Behavioural differences between populations of *Nassarius pauperatus* (Mollusca : Prosobranchia)

by

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A thesis submitted for the degree of
Doctor of Philosophy, November, 1979.

Awarded 5th Aug 1980

Contents

Summary

The whelk *Nassarius pauperatus* (Lamarck) is common on intertidal sandflats in southern Australia. A behaviour described and defined as being a "Twister" was observed in some individuals. Upon contacting a member of their own species, Twisters gave a very specific response, rotating their shell from side to side in a way similar to the avoidance responses displayed by many gastropods to predators. However, some individuals, defined as "Non Twisters", never gave this response after contacting another *N. pauperatus*. The frequency of Twisters varied between populations of *N. pauperatus*, and was negatively correlated with food availability (as estimated by measuring hunger) and positively correlated with the population density of juvenile *N. pauperatus*.

Some possible reasons for the differences in the frequency of Twisters between populations were investigated in terms of whether they could be attributed to either selection or stochastic factors. Breeding experiments suggested that being a Twister was heritable. Three hypotheses were proposed to account for the association between the percentage of Twisters and food availability, and these were tested in the field and the laboratory. It was found in the laboratory that Twisters were better than Non Twisters at competing for space to feed, when food was in short supply and distributed as discrete patches. There was also evidence that competition for space to feed occurred when food was in short supply in the field and it was postulated that Twisters thus had a selective advantage compared to Non Twisters in such situations. A possible disadvantage of being a Twister was not adequately tested.

Declaration

This thesis contains no material accepted for the award of any other degree or diploma in this or any other University.

To the best of my knowledge this thesis contains no material previously published or written by another person, except where due reference is made in the text.

Stephen C. McKillup.
16/XI/1979

Acknowledgements.

Alan Butler patiently supervised this project, offering encouragement, criticism and advice. During Alan's absence of nine months, Mike Coates provided stimulating supervision, comment and criticism.

The following persons helped in the field: Bob Inns, Paul Zed, Ruth Bentley and Neil Piller. Mick and Deirdre Keough collected *Nassarius pauperatus* from Streaky Bay and Smoky Bay.

Ruth Bentley assisted with the tedious task of proof reading the manuscript, which was made easier by the competent typing of Mrs. A. Briedis.

I am grateful to the limited number of people in the Department of Zoology with whom I had meaningful scientific and social interaction and especially for Alice Kay's tolerance, encouragement, criticism and companionship.

1. The usefulness of studying differences in behaviour within a species.

1.1 Introduction.

There have been very few studies of variation in behaviour, compared to studies of differences in morphology, or the structure of chromosomes or proteins, within a species.

This may be because behavioural differences are more subtle and also more difficult to experiment with compared to other traits (Seghers, 1974b). Behavioural phenotypes result from the interaction between the genotype of an organism and the environment (Hinde, 1966). Whilst the classification of behaviour as either "learned" or "innate" has been recognised as unrealistic, patterns of behaviour will have varying degrees of heritability (see Fox 1978). Whatever their heritability, behavioural patterns may affect the probability of their possessor surviving and reproducing (see Eibl-Eibesfeldt, 1970, for numerous examples). Tinbergen (1963, 1965) has especially stressed the usefulness of studies aimed at determining the selective value of behaviour patterns since these clarify the importance of natural selection in the evolution of behaviour.

Differences in behaviour between individuals of the same species may provide unique opportunities for the study of the selective value of different behavioural phenotypes, since individuals are available which either possess or lack a particular pattern of behaviour but are otherwise very similar, whereas different species may also differ greatly with respect to other traits as well. Liley and Seghers (1975) argue that behavioural differences between natural populations of the same species provide an especially powerful means of investigating the selective values of such intra-specific behaviour since differences in behaviour between populations can be correlated with differences in the environment, giving rise to testable hypotheses accounting for these observed associations.

Behaviour is only one of the large set of characters which will vary between local populations of a species. As well as being considered important in the process of speciation, the extent to which such differences can be accounted for by natural selection, relative to stochastic factors, is an important question for evolutionary biologists (Dobzhansky, *et al*, 1977). This thesis concentrates primarily upon this question in respect to behavioural differences between natural populations of *Nassarius pauperatus* (Lamarck).

1.2 Previous studies of differences in behaviour between populations of the same species.

Curio (1961) observed that German Pied Flycatchers (*Ficedula hypoleuca hypoleuca*) mobbed both the Redbacked Shrike (*Lanius collurio*) and Owls, but that Spanish Pied Flycatchers (*Ficedula hypoleuca iberiae*) only mobbed Owls. Curio noted that *Lanius collurio* did not naturally occur in the habitat of *Ficedula hypoleuca iberiae*, but did not further investigate this difference in behaviour.

McPhail (1969) described differences in the nuptial colours of male sticklebacks (*Gasterosteus* species). The nuptial colour of males was either red or black and these differences were heritable. Females from populations containing either all red or all black males preferred to mate with red males, yet populations were found that contained all, or a high proportion, of black males. These were in habitats containing *Novumbea* species, a predator of juvenile sticklebacks. McPhail found that although this predator could not consume adult sticklebacks, it oriented towards red males in preference to black males and since male sticklebacks tend their progeny, this predator may consume a greater proportion of progeny from red males. McPhail also found that the escape responses of larvae raised in the laboratory differed according to whether their parents came from populations with or without predators. Larvae from black parents (from a population experiencing predation) were observed to be better able to elude individual *Novumbea* than larvae from red parents (from a population which had not experienced predation), and significantly less black larvae were eaten in laboratory experiments.

McPhail concluded that "there is an innate escape response in black larvae that makes them less susceptible to predation". However this difference in behaviour may have been completely independent of colouration, since all larvae from the population suffering predation were from black parents and *vice versa*.

Whilst McPhail has not shown that differences in behaviour are causally associated with black colouration, he has shown that a difference in behaviour associated with the presence of a predator in the field can decrease the probability of an individual stickleback being consumed by the same predator in the laboratory, and also that the difference appears to be heritable.

Krebs (1970) described changes in aggressive behaviour that occurred during changes in population density in two species of vole, *Microtus ochrogaster* and *Microtus pennsylvanicus*. Krebs emphasised that it was not known whether these behavioural differences were genetically determined or not, nor whether they were actually caused by changes in population density.

Strong (1973) found that the proportion of the female moult cycle and the total time spent in amplexus by the amphipod *Hyalella azteca* varied between geographically isolated populations and was negatively correlated with the presence of species known to prey upon *H. azteca*, in the habitats of these populations. A pair of *H. azteca* spends up to several days continuously amplexed and cultures established in the laboratory from different populations maintained their respective differences in the proportion of the moult cycle and total time spent amplexed, suggesting that the differences in behaviour were heritable. Strong postulated that amplexed pairs of *H. azteca* were more vulnerable to predators, since they were larger (hence more visible) and also more slower moving than individuals. Consequently he argued that selective predation upon amplexed pairs had selected for individuals which spent less time in amplexus. However, Strong did not test this hypothesis.

Barash (1973) observed differences in social behaviour between populations of the marmot *Marmota flaviventris* along an altitudinal gradient. He postulated that these differences in social behaviour "were real and reflected a complex series of adaptations to different lengths of growing seasons". Barash did not attempt to establish the reality or advantages of these differences by attempting to demonstrate whether they were heritable, or how they were related to differences in growing season.

Yeaton and Cody (1974) found that territory size in the song sparrow *Melospiza melodia* was correlated with the number of other avian species present on different islands, which were thought to compete with *Melospiza melodia* for food. They argued that this correlation illustrated "competitive release" wherein the lack of competing species enabled *M. melodia* to exploit the ecological niches thus left vacant and hence obtain sufficient resources from a smaller area of territory. Yeaton and Cody did not test this hypothesis by experimentally manipulating these populations.

Seghers (1974a) described how the degree to which guppies (*Poecilia reticulata*) formed schools varied between geographically isolated populations, and that the tendency to form schools was positively correlated with the presence of species known to prey upon P. reticulata. Seghers cultured guppies from these different populations for 3-4 generations in the laboratory without predators and found that these retained the same differences in schooling behaviour that he had observed in the field. Consequently he assumed that these differences in behaviour were heritable. However, these guppies were raised in laboratory populations together with their parents so it is possible that the differences in schooling behaviour may have been learned. Seghers postulated that individuals in a school would not be so heavily preyed upon as those swimming alone (see Milinski, 1977) and demonstrated experimentally that predators consumed significantly more guppies from "non schooling" than from "schooling" stocks, but emphasised that since guppies from these different stocks also differed with respect to other behaviour patterns, the differences in predation were also probably partly due to other behavioural differences. Nevertheless Seghers has shown that the presence of predators in the habitat of some populations of a species does result in differences in the behaviour of individuals in these populations which reduce their probability of being preyed upon.

Seghers also (1974b) showed that the laboratory raised progeny from two of the populations previously studied also displayed different responses to shapes resembling aerial avian predators, and postulated that these differences had arisen as a result of "differences in the selection pressures exerted by aquatic versus aerial predators" between these populations. This hypothesis was not tested.

Similarly, Farr (1975) observed a correlation between differences in the frequency and type of male courtship display by Poecilia reticulata and the presence of fish which prey upon this species in the field. The number of courtship displays per five minutes by males in geographically isolated populations was significantly less in populations which were preyed upon by Rivulus hartii. Farr proposed that selective predation of courting male P. reticulata and also sexual selection for courting males were responsible for maintaining these differences in behaviour, but these hypotheses were not tested.

Caldwell and Dingle (1977) observed that there were differences in the frequency of agonistic behaviour shown by individuals of the stomatopod crustacean Haptosquilla glyptocercus from two natural populations, when observed in the laboratory. H. glyptocercus occupies natural cavities in the substratum, for which it competes with H. glyptocercus as well as other stomatopod species (Caldwell and Dingle, 1975). The frequency of raptorial strikes and spreading of the raptorial meri, both of which are exhibited during competitive interactions for cavities, were significantly higher in individual H. glyptocercus from one of these populations. Different sets of stomatopod species compete for cavities with H. glyptocercus at each location and Caldwell and Dingle hypothesised that this may be responsible for the observed differences in behaviour, by selecting for more aggressive individuals when competition is more intense. Neither this hypothesis, nor Caldwell and Dingle's assumption that the behaviour was heritable, were tested.

In summary, only McPhail (1969) and Seghers (1974a) have actually demonstrated how different patterns of behaviour can affect the probability of an individual surviving in different environments. Others have observed differences in behaviour between populations of the same species and have proposed various untested hypotheses to account for these. With the exception of Curio, (1961) and Yeaton and Cody, (1974) all of these authors discuss the behavioural differences in terms of natural selection for these specific differences in behaviour, yet only McPhail (1969), Strong (1973) and Seghers (1974a) have evidence to suggest that the behaviour in question is heritable.

1.3 Behavioural differences between populations of Nassarius pauperatus.

This thesis describes and defines a behaviour pattern displayed by some individuals of the marine whelk Nassarius pauperatus (Lamarck). The proportions of individuals showing this behaviour vary between populations. The heritability of this behaviour is investigated and hypotheses concerning its survival value are proposed and tested.

2. Taxonomy, distribution and diet of Nassarius pauperatus, description and definition of "twisting" and of being a "Twister" and initial observations of differences in this behaviour between populations.

2.1 Distribution, taxonomy and habitat of Nassarius pauperatus.

2.1.1 Distribution and taxonomy.

The marine snail Nassarius pauperatus (Lamarck) occurs in Western Australia (Smith, 1975), Queensland, (from labelled museum specimens), Victoria, New South Wales and Tasmania as well as South Australia(MacPherson and Gabriel, 1962). The taxonomy of the Indo-Pacific Nassariidae has recently been revised by Cernohorsky (1972) and the history of the classification of N. pauperatus detailed by Smith (1975)* who also described the radula of this species and compared it with that of Nassarius burchardi (Dunker in Philippi) which often occurs sympatrically with N. pauperatus.

Therefore I shall not comment further upon the classification of this species, except to note that the Nassarius pauperatus sampled in South Australia were easily distinguished morphologically from Nassarius burchardi (Dunker in Philippi) and Nassarius pyrrhus (Menke), these being the only other Nassariidae found with N. pauperatus, and that radulae from both juvenile and adult N. pauperatus fitted the description given by Smith.

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* Throughout his thesis Smith refers to Nassarius pauperatus as Nassarius pauperata, misquoting Cernohorsky (1972).

2.1.2 The habitat of Nassarius pauperatus.

MacPherson and Gabriel (1962) state that Nassarius pauperatus may be found "alive on sandbanks at low tide" and Cernohorsky (1972) describes its habitat as being "in rock pools, among weed, intertidal". Womersley and Edmonds (1958) found South Australian N. pauperatus in the lower to mid littoral zone on "coasts of slight wave action, with sandy or muddy flats or beaches". I likewise found N. pauperatus on sheltered intertidal sandflats, often in pools, on beaches with slight wave action and in the mouth of the Onkaparinga estuary. At all locations where it occurred, N. pauperatus was always the most abundant species of Nassarius. On steeply sloping but sheltered beaches with extensive lower littoral sand or mudflats at low tide, N. pauperatus was restricted to a narrow band on the shelving part of the beach corresponding to the mid to lower littoral zone and was often not found at all on very steep but nevertheless sheltered beaches (e.g. Pine Point, South Australia).

2.2 The diet of Nassarius pauperatus.

2.2.1 The belief that Nassarius pauperatus is a predatory carnivore.

Nassarius pauperatus is described by MacPherson and Gabriel (1962), pp 192-3, as "carnivorous, readily boring through the shells of other molluscs and feeding on them". I found this to be a common belief among South Australian malacologists (D.P. Thomas, personal communication). However, Johnston and Mawson (1946) describe N. pauperatus as "carnivorous, readily coming to the surface when food is available" suggesting that it is a carrion feeder, and Cernohorsky (1972) has categorised the Nassariidae as being mainly carrion feeders.

2.2.2 Observations of the feeding behaviour of *Nassarius pauperatus* in the field and laboratory.

Nassarius pauperatus were never observed drilling other molluscs in the field but were found feeding upon dead molluscs, crustaceans, and annelids as well as appearing to graze upon algal film coating the surface of the sandflat. Many *N. pauperatus* rapidly congregated around and fed upon living bivalves (e.g. *Katelysia scalarina* Lamarck) if the latter were forced open and placed on the sand. Hence my field observations did not suggest that *N. pauperatus* preys upon other molluscs by drilling them.

To further confirm this, a total of 50 adult and juvenile *N. pauperatus* were collected from Port Gawler and kept in the laboratory for three months at 19°C together in an aquarium with several *K. scalarina*, also collected from Port Gawler. The latter ranged in shell length from 1.5 cm up to 4.6 cm, thus providing the snails with a range of shell thicknesses to drill. However, even though all the *N. pauperatus* eventually died (presumably of starvation, since those which died were consumed without having been drilled, unless quickly removed) none of the bivalves were drilled. Bivalves had to be occasionally replaced since these were not fed either, in case the snails were able to utilise this food for themselves. When a bivalve died, snails gathered around it and were observed intruding their proboscises through the open shell valves and feeding on the dead bivalve.

2.2.3 Examination of gut contents.

The gut contents from 25 adult and 25 juvenile *Nassarius pauperatus* which had been freshly collected and dissected in the field were examined under a binocular microscope at 40X magnification, revealing what appeared to be a mixture of both plant and animal material, the proportions of which varied between individuals.

A crystalline style, characteristic of species feeding primarily upon plant material (Jenner, 1956) was never found, even in specimens dissected in the field only minutes after collection. Bottom sediment was rarely observed in the gut and then only in very small quantities, of usually less than 5% of the total contents.

2.2.4 Conclusion

From these observations I conclude that this species is an omnivore, eating both plant material and animal carrion. This was later confirmed by the finding that N. pauperatus could be maintained in the laboratory for long periods whilst being fed only algae or only animal carrion (see Appendices 1 and 2).

2.3 Description and definition of "twisting" and of being a "Twister".

2.3.1 Initial observations of twisting.

Whilst watching a sample of 50 adult and juvenile Nassarius pauperatus encountering each other in an aquarium in the laboratory, a rather striking behaviour was observed* and it was decided to investigate this further.

Initially this behaviour was called an "avoidance response" since it resembled the avoidance responses that many species of gastropod exhibit following an encounter with species which prey upon them (e.g. see Feder, 1963). However, the response by N. pauperatus differed inasmuch as the "avoider" did not always change

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* Since it was commonly believed that Nassarius pauperatus was a predatory species which drilled other molluscs it was intended to use it to test an hypothesis relating spatial heterogeneity and predator prey interactions. The snails being watched were those used in the previous section (2.2.2) in which it was concluded that this species was not predatory.

direction, move away from the individual it had encountered or lose contact with the substratum, but instead often continued in the same direction and remained in bodily contact with the other snail. For these and other reasons I later renamed the behaviour "twisting".

Twisting was displayed by some individuals after they had touched any accessible part of the body of another N. pauperatus with either, or any combination of, their siphon, tentacle(s) or proboscis, but not if they only touched the shell of the other snail. Individuals also twisted if another snail touched them, especially if this contact occurred on the rear of their foot.

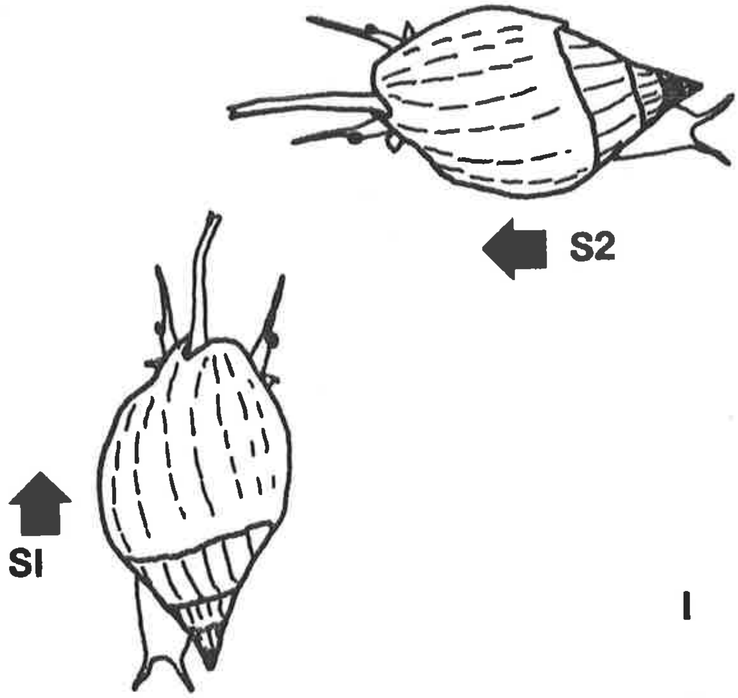
Touching the body of another with either siphon, tentacle(s) or proboscis was defined as "contact" and this term will be used solely in this context throughout this thesis. The snail which initiated contact was referred to as Snail 1 (one) and the snail thus contacted, Snail 2 (two). Twisting, which consisted of swinging the shell from side to side (see Figures 2.1 and 2.2), usually commenced very shortly after contact occurred, and continued whilst contact was maintained and often for one or two seconds after contact ceased. Not all contacts resulted in twisting - either both, Snail 1, Snail 2, or neither snail twisted following contact. Twisting by Snail 2 appeared to differ from twisting by Snail 1 in that Snail 2 frequently changed direction and became detached from the substratum whilst twisting, but this was rarely observed by Snail 1. Thus twisting by Snail 2 more resembled the classical "avoidance response" described by Feder (1963), and this difference will be dealt with in greater detail later.

Twisting by N. pauperatus, characteristic of Snail 2, could also be elicited by mechanically damaging individuals with a needle on the rear of their foot, or by forcibly restraining their shells for a few seconds.

Also, following several hours of mechanical disturbance (e.g.

Figure 2.1 Twisting by Snail 1, after having contacted another (Snail 2).

1. Snail 1 and 2 approaching.
2. Snail 1 contacts Snail 2.
3. Snail 1 commences twisting. Snail 2 contacts Snail 1.
4. Both snails remain in contact. Snail 1 continues twisting, Snail 2 does not twist.
5. Snail 1 ceases contact with Snail 2 but continues twisting.
6. Snail 1 ceases twisting.



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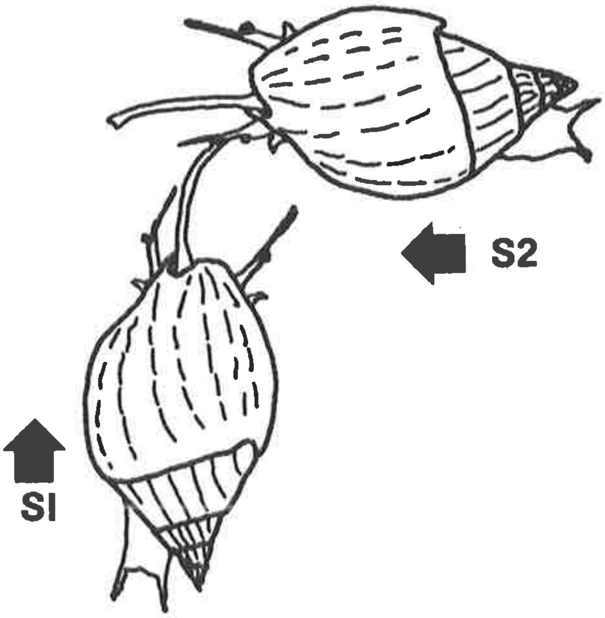

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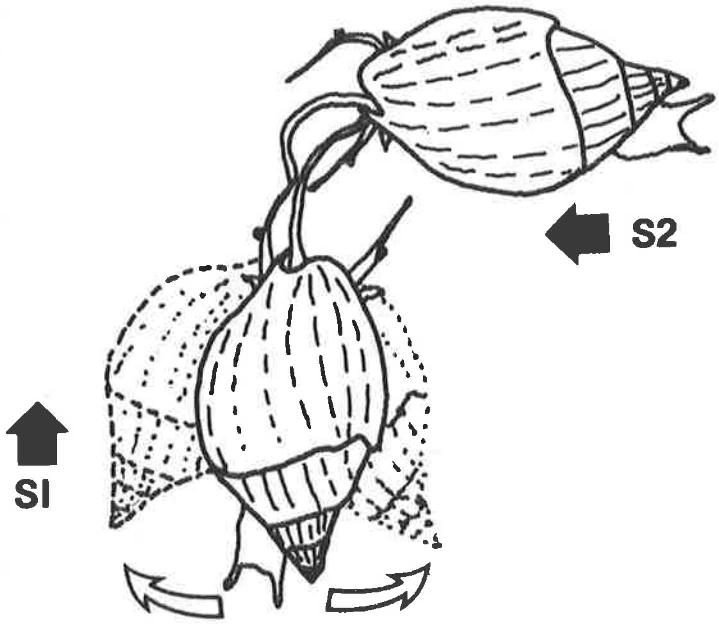

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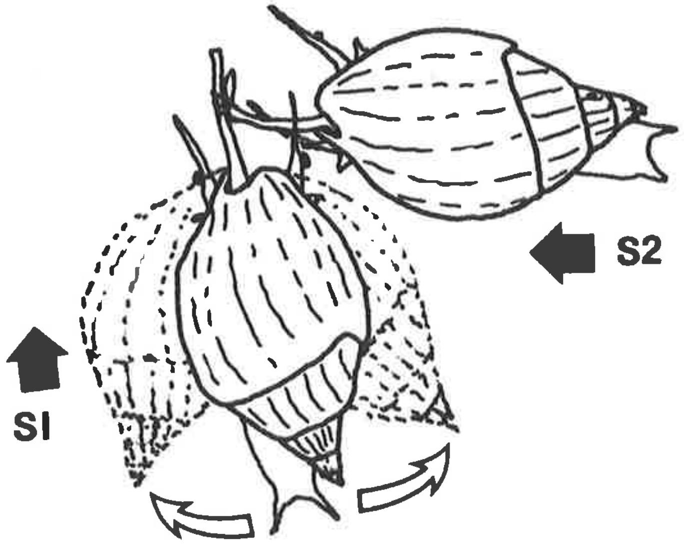

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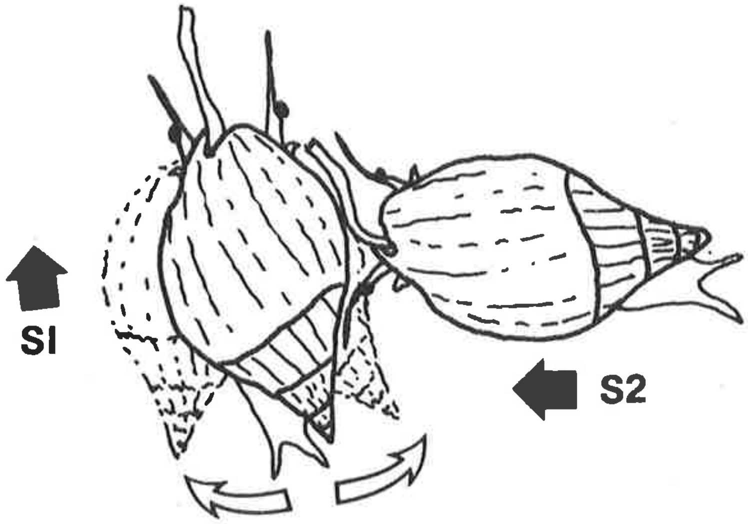

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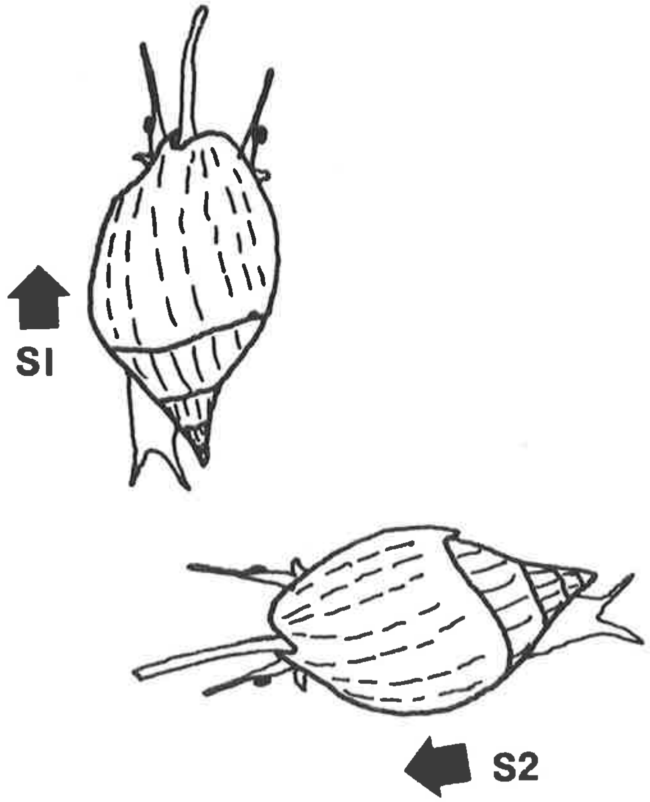

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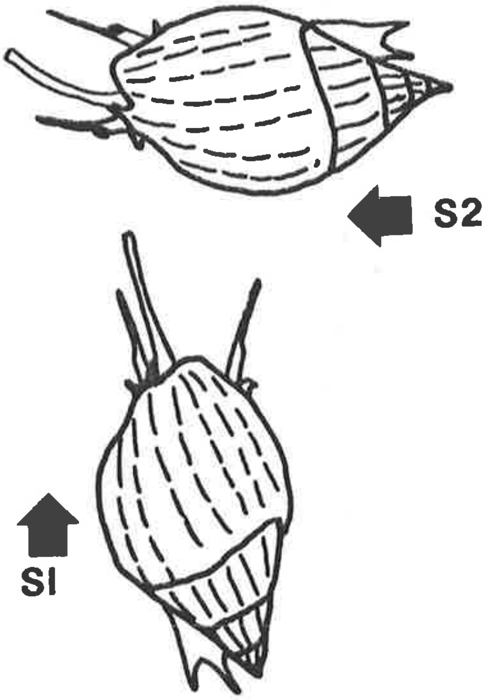
Figure 2.2 Twisting by Snail 2 after being contacted by Snail 1.

1. Snails 1 and 2 approaching.
2. Snail 1 approaches hind part of Snail 2's foot.
3. Snail 1 contacts Snail 2 with proboscis. Snail 1 does not twist.
4. Snail 2 begins twisting. Contact ceases.



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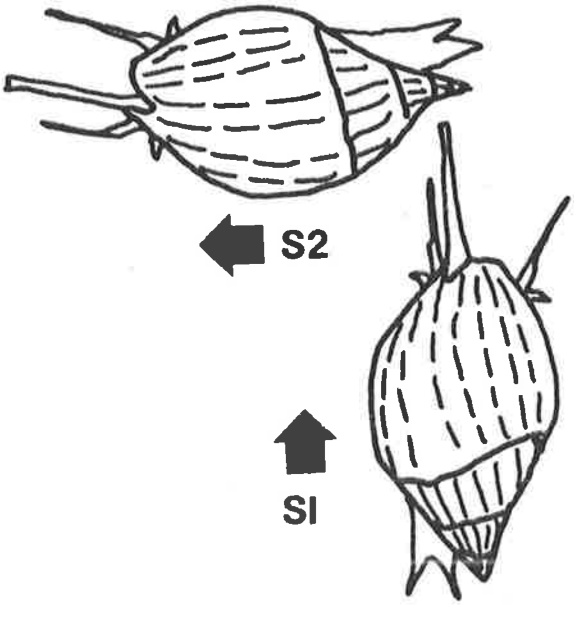

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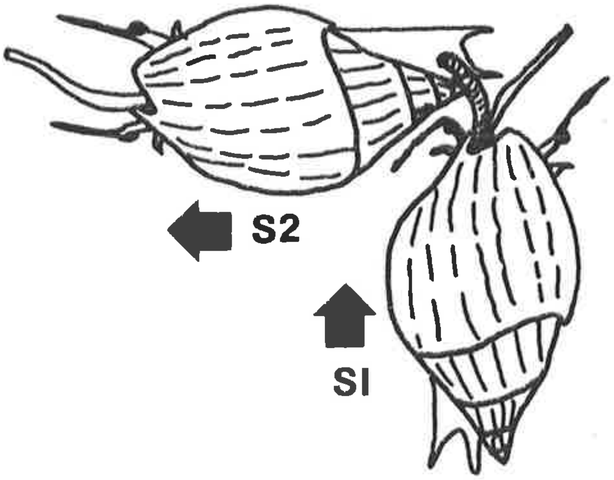

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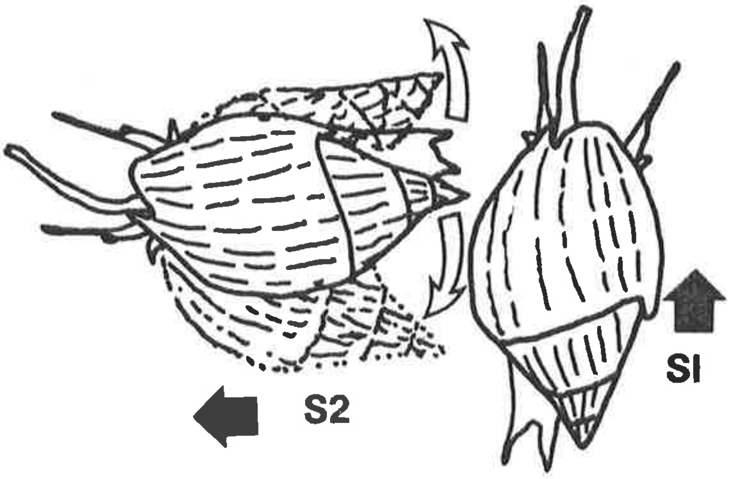

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that experienced during travel in a motor vehicle) many individuals were observed swinging their shells from side to side without having had any recent contact with another snail. However, this behaviour ceased within an hour of being undisturbed, and was not investigated further.

2.3.2 Initial survey of other locations.

Following these casual observations in the laboratory of twisting by Nassarius pauperatus from Port Gawler I decided to sample N. pauperatus from other locations in South Australia, to see if individuals from these also displayed this behaviour. Port Gawler, Middle Beach, Parham and Port Clinton (see Figure 3.1) were visited on either 12/V/76 or 13/V/76. At each site qualitative observations were made of the frequency of twisting responses following contact between pairs of N. pauperatus.

High frequencies of twisting following contact were observed at Port Gawler, Port Parham and Middle Beach. However, at Port Clinton, far fewer contacts were followed by twisting by either Snail 1 or 2. Consequently, samples of 80 adult N. pauperatus were collected from each of Port Gawler and Port Clinton. To ensure that all (or most) of the N. pauperatus per area of substratum were collected, the surface of the sandflat was disturbed and searched, rather than just collecting snails visible on the surface. N. pauperatus were never found any deeper than 3.0 cm, so the substratum was searched to this depth.

Snails were placed in polyurethane foam containers and transported to the laboratory where they were kept without food in white polystyrene trays (dimensions 54 x 32 x 12 cm deep) which were filled with aerated seawater to a depth of 4 cm, at a constant temperature of 17°C and current daylength.

To observe contacts between snails, the airstone was removed from the tray and the usually inactive snails pushed into a pile in the centre of the tray. Following this disturbance, many snails then began to crawl outwards from the heap, contacting others as they did so. Snails from both locations were observed for two hours each day on the second, sixth and eighth day following collection. Every 20 minutes during the observation period, snails were returned to the centre of the container since most usually became inactive after 15-20 minutes.

The snail which initially contacted another was referred to as Snail 1, and the contacted snail, Snail 2 (as defined previously). If both snails contacted each other (i.e. each touched the other with either siphon, tentacle(s) or proboscis), two contacts were recorded, with Snail 1 becoming Snail 2 and vice versa. The results of Snail 1 contacting Snail 2 were scored in one of the four following categories:

(1) NTR No twisting response by either snail.

(2) TRBS1 Twisting response by Snail 1.

(3) TRBS2 Twisting response by Snail 2.

(4) TRBB Twisting response by both snails.

Results appear in Table 2.1. The proportion of responses in each of the four categories did not differ significantly between days without food following collection for snails from Port Gawler but did for those from Port Clinton (see Table 2.1). Examination of the data from Port Clinton in Table 2.1 reveals that the changing proportions in each category with time are due to an increase of the proportion of contacts resulting in TRBS2 (and a decrease in those resulting in NTR). Observations showed that contacts resulting in

Days without food following collection	Number of contacts resulting in NTR	TRBS1	TRBS2	TRBB	Total
Port Gawler					
2	6	20	6	3	35
6	14	31	13	21	79
8	20	34	10	9	73

4 x 3 contingency table for independence, $\chi^2_6 = 10.40$, $p > 0.05$

Days without food following collection	NTR	TRBS1	TRBS2	TRBB	Total
Port Clinton					
2	32	4	1	0	37
6	28	8	31	0	67
8	29	6	26	3	64

4 x 3 contingency table for independence, $\chi^2_6 = 29.43$ $p < 0.001$

Table 2.1. Results of contacts observed between 80 adult *Nassarius pauperatus* from each of Port Gawler and Port Clinton in the laboratory, 2, 6 and 8 days after collection. For definition of responses to contact, see text.

twisting by Snail 2 (i.e. TRBS2 and TRBB) were due to Snail 1 touching the rear of the foot of Snail 2, and the response usually appeared different from twisting by Snail 1, (see Section 2.3.1). Consequently I recategorised the results of contact into those resulting in twisting by Snail 1 (TRBS1 and TRBB) and those not resulting in twisting by Snail 1 (TRBS2 and NTR). These sums appear in Table 2.2. The proportion of responses in each of the two categories was independent of days without food following collection for snails from both Port Gawler and Port Clinton (see Table 2.2). Considering this homogeneity within samples, all observations from Port Clinton and all from Port Gawler were summed between days following collection within each location (see Table 2.2). The proportion of contacts resulting in twisting by Snail 1 differed significantly between snails from Port Gawler and Port Clinton (see Table 2.2).

2.3.3 Observations of twisting in the field.

Although it had been casually observed that the frequency of contacts resulting in twisting differed between locations in the field, no quantitative measurements had been made. To compare the behaviour of N. pauperatus between populations in the field and also between the field and the laboratory, observations of unstaged contacts were made at Port Gawler and Port Clinton on several occasions during the latter part of 1976. Results of these appear in Table 2.3(a). The data were treated similarly to those in Table 2.2, and categorised as either resulting in twisting by Snail 1, or not resulting in twisting by Snail 1. Data in Table 2.3(b) were compared to those in Table 2.2. χ^2 tests did not lead to rejection of the hypothesis that the frequency of twisting by Snail 1 following contact was independent of whether observations were made in the laboratory or

Days without food following collection	Contacts resulting in	
	Twisting by Snail 1 (TRBS1 + TRBB)	No Twisting by Snail 1 (TRBS2 + NTR)
Port Gawler		
2	23	12
6	52	27
8	43	30
Total:	118	69
Port Clinton		
2	4	33
6	8	59
8	9	55
Total:	21	147

3 x 2 contingency table for independence, Port Gawler, $\chi^2_2 = 0.91$, $p > 0.05$

3 x 2 contingency table for independence, Port Clinton, $\chi^2_2 = 0.26$, $p > 0.05$

2 x 2 contingency table for independence between totals for Port Clinton and Port Gawler $\chi^2_1 = 95.11$, $p < 0.001$.

Table 2.2. Results of contacts observed between 80 adult *Nassarius pauperatus* from Port Gawler and Port Clinton in the laboratory. Data from Table 2.1.

(a)	Number of contacts resulting in				
	NTR	TRBS1	TRBS2	TRBB	Total
Port Gawler	12	32	11	14	69
Port Clinton	29	5	19	1	54

(b)	Number of contacts resulting in		
	Twisting by Snail 1	No Twisting by Snail 1	Total
Port Gawler	46	23	69
Port Clinton	6	48	54

2 x 2 contingency table comparison between contacts resulting in twisting by Snail 1, by snails from Port Gawler in the laboratory (from Table 2.2) and in the field (from Table 2.3(b)), $\chi^2_1 = 0.28$, $p > 0.05$.

