Morphometrics and preliminary biology of the caridean shrimp *Nauticaris marionis* Bate, 1888, at the Prince Edward Islands (Southern Ocean)

37°50'E, 46°45'S

by

Paddy Kuun

Submitted in fulfilment of the requirements for the
degree of Master of Science at Rhodes University

Supervisors:
Dr Evgeny Pakhomov
&
Prof. Christopher McQuaid

March 1998

For:

My son, Finbar, so that I can one day offer him a better deal than I had.

Dedicated to:

Evgeny Pakhomov, who showed me that, like a bottle of urine, a bottle of *N. marionis* can contain an incredible amount of information.


Allegro non troppo.
Kl. Flöte.
2 gr. Flöten.
2 Hoboen.
2 Clarinetten in B.
2 Fagotte.
2 Hörner in D.
2 Hörner in F.
2 Trompeten in D.
3 Posaunen und Baßtuba.
Pauken in D.A.
Violine I.
Violine II.
Bratsche.
Violoncell.
Contrabaß.
ff
f
fp
pp
p sotto voce
p
Allegro non troppo.


a 2
cresc.
molto cresc.
f
sf
ff
p cresc.
molto cresc.

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Acknowledgements

"THE RESPONSIBILITIES OF THE CANDIDATE ARE (DURING THE COURSE OF THE PROJECT) H) TO ACKNOWLEDGE ACCURATELY ALL SOURCES OF INFORMATION USED AND ASSISTANCE RECEIVED" (RHODES SENATE MINUTE NO. 03.1.3 OF 1 NOVEMBER 1991).

Normally, acknowledgements are never read by anyone other than the author and those he has acknowledged (note the political incorrectness). I intend this section to be a list of names that future Rhodes students can refer to in times of need, and so make it worth their while to read through.

About Evgeny Pakhomov I could write a book. I'm going to practice classical British understatement by simply pointing out that this thesis has been dedicated to him. It was not done lightly. Others obviously feel the same way I do, judging from the fact that Evgeny won the Vice-Chancellor's Award for Distinguished Research here at Rhodes whilst I was studying under him. I am proud to have his name on my thesis. I only hope that this dissertation rises to his expectations.

I am very grateful to Christopher McQuaid for the concern he has shown over me, particularly after my first project (in rocky shore ecology) was repeatedly vandalised, which led him to refer me to Evgeny. It must be pointed out that I specifically relocated to Grahamstown with wife and babe in arms to study under him as a man of formidable reputation. The project would have been a good one. It was such a pity that it never could be. His sound advice on the initial draft of this thesis has led, I believe, to a much more robust presentation, particularly for Chapter 3, with which even I was originally vaguely displeased with, although I couldn't quite put my finger on the reasons for this at the time.

Another rare person who cannot go unacknowledged is Sarah Radloff, who became Head of the Department of Mathematical Statistics while I was at Rhodes, an acknowledgement by her peers which she richly deserves. I'm afraid that I somewhat overused her expertise, although I was never made to feel as if I was. I owe her more than I can put into words, as do many other zoologists.

Two other people whom I strongly suggest future students in the department get to know well are Martin Villett and Terry Butterworth. They have both helped me with numerous professional problems, as well as many personal ones which definitely did not fall within their job descriptions. Kindness of heart is a human quality I appreciate immensely.

During moments of personal crises various people offered me support. I cannot thank Pat Hulley and Pete Illgner enough in this regard. Martin also does a pretty good impersonation of a psychologist. Also keen to help with practical solutions to personal problems were Prof. Randall Hepburn and Ric Bernard. I greatly appreciate what all these gentlemen did for me.

Other people who have offered me professional advice and help are Nikita Muller, Lil Haig, Alan Hodgson and Chris Brown (Zoology Dept.); Prof. Roy Lubke (Botany Dept.); Graham Shepherd and Mike Burton (Maths Dept.); Warren Snowball (Classics Dept.) and Neil Cannon (sadly no longer at the Electron Microscopy Unit). I am particularly grateful to Louise Page for proofreading this thesis when time was not readily available to either of us. Whilst I express my gratitude to all these people, all errors in this thesis reflect on me and me alone. Hopefully I shall be forgiven for them. "As the famous ichthyologist J.L.B. Smith remarked, there is only one sure-fire way to avoid errors - don't publish!" (Branch & Branch 1988: 11).

This thesis, and the aborted one that preceded it, were made possible through funding from the F.R.D., SANCOR, the Department of Environmental Affairs and Tourism (through the Southern Oceans Group at Rhodes University) and Rhodes University itself.

Abstract

Carapace length, as the best measure of *N. marionis* body size, is precisely defined. It is shown that *N. marionis* is a partially protandric hermaphrodite. *N. marionis* appear to hatch just before April each year, with a little hatching persisting until May. The vast majority of juveniles develop into males. The majority of males transmutate into females in their third year. By April/May the transmutation is probably complete. Reproduction can occur before all male secondary characteristics have been lost. A small minority of individuals develop directly into females without passing through a male phase. At least some of these females can be initially recognized after they have developed mature ovaries by the presence of *appendices internae* on their first pleopods, a male copulatory structure which all juvenile *N. marionis* possess. Too few gravid females were recovered to make any statement on whether spawning can occur before this structure is lost. Such females may lose their first pleopod *appendices internae* in one moult, possibly just before spawning, which may be in late April/early May. Such individuals seem to mature into ovigerous females at a slightly smaller carapace length than do the majority of females which have had a male-phase past. A few females which have passed through a male phase seem to begin developing ovaries at about this small carapace length as well. Once the *appendices internae* have been lost there appears to be no way of identifying any given female's past life-history. It would seem that during the first year of life *N. marionis* survive in undetected localities, moult into juveniles, and then settle amongst the benthos from the plankton. Diurnal vertical migration then occurs up to an unknown larger size. It is not known whether the larvae are initially planktonic or not. It is possible that settling of small *N. marionis* onto the benthos only begins after November. Whether the *appendices masculinae* of some males only begin growing after they have settled onto the benthos is unknown, but for the majority at least this begins whilst they are planktonic juveniles. Individuals older than five years are undetectable using samples of the sizes analyzed in this thesis, but they may well persist until quite an advanced age. Niche separation between smaller and larger *N. marionis* individuals may occur. Diel vertical migration may occur to some extent amongst large *N. marionis*. Itinerant euphausiids may contribute substantially to the maintenance of top predator populations at the archipelago, either through direct predation by those predators or via predation by *N. marionis*, which in turn are consumed by those predators. *N. marionis* itself is an opportunistic feeder, although the majority of its prey seem to be suspension feeders, both benthic and pelagic. In multisample situations, ageing of *N. marionis* cohorts is made less subjective if one utilizes the phenomenon of synchronized sexual inversion. The von Bertalanffy growth parameters for *N. marionis* are tentatively identified as $k = 0.2353$/year, $L_\infty = 12.69$mm, $t_0 = -0.2828$ years and $WW_\infty = 2.03$g. The programme FiSAT is discussed, having been found to be extremely useful, but having also been found to have certain faults. Various hypotheses are proposed and are put forward as suggestions for future studies.

Chapter 1

Introduction

> "In making both the descriptions and the drawings I have always felt that I was dealing with specimens of more than specific interest, since they were in many instances obtained from localities which are not likely to be explored again for some time, and which are scattered over a vast area of the Atlantic, Pacific and Indian Oceans" (Bate 1888: i).

1.1 General background:

The specimens referred to in the introductory quotation (part of that author's preface) included *Nauticaris marionis* Bate, 1888. Today, over a century later, publications dealing with this shrimp are sparse, most references being incidental observations to the effect that it has been found to be a component of the diets of certain top predators. Very little is known about its actual biology.

N. marionis is a caridean shrimp of the family Hippolytidae (Bate 1888: 577), although Christoffersen (1987) prefers his new family Nauticaridae. It is found over a wide area, from southern New Zealand in the east to the Prince Edward Islands in the west (Perissinotto & McQuaid 1990). Bate (1888: 610) refers to one specimen from Hong Kong which he called *N. unirecedens*, which he says was almost indistinguishable from *N. marionis*. Bate (1888:

606) also observes that although *N. marionis* is a bottom dweller, the species does not inhabit deep water (see also Branch *et al.* 1991 and Branch *et al.* 1993).

N. marionis made up most of the crustacean biomass dredged at the Prince Edward Islands in 1983-84 (Parker 1984: 67). Only bryozoans contribute more to the overall dredged biomass at this locale (Perissinotto & McQuaid 1990). However, *N. marionis* is not as predominant in other ecosystems where it is found (e.g. Ledoyer 1979, cited in Branch *et al.* 1991). Because it is so numerous at the Prince Edward Islands, *N. marionis* has been implicated as being important in making the phytoplanktonic primary production available to the numerous top predators on the islands (Perissinotto & McQuaid 1990). It has been suggested that these phytoplankton stocks are maintained by an 'island mass effect' (Parker 1984: 2, Allanson *et al.* 1985: 44, Boden 1988).

Predators of *N. marionis* which have been identified around this archipelago include the fishes *Notothenia macrocephala* (Blankley 1982) and to a lesser degree the congeneric *Notothenia magellanica* (Blankley 1981, 1984, both cited in Blankley & Grindley 1985: 634). Slight predation of the caridean shrimp by the starfish *Anasterias rupicola* (ibid.) and the King penguin *Aptenodytes patagonicus* (Adams & Klages 1987, Adams & Brown 1989) has been recorded, although the *N. marionis* remains recovered from the King penguin guts may have originally been taken by their fish or squid prey (Adams & Klages 1987). *N. marionis* has also been shown to be a prey item of the Gentoo penguin *Pygoscelis papua* (Adams & Brown 1989, Adams & Wilson 1987, both cited in Ridoux 1994), *P. papua* having been shown to make up to between 50% to 70% of the wet weight of its diet with *N. marionis* in some months (Adams & Klages 1989). 6% of the total wet weight of the diet of *P. papua* was juvenile *N. marionis* in one month's sample (La Cock *et al.* 1984). Juvenile *N. marionis* were furthermore found to be the main crustacean prey item of the Macaroni penguin *Eudyptes chrysolophus chrysolophus* and the Rockhopper penguin *Eudyptes chrysocome chrysocome* in 1983-84 (crustaceans having formed 98.3% and 99.7%% of that year's total diet wet weight respectively), although over the following year no *N. marionis* were found in *E. chrysolophus chrysolophus* stomachs, and very few were consumed by *E. chrysocome chrysocome* (Brown & Klages 1987). The Imperial cormorant *Phalacrocorax atriceps* has also been recorded as a shrimper (Blankley 1981, Espitalier-Noel *et al.* 1988, both cited in Ridoux 1994), *N. marionis* making up 10% of the mass of its diet at the islands (Blankley 1981, 1984, both cited in Blankley & Grindley 1985: 634). The complexity of the food web is such, however, that the contribution *N. marionis* makes to the diet of *P. atriceps* must take into account the preference this bird has for fish *Notothenia magellanica* (56% of the total dietary wet weight), which itself also feeds on *N. marionis* (see above).

Predation on *N. marionis* has also been recorded at other localities. *N. marionis* has been described as "one of the most important food-organisms (for vertebrates including fishes) in the Auckland Island waters" (Hefford 1949, cited in Yaldwyn 1958). At that locality *N. marionis* has been shown to be preyed upon by the Hooker's sea lion *Phocarctos hookeri* and the Auckland Island shag *Phalacrocorax campbelli colensoi* (Yaldwyn 1958). However, a review by Croxall (1987) has been cited by Perissinotto & McQuaid (1990) who have, referring to the Prince Edward Island group, noted that "to our knowledge, in no other Southern Ocean ecosystem has *N. marionis* been found to be an important component of the diet of seabirds". More recently, *N. marionis* has been found to be an important component of the diet of the Imperial cormorant *Phalacrocorax atriceps* at the Crozet Islands (Ridoux 1994), and is also preyed upon by the Gentoo Penguin *Pygoscelis papua* at this locale (ibid.).

1.2 The objects and basic structure of this thesis:

The importance of *N. marionis* to the Prince Edward Island group ecosystem appears to extend onto the land mass because of its rôle in coupling the marine and terrestrial food webs. Given this, and the fact that very little work on the ecology and biology of *N. marionis* has been done, it was decided to investigate certain fundamental aspects of the biology and ecology of this hitherto poorly studied animal. Morphometric relationships were therefore calculated to allow for future integration of studies in which this hippolytid plays a part, particularly those in which it is identified as a prey item.

The diet and life-history of *N. marionis* were also examined. During the analysis of the life-history of *N. marionis*, data were obtained which allowed for a preliminary calculation of its growth parameters. *N. marionis* is not a commercially harvested species, although there exists the possibility that this may change in the future (Pakhomov pers. comm.). Pre-harvest data may prove to be important in the construction of realistic future maximum sustainable yield models should this eventuality become reality, a luxury not afforded to many historical fisheries which have had to rely on growth parameter estimates which have only been derived well after exploitation of the stock in question had already commenced.

The aims of this thesis consequently are:

1) To present conversion formulae to facilitate energy transfer studies involving this shrimp at the Prince Edward Islands.
2) To estimate preliminary length-based and wet weight-based growth parameters for *N. marionis* for the first time in order to estimate the species' natural longevity.
3) To make further observations about the biology of *N. marionis*, and in so doing to present evidence that the species is predominantly protandrous.
4) To make observations on the diet of *N. marionis* as a further aid to understanding its rôle in the food web at the island group.

1.3 Samples:

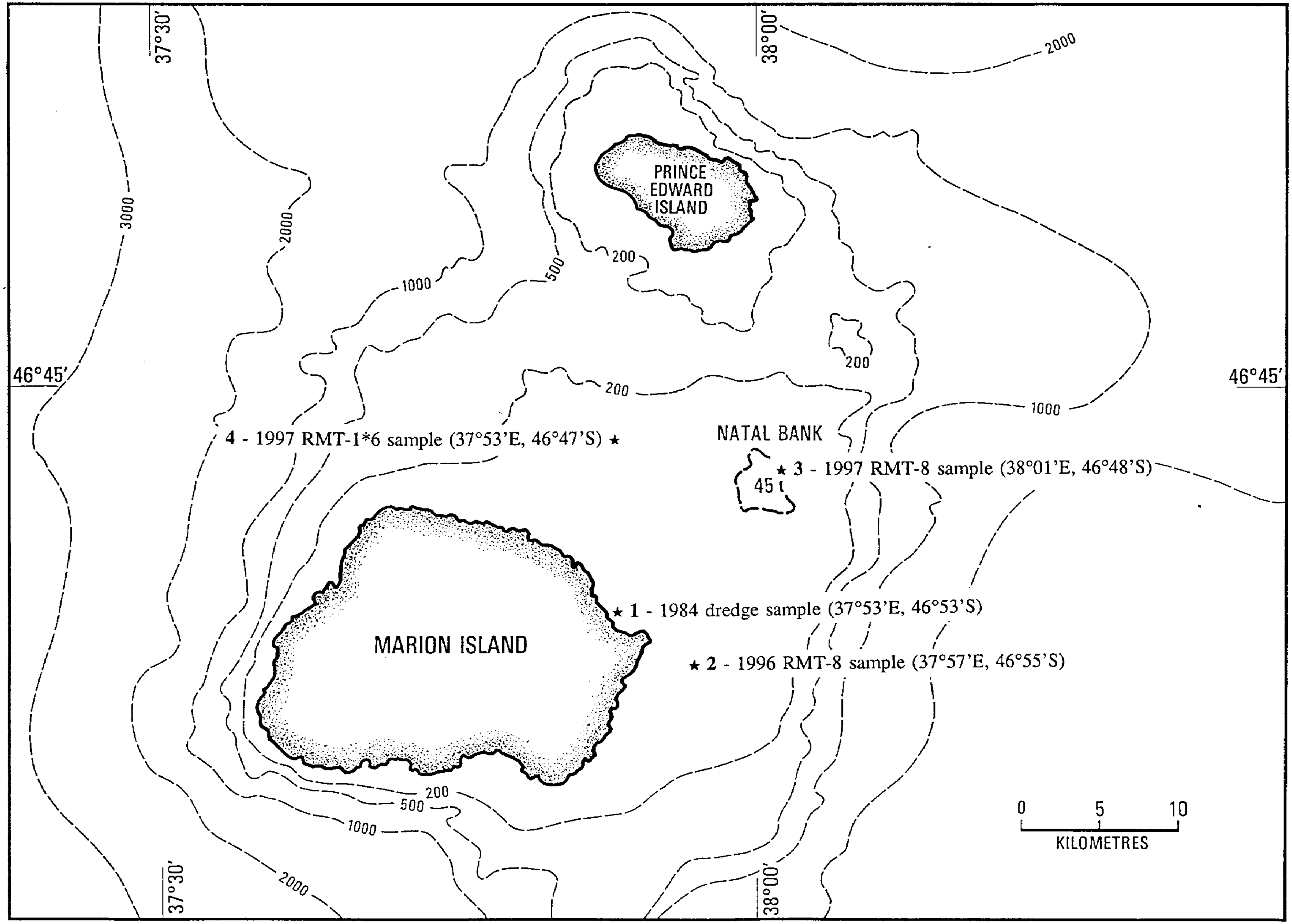
The shrimps examined came from four stratified random samples which had been preserved in 10% buffered formalin: **1** - The majority from a dredge in 1984 taken north of East Cape at Marion Island; **2** - a small sample (only 28) from an RMT-8 mesh of 5mm in 1996, trawled between 5m and 10m above the bottom; **3** - a larger sample from an RMT-8 mesh of 5mm north of Natal Bank which struck the bottom at about 90m; and **4** - another small sample (only 21) from an RMT-1*6 mesh of 0.2mm taken in an oblique tow from ~150m to ~50m from the surface, at a point where the sounding was 165m. A mechanical malfunction prevented a larger sample being taken with the RMT-1*6 mesh. Attention is drawn to the fact that different sampling techniques were employed, and that the largest sample was taken 12 or 13 years earlier than the other three samples. The samples were also taken at different times of the day. The samples were treated as if they had been taken in the same season of different years, the 23 day difference between 26 April and 19 May being regarded as insignificant.

Sample number	Year	Sampling technique	Date of sample	Sampling time (GMT)	Sampling depth (m)	Sample station co-ordinates
1	1984	Dredge	26/4/84	11H00	97m	37°53'E 46°53'S
2	1996	RMT-8 net	8/5/96	00H39	120m-130m (5m-10m above bottom)	37°57'E 46°55'S
3	1997	RMT-8 net	19/5/97	19H17	80m-100m (struck bottom at ~90m)	38°01'E 46°48'S
4	1997	RMT-1*6 net	5/5/97	22H57	150m-50m (oblique tow to surface)	37°53'E 46°47'S

Table 1.1: Details of the four stratified random samples examined for this dissertation (see also Figure 1.1 and Appendix 1).

Overleaf:

Figure 1.1: Map of the Prince Edward Islands showing the sample station positions (see also Table 1.1). Isobars show the depth in metres. Map courtesy of the Southern Oceans Group at Rhodes University.


37°30'
38°00'
2000
3000
2000
1000
500
200
PRINCE EDWARD ISLAND
200
46°45'
46°45'
200
1000
NATAL BANK
45
4 - 1997 RMT-1*6 sample (37°53'E, 46°47'S)
3 - 1997 RMT-8 sample (38°01'E, 46°48'S)
MARION ISLAND
1 - 1984 dredge sample (37°53'E, 46°53'S)
2 - 1996 RMT-8 sample (37°57'E, 46°55'S)
200
500
1000
2000
0 5 10
KILOMETRES
37°30'
38°00'

Chapter 2

Morphometric relationships

"IN THE SCIENCES, EQUATIONS MAY INACCURATELY REPRESENT NATURAL PROCESSES YET MAY BE EMPLOYED ADVANTAGEOUSLY TO PREDICT THE VALUE OF ONE VARIABLE GIVEN THE VALUE OF AN ASSOCIATED VARIABLE." (ZAR 1984: 278)

2.1 Introduction:

2.1.1 The necessity for conversion formulae:

Analyses of the diets of predators feeding on *N. marionis* have often taken the form of assessing the proportion of total gut content weight formed by identifiable *N. marionis* remains (e.g. Blankley 1982, La Cock *et al.* 1984, Adams & Klages 1989, Adams & Brown 1989). Such an approach leads to an overestimation of prey items which digest more slowly (Adams & Klages 1987).

This approach is necessary in the case of crustacean prey because digestive processes often do not allow for their body sizes to be measured (Adams & Klages 1987). Furthermore, formulae for calculating total body length and/or mass from partial body part measurements are usually unavailable (Ridoux 1994). In a study of the diets of 27 seabird species at the Crozet Islands, Ridoux (1994) tried to address this bias by developing his own equations from intact crustacean specimens found in the stomachs of the predatory seabirds. These specimens had already been subjected to some form of digestive action. In the case of *N. marionis*, Ridoux used 30 specimens to calculate a formula converting a length measurement into wet weight. This formula is, to the best of my knowledge, the only such conversion formula for *N. marionis* in the literature.

Ridoux unfortunately had a very narrow range and a small sample size, even though his coefficient of determination was 0.98. More importantly, he only supplies one with an equation relating body mass in grams (what I shall call 'wet weight') to *body length* in centimetres. The "body length" measurement for *N. marionis* which Ridoux uses is not defined, although it would seem from his diagrams of euphausiids that it is probably the length from the anterior tip of the rostrum to the posterior edge of the telson (I have abbreviated this to RT). It is not clear how he subsequently dealt with *N. marionis* remains for which the body length was not measurable.

Given the probable importance of *N. marionis* in the food web at the Prince Edward Islands (see section 1.1), it will at some stage become necessary to have better equations available for the estimation of energy transfer and for other studies at this locality, and this is the primary objective of this chapter.

2.1.2 Measures of body size and their usefulness:

The RT measurement is unreliable as an indicator of *N. marionis* body size for several reasons. *N. marionis* often adopts a foetal-like position making measurement of the straight-line length difficult (an observation made on the ovigerous females of the caridean genus *Crangon* by Kuris & Carlton (1977), when they recalled that Israel (1936) had reported a 6-8% variation in this measurement depending on how the specimen was held). It is a measurement unlikely to be available for many ingested *N. marionis* remains after capture, swallowing and partial digestion. There is also a vast disparity in size between the largest and smallest specimens, necessitating the usage of different methods of measurement for the two groups (a problem not faced by Ridoux (1994) because he was only looking at larger specimens). Hoffman (1972) has also reported the lengths of the rostrum and abdomen varying in caridean shrimps (*Pandalus platyceros*) of identical carapace lengths.

For this dissertation, therefore, I have chosen to follow the advice of Sparre & Venema (1992: 11) that "the most accurate measure for shrimps and lobsters is the "carapace length"". The carapace is a relatively long and inflexible body part and therefore suited as a measure of body size (Kuris & Carlton 1977).

2.1.3 The need for an exact definition of carapace length:

A number of different definitions of 'carapace length' of shrimps can be found in the literature, many of them ambiguous. In at least one instance it was not defined at all. Perissinotto & McQuaid (1990) published, in histogram form, *N. marionis* 'carapace length' data showing size distribution without defining this measurement. The data originally comes from Parker (1984: 74), but a definition of the measurement used is not available from either that source or its author. It is impossible to gauge how the measurement was taken. Probably, however, it did not coincide with my own definition of the term (section 2.2.1.5 below), for his sample would then have included an unrealistic number of large animals. Some would have been much larger than those recorded by Ridoux (1994) who, it should be noted, dealt with the gut contents of seabirds which had no doubt fed selectively on larger specimens. It is also unlikely that Parker (1984) included the rostrum in his measurement, for his sample would then have included benthic *N. marionis* specimens about a third smaller than the planktonic specimens sampled for this dissertation. It must be pointed out here, though, that his sample was numerically well in excess of both Ridoux's (1994) and my own, so his range is likely to be greater. However, "a really important thing is to specify exactly what kind of length measurement has been used, as one may otherwise run into difficulties when comparing results with those of other investigations" (Sparre & Venema 1992: 11).

I have therefore concluded that Parker's (1984) data are not useful for any comparative study. It is unfortunate that such potentially valuable data had to be discarded for want of a proper definition of the size measurement used, as the data set was large and, to the best of my knowledge, this is the only study in which a size-frequency distribution of *N. marionis* is presented.

Different definitions of carapace length in the literature include that of Hoffman (1972) who, while working on the caridean shrimp *Pandalus platyceros*, defined it as "the measurement ... along the left side of the carapace from the base of the eyestalk to the posterior edge of the carapace", although he does not define whether the posterior edge of the carapace is dorsal or not. Bauer (1976), on the other hand, while studying the related Hippolytid shrimp *Heptacarpus pictus*, refers to 'carapace length' as "the **mid-dorsal** distance from the posterior edges of the eye orbits to the posterior edge of the carapace". In 1977 Kuris & Carlton, while working on a comparison between seven different caridean species, redefined the carapace length as "*the posterior margin of the orbit to the dorsal midline of the posterior margin of the carapace*" (my italics, possibly the same measurement used by Hoffman 1972). In 1986, Bauer adopted the italicized definition when studying the caridean shrimp *Thor manningi* (cf.

the definition in Bauer 1976), and this definition has subsequently been used on the two caridean shrimps *Chorismus antarcticus* (Gorny *et al.* 1993) and *Nematocarcinus lanceopes* (Gorny & George 1997). It is obvious that some uniform agreement in the measure of this important variable should be reached to facilitate future comparative studies.

I found Kuris & Carlton's (1977) definition more reliable than that of Bauer (1976) because under the microscope the decision as to 'how the dorsal line runs' is less subjective. This measurement is also more reliable than any including the length of the rostrum because many rostra in my sample were either broken or bent out of shape. Kuris & Carlton's (1977) definition covers the longest hard body part that is least likely to be bent out of shape under stress (such as that which occurs when the animal is captured by net or predators), and has defined beginning and end points of measurement. Errors associated with different researchers taking this measurement are therefore minimized.

2.2 Materials and methods:

Morphometric measurements were obtained from samples 1 and 2 (the 1984 dredge and the 1996 RMT-8 samples respectively; see section 1.3). Only 1984 dredge sample specimens were used for the dry weight and ash-free dry weight measurements. The specimens used for these two mass measurements were randomly selected. Therefore, relatively few large animals were represented. Because specimens were destroyed during the weighing process (see sections 2.2.1.2 & 2.2.1.3 below), larger specimens could not be selectively removed from the greater sample which remained to improve this situation. These larger specimens were necessary for later gut-contents analysis, which required whole animals (see Chapter 5). All other morphometric measurements could be measured without destroying specimens. Therefore, where possible, larger, non-randomly selected animals were included when measuring variables. This somewhat alleviated the lack of representation of larger animals in most cases. The sum of squares of X_i values were also calculated to allow the reader to compute the confidence and prediction limits over the entire ranges examined for all independent variables, including those for which there were sparse data at their extreme ends. The sum of squares of X_i values is defined as $\Sigma_{i=1}^{n} X_i^2 - (\Sigma_{i=1}^{n} X_i)^2/n$ (Zar 1984: 264), and the definitive formulae for calculating the confidence and prediction limits are given by Zar (1984: 274, equations 17.26 & 17.29).

Relationships between selected measurements were calculated, and where non-linear were transformed to ln or lnln equations which are amenable to linear regression techniques (Zar 1984: 287). During regression no unexplained outliers were excluded from any of the data sets. The slope and intercept of Ridoux's (1994) length/wet weight equation were compared to a corresponding equation calculated from this data set using a t-test. Parasitized *N. marionis* were included when calculating these relationships (see Mathews *et al.* 1987: 163 for a discussion on their effects on data sets) because the equations are intended to be of use in situations where parasitized shrimps are present.

2.2.1 Definitions:

Three of the size measurements are further illustrated in Figure 2.1 and equation numbers refer to those in Table 2.1. Abbreviations given in this section (such as 'WW') are used throughout this thesis. Apart from the RT measurement (section 2.2.1.7 below), length measurements were made using a dissecting microscope with an ocular micrometer.

2.2.1.1 WET WEIGHT (WW): *Equations 1, 4, 7, 10, 18 & 19.*

Shrimps were removed from the formalin and lightly pressed into tissue paper to remove excess moisture. They were then weighed in grams on a Mettler PE 1600 scale to *two* decimal places i.e. to the nearest centigram. Unfortunately a more accurate scale was not available and about a third of the specimens were weighed to only one significant figure. Most specimens, however, were weighed to two or more significant figures.

2.2.1.2 DRY WEIGHT (DW): *Equations 2, 5, 8, 18 & 20.*

Shrimps were dried in a Labotec Term-O-Mat oven at 60°C for 48 hours and then weighed on a Cahn C-31 Microbalance after first being allowed to cool to ambient temperature in a desiccator for one hour. It was found that cooled specimens did not absorb moisture from the atmosphere as readily whilst they were being weighed. They were weighed in small batches of about five at a time to minimize the time spent in the desiccator for each specimen. Once removed from the desiccator, each specimen was allowed to settle on the scale for exactly two minutes and was then immediately weighed. During these two minutes the specimens absorbed moisture from the atmosphere, probably not randomly as larger animals have a smaller surface-area to body-volume ratio (Schmidt-Nielsen 1990: 195). It was noticed, however, that the masses recorded on the Cahn Microbalance of even the larger animals stabilized at around two minutes. The Cahn Microbalance was too sensitive to measure the wet weight of the larger shrimps. The dry weight was measured to the nearest hundredth of a milligram.

2.2.1.3 ASH-FREE DRY WEIGHT (CC): *Equations 3, 6, 9, 19 & 20.*

Dried shrimps were placed in a muffle furnace at 500°C for 24 hours, and after ashing were weighed using the same procedure as for weighing the dried animals. The ash weight was subtracted from the dry weight to yield the ash-free dry weight to the nearest hundredth of a milligram.

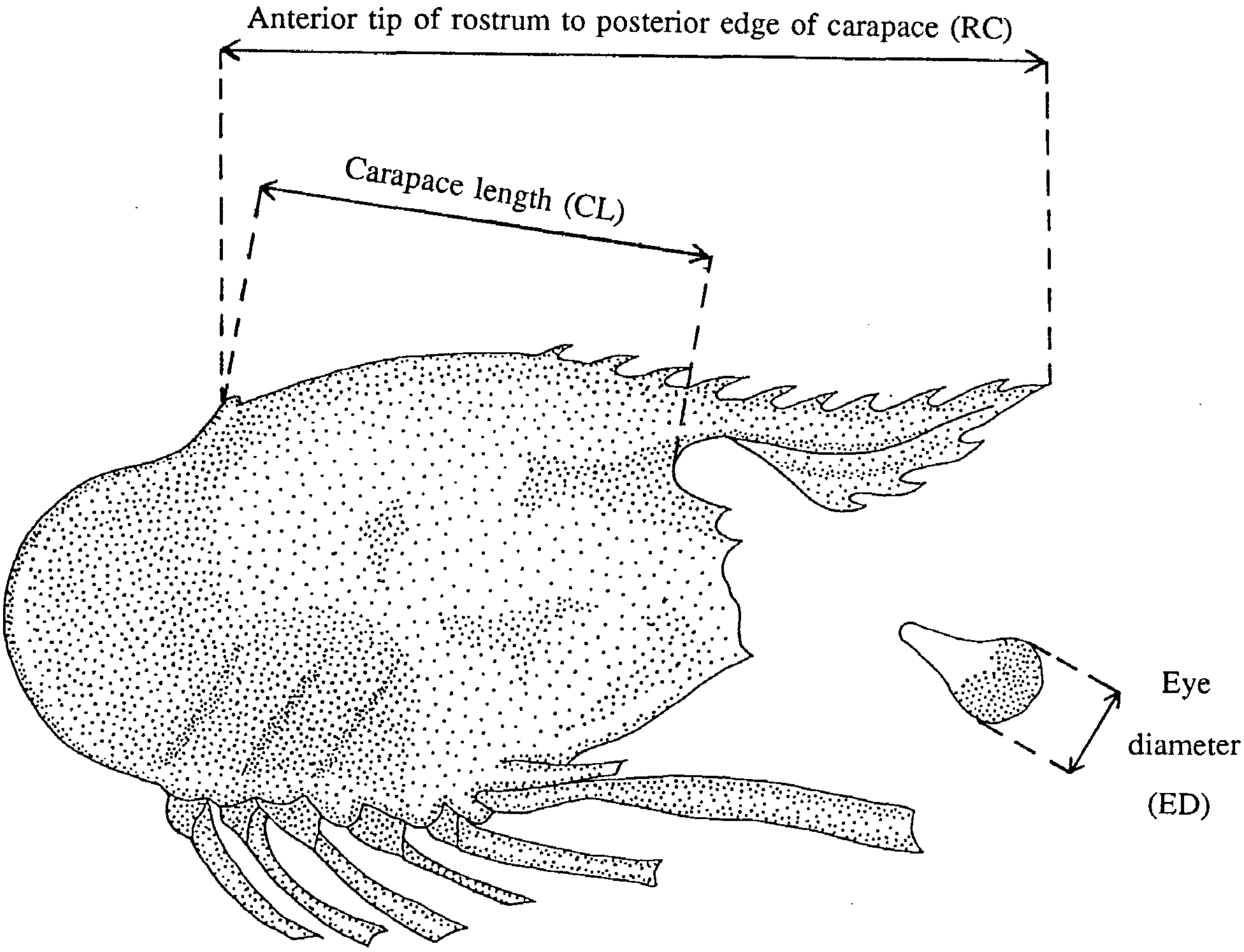


Figure 2.1: Diagrammatic representation of three of the measurements referred to in section 2.2. Eye diameter (ED, section 2.2.1.4); Carapace length (CL, section 2.2.1.5); Anterior tip of rostrum to posterior edge of carapace (RC, section 2.2.1.6). All three measurements were made in mm. Range: (♂) CL = 2.46mm to 8.17mm; (♀) CL = 5.17mm to 12.67mm. Loosely based on a drawing by Bate (1888, Plate CVIII).

2.2.1.4 EYE DIAMETER (ED): *Equations 1, 2, 3, 14, 15, 16 & 17.*

Crustacean eyes are sometimes found separately in predator stomachs (Ridoux 1994). Eyes were removed from all specimens on the right-hand side in order to facilitate the later measurement of carapace length, which needed an unimpeded view of the posterior edge of the eye orbit. They were then placed under a dissecting microscope at 500X magnification and the maximum diameter to the nearest hundredth of a millimetre was measured using a micrometer scale. The spherical end of the eye is slightly oblate, and its *maximum* width (with the pole facing the viewer) was measured after removal from the shrimp to realistically simulate future comparisons where only the eye is present. See Figure 2.1.

2.2.1.5 CARAPACE LENGTH (CL): *Equations 4, 5, 6, 11, 12, 13, 14 & 16.*

Defined above in section 2.1.3 as 'the posterior margin of the orbit to the dorsal midline of the posterior margin of the carapace', carapace length was measured on the right-hand side of all individuals to the nearest hundredth of a millimetre after the eye had been removed, and I shall use this measure in the rest of this thesis more than any other. Figure 2.1 shows a diagrammatic representation of how the measurement was taken under a dissecting microscope, which had a magnification range of 60X to 500X. The sizes of the specimens varied considerably, and the greatest magnification possible was used on each specimen.

2.2.1.6 ANTERIOR TIP OF ROSTRUM TO POSTERIOR EDGE OF CARAPACE (RC): *Equations 7, 8, 9, 12, 13, 15 & 17.*

This measurement is what Parker (1984) may or may not have called 'carapace length' (see section 2.1.3 above), measured to the nearest hundredth of a millimetre under a dissecting microscope as described above in section 2.2.1.5. It should not be confused with my own definition of carapace length, which, amongst other things, did not include the length of the rostrum (see section 2.2.1.5 above and Figure 2.1). This is not a terribly useful partial body part measurement (see section 2.1.3 above).

2.2.1.7 ANTERIOR TIP OF ROSTRUM TO POSTERIOR END OF TELSON (RT): *Equations 10 & 11.* This measurement is probably what Ridoux (1994) called the "body length", a term also used in this thesis. I do not recommend its usage for reasons outlined in sections 2.1.2 above and 2.3 below. The measurement was made to the nearest tenth of a millimetre using a Vernier calliper.

2.3 Results and discussion:

The equations in Table 2.1 are presented here solely as a means of predicting the values of other variables. Relationships between various fragmentary body measurements and the three measures of mass viz. wet weight, dry weight and ash-free dry weight (see equations 1 to 10) are included. I have considered the possibility that it may be necessary to compare two separate *N. marionis* body parts in the same predator's stomach and make a decision as to whether they came from the same individual or not (equations 14 to 17). It may also be useful to have some measure of dry weight and ash-free dry weight in a sample given just the wet weights of individual specimens (equations 18 and 19). I have also included a formula to calculate the ash-free dry weight from a measured dry weight (equation 20). Furthermore, it became obvious during this study that some method of comparing the relationships between the various length measurements was necessary, in order to facilitate comparisons between different studies, a necessity also identified by Sparre & Venema (1992: 11). This is done in equations 11 to 13. I have not related Adams' & Klages' (1989) measurement of length to the definition of carapace length used in this study, as it was not used to calculate body mass in that study.

No doubt due to the time of year of sampling, no specimens in berry were used in deducing the equations. It should therefore be borne in mind that when using other data sets deviations from the expected relationships may occur because of this. However, it may be noted that the wet weight of the single gravid female recorded by Pakhomov *et al.* (in review: Table 1) fell within the 95% confidence interval of equation 4 in Table 2.1, as did the wet weights of all eighteen specimens presented in that study. The carapace lengths of all eighteen of those specimens fell within the range covered by equation 4. The researcher who measured the carapace lengths of those specimens also measured those for this study.

Overleaf:

Page 19, Table 2.1: Standard linear relationships between partial body part measurements, biomass and other partial body part measurements. ED = eye diameter in mm; CL = carapace length in mm; RC = posterior tip of rostrum to anterior tip of carapace in mm; RT = posterior tip of rostrum to anterior tip of telson in mm; WW = wet weight in grams; DW = dry weight in milligrams; CC = ash-free dry weight in milligrams; ln = natural logarithm; Ind. variable = independent variable; Dep. variable = dependant variable; S.E. = standard error; Range = untransformed independent variable range (see section 2.3); SS = sum of squares for independent variable values (see section 2.2 for a precise definition). Both the SS and sample means were calculated from the sometimes transformed linear independent variables indicated above e.g. '-2.1403' is the average natural logarithm of the wet weight, not the average wet weight. None of the specimens were in berry; for such specimens different correlations may hold true.

