Growth, feeding and sex change in the sequential protandric shrimp *Nauticaris marionis* Bate 1888 at the Prince Edward Islands (Southern Ocean)

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Lukhanyiso Unam Vumazonke

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Abstract

Demographic parameters and the general biology of the subantarctic shrimp *Nauticaris marionis* from the Prince Edward Islands were investigated. The carapace length is the most accurate indicator of body size and it was confirmed that *N. marionis* is a partially protandric hermaphrodite. Gravid females of *N. marionis* observed mainly in March with negligible hatching persisting until April/May. The majority of juveniles develop into males. Juveniles are characterised by an appendage on the endopodite of the first pleopod called the *appendix interna* or a.i.1. Juveniles that develop into males do so by growing a further appendage on the endopodite of the second pleopod. This appendage characterises males and is called the *appendix masculina* (or a.m.). Juveniles may develop directly into primary females through the development of an ovary and the loss of the a.i.1. The sequence differs among individuals, with some developing the ovary before losing the a.i.1 and others losing the a.i.1 first. Loss of the a.i.1 appears to be by shedding during a single moult, rather than by atrophy. Females can also develop by an alternative route as secondary females. Such animals first become males with an a.m. and then develop an ovary, thus forming an intermediate form called a *tertium quid*, or "third thing". Again, there are two forms of *tertium quid*. *Tertia quae a* have the a.m. as well as the a.i.1. *Tertia quae b* have the a.m. in combination with an ovary but no a.i.1. Either form of *tertium quid* can develop into a secondary female through loss of the a.m., and in the case of the *tertium quid a*, loss of the a.i.1. It is unclear whether sexual differentiation occurs in the plankton or just after *N. marionis* settles on the benthos.

The results of gut content analysis suggest that *N. marionis* is an opportunistic feeder, preying on a variety of prey with a preference for detritus, benthic amphipods and gastropods. Cannibalism of conspecific pleopods by large individuals of *N. marionis* occurred mostly in females, particularly in incubation containers. Cannibalism also occurs in males, and its occurrence depends on individual size, not gender. No diel patterns were observed in the feeding activity of *N. marionis*. *In situ* daily rations of males (carapace length <7mm) and females (>7mm) were equivalent to ≈10% and ≈5% of body dry weight, respectively. The von Bertalanffy growth curve parameters were empirically identified, by cohort analysis of data collected during 4 years, as K = 0.22239/year, L_∞ = 14.05789mm, t_0 = -0.05174, L_0 = 0.16083mm. *N. marionis* can survive up to seven years under natural conditions.

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Preface

This study is part of the ongoing broader research study in the Prince Edward Islands and part of it has been published in Polar Biology –

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This dissertation is my own unaided work and is being submitted for the Master of Science in Marine Biology at the Department of Zoology and Entomology, Rhodes University in Grahamstown. It has not been submitted in whole or in part for any degree or examination in any university.

Lukhanyiso Unam Vumazonke

Chapter One

General Introduction

1.1 Prince Edward Islands (Southern Ocean)

The Prince Edward Islands (46^{o} 45' S: 37^{o} 50' E) consist of two volcanic islands, Marion and Prince Edward Islands. The islands are situated directly in the path of the easterly flowing Antarctic Circumpolar Current in the roaring forties, approximately 2000km southeast of Cape Town. Both islands arose about 250 000 years ago (McDougall 1971, cited in Branch et al. 1993). Marion Island is $290km^2$ while Prince Edward Island is one-eleventh its size and lies 22km NNE of Marion.

The Prince Edward Islands are among the most isolated of terrestrial and shallow marine environments around the globe (Branch et al. 1993). Isolated subantarctic islands within the Southern Ocean are characterized by the presence of enormous populations of predatory flying seabirds, penguins and seals (Pakhomov and Froneman 1999). These predators rely on the surrounding waters for their food (Froneman and Ansorge 1998). The scenic beauty and the plentiful wildlife on the islands, as well as their importance for meteorological observations and ecological research, have attracted much attention to the islands since their annexation in 1947/48 by the Union (now Republic) of South Africa (Prince Edward Islands Management Plan 1996). Currently, the islands are recognized as an important South African natural laboratory, enabling pioneering research, and they are protected as a 'Special Natural Reserve' under the Environmental Conservation Act No. 73 of 1989 (Prince Edward Islands Management Plan 1996).

The uniqueness of the islands' ecosystem is the result of a close marine-terrestrial interaction known as the 'life-support system', which ultimately provides food for the entire community of seabirds and mammals (Perissinotto and McQuaid 1992, Pakhomov et al. 1998, Pakhomov and Froneman 1999).

Among the major components in the diet of the community of land-based predators on the island are demersal fish and the caridean shrimp *Nauticaris marionis* (Condy 1981, Brown 1989, Adams 1990, Pakhomov and Froneman 1999). Since the earliest benthic surveys of the Prince Edward Islands seas, the local population of *Nauticaris marionis* has been noted for its high abundance, compared to populations at other subantarctic islands (Ledoyer 1979, cited in Perissinotto and McQuaid 1990). Previous studies have suggested that *Nauticaris marionis* plays a key role in making primary production and allochthonous secondary production available to selected top predators at the islands (Perissinotto and McQuaid 1990, Pakhomov and Froneman 1999).

1.2 The swimming prawn *Nauticaris marionis*

Nauticaris marionis is a caridean shrimp of the family Nauticaridae (Christoffersen 1987). *N. marionis* or the swimming prawn is an endemic sub-Antarctic species inhabiting shelf regions from southern New Zealand in the east to the Prince Edward Islands in the west (Yaldwyn 1965, 1966, Perissinotto and McQuaid 1990). Although, Bate (1888) refers to *N. marionis* as a bottom dweller, the species does not inhabit deep waters. *N. marionis* has been found to be abundant from 1 to over 30m depth at the Snares Island off New Zealand, especially in crevices in some underwater caves (Fenwick 1978). At the Prince Edward Islands it has been recorded at depths of 5 to 697m, but is most abundant between 50 and 100m (Branch et al. 1991, Branch et al. 1993).

N. marionis is one of the most important 'bottom-dwelling' invertebrates at the Prince Edward Islands, ranking after bryozoans in terms of total shelf benthic biomass (Parker 1984, Perissinotto and McQuaid 1990). *N. marionis* is regarded as an important food source for many of the top predators at the islands, and has been found to be a staple diet of Gentoo penguins and Imperial cormorants (La Cock et al. 1984, Espitalier-Noel et al. 1988, Adams and Klages 1989). It has been recorded in the stomachs of King penguins (Adams and Klages 1987, Adams and Brown 1989), and in some seasons, may contribute substantially to the diets of Macaroni and Rockhopper penguins (Adams and Klages 1987). *N. marionis* has also been found in

the stomachs of some nototheniid fishes, including *Notothenia macrocephala* and *N. magellanica*, and the starfish *Anasterias rupicola* (Blankley 1982, Blankley and Grindley 1985). Because it is so numerous at the Prince Edward Islands, *N. marionis* has been indicated as being important in making phytoplanktonic primary production available to the numerous top predators on the islands (Perissinotto and McQuaid 1990, Pakhomov and Froneman 1999).

Over a century after its discovery, publications dealing with the shrimp remain 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 on the Prince Edward Islands. Only a few aspects of its biology are known to date (Perissinotto and McQuaid 1990, Kuun et al. 1999, Pakhomov et al. 1999, 2000). Given its importance in the food web, more knowledge of the fundamental aspects of its biology is required to understand the role of *N. marionis* in coupling marine and terrestrial food webs. Data on its life cycle, longevity, distribution, reproduction and mortality are crucial in the construction of realistic future maximum sustainable yield models for this species. *N. marionis* is not commercially exploited at present (Pakhomov et al. 2000), although this may change in the future.

1.3 Objectives of the Project

The objectives of this project are to study the demographic parameters (e.g. growth rates) and general biology (e.g. reproduction, development, feeding) of the swimming prawn *Nauticaris marionis* at the Prince Edward Islands.

Nauticaris marionis is thought to be one of the key species of the islands' benthic community that makes pelagic production directly (via larvae) or indirectly (via adults) available to large predators (Perissinotto and McQuaid 1990, Pakhomov et al. 1999, 2000). Given its importance in this food web, this project will form a contribution to the ongoing, broader research programme around the Prince Edward Islands.

In Chapter Two of this thesis, the life cycle of the swimming prawn at Marion Island shelf is presented. A multi-year cohort analysis of *N. marionis* is conducted, and several demographic parameters and long-term variability in recruitment are investigated.

In Chapter Three, the gonadal development in different sampling years is described. Initial larval development in the eggs and the mean egg diameter for females of different sizes are estimated to complement the fecundity data.

Chapter Four provides additional information on the diet and assesses the *in situ* daily ration of males and females of *N. marionis*. A summary of findings of this project is provided in Chapter Five and suggestions for further research are presented.

1.4 Samples and sample sites

Specimens of the swimming prawn *Nauticaris marionis* were collected using dredges, grabs or an Agassiz trawl in the vicinity of Marion Island. Samples came from four major collections (Table 1.1). These were;(i) the University of Cape Town Surveys in 1984 (August to September), in April 1985 and 1987, and September 1988; (ii) the fourth and fifth Rhodes University Marion Island Oceanographic Surveys (MIOS) conducted from April to May 1999 and 2000; (iii) the first and second Rhodes University Marion Offshore Variability Ecosystem Study (MOVES) conducted from April to May 2001 and 2002; (iv) the 2003 samples were collected from late March to April during the Dynamics of Eddy Impacts on Marion's Ecosystem Study (DEIMEC) II Programme (Table 1.1). Collection (i) was provided by the South African Museum in Cape Town, collections (ii) to (iv) were stored at Rhodes University, Grahamstown. The author collected samples from 2000 to 2003 himself.

All specimens were preserved in either 4% buffered formalin or 70% ethanol seawater solutions, except for specimens collected during 2001 and 2002 which were frozen at –18^{o}C for storage. Samples were taken at different times and sites in the vicinity of Marion Island (Fig. 1.1). Most stations were sampled near the Marion Island base and at Natal Bank (see Fig. 1.1 for stations, and Table 1.1 for co-ordinates).

Table 1.1 Details of the samples collected at Marion and Prince Edward Islands (see also Fig. 1.1).

Sample number	Dates	Sampling Station co-ordinates (South – East)	Sampling time (GMT)	Sampling details and sample pre-treatment	Sampling depth (m)
1	06/09/1984	46° 83' – 37° 78'	15:11	Dredge, mesh size 7mm, 70% ethanol	36-52
2	29/08/1984	46° 89' – 37° 89'	08:45	Dredge, mesh size 7mm, 70% ethanol	90
3	21/04/1985	46° 58' – 37° 93'	09:30	Dredge, mesh size 7mm, 70% ethanol	48-50
4	26/04/1985	46° 81' – 37° 69'	08:12	Dredge, mesh size 7mm, 70% ethanol	34-42
5	04/05/1987	46° 89' – 37° 91'	16:30	Dredge, mesh size 7mm, 70% ethanol	42-85
6	26/04/1987	46° 78' – 38° 00'	15:05	Dredge, mesh size 7mm, 70% ethanol	92-133
7	02/09/1988	46° 67' – 37° 85'	09:39	Dredge, mesh size 7mm, 70% ethanol	350-600
8	05/09/1988	46° 97' – 37° 86'	15:25	Dredge, mesh size 7mm, 70% ethanol	92-105
9	10/04/1999	46° 88' – 37° 92'	08:17	Dredge, mesh size 7mm, 4% formalin	111-123
10	10/04/1999	46° 85' – 37° 92'	11:35	Agassiz trawl, 4% formalin	130
11	10/04/1999	46° 92' – 37° 89'	22:00	Agassiz trawl, 4% formalin	100-105
12	11/04/1999	46° 95' – 37° 89'	16:24	Agassiz trawl, 4% formalin	111
13	28/04/1999	46° 30' – 37° 75'	10:32	Dredge, mesh size 7mm, 4% formalin	119
14	28/04/1999	46° 80' – 37° 70'	10:55	Dredge, mesh size 7mm, 4% formalin	142
15	28/04/1999	46° 82' – 37° 66'	12:00	Dredge, mesh size 7mm, 4% formalin	107
16	29/04/1999	46° 87' – 37° 86'	08:02	Grab, 0.25m^2, 4% formalin	39.7
17	30/04/1999	46° 62' – 37° 85'	16:11	Dredge, mesh size 7mm, 4% formalin	125
18	10/04/2000	46° 87' – 37° 88'	15:54	Dredge, mesh size 7mm, 4% formalin	97
19	11/04/2000	46° 86' – 37° 91'	09:31	Dredge, mesh size 7mm, 4% formalin	125
20	11/04/2000	46° 88' – 37° 88'	12:18	Dredge, mesh size 7mm, 4% formalin	50-60
21	13/04/2000	46° 85' – 37° 94'	09:54	Dredge, mesh size 7mm, 4% formalin	130
22	13/04/2000	46° 79' – 38° 01'	10:44	Dredge, mesh size 7mm, 4% formalin	90
23	13/04/2000	46° 79' – 38° 02'	10:50	Dredge, mesh size 7mm, 4% formalin	90
24	13/04/2000	46° 79' – 38° 00'	11:45	Dredge, mesh size 7mm, 4% formalin	55
25	13/04/2000	46° 95' – 38° 01'	12:15	Dredge, mesh size 7mm, 4% formalin	114
26	16/04/2000	46° 86' – 37° 88'	11:06	Dredge, mesh size 7mm, 4% formalin	115-117
27	18/04/2000	46° 88' – 37° 89'	06:49	Dredge, mesh size 7mm, 4% formalin	90
28	18/04/2000	46° 88' – 37° 89'	07:01	Dredge, mesh size 7mm, 70% ethanol	75

Table 1.1…continued

Sample numbers	Dates	Sampling Station co-ordinates (South – East)	Sampling time (GMT)	Sampling details and sample pre-treatment	Sampling depth (m)
29	18/04/2000	46° 88' – 37° 89'	07:01	Dredge, mesh size 7mm, 4% formalin	75
30	18/04/2000	46° 88' – 37° 93'	08:13	Dredge, mesh size 7mm, 4% formalin	124
31	22/04/2000	46° 87' – 37° 88'	11:28	Dredge, mesh size 7mm, 4% formalin	80-90
32	23/04/2000	46° 87' – 37° 95'	10:35	Dredge, mesh size 7mm, 4% formalin	127
33	13/04/2001	46° 88' – 37° 89'	16:36	Dredge, mesh size 7mm, frozen at –18°C	70
34	15/04/2001	46° 87' – 37° 88'	13:14	Dredge, mesh size 7mm, frozen at –18°C	100
35	15/04/2001	46° 86' – 37° 87'	15:26	Dredge, mesh size 7mm, frozen at –18°C	90
36	15/04/2001	46° 86' – 37° 87'	16:07	Dredge, mesh size 7mm, frozen at –18°C	85
37	23/04/2002	46° 81' – 37° 93'	18:30	Dredge, mesh size 7mm, frozen at –18°C	100
38	23/04/2002	46° 87' – 37° 87'	13:55	Dredge, mesh size 7mm, frozen at –18°C	50
39	30/03/2003	46° 82' – 37° 89'	22:00	Dredge, mesh size 5mm, 4% formalin	90-110
40	01/04/2003	46° 82'-37° 91'	14:45	Dredge, mesh size 5mm, 4% formalin	140
41	01/04/2003	46° 85' – 37° 91'	15:25	Dredge, mesh size 5mm, 4% formalin	120
42	01/04/2003	46° 84' – 38° 03'	21:15	Dredge, mesh size 5mm, 4% formalin	154
43	01/04/2003	46° 87' – 37° 89'	22:47	Dredge, mesh size 5mm, 4% formalin	111
44	03/04/2003	46° 65' – 38° 02'	11:05	Grab, 0.25m^2, 4% formalin	130
45	03/04/2003	46° 78' – 38° 06'	19:15	Dredge, mesh size 7mm, 4% formalin	180
46	03/04/2003	46° 80' – 37° 99'	20:35	Dredge, mesh size 5mm, 4% formalin	77
47	03/04/2003	46° 82' – 37° 97'	21:50	Dredge, mesh size 5mm, 4% formalin	142
48	03/04/2003	46° 84' – 37° 94'	23:50	Dredge, mesh size 5mm, 4% formalin	133
49	03/04/2003	46° 85' – 37° 99'	00:30	Dredge, mesh size 5mm, 4% formalin	129
50	20/04/2003	46° 75' – 37° 84'	22:15	Dredge, mesh size 5mm, 4% formalin	182
51	20/04/2003	46° 84' – 37° 93'	23:50	Dredge, mesh size 5mm, 4% formalin	135
52	21/04/2003	46° 86' – 37° 86'	11:55	Dredge, mesh size 5mm, 4% formalin	55
53	21/04/2003	46° 86' – 37° 87'	12:06	Dredge, mesh size 5mm, 4% formalin	77
54	21/04/2003	46° 88' – 37° 89'	16:30	Dredge, mesh size 5mm, 4% formalin	97-105

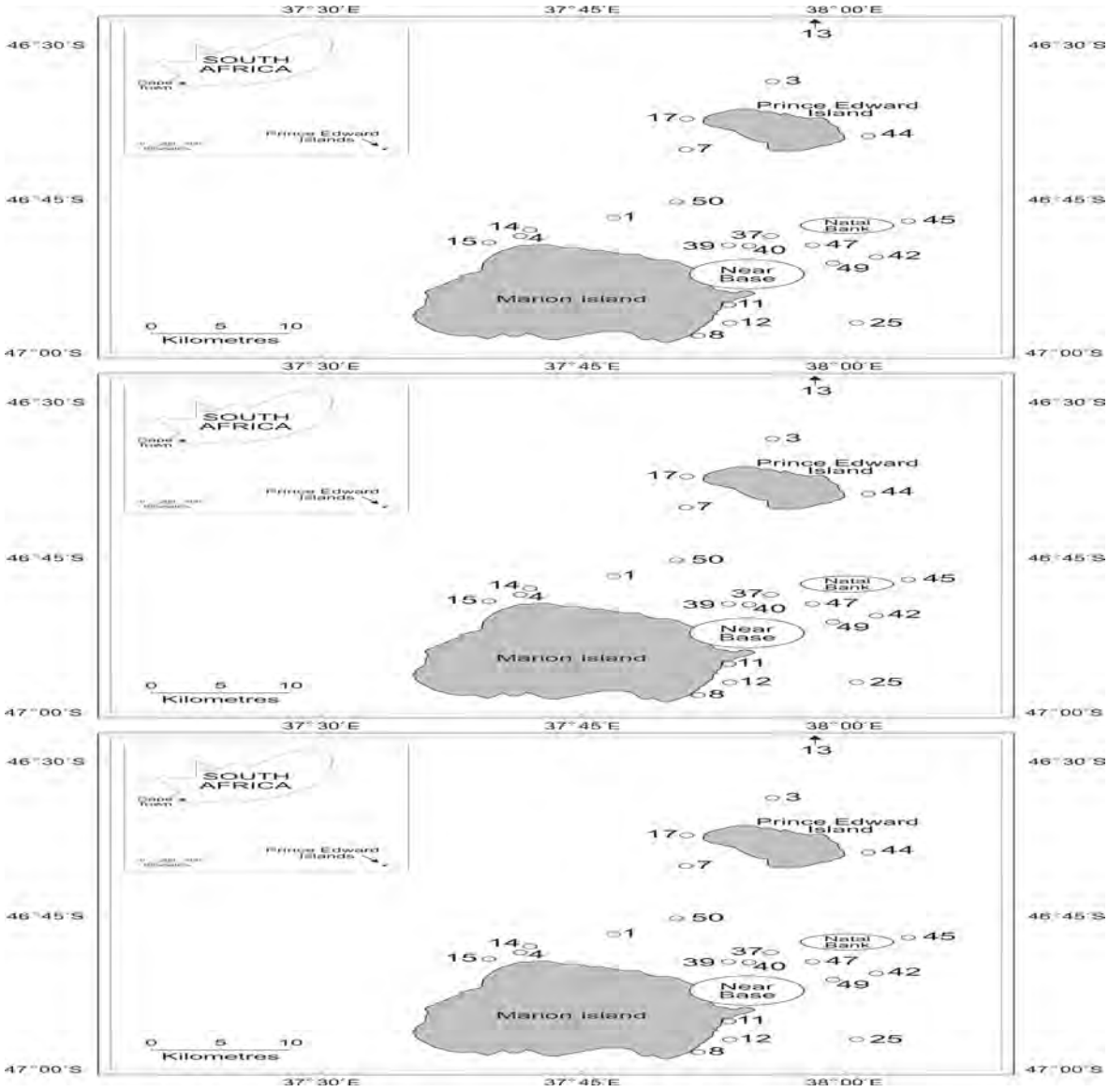

Fig. 1.1 Location of sampling sites at Marion and Prince Edward Islands between 1984 and 2003. Most samples were collected near the base (for example samples 2, 5, 9, 10, 16, 18-21, 26-36, 38, 41, 43, 48 and 51-54), and at Natal Bank (6, 22-24 and 46). The arrow above sample 13 represents sampling away from the islands (for positions see Table 1.1).