2 x 2 contingency table comparison between contacts resulting in twisting by Snail 1, by snails from Port Clinton in the laboratory (from Table 2.2) and in the field (from Table 2.3(b)), $\chi^2_1 = 0.07$, $p > 0.05$.

2 x 2 contingency table comparison between contacts resulting in twisting by Snail 1 in the field between Port Gawler and Port Clinton (from Table 2.3(b)), $\chi^2_1 = 38.31$, $p < .001$.

Table 2.3(a) Results of unstaged contacts observed between *Nassarius pauperatus* in the field at Port Gawler and Port Clinton, and 2.3(b) Results from Table 2.3(a) classified as resulting in twisting by Snail 1 or not resulting in twisting by Snail 1.

the field (Table 2.3(b)), for snails from either Port Gawler or Port Clinton. However, the frequency of twisting responses by Snail 1 differed significantly between Port Gawler and Port Clinton (Table 2.3(b)).

2.3.4 Specificity of twisting by Snail 1.

Whilst observing contacts between Nassarius pauperatus in the laboratory it was noticed that specific individuals, recognisable by shell pattern and size, could be relied upon either always to twist, or never to twist, after they had contacted another N. pauperatus.

To test the hypothesis that individual N. pauperatus always twisted or never twisted after they had contacted another snail, further samples of 70 adult plus juvenile N. pauperatus were collected from both Port Gawler and Port Clinton on 4/VI/76 and 5/VI/76. These were kept in the laboratory in separate polystyrene trays as before. All snails which twisted after they had contacted another, (that is, all Snail 1's involved in contacts resulting in TRBS1 or TRBB) were marked on the visible part of their shell with red fingernail polish. Likewise, individuals which did not twist after having contacted another (that is, all Snail 1's which were involved in contacts resulting in either TRBS2 or NTR) were marked with blue fingernail polish. Once all the snails from each location had been marked, each was observed contacting another snail on a minimum of two more occasions, to see if it displayed different behaviour to that initially observed.

From these observations it was apparent that the behaviour of either twisting or not twisting after contacting another snail was very constant in individuals. In both samples, all snails which had twisted initially after having contacted another snail also did so on both of the later occasions. Two of the snails which had initially not exhibited a twisting response after they had appeared to contact another snail did twist when later observed. This may have been due to their behaviour having changed, or because they did not actually contact the other snail when initially observed. All snails were kept in the laboratory for the following six months, and occasionally fed Katelysia scalarina. No change in the twisting behaviour of the snails was observed during this period. Furthermore, later in this study other samples of N. pauperatus were collected, observed and scored for whether they twisted after contacting another, and I soon became skilled at observing whether contact between snails had occurred or not. Under these circumstances, all snails in a sample were almost invariably classified correctly, suggesting that the behaviour of twisting after contacting another snail was not labile, and that snails incorrectly classified were a result of faulty observations.

2.3.5 Definition of a "Twister" and a "Non Twister".

From the observations above, the following types of Nassarius pauperatus were defined. Snails which always twisted after they had contacted another were named "Twisters" and those which did not were named "Non Twisters". Twisters always twisted after contacting another N. pauperatus and Non Twisters did not, although both Twisters and Non Twisters often twisted if they were mechanically damaged or contacted on the rear of their foot by another N. pauperatus, but the latter twisting response more resembled a classic "avoidance response". Therefore, in a mixed sample of Twisters and Non Twisters the four possible outcomes of Snail 1 contacting Snail 2 will result from the following combinations of Twisters and Non Twisters:

(1)	NTR	No twisting response by either snail. (Snail 1 must be a Non Twister, Snail 2 could be either a Twister or a Non Twister).
(2)	TRBS1	Twisting response by Snail 1. (Snail 1 must be a Twister, Snail 2 could be either a Twister or a Non Twister).
(3)	TRBS2	Twisting response by Snail 2. (Snail 1 must be a Non Twister, Snail 2 could be either a Twister or a Non Twister).
(4)	TRBB	Twisting response by both snails. (Snail 1 must be a Twister, Snail 2 could be either a Twister or a Non Twister).

2.3.6 Differences in the frequency of Twisters between populations.

The final numbers of Twisters and Non Twisters (following the reclassification of two Non Twisters as Twisters) in the sample collected during June, 1976 (Section 2.3.4) were: Port Gawler, 54 Twisters, 16 Non Twisters; Port Clinton, 12 Twisters, 58 Non Twisters. A (somewhat unnecessary) χ^2 test led me to reject the hypothesis that the frequency of Twisters was independent of location ($\chi^2_1 = 50.57$ $p < 0.001$).

2.3.7 Association of being a Twister with sex or maturity.

Following the observation that the behaviour of Twisters and Non Twisters remained constant, snails were examined to see if being a Twister was associated with sex or with being an adult or a juvenile.

Each adult snail was sexed by observing the underside of its foot as it moved up the side of a glass beaker of seawater. Adult male N. pauperatus have a penis and adult females a pedal gland opening (Smith 1975), the latter of which could thus be observed. Snails lacking a pedal gland opening were assumed to be males and later dissection of individuals with and without pedal gland openings confirmed the reliability of this method of sexing. Juvenile snails could not be sexed since they did not possess either a pedal gland opening or a penis.

The numbers of adult and juvenile Twisters and Non Twisters from Port Gawler and Port Clinton are in Table 2.4. The proportion of Twisters did not differ significantly between sexes, nor between adult or juvenile snails from either Port Gawler or Port Clinton (Table 2.4).

2.4 Discussion.

These observations have shown that significantly fewer Nassarius pauperatus from Port Clinton compared to Port Gawler display a behaviour defined as being a "Twister". Being a Twister remained remarkably constant within individuals, with only two changes, from Non Twister to Twister occurring out of 140 initial classifications. These may have been either real changes in behaviour, or cases in which the initial encounter did not actually involve contact. Following more experience of observing cases of contact it was common to initially be able to classify all snails correctly. Furthermore, the few cases of misclassification were of snails classified as Non Twisters later becoming Twisters. A change in behaviour from Twister to Non Twister would be a far more powerful test of the above hypothesis, but was not observed.

Port Gawler

	Twisters	Non Twisters
Adults:		
Males	12	4
Females	30	9
Juveniles:	12	3

Port Clinton

	Twisters	Non Twisters
Adults:		
Males	3	16
Females	4	23
Juveniles:	5	19

Port Gawler:

2 x 2 contingency table for independence between Twisters and Non Twisters and sex, $\chi_1^2 = 0.02$, $p > 0.05$.

2 x 2 contingency table for independence between being adults or juveniles and Twisters or Non Twisters, $\chi_1^2 = 0.09$, $p > 0.05$.

Port Clinton:

2 x 2 contingency table for independence between Twisters or Non Twisters and sex, $\chi_1^2 = 0.01$, $p > 0.05$.

2 x 2 contingency table for independence between being adults or juveniles and Twisters or Non Twisters, $\chi_1^2 = 0.35$, $p > 0.05$.

Table 2.4. Numbers of adult and juvenile Twisters and Non Twisters from Port Gawler and Port Clinton.

Results of encounters between snails in trays in the laboratory are also consistent with the hypothesis that the behaviour of Twisters and Non Twisters did not change, in that the proportion of contacts resulting in twisting by Snail 1 did not vary significantly over time within samples from both Port Gawler or Port Clinton (see Tables 2.1 and 2.2), suggesting that there is a fixed proportion of snails that will twist after they have contacted another.

Being a Twister did not appear to be related to the sex of adult *N. pauperatus*, nor to whether snails were adult or juvenile. During this study it was also observed that Twisters did not twist after having contacted other species common on the sandflat (*Katelysia scalarina*, *Nassarius burchardi*, the crab *Philyra laevis* (Bell), or the gastropod *Bedeva hanleyi* Angas).

To be able to establish whether or not a behaviour has selective value and how this operates, it is necessary to postulate various hypotheses and test these. Considering that the frequency of Twisters varied between Port Gawler and Port Clinton, sampling of *N. pauperatus* from other locations may provide clues to the factors responsible for this, and this survey is described in the next chapter.

3. Survey of populations of Nassarius pauperatus in South Australia.

3.1 Introduction.

Populations of Nassarius pauperatus at Port Gawler and Port Clinton did not only contain different proportions of Twisters. It was also observed that firstly, the population density of N. pauperatus, especially with respect to juvenile snails, appeared to be much higher at Port Gawler than at Port Clinton and, secondly, that adults seemed larger at Port Clinton than at Port Gawler.

When N. pauperatus become sexually mature they develop a lip on their shell and cease growing, then being classified as adults (Smith, 1975). The shell lengths of both adult males and females appeared to be normally distributed in samples from Port Gawler and Port Clinton, with the sex ratio exceeding 2:1 in favour of females at both locations. Therefore a two way analysis of variance, with unequal but proportional subclass sizes (Sokal and Rohlf, 1969), was performed upon the shell lengths of 40 adult females and 20 adult males from each location, which were randomly selected from samples used in the previous chapter. (The variances of these samples were not significantly heterogeneous: $F_{max\ (4,19)} = 1.97$, $p > 0.05$). Results of the ANOVA (see Table 3.1) confirmed that there were significant differences in size between location and sex, but no significant interaction.

By inspection (Table 3.1) the mean length of male and female snails is greater at Port Clinton than at Port Gawler. This significant difference in size may be due to a variety of factors, one of which is the availability of food.

Considering these observations, it was decided to sample other populations of N. pauperatus for the density of adult and juvenile snails, the shell lengths of adults and to estimate food availability,

	Population			
	Port Gawler		Port Clinton	
	Males	Females	Males	Females
$\bar{x}$	1.47	1.42	1.56	1.48
s_x	0.116	0.111	0.091	0.128
n	20	40	20	40

Source of variation	df	SS	MS	$F_{(1,116)}$
Subgroups	3	0.2811	0.0937	
Sex	1	0.1281	0.1281	9.7045***
Location	1	0.1466	0.1466	11.1061***
Interaction	1	0.0064	0.0064	0.4848 N.S.
Error	116	1.5349	0.0132	
Total	119			

$\bar{x}$: mean, s_x : standard deviation, n : sample size,

N.S. : $p > 0.05$, *** : $p < .001$

Table 3.1 Mean shell length of 20 male and 40 female *Nassarius pauperatus* from both Port Clinton and Port Gawler, and results of two-way ANOVA, with unequal but proportional subclass sizes.

in addition to the percentage of Twisters.

3.2 Methods.

3.2.1 Locations at which *Nassarius pauperatus* were sampled.

The South Australian coastline from Coffin Bay to Robe, including that of Kangaroo Island (see Figure 3.1), was surveyed for *Nassarius pauperatus* during 1976-1978, and snails from two populations west of Coffin Bay were collected by others.

N. pauperatus occur continuously for several kilometres on each side of the Gulf St Vincent (Figure 3.1) and snails were sampled from several locations within these populations. Altogether, 20 locations were sampled for the size and sex of adults and the percentage of Twisters. Food availability was indirectly estimated (see Section 3.3.1) at 17 locations, and the population densities of juvenile and adult snails estimated at 10 locations. Only one known population of *N. pauperatus* (Ceduna) in South Australia was not sampled.

3.2.2 Estimation of population density.

It was soon noticed that the population density of adult and juvenile *Nassarius pauperatus*, as well as the proportion of juveniles, varied greatly between locations sampled at the same time of year. *N. pauperatus* lay eggs in the field during late October and November. Juveniles from these have reached approximately 0.5 cm in length by March of the following year and continue growing until they reach sexual maturity between May and September of the year after that (Smith, 1975, also confirmed by my collections). Collection in the field of juveniles of shell length less than 0.5 cm was not representative of the total numbers present, since snails of such small size were easily overlooked, especially when buried in the substratum. However, by November of each year, juvenile *N. pauperatus*

Figure 3.1 *Nassarius pauperatus* populations studied in South Australia, and locations sampled.

Gulf Saint Vincent

1. Mouth of the Onkaparinga River.
2. Mouth of Swan Alley Creek, Port Adelaide.*+
3. Port Gawler.
4. Middle Beach.
5. Port Prime.
6. Parham.
7. Port Wakefield.*
8. Port Clinton.*
9. Rogues Point.*
10. Stansbury.
11. Coobowie: "Bag Wash" on Salt Creek Bay.
12. Sultana Point: 1.0 km south of Edithburgh on the Sultana Point Road.

Spencer Gulf

13. Moonta Bay: north side of jetty.
14. Yatala Harbour.
15. Lucky Bay: 0.5 km north of holiday shacks.
16. Port Lincoln I: Beach by Flinders Cairn.
17. Port Lincoln II: Entrance to Sleaford Mere.

West Coast

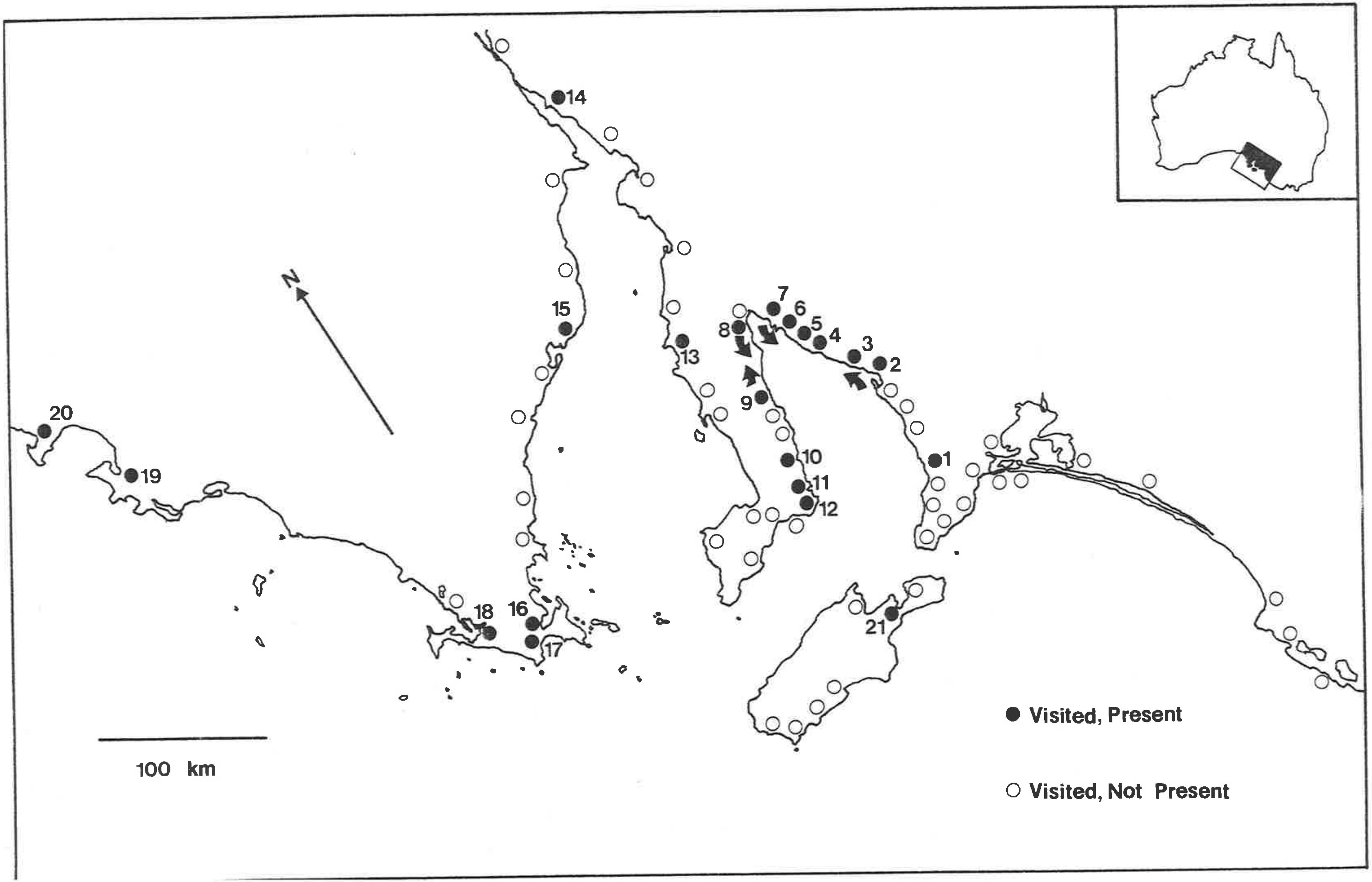
18. Coffin Bay: 100 metres north of boat ramp.
19. Streaky Bay.
20. Smoky Bay.

Kangaroo Island

21. American River.

* Distribution continuous between locations 2 to 7 and 8-9, indicated by arrows on Figure 3.1.

\+ Only two snails found at this location.


14
15
13
7
6
5
4
3
2
8
9
1
10
11
12
20
19
18
16
17
21
N
100 km
● Visited, Present
○ Visited, Not Present

resulting from breeding during the previous year were easily noticed, being larger than 0.7 cm in all populations but lacking lips on their shells. The proportion of juveniles in November of each year was taken as representing the yearly recruitment of juveniles, and this was sampled for more than one year at several locations.

Population densities of adult and juvenile *N. pauperatus* were estimated at ten locations (numbers 1,3,6,8,9,10,11,12,13 and 15 in Figure 3.1). At each, a minimum of 10 random 60 x 60 cm quadrat samples were taken from the zone in which *N. pauperatus* occurred. Samples were randomised by tossing the quadrat between 2 and 3 metres forward every 10 paces (approximately 8 metres) with my eyes shut. The first sample was taken in the approximate centre of the zone in which *N. pauperatus* occurred and successive samples taken by walking ten paces away from the same side of the quadrat every time. Since the quadrat spun when thrown, the positions of all samples following the first were randomised. At locations where the width of the zone containing *N. pauperatus* was narrow (less than 10 metres) the quadrat was tossed forward,with my eyes shut,approximately parallel to the shore thus ensuring that it fell within the zone but still randomly so.

The quadrat was constructed of galvanised iron sheet and had a 5 cm lip which was pushed 3.5 cm down into the substratum as soon as the quadrat was reached after being thrown. This prevented any snails from entering or leaving the sample area whilst it was being searched and was necessary since snails were frequently aroused, either by the quadrat striking the substratum or the subsequent disturbance during collection. *N. pauperatus* were never found any deeper than 3.0 cm in the substratum, so this was searched to the depth of the quadrat.

3.2.3 Size and sex of adults and the percentage of Twisters.

Considering that in some populations the proportion of juvenile snails was very small, and that the proportion of Twisters was independent of whether snails were juvenile or adults (Section 2.3.7), samples of adult snails were used for all estimates of the percentage of Twisters.

A minimum sample of 80 adults was collected at random from each location, except in two instances where population densities were so low that this number could not be collected within one hour, and also in the two cases when snails were collected by others. Snails were packed in polyurethane foam containers and transported to the laboratory within 24 hours, where they were placed in polystyrene trays (32 x 54 x 12 cm deep) containing 4-6 cm depth of aerated seawater, with a maximum density of 50 snails per tray. Pairs of snails were observed contacting each other and Snail 1's classified and marked as either Twisters or Non Twisters, as described and defined in Section 2.3.5. The behaviour of all snails was subsequently checked twice. Snails were then sexed by either examination or dissection (as in Section 2.3.7) and their axial shell length measured to the nearest 0.005 cm. The proportion of juvenile snails, the shell length of adults and the percentage of Twisters were measured more than once for some locations during three years.

3.3 Methods: Food availability.

3.3.1 Introduction.

It was earlier concluded that Nassarius pauperatus was an omnivore, feeding upon both plant material and animal carrion (Section 2.2.4). However, sampling the sandflat at low tide for the density of carrion and various types of algae was not considered to be a valid, nor reliable, estimate of the amount of food available to *N. pauperatus*. Firstly, carrion is rapidly scavenged by other species as well as *N. pauperatus*, secondly, the sandflat could only be sampled during the day at low tide and thirdly, it was not known which species of algae were eaten, nor their food value.

Instead it was attempted to estimate relative food availability by measuring how hungry samples of *N. pauperatus* were from different locations by observing the behaviour of snails when offered food. Although this is an indirect measure of food availability, it was considered to be more reliable than any sampling of food for the above reasons, and may also provide information about the behaviour of *N. pauperatus* in relation to the availability of food, which may help to explain any association observed between the percentage of Twisters and hunger.

3.3.2 Estimation of hunger - Method 1.

3.3.2.1 Procedure.

Two methods were designed to measure hunger. In Method 1, one hundred *Nassarius pauperatus* were collected at random at each location by searching the substratum to a depth of 3.0 cm (as described in Section 2.3.2). Snails were arranged in a ring of 14 cm diameter upon a 60 x 60 cm sheet of fibreglass mesh (hole size 2 mm x 2 mm) which was laid on top of the sandflat at each location. A living bivalve (*Katelysia scalarina*) was forced open and placed in the centre of the ring of snails. The behaviour of each snail during the next 15 minutes was recorded in one of the following three categories:

"Feeding" - snail fed upon *K. scalarina*

"Active" - snail moved about but did not feed

"Sedentary" - snail did not move at all.

Any snails which commenced feeding during this time were immediately removed from the bivalve, to ensure that there was space available for others to feed, and placed in a separate container. At the end of 15 minutes, all the sedentary snails were counted and the number of active snails obtained by subtraction.

Trials at Port Gawler and Port Clinton showed that all *N. pauperatus* that were going to feed during the next hour usually commenced during the first 10 minutes, and that those classified as being active during the next hour usually became so before 15 minutes had elapsed.

3.3.2.2 Differences between *Nassarius pauperatus* collected from upon and within the substratum.

Nassarius pauperatus were found both upon the surface of, and buried within, the substratum (Section 2.3.2). Adult *N. pauperatus* were collected at the same time from the same area of substratum at Port Clinton and separate samples from the surface and from within the substratum were offered *Katelysia scalarina* (as described in the previous Section) and the proportion of snails feeding were compared.

3.3.2.3 Differences between adult and juvenile *Nassarius pauperatus.*

One hundred juvenile and 100 adult *Nassarius pauperatus* were collected at each of Port Gawler and Port Clinton on adjacent days. Both adult and juvenile snails were separately offered *Katelysia scalarina* and the proportions of snails that fed were compared within locations. (It was found that the proportion of snails feeding did not differ significantly between adults and juveniles at either location (see Results, Section 3.4.2.3). However, the proportion of snails feeding did differ significantly between samples of adults collected from upon and within the substratum (see Section 3.4.2.2). Considering that smaller juveniles buried within the substratum were likely to be overlooked, and that the proportion of juveniles varied between locations, only adult snails, collected from both the surface and from within the substratum (see Section 2.3.2) were used for subsequent estimates of hunger .

3.3.2.4 Differences between samples offered plant and animal material.

A difference in the proportion of Nassarius pauperatus feeding upon Katelysia scalarina between locations may be due to a difference in the response of snails to carrion rather than a difference in hunger. Therefore, 200 adult snails were collected at each of Port Clinton and Port Gawler on adjacent days. . One hundred snails from each location were offered K. scalarina and the other 100 a piece of cotton wool, soaked with a pure culture of an alga that N. pauperatus was known to feed upon (see Appendices 1 and 2), using the procedure described in Section 3.3.2.1, and the proportions of snails feeding were compared.

3.3.2.5 The effects of food availability upon the feeding and growth of Nassarius pauperatus.

To examine the effects of different levels of food availability upon the feeding behaviour and growth of Nassarius pauperatus, 50 juveniles were chosen from a larger sample collected from Port Gawler. These juveniles formed 25 matched pairs, matched for being Twisters or Non Twisters and also shell length to within 0.015 cm (and in most cases to within 0.005 cm). The 50 snails were assigned to two groups of 25, with one member of each matched pair in each group.

It was not possible to match snails for sex since sexual differences were not apparent until after individuals had matured. Matched pairs were numbered from 1 to 25 with a "Ball Pentel Extra Fine R 56" pen on their shells and the number was then covered with clear fingernail polish to protect it. One group was then designated "high food" and the other "low food" and these were marked distinctively with spots of differently coloured fingernail polish on their shells.

Both groups were initially kept together in the same tray of aerated seawater at 20°C with a "long day" photoperiod and otherwise as described in Section 2.3.2, until some snails showed signs of becoming mature, whereupon groups were transferred to separate trays, for reasons described in Section 4.2.

The high food group was offered *ad lib Katelysia scalarina* every day and the low food group was offered *ad lib K. scalarina* every 14 days. Different frequencies of such feeding "pulses" have been shown to simulate different levels of food availability to other gastropods (e.g. see Calow, 1973; Moriarty, 1978).

Snails were normally inactive unless food was added to their tray. Before being offered food, snails were arranged in a ring of 14 cm diameter in the middle of their tray and several frozen *K. scalarina*, (to ensure that all snails had space to feed) were placed in the centre of the ring. The behaviour of each snail in each group when offered food was classified as either feeding, active or sedentary (as in Section 3.3.2.1) and was noted daily for two periods; one of 53 days duration while all snails were juvenile and one of 48 days duration when all snails had become adult.

Interruptions to the daily feeding of the high food group outside these periods, whilst I was away on fieldwork provided an opportunity to examine the effects of various periods of starvation upon the proportion of snails feeding.

3.3.3 Estimation of hunger - Method 2.

3.3.3.1 Procedure.

The second method for attempting to measure how hungry Nassarius pauperatus were, consisted of collecting a random sample of a minimum of 80 snails from each location. These were transported to the laboratory as quickly as possible, in polyurethane foam containers, to minimise temperature changes during travel. Snails were placed in polystyrene trays of aerated seawater at a constant temperature of 17°C and current day length, at a maximum density of 50 per tray. Each day following collection all snails were offered Katelysia scalarina in their trays (as previously described for the high and low food group) and those snails that commenced feeding during the next 15 minutes were removed and counted. The number of snails feeding each day thus provided an estimate of the mean time taken for all snails in a sample to commence feeding. An initial trial showed that snails fed significantly sooner at Port Gawler than Port Clinton (see Results, Section 3.4.3.1). This may have been due to snails actually being hungrier at Port Gawler, to a difference in preference for K. scalarina between locations, to having been transported for different distances and times (see Figure 3.1) or other unknown factors differing between locations, so attempts were made to differentiate between such factors and differences in hunger.

3.3.3.2 Differences between samples offered plant and animal material.

Differences in response to plant and animal food were examined by offering two separate samples of 100 adult snails from each of Port Gawler and Port Clinton either Katelysia scalarina or the alga offered in Section 3.3.2.4, (using the procedure described above in

Section 3.3.3.1), and differences in the mean days to feed compared between foods, within locations.

3.3.3.3 Starvation, satiation and re-feeding.

If the difference in mean days to feed between snails from Port Gawler and Port Clinton was due to a difference in hunger, then snails from different locations having experienced the same levels of food availability should not differ in the time taken to feed. One hundred adult *Nassarius pauperatus* were collected on adjacent days from each of Port Gawler and Port Clinton and offered *Katelysia scalarina* daily in the laboratory, as described previously. As soon as any snail in either group attempted to feed, it was removed to another tray without food. All snails in both samples had attempted to feed by 16 days after collection.

Both samples were then starved for a further 14 days (thus, all snails in both samples had effectively been starved for 30 days) and then satiated on day 30 with *ad lib K. scalarina*, after which they were offered *K. scalarina* every following day and the snails first feeding upon each day were removed and counted.

3.3.3.4 The effects of differences in travelling time.

To examine the effects of being transported upon the time taken to first feed, 200 adult snails were collected on adjacent days from each of Port Clinton and Port Gawler. 100 snails from each location were driven directly to the laboratory and transferred to trays of aerated seawater at 17°C, and the other 100 from each location were driven about for an additional hour. Temperatures in the polyurethane foam containers used to transport snails were kept as close as possible to those of the seawater that snails were collected from by the addition of plastic bags containing ice, for various lengths of time. These snails were then transferred to separate laboratory trays and the average time elapsing before they fed upon K. scalarina compared to that of snails driven directly to the laboratory.

3.3.4 Comparison of hunger between other locations.

Method 1 was chosen to estimate hunger, following the finding that Method 2 was affected by the duration of time snails had spent in transit between the field and the laboratory (see Results, Section 3.4.3.3). Hunger was estimated at Port Gawler and Port Clinton on adjacent days every month for 13 months. During this time most of the other locations were also sampled and hunger estimated. The estimates of hunger at Port Gawler and Port Clinton varied between months so the values obtained for other locations were expressed in relation to the value for Port Gawler that they were sampled closest to, in time. If a location was sampled outside the 13 month period, a sample was also taken from Port Gawler for comparison. In no case was any location sampled more than 12 days apart from its reference sample at Port Gawler, and this difference was usually less than six days (see Table 3.22).

3.4 Results.

3.4.1 Population densities and the percentage of Twisters.

Population densities of adult and juvenile Nassarius pauperatus and the percentage of Twisters from 10 locations appear in Table 3.2. Whilst collecting snails and estimating population densities it was observed that the distribution of N. pauperatus appeared to be clumped at some locations, namely Port Gawler, Moonta Bay and Lucky Bay (which is not surprising, considering the distribution of carrion and intertidal pools on the sandflat), and the distribution

Sample size (quadrats)	Date	Location	Adults plus juveniles		Adults		Juveniles		Percent Twisters
			$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x	
10	IV/77	Onkaparinga	9.40	2.02	1.20	2.00	8.20	1.60	86.25
23	XI/76	Port Gawler	8.96	4.51	1.57	1.53	7.39	3.80	77.14
10	"	Parham	8.20	3.20	1.70	2.76	6.60	1.01	83.75
20	"	Port Clinton	1.05	1.00	0.85	0.88	0.30	0.73	17.50
10	"	Rogues Pt.	1.70	1.00	1.70	1.00	None found		15.79
25	"	Stansbury	0.80	0.87	0.80	0.87	None found		16.25
25	"	Coobowie	8.60	3.33	3.80	2.06	4.80	2.71	16.32
25	"	Sultana Pt.	1.20	1.12	1.20	1.12	None found		7.50
10	"	Lucky Bay	11.80	6.59	10.70	6.19	1.10	1.13	11.25
10	II/79	Moonta Bay	6.30	2.67	3.30	1.66	3.00	1.43	62.00

Table 3.2 Densities of adult and juvenile *Nassarius pauperatus* and the percentage of Twisters at each location. $\bar{x}$: mean, s_x : standard deviation.

of snails per 3600 cm^2 was highly skewed to the right at these locations. Therefore the non parametric Spearman rank correlation coefficient (Siegel, 1956) was used to compare population densities and the percentages of Twisters for the eight locations sampled during November 1976. Only the population density of juvenile N. pauperatus was significantly correlated with the percentage of Twisters (Juveniles, $r_s = 0.74$, $0.01<p<0.05$; Adults, $r_s = 0.08$, $p>0.05$; Adults plus juveniles, $r_s = 0.10$, $p >0.05$).

Some locations were sampled several times during this study for the proportion of adult and juvenile snails, the mean size of adults and the percentage of Twisters. Considering the life history pattern of this species, with juvenile snails recruited into the adult population during May to September each year, data for adult shell length are only presented for periods after successive recruitments had occurred. Descriptive statistics for the shell length of male and female N. pauperatus, the proportion of juvenile snails, the sex ratio of adult snails and the percentage of Twisters appear in Tables 3.3 to 3.7. From these data it can be seen that the variables differ between locations, but that values remained remarkably similar within the locations sampled more than once during 1976-1978.

3.4.2 Estimation of hunger - Method 1.

3.4.2.1 Behaviour of Nassarius pauperatus before and after feeding.

Observations of snails in the high food group in the laboratory showed that generally after a period of feeding, individual snails were then sedentary for one or more days, followed by a period of 1-2 days activity, after which they fed again. However, these events did not always occur in this sequence, with snails being sometimes active after feeding and then sedentary before feeding

Location	Males	Females
Onkaparinga	29	51
Port Gawler	43	157
Middle Beach	23	57
Port Prime	20	60
Parham	23	57
Port Wakefield	16	64
Port Clinton	57	123
Rogues Point	9	29
Stansbury	32	68
Coobowie	24	86
Sultana Point	25	78
Moonta Bay	11	89
Yatala Harbour	3	18
Lucky Bay	27	53
Port Lincoln I	14	66
Port Lincoln II	19	61
Coffin Bay	21	59
Streaky Bay	20	54
Smoky Bay	9	35
American River	14	66

$\chi^2_{19} = 37.26,\ 0.005 < p < 0.01$

Table 3.3 Numbers of male and female Nassarius pauperatus in samples from 20 locations in South Australia. The proportions of each sex are significantly different between locations.

Location	Shell length					
	Males			Females		
	$\overline{x}$	s_x	n	$\overline{x}$	s_x	n
Onkaparinga	1.49	0.137	29	1.40	0.197	51
Port Gawler	1.44	0.112	43	1.38	0.124	157
Middle Beach	1.48	0.134	23	1.38	0.124	57
Port Prime	1.48	0.126	20	1.40	0.119	60
Parham	1.46	0.070	23	1.31	0.195	57
Port Wakefield	1.50	0.125	16	1.39	0.184	64
Port Clinton	1.51	0.111	57	1.47	0.144	123
Rogues Point	1.61	0.104	9	1.47	0.135	29
Stansbury	1.62	0.092	32	1.55	0.127	68
Coobowie	1.42	0.091	24	1.28	0.153	86
Sultana Point	1.55	0.098	25	1.48	0.109	78
Moonta Bay	1.51	0.094	11	1.36	0.115	89
Yatala Harbour	1.40	0.121	3	1.36	0.102	18
Lucky Bay	1.73	0.217	27	1.62	0.257	53
Port Lincoln I	1.65	0.134	14	1.59	0.142	66
Port Lincoln II	1.43	0.108	19	1.34	0.099	61
Coffin Bay	1.66	0.094	21	1.53	0.095	59
Streaky Bay	1.70	0.100	20	1.57	0.127	54
Smoky Bay	1.78	0.095	9	1.62	0.103	35
American River	1.46	0.109	14	1.37	0.133	66

$\overline{x}$: mean, s_x : standard deviation, n : sample size

Table 3.4 Shell lengths of male and female adult *Nassarius pauperatus* from 20 locations in South Australia.

Location and dates sampled	Adult shell length					
	Males			Females		
	$\bar{x}$	s_x	n	$\bar{x}$	s_x	n
Port Gawler						
June 1976	1.47	0.117	20	1.42	0.111	40
November 1976	1.44	0.112	43	1.38	0.124	57
November 1977	1.46	0.120	50	1.40	0.139	150
November 1978	1.49	0.114	30	1.41	0.116	100
Port Clinton						
June 1976	1.56	0.091	20	1.48	0.128	40
November 1976	1.51	0.111	57	1.47	0.144	123
November 1977	1.57	0.117	57	1.45	0.132	154
November 1978	1.53	0.099	30	1.49	0.137	109
Stansbury						
November 1976	1.62	0.092	32	1.55	0.127	68
October 1977	1.63	0.104	25	1.55	0.133	70
November 1978	1.66	0.079	20	1.56	0.120	60
Coobowie						
November 1976	1.42	0.091	24	1.28	0.153	86
October 1977	1.39	0.103	35	1.30	0.126	90
November 1978	1.40	0.085	30	1.33	0.141	85
Sultana Point						
November 1976	1.55	0.098	25	1.48	0.109	78
October 1977	1.53	0.100	36	1.45	0.095	114
November 1978	1.58	0.120	29	1.44	0.101	73

$\bar{x}$: mean, s_x : standard deviation, n : sample size

Table 3.5 Shell lengths of male and female, adult Nassarius pauperatus, sampled from five locations during 1976-1978, following successive recruitment of adults.

Location	Date(s) sampled	Proportion of juveniles
Port Gawler	XI/76	0.82
	XI/77	0.75
	XI/78	0.72
Middle Beach	XI/76	0.41
Port Prime	XI/76	0.42
Parham	XI/76	0.35
Port Wakefield	XI/76	0.68
Port Clinton	XI/76	0.26
	XI/77	0.15
	XI/78	0.23
Rogues Point	XI/76	0.09
Stansbury	XI/76	0.10
	XI/78	0.05
Coobowie	XI/76	0.49
	XI/78	0.52
Sultana Point	XI/76	0.06
	XI/78	0.07
Lucky Bay	XI/76	0.11
Port Lincoln I	XI/77	0.08
Port Lincoln II	XI/77	0.39
Coffin Bay	XI/77	0.09

Table 3.6 Proportion of juveniles in samples of *Nassarius pauperatus* from various locations during November of each year.

Locations and dates sampled	Proportion of Twisters (adults)
Port Gawler	
VI/76	0.77
XI/76	0.77
III/77	0.83
VI/77	0.76
VIII/77	0.84
XI/77	0.79
II/78	0.85
Port Clinton	
VI/76	0.14
XI/76	0.17
XI/77	0.22
XI/78	0.12
Stansbury	
XI/76	0.16
V/77	0.19
X/77	0.16
XI/77	0.17
XI/78	0.13
Coobowie	
XI/76	0.16
V/77	0.15
X/77	0.19
XI/77	0.13
XI/78	0.13
Sultana Point	
XI/76	0.08
V/77	0.09
X/77	0.07
XI/77	0.05
XI/78	0.11

Table 3.7 Proportion of Twisters in samples of adult *Nassarius pauperatus* at five locations sampled more than once during 1976-1978.

again. Accordingly snails were only classified as either "feeding" or "not feeding" when offered food, with snails formerly classed as "sedentary" being included in the latter category.

3.4.2.2 Differences in feeding between *Nassarius pauperatus* collected from upon and from within the substratum.

A significantly greater proportion of snails collected from the surface of the substratum fed in the field when offered *Katelysia scalarina* compared to snails collected from within the substratum (Table 3.8). This result is not surprising considering that snails active on the surface of the sandflat are probably those that are hungry and seeking food.

