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Ecology of wild and cultured juvenile *Trochus niloticus* relevant to the use of juveniles for population enhancement

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Thesis submitted for the degree of Doctor of Philosophy in the
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ABSTRACT

Natural stocks of the topshell *Trochus niloticus* L. (hereafter referred to as *Trochus* and trochus) have decreased significantly as a result of commercial fishing. This has led to the release of hatchery-reared juveniles being viewed as a potential tool to enhance populations. However, most experimental releases have resulted in low survival rates, with little known of the reasons for these results and how to improve them. The objectives of this study were to understand better the ecology of juvenile *Trochus* on the reef and to use this information to identify factors affecting their survival and growth.

Density, natural distribution, habitat characteristics and growth of juvenile *Trochus* and other gastropods on the intertidal reef flat at Orpheus Is., Australia, were sampled over a two-year period. *Trochus* between 1.5 mm and 62 mm shell width (SW) were found across the reef flat, but were most abundant in the middle section of the reef, 50-150 m off shore. There was no evidence of a size gradient. Mean density of *Trochus* was 0.178m^{-2} in 1993 and 0.115 m^{-2} in 1994. *Trochus* occurred in groups of 2-4 per m^2 more frequently than expected by random, but higher densities were very rarely observed. As *Trochus* size increased, there was a change from tending to occupy small rubble to occupying rock and coral bench, and from shallow to deeper pools. Growth rates estimated by progression of modal size classes ranged between 2.3 and 2.6 mm.month^{-1}.

The interaction between juvenile *Trochus* and three common invertebrate predators, the portunid crabs, *Thalamita admete* and *T. stimpsoni*, and the carnivorous gastropod, *Thais tuberosa*, were examined. Interactions with crabs were studied in the laboratory. The size of trochus eaten increased with crab size, but larger crabs continued to eat the smallest trochus offered. Crabs also attacked relatively large trochus, up to 24 mm SW with low probability of success. The combined action of *Th. admete and Th. stimpsoni* may have a significant effect on the survival of *Trochus* < 20 mm SW, but mainly on smaller individuals (< 13 mm SW). Crabs responded to an increase in trochus density from 5 to 30 individuals.container^{-1} by increasing their predation rate so that the proportion of trochus eaten after 24 h was significantly greater at higher density. When offered three patches with trochus at different densities (5, 15 and 30 trochus.patch^{-1}) in large raceways, crabs did not identify the patches of high density, but instead moved frequently around all patches. After 48h, the proportion eaten was not significantly different among the three densities. These observations of crab behaviour suggest there was no response to increased prey densities due to the crabs' mobile foraging behaviour.

Field distributions of *Trochus*, *Turbo brunneus* (another herbivorous gastropod) and *Thais* (a predatory gastropod) were compared, and the frequency of recently dead undamaged shells was used as an indication of mortality by non-crushing predators (e.g. *Thais*). Distributions of *Trochus*, *Turbo* and *Thais* overlapped and all were often found in close proximity. The proportions of undamaged shells that were recently killed was 10% for *Trochus* and 28% for *Turbo*. The behaviour of *Trochus* and *Turbo* (potential prey) to the presence of *Thais* was observed in laboratory experiments. *Thais* elicited a response from both prey species, but these differed considerably: *Turbo* showed a conventional flight escape response, whereas *Trochus* did not change speed but instead released white mucus. Cultured and wild *Trochus* showed the same response when exposed to *Thais*. *Thais* showed a strong preference for *Turbo* as prey, but the capture of *Turbo* was inhibited in water containing mucus released by *Trochus*. The mucus response of *Trochus* provides protection from predation by *Thais*.

The difficulty associated with finding trochus on the reef and how this may affect estimates of survival was examined in two experiments, one at Orpheus Is., Australia and another at Moso Is., Vanuatu, using flagged trochus (with a bright tag and easier to see) and unflagged trochus. Recapture of flagged trochus 2-3 days after the release was significantly higher than for unflagged trochus in both experiments, supporting the hypothesis that a significant proportion of trochus are overlooked by the observer. Mean sighting probability of trochus at Orpheus Is. was 0.69 for an average size of 23 mm SW and 0.81 at Moso Is. for an average size of 30 mm SW.

A series of field experiments directly related to seeding were conducted. Two field techniques were used: releasing trochus freely onto the reef and tethering trochus to rods hammered into the reef substratum. Free releases lasted between a few days to a maximum of 4 months and tether experiments lasted a maximum of eight days.

In two experiments I examined the effect of seeding density on survival and growth, one experiment at Orpheus Is. and one at Moso Is. The effect of seeding density on trochus growth was also examined at Orpheus Is. Survival was very similar between low and high seeding densities 2-3 days after the release, in both experiments. The effect of seeding density on survival after a few days was not significant at any site, but at Moso Is., significantly more trochus were recaptured in the deeper zone. One to four months after the release, survival was again not significantly different between seeding densities but growth rates of trochus released at low density were significantly greater than those of trochus released at high density. Individual growth rates ranged between 1- 5 mm.month^{-1}. The detrimental effect on growth rate suggests that trochus should be released at low rather than at high density.

In five experiments at Orpheus Is. I examined the effect of trochus size and habitat on survival. Two main patterns emerged with high consistency in the results. First, survival of larger trochus was always greater; however, survival estimates varied among experiments. Small trochus (4-12 mm SW) and medium-large trochus were lost at a rate of 8-35% and 3-8% per day, respectively, over the first few days after the release. Second, trochus survival at different depth levels on the intertidal reef flat did not vary significantly. There was high variability among replicates, even five metres apart. Such variability is likely to be due to small scale differences in habitat characteristics which affect the distribution of predators and probability of trochus being detected. Analysis of trochus loss in a tether experiment showed that in 78% of cases where the number of trochus tethered to a rod decreased, only one trochus was lost at a time. This suggests that, after encountering and eating a trochus, predators did not remain in the area long enough to find another prey.

Mortality of cultured trochus immediately after release is very high but decreases with time, as is also the case for other species where cultured individuals are released for population enhancement. The results of this study emphasise the importance of increasing survival during that first period after release. The following procedures are likely to improve survival of released trochus:

1. It is better to release larger trochus. If possible, juveniles 20 mm SW or larger should be used in seeding reef environments.
2. It is better to spread trochus over a large section of the reef to reduce the risk of releasing them in an unfavourable area.
3. It is better to release trochus at low rather than high densities.
4. In monitoring survival through time, based on recapture rates, the probability of sighting a juvenile should be determined. This probability will depend on the size of trochus used and the characteristics of the habitat.
5. Great care should be given to the condition of the seed. Behaviour can be affected by disease or poor condition. Inappropriate behaviour (such as poor antipredator responses) could have important consequences on the susceptibility of trochus to attack by predators.

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CHAPTER 1

1.1 GENERAL INTRODUCTION

Trochus niloticus L. is a herbivorous, prosobranch gastropod and the largest member of the family Trochidae, reaching a maximum shell diameter of 160 mm. It is found in the tropical and subtropical waters of the eastern Indian and western Pacific oceans (Nash, 1993). Its latitudinal distribution extends from southern Japan in the north to the southern reefs of the Great Barrier Reef in Australia (Nash, 1993).

Commercial harvest of *T. niloticus* commenced early this century (Nash, 1985) and, today, commercial trochus fisheries exist in Australia, Fiji, French Polynesia, Indonesia, Japan, New Caledonia, Papua New Guinea, Philippines, Vanuatu, and in the Cook, Caroline, Mariana, Marshall and Solomon Islands. The pearly nacre inside the trochus shell is used to make 'pearl' buttons and handicrafts, while the meat of the muscular foot is eaten fresh or dried. Total annual production of trochus shells is estimated to be around 3000 to 6000 tonnes (Bour,1992; Fao, 1992), 60-70% of which comes from Pacific and the rest from Asia (Bour, 1992). Most of the raw materials are exported to Japan, South Korea, Singapore, Hong Kong, Taiwan and Europe (Fao, 1992; Clarke and Ianelly, 1995), although processing factories have recently opened in Fiji, Vanuatu and Solomon Islands (Nash, 1993; Jimmy, 1995). In some Pacific islands, *T. niloticus* has for many years been the main source of family income (Heslinga and Hillmann, 1981; Preston, 1992). Export prices for unprocessed shell vary between years and localities, but are generally in the range of US\$2.80 to US\$10.00.kg^{-1} (Fao, 1992). To give some examples, estimated production of trochus in the Marshall Islands between 1987 and 1992, varied between 100 - 150 tonnes a year producing a revenue of US\$179,000 - 467,000 each year (Clarke and Ianelli, 1995). Shell prices in Australia were in the order of A\$1,795 per tonne in 1985 (Nash, 1985), reaching A\$9,000 per tonne in 1990 and falling by 1993 to A\$2,500 - 4,500 per tonne (Larcombe, 1993).

Decreases in trochus stocks shortly after the beginning of commercial fisheries indicate that the resource is vulnerable to overfishing (Heslinga,1981; Nash, 1985). This is most likely due to a combination of factors: 1) their intertidal and shallow subtidal habitat, which allows collection with basic diving gear (i.e. mask and snorkel) or even while walking on the reef flat, 2) their slow movement and large size which make them easily targeted by an experienced fisher, and 3) apparent slow recovery rates of depleted populations (Nash, 1985, 1993).

Management has been practiced for a long time within island communities, e.g., traditional ownership of nearshore areas, which results in restricted fishing for trochus (e.g. Amos, 1995); and the sari system in Indonesia, which consists of closing a reef for several years, and then open it for several weeks (Arifin and Purwati, 1993). However, intense overfishing has led to the implementation of other management options at a governmental level, which generally apply to whole regions. Nash (1993) reviews present options and discusses their effectiveness based on biological knowledge of the species, and Clarke and Ianelli (1995) discuss the costs and benefits of various management tools applied throughout Micronesia. The most common are:

1. Size limits: there is a minimum legal size below which trochus shells cannot be legally collected. This size limit varies among regions and is set somewhere between 60 and 90 mm of shell width (SW) (see Nash, 1993). It is the most widely used management tool. In some countries, including Australia, there is also an upper size limit, above which trochus can not be collected. These largest trochus have poorer quality shells because of boring organisms, but can still make an important contribution as part of the reproductive stock.
2. Total allowable catch: a maximum catch is designated either to individual fishers or to sections or whole regions. It is practiced in Australia, the Cook Islands and French Polynesia (Nash, 1993).
3. Limited fishing season: the time during which fishing is permitted is restricted. A number of countries practice this method. An extreme example of this practice is in Aitutaki, Cook Islands, where fishing is allowed for only few days every year (Nash et al., 1995).
4. Sanctuaries: areas are set as natural reserves were fishing is not allowed at any time. They have been established in several countries, but not in Australia (Heslinga et al., 1984; Nash, 1993).
5. Stock replenishment by adults: it consists of transplanting adult trochus to reefs where a population is either very small or completely absent. The most successful record of a transplant is that of Aitutaki, where an initial group of 40 adults was released in 1957 and seven years later the standing stock was estimated as 35,000 trochus (Marsters and Wicham, unp. data cited in Sims, 1988).

6. Stock replenishment by hatchery-reared juveniles (also referred to as seeding): consists of releasing hatchery-reared juveniles onto reef areas. The use of cultured juveniles for stock enhancement is the subject of this thesis. Observations on spawning of *T. niloticus* date back to 1932 (Moorhouse, 1932; Asano, 1991), but it was in the early 1980s that juveniles were successfully reared in tanks (Heslinga, 1981; Heslinga and Hillmann, 1981). Since then, culture methods have improved and it is now possible to produce batches of tens to hundreds of thousands of juveniles (Nash, 1989; Murakoshi, 1991). The option of seeding is appealing to many because trochus aquaculture is relatively easy and inexpensive once a hatchery has been established. Spontaneous spawning can be obtained throughout most of the year (Nash, 1985, Murakoshi, 1991, Castell, pers. obs.), and research into artificial induction is presently under way in Japan and Australia (Murakoshi, 1991; C. Lee, personal communication). Trochus have a short larval life of 3-7 days at 27-29 °C, and the larvae are lecithotrophic and do not require external food supply (Heslinga, 1981). Once they settle, resulting juveniles feed on bacteria and the microscopic and filamentous algae that grow on various substrates (Nash, 1989; Murakoshi, 1991; Castell, pers.obs). As juveniles grow, their food and space requirements rapidly increase and it soon becomes difficult to keep large numbers in tanks. Thus, growing trochus in tanks to the adult or marketable size is currently not economically feasible.

1.2 History of seeding

Population enhancement through the release of hatchery-reared seed stock has been practiced for many years with a variety of species, including fishes, crustaceans and various molluscs such as abalone, clams, conch, oysters and scallops (e.g. Flagg and Malouf, 1983; Saito, 1984; Liu, 1990; Solazzi et al., 1991; Shield, 1993; Stoner, 1994).

Seeding with juvenile *T. niloticus* was first attempted in Palau with no success, since 100% mortality occurred within the first few days (Heslinga cited in Nash, 1988). Research on seeding in New Caledonia commenced in the late 1980s (Hoffschir et al., 1989) and has extended more recently to Japan, Vanuatu, Kosrae, Indonesia and Australia, (Amos, 1991; Kubo, 1991; Tsutsui and Sigrah, 1994). Estimates of survival have been highly variable. Possibly the best results were obtained in Vanuatu, where a total of 1,400 juveniles of 20 mm mean SW were released at Erakor Is. Twenty eight percent were estimated to have survived after 13 months (Amos, 1991; Nash, 1993). Hoffschir et al. (1989) released a total of 5,000 juveniles between 18-23 mm mean SW in 20 different locations in New Caledonia. Their estimates of survival three months after the release ranged between 0 - 47%, varying with location.

The importance of understanding the ecological processes that determine the fate of released populations prior to engaging in large scale releases has been emphasised by various authors (e.g. Hatcher et al., 1993; Stoner, 1994). Experimental seeding with juvenile *T. niloticus* has consisted largely of releasing trochus of certain sizes at one or more locations and monitoring the areas periodically to estimate survival. This trial and error method results in failures as well as successes, but, more importantly, it does not provide an ecological understanding leading to a more predictable increase in success rate (Hatcher et al., 1993). As a logical first step in research on seeding we need to ask the general question of how best to release juveniles so as to achieve the highest survival. More specifically, we need to determine what size of juvenile to release, what habitat characteristics and at what density. Research on other species has shown that a variety of factors can have significant effects on the outcome of releases, including size, density, habitat, substrate type and inappropriate behaviour of cultured individuals (e.g. Flagg and Malouf, 1983; Boulding and Hay, 1984; Stoner, 1989; Schiel, 1992; Ray et al., 1994).

Some experiments have tried to determine if releasing juvenile *T. niloticus* already attached to a substratum, such as branching dead coral and flat stones (Kubo, 1991), or if providing cover from potential predators (Moses, 1991) improved survival. Kubo (1991) found that survival of juveniles attached to dead branched coral was higher than of those attached to flat stones in one site but lower in another site. He suggested rough weather conditions as an explanation of the lower survival in one of the sites (Kubo, 1991). It is generally considered that the larger the trochus released, the greater survival. Laboratory experiments on predation in Japan found that most predators consumed small trochus (10 mm SW) at a higher rate than larger trochus (17 mm SW) (Kubo, 1991). In an experiment where large (27-32 mm SW) and medium (20 mm) size juveniles were released, the larger juveniles survived better (Kubo, 1992). However, in most other experiments where different sizes of juveniles were used or juveniles were released at different intertidal levels, it has been difficult to separate potentially confounding effects such as time of release and location (i.e. different reefs).

There is still much unknown about what factors affect survival of juvenile *Trochus* and what are favourable conditions to release juveniles. This lack of knowledge, combined with the highly variable results of seeding trials, leaves open to question the application of seeding (Sims, 1988; Larcombe, 1993; Nash, 1993; Clarke and Ianelli, 1995).

1.2 Outline of this thesis

This study pursued the question of how to release juvenile *Trochus niloticus* from a number of perspectives. One perspective, described in Chapter 2, consisted of monitoring a natural population of juveniles. Detailed information on the distribution and natural densities of juvenile *T. niloticus* is scarce, largely because of the difficulty in finding them. Such information would provide essential ecological information of relevance to seeding (e.g. Stoner and Sandt, 1991). A second approach examined the interaction between three predators of juvenile *T. niloticus*, the portunid crabs *Thalamita admete* and *T. stimpsoni*, and the carnivorous gastropod *Thais tuberosa*. This study is reported in Chapters 3,4 and 5. Chapter 3 describes laboratory experiments with crabs to determine their capacities to prey upon trochus and Chapter 4 describes the effect of trochus density on their predatory behaviour. Chapter 5 reports laboratory and field observations on the interaction of *Thais tuberosa* with *Trochus niloticus* and *Turbo brunneus*, another common inhabitant of the intertidal reef zone. This study considered the behavioural response of these two herbivorous gastropods to their predator, *Thais*, and their effectiveness. The material in this chapter has been accepted for publication (see Appendix 1). The choice of predators was based on their relative abundance and the logistics associated with the work. A third approach was directed towards the question of how to release trochus and includes Chapters 6, 7 and 8. Chapter 6 addresses the problem of finding trochus on the reef and how this may affect estimates of survival in seeding experiments. I did this by comparing recapture of inconspicuous and conspicuously marked trochus in two experiments. The material in this chapter has been accepted for publication (see Appendix 1). Chapters 7 and 8 deal with the questions of at what size, at what density and where to release trochus. Chapter 7 examines the effect of seeding density on survival and describes two field experiments. Chapter 8 examines the effect of trochus size and location of release in a series of five field experiments. Throughout the chapters I have tried to interpret the observations and experimental results within the framework of trochus seeding. Chapter 9 presents an overall discussion of the study.

To simplify reading for the next chapters, I will refer to *Trochus niloticus* as *Trochus* except when mentioning other species within the genus, in which case the name *T. niloticus* will be used. The term trochus will be used as a common name for *Trochus niloticus*.

CHAPTER 2

Population studies of juvenile *Trochus niloticus* and other gastropods on a reef flat on the North Queensland Coast, Australia.

2.1 Introduction

Some aspects of the biology of *Trochus* have been well studied, including growth rates of adults (> 60-70 mm maximum shell width) (SW), adult habitat, spawning behaviour and larval development in the laboratory (e.g. Rao, 1937; Asano, 1963; Heslinga, 1981; Nash, 1985; Bour, 1992). Adult *Trochus* grow to 160 mm SW and are generally found subtidally on the windward side of coral reefs (Bour, 1992; Nash, 1993), whereas juveniles (< ca. 60 mm SW) live on the reef flat, sometimes exposed at low tide (Heslinga et al., 1984; Nash, 1985; Bour, 1992). Very little is known about the ecology of juvenile *Trochus*. Our knowledge of wild juvenile *Trochus* is limited to a few studies (i.e. Heslinga et al., 1984; Nash, 1985; Smith, 1987; Bour, 1992) and there is no detailed information on aspects of their ecology. In particular, very little is known about the smaller juveniles (< 20 mm), which may represent the most economic option for population enhancement. Many authors have commented that, in spite of specific attempts to search for juveniles, individuals smaller than 30 mm SW are rarely found (Rao, 1937; Heslinga et al., 1984, Nash, 1985; Smith, 1987; Kubo, 1991; Arifin and Purwati, 1993; Gillet, 1994). This is partly due to their size, colour and behaviour, all making them cryptic (Heslinga et al., 1984; Nash, 1985).

The importance of basic information on juvenile ecology for population enhancement is well recognised (i.e. Shepherd and Turner, 1985; Stoner and Sandt, 1991; Hatcher et al., in press). Where do juveniles live? what are their natural densities? what other organisms share their habitat? are questions that come to mind when considering the minimum information needed for stock enhancement.

This chapter describes the distribution and natural densities of wild juvenile *Trochus* and other common gastropod species inhabitants of a reef flat. Recruitment and growth rates of *Trochus* are also described. The applicability of these results for stock enhancement are then discussed.

2.2 Study area

Observations were made between June 1993 and May 1995 on an intertidal reef flat on the north east side of Orpheus Is., a continental island located approximately 15 km off the North Queensland Coast, Australia (18°34'S, 146°29'E) (Fig. 2.1). The reef is exposed to south-east trade winds between March and October and north-east monsoon winds between October and March.

Adult *Trochus* live subtidally and are relatively abundant along a stretch of this coast approximately 800 m long (e.g. two snorkel divers can collect 60 adults in approximately 15 minutes). The population is not fished. Juvenile *Trochus* live on the intertidal reef flat, which consists of abundant coralline rubble and coral bench (massive flat coral rock), with sand and colonies of live coral patchily distributed. The average width of the reef flat is 170 m (range 120 - 220 m), with a very shallow slope. It is approximately 1.5 km long and is exposed for periods of up to five hours in a semi-diurnal tidal cycle. The maximum tidal range during the study was 3.69 metres. At the beach edge of the reef there is a clear change in substrata from igneous boulders to coralline rubble.

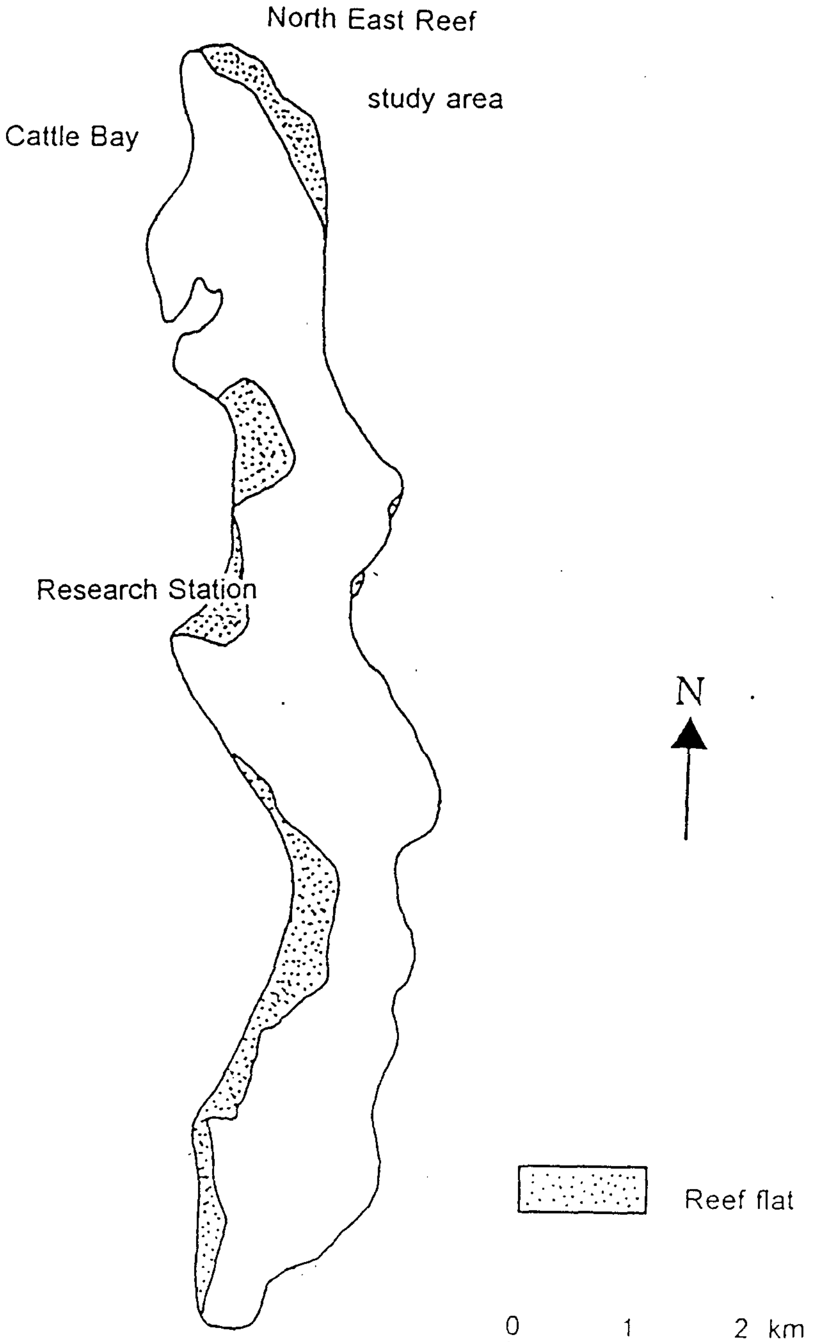
2.3 Methods

Distribution and density of *Trochus* along the intertidal reef were determined using transects 100 - 200 metres long, perpendicular to the shoreline, during June to September 1993 (N=7 transects) and July to August 1994 (N=9 transects). All sampling was done while walking on the reef within two hours of low tide. The length of the transects was not constant because of natural variation of the reef flat. Distance between transects ranged 20-50 m and they were systematically spread to cover most of the reef flat. Transects started at the beginning of the reef flat, after the boulder zone on the beach end, and extended to about 40 m from the reef crest. The last 50 metres were not included in this study because the area was generally covered with water resulting in inefficient searching. Whenever possible the area was quickly checked for *Trochus*, which seemed to be uncommon in this area. In each transect, one quadrat ($1m^2$) was sampled every five metres. The number of quadrats was increased to two in 1994 in an effort to increase sample size of recorded *Trochus*. Sampling consisted of a thorough search of the substrata, picking up and examining individual pieces of rubble of all sizes, and searching through algal mats and rock crevices. All *Trochus* found were measured (maximum shell width (SW)) and the following characteristics of the microhabitat they occupied were noted: 1) type of substratum where *Trochus* were attached: small coral rubble (< 10 cm longest axis), rock (> 10 cm) or coral bench; 2) position of attachment to the substratum: on top, sides or underneath and, 3) depth of pool where *Trochus* were found: < 10 cm or > 10 cm. Although there were tidal changes throughout each sampling period, it was easy to distinguish the intertidal pools, since they remained filled with water even at times of very low tides. A general description of the reef habitat was obtained in 1994 by measuring five characteristics within all quadrats: sand, algae, live coral, rubble (i.e. not coral bench) and intertidal pools. A qualitative index from 0 to 5 was used to estimate abundance: 0 = none (0%), 1 = very little (5 - 10%), 2 = little (15 - 25%), 3 = medium (30 - 60%), 4 = abundant (65 - 80%), 5 = very abundant (85 - 100%). Thus, there were characteristics to describe broadly the reef habitat and characteristics to describe the localised area where an individual *Trochus* was found. All dead trochus were counted and the condition of the shell recorded (long exposed to weathering, recently dead, crushed, undamaged, drilled, chipped aperture lips) to use as an indication of the cause of mortality. Trochus were grouped into 10 mm size classes for the distribution analysis and in 5 to 10 mm size classes for the habitat analysis.

The frequency distribution of *Trochus* per 1m^2 quadrat was used to determine if they were randomly distributed or aggregated. To do this I used a randomisation (Crowley, 1992) to test the null hypothesis that *Trochus* were randomly and independently distributed. I generated 4999 sets of 799 random numbers (representing the 799 quadrats in the field study). The random numbers were drawn from a Poisson distribution with mean set to the average density of *Trochus* over the 2 years of sampling. For each sample set, I recorded the number of quadrats containing more than one *Trochus* and found the proportion of the random model samples that had more such quadrats than occurred in the field data.

To determine if *Trochus* abundance was random along the reef flat, the reef was arbitrarily divided into four sections: 0-45 m (adjacent to the boulder beach), 50- 95 m, 100 - 145 m and > 150 m (approximately 40-50 m from the reef crest), and the density of *Trochus* within each zone was estimated for each transect. Mean densities among zones were compared using a single factor analysis of

Fig. 1 Study site at Orpheus Is.



variance, and following significant difference from the anova test, treatment means were compared using the T3 method (Day and Quinn, 1989).

Other gastropod species common in the area were also sampled: 1) herbivorous gastropods: *Turbo brunneus*, cowries (*Cypraea annulus* and *C. erroneus* being the most abundant), spider shell (*Lambis scorpius*), abalone (*Haliotis* spp.); 2) carnivorous gastropods: *Thais tuberosa*, *Morula biconica*, *Peristernia australiensis*, *Morula granulata* and *Conus* spp. Other very small and common species, such as small Cerithiidae and *Engina* sp. were not included because their analysis would have increased the sampling time to impractical levels. Densities between years were compared individually by species using t tests.

Additional information on recruitment and distribution of *Trochus* was obtained throughout the year by monthly or bimonthly sampling. The areas sampled during these visits were not randomly chosen since they formed part of a concurrent study but they were still located within the general study area. Sampling effort was not constant during these visits and consequently they were not used to estimate relative abundance of trochus across the reef. The areas sampled were located in the general study area and in most cases overlapped but sampling intensity was not constant. Whenever *Trochus* were found, they were measured, the distance of their location to the beach was recorded, and characteristics of the substratum to which they were attached, position and water depth if in an intertidal pool (as explained above) were also recorded.

To compare abundance of *Trochus* recruits in particular areas of the reef, rubble samples were taken from the reef slope, reef crest and intertidal flat, and treated using an anaesthetic technique developed for abalone (Prince and Ford, 1985). Briefly, all rubble present inside a 50 x 50 cm quadrat was collected in a plastic bag for later processing. In the laboratory, the contents of the bag were immersed in 10% ethanol for 10 minutes. Ethanol anaesthetised all fauna associated with the rubble and these animals fell to the bottom of the container. The dislodged material was collected with a sieve and examined under the microscope. Samples were taken during November and December 1992, January and February 1993. Eight replicate quadrats were sampled in each of the three areas. During December 1992 only the intertidal flat and the reef crest were sampled, and in January 1993 only the reef slope was sampled, due to rough weather.

The size frequency distribution of *Trochus* on the reef flat between June 1993 and May 1995 was analysed using modal progression analysis (NORMSEP method in FISAT). This information was used to identify periods of more intense recruitment during a year, and to estimate growth rates. Regression lines were fitted to individual modal classes through time to obtain growth rates. To look at the pattern of recruitment with respect to water temperature, records were obtained by the Great Barrier Marine Park Authority from data loggers located on the reef flat at Cattle Bay, adjacent to the study site.

2.4 Results

2.4.1 Distribution and density of *Trochus niloticus*

Macrohabitat characteristics of two transects are shown in Fig. 2.2. These two transects were chosen because they illustrate the variability among transects and represent effectively the observed pattern. The first 20-40 metres width of the reef flat were characterised by a large intertidal pool which remained under water even during the lowest tides of the year. The substrata in this pool area consisted of abundant small and large rubble (a few centimetres to 40 cm longest axis) on top of a layer of sand. Algae were abundant over the reef flat, in the sense that they were always present covering the coral rock but not forming dense mats. The presence of live corals was limited to a very few small colonies of massive or branching coral patchily dispersed along most of the reef flat and becoming more abundant towards the reef crest. The last 40 - 50 m of the reef crest were characterised by large patches of coarse sand between live colonies of the branching coral, *Acropora* sp. (Fig. 2.2a)

The transect surveys showed that trochus were not randomly distributed along the shore, but they were most common in the middle section of the reef flat, between 50 - 145 metres from the beach (one way anova $F(3,56)=4.09$, $p=0.011$) (Table 2.1, Figs. 2.2 and 2.3). Densities were not significantly different between the internal and external edges of the reef flat and between the two intermediate zones ($p>0.05$ for both comparisons), but they differed significantly between the edge and the middle section ($p<0.05$). The size range of individuals found on the intertidal reef flat was 1.5 - 62 mm SW. There was no evidence of a gradient in juvenile size from the shore to the reef crest (Fig. 2.3).

Fig. 2.2 Habitat characteristics along two transects (a and b) perpendicular to the shoreline on the reef flat at Orpheus Is. See methods for explanation on the index of relative abundance. Top graph corresponds to the distribution and number of juvenile *Trochus niloticus* found along the transect.

Transect a

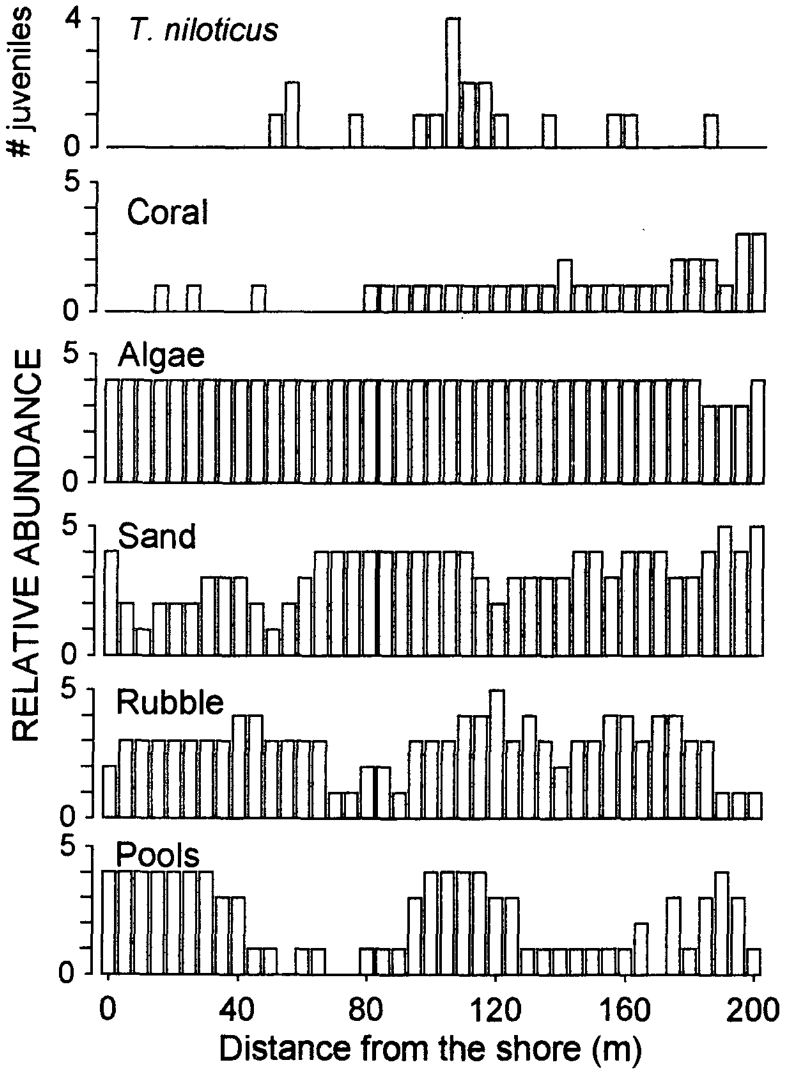


Fig. 2.2 cont.

Transect b

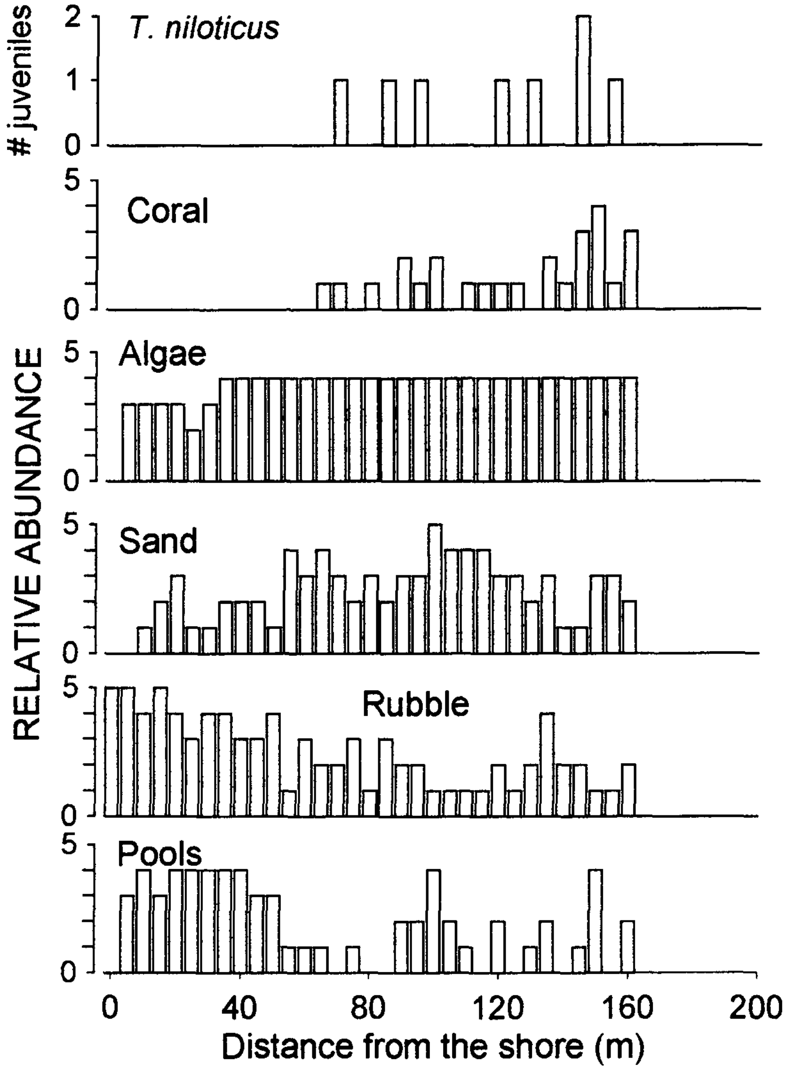


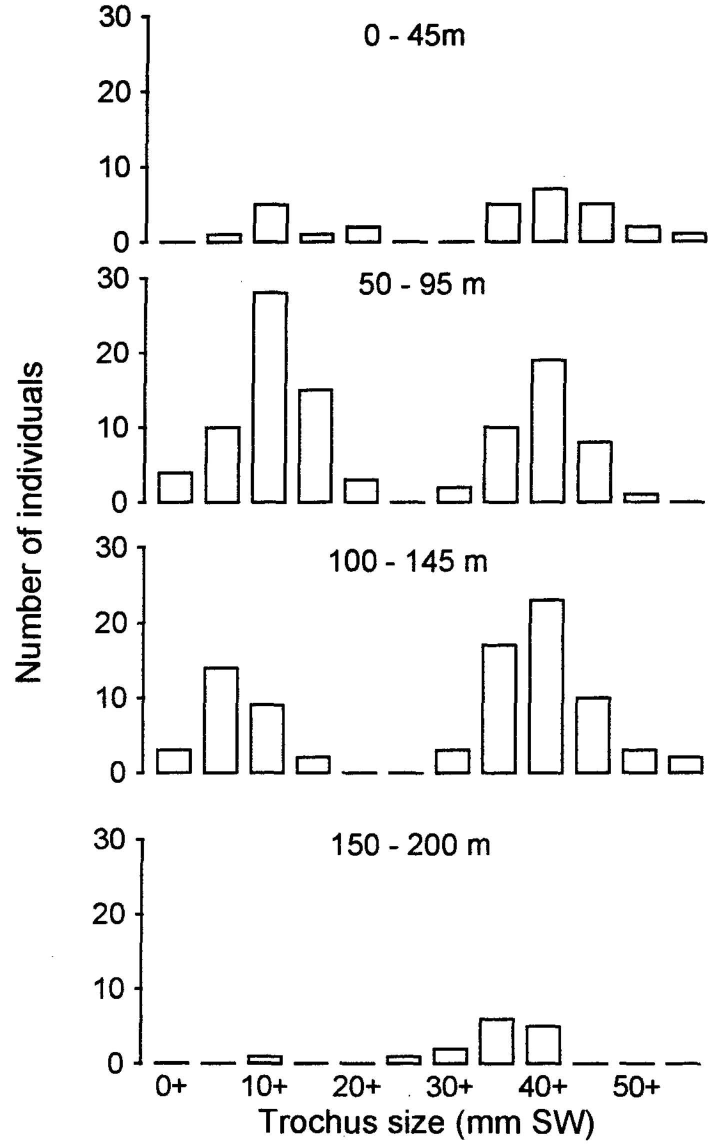
Table 2.1 Density of juvenile *Trochus niloticus* on different sections of the reef flat at Orpheus Is., divided according to their distance from the shore. The data from transect surveys in 1993 and 1994 were pooled. N=number of estimates of density for each section.

