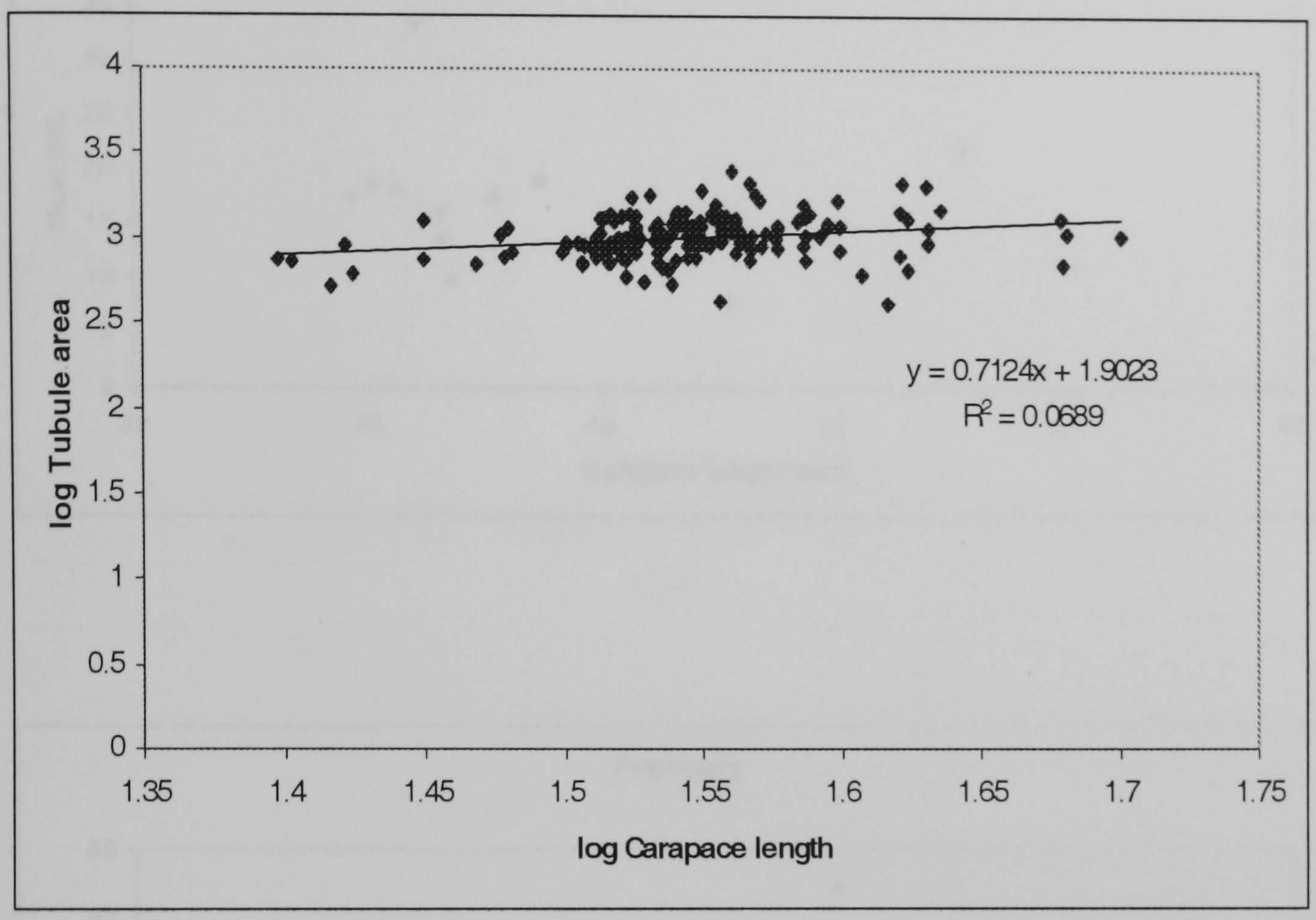
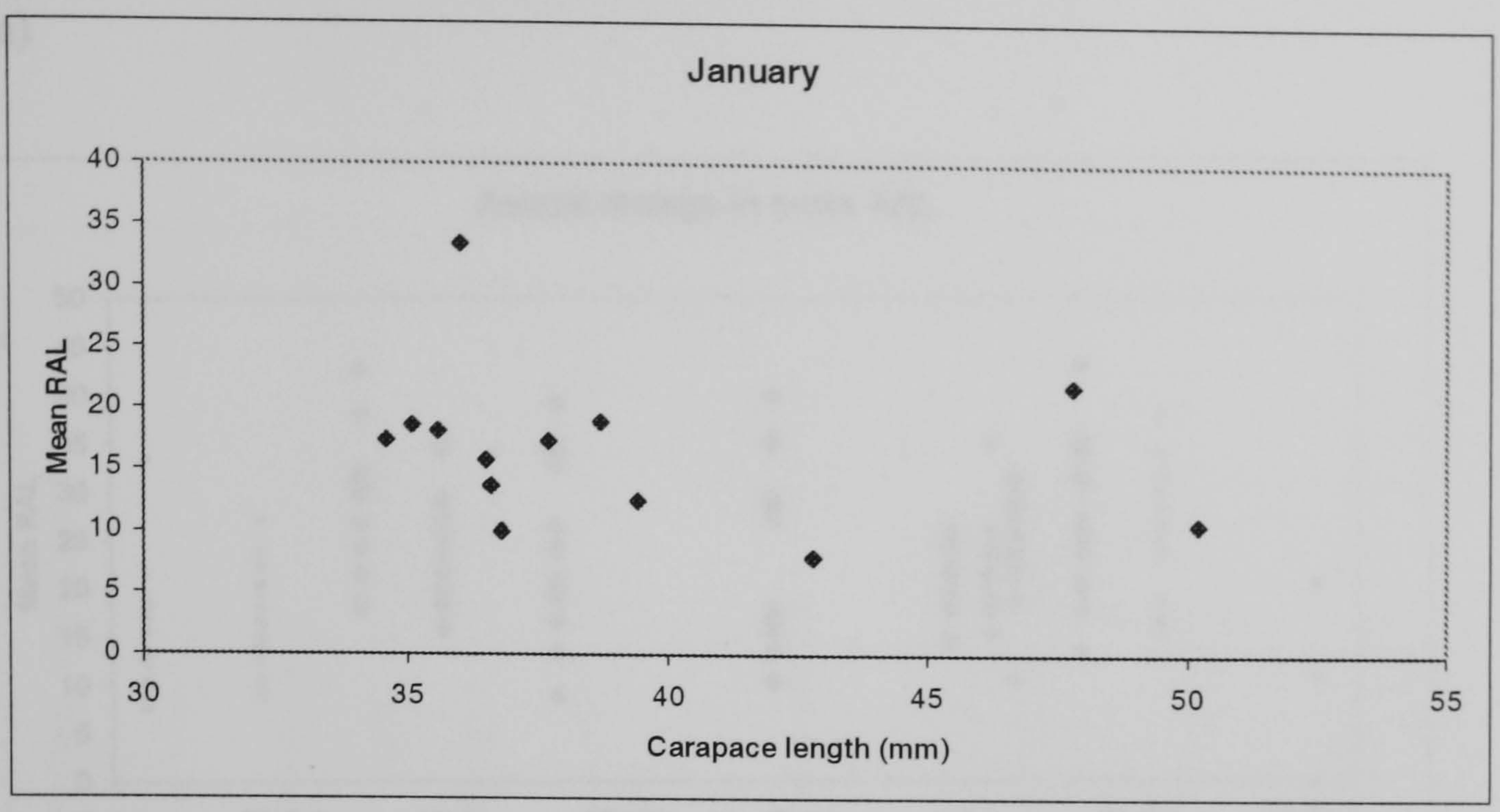


Figure 3.5: The log-log relationship between seminiferous tubule area (in μm^2, as defined by Minegawa, 1999) and animal size (mm carapace length) in *Nephrops norvegicus*.



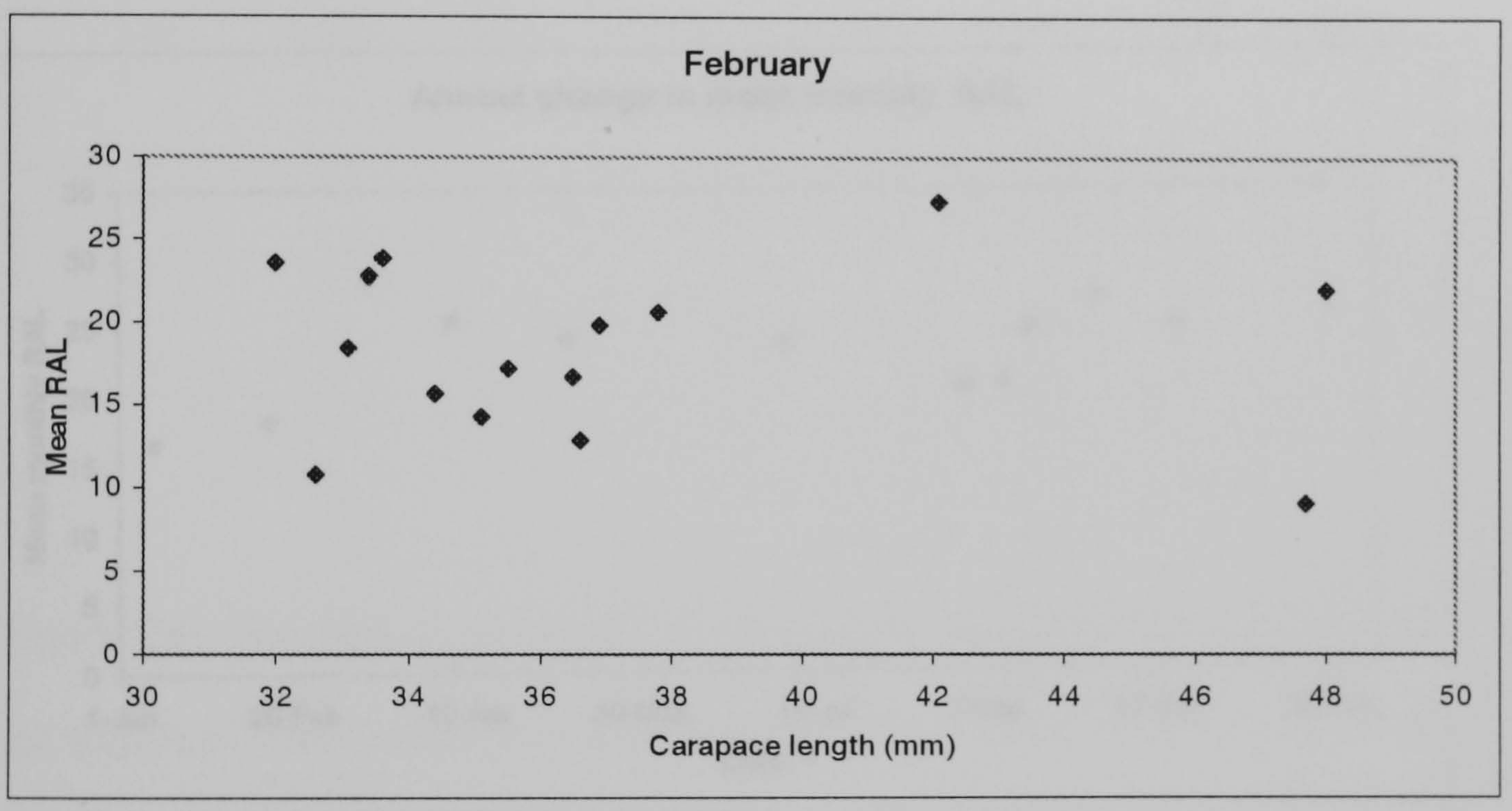
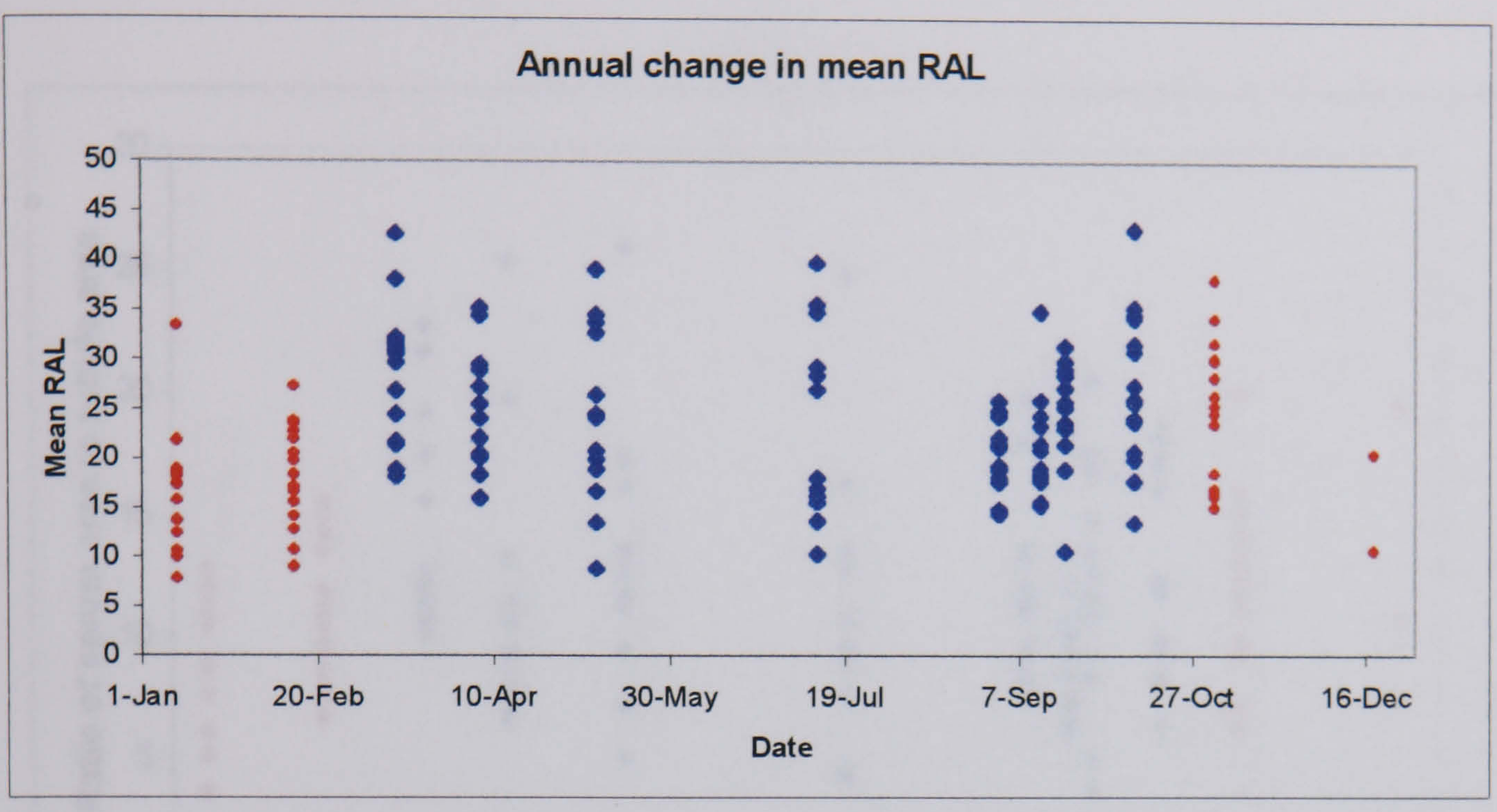


Figure 3.6: The relationship between the mean ratio of the area of the lumen to the area of the seminiferous tubule (RAL) and carapace length for *Nephrops norvegicus* collected in January and February 2002, the data for all other months can be found in Appendix 1.

(a)



(b)

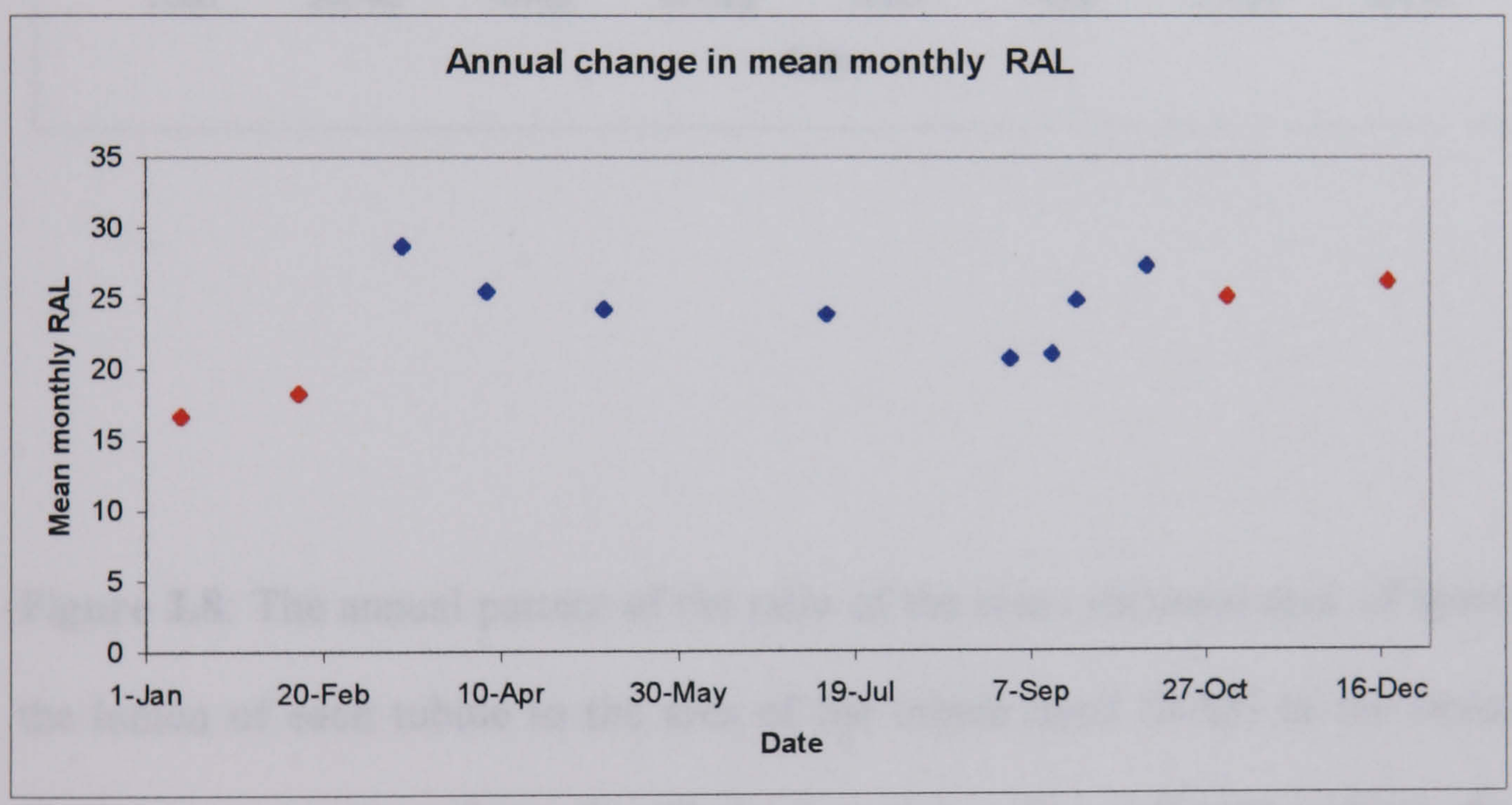


Figure 3.7: The annual change in the mean ratio of the area of the seminferous tubule to the area of its lumen (RAL) in *Nephrops norvegicus* (a) represents the mean for individual animals within a month and (b) the mean value for each month (red = Nov. 2001 – Feb. 2002, Blue = March 2000 – Oct. 2000). See Appendix 2 for the non-linear regression output.

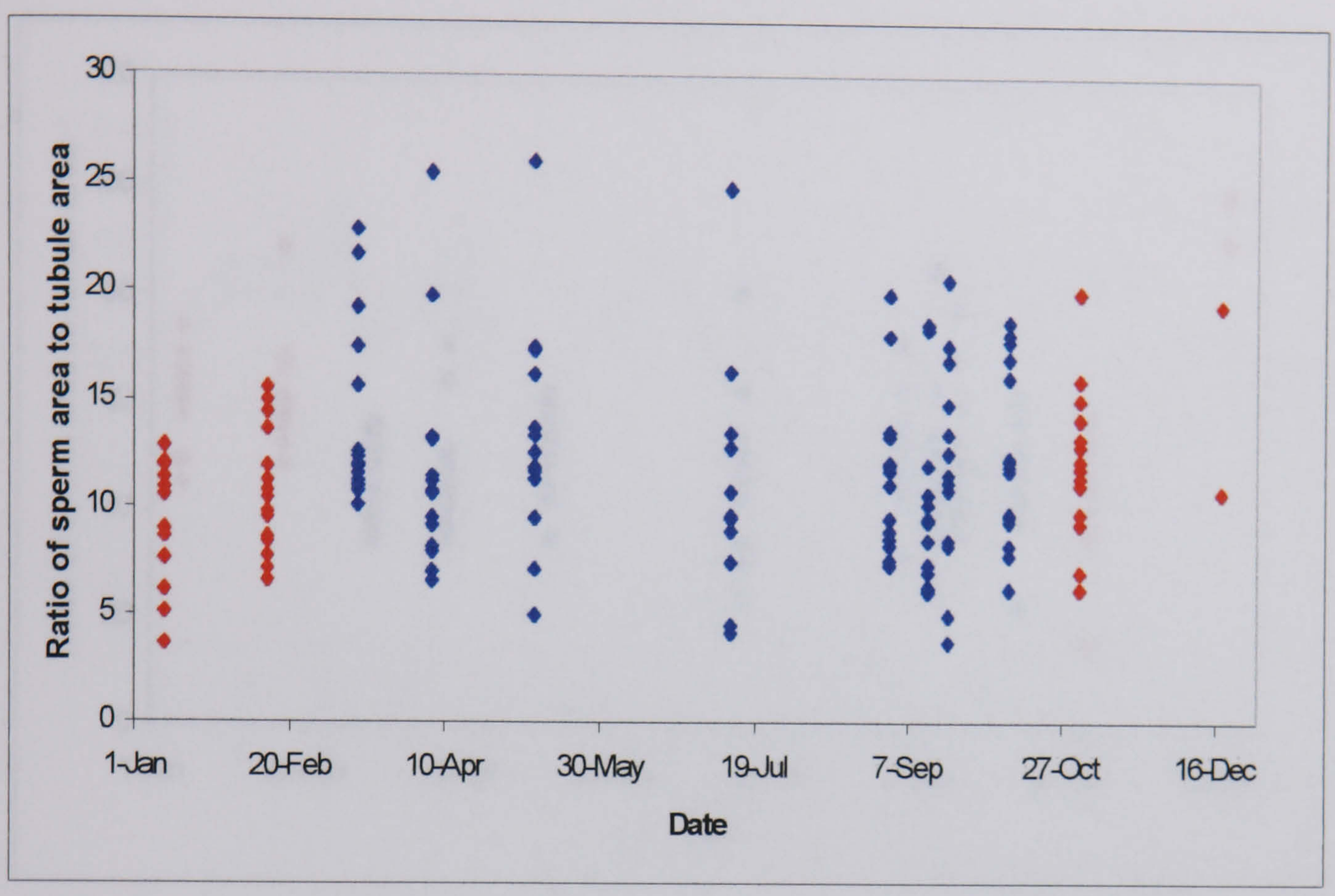


Figure 3.8: The annual pattern of the ratio of the cross sectional area of sperm in the lumen of each tubule to the area of the tubule itself (RAS) in the testes of *Nephrops norvegicus* form the Clyde sea area. The different colours depict separate series of samples (red = November 2001 – February 2002, Blue = March 2000 – October 2000). See Appendix 2 for the non-linear regression output.

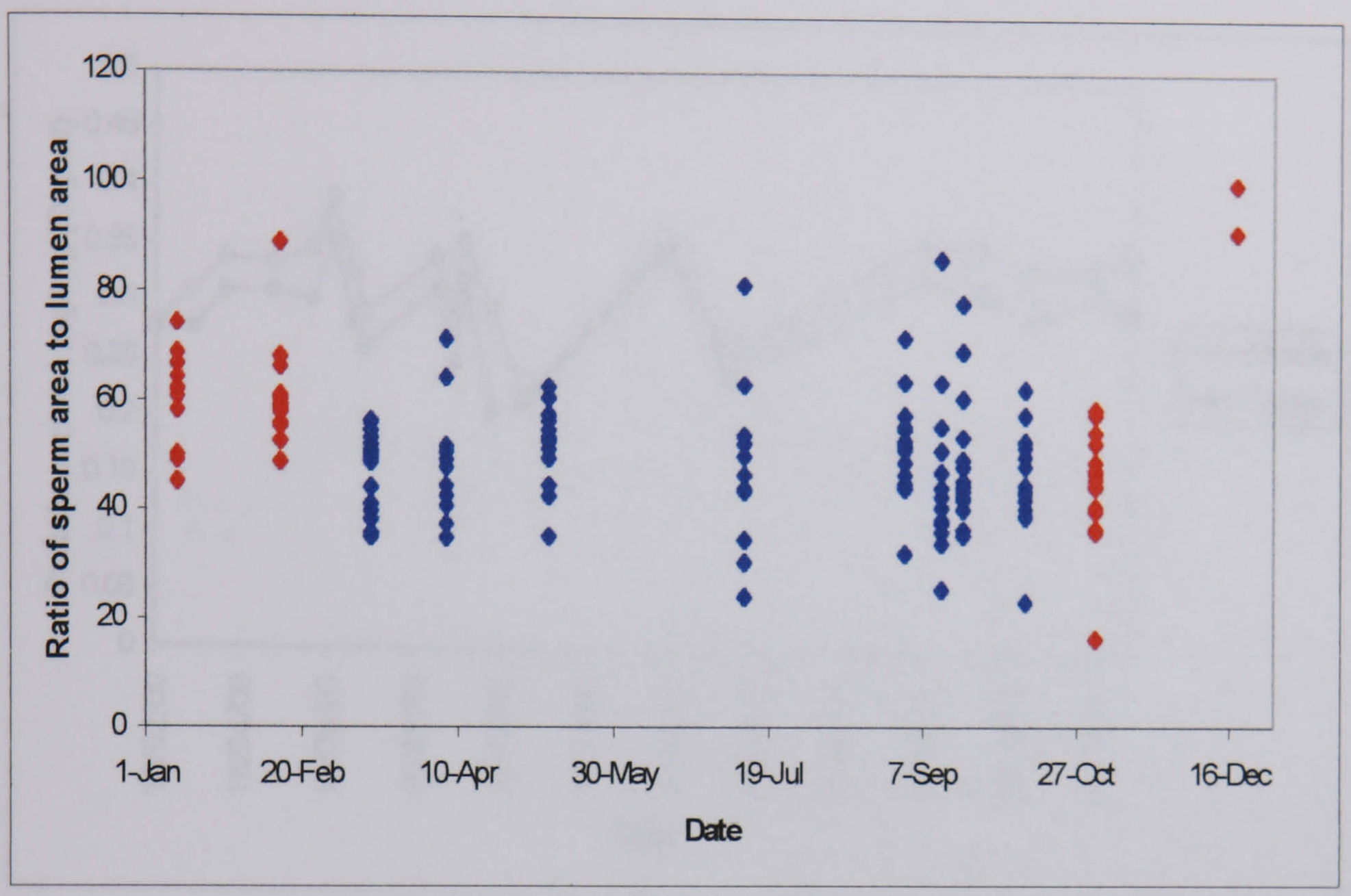


Figure 3.9: The ratio of the area of sperm in the lumen of the seminiferous tubule to the cross sectional area of the lumen itself (RLS) in the testes of *Nephrops norvegicus* from the Clyde sea area. The different colours depict separate series of samples (red = November 2001 – February 2002, Blue = March 2000 – October 2000). See Appendix 2 for the non-linear regression output.

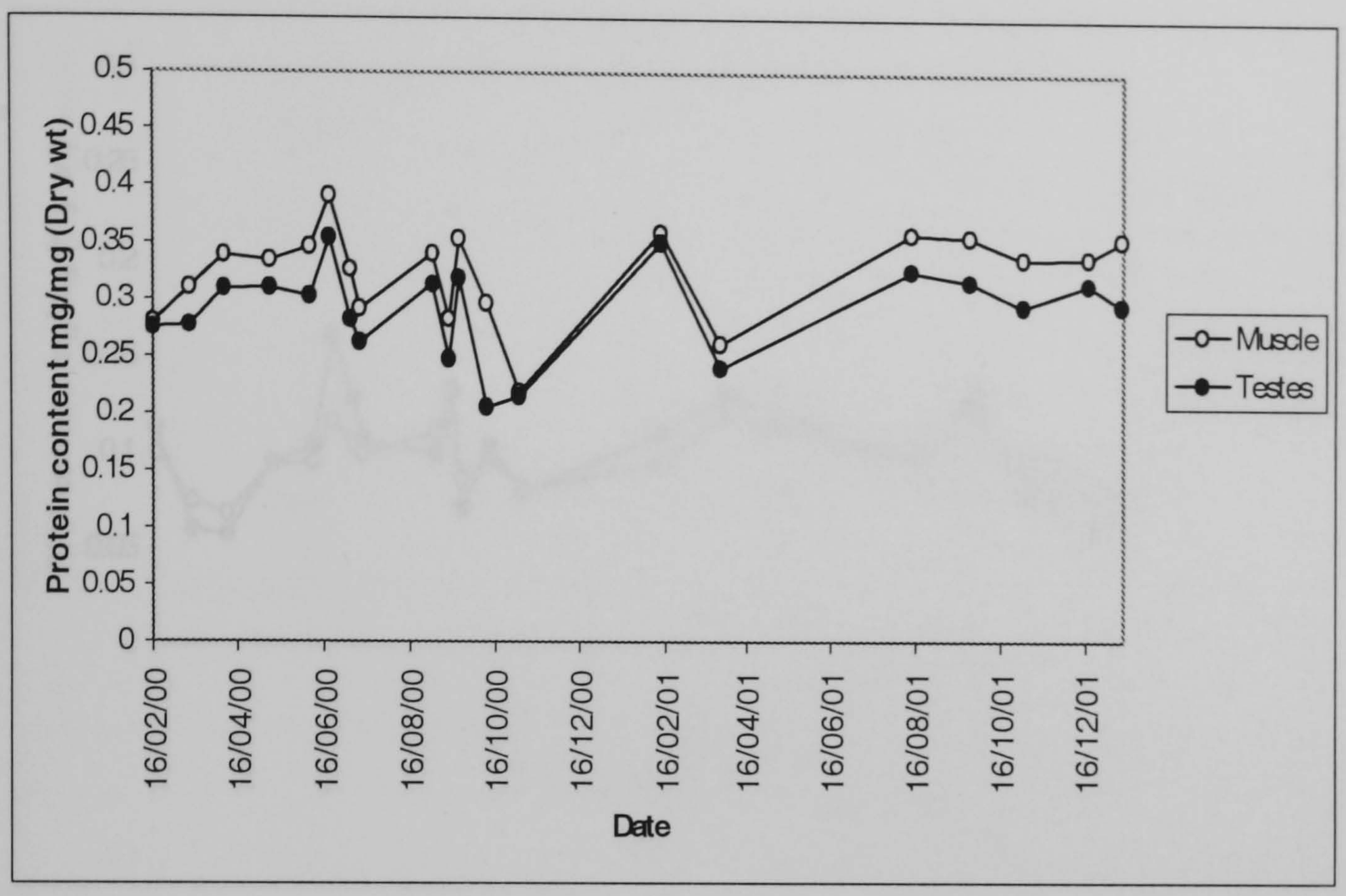


Figure 3.10: Seasonal changes in the protein content of the abdominal flexor muscle and the testes of *Nephrops norvegicus* from the Clyde sea area. Each value is a mean of 15 individuals from each sampling date. See Appendix 2 for the non-linear regression output.

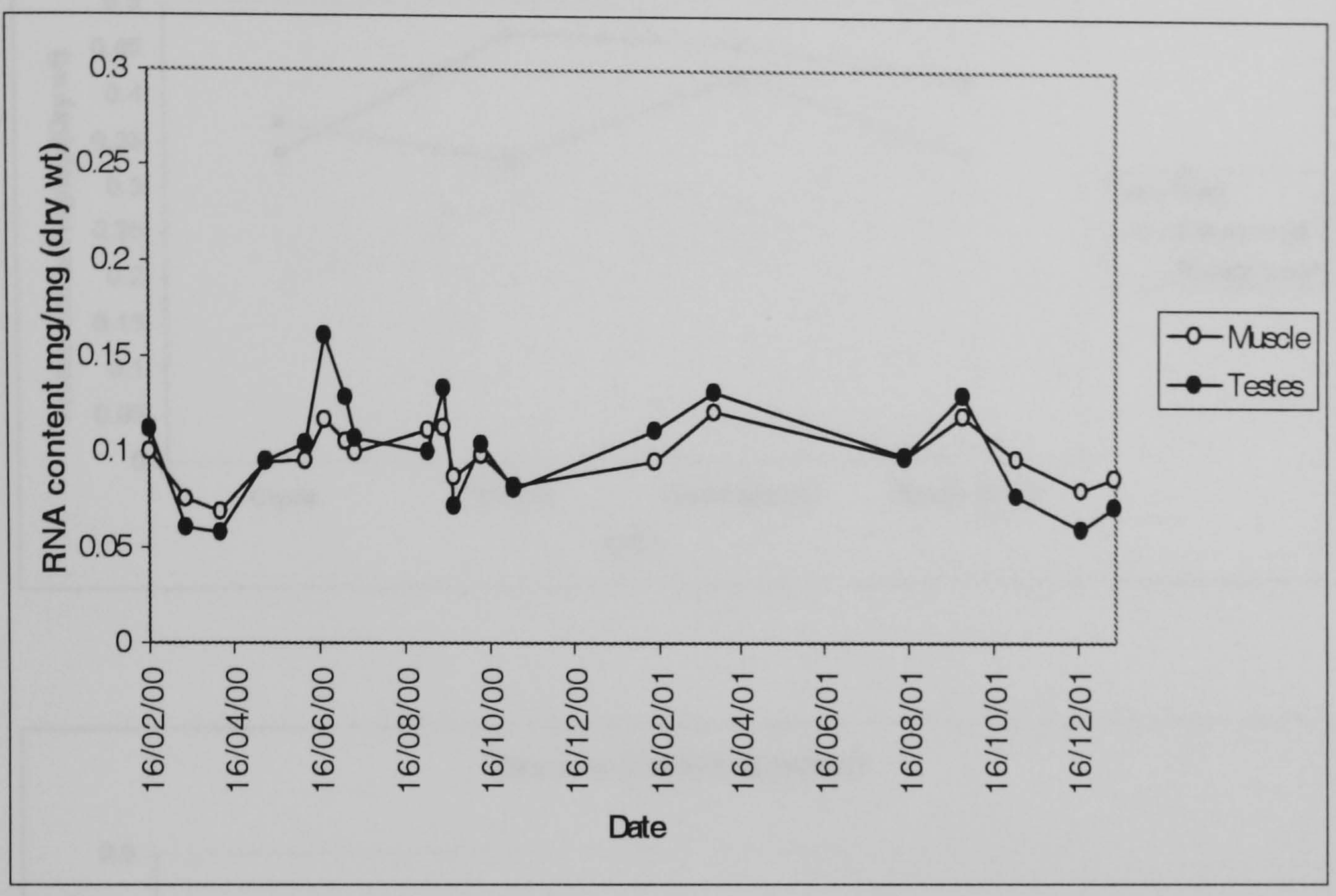
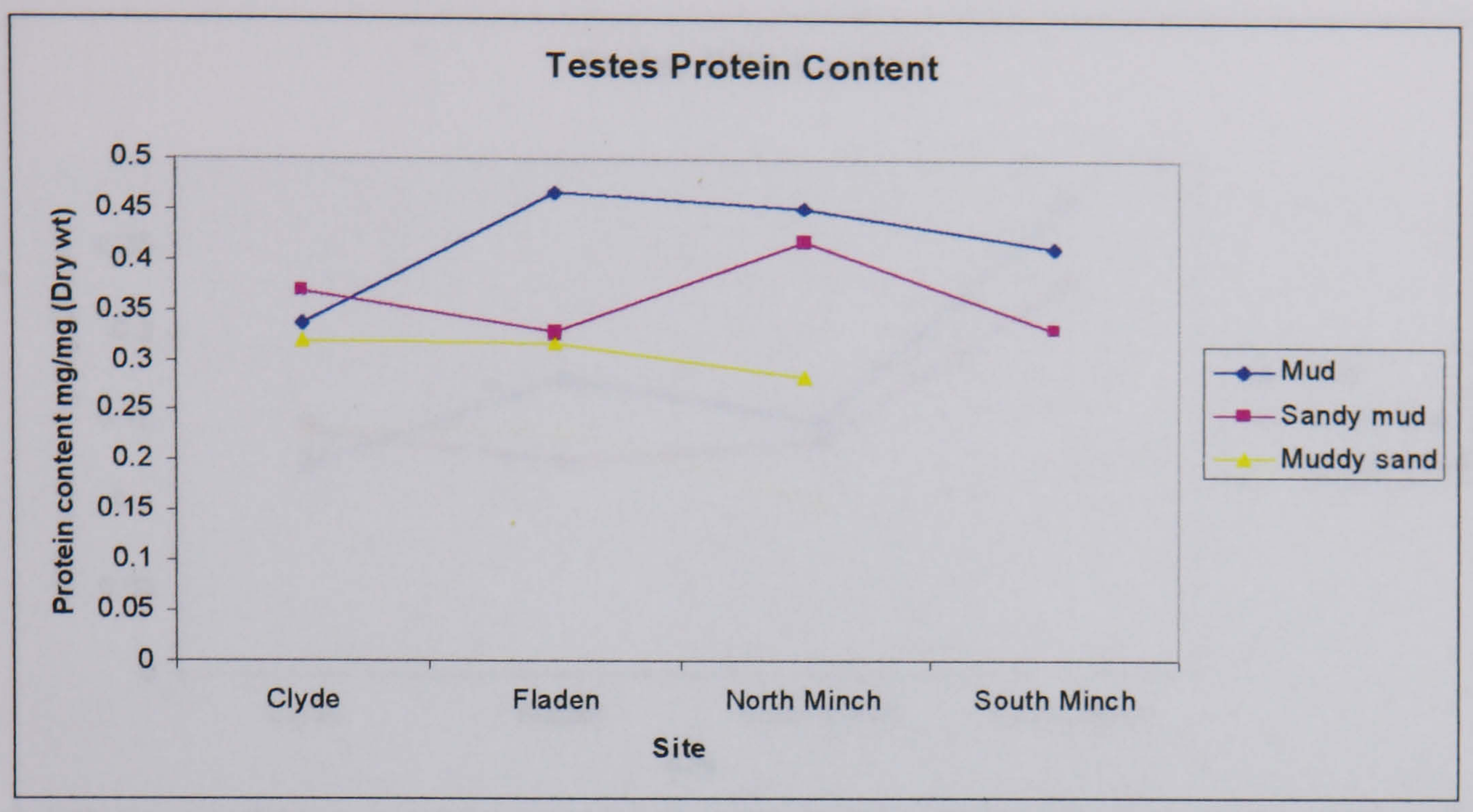


Figure 3.11: Seasonal changes in RNA content of the abdominal flexor muscle and testes of *Nephrops norvegicus* from the Clyde sea area. Each value is a mean of 15 individuals from each sampling date. See Appendix 2 for the non-linear regression output.



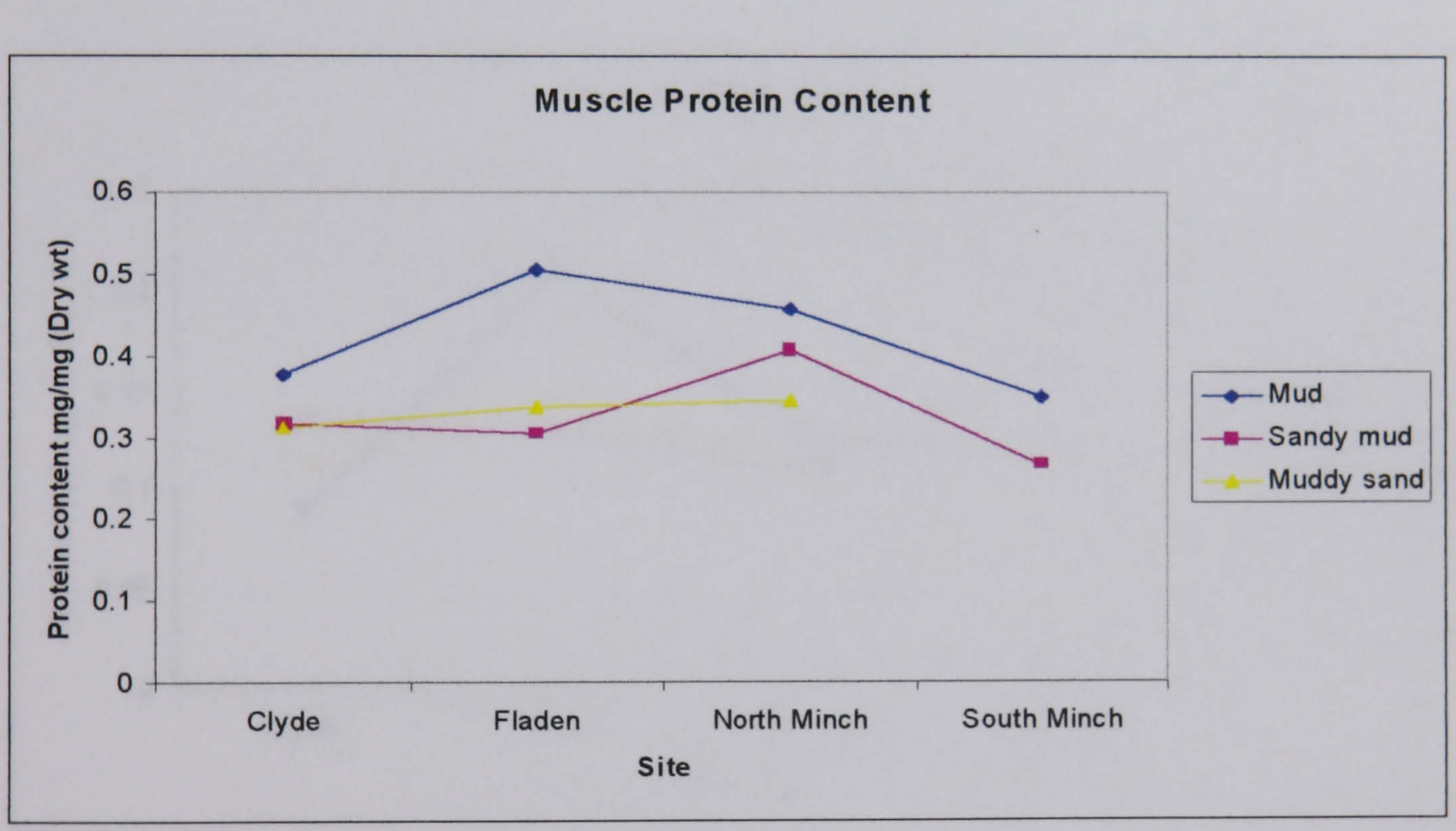
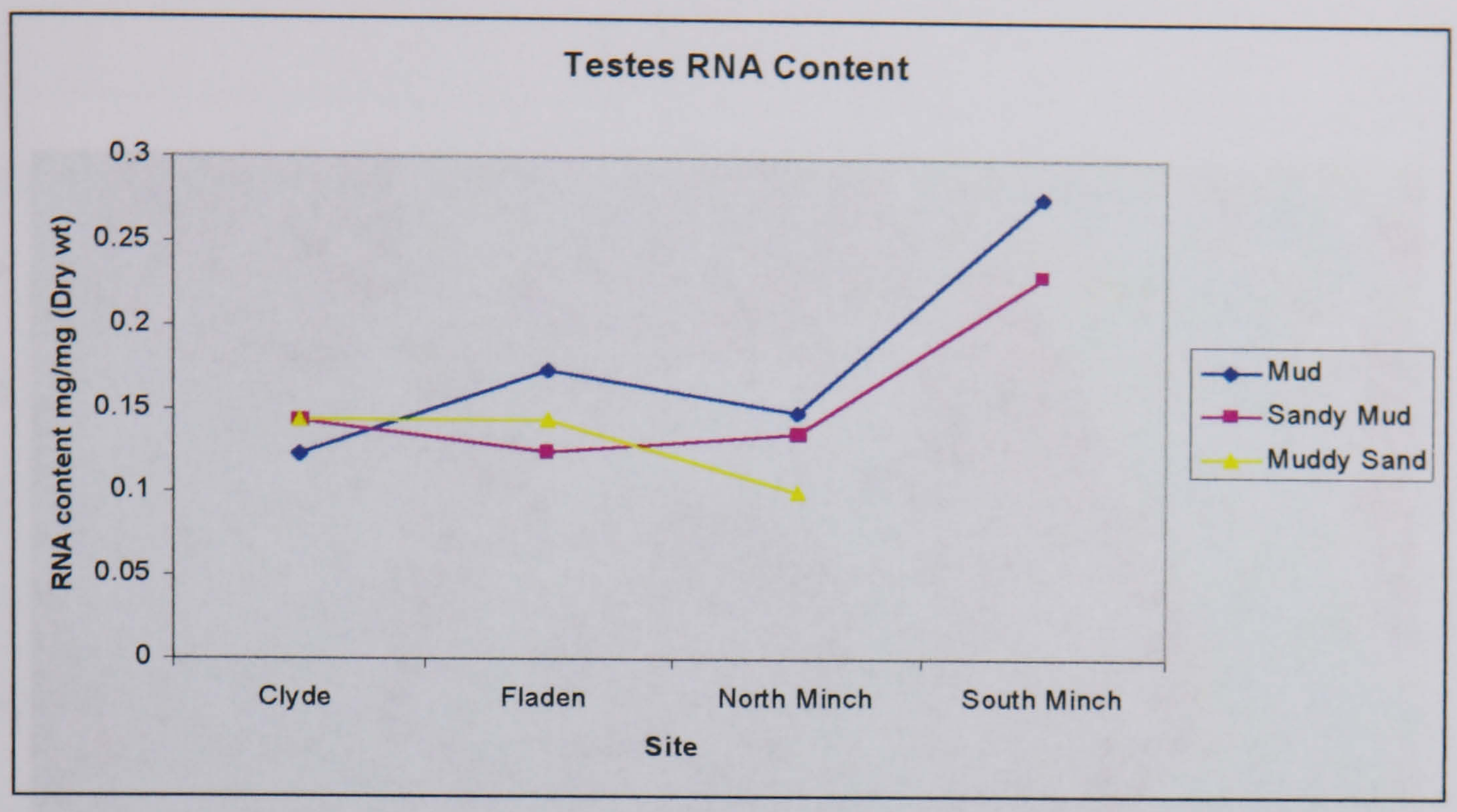


Figure 3.12: The mean protein content of the testes and abdominal muscle of male *Nephrops norvegicus* from around the Scottish coast displayed according to sediment type (a mean of the two intermediate Fladen sediment samples was taken).



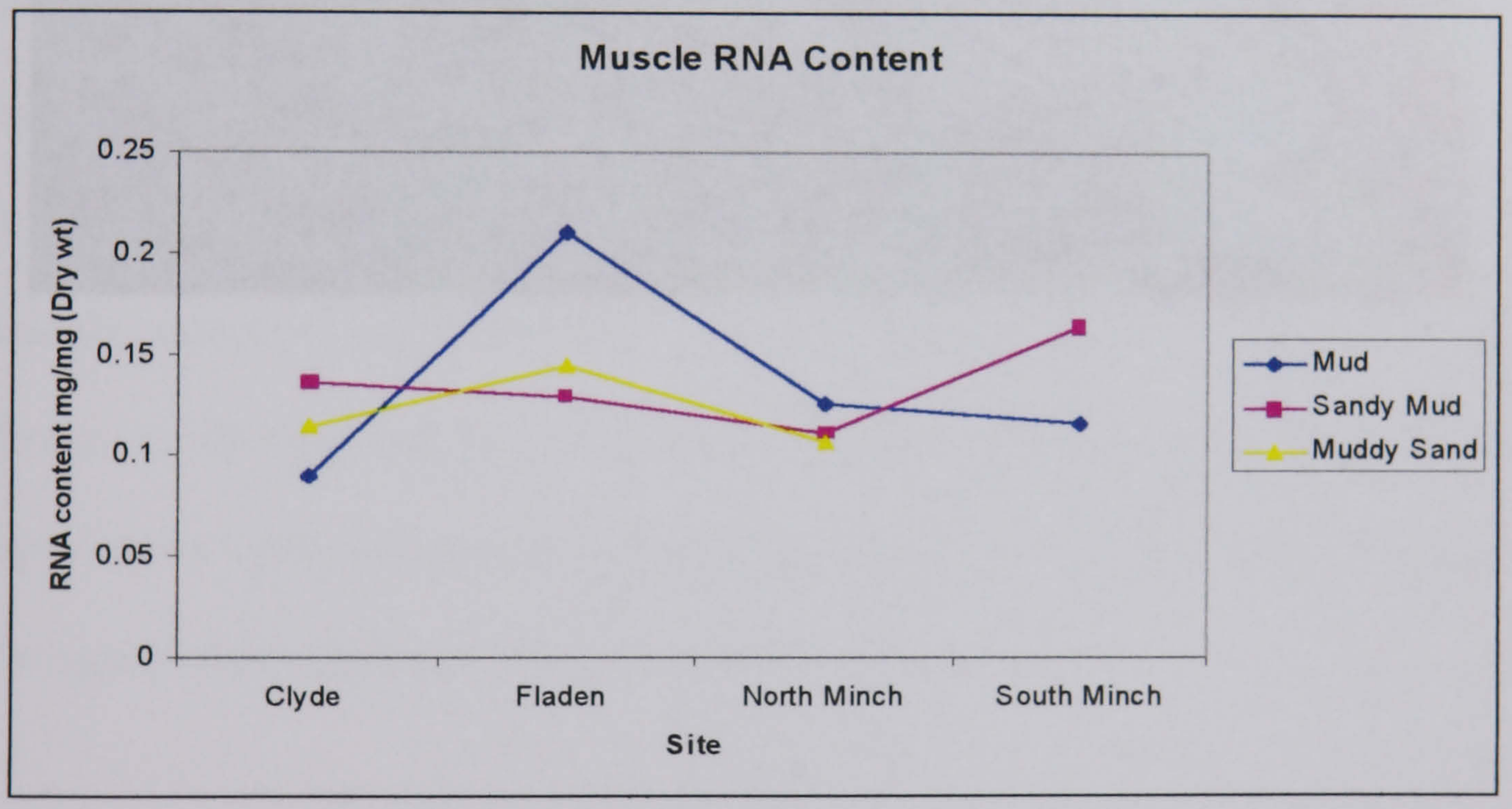


Figure 3.13: The mean RNA content of the testes and abdominal muscle of male *Nephrops norvegicus* from around the Scottish coast displayed according to sediment type (a mean of the two intermediate Fladen sediment samples was taken).

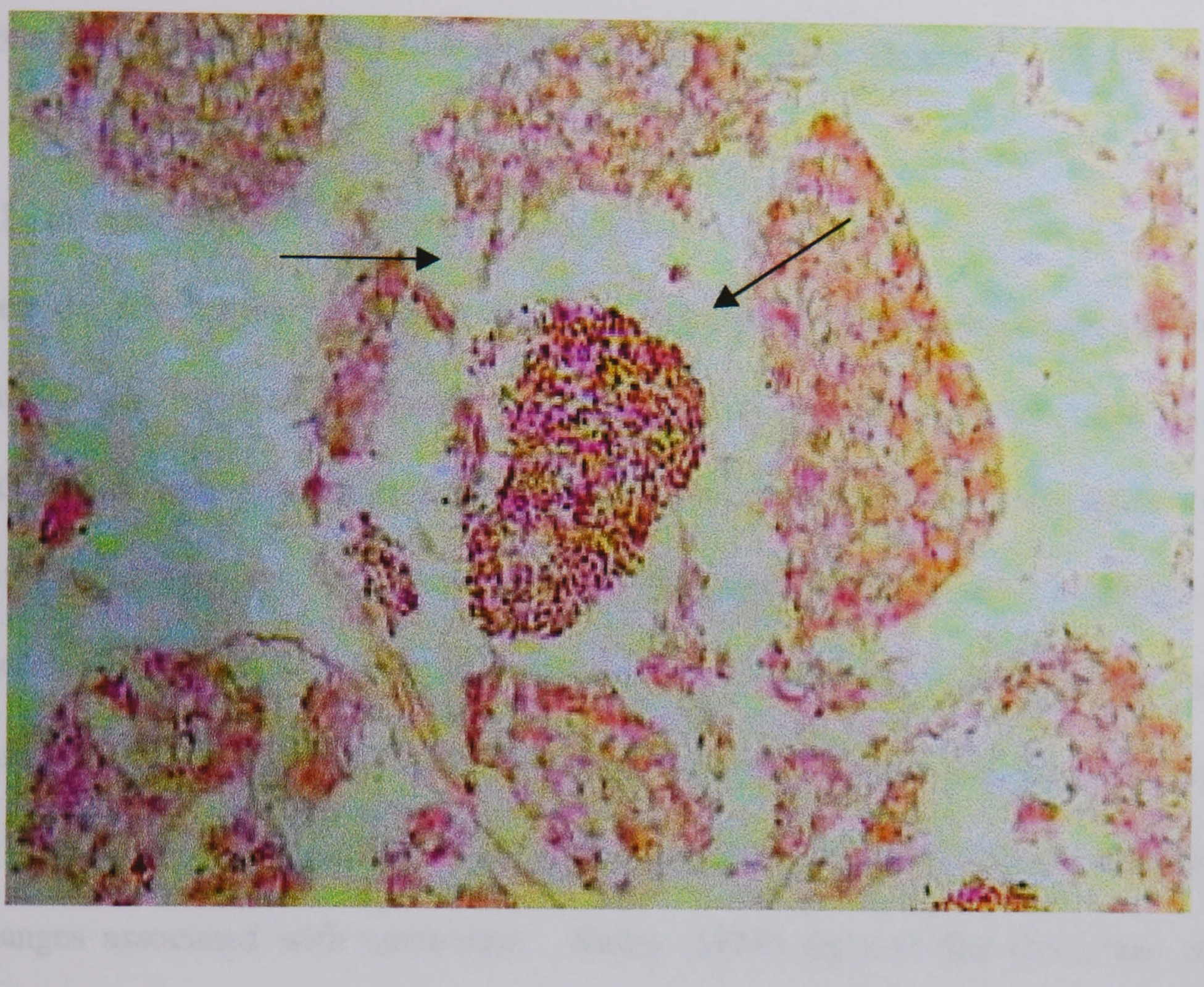


Figure 3.14: A section of testis tissue prepared for sectioning using paraffin wax embedding and stained using Hematoxylin and Eosin (x40). The arrows show areas of tissue shrinkage, which is an artefact caused by histological processing.

Chapter 4

Assessing size at maturity in male *Nephrops norvegicus* (L.).

4.1 Introduction

Determination of size at sexual maturity in female decapod crustaceans can be carried out relatively easily by recording the size of the smallest ovigerous individuals in the population (e.g. Figueiredo & Barraca, 1963; Thomas, 1964). In some species e.g. the ghost shrimp (*Lepidophthalmus* (as *Callianassa) louisianensis*) it is even possible to observe ovary maturation *in vivo* through the carapace (Felder & Lovett, 1989). It is much more difficult to determine the size at sexual maturity in males, as the testes often do not show any macroscopic changes associated with maturation. Bailey (1984) showed that there was no change in the dry weight of the testes of *Nephrops norvegicus* during the reproductive cycle and mature and immature males of a similar size cannot be distinguished through any obvious external characteristics.

Before assessments of male maturity can be carried out, maturity must be clearly defined. One definition is that of physiological maturity, which represents the point at which the testes become active in the production of sperm, or when spermatophores are present in the vasa deferentia. Animals may become physiologically mature at much smaller sizes than morphometric analysis would indicate as has been found in *N. norvegicus*. Morphometric analyses use differences in growth rates between mature and immature animals; for example, in

the American lobster adult males have enlarged chelae. Such differentiation is most obvious in the larger size classes. Physiologically mature male *N. norvegicus* of 18 mm CL have been recorded in the Irish Sea (Farmer, 1974a) and more recently physiologically mature individuals as small as 15 mm CL have been reported (N. McQuaid, pers. comm.). These animals are physiologically capable of reproduction but there is no evidence to suggest that they take part in the reproductive output of the population. The use of morphometric data for the analysis of maturity in crustaceans is often quoted as a measure of functional maturity. This relies on the assumption that the increased size of the claws of male animals will allow them to compete with other males for females and that they will be of a suitable size to mate successfully with a female. These assumptions, however, can contradict data on behaviour at maturity (e.g. Paul, 1992). Morphological maturity calculated for female American lobsters has often been shown to occur after egg laying (Swires, 1970). The use of morphometric data could, therefore, be meaningless when applied to *N. norvegicus* in the wild. If, however, it provided a good rough estimate of maturity this technique would be a very useful tool for rapid fisheries assessment that could be carried out at sea. Morphological maturity has been estimated at 26 mm CL for males in the Irish Sea (Farmer, 1974c) and more recently 25.9 – 31.0 mm CL from cutter claw analysis (N. McQuaid, pers. comm.); however, there are no indications that these animals are or are not capable of copulation. Maturity based on changes in the allometry of the appendix masculina have also been carried out on males from the Irish Sea giving values of 24.3 – 26.9 mm CL (N. McQuaid, pers. comm). These may be better related to copulatory ability although no studies have been carried out thus

far. A further definition of maturity that could also be considered is that of behavioural maturity, which would indicate that an individual was able to out-compete opponents for mating opportunities.

A method for the determination of size at the onset of maturity (SOM) that has been used for a number of decapod crustaceans is measurement of the allometric growth of secondary sexual characteristics following maturity. For example, claw volume has been used to assess SOM for the American lobster, *Homarus americanus* (Aiken & Waddy, 1989); and claw height in the stone crab, *Hapalogaster dentata* (Goshima *et al.*, (2000). Relative growth of the major chela has been used for the ghost shrimp, *Lepidophthalmus louisianensis* (Felder & Lovett, 1989) and the swimming crabs, *Aareanus cribarius* and *Liocarcinus depurator* (Muiño *et al.*, 1999). Other methods include relative growth of the appendix masculina in *Parapandalus narval* (Thessalou-Lageaki, 1989), and in *N. norvegicus* (Hillis, 1981), and of the pleopods and pereiopods in the spiny lobster, *Panulirus japonicus* (Minegawa & Higuchi, 1997).

Farmer (1974b) has shown that there is a definite change from isometric to positive allometric growth in the claws of male *N. norvegicus* and described the distinct sexual dimorphism in propodus form between the sexes. The aim of this study was to determine the change in propodus growth from isometric to positively allometric, and this is taken to indicate the size at which males become sexually mature.

Several methods can been applied in order to assess morphometric maturity. Power curves for male and female individuals can be examined to determine if they follow the pattern of allometric growth (see Watters & Hobday, 1998). The equation $y=\beta x^{\alpha}$ represents the measure of organ size where y = variable dimension, x = reference dimension, α = regression coefficient, and β is the y-intercept (from Hartnoll, 1978). For volumetric measurements, if the value of α is equal to 3 then growth is isometric with values of less than 3 and greater than 3 indicating negative and positive allometric growth, respectively. It has been noted, however, that power curves are not necessarily the best way to assess SOM because allometry indicates only that the change in the measured value is not constant (Watters & Hobday, 1998).

Further methods for the determination of SOM include a least squares method (e.g. Minegawa & Higuchi, 1997). This method requires a logarithmic transformation of the crusher propodite volume (CPV) data. This is a Model I type regression, which assumes that x values are measured without error and that variation in y is the same for any given value of x. This type of regression may not be appropriate for use with morphometric data (Tuck *et al.*, 2000). Application of a reduced major axis technique (RMA), as described by Lovett & Felder (1989) can be carried out to determine an inflection point in data sets. This is a Model II regression which does not make the same assumptions of the Model I and is therefore more appropriate for use in morphometric analyses. The RMA technique has previously been applied to claw length data of *N. norvegicus* (Tuck *et al.*, 2000).

Aiken and Waddy (1989) used the principle of a CPV index (CPI) to obviate the need for mathematical transformation of the data. They derived their equation from the power curve of data collected on female American lobsters to produce the following equation $y=0.22x^{2.98}$, which is comparable with data from immature males. As the pattern of growth for immature males should be similar to that of the females, any deviation from this would indicate an inflection in the data, which should coincide with male maturity.

Size at first maturity in female *N. norvegicus* shows large geographical variation (Anon, 1982) and relatively local variation (Bailey, 1984). For example females from part of the Portuguese fishery reach 50% maturity at 33 mm CL (Figueiredo & Thomas, 1967b), whereas in Scottish waters, 50% of female *N. norvegicus* are mature at 23 mm CL and 100% mature at 29 mm CL (Thomas, 1964). Bailey (1984), however, has found ovigerous females of 20 mm CL in the Sound of Jura. It has been reported by Figueiredo & Thomas (1967b) that there is no apparent variation in size of males associated with latitude, however, they did find that there was some geographical variation in the size of the largest males present. It is possible, therefore, that male size at maturity in Scottish populations will vary between different areas, both on a local and geographical scale. The reasons for these differences are likely to be related to differences in environmental parameters, for example, depth (Tully *et al.*, 1989), temperature (Froglia & Gramitto, 1988), and factors that correlate with sediment type (Hillis, 1988b). It is probable that these environmental factors are related to each other through hydrography and other factors that will affect sediment type. Maynou & Sarda (1997) found that

organic content and grain size, were both important in determining the density of *N. norvegicus* in a particular area, and that *N. norvegicus* preferred well oxygenated sediments. Afonzo-Dias (1997) reports that the majority of spatial variation between *N. norvegicus* population characteristics can be related to the particle mean size of the sediments on which they are found.

One of the main factors that can affect size at first maturity is growth rate (Bailey, 1986; Wenner, *et al.*, 1974). In populations where there is a higher growth rate animals could reach maturity at a greater size than those in slow growing populations. In *N. norvegicus* populations, growth is thought to be density dependent with animal size being inversely related to density (Chapman & Rice, 1971; Chapman, 1980; Bailey & Chapman, 1983). Density is associated with the sediment type on *N. norvegicus* grounds (Hillis, 1988b) and thus marginal areas with a lower density will contain animals with higher growth rates than areas with a greater density of animals. *N. norvegicus* are found on sediments from 20 – 100% silt plus clay (Bailey *et al.*, 1986). It may be that the structural integrity of larger burrows requires more cohesive sediment (Chapman & Howard, 1988). This could affect the composition of populations restricting larger animals to specific mud patches. It is also possible that the settlement of larvae and, therefore adult density, is affected in some way by the hydrographic conditions that affect the sediment types.

It is thought that maturity is related to age (Bailey, 1984; Tuck *et al.*, 2000). Therefore, differences in growth will produce differences in the SOM related to

sediment type as seen in females (Chapman & Bailey, 1983; Afonso-Dias, 1997). Chapman & Howard (1988) showed differing length frequencies from two west coast populations, which had the same age structures. This suggests that the differences were caused by differences in growth rate. Investigation of *N. norvegicus* biometrics from different sediment types from different grounds should provide information on the effects of habitat on the reproductive characteristics of this species. The relationship between animal size and sediment type is not linear, however. It has been shown for females that mean length is smallest in areas of sediment with an intermediate mean particle size and that there is a higher mean length for populations from sediments with low and high mean particle size (Afonso-Dias, 1997). The relationship between female first maturity and mean particle size is much less pronounced but there is an increase in size at first maturity at sites with higher mean sediment particle size (Phi values). These results indicate that there is higher growth on both coarse and fine sediments and a lower growth rate on sediments with an intermediate particle size. This was also shown in the Clyde Sea area where in the soft sediments of the Cumbrae main channel (~8.5 Phi), much higher growth was seen than at a site near Ailsa Craig which had sediments with an intermediate particle size composition (~6 Phi) (Afonso-Dias, 1997).

This study investigated two methods for the determination of SOM in *N. norvegicus* using morphometric data. Collection of animals from different areas and different sediment types allowed comparisons to be made. This is particularly important in relation to the fishery of *N. norvegicus* where male mean size is being

reduced in fished populations due to differences in fishing mortality between the sexes. Ovigerous females are largely unavailable to the fishery, as they tend to remain mainly in the burrow until their eggs hatch. It is also valuable to determine estimates of size of maturity within populations of *N. norvegicus* so that informed estimation of Minimum Legal Sizes (MLS) can be provided. The MLS relates to the smallest sized animal that can legally be landed and in trawl fisheries is related to the mesh size of the nets used.

4.2 Methods

The main sampling area for this study was south of Little Cumbrae in the Clyde Sea area (55.41°N, 4.56°W). The samples of *Nephrops norvegicus* were collected between September 1999 and November 2000. Samples were collected by trawling using a standard 70mm mesh otter trawl, with smaller individuals caught using a small 20mm mesh net. Comparative studies were also carried out on animals from the vicinity of Ailsa Craig (1999 – 2002), and the North and South Minch on the west coast of Scotland, and from the Fladen ground in the North Sea (2000 - 2002) (see Figure 1.1). A Scotnet 50mm prawn trawl (headline 176`) was used in the Fladen and the Minches. Sediment samples were taken using a Day grab and were classified from British Geographic Survey data (BGS, 1987). Sediment type was divided into three categories (four in the Fladen) based on the silt clay content of the substratum. The three main categories used are Mud (M>90% silt clay), sandy mud (Sm 50 – 90% silt clay) and muddy sand (mS 10 – 50% silt clay) (BGS, 1987).

For each animal, measurements of carapace length and crusher propodus length, height and width were taken to the nearest 0.1 mm (Figure 4.1). Observation of the lateral position of the crusher claw also allowed an assessment of handedness of animals from the Cumbrae site. Claws that appeared to have been regenerated were excluded from the analyses. Carapace length (the distance from the posterior margin of the orbit to the midline of the carapace edge) was measured as an indicator of body size while propodus measurements were used to calculate the Crusher Propodite Volume (CPV) as described by Aiken & Waddy (1989).

$$CPV = Claw\ Length \times Width \times Height$$

A total of 474 males and 243 females (size range: males 9.8 – 52.6 mm CL, females 9.8 – 64.2 mm CL) were collected from south of Little Cumbrae.

The methods used for morphometric analysis were the Crusher Propodite Index, because of its transferability between sites and the Reduced Major Axis technique, which was applied to both log-transformed volumetric data and claw length data. Analysis of the morphometric data was carried out on claw length data and following the calculation of CPV. Power curves were used to describe the relationship between CPV and CL for males and females. The Crusher Propodite Index (CPI) was then calculated for males and females from the Clyde Sea area using the methodology of Aiken & Waddy (1989).

$$CPI = 100(CPV)/Carapace\ Length^3$$

The Reduced Major Axis (RMA) technique (Tuck *et al.*, 2000), based on the methodology of Lovett & Felder (1989) was also used to determine the inflection point in the male data. The original data set was then split into immature and mature individuals (as estimated by each of the previously described methods) and power curves were again examined.

The RMA analysis was used to determine size at maturity from the comparative sites from around Scotland. To investigate the effects of factors relating to

sediment type on the data, various analyses (SYSTAT, 1992) were carried out to determine if the patterns of claw growth were the same at all sites. The term 'maturity' is used throughout to refer to morphometric maturity.

4.3 Results

4.3.1 Handedness

In animals studied from the Clyde there was no evidence of handedness in male *Nephrops norvegicus* (51.14% left, 48.83% left); however, females appear to be slightly right handed (45.63% left, 54.37% right), however, there was no significant relationship between sex and handedness (χ^2 DF=1; p=0.333) (Figure 4.2). Few animals were seen with two claws of the same type and none of those observed had two crusher claws.

4.3.2 Crusher propodite volume

The crusher propodite volume data for females (Clyde only) (Figure 4.3) showed that growth was slightly positively allometric (i.e. $\alpha > 3$). However, the growth curve for females showed a slower increase in CPV with carapace length than that for males (Figure 4.4) ($y=0.0247x^{3.3867}$ females, $y=0.0032x^{4.0842}$ males). These data are similar to those described for female American lobsters. A double log transformation of the data was carried out (Figure 4.5) in order to determine any possible relationships between juvenile and adult phase lines (Somerton, 1980). It can be seen that there is a good linear relationship between log CPV and log CL for both female and male data; however, there is no clear indication of any inflection in the line that might indicate the size at maturity for males. There is a slight deviation of the male from the female data that occurs between 1.3 and 1.4 log CL, which represents a carapace length of approximately 20 - 25.5 mm.

Claw length data from male and female *N. norvegicus* was also compared (Figure 4.6a & b) and there appeared to be a slight inflexion in the male data, and there was also a distinct deviation of the male data from the female data. This deviation of the male data from the female occurred at approximately 25 mm CL (Figure 4.6a), which was very similar to the log-log relationship between CPV and carapace length. Examination of the data grouped into 5 mm size classes, however, shows that there is no significant deviation until approximately 40 mm CL. It is interesting to note that in smaller size classes female *N. norvegicus* appear to have longer crusher propodites than males until a size of about 25 mm carapace length (Figure 4.6b).

4.3.3 Maturity in male *Nephrops norvegicus* from the Clyde Sea area

Calculation of crusher propodite volume for male *N. norvegicus* collected from the Clyde showed a power relationship to carapace length (CL) (Figure 4.4). The relationship between CPV and CL for all other sites sampled can be seen in Appendix 2. Fitting a power curve to the data obtained for male *N. norvegicus* showed that there is a strong positive allometry ($y = 0.0032x^{4.08}$). The increase in CPV with carapace length appears to change most rapidly between the sizes 30 mm and 40 mm carapace length. These data were log transformed in order to carry out Reduced Major Axis (RMA) analyses, which produced a highly significant value ($p<0.001$) of 27.9 mm CL for SOM (Table 4.1). Claw length was also analysed and also produced a highly significant ($p<0.001$) value of 30.6 mm CL for SOM (Table 2). When the residual sum of squares (RSS) was plotted for each of these analyses it was observed that neither log claw volume nor claw

length provided a plot with a well defined minimum RSS providing a conclusive estimate of SOM. Figure 4.7a shows more than one local minima and Figure 4.7b shows a broad range of sizes with generally low values. It can be seen, however, that claw length provides the best fit for analysis of SOM (Figure 4.7b).

The index of crusher propodite volume for males showed a positive linear relationship with size, although there was some scattering of points (Figure 4.8). The crusher propodite indices (CPI) of females and small males are very similar; therefore the deviation of the male CPI from the female CPI should indicate the size at maturity for males. Use of this technique shows a significant deviation of the male curve from the female data at 25 mm CL (Mann-Whitney U; $p<0.001$). The corresponding CPI value for the size at maturity at the Cumbrae site was 8.5 (Figure 4.9).

Using the SOM produced by the CPI and RMA techniques the data on male claw length were separated into mature and immature, and the power curves were further examined. This showed that the juvenile males i.e. those smaller than the estimated SOM, had a power function close to that of females (CPI $y= 0.0242x^{3.39}$, RMA $y = 0.0068x^{3.85}$). Mature males showed a power curve with α greater than that of the whole male data set (CPI $y=0.0025x^{4.16}$, RMA $y = 0.001x^{4.41}$). The change in power relationships between the estimated juvenile and adult phases of growth were of a similar magnitude using both methods although the power functions were slightly higher using the RMA method where the inflection point was determined at a larger carapace length.

4.3.4 Variation according to sediment type

The RMA analysis was used to determine any differences in SOM between different areas and different sediment types (see also Appendix 2). Significant results were obtained for SOM at most sites using log CPV (Table 4.1); however, all RMA analyses on claw length produced significant results on SOM for all sites (Table 4.2). These data were analysed to determine if the pattern of claw growth was the same for all sites, a homogeneity of slopes test indicated that the sites were significantly different from one another ($p<0.001$). There does not appear to be any relationship between the sediment type (Table 4.3) and SOM. The highest values of SOM were for *N. norvegicus* from mud sediments in the Clyde and the North Minch, but they occur on muddy coarse and sandy mud in the Fladen and South Minch. The smallest SOM's are seen on muddy sand (Clyde), mud (Fladen), sandy mud (North Minch) and muddy sand in the South Minch. The SOM's calculated from the Fladen appear to indicate that male *N. norvegicus* are maturing at a larger size overall, than at other sites. The RSS plots for all sites sampled can be seen in Appendix 3.

Comparisons were made between male and female claw length data from all sites in the Clyde. This showed that there are differences in SOM from the Cumbrae site (Figure 4.6) where the SOM was estimated at 25 mm CL, Ailsa Craig (Figure 4.10a), where the SOM was estimated at 21 mm CL, and for the Clyde 3 site (Figure 4.10b) where the SOM was estimated at approximately 23 mm CL. The relationship between SOM and sediment type was the same for this method as

using the RMA technique, although the actual results were much higher for RMA analyses.

The use of the Crusher Propodite Index (CPI) on data from the Clyde (Figures 4.8, 4.9 & 4.11) provided similar results to that of the analysis of male and female claw length. It was shown that SOM occurred between 20 and 25 mm CL for all three sediment types. Again this analysis produced SOM results that were much lower than those produced using the RMA technique. The CPI values for SOM were not the same as that seen or animals from south of Little Cumbrae (Ailsa Craig CPI = 9.9; Clyde 3 CPI = 11.1). Female data were not available from the other sites sampled.

The data from each area were split into immature and mature males, based on the results of the RMA analysis on claw length (Table 4.2), to assess if there were any differences between the two growth phases between sites. Analysis of the homogeneity of slopes showed that there was a significant difference between slopes for immature ($p<0.001$) and mature ($p<0.001$) individuals. It was therefore not possible to carry out covariance analysis. The data from immature and mature animals were plotted together to determine the pattern of any inflection calculated by the RMA technique, the results of this are shown in Figure 4.12a, b & c (the plots for all other sites can be seen in Appendix 4).

4.4 Discussion

The incidental data collected on handedness in *Nephrops norvegicus* were similar to those collected by Farmer (1974b), who showed that there was no significant difference between the claws in males. For females, Farmer (1974b) did not find a significant result as in this study; however, there did appear to be a slight bias towards the crusher claw occurring on the right hand side of the body. Farmer (1974b) also indicated that there was a low occurrence of animals with both claws the same, especially two crushers, which concurs with the data presented in this study. The low incidence of homochelous animals reported with two crushers could be caused by previous autonomy of the crusher claw, which in some cases may never be replaced (Smith, 1990). What was interesting in the differences between males and females from south of Little Cumbrae, was that claw length appeared to be greater in females until about 25 mm CL. There is no apparent reason for this difference in the size of the propodus and it was not reported in a previous investigation in the Irish Sea by Farmer (1974b).

Power curves showed that positive allometric growth in CPV is present in both male and female *N. norvegicus*. Positive allometric growth along a power curve would be expected if the growth of the claw was a function of body size, a problem noted by Watters & Hobday (1998). In this case, however, it has been shown that the positive allometric growth in males is greater than that of females and immature males indicating that crusher propodite volume can be used as an indicator of size at maturity in male *N. norvegicus*. A further problem outlined by Watters & Hobday (1998) is that it could be difficult to determine more than one change in

the growth of the chelae. The use of both the crusher propodite index (CPI) and crusher propodite length enable the growth of the male chelae to be compared with that of the female. This allows observations to be made on the positive allometry of male growth defining sexual maturity, from that of the growth of the female chelae. In this study, both these approaches showed that there was a definite single departure in the growth rate of the claw of males from that of females, although the transferability of CPI data may not be straightforward in *N. norvegicus*.

Watters & Hobday (1998) suggested that outliers can have a significant effect when using linear or log-transform models to describe morphometric size at maturity. They suggested that outliers in *X* space (i.e. those with very large or very small carapace lengths) can have a large effect on the slope of lines fitted to the data. Smith (1980), however, states that the use of a log-log transformation will benefit analysis of a data set containing outliers since the relationship described will change less through the inclusion or removal of these outliers. The Clyde male data set contains no such outliers and therefore the use of log transformed data should be unaffected by leverage. Watters & Hobday (1998) also suggested that chelae that are slow growing could prove difficult to separate from fast growing, regrown chelae in *Y* space. The calculated values of CPV transformed for the least squares method showed little scatter and it was therefore considered that regrown chelipeds had been identified and successfully removed from the analyses.

The reduced major axis method is also only applicable where all animals are maturing at the same size and the two lines meet (Somerton, 1980). If there is a

large overlap of the two lines this method would not be applicable. The data in this study provided, in the majority of cases, highly significant results using the RMA technique, which would suggest that it is appropriate to fit two lines to the data.

The use of claw volume has been shown to provide a better relationship than that derived from claw length as shown from comparisons between the regression equations shown here and those produced by Farmer (1974b) (where values of α were much closer to 1). In this study, however, it was found that claw length gave a better output than claw volume when using the Reduced Major Axis (RMA) technique (Figure 4.7), and it was therefore used for further manipulations of the data. As the relationship between crusher propodus length and carapace length is linear this also negated the need for transformation of the data. The highly significant results obtained using this method, should be interpreted with some caution, however, as the plots of the residual sum of squares (Figure 4.7 and Appendix 3) indicated that there was often not a clear relationship obtained from the output of this test.

The benefit of using CPI as a determinant of male maturity is that the CPI index can, in theory, be applied to any stock. Animals from stocks that mature at different sizes will still have similar CPI values on reaching maturity. Aiken & Waddy (1989) suggested that it should be possible to determine the SOM of a population from the calculation of male CPI only. This would be particularly appropriate for *N. norvegicus* stocks, which vary in characteristics from site to site. When this technique was applied to three different populations from the Clyde,

however, the same CPI value was not produced each time. In addition, it was found that the CPI value for maturity from the Cumbrae site was lower than the range of CPI values calculated for some data sets.

Different methods for the estimation of morphological maturity have been shown to produce very different results in this study. While Aiken & Waddy's (1989) index provided a SOM only slightly greater than the estimated size at physiological maturity, the RMA technique using claw length produced an SOM larger than the previous estimate of maturity made for *N. norvegicus* in the Irish Sea. Farmer (1974c) suggested that from observations of the allometric growth of the propodus of males that *N. norvegicus* become sexually mature at a size of 26.5 mm CL. This difference in the estimation of size at the onset of maturity SOM could be due to differences between the stocks; it has been reported that Irish Sea *N. norvegicus* mature at a smaller size than individuals from other areas (Briggs, 1988). The CPV technique used by Aiken and Waddy (1989) may not translate well to *N. norvegicus*, as their claws are much narrower and, therefore, do not undergo such large changes in volume as occurs in *H. americanus*. The comparison of claw length between female and male *N. norvegicus* provided an SOM larger than that of the CPI analysis and yet smaller than that produced using the RMA analysis. It is possible that the calculation of male maturity from morphometric characteristics of the claw cannot be carried out without reference to the female claw as a control.

When using the results from CPI and RMA of claw length to divide the results into those for immature and mature animals, the power functions for each method were

similar. As the SOM for male *N. norvegicus* estimated from these techniques was quite different, this could suggest that these methods are not satisfactory for the assessment of morphometric maturity in a species such as *N. norvegicus*. It is possible that there is a relatively wide size range over which males become mature and it is therefore not possible to pinpoint a particular SOM that will be appropriate for each study area. It may therefore be appropriate to select a size within a variance. It is also possible that some of the data sets may have been restricted in the numbers of small animals sampled. For most data sets, with the exception of Fladen 3 and perhaps South Minch 2 & 3 (see Appendix 2) there was a good size range of animals and, for comparison, of these some were below the estimated size at maturity in the Irish Sea (Farmer, 1974b).

The use of morphological techniques has shown that there are differences in SOM between different sediment types and also between different areas, however, no distinct patterns were seen. Differences in biometrics between areas have previously been reported between sites in the North Minch and the Sound of Jura (Howard & Hall, 1983). The patterns seen in this study in North Minch and Clyde grounds followed that expected from predictions based on growth and density; with larger sizes at maturity for sediments at either end of the particle gradient and intermediate sediments with the smallest size at maturity. The results from the other sites were less clear, however. It is unlikely, however, that sediment type is the only factor affecting these populations. Hillis & Tully (1993) showed that the temperature at the seabed varied across their sampling area in the Irish Sea. Areas of fine sediment were associated with strong stratification, and hence had low

seabed temperatures, whereas areas of coarser sediment were more associated with weaker stratification. These differences in temperature could well affect growth (Lipcius, & Herrnkind, 1987; Froglia & Gramitto, 1988; Hillis & Tully, 1993). It is also possible that larval settlement, and therefore density, could be affected by stratification in the water column overlying a population.

Size differences between males from different geographical locations have previously been related to fishing intensity and predation (Figueiredo & Thomas, 1967b), thus differences in fishing pressure between sites could have an impact on SOM. The Clyde sea area results produced a relatively small SOM compared with the larger SOM recorded for males in the Fladen ground. The Clyde is heavily trawled and has been fished since the 1950s whereas the Fladen has only recently been developed as a fishery. It could be hypothesised that these differences in SOM are as a result of the duration and intensity of fishing pressure since the Clyde is subject to greater effort than the Fladen (Marrs *et al.*, 2002a; Marrs *et al.*, 2002b). It has also been reported that a reduction in the size of animals has occurred in stocks from the south of Portugal because of high fishing pressure (Arrobas, 1982). Tully & Hillis (1995) suggest that sediment type alone cannot describe all of the variation they found in Irish Sea populations and suggest that spatially variable fishing pressure may be the cause, as female length frequency distributions correlate more closely with the sediment types than those of males. This could well be a result of the differences in fishing mortality between the sexes. Both Atkinson (1989) and Tuck *et al.* (1994) showed changes in the burrow density at sites in Loch Sween, which could be attributed to increased fishing

pressure. Bas & Sarda (1998) postulated that long term changes (over 30 years) in morphometric characters of the deep-sea shrimp, *Aristeus antennatus*, can take place as a result of increased fishing pressure.

When the relationship between carapace length and crusher propodus length was divided into immature and mature data sets using the value obtained through the RMA on claw length, it was seen that there were significant differences between the slopes for both immature and mature. There were also significant differences when comparing the complete data set between sites of differing sedimentology. These results are perhaps not surprising, however, as at sites with animals that display a higher growth rate, the slope of the regression will be closer to 1 than at those with a lower growth rate, resulting in differences in the slope of the relationships.

Farmer (1974b) suggested that the differences in body proportions between populations could occur because of partial reproductive isolation from neighbouring populations. The differences between areas, particularly when comparing the Fladen ground, could be due to reproductive isolation. It is perhaps unlikely that such differences would occur within areas unless there were severe hydrogeographic conditions, which isolated each population from larval input from the others.

Morphometric analyses of maturity have been shown to overestimate the size at maturity in both tanner (*Chionoecetes baridi*) and king (*Paralithoides*

camtschaticus) crabs (Paul, 1992). In this study it seems likely that the use of RMA analyses have overestimated the SOM, when compared to the analyses that used female data as a comparison. Morphometric analysis may prove to be a better tool in combination with behavioural analyses to determine the competitive ability of individuals rather than as an absolute measure of maturity. It has been shown that crustaceans with larger claws are more likely to succeed in agonistic encounters (Sneddon *et al.*, 1997). Claws could also be important in displaying to females as is seen in the fiddler crab (e.g., Schöne, 1968; Salmon, 1983; Hinsch, 1992) although this is unlikely to be the case in *N. norvegicus* where mating occurs during the hours of darkness (Farmer, 1974a). Large claw size could also be an advertisement of fitness to prospective mates (Dingle, 1983; Kodric-Brown & Brown, 1984; Andersson, 1986; Sneddon *et al.*, 1997). It has been shown that shore crabs (*Carcinus maenas*) with larger claws had a greater energy intake than males of a similar size with smaller claws (Elner, 1980), although these animals were feeding on mussels. The relationship between claw size and reproductive behaviour is, as yet, unclear in *N. norvegicus* and it may be wise therefore to use estimations of functional maturity based on morphometrics with caution.

Farmer (1974b) suggested that female *N. norvegicus* matured at a smaller size than males. The onset of physiological maturity will impose costs on males at a relatively small size when compared with functional maturity. This would suggest that there must be a payoff for precocious maturation in males. Precocious parr are often seen fertilising eggs in the redds of Atlantic salmon, in red deer young subordinate males are seen to 'sneaker' mate with females belonging to a dominant

male's harem. The lack of post-copulatory mate guarding in *N. norvegicus* could provide an opportunity for small males to take advantage of a vulnerable moulting female in the absence of a larger functionally mature male. Thus providing an evolutionary advantage to early physiological maturity. Gonadosomatic indices have been used to assess the onset of physiological maturity in crustaceans, however, the difficulties of accurately weighing the small testes of animals around the point of physiological maturity; which can be as small as 15 mm CL as previously mentioned, rendered this method unsatisfactory in this species.

Sperm limitation is a problem that could affect the fished populations of *N. norvegicus* as males are preferentially removed from the system. The ability of males to produce sperm from a small size could help to reduce sperm limitation through larger males being removed from the fishery. It is possible that this is the case in the Clyde Sea area, as a significant number of sexually active females are inseminated (Chapter 6). Small males, although sexually mature, may not be able to produce sufficiently large spermatophores, which could result in reduced egg production from the larger females, a problem that has been found in species of spiny lobsters (MacDiarmid & Butler, 1999). The size range of males within the population may also affect the reproductive cycle; in *Panulirus argus*, for example, the smaller individuals were observed to be breeding earlier in the season than the larger individuals (Quackenbush, 1994). There is no evidence to suggest that this is occurring in *N. norvegicus*.

There are potential implications for the management of *N. norvegicus* fisheries from these findings. One approach to ensure continuing recruitment occurs is to set a Minimum Legal Size (MLS) above the size at which maturity occurs. It is therefore important to determine the size at first maturity in *N. norvegicus* so that an appropriate minimum landing size could be implemented. If it is proved that different populations have different sizes at maturity, this could complicate management issues further by necessitating minimum landing sizes applicable in all fished populations. Calculation of size at first maturity is particularly important in populations with respect to the reproductive output of the population as a whole.

The Norway lobster is the subject of important fisheries in the Mediterranean and Northeast Atlantic. It is possible that fishing pressure may affect the size of the population. Removal of larger animals from the population could result in a reduction in the mean size of animals in the population through increased recruitment (as discussed by Tully *et al.*, 1989). The presence of higher densities of animals could then reduce growth rate. This reduction in the overall size of individuals could affect reproduction. Changes in breeding patterns could occur through reduced potential reproductive rate in males and perhaps a reduction in the variability in the quality of mates available to females. In trawled populations, the reproductive biology of male *N. norvegicus* is especially important since they are subjected to greater fishing pressure than females over much of their distribution. Females become largely unavailable to the fishery for six months of the year in Scottish waters, remaining mainly in the burrow while they incubate their eggs. The relationship between *N. norvegicus* and sediment type is also important as

populations can be constrained by the limits of the mud patch which they inhabit (Hill & White, 1990).

It would appear from the comparison of morphometric analyses carried out in this study, that using data from males alone can be misleading in interpreting SOM data. When analyses used data from both males and females the estimate of SOM was relatively small, and closer to the size at physiological maturity, when compared with the analyses where males were analysed independent of female data. A further recommendation would be that data from sites of differing sedimentology should be considered separately, this could be carried out relatively easily through claw length measurements from a wide size range of males and females from particular fishing grounds.