3.4.2.3 Differences in feeding between adult and juvenile *Nassarius pauperatus*.

Results of offering *Katelysia scalarina* to separate groups of 100 adult and 100 juvenile *Nassarius pauperatus* on adjacent days at Port Clinton and Port Gawler are in Table 3.9. There was no significant difference in the proportion of adult or juvenile snails feeding within either location, even though the proportions of total snails feeding differed significantly between locations (Table 3.9).

3.4.2.4 Proportion of *Nassarius pauperatus* feeding when offered plant and animal material.

There was no significant difference between the proportion of adult *Nassarius pauperatus* feeding when separate samples were offered either *Katelysia scalarina* or an alga at Port Clinton or Port Gawler, even though the proportions of snails feeding differed significantly between the above locations (Table 3.10).

	Snails collected from	
	Sandflat surface	Within substratum
Number feeding	26	6
Number not feeding	17	51

2 x 2 contingency table for independence: $\chi^2_1 = 28.09$, $p < .001$.

Table 3.8 Numbers of adult *Nassarius pauperatus* feeding or not feeding, in samples collected from the sandflat surface or from within the substratum, at Port Clinton.

	Population			
	Gawler		Clinton	
	Juveniles	Adults	Juveniles	Adults
Feeding	55	48	10	13
Not feeding	45	52	90	87

2 x 2 contingency tables for independence between:

(a) proportion of adults and juveniles feeding;

Port Gawler, $\chi^2_1 = 0.98$, $p > 0.05$

Port Clinton, $\chi^2_1 = 0.44$, $p > 0.05$

(b) proportion of total snails feeding at Port Gawler and Port Clinton, $\chi^2_1 = 74.15$, $p < .001$.

Table 3.9 Numbers of adult and juvenile *Nassarius pauperatus* feeding or not feeding when offered *Katelysia scalarina* at Port Gawler and Port Clinton.

3.4.2.5 Food availability and feeding behaviour in the laboratory.

Observations made in the laboratory (not surprisingly) suggested that the proportion of *Nassarius pauperatus* feeding was correlated with the length of time a sample of snails had been deprived of food. All snails in the low food group fed when offered food every fortnight. By design the data are extremely scanty for periods other than zero or 14 days spent without food. However, seven interruptions occurred in the usual daily feeding regime of the high food group whilst I was away on fieldwork. The number of snails feeding when offered *K. scalarina* following four of these occasions are in Table 3.11, and support the hypothesis that the proportion of a sample of snails feeding is related to the length of time they have been deprived of food. Table 3.12 shows the mean number of snails feeding per day in the high food group whilst all snails were juvenile and later when all snails were adult. No interruptions to feeding occurred during either of these periods. The total number of snails is less during the second period of observation, due to five snails having died whilst I was away on fieldwork between weeks 21 and 23. Data for all juvenile snails were included in the analysis, this observation period having been between weeks zero and eight. Because the total number of snails differed between the observation periods a comparison of the distributions of the percentage of snails feeding per day was made using a Kolmogorov-Smirnov two tailed, two sample test (Siegel, 1956) which showed that there was no significant difference between the distributions of the percentage of snails feeding whilst either juvenile or adult (D_{max} observed = 0.1679; $D_{0.05}$ = 0.2710), see Figure 3.2.

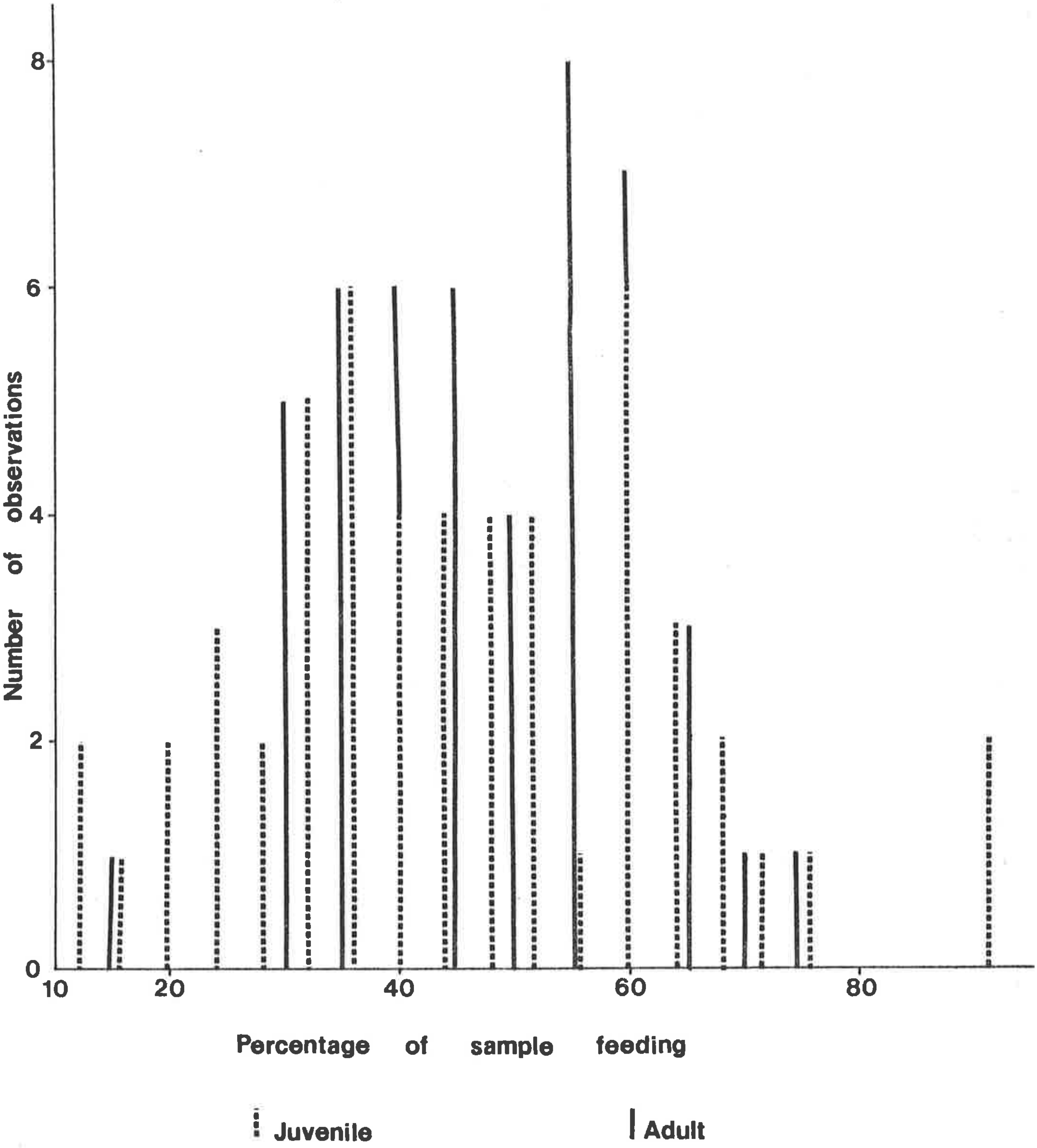


Figure 3.2 The percentage of <u>Nassarius</u> <u>pauperatus</u> feeding whilst juvenile and adult. The two distributions do not differ significantly (see text).

	Population			
	Port Gawler		Port Clinton	
	Alga	Cockle	Alga	Cockle
Feeding	32	25	21	16
Not feeding	68	75	79	84

2 x 2 contingency tables for independence between:

(a) whether feeding, versus food type;

Port Gawler, $\chi^2_1 = 1.20$, N.S.

Port Clinton, $\chi^2_1 = 0.83$, N.S.

(b) whether feeding versus location: $\chi^2_1 = 5.56$, *

N.S. : $p > 0.05$, * : $.01 < p < .05$

Table 3.10 Numbers of adult Nassarius pauperatus feeding or not when offered cockle (Katelysia scalarina) or alga, at Port Clinton and Port Gawler.

Days without food	Number of snails feeding
1	12
1	14
2	19
10	25

Table 3.11 Number of Nassarius pauperatus feeding out of 25 in the high food group, following interruptions to feeding.

Snails feeding	Juvenile	Adult
$\bar{x}$	11.30	9.52
s_x	4.59	2.55
n (observations)	53	48
n (snails)	25	20
range	3-23	3-15

$\bar{x}$: mean, s_x : standard deviation, n : sample size

Table 3.12 Mean, range and standard deviation of the number of *Nassarius pauperatus* feeding per day in the high food group from observations made whilst snails were both juvenile and adult. Note that the total number of snails has decreased in the adult group.

Data for the number of days spent feeding and not feeding for individuals in the high food group whilst juvenile and adult are in Tables 3.13 and 3.14. The total days spent feeding plus not feeding differ between individual snails, because data from the beginning and end of each observation period were excluded, since it was not known how long a snail had been either feeding or not before observations commenced or after they ceased.

All combinations of behavioural types and sexes are present in the high food group. Heterogeneity of the proportions of days spent feeding between snails of different sizes, ages, sexes or behaviour will mean that the technique of estimating hunger from the proportion of snails feeding at any location will be biased by the proportion of each sex, size, age or behaviour in each sample. Chi - squared tests of homogeneity showed that there were no significant differences in the proportions of days spent feeding between individuals whilst either adult or juvenile (see Tables 3.13 and 3.14). Therefore, the data were summed within each of the two observation periods and the proportion of days spent feeding or not compared between juveniles and adults. These did not differ significantly (2 x 2 contingency table, $\chi^2_1 = 2.32$, $p>0.05$).

Considering these results it appears that there are no significant differences in the proportion of time spent feeding between snails whether they are any combination of Twister, Non Twister, adult or juvenile, male or female. However, differences in the distribution of the days spent feeding per individual will also affect estimates of hunger made by sampling the proportion of snails feeding if some synchrony of feeding occurs. This is quite likely because the availability of carrion on the sandflat may vary over time.

Snail number	Sex	Behaviour	Shell length (cm)	Number of days	
				Feeding	Not feeding
1	NK	T	0.980	22	23
2	NK	T	1.125	25	27
3	F	T	1.200	21	28
4	NK	N	1.190	25	26
5	F	T	1.200	21	23
6	F	N	1.140	23	27
7	F	T	1.170	19	27
8	F	N	1.050	17	28
9	NK	N	1.295	15	28
10	F	T	1.225	19	29
11	M	N	1.265	21	22
12	F	T	1.100	27	22
13	NK	T	1.040	23	22
14	M	N	1.205	21	18
15	F	T	1.110	26	20
16	F	T	1.150	21	29
17	M	T	1.210	26	24
18	M	N	1.240	18	32
19	F	T	1.125	24	23
20	F	T	1.165	19	26
21	F	T	0.980	11	26
22	F	N	1.040	25	24
23	F	T	1.040	18	31
24	F	T	1.250	20	26
25	F	N	1.070	22	25

M : male, F : female, NK : sex not known since snail died before becoming adult, T : Twister, N : Non Twister

25 x 2 contingency table for homogeneity between snails with respect to proportion of days spent feeding or not, $\chi^2_{24} = 20.45$, $p > 0.05$.

Table 3.13 High food treatment, whilst snails juvenile. Snail number, behaviour, sex, shell length and days spent feeding and not feeding.

Snail number	Sex	Behaviour	Shell length (cm)	Number of days	
				Feeding	Not feeding
1	Died				
2	Died				
3	F	T	1.300	24	28
4	Died				
5	F	T	1.585	24	28
6	F	N	1.450	27	25
7	F	T	1.360	25	27
8	F	N	1.410	27	25
9	Died				
10	F	T	1.375	26	26
11	M	N	1.440	26	26
12	F	T	1.350	22	30
13	Died				
14	M	N	1.625	25	27
15	F	T	1.200	25	27
16	M	T	1.520	24	28
17	M	T	1.580	26	26
18	F	N	1.300	24	28
19	F	T	1.255	25	27
20	F	T	1.430	23	29
21	F	T	1.300	29	23
22	F	N	1.280	29	23
23	F	T	1.505	25	27
24	F	T	1.445	25	27
25	F	N	1.495	25	27

M : male, F : female, T : Twister, N : Non Twister.

20 x 2 contingency table for homogeneity between snails with respect to proportion of days spent feeding or not, $\chi^2_{19} = 4.48$, $p > .995$!

Table 3.14 High food treatment after snails became adult. Snail number, behaviour, sex, shell length and days spent feeding and not feeding.

The distributions of the length of time spent feeding for individual snails were not normal (see Figure 3.3) so were compared within groups of adult and juvenile snails using a Kruskal-Wallis one way analysis of variance (Siegel, 1956) and did not differ between individuals whilst juvenile or adult (Juveniles, $H_{24} = 31.41$, $p>0.05$; Adults, $H_{19} = 5.96$, $p>0.05$). Therefore the data for snails whilst juvenile and adult were combined and the two age groups compared using a Kolmogorov-Smirnov, two tailed, two sample test. There were no significant differences between the distributions of days spent feeding whilst snails were juvenile or adult ($D_{max} = 0.0480$, $D_{0.05} = 0.1084$).

Therefore these data suggest that whether a snail is any combination of a Twister or Non Twister with male or female whilst adult or juvenile, these differences themselves will not affect its pattern of feeding.

3.4.2.6 Food availability and growth of *Nassarius pauperatus* in the laboratory.

The axial shell lengths of snails in the high and low food treatments were measured weekly to the nearest 0.005 cm with vernier calipers, until the individual became sexually mature and ceased growing. The mean shell lengths of snails in both the high and low food treatments were plotted versus time (Figure 3.4). It is possible that snails that died during the course of the experiment had different rates of growth prior to dying, although I consider it unlikely that I would have recorded these, since all snails that died did so during a period of 14 days starvation between weeks 21 and 23. Nevertheless, data from the six snails that died have been excluded to eliminate the possibility that differences in the shape of the growth curves between the high and low food groups were an artefact

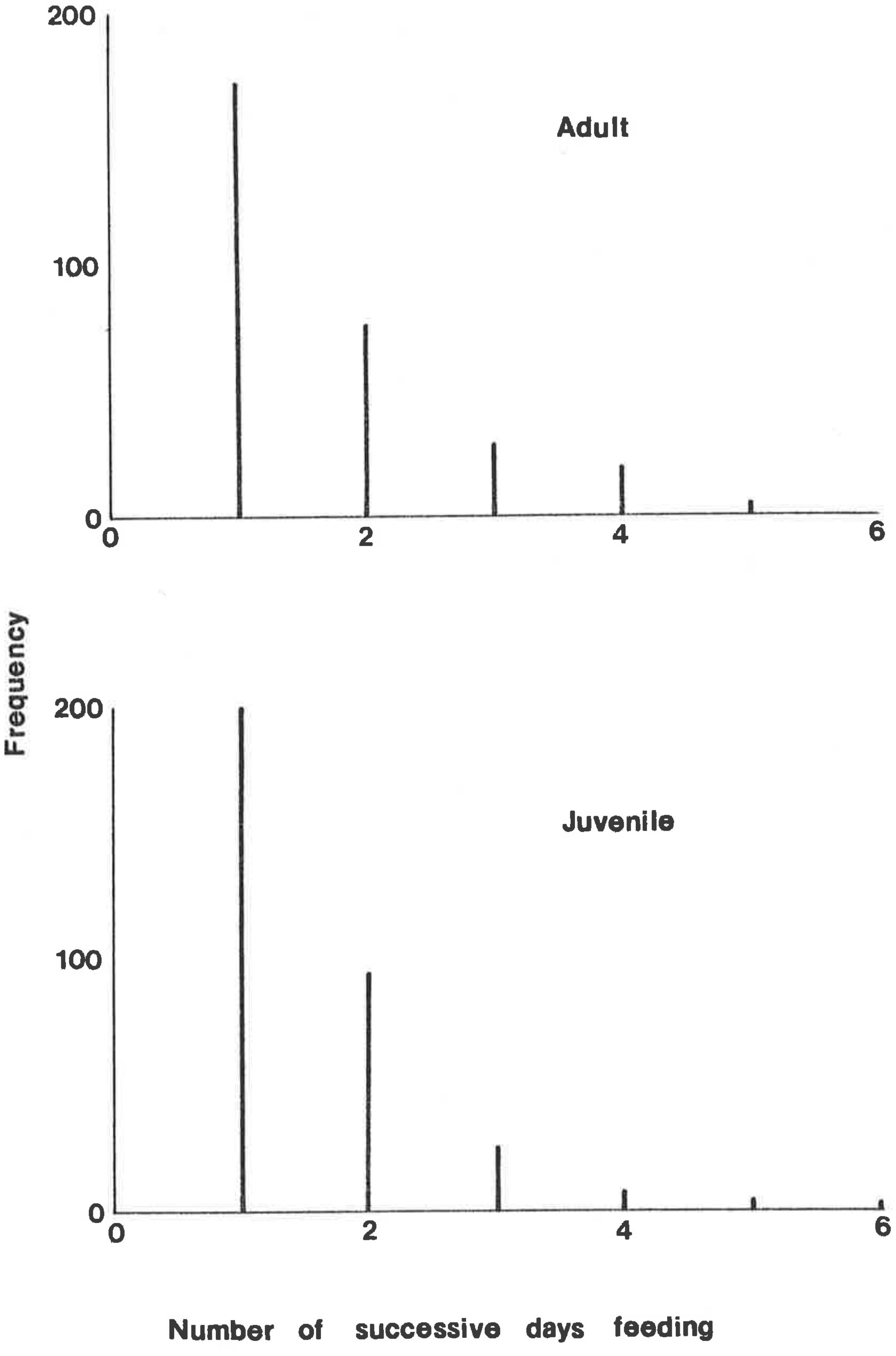


Figure 3.3 Distributions of the number of successive days spent feeding for 25 Nassarius pauperatus whilst juvenile, and for 20 of these whilst adult.

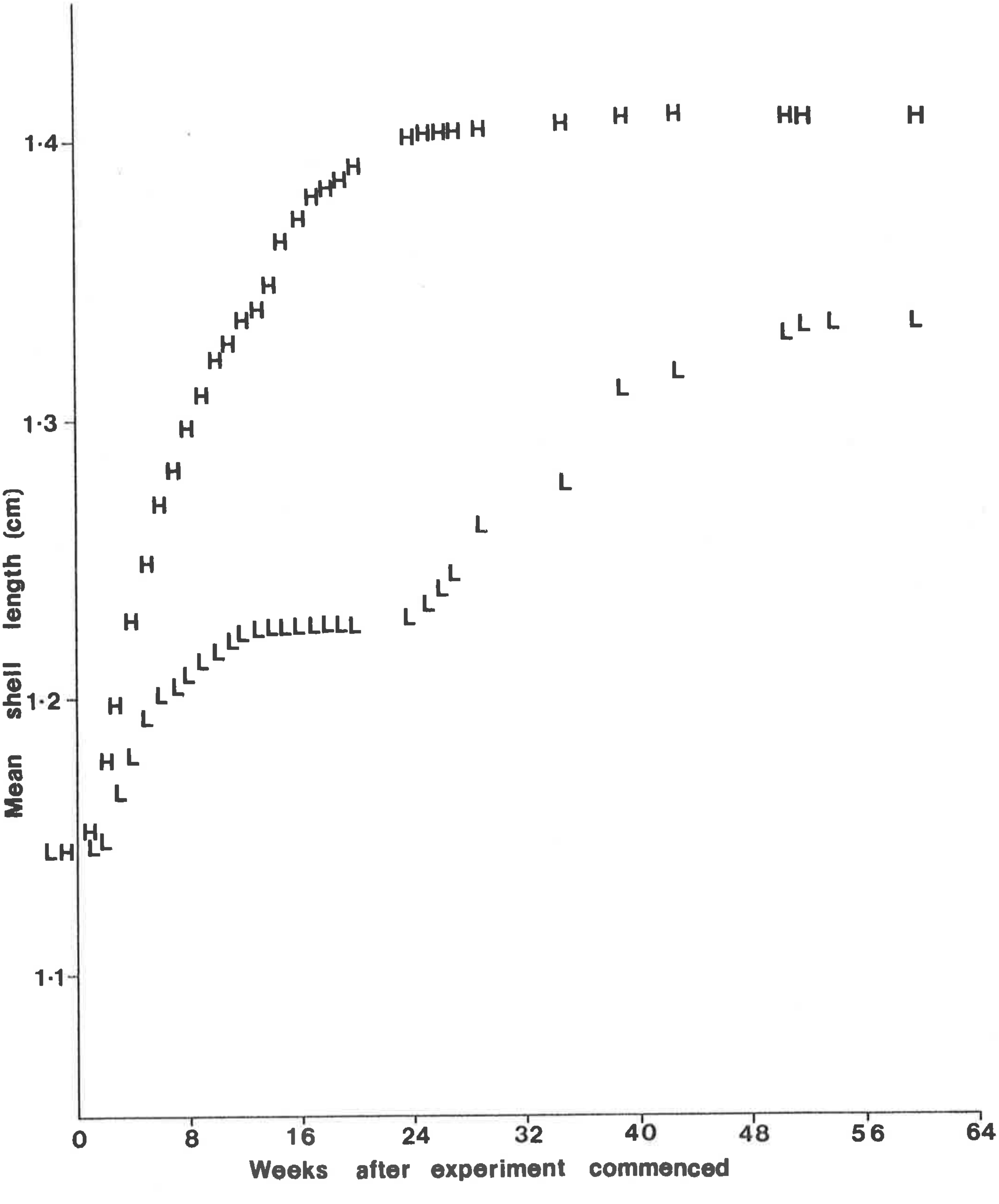


Figure 3.4 The mean shell length of Nassarius pauperatus in both high and low food treatments, versus the number of weeks after the experiment commenced. H = high food treatment, L = low food treatment.

of their containing snails which eventually died. By inspection it does not seem appropriate to compare the rates of growth between the high and low food treatments using a mathematical model of growth. Smith (1975) used the von Bertalanffy model (see Beverton and Holt, 1957) to describe the growth of *Nassarius pauperatus*. However, although the data for snails in the high food treatment resembled the curve predicted by this model (see Figure 3.4), data from the low food group did not.

From Figure 3.4 it appears that snails in the low food group grew more slowly, but for longer, than their counterparts in the high food treatment. All snails that died during the experiment did so before becoming mature and thus were excluded from the data, and the time elapsing before snails of matched pairs developed a lip on their shells were compared, using a one tailed Wilcoxon matched-pairs signed-ranks test (Siegel, 1956). This confirmed that snails in the low food group took significantly longer to develop lips on their shells and hence to cease growing (Table 3.15).

The distribution of the shell lengths of juvenile *N. pauperatus* is not normal, although the shell lengths of adult males and females in the field appear to be normally distributed (Smith, 1975 and also my data). In the high and low food experiments snails were chosen as matched pairs of juveniles and since their sex could not be distinguished, males were paired with females of the same initial size (see Tables 3.13, 3.14, 3.16 and 3.17). Also the pairs of snails were not chosen at random, instead reflecting the most common sizes in the sample of juvenile snails. Therefore samples of males and females in the high and low food treatments cannot be regarded as random samples from a normal distribution, so a non parametric comparison was made between the lengths of the matched pairs of snails in each treatment. The number of times males in the low food group

Snail number	Week following commencement of experiment upon which first sign of lip formation noticed	
	High food	Low food
1	DIED	51
2	DIED	51
3	11	24
4	DIED	18
5	25	11
6	29	29
7	14	11
8	18	29
9	DIED	29
10	13	13
11	16	12
12	15	43
13	DIED	43
14	27	39
15	27	51
16	13	39
17	25	13
18	29	43
19	11	12
20	27	35
21	29	51
22	26	43
23	39	52
24	43	DIED
25	25	39

Wilcoxon matched-pairs signed-ranks test (one tailed),
$n = 17$, $T = 22.5$, $p < 0.01$.

Table 3.15 Number of weeks after experiment commenced before individual snails formed a lip on their shells and ceased growing.

Snail number	Sex	Behaviour	Shell length (cm)
1	F	T	0.980
2	F	T	1.120
3	F	T	1.200
4	F	N	1.195
5	F	T	1.200
6	F	N	1.135
7	F	T	1.175
8	F	N	1.050
9	M	N	1.290
10	F	T	1.225
11	F	N	1.265
12	F	T	1.100
13	F	T	1.040
14	M	N	1.205
15	F	T	1.110
16	F	T	1.150
17	F	T	1.210
18	M	N	1.240
19	F	T	1.125
20	F	T	1.165
21	M	T	0.980
22	F	N	1.040
23	F	T	1.040
24	NK	T	1.240
25	M	N	1.070

M : male, F : female, NK : snail died before becoming adult,
T : Twister, N : Non Twister.

Table 3.16 Low food treatment whilst snails juvenile. Snail number, behaviour, sex and shell length.

Snail number	Sex	Behaviour	Shell length (cm)
1	F	T	1.100
2	F	T	1.365
3	F	T	1.330
4	F	N	1.420
5	F	T	1.320
6	F	N	1.275
7	F	T	1.255
8	F	N	1.185
9	M	N	1.555
10	F	T	1.315
11	F	N	1.375
12	F	T	1.280
13	F	T	1.190
14	M	N	1.330
15	F	T	1.325
16	F	T	1.485
17	F	T	1.350
18	M	N	1.545
19	F	T	1.230
20	F	T	1.275
21	M	T	1.585
22	F	N	1.165
23	F	T	1.275
24	Died		
25	M	N	1.450

M : male, F : female, T : Twister, N : Non Twister

Table 3.17 Low food treatment after all snails adult. Snail number, behaviour, sex and shell length.

were matched with females in the high food group and vice versa was fortunately the same, namely four, in both cases so this should eliminate any bias caused by there being unequal pairings of sexes between groups, when a matched pairs test is used. Matched pairs containing a snail which died during the experiment were not used, resulting in the shell lengths of pair numbers 3, 5, 6, 7, 8, 10, 11, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 and 25 being compared. Prior to the experimental treatments there were measurable differences between the shell lengths of only two pairs of juvenile snails and these were both within 0.010 cm and effectively cancelled each other (compare Tables 3.13 and 3.16). After the treatments, adult snails in the high food group were significantly larger than their counterparts in the low food group, when compared using a Wilcoxon matched-pairs signed-ranks test (compare Tables 3.14 and 3.17; $n = 19$, $T = 46$, $p<0.01$).

3.4.3 Estimation of hunger - Method 2.

3.4.3.1 Differences between Nassarius pauperatus from Port Gawler and Port Clinton.

Both the mean and standard deviation of the days elapsing before individual Nassarius pauperatus fed upon Katelysia scalarina were significantly greater in samples collected from Port Clinton than Port Gawler (Table 3.18). The distributions of the number of snails first feeding per day had significantly different variances, and by inspection, different shapes (see Figure 3.5) so a non-parametric Randomisation Test (Siegel, 1956) was used to compare the mean days to feed between these samples. After being starved for 30 days, snails were then satiated for one day and offered K. scalarina on every subsequent day. The time taken to re-feed after satiation did not then differ significantly between snails from different

Days before feeding, following collection	Population	
	Gawler	Clinton
$\bar{x}$	4.08	8.55
s_x	1.66	3.38
n	93	91

$F_{90,92} = 4.12$, $p < .001$

t_{182} (Randomisation Test) = 11.44, $p < .001$

Days before feeding, after starvation for 30 days and satiation	Population	
	Gawler	Clinton
$\bar{x}$	1.83	1.92
s_x	0.81	0.74
n	92	83

$F_{91,82} = 1.20$ $p > 0.05$

t_{173} (Randomisation Test) = 0.76, $p > 0.05$

$\bar{x}$: mean, s_x : standard deviation, n : sample size.

Table 3.18 Days before attempting to feed on *Katelysia scalarina* for adult *Nassarius pauperatus* from Port Gawler and Port Clinton, following collection and also following 30 days starvation and satiation on day 30. Sample sizes differ as a result of snails dying.

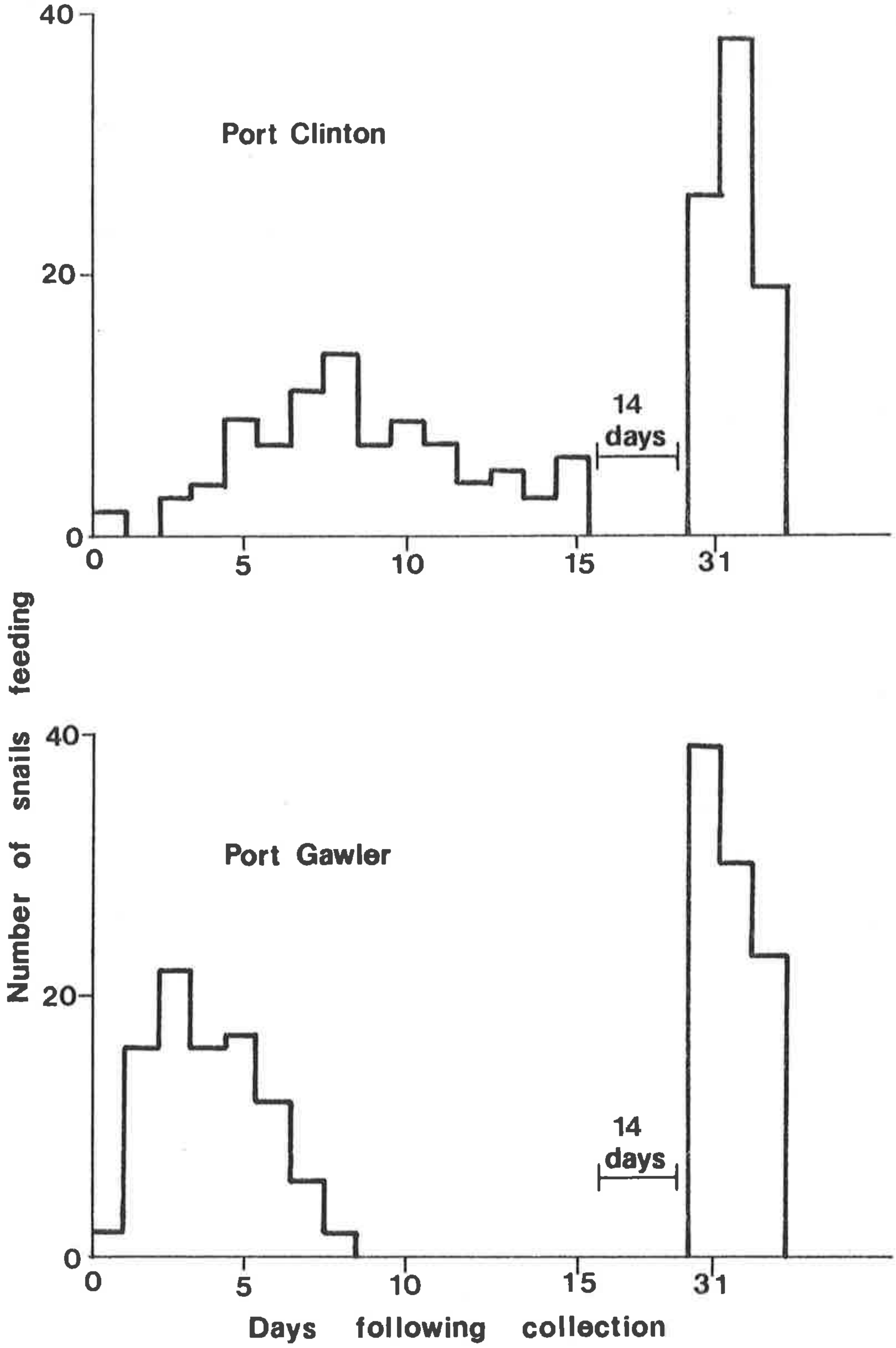


Figure 3.5 Days before attempting to feed upon *Katelysia scalarina* for *Nassarius pauperatus* from Port Gawler and Port Clinton following collection, and then following starvation for 30 days following collection and satiation upon *K. scalarina* on day 30.

locations (see Table 3.18) suggesting that the initial difference observed was due to a difference in hunger, resulting from previous experience of different levels of food availability at Port Gawler and Port Clinton.

3.4.3.2 Proportion of Nassarius pauperatus feeding when offered plant and animal material.

Results of offering Katelysia scalarina or an alga to separate groups of Nassarius pauperatus from Port Gawler and Port Clinton are in Table 3.19. Neither the mean nor the variance of the days elapsing before snails fed differed significantly between foods within each location, when compared using a Randomisation Test and F test.

3.4.3.3 The effect of being transported upon feeding.

Data showing the effects of being transported upon the time taken for snails to feed are in Table 3.20. From these it is obvious that an increased time spent in transit greatly reduces the mean and standard deviation of days elapsing before snails feed. Therefore, as the distance between the laboratory and the location sampled increases, then so will the estimate of hunger, when Method 2 is used.

3.4.4 Comparison between other locations.

The numbers of adult Nassarius pauperatus either feeding or not feeding when offered food on adjacent days every month at Port Clinton and Port Gawler for 13 consecutive months are in Table 3.21. In 12 of the 13 cases a greater proportion of snails fed at Port Gawler than at Port Clinton, and Chi-squared comparisons (2 x 2 contingency tables) showed that all but three of these differed at the 5% level or less. Only once did a significantly greater proportion of snails

	Population			
	Port Gawler		Port Clinton	
	Cockle	Alga	Cockle	Alga
Days before feeding				
$\bar{x}$	7.50	7.42	10.00	10.42
s_x	2.52	2.86	3.20	3.33
n	50	50	50	50
	$F_{49,49} = 1.29$, $p > 0.05$		$F_{49,49} = 1.08$, $p > 0.05$	
	t_{98} (Randomisation test) $= 0.15$, $p > 0.05$		t_{98} (Randomisation test) $= 0.64$, $p > 0.05$	

Table 3.19 Days before first feeding on cockle(*Katelysia scalarina*) or alga by adult *Nassarius pauperatus* from Port Clinton and Port Gawler.

	Population			
	Port Gawler		Port Clinton	
	Distance travelled		Distance travelled	
	Short	Long	Short	Long
Days before feeding				
$\bar{x}$	5.37	1.43	9.74	2.37
s_x	2.95	0.78	2.85	1.15
n	100	102	100	100

$\bar{x}$: mean, s_x : standard deviation, n : sample size

Table 3.20 The effect of different times spent *in transit* between the field and the laboratory upon the mean and standard deviation of days elapsing before *Nassarius pauperatus* fed upon *Katelysia scalarina*.

Port Gawler			Port Clinton				
Date	Number feeding	Number not	Date	Number feeding	Number not	χ^2_1	p
2/XII/76	39	61	3/XII/76	32	68	1.07	N.S.
9/I/77	23	77	10/I/77	3	97	17.68	***
8/II/77	13	87	9/II/77	28	72	6.90	**
8/III/77	33	67	9/III/77	21	79	3.65	N.S.
6/IV/77	4	96	5/IV/77	12	88	4.35	*
4/V/77	39	61	5/V/77	17	83	12.00	***
2/VI/77	32	68	3/VI/77	23	77	2.03	N.S.
7/VII/77	51	49	8/VII/77	22	78	18.14	***
4/VIII/77	40	60	5/VIII/77	18	82	11.75	***
15/IX/77	43	57	14/IX/77	11	89	25.98	***
12/X/77	60	40	13/X/77	21	79	31.56	***
11/XI/77	35	65	10/XI/77	6	94	25.80	***
2/XII/77	27	73	3/XII/77	3	97	22.59	***

Table 3.21 Numbers of Nassarius pauperatus either feeding or not on adjacent days at Port Gawler and Port Clinton, when offered Katelysia scalarina.

N.S. : not significant ($p > 0.05$), * : $0.01<p<0.05$, ** : $0.001<p<0.01$, *** : $p<.001$.

feed at Port Clinton compared to Port Gawler. Results from other locations and their comparison with reference samples taken from Port Gawler within 12 days are in Table 3.22. To simplify the interpretation of these data, the proportion of snails feeding was expressed as a percentage of the total snails in a sample (see Table 3.23). This percentage was then subtracted from the percentage of snails feeding in the equivalent reference sample from Port Gawler. Thus, samples with a greater proportion of snails feeding than at Port Gawler had indices of hunger greater than zero, and those with a smaller proportion of snails feeding than at Port Gawler had indices of hunger of less than zero.

Table 3.24 shows the percentage of Twisters and index of hunger relative to that at Port Gawler (from Table 3.23) for each location sampled.

The sex ratio differed significantly between locations (Table 3.3) with female *N. pauperatus* outnumbering males by 1.76 or more at all locations. Male *N. pauperatus* were larger than females at all locations.

The mean shell lengths of both female and male *N. pauperatus* were significantly correlated with hunger (Figure 3.6; Table 3.25). (A non-parametric Spearman rank correlation coefficient was used, in view of the computations undertaken to obtain a relative index of hunger). This provides evidence that Method 1 is a valid estimate of hunger and hence of relative food availability, considering the result of the laboratory experiment showing that food availability significantly affected final adult size.

The percentage of Twisters is plotted against the above estimate of hunger in Figure 3.7, against the mean shell length of males in Figure 3.8, and females in Figure 3.9. It can be seen that there appears to be a relation between the percentage of Twisters and both

Table 3.22 Number of Nassarius pauperatus feeding or not when offered Katelysia scalarina at various locations, together with reference sample from Port Gawler, and χ^2 (2 x 2 contingency table) comparisons between each location and Port Gawler.