Reef Section	Density (ind.m^{-2})	SE	N
0 - 45 m	0.073	0.022	15
50 - 95 m	0.172	0.029	16
100 - 145 m	0.199	0.055	16
$\geq$ 150 m	0.049	0.020	13

Table 2.2 Density of juvenile *Trochus niloticus* on the intertidal reef flat at Orpheus Is. estimated by transect and quadrat surveys. Total area searched was 209 m^2 (June - September) in 1993 and 590 m^2 (July - August) in 1994.

	Individuals.m^{-2}	
Transect	1993	1994
1	0.108	0.133
2	0.120	0.050
3	0.091	0.117
4	0.389	0.100
5	0.190	0.031
6	0.348	0.244
7	0.000	0.192
8	--	0.109
9	--	0.058
Mean (SE)	0.178 (0.054)	0.115 (0.023)

Fig. 2.3 Distribution and abundance of juvenile *Trochus niloticus* on the intertidal reef flat at Orpheus Is. The reef was divided into four sections based on distance from the shore. These data are pooled observations between 1993 and 1994. SW= shell width. Individuals were grouped in 5 mm size classes.



Mean density of *Trochus* was 0.178 individuals.m^{-2} in 1993 and 0.115 individuals.m^{-2} in 1994 (Table 2.2). There was no significant difference in density between 1993 and 1994 (t test df[2,14] p=0.3081). Variability was high among replicate transects.

Of a total of 799 m^2 quadrats sampled in transects in both years, 104 quadrats (13%) had juvenile *Trochus*. Of these, 89 (86%) had only one individual, 14 (13%) had two individuals and one quadrat (1%) had four individuals. The proportion of quadrats that had more than one individual was significantly higher than expected by random (Randomisation test, p(n>1)= 0.0024). Additional sampling done outside transects showed the same pattern: of 129 quadrats with Trochus, 97 (75%) had only one individual, 24 (19%) had two individuals and 8 (6%) had three individuals. Small and large trochus were not found together in 94% of cases: of 47 quadrats with more than one trochus, 32 quadrats (68%) had all of them > 30 mm SW, 12 quadrats (26%) had all trochus < 30 mm SW and three quadrats (6%) had a combination of small and large trochus.

There were some differences in the microhabitat occupied by trochus of different size classes (Figs. 2.4, 2.5 and 2.6). Trochus < 15 mm SW were found most frequently attached to very small fragments of coralline rubble (Fig. 2.4). It was not unusual to find them on rubble as small as two centimetres longest axis (e.g. an old mussel shell covered with coralline algae). As they increased in size the preferred substratum shifted towards larger rocks and coral bench: trochus 15-29.5 mm SW were found in similar proportions in all three types of substrata, but trochus $\geq$ 30 mm were very rarely found on small rubble (Fig. 2.4). A similar shift in habitat from small to large juveniles is shown by the data on intertidal pools: trochus < 15 mm were most common in areas covered by only few centimetres of water at low tide (Fig. 2.5). With increased size, they were mostly found on deeper intertidal pools. Areas that appeared favourable for large trochus were ledges in the coral bench in deeper pools. With respect to position, trochus of all sizes were found either on the top, sides or underneath of rocks, rubble or coral bench, with no clear pattern of preference or change among size classes (Fig. 2.6).

Eighty four dead *Trochus* were found within the transects. They ranged from 4.5 mm to 60 mm SW. Forty one were relatively new. Most of the shells were either crushed, with chipped lips or with large holes. Of the shells that were relatively new, 4 (10%) had no damage to the shell and 37 (90%) were damaged.

Fig. 2.4 Frequency distribution of juvenile *Trochus niloticus* across sizes found on small coral rubble (< 10cm longest axis)(white bars); on rocks (> 10 cm)(shaded bars) and on the coral bench substratum (black bars), on the reef flat at Orpheus Is. Numbers above bars are sample sizes. SW= shell width

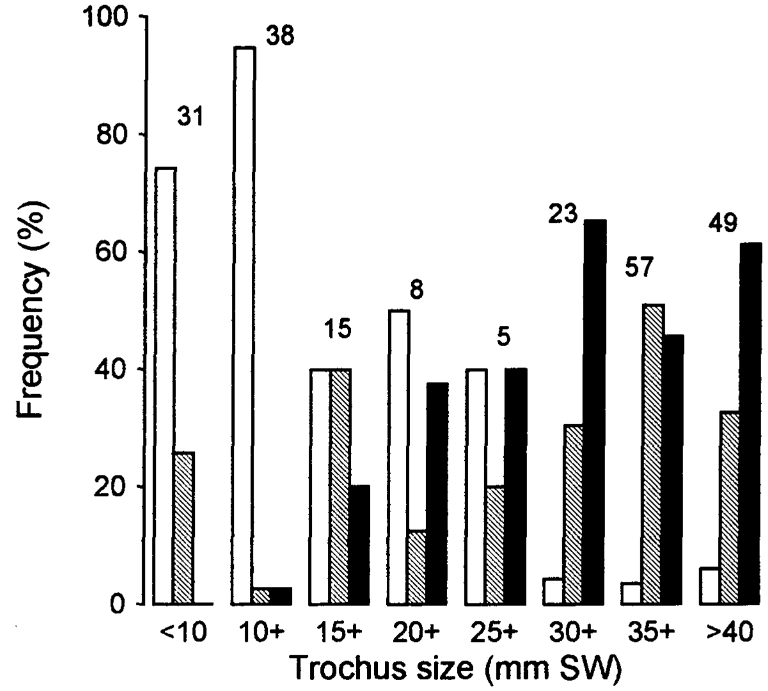


Fig. 2.5 Frequency distribution of juvenile *Trochus niloticus* across sizes found in shallow (< 10 cm of water at low tide)(black bars) and deep (> 10 cm)(white bars) intertidal pools on the reef flat at Orpheus Is. Numbers above bars are sample sizes. SW= shell width.

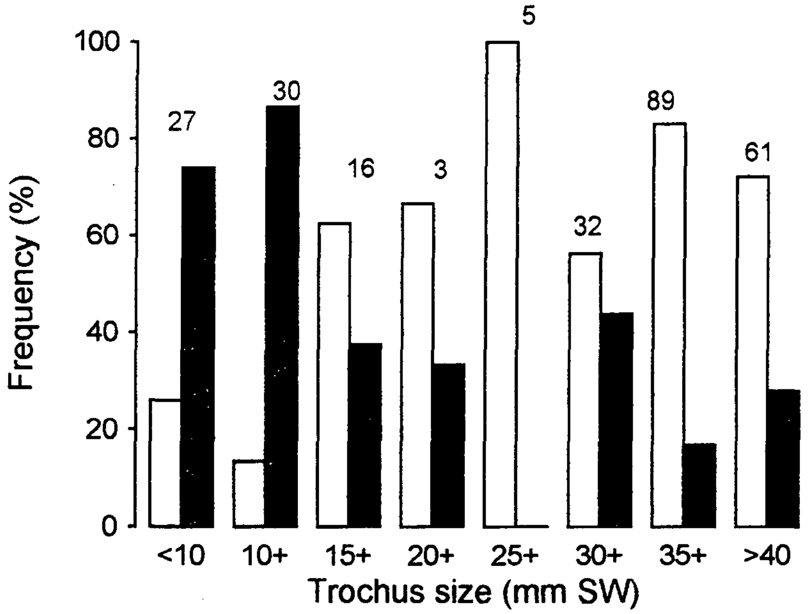
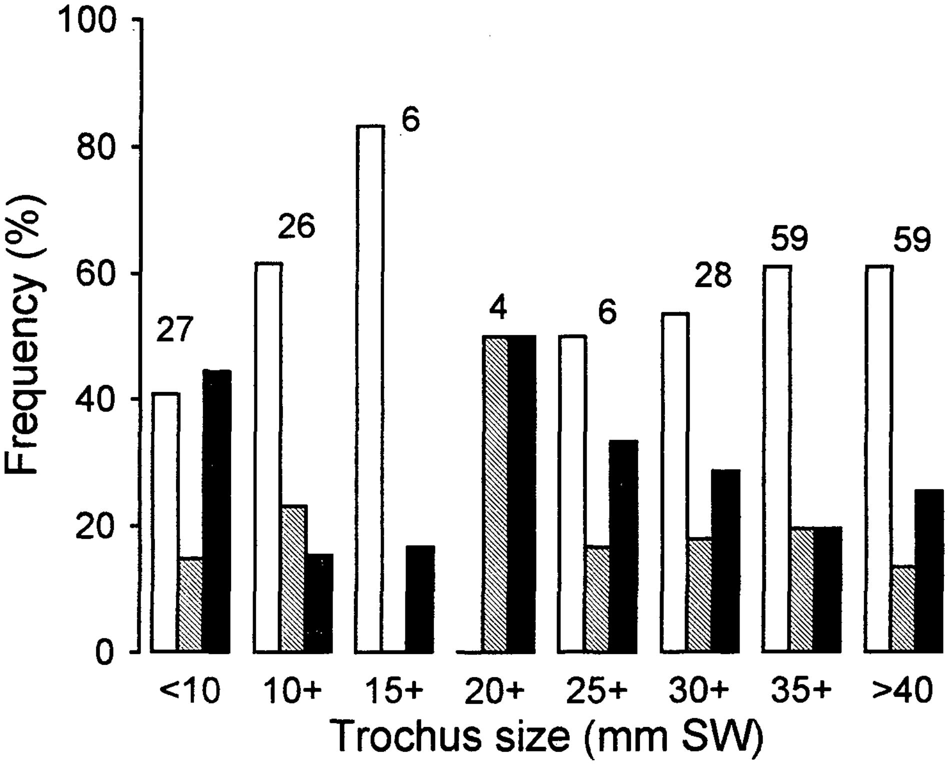


Fig. 2.6 Frequency distribution of juvenile *Trochus niloticus* across sizes found attached on the top (white bars), sides (shaded bars) and underneath (black bars) of the substratum, on the reef flat at Orpheus Is. Numbers above bars are sample sizes. SW= shell width



2.4.2 Density and Distribution of other gastropods

All other herbivorous gastropods studied had similar distributions to *Trochus*, with fewer individuals within the most inshore 40 metres of reef flat (Fig. 2.7). Occasionally, individuals of *Trochus pyramis* and *Trochus sp.* were found on the reef flat, apparently sharing the same habitat as *T. niloticus*. Carnivorous gastropods showed more variable distribution, with some species showing clear zonation patterns (Fig. 2.7). *Thais tuberosa* was rare in the first 40 metres; *Conus* spp. were distributed all across the reef but most commonly in the central section. *Morula biconica*, *Peristernia australiensis* and *Morula granulata* were most typical of the inshore section of the reef flat. *M. biconica* and *P. australiensis* were very abundant within this zone: the maximum density found was 25 individuals.m^{-2}, although typical densities were 2 - 6 individuals. m^{-2}. *Morula fiscella*, a carnivorous gastropod of similar size to the previous three species, was practically absent in the first 80 m of reef flat where *M. biconica* and *P. australiensis* were common. These three species were observed feeding on *Cerithium* spp. 6 - 16 mm SL, *Turbo brunneus* 8 mm SL and an unidentified small Turbinidae (5 - 6 mm SW).

Cowries were the large herbivorous gastropods which were most abundant in both years (Table 2.3). *Turbo brunneus* had a higher density than *Trochus* in 1993, but densities were not significantly different in 1994 ($p > 0.1$, Table 2.3). *Conus* spp. were the most abundant carnivorous gastropods in both years, followed by *M. biconica* and *P. australiensis*. Densities of *Thais tuberosa* and *M. fiscella* were similar in both years. Overall, Cypraeidae, Muricidae and Buccinidae were the most abundant large-sized gastropod families on the reef flat, followed by Turbinidae and Trochidae (but see previous comment about Cerithiidae and *Engina* sp.). Mean density of all herbivorous gastropods sampled was significantly lower in 1994 than in 1993 ($p= 0.03$, Table 2.3). This difference was driven by the declines in *T. brunneus*, *Haliotis* spp. and *Cypraea* spp. Mean density of carnivorous gastropods did not differed significantly between years.

Fig. 2. 7 Distribution and abundance of *Trochus niloticus* and other common gastropods on the intertidal reef flat at Orpheus Is., during 1993 (a) and 1994 (b).

(a)

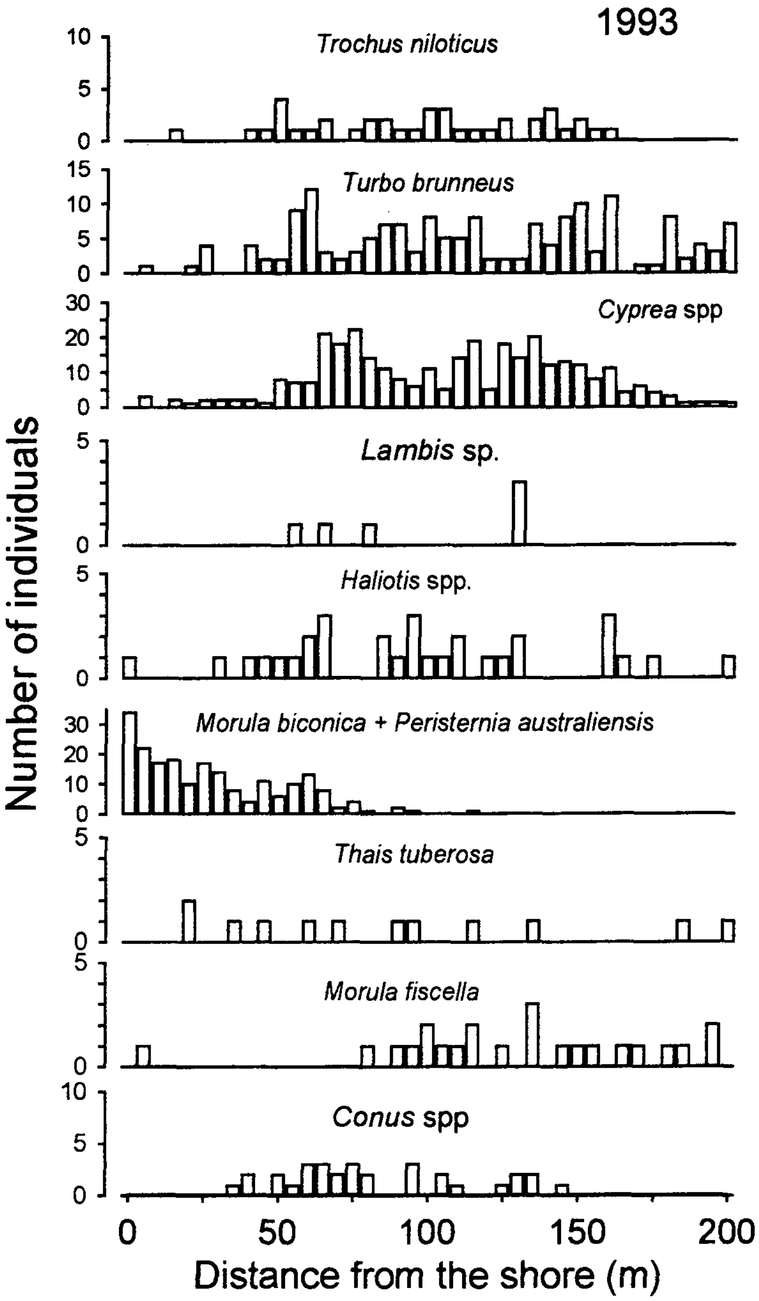


Fig. 2.7 Cont. (b)

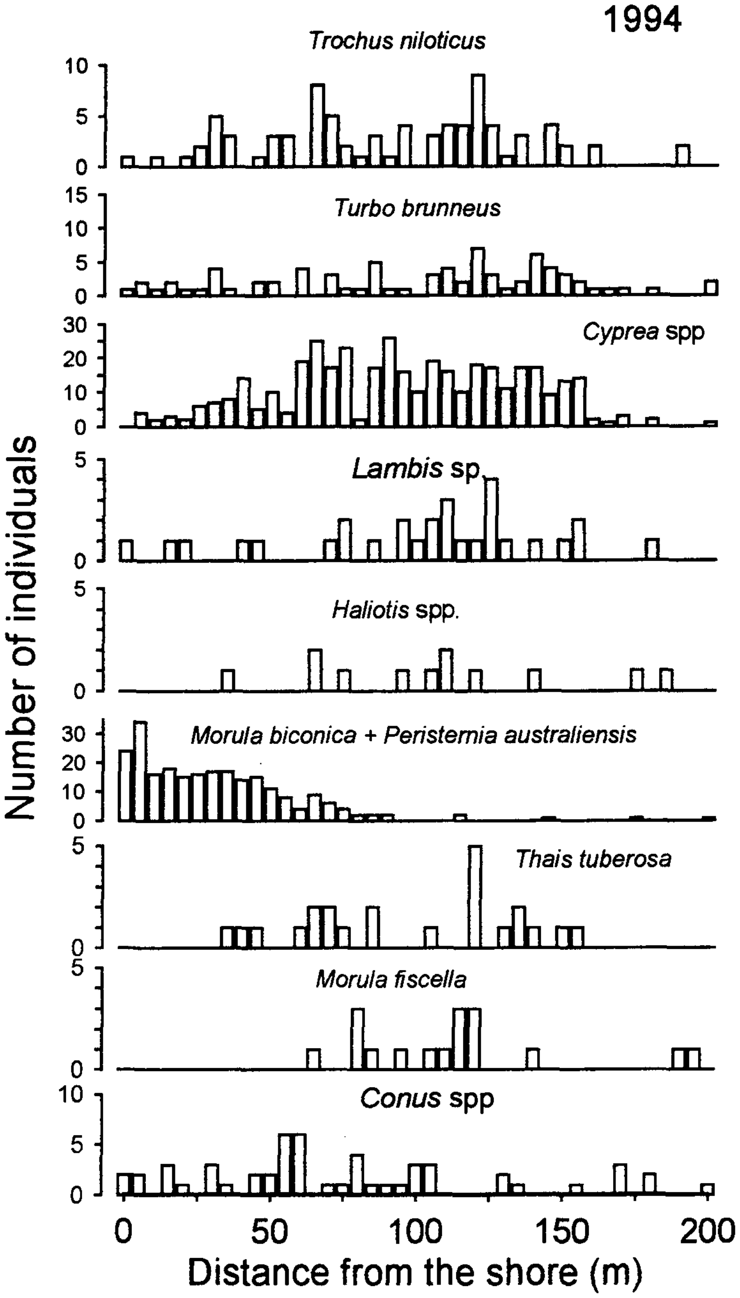

1994
Trochus niloticus
Turbo brunneus
Cyprea spp
Lambis sp.
Haliotis spp.
Morula biconica + Peristernia australiensis
Thais tuberosa
Morula fiscella
Conus spp
Number of individuals
Distance from the shore (m)
0
50
100
150
200
10
5
0
15
10
5
0
30
20
10
0
5
0
5
0
30
20
10
0
5
0
5
0
10
5
0

Table 2.3 Mean density (individuals.m^{-2}) (± se) of *Trochus niloticus* and other common gastropods of the intertidal reef flat at Orpheus Is., during 1993 and 1994. p= probability value of t test to compare density between years, N=7 transects for 1993 and N=9 for 1994, * significant difference at α=0.05.

SPECIES	1993	1994	p (t test)
HERBIVOROUS			
Trochus niloticus	0.18 ± 0.05	0.12 ± 0.02	0.31
Turbo brunneus	0.77 ± 0.10	0.13 ± 0.02	< 0.01 *
Cypraea spp.	1.54 ± 0.36	0.69 ± 0.14	0.05 *
Haliotis spp.	0.14 ± 0.03	0.02 ± 0.01	< 0.01 *
Lambis sp.	0.03 ± 0.01	0.05 ± 0.02	0.24
all Herbivorous	2.16 ± 0.39	1.08 ± 0.18	0.03 *
CARNIVOROUS			
Thais tuberosa	0.08 ± 0.02	0.04 ± 0.01	0.06
Morula fiscella	0.09 ± 0.04	0.03 ± 0.01	0.19
M. biconica and *Peristernia australiensis*	0.97 ± 0.38	0.40 ± 0.06	0.19
M. granulata	0.02 ± 0.02	0.05 ± 0.01	0.28
Conus spp.	0.15 ± 0.03	0.08 ± 0.01	0.06
all Carnivorous	1.09 ± 0.31	0.67 ± 0.09	0.23

2.4.3 Recruitment and growth of *Trochus*

The size frequency distribution of *Trochus* on the reef flat was often bimodal, with two cohorts clearly differentiated between June and September 1993 and in June and July 1994 (Fig. 2.8). In June 1993, when sampling began, the population consisted of one cohort of small juveniles with a modal mean of 10.5 mm SW and a second cohort with modal mean size of 39.9 mm SW. Modal mean size of the first cohort of small juveniles was 32.7 mm by February 1994 (Fig. 2.9). The larger cohort, which could only be followed for the first few months, had a modal mean size of 49.2 mm by September 1993. A third cohort of new recruits appeared in March 1994 and was well developed by July 1994 (modal mean 8.7 mm SW). Estimates of growth rate obtained by regression analysis were 2.27 and 2.35 mm.month^{-1}, respectively, for the first and third cohorts (small juveniles) and 2.57 mm.month^{-1} for the larger second cohort (Fig. 2.9).

Only five *Trochus* recruits between 1-2 mm SW were found in 79 rubble samples analysed microscopically: two recruits of 1.2 mm SW were found in samples from December 1992 and three recruits between 1.0 and 1.5 mm SW were found in the samples from February 1993. All recruits came from samples collected in the intertidal reef flat. Five newly recruited individuals were also found in quadrats visually censused between March and July 1994 (Fig. 2.8).

Fig. 2.10 shows the monthly average water temperature in Cattle Bay, at Orpheus Is. during 1993 and 1994. During the winter months, between May and September, temperatures were between 21 - 24 °C.

Fig. 2.8 Size frequency distribution of juvenile *Trochus niloticus* during June 1993 to May 1995, on the intertidal reef flat at Orpheus Is. Numbers in parenthesis indicate the size of the area sampled in m^2.

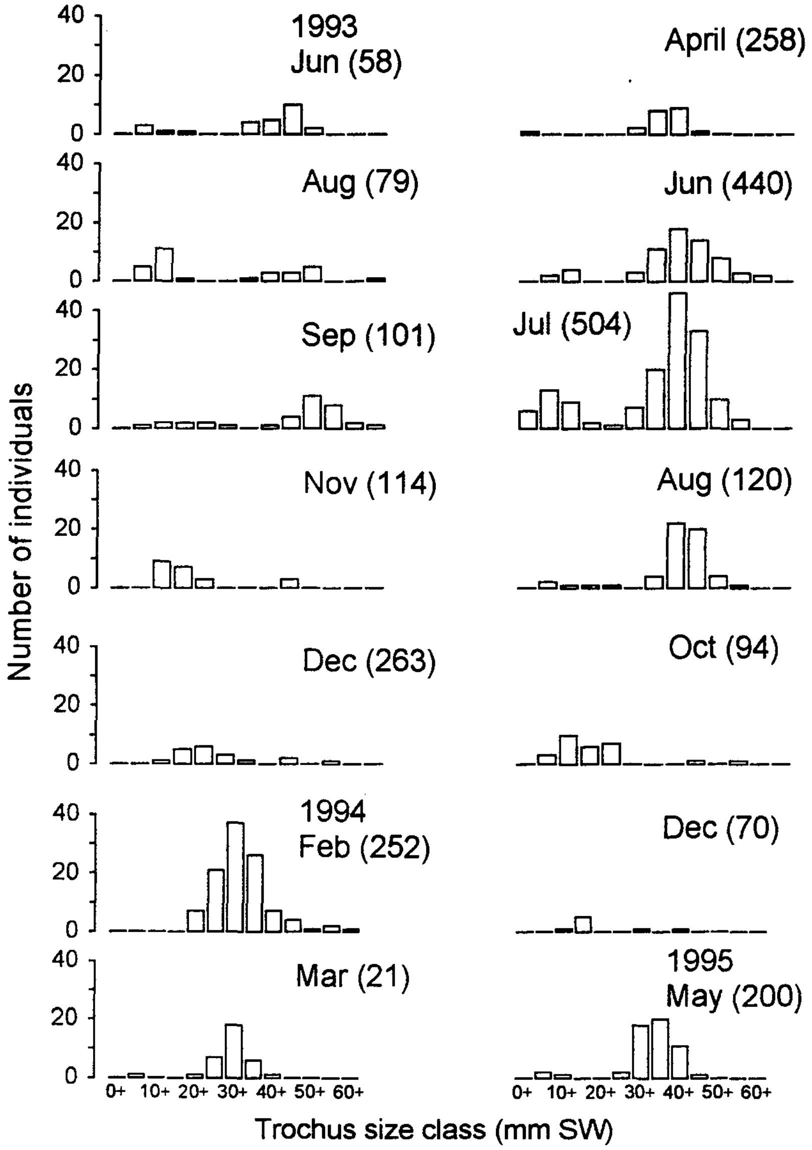


Fig. 2.9 Progression of modal size classes of juvenile *Trochus niloticus* from June 1993 to May 1995 on the intertidal reef flat at Orpheus Is. Regression lines were fitted to the data. Y=trochus size (mm shell width (SW)), X= time in days. Raw data is shown in Fig. 2.8.

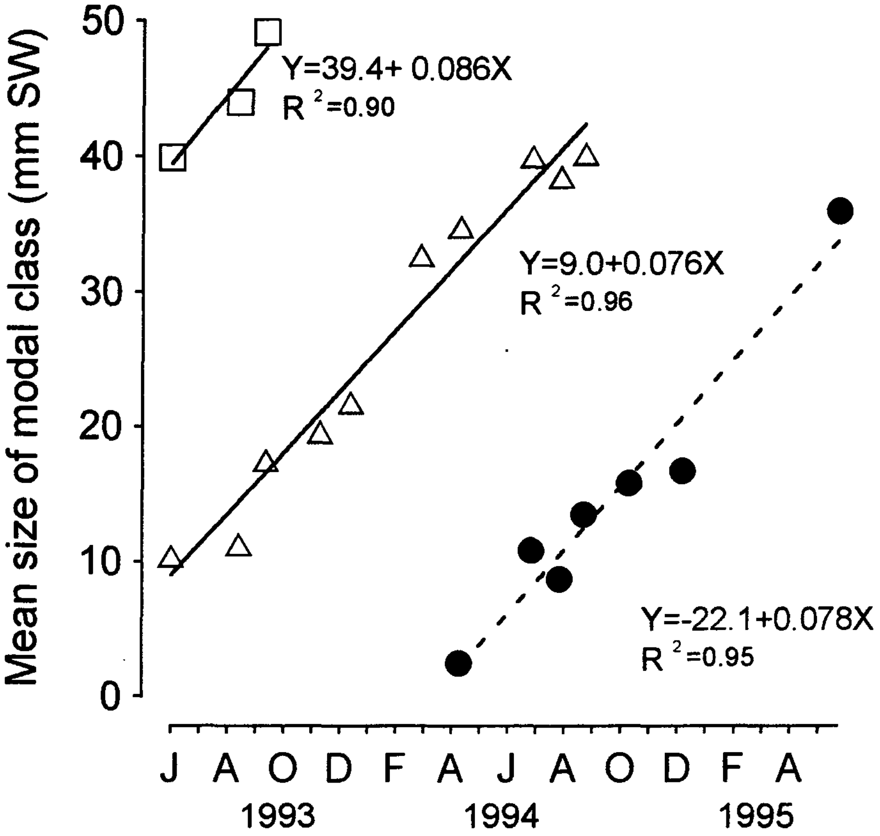
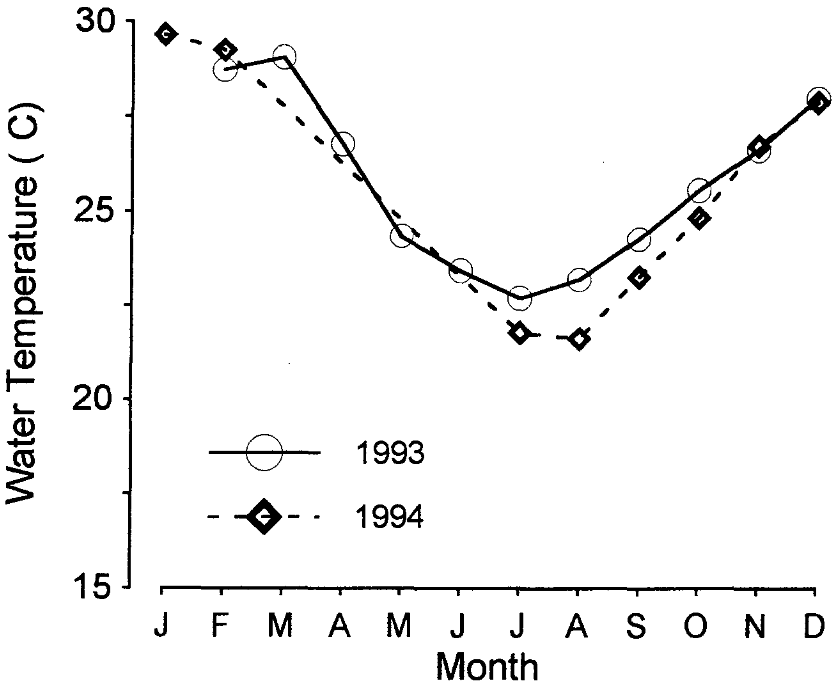


Fig. 2.10 Monthly average water temperature on the reef flat at Cattle Bay, Orpheus Is (provided by the Great Barrier Reef Marine Park Authority).



2.5 Discussion

2.5.1 Distribution and density of *Trochus*

Small and large juvenile *Trochus* shared the same macrohabitat on the reef flat at Orpheus Is., but there were differences in the microhabitat they occupied. This was indicated by two facts: 1) only 6% of the quadrats with more than one trochus had small and large individuals present together; 2) there appeared to be a shift in microhabitat characteristics with which juveniles were associated, with a change from small rubble to larger rocks or coral bench (Fig. 2.4), and from very shallow to deeper intertidal pools (Fig. 2.5) as trochus increased in size. Bour (1992) found a positive correlation between size of *Trochus* (10 - 85 mm SW) and the presence of massive dead corals and coral bench.

Juveniles were not restricted to a particular position on rocks (Fig. 2.6). This observation is possibly influenced by time of day and tidal height, if for example, feeding activity is related to these factors. During searches, the small juveniles were never observed moving actively, independent of their position on the rock, but most of the larger juveniles seemed to be feeding. I snorkelled at high tide during the day and only occasionally observed large juveniles, which were always apparently inactive under rocks and never on top or away from shelter. As the tide went out, these juveniles became much more visible and active.

Microhabitat differences between small and large juvenile *Trochus* could be a consequence of active selection or differential survival between zones. The reef flat holds a large number of benthic predators, such as stomatopods and portunid crabs, which actively search for prey at low tide (Cardwell et al., 1989; Castell, pers. obs.). These crustaceans are unlikely predators of *Trochus* > 30 mm SW, but can be very important predators of the smaller juveniles (Kubo, 1991; Chapter 3). Shallower areas with small rubble may be safer for small trochus if they offer risk to potential predators, such as low availability of refuges to escape from other low tide predators (i.e. eels and birds), or high risk of getting stranded as the tide goes out. Food preferences could also result in differential choice of substrata. Shepherd and Cannon (1988) found differences in the habitat occupied by small and large juvenile *Haliotis laevigata* and *H. scalaris* and these differences were related to their different diets. Small *Trochus* were generally found on small fragments of coralline rubble without or with very little fleshy algae. Coralline algae and a thin film of microscopic algal cells are a favourable substratum for settlement of *Trochus* larvae

(Heslinga, 1981). The larger rocks more commonly inhabited by larger trochus, had both coralline and fleshy algae. Research into natural diets of juvenile Trochus is needed to determine if food preferences vary with juvenile size.

Population density estimates of *Trochus* are available from a number of studies (e.g. Heslinga et al., 1984; Nash, 1985; Smith, 1987; Bour, 1992; Tsutsui & Sigrah, 1994; Nash et al., 1995), however, the variety of methods used and the fact that in most cases only individuals > 70 mm SW were found, precludes comparison with the results obtained in this study. Smith (1987) found mostly juveniles between 20 - 50 mm SW on the outer reef flat of reefs around Guam and mean estimates of density for this section on four reefs ranged between 0.003 - 0.123 individuals.m^{-2} (Smith, 1987). The mean densities of 0.115 and 0.178 individuals.m^{-2} obtained for Orpheus Is. in 1993 and 1994 are comparable to the higher value obtained by Smith (1987). Due to differences in the methods used this comparison may not be completely valid, but it provides an idea of the natural range of densities for juvenile *Trochus*. It is, however, likely that density estimates of small juvenile *Trochus* represent underestimates of natural densities. Juveniles are very cryptic and, in spite of careful searching, a proportion of them are missed by the observer (Chapter 6).

At Orpheus Is., juvenile *Trochus* occurred in groups of two to four individuals per m^2 more frequently than expected by random, but higher density groups were very rarely observed. In larger areas (~6 m^2) that had apparently favourable characteristics, such as pools, coral bench or large rocks with ledges, it was not unusual to find about 10 large juveniles. The scale of sampling used in this study only allowed description of the distribution of *Trochus* at a scale of $1m^2$. However, it is possible that if examined at a larger scale of tens of square metres their distribution would appear more aggregated. The scallop *Patinopecten yessoensis* had an aggregated distribution when assessed on a scale of kilometres, but on a smaller scale of 0.5 km^{-2} their distribution was random (Goshima and Fujiwara, 1994). Bour (1992) found a positive correlation between size and density of *Trochus*, i.e. as individuals became larger they formed denser groups. As juveniles grow, their ability to disperse to favourable sites is likely to increase and thus they may form more dense areas.

The large pool area close to the boulder beach had low numbers of *Trochus* and most of the gastropod species sampled, with the exception of the two carnivorous species that were most abundant. The reasons for this pattern are unclear. Most of this area remained submerged at low tide, and on hot and calm days water could reach high temperatures and become stagnant in some sections. Other invertebrate predators were also abundant, and stomatopods and portunid crabs were frequently observed. *Morula biconica* and *P. australiensis* were observed in groups of up to nine individuals feeding on tethered juvenile *Trochus* in this pool area (Castell, unpublished data). Kubo (1991) attributed the low survival of released cultured trochus at a particular site to the presence of a large tide pool and high numbers of predators. Emergence as the tide falls, as observed for the large trochus, could be an adaptive behaviour to avoid visual predators restricted to the high tides (i.e. fishes). Small trochus are also vulnerable to predation at low tide, so a similar behaviour would not be necessarily expected. Nash (1988) hypothesised that less intense predation on juveniles on high reefs that dry at low tide could explain the general observation that high reefs have greater densities of *Trochus* than low reefs that remain submerged. Similarly, within an intertidal reef flat, small trochus in areas that remain underwater at low tide may also suffer higher predation intensity.