Chapter Two

Growth and cohort analysis

2.1 Introduction

Many ecosystem and most population studies require that the ages of animals be determined, or at least estimated (Grant et al. 1987). Important aspects of the populations of marine invertebrates include: the number of age classes, growth rates, recruitment and mortality as a function of age. At least three approaches can be used to estimate the age structure of a population.

One approach is aging of individual invertebrates using growth rings on the hard exoskeleton. The hard parts of many groups of invertebrates show more or less distinct striations, which are often believed to be annual (Grant et al. 1987). The estimation of demographic parameters is relatively straightforward when aging of individuals is based on annual patterns in their hard structures. In the absence of hard body structures with annuli, the estimation of age, growth and, mortality for invertebrates such as crustaceans can be very difficult. Crustaceans have no permanent hard body parts, such as fish otoliths or molluscan shells, to carry a record of seasonal or annual changes in growth rates, and allow actual ageing or validation of indirect age methods. Although some crustaceans may have hard body structures, growth estimation still remains difficult due to loss of the age marks during moulting.

Another method is direct observation of individuals, either held in confinement or from mark/recapture experiments. Tagging or marking of individuals can also yield information on age structure, and is sometimes feasible in invertebrates, using fluorescent markers, coloured dyes or physical marks on shells (Ropes et al. 1984, Kaehler and McQuaid 1999, McQuaid and Lindsay 2000). Recapture rates can, however, be very low (Ropes et al. 1984), and also use of growth markers such as fluorochromes can cause problems such as high post-treatment mortality (Kaehler and McQuaid 1999).

The third approach is the identification of cohorts based on length frequency distributions from samples taken at different times. This approach is based on the observation that the length composition of a population often exhibits modes (peaks) among the younger age groups. The particular shape of a size frequency distribution is the result of recruitment, growth, mortality and sampling bias. Bias occurs if the sampling method, (eg. the gear employed) is selective with respect to size or age. Several graphical methods have been developed to perform this task (Harding 1949, Cassie 1954 both cited in Grant 1989), and are still in use (Henmi 1992). These methods commonly depend on some sort of graphical interpretation that requires sample sizes to be large enough to yield clear modes in size/frequency histograms (Grant 1989). The results of these methods "are not reproducible and it is quite possible for two workers to obtain very different answers from the same set of data" (Macdonald and Pitcher 1979: 989). As a result, these methods can lead to misleading conclusions (Grant 1989).

Recently, several advances have been made in the analysis of length frequency data (Macdonald and Pitcher 1979, Macdonald and Green 1988, Fournier et al. 1990, 1991). The MIX algorithm, a maximum likelihood-principle based procedure to decompose a time series of length frequency data into year classes, was developed by Macdonald and Pitcher (1979), and a MULTIFAN algorithm, also a likelihood-principle based procedure to decompose a single collection of length-frequency data into year classes, was developed by Fournier et al. (1990, 1991).

In this study, analyses have been done using the new Rmix program (Du 2002), adapted from the original version of MIX by Macdonald and Pitcher (1979) and Macdonald and Green (1988). This program has been used to group size classes from a time series of length-frequency distributions into age classes. A mixture distribution is a compounding of statistical distributions, which arises from sampling of non-homogeneous populations (or mixed populations) with a different probability density function for each component (Du 2002). Finite mixture distributions are important modelling tools in many diverse fields of science and have been used since the time of

Pearson in 1894 (Hathaway 1983, Titterington et al. 1985) and are still used today as a major tool in the development of good estimation techniques. Size-frequency distributions in animal populations with distinct age-groups, the distribution measure of percentage of total weight in various ranges of grain size, the distribution of some diagnostic measure in a population of patients, some of whom have a given disease and some of whom do not, and the distribution of the earth's surface area in terms of elevation, the hypsometric curve of the earth, are all examples of mixed distributions given by Titterington et al. (1985) and Du (2002). A finite mixture has a finite number of components (Du 2002). For this chapter, the current method applied is the standard maximum likelihood estimation (MLE) method, although such methods give rise to theoretical and computational difficulties. For example, estimating the parameters of a mixture distribution is difficult when the components overlap heavily. An approach designed by Macdonald and Pitcher (1979) to circumvent these difficulties is to apply constraints on individual parameters.

The new version of MIX, called Rmix, for the R statistical computing environment has most of the functionality of Macdonald and Pitcher's MIX software, but with greatly improved numerical methods and graphics. Rmix's improved numerical methods are based on a combination of the EM algorithm and a Newton-type method implemented in the function `nlm` provided by the R system (Du 2002). R is a language and environment for statistical computing and graphics. R implements a dialect of the S language developed by Becker et al. (1988, cited in Ihaka and Gentleman 1996). Ross Ihaka and Robert Gentleman initially developed R in 1996; now the R Development Core Team oversees its development. R is open-source and runs on UNIX, Windows and Macintosh, and can be downloaded at no cost.

In this chapter cohort analysis is carried out using samples from different years. The aims are to acquire information about the population structure and the growth of age classes using the Rmix algorithm program to estimate growth parameters.

2.2 Body size measurement

Ridoux's (1994) study of diet in 27 species of seabirds at Crozet Island used the body length and eye diameter of the crustacean prey, including *Nauticaris marionis*, found in the stomachs of seabirds as a measure of body size. Although the body length used in Ridoux (1994) was not well defined, one can infer from the diagram of the euphausiid given that it was most likely the distance from the anterior tip of the rostrum to the posterior end of the telson (definition from Kuun et al. 1999). Total length can be of use when measuring living shrimps. For example, a laboratory study on the caridean shrimp *Lysmata wurdermanni* by Lin and Zhang (2001) used the total length, because it is much easier to use than carapace length (discussed below), especially for small living individuals. However, a recent report by Kuun et al. (1999) showed that the body length or total length measurement is unsuitable as an indicator of body size for *N. marionis* for several reasons. Firstly, *N. marionis* adopts a foetal-like position making the measurement of the straight-line length difficult, a problem also experienced by Kuris and Carlton (1977) working on ovigerous females of the caridean genus *Crangon*. Secondly, if *N. marionis* is captured, swallowed and partially digested, its measurement is unlikely to be available (Kuun 1998). Lastly, a study of the caridean shrimp, *Pandalus platyceros* by Hoffman (1972) stated that lengths of the rostrum and the abdomen vary in caridean shrimps of identical carapace lengths.

An alternative measure of body size, the carapace length, has been used for decapod crustaceans (Hoffman 1972, Bauer 1976, Kuris and Carlton 1977, Bauer 1986, Gorny et al. 1992, Mascetti et al. 1997, Maiorano et al. 2002), including studies of *N. marionis* (Parker 1984, Perissinotto and McQuaid 1990, Ridoux 1994). Although carapace length is widely used, a number of different definitions are found in the literature, many of them ambiguous (Kuun 1998). The first study on size-frequency distributions of *N. marionis* by Parker (1984) failed to provide a definition of carapace length. In a study of the ecological role played by *N. marionis* at the Prince Edward Islands, Perissinotto and McQuaid (1990) used a histogram from original data of Parker (1984) without providing a clear definition of carapace length.

An early study by Kuris and Carlton (1977) described the carapace length as "a measure from the posterior margin of the orbit to the dorsal midline of the posterior margin of the carapace". Using this definition, Kuun et al. (1999) suggested that carapace length is the most accurate indicator of body size in *N. marionis*. This is due to the fact that the carapace is a relatively long and inflexible body part that is likely to survive the stresses of digestion (Kuris and Carlton 1977), making it the preferred indicator of body size. Therefore, later studies on *Nauticaris marionis* used the well-defined carapace length (eg. Pakhomov et al. 1999, 2000).

For this thesis, the carapace length (CL) defined after Kuris and Carlton (1977) as "a measure from the posterior margin of the orbit to the dorsal midline of the posterior margin of the carapace" will be used as an indicator of body size for all samples of *Nauticaris marionis*.

2.3 Materials and methods

2.3.1 Data handling

Carapace length (CL), described above, was measured on the right-hand side of all individuals collected between 1999 and 2003 (for sampling details see section 1.4; Table 1.1). Measurements were taken to the nearest hundredth of a millimetre under a Wild-M5 dissecting microscope equipped with an ocular micrometer. See Figure 2.1 for a diagrammatic representation on how the carapace length was measured in millimetres. Although sizes of individuals varied quite considerably, a magnification of X6 was used on all specimens. Separate size-frequency histograms for each sample were plotted using 0.25mm size class intervals. After carapace length measurement, the sexes of individuals were determined following Pakhomov et al. (2000). For full details see the following chapter, section 3.2. An exception for sexing specimens was made for animals collected in 2001, due to the high number of damaged animals (details in Chapter 3 section 3.2.2).

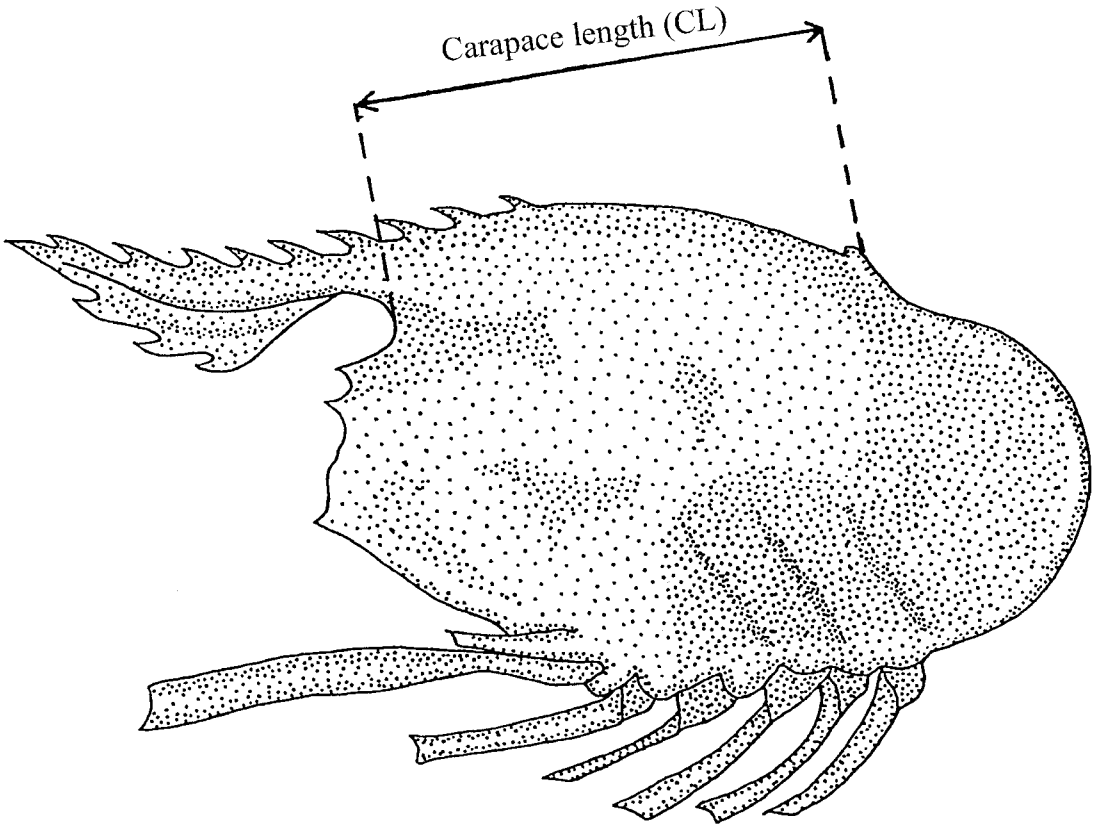

Fig. 2.1 Diagrammatic representation of the measurement of the carapace length (CL) from Kuun et al. (1999).

Some specimens examined were found to have a bulge under the carapace caused by a parasitic isopod of the family Bopyridae (see Plate 1 and 2). Although there were also some loose carapaces found, none of them possessed the bulging characteristic caused by these parasites. All parasites found growing under the carapace of *N. marionis* appeared to be large female bopyrids, each usually carrying a dwarf male attached to its abdomen (Plate 3). In some specimens, bulged carapaces were detected without the presence of the parasite, presumably indicating that the shrimp had survived infestation. Specimens demonstrating characteristics of bulging were treated as infected, with or without the presence of the parasite. Infected specimens were excluded for this chapter because parasites may bias growth rate estimates (Mathews et al. 1987).

2.3.2 Rmix for fitting mixture distribution

To run Rmix, R must first be installed in the system, and as stated above, it can be freely downloaded from the nearest Comprehensive R Archive Network (CRAN) site. R has been started at version 0.90.0 and is rapidly developing. At the time of writing, the latest version is 1.6.2. After installing R, Rmix can then be installed as a package (i.e. library) to the R environment.

The mixture analysis method (Rmix; Du 2002) was used to identify cohorts (population components) within length-frequency distributions constructed from population samples. Preparation of the data, the initial values of parameters and constraints on parameters are required before embarking on any analysis. Rmix assumes that the data are grouped over successive intervals. Size-frequency data are the most common form of grouped data. Samples are presented as size-frequency data because the appearance of separate modes that the data reveal is generally interpreted as an indication of age groups. Although Rmix deals with either grouped data or conditional data, grouping greatly simplifies the calculation of maximum likelihood estimates (Macdonald and Pitcher 1979). Grouped observations are often given in the form of a histogram. The program computes a mixture of normal distributions and compares it to the length-frequency data of a sample. The

appearance of modes in the length-frequency plot depends upon a combination of the distances between means, the magnitude of the variances, the proportion of the population in each age-group and, the overall sample size (Macdonald and Pitcher 1979). This program then requires that the user specify the number of components within the distribution.

2.3.3 Estimating components and applying constraints on the parameters

The parameters of the components will not be known initially; only the histogram is available. If the number of components is unknown, the user can use his discretion to look at the different modes because components do not all form distinct modes and there is therefore no indication from the histogram alone as to the number of components to expect. With practise, one is able to estimate the number of components in the original histogram.

Fitting a series of curves to the data requires the user to estimate the mixing proportions and the parameters of the component distribution. Firstly, the user needs to determine which parameters of the mixture can be estimated and which cannot. The initial parameter values should be in the form of three variables, namely, the proportions (π_i), means (μ_i), and standard deviations (σ_i). In this study, starting values for means and standard deviations were determined by inspecting the histograms provided by an Rmix function that plots the histogram. These values should be properly assigned, because the iterations employed will not converge if they are not well chosen. The starting values for proportions are less critical, since they can usually be improved very efficiently. For each sample analysed, a histogram was plotted with the components using the starting values to check if the means and standard deviations were reasonable.

The program allows iterative alterations of standard deviations and means to optimise the “goodness-of-fit” for each plot. Altering the mean will shift the corresponding component to the left or to the right. Increasing the standard deviation of the mode

will cause the corresponding component to spread or flatten, while decreasing a standard deviation will produce a narrower and higher peak. It is better to choose initial values of standard deviations that are too small, rather than too large. Large standard deviations cause components to overlap more than necessary, obscuring the resolution of the means. To avoid bias in the number of components chosen, each sample was analysed several times with different numbers of components so that the data were reproducible. Choosing a constraint mainly depends on some form of prior information about the parameters or the relations among them.

The function `mixconstr` allows the user to organize the constraints in the form Rmix requires and to check if the constraints meet these requirements. Rmix allows a combination of the constraints on the proportions, means and standard deviations to be applied according to various practical situations. One should note that not all combinations are allowed, theoretical and practical restrictions have to be considered before choosing constraints to apply on the parameters. For this study, each component of the mixture representing a cohort, the mean carapace length (and standard deviation) and the proportion of the total sample represented by that component (cohort) were calculated. After running the function `mix`, an optimal combination of these parameters was achieved for the entire mixture. For each combination of solutions, an R object containing the estimated parameters, standard errors of the parameters estimated, the goodness-of-fit, chi-square statistic and, the P-value (probability of the null hypothesis of no difference between the mixture distribution and the original size-frequency distribution) were generated by the Rmix program. The solution will yield the highest P-value corresponding to the lowest chi-square (χ^2) value. The solution has to be one with a P-value greater than the level of significance that is preset to 95 percent.

After cohorts were identified from the analysis, the resulting size-frequency plots of all samples were compared. Beginning with cohorts on the far right of the histogram, cohorts of a sample were matched with what appeared to be the same cohorts in samples before and after following Baldwin and Bauer (2003). In this study cohorts were matched using the Kolmogorov-Smirnov test for two independent samples using

R. Recruitment is defined as the entry of individuals into the juvenile and adult benthic population after settlement from the planktonic larval stage following Connell (1985). For this study, recruitment was seen as the entry of individuals into the smallest size classes (cohort) of the annual size-frequency distribution (see results).

2.3.4 Fitting the von Bertalanffy growth curve (VBGC)

Estimation of growth is assumed to follow the von Bertalanffy growth function (von Bertalanffy 1938, cited in Ulmestrand and Eggert 2001):

$$L_t = L_\infty [1 - e^{-K(t - t_0)}] \qquad (1)$$

where L_t is length at age t, L_∞ is the asymptotic length, K (yr^{-1}) is the rate at which L_∞ is approached, and t_0 is time zero.

The asymptotic length, is the mean length that an individual would reach if it grew for an infinite number of years, and was estimated from the mean of a selection of the largest carapace lengths in the samples. Thereafter, a nonlinear least-square method from R library (`nls`) was used to estimate the parameters K and t_0.

Each cohort consisted of specimens of the same size group from the same stock, and this was termed a generation, following Pakhomov et al. (2000). The average of the mean lengths of cohorts of the same generation was used to estimate the mean carapace length of the generation. The estimated means for each generation were used to compute the von Bertalanffy growth curve parameters. In this study *N. marionis* was assumed to spawn once annually following Bauer (1989) and Pakhomov et al. (1999). Spawning more than once a year was considered improbable for *N. marionis* because of the strong seasonality experienced at the latitudes of the Prince Edward Islands group (Kuun 1998). For this reason it was assumed for the purposes of the calculation that the first generation was one year old.

2.4 Results

2.4.1 Cohort analysis

Five cohorts were identified in the 1999 sample and assigned letters in alphabetical order. The cohort on the far right of the 1999 sample, the oldest cohort in the sample, was assigned letter “A” (Fig. 2.2). Cohort A from the 1999 sample did not match with any cohort from the 2000 sample and was presumed to have gone extinct (Fig. 2.2). In the 2001 sample, cohort B also disappeared and an additional cohort (F) was introduced. Cohort F did not match with any cohort from the previous samples, and was therefore considered to be a cohort of recruits with a mean carapace length of 3.103mm. Working on the assumption that the specimens are normally distributed, the mean carapace length for cohort F was used to calculate a threshold carapace length for recruits, by taking the upper limit of the 95% confidence interval. This gave a value of 3.15mm. By this definition, cohort E having a mean carapace length below this threshold was considered to be a cohort of recruits. Cohort C from the 2001 sample did not match with any cohort from the 2002 sample and was also considered to have gone extinct. A new cohort, with mean carapace length 13.165mm, appeared in the 2002 sample and did not match with any cohort from the 2001 sample. This cohort was then assigned letter “Z” to symbolize its uniqueness from other cohorts (Fig. 2.2). Cohort Z could not be matched with the 2001 sample and may be artifactual as the sample size for the 2002 sample was very small. This resulted in only two cohorts (D and E) matching with the previous sample. When matching the 2002 and 2003 samples, only cohorts D and E matched (Fig. 2.2). The mean carapace length of the new cohort was too large to be considered recruitment in that sample. Therefore, this cohort was matched with cohort F from the 2001 sample. The reappearance of cohort F in the 2003 sample was due to the small sample size in the 2002 sample (Fig. 2.2).