				Reference sample at Port Gawler				
Location	Date	Number feeding	Number not feeding	Date	Number feeding	Number not feeding	χ^2_1	p
Onkaparinga	22/IV/77	31	69	4/V/77	39	61	1.41	N.S.
Middle Beach	26/XI/76	51	29	2/XII/76	39	61	10.89	***
Port Prime	26/XI/76	32	48	2/XII/76	39	61	0.02	N.S.
Parham	26/XI/76	44	36	2/XII/76	39	61	4.58	*
Port Wakefield	28/XI/76	40	40	2/XII/76	39	61	2.18	N.S.
Rogues Point	28/XI/76	4	30	2/XII/76	39	61	8.64	**
Stansbury	20/III/77	3	97	8/III/77	33	67	30.49	***
Coobowie	21/I/77	74	26	9/I/77	23	77	52.07	***
Coobowie	20/III/77	62	38	8/III/77	33	67	16.86	***
Sultana Point	20/III/77	11	89	8/III/77	33	67	14.10	***
Moonta Bay	7/II/79	35	65	8/II/79	13	87	13.27	***
Yatala Harbour	29/XI/76	10	11	2/XII/76	39	61	0.54	N.S.
Lucky Bay	5/I/77	0	100	9/I/77	23	77	25.99	***
Port Lincoln I	29/XI/76	7	93	2/XII/76	39	61	28.91	***
Port Lincoln II	29/XI/76	53	47	2/XII/76	39	61	3.95	*
Coffin Bay	29/XI/76	0	100	2/XII/76	39	61	48.45	***

N.S. : not significant (p>0.05), * : .05<p<.01, ** : .001<p<.01, *** : p<.001

Location	Percentage of snails feeding	Value relative to Port Gawler
Onkaparinga	31	-8
Middle Beach	63.75	+24.75
Port Prime	40	+1
Parham	55	+16
Port Wakefield	50	+11
Rogues Point	11.76	-27.24
Stansbury	3	-30
Coobowie	74	+51
Coobowie	62	+29
Sultana Point	11	-22
Moonta Bay	35	+22
Yatala Harbour	47.62	+8.62
Lucky Bay	0	-23
Port Lincoln I	7	-32
Port Lincoln II	53	+14
Coffin Bay	0	-39
Port Clinton[a]	16.69	-17.08
Port Gawler[a]	33.77	0

[a] Mean of 13 values (from Table 3.21)

Table 3.23 Percentages of *Nassarius pauperatus* feeding when offered *Katelysia scalarina* at various locations, and expressed relative to reference samples from Port Gawler (from Tables 3.21 and 3.22).

Location	Hunger (assessed by Method 1, and relative to Port Gawler).	Percentage of Twisters	n
Onkaparinga	-8	86.25	80
Port Gawler	0[a]	77.14	350
Middle Beach	+24.75	78.75	80
Port Prime	+1.0	66.25	80
Parham	+16.0	83.75	80
Port Wakefield	+11.0	68.75	80
Port Clinton	-17.08[b]	17.50	200
Rogues Point	-27.24	15.79	38
Stansbury	-30.0	16.25	160
Coobowie	+40.0[c]	16.32	190
Sultana Point	-22.0	7.50	160
Moonta Bay	+22.0	62.0	100
Yatala Harbour	+8.62	80.95	21
Lucky Bay	-23.0	11.25	80
Port Lincoln I	-32.0	0	80
Port Lincoln II	+14.0	12.50	80
Coffin Bay	-39.0	11.25	80
Streaky Bay	not measured	0	74
Smoky Bay	not measured	0	44
American River	not measured	67.50	80

a : by definition

b : mean of 13 values

c : mean of 2 values

Table 3.24 Hunger, as assessed by Method 1, relative to Port Gawler (from Table 3.23) and the percentage of Twisters.

Comparison	r_s	z	n	p
Hunger, mean female shell length, all locations	-0.89	-3.58	17	$p<.001$
Hunger, mean male shell length, all locations	-0.78	-3.11	17	$p<.001$

Table 3.25 Spearman rank correlation coefficients (r_s), and standardized normal conversions of these (z) for hunger versus both male and female adult shell length, with one tailed probabilities for z values.

	All locations			Excluding Coobowie and Port Lincoln II		
Comparison	r_s	z	n	r_s	z	n
Hunger relative to Port Gawler, % Twisters	+0.58	+2.33	17	+0.74	+2.78	15
Mean shell length of adult females, % Twisters	-0.59	-2.38	17	-0.81	-3.02	15
Mean shell length of adult males, % Twisters	-0.72	-2.86	17	-0.85	-3.19	15

Table 3.26 Spearman rank correlation coefficients (r_s), and standardized normal conversions of these (z) for hunger versus the percentage of Twisters, and also the shell lengths of adult males and females versus the percentage of Twisters, both with, and excluding, data from Coobowie and Port Lincoln II. (One tailed probabilities for z are all less than .01).

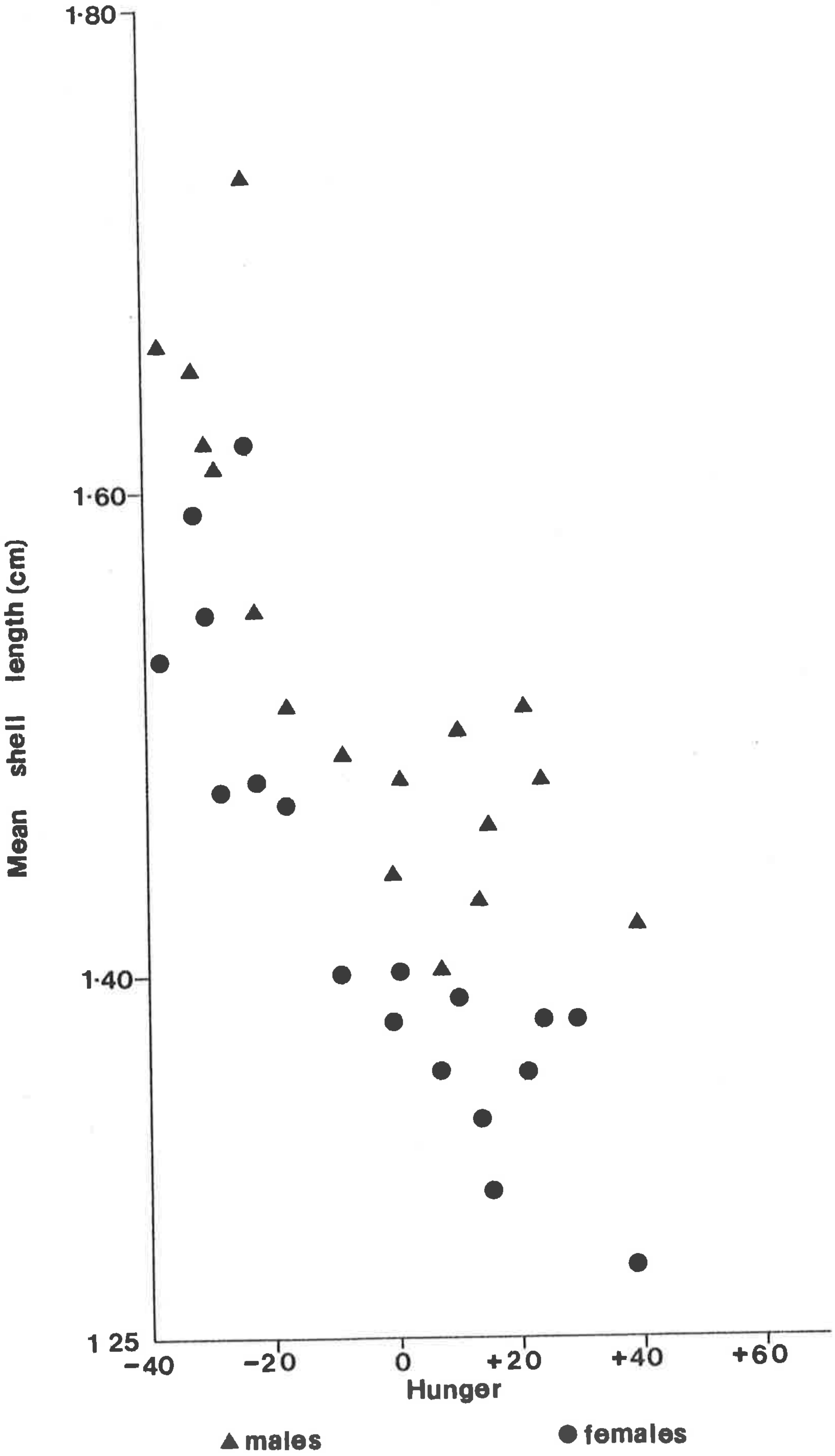


Figure 3.6 The mean shell length of adult male and female Nassarius pauperatus versus hunger assessed by Method 1, relative to Port Gawler.

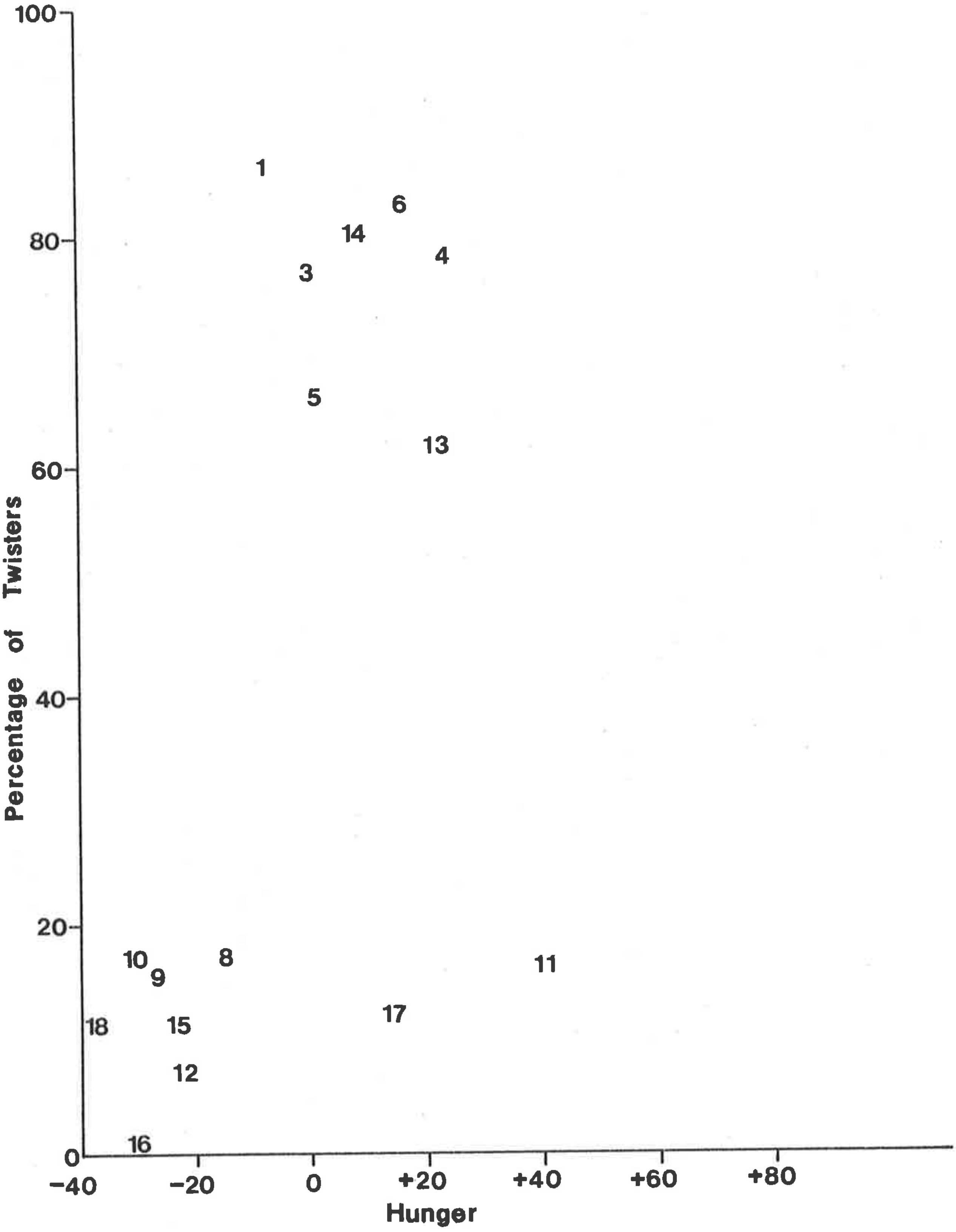


Figure 3.7 The percentage of Twisters at each location, versus hunger, as assessed by Method 1, relative to Port Gawler. Numbers indicate locations as in Figure 3.1.

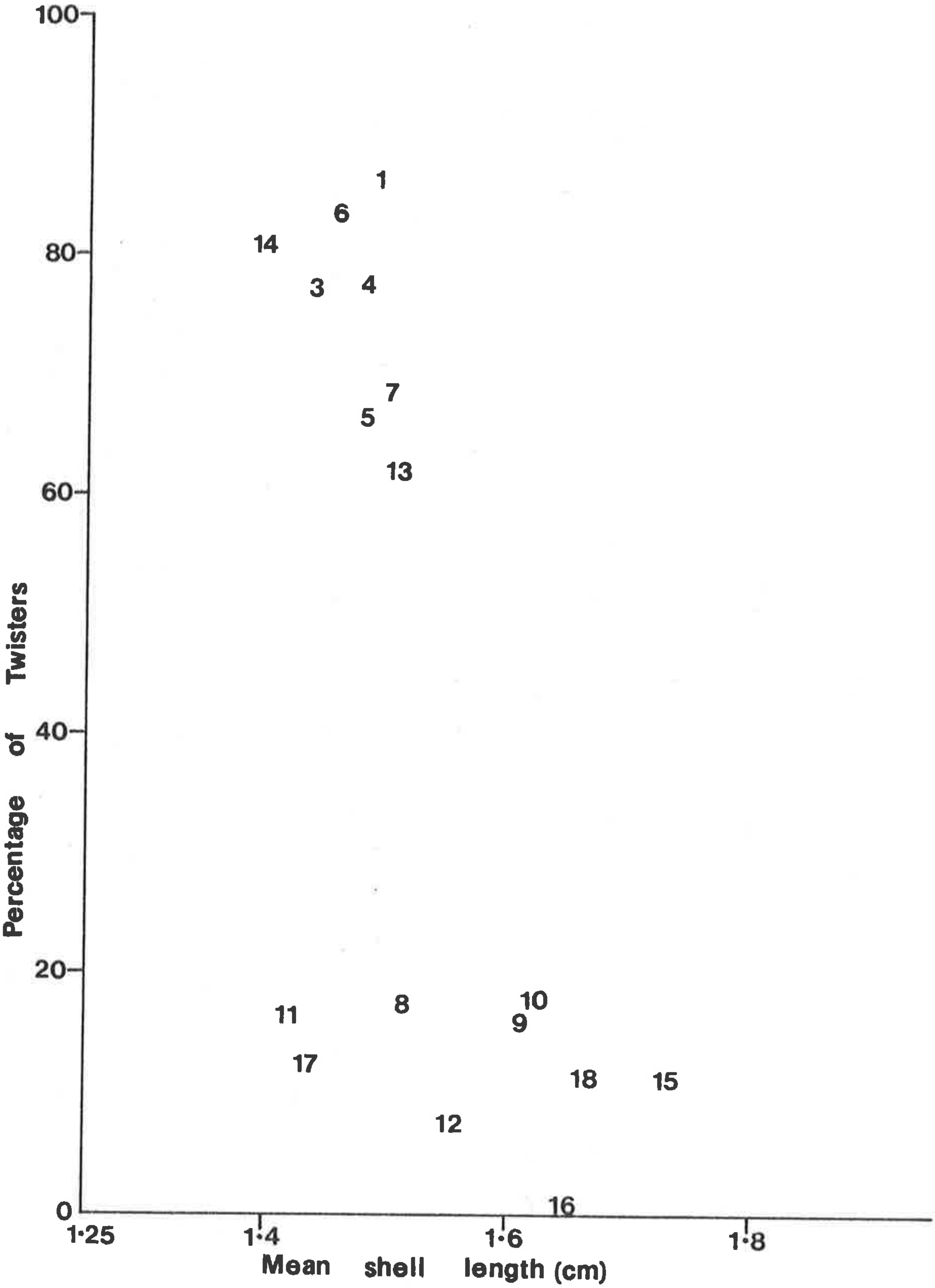


Figure 3.8 The percentage of Twisters versus the mean shell length of male Nassarius pauperatus. Numbers refer to locations, as in Figure 3.1.

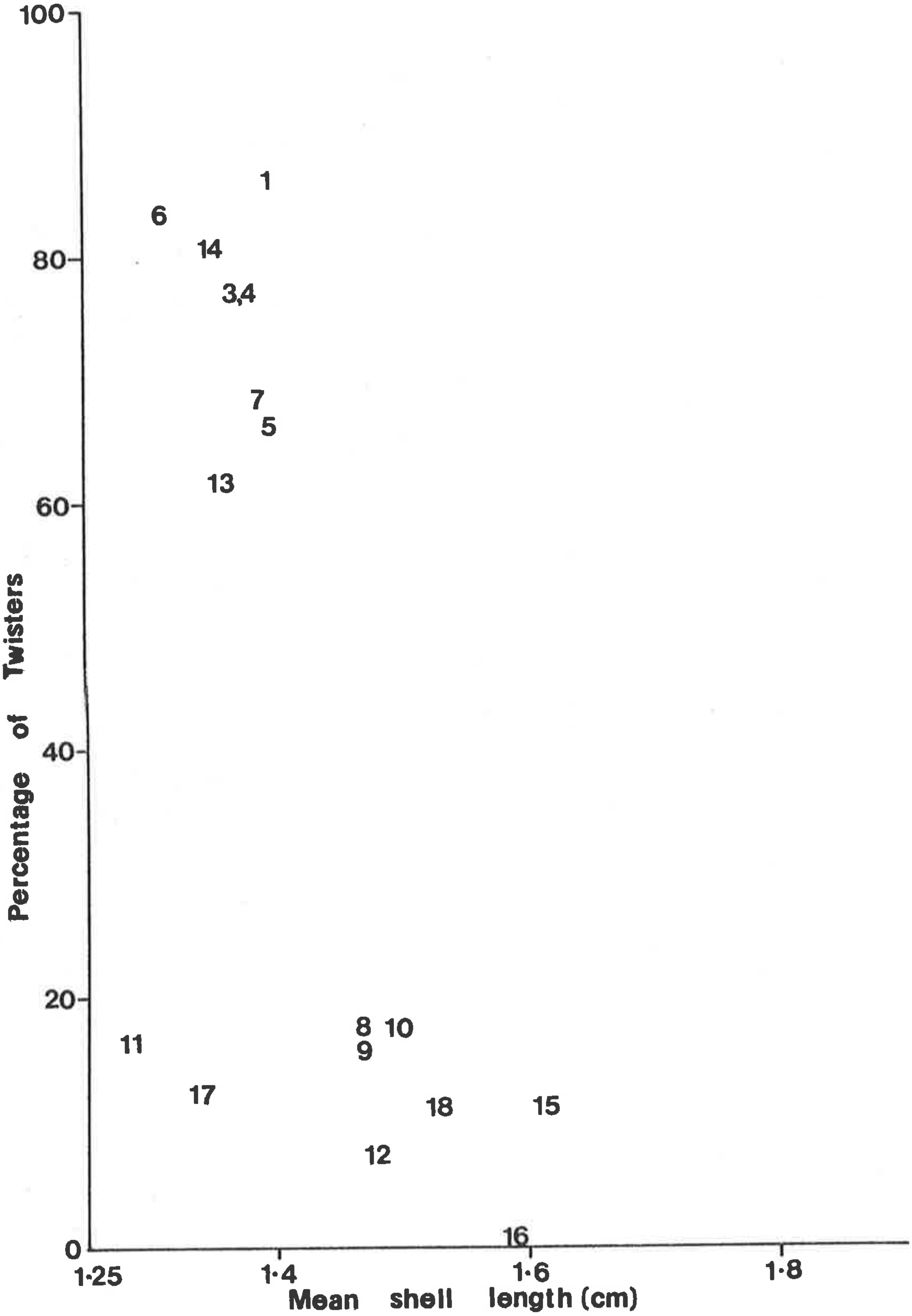


Figure 3.9 The percentage of Twisters versus the mean shell length of female Nassarius pauperatus. Numbers refer to locations, as in Figure 3.1.

hunger and shell length except for two points - Coobowie and Port Lincoln II and this was confirmed statistically; see Table 3.26. Both the mean shell length of snails and hunger are significantly correlated with the percentage of Twisters. The sample sizes of the data are large enough to convert the Spearman r_s values to those of the standard normal deviate (z) (Gibbons, 1971), and these values are increased if the data for Coobowie and Port Lincoln II are excluded.

3.5 Discussion.

3.5.1 Population density and the percentage of Twisters.

The percentage of Twisters is only significantly correlated with the population density of juvenile *Nassarius pauperatus* (Section 3.4.1 and Figure 3.10). This correlation is from data with most points clustered around zero, but does nevertheless suggest that the population density of juvenile snails may somehow be associated with the percentage of Twisters. From Tables 3.5, 3.6 and 3.7 it can be seen that the proportion of juvenile *N. pauperatus*, the percentage of Twisters and the mean shell lengths of adult snails remained very similar within all locations sampled more than once during 1976-1978. Adult *N. pauperatus* survived for more than three years in the laboratory suggesting that members of this species may live for at least 4.5 years, including the time spent as a juvenile. Therefore, a sample of adult snails may include individuals which became adult at least three years before. In the laboratory, the availability of food was shown to affect significantly the mean adult size of *Nassarius pauperatus*. Assuming that this is also the case in the field, the distribution of the shell lengths of adults will reflect food availability for the previous 4.5 years. Thus, from the data gathered during the years 1976-1979, food availability can be

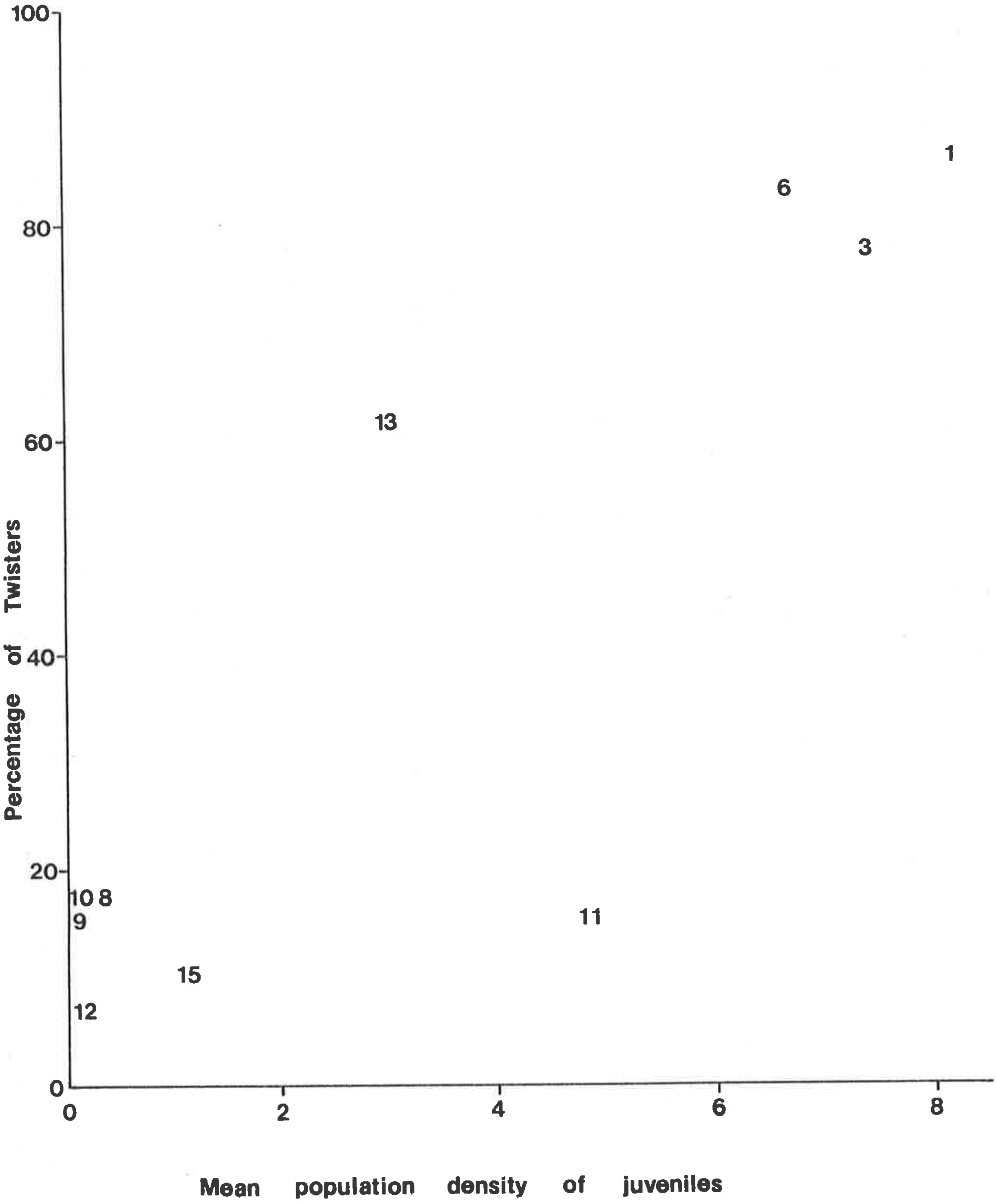


Figure 3.10 The mean population density of juvenile Nassarius pauperatus per 3600 cm^2 versus the percentage of Twisters. Numbers refer to locations, as in Figure 3.1.

inferred for 7.5 years at least, and it is notable that all locations sampled had a monomodal, apparently normal distribution of the shell lengths of both sexes, and that the mean shell length remained similar within each location sampled more than once during 1976-1978. These data suggest that food availability has remained at very similar levels within locations for at least 7.5 years.

This argument can be criticised in that other factors may also affect final adult size, or that adults may not survive for as long in the field as in the laboratory and that survival may also vary between locations. Nevertheless, the data do suggest that food availability has remained relatively constant both between and within locations for at least three years.

3.5.2 Estimation of hunger - Method 1.

There was no evidence to suggest that Method 1 was not a valid estimation of hunger and hence of the relative availability of food for Nassarius pauperatus at different locations in the field. No significant differences were found between the proportion of snails feeding when offered plant or animal material, nor between the proportion of adult or juvenile snails feeding when offered Katelysia scalarina in the field.

The feeding patterns of a group containing all four combinations of Twisters or Non Twisters with males or females was examined in the laboratory, and were not found to be significantly heterogeneous between individual snails fed ad lib K. scalarina whilst adult or juvenile, nor between pooled data for adults and juveniles.

Furthermore, in the laboratory the availability of food was found to affect significantly the final adult size of N. pauperatus and there was a significant correlation between hunger assessed by Method 1 relative to Port Gawler and the mean shell lengths of both male and female snails in the field. This is good evidence that relative food availability in the field can be inferred from Method 1.

3.5.3 Estimation of hunger - Method 2.

The time taken to feed in the laboratory by snails from Port Gawler and Port Clinton was concordant with results obtained by Method 1 and suggested that *Nassarius pauperatus* were hungrier at Port Gawler than Port Clinton. However, the time elapsing before snails fed upon *Katelysia scalarina* in the laboratory decreased as the time spent *in transit* to the laboratory increased and therefore Method 2 has little use as a comparative estimate of hunger between locations. However, the other tests performed upon Method 2, namely the comparison of the mean days elapsing before snails fed following collection, and after being starved for 30 days, satiated, and then re-offered food, do suggest that the initial differences were a result of snails being hungrier at Port Gawler than Port Clinton.

3.5.4 The percentage of Twisters, population density and food availability - An hypothesis.

Results have shown there to be significant correlations between the population density of juvenile *Nassarius pauperatus* and the percentage of Twisters, as well as between food availability (as estimated by both hunger and the mean shell length of adults) and the percentage of Twisters.

Differences between locations, with respect to the percentage of Twisters, the proportion of juveniles and the shell length of adults, persisted for all locations sampled several times during the three year study. Data for the shell length of adults suggested that different levels of food availability had persisted between locations for at least three years, and possibly as long as seven. Later

(Chapter 7) an hypothesis is proposed, relating differences in the morphology of the shore to the above data and it is then postulated that the differences in food availability described above have persisted for many generations.

If the values for Coobowie and Port Lincoln II were removed, the correlations of both hunger and mean adult shell lengths with the percentage of Twisters were improved (see Table 3.26 and also Figures 3.7, 3.8 and 3.9). Both Coobowie and Port Lincoln II are populations containing small hungry snails and both of these are adjacent to populations containing larger, less hungry snails. Nassarius pauperatus has a planktonic larval stage which is pelagic for a minimum of 21 days in laboratory cultures at 26°C (see Section 4.1). Assuming that as food availability decreases then so does the number of offspring produced (see Krebs, 1972), then snails at Coobowie and Port Lincoln II will contribute relatively fewer larvae to the total larval "pool" in their area than snails from the surrounding populations. Therefore, assuming that larvae from adjacent populations can be transported to both Coobowie and Port Lincoln II, most of the larvae recruited at the latter two locations might be expected to have originated from surrounding populations. If it is also postulated that being a Twister is heritable, non labile and also selected for when food is in short supply and/or selected against when food is not in short supply, the unexpectedly low percentages of Twisters at Coobowie and Port Lincoln II can be accounted for, since snails recruited to these populations will mostly be Non Twisters. The percentages of Twisters at Coobowie and Port Lincoln II are consistent with this hypothesis in that they are higher than those of the populations closest to them (Sultana Point and Port Lincoln I respectively) (see Figure 3.1 and Table 3.24), but still lower than expected for locations with such low food availability. It is also notable that the data from Coobowie (Port Lincoln II was not sampled) for the population density of juvenile snails was characteristic of locations having a higher frequency of Twisters (see Figure 3.10). Therefore, the argument outlined above can also be proposed in relation to the population density of juvenile snails and the percentage of Twisters.

In the following chapter I examine the hypotheses and assumptions above, namely that being a Twister is heritable, that reproductive output decreases as food availability decreases, and that transport of larvae is possible from adjacent populations to Coobowie and Port Lincoln II.

4. The assumptions; That being a Twister is heritable, that reproductive output decreases as food availability decreases and that transport of larval Nassarius pauperatus is possible between certain locations.

4.1 The heritability of being a Twister.

4.1.1 Introduction.

A great deal of anecdotal evidence was accumulated which suggested that being a Twister was constant in individuals. As well as the observations described in Chapter 2 of the behaviour of individuals remaining the same, observations made whilst scoring samples of snails from various locations for the percentage of Twisters (see Chapter 3) also showed that individuals did not appear to change their behaviour. Also, in experiments described later, snails were subjected to various selective pressures and although some snails eventually died, their behaviour did not change.

Whilst being a Twister appeared to be constant in individuals, the only way to establish the heritability of this behaviour is to cross all combinations of Twisters and Non Twisters and to observe the results of raising the progeny of these crosses in similar environments (Caspari, 1958).

4.1.2 Materials and Methods.

4.1.2.1 Introduction.

Members of the genus Nassarius have separate sexes (Fretter and Graham, 1962) and planktonic larvae (Scheltema, 1962). Scheltema described a technique modified from Loosanoff (1954) with which he was able successfully to raise two species of Nassarius. I initially attempted to establish whether Scheltema's technique was suitable for raising Nassarius pauperatus.

4.1.2.2 Induction of egg laying.

Adult Nassarius pauperatus can be induced to copulate and produce eggs prior to their usual breeding season in late October - November, if brought in from the field after late April and kept at or above 17°C (Smith, 1975). Samples of adult females treated similarly but kept without males did not produce eggs or egg capsules, suggesting that sperm storage does not occur between breeding seasons and therefore that crosses performed in the laboratory will contain only the progeny of the chosen male.

Thirty adult N. pauperatus were collected from Port Gawler, brought into the laboratory and kept at 19°C in polystyrene trays (dimensions 54 x 32 x 12 cm deep) containing a minimum of 10 litres of aerated seawater which had been filtered through a "Whatman GF/C" glass fibre filter of maximum pore size approximately 1.3 µm. Snails began copulating at the end of the second day following collection and egg production commenced on the third or fourth day. Eggs were encased in transparent dome shaped capsules which were laid on the sides and bottom of the tray. These were removed by sliding a razor blade between the base of the capsule and the tray. Few capsules were damaged by this method of removal.

4.1.2.3 Seawater.

A reticulated supply of seawater was not available. Seawater was collected from metropolitan Adelaide beaches on infrequent occasions and stored until use in a tank lined with fibreglass. Filtered and aerated seawater from this source was found to be toxic to larvae, causing them to retract their vela and sink to the bottom of the container. These usually did not recover and eventually died. Seawater was also collected from various locations in the Gulf St Vincent and transported to the laboratory in 25 litre polyethylene containers. However, seawater collected in this way also proved to be toxic to larvae, especially after being stored for several days, and the distance between the laboratory and the sea made it impractical to collect seawater daily.

The toxicity of seawater to N. pauperatus larvae could be removed by keeping it in a shallow polystyrene tray with constant aeration under a light bank of Gro-Lux fluorescent tubes for more than 12 hours. Trays were maintained for this purpose and soon developed a dense growth of algae on their sides and bottom. Seawater was kept in these trays for at least 24 hours, after which it was filtered and added to the culture trays as required.

4.1.2.4 Food.

Larvae are usually offered a range of laboratory cultures of phytoplankton (Pilkington and Fretter, 1970) without attempting to establish the algal species consumed naturally, an exception being Fretter and Montgomery (1968) who recognised algal cells in the gut of larvae from plankton hauls.

The larvae of Nassarius pauperatus were offered two different species of "small local flagellates" (isolated from local seawater by I.M. Thomas) which were cultured in one litre flasks of sterile Modified Schreiber solution (Stein, 1973) under a light bank of Gro-Lux fluorescent tubes. The density of algal cells in the culture trays of larvae was maintained at 200,000 per ml, by estimating densities using an "Assistent" haemocytometer and adding the appropriate volume of algal culture. Using either or both of the small local flagellates as food, no larvae survived to metamorphosis or even grew to the intermediate stage described by Scheltema.

Since larvae collected in plankton hauls sometimes have freshly ingested algal cells in their gut (Fretter and Montgomery, 1968), ten larvae and six recently settled postlarvae of *N. pauperatus* were collected from intertidal pools at Port Gawler, during November 1977. These were each washed four times in sterile seawater and crushed onto a welled microscope slide. Algal cells from the stomach of each larva were collected in 5 µl micropipettes, under a binocular dissecting microscope at 40 X magnification. This took place no more than 15 minutes after collection to minimise the effect of larval digestive processes upon the viability of algal cells. The contents of each micropipette were then added to separate 50 ml flasks of sterile Modified Schreiber solution (Stein, 1973) and incubated at 19°C under a light bank for 14 days.

A flagellate, tentatively identified as *Dunaliella tertiolecta* Butcher (R. Rose, personal communication) was thus isolated from two larvae and one postlarva and maintained in Modified Schreiber solution. By offering this alga to the larvae of *N. pauperatus* it was possible to raise them past metamorphosis.

4.1.2.5 Temperature.

Initial attempts at culture were carried out at both 19°C and 28°C. Larvae kept at 28°C and offered the small local flagellates died far sooner than those kept at 19°C. Further attempts thus took place at 19°C which is also the spring seawater temperature in South Australia. A culture of larvae was established using the above techniques and egg capsules were added whenever available. Fifty five days after establishment of the culture, metamorphosed juveniles were visible on the sides of the rearing tray. However, there were very few of these (less than 1% of the number of eggs added initially to the culture tray) and need not have developed from eggs initially added. It was suggested that I culture at higher temperatures (Scheltema, personal communication, also see

Scheltema, 1967) so temperatures were increased to 26°C. At this temperature development from metamorphosis to hatching took a minimum of 21 days and mortality was so reduced that larvae from a group of egg capsules added to the culture could be followed through their various developmental stages.

4.1.2.6 Technique used for *Nassarius pauperatus*.

Separate pairwise crosses of all combinations of male and female Twisters and Non Twisters were attempted. Samples of up to 100 adult *Nassarius pauperatus* were collected from Port Gawler, prior to the breeding season, and snails separated with respect to sex on the evening following collection. During the following 1-2 days snails were scored as Twisters or Non Twisters using the methods described in Chapters 2 and 3. Pairs of male and female snails were then placed together in polystyrene tubs containing approximately 400 ml of aerated seawater at 26°C and offered *ad lib Katelysia scalarina*. However, from 26 attempts, only two pairs of snails produced any eggs at all. Considering that groups of many males and females kept together in the same tray produced a much greater number of egg capsules per female, pairs of snails were caged together in cylinders constructed of fibreglass mesh, (hole size 2 mm x 2 mm) which were suspended in larger polystyrene trays of aerated seawater, containing several other caged pairs. This did not improve the frequency of egg production and even though fertilisation is internal, it may possibly have occurred via water borne sperm from other than the proximal male.

Considering that it was easy to obtain eggs from groups of snails maintained together in the same tray without restriction, "mass crosses" within groups of Twisters and Non Twisters were carried out. Such crosses may also yield useful information about whether a character is heritable.

Egg capsules were removed from both individual and mass crosses as soon as they were observed. Those from individual crosses were transferred to identical tubs containing approximately 450 ml of filtered and aerated seawater, and those from mass crosses were transferred to larger polystyrene trays containing a minimum of 10 litres of seawater. Approximately 25% of the seawater in both trays and tubs was changed daily. Several "scoops" of up to 400 ml were removed from the trays, using a white polystyrene tub. Any larvae included with this water could easily be seen against the white background of the tub and were pipetted back to the tray. Similarly, water was decanted from the pairwise crosses maintained in tubs, and any larvae included were returned to their original tub. Larvae were offered the alga previously isolated from wild *N. pauperatus* larvae. Following metamorphosis, postlarvae were maintained under identical conditions in their respective culture trays except that they were also offered small pieces of *Katelysia scalarina*. Some 8 weeks after metamorphosis snails commenced feeding upon the latter food in large numbers, before this seeming unable to locate the pieces of *K. scalarina*, and instead feeding upon the film of alga on the walls of their containers.