Migration of juveniles to deeper water as they reach maturity has been suggested to explain the increase in shell diameter with depth and the absence of juveniles in the subtidal area where adults live (Heslinga et al., 1984; Nash, 1985; Smith, 1987; Bour, 1992). My results further support this: only individuals < 62 mm were found on the reef flat, whereas individuals between 70 - 125 were always found subtidally (Castell, personal observation). However, the lack of a size gradient of trochus along the intertidal flat as found in this study, suggests that migration towards the reef crest may not necessarily progress as juveniles grow. The change of habitat from intertidal to subtidal may be triggered by reaching a threshold size or reproductive maturity.

2.5.2 Density and distribution of other gastropods

Cypraeidae, Muricidae and Buccinidae were the most common gastropod families on the reef, representing approximately 71% of the groups sampled, which excluded small species of the genera *Cerithium* (Cerithiidae, herbivorous) and *Engina* (Buccinidae, carnivorous). On Kenyan reef flats, Cypraeidae and Muricidae were also found to be among the most common families, representing between 50-

60% of the gastropod population (McClanahan, 1989). Similar to this study, *Cypraea annulus* was the most common cowry and also one of the most abundant species. However, the families Trochidae and Turbinidae represented on average 20% of the gastropods at Orpheus Is., whereas they were very uncommon on Kenyan reef flats (Fig. 2 in McClanahan, 1989). *Cypraea errones*, the second most common cowry on the study site at Orpheus Is., was among the commonest gastropods sharing the shallow reef with *T. niloticus* in New Caledonia (Bour, 1992). Densities of *Conus* species at Orpheus Is. were comparatively lower than those reported for other sites: Leviten and Kohn (1980) reported values between 0.2 - 8.6 ind.m^{-2} for a number of localities, and Reichelt (1982) reports a mean density of 0.3 ind.m^{-2} at Heron Is.

Herbivorous gastropods were more abundant than carnivorous gastropods, representing 64% of all species sampled, compared to 36% for carnivorous gastropods. In Kenya, more herbivorous than carnivorous gastropods were found on reefs protected from human gathering, but the opposite pattern was found in unprotected reefs (McClanahan, 1989).

2.5.3 Recruitment and growth of *Trochus*

Spawning at Orpheus Is. during the study may not occur uniformly throughout the year. This is suggested by the bimodal distribution of size classes, possibly representing two cohorts per year (Fig. 2.8). Observations on spawning and microscopic analysis of gonads indicate that *Trochus* spawns throughout the year at low latitudes (Heslinga and Hillman, 1981; Nash, 1985) and seasonally at higher latitudes (Tourrel and Carlot, 1988; Kubo, 1991; Bour, 1992). There is some confusion regarding the pattern of reproduction at higher latitudes, for example Hahn (1993), who found ripe and spent gonads in all monthly samples of trochus from French Polynesia (17-18°S) and suggested that spawning occurred throughout the year.

New recruits were found between December 92 - February 93 in rubble samples and between March 94 - July 94 in visual surveys. Rubble samples were only taken in four months and thus do not provide information on recruitment intensity throughout the year. Visual surveys, however, were done periodically in both years. Based on estimates of growth rate from laboratory cultures at Orpheus Is., the estimated spawnings to produce these recruits would have occurred in

October 1992, December 92, January 93, February 94 and May 94, i.e. during the warmer months of October and May. Observations of spontaneous spawning from September to May in tanks at Orpheus Is. show a similar pattern (Castell, unpublished data). Only after induction with UV water treatment and water temperature rise did a few females spawn in August 1992, but the larvae did not complete development at the low water temperatures of the time (18 - 23°C). However, normal development of larvae from the same batch reared at 27-28°C proved that the fertilised eggs were viable (Castell, unpublished data). This spawning period coincided with the months when water temperature was on average greater than 23 °C (Fig. 2.10). Bour (1992) suggested a minimum threshold temperature of 22 °C to trigger maturation of the gonads in New Caledonia. It seems possible that the lower water temperatures between June and August prevent most spawning activity at Orpheus Is.

In this study, growth rates of trochus between 2 - 50 mm SW ranged between 2.3 - 2.6 mm.month^{-1}, suggesting that juveniles reach 23 - 26 mm SW in their first year and that they spend about 2 -2.5 years on the reef flat before migrating to the subtidal adult habitat. Visual inspection of the data does not show a differential pattern of growth due to juvenile size or time of year (Fig. 2.9). Growth rates of individually marked trochus between 25 - 68 mm SW averaged 2.1 mm.month^{-1} (SE 1) at Green Is. (~17°S)(Nash, 1985), and, as in this study, growth rate was linear within that size range.

2.5.4 Application to seeding

A number of observations have implications for population enhancement using juvenile *Trochus*:

1) Mean natural densities were about one juvenile every five square metres but *Trochus* formed groups of 2-4 individuals more frequently than expected by random. Density was not measured on a larger scale, greater than 1m^2, but informal observations in the field suggest that in some areas were trochus were common their density would be higher than measured if a larger area, of for example 5 m^2 had been considered. Densities between 1-4 individuals.m^{-2} could be considered for seeding experiments. Seeding density has not received much attention in studies on population enhancement with *Trochus*. In some cases, juvenile *Trochus* have been released at densities between 10 - 112 per m^2 (Kubo,

1991; Nguyen, 1992). If such high densities are used, its potential effect on juvenile survival should be investigated.

2) The macrohabitats where juvenile *Trochus* were found included most of the reef flat, although juveniles were more abundant in the middle section of the reef. Small scale variation in habitat may be of critical importance to the survival of released trochus and consequently should be considered in seeding experiments. Further research into the distribution of small and large trochus is needed to determine if the observed patterns represent a general characteristic and, if so, what are the processes producing them (i.e. differential choice or mortality). This information could be very important in choosing release sites. Based on the results for Orpheus Is., if large juveniles are released, small intertidal pools with large rocks should be targeted, but if small juveniles are released, then shallow areas of small rubble should be favoured. Contrary to expected, adding rocks to provide shelter from wave action and protection from predators may have negative effects on the small juveniles. This seemed to have been the case in a seeding experiment using cultured *Trochus* between 9-25 mm SW, where most juveniles moved out of the additional shelter provided (Amos, 1991). Shepherd and Turner (1985) also found that boulders that had been caged or covered with a plastic net had fewer abalone than uncovered boulders.

Judging from the relative ease in finding large juveniles and adults, the reef studied at Orpheus Island seems to offer a favourable habitat for juvenile and adult *Trochus*. The reef is within a National Park and collection of any species is not allowed except for research purposes. Thus, the results of this study describe a population of juvenile *Trochus* affected only by natural levels of mortality and recruitment variability, providing an example of natural densities, natural distribution and growth. This study provides an initial source of reference for various aspects of juvenile ecology which are important in designing seeding experiments and interpreting their results. Similar studies on wild juveniles in other areas are needed to determine the extent of variation among populations.

CHAPTER 3

Laboratory studies of predation by portunid crabs on juvenile *Trochus niloticus*: prey size range, prey selection and vulnerability of prey.

3.1 Introduction

Predation is one of the most important sources of mortality for early life history stages in many organisms. An understanding of the effect that predators may have on their prey is particularly important in studies of population enhancement for fishery resources. Large numbers of potential prey are released into the natural environment creating conditions that could lead to intense predation and low survival of the released population. The predatory activity of crabs can have a significant impact on their prey populations, influencing the abundance, distribution and size frequency of their prey (Chilton and Bull, 1984; Hines et al., 1990; Eggleston et al., 1992; Sousa, 1993; Schindler et al., 1994). High rates of crab predation have been documented for seeded scallops (Minchin, 1991; Hatcher et al., 1996), oysters (Elner and Lavoie, 1983) and clams (Boulding and Hay, 1984; Peterson, 1990). Predator exclusion by cages or manual removal is an unlikely option for large scale seeding (Lawton and Zimmer-Faust, 1992). There are other mechanisms that regulate natural populations, such as size-specific predation (Sousa, 1993), effect of substrate type on foraging performance (Lipcius and Hines, 1986; Sponaugle and Lawton, 1990) and low and high prey density refuges (Eggleston et al., 1992; Peterson and Black, 1993). A close look into the interaction between prey and predators may provide clues into ways of reducing predation intensity in the field.

There is some evidence that predation is a major cause of mortality of juvenile *Trochus* shortly after release (Kubo, 1991; Nguyen, 1992; Chapter 8), although little is known of the interaction between juvenile *Trochus* and their predators (Kubo, 1991; Nguyen, 1992). Portunid crabs are likely to be important predators: Bour (1992) mentioned that *Thalamita* spp. were common in trochus habitat in New Caledonia, and I found that two species, *Thalamita admete* (Herbst) and *T. stimpsoni* A. Milne Edwards, were very common on an intertidal reef flat also holding a population of juvenile *Trochus*.

While there are numerous studies on the feeding behaviour of portunid crabs from temperate areas (e.g. Hughes and Seed, 1981; Eggleston et al., 1992;

Kaiser et al., 1993), studies on tropical portunid crabs are rarer (Bertness and Cunningham, 1981; Hughes, 1989). In particular, there are few studies on the feeding ecology of *Thalamita* spp., in spite of the abundance of this genus in Indo-Pacific waters (Yap, 1977; Seed, 1990).

Information on what sizes of prey are eaten by predators and an understanding of the mechanisms through which predators select their prey should be used to base decisions on the size of seed to release for population enhancement (Peterson, 1990). Another aspect in the study of prey selection is to determine if predators behave "optimally", i.e. if they select prey that provide the highest nutritional benefit for the lowest energy expenditure (Stephens and Krebs, 1986). In most interactions between predatory crabs and their shelled prey (bivalves and gastropods), once the prey is recognised and grabbed by the predator, there is little chance of the prey actively escaping (with some exceptions, such as swimming scallops, e.g. Barbeau et al., 1994). In such conditions, an ability of the predator to assess prey nutritional value during the handling process would be advantageous. Many crabs seem to prey selectively on their molluscan prey (review by Juanes, 1992), but the mechanisms by which crabs select their prey appear variable and complex (e.g. Jubb et al., 1983; Davidson, 1986; Kaiser et al., 1993).

The objectives of this study were therefore twofold: to describe the interaction between *Thalamita stimpsoni* and *T. admete* with their prey juvenile *Trochus* and to use this information to determine the most appropriate size of cultured trochus, to reduce predation by portunid crabs after release. I examined: 1) the relationship between size of predator, size of trochus taken as prey and prey handling time; 2) at what size *Trochus* reaches a refuge from predation by these crabs and the relationship between prey vulnerability and prey size (Palmer, 1990); and 3) if crabs are selective in their choice of trochus when offered a range of prey sizes.

3.2 Materials and Methods

Experiments were conducted at Orpheus Is. Research Station between June 1993 and October 1994. Portunid crabs, *T. admete* and *T. stimpsoni*, were collected from the reef flat at low tide and used in experiments within three days of collection. They were kept individually in plastic containers: crabs >22 mm carapace width (CW) were kept in 10 L containers (30x27.5x14 cm) and crabs < 22 mm CW were kept in 1.5 L containers (27x11x9 cm) supplied with continuous water flow (0.5 $L.min^{-1}$). Size ranges of crabs used were 11 - 39 mm CW for *T. admete* and 35 - 61 mm CW for *T. stimpsoni*. These represent the most abundant sizes observed on the reef during the study. Crabs were fed once a day with prawn meat and no food was provided for 24 hours prior to an experiment. Both male and female crabs were used in experiments. The results were not analysed separately by sex after preliminary inspection of the data suggested that crabs of both sexes behaved similarly (overlap in size between the two species was restricted to few individuals in only one size class). Crabs missing a chela or legs, those that were about to moult (i.e. with algal growth on their carapaces) or that had recently moulted were not used. Water temperature ranged between 21-28°C. Activity of crabs on the intertidal flat and in the laboratory did not appear to vary within this temperature range.

Juvenile *Trochus* were cultured in tanks at the research station. *Trochus* broodstock were collected from the same reef as the predators. Juvenile *Trochus* used during the study were products of several spawnings, involving 3-4 spawning females and a minimum of six spawning males in each spawning event. New broodstock were collected for each spawning.

3.2.1 Handling time.

Observations were made at night using a torch with a red filter. Crabs were offered trochus of different sizes, one at a time and in random order of size. Offerings to the same crab were spaced by a minimum of 30 min and up to 72 h. Single prey were gently dropped in front of the crab, which almost always responded by grabbing the trochus. Handling time extended from the moment the prey was grabbed to the moment the crab dropped the prey or finished manipulating shell fragments. An attack was categorised as unsuccessful if the crab grabbed and manipulated the prey for a minimum of five seconds but then dropped it and either walked away or pushed the prey away with its chelae. I recorded if the shell of the prey was undamaged or damaged after manipulation.

As an index of profitability of prey, I divided flesh dry weight by handling time. Dry weight of flesh was determined from shell width according to the formula $\ln \text{dry mass} = 0.23 * \ln SW - 0.06$, $R^2=0.93$ (Chapter 5). Crabs were grouped into three size classes: 20-29.5 mm CW (N=6 *T. admete*); 30-39.5 mm (N=17 *T. stimpsoni* and N=2 *T. admete*), and 40-49.5 mm (N=19 *T. stimpsoni*).

3.2.2 Size range of prey

The sizes of trochus eaten by particular crab sizes were recorded throughout the study from observations on prey handling, size selection experiments (described below) and by offering crabs trochus of various sizes. Observations included recording the sizes of trochus that were attacked unsuccessfully (i.e. where the crab attempted to crush the prey) by crabs. These were easily identified by damage to the shell, mostly as chipped apertures and broken ornamentation around the base of the shell. As a difference from the previous section, only those cases where the trochus shell was damaged were classified as unsuccessful. These data were used to estimate a vulnerability function (Palmer, 1990) describing the relationship between trochus size and the probability of being eaten (i.e. its vulnerability) by crab predators of various sizes. Only data on prey that were eaten and or not eaten but definitely damaged were included in the analysis, making the probability of being eaten conditional on the decision of the predator to attack. I used the expression:

$$\mathit{Probability\ of\ being\ eaten} = \frac{\mathit{N\ eaten}}{\mathit{N\ eaten} + \mathit{N\ damaged\ but\ no\ eaten}}$$

An useful outcome of this function is the estimate of the size of prey at which attacks from predators are successful only 50% of the time (SV_{50} , Palmer, 1990).

To determine the largest size of prey that an individual crab could eat, crabs were offered prey of a range of sizes and if all eaten, larger prey were offered until attacks were unsuccessful, during the course of 4-5 days. I considered that a crab was unable to eat a particular size of prey if the prey survived an attack (evident from shell damage) or if the prey was apparently not attacked (i.e. no shell damage occurred) in more than 12 hours. In the latter case, crabs were offered another smaller prey to rule out that satiation had occurred. The size of the largest prey eaten was averaged within predator size groups of 10 mm intervals (20-29.5, 30-

39.5, 40-49.5 and 50-61 mm CW).

I recorded sizes of trochus unsuccessfully attacked, but did not attempt to estimate the largest trochus that a predator would attack. This means that the size range of trochus attacked does not necessarily include the maximum sizes that these predators might unsuccessfully attempt to attack.

3.2.3 Prey size choice

Thalamita admete 16 - 29 mm CW (N= 16) and *T. stimpsoni* 34 - 55 mm CW (N=16) were individually provided with 10 juvenile *Trochus* 4 - 21 mm SW, placed on top of a small piece of coral rubble. Rubble was used because without it trochus moved around in the container and climbed up the walls. This movement was not typical behaviour in the wild and could have affected the incidence of predation. All crabs were offered small trochus 4 - 5 mm SW, but the largest size of trochus offered to each crab increased with predator size to reduce the possibility that crabs would ignore prey far too large for them (e.g. Hughes, 1989). This experiment was run in two consecutive trials. The first used 18 crabs and the containers were checked after 6 and 24 hours. Since very few crabs had attacked any prey after 6 hours, containers were only checked at 24 hours in the second trial using another 8 crabs. The sizes of all prey eaten and attacked, but not eaten, were recorded. A prey was classified as attacked if the shell was damaged. The sizes of trochus found upside down but undamaged on the floor of the container were also recorded, since these had been handled by the predator. Overturned trochus eventually right themselves, meaning that those found overturned at one particular time are likely to represent an underestimate of the total number of prey handled (without damage) by the predator. Only those trials in which crabs ate or attacked at least one prey were analysed (N=24, 92%: 11 *T. admete* and 13 *T. stimpsoni*).

To analyse results for all crabs, prey size was standardised by expressing it as a proportion of the estimated mean largest prey eaten by crabs of an equivalent size (i.e. prey size offered / mean largest prey eaten). The data were analysed in two ways. First, all prey attacked (those eaten and those only damaged) by each crab were considered together and their standardised sizes were summed to provide an overall value. A randomisation test was performed to select randomly a number of prey equal to the number attacked (from the range of sizes offered to each individual crab). The sum of 5000 sets of such randomly chosen sizes were used to generate a probability distribution of expected values if prey size was chosen at random. The test determined the probability that the observed value would be larger than the expected value, or in other words, that crabs attacked larger or smaller trochus in higher proportion than expected by random selection.

The second analysis used a similar simulation but included only the sizes of prey eaten, ignoring those that had been unsuccessfully attacked.

3.3 Results

3.3.1 Handling time and Profitability

Handling time involving successful attacks increased with trochus size and decreased with predator size (Fig. 3.1). Handling time was highly variable, especially as the size of trochus increased. The data were best described by power functions, which explained 77% of the variation for crabs 40-49 mm CW , but only 35% for crabs 30-39.5 mm ($p<0.001$ for both size classes) (Fig. 3.1). The slope of the regression line (LN Handling time vs. LN prey size) was not significant for the smallest crabs 20-29.5 mm ($p=0.22$).

The time spent handling a trochus was positively correlated with shell damage in unsuccessful attacks. In 92% (22) of cases in which the prey was not damaged, handling time was less than two minutes and in only one case was a trochus handled for over four minutes without damage (Fig. 3.2). In 78% of these cases the prey was within the crushing possibilities of the crab (compared to the size range of prey eaten by predators of similar sizes). Alternatively, in 13 of 14 attacks where the prey was damaged, handling time was greater than three minutes and most were over five minutes (Fig. 3.2). In 85% of these cases the prey was of the same size or larger than the mean size of the largest trochus eaten by a crab of the corresponding size.

According to the index of profitability used (prey dry mass. handling time^{-1}), small *Trochus* were the least profitable prey for crabs in the medium size class (30-39.5 mm, p(slope)<0.001, N=41observations) but profitability did not change with prey size for the largest crabs (40-49.5 mm, p(slope)=0.23, N=66 observations) (Fig. 3.3). A similar result was obtained for the smallest crabs (20-29.5 mm, p(slope)=0.42, N=6 observations) but sample size in this case was very small.

Fig. 3.1 Handling time of *Trochus niloticus* prey by crabs of three size classes: 20-29.5 mm CW (N= 6 *Thalamita admete*); 30-39.5 mm CW (N=2 *T. admete* and N=17 *T. stimpsoni*); 40-49.5 mm CW (N= 19 *T. stimpsoni*). Successful attacks (▲, *T. stimpsoni*; X, *T. admete*), unsuccessful attacks (○, *T. stimpsoni*, □, *T. admete*), handling time (Y) and prey size (X). SW= shell width, CW= carapace width. Exponential curves were fitted to the data on successful attacks, Y=handling time in minutes, X=prey size in mm. Ns= No. of observations of successful attacks and Nu for unsuccessful attacks.

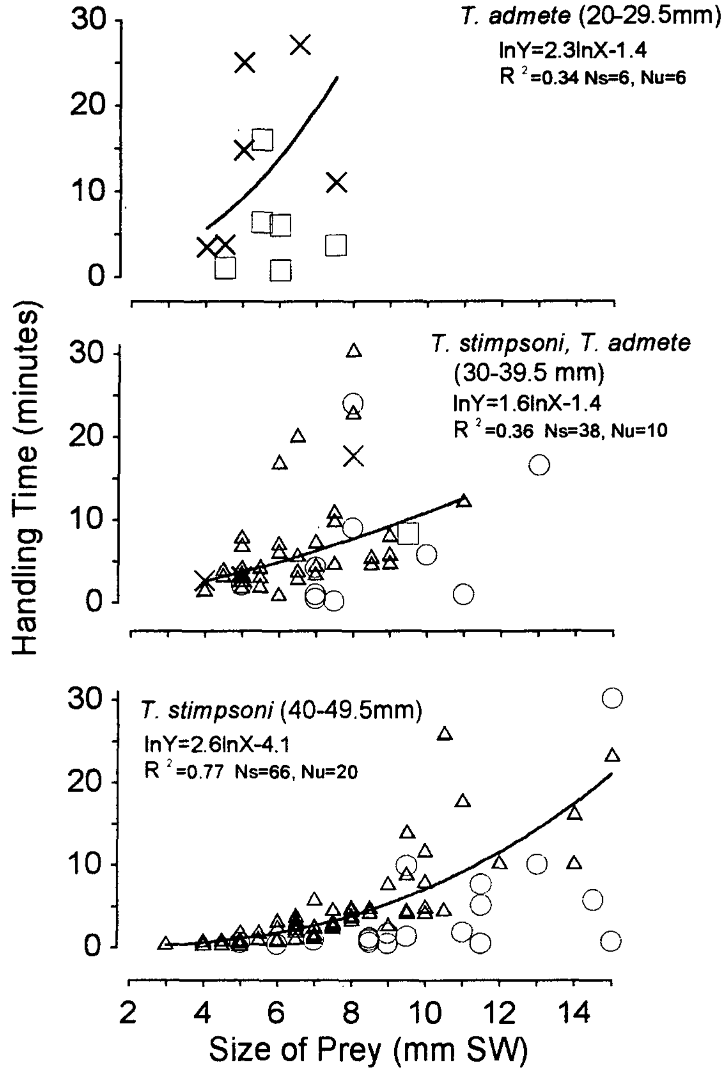


Fig. 3.2 Handling time of prey *Trochus niloticus* in unsuccessful attacks by *Thalamita admete* and *T. stimpsoni*, when the prey was left undamaged (white bars, N=24) and damaged (filled bars, N=14). Frequency distribution was calculated separately for each group.

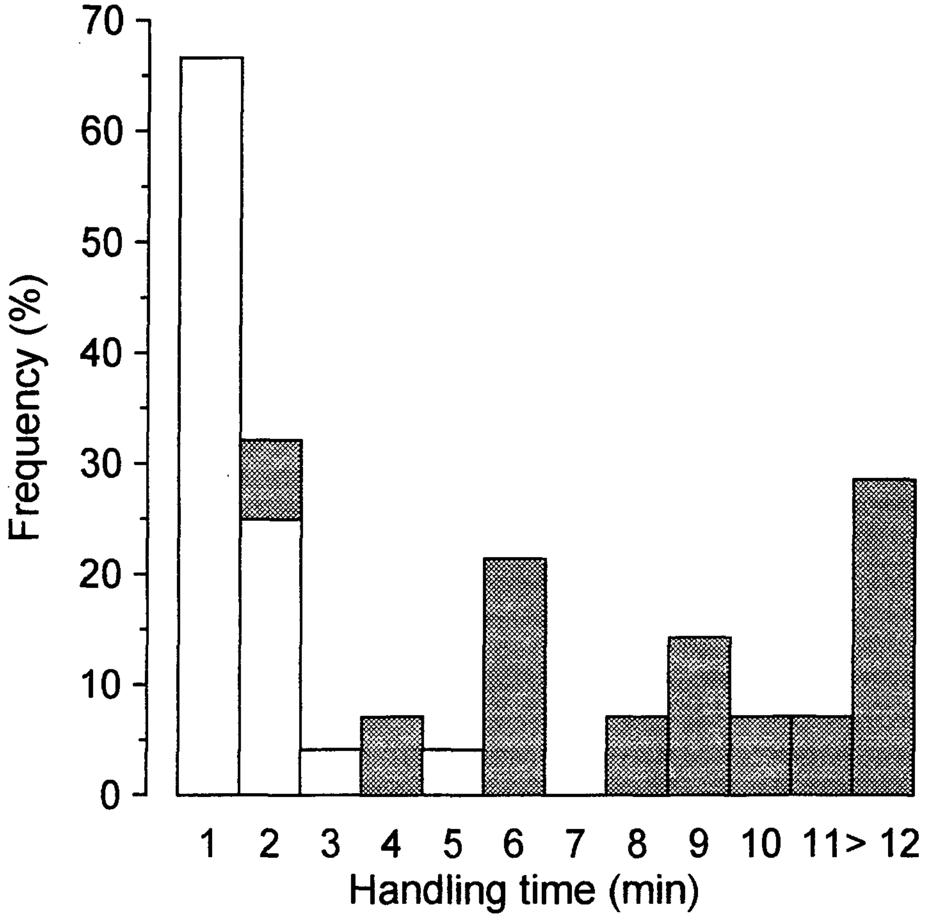
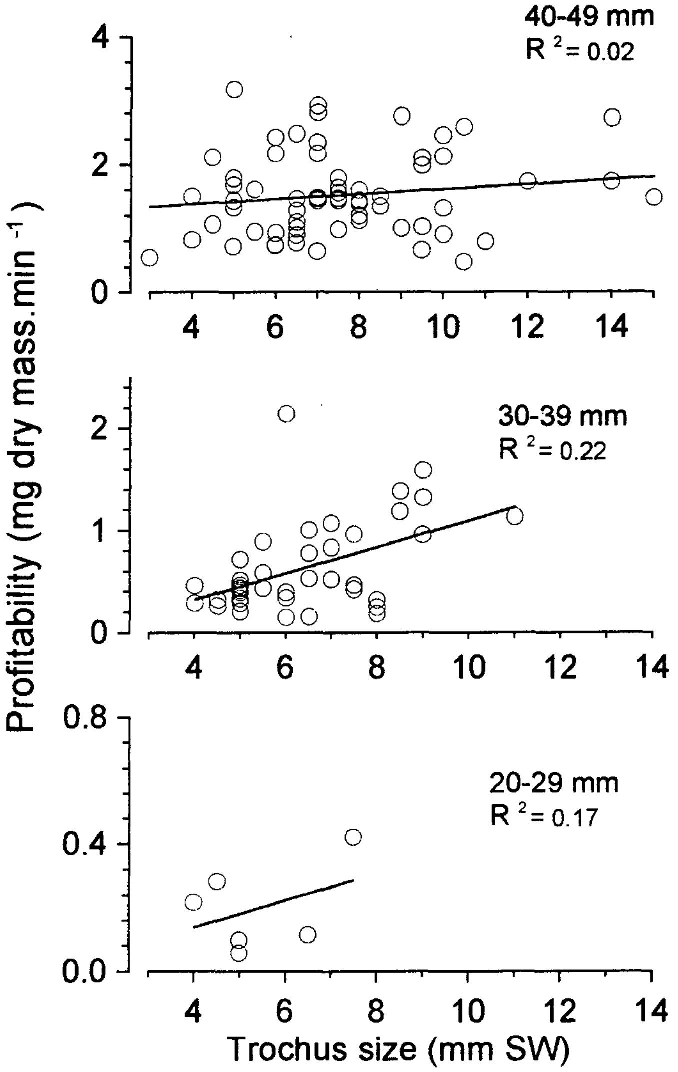


Fig. 3.3 Relationship between profitability of *Trochus niloticus* and prey size for three size classes of predatory crabs: 20-29.5 mm (6 *Thalamita admete*, 6 observations), 30-39.5 (17 *T. stimpsoni*, 41 observations) and 40-49.5 (19 *T. stimpsoni*, 66 observations). Estimated curves: 20-29.5 mm: lnY= -0.65lnX + 7.01, p(slope)=0.42; 30-39.5 mm: lnY= -1.26lnX + 7.07, p(slope)<0.001; 40-49.5 mm: lnY= -0.22lnX + 4.21, p(slope)=0.23, where Y= profitability and X= prey size. SW= shell width.



3.3.2 Prey size range and vulnerability

The size range of trochus consumed increased with crab size. Larger crabs were able to eat larger trochus, but they also continued eating the smallest trochus offered (Fig. 3.4). The size range of trochus left alive but with damaged shells included both small and large individuals, although crabs larger than 37 mm CW only occasionally failed to eat trochus smaller than 6 mm once it had been attacked. The maximum size of trochus eaten was highly variable among crabs of similar size (Figs. 3.5 and 3.6). The line of best fit (ln trochus size= 0.035.crab size +0.837, R^2= 0.69) indicates that the size of the largest trochus eaten increased exponentially with crab size (Fig. 3.5). The mean largest trochus eaten also increased exponentially with crab size class (Fig. 3.6). The largest trochus eaten was 20 mm SW by a crab 61 mm CW. The largest trochus unsuccessfully attacked but damaged was 24 mm SW. Crabs attacked trochus well above the maximum size they ate, up to 2.4 times larger than the average largest trochus eaten (Fig. 3.5).

The relationship between prey size and the probability of being eaten once an attack had commenced, is shown in Fig. 3.7 for each of five size classes of crabs. This relationship is based on 2310 observations of predation and 211 unsuccessful attacks involving 126 crabs. Prey vulnerability decreased as a continuous function of prey size and this decrease was best described by a quadratic function for the two smallest and the largest of predator size classes (Fig. 3.7, Table 3.1). For predator size classes 10-20 mm, 31-40 mm and 41-50 mm the quadratic component of the function explained only an extra 1-5% of the variation, indicating a mainly linear relationship (Table 3.1). Estimates of SV_{50} are in Table 3.1. Smallest trochus, 3 - 5 mm SW, were the most vulnerable because they were attacked by predators of all sizes and rarely survived an attack.

3.3.3 Prey size choice

Twenty four crabs (13 *T. stimpsoni* and 11 *T. admete*) attacked prey. The standardised sizes (trochus size / mean largest trochus eaten) of prey offered are shown in Fig. 3.8. The sizes of prey attacked (eaten or not) were not significantly different from what would be expected by random selection (Randomisation test p=0.272). Sixteen crabs out of 24 (10 *T. stimpsoni* and 6 *T. admete*) consumed prey. The sizes of trochus eaten were significantly smaller than expected if they

Fig. 3.4 Size range of juvenile *Trochus niloticus* taken as prey by *Thalamita admete* and *T. stimpsoni* in laboratory studies. Successful attacks (▲) and unsuccessful attacks (O). Successful attacks: 66 *T. stimpsoni*, 300 observations; 38 *T. admete*, 107 observations. Unsuccessful attacks: 48 *T. stimpsoni*, 103 observations; 32 *T. admete*, 90 observations. CW= carapace width, SW= shell width.

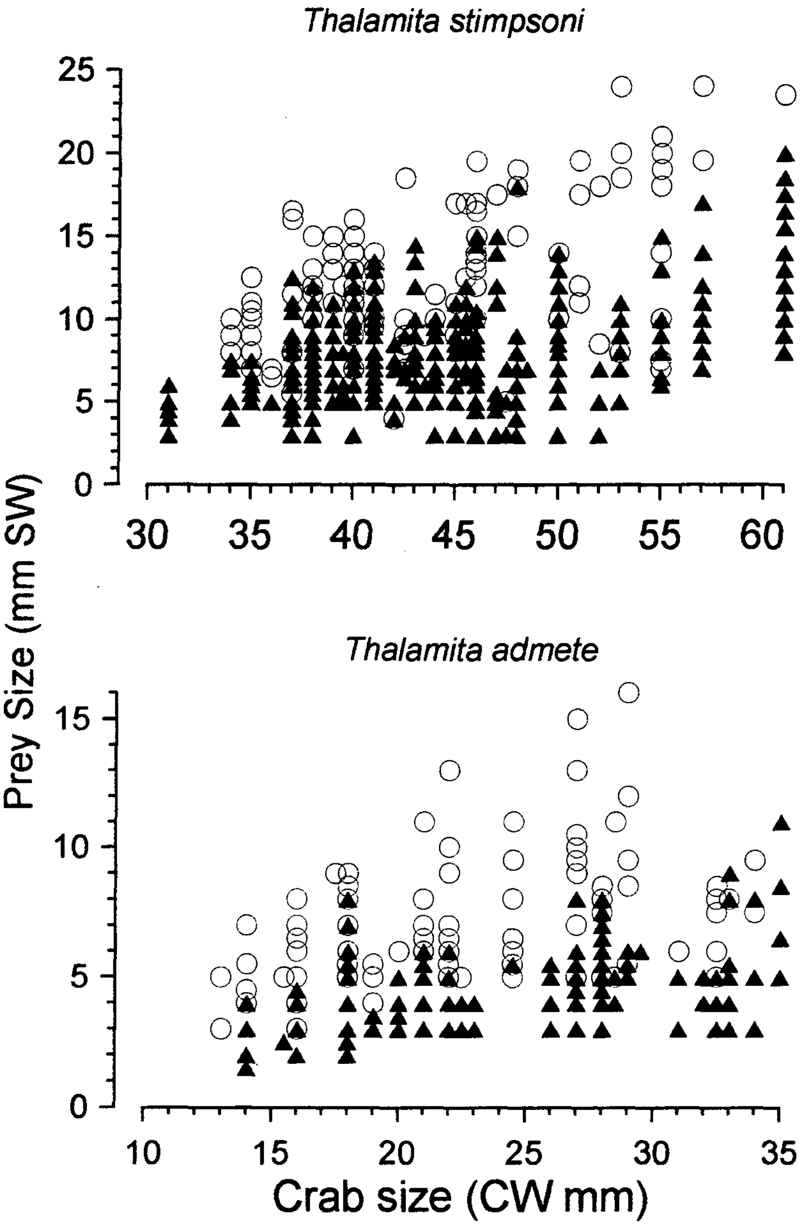


Fig. 3.5 Relationship between predator size and largest size of juvenile *Trochus niloticus* eaten (●) and attacked but left alive with a damaged shell (O), in laboratory studies. Data for *Thalamita admete* and *T. stimpsoni* have been pooled (N=25 and 28 respectively). The solid line represents the function that best describes the relationship between crab size (X) and largest eaten prey (Y)(N=53 observations, β<0.001). CW= carapace width.

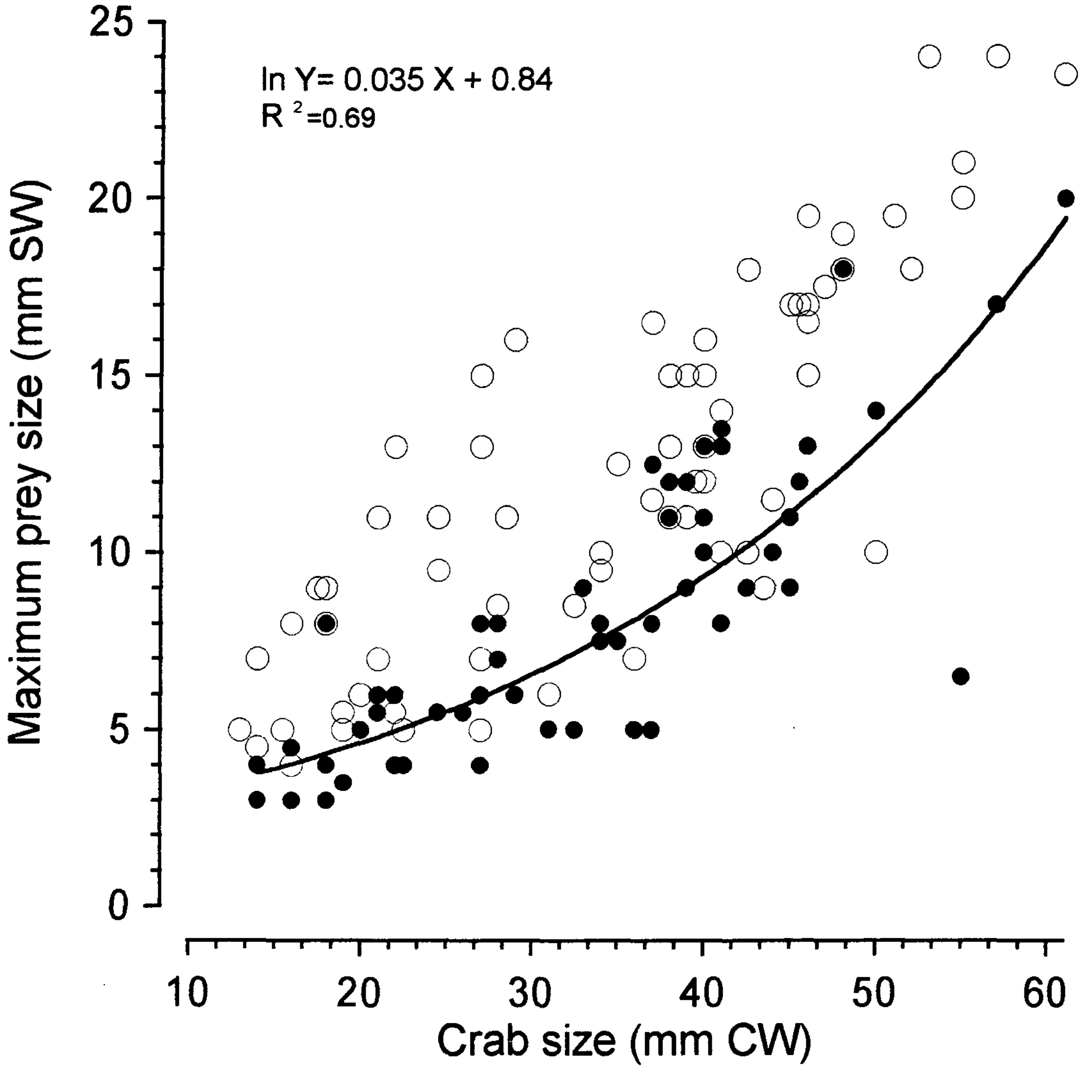


Fig. 3.6 Mean largest juvenile *Trochus niloticus* eaten by *Thalamita admete* and *T. stimpsoni* grouped in size classes. Vertical bars represent the range of values. The dotted line represents the best fit to the mean values (Y= maximum prey size, X=crab size class 1-5, β<0.001). CW= carapace width, SW= shell width.