Pages 20, 21, Figure 2.2: Three measures of weight regressed against two measures of length. Equation numbers refer to those in Table 2.1.

Equation number	Ind. variable	Dep. variable	Linear relationship	Linear S.E.	r^2	Range	n	Sample mean	SS
1	ED	lnWW	lnWW=(3.2870*ED)-6.3465	0.2137	0.946	0.82mm - 2.18mm	457	1.3436	33.6119
2	ED	lnDW	lnDW=(3.5889*ED)-1.5512	0.2096	0.947	0.90mm - 1.90mm	212	1.2846	12.8771
3	ED	lnCC	lnCC=(3.5872*ED)-1.6819	0.1967	0.953	0.90mm - 1.90mm	211	1.2871	12.7955
4	lnCL	lnWW	lnWW=(2.6520*lnCL)-6.3154	0.1591	0.970	2.46mm - 12.58mm	460	1.6524	54.2473
5	lnCL	lnDW	lnDW=(2.8471*lnCL)-1.4548	0.1751	0.963	2.76mm - 10.42mm	212	1.5846	20.6095
6	lnCL	lnCC	lnCC=(2.8188*lnCL)-1.5459	0.1540	0.970	2.76mm - 10.42mm	210	1.5850	20.2267
7	lnRC	lnWW	lnWW=(2.9854*lnRC)-8.7896	0.1500	0.974	5.50mm - 22.33mm	375	2.3088	35.9401
8	lnRC	lnDW	lnDW=(3.3051*lnRC)-4.3222	0.1650	0.968	5.75mm - 19.50mm	190	2.2330	13.9547
9	lnRC	lnCC	lnCC=(3.2917*lnRC)-4.4283	0.1497	0.973	5.75mm - 19.50mm	188	2.2343	13.7388
10	lnRT	lnWW	lnWW=(3.3090*lnRT)-12.2286	0.1494	0.951	21.1mm - 48.1mm	188	3.3193	7.4363
11	RT	CL	CL=(0.2814*RT)-1.0512	0.3509	0.959	21.1mm - 48.1mm	187	28.2497	6751.607
12	CL	RC	RC=(1.7271*CL)+0.9272	0.3948	0.987	2.46mm - 12.58mm	378	5.5948	1517.019
13	RC	CL	CL=(0.5716*RC)-0.4585	0.2271	0.987	5.50mm - 22.50mm	378	10.5900	4583.771
14	lnED	lnCL	lnCL=(1.6167*lnED)+1.2077	0.0819	0.943	0.82mm - 2.18mm	455	0.2771	19.0825
15	ED	lnRC	lnRC=(1.1003*ED)+0.8257	0.0677	0.953	0.88mm - 2.18mm	378	1.3489	28.9746
16	lnCL	lnED	lnED=(0.5830*lnCL)-0.6881	0.0492	0.942	2.46mm - 12.58mm	455	1.6557	52.9204
17	lnRC	lnED	lnED=(0.6431*lnRC)-1.2075	0.0489	0.944	5.50mm - 22.50mm	378	2.3100	36.8027
18	lnWW	lnDW	lnDW=(1.0711*lnWW)+5.3507	0.1252	0.981	0.03g - 1.15g	214	-2.1430	150.226
19	lnWW	lnCC	lnCC=(1.0666*lnWW)+5.2078	0.1186	0.983	0.03g - 1.15g	213	-2.1341	149.681
20	DW	CC	CC=(0.8648*DW)+0.1183	1.8037	0.997	4.41mg - 253.55mg	211	33.1462	339183.9

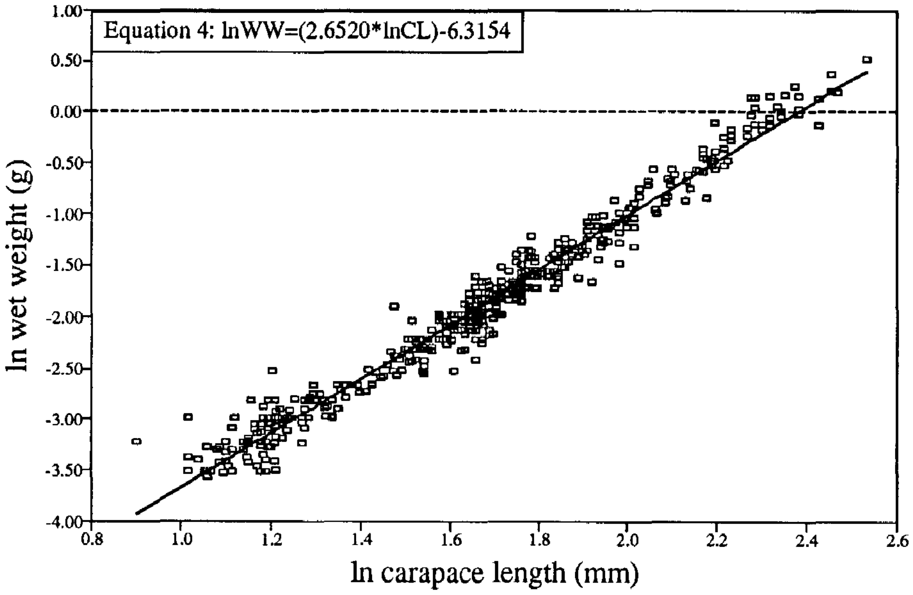


Figure 2.2A: ln wet weight (lnWW) as a function of ln carapace length (lnCL); $r^2 = 0.97$, $n = 460$.

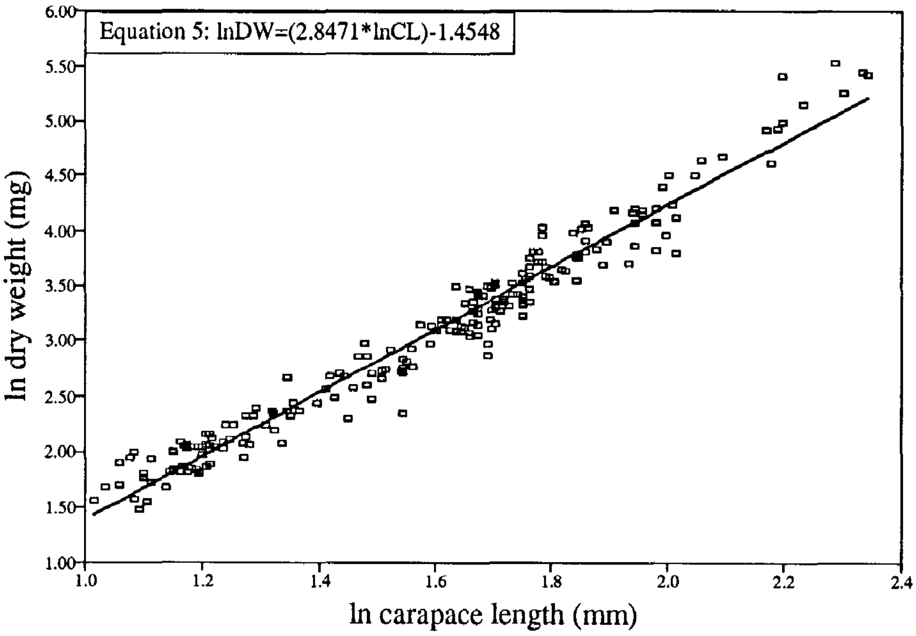


Figure 2.2B: ln dry weight (lnDW) as a function of ln carapace length (lnCL); $r^2 = 0.96$, $n = 212$.

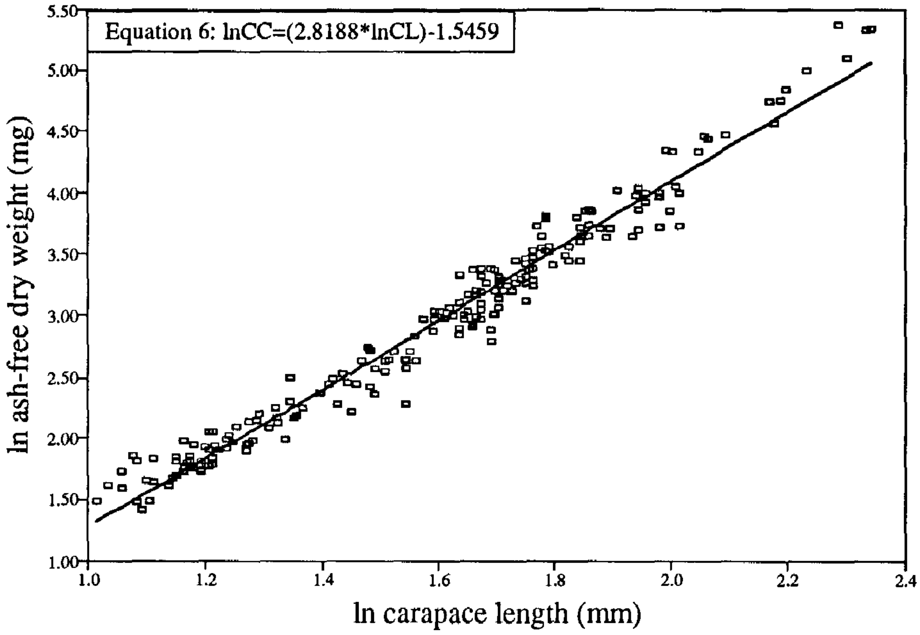


Figure 2.2C: ln ash-free dry weight (lnCC) as a function of ln carapace length (lnCL); $r^2 = 0.97$, $n = 210$.

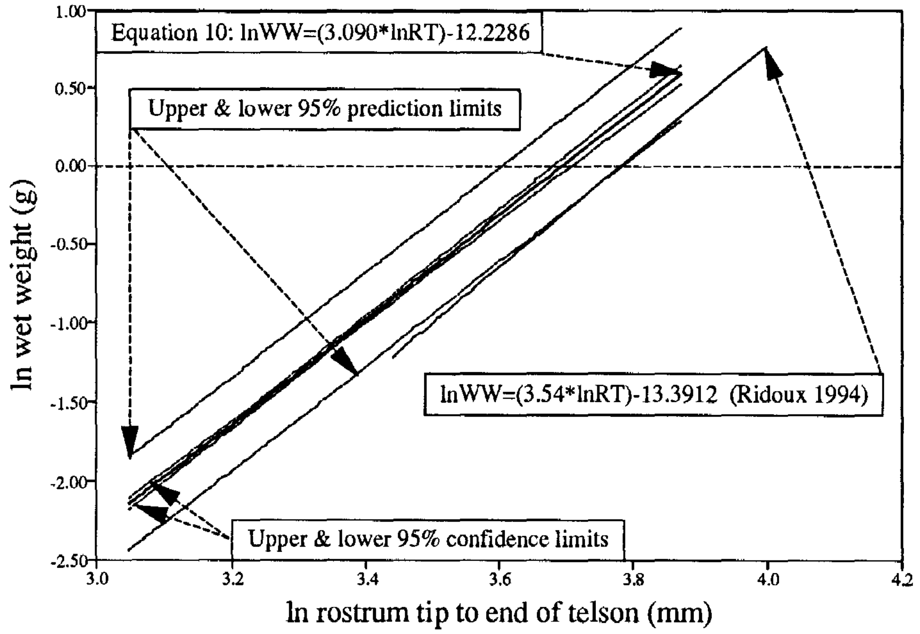


Figure 2.2D: ln wet weight (lnWW) as a function of ln 'body length' (lnRT), with confidence and prediction limits; $r^2 = 0.95$, $n = 188$; Ridoux's (1994) regression, with a different range has been transformed and superimposed.

In Table 2.1 equation constants are given to four decimal places, although calculated values using these equations should only be reported to two decimal places (except for 'body length' (RT) which should be reported to only one decimal place). The extra decimal places were reported in this table, and indeed in others throughout the following chapters, because "in doing a calculation of two or more steps, it is desirable to retain additional digits for intermediate answers. This ensures that small errors from rounding do not appear in the final result" (Ebbing 1987: 9). Similarly, Zar (1984: 340) points out that "round-off errors at an early computational stage will be exacerbated during successive calculations". These authors recommend rounding off final results, not intermediate ones. This point, not completely understood by many researchers in different disciplines, is further subtly emphasized by Zar (1984: 485).

In the case of comparing an eye and one of the two carapace measurements, both found separately in a predator's stomach, with the intention of deciding whether they could have come from the same animal or not, it is best to use the harder body part (if this is relatively undamaged) as the independent variable. Harder body parts are less subject to measurement error. If, however, this is not possible, then one should use the least damaged body part as the independent variable and make a judgement as to whether the more damaged body part *could* have fallen within the predicted 95% prediction interval (given the size of the other variable, the reported standard error and sum of squares of the independent variable values for that equation, see equations 14 to 17). This procedure would minimize measurement error of the independent variable, which is necessary because during regression analysis "what we are doing in practice is assuming that the errors in the *X* [independent variable] data are negligible" (Zar 1984: 268, see also Walpole 1990: 353).

Equation 14, which calculates the expected carapace length from a given eye diameter, could have read lnCC = (1.2127 * ED) + 0.0223 (standard error = 0.0805; $r^2 = 0.944$; $\overline{x} = 1.3470$; sum of squares for the independent variable values = 33.9872; n and range remain the same). This is a slightly better correlation co-efficient than the one reported in Table 2.1, but the plotted data, although more compact, showed a slight but consistent downward trend. An exponential relationship such as this might have been expected, rather than the logarithmic relationship reported in equation 14, because of the exponential relationship in equation 15 which calculates another expected length measurement from eye diameter (in this case the carapace length including the rostrum, defined in section 2.2.1.6 above, which is itself linearly related to the carapace length in equations 12 & 13), and so it is reported here.

Likewise, one might have expected a logarithmic relationship between the two mass measurements of dry weight and ash-free dry weight in equation 20, given the other two logarithmic mass/mass relationships (equations 18 & 19). In fact, such a relationship does exist at an imperceptibly smaller r^2 value of 0.9970 as opposed to the 0.9973 of equation 20. This relationship is lnCC = (0.9899 * lnDW) - 0.1033 (standard error = 1.8037; $\overline{X} = 3.0547$; sum of squares for the independent variable values = 173.216; n and range remain the same). All relationships examined which had correlation coefficients of less than 0.950 (but never less than 0.942) involved eye diameter measurements. This must be attributed to the greater difficulty in measuring the diameter of a soft body part which can easily lose a bit of shape.

It is obviously not possible to use equations 18, 19 or 20 to convert total reported wet weight or dry weight measurements (from other studies) to other biomass equivalents as such grand totals would fall outside my measured range. However, in the absence of individual data from other studies, as a *rough* guide it may be pointed out that the total **dry weight** of the specimens used in this study was 19.54% of the total wet weight and the total **ash-free dry weight** was 86.83% of the dry weight and 17.03% of the wet weight. These last percentages should be used circumspectly, but they can give an idea, though, of *approximately* how much dry weight and ash-free dry weight was present in previous studies where total mass has been reported but individual data sets are unavailable. For instance, in Ridoux's (1994) gut samples from 129 Imperial cormorants (*P. atriceps*) at the Crozet Islands the reported *total* reconstituted wet weight of *N. marionis* was 326.8g, so the dry weight would have be about 63.86g and the ash-free dry weight about 55.65g (ash-free dry weight calculated from the reported wet weight measurement). The assumptions that these percentage relationships hold for data like his which fall outside my measured range and that they can be applied to data obtained from the guts of predatory seabirds, which may be feeding preferentially on certain size classes is, however, tenuous. So too is the assumption that his original figure of 326.8g is accurate, given the different relationship between the two variables uncovered in this thesis (using a larger sample size), as well as what has been mentioned hereto regarding the acceptability of the RT measurement.

Total body length data were primarily recorded to allow a conversion between Ridoux's (1994) definition of the term and what I have called 'carapace length' (defined in section 2.2.1.5), assuming that the same relationships hold at both localities. This is, I think, a reasonable assumption (the same species at two relatively closely situated sub-Antarctic Island groups at more or less the same latitudes which, *inter alia* "form part of the Kerguelen Province of sub-antarctic islands" (Branch *et al.* 1991).

In comparing my own and Ridoux's (1994) equations relating body length to wet weight, I only used animals of 0.11g and more. This was done because I found when correlating my RT data to other measurements that a distinct kink appeared in my graphs exactly at the point where I stopped measuring the smaller specimens under a dissecting microscope at 60X magnification and began measuring the larger specimens using a Vernier calliper. I consequently discarded those data points smaller than 21mm (i.e. those measured under the microscope) to concentrate on a comparison between the two sets of data (see section 2.2.1.7 above for a description of how the measurement was taken). Ridoux reports his range, incidentally, as the "*Y* range". All sample ranges reported by me in Table 2.1 are untransformed **independent** variable ranges, following Zar (1984: 267) that "what the linear regression describes is *Y* as a function of *X within the range of observed values of X*" (his italics).

Ridoux's (1994) equation relating body length (RT) to wet weight (WW) is, after transformation, lnWW = (3.54 * lnRT) - 13.3912. The slope and intercept of my equation 10 (lnWW = (3.3090 * lnRT) - 12.2286) are both significantly different to the slope and intercept of the former equation (t-test, $p<<0.001$ in both cases). Figure 2.2D shows that when plotted, Ridoux's (1994) equation lies outside my 95% confidence limits for the comparable overlapping ranges, but inside my *prediction* limits for *part* of the overlapping range. Ridoux's (1994) range did not extend below 31mm carapace length, although it did extend higher than my own. My wet weight (or *dependent variable*) range was 0.03g to 1.68g, whereas Ridoux's was 0.3g to 2.2g.

I do not recommend the use of either of these last two equations for reasons outlined in section 2.1.2. It is, in any event, difficult to imagine many individuals in any sample of this shrimp where both the total body length is available and the more durable carapace length is not. Why Ridoux chose this measure in favour of some form of carapace length measurement is unclear, although it must be mentioned that Zupo (1994) also used this measurement on the related Hippolytid shrimp *Hippolyte inermis*. It is strongly recommended that the carapace length measurement used in this thesis (see section 2.2.1.5) be used in future studies of *N. marionis* because it seems to be the most accurate measurement of body size, and it is the measurement used on this, the first extended study of the animal.

Chapter 3

Preliminary length-based analysis

"HYPOTHESES ARE NETS; ONLY HE WHO CASTS WILL CATCH" (NOVALIS, CITED IN POPPER 1983: 11)

3.1 Introduction:

Stock assessment methods depend on proper ageing techniques (Sparre & Venema 1992: 10). One of the oldest methods of ageing stocks dates from Petersen (1891, cited in Pauly 1987: 10). The basic assumption behind it is that because length and age are related (Sparre & Venema 1992: 10), revealed modes from size-frequency distributions represent different age groups (Macdonald & Pitcher 1979). It is now possible to age some fish stocks using daily rings (Kelly & Wolf 1959, cited in Pannella 1971), and therefore the possibility of cross-validation of ageing for certain stocks exists. Ageing of fish is most often done by counting rings in the otoliths or scales (Sparre & Venema 1992: 10). Length-based methods will, however, continue to remain important even in fisheries where individual ageing techniques have been developed. This is because length-based methods are a relatively inexpensive and less time-consuming process, and often form the only type of historical data available (Pauly 1987: 10).

Although cross-validation techniques should be used when possible (ibid.), this is often not possible for organisms such as shrimp (Mathews *et al.* 1987: 147), where the only individual ageing technique available is the expensive mark-recapture method (Pauly 1987: 10). It is fortunate, then, that when using length-based methods what one is often interested in are *size*-related processes, such as predation, food conversion efficiency (Ursin 1973 and Pauly 1981 respectively, both cited in Pauly 1987: 10), gear (mesh) selection, marketability, price (Pauly 1987: 10) and fecundity (Gulland 1987: 335).

Despite the fact that length-based frequency analyses are extremely important for ageing various species, the principles behind them contain a number of inherent weaknesses (see Pauly & Morgan 1987: 9-10 for a balanced commentary). Some of the difficulties which present themselves with length-based analyses include the problem of subjectivity (Macdonald & Pitcher 1979), and the basic assumption that the cohort components of the size-frequency distribution are normally distributed (Pauly 1987: 8). Macdonald & Pitcher (1979) have developed a method which they believe is superior, and whilst this method has been lauded (Shepherd *et al.* 1987: 354), it has been mentioned that it too requires "considerable interpretative skill" (ibid.). It can also result in negative numbers being estimated for cohorts (Sparre 1987: 75).

I have rather opted to use the FiSAT programme (Gayanilo *et al.* 1996), which incorporates the maximum-likelihood principle "which is closely related to the so-called "chi-squared criterion"" (Sparre & Venema 1992: 104). The precursors of FiSAT are Compleat ELEFAN (Electronic LEngth Frequency ANalysis) and LFSA (Length-based Fish Stock Assessment). FiSAT was only developed from its two predecessors after years of effort (Gayanilo *et al.* 1996: iii to vi). This programme separates size-frequency data into cohorts using the "Bhattacharya" and "Normsep" procedures in sequence, from which length-based and weight-based growth parameters can subsequently be estimated. Through the calculation of *N. marionis*' growth parameters it is possible to estimate its natural longevity. This is the primary goal of this chapter.

3.2 Materials and methods:

Carapace lengths of the randomly selected individuals from samples 1, 3 and 4 (the 1984 dredge, the 1997 RMT-8 and the 1997 RMT-1*6 samples respectively, see section 1.3) were measured, and separate size-frequency histograms for each sample were plotted using 0.25mm class sizes. The small 1996 RMT-8 sample (sample 2) covered too wide a range of sizes with too few animals in each size class to allow any χ^2 test to be performed on it, and so it is not considered in this chapter. Although the 1997 RMT-1*6 sample was approximately the same size as the 1996 RMT-8 sample, it was possible to run a χ^2 test on the former sample's data as they were concentrated in sufficient numbers in the represented class intervals.

Some of the specimens examined were obviously parasitized by isopods of the family Bopyridae. Female bopyrids are easily identifiable as they are large and the carapace of *N. marionis* appears to grow around them, leaving quite a noticeable bulge. The much smaller and consequently less noticeable males tend to be attached to the females, although one male was found in isolation on one specimen without the female present and with no characteristic bulge on the shrimp. It may have been dislodged from another specimen in the same jar, but the shrimp was recorded as infested. Shrimps with no bopyrids present, but with the typically swollen carapace which accompanies such infestation, were also recorded as infested. Many loose carapaces were deemed not to have resulted from infestation, however, because the characteristic bopyrid body shape was absent. Because parasites may affect growth (Mathews *et al.* 1987: 163), infested specimens were excluded from this chapter's analyses.

Length class intervals were successively increased in FiSAT until an optimum was found for each of the samples, because "when doing the Bhattacharya analysis on the computer ... always, as a matter of routine, try out different length class intervals ... since it often happens that the structure of the points on the Bhattacharya plot emerges only for an optimal length class interval" (Sparre & Venema 1992: 87). FiSAT contains a routine specifically for this purpose. One of the effects of this routine is to smooth out irregularities in a data set (Gayanilo *et al.* 1996: 33).

No smoothing was done on the data despite the recommendations from Laurec & Mesnil (1987: 253) and Majkowski *et al.* (1987: 367) that this be done in preference to grouping over larger intervals; and despite the fact that this function is available on FiSAT. This procedure was deliberately not used because, given that class interval adjustment had already smoothed the data, it was felt that using the smoothing function as well would lead to some sort of 'double smoothing'. One is warned against 'double smoothing' by Gayanilo *et al.* (1996: 34) who, writing in a different sense, say that although "FiSAT will not prevent users from reapplying the smoothing function ... this is not recommended, as repeated smoothing leads to loss of information". To test my decision, I experimentally performed both the smoothing and class interval adjustment procedures on the data from the 1997 RMT-8 sample and compared the results with unsmoothed data from the same sample. I also attempted to perform the cohort analysis on the original 1997 RMT-8 sample raw data without any class interval adjustment, both with and without the smoothing function.

Genders are precisely defined in the next chapter. Except for comparative purposes, no attempt was made to separate the data into the different genders as seemingly recommended by Sparre & Venema (1992: 69). This was because, although males and females may experience different growths and mortalities, it was only possible to unambiguously identify two male cohorts and two female cohorts. The male cohorts were both from the 1984 dredge sample and the female cohorts both from the 1997 RMT-8 sample. Too few males were present in the 1997 RMT-8 and RMT-1*6 samples and too few females were present in the 1984 dredge and 1997 RMT-1*6 samples to perform the χ^2 test successfully on their isolated frequencies in these samples. It was not deemed wise to try to plot two gender-specific von Bertalanffy curves using only two data points for each curve.

Males do not require much energy to produce sperm (Hoffman 1972). Females, on the other hand, must convert some of their potential growth energy into gonadal production. Different growth rates can therefore be expected for similarly sized males and females. Evidence for predominant protandry in *N. marionis* is, however, presented in the next chapter, with the majority of individuals going through the cycle of juvenile-male-transitional-female stages. The assumption was therefore made that sufficient numbers of juveniles, males, transitional individuals and females of similar sizes experience the same mortality and growth parameters (an impossible assumption for a size-based sexually dimorphic dioecious species) for pooling of data to be potentially justifiable. This could be done by assuming that at any given size the vast majority of individuals were of one gender and were passing through the dominant protandric life-cycle. The existence of a growth curve was therefore assumed which represented the average growth of a typical individual *N. marionis*, with parameters which reflect an adjusted growth rate at a certain point (where the sexual inversion takes place).

Cohorts in the three different samples were identified by the "Normsep" routine in FiSAT for situations where their cumulative frequencies were not significantly different to the observed frequencies in the relevant data sets, using a χ^2 'goodness-of-fit' test at 95%. The "Bhattacharya" routine was only used to estimate the initial parameters entered into the "Normsep" procedure as suggested by Gayanilo *et al.* (1996: 53). Only results from the "Normsep" procedure which yielded separation indices between cohorts of >2 were ultimately used (Sparre & Venema 1992: 108).

No modal progression analysis was attempted with so few samples, as repeatedly entering the same data set (as suggested by Sparre 1987: 77) would have required the assumption of a steady-state condition. The different demographics of the 1997 RMT-8 and 1984 dredge samples suggested that this was not the case. Furthermore, it was noted by Brown & Klages (1987) that over one year almost no *N. marionis* were recovered from the guts of penguins which had used them as a major food source the previous year, prompting the suggestion that *N. marionis* may have been scarce during that year. It is therefore possible that the *N. marionis* population at the Prince Edward Islands oscillates quite markedly from year to year, making the steady-state assumption tenuous.

I then defined the term *generation* as a group of *N. marionis* of the same age from the same stock, but from different years. Relatively same-aged cohorts from the different samples were linked to different generations, based on the premise that two cohorts, one in each of the 1984 dredge and the 1997 RMT-8 samples, were of the same generation. The assumption was that protandric sex change occurs at the same age for the majority of individuals. Both these cohorts (they are identified as cohorts 3 and 2 from the 1984 dredge and 1997 RMT-8 samples respectively in section 3.3.2 below) were the smallest cohorts dominated by females in both their respective samples i.e. they were both preceded by cohorts dominated by males.

Many unrealistic linkages between the remaining cohorts could then be eliminated because any cohorts smaller than these two (where they occurred in the same sample) had to be from generations younger than the female-dominated one. This was also assumed for the only cohort from one sample which had a substantially smaller mean carapace length. In addition, care was taken not to link a male-dominated cohort with a female-dominated cohort. Furthermore, because "only resolutions of the length-frequency samples into components whose mean values correspond to a von Bertalanffy growth-curve are accepted" (Sparre 1987: 76), the von Bertalanffy growth assumption that growth rate decreases with increasing size meant that differences between mean-sizes-at-age of succeeding generations had to decrease. This disallowed many linkages as well.

A few of the possible cohort linkages which remained were then eliminated by assuming the fewest number of undersampled generations (so that as few parameters as possible had to be estimated). In doing this I assumed what I felt to be the simplest hypothesis explaining the observed data (*non sunt multiplicanda entia præter necessitatem*, or Ockham's razor). See Lambert & Brittan (1979: 73-77) for a philosophical discussion on the benefits and problems associated with this general approach.

Where one generation remained unidentified, a cohort was assumed to exist at a modal point that was weakly identified between cohorts taken to represent the subsequent and preceding generations in the 1997 RMT-8 sample. This cohort was not identified by FiSAT. The mid-point of this mode was deemed to be the *best* estimate of the mean size of that cohort. In assuming this I used the historical, imprecise and non-statistical technique of visually identifying modal points as being representative of cohorts that Macdonald & Pitcher (1979) called "the 'Petersen' method". This method has not, to the best of my knowledge, ever been used *in conjunction* with any of the more developed methods.

The cohort mean sizes in the resultant length-frequency histograms calculated by FiSAT were then used to estimate the mean sizes of each *generation*. This was done by taking a non-weighted average of the means of the cohorts linked to those generations. *Average* means, rather than total means, were considered to be a truer reflection of what could be expected in the future under different conditions. The modal mid-point of the carapace length class taken to represent the undersampled cohort was accepted as the best estimate of the mean size of the generation with which it was linked, there being no other cohort linked to this generation.

The mean-size-at-age and standard deviations for the different cohorts were then compared to each other, and also to the resultant generation mean sizes. This subjectively imprecise comparison (Zar 1984: 108) was done only *after* the various identified cohorts had been aged using the method described above i.e. comparing the cohort means and standard deviations was *not* the initial method used to link different cohorts from the different samples. FiSAT calculates the approximate numbers of specimens in each cohort, but obviously cannot ascertain *which* individuals were included in this cohort as its input data must be in histogram form. Because the constituent data of each identified cohort were not available, it was impossible to make proper statistical comparisons between different cohort and generation mean sizes (Radloff pers. comm.). The comparisons between the means and standard deviations of the different cohorts were therefore made purely as an attempt to subjectively decide whether the associations made between the different cohorts via a different method were realistic or not.

The mean-size-at-age and standard deviations for the four gender-specific cohorts identified above were then also compared to those of all the sample cohorts calculated from the combined data as well as to the different generation mean sizes. This was done to test whether the assumption that the different generations were dominated by one or other gender was reasonable or not. No attempt was made to link cohorts using FiSAT because I did not have time-series data, and "linking [in FiSAT] should not be attempted if the mean lengths do not display a clear progression through time" (Gayanilo *et al.* 1996: 54).

The subsequently calculated or estimated means for each generation were used to compute the length-based von Bertalanffy growth curve parameters using the technique shown in Table 3.4 (Pakhomov pers. comm.). For this it was assumed that the shrimp spawned only once annually because carideans seem to breed seasonally at higher latitudes (Bauer 1989). Spawning more than once a year was considered improbable for *N. marionis* because of the strong seasonality experienced at the Prince Edward Island group latitudes. After a review of the scant literature available on *N. marionis*, I decided to work on the assumption that the first generation was one year old for this calculation (see section 3.4.6).

The parameters for the length-based von Bertalanffy growth equation are defined by Sparre & Venema (1992: xi & xii) as L_∞ (the asymptotic length), t_0 (the initial condition parameter in years) and k (the curvature parameter). The value for L_0 (the size at hatching, see Sparre & Venema 1992: 46) was calculated by entering t = 0 into the equation, which has the general form $L_{(t)} = L_\infty * [1 - e^{-k*(t-t_0)}]$ (Sparre & Venema 1992: 44).

A weight-based von Bertalanffy equation was then also calculated based on the length-based equation above as well as the relationship between carapace length and *wet* weight (equation 4, Table 2.1). In the description that follows, the normally accepted abbreviation 'W' to indicate weight is changed to read 'WW' (for 'wet weight').

As outlined in Sparre & Venema (1992: 37), a t-test was used to calculate the 95% confidence intervals of the slope in equation 4, which was 2.6520 ± 0.04245. The slope therefore lay (with 95% confidence) between the values of 2.60955 and 2.69445. It could only be *precisely* measured at that confidence interval to a maximum of one significant figure (i.e. 3). See Zar (1984: 4) for a definition of precision as opposed to accuracy. One would expect the value to be close to 3 because volume is often proportional to length cubed (Sparre & Venema 1992: 37, see also Mendenhall *et al.* 1990: 551). With this 'new b' (or 'b'), a 'new a' (or 'a') was calculated (the original form of equation 4 was $Y = a + b*X$, where $Y = \ln WW$ and $X = \ln CL$) from the relationship $\text{'a'} = \overline{Y} - (\text{'b'} * \overline{X})$. 'a' thus became -6.8951. With 'q' being equal to $e^{\text{'a'}}$ (from the length/weight equation of $WW_{(i)} = q*CL_{(i)}^{b}$, a derivative of the form presented in equation 4), the condition factor, 'q', became 0.001013. The final form of the length/weight relationship used in the calculation of the von Bertalanffy weight-based growth curve became $WW = 0.001013*CL^3$ (using the same units as in Table 2.1). Substituting the general form of the length-based von Bertalanffy growth equation into the general form of the length/weight relationship yields the weight-based von Bertalanffy growth equation $WW_{(t)} = WW_{\infty} * [1 - e^{-k*(t-t_0)}]^3$, the parameters for which are WW_{∞}, k and $t_{(0)}$ (Sparre & Venema 1992: 50). The only unknown weight-based von Bertalanffy growth parameter, which was WW_{∞} (the asymptotic weight), was calculated by substituting L_{∞} into the length/weight relationship.

WW_0 (the wet weight at hatching) was then determined from the weight-based von Bertalanffy growth equation via the same procedure that $L_{(0)}$ was determined i.e. by entering $t = 0$ into the weight-based von Bertalanffy growth equation.

3.3 Results:

3.3.1 Cohort parameter estimates using FiSAT:

For the 1984 dredge sample it was found that the optimal class interval was 0.35mm, and for the 1997 RMT-8 sample it was found to be 0.39mm. The small 1997 RMT-8 sample needed only a small class interval adjustment to 0.26mm in order to perform the 'goodness-of-fit test' successfully. The original 0.25mm class interval yielded the desired 'no significant difference' for the 1997 RMT-8 sample on FiSAT, but the χ^2 value (3.578), degrees of freedom (1) and significance level (95%) at this class interval did not correspond to the χ^2 distribution critical values used in this thesis to calculate *p* (Zar 1984: 479), nor did they conform to any other standard text examined (Underhill 1987: 419, Walpole 1990: 482, Mendenhall *et al.* 1990: 762).

The experimental use of both class interval adjustment and smoothing on the 1997 RMT-8 data set led to a visibly obvious, very small, first cohort in that sample being completely absorbed by a much larger second cohort. This also happened to the small modal point between cohorts 2 and 3 in that sample (Figures 3.2A, B & C and Tables 3.2A & B). These unwelcome consequences still yielded a significant result, as did a subsequent attempt using a different class interval (and no smoothing) which identified both the first cohort and the small modal point. Re-runs of the 'Bhattacharya' and 'Normsep' routines in FiSAT using unsmoothed data did not allow for the unambiguous identification of another cohort in the 1997 RMT-8 sample. Data smoothing without class interval adjustment failed to yield significant results for the 1997 RMT-8 sample data set, and neither did different initial estimates with the original, unsmoothed data.

Eight cohorts were identified in all the samples using the size classes above. Four cohorts were isolated in the 1984 dredge data set, three in the 1997 RMT-8 data set, and one in the 1997 RMT-1*6 data set (see Figures 3.1B, 3.2B & 3.3B and Tables 3.1A, 3.2A & 3.3A.).

3.3.2 Ageing of cohorts assuming a relationship between sexual inversion and age:

Cohorts three and two in the 1984 dredge and 1997 RMT-8 samples respectively were assigned a common age because of the predominance of females in both of them (see section 3.2 and Figures 3.1A & 3.2A). Of the remaining six cohorts identified in all the samples, four had mean carapace lengths smaller than those of the two female-dominated cohorts. Three were the first (or only) cohort in each of the three samples in which they occurred, whilst the fourth was the second cohort identified in the 1984 dredge sample. Of these four cohorts, three were undeniably younger than the female-dominated generation because of their occurrence in the same sample as one of the cohorts linked to that generation (Figures 3.1B and 3.2B). The exception was the single cohort from the 1997 RMT-1*6 sample, and this cohort had the smallest mean carapace length of all the samples. It was also a planktonic cohort. This cohort was therefore assumed, along with the other three cohorts, to be younger than the female-dominated generation.

Had the two smallest cohorts from the 1984 dredge and 1997 RMT-1*6 (Figures 3.1B & 3.3B) been from different generations, and had their calculated means been reasonable estimates of those generation's means (Tables 3.1A & 3.3A), then the carapace length growth rate between these two generations would have been either 0.11mm/generation or less. Such a small growth rate between the first and second generations would have necessitated the assumption of a large number of unsampled generations in the three samples because of the von Bertalanffy assumption of decreasing growth rates in older generations. Also, it would have been difficult to separate these two cohorts because the cohort with the larger mean size (the 1984 dredge sample cohort) noticeably included the smallest animals sampled. Therefore the only cohort identified in the 1997 RMT-1*6 data set and the first cohort in the 1984 dredge data set were identified as being from the first sampled generation. One of these cohorts (from the planktonic 1997 RMT-1*6 sample, n = 21) was dominated by juveniles (Figure 3.3A).