Snails were scored as Twisters or Non Twisters following 5.5 months of post-metamorphic growth, by which time they were about 0.5 cm in shell length. The availability of time did not permit the keeping of snails until they were adults so they could not be sexed, and nor could their behaviour be observed whilst they grew. Lack of time also prevented very many data being obtained.

4.1.3 Results.

Juveniles were only obtained from four individual crosses (two from pairs of snails in separate tubs, and two from snails caged in cylinders). These results appear in Table 4.1. Very little can be deduced from the separate pairwise crosses, since not all combinations of Twister and Non Twister parents are present, and the sample sizes of progeny are extremely small.

Data from the mass crosses (Table 4.2) do suggest that being a Twister or Non Twister is heritable. Non Twisters only produced Non Twister progeny and Twisters produced both Twisters and Non Twisters.. The probability of obtaining this difference by chance was 3.02×10^{-12}, using a Fisher exact probability test (Siegel, 1956).

4.1.4 Discussion.

Results of the mass crosses are in accordance with Twister being heritable and dominant to Non Twister. Results of the individual crosses do not provide evidence to either refute or support this, in that sample sizes are too small and no data are available from crosses between Non Twisters.

Therefore, whilst these results do suggest that being a Twister or Non Twister is heritable, the data are scanty and the actual mode of inheritance cannot be established with any certainty. It was unfortunate that the difficulties encountered with obtaining suitable seawater, food and offspring from paired crosses plus the high mortality of larvae, prevented the gathering of more conclusive results.

Parents	Progeny	
	Twisters	Non Twisters
♂ Non Twister x ♀ Twister	0	2
♂ Non Twister x ♀ Twister	1	3
♂ Non Twister x ♀ Twister	3	0
♂ Twister x ♀ Non Twister	5	4

Table 4.1 Results of successful paired crosses between Twisters and Non Twisters.

Parents

3 ♂ Twisters x 14 ♀ Twisters

Progeny

Twisters	Non Twisters
26	22

Parents

7 ♂ Non Twisters x 15 ♀ Non Twisters

Progeny

Twisters	Non Twisters
0	71

Table 4.2 Results of "mass crosses" between several male and female <u>Nassarius pauperatus</u>, all of which were Twisters or all Non Twisters.

4.2 Food availability and egg production.

4.2.1 Introduction.

When postulating that being a Twister was heritable and non labile (see Section 3.5.4) it was assumed that the number of offspring produced decreased as the availability of food decreased, to account for the unexpectedly low percentages of Twisters at Coobowie and Port Lincoln II. This assumption was tested by comparing egg production between *Nassarius pauperatus* collected from Coobowie, Stansbury and Sultana Point (see Figure 3.1), as well as between groups of *N. pauperatus* from the same population maintained with either high or low levels of food in the laboratory.

4.2.2 Materials and Methods.

From late April (Autumn) adult *Nassarius pauperatus* can be induced to breed prior to their usual breeding season in late October and November, as described in Section 4.1.2.2.

Twenty male and 60 female adult *N. pauperatus* were each collected from Stansbury, Coobowie and Sultana Point (see Figure 3.1) on the same day during May 1977 and again during October 1977. All samples were brought back to the laboratory on the same day and kept without food in separate polystyrene trays (dimensions 54 x 32 x 12 cm) containing 4 cm depth of aerated seawater at 20°C, with photoperiod adjusted daily to current daylength. The numbers of egg capsules produced per day were counted until egg production ceased, and an estimate of the average number of eggs per capsule in each group was made by counting the number of eggs per capsule in a maximum of 45 capsules, or all capsules if less than 40.

It was hoped that this would provide an estimate of how egg production varied between populations in the field having very different levels of hunger. (Snails at Coobowie being the smallest and hungriest samples, and both Stansbury and Sultana Point having much lower values for hunger and containing larger snails: see Section 3.4.4). Samples of snails were not fed in the laboratory since hunger was known to vary between populations and this difference would be difficult to simulate.

The effect of food availability upon egg production was also investigated in a laboratory experiment. Two groups, each consisting of 25 juvenile N. pauperatus were chosen from a larger sample collected from Port Gawler during November 1977. These groups of snails were the same ones previously used to investigate the effect of differences in food availability upon the growth and feeding behaviour of N. pauperatus (Section 3.3.2.5) and consisted of 25 pairs of snails, matched for being Twisters or Non Twisters, and for initial shell length to within 0.015 cm and usually to within 0.005 cm. One group was designated "high food" and fed ad lib K. scalarina daily, whilst the other "low food" group was fed ad lib K. scalarina every fortnight. Both groups were initially kept together in the same tray.

Once N. pauperatus become sexually mature they develop a lip on their shell and cease growing. As soon as the first individual showed signs of becoming mature the high and low food groups were transferred to separate trays to enable egg production to be quantified without error. After all members showed signs of forming lips on their shells, snails began laying eggs (on 17/VIII/78 in the high food group and on 28/X/78 in the low food group). The difference in time before egg production commenced between the high and low food groups is in accordance with the observation in Section 3.4.2.6 that snails in the low food group took significantly longer to become sexually mature than their counterparts in the high food group.

Considering that in some samples of adult snails collected and brought back to the laboratory, less egg capsules were produced than there were females (see Table 4.3), variation in egg production between populations may be a result of different proportions of females actually laying eggs rather than variation in egg production per female. Consequently snails in both the high and low food treatments were watched, and the identifying numbers of all those laying eggs were recorded. The numbers of egg capsules produced per day were counted by marking the locations of new egg capsules with a chinagraph pencil, until egg production ceased. The average number of eggs per capsule produced by each group was estimated by counting the number of eggs per capsule in 40 randomly selected capsules from each group, and the lengths and widths of these capsules measured in microns using an ocular micrometer attached to an Olympus monocular microscope at 100x magnification. The diameters of all eggs were usually uniform within each capsule, so the diameter of one egg from each of the above capsules was also measured, in microns.

Cannibalism of egg capsules was frequently observed in the low food treatment and this was estimated in both treatments by counting the number of egg capsules that survived intact until the developing larvae could be clearly distinguished within them. This method under-estimated the degree of cannibalism, but was the only one possible since hatching of larvae would have confounded estimates made later in larval development. Once egg production had ceased, the snails in both groups were sexed and their shell lengths measured as described previously.

4.2.3 Results.

For all three samples collected in May, egg production commenced two days after collection. As expected, egg laying was observed whilst collecting snails from all three locations during October.

Results appear in Table 4.3. 99% confidence limits for the mean number of eggs per capsule were calculated in the usual way assuming normality. For the total number of eggs produced a standard deviation was estimated by multiplying the standard deviation of eggs per capsule by the number of capsules. The confidence intervals show that there are no significant differences between mean eggs per capsule or total eggs produced on either occasion between Stansbury and Sultana Point. However, results from Coobowie differ from the above locations on both dates. Snails from Coobowie produced significantly more eggs and significantly less eggs per capsule than those from Stansbury or Sultana Point.

Results from the laboratory experiment are shown in Table 4.4. A t test comparison showed that the mean number of eggs per capsule was significantly lower in the low food treatment than in the high food one ($t_{78} = 14.34$, $p < .001$). The mean number of eggs produced per female was much higher in the low food than in the high food treatment, and the 99% confidence intervals do not overlap.

Most of the females in each treatment were observed mating and all were seen laying eggs. This result suggests that differences in egg production between populations of _N. pauperatus_ in the field are not caused by differences in the proportions of females reproducing.

A comparison of mean egg diameters for eggs from capsules selected at random showed that those from the low food treatment

		Population	
	Stansbury	Coobowie	Sultana Point
(1) May 1977			
Total capsules	40	267	45
Eggs per capsule			
$\bar{x}$	28.03	13.00	29.27
s_x	3.94	4.65	4.83
(n)	40	40	45
99% C.I.	26.34-29.72	11.01-14.99	27.33-31.21
Estimated total eggs produced (or total eggs produced)	1121	3473.67	1317
99% C.I. for total eggs*	1053.6-1188.8	2939.30-4002.33	1229.9-1404.4
(2) October 1977			
Total capsules	14	108	15
Eggs per capsule			
$\bar{x}$	37.29	24.55	36.73
s_x	4.54	5.03	4.64
(n)	14	40	15
99% C.I.	33.64-40.94	24.21-24.89	33.49-39.97
Estimated total eggs produced	522	2651.40	551
99% C.I. for total eggs*	470.9-573.2	2426.8-2876.0	502.35-599.59

$\bar{x}$: mean, s_x : standard deviation, n : sample size

* This estimate ignores variability associated with the total capsules, of which I have no estimate.

Table 4.3 Total egg capsules, mean eggs per capsule and estimated total number of eggs produced, with 99% confidence intervals, from 60 females and 40 males from Stansbury, Coobowie and Sultana Point during May and October 1977.

	Treatment	
	High food	Low food
Total capsules	130	437
Number of females	16	19
Capsules per female	8.13	23
Eggs per capsule		
$\bar{x}$	27.08	15.33
s_x	3.49	3.83
(n)	40	40
Eggs per female		
$\bar{x}$	220.16	352.59
s_x (est)	28.37	88.09
99% C.I.	208.44-231.88	316.55-388.63
Egg diameter (in μm)		
$\bar{x}$	143.25	147.20
s_x	4.51	5.04
(n)	40	40
Capsule dimensions (in μm)		
length		
$\bar{x}$	1078.03	1067.18
s_x	44.17	87.76
width		
$\bar{x}$	904.75	916.67
s_x	28.93	63.51
(n)	40	40

$\bar{x}$: mean, s_x : standard deviation, n : sample size

Table 4.4 Total capsules, mean eggs per capsule, estimated total eggs, mean eggs per female and egg and egg capsule dimensions for high and low food treatments.

were significantly larger than those in the high food treatment ($t_{78} = 3.69$, $p < .001$), but neither the mean lengths nor the mean widths of capsules from each treatment differed significantly, when compared using a Randomisation test (Siegel, 1956) (lengths, $t_{78} = 0.70$ $p > 0.1$) (widths, $t_{78} = 1.08$ $p > 0.1$). It was estimated that cannibalism in laboratory trays destroyed 309 out of 397 capsules in the low food group while no capsules showed signs of being eaten in the high food group. Cannibalism or predation of egg capsule contents in the field must invariably be underestimated by any simple survey, since attacked capsules can only be distinguished from those which have hatched if some undifferentiated or slightly differentiated eggs remain in them.

However, during October 1977, no egg capsules of this type were found at either Stansbury or Sultana Point despite the presence of many capsules containing eggs or developing larvae. In contrast, at Coobowie no full capsules were found and over 50% of empty ones found appeared to have been attacked. Considering that this is an underestimate and that no intact egg capsules were found at Coobowie this suggests that cannibalism or predation of eggs is high when food is scarce in the field, as well as the laboratory.

During 1977-1979 both Port Gawler and Port Clinton were searched for egg capsules. Intact capsules were found at both locations, and also some capsules at Port Gawler which appeared to have been attacked.

4.2.4 Discussion.

I conclude that as food availability decreases, Nassarius pauperatus produce more eggs and egg capsules per female, per breeding season. This somewhat paradoxical result is interpreted in terms of the selective advantages of semelparous and iteroparous patterns of reproduction in different environments, in Appendix 3.

However, despite this result, the assumption that effective reproductive output (that is, the number of eggs that actually hatch) decreases as food availability decreases still stands, since there is good evidence that predation upon the contents of egg capsules is extremely high at Coobowie, compared to the adjacent populations at Stansbury and Sultana Point. The amount of actual predation upon the contents of egg capsules was impossible to quantify because attacked capsules could not always be distinguished from ones which had hatched. Predation upon the contents of egg capsules in the laboratory was estimated as being 77.83%, up to the time when larvae in capsules began to be indistinguishable from veligers that had hatched, since a loss of the contents of a capsule containing larvae in later stages of development may have been due to hatching. The former period lasted for a maximum of 6/7 of the total time larvae spend in capsules, since capsules containing apparently fully developed larvae often remained for several days before hatching. Therefore, cannibalism in the laboratory was estimated as being a minimum of 90.81%, assuming a constant probability of attack during the development of larvae. The intensity of predation upon the contents of egg capsules in the field cannot be inferred from these data. However, despite extensive searching, no full capsules were found at Coobowie at the time N. pauperatus were known to be laying eggs, but capsules containing eggs or developing larvae were common at Stansbury and Sultana Point. This is very good evidence that the effective reproductive output of the population of N. pauperatus at Coobowie is close to zero.

Predation upon the contents of egg capsules was not estimated at Port Lincoln II (because this location was visited before the importance of predation in determining effective reproductive output was realised). Port Lincoln II had an index of hunger of +14 which was significantly higher than that for Port Gawler (see Table 3.22) but less than the mean of +40 for Coobowie. The mean shell lengths of adult males and females at Port Lincoln II were 1.43 and 1.34 cm respectively, compared to 1.42 and 1.28 cm at Coobowie and 1.44 and 1.38 cm at Port Gawler (Table 3.4). Observations in the field suggested that effective reproductive output was not close to zero at Port Gawler.

Even though the values for hunger and shell length of adults at Port Lincoln II are between those of Coobowie and Port Gawler, the same cannot be assumed for the effective reproductive output of this population. Both predation and egg production determine effective reproductive output and it is unlikely that this will be linearly related to food availability and hunger. Therefore I only have evidence that effective reproductive output is close to zero at Coobowie. However, considering the index of hunger and size of adults at Port Lincoln II, plus the observation that some egg capsules appeared to have been attacked at Port Gawler, it is postulated that effective reproductive output is relatively lower at Port Lincoln II than in adjacent populations.

4.3 Circulation.

4.3.1 Introduction.

To account for the unexpectedly low percentages of Twisters at Coobowie and Port Lincoln II it was assumed that transport of veliger larvae from adjacent populations to these was possible.

It is not feasable to mark and recapture larvae in order to make direct observations of transport (Crisp, 1978) thus the likelihood of larvae being transported from one population to the other has to be inferred from a consideration of the direction and velocity of currents, the length of pelagic life, and probabilities of diffusion.

There have been few actual measurements of the direction or velocity of water circulation in both of the South Australian gulfs, although several models have been proposed (Bullock, 1975; Bye, 1976; Easton, 1978; Sharobeam and Sag, 1977; and Tronson, 1974, 1975). The need for more data of circulation, or parameters from which circulation can be inferred, has often been stressed (e.g. Bye, 1976; Bullock, 1975; Tronson, 1974).

I shall initially describe the circulation known from actual measurements of currents, or that which can be inferred from the distribution of sediments, salinity, temperature and density of water in the gulfs. Finally I shall consider the predictions made by various models.

4.3.2 Gulf Saint Vincent.

The distribution of sediments in the Gulf St Vincent (Walters, unpublished, quoted in Tronson, 1974)

> "suggests that there exists a northward transport along the western shore of St Vincent Gulf and a circulation cell at the head of the gulf. It also seems that the circulation along the eastern shore is separated from the remainder of the gulf and that this circulation may be divided into cells with a null point south of Adelaide".

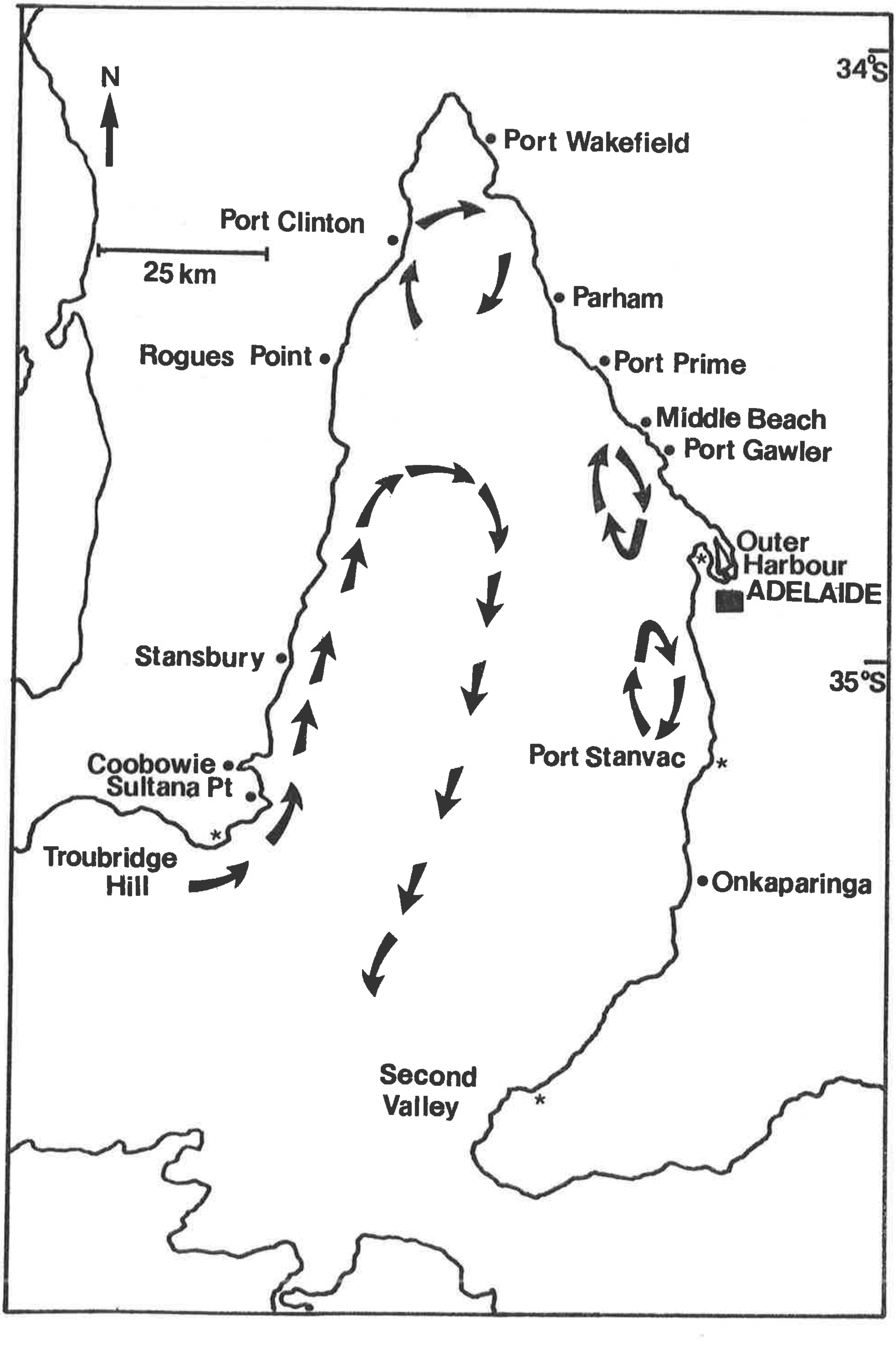
The pattern of temperature, salinity and density of the seawater in the Gulf St Vincent provides evidence of circulation that is concordant with the distribution of sediments (Bye, 1976). A tongue of cold, low salinity water from the Great Australian Bight extends northwards up the west coast of the Gulf St Vincent and salinity, temperature and density of the seawater increase rapidly in a northerly direction, indicating that water in the upper part of the gulf is only slowly being exchanged with that further south.

No measurements have been made of the non tidal circulation in the Gulf St Vincent. However, several models of circulation and the velocities of currents have been proposed. Tronson (1974) modelled circulation due to winds of various velocities and directions and his predictions agreed well with data from the distribution of sediments and seawater characteristics when he simulated the direction of the prevailing wind. Similar predictions were also made by Bye (1976) using values of thermohaline forcing calculated from known density values plus average seasonal wind stresses, from Eyre (1973). Bye modelled the velocity of circulation during October (when Nassarius pauperatus veligers should be released from capsules in the field) and predicted a current of velocity 0.05 m/second flowing northwards up the western shore of the Gulf St Vincent, which then circulates in a clockwise direction at about the latitude of Adelaide (see Figure 4.1) and flows southward out of the centre of the gulf. There is also a separate, clockwise circulation at the head of the gulf, with velocities of between 0.02 to 0.05 m/second (Figure 4.1).

Currents also result from tidal inflow and outflow in both gulfs. Tidal transport is northward towards the top of Gulf St Vincent on the flood tide and vice versa on the ebb.

Currents having velocities greater than 1.7 m/second have been recorded offshore from Edithburgh during both ebb and flood tides (Bye, 1976). However, to calculate how far water may be transported by these currents, variation in velocity between high and low water needs to be known. Radok and Raupach (1977) made continuous measurements of currents in Gulf St Vincent and integrated these to obtain values for transport during ebb and flood tides. Maximum daily values were: 4 km at Troubridge Hill (both northward and southward), 4.8 km at Second Valley (southerly flow only recorded)

Figure 4.1 Gulf St Vincent: The pattern of non tidal circulation which can be inferred from the distribution of sediments, seawater characteristics and actual measurements of currents. Arrows indicate circulation, stars the location of current recorders mentioned in the text.


N
34°S
Port Wakefield
Port Clinton
25 km
Parham
Rogues Point
Port Prime
Middle Beach
Port Gawler
Outer Harbour
ADELAIDE
Stansbury
35°S
Coobowie
Sultana Pt
Port Stanvac
Troubridge Hill
Onkaparinga
Second Valley

and 5 km at Port Stanvac (northward and southward). (Locations of the abovementioned current recorders are indicated in Figure 4.1).

Currents have also been measured during an ebb tide, offshore from Outer Harbour, Port Adelaide (see Figure 4.1) by the Engineering and Water Supply Department of the South Australian Government (1973). The area under their velocity versus time graph was equivalent to a southerly transport of 6.48 km. Although the currents during a flood tide were not measured, it was postulated that these were similar and opposite in direction to those during the ebb tide, thus resulting in a northerly transport of similar magnitude.

4.3.3 Discussion.

Water movement resulting from tidal changes should result in transport of larvae in a north-south direction on either side of the gulf, but not between shores. The pattern of non tidal circulation in Gulf St Vincent suggests that larvae should be able to travel northwards up the western shore, but only be exchanged between shores near the head of the gulf. Velocities from Bye's model give a daily transport of 4.32 km per day up the western shore. Considering that *Nassarius pauperatus* has a veliger larva which is planktonic for a minimum of 21 days in the laboratory at 26°C, veligers from Sultana Point should easily be transported 5 km to Coobowie from Sultana Point and would probably be transported much further by the non tidal circulation alone. Tidal transport is also sufficient to transport larvae from Sultana Point to Coobowie and *vice versa*.

In addition to Coobowie, there are other locations in Gulf St Vincent having high indices of hunger and containing relatively small adults, which the pattern of circulation suggests could exchange larvae with locations having lower indices of hunger, larger adults, and relatively low percentages of Twisters. All populations of N. pauperatus on the eastern shore of the Gulf St Vincent have higher indices of hunger and contain smaller adults than those on the western shore, with the exception of Coobowie (Tables 3.4 and 3.24). The inferred pattern of circulation described above suggests that exchange of larvae is possible between shores at the head of the gulf yet the percentage of Twisters increases abruptly from 17.50% at Port Clinton (on the western shore) to 68.75% at Port Wakefield (on the eastern shore) (Table 3.24 and see Figure 3.1).

Although Port Wakefield has a higher index of hunger than Port Gawler, the difference is not statistically significant (Table 3.22). Also the mean shell lengths of adult males and females from Port Wakefield were very similar to those from Port Gawler (by inspection of Table 3.4). I have argued previously that effective reproductive output cannot be reliably determined from such data (Section 4.2.4). However, since values for hunger and adult shell length at Port Wakefield are similar to those at Port Gawler, it is likely that effective reproductive output is also very similar at both locations and thus is not close to zero at Port Wakefield. If so, the abrupt change in the percentage of Twisters between Port Clinton and Port Wakefield is not inconsistent with the pattern of circulation in the gulf. N. pauperatus at Port Clinton and Port Wakefield will both contribute to their local pool of larvae and differentiation between populations between which there is substantial gene flow is not unknown (Ehrlich and Raven, 1969).

4.3.4 Spencer Gulf.

Bullock (1975) measured the temperature and salinity of water in Spencer Gulf in the period from February to May during 1961 to 1966. From this he inferred that the pattern of water flow in the gulf was clockwise, flowing northwards up the western shore and southwards down the eastern shore, with little exchange occurring between water north of $33^{o}45'$ and the rest of the gulf (see Figure 4.2). Bullock also modelled the circulation produced by thermohaline and wind forcing. Unlike Bye (1976), Bullock did not use data of actual wind stress, but simulated winds of varying directions. Bullock's predictions of the direction of circulation agree well with those inferred from other data, but his predictions of velocity should be treated with caution since winds of the velocities used in his model do not usually occur for more than one or two days (Easton, 1978).

Tronson (1974) also modelled the direction of circulation driven by winds of particular directions, and obtained results similar to those of Bullock.

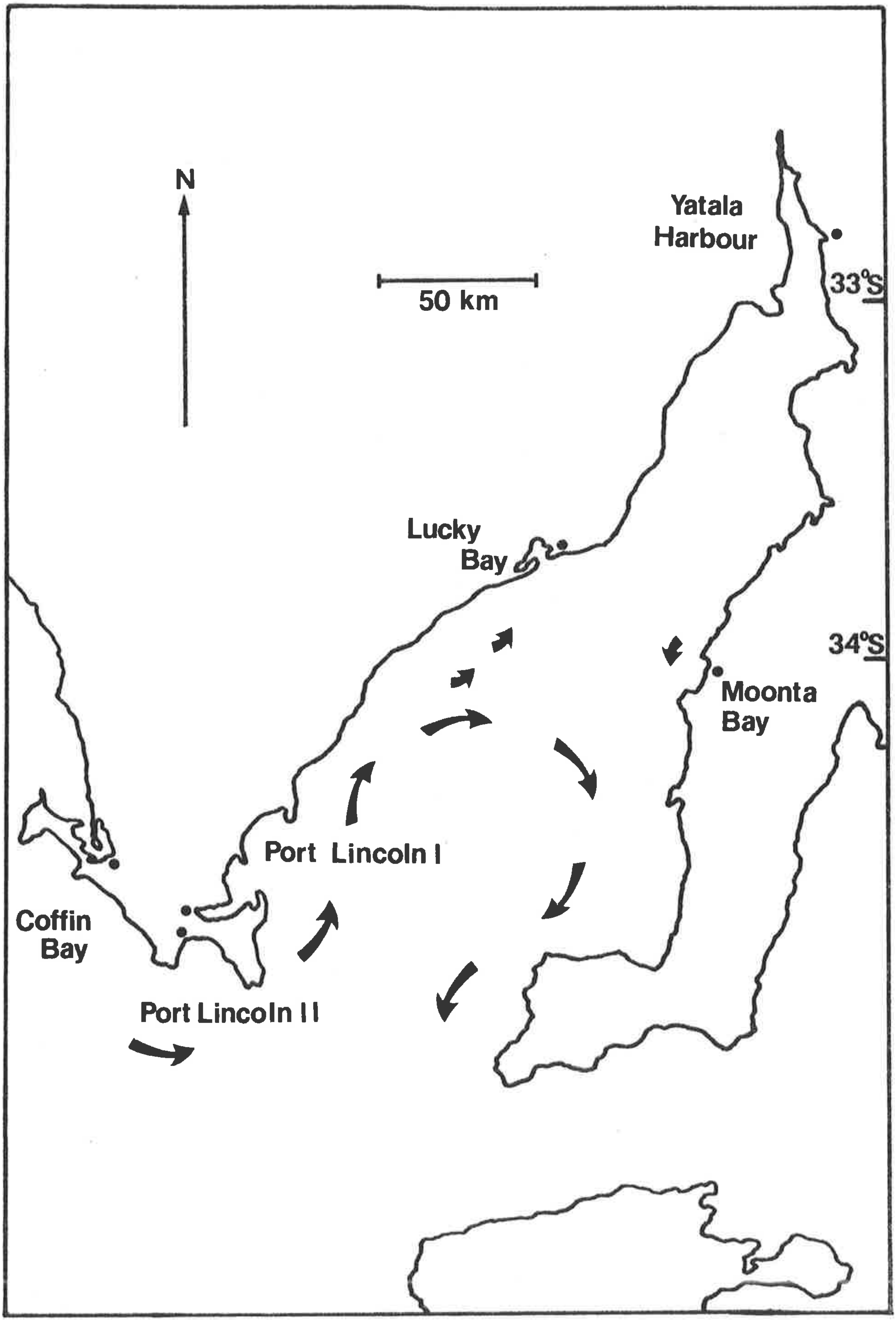
Water movement also occurs in Spencer Gulf due to tides. Bullock (1975) observed that the change in salinity in the northern part of the gulf during a tidal cycle was equivalent to a tidal excursion "of a few kilometres". Easton (1978) modelled the currents resulting from tidal changes and predicted average velocities of about 1 metre per second. This corresponds to a tidal excursion of ± 21.6 kilometres, assuming the time between high and low water to average 6 hours.

4.3.5 Discussion.

The data from Spencer Gulf are as reliable as those from the Gulf St Vincent with respect to the direction of circulation. However, no measurements of current velocities due to either tidal or non tidal circulation have been made and the model predicting non tidal velocities (Bullock, 1975) has been criticised by Easton, (1978).

Bullock's estimate of the tidal excursion of "a few kilometres" in the upper part of Spencer Gulf may not be the same as the excursion

Figure 4.2 Spencer Gulf: The pattern of non tidal circulation which can be inferred from the distribution of sediments and seawater characteristics.


N
50 km
Yatala Harbour
33°S
Lucky Bay
34°S
Moonta Bay
Port Lincoln I
Coffin Bay
Port Lincoln II

in the vicinity of Port Lincoln. Easton's predicted average tidal velocity of 1 metre per second makes transport of larvae to Port Lincoln II from Port Lincoln I quite possible during the 6 hour period from high to low tide.

From the above models and data it appears possible that larvae from Port Lincoln I could be transported to Port Lincoln II, but until there are data for actual velocities and direction of water movement, this transport cannot be assumed.

Despite this, the known pattern of circulation can be used to predict which populations are unlikely to receive larvae from others. From the distribution of density, temperature and salinity recorded by Bullock (1975), larvae from Coffin Bay, Port Lincoln I, Port Lincoln II and Lucky Bay could not be transported to Yatala Harbour since the water in the upper region of the gulf is thought to be separated from the current flowing northwards up the western shore of Spencer Gulf. Also, Moonta Bay appears to be separated from all populations on the western shore by a tongue of high salinity water extending southwards down the eastern coast, but may be able to receive larvae from Yatala Harbour. Therefore, (see Figure 4.2 and Table 3.24) apart from Port Lincoln I and Port Lincoln II, there are no other populations having greatly dissimilar levels of food availability, between which transport of larvae appears possible.

4.4 Discussion.

There is evidence from breeding experiments to suggest that being a Twister is heritable, although data are scanty. The assumption that effective reproductive output decreases as food availability decreases was also shown to be correct. Even though reproductive output (in terms of eggs per female) increased with decreased food availability, predation upon the contents of egg capsules when food was in extremely short supply appeared sufficient to overcome this. The shape of the curve of effective reproductive output versus food availability is not known, since the situation involved only two treatments (high and low food). However, there is no reason to assume that the relation is linear, considering that it is a product of both egg production and predation, both of which increase as food availability decreases. Nevertheless, when food is in very short supply, effective reproductive output appears to be close to zero.

There are sufficient data to suggest that larvae of *Nassarius pauperatus* could be transported to Coobowie from adjacent populations but not enough to decide whether the same could occur at Port Lincoln II, although models of circulation suggest that this is likely.

Characterisation of populations of *N. pauperatus* at and adjacent to Coobowie and Port Lincoln II using electrophoretic techniques need not provide conclusive evidence for or against the assumption that *N. pauperatus* at Coobowie and Port Lincoln II are largely recruited from other populations. Differences in the frequency of protein polymorphisms between populations do not necessarily indicate the extent of gene flow, being also influenced by selective pressures (Gaines, *et al*, 1974, Williams, 1975).

Tentatively accepting all three assumptions on the basis of the evidence examined in this chapter, I shall now propose and test hypotheses about the association between the percentage of Twisters, hunger, and the population density of juvenile *N. pauperatus*.

5. The first hypothesis ; That Twisters are at a selective advantage compared to Non Twisters when _Nassarius pauperatus_ are so hungry that they attempt to eat each other.

5.1 Introduction.

Whether or not a snail is a Twister appears to be non-labile and inherited. The percentage of Twisters is significantly correlated with hunger, the mean shell length of males and females, and the population density of juvenile _Nassarius pauperatus_ (see Chapter 3).

The first hypothesis accounting for these correlations was proposed as a result of observations made in the laboratory whilst differences in the percentage of Twisters between Port Gawler and Port Clinton were being examined (see Section 2.3.4).

In Section 2.3.5, Twisters and Non Twisters were defined. Twisters always twisted after contacting another snail and Non Twisters did not, although both Twisters and Non Twisters often twisted if they were contacted by another snail, or physically damaged on the rear of their foot. Therefore, in a mixed sample of Twisters and Non Twisters, there are four possible outcomes of an encounter involving contact (this having been previously defined in Section 2.3.1 as Snail 1 touching any part of Snail 2 except the latter's shell, with its siphon, tentacle(s) or proboscis). These are repeated from Section 2.3.5:

(1) NTR No twisting by either snail. (Snail 1 must be a Non Twister, Snail 2 could be either a Twister or a Non Twister).

(2) TRBS1 Twisting by Snail 1. (Snail 1 must be a Twister, Snail 2 could be either a Twister or a Non Twister).

(3) TRBS2 Twisting by Snail 2. (Snail 1 must be a Non Twister,

Snail 2 could be either a Twister or a Non Twister).

(4) TRBB Twisting by both snails. (Snail 1 must be a Twister, Snail 2 could be either a Twister or a Non Twister).

In Section 2.3.2 it was found that the frequency of contacts resulting in twisting by Snail 1 did not differ significantly as the number of days without food following collection increased (and this observation was concordant with the finding that individual snails were either Twisters or Non Twisters).

The proportion of contacts resulting in twisting by Snail 2 can be obtained by summing the frequencies of contacts resulting in TRBS2 and TRBB. This has been performed on the data in Table 5.1, which are taken from Table 2.1 - the number of contacts resulting in each of the four possible outcomes described above, by samples of snails in laboratory trays, collected from Port Gawler and Port Clinton.

It can be seen from Table 5.1 that the proportion of contacts resulting in twisting by Snail 2, two days after collection, is significantly lower in the sample from Port Clinton than in the sample from Port Gawler (0.03 compared to 0.26) but that by six days after collection, the proportions have risen in both samples and are not significantly different (0.46 compared to 0.43; see Table 5.1). By eight days after collection, the proportion of contacts resulting in twisting by Snail 2 has declined to 0.26 in the Port Gawler sample, but has remained virtually unchanged at 0.45 in the sample from Port Clinton.

Observations of unstaged contacts in the field at Port Gawler and Port Clinton (from Table 2.3) have been similarly treated in Table 5.2).

Port Gawler

Days without food since collection	NTR + TRBS1		TRBS2 + TRBB		Total
	Frequency of contacts resulting in:				
	n	f	n	f	n
2	26	0.74	9	0.26	35
6	45	0.57	34	0.43	79
8	54	0.74	19	0.26	73

Port Clinton

Days without food since collection	NTR + TRBS1		TRBS2 + TRBB		Total
	Frequency of contacts resulting in:				
	n	f	n	f	n
2	36	0.97	1	0.03	37
6	36	0.54	31	0.46	67
8	35	0.55	29	0.45	64

2 x 2 contingency tables for independence between locations on each day:

Day 2, $\chi^2_1 = 7.96$, $p < .005$

Day 6, $\chi^2_1 = 0.15$, $p > 0.05$

Day 8, $\chi^2_1 = 5.57$, $p < .05$

n : sample size and number in each category

f : relative frequency

Table 5.1 Numbers and frequencies of contacts either resulting in twisting by Snail 2, or not resulting in twisting by Snail 2, in samples from Port Gawler and Port Clinton, kept without food in the laboratory. Data from Table 2.1.

Population	TRBS1 +	NTR	TRBB +	TRBS2	Total
	n	f	n	f	n
Port Gawler	44	0.64	25	0.36	69
Port Clinton	34	0.63	20	0.37	54

2 x 2 contingency table for independence between Port Gawler and Port Clinton : $\chi^2_1 = 0.01$, $p > 0.05$.

n : number in each category, f : relative frequency.

Table 5.2 Number and frequency of contacts occurring in the field at Port Gawler and Port Clinton resulting or not resulting in twisting by Snail 2, (from Table 2.3).

The proportion of contacts resulting in twisting by Snail 2 did not differ significantly between locations (see Table 5.2) being 0.36 and 0.37 at Port Gawler and Port Clinton respectively.

It was noted in Section 2.3.1 that twisting by Snail 2 almost invariably occurred after Snail 1 had contacted Snail 2 on the rear of the latter's foot with its proboscis rather than its siphon or tentacles. When feeding upon carrion, N. pauperatus rasps away pieces of flesh with the radula at the end of its proboscis. Both living and dead snails were often eaten in the laboratory if samples of snails were not fed. It was therefore postulated that when snails extend their proboscis they are attempting to feed, and when doing this to another snail they damage it, causing the latter snail to twist, which terminates contact and prevents further damage. (It is notable that twisting by Snail 2 resembled the avoidance response shown by various gastropods to predators (see Section 2.3.1)). It was also postulated that the reason why no significant differences were observed in the proportion of contacts resulting in twisting by Snail 2 between Port Gawler and Port Clinton in the field (Table 5.2) was because these were unstaged encounters between hungry snails (see Section 3.4.2.2). In contrast, contacts observed in the laboratory were between all the snails from an area of substratum - not just those visibly active on the surface of the sandflat at the time of collection (see Section 2.3.2 for details of how snails were collected); also, contacts between these snails were observed after artificially disturbing them in their laboratory tray. Therefore it was argued that on day 2, a lower proportion of contacts resulted in twisting by Snail 2 in the sample collected from Port Clinton, compared to that from Port Gawler, because fewer snails in the former were hungry enough to attempt to feed upon others. However, by day 6 the proportion of contacts resulting in twisting by Snail 2 had risen in the sample from Port Clinton to a frequency similar to that in the Port Gawler sample, presumably because snails became hungrier. I cannot explain the decline in the frequency of twisting by Snail 2 on day 8 in the Port Gawler sample, except that it may have been a result of snails being so starved that they were incapable of attempting to feed - this was supported by the death of several snails in this sample during the next few days, compared to no deaths in the sample from Port Clinton.