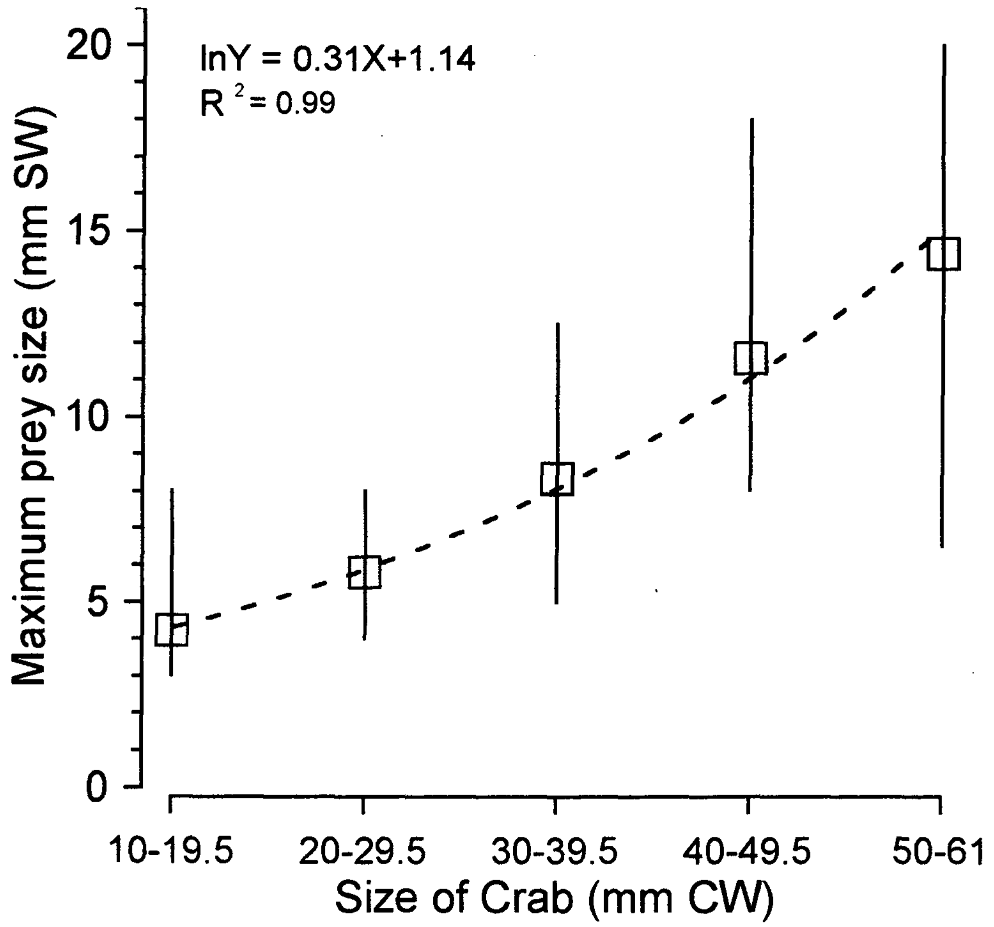


Fig. 3.7 Relationship between size of juvenile *Trochus niloticus* and its probability of being eaten by *Thalamita admete* and *T. stimpsoni* once an attack is initiated. Dotted lines represent fitted curves for predators of five size classes: 10-19.5 mm CW (+), 20-29.5 mm (◊), 30-39.5 mm (□), 40-49.5 mm (O), 50-61 mm (▲). Data from both crab species has been pooled. See Table 3.1 for equations. CW= crab carapace width, SW=trochus shell width.

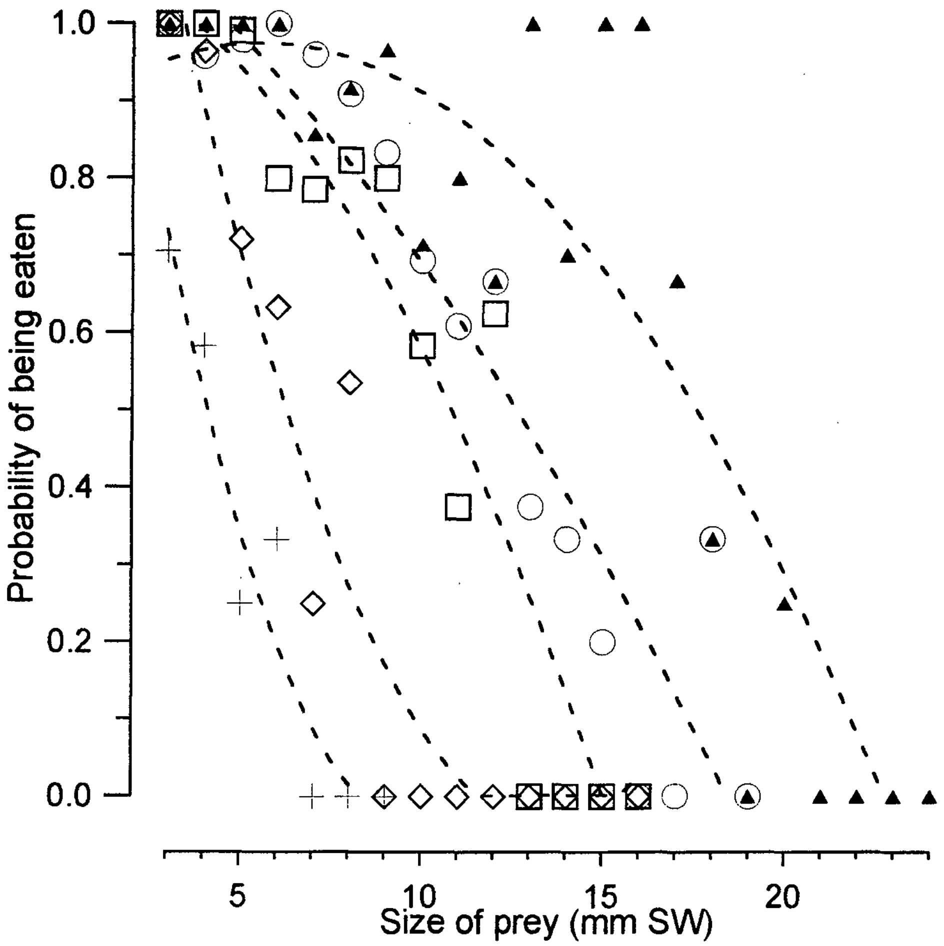
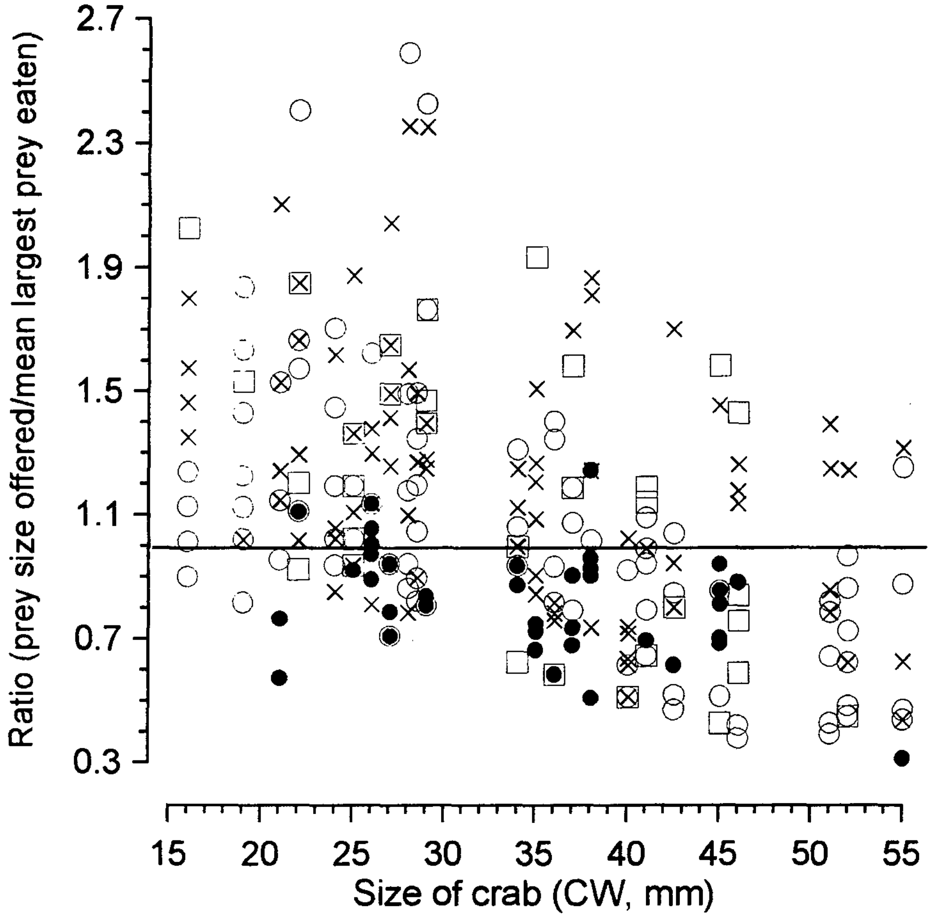


Table 3.1 Parameters of trochus prey vulnerability functions for each crab predator size class. Vulnerability = $a+bX+cX^2$ where X= prey size in mm. R^2 is the coefficient of determination for the first (1) and the second grade interactions (2). SV_{50} is the size of juvenile *Trochus niloticus* (mm shell width) at which predators were 50% successful in an attack, for that particular predator size class. CW= carapace width.

Crab size (mm CW)	**Equation Parameters**					**SV_{50}**
	a	**b**	**c**	**R^2 (1)**	**R^2 (2)**	
10 - 20	1.578	-0.334	0.017	0.87	0.92	4.5
21 - 30	1.833	-0.277	-0.010	0.77	0.92	6.5
31 - 40	1.098	-0.010	-0.004	0.87	0.90	10.1
41 - 50	1.198	-0.034	-0.002	0.89	0.90	12.1
51 - 61	0.874	0.037	-0.003	0.69	0.80	18.5

Fig. 3.8 Standardised sizes (prey size/mean size of largest prey eaten by corresponding predator size) of juvenile *Trochus niloticus* offered (black dots) as prey to *Thalamita admete* and *T. stimpsoni* , eaten (●), damaged but not eaten (X), handled but not damaged (□) and not handled (O), in prey size choice experiments.



were chosen randomly (Randomisation test p=0.0001). Thus, when only prey that were eaten were considered, crabs appeared to select the smallest prey offered, but when all prey attacked were considered, crabs did not show selection.

Observations of prey overturned undamaged showed that in at least 67% of the trials, crabs came into contact with a greater number of prey than they attacked, implying that prey were not necessarily attacked as encountered. In 13 trials (54%), crabs handled at least one prey smaller or of the same size than the smallest prey attacked. This means that these crabs encountered but did not attack some trochus that they were more likely to eat (because of smaller size). In six trials (25%) a trochus larger than all those attacked was handled.

3.4 Discussion

3.4.1 Prey size range and prey size selection

Thalamita admete preyed upon *Trochus* 1.5 - 11 mm and *T. stimpsoni* preyed upon *Trochus* 2.5 - 20 mm SW. The lower limits of these ranges correspond to the smallest trochus offered. The size of trochus successfully attacked increased with predator size (Fig. 3.4). The profitability index used in this study (prey dry tissue.handling time^{-1}) indicates that small trochus have the lowest profitability for the medium size class of predators examined (Fig. 3.2), but profitability did not change with size for the smallest and largest crabs. If other energetic costs of breaking shells are considered, such as metabolic expenditure, and damage to and wearing of the chelae (Juanes and Hartwick, 1990; Juanes, 1992), the profitability of small trochus is likely to be higher and that of larger trochus lower than shown in Fig. 3.2. This could likely result in a more even distribution of profitability with trochus size, as observed for crabs 40-49 mm.

If prey selection is occurring and predators forage optimally (i.e. they maximise their food intake per unit time (Stephen and Krebs, 1986)), one would predict that *T. admete* and *T. stimpsoni* would prefer either the largest prey offered or would show no preference, provided that trochus sizes are within the crab's crushing capabilities. When offered snails individually, larger crabs consumed larger trochus but continued to eat the small trochus offered (Fig. 3.4). From experiments on prey size selection, I found no evidence that *T. admete* and *T. stimpsoni* select for trochus of any particular size. However, if unsuccessful attacks had been unnoticed, crabs would have appeared to select for small trochus. An apparent selective behaviour was due to differential vulnerability of the prey rather than active prey selection. Other studies that analysed predator attack behaviour have also found that the observed selection for small prey resulted from unsuccessful attacks on larger prey (e.g. in predatory gastropods, Palmer, 1990; crabs, Brown and Haight, 1992; insect larvae, Pastorok, 1981; and fishes, Hart and Hamrin, 1990). A mechanism of passive selection, based on the predator's ability to catch or crush its prey, has been proposed to distinguish this behaviour from that where predators actively select for their prey (Brown and Haight, 1992; Juanes 1994). The behaviour of attacking large shelled prey unsuccessfully appears to be widespread among crabs (Vermeij, 1982), even in cases where crabs preyed more frequently on a particular size class or prey species (Seed, 1980; Jubb et al., 1983; ap Rheinallt, 1986; Davidson, 1986; Juanes and Hartwick, 1990; Ebersole and Kennedy, 1995).

The randomisation test indicated that the observed pattern of attack was not different from random, suggesting that attack rate did not differ significantly among prey sizes. Larger prey may be more frequently encountered (e.g. Davidson, 1986; Barbeau and Scheibling, 1994) and consequently more frequently attacked. The fact that some small trochus were handled but not attacked (Fig. 3.7) further indicates that prey encounter was not a major factor determining the size of prey attacked. Attacking large prey is favourable only if the predator can crush it. *T. admete* and *T. stimpsoni* attacked trochus that were very unlikely to be crushed, as measured by the corresponding mean largest trochus eaten (Fig. 3.8). The significance of this behaviour is unclear. Shell crushing involves energetic costs and the risk of damaging and even breaking the chela (Juanes and Hartwick, 1990). Missing a limb can have important consequences in the foraging ability and interactions with other individuals (Sekkelsten, 1988; Juanes and Hatrwick, 1990). *T. admete* and *T. stimpsoni* with a missing chela were not able to eat even the smallest trochus offered (Castell, pers. obs.).

One possible explanation for the relative frequency of unsuccessful attacks is that an attack is part of the predator's mechanism to decide whether to accept or reject the prey. If selection is based on information on shell strength, an attempt to crush the shell may be necessary and rejection would occur after an attack. Evidence for shell strength selection was found in *Carcinus maenas*, which attacked all *Nucella lapillus* but rejected those that did not yield after a maximum of 2.75

minutes (Hughes and Elner, 1979). The authors suggested that attacking all encountered prey, but prompt rejection on failing to break the shell, may be the most appropriate foraging tactic for prey types that are only rarely encountered. Jubb et al. (1983) proposed a size-based mechanism for selection of small mussels by *Carcinus maenas*, but a strength-based mechanism for selection of medium and large mussels. Decisions based on prey size may be inadequate when handling time is highly variable (Juanes and Hartwick, 1990), a common pattern for prey whose size is close to the maximum size that predators can eat (e.g. Hughes and Seed, 1981; Sanchez-Salazar et al., 1987; Seed 1990; Hughes 1989; this study Fig. 3.1). The way prey are encountered may also affect the predator's behaviour (Jubb et al., 1983). Hughes and Seed (1995) suggested that when shelled prey are encountered sequentially, each one is attacked independent of size, but when different prey are encountered simultaneously, the time spent attacking one prey will be affected by the strength of other stimulus, such as contact with more prey. If crabs select their prey based on their appreciation of size, rejection should occur after handling the prey but before attacking it (Hughes and Elner, 1979; Hughes, 1980). *T. admete* and *T. stimpsoni* appeared to reject some prey prior to an attack, but others were attacked before they were rejected. It is likely that some prey were also encountered simultaneously, explaining rejection on sizes that would have yielded after some more persistence.

Prey handling observations indicate that crabs must spend time to cause damage to a shell and that this time was highly variable, with some attacks lasting up to 30 minutes in one continuous bout (Fig. 3.1). However, these measurements corresponded to attacks on trochus close to the maximum size that predators could crush. Handling time of unsuccessful attacks was not determined in prey choice experiments (crabs were not observed). It is possible that persistence time varies with prey size: predators may spend more time attacking prey close to the critical size than prey too large to be crushed. A variable handling time depending on the vulnerability of the prey to be crushed implies an ability of the predator to recognise vulnerable shells during an attack. Davidson (1986) suggested such ability to explain greater persistence on some mussels, 26% of which were eventually opened. The crab *Oxius verreauxii* spent up to 50 min handling (and presumably trying to crush) the largest prey they could crush, but did not attack prey above that size (Hughes, 1989).

Other factors, such as predation risk (Godin 1990; Lima and Dill, 1990), hunger (Lucas, 1983; Stephens and Krebs, 1986) and experience (Cunningham and Hughes, 1984; Kaiser et al., 1993; Micheli, 1995), can affect a predator's decision to feed. In this study, crabs were most likely to be hungry and probably inexperienced with trochus shells. Juvenile *Trochus* were present in the area where predators were collected but at low densities (0.143 individuals.m^{-2}, SD 0.108) (Chapter 2). *Thalamita stimpsoni* and *T. admete* are generalist predators (Castell, pers. obs.), as are many other portunid crabs (Laughlin, 1982; Choy, 1986; Cerda and Wolff, 1993) and even if encounters with *Trochus* are infrequent, they will encounter other gastropods on the reef. A flexible foraging behaviour allowing predators to modify their choices according to the environment (Creswell and McLay, 1990; Micheli, 1995) and a general "effective" prey choice mechanism (Hughes and Seed, 1995) are predicted as favourable for predators with an opportunistic diet. This study was designed to determine if *T. stimpsoni* and *T. admete* were selective in the choice of sizes of trochus prey. Further research is necessary to determine the mechanisms underlying the observed unselective behaviour and, furthermore, to explain the high frequency of unsuccessful attacks. Unsuccessful attacks appear to have been largely excluded from analysis of prey selection, but quantitative studies of attack behaviour may be a key component to establish the mechanisms that lead to prey consumption (Jubb et al., 1983; Palmer, 1990, Juanes, 1994; Hughes and Seed, 1995).

From the point of view of the prey, unsuccessful predation has some negative effects, since the prey are dislodged and in most cases damaged. Prey with damaged shells are likely to be more vulnerable to crushing by other predators, because of weakening of their shells (Boulding, 1984) and breakage of protective knobs (Palmer, 1979). Juvenile *Trochus* have characteristic knobs in their shells that are frequently broken during a crab attack, sometimes reducing shell diameter by 2 - 3 mm, which could represent a decrease of 15 - 40% in shell size depending on juvenile size. Dislodged shells can be washed away by waves to less favourable habitats.

3.4.2 Vulnerability function and application to seeding

The small trochus offered to all crabs (3-5 mm SW) were the most vulnerable because they were readily eaten by a wide range of crab sizes (11- 61 mm CW) and because their chances of surviving and attack by a crab predator were minimal (Figs. 3.4 and 3.7). As indicated by Palmer (1990), prey vulnerability was a continuous function of prey size for each predator size class. For all except the smallest crab size class (10-20 mm SW), the estimated average size of the largest trochus eaten were smaller than estimates of SV_{50} (the size at which the probability of surviving an attack is 0.5) estimated by the vulnerability function (Fig. 3.6, Table 3.1). Thus, SV_{50} estimates provide a more conservative base to decide on what size of trochus for release.

It must be stressed that the relationship between the probability of surviving an attack and trochus size is not necessarily equivalent to the probability of juvenile *Trochus* surviving on the reef. To estimate survival probabilities it is necessary to know the rate of prey-predator encounters, the size-structure of populations of predators and range of predators. My numerous observations of predation on small trochus by crabs of various sizes, in laboratory conditions where prey were attached to coral rubble typical of their natural habitat, suggest that small trochus are likely to be encountered and attacked in the wild by portunid crabs of most sizes. Attack behaviour of crabs suggests that juveniles will be unsuccessfully attacked and left damaged in some encounters. The extent of the effect that unsuccessful attacks may have on *Trochus* survival can be important and could result in greater vulnerability than predicted from individual attack success.

The results of this study strongly suggest that large rather than small trochus should be preferred for release on reefs. Trochus larger than 19 mm SW will have a much greater chance of surviving an attack by *T. stimpsoni* or *T. admete*.

This study focused on portunid crabs, one of the numerous groups of potential predators of juvenile *Trochus* on the reef. Stomatopods, other crabs, flatworms, carnivorous gastropods, octopus and fishes are known to eat juvenile *Trochus* (Zipser and Vermeij, 1978; Kubo, 1991; Nguyen, 1992; Chapter 5; Castell, pers. obs.). Thus, recommendations on size for release are relevant to the crabs studied and may, or may not, overlap with the results of studies with other predators. However, this is a necessary step to understand outcomes of experimental releases and to improve those conditions under which survival of released trochus would be highest.

CHAPTER 4

Predation response of portunid crabs to different densities of juvenile *Trochus niloticus*

4.1 Introduction

Prey and predators coexist in nature through various regulatory processes (Murdoch and Oaten, 1975; Sih, 1987), although local extinction of prey does occur (Murdoch and Oaten, 1975; Kats, 1985). Density dependent predation is invoked as one of the mechanisms through which coexistence is achieved (Solomon, 1949; Hassell and May, 1973; Murdoch and Oaten, 1975).

Predation is thought to be an important source of mortality among seeded populations (e.g. Flagg and Malouf, 1983; Hatcher et al., 1996). Associated with releases is the creation of areas with high densities of potential prey (e.g. Kubo, 1991; Hatcher et al., 1996). Predators can respond to an increase in prey density by increasing their numbers (numerical response) or by modifying their predation rate (functional response) (Solomon, 1949). If releasing individuals at high density results in more intense predation, the benefits of releasing larger number of individuals can be overturned by an increase in predation pressure.

Field experiments have shown that crabs can respond with increased predation when exposed to high prey densities (Boulding and Hay, 1984; Auster and Malatesta, 1991; Barbeau et al., 1994). Furthermore, the existence of a low density refuge below which crab predation is highly reduced has been demonstrated for some crab-bivalve populations and proposed as a mechanism by which prey and predators coexist (Lipcius and Hines, 1986; Sponaugle and Lawton, 1990; Eggleston et al., 1992; Mansar and Lipcius, 1992; Iribarne et al., 1995).

This chapter examines the effect of density of juvenile *Trochus* on predation by *Thalamita admete* and *T. stimpsoni*. Two main questions were addressed. The first question addressed the response of crabs exposed simultaneously to patches of trochus prey of different densities. In particular, I was interested to determine if predators concentrated their feeding activity on patches with high prey density while at the same time decreasing their effect on patches with low prey density. The second question was whether predation rate is affected by prey density. Comparison of the proportion of prey consumed at different densities, allows detection of a change in foraging rate (e.g. Lipcius and Hines, 1986; Eggleston, 1990).

4.2 Materials and Methods

The study was carried out at Orpheus Island (see Chapter 2 for detailed description). Portunid crabs, *Thalamita admete* and *T. stimpsoni*, were collected from the intertidal reef flat at low tide and used within four days of collection. They were maintained individually in 10 L plastic containers supplied with continuous water flow. Crabs were fed once daily with prawn meat and no food was provided for 24 hours prior to an experiment. Both males and females were used. Crabs missing a chela or legs and crabs that were about to moult (i.e. with algal growth on their carapaces) or had recently moulted, were not used in experiments. The size range of crabs was chosen based on preliminary observations of their abundance on the reef. Crabs *T. admete* were grouped in two size classes: medium (16 - 22 mm CW) and large (23 - 35 mm CW) and crabs *T. stimpsoni* were grouped in one class, large individuals (35 - 55 mm CW). Individuals of *T. admete* smaller than 16 mm carapace width (CW) were not included because they could only eat very small trochus (< 2 mm shell width)(SW), which were not available at the time of experiments. Individuals *T. stimpsoni* smaller than 35 mm were only occasionally observed.

Juvenile *Trochus* were cultured in tanks at the station using broodstock from the same reef where predators were collected (see Chapter 3 for details on culture of trochus). Fresh coral rubble was collected from the intertidal reef flat and provided as substrata for the trochus to attach. Any visible animals such as other shells and invertebrates were removed prior to use. Trochus size was selected based on studies that determined the size range of trochus taken as prey by crabs of various sizes (Chapter 3), to ensure that crabs could easily handle and eat the trochus offered if desired. *Thalamita stimpsoni* crabs 35 - 55 mm CW were offered trochus between 6 - 11 mm SW; *T. admete* 24 - 35 CW were offered trochus between 3.5 - 6 mm SW and *T. admete* 16 - 23 mm CW were offered trochus between 2 - 3.5 mm SW.

Experiments were conducted at various times between September 1993 and August 1994. Water temperature varied between 21 - 26 °C throughout the day for most experiments. Four trials were conducted in November 1993 when water temperature was 25 - 28 °C. Temperature has been shown to affect predation rates of some crab species (Eggleston, 1990); however, informal observations and visual inspection of the data indicated that crab behaviour and feeding rates in the November trials were similar to those observed at the lower temperatures.

4.2.1 Response to prey density in patches

Large *T. admete* and large *T. stimpsoni* were used in these experiments. Three patches of coral rubble, 20 cm in diameter (314 cm^2), were built inside a rectangular raceway 4.2x1x0.7 m filled to a depth of 10-15 cm and provided with continuous water flow (0.8 L. min^{-1}). Distance between patches was 0.9 m. Six small clam shells were placed 0.5 m around the rubble patches to provide an optional refuge for the crabs. Cultured trochus were released onto the rubble at three densities, 5, 15 and 30 individuals per patch and left to settle for two hours. The density assigned to each patch was randomised among trials. After trochus were settled, one crab was released into the raceway. Trochus were never observed moving out of the rubble along the floor of the raceway. Each experiment ran for 48 hours, after which all patches were searched for trochus and the number surviving counted. There were 15 replicate trials for *T. admete* and 26 for *T. stimpsoni*. Individual crabs and trochus were used only once in experiments. In all 15 trials with *T. admete* and in 13 with *T. stimpsoni*, the patches were also checked for surviving juveniles after 24 hours with the minimum possible disturbance. These checks were done during the day while crabs were generally inactive in one of the refuges or in one of the patches. Two control trials were run to estimate mortality rate of juveniles in the absence of a crab.

The proportions of trochus eaten within each patch were compared using analysis of variance, with prey density as the main factor and crab as a blocking factor. Analysis were done separately for each species. The assumption of homogeneity of variances among density treatments was tested using the Brown-Forsythe's procedure (Keppel, 1991). All three densities of prey were simultaneously offered to each predator and consequently they do not represent independent estimates of predation rate. The purpose of the experiment was precisely to examine the response of predators under a choice situation. By making the factor crabs a blocking factor, differences among crabs (e.g. hunger, agressiveness) were taken into account.

To investigate if trochus reach a low density refuge from crab predation, I determined the frequency of patches left with 0, 1, 2, 3, 4 and 5 individuals in the patch with five trochus, and the frequency of patches left with 0, 1-5, 6-10 and >11 in those with 15 and 30 trochus. I then estimated the proportion expected by random assuming a Poisson distribution and using the mean number of trochus left at each patch density. If there is a low density refuge one would predict the number of patches left with few trochus to be higher than expected by random.

Observations on behaviour.

Three *T. stimpsoni* were observed for 0.5 - 2 h during the first night of the experiment using a torch with a red light. While observing a crab, all movements were recorded, such as patches visited, time spent at the patch and if the crab ate any trochus during a visit.

In another set of observations, the positions of four *T. admete* between 24 - 26 mm CW were recorded on six occasions during the first 24 h of the experiment (12pm, 19pm, 22pm, 1am, 6am and 9am).

4.2.2 Effect of prey density on predation rate

Experiments were run using plastic containers provided with continuous water flow (0.8 $L.min^{-1}$): for crabs 16 - 22 mm CW containers were 27x11x9cm (275 cm^2 area) filled with water to 7cm depth; for all the other size classes the containers used were 30x27.5x14 cm (825 cm^2 area) and 10 cm water depth.

The floor of the container was covered with freshly collected coral rubble to form a single layer. Either five or 30 trochus were released haphazardly onto the rubble and left for two hours to settle. After that period, a single crab was released. The rubble was searched after 24 hours and the number of trochus eaten was recorded. The crabs were kept without food for the following 24 hours and the experiment was repeated the next day with the same crab but using the alternative number of trochus prey. In this way, each individual crab was exposed to both prey densities with a period of 24 h in between. Two control trials were run for each density. The results were analysed using a paired t-test to compare the proportion of trochus eaten by each crab at each prey density, separately by size class and species.

To determine the existence of low density refuge I followed the same procedure as explained in the raceway experiments

4.3 Results

4.3.1 Patch Foraging

The number of trochus eaten increased significantly with prey density but the proportion eaten did not differ significantly among prey densities (Fig 4.1, Table 4.1). After 24 hours, *T. admete* consumed between 16-26% of the prey in each patch, and *T. stimpsoni* consumed almost the same proportion of prey in each patch, 32 - 35% (Fig. 4.1). After 48 hours, *T. admete* had eaten on average the same proportion (25%) of trochus in the low and intermediate density patches and 41% in the high density patches, whereas *T. stimpsoni* had eaten between 47 - 57% of the prey in each patch (Fig. 4.1). There was high variability among crabs and after 48h differences among individual crabs of both species were statistically significant (Table 4.1).

The mean number of trochus eaten during the first 24 hours was 11 for *T. admete* and 17 for *T. stimpsoni* (Table 4.2). In the following 24 hours the mean number of prey eaten decreased by almost half for *T. admete*, whereas it remained similar for *T. stimpsoni* . The difference in predation rate between the first and second days was marginally significant for *T. admete* (Paired t test $_{(0.05,\ 13)}$, p=0.051) and not significant for *T. stimpsoni* (Paired t test $_{(0.025,11)}$, p= 0.726). The total number of prey eaten after 48 hours was highly variable among individual crabs of each species (note the large SE in Fig. 4.1), but this variability was poorly related to crab size for *T. admete* (R^2 0.24, p(slope)= 0.04) and not related to size for *T. stimpsoni* (R^2 0.01, p(slope)=0.29) (Fig. 4.2).

The number of trochus left alive in low density patches was higher than expected if prey removal had occurred randomly in *T. admete*; but observed and expected values were more similar in *T. stimpsoni* (Fig. 4.3 top figure). Total depletion of trochus in patches did not occur in experiments with *T. admete*, but between 4 and 12% of the patches at each density treatment were completely depleted by *T. stimpsoni* . These percentages were higher than predicted.

Fig. 4.1 Response of crabs to three densities of trochus prey offered simultaneously in patches, in raceways experiments. Mean number of trochus eaten and mean proportional mortality at three patch densities, after 24 h (dashed lines) and 48 h (solid lines). Vertical bars represent standard errors. Data analysis shown in Table 4.1.

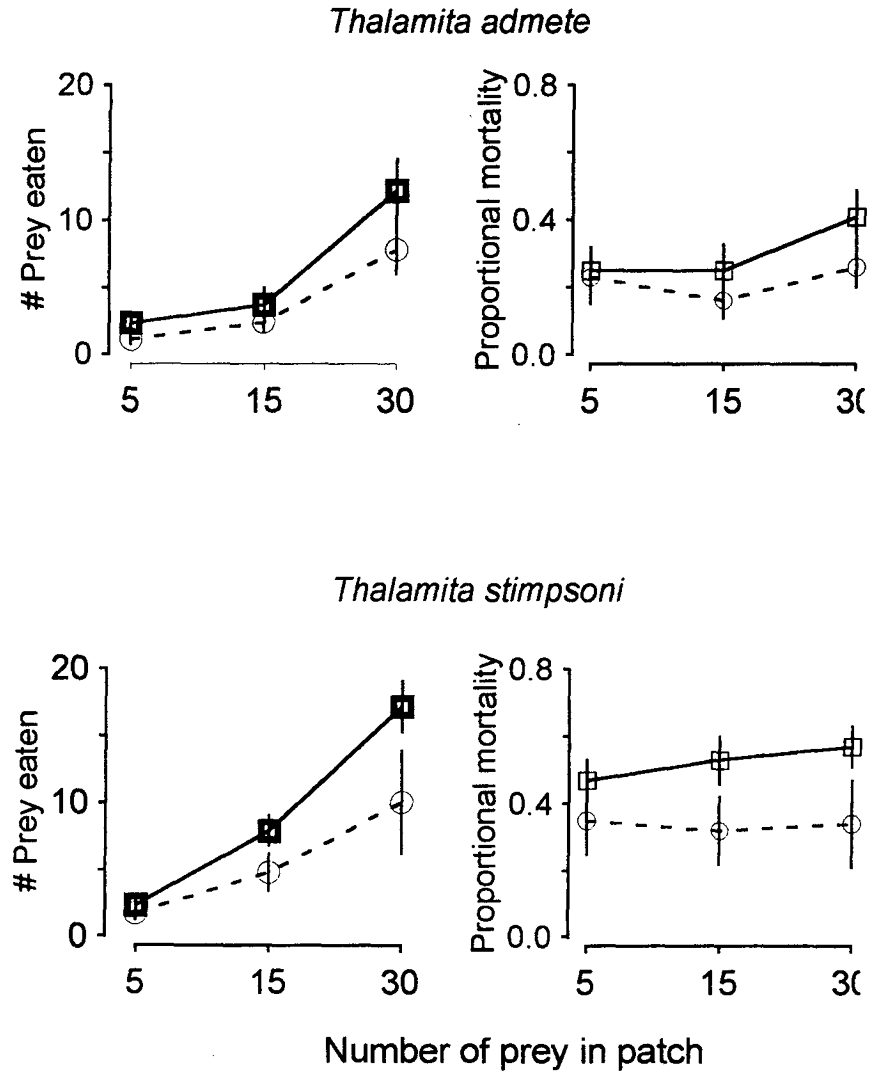


Table 4.1 Results of analysis of variance to determine the effect of prey patch density on the number and the proportion of trochus eaten by *Thalamita admete* and *T. stimpsoni* when offered simultaneously, after 24 and 48 hours.

		Number of trochus eaten			**Proportion eaten**		
Thalamita admete	**df**	**MS**	**F**	**p**	**MS**	**F**	**p**
24 HOURS							
Patch density	2	188.0	10.4	<0.001	0.039	0.68	0.517
Crab	14	25.0	1.4	0.223	0.075	1.31	0.265
Error	28	18.1			0.058		
48 HOURS							
Patch density	2	229.4	7.7	0.003	0.121	1.80	0.183
Crab	14	48.7	1.6	0.146	0.136	2.03	0.054
Error	28	29.7			0.067		
Thalamita stimpsoni							
24 HOURS							
Patch density	2	493.3	17.5	<0.001	0.003	0.06	0.941
Crab	12	54.1	1.92	0.069	0.139	2.62	0.022
Error	24	28.2			0.053		
48 HOURS							
Patch density	2	1454.2	63.4	<0.001	0.060	1.70	0.194
Crab	25	76.1	3.3	<0.001	0.255	7.15	<0.001
Error	50	22.9			0.036		

Table 4.2 Mean number (± standard error) of juvenile *Trochus niloticus* eaten by *Thalamita admete* and *T. stimpsoni* in (a) raceway experiments, after 24 and 48 h, and in (b) small container experiments, after 24 h. CW= carapace width.

a) Multiple patch experiments (raceways)

	Thalamita admete (23-35 mm CW, N=15)	*Thalamita stimpsoni* (35-55 mm CW, N=13)
Day 1	11.0 (2.0)	16.7 (3.4)
Day 2	6.2 (2.6)	15.0 (4.2)

b) Single patch experiments (containers)

		Prey density		
Predator	Size (mm CW)	5 ind	30 ind	N
T. admete	16 - 22	2.6 (0.5)	16.1 (3.1)	16
T. admete	23 - 35	2.0 (0.4)	19.5 (2.3)	20
T. stimpsoni	35 - 50	2.9 (0.4)	21.0 (2.4)	21

Fig. 4.2 Relationship between predation rate and predator size. Total number of trochus eaten after 48 hours in raceway experiments (N offered = 50). The line represents the best fit to the data. $R^2 = 0.24$ p(slope)= 0.04 for *T. admete* and $R^2 = 0.01$, p(slope)=0.29 for *T. stimpsoni*. CW= carapace width.