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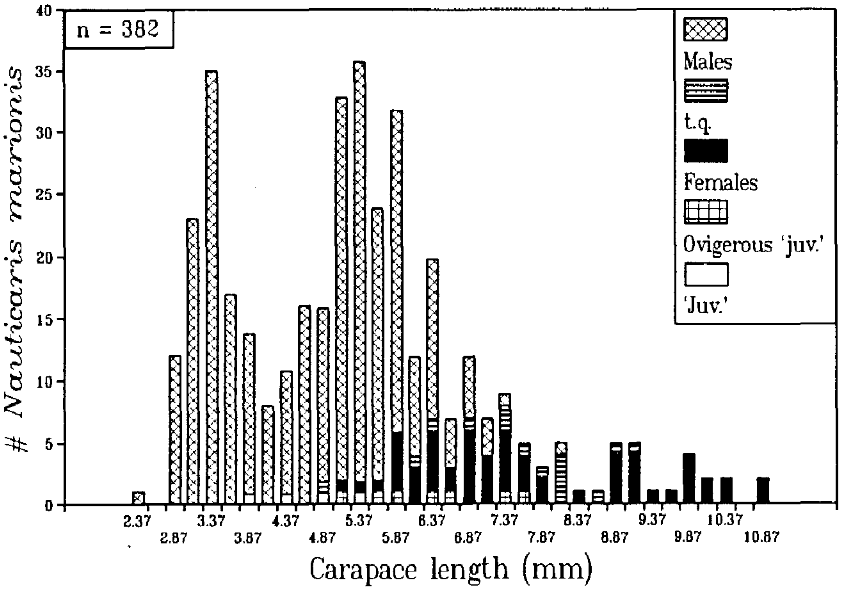

Figure 3.1A: 1984 dredge sample carapace length-frequency distribution, showing the proportional contribution of the different genders to the different size classes. Class interval = 0.25mm. Compare the occurrence of males and females to the cohort resolution in Figure 3.1B. Genders are precisely defined in section 4.2. t.q. = tertia quae (section 4.2.4) and 'juv' = 'juveniles' (sections 4.2.1 and 4.2.2). An explanation for the occurrence of 'juveniles' in relatively large class intervals is given in the appropriate place in section 4.5.2.

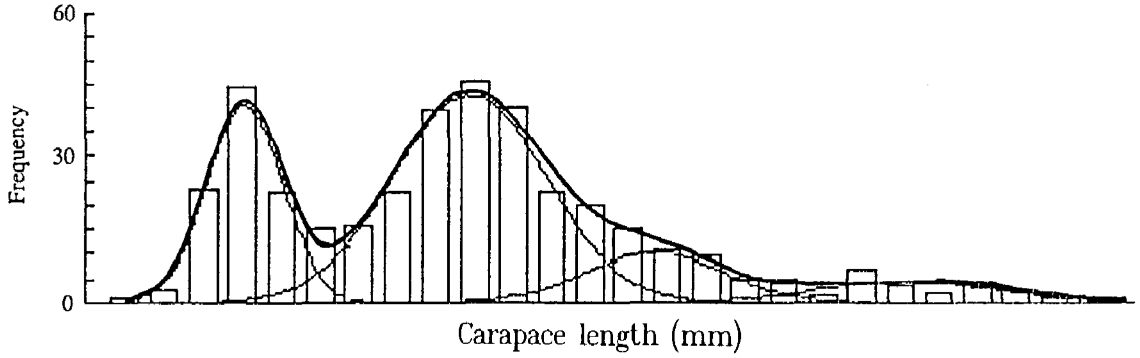

Figure 3.1B: The data in Figure 3.1A resolved into cohorts using FiSAT. See Table 3.1A for the relevant statistics.

Generation	Cohort	n	Mean size (mm)	Standard deviation (mm)	Separation index	Cohort mean size differences (mm)
1	1	98.35	3.342	0.33898	-	-
2	2	210.87	5.376	0.69244	3.94500	2.034
3	3	46.27	7.072	0.62899	2.56620	1.696
5	4	26.51	9.357	0.82775	3.31373	2.285

Carapace length class interval = 0.35mm, $\chi^2 = 28.422$, df = 18, $p \approx 0.058$,
At the 95% confidence interval, the composite distribution is not significantly different from the observed values.

Table 3.1A: Resolution of the total 1984 dredge length-frequency data into age groups by FiSAT. Generations were assigned to the cohorts above independently of the programme (see Figure 3.1B and section 3.3.2).

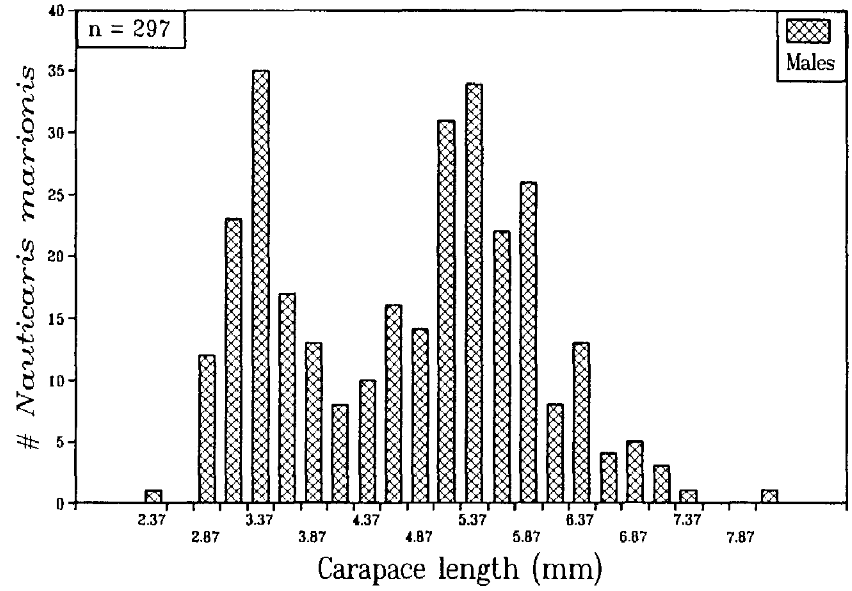

Figure 3.1C: 1984 dredge sample male carapace length-frequency distribution. Class interval = 0.25mm. Males are precisely defined in section 4.2.3.

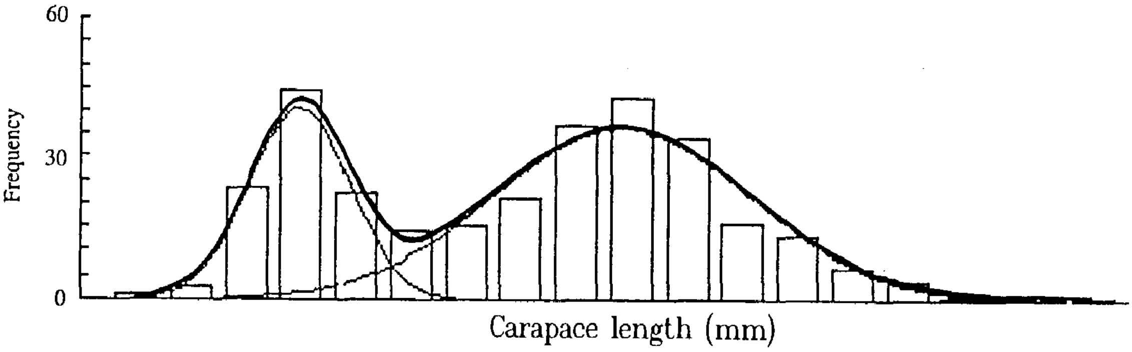

Figure 3.1D: The data in Figure 3.1C resolved into cohorts using FiSAT. See Table 3.1B for the relevant statistics.

Generation	Cohort	n	Mean size (mm)	Standard deviation (mm)	Separation index	Cohort mean size differences (mm)
1	1	91.44	3.310	0.31443	-	-
2	2	205.56	5.362	0.79965	3.68330	2.052
Carapace length class interval = 0.35mm, χ^2 value = 18.811, df = 12, $p \approx 0.095$, At the 95% confidence interval, the composite distribution is not significantly different from the observed values.						

Table 3.1B: Resolution of the male 1984 dredge length-frequency data into age groups by FiSAT. Generations were assigned to the cohorts above independently of the programme (see Figure 3.1D and section 3.3.2).

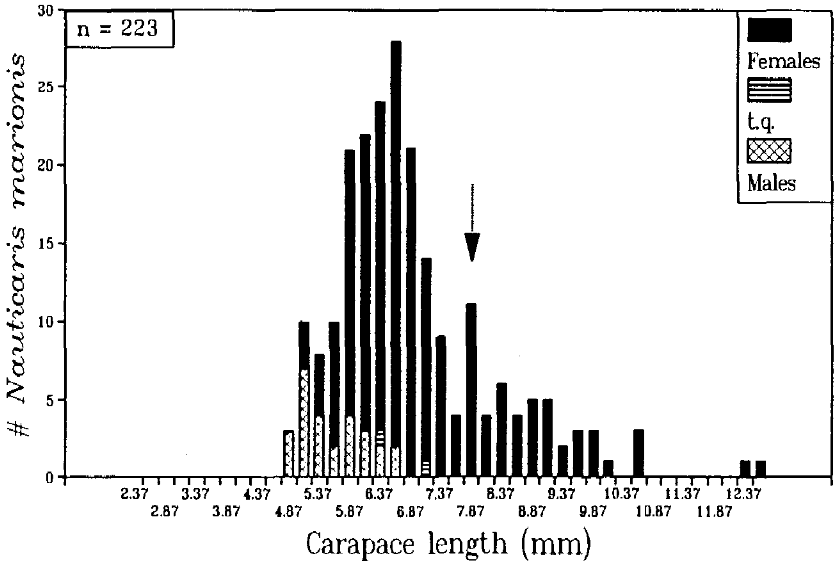

Figure 3.2A: 1997 RMT-8 sample carapace length-frequency distribution, showing the proportional contribution of the different genders to the different size classes. Class interval = 0.25mm. Compare the occurrence of males and females to the cohort resolution in Figure 3.2B. Genders are precisely defined in section 4.2. t.q. = tertia quae (section 4.2.4). Note the highlighted weak modal point at 7.87mm.

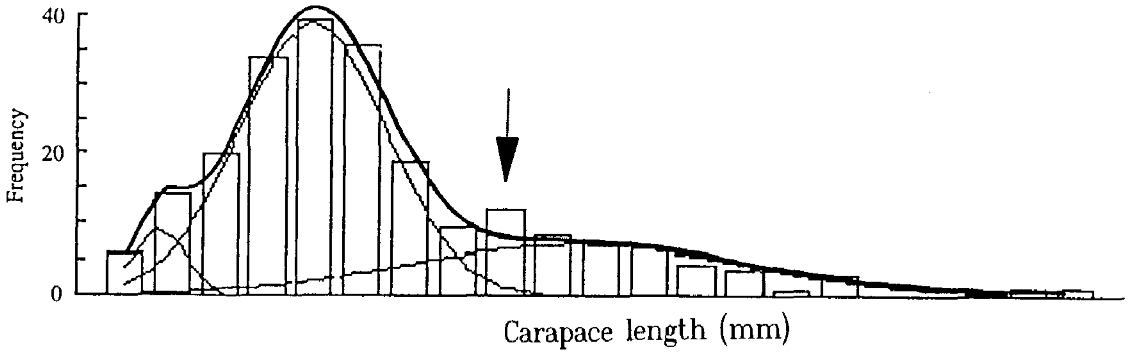

Figure 3.2B: The data in Figure 3.2A resolved into cohorts using FiSAT. Note the highlighted weak modal point at 8.00mm. See Table 3.2A for the relevant statistics.

Generation	Cohort	n	Mean size (mm)	Standard deviation (mm)	Separation index	Cohort mean size differences (mm)
2	1	12.35	5.158	0.21764	-	-
3	2	149.53	6.412	0.59818	3.07340	1.254
5	3	61.11	8.580	1.36277	2.21130	2.168
Carapace length class interval = 0.39mm, $\chi^2 = 13.889$, df = 13, $p \approx 0.39$, At the 95% confidence interval, the composite distribution is not significantly different from the observed values.						

Table 3.2A: Resolution of the total 1997 RMT-8 length-frequency data into age groups by FiSAT. Generations were assigned to the cohorts above independently of the programme (see Figure 3.2B and section 3.3.2).

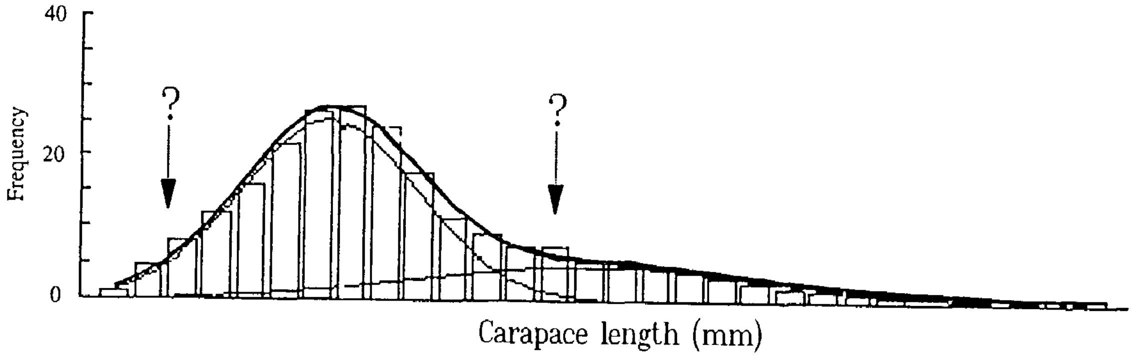

Figure 3.2C: The data in Figure 3.2A resolved into cohorts using FiSAT, after smoothing. Note how the first cohort and the modal peak identified in Figures 3.2A and 3.2B have disappeared, illustrating the dangers of smoothing one's data even once (section 3.4.2). See Table 3.2B for the relevant statistics.

Generation	Cohort	n	Mean size (mm)	Standard deviation (mm)	Separation index	Cohort mean size differences (mm)
3	1	163.65	6.343	0.72282	-	-
5	2	59.22	8.475	1.38678	2.02120	2.132
Carapace length class-interval = 0.278mm, smoothed using running average by three classes, $\chi^2 = 19.191$, df = 17, $p \approx 0.33$, At the 95% confidence interval, the composite distribution is not significantly different from the observed values.						

Table 3.2B: Resolution of the total 1997 RMT-8 length-frequency data into age groups by FiSAT, after smoothing the data. Note the significance of the χ^2 test (see Figure 3.2C and section 3.3.1).

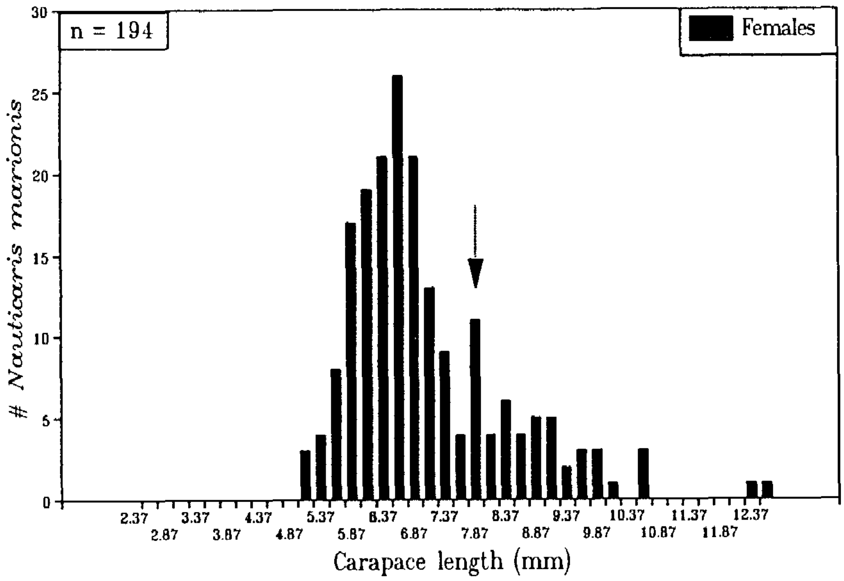

Figure 3.2D: 1997 RMT-8 sample female carapace length-frequency distribution. Class interval = 0.25mm. Note the highlighted weak modal point. Females are precisely defined in section 4.2.5.

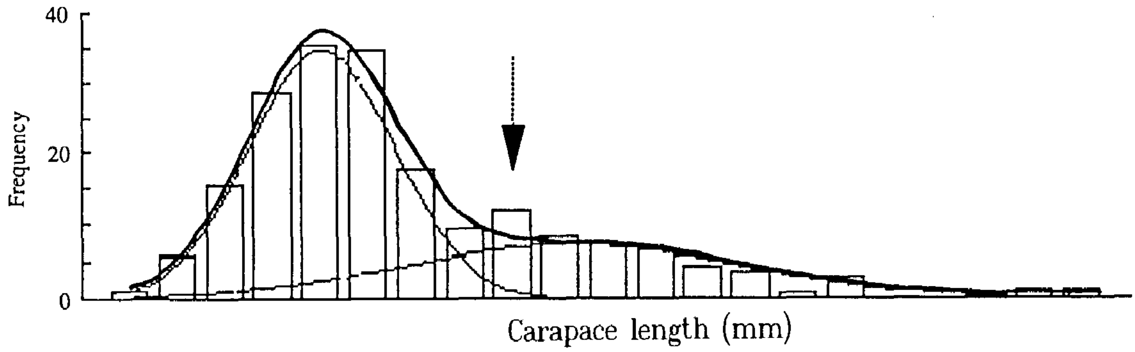

Figure 3.2E: The data in Figure 3.2D resolved into cohorts using FiSAT. Note the highlighted weak modal point. See Table 3.2C for the relevant statistics.

Generation	Cohort	n	Mean size (mm)	Standard deviation (mm)	Separation index	Cohort mean size differences (mm)
3	1	130.72	6.449	0.58690	-	-
5	2	63.28	8.495	1.37942	2.08150	2.046
Carapace length class-interval = 0.39mm, $\chi^2 = 12.863$, df = 12, $p \approx 0.39$, At the 95% confidence interval, the composite distribution is not significantly different from the observed values.						

Table 3.2C: Resolution of the female 1997 RMT-8 length-frequency data into age groups by FiSAT. Generations were assigned to the cohorts above independently of the programme (see Figure 3.2E and section 3.3.2).

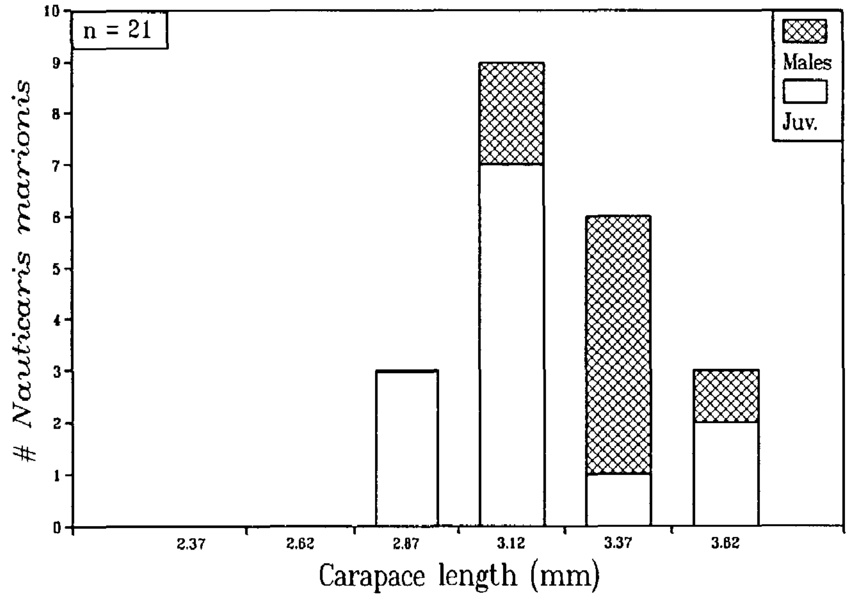

*Figure 3.3A: 1997 RMT-1*6 sample carapace length-frequency distribution, showing the proportional contribution of males and juveniles to the different size classes. Class interval = 0.25mm. Genders are precisely defined in section 4.2.*

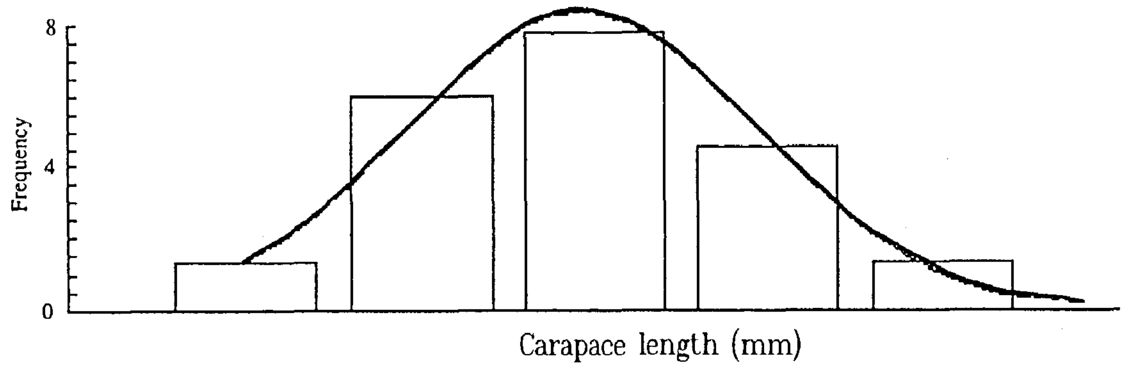

Figure 3.3B: The data in Figure 3.3A resolved into a cohort using FiSAT. See Table 3.3A for the relevant statistics.

Generation	Cohort	n	Mean size (mm)	Standard deviation (mm)	Separation index	Cohort mean size differences (mm)
1	1	21.00	3.232	0.26018	-	-
Carapace length class interval = 0.26mm, $\chi^2 = 3.227$, df = 2, $p \approx 0.077$, At the 95% confidence interval, the composite distribution is not significantly different from the observed values.						

*Table 3.3A: Resolution of the total 1997 RMT-1*6 length-frequency data into an age group by FiSAT. The identified cohort was linked to the first generation independently of the programme (see Figure 3.3B and section 3.3.2).*

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Figures 3.2B & C show how easily the small first male-dominated cohort in the 1997 RMT-8 sample was absorbed by the next largest cohort (which was female-dominated) when the smoothing function was used. It was reasoned that if an intermediate and undersampled generation had existed between these two cohorts, then they probably would have been more distinct and therefore less susceptible to assimilation. It was therefore assumed that no intermediate cohort existed between cohorts one and two of the 1997 RMT-8 sample. Because the second cohort from this sample was dominated by females, this assumption meant that the first, male-dominated cohort in the 1997 RMT-8 sample was from the last generation before sexual inversion took place. The 1984 dredge sample had already revealed that there were at least two male-dominated generations (Figures 3.1A & B). The first cohort from the 1997 RMT-8 sample therefore could not have represented the first generation. Identifying it with the first generation in any case would have resulted in a larger first generation mean size. Given the other assumptions for the von Bertalanffy curve made in this study (see section 3.2), this would have in turn resulted in an estimated L_∞ well in excess of any specimen sampled in this or any other study. The smallest cohort in the 1997 RMT-8 sample was therefore identified as being from the second generation.

As was the case with the two first generation cohorts, separating the second cohort of the 1984 dredge sample and the first cohort of the 1997 RMT-8 sample on the basis of age would have meant that many generations had not been sampled because of the von Bertalanffy assumption that growth rates decrease with increasing age. This would have made the calculation of a von Bertalanffy growth curve with the available data impossible. The second cohort of the 1984 dredge sample was therefore also linked to the second generation.

The next two largest cohorts observed in all the samples were the third cohort in the 1984 dredge sample and the second cohort in the 1997 RMT-8 sample (Figures 3.1A & B and 3.2A & B). These two cohorts had already been linked *a priori* to the same undefined generation. This was the generation in which sexual inversion was assumed to have taken place. It was the third sampled generation, and because I assumed as few undersampled generations as possible, it was judged to be the third generation overall.

It was observed that the mean-size-at-age of the 1997 RMT-8 sample cohorts representing the second and third generations had, for whatever reason, a consistently smaller mean size than their 1984 dredge cohort counterparts (Tables 3.1A and 3.2A). Because of this, it was assumed that other linked cohort pairs from these two samples would show the same relationship. This allowed for the fourth and third cohorts in the 1984 dredge and 1997 RMT-8 samples respectively to be linked to the same generation, judged to be the fifth and not the fourth generation because of the assumption of decreasing growth rates with increasing size.

The mean-size-at-age of the unknown fourth generation was taken to be represented by the weak modal peak between the third and fifth generation cohorts identified in the 1997 RMT-8 sample. The modal peak mid-point was 7.87mm in the original data set (Figure 3.2A), but was better shown in the 0.39mm adjusted class-size-frequency distribution at the modal peak mid-point of 8.00mm (Figure 3.2B). The 0.39mm adjusted size-frequency distribution was the one best suited to determine the mean sizes of the cohorts in that sample. It was decided, therefore, to accept the figure of 8.00mm as an estimate for the mean size of that generation. The assumption of decreasing growth rates held.

The data was such that all calculated generation means were ultimately derived from two cohort means identified in different samples by FiSAT. Other than one unrepresented generation (the fourth), in no case was a mean generation size calculated from anything *other* than two data points from two different samples.

Five generations in total were identified using all the samples. Tables 3.1A, 3.2A & 3.3A assign generations to the various cohorts of the three different samples.

3.3.3 Cohort parameter comparisons:

It was noted *after* the procedure above had been followed, that most linked cohort pairs from the different samples fell within one *sample* standard deviation of each other. The only exceptions were the two cohorts linked to the third generation; these means fell just outside one, but well within two, standard deviations of each other. None of the cohort means fell within less than one standard deviation of any cohort linked to any other generation. Calculated mean generation sizes all fell within one standard deviation of both cohorts linked to them. Furthermore, apart from the undersampled fourth generation mean which had been estimated using the 'Petersen' method, none of the *generation* mean lengths (Table 3.4) fell within less than one standard deviation of any cohort linked to any other generation. The estimated fourth generation mean fell within one standard deviation of the fifth generation cohort sampled in the 1997 RMT-8 sample, the same sample from which the fourth generation mean was estimated (Tables 3.1A, 3.2A, 3.3A & 3.4).

The first and second generation mean sizes (Table 3.4) fell within one standard deviation of the first and second male cohorts respectively (Table 3.1B). The smaller male cohort mean size fell within one standard deviation of both the cohorts linked to the first generation and the larger male cohort mean size fell within one standard deviation of both the cohorts linked to the second generation. The mean sizes of both cohorts linked to the first generation fell within one standard deviation of the first male cohort, and the mean sizes of both cohorts linked to the second generation fell within one standard deviation of the second male cohort.

Similarly, the third and fifth generation mean sizes (Table 3.4) fell within one standard deviation of the first and second female cohorts respectively (Table 3.2C). The smaller female cohort mean size fell within one standard deviation of both of the cohorts linked to the third generation. The larger female cohort mean size fell within one standard deviation of both the cohorts linked to the fifth generation. The mean sizes of one of the cohorts linked to the third generation fell within one standard deviation of the first female cohort, while the mean size of the other third generation cohort fell just outside this range. The mean sizes of both cohorts linked to the fifth generation fell within one standard deviation of the second female cohort.

3.3.4 von Bertalanffy growth estimates:

The parameters for both von Bertalanffy growth curves are given in Table 3.4, as is the method used to calculate them. The generation mean sizes coincided well with the calculated von Bertalanffy length-based growth curve, the parameters for which were k (0.2353 per year), t_0 (-0.2828 year) and L_∞ (12.60mm). L_0 was calculated as 0.8111mm. Given the calculated size at one year old (3.29mm carapace length, see Table 3.4), if one tentatively accepts both that L_0 is approximately accurate as an estimate of the carapace length at birth and that the von Bertalanffy growth curve is applicable to larval stages (see section 3.4.5), then the growth rate during the first year should be about 2.47mm CL/year, or about 0.20mm CL/month. WW_∞ and WW_0 were 2.03g and 0.00054g respectively.

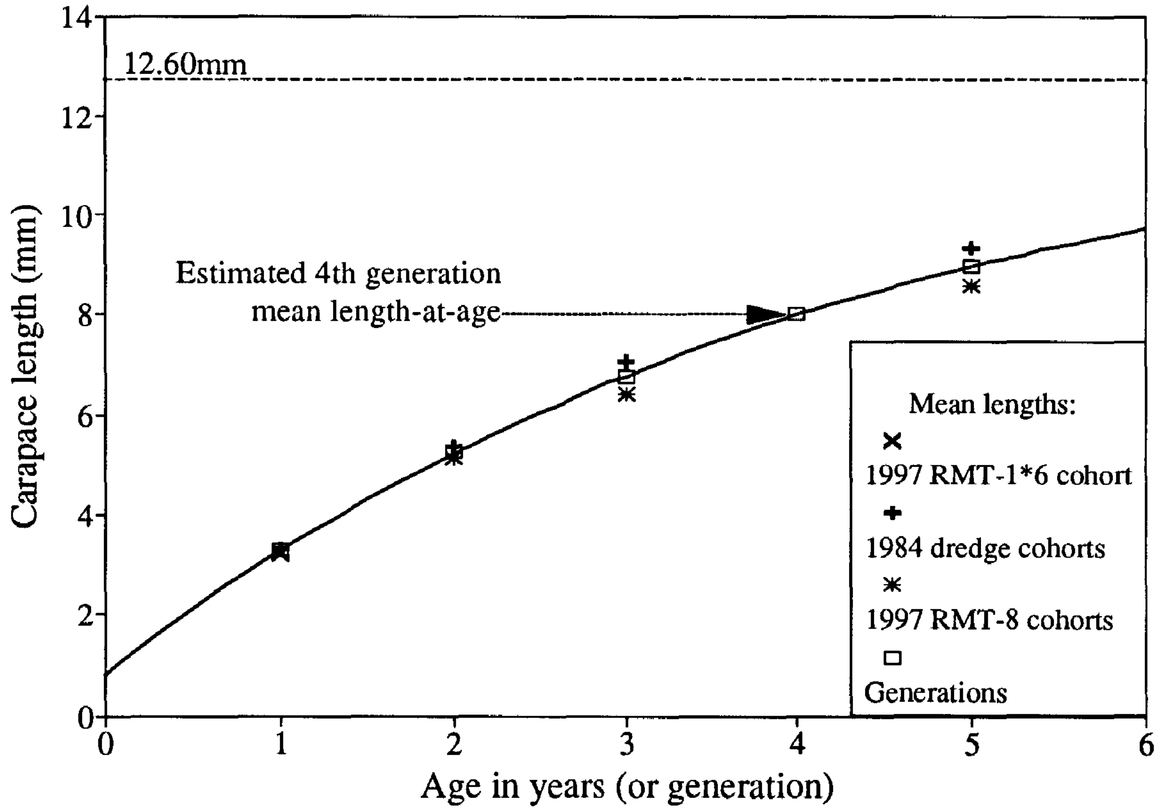


Figure 3.4: The von Bertalanffy length-based growth curve (see also Table 3.4). L_∞ is represented by the dashed line. Data points indicate sample cohort and generation mean sizes. The fourth generation mean size was an estimate.

Generation	$t_{(years)}$	$L_{(t)}$ (mm)	$L_{(t)}$* $L_{(t+1)}$	$L_{(t)}^2$	L_∞-$L_{(t)}$ (mm)	ln (L_∞-$L_{(t)}$)	$L_{(t)}$ from length-at-age equation	Growth per year (mm)
1	1	3.287	17.31	10.80	9.31	2.2313	3.2822	—
2	2	5.267	35.51	27.74	7.33	1.9922	5.2353	1.9531
3	3	6.742	53.94	45.46	5.86	1.7676	6.7790	1.5437
4	4	8	71.74	64	4.60	1.5258	7.9990	1.2200
5	5	8.968	C =	D =	3.63	1.2895	8.9633	0.9643
Σ			178.5	148.0		E=8.806		

n = 5	*Number of generations*
$t_{(i+1)}$ - $t_{(i)}$ = 1	*Difference in age (in years) between generations*
A = 23.296mm	$\Sigma_{t=1}^{4} L_{(t)}$
B = 28.977mm	$\Sigma_{t=2}^{5} L_{(t)}$
b = 0.790364	*[(v*C)-(A*B)]/[(v*D)-(A^2)]; where v = n-1*
k = 0.235262/year	*-lnb*
a = 2.641171mm	*[B-(b*A)]/v; where v = n-1*
L_∞ = 12.59883mm ≈ 12.60mm	*a/(1-b)*
t_0 = -0.28284 years	*{[((1/n)*E)-(lnL_∞)]/k}+{(n+1)/2}*
L_0 = 0.811061mm ≈ 0.81mm	*Put t = 0 in von Bertalanffy equation*
WW_∞ = 2.025818g ≈ 2.03g	*WW_∞ = 0.001013*L_∞^3*
WW_0 = 0.00054g	*Put t = 0 in weight-based growth curve*

Table 3.4: Calculation of the von Bertalanffy parameters (in bold). Estimated 4th generation mean size highlighted. Calculations were done to more significant figures than are shown, then rounded off at the end. Italicized right hand column explains its left hand equivalent. $L_{(t)}$ values are the mean sizes of each generation calculated as the averages of linked cohort mean lengths (in Tables 3.1, 3.2 & 3.3. See also section 3.2).

3.4 Discussion:

3.4.1 Parasitism:

Bopyrid parasites have been previously recorded on *N. marionis* at the Prince Edward Islands by Branch *et al.* (1991). Not many specimens were excluded from the length-frequency analysis because of parasitism, possibly because only very noticeably parasitized individuals were identified as such. See Mathews *et al.* (1987: 159) for some of the problems associated with parasitized length-frequency data, including the problem of historically infested specimens. See also Plates 19 to 21, as well as section 4.5.3 which discusses the possibility that bopyrids castrate *N. marionis.*

3.4.2 The programme FiSAT:

When entering data into FiSAT it should be borne in mind that large class intervals have an extremely negative impact on results (Laurec & Mesnil 1987: 239) because they can obscure trends (Zar 1984: 8). Despite the fact that FiSAT allows one to reduce the class interval of a data set once it has been entered, and despite the fact that Daniel Pauly (pers. comm.) has intimated that one may legitimately do this, Sparre & Venema (1992: 96) note that nothing can be done if one's class intervals are initially too large. Reducing class intervals is therefore probably controversial. One should therefore rather enter data in as small an interval width as possible, in order to allow for a later upward adjustment to an optimal width during the analysis (Hoenig *et al.* 1987: 345).

Smoothing *and* class interval adjustment on the 1997 RMT-8 data set gave erroneous results, which validated my concerns about using both together. Smoothing of the data in FiSAT alone did not yield usable results, and neither did different mean size estimates using the original class intervals. It therefore appears that experimentation with adjusted length class intervals at the very least are essential when using FiSAT, but that the smoothing option should be avoided. This conclusion was reached despite criticism of all forms of preliminary data treatment (Shepherd 1987: 113) and antithetical advice to avoid class interval adjustment in favour of smoothing (Laurec & Mesnil 1987: 253 and Majkowski *et al.* 1987: 367).

No explanation is offered as to why FiSAT should have calculated no significant difference between the accumulated frequency and the observed data from the small 1997 RMT-1*6 sample when the reported degrees of freedom, significance level and χ^2 value did not correlate with those of a number of standard texts. It would seem advisable, therefore, to consult a χ^2 table from a standard text when using FiSAT in order to correlate the programme's significance tests with accepted χ^2 values. It is, in any case, advisable to regularly consult a χ^2 table when obtaining initial parameter estimates using the "Bhattacharya" procedure, because it was found that viable class interval adjustments always gave initial results which were *nearly* significant. FiSAT does not provide p values for the user, which, if known, reduces the amount of time spent trying out different initial estimates.

The χ^2 procedure on FiSAT uses the null hypothesis of no difference between the observed values and the cumulative curve resulting from the cohort identification. Usually the null hypothesis is one which one hopes to *disprove* (Underhill 1987: 241), which is not the case here. This may perhaps be what Shepherd (1987: 113-114) means when pointing out that, in

ELEFAN I (one of the precursors to FiSAT), "the basis of the goodness-of-fit criterion ... is arguably not ideal" because, as Johnson (1992) has mentioned, "one must always bear in mind that our manner of hypothesis testing is developed to detect differences, not similarities". Setting the test at 95% is thus equivalent to setting the probability of rejecting the hypothesis that there is no difference when it is true (a Type I error) at 5%. The probability of a Type II error - that is, accepting the null hypothesis of no difference when it is false (i.e. a difference does exist) - is unknown. It is the latter hypothesis which one is strictly speaking interested in when one asks whether the calculated cumulative distribution is the same as the observed distribution. Accordingly, when FiSAT indicates that a cumulative curve is statistically the same as the observed values, there may still be a difference.

3.4.3 Identifying undersampled cohorts using the 'Petersen' method:

The use of the 'Petersen' method to calculate the mean size of the unrepresented fourth generation seemed to be justified because of the unknown probability that there was a difference between the cumulative curve and the observed data. The missing generation might therefore have been imperfectly represented in one of the histograms by a weak modal point between cohorts representing the preceding third and subsequent fifth generations in a sample covering this range (of generations). The anomalous mode found in the 1997 RMT-8 sample conformed to these expectations, and so its mid-point was implicated as the best estimate of that cohort's (and subsequently generation's) mean size (see section 3.3.2 above).

The validity of using the 'Petersen' method in conjunction with FiSAT has not been commented upon in the literature. One's instinct suggests, though, that if such a cohort could not be revealed as a discreet entity in subsequent analyses, then the effect of including that cohort's data (which are presumably few) when calculating the parameters of adjacent cohorts within the same sample should be negligible. Certainly all the generation mean length-at-age values, including that of the estimated fourth generation, fitted well onto the von Bertalanffy length-based growth curve in this study (Figure 3.4). This is a situation which one might perhaps not have expected had the mean estimate been significantly incorrect.

The accuracy of the fourth generation mean-size-at-age *estimate* was slightly threatened by the fact that it fell within one standard deviation of the fifth generation cohort mean from the same sample (the 1997 RMT-8 sample). None of the *calculated* generation mean sizes fell within the standard deviations of any cohort from any other generation. It must be borne in mind, though, that "standard deviations usually increase with lengths" (Gayanilo *et al.* 1996: 53). At the same time mean size differences decrease between larger cohorts because of the von Bertalanffy assumption of decreasing growth with increasing length. This makes it more likely that overlaps such as this will occur between older cohort and generation parameter estimates. The inconsistent relationship between the two oldest sampled generations' parameters should therefore have no special significance attached to it.

The use of the 'Petersen' method to calculate the parameters of underrepresented cohorts is an aspect of FiSAT which needs to be examined in future studies. It was accepted as valid here out of necessity and because, as Aaron Levenstein (cited in Peter 1991: 452) has aptly illustrated the point: "Statistics are like a bikini. What they reveal is suggestive, but what they conceal is vital". Nonetheless, the data have been presented here in such a way as to facilitate their incorporation into future studies by excluding the fourth generation mean size estimate.