As suggested above, when hunger in a population increases, so should the proportion of snails that will become active and seek food, in response to the appropriate chemical stimulus. If this assumption is true, then the number of N. pauperatus aggregated around items of carrion should be correlated with hunger. (Hunger need not be correlated with the population density of N. pauperatus; see Table 3.2, Lucky Bay). Consider now the two extremes of the above continuum : (1) A population in which there is so much food available that snails need only spend a very small proportion of their time seeking food and (2) The opposite extreme in which so little food is available that snails spend most of their time seeking food.

(1) In a population in which food is plentiful, snails seeking carrion should have a low probability of contacting and being contacted by other hungry snails whilst moving towards food, or when feeding. Therefore twisting by Snail 2 should not occur frequently.

(2) In a population in which food is not plentiful, there should be a high probability that snails seeking carrion will contact, and also be contacted by, other hungry snails.

A snail moving towards a source of food should contact other snails in front of it with its proboscis (because it is hungry) and also be contacted from behind by the proboscises of other snails (because they too are hungry). When finally reaching the item of food a snail should be in tentacular or siphonal contact with snails feeding on each side of it, and also be contacted from behind by the proboscises of other snails. In such situations a Twister which twists as long as it is in contact with other snails should have a much lower probability of being damaged than a Non Twister which only twists after damage commences, and which ceases twisting soon after contact ceases (see Section 2.3.5). Assuming that being damaged incurs an increased probability of dying, it is therefore postulated that Twisters should have a selective advantage over Non Twisters, the magnitude of which should be correlated with the probability of contacting other hungry snails, and hence, hunger of a population. (At this stage the possible disadvantages of being a Twister or the advantages of being a Non Twister when food is not in short supply were not considered).

From the above hypothesis it was predicted that, if Twisters and Non Twisters are caged together without food, Twisters should survive for longer than Non Twisters because Twisters should be less likely to be damaged or cannibalised by other snails. This hypothesis was tested in the field and the laboratory.

5.2 Materials and Methods - Survey of locations for aggregations of *Nassarius pauperatus*.

Initially the sandflat was searched at seven locations for naturally occurring aggregations of *Nassarius pauperatus* around items of carrion. These were found to be very uncommon, presumably because any carrion was rapidly consumed. Also, naturally occurring aggregations of *N. pauperatus* around items of carrion were usually only noticed after snails had actually gathered, thus many interactions between snails moving towards the carrion were not observed. Consequently, between 17 and 21 baits of single Katelysia scalarina, all of a similar size, which had been previously collected from Port Gawler were placed in a non-random pattern, consisting of a grid of points each 5 metres apart, across the zone in which N. pauperatus occurred, at each location. This non-random pattern was chosen instead of a random distribution to eliminate possible variation between locations caused by some baits being so close that the number of snails gathering around these were reduced. Positions of baits were marked with white pipecleaners pushed into the substratum. The length of time a bait remained on the sandflat until completely consumed varied from a few minutes at locations where snails were relatively hungry, to over 20 minutes at locations where snails were less hungry. The maximum number of N. pauperatus aggregated around each bait was recorded. Other species also were infrequently found feeding upon the baits and the numbers and types of these were also recorded.

5.3 Materials and Methods - Testing the first hypothesis.

5.3.1 Treatment of snails prior to all experiments.

Both juvenile and adult Nassarius pauperatus were collected from Port Gawler, South Australia and maintained in the laboratory at 19°C and current day length, in white polystyrene trays of aerated seawater (as described in previous sections) at a maximum density of 50 snails per tray. Contacts between pairs of snails were observed to distinguish between Twisters and Non Twisters and adult snails were sexed (as in Section 2.3.7). Snails of each sex and behaviour were marked on their shells with different coloured spots of fingernail polish and their behaviour reobserved twice to confirm the initial categorisation. Less than 1% of snails were misclassified and those were all Twisters initially typed as Non Twisters, presumably because they did not make contact with the other snail during the initial encounter.

Once scored as either Twisters or Non Twisters and, if adult, males or females, snails were satiated daily on Katelysia scalarina. All the snails used in the same experimental "run" were collected on the same day and both starved and satiated for the same length of time, prior to their use in experiments.

Two treatments were run; these were named "interacting" and "separate". The "interacting" treatment consisted of caging several pairs of Twisters and Non Twisters matched for shell length and sex, together without food. These snails were thus able to contact one another. The "separate" treatment contained several similarly matched pairs of Twisters and Non Twisters caged together without food but these were glued down by the back of their shells (with their opercular openings facing upwards) to a 30 x 30 cm flat sheet of glass, with "Repco Woodhill E.POX.E putty ribbon". If the hypothesis is correct, Twisters would be expected to survive longer than Non Twisters in the "interacting" treatments but not in the "separate" treatments, and if not, there should be no differences in the length of time Twisters and Non Twisters survived within either treatment.

5.3.2 Field experiments.

Port Gawler was chosen for all field experiments because multiple re-examination of cages could not be afforded at sites further away from Adelaide.

Cage frames, dimensions 60 x 60 x 16 cm deep were constructed from 0.625 cm diameter welded steel rod which was then dipped in zinc. These were covered with fibreglass coated zinc flywire, of hole size 2 mm x 2 mm, which was sewn onto the frames with nylon fishing line. A slit of about 55 cm was left in the "top" 60 x 60 cm side of the cage (see Figure 5.1) so that snails could be added and later removed and examined to see if they had died. Cages could be completely closed by sewing this opening shut with nylon fishing line.

Cages were placed in the centre of the zone in which N. pauperatus occurred in the field, sunk 8 cm into the substratum (see Figure 5.1), and filled from the outside by straining substratum through the flywire covered sides, thereby ensuring that food items of greater than 4 cm^2 cross sectional area were excluded. Once filled, the cages were anchored in place with four 2 cm x 2 cm x 1 metre jarrah-wood stakes, buried 92 cm in the substratum and angled inwards over each of the four sides of the cage (Figure 5.1). The cages were then left closed for a minimum of 10 days before snails were added. A density of 10 snails per cage of floor area 3600 cm^2 was chosen, since the mean density of N. pauperatus at Port Gawler was 8.96 snails per 3600 cm^2. Each cage contained 5 Twisters and 5 Non Twisters, as five pairs matched for shell length (usually to within .005 cm, with a maximum difference of .025 cm) and, if adult, sex. All snails were individually numbered. Snails were simply added to the "interacting" treatments and those in the "separate" treatments glued to their glass sheet during the day on which they were placed out in the field, and this plate laid on the substratum inside the cage, with the snails facing upwards. Both types of treatment cages were then sealed by sewing their tops closed with fishing line, and visited upon various days after this to score snails as being alive or dead.

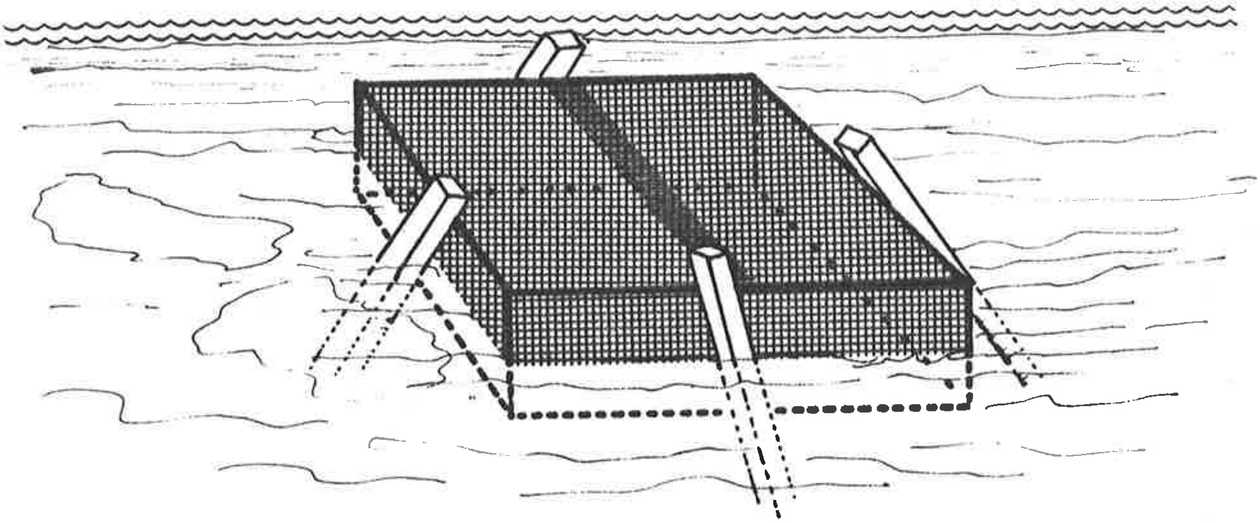


Figure 5.1 Cage type used to test the first hypothesis in the field, shown in position on the sandflat. Cage dimensions were 60 x 60 x 16 cm deep and cage is shown sunk 8 cm into the substratum and anchored in place with four jarrah-wood stakes, dimensions 2 cm x 2 cm x 1 metre.

5.3.3 The pilot experiment.

A pilot experiment, consisting of one replicate of each of the "separate" and "interacting" treatments was run at Port Gawler, commencing on the 21/IV/77 and lasting for 112 days. Cages were visited and snails examined to see if they were dead or alive on 4, 10, 24, 42 and 51 days after the experiment commenced, by which time all the snails in the "interacting" treatment had died. The "separate" treatment was re-examined on days 61 and 112, after which the pilot experiment was terminated, even though none of the snails in this treatment had died.

5.3.4 Experimental runs.

Using the pilot experiment as a guide to time the examination of cages to score snails as dead or alive, the following treatments were run at Port Gawler, in two consecutive runs, each consisting of six separate cages.

Adult females: 4 "interacting" replicates, and 2 "separate" replicates, comprising the first run. Juveniles: 3 "interacting" replicates, 1 "separate" replicate; Adult males: 1 "interacting" replicate and 1 "separate" replicate, comprising the second run. Fewer "separate" treatments were run than initially planned since the results from the pilot experiment showed that all snails in this treatment were still alive on day 112 even though all snails in the "interacting" treatment had died by day 51, suggesting that the "separate" treatment was inappropriate (see Discussion below).

5.3.5 Laboratory experiments.

Laboratory experiments were more extensive, could not be interfered with by others (see below) and since trays could be examined daily, the exact day on which a snail died was known.

A similar experimental design to that of the field experiments was used, except that snails were caged at higher population densities than in the field experiment - of 10 per polystyrene tray of dimensions 54 x 32 x 12 cm deep, filled with seawater to a depth of 9 cm, thus having a total area used by snails of 3276 cm^2, compared to that of 3600 cm^2 in the field cages. However this was still less than the highest mean population density observed in the field and within the standard deviation of population density at Port Gawler (see Table 3.2).

Trays were filled with aerated seawater and kept at a constant temperature of 19°C with photoperiod adjusted to current daylength. Seawater in trays was changed fortnightly. The following treatments were run;

Juveniles: 4 "interacting" replicates and 3 "separate" replicates;
Adult females: 4 "interacting" replicates and 3 "separate" replicates;
Adult males: 2 "interacting" replicates and 2 "separate" replicates.

5.4 Results.

5.4.1 Aggregations of snails.

The mean of the maximum numbers of Nassarius pauperatus gathered around baits of Katelysia scalarina, as well as hunger as assessed by Method 1, relative to Port Gawler (from Table 3.24) appear in Table 5.3 and are graphed in Figure 5.2. It is noteworthy that these data show that Lucky Bay, which had the highest population density of adults + juveniles of all locations sampled (see Table 3.2) had the second lowest mean number of N. pauperatus per aggregation. Furthermore, the mean of the maximum number of snails per bait is significantly correlated with hunger (Spearman r_s = 0.89; $.01 < p < .05$). Only three other species were recorded feeding in the naturally occurring aggregations and around the

Location	Aggregations around *K. scalarina*			Hunger, as assessed by Method 1, relative to Port Gawler	Other species present in aggregations:		
	$\bar{x}$	s_x	n		*Nassarius burchardi*	*Bedeva hanleyi*	*Philyra laevis*
Port Gawler	21.35	5.36	20	0*	0	1(1)	3(1)
Port Clinton	5.40	3.61	20	-17.08*	6(1),2(2),1(3)	4(1)	1(1),1(2)
Rogues Point	3.65	4.73	20	-27.24	1(1),2(3),1(4)	0	0
Stansbury	2.20	3.49	20	-30	5(1),3(2)	0	2(1)
Coobowie	27.12	8.41	17	+40†	0	3(1),2(2)	2(1)
Sultana Point	3.57	3.17	21	-22	4(1),1(3),1(4),1(5), 1(7),1(9),1(12)	0	1(1)
Lucky Bay	2.80	1.05	20	-23	0	0	0

* : mean of 13 values
† : mean of 2 values
$\bar{x}$: mean
s_x : standard deviation
n : sample size

a (b) : a: number of aggregations also containing other species,
b: number of individuals of that species

Spearman rank correlation coefficient between mean number of snails per aggregation and hunger as assessed by Method 1, relative to Port Gawler: $n = 7$, $r_s = 0.89$, $p < 0.05$.

Table 5.3 The mean of the maximum number of *Nassarius pauperatus* in aggregations around baits of *Katelysia scalarina* in the field, with hunger as assessed by Method 1, relative to Port Gawler, and the numbers of other species in aggregations.

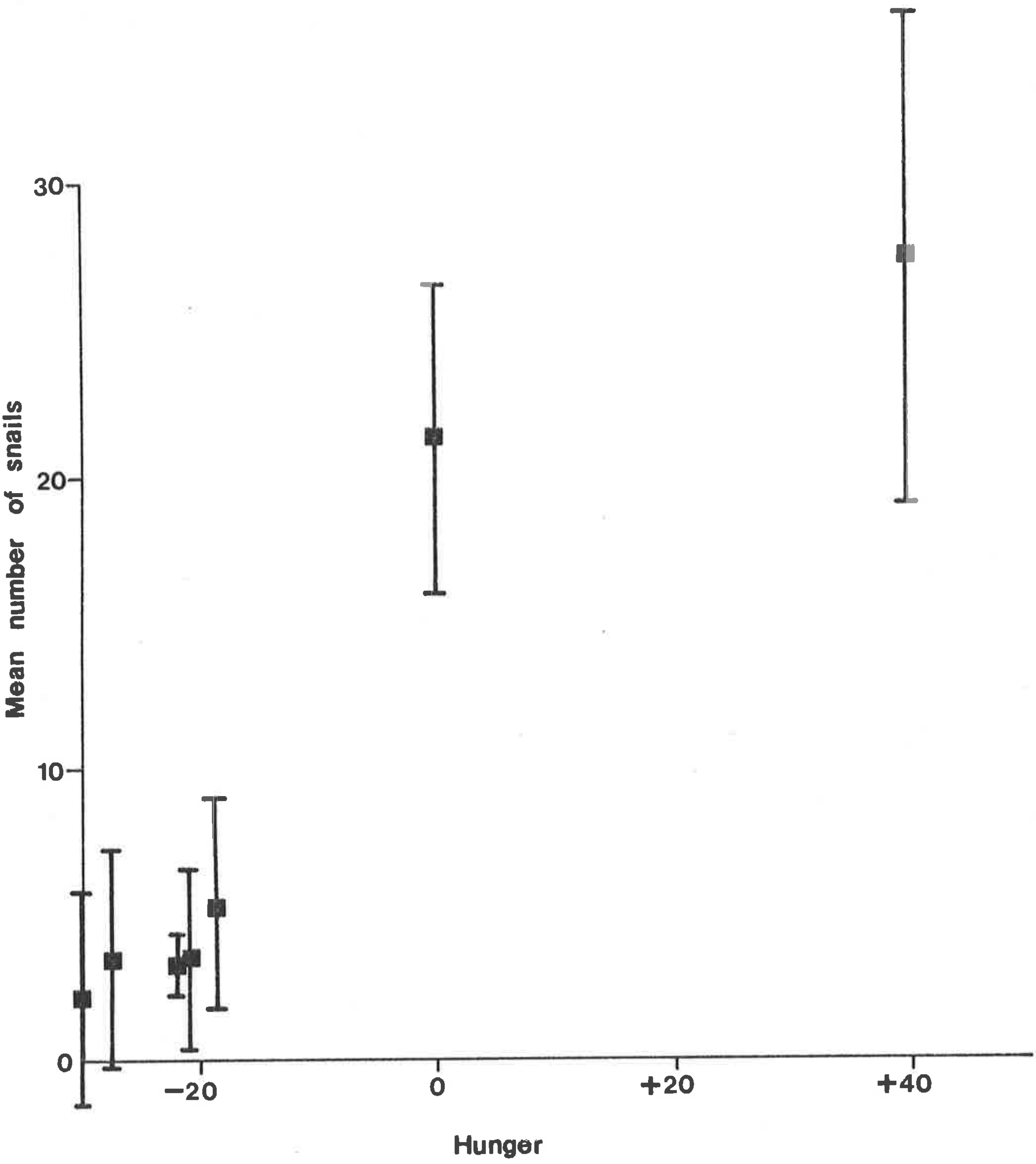


Figure 5.2 The mean of the maximum number of <u>Nassarius pauperatus</u> in aggregations around baits of <u>Katelysia scalarina</u> versus hunger as assessed by Method 1, relative to Port Gawler. Standard deviations are also indicated.

artificial baits of *K. scalarina*; these being the predatory whelk *Bedeva hanleyi*, the snail *Nassarius burchardi* and the crab *Philyra laevis*. However, a large proportion of aggregations at each location consisted only of *N. pauperatus*, and *N. pauperatus* did not twist when contacting any of the above species.

5.4.2 Experimental results.

Results of the pilot experiment are in Table 5.4. Five snails in the "interacting" treatment were dead when the cages were visited 24 days after the experiment was started and all had died by day 51. However, none of the snails in the "separate" treatment had died by day 112 and these were observed extending their proboscises and grazing on algal film that grew on their shells and the glass they were stuck to - this may have been sufficient food to have enabled snails to have survived. Considering the lack of mortality in the "separate" treatment, fewer were used in the subsequent field experiments than were initially intended.

Results from the other field experiments are in Tables 5.5 to 5.7. Interference with some of these cages (presumably by *Homo sapiens* seeking edible crabs) during the spring of 1977 reduced the results obtained in the field and resulted in termination of the field experiments on 11/11/77.

Only one of the snails in any of the "separate" replicates died prior to the experiment being terminated. Without comparison between the "separate" and "interacting" treatments, the results are meaningless. However, in the laboratory experiments, snails in the "separate" treatments did die, permitting comparison between both types of treatments.

Results from the laboratory experiment are in Tables 5.8 to 5.10. Treatments were continued until all snails in both treatments

	Twisters			Non Twisters	
Sex	Shell length (cm)	Day on which found dead	Sex	Shell length (cm)	Day on which found dead
		"interacting"			
M	1.525	24	M	1.520	24
F	1.265	24	F	1.270	24
F	1.170	42	F	1.175	51
M	1.630	42	M	1.635	24
F	1.050	51	F	1.050	42
		"separate"			
M	1.520	*	M	1.520	*
F	1.265	*	F	1.265	*
F	1.180	*	F	1.170	*
M	1.630	*	M	1.615	*
F	1.050	*	F	1.050	*

* All snails still alive on day 112.

Table 5.4 The pilot experiment. Shell length, behaviour, sex, and day upon which snails found dead. All snails in the "interacting" treatment had died by day 51, but all in the "separate" treatment were still alive on day 112, upon which the experiment was terminated.

Twisters Shell length (cm)	Twisters Day on which found dead	Non Twisters Shell length (cm)	Non Twisters Day on which found dead
	"interacting" : F1		
1.235	36	1.235	36
1.340	54	1.340	29
1.065	36	1.060	71
1.045	54	1.045	54
1.265	54	1.270	54
	"interacting" : F2		
1.610	29	1.605	29
1.575	29	1.575	29
1.440	54	1.445	29
1.515	36	1.520	54
1.320	36	1.320	36
	"interacting" : F3		
1.265	36	1.245	71
1.230	71	1.230	54
1.100	71	1.105	77
1.475	71	1.470	29
1.290	36	1.290	71

("Interacting" treatment F4 was broken into after day 29).

One tailed, Wilcoxon matched-pairs signed-ranks test, comparing time elapsing before Twisters and Non Twisters were discovered dead : $n = 9$, $T = 20$, $p > 0.05$.

Table 5.5(a) Field experiments in cages without food: Adult females in "interacting" treatments, shell length, behaviour, and day upon which snails first discovered dead.

Twisters Shell length (cm)	Day on which found dead	Non Twisters Shell length (cm)	Day on which found dead
	"separate" : F5		
1.235	*	1.235	*
1.340	*	1.345	*
1.060	*	1.065	*
1.055	*	1.050	*
1.400	*	1.400	*
	"separate" : F6		
1.445	*	1.450	*
1.520	*	1.525	*
1.575	*	1.570	6
1.605	*	1.605	*
1.305	*	1.305	*

Table 5.5(b) Field experiment in cages without food: Adult females in "separate" treatments, shell length and behaviour.
* Snails were still alive upon day 77, after which experiment was terminated.

Twisters Shell length (cm)	 Day on which found dead	Non Twisters Shell length (cm)	 Day on which found dead
"interacting" : F7			
0.990	40	0.980	31
0.845	31	0.845	31
0.990	31	0.990	31
1.065	40	1.060	40
1.165	40	1.165	61
"interacting" : F8			
0.945	10	0.950	40
0.990	40	0.995	40
1.075	31	1.075	40
1.130	31	1.130	10
1.175	10	1.175	40
"interacting" : F9			
1.265	61	1.265	40
0.965	31	0.950	31
1.100	40	1.105	40
0.870	31	0.865	31
1.090	40	1.090	31

One tailed, Wilcoxon matched-pairs signed-ranks test comparing time elapsing before Twisters and Non Twisters were discovered dead: $n = 8$, T (+ ranks) = 14.00, T (- ranks) = 22.00, $p > 0.05$ in both cases.

Table 5.6(a) Field experiments in cages without food: Juvenile snails in "interacting" treatments, shell length, behaviour and day upon which snails first discovered dead.

Twisters		Non Twisters	
Shell length (cm)	Day on which found dead	Shell length (cm)	Day on which found dead
"separate" : F10			
1.230	*	1.235	*
1.175	*	1.175	*
0.920	*	0.920	*
1.020	*	1.020	*
0.960	*	0.975	*

Table 5.6(b) Field experiments in cages without food: Juvenile snails in "separate" treatment F10

* All snails were alive on day 40, after which cage was broken into.

Twisters Shell length (cm)	 Day on which found dead	Non Twisters Shell length (cm)	 Day on which found dead
"interacting" : F11			
1.575	40	1.575	*
1.605	40	1.610	4
1.450	40	1.440	31
1.525	40	1.520	31
1.450	31	1.440	40

* cage thrown about by crabbers after day 40, and snails emptied out.

Twisters Shell length (cm)	 Day on which found dead	Non Twisters Shell length (cm)	 Day on which found dead
"separate" : F12			
1.600	*	1.595	*
1.580	*	1.580	*
1.450	*	1.445	*
1.445	*	1.445	*
1.520	*	1.520	*

* alive at day 40. Snails removed after day 40 by crabbers.

Table 5.7 Field experiments in cages without food: adult males in both "interacting" and "separate" treatments, shell length, behaviour and day upon which snails first discovered dead. Cages were broken into or otherwise disturbed after day 40. (Sample sizes in "interacting" treatment are too small to compare statistically).

Twisters		Non Twisters	
Shell length (cm)	Day snail died	Shell length (cm)	Day snail died
"interacting" : L1			
0.945	58	0.940	32
1.035	24	1.040	27
1.050	47	1.045	43
1.135	46	1.140	59
1.235	32	1.240	21
"interacting" : L2			
1.060	62	1.060	76
1.180	61	1.180	82
1.350	81	1.345	60
1.255	97	1.260	84
0.985	91	0.990	81
"interacting" : L3			
1.220	31	1.220	49
1.130	39	1.130	30
1.160	44	1.155	46
1.160	27	1.165	42
0.920	35	0.920	55
"interacting" : L4			
0.930	23	0.930	16
0.825	37	0.825	41
0.810	32	0.805	28
0.750	12	0.745	28
0.740	45	0.740	37

One tailed, Wilcoxon matched-pairs signed-ranks test comparison between days elapsing before Twisters and Non Twisters died:

$n = 20$, T (+ ranks) = 98, $p > 0.05$

$n = 20$, T (- ranks) = 112, $p > 0.05$

Table 5.8(a) Laboratory experiments in trays without food: juvenile snails in "interacting" treatments, behaviour, length and days elapsing before snails died.

Twisters		Non Twisters	
Shell length (cm)	Day snail died	Shell length (cm)	Day snail died
"separate" : L5			
0.925	31	0.915	6
0.825	33	0.810	34
0.785	28	0.785	20
0.855	9	0.855	14
0.915	13	0.915	33
"separate" : L6			
0.855	19	0.860	11
0.850	33	0.850	8
0.895	28	0.900	41
0.790	33	0.790	8
1.225	27	1.225	10
"separate" : L7			
0.945	22	0.945	13
0.945	16	0.950	13
0.890	12	0.890	32
1.140	29	1.140	46
1.060	5	1.060	26

One tailed, Wilcoxon matched-pairs signed-ranks test comparison between days elapsing before Twisters and Non Twisters died:

$n = 15$, $T = 52.5$, $p > 0.05$.

Table 5.8(b) Laboratory experiments, in trays without food: juvenile snails in "separate" treatments, behaviour, length and days elapsing before snails died.

Twisters		Non Twisters	
Shell length (cm)	Day snail died	Shell length (cm)	Day snail died
"interacting" : L8			
1.460	22	1.460	34
1.405	26	1.400	78
1.415	13	1.415	59
1.485	27	1.485	39
1.410	30	1.400	48
"interacting" : L9			
1.490	55	1.490	33
1.520	28	1.520	83
1.525	11	1.525	47
1.240	22	1.240	46
1.565	20	1.565	43
"interacting" : L10			
1.250	80	1.250	42
1.400	46	1.400	12
1.235	23	1.235	55
1.285	16	1.280	7
1.455	80	1.450	26
"interacting" : L11			
1.610	20	1.610	38
1.535	19	1.530	33
1.470	7	1.470	50
1.435	48	1.435	6
1.585	65	1.585	67

One tailed Wilcoxon matched-pairs signed-ranks test comparison between days elapsing before Twisters and Non Twisters died:
$n = 20$, $T = 70$, $p > 0.05$.

Table 5.9(a) Laboratory experiments in trays without food: adult female snails in "interacting" treatments, behaviour, length and days elapsing before snails died.

Twisters		Non Twisters	
Shell length (cm)	Day snail died	Shell length (cm)	Day snail died
"separate" : L12			
1.495	48	1.495	36
1.410	67	1.410	85
1.480	38	1.485	29
1.520	42	1.520	11
1.415	43	1.410	45
"separate" : L13			
1.220	33	1.220	49
1.510	47	1.510	9
1.235	8	1.235	5
1.435	8	1.435	51
1.195	9	1.190	80
"separate" : L14			
1.585	64	1.580	75
1.345	33	1.345	15
1.470	54	1.470	41
1.460	58	1.465	22
1.330	87	1.330	36

One tailed Wilcoxon matched-pairs signed-ranks test comparison between days elapsing before Twisters and Non Twisters died:
$n = 15$, $T = 48.50$, $p > 0.05$.

Table 5.9(b) Laboratory experiment in trays without food: adult female snails in "separate" treatments, behaviour, length and days elapsing before snails died.

Twisters Shell length (cm)	 Day snail died	Non Twisters Shell length (cm)	 Day snail died
	"interacting" : L15		
1.300	40	1.300	12
1.500	16	1.500	31
1.265	51	1.260	37
1.585	19	1.595	20
1.600	78	1.600	55
	"interacting" : L16		
1.560	13	1.550	38
1.560	89	1.555	11
1.445	27	1.445	40
1.480	53	1.480	38
1.670	8	1.670	47

One tailed Wilcoxon matched-pairs signed-ranks test comparison between days elapsing before Twisters and Non Twisters died:
$n = 10$, $T = 23.50$, $p > 0.05$.

Table 5.10(a) Laboratory experiments in trays without food: adult male snails in "interacting" treatments, behaviour, length and days elapsing before snails died.

Twisters Shell length (cm)	 Day snail died	Non Twisters Shell length (cm)	 Day snail died
	"separate" : L17		
1.615	47	1.610	20
1.610	7	1.610	58
1.565	36	1.565	71
1.485	16	1.485	61
1.525	78	1.525	52
	"separate" : L18		
1.475	75	1.475	68
1.300	14	1.300	75
1.580	15	1.580	87
1.430	47	1.435	59
1.425	80	1.425	22

One tailed Wilcoxon matched-pairs signed-ranks test comparison between days elapsing before Twisters and Non Twisters died:

$n = 10$, $T = 16$, $p > 0.05$.

Table 5.10(b) Laboratory experiments in trays without food: adult male snails in "separate" treatments; behaviour, length and days elapsing before snails died.

had died, and in contrast it can be seen by inspection that snails in both treatments died at similar times. It was noted that algae did not grow on the shells of snails in the "separate" treatments nor to the glass plates to which they were affixed, in laboratory experiments. One tailed, Wilcoxon matched-pairs signed-ranks tests were used to compare the time taken to die between the matched pairs of Twisters and Non Twisters within both the "separate" and "interacting" treatments. No significant difference was found between the time elapsing before Twisters or Non Twisters died within either the "separate" or "interacting" treatments (see Tables 5.8 to 5.10). Similarly, one tailed Wilcoxon matched-pairs signed-ranks test comparisons between the time elapsing before snails were discovered dead in "interacting" treatments in the field did not reveal any significant differences between Twisters and Non Twisters (see Tables 5.5 and 5.6).

5.5 Discussion.

Results from the survey of seven locations for the maximum numbers of Nassarius pauperatus in aggregations around baits of Katelysia scalarina supported the assumption that as hunger increases so does the probability of contacting other hungry snails, in that the mean number of snails in aggregations around baits was correlated with hunger. These results also confirmed that the number of snails in aggregations around baits was primarily a function of hunger, and that the latter was usually, but not always, predictable from population density.

Results from the field experiments were inconclusive since none of the snails in the "separate" treatments died. In the laboratory there were no significant differences between the time elapsing before Twisters and Non Twisters died within either the "separate" or "interacting" treatments. If there had been a difference between Twisters and Non Twisters in the "interacting" treatments, then differences between Twisters and Non Twisters in the "separate" treatments would have been of interest, to test whether interaction between snails had been responsible for this.

Results from the laboratory experiments did not support the prediction that Twisters should survive for longer than Non Twisters when snails are so hungry that they attempt to eat each other. Even though contacts resulting in twisting by both Snail 1 and Snail 2 were observed occurring in field cages and in the laboratory, Twisters did not survive for significantly longer than Non Twisters in the "interacting" or the "separate" treatments. Therefore the observed correlation between the percentage of Twisters and hunger cannot be accounted for by the hypothesis that Twisters are selected when snails are hungry because they are less likely than Non Twisters to be damaged or eaten by others.

Considering that most cases of snails twisting were observed both in the field and the laboratory when carrion was made available, a second hypothesis was proposed to explain the correlation between the percentage of Twisters and hunger. This is described and tested in the following chapter.

6. The second and third hypotheses; That Twisters are better than Non Twisters at competing for space to feed, and that Twisters are more conspicuous to predators than Non Twisters.

6.1 The second hypothesis; That Twisters are better than Non Twisters at competing for space to feed.

The hypothesis that Twisters are better than Non Twisters at competing for space to feed was proposed as a result of observing the behaviour of *Nassarius pauperatus* in aggregations around naturally occurring items of carrion and baits of *Katelysia scalarina* in the field. The terms "competition" and "compete" are used in the sense defined by Milne (1961) (and similarly by Birch in an earlier paper (Birch, 1957)) as

> "the endeavour of two (or more) animals to gain the same particular thing, or to gain the measure each wants from the supply of a thing when that supply is not sufficient for both (or all)".

In the previous Chapter it was found that Twisters did not appear to have a selective advantage compared to Non Twisters, in terms of being damaged by other snails when they were hungry. Observations made in the field revealed that many encounters involving contact and twisting occurred between *N. pauperatus* moving towards, or gathered around, items of carrion. It was also noted that in some populations there were often so many snails gathered around a piece of carrion that some of those on the outside of the aggregation could not reach the carrion with their proboscises and hence could not feed. The data from Table 5.3, of the mean number of snails gathered around artificial baits are repeated in Table 6.1, along with observations of whether or not all snails present were able to feed. Other populations were also surveyed for whether or not all the snails gathered around naturally occurring items of carrion were able to feed, and these results appear in Table 6.2, along with the names and

Date sampled	Location	Snails per aggregation $\bar{x}$	s_x	n	Description of aggregation	Hunger, relative to Port Gawler
November 1977	Port Gawler	21.35	5.36	20	Not all snails capable of feeding	0
" "	Port Clinton	5.40	3.61	20	All snails feeding	-17.08
" "	Lucky Bay	2.80	1.05	20	All snails feeding	-23
" "	Rogues Point	3.65	4.73	20	All snails feeding	-27.24
" "	Stansbury	2.20	3.49	20	All snails feeding	-30
" "	Coobowie	27.12	8.41	17	Not all snails capable of feeding	+40
" "	Sultana Point	3.57	3.17	21	All snails feeding	-22

Table 6.1 The mean maximum number of snails present per bait of Katelysia scalarina and whether or not all the snails present in an aggregation are capable of feeding. Populations were all sampled during November 1977. Data from Table 5.3.

($\bar{x}$: mean, s_x : standard deviation, n : sample size)

Location	Number of natural aggregations examined	Those with All snails feeding	Those with Some snails unable to feed	Those with other species *N. burchardi*	Those with other species *P. laevis*	Those with other species *B. hanleyi*
Onkaparinga	3	0	3	0	0	0
Port Gawler	11	2	9	1 (5)	3 (1)	0
Middle Beach	1	0	1	0	0	0
Port Clinton	17	17	0	1(2), 1(3)	0	6(1), 2(1)
Stansbury	2	2	0	0	0	1 (2)
Coobowie	1	0	1	0	1 (1)	0
Sultana Point	4	4	0	1(4),1(7),1(8)	0	0
Moonta Bay	2	0	2	1 (1)	0	0

a (b) : a : number of aggregations containing this species, b : numbers of this species.

Table 6.2 Naturally occurring aggregations of *Nassarius pauperatus* around items of carrion in the field. Whether all snails in an aggregation were capable of feeding is indicated, plus the number of aggregations containing other species, and the types and numbers of these (e.g. 2(3) indicates two aggregations containing three of the species in question). Populations were sampled on several dates during late 1977 and early 1978. Other species found in aggregations were the crab *Philyra laevis*, *Nassarius burchardi* and the whelk, *Bedeva hanleyi*.

numbers of all other species found in the aggregations.

It can be seen from Table 6.2 that few naturally occurring aggregations contained species other than Nassarius pauperatus, and that other species, when present, were usually in low numbers. This was also the case in aggregations of N. pauperatus around baits of Katelysia scalarina (see Table 5.3).

Observations of naturally occurring aggregations around carrion and those around baits of K. scalarina showed that at locations that were relatively hungry (see Table 3.24) the number of N. pauperatus in aggregations was often so large that there was insufficient space around the food for all snails to feed. Aggregations of N. pauperatus around algae were never observed naturally in the field, presumably because algae were distributed as a film on the surface of the sandflat. However, aggregations of snails did occur both in the field and the laboratory around pieces of cotton wool soaked with algal cells that N. pauperatus was known to feed on (see Appendix 1). Therefore, food that is available as a discrete "patch" was defined as a "point source" of food. In the field, carrion was usually available as point sources, but algae were not.

It is easy to visualise how competition for space to feed may occur around point sources when food for N. pauperatus is in short supply. If food is not in short supply (that is, there is more than enough available for all the N. pauperatus in a specific area) it is unlikely that there will be competition for space to feed, even if the food is distributed as point sources. If food is in short supply (that is, there is insufficient food available in a given area for all the snails) and some or all is distributed as point sources, there will be competition for space to feed around these. However, if food is in short supply but not distributed as point sources, there will not be competition for space to feed.

Whilst aggregations of snails around point sources of carrion were being examined and counted in the field it was noticed that some of the snails unable to feed on the outside of an aggregation appeared, by twisting, to be able to force their way into the aggregation and feed. It was therefore postulated that a Twister, approaching an aggregation of snails around a point source of food at which there was no more space available for any more snails to feed may, by twisting, be able to force its way into the group and feed. A Non Twister would not usually twist when reaching snails aggregated around food and even if another snail approached and contacted a Non Twister from behind, causing the latter to twist, the Non Twister would not necessarily force its way into the group of snails gathered around the carrion (see Section 2.3.5). This hypothesis can account for the correlation between hunger and the percentage of Twisters in a population, assuming that as snails become hungrier, then so does the probability of their dying of hunger increase, and that carrion is an important source of food in the field. There was evidence for this assumption, namely that both juvenile and adult *N. pauperatus* from Coobowie (the hungriest population sampled) often died whilst being transported to Adelaide, but deaths could be prevented by feeding the snails *K. scalarina* as soon as they were collected. Furthermore, plant material is generally a poor source of nitrogen, compared to animal tissue (White, 1978) therefore *N. pauperatus* may be surrounded by a lot of algal food of poor quality, with carrion being an important component of their diet for survival, growth and reproduction. Therefore these snails may face a relative shortage of nitrogenous material available from plant tissue, and in some populations, an absolute shortage of nitrogenous material available from animal carrion (see Andrewartha and Browning, 1961).