4.5 Conclusions

- There was no evidence for handedness in male or female *Nephrops norvegicus*, based on the position of the crusher claw.
- At the Little Cumbrae site, female crusher propodite length appeared to be greater than that of the males until a carapace length of approximately 25 mm.
- Male *N. norvegicus* show higher positive allometric growth of the crusher claw than females based on the measurement of crusher propodite volume (CPV).
- The use of crusher propodus length in reduced major axis (RMA) analyses produced better results for the calculation of size at onset of maturity (SOM) than CPV data, although for both measurements the residual sum of squares output from the RMA analysis was not a good fit. This may have been due to the restricted size range in some samples.

- The use of a crusher propodite volume index (CPI) did not appear to have any transferability between sites as suggested for the American lobster (*Homarus americanus*). This method also produced a much smaller SOM than for both CPV and claw length using the RMA technique.
- There did not appear to be any relationship between sediment type and SOM in males based on morphometric analyses, nor any relationship between area of sampling and SOM, although animals sampled from the Fladen ground appeared to be larger overall in terms of SOM than from other areas (Claw length SOM range = 28.1 – 40.6 mm CL). Males from the Clyde appeared to have somewhat smaller SOM's (claw length SOM range = 25.6 – 30.6 mm CL).
- It appears that a greater accuracy can be achieved in the estimation of SOM in male *N. norvegicus* by using female data as a comparison.

Area	CL	Lower group		Upper Group		F	N	P
		Slope	Constant	Slope	Constant			
Clyde	27.9	3.963	-2.323	4.657	-3.379	20.00	474	<0.001
Ailsa	24.0	4.340	-2.782	4.566	-3.217	1.8027	150	n.s.
Clyde 3	36.5	3.931	-2.242	5.311	-4.510	0.310	85	n.s.
Fladen 1	40.6	2.803	-0.624	4.10	-2.504	6.991	90	<0.005
Fladen 2	33.3	4.024	-2.427	5.606	-5.052	8.139	43	<0.005
Fladen 3	39.9	4.286	-2.824	11.664	-14.842	1.775	21	n.s.
Fladen 4	33.1	4.225	-2.724	4.323	-2.961	4.515	115	<0.025
North Minch 1	31.4	3.772	-2.092	3.748	-2.030	2.683	133	n.s.
North Minch 2	43.2	3.978	-2.358	2.976	-0.721	3.920	125	<0.025
North Minch 3	30.1	3.727	-1.998	4.375	-2.957	5.923	122	<0.005
South Minch 1	33.5	3.588	-1.842	4.602	-3.351	8.335	91	<0.001
South Minch 2	26.5	47.384	-64.030	4314	-2.879	8.2857	59	<0.001
South Minch 3	23.1	10.334	-10.966	4.139	-2.636	0.155	98	n.s.

Table 4.1: Summary of regression statistics from reduced major axis analysis of male crusher propodite volume from areas of differing sedimentology (See Table 4.3 for sedimentology).

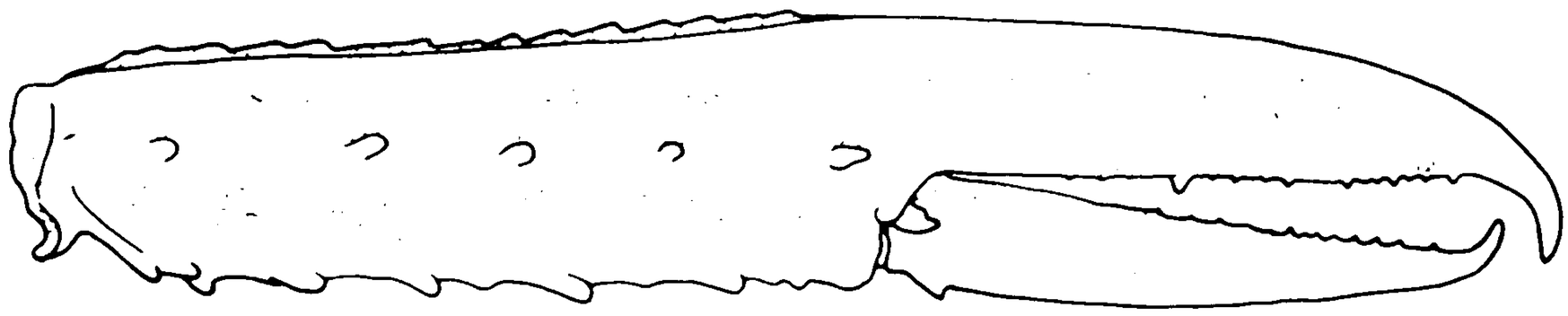
Area	CL	Lower group		Upper Group		F	N	P
		Slope	Constant	Slope	Constant			
Cumbrae	30.6	1.670	-4.920	2.622	-35.05	87.948	474	<0.001
Ailsa	26.4	1.556	-2.183	2.599	-32.0102	27.33	150	<0.001
Clyde 3	25.6	1.555	-2.198	2.302	-22.545	11.005	85	<0.005
Fladen 1	28.1	1.583	-2.147	2.456	-29.121	9.798	90	<0.005
Fladen 2	37.9	1.620	-3.417	3.200	-65.224	15.549	43	<0.001
Fladen 3	40.6	2.099	-17.415	11.727	-422.105	5.593	21	<0.05
Fladen 4	32.5	1.667	-4.989	2.379	-30.202	15.559	115	<0.001
North Minch 1	44.8	1.794	-8.251	3.690	-93.700	21.298	133	<0.001
North Minch 2	26.2	1.602	-27.322	2.140	-18.606	9.383	125	<0.001
North Minch 3	29.1	1.557	-1.653	2.342	-25.789	17.550	122	<0.001
South Minch 1	31.5	1.832	-9.734	2.405	-29.844	5.632	91	<0.01
South Minch 2	34.5	1.639	-2.322	2.611	-37.746	12.546	59	<0.001
South Minch 3	24.4	1.446	-0.330	2.068	-17.151	133.226	98	<0.001

Table 4.2: Summary of regression statistics from reduced major axis analysis of male crusher propodite length from areas of differing sedimentology (See Table 4.3 for sedimentology).

Site	Sediment Type	% Silt clay content	Claw Length SOM	Claw volume SOM
Cumbrae	Mud	90 – 100%	30.6	27.9
Ailsa Craig	Sandy Mud	50 – 90%	26.4	24.0
Clyde 3	Muddy Sand	10 – 50%	25.6	36.5
Fladen 4	Mud	80 – 100%	28.1	40.6
Fladen 2	Muddy Fine	55 – 79%	37.9	33.3
Fladen 1	Muddy Coarse	40 – 54%	40.6	39.9
Fladen 3	Muddy Sand	10 – 39%	32.5	33.1
North Minch1	Mud	90 – 100%	44.8	31.4
North Minch 2	Sandy Mud	50 – 90%	26.2	43.2
North Minch 3	Muddy Sand	10 – 50%	29.1	30.1
South Minch 1	Mud	90 – 100%	31.5	33.5
South Minch 2	Sandy Mud	50 – 90%	34.5	26.5
South Minch 3	Muddy Sand	10 – 50%	24.4	23.1

Table 4.3: The relationship between sedimentology and the estimated size at maturity (SOM) using the Reduced Major Axis (RMA) technique on claw length and claw volume from male *Nephrops norvegicus*.

a)



b)

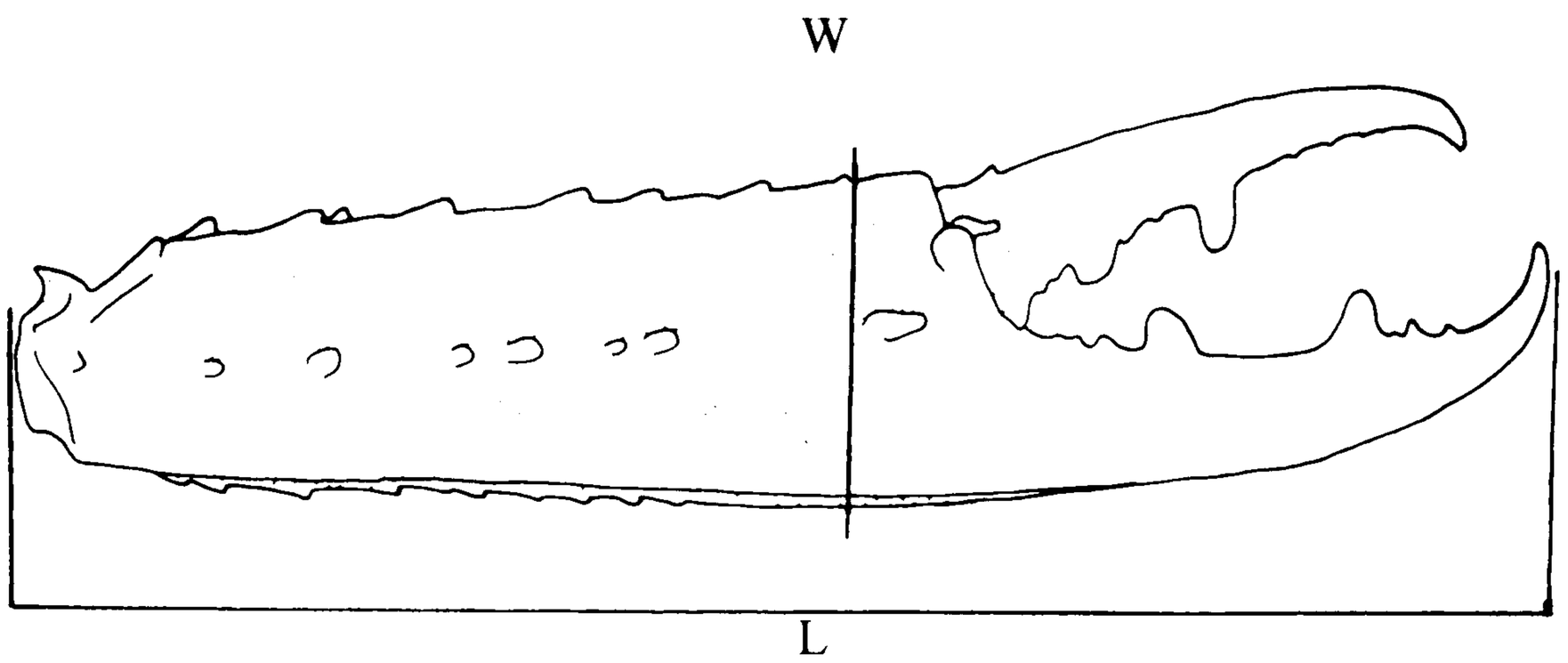


Figure 4.1: The claws of a mature male *Nephrops norvegicus* a) cutter and b) crusher. Claw measurements were carried out on the crusher propodite as shown. L = length, W = width, depth of the claw was measured at the same position as width. Claws redrawn from Farmer (1974).

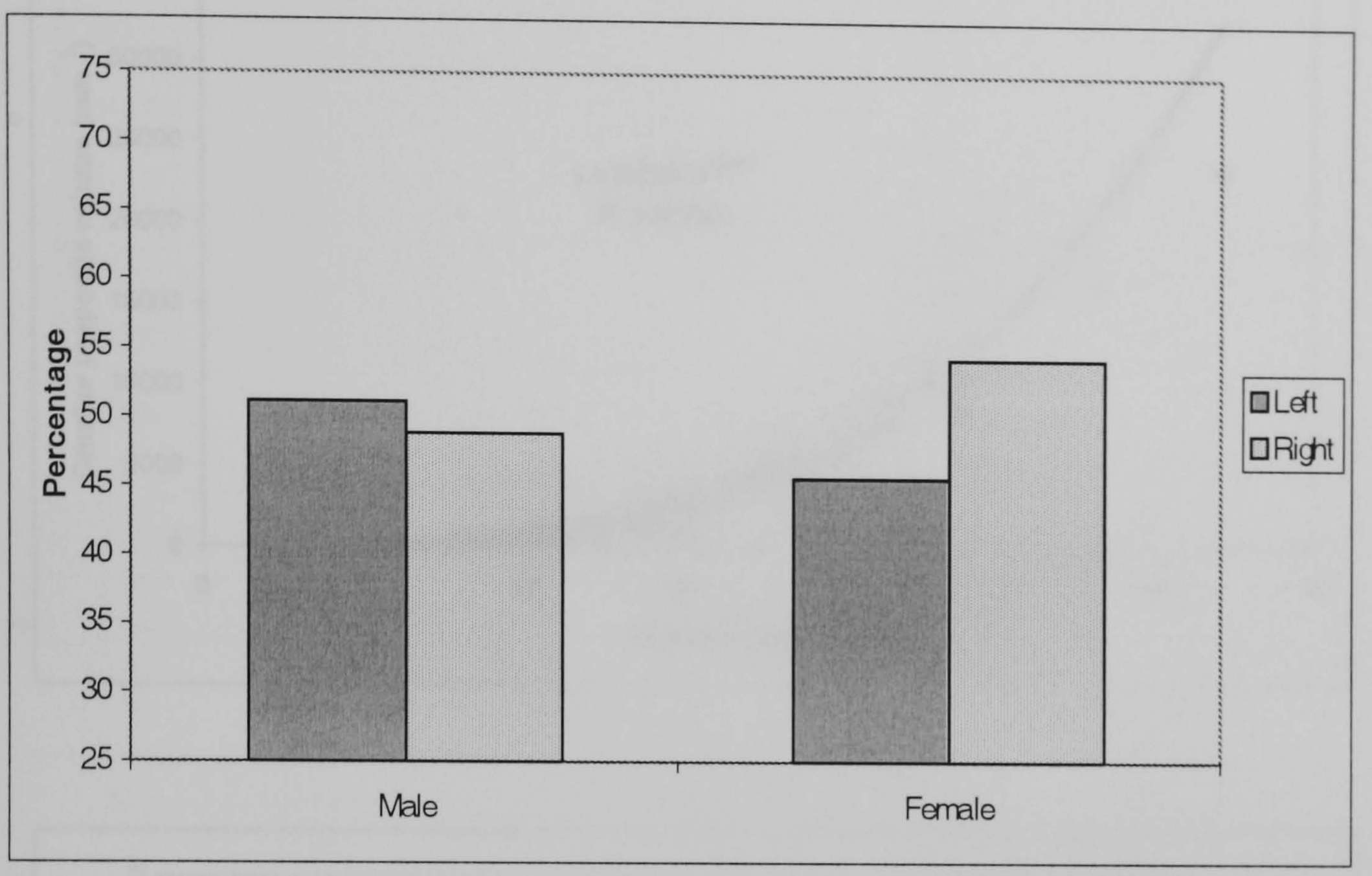
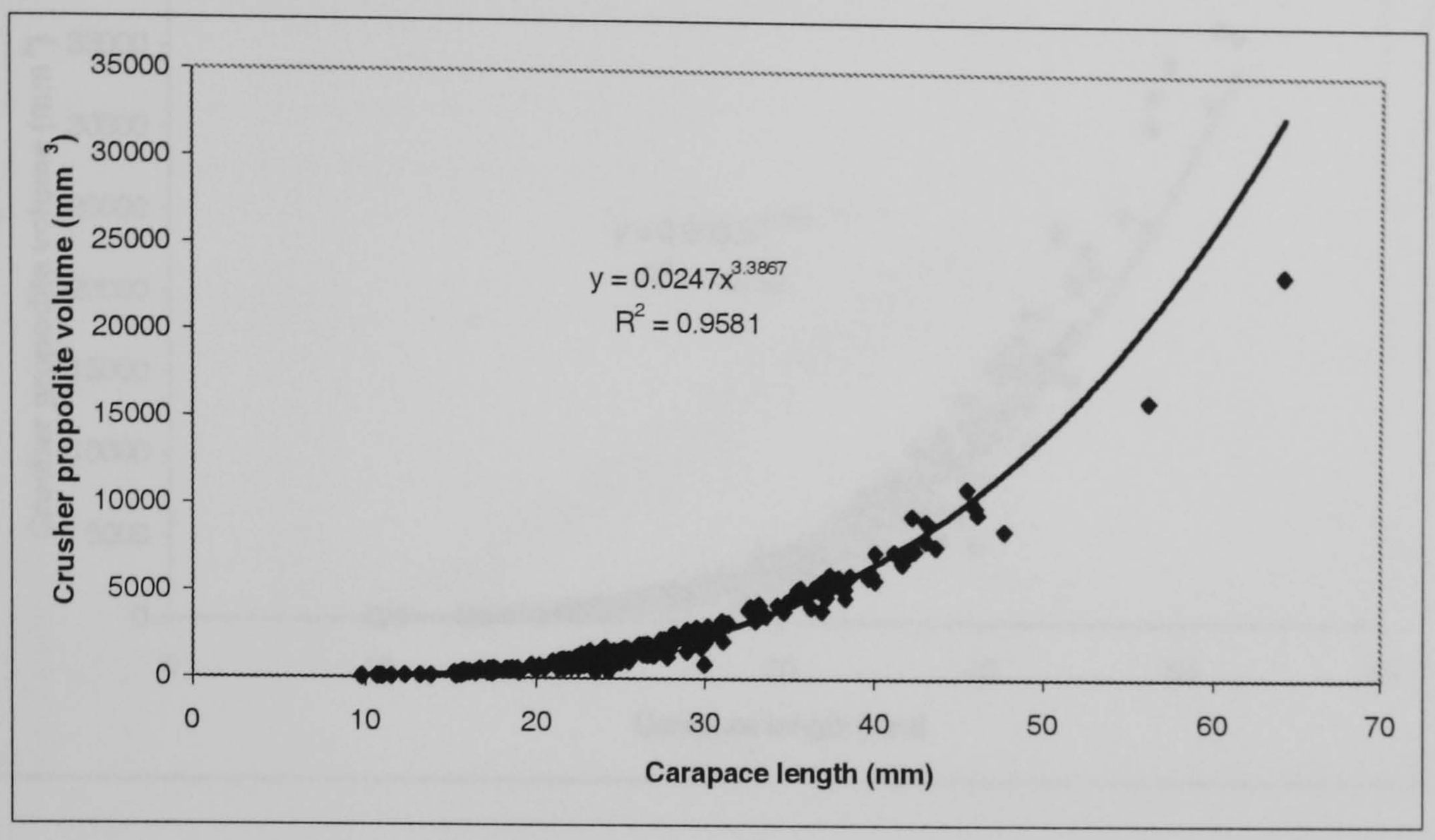


Figure 4.2: Handedness in male and female *Nephrops norvegicus* based on the position of the crusher claw. All animals were sampled from south of Little Cumbrae in the Clyde sea area (χ^2 DF=1; p=0.333).



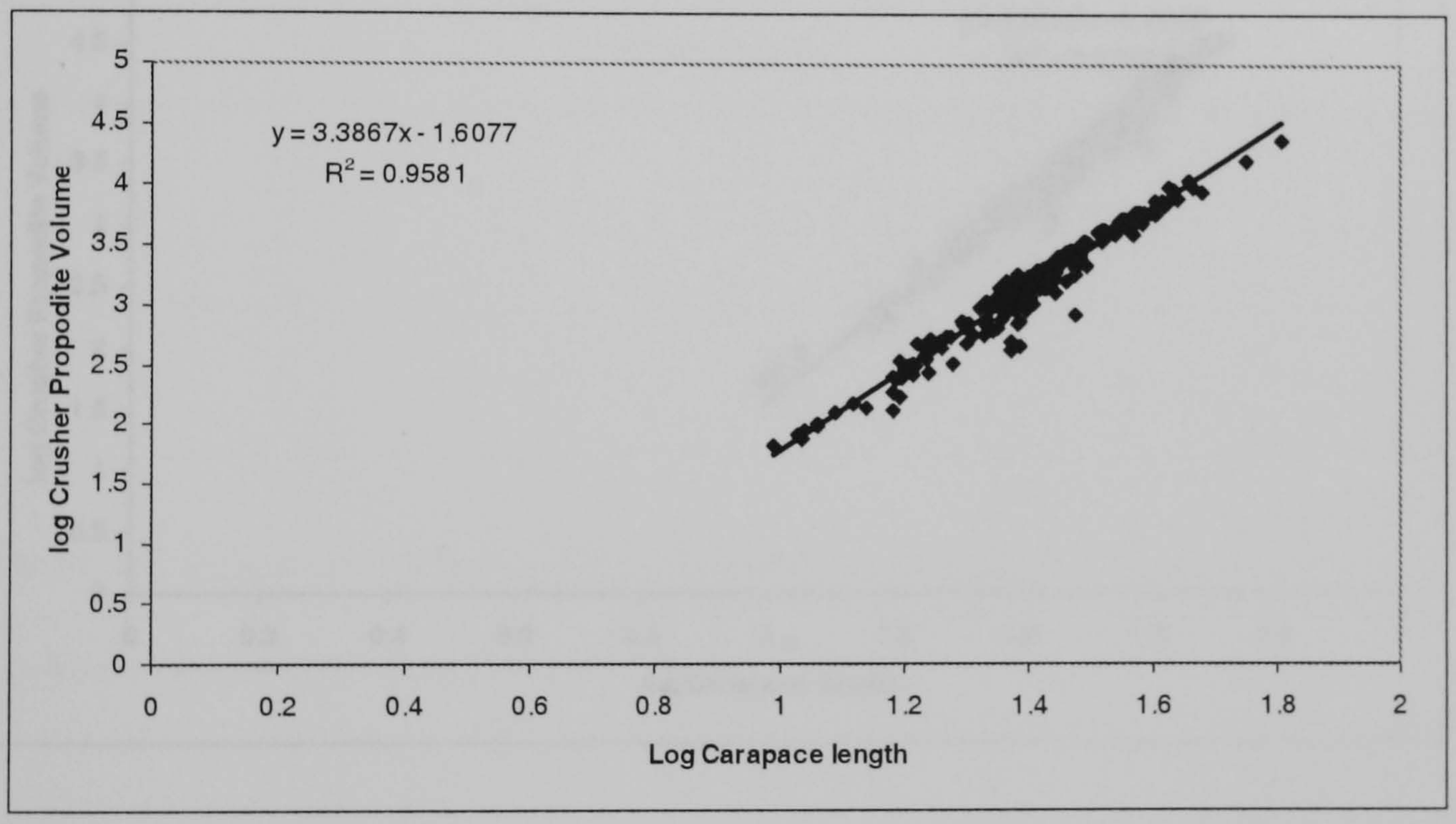
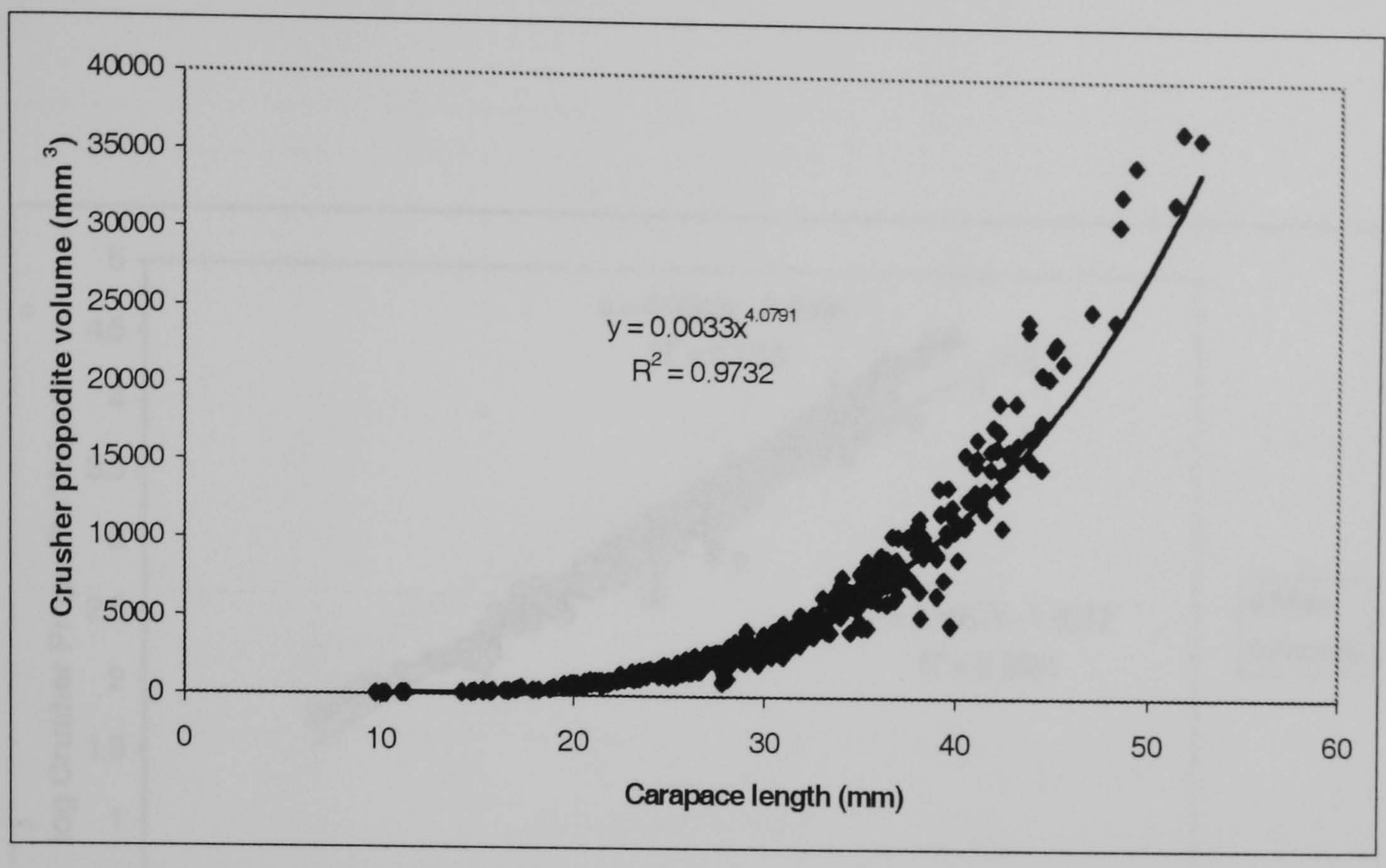


Figure 4.3: The relationship between crusher propodite volume and the carapace length of female *Nephrops norvegicus* sampled from south of Little Cumbrae in the Clyde Sea area.



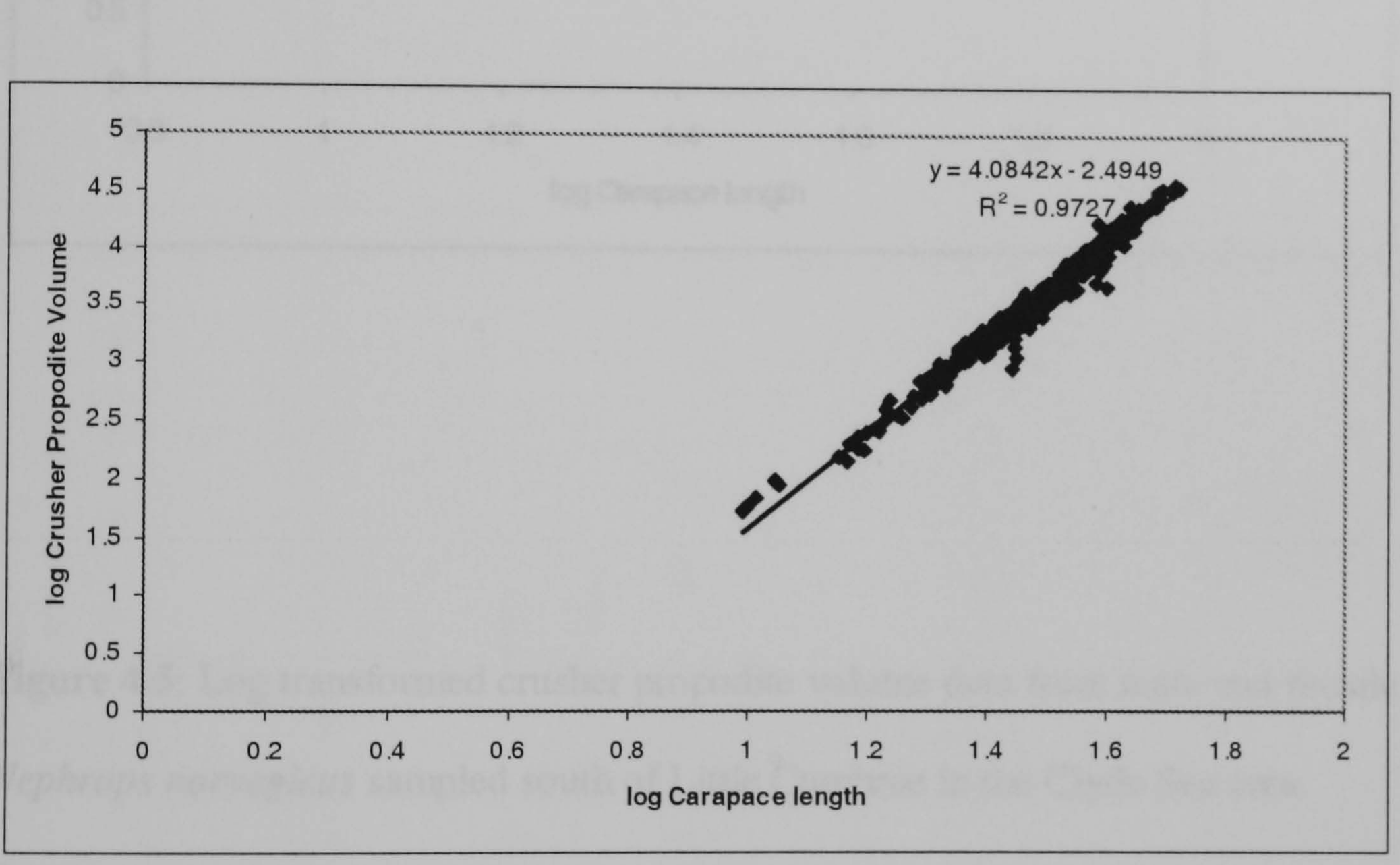


Figure 4.4: The relationship between crusher propodite volume and carapace length for male *Nephrops norvegicus* sampled from south of Little Cumbrae in the Clyde Sea area. The relationships between crusher propodite volume and carapace length for males from the other sites sampled can be found in Appendix 2.

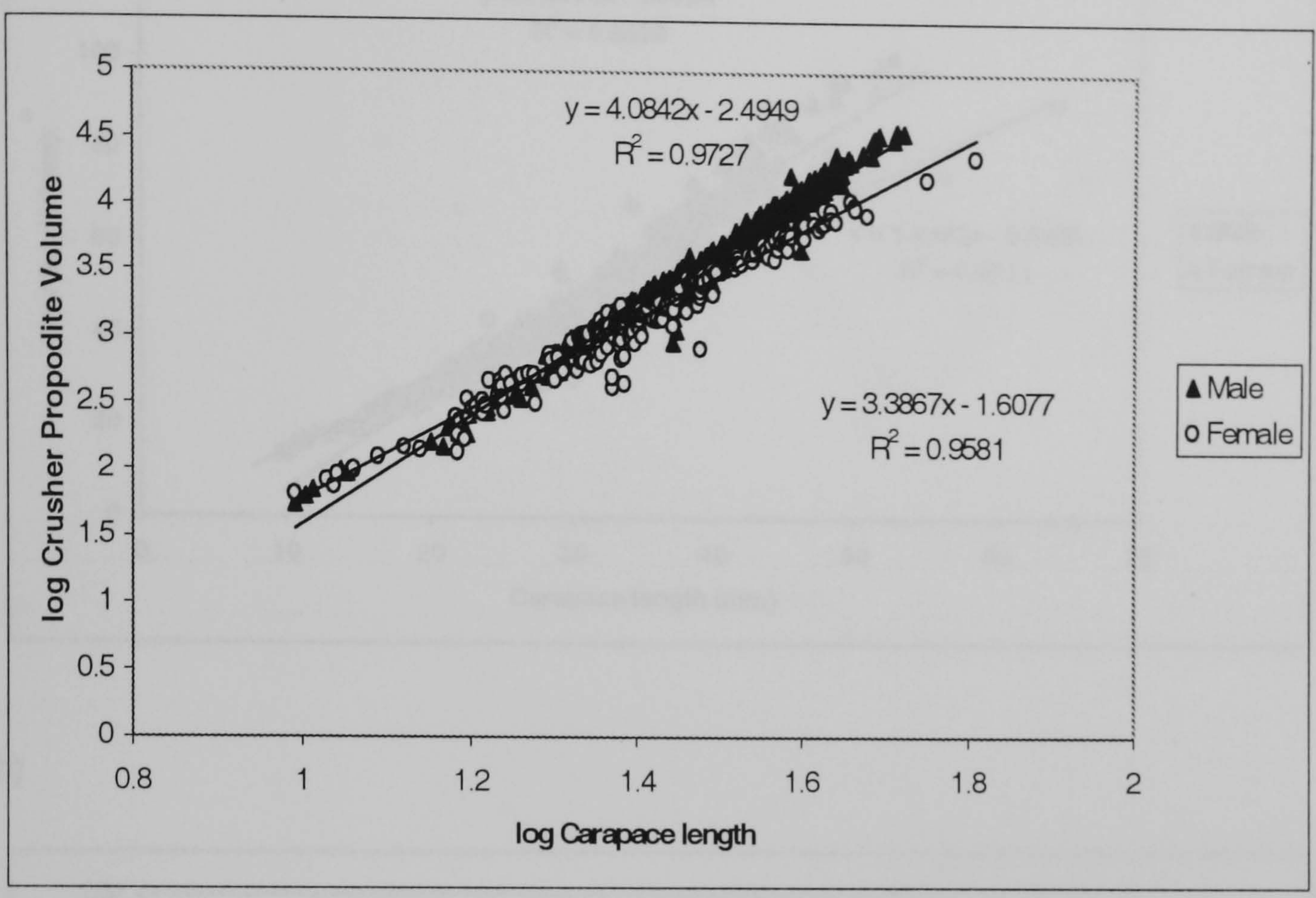
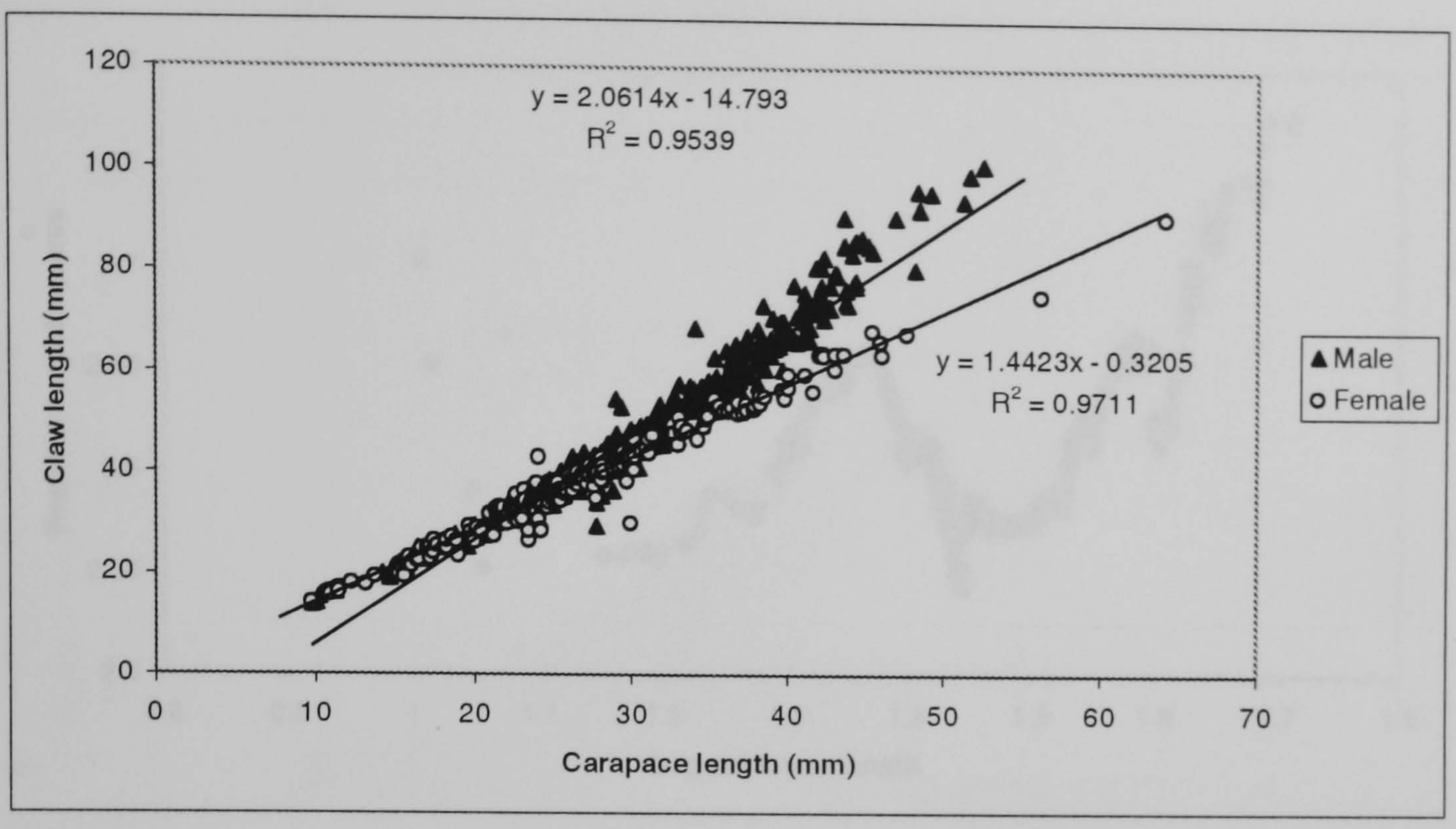


Figure 4.5: Log transformed crusher propodite volume data from male and female *Nephrops norvegicus* sampled south of Little Cumbrae in the Clyde Sea area.

a)



b)

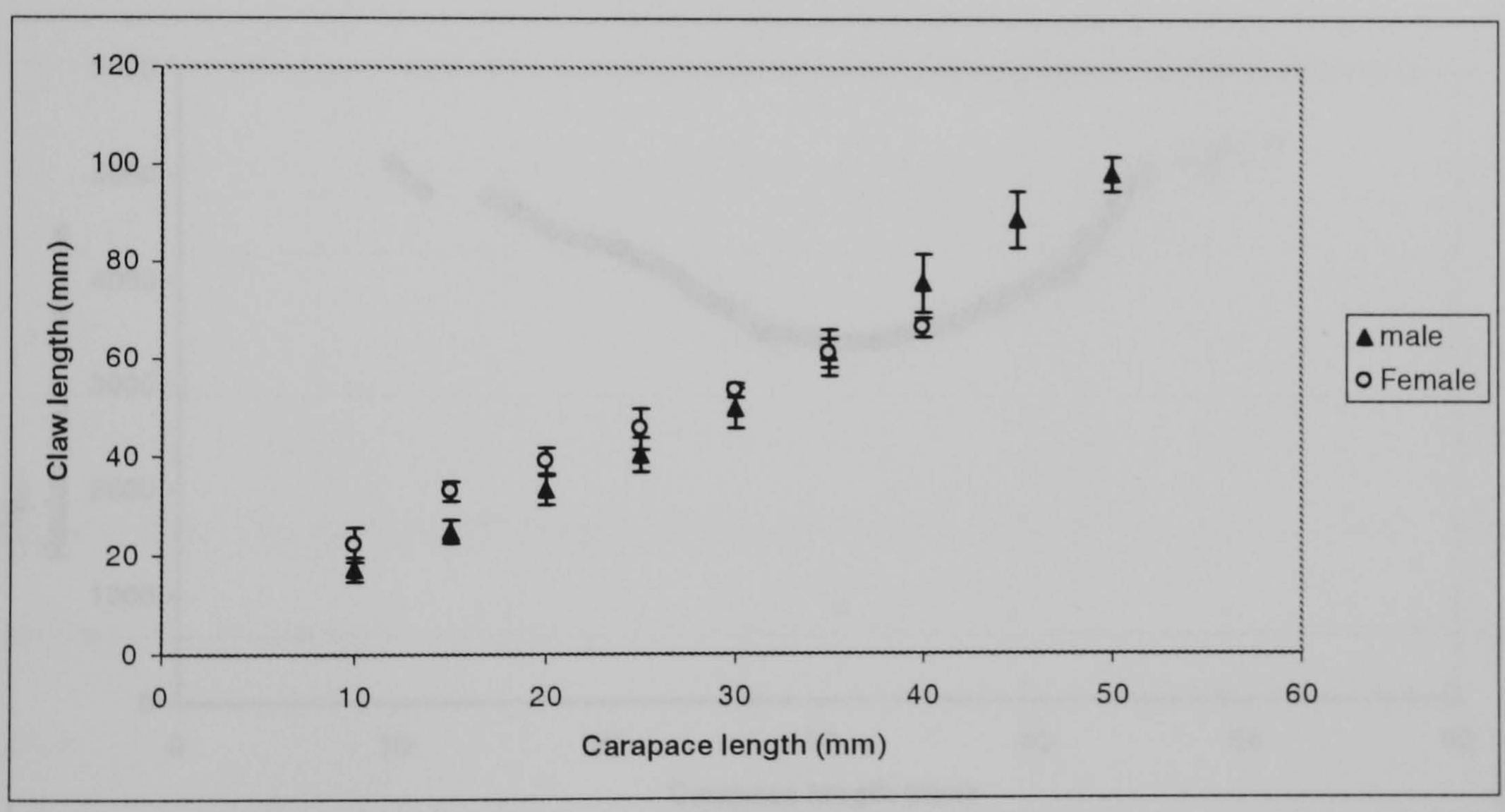
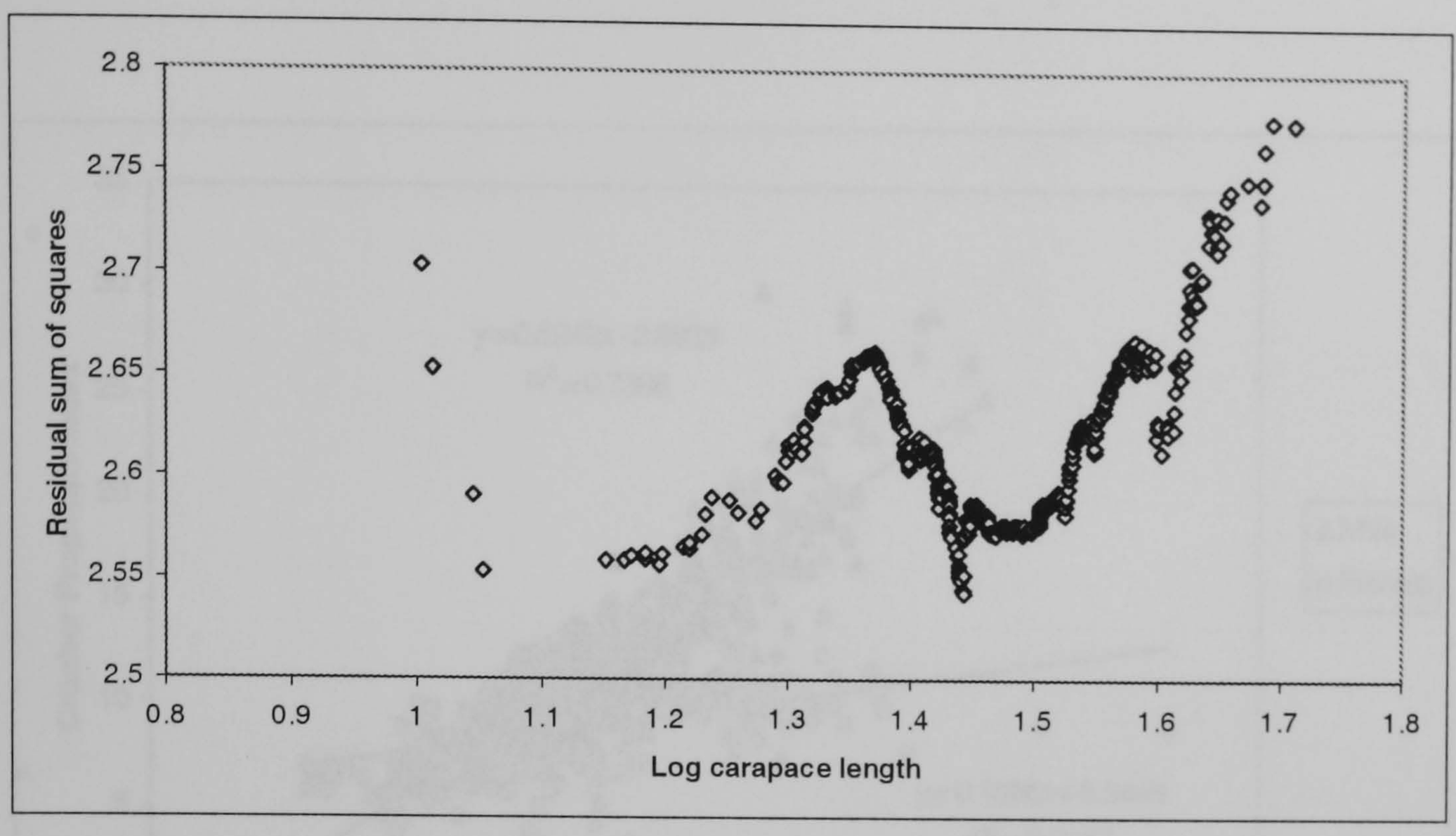


Figure 4.6: The relationship between a) claw length (mm) and carapace length (mm) and b) mean claw length (± SD) in 5 mm size classes for male and female *Nephrops norvegicus* sampled from south of Little Cumbrae in the Clyde Sea area.

a)



b)

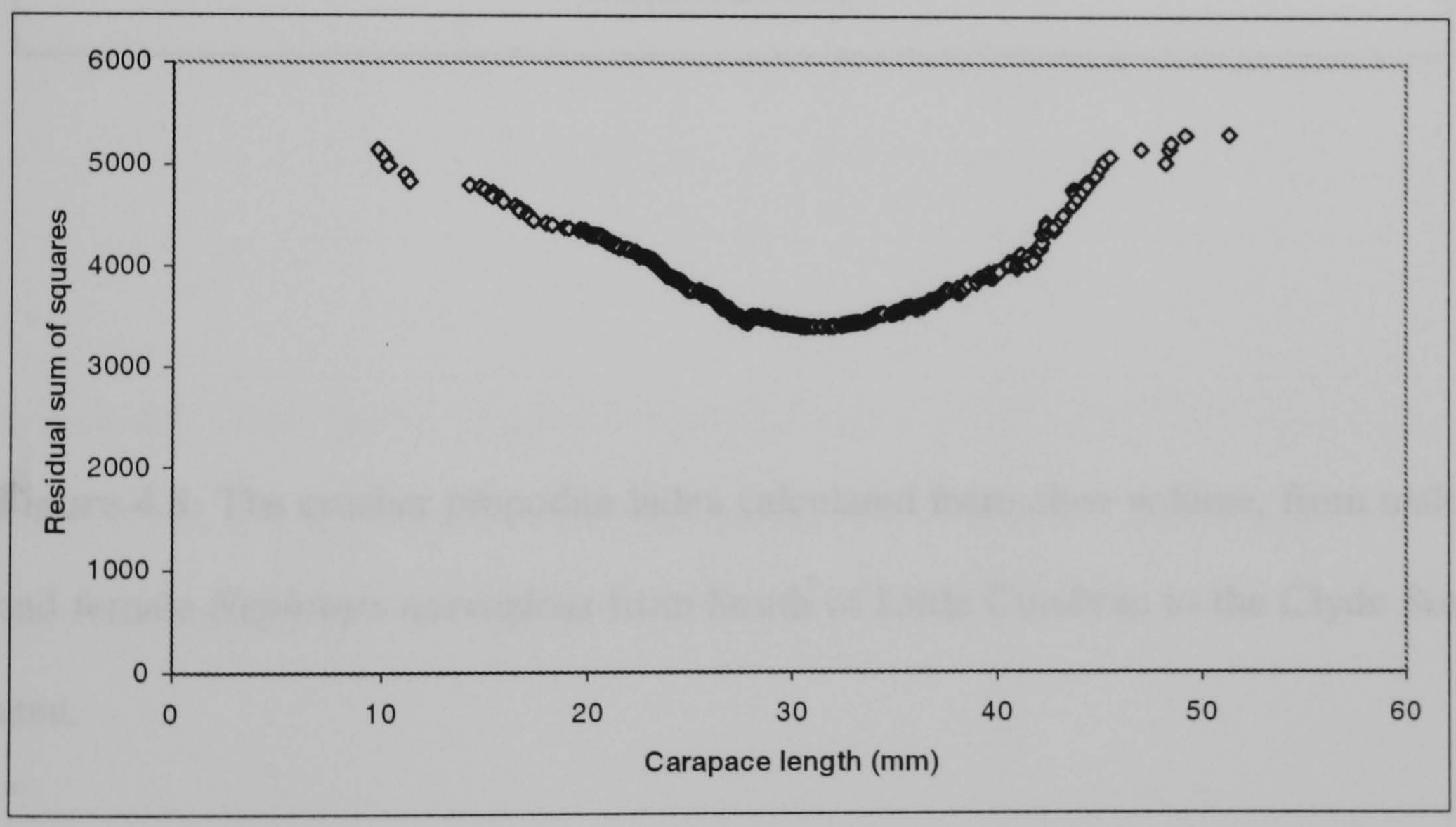


Figure 4.7: Plots of the residual sum of squares output from the Reduced Major Axis (RMA) analysis of a) log crusher propodite volume and b) claw length of male *Nephrops norvegicus* sampled from south of Little Cumbrae in the Clyde Sea area.

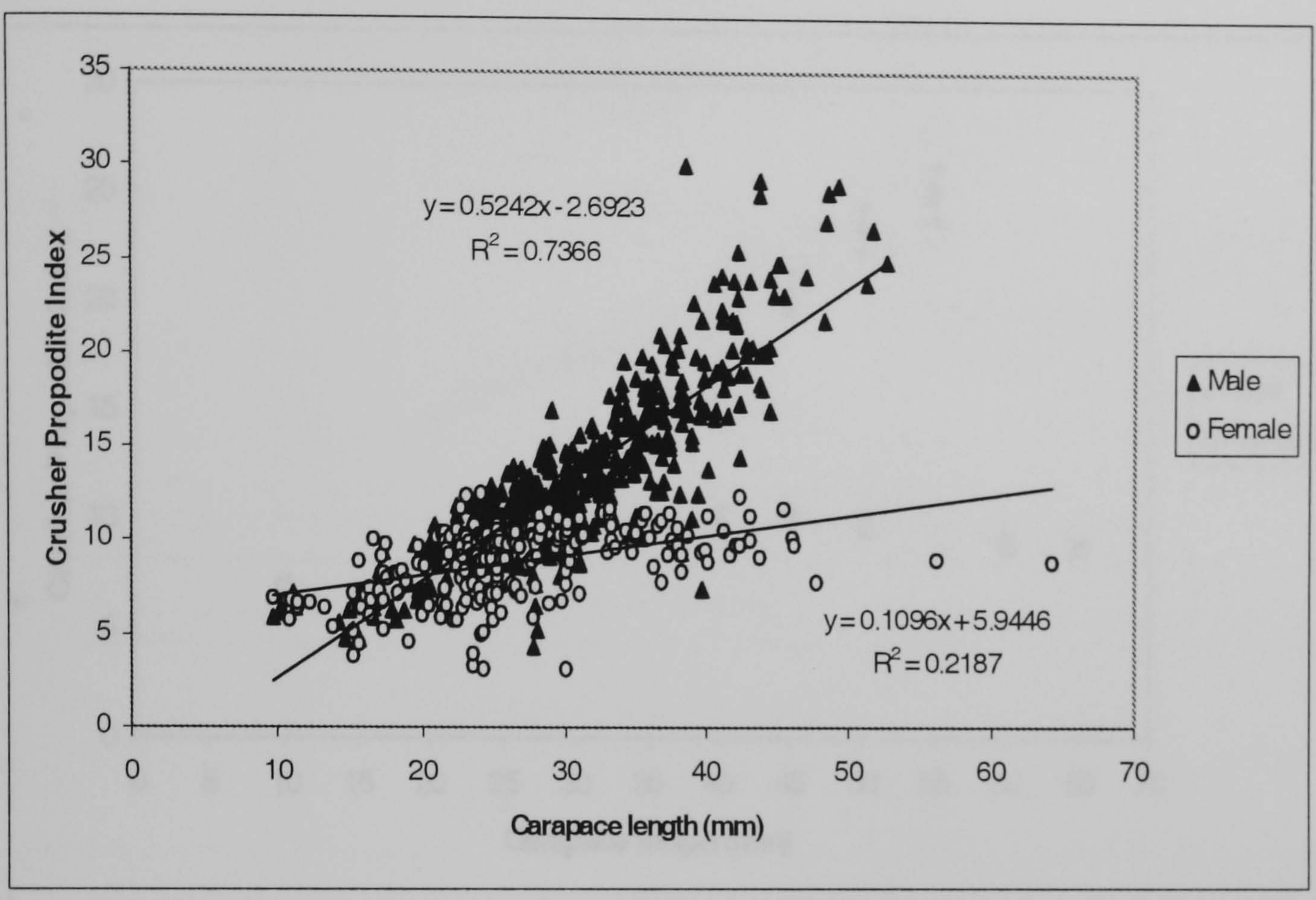


Figure 4.8: The crusher propodite index calculated from claw volume, from male and female *Nephrops norvegicus* from South of Little Cumbrae in the Clyde Sea area.

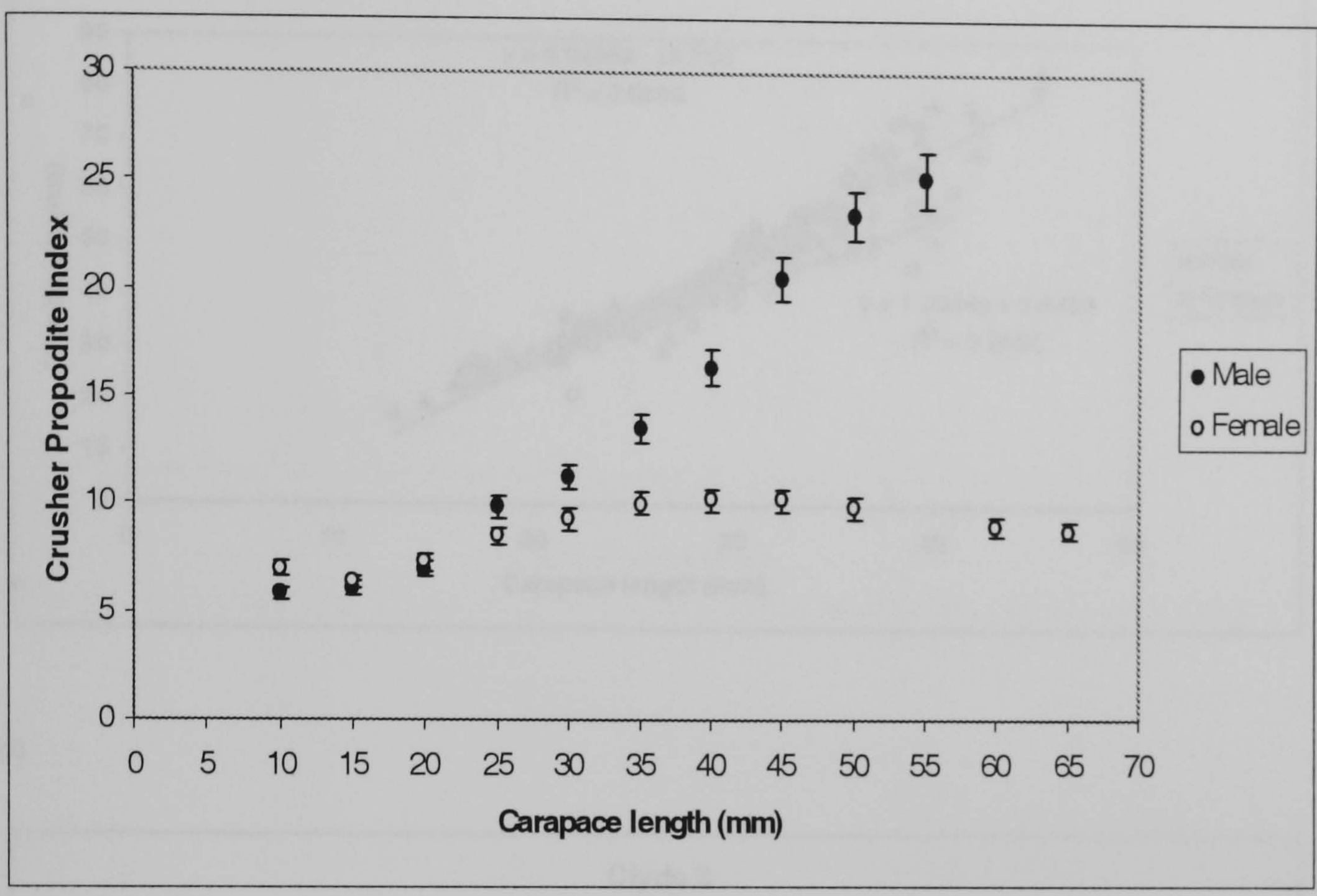
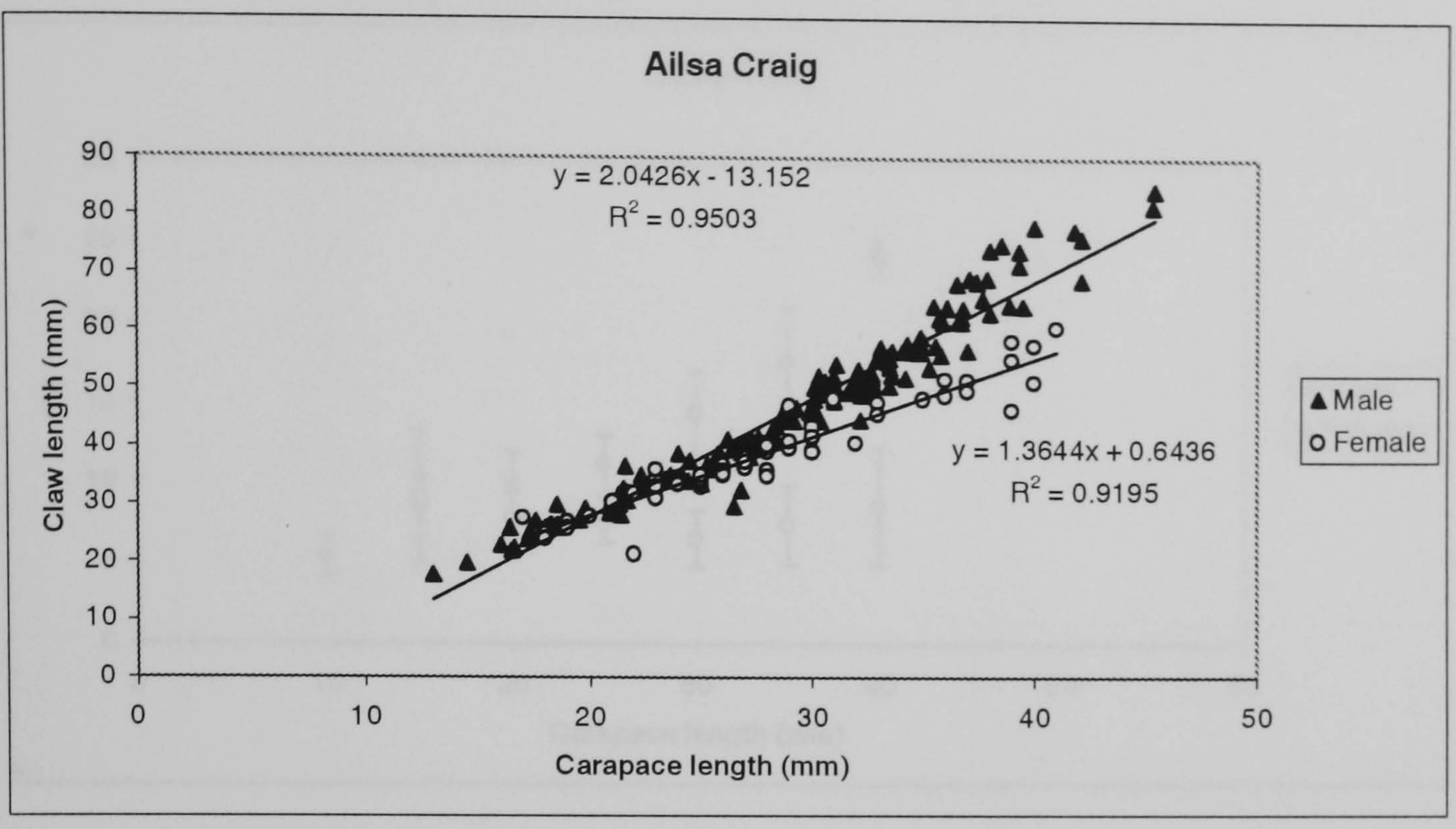


Figure 4.9: Mean (±SD) Crusher Propodite Index (CPI) for 5mm size classes of male and female *Nephrops norvegicus* sampled from south of Little Cumbrae in the Clyde Sea area.

a)



b)

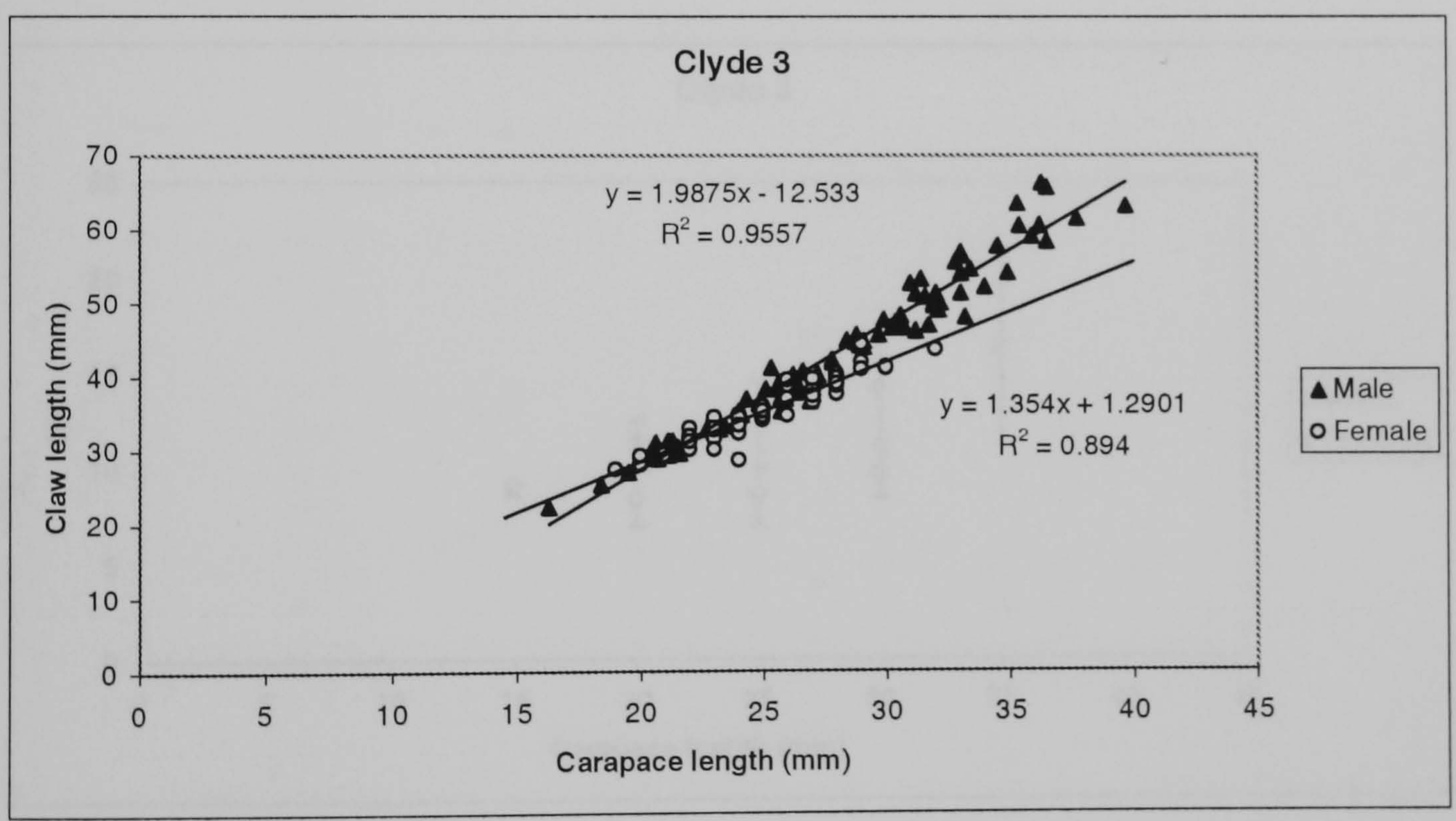
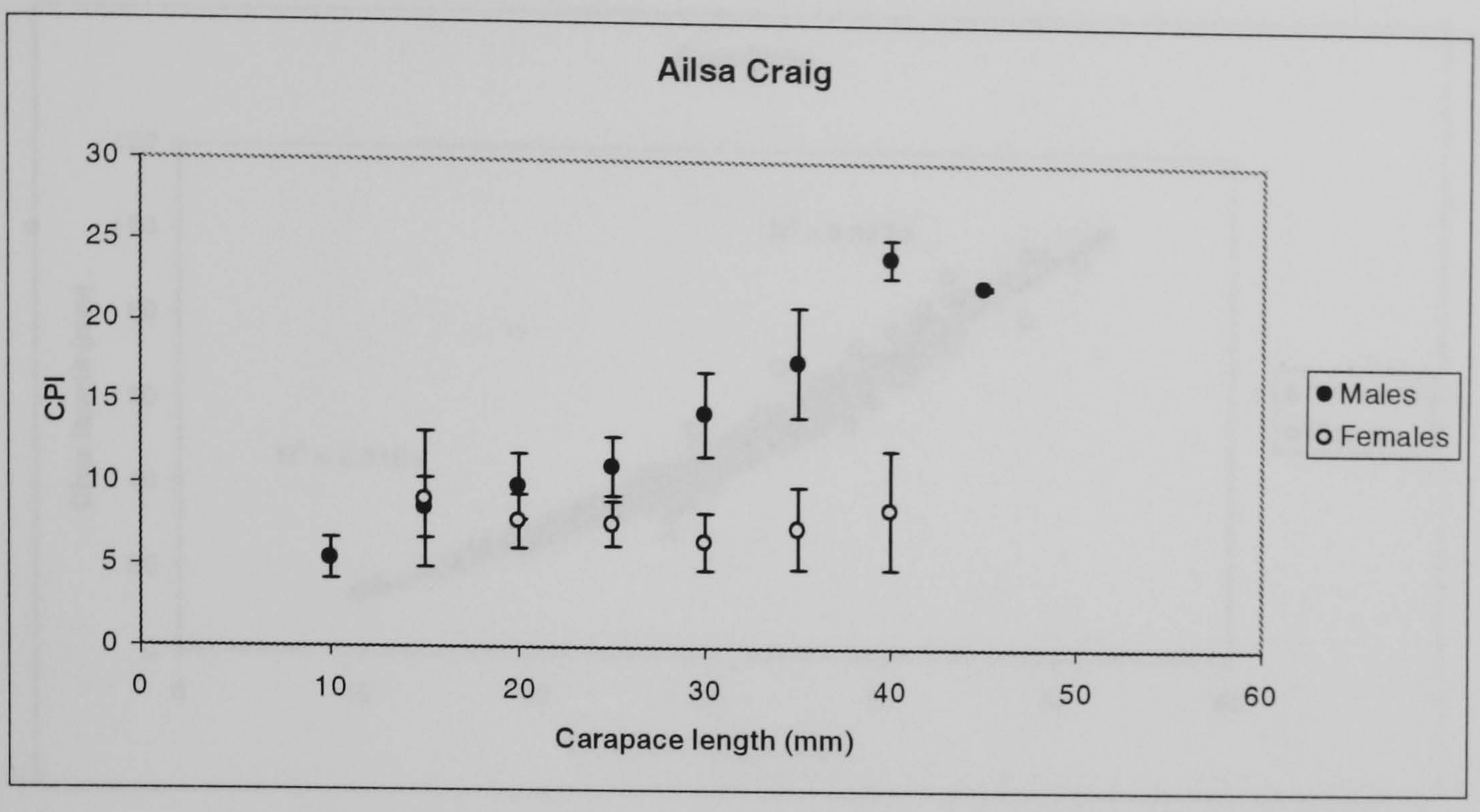


Figure 4.10: The relationship between male and female carapace length and claw length in *Nephrops norvegicus* from a) Ailsa Craig and b) Clyde 3 in the Clyde Sea area.

a)



b)

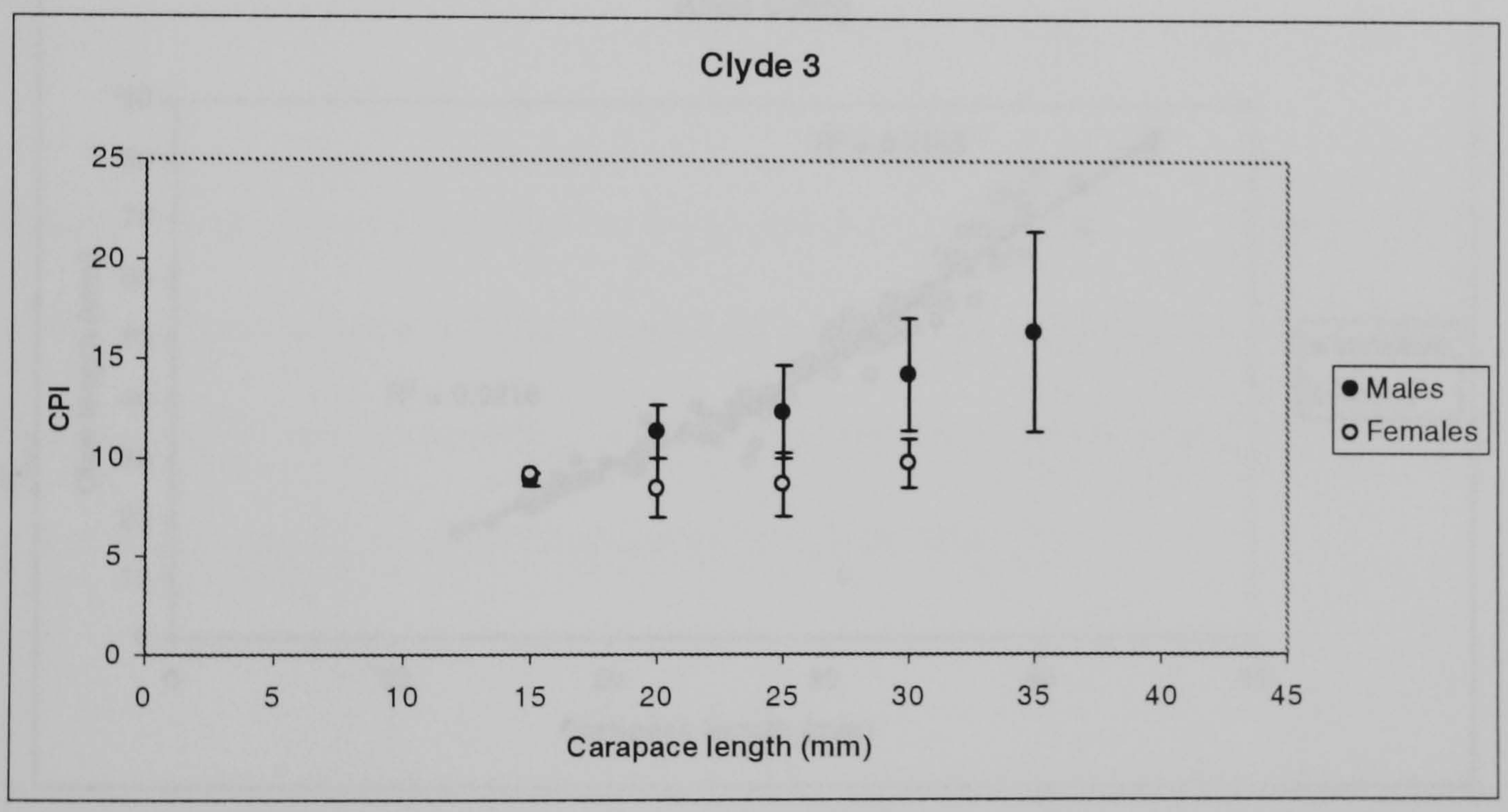
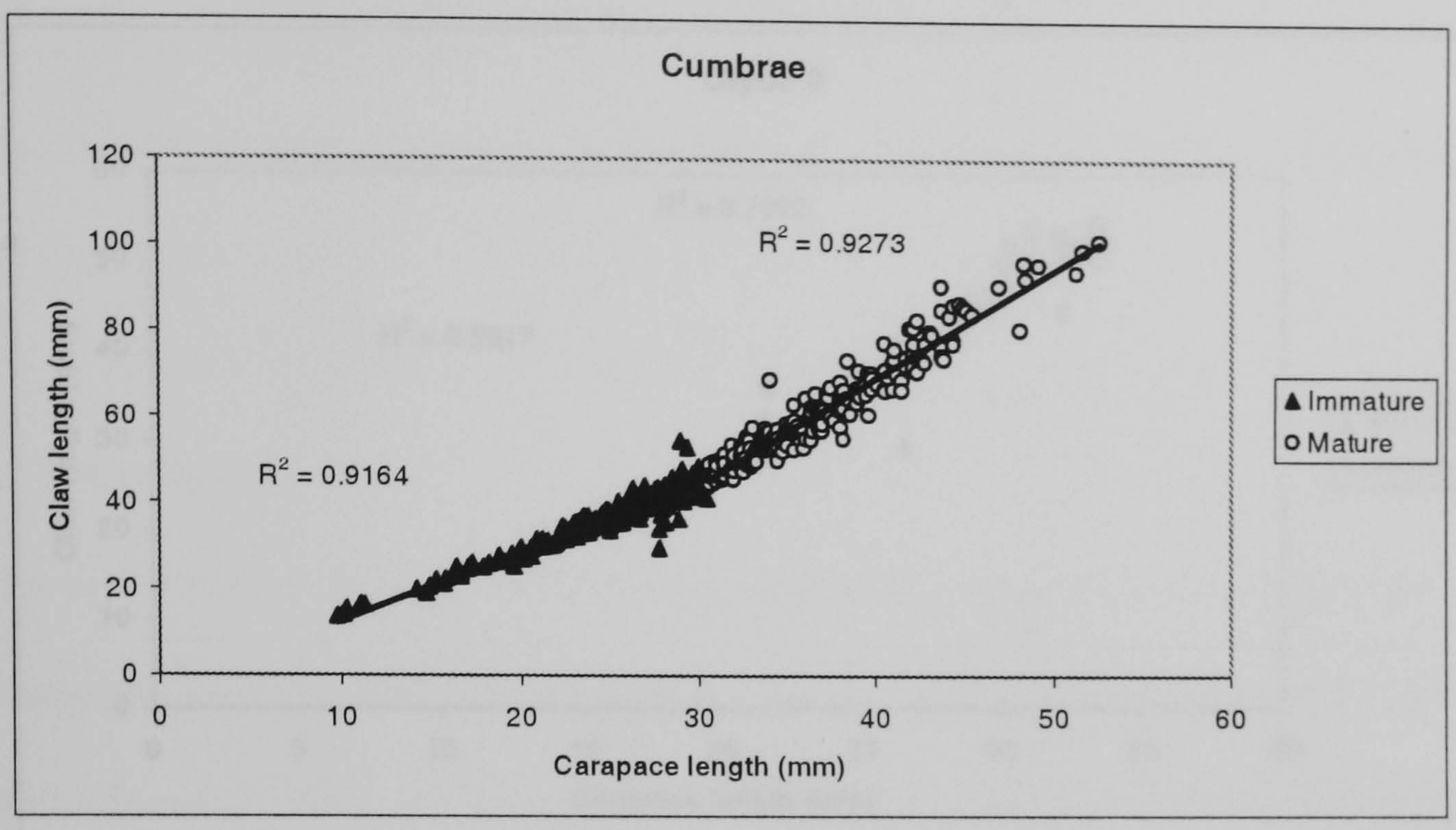


Figure 4.11: The mean (± SD) crusher propodite index in 5mm size classes for male and female *Nephrops norvegicus* from two sites in the Clyde Sea area.

a)



b)

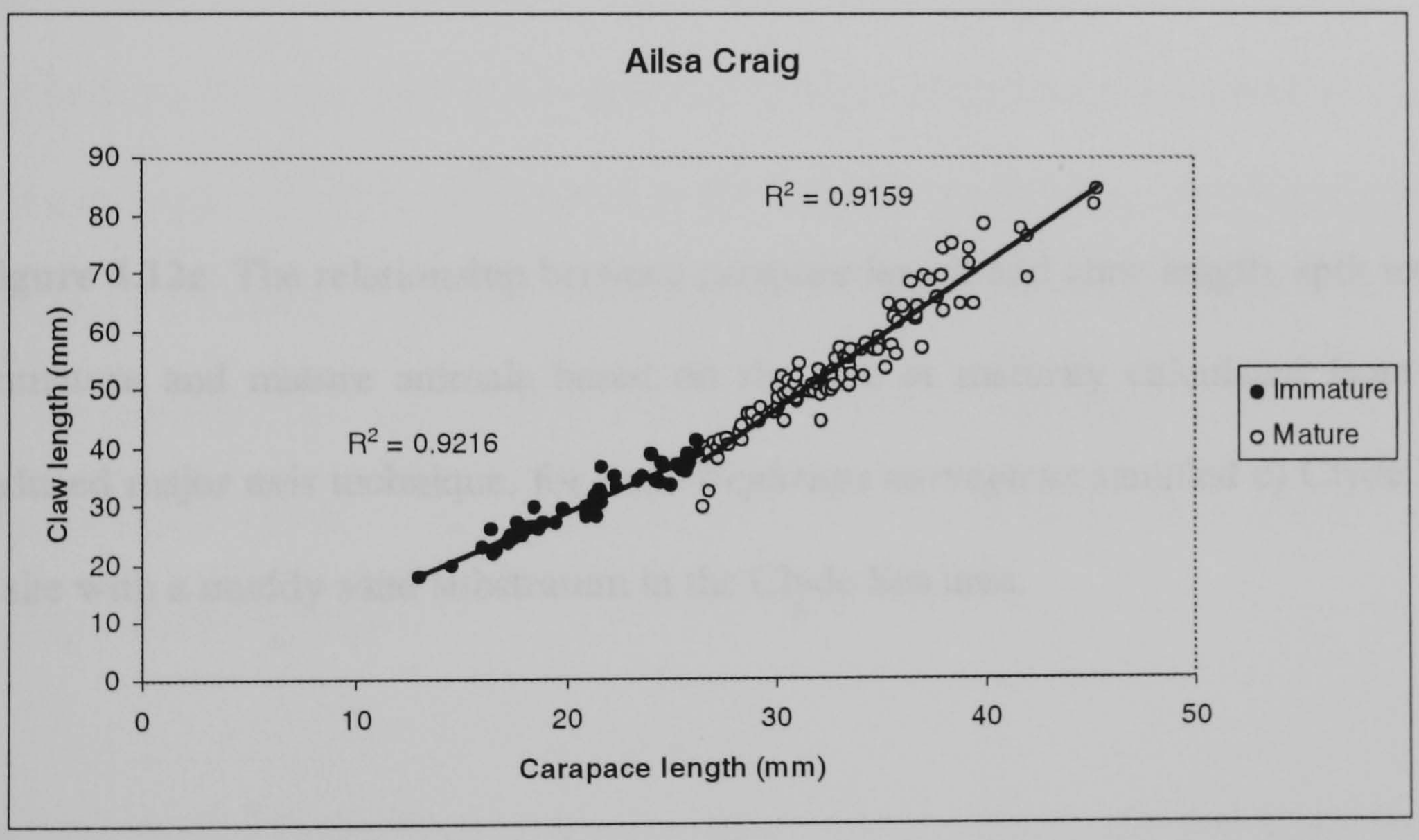


Figure 4.12a & b: The relationship between carapace length and claw length, split into immature and mature animals based on the size at maturity calculated from a reduced major axis technique, for male *Nephrops norvegicus* sampled a) south of Little Cumbrae and b) south west of Ailsa Craig in the Clyde Sea area.

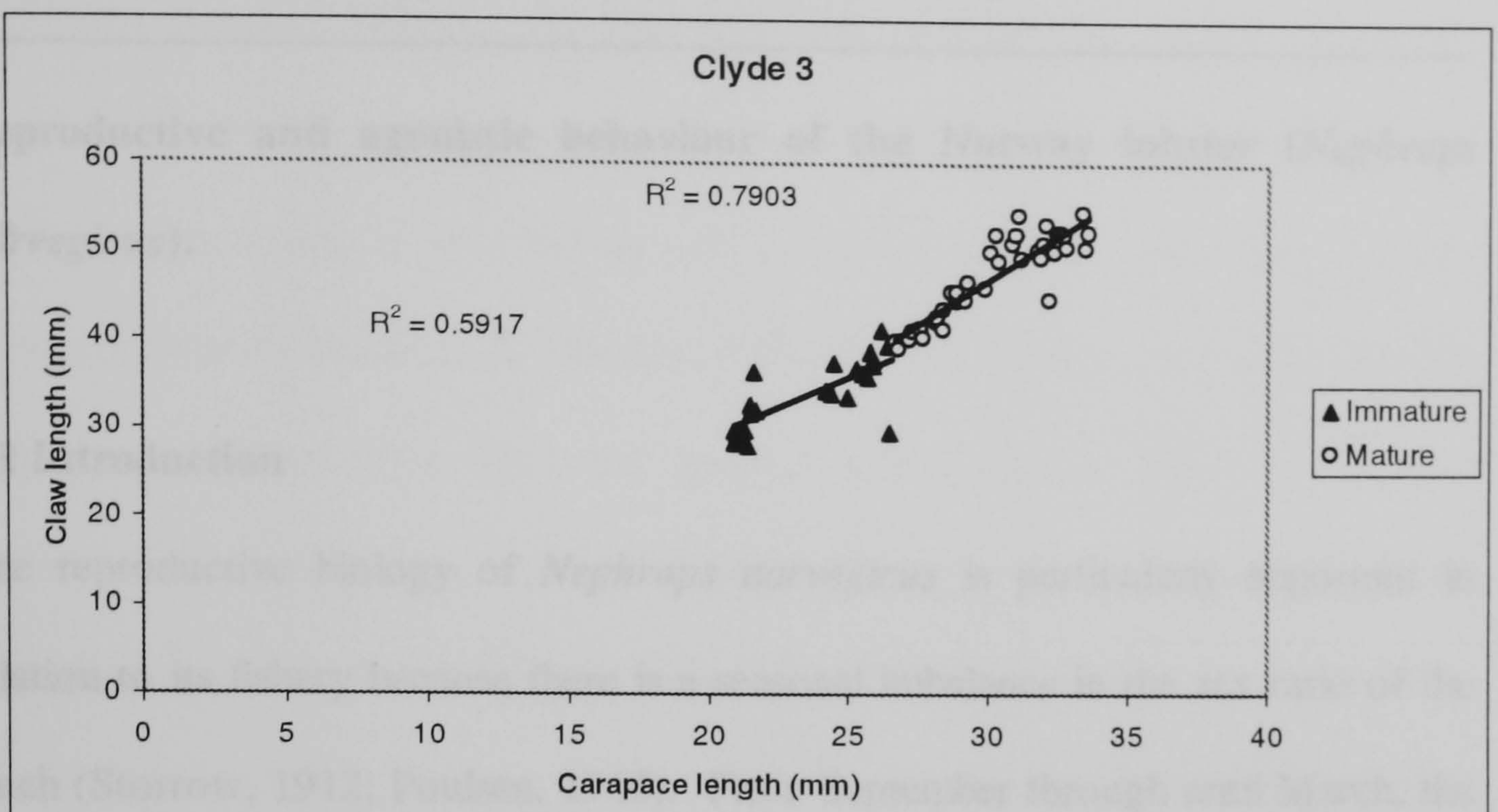


Figure 4.12c: The relationship between carapace length and claw length, split into immature and mature animals based on the size at maturity calculated from a reduced major axis technique, for male *Nephrops norvegicus* sampled c) Clyde 3, a site with a muddy sand substratum in the Clyde Sea area.

Chapter 5

Reproductive and agonistic behaviour of the Norway lobster (*Nephrops norvegicus*).

5.1 Introduction

The reproductive biology of *Nephrops norvegicus* is particularly important in relation to its fishery because there is a seasonal imbalance in the sex ratio of the catch (Storrow, 1912; Poulsen, 1946). From September through until March, the trawl catch consists mainly of males. During this period, female *N. norvegicus* remain largely in their burrows incubating their eggs (which are carried on the pleopods on the underside of the abdomen) and are thus unavailable to the fishery. It is important, therefore, to understand the patterns of behaviour displayed by this species to determine if relatively higher fishing mortality in males is likely to impact on the reproductive output of populations. This is especially important for a species that is patchy in its distribution, since it is restricted by the availability of suitable muddy substrata.

Farmer (1975) reported that, in the laboratory, mating in *N. norvegicus* occurred at dusk or in the hours of darkness. Mating is directly related to the female moult cycle. The female becomes receptive once she has moulted and while her exoskeleton is still soft. It is thought that pheromones produced by the female advertise her availability during this vulnerable period. Courtship involves the male approaching the female from behind and stroking her with his antennae. The male

then straddles the female and turns her with the third maxillipeds and the second periopods (Farmer 1974a). At this stage, the animals are usually positioned horizontally with the male lying above the female (Aiken & Waddy 1980; Farmer, 1974a). Penetration is brief lasting only 2 seconds during which time a single spermatophore is transferred (Farmer, 1974a). By flexing the abdomen, the spermatophore, extruded from the genital opening at the base of the fifth pereiopods, is passed along a groove in the first pair of pleopods by the appendix masculina on the second pair of pleopods.

During copulation, the male passes a spermatophore into the seminal receptacle, also called the thelycum or spermatheca, of the female. The spermatophore consists of spermatozoa embedded in a gelatinous matrix. This gelatinous mass then hardens within a few hours (Farmer, 1974a) to protect the sperm during the period of storage before spawning. In the mating of the American lobster, *Homarus americanus*, Templeman (1934) observed that this sperm plug hardened to such a degree that it was determined that no other male would be able to inseminate the female, although multiple mating was induced under enforced conditions. This would suggest that paternity is guaranteed to any male able to successfully compete for, and mate with, a female. Multiple mating has been observed in *N. norvegicus* (Salerno, 2000) and in the American lobster (Templeman, 1934), in the laboratory where males were introduced to the female in succession. Templeman (1934) suggested, however, that it was unlikely that the same male would inseminate the female more than once. Indeed Salerno (2000) reported that following copulation the males showed no further interest in the

female. The female spiny lobster *Jasus lalandii* has also been shown to mate only once with no multiple sperm masses reported (Atema & Cobb, 1980). Templeman (1934) suggested that a single sperm mass from *H. americanus* contained more than one spermatophore, contrary to the description of *N. norvegicus* mating given by Farmer (1974a).

Following mating, there is no further association between male and female *N. norvegicus* (Farmer, 1974a) unlike the pre- and post- copulatory mate-guarding behaviour reported in the American lobster (e.g. Atema *et al.*, 1979) and which is also common in crabs (e.g. the blue crab, *Callinectes sapidus* (Jivoff & Hines, 1998)). The behaviour of the female *N. norvegicus* during the mating process was described as mainly passive (Farmer, 1974a) although this would be expected during this vulnerable stage in the moult cycle. In species where it occurs, mate guarding is beneficial to the female during this vulnerable time and is also thought to benefit the male in preventing other males from copulating. The timing of the single mating in the American lobster has been reported as occurring around 30 minutes after the female moult (Atema, 1986). After a period of about 12 – 20 hours, the female American lobster will become less submissive and may attempt to tail flip away from an approaching male, and after 2 – 3 days mating did not take place at all (Templeman, 1934).