3.4.4 Cohort ageing:

Cohorts from different samples were linked via two different lines of reasoning which complemented each other. The first assumed that sexual inversion takes place at the same age. Assuming that growth rates decrease with increasing age, a logical and objective process based on clearly defined assumptions subsequently allowed the cohorts to be aged.

The second line of reasoning compared calculated means and standard deviations of the different cohorts. It is important to note here that, while it might be tempting to age cohorts using this method alone, checking whether the standard errors of means overlap as a method of determining differences *"is not generally valid"* (Zar 1984: 108, my emphasis). The linking of cohorts can be problematic (Gayanilo *et al.* 1996: 54). When using FiSAT, for instance, one is advised not to link cohorts from different samples unless certain criteria are met, and even then it is stressed that the process is subjective (ibid.). In the case of *N. marionis* (and possibly other protandric species), there is a method which allows for a much less subjective and therefore relatively more rigorous way of ageing cohorts (see sections 3.3.2 and 3.4.4). This method should be preferentially employed where possible.

The assignation of generations to cohorts using the phenomenon of sexual inversion did, however, result in cohort pairs which were, by virtue of their parameters, linked in ways in which they were not to any other cohorts. Each calculated generation mean size was also similarly linked to its two constituent cohorts and no other. Each gender-specific cohort was also linked to one overall generation and no other by its parameters. This supported the premise of the initial ageing method, which was that sexual inversion is synchronized with age, and because of this, similarly aged cohorts will be characterized by similar gender proportions.

As the undersampled fourth generation fell between two groups dominated by females, and the size-frequency distribution for the female data included the enigmatic modal point associated with this cohort (see Figures 3.2D & E), it must be assumed that it too was predominantly female. Given the evidence for protandry which shall be led in the next chapter, as well as the assumption that the first generation sampled was one year old, it can be concluded that the majority of *N. marionis* undergo a sex change from male to female between their second and third years.

3.4.5 Assessment of the reported von Bertalanffy curve:

If these data are to be used in future refinements of estimates of mean-size-at-age data for *N. marionis*, such a procedure should bear in mind that the largest cohorts in the 1997 RMT-8 and 1984 dredge samples may be from the fifth and sixth non-larval generations respectively. This idea was considered but abandoned in the present study because such an assumption would have dissociated the resultant von Bertalanffy growth curve from the observed data. Nevertheless, the idea is not an implausible one. The data available and the method in Table 3.4, however, indicate that *N. marionis* survives in significant numbers for 5 years under natural conditions. Older cohorts were surely also present in the length-frequency distributions, but could not be detected because of their depleted numbers. It may be possible to detect older generations with larger sample sizes than were used in this study.

Given the generation mean sizes and the various assumptions above, the von Bertalanffy growth parameter L_∞ (the asymptotic length) was calculated to be 12.60mm. L_∞ can be regarded as "the mean length of very old (strictly: infinitely old)" individuals (Sparre & Venema 1992: 46). Specimens of length L_∞ and greater are not necessarily this old though, as they may well be younger, faster growing individuals. However, given that in smaller samples such as my own L_∞ may be approximated by using the size of the largest specimens (Sparre & Venema 1992: 66), it is satisfying to note that the longest specimen observed in this study was quite close to the calculated L_∞ at 12.67mm. This lends credibility to the reported length-based growth parameters.

In very large samples, on the other hand, one should take the average of the lengths of the largest specimens (ibid.), because of variable growth rates of specimens of the same age (i.e. there should always be some specimens in any population larger than L_∞, because of this variable growth rate). Assuming that my carapace length/wet weight relationships can be extrapolated to cover Ridoux's (1994) entire *dependent variable* range, which included at least one specimen of 2.2g, then that (those) specimen(s) must have been about 14.1mm in carapace length. If this assumption is invalid, then it (they) must certainly have been longer than my longest specimen. I do not present my equation converting 'wet weight' to 'carapace length' in this thesis, it is *not* a simple matter of rearranging equation 4 in Table 2.1. It must be borne in mind that Ridoux's (1994) sample was not random; it came from the guts of predatory seabirds which are no doubt more efficient at 'sampling' the largest shrimps in the population than any human mechanism currently available. Therefore, even though Ridoux's sample consisted of only 30 undamaged specimens, those specimens may in a sense be regarded as being comparable in size to the largest specimens from an extremely large random sample, and therefore indicative of the size of the largest specimens in the population.

However, the possibility that those birds cannot dive to the depths at which the largest specimens occur must also be considered, but it is equally possible that they can. Ridoux's sample was taken in the southern summer months between November and February, between 1980 and 1983; those months being the time of year when it is assumed in this thesis that *N. marionis* hatching should soon occur (see section 3.4.6). There is the possibility, for instance, that the largest females which are not normally accessible to diving sea birds migrate to more ornithologically vulnerable areas for reproductive reasons during these months. Because there are so many imponderables, I decided to ignore the possibility that Ridoux's sample did not access the largest specimens in the population and accept Ridoux's maximum sized *N. marionis* as indicative of the maximum size attained. The expectation that the calculated L_∞ should be smaller than the largest specimens in the population was therefore fulfilled.

The calculated WW_∞ (2.03g), on the other hand, was noticeably higher than the heaviest specimen from my samples (1.68g). This may be a result of the approximations used to convert the length-based von Bertalanffy growth curve to its weight-based equivalent. This difference may indicate that the weight-based von Bertalanffy growth curve presented in this chapter is relatively less reliable than the length-based version. However, the maximum *N. marionis* wet weight recorded by Ridoux (1994) is, as expected, much higher than WW_∞ at 2.2g. For the same reasons that the largest specimens in a population should be larger than L_∞, so too should the heaviest specimens weigh more than WW_∞. This condition was fulfilled, so one may still regard the wet-weight based von Bertalanffy growth equation given in this chapter to be approximately accurate.

k, the calculated curvature parameter, indicates the rate at which the shrimp approaches L_∞ (Sparre & Venema 1992: 46), and therefore also WW_∞. The other von Bertalanffy growth parameter t_0, which is the time at which the shrimp has zero length, has no meaning biologically (ibid.). As with the estimate for t_0, the estimates for the carapace length and wet weight at birth (L_0 and WW_0 respectively) are not realistic. This is because the gross morphology of a caridean zoea is completely different to that of an adult. Larvae may also display a completely different growth curve to the adults (Sparre & Venema 1992: 46). In the case of the calculated WW_0 (0.00054g) these factors are compounded by intervening calculations and the fact that the wet weights from which the original carapace length/wet weight relationship was derived were measured to the nearest hundredth of a gram (centigram), whereas WW_0 could only be calculated to the nearest hundred thousandth of a gram.

However, an estimate of the carapace length at birth (L_0) may give one a better idea of the size of *N. marionis* when it hatches than other measurements which include the rostrum, such as Ridoux's (1994) "body length". The rostrum is barely present, for instance, in stage one of the life-cycle of the related Hippolytid shrimp *Hippolyte pleuracantha* (see diagram in Gurney 1960: 210; the larvae of *N. marionis* do not appear to be described). The *H. pleuracantha* rostrum therefore undergoes a more obvious morphological change from zoea to adult than does the carapace. It is quite possible that the same applies to the closely related *N. marionis*.

Nonetheless, because of the unreliability associated with extrapolating the von Bertalanffy growth curves, the calculated first year growth rate is probably inaccurate. The von Bertalanffy growth parameters should also be used with caution, albeit to a lesser degree where they are applied over the range of data collected. This is because they are estimates based on only three samples, one of them very small and one temporally separated from the other two by thirteen years.

One obvious potential problem with the von Bertalanffy growth parameters calculated in this thesis is that they indicate the average carapace length of the oldest identified *N. marionis* as being only ~71% of L_∞. However, one may expect that a population of animals which is a "key" conduit of energy to top predators in an ecosystem (Perissinotto & McQuaid 1990) to have a high mortality rate. If one accepts that *N. marionis* life-expectancy and predation pressures at the Crozet and Prince Edward Islands are similar, one can then compare Ridoux's (1994) small sample which was obtained from the guts of top predators to my larger samples. Ridoux's range was not as wide as mine, but included much larger specimens (Figure 2.2D). This suggests that certain top predators feed preferentially on larger *N. marionis.* One may expect a low survival amongst older cohorts because of this, perhaps resulting in their decimation as they approach the age at which L_∞ is attained. This would make their recognition in samples difficult. The fact that the oldest generation recognized from my data still had some way to grow to reach L_∞ should therefore not be surprising.

What was surprising was that the curve suggested a potentially long individual life-span in optimal circumstances (if the curve parameters are applicable to specimens older than five years). This suggests that undetectable remnants of cohorts much older than five years may be present in the ecosystem. Whether this is indicative of a problem with the curve or not is unknown. However, it is stressed here that the results obtained were preliminary, which is why they have been presented in such a way so as to facilitate their incorporation into other data sets.

In keeping with this philosophy, the means and standard deviations of the carapace length ranges for each of the genders in all the samples (including the sample not analyzed in this chapter) are presented in Appendix 1.

3.4.6 The age and ecology of the first sampled generation:

In calculating the age of the first generation, an attempt was made to establish an approximate hatching time for the species. It was noted that in the sample Parker (1984: 73) analyzed (n = 2295, apparently including males), "many of the nauticarids were in berry". It is unclear from Parker's study exactly when this sample was taken, but it was *probably* taken in March 1984 (Parker pers. comm.). It should be noted here that the *N. marionis* size-frequency data from Parker's thesis were *not* from April 1984, as was reported by Perissinotto & McQuaid (1990); no such benthic sample was analyzed in that work. Adams (1990: 66) makes the incidental observation that "some" ovigerous females were recovered in the guts of different penguins between April and September 1984 at the Prince Edward Islands. This is too wide a time range to draw any conclusions about the time of year of egg production.

Parker (1984) and Adams (1990) are theses. The only reference to the reproductive status of female *N. marionis* found in the literature is Bate (1888: 604), who observes that in a sample dredged off Marion Island in December 1873 (n = 67) where males "apparently" dominated, "some of the females were taken with ova attached, in which the embryo was far in development". I found only six individuals in all my samples that were in berry, and they were all from the May 1997 RMT-8 sample. This may have been because the 1997 RMT-8 sample included many more females than did the 1984 dredge sample, despite the former sample being smaller overall.

It was assumed that hatching times do not vary greatly between years, and therefore, because a) Parker (pers. comm.) suggests that *N. marionis* females are gravid in large numbers during March, b) Bate (1888: 604) observes that the eggs are in an advanced developmental stage during December, and c) my own observations were that there are only a few gravid females in May and that their numbers were not high in April (I found none during that month), I assumed that most hatching occurs just before April, with fewer individuals hatching between that time and May. The length of the hatching period is unknown. The females observed by Adams (1990: 66) may have been late-hatching females from April/May 1984, or females which were due to release young in March/April 1985, or both.

The assumption that the shrimps spawned only once annually (see section 3.2 above) meant that if this first generation was less than one year old then the von Bertalanffy curve would have differed markedly from the observed data. The March/April hatching time and the April/May sampling time therefore meant that the first generation sampled must have been at least one year old. If the first generation had been two or more years old then the von Bertalanffy assumption of a decreasing growth rate with increasing size would have necessitated the assumption of many unsampled generations. This would have not allowed for the calculation of the von Bertalanffy growth parameters with the available data. In addition, juveniles dominated the first generation cohort taken from the planktonic 1997 RMT-1*6 sample, and therefore it would seem unlikely that the first sampled generation was very old - probably the first non-larval generation. The assumption that the first generation was one year old allowed a better fit of the von Bertalanffy growth curve to the observed data (Figure 3.4) than did the assumption of one year and one month old. In the absence of any other data, it was therefore assumed that the first generation was one year old.

Because the first generation was observed to be partly planktonic, it would appear that settling from the plankton occurs during the first generation about a year after birth. Some diurnal migration may occur up to an unknown larger size (see Perissinotto & McQuaid 1990). This then begs the question of where the missing *N. marionis* larvae are to be found during their first year. It is not known whether the larvae are initially planktonic or not. The planktonic juveniles recorded in this thesis had fully developed rostral teeth, and were, in all gross morphological aspects other than size, identical to adults. This differentiated them from larvae, none of which were sampled in this study. If the age assumption for the first generation is correct, then *N. marionis* larvae must exist for up to a year in substantial numbers at a hitherto undiscovered locality. None have been sampled at the Prince Edward Islands in the past (Pakhomov pers. comm.). Although reference has been made by Perissinotto & McQuaid (1990) to the presence of *N. marionis* "megalope" larvae at the Prince Edward Islands in March/April, these were probably juveniles. This is because the "megalope" larvae specimens referred to by these authors were sampled from both midwater RMT-2 trawls *and* dredge samples in that study. Their occurrence in dredges taken in the same year as that of the dredge sample in this study makes one suspect that they were a group of postlarval individuals, of recognizable adult form, such as were observed in my samples. The normal caridean protozoea and zoea stages (Barnes 1987: 631) for *N. marionis* have not yet been specifically recorded.

Interestingly, no "megalope" larvae (or first generation post-larval individuals) were recorded by Perissinotto & McQuaid (1990) from a November 1983 dredge sample. This suggests that by November, individuals that have already hatched have not yet grown to a size where they start settling amongst the benthos. This may be because some or most of them are still in their larval forms at some unknown retreat. However, it must be remembered that from the gut contents of their predators there may be great variability in *N. marionis* recruitment from year to year, and it is therefore possible that November 1983 merely formed part of a low recruitment year. This would seem unlikely, though, because the following April the first generation was well represented amongst the benthos (Figure 3.1A and Table 3.1A). It is also possible that no juveniles were recovered in November 1983 because of the sample site choice, sampling times or even that they were recovered but not recognized as such because of a lack of exact definition.

The exact location of the larval feeding grounds remains a mystery. There is a substantial risk that any unsheltered surface larvae at this island group will be swept into the open ocean. However, it is known that changes in water temperature, as well as variations in salinity and photoperiod have *inter alia* been implicated in triggering the onset of the reproductive period in benthic invertebrates (Sastry 1983, cited in Bauer & Rivera Vega 1992). These are all factors which vary in shallower, less saline waters, such as are found in estuaries. It is also known that the ecologically similar shallow-water Penaeoidea *all* "have a life-cycle which includes a marine and an estuarine phase" (de Freitas 1989: 82).

Although *N. marionis* does not belong to the suborder Penaeoidea (Barnes 1987: 629), it is a shallow-water decapod in the truest sense of de Freitas' (1989: 82) definition. Similar evolutionary pressures may have resulted in a tendency towards estuarine utilization. There are, however, no real estuaries at the Prince Edward Islands (Brown pers. comm.). The area between the substantial kelp beds at these islands and the shore may, therefore, serve as a satisfactory substitute location for *N. marionis.* This area has not been sampled (Pakhomov pers. comm.). It has a low salinity resulting from run-off from the islands which does not mix readily with the outer reaches because the kelp beds affect local currents (ibid.). With very little interchange between these two bodies of water, any planktonic larvae in this zone would be protected from being swept away by oceanic currents. The area is also relatively shallow and "it appears to be a general feature of the distribution of many species of Caridea that the larger (older) individuals of the population are to be found in deeper water than the smaller" (Allen 1966: 56). I believe that this zone should be sampled both for gravid females and larvae during March/April i.e. the breeding period tentatively identified in section 3.4.6.

3.4.7 Potential sample bias:

Each succeeding cohort in any sample should have fewer members than the one preceding it unless a) the younger cohort had experienced much lower recruitment or higher mortality than the older one, or b) the younger cohort was undersampled. Undersampling of selected cohorts may occur when different sampling techniques are used, such as sampling different areas and sampling at different times of the day. During this study it was noticed that the first (youngest) cohorts in both the 1984 dredge and 1997 RMT-8 samples contained fewer members than the second (slightly older) cohorts in these samples.

The data presented here strongly suggest that the first generation is partly planktonic during March/April. The first cohort in the 1984 dredge sample was therefore probably undersampled because some of its members were in the plankton, which would explain its relatively low abundance. However, the undersampling of the first cohort from the midday 1984 dredge sample was perhaps not as great as might have occurred had that benthic sample been taken at night. *N. marionis* "megalope larvae" (presumably post-larval individuals of a carapace length which I would have associated with the first generation) have been shown to exhibit diurnal migration in the water column, feeding amongst the plankton at night, and are also present amongst the benthos during the day (Perissinotto & McQuaid 1990). This could explain why the 1984 day-time dredge sample first generation cohort contained individuals comparable in size to those found in the first generation cohort recovered from the (much less numerous) 1997 RMT-1*6 planktonic night-time sample. This observation was contrary to the expectation that settling amongst the benthos by first generation individuals is solely related to their attaining a certain size.

Although the 1997 RMT-8 sample was taken at night (when migration to the plankton might be expected), the first cohort recovered from it was part of the second generation. There was no indication that a substantial part of the second generation exists in the plankton at that time of year as is seemingly the case with the first generation. The first 1997 RMT-8 cohort was so small ($n \approx 12$) that it disappeared when the data were smoothed, whereas the third generation cohort from that sample (the first female-dominated cohort) made up ~67% of the total sample size. The third generation cohort from the 1984 dredge sample made up only ~12% of that sample. This makes one suspect either horizontal (rather than planktonic) migration of different generations or variable recruitment and/or mortality in different years.

The variable annual recruitment that has been suggested by Brown & Klages (1987) was based only on the fact that certain penguin diets were dominated by euphausiids in one year whereas they were dominated by *N. marionis* the previous year. This could just as easily have been the result of a high euphausiid presence which was more accessible to the penguins which they therefore preyed upon preferentially. It was therefore unclear whether undersampling or differential recruitment/mortality was the cause of the observed 1997 RMT-8 sample demographics, and therefore no possible reason for them is suggested here.

However, undersampling of selected cohorts would not have affected the calculations done in this chapter, as long as the cohort statistics were not *biased.* Bias should have been at least partially compensated for by sampling different areas at different times using different techniques. I assumed that none of the statistics were biased because of this. Current knowledge of *N. marionis* biology does not allow for an analysis of this assumption.

3.4.8 Differences between same-aged cohort mean sizes:

Although the same-aged cohorts from the different samples were interestingly similar in size, there is no obvious reason why the 1984 dredge sample cohort mean sizes were *consistently* slightly larger than their same-aged 1997 RMT-8 sample counterparts. The differences in size within the second, third and fifth generation cohort pairs cannot be explained on the basis of the 23 day difference between the times of year of sampling (which may have resulted in extra growth for the cohorts sampled in May), as the larger-sized constituent cohorts for these generations were the ones sampled earlier in the year in April.

These differences in size between the 1984 dredge sample cohorts and coupled 1997 RMT-8 sample cohorts may have arisen for a variety of reasons. Different diets, caused by temporal and spatial variation in the sampling, may have affected growth rates. Differentially-sized generations, or genders, may also have migrated to different regions, resulting in biased samples. Temporal variation of recruitment and mortality rates may be indicated. It has also been recorded that sea surface temperatures at the Prince Edward Islands have been changing over the last three decades (Smith 1991). Effects resulting from different sampling years may have been compounded by temperature changes over the intervening 13 year period. Temperature changes may also affect the life-cycle of *N. marionis* (see next chapter).

Diurnal variation in sampling may have also resulted in consistently smaller-sized, same-aged 1997 cohorts *vis-à-vis* those taken in 1984. Different sampling techniques may have resulted in the undersampling of certain cohorts in the different samples (although it is also possible that if this were the case, then different cohorts that would have remained undiscovered with a consistent sampling technique could have been detected). Alternatively, any combination of these factors could have caused the differences.

It is unlikely, however, that the differences observed between the same-aged cohorts from the 1984 dredge and 1997 RMT-8 samples were purely the result of random sampling, because this explanation would not account for the consistency observed.

3.5 Conclusions:

The rationale behind this chapter is to present preliminary results that can be incorporated into future studies. It is emphasized that the calculation of a realistic growth curve requires more data than was available for this hitherto poorly studied animal. In particular more random samples should be taken. The methods used in arriving at the conclusions in the preceding section have been carefully outlined, so as to stress their potential pitfalls, and also to facilitate their reproducibility. The data have been presented in such a manner as to make them useful addenda to future research. Even with more data, though, Sparre (1987: 76) "doubts that any objective method based entirely on length-frequency data will ever be developed". Sparre also opines that, with respect to iterative processes which require initial guesses of the results (such as in FiSAT), "one may say that the method attempts to improve the user's opinion". But see also Novalis' introductory quote at the beginning of this chapter.

Briefly then, the conclusions of this chapter are:

1) *N. marionis* appear to hatch just before April each year, with minimal hatching persisting until May.

2) It would seem that during the first year of life, *N. marionis* survive in undetected localities, moult into juveniles (probably what other researchers have called "megalope larvae"), and then settle amongst the benthos from the plankton. Diurnal vertical migration then occurs until the animals are an unknown larger size. It is not known whether the larvae are initially planktonic or not. It is possible that settling of small *N. marionis* onto the benthos only begins

after November. Future attempts should be made to discover the refuge of *N. marionis* during its first year, in order to better understand the ecology of this important animal in the Prince Edward Island group ecosystem. The suggestion is made here that the undetected refuge of the larvae *may* be in the shallower waters surrounding the islands, between the shore and the substantial kelp beds. Migrating gravid females *may* also occur there during the breeding period.

3) *N. marionis* survive for a total of five years in significant numbers, spending the first two years as males. Thereafter, they become female, but female population numbers become undetectable after three years using the samples of the sizes analyzed in this thesis. The difference in size between L_∞ and the average carapace length of the oldest generation sampled suggests that undetectable remnants of cohorts much older than five years may be present in the ecosystem.

4) In multisample situations, ageing of *N. marionis* cohorts is made less subjective if one utilizes the phenomenon of synchronized sexual inversion. This may be true of other protandric species as well. Ageing in future studies should use this fact where possible.

5) The von Bertalanffy length-based growth parameters presented in Table 3.4 seem viable because of the independent estimate of L_∞. The weight-based growth curve should be used with more caution. I wish to stress again, however, that the parameters for both curves are estimates based on only three samples, one of which was very small and another of which was temporally separated from the other two by thirteen years; and also that one generation mean size was estimated using an arguably less than ideal method. Despite showing potential,

the parameters in Table 3.4 are preliminary and are intended to facilitate the calculation of better curves. The growth parameters are tentatively identified as k = 0.2353/year, L_∞ = 12.69mm, t_0 = -0.2828 years and WW_∞ = 2.03g.

6) The programme FiSAT, although extremely useful, was found to have certain faults; the most important of which appears to be the basis of the χ^2 test, allowing for differences in data sets which the programme indicates do not exist. This may allow one to isolate unidentified cohorts using the 'Petersen' method. Future commentary on the validity of using the historical "Petersen" method in conjunction with FiSAT to isolate cohort means is necessary. Upward class interval adjustment after entering data into FiSAT seems to be a prerequisite for its use, even though this adjustment may be controversial. There was also an implicit recommendation in the user's manual that "smoothing" be done on data after class interval adjustment has been performed. This procedure is not recommended. Consultation of standard χ^2 tables when using FiSAT is recommended, as the values released by the programme may be inaccurate. Using the tables also helps identify viable initial estimates with the "Bhattacharya" procedure.

Chapter 4

Protandry and peripheral aspects of reproduction

"BISEXUALITY IMMEDIATELY DOUBLES YOUR CHANCES FOR A DATE ON SATURDAY NIGHT"

(WOODY ALLEN, CITED IN PETER 1991: 440).

4.1 Introduction:

There are many dioecious hippolytids which show size-based sexual dimorphism (Bauer 1986) because large females have a selective advantage (Hoffman 1972). Although larger dioecious females must at some stage pass through smaller size-classes, smaller females may be absent at certain times of the year. For example, there is a clear separation in the size distribution frequencies of males and females of the dioecious hippolytid *Heptacarpus pictus* at the beginning of the breeding season which does not persist into the non-breeding season (Bauer 1976). The assumption that the first generation of *N. marionis* identified in Chapter 3 was one year old suggests that this thesis' samples (taken in April and May) were taken just after hatching. This could account for the pronounced sexual dimorphic sizes observed (Plate 1). Considered in isolation, therefore, the sexual dimorphism found in *N. marionis* reveals nothing about the sexual strategies of this caridean.

It was, however, on the basis of similar data, albeit with more samples, that Chace (1972, cited in Bauer 1986) suggested the possibility of protandric hermaphroditism in the hippolytid genus *Thor*, having noticed that males consistently composed the smaller size-classes and females the larger. Subsequent examination confirmed Chace's intuition, with *Thor manningi* being shown to be the only known protandric decapod species where primary males i.e. males which never transmutate into females, persist (Bauer 1986).

Combining data from three review articles, Bauer reports that there are 32 known sex-changing decapods, of which 26 are caridean shrimps. He describes three strategies used by sex-switching carideans: 1) all individuals pass through a small male stage to transmutate into females (I shall refer to females with such a history as secondary females); 2) a variable proportion of individuals (less than 50%) matures directly into females (what Hoffman (1972) called primary females), the majority of individuals following the route as in (1); and 3) the sole case of *T. manningi*, where primary and secondary females occur, as in (2), but where primary males persist as well.

The presence and stage of development of the male copulatory organs is generally used to determine gender in decapods (Mascetti *et al.* 1996). Male copulatory organs can be found on the first two pairs of pleopods in "natantian decapods" (Hoffman 1972). The suborder 'Natantia' is not normally used anymore (e.g. Barnes 1987: 629), although Branch *et al.* (1991) and Arntz & Gorny (1991) have used this name recently. This taxon (*sensu lato*) included the two families Caridea and Penaeidea, which are sometimes inexactly referred to as 'shrimps' and 'prawns' respectively (de Freitas 1989: 81).

One feature which distinguishes carideans from penaeids is the specialized modification of the pleopods of the latter family for copulation. The following comparison between these two families is based on Bauer (1976).

The male copulatory organs of carideans are modest in comparison to the petasmae of male penaeids. The caridean copulatory organs consist of two sets of rami on the pleopods; one set on the endopod of the second pleopod called the *appendices masculinae* (am) and one on the endopod of the first pleopod called the *appendices internae* (ai1) (my abbreviations, see Plates 2-10). External fertilization occurs in both the caridean and penaeid genera, but whereas the penaeid spermatophore is deposited in the ventral thelyca of the female, the caridean spermatophore is merely attached to some ventral part of the female where it will fertilize the eggs as they are transferred to the pleopods, to which they finally adhere. The am and ai1 are both necessary for the proper transfer of the caridean sperm. Without them, mating proceeds normally but the females often reject the probably ill-positioned spermatophore. Male *Heptacarpus pictus*' which had had both pairs of copulatory rami ablated displayed a significantly lower number of successful spermatophore transfers than those which had had only one pair ablated (which pair, the am or ai1, was not specified). Bauer concluded that the am and ai1 were vital to caridean male reproductive success as spermatophore placement structures.

During the sexing of individual *N. marionis* for this study, it was noted that different combinations of the male and female primary and secondary sexual characteristics occurred in certain individuals, with some rudimentary am observed in relatively large individuals (Plates 8-10). Also noted was the presence of the ai1 in the smallest of individuals, even those without any other sign of sexual differentiation in the plankton. Protandric carideans lose the am during sex-change (Bauer 1986). The ai1 has been recorded as absent in the juveniles and females of the dioecious species *H. pictus* (Bauer 1976). It was not seen on obviously female *N. marionis* (Plate 6), but all juvenile *N. marionis* that were observed possessed this male copulatory organ. Together, these observations suggested some form of hermaphroditism in *N. marionis*, probably protandric because the male characteristic was seen on the smallest of individuals.

Although protandry amongst carideans and especially hippolytids is rare (Bauer 1986), it is usual in some caridean families amongst certain genera (ibid.). In the family Hippolytidae, protandry has been recorded in *Lysmata seticaudata* (Dohrn 1950, cited in Bauer 1986), *Hippolyte inermis* (Reverberi 1950, cited in Zupo 1994), and *Chorismus antarcticus* (Yaldwyn 1966, cited in both Mascetti *et al.* 1996 and Gorny *et al.* 1993). *C. antarcticus* is the most common benthic caridean on the Antarctic continental shelves (Kirkwood 1984, cited in Mascetti *et al.* 1996). This may indicate that it fulfils an important ecological rôle in certain locations (Gorny *et. al* 1993), much like *N. marionis* at the Prince Edward Islands (Perissinotto & McQuaid 1990). *C. antarcticus* is also found in the area surrounding the Prince Edward Islands, although not at population sizes comparable to those of *N. marionis* (Branch *et al.* 1991). *C. antarcticus* is therefore both an Antarctic and a sub-Antarctic species (Arntz & Gorny 1991, Gorny *et al.* 1992), whereas *N. marionis* is exclusively sub-Antarctic (Perissinotto & McQuaid 1990).

Protandric hermaphroditism therefore occurs sporadically amongst other related hippolytid genera, one of them, at least, showing certain ecological similarities to *N. marionis*. Furthermore, a clearly hermaphroditic condition was observed in some *N. marionis* specimens. This chapter therefore primarily examines the nature and implications of protandric hermaphroditism in *N. marionis*, but also includes observations relating to peripheral aspects of its reproduction.

4.2 Gender definitions:

Gorny *et al.* (1993) used ovary development and am structure to sex individual *Chorismus antarcticus*. Mascetti *et al.* (1996), who also studied *C. antarcticus*, have suggested a more accurate definition of the different genders which takes into account the development of the gonopores, noting that females of this protandric hippolytid sometimes possess am. In this study, only the *presence or absence* of am, ai1, ovaries and abdominal eggs were used to define these genders. Five 'genders' (*sensu lato*) were recognized and are defined below.

N. marionis specimens possessing ovaries as well as am were recovered from two of the samples (see section 4.3). These specimens are provisionally referred to as *tertia quae* (*pl.* of *tertium quid*) to distinguish them from male and female specimens. A fourth type of specimen which possessed an ovary and the other male copulatory organ (the ai1), but no am, was also defined. The last mentioned type of individual was indistinguishable from juveniles in respect of the features mentioned above except for the presence of an ovary. No prehensile third pereiopods were observed in any of the males - such pereiopods being characteristic of primary male *Thor manningi* (Bauer 1986).

The gender definitions used in this thesis are designed to be as simple as possible, to facilitate the minimally acceptable sexing of individuals with the least possible effort. Such an approach is useful when large numbers of animals have to be considered. Size was not used as a criterion because there was some overlap in size between males and females (see previous chapter, Figures 3.1A & 3.2A, cf. also Bauer (1989), where size *was* used). Accordingly, am setae (presumably used in the transfer of the spermatophore in sexually mature males, Bauer

(1976)) and gonopore structures were not used. Bate (1888: 604) made the anecdotal observation that male *N. marionis* seem to have more upper rostral teeth than females. It should be mentioned here, *en passant*, that there does not seem to be any truth to this. Rostral teeth should consequently not be used as a criterion for sexing individuals.

In cases of uncertainty, ovaries were recorded as absent. Ovaries were only identified when they were undeniably present i.e. when they were large and yellow or orange. Such is the definition of a mature ovary given by Bauer & Rivera Vega (1992). Immature ovaries are described by these authors as colourless or transparent. They were therefore more difficult to identify. Their presence in *N. marionis* was not recorded after one aborted attempt at this, because of the inexperience of the researcher and the resultant ambiguities. Presumably because of the conservative definition of ovary presence, no specimens were recorded which had both eggs and ovaries. The ovaries in the few gravid specimens that were recovered were no doubt spent, if only temporarily, and did not display the full-bodiedness used as a criterion for identification in this study. Only six such gravid individuals were found.

Mascetti *et al.* (1996) found remarkable differences between the structures of the left and right pleopods in a minority of *C. antarcticus* specimens. Accordingly, the secondary sexual characteristics referred to in this study (the male copulatory organs am and ai1) were identified using only the right-hand side pleopods after they had been amputated and then examined under a compound microscope. Ovary presence (the primary sexual characteristic used) was recorded when obvious through the dorsal carapace (Plates 13-18). Eggs, when present, were carried under the abdomen and were easily visible.

All specimens sampled, including those from the 1997 RMT-1*6 planktonic sample, had well developed rostral teeth, which differentiated them from larvae (see Gurney 1937). The following gender definitions, which between them covered every specimen observed, were designed for preliminary separation into distinct groups. These definitions are redefined in the discussion, based on the results.

4.2.1 Juvenile:

Not ovigerous, not gravid, ai1 present, am absent.

4.2.2 'Juvenile' with ovary:

Ovigerous, not gravid, ai1 present, am absent.

4.2.3 Male:

Not ovigerous, not gravid, ai1 present, am present.

4.2.4 *Tertium quid (pl. tertia quae)*:

Two subtypes; a) ovigerous or gravid, ai1 present, am present.

b) ovigerous, not gravid, ai1 absent, am present.

4.2.5 Female:

ai1 absent, am absent, presence/absence of ovaries or eggs not diagnostic.

4.3 Materials and methods:

4.3.1 *Appendix* measurements:

In addition to sexing all the specimens from all the samples (see section 1.3) using the procedures described above, the right-hand side am and ail of each individual in the 1997 RMT-1*6 planktonic and the 1984 dredge samples, including a number of non-random specimens from the latter sample, were measured under a dissecting microscope at 500X magnification using an ocular micrometer. The am and ail measurements were taken from the apex to the middle of the base. Am length measurements did not include any setae.

The bases of each am and ail measured were exposed under the microscope after cautiously bifurcating these appendages from the amputated pleopods on which they were found. This was found to be problemic however, because after separation these soft appendages (especially the smaller ones) tended, when moist, to readhere quickly to the endopods to which they were attached. This resulted in their base attachments becoming concealed again. The problem could be partially overcome by allowing the Petri dish to dry slightly, which caused the base attachment to remain exposed. This in turn, however, often caused quick and pronounced shrinkage in the *appendix* of interest because of the high surface-area to body-volume ratio of these small tubular structures. The problem was never fully resolved, but I believe that fairly representative measurements were obtained through careful timing, although the accuracy of the measurements and their compatibility with those taken by other researchers remains doubtful.

The am length measurements for the relevant gender groups in the 1984 dredge sample were plotted against carapace length (defined in section 2.2.1.5) to reveal trends, as was done by Mascetti *et al.* (1996). Concurrent carapace and am lengths below ~4.00mm and ~0.10mm respectively were unrepresented in the 1984 dredge sample. In order to give an indication of trends in this area, the 1997 RMT-1*6 planktonic sample data were tentatively superimposed on the 1984 dredge data, bearing in mind that there were differences in size between same-aged cohorts from these two samples, which are discussed in the previous chapter. The ai1 measurements were similarly compared to the carapace lengths.

4.3.2 Ovary development:

The ovary appears to develop sequentially from posterior to anterior under the carapace. Ovary development was subjectively classified by taking the most posterior upper rostral tooth as the point of discrimination, using a system loosely adapted from Bauer (1986). Classification was as follows: 0 = no ovary clearly visible; 1 = ovary present but anterior point of ovary posterior to point of discrimination; 2 = ovary present, anterior point of ovary anterior to point of discrimination, but posterior to the posterior margin of the eye orbit; 3 = as in (2), but ovary in contact with posterior margin of the eye orbit. See Plates 12-18 for examples of stages 1, 2 and 3 of ovary development. Ovary development was recorded in all the samples, except one large random subsample from the 1984 dredge sample, in which only presence/absence was recorded. An aborted attempt to record immature ovary presence was made in this subsample, using a different classification system.

The total numbers of each of the different ovary developmental stages in the different genders where recorded were plotted in histogram form. Six gravid animals with no discernible ovaries, all females apart from one *tertium quid* type 'a', and all from the 1997 RMT-8 sample, were included in the histogram by simply treating them as another ovary developmental stage (4). As there were three potentially ovigerous or gravid genders, the levels of ovary development between them were then compared using χ^2 tests of association for the samples in which at least two such genders occurred (i.e. the 1997 RMT-8 and part of the 1984 dredge samples). An extra, random subsample from the 1997 RMT-8 sample was taken for this comparison, to try and include more *tertia quae* in this sample, but it only yielded two more such specimens, both type 'a'. In order to deal with the existence of non-ovigerous females for these two tests, it was decided to allow for the existence of non-ovigerous specimens of the other two genders (which were defined by the presence of ovaries). This was done by first pooling all 'juvenile' data (with and without ovaries) into one group, and second by recording zero observations of *tertia quae* without ovaries. It was recognized that the results of these tests of association would need to be cautiously interpreted because a) the majority of individuals from the 1984 dredge sample only had ovary presence or absence measured, resulting in the χ^2 test for that sample being done on few potentially ovigerous specimens, b) the other sample tested (the 1997 RMT-8 sample) only contained four non-female potentially ovigerous specimens, and c) there was a likelihood of finding a difference where none existed because an artificial difference between observed and expected frequencies of non-ovigerous *tertia quae* would have been created, *tertia quae* having being defined by ovary presence.

4.3.3 Differences in size between genders:

Gender-specific carapace lengths in all the samples, including those for which the am and ail lengths were not measured, were statistically compared within random samples. The data were not normally or lognormally distributed (see Zar 1984: 239), in fact the histograms of Figure 3 in the previous chapter visually suggest that the different gender-specific data from the two largest samples were heterogenous and were polymodally distributed. Polymodally distributed raw data cannot be normally transformed (Radloff pers. comm.). The gender subsample sizes from each sample were vastly different. Some were quite small, so the robustness of parametric ANOVA to heterogeneous data when sample sizes are equal or nearly equal (Zar 1984: 170); and non-normal data when sample sizes are large (ibid.) could not be relied upon. Consequently parametric analysis could not be done on these data and a less powerful non-parametric method (Zar 1984: 138) had to be chosen. The Kruskal-Wallis test with tied ranks (Zar 1984: 178) was therefore used to compare gender sizes. After significant size differences between genders were revealed in samples where more than two genders were sampled, nonparametric multiple comparisons for unequal sample sizes were done using the technique described in Zar (1984: 200), with the aid of a simple spreadsheet. p values were calculated using linear interpolation (Zar 1984: 477).