From the hypothesis that Twisters were better than Non Twisters at competing for space to feed, several predictions were made. These were:

(a) That juvenile Twisters should grow at a faster rate than juvenile Non Twisters when food is in short supply and distributed as point sources, but that there should be no difference in the rates of growth of juvenile Twisters and Non Twisters when food is not in short supply, or not distributed as point sources.

(b) That both adult and juvenile Non Twisters should have a greater probability of dying than Twisters when food is in short supply and distributed as point sources, but there should be no differences in probability of dying between Twisters and Non Twisters when food is not in short supply, or not distributed as point sources.

(c) That Non Twisters should be hungrier than Twisters in populations in which food is in short supply and distributed as point sources, but there should be no differences in hunger between Twisters and Non Twisters when food is not in short supply or not distributed as point sources.

(d) That Twisters should have a greater probability than Non Twisters of being able to displace other snails from point sources of food when there is limited space available for snails to feed.

These predictions were tested in the laboratory and the field.

6.2 Growth, mortality and behaviour of *Nassarius pauperatus* when food is in short supply and distributed as point sources.

6.2.1 Introduction.

This experiment compared the growth and mortality of Twisters and Non Twisters when offered less food than they would normally consume, as a point source around which they had to compete for space to feed. Controls were run in which snails were offered the same amount of food as in the above treatment, but did not have to compete for space to feed.

6.2.2 Materials and Methods.

6.2.2.1 Treatment of snails prior to the experiment.

Prior to their use in the experiment, snails were treated identically to those used in the experiments described in Chapter 5. Both adult and juvenile *Nassarius pauperatus* were collected from Port Gawler and kept in covered trays of aerated seawater at 19°C and current daylength without food. Snails were observed contacting each other and the shells of Twisters and Non Twisters marked with differently coloured spots of fingernail polish. Adult snails were also sexed (as described previously) and marked with fingernail polish. The behaviour of all snails was checked twice within seven days following collection and all snails were then satiated daily on *Katelysia scalarina*, until used. All snails used in the same experimental "run" were collected on the same day and were starved and satiated for the same number of days before being used in the experiment.

6.2.2.2 Choice of food.

A food source was required which could be readily obtained, varied little in quality and did not change weight or disperse when immersed in seawater. The adductor muscle of *Katelysia scalarina* was chosen, since *Nassarius pauperatus* was known to feed upon this tissue, it could be easily isolated from frozen *K. scalarina* and was a relatively dense, seemingly homogeneous and well defined tissue (by comparison with other tissues of *K. scalarina*) which was easily handled and weighed. Also the adductor muscles were attached to pieces of *K. scalarina* shell, which prevented snails from moving the source of food around, thus making these ideal as point sources, around which snails had to compete for space to feed.

6.2.2.3 Experimental design.

Feeding experiments were all performed in the laboratory since it was impossible to quantify the weight of food offered to snails in the field, or to visit cages frequently enough to perform the experiments adequately. The following design was used: A treatment named "*ad lib*" and consisting of five numbered pairs of a Twister and Non Twister, matched for shell length to within 0.015 cm, was offered *ad lib* *K. scalarina* adductor muscle every day, as three separate pieces, thus ensuring that individuals did not have to compete for space to feed and that they had food in excess of their requirements. The pieces of shell to which the muscle was attached were scraped clean of all flesh except for one of the two adductor muscles, rinsed in seawater, blotted on a "Kimberly Clark kimwipe" tissue and weighed to the nearest 0.0001 gram, using a "Mettler" balance. After all snails had ceased feeding (usually in less than 15 minutes) the pieces of shell and remaining muscle were removed from the tray, blotted and re-weighed to establish, by subtraction, the weight of muscle consumed. To assess how accurate this procedure was, twenty pieces of muscle attached to their shells were scraped, rinsed in seawater, blotted, weighed, immersed for 20 minutes in seawater, then re-blotted and re-weighed.

The weight of adductor muscle consumed daily by the "*ad lib*" group was recorded. This was used as a standard to feed half this weight, on average, to the experimental treatments. Exactly half the weight of muscle consumed by the "*ad lib*" treatment could not be offered to the other experimental treatments since the muscle was attached to pieces of shell, so the weight of muscle required was estimated by eye and the exact weight consumed by the snails in a treatment found after the snails had fed, by subtraction, as above. Since snails in the experimental treatments were never offered exactly half the weight of muscle consumed by the "ad lib" group, balance sheets for each treatment were kept. The daily weight of muscle consumed by each replicate was recorded and this was adjusted as closely as possible to half that consumed by the "ad lib" group, by increasing or decreasing the weight offered the next day, so that on average over several days, snails were fed half the weight consumed by the "ad lib" group.

It was predicted that Twisters should grow faster and survive for longer than Non Twisters, when food is in short supply and snails are forced to compete for space to feed. However, Twisters may grow faster than Non Twisters for other reasons when food is in short supply. Therefore the following treatment termed "spread evenly" was used as a control. In the "spread evenly" treatment, snails were offered the same amount of adductor muscle on average as the "point source" treatments. However, the food was offered every seven days, as several pieces, so there was no competition for space to feed in the "spread evenly" treatment.

6.2.2.4 Limitations of the experimental design.

There were two limitations of the experimental design. Firstly, as soon as more than two or three snails died in a treatment there was usually sufficient space around the food for all snails to feed, so the "point source" treatments ceased to be effective. As a result, all of the snails did not die in any of the "point source" treatments, thus limiting the comparisons that could be made of the time elapsing before a snail died. Secondly, it was found that snails in the "spread evenly" treatments did not die, even after several months, again preventing comparisons on the basis of days elapsing before death, both between and within treatments.

6.2.2.5 Replicates, and snails per treatment.

Nine replicates of each of the "ad lib", "point source" and "spread evenly" treatments were run, consisting of three sets of adult and six sets of juvenile *N. pauperatus*. Each replicate contained 10 snails, being 5 numbered pairs of a Twister and Non Twister matched for shell length usually to within 0.010 cm, and never more than 0.020 cm. The shell lengths of the matched pairs were also matched between each of the 9 sets of treatments (e.g. replicates L1 of the "ad lib", "spread evenly" and "point source" treatments contained 5 pairs of Twisters and Non Twisters and these were matched for shell length to the nearest 0.020 cm between treatments as well). Each "point source" and "spread evenly" replicate was offered half the weight, on average, of adductor muscle consumed by their particular reference "ad lib" replicate. Freshly isolated pieces of muscle were offered to all treatments. Treatments were examined daily to see if individuals were dead or alive and the shell lengths of snails in the six juvenile treatments were measured weekly to the nearest 0.005 cm, using NSK vernier calipers, as described previously. Experiments commenced in November 1977 and ran until March 1978. It was ensured that juvenile snails of the same year-class were used, by collecting only those that were larger than 0.8 cm (see Section 3.2.2).

6.2.2.6 Behaviour of Twisters and Non Twisters while competing for space to feed.

Snails in the "point source" treatments were observed as they competed for space around food. If a snail on the outside of an aggregation successfully forced its way into the aggregation and forced another snail out, this was taken to indicate that space to feed was limited. Whether or not the displacer was a Twister or a Non Twister and its number was recorded, enabling its size to be known. Similarly, the behaviour and size of the snail displaced from the aggregation was also recorded. To ensure that size differences between Twisters and Non Twisters did not have an effect, only adults, or juvenile replicates during the first week of the experiment were observed. The same individual was often recorded as being a displacer or as being displaced more than once.

6.2.3 Results.

6.2.3.1 Accuracy of the re-weighing procedure.

The mean difference in weight of Katelysia scalarina muscle before and after immersion in seawater for 20 minutes was +0.0004 grams and the standard deviation of this difference (from 20 samples) was 0.0062 grams. The weight of K. scalarina muscle consumed by the nine "ad lib" treatments ranged from means of 0.0385 gm per day to 0.1511 gm per day between treatments therefore the error associated with re-weighing was considered of little importance, compared to the magnitude of the quantities being measured.

6.2.3.2 Accuracy of the "point source" and "spread evenly" feedings.

To illustrate the accuracy of the method of feeding the "point source" and "spread evenly" treatments, the mean weight of K. scalarina consumed by the juvenile L1 "point source" and juvenile L1 "spread evenly" treatments, compared to the weight of muscle consumed by the juvenile L1 "ad lib" treatment are in Table 6.3. It can be seen that the "point source" and "spread evenly" treatments received and consumed close to half the weight of adductor muscle on average

Treatment	Weight of *Katelysia scalarina* muscle consumed, in grams		
	$\bar{x}$	s_x	n
"*ad lib*"	0.0703	0.0615	30
"point source"	0.0320	0.0284	30
"spread evenly"	0.0343	0.0843	30

Table 6.3 Weight of *Katelysia scalarina* muscle consumed by the L1 "*ad lib*", "point source" and "spread evenly" treatments. The standard deviation of the mean weight of muscle consumed by the "spread evenly" treatment is high due to snails not being fed for six days out of seven.

$\bar{x}$: mean, s_x : standard deviation, n : sample size

consumed by their equivalent "ad lib" treatment.

6.2.3.3 Analysis of data.

In Section 3.4.2.6 it was observed that the growth of juvenile Nassarius pauperatus did not appear to fit the von Bertalanffy growth curve, or any other mathematical models of growth, when snails were not fed ad lib K. scalarina. Also the frequency distribution of juvenile shell lengths of N. pauperatus from the same year class does not appear to be normal (see Smith, 1975). Therefore, the following analysis of growth was carried out.

At the start of the experiment, snails of the same matched pair differed by a maximum of 0.015 cm. Attempts were made to equalise these differences within replicates of each treatment by choosing snails of other pairs which varied in length in the opposite direction.

An estimate of the difference in rate of growth between snails of a matched pair, which is independent of the form of the growth curve, is the magnitude and direction of the difference in shell length between snails of a matched pair at the conclusion of an experimental treatment. Non parametric Wilcoxon matched-pairs signed-ranks tests were used to compare the lengths of matched pairs of Twisters and Non Twisters both before and after the experiment. To make as many comparisons as possible, and to avoid "point source" treatments which had been transformed into "ad lib" treatments after some snails had died, comparisons of shell lengths were made on the last occasion all snails were measured whilst still alive. This varied between replicates of the "point source" treatment. None of the snails in the "ad lib" replicates died and only one in all of the "spread evenly" replicates and this occurred several weeks after three snails had died in the equivalent "point source" replicate. The length of time elapsing before the first snails died varied from 25 to 44 days, between "point source" replicates.

6.2.3.4 Comparisons of growth.

Results of the growth experiments appear in Tables 6.4 ("ad lib" treatments); 6.5 ("spread evenly" treatments); and 6.6 ("point source" treatments). The hypothesis being tested predicted that Twisters should grow faster than Non Twisters in the "point source" treatments so one tailed probabilities were used for all analyses. Results (see Tables 6.4-6.6) did not lead to rejection of the hypothesis. Twisters were significantly larger than their Non Twister counterparts at the conclusion of the "point source" experiment ($p < .001$), but there were no significant differences in length between matched pairs of Twisters and Non Twisters at the commencement of any of the three treatments ($p > 0.05$), nor at the conclusion of the "ad lib" ($p > 0.05$) or "spread evenly" ($p > 0.05$) treatments, even though these ended at the same time as their equivalent "point source" treatments.

Data for the mortality of juvenile snails in the six replicates of the "point source" treatment are in Table 6.7, and for adult snails in the three replicates of the "point source" treatment in Table 6.8. Significantly more adult ($p < 0.05$) and juvenile ($p < 0.005$) Non Twisters than Twisters died in this treatment, therefore results are again in accordance with the prediction that Non Twisters should die sooner than Twisters, when forced to compete for space to feed around a point source. None of the snails in any of the "spread evenly" treatments died, so whilst comparisons on the basis of the number of days elapsing before a snail died cannot be made between treatments, these results do suggest that Twisters do not have a selective advantage compared to Non Twisters when food is in short supply, unless they have to compete for space to feed. In

Treatment number	Before		After	
	Twisters	Non Twisters	Twisters	Non Twisters
L1	0.985	0.980	1.055	1.060
	0.975	0.975	1.125	1.105
	1.325	1.325	1.370	1.395
	1.000	1.000	1.220	1.165
	1.015	1.115	1.130	1.085
L2	1.120	1.125	1.295	1.285
	1.155	1.150	1.275	1.335
	1.070	1.065	1.255	1.235
	0.925	0.930	1.125	1.190
	1.145	1.145	1.315	1.185
L3	0.920	0.920	1.005	1.030
	1.145	1.145	1.200	1.270
	1.250	1.245	1.355	1.310
	1.330	1.330	1.340	1.345
	0.960	0.955	1.085	1.025

Table 6.4 Shell lengths of juvenile snails in the "*ad lib*" treatments L1, L2 and L3 both before and after the experiment. (L1, L2, 16/XI/77 - 25/XII/77; L3, 16/XI/77 - 18/XII/77).

Treatment number	Before		After	
	Twisters	Non Twisters	Twisters	Non Twisters
L4	0.995	0.990	1.105	1.115
	1.025	1.030	1.235	1.255
	1.085	1.085	1.245	1.265
	1.175	1.180	1.230	1.190
	0.925	0.925	1.205	1.195
L5	0.945	0.945	1.180	1.220
	1.010	1.015	1.215	1.065
	1.085	1.080	1.315	1.300
	1.140	1.145	1.220	1.315
	1.240	1.245	1.390	1.295
L6	1.180	1.180	1.375	1.260
	0.970	0.975	1.185	1.120
	1.320	1.325	1.345	1.400
	1.300	1.310	1.395	1.325
	0.960	0.965	1.095	1.150

Table 6.4 (continued) Shell lengths of juvenile snails in the "*ad lib*" treatments L4, L5 and L6 both before and after the experiment. (L4, 30/XII/77 - 12/II/78; L5, 30/XII/77 - 29/I/78; L6, 23/II/78 - 19/III/78). Wilcoxon matched-pairs signed-ranks test for all treatments:

Before, $n = 17$, $T = 51$, $p > 0.05$

After, $n = 30$, $T = 274.5$, $z = 0.86$, $p > 0.05$

Treatment number	Before		After	
	Twisters	Non Twisters	Twisters	Non Twisters
L1	0.985	0.985	1.020	1.050
	0.975	0.975	1.000	1.010
	1.325	1.325	1.325	1.340
	1.000	1.005	1.055	1.025
	1.015	1.015	1.060	1.030
L2	1.120	1.120	1.195	1.180
	1.145	1.145	1.200	1.195
	1.065	1.065	1.065	1.100
	0.925	0.930	1.040	1.075
	1.150	1.155	1.195	1.190
L3	0.920	0.920	0.925	0.940
	1.140	1.140	1.160	1.145
	1.245	1.245	1.280	1.285
	1.330	1.340	1.340	1.345
	0.950	0.955	1.040	1.090

Table 6.5 Shell lengths of juvenile snails in the "spread evenly" treatments L1, L2 and L3. Each replicate ran for the same time as its equivalent "*ad lib*" treatment (see Table 6.4).

Treatment Number	Before		After	
	Twisters	Non Twisters	Twisters	Non Twisters
	0.995	0.995	1.005	1.005
	1.025	1.025	1.085	1.050
L4	1.085	1.080	1.095	1.115
	1.175	1.180	1.200	1.295
	0.925	0.920	0.960	0.960
	0.945	0.950	1.005	1.000
	1.010	1.000	1.035	1.080
L5	1.080	1.075	1.105	1.090
	1.140	1.140	1.245	1.200
	1.245	1.245	1.250	1.260
	1.180	1.175	1.180	1.180
	0.970	0.975	0.975	0.990
L6	1.320	1.320	1.330	1.345
	1.300	1.315	*BROKEN	1.315
	0.965	0.955	0.990	1.020

Table 6.5 (continued) Shell lengths of juvenile snails in the "spread evenly" treatments L4, L5 and L6. Each replicate ran for the same time as its equivalent "_ad lib_" treatment (see Table 6.4).

Wilcoxon matched-pairs signed-ranks test for all treatments;

Before, $n = 15$ $T = 50$ $p > 0.05$

After, $n = 26$ $T = 121.5$ $z = 1.37$ $p > 0.05$

* Tip of shell broken off, preventing shell length from being measured.

Treatment number	Before		After	
	Twisters	Non Twisters	Twisters	Non Twisters
L1	0.985	0.985	0.990	1.020
	0.975	0.975	0.995	1.000
	1.325	1.325	1.390	1.385
	1.000	1.000	1.150	1.055
	1.015	1.020	1.190	1.180
L2	1.120	1.125	1.220	1.180
	1.150	1.145	1.250	1.220
	1.070	1.060	1.125	1.110
	0.925	0.930	0.980	1.000
	1.150	1.155	1.250	1.220
L3	0.920	0.920	0.940	0.945
	1.145	1.140	1.245	1.180
	1.245	1.245	1.335	1.265
	1.330	1.340	1.410	1.340
	0.960	0.955	0.980	0.955

Table 6.6 Shell lengths of juvenile snails in the "point source" treatments L1, L2 and L3. Each replicate ran for the same time as its equivalent "*ad lib*" treatment (see Table 6.4).

Treatment number	Before Twisters	Before Non Twisters	After Twisters	After Non Twisters
	0.995	0.995	1.080	1.000
	1.010	1.015	1.085	1.085
L4	1.080	1.080	1.145	1.140
	1.175	1.180	1.210	1.200
	0.925	0.925	0.950	0.930
	0.945	0.945	1.020	0.950
	1.010	1.015	1.045	1.050
L5	1.080	1.080	1.125	1.120
	1.140	1.145	1.205	1.190
	1.250	1.245	1.330	1.260
	1.180	1.180	1.185	1.190
	0.970	0.970	0.985	0.990
L6	1.320	1.315	1.330	1.325
	1.300	1.310	1.355	1.310
	0.965	0.965	0.980	0.965

Table 6.6 (continued) Shell lengths of juvenile snails in the "point source" treatments L4, L5 and L6. Each replicate ran for the same time as its equivalent "*ad lib*" treatment (see Table 6.4). Wilcoxon matched-pairs signed-ranks test for all treatments:

Before, $n = 16$, $T = 50$, $p > 0.05$

After, $n = 29$, $T = 59$, $z = 3.43$, $p < 0.001$

Replicate number	Number of snails dying: Twisters	Non Twisters
L1	0	2
L2	1	2
L3	1	3
L4	1	4
L5	1	3
L6	0	2
Total dead:	4	16
(Number alive):	26	14

2 x 2 contingency table for independence between proportions of Twisters and Non Twisters dying: $\chi_1^2 = 10.80$, $p < .005$

Table 6.7 Numbers of juvenile Twisters and Non Twisters dying in the "point source" treatments.

Replicate number	Number of snails dying: Twisters	Non Twisters
L7	0	2
L8	1	2
L9	0	2
Total:	1	6
(Number alive) :	14	9

2 x 2 contingency table for independence between proportion of Twisters and Non Twisters dying: $\chi_1^2 = 4.66$, $p < 0.05$

Table 6.8 Numbers of adult Twisters and Non Twisters dying in the "point source" treatments.

this context it is also notable that there was no significant difference between the number of days elapsing before either Twisters or Non Twisters died, when kept together without food (see Section 5.4.2).

6.2.3.5 Displacements in laboratory trays.

The numbers of Twisters and Non Twisters successfully joining an aggregation of snails, and the numbers of Twisters and Non Twisters displaced, are in Table 6.9.

As a null hypothesis, the proportion of Twisters displaced from an aggregation should be the same as the proportion joining. Therefore a 2 x 2 contingency table χ^2 comparison for homogeneity was performed on the data in Table 6.9. By inspection the proportion of Twisters capable of joining an aggregation is much greater than the proportion displaced, and these differed significantly ($\chi^2_1 = 48.03$, $p < .001$). Observations of snails joining and being displaced from aggregations revealed that the displaced snail was often several snails distant from the one joining the aggregation. Twisters, by twisting, seemed better able to force their way in between snails than Non Twisters. Once an additional snail had entered an aggregation, another was usually displaced within the next minute. Although it would be interesting to compare the lengths of snails displaced with those of snails joining aggregations, the observations of displacements were made in laboratory trays in which there was a limited size range of snails and also different frequencies of small and large snails in different replicates of the "point source" treatment. Therefore the data in Table 6.9 have not been further analysed. It is interesting to note that larger as well as smaller snails were often displaced from aggregations, and that the size of the displaced snail did not appear to depend upon the size of the

		Twisters	Non Twisters	Total
Snails observed capable of displacing others	n	86	14	100
	$\bar{x}$	1.18	1.20	
	s_x	0.128	0.143	
Snails displaced	n	38	61	99
	$\bar{x}$	1.13	1.07	
	s_x	0.149	0.181	

2 x 2 contingency table for independence, $\chi_1^2 = 48.03$ $p < .001$

$\bar{x}$: mean length of snails in each category

s_x : standard deviation

n : sample size, and number in each category.

Table 6.9 The behaviour of 100 snails observed capable of displacing others from around point sources, and behaviour of 99 snails displaced (one of the snails displaced was not removed and scored for behaviour or size).

snail entering the aggregation. This is not surprising, considering that the snail displaced was often several snails removed from the one entering the aggregation.

6.2.4 Discussion.

Results from the laboratory experiment did not lead to rejection of the hypothesis that Twisters were better at competing for space to feed than Non Twisters. Juvenile Twisters grew significantly faster than juvenile Non Twisters in the "point source" treatment but not in the "ad lib" or "spread evenly" treatments. Also, significantly less Twisters died than Non Twisters in the "point source" treatment and although no snails died in either of the other two treatments, this difference in mortality is in accordance with the difference in growth of Twisters and Non Twisters in the "point source" treatment.

Observations of snails forcing their way into aggregations and displacing other snails are also consistent with the hypothesis that Twisters are better at competing for space to feed than Non Twisters, in that a significantly greater proportion of Twisters were able to enter aggregations than were displaced from aggregations.

Therefore, these data clearly show that Twisters are better than Non Twisters at competing for space to feed and that this significantly affects their growth and mortality when food and space to feed are in short supply.

These differences may result in Twisters having a selective advantage over Non Twisters in such a situation, and may thus account for the observed correlation between the percentage of Twisters and hunger in natural populations. However, the differences in growth and mortality between Twisters and Non Twisters have only been demonstrated in the laboratory.

These feeding experiments would have been virtually impossible to carry out in the field. However, from the hypothesis tested in the laboratory, and results of the laboratory experiments, testable predictions can be made about populations of Nassarius pauperatus in different situations in the field.

6.3 Predictions about populations of Nassarius pauperatus in the field.

6.3.1 Introduction.

Two predictions from the hypothesis that Twisters were better than Non Twisters at competing for space to feed could be easily tested by surveying different populations. These predictions were:

(a) That Non Twisters should be hungrier than Twisters in populations in which food is in short supply and distributed as point sources such that competition for space to feed occurs. Conversely there should be no difference in hunger between Twisters and Non Twisters when food is not in short supply, and/or not distributed as point sources.

(b) In populations where Non Twisters are hungrier than Twisters, juvenile Twisters should grow at a faster rate than juvenile Non Twisters. Therefore, juvenile Twisters should be larger than juvenile Non Twisters of the same year class. However, this may not result in differences in size between adult Twisters and Non Twisters since snails receiving less food than others are able significantly to delay the onset of lip formation, at which time they cease growing (see Section 3.4.2.6). When there are no differences in hunger between Twisters and Non Twisters, there should be no significant differences in shell length between juvenile Twisters and Non Twisters of the same year class.

6.3.2 Materials and Methods.

6.3.2.1 Choice of populations.

Two populations were required, each containing both Twisters and Non Twisters and also a collectable proportion of juvenile snails. In one there had to be evidence of a shortage of food and competition for space to feed, but not in the other. Also the number of snails feeding when offered food needed to be an intermediate value between "all snails feeding" and "all snails not feeding". With these considerations in mind, as well as the need for economy, populations at Port Clinton and Port Gawler were sampled. Both contained more than 12% of each behavioural phenotype (see Table 3.24) more than 15% of each population were juvenile snails (Table 3.6) and the percentage of snails feeding was not usually close to either zero or 100 percent (Table 3.21). Snails at Port Gawler were significantly hungrier than those at Port Clinton on 9 out of 13 occasions during 1977 (see Section 3.4.4). Furthermore there was evidence of competition for space to feed at Port Gawler but not at Port Clinton (see Tables 6.1 and 6.2), and the latter population was the closest to Adelaide in which there was no evidence of competition for space to feed around natural or artificial point sources of food.

6.3.2.2 Hunger and size of juvenile Twisters and Non Twisters.

The usual method of estimating hunger, by offering snails Katelysia scalarina in the field (see Section 3.3.2) was used at both locations. As soon as a snail commenced feeding it was removed from the bait to ensure that there was always space available for other snails to feed. After 15 minutes had elapsed, all snails that had fed and all those that had not, were collected, placed in separate polyurethane containers, and transported to the laboratory where the snails in each group were marked with differently coloured spots of fingernail polish on their shells and placed in covered trays of aerated seawater at 19°C, without food. The behaviour of all snails was classified within the next 3 days (to minimise growth of juveniles in the laboratory) and then the shell lengths of all snails were measured.

6.3.3 Results.

Results of offering Katelysia scalarina to both adult and juvenile Nassarius pauperatus at Port Gawler and Port Clinton are in Table 6.10. There is no significant difference between the proportions of either adult or juvenile Twisters and Non Twisters feeding at Port Clinton. However, at Port Gawler, a significantly greater proportion of both adult and juvenile Non Twisters, compared to Twisters, fed when offered food, indicating that Non Twisters are significantly hungrier than Twisters at Port Gawler.

The lengths of both juvenile and adult Twisters and Non Twisters from Port Gawler and Port Clinton are in Tables 6.11 and 6.12. The shell lengths of juvenile N. pauperatus did not appear to be distributed normally (Smith 1975, also my data) so a non parametric Randomisation test for use with large samples was used to compare the shell lengths of juveniles. The proportion of adult Twisters and Non Twisters was previously found to be independent of sex at Port Gawler and Port Clinton, so the shell lengths of samples of Twisters and Non Twisters containing both males and females were also compared using a Randomisation test (Siegel, 1956). Considering that it was postulated that Twisters would be larger than Non Twisters in some samples, one tailed probabilities were used.

There were no significant differences in shell length between either adult or juvenile Twisters or Non Twisters at Port Clinton (Table 6.11). However, both adult and juvenile Twisters were

Adults, Gawler

	Feeding	Not Feeding
Twisters	10	67
Non Twisters	13	14

χ^2_1 (contingency table) = 14.35, $p < 0.001$

Adults, Clinton

	Feeding	Not Feeding
Twisters	8	21
Non Twisters	20	49

χ^2_1 (contingency table) = 0.02, $p > 0.05$

Juveniles, Gawler

	Feeding	Not Feeding
Twisters	70	106
Non Twisters	44	14

χ^2_1 (contingency table) = 22.74, $p < 0.001$

Juveniles, Clinton

	Feeding	Not Feeding
Twisters	7	22
Non Twisters	35	63

χ^2_1 (contingency table) = 1.35, $p > 0.05$

Table 6.10 The number of adult and juvenile Twisters and Non Twisters, either feeding or not feeding when offered *Katelysia scalarina* at Port Gawler and Port Clinton. Populations were sampled on adjacent days, during February 1978.

Port Gawler

	Twisters	Non Twisters
$\bar{x}$	1.12	1.04
s_x	0.143	0.135
n	295	91

t_{384} (randomisation test) = 4.24 $p < 0.001$

Port Clinton

	Twisters	Non Twisters
$\bar{x}$	1.14	1.11
s_x	0.160	0.262
n	29	98

t_{125} (randomisation test) = 0.59 $p > 0.05$

$\bar{x}$: mean,

s_x : standard deviation,

n : sample size

Table 6.11 Shell lengths of juvenile Twisters and Non Twisters of the same year-class from Port Gawler and Port Clinton. Samples were collected on adjacent days, during February 1978.

significantly larger than Non Twisters at Port Gawler, as predicted (Table 6.12).

6.3.4 Discussion.

These results which show that both juvenile and adult Non Twisters are significantly smaller than Twisters at Port Gawler but not at Port Clinton, are in accordance with the hypothesis that food and space to feed are only in short supply at Port Gawler and that Twisters are better than Non Twisters at competing for these resources. At Port Clinton there was no evidence of competition for space to feed, presumably because although carrion is distributed as point sources there is sufficient available to prevent space to feed from being in short supply. However, at Port Gawler (where snails are significantly hungrier than at Port Clinton) there is also evidence of competition for space to feed. Therefore, Port Gawler appears to be equivalent to a "point source" and Port Clinton an "_ad lib_" situation, although this categorisation is undoubtedly a simplification, since in reality the amount of competition for space to feed should vary continuously, between the case where there is always competition for space to feed, and that where there is never competition for space to feed. Port Gawler and Port Clinton are only two points on this continuum and their relative positions no doubt vary seasonally as their relative indices of hunger change (see Table 3.21).

Also, the intensity of competition for space to feed is not necessarily a function of hunger. It is possible to envisage a hungry population in which food is not distributed as point sources, or a population at such low density that even though food is available as point sources and snails are extremely hungry, there is not competition for space to feed. Nevertheless, in most populations

Port Gawler

	Twisters	Non Twisters
$\bar{x}$	1.33	1.29
s_x	0.097	0.137
n	169	42

$F_{(41,168)} = 2.00 \quad p < 0.001$

$t_{(209)}$ (randomisation test, one tailed) = 2.19, $.01 < p < .05$

Port Clinton

	Twisters	Non Twisters
$\bar{x}$	1.46	1.48
s_x	0.143	0.121
n	56	158

$F_{(55,157)} = 1.40, \quad p > 0.05$

$t_{(212)}$ (randomisation test, one tailed) = 1.24, $p > 0.05$

$\bar{x}$: mean,

s_x : standard deviation,

n : sample size

Table 6.12 Shell lengths of adult Twisters and Non Twisters from Port Gawler and Port Clinton. Populations sampled on adjacent days during February 1978.

containing relatively hungry snails it is likely that there will be competition for space to feed since *Nassarius pauperatus* has the ability to locate food on the sandflat in response to chemical cues from a distance of up to several metres, making the occurrence of aggregations in which some snails cannot feed possible over a wide range of population densities. Furthermore, in populations where snails are not hungry there should not be competition for space to feed, whatever the distribution of food.

As a result there may be a correlation between hunger and the intensity of competition for space to feed in the populations of *N. pauperatus* studied. It has previously been postulated that differences in the ability of Twisters and Non Twisters to compete for space to feed will result in Twisters having a selective advantage over Non Twisters when food is in short supply and space to feed is limited (Section 6.2.4). If so, this may account for the correlation observed between the percentage of Twisters and hunger.

The above argument appears contradictory to the previous finding that egg production by *N. pauperatus* per breeding season, was inversely related to food availability (Section 4.2). It could be argued that if Non Twisters are hungrier than Twisters at Port Gawler then they will produce more eggs than Twisters and this may overcome any selective advantage related to growth and mortality that Twisters may have compared to Non Twisters. However, the difference in shell length between adult Twisters and Non Twisters at Port Gawler is small compared to that between snails from locations where egg production differs greatly (compare Stansbury with Sultana Point and then Coobowie in Table 1, Appendix 3 with the difference between adult Twisters and Non Twisters from Port Gawler in Table 6.12). Also, considering current theories about semelparous and iteroparous reproductive patterns (Appendix 3) it is likely that egg production will not be linearly related to hunger, but instead will suddenly increase once hunger exceeds a certain value. Therefore, although I did not compare relative egg production in a low food situation where there was competition for space to feed, it is postulated that this is similar for Twisters and Non Twisters.

It is unlikely that a shortage of food and space to feed is the only selective pressure acting upon Twisters and Non Twisters. An hypothesis relating to the relative conspicuousness of Twisters and Non Twisters is described below.

6.4 The third hypothesis; that Twisters are more conspicuous to predators than Non Twisters.

6.4.1 Introduction.

Whilst collecting snails on the sandflat I postulated that Twisters, because they twisted, might be more conspicuous to predators which relied upon visual location of prey. It was not known if Nassarius pauperatus had any predators, but rather than trying to establish the identity of these I went ahead with experiments designed to test whether there were differences in the proportion of Twisters and Non Twisters removed from open enclosures in the field.

6.4.2 Materials and Methods.

The design of the predation experiment consisted of the following two treatments:

(a) "Open" : Open cage, predators not excluded.

(b) "Excluded" : Open cage, but with canopy to exclude predators.

Cage frames of dimensions 60 x 60 x 16 cm were constructed (as described previously in Section 5.3) of welded steel rod which was then dipped in zinc. Cages were covered on all sides but for one 60 x 60 cm panel, (which became the open top when the cage was in position) with fibreglass coated zinc mesh of hole size 2 mm x 2 mm. To prevent snails leaving, or any animals entering the open cages by crawling up the sides, the top edge of the cage was surrounded by a double sided fringe of fibreglass covered mesh which had been frayed so that wires extended 5 cm outwards (see Figure 6.1). Trials in the laboratory showed that such a fringe was effective at preventing *N. pauperatus* from leaving a container, even though food was available outside the container.

To exclude predators from "excluded" treatments a square 80 x 80 cm mesh canopy was suspended over, but not touching the top of the above cages. The canopy was approximately 3.0 cm above the fringed edge of the top of the cage, and was secured by its corners to four jarrah-wood stakes (see Figure 6.1). Therefore, both treatments were identical, except that one had a canopy suspended over it, which was designed to reduce predation, but should not affect the probability of snails leaving the cage of their own accord. Cages were buried 4 cm into the substratum, and filled by straining substratum through the mesh sides.

Snails were offered *ad lib Katelysia scalarina* every time the cages were visited. Ten snails, being 5 pairs of a Twister and Non Twister matched for shell length to within 0.015 cm were added to each cage. Experiments were run at both Port Gawler and Port Clinton, commencing in May 1978 and ran until August 1978. Cages were visited every month or less and the number of snails per cage were counted. Two replicates of each treatment were run at each location.

6.4.3 Results.

In all the time cages were in the field no snails either died or disappeared from any of either the "open" or the "excluded"

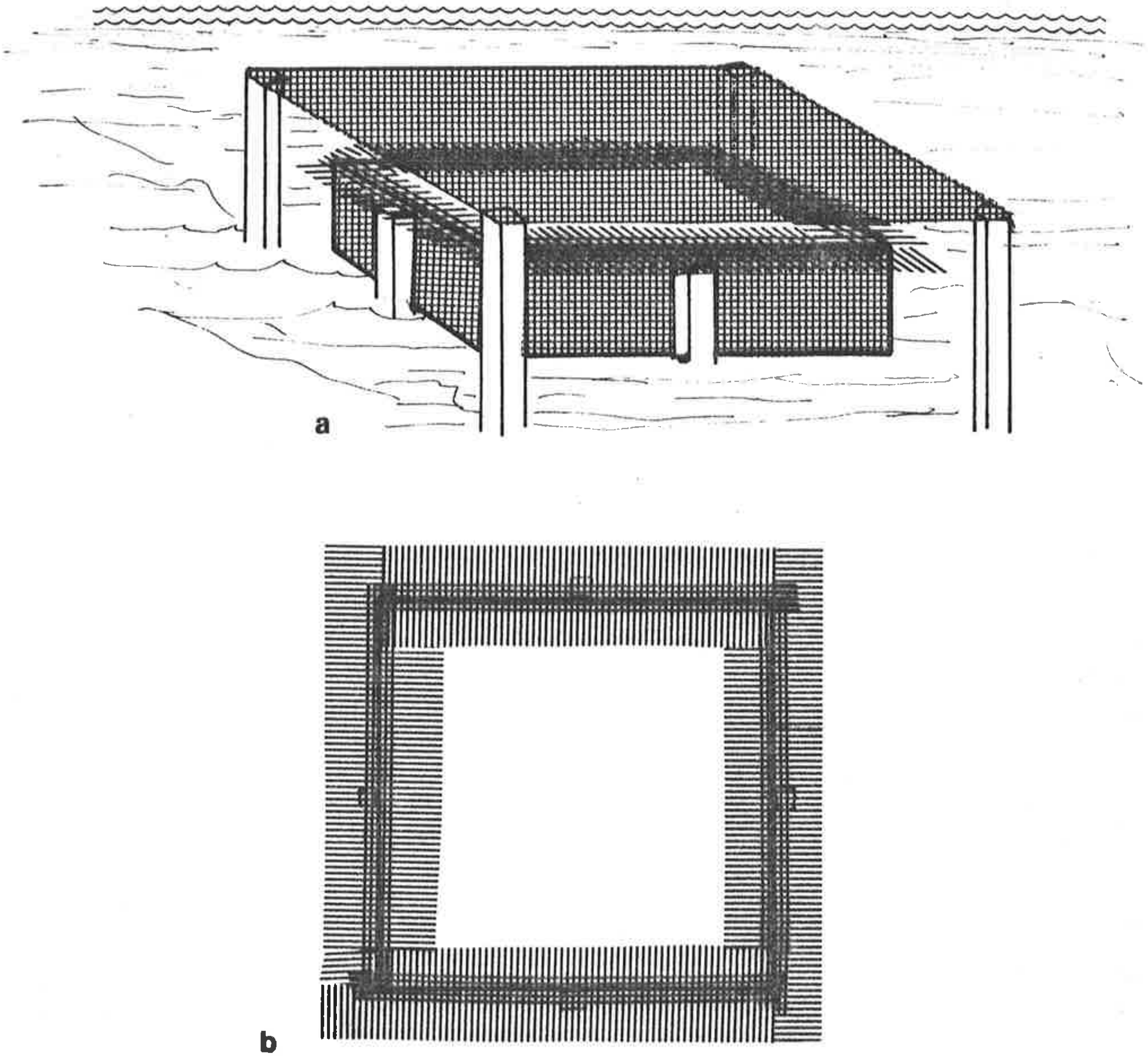


Figure 6.1 Cage types used to test the third hypothesis. (a)"Excluded"treatment: Open topped cage of dimensions 60 x 60 x 16 cm deep, anchored to the substratum with four jarrah-wood stakes with an 80 x 80 cm canopy of fibreglass coated zinc mesh suspended over, but not touching, the open top. (b) Detail of the fringe containing snails within the cage, from above; see text for details.

replicates. Cages of the "open" replicates frequently became clogged with Ulva species which often had covered the snails to a maximum thickness of about 3.0 cm. Therefore, even if there had been predators of Nassarius pauperatus present they may not have seen, or been able to reach, the snails in the "open" treatments.