2.4.2 Estimation of growth parameters

The estimated growth parameters were L_∞ = 14.05789mm, K = 0.22239 per year (P = 0.0135) and t_0 = - 0.05174. L_0 was calculated as 0.16083mm. Using the estimated parameters, the von Bertalanffy growth curve (Fig. 2.3) was fitted (χ^2 = 14.8823; P = 0.0211192) from data presented in Table 2.1.

Table 2.1 Mean carapace lengths and standard deviations for generations of *N.marionis*.

Generation	Mean Carapace length	Standard deviation
1	4.1790	1.521693793
2	4.6026	1.565652675
3	6.0615	2.126258639
4	6.7494	1.30484980
5	8.1155	0.757311363
6	12.2320	-
7	13.1650	-

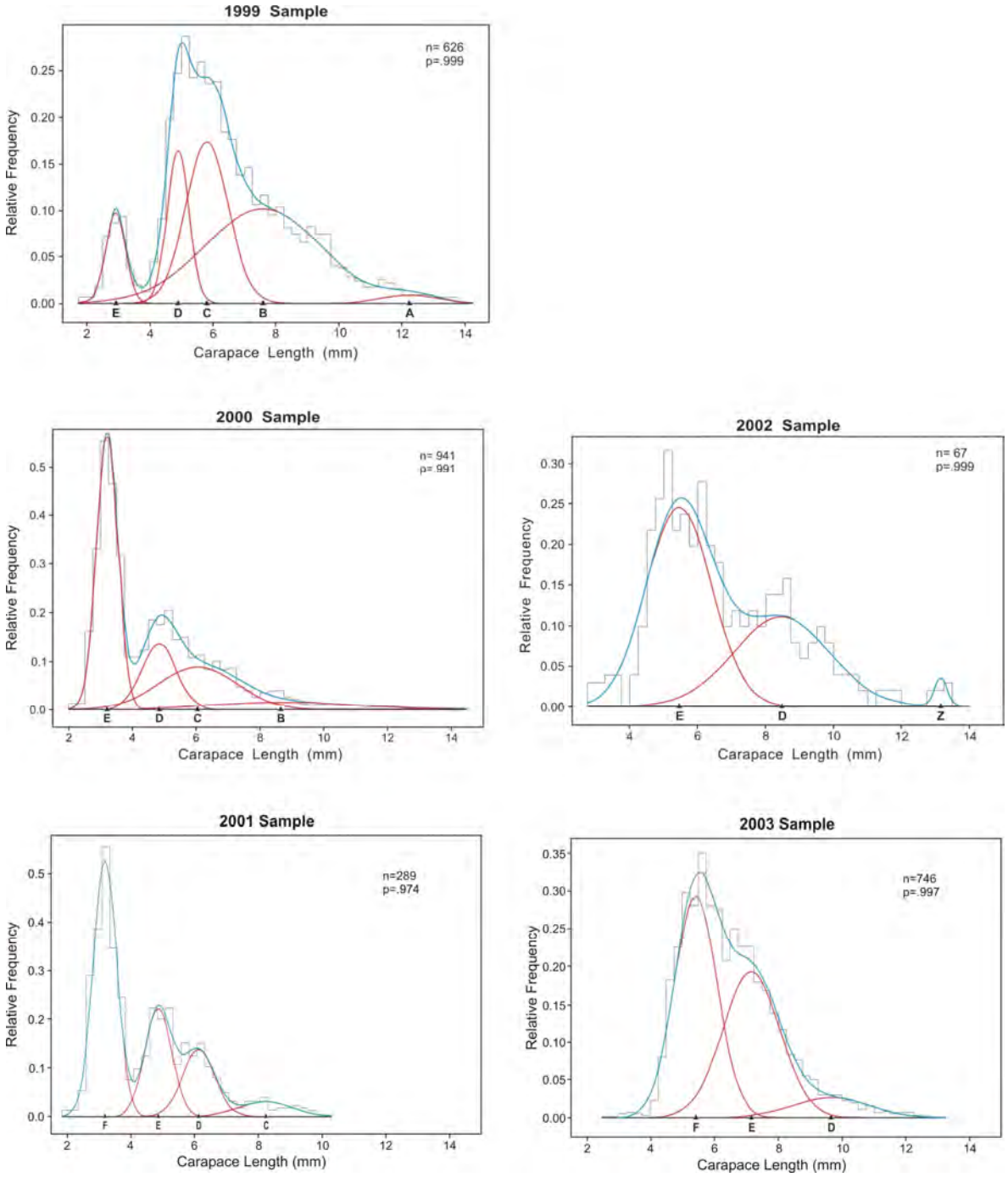

Fig. 2.2 Cohorts of *N. marionis* identified from size-frequency distributions for samples collected between 1999 and 2003 at the Prince Edward Islands, using mixture analysis (Rmix). *Upper case letters* are used for identified cohorts; *n* = number of individuals in the sample; *p* = probability of goodness-of-fit of the mixture distribution (cohort curves) with the observed size-frequency distribution. The blue line = observed curve and the red line = expected curve.

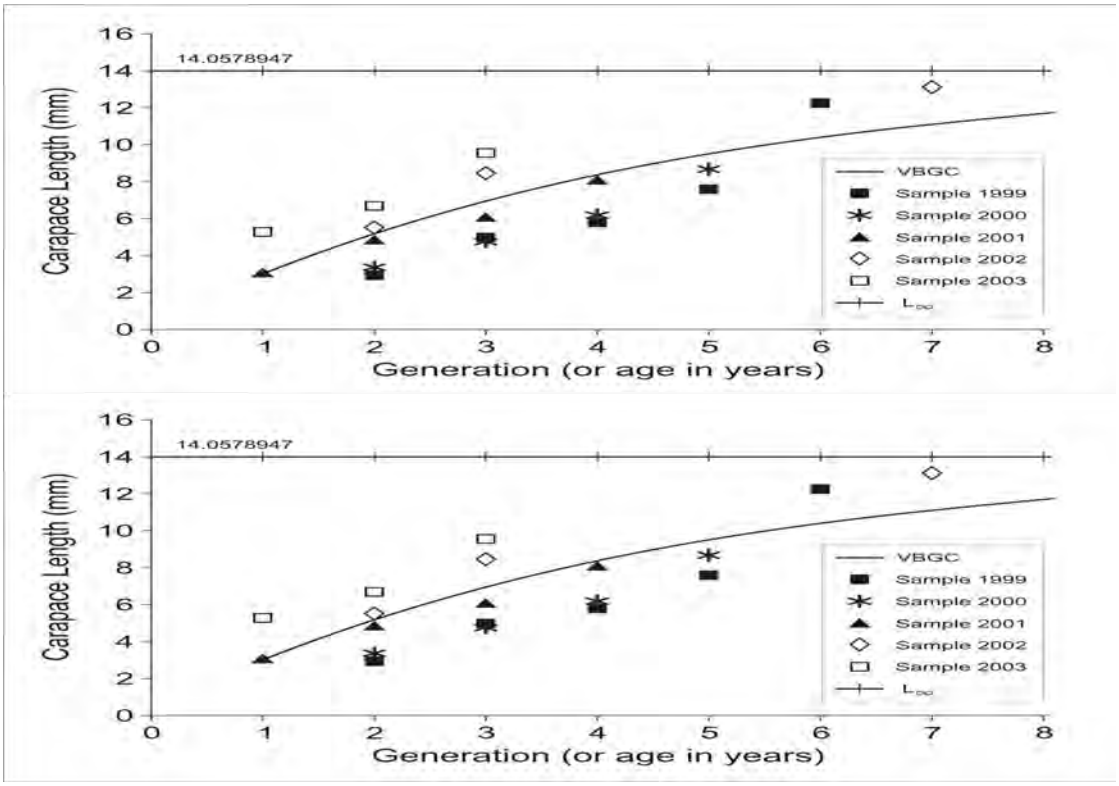

Fig. 2.3 The von Bertalanffy length-based growth curve (VBGC). Data points indicate sample cohort and generation mean sizes. The value (14.0578947) on the horizontal line represents L_∞.

2.5 Discussion

2.5.1 Using the program Rmix

The program Rmix from Mix is an interactive program for fitting mixtures of distributions to single length-distributions. Rmix analyses histograms as mixtures of statistical distributions by finding a set of overlapping component distributions, which can be normal, lognormal, exponential or gamma distributions, that give the best fit to the histogram and the statistical method used is maximum likelihood estimation for grouped and conditional data.

Rmix has the ability to accept raw data as input without first organizing it into class intervals. The program has an inbuilt function (`mixgroup`) that allows the user to have control of the choice of the best class interval from the raw data entered. When estimating the number of components, which is usually at the user's discretion, the program is able to guide the user if, for example, a component has been missed in the selection. The program allows the user to alter the means and standard deviations to get the best possible fit for cohorts. The applied constraints can also be tested using the function (`testconstr`) to verify their validity.

The first and only reliable study of length frequency distributions in *N. marionis* was reported in Pakhomov et al. (2000) using a different program called FAO-ICLARM Fish Stock Assessment Tools (FiSAT). Although Perissinotto and McQuaid (1990) also reported on size distributions using Parker's (1984) diagram, because of the lack of a clear definition of the carapace length used this is disregarded in this thesis and not considered in the discussion. Although FiSAT provides the degrees of freedom from the observed data, significance level and χ^2 values, its decisions on significant differences between the accumulated frequency and the observed data do not correlate with those reached when using the standard χ^2 tables (Kuun 1998 MSc thesis, and subsequently published in Pakhomov et al. 2000). Therefore, Kuun (1998) states that, when using FiSAT, the user needs to consult the standard χ^2 tables as the program may provide inaccurate values. In contrast, Rmix provides accurate p values.

2.5.2 Growth in cohort analysis

Initially, five cohorts of *N. marionis* were identified in the 1999 sample, although four were expected from the inspection of the original data. The oldest cohort (cohort five) would not have been detected easily because, as shrimps age the mean carapace length of cohorts become closer, but the spread (standard deviations) often increases and aging becomes more uncertain. Using Rmix, this cohort was detected even though it had low numbers. Inability to detect far right hand side cohorts (older cohorts) may result in these cohorts being ignored or underestimated.

A nonparametric Kolmogorov-Smirnov test for two independent samples was used to match cohorts instead of the standard comparison of the means and standard deviations of the cohorts. The reason is that the Kolmogorov-Smirnov test takes into account shifts in either of the distributions being compared. In this study such shifts are clearly temporal effects because cohorts are matched in subsequent years, but shifts could also reflect phenotypic responses (pers. obs.). In Pakhomov et al. (2000), cohorts were compared using the standard comparison test mentioned above, which I believe could have led to inappropriate matching of cohorts. One of the reasons for matching cohorts in the manner described above might be that Pakhomov et al. (2000) examined only three samples, which were not in sequential order. Another reason is that the study had samples taken 12/13 years earlier than others and only a small sample collected from a 1997 RMT*6 sample.

Zupo (1994) used sex inversion as a strategy for matching cohorts in the shrimp *Hippolyte inermis*, based on the relationship between the growth rates and the sex inversion. It was suggested that *H. inermis* displays an unusual sexual inversion strategy that could be in synchrony with environmental factors. The sexual inversion strategy assumes that when matching cohorts the gender proportions in that cohort are the same as the matched one. It then follows that the gender proportion of the same age groups is assumed to be relatively constant. Using the *t*-test to confirm matching results from the

table of generations in Pakhomov et al. (2000), all presented generations were consistent with results of cohort analysis, with the exception of generation three.

Presented histograms in the previous study suggest that cohorts (from the 1984 dredge and the 1997 RMT-8) from generation three were matched, based on the gender proportions that were predominantly females. Therefore, based on these findings the authors might have used the sexual inversion strategy to match their cohorts into generations. The sexual inversion method when applied, requires a very large data set for all samples collected during that sampling period. This requirement is in accordance with Bernoulli's law of large numbers (Shiryayev 1984), which states that (gender) proportions converge in probability to true proportions, as *n* (number of observations) approaches ∞. Given the data set from Pakhomov et al. (2000), the number of observations in some cohorts was not large enough to confirm the matching of cohorts by using gender proportions. For example, in the 1984 dredge sample the number of observations in cohort three calculated using the proportional contribution to overall sampling (12.1%) was 47, as opposed to 150 individuals from cohort 2 of the1997 RMT-8 sample.

After cohorts were matched in the present study, the disappearance of the older cohort in the following length frequency distribution of that sample was referred to as having gone to extinction. The term was loosely used and did not necessarily imply that the age group was entirely wiped out, as it may have been highly misrepresented. This misrepresentation of the largest cohorts was thought to be related to the sampling gear used as it was only in the 1999 sample that the Agassiz trawl was used (see section 1.4; Table 1.1). In this sample specimens with carapace lengths greater than 11mm were more strongly represented. This was thought to be one of the factors that resulted in cohort A (oldest cohort) being identified in the 1999 sample. Nevertheless, although other gear (dredge) was also used, a test of association indicated that there was insufficient evidence to suggest that sampling results were dependent ($\chi^2 = 0.2123$, $p > 0.05$). This therefore rules out the possibility that the sampling method was biased, or that extinction of the old cohort in the length frequency distributions was artifactual. Using

the stratified sampling method ensures the statistical computations were not biased in this study.

The first generation is usually regarded as equivalent to a year class belonging to an age group with a minimum mean carapace length. Pakhomov et al. (2000) assumed the age of the first generation by attempting to establish the hatching time of this species. Only six gravid females were observed in May 1997 by Pakhomov et al. (2000), and compared with other reported studies. To summarise these findings; 1) Parker (1984) reported that many individuals analysed in that study were in berry in March 1984; 2) Adams (1990) recovered ovigerous females from the guts of penguins at the Prince Edward Islands between April and September 1984; lastly 3) in December 1873, Bate (1888) observed some females carrying ova in which the embryos were fully developed. In this study, a total of 30 females in berry were observed; 20 in August 1984 and 8 in September 1984 and 2 gravid females were taken in April 1999 (for full details see Chapter 3 on section 3.2.4). In the August 1984 sample, 11 females were carrying eggs in which the embryo was fully developed (stage 3) and the other 9 females carried eggs that were in stage 1 (full details on egg staging on section 3.2.4). Seven ovigerous females collected in September 1984 were carrying eggs in stage 3, but only one female in the same sample had eggs in stage 1. Two females collected in April 1999 were found to be carrying eggs with well-developed embryos (stage 3).

With the assumption that shrimps spawn once annually (Bauer 1989), spawning of *N. marionis* is likely to be seasonal and the hatching time would not vary significantly between years (Pakhomov et al. 2000). Reports gathered on gravid females (Parker 1984, Adams 1990, and Bate 1888) suggest hatching probably occurs just before April. In this study few females in berry were found in the April/May samples while two gravid females were found in April. This supports the suggestion of Pakhomov et al. (2000) that hatching mostly occurs earlier (March) but may continue until April/May.

Pakhomov et al. (2000) reported that the duration of the hatching period of *N. marionis* at the Prince Edward Islands is unknown. These authors, therefore, assumed that the first

generation of *N. marionis* was 1 year old. The first generation in Pakhomov et al. (2000) was observed to be partly planktonic, originating from the planktonic 1997 RMT-1*6 sample. In this study, the first generation was observed to be on average 1 year old. This is in agreement with previous work on *N. marionis*, although the first generation from this study did not originate from a planktonic sample but from a bottom dredge. The larvae of *N. marionis* were not observed in this study or by Pakhomov et al. (2000). Reports of megalope larvae in Perissinotto and McQuaid (1990) were probably juveniles (Kuun 1998). Kuun argued that these "larvae" were sampled from both midwater RMT-2 trawls and dredges sample in that study. The occurrence of these "larvae" in dredges taken in the same year as that of the dredge sample in Kuun's study makes one suspect that they were a group of postlarval individuals, of recognizable adult form, such as those observed in Kuun (1998). Pakhomov et al. (2000) suggest that the kelp beds may be used as a refuge habitat by *N. marionis* larvae. To my knowledge no larvae of *N. marionis* have been recorded from the Prince Edward Islands. Pakhomov et al. (2002) sampled the kelp beds of the islands as a possible refuge of these larvae, but none were found.

The data presented in this study indicate that, *N. marionis* can survive for at least 7 years under natural conditions. These results complement those obtained by Pakhomov et al. (2000) which suggest that *N. marionis* survives up to at least 5 years with the provision that older cohorts may have been missed because of the low numbers collected. Growth performance was compared with previous studies using the index of overall growth performance $\mathbf{\Phi}' = \log(k) + 2\log(CL_\infty)$ from Pauly and Munro (1984, cited in Pakhomov et al. 2000). The growth rate performance has increased from 1.57 (Pakhomov et al. 2000) to 1.64, in the present study. The average carapace length of the oldest *N. marionis* generation found was ~ 94% of L_∞. In the study by Pakhomov et al. (2000), the oldest shrimp was found to be ~ 71% of L_∞. Their low average carapace length might have been the result of undetected cohorts (older cohorts) on the far right hand side in the length frequency distributions.

2.5.3 Parasitism on growth

Parasitism by isopods of the family Bopyridae has been recorded on *N. marionis* at the Prince Edward Island (Branch et al. 1991, Pakhomov et al. 2000) and in New Zealand (Page 1984). Bopyrid isopods are haematophagous ectoparasites of decapod crustaceans. A review of the Bopyridae in New Zealand (Page 1984) showed that three subfamilies occupy three different areas in their host; most bopyrids occupy the branchial chamber, while the subfamilies Phyllodurinae, Hemiarthrinae, and Athelginae attach themselves to the host abdomen and the subfamily Entophilinae, resides within the host's visceral cavity. In *N. marionis* the parasite occupies the branchial chamber (Branch et al. 1991, present study) under the thin layer of the carapace causing it to bulge. Infected *N. marionis* with bulging carapaces were discarded in the study, because parasitism may affect growth rates (Mathews et al. 1987). Due to lack of expertise by this researcher, the parasite was not identified to species level.

According to Page's (1984) review, *N. marionis* from New Zealand is infested by the bopyrid *Pseudione affinis*. *P. affinis* occurs in Europe, Indonesia and South Africa (Barnard 1920, Bourdon 1968, 1972, both cited in Page 1984). *Nauticaris marionis* is the first record of the Pseudionidae parasitizing the genus *Nauticaris*. *Pseudione affinis* has subsequently been thought to infest the congeneric species, *Nauticaris magellanica* (Roman-Conteras and Wehrtmann 1997). These authors found that the species infecting *N. magellanica* is not *Pseudione affinis* as previously believed, but a new species *Pseudione chiloensis*. Although *P. affinis* occurs in South Africa (Barnard 1920), to my knowledge there is no literature available on the identification of this parasite of *N. marionis* around the Prince Edward Islands. The presence of *P. affinis* in the branchial chambers of its host is thought to affect the reproduction of the shrimp (Al-Adhub and Bowers 1977), which will be discussed further in the next chapter.

2.6 Conclusions

1. The program Rmix allowed the detection of older cohorts and produced high P values.

2. When matching cohorts that were in sequential order, a nonparametric test method was effective in identifying cohorts. Use of the sexual inversion strategy to identify cohorts is discouraged, as one requires very large sample sizes to match cohorts using gender proportions.

3. Some gravid individuals were found in April/May, indicating that, although earlier studies have suggested that hatching occurs mainly in March, some hatching will continue into autumn.

4. *N. marionis* can survive up to seven years under natural conditions. More sampling is required to detect larvae of this species in order to get a better estimation of age.

5. The von Bertalanffy growth curve parameters are empirical identified as K = 0.22239/year, L_∞ = 14.05789mm, t_0 = -0.05174, L_0 = 0.16083mm.

6. Parasitism of *N. marionis* by bopyrid isopods occurs, but the species involved has not been identified. The effects of parasitism are unknown, but it may reduce the growth rate of *N. marionis*.

Chapter Three

Reproduction and fecundity estimates in *N. marionis*

3.1 Introduction

Animals and plants that change sex during their lives are widely distributed geographically and taxonomically, but are a minority of sexual organisms (Policansky 1982). The term sex change defined by Policansky (1982), applies when an organism functions during one breeding season or episode as one sex, and as the other sex during another. Animals that carry both male and female sex organs at once are referred to as hermaphrodites, while those that bear only one set of sex organs at a time, but change sex during their lifetime are described to as gonochoristic.