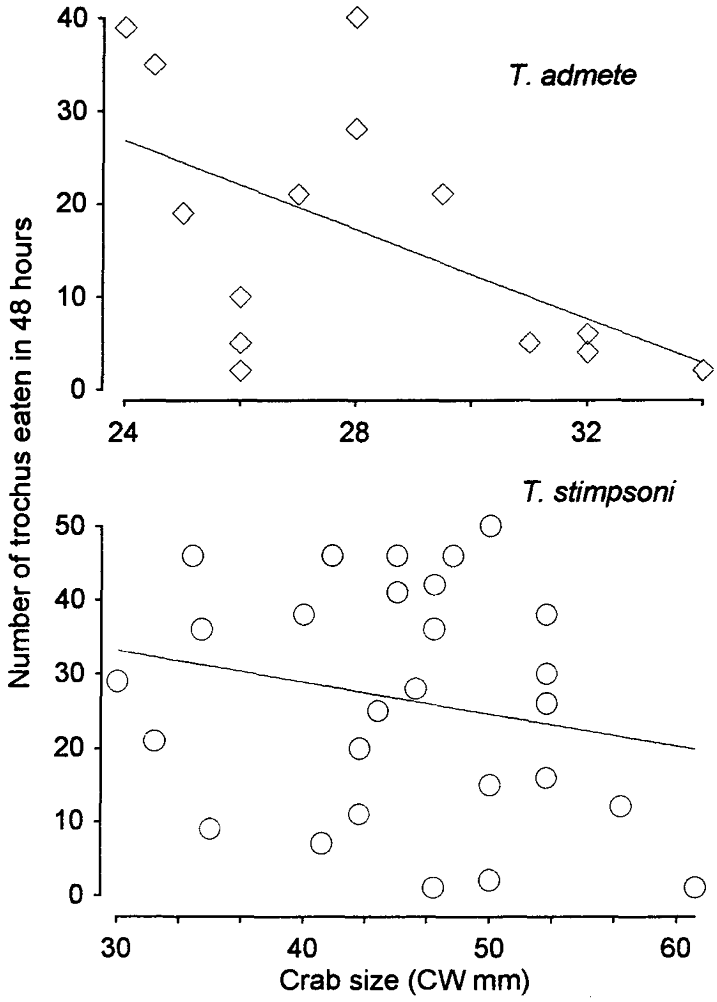
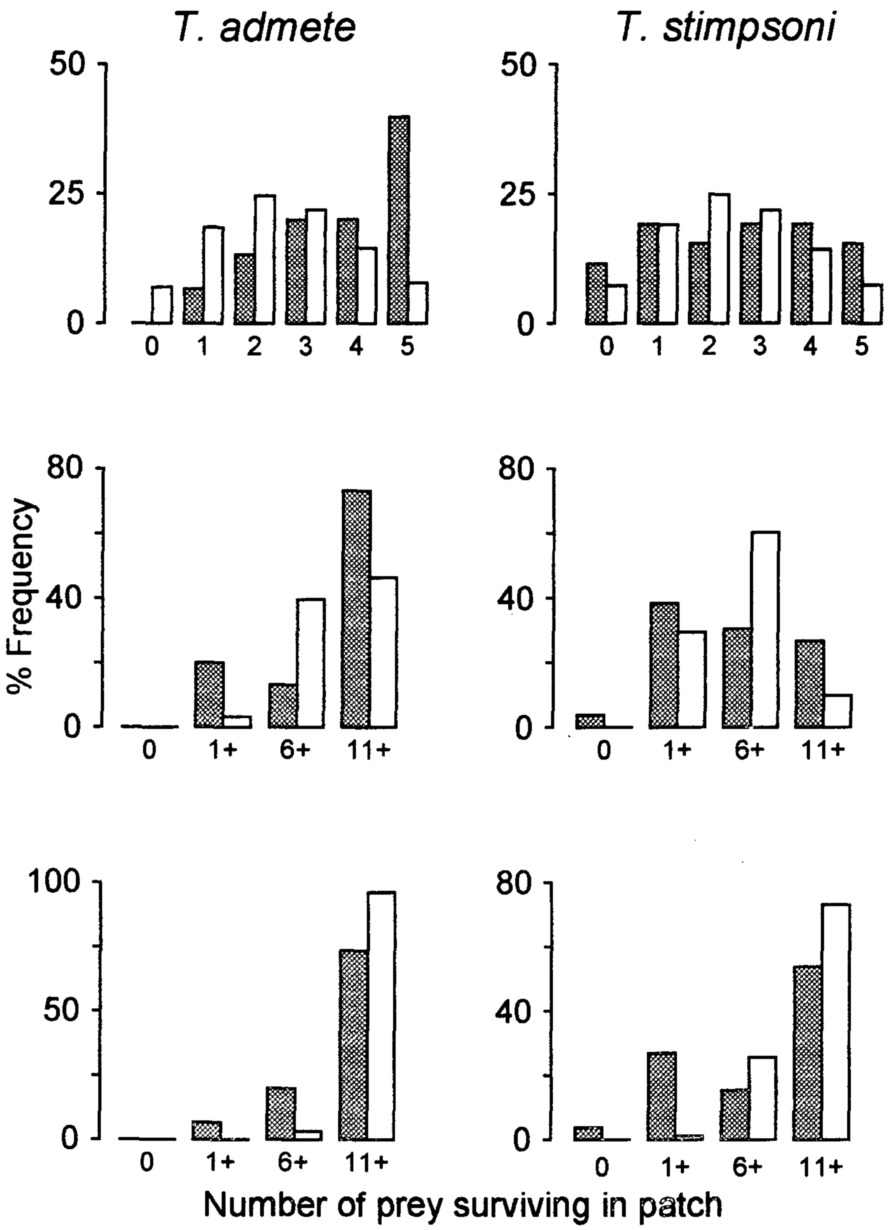


Fig. 4.3 Frequency distribution of the number of trochus surviving in a patch after 48 h in raceway experiments. Initial number of trochus in the patches were 5 (top figure), 15 (mid figure) and 30 (bottom figure) . Observed values (filled bars) and expected values (open bars). Expected values were estimated assuming a Poisson distribution and using the corresponding mean number of surviving trochus. N=15 for *T. admete* and N=26 for *T. stimpsoni*.



Behavioural observations.

Crabs were observed in patches and away from patches. The positions where crabs were observed was unrelated to the final outcome of the experiment (Figs. 4.4 and 4.5). Observations on crabs that were followed for some time indicate that crabs moved about even within the short period of observation (30min to 2h) (Fig. 4.4). Visiting a patch did not always result in encountering a prey (e.g. Fig. 4.4 top figure). Crabs stayed longer in patches where they captured prey: they remained in the patch for a total of 9, 10, 12 and 22 min in cases where they encountered prey, but visits when no prey was encountered lasted 2, 3 and 4 min, after which the crab moved away. The maximum number of trochus eaten in the same visit to a patch was two, and this corresponded to the intermediate patch density (Fig. 4.4, middle figure).

4.3.2 Effect of density on predation rate in container experiments

The number of trochus eaten increased significantly with trochus density for both crab species and size classes (Fig. 4.6) (Paired t test $p<0.001$ for all three treatments). Proportional mortality also increased significantly with prey density for *T. stimpsoni* (35-55 mm) and *T. admete* (23-35 mm), but not for the smallest *T. admete* (16-22 mm) (Fig. 4.6) (Table 4.3).

Predation rate at each prey density was very similar for both size classes of *T. admete* and slightly higher for *T. stimpsoni* (Table 4.2). Total consumption of the available trochus occurred in both density treatments and in all three categories of predators. Total depletion of trochus occurred more frequently than predicted by random (Fig. 4.7). There was a general pattern for a higher than expected frequency of containers left with either many or few trochus, and a lower than expected frequency of containers left with an intermediate number of trochus.

Fig. 4.4 Short-term observations on three *T. stimpsoni* foraging at night. The bars show the percentage of the observation period that the crab spent in or outside a patch. t= period of observation. The solid line and solid circles (right y axis) show the proportion of prey consumed per patch by the end of the experiment, after 48 h. NIP= not in a patch. Low, medium and high correspond to the density of trochus in the patch. CW= carapace width.

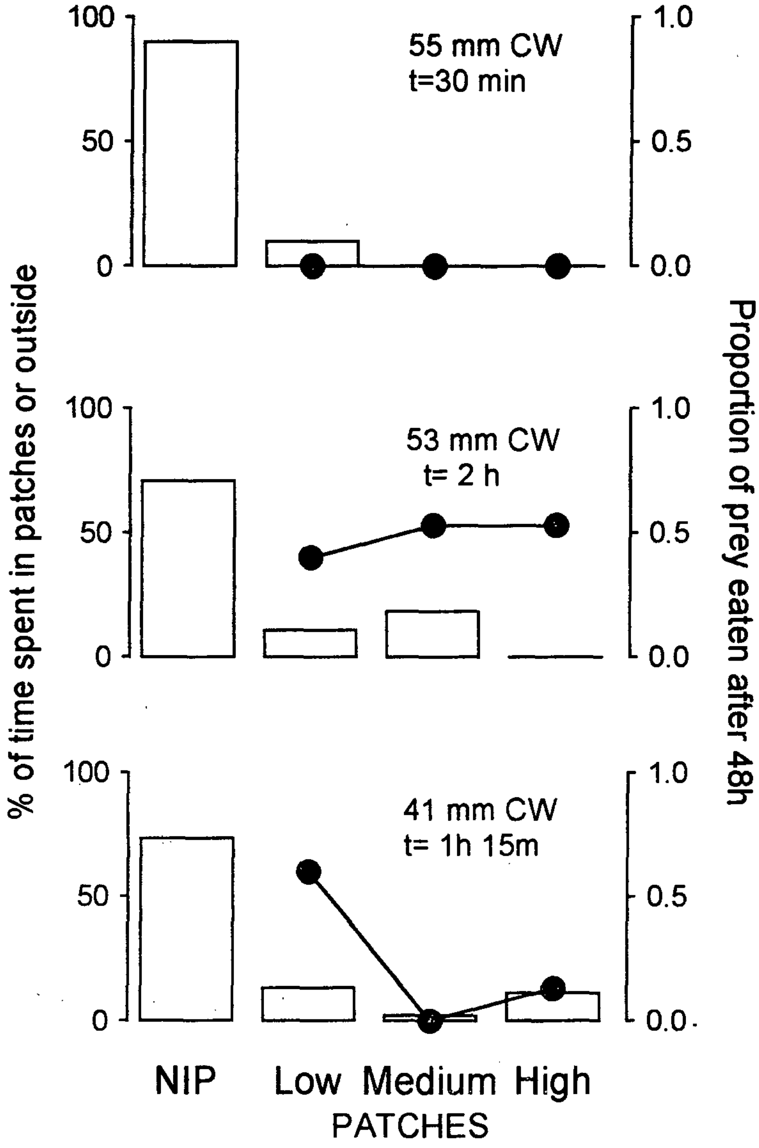


Fig. 4.5 Position of four *T. admete* at different times throughout the day (N=6 times). Bars show the number of times (total N=6) that a crab was observed in or outside a patch. The solid line (right y axis)shows the proportion of prey consumed per patch at the end of the experiment. NIP= not in a patch. Low, medium and high correspond to the density of trochus in the patch. CW= carapace width.

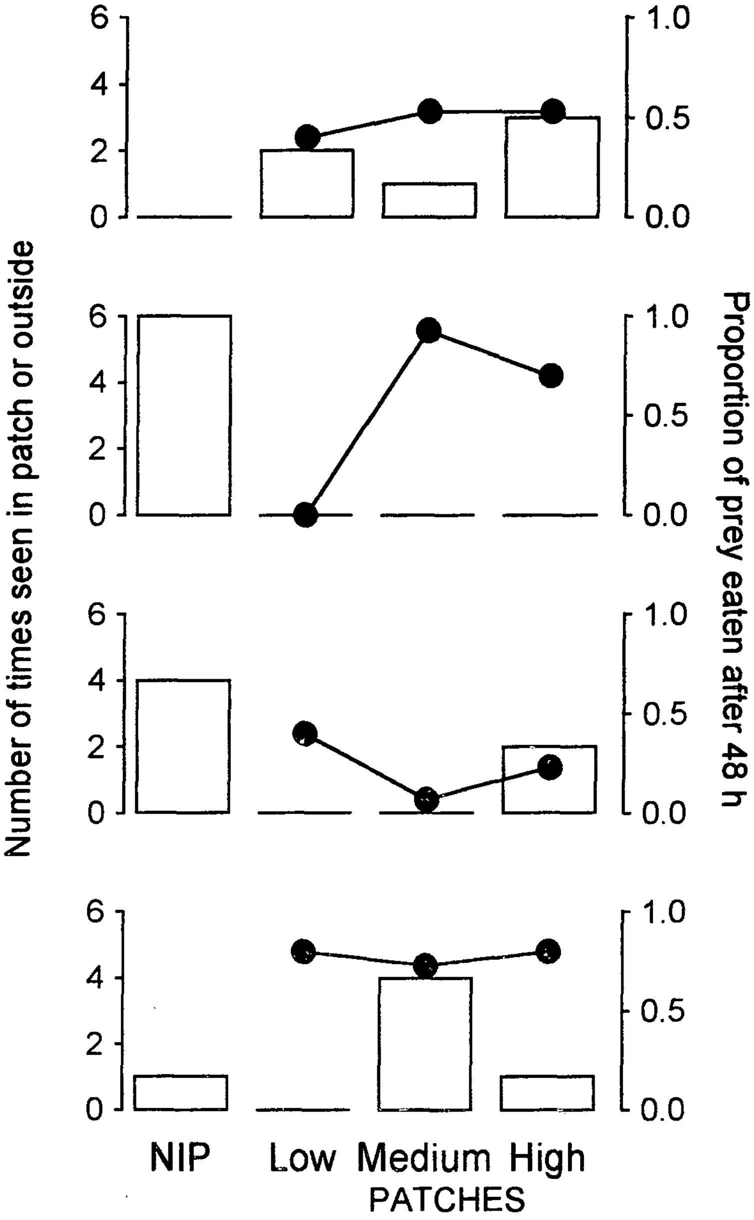


Fig. 4.6 Mean number of prey eaten and mean proportional mortality in containers with two densities of prey *Trochus niloticus*. For *T. admete* circles corresponds to the smallest size class (16-22 mm CW, N=16) and squares to the larger size class (23-35 mm CW, N=21). For *T. stimpsoni* crab size was 35-55 mm CW, N=22. CW= carapace width, vertical bars are standard errors. Data analysis shown in Table 4.3.

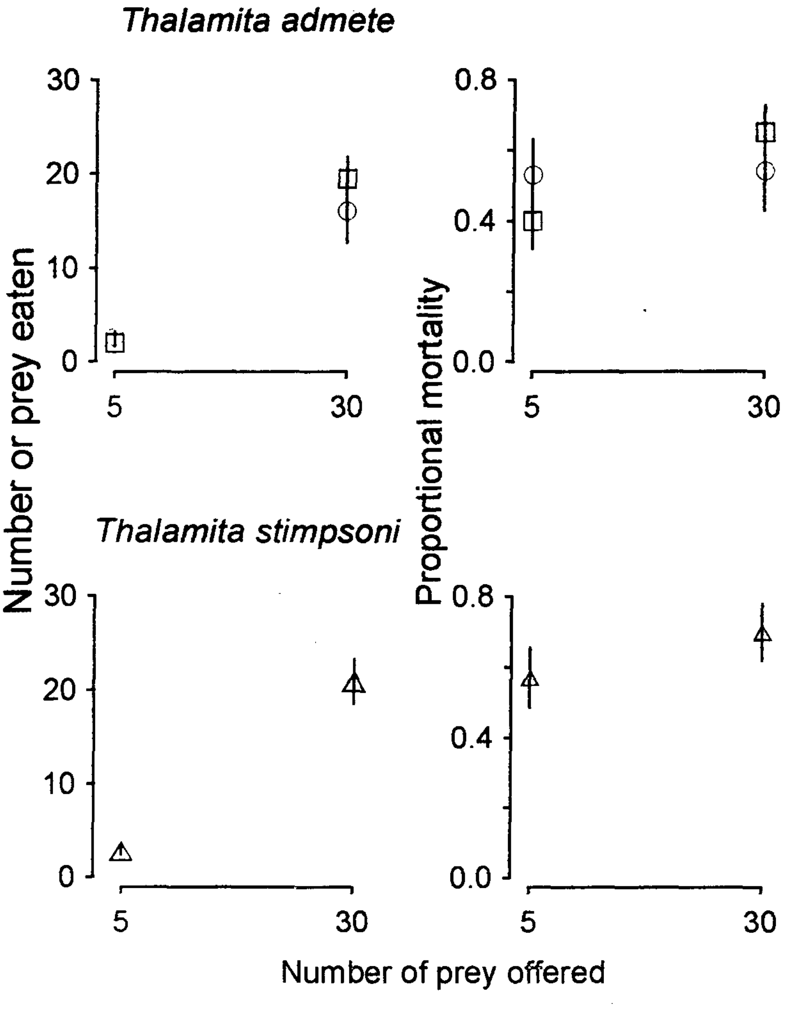
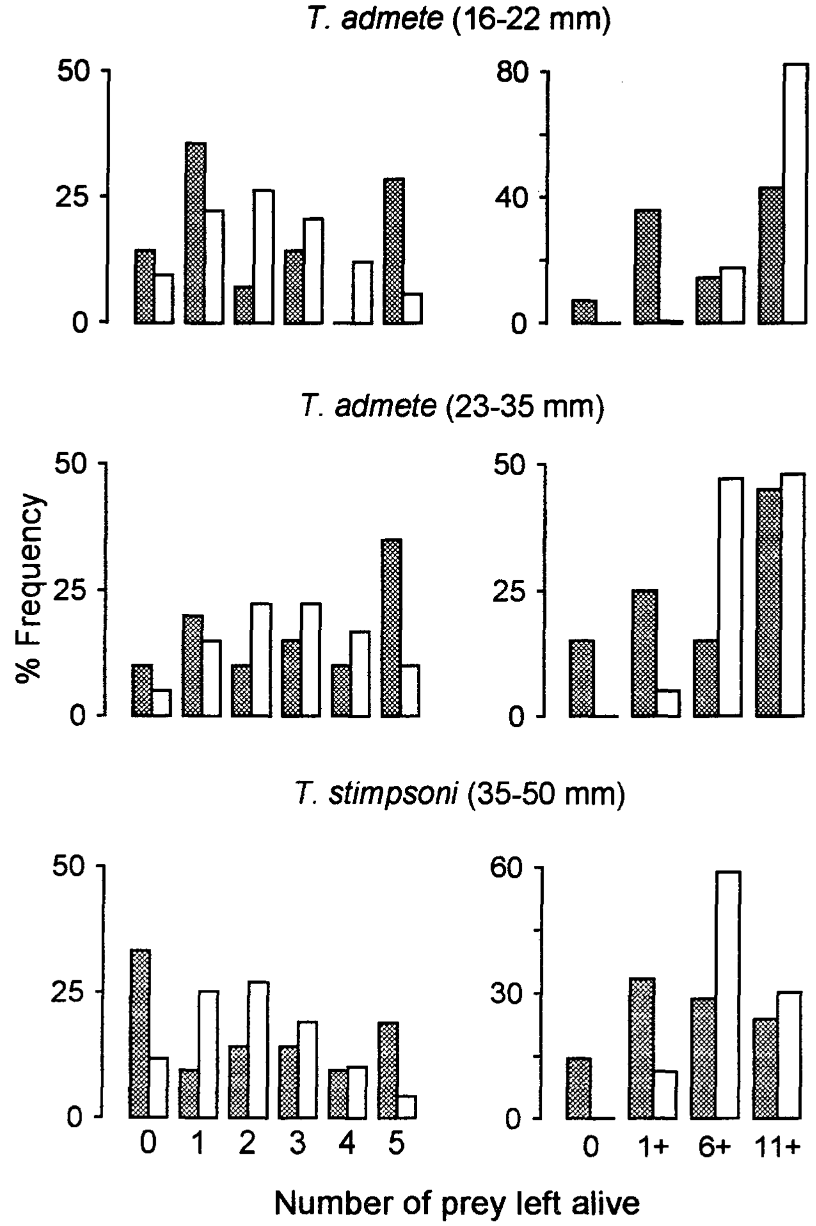


Table 4.3 Results of paired t tests to compare the number and the proportion of juvenile *Trochus niloticus* eaten after 24 h in containers, by *Thalamita admete* and *T. stimpsoni* at two prey densities, 5 and 30 trochus.container^{-1}. CW= carapace width. Data shown in Fig. 4.6

Predator	Predator size (mm CW)	N	p Number eaten	p Proportion eaten
T. admete	16 - 22	16	< 0.001	0.39
T. admete	23 - 35	20	< 0.001	< 0.001
T. stimpsoni	35 - 50	21	< 0.001	0.016

Fig. 4.7 Frequency distribution of the number of trochus surviving in container experiments, after 24 h when at low (left) and high (right) densities. Observed values (filled bars) and expected values (open bars). Expected values were estimated assuming a Poisson distribution and the corresponding mean number of surviving trochus.



4.4 Discussion

Increased trochus density was associated with greater numbers of trochus eaten in both containers and raceways experiments (Figs. 4.1 and 4.6). However, the increase in predation rate was proportionally higher in the containers but not in the raceways. An effect of prey density on proportional mortality in raceway experiments, would indicate that crabs foraged preferentially between patches. This would imply a mechanism by which crabs gained information about the distribution of prey (Stephen and Krebs, 1986). Crabs consumed more trochus in patches with higher density, but the proportion eaten did not differ significantly between patches for any of the two crab species, *T. admete* and *T. stimpsoni*. The power of the statistical tests for the effect of prey density was < 30% in all four analysis, however, more than an indication for the need of larger replicates, this result indicates that the effect of prey density on predation rate, if any, was very small, and this is particularly evident for the results after the first 24h of the experiment. Observations on crab behaviour indicate that crabs moved actively around the experimental area and spent time inside as well as outside the patches (Figs. 4.4 and 4.5). Crabs did not seem to identify patches with highest density of prey. A probable mechanism leading to the result of no proportional difference in mortality between patches is that crabs visited patches randomly and encountered more trochus in patches with higher density, if encounter rate was a linear function of prey density (e.g. Streams, 1994).

The results of experiments in containers showed, however, that trochus density had a greater than proportional effect on predation rate of two crab size-classes (the same ones also used in raceways experiments) (Fig. 4.6). Higher predator activity, leading to increased encounter with prey (Smith, 1974) and increased efficiency in handling prey (Cunninghan and Hughes, 1984), are mechanisms that could lead to increased predation rate at higher prey densities (Lipcius and Hines, 1986, Abrams et al., 1990). Total encounter rate increased significantly with prey density in the blue crab *Callinectes sapidus*, but this increase was related to a decrease in attack success rate and in persistence time at higher prey density (Eggleston, 1990). Sizes of trochus offered to *T. admete* and *T. stimpsoni* were well within the range of sizes they could eat and, judging from the complete absence of trochus lying upside down (an indication of handling by the predator) or damaged but alive (an indication of unsuccessful attack), trochus were most likely eaten as encountered and crabs were always successful in an attack. Thus, persistence time is not likely to have been important in this study.

Experiments in containers provide an idea of the potential response of predators foraging at high prey densities. Prey density in containers and raceways was high in comparison to natural densities and this may have affected the overall predation rate of crabs, if compared to the field situation, i.e. predation rates may have been greater than in the field. However, because of the crab's foraging behaviour, patches were depleted at a rate proportional to their density in the raceways. Thus, even if density affected predation rate, it did not result in crabs consuming proportionally more prey in the patches at high density, as could have been predicted from the experiments in containers. The implications of such behaviour on seeding will be discussed later.

The decision to leave a patch may be determined by 1) a number rule: leave after catching N prey, 2) a time rule: leave after t seconds and 3) a giving up time rule: leave after g seconds of unsuccessful search (Stephens and Krebs, 1986). Which rule is best varies according to the distribution of the prey (Iwasa et al., 1981; Stephens and Krebs, 1986). The limited number of behavioural observations suggest that the decision to leave a patch was not based on a number rule, since crabs were observed leaving a patch even if no trochus had been captured. Crabs stayed longer in patches where they captured prey, favouring the hypothesis of a giving up time rule rather than a time rule; however, this would need to be examined further.

Iwasa et al. (1981) showed that when the number of prey per patch has a high variance a giving up time rule would do best, and when the number of prey per patch follows a Poisson distribution a time rule would do best. The distribution of *Trochus* on the intertidal reef where predators were collected follows approximately a Poisson type (i.e. low density and random), and although they formed groups of 2-4 individuals.m^{-2} more frequently than expected by random, these were only occasional and, overall, their density was low (mean 0.143 m^{-2}, SD= 0.108, which includes juveniles too large to be preyed upon by these crabs) (Chapters 2 and 3). Density of other gastropods on the reef were also low and variable with the exception of two carnivorous gastropods *Morula biconica* and *Peristernia australiensis*, which occurred at high densities in the high intertidal area (Chapter 2). This study of the distribution of *Trochus* and other gastropod species on the reef indicates that the distribution and abundance of most species were variable. Based on these observations one would predicted that a time rule would be appropriate for *T. admete* and *T. stimpsoni*.

The difference in results between container and raceway experiments shows the importance of the crab's foraging behaviour in unrestrained conditions. Field studies have shown that some large crabs are mobile and can cover tens or hundreds of metres a day (Hill, 1978; Boulding and Hay, 1984; Hines et al., 1995), or they may move in slow meandering patterns (Hines et al., 1995) or spend several days in one place (Boulding and Hay, 1984). The intertidal xanthid, *Eriphia smithi*, moves up to 50m from its home refuge to search for food (Vannini and Gherardi, 1988). To my knowledge, there are no published studies on foraging behaviour in the field of members of the genus *Thalamita*, but Vezzosi et al. (unpubl. data cited in Cannicci et al., 1995) report that *Thalamita crenata* confined its activity to an area of about five metres radius around refuges in an intertidal mangrove swamp habitat. During casual observations at low tide, one individual *T. stimpsoni* ~40 mm CW moved a total of three metres in 15 min, five of which were spent under a rock. However, the crab remained within a 1m^{-2} area. Another *T. stimpsoni* ~40mm CW moved about one metre in a circular pattern about half a metre from a rock where it remained for 10 min. In many occasions large crabs (40-60 mm CW) were found out in the open and apparently far from a refuge. These observations indicate a degree of mobility and would predict the mobile behaviour observed in the raceways.

A minimum estimate of portunid crab density obtained using transects perpendicular to the coast line was 0.13.m^{-2} (SD 0.08) (Castell, unpublished data). This is a minimum estimate because large individuals were seen moving away from the sampled areas. Most crabs were solitary but two individuals were occasionally found together, one generally of greater size. These pairs were likely reproductively active, since they were found mating on several occasions. Solitary individuals were highly aggressive when put with other crabs in a container. These observations and estimates of density suggest that *T. admete* and *T. stimpsoni* do not live in high density aggregations.

Intraspecific interactions among predators can modify their impact on prey populations. For example, juvenile crabs, *Cancer magister*, emigrated from areas of high conspecific densities irrespective of whether or not food had been enhanced or predation risk increased (Iribarne et al., 1994). Laboratory experiments with *Callinectes sapidus* showed that predation rate per capita decreased as crab density increased (Mansour and Lipcius, 1991). Hatcher et al. (1996) found that an increase in crab density lasted for 16 h following the release of scallops but then crab density decreased to consistent low levels for the next months. Lawton and Zimmer-Faust (1992) stressed the difference between "fundamental foraging scope", which refers to the predator's inherent capacity, and the "realised foraging pattern" which is seen in nature under the influence of other factors that modify their behaviour. The difference observed between container and raceway experiments is an example of how behaviour can influence predation. More research is needed to determine the extent of similarity between foraging behaviour in raceways and in the field.

The abundance and distribution of prey can affect predator foraging behaviour and consequently have important applications for determining how to release individuals when seeding. These results show that *T. admete* and *T. stimpsoni* exposed to continuous high prey densities can increase their predation rates in a density-dependent manner. This could be the scenario if trochus are released at high densities. However, by releasing them in small patches, the intensity of predation per patch may not be density-dependent, as was observed in the raceway experiments. Because overall high density of prey can result in more intense predation, releasing trochus at low densities and in patches may result in higher survival. This topic of the possible influence of spatial patterns of trochus release on survival is discussed in more detail in the general discussion (Chapter 9).

CHAPTER 5

Predator - prey interactions among *Trochus niloticus*, *Turbo brunneus* and *Thais tuberosa*

5.1 Introduction

Behavioural defences have been shown to play an important role in interactions between prey and predators. As well as affecting the direct interaction between prey and predator (Sih, 1987), they also influence the competitive interaction between prey species, as has been demonstrated in larval salamanders (Walls, 1995), juvenile fish (Persson, 1991) and marine gastropods (Schmitt, 1982). Numerous studies have described antipredator responses of animals (see review by Edmunds, 1974). Many marine gastropods show such responses, varying widely from simple flight to sophisticated chemical defences (see reviews by Bullock, 1953; Ansell, 1969; Thompson, 1988; Pawlik, 1993).

Critical to the process of stock enhancement with hatchery-reared individuals is that they must respond appropriately to predators when released. Behavioural differences between cultured and wild juveniles of other gastropods species are a potential cause for increased mortality of cultured juveniles in the field (Shield and Welden, 1987; Stoner and Davis, 1994). Hence, I compared the antipredator response of cultured and wild juvenile *Trochus* to determine if lack of previous exposure to the predator had any effect on the behaviour of cultured juveniles.

The comparison of antipredator responses in closely related species exposed to a shared predator illustrates the development of different strategies to address a common problem. Differences in antipredator responses have been related to differences in the susceptibility of the species to capture by the predator, for example in sea urchins (Moitoza and Phillips, 1979), marine gastropods (Fishlyn and Phillips, 1980; Harrold, 1982; Schmitt, 1982; Watanabe, 1983), mayflies (McIntosh and Townsend, 1994) and amphibian larvae (Kats et al., 1988).

Trochus niloticus and *Turbo brunneus* Röding are gastropods belonging to closely related families, Trochidae and Turbinidae (Hickman and McLean, 1990). In North Queensland, *Turbo brunneus* (hereafter referred to as *Turbo*) and juvenile *Trochus* share the same intertidal habitat with the predatory snail *Thais tuberosa* Röding (hereafter referred to as *Thais*). A second aim of this study was to compare the interaction between these two potential prey species and a shared predator, with emphasis on the role of antipredator responses. I examined various components: 1) the distributional pattern of these three species in the field; 2) the proportion of mortality in *Turbo* and *Trochus* caused by non-crushing predators (i.e. *Thais tuberosa*); 3) the antipredator response of *Turbo* and *Trochus* to *Thais* in laboratory and field experiments and 4) the choice of prey by *Thais*.

5.2 Methods

The study site was the same intertidal reef flat described in Chapter 2. Observations were made between December 1993 and June 1995.

5.2.1 The Prey

Juvenile *Trochus* (1-~60 mm) live intertidally on reefs that dry during low tide, while larger individuals live subtidally (Smith, 1987; Nash, 1993). Cultured juvenile *Trochus* were reared in tanks at Orpheus Is. Research Station (Chapter 3).

Turbo brunneus reaches a maximum size of 50 mm shell length (SL) (Wilson, 1993). Individuals up to 35 mm SL were found intertidally in the study area. Maximum width was used for *Trochus* and maximum length for *Turbo* because individuals of the two species with similar values of the respective measures had similar dry flesh weights (see Results).

The Predator

Thais tuberosa is a carnivorous muricid gastropod that feeds on other gastropods. It attacks prey through the aperture, leaving the shell clean and undamaged, with the operculum lying loose inside. Abundant transparent mucus is produced while eating prey. Experimental individuals ranged in size from 42-51mm SL, representing the most abundant size range in the field. Other predatory gastropods present in the area were *Morula fiscella*, *Morula biconica* and *Peristernia australiensis*. Observed predation by these species most frequently involved drilling through the prey's shell, although prey were occasionally attacked through the aperture and left with undamaged shells.

For laboratory experiments, *Trochus*, *Turbo* and *Thais* were collected from the reef and kept in containers with flowing seawater at the field station. They were used in experiments within four days of collection. Cultured *Trochus* were moved from the culture tanks to containers.

5.2.2 Distribution

Natural densities of *Trochus*, *Turbo* and *Thais* were determined using 100 - 200 m transects perpendicular to the shore, covering most of the intertidal reef flat. Every five metres, one 1m^2 quadrat in 1993 and two quadrats in 1994 (due to low densities sampling intensity was increased) were sampled by counting and measuring all *Turbo*, *Trochus* and *Thais* present (see Chapter 2). I also counted and measured recently dead individuals (judged by the lack of algal overgrowth and wear of the shell) and inferred the cause of mortality by the presence of chipping, holes, crushing, drilling or no damage. When several shell fragments of the same species were found within one quadrat, the fragments were scrutinised to determine if components of one or more individuals were present. Undamaged shells were taken as an indication of mortality by non-drilling predatory gastropods. Seven transects were sampled in July-September 1993 and 10 more in July-August 1994. Sampling was within 2 hours of low tide.

To get an indication of the relative frequency of interaction between the two prey and the predator in the field, I estimated the proportion of 1m^2 quadrats where either *Turbo*, *Trochus* or both prey species were present together with the predator.

5.2.3 Prey Responses

5.2.3.1 Laboratory experiments

Three types of prey were used: *Turbo* and *Trochus* collected from the field, and cultured *Trochus*, which had never been exposed to the predator. A single prey was measured and placed on a small piece of coral rubble in the middle of a rectangular plastic container (27x11x9cm) supplied with continuous water flow (0.5 L.min^{-1}). The bottom and walls of the container were ruled into 3x3 cm numbered squares. Preliminary observations showed that, in the absence of rubble, the gastropods moved around excessively. Intended prey were left for 5-10 min. In each trial, one predator was introduced and the position of the prey was recorded every 30 s for 5 min. Prey behaviour, such as tentacle waving, raising the shell and secretion of substances, was also recorded. Prey were then subjected to either of two treatments: (1) To determine the reaction of the prey to the presence of the predator, the predator was added to the container approximately 10 cm from the prey. (2) To determine the reaction of the prey to contact with the predator, the predator was placed in the container and the prey was moved to be millimetres from the predator, so that, when the prey re-emerged, its tentacles made contact with the predator. Different prey individuals were used in control trials which also included presence and contact with a novel object, but in these cases the predator was replaced by a piece of recently collected coral rubble of similar size. Individual prey were used only once. Predators were used several times but never consecutively. Sample sizes are given in Table 5.3. Sizes of prey used in these experiments were: cultured *Trochus* 7-30 mm SW, wild *Trochus* 11-35mm SW, and *Turbo* 13-33 mm SL. I measured the pH of mucus released by *Trochus* in situ on four different individuals.

The data for each species were subject to analysis of variance. A two factor anova with predator (*Thais* or rock) and type of exposure (presence or contact) as main factors was used for *Turbo*. An additional factor was included for *Trochus*: juvenile type (wild or cultured). The frequencies of mucus release between wild and cultured *Trochus* and between presence and contact with the predator, were expressed as proportions (due to differences in sample size) and compared using logistic regression analysis.

5.2.3.2 Field experiments

Forty nine trials examined prey response to the predator in the field. As in laboratory experiments, a single prey, either *Turbo* or wild *Trochus*, was placed in a small tide pool and left for 5 minutes while activity was recorded (i.e. waving of cephalic tentacles, movement). After 5 min, a *Thais* was added to the pool approximately 10 cm from the prey (predator presence treatment) or a few millimetres from the prey (predator contact treatment). The reactions of both prey and predator were recorded and the net distance moved by the prey after 5 and 10 minutes was measured. Control observations were made for each species and for each type of interaction (presence or contact) using a piece of rubble instead of the predator. Individual prey were used only once and predators were used several times but never consecutively. Preliminary observations indicated that a response by the prey could be observed irrespective of the activity of the predator. Size range was 11 - 29 mm SL for *Turbo* and 22 - 44 mm SW for *Trochus*.

5.2.4 Prey Choice

Two types of experiments were used to examine prey choice by the predator. In the first, a single *Thais* was placed into a plastic container (27x11x9cm) with one *Trochus* and one *Turbo*. Water was supplied continuously at a rate of 0.5 L.min^{-1}. In the initial 30 trials, prey sizes were matched to represent similar dry flesh weight; but after initial results, 15 more trials were run where *Trochus* were 2-3.5 times larger than *Turbo*. The container had a top to prevent the prey escaping. In the second type of experiment, four different prey were offered to the predator, one each of *Trochus*, *Turbo*, the cowry *Cypraea annulus* (Linnaeus) and either the muricid *Morula biconica* (Blainville) or the fasciolariid *Peristernia australiensis* (Reeve). These species were chosen because they were relatively abundant in the study area. Experiments lasted 16 hours or until one prey was captured. The relationship between dry weight of flesh and shell dimension was determined by extracting and weighing the dry flesh of 42 cultured *Trochus*, 26 *Turbo*, 29 *C. annulus*, 16 *P. australiensis* and 9 *M. biconica*. The frequency of capture was compared among species using Fisher's exact test, with the null hypothesis being that all species had the same probability of capture.