Visual, tactile, acoustic and chemical communications have all been reported in the transmission of information on gender and reproductive condition of crustaceans (see Sastry, 1983; Salmon & Hyatt, 1983). It appears that visual communication is

more important in terrestrial and semi-terrestrial crustaceans. For example, visual cues are important in the mating of fiddler crabs where females choose a mate based on claw displays (e.g., Salmon, 1983; Hinsch, 1992). Vision has also been shown to be used in size assessment which is important in pair formation of the snapping shrimp, *Alpheus heterochaelis* (Hughes, 1996). Acoustic signals have been recorded in the communication of six genera of the Palinuridae and also in *H. americanus* (Phillips *et al.*, 1980). Acoustic displays have been related to both aggressive and reproductive behaviour (e.g. *Palinurus vulgaris*; Mercer, 1975). Such displays are likely to be useful over longer distances whereas tactile and chemical communications are used in close interactions. The tactile sense is well developed in lobsters with receptors located all over the body (Phillips *et al.*, 1980). Tactile behaviour is important during courtship and copulation as previously mentioned and also has a major role in aggressive interactions.

It is thought that chemical communication in the form of pheromones is important in the reproductive and other behaviours of aquatic crustaceans (see Dunham, 1988, for review). Females are thought to produce pheromones not only to attract a mate but also to subdue the natural aggressive instincts of potential partners (Atema & Engstrom, 1971). This would be especially important to the soft female following the moult when she would be vulnerable to predation or even cannibalism. It has been shown that newly moulted eyestalk-ablated *Homarus americanus* females have been attacked by males despite showing normal female mating behaviour. It is thought that such females may not have produced sufficient quantities of pheromone to subdue the male (Atema *et al.*, 1979).

There is some evidence to suggest that male crustaceans may also produce hormones to advertise themselves (Atema & Cowan, 1986). This communication is likely to be transmitted in the urine and it has been reported that male *H. americanus* increase their urine output in response to a female attempting to enter the shelter (Bushmann & Atema, 2000). Bushmann & Atema (2000) also found that preventing the release of urine from male *H. americanus* caused a reduction in the number of female visits to the shelter entrance and the duration of entry attempts. The response of the female was also assessed in relation to the dominance of the males and it was found that premoult females preferentially approached dominant males (Bushmann & Atema, 2000).

Behavioural activities that are associated with pheromones include fanning by the males and what is termed 'knighting' which is a behaviour shown by the female just prior to the moult (Atema, 1986). During knighting, the female places her claws on the male, which will result in her gill current, containing possible pheromones, being directed at the male. The urine of lobsters is stored in bladders which suggests that controlled release of urine containing pheromones is possible (Atema & Cowan, 1986). Such controlled urine release has been seen in agonistic encounters between male *H. americanus* (Breihaupt & Atema, 2000) and between male crayfish (Breithaupt & Eger, 2002), where directional control of the urine stream was also observed. Male American lobsters have been seen to display fanning behaviour, which is thought to draw any pheromones produced by the female through the shelter; however, this is usually associated with cohabitation behaviours. Mating in *N. norvegicus* has been seen to occur on the sediment

surface in laboratory studies (Farmer, 1974a). This could suggest that the shelter may be of less importance in this species. However, these results are likely to be caused by the experimental protocol and do not necessarily reflect the natural condition.

Changes in the behaviour patterns of individuals can be used to determine the onset of mating. In the crayfish *Austropotamobius pallipes*, individuals become more active with an increase in dispersal, and males more aggressive (Villanelli & Gherardi, 1998). In populations of *N. norvegicus* this type of movement would be constrained, to a certain extent, by the size of the mud patch upon which the animals were found, since *N. norvegicus* do not show any migratory behaviour (Chapman, 1980). Differences in the behaviour of male and female *N. norvegicus* have been described previously. Using an acoustic tagging technique, Chapman *et al.* (1975), found that males were much more mobile than females and that males did not return to the same burrow more than once during the course of the study. Females, however, tended to remain in the same burrow. Although these observations were carried out on only a small number of animals in a restricted area, they may provide evidence of distinct differences in the behaviour of male and female *N. norvegicus*.

Villanelli & Gherardi (1998) discovered that there were differences in the behaviour of male crayfish (*Austropotamobius pallipes*) of different sizes. They found larger males to be more receptive to females: in this species both males and females must be receptive for mating to occur. Male size also affected female mate

choice with smaller males being less successful. Larger males were also more successful in agonistic encounters. Males did not show any preference for female size (Woodlock & Reynolds, 1988) although large males were unsuccessful in inseminating small females. Pairings in *H. americanus* have been shown to occur mostly between females and a slightly larger male although pairings with smaller and larger males have been observed (Aiken & Waddy, 1980). The effects of male size on the mating ability of *N. norvegicus* is unknown; however, it is likely that there will be a certain size difference between large females and small males (and *vice versa*) that will prohibit successful copulation.

In the presence of a dominant male, female American lobsters have been shown to stagger their moult (Atema, 1986), thus enabling all females to mate with the dominant male in succession. The succession does not appear to be related to any dominance hierarchy among females. This process is linked to mate guarding, which involves a period of cohabitation in *H. americanus*. Although mate guarding has never been observed in N. norvegicus it is still possible that it could influence moulting.

Female *N. norvegicus* invest a great deal in the production of offspring, through ovarian maturation and egg production, and by carrying and protecting the eggs for an extended period of time, during which they rarely, if ever, leave their burrows to feed. It might be expected, therefore, that female *N. norvegicus* would carry out mate selection. The contribution of gametes by the male is in the form of spermatophores, which can also be considered a costly investment (Villanelli &

Gherardi, 1998). Males, however, have the ability to mate with more than one female should they be available. It would be reasonable to hypothesise, therefore, that the mating system of *N. norvegicus* could be based around 'choosy' females and relatively promiscuous males. Farmer (1975) has previously described mating in *N. norvegicus* as promiscuous. This statement, however, was based on observations of individual pairings. Whether or not males are promiscuous in the field will depend on various factors, which will be related to the mating behaviour of the population. Aspects such as mate choice, inter-male competition and physiological condition are likely to be important.

The sex ratio of a population can affect the mating behaviour of a species in terms of sexual competition. The operational sex ratio (OSR) represents the number of fertilisable females compared with the number of sexually active males present in the population at a given time. For species in which males contribute relatively less to the next generation than females, OSR is biased towards males because they have a higher potential rate of reproduction (PRR). This bias causes greater variability in male mating success (Debuse *et al.*, 1999). In a species such as *N. norvegicus*, where males have a higher PRR than females, it would be expected that male-male competition would be greater than that between females. Using these principles, models can be created to predict the direction of sexual competition. Factors such as the adult sex ratio; the time out, when an individual is unable to participate in mating; the occurrence of 'collateral' input, where more than one male will inseminate a female, can be used to predict the direction of

sexual competition (Parker & Simmons, 1996). These factors are also considered to be important for influencing the intensity of sexual selection.

Species in which the time available for mating to take place is controlled by the female moult cycle often show precopulatory mate guarding as a method of increasing the chances of mating (Parker, 1974; Grafen & Ridley, 1983). Mate guarding will decrease the PRR of males by increasing their 'time out' when they are unavailable to mate with other females, and therefore the OSR will be less biased. In the American lobster pre- and post-copulatory mate-guarding forms an important part of the mating behaviour with the male and female cohabiting a burrow for perhaps as long as a week. The female is protected during the vulnerable soft stage of her moult and the male is protecting his investment in the next generation, or indeed several generations in some cases, from other males. Disruption of mate guarding in the Jonah crab, *Cancer borealis*, by replacing the male (Elner *et al.*, 1985) has indicated that mate guarding is a mechanism by which the guarding male can prevent displacement of his sperm by another male. Although mating in *N. norvegicus* is very similar to that of *H. americanus* (Aiken & Waddy, 1980), it is not thought that *N. norvegicus* shows any guarding behaviour. Mate guarding involves costs to both the male and female and the lack of mate guarding in *N. norvegicus* points to a promiscuous mating system with a high male PRR and male-biased OSR.

As Atema *et al.* (1977) report, pair formation is common in decapod Crustacea; however, it is not synonymous with monogamy. It is possible that mating with the

dominant male is more important in species where mate guarding occurs. A female would be assured of adequate protection if guarded by the dominant male while subordinate males would be less able to defend a female, a situation evident in laboratory observations of the American lobster (Atema, 1986). Similarly, in pair formations of the blue crab, *Callinectes sapidus*, larger males were more successful in removing and replacing a smaller mate-guarding male (Jivoff & Hines, 1998).

Female American lobsters undertake mate-searching behaviour (Atema *et al.*, 1977; Atema, 1986), choosing locally dominant males. Atema (1986) also suggests that intrasexual agonistic behaviour and chemical cues influence the choice of females. Although the act of mating is very similar in both *H. americanus* and *N. norvegicus*, their habitats are very different with mating in American lobsters taking place in the shelter and *N. norvegicus* mating appears, from laboratory observations, to take place on the surface of the sediment (Farmer, 1974a). The female is likely to be better protected from predation and competition from other lobsters during mate guarding if she is cohabiting with a dominant male (Atema, 1986). It is unclear what role the provision of shelter would have on the mating behaviour of *N. norvegicus*.

Female mate choice is likely to induce intrasexual competition in males. Male *N. norvegicus* show positive allometric growth of the chelipeds following maturity (Farmer, 1974c; Chapter 4) and this may be important when competing with other males for females. In the American lobster, small differences in claw size between protagonists can have a highly predictive value on the outcome of antagonistic

encounters (Scrivener, 1971; Atema & Cobb, 1980). In the shore crab, *Carcinus maenas*, it has been shown that claw size has a greater importance than body size in determining the outcome of an agonistic encounter (Sneddon *et al.*, 1997). Claw size could be an important factor in *N. norvegicus* behaviour also. The larger claws of males could be used in displays of dominance to other males and to advertise size to potential mates. The absence of chelae may also affect a male's ability to mate. In the shore crab it has also been found that males with such handicaps were not as successful at acquiring and guarding females (Sekkelsten, 1988). This was not as a result of female mate choice but rather due to a reduced competitive ability.

In the crayfish, *Orconectes propinquus*, Stein (1976) found that males were most successful at mating with a female 2mm smaller in size (carapace length) than themselves and that males in the wild would actively search out females of the appropriate size. In this case, mating would be more likely to be successful on finding a partner of a similar size. In the American lobster, it is suggested that the male must be larger than, or of a similar size to, the female in order to dominate the most desirable (largest) females (Aiken, 1986). In the spiny lobster, *Jasus edwardsii*, it has been shown that large males will have a greater chance of mating with females of all sizes than small mature males (MacDiarmid & Butler, 1996). The effects of male size on the reproductive output of the female were also examined, and it was found that mating with small males resulted in the reduction of egg production to one third of that produced when mating with a large male (MacDairmid & Butler, 1999a). Bigger females are capable of producing larger

broods as they have higher fecundity and a greater area of abdomen for carrying the developing eggs. This would suggest that males should compete for larger females to increase their contribution to the next generation. This would be more important in species that showed serial polygyny and may not be as important in promiscuous species such as *N. norvegicus*.

Dominance within populations is likely to be driven by animal size. Although it has been shown that aggression and dominance are both affected by the moult cycle (Tamm & Cobb, 1978, 1980; Cromarty *et al.*, 2000), there may be a number of factors, both physiological and social, which affect the dominance of an individual. Karnofsky & Price (1989) noted that smaller individuals sometimes outranked large animals and that ovigerous females had a higher level of dominance than would be expected. In the American lobster, dominant males will mate almost exclusively with all the females in the area. It is possible that dominance hierarchies control the mating systems of *N. norvegicus*; however, the lack of mate guarding in this species suggests that the reproductive behaviour, at the population level, may be more complex than that of *H. americanus*.

Agonistic behaviour is likely to be a key driving force in the formation and maintenance of any dominance infrastructure within a population, with larger and more aggressive individuals gaining access to a greater proportion of the available resources. During observations made of natural populations of *N. norvegicus*, only large males were seen to engage in agonistic behaviour (Chapman & Rice, 1971). Atema & Cobb (1980) suggest that the majority of agonistic encounters among

lobsters tend to be simple approach - avoid sequences, which do not involve physical contact. Karnofsky & Price (1989) also described this behaviour in laboratory mesocosm studies. Display behaviour is likely to be important in avoiding costly aggressive interactions, as is seen in the behaviour of the stomatopod, *Odontodactylus scyllarus*, which rarely resorts to physical combat because of the entailed risks (Caldwell & Dingle, 1976). For example, the most probable use of meral spread behaviour is to increase the apparent size of an individual towards an opponent. Dingle (1983), in a review of aggressive behaviour in crustaceans, suggests that such behaviour would be most often used in smaller individuals, probably as a bluff tactic. It has also been found that chemical displays may be important in the types of behaviour shown in agonistic encounters (Karavanich & Atema, 1998; Breithaupt & Atema, 2000; Breithaupt & Eger, 2002).

One of the main difficulties in carrying out behavioural investigations on *N. norvegicus* is that the visual pigments and dioptric apparatus of *N. norvegicus* are easily damaged through exposure to daylight (e.g. Loew, 1976; Shelton *et al.*, 1985; Gaten, 1988; Gaten *et al.*, 1990). Although most types of behaviour appear to be unaffected by this damage (Richardson, 1996), it is possible that reproductive behaviour may be disrupted. Chapman *et al.* (2000) reported a decline (10%) in the reproductive success of eye damaged females from a mark recapture experiment. Although this result was not statistically significant it indicates that there may be an effect of blindness on the reproductive behaviour of *N. norvegicus*.

The aims of this study were to investigate the reproductive behaviour of *N. norvegicus* both at the level of the individual and the population. The mating behaviour of *N. norvegicus* has been previously investigated on an individual scale using small tanks (e.g. Farmer, 1974a). This study aimed to observe the mating behaviour of *N. norvegicus* on a larger scale using small replicated populations. Observations of laboratory populations were carried out to ascertain the importance of dominance hierarchies to the functioning population structure, to determine the level and direction of agonistic behaviour in reproduction and to look for the presence of any patterns in mate searching behaviour. An investigation into reproductive behaviour was carried out, and a study was made of activity patterns and the levels of agonistic and non-agonistic encounters within experimental populations in order to determine the formation of any social structure.

Small-scale experiments were also carried out to determine the importance of male size on the outcome of agonistic encounters against intact and damaged (one claw missing) opponents, and to determine the driving force behind the formation of dominance hierarchies. These factors are likely to be very important to the mating system and reproductive output. It is hypothesised that larger males are more likely to be successful in agonistic encounters and will therefore be more likely to mate with females in the area. The reduction in the size and numbers of males within a population could have implications for the mating opportunities of females, particularly large females. It is therefore important to determine the ability of small males to mate with large females.

5.2 Methods

5.2.1 Experiment 1

Animals were creel caught from the Clyde Sea area south of Little Cumbrae and from Loch Torridon at depths of 70 – 90 m from April to June 2001. All animals were collected at night, with the aid of dim red lighting, and immediately placed in light-proof containers to avoid any damage to their eyes. All subsequent handling was carried out under dim red light. All of the animals were measured with vernier callipers to the nearest 0.1 mm using the standard measure of carapace length (distance from the posterior orbit to the midline of the posterior carapace edge) and females were moult-staged using the pleopod method (Aiken, 1980). Each individual was tagged using waterproof tape and cyanoacrylate glue, with a number corresponding to the artificial burrow to which it was allocated. Tagging did not have any discernible effect on the animals. Prior to the experiment, animals were kept in visual and tactile isolation at 10°C in a separate holding tank. They were not fed during this time.

Animals were observed at the Fisheries Research Services' marine aquarium facility in Aberdeen, using a large round tank (approximately 10m diameter) divided into quarters using cloth screens which allowed through-flow of water but prevented visual or physical contact between animals in the different quarters. There was a constant supply of filtered seawater at 10°C to the tank, which contained a depth of water of approximately 30cm for the duration of the experiment. A natural light/dark cycle was maintained; green light represented day (c. 1.0 $\mu E. s^{-1} m^{-2}$) and red light was used for filming during the hours of darkness. Red lighting was used

as *N. norvegicus* are not sensitive to red wavelengths of light, having a λ_{max} at 498nm (Loew, 1976). It was not possible to add any form of substratum to the tank, which was painted concrete. The animals were fed on sandeels two to three times a week to satiation. Each section of the tank was filmed individually using digital video cameras connected to a monochrome duplex multiplexer. Continuous recording was carried out using a time-lapse recorder.

Each section of the tank was approximately $18m^2$ in area and animals were placed at a density of no greater than $1.m^{-2}$. Artificial burrows (inverted sections of plastic guttering 25cm in length) were positioned using a random number generator in SYSTAT (SYSTAT, 1992); the pattern produced was also used to allocate animals to each 'burrow'. The density and positioning of burrows in the field varies greatly in relation to sediment type (Tully & Hillis, 1995) and time of year. Chapman and Rice (1971) found that the burrows of *N. norvegicus* in upper Loch Torridon occurred at a density of approximately $1.m^{-2}$, however, the burrows were not evenly spaced, varying from 0 to $3.5.m^{-2}$. Similar observations have been made in the Irish Sea (Hillis, 1974). It has been suggested that the clustering of burrows is related to the settlement of juvenile *N. norvegicus* into branches of the burrows of larger individuals (Tuck *et al.*, 1994). Reproduction of the natural burrow pattern in the laboratory was not attempted. It is probable that the density of $1.m^{-2}$ was higher than would have been experienced in the habitat where they were originally creel-caught. These animals tend to be fished on less productive grounds, from populations of relatively fast growing, large animals that occur at

quite low densities. The higher density chosen for this study was to allow an adequate number of animals to be used in each replicate.

On introduction to the tank, animals were confined to a small area around an artificial burrow. This was to allow each individual to become accustomed to the 'burrow', which would then be more readily used for shelter. It was observed in a pilot study that *N. norvegicus* do not adapt to artificial burrows quickly and this method improved the association. The animals were confined for a period of at least two days and then were filmed on release.

Identification of individual animals was not possible from the video recordings due to the high placement of the cameras above the tank that was necessary for a complete field of view. The position of individual animals was therefore recorded by direct observation. This was generally carried out in the morning, and always during experimental daylight. Analysis of the behaviour was carried out for a period of one hour from every four hours, beginning at 2400 h; this also allowed observations to be made around the times of dawn and dusk. These times would be expected to represent the periods of peak activity for animals sampled from the depth of 70 – 90m. Chapman *et al.* (1975) suggested that deep populations are active diurnally, while shallow populations are nocturnal, and those populations found at intermediate depths are crepuscular.

Within each hour of observation the number of animals not in 'burrows' was recorded at 0, 30 and 60 minutes. Only active animals were included in the count,

as some individuals did not use 'burrows' as shelter. These values were used to calculate the average proportion of active animals during the time analysed. Counts of agonistic and non-agonistic encounters were also made, as well as observations of any mating behaviour. Individual behavioural components were described with reference to the descriptions of Richardson (1996); however, counts were made of bouts rather than of individual behavioural components. An individual bout was defined with reference to Hyatt (1983) as an approach followed by agonistic interactions and then a withdrawal of one individual and a cessation of agonistic interaction between the protagonists. Agonistic encounters lasted for various lengths of time; however, the count remained one unless the animals separated and began the agonistic display once more. Non-agonistic behaviour was counted if animals met and then withdrew without any display of aggression or prolonged contact. During the initial period of the study, counts were duplicated (by the same observer) in order to determine the degree of observer error. Because the counts were consistent this was not continued throughout the analysis. It was not possible to carry out observations on tank 3 during the hours of darkness due to poor visibility. Therefore, there were no readings from 2400 h – 0100 h and the results from 2000 h – 2100 h are only for the first 30 minutes of this time period.

Data from the first 24 hours following the release of animals from their temporary enclosures were carefully examined prior to inclusion in the analyses. This was to avoid the inclusion of any possible hyperactivity following release. As a control for

breeding behaviour, females that were not at a reproductive moult stage (i.e. those that had already moulted or were at stage 0) were also placed in the tank.

Initially the sex ratio was set at 1:1, which is similar to that which would be found in the catch during the breeding season. Males can, however, represent up to 95% of landings by weight (Chapman, 1980), due to the reduced availability of females while they are incubating eggs within the confines of their burrows. This ratio changed with mortalities in the tank, since animals were not replaced. The results were adjusted for changes in density and are therefore comparable between tanks.

Following the removal of all animals from the tank the females were examined for any evidence of mating. The spermathecae of those females that were in premoult at the beginning of the experiment were dissected to determine the presence of a spermatophore, which would indicate that the female had mated following the moult.

5.2.2 Experiment 2

N. norvegicus were collected from the Clyde Sea area using a standard prawn trawl south of Little Cumbrae and a beam trawl in the Cumbrae-Bute channel from a depth of 70 – 90m. Animals were stored in a communal tank prior to experimentation. The moult stage of females was assessed using the scale developed by Aiken (1980). Those individuals that were close to moulting were placed in circular tanks (Ø 1.5m, depth 0.75m) containing ~50 cm of well-settled, consolidated sediment from *N. norvegicus* grounds in the Clyde Sea area. Each

tank was divided into two equal areas, each area initially containing an individual premoult female. Unfiltered seawater at ambient temperatures (average 13.8°C) was continually provided to both the mud tanks and the holding tank. Animals were not fed during the experiments; however, they may have obtained food from suspension feeding and from small animals in the sea water supply. A natural light dark cycle was maintained with diffuse green lighting (c. 1.0 μE. s^{-1} m^{-2}) during the 'daylight' hours and red light for filming in the hours of darkness. The animals used had not been fished during the hours of darkness and the holding tanks wer subject to ambient light and as such could have been visually impaired on contact with daylight as previously noted. Chapman *et al.* (2000) reported that damage by natural light is very variable and can vary from 0 – 100% of the retina.

The carapace length (CL) of all animals was measured to the nearest 0.1mm using dial callipers and each animal was labelled using the method previously described. Pairings of animals of the same size (no more than 1.0mm difference in carapace length), and of differing size (no less than 5.0mm difference in carapace length) were used. Further experiments were carried out in which animals with only one claw were matched with intact individuals. Again, these pairings were divided into those between animals of the same size and animals of differing size. The position and type of the missing claw was also noted. In the pairings between animals of different size the animal missing the claw was always smaller than it opponent, although this was due to the small size of individuals missing appendages rather than the experimental design.

Pairs of animals were observed in a glass tank (80 x 40 x 40 cm with 20cm depth of water). The initial use of mud tanks was found to be unsuitable due to the suspension of silt by moving animals, making detailed observations difficult. Following placement in the tank, contact between individuals was observed and categorised. Bouts of aggressive behaviour could not be defined as they were for experiment 1, since much less agonistic behaviour was seen. However, Maynard Smith (1974) suggests that the winner is the individual that displays/engages longest. Individuals were therefore classed as losers following three retreats from an interaction with the opposing individual. The time taken (duration) from placement in the tank to the 'defeat' of an individual was also recorded. If no loser had been identified after 30 minutes either due to inactivity or because no clear winner emerged, the experiment was terminated. Each male was used only once where possible, and where animals were used more than once care was taken to ensure they were not observed twice in the same day.

5.3 Results

5.3.1 Experiment 1

5.3.1.1 Activity levels

Within the tank, the majority of animals used the artificial burrows, although some of the larger animals may have had more difficulty negotiating them and did not use them for shelter. While in the 'burrows' animals were often seen to move at the entrance and would often exit the 'burrow', turn, and re-enter so that they were facing out of the opposite opening. Animals did not remain in a single home 'burrow', but moved freely amongst the 'burrows'. The duration of 'burrow' occupancy was often quite short causing difficulties in determining the position of individual animals.

The animals showed a diel pattern of activity. Generally, more animals were active during the hours from experimental dusk until dawn (1930 h – 0415 h) (Figures 5.1 & 5.2). In tanks 1 and 2 the changing activity patterns were quite distinct, especially in tank 2 (Figure 5.1). In tanks 3 and 4, however, the activity patterns were not as pronounced (Figure 5.2). The animals in tank 3 showed highest activity levels between 0400 – 0500 h although there was not a great deal of variability in activity between each of the observation times. The animals in tank 4 showed a similar pattern of activity to those in tanks 1 and 2, however, there was not a great deal of difference between each observation time. It can be seen from Figures 1 and 2 that some animals were active during the hours of daylight in each tank.

The movement of animals between 'burrows' was also associated with eviction behaviours. Evictions were seen frequently and often involved a large animal evicting a smaller animal. The evicting animal often did not remain in the 'burrow' for long. Larger animals appeared to have more success during evictions; they were better able to evict others and to resist the eviction attempts of a smaller animal.

5.3.1.2 Agonistic encounters

Several different types of agonistic behaviour were discernible from the time lapse video recordings. These agree with the descriptions of agonistic behaviour described by Richardson (1996) involving approach, non-contact cheliped display and contact cheliped attack. Retreat behaviour was also seen, with a full range of behaviour from a tail flip swimming escape to slow backward walking. Retreat pursuit behaviour was also observed where the aggressor would chase, and sometimes attack, the retreating individual. Similar descriptions were also given by Salerno (2000), for *N. norvegicus*, and by Scrivener (1971) for the American lobster; however, for the purpose of these descriptions the terminology of Richardson (1996) will be used.

Within the aggressive categories described by Richardson (1996) there were three types of approach behaviour: direct approach, turn on axis and chase, which involve the attacking animal moving with the chelipeds raised either parallel or spread (not as widely held as in a meral spread). Although it was not possible to determine the degree to which agonistic individuals raised their claws, all of these

approach behaviours were observed in agonistic encounters. Richardson (1996) also divided the non-contact display behaviour into three categories: meral spread, thrust and raised chelipeds. Again it was not possible to determine from the video analysis if the claws were raised; however, meral spread and thrust behaviours were observed. The contact cheliped attack has been described in great detail by Richardson (1996) who divided this agonistic behaviour into seven types. It was often difficult to determine the level of contact between individuals, especially in terms of the interlock descriptions used to describe the use of the chelae propodus by both opponents to grasp the other's chelae. It is very likely that this behaviour did occur, however, since all other types of contact aggression were observed. These included single and double cheliped contact, and pushing behaviour, where both animals hold the claws in a meral spread facing each other and tried to force each other backwards. Wrestling behaviour, where the claws are used to hold the opponent in a position where they are touching the carapace of the opponent, and strike behaviour, where the chelipeds were used to strike any part of the opponent, were also seen. Further agonistic behaviour observed included the chasing of defeated individuals by their successful opponent. In some cases this constituted the aggressor following the defeated individual around the tank and at times there was further aggression directed at the abdomen of the retreating loser.

Richardson (1996) classified four different types of retreat behaviour and measured the speed of certain retreat types. Although it was not possible to determine the speed of movement in this study these different behaviour types were all observed. These included 'move backward', where one animal walks away backwards from a

stationary opponent. 'Fleeing' is a faster movement where the retreating animal runs in a forward direction away from its opponent. 'Tail-flip behaviour' where the retreating animal 'swims' away using rapid flexions of the abdomen, and 'turn away behaviour', where one opponent moves to face away from the other, were also observed.

Observations of the mean number of agonistic encounters over the 24-hour period produced almost identical patterns in tanks 1 and 2 (Figure 5.3). It can be seen that the patterns of agonistic encounters reflect the activity of the animals over time (Figure 5.1). There was a peak in agonistic activity between 2000 h – 2400 h in tank 1 and between 2400 h – 0100 h in tank 2. Agonistic encounters were at their lowest between the hours of 0800 h – 1200 h in both tanks. In tank 3 there was a peak in agonistic activity at 0400 h – 0500 h (Figure 5.4) although this may not be representative due to the lack of observations available during the hours of darkness. In tank 4 the peak in agonistic behaviour occurred at 2400 h – 0100 h with the lowest level of encounters seen at 1600 h – 1700 h. The occurrence of agonistic behaviour during the 24h period reflects the overall patterns of activity with increased aggression during the hours of darkness (Figures 5.3 & 5.4).

The total number of agonistic encounters in tanks 1 and 2 followed a similar pattern (Figure 5.5). During the first three weeks of the experiment there was a decrease in the number of agonistic interactions observed. The total number of agonistic encounters then levelled off somewhat, at between 10 and 20 for tank 1, and 20 and 30 per day for tank 2. In tank 3 there was an overall decline in

agonistic behaviour over duration of the experiment (Figure 5.6). In tank 4 there was little change in the level of agonistic encounters during the period of observation (Figure 5.7) but levels were generally higher.

5.3.1.3 Non-agonistic Encounters

Non-agonistic behaviour was not classified into behaviour types (as the more ritualised agonistic behaviour was). Rather, it was observed where there was a lack of agonistic behaviour. When two individuals encountered one another in the tank and did not show any approach behaviours, display or engage in any aggressive physical contact, behaviour was considered to be non-agonistic. This type of behaviour was seen most frequently when individuals moved around the tank, came into contact with others and simply moved on. The animals moved on both before and after physical contact had occurred, although physical contact was generally a brief contact with the chelae. Prolonged non-agonistic contact was not seen.

In tanks 1 and 2 the peak in non-agonistic encounters was seen between 2000 - 2100 h although this was more pronounced in tank 1 (Figure 5.8). The period when least non-agonistic activity was observed at 1200 – 1300h in tank 1 and at 0800 - 0900 h in tank 2, although there was little difference between either of these times in both tanks. In tank 3 there was a peak in non-agonistic encounters at 0400 – 0500h (Figure 5.9) although this may not be representative due to the lack of observations during the hours of darkness. In tank 4 the pattern of non-

agonistic encounters followed that of the average number of active animals with a peak at 0000 – 0100 h (Figure 5.9).

The pattern of total non-agonistic encounters over time was similar for both tanks 1 and 2. There was an initial period of increase in the number of non-agonistic encounters during the first 10 days of the experiment. This was then followed by a decrease and levelling off at around 10 encounters per day for both tanks at the termination of the experiment (Figure 5.10). In tank 3 there appeared to be an overall decrease in the total number of non-agonistic encounters (Figure 5.11). In tank 4, however, there did not appear to be any pattern of change in the total number of non-agonistic encounters (Figure 5.12) over the course of the experiment, as was seen for the pattern of agonistic encounters (Figure 5.7).

Looking at the levels of encounters from the longer and more complete data sets from tanks 1and 2, the patterns of interactions can be seen more clearly (Figures 5.13 & 5.14). In tank 1 (Figure 5.13) the initial increase in non-agonistic activity, which was accompanied by the decrease in agonistic activity, can clearly be seen. The total number of interactions showed a slight increase during this period and then decreased to a stable level towards the end of the experiment. During this latter period of relative stability, the frequency of agonistic and non-agonstic behaviours was similar. In tank 2 (Figure 5.14) there was a slight decrease in the total number of agonistic interactions at the beginning of the experiment; however, this was not as pronounced as in tank 1. The total number of non-agonistic interactions did not appear to show any change in pattern over the course of the

experiment apart from a large peak in non-agonistic encounters around Day 18. This peak was not reflected in the agonistic encounters and is unexplained.

5.3.1.4 Mating

Although no mating behaviour was observed, there was evidence to suggest that mating had occurred. At the beginning of the experiment the animals were each confined with an artificial burrow until they were familiar with them as shelters. During this period all the premoult females moulted. The females were all active on release from isolation with the burrow. Following the experiment, all females were dissected to determine if they had been inseminated. Spermatophores were found in the spermathecae of every female (Tank 2 result), indicating that although the moult had taken place during confinement they were still receptive to males at the time of release.

5.3.2 Experiment 2

None of the females placed in the mud tanks survived the moult and only one animal displayed burrowing behaviour. This burrow was shallow, and was shorter in relation to the animal's body length than those reported from field observations (Rice & Chapman 1971). The burrow later collapsed and the female made no attempt to re-excavate it. Attempts to encourage the animals to dig burrows through the creation of craters in the sediment were unsuccessful. It was not possible, therefore, to determine the mating behaviour of females in relation to different sized males. Nor was it possible to determine the role of the burrow in mating.

5.3.2.1 Agonistic Behaviour

When two individuals were placed into the experimental tank little agonistic activity was observed, even when those animals differed in size. When placed in the tank two general behaviours were seen. Some animals would remain inactive in one area of the tank, typically a corner, occasionally showing cleaning behaviour, as has also been described by Salerno (2000). In contrast, active animals would explore the tank, often for the duration of the experiment. This type of behaviour involved the individual walking extensively around the tank. Such active animals were often seen trying to escape from the tank by attempting to climb up the sides of the tank. In these attempts the pleopods were often used. When two inactive animals were placed together in the tank there was generally no conclusive outcome as the number of interactions seen was minimal. Where one active animal was placed with one inactive animal, the active animal was usually the initiator of any interactions; however, these interactions were rarely considered agonistic and the initiator was not always deemed the winner. When two active animals were present in the tank interactions appeared to occur by chance with animals encountering one another as they moved around the tank. Again, these interactions were rarely agonistic and it was often difficult, if not impossible, to determine if either animal had initiated the contact.

Based on these behaviour types, the first individual to withdraw from contact with its opponent three times was considered to be the loser. Where animals differed in size, the larger individual present won every time except one (n=8). The average time until a winner was seen was 17.3 ± 8.1 (SD) minutes and there was only 1

pairing that was inconclusive. The average time until a winner was decided in interactions between animals of the same size was 13.2 ± 7.1 (SD) minutes (n=7) (Figure 5.15). Three encounters were concluded without a winner being assigned. There were fewer interactions 5.4 ± 1.6 (SD) per pairing for different sized pairs than for pairs of the same size 6.0 ± 1.5 (SD) (Figure 5.16). There was no significant difference between the duration of interactions between animals of differing and similar size (Mann Whitney U; p = 0.18), nor between the number of encounters in each pairing (Mann Whitney U; p = 1.0) although the data set was small. In some cases where there was a large difference in size, the smaller animal was seen to display the tail flip response in reaction to contact with the larger individual. This was uncommon, however.

The behaviour of those individuals with only one claw was slightly different from that of the intact individuals. Where animals of the same size were paired, the intact animal always appeared to dominate the damaged individual (n=2). Where the damaged animal was smaller than its opponent, only one smaller individual won (n=5). All pairings were conclusive. The mean duration of encounters (Figure 5.15) between individuals of differing size was 16.2 ± 7.0 (SD) minutes, while between animals of a similar size the mean duration was greater 27.7 ± 2.3 (SD) minutes. The mean number of interactions per pairing (Figure 5.16) was less for different size pairings (7.4 ± 3.2 SD) than for pairs of the same size (10.5 ± 4.9 SD). The mean number of interactions per pairing was greater for pairs that included an animal with only one claw than for pairings of intact animals, of both

different and the same size. There was no significant difference between the duration of interactions of different sized and similar sized animals missing a claw (Mann Whitney U; $p = 0.8$), nor between the number of encounters in each bout (Mann Whitney U; $p = 0.44$) although the data sets were very small. There were some displays of agonistic behaviour between pairs of individuals including one with a missing claw. In one case, the individual missing a claw was aggressive towards a larger opponent using its remaining claw to direct an attack on its opponent's eyes.

A comparison was made of the data on encounters between intact animals and encounters containing an animal missing a claw. Again, there was no significant difference in the duration of bouts between intact and damaged animals of differing size (Mann Whitney U, $p = 0.61$), nor between animals of the same size (Mann Whitney U, $p = 0.057$). There were no significant differences in the numbers of encounters between animals of differing size (Mann Whitney U, $p = 0.27$), nor animals of the same size (Mann Whitney U, $p = 0.24$).

5.3.2.2 Moulting

During the course of this experiment no female survived through the moult so it was not possible to observe the effects of newly moulted females on the behaviour or physiology of males. In one case, however, a male survived moulting in captivity and was stored in a small perspex tank containing 2L of still seawater for an hour. Another male of a similar size was then introduced to the tank. The presence of a newly moulted male did not elicit a response from the intermoult

male; however, the soft animal did react to the presence of the introduced animal, retreating into a corner of the tank. A further sample of 60ml of the still water from the tank containing the soft male was removed and introduced to a male in a larger tank, again there was no response to the added water.

5.4 Discussion

5.4.1 Experiment 1

The experimental design used here was far removed from the conditions experienced by the animals in their natural environment; there was a lack of any muddy substratum in which to burrow and the animals could only use artificial shelters. The lighting regime was set to be as natural as possible in terms of the light:dark cycle; however, the level of illumination was higher than would have been experienced in the field (between 10^{-5} and 1 lux, see Loew, 1976) to facilitate filming and observation. A further problem with the experimental set-up was that the water supply for all four areas of the tank was from two inflow pipes in one half of the tank (in different quarters), with two outflow pipes in the opposite half of the tank (again in separate quarters). This combined with the cloth dividers, which allowed through-flow of water could have allowed the transfer of pheromones from one 'population' of individuals to other 'populations'. The system did, however, allow for the development of home ranges around a particular shelter. The artificial burrows were used for shelter by most animals. A further benefit of the set-up was that it allowed observations to be made on the behaviour of individuals within a group rather than looking at individuals or pairs of animals in small aquaria. This had never been attempted before in the description of *N. norvegicus* behaviour.

The activity patterns shown by the *N. norvegicus* in this experiment (Figures 5.1 & 5.2) showed that *N. norvegicus* are mostly nocturnal but are also quite active during the periods of dawn and dusk. The patterns of activity seen in tank 3 were

not typical and this was probably due to poor lighting levels during the hours of darkness, which prevented the collection of data from 2030 h – 0400 h. It would be expected that animals collected from 70 – 90m would be most active at dusk and dawn, as is suggested for populations at 60m depth by Chapman *et al.* (1975). *N. norvegicus* have been shown to emerge from their burrows at certain levels of light intensity (between 10^{-5} and 1 lux, see Loew, 1976). It is possible that the lighting conditions used in this study induced behaviour that is more representative of populations that are found at shallower depths. The majority of animals in such populations are active during the hours of darkness between dusk and dawn. A further explanation for the increased nocturnal activity seen could be the artificial, and quite short, transition between daytime and night lighting regimes. The transition lasted approximately 20 minutes, which is much shorter than natural dusk and dawn periods. Aréchiga & Atkinson, (1975) reported that animals subjected to abrupt changes in lighting were seen to restrict their behaviour to the period of darkness and they suggest that the gradually changing light conditions around dawn and dusk could be a key environmental variable for *N. norvegicus*.

The occurrence of relatively normal locomotor activity suggests that the animals adapted reasonably well to the aquarium environment. It can be seen that the activity patterns of individuals (in terms of numbers that were active) were very similar to those measured as encounters between animals. This indicates that there was little or no change in the level of activity shown by animals out of the burrow, it is just that more individuals were active during the hours of darkness. The presence of active animals during the day may not be expected of a species that is

normally considered to be nocturnal or crepuscular; however, a small part of the population is active during the day in the field (Chapman & Rice, 1971).

Movement between burrows by animals of both sexes has been seen in tagged populations observed in Loch Torridon (Chapman & Rice, 1971). They do not, however, appear to be compatible with observations of the acoustically tagged animals followed by Chapman *et al.* (1975). If those results were applied to the data obtained during the present study, then it would be expected that the females would largely remain associated with one burrow, while males would move from burrow to burrow. During studies of agonistic behaviour, Salerno (2000) noted that female *N. norvegicus* appeared to show more interest in using artificial burrows than males. It was also noted that burrow ownership was always the cause of aggressive interactions between females. Unfortunately, it was not possible to determine the sex of evicting individuals from the video recordings made during the present study. The experimental surroundings may have induced movement between burrows. The confined space and the availability of alternative shelters in this study could explain the differences in these patterns. Increased general activity among the animals following release would increase the chances of finding an unoccupied shelter.

Eviction behaviours may be used in establishing and maintaining a dominance hierarchy. In many cases, the evicting animal did not remain long in the 'burrow' it had won and in some cases the evicting animal would simply push the resident animal out of the 'burrow' by walking through it. It was often seen that the former

resident of the 'burrow' would almost immediately re-enter the 'burrow'. This behaviour is unlikely to be resource motivated or the evicting animal would remain in the 'burrow'. It is more probable that this behaviour is associated with assertions of dominance as has been reported in the American lobster (Atema, 1986, Karnofsky & Price, 1989). The relative success of larger animals during evictions would suggest that this is an agonistic assertion of the dominance of larger animals. This could also indicate that body size is important in these encounters since the evicting animal often entered the 'burrow' from behind the occupant, preventing any observation of claw size. The confined space of the burrow would prevent some aggressive displays such as a meral spread.

It was not possible to assess quantitatively the impact of the size of an individual on its ability to evict others, or to be successful in agonistic encounters since it was not possible to identify individual animals from the recordings. A qualitative analysis based on observations from the recordings indicated that larger animals tended to be successful. However, it was not possible to determine from the tapes the relative size difference when two animals were of a similar size.

The agonistic behaviour that was observed is similar to that seen by Richardson (1996), who developed an ethogram of the aggressive behaviour of *N. norvegicus*. The pattern for *N. norvegicus* fitted that of the generalised decapod template of agonistic behaviour as described by Hyatt (1983) in a review of published data on the aggressive behaviour of decapod crustaceans.

The mean numbers of agonistic encounters in each of the tanks was similar to that of the general activity patterns seen (Figures 5.3 & 5.4). The results from tank 3 were not the same as those in other tanks and this was probably due to the lack of data during the hours of darkness, which prevented the collection of data from 2030 h – 0400 h. The data on total agonistic encounters from the more complete and longer data sets from tanks 1 and 2 were compared to determine any patterns in agonistic activity over the duration of the experiment. This showed an initial decrease in the amount of aggression, which then levelled off towards the end of the experiment. This could be a result of the formation of a dominance hierarchy following a high number of agonistic encounters after the introduction of animals into the tank. During this initial period, individuals would be assessing and asserting dominance status through agonistic behaviour. The period of reduced aggressive behaviour that followed would suggest that, once a dominance hierarchy had become established, individuals would be less likely to engage in agonistic encounters with dominant individuals. Indeed, upon the establishment of a dominance hierarchy it could be expected that less competition would take place and more ritualised behaviours would be seen, thus reducing the chances of injury (Atema & Cobb, 1980). It has been reported that isolation can augment aggression in hermit crabs, *Pagurus samuelis* (Courchesne & Barlow, 1971), therefore the higher levels of aggression at the beginning of the experiment could be due to the short periods of isolation prior to the experiment and during burrow familiarisation.

The patterns of non-agonistic behaviour over the 24-hour period were again similar to the general activity patterns seen within the tanks (Figures 5.8 & 5.9). The total number of non-agonistic encounters was compared between tanks 1 and 2 to determine any patterns over the course of the experiment (Figure 5.10). There appeared to be an increase in the non-agonistic behaviour during the initial stages of the experiment, although this was much more pronounced in tank 1. This increase was then followed by a decrease and levelling off in the total number of non-agonistic encounters. This might be expected along with the establishment of a dominance hierarchy as individuals begin to recognise the dominance status of others within the tank and do not engage in agonistic behaviour. Again, the data from tanks 3 and 4 were largely inconclusive (Figures 5.11 & 5.12).

The total number of interactions within each tank (including both agonistic and non-agonistic interactions) showed an overall decrease in tank 1 following a slight initial increase (Figure 5.13). These data support the theory of the formation of a dominance hierarchy, but also suggest that there was a period of greater activity at the beginning of the experiment. This could be a familiarisation period when the animals are becoming more accustomed to their surroundings and each other. This pattern is not obvious in tank 2 (Figure 5.14), where there is a small initial decrease in activity as a result of a reduction in agonistic encounters. This is followed by a relatively stable pattern of interaction, except for a large peak in non-agonistic encounters around day 18. There is no apparent cause of this increase in this behaviour.

Unfortunately, it was not possible to observe females during the process of moulting as they were still confined with their artificial burrows. It was therefore not possible to determine any changes in the activity patterns or general behaviour of the other individuals within the population in reaction to the presence of a newly moulted female. Nor was it possible to record the behaviour of other females to the presence of a soft female. The presence of spermatophores in the spermathecae of all the females that had moulted just prior to the experiment indicates that there must be a relatively long period following the moult in which females are still soft enough to be inseminated. This does raise questions about the production of pheromones. It is possible that a female that had not been inseminated would continue to produce pheromones and be receptive to males for longer than a female that had mated immediately following the moult. In this same way it could be possible for a female to control the number and possibly the quality of males that mate with her, through selective release of pheromones.

The nature of the particular time-lapse video system did not lend itself well to recording mating behaviour, and the details of behaviour were not clear. The act of copulation is over very quickly and could easily be overlooked on tape. The probable occurrence of mating during the hours of darkness also hampered observations as some areas of the tank were in shade and it was only possible to make out animals that were moving around. Similarly, it was not possible to tell if females were being inseminated by more than one male. A further problem caused by the delayed start to the experiment was that recently moulted females were present before any form of dominance hierarchy had been established.

5.4.2 Experiment 2

The behaviours observed in this part of the experiment may have been influenced by blindness in the animals used. It was not possible to collect the animals during the hours of darkness, nor were the holding tanks light-proof. It has been shown that prolonged exposure to light causes irreparable damage to the eye (Loew, 1976; Shelton *et al.*, 1985; Gaten, 1988). The effects of blindness on behaviour are limited, however, with no significant effects on emergence activity, agonistic contests, and ability to gain shelter and food (Richardson, 1996). It has also been shown through mark recapture experiments, that eye damage does not appear to affect long term growth and survival (Chapman *et al.*, 2000). Although there was a reduction in the proportion of ovigerous females in the recapture group that pointed to a 10% reduction in the reproductive success of eye damaged females, this was not significant (Chapman *et al.*, 2000). The effects of light induced eye damage on reproductive behaviour have yet to be assessed.

The lack of burrowing behaviour displayed by the premoult females introduced to mud tanks was surprising. Rice & Chapman (1971) found that most animals would begin to excavate a burrow immediately upon entry to a tank containing mud, although they noted that male animals were often seen to use shallow craters rather than excavating a burrow. Salerno (2000) observed females moulting within the burrow, which would provide shelter from predation during this vulnerable period. Females close to the moult, as used in this experiment, might therefore be expected to secure burrows to ensure that they were protected during the moult. Richardson (1996) found that there was no effect of blindness on artificial burrow

use and the ability of one female to excavate a burrow indicates that blindness was not a problem for the excavation of a mud burrow. When males were held in mud tanks they also showed little desire to burrow. This could be due to the lack of any predator stimulus. It has been found that *N. norvegicus* will show decreased emergence behaviour, from artificial burrows, in the presence of either a predator (e.g. the cod *Gadus morhua*) or the odour of a potential predator (Richardson, 1996).

In the assessment of agonistic encounters between individuals, the behaviour patterns observed were not as expected. The individuals used did not respond to each other in an aggressive manner and agonistic behaviour was rare. There were few incidences of threat behaviour, for example the meral spread, and few animals showed the tail flip escape response. Under these circumstances, a subordinate animal was defined as the first animal to withdraw from contact with its opponent. The results obtained were from small sample sizes and this rendered statistical analysis of the data difficult. The results appeared to show, however, that encounters between animals of a similar size took slightly less time to resolve than those between animals of a different size (Figure 5.15) although this was not significant. This result is unexpected, as a larger animal in a pair would be expected to take less time to assert its dominance over a smaller opponent. Farmer (1974e) observed that two animals of dissimilar size would display for only a short time while similarly sized mature males would fight for periods of up to 10 minutes. When paired with an animal of a similar size, the dominant individual would not necessarily be immediately apparent, resulting in a series of interactions

between the animals to assert dominance. The shorter duration of encounters between animals of a similar size has been reported for the velvet swimming crab, *Necora puber* (Thorpe *et al.*, 1994). The use of visually impaired animals could have affected the results through a reduced ability of individuals to assess effectively the size of their opponent. It is possible that this could have influenced the duration of encounters.

Richardson (1996) found that blinding either the dominant or subordinate animal from a previous encounter did not affect the duration or outcome of agonistic interactions. The same pairs of individuals were used in these experiments both before and after blinding. Each individual would therefore have had the opportunity to carry out size assessment prior to blinding, and it is possible that they would recognise their opponent's dominance status from previous encounters (Karavanich & Atema, 1991). It has been shown for the American lobster that the duration of agonistic encounters was reduced when animals were familiar with one another and it is suggested that successive fights may be mediated by chemical communication of dominance (Karavanich & Atema, 1998). Chemical communication may be important but it must be acknowledged that in this study the outcome and duration of interactions between unfamiliar pairs of individuals could have been affected by any light-induced retinal damage in both animals.

The number of interactions seen in the pairings showed that encounters between animals of the same size involved more contact between the individuals than those between different sized opponents (Figure 5.16) although this was not statistically

significant. It could be the case that with different sized opponents the dominant animal is immediately apparent and does not therefore require to assert its position immediately, whereas animals of the same size would perhaps require more physical interactions to assess their opponent's status.

In the pairings that contained an individual with only one claw, the contests with animals of similar size took slightly longer to resolve than those between animals of differing size, as would be expected (Figure 5.15) although the result was not significant. It was unexpected, however, that those encounters involving a damaged individual took longer, on average, than those involving intact animals only, although there was a very small sample size and the results were not significant. It would be expected that animals with only one claw would avoid contact with intact individuals. In all the pairings of different sized animals the damaged individual was smaller than its opponent, and it would be logical to assume that the larger intact individual would quickly assert its dominance. It could be that the loss of a claw causes an increase in the aggressiveness of an individual to compensate for the lack of the second appendage. Lang *et al.* (1977) found that American lobsters that had no claws did not engage in any defensive activity but rather showed a tail flip response when threatened. Although there was not a great deal of typically agonistic behaviour recorded in this study, it would seem possible that one claw is sufficient for engagement in agonistic behaviour in *N. norvegicus*, although this is not reflected in the literature (see Juanes & Smith, 1995 for review). For example, Villanelli & Gherardi (1998)

found that the loss of a major cheliped resulted in a reduced success during inter-male competition in the crayfish, *Austropotamobius pallipes*.

When looking at the average number of encounters in each pairing, it can be seen that for those involving animals missing a claw there were more encounters (Figure 5.16) although the result was not significant. This was also unexpected as damaged individuals might be expected to avoid contact with intact animals. Again, this could point to an increased level of aggression in animals missing a claw. It can also be seen from Figure 5.16, that during encounters involving intact and damaged animals of the same size, there were higher frequencies of contact, indicating that contests between individuals of a similar size were more closely contested.

The reluctance of male *N. norvegicus* to engage in agonistic behaviour has previously been noted (Salerno, 2000) although this was not the case in the studies carried out by Richardson (1996). One reason for the lack of overt aggression between individuals in this study could have been that the experiments were carried out during the day. During this time *N. norvegicus* in the field, would largely be found in their burrows becoming active between dawn and dusk (depending on the depth of the population). It is possible, therefore, that the individuals were not responsive during the day, although agonistic behaviour and general activity were both observed during the hours of daylight in experiment 1. It is highly unlikely that the low levels of aggression seen were a result of the animals being blinded since Richardson (1996) has shown that blinding male *N. norvegicus* has no effect

on their agonistic behaviour. A further reason for a lack of aggression could be the lack of a defensible resource, for example a burrow. This is unlikely, however, as individuals did not use artificial burrows successfully without a period of acclimatisation and did not generally compete for burrows other than through eviction (experiment 1). Starvation of the animals prior to the experiment and using food as a cue might have been more successful. A possible explanation for the lack of inactivity between animals of different size is that the dominant individual is clearly recognisable and therefore no interaction is required. This has been reported in the interactions of both American lobsters (Karnofsky & Price, 1989) and stomatopods (Dingle & Caldwell, 1969). This does not, however, explain the lack of activity in pairs of animals of a similar size.

It is most likely that the lack of agonistic encounters observed was due to the conditions of holding the animals were exposed to prior to the analyses. Agonistic behaviour has been recorded in the field between *N. norvegicus* individuals and also with other species (Conan *et al.*, 1984). In the laboratory, agonistic encounters have been observed in both sighted and blind *N. norvegicus* that had been held in visual and tactile isolation prior to observation (Richardson, 1996). In this study, the holding tank in which animals were stored was quite crowded and individuals were freely in contact with each other. It is probable that the constant contact with other animals desensitised each individual to the presence of a possible competitor in the experiments. Such a reduction in aggressive behaviour following group holding conditions has previously been observed in the American lobster (Dunham, 1972; Hoffman *et al.*, 1975). Dunham (1972) also noted that

animals that had been housed communally did not use any preliminary display behaviours that were seen in those housed individually. It is also possible that the artificial conditions in the experimental tank affected the results; however, agonistic behaviour was observed in the artificial conditions of experiment 1, where animals were held individually prior to the experiment. It would therefore appear that the development of individual storage for each animal prior to experimentation would be desirable for any behavioural analysis of *N. norvegicus*.

Care was taken to avoid the use of individuals more than once in the agonistic experiments and this greatly reduced the sample sizes for each type of pairing, especially for those animals with missing chelipeds. In a system where individual storage would be possible, it would be worthwhile increasing the numbers of individuals used in the experiment, and to examine the effects of moult stage on the outcome of aggressive interactions.

These results can, of course, only be interpreted cautiously due to the quiescent nature of the animals observed and the small sample sizes. They do, however, raise some very interesting questions about the agonistic interactions of *N. norvegicus* and the effects of claw loss on the dominance status of an individual.

5.4.2.1 Moulting

Unfortunately, the lack of newly moulted individuals of either sex prevented the examination of the influence of pheromones on the physiology and behaviour of *N. norvegicus*. The presence of one moulted male did, however, allow some

assumptions to be made about the presence of a newly moulted individual. The lack of response of males to either the presence of a newly moulted male or the introduction of a sample of water in which the moulted animal had been kept (moult water) indicates that the presence of a moulting individual alone does not induce reproductive behaviour. It had previously been suggested that moulting hormones might have been responsible for mating behaviour in crustaceans (Kittredge *et al.*, 1971), although this has since been disproved, for example in the American lobster (Atema & Gagosain, 1973) and the shore crab, *Carcinus maenas* (Bamber & Naylor, 1997). The lack of response of a male to the moult hormones produced by another male could indicate that in *N. norvegicus* a sex specific hormone is likely to be responsible for behavioural responses as reported by Farmer (1974a). Mating has been observed in *N. norvegicus* without any of the display behaviour described as a response to the presence of a newly moulted female (Salerno, 2000). The differences between male and female moult water has previously been described in the American lobsters where it was seen that animals would only respond to moult water from an individual of the opposite sex (Atema & Cowan, 1986).

It is possible that the lack of response from the introduced male was because the length of time that the moulted male was left in the tank following handling was insufficient. Eales (1974) showed that newly moulted females did not induce a behavioural response in males unless they had been allowed at least two hours recovery time following handling. It is unlikely this is the case in *N. norvegicus*, which is a cannibalistic species (Wieczorek *et al.*, 1999). It would be expected that

newly moulted males would not wish to advertise their vulnerable state to other males within the vicinity due to the risk of predation. In the case of females, the length of time that pheromones are used could also be limited, although Kittredge *et al.* (1971) found that female *Cancer magister* produced pheromones for up to two weeks following the moult. In the study of *N. norvegicus* carried out by Salerno (2000) successive males were introduced to a single soft-shelled female. Display behaviour (Farmer, 1974a) was only shown by the first male that was introduced to the soft female, although copulation did not take place. When subsequent males were introduced to the female they did not show any reproductive display behaviour, but rather grabbed and mated with the female. Mating could have occurred as a result of close proximity within the tank rather than as a direct result of the female advertising with pheromones. This could indicate that females do not display with chemical signals for long after moulting.

Although the results of these behavioural analyses are largely inconclusive they do point towards the existence of a mating system based on dominance hierarchies. Individual dominance would appear to be driven by the size of an animal. It is not clear if the female actively takes part in the reproductive behaviour. The substantial investment made by females in terms of energy and time would suggest that female mate choice might be important; however, it is not clear how this would occur in natural populations. It could be that pheromones are important since they enable the female to advertise her status to the dominant male prior to the moult; however, the lack of any kind of mate guarding would point to a promiscuous mating system. This area of *N. norvegicus* biology requires further

investigation in order to determine the possible effects of a male biased fishery on the mating systems and reproductive output of populations.

5.5 Conclusions

5.5.1 Experiment 1

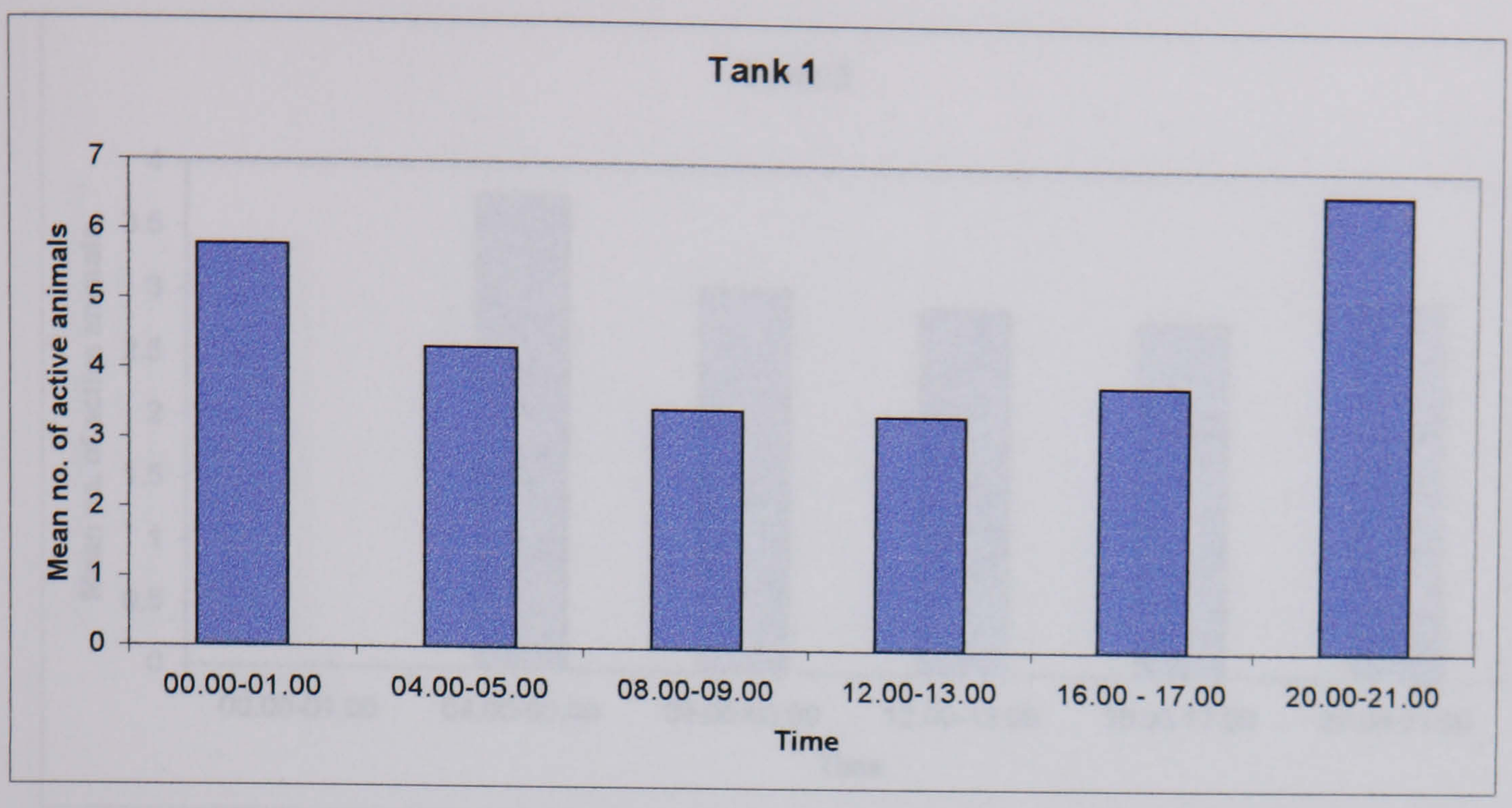
- The activity patterns of sighted *N. norvegicus* held in laboratory populations match those that would be expected from natural populations at the depth from which the experimental animals were sampled.
- A high degree of movement between artificial burrows was seen for individuals of both sexes.
- Eviction behaviours were seen often. Evictions were probably related to agonistic behaviour and dominance rather than being resource motivated as burrows were not limited and evicting animals often did not remain in the burrow following the eviction of the original occupant.
- Within the laboratory populations there was an initial decrease in the levels of agonistic behaviour seen, coupled with an increase in non-agonistic encounters. The overall number of interactions decreased over the course of the observations. This could indicate the formation of a dominance hierarchy.

5.5.2 Experiment 2

- In observations of agonistic interactions between pairs of animals it was noted that aggressive behaviour was minimal. It is likely that the group holding facility in which the animals were placed prior to observation suppressed agonistic behaviour.

- The duration of bouts between individuals of the same size was seen to be shorter than the duration of bouts between animals of differing size, this was unexpected, although there were more individual interactions between animals of a similar size. These results were not statistically significant however, and any interpretation is therefore tentative.

- In bouts between intact individuals and individuals that were missing a claw, bouts between animals of a similar size were longer than between animals of differing size and in all cases there were more interactions recorded before the bout was concluded. Again, these results were not statistically significant however, and any interpretation is therefore tentative.



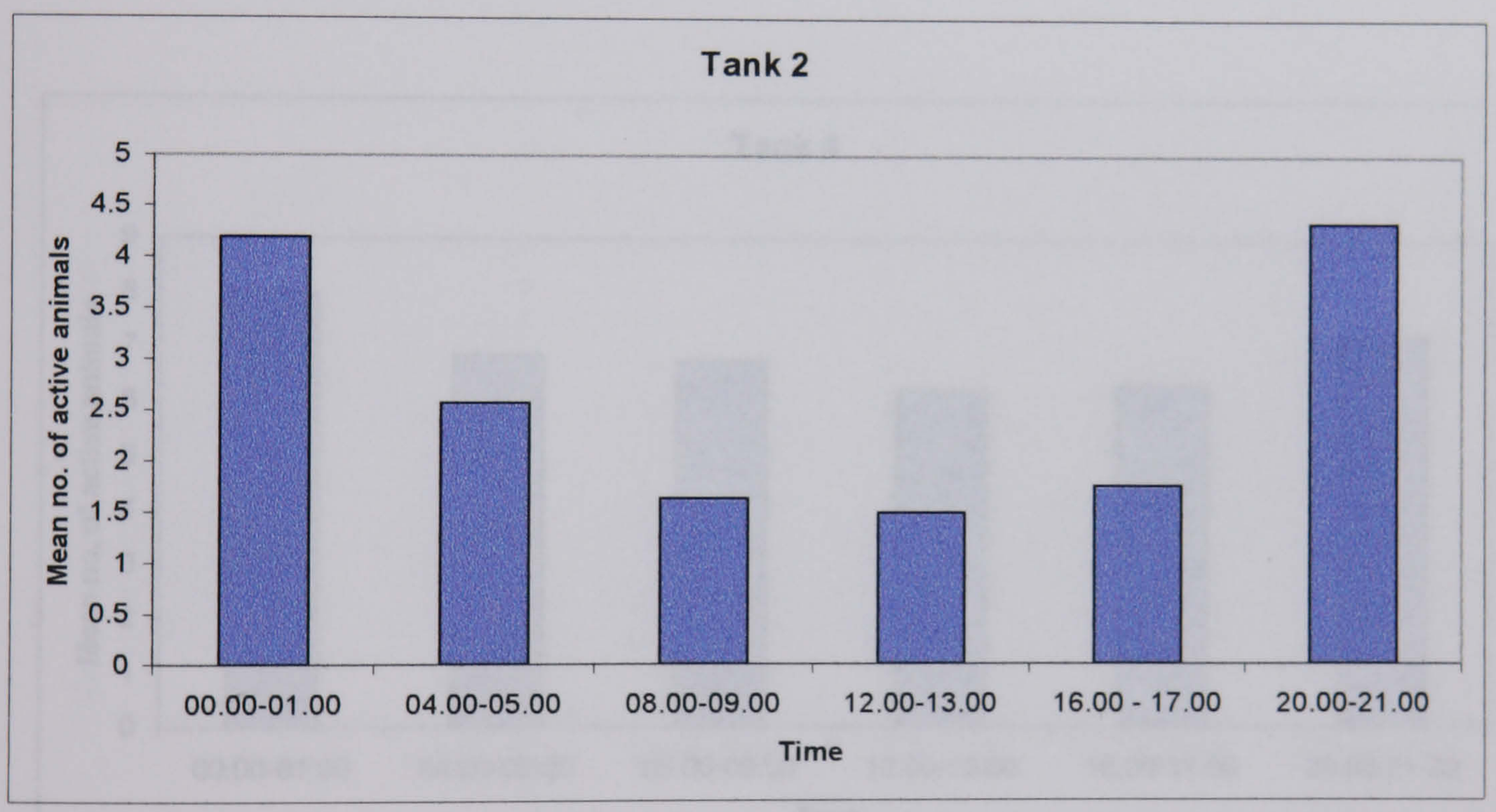
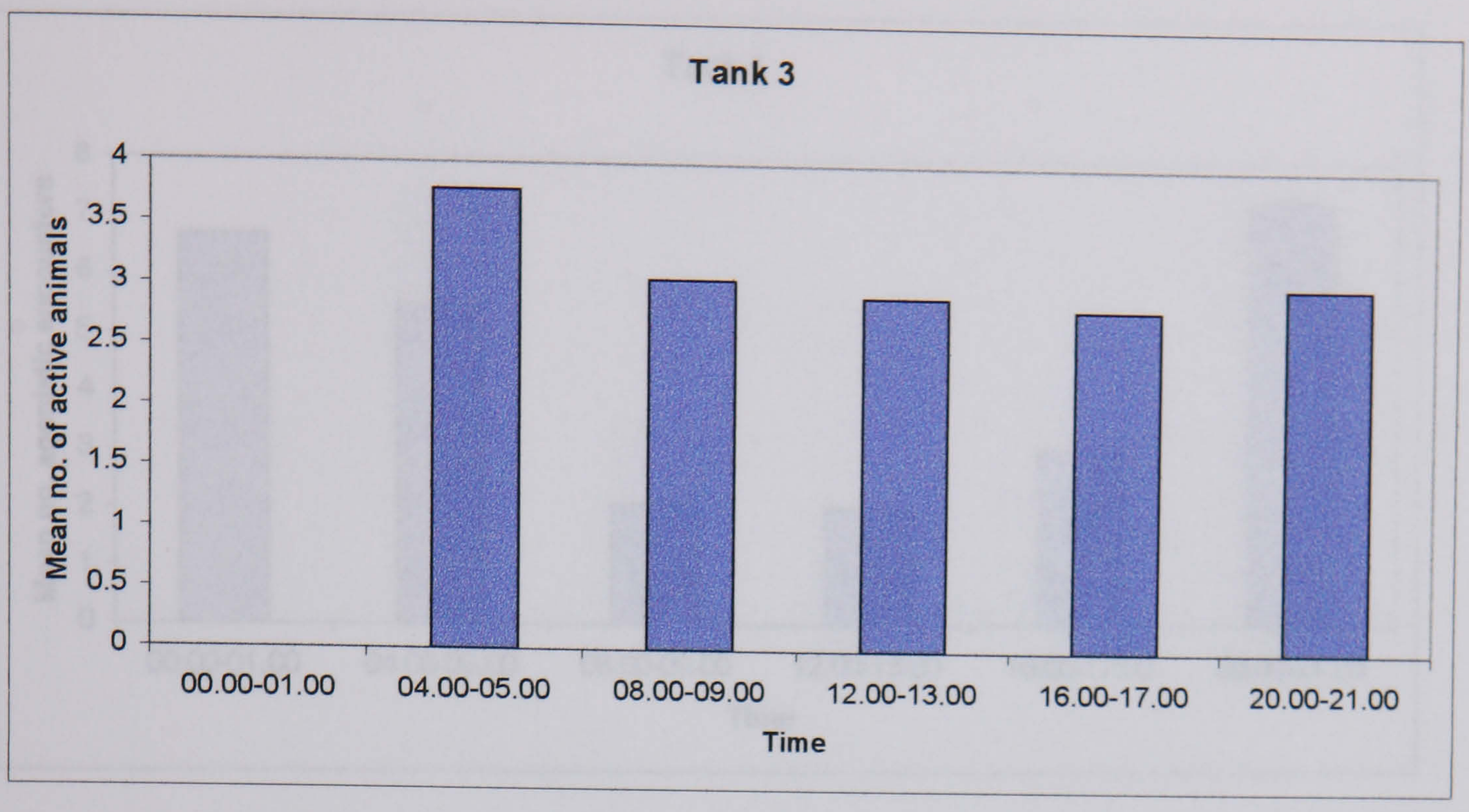


Figure 5.1: The mean number of active *Nephrops norvegicus* in tanks 1 and 2, observed periodically throughout the 24 h period over 33 days.



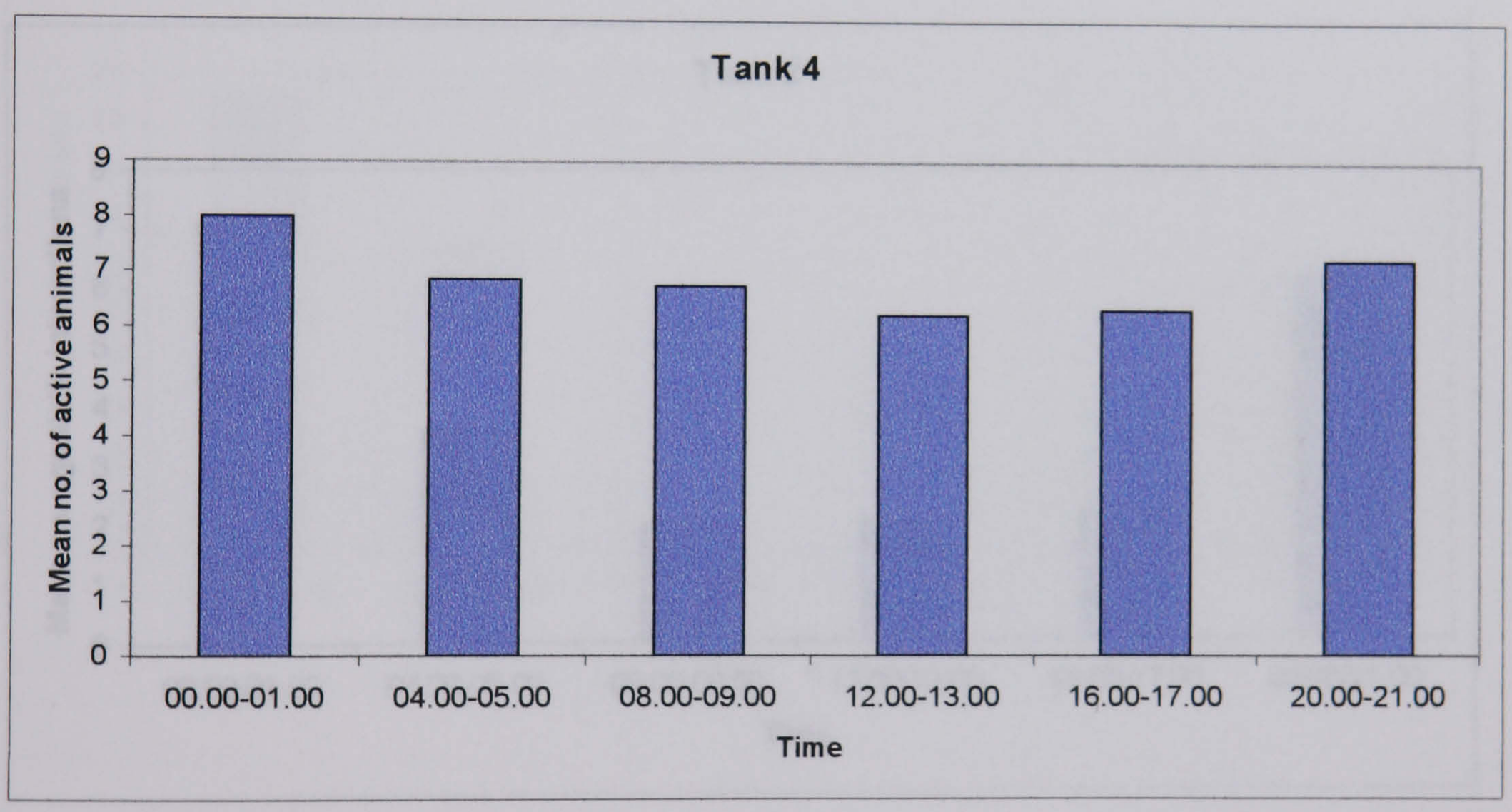
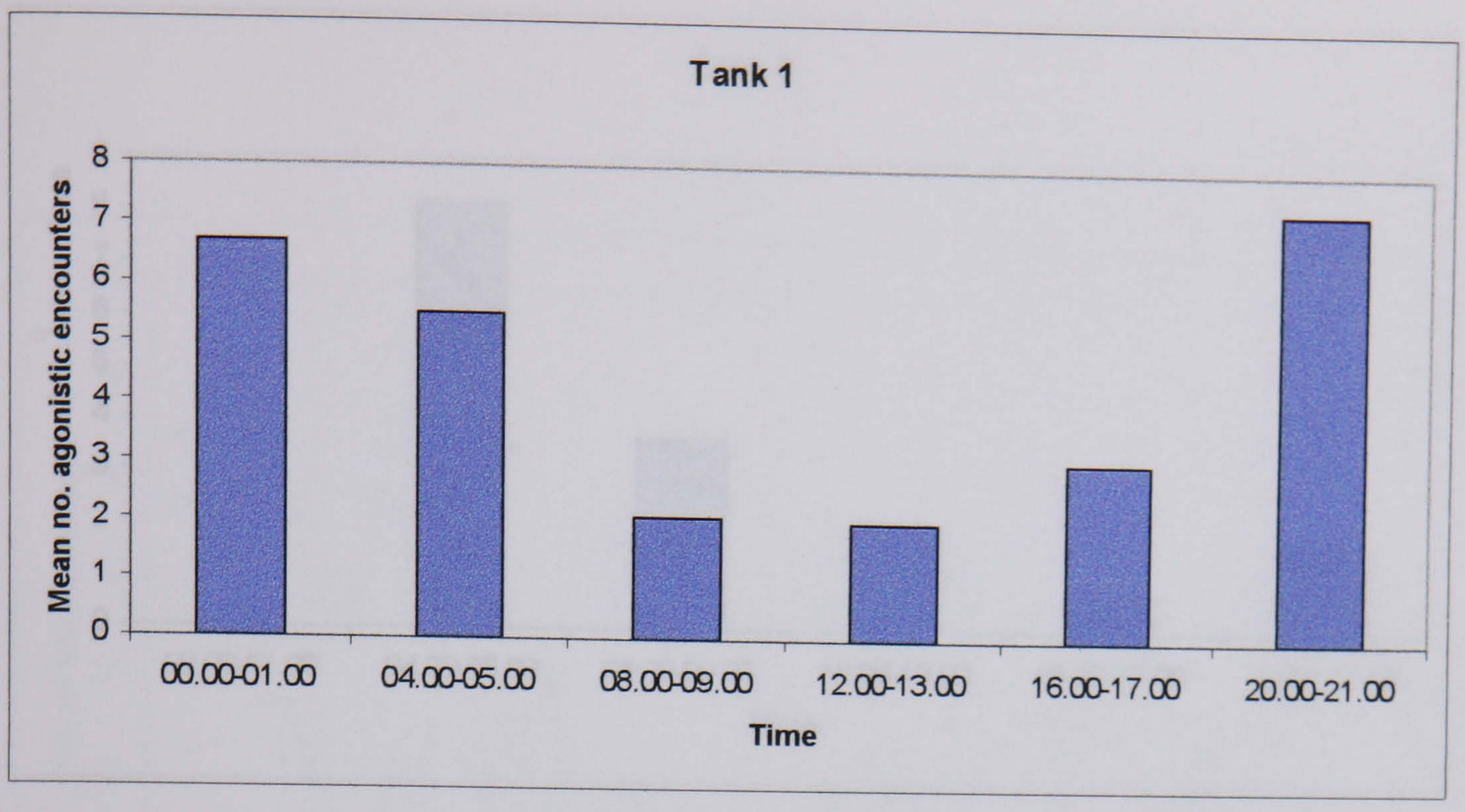


Figure 5.2: The mean number of active *Nephrops norvegicus* seen in tanks 3 & 4, observed periodically through the 24 h period, for 16 days. Light was not sufficient to count animals in tank 3 during the hours of darkness.



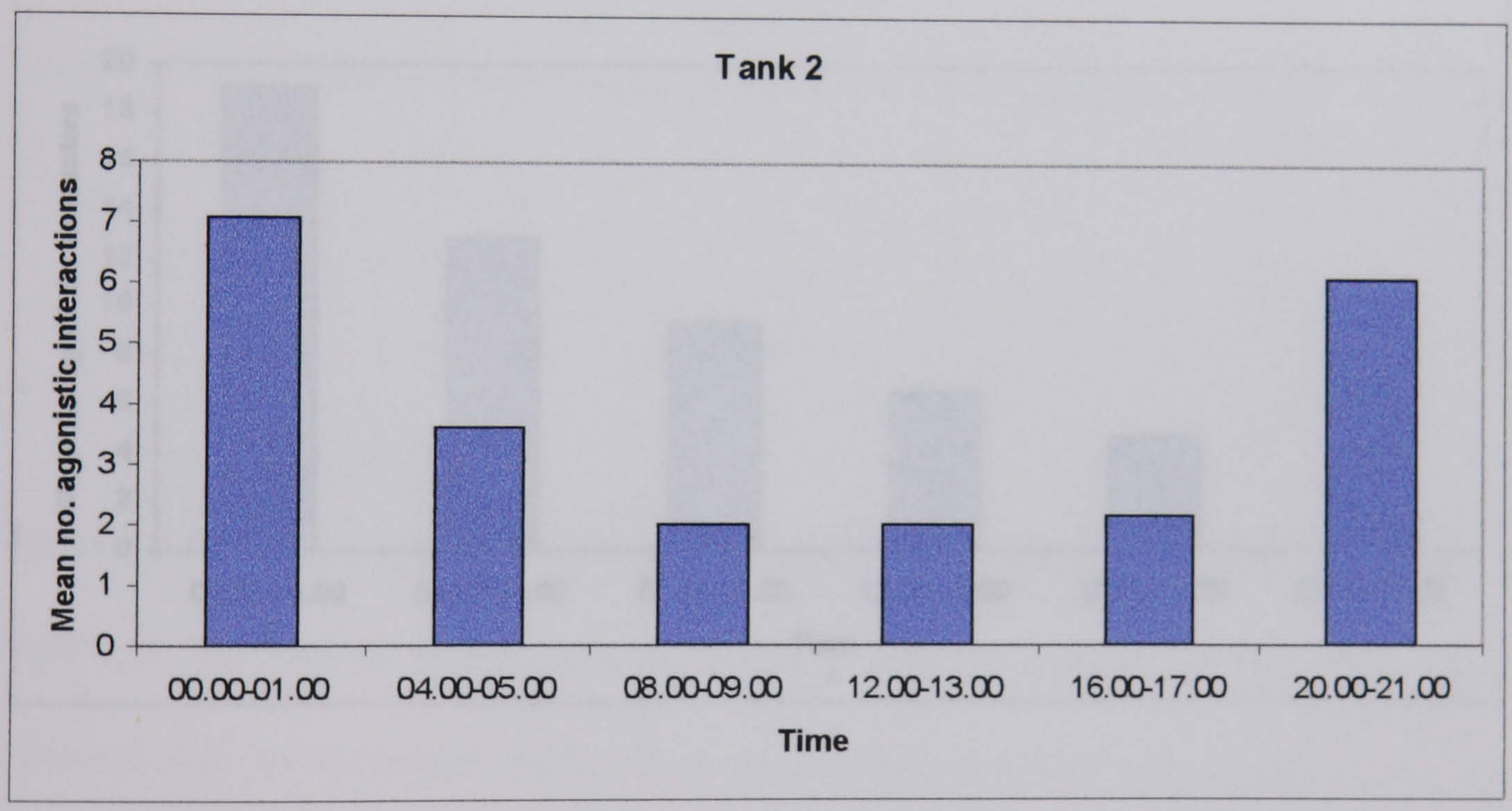
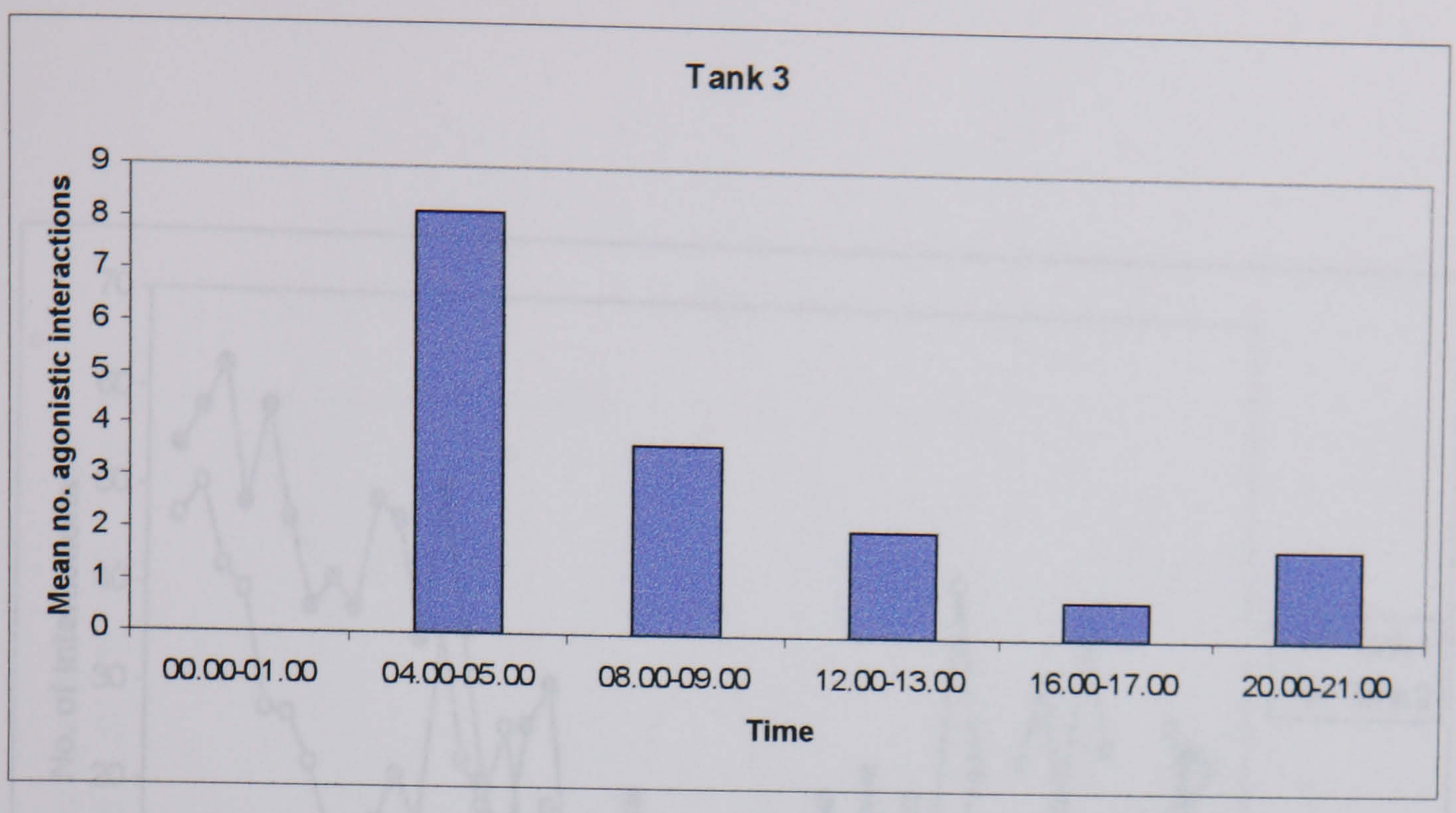


Figure 5.3: The mean number of agonistic encounters observed between *Nephrops norvegicus* individuals in tanks 1 and 2 over the 24 hour period, observed over 33 days.



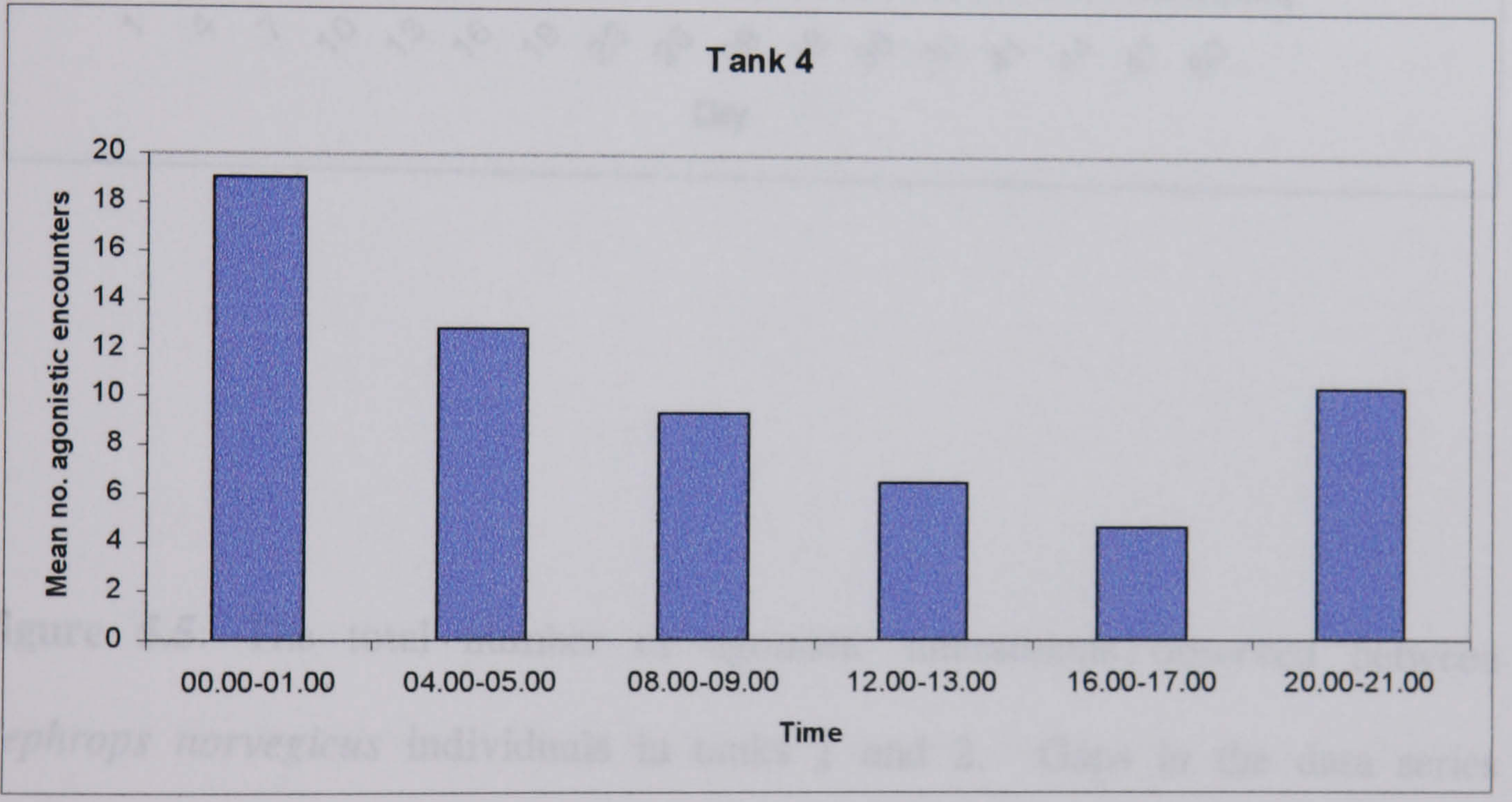


Figure 5.4: The mean number of agonistic interactions observed between *Nephrops norvegicus* individuals in tanks 3 and 4 over the 24 h period, observed over 18 days. It was not possible to observe interactions in tank 3 during the hours of darkness.