Hermaphroditism occurs in most animal phyla (Ghiselin 1969, Warner 1975, Policansky 1982). It is found in at least 18 families of teleost fishes scattered through five orders (Artz 1964, cited in Warner 1975), and is also widespread among invertebrates (Ghiselin 1969). Although most species of decapod crustaceans have separate sexes, sequential hermaphroditism in decapod crustaceans has been documented in a number of species, particularly caridean shrimps (Yaldwyn 1966, Policansky 1982, Bauer 1986, Bauer and Holt 1998, Pakhomov et al. 2000). Only 37 cases of sex-changing decapods exhibiting sequential hermaphroditism have been documented; of these, 31 are caridean shrimps (for reviews, see Policansky 1982, Bauer 1986, 2000; for recent examples, see Bauer and VanHoy 1996, Pakhomov et al. 2000, and Lin and Zhang 2001). In species exhibiting sequential hermaphroditism, the individual changes sex at some point in its life history.

Forms of hermaphroditism in which individuals first mature as males and then with increasing age and size, change sex to females are referred to as protandry. Several

variations of protandry have been recorded among the carideans (Bauer 2000). One of the best-studied cases is that of *Lysmata seticaudata* in the family Hippolytidae (Charniaux-Cotton 1975). *L. seticaudata* is regarded as the first decapod in which protandry was recognised in 1912 by T. Spitschakoff (see Yaldwyn 1966). In this species, all individuals are believed to transform from males into females as they grow; such individuals are referred to by Pakhomov et al. (2000) as secondary females. Although this simplest form of protandry is not common among the Hippolytidae, it has also been recorded in several pandalid genera including *Pandalus* and *Pandalopsis* (Bauer 1986). This form of protandry has a variation, in which a variable proportion of the population (usually less than 50%) matures directly as females (termed primary females, after Bauer 1986), without first passing through a male phase. This form of protandry has been reported in the crangonid *Crangon crangon* (Boddeke et al. 1991) and also in the pandalid *Pandalus borealis* (Bergström 1997). A third form of protandry occurs as a unique case in the hippolytid, *Thor manningi*. Primary and secondary females occur in *T. manningi*, but primary males also persist at high frequencies (Bauer 1986). Bauer (2000) refers to this unique form of protandry as protandry with primary males.

The sexual system of caridean shrimps described above has been considered as demonstrating three forms of protandry in a review by Bauer (1989). Recent studies on the sexual system of the genus *Lysmata*, which was thought to demonstrate simple protandry, have shown that in at least two species, individuals in the female-phase are actually outcrossing simultaneous hermaphrodites (Bauer and Holt 1998, Fiedler 1998). Histological examinations conducted by Fiedler (1998) on each female-phase individual *Lysmata amboinensis* confirmed this unique sexual system by the presence of vitellogenic oocytes and sperm in the same gonad. Bauer and Holt (1998) observed the sexual system of seventeen pairs of female-phase *Lysmata wurdermanni* using time-lapsed video. The results of this study suggested that this species is a true outcrossing hermaphrodite, with the ability to copulate as females with other female-phase individuals, resulting in successful spawning and development of embryos (Bauer and Holt 1998). Therefore, separate experiments conducted on *Lysmata* spp. imply an outcrossing functional simultaneous hermaphroditism in *L. wurdermanni* and *L.*

amboinensis (Bauer and Holt 1998, Fiedler 1998; respectively). This gave rise to the concept of a new form of sexual system, protandric simultaneous hermaphroditism (PSH), described by Bauer (2000).

Early reports on sub-Antarctic decapods have found the caridean shrimp *Nauticaris marionis* occurring in shallow waters around Marion and Prince Edward Islands, and also Auckland and Campbell Islands off New Zealand (Yaldwyn 1965, 1966). Yaldwyn (1966) was the first to report on the sexual system of *Nauticaris marionis*, using specimens collected from Auckland and Campbell Islands. In Yaldwyn's samples, the female specimens examined were larger in size, and mostly ovigerous, while males were mostly smaller. Further examination of the males led Yaldywn to suggest that the species has a well-developed male copulatory organ. Yaldwyn's failure to recognize the intersex specimens (which in this thesis will be referred to as transitionals or *tertia quae*), led him to conclude that "This species (*Nauticaris marionis*) then, *does not show sex reversal*, but mature females do reach a much larger size than males" (Yaldwyn 1966: 1366).

Until recently, thirty years after Yaldwyn's studies of the sexual system of the *Nauticaris marionis*, work by Kuun (1998, MSc thesis) and Pakhomov et al. (2000) suggested that *Nauticaris marionis* is a partially protandric hermaphrodite. These studies further suggested that the vast majority of *Nauticaris marionis* juveniles develop into males. Juveniles of *N. marionis* tend to grow to a certain carapace length before they change sex to females (Pakhomov et al. 2000). A review of sexual morphs in the hermaphroditic reproductive system of the family Caridea by Bauer (2000) would classify the sexual system of *N. marionis* as, not simple protandry because not all individuals develop into females, but protandry with primary females and/or with early maturing males.

Criteria generally used to determined sex in decapod crustaceans are the presence and development of the male copulatory organ, the *appendix masculina* (a.m.). The a.m. is located on the endopod of the second pair of pleopods. Another criterion used to determine sex is the shape of the endopod of the first pleopod (Mascetti et al. 1997), in the case of *N. marionis* this endopod forms the *appendix interna* (a.i.1). Both these sets

of rami (a.m. and a.i.1) are referred to as male copulatory organs. In protandric carideans, the transformation from male to female is marked externally by loss of the *appendix masculina* (shown to be the copulatory structure by Bauer 1979, 1986), and by modification of the endopodite of the first pleopod (Mascetti et al. 1997), which is the *appendix interna* (a.i.) in *N. marionis*.

Fecundity has been long recognized as an ecologically important aspect of the population biology and life history of a species (Corey and Reid 1991). Fecundity and egg size are of considerable evolutionary and ecological importance. Considering the latter, egg size of aquatic invertebrates varies widely between quite closely related species (Mauchline 1988, Clarke 1993) and even within species. For this thesis, I define fecundity as the total number of eggs produced by a female in one brood, and these eggs are estimated by counting the number of externally attached eggs on the pleopods of each gravid *N. marionis* female.

This chapter entails a further study of the reproductive biology of the caridean shrimp *Nauticaris marionis* with the aim of providing a first estimate of individual fecundity, and investigating development. Samples from different years and seasons are used to establish gonadal development, examining the two sets of rami on the endopods of *N. marionis* pleopods.

3.2 Materials and methods

All specimens of the swimming prawn were collected in the vicinity of Marion Island between 1984 and 2003. Specimens were either preserved in 70% ethanol or 4% buffered formalin seawater solution, except for samples collected in 2001 and 2002, which were frozen at -18^{o}C for storage (see section 1.4; Table 1.1). To avoid errors associated with measuring a flexible abdomen, the carapace length (CL) was used as a standard measure of length of the shrimp (see section 2.2 from preceding chapter). The carapace length was measured using a Wild M-5 dissecting microscope to the nearest hundredth of a millimetre. Gender of individuals was determined, and five gender

definitions were recognised following Pakhomov et al. (2000). The definitions were based on examination of the first and second pleopods (swimmerets) to determine the presence or absence of the two sets of rami, the a.m. and the a.i.1. Ovaries lying under the carapace and eggs attached to the pleopods in the abdomen were also used to assist in sex determination. *N. marionis* specimens possessing ovaries as well as a.m. were conditionally referred to as *tertia quae* (*pl*. of *tertium quid*) or simply transitionals. This was done to distinguish them from male and female specimens. Two types of *tertia quae* were identified; type (a) ovigerous or gravid with both male copulatory organs (a.i.1 and a.m.), and type (b) ovigerous but not gravid with only one male copulatory organ present, the a.m. The last type of specimen is identical to juveniles except for the presence of an ovary. This type of individual was defined by the possession of an ovary and the other male copulatory organ (the a.i.1), while the a.m. was absent. The gender definitions adopted from Pakhomov et al. (2000) and used for this chapter, are given below.

3.2.1 Gender definitions

3.2.1.1 Juvenile

Neither ovigerous nor gravid, a.i.1 present but a.m. absent.

3.2.1.2 Juvenile with ovaries

Ovigerous, not gravid with a.i.1 present and a.m. absent.

3.2.1.3 Male

Both a.m. and a.i.1 present, but neither ovigerous nor gravid.

3.2.1.4 *Tertium quid*

Transitional individuals, two types: (a) ovigerous or gravid with both a.m.
and a.i.1 present, (b) ovigerous but not gravid, with a.i.1 absent but a.m. present.

3.2.1.5 Female

The rami, a.i.1 and a.m. both absent. The presence or absence of ovaries or eggs is not itself diagnostic.

3.2.2 Sex determination and gonodal development

On the pleopods of each specimen, two sets of rami were examined. The *appendices internae* (a.i.1) is on the endopod of the first pleopod, and the *appendices masculinae* (a.m.) occurs on the endopod of the second pleopod in *N. marionis*. The *appendix masculina* is a small squamiform protuberance on the endopod of the second pleopod of males. It can also be found in transitionals or *tertia quae*. Females and juveniles lack this appendix (see Plates a to g).

Mascetti et al. (1997) reported a significant difference when comparing the structures on the left and right pleopod of an Antarctic shrimp *Chorismus anatrcticus*. However, this difference was obvious in only four specimens of *C. anatrcticus*. Therefore, for the purpose of this chapter the right-hand pleopod was used to measure the a.m. and a.i.1 after they were amputated from each individual and examined under a Wild M-5 dissecting microscope equipped with an ocular micrometer at 25X magnification. All specimens examined were from samples collected between 1984 and 2003 (see section 1.4; Table 1.1). After defrosting specimens from the 2001 samples (sample 33 to 36) in the laboratory, a large number of these specimens were found to be damaged, some lacking pleopods. This made sex determination impossible for the 2001 samples. The a.m. and a.i.1 measurements were taken from the apex of the appendage to the middle of its base following Mascetti et al. (1997). A.m. length measurements did not include any setae. In all the relevant gender groups, the a.m. length measurements were plotted against carapace length to examine possible trends. The a.i.1 measurements were also compared to the carapace lengths for each sample.

The a.m. and a.i.1 were each amputated from the pleopods and measured from the base to the tip. Measurements were taken by cautiously separating each appendage from the amputated pleopods. A problem was encountered when separating these appendages (especially the small ones), as they tend to realign to their original position, creating a problem in measuring the base. Kuun (1998) suggested that allowing the Petri dish to dry slightly, which causes the base attachment to remain exposed partially, overcomes the

problem. But this in turn, however, often causes quick and pronounced shrinkage in the tubular structures. The pleopods cannot be examined under water because water does not allow the appendages to separate.

The Kruskal-Wallis test with tied ranks (Zar 1999) was used to compare gender sizes in all samples where more than two genders were sampled. If significant size differences were revealed between genders, a nonparametric multiple comparison for unequal sample size test was carried out (Zar 1999) to identify pairs of genders with significant differences. Sample 7 and 8 from the 1988 sample were found to be very small (n = 8), and were pooled with samples 5 and 6 collected in 1987 (see table 1.1 in section 1.4). In all samples used for statistical tests, both *tertium quid* types were pooled because of the small number of representatives of *tertia quae b*. Although *tertia quae a* and *b* are different, the aim of the test was to compare the sizes of 5 genders that were recognized.

3.2.3 Ovary development

The developing ovary of the swimming prawn was observed through the thin carapace under a dissecting microscope, and appears to develop sequentially from posterior to anterior. The degree of ovarian development was estimated on a subjective scale from 0 – 4, initially adapted from Bauer (1986) for *N. marionis* by Pakhomov et al. (2000) by taking the most posterior upper rostral tooth as the point of discrimination. Classification was based on the amount of space above the cardiac stomach occupied by the ovary; Stage 0 – no ovary clearly visible; Stage 1 – ovary present but anterior point of ovary posterior to point of discrimination; Stage 2 – ovary present, anterior point of ovary anterior to point of discrimination, but posterior to the posterior margin of the eye orbit and; Stage 3 – as in (2), but ovary in contact with posterior margin of the eye orbit. See Plates h to w for examples of stage 1, 2 and 3 of ovary development. Stage 4 was assigned to gravid females.

Bauer and Rivera Vega (1992) were able to recognize immature and mature ovaries based on size and colour, where immature ovaries were colourless and mature ovaries were yellow in colour. Ovarian maturity was almost impossible to identify in *N. marionis* due to loss of colour caused by the different means used to preserve these animals. This resulted in difficulties in differentiating between immature and mature ovaries. Therefore, due to lack of clear ovarian maturity, all ovaries were assumed to be mature. In small specimens where ovaries are not clear when viewed through the thin carapace, lifting the carapace off the specimen without completely removing it from the animal exposes the ovaries. However, it is important not to remove the carapace completely as this makes it impossible to classify the ovary by its position. In cases of uncertainty, ovaries were recorded as absent (n = 12).

Three potential ovigerous or gravid genders were identified using the presence or absence of ovaries. These were: female, juvenile with ovaries and both types of *tertia quae*. Ovary development within the three ovigerous genders was then compared using χ^2 tests of association for all samples in which at least two such genders occurred. In samples collected in 1984, 1985, 1987 and 2002 there were no juveniles with ovaries. In this study only ovigerous or gravid genders were used, in contrast to Pakhomov et al. (2000) who also used non-ovigerous juveniles to test the level of ovary development. Stage 0 (see above) was also excluded because it is usually not advisable to employ the test when expected frequencies are far less than 5 (Mann 1995). Both *tertium quid* types were pooled to include more representatives of that gender.

3.2.4 Egg size and fecundity

Estimates of fecundity were made for 28 ovigerous females from the 1984 sample and another 2 gravid females from the 1999 sample (see section 1.4; Table 1.1). In the laboratory, eggs were carefully stripped from the pleopods of ovigerous females (females carrying eggs externally on the pleopods) with a spatula and any setal material and extraneous matter was removed. Eggs were then placed in a small Petri dish with distilled water and examined under a dissecting microscope. Every egg carried by the

ovigerous female was counted. The degree of embryonic development of the egg was measured on a subjective scale modified from Wehrtmann and Kattner (1998) for a congeneric species, *Nauticaris magellanica*: Stage I – eggs recently produced, uniform yolk, no eye pigments visible; Stage II – beginning of embryo development is visible, eye pigment barely visible, yolk occupying more than half of the egg; Stage III – eyes clearly visible and fully developed, abdomen free.

A random subsample from each ovigerous female was used for measurement using a Wild M-5 dissecting microscope equipped with an ocular micrometer. Assuming the eggs to be ellipsoid, the *z*- and *x*-axes of the eggs were measured. Thomas and Finney (1988) state that *x*- and *y*-axes of an ellipsoid are identical. The formula for egg volume (EV) is:

$$EV = {}^{4}/_{3}\,\pi\, a^{2}\, b \qquad (2)$$

Where $a = x/y$-axis length (mm)/2 and $b = z$-axis length (mm)/2. Total egg volume in each ovigerous female was estimated by multiplying the female's average egg volume by the total number of eggs counted.

Egg number (EN) data was not normally distributed (Kolmogorov-Smirnov test, $P < 0.05$). Data were normally distributed and passed the homogeneity test (Levene's test; $P > 0.05$) after square-root transformation. Analyses of covariance (ANCOVA) were run on transformed data using stage as a fixed factor and carapace length as the co-variate.

3.2.5 Parasitism

Parasitism by bopyrid isopod was recorded in five samples collected between 1984 and 2000. Bopyrid parasites were observed under the thin layer of the carapace causing it to bulge. For full details on bopyrid infestation see preceding chapter, section 2.3.1. Infected specimens were sexed and measured to determine the rate of infection.

3.3 Results

3.3.1 Differences in genders and gonad development

Kruskal-Wallis test showed that there was a significant effect of gender on size ($p < 0.05$) in all samples. A nonparametric multiple comparison test further revealed a significant difference between male and female specimens in all samples examined. For instance in the 1984 sample, males were smaller than females, and *tertia quae* ($Q = 9.76$, $p << 0.001$; $Q = 3.8$, $p < 0.01$, respectively; Fig. 3.1).

In the years examined, all five genders occurred only in samples from 1999 and 2000 (Fig. 3.2). Ovigerous juveniles (juveniles with ovaries) were smaller than males in the 1999 sample ($Q = 4.99$, $p < 0.001$), but larger in the 2000 sample ($Q = 3.99$, $p < 0.001$). In the 2003 sample only three genders were identified, and ovigerous juveniles were found to be larger than males (Fig. 3.2). Females proved to be larger than ovigerous juveniles in both 1999 and 2000 ($Q = 3.79$, $0.001 < p < 0.005$; $Q = 2.87$; $p < 0.05$). Only in the 2001 sample were *tertia quae* found to be larger than females ($Q = 3.92$, $p < 0.001$).

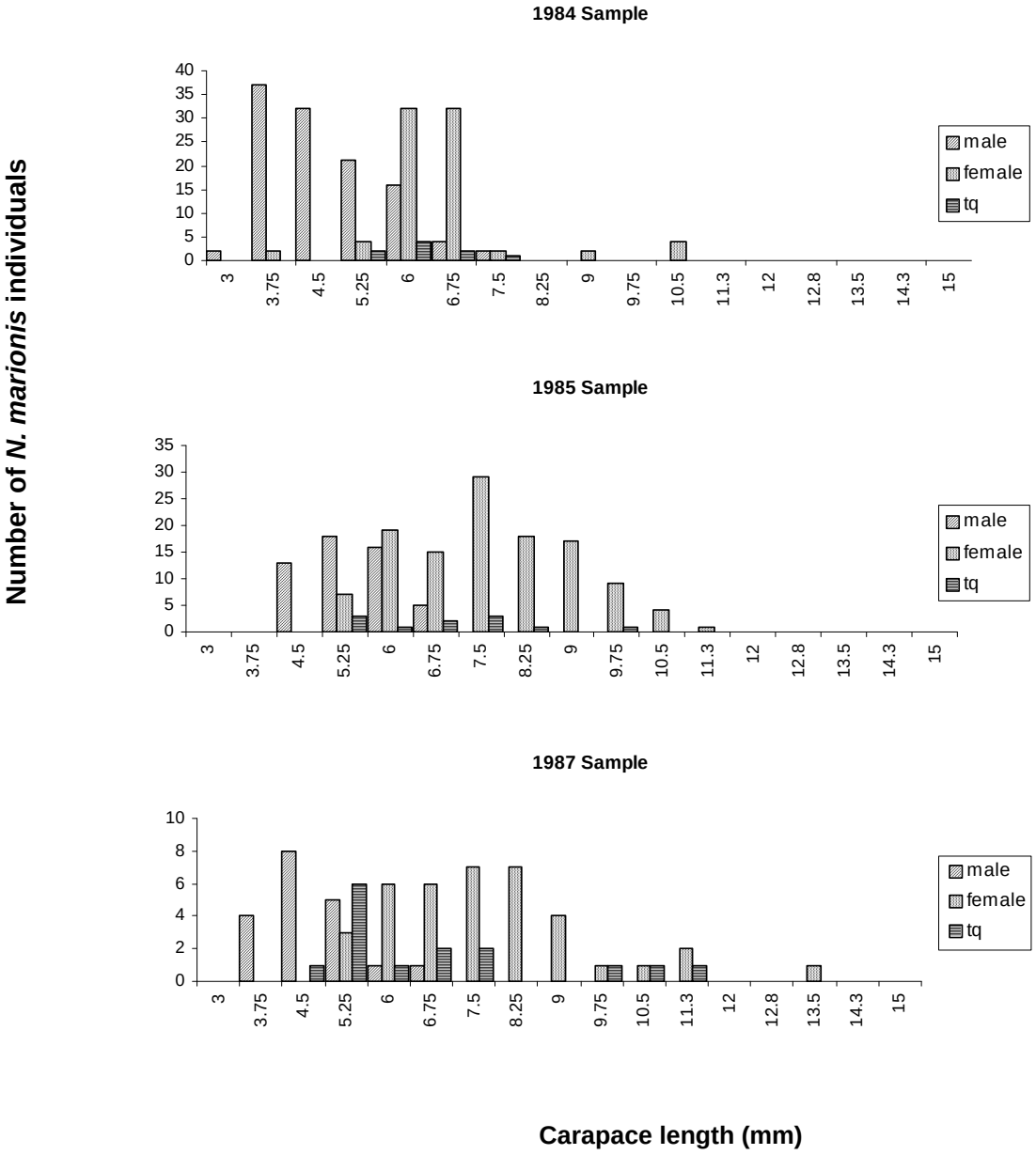

Fig. 3.1 Relationship between carapace length of *Nauticaris marionis* and the frequency of different genders collected between 1984 and 1987. tq = *tertia quae*.