Both *tertium quid* types in both the samples in which they were found were combined for all statistical tests, there being only two 'b' type *tertia quae* in the 1984 dredge sample and one in the 1997 RMT-8 sample.

4.3.4 Egg size:

The six gravid individuals all came from one sample (the 1997 RMT-8 sample). The low number of eggs in most of the gravid individuals suggested that substantial egg loss had occurred (one specimen was carrying only eight eggs), making any estimate of average egg size for those specimens quite dubious. Two specimens had an acceptable number of eggs, one of them seemingly not having lost any. Eggs seemed to adhere quite strongly to the pleopods, even after tearing the pleopods from the abdomen (Plate 10). *Every* egg carried by these two animals was counted.

The eggs were ellipsoid in shape. A random subsample from each of the two animals was taken and the ellipsoid z and x axes of all subsample eggs were measured under a dissecting microscope using an ocular micrometer at 500X magnification. The x and y axes of an ellipsoid are identical (Thomas & Finney 1988: 845). Egg volume (V) was calculated using the formula $V = \frac{4}{3}\pi a^2 b$, where $a = x$ (or y) axis length (mm)/2 and $b = z$ axis length (mm)/2 (Graham Shepherd pers. comm.). Total egg volume carried by both animals was estimated by multiplying that animal's average egg volume by the total number of eggs counted.

4.3.5 Other observations:

Other peripheral observations relevant to the discussion which follows were recorded for the two largest samples (the 1984 dredge and 1997 RMT-8 samples). These included parasitism by bopyrid isopods and some aspects of egg development.

4.4 Results:

4.4.1 Differences between genders:

The five genders represented in the 1984 dredge sample were significantly different in size ($\chi^2=153.65$, $p<<0.001$), but the inclusion of the four non-ovigerous juvenile data points as a separately comparable group created inconsistencies when assessing the subsequent multiple comparisons. The test statistic Q calculated during these comparisons was greatly decreased by very small sample sizes, and it was observed that other trends were obscured by the inclusion of that small subsample's data. They were consequently omitted from the size comparison. Gender carapace length sizes remained significantly different (χ^2=152.67, p<<0.001), with the average size ranking being, from smallest to largest, males-'juveniles' with ovaries-*tertia quae*-females. Males were of a smaller size than females, *tertia quae*, and 'juveniles' with ovaries (Q=11.03, p<<0.001; Q=5.96, p<<0.001 and Q=3.33, p≈0.005 respectively). None of the three potentially ovigerous or gravid genders could be separated by size using the Q statistic (p>>0.50 for all three comparisons).

The three genders in the 1997 RMT-8 sample were significantly different in size (χ^2=45.58, p<<0.001), females being larger than males (Q=6.75, p<<0.001), and neither males nor females different in size to the two *tertia quae* specimens in the sample (Q=0.03 and Q=1.86 respectively, p>>0.50 in both cases). Unsurprisingly, discarding the *tertium quid* data because of the small subsample size did not alter the significance of the size differences between males and females (χ^2=45.37, p<<0.001).

The other two samples, in each of which only two genders were represented apiece, were quite small. The females in the 1996 RMT-8 sample were significantly larger than the males in that sample (χ^2=15.37, p<<0.001). The juveniles (without ovaries) in the 1997 RMT-1*6 sample were smaller than the males at the 95%, but not 99% significance level (χ^2=5.22, p≈0.02). Larger planktonic samples are needed to clarify the size-differences (or lack thereof) between planktonic juveniles and males, but it would seem that, despite the fact that the largest individual in this sample was juvenile, on average males were larger.

Nine non-ovigerous females were recovered in total, all from these two samples. Only one (from the 1997 RMT-8 sample) fell outside the upper limit of the 'juvenile' with ovary size-range. Using tests of association, no relationship was found between potentially ovigerous genders and ovary development (or egg presence) in either the 1984 dredge sample (χ^2=9.63, $p\approx0.39$, n=37) or the 1997 RMT-8 sample (χ^2=7.79, $p\approx0.10$, n=218).

Sample	n	Size comparisons	Significance level	Omitted data
1984 dredge	378	M < 'j'+o = *tq* = F	99%	juv
1996 RMT-8	28	M < F	99%	
1997 RMT-8	221	M < F	99%	*tq*
1997 RMT-1*6	21	M > juv	95%	

Table 4.1: Size comparisons between the different genders in the different samples. F = females, M = males, tq = tertia quae, 'j'+o = ovigerous 'juveniles', juv = non-ovigerous juveniles. Selected data were omitted where genders were underrepresented.

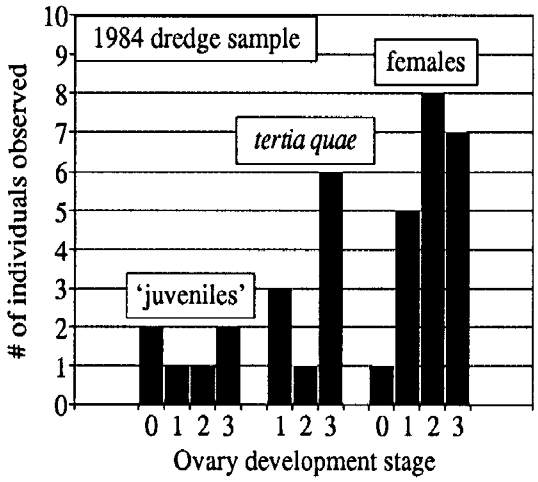


Figure 4.1: Ovary development in three genders recovered from the 1984 dredge sample. A small random subsample was used (n=37) for the χ^2 test of association.

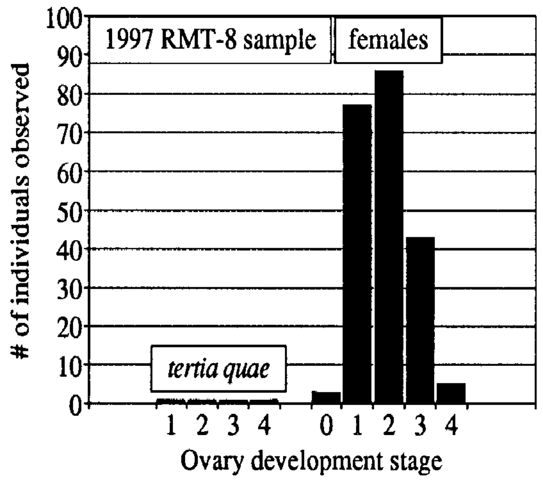


Figure 4.2: Ovary development in two genders recovered from the 1997 RMT-8 sample (n=218). Stage 4 indicates gravid specimens (see text).

'Juveniles' with ovaries, *tertia quae* and females were therefore not properly separable in the 1984 dredge sample by ovary development or size (although these two tests were done separately on partially different data sets, so no interaction effect was measured). The three potentially ovigerous genders were, however, consistently found to be larger than males in the samples where they were compared (Table 4.1).

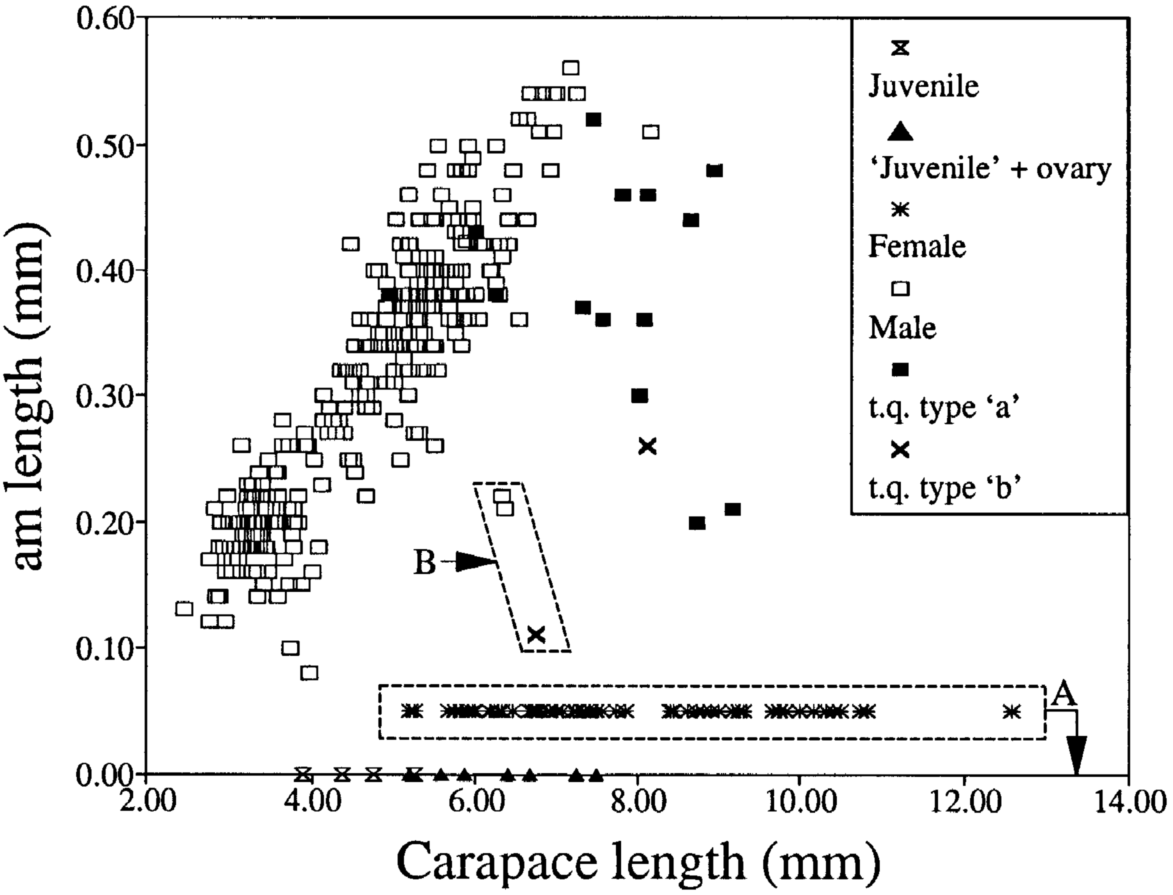


Figure 4.3: Appendix masculina length plotted against carapace length for the 1984 dredge data. Tertium quid has been abbreviated to 't.q.' above. A - Female data points have been artificially raised for clarity; B - see text.

When plotted, the 1984 dredge sample carapace lengths and am lengths demonstrated noticeably cohesive gender specificity (Figure 4.3). The lower size limit of the females overlapped completely with the ovigerous 'juvenile' gender (note that the female data points have been artificially raised from the x-axis in Figure 4.3 to make this clear). There was almost no overlap in size between ovigerous 'juveniles' and the sparsely represented gender I have called 'juveniles'. This suggested that there was a difference in size between the two 'juvenile' genders despite the fact that there were too few juvenile data points to make any statistical comparison.

In the 1984 dredge sample the length of the am increased with carapace length as this rose from 2.46mm to ~7.00mm. Data points in this carapace length range were predominantly male. At carapace lengths larger than ~7mm, *tertia quae* began emerging strongly. The data points scattered somewhat at carapace lengths greater than 7mm, although a clear reduction in am size could be observed. It is clear from Figure 4.3 that the carapace length at which the am showed signs of becoming smaller was also the point at which the males disappeared and the *tertia quae* began manifesting themselves, although three *tertium quid* type 'a' individuals were recorded at carapace and am lengths more commensurate with males. Also in Figure 4.3 one can see two males and one *tertium quid* type 'b' with small am relative to their carapace lengths (see the dashed-line rhomboid marked 'B' in Figure 4.3).

The longest am measured (0.56mm) was from a relatively large male from the 1984 dredge sample (CL=7.17mm). Larger males and all *tertia quae* (whether larger than 7mm in carapace length or not) had am of lengths less than 0.56mm (the longest am observed in a *tertium quid* was 0.52mm, CL=7.46mm). A steep decrease in am length with increasing carapace length was clear amongst *tertia quae*.

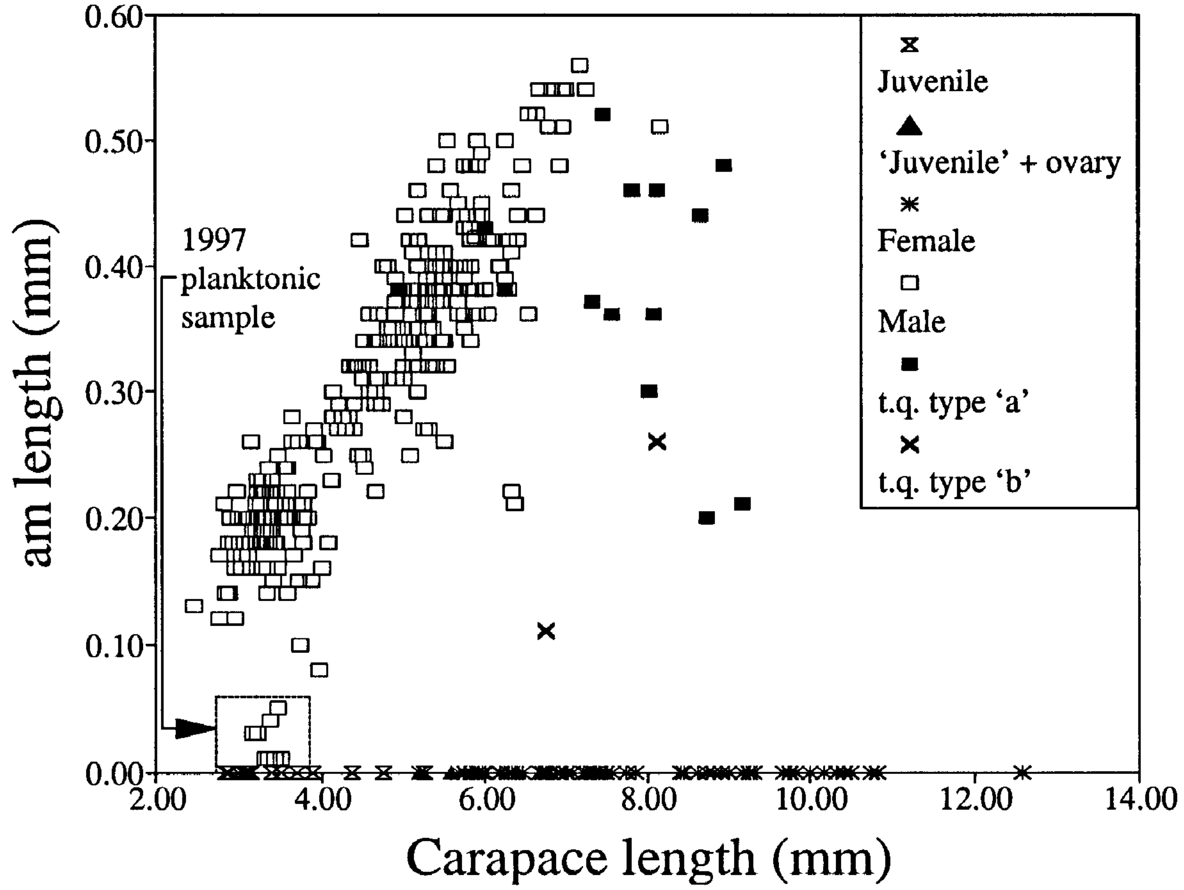


*Figure 4.4: Appendix masculina length plotted against carapace length, with superimposition of the highlighted 1997 RMT-1*6 data onto the 1984 dredge data. cf. Figure 4.3, where the female data was artificially raised from the x-axis.*

In Figure 4.3, concurrent carapace and am lengths below ~4.00mm and ~0.10mm respectively were unrepresented. The 1997 RMT-1*6 planktonic sample CL and am lengths (which covered this area) were superimposed onto Figure 4.3 to yield Figure 4.4. Development of am seemed to have occurred over almost the whole carapace length of that planktonic sample, from ~3.1mm to ~3.5mm. The largest individual taken in this sample (CL=3.70mm), however, had not developed an am.

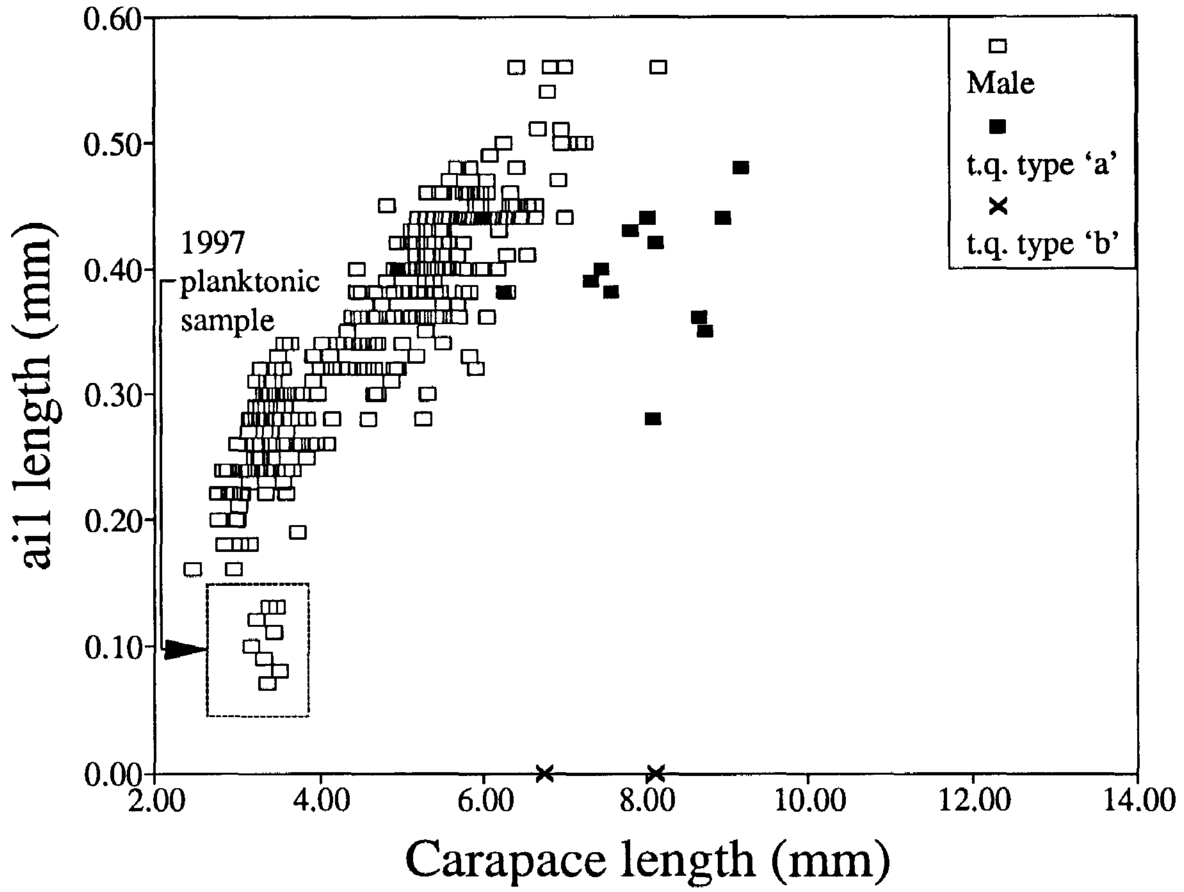


Figure 4.5: Appendix interna length (1st pleopod) plotted against male and tertium quid carapace length, with female and 'juvenile' data excluded for clarity.

Similar results were obtained when plotting male and *tertia quae* ail lengths against carapace lengths (Figure 4.5). Carapace lengths at which ail lengths began decreasing largely coincided with those at which the am began decreasing. As a result of this, individuals in which the decreasing ail tendency was noticed all possessed ovaries i.e. were *tertia quae.* Ovaries began developing in a few individuals at carapace and ail lengths more commensurate with males. In contrast, however, apart from one ovigerous and one non-ovigerous juvenile (carapace and ail lengths 5.25mm, 0.10mm & 7.25mm, 0.02mm respectively), the scattered data points from all 'juveniles' gave no discernible indication that this male copulatory structure decreased in length with an increase in carapace length in those individuals (Figure 4.6).

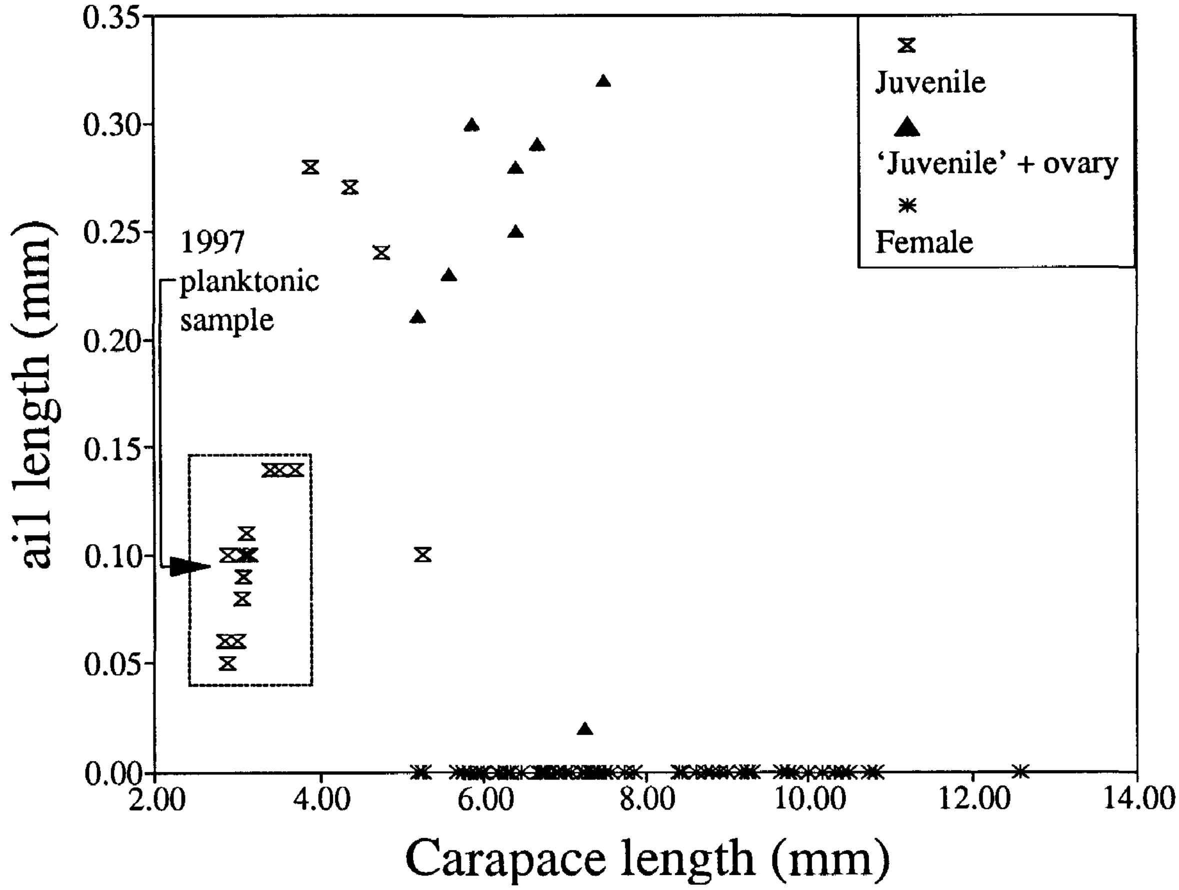


*Figure 4.6: Appendix interna length (1st pleopod) plotted against 'juvenile' and female carapace length in the 1984 dredge and highlighted 1997 RMT-1*6 samples. This structure did not seem to decrease in length in larger specimens.*

4.4.2 Parasitism:

One juvenile (CL=5.83mm), six males (mean CL=5.84mm, standard deviation=1.42mm, range=4.50mm-7.75mm) and three females (CL= 6.33, 7.08 & 9.67mm) from the April 1984 dredge sample were found to be infected by parasitic isopods of the family Bopyridae. No other sample showed any infestation, although it must be stressed that only obviously parasitized individuals were recorded, and that the 1984 dredge sample was the largest of all the samples analyzed. See Plates 19-21 for examples of Bopyridae. No *N. marionis* parasitized by Bopyridae were ovigerous or gravid.

4.4.3 Incidental comparisons between the 1984 dredge and 1997 RMT-8 samples:

Apart from the occurrence of parasitized individuals, the 1984 dredge and 1997 RMT-8 samples displayed other differences. The male:female ratio in the 1984 dredge sample was 83:17, whereas it was 3:22 in the 1997 RMT-8 sample. 'Juveniles' made up 3% of the total random April 1984 dredge sample, 1% non-ovigerous and 2% ovigerous. No 'juveniles' were sampled in the May 1997 RMT-8 sample. 2% of the May 1997 RMT-8 sample specimens were gravid, whereas no such specimens were recovered in 1984. Two of the five gravid females from the May 1997 RMT-8 sample (CL = 5.17mm & 6.17mm) were smaller than or approximately equal in size to the mean female carapace length of the first cohort of that sample, identified in the previous chapter (which excluded larger, older females; total female data for cohort: Mean carapace length = 6.449mm, standard deviation ≈ 0.587mm, n≈150, see Table 3.2C). Two of the four *tertia quae* from the two combined 1997 RMT-8 subsamples were unusual specimens. One (CL=7.00mm) was of type 'b' (which had lost the ail, see section 4.2.4 b), of which only two others were recorded in all the samples (both from the 1984 dredge sample). Like the two recorded in the 1984 dredge sample (CL=6.75mm & 8.13mm), it possessed a well-developed stage 3 ovary. The second was of type 'a' (section 4.2.4a), the only gravid *tertium quid* taken in all the samples (CL=6.42mm).

4.4.4 Egg data:

Five gravid females (carapace lengths 5.17mm, 6.17mm, 6.96mm, 7.17mm & 9.25mm) and one gravid *tertium quid* (CL=6.42mm) were recovered. All were from the May 1997 RMT-8 sample. None of the eggs observed seemed to be in an advanced stage of development. Only two females were used to record egg volumes in Table 4.2 (see section 4.3.4).

Carapace length (mm)	Total # eggs	Sample size	Average egg volume (mm^3)	Standard deviation (mm^3)	Total egg volume (mm^3)
7.17	107	12	0.067	0.0049	7.14
9.25	783	21	0.10	0.0098	78.0

Table 4.2: Egg volumes in two female specimens from the 1997 RMT-8 sample.

4.5 Discussion:

4.5.1 Gender definitions:

The sexing procedure used in this study is arguably not ideal, potentially leading to an underestimation of the number of 'juveniles' with ovaries, males and females; and an overestimation of the numbers of juveniles and *tertia quae*. The procedure allows for immature males to be regarded as distinct from juveniles based on the possession of am without setae, which may cause a problem in assessing samples containing small juveniles. This is perhaps not as serious, however, as the existence of breeding individuals which are classified as *tertia quae*, which may be fully-fledged females that merely retain vestiges of a male-phase past (at least one such individual was found which was in berry). The lack of recognition of immature ovaries also allows for individuals which perhaps should be regarded as *tertia quae*, to be classified as males. Some individuals which should be classified as ''juveniles' with ovaries' (identical to juveniles except for the presence of ovaries) may also be classified as juveniles for the same reason. It is also idiosyncratic to define individuals which clearly possess mature ovaries as juveniles.

However, no males would be classified as being of any other gender, and all females identified would be true females. Note that this procedure recognizes three potentially ovigerous genders. Attempts to redefine these genders, and in so doing to solve some of the problems of definition mentioned above, are made below.

Sexing protandric species can be problematic, even when protandry has been recognized (Mascetti *et al.* 1996). This raises the possibility that the two previous studies on *N. marionis* in which some sexing was done (Bate 1888 and Perissinotto & McQuaid 1990) may have used completely different definitions. Unfortunately, despite attempts by this researcher, it has proved impossible to verify this. More than likely the "megalope larvae" of Perissinotto & McQuaid (1990) were both juveniles and small males (by this thesis' definitions), especially given their reported occurrence in the benthos in March/April 1984. Therefore size was probably some sort of criterion in that study. Males and large non-ovigerous juveniles were possibly classified together as males. The larval stages of *N. marionis* still remain to be described, reports of "megalope larvae" in the literature notwithstanding.

On the definition of females, Bate (1888) and Perissinotto & McQuaid (1990) probably recognized what I have called *tertia quae*, ovigerous 'juveniles' and females as "females" because of the presence of ovaries in all three of these groups. I shall argue in this discussion that this is probably not far off the mark, although I recognize two types of females, not always separable. It is possible, however, that a few large males were also regarded as females in those studies, and smaller, non-ovigerous females were regarded as male.

Nonetheless, differences in definition aside, with the precise definitions presented in this study, the results obtained herein are potentially reproducible, and therefore the errors potentially measurable. This will facilitate future comparisons. I therefore recommend future studies which utilize more accurate characteristics to sex individual *N. marionis* given the evidence for protandry which has been uncovered in this study. Recording gonopore and ovary development *à la* Mascetti *et. al* (1996) and am setae development *à la* Gorny *et. al* (1993) respectively are especially recommended.

4.5.2 Sex change:

The male and *tertia quae* data in Figure 4.3 showed the same broad trends as those from the hippolytid *Chorismus antarcticus* (Mascetti *et al.* 1996), in which species protandry has already been established. However, unlike that species, a small number of *N. marionis* without am extended over the whole size range covered by males in the 1984 sample. These specimens were those originally classified as juveniles and 'juveniles' with ovaries. Such findings are consistent with a sex-reversal strategy identical to the second mentioned in section 4.1 above i.e. a variable proportion of individuals (less than 50%) maturing directly into females, the majority of individuals passing through a male stage to transmutate into females (see Bauer 1986). The only possible explanation for the trends seen in Figures 4.3 & 4.4 is that two types of *N. marionis* females exist, one developing out of the *tertia quae*, and the other from the juveniles. Sex change in the first group would be characterized by atrophy of the am and ai1. If this is the case, then the *tertia quae*-evolved females should properly be regarded as secondary females, and the 'juvenile with ovary'-evolved females as primary females. The possibility that primary males exist has to be discounted because of lack of evidence (no prehensile third pereiopods were observed). *N. marionis* is therefore a partially protandric hermaphrodite.

There was a complete overlap between the size range of the ovigerous 'juveniles' and the smaller females. This size range was smaller than what might have been expected from *tertia quae*-evolved females. It would seem from this that at least some of the females in this size-range (which had lost the ai1) had evolved from ovigerous 'juveniles'. Most females sampled were ovigerous. Because the ovigerous 'juveniles' were defined by the presence of mature ovaries, one feels that they should be recognized as primary females in their own right as well. This would explain why the primary females which were distinguished by the presence of the ai1 were, as a group, not statistically smaller than the rest of the females. However, there is no evidence for any hitherto unrecorded and unambiguous distinction between the two types of females using the presence/absence of the ai1 in protandric caridean shrimps. The possibility that *all* primary and secondary *N. marionis* females can be so distinguished at all times of the year is unlikely. In the related partially protandric hippolytid *Thor manningi* the two types of females are indistinguishable after embryo development has begun (Bauer 1986).

The question of whether the *tertia quae* were already secondary females is more complex, even if it seems certain that they were *en route* to becoming such. The ovotestis development of the protandric caridean *Pandalus platyceros* has been thoroughly investigated by Hoffman (1972). The sperm ducts in this shrimp are filled with spermatozoa through a process which sees the testicular gonadal epithelium lose its ability to form more spermatogonia. The transformation from male to female (in terms of primary sexual characteristics) then begins with the sperm ducts still filled with spermatozoa, during which time the transforming individuals are still functional males. It would appear, from Hoffman's descriptions, that at some point a few of the transmutating individuals become functional females (and no longer males) whilst still possessing male secondary sexual traits, but also that some individuals develop female primary characteristics whilst still being functional males (with the sperm ducts still containing sperm). No hint of bisexuality is suggested; at any given time the animals seem to function only as males or females. Assuming that the same system functions within secondary females of the related *N. marionis*, then what I have referred to as *tertia quae* may include both functional males and functional females (one of them gravid in the 1997 RMT-8 sample).

This study probably erred in emphasis on the side of female characters, using, as it has, only the presence of *mature* ovaries to define *tertia quae.* This strict criterion may have mitigated against *tertia quae* (as defined herein) as being male in any functional sense. One of them had already spawned in the May sample and so must surely be regarded as already female. If the April 1984 dredge and May 1997 RMT-8 samples can be regarded as representative of those months, it would seem that the *tertium quid* transformation into secondary females is almost complete by May. By this time only a nominal presence was recorded and at least one *tertium quid* was already a secondary female which retained remnants of its male history.

The incorporation of fictitious '*tertia quae* without ovaries or eggs' data in the tests of association between gender and ovary development when *tertia quae* were defined as being ovigerous or gravid meant a greater likelihood of a difference between observed and expected frequencies being calculated. The opposite deduction for these tests i.e. the acceptance of H_o: *no difference between the ovary development of the different potentially ovigerous genders* should therefore not have been affected by this decision. However, one must bear in mind the other *a priori* concerns over these tests mentioned in section 4.3.2. I suggest that future studies compare the ovary development of the three ovigerous genders using sample sizes which include larger numbers of *tertia quae* and ovigerous 'juveniles'. This would allow for a more accurate comparison between them than was possible with the samples used in this study. These samples should ideally have been taken at different times of the year, and the comparisons may help isolate at what time of the year ovigerous 'juveniles' and *tertia quae* may be regarded as female. Nonetheless, in this study ovigerous 'juveniles' and *tertia quae* could not be statistically separated from females by size or ovary development and were statistically larger than males. This would not be expected from early-stage transitional specimens. It seems, then, that both *tertia quae* and ovigerous 'juveniles' are well advanced in their development towards becoming female by April/May, and may therefore profitably be regarded as having achieved that goal.

For part of the year at least, then, some primary females can be recognized by the presence of the ail, absence of the am and presence of ovaries. Some secondary females can be recognized for part of the year by the presence of the am as well as eggs or mature ovaries. At some point, not necessarily related to embryo development, no way of differentiating between primary and secondary females is obvious, although the vast majority of such females are almost certainly secondary, judging from the numbers of *tertia quae* and males *vis-à-vis* 'juveniles' (ovigerous and non-ovigerous) in the 1984 dredge sample.

Non-ovigerous specimens (undeniably female) were sampled in the 1984 dredge and 1997 RMT-8 samples, although whether primary or secondary was unclear. The non-ovigerous 'juveniles' in the 1984 dredge sample could therefore possibly be regarded as either non-ovigerous (probably immature) primary females or as large juveniles *en route* to becoming primary females. The difference in definition with regard to that sample is probably needlessly pedantic. A problem could arise, though, when one has to differentiate between juveniles *en route* to becoming males, and those *en route* to becoming primary females, a problem not faced in this study, because the size of the juveniles in the 1984 dredge sample almost certainly suggested that those individuals were already committed to becoming primary females. The only other sample in which juveniles were sampled was the 1997 RMT-1*6 planktonic sample, and no attempt was made there to differentiate between juveniles destined to become primary females and those destined for the secondary route. No suggestion is offered herein on how to solve this problem, if indeed there needs to be one.

Figure 4.4 suggests that at carapace lengths of about ~3.1mm many of the 1997 planktonic specimens had been committed to the growth of an am, indicating that sexual differentiation takes place before individuals settle amongst the benthos. There were, however, relatively larger specimens with relatively small am sampled from the plankton, suggesting that individuals began developing am over almost the entire size-range of the 1997 RMT-1*6 sample. The largest individual taken in this sample, however, had not developed an am, indicating that it (and possibly others) was destined to develop into a female directly without passing through an intermediate male stage. Whether individual *N. marionis*' life-history is determined before the planktonic juvenile phase or not is unknown, but the highlighted data in Figure 4.4 do indicate that the am only begins growing *after* the larvae have differentiated into this phase. Whether some males exist which only begin developing am after having settled onto the benthos is unknown. The carapace length at which the am begins growing was hard to pin-point and may well also differ between years and locality.

The superimposition of the 1997 RMT-1*6 planktonic sample data onto the 1984 dredge data in Figure 4.4 seems to dispel the possibility that the juveniles recorded in the 1984 dredge sample had previously been males, although the carapace lengths recorded in the planktonic sample were larger than one might have expected. One may have suspected such a scenario from the fact that all juveniles possessed the male copulatory ai1. However, this always seemed unlikely for *N. marionis* because it would have involved males undergoing a sex-change at a very small size (about the average carapace length of the first generation, see Table 3.1A). Rather than indicating a male-phase past, the presence of the ai1 in the smallest of the juveniles observed in the 1997 RMT-1*6 planktonic sample would be better regarded as indicating that all *N. marionis* have the *potential* to become male (and ultimately secondary female).

The innate ability of female malacostracans to differentiate into males has already been reported (Hoffman 1972, citing the experiments of Berreur-Bonnefant 1963 and Charniaux-Cotton 1959). According to Hoffman's hypothesis, protandric male malacostracans probably originally evolved from a line of females which "autodifferentiated" into males at a small size, the original stock being gonochoristic. One may therefore envisage small *N. marionis en route* to becoming female, but with the innate potential to differentiate into males, which most of them do.

What causes most individuals to develop into males may be due to genetic, environmental and/or social factors. There are indications that the numbers of *N. marionis* may fluctuate greatly from year to year (see previous chapter), possibly indicating unpredictable environmental conditions. The numbers of primary female *Pandalus borealis* (Caridea: Pandalidae) increase as a proportion of the total population when temperature rises (Allen 1959, cited in Allen 1966: 57). Environmental factors (possibly interacting with genetic or other factors) may therefore determine the sex-change strategy in some carideans. If this is true for *N. marionis*, then the numbers of primary females in this species may vary under different environmental conditions. One may therefore find different emphases placed on the two different sex change strategies in *N. marionis* from year to year due to changing environmental (and/or other) circumstances, although Bauer (1986) suggests that amongst carideans displaying this type of protandry, primary females never make up more than 50% of the total female population, presumably irrespective of environmental conditions.