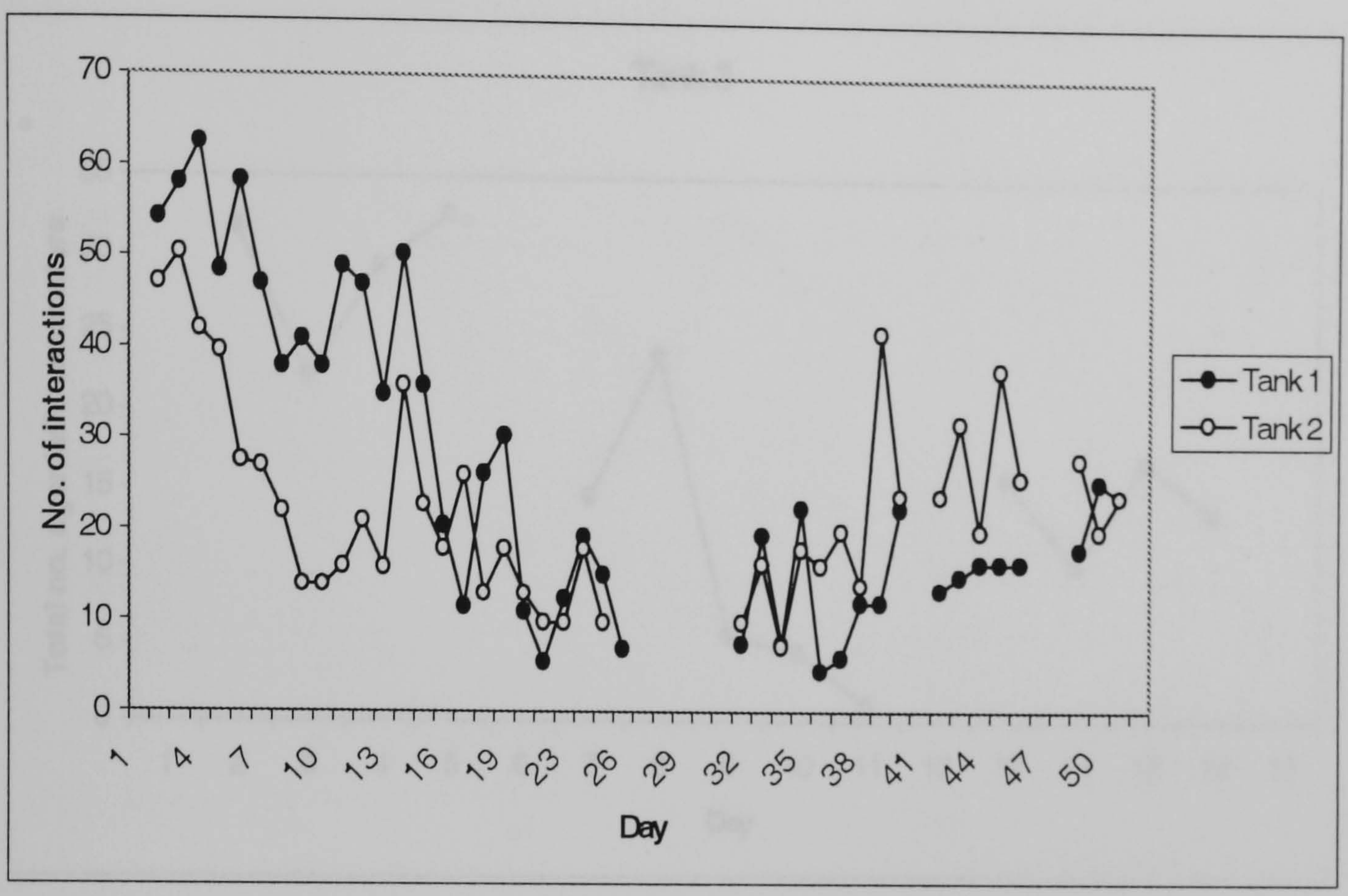


Figure 5.5: The total number of agonistic interactions observed between *Nephrops norvegicus* individuals in tanks 1 and 2. Gaps in the data series occurred due to technical difficulties and were included where there was an incomplete data set for a particular day.

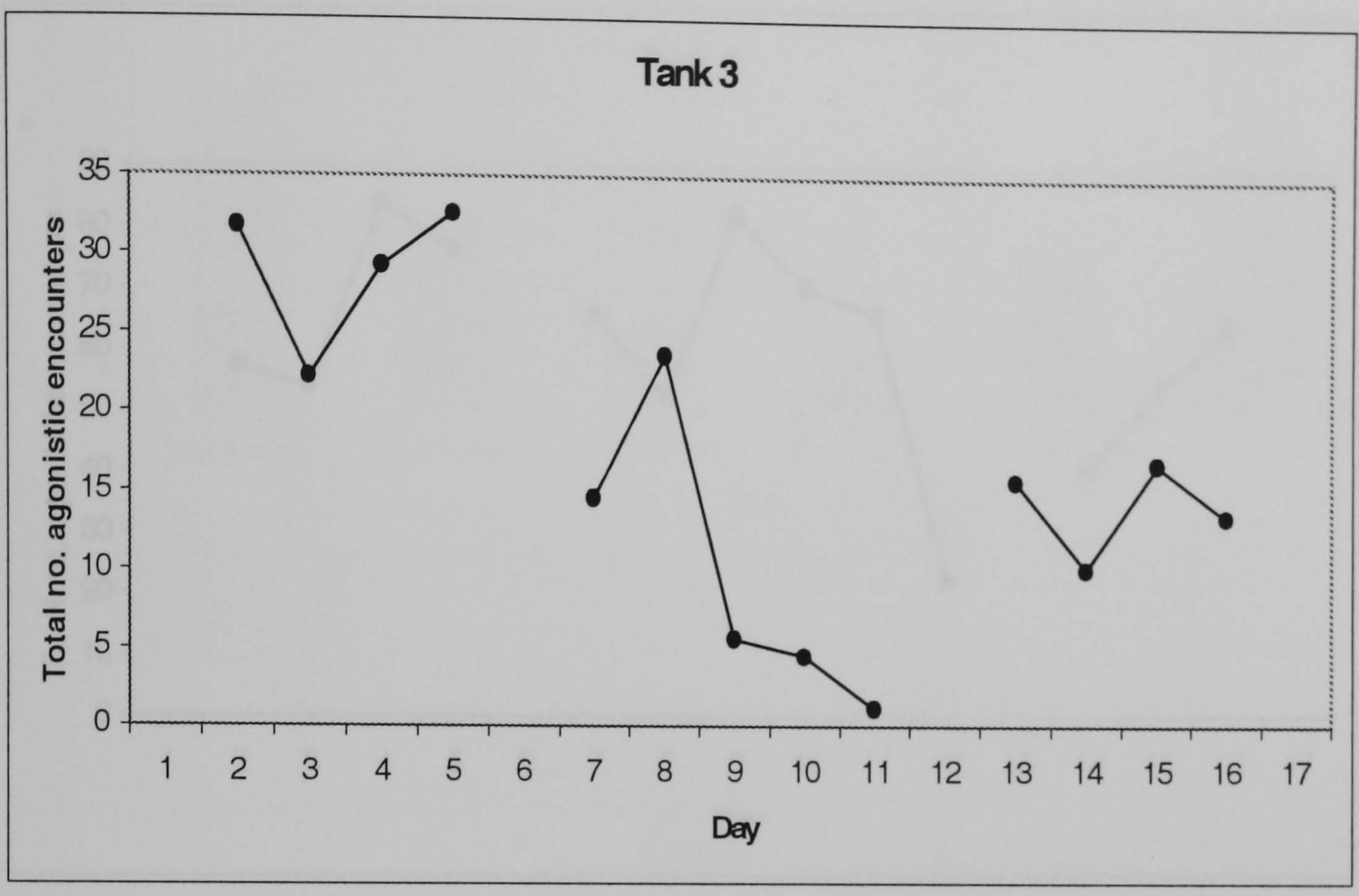


Figure 5.6: The total number of agonistic encounters observed in tank 3 over the course of the experiment. Gaps in the data set are due to incomplete data for particular dates (behaviour during the hours of darkness was not observed).

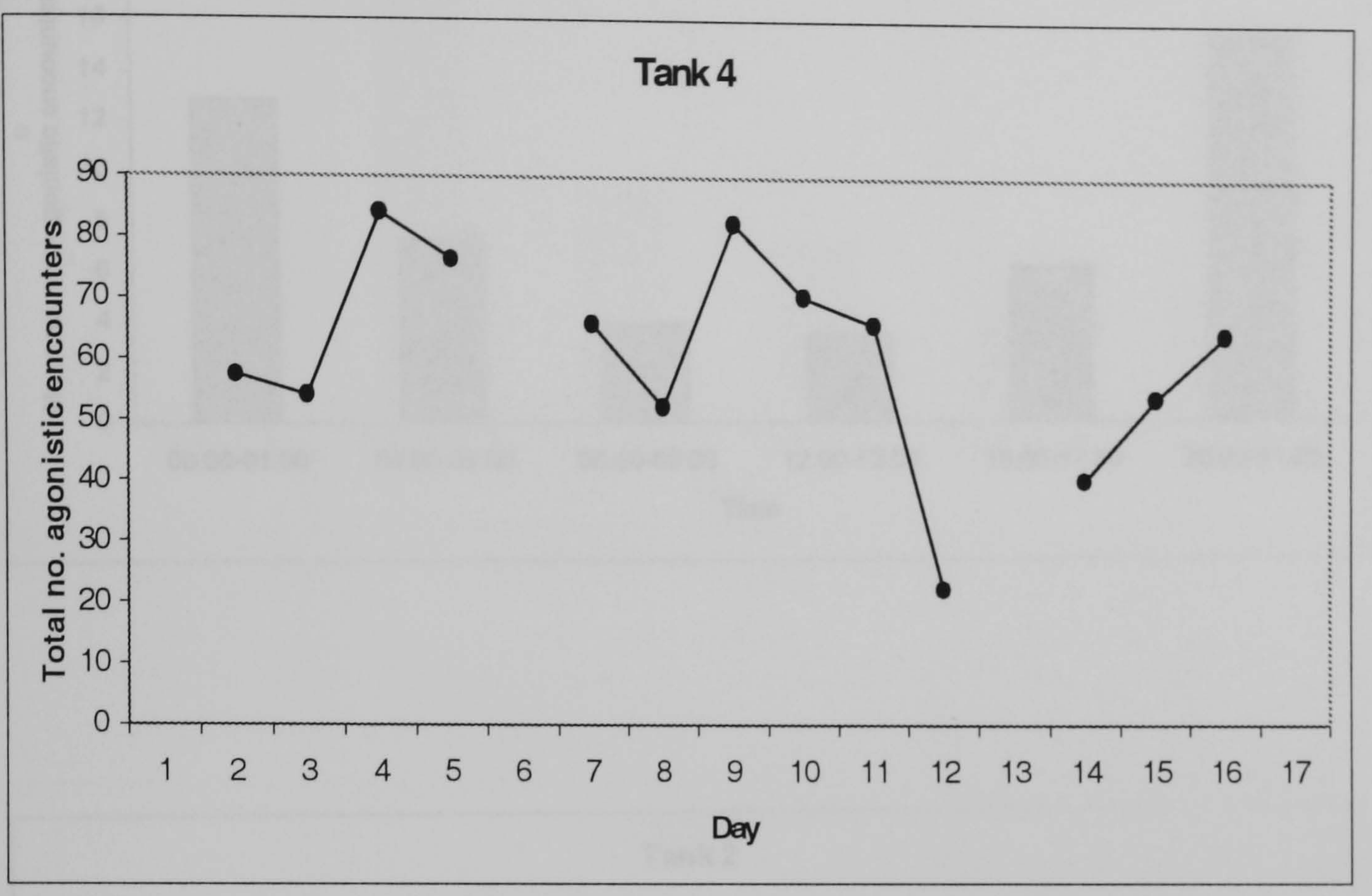
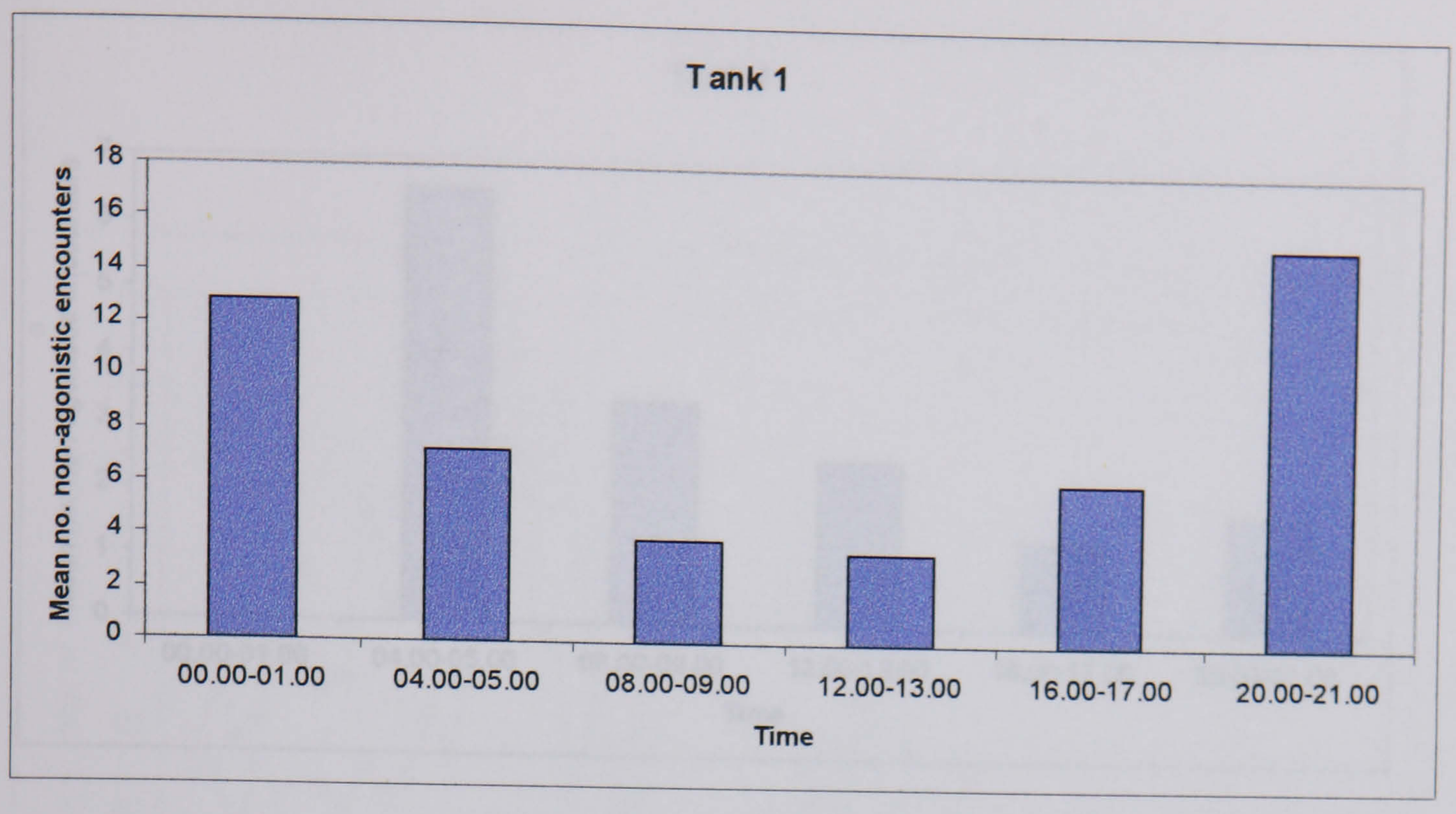


Figure 5.7: The total number of agonistic interactions observed in tank 4, gaps in the data are due to incomplete data sets for some dates.



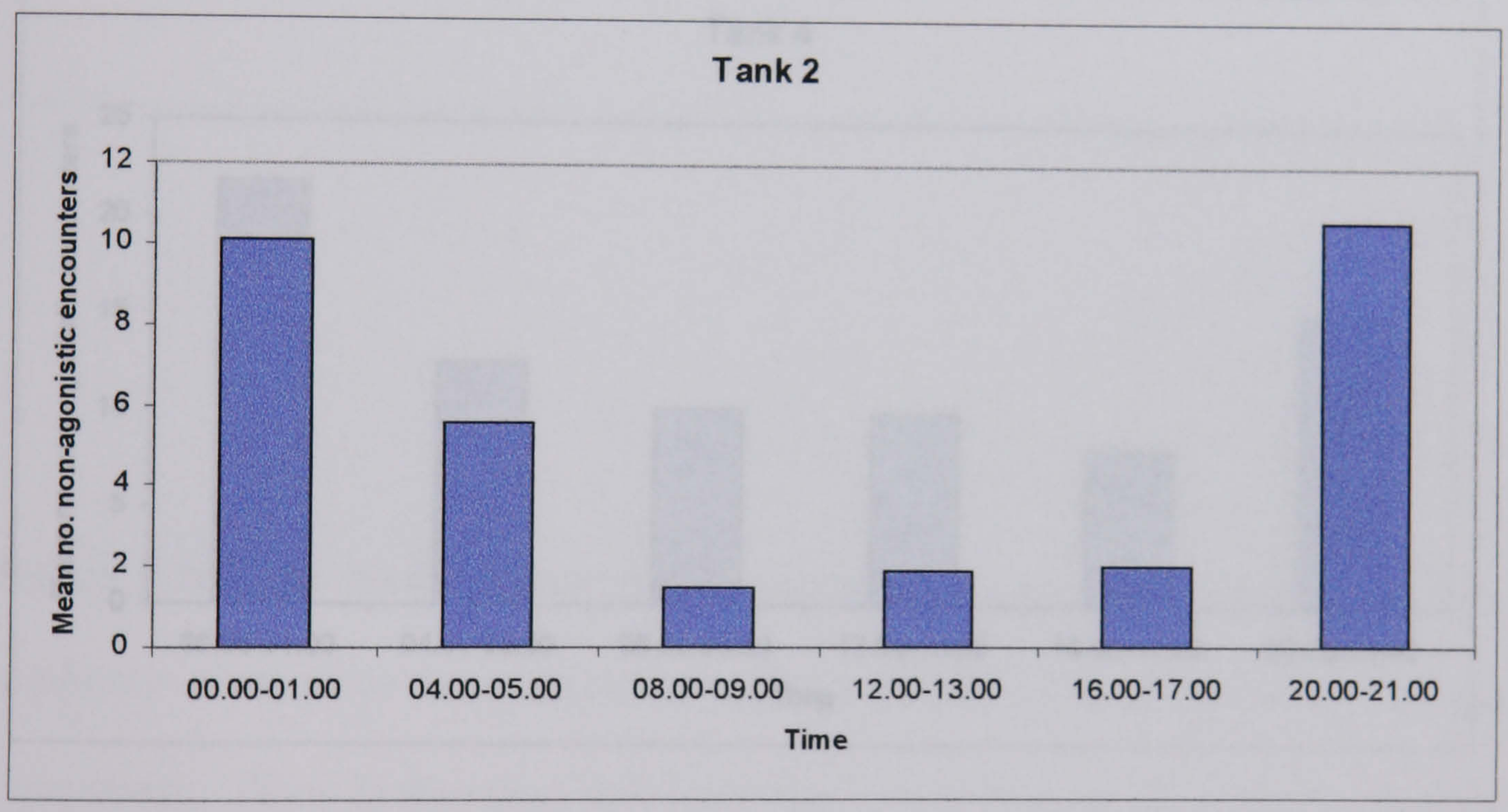
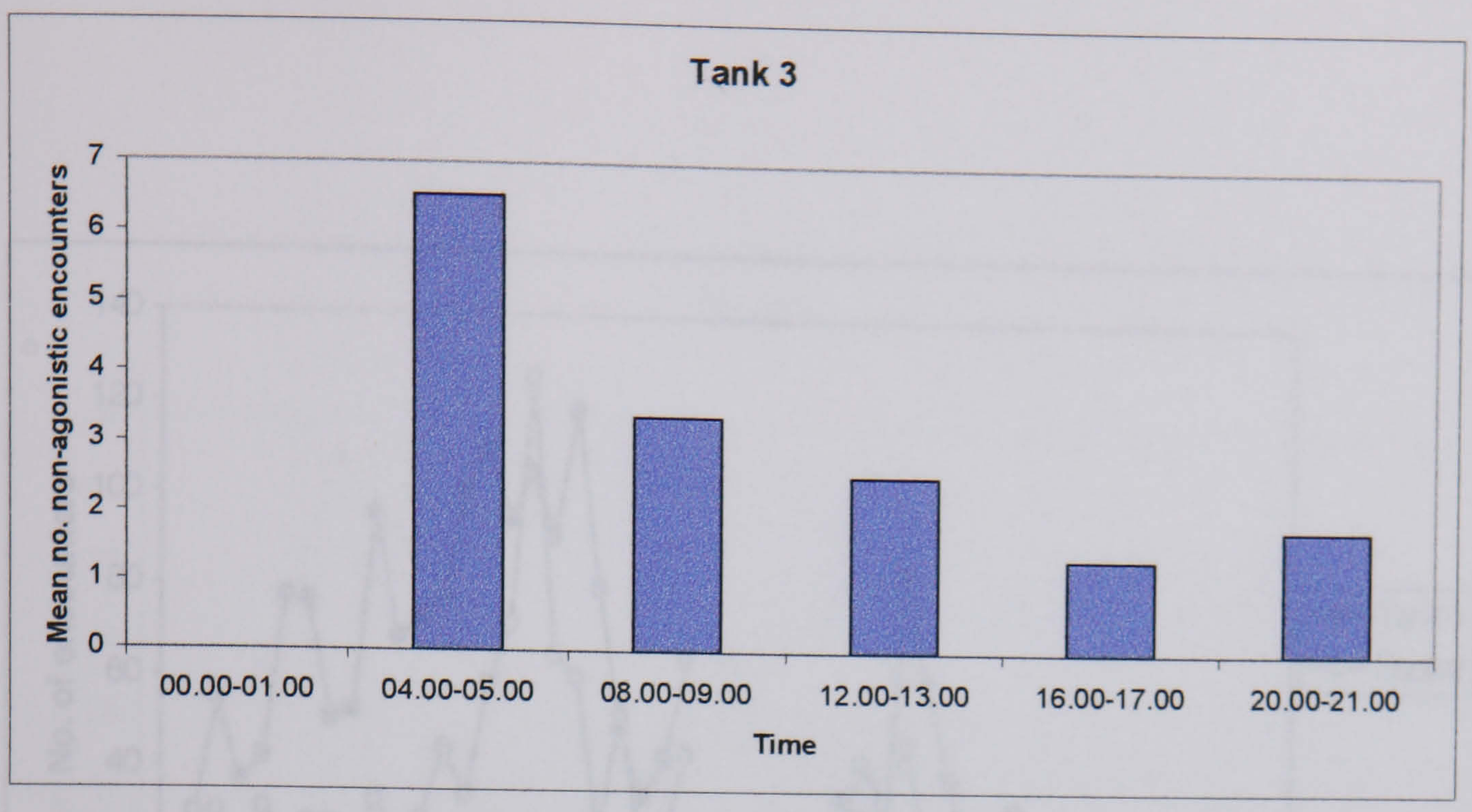


Figure 5.8: The mean number of non-agonistic encounters observed between *Nephrops norvegicus* in tanks 1 and 2 over the 24 hour period, recorded over 33 days.



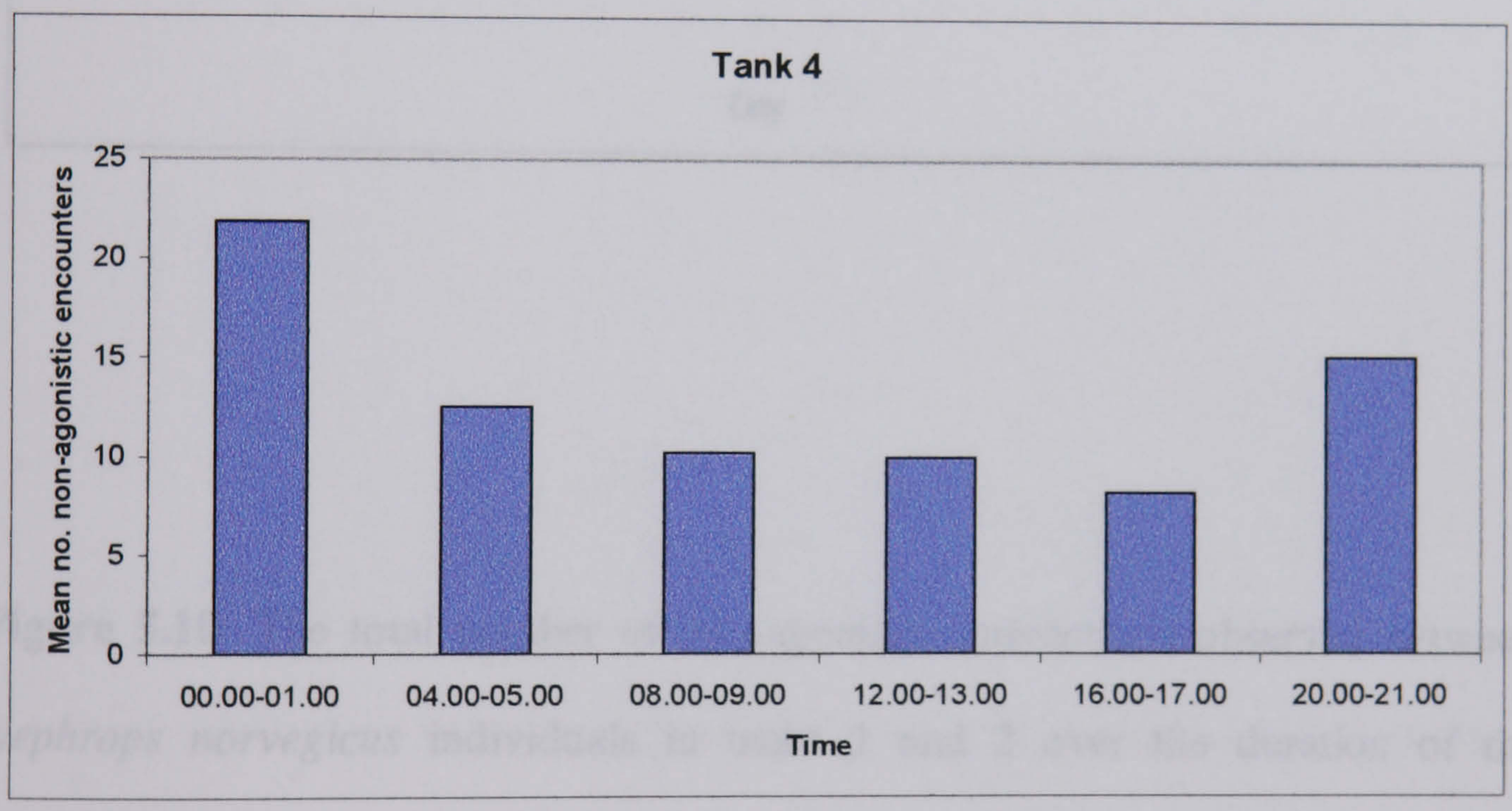


Figure 5.9: The mean number of non–agonistic interactions observed between *Nephrops norvegicus* individuals during the 24h period in tanks 3 and 4. It was not possible to record behaviour during the hours of darkness in tank 3.

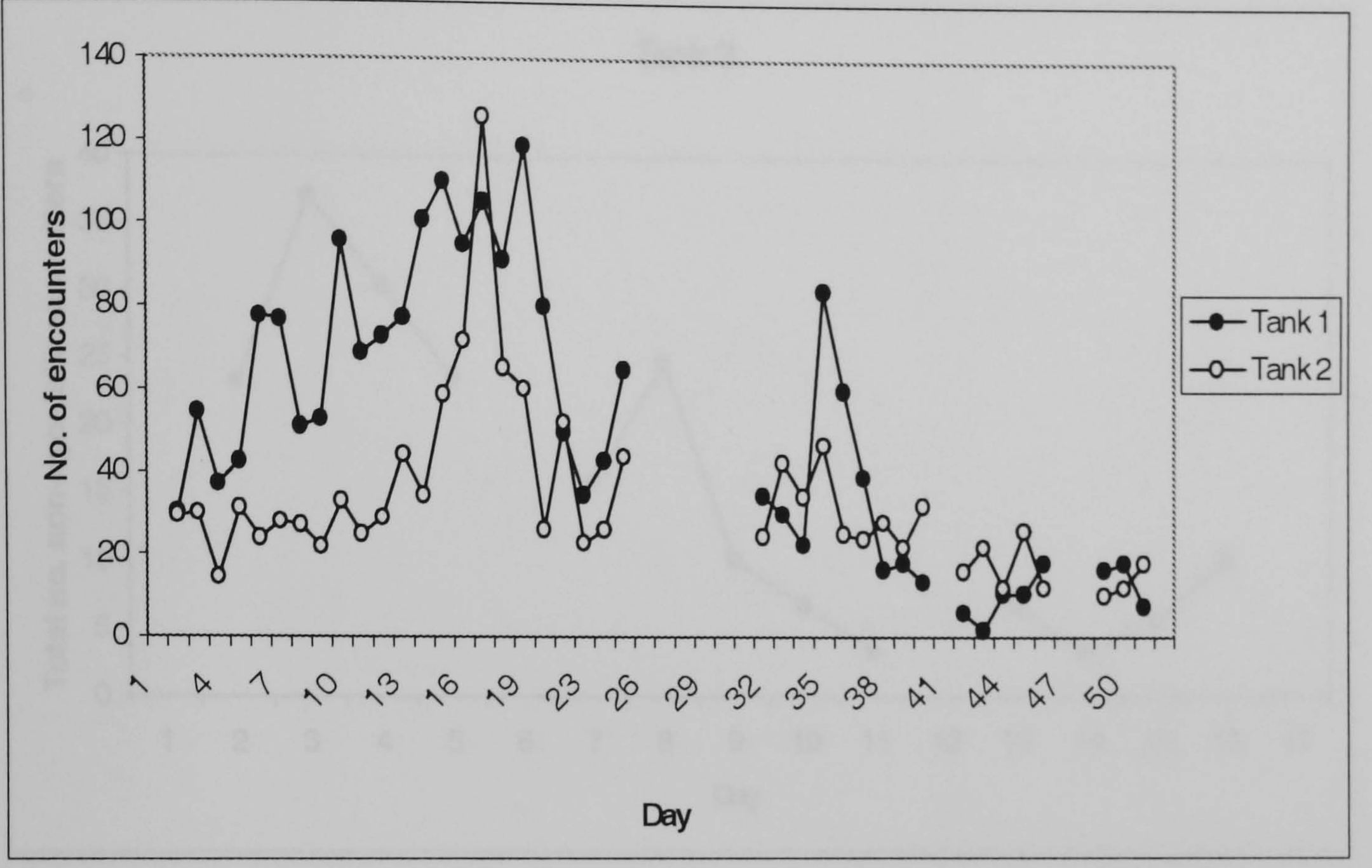


Figure 5.10: The total number of non-agonistic interactions observed between *Nephrops norvegicus* individuals in tanks 1 and 2 over the duration of the experiment. Gaps in the data series are due to incomplete data sets for certain dates.

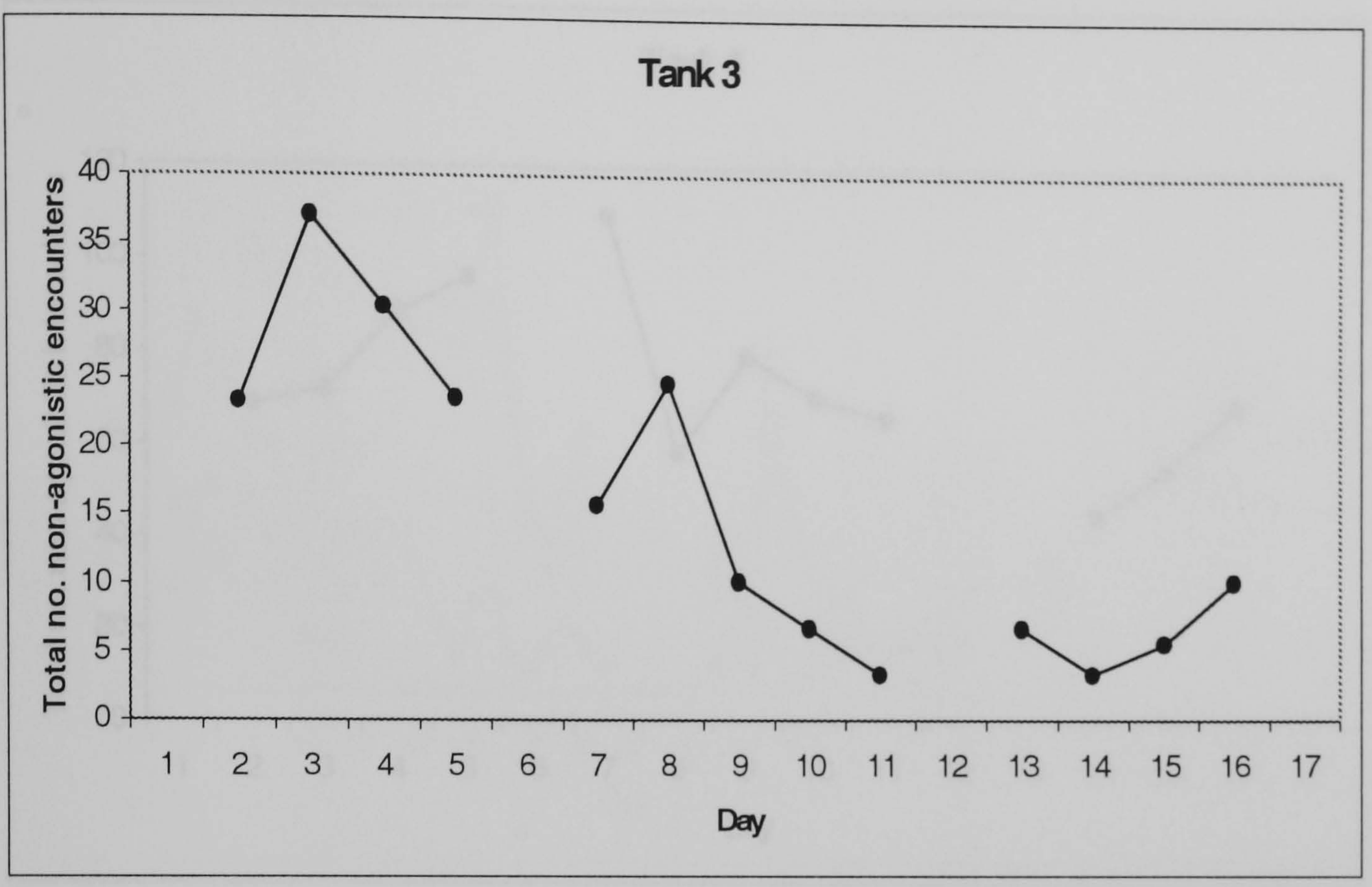


Figure 5.11: The total number of non-agonistic interactions observed between *Nephrops norvegicus* individuals in tank 3 over the duration of the experiment. Gaps in the data are due to incomplete data sets for some dates (it was not possible to obtain data during the hours of darkness).

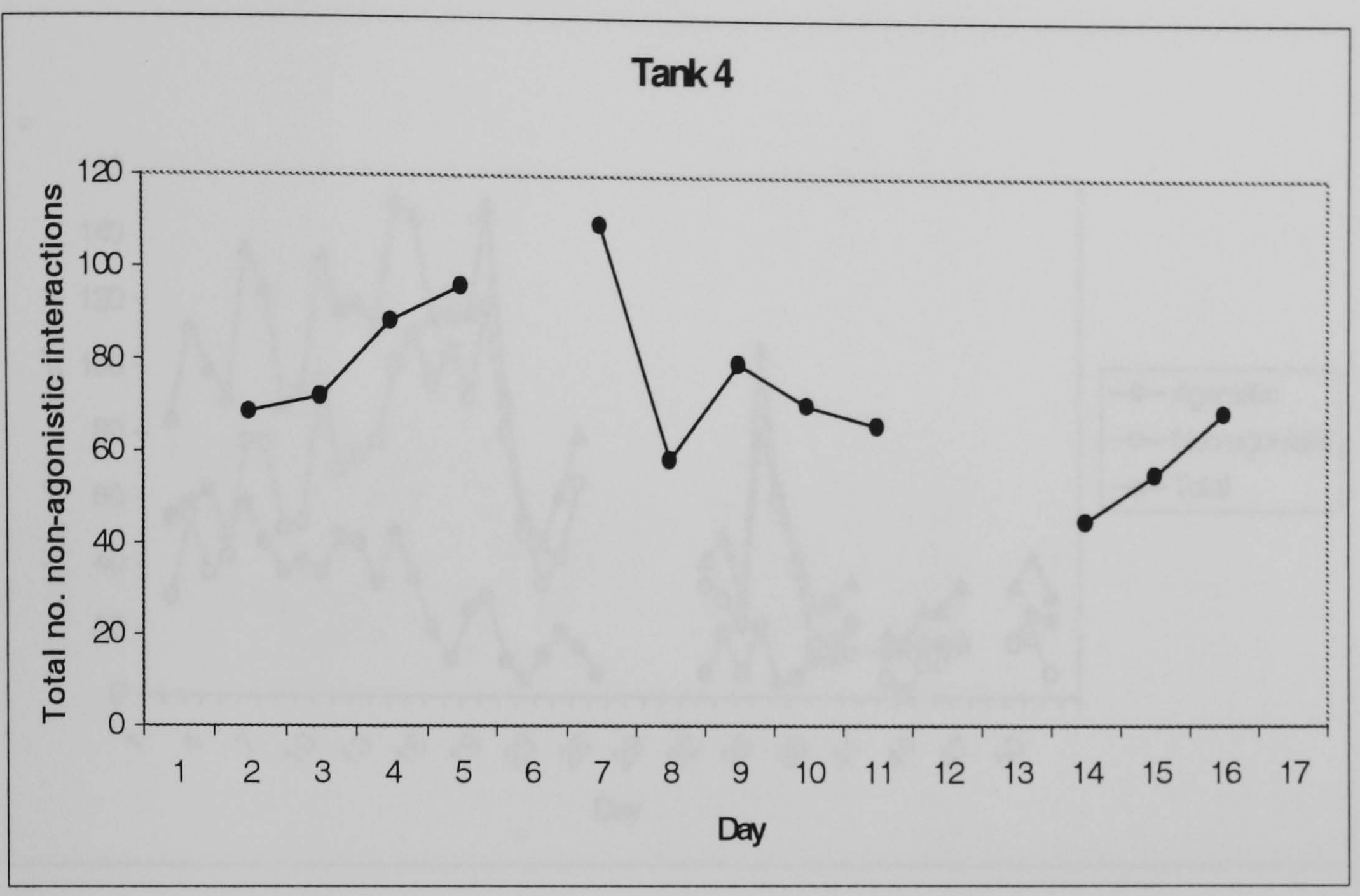


Figure 5.12: The total number of non-agonistic interactions between *Nephrops norvegicus* individuals observed daily in tank 4. Gaps in the data series are due to incomplete data on certain dates.

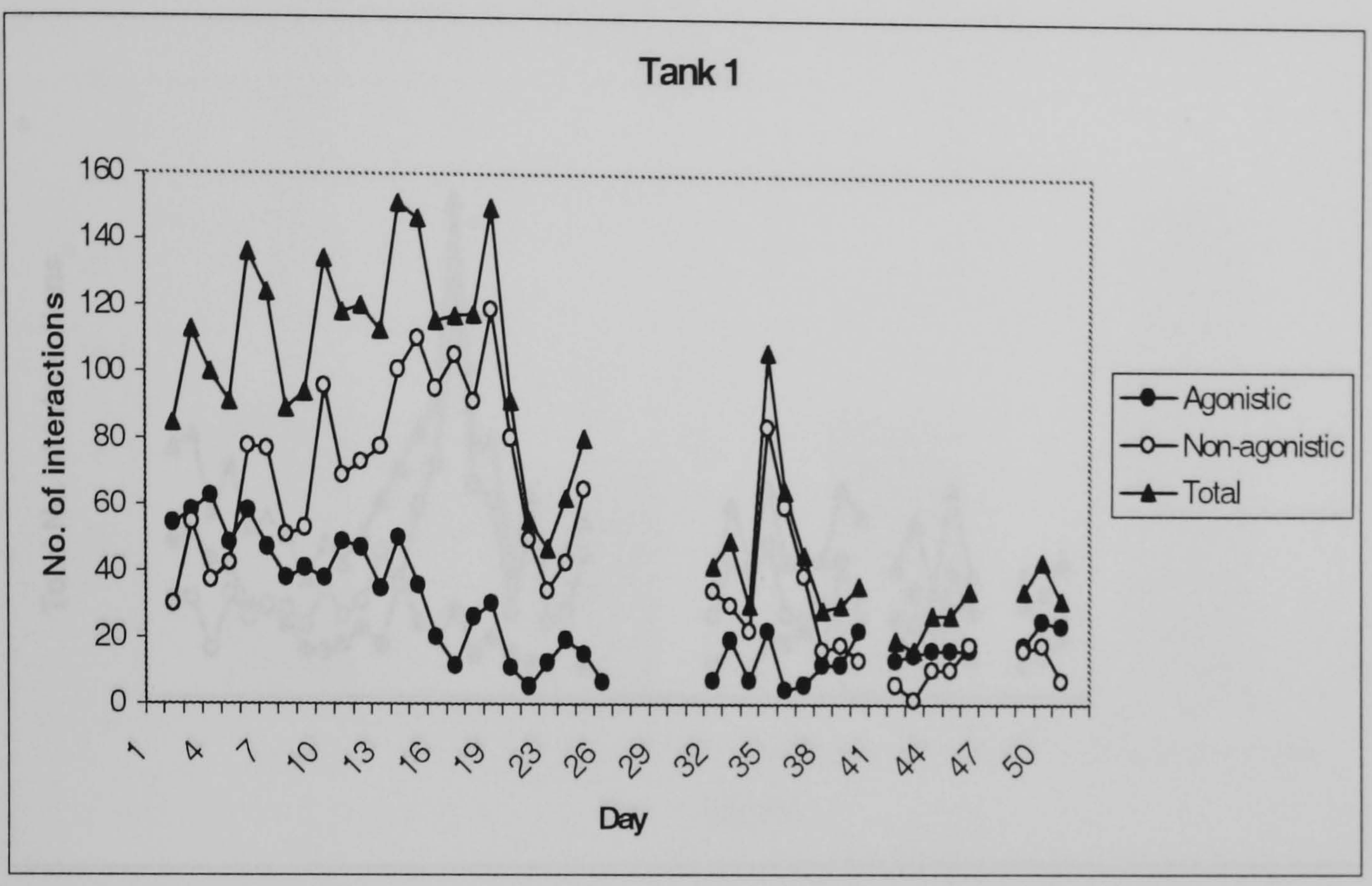


Figure 5.13: Variations in the total number of interactions between *Nephrops norvegicus* individuals observed over the period of observation (51 days) in tank 1. The data are further broken down into both agonistic and non-agonistic behaviours.

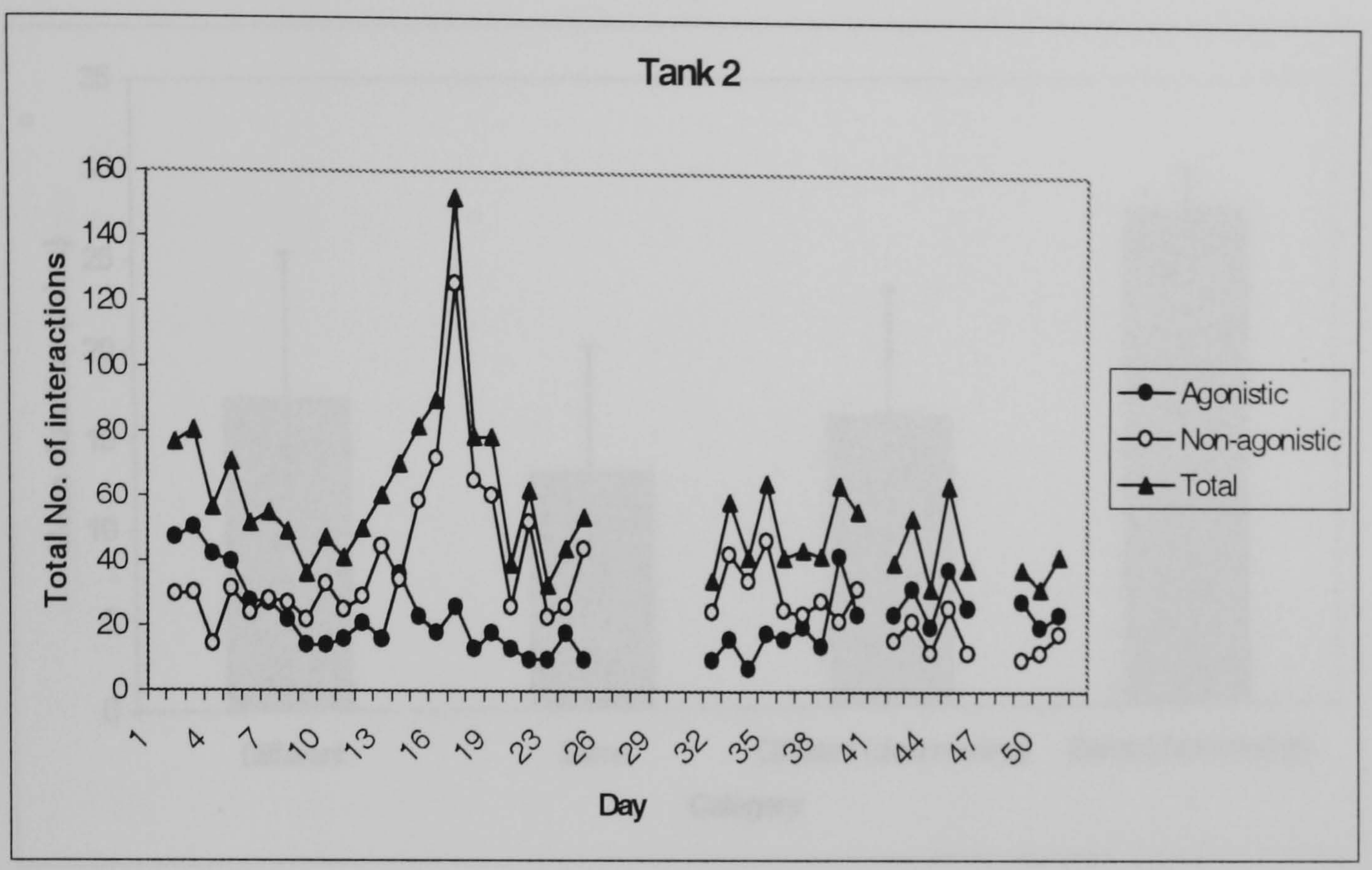


Figure 5.14: Variation in the total number of interactions observed between *Nephrops norvegicus* individuals throughout the period of observation (51days) in tank 2, further broken down into agonistic and non-agonistic behaviours.

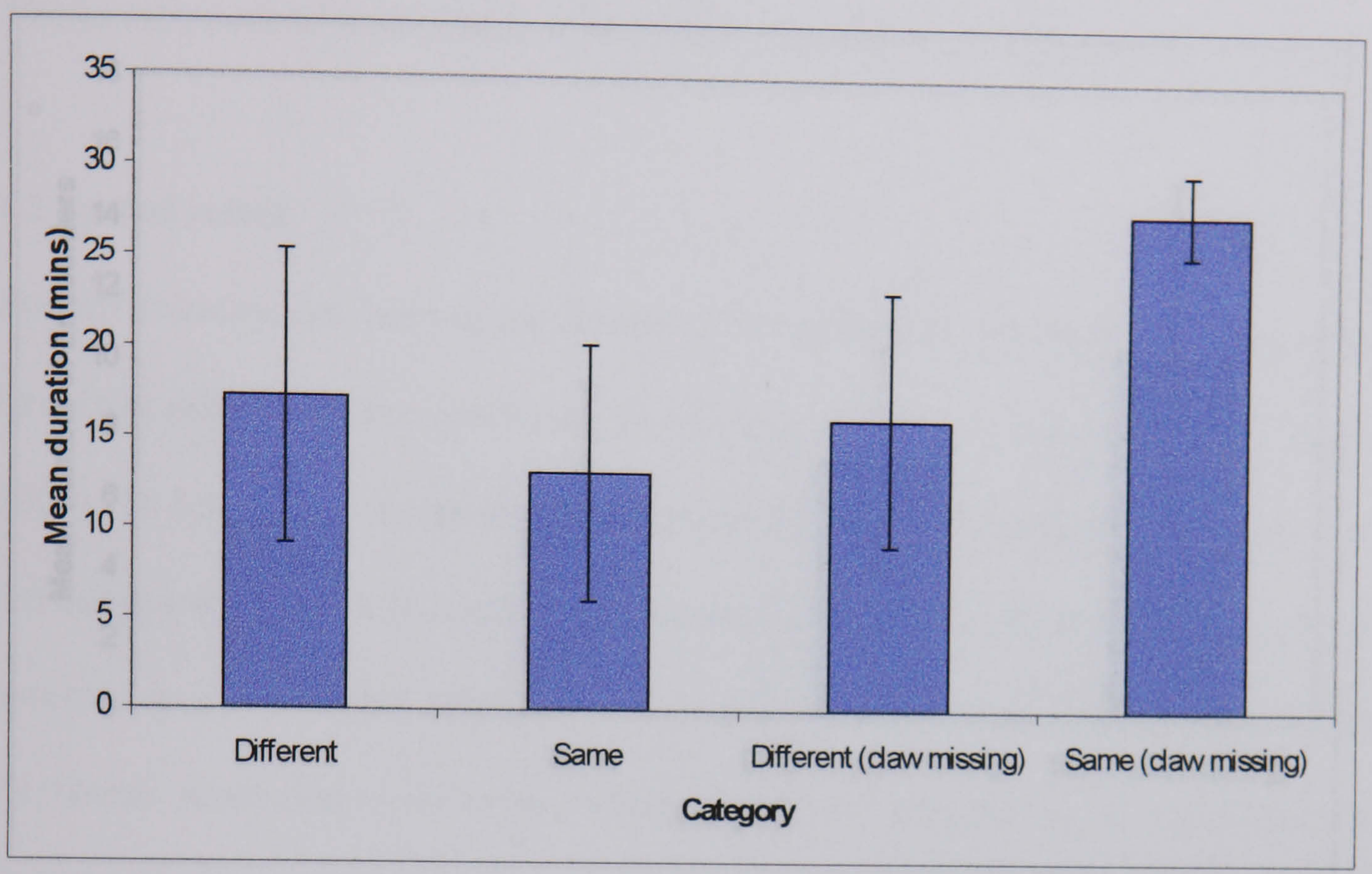


Figure 5.15: The mean duration (± S.D.), in minutes, of encounters between pairs of male *Nephrops norvegicus* of the same size and different sizes, with both intact individuals and males with a missing cheliped. Where opponents were of differing sizes it was always the larger individual that was intact.

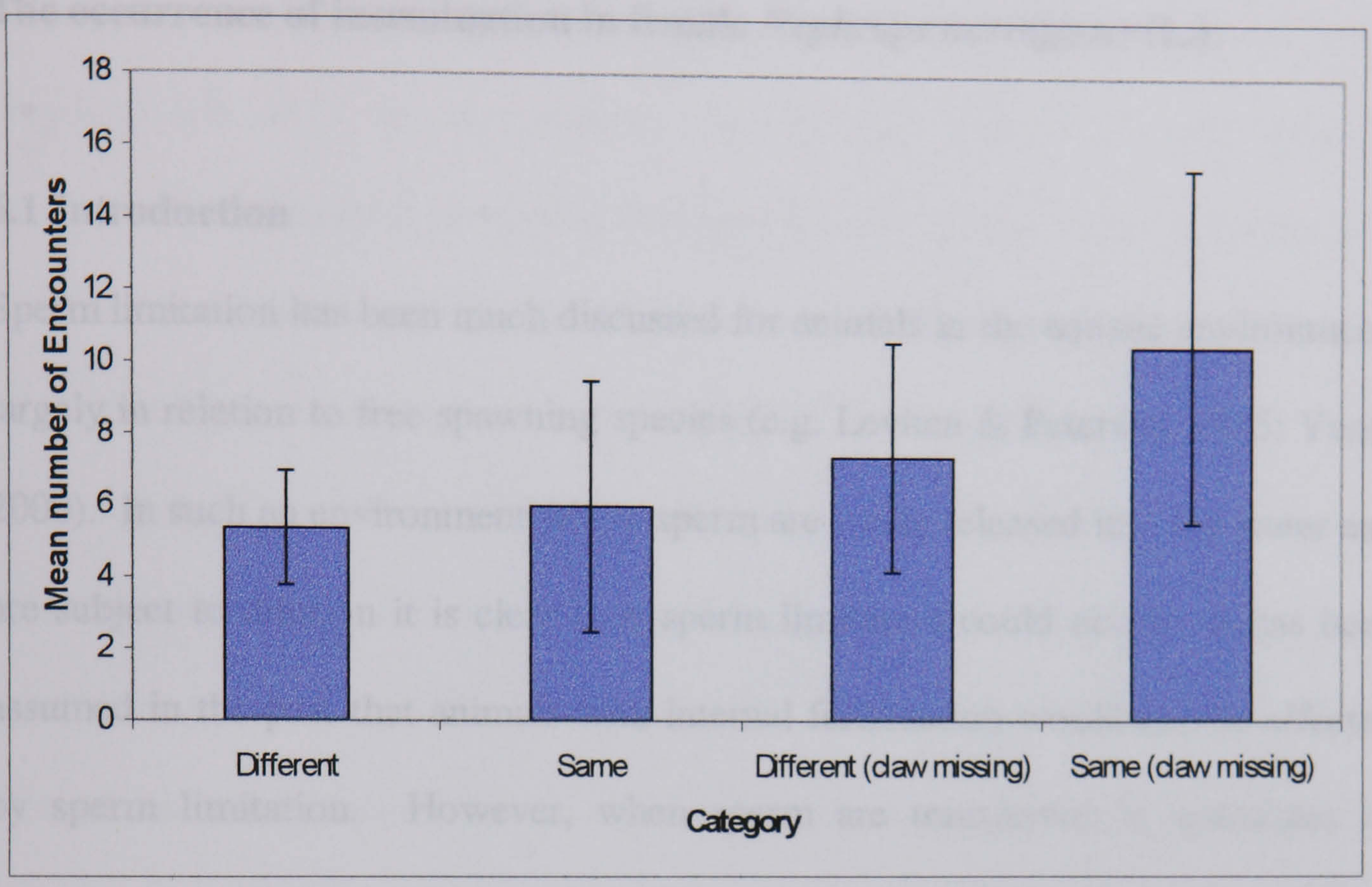


Figure 5.16: The mean number (± S.D.) of encounters observed between pairs of male *Nephrops norvegicus* of the same size and different sizes, with both intact individuals and males with a missing cheliped. Where opponents were of differing sizes it was always the larger individual that was intact.

Chapter 6

The occurrence of insemination in female *Nephrops norvegicus* (L.).

6.1 Introduction

Sperm limitation has been much discussed for animals in the aquatic environment, largely in relation to free spawning species (e.g. Levitan & Petersen, 1995; Yund, 2000). In such an environment where sperm are freely released into the water and are subject to dilution it is clear how sperm limitation could occur. It has been assumed in the past that animals with internal fertilisation would not be affected by sperm limitation. However, when sperm are transferred in ejaculates or spermatophores, sperm limitation could become an issue. Dewsbury (1982) states, "In theory males may daily produce sufficient sperm to inseminate millions of females, in practice they can inseminate but few". Factors such as the cost of ejaculate or spermatophore production, and behavioural aspects of reproduction can therefore cause sperm limitation. In this context this subject has received recent attention in relation to exploited populations.

Sperm limitation is of particular relevance in populations exposed to sex-biased harvesting. For example, in ungulate populations hunting may involve the removal of only large males, and it has been shown that this could result in reduced female fecundity and population collapse (Ginsberg & Milner-Gulland, 1994). Sperm limitation can occur in fished populations through selective fishing pressure on males. For example, many Alaskan crab fisheries are sex-selective

for males (Orensanz *et al.*, 1998) as in most cases females cannot legally be landed. Brown & Powell (1972) noted that in the tanner crab (*Chionoecetes bairdi*) fishery there was a potential for what they called a 'depletion of male breeding stocks'. In the collapse of the red king crab (*Paralithoides camtschaticus*) fishery it was noted that there was an increase in female crabs with smaller than expected brood sizes (Powell *et al.*, 1974). In some areas up to 25% of sampled females were barren in the 1968/69 season increasing to 47% in the 1987 survey (Orensanz *et al.*, 1998). Powell *et al.* (1974) linked the decline in fecundity of female red king crabs with the reduction in numbers of male crabs in the fished areas. This indicates the presence of recruitment overfishing. In the Dungeness crab fishery (*Cancer magister*), only male crabs are taken and the minimum landing size is set to allow males at least one breeding season prior to capture. This was thought to be a sound management scheme because of the ability of males to mate with more than one female and the ability of females to store sperm to fertilise broods over a number of breeding seasons. This could be a misconception, however, as female Dungeness crabs appear to require mates larger than themselves (Butler, 1960; Snow & Neilsen, 1966), therefore larger females could go unmated. Smith & Jamieson (1991) also suggested that where there is high fishing pressure on populations of Dungeness crabs, both the numbers of males of legal landing size and their opportunities to mate could be substantially reduced. Hankin *et al.* (1997) also examined the effects of the fishery on this species and estimated that the egg production could be reduced by 2 – 25% through sperm limitation.

The problem of higher fishing mortality in males affecting reproduction is not restricted to Alaskan crab fisheries. Similar observations have been made on the fishery for blue crab (*Callinectes sapidus*) in South Carolina where Wenner (1989) found that although only 3% of the females examined were uninseminated over the course of one year; 57% of these females had developing ovaries.

Sperm limitation has also been reported in two genera of spiny lobsters (MacDiarmid & Butler, 1999a, 1999b). They showed that small males were unable to regulate the size of their spermatophores; however, larger males were shown to vary spermatophore size in relation to the size of the female mated. This regulation of spermatophore size could be in response to sperm competition, as has been found in bushcrickets (Wedell, 1997), and the bluehead wrasse (*Thalassoma bifasciatum*) (Shapiro *et al.*, 1994). It is also found in the blue crab (*C. sapidus*), where males produced larger ejaculates when there were other males in attendance and also when there were spermatophores present from previous matings (Jivoff, 1997). It has been shown in spiny lobsters that females that mated with smaller males produced smaller broods than those females that mated with large males (MacDiarmid & Butler, 1999a; 1999b). A further problem is that female spiny lobsters have only a short window of opportunity for mating and should there be a lack of suitable mates the female will resorb her eggs (MacDiarmid & Butler, 1999b). This resorbtion of ovarian tissue has repercussions in the following breeding season where females were seen to have largely atrophied ovaries, and the small amount of normal egg development resulted in very small brood sizes (MacDiarmid & Butler, 1999b).

It can therefore be seen that within fished populations with male-biased fishing mortality, sperm limitation can occur by two different routes. Firstly, the reduction in the numbers of males within a population can result in the remaining males not being able to produce enough sperm to fertilise all the receptive females. The assumption that a reduction in the numbers of males will be compensated for by male polygamy is not sound. Male red king crabs (*P. camtschaticus*) have been shown to be able to mate with several successive females at intervals of about 5 days. However, mating ability in terms of percentage of eggs fertilised and the relative fullness of the brood chamber was seen to decline after the seventh to ninth mating (Powell *et al.*, 1974). Furthermore, the use of sperm from a single breeding season by a female for fertilising multiple broods could also be limiting. It has been shown that the quality, as well as the numbers, of stored sperm could be reduced over time (Paul, 1984). This has been shown to result in poor or no egg production in female *Chionoecetes bairdi* (Paul & Paul, 1992).

Secondly, sperm limitation can occur through a lack of males of a suitable size for mating. If there are specific size constraints on a mating couple, as has been shown for the amphipod *Gammarus duebeni* (Hatcher & Dunn, 1997), the removal of large males from the population could leave larger females without a suitable mate. Likewise, if there is a short window of opportunity for mating, females may not be able to find a suitable mate in a fishery depleted population. Changes in population structure due to fishing will affect the operational sex ratio

of a population and it is likely that this will have effects on the reproductive biology and mating systems of exploited populations.

The aim of this study was to examine the spermathecae of a wide size range of females throughout the breeding season of *N. norvegicus* to determine if there was any evidence of sperm limitation through the presence of unmated females. The male bias in fishing mortality in the fishery for *Nephrops norvegicus* is not as severe as in the Alaskan crab fisheries since females also make up a proportion of the catch; however, sperm limitation could still occur. The increased fishing mortality for males reduces both the number and size of males within a population and this could cause sperm limitation if there is a major reduction in the number of sexually mature males in a given area, resulting in some females going unmated. As most of the mating occurs within a distinct breeding season this effect could be further exacerbated by the length of time required for a male to produce spermatophores. If the mean size of males in a population is also reduced the quantity of sperm delivered to females could be diminished, and this could then be further reduced through individual males carrying out multiple matings. Female *N. norvegicus*, however, are unlikely to be able to use sperm from a single season's mating to fertilise more than one batch of eggs since, for most size classes, moulting occurs annually and any stored sperm would be shed with the old exoskeleton. It is thought that biennial moulting and spawning occurs in some of the larger size classes of females; however, this could be due to the energetic constraints of ovarian development in larger animals, or it is possible that females are producing two broods from one mating (C. Chapman, pers. comm.).

6.2 Methods

Females were collected from the Clyde Sea area south of Little Cumbrae (55.41°N, 4.56°W) using a standard prawn trawl during the breeding seasons in July 2001 and September 2002. The carapace length (CL) was recorded, and moult stage allocated according to the pleopod method described by Aiken (1980). Further to these observations, ovarian maturation was assigned on a 5 point scale (Bailey, 1984) from visual inspection of the tissue, prior to dissection of the spermathecae.

Ovary Stage	Description
0	White
1	Cream
2	Pale green
3	Dark green
4	Dark green swollen

The spermatheca of each female was then dissected to ascertain whether a spermatophore was present indicating that a female had mated. The sperm plug associated with the spermatophore was easily identified by eye as a gelatinous mass stored in the area of the spermatheca furthest from its opening (Farmer, 1974a). The absence of a spermatophore in the spermatheca of a female that had moulted and possessed developing ovaries indicated a female that had not mated that season. A wide size range of females was sampled in order to determine the incidence of sperm limitation. Ovigerous females were also sampled and the carapace length of each recorded.

Bailey (1984) and Tuck *et al.* (2000) have previously investigated size at onset of maturity (SOM), for females from this site south of Little Cumbrae. It has been shown that the estimated size at maturity, based on ovarian maturation studies, increases over the period of the spawning season. Bailey (1984) hypothesised that this was due to smaller females breeding earlier in the season and then disappearing from the catch. Bailey (1984) gave an SOM of 26.1 mm CL in July 1981, and 28.2 and 26.6 mm CL in August 1981 and 1982, respectively. The study by Tuck *et al.* (2000) recorded an SOM of 33.5 mm CL for females sampled in August 1991. Based on the size range of females sampled and the numbers of ovigerous females and females with developing ovaries in the smaller size classes, it was decided that the maturity estimate (26.1 mm CL) calculated by Bailey (1984) was the most appropriate reference point for this study.

6.3 Results

A wide size range of individuals was sampled on each sampling date (Figure 6.1). The samples were analysed on a monthly basis, because of the small individual sample sizes in July and the similarity of the length distributions from both samples in September (Figure 6.1).

Of the females sampled in July (n = 110 see Table 6.1) 91.8% had been inseminated (n = 101). Of the inseminated females 92 had developing ovaries (83.6% of the total) and nine did not show any ovarian development (8.2%). There was a small number of uninseminated females (n = 9, 8.2%) and of these only 2.7% (n = 3) showed any ovarian development. The females sampled in September (n = 322) showed a higher level of insemination (97.5%) and there was a higher proportion of the inseminated individuals that had maturing ovaries (97.2%). Of the lower proportion of uninseminated females only two showed ovarian development. The majority of females were in intermoult when sampled; however, there were two animals in July and one in September that were in premoult. These animals showed no ovarian development and were uninseminated.

The animals collected included individuals that were below the estimated size of maturity for female *N. norvegicus* as estimated by Bailey (1984). In September, five of the females (size range 17.0 – 25.0 mm CL) were in intermoult, but did not show any ovarian development and were not inseminated. These were all below the estimated size at maturity (26.1 mm CL). Those individuals that showed

ovarian development but which had not been inseminated were all greater than or around the estimated size at maturity in July (26, 40 and 52.5 mm CL). This was also the case in September, when the uninseminated females that did not show ovarian maturation were all below the size of maturity (17.0 – 25.0 mm CL) and those with developing ovaries were above it (30.4 and 64.3 mm CL).

The number of inseminated females was also adjusted to include only those individuals that showed ovarian development. The adjusted percentages were then calculated as; July inseminated – 96.8%, and uninseminated – 3.2%; September inseminated 99.4%, and uninseminated 0.6%. Several of the females with developing ovaries sampled in September (n = 7) were shown to have recently moulted through the presence of a 'paper-like' carapace, which had not yet fully hardened following the moult. These individuals were all similar in size (26.6 – 31.0 mm CL; mean 27.87 ± 1.47).

In order to assess the effects of size on ovary maturation the average size of animals in each ovarian development stage was assessed for the September samples (Figure 6.2). This showed that there was a slight increase in the average size of female with increasing ovarian development, this trend was shown to be significant for both July and September samples ($F = 29.10$; $p<0.001$; ANOVA). Post hoc tests (Fishers pairwise comparisons; critical value = 1.97), however, revealed that there were no significant differences between ovary stage 2 and 3 nor stages 2 and 4 in July. In September there were no significant differences in the mean size of females between ovary stages 1 and 2 ($p>0.05$), and between

ovary stages 2 and 3 ($p>0.05$). No stage 1 ovaries were seen in the July sample, although it is probable that this was due to the smaller sample size than to any underlying trend.

From the females sampled in September 2002, a sample of ovigerous females was taken (n=169), all of which were brooding recently extruded dark green eggs. The size range of these females was 22.0 – 43.3 mm CL. The mean size was 29.16 mm CL (Figure 6.3). The frequency distribution was very similar to that seen in the non-ovigerous females sampled.

6.4 Discussion

The low incidence of uninseminated females showing ovarian maturation indicated that there were sufficient males to inseminate females during the breeding season. Of the uninseminated females, all but one were at an advanced stage of ovarian development and all were in intermoult. This strongly suggests that these females had moulted that season but had been unable to find a suitable mate. Although two of the uninseminated females were comparatively large, size did not appear to be a restricting factor on mating, as several females of a similar size or larger had been inseminated. There were very few premoult females in either sample and these animals were all smaller than the size at maturity reported by Bailey (1984) and are therefore unlikely to be reproductively active.

The numbers of unmated mature females with developed ovaries in the samples did not appear to be significant in this population, which indicates that the fishery is not presently affected by recruitment overfishing. At the population level, however, there could be effects of sperm limitation on reproductive output. Large females contribute substantial numbers of eggs and any reduction in their production across a fished population could result in a decrease in subsequent larval settlement and therefore recruitment.

The relationship between ovary stage and the carapace length of female *N. norvegicus* (Figure 6.2) shows that in both months larger females are more likely to have ovaries at an advanced stage of development, indicating that they may have moulted and mated earlier than smaller females. This contradicts data

presented by Bailey (1984) who suggested that the recovery time taken for larger females following breeding would cause them to moult progressively later each year, eventually becoming out of synchrony with the breeding season. These females would then take a gap year from breeding to 'reset the clock'. It should be noted, however, that the larger individuals sampled during this study were not as large as those sampled by Bailey (1984). It is possible that the increase in the size range of females with more developed ovaries is as a result of the maturity ogive, whereby larger females have a higher probability of being mature. It was noted that a small number of individuals that had recently moulted, possessing a paper-like carapace, showed ovarian development up to a stage 2. This suggests that ovarian development may have been initiated prior to the moult or that only a brief recovery time was required following the moult before the initiation of ovarian development. A further possible reason for ovarian development soon after the moult could be that the females were sampled towards the end of the breeding season and would therefore have a shorter time available for egg-laying. The females observed were also not much larger than the size at maturity which suggests again that smaller females may be moulting and mating later in the year than larger females.

Bailey (1984) suggested that large females may develop the ovaries to stage 4 and then resorb them to an intermediate stage of development and therefore do not reproduce annually. It is also possible that in exploited populations these females are in fact resorbing the ovaries because they have not been mated. There is no explanation why either unmated females or females taking a gap year in

reproduction would invest in ovarian development. It may be that there are exogenous factors at work which control the hormonal cycle causing ovarian development and these cannot be countered until some ovarian development has occurred. Unmated female spiny lobsters (*Jasus edwardsii*) have been reported to extrude their eggs in some cases, although the eggs did not appear to attach to the pleopods (MacDairmid *et al.*, 1999). It is therefore possible that those large females with developing ovaries and which were uninseminated had in fact not moulted that year and were taking a gap year. However, both the sampling dates were within the normal breeding season, and it is therefore unlikely that any large females with maturing ovaries could be considered to be out of synchrony with the rest of the breeding population. It is more likely that these females were in fact unmated.

It was not possible to determine the volume of the spermatophores contained within the spermathecae of the females examined. Sperm limitation could, therefore, be occurring in larger females due to an insufficient quantity of sperm being transferred from a small male. In this way sperm limitation could affect *N. norvegicus* fisheries, where a reduction in the mean size of males could reduce the brood sizes of large females. Measuring the area or volume of the spermatophores contained within spermathecae is unlikely to provide an indication of male size if larger males are able to vary spermatophore size relative to female size or the presence of competitors. Wedell *et al.* (2002), in a review of sperm competition and limitation, suggested that nutritional constraints on sperm production may be the cause of ejaculate size modification in males. Further

studies relating breeding behaviour to factors such as ejaculate size and multiple mating would provide an insight into the reproductive dynamics of *N. norvegicus*.

If sperm limitation through reduced numbers of males were to occur in the sampled population it would be expected that a number of individuals from all size classes would have moulted and have maturing ovaries, yet would remain unmated. In such a scenario the sperm limitation may not be evident at the beginning of the breeding season when most males would have a store of spermatophores, as seen in *N. norvegicus* by Welsh (2000). Sperm limitation may therefore become more apparent at the height of the season when more females are moulting. Towards the end of the season when there were fewer females moulting sperm limitation might be expected to be less prevalent. The results of this study indicate that the occurrence of unmated females was slightly higher in the middle of the breeding season (from the July sample) than at the end of the breeding season in September; however, this difference was slight.

If sperm limitation was caused by the reduction in the size of males in the population it may not be as apparent in terms of insemination rates. Samples from July (1998 – 2000) have shown that the mean size of females was greater than that of males (Stentiford, 2000). It is probable that there are size limitations on the mating success of a disproportionately size-matched pair and under such circumstances the largest females may go unmated due to a lack of suitable mates. A further impact of a reduction in the size of males would be that females might not receive sufficient quantities of sperm from a small male to fertilise all the eggs

produced. This could result in decreased individual fecundity and a reduced reproductive output from the population. Males could be expected to increase the size of the spermatophore delivered to a large, and therefore potentially highly fecund, female. Small males, however, may not have the ability to regulate spermatophore size to this degree, or indeed produce enough to prevent sperm limitation in a large female.

Sperm limitation through the reduction in size of males could be occurring in the studied population of *N. norvegicus*. However, the effects could be reduced, through multiple mating of females (M. Hughes pers. comm. and Salerno, 2000) possibly by small yet physiologically mature males. Sperm limitation is more likely to affect the larger, more fecund females in the population, although the results here show that the numbers of uninseminated females is low, large females were shown to be affected. Continued exploitation of *N. norvegicus* populations could result in increasing sperm limitation in the larger size classes of females.

The ability of males to regulate ejaculate size could be of value in increasing the number of potential copulations by reducing the volume, and by increasing ejaculate size in response to sperm competition, as discussed by Wedell *et al.* (2002). A reduction in the numbers of sperm allocated may allow the male to fertilise a larger number of females but it is unlikely to be in the best interests of the females who may suffer reduced fertilisation levels due to sperm limitation (Marconato *et al.*, 1995). Similarly, increasing ejaculate size will limit the number of females that a male will be able to inseminate. If males are scarce,

however, sperm competition through multiple inseminations would be less likely and therefore sperm limitation through a reduction in the number of females a male can inseminate is improbable.

Although the level of sperm limitation the studied population was not significant there are other implications of the removal of male *N. norvegicus*. It is possible that the fishery may also be causing selective effects on the population as suggested by Jamieson *et al.* (1991) in the case of the Dungeness crab. For example, it is possible that reducing the size of the males that are mating in a population could be selecting for smaller males and thus any effects of sperm limitation would be compounded. Such compensatory and density dependent effects have been reported (Chubb, 1994).

6.5 Conclusions

- There did not appear to be significant numbers of uninseminated reproductively active females although they were recorded in small numbers during the breeding season.
- There appeared to be a relationship between female size and ovarian development, which could indicate that larger females were more likely to have well developed ovaries. It is also possible, however, that this is related to the maturity ogive and the restricted size range of females.
- There is evidence to suggest that some females begin ovarian maturation prior to the moult and that unmated females continue ovarian development in the absence of a spermatophore.

Date	July 2000	September 2002
Total Sampled	110	322
Inseminated	101 (91.8%)	314 (97.5%)
- Developing ovaries	92 (83.6%)	313 (97.2%)
- Undeveloped ovaries	9 (8.2%)	1 (0.3%)
Uninseminated	9 (8.2%)	8 (2.5%)
- Developing ovaries	3 (2.7%)	2 (0.6%)
- Undeveloped ovaries	6 (5.5%)	6 (1.6%)

Table 6.1: The numbers of inseminated and uninseminated female *Nephrops norvegicus* from each sampling date. The values in parenthesis represent the percentage of the total sample. These values do not include any individuals that were premoult (two from the month of July and one from the September sample).

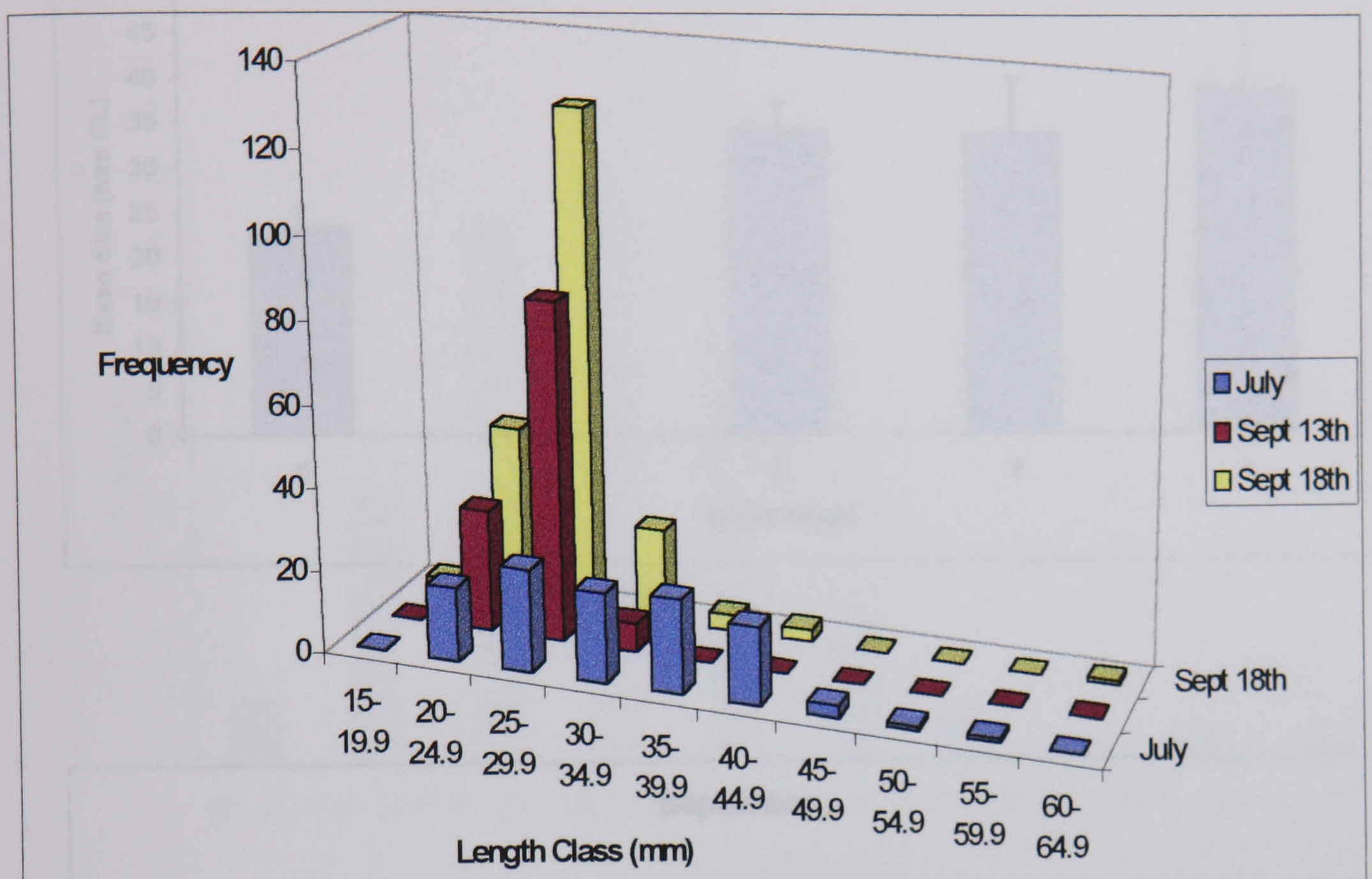
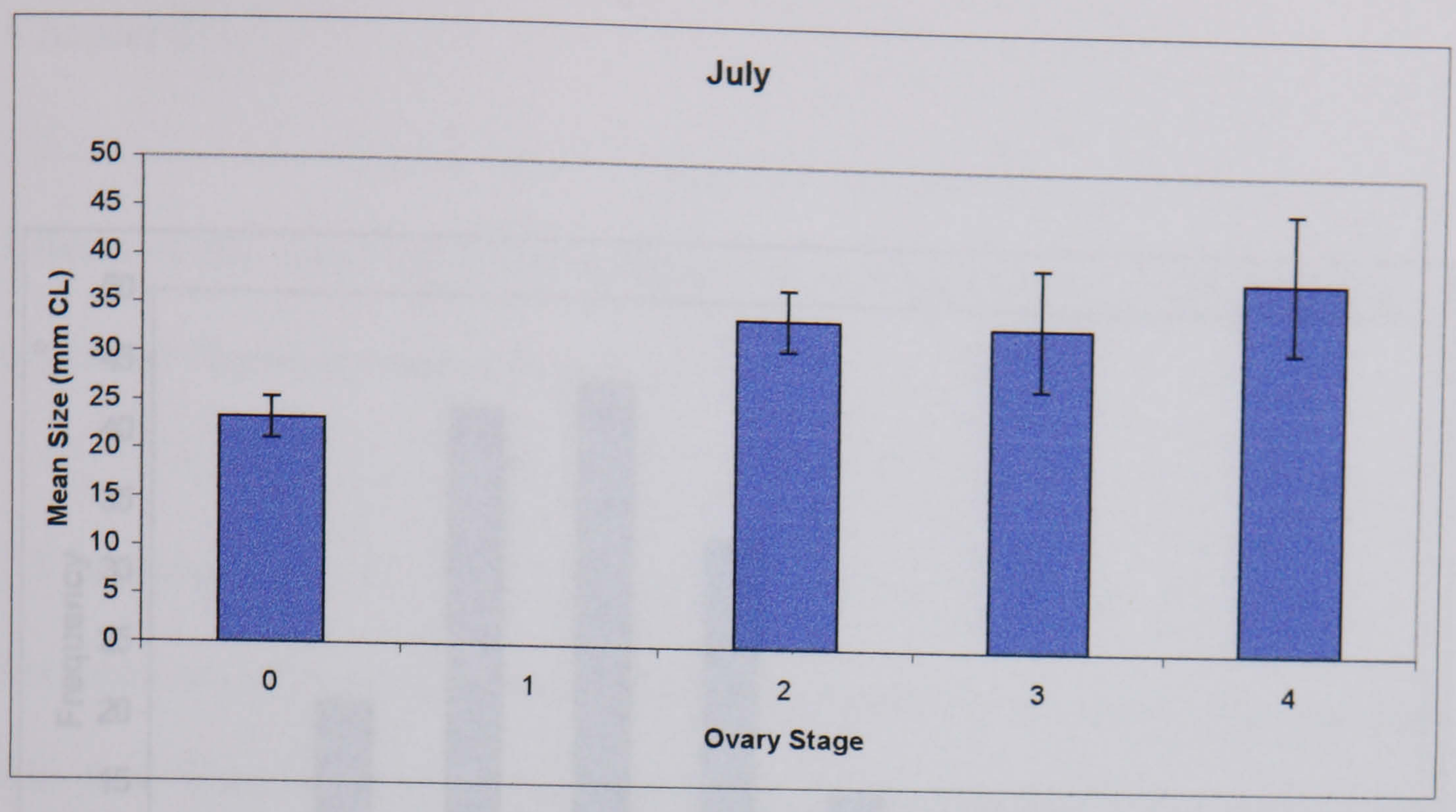


Figure 6.1: Length frequencies (mm carapace length) of female *Nephrops norvegicus* sampled from the Clyde Sea area during the breeding season of 2001. These values do not include uninseminated premoult females.



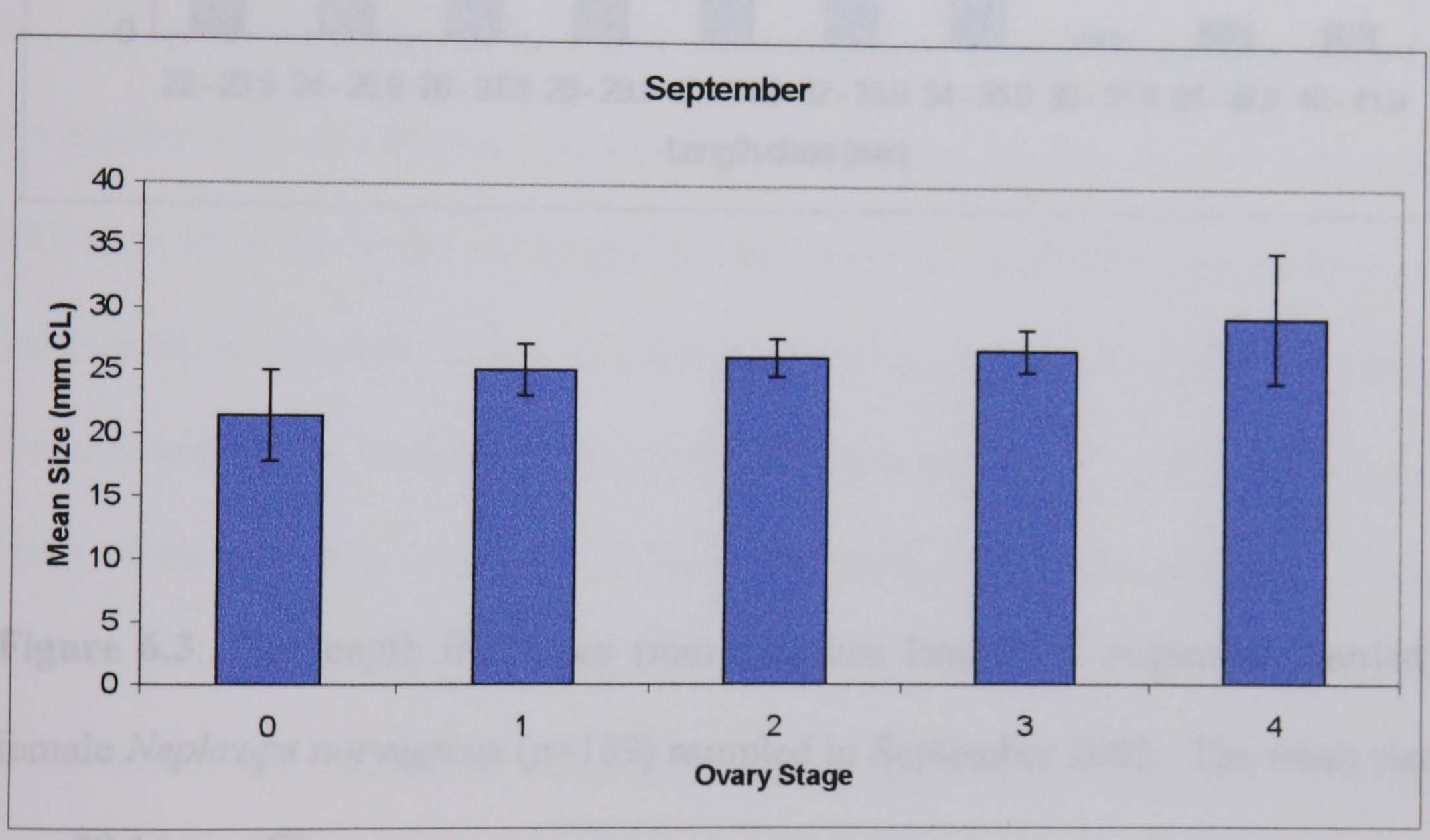


Figure 6.2: The mean (±SD) carapace length of female *Nephrops norvegicus* at each ovary stage as sampled in July and September 2002.

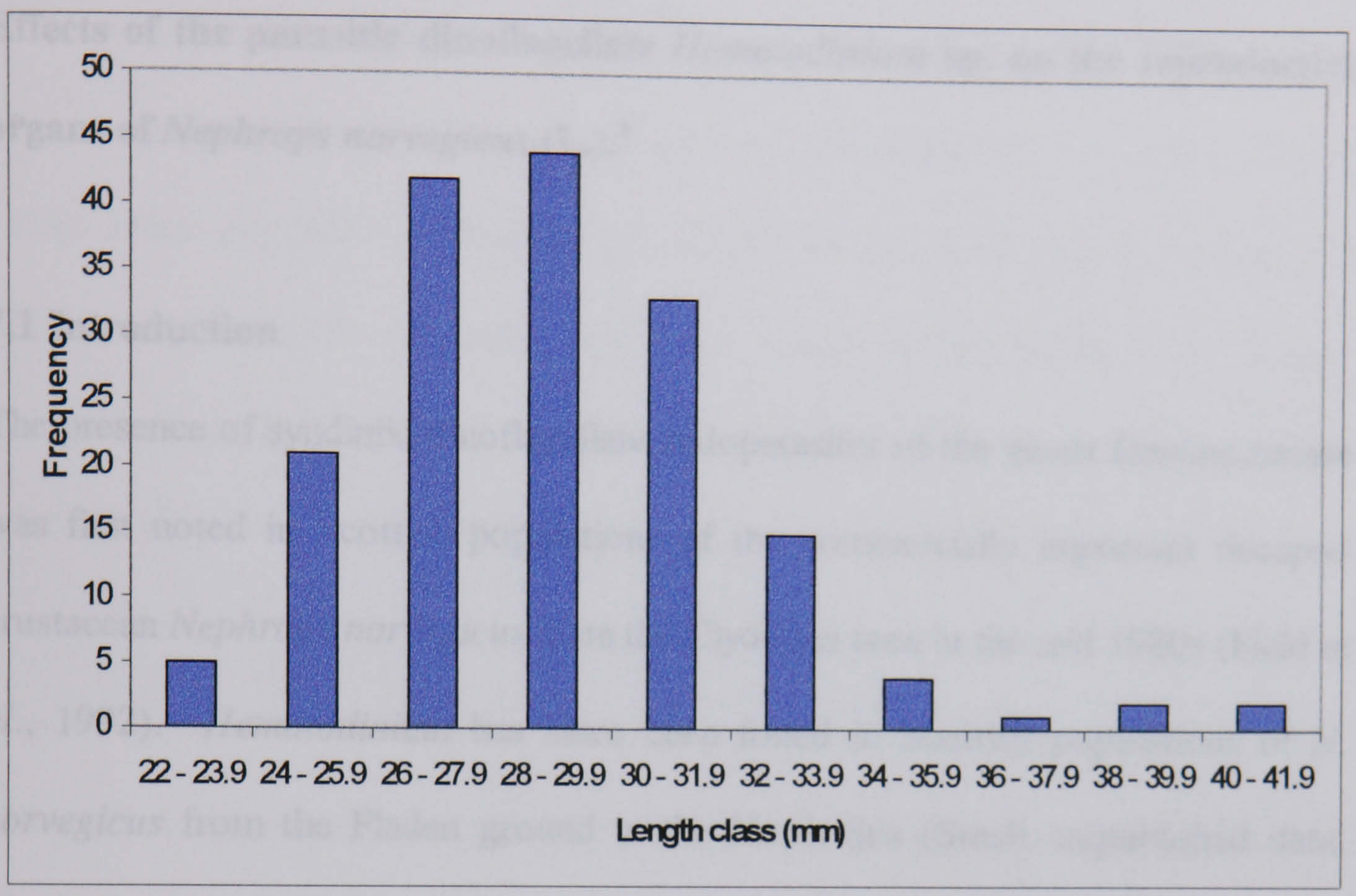


Figure 6.3: The length frequency (mm carapace length) of ovigerous (berried) female *Nephrops norvegicus* (n=169) sampled in September 2002. The mean size was 29.16 mm CL.