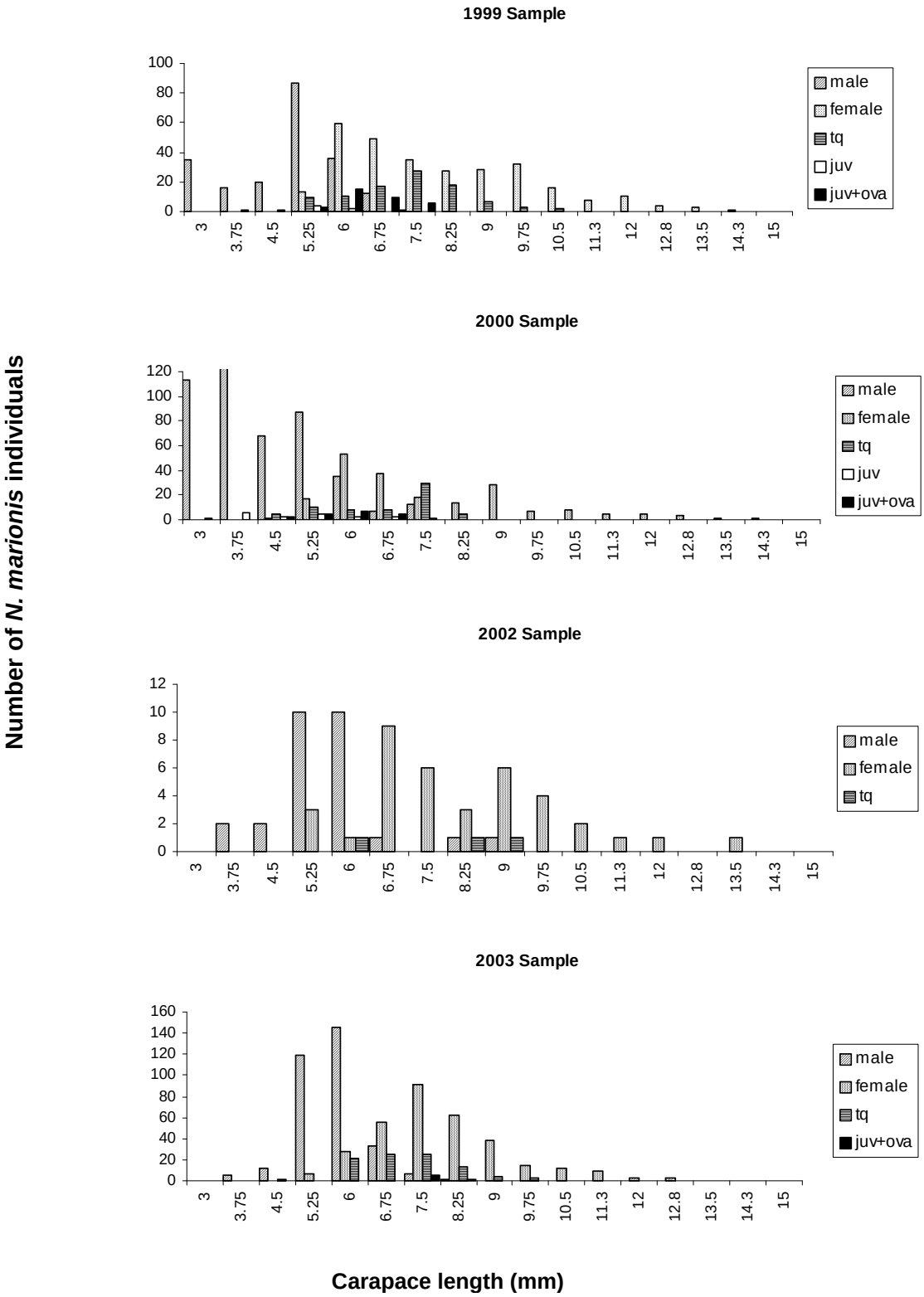

Fig. 3.2 Relationship between carapace length of *Nauticaris marionis* and the frequency of different genders collected between 1999 and 2003. tq = *tertia quae*, juv = non-ovigerous juveniles and juv + ova = ovigerous juveniles.

The χ^2 tests showed no association between stage of ovary development and ovigerous genders in any of the samples ($P > 0.05$). For both females and *tertia quae*, eggs were mostly at stage 1 followed by stage 2 in all years except in 2002 when most were at stage 2 (females and *tertia quae* only; Table 3.1). Stage 3 was rare in all years except for 2003 when, for both female and *tertia quae*, stage 3 was almost as common as stage 2. For example in samples collected between 1984 and 1987, only females were found with stage 3 in 1985 ($\chi^2 = 0.911$; df = 2; $P = 0.633$; n = 2) and 1987 ($\chi^2 = 0.851$; df = 2; $P = 0.653$; n = 3). For juveniles, stage 3 was not represented in the 2000 sample, and was found to be < 3% in the 1999 sample (Table 3.1). Due to the small sample size from 2002, the chances of ovigerous juveniles being represented were remote.

Table 3.1 Percentage frequency of each ovary developmental stage for each ovigerous sex.

Sample	**Females**				***Tertia quae***				**Juveniles with ovaries**			
year	**1**	**2**	**3**	**n**	**1**	**2**	**3**	**n**	**1**	**2**	**3**	**n**
1984	83	17	-	78	89	11	-	9	-	-	-	-
1985	80	18	2	119	70	30	-	10	-	-	-	-
1987	68	24	8	37	78	22	-	9	-	-	-	-
1999	58	19	13	283	53	31	16	92	64	33	3	33
2000	63	28	9	193	64	23	13	61	67	33	-	18
2002	33	51	16	37	33	67	-	3	-	-	-	-
2003	52	26	22	320	69	14	17	86	57	14	29	7

The relationship between a.m. and carapace length differed between males and *tertia quae*. In most cases (not 1985 or 2002), there was a significant correlation for males and r^2 values were mostly around 0.4 – 0.7 (Table 3.2; Fig. 3.3). For *tertia quae a*, correlations were often significant, but unconvincing. For one of the three significant relationships (2000) the slope of the regression line was not significantly different from zero (Fig. 3.4), while for 2002 and 1987, the significant relationships were based on

sample size of less than 10. There were only two samples for which *tertia quae b* could be examined and both had very small sample sizes (Table 3.2). *Tertia quae* started appearing at carapace lengths greater than 4mm (Fig. 3.4), therefore, to avoid a size bias, male individuals greater than 4mm CL were regressed separately, omitting smaller males (Table 3.3, Fig. 3.5). This had no obvious effect on the patterns recorded and the results were the same as for all males, indicating that bias due to size differences between *tertia quae* and males was not a problem.

Table 3.2 Results of regression analyses of a.m. on carapace length for males, *tertia quae a* and *b*. * = slope of the regression line is not significantly different from zero, # = sample size extremely low (n < 7) and ns = non-significant. Note sample sizes differ from Table 3.1 because some individuals could not be analysed due to damage to the pleopods.

Sample year	**Males**			***Tertia quae a***			***Tertia quae b***		
	n	**r^2**	**P**	**n**	**r^2**	**P**	**n**	**r^2**	**P**
1984	114	0.4947	P<0.01	9	0.0114	ns	-	-	-
1985	52	0.0414	ns	11	0.1783	ns	-	-	-
1987	25	0.4254	P<0.01	9	0.5887	P<0.05	-	-	-
1999	207	0.6295	P<0.01	91	0.0596	ns	-	-	-
2000	640	0.6839	P<0.01	59	0.1636	P<0.05*	4	0.0165	ns
2002	27	0.0185	ns	3	0.9530	P<0.05#	-	-	-
2003	317	0.2155	P<0.01	83	0.0866	ns	6	0.752	P<0.05#

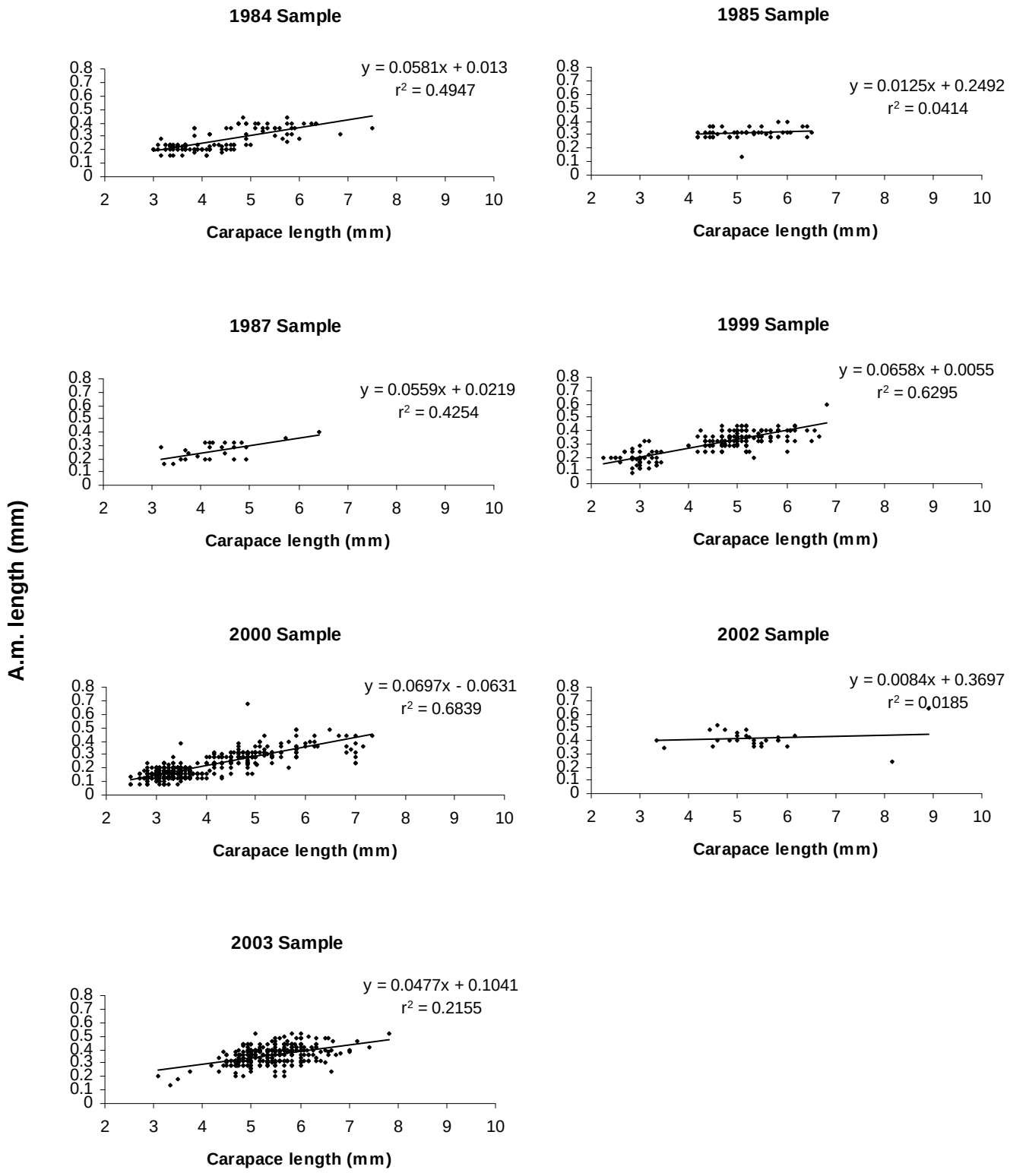

Fig. 3.3 Relationship between carapace length and a.m. length for males.

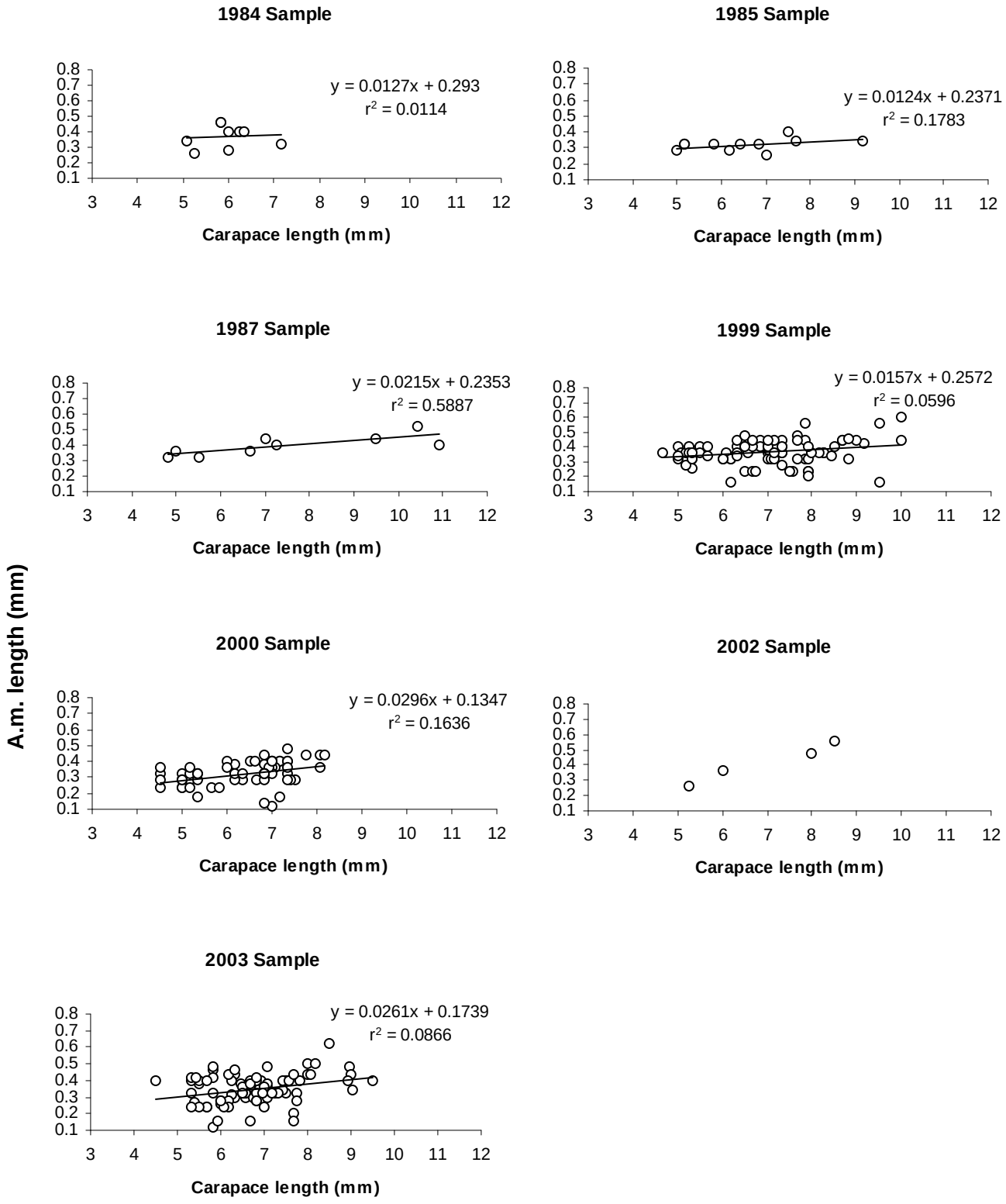

Fig. 3.4 Relationship between carapace length and a.m. length for *tertia quae a*.

Table 3.3 Results of regression analyses of a.m. for males on carapace length for individuals > 4mm. ns = non-significant.

Sample	Males greater than 4mm CL		
	n	**r^2**	**P**
1984	62	0.3529	P<0.01
1985	52	0.0414	ns
1987	17	0.2756	P<0.01
1999	154	0.1983	P<0.01
2000	191	0.2656	P<0.01
2002	25	0.0338	ns
2003	313	0.1604	P<0.01

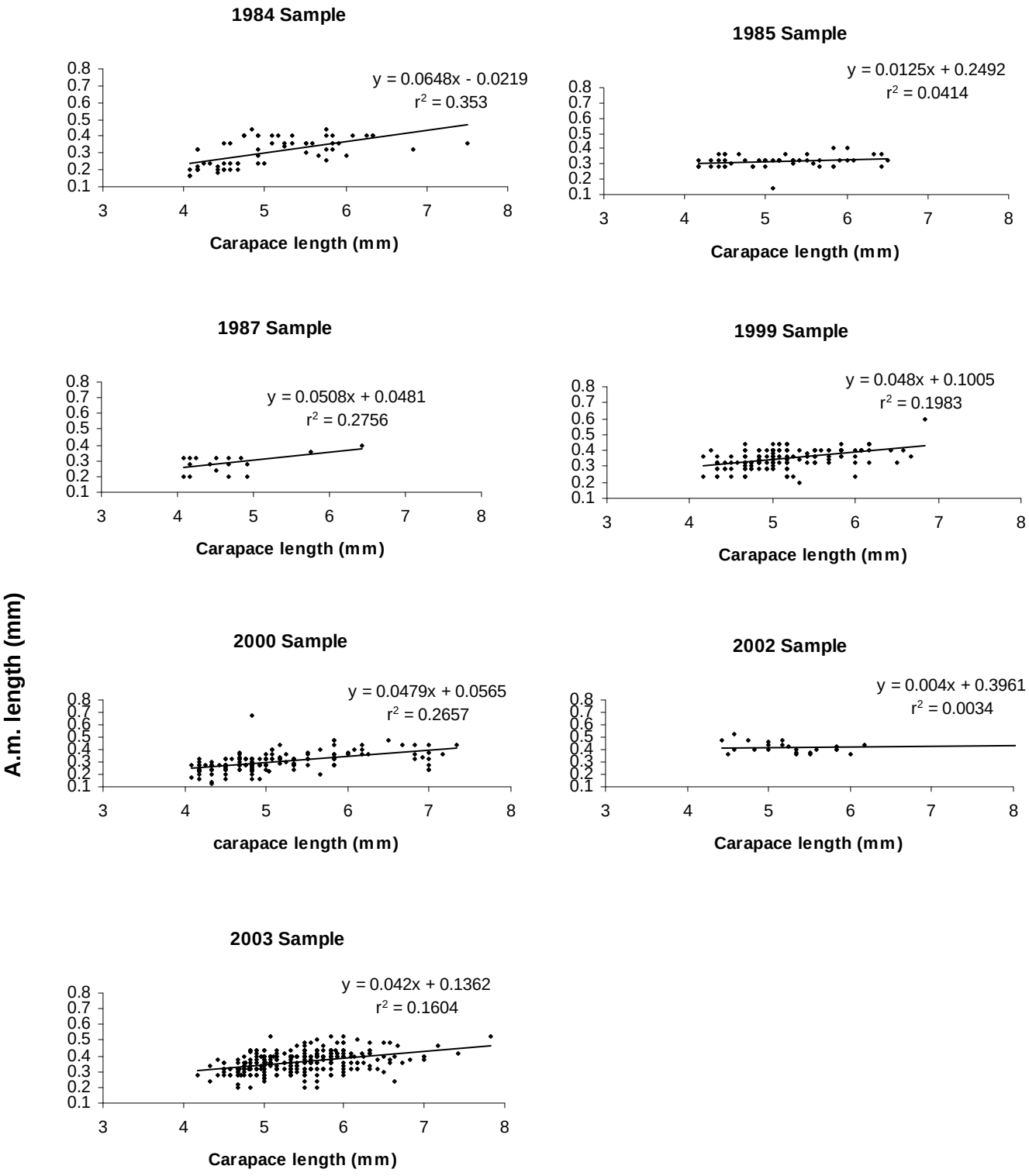

Fig. 3.5 Relationship between carapace length and a.m. (*appendix masculina*) for males of > 4mm.

The length of the a.i.1 showed a significant correlation with carapace length for all males except in the 2002 sample (Table 3.4, Fig. 3.6). For *tertia quae a*, the length of the a.i.1 rarely showed a significant correlation with CL (Fig. 3.7). Only two significant relationships (1987 and 2003) were recorded for *tertia quae a* plus one significant relationship (2002) with an extremely low sample size (n = 3; Table 3.4). Non-ovigerous and ovigerous juveniles were only represented in two and three samples respectively (Fig. 3.8). In three out of these five samples there was a significant correlation between the a.i.1 and CL, with one non-significant relationship each for non-ovigerous juvenile (1999) and ovigerous juvenile (2003; Table 3.4). For all juveniles, r^2 values were relatively low, between 0.3 and 0.4.

Table 3.4 Results of regression analyses of a.i.1 on carapace length for males, *tertia quae a*, juveniles and juveniles with ovaries. # = sample size extremely low (n < 5) and ns = non-significant. Note sample sizes differ from Table 3.1 because some individuals could not be analysed due to damage to the pleopods.