Changing climate and sea surface temperatures around Marion Island over the past three decades have been reported by Smith (1991). This suggests that *N. marionis*' present sex-reversal pattern at Marion Island is being affected. The interdisciplinary project referred to by Smith, which is investigating the effects of climate change on the biology and ecology of the Prince Edward Islands, may perhaps fruitfully consider possible effects that such change may have on the life-history of *N. marionis*. Such a study may be imperative, given the seemingly important rôle that *N. marionis* plays in making planktonic primary production available to top predators on the islands. It is worth recalling here that the sex-change route for individual *N. marionis*' may be determined in the plankton i.e. during a time of any individual *N. marionis*' life-history when it is relatively more exposed to changes in sea surface temperatures.

Assuming that the trends seen in *P. borealis* apply to *N. marionis*, one would expect that more recent, warmer temperatures would affect the percentage occurrence of different sex-reversal strategies within the species, resulting in *more*, rather than fewer, primary females (including ovigerous 'juveniles') in more recent samples. Precisely the opposite was seen in my samples. For the present, therefore, I shall suggest below that the absence of 'juveniles' (or developing primary females) in May 1997 *vis-à-vis* April 1984 was due to the time, rather than the average temperature, of that year.

4.5.2.1 THE PRIMARY ROUTE:

The emergence of ovigerous 'juveniles' seems to begin at about the same size that a small number of *tertia quae* begin appearing. The transformation of 'juveniles' to female seems complete by May, there having been a complete lack of 'juveniles' (whether ovigerous or not) in the 1997 RMT-8 sample. Only a minority of individuals follow this route.

Compared to the majority of secondary females, primary females seem to develop mature ovaries at relatively small carapace lengths. This may be because of a lower growth rate in primary females which need to direct energy towards ovary development at a smaller size. Another contributing factor may be that a delay in developing primary female characteristics is a better reproductive strategy for most males of comparable size.

The number of primary females in the population would appear to be very low, given the numbers of 'juveniles' with ovaries and large juveniles in the April 1984 dredge sample. This suggests that the numbers of primary females may be regarded as insignificant for analyses of age groups as done in the previous chapter. The fact that the mean size at which sexual differentiation occurred in the 1984 dredge sample (the third cohort in Table 3.1A) coincided with the size at which secondary females (called *tertia quae*) emerged in Figures 4.3 & 4.4 certainly supports such an interpretation.

One would have expected a decline in ail size in individuals *en route* to becoming females, which do not have this structure. This was not supported by Figure 4.6. One explanation for the absence of primary females with an ail in May may be that this structure (for which they would appear to have no use once they are committed to the primary route) is lost in one moult, rather than atrophying over a period of time. Spawning in female dioecious hippolytid *Heptacarpus pictus* is preceded by a moult, the females being attractive to males for only a few days following this (Bauer 1976). An association between the smaller, highly mature primary females in April which possess an ail and the smaller gravid females in May may be made by hypothesizing a pre-spawning moult of primary females which takes place in late April/early May. A relatively recent spawning was indicated for the May 1997 gravid specimens by undeveloped embryos carried by those specimens, and perhaps the data in Table 4.2 could support or help refute this hypothesis when more egg data is gathered. Such an hypothesis may explain why the proportions of 'ovigerous 'juveniles'' in April and gravid females in May were so similar, and the fact that no 'juveniles' were taken in May 1997. Furthermore, such a strategy could account for the fact that some gravid females from the *May* 1997 RMT-8 sample were inexplicably small in carapace length if one expects that gravid females should on average be larger than non-gravid females. The whole process of protandry implies that reproductive success as a female is some function of size, perhaps synergistic with other factors.

I therefore tentatively propose the hypothesis that a few relatively small primary females are capable of spawning during late April or May when the bulk of similarly-aged females are not (i.e. the majority of secondary females of the same carapace length).

A colleague's sample of *N. marionis* found in the gut contents of Gentoo penguins (*Pygoscelis papua*) in September 1982 to 1984, very late March to July 1984, and October to November 1984, was briefly examined during the closing days of this thesis (although they were not counted or measured). It was noted that only one gravid specimen was present, *and as with the gravid specimens recovered for this thesis, it was taken in the month of May.* Gravid females would surely have been taken by the penguins *if* they were accessible, given that they would have been larger. This is compatible with my hypothesis insofar as it suggests that in May 1984, as well as May 1997, there were few gravid females.

It is unlikely that hatching times differ greatly between broods from primary and secondary females, because of the highly seasonal climate. If my hypothesis is true, then the first brood produced by the primary females may hatch slightly earlier and/or at a comparatively advanced stage of development than those from secondary females (having had longer to develop inside the egg, even if more slowly). The latter possibility may be regarded as a more k-selected strategy, perhaps one which compensates for the inability to carry the number of eggs which larger females are able to due to their size. It could also perhaps compensate primary females to some extent for their inability to reproduce at the very small sizes that the vast majority of individuals do as males. It is even possible that while total lifetime reproductive success for the smaller primary females may be lower than that of animals following the secondary route, their reproductive success *as females* may be higher through this strategy. This is extremely speculative, but worthy of future investigation.

4.5.2.2 THE SECONDARY ROUTE:

The vast majority of juveniles follow this route. The majority of males in the 1984 dredge sample became secondary females at ~7mm, if one accepts that *tertia quae* are females. The decline of am lengths seemed relatively rapid compared to their growth once the (former) males had developed mature ovaries. From Table 3.1A, the mean carapace length at which sex-change occurred was also ~7mm, being the mid-point of the third cohort in the same sample (the cohort where sex-change was assumed to have occurred). Production of mature ovaries at about the third generation average carapace length may therefore yield optimal reproductive success for the majority of individuals already male. This size may differ between years (cf. the third generation mean sizes in the 1997 RMT-8 and 1984 dredge samples in Table 3, see also section 3.4.8).

There is very little evidence in Figure 4.3 that the transformation of any of the 1984 dredge sample males into secondary females began much *above* 7mm carapace length, although there were a few specimens that had begun the transformation *below* this size. All *tertia quae* possessed am of lengths less than that of the maximum recorded in a male of 7.17mm carapace length. Most *tertia quae* could easily be regarded as having begun the transformation at ~7mm. It is difficult to say this with certainty, however, because of the relative scatter of *tertia quae* data points in Figure 4.3. The bulk of *tertia quae* had carapace lengths above 7mm, however, indicating that the secondary females into which they were destined to transmutate would be larger.

As was seen with the protandric *Chorismus antarcticus*, intermediate individuals began manifesting themselves at carapace and am lengths more commensurate with males (Mascetti *et al.* 1997), although there were relatively few such individuals in the 1984 dredge sample. Apart from the fact that these are two totally different species, this was probably due, in part at least, to the fact that more accurate gender determinants (gonopore development) were examined by those authors. Some male *N. marionis*, therefore, begin sex-change earlier than the majority.

These few, small *tertia quae* in the April 1984 sample were of about the same carapace length as the smallest identifiable primary females in that sample. The gravid *tertium quid* from the May 1997 sample was certainly of secondary stock, and whilst two of the gravid females from the latter sample seemed to be primary (from their size), there was no indication whether the other gravid females were primary or secondary. The gravid *tertium quid* was also relatively small in size. This suggests that a minority of males may, therefore, undergo sex-change at about the same size as, or slightly larger than, the size at which the primary females mature, perhaps as a strategy designed to get the best of both worlds.

Unlike the case of primary females, no observations from this thesis' data support any hypothesis (tentative or otherwise) as to what time of the year the bulk of secondary females first spawn, although, given that primary females and early-maturing secondary females spawn in about May, it might be supposed that it would not be too much longer after this.

4.5.3 Parasitism:

Bopyrid isopods possibly castrate individual *N. marionis* on which they occur, although the recorded rate of infestation was not high in this study and no conclusions can be reached from the paucity of data and conservative identification. However, none of the parasitized *N. marionis* showed any sign of ovary development, and bopyrids have been shown to castrate other hippolytids (e.g. the dioecious hippolytid *Heptacarpus paludicola*, Bauer (1979)). Parasites may also affect growth rates (see Mathews *et al.* (1987: 159) for an interesting general commentary on bopyrid parasitism). If their presence on *N. marionis* inhibits the development of ovaries, then some of the six males may well have been better regarded as castrated *tertia quae*, and the one parasitized juvenile may also have been better classified as a castrated primary female. These individuals' data, given in section 4.4.2, may be compared to the statistics given in Appendix 1, but deductions from so few animals are not possible, except for the observation that parasitism does not appear to be gender-specific. Moulting can facilitate the loss of bopyrid parasites in shrimps (Mathews *et al.* 1987: 159). The fact that no parasitized individuals were found in the 1997 RMT-8 sample (the other two samples being very small) may therefore indicate a moult at about the end of April. Bopyrid parasitism of *N. marionis* is potentially an area of fruitful future research.

4.5.4 Egg volumes:

The egg volumes for the two female specimens in Table 4.1 are the only data on *N. marionis* egg volumes reported to date. The smaller female with fewer eggs may have lost eggs differentially, making the estimate of average egg volume for that animal inaccurate. The estimated average egg volume for the larger animal is probably fairly accurate, given the sample size and its calculated standard deviation. However, it was not compared to other gravid individuals, and so in itself constitutes only one datum point. The carapace lengths of these two animals do not hint at whether they may have been primary or secondary females. The egg volumes of these two types of females may be different, especially if relatively different selection strategies are employed by them. No further commentary on only two data points is offered.

4.6 Conclusions:

1) *N. marionis* is a partially protandric hermaphrodite. The vast majority of juveniles develop into males. Once having grown to a certain carapace length they transmutate into females. The size at which this begins for the majority is coincident with their third year, and may therefore differ between years. For a period, at least some of them retain their am after developing ovaries. By April/May the transmutation is probably complete. Reproduction can occur before all male secondary characteristics have been lost. A small minority of individuals develop directly into primary females without passing through a male phase. At least some ovigerous primary females can be initially recognized by the presence of ai1, male copulatory structures which *all* juvenile *N. marionis* possess. These primary females may lose their ai1 in one moult, possibly just before spawning, which may be in late April/early May. Too few

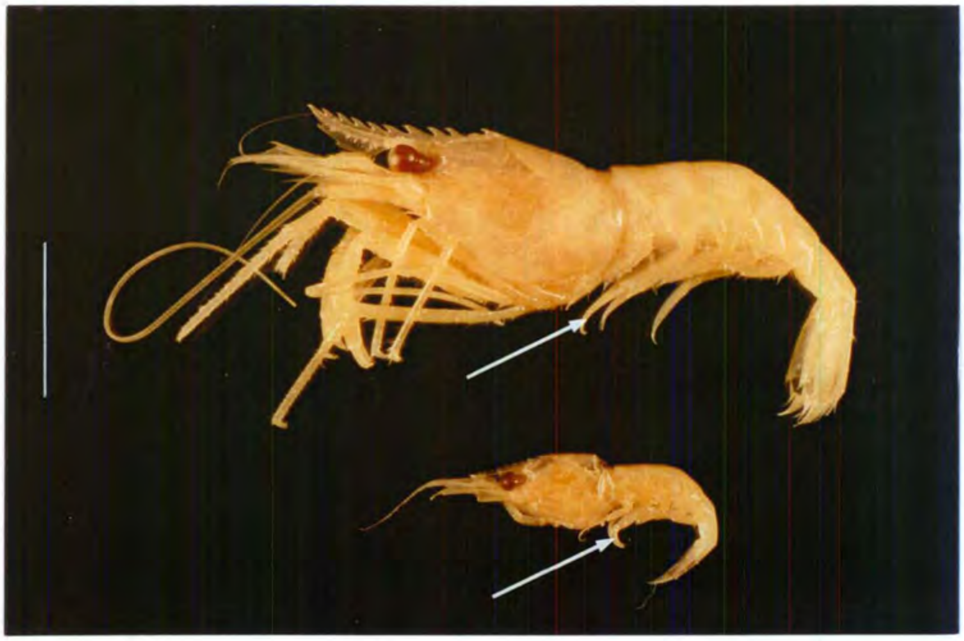
gravid females were recovered to make any statement on whether spawning can occur before the ail is lost. Primary females seem to become ovigerous at a slightly smaller carapace length than do the majority of secondary females. A few secondary females seem to begin developing ovaries at this small carapace length as well. Once a certain level of maturity has been reached, primary and secondary females are probably indistinguishable. More accurate gender definitions, possibly using gonopore, ovary and am setae development are required now that protandry has been established.

2) It would seem that *N. marionis* juveniles have the potential to differentiate into either males or females and that this separation is done in the plankton. It is possibly influenced by environmental factors like sea temperature. It is unknown whether individual *N. marionis* life-histories are determined at conception or in the larval or juvenile phases, but it seems that am are only developed after the juvenile phase has been attained, and this begins whilst they are planktonic. Whether the am of some males only begin growing after they have settled onto the benthos is unknown. Climate change, which has already been recorded at these islands, may affect *N. marionis*' life-history whilst it is still in a planktonic phase. As the species has been implicated as being quite important in the functioning of the Prince Edward Island ecosystem, this may be affecting the ecosystem as a whole. This aspect requires further study.

3) There are reproductive trade-offs involved in individuals becoming primary or secondary females. It needs to be established whether spawning for the first time occurs slightly earlier for primary females than for secondary females. Some circumstantial evidence for this is presented in this chapter and it is proposed as a tentative hypothesis. If this is found to be the case, the implications should be investigated. One possible implication might be that the total

reproductive success of primary females *as females* is higher than that of secondary females *as females*, although total *lifetime* reproductive success may be greater for secondary females.

4) Bopyridae are possibly implicated in the castration of individuals, although their rate of infestation does not appear to be high from the few data available. Moulting may possibly facilitate the loss of bopyrid parasites. The effects of parasitism on the reproduction and growth rates of *N. marionis* need to be examined in the future.



1

SEXUALLY DIMORPHIC SIZES IN N. MARIONIS:

1. *A female (top, CL = 11.33mm) and a male (bottom, CL = 4.58mm). Arrows indicate the positions of the pleopods. Scale bar = 10.50mm.*

Overleaf:

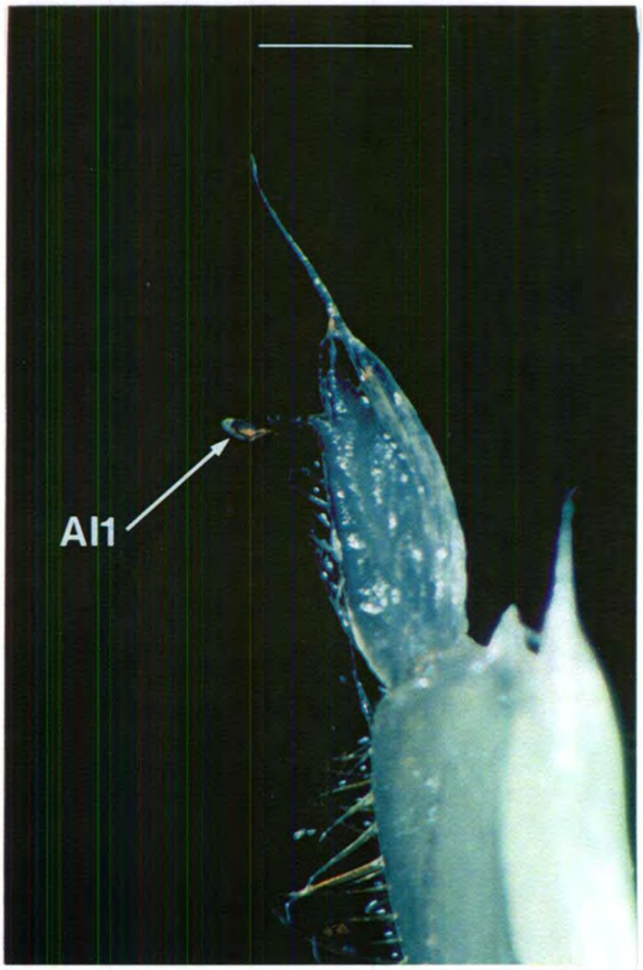
MALE COPULATORY ORGANS:

2. *An appendix interna on the first pleopod* (AI1), *from a tertium quid type a (CL = 7.50mm, type 2 ovary). Scale bar = 0.56mm.*

3. *An appendix masculina on the second pleopod* (AM), *from the same specimen as in* **2**. *Scale bar = 1.11mm.*

4. *An appendix interna on the first pleopod* (AI1), *from a tertium quid type a (CL = 6.25mm, type 1 ovary, ai1 = 0.38mm). Scale bar = 0.49mm.*

5. *An appendix masculina on the second pleopod* (AM), *from the same specimen as in* **4** *(am = 0.38mm). Scale bar = 0.69mm.*



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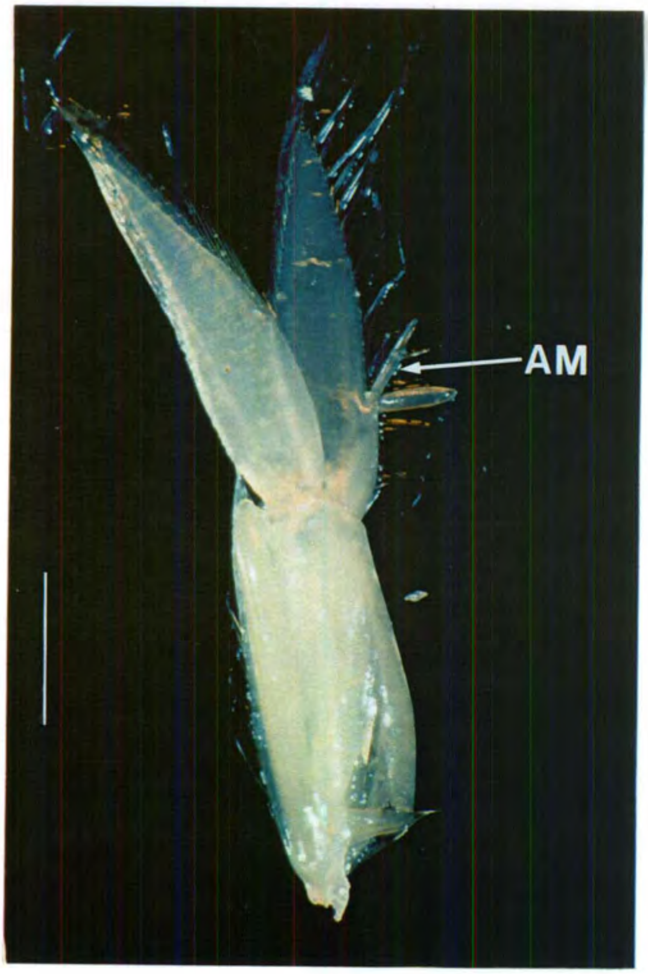

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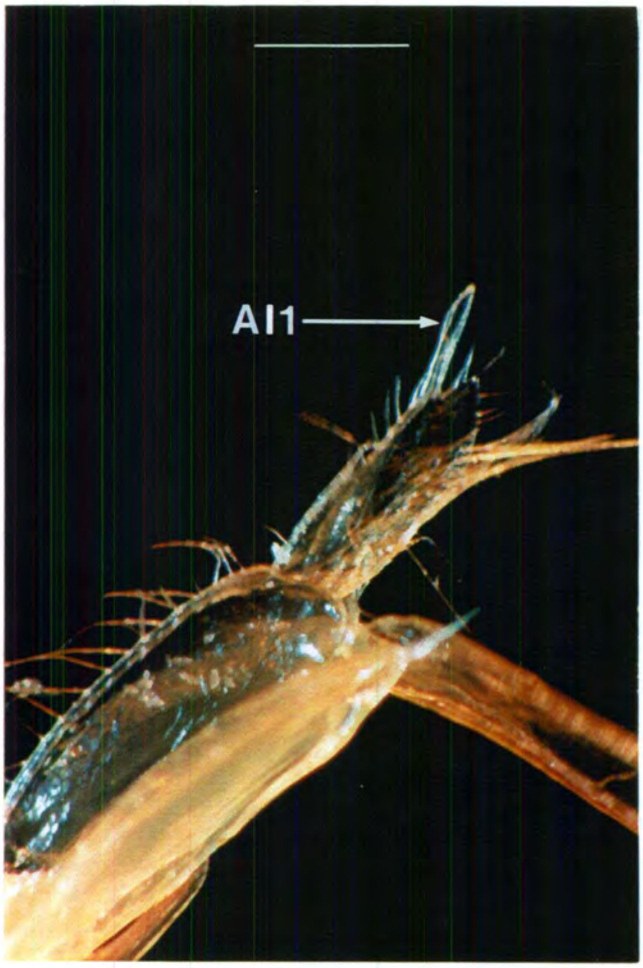

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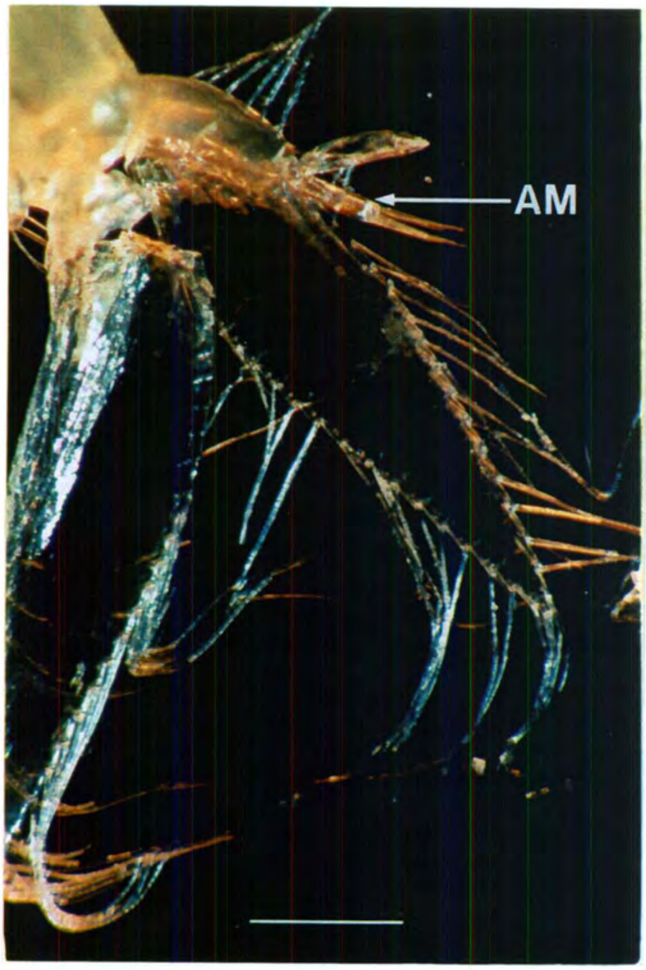

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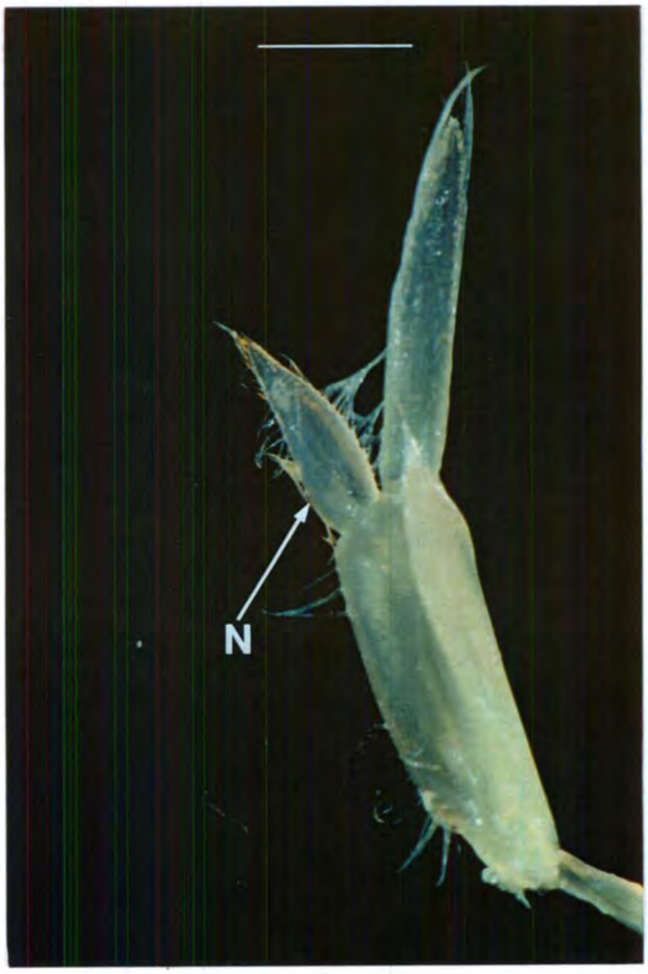

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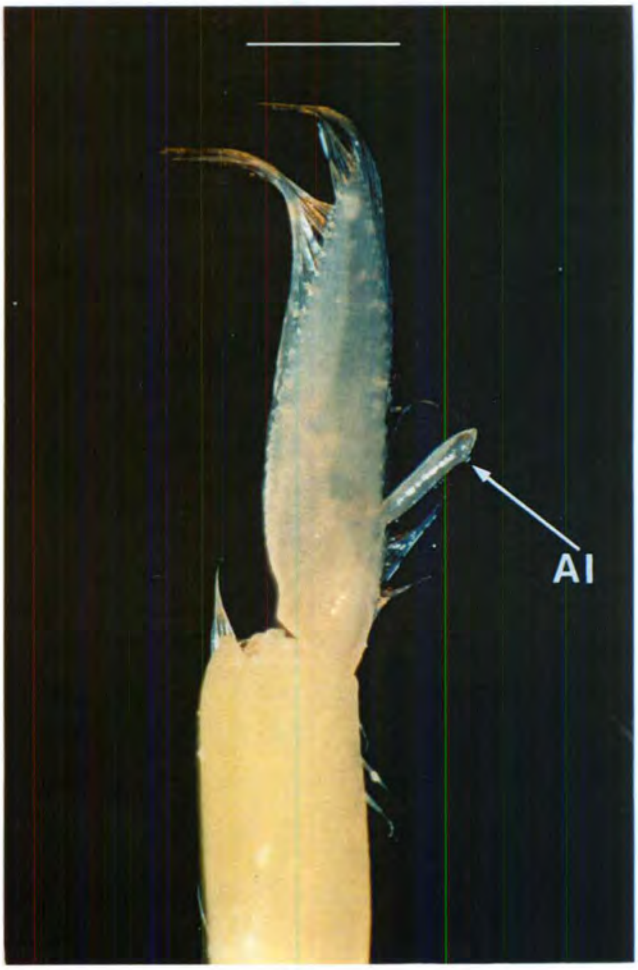


7

FEMALE ENDOPODS:

6. *First pleopod (female, CL = 10.17mm, type 3 ovary). Note the absence of an appendix interna on the endopod* (N). *Scale bar = 1.18mm.*

7. *Second endopod (female, CL = 11.33mm, type 3 ovary), showing an appendix interna* (AI) *and no am. Scale bar = 1.13mm.*

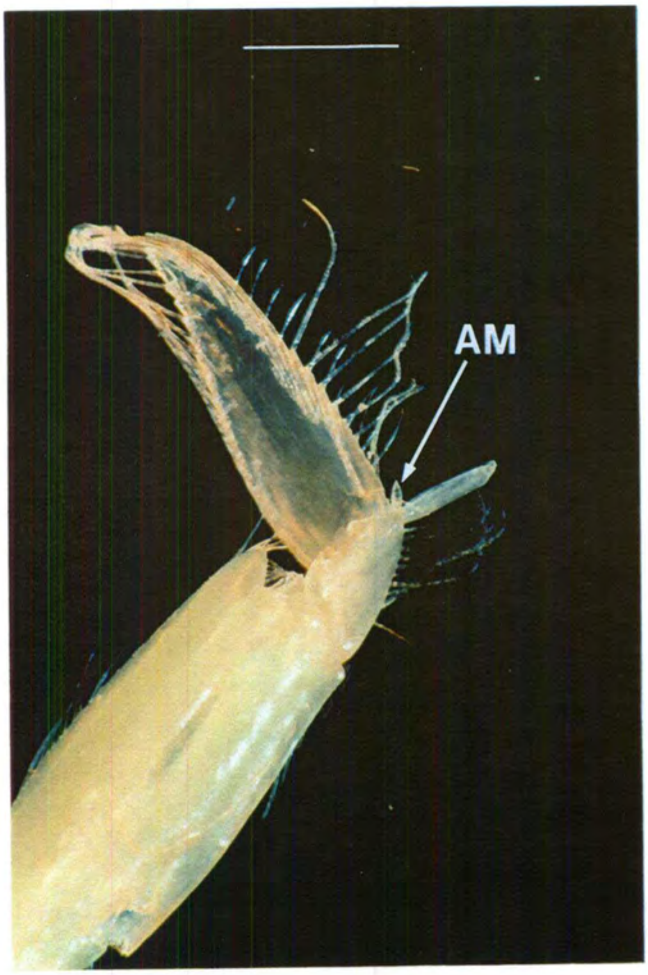
Overleaf:

TERTIA QUAE:

8. *A second endopod showing a rudimentary appendix masculina* (AM) *with an appendix interna (0.20mm and 0.74mm respectively, CL = 8.75mm, type 3 ovary, see Plate* **15***). Scale bar = 1.10mm.*

9. *The second endopod of the same specimen as in* **8**, *showing an appendix masculina* (AM). *Scale bar = 0.70mm.*

10. *The second pleopod of the only gravid tertium quid taken (CL = 6.42mm, no discernible ovary). Note the rudimentary appendix masculina* (AM) *as well as the eggs* (E) *which adhered to the endopod after it was torn from the abdomen. Scale bar = 1.43mm.*



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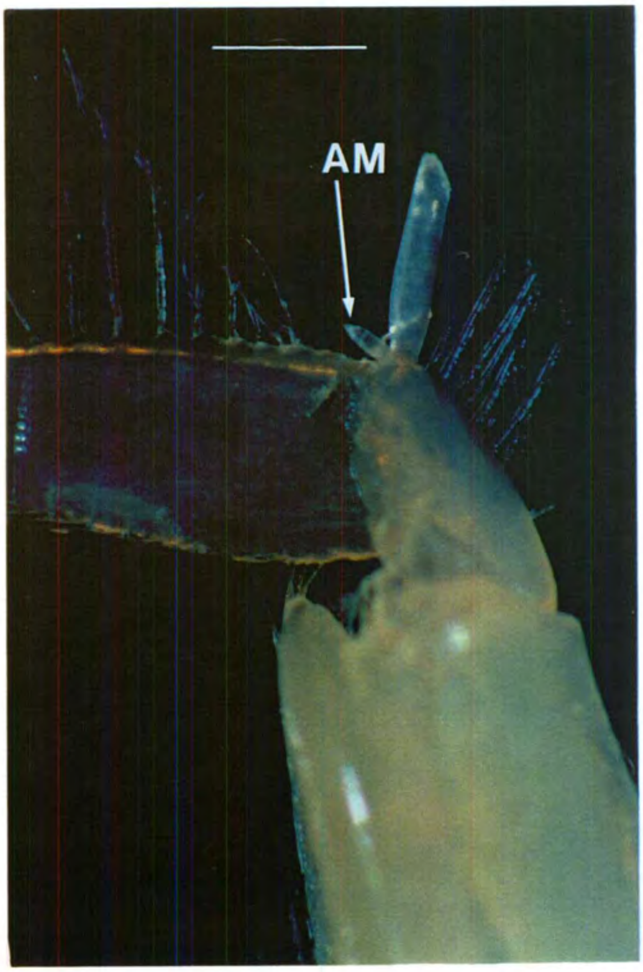

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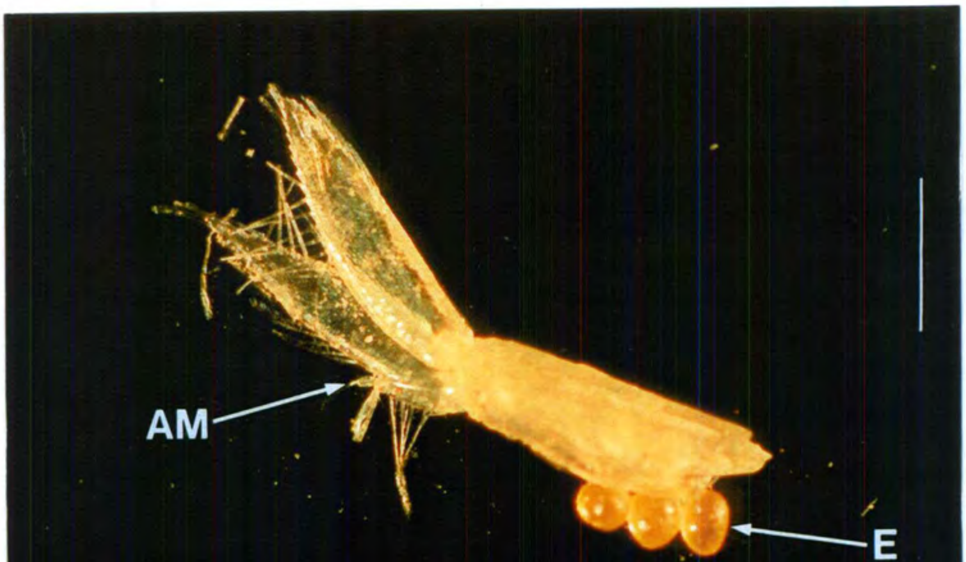

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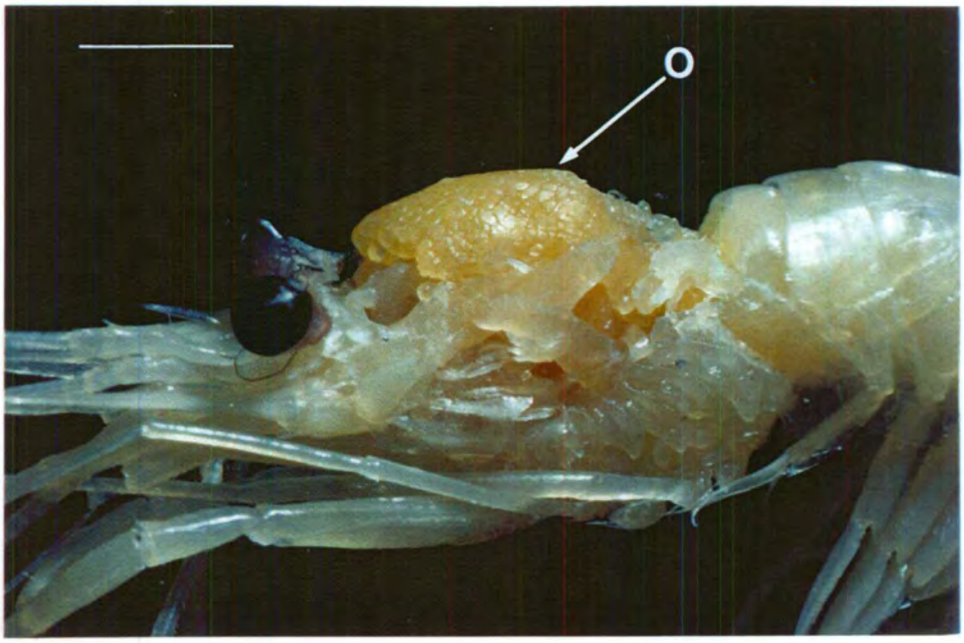

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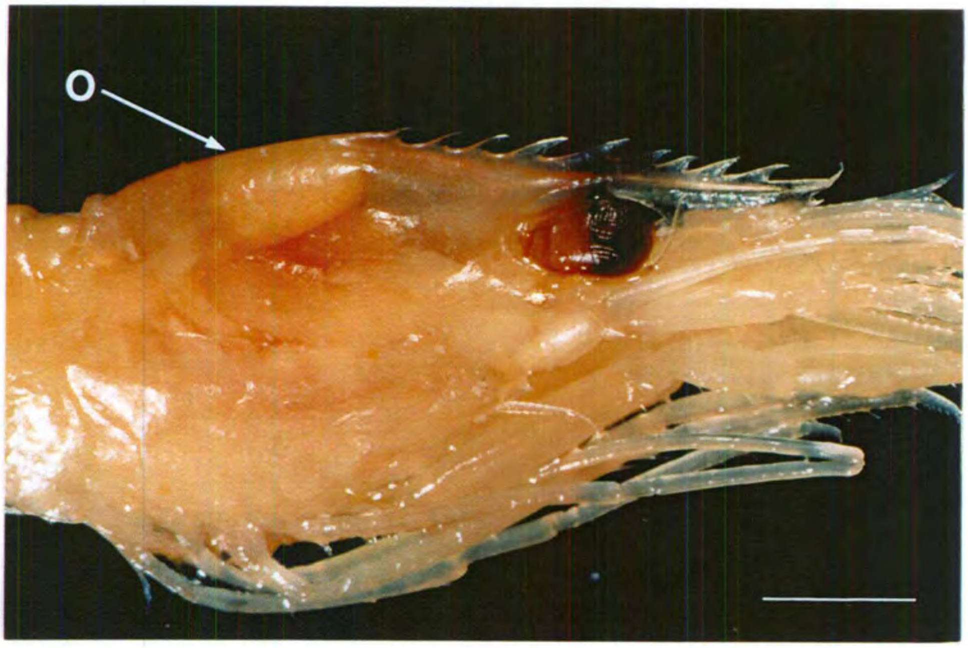
OVARIES:

11. *A female (CL = 7.13mm) with its carapace removed, showing a type 2 ovary* in situ (O). *Scale bar = 3.17mm.*

12. *The displaced type 2 ovary from the same specimen as* **11**. *Scale bar = 1.60mm.*