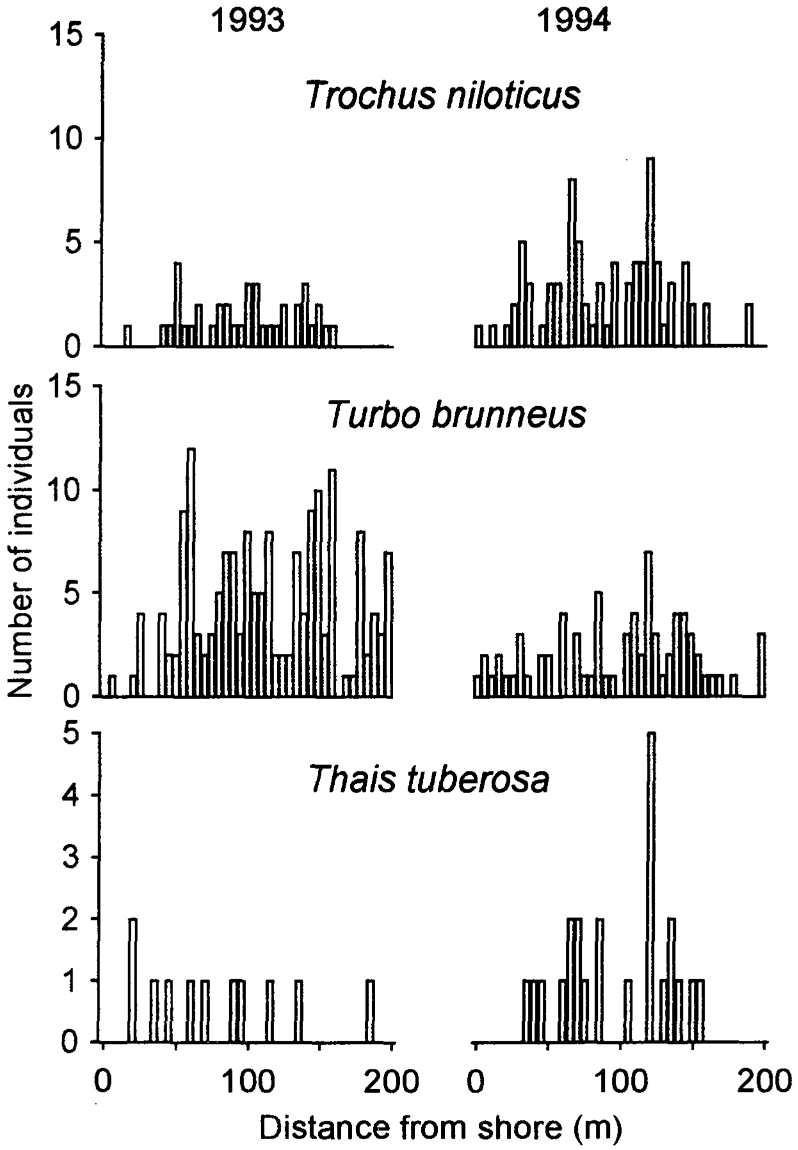
These experiments indicated that *Thais* preferred *Turbo* over *Trochus* so I tested whether the mucus released by *Trochus* was related to its lower preference as prey in a third experiment. A single *Thais* was placed inside a plastic container with standing water and left for 5-10 minutes. A single *Turbo* was then placed a few millimetres from the predator and held in place with a rod. Preliminary observations indicated that holding the prey was an effective way to induce a response from the predator within few minutes. If the predator moved away the prey was moved close again. The experiment ran for 10 minutes and the outcome of the experiment was classified as capture or no capture. In half of 34 trials, water containing mucus freshly released by *Trochus* was added at the same time as the *Turbo*. Individual prey and predators were used only once. The frequency of capture in water with and without mucus was compared using Fisher's exact test.

5.3 Results

5.3.1 Distribution

The distributions of *Trochus*, *Turbo* and *Thais* showed a high degree of overlap in both years of sampling (Fig. 5.1). *Turbo* and *Trochus* were found all over the intertidal shore, although they were more abundant 50 - 150 m from the shore. Most *Thais* were also found 30 - 150 m from the shore. Densities of *Trochus* and *Thais* remained similar between years, but the density of *Turbo* decreased significantly from 1993 to 1994 (Table 5.1). The density of *Trochus* was

Fig. 5.1 Abundance and distribution of *Trochus niloticus*, *Turbo brunneus* and *Thais tuberosa* across the intertidal reef flat at Orpheus Is. during 1993 and 1994. Data from all transects combined. Total area searched was 209 m^2 in 1993 and 590 m^2 in 1994.



significantly lower than the density of *Turbo* in 1993 ($p<0.0001$), but not significantly different in 1994 ($p>0.1$). *Turbo* were generally found inside holes and crevices in the rocks and were often difficult to reach by hand. *Trochus* were found on the sides or underneath of rocks and were generally more accessible.

Predators and prey were found in the same places at the same time. Of 36 *Thais* found in the transects, 23 (64%) were in the same quadrat ($1m^2$) as *Turbo* or *Trochus*: ten were found with one or two *Turbo*; eight were found with one or two *Trochus*, and five were found with both prey species: one to three *Trochus* and one or two *Turbo*. Thirteen *Thais* (36%) were found in quadrats with no *Turbo* or *Trochus* present but, in most of these cases, potential prey were present in nearby quadrats. The probability of finding each species was estimated from the total number of quadrats where each was present assuming that the three species were distributed independently. Fifty quadrats had more than one of the three species present and 20 quadrats had the predator *Thais* with a potential prey species (i.e. *Turbo*, *Trochus* or both). Species were found together more frequently than expected if the distributions were independent (Binomial test $p<0.001$ for more than one species together, and $p=0.002$ for finding *Thais* with *Turbo* and/or *Trochus*). While sampling the transects, it was never evident that prey were responding to the presence of *Thais* or that the predator was actively searching for prey.

Thais were observed eating *Turbo* in the field on four occasions. In three of these, a single *Thais* was feeding on a single *Turbo* (10, 12 and 21mm SL). In a fourth instance, three *Thais* (43-45mm SL) were all feeding on a *Turbo* 30mm SL. Predation by *Thais* on wild *Trochus* was observed only once in the field: one *Thais* 43 mm SL was eating one *Trochus* 32 mm SW. The same two individuals had been observed less than half metre apart the previous day. Predation on cultured *Trochus* was observed once in the laboratory in a pilot study, and once in the field during a seeding experiment. The presence on the reef flat of two empty cultured *Trochus* shells close to two *Thais* suggests that these juveniles may also have been eaten by *Thais*. *Thais* were also observed feeding on small (< 10 mm SL) *Cerithium* sp., a whelk *Engina* sp. (26 mm SL) and a mussel (20 mm SL) on the reef flat.

Approximately one third (29 %) of the recently dead *Turbo* shells found in the transects were undamaged and hence possible victims of *Thais*. About 10 % of dead *Trochus* shells were undamaged (Table 5.2).

5.3.2 Prey Response

Both *Turbo* and *Trochus* showed an antipredator response to *Thais* but the type of response differed between species. *Turbo* showed a flight response, moving approximately nine times faster in the presence of the predator. The intensity of the response was the same whether the interaction involved presence of or physical contact with the predator (Tables 5.3 and 5.4, Fig. 5.2). All *Turbo* responded within the first 30 seconds of exposure to the predator, raising their heads, extending and waving their cephalic tentacles very vigorously while moving very fast with the shell slightly raised. Wild and cultured *Trochus* did not move significantly faster when exposed to *Thais* (Tables 5.3 and 5.4, Fig. 5.2). Contact, whether with a rock or with *Thais*, resulted in a significant increase in speed in both wild and cultured trochus (Table 5.4, Fig. 5.2). In three of the four treatments, cultured trochus were more active than wild trochus, but the difference was not statistically significant (Table 5.4, Fig. 5.2).

While neither cultured nor wild *Trochus* moved more in the presence of the predator, they did change their behaviour. They oriented towards the predator with the shell slightly raised and extended, and gently waved their cephalic and epipodial tentacles. In 45% of all the trials with *Trochus* (wild and cultured), a thick white mucus was released after exposure to *Thais* . Mucus was produced significantly more often when there was contact with the predator (Table 5.3). A logistic regression analysis indicated that the incidence of mucus production differed significantly between presence or contact with a predator (Change in deviance= 16.44, df 1, $p<0.001$), but it was not significantly different between wild and cultured trochus (Change of deviance = 1.37, df 1, $p=0.639$). The mucus seemed to be released from the anterior side of the mantle fold, to the right of the head and it was produced one to three times during the five minutes observation period. Fifty % of cultured trochus released mucus only once, 39% released it twice and 11% released it three times. Eighty six % of wild trochus released mucus once and 14% released it twice. The time at which mucus was first released ranged from 11 seconds to 4.2 minutes in cultured juveniles and from 30 seconds to 4 minutes in wild juveniles. The size range of *Trochus* that released mucus was 8-30 mm for cultured and 17-30 mm SW for wild juveniles. The pH of the mucus produced by *Trochus* did not differed from the surrounding water (pH~7.5).

Table 5.1 Natural densities of prey and predator: mean numbers per squared metre ± SE. Total area sampled was 209 m^2 in 1993 and 590m^2 in 1994. p= probability value of t-test using Satterthwaite approximation to compare densities between years.

SPECIES	Mean Density		p
	1993	1994	
Trochus niloticus	0.19 ± 0.05	0.11 ± 0.02	0.308
Turbo brunneus	0.77 ± 0.10	0.13 ± 0.02	0.001
Thais tuberosa	0.08 ± 0.02	0.04 ± 0.01	0.062

Table 5.2 Frequency of damaged and undamaged recently dead shells of *Turbo brunneus* and *Trochus niloticus* found in transect surveys. The data for 1993 and 1994 were pooled.

	Trochus niloticus	*Turbo brunneus*
Number of dead individuals	41	294
Damaged (%)	90.2	71.8
Undamaged (%)	9.8	28.2

Table 5.3 Prey response to *Thais tuberosa* in 5 minutes laboratory observations and 10 minutes field observations. An individual *Thais* was placed 10 cm away from the prey in the predator presence treatment and few millimetres away in the predator contact treatment. [1] The response was classified as more active if increased tentacles waving, shell raising or slow movement was observed.

PREY SPECIES	PREDATOR	N	RESPONSE TO *Thais tuberosa* (Number of individuals) Flight	More active [1]	None	Mucus	Capture
LABORATORY OBSERVATIONS							
Turbo	Presence	10	10	0	0	0	0
	Contact	11	11	0	0	0	3
Trochus (cultured)	Presence	19	0	16	3	3	0
	Contact	20	0	19	1	15	0
Trochus (wild)	Presence	8	0	8	0	2	0
	Contact	9	0	9	0	5	0
FIELD OBSERVATIONS							
Turbo	Presence	12	2	5	2	0	4
	Contact	11	9	0	2	0	2
Trochus (wild)	Presence	13	0	7	6	1	0
	Contact	13	0	10	3	4	0

Table 5.4 Results of analysis of variance to determine the effect of *Thais tuberosa* on total distance moved by *Trochus niloticus* and *Turbo brunneus*. df degrees of freedom, MS mean sum of squares, $^*p \leq 0.05$. Corresponding mean values are shown in Fig. 5.2.

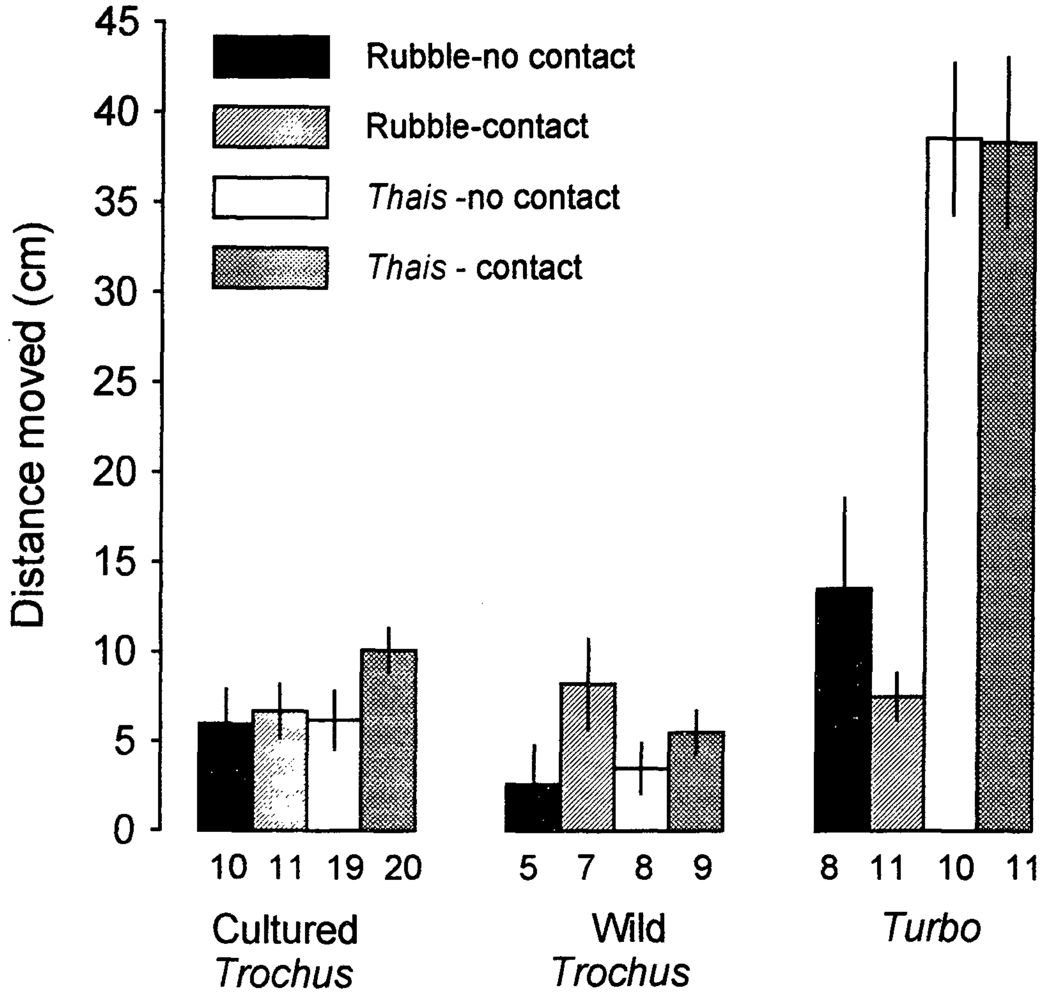
Trochus niloticus

Source	df	MS	F	p
Predator (*Thais* vs.rock)	1	4.40	0.13	0.715
Exposure level (presence vs.contact)	1	196.75	6.01	0.016*
Type (wild vs.cultured)	1	111.87	3.42	0.068
Predator*Exposure	1	0.05	0.00	0.968
Predator*Type	1	37.35	1.14	0.288
Exposure*Type	1	11.24	0.34	0.559
Predator*Exposure*Type	1	63.09	1.93	0.168
Error	87	32.71		

Turbo brunneus

Predator (*Thais* vs.rock)	1	7661.1	49.42	0.000*
Exposure level (presence vs.contact)	1	94.2	0.61	0.441
Predator*Exposure	1	84.9	0.55	0.464
Error	36	155.0		

Fig. 5.2 Mean total distance (±SE) moved by prey after 5 minutes when initially 10 cm from (no contact) or when in contact with the predator. Control treatments had a piece of rubble in place of the predator. Numbers along the x axis are sample sizes.



No prey showed an increase in activity in field trials with the control stimulus. The response of *Turbo* to the presence of *Thais* 10 cm away was much less intense in the field than in the laboratory, with only two of the 12 individuals showing a flight response (Table 5.3). In four other cases, the predator moved towards the prey, which responded only when the predator was about to capture it. All four ended in capture. Nine (82%) individuals responded with flight when in contact with *Thais* (Table 5.3), and this response was evident within the first 30 seconds. Two individuals did not show any response and these were captured by the predator within 10 minutes. Shortly after being captured, each *Turbo* released a greenish-transparent substance that was not as thick as the mucus released by *Trochus*. In each case, the predator seemed to react by extending its proboscis temporarily away from the released substance, but held onto the prey with its foot and eventually the *Turbo* was engulfed.

The response shown by *Trochus* in the field was similar to that observed in the laboratory experiments, with a stronger response to contact with the predator. When *Thais* was present but not in contact, half of the juveniles did not respond and the other half showed some increase in activity, but only one individual released mucus (Table 5.3). When in contact with *Thais*, 77% of the juveniles showed an increase in activity compared to 23% that did not show any response. Thirty percent of the individuals released mucus. In no case did the predator attempt to capture a *Trochus*.

5.3.3 Prey Choice

Thais showed a strong preference for *Turbo*, both when it was offered with only *Trochus* and when it was offered with three other species (Table 5.5, $p<0.0001$). For individuals of the same maximum shell dimension, *Turbo* had slightly more flesh than *Trochus* (Fig. 5.3). However, *Turbo* was the preferred prey even when flesh weight was lower than for the other available prey. Of 22 trials in which *Turbo* was captured, 10 were 1-5mm smaller than *Trochus*, three were the same size and nine were 1-4mm larger than *Trochus*. In 15 trials where *Turbo* was 2-3 times smaller than *Trochus* no prey was captured.

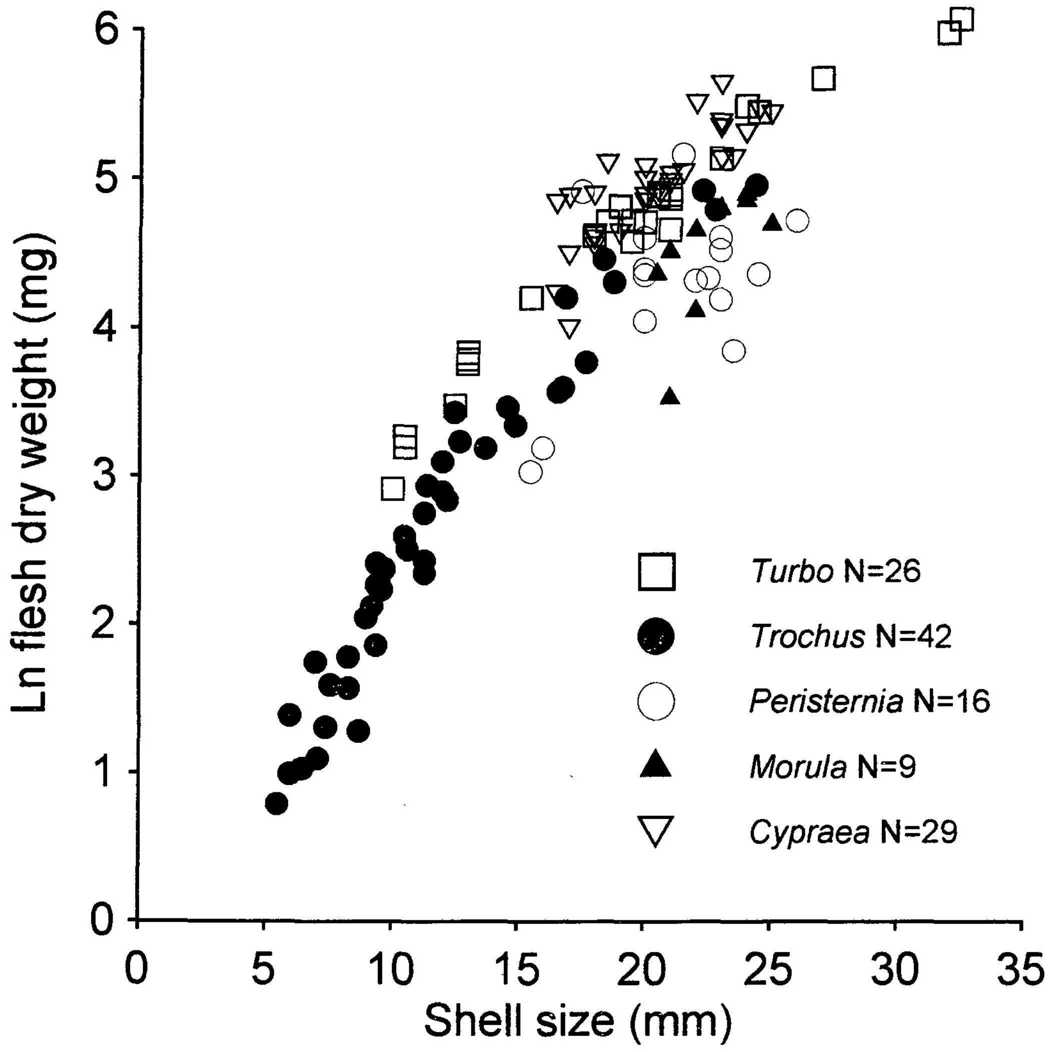
Capture rate of *Turbo* was greatly reduced when mucus from *Trochus* was added to the water. In eight of the 17 trials (47%) where *Turbo* was held close to *Thais* in water without mucus, the predator grabbed the prey, whereas no *Turbo*

Table 5.5 Frequency of prey capture by *Thais tuberosa* in choice experiments. One individual of each species was offered at a time. Exp. 1b was as Exp. 1a but flesh weight of Trochus was 2-3 times greater than flesh weight of Turbo (see methods for details).

	PREY SPECIES			
	Turbo brunneus	*Trochus niloticus*	*Cypraea annulus*	*Morula biconica* or *Peristemia australiensis*
EXP. 1a				
Offered	30	30		
Captured	12	0		
EXP. 1b				
Offered	15	15		
Captured	0	0		
EXP. 2				
Offered	12	12	12	12
Captured	11	0	0	0

Fig. 5.3 Relationship between shell dimensions (X=ln shell width or height) and dry flesh weight (Y= ln dry weight) of species offered as prey in prey choice experiments. Shell size is maximum shell width for *Trochus niloticus* and maximum shell length for all other species. Note y axis has a logarithmic scale. $p<0.0001$ for all slopes.

Trochus $Y= 2.89X - 4.31$ $R^2= 0.95$
Turbo $Y= 2.56X - 2.85$ $R^2= 0.98$
Cypraea $Y= 2.50X - 2.55$ $R^2= 0.70$



was captured in trials with mucus in the water (Table 5.6). This difference was statistically significant (Fisher's exact test p= 0.001). The predator seemed to be aggravated by the presence of mucus: it either repeatedly turned away from the prey, or it became inactive but with its siphon extended. The predators always became active again when transferred to clean water.

Table 5.6 Frequency of capture of *Turbo* by *Thais tuberosa* in water with and without mucus freshly released by *Trochus*.

EXP. 3	*Turbo* in water without mucus	*Turbo* in water with mucus
Offered	17	17
Captured	8	0

5.4 Discussion

Trochus and *Turbo* differed in their behavioural responses to the predator *Thais*. *Turbo* showed the characteristic "flight" response of many gastropods responding to carnivorous gastropods and starfishes, whereas *Trochus* showed a less common behaviour of mucus production. Fleeing seems to be the most common antipredator behaviour in archaeogastropods (e.g. Bullock, 1953; Feder, 1963; Ansell, 1969; Hoffman and Weldon, 1978; Hoffman, 1980; Schmitt, 1981; Engstrom, 1982), whereas chemical defences are most common among more advanced gastropods such as mesogastropods and opisthobranchs (Thompson, 1988; Pawlik, 1993). To the best of my knowledge, trochid gastropods have not been reported to release mucus in response to predators. I found only three references to antipredator behaviour within the genus *Trochus*: both *Trochus viridis* (Clarke, 1958) and *T. pyramis* (Kohn and Waters, 1966) showed increased speed of locomotion in the presence of a predator, but mucus release was not mentioned. *Trochus intexus* twisted its shell by a strong movement of the foot when attacked by a hermit crab (Hazlett, 1989). I observed *T. niloticus* showing a similar behaviour when having on top of its shell an individual of the carnivorous gastropod *Morula fiscella*. Some haliotid species (archaeogastropods) release mucus as they flee from predatory starfishes (Montgomery, 1967). Acmaeid limpets also have chemical defences (Fishlyn and Phillips, 1980; Pawlik et al., 1986). However, the response shown by *Trochus* in this study is most similar to that of some pulmonate limpets such as *Trimusculus reticulatus* and *Siphonaria capensis*, which produce white mucus when irritated (Branch and Cherry, 1985; Rice, 1985). Mucus release in *Trimusculus reticulatus* was, as in *Trochus*, associated with lower predation rate (Rice, 1985). Faulkner and Ghiselin (1983) suggested that chemical defences developed in association with shell loss, either as a preadaptation or a postadaptation. The presence of a mucus response in *Trochus* is another example of the existence of chemical defences in shelled gastropods.

Three facts indicate that mucus production in *Trochus* is a mechanism to discourage an attack by predators such as *Thais*. Firstly, feeding observations show that *Thais* can feed upon *Trochus* and that the meat is palatable. Secondly, mucus release was only observed in response to predatory gastropods, particularly *Thais*, but also *Morula fiscella*. Thirdly, the decrease from 47% to no capture of *Turbo* in experiments where the water contained mucus produced by *Trochus* links the antipredator function of this mucus to the lower preference of *Thais* for *Trochus* as prey. How the mucus affects the predator is not yet clear. The predator showed repulsion by turning away from the source of mucus and in some cases becoming inactive. If the mucus served to disturb the predator, allowing the prey to gain time for escape, prey movement should increase after mucus release, which was not the case. Alternatively, if the function of mucus was to prevent detection of the prey, as in the limpet *T. reticulatus* (Rice, 1985), movement should have been minimal after mucus release to decrease dispersion of mucus away from the prey. This also was not the case.

Thais preferred *Turbo* out of the four species offered in laboratory experiments. This preference seemed to be independent of prey size. Flesh dry weight alone does not provide enough information to determine if *Thais* preferred the most profitable prey. A proper estimate of profitability should include flesh caloric content and handling time. However, *Trochus* was not selected as prey even in cases where its flesh weight was 2-3 times greater than the flesh weight of *Turbo* offered at the same time. This suggests that difference in profitability between the two prey species was not the reason for the observed preference. Differences in prey abundance may also influence prey selection by conditioning the predator (Dunkin and Hughes, 1984; Palmer, 1984), but this is unlikely to be important in this study. Estimated density of *Turbo*, the preferred prey, decreased significantly in the study area between 1993-1994. Although *Turbo* was more abundant than *Trochus* in 1993, densities of the two prey were similar in 1994. Since predators that were exposed to different field densities of *Turbo* preferred this species in both years, prior experience is not likely to have caused the observed preference. Field observations indicate that *Thais* feeds upon a variety of gastropods and bivalves, making it unlikely that *Trochus* was not recognised as potential prey. Of the factors examined, the difference in antipredator response between *Turbo* and *Trochus* is most compatible with the results.

Predators and prey coexisted within relatively small areas ($1 m^2$) and were found together more frequently than expected by random. This was surprising given the strong and consistent escape response shown by *Turbo* in laboratory experiments and the relatively low density of *Thais* on the intertidal reef. Field observations indicated that *Turbo* did not respond to *Thais* with the same intensity as in the laboratory. All *Turbo* showed a flight response in laboratory experiments whether the predator was 10 cm away or in contact. In field observations, the corresponding figures were 17% and 82% (Table 5.3). Wild *Trochus* also responded more strongly in the laboratory than in the field. The reasons for this difference are not known but it would explain the coexistence of prey and predators within small areas. Coexistence of prey and predators in close proximity has been noted in other studies: Feder (1963) observed tide pools with both starfish predators and gastropod prey present and sometimes the prey were even grouped around the predator. Garrity and Levings (1981) observed that the gastropod *Nerita scabricosta* seemed to acclimate to the presence of a predator after 10 minutes. Prey may need a closer contact with the predator to elicit a response in the field. Both *Turbo* and *Trochus* responded much more strongly when in contact with than in the presence of *Thais* in the field. Similarly, the snail *Stramonita* reduced feeding in response to the presence of nearby crabs in laboratory experiments, but a direct contact seemed to be necessary in the field (Richardson and Brown, 1992). If leaving a refuge to avoid a predator can result in increased risks from other sources, prey may only respond when the predator is very close. However, similar behaviour in field and laboratory experiments would still be expected. One explanation for the observed differences may be that the concentration of the predator's chemical cues in the water was stronger in laboratory experiments.

The larger proportion of freshly dead undamaged *Turbo* compared with *Trochus* (Table 5.2), combined with the preference of prey shown by *Thais* in laboratory experiments (Table 5.5), suggest that mortality due to predation by *Thais* is more important in the population dynamics of *Turbo* than of *Trochus*. Adding mucus gave extra protection to *Turbo*, indicating that the antipredator response of *Trochus* is more effective against *Thais* than is the flight response of *Turbo*. Thus, differences in vulnerability to predation by *Thais* between *Turbo* and *Trochus* are likely to be related to differences in response. The extent of competitive interactions between *Turbo* and *Trochus* is not known. The species share the same habitat and are herbivorous, and so are potential competitors for food and space, but their diets and their microhabitat requirements have not yet been described.

5.5 Comparison of cultured vs. wild *Trochus*

Cultured and wild juvenile *Trochus* responded similarly to *Thais* in laboratory experiments. It appears that the response is genetically based and is not affected by rearing conditions. Both wild and cultured *Trochus* had the capability to release mucus. This study indicates that the response of *Trochus* to *Thais* and possibly to other carnivorous gastropods, may reduce the susceptibility of this species to predation by gastropods. A reduction in response of cultured trochus could increase their mortality after release. In other gastropod species (Geller, 1982; Thomas and Himmelman, 1988), as well as in other animal groups (e.g. Kats et al., 1988), populations from areas with lower predator densities showed reduced antipredator response. This means that the source of broodstock may influence the presence and intensity of antipredator responses in cultured juvenile *Trochus*. The broodstock used in this study came from the same reef as the wild trochus and predators. This kind of study should be extended to include broodstock or juvenile *Trochus* from other areas where carnivorous gastropods are at very low densities.

To conclude, this study showed that the interaction with the predator *Thais* differs between *Turbo* and juvenile *Trochus*. Distributional patterns indicate that *Turbo* and *Trochus* interact with their predator *Thais* and that encounters between prey and predator are common. Relative proportions of freshly dead damaged and undamaged shells and results of prey choice suggest that *Thais* preys upon *Turbo* more intensively than it does on *Trochus*. The reasons for this difference appear to be strongly related to the different antipredator responses shown by these two prey species. The release of mucus by *Trochus* seems to be more effective in avoiding predation by *Thais* than is the flight behaviour of *Turbo*. The results stress the significance of behaviour in interactions between species and its potential importance to the success of population enhancement programs.

CHAPTER 6

Detectability of cryptic juvenile *Trochus* in stock enhancement experiments.

6.1 Introduction

Seeding experiments involve the release of tagged or untagged juveniles, followed by periodic censuses to determine survival rates (e.g. Hoffschir et al., 1989; Amos, 1991; Kubo, 1991; Schiel, 1993). The feasibility of seeding will be determined by the economic balance between costs of producing hatchery-reared juveniles of various sizes and the proportion that reach maturity after release. Survival estimates are a key measure in assessing whether seeding is a viable tool for population enhancement.

Small trochus (< 30 mm maximum shell width) (SW) are difficult to find (Heslinga et al., 1984; Smith, 1987; Kubo, 1991). They are cryptic because of their small size, the colours of their shells are similar to those of the coralline algae of the rubble on which they live and because the heterogeneous substrata provide many places where juveniles can hide. The effect of their cryptic nature on recapture and survival estimates are likely to be important. Studies of small abalone, another cryptic gastropod, indicate that up to 70% of the population can be missed during a census (Shepherd et al., 1982; Shepherd, 1990; Tanaka et al., 1991). Several authors (Heslinga et al., 1984; Smith, 1987; Kubo, 1991) have noted the difficulty of finding juvenile *Trochus*, but the question of how this may affect estimates of survival has not been addressed.

This chapter focuses on how searching success affects estimates of survival in *Trochus* given the cryptic nature of the juveniles. I describe two experiments in which I measured the proportion of trochus that could have been overlooked in the search area. More specifically I asked: 1) How much more frequently are conspicuously marked trochus recovered than inconspicuous trochus? 2) By what magnitude overlooking trochus will affect estimates of survival?; and 3) Is the effect of flagging similar between live and dead trochus?

6.2 Methods

6.2.1 Study sites

Experiments were carried out in Australia and Vanuatu during 1994. One study site was on the north-east side of Orpheus Is., described in detail in Chapter

2. The second site was on the north-east side of Moso Is. (17° 6' S, 168° 18' E), a coral island NW of the main island of Efate in Vanuatu. Both sites have intertidal reef flats 150 - 200 m wide and the substrata are composed largely of coralline rubble, coralline rock bench and calcareous sand. The reef flat at Moso Is. is more protected from wave action and it is sandier than the reef flat at Orpheus Is.

6.2.2 Experiment 1 (Orpheus Is.)

This experiment commenced on June 22, 1994. There were three replicate plots 2x2 m, at each of three intertidal levels, high, mid and low. The levels corresponded to different distances from the beach (20 m, 70 m and 120 m, respectively). Juvenile *Trochus* used in this experiment were cultured in tanks at Orpheus Is. Research Station (see Chapter 3). Forty trochus were released per plot to give a density of 10 m^{-2}. Half of them were marked with a numbered tag glued to the shell (Hallprint Inc., SA), and the other half were marked with a tag plus a "flag" (1x1 cm piece of fluorescent forestry pink tape) glued to the shell. Shells with the brightly coloured flag were easily picked out by an observer walking on the reef. Hereafter, I will refer to trochus with the pink tape as "flagged" and trochus with only tags as "unflagged". The mean size of released trochus was 23.3 mm (2.1 std, range 17-30 mm). A total of 360 trochus was released In this experiment. Six plots were censused on June 24 and the other three on June 25. An area 6x6 m centred on the original release site of each plot was sampled using 1m^2 quadrats and all living and dead juveniles were recorded.

6.2.3 Experiment 2 (Moso Is.)

Trochus used in this experiment were cultured in the Vanuatu Fisheries Department hatchery. The experiment commenced on May 9, 1994. Trochus were released in two intertidal levels, high (approximately 40 m from the beach) and low (120 m from the beach). There were six plots 2x2 m per level, three were seeded with trochus at a density of 5 m^{-2} and the other three with a density of 20 m^{-2}. Half of the trochus released in each plot had only tags and the other half had tags plus the "flags". The mean size of experimental trochus was 30.3 mm SW (3.2 std, range 23 - 48 mm SW). A total of 600 trochus was released. All plots were censused on May 11 by searching an area of 6x6m around the centre of each plot.

6.2.4 Data Analysis

In both experiments, data for live and dead individuals were analysed separately using a factorial analysis of variance. For Exp. 1 (Orpheus Is.) the main factors of the analysis were flagging, intertidal level (both fixed factors) and plot (nested within intertidal level). For Exp. 2 (Moso Is.) the numbers of live and dead individuals recaptured were expressed as proportions of the number initially released. In this case flagging, seeding density and intertidal level were the main fixed factors of the analysis and the factor plot nested within intertidal level and density. The Brown-Forsythe's procedure was used to test for homogeneity of variances (Keppel, 1991).

The probability of sighting a trochus was estimated using the expression:

$$\textit{Sighting probability} = \frac{\textit{proportion of live unflagged trochus recaptured}}{\textit{proportion of live flagged trochus recaptured}}$$

6.3 Results

6.3.1 Experiment 1 (Orpheus Is.)

In eight of the nine plots the total number of flagged juveniles recaptured alive was higher than that of unflagged juveniles (Fig. 6.1). In the only plot where this was not the case, number of flagged and unflagged trochus recaptured differed by only one individual (Fig. 6.1). Mean recapture of flagged trochus was 76 % (SE= 4) compared to 52 % (SE= 5) of unflagged trochus. The difference was statistically significant (Table 6.1). Mean sighting probability of live trochus was estimated to be 0.69 (95% CI= 0.56 - 0.82).

Mean recapture of dead flagged trochus was 9% (SE= 3) compared to 7% (SE= 2) of unflagged trochus. There was high variability among intertidal levels and within replicate plots (Fig. 6.2, Table 6.2 p(plots)<0.05). Overall, the mean number of flagged and unflagged trochus recovered dead was not significantly different (Table 6.1); however, the power of the test to detect the difference found as significant was < 10% (Effect size= 0.01). There was also a significant interaction between intertidal level and flagging (Table 6.1). Analysis of simple effects showed that the number of dead flagged trochus was significantly higher than that of unflagged trochus in the high intertidal level (p=0.0134) but not significantly different in the mid (p=0.222) and low (p=0.529) levels (Fig. 6.2).

Fig. 6.1 Total number of flagged (open bars) and unflagged (filled bars) juvenile *Trochus niloticus* recaptured alive after two-three days at Orpheus Is., Australia. There were three replicate plots within each of three intertidal levels (high, mid and low) defined by their distance to the beach.

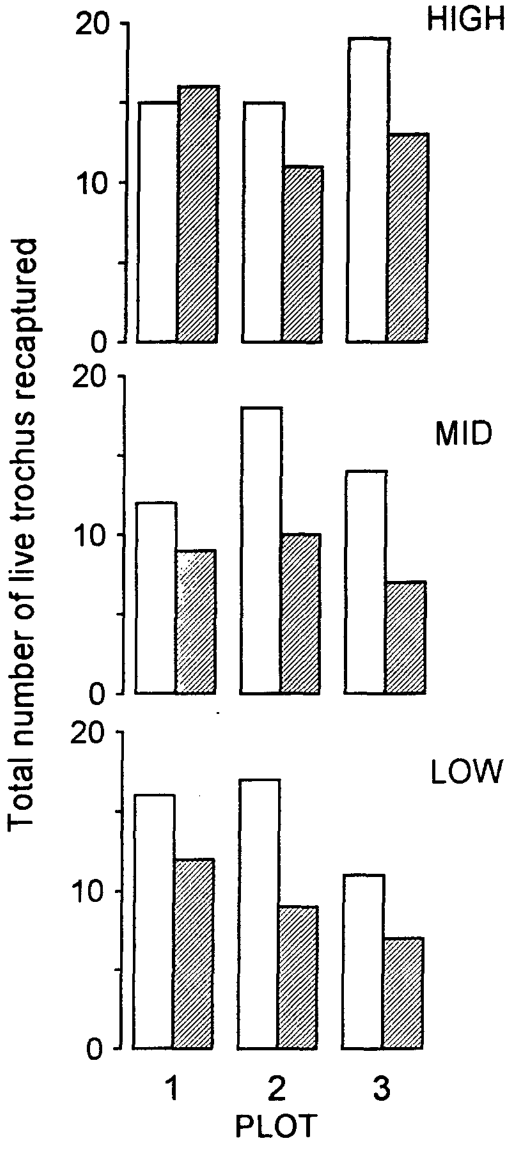


Table 6.1 Results of Experiment 1, Orpheus Is. Analysis of variance to examine effect of flagging and intertidal level on the number of live and dead juvenile *Trochus niloticus* recovered two-three days after the release.