Sample year	**Males**			***Tertia quae a***			**Juveniles**			**Juveniles with ovaries**		
	n	**r^2**	**P**	**n**	**r^2**	**P**	**n**	**r^2**	**P**	**n**	**r^2**	**P**
1984	114	0.4577	P<0.01	9	0.0898	ns	-	-	-	-	-	-
1985	52	0.2252	P<0.01	11	0.0696	ns	-	-	-	-	-	-
1987	25	0.4159	P<0.01	9	0.5439	P<0.01	-	-	-	-	-	-
1999	207	0.6016	P<0.01	97	0.0106	ns	8	0.0036	ns	33	0.4292	P<0.01
2000	643	0.6946	P<0.01	59	0.0925	ns	16	0.3362	P<0.01	18	0.3958	P<0.01
2002	27	0.0065	ns	3	0.725	P<0.05#	-	-	-	-	-	-
2003	302	0.1469	P<0.01	100	0.1316	P<0.01	-	-	-	7	0.088	ns

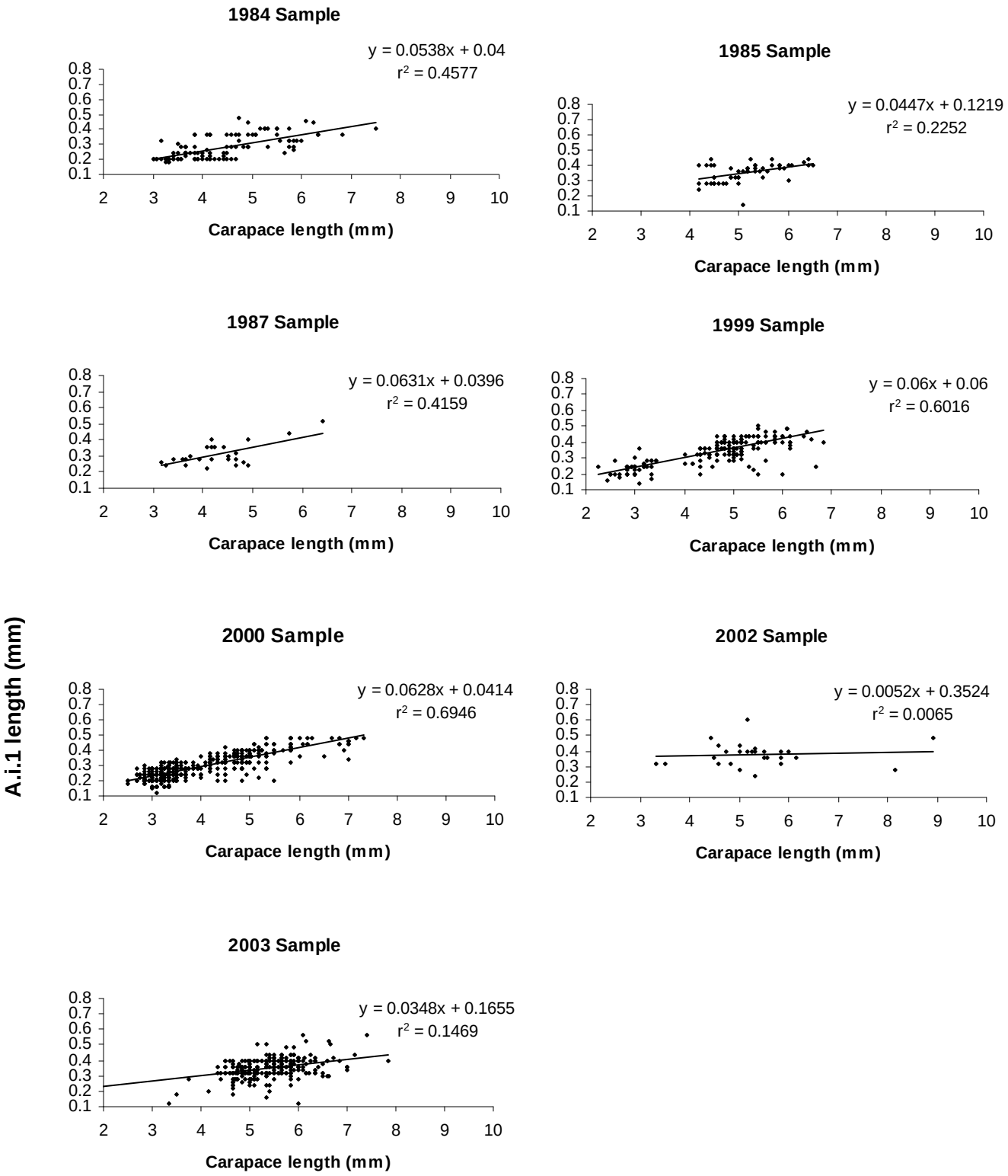

Fig. 3.6 Relationship between carapace length and a.i.1 for males collected between 1984 and 2003.

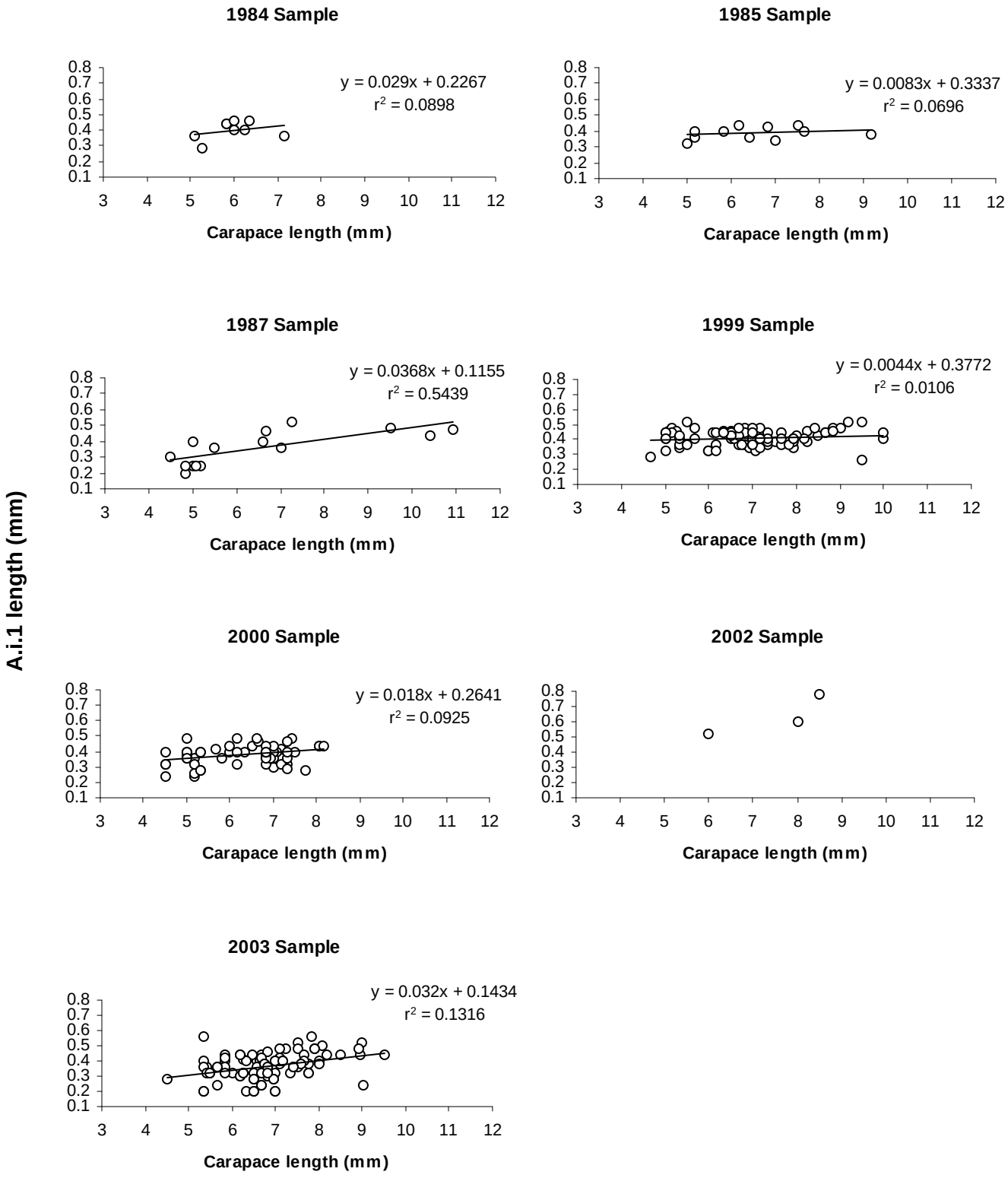

Fig. 3.7 Relationship between carapace length and a.i.1 for *tertia quae* collected between 1984 and 2003.

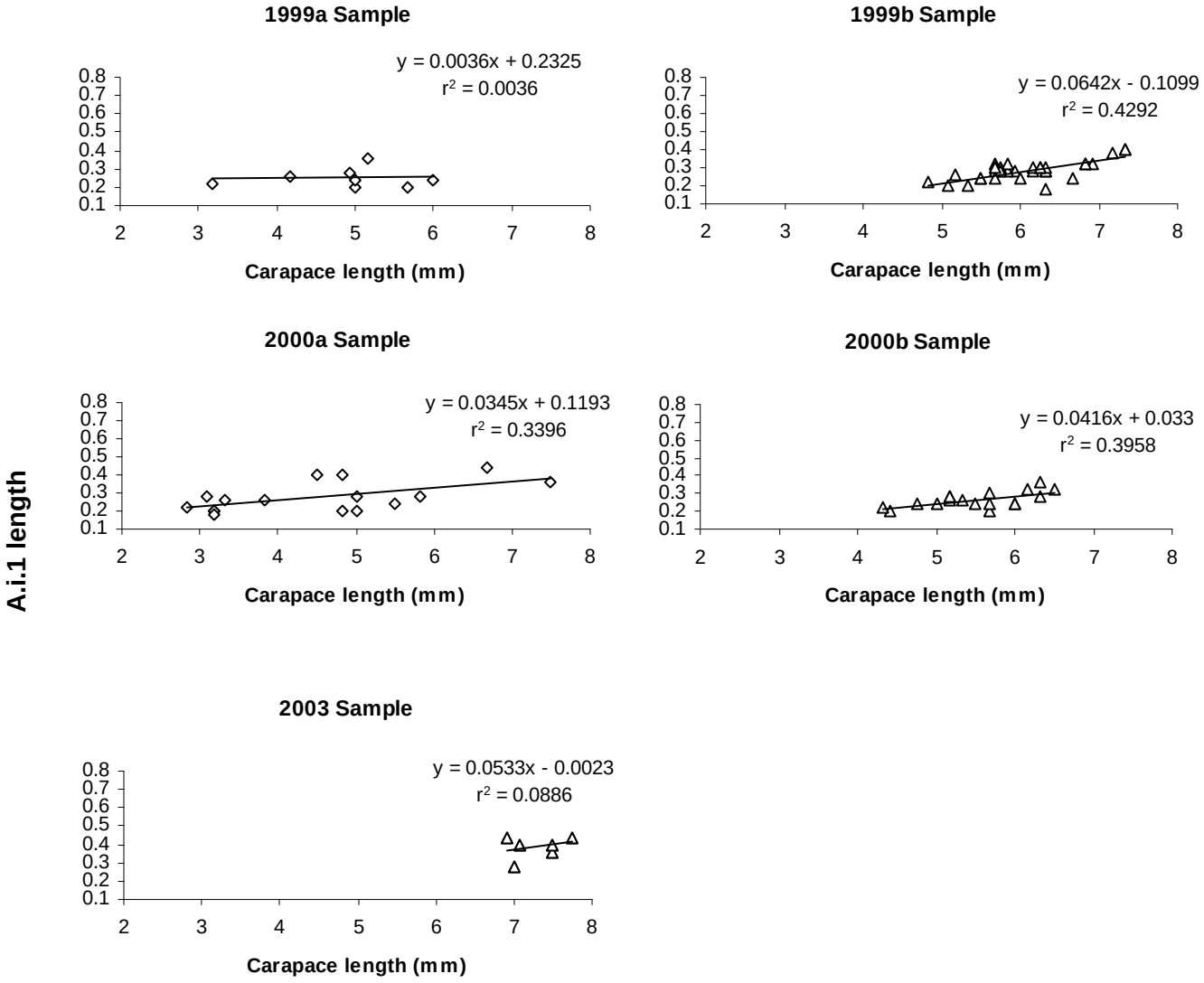

Fig. 3.8 Relationship between carapace length and a.i.1 for juveniles (diamond) and ovigerous juveniles (triangle) collected between 1999 and 2003.

3.3.2 Egg data

Analysis of co-variance (ANCOVA) showed that carapace length (CL) has a significant effect on egg number (EN) ($P < 0.001$), while stage of development has no significant effect ($P > 0.05$, Table 3.5).

Table 3.5 ANCOVA on the relationship between the egg number (EN) in two different stages and the carapace length (CL).

Factor	df	MS	F-ratio	P
Co-variate CL	1	952.769	19.5554	0.00014
Stage	1	0.0007	0.00002	0.9969
Error	27	48.7216		
Total	29			

3.3.3 Bopyrid parasitism

A total of twenty specimens were infected by parasitic isopods of the family Bopyridae. The infection came from a range of genders and several years, but some samples showed higher rates of infestation than others. Two males, two *tertia quae a*, and four females (mean CL = 6.45mm, standard deviation = 0.73, range = 5.75mm-7.33mm) were observed from the 1984 sample. Three females were observed, two from the 1985 sample and one from the 1988 sample (CL = 10.08, 6.67 and 9.33mm, respectively). In the 1999 sample there were five males and one *tertium quid a* infested with the parasite (mean CL = 6.57mm, standard deviation = 1.27, range = 5mm-8.83mm). Only three males from the 2000 sample were found to be infested by the bopyrid parasite (CL = 4.83, 5.33 and 5.83mm). No other sample showed any infestation and no *N. marionis* parasitized by the bopyrids were gravid, although there were some that were ovigerous. Infected ovigerous females and *tertia quae* were found to be in stage 1 of ovarian development (see section 3.2.3), except for one female from the 1988 sample that was in

stage 2. In one female from the 1985 sample (CL = 10.08mm) there were no ovaries or parasite visible, but the carapace showed clear bulging, suggesting infestation had occurred.

Table 3. 6 Bopyrid parasitism on different genders (years pooled).

Gender	**Infection rate (%)**
Males	**0.7**
Females	**0.65**
Tertia quae	**1.1**
Juveniles	**0**
Juveniles with ovaries	**0**

3.4 Discussion

3.4.1 Sex change

Sexing protandric species can be problematic (Mascetti et al. 1997). In the case of *Nauticaris marionis* Pakhomov et al. (2000) recognised two types of females, one developing out of *tertia quae* and the other from juveniles. Before comparing the previous study to the present, there is a need for a clear definition of genders. Centuries after its discovery by Bate (1888), gender definitions for *N. marionis* were not well defined. An early study (probably the first one) by Yaldwyn (1966) of the sexual system of *N. marionis* stated that the males posses a male copulatory organ, the *appendix masculina* (a.m.), and that there are differences in size between males and females. Yaldwyn (1966: 1366) also states, "*all* the larger specimens were female, mostly ovigerous, and the smaller specimens were largely male" (my italics). Judging from the description and the use of the word "all", I therefore, assume that size was probably used as a criterion in determining the sex of individuals in that study. The reason for suggesting this is because of the distinct sexual dimorphism displayed by this species, with females tending to be larger than males.

Studies on size-dependent diet of *N. marionis* (Perissinotto and McQuaid 1990, Pakhomov et al. 1999) may be related to the sexual dimorphism exhibited by this species. To date, the feeding dynamics of different sexes have not been appropriately addressed (Perissinotto and McQuaid 1990, Pakhomov et al. 1999), see following chapter or Vumazonke et al. (2003) for details. Size alone is an unreliable method of sexing *N. marionis* as it may lead to identification of larger males as females, and small non-ovigerous females as males. The earlier studies cited above reported differences in feeding patterns in males and females of *N. marionis* without providing any definitions as to how the specimens were sexed. After the first clear gender definitions were reported in Pakhomov et al. (2000), later studies on *N. marionis* employed these definitions (eg. Vumazonke et al. 2003, present study). Kuun (1998) stated the sexing procedures used in his study, and the present one, might result in underestimation of the number of ovigerous juveniles, males and females and overestimation of the numbers of juveniles and *tertia*

quae. Lack of recognition of immature ovaries can lead to individuals which perhaps should be regarded as *tertia quae* or ovigerous juveniles being classified as males and non-ovigerous juveniles, respectively. However, no females would be classified as being of any other gender; this also applies to males.

Previous studies suggest many specimens with a carapace length ~ 3mm have begun growing the a.m. (Pakhomov et al. 2000). Because of this, and the fact that these individuals were collected from the plankton, these authors suggest sexual differentiation takes place before settlement into the benthos. However, in the present study, animals of less than 3mm CL were collected in benthic samples, so it remains unclear whether sexual differentiation, which does seem to depend on size, occurs planktonically or after settlement.

The results from this study confirm the findings of (Pakhomov et al. 2000) that there is no difference in ovary development among the different potentially ovigerous genders. Although 1984 was sampled in spring (August/September) from the other years, there was no obvious difference from samples collected in austral summer (April/May). In fact the only years that looked different in ovarian development were 2002 and 2003, which were also collected in austral summer (see Table 3.1). Ancova showed significant effect of carapace length on egg number (Table 3.5). Large gravid female *N. marionis* tend to carry more eggs, as they have larger pleopods to which these eggs are attached. However, there was no significant effect of stage on egg number, suggesting that there is no mortality or loss of eggs as they develop.

3.4.2 *En route* to becoming a female

In essence there are five recognisable genders, two of which can be subdivided: *tertia quae* (*a* and *b*) and females. Pakhomov et al. (2000) described two types of female, primary females that develop from juveniles and secondary females that first pass through a male phase. These two forms of female were originally described by Kuun (1998), but were based on a rather small sample size and are confirmed in the present

study. However, this thesis has in addition recognise three routes by which these females develop, based on the relationship between ovary development and the presence or absence of the a.m., the a.i. and the a.i.1 (Fig. 3.9). Primary females form from juveniles either (a) by losing their a.i.1 and then develop ovaries or (b) by first developing ovaries (ie. becoming ovigerous juveniles) and then losing the a.i.1. Thirdly, secondary females develop from *tertia quae* which lose the a.m. and the a.i.1. The results of sex change in juveniles and *tertia quae* confirm the findings of previous studies which indicated that *N. marionis* is a partially protandric hermaphrodite (Kuun 1998, Pakhomov et al. 2000). This is obvious because there is no evidence from either previous or the present studies to support the existence of primary males such as in the hippolytid *Thor manningi* (Bauer 1986).

Juveniles were recorded in 1999, 2000 and 2003 with no juvenile samples from the 1980s, or in 2001 or 2002. This low frequency of juveniles suggests that the number of primary females in the population is very low, given the total number of ovigerous and non-ovigerous juveniles in all samples. This, therefore, is in agreement with Pakhomov et al. (2000), that a minority of individuals follow the primary route.

Pakhomov et al. (2000) observed neither ovigerous nor non-ovigerous juveniles in samples collected in May, and then implied that transformation from juvenile to primary females must be complete by then. This study, therefore, is in agreement with the previous study that transmutation from juveniles to primary females is probably completed in April/May. To achieve the status of primary female, one would expect a decline in a.i.1 size in individuals *en route* to becoming female.