Chapter 7

Effects of the parasitic dinoflagellate *Hematodinium* sp. on the reproductive organs of *Nephrops norvegicus* (L.).[1]

7.1 Introduction

The presence of syndiniid dinoflagellate endoparasites of the genus *Hematodinium* was first noted in Scottish populations of the commercially important decapod crustacean *Nephrops norvegicus* from the Clyde sea area in the mid 1980s (Field *et al.*, 1992). *Hematodinium* has since been found in Scottish populations of *N. norvegicus* from the Fladen ground in the North Sea (Small, unpublished data, 2001) and from the North and South Minches (Field *et al.*, 1998) and elsewhere on the Scottish west coast. It has also been recorded in the Irish Sea (Briggs, 1996) and in Scandinavian waters (Anon, 2000). Similar dinoflagellate infections of the genus *Hematodinium* have been reported in other crustacean fisheries. The Alaskan fishery for tanner crab, *Chionecetes bairdi*, has been affected by 'bitter crab disease' (e.g. Meyers *et al.*, 1987; Love *et al.*, 1993), which has, in some areas, been reported by fishermen to reach a prevalence of almost 100%. Bitter crab disease has also been described in the *C. opilio* (Taylor & Khan, 1995) fishery. *Hematodinium perezi* has been found in the blue crab (*Callinectes sapidus*) fishery (e.g. Newman & Johnson, 1975, Messick, 1994; Messick & Shields, 2000) which is commercially important along the Atlantic and Gulf coasts

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[1] This work was carried out in collaboration with Hamish Small, who carried out the molecular and immunological analyses.

of the United States of America. *Hematodinium australis* has been found in the sand crab (*Portunus pelagicus*) an important Australian fishery (Shields, 1992; Hudson & Shields, 1994). In European waters, a dinoflagellate of the genus *Hematodinium* is thought to be responsible for the decline of populations of the velvet swimming crab, *Necora puber*, around the coast of France (Wilhelm & Mailhe, 1996). More recently, pink crab disease in the edible crab, *Cancer pagurus*, from the English Channel has been attributed to a *Hematodinium*-like infection (Stentiford *et al.*, 2002).

The life cycle of *Hematodinium* is complex and not completely understood. The cell types which were first reported by Field *et al.* (1992) in haemolymph from *N. norvegicus* were mostly non-motile rounded forms as described by Appleton & Vickerman (1998) who suggested that they represented the sporoblasts that are characteristic of late stage infection. These cells can appear in large numbers and can be uni- or multi-nucleate. *In vitro* cultivation of *Hematodinium* from *N. norvegicus* (Appleton & Vickerman, 1998) has shown that there is a complex life cycle about which nothing is known of the early stages *in vivo*.

Appleton & Vickerman (1998) suggested that infection is likely to occur through ingestion of a dinospore stage during suspension feeding (Loo *et al.*, 1993) or by cannibalism of deceased, infected individuals (e.g. Shields, 1994; Meyers *et al.*, 1990). It is also possible that there may be an intermediate host species, for example, gammaridean amphipods (Messick & Shields, 2000). Infection may occur through one of two types of motile dinospores that have been seen *in vitro*

within cultures of *Hematodinium* taken from *N. norvegicus* (Appleton & Vickerman, 1998), and *in vivo* from *C. bairdi* (Meyers *et al.*, 1987). These dinospores have been shown to be viable in seawater for up to 2 to 3 months (Meyers *et al.*, 1987). A further explanation is that dinospores may be able to infect moulting individuals (Meyers *et al.*, 1987; Eaton, *et al.*, 1991; Field *et al.*, 1992; 1998). Wilhelm & Mialhe (1996), however, found higher levels of infection by a *Hematodinium*-like parasite in soft-shelled *Necora puber* than in those that had not moulted. How the dinospores are released into the environment is as yet unclear although Love *et al.* (1996) recorded motile dinospores exiting through the lamellae of the gills of infected *C. bairdi*. This process has also been described in *N. norvegicus* (Appleton *et al.*, 1997). It has also been suggested that the death of the host may liberate the parasites within.

The symptoms of heavy infection by *Hematodinium*, include moribund animals with dull orange coloration, milky white body fluids, and 'watery muscles' (as described by Field *et al.*, 1992). These symptoms had previously been attributed to moulting animals. Internal effects of parasitic infection include acute enlargement of the haemal spaces and the haemocoel through the presence of *Hematodinium* cells. This is combined with a reduction in the number of haemocytes (Field & Appleton, 1995; Love *et al.*, 1996) and low haemolymph pressure.

There are a number of effects of *Hematodinium* infection on the haemolymph of infected animals including increased osmolarity (Love *et al.*, 1996), lactate concentration, and oxygen consumption (Taylor *et al.*, 1996). There is also a

reduction in PO_2, pH, and glycogen content (Taylor *et al.*, 1996), and lowered haemocyanin concentrations (Field *et al.*, 1992; Love *et al.*, 1996; Taylor *et al.*, 1996). The reduction in haemocyanin concentration is the most probable cause of the decreased copper concentrations in the haemolymph of infected animals, causing a reduced oxygen carrying capacity (Field *et al.*, 1992; Taylor *et al.*, 1996). This is combined with elevated oxygen consumption of the whole animal, which occurs with increased infection (Taylor *et al.*, 1996). The problem is further exacerbated by the presence of parasite cells in the branchial sinuses of the gill filaments (Field *et al.*, 1992; Field & Appleton, 1995). This has also been seen in *Chionecetes bairdi* (Meyers *et al.*, 1897) and in *C. opilio* (Taylor & Khan, 1995), and is likely to reduce the efficiency of gas exchange between the host and its environment. The respiratory burden of the parasite on the host can be severe. Taylor *et al.* (1996) reported an increase in L-lactate in the haemolymph of infected animals indicating that some of the affected individual's tissues have resorted to anaerobic respiration in response to internal hypoxia caused by the parasites.

Infection by *Hematodinium* sp. is, in most cases, likely to prove fatal to the host animal (Newman & Johnson, 1975; Meyers *et al.*, 1987; Field *et al.*, 1992; Love *et al.*, 1996). The probable causes of death are organ and/or respiratory dysfunction (Meyers *et al.*, 1987; Field *et al.*, 1992), disruption of digestion (Field *et al.*, 1992), and parasite toxins (Meyers *et al.*, 1987). Other possibilities could be related to tissue hypoxia and the resulting acidosis (Taylor *et al.*, 1996). A

combination of these factors may be the ultimate cause of death; however, the exact mechanism remains unknown.

Infection of *N. norvegicus* with *Hematodinium* has implications for the behaviour of affected individuals. The parasite induces lethargy, which increases with the severity of infection (Field *et al.*, 1992). Progressive lethargy has also been reported in *C. bairdi* (Meyers *et al.*, 1987). Animals have also been shown to spend significantly more time on the surface of the sediment rather than in the burrow (Stentiford *et al.*, 2001a), and are therefore more susceptible to both natural and fishing mortality. This vulnerability is increased by the decrease in efficiency of the tail flip swimming escape response which is severely compromised by the effects of *Hematodinium* on the musculature of the tail (Stentiford *et al.*, 2000a; Stentiford *et al.*, 2000b). It has also been shown that there are sex-related differences in the susceptibility of infected *N. norvegicus* to capture by trawling. Field *et al.* (1998) showed that the time of trawling had no effect on the presence of infected females within the catch; however, male *N. norvegicus* were most prevalent in catches taken during the middle of the night. This indicated that infected male *N. norvegicus* spend more time out of the burrow during the hours of darkness than uninfected males. This was confirmed by Stentiford *et al.* (2001a).

The prevalence of this parasite within the west-coast populations of *N. norvegicus* is very seasonal and by far the highest levels of infection are seen in the Clyde Sea area (Field *et al.*, 1998). Seasonality appears to be a feature of *Hematodinium* infections (e.g. Love et al., 1993; Wilhelm & Mialhe, 1996; Briggs & McAliskey

2002). In the Clyde Sea area the prevalence is lowest during the months of July to December but increases to peak during May (Stentiford *et al.*, 2001b). During the infection period Stentiford *et al.* (2001b) recorded prevalence at 20 – 25% in the population south of Little Cumbrae. Field *et al.* (1992), however, stated that as many as 70% of the population could be infected. At the Little Cumbrae site, Field *et al.* (1998) found that *Hematodinium* was most prevalent in medium sized animals, males of 24 – 48 mm carapace length (CL) and females of 24 – 44 mm CL. Stentiford *et al.* (2001b), however, suggest that the infection is more prevalent in smaller animals, for females a mean carapace length of 28.1 (±0.67) mm, and for males 30.9 (± 0.50) mm. The difference in the findings of these authors probably reflects an increase in the numbers of smaller individuals in the Clyde fishery during this time (Anon, 2001). Interestingly, *Hematodinium* infection is more common in females than in males (Field *et al.*, 1998; Stentiford *et al.*, 2001b). As yet there is no explanation for why this is the case.

Some investigations have been carried out into environmental influences on *Hematodinium* infection. Briggs & McAliskey (2002) found that there was no correlation of infection in *N. norvegicus* from the Irish Sea with changes in either depth or temperature. They did, however, report a positive correlation between average infection levels and salinity, although variations in salinity were small during the period of investigation. Newman & Johnson (1975) have also noted the effect of salinity on infection. They examined blue crabs (*C. sapidus*) from salinities ranging from 0 – 36‰, but found infection only in animals living at a salinity 11‰ or above, an effect which was also confirmed by Messick *et al.*

(1999). Messick & Shields (2000) also found that infections of *C. sapidus* were significantly associated with salinity over a wide range. Messick *et al.* (1999) have also related infection intensity to temperature. They found that crabs held at or below 9°C showed a decrease in infection intensity while those in ambient seawater and higher temperatures presented an increase in intensity. Temperature may be related to sporulation events as suggested by Meyers *et al.* (1987).

The implications of *Hematodinium* infection for the fishery can be severe. In the case of the Alaskan tanner crab, *C. bairdi*, 'bitter crab' disease led to economic losses of around $150000 per annum, since 4% of the landings were unmarketable through poor flesh quality (Love *et al.*, 1996). The suggested losses of pre-recruit and legal size crabs were also high (Taylor & Khan, 1995). The fishery for *Necora puber* off Southern Brittany declined by 96% between 1984 and 1988 and was accompanied by a decrease of only 60% in fishing effort. This discrepancy has been attributed to a *Hematodinium* infection (Wilhelm & Mialhe, 1996).

As previously stated, the peak prevalence of *Hematodinium* infection of *N. norvegicus* can be as high as 70%. As smaller animals are more likely to be infected, this could have serious implications for the processing industry, which uses mostly tailed smaller animals. The prevalence of infection within populations also has a more serious effect on the fishery, since the majority of infected animals are unlikely to survive. During the peak of infection a large part of the breeding population could be lost, which could result in a reduction in reproductive potential.

Little is known about the effects of *Hematodinium* on reproduction. Therefore, the aim of this study was to investigate the effects of *Hematodinium* infection on the gonads of *N. norvegicus* using different techniques. Quantification of the parasite cells within the tissue was carried out to gauge the level of infection in each individual. This was combined with qualitative analysis using immunological and molecular techniques. The data obtained from these analyses were used to predict the possible outcome of infection on reproduction.

7.2 Methods

Nephrops norvegicus were collected using a standard otter trawl, from south of Little Cumbrae in the Firth of Clyde (55.41°N, 4.56°W) during the peak of the *Hematodinium* infection season in March 2002. Infected animals were selected visually from the catch using a body colour field diagnosis (Field *et al.*, 1992) together with animals, which showed no outward signs of infection. Although this method is insensitive to early infections (Stentiford *et al.*, 2001c) it was used rather than the pleopod method of diagnosis (Field *et al.*, 1992; Field & Appleton, 1995), a five point scale based on aggregation of parasites under the cuticle of the pleopod. Field & Appleton (1995) noted that systemic infection is more severe than would be indicated by haemolymph infection alone, which in turn renders the pleopod method of infection staging somewhat unreliable; hence the simpler method of body colour diagnosis was used in this study. Both methods, however, occasionally fail to identify low levels of infection.

Animals were transported live to the laboratory where they were kept in holding tanks overnight (10°C, salinity 33‰) prior to dissection. The carapace length (CL) of each animal was recorded and ovarian maturation assigned on a 5 point scale (Bailey, 1984) from visual inspection of the tissue. Haemolymph samples were taken from the base of the fifth pereiopod using a 1ml syringe with a 25-gauge needle, and samples of testes and ovary were dissected from male (n = 9) and female (n = 11) *N. norvegicus*, for histological, enzyme-linked immunosorbant assay (ELISA), and polymerase chain reaction (PCR) diagnostic analyses. Of the immunological methods available for diagnosis of *Hematodinium* ELISA was

selected because it is more sensitive than western blotting, with a detection limit of 5×10^4 parasites ml^{-1} haemolymph, and is much less complex than the indirect fluorescent antibody technique (Small *et al.*, 2002).

Testes were fixed in 10% formalsaline prior to histological analyses. The ovarian tissue was fixed in Bouin's for no longer than 18 hours prior to sectioning. Tissues were prepared through a standard alcohol dehydration routine and embedded in paraffin wax. Sections of 7μm thickness were stained in Haematoxylin and Eosin (H&E) prior to examination. For each ovary, 9 images were captured, at random, using a digital camera attached to a compound microscope and using the PCImage programme. The area of the frame was measured using a computer package (Scion Image) and the number of *Hematodinium* cells in each picture was counted. The average count of parasite cells was used as an index of infection in the tissue of each animal sampled. Counts were not expressed as cells per unit area, however, due to their patchy distribution within the haemal spaces of the tissue. The method of counting *Hematodinium* cells in the tissues of infected animals was used as opposed to counting parasites in the blood to avoid the problems caused by blood coagulation and conglomeration of parasite cells.

Haemolymph samples taken for ELISA analysis were stored immediately at -20°C and thawed only once; the experimental temperature thereafter was 22°C. After thawing, the haemolymph was vortexed prior to the removal of an aliquot of 15μl from each sample. This was then diluted with 285μl distilled water and 100μl transferred to a 96-well microtitre plate (Immulon 4 HBX). Each plate was

incubated for 30 minutes prior to 4 washes in PBS (pH 7.2) with 0.05% v/v Tween 20. The plates were incubated for a further 30 minutes following the addition of 100µl/well of rabbit anti-*Hematodinium* antiserum (1/2000 dilution) (see Field & Appleton, 1996 for antiserum production). Prior to a third 30 minute incubation, each plate was washed 4 times and 100µl/well of goat anti-rabbit horseradish peroxidase conjugated antibody (1/500 dilution) (Diagnostics Scotland). Each plate was then washed 4 times and 100µl TMB substrate (3, 3', 5, 5'-tetramethyl benzidine) (Dynex Technologies) was added to each well for colour development following 20 minutes incubation in darkness. The optical density of the wells was measured using a microplate reader (Titretech Multiscan) at 690nm. For each sample the ELISA was duplicated.

DNA extraction and purification was carried out on tissue samples of both ovary and testis. The tissue was homogenised in 250µl of extraction buffer (50mM Tris, 5mM EDTA, 100mM NaCl, pH 8), 100 µm of 10% SDS and 10µl Proteinase-K (10µl/ml). The samples were then incubated at 57°C for 18 – 24 hours. For haemolymph samples the procedure was the same, only 100µl of haemolymph was used. DNA was purified using a standard phenol/chloroform extraction, ethanol precipitated and re-suspended in sterile de-ionised water. DNA concentration and purity were estimated by measuring the A260 – 280 optical density ratio using a spectrophotometer and adjusted accordingly.

The PCR was performed using the forward and reverse oligonucleotide primers NnHem For (5'-CAGTTTCTGGAAGTGGCAGCTG-3') and NnHem Rev (5'-GAAGGGAAGGGGAGAAGAAGC-3'). The primer sequences were designed by Small *et al.* (unpublished) and were shown to be *Hematodinium* sp. specific through the creation of a 380 bp amplification product. PCR reactions were performed in 20µl total reaction volume by adding 2µl of 10 x reaction buffer (final concentration 10mM Tris-HCL, pH 9, 50mM KCL, 0.1%Triton x-100), 1.2µl $MgCl_2$ (final concentration 1.5mM), 1µl dNTP mix (final concentration 100µm), 1µl for each of the forward and reverse primers (final concentration 0.5 pmol μl^{-1}), 50-100ng template DNA, 1 unit of Taq polymerase (Promega), and sterile de-ionised water to a final volume of 20µl. Reactions were overlaid with 10µl mineral oil. Thermal cycling conditions were as follows: denaturing at 94°C for 1 minute, primer annealing at 52°C for 1 minute, and chain extension at 70°C for 3 minutes. This was carried out for 35 cycles with a final cycle incorporating a 7 minute extension. Amplification products were run on a 1.5% [w/v] agarose gel, stained with ethidium bromide and viewed under a UV light source. Following this, a molecular weight standard (100 bp: adder, Gibco BRL) was used to estimate the size of the products.

7.3 Results

Individuals from the catch diagnosed using the body colour index as infected showed clear symptoms of the presence of *Hematodinium.* The body cavity was filled with a milky fluid (a suspension of parasite cells), as were tissues such as the heart. The tail muscle was opaque and the tail flip response in such individuals was weak or not present at all. Of those females that were sampled, those presenting external symptoms of infection did not show any ovarian development (Table 7.1).

The uninfected females of similar size all showed ovarian development and sections of ovarian tissue showed developing vitellogenic oocytes (Figure 7.1). In infected females, parasite cells could clearly be seen filling the haemal spaces of the ovaries (Figure 7.2). Developing vitellogenic oocytes can be seen; however, these and previtellogenic oocytes are degraded and showing signs of possible pressure necrosis. Figure 7.3a shows the effects of massive infection on the oocytes in greater detail; condensed yolk bodies and necrotic membrane fragments can be seen in the degenerating oocytes. In Figure 7.3b the effects of infection on previtellogenic oocytes and oogonal germ cells are shown. It appears that the infection is causing apoptosis in the germ cells as well as degradation of the oocytes. The presence of developing oocytes indicates that ovarian maturation had begun prior to patent infection. The degradation of the developing oocytes is severe and in some cases the necrotic oocytes were broken down and their contents released into haemal spaces as can be seen in Figure 7.4 where vitellogenin is visible in the haemolymph matrix. An interesting factor in the

pathology of the infections seen was the presence of multinucleate filamentous plasmodial cells attached to basal laminae in the ovaries (Figure 7.5).

The pathology of *Hematodinium* in the testes of *N. norvegicus* was somewhat different from that described in the ovaries. Even in an animal with a patent infection, the parasites do not cause degradation of the testes as was seen in infected ovary. The collecting tubules remain intact and sperm can still be seen in the lumen (Figure 7.6). Parasitic infiltration of the haemal spaces does not appear to affect the functioning of the testes. One aspect of *Hematodinium* infection in the testes, similar to that seen in the ovary, was the presence of filamentous multinucleate plasmodial cells attached to basal laminae of the tissue (Figure 7.6). In the testes, parasite cells were seen attached to both the external and internal surfaces of the collecting tubules (Figure 7.7). The testicular basal laminae of an uninfected male are shown in Figure 7.8. It can also be seen that there are glycogen containing reserve inclusion cells associated with the membranes in the testes.

Females identified as infected had mean parasite counts ranging from 87.91 (± 57.5 SD) cells to 1710.8 (± 396 SD) cells in the ovarian tissue. There is, however, a high degree of variability of cell counts for each infected animal (Figure 7.9). There was an exceptionally high cell count for female 10 (Table 7.1, Figure 7.9). ELISA analysis of haemolymph samples from each of the females produced positive results for all those animals identified by the body colour as being infected (Table 7.1). The results, however, did not mirror those from the parasite cell

counts from the tissue. The individuals which showed high cell counts registered low levels of infection in the ELISA, whereas animals with low cell counts showed heavy infection in the haemolymph. Unfortunately, blood parasite cell counts were not possible due to agglutination of parasite cells and clotting of the haemolymph.

Tissue parasite cell counts from the testes ranged from 7 (± 7.7) to 362.6 (± 143.3) and again there was a high degree of variability seen in the counts (Figure 7.10). The ELISA analysis of testicular tissue gave a positive result for all animals that were visually assessed as infected (Table 7.2). The levels of infection in the haemolymph recorded using this technique did not match the levels of infection in the tissue as measured by the parasite cell counts. The individuals that produced the highest parasite cell counts (animals 1, 3, & 10; see Table 7.2) scored a low infection from the haemolymph ELISA and those with a heavy infection in the ELISA (animals 4 & 9, see; Table 7.2) had an intermediate cell count in the testicular tissue. The PCR analyses confirmed the presence of *Hematodinium* in the infected individuals although it was not possible to quantify this result.

7.4 Discussion

In this study the method of counting parasite cells in the tissue of infected *N. norvegicus* proved more successful than counts made on infected blood. The presence of a greater number of cells in the ovary of animal 10 could be a relative difference due to changes in the parasite life cycle, or it could be an absolute change in the prevalence of the parasite in the tissue. Meyers *et al.* (1987) reported a change in the size of parasite cells seen in *C. bairdi*. In crabs that had died, the cell sizes were smaller than the previously observed vegetative cells seen in haemolymph smears. This could indicate that during late infection or around the sporulation event there is a reduction in parasite cell size. In *N. norvegicus*, the nuclei of the parasite cells in female 10 did not, however, show the same lobular structure as those reported by Meyers *et al.* (1987), nor were any dinospore stages seen. The high degree of variability seen in the numbers of parasites recorded from tissue cell counts is probably due to the discrete distribution of the parasite cells within the haemal spaces.

It is apparent from the data collected that *Hematodinium* infection in females prevents the development of maturation of the ovary. It is clear that ovarian maturation has begun, through the presence of vitellogenin in the ovaries (Figure 7.4); however, the continuation of maturation is prevented through the breakdown of ovarian tissue. It remains unclear, however, if the breakdown of the ovaries is caused by active resorption of tissue by the female or by tissue breakdown through pressure necrosis and apoptosis due to infection. The presence of green pigmentation in the hepatopancreas of infected females could indicate recent

resorption of ovarian products. This coloration has, however, been related to the disruption of haemocyanin by the parasite resulting in increased concentrations of copper in the hepatopancreas (Field *et al.*, 1992). It is also unlikely that females sampled in March would have well-developed ovaries, which are dark green in colour. The ovaries of infected individuals appeared to be undeveloped; resorbed ovaries, as sampled from natural populations, are distinguished by being dark and often with a mosaic of white and green oocytes (Figueiredo, 1982). This stage represents resorption just prior to spawning, however, and there does not appear to be any evidence for, or descriptions of, resorption in ovaries at an earlier stage of development.

Briggs & McAliskey (2002) suggested that the lack of ovarian development in smaller animals indicates a greater susceptibility to *Hematodinium* infection in immature animals or that mature animals may have some immune response that provides a degree of protection. This is unlikely to be the case, however, since the smaller infected individuals in this study were larger than the estimated size of maturity for this area (Bailey, 1984). As such they would be reproducing annually and should have had developing ovaries. Field *et al.* (1998) reported that in males the prevalence of infection was very similar for all sizes; however, in females the peak in prevalence was in medium sized animals, between 28 and 38 mm CL. The presence of infected ovigerous females has been recorded in the Clyde Sea area (H. Small, pers obs), although these individuals are comparatively rare they clearly indicate that mature females are susceptible to infection. There also does not

appear to be any evidence to suggest that maturity or body size will affect the immune responses of crustaceans.

Resorption of the ovaries occurs naturally within populations and has been shown to be inversely related to animal size in Portuguese populations (Figueiredo, 1982). It is possible that the high rates of resorption reported in smaller size classes by Figueiredo (1982) are due to small females undergoing ovarian development but undertaking resorption as they were not fully mature. It is possible that resorption in larger females could be associated with the occurrence of biennial spawning (Bailey, 1984; Sterk & Redant, 1989). The infected animals in the current study were of an intermediate size and would be expected to moult and mate annually. One previously reported cause of resorption of ovaries in crustaceans is pollution. Atresia of the ovaries of the crayfish *Procambarus clarkii* has been shown to occur in relation to exposure to some heavy metals and aromatic hydrocarbons (Sarojini *et al.*, 1995; Reddy *et al.*, 1997). It is unlikely that *N. norvegicus* from the areas sampled would be exposed to levels of pollutant high enough to cause resorption of the ovary. Canli & Furness (1993) found no evidence that ambient levels of heavy metals in the Clyde Sea exceeded levels that could be regulated by *N. norvegicus* and therefore suggested that they were unlikely to present a toxic hazard to the population.

There are many recorded occurrences of ovarian atresia in response to poor nutritional status, for example in Atlantic cod, *Gadus morhua* (Rideout *et al.*, 2000), and through enforced reduced feeding as carried out with Northern

anchovy, *Engraulis mordax* (Hunter & Macewicz, 1985) and a bug *Leptocoris coimbatorensis* (Hemiptera: Coreidae) (Kaur *et al.*, 1987). The lack of ovarian development seen in infected animals could be a result of the nutritional constraints placed upon the host by the parasite burden. It has been shown that *Hematodinium* infections in *N. norvegicus* result in elevated plasma concentrations of crustacean hyperglycaemic hormone (CHH) (Stentiford *et al.*, 2001c). CHH is part of a negative feedback loop, which results in the liberation of glucose from tissue glycogen reserves. Stentiford *et al.* (2001c) found that, in heavily infected individuals, although plasma CHH concentrations were elevated, glucose concentrations remained low. This indicates disruption of the feedback loop as the parasite cells consume the liberated glucose, ensuring the further release of CHH. Hunter & Macewicz (1985) state that atresia of the ovaries may also be related to the state of food reserves present. *Hematodinium* infection has been shown not only to reduce plasma glycogen but also to attack the spongy connective tissues of the host (Love *et al.*, 1996) which are sites of glycogen storage. The physiological impacts of *Hematodinium* infection would appear sufficient to cause atresia in the ovaries of infected *N. norvegicus*.

The effects of infection by *Hematodinium* on the male reproductive system were not as severe as that seen in females and the tissue appeared to continue to function. No parasite cells were seen to have infiltrated the lumen of any tubules within the testes. This is contrary to what was observed in the ovaries where tissue breakdown was seen. This could be due to the lack of a seasonal cycle in the testes (Bailey, 1984; Chapter 3). In the females, there is a clear mobilisation of

resources contributing to the annual development of the ovaries. The relative weight of the ovary increases by a factor of three during this time (Tuck *et al.*, 1997). These physiological changes are clearly of benefit to an endoparasite, and as there are no such changes occurring in the physiology of males, there is likely to be a difference in the patency of the infection. The greater prevalence of infection in females may well be due to the greater availability of nutrients to the parasite during ovarian development.

Field & Appleton (1995) reported that there was severe disruption of many of the tissues in infected individuals including the hepatopancreas and midgut. Tissues such as the antennal gland and heart, however, were largely unaffected in all but the most severely infected individuals. It could be hypothesised, from Field & Appleton (1995) and the data presented in this study, that the degree of tissue breakdown might be related to its nutritional value to the parasite.

The presence of glycogen-storing reserve inclusion (RI) cells associated with membrane systems of the connective tissue in the testes (Figure 7.8) could indicate that glycogen from these cells is being utilised by the parasite for growth and reproduction. It could be hypothesised that the parasite cells associated with the basal laminae are targeting RI cells during the initial stages of infection, as a potential energy source on development of patent infection. The absence of RI cells in animals with patent *Hematodinium* infection has previously been reported in *N. norvegicus* (Stentiford *et al.*, 1999, 2000b, 2001c), in *Cancer pagurus* (Stentiford *et al.*, 2002) and in the blue crab, *Callenecetes sapidus* (Whittingdon *et*

al., 1997). Claw musculature is often a site of heavy infection by *Hematodinium* and it contains many RI cells.

The association of filamentous multinucleate cells with the basal lamina of the tissues observed (Figures 7.5, 7.6, & 7.7) has been seen previously in *N. norvegicus*. Field (1992) reported that vermiform cells were attached to the tissues of the heart, antennal gland, midgut wall and haemopoietic tissues. These were not specified as filamentous but on re-examination of the histological plates it can be seen that filamentous forms are present in the heart and antennal gland. They have also been observed attached in the lumen of the midgut (Field *et al.*, 1992) and hepatopancreas (Field & Appleton, 1995). These filamentous forms were only seen on the external surfaces of the tissues, however, unlike those seen in the testes in this study, where parasites were associated with both sides of the basal lamina (Figures 7.6 & 7.7). The filamentous forms observed resemble the filamentous trophonts seen by Appleton & Vickerman (1998) during *in vitro* cultivation of *Hematodinium* from *N. norvegicus*. These filamentous trophonts have not been seen in the haemolymph of *N. norvegicus*.

The result of ELISA analyses on haemolymph from infected individuals provided an interesting disparity with the results of tissue parasite cell counts. In each of the infected animals, where there was a high tissue parasite load, the ELISA indicated a low blood parasite level and *vice versa*. This was especially apparent in ovary and haemolymph samples from females (Table 7.1). This could indicate movement of the parasite cells from the tissue to the haemolymph at high levels of infection.

Immunological diagnostic techniques have proven to be highly sensitive in the detection of *Hematodinium* infection, and able to demonstrate the existence of latent infection in the tissue and sub-patent infections in the haemolymph of apparently healthy *N. norvegicus* (Field & Appleton, 1996). The location of parasite cells in the tissues during latent infection has previously been discussed (Field & Appleton, 1995, 1996; Stentiford *et al.*, 2001d, 2002) although detailed examinations have yet to be carried out. It is possible that the connective tissues and basal laminae of tissues provide the seat of latent *Hematodinium* infection with multinucleate filamentous forms attaching to the tissue surfaces. These filamentous plasmodial forms could then give rise to the blood-borne infection through budding off both uni- and multinucleate forms into the haemal spaces during sub-patent and patent infection. The cells found in the haemal spaces may represent the sporoblasts typical of patent infection.

Although infection by *Hematodinium* sp. is almost certain to cause death, Love *et al.* (1996) suggest the parasite host cycle is highly evolved. They report that, in the case of *Chionecetes bairdi*, the seasonal timing of peak infection occurs such that infected females would have been able to spawn prior to death. The same could also be said for *Hematodinium* sp. infection in *N. norvegicus*, where peak infection appears to occur after hatching and following the moult. However, in a fished population where a large proportion of the breeding animals may succumb to infection annually, there may be repercussions for the average size of individuals in the population. Both fishing and natural mortality may be influencing the size structure of the population through a reduction in the mean size of individuals.

7.5 Conclusions

- *Hematodinium* infection in female *N. norvegicus* causes a cessation in ovarian development and degradation of the ovarian tissue.
- Infection of the testes of *N. norvegicus* did not cause any severe breakdown of the tissue, although this may occur in late infection just prior to the sporulation event. This could be related to the lack of any cyclical activity in the testes (see Chapter 3).
- There was a disparate relationship between tissue parasite cell counts and ELISA analysis of the haemolymph of infected animals, whereby high tissue counts were accompanied by low ELISA readings. This may indicate a movement of parasite cells from the tissue to the blood during late infection.
- Filamentous plasmodial cells were seen attached to the basal laminae of both the ovary and testes. In the testes, filaments were seen attached to both the external and internal surfaces of a tubule membrane. It is hypothesised that these filaments could be the source of latent infection, budding off both uni- and multinucleate cells into the haemal spaces.

Animal	Carapace Length (mm)	Infected	Ovary stage	Parasite Cell Count (±SD)	ELISA result
1	28.5	Yes	0	516.3 ± 119.1	Low
2	29.1	Yes	0	372.5 ± 121.5	High
3	27.2	Yes	0	87.9 ± 25.7	High
4	29.9	No	3	0	Uninfected
5	28.1	No	2	1.00 ± 1.33	Uninfected
6	32.6	No	3	0.8 ± 1.0	Uninfected
7	26.5	No	1	1.2 ± 1.3	Uninfected
8	27.6	No	4	0.8 ± 2.1	Uninfected
9	28.6	No	3	0	Uninfected
10	26.8	Yes	0	1710.8 ± 177.1	Low
11	23.6	Yes	0	94.6 ± 22.2	High

Table 7.1: The results of tissue cell counts (± SD) from the ovaries and haemolymph ELISA analysis on a sample of female *Nephrops norvegicus* collected from the Clyde sea area during peak *Hematodinium* sp. infection. The parasite cell count represents the number of cells seen in the field of view. Due to the distribution of parasite cells, in the haemal spaces of the tissue, a cell count per unit area was deemed inappropriate.

Animal	Carapace Length (mm)	Infected	Parasite Cell Count (±SD)	ELISA result
1	30.2	Yes	362.6 ± 143.3	Low
2	27.3	Yes	101.4 ± 55.3	Low
3	29.6	Yes	225.6 ± 138.2	Low
4	35.2	Yes	148.8 ± 110.3	High
5	34.6	No	0	Uninfected
6	25.8	No	7 ± 7.7	Uninfected
7	28.3	Yes	88.6 ± 61.0	Low
8	31.4	No	14.6 ± 19.1	Uninfected
9	31.5	Yes	148.9 ± 174.7	High
10	30.5	Yes	200.9 ± 165.7	Low

Table 7.2: The results of tissue cell counts (± SD) from the testes and haemolymph ELISA analysis from a sample of male *Nephrops norvegicus* collected from the Clyde sea area during peak *Hematodinium* sp. infection. The parasite cell count represents the number of cells seen in the field of view. Due to the distribution of parasite cells, in the haemal spaces of the tissue, a cell count per unit area was deemed inappropriate.

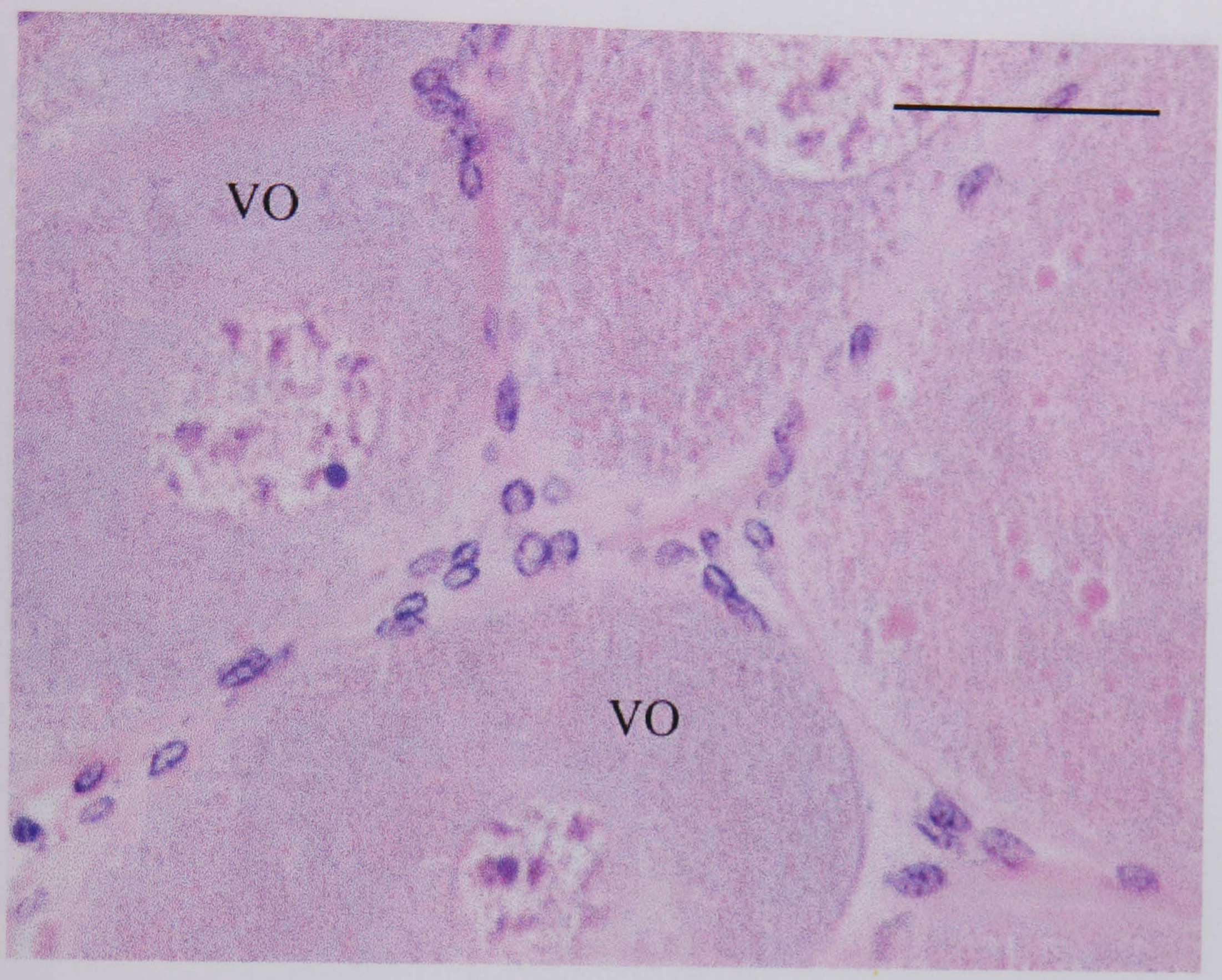


Figure 7.1: Developing ovarian tissue (stage 2) from a healthy female *Nephrops norvegicus* showing large tightly packed vitellogenic oocytes (VO). H&E (bar = 50μ).

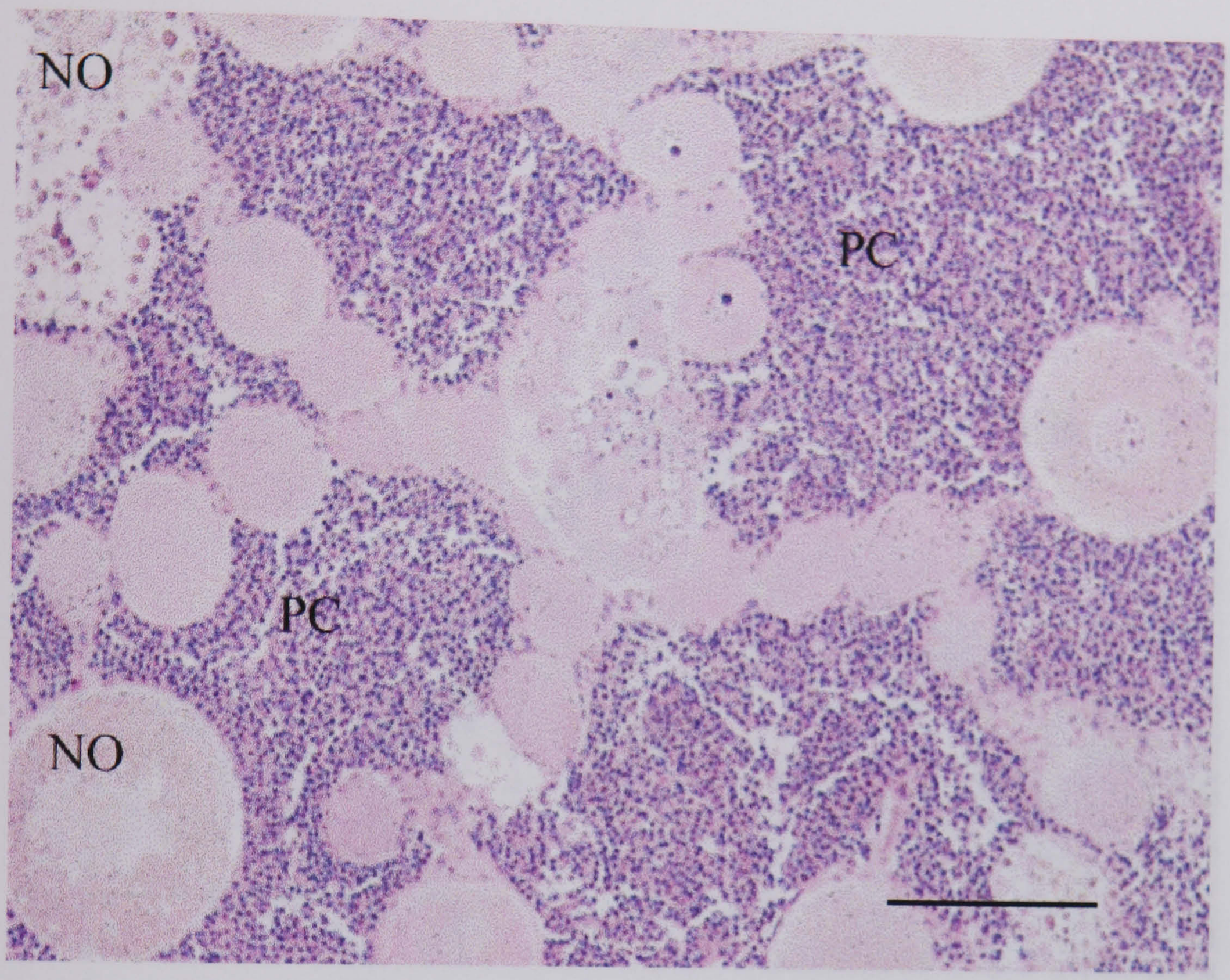


Figure 7.2: Ovarian tissue from a Norway lobster (*Nephrops norvegicus*) with a patent *Hematodinium* sp. infection. The ovarian follicle is infiltrated by masses of parasitic plasmodial cells (PC) causing possible pressure necrosis to the remaining oocytes (NO) and connective tissue. H&E (bar = 200μm).

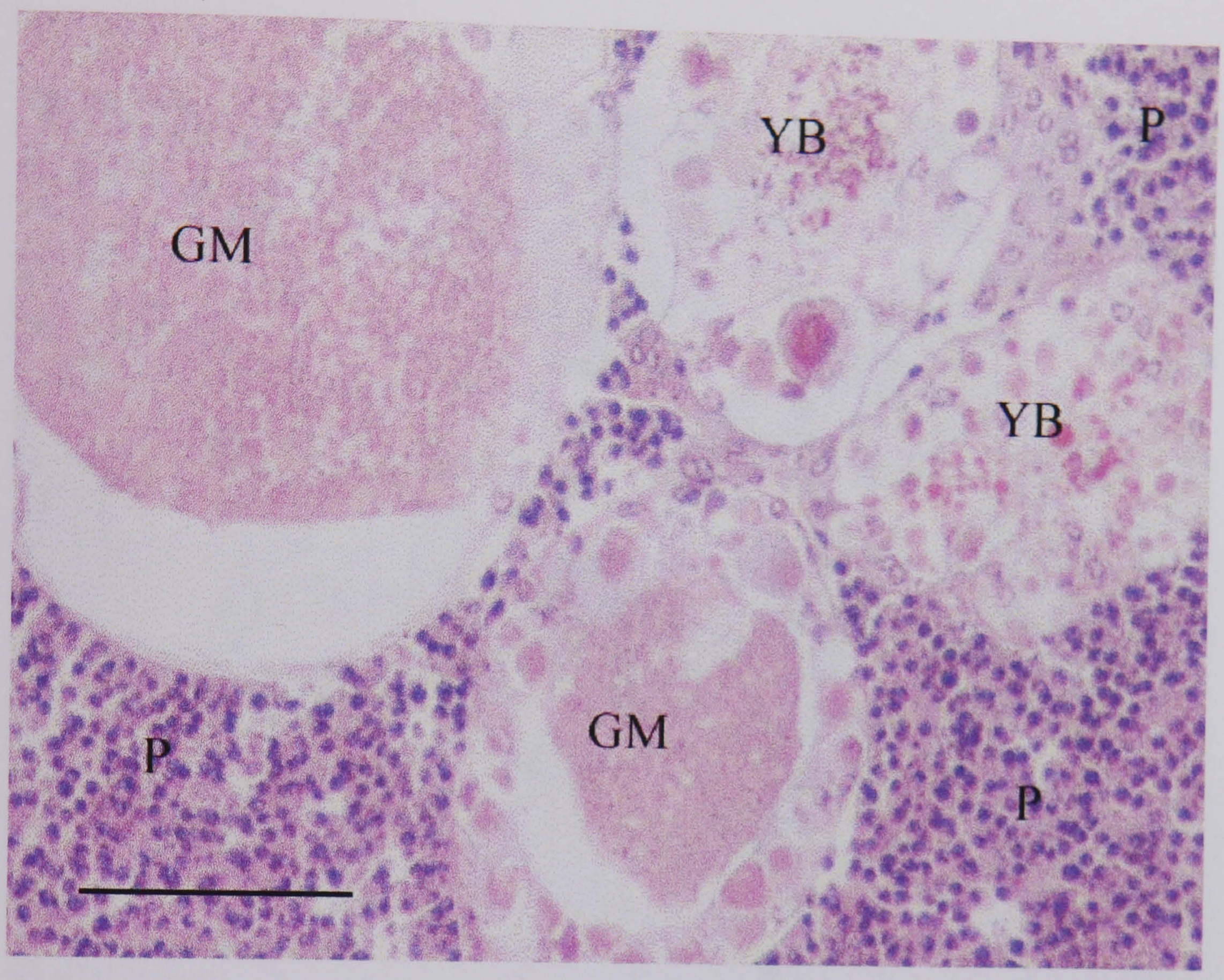


Figure 7.3a: Degenerating secondary oocytes from the ovary of a female *Nephrops norvegicus* with a patent *Hematodinium* sp. infection. Parasite cells (P) can be seen surrounding necrotic oocytes containing either a fine granular matrix (GM) or condensed yolk bodies (YB). H & E (bar = 50μm).

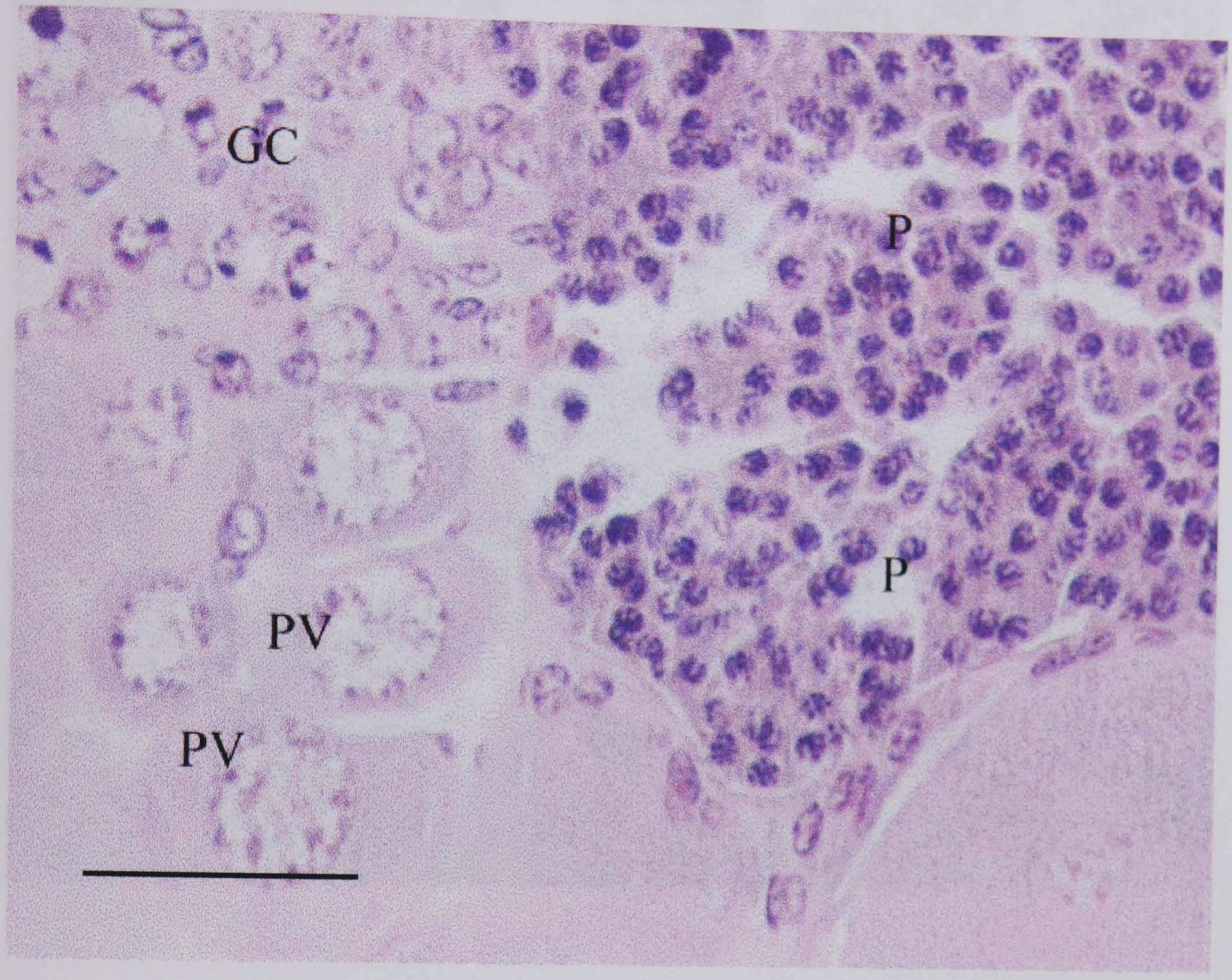


Figure 7.3b: Degenerating previtellogenic oocytes (PV) from the ovary of a female *Nephrops norvegicus* with a patent *Hematodinium* sp. infection. Apoptotic germ cells (GC) and parasite cells (P) can also be seen. H & E (bar = 50μm).

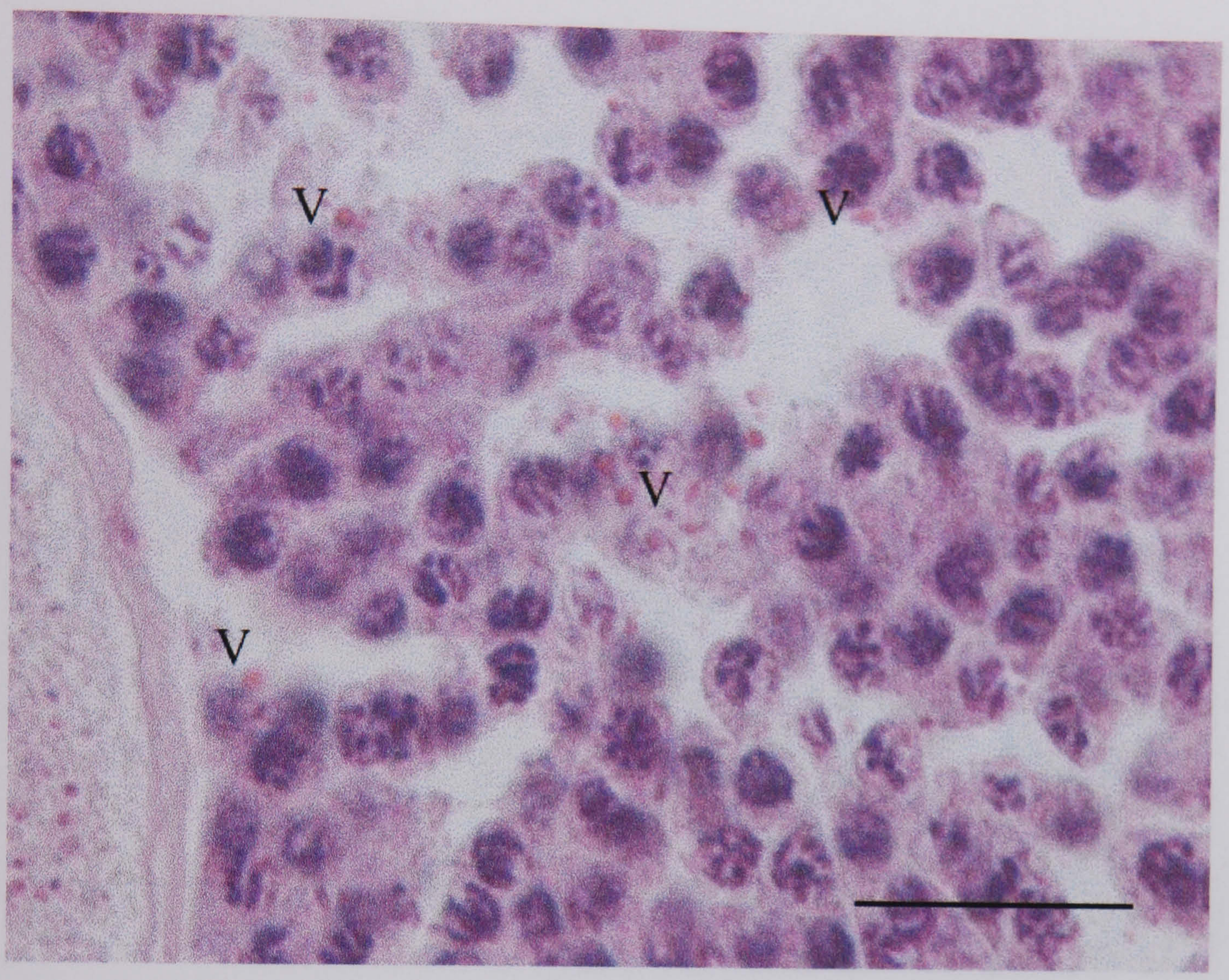


Figure 7.4: Free globules of vitellogenin (V) in the ovarian follicle of *Hematodinium* sp. infected female *Nephrops norvegicus* as a result of the oocyte degeneration. H & E (bar = 25μm).

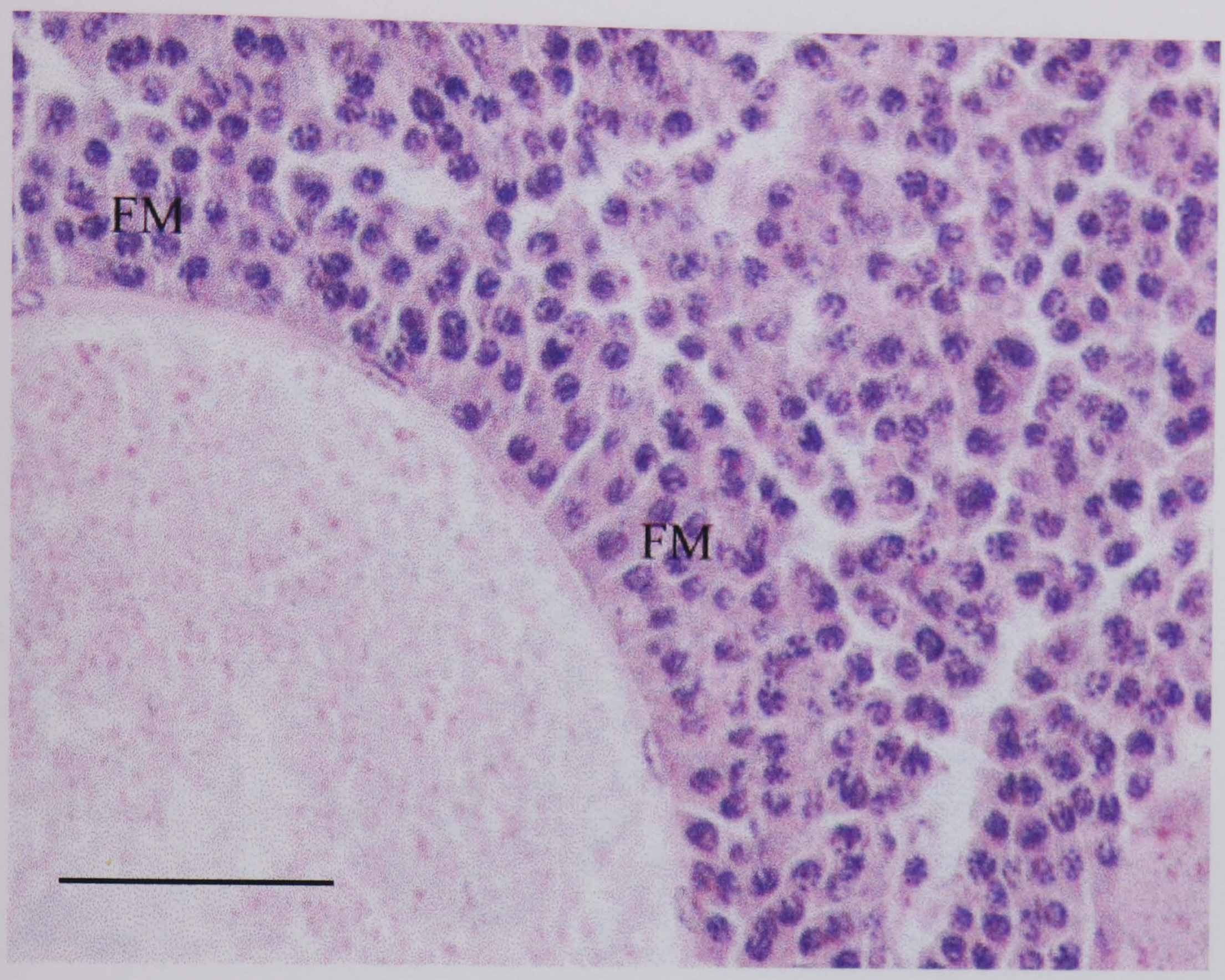


Figure 7.5: Filamentous multinucleate plasmodial *Hematodinium* sp. cells (FM) attached to the basal lamina of a degrading vitellogenic oocyte in the ovary of *Nephrops norvegicus* during patent infection. H & E (bar = 50µm).

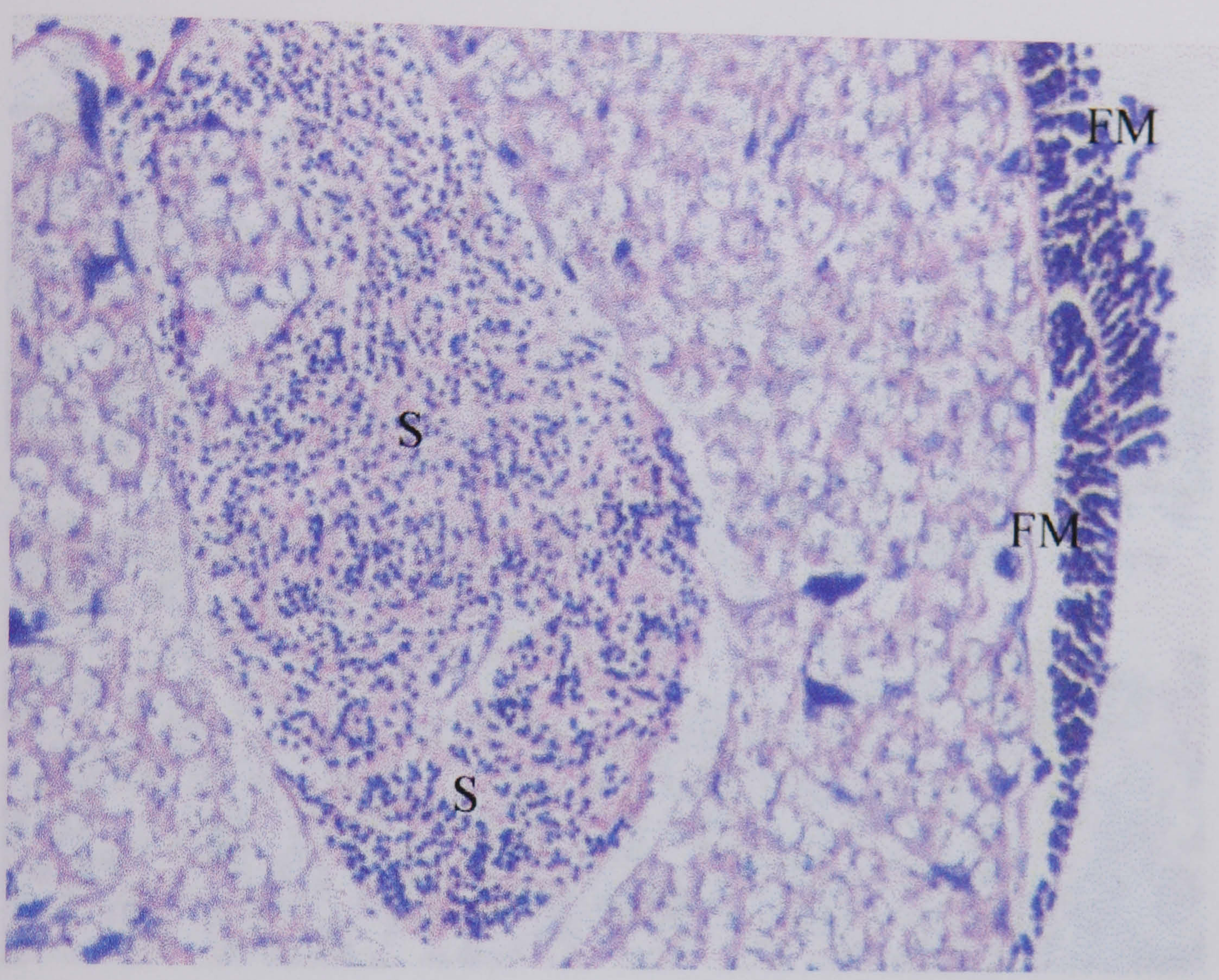


Figure 7.6: A collecting tubule from the testis of a male *Nephrops norvegicus* infected with *Hematodinium* sp. Filamentous multinucleate parasites (FM) can be seen attached to both sides the basement membrane of the tubule wall, the tubule itself is intact and filled with developing spermatozoa (S). H & E.

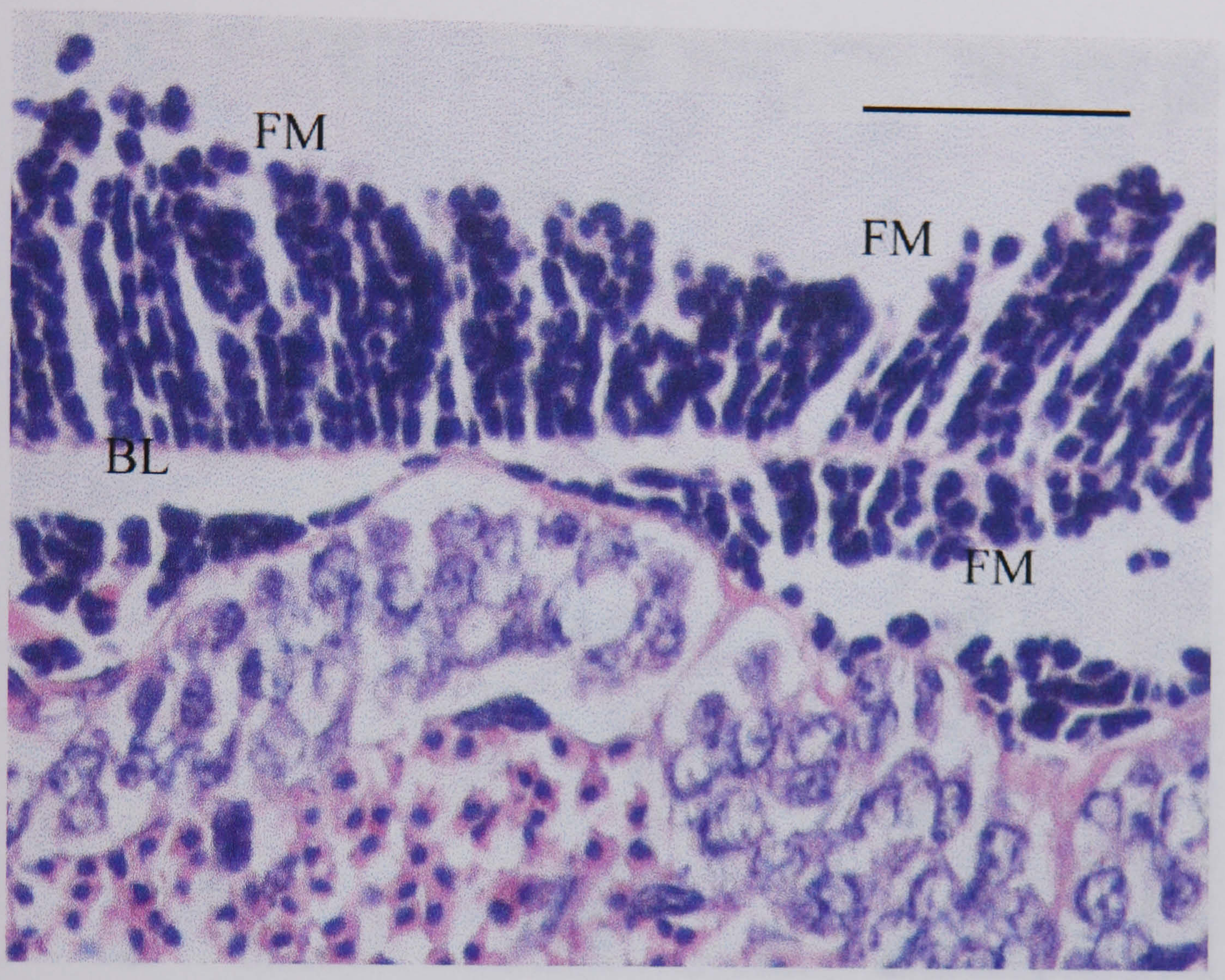


Figure 7.7: Filamentous multinucleate plasmodia (FM) *Hematodinium* sp. attached to both sides of the basal lamina (BL) of a collecting tubule in the testes of a patently infected *Nephrops norvegicus*. H & E (scale bar = 50μm).

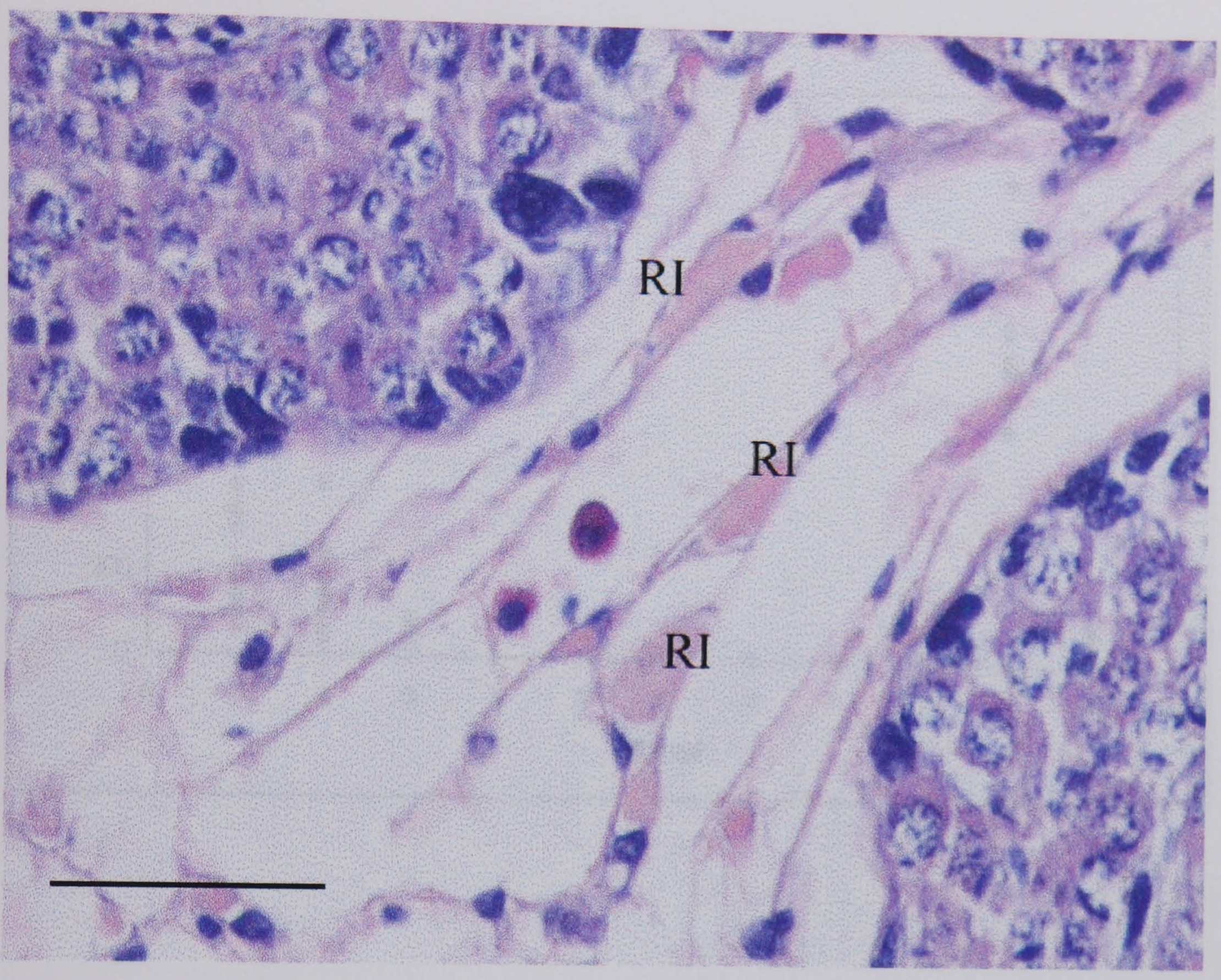


Figure 7.8: Testis tissue from an uninfected male *Nephrops norvegicus* showing the membrane systems associated with the connective tissue. Glycogen containing reserve inclusion (RI) cells can be seen (pale pink). H & E (bar = 50μm). Further pictures of uninfected testes can also be seen in Chapter 2.

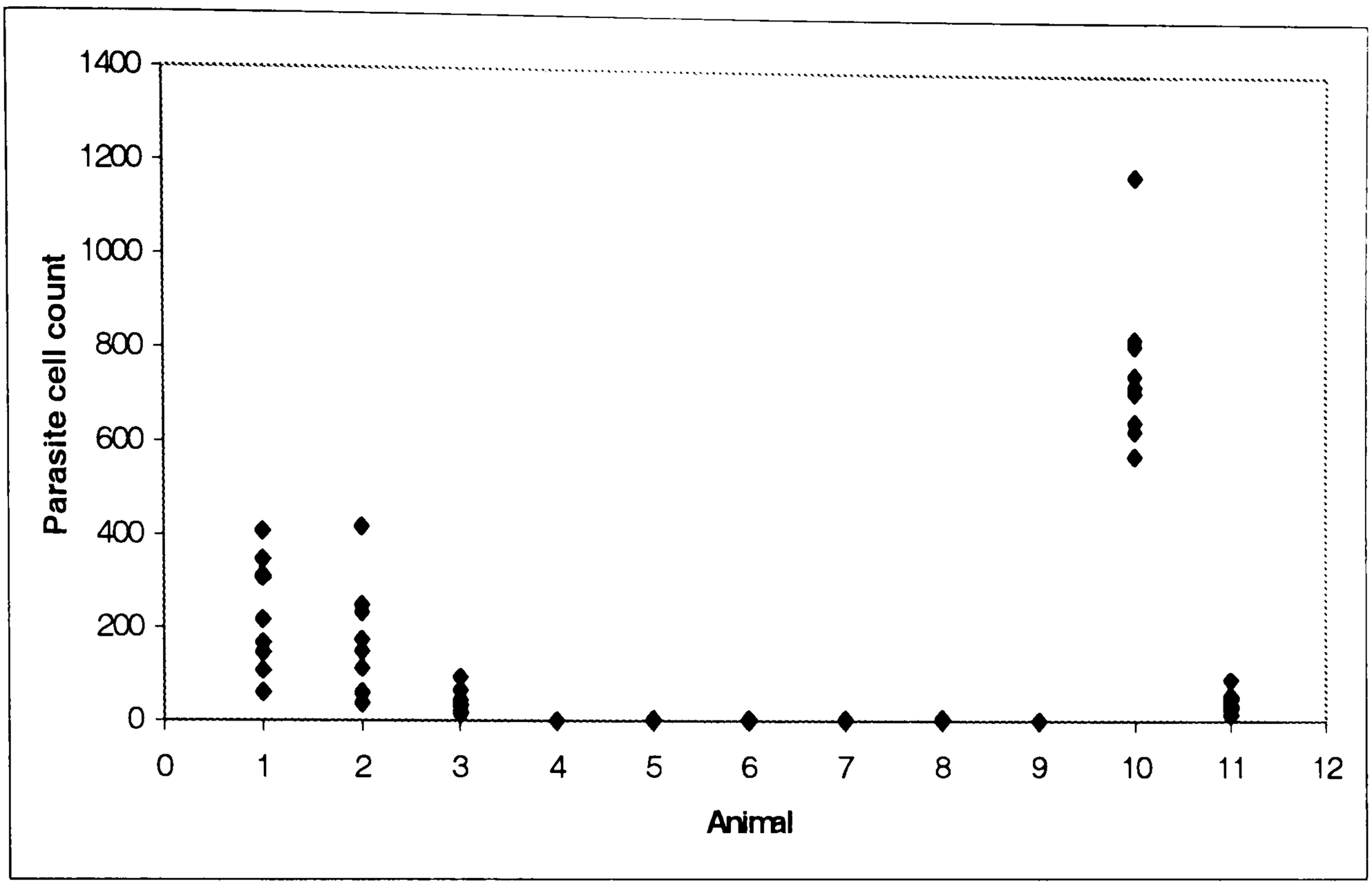


Figure 7.9: *Hematodinium* sp. cell counts from the ovaries of female *Nephrops norvegicus* sampled south of Little Cumbrae during the peak infection season. The parasite cell count represents the number of cells seen in the field of view. Due to the distribution of parasite cells, in the haemal spaces of the tissue, a cell count per unit area was deemed inappropriate.

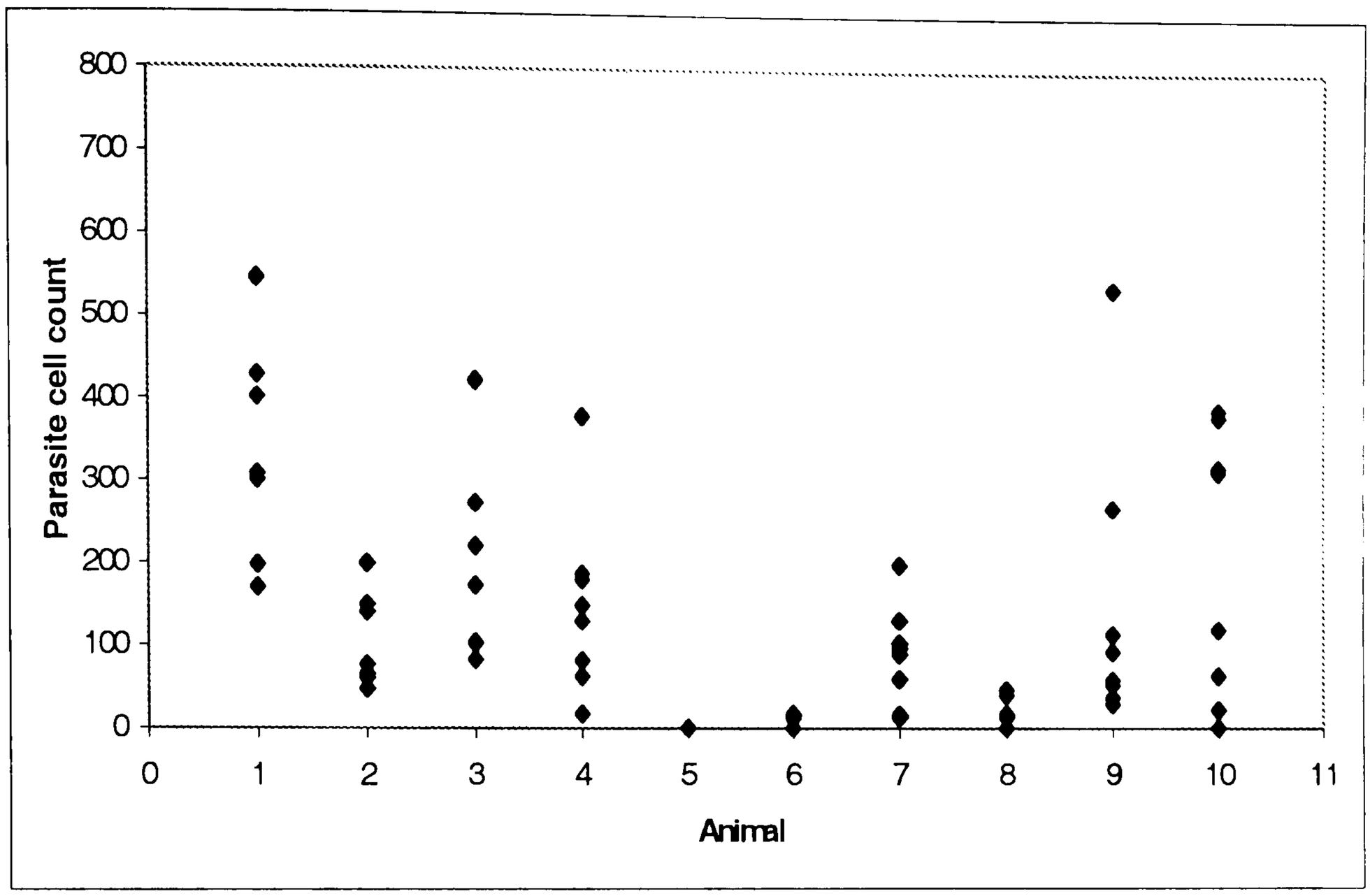


Figure 7.10: *Hematodinium* sp. cell counts from the testes of *N. norvegicus* sampled from south of Little Cumbrae during the peak infection season. The parasite cell count represents the number of cells seen in the field of view. Due to the distribution of parasite cells, in the haemal spaces of the tissue, a cell count per unit area was deemed inappropriate.

Chapter 8

General Discussion

The aim of this investigation into the reproductive dynamics of the male Norway lobster, *Nephrops norvegicus* (L.), was to determine any possible impacts that increased fishing pressure on males may have on the reproductive output of the population. To this end, investigations of the structure and functioning of the testes were carried out along with studies on size at maturity and reproductive behaviour. The incidence of insemination in females was also investigated along with the impact on the gonads of infection by parasitic dinoflagellates of the genus *Hematodinium*.

The results of this study have improved current knowledge about the male reproductive system of *Nephrops norvegicus*. Histological investigations of the testis have revealed an acinar structure, within which there are individual cycles of production within each acinus. This indicated that production in the testes was likely to be continuous, a theory supported by previous work (Bailey, 1984). Observations of changes in the testes over the course of the year confirmed this, as there was no seasonal change in the levels of RNA and protein within the testes. A technique to determine changes in the productivity of the seminiferous tubules was also used and again did not show any seasonal change, although this was not surprising as the tubules in the testes of *N. norvegicus* do not appear to have any germinative function. The lack of any seasonality was also confirmed

through the presence of spermatophores in the vasa deferentia throughout the year, as was previously reported for *N. norvegicus* in the Irish Sea (Farmer, 1974a), but not in Portuguese waters (Figueiredo & Barraca, 1963). These differences in productivity and seasonality between different geographical areas raise some interesting issues for fisheries management, as females in areas where males do not produce spermatophores all year round may be more susceptible to sperm limitation.

The observed lack of cyclical activity does, however, indicate that male *N. norvegicus* in the Clyde Sea Area are capable of mating throughout the year. This would be beneficial to the males in that they would be able to mate with any available moulting female, and some mating may occur outwith the main breeding season. In terms of the fishery, this would also be of benefit, because all mature males present within a population would potentially be able to mate with receptive females at any given time. Indeed females have been recorded carrying eggs outwith the expected reproductive periods (Sterk & Redant, 1989).

In histological terms it would be very useful to gain better knowledge about the spermatophores of male *N. norvegicus*, both in the reproductive tract of the male and also in the spermatheca of the female. Research on the crab *Scylla serrata* has shown that the composition of spermatophores changed with male maturity (Jeyalectumie & Subramonaim, 1991). The authors found that the spermatophores of immature crabs contained less organic material than the spermatophores of mature animals. This could be an area that merits further

investigation in *N. norvegicus*. Not only would it provide valuable information on the chemical composition of the spermatophore, but it would also allow investigation of the storage environment of the spermatozoa within the female, including aspects of respiration and nutrition. The increase in organic material in the spermatophores of *S. serrata* was also combined with an increase in the size of spermatophores and this also could be an interesting area for study, in relation to assessing size at maturity.

It is possible that other compounds could signify reproductive cycle state. For example, analyses of methyl farnesoate levels could be useful in determining any differences in the reproductive cycle of male *N. norvegicus* between populations. Methyl farnesoate (MF) is a hormone produced by the mandibular organ; it is the unepoxidated form of the juvenile insect hormone and it has been shown to vary cyclically in the spider crab *Libinia emarginata* (Takac *et al.*, 1997). It has been isolated from *N. norvegicus* haemolymph in both sexes, and linked to the reproductive cycle of females (Rotllant *et al.*, 2001). This technique would be a more sophisticated method for the determination of any cyclical activity as MF has also been shown to affect mating strategies in the spider crab (Laufer *et al.*, 1994). This method could also be used to determine size at maturity as MF has also been shown to be important in allometric growth and differentiation in male *L. emarginata* (Laufer *et al.*, 2002).

It is possible that the small size at physiological maturity found in *N. norvegicus* would be of great benefit in reducing the possible impacts of sperm limitation. If

the numbers of large males were reduced due to fishing the smaller individuals would still be theoretically capable of inseminating females. The limiting factor in this scenario may be the size of the individual mating pair. It is unlikely that a small male would be able to inseminate a very large female. This could cause sperm limitation if the largest, and therefore most fecund, females in the population were unmated. It would appear that there is no sperm limitation caused by the sizes of individuals in mating pairs in the Little Cumbrae population as there was a large proportion of inseminated females from this population and the larger females had spermatophores in their spermathecae. There was no evidence obtained on the quantity, or indeed quality, of the sperm transferred to females within the population, so this may yet prove to be a limiting factor.

Variation in the size at maturity between sites and sediment types was observed using the Reduced Major Axis technique; however, there were no obvious trends in the data. Males from the Fladen ground did appear to mature at larger size than those in the Clyde; however, the differences were not great and could be related to differences fishing effort between these grounds rather than any underlying geographic variation. For example, in areas such as the Clyde, sustained fishing pressure can reduce the size ranges of animals in the population. Morphometric techniques are useful tools for a rapid assessment of functional maturity within a *N. norvegicus* population and are easy to use in the field; however, there is no direct link between functional maturity as suggested by these techniques and any accurate assessment of actual maturity. In this study it was not possible to determine the effects of size on mating and therefore assign a behavioural

component to maturity for the Little Cumbrae population. It would be very beneficial to determine the size at behavioural maturity for a single population and to assess it in relation to methods that could be carried out at sea, for example. The results could then be applied to other populations. This could be a very useful tool for fisheries management.

It appears likely from the lack of success in pinpointing an exact size at morphometric maturity using the different techniques discussed, that there is a size range at which males become mature within each population. It would therefore be more appropriate to allocate a size range for each population that would encompass this. Such a size range could also be incorporated into areas containing different sediment types (indicative of population differences) to provide a more functional approach to providing a minimum legal size (MLS) to fisheries. In this way each fished area could be assessed separately to maximise the benefits to fisheries management within each management unit. A problem with this approach, however, is that it would be extremely difficult to enforce and therefore a single conservative value for each fishery may be more appropriate. There is a further difficulty in applying MLS to trawl fisheries as many undersized individuals are caught in the nets due to clogging of the meshes. The survival rates of these individuals on return to the sea is not high (Chapman, 1981).

The use of morphological data from males alone produced quite large SOMs in *N. norvegicus*, much greater than the estimated size at physiological maturity. It

would appear prudent therefore, to use the deviation of male and female data to estimate functional maturity in males. This gives a more thorough estimation for each site sampled. Use of such a technique may underestimate the size at behavioural maturity. It seems likely, however, that small males are able to mate with females in the absence of a larger competitor and are therefore contributing to the reproductive output of the population. This contribution may not be substantial in the presence of larger males; however, in heavily fished populations the contributions of smaller males may be increasingly important.

Due to the artificial nature of the environment in which the behaviour of 'populations' of *N. norvegicus* were observed, there are many improvements that could be made to this experimental methodology. The use of artificial burrows was quite successful when animals were given a period of time to acclimatise; however, larger animals did not utilise the 'burrows' as shelters. This could be due to the 'burrows' being too small for these individuals. It was also noted that animals would often change position in the artificial burrows so that they were facing the opposite direction. Observations on animals digging burrows in mud in the laboratory have shown that individuals use both the original entrance and exit of the burrow with equal frequency, although the openings initially join the mud surface at different angles (Farmer, 1974e). An improvement in the use of artificial burrows would perhaps be to provide a variety of burrow sizes and shapes. This may well affect the eviction rates of *N. norvegicus*. In most cases evictions occurred when an animal pushed though a 'burrow' ejecting the occupant. It is unlikely that evictions would be as easily carried out from mud

burrows. Rice & Chapman (1971) suggest that burrow occupation by two individuals, which was observed frequently in the field, could occur as a result of a more powerful individual entering an already occupied burrow. It would appear likely that this scenario would also occur should two animals of similar size contest burrow ownership and neither animal succeed in defeating the other. The small relative size of the artificial burrows used may have precluded this type of behaviour from occurring often. Therefore modification of the artificial burrows would be an obvious improvement, although they would still be a crude representation of naturally excavated burrows in mud.

Another method by which the tank environment could be improved is by landscaping. It was noted that many active animals spent a lot of time at the edges of the tank. These could be landscaped using rocks to provide less of an artificial environment. This type of behaviour has also been seen in *H. americanus*, where it was interpreted as a possible attempt to migrate away from the area (Karnofsky & Price, 1989). It could be that this behaviour is a reflection of the high density of animals in the study. The use of mud tanks in behavioural experiments is very problematic as any disturbances in the tank cause the mud to become suspended in the water column. It would perhaps be better to have some form of substratum in the tanks, probably sand or fine gravel, which would create a more natural environment without the problems of mud suspension.

Although the studies on *N. norvegicus* reproductive behaviour have been largely inconclusive, the proposed formation of a dominance hierarchy over time could

indicate that the larger males within a population are indeed carrying out much of the reproductive activity. The enlarged claws of adult male *N. norvegicus* are a prominent secondary sexual characteristic and are likely to play a large role in the development of dominance hierarchies and also in competition for breeding.