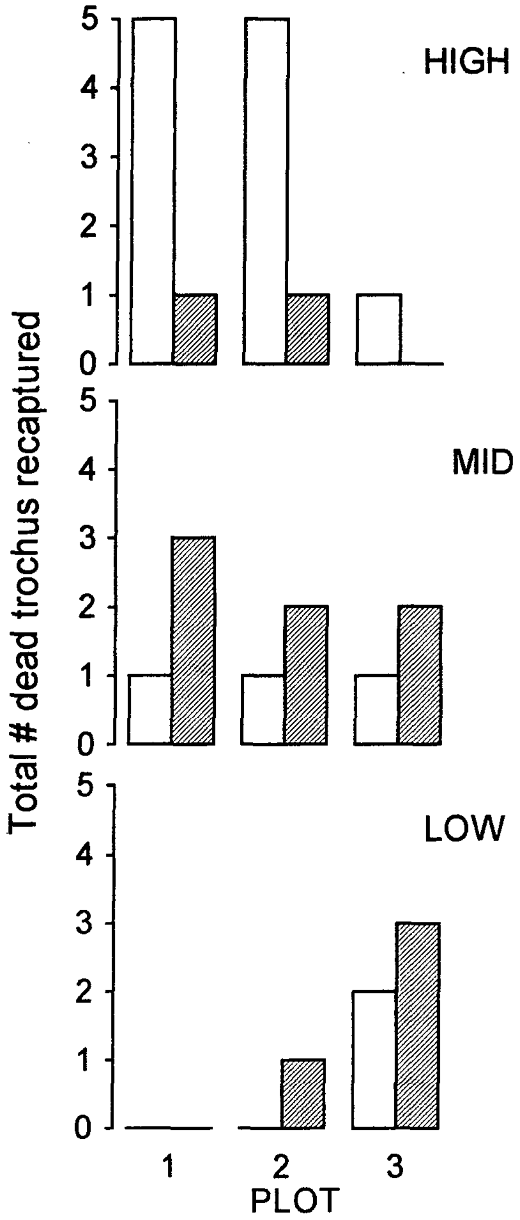
NUMBER OF LIVE TROCHUS

Source	**MS**	**df**	**F**	**p**
Flagging	102.72	1	24.33	0.002
Intertidal level	18.17	2	1.99	0.217
Plot (level)	9.11	6	2.16	0.186
Flagging*level	3.72	2	0.88	0.462
Error	4.22	6		

NUMBER OF DEAD TROCHUS

Source	**MS**	**df**	**F**	**p**
Flagging	0.50	1	0.82	0.401
Intertidal level	2.06	2	0.79	0.497
Plot (level)	2.61	6	4.27	0.051
Flagging*level	8.17	2	13.37	0.006
Error	0.61	6		

Fig. 6.2 Total number of flagged (open bars) and unflagged (filled bars) juvenile *Trochus niloticus* found dead after two-three days at Orpheus Is., Australia. There were three replicate plots within each of three intertidal levels (high, mid and low).



6.3.2 Experiment 2 (Moso Is.)

There was a consistent pattern of higher recapture of flagged live trochus among replicate plots: more flagged trochus were found in 10 of the 12 plots, one plot had the same number of both types and one plot had slightly more unflagged trochus (Fig. 6.3). The ratios between flagged and unflagged trochus ranged from 1.13 to 1.33 (Table 6.2). Statistically, the difference between the proportion of live flagged and unflagged trochus recaptured was significant ($p=0.025$, Table 6.3). Mean sighting probability of live trochus was 0.81 (95% CI= 0.69 - 0.93).

A total of 18 trochus were recovered dead after two days. The mean proportion of flagged dead trochus recaptured was 3.1% (SE= 1.1) compared to 3.0% (SE= 1.7) for unflagged trochus, with the difference being not statistically significant (Tables 6.3 and 6.4). The power of this test was < 10% (Effect size=0.13).

6.3.3 Estimates of survival (Exps. 1 and 2)

Data for recovery of trochus in each experiment two-three days after release are summarised in Fig. 6.4. Survival estimates at Orpheus and Moso Is. based on total recapture of flagged trochus were 1.5 and 1.2 times higher respectively, than estimates based on recapture of unflagged juveniles. Similarly, when using the results for unflagged trochus, the proportion of juveniles unaccounted for was 2.7 times higher for Orpheus Is. and 1.4 times greater for Moso Is. than when using results of flagged juveniles. Estimates of mortality based on recovery of flagged and unflagged shells were very similar in both study sites.

6.4 Discussion

The proportion of flagged trochus recovered alive two to three days after the release was significantly higher than the proportion of unflagged trochus (Figs. 6.1 and 6.3). The pattern for more flagged than unflagged trochus was consistent between the two experiments and among experimental plots within each experiment. The most likely explanation for these results is that flagged trochus were easier to detect by observers censusing the area. Thus, the results support the hypothesis that a significant proportion of trochus in the censused area will be overlooked by the observer simply because they are cryptic and live in a complex habitat.

Fig. 6.3 Percentage of flagged (open bars) and unflagged (filled bars) juvenile *Trochus niloticus* recaptured alive two days after released at Moso Is., Vanuatu. There were three replicate plots for each of two seeding densities, 5 and 20 individuals.m^{-2}, within the deep and shallow intertidal levels.

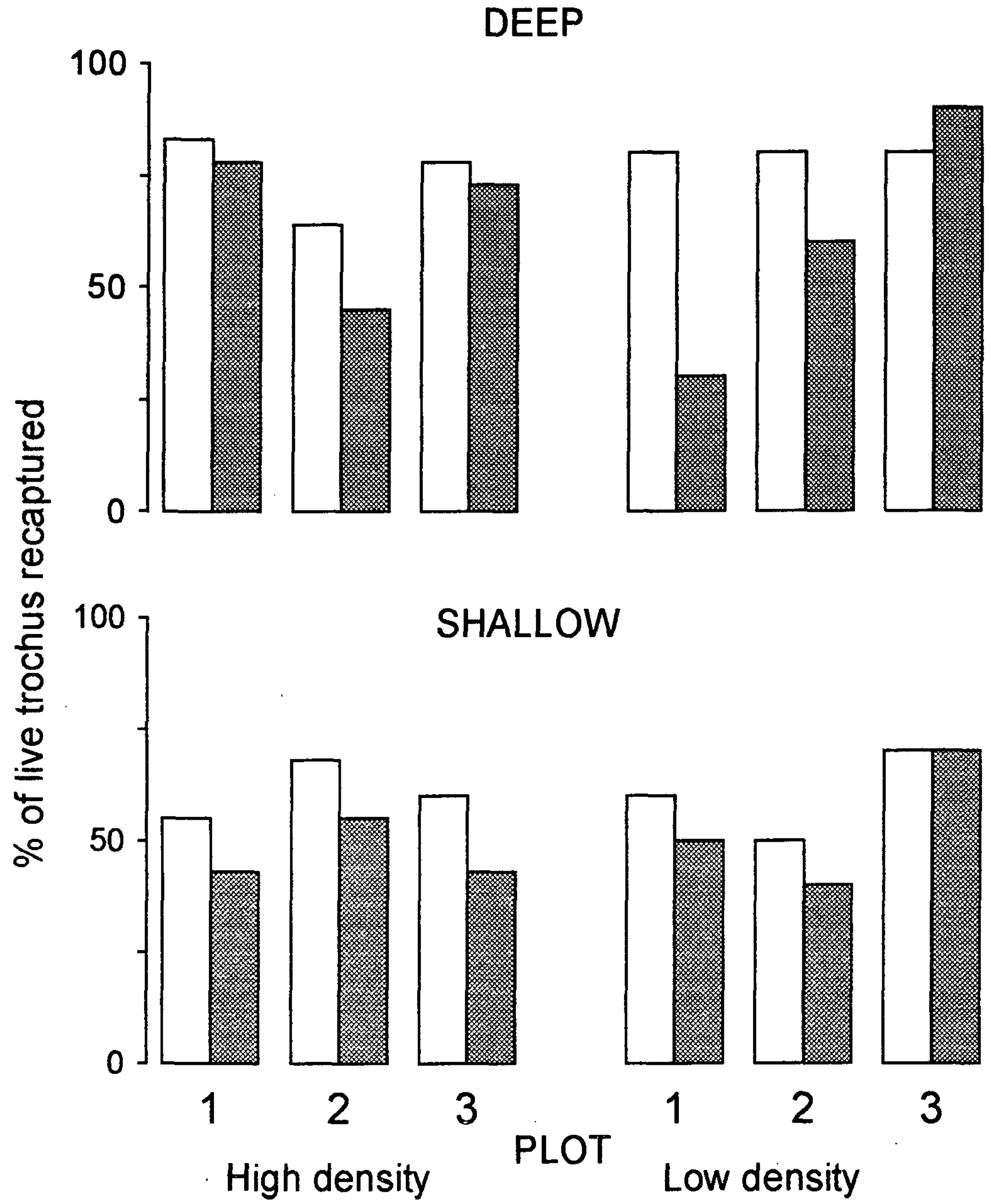


Table 6.2 Mean % of cultured flagged and unflagged juvenile *Trochus niloticus* recaptured alive after two days of release, at Moso Is., Vanuatu. Juveniles were released at two densities (5 -low and 20-high individuals.m^{-2}) in two intertidal levels. se= standard error.

	% FLAGGED (se)	**% UNFLAGGED** (se)
DEEP LEVEL		
High density	74.7 ± 5.5	65.0 ± 10.1
Low density	80.0 ± 0.0	60.0 ± 17.3
SHALLOW LEVEL		
High density	60.8 ± 3.6	46.7 ± 4.2
Low density	60.0 ± 5.8	53.3 ± 8.8

Table 6.3 Results of Experiment 2, Moso Is. Analysis of variance to examine effect of flagging, seeding density and intertidal on the proportion live and dead juvenile *Trochus niloticus* recaptured two days after the release.

% OF LIVE TROCHUS

Source	**MS**	**df**	**F**	**p**
Flagging	0.10	1	7.60	0.025
Tidal level	0.13	1	4.26	0.073
Density	<0.01	1	0.05	0.829
Plot (level, density)	0.03	8	2.42	0.117
Level*Density	<0.01	1	0.04	0.847
Flagging*Level	<0.01	1	0.24	0.637
Flagging*Density	<0.01	1	0.02	0.883
Flagging*Level* Density	0.01	1	0.94	0.361
Error	0.02	8		

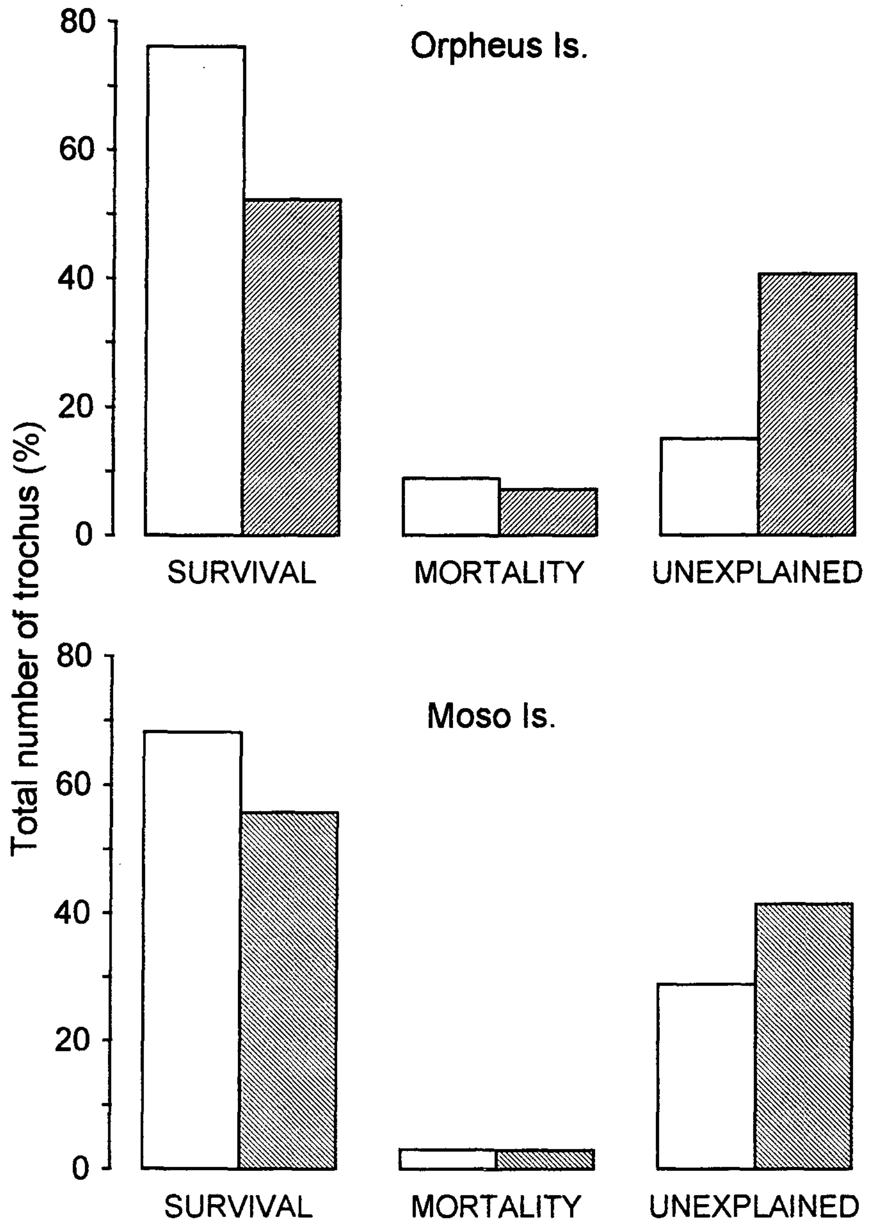
% OF DEAD TROCHUS

Source	**MS**	**df**	**F**	**p**
Flagging	<0.001	1	<0.01	0.951
Tidal level	0.012	1	9.88	0.014
Density	<0.001	1	0.16	0.702
Plot (level, density)	0.001	8	0.31	0.939
Level*Density	0.003	1	2.48	0.154
Flagging*Level	<0.001	1	0.07	0.797
Flagging*Density	<0.001	1	<0.01	0.951
Flagging*Level* Density	<0.001	1	0.07	0.797
Error	0.004	8		

Table 6.4 Mean proportion (%) (± se) of flagged and unflagged juvenile *Trochus niloticus* found dead two days after released at Moso Is., Vanuatu. There were three replicate plots for each of two seeding densities, 5 and 20 individuals.m^{-2}, within the high and low intertidal levels.

	HIGH LEVEL		LOW LEVEL	
Seeding density	Flagged	Unflagged	Flagged	Unflagged
HIGH	3 ± 2	4 ± 2	3 ± 1	1 ± 1
LOW	7 ± 3	7 ± 7	0	0

Fig. 6.4 Estimates of survival and mortality for cultured juvenile *Trochus niloticus* two-three days after released, based on total recapture of flagged individuals (open bars) and unflagged individuals (filled bars), for Experiment 1 at Orpheus Is. and Experiment 2 at Moso Is.



An assumption of the flagging technique is that there are no differences, for instance in behaviour and survival, between flagged and unflagged trochus other than the presence of the flag. Differences in cryptic behaviour between flagged and unflagged trochus are very unlikely: all trochus were cultured and handled in the same way and at the same time; and the tape was very light. The assumption of equal survival between flagged and unflagged trochus was not tested directly; this would have involved restraining trochus artificially, either by tethers or open cages. A number of visual predators such as stomatopods and fishes are known to prey upon juvenile *Trochus* (Kubo, 1991; Castell, unpublished data); it is possible that flagged trochus were more vulnerable to such predators. This would reduce the advantage of flagging as a means to estimate sighting probability, but by running the experiment for a short period the potential effect of flagging on survival was minimised. The advantages of a census few days after the release instead of after only few hours is to allow trochus to settle in their new reef habitat and show a more natural behaviour, possibly different from the behaviour shown in cultured tanks were the substrata and wave action are noticeably different. However, even in the worst case where survival of flagged trochus was artificially decreased, there was still a consistently higher proportion of flagged trochus.

From this, I suggest that the use of flagged trochus provides a reasonable estimate of the proportion of trochus overlooked by the observer in experiments lasting a few days. This is not necessarily true in the longer term. A later census at Moso Is. found that significantly more unflagged trochus were recovered alive in one of the intertidal levels, although this was not the case at Orpheus Is. (Chapter 7, unpublished data). If flagged trochus are more vulnerable to visual predators, this effect would be more pronounced with time. Also, as more time is available for natural processes such as dispersion and mortality to occur, juvenile recapture will decrease (e.g. Amos, 1991, Kubo, 1991, Chapters 7 and 8) resulting in low sample sizes and higher variability among replicates (as found for dead juveniles), both conditions that lower the power of associated statistical tests (Peterman, 1990). The objective of the experiment was that by determining the difference in proportions of flagged and unflagged trochus recaptured alive, the probability of sighting juveniles could be estimated and included in calculations of survival. The proportions of trochus surviving may well change in time but the proportion visible to the observer will remain similar within the first 2-3 months when the change of size due to growth is relatively small. With time, as trochus attain larger sizes, sighting probability will increase and correspondingly, the proportion missed by the observer will decrease.

According to the results, the average probability of sighting a juvenile *Trochus* (23 mm average SW) at Orpheus Is. was 0.69 and that of sighting a juvenile (30 mm average SW) at Moso Is. was 0.81. The expression used to estimate sighting probability (see Methods) assumes that all flagged trochus present in the search area were found. This may not always be true: some flagged trochus in inaccessible crevices could be overlooked. Missing a proportion of the flagged trochus will result in a slight overestimation of sighting probability. As an example, if 5% of the flagged trochus were overlooked at Orpheus Is. sighting probability would be 0.65 instead of 0.69.

Sighting probability of trochus is likely to vary among study sites because finding them will depend on particular characteristics of the reef substrata (abundance of crevices, coral rubble, algal mats, etc). It is also likely to vary with juvenile size, because larger trochus are easier to detect. I found indications of both of these sources of variation in the results: at Orpheus Is., flagged trochus were on average 1.46 times more frequently found than flagged trochus, but within intertidal levels these averages were 1.3, 1.6 and 1.7, respectively, for the high, medium and low levels (Fig. 6.1). At Moso Is. flagged trochus were found on average 1.24 times more frequently than unflagged trochus, but the averages within experimental treatments ranged between 1.13 and 1.33 (Table 6.2). Trochus used at Moso Is. were slightly larger than those used at Orpheus Is., and the sighting probability was correspondingly higher than at Orpheus Is. In another cryptic gastropod, the abalone *Haliotis laevigata*, mean sighting probability was estimated to be 0.35 for juveniles between two and three years old (approximately between 40-70 mm shell length) (SL) and 0.62 for larger juveniles (Shepherd, 1990).

The cryptic nature of trochus may vary depending on whether they are alive or dead. Dead shells were found on the substratum, sometimes upside down and visible, but also partially buried and inside holes, or even as crushed pieces lying around on the substratum. Partially buried flagged shells or shell fragments with a flag still attached are more likely to be found than unflagged shells in similar conditions. However, flagging would not have an effect in cases where only small shell fragments are left or when shells are taken far away from the experimental area, either by predators or water movement. Further more, recovery of dead trochus was low and variable among plots (a common occurrence in experiments with *Trochus*, e.g. Amos, 1991; Kubo, 1991) and the power of the tests to detect the differences observed were unacceptably low. Consequently, the difference in proportion of dead flagged and unflagged shells recovered is not likely to represent the proportion of dead trochus overlooked by the observer.

To conclude, estimates of juvenile survival based on recapture varied considerably depending on whether data from flagged or unflagged individuals was used (Fig. 6.4). I suggest that survival estimates based on recapture of flagged individuals are closer to true survival. Those obtained using recapture of unflagged trochus underestimate survival. In every case there was a percentage of the population which was not accounted for (see the larger proportion of unexplained disappearance for this group, in both experiments, Fig. 6.4). This is expected, since in both situations, trochus are dispersing, are being eaten and are being missed by the observer during the census.

As found in this study, sighting probabilities are likely to vary among sites and with trochus size. I recommend a short-term experiment to estimate the sighting probability of trochus of the same size and in the same experimental area where seeding experiments are planned, as a necessary precursor to estimate survival from visual censuses. The method described in this study seems appropriate for that specific goal. It should not be used as a means to follow trochus survival in time unless it can be shown that flagging does not influence survival in the longer term.

CHAPTER 7

Effect of seeding density on survival and growth of juvenile *Trochus*

7.1 Introduction

Many factors could potentially affect survival of cultured juveniles after release, including seed quality, behavioural differences between cultured and wild juveniles, habitat characteristics, juvenile size, time of release and seeding density (i.e. Brand, 1991; Schiel, 1992, 1993; Ruzzante, 1994; Stoner, 1994; Stoner and Davis, 1994).

Limited data on the natural densities of juvenile *Trochus* indicate that these are low. Smith (1987) reported a density range between 0.003 - 0.123 individuals.m^{-2} for 20-40 mm SW trochus in four reefs around Guam. A two-year study at Orpheus Is., Australia, found a mean density of juvenile *Trochus* 2 - 60 mm SW of 0.143 individuals.m^{-2} (± 0.108 SD) (Chapter 2). However, in enhancement experiments using cultured *Trochus*, where information on seeding density was provided or could be extrapolated from the text, values ranged between 10 - 100 individuals.m^{-2} (e.g. Kubo, 1991; Nguyen, 1992), i.e. two and three orders of magnitude higher than reported natural values. In spite of such large differences between natural and seeding densities and their potential effect on the outcome of releases, the effect of seeding density on survival of released trochus has not been examined.

Increasing seed density can affect survival in several ways: 1) by increasing predation rate when prey density increases, either by an increase in predator feeding rates (i.e. Barbeau et al., 1994) or by an increase in the number of predators (i.e. Moran, 1985); 2) by decreasing predation rates at high seed densities due to an inverse relationship between the probability of being preyed upon and the number of alternative prey (Peterson and Black, 1993; Ray and Stoner, 1994); and 3) by intensifying intraspecific competition for food resources, resulting in seed growing more slowly or becoming weakened as a result of poor nutrition in high density conditions (Stoner, 1989; Fagerlund et al., 1981).

This chapter addresses the effect of seeding density on short-term survival of juvenile *Trochus*. More specifically, I tested whether releasing trochus at high or at low density had an effect on their short-term survival, mortality and growth.

7.2 Methods

Experiments were carried out in Australia and Vanuatu. One study site was at Orpheus Is. and another site was on the north-east side of Moso Is. in Vanuatu (see Chapters 2 and 6 for detailed description).

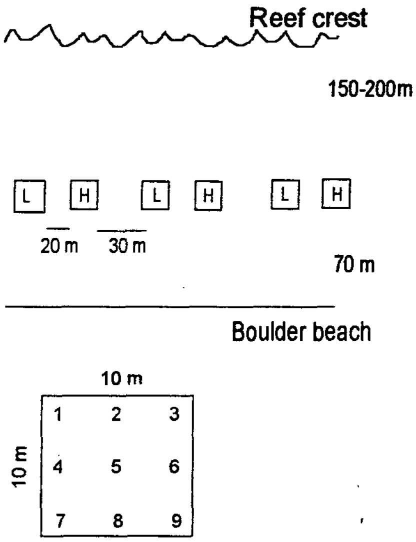
Juvenile *Trochus* used in Australia were cultured in tanks at Orpheus Is. Research Station and trochus used in Vanuatu were cultured in tanks at Vanuatu Fisheries Department hatchery. Densities used in this study ranged from 5 to 30 individuals.m^{-2}. All work involved walking on the reef within two hours of the low tide. When released, trochus were individually placed in an upright position on the substratum, avoiding obviously unfavourable areas such as sand and live coral.

7.2.1 Experiment 1, Orpheus Is.

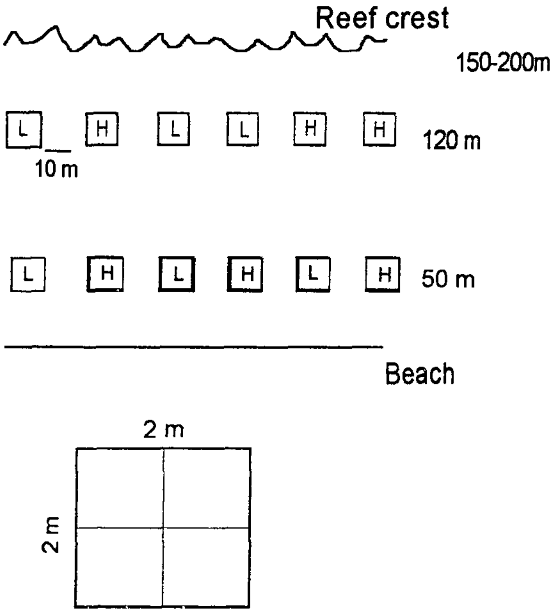
Six 10x10 m plots were set up parallel to the shoreline 70-80 m from the beach. A total of 943 cultured juvenile *Trochus* 13 - 44 mm SW (mean 18.2 ± 4.7 SD) were individually marked using numbered plastic tags (Hallprint Inc., South Australia) glued onto the shell or spots of coloured paint (for the smallest individuals). Size distribution of trochus was constant among plots. Trochus were released at nine places within each plot (each of 1m^2), eight of them located around the perimeter of the plot and one in the centre (Fig.7.1a), at a density of either 5 m^{-2} (low density) or 30 m^{-2} (high density). A rod was placed in the centre of each plot for later identification. Density treatments alternated along the line of plots. Trochus were released on November 9, 1993 and the areas were sampled 3, 30 and 111 days after the release using 1m^2 quadrats. All live and dead trochus were recorded and live trochus were measured in the last two censuses to determine growth rates. After three days, 19 quadrats were searched within each plot: nine of them corresponded to the nine points where juveniles were released and the other 10 quadrats were haphazardly spread. This sampling was adopted after observing that most trochus stayed close to the release point and because searching was time consuming. Areas surrounding the quadrats inside and outside the plots were also quickly searched at each census by turning rubble and looking into accessible cavities. After 30 and 111 days, a total of 40 quadrats were searched per plot, 20 inside and 20 outside of the plot, covering an approximate area of 400 m^2 per plot. At 30 and 111 days, additional searches for trochus were made haphazardly while walking on the reef flat, tens of metres away from the

Fig. 7.1 Experimental design of trochus release: a) Orpheus Is. , b) Moso Is. L= low seeding density, 5 individuals.m^{-2}, H= high seeding density, 20 or 30 individuals.m^{-2} (see text for explanation). The insert shows the experimental plot. In a) numbers show the locations within a plot where juveniles were released.

(a) Exp. 1 Orpheus Is.



(b) Exp. 2, Moso Is.



experimental area.

7.2.2 Experiment 2, Moso Is.

Size range of trochus used was 25.5 - 39 mm SW (mean 29.6 ± 2.3 SD). They were released at two intertidal depths: "shallow", approximately 50 m from the beach and "deep", approximately 120 m from the beach. The shallow zone had more coral bench with cavities than the deep zone, which had rocks (< ~ 50 cm longest axis) and rubble sparsely distributed over a layer of coarse sand. There were six plots, 2 x 2 m, per intertidal level. Three were seeded with trochus at a density of 5 m^{-2} and the other three at a density of 20 m^{-2} (Fig. 7.1b). Distance between plots was 10 m. The high density treatment had less trochus than in Exp. 1 because less numbers were available at the time, but trochus were of greater size than in Exp. 1. A rod fixed into the substrata was used to mark the centre point where trochus were released. The total number of trochus released per replicate plot was 20 in those seeded at low density and 80 in plots seeded at high density. Overall, 600 trochus, all individually marked with numbered tags, were released on May 9, 1994. Half of them had an additional marking as part of another study to determine searching efficiency; these juveniles will be referred to as flagged and unflagged (Chapter 5). The plots were censused two and 40 days after the release and all live and dead trochus were recorded. An area of 6x6m around the centre of each plot was checked on the first census. The area searched was increased to 20x20 m on the second census,. A third census made five months later failed to find any tagged trochus. Recaptured individuals were not measured and growth data were not obtained in this experiment.

7.2.3 Data analysis

The numbers of trochus recaptured per area were expressed as proportions of the number initially released. The Brown-Forsythe's procedure was used to test for homogeneity of variances (Keppel, 1991). For Exp. 1, variances were homogeneous among censuses and a repeated measures analysis of variance was used to examine the effect of seeding density on trochus recapture. In Exp. 2, variances were homogeneous within a census but non-homogeneous among censuses and the data were analysed separately for each census. The main factors in this experiment were seeding density, intertidal level (shallow and deep) and flagging, with plot as a factor nested within density and intertidal level..

To estimate survival, numbers of trochus recaptured in Exp. 1 were corrected for area (only a fraction of the area censused was sampled) and for sighting probability (estimated as 0.69, Chapter 6). In Exp. 2 the data were corrected for sighting probability (estimated as 0.81, Chapter 6), but in this case all the designated area was sampled.

At Orpheus Is. proportional means of dead trochus recovered after three days were compared with a t test using Satterthwaite's approximation. Recovery in the other two censuses was too low for analysis. At Moso Is. the numbers of dead shells recovered were compared as described for live individuals.

In Exp. 1, analysis of covariance was used to test for the effect of seeding density on growth rate, using trochus size as the covariate, after confirming homogeneity of slopes.

7.3 Results

7.3.1 Experiment 1. Orpheus Is.

Three days after released, the percentage of trochus recaptured alive averaged 35 % (± 4 SE) at low density and 36 % (± 6 SE) at high density. Repeated measures analysis showed that seeding density did not have a significant effect on the percentage of trochus recaptured at any of the three censuses (Table 7.1). The change in recapture rate with time was similar for both seeding densities, although a pattern developed in time, with a slightly higher recapture rate of trochus in the areas seeded at low density (Fig. 7.2a). Differences between seeding densities with time were more pronounced when recapture data were translated into survival estimates (Fig. 7.2b). By the last census at 111 days, the estimated mean survival was 38% for trochus released at low density and 10% for trochus released at high density (Fig. 7.2b). Mean density of trochus in the experimental area 111 days after the release was 1.3 times higher in the area seeded at high density: $0.84.m^{-2}$ (SE 0.08) for the areas seeded at low density and $1.10.m^{-2}$ (SE 0.31) for the areas seeded at high density.

Growth rates of trochus released at low density were significantly higher than growth rates of trochus released at high density (Fig. 7.3, Table 7.2). Growth rate was inversely related to trochus size (Table 7.2), indicating that smaller trochus grew faster than larger trochus (Fig. 7.3). The slopes of the regression lines were

Table 7.1 Exp. 1, Orpheus Is. Results of repeated measures analysis of variance to examine effect of seeding density on recapture of live cultured juvenile *Trochus niloticus*, at 3, 30 and 111 days after released at Orpheus Is.

Tests of Between-Subjects Effects

SOURCE	DF	MS	F	p
Density	1	<0.01	0.45	0.539
Within + Residual	4	<0.01		

Tests involving TIME Within-subject Effect

SOURCE	DF	MS	F	p
Time	2	0.18	84.54	< 0.001
Density*Time	2	<0.01	0.50	0.626
Within + Residual	8	<0.01		

Fig. 7.2 a) Mean % of juvenile *Trochus niloticus* recaptured alive at Orpheus Is, on three censuses after released. Juveniles were released at low (5 individuals.m^{-2}) and high (30 individuals.m^{-2}) density. Vertical bars represent standard error of the mean. b) Same as above but data represent survival estimates, obtained taking into account area censused and sightibility of trochus. Lines were fitted by eye.

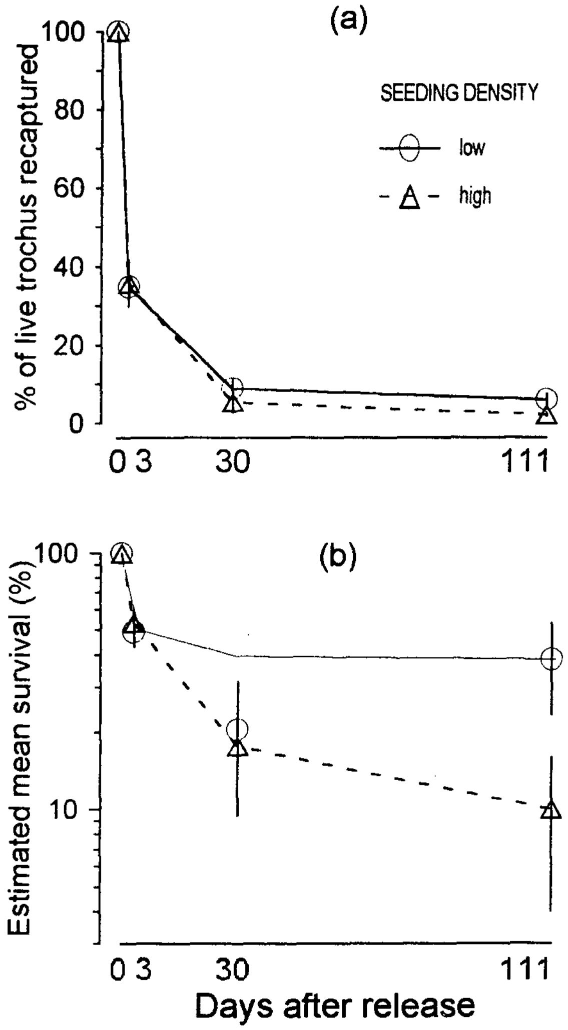


Fig. 7.3 Individual growth rates of cultured juvenile *Trochus niloticus* released at Orpheus Is., at low (5 individuals.m^{-2} , N=10)(●) and high (30 individuals.m^{-2}, N=50) (○) densities. Fitted regression lines for low (dotted line) and high density (solid line). p(slope)<0.001 for high density and p=0.009 for low density.

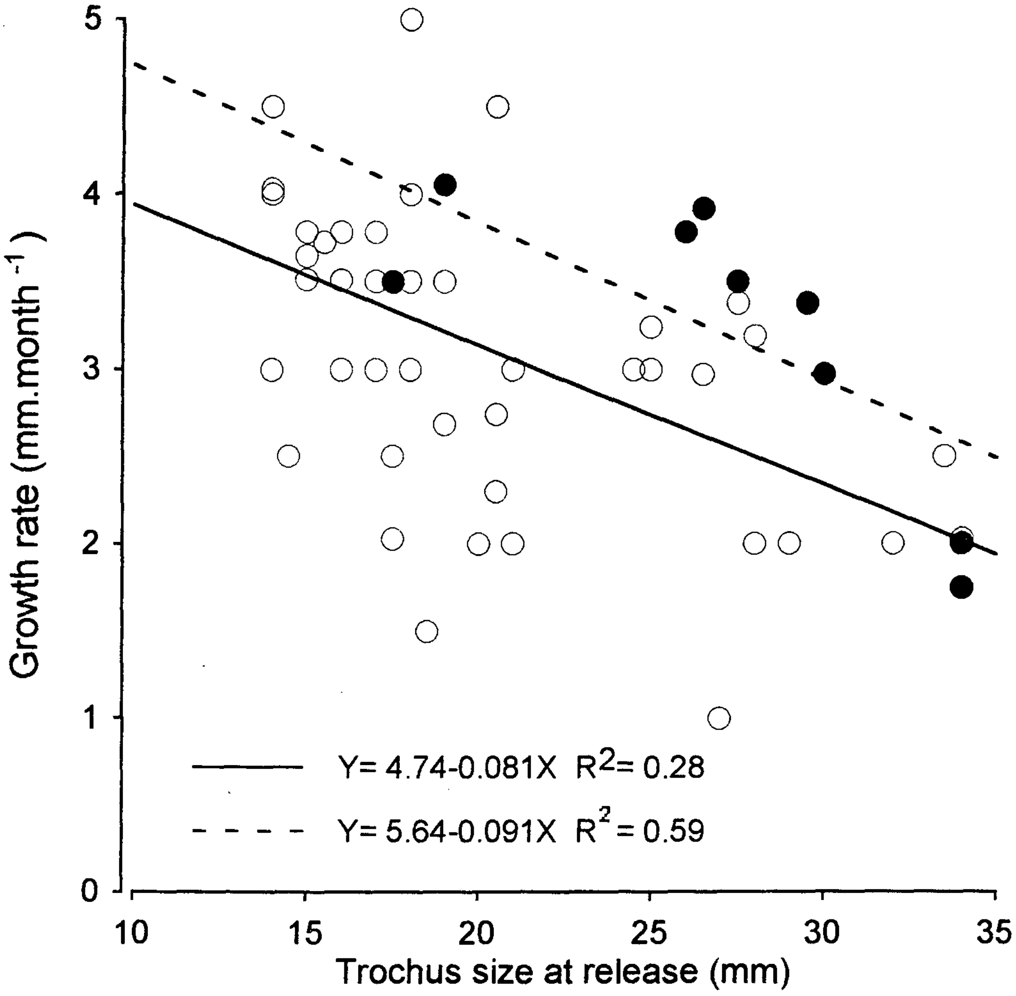


Table 7.2 Analysis of covariance to determine effect of seeding density on growth rate of released juvenile *Trochus niloticus* at Orpheus Is.