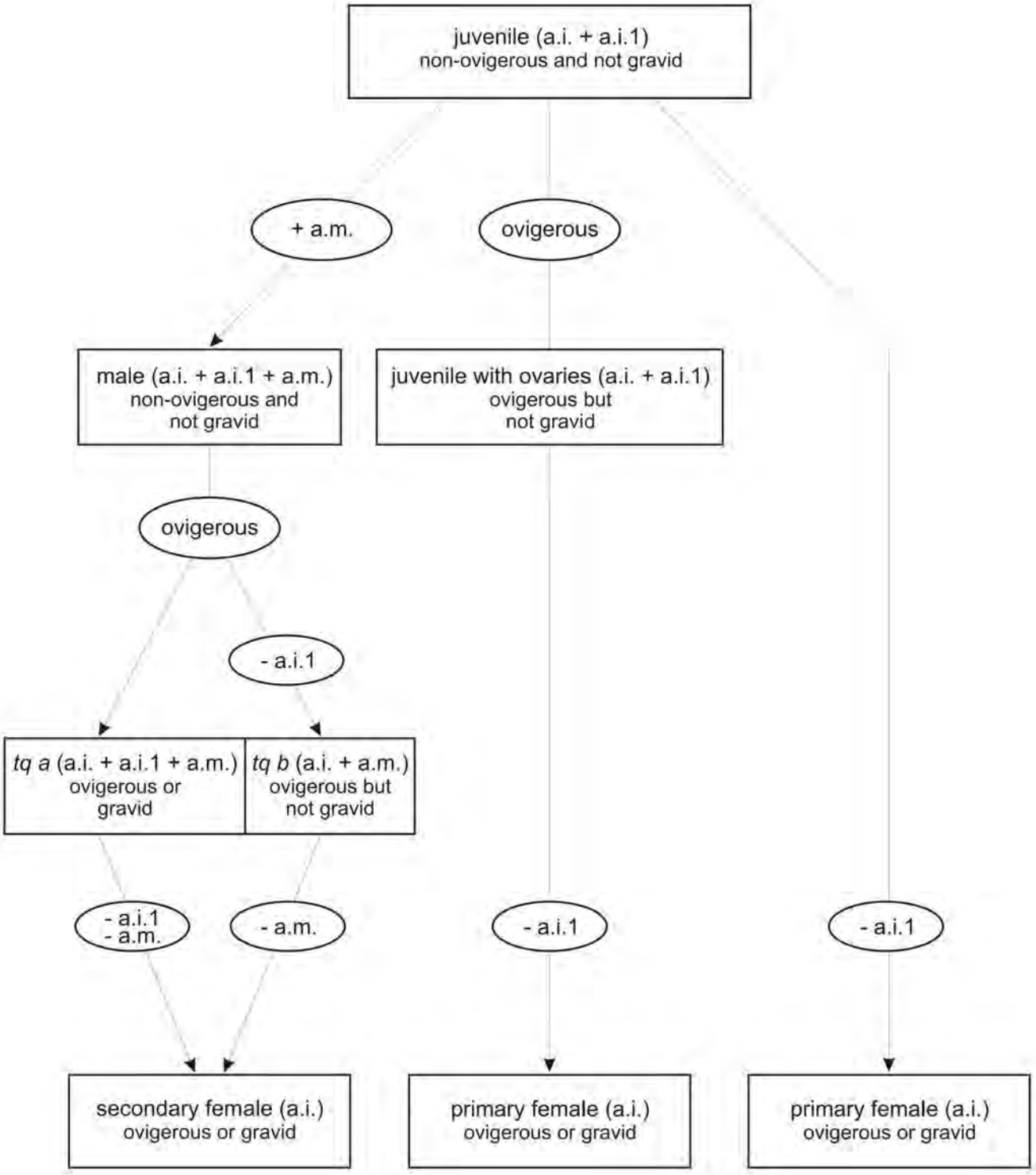

Fig. 3.9 Diagrammatic representation of sex change in *N. marionis*.

According to Kuun (1998), at least some ovigerous primary females can be recognised by the presence of the a.i.1, a male copulatory structure that all juvenile *N. marionis* possess. Because of the low numbers of primary females, no primary female with an a.i.1 was observed in this study and subsequently, as in Kuun's study, the expected decline in a.i.1 was not evident. The loss of the a.i.1 from the juveniles *en route* to becoming female is thus apparently rapid, and probably occurs during moulting (Pakhomov et al. 2000). Kuun (1998) also suggested the loss of a.i.1 by the primary females may occur in a single moult, possibly just before spawning, which may be in late April/early May as transmutation is completed.

While primary females occur, it seems that the vast majority of juveniles follow the secondary route as described by Pakhomov et al. (2000). However, while Pakhomov et al. (2000) found most *tertia quae* were greater than 7mm carapace length, in the present study, *tertia quae* were often between 4-7mm carapace length (Figs. 3.4 and 3.7). Thus, in their study primary females were believed to develop ovaries at a relatively small carapace length while secondary females, developing from *tertia quae* of >7mm CL would develop ovaries only at a comparatively large size. Pakhomov et al. (2000) further suggested that secondary females (developing from large *tertia quae*) channel their energy into overall growth, with primary females (developing from small juveniles) channel energy into ovary development at a smaller size. However, *tertia quae* in the present study were often smaller than indicated by Pakhomov et al. (2000), who dealt with a much smaller sample size. Consequently my results indicate that secondary females need not be large, but may complete transmutation at 5mm CL or so, at any rate, at < 7mm CL.

There are also conflicting suggestions as to how secondary females develop. This development involves loss of the a.i.1 and the a.m. from the *tertia quae en route* to becoming a secondary female. As suggested above for the primary route, the rapid loss of these appendages is not by atrophy, but through moulting. Although Pakhomov et al. (2000) suggested some secondary females could be recognised for part of the year by the presence of the male organs, the a.i.1 and the a.m., in combination with female

characteristics, no secondary females possessing an a.m. were observed in this study. I therefore recommend future studies on this matter, as it appears that the a.m. may be present in secondary females, which might have been missed in the present study.

The actual switch between genders may be driven by environmental conditions. For example Allen (1966, cited in Pakhomov et al. 2000), suggested that the proportion of primary females in populations of the pandalid shrimp, *Pandalus borealis*, may increase at higher water temperatures. Although this may be relevant to the situation at the Prince Edward Islands, as sea temperatures there have shown a marked tendency to increase over the last 30 years (Smith and Steenkamp 1990, Smith 1991, Bergstrom and Chown 1999), there is no clear pattern of change in sex ratios in the samples examined in this study. Alternatively, sexual differentiation may be driven by biological factors. It has been suggested by Ghiselin (1969) that evolutionary pressures will favour the ability of populations to alter sex ratios by increasing or decreasing rates of sex change from males to secondary females in response to imbalances between the genders, although as with proposed temperature-based switches, no actual mechanism was proposed.

3.4.3 Bopyrid effects on reproduction

Nauticaris marionis is the first recorded host of the bopyrid *Pseudione affinis* that does not belong to the Pandalidae, and *P. affinis* has been described only in hippolytid *N. marionis* occurring off New Zealand (Page 1984; see also preceding chapter). Although this parasite may affect growth rates (Mathews et al. 1987), it is also suggested that decapods infected with bopyrids may undergo "parasitic castration" (Reinhard 1956, cited in Page 1984, Al-Adhub and Bowers 1977). This effect may involve atrophy of the gonads, inhibition of spawning, or reduced fecundity. The recorded rate of infestation in this study was not high and there were no signs of gonad atrophy.

Kuun (1998) observed three females from a small sample of ten *N. marionis* specimens without any signs of ovary development. This led Kuun to suggest that these females had been castrated as occurs in other hippolytids like the *Heptacarpus paludicola* (Bauer

1979). In this study, the ovarian development in the infested females and *tertia quae* were mostly found to be in stage 1. This suggests that the infestation might have occurred just after the initial stages of ovarian development. When female penaeid prawns from Kuwait, *Penaeus semisulcatus*, are infested by bopyrid parasite in the gill chambers, the infected prawns do not reach gonad stages III and IV (Mathews et al. 1987). This may also be the case in the ovary development observed in female and *tertia quae* in this study, where these infected individuals, if given a chance to live, may not reach later stages of ovarian development, although a single female from the 1988 sample had ovaries at stage 2 of development.

The bopyrid parasite, *Pseudione affinis*, has been found in the branchial cavity of the pink shrimp *Dichelopandalus bonnieri* and appears to completely inhibit breeding in that shrimp (Al-Adhub and Bowers 1977). This bopyrid occurs just under the carapace of *N. marionis*, but to my knowledge there is no literature available on its effects. If *P. affinis* does inhibit ovary development in *N. marionis*, then males observed in this study may be regarded as castrated *tertia quae*. There were no parasites found in other samples. Parasites may be lost or shed during moulting. When shrimps moult, all signs of infestation may disappear (Mathews et al. 1987), except for the fact that their previous history will be influenced by the parasite. Parasites found in the 1999 and 2000 samples occurred on shrimps belonged to cohorts E to B and D to B, respectively (see preceding chapter on section 2.4.2). The observed data suggest that parasitism in *N. marionis* occurs between 3 to 9mm carapace lengths. Specimens greater than 9mm CL were not affected by this parasite or they may have lost it through moulting. These data suggest that bopyrid parasitism may be size-specific, but not gender-specific.

3.5 Conclusions

1. This study confirms that *N. marionis* is a partially protandric hermaphrodite with no direct evidence of primary males.

2. Three routes exist by which females can develop, with a vast majority passing through the *tertia quae* linked secondary route. A small minority of ovigerous and non-ovigerous juveniles transmutate to primary females. Transmutation to females probably occurs in early April/late May. During sex change, loss of the male copulatory organs, a.i.1 and a.m., appears to be a distinct event happening during a single moult rather than by atrophy.

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3. It is still unclear whether sexual differentiation occurs in the plankton or just after *N. marionis* settles on the benthos.

4. Bopyrid parasitism appears to be size-specific rather than gender-specific.

Chapter Four

Diet and daily ration of male and female *N. marionis*

4.1 Introduction

Studies of food consumption in marine species are typically carried out by daily ration methods. Daily ration methods were originally developed for fishes (Durbin et al. 1983, Worobec 1984, Bulman and Koslow 1992, Pakhomov et al. 1996), and are used to determine the amount of food consumed by marine organisms under natural conditions (Maynou and Cartes 1997, Pakhomov et al. 1999). These methods are now well established, for use in both the laboratory and the field. Although such methods have rarely been applied to crustaceans in the laboratory (exceptions are Yamashita et al. 1985, Sardà and Valladares 1990), or in the field (except in Pakhomov and Perissinotto 1996, Perissinotto and Pakhomov 1996, Froneman et al. 2000), this methodology has been applied to some decapod crustaceans.

Previously, studies on decapod crustaceans using daily ration methods were based on data obtained in the laboratory (Sardà and Valladares 1990). More recently, studies of food consumption and evacuation rates in decapod crustaceans were conducted in the field (Maynou and Cartes 1997, 1998, Pakhomov et al. 1999). The application of daily ration methods to shallow water animals reared in the laboratory is difficult or sometimes impossible in other species due to the difficulties of keeping animals in the laboratory. Studies based on field sampling have the advantage of working within the environmental conditions in which the animal lives, and are preferable when trying to quantify food consumption under natural conditions.

Field estimates of food consumption in decapods have mainly been carried out on coastal and shallow water species (del Norte-Campos and Temming 1994, Oh et al. 2001). Although daily ration methods have been widely used in other deep-sea areas, they have been only conducted on fishes (Gorelova 1985, Bulman and Koslow 1992, Pakhomov et al. 1996). Maynou and Cartes (1997) were the first to produce an estimate of food consumption for a deep-sea penaied shrimp, *Aristeus antennatus*, based on field measurements. Despite the scant literature on field estimates of food consumption in deep-sea decapods, Maynou and Cartes (1998) continued investigating daily ration methods in deep-water shrimps. In the NW Mediterranean, a comparative study of food consumption and daily ration in nine deep-sea shrimps was conducted (Maynou and Cartes 1998). This pioneered other field estimations of deep-sea decapods.

One particular species that has benefited from previous field estimates is the swimming prawn, *Nauticaris marionis*, around the Prince Edward Islands. The shallow inter-shelf region separating Marion and Prince Edward Islands has a diverse and biomass rich benthic community (Branch et al. 1993), and the presence of such a large community between the islands indicates a high rate of food supply (Pakhomov and Froneman 1999). Feeding studies conducted on several top predators indicate that *N. marionis* is an important component in their diet (Blankley and Grindley 1985, Adams and Klages 1989). As a consequence, *N. marionis* represents an important link between the pelagic environment and the top predators found at the Prince Edward Islands (Pakhomov and Froneman 1999).

Observations on the prey consumed by *N. marionis* suggest that prey composition changes with body length and may also differ between males and females (Perissinotto and McQuaid 1990, Pakhomov et al. 1999). Previous studies on the feeding dynamics of *N. marionis* suggest that it is an opportunistic feeder, preying on a wide range of pelagic and benthic organisms (Pakhomov et al. 1999). Pelagic prey, mainly comprising copepods and euphausiids, dominated the diets of smaller males, while hydrozoans, bryozoans and polychaetes were the main components of the benthic prey found in the stomachs of larger females (Pakhomov et al. 1999). Previous studies on differences

between male and female *N. marionis* prey preferences were ascribed to the difference in size between two sexes (Perissinotto and McQuaid 1990). Estimates of the daily ration of *N. marionis* range between 1.9% and 6.4% body weight (Pakhomov et al. 1999). Although size dependent diet composition and daily ration have been documented for *N. marionis* (Perissinotto and McQuaid 1990, Pakhomov et al. 1999), to date the differences in the feeding dynamics of males and females have not been appropriately addressed (Perissinotto and McQuaid 1990, Pakhomov et al. 1999). Recently, *N. marionis* is thought to be partially protandrous hermaphrodite (Pakhomov et al. 2000) and to demonstrate distinct sexual dimorphism.

This chapter aims to provide additional information on the diet and to assess *in situ* daily rations of male and female *N. marionis* in the shelf region of Prince Edward Islands to understand its role in the food web at the island's group better.

4.2 Materials and Methods

Specimens were selected randomly from dredge samples 37 and 38, collected in April/May 2002 (see section 1.4; Table 1.1). All specimens collected were immediately frozen at -18^{o}C and transported to the laboratory for analysis. In the laboratory, a random subsample of 82 individuals from sample 37 and the whole of sample 38 (97 individuals) were used to measure the carapace length (CL) was measured as in Chapter Two (see section 2.1.1). For each specimen the sex was determined using the first two sets of rami on the endopod of the pleopod (see previous chapter on section 3.2). This resulted in the identification of 43 males and 39 females in total the subsample from sample 37, with 51 males and 46 females from sample 38.

4.2.1 Stomach content analysis

Stomachs of male and female *N. marionis* were dissected under a dissecting microscope at 6X to 50X magnification and prey items were identified to the lowest possible taxon. Prey items of dissected stomachs were assigned to the following groups: detritus, benthic

and pelagic. All unidentifiable, amorphous biological material was classified as detritus. Prey items that could be taken from either the benthos or the water column were recorded as “unclassified”. Foraminiferan shells were also recorded as “unclassified” mainly because pelagic forms could have sunk to the benthos. Gut evacuation experiments conducted by Pakhomov et al. (1999) have shown remains of conspecifics in the guts of *N. marionis*. In this study pleopod of *N. marionis* were identified in the stomachs of some specimens. This occurrence was referred to as cannibalism. Cannibalism was thought to occur in experimental containers, but no animals went missing due to cannibalism during this study.

Unfortunately, the percentage volume contribution of each prey item to the total stomach content of each specimen was not estimated for samples 37 and 38. However, a non-random subsample from sample 37 (n = 31) was used to provide this information. The non-random subsample consisted of 14 males and 17 females. In the non-random subsample, prey items were counted and their contribution to the total volume of food bolus was estimated visually with an accuracy of ± 5%. Prey items that were sparsely represented were recorded as making up 5% of those gut’s volume. Detritus, unlike other prey items, could not be counted; therefore the total volume of stomach and the volume of each of the separable components were estimated. The percentage of specimens with no food in their stomachs was also calculated after Cortez et al. (1995), and was termed emptiness index or EMI.

The occurrence index (OI) was calculated for each food item as the percentage of *N. marionis* stomachs in which food items were recorded. Values were calculated for samples from the two dredged samples, with both subsamples from sample 37 included. The mean percentage composition (MPC) was calculated for the non-random subsample. This was done for the entire non-random subsample (size classes pooled) and again for each size class separately (Pakhomov et al. 1999). Only specimens with food in their stomachs were used to calculate MPC and OI. In the case of the OI, size classes were pooled (Pakhomov et al. 1999).

4.2.2 Stomach Fullness Index (SFI)

Stomach contents were collected onto pre-weighed GF/C filters and dried at 60^{o}C for 24h; the shrimps themselves were dried at 60^{o}C for 36h. Both stomach contents and shrimps were weighed on a Sartorius MC-5 model microbalance to the nearest hundredth of a milligram. Stomach fullness index (SFI), expressed as the percentage of body weight, was then calculated by dividing the dry weight (dry wt) of the stomach contents by the total dry wt of the shrimp. Daily rations of *N. marionis* males and females (*I*) were estimated using Baikov's relationship: $I = 24*G*k$ (Eggers 1977), where *G* is the average value of SFI over 24h (expressed as percentage of body dry wt) and *k* is the gut evacuation constant. Multiple range tests indicated that there were no significant differences in SFI between day and night samples ($P > 0.05$). As a consequence, integrated SFI values over a day were calculated by multiplying the average SFI by 24h (Pakhomov et al. 1999).

4.2.3 Gut evacuation rate constant calculations

The gut evacuation constant (*k*) was estimated at the islands *in vitro*. Measurements were made separately for males and females. A total of 170 (88 males and 82 females) undamaged, active *N. marionis* were transferred in two 100*l* containers filled with seawater obtained from the scientific water supply. Experimental containers with animals were left on deck at an ambient temperature of ≈ 6^{o}C and were kept covered to prevent animals from being exposed to light. At hourly intervals 2 to 7 individuals were sacrificed, and immediately frozen for later laboratory analysis as described above. The gut evacuation constant ($k.h^{-1}$) was derived from the negative exponential slope of the SFI versus time (Perissinotto and Pakhomov 1996, Pakhomov et al. 1999).

4.3 Results

4.3.1 Differences in diet between sexes

The EMI in sample 37 was 8.2% and 10.1% for male and female *N. marionis*, respectively (Table 4.1). In sample 38, the EMI for males was recorded in 13.3% and for females was 15.7% (Table 4.1). In the non-random subsample there were no empty stomachs observed for either male or female *N. marionis*.

Among the various prey items identified in the stomachs of male and female *N. marionis*, detritus was recorded most often. In males, detritus was recorded in 51.8% and 48% of all stomachs examined in samples 37 and 38, respectively (Table 4.1). Detritus was recorded in 72.7% of females from sample 37, and in 80.4% in females from sample 38 (Table 4.1). In the non-random subsample from sample 37, the total food contribution of detritus was 86.8% in male and 66.6% in female *N. marionis* (Table 4.1).

The second most important food type observed in the stomachs of male and female *N. marionis* was benthic material. The contribution and species of benthic organisms consumed differed between males and females. The contribution of benthic prey to males from both sample 37 and 38 was almost identical (OI = 32.2% and 32%, respectively; Table 4.1). Among the prey consumed bivalves and gastropods, including gammarid amphipods were the best represented in the stomachs of male *N. marionis* (Table 4.1). In female stomachs, benthic material occurred more in sample 37 (OI = 49.1%) than in sample 38 (OI = 39.2%) in all examined stomachs (Table 4.1). Bivalves, gastropods and amphipods were well represented in the stomachs of females. Brachyurans were found in one female stomach from the non-random subsample. Benthic prey food volume contribution from the random subsample in males and females was 10.7% and 9.7%, respectively (Table 4.1).

Pelagic prey were only encountered in 1.8% of males from the random subsample 37 and 2% from sample 38, contributing to < 2% of prey volume in male stomachs (Table 4.1). In females, pelagic prey occurrence was higher in the random subsample 37 (OI = 3.6%)

than in sample 38 (OI = 2.2%). Unclassified material occurred in 8.9% of males in the random subsample 37 and 10% in sample 38, and the total food volume contribution was 1.1%. For females, unclassified prey accounted for 3.6% and 6.5% in the random subsample 37 and sample 38, respectively. The total food volume contributed by unclassified prey in female *N. marionis* was < 1% (Table 4.1).

The results show cannibalism occurring mostly in female *N. marionis*. Two exceptions were large males from sample 38, with carapace lengths of 6.16mm and 6.83mm (OI = 4%, Table 4.1). Only pleopod remains were found in stomachs of *N. marionis*. Cannibalised pleopods were up to 30% in females from the random subsample 37 and 30.4% from sample 38. Cannibalism contributed 23.5% of stomach contents by volume in females from the non-random subsample. Diet composition of different size classes showed cannibalism was restricted to the largest female individuals (CL > 7mm), with the exception of one female of 6.6mm (Fig. 4.1). The contribution of detritus was greatest (up to 98%) in the stomachs of the smallest shrimps and decreased to ≈ 38% in the diet of individuals with CL > 9mm (Fig. 4.1).