6.4.4 Discussion.

This experiment did not adequately test the hypothesis that Twisters may be more conspicuous to visual predators than Non Twisters and hence have an associated selective disadvantage. Because there was little additional time available, the hypothesis was not tested further, and experiments were discontinued.

7. Discussion.

7.1 Behavioural differences between populations of Nassarius pauperatus, considering the selective advantage of being a Twister when food and space to feed are in short supply.

This study has described differences in the frequency of individuals having a behavioural phenotype defined as being a "Twister", between natural populations of the marine snail Nassarius pauperatus in South Australia. Being a Twister was found to be constant in individuals (see Section 2.3.4) and results from breeding experiments, although scanty (Section 4.1.4), did suggest that being a Twister was heritable. Twisters were found to be better than Non Twisters at competing for space to feed when this and food were in short supply in the laboratory, and there was evidence that these selective pressures were also operating in the field (Section 6.3.4). The following discussion, therefore, is based on the conclusion that the difference between Twisters and Non Twisters is not selectively neutral.

If the lack of food and of space to feed were the only selective pressures acting upon natural populations it would be expected that eventually most of the snails in these populations would be Twisters. There are two alternative explanations for the present situation. Firstly, the polymorphism may be transient; the frequency of Twisters may indeed be proceeding to fixation and the differences between populations containing Twisters may be a result of differences in the intensity of selection for Twisters. Secondly, the polymorphism may be a balanced one; there may be other selective pressures acting upon Twisters and Non Twisters such that the observed frequencies of Twisters in the populations studied are stable and maintained by different intensities of opposing selective pressures

(Dobzhansky,1970).

One possible disadvantage of being a Twister was postulated, but was not tested adequately (Section 6.4), and it is quite possible that there are several selective pressures acting upon Twisters and Non Twisters. During the three years of this study there were no noticeable trends in the frequency of Twisters in several populations sampled (Section 3.5.1), but this period may not have been long enough to detect a change.

7.2 Recruitment of Nassarius pauperatus in South Australia; An hypothesis.

In Section 3.5.1 it was described how the size of adult Nassarius pauperatus, hunger and the proportion of juvenile snails remained similar within all locations sampled more than once during the three years of this study.* Although not essential, this apparent constancy makes more plausible the explanation developed here for differences in the frequency of Twisters between populations. It merits brief discussion because it is an unusual observation.

Other studies have found that there are often great fluctuations in recruitment of benthic marine invertebrates at the same location (e.g. Loosanoff, 1964, 1966; Bowman and Lewis, 1977). One example is a congener of the species studied here, namely Nassarius obsoletus (Scheltema, 1964).

It was noticed whilst collecting N. pauperatus that hungry, relatively dense populations, having a relatively high proportion of juvenile snails, were found on very sheltered sandflats with many pools of standing water at low tide within the zone in which N.

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* I attempted to obtain more data for the shell length of adult Nassarius pauperatus by examining collections held in the South Australian Museum. However, although large numbers of adult shells had been collected during the early part of this century in the "Gulf St Vincent", these collections were not labelled any more specifically.

pauperatus occurred, and that this zone was relatively wide. Conversely, populations of less hungry N. pauperatus, at relatively low density, and with a lower proportion of juveniles, were found on steeply sloping beaches which often completely lacked intertidal pools in the zone where N. pauperatus were found, which was usually relatively narrow. These qualitative observations are summarised in Appendix 4.

From the above observations it is postulated that the most important factor affecting the recruitment of Nassarius pauperatus in South Australia is the morphology of the shore at each location, rather than less predictable variables such as weather or larval availability. There are many possible mechanisms which may explain the importance of shore morphology. For example, larvae may selectively settle in response to sediments characteristic of certain locations, or to chemical stimuli from metamorphosed N. pauperatus, or the mortality of postlarvae may simply be higher upon steeply sloping shores. Scheltema (1961) found that Nassarius obsoletus, primarily a deposit feeder having a crystalline style, but also a scavenger of carrion, metamorphosed in response to particular substrata. In contrast, Nassarius vibex, which is only a carrion feeder, did not. Nassarius pauperatus is an omnivore, does not ingest noticeable quantities of detritus, and lacks a crystalline style. The substratum preferences of larvae that were competent to metamorphose were not investigated. Whatever the cause of these differences in recruitment, the situation is interesting since the population density of juvenile snails was significantly correlated with hunger (Section 3.5.1), and by inspection of Appendix 4, locations with a high proportion of juveniles also contained hungry snails.

Therefore I postulate that certain features of shore morphology result in a high density of N. pauperatus larvae either settling or subsequently surviving per area of sandflat. This results in there being a high population density and proportion of juvenile snails at such locations , causing food for N. pauperatus to be in short supply.

Such a situation may appear paradoxical, since it might be expected that there would be selection for larvae to settle at locations where food for juveniles and adults is less likely to be in short supply. However, I propose that in such locations (i.e. on steeply sloping drained shores) either larvae cannot settle, or their subsequent survival is so low (due possibly to physical factors) that this far outweighs any selective advantage conferred by having adequate food following metamorphosis. It is obvious that this situation would repay further investigation, which could commence with a study of the substratum preference in metamorphically competent N. pauperatus larvae.

7.3 Conclusion.

Of the other studies of differences in behaviour between populations of the same species, only those of McPhail (1969) and Seghers (1974a) have tested hypotheses about the functional advantages of different behavioural phenotypes.

This study of variation in behaviour between populations of Nassarius pauperatus differed from those of McPhail and Seghers in that the behaviour of being a Twister was well defined and the frequency of individuals showing this behaviour was measured in several populations. In contrast, both Seghers and McPhail described differences in behaviour in terms of populations, and made no attempt to estimate the frequency of individuals having a particular behaviour in each population (e.g. Seghers described schooling behaviour of populations in categories ranging from "well developed" to "absent"). This difference is unimportant except that it illustrates how differences in behaviour often cannot be defined with such precision as can morphological characters, presumably because patterns of behaviour are usually more complex and have great inherent variability.

This study of twisting in Nassarius pauperatus supports the suggestion by Seghers (1974b), that differences in behaviour may be more subtle, and more difficult to work with, than morphological traits. Twisting was initially observed by chance in laboratory trays, and it is extremely unlikely the crucial observation that being a Twister appeared to be constant in individuals would have been made in the field, since it required several observations of the same individual initiating contact with others. The behavioural phenotypes were also inherently more difficult to experiment with. Individual snails had to be observed contacting others, be marked, and then their subsequent behaviour observed, unlike differences in morphology which are often readily apparent.

In terms of assessing whether these differences in the frequency of a behavioural polymorphism were a result of selection or simply stochastic factors, this study has provided evidence that these differences can be accounted for at least partly by selection in favour of Twisters; the possible selective pressures against Twisters were not sufficiently investigated. Tinbergen (1965) has stressed that even though the function of a behaviour is not readily apparent, it cannot thus be presumed to be selectively neutral, and also emphasised the need for studies wherein hypotheses are proposed from observations of behaviour, and then tested. This study illustrates the success of such an approach.

Apart from providing rather limited information about the selective value of a particular difference in behaviour, two somewhat unrelated hypotheses were proposed from this study. These were, firstly, that the relative recruitment by some species of benthic marine invertebrates may be predictable, given shore morphology (Section 7.2 and Appendix 4) and, secondly, that the flexibility of reproductive pattern is correlated with dispersive ability (Appendix 3). These two hypotheses emphasise how little is yet known about the ecology of benthic marine invertebrates.

8. Appendices.

Appendix 1. Keeping Nassarius pauperatus in the laboratory.

During all laboratory experiments and while they were being prepared for experimental treatments in the field, all Nassarius pauperatus were kept in rectangular polystyrene trays, dimensions 32 x 54 x 12 cm deep, which were filled with aerated seawater of various depths, as specified in each experiment. Trays were covered with a transparent sheet of plastic sewn on to a rigid iron or wooden frame, dimensions 38 x 62 cm, to reduce evaporation and to prevent snails from escaping.

Seawater was replaced every 14 days, or sooner if required. The temperature of water in trays only usually varied within ± 0.5°C of the set temperature, even though the air temperature in the constant temperature room oscillated ± 2°C around the set temperature every 15 minutes.

Illumination was provided either by a bank of six, 40 watt "Gro-Lux" fluorescent tubes positioned 1 metre above the tray tops, or by natural daylight.

Both adult and juvenile N. pauperatus were maintained for over a year upon diets of either Katelysia scalarina flesh (minus the algae-filled gut which tended to make the water cloudy and foul smelling), or a flagellate alga which N. pauperatus was known to feed upon (see Appendix 2). Washing K. scalarina in either seawater or tapwater before feeding and removing any that remained uneaten after 30 minutes greatly reduced the incidence and intensity of fouling of seawater.

Deaths of N. pauperatus in the laboratory appeared to result from starvation or from being kept in fouled seawater.

Aquaculture, 16 (1979) 361—362

Short Communication

A TECHNIQUE FOR THE ISOLATION OF MICROSCOPIC ALGAE SUITABLE FOR FEEDING TO SPECIFIC INVERTEBRATE LARVAE

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(Accepted 24 December 1978)

ABSTRACT

McKillup, S.C., 1979. A technique for the isolation of microscopic algae suitable for feeding to specific invertebrate larvae. *Aquaculture*, 16: 361—362.

A technique is described for extracting algal cells from the gut of wild *Nassarius pauperatus* larvae. These cells were subsequently cultured and proved a suitable food for *Nassarius pauperatus* larvae cultured in the laboratory. It is suggested that the application of this technique may supply suitable food species for many species of herbivorous zooplankton, and therefore be important aquaculturally.

Unsuccessful attempts to culture herbivorous planktonic larvae of molluscs are occasionally reported and then often attributed to a failure to provide the larvae with suitable food (e.g. Bebbington and Thompson, 1969; Fish and Fish, 1977). Larvae are usually offered a range of laboratory cultures of phytoplankton (Pilkington and Fretter, 1970) without attempting to establish the algal species consumed naturally, an exception being Fretter and Montgomery (1968) who recognised algal cells in the gut of larvae from plankton hauls. An attempt to raise larvae of the prosobranch snail *Nassarius pauperatus* (Lamarck), using locally available laboratory cultures of phytoplankton in conjunction with the technique developed by Scheltema (1962) for other *Nassarius* species was unsuccessful. Newly hatched larvae were offered either of two unidentified flagellates previously isolated from South Australian waters, but no larvae survived to metamorphosis or even grew to the 'intermediate stage' described by Scheltema.

Since larvae caught in plankton hauls sometimes have freshly ingested algal cells in their gut (Fretter and Montgomery, 1968), ten larvae and six recently settled postlarvae of *Nassarius pauperatus* were collected from intertidal pools at Port Gawler, South Australia. These were each washed four times in sterile sea water and crushed onto a welled microscope slide. Algal cells from the stomach of each larva were collected in 5-μl micropipettes, under a binocular dissecting mocroscope at 40 × magnification. This took place no more than

15 min after collection to minimise the effect of larval digestive processes upon the viability of algal cells. The contents of each micropipette were then added to separate 50-ml flasks of sterile Modified Schreiber solution (Stein, 1973) and incubated at 19° C under a light bank for 14 days.

An as yet unidentified flagellate was thus isolated from two larvae and one postlarva and maintained in Modified Schreiber solution. By offering this flagellate to larvae of *Nassarius pauperatus* hatched in the laboratory and otherwise using the same culture technique as before, it was possible to raise them beyond metamorphosis.

This technique should be useful for obtaining suitable food species for many herbivorous zooplankton and thus be useful aquaculturally, especially since it is not necessary to identify the cells found in the gut, which may be unknown species. However, since cells of undigestible algal species often remain intact and therefore probably viable whilst passing through the gut of zooplankton (Fretter and Montgomery, 1968), the technique relies upon the presence of recently ingested algal cells in the gut and their rapid extraction.

ACKNOWLEDGEMENTS

The work was supported by a Commonwealth of Australia Postgraduate Research Award. I wish to thank Mr I.M. Thomas for supplying the algal species used in the initial attempt at larval culture.

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Appendix 2. A technique for the isolation of microscopic algae suitable for feeding to specific invertebrate larvae.

Appendix 3. *Modification of egg production and packaging in response to food availability by *Nassarius pauperatus*.

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* Similar material is currently in press: McKillup and Butler (1979) *Oecologia*.

Introduction

Several models of life history patterns[1] related to differences in the environment of a species have been proposed (see Stearns, 1976). One prediction from some of these models is that in environments in which the probability of an adult surviving from one breeding season to the next is low there will be selection for a single massive reproductive effort, even though this results in the death of the reproducing individual (semelparity). Conversely, when the probability of adult survival is high, there will be selection for individuals that breed more than once and produce a smaller number of offspring per breeding season (iteroparity).

These predictions have been derived from the assumption that the advantage of producing more offspring at any one time is countered by an increasing probability of dying before reproducing again during the next breeding season because of the energetic cost of reproduction (Williams, 1966; Gadgil and Bossert, 1970). Similar theoretical models also predict that semelparity will be selected for when the probability of adult survival is low compared to that of juveniles, and *vice versa*

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[1] In the literature variables such as the number of offspring produced per reproductive season, the number of clutches, litters etc. per reproductive season and the number of reproductive periods per lifetime have been called "life history parameters" (Haukioja and Hakala, 1978) "life history characteristics" (Dearn, 1977) or, taken as a set, the "reproductive strategy" (Demetrius, 1975), "life history strategy" or "life history tactics" (Stearns, 1976) or simply "life history" (Schaffer and Elson, 1975). The terms "tactics" and "strategies" are unlikely to be strictly correct for most organisms (Fowler and Fowler, 1964, pp. 1273, 1317) and I see no need for their use as a metaphor. "Parameter" has a strict meaning in mathematical statistics (e.g. Zar, 1974, p. 17) and its use for what must almost always be considered as variates is confusing. "Life history" or "life cycle" traditionally includes more than just the distribution of reproductive effort (e.g. Kennedy, 1975). We need a simple term for that set of variables which describes how reproductive effort is distributed physically amd temporally. In this paper I shall call it the "reproductive pattern" or when the meaning is clear, simply "pattern".

(Charnov and Schaffer, 1973). These predictions are supported by evidence that there is a real cost associated with reproducing (Stearns, 1976).

Thus in harsh environments (e.g. where a resource essential to adult survival is in very short supply) the probability of an adult female surviving to reproduce more than once is slight. This should select for semelparity. Conversely, in less harsh environments (e.g. when the same resource is not in such short supply) the probability of an individual surviving to reproduce again is high, provided that the effort expended upon reproducing does not endanger the individual's immediate chances of survival, hence selecting for iteroparity. Studies of variation in reproductive patterns between populations in increasingly harsh environments have tended to confirm these predictions (e.g. Dearn, 1977; Spencer and Steinhoff, 1968).

All models of life histories assume that they are heritable and therefore capable of being naturally selected. Most also assume constancy of the environment and exceptions (see Stearns, 1976) do not include the case where different successive generations encounter different environments. Many plant and animal species have developmental stages which are capable of being dispersed widely and therefore different successive generations of a species may encounter environments in which different life histories are advantageous. Evolution in these circumstances should produce individuals with not one particular life history pattern but a repertoire of different patterns which they are capable of adopting in response to different environments.

The whelk Nassarius pauperatus (Lamarck) is common on intertidal sandflats in both South Australian Gulfs (see Figure 3.1). This iteroparous species has a larval stage which is planktonic for a minimum of 21 days at 26°C. Populations of N. pauperatus occurring less than 10 kilometres apart were found to differ significantly in the amount of food available to them. I present data showing that different reproductive patterns are evident in these different populations and also that individuals from the same population are capable of displaying different reproductive patterns in different environments.

Materials and Methods.

1. Population densities, size and sex of adults.

Populations of *Nassarius pauperatus* were surveyed from Coffin Bay to Robe, South Australia (Figure 3.1) and it was observed that differences in population density, food availability and the size of adult *N. pauperatus* were common between location (Chapter 3).

Three populations; at Stansbury, Coobowie ("Bag Wash" on Salt Creek Bay) and between Edithburgh and Sultana Point (see Figure 3.1) were chosen for this study because as well as the abovementioned differences between them, they were also near to each other and easily accessible making it possible to sample all three on the same day. These three populations will henceforth be referred to as "Stansbury", "Coobowie" and "Sultana Point".

Between 100 and 125 adult *Nassarius pauperatus* were collected from each population on the same day and their shell lengths measured in cm with NSK vernier calipers to the nearest 0.005 cm. Each snail was sexed by observing the underside of its foot as it moved up the side of a glass beaker of seawater. Adult male *N. pauperatus* have a penis and adult females a pedal gland opening (Smith, 1975), the latter of which could thus be observed. Snails lacking a pedal gland opening were assumed to be males and later dissection of individuals with and without pedal gland openings confirmed the reliability of this method of sexing.

Population densities were estimated by taking 25 random 60 x 60 cm quadrat samples at each of the three locations. The quadrat used had a 5 cm lip which was pushed 3.5 cm down into the substratum. This prevented any *N. pauperatus* from entering or leaving the sample area while it was being searched. *N. pauperatus* were never found any deeper than 3.0 cm in the substratum so this was searched to the depth of the quadrat.

2. Estimation of relative food availability between populations.

Nassarius pauperatus is omnivorous, feeding on both plant material (see Appendices 1 and 2) and animal carrion. However, sampling the sandflat for the density of carrion and various types of algae was not considered to be a valid estimation of the amount of food available to *N. pauperatus*, since carrion is rapidly scavenged by other species as well as *N. pauperatus* and also it was not known which species of algae were actually eaten, nor their food value.

Instead, an attempt was made to compare how hungry samples of *N. pauperatus* were from each location. A random sample of 100 *N. pauperatus* were collected from each of the three populations on the same day. These were arranged in a ring of 14 cm diameter on a 60 x 60 cm sheet of fibreglass mesh (hole size 2 mm x 2 mm), which was laid on top of the sandflat at each location. A living bivalve (*Katelysia scalarina* Lamarck) was forced open and placed in the centre of the ring and the number of snails feeding during the next 15 minutes recorded. Previous trials had shown firstly that all the snails that fed during the first hour commenced within the first 10 minutes, secondly that the number of snails feeding was correlated with the amount of time they had been deprived of food in the laboratory and that this was independent of size, age or sex of individuals, and finally, that *N. pauperatus* do not display a preference for K. scalarina compared to algae they are known to feed on (see Section 3.5.2). Therefore, the offering of food in the field appeared to be a reliable comparative estimate of how hungry individuals in different populations were, and hence of relative food availability.

3. Comparison of egg production between populations.

From late April (Autumn) adult Nassarius pauperatus can be induced to breed prior to their usual breeding period in late October-November (Spring) by raising the temperature (Smith, 1975). Twenty male and 60 female adult N. pauperatus were collected from each location (Stansbury, Coobowie and Sultana Point) during May 1977 and again during October 1977. All samples were brought back to the laboratory on the same day and kept without food in polystyrene trays, dimensions 54 x 32 x 12 cm containing 4 cm depth of aerated seawater, at 20°C with photoperiod adjusted daily to current day length. The numbers of egg capsules produced per day were counted until egg production ceased and an estimate of the average number of eggs produced per capsule in each group was made by counting the number of eggs per capsule in a maximum of 45 capsules, or all capsules if less than 40.

4. Comparisons of egg production and average adult size of groups with high and low food availability.

Two groups, each consisting of 25 juvenile Nassarius pauperatus were chosen from a larger sample collected from Port Gawler, South Australia (a suitable source of large numbers of juveniles, near to Adelaide) during November 1977. These juveniles had settled during November-December 1976 and ranged in size from 0.980 cm to 1.295 cm.

Each snail in one group was matched for shell length to within .015 cm of its counterpart in the other group. It was not possible to match the snails for sex since sexual differences were not apparent until after individuals matured. Matched pairs were numbered from 1 to 25, with a black "Ball Pentel Extra Fine R56" pen on their shells and the number was then covered with clear fingernail polish to protect it. One group was then designated "high food" and the other "low food" and these were marked distinctively with spots of differently coloured fingernail polish on their shells.

Both groups were kept together in the same tray of aerated seawater at 20°C with a "long day" (16L:8D) photoperiod, and otherwise as described previously. The high food group was separated and offered *ad lib* frozen *Katelysia scalarina* every day and the low food group was offered *ad lib* *K. scalarina* every 14 days. Different frequencies of such feeding "pulses" have been shown to simulate different levels of food availability to other molluscs (Calow, 1973; Moriarty, 1978). The only interruptions to this feeding regime occurred when the feeder was away on fieldwork and this only happened on seven occasions, none of which exceeded 14 days. However, on one of these, five individuals in the high food group, and one in the low food group died.

Once *N. pauperatus* become sexually mature they develop a lip on their shell and cease growing. As soon as the first individual showed signs of becoming mature the high and low food groups were transferred to separate trays to enable egg production to be quantified without error. Egg production commenced in both groups after all members showed signs of forming lips on their shells.

Considering that some groups produced less egg capsules than there were females (see Table 3), variation in egg production between populations may be a result of different proportions of females actually laying eggs rather than variation in egg production per female. Consequently snails in both the high and low food treatments were watched, and the numbers of all those laying eggs were recorded. The numbers of egg capsules produced per day were counted by marking the locations of new egg capsules with a chinagraph pencil, until egg production ceased. The average number of eggs per capsule produced per group was estimated by counting the number of eggs per capsule in 40 randomly selected capsules from each group, and the lengths and widths of these capsules measured in microns using an ocular micrometer attached to an Olympus monocular microscope at 100x magnification. The diameters of all eggs were usually uniform within each capsule, so the diameter of one egg from each of the above capsules was also measured, in microns.

Cannibalism of egg capsules was frequently observed in the low food treatment and this was estimated in both treatments by counting the number of egg capsules that survived intact until the developing larvae could be clearly distinguished within them. This method under-estimated the degree of cannibalism, but was the only one possible since hatching of larvae would have confounded estimates made later in larval development. Once egg production had ceased, the snails in both groups were sexed and their shell lengths measured, by the methods previously described.

Results.

1. Population densities, size and sex of adults.

Results appear in Table 1. The sex ratio was not significantly heterogeneous between locations (contingency table: $\chi^2_2 = 3.04$, $p > .05$). To simplify comparison of shell lengths of males and females between locations, values were randomly removed from the data from Sultana Point and Coobowie so that a two way analysis of variance with unequal but

Table 1. Shell lengths of males and females, sex ratios and population densities per 3600 cm^2 for <u>Nassarius pauperatus</u> at Stansbury, Coobowie and Sultana Point.

	Population		
	Stansbury	Coobowie	Sultana Point
Shell length (cm)			
Males			
$\bar{x}$	1.62	1.43	1.55
s_x	0.092	0.091	0.098
n	24	24	24
Females			
$\bar{x}$	1.55	1.28	1.48
s_x	0.127	0.152	0.109
n	68	68	68
Sex ratio			
Males	32	24	25
Females	68	86	78
Population density (adults + juveniles)			
$\bar{x}$	0.800	8.600	1.200
s_x	0.87	3.33	1.12
n	25	25	25

$\bar{x}$: mean, s_x : standard deviation, n : sample size

proportional sample sizes could be performed (Sokal and Rohlf, 1969).

This analysis showed that shell length varied significantly between locations ($F_{2,271} = 61.27$, $p < .001$) and between sexes ($F_{1,271} = 18.68$, $p < .001$) but that there was no significant interaction between location and sex ($F_{2,271} = 1.13$, $p > .05$).

Comparison between the mean shell lengths of males and females at each location using a t test, showed that males were significantly larger ($p < .05$) then females at all locations. Also a SNK comparison of mean shell lengths between locations, for males and females separately, showed significant differences between all locations ($p < 0.05$) in each case. By inspection it can be seen that for both sexes snails at Stansbury are largest and those at Coobowie smallest. Population density estimates were also found to differ significantly between locations (one-way analysis of variance: $F_{2,72} = 110.60$, $p < .001$). By inspection it is clear that this significant result is due to the population density at Coobowie being much greater than at either Stansbury or Sultana Point.

2. Estimation of relative food availability between populations.

The numbers of _Nassarius pauperatus_ feeding or not feeding when offered _Katelysia scalarina_ appear in Table 2. The proportion of snails feeding is significantly different between locations (3 x 2 contingency table: $\chi^2_2 = 108.31$, $p < .001$) and this is because the proportion of snails feeding differ significantly between Coobowie and Stansbury ($\chi^2_1 = 79.34$, $p < .001$) and between Coobowie and Sultana Point ($\chi^2_1 = 56.11$, $p < .001$) but not between Sultana Point and Stansbury ($\chi^2_1 = 4.92$, $p > .05$). Therefore, I conclude that _N. pauperatus_ are significantly hungrier at Coobowie than at Stansbury or Sultana Point and this agrees well with the data for shell lengths of adults in Table 1. (It may be noted that the non-significant difference in "hunger" between Sultana Point and Stansbury

Table 2. Number of _Nassarius pauperatus_ feeding out of 100 when offered _Katelysia scalarina_ at Stansbury, Coobowie and Sultana Point.

	Population		
	Stansbury	Coobowie	Sultana Point
Feeding	3	62	11
Not feeding	97	38	89

accords with the significant difference in shell length between those locations).

3. Comparison of egg production between populations.

Results for Nassarius pauperatus collected in the field from each population during May 1977 and October 1977 appear in Table 3. 99% confidence limits for the mean eggs per capsule were calculated in the usual way assuming normality. For the total number of eggs produced a standard deviation was estimated by multiplying the standard deviation of eggs per capsule by the number of capsules. The confidence intervals show that there are no significant differences between mean eggs per capsule or total eggs produced on either occasion between Stansbury and Sultana Point. However, results from Coobowie differ from the above locations on both dates. Snails from Coobowie produced significantly more eggs and significantly less eggs per capsule than those from Stansbury or Sultana Point.

4. Food availability and egg production.

Results from the laboratory experiment are shown in Table 4. A t test comparison showed that the mean number of eggs per capsule was significantly lower in the low food treatment than in the high food one ($t_{78} = 14.34$, $p < .001$). The mean number of eggs produced per female was much higher in the low food than in the high food treatment, and the 99% confidence intervals do not overlap.

Most of the females in each treatment were observed mating and all were seen laying eggs. This result suggests that differences in egg production in the field are not caused by differences in the proportions of females reproducing.

Table 3. Total egg capsules, mean eggs per capsule and estimated total number of eggs produced, with 99% confidence intervals, from 60 females and 40 males from Stansbury, Coobowie and Sultana Point during May and October 1977.

	Population		
	Stansbury	Coobowie	Sultana Point
(1) May 1977			
Total capsules	40	267	45
Eggs per capsule			
$\bar{x}$	28.03	13.00	29.27
s_x	3.94	4.65	4.83
n	40	40	45
99% C.I.	26.34-29.72	11.01-14.99	27.33-31.21
Estimated total eggs produced (or total eggs produced)	1121	3473.67	1317
99% C.I. for total eggs*	1053.6-1188.8	2939.30-4002.33	1229.9-1404.4
(2) October 1977			
Total capsules	14	108	15
Eggs per capsule			
$\bar{x}$	37.29	24.55	36.73
s_x	4.54	5.03	4.64
n	14	40	15
99% C.I.	33.64-40.94	24.21-24.89	33.49-39.97
Estimated total eggs produced	522	2651.40	551
99% C.I. for total eggs*	470.9-573.2	2426.8-2876.0	502.35-599.59

$\bar{x}$: mean, s_x : standard deviation, n : sample size

*This estimate ignores variability associated with the total capsules, of which I have no estimate.

Table 4. Total capsules, mean eggs per capsule, estimated total eggs, mean eggs per female and egg and egg capsule dimensions for high and low food treatments.

	Treatment	
	High food	Low food
Total capsules	130	437
Number of females	16	19
Capsules per females	8.13	23
Eggs per capsule:		
$\bar{x}$	27.08	15.33
s_x	3.49	3.83
(n)	40	40
Eggs per female:		
$\bar{x}$	220.16	352.59
s_x (est)	28.37	88.09
99% C.I.	208.44-231.88	316.55-388.63
Egg diameter (in μm):		
$\bar{x}$	143.25	147.20
s_x	4.51	5.04
(n)	40	40
Capsule dimensions (in μm)		
length		
$\bar{x}$	1078.03	1067.18
s_x	44.17	87.76
width		
$\bar{x}$	904.75	916.67
s_x	28.93	63.51
(n)	40	40

$\bar{x}$: mean, s_x : standard deviation, n : sample size

A comparison of mean egg diameters for eggs from capsules selected at random showed that those from the low food treatment were significantly larger than those in the high food treatment ($t_{78} = 3.69$, $p < .001$), but neither the mean lengths nor the mean widths of capsules from each treatment differed significantly, when compared using a Randomisation Test (Siegel 1956) (lengths, $t_{78} = 0.70$ $p > 0.1$) (widths, $t_{78} = 1.08$ $p > 0.1$). It was estimated that cannibalism in laboratory trays destroyed 309 out of 397 capsules in the low food group while no capsules showed signs of being eaten in the high food group. Cannibalism or predation of egg capsule contents in the field must invariably be underestimated by any simple survey, since attacked capsules can only be distinguished from those which have hatched if some undifferentiated or slightly differentiated eggs remain in them. However, during October 1977, no egg capsules of this type were found at either Stansbury or Sultana Point despite the presence of many capsules containing eggs or developing larvae. In contrast, at Coobowie no full capsules were found and over 50% of empty ones found appear to have been attacked. Considering that this is an underestimate and that no intact egg capsules were found at Coobowie this suggests that cannibalism or predation of eggs is high when food is scarce in the field as well as the laboratory.

The distribution of the shell lengths of juvenile N. pauperatus is not normal, although the shell lengths of adult males and females in the field appear to be normally distributed (Smith, 1975). In the high and low food experiments snails were chosen as matched pairs of juveniles and since the sex of juveniles cannot be distinguished, males were paired with females of the same initial size. Also, the pairs of snails were not chosen at random, instead reflecting the most common sizes in the sample of juvenile snails. Therefore, samples of males and females in the high and low food treatments cannot be regarded as random samples from a normal distribution, so a non parametric comparison was made between the lengths of the matched pairs of snails in each treatment. Pairs containing a snail which died during the experiment were not used. Prior to the experimental treatments there were only measurable differences between the shell lengths of only two pairs of juvenile snails used in the subsequent analysis and these were both within 0.010 cm and effectively cancelled each other. After the treatments, adult snails in the high food group were significantly larger than their counterparts in the low food group, when compared using a one tailed Wilcoxon matched-pairs signed-ranks test ($n = 19$, $T = 46$, $p < 0.01$). Thus it appears that food availability significantly affects the size of adult _N. pauperatus_.

Discussion.

I conclude that as food availability decreases, _Nassarius pauperatus_ produce more eggs and more egg capsules per female, but less eggs per individual capsule. Increased egg production as food availability decreases can be explained in terms of the advantages of semelparity and iteroparity in different environments (Stearns, 1976). As food availability decreases, so does the probability of an iteroparous adult surviving for more than one breeding season. Therefore the "big bang strategy" (Gadgil and Bossert, 1970) of producing as many eggs as possible is more advantageous than that of producing fewer eggs. With higher food availability the probability of adult survival is higher, so that the number of eggs produced should optimally be the maximum possible without endangering an individual's chances of survival (Stearns, 1976). No data on the mortality of _N. pauperatus_ in the field exist but it has been observed that non reproductive juvenile _N. pauperatus_ from Coobowie died significantly sooner when brought into the laboratory and kept without food than those from Sultana Point or Stansbury and also that snails collected from Coobowie often died whilst being transported to the laboratory, and this did not occur if snails were offered *K. scalarina* when collected.

Females in low food situations also produced significantly less eggs per capsule than those in high food situations, even though egg capsules are not significantly smaller when food is scarce. Observations confirmed that egg capsules produced by the low food group were less tightly packed with eggs. This difference may simply be an artefact of producing many eggs when food is in short supply, but such a response would have a selective advantage when predation upon eggs is high (see Gillespie, 1974; Wilbur, 1977).

Apart from confirming that the level of food availability is responsible for the differences in egg production observed between populations of *N. pauperatus*, the laboratory experiment also showed that *N. pauperatus* from the same population can vary their reproductive patterns in response to different levels of food availability. Because *N. pauperatus* has a planktonic larval stage and adjacent habitats differ significantly in the availability of food, different successive generations may be exposed to selection for very different reproductive patterns. Therefore I postulate that there has been selection for individuals with a repertoire of reproductive patterns, rather than any particular one. Conversely, in species which do not have a stage capable of wide dispersal (e.g. gastropods with direct development) each population may experience selection for a particular reproductive pattern for many generations. Thus individuals of such species may not be capable of displaying different patterns in different environments.

This prediction relies on the assumption that reproductive patterns are heritable and that environments often differ considerably over the range of a species.

A more general prediction from this hypothesis is that species capable of wide dispersion will be better able to cope with environmental change than non dispersive ones since the dispersive species will be more likely to have experienced selection for a repertoire of adaptations. This, if true, might help explain the relationship noted by Scheltema (1977) between possession of a pelagic stage and persistence through geological time.

Appendix 4. Observations of shore morphology, hunger, population density and the ratio of juvenile to adult snails.

Whilst collecting snails, I made brief qualitative notes of shore morphology, substrate type etc. These are presented below along with other characteristics of each population of Nassarius pauperatus, taken from other sections of this thesis.

Location	Description of shore	Hunger (from Table 3.24)	Proportion of juvenile snails during November (from Table 3.6)	Mean population density of juveniles per 3600 cm^2 (Table 3.2)
Onkaparinga	Snails in sheltered tidal estuary, not facing open sea. Shallow pools at low tide.	-8	-	-
Mouth of Swan Alley Creek	Sheltered with intertidal pools. Tidal flow through channels may "scour" surface of sandflat.	(only two snails found at this location)		
Port Gawler	Extremely extensive intertidal pools, zone with snails about 1 km wide.	0	0.82 0.75 0.72	7.39
Middle Beach	As Port Gawler	+24.75	0.41	-
Port Prime	As Port Gawler	+1.0	0.42	-
Parham	As Port Gawler	+16.0	0.35	6.61
Port Wakefield	Zone with snails narrower than Port Gawler, still 0.5 km, still extensive intertidal pools.	+11	0.68	-
Port Clinton	Zone with snails narrower than Port Wakefield, beach more sloping, far fewer intertidal pools.	-17.08	0.26 0.15 0.23	0.30
Rogues Point	Steeply sloping shore. Zone with snails only about 2 metres. No intertidal pools.	-27.24	0.09	none found in 10 samples
Stansbury	As Rogues Point.	-30	0.10 0.05	none found in 25 samples

Location	Description of shore	Hunger (from Table 3.24)	Proportion of juvenile snails during November (from Table 3.6)	Mean population density of juveniles per 3600 cm^2 (Table 3.2)
Coobowie	"Port Gawler" type. Zone with snails about 600 metres. Extensive intertidal pools.	+40	0.49 0.52	4.80
Sultana Point	Stansbury type. No intertidal pools. Zone with snails about 2 metres.	-22	0.06 0.07	none found in 25 samples
Moonta Bay	Port Gawler type. Intertidal pools. Zone with snails 200-300 metres.	+22	-	-
Yatala Harbour	Intertidal pools. Zone with snails about 120 metres. Similar to Port Gawler type.	+8.62	sample too small	-
Lucky Bay	Unusual. Steeply sloping shore with no intertidal pools in *N. pauperatus* zone, but pools higher on shore, containing some adult snails.	-23	0.11	1.10
Port Lincoln I	No intertidal pools. Zone with snails 2-3 metres.	-32	0.08	-
Port Lincoln II	Very similar to Coobowie and Port Gawler. Zone with snails about 80 metres. Intertidal pools.	+14	0.39	-
Coffin Bay	Zone about 6 metres, few intertidal pools.	-39	0.09	-
American River	Extremely sheltered embayment. Intertidal pools similar to Port Gawler. Zone with *N. pauperatus* 15-20 metres.	-	-	-
Streaky Bay	Snails collected by others.	-	-	-
Smoky Bay		-	-	-

By comparing observations of shore morphology with hunger, the ratio of juvenile to adult snails and the population density of juvenile snails, it is apparent that populations having a high density of juvenile snails, a high proportion of juvenile snails and in which snails are relatively hungry, are usually found on shores which have extensive intertidal pools and where the zone containing *N. pauperatus* is wide. From these observations I propose that factors associated with the presence of "undrained" sandflats and a wide zone in which *N. pauperatus* can live are somehow responsible for promoting the settlement and/or subsequent survival of larval *N. pauperatus*. This results in a large number of juveniles being recruited each year at such locations. Snails at locations where recruitment of juveniles is relatively high are, simply because of the high density of juveniles, more likely to face a shortage of food and selection for Twisters. This may explain the significant correlation between the population density of juvenile *N. pauperatus* and the percentage of Twisters (Section 3.5.1) and is still consistent with the observation in Section 5.4.1, that hunger need not be correlated with the population density of adult, or of adult plus juvenile *N. pauperatus*.

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