Source	**MS**	**df**	**F**	**p**
Density	2.98	1	5.67	0.021
Regression	14.07	1	26.76	<0.001
Error	0.53	57		

COVARIATE	**t**	**p**	**95% C.I.**
Initial size	-5.17	<0.001	-0.115 , -0.051

not significantly different between seeding densities (p=0.13). Individual growth rates obtained for cultured trochus in this study ranged between 1.0 - 5.0 $mm.month^{-1}$ (Fig. 7.3).

Few dead trochus were recovered and the rate of recovery decreased with time. After three days, the mean percentage of released trochus found dead was 1.5% (SE= 0.7) in the areas seeded at low density and 1.1% (SE= 0.4) in the areas seeded at high density. The difference between the two densities was not significant (t test p=0.39). Four dead trochus were recovered after 30 days and only two after 111 days.

7.3.2 Experiment 2, Moso Is.

After 2-3 days, recapture of live trochus within each intertidal level was very similar for the two seeding densities: in the deep zone, 70% trochus were recaptured in both high and low seeding density areas, and in the shallow zone, recapture was 57% and 54%, respectively, for the high and low density treatments (Fig. 7.4). Overall, the number of trochus recaptured in the deep zone was 1.27 times higher than in the shallow zone and this difference was marginally nonsignificant (Table 7.3).

After 40 days, recapture of trochus was low and variable among experimental plots. Interpretation of the results is further complicated by the fact that flagging had an effect on trochus survival (Table 7.3). As in the first census, recapture was higher in the deeper level, but no clear pattern of the effect of seeding density was evident. Mean recapture of trochus in the deeper level was 10% (SE 3.2), and 2.3% (SE 1.1) for shallow. The greatest recapture corresponded to the low density treatment in the deep level (Fig. 7.4).

After 40 days, mean density of *Trochus* was 3.4 times higher in the areas seeded at high density: 0.005 (SE 0.002) and 0.017 (SE 0.003) $individuals.m^{-2}$, respectively, for low and high density.

On average, the percentage of dead trochus found after 2-3 days was significantly higher in the deep level (5%, N=16, compared to 2%, N=6 in the shallow zone, Fig. 7.5, Table 7.4), but within each zone, percentage of dead trochus did not differ significantly between high and low density (Fig. 7.5, Table 7.4). Forty days later, the opposite pattern was observed, with more dead trochus found in the shallow zone (8% compared to 3%)(Fig. 7.5); but, as in the first

Fig. 7.4 Mean % of juvenile *Trochus niloticus* recaptured alive at Moso Is. after two-three and 40 days. Trochus were released at low density (Sparse, 5 individuals.m^{-2}) and high density (Dense, 20 individuals. m^{-2}), at two intertidal depths. Open bars correspond to flagged trochus and filled bars to unflagged trochus. Vertical bars represent standard error of the mean.

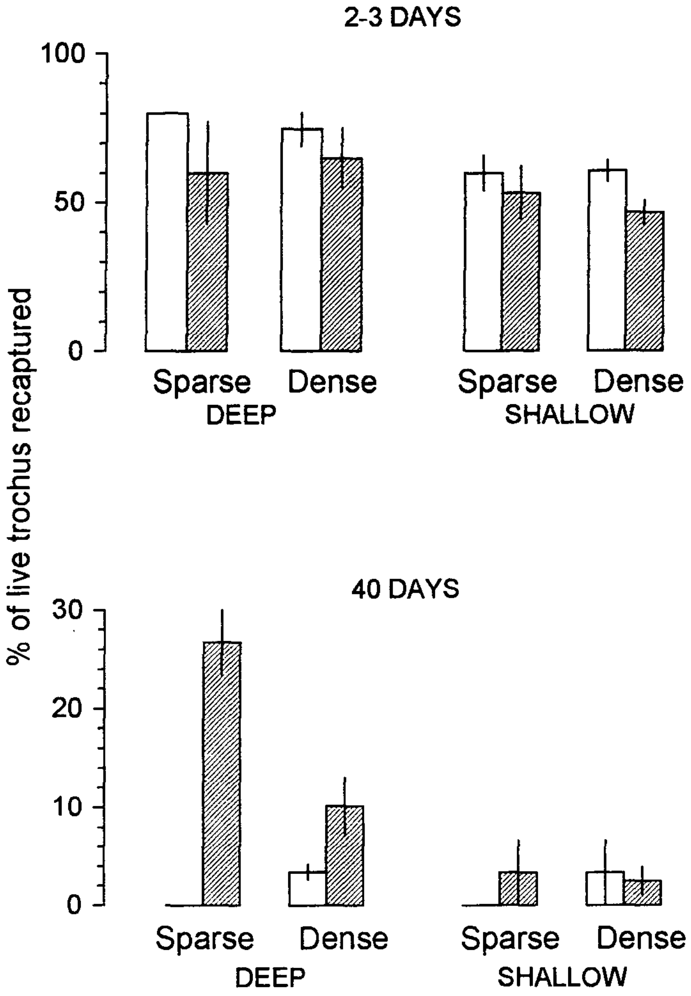


Table 7.3 Exp. 2, Moso Is. Results of analysis of variance to examine effect of seeding density, flagging and intertidal level on the proportion of live juvenile *Trochus niloticus* recaptured two and 40 days after the release.

CENSUS 1, Two-three days after release

Source	MS	df	F	p
Density	0.001	1	0.07	0.829
Flagging	0.096	1	7.60	0.025
Intertidal level	0.130	1	4.26	0.073
Plot (density,level)	0.030	8	2.42	0.117
Flagging*Density	<0.001	1	0.02	0.883
Level*Density	0.001	1	0.04	0.847
Flagging*Level	0.003	1	0.24	0.637
Flagging*Level* Density	0.012	1	0.94	0.361
Error	0.013	8		

Census 2, 40 days after release

Source	MS	df	F	p
Density	0.004	1	3.62	0.094
Flagging	0.048	1	22.57	0.001
Intertidal level	0.036	1	30.03	<0.001
Plot (density,level)	0.001	8	0.56	0.786
Flagging*Density	0.022	1	10.22	0.013
Level*Density	0.009	1	7.77	0.024
Flagging*Level	0.036	1	16.72	0.003
Flagging*Level* Density	0.009	1	4.38	0.070
Error	0.002	8		

Fig. 7.5 Mean % of juvenile *Trochus niloticus* recaptured dead after two-three and 40 days, at Moso Is. Trochus were released at low density (Sparse, 5 individuals.m^{-2}) and high density (Dense, 20 individuals. m^{-2}), at two intertidal depths. Open bars correspond to flagged juveniles and filled bars to unflagged juveniles. Vertical bars represent standard error of the mean.

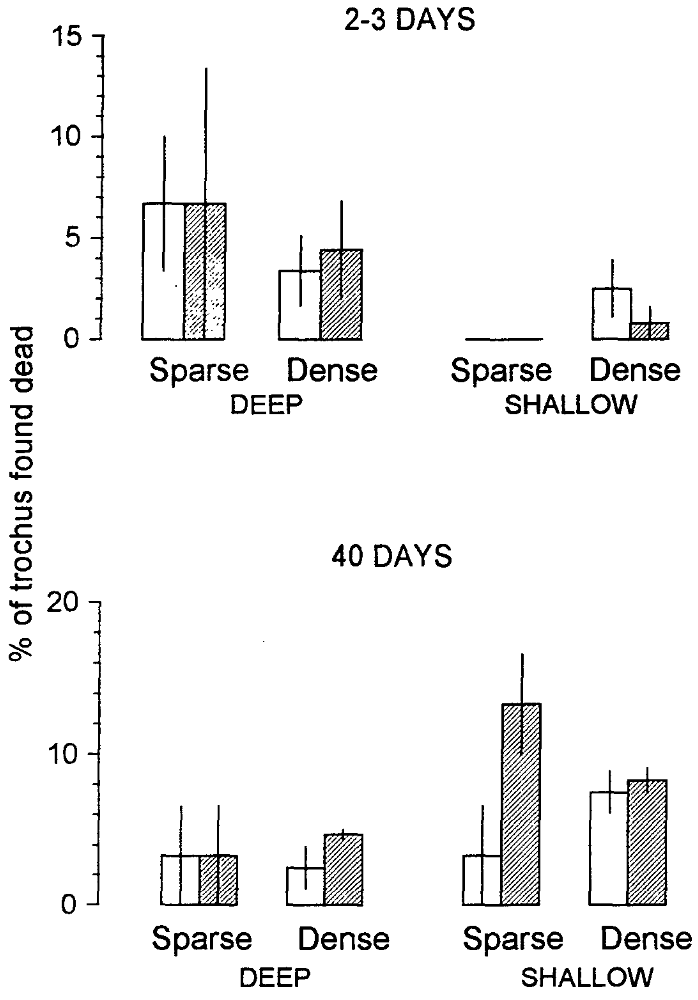


Table 7.4 Exp. 2, Moso Is. Results of analysis of variance to examine effect of seeding density, flagging and intertidal level on the proportion of dead juvenile *Trochus niloticus* recaptured two and 40 days after the release.

CENSUS 1, Two-three days after release

Source	MS	df	F	p
Density	<0.001	1	0.157	0.702
Flagging	<0.001	1	0.004	0.951
Intertidal level	0.012	1	9.877	0.014
Plot(density, level)	0.001	8	0.314	0.939
Flagging*Density	<0.000	1	0.004	0.951
Level*Density	0.003	1	2.482	0.154
Flagging*Level	<0.001	1	0.071	0.797
Flagging*Level* Density	<0.001	1	0.071	0.797
Error	0.004	8		

Census 2, 40 days after release

Source	MS	df	F	p
Density	<0.001	1	0.001	0.973
Flagging	0.006	1	4.79	0.061
Intertidal level	0.013	1	5.48	0.047
Plot(density, level)	0.002	8	1.79	0.215
Flagging*Density	0.002	1	1.37	0.276
Level*Density	<0.001	1	0.03	0.869
Flagging*Level	0.003	1	2.10	0.185
Flagging*Level* Density	0.005	1	3.64	0.093
Error	0.001	8		

census, there were no significant differences in the percentage of dead trochus found in the density treatments within each zone (Table 7.4).

7.4 Discussion

7.4.1 Sources of juvenile loss.

Survival of seeded trochus was proportional to their seeding density up to four months after the release. There are at least five possible sources of juvenile loss: mortality, dispersion, tag loss, juvenile crypsis and transport of loosely attached shells away from the area. Of these, the first two, mortality and dispersion, could be affected by trochus density, whereas tag loss, crypsis and dislodgment should logically be independent of density. Since the objective was to determine the effect of seeding density on survival, I will first examine the data from this and other studies for indication of differences in sources of juvenile loss between density treatments.

a) **Tag loss**.

The reef at Orpheus Is. holds a population of wild trochus. The number of trochus found without tags during each census during this study increased significantly with time (repeated measures analysis of variance for time, p=0.032), suggesting that tag loss may have occurred. However, as expected under the assumption of no effect of density on tag loss, there was no significant difference between the number of unmarked trochus in low and high density areas (p=0.604).

b) **Juvenile crypsis**.

Habitat characteristics are likely to be the most important factor that determines how difficult it is to find trochus. I used flagged trochus to measure crypsis (Chapter 6). The results from Moso Is., where half of the trochus were flagged (i.e. easier to detect), indicated that the probability of finding trochus was not affected by their density during the first census. If such had been the case, one would have expected a significant interaction between density and intertidal level (Table 7.3). The same did not hold for the second census, but rather than an effect of density on crypsis, the results suggest that flagging had a detrimental effect on trochus survival in the longer term.

c) **Dispersion**.

Transplanting individuals to new places can result in increased dispersion simply as a result of disturbance (Underwood, 1988). By transporting trochus from culture tanks into a new reef environment, the potential was for increased movement of disturbed trochus. One would expect this effect to be similar for all trochus since they were handled at the same time and in the same way. However, dispersion rate could differ between trochus in low or high density conditions, as has been found for scallops and queen conch, where individuals moved faster at higher densities (Siddall, 1984; Kalashnikov, 1991). If trochus moved more and dispersed more rapidly in areas of high density, lower recapture in these areas could have resulted because of higher dispersion. Some evidence suggests that this was not the case. At Orpheus Is., three days after the release, 93-100% of trochus recaptured in plots seeded at low density were within the same squared metre where initially released and the corresponding value for plots seeded at high density was 96-100%. In this census, the area inside the plot was most extensively sampled, but the point to notice is that within the plot, dispersion was minimal at both seeding densities. At 30 days, only one trochus was found in the area outside one of the high density plots and none outside the low density plots. Searching at various times covering large areas of the reef as part of concurrent studies, resulted in 12 encounters with marked trochus from this release: two from the group released at low density and ten from the group released at high density. These represent 1.5 % and 1.2 %, respectively, of the number initially released. An example of low dispersion at very high densities is from an experiment in Okinawa. Trochus were released at densities greater than 100 m^{-1}, but after two days all those recaptured were within 0 - 4 m from the release area and the spread increased by only 2 m after 17 days (Kubo, 1991). Thus, there is circumstantial evidence that trochus recapture was not seriously affected by differential dispersion rate between high and low seeding densities. The data suggest that those trochus that remained in the area, dispersed slowly.

d) **Mortality**.

The results indicate that, as with live trochus, the number of dead trochus recovered was proportional to density (Fig. 7.5, Table 7.4). Recovery of dead shells was very low, accounting for less than 2% of the missing trochus at Orpheus Is. and up to 8% at Moso Is. These are likely to be underestimates of true mortality since they are based on the recovery of identifiable individuals (i.e. whole shells, shell fragments with the tag still attached and other fragments such as shell tops and bases, that can be translated into number of individuals). Fish predators can crush shells to small pieces and transport them away from the capture area. Stomatopod predators of juvenile *Trochus* (Kubo, 1991; Castell pers. obs.) generally grab their prey and take it to their home cavity to feed (Caldwell and Childress, 1990). Thus, a proportion of the dead individuals will be either unrecognisable against the reef substratum or will be moved farther from the sampling area. However, in spite of a low sample size, the evidence from both experiments suggests that mortality is proportional to density.

To summarise, tag loss, crypsis and trochus dispersion did not appear to vary depending on whether trochus were released at high or low densities. Differences in the number of trochus recaptured between density treatments are more likely a reflection of differences in survival. This is supported by the result that recovery of dead shells was proportional to seeding density.

7.4.2 Short-term vs. long-term effects of initial seeding density

7.4.2.1 Survival

Trochus loss was density-independent during the first few days of release at both study sites, within a range of densities between 5 - 30 trochus.m^{-2}. Shortly after release is likely to be the most vulnerable period for trochus because of handling and exposure to a new environment (Schiel, 1993). Generally, survival of individuals decreases rapidly immediately after release (Kubo, 1991; Barbeau et al., 1994; Hatcher et al., 1996) and this was true in this study (Fig. 7.2). Even though mortality based on shell recovery usually explains only a small proportion of this decrease (Kubo, 1991; this study), predation is thought to be an important source of mortality (e.g. Hatcher et al., 1996). Predator densities and predator feeding rates can both be influenced by prey density (Murdoch, 1973), and predator responses in hours to days of changes in prey density have been documented (Hatcher et al., 1996). An effect of density on survival is possible even within days of release. I did not find such effect.

The difference in proportional recapture of live trochus between density treatments was greater by the time of the later censuses, 1-4 months after release. There was a trend towards greater recapture at low density for Orpheus Is. (Fig. 7.2) and at the deeper area at Moso Is, but the observed differences were not significant.

Density has been shown to affect mortality in some studies but not in others.

Mortality did not vary significantly among seeding densities of 0.5 to 2 conch.m^{-2} over three months (Stoner and Davis, 1994), but increased from 1.6% to 38% with a change in density from 1 conch.m^{-2} to 8 conch.m^{-2} over a period of four and a half months in another study (Stoner, 1989). Among other examples, clam mortality was 20-30% higher in patches with four times greater density after six months of planting (Boulding and Hay, 1984), and a threefold increase in scallop density corresponded with an increase in mortality rate due to crushing predators from 0.05 to 0.2 scallops.m^{-2}.d^{-1} over a nine days period, although no effect was evident for non-crushing predators (Fig. 2 in Barbeau et al., 1994). In some of these studies, density was maintained constant throughout the experiment (Stoner 1989, Barbeau et al., 1994). In this study, low and high density treatments differed by a factor of 4 for Moso Is. and 6 for Orpheus Is., which are comparable to values in other experiments where effects were observed. I did not replace dead or missing trochus, so densities varied over the experimental period. Given the large drop in recapture after only a few days, the densities of trochus must have dropped considerably, but continued to be higher than natural densities. In both sites, mean densities of trochus in areas seeded at high density were still greater than corresponding estimates for areas seeded at low density by the end of the experiments. Short-term replacement is unlikely to be part of an enhancement program. Studies where an effect of density was observed involved constant densities or longer time scale than this study (Boulding and Hay, 1984; Stoner, 1989; Barbeau et al., 1994). If densities are kept high for prolonged periods of time, there is more chance for an effect to occur through processes such as prey-switching (Murdoch, 1969) and learning of predatory skills (Cunningham and Hughes, 1984; Kaiser et al., 1993).

7.4.2.2 Growth rate

Trochus grew faster at low density than at high densities. Juvenile *Trochus* are grazers, feeding upon small filamentous algae growing on the rock surface. Food limitation is then a potential outcome of high density conditions. An initial six-fold increase in seeding density corresponded to an average decrease in growth rate by 6%. Although the change was relatively small, the results stress the potential impact that releasing juveniles in high densities could have on growth and consequently on survival. Vulnerability to predation decreases with trochus size for a large range of predators (Kubo, 1991; Chapter 3), as also found for other species (e.g. Appeldoorn, 1984; Schiel, 1992; Barbeau et al., 1994). Larger trochus survive better in the field than smaller trochus (Chapter 8). Thus, the more rapidly trochus grow, the greater chances they have to survive.

Density-dependent growth rates may be indicative of the carrying capacity of an environment. Juvenile conch *Strombus gigas* grew progressively slower as density increased from one to four times their natural density (Stoner, 1989). Reduced growth corresponded with lower indices of stomach fullness, suggesting that food was a limiting factor and that natural densities may have been near the carrying capacity of the habitat (Stoner, 1989). In this study, densities of juvenile *Trochus* in the experimental area at Orpheus Is. after four months, were 6-7 times greater than the initial mean density of wild juveniles before the experiment. Individual growth rates were, however, comparable to those of wild trochus on the same reef (Chapter 2). This suggests that natural densities on the reef flat at Orpheus do not represent the carrying capacity of this environment, which is not surprising considering the mean of one trochus per 5m^2 of reef surface (Chapter 2).

7.5 Application to seeding

The basis to select a seeding density for releasing trochus should take into account its effect on survival and health. Releasing trochus at 5 or 20-30 individuals.m^{-2} did not affect their survival. This result was clear for a time scale of 2-3 days, but not as clear for the longer time scale of 1-4 months. The results also indicate that growth rate was lower among trochus released at high density. Thus, on these criteria it is more appropriate to release trochus at low rather than high densities. It must be stressed that these results apply to a range of densities between 5-30 individuals.m^{-2}. The use of higher densities is likely to enhance the importance of density on trochus survival.

CHAPTER 8

Effect of juvenile size and location of release on survival of juvenile *Trochus* in field releases

8.1 Introduction

The size of seed for release is probably one of the most important factors affecting the decision of whether or not seeding will be a viable option for managing and restocking a commercially exploited species. The size at which individuals should be released will be a compromise between size-specific survival and the cost of producing individuals of different sizes (Schiel, 1993; Ray et al., 1994). On one hand, smaller individuals are generally cheaper to produce in large numbers (Murakoshi, 1991; Schiel, 1992). On the other hand, decreasing mortality with increasing size is a pattern widely observed in nature (Paine, 1976; Palmer, 1990; Sousa, 1993). Experimental releases have shown that larger individuals survive better than small ones for a variety of organisms, including gastropods (Ray et al., 1994), giant clams (Heslinga et al., 1984) and scallops (Minchin, 1991). Therefore, the choice of size for release is affected by two, sometimes opposing, forces: from the economic point of view, the smaller the seed the better, whereas from the biological point of view, the larger the seed the better. Determining the relationship between survival and size is then an important component of seeding research.

Little is known of the relationship between survival and juvenile size for *Trochus*. Experimental releases in Palau using trochus ~10 mm shell width (SW) resulted in 100% mortality after only a few days (Heslinga cited in Nash, 1993). Kubo (1991) concluded that survival did not differ between trochus 8 mm and 16 mm SW. In Vanuatu, release of 20 mm (mean size) trochus resulted in survival up to 55% after 49 days (Amos, 1991) and up to 28% after 13 months (Nash, 1993).

Another aspect of seeding is where to release them. Reef flats with abundant coral rubble and some degree of wave exposure are considered favourable characteristics for juvenile *Trochus* (Heslinga and Hillman, 1981; Nash, 1985). The observations that larger trochus live subtidally, whereas smaller individuals are found shallower on the reef flat (Smith, 1987; Bour, 1992), and that *Trochus* are generally more abundant on reefs that dry at low tide, suggest that the intertidal flat provides the best conditions for juveniles (Nash, 1988). Besides these observations, very little is known about the habitat of juvenile *Trochus*. Areas with apparently similar characteristics are not necessarily equally favourable. Research on the queen conch, *Strombus gigas*, showed that growth rates and mortality of transplanted conch differed significantly among three habitats with similar physical characteristics (see review by Stoner, 1994).

The two main objectives of this chapter were to determine size-related survival of released trochus and the importance of habitat on survival.

8.2 Study site

All experiments were conducted on the intertidal reef flat located on the north-east side of Orpheus Is. (see Fig. 2.1, Chapter 2). This reef has been described in Chapter 2.

8.3 General methods

Five experiments were conducted between November 1993 and December 1994. There were two types of experiments: 1) release of trochus freely onto the reef, and 2) release of trochus tethered to fixed rods. Experiments of the first type lasted a few days to a maximum of four months. The length of the experiments was determined by trochus size, recapture rate and experiment type. Searching time for very small trochus becomes impractical after a few days. Recapture rate decreased rapidly in most experiments. Experiments which used tethered trochus lasted for a maximum of eight days. The technique proved useful and satisfactory for trochus > 8 mm SW, but, given the potential for artefacts associated with tethering (Barbeau and Scheibling, 1994; Peterson and Black, 1994), I opted for minimising the duration of these experiments.

All work was done during the hours of tidal exposure, within approximately two hours of low tide. Due to wave action it was extremely difficult to either release or search for juveniles while swimming.

8.3.1 Release Experiments

Experiment 1

In this experiment I examined the effect of seeding density and trochus size on recapture. The experimental design and the results with respect to seeding density have already been described in detail in Chapter 7. Here, I will detail the methods focusing on juvenile size as the factor of interest. A total of 943 cultured trochus between 13 and 44 mm SW (mean 18.2 ± 4.7 SD) were released. They were grouped into five size classes: 10-14.5 mm, 15-19.5 mm, 20-24.5 mm, 25-29.5 mm and ≥30 mm SW. Size distribution of trochus was constant among experimental plots and among releasing points within each plot (see Fig. 7.1). The areas were censused at three, 30 and 111 days after the release.

I used simulation tests to determine if survival of trochus differed among size classes at any of the three censuses. The data from all experimental plots were pooled and the size frequency distribution of the whole population released was compared to that of the population recaptured. Pooling the data was necessary because of low recapture, especially in the censuses 30 and 111 days after the release. Since recaptures were relatively high in the first census, I also analysed these data separately by experimental plot (N=6). Each simulation consisted of drawing 5000 random samples from the original distribution of size frequencies released, of size equal to the number recaptured alive. Trochus were assigned to the size class to which they belonged at the moment of release. The test provided the probability that the observed size frequency distribution could have been the result of random disappearance of trochus, and thus, independent of their size. The null hypothesis was that the size frequency distribution of the population recaptured was not significantly different to that of the population released.

Experiment 2

In this experiment I examined survival of very small trochus within the first days of release. On October 3, 1994, trochus between 4-8 mm SW were released unmarked in groups of 10 individuals each, next to a fixed rod marking a site. Care was taken to place each individual in a suitable substratum (e.g. avoiding sand) and in upright position. There were 22 replicate rods, each set at every five metres along a transect parallel to the shoreline. This set up was repeated at two habitat levels on the intertidal reef, high, at 20 m from the shore, and mid, 70 m from the shore (there were 21 rods in this level). The area adjacent to each rod was carefully searched after one and three days, when the experiment ended. Handling of trochus was kept to a minimum to minimise disturbance during the search. Because trochus were so small, they all had to be placed very close to the marking rod. Searching was concentrated on the immediate vicinity of the rod, covering a circular area of approximately 30 cm in diameter with the rod in the centre. With time, trochus are likely to disperse and increasing the searching area became rapidly impractical.

Experiment 3

This experiment had the same objective as Exp. 2 but was conducted at a different time. Trochus between 4-8 mm SW were released on December 1, 1994, following the same protocol as described above, but in this case there were 12 replicate rods (i.e. sites) at each of the two intertidal habitat levels. The sites were checked after one and two days. On December 2, a new set of juveniles was released to increase sample size in each intertidal level from 12 to 23, but these were checked after only one day.

Sighting probability

Since juvenile *Trochus* are very cryptic, it is likely that a proportion of the juveniles present in the searched area will be overlooked by the observer (see Chapter 6). To estimate the probability of sighting a trochus in experiments 2 and 3, the proportion of individuals known to have been missed (i.e. when the number found in the second census was greater than the number found in the first census) were averaged over the total number of sites.

$$\text{Mean proportion trochus missed per site} = \frac{\sum \text{trochus missed per site on first census}}{\text{No. sites} \times \text{Total No. trochus per site}}$$

$$\text{Sighting probability} = 1 - \text{Mean proportion trochus missed per site}$$

The actual values of trochus recaptured were then corrected to obtain survival estimates, using the expression:

$$\text{Survival} = \frac{\text{No. of trochus recaptured}}{\text{sighting probability}}$$

These estimates of survival represent minimum estimates. The method assumes that in sites where more trochus were found on the first than on the second census no trochus were overlooked.

The data were analysed using a repeated measures analysis of variance, with intertidal level and time as the main factors. Estimates of survival (i.e. corrected for sighting) were used in these analysis.

8.3.2 Tether Experiments

To tether trochus, I used 0.5m of mercerised cotton thread, attached to the top of the shell using commercial "super glue" and tied at the other end to a rod hammered into the reef substratum. Preliminary observations suggested that movement was not restricted and trochus remained as cryptic as usual (some could only be found after following the thread to its end). Trochus smaller than 8 mm SW were not used in these experiments because in a pilot study they appeared to be susceptible to overturning, possibly because of the pulling strength of the thread relative to the weight of the shell. Rods were checked at each census, counting the number of trochus alive, dead and missing, and observations on sources of mortality. In the case of missing trochus, I recorded if the thread had been shredded or if it was intact. Shredded threads could be the result of predators such as fishes and crustaceans. Some loose ends could result if the glue fails to stick to the shell. However, the proportion of missing trochus due to tethers coming undone was expected to be low. Once the glue had dried, significant force had to be applied to pull the juvenile away. The fact that shell fragments were still attached to the tether in numerous cases is indicative of the glue's efficiency.

Estimates of survival were based on the number of live trochus. This may result in a slight underestimation of survival if some of the missing trochus were detached and alive.

Tether Exp. 1

In this experiment I examined trochus size and habitat. Four trochus, two small (mean 11.9 mm SW ± 2.0 SD) and two of medium size (mean 22.7 mm ± 1.5 SD), were tethered to a rod fixed into the reef substratum. There were five replicate rods, with a distance of five metres between. Rods were placed at three levels on the intertidal reef: low (120 m from the beach), mid (70 m from the beach) and high level (20 m from the beach). The experiment lasted 8 days, from February 28 to March 7, 1994, when all rods were checked.

The data were analysed using an analysis of variance with two main factors, intertidal level and juvenile size and the factor site nested within intertidal level.

Tether Exp. 2

Given the success of the tethering technique and observed high variability within experimental treatments in the previous experiment, a second experiment was conducted between June 21 - 25, using a larger number of replicates. Again, four trochus were tethered to each rod, two of medium size (mean 19.8 ± 0.2 mm SW) and two of large size (mean 27.1 ± 0.2 mm SW). There were 20 replicate rods aligned in two rows of 10 rods each and separated by five metres. Rods were placed at three levels on the intertidal reef flat: low (120 m from the beach), mid (70 m from the beach) and high (20 m from the beach).

All rods were checked 1.5 hours after the initial set up and then daily for four days, when the experiment was finished. The numbers of trochus surviving, missing and dead were recorded. I also made observations on the type of substrata (sandy, abundant rubble) and the presence or absence of intertidal pools at each site (i.e. rod). I used a qualitative index to describe the relative abundance of each factor: 0 = none or very little (0-10%), 1= medium (20-60%), 2= abundant (60 - 100%).

An analysis of variance was used to test if trochus size and intertidal level (fixed factors) affected the number of trochus found alive after four days. The factor site (i.e. rod) was nested within intertidal level.

8.4 Results

8.4.1 Release Experiments

Release Exp. 1

The size frequency distribution of the whole population recaptured after three days had a greater proportion of juveniles in the larger size classes than expected by random (Randomisation test p= 0.044, Fig. 8.1). Inspection of the data by individual plots shows an overall pattern of increase in the proportion of trochus in the three larger size classes and a decrease in the smallest two size classes (Fig. 8.2). However, when the data were analysed separately by plot, the difference in size frequency distribution of the population was significant in only one plot (Plot C, Fig. 8.2). After 30 and 111 days, the size frequency distribution of the population recaptured included significantly more of the larger trochus than

Fig. 8.1 Release Exp. 1. Size frequency distribution of the population of juvenile *Trochus niloticus* released and recaptured at different times, on the intertidal reef flat at Orpheus Is. N= number released and recaptured; p is the probability that the observed frequency was the result of random disappearance of juveniles irrespective of their size.

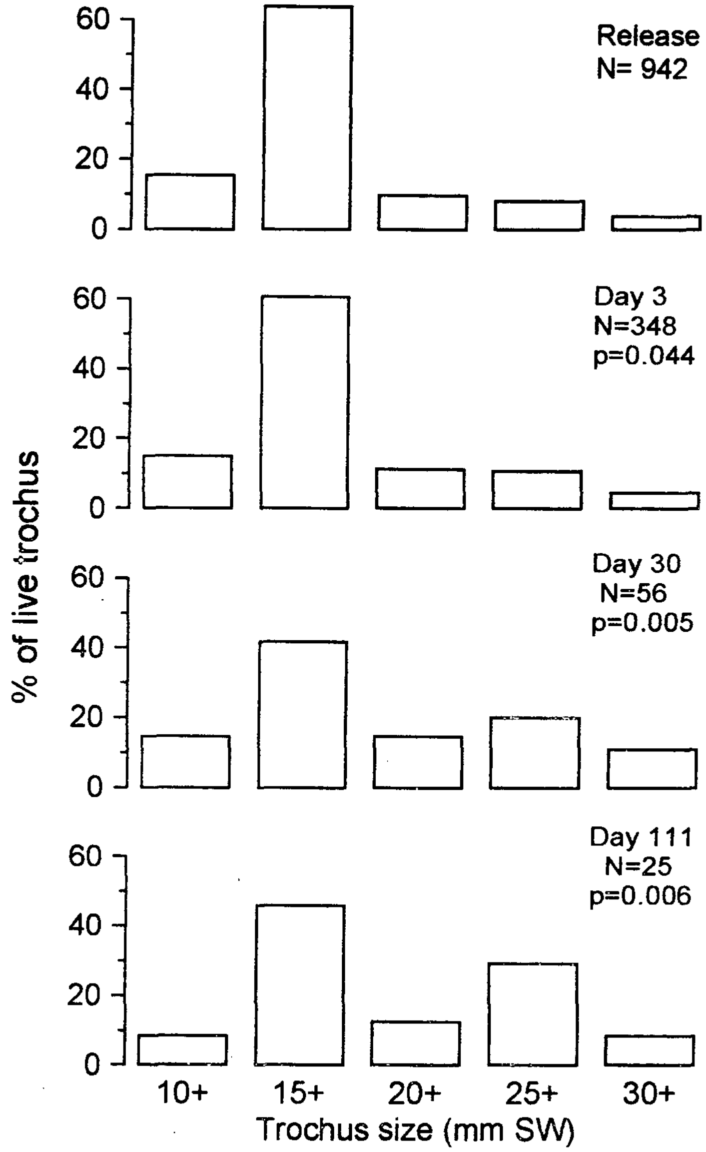
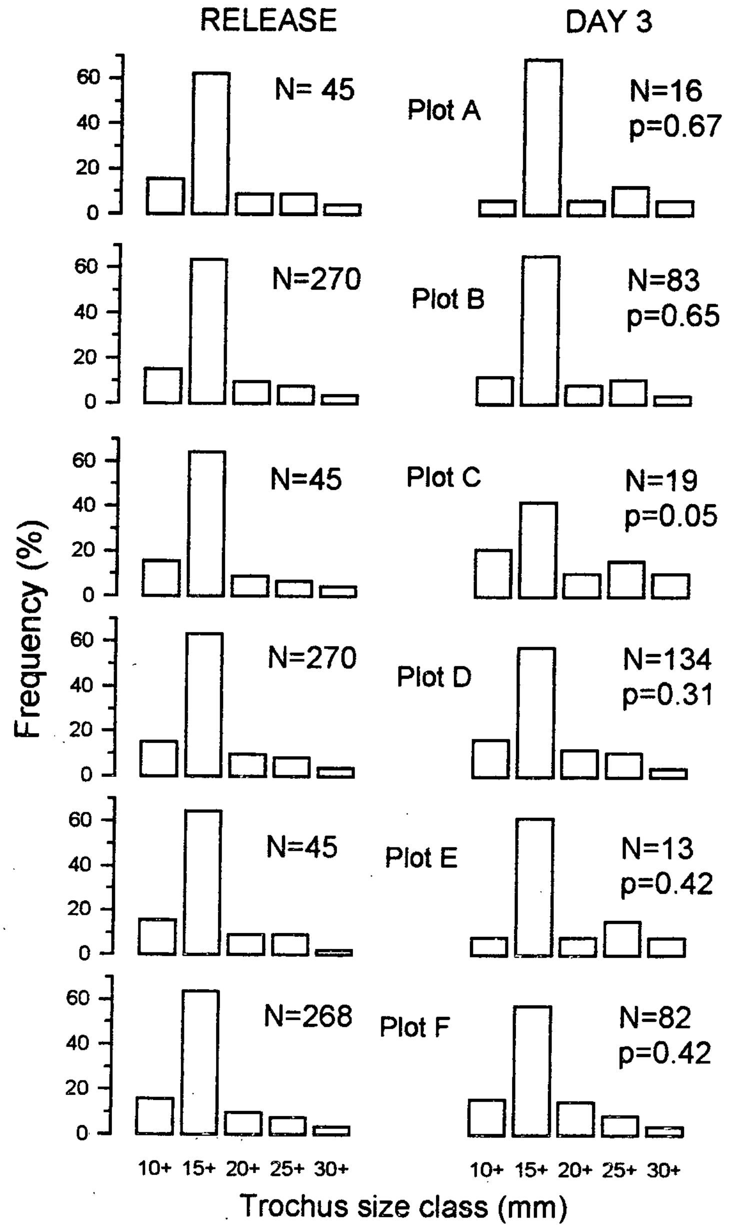


Fig. 8.2 Release Exp. 1. Size frequency distribution of the population of juvenile *Trochus niloticus* released and recaptured in each experimental plot after 3 days of release at Orpheus Is. N= number released and recaptured; p is the probability that the observed frequency was the result of random disappearance of trochus irrespective of their size.



expected by random (Randomisation test $p<0.001$ for 30 days and $p=0.006$ for 111 days) (Fig. 8.1).

Twelve trochus were found dead three days after the release, representing only 1.3% of the total population released. These trochus were of the three smaller size classes: 2 were 10-14.5 mm, 9 were 15 - 19.5 mm and one was 20 - 24.5 mm. Six of the dead shells had chipped apertural lips, two where undamaged, one had two large holes 3-4 mm diameter, and three had been crushed.

Five trochus were found dead during the second census, 30 days after the release. Four were between 15 - 19.5 mm SW and one was 25 mm in size. All of them had chipped lips.

Experiment 2

The number of trochus found on day three was greater than the number previously found on day one in nine sites (15%), proving that some trochus were missed during the first census even though they were present. From these data the probability of sighting one of these small trochus was estimated as 0.97 (SE 0.01).

The mean numbers of trochus estimated to be alive after one day were 5.8 individuals.site^{-1} for the mid intertidal level and 6.4 individuals.site^{-1} for the high intertidal level. By day three, mean values were 4.3 individuals.site^{-1} for the mid level and 5.6 individuals.site^{-1} for the high level. In terms of overall survival, 58% of the trochus released in the mid intertidal level were estimated to be alive on day one and 43% on day three, and respective values for the high intertidal level were 64% and 56% (Fig. 8