A further area of interest in behavioural studies is the possible use of contrast banding on the claws for communication between opposing individuals. At low light levels it has been suggested that the colouration of the claws causes banding patterns that would become apparent to an opponent during a meral spread display of aggression (Farmer, 1974e). The importance of these banding patterns is as yet unknown, but they may serve as indicators of the size of the animal or emphasise the size of the claw or positions of the cutter and crusher claws. They could also act to intimidate opponents. In the Hawaiian hermit crab (*Calcinus laevimanus*) there is a white patch on the major chela. In agonistic encounters between animals of a similar size the crab with the largest area of white on the claw was more likely to be successful (Dunham, 1978). It was suggested that the white patches were used in size assessment of opponents.

These studies could include observations on male competition, looking at the effects of body size, claw size, presence or absence of claws and also the presence of a newly moulted female (or pheromones). It would also be very useful to look at the effects of size of both males and females on mating success. This would involve presenting a newly moulted female with males of differing sizes and observing mating attempts. This could be carried out using relatively few females

as multiple mating of the same female by different males has been induced in the laboratory. Success of insemination could also be observed although this would require more females of the correct moult stage. It may also be appropriate to determine whether or nor the same male would attempt to mate more than once with a female if they were confined together, although this was not observed by Salerno (2000). This could be part of a study looking at the ability of males to transfer multiple spermatophores and hence their ability to be promiscuous.

Little is known of the reproductive behaviour of *N. norvegicus* in the field and it is therefore difficult to postulate the possible functions of pheromones in relation to specific behaviours. In the courtship of the American lobster, the dominant male remains within his shelter while the premoult females visit its entrance. During these visits Atema (1986) suggested that the female is directing a plume of pheromones into the male shelter while the male beats his pleopods to draw the pheromones through the burrow. As mating in *N. norvegicus* has only been observed on the sediment surface in laboratory experiments and no mate guarding has been recorded, it is less likely that this kind of behaviour would occur. The mechanism by which a male finds a newly moulted female is unclear. Perhaps mate-searching behaviour occurs on the surface during the breeding season, or perhaps males are able to detect pheromones from within the burrow. In a study on burrow irrigation behaviour by *N. norvegicus*, Gerhardt & Baden (1998) showed that males have a significantly higher pleopod activity than females under normoxic conditions. They suggest the difference between the sexes is related to the fact that ovigerous females do not often leave the burrow and are therefore

better able to cope with hypoxic conditions that may prevail. It has been shown, however, that ovigerous females inhabit well-ventilated burrows (Rice & Chapman, 1971). It could also be the case that males would be more actively seeking information on the environment outside the burrow, including odour plumes from food items, and pheromones produced by newly moulted females.

It is perhaps likely that the female remains in the burrow following the moult when she is vulnerable and that males undertake mate-searching behaviour on the surface. Salerno (2000) observed moulting females in mud tanks and found that the moult took place in the burrow. The moulting process would render an individual particularly susceptible to attack and predation and therefore moulting in the burrow would provide the female with some protection. It is still not known whether or not mating would take place in the burrow, which is big enough to accommodate two individuals (R. J. Atkinson, pers. comm.). It is possible that a soft female could use pheromones to attract possible mates by utilising irrigation currents to eject pheromones from the burrow. Pheromones have been described to reduce the cannibalistic nature of males during the mating process (Atema & Engstrom, 1971), is it therefore possible that they would have the same effect on females. A female using pheromones to attract a male is also advertising her presence and vulnerability to other females who could evict her to gain access to the burrow or cannibalise the advertising resident.

Further work into the function and behavioural context of pheromone use is required to increase knowledge on the reproductive biology of *N. norvegicus*.

Determination of the longevity and threshold of detection of pheromones in the environment, both in terms of concentration and distance from source, would be especially useful. To facilitate these investigations more work would need to be carried out on the source of pheromones from females; for example urine could be collected directly from the female for introduction to the male. The development of a sound bioassay would also be of importance.

Although there was no evidence from this study to suggest that larger females are being significantly reproductively compromised by the probable reduction in the size of males in the population due to fishing, there is a possibility of sperm limitation through insufficient gamete transfer. Studies have been carried out on the spiny lobsters *Jasus* sp. to examine the egg production of large females mated with small males (MacDairmid & Butler, 1999a). This technique could be used in the laboratory to study sperm limitation in female *N. norvegicus*. There are several possible problems with this method of research, however, in terms of the survival of females over the duration of the experiment and the probability of egg loss, which is quite common in laboratory animals (pers. obs.). A further method that could be used to determine the quantity of sperm passed to females is to measure the area or volume of the spermatophore.

It is possible that, in the apparently promiscuous mating system of *N. norvegicus*, large females may make up any shortfall in the sperm transferred from a small male, by mating with many males. There is little information regarding the possibility of multiple mating in the wild although spermatophores from several

males have been found in the spermathecae of females from the Irish Sea and there is some evidence for multiple paternity (M. Hughes, pers. comm.). It could be that *N. norvegicus* are not susceptible to sperm limitation in this way due to the lack of any mate guarding and the vulnerability of recently moulted females.

In relation to the behaviour of female *Nephrops norvegicus* during reproduction it is entirely possible that more than one male could mate with a female. Females are apparently passive during the mating process and are vulnerable due to their soft-shelled state. It is possible that more than one male would be attracted by the pheromones of a newly moulted female and as there appears to be no mate guarding, more than one copulation could take place. For example, the largest male in the vicinity is likely to out compete any smaller males for the female. However, once he had copulated 'sneaker males' could then proceed to mate with the female. This could reduce the effects of sperm limitation in the population providing the densities of males were not so severely reduced that a moulting female was unlikely to come into contact with one or more males. In the case of multiple mating the position of the spermatophore within the spermatheca could be of vital importance (Urbani *et al.*, 1998).

If larger females were unable to find a partner it is possible that the ovaries would be resorbed, or that the female would lay eggs that would then be lost (Woodlock & Reynolds, 1988). If the latter were true it would place quite a high physiological burden on the female and could affect her reproductive ability in the following year. It has been reported that larger females, under certain

circumstances, may reproduce biennially (Bailey, 1984) if their reproduction was additionally compromised by a failed breeding season, spawning could be occurring every three years or at greater intervals. However, the high proportions of ovigerous females present in autumn creel catches in Scottish waters do not support this. Further study is required into the fate of the developed ovary of an uninseminated female. This could easily be achieved by maintaining a size range of females in isolation prior to the moult.

Although the incidence of uninseminated females was not significant in this investigation, such females did occur. This could represent a substantial reduction in egg production and therefore subsequent recruitment at the population level. Current estimates, from burrow counts, suggest that there are approximately 1507 million *N. norvegicus* in the Clyde Sea area (Dr I. D. Tuck, pers. comm.). Of these, the sex ratio was estimated as 1:1 for immature individuals and 1:2 in favour of females for mature animals (Tuck *et al.*, 1997c). The proportion of all females that are mature in the Clyde is 45% (Smith, 1987), giving an overall sex ratio of 1:1.29 (Tuck *et al.*, 1997c) and indicating that within the Clyde fishery there are 382 million mature females. If, as in July 2000, 2.7% of females were uninseminated yet showed ovarian development, an approximate total of 10,314,000 females would be removed from the breeding population because of a lack of suitable mates. Actual fecundity of female *N. norvegicus* has been shown to vary considerably; for example, for females of 27 mm CL, sampled from the Clyde Sea area, egg counts varied from 347 – 1050 (Tuck *et al.*, 2000). Estimated size dependent larval production has been estimated at 250 – 2000 larvae per

female in each breeding season (Chapman, 1980). The mean size of uninseminated individuals sampled in July 2000 was 39.5 mm. Using the potential fecundity parameters for the Little Cumbrae site (Tuck *et al.*, 2000) the potential fecundity of a female of this size would be 3544 eggs. Although this figure will be affected by egg loss, and the relative number of larvae surviving to settlement is likely to be small, it can be seen that the removal of even a small percentage of females from the reproductive population could have implications for recruitment to the fishery.

The effects of infection by parasitic dinoflagellates of the genus *Hematodinium* on the gonads of *N. norvegicus* showed a marked difference between the sexes. It would seem that in heavily infected males the testes are still able to produce sperm; however, infection in females with developing ovaries causes a cessation in development. More females are infected than males and are therefore prevented from breeding. It is possible that infected males are still able to mate with females, although perhaps not in the advanced stages of infection when there are behavioural implications of parasite burden. This could have implications for the transmission of the parasite. It is possible that infection could be passed on horizontally and/or vertically in the spermatophores. Although parasite cells were never seen in the lumen of the testes, an investigation of the vasa deferentia of infected males could identify a possible route of infection. Females could be particularly vulnerable to infection following the moult when mating would take place. It would be especially interesting to investigate the presence or absence of

parasite cells in the eggs of *N. norvegicus*, to determine the presence of vertical transmission.

Females have been shown to be more susceptible to infection by *Hematodinium*. This combined with the resorption of developing ovaries during infection could have implications for the reproductive output of the population. The prevalence of infection in females at the sampling site south of Little Cumbrae has been reported to reach values as high as 98.3% in 1990 and 77.8% in 1991 by Field *et al.* (1992). More recently, however, the prevalence in female *N. norvegicus* at this site has been reported at 35% (Stentiford *et al.*, 2001a). These figures should be taken with some caution as the behavioural consequences of infection cause increased catchability of infected animals (Stentiford *et al.*, 2000a). This does represent, however, a substantial proportion of the breeding population and if all of these females are prevented from breeding it could have a significant effect on the reproductive output of the population, and on subsequent recruitment.

The prevalence of *Hematodinium* infection has also been shown to be higher in smaller individuals (Field *et al.*, 1998; Stentiford *et al.*, 2001a). This could have implications on the numbers of males affected in fished populations. Should there be a reduction in the size of males within a population, there may be a resultant increase in the prevalence of infection in male *N. norvegicus*. This combined with the higher fishing mortality sustained by males could confound the problem of fishing-induced imbalance in the sex ratio by further reducing the numbers of males.

8.1 Conclusions

The results of this study have indicated that the reproductive biology of *Nephrops norvegicus* is complex. When examining the reproductive biology of males in particular, it is surprising that there are geographical differences in the seasonal reproductive output. In terms of the reproductive output of a fished population, it is perhaps advantageous that males in some populations are capable of breeding throughout the year. For example, in the Clyde Sea area it might be possible that mating outwith the breeding season could augment larval production.

Although there were no direct observations on the mating behaviour of *N. norvegicus*, it can be hypothesised, that the mating behaviour is based on vulnerable postmoult females within burrows and a dominance hierarchy of males competing to mate. Males would undertake mate searching in such a system, and in this way smaller males would be able to mate with females in the absence of more dominant individuals, and this could result in the multiple paternity that has been recorded (M. Hughes, pers. comm.). The impacts of fishing on this system in reducing the number and size of males within the population could break down the hierarchical structure of populations and provide mating opportunities for a greater number of males. This must, however, be balanced by the possibility that sperm limitation could occur and reduce the reproductive output of the population.

The use of different techniques for the analysis of size at onset of maturity (SOM) has indicated that there are variations in SOM between different areas and sediment types, and that at least some of this variation could be due to differences

in fishing pressure (Marrs *et al.*, 2002b). It may therefore be beneficial to apply assessments of maturity to different areas in order to determine the most appropriate management strategy for individual fisheries, in relation to the level of exploitation and the seabed characteristics.

It would appear that the study population south of Little Cumbrae is not affected in a statistically significantly way by sperm limitation; however, with continued fishing pressure the population structure may be subject to change and the numbers of uninseminated females could become higher. This combined with the effects of *Hematodinium* infection on the ovaries of females could represent the removal of between 30 – 40% of breeding females from contributing to reproduction in any given year. This could represent a serious threat to future recruitment of the *N. norvegicus* fishery in the Firth of Clyde.

References

Afonso-Dias, M. (1997). Variability in *Nephrops norvegicus* (L.) populations in Scottish waters in relation to the sediment characteristics of the seabed. PhD Thesis, University of Aberdeen.

Afzelius, B. A. (1995). Gustaf Retzius and spermatology. *Int. J. Dev. Biol.* **39**: 675 – 685.

Aiken, D. E. (1980). Moulting and Growth. Chapter 2 in The Biology and Management of Lobsters. Volume 1: Physiology and behaviour. Cobb, J. S. & Phillips, B. F. (Eds.), Academic Press Inc., New York, pp. 91 – 163.

Aiken, D. E. & Waddy, S. L. (1980). Reproductive Biology. Chapter 4 in The biology and management of lobsters. Volume 1: Physiology and behaviour. Cobb, J. S. & Phillips, B. F. eds. Academic Press Inc., New York. pp 215 – 276.

Aiken, D. E. & Waddy, S. L. (1989). Allometric growth and onset of maturity in male American lobsters (*Homarus americanus*): crusher propodite index. *J. Shellfish Res.* **8**:7 – 11.

Andersson, M. B. (1986). Evolution of condition-dependant sex ornaments and mating preferences: sexual selection based on viability differences. *Evolution.* **40**:804 – 816.

Anilkumar, G., Sudha, K., Anitha, E. & Subraminiam, T. (1996). Aspects of sperm metabolism in the spermatheca of the brachyuran crab *Metopograpsis messor* (Forskål). *J. Crust. Biol.* **16**:310 – 314.

Anon. (1982). Report of the *Nephrops* working group. Brest, 24 - 27 May, 1982. *ICES C.M. 1982/K:8.*

Anon. (1984). Compositional changes in the *Nephrops* catch in the western Irish Sea, 1969 – 84. *ICES C. M. 1984/K:37.*

Anon. (2000). Report of the study group on life history of *Nephrops*. Reykjavik, Iceland, 2-5 May 2000. *ICES C.M. 2000/G:06, 148pp.*

Anon. (2001). Report of the working group on *Nephrops* stocks. *ICES C. M. 2001 ACFM:16.*

Appleton, P. L. & Vickerman, K. (1998). *In vitro* cultivation and developmental cycle in culture of a parasitic dinoflagellate (*Hematodinium* sp.) associated with mortality of the Norway lobster (*Nephrops norvegicus*) in British waters. *Parasitology*. **116**: 115 – 130.

Appleton, P. L., Field, R. H., Vickerman, K., Atkinson, R. J. A., Taylor, A. C., Rogerson, A. & Neil, D. M. (1997). Mortality of *Nephrops norvegicus* on the west coast of Scotland. Project Ref: MFO218. Final report to MAFF CSG. March 1997.

Aréchiga, H. & Atkinson, R. J. A. (1975). The eye and some effects of light on locomotor activity in *Nephrops norvegicus. Mar. Biol.* **32**:63 – 76.

Aréchiga, H., Atkinson, R. J. A. & Williams, J. A. (1980). Neurohumoral basis of circadian rhythmicity in *Nephrops norvegicus* (L.). *Mar. Behav. Physiol.* **7**:185 – 197.

Arrobas, I. (1982). Some aspects on the biology and fishery of *Nephrops norvegicus* (L.) form the south Portuguese coast. *ICES C.M.1982/K:7.*

Atema, J. (1986). Review of sexual selection and chemical communication in the lobster, *Homarus americanus. Can. J. Fish. Aquat. Sci.* **43**:2283 - 2290.

Atema, J. & Engstrom, D. G. (1971). Sex pheromone in the lobster, *Homarus americanus*. *Nature*. **232**:261 – 263.

Atema, J. & Gagosian, R. B. (1973). Behavioural responses of male lobsters to ecdysones. *Mar. Behav. Physiol.* **2**:15 – 20.

Atema, J. & Cobb, J. S. (1980). Social Behaviour. Chapter 8 in The biology and management of lobsters. Volume 1: Physiology and behaviour. Cobb, J. S. & Phillips, B. F. eds. Academic Press Inc., London.

Atema, J. & Cowan, D. (1986). Sex-identifying moult and urine signals in lobster (*Homarus americanus*). *J. Chem. Ecol.* **12**: 2065 – 2080.

Atema, J., Jacobson, S. & Stein, L. (1977). Pair formation and mating behaviour in the lobster, *Homarus americanus*. *CSIRO. Div. Fish. Oceanogr. (Aust). Circ No. 7*: 19 – 20.

Atema, J., Jacobson, S., Karnofsky, E., Olsezko-Szuts, S. & Stein, L. S. (1979). Pair formation in the lobster, *Homarus americanus*: Behavioural development, pheromones and mating. *Mar. Behav. Physiol.* **6**:277 – 296.

Atkinson, R. J. A. (1989). Baseline study of burrowing megafauna of Loch Sween PMNR and an investigation of the effects of trawling on the benthic megafauna. Reoprt to the NCC. Peterborough, from UMBSM. 65pp.

Atkinson, R. J. A. & Naylor, E. (1976). An endogenous activity rhythm and the rhythmicity of catches of *Nephrops norvegicus* (L.) *J. exp. Mar. Biol. Ecol.* **25**:95 – 108.

Bailey, N. (1984). Some aspects of reproduction in *Nephrops*. *ICES CM 1984/K:33.*

Bailey, N. (1986). Why does the size of *Nephrops* vary? *Scot. Fish. Bull.* **49**:31 – 36.

Bailey, N. & Chapman, C. J. (1983). A comparison of length, density and growth of two *Nephrops* populations off the west coast of Scotland. *ICES C. M. 1983/K:42.*

Bailey, N., Howard, F. G., and Chapman, C. J. (1986). Clyde *Nephrops*: biology and fisheries. *Proc. Roy. Soc. Edinb B.* **90**:501 - 518.

Bamber, S. D. & Naylor, E. (1997). Sites of putative sex pheromone and sexual behaviour in female *Carcinus maenas* (Crustacea: Decapoda). *Estuar. Coast. Mar. Sci.* **44**:195 – 202.

Barnes, R. S. K., Calow, P. & Olive, P. J. W. (1993). The invertebrates a new synthesis. Second Edition. Blackwell Science.

Bas, C. & Sardà, F. (1998). Long-term moprhometric variations in a population of the deep-sea shrimp *Aristeus antennatus* (Risso, 1816) (Decapoda:Aristeidae). *Cruststaceana.* **71**: 369 – 377.

Batoy, C. B., Sarmago, J. F. & Pilapil, B. C. (1987). Breeding season, sexual maturity and fecundity of the blue crab, *Portunus pelagicus* (L.) in selected coastal waters in Leyte and vicinity, Philippines. *Ann. Trop. Res.* **9**:157 – 177.

Benhalima, K. & Moriyasu, M. (2001). Prevalence of bacteria in the spermathecae of female snow crab, *Chionoecetes opilio* (Brachyura: Majidae). *Hydrobiologica.* **449**:261 – 266.

Berry, P. F. (1969). The Biology of *Nephrops andamanicus* Wood-Mason (Decapoda, Reptantia). *Oceanogr. Res. Inst. (Durban), Invest.Rep.* **22**:1 – 55.

BGS. (1987). Seabed sediments around the United Kingdom. Sediment maps. British Geological Survey. Natural Environment Research Council, Edinburgh.

Black, J. B. (1966). Cyclic male reproductive activities in the Dwarf crawfishes *Cambarellus shufeldti* (Faxon) and *Cambarellus puer* Hobbs. *T. American Micros. Soc.* **85**:214 – 232.

Breithaupt, T. & Atema, J. (2000). The timing of chemical signalling with urine in dominance fights of male lobsters (*Homarus americanus*). *Behav. Ecol. Sociobiol.* **49**:67 – 78.

Breithaupt, T. & Eger, P. (2002). Urine makes the difference: chemical communication in fighting crayfish made visible. *J. Exp. Biol.* **205**:1221 –1231.

Briggs, R. P. (1988). A preliminary analysis of maturity data for northwest Irish Sea *Nephrops*. *ICES C.M. 1988:K/21.*

Briggs, R. P. (1997). The Dublin Bay Prawn. *Biologist* **44:**246 - 248.

Briggs, R. P. & McAliskey, M. (1996). The prevalence of *Hematodinium* in Irish Sea *Nephrops*. ICES *Nephrops SG*, Lorient. 4pp.

Briggs, R. P. & McAliskey, M. (2002). The prevalence of *Hematodinium* in *Nephrops norvegicus* from the western Irish Sea. *J. Mar. Bio. Ass. U.K.* **82**:427 – 433.

Brown, R. B. & Powell, G. C. (1972). Size at maturity in the male Alaskan tanner crab, *Chionoecetes bairdi*, as determined by chela allometry, reproductive tract weights and the size of precopulatory males. *J. Fish. Res. Board Can.* **29**:423 – 427.

Burton, T. E. (1995). The spermatid pathway and associated reproductive structures of the squat lobster *Thenus orientalis* (Lund, 1793). *Invertebr. Reprod. Dev.* **28**:53 – 61.

Bushmann, P. J. & Atema, J. (2000). Chemically mediated mate location and evaluation in the lobster, *Homarus americanus*. *J. Chem. Ecol.* **26**:883 – 899.

Butler, T. H. (1960). Maturity and breeding of the Pacific edible crab, *Cancer magister* Dana. *J. Fish. Res. Board Can.* **17**:641 - 646

Caldwell, R. L. & Dingle, H. (1976). Stomatopods. *Sci. Am.* **234**: 81 – 89.

Canli, M & Furness, R. W. (1993). Heavy metals in tissues of the Norway lobster *Nephrops norvegicus*: effects of sex, size and season. *Chem. Ecol.* **8**:19 – 32.

Castille, F L. & Lawrence, A. L. (1989). Relationship between maturation and biochemical composition of the gonads and digestive glands of the shrimps *Penaeus aztecus* Ives and *Penaeus setiferus* (L.). *J. Crust Biol.* **9**:202 - 211.

Chapman, C. J. (1980). Ecology of juvenile and adult *Nephrops*. Chapter 4 In The biology and management of lobsters. Volume 11 Ecology and management. Cobb, J. S. & Phillips, B. F. (Eds.). Academic Press, London.

Chapman, C. J. (1981). Discarding and tailing *Nephrops* at sea. *Scot. Fish. Bull.* **46**:10 – 13.

Chapman, C. J. & Rice, A. L. (1971). Some direct observations on the ecology and behaviour of the Norway lobster *Nephrops norvegicus*. *Mar. Biol.* **10**:321 – 329.

Chapman, C. J. & Priestley, R. (1972). Observations on the diurnal activity of the Norway lobster, *Nephrops norvegicus* (L.). *ICES C.M. 1972/K:20.*

Chapman, C. J. & Howard, F. G. (1979). Field observations on the emergence rhythm of the Norway lobster, *Nephrops norvegicus*, using different methods. *Mar. Biol.* **51**:157 – 165.

Chapman, C. J. & Bailey, N. (1987). Biological research on fish and shellfish stocks. Recent progress in Norway lobster research, pp 99 – 111 in Developments in fisheries research in Scotland. Bailey, R. S. & Parrish, B. B. (Eds). Fishing News Books, Farnham.

Chapman, C. J. & Howard, F. G. (1988). Environmental influences on Norway lobster (*Nephrops norvegicus*) populations and their implications for fishery management. *Symp. Zool. Soc. Lond.* **59**:343 - 353.

Chapman, C. J., Johnstone, A. D. F. & Rice A. L. (1975). The behaviour and ecology of the Norway lobster, *Nephrops norvegicus* (L.). *Proceedings of the 9th European Marine Biology Symposium.* 1975: 59 – 74.

Chapman, C. J., Shelton, P. M. J., Shanks, A. M. & Gaten, E. (2000). Survival and growth of the Norway lobster *Nephrops norvegicus* in relation to light-induced eye damage. *Mar. Biol.* **136**:233 – 241.

Chevaillier, P. (1966). Contribution à l'étude du complexe ADN-histone dans le spermatozoïde du pagure *Eupagurus bernhardus* L. (Crustacé Décapode). *J. du Microsc.* **5**:739 – 758.

Chubb, C. F. (1994). Reproductive biology: issues for management. In: Phillips, B. F., Cobb, J. S., Kittaka, J. (Eds.). Spiny lobster management: current situation and perspectives. Blackwell, Oxford. pp 181 – 212.

Conan, G. Y., Buestel, D., Guennegan, Y. & Hillis, J. P. (1984). Diving and underwater television observations on *Nephrops norvegicus* in Bantry Bay, Ireland. *ICES C. M. 1984/K:48.*

Courchesne, E. & Barlow, G. W. (1971). Effect of isolation on components of aggressive and other behaviour in the hermit crab, *Pagurus samuelis*. *Z. vergl. Physiologie*. **75**:32 – 48.

Cromarty, S. I., Mello, J. & Kass-Simon, G. (2000). Molt-related and size-dependent differences in the escape response and post-threat behaviour of the American lobster, *Homarus americanus*. *Biol. Bull.* **199**:265 – 277.

Cronin, L. E. (1947). Anatomy and histology of the male reproductive system of *Callinectes sapidus* Rathbun. *J. Morph.* **81**:209 – 239.

Debuse, V. J., Addison, J. T. & Reynolds, J. D. (1999). The effects of sex ratio on sexual competition in the European lobster. *Anim. Behav.* **58**: 973 – 981.

Dewsbury, D. A. (1982). Ejaculate cost and male choice. *Am. Nat.* **119**:601 – 310.

Dingle, H. (1983). Studies of agonistic behaviour in crustacea. Chapter 4 in Studies in adaptation. The behaviour of higher Crustacea. Rebach, S & Dunham, D. W. (eds.). John Wiley and Sons, New York.

Dingle, H. & Caldwell, R. L. (1969). The aggressive and territorial behaviour of the mantis shrimp *Gonodactylus bredini* Manning (Crustacea: Stomatopoda). *Behaviour*. **33**:115 – 136.

Diwan, A. D. & Nagabhushnam, R. (1974). Reproductive cycle and biochemical changes in the gonads of the freshwater crab *Barytelphusa cunicularis* (Westwood, 1836). *Indian Jnl. Fish.* **21**:164 – 176.

Dunham, D. W. (1972). Some effects of group housing upon the aggressive behaviour of the lobster *Homarus americanus*. *J. Fish. Res. Board Can.* **29**:598 – 601.

Dunham, D. W. (1978) Effect of chela white on agonistic success in a diogenid hermit crab (*Calcinus laevimanus*). *Mar. Behav. Physiol.* **5**:137 – 144.

Dunham, P. J. (1988). Pheromones and behaviour in Crustacea. Chapter 9 in Invertebrate endocrinology Vol. 2. Endocrinology of selected invertebrate types (Laufer, H. & Downer, R. G. H. Eds.). Alan R. Liss, Inc. New York.

Dunthorn, A. A. (1967). Some observations on the behaviour and development of the Norway lobster. *ICES C. M. 1967/K:5.*

Eales, A. J. (1974) Sex pheromone in the shore crab *Carcinus maenas*, and the site of its release from females. *Mar. Behav. Physiol.* **2**:345 – 355.

Eaton, W. D., Love, D. C., Botelho, C., Meyers, T. R., Imamura, K. & Koeneman, T. (1991). Preliminary results on the seasonality and life cycle of the parasitic dinoflagellate causing bitter crab disease in Alaskan tanner crabs (*Chionecetes bairdi*). *J. Invertebr. Pathol.* **57**:426 – 434.

Eiriksson, H. (1970). On the breeding cycle and fecundity of the Norway lobster at south-west Iceland. *ICES C.M. 1970/K:6.*

El Haj, A. J. & Houlihan, D. F. (1987). *In vitro* and *in vivo* protein synthesis rates in a crustacean muscle during the moult cycle. *J. Exp. Biol.* **127**:413 – 426.

Elner, R. W. (1980). The influence of temperature, sex and chela size in the foraging strategy of the shore crab *Carcinus maenas* (L.). *Mar. Behav. Physiol.* **7**: 15-24.

Elner, R. W., Gass, C. A. & Campell, A. (1985). Mating behaviour of the Jonah crab, *Cancer borealis* Stimpson (Decapoda, Brachyura). *Crustaceana*, **48**:34 – 39.

Farmer, A. S. D. (1974a). Reproduction in *Nephrops norvegicus* (Decapoda: Nephropidae). *J. Zool.* **174**:161 – 183.

Farmer, A. S. D. (1974b). Relative growth in *Nephrops norvegicus* (L.) (Decapoda:Nephropidae). *J. Nat. Hist.* **8**:605 - 620.

Farmer, A. S. D. (1974c). The development of the external sexual characters of *Nephrops norvegicus* (L.) (Decapoda: Nephropidae). *J. Nat. Hist.* **8**:241 - 255.

Farmer, A. S. D. (1974d). Field assessments of diurnal activity in Irish Sea populations of the Norway lobster, *Nephrops norvegicus* (L.) (Decapoda: Nephropidae). *Estuar. Coast. Mar. Sci.* **2**:37 – 47.

Farmer, A. S. D. (1974e). Burrowing behaviour of the Norway lobster, *Nephrops norvegicus* (L.) (Decapoda: Nephropidae). *Estuar. Coast. Mar. Sci.* **2**:49 – 58.

Farmer, A. S. D. (1975). Synopsis of biological data on the Norway lobster *Nephrops norvegicus* (Linnaeus, 1758). *FAO Fisheries Synopsis.* **112**, 97pp.

Fasten, N. (1926). Spermatogenesis of the black-clawed crab, *Lophopanopeus bellus* (Stimpson) Rathbun. *Biol. Bull.* **50**:277 – 293.

Felder, D. L. & Lovett, D. L. (1989). Relative growth and sexual maturation in the estuarine ghost shrimp *Callanasa lousianensis* Schmitt, 1935. *J. Crust. Biol.* **9**:540 – 553.

Field, R. H. (1992). The control of escape behaviour in, and the histolpathology of, the Norway lobster, *Nephrops norvegicus* (L.). PhD Thesis, University of Glasgow.

Field, R. H. & Appleton, P. L. (1995). A *Hematodinium*-like infection of the Norway lobster *Nephrops norvegicus*: observations of pathology and progression of infection. *Dis. Aquat. Org.* **22**:115 – 128.

Field, R. H., Chapman, C, J., Taylor A. C., Neil, D. M. & Vickerman, K. (1992). Infection of the Norway lobster *Nephrops norvegicus* by a *Hematodinium*-like species of dinoflagellate on the west coast of Scotland. *Dis. Aquat. Org.* **13**: 1 – 15.

Field, R. H., Chapman, C. J. Taylor, A. C. Neil, D. M. & Vickerman, K. (1998). Distribution and seasonal prevalence of *Hematodinium* sp. infection of the Norway lobster (*Nephrops norvegicus*) around the west coast of Scotland. *ICES J. Mar. Sci.* **55**: 846 – 858.

Figueiredo, M. J. (1982). The occurrence of the resorbtion in the ovaries *Nephrops norvegicus* (L.) in Portuguese waters. *ICES C.M. 1982/K:28.*

Figueiredo, M. J. & Barraca, I. F. (1963). Contribuição para o conhecimento da peaca e da biologia do lagostim (*Nephrops norvegicus* L.) na costa Portuguesa. *Notas. Estuo. Inst. Biol. Mar., Lisb.* **28**:1 – 46.

Figueiredo, M. J. and Thomas, H. J. (1967a). *Nephrops norvegicus* (Linnaeus, 1758) Leach - a review. *Oceanogr. Mar. Biol. Ann .Rev.* **5**:371 - 407.

Figueiredo, M. J. & Thomas, H. J. (1967b). On the biology of the Norway lobster, *Nephrops norvegicus* (L.). *J. Cons. perm. Int. Explor. Mer.* **31(1)**: 89 – 101.

Frogila,C. & Gramitto, M. E. (1988). An estimate of growth and mortality parameters for Norway lobster (*Nephrops norvegicus*) in the Central Adriatic Sea. Report of the 5th technical consultation on stock assessment in the Adriatic and the Ionian Seas, Bari, (1987). *FAO Fisheries Report.* **394**:189-203.

Gardner, C. & Williams, H. (2002). Maturation in the male giant crab, *Pseudocarcinus gigas*, and the potential for sperm limitation in the Tasmanian fishery. *Mar. Freshwater Res.* **53**:661 – 667.

Gaten, E. (1988). Light-induced damage of the dioptric apparatus of *Nephrops norvegicus* (L.) and the quantitative assessment of the damage. *Mar. Behav. Physiol.* **13**:169 – 183.

Gaten, E., Shelton, P. M. J. Chapman, C. J. & Shanks, A. M. (1990). Depth related variation in the structure and functioning of the compound eye of the Norway lobster *Nephrops norvegicus*. *J. mar. biol. Ass. U.K.* **70**:343 – 355.

Gerhardt, L. & Baden, S. P. (1998). Gender- and oxygen-related irrigation behaviour of the decapod *Nephrops norvegicus*. *Mar. Biol.* **131**:553 – 558.

Ginsberg, J. R. & Milner-Gulland, E. J. (1994). Sex biased harvesting and population dynamics in ungulates: implications for conservation and sustainable use. *Conserv. Biol.* **8**:157 – 166.

Goshima, S., Kanazawa, M., Yoshino, K. & Wada, S. (2000). Maturity in the stone crab *Hapalogaster dentata* (Anomura: Lithoididae) and its applications for fishery management. *J. Crust. Biol.* **20**:641 – 646.

Grafen, A. & Ridley, M. (1983). A model of mate guarding. *J. Theor. Biol.* **102**:549 - 567.

Greenwood, J. G. (1972). The male reproductive system and spermatophore formation in *Pagurus novae-zealandiae* (Dana) (Anomura: Paguridea). *J. Nat. Hist.* **6**:561 – 274.

Haley, S. R. (1984). Spermatogenesis and spermatophore production in the Hawaiian red lobster *Enoplometopus occidentalis* (Randall) (Crustacea, Nephropidae). *J. Morphol.* **180**:181 – 193

Hankin, D. G., Butler, T. H., Wild, P. W. & Xue, Q.-L. (1997). Does intense fishing on males impair the mating success of female Dungeness crabs? *Can. J. Fish. Aquat. Sci.* **54**:655 – 669.

Harris, R. R., Lowry, N., Moth-Poulsen, T., Sangster, G. Soldal, A. V. Ulmestrand, M. & Valentinsson, D. (1997). Damage and physiological analysis of *Nephrops* cod-end escapees and discards. ICES Working Group on Fishing Technology and Fish Behaviour, Working Group Meeting, Hamburg 14 – 17 April 1997.

Hartnoll, R. G. (1978). The determination of relative growth in Crustacea. *Crustaceana*. **34**: 281 – 293.

Hatcher, M. J. & Dunn, A. M. (1997). Size and pairing in *Gammarus duebeni*: can females be too big? *Anim. Behav.* **54**:1301 - 1308.

Hill, A. E. & White, R. G. (1990). The dynamics of Norway lobster (*Nephrops norvegicus* L.) populations on isolated mud patches. *J. Cons. Int Explor. Mer.* **46**:167-174.

Hillis, J. P. (1974). A diving study on the Dublin bay prawns *Nephrops norvegicus* (L.) and their burrows off the east coast of Ireland. *Irish Fish. Invest. B.* **12**:3 – 9.

Hillis, J. P. (1981). Maturity in male *Nephrops norvegicus*: a study in secondary sexual characters. *ICES C. M. 1981/K22*.

Hillis, J. P. (1988a). Changes in the Irish Sea *Nephrops* catch since 1969. *C.M. 1988/K:38.*

Hillis, J. P. (1988b). Relation of *Nephrops* catch characteristics to sea-bed sediment type. *ICES CM 1988/K:37.*

Hillis, J. P. & Tully, O. (1993). Growth rate, mortality and small mean size in Irish Sea *Nephrops. ICES CM 1993/K:65.*

Hinsch, G. W. (1988). Morphology of the reproductive tract and seasonality of reproduction in the golden crab *Geryon fenneri* from the eastern Gulf of Mexico. *J. Crust. Biol.* **8**:254 – 261.

Hinsch, G. W. (1991). Structure and chemical content of the spermatophores and seminal fluid of reptantian decapods. Chapter 16 in Crustacean Sexual Biology. Bauer, R. T. and Martin, J. W. (eds.). Columbia University Press, New York.

Hinsch, G. W. (1992). Arthropoda – Crustacea: Sexual behaviour and receptivity. Chapter 18 in Reproductive Biology of Invertebrates. Volume V. Sexual Differentiation and Behaviour. Adiyodi, K. G. & R. G. (Eds.). John Wiley & Sons. Chichester.

Hoffman, R. S., Dunham, P. J. & Kelly, P, V. (1975). Effects of water temperature and housing conditions upon the aggressive behaviour of the lobster *Homarus americanus. J. Fish. Res. Board Can.* **32**:713 – 717.

Howard, F. G. & Hall, W. B. (1983). Some observations on the biometrics of *Nephrops norvegicus* (L.) in Scottish waters. *ICES CM 1983/K:36.*

Hudson, D. A. & Shields, J. D. (1994). *Hematodinium australis* sp., A parasitic dinoflagellate of the sand crab *Portunus plagicus* from Moreton Bay, Australia. *Dis. Aquat. Org.* **19**:109 – 119.

Hughes, M. (1996). Size assessment via a visual signal in snapping shrimp. *Behav. Ecol. Sociobiol.* **38**:51 – 57.

Hunter, J. R. & Macewicz, B. J. (1985). Rates of atresia in the ovary of captive and wild Northern anchovy, *Engaulis mordax. Fish. Bull.* **83**:119 – 136.

Hyatt, G. W. (1983). Qualitative and quantitative dimensions of crustacean aggression. In Studies in Adaptation. The behaviour of higher Crustacea. (Rebach, S. & Dunham, D. W. Eds.). pp 113 – 139. Wiley Interscience, New York.

Jamieson, G. S., Phillips, A. & Smith, B. D. (1991). Implications of selective harvests in Dungeness crab (*Cancer magister*) fisheries. *Can. Spec. Publ. Fish. Aquat. Sci.* **125**:309 – 321.

Jayasankar, V. & Subramoniam, T. (1999). Antibacterial activity of seminal plasma of the mud crab *Scylla serrata* (Forskål). *J. Exp. Mar. Biol. Ecol.* **236**:253 – 259.

Jeyalectumie, C. & Subramoniam, T. (1991). Biochemistry of seminal secretions of the crab *Scylla serrata* with reference to sperm metabolism and storage in the female. *Mol. Reprod. Dev.* **30**:44 – 55.

Jivoff, P. (1997). The relative roles of predation and sperm competition on the duration of post-copulatory association between the sexes in the blue crab, *Callinectes sapidus. Behav. Ecol. Sociobiol.* **40**:175 – 185.

Jivoff, P. & Hines, A. H. (1998). Female behaviour, sexual competition, and mate guarding in the blue crab, *Callinectes sapidus. Anim. Behav.* **55**:589 – 603.

Joshi, P. C. & Khanna, S. S. (1982). Structure and seasonal changes in the testes of a freshwater crab, *Potamin kooloense* (Rathbun). *Proc. Indian Acad. Sci.* **91**:439 – 450.

Juanes, F. & Smith, L. D. (1995). The ecological consequences of limb damage and loss in decapod crustaceans: a review and prospectus. *J. Exp. Mar. Biol. Ecol.* **193**:197 – 223.

Karavanich, C. & Atema, J. (1991). Role of olfaction in recognition of dominance in the American lobster, *Homarus americanus. Biol. Bull.* **181**:359-360.

Karavanich, C. & Atema, J. (1998). Olfactory recognition of urine signals in dominance fights between male lobster, *Homarus americanus. Behaviour.* **135**:719 – 730.

Karlovac, O. (1953). An ecological study of *Nephrops norvegicus* of the high Adriatic. *Izv. Inst. Oceanogr. Ribarst.* **5(2C)**:1 - 51.

Karlovac, O. (1955). A summary of our present knowledge regarding the Norway lobster (*Nephrops norvegicus* L.) in the Adriatic. *Proc. Tech. Pap. GFCFM.* **46**:417 – 421.

Karnofsky, E. B. & Price, H. J. (1989). Dominance, territoriality and mating in the lobster, *Homarus americanus*: A mesocosm study. *Mar. Behav. Physiol.* **15**:101 – 121.

Kaur, A., Kishen Rao, B., Thakur, S. S. & Raja, S. (1987). Factors inducing oocyte resorption in *Leptocoris coimbatorensis* Gross (Hemiptera:Coreidae). *Journal of the Kansas Entomological Society.* **60**:353 – 360.

Kittredge, J. S., Terry, M. & Takahashi, F. T. (1971). Sex pheromone activity of the molting hormone, crustecdysone, on male crabs (*Pachygrapsus crassipes, Cancer antennarius*, and *C. anthonyi*). *Fish. Bull.* **69**:337 – 343.

Kodric-Brown, A. & Brown, J. H. (1984). Truth in advertising: the kinds of traits favoured with sexual selection. *Am. Nat.* **124**:305 – 322.

Lang, F., Govind, C. K., Costello, W. J. & Greene, S. I. (1977). Developmental Neuroethology: Changes in defensive behaviour during growth of the lobster. *Science*. **197**:682 – 685.

Laufer, H., Sagi, A. & Ahl, J. S. B. (1994). Alternate mating strategies of polymorphic males of *Libina emarginata* appear to depend on methyl farnesoate. *Invertrebr. Reprod. Dev.* **26**:41 – 44.

Laufer, H., Ahl, A., Rotllant, G. & Baclaski, B. (2002). Evidence that ecdsteriods and methyl farnesoate control growth and differentiation in a crustacean. *Insect Biochem. Molec.* **32**:205 – 210.

Levitan, D. R. & Petersen, C. (1995). Sperm limitation in the sea. *Trends Ecol. Evol.* **10**:228 – 231.

Lipcius, R. N. & Herrnkind, W. F. (1987). Control and coordination of reproduction in the spiny lobster *Panulirus argus*. *Mar. Biol.* **96**:207 – 214.

Loew, E. R. (1976). Light, and photoreceptor degeneration in the Norway lobster, *Nephrops norvegicus* (L.). *Proc. R. Soc. Lond. B.* **193**:31 – 44.

Loo, L. -O., Baden, S. P. & Ulmestrand, M. (1993). Suspension feeding in adult *Nephrops norvegicus* (L.) and *Homarus gammarus* (L.) (Decapoda). *Netherlands Journal of Sea Research*. **31**:291 – 297.

Love, D. C., Rice, S. D., Moles, D. A. & Eaton, W. D. (1993). Seasonal prevalence and intensity of bitter crab dinoflagellate infection and host mortality in Alaskan tanner crabs *Chionoecetes bairdi* from Auke Bay, Alaska, USA. *Dis. Aquat. Org.* **15**:1 – 7.

Love, D., Thomas, R. & Moles, A. (1996). Bitter crab haemolymph studies: indications of host physiological condition. Symposium on High Latitude Crabs: Biology, Management and Economics.

Lovett, D. L. & Felder, D. L. (1989). Application of regression techniques to studies of relative growth in crustaceans. *J. Crust. Biol.* **9**:529 – 539.

MacDiarmid, A. & Butler, M. (1996). Effects of mate size on reproduction in spiny lobsters. p 57 in Twenty Fourth Annual Benthic Ecology Meeting, held in Columbia, South Carolina, March 7 – 10.

MacDairmid, A. B. & Butler, M. (1999a). Sperm economy and limitation in spiny lobsters. *Behav. Ecol. Sociobiol.* **46**:14 – 24.

MacDairmid, A. B. & Butler, M. J. Jr. (1999b). Sperm limitation in exploited spiny lobsters. *The Lobster Newsletter.* **12**:2 – 3.

MacDairmid, A. B., Stewart, R. & Oliver, M. (1999). *Jasus* females are vulnerable to mate availability. *The Lobster Newsletter.* **12**:1 – 2.

Marconato, A., Tessari, V. & Marin, G. (1995). The mating system of *Xyrichthys movacula*: sperm economy and fertilisation success. *J. Fish. Biol.* **47**:290 – 301.

Marrs, S. J., Tuck, I. D., Atkinson, R. J. A., Stevenson, T. D. I. & Hall, C. (2002a). Position data loggers and logbooks as tools in fisheries research: result of a pilot study and some recommendations. *Fish. Res.* **58**:109 – 117.

Marrs, S. J., Tuck, I. D., Arneri, E., La Mesa, M., Atkinson, R. J. A., Ward, B. & Santojanni, A. with technical support from Stevenson, T. D. I. & Belardinelli, A. (2002b). Technical improvements in the assessment of Scottish *Nephrops* and Adriatic clam fisheries. Final Report to the European Commission. Reference 99/077. Study Project in support of the Common Fisheries Policy (call 99/OJ C122). 276pp + appendices.

Matthews, D. C. (1951). The origin, development, and nature of the spermatophoric mass of the spiny lobster, *Panulirus pencillatus* (Oliver). *Pac. Sci.* **5**:359 – 371.

Maynard Smith, J. (1974). The theory of games and the evolution of animal conflicts. *J. Theor. Biol.* **47**:209 – 221.

Maynou, F. & Sardà, F. (1997). *Nephrops norvegicus* population and morphometrical characteristics in relation to substrate heterogeneity. *Fish. Res.* **30**: 139 – 149.

Melville-Smith, R. (1987). The reproductive biology of *Geryon maritae* (Decapoda, Brachyura) off South West Africa/Namibia. *Crustaceana.* **53**: 259 – 275.

Mercer, J. P. (1975). Spiny lobster *Palinurus vulgaris* (Latreille) on the west coast of Ireland. *Proc. Challenger Soc.* **4**:124 – 125.

Messick, G. A. (1994). *Hematodinium perezi* infections in adult and juvenile blue crab *Callinectes sapidus* form coastal bays of Maryland and Virginia, USA. *Dis. Aquat. Org.* **19**:77 – 88.

Messick, G. A. & Shields, J. D. (2000). Epizootiology of the parasitic dinoflagellate *Hematodinium* sp. in the American blue crab *Callinectes sapidus*. *Dis. Aquat. Org.* **43**:139 – 152.

Messick, G. A., Jordan, S. J. & Van Heukelem, W. F. (1999). Salinity and temperature effects on *Hematodinium* sp. in the blue crab *Callinectes sapidus*. *J. Shellfish Res.* **18**:657 – 662.

Meyers, T. R., Koeneman, T. M., Bothelo, C. & Short, S. (1987). Bitter crab disease: a fatal dinoflagellate infection and marketing problem for Alaskan tanner crabs *Chionoecetes bairdi*. *Dis. Aquat. Org.* **3**:195 – 216.

Minagawa, M. (1999). Quantitative analysis of the seasonality of male reproduction in the spiny lobster *Panulirus japonicus* (Decapoda: Palinuridae). *J. Crust. Biol.* **19**: 276 – 282.

Minegawa, M. & Higuchi, S. (1997). Analysis of size, gonadal maturation, and functional maturity in the spiny lobster *Panulirus japonicus* (Decapoda: Palinuridae). *J. Crust. Biol.* **17**:70 – 80.

Moller, T. H. & Naylor, E. (1980). Environmental influence on locomotor activity in *Nephrops norvegicus* (Crustacea: Decapoda). *J. mar biol. Ass. U.K.* **60**:103 – 113.

Muiño, R., Fernandez, L. González-Gurriarán, E. Freire, J. & Vilar J. A. (1999). Size at maturity of *Liocarcinus depurator* (Brachyura:Portunidae): a reproductive and morphometric study. *J. Mar. Biol. Ass. UK.* **79**:295 – 303.

Munro, H. N. & Fleck, A. (1966). The determination of nucleic acids. *Method. Biochem. Anal.* **14**:113 – 176.

Newman, M. W. & Johnson, C. A. (1975). A disease of blue crabs (*Callinectes sapidus*) caused by a parasitic dinoflagellate, *Hematodinium* sp. *J. Parasitol.* **63**:554 – 557.

Nicolajsen, Á. & Eiriksson, H. (1990). A preliminary report on the reproductive cycle of *Nephrops norvegicus* at the Faeroes. *ICES C.M. 1990/K:3.*

O'Neill, M. (1992). Physiological and morphological maturity in male *Nephrops norvegicus* (L.). BA Mod. Thesis University of Dublin.

Orensanz, J. M., Armstrong, J., Armstrong, D. & Hilborn, R. (1998). Crustacean resources are vulnerable to serial depletion – the multifaceted decline of crab and shrimp fisheries in the Greater Gulf of Alaska. *Rev. Fish Biol. Fisher.* **8**:117 – 176.

Orsi Relini, L., Zamboni, A., Fiorentino, F. & Massi, D. (1998). Reproductive patterns in Norway lobster *Nephrops norvegicus* (L.), (Crustacea Decapoda Nephropidae) of different Mediterranean areas. *Sci. Mar.* **62(Suppl.1)**:25 – 41.

Parker, G. A. (1974). Courtship persistence and female guarding as male time investment strategies. *Behaviour* **48**:157 – 184.

Parker, G. A. & Simmons, L. W. (1996). Parental investment and the control of sexual selection: predicting the direction of sexual competition. *Proc. Roy. Soc. Lond. B.* **263**:315 – 321.

Parslow-Williams, P. J. (1998). Nutritional limitation in populations of the Norway lobster, *Nephrops norvegicus* (L.) in the Firth of Clyde, Scotland. PhD Thesis, University of Glasgow, Scotland.

Parslow-Williams, P. J., Atkinson, R. J. A. & Taylor, A. C. (2001). Nucleic acids as indicators of nutritional condition in the Norway lobster *Nephrops norvegicus*. *Mar. Ecol. Prog. Ser.* **211**:235 – 243.

Paul, A. J. (1984). Mating frequency and viability of stored sperm in the tanner crab, *Chionoecetes bairdi* (Decapoda, Majidae). *J. Crust. Biol.* **4**:375 – 381.

Paul, A. J. (1992). A review of size at maturity in male tanner (*Chionoecetes bairdi*) and king (*Paralithoides camtschaticus*) crabs and the methods used to determine maturity. *Am. Zool.* **32**: 534 – 540.

Paul, A. J. & Paul, J. M. (1992). Second clutch viability of *Chionoecetes bairdi* Rathbun (Decapoda: Majidae) inseminated only at the maturity molt. *J. Crust. Biol.* **12**:438 – 441.

Phillips, B. F., Cobb, J. S. & George, R. W. (1980). General Biology. Chapter 1 in The Biology and Management of Lobsters Volume 1. Physiology and Behaviour. Cobb & Phillips (Eds). Academic Press, New York.

Poulson, E. M. (1946). Investigations on the Danish fishery for and biology of the Norway lobster and the deep sea prawn. *Rep. Dan. Biol. Stn.* **48**:27 – 49.

Powell, G. C., James, K. E. & Hurd, C. L. (1974). Ability of male king crab, *Paralithoides camtschatica*, to mate repeatedly, Kodiak, Alaska, 1973. *Fish. Bull.* **72**:171 – 179.

Quackenbush, L. S. (1994). Lobster Reproduction: A Review. Proceedings of the Fourth International Workshop on Lobster Biology and Management, 1993.

Reddy, P. A., Tuberty, S. R. & Fingerman, M. (1997). Effects of cadmium and mercury on ovarian maturation in the red swamp crayfish, *Procambarus clarkii*. *Ecotox. Environ. Safe.* **37**:62 – 65.

Rice, A. L. & Chapman, C. J. (1971). Observations on the burrows and burrowing behaviour of two mud-dwelling decapod crustaceans, *Nephrops norvegicus* and *Goneplax rhomboides*. *Mar. Biol.* **10**:330 – 342.

Richardson, G. (1996). The effect of light-induced eye damage on the behaviour of *Nephrops norvegicus*. PhD Thesis, University of Leicester.

Rideout, R. M., Burton, M. P. M. & Rose, G. A. (2000). Observations on mass atresia and skipped spawning in northern Atlantic cod, from Smith Sound, Newfoundland. *J. Fish Biol.* **57**:1429 – 1440.

Rosa, R. & Nunes, M .L. (2002). Biochemical changes in the reproductive cycle of the deep-sea decapod *Nephrops norvegicus* on the south coast of Portugal. *Mar. Biol.* **141**: 1001 – 1009.

Rotllant, G., Pascual, N., Sardà, F., Takac, P. & Laufer, H. (2001). Identification of methyl farnesoate in the haemolymph of the Mediterranean deep-sea species Norway lobster, *Nephrops norvegicus. J. Crust. Biol.* **21**:328 – 333.

Salerno, F. (2000). *Nephrops norvegicus.* Some aspects of its behaviour over its life cycle. Report. Univesirta delgi studi di Bologna, Italy, and UMBS Millport.

Salmon, M. (1983). Courtship, mating systems, and sexual selection in decapods. In Studies in Adaptation. The behaviour of higher Crustacea. Rebach, S. & Dunham, D. W. (Eds.). John Wiley & Sons, New York.

Salmon, M. & Hyatt. G. W. (1983). Communication. Chapter 1 in The biology of Crustacea. Vol. 7 Behaviour and Ecology. (F. J. & W. B. Vernberg Eds.) Academic Press, New York.

Sardà, F. (1991). Reproduction and moult synchronism in *Nephrops norvegicus* (L.) (Decapoda, Nephropidae) in the western Mediterranean: is spawning annual or biennial. *Crustaceana.* **60**:186 – 199.

Sardà, F. (1995). A review (1967 – 1900) of some aspects of the life history of *Nephrops norvegicus. ICES mar. Sci. Symp.* **199**:78 – 88.

Sardà, F. (1998). *Nephrops norvegicus* (L.): Comparative biology and fishery in the Mediterranean Sea. Introduction, conclusions and recommendations. *Sci. Mar.* **62 (Suppl. 1)**:5 – 15.

Sarojini, R., Nagabhushanam, R. & Fingerman, M. (1995). Naphthalene-induced atresia in the ovary of the crayfish, *Procambarus clarkii*. *Ecotox. Environ. Safe.* **31**:76 – 83.

Sastry, A. N. (1983). Ecological aspects of reproduction. Chapter 5 in Environmental Adaptations. The Biology of Crustaceans (Series eds. Vernberg, F. G. & Vernberg, W. B.). Academic Press, New York.

Schöne, H. (1968). Agonistic and sexual display in aquatic and semi-terrestrial brachyuran crabs. *Am. Zool.* **8**:611 – 654.

Scrivener, J. C. E. (1971). Antagonistic behaviour of the American lobster, *Homarus americanus* (Milne-Edwards). *Fish. Res. Board Can. Tech. Rep.* **235**: 1 – 128.

Sekkelsten, G. I. (1988). Effect of handicap on mating success in male shore crabs *Carcinus maenas*. *Oikos*. **51**:131 – 134.

Shapiro, D. Y., Marconato, A. & Yoshikawa, T. (1994). Sperm economy in a coral reef fish, *Thalassoma bifasciatum*. *Ecology*. **75**:1334 – 1344.

Shelton, P. M. J., Gaten, E. & Chapman, C. J. (1985). Light and retinal damage in *Nephrops norvegicus* (L.) (Crustacea). *Proc. Roy. Soc. Lond. B.* **226**:217 – 236.

Shields, J. D. (1992). Parasites and symbionts of the crab *Portunus pelagicus* from Moreton Bay, Eastern Australia. *J. Crust. Biol.* **12**:94 – 100.

Shields, J. D. (1994). The parasitic dinoflagellates of marine crustaceans. *Ann. Rev. Fish Dis.* **4**:241 – 271.

Silberbauer, B. I. (1971). The biology of the South African rock lobster *Jasus lalandii* (H. Milne-Edwards). 2. The reproductive organs, mating and fertilisation. *S. Afr., Div. Sea Fish., Invest. Rep.* **93**:1 – 46.

Small, H. J., Wilson, S., Neil, D. M., Hagan, P. & Coombs, G. H. (2002). Detection of the parasitic dinoflagellate *Hematodinium* in the Norway lobster *Nephrops norvegicus* by ELISA. *Dis. Aquat. Org.* **52**:175 - 177.

Smith, R. J. (1980). Rethinking allometry. *J. Theor. Biol.* **87**:97-111.

Smith, R. S. M. (1987). The biology of larval and juvenile *Nephrops norvegicus* (L.) in the Firth of Clyde. PhD Thesis, University of Glasgow.

Smith, L. D. (1990). Patterns of limb loss in the blue crab, *Callinecetes sapidus* Rathbun, and the effects of autonomy on growth. *Bull. Mar. Sci.* **46**:23 – 36.

Smith, B. D. & Jamieson, G. S. (1991). Possible consequences of intensive fishing for males on the mating opportunities of Dungeness crabs. *T. Am. Fish. Soc.* **120**:650 – 653.

Sneddon, L. U., Huntingford, F. A. & Taylor, A. C. (1997) Weapon size versus body size as a predictor of winning in fights between shore crabs, *Carcinus maenas* (L.). *Behav. Ecol. Sociobiol.* **41**:237 – 242.

Snow, C. D. & Neilsen, J. R. (1966). Premating and mating behaviour of the Dungeness crab (*Cancer magister* Dana). *J. Fish. Res. Bd. Canada.* **23**:1319 – 1323.

Somerton, D. A. (1980). A computer technique for estimating the size of sexual maturity in crabs. *Can. J. Fish. Aquat. Sci.* **37** 1488 – 1494.

Stein, R. A. (1976). Sexual dimorphism in crayfish chelae: functional significance linked to reproductive activities. *Can. J. Zool.* **54**:220 – 227.

Stentiford, G. D. (2000). Effects of *Hematodinium* infection on the Norway lobster, *Nephrops norvegicus* (L.). PhD Thesis, University of Glasgow.

Stentiford, G. D., Neil, D. M. & Coombs, G. H. (1999). Changes in the plasma free amino acid profile of the Norway lobster *Nephrops norvegicus* at different stages of infection by a parasitic dinoflagellate (genus *Hematodinium*). *Dis. Aquat. Org.* **38**:151 – 157.

Stentiford, G. D., Neil, D. M., Atkinson, R. J. A. & Bailey, N. (2000a). An analysis of swimming performance in the Norway lobster, *Nephrops norvegicus* L. infected by a parasitic dinoflagellate of the genus *Hematodinium*. *J. Exp. Mar. Biol. Ecol.*. **247**: 169 – 181.

Stentiford, G. D., Neil, D. M. & Coombs, G. H. (2000b). Alterations in the biochemistry and ultrastructure of the deep abdominal flexor muscle of the Norway lobster *Nephrops norvegicus* during infection by a parasitic dinoflagellate of the genus *Hematodinium*. *Dis. Aquat. Org.* **42**:133 – 141.

Stentiford, G. D., Neil, D. M. & Atkinson, R. J. A. (2001a). The relationship of *Hematodinium* infection prevalence in a Scottish *Nephrops norvegicus* population to season, moulting and sex. *ICES J. Mar. Sci.* **58**:814 – 823.

Stentiford, G. D., Neil, D. M. & Atkinson, R. J. A. (2001b). Alteration of burrow-orientated behaviour of the Norway lobster, *Nephrops norvegicus* (L.) during infection by the parasitic dinoflagellate *Hematodinium*. *Mar. Fresh. Behav. Physiol.*. **34**:139 – 156.

Stentiford, G. D., Chang, E. S., Chang, S. A. & Neil, D. M. (2001c). Carbohydrate dynamics and the crustacean hyperglycemic hormone (CHH): Effects of parasitic infection of the Norway lobster (*Nephrops norvegicus*). *Gen. Comp. Endocr.* **121**:13 – 22.

Stentiford, G. D., Neil, D. M. & Coombs, G. H. (2001d). Development and application of an immunoassay diagnostic technique for studying *Hematodinium* infections in *Nephrops norvegicus* populations. *Dis. Aquat. Org.* **46**:223 – 229.

Stentiford, G. D., Green, M., Bateman, K., Small, H. J., Neil, D. M. & Feist, S. W. (2002). Infection by a *Hematodinium*-like parasitic dinoflagellate causes pink crab disease (PCD) in the edible crab *Cancer pagurus*. *J. Invertebr. Pathol.* **79**:179 – 191.

Sterk, W. & Redant, F. (1989). Further evidence on the biennial spawning of the Norway lobster, *Nephrops norvegicus*, in the central North Sea. *ICES C.M. 1989:/K:2*.

Storrow, B. (1912). The prawn (Norway lobster) *Nephrops norvegicus* and the prawn fishery of North Shields. *Rep. Dove. Mar. Lab. (N.S.).* **1**:10 – 31.

Strenth, N. E. & Longley, G. (1990). Reproductive patterns of the subterranean shrimp *Palaemonetes antrorum* Benedict (Decapoda, Palaemonidae) from Central Texas. *Stygologia*. **5**:221 – 224.

Subrahmanyam, C. B. (1963). A note on the annual reproductive cycle of the prawn *Penaeus indicus* (M.-Edw.) of Madras Coast. *Curr. Sci.* **4**: 165 – 166.

Subramoniam, T. (1991). Chemical composition of spermatophores in decapod crustaceans. Chapter 17 in Crustacean sexual biology. Columbia University Press, New York.

Swires, H. J. (1970). Lobster (*Homarus americanus*) fishery and ecology in Port au Port Bay, Newfoundland 1960 – 1965. *Proc. Nat. Shellfish. Ass.* **60**:22 – 39.

SYSTAT (1992). SYSTAT: Statistics. Evanston, Illinois:SYSTAT.

Takac, P., Ahl, J. S. B. & Laufer, H. (1997). Seasonal differences in methyl farnesoate esterase activity in tissues of the spider crab *Libinia emarginata*. *Invertebr. Reprod. Dev.* **31**:311 – 316.

Tamm, G. R. & Cobb, J. S. (1978). Behaviour and the crustacean moult cycle: changes in aggression of *Homarus americanus*. *Science*. **200**:79 – 81.

Tamm, G. R. & Cobb, J. S. (1980). Aggression, dominance and the crustacean moult cycle. *Am. Zool.* **20**:727 – 727.

Taylor, D. M. & Khan, R. A. (1995). Observations on the occurrence of *Hematodinium* sp. (Dinoflagellata: Syndinidae), the causative agent of bitter crab disease in Newfoundland snow crab (*Chionecetes opilio*). *J. Inverbrt. Pathol.* **65**:283 – 288.

Taylor, A. C., Field, R. H. & Parslow-Williams, P. J. (1996). The effects of *Hematodinium* sp.-infection on aspects of the respiratory physiology of the Norway lobster, *Nephrops norvegicus* (L.). *J. Exp. Mar. Biol. Ecol.* **207**:217 – 228.

Templeman, W. (1934). Mating in the American lobster. *Contrib. Can. Biol. Fish Inst.* **8**: 423 – 437.

Thessalou-Legaki, M (1989). Allometric growth of male secondary sexual characters and dioecy in *Parapandalus narval* (Decapoda: Pandalidae). *J. Crust. Biol* .**9**:595 – 600.

Thomas, H. J. (1964). Spawning and fecundity of the Norway lobster (*Nephrops norvegicus* L.) around the Scottish Coast. *J. Cons. Ciem.* **29**:221 – 229.

Thomas, H. J. (1965). The distribution of the Norway lobster around Scotland and the stock composition in areas of different fishing intensity. *Rapp. P-V. Reun. Cons. Perm. Int. Explor. Mer.* **156**:176 – 182.

Thorpe, K. E., Huntingford, F. A. & Taylor, A. C. (1994). Relative size and agonistic behaviour of the female velvet swimming crab, *Necora puber* (L.) (Brachyura, Portunidae). *Behav. Process.* **32**:235 – 246.

Tuck, I. D., Atkinson, R. J. A. & Chapman, C. J. (1994). The structure and seasonal variability in the spatial distribution of *Nephrops norvegicus* burrows. *Ophelia.* **40**:13-25.

Tuck, I. D., Chapman, C. J. & Atkinson, R. J. A. (1997a). Population biology of the Norway lobster, *Nephrops norvegicus* (L.) in the Firth of Clyde, Scotland – I: growth and density. *ICES J. Mar. Sci.* **54**:125 – 135.

Tuck, I. D., Taylor, A. C., Atkinson, R. J. A., Gramitto, M. E. & Smith, C. (1997b). Biochemical composition of *Nephrops norvegicus*: changes associated with ovary maturation. *Mar. Biol.* **129**:505 – 511.

Tuck, I. D., Chapman, C. J., Atkinson, R. J. A., Bailey, N. & Smith, R. S. M. (1997c). A comparison of methods for stock assessment of the Norway lobster, *Nephrops norvegicus*, in the Firth of Clyde. *Fish. Res.* **32**:89 – 100.

Tuck, I. D., Atkinson, R. J. A. & Chapman, C. J. (2000). Population biology of the Norway lobster, *Nephrops norvegicus* (L.) in the Firth of Clyde, Scotland – II: fecundity and size at onset of sexual maturity. *ICES J. Mar. Sci.* **57**:1227 – 1239.

Tully, O. & Hillis, J. P. (1995). Causes and spatial scales of variability in population structre of *Nephrops norvegicus* (L.) in the Irish Sea. *Fish. Res.* **21**:329 – 347.

Tully, O., Hillis, J. P. & McMullan, D. (1989). Fitting normal curves to polymodal length frequencies to assess growth in *Nephrops*. *ICES C.M. 1989/K:32.*

Urbani, N., Sainte-Marie, B., Sévigny, J.-M., Zadworny, D. & Kuhnlein, U. (1998). Sperm competition and paternity assurance during the first breeding period of female snow crab (*Chionoecetes opilio*) (Brahyura: Majidae). *Can. J. Fish. Aquat. Sci.* **55**:1104 – 1113.

Villanelli, F. & Gherardi, F. (1998) Breeding in the crayfish, *Austropotamobius pallipes*: mating patterns, mate choice and intermale competition. *Freshwater Biol.* **40**: 305 – 315.

Watters, G. & Hobday, A. J. (1998). A new method for estimating the morphometric size at maturity of crabs. *Can. J. Fish. Aquat. Sci.* **55**:704 – 714.

Wedell, N. (1997). Ejaculate size in bushcrickets: the importance of being large. *J. Evol. Biol.* **10**:315 – 325.

Wedell, N., Gage, M. J. G. & Parker, G. A. (2002). Sperm competition, male prudence and sperm-limited females. *Trends Ecol. Evol.* **17**:313 – 320.

Welsh, J. (2000). Reproduction in *Nephrops norvegicus*: a study of the vas deferens. BSc Hons Thesis, University of Glasgow.

Wenner, A. M. Fusaro, C. & Oaten, A. (1974). Size at onset of maturity and growth rate in Crustacean populations. *Can. J. Zool.* **52**:1095-1106.

Weiczorek, S. K., Campagnuolo, S., Moore, P. G., Froglia, C., Atkinson. R. J. A., Gramitto, E. M & Bailey, N. (1999). Final Report: The composition and fate of discards from *Nephrops* trawling in Scottish and Italian waters. Reference No. 96/092 (Study project in support of the Common Fisheries Policy, call for proposals 96/C 75) pp 323.

Wilhelm, G. & Mialhe, E. (1996). Dinoflagellate infection associated with the decline of *Necora puber* crab populations in France. *Dis. Aquat. Org.* **26**:213 – 219.

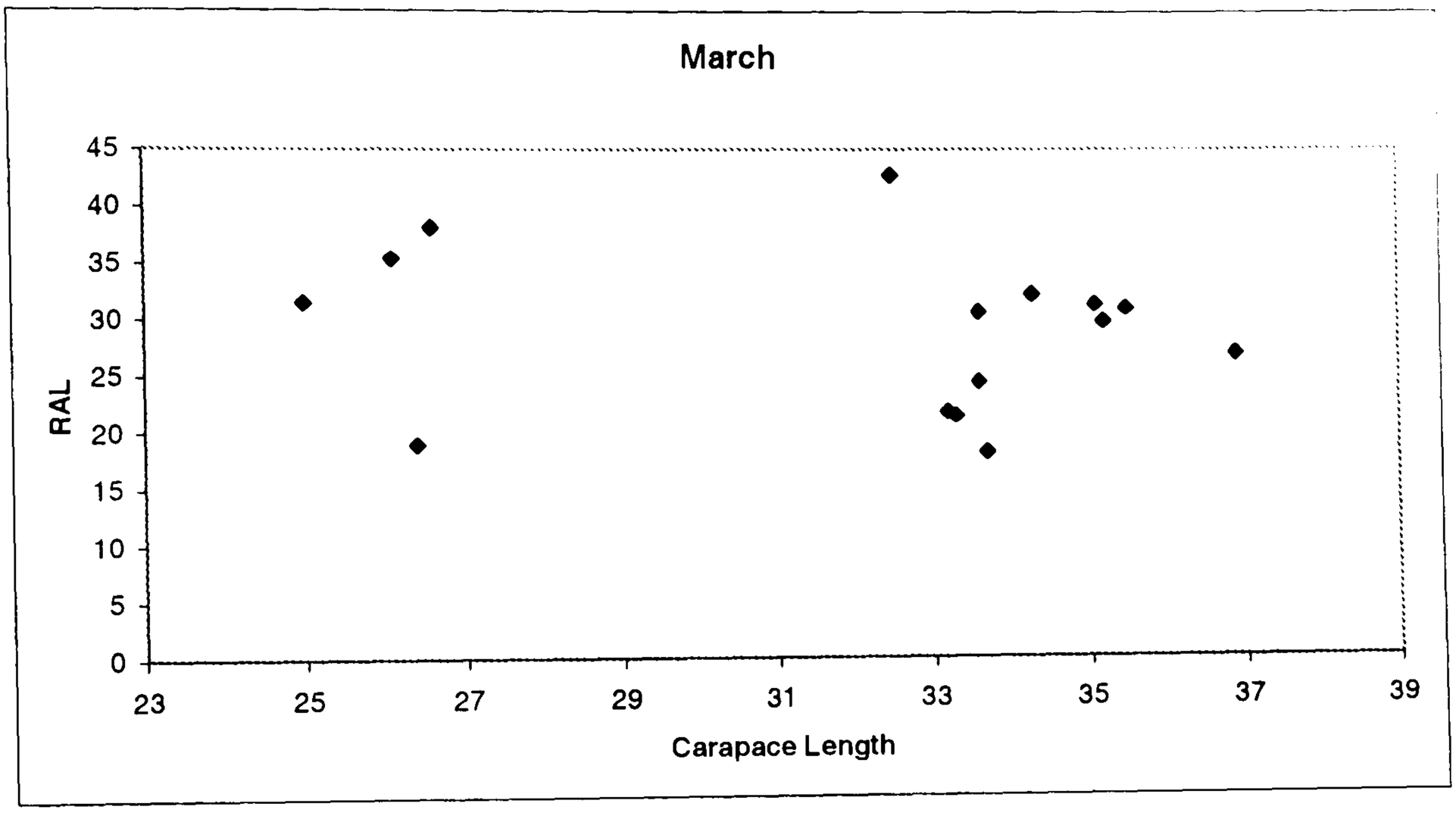
Woodlock, B. & Reynolds, J. D. (1988). Laboratory breeding studies of the freshwater crayfish, *Australopotamobius pallipes* (Lereboullet). *Freshwater Biology*. **19**:71 – 78.

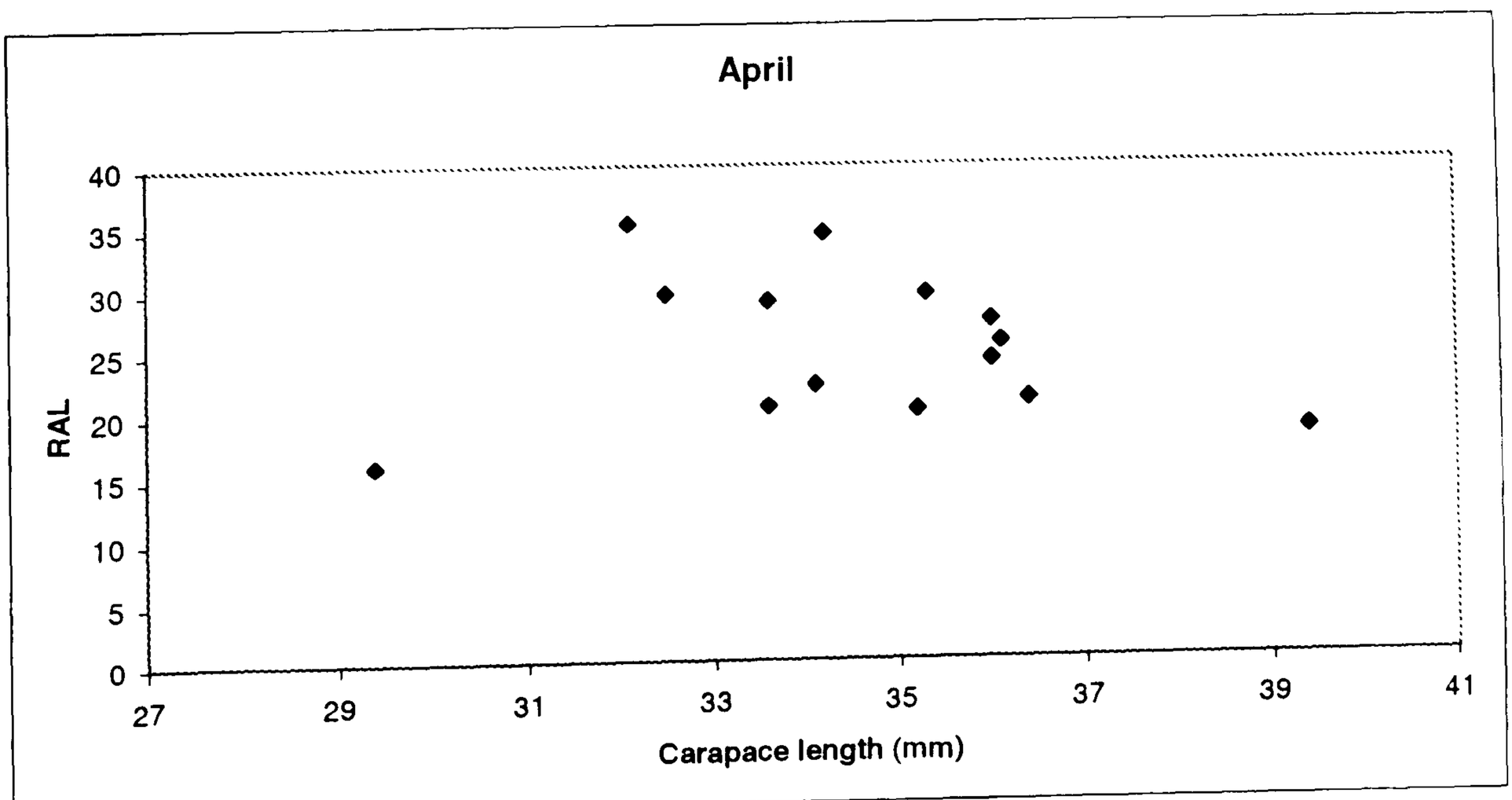
Yund, P. O. (2000). How severe is sperm limitation in natural populations of marine free-spawners? *Trends Ecol. Evol.* **15**:10 – 13.

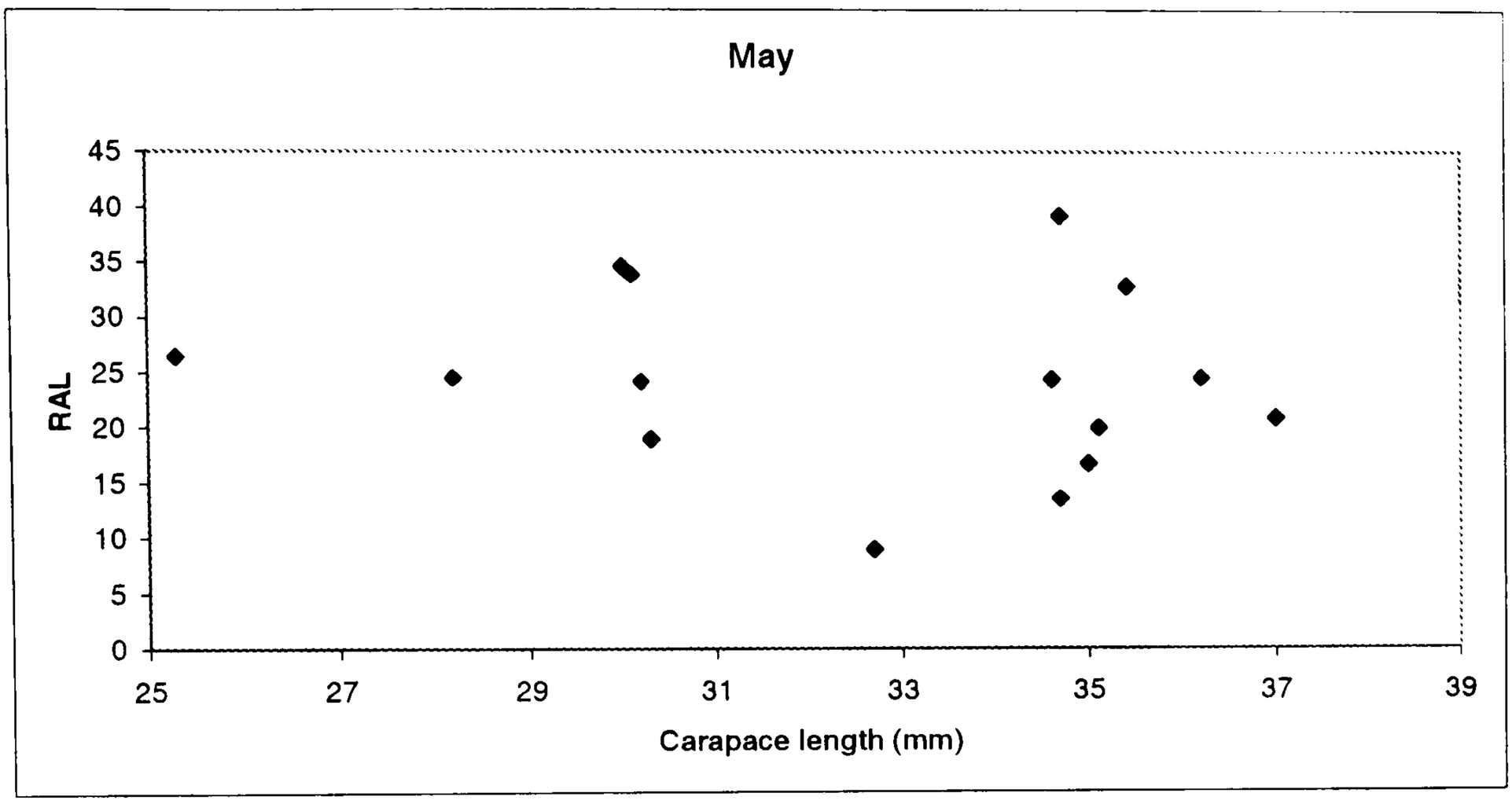
Appendices

The following appendices contain information additional to that displayed in chapters 3 and 4. Appendix 1 contains monthly data from Chapter 3, obtained using measurements of area from the tubules of the testis of *Nephrops norvegicus*. The figures within contain data on the ratio of the area of the lumen of the seminiferous tubule to the area of the tubule itself. Appendix 2 contains graphical results from the fifth order regression analyses carried out on all seasonal data (histological and biochemical) from chapter 3. Appendices 3 – 5 contain data further to that displayed in Chapter 4 on methods for determining the size at first maturity in male *Nephrops norvegicus*, from areas of differing sedimentology. Appendix 3 contains data on the crusher propodite volume of male *N. norvegicus* in relation to carapace length, from different sites within several fishing grounds. Appendix 4 shows the output of reduced major axis analyses on crusher propodite length from the areas of differing sedimentology within the different areas sampled. The figures in Appendix 5 show data on the relationship between claw length and carapace length for male *N. norvegicus* from different sites. This data is divided into mature and immature, based on the results of reduced major axis analyses.

Appendix 1: Monthly data for the ratio of the area of the lumen of the seminiferous tubule to the area of the tubule itself (RAL) data from December is not included as data from two animals only was available.