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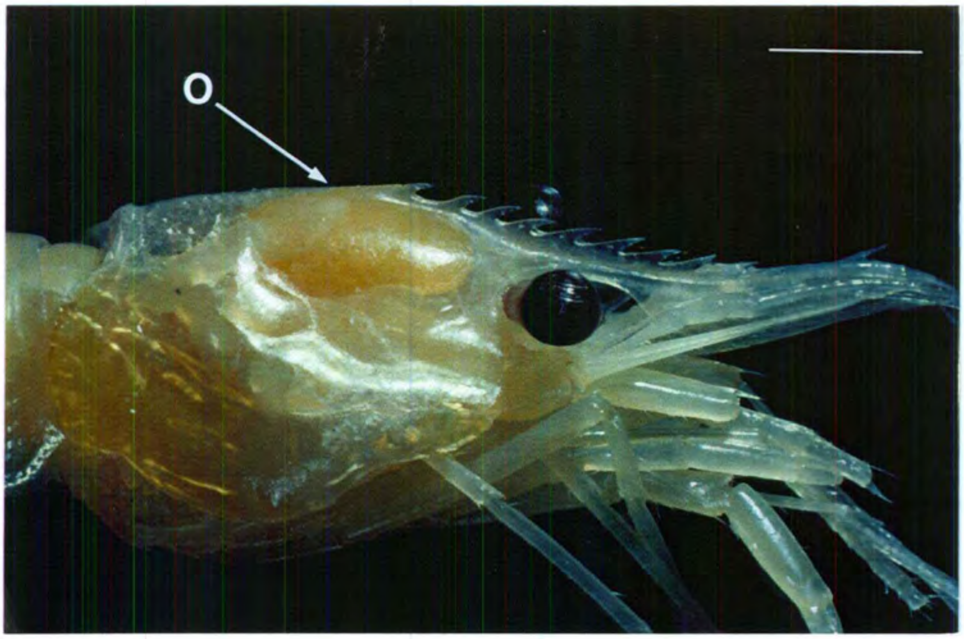

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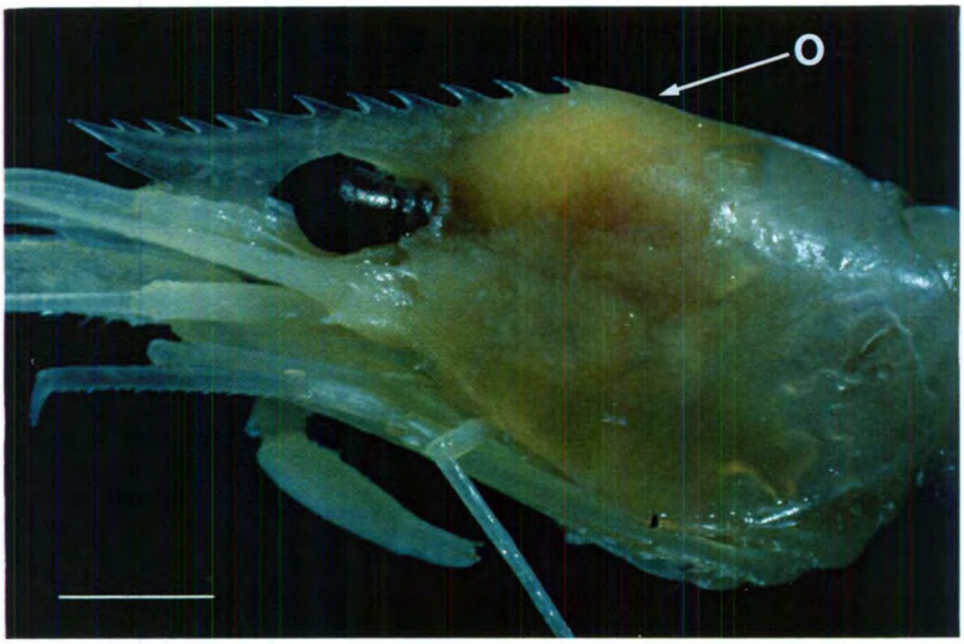
THE THREE TYPES OF OVARY RECORDED:

13. *A type 1 ovary* (O) *from a female; CL = 7.00mm. Scale bar = 2.50mm.*
14. *A type 2 ovary* (O) *from a female; CL = 7.13mm. Scale bar = 2.74mm.*
15. *A type 3 ovary* (O) *from a tertium quid; CL = 8.75mm (see Plates* **8** *&* **9***). Scale bar = 3.00mm.*



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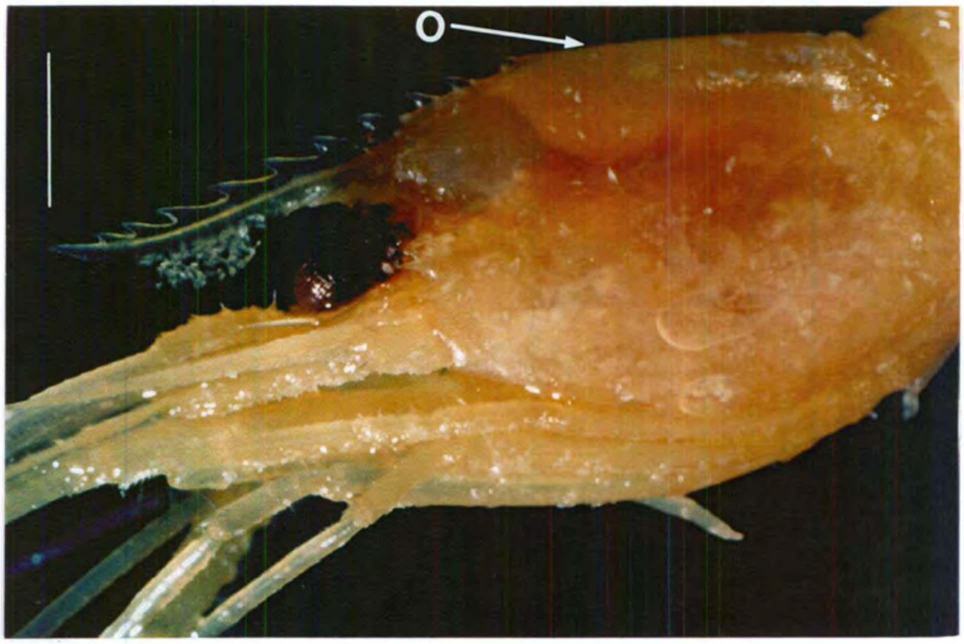

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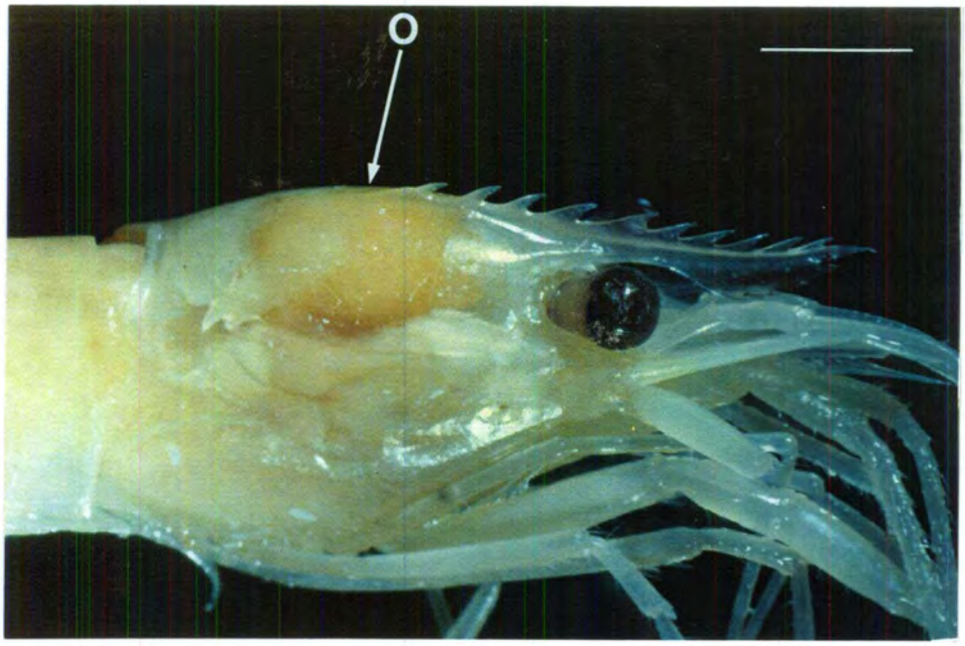

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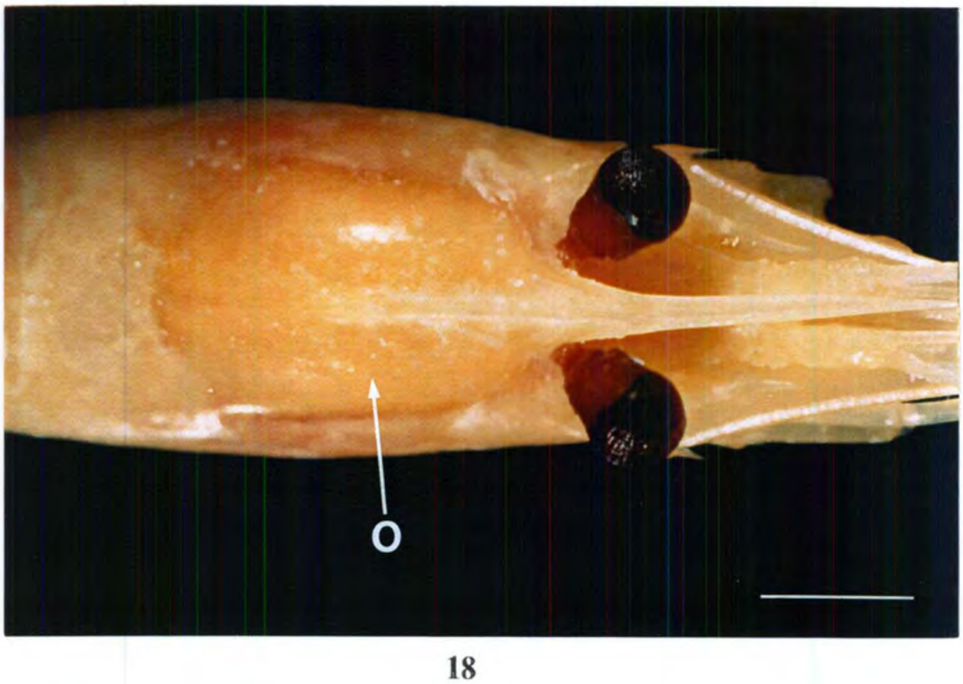
FURTHER EXAMPLES OF OVARY CLASSIFICATION:

16. *A female (CL = 7.88mm); note how the ovary* (O) *has developed just past the point of discrimination (the most posterior upper rostral tooth), causing it to be classified as type 2. Scale bar = 2.50mm.*

17. *A better developed ovary* (O) *than in* **16**, *but still of type 2 (tertium quid, CL = 7.50mm). Scale bar = 2.83mm.*

18. *The dorsal view of a female (CL = 10.17mm) showing a type 3 ovary* (O). *Scale bar = 2.74mm.*



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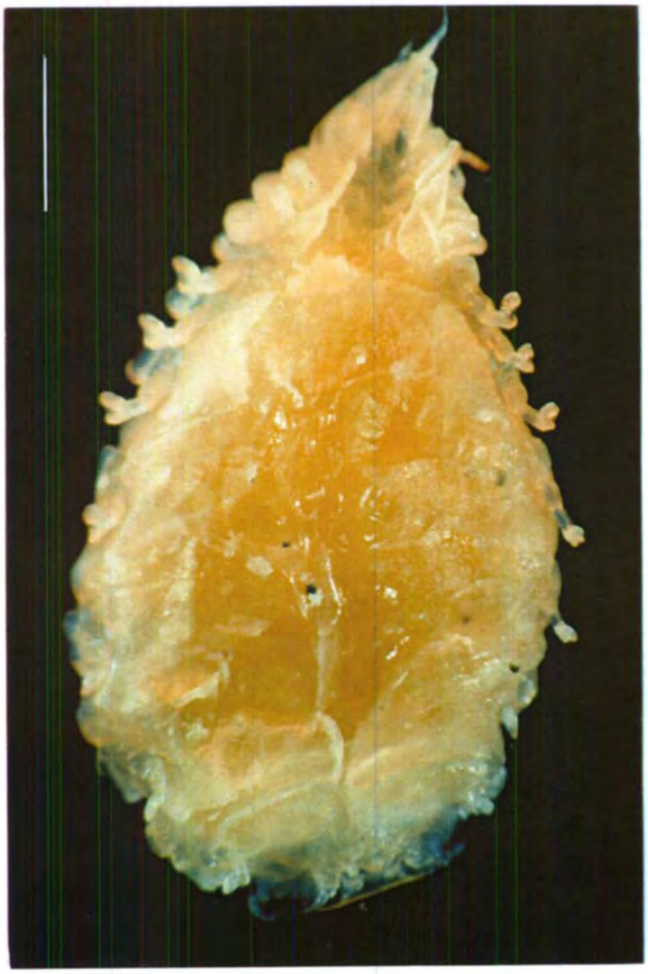

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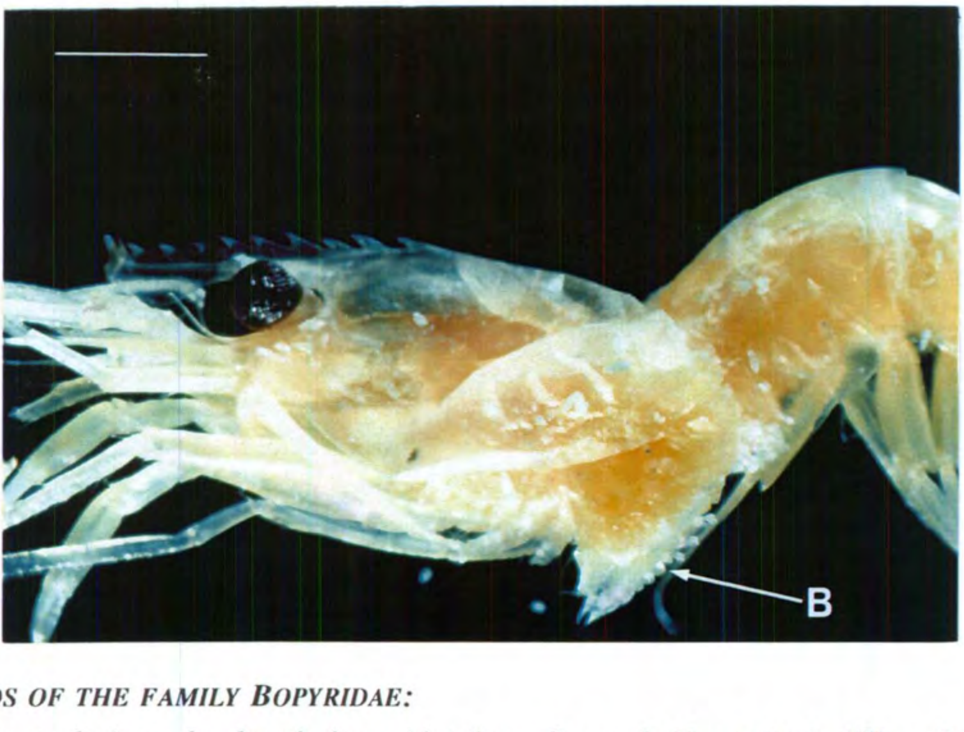
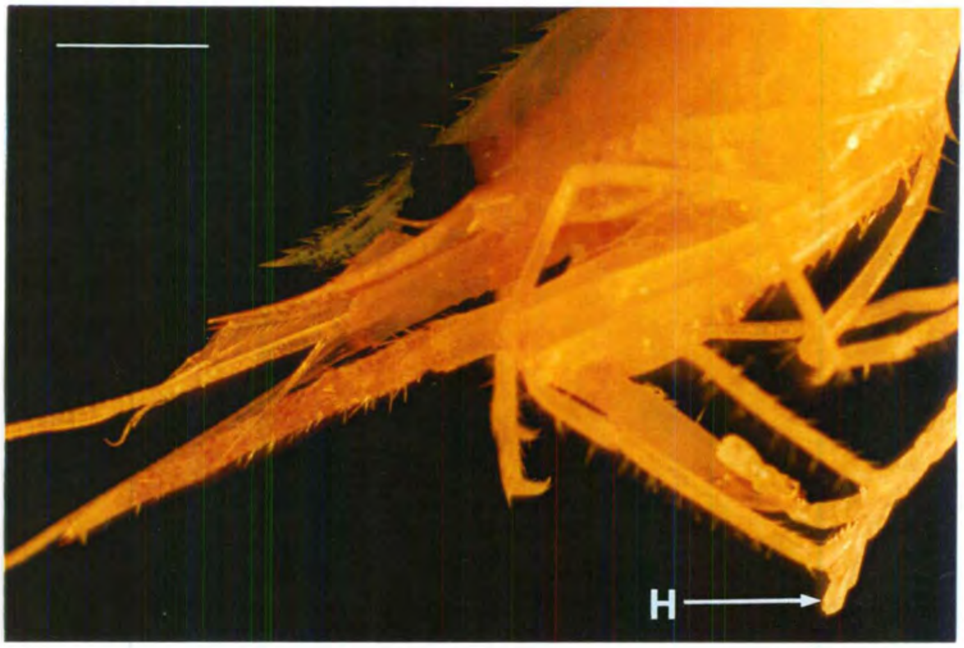


Isopods of the family Bopyridae:

19. *A ventral view of a female bopyrid, taken off a male N. marionis (CL = 5.50mm). See Plate* **21**. *Scale bar = 0.85mm.*

20. *A dorsal view of a female bopyrid, taken off a male N. marionis (CL = 4.63mm). Note the small, bilaterally symmetrical male (♂) attached to the female. Scale bar = 0.85mm.*

21. *A lateral view of a male N. marionis (CL = 5.50mm) showing the bopyrid* (B) *in Plate* **19** *partially withdrawn from under the carapace. Note the bulge of the carapace which formed around the isopod. Scale bar = 2.62mm.*



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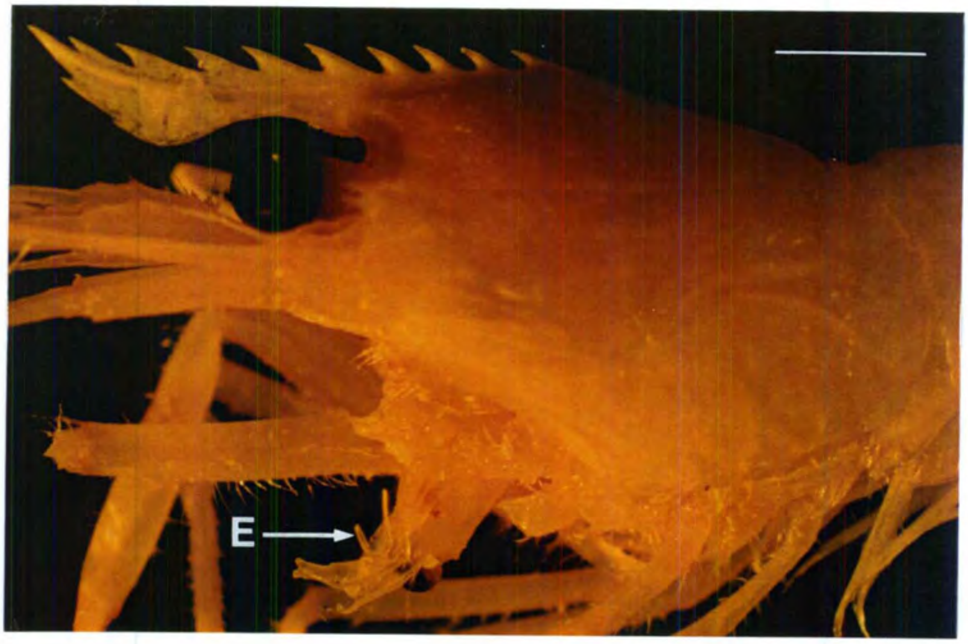

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THE ECLECTIC PREDATOR <u>N. MARIONIS</u>:

22. *A female (CL = 6.46mm) firmly clasping a hydrozoan* (H) *with its right pereiopod. Scale bar = 3.17mm.*

23. *A female (CL = 8.83mm) with a half ingested <u>Euphausia vallentini</u>* (E). *Left pereiopods removed for clarity. Scale bar = 3.17mm.*

Chapter 5

Feeding biology

"THE RELATION OF ANY SPECIES TO OTHERS IN ITS FOOD WEB IS AN IMPORTANT DIMENSION OF ITS ECOLOGICAL NICHE" (CURTIS 1986: 968).

5.1 Introduction:

Many studies have noted the often significant contribution of *N. marionis* to the diets of predators at the Prince Edward Islands (Adams & Brown 1989, Adams & Klages 1987, Blankley 1982, Blankley & Grindley 1985, La Cock *et al.* 1984, *inter alia*). However, little is known about its diet, which forms an integral part of its position within the food web at this locale.

Given the numbers of *N. marionis* at the archipelago, the prey biomass consumed by this hippolytid must be large. From this it has been inferred that *N. marionis* may be a key component in making primary production at the islands available to top predators (Perissinotto & McQuaid 1990), although this suggestion was conjectural. If it is true, then one would need to understand *N. marionis*' diet in order to describe that process. As in the Perissinotto & McQuaid study, this short chapter shall explore the position of *N. marionis* within the food web at the island group by examining the gut contents of selected individuals, and in so doing add to the knowledge of the feeding ecology of *N. marionis*. The methods used are qualitative, and the results should be used to facilitate future studies on the functional rôle of

N. marionis within the food web at the archipelago. Future studies can only achieve this by determining the strengths of the various trophic interactions (Paine 1980).

5.2 Materials and methods:

Subsamples were chosen *randomly* from samples 1 and 3 (the 1984 dredge and 1997 RMT-8 samples respectively, see section 1.3) in order to allow for unbiased data to be presented on the percentage of empty guts in each of the samples (n = 157 & 26 respectively). The entire 1996 RMT-8 sample (sample 2, section 1.3), being small (n=28), was also used for this purpose. Empty guts were also recorded for those specimens whose gut contents were insignificant.

After it became apparent that the inexperience of this researcher had led to misleading identifications of prey items in the random 1984 dredge subsample, a smaller, non-random subsample (n=23) was taken from that sample which included scarcer, larger individuals. Only specimens whose gut contents were visible through their carapace were chosen for the non-random 1984 dredge subsample. Data from the *random* 1984 dredge subsample were retained only to calculate the ratio of empty and full guts in this sample, and also to list selected prey items, the identification of which I was sure about.

Prey items were identified to the lowest possible taxon in the non-random 1984 dredge and random 1996 and 1997 RMT-8 samples using a dissecting microscope at 60X to 500X magnification. The percentage volume contribution of each prey item to the total gut content of each specimen was also visually estimated to a precision of ±5%. Identification of prey items and estimates of their percentage volume contribution to the gut contents were verified by another (experienced) researcher. Prey items that were sparsely represented were recorded as making up 5% of those gut's volumes.

Specimens from all the samples were divided into 2.50mm carapace length classes because *N. marionis* diet may be determined by its size (Perissinotto & McQuaid 1990). The proportion of empty to full stomachs in each size class was calculated for the random 1984 dredge, 1996 RMT-8 and 1997 RMT-8 samples. The *total* average percentage of empty stomachs for each of those random samples was also calculated, which corresponds to the "emptiness index" or "EMI" of Cortez *et al.* (1995).

Gut contents were divided into five overall categories i.e. pelagic prey items, benthic prey items, detritus, unclassified prey items and stones. Detritus was recorded for all unidentifiable, amorphous biomass. Prey for which it could not be said were taken benthically or pelagically were recorded as 'unclassified'. In particular, foraminiferan shells (mainly pelagic forms, the shells of which could have sunk to the benthos) were categorized as 'unclassified'.

Because the data from the random 1984 dredge sample were imprecise, only prey items recovered from the non-random 1984 dredge and the random 1996 and 1997 RMT-8 samples were arranged into the abovementioned gut content categories. Within those categories they were then arranged into a taxonomic hierarchy, with individual prey items forming the lowest level within the hierarchy.

The mean percentage composition (MPC) of each of the five categories was calculated for each size class in each sample. This was done by taking the average percentage composition by volume of the combined prey items that fell under each category. Size classes were then disregarded, and the total MPC for each of the five main gut content categories for each of the relevant samples was also calculated by the same method. The MPCs of lower hierarchical levels were calculated separately by taking the average percentage composition by volume of all the prey items from those levels. MPCs of lower hierarchical levels were not calculated for the different size classes. The MPC was regarded as an estimate of the "importance in weight index" or "IWI" of Cortez *et al.* (1995).

The occurrence index (OCI) was calculated for each level as the percentage of *N. marionis* guts in which that level's presence was recorded, and is a term used in the same sense by Cortez *et al.* (1995). Size classes were not used to calculate the OCI.

All specimens from the relevant samples which did not have empty guts were used to calculate MPC and OCI. These included those whose guts were full, but did not contain prey from that level.

5.3 Results:

The EMIs for the random 1984 dredge, 1996 RMT-8 and 1997 RMT-8 samples were 28%, 43% and 23% respectively. Most specimens therefore had food in their guts, although a high proportion appeared not to have fed shortly before capture, especially those from the pelagic, nighttime 1996 RMT-8 sample.

Overall, more detritus was observed in the full guts taken from non-randomly sampled 1984 dredge specimens than those taken from the 1996 or 1997 RMT-8 samples (MPC = 51.2%, 26.6% & 17.8% respectively, Table 5.1). Benthic prey items, however, dominated overall by presence, and, after detritus, by volume in the 1984 dredge sample (OCI = 82.6% & MPC = 37.2%, Table 5.1). Generally, benthic prey contributed more towards the gut contents of larger specimens from this sample (Figure 5.2A). In the 1984 dredge sample benthic prey exceeded the total contribution by volume of all other 'food classes' together (including detritus) in specimens >7.50mm carapace length. Pelagic prey items from this sample were confined to size classes <7.50mm carapace length, although most of the gut content recovered from specimens below this size was detritus (Figure 5.2A).

N. marionis specimens from all size classes from the 1996 RMT-8 sample had consumed pelagic prey (Figure 5.2B). Overall, most of the prey volume from this sample was pelagic (MPC = 55%, Table 5.1). Pelagic prey also occurred more often than any other food category in the 1996 RMT-8 sample except for unclassified prey (OCI = 68.7%, Table 5.1). The unclassified prey was made up primarily of trace volumes of foraminiferans, many of which were no doubt pelagic as well. The two size classes from which pelagic prey items were recovered in 1984 coincided with the two size classes in which pelagic prey items were dominant in the 1996 RMT-8 sample (carapace length class mid-points 3.75mm and 6.25mm, cf. Figures 5.2A & 5.2B). Proportionately less pelagic biomass (by volume) was recovered from these two smaller size classes in the 1984 dredge sample than from the equivalent-sized 1996 RMT-8 sample size classes. However, as seen in the 1984 dredge sample, specimens >7.50mm carapace length from the 1996 RMT-8 sample had consumed more benthic prey (by volume) than any other food category. Only two *N. marionis* specimens of >7.50mm carapace length were, however, available from the 1996 RMT-8 sample for this comparison (Figure 5.2B).

Analogous to the 1996 RMT-8 sample, guts from all size classes represented in the 1997 RMT-8 sample contained pelagic prey items (Figure 5.2C). In the stomach of the single specimen from the largest size class in the 1997 RMT-8 sample only benthic and pelagic prey were found. The pelagic prey component was represented by a single *Thysanöessa* that could have been taken during capture. However, three of the six specimens >7.50mm recovered from this sample had consumed *Thysanöessa*. The other size classes were dominated by benthic prey, while *N. marionis* specimens <5.00mm carapace length were unrepresented. Overall, benthic prey dominated by volume and occurrence in the guts from this sample (MPC = 68.8% & OCI = 90%, Table 5.1), more so than in the benthic 1984 dredge sample.

Common prey items from all the samples (apart from the imprecisely analyzed *random* 1984 dredge sample) were thecostomatans, euphausiids (the dominant biomass volume in the 1996 RMT-8 sample, MPC = 46.6%), hydrozoans (the dominant biomass volume in the 1997 RMT-8 sample, MPC = 60.5%), tube-dwelling polychaetes, and foraminiferans. Foraminiferans were present more often in guts from the 1996 RMT-8 and *non-randomly* chosen 1984 dredge sample specimens than any other prey item observed (OCI = 73.9% and 93.8% respectively), but they contributed only insubstantially to the total volume of biomass consumed in all three samples (MPC ≤ 5% in all cases, Table 5.1). Of these five taxa, hydrozoans and polychaetes have not previously been identified as *N. marionis* prey (see Perissinotto & McQuaid 1990). Other *N. marionis* prey items discovered in one or more of the sample guts which were not recorded by Perissinotto & McQuaid (1990) were radiolarians, small bivalve shells, poriferans, brachyurans, *Apseudes sp.*, and, quite interestingly, *N. marionis* (Table 5.1).

From the imprecisely analyzed *random* 1984 dredge sample, *N. marionis* was again positively identified as a prey item in another stomach. Cannibalism was therefore rare, with only two *N. marionis* recovered from the guts of two females taken in the two 1984 dredge subsamples. Both cannibals had carapace lengths of 9.83mm, and from the remains in their stomachs, both prey individuals' carapace lengths were independently and tentatively estimated at 1.80mm by extrapolating the equations from Chapter 1.

Brachyura, some of which were surprisingly large and fairly intact, were again found a number of times in the gut contents from the random 1984 dredge sample. There was one example of a fairly large fragment of a bird feather in a female *N. marionis* gut from this sample. One case was noted from the 1997 RMT-8 sample of a large *N. marionis* specimen firmly clutching an arm of an *Octopus sp.* with its left pereiopod. The capture may have occurred during sampling, but is recorded here as large *N. marionis* and *Octopus* are both found amongst the benthos.

Gammarids were not found in any 1996 or 1997 RMT-8 sample guts. These amphipods were only recovered from three male guts from the non-random 1984 dredge sample (CL = 3.42mm, 6.33mm & 6.83mm). The only specimen from which an euphausiid was recovered in the non-random 1984 dredge sample was male (CL = 3.00mm). Euphausiids were also recovered from female *N. marionis* guts in the 1997 RMT-8 sample (mean CL = 8.38mm, standard deviation = 2.16mm, range = 6.83mm to 12.17mm, n = 5). Both male and female *N. marionis* had consumed euphausiids in the 1996 RMT-8 sample (female euphausiid predators: mean CL = 6.78mm, standard deviation = 0.81mm, range = 6.25mm to 8.21mm, n = 5; male euphausiid predators: CL = 4.54mm, 4.75mm, 5.08mm & 5.17mm).

Overleaf:

Table 5.1: Prey items recovered from the non-random 1984 dredge and random 1996 and 1997 RMT-8 samples, arranged into food classes (see text). The MPC and OCI for each hierarchical level are shown.

	1984 dredge, n = 23		1996 RMT-8, n = 16		1997 RMT-8, n = 20	
FOOD ITEMS	MPC	OCI	MPC	OCI	MPC	OCI
PELAGIC PREY (TOTAL)	1.09	13.04	55.00	68.75	9.50	30.00
Chaetognatha	0.22	4.35	0.94	12.50	0.00	0.00
Thecostomata	0.43	4.35	0.94	18.75	0.50	5.00
Euphausiacea (total)	0.43	4.35	46.56	56.25	9.00	25.00
Thysanöessa (Euphausiacea)	0.00	0.00	33.75	37.50	7.00	20.00
Unidentified euphausiacea	0.43	4.35	12.81	18.75	2.00	5.00
Radiolaria	0.00	0.00	0.31	6.25	0.00	0.00
Copepoda (total)	0.00	0.00	6.25	12.50	0.00	0.00
Unidentified copepoda	0.00	0.00	5.94	6.25	0.00	0.00
Oncaea (Cyclopoida)	0.00	0.00	0.31	6.25	0.00	0.00
BENTHIC PREY (TOTAL)	37.17	82.61	12.50	43.75	68.75	90.00
Cnidaria (total)	4.78	39.13	1.88	25.00	63.50	85.00
Hydrozoa	4.78	39.13	1.88	25.00	60.50	85.00
Madreporaria (Anthozoa)	0.00	0.00	0.00	0.00	3.00	5.00
Bryozoa	3.04	17.39	0.00	0.00	0.00	0.00
Gastropoda (total)	2.39	8.70	1.88	12.50	0.00	0.00
Bivalvia	2.39	8.70	0.63	6.25	0.00	0.00
Unidentified gastropoda	0.00	0.00	1.25	6.25	0.00	0.00
Porifera	1.30	4.35	0.00	0.00	0.00	0.00
Polychaeta (total)	14.78	30.43	8.13	12.50	5.25	10.00
Tube-dwelling polychaeta	5.65	8.70	8.13	12.50	5.25	10.00
Unidentified polychaeta	9.13	21.74	0.00	0.00	0.00	0.00
Ostracoda	0.00	0.00	0.63	12.50	0.00	0.00
Crustacea (total)	10.87	39.13	0.00	0.00	0.00	0.00
Brachyura	0.22	4.35	0.00	0.00	0.00	0.00
Unidentified decapoda	0.43	4.35	0.00	0.00	0.00	0.00
Apseudes (Tanaidacea)	3.26	4.35	0.00	0.00	0.00	0.00
Unidentified crustacea	2.61	13.04	0.00	0.00	0.00	0.00
Gammaridae (Amphipoda)	4.35	13.04	0.00	0.00	0.00	0.00
UNCLASSIFIED PREY (TOTAL)	10.57	78.26	5.94	93.75	3.50	60.00
Foraminifera	4.48	73.91	5.00	93.75	3.50	60.00
Crustacea (total)	3.48	8.70	0.94	12.50	0.00	0.00
Unidentified crustacea	0.87	4.35	0.94	12.50	0.00	0.00
Nauticaris marionis	2.61	4.35	0.00	0.00	0.00	0.00
Unidentified "calcium bulb"	2.61	8.70	0.00	0.00	0.00	0.00
DETRITUS	51.17	78.26	26.56	31.25	17.75	20.00
STONES	0.00	0.00	0.00	0.00	0.50	5.00

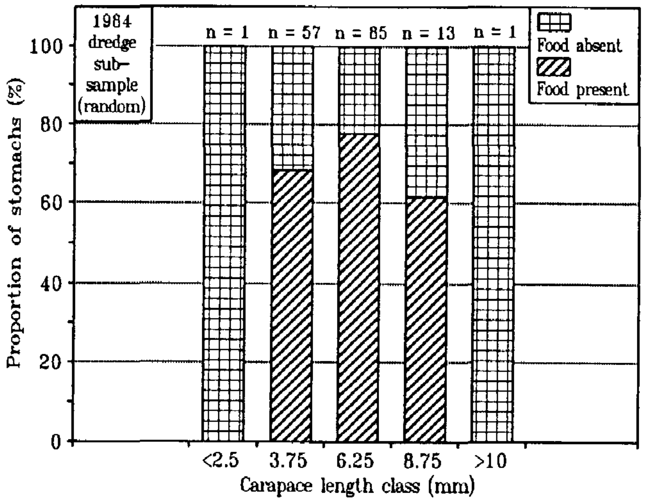

Figure 5.1A: Percentages of randomly selected N. marionis specimens from the 1984 dredge sample which had food in their guts, divided into carapace length classes. Percentages per carapace length class mid-point read as follows: 3.75mm = 68.42%, 6.25mm = 77.65%, 8.75mm = 61.54%. Too few specimens <2.50mm and >10.00mm were recovered to allow for any analysis of trends in these two size classes.

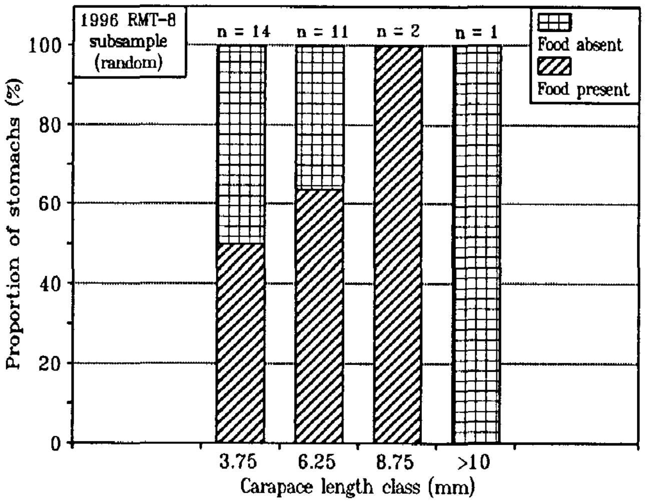

Figure 5.1B: Percentages of randomly selected N. marionis specimens from the 1996 RMT-8 sample which had food in their guts, divided into carapace length classes. Percentages per carapace length class mid-point read as follows: 3.75mm = 50.00%, 6.25% = 63.64%. No specimens <2.50mm carapace length were recovered. No trends amongst specimens >7.50mm could be discerned as too few specimens in this range were recovered.

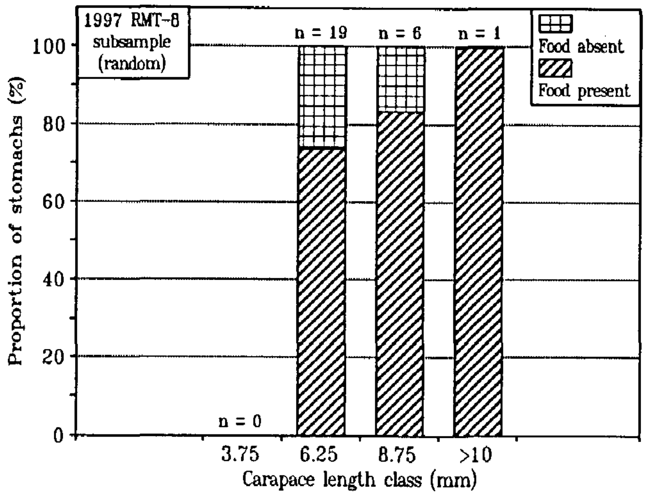

Figure 5.1C: Percentages of randomly selected N. marionis specimens from the 1997 RMT-8 sample which had food in their guts, divided into carapace length classes. Percentages per carapace length class mid-point read as follows: 6.25mm = 73.68%, 8.75mm = 83.33%. No specimens <5.00mm and only one >10.00mm carapace length were recovered.

Figure 5.1: Proportion of full guts recovered from different carapace length classes found in the randomly selected 1984 dredge (Figure 5.1A), 1996 RMT-8 (Figure 5.1B) and 1997 RMT-8 (Figure 5.1C) subsample specimens. N. marionis specimens were divided into 2.50mm carapace length classes.