Table 4.1 Diet composition of male and female *Nauticaris marionis* from dredge random subsample 37 and sample 38 and the non-random subsample from dredge sample 37 arranged into food classes, OI: occurrence index, MPC: mean percentage

Sample	**Random subsample 37 (n=82)**		**Sample 38 (n=97)**		**Non-random subsample 37 (n=31)**	
Prey item	**O.I. males n=43**	**O.I. females n=39**	**O.I. males n=51**	**Prey item n=46**	**O.I. males n=14**	**O.I. females n=17**
Pelagic prey	**1.8**	**3.6**	**2**	**2.2**	**1.4**	**0**
Euphausiacea	1.8	3.6	2	2.2	1.4	0
Benthic prey	**32.2**	**49.1**	**32**	**39.2**	**10.7**	**9.7**
Hydrozoa	1.8	5.5	2	0	0	0.2
Anthozoa	0	0	2	2.2	0	0
Bryozoa	1.8	0	0	0	0.7	0
Bivalvia	5.4	9.1	14	19.6	0	1.5
Gastropoda	12.5	12.7	6	8.7	2.9	0.6
Polychaeta	0	3.6	0	2.2	0	1.2
Brachyura	0	1.8	0	0	0	2.4
Gammaridea	10.7	9.1	8	6.5	7.1	0
Isopoda	0	5.5	0	0	0	2.9
Crustacea unident.	0	1.8	0	0	0	0.9
Unclassified prey	**8.9**	**3.6**	**10**	**6.5**	**1.1**	**0.2**
Foraminiferans	8.9	3.6	10	6.5	1.1	0.2
Detritus & gravel	**51.8**	**72.7**	**48**	**80.4**	**86.8**	**66.6**
Cannibalism *N. marionis* (mainly pleopods)	**0** 0	**29.1** 29.1	**4** 4	**30.4** 30.4	**0** 0	**23.5** 23.5
Percentage of empty stomachs	**8.2**	**10.1**	**11.3**	**15.7**	**0**	**0**

The opposite pattern was observed for the consumption of benthic prey (Fig. 4.1). Unclassified and pelagic prey items, both accounted for only 1% by volume in specimens with carapace lengths 5-6mm.

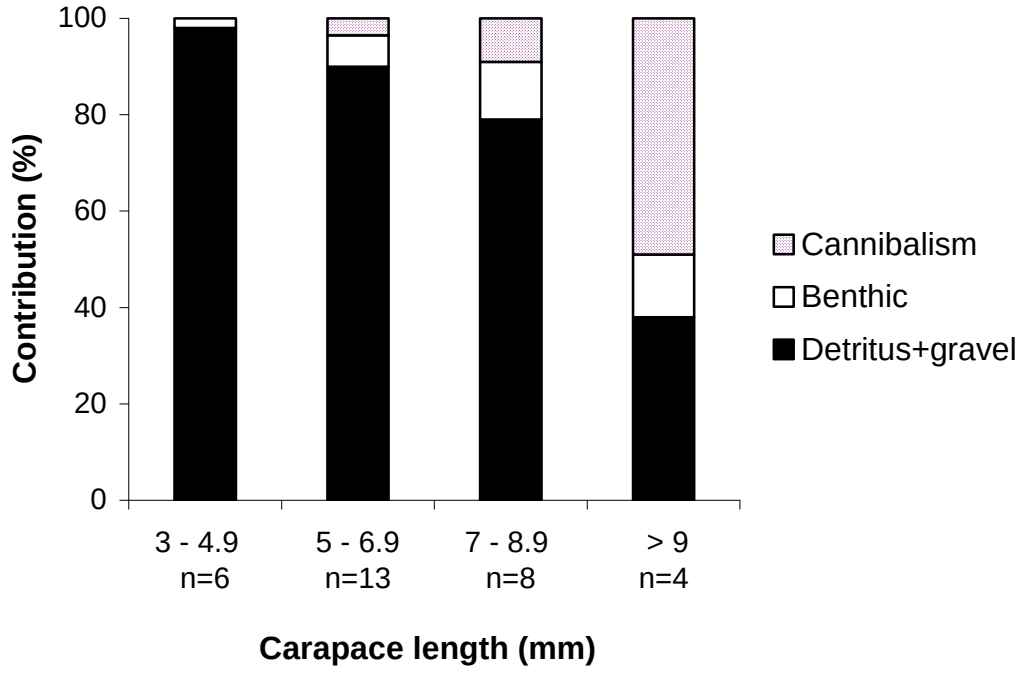

Fig. 4.1 Diet composition (by volume) of different size classes of *N. marionis*. Figures below the bars represent numbers of stomachs analysed.

4.3.2 Gut evacuation rate and *in situ* daily ration

The average SFI of neither sex differed significantly between day and night time ($P > 0.05$, Fig. 4.2). In males, the average SFI was 3.05% and 1.08% of body dry wt for samples 37 and 38, respectively. Average SFI in females was 3.45% of body dry wt for sample 37, while in sample 38 it was 2.37% of body dry wt.

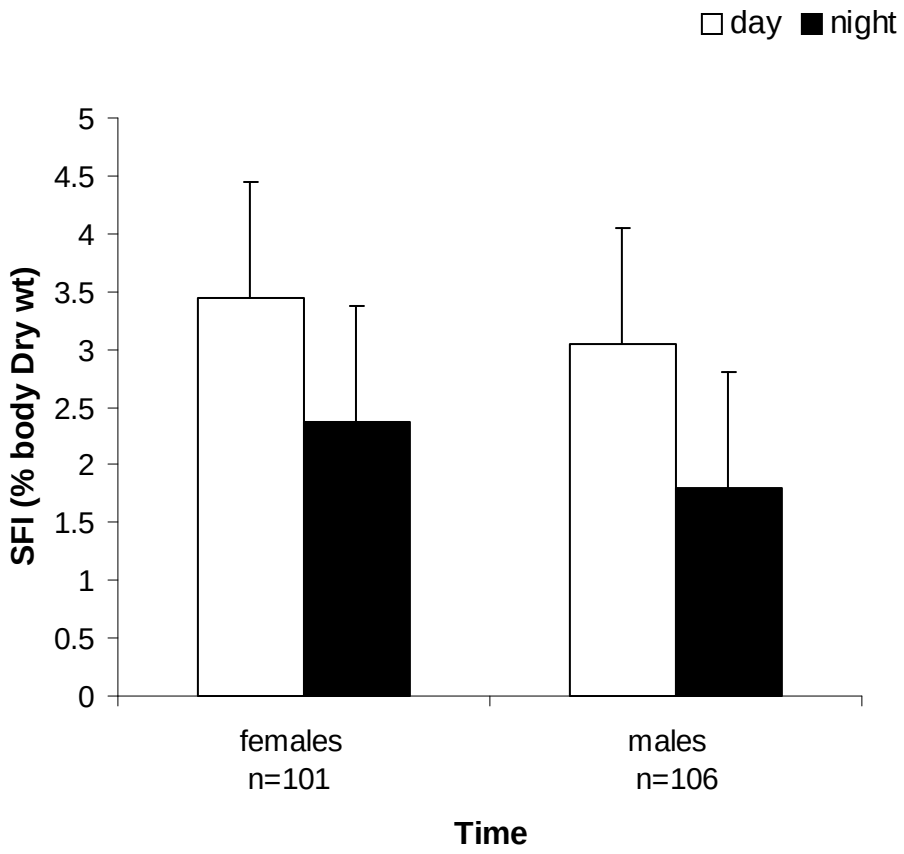

Fig. 4.2 Average *in situ* stomach fullness indices over a 24-h cycle in male and female *Nauticaris marionis*. The error bars represent one standard deviation.

Data obtained for both male and female *N. marionis* in the gut evacuation rate experiments were best fitted by a negative exponential model of SFI versus time (Fig. 4.3). The gut evacuation rate constant (*k*) and the gut passage time (1/k) of males were $0.173h^{-1}$ and 5.8h respectively (Fig. 4.3). The female gut evacuation rate constant value was $0.065h^{-1}$ and, the gut passage time 15.3h (Fig. 4.3). The daily food intake of males and females were estimated at 10.1% and 4.6% of body dry wt, respectively.

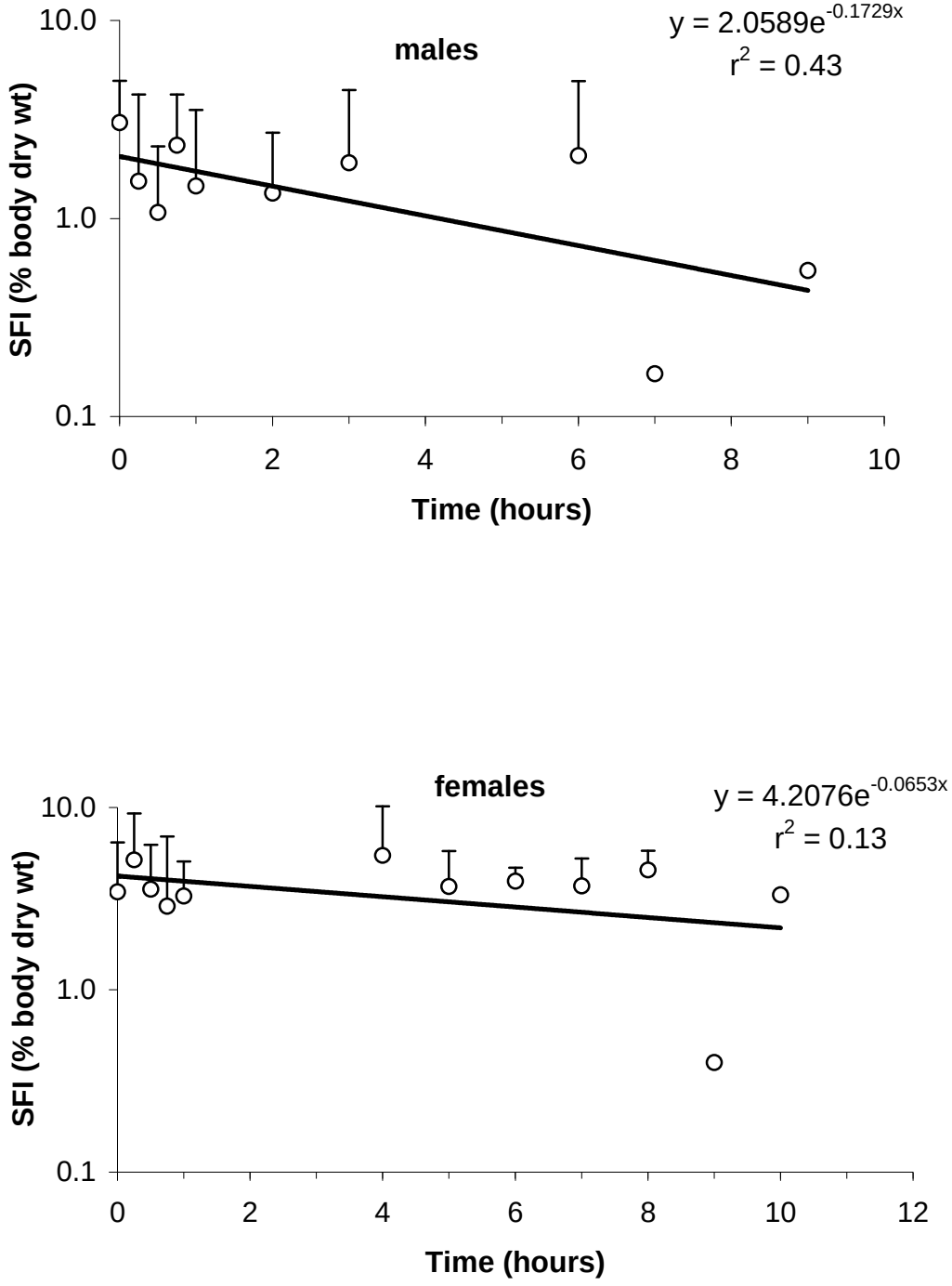

Fig. 4.3 Gut evacuation rate of males (carapace length < 7mm) and females (> 7mm) of *Nauticaris marionis.*

4.4 Discussion

No diel patterns in the feeding activity of the bottom-dwelling shrimp *N. marionis* at the Prince Edward Islands (Fig. 4.2). These results are in agreement with a recent study conducted on *N. marionis* (Pakhomov et al. 1999). The results of stomach content analyses of males and females in agreement with the findings of previous studies which indicated that *N. marionis* can be regarded as an opportunistic feeder (Perissinotto and McQuaid 1990, Pakhomov et al. 1999).

Differences in prey items consumed by male and female *N. marionis* have been previously ascribed to the sexual dimorphism exhibited by this species (Perissinotto and McQuaid 1990). Pelagic prey has been shown to dominate the stomachs of smaller males and benthic prey in larger females (Pakhomov et al. 1999). In contrast to previous studies, the present data revealed a very low contribution of pelagic prey. Although the MPC values obtained in this study are in the same range as the values for dredged samples from 1984 (Pakhomov et al. 1999), they are very low compared to the two RMT-8 samples from 1996 and 1997 (Pakhomov et al. 1999).

Nevertheless, it is evident that both males and females feed predominantly on detritus. The origin of this detritus could not be ascertained. In a recent study, conducted in the vicinity of the islands, the origins of detritus were determined using stable isotope analysis. The results of that study indicated that detritus was largely derived from the kelp beds recorded on the shallow island shelf on the two islands (Kaehler et al. 2000). These data suggest that carbon derived from the kelp beds represents an important carbon source for *N. marionis* (Kaehler et al. 2000).

In previous studies, cannibalism in *N. marionis* was rare and limited to large female individuals (Pakhomov et al. 1999). This thesis has presented the first evidence of cannibalism demonstrated by two male *N. marionis* from dredged sample 38. Cannibalised prey seemed to be of male or juvenile origin, this is based on the gender definitions using the rami on the endopod of the pleopods (Pakhomov et al. 2000) found

in the stomachs of *N. marionis*. It has previously been hypothesized that cannibalism might be density depended (Pakhomov et al. 1999). Although precautions were taken to minimize cannibalism in experimental containers, one cannot discount the possibility that cannibalism may have occurred during dredging when animal densities would be high. The freshly consumed pleopods remains often found in the stomachs of *N. marionis* support the abovementioned hypothesis.

Estimates of *k* and 1/k presented here are in the same range as a single study conducted in April/May 1997 with *N. marionis* in the vicinity of the islands (Pakhomov et al. 1999). Pakhomov et al. (1999), employing the same technique, estimated that the daily rations of *N. marionis* ranged from 1.9% to 7.5% of body dry wt, with an absolute maximum of 17% of body dry wt (Pakhomov et al. 1999). The present estimates of daily ration were 10.1% for males and 4.6% for females of body dry wt. Thus, daily rations estimated in 1997 and 2002 are within the same range.

Bromley (1994) concluded that *k* (and hence daily food intake) is mainly influenced by the composition of prey and water temperature, while predator, prey size, meal size and feeding frequency are less important. It is likely that the higher estimates presented here reflect differences in prey composition between the present study and that of Pakhomov et al. (1999). It is also possible that the higher daily rations found in this study are the result of a predominance in the diet of *N. marionis* of low energetic quality food, such as detritus during this study.

In this study, my estimates were, however, high compared to daily rations of the deep-water shrimp *Aristeus antennatus* (< 1% of body dry wt; Maynou and Cartes 1997), and lower than (≈ 16% of body dry wt) of the shallow water brown shrimp *Crangon crangon* (del Norte-Campos and Temming 1994). A study of nine species of deep-water decapod crustaceans of the NW Mediterranean suggests that actively swimming shrimps have higher consumption rates than less mobile benthic decapod crustaceans (Maynou and Cartes 1998). This pattern of increasing food intake with increasing mobility is equivalent to that identified by Koslow (1996) in a review of studies on metabolism and

daily ration in deep-water fishes. Koslow (1996) found that slow-moving deep-sea fish had low metabolic rates and low food intake, while fast-swimmers had a higher metabolic rates and daily rations. The higher daily ration obtained for males in this study are thus probably a reflection of the increased metabolic rates of the smaller individuals.

4.5 Conclusions

1. The results are in agreement with previous studies that *N. marionis* is an opportunistic feeder, which feeds on the food that is available at a particular time or area.

2. Cannibalism has been observed in female *N. marionis.* Cannibalism also occurs in males, and its occurrence depends on individual size, not gender.

3. There appear to be differences in daily ration between males and females. The higher daily rations for males appear to be related to a higher metabolic rate linked to their smaller size.

Chapter Five

Summary

5.1 Conclusions

1. The carapace length was used as the measurement of size for *Nauticaris marionis*. A clear definition of this measurement was given in the thesis as "a measure from the posterior margin of the orbit to the dorsal midline of the posterior margin of the carapace". Using this definition, the carapace length is the most accurate indicator of body size in *Nauticaris marionis*, and indeed most decapod crustaceans. This is due to the fact that the carapace is a relatively long and inflexible body part that is likely to survive the stresses of digestion, therefore, making it the preferred indicator of body size in studies where the crustacean is a prey item.

2. The program Rmix was useful in studying length frequency distribution as it had the ability to detect older cohorts which, if not detected, may have resulted in these cohorts being ignored or underestimated. The Rmix ability allows the program to obtain a good fit between calculated curves and the actual data, resulting in high P values. When matching cohorts that are in sequential order, a nonparametric test method was effective in identifying cohorts. Another method used to match cohorts using gender proportions is the sexual inversion method. The use of this method is discouraged, as it requires a large data set for all samples collected during the sampling period.

3. The von Bertalanffy growth curve parameters for *N. marionis* were empirically identified as K = 0.22239/year, L_∞ = 14.05789mm, t_0 = -0.05174, L_0 = 0.16083mm.

4. Previous work suggests that *N. marionis* hatches mainly in March (Pakhomov et al. 2000), with negligible hatching persisting until May. The finding here of gravid females in samples from April and May supports this suggestion.

5. *N. marionis* survives for a maximum of seven years in significant numbers under natural conditions.

6. This study confirms that *N. marionis* is a partially protandric hermaphrodite. The majority of juveniles develop into males. A small minority of individuals develop directly into primary females, without passing through a male phase. Once having grown to a certain carapace length they transmutate into females. By April/May the transmutation is probably complete. Juveniles developing into primary females do so by losing the *appendices internae* before developing ovaries or may grow ovaries first then lose the appendage to become primary females.

The vast majority of juveniles first become males before entering a transitional stage, *tertia quae,* to become secondary females. Sex change is achieved by a rapid loss through a single moult of two male copulatory organs, the *appendices internae* and the *appendices masculinae*. It is unknown whether sex differentiation occurs in plankton or just after larvae settle in the benthos to become juveniles.

7. Parasitism by an isopod of the family Bopyridae noted off New Zealand, such parasite is by *Pseudione affinis* but the species attacking *N. marionis* at the Prince Edward Islands has not been identified. The effects of the parasite are unknown, but it may reduce the growth rate of *N. marionis*. Bopyrid parasitism appears to be size-specific rather than gender-specific and observed in males, females and *tertia quae*, but always in individuals between 3 and 9mm carapace length.

8. *N. marionis* appears to be an opportunistic feeder preying on a wide variety of prey, with a preference for detritus, benthic amphipods and gastropods. Cannibalism of the pleopods of conspecifics was been observed in female *N. marionis*, as well as a few males. The occurrence of cannibalism in *N. marionis* appears to depend on individual size, not gender.

9. The differences in *in-situ* daily ration between male and female *N. marionis* suggest that males have a higher daily ration than females. The higher daily rations of males appear to be related to their higher metabolic rate that is linked to their smaller size.

5.2 Suggestions for future studies

1. Using appendices to identify genders is an indirect approach and it would be useful to take a more direct method, in examining the development of gonads themselves and of the gonopore. Therefore a study of gonopore development is required for more accurate gender definition.

2. Larvae of *N. marionis* have yet to be caught in either dredges or trawls, suggesting that larval habitats have not been sampled. This is a serious weakness in our understanding of the biology of *N. marionis* and further attempts should be made to discover larval refuges. Good sampling of larvae could improve our understanding of not only population dynamics, but also of juvenile development and sex change

3. Existing evidence of long term climatic change at the Prince Edward Islands and the link between environmental factors and sex changes in other decapods suggest that decadal trends of high sea temperature may affect sex change in *N. marionis*, especially during larval stages since sexual differentiation is presumed to occur in the plankton.

4. The effects of parasitism on growth and reproduction of *N. marionis* require further study. A description of the parasite to species level is also required.

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