May
RAL
45
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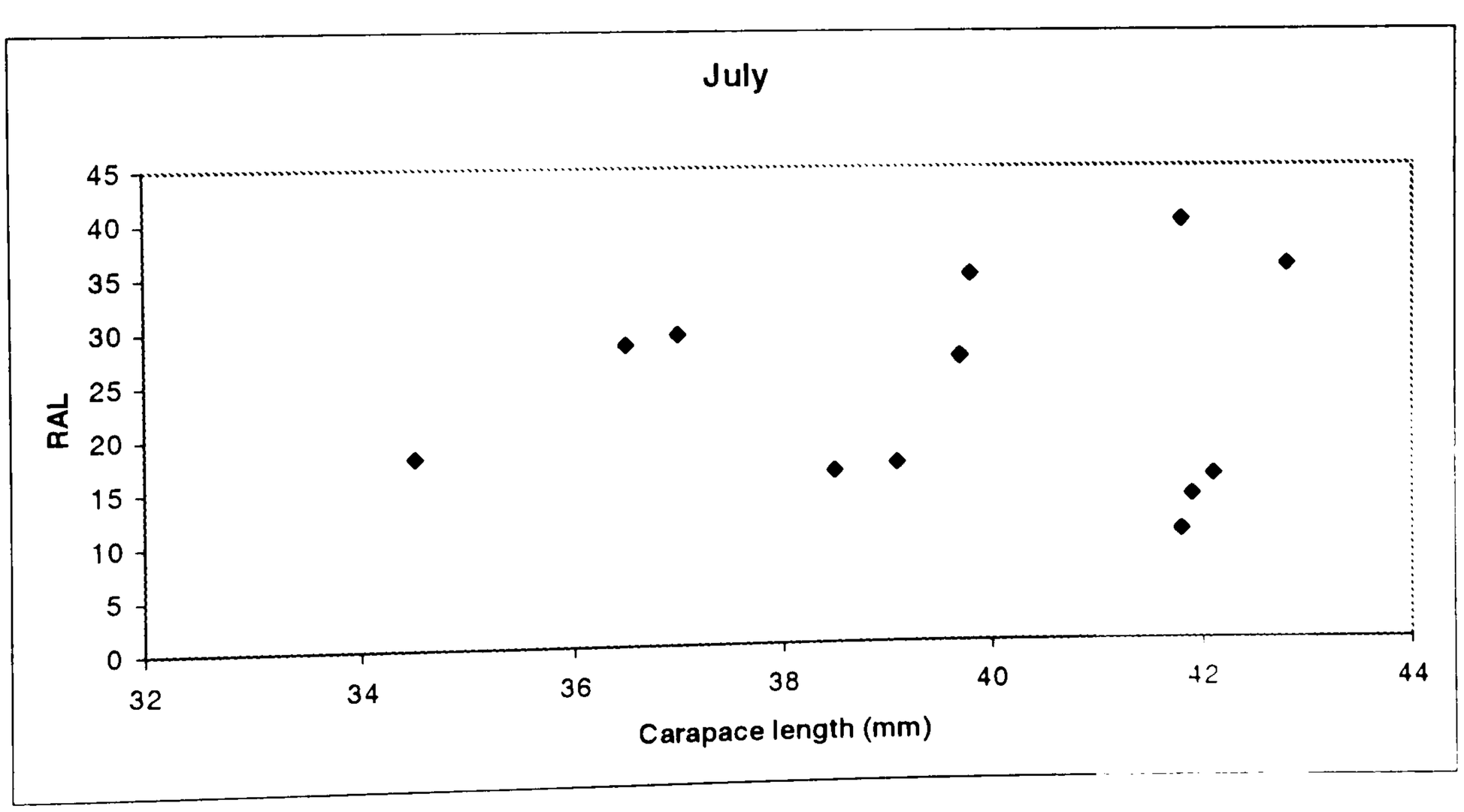
27
29
31
33
35
37
39
Carapace length (mm)


July
RAL
45
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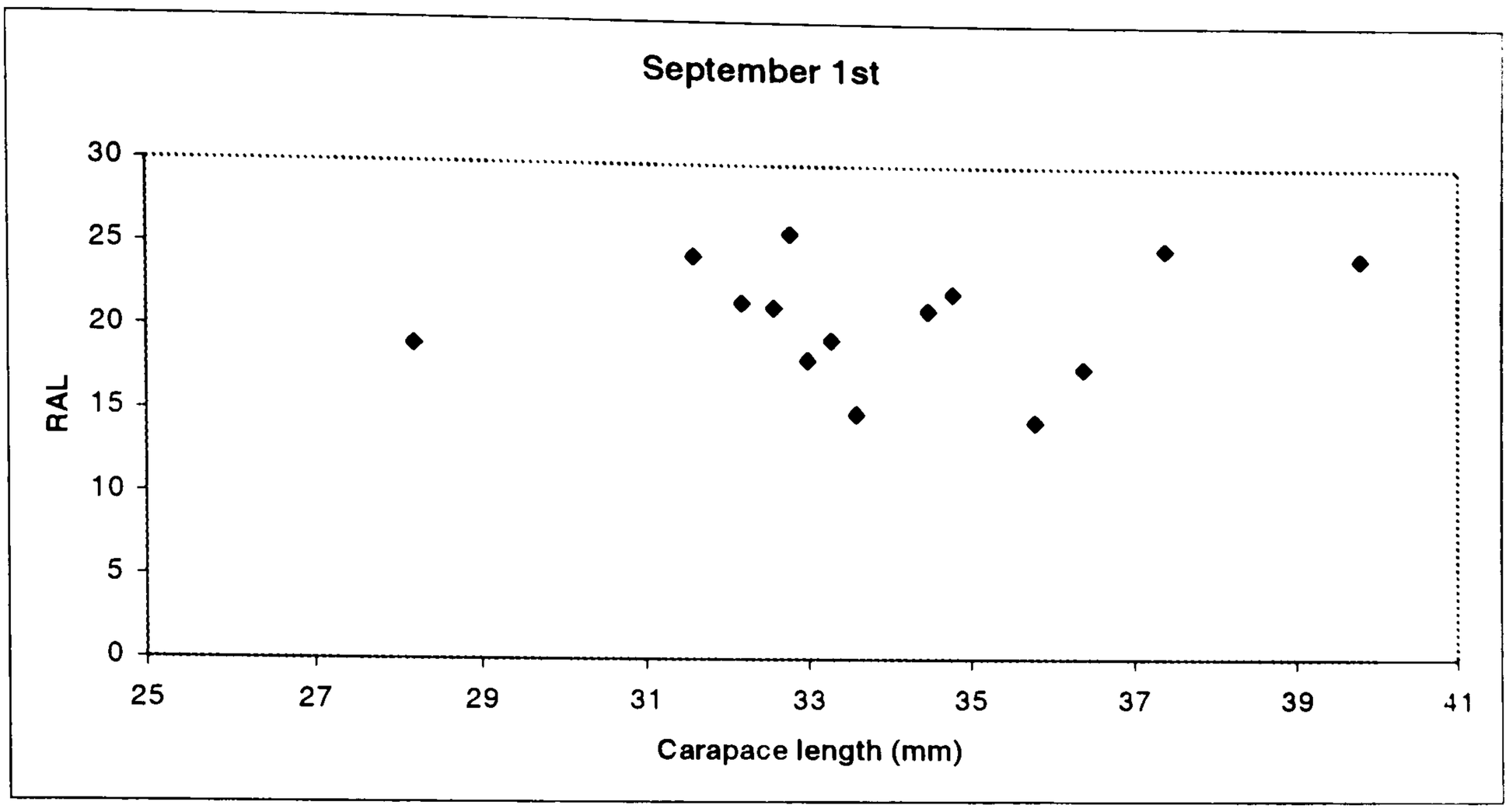
42
44
Carapace length (mm)


September 1st
RAL
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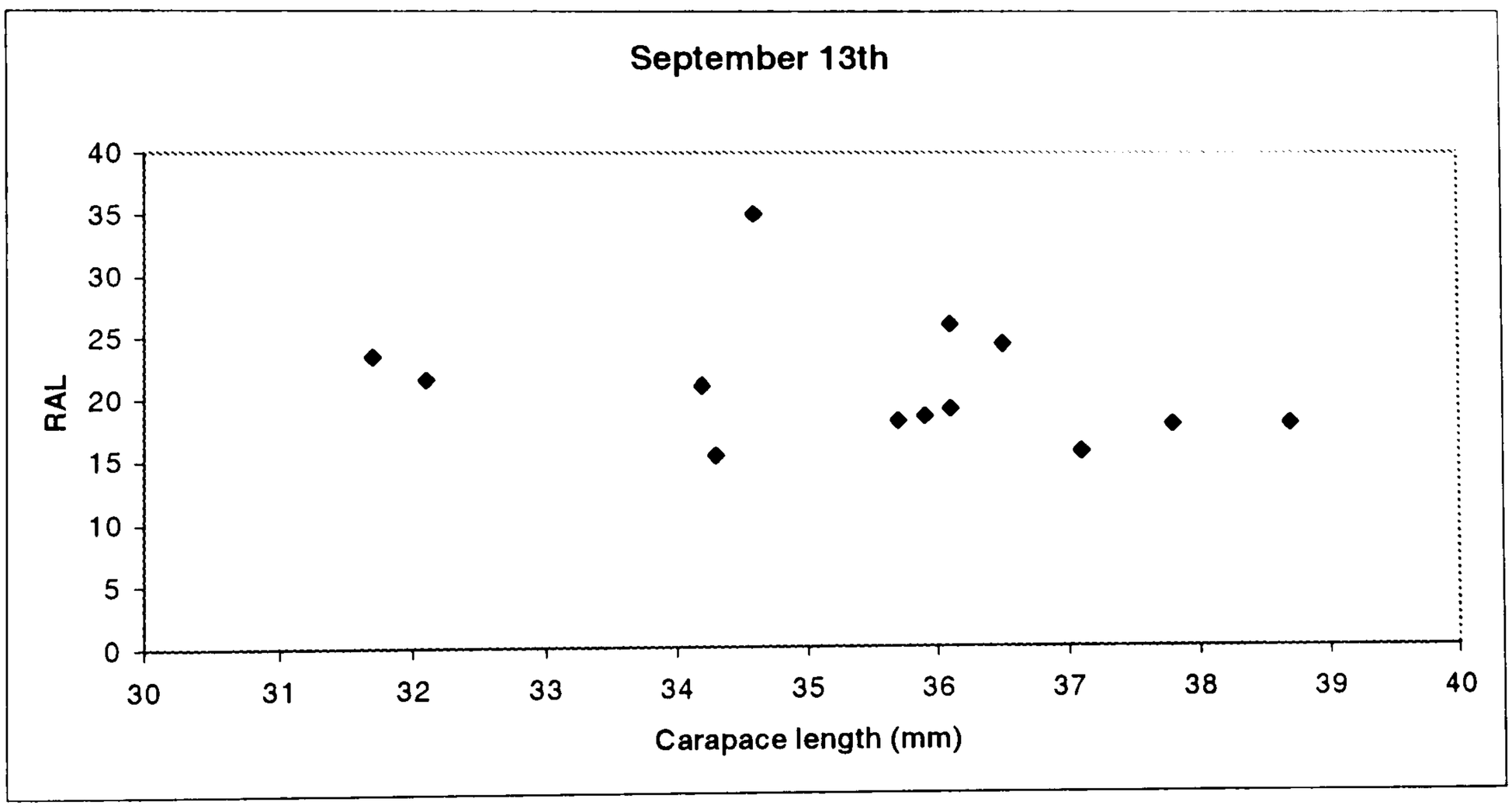
41
Carapace length (mm)


September 13th
RAL
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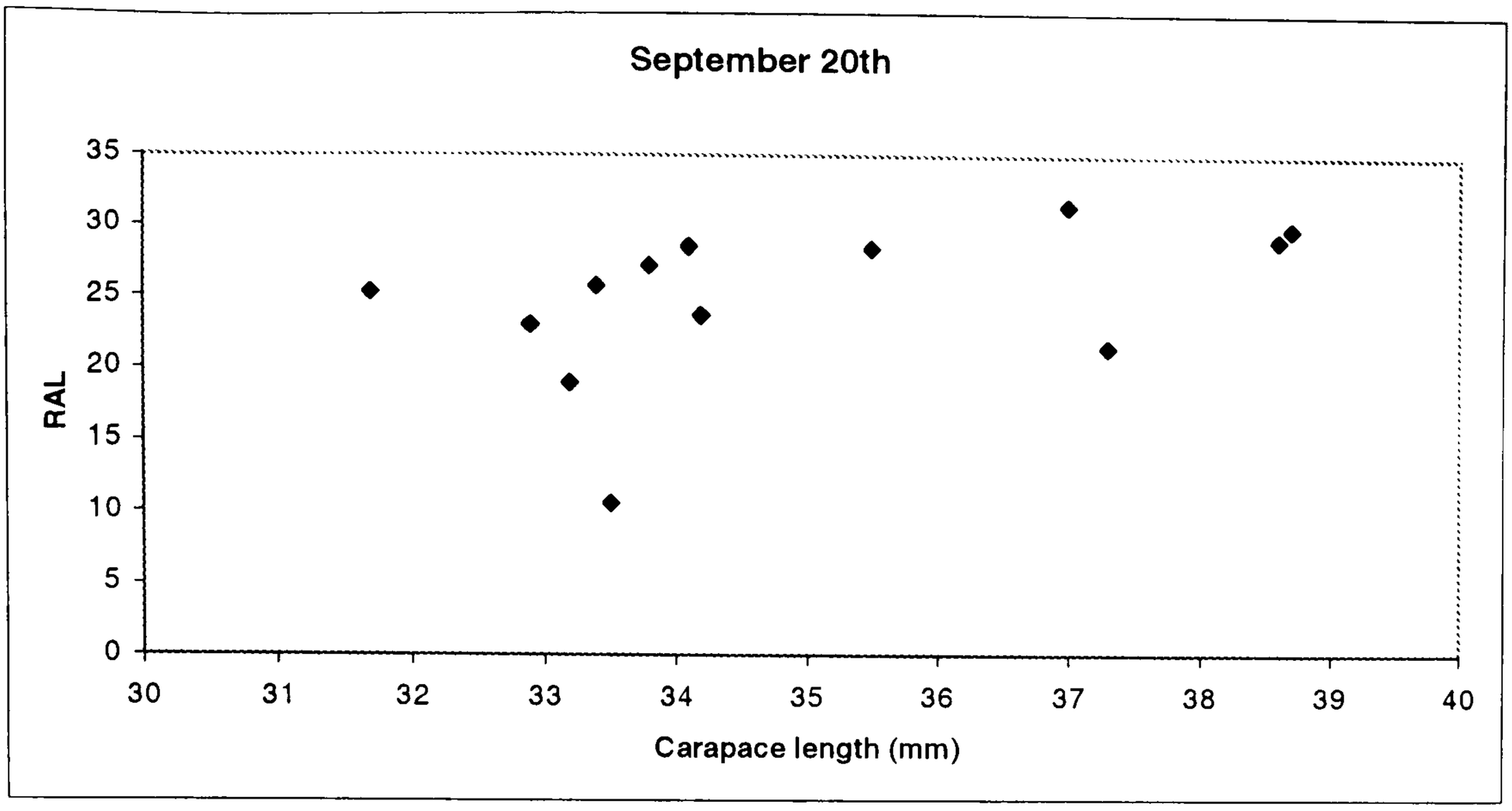
Carapace length (mm)


September 20th
RAL
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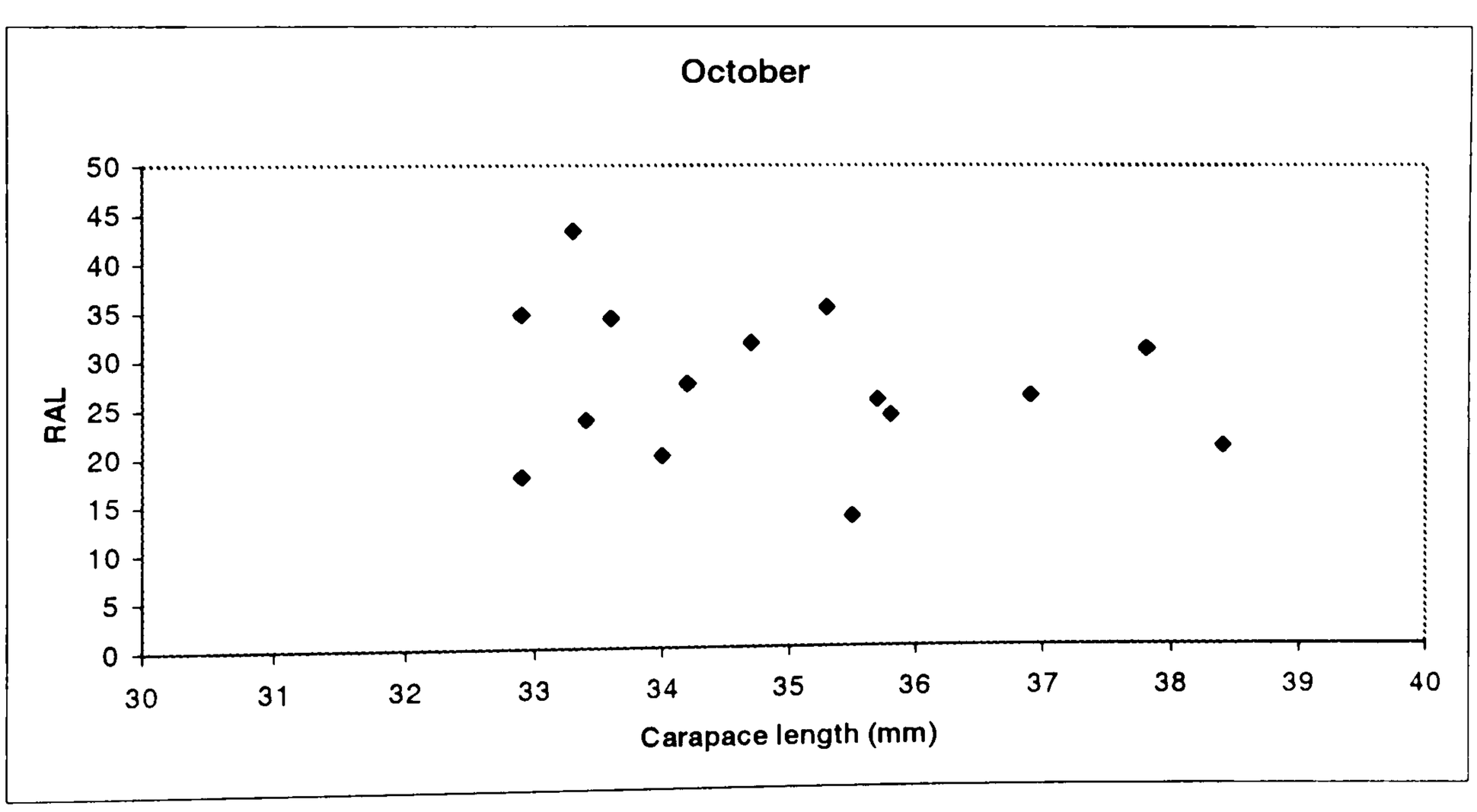
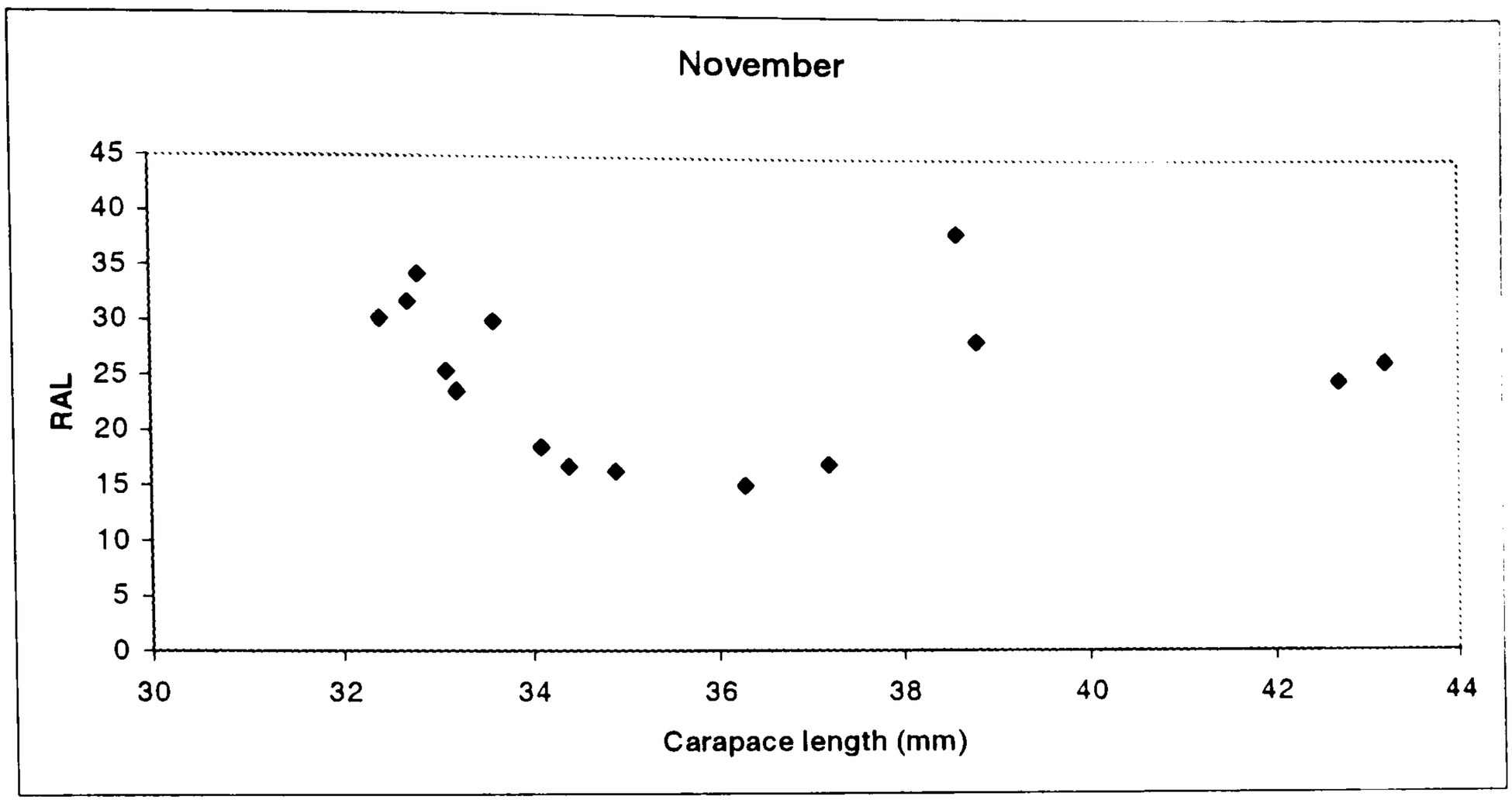
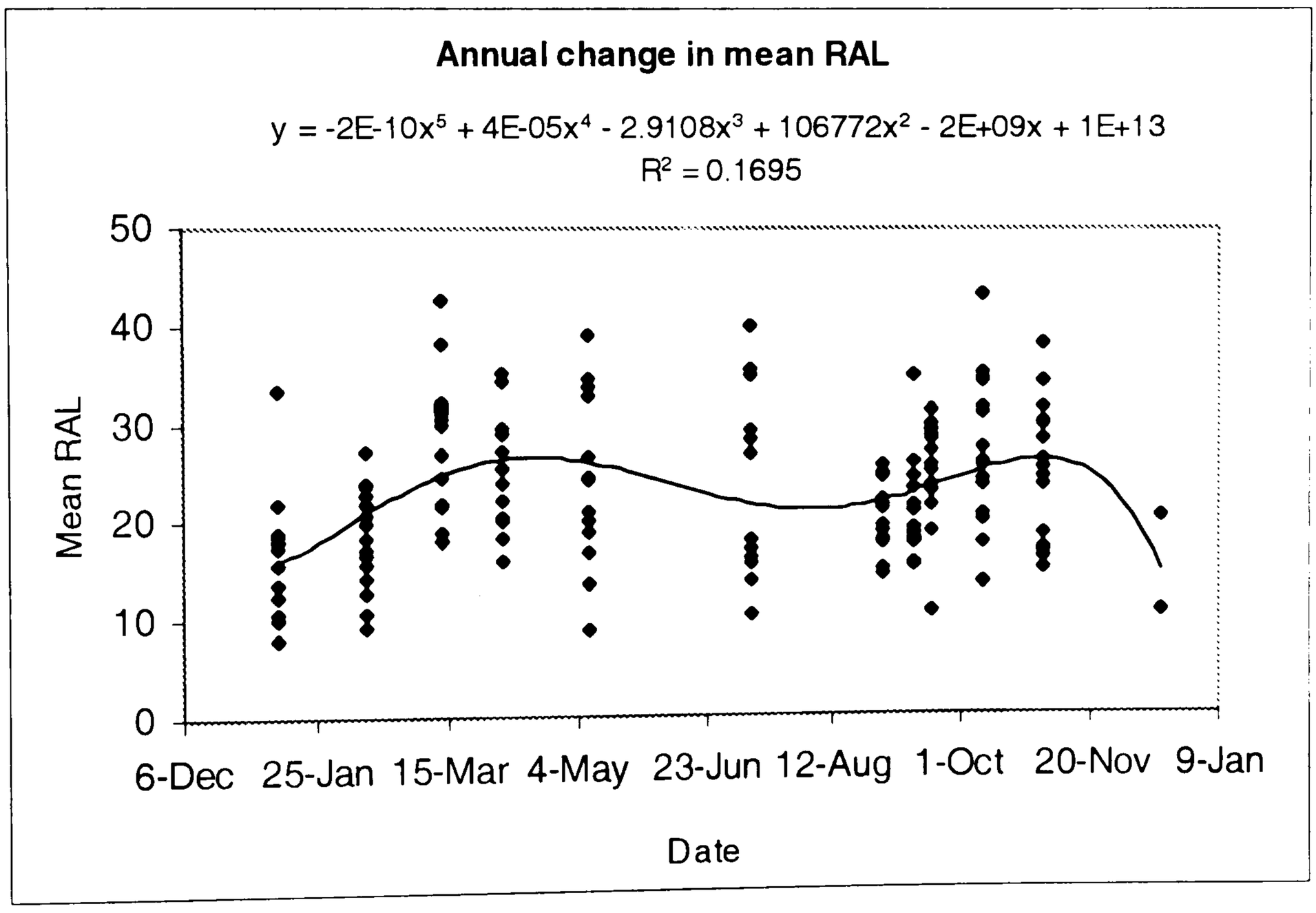
40
Carapace length (mm)

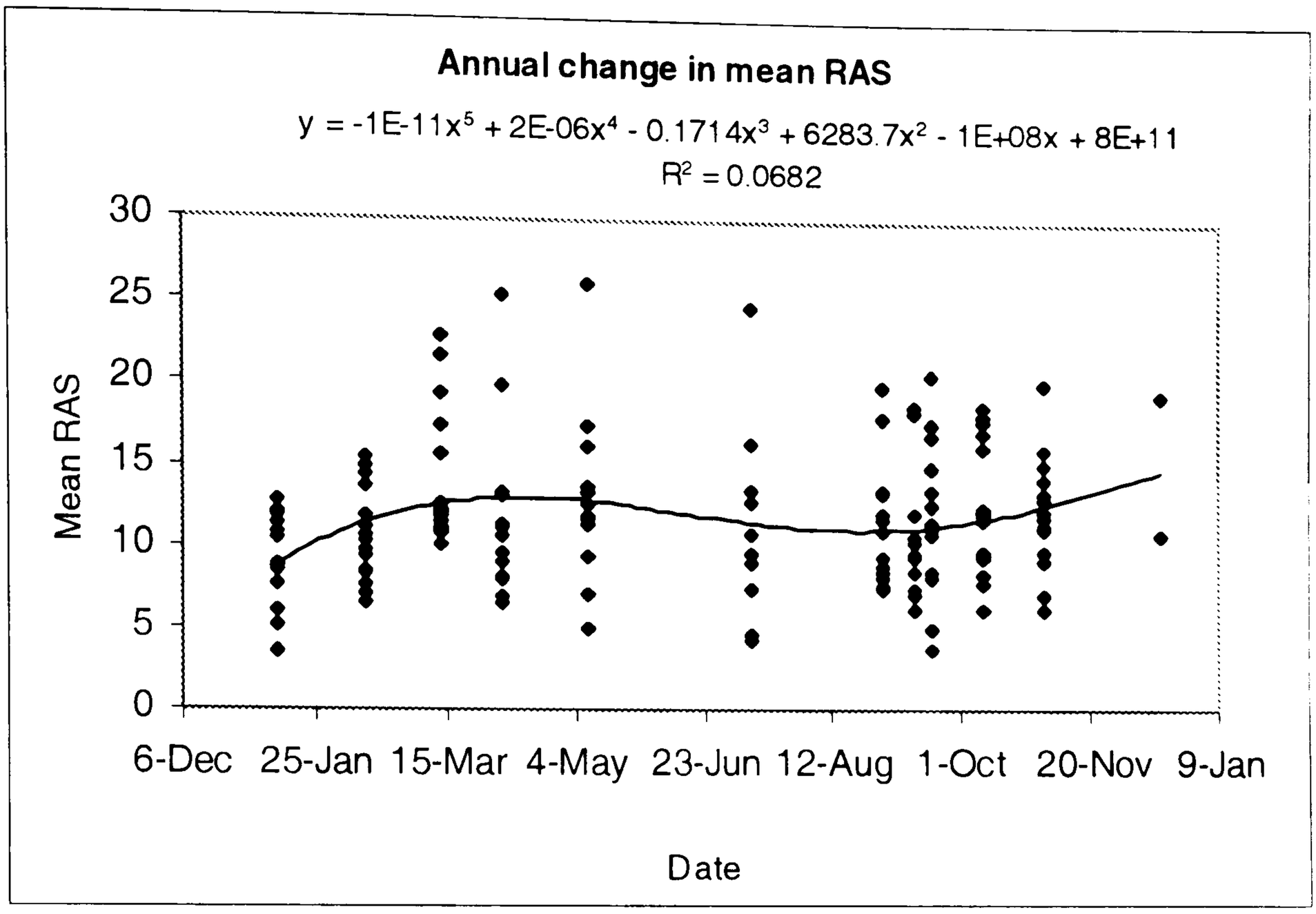

October
RAL
50
45
40
35
30
25
20
15
10
5
0
30
31
32
33
34
35
36
37
38
39
40
Carapace length (mm)

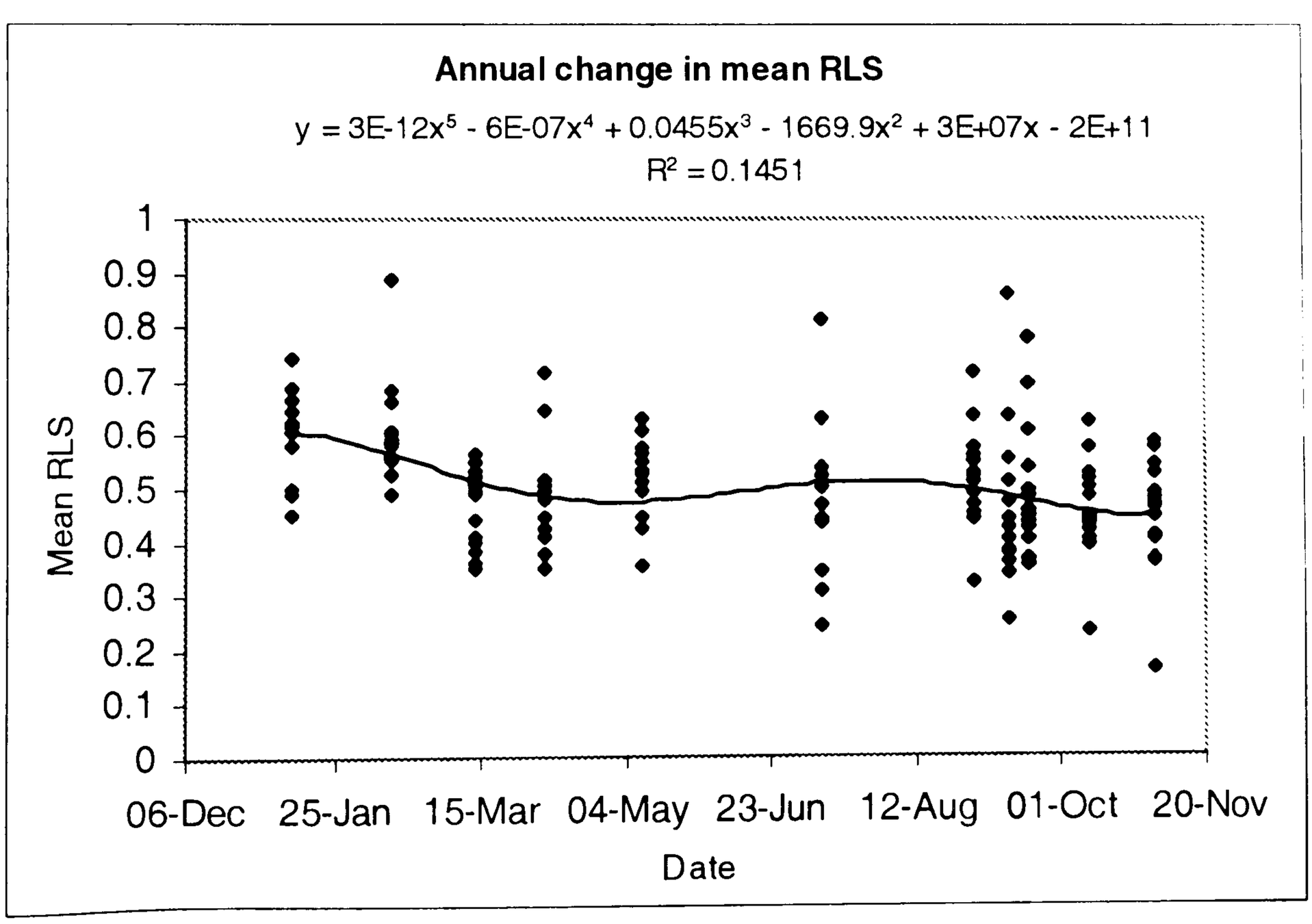

November
RAL
45
40
35
30
25
20
15
10
5
0
30
32
34
36
38
40
42
44
Carapace length (mm)

Appendix 2: Non-linear regressions for all seasonal data presented in Chapter 3, including both histological and biochemical data. Fifth order regressions carried out as these best fitted the data, however, the R^2 values produced using this technique are very small indicating that only a small proportion of the variance is described by the regression.

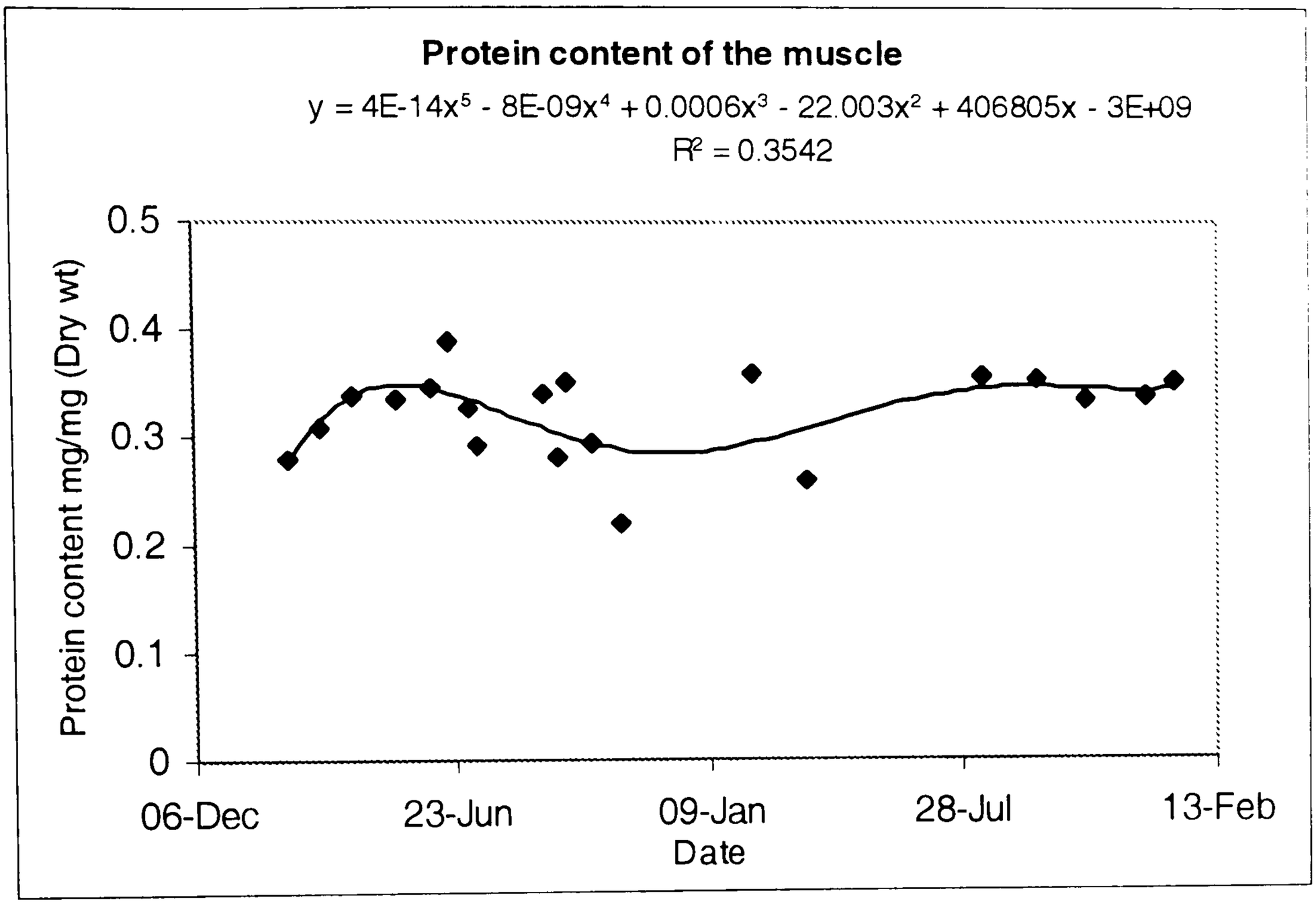
Histological Results

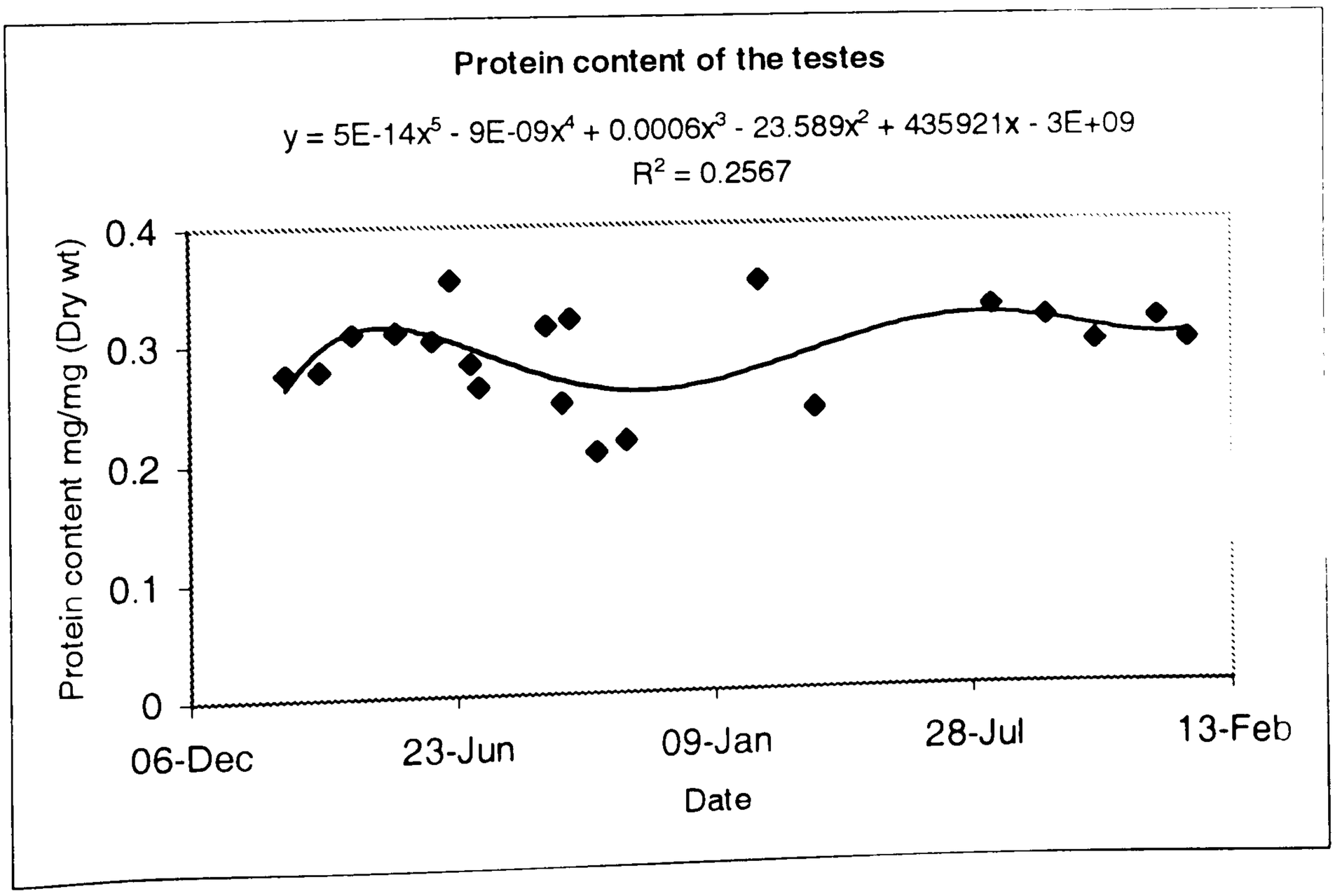


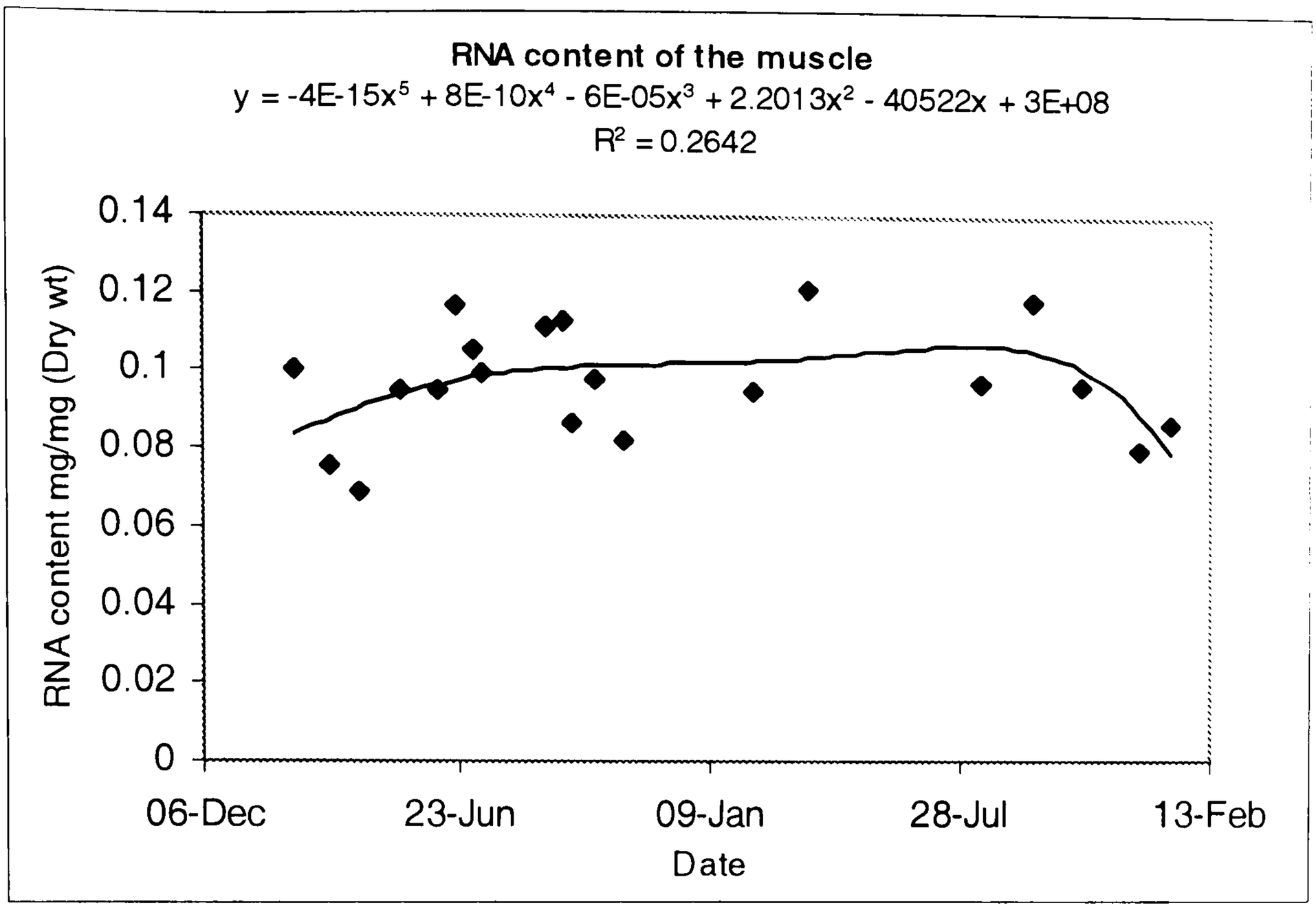

Annual change in mean RAS
y = -1E-11x5 + 2E-06x4 - 0.1714x3 + 6283.7x2 - 1E+08x + 8E+11
R2 = 0.0682
Mean RAS
30
25
20
15
10
5
0
6-Dec
25-Jan
15-Mar
4-May
23-Jun
12-Aug
1-Oct
20-Nov
9-Jan
Date

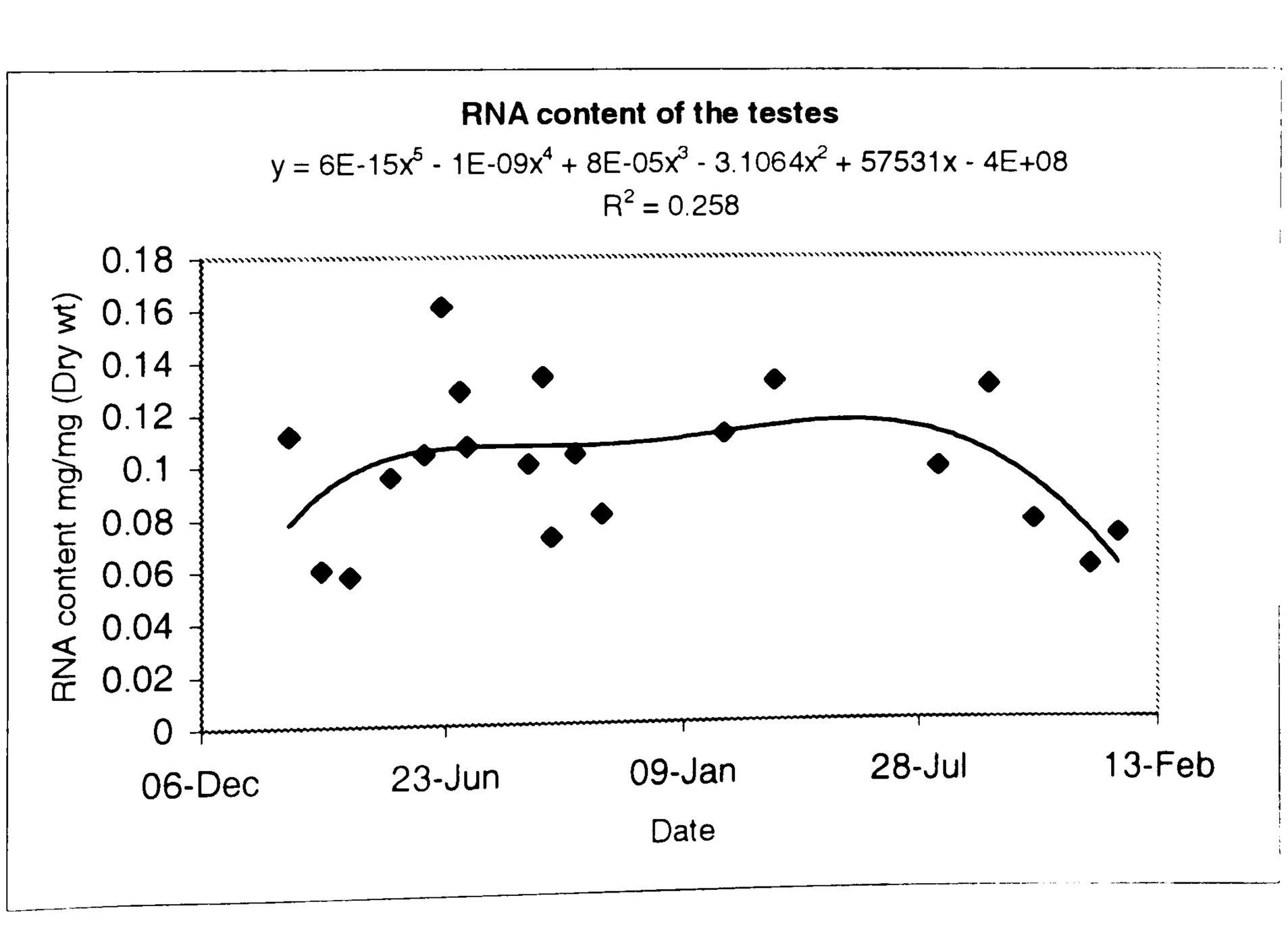

Annual change in mean RLS
y = 3E-12x5 - 6E-07x4 + 0.0455x3 - 1669.9x2 + 3E+07x - 2E+11
R2 = 0.1451
Mean RLS
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
06-Dec
25-Jan
15-Mar
04-May
23-Jun
12-Aug
01-Oct
20-Nov
Date

Biochemical Results

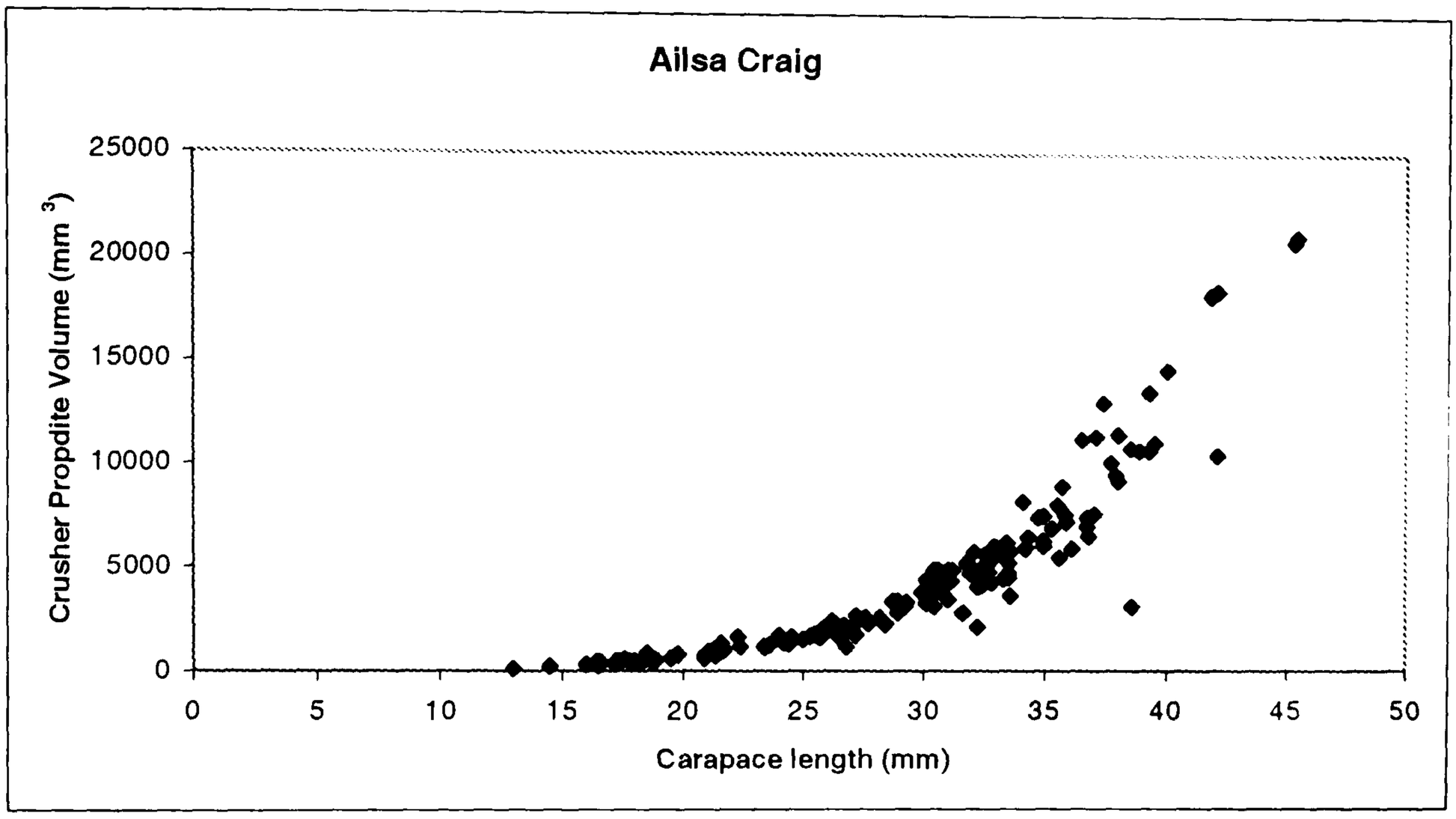


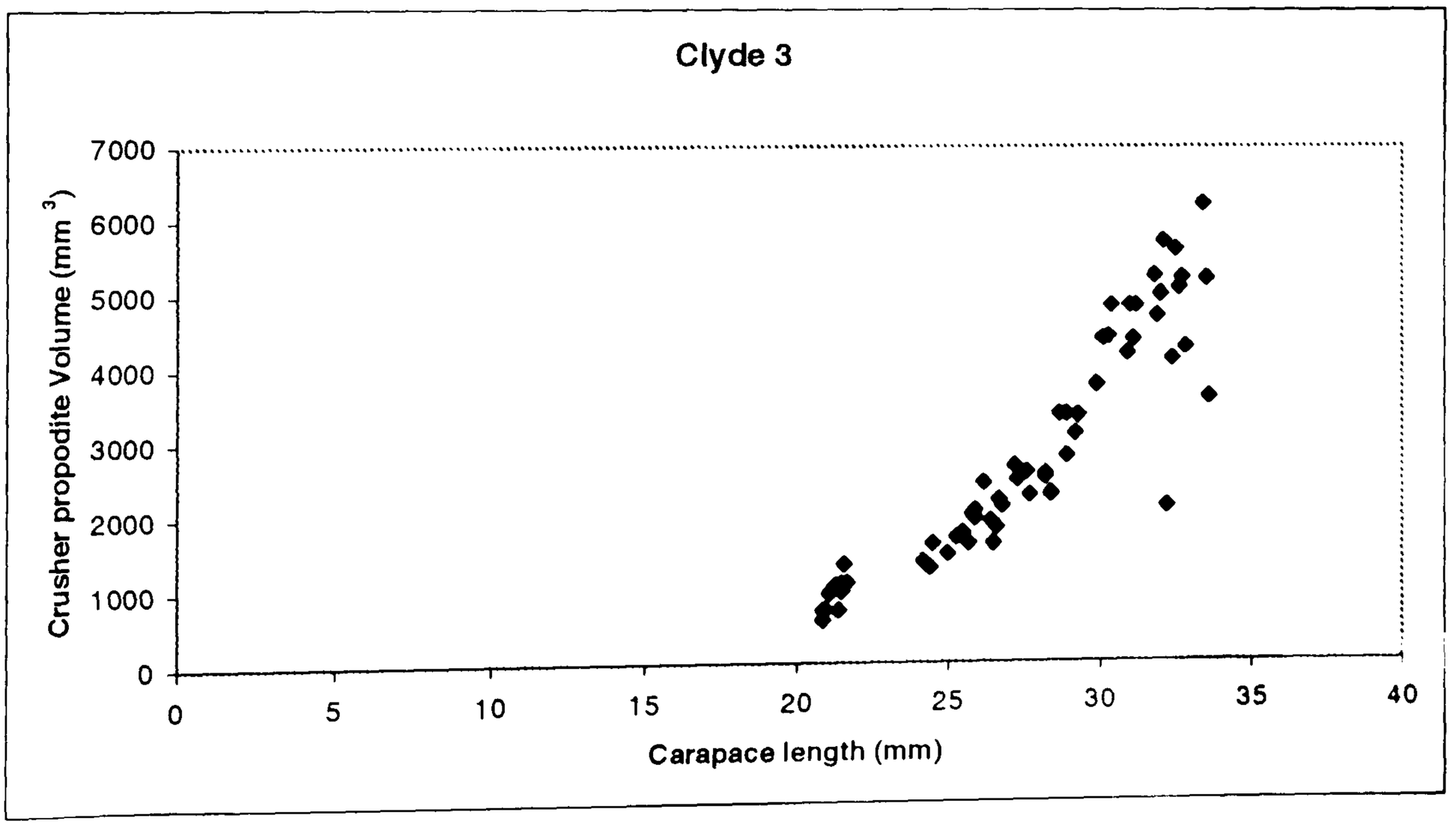


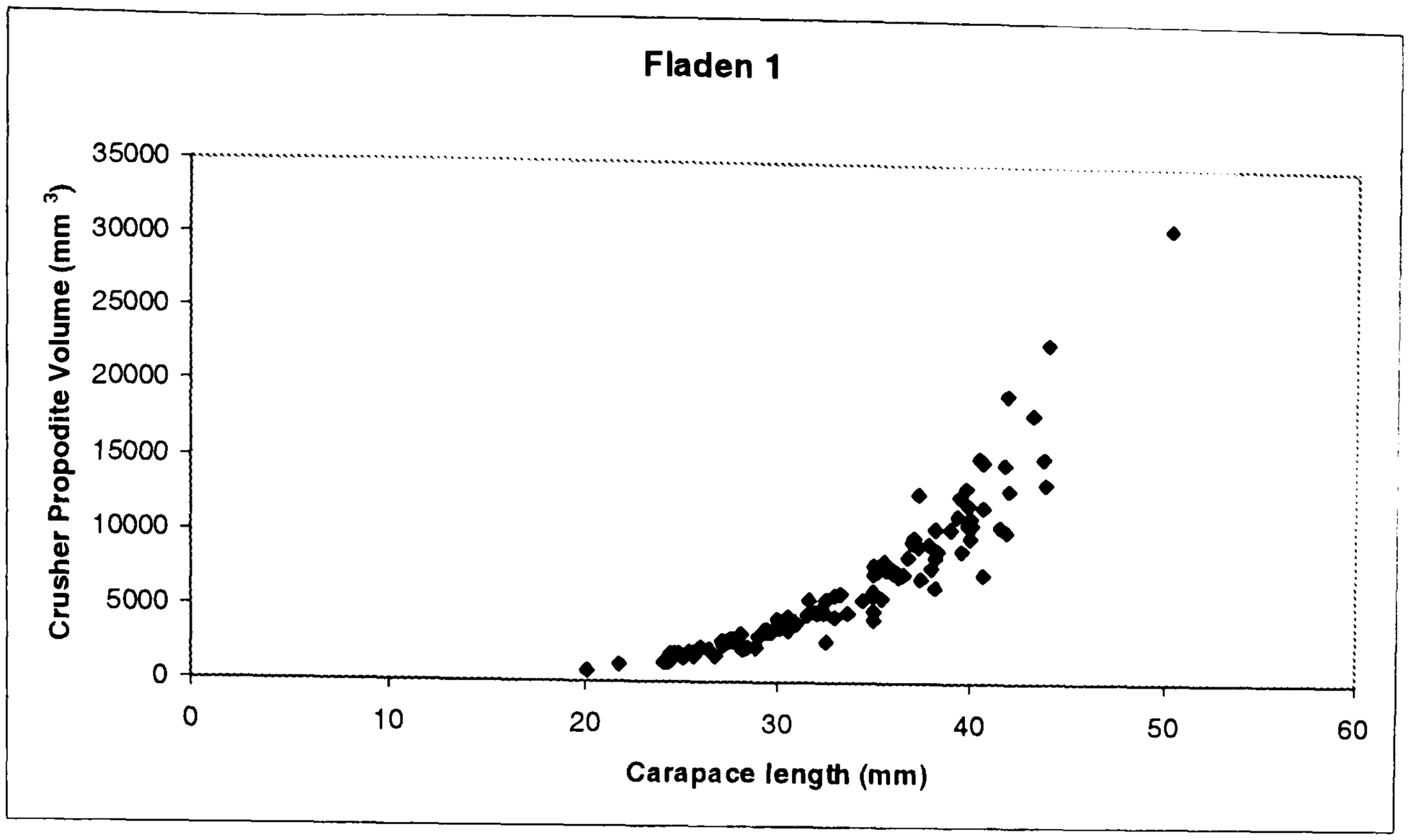

RNA content of the muscle
y = -4E-15x5 + 8E-10x4 - 6E-05x3 + 2.2013x2 - 40522x + 3E+08
R2 = 0.2642
RNA content mg/mg (Dry wt)
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
06-Dec
23-Jun
09-Jan
28-Jul
13-Feb
Date

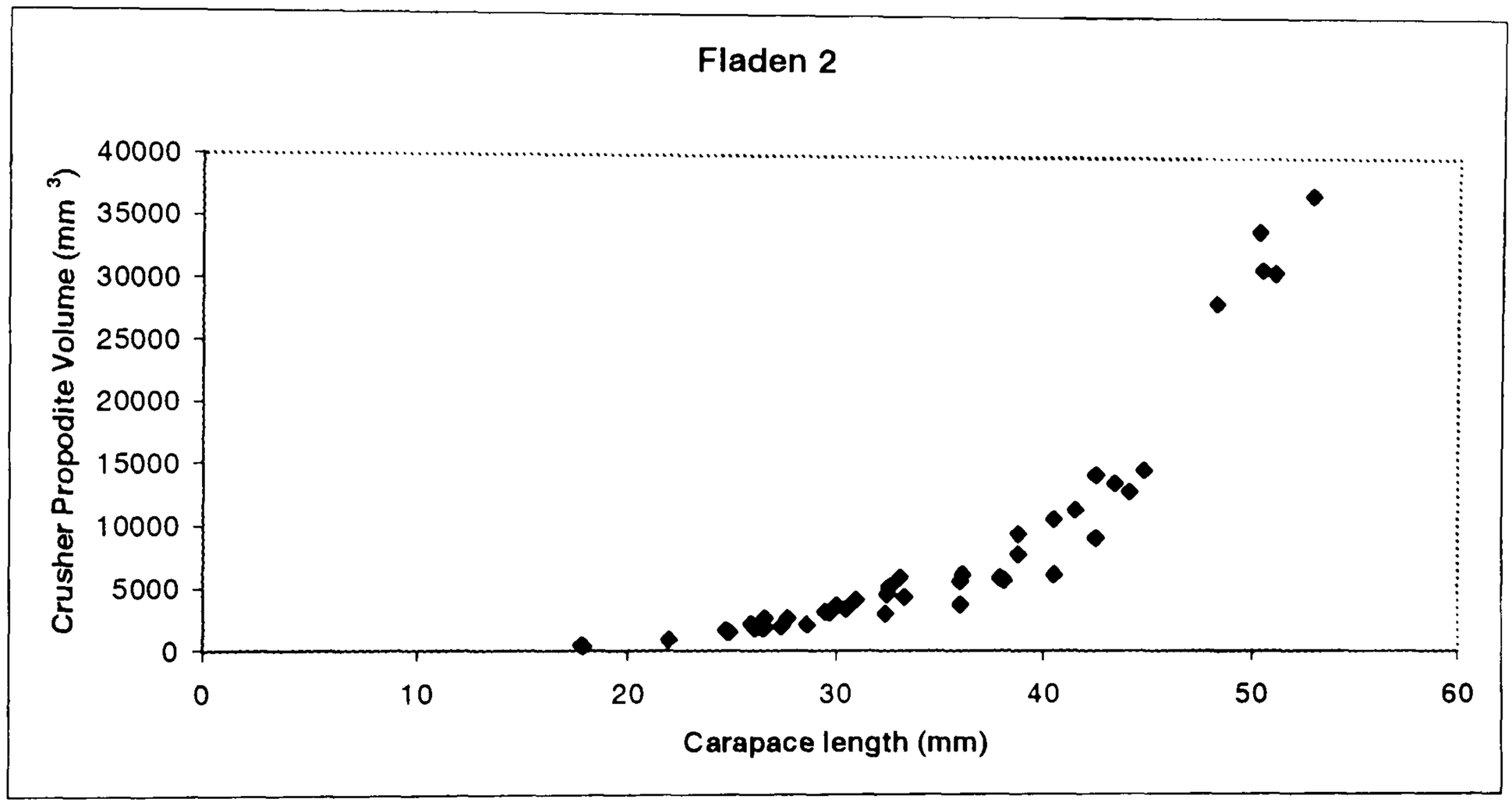

RNA content of the testes
y = 6E-15x5 - 1E-09x4 + 8E-05x3 - 3.1064x2 + 57531x - 4E+08
R2 = 0.258
RNA content mg/mg (Dry wt)
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
06-Dec
23-Jun
09-Jan
28-Jul
13-Feb
Date

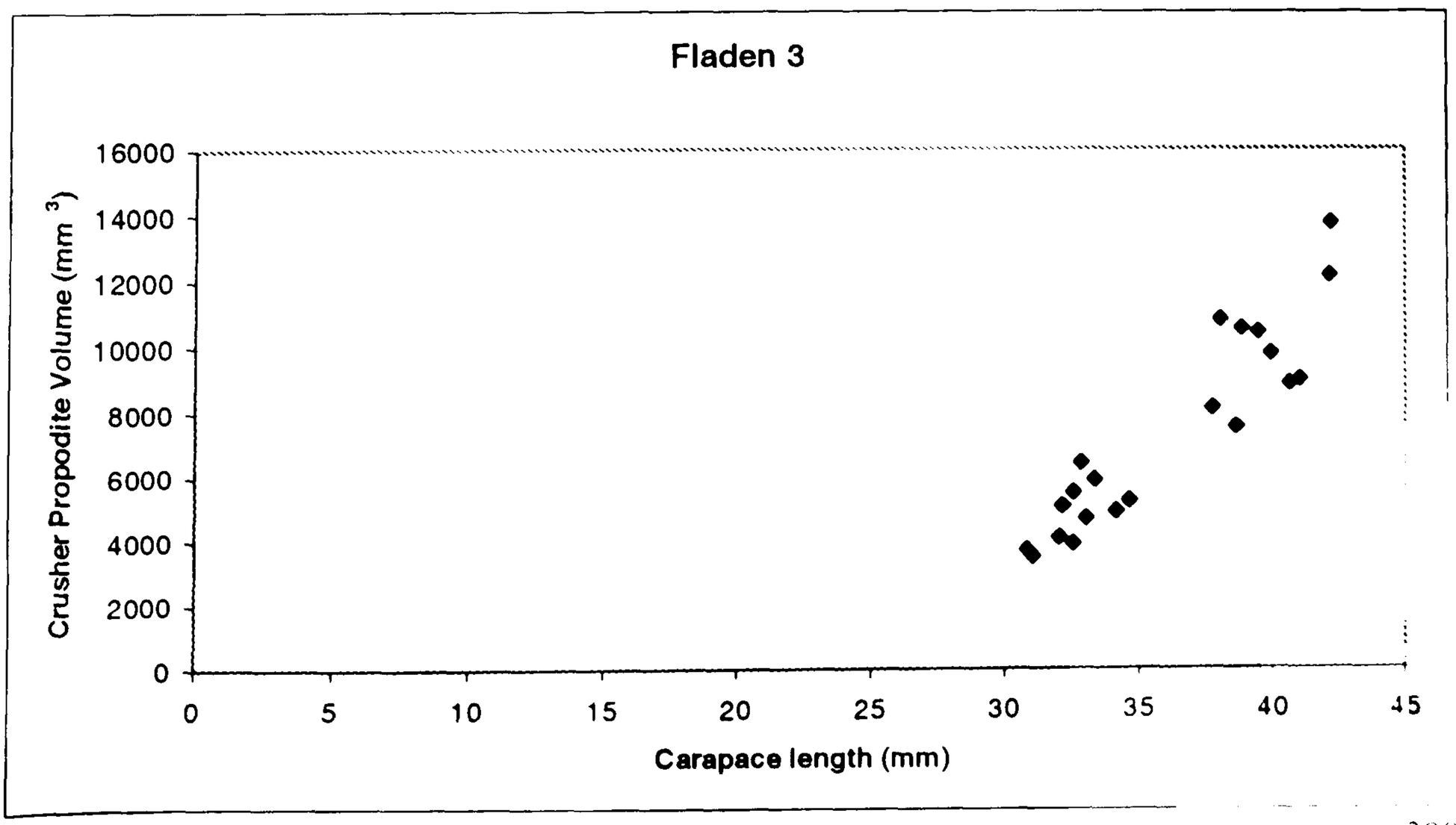
Appendix 3: The relationship between crusher propodite volume and carapace length for male *Nephrops norvegicus* sampled from the Clyde, the Fladen ground and the North and South Minches. The data is further to that displayed in Chapter 4.

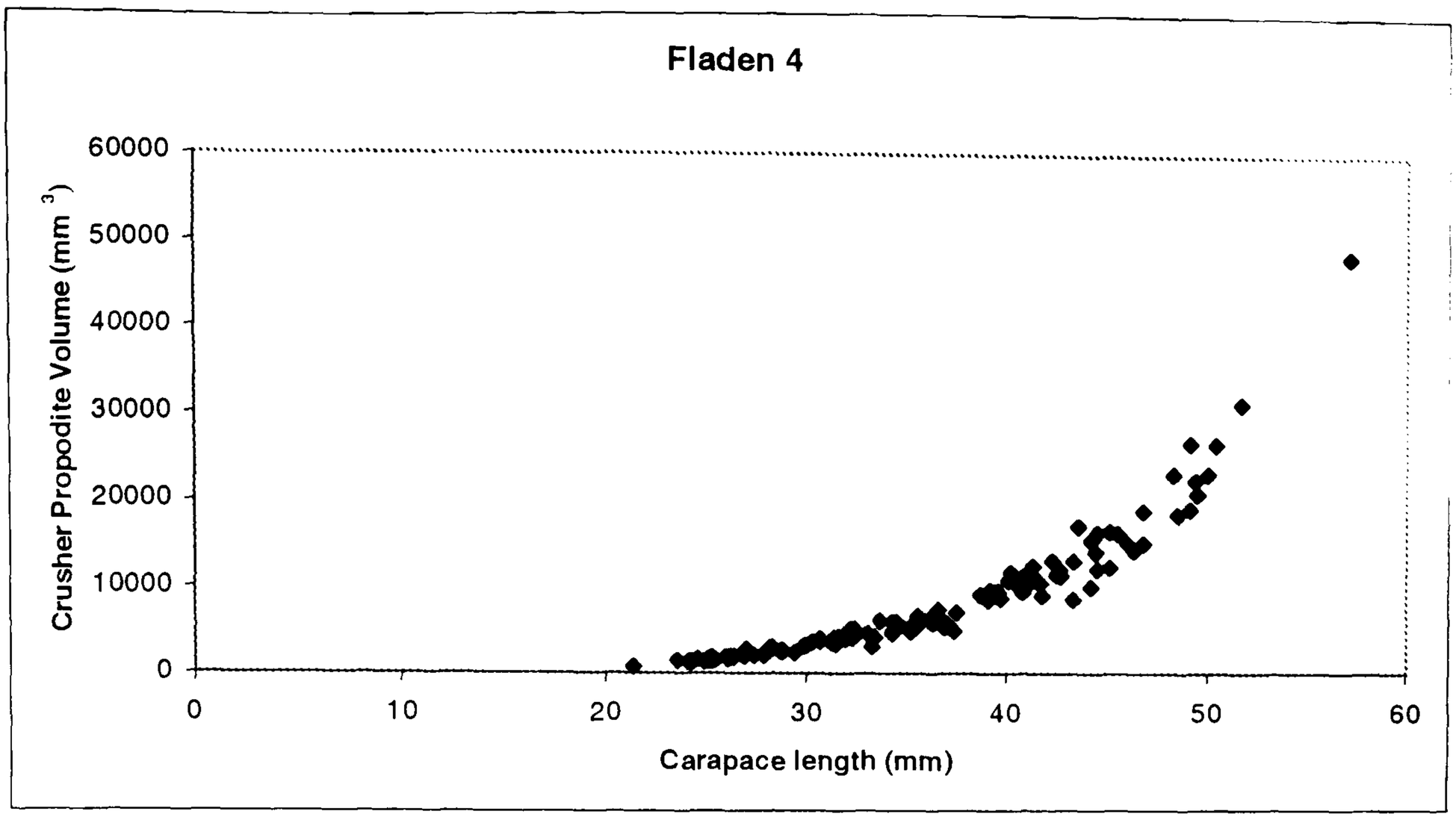


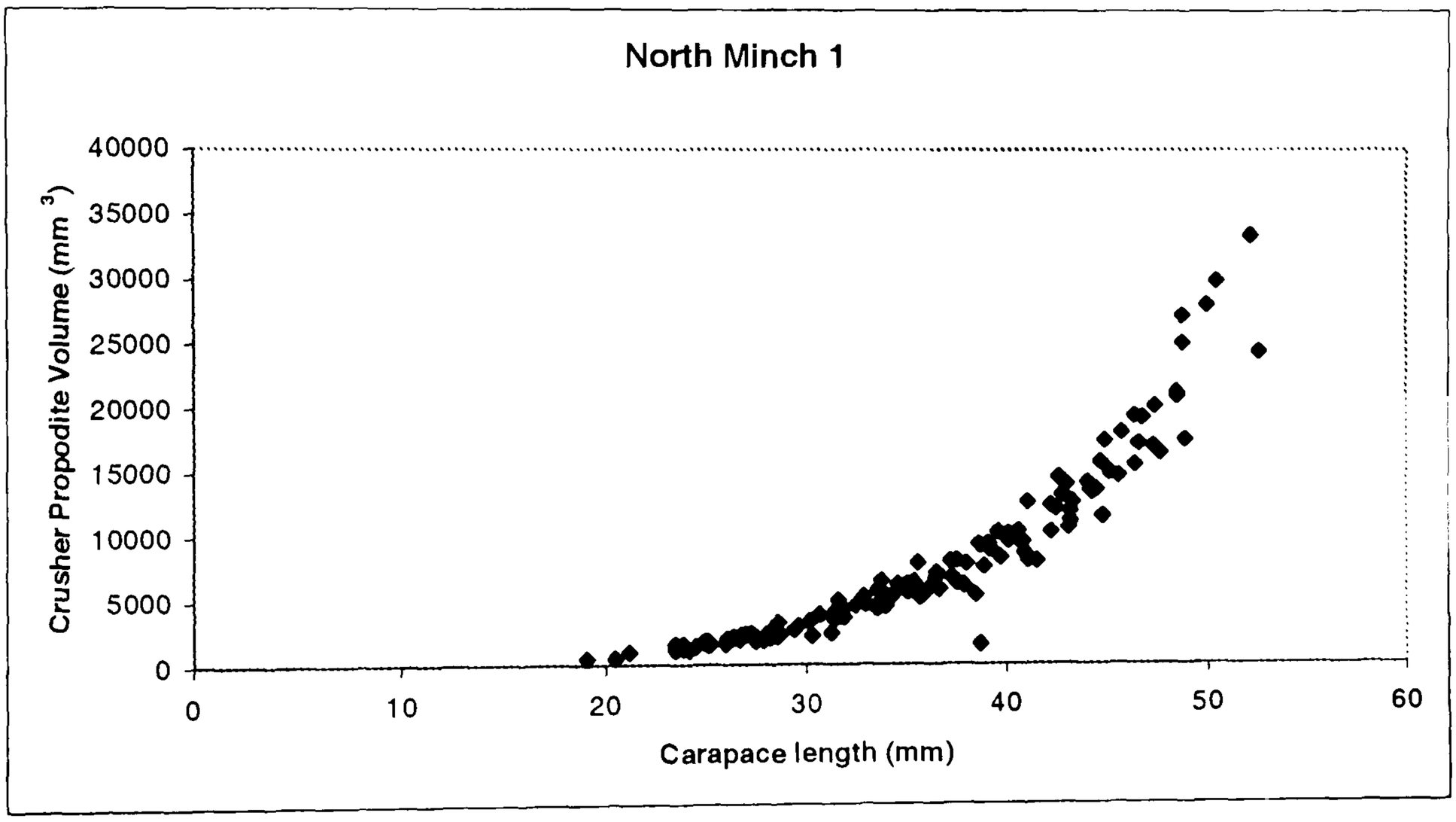


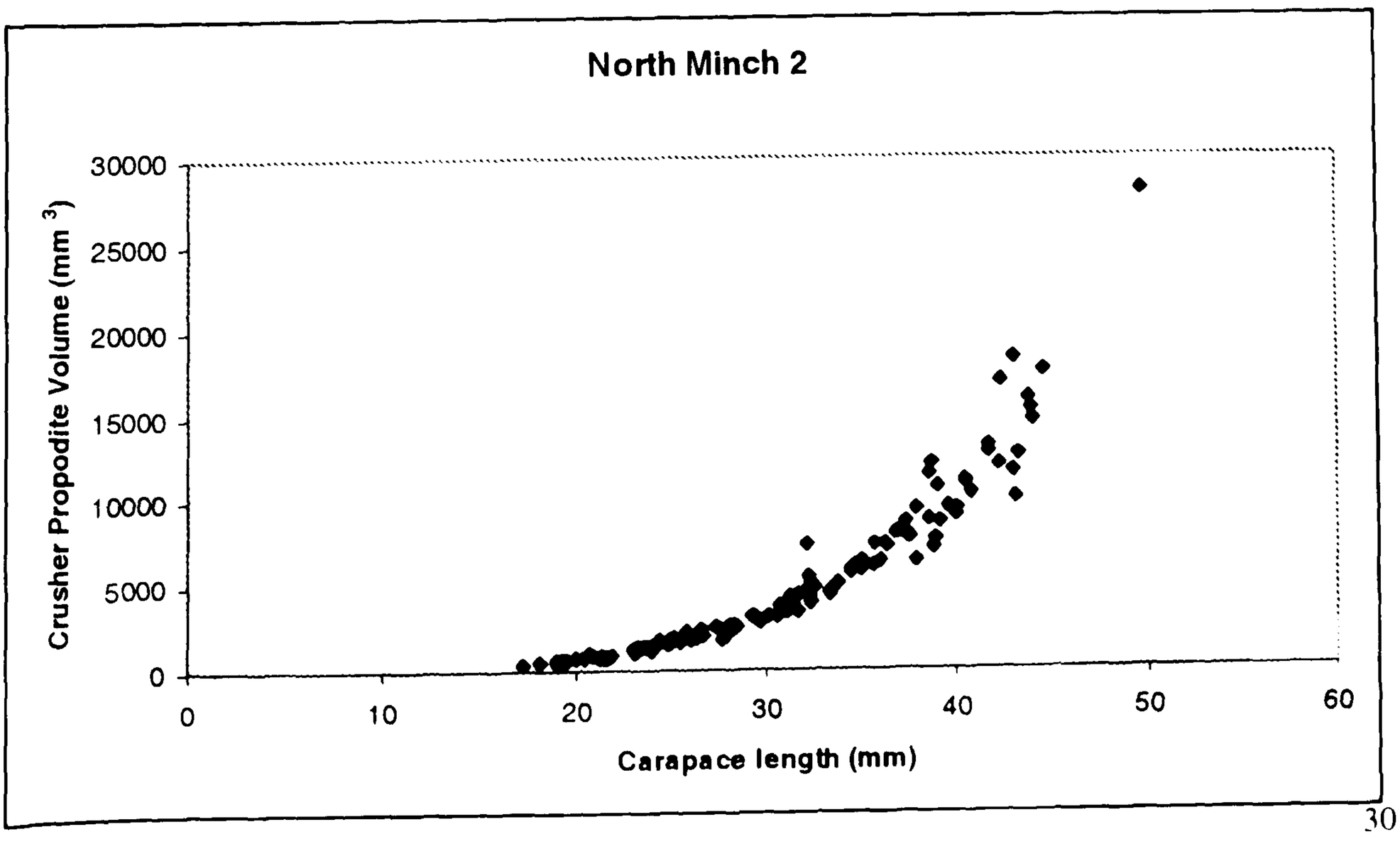

Fladen 1
Crusher Propodite Volume (mm 3)
35000
30000
25000
20000
15000
10000
5000
0
0
10
20
30
40
50
60
Carapace length (mm)

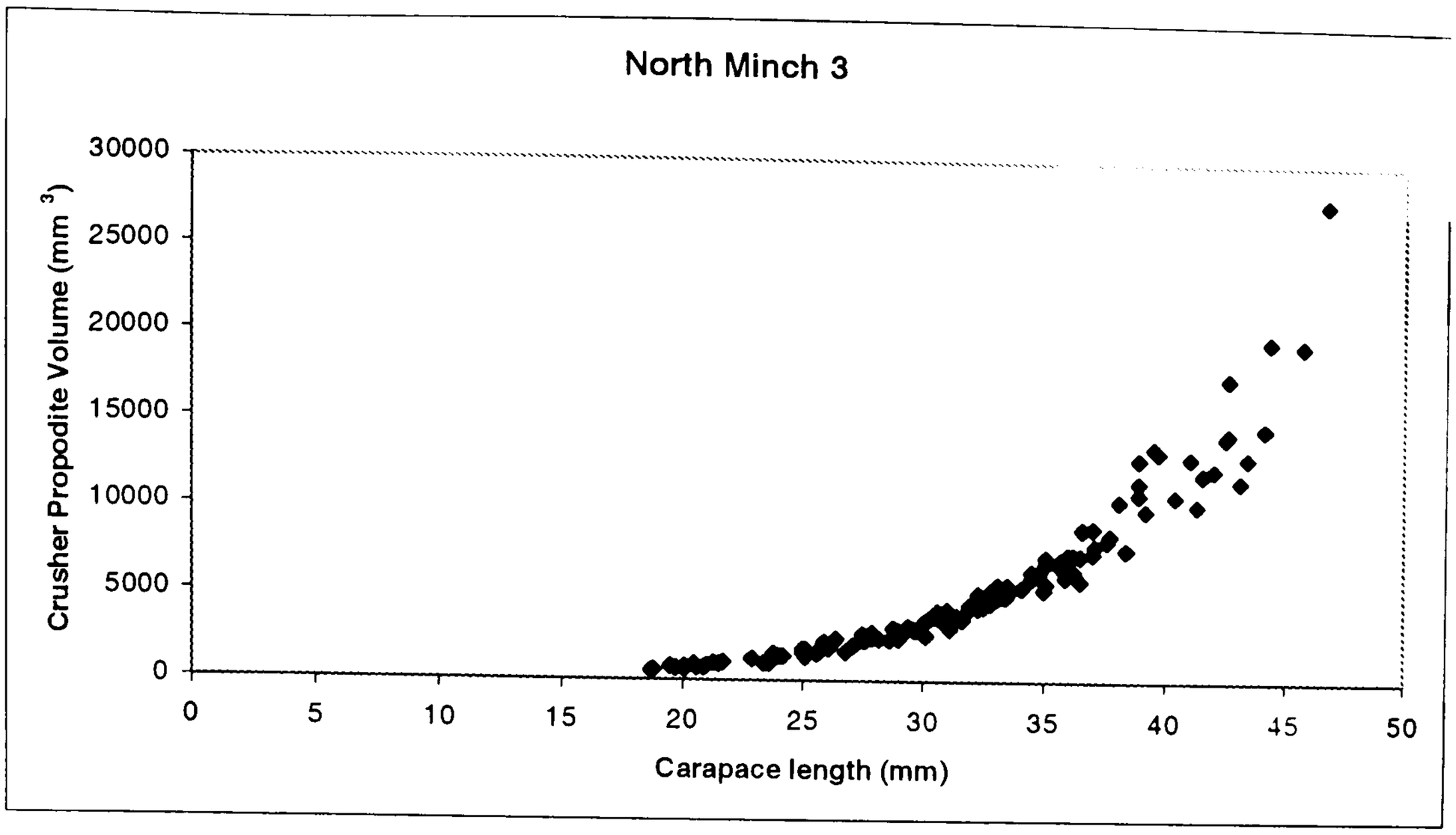

Fladen 2
Crusher Propodite Volume (mm 3)
40000
35000
30000
25000
20000
15000
10000
5000
0
0
10
20
30
40
50
60
Carapace length (mm)

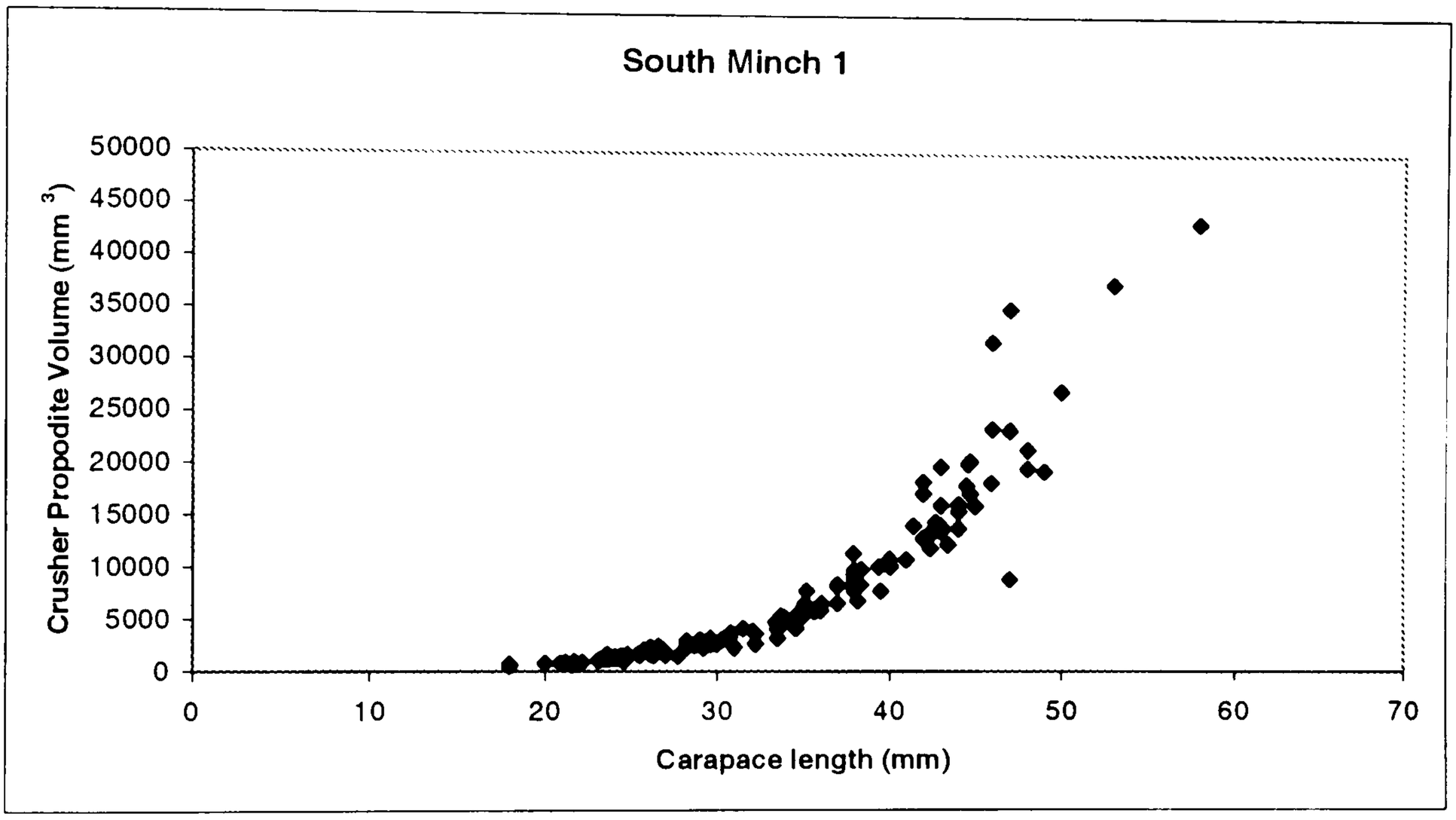

Fladen 3
Crusher Propodite Volume (mm 3)
16000
14000
12000
10000
8000
6000
4000
2000
0
0
5
10
15
20
25
30
35
40
45
Carapace length (mm)

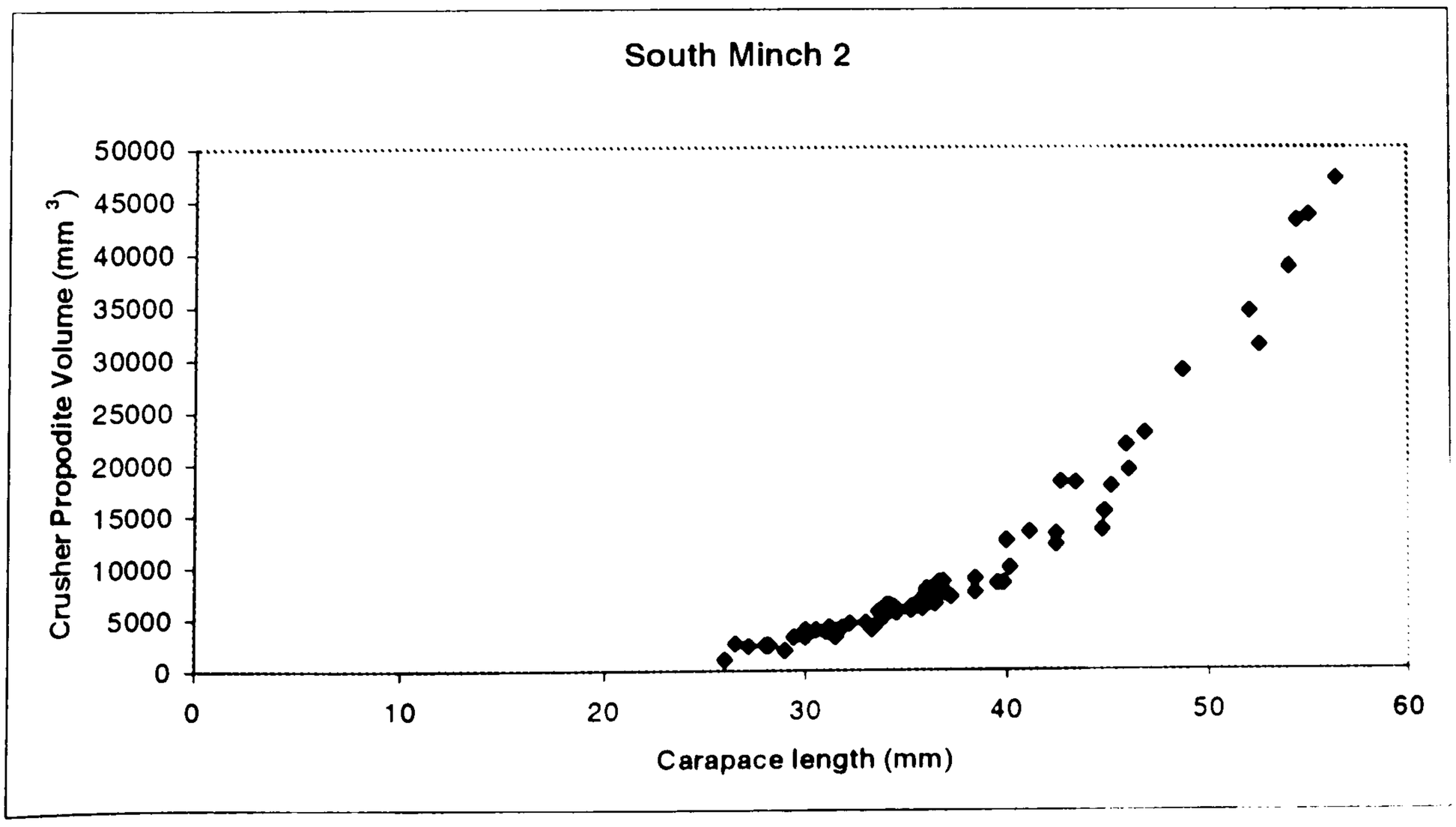

Fladen 4
Crusher Propodite Volume (mm 3)
60000
50000
40000
30000
20000
10000
0
0
10
20
30
40
50
60
Carapace length (mm)

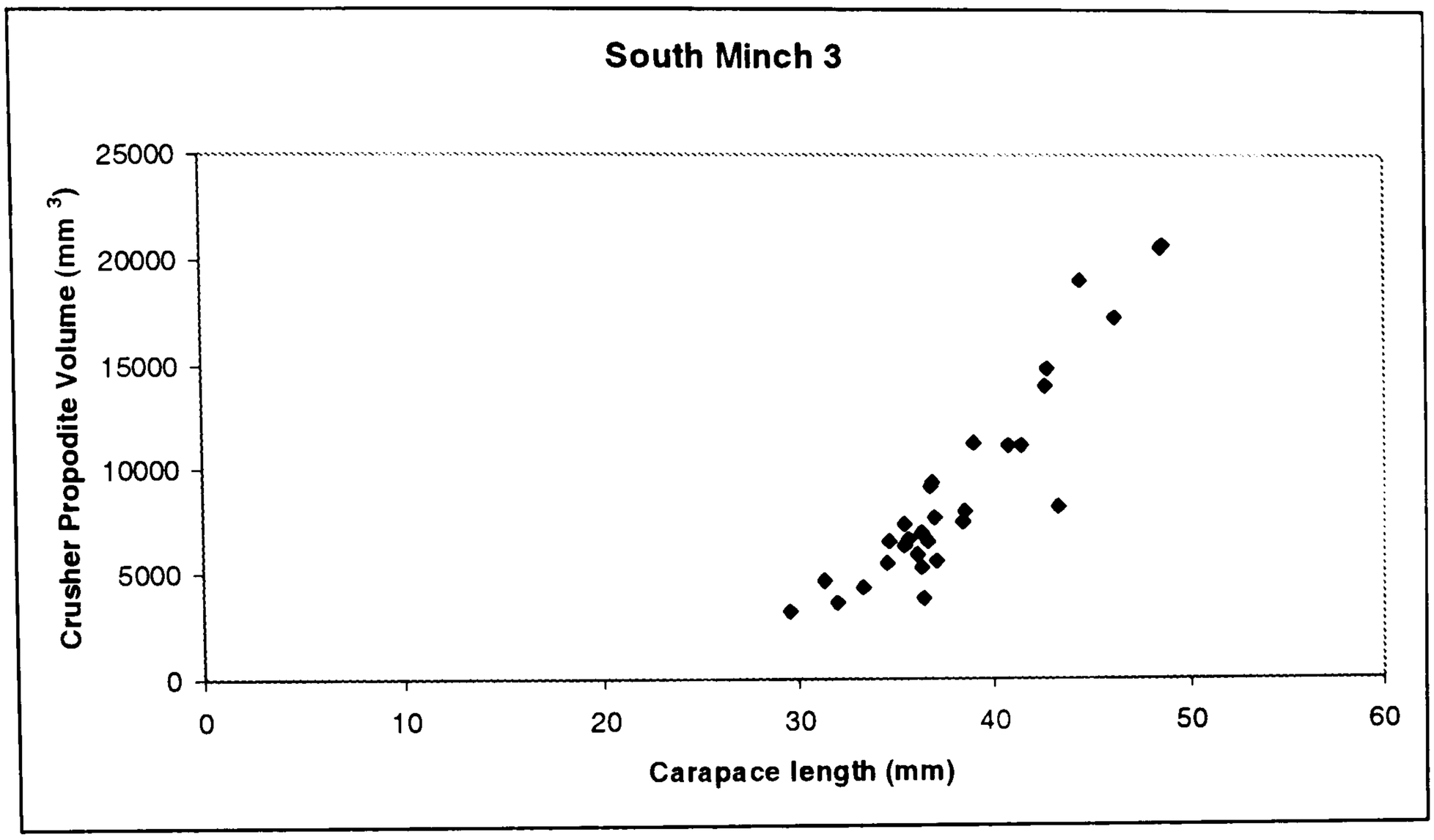

North Minch 1
Crusher Propodite Volume (mm 3)
40000
35000
30000
25000
20000
15000
10000
5000
0
0
10
20
30
40
50
60
Carapace length (mm)