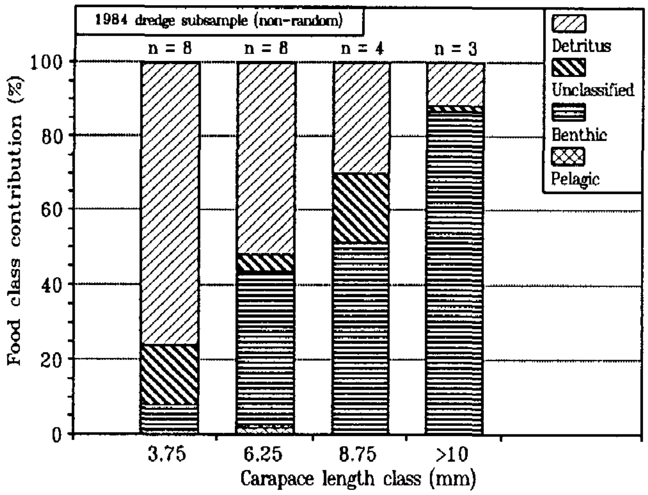

Figure 5.2A: Prey item categories recovered from the guts of differentially sized N. marionis specimens taken in the 1984 dredge sample. These specimens were not randomly selected.

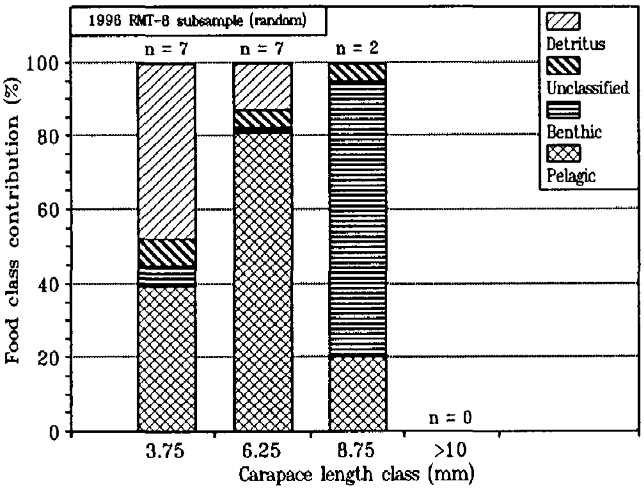

Figure 5.2B: Prey item categories recovered from the guts of differentially sized N. marionis specimens taken in the 1996 RMT-8 sample. These specimens were randomly selected.

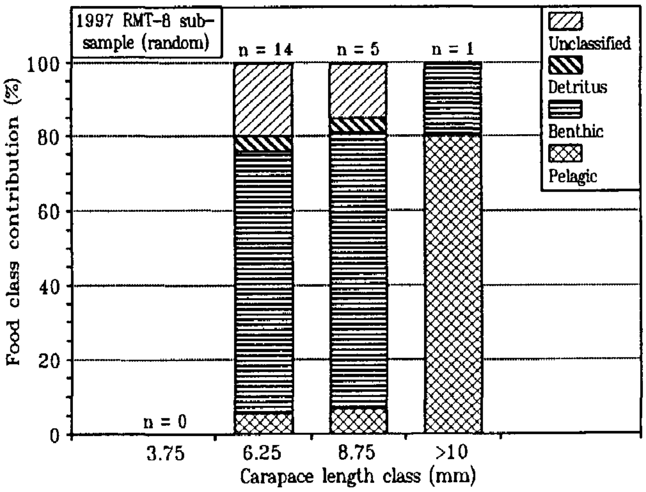

Figure 5.2C: Prey item categories recovered from the guts of differentially sized N. marionis specimens taken in the 1997 RMT-8 sample. These specimens were randomly selected. 0.71% of the 6.25mm carapace length class gut contents were stones (not shown here for clarity). The gut contents recovered from the single specimen >10.00mm carapace length should not be regarded as representative of that size class.

Figure 5.2: Contribution of different prey item categories to the gut contents recovered from the non-randomly selected 1984 dredge and randomly selected 1996 and 1997 RMT-8 subsample specimens. N. marionis specimens were divided into 2.50mm carapace length classes.

5.4 Discussion:

5.4.1 Opportunistic feeding in *N. marionis*:

Cannibalism in *N. marionis* during gut evacuation experiments was recently noted by Pakhomov *et al.* (in review). In the experiments conducted by these authors, *N. marionis* began appropriating the antennal flagella, eyes and other body appendages of relatively large conspecifics. They also preyed directly on smaller *N. marionis*. This thesis is, however, the first study which presents direct evidence of cannibalism in this species in nature. The two cannibalized *N. marionis* were smaller than any specimen recovered from any of the samples described in section 1.3. It was probably coincidental that the two cannibalistic *N. marionis* specimens both had carapace lengths of 9.83mm, although their similarly-sized conspecific prey may have been due to prey size preference. However, the recovered body parts of the cannibalized *N. marionis* were in an advanced state of digestion. The prey carapace length estimates were also made by extrapolating the equations of Chapter 1. The prey size estimates should, therefore, be regarded as inexact at best. The occurrence and rarity of cannibalism may be indicative of some sort of niche separation between larger and smaller *N. marionis*.

The variety of prey items identified by Perissinotto & McQuaid (1990) and this chapter, which includes conspecifics, leads one inescapably to the conclusion that *N. marionis* is an opportunistic feeder. Items seem to have been indiscriminately ingested, including one example of a bird feather fragment. The dominance of benthic hydrozoans in the guts of the 1997 RMT-8 sample specimens compared to their incidental occurrence in the benthic 1984 dredge sample specimen guts strongly suggests that *N. marionis*' prey is determined by whatever is in abundance at the time. This should be regarded as a word of caution to future researchers who may wish to measure the strength of trophic interactions at the Prince Edward Islands, as these interactions may vary substantially between different sampling sites and sampling years when different prey items may be more readily available. Growth rates and other aspects of *N. marionis* biology may also be affected by diets of different quality. However, such eclectic feeding could make *N. marionis* an ideal conduit of energy in any heterogenous ecosystem in which it dominates, especially given its importance as a prey item for higher predators.

5.4.2 Prey choice:

Differences between male and female *N. marionis* prey item preference has been ascribed to the difference in size between the two genders (Perissinotto & McQuaid 1990). There is some suggestion from the daytime 1984 dredge sample that larger individuals feed more on benthic prey items than do their smaller counterparts. However, most of the gut contents of the smaller size classes from the 1984 sample were made up of detritus i.e. gut contents which may have been benthic or pelagic in origin. The apparent preference for benthic prey by larger individuals may have been an artifact caused by larger individuals consuming larger prey or portions of prey which could be more easily identified. Nonetheless, in the non-random 1984 dredge sample pelagic prey were exclusive to the smaller size classes, albeit in small quantities. In addition, although the number of larger *N. marionis* individuals from the nighttime 1996 RMT-8 sample was low, the volume of pelagic prey contributed more to the diets of smaller specimens in this sample. This could suggest that pelagic prey contributes more to the diets of smaller *N. marionis*, and benthic prey to the diets of larger specimens.

However, the trend towards more pelagic prey from the guts of smaller *N. marionis* was not observed in the 1997 RMT-8 sample. One cannot say that the opposite was true in this sample, because the single specimen >10.00mm carapace length cannot be regarded as indicative of the norm in this size range. Pelagic prey were recovered from all sampled size classes from the 1997 RMT-8 sample. Therefore this study draws no conclusions regarding food class preferences of the different size classes in any of the samples.

A number of hitherto unrecorded *N. marionis* prey items have been identified in this study, most notably the benthic suspension feeding hydrozoans and to a lesser extent deposit feeding tube-dwelling polychaetes. These two taxa constituted the main benthic prey items, the former surely feeding primarily from descending and the latter from settled pelagic prey. Another noteworthy portion of the ingested biomass from the 1984 dredge sample was from benthic crustaceans.

Perhaps this chapter's most important observation is, however, that a large euphausiid biomass was consumed by *N. marionis* specimens recovered from the nighttime 1996 RMT-8 sample. Unlike Perissinotto & McQuaid's study, euphausiids were not only recovered from female *N. marionis* guts, but were present in the guts of some relatively small males. Euphausiids, mostly identified as *Thysanöessa*, were found in the guts of specimens from all size classes represented in the 1996 RMT-8 sample, and were more important than benthic prey both in volume and occurrence in this sample. All euphausiids consumed by *N. marionis* were relatively small, and at this size are regarded as filter feeders (Pakhomov pers. comm.).

Therefore, despite evidence for opportunistic feeding in *N. marionis*, it may be said that this species' main prey items seem to be both benthic *and pelagic* suspension feeders.

5.4.3 Predation strategies:

The high percentage of empty guts recovered from the nighttime 1996 RMT-8 pelagic sample could be a reflection of the fact that their predominant prey were euphausiids, which are fast swimmers (Perissinotto 1989) and therefore have to be hunted. Such a predation strategy would not allow for continuous feeding. It also suggests some agility in the water column by *N. marionis*.

The smaller percentage of empty guts in the 1997 RMT-8 sample may have been because that sample was taken earlier in the evening, possibly when benthic prey had not yet been fully digested. It is presumably easier to locate benthic prey items taken during the day than pelagic prey items taken at night, because of the relative immobility of benthic fauna. However, the large proportion of detritus in the 1984 dredge sample may suggest that feeding is not as intense during the day (detritus perhaps representing well digested nighttime prey), or it may reflect a lower benthic prey quality during the day.

N. marionis specimens >7.50mm carapace length which had consumed pelagic prey were taken in the 1996 RMT-8 sample. This sample was taken at night with a net which was towed 5m-10m above the bottom, never touching it. Although the similarly-sized *N. marionis* specimens taken in the nighttime 1997 RMT-8 sample could have been benthic specimens because the net did touch the bottom, pelagic prey items (*Thysanöessa*) were recovered from the guts of three of the six specimens >7.50mm carapace length in this sample. The 1997

RMT-8 sample was taken earlier in the evening than the 1996 RMT-8 sample. Unlike the nighttime 1996 RMT-8 sample, the nighttime 1997 RMT-8 sample prey were largely benthic. Perhaps this was because the net struck the bottom in 1997, or perhaps it was due to the earlier time of night that the 1997 RMT-8 sample was taken. However, unlike the daytime, completely benthic 1984 dredge sample, pelagic prey items were recovered from specimens >7.50mm carapace length from the 1997 RMT-8 sample. These observations suggest that some level of pelagic feeding by large individual *N. marionis* occurs, possibly only at night.

Pelagic prey items were also recovered from female guts by Perissinotto & McQuaid (1990), females being larger than males (see Chapter 3). It is not clear whether the 1989 sample examined by Perissinotto & McQuaid was benthic or not, as they refer to both a benthic and a suprabenthic sample taken during that year, but this does suggest that large *N. marionis* prey on pelagic fauna, and hence at some time do leave the benthic environment. How far up the water column large *N. marionis* may migrate cannot be surmised from its diet, because diel vertical migration of pelagic organisms such as euphausiids and copepods has been demonstrated (Perissinotto & McQuaid 1992). These authors have also shown diel vertical migration of small *N. marionis*, which they called megalope larvae. However, the acoustic data gathered by these authors may well have also included large *N. marionis*. If larger *N. marionis* do feed amongst the plankton at some time, more than likely this is done at night as well. This suggests that some diel vertical migration may also occur amongst larger specimens.

5.4.4 *N. marionis*' place in the food web at the archipelago:

Previous studies have shown that there is a direct link between allochtonous macrozooplankton/micronekton and the offshore feeding land-based top predators in the vicinity of the Prince Edward Islands (Perissinotto & McQuaid 1992). It has been suggested that *N. marionis* is an important component of an indirect link which may make the local primary production available to the selected land-based top predators in this region (Perissinotto & McQuaid 1990).

Benthic suspension feeders have already been shown to be an important component in the diet of *N. marionis* (Perissinotto & McQuaid 1990). These organisms may, thus, form a direct link between the local primary production in the upper layers of the water column (which sinks) and *N. marionis* (ibid.). Therefore, the number of trophic levels through which a substantial amount of the energy from local primary production is transferred to top predators via benthic suspension feeders would seem to be few. Indeed, it has been noted that a simple food web might be expected at the archipelago (Blankley & Grindley 1985) as it is a relatively young ecosystem (McDougall 1971, cited in Blankley & Grindley 1985). The short, simple food chain previously suggested by Perissinotto & McQuaid (1990), i.e. primary producers → benthic filter feeders → *N. marionis*, would therefore allow an efficient transfer of local primary production to selected top predator populations within the region.

Results of the gut content analyses of *N. marionis* indicate that small pelagic prey, e.g. calanoid and cyclopoid copepods (Perissinotto & McQuaid 1990), as well as euphausiids (Table 5.1), are especially important components in the diet of *N. marionis*. It is well documented that the mesozooplankton in the vicinity of the Prince Edward Islands is derived from an allochtonous source (Perissinotto 1989) representing energy primarily accumulated outside the island ecosystem. It has also been shown that allochtonous mesozooplankton do not appear to represent another significant trophic level between the locally enhanced primary production and *N. marionis*. This is because the imported zooplankton does not have a significant grazing impact on local phytoplankton assemblages (Perissinotto 1992).

However, by feeding on small pelagic prey, *N. marionis* appears to represent a valuable link between smaller zooplankton, which would not otherwise be fed on by top predators, and selected top predators in the islands. *N. marionis* may also feed indirectly on the smaller zooplankton because some of their prey, i.e. hydrozoans, are known to consume small zooplankton as well (Barnes 1987: 102), further facilitating this transfer. This suggests that pelagic mesozooplanktonic prey may be a more important energy source for the land-based top predators than previously thought. Given the fact that the Prince Edward Islands are regarded as a flow-through system, the source of allochtonous mesozooplankton should be plentiful and stable (Pakhomov & Froneman, in press).

5.5 Conclusions:

1) *N. marionis* is an opportunistic feeder, although the majority of its prey seem to be suspension feeders, both benthic and pelagic in origin. Future studies on the strengths of trophic interrelationships involving *N. marionis* should bear in mind that their importance may vary between sampling sites and years, depending on what prey is dominant at the time. Growth rates and other aspects of *N. marionis* biology may be affected by diets of different quality.

2) Itinerant euphausiids and other crustaceans may contribute substantially to the maintenance of top predator populations at the archipelago, either through direct predation by these predators or via predation by *N. marionis*, which is in turn consumed by these predators. Predation on this biomass may also occur by *N. marionis*' prey. A short, simple food web consisting of primary producers, benthic suspension feeders and *N. marionis* may simultaneously allow a substantial percentage of local primary production to become available to higher trophic levels.

3) Niche separation between smaller and larger *N. marionis* individuals may occur, evidenced by the small occurrence of cannibalism.

4) Diel vertical migration may occur to some extent amongst large *N. marionis*, as has already been shown for small *N. marionis* during a previous study.

Summary

Conclusions:

1. The best measurement of size for *N. marionis*, and indeed any crustacean, is carapace length. When used, this measurement should be properly defined to facilitate comparisons between different studies. The definition used in this thesis i.e. the posterior margin of the orbit to the dorsal midline of the posterior margin of the carapace (Kuris & Carlton 1977) has been used in other studies of crustaceans and should be preferentially adopted as a standard when studying *N. marionis*, given that it seems to be the most accurate and that it has been used in this, the first extended study of the animal.

2. *N. marionis* is a partially protandric hermaphrodite. It would seem that *N. marionis* juveniles have the potential to differentiate into either males or females and that this separation is done in the plankton. It is unknown whether individual *N. marionis*' life-histories are determined at conception or in the larval or juvenile phases, but it seems that male *appendices masculinae* are only developed after the juvenile phase has been attained, and that this begins whilst they are planktonic.

N. marionis appear to hatch just before April each year, with minimal hatching persisting until May. It would seem that during the first year of life, *N. marionis* survive in undetected localities, moult into juveniles, and then settle amongst the benthos from the plankton. Diurnal vertical migration then occurs until the animals are an unknown larger size. It is not known whether the larvae are initially planktonic or not. It is possible that settling of small *N.*

marionis onto the benthos only begins after November. Whether the *appendices masculinae* of some males only begin growing after they have settled onto the benthos is unknown.

The vast majority of juveniles develop into males. Once having grown to a certain carapace length they transmutate into females. The size at which this begins for the majority is coincident with their third year, and may therefore differ between years. It may also differ between localities. During transition from male to female, at least some *N. marionis* retain their *appendices masculinae* after developing ovaries. By April/May the transmutation is probably complete. Reproduction can occur before all male secondary characteristics have been lost.

A small minority of individuals develop directly into primary females without passing through a male phase. At least some ovigerous primary females can be initially recognized by the presence of *appendices internae* on their first pleopods, male copulatory structures which all juvenile *N. marionis* possess. These primary females may lose their first pleopod *appendices internae* in one moult, possibly just before spawning, which may be in late April/early May. Too few gravid females were recovered to make a statement on whether spawning can occur before this structure is lost. Primary females seem to become ovigerous at a slightly smaller carapace length than do the majority of secondary females. A few secondary females seem to begin developing ovaries at about this small carapace length as well. After the *appendices internae* have been lost primary and secondary females are probably indistinguishable.

Individuals older than five years are undetectable using samples of the sizes analyzed in this thesis, but they may well persist until quite an advanced age.

3. In multisample situations, ageing of *N. marionis* cohorts is made less subjective if one utilizes the phenomenon of synchronized sexual inversion. This may be true of other protandric species as well. Ageing of cohorts in future studies should use this fact where possible.

4. The von Bertalanffy growth parameters for *N. marionis* are tentatively identified as $k = 0.2353$/year, $L_\infty = 12.69$mm, $t_0 = -0.2828$ years and $WW_\infty = 2.03$g.

5. The programme FiSAT, although extremely useful, was found to have certain faults, the most important of which appears to be the basis of the χ^2 test, allowing for differences in data sets which the programme indicates do not exist. Upward class interval adjustment after entering data into FiSAT seems to be a prerequisite for its use, even though this adjustment may be regarded as controversial. "Smoothing" of the data after class interval adjustment is not recommended. Consultation of standard χ^2 tables when using FiSAT is recommended, as the values released by the programme may be inaccurate, and also to help identify viable initial estimates with the "Bhattacharya" procedure.

6. Niche separation between smaller and larger *N. marionis* individuals may occur, evidenced by the small occurrence of cannibalism.

7. Diel vertical migration may occur to some extent amongst large *N. marionis*, as has already been shown for small *N. marionis* during a previous study.

8. Itinerant euphausiids may contribute substantially to the maintenance of top predator populations at the archipelago, either through direct predation by these predators or via predation by *N. marionis*, which in turn are consumed by these predators. *N. marionis*' prey items may also prey on portions of this pelagic biomass. *N. marionis* itself is an opportunistic feeder, although the majority of its prey seem to be suspension feeders, both benthic and pelagic in origin.

Suggestions for future studies:

1. Future attempts should be made to discover the refuge of *N. marionis* during its first year, in order to better understand the ecology of this important animal in the Prince Edward Island group ecosystem. The suggestion is made here that the undetected refuge of the larvae *may* be in the shallower waters surrounding the islands between the shore and the substantial kelp beds. Migrating gravid females *may* also occur there during the breeding period.

2. Data are presented in this dissertation in such a way as to facilitate their incorporation into other data sets, which should be gathered in order to refine this thesis' growth parameter estimates. Future commentary on the validity of using the historical "Petersen" method in conjunction with FiSAT to isolate undersampled cohort means is necessary.

3. More accurate gender definitions, possibly using gonopore, ovary and *appendix masculina* setae development are required now that protandry has been established.

4. Climate change, which has already been recorded at these islands, may affect *N. marionis*' life-history whilst it is still in a planktonic phase. Given that *N. marionis* has already been implicated as being quite important in the functioning of the Prince Edward Island ecosystem, this may be affecting the ecosystem as a whole. This aspect requires further study.

5. There are reproductive trade-offs involved in individuals becoming primary or secondary females. It needs to be established whether spawning for the first time occurs slightly earlier for primary females than for secondary females. Some circumstantial evidence for this is presented in this dissertation and it is proposed as a tentative hypothesis. If this is found to be the case, the implications should be investigated. One possible implication might be that the total reproductive success of primary females *as females* is higher than that of secondary females *as females*, although total *lifetime* reproductive success may be greater for individuals following the secondary route.

6. Bopyridae are possibly implicated in the castration of individuals, although their rate of infestation does not appear to be high from the few data available. Moulting may possibly facilitate the loss of bopyrid parasites. The effects of parasitism on the reproduction and growth rates of *N. marionis* need to be examined in the future.

7. Because *N. marionis* is an opportunistic feeder, future studies on the strengths of trophic interrelationships involving *N. marionis* should bear in mind that prey importance may vary between sampling sites and years, depending on what is available at the time. The importance of *N. marionis* in making primary production and imported allocthonous pelagic biomass

available to top predators at the archipelago needs to be investigated by measuring the strengths of the various trophic interactions.

References cited

All periodical titles have been abbreviated as recommended by Alkire (1996), except for three which are not cited amongst his some 159 000 entries. In these three cases the periodical titles have been reported in unabbreviated format. The relevant page numbers of non-journal citations are given in the text.

ADAMS N.J. 1990. Feeding biology and energetics of King *Aptenodytes patagonicus* and Gentoo *Pygoscelis papua* Penguins at sub-Antarctic Marion Island. Ph.D. Thesis. University of Cape Town.

ADAMS N.J. & BROWN C.R. 1989. Dietary differentiation and trophic relationships in the sub-Antarctic penguin community at Marion Island. *Mar Ecol Prog Ser* 57: 249-258.

ADAMS N.J. & KLAGES N.T. 1987. Seasonal variation in the diet of the king penguin (*Aptenodytes patagonicus*) at sub-Antarctic Marion Island. *J Zool (Lond)* 212: 303-324.

ADAMS N.J. & KLAGES N.T. 1989. Temporal variation in the diet of the Gentoo Penguin *Pygoscelis papua* at sub-Antarctic Marion Island. *Colon Waterbirds* 12(1): 30-36.

ALKIRE L.G. JR. 1996. Periodical title abbreviations. 10th edition. Gale Research Inc. New York, London.

ALLANSON B.R., BODEN B., PARKER L. & DUNCOMBE RAE C. 1985. A contribution to the oceanology of the Prince Edward Islands. In: Antarctic nutrient cycles and food webs, eds. Siegfried W.R., Condy P.R. & Laws R.M. Springer-Verlag. Berlin, Heidelberg.

ALLEN J.A. 1966. The dynamics and interrelationships of mixed populations of Caridea found off the north-east coast of England. In: Some contemporary studies in marine science, ed. Barnes H. George Allen & Unwin Ltd. London.

ARNTZ W.E & GORNY M. 1991. Shrimp (Decapoda, Natantia) occurrence and distribution in the eastern Weddell Sea, Antarctica. *Polar Biol* 11: 169-177.

BARNES R.D. 1987. Invertebrate Zoology, 5th edition. Saunders College Publishing. New York.

BATE C.S. 1888. Report on the Crustacea Macrura collected by H.M.S. Challenger during the years 1873-76. In: Report on the scientific results of the voyage of H.M.S. Challenger during the years 1873-76, Zoology, 24, eds. Thompson C.W. & Murray J. Neil & Co. London.

BAUER R.T. 1976. Mating behaviour and spermatophore transfer in the shrimp *Heptacarpus pictus* (Stimpson) (Decapoda: Caridea: Hippolytidae). *J Nat Hist* 10: 415-440.

BAUER R.T. 1979. Sex attraction and recognition in the caridean shrimp *Heptacarpus paludicola* Holmes (Decapoda: Hippolytidae). *Mar Behav and Physiol* 6: 157-174.

BAUER R.T. 1986. Sex change and life history pattern in the shrimp *Thor manningi* (Decapoda: Caridea): a novel case of partial protandric hermaphroditism. *Biol Bull* 170: 11-31.

BAUER R.T. 1989. Continuous reproduction and episodic recruitment in nine shrimp species inhabiting a tropical seagrass meadow. *J Exp Mar Biol Ecol* 127: 175-187.

BAUER R.T & RIVERA VEGA L.W. 1992. Pattern of reproduction and recruitment in two sicyoniid species (Decapoda: Penaeoidea) from a tropical seagrass habitat. *J Exp Mar Biol Ecol* 161: 223-240.

BLANKLEY W.O. 1982. Feeding ecology of three inshore fish species at Marion Island (Southern Ocean). *S Afr J Zool* 17(4): 164-170.

BLANKLEY W.O. & GRINDLEY J.R. 1985. The intertidal and shallow subtidal food web at Marion Island. In: Antarctic nutrient cycles and food webs, eds. Siegfried W.R., Condy P.R. & Laws R.M. Springer-Verlag. Berlin, Heidelberg.

BODEN B.P. 1988. Observations of the island mass effect in the Prince Edward archipelago. *Polar Biol* 9: 61-68.

BRANCH G.M., ATTWOOD C.G., GIANAKOURAS D. & BRANCH M.L. 1993. Patterns in the benthic communities on the shelf of the sub-Antarctic Prince Edward Islands. *Polar Biol* 13: 23-34.

BRANCH M.L. & BRANCH G.M. 1988. The living shores of southern Africa. C. Struik Publishers. Cape Town.

BRANCH M.L., GRIFFITHS C.L., KENSLEY B. & SIEG J. 1991. The benthic Crustacea of subantarctic Marion and Prince Edward Islands: Illustrated keys to the species and results of the 1982-1989 University of Cape Town Surveys. *S Afr J Antarct Res* 21(1): 3-44.

BROWN C.R. & KLAGES N.T. 1987. Seasonal and annual variation in diets of Macaroni (*Eudyptes chrysolophus chrysolophus*) and southern Rockhopper (*E. chrysocome chrysocome*) penguins at sub-Antarctic Marion Island. *J Zool (Lond)* 212: 7-28.

CHRISTOFFERSEN M.R. 1987. Phylogenetic relationships of hippolytid genera, with an assignment of new families for the Crangonoidea and Alpheoidea (Crustacea, Decapoda, Caridea). *CLADEC* 3(4): 348-362.

CORTEZ T., CASTRO B.G. & GUERRA A. 1995. Feeding dynamics of *Octopus mimus* (Mollusca: Cephalopoda) in northern Chile waters. *Marine Bio* 123: 497-503.

CURTIS H. 1986. Biology, 4th edition. Worth Publishers Inc. New York.

DE FREITAS A.J. 1989. Shrimps and prawns. In: Oceans of life off southern Africa, eds. Payne A.I.L. & Crawford R.J.M. Vlaeberg Publishers CC. Cape Town.

EBBING D.D. 1987. General chemistry. Houghton Miffler Co. Boston.

GAYANILO F.C. JR., SPARRE P. & PAULY P. 1996. FAO-ICLARM stock assessment tools: User's manual. Food and agriculture organization of the United Nations. Rome.

GORNY M. & GEORGE M.R. 1997. Oocyte development and gonad production of *Nematocarcinus lanceopes* (Decapoda: Caridea) in the eastern Weddell Sea, Antarctica. *Polar Biol* 17: 191-198.

GORNY M., ARNTZ W.E., CLARKE A. & GORE D.J. 1992. Reproductive biology of caridean decapods from the Weddell Sea. *Polar Biol* 12: 111-120.

GORNY M., BREY T., ARNTZ W. & BREWS T. 1993. Growth, development and productivity of *Chorismus antarcticus* (Pfeffer) (Crustacea: Decapoda: Natantia) in the eastern Weddell Sea, Antarctica. *J Exp Mar Biol Ecol* 174: 261-275.

GULLAND J.A. 1987. Length-based methods in fisheries research: From theory to application. In: Length-based methods in fisheries research: Proceedings of the International Conference on the theory and application of length-based methods for stock assessment, eds. Pauly D. & Morgan G.R. International Center for Living Aquatic Resources Management and Kuwait Institute for Scientific Research. Safat, Kuwait.

GURNEY R.G. 1937. Larvae of decapod crustacea. Part 4. Hippolytidae. *Discovery Rep* 14: 351-404.

GURNEY R. 1960. Bibliography of the larvae of decapod Crustacea. In: Historiæ Naturalis Classica, eds. Cramer J. & Swann H.K. H.R. Engelmann (J. Cramer) and Wheldon & Wesley Ltd. Weinheim.

HOENIG J.M., CSIRKE J., SANDERS M.J., ABELLA A., ANDREOLI M.G., LEVI D., RAGONESE S., AL-SHOUSHANI M. & EL-MUSA M.M. 1987. Data acquisition for length-based stock assessment: Report of working group 1. In: Length-based methods in fisheries research: Proceedings of the International Conference on the theory and application of length-based methods for stock assessment, eds. Pauly D. & Morgan G.R. International Center for Living Aquatic Resources Management and Kuwait Institute for Scientific Research. Safat, Kuwait.

HOFFMAN D.L. 1972. The development of the ovotestis and copulatory organs in a population of protandric shrimp, *Pandalus platyceros* Brandt from Lopez Sound, Washington. *Biol Bull* 142: 251-270.

JOHNSON L.E. 1992. Potential and peril of field experimentation: the use of copper to manipulate molluscan herbivores. *J Exp Mar Biol Ecol* 160: 251-262.

KURIS A.M. & CARLTON J.T. 1977. Description of a new species, *Crangon handi*, and new genus, *Lissocrangon*, of crangonid shrimps (Crustacea: Caridea) from the California coast, with notes on adaptation in body shape and coloration. *Biol Bull* 153: 540-559.

LACOCK G.D., HECHT T. & KLAGES N. 1984. The winter diet of Gentoo Penguins at Marion Island. *Ostrich* 55: 188-191.

LAMBERT K. & BRITTAN G.G. JR. 1979. An introduction to the philosophy of science. 2nd edition. Ridgeview Publishing Co. Reseda, California.

LAUREC A. & MESNIL B. 1987. Analytical investigations of errors in mortality rates estimated from length distributions of catches. In: Length-based methods in fisheries research: Proceedings of the International Conference on the theory and application of length-based methods for stock assessment, eds. Pauly D. & Morgan G.R. International Center for Living Aquatic Resources Management and Kuwait Institute for Scientific Research. Safat, Kuwait.

MACDONALD P.D.M. & PITCHER T.J. 1979. Age-groups from size-frequency data: A versatile and efficient method of analyzing distribution mixtures. *J Fish Res Board Canada* 36: 987-1001.

MAJKOWSKI J., HAMPTON J., JONES R., LAUREC A. & ROSENBERG A.A. 1987. Sensitivity of length-based methods for stock assessment: Report of working group 3. In: Length-based methods in fisheries research: Proceedings of the International Conference on the theory and application of length-based methods for stock assessment, eds. Pauly D. & Morgan G.R. International Center for Living Aquatic Resources Management and Kuwait Institute for Scientific Research. Safat, Kuwait.

MASCETTI P., FERNANDEZ DE LA REGUERA R., ALBORNOZ L., OYARZUN S., GORNY M. & WEHRTMANN I. 1966. Gonopore development and sex change in the Antarctic shrimp *Chorismus antarcticus* (Caridea: Hippolytidae). *Polar Biol* 17: 384-388.

MATHEWS C.P., AL-HOSSAINI M., ABDUL GHAFFAR A.R. & AL-SHOUSHANI M. 1987. Assessment of short-lived stocks with special reference to Kuwait's shrimp fisheries: A contrast of the results obtained from traditional and recent size-based techniques. In: Length-based methods in fisheries research: Proceedings of the International Conference on the theory and application of length-based methods for stock assessment, eds. Pauly D. & Morgan G.R. International Center for Living Aquatic Resources Management and Kuwait Institute for Scientific Research. Safat, Kuwait.

MENDENHALL W., WACKERLY D.D. & SCHEAFFER R.L. 1990. Mathematical statistics with applications. 4th edition. Duxbury Press. Belmont.

PAINE R.T. 1980. Food webs: Linkage interaction strength and community infrastructure: The third Tansley lecture. *J Animal Ecology* 49: 667-685.

PAKHOMOV E.A. & FRONEMAN P.W. In press. The Prince Edward Islands pelagic ecosystem, South Indian Ocean: A review of achievements, 1976-1990. *Journal of Marine Systems.*

PAKHOMOV E.A., FRONEMAN P.W. & BALARIN M. In press. Respiration and daily food intake of the bottom-dwelling caridean shrimp *Nauticaris marionis* Bate, 1888 (Crustacea: Decapoda) in the vicinity of Marion Island (Southern Ocean). *J Exp Mar Biol Ecol.*

PARKER L.D. 1984. A contribution to the oceanology of the Prince Edward Islands. M.Sc. thesis. Rhodes University.

PANNELLA G. 1971. Fish otoliths: Daily growth layers and periodical patterns. *Sc* 173(4002): 1124-1127.

PAULY D. 1987. A review of the ELEFAN system for analysis of length-frequency data in fish and aquatic invertebrates. In: Length-based methods in fisheries research: Proceedings of the International Conference on the theory and application of length-based methods for stock assessment, eds. Pauly D. & Morgan G.R. International Center for Living Aquatic Resources Management and Kuwait Institute for Scientific Research. Safat, Kuwait.

PERISSINOTTO R. 1989. The structure and diurnal variations of the zooplankton of the Prince Edward Islands: Implications for the biomass build-up of higher trophic levels. *Polar Biol* 9: 505-510.

PERISSINOTTO R. & MCQUAID C. 1990. Role of the sub-Antarctic shrimp *Nauticaris marionis* in coupling benthic and pelagic food webs. *Mar Ecol Prog Ser* 64: 81-87.

PERISSINOTTO R. & MCQUAID C.D. 1992. Land-based predator impact on vertically migrating zooplankton and micronekton advected to a Southern Ocean archipelago. *Mar Ecol Prog Ser* 80: 15-267.

PETER L. 1991. 5,000 gems of wit and wisdom: Memorable quotations for all occasions. Treasure Press. London.

POPPER K. 1983. The logic of scientific discovery. Hutchinson & Co. (Publishers) Ltd. London.

RIDOUX V. 1994. The diets and dietary segregation of the seabirds at the subantarctic Crozet Islands. *Marine Ornithology* 22(1): 1-192.

SCHMIDT-NIELSEN K. 1990. Animal physiology: Adaptation and environment. 4th edition. Cambridge University Press. Cambridge.

SHEPHERD J.G. 1987. A weakly parametric method for estimating growth parameters from length composition data. In: Length-based methods in fisheries research: Proceedings of the International Conference on the theory and application of length-based methods for stock assessment, eds. Pauly D. & Morgan G.R. International Center for Living Aquatic Resources Management and Kuwait Institute for Scientific Research. Safat, Kuwait.

SHEPHERD J.G., MORGAN G.R., GULLAND J.A. & MATHEWS C.P. 1987. Methods of analysis and assessment: Report of working group 2. In: Length-based methods in fisheries research: Proceedings of the International Conference on the theory and application of length-based methods for stock assessment, eds. Pauly D. & Morgan G.R. International Center for Living Aquatic Resources Management and Kuwait Institute for Scientific Research. Safat, Kuwait.

SMITH V.R. 1991. Climate change and its ecological consequences at Marion and Prince Edward Islands. *S Afr J Antarct Res* 21(2): 223.

SPARRE P. 1987. A method for the estimation of growth, mortality and gear selection/recruitment parameters from length-frequency samples weighted by catch per effort. In: Length-based methods in fisheries research: Proceedings of the International Conference on the theory and application of length-based methods for stock assessment, eds. Pauly D. & Morgan G.R. International Center for Living Aquatic Resources Management and Kuwait Institute for Scientific Research. Safat, Kuwait.

SPARRE P. & VENEMA S.C. 1992. Introduction to tropical fish stock assessment: Part 1 - Manual: FAO fisheries technical paper 306/1 Rev. 1. Food and agriculture organization of the United Nations. Rome.

THOMAS G.B. JR. & FINNEY R.L. 1988. Calculus and analytic geometry. 7th edition. Addison-Wesley Publishing Co. Reading, Massachusetts.

UNDERHILL L.G. 1987. Introstat. 4th edition, revised second impression. Juta & Co., Ltd. Cape Town.

WALPOLE R.E. 1990. Introduction to statistics. 3rd edition. Macmillan Publishing Co., Inc. New York.

YALDWYN J.C. 1958. Decapod Crustacea from subantarctic seal and shag stomachs. *Rec Dom Mus (Wellington)* 3(2): 21-27.

ZUPO V. 1994. Strategies of sexual inversion in *Hippolyte inermis* Leach (Crustacea, Decapoda) from a Mediterranean seagrass meadow. *J Exp Mar Biol Ecol* 178: 131-145.

ZAR J.H. 1984. Biostatistical analysis. 2nd edition. Prentice Hall Inc. Englewood Cliffs, New Jersey.

Sample 1	1984 dredge sample (Chapters 2, 3, 4 & 5)			
Gender	range	n	mean (mm)	s (mm)
juveniles	3.90mm - 5.25mm	4	4.57	0.57
'juveniles' with ovaries	5.21mm - 7.50mm	8	6.37	0.79
males	2.46mm - 8.17mm	297	4.72	1.15
tertia quae	4.96mm - 9.17mm	15	7.54	1.14
females	5.20mm - 10.83mm	59	7.70	1.56
		382		

Largest male in the non-random subsample: 8.08mm
Largest *tertium quid* in the non-random subsample: 8.96mm
Largest female in the non-random subsample: 12.58mm

Sample 2	1996 RMT-8 sample (Chapters 2, 4 & 5)			
Gender	range	n	mean (mm)	s (mm)
males	2.92mm - 5.58mm	20	4.52	0.76
females	5.17mm - 11.50mm	8	7.32	1.95
		28		

Sample 3	1997 RMT-8 sample (Chapters 3, 4 & 5)			
Gender	range	n	mean (mm)	s (mm)
males	4.92mm - 6.50mm	27	5.57	0.52
tertia quae	6.42mm - 7.00mm	2	6.71	0.41
females	5.17mm - 12.67mm	194	7.08	1.30
		223		

Sample 4	1997 RMT-1*6 sample (Chapters 3 & 4)			
Gender	range	n	mean (mm)	s (mm)
juveniles	2.86mm - 3.70mm	13	3.14	0.25
males	3.18mm - 3.52mm	8	3.37	0.12
		21		

Appendix 1: Carapace length means, standard deviations (s) and ranges of the different genders recovered from the various samples, showing the chapters in which they were considered. Sample numbers are those allocated in Table 1.1 and section 1.3. Genders are as defined in section 4.2. Non-randomly selected specimens from the 1984 dredge sample (1) were removed for morphometric analyses in Chapter 2, otherwise all statistics refer to stratified random samples.