North Minch 2
Crusher Propodite Volume (mm 3)
30000
25000
20000
15000
10000
5000
0
0
10
20
30
40
50
60
Carapace length (mm)

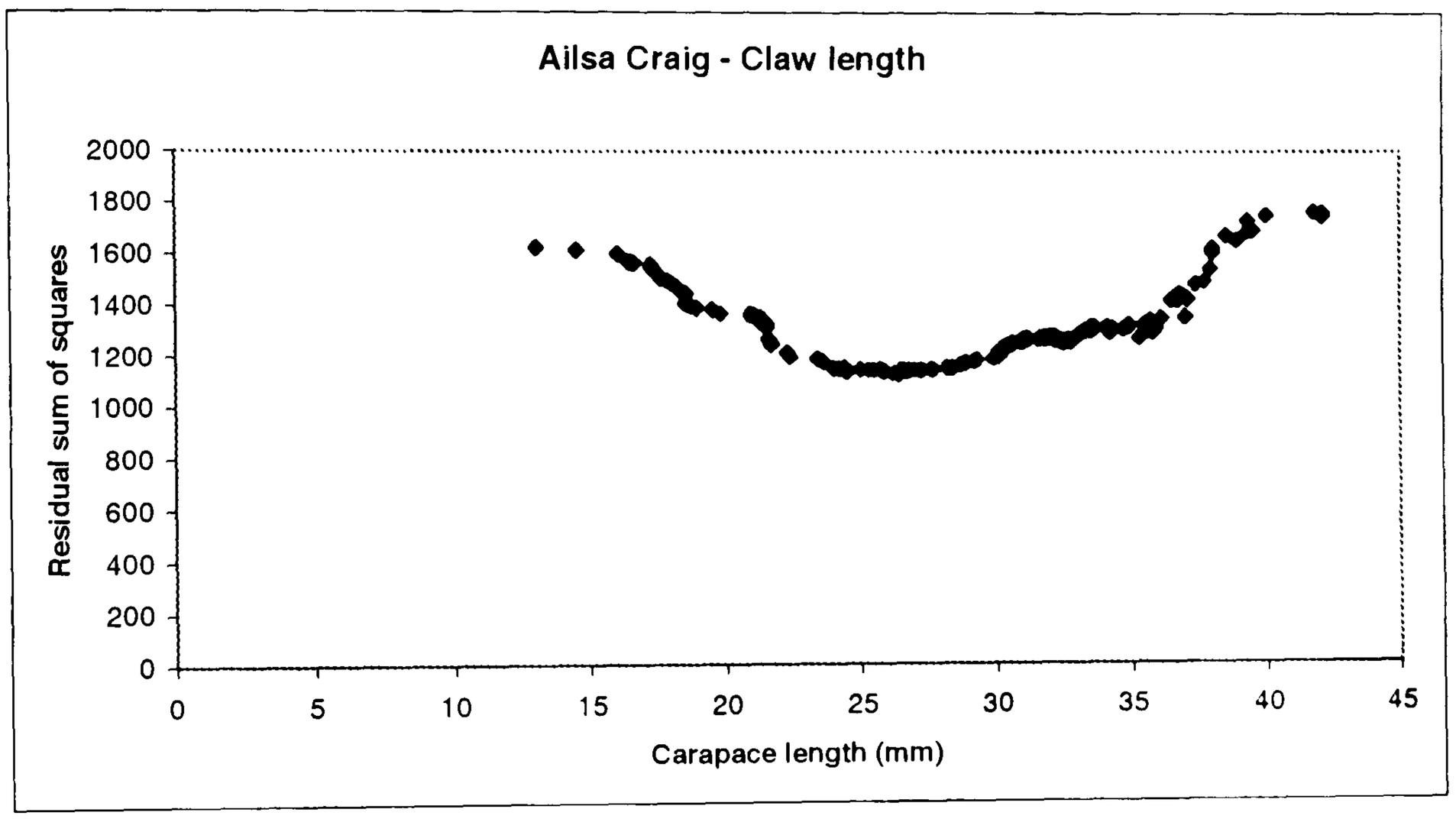

North Minch 3
Crusher Propodite Volume (mm 3)
30000
25000
20000
15000
10000
5000
0
0
5
10
15
20
25
30
35
40
45
50
Carapace length (mm)

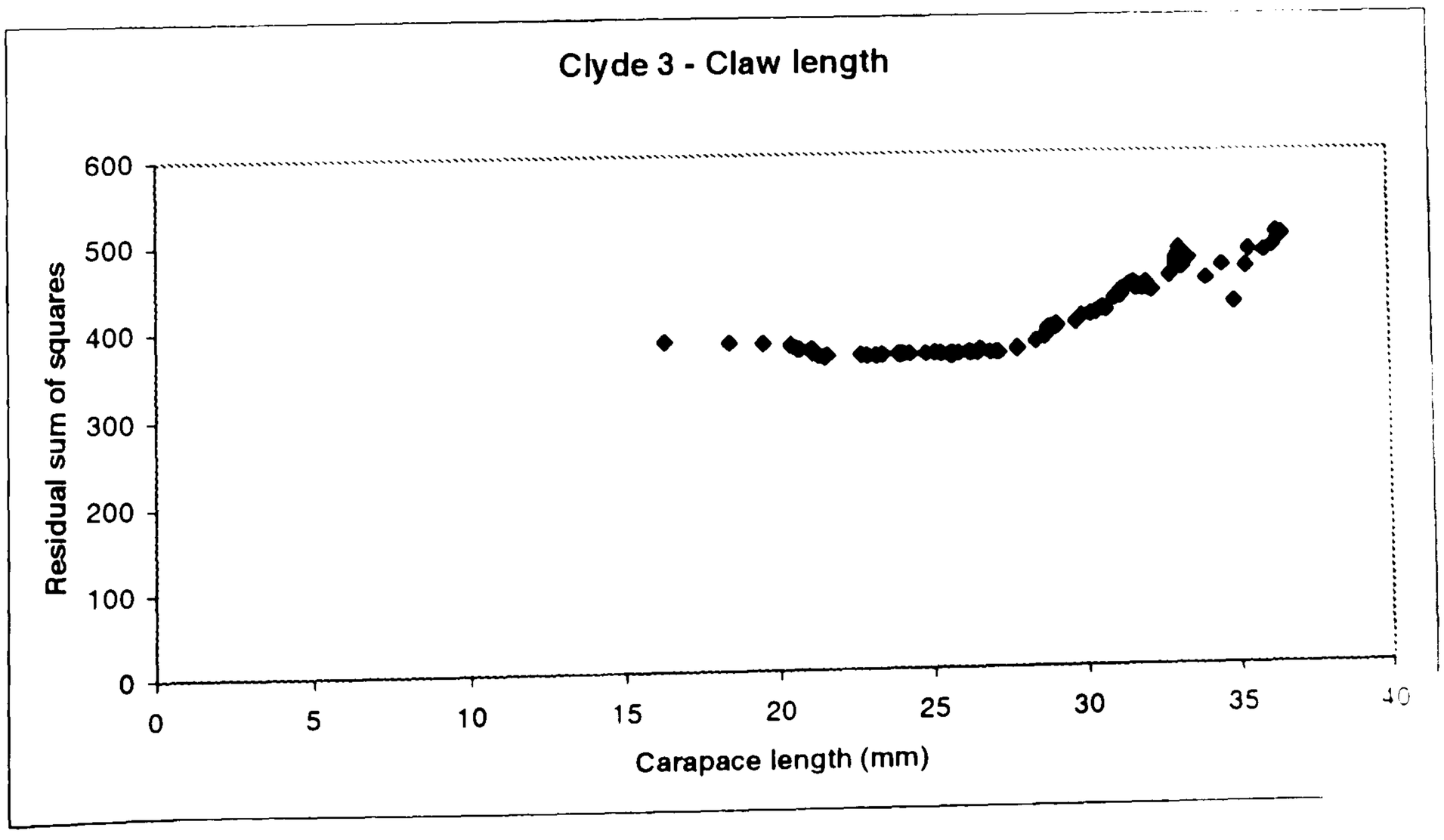

South Minch 1
Crusher Propodite Volume (mm 3)
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
0
10
20
30
40
50
60
70
Carapace length (mm)

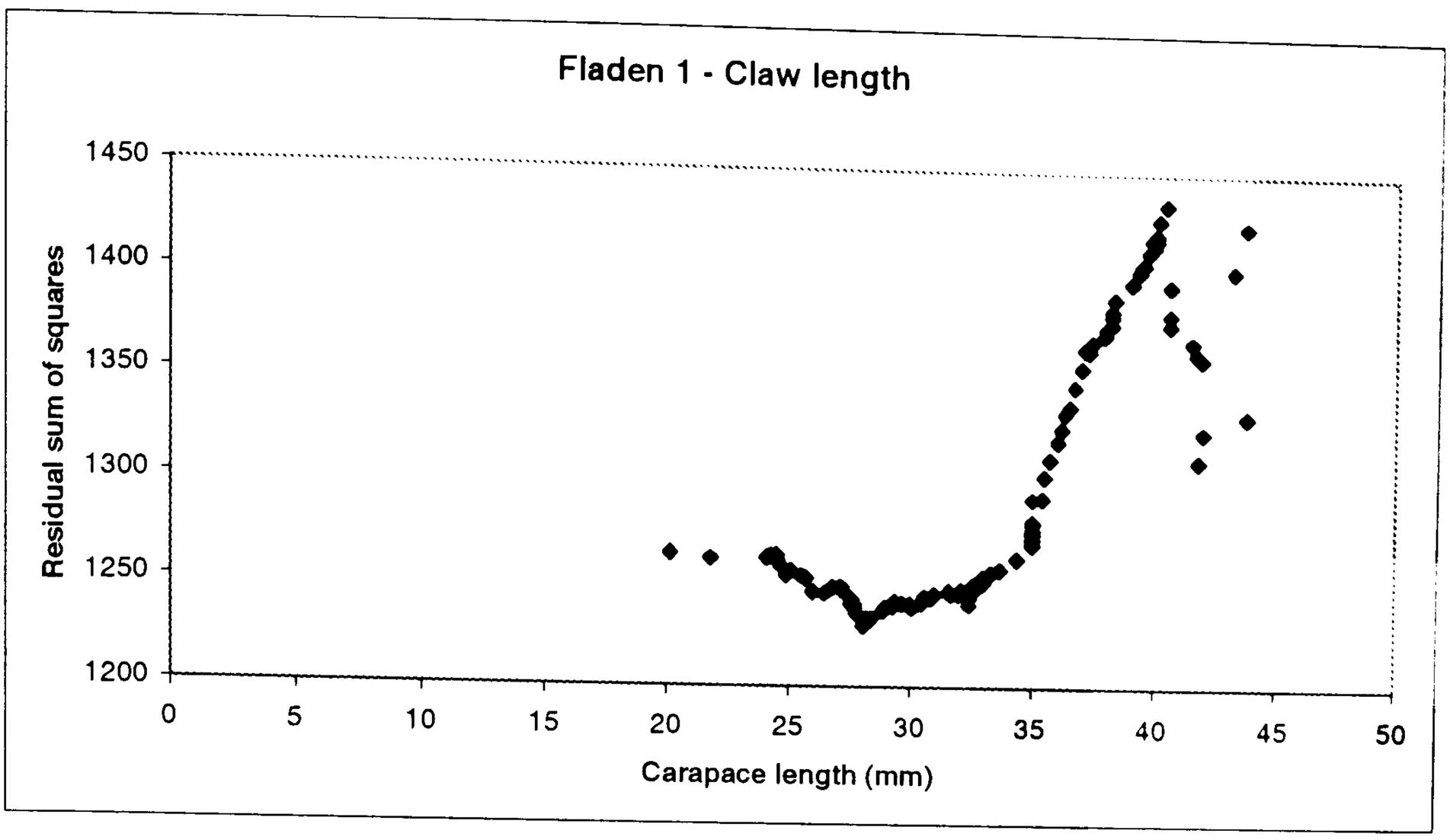

South Minch 2
Crusher Propodite Volume (mm 3)
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
0
10
20
30
40
50
60
Carapace length (mm)

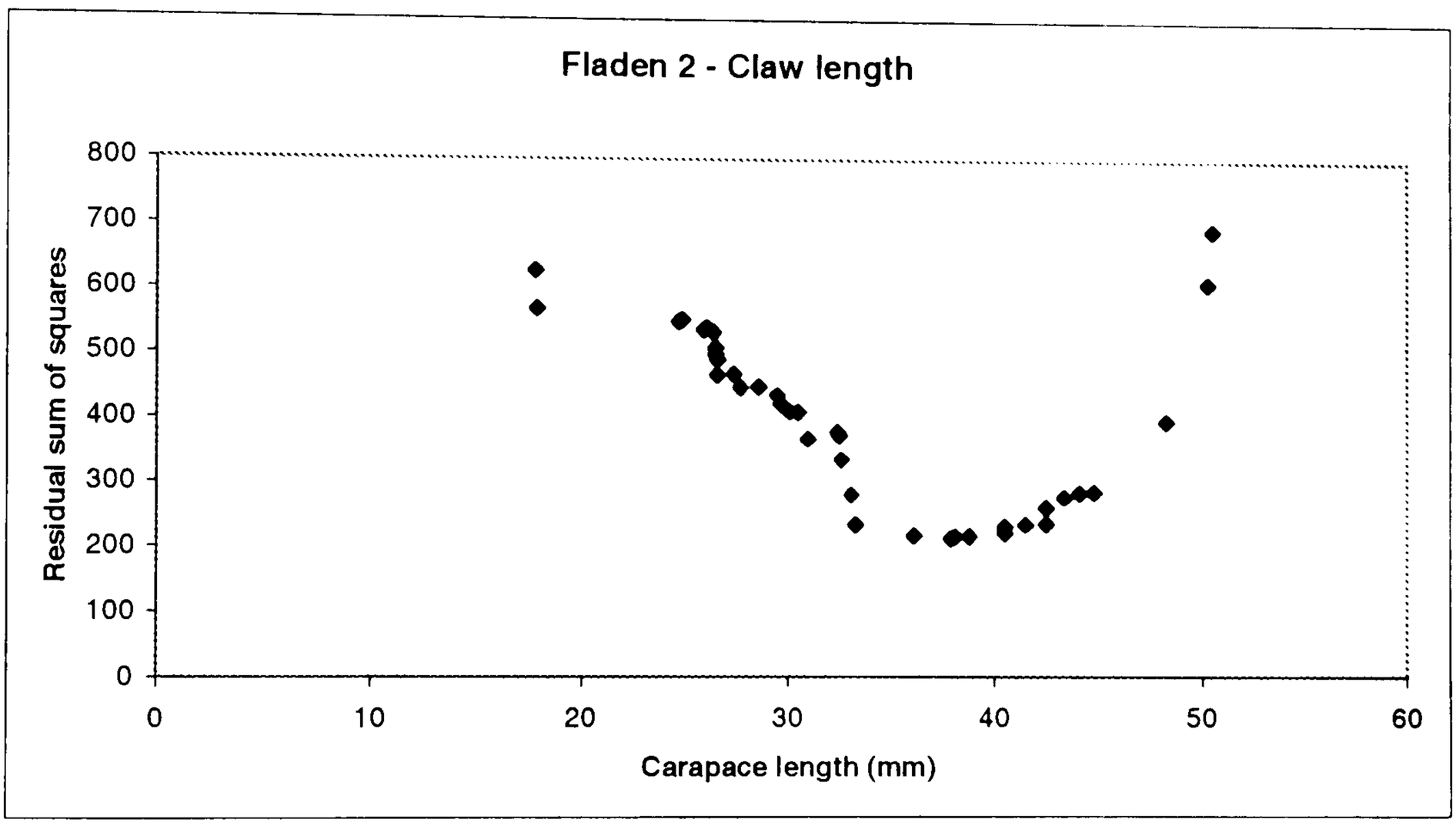

South Minch 3
Crusher Propodite Volume (mm 3)
25000
20000
15000
10000
5000
0
0
10
20
30
40
50
60
Carapace length (mm)

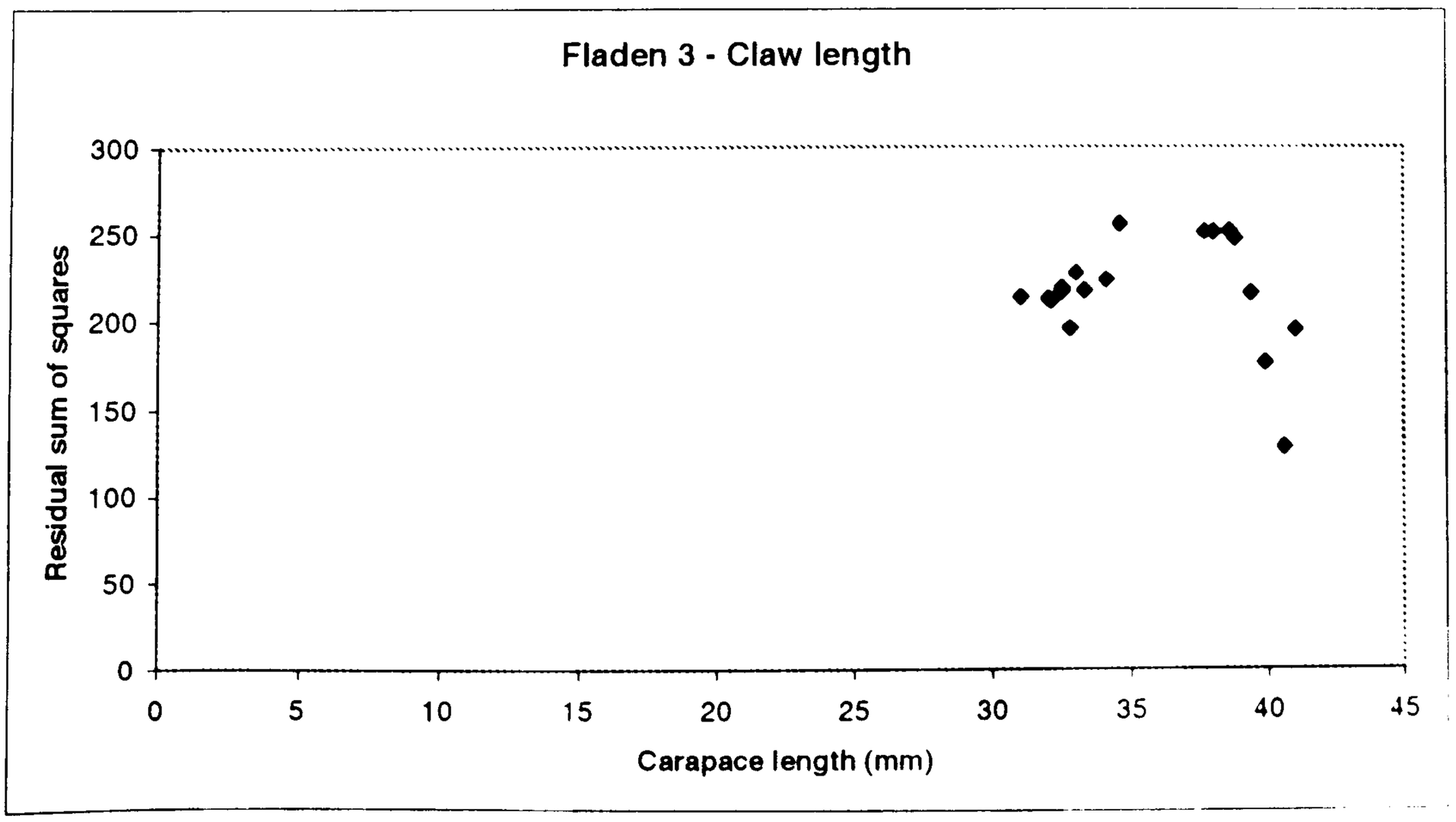
Appendix 4: Plots of the residual sum of squares calculated from the reduced major axis analyses of crusher propodus length from male *Nephrops norvegicus* sampled at sites in the Clyde, Fladen, and the North and South Minches. This data is further to that displayed in Chapter 4.

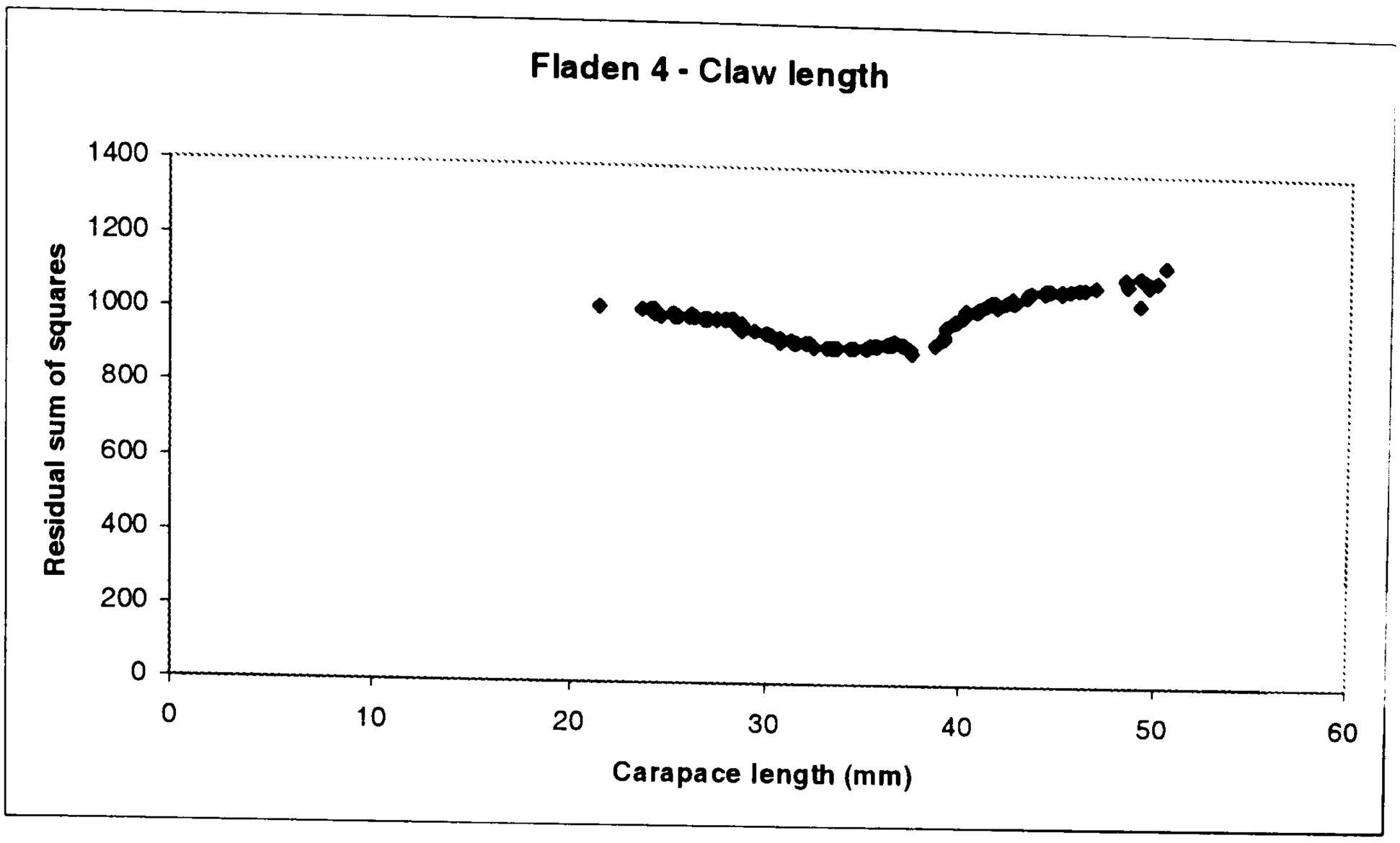


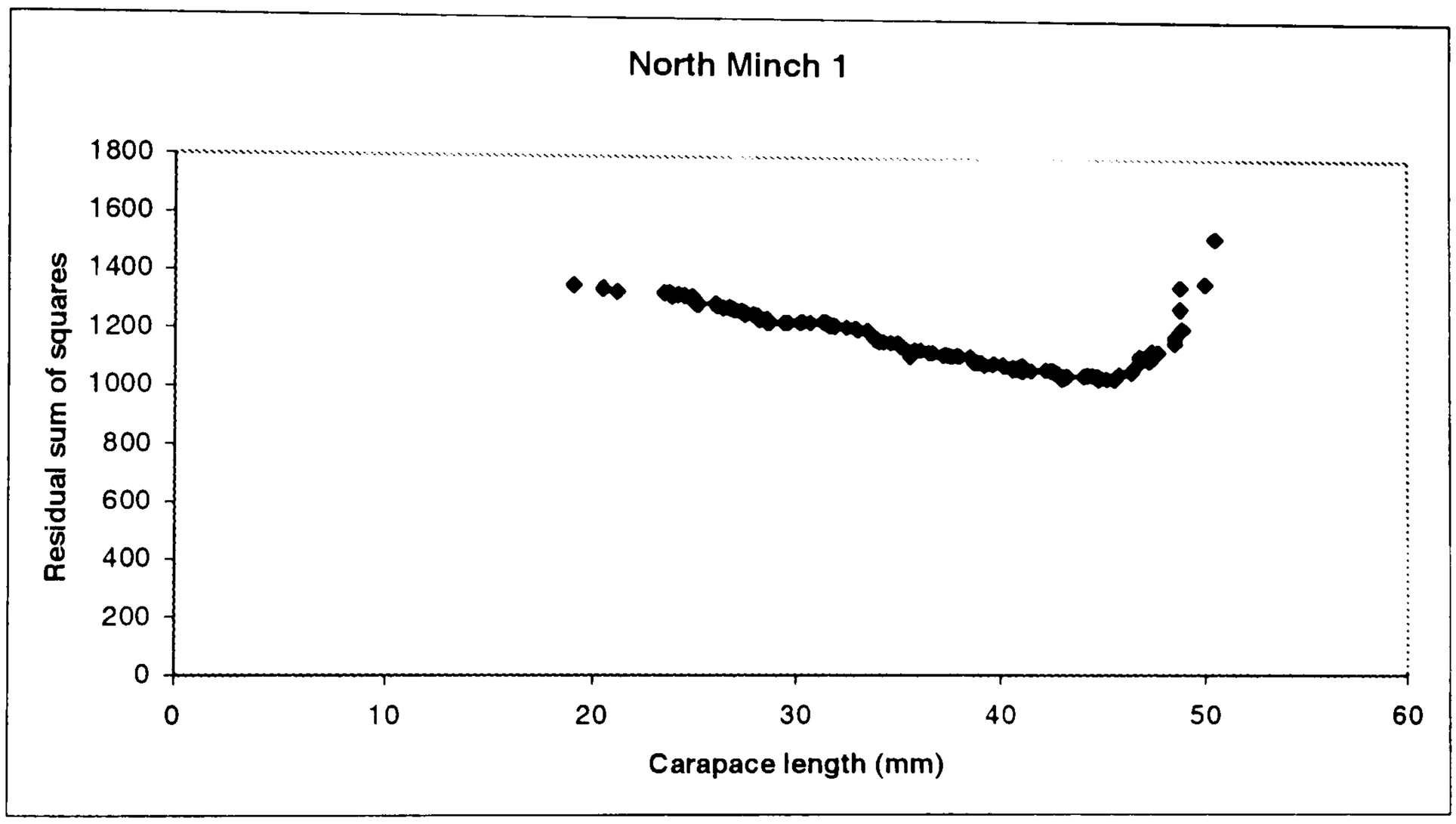


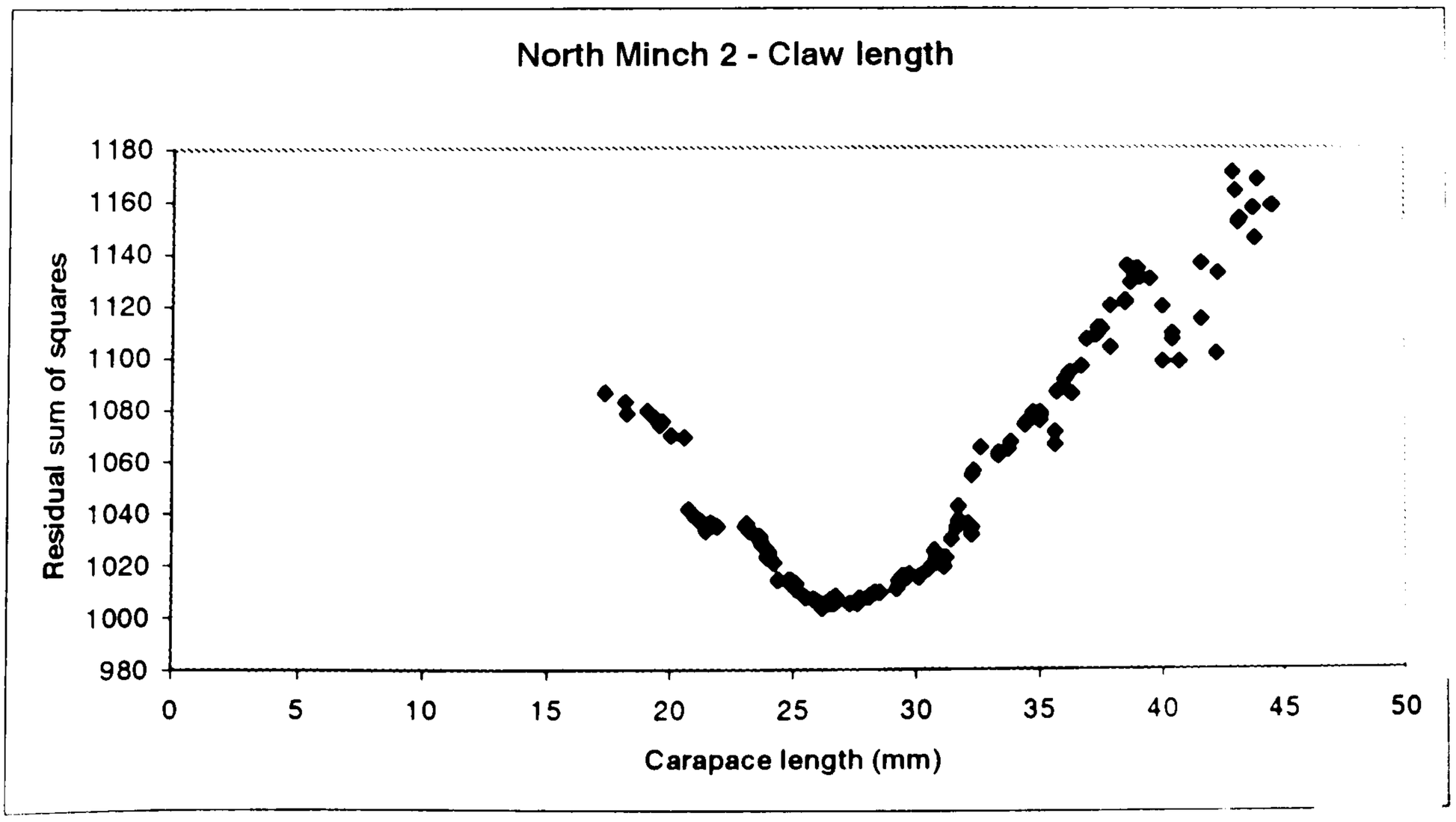

Fladen 1 - Claw length
Residual sum of squares
1450
1400
1350
1300
1250
1200
0
5
10
15
20
25
30
35
40
45
50
Carapace length (mm)

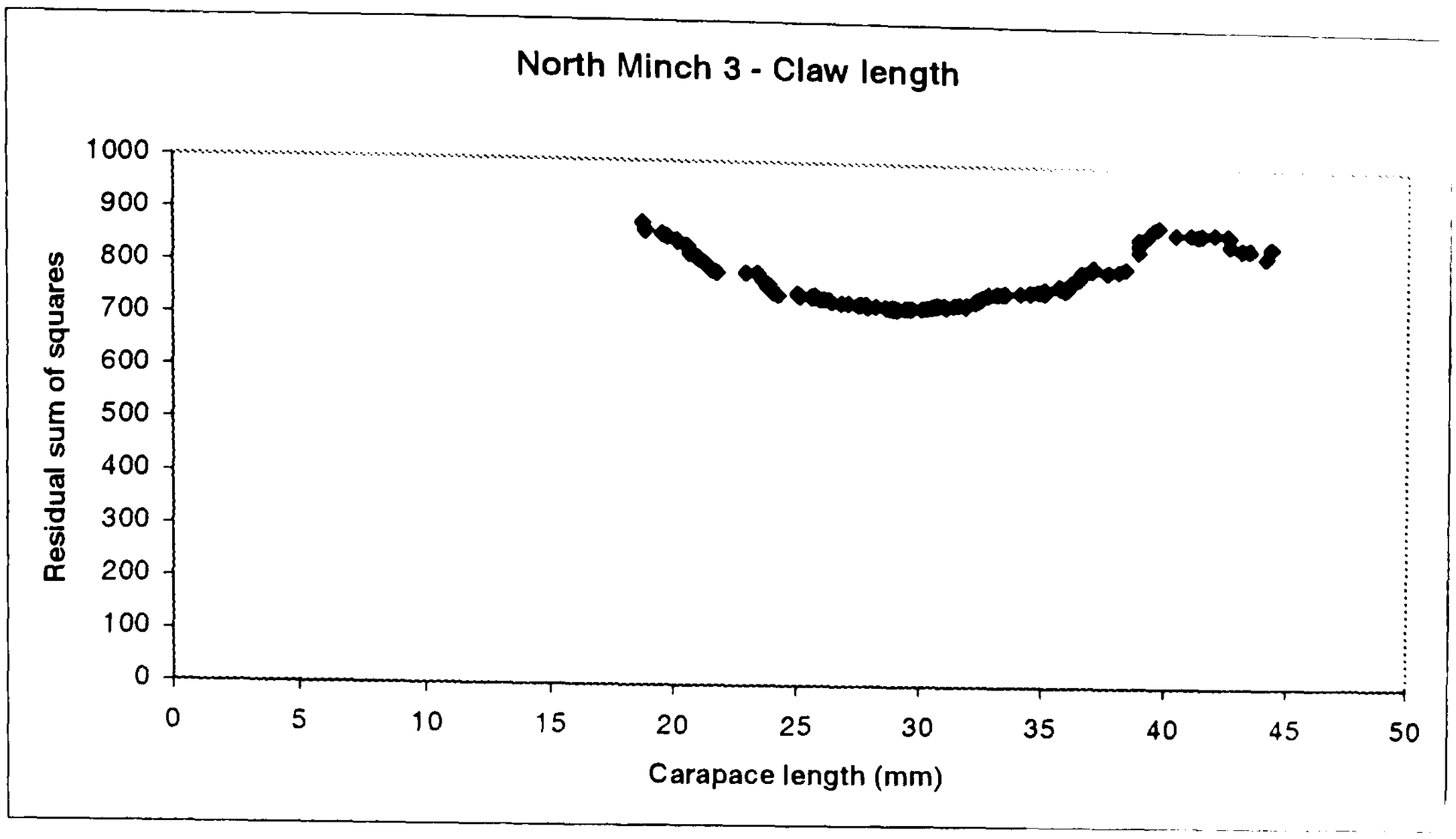

Fladen 2 - Claw length
Residual sum of squares
800
700
600
500
400
300
200
100
0
0
10
20
30
40
50
60
Carapace length (mm)

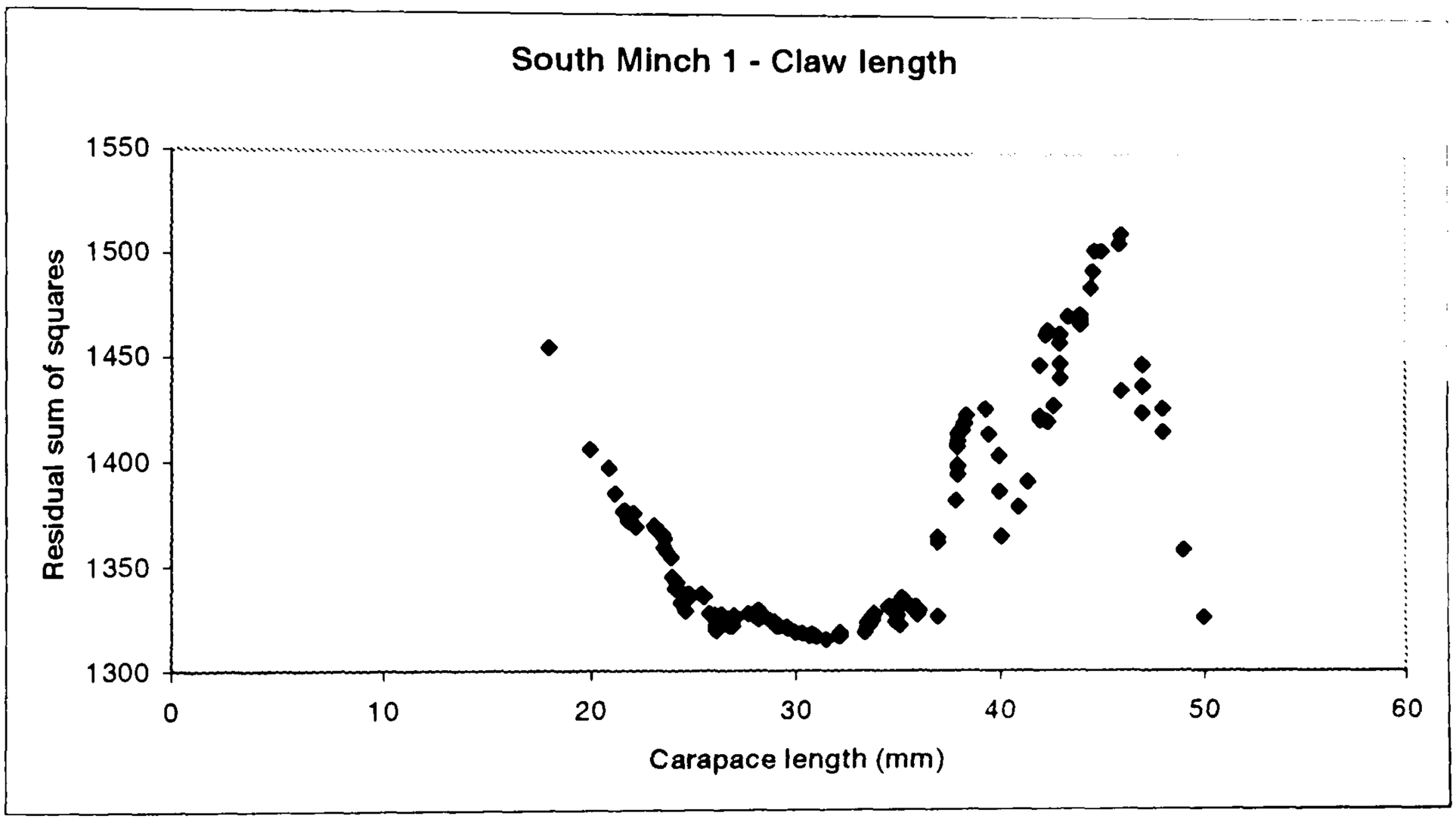

Fladen 3 - Claw length
Residual sum of squares
300
250
200
150
100
50
0
0
5
10
15
20
25
30
35
40
45
Carapace length (mm)

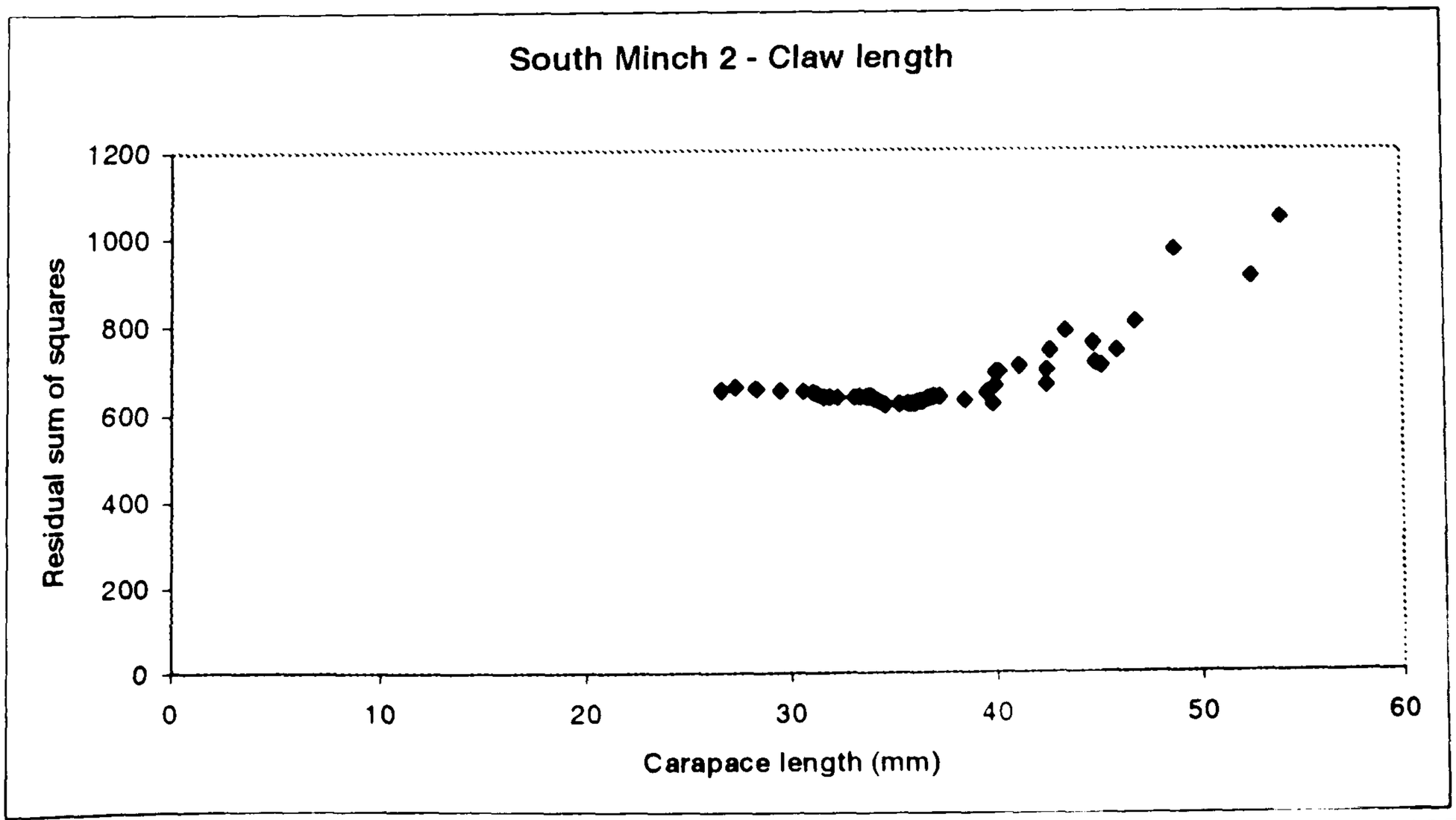

Fladen 4 - Claw length
Residual sum of squares
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
60
Carapace length (mm)

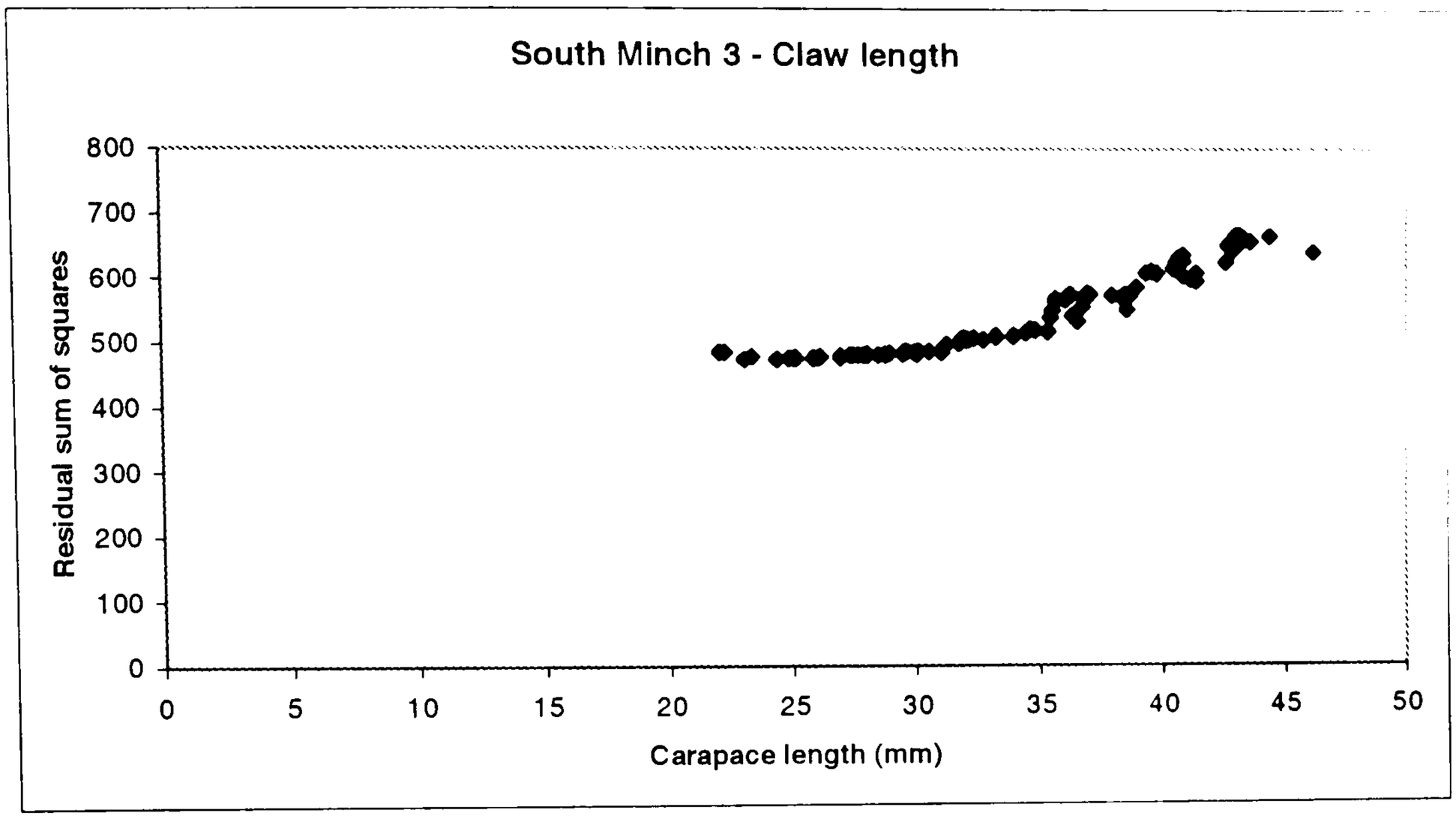

North Minch 1
Residual sum of squares
1800
1600
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
60
Carapace length (mm)


North Minch 2 - Claw length
Residual sum of squares
1180
1160
1140
1120
1100
1080
1060
1040
1020
1000
980
0
5
10
15
20
25
30
35
40
45
50
Carapace length (mm)

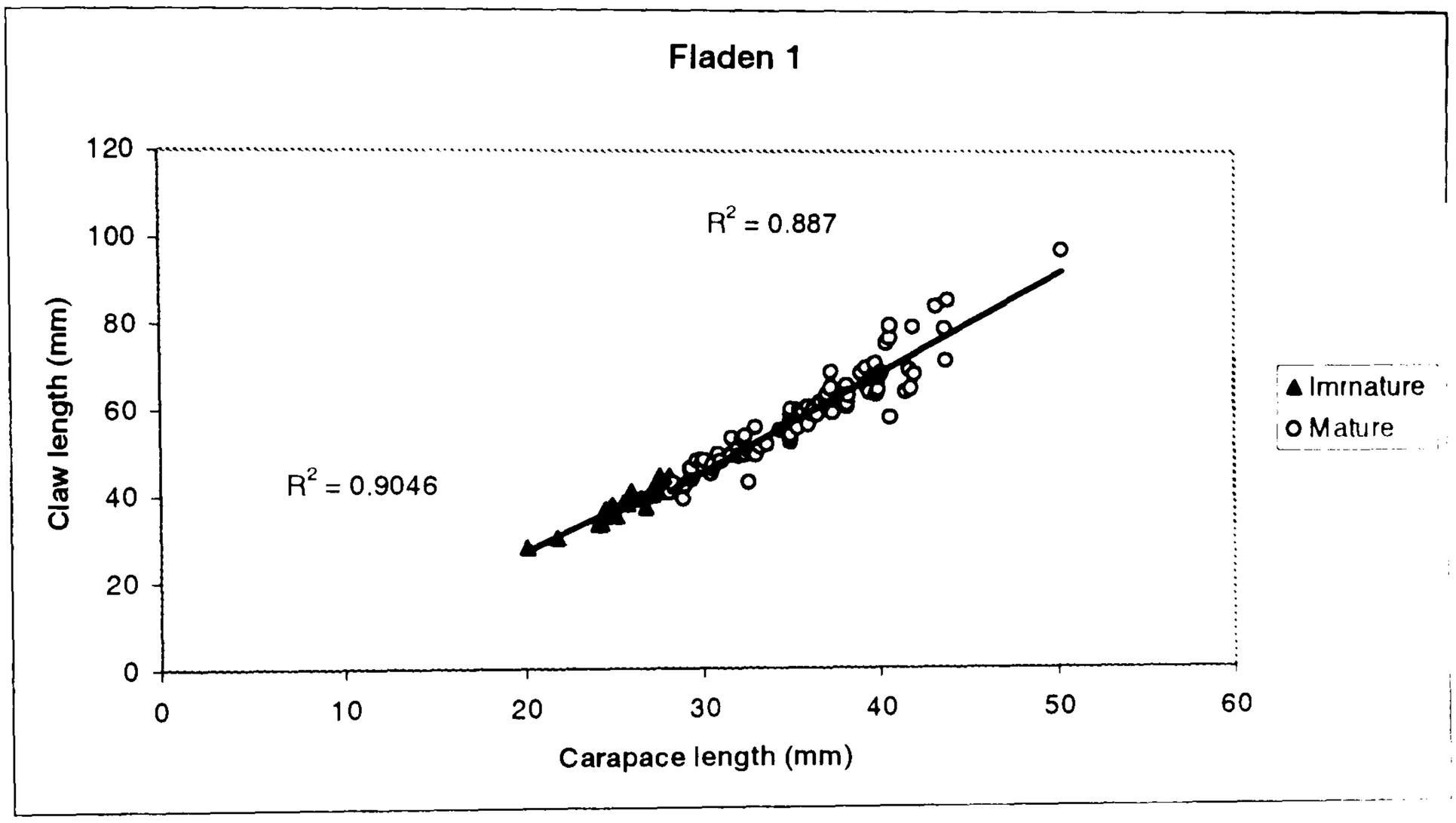

North Minch 3 - Claw length
Residual sum of squares
1000
900
800
700
600
500
400
300
200
100
0
0
5
10
15
20
25
30
35
40
45
50
Carapace length (mm)

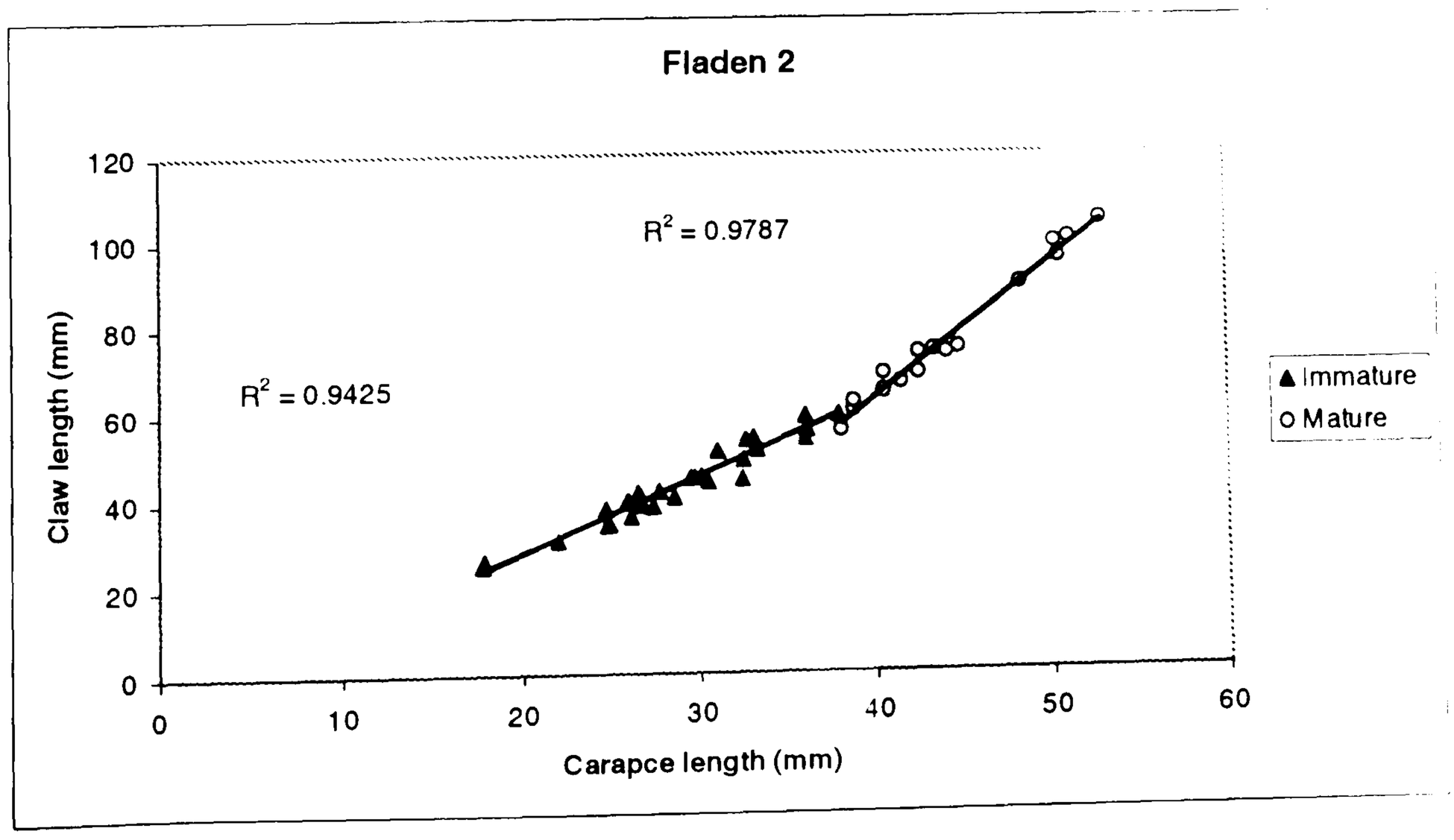

South Minch 1 - Claw length
Residual sum of squares
1550
1500
1450
1400
1350
1300
0
10
20
30
40
50
60
Carapace length (mm)

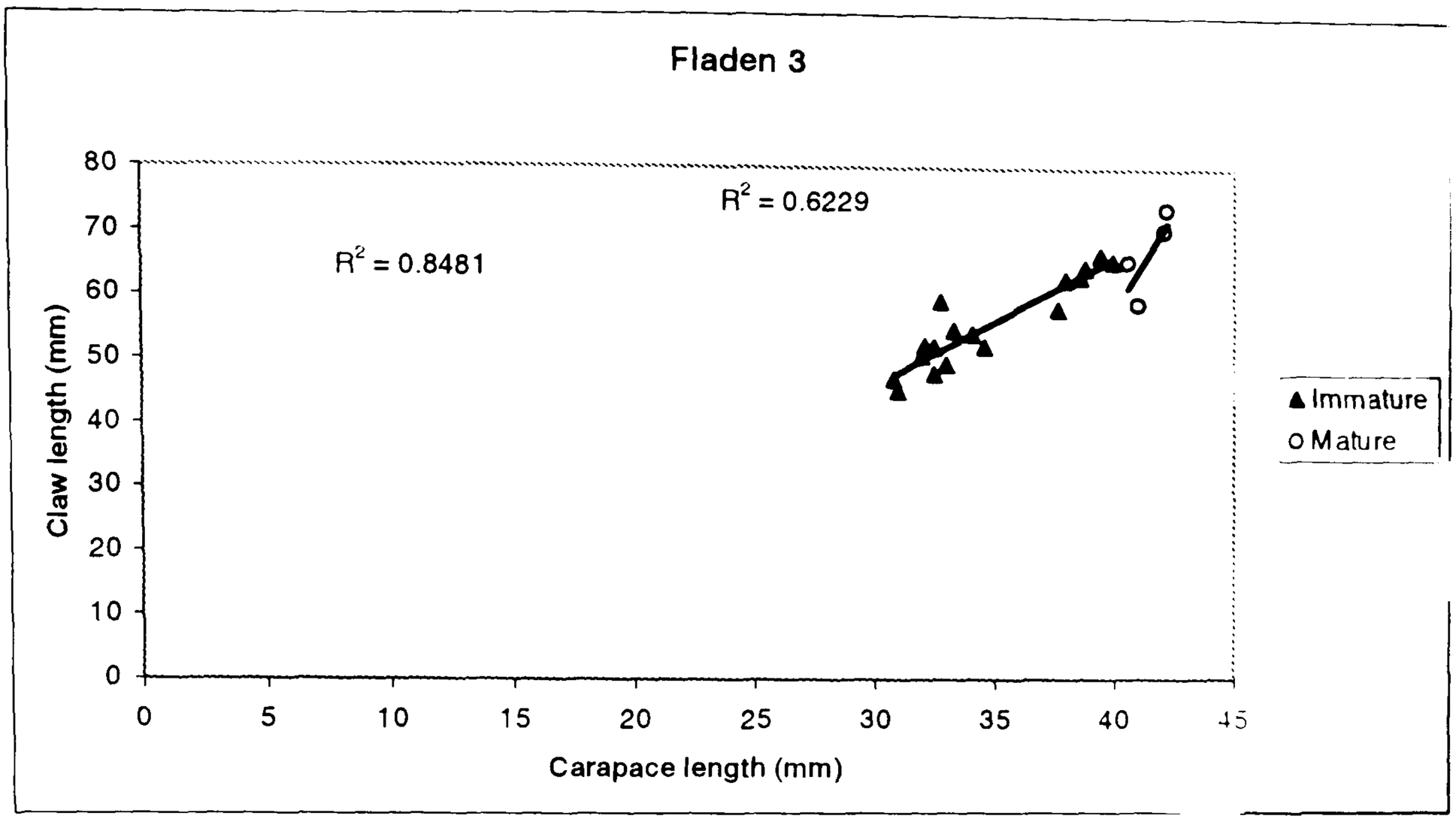

South Minch 2 - Claw length
Residual sum of squares
1200
1000
800
600
400
200
0
0
10
20
30
40
50
60
Carapace length (mm)

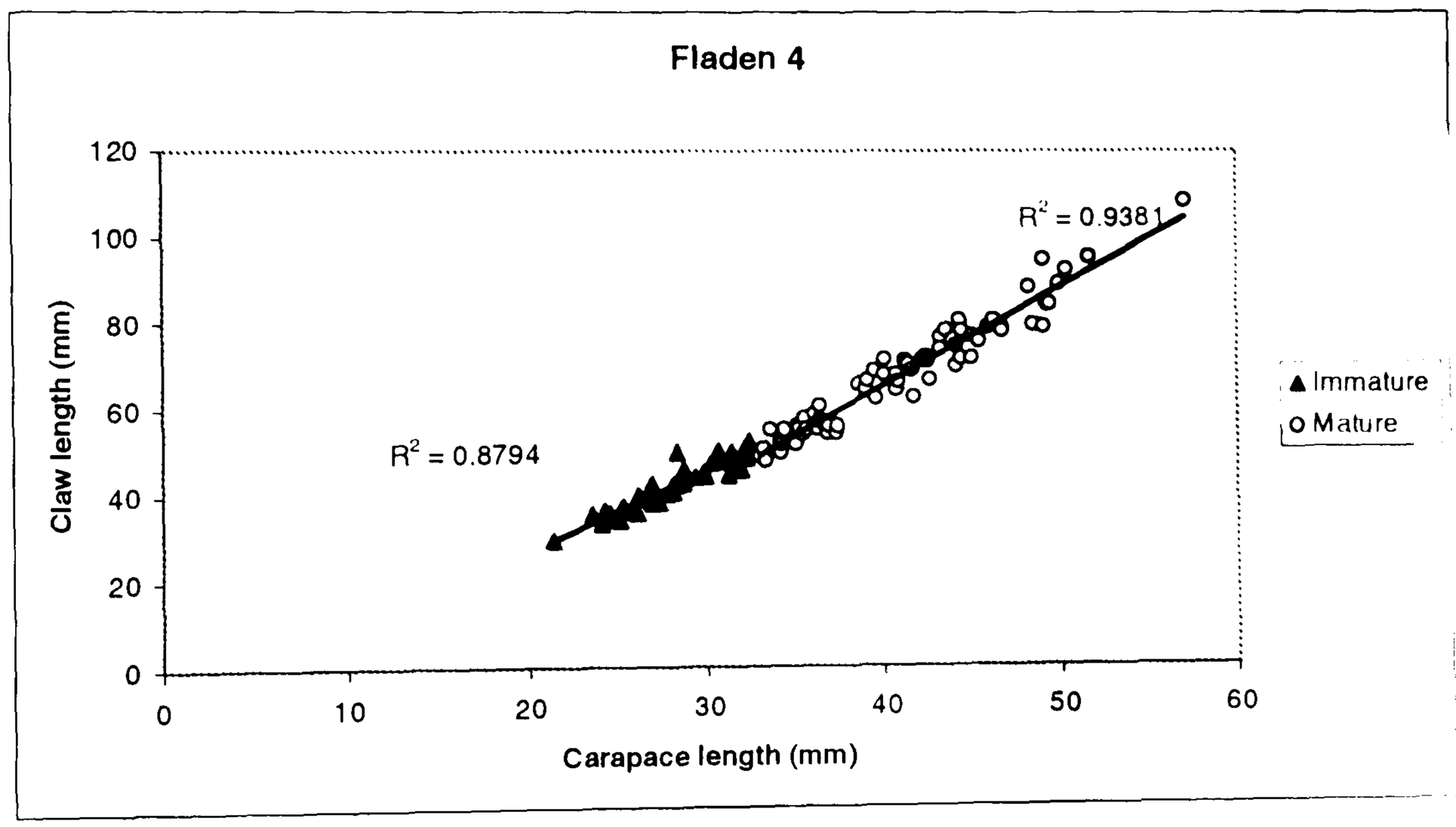

South Minch 3 - Claw length
Residual sum of squares
800
700
600
500
400
300
200
100
0
0
5
10
15
20
25
30
35
40
45
50
Carapace length (mm)

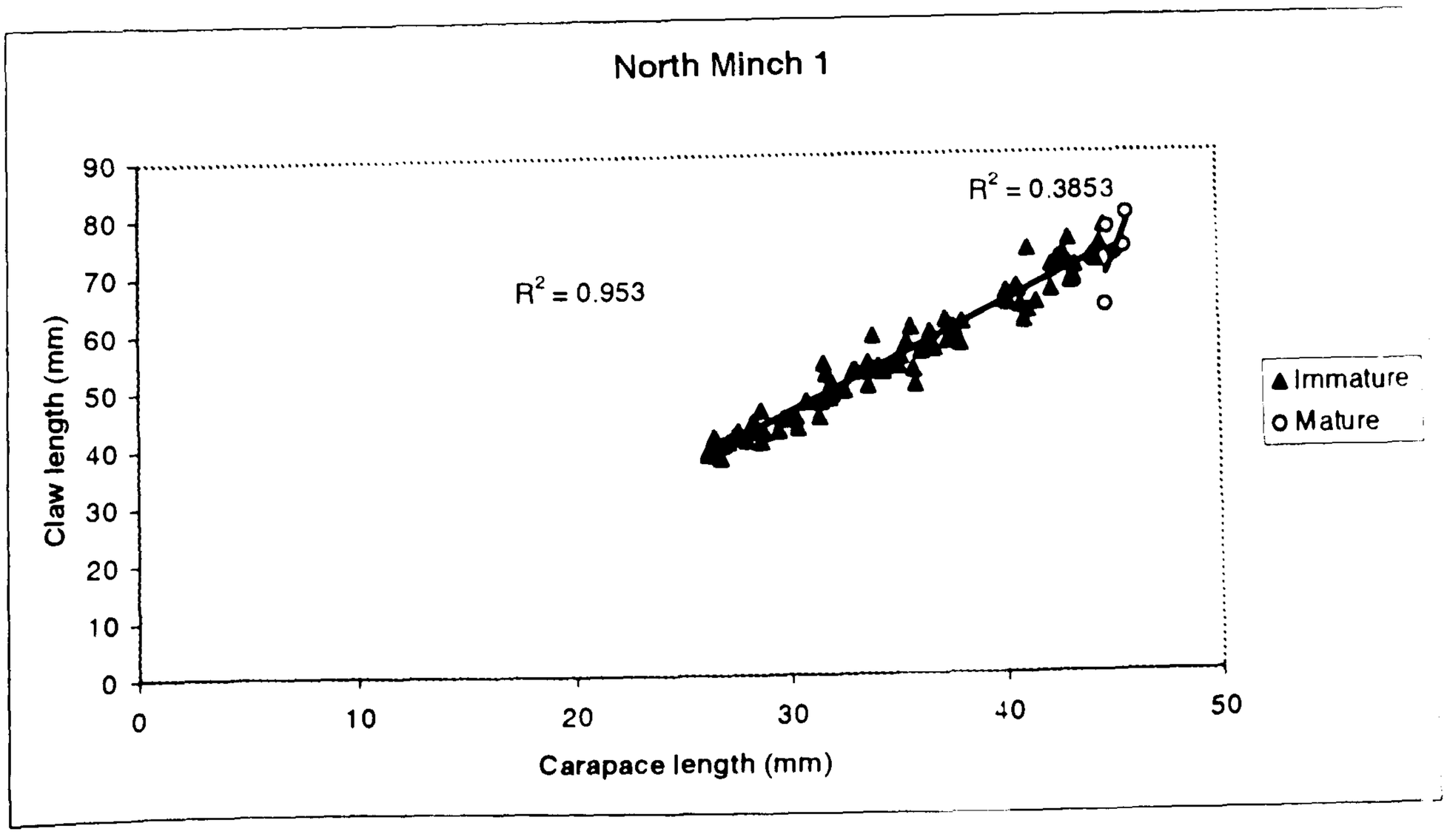
Appendix 5: The relationship between claw length and carapace length of immature and mature animals classified using a size at first maturity calculated using a reduced major axis technique. Data from the Fladen ground and the North and South Minches are shown further to the data in Chapter 4.

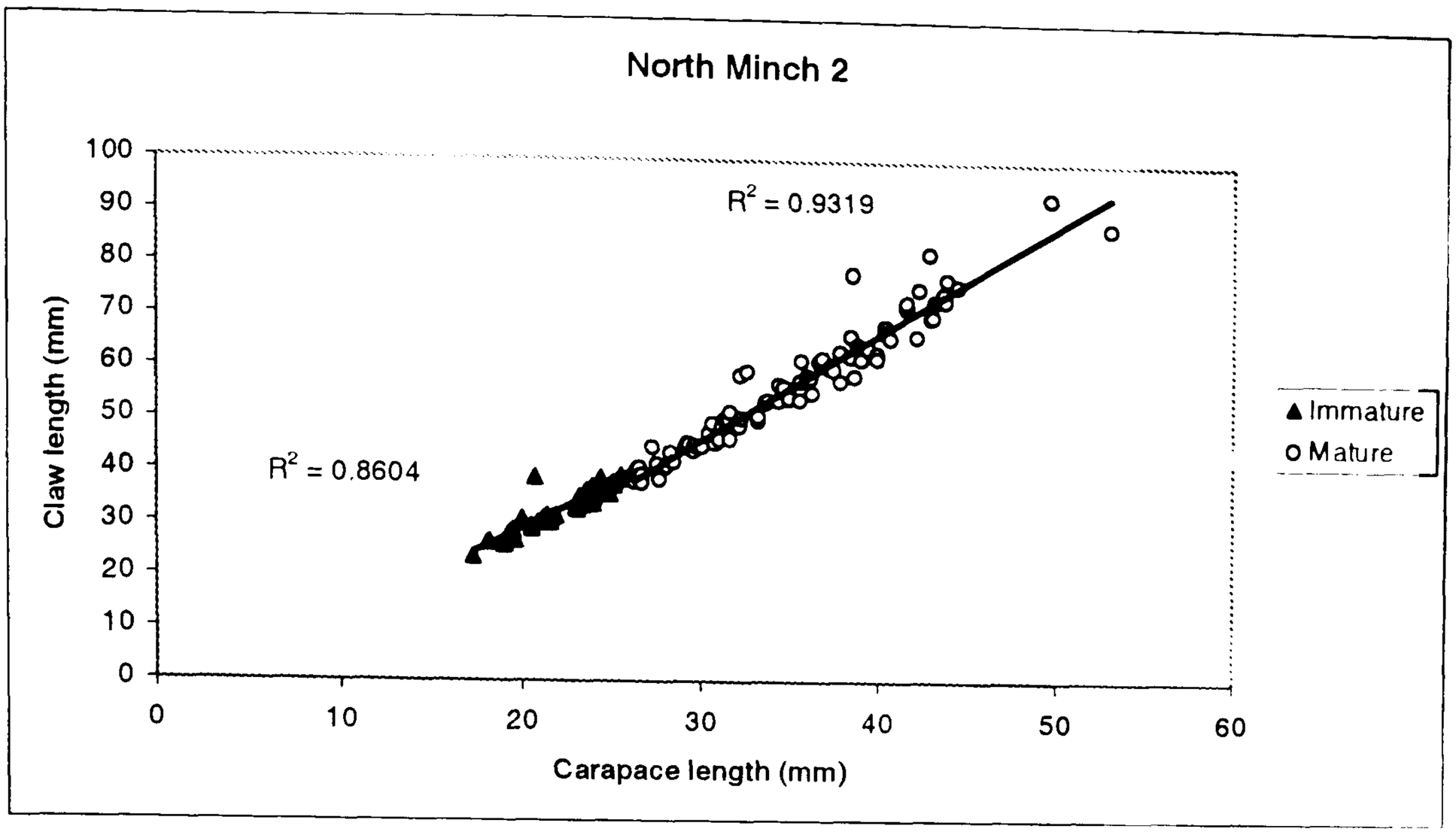


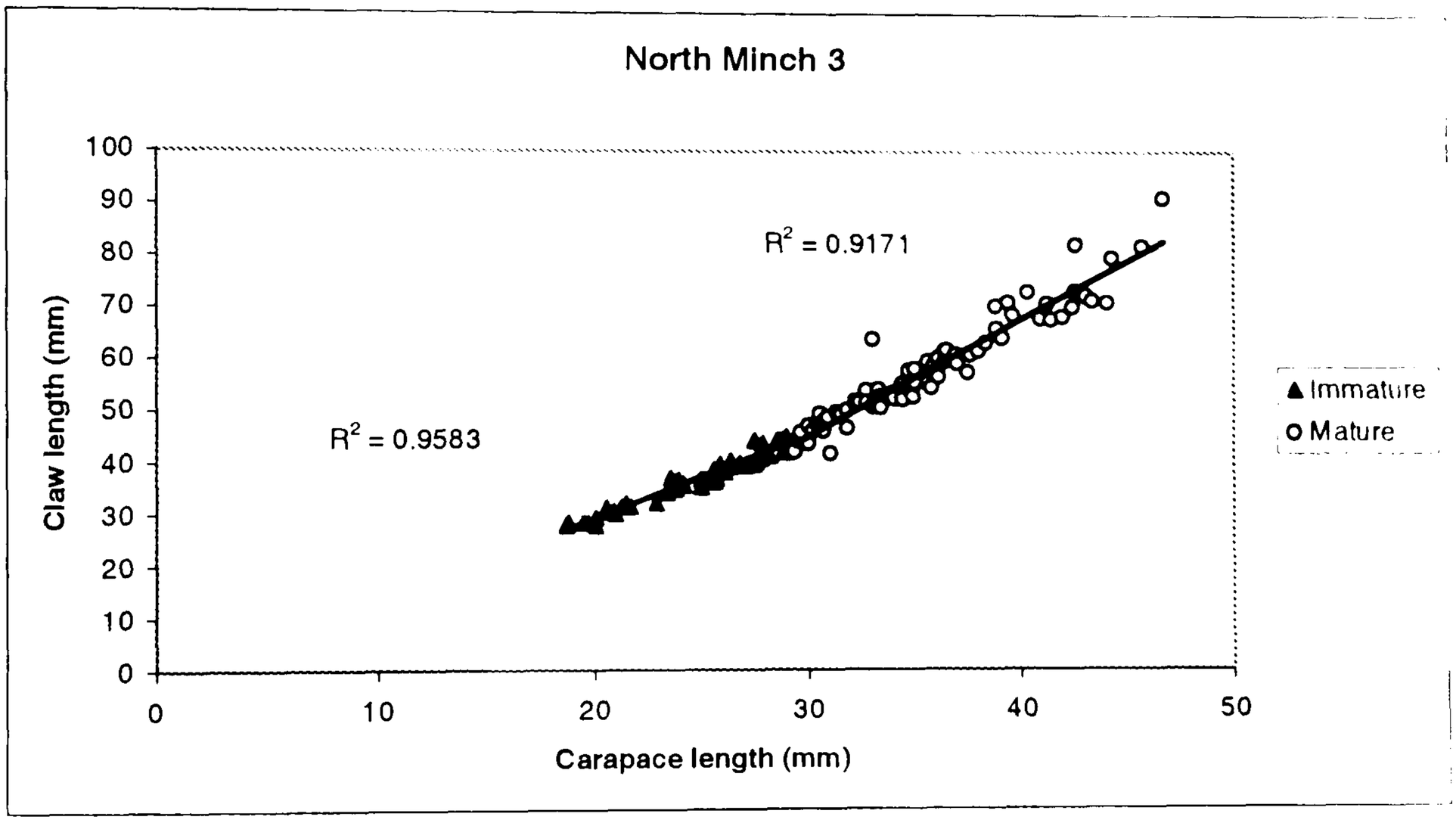


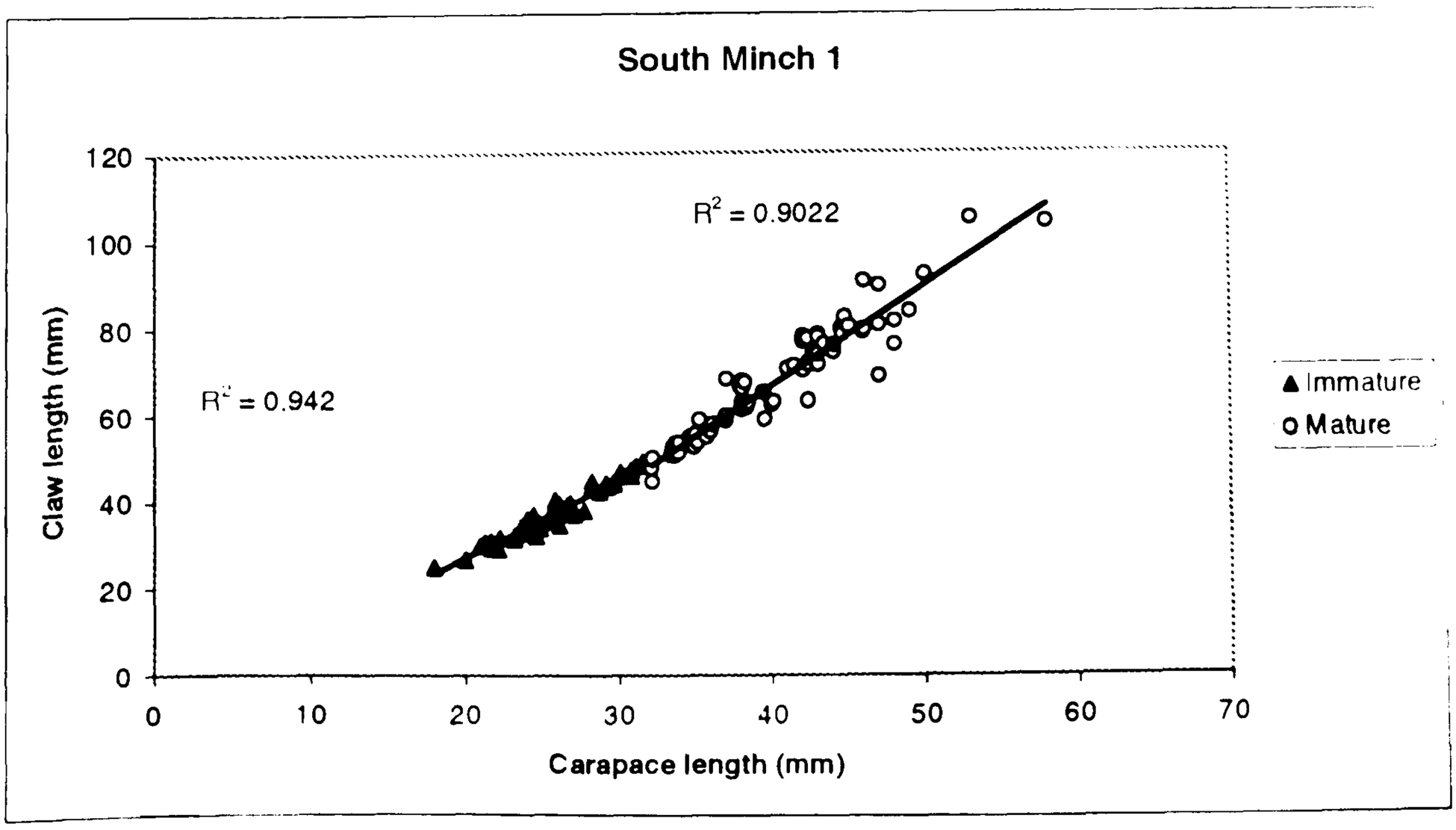

Fladen 3
Claw length (mm)
Carapace length (mm)
R^2 = 0.8481
R^2 = 0.6229
Immature
Mature
0
10
20
30
40
50
60
70
80
0
5
10
15
20
25
30
35
40
45

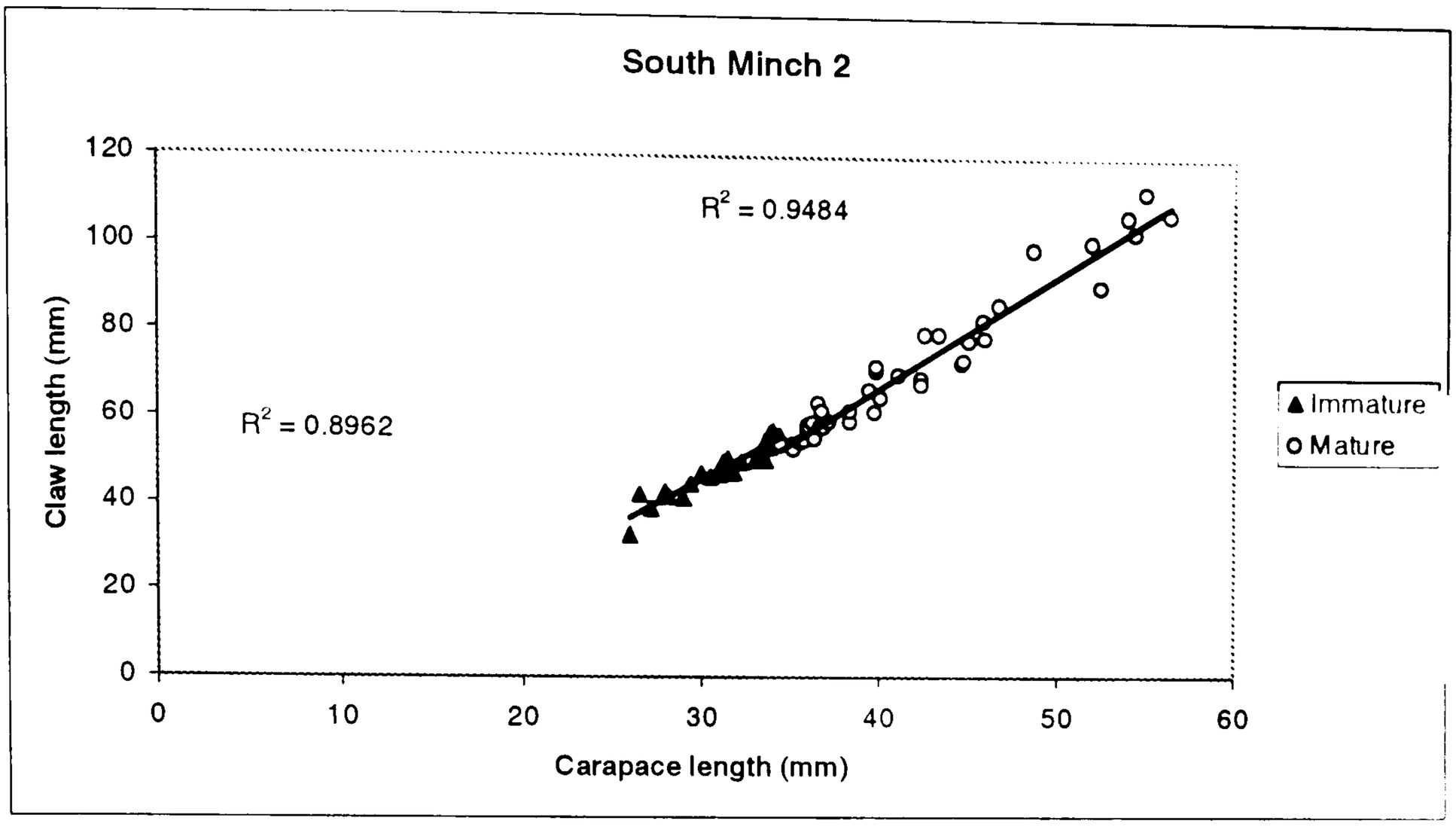

Fladen 4
Claw length (mm)
Carapace length (mm)
R^2 = 0.8794
R^2 = 0.9381
Immature
Mature
0
20
40
60
80
100
120
0
10
20
30
40
50
60

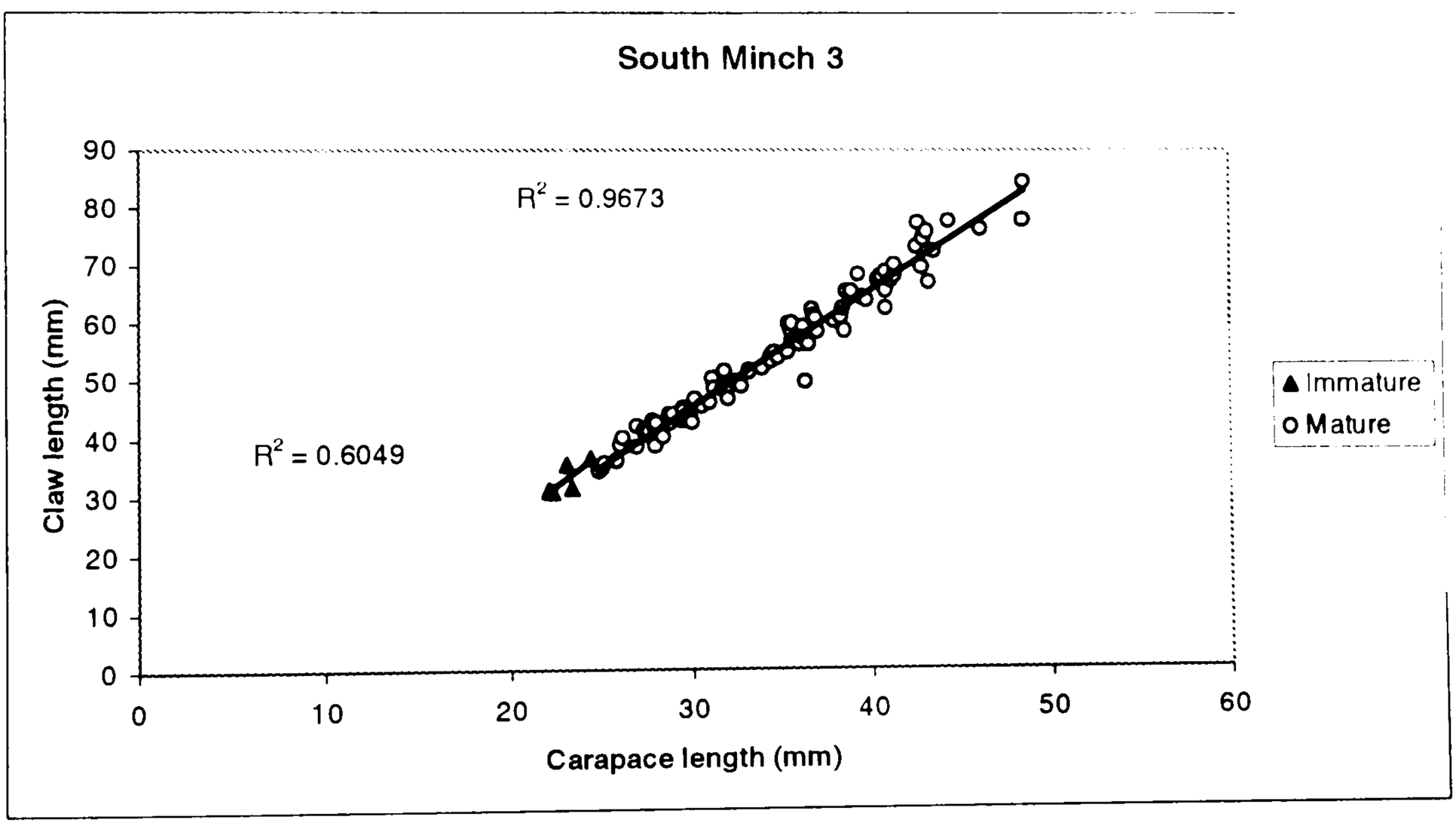

North Minch 1
Claw length (mm)
Carapace length (mm)
R^2 = 0.953
R^2 = 0.3853
Immature
Mature
0
10
20
30
40
50
60
70
80
90
0
10
20
30
40
50


North Minch 2
Claw length (mm)
Carapace length (mm)
R^2 = 0.9319
R^2 = 0.8604
Immature
Mature
0
10
20
30
40
50
60
70
80
90
100


North Minch 3
Claw length (mm)
Carapace length (mm)
R^2 = 0.9171
R^2 = 0.9583
Immature
Mature
0
10
20
30
40
50
60
70
80
90
100


South Minch 1
Claw length (mm)
Carapace length (mm)
R^2 = 0.9022
R^2 = 0.942
Immature
Mature
0
10
20
30
40
50
60
70
80
100
120


South Minch 2
R^2 = 0.9484
R^2 = 0.8962
Claw length (mm)
Carapace length (mm)
Immature
Mature
0
20
40
60
80
100
120
0
10
20
30
40
50
60


South Minch 3
R^2 = 0.9673
R^2 = 0.6049
Claw length (mm)
Carapace length (mm)
Immature
Mature
0
10
20
30
40
50
60
70
80
90
0
10
20
30
40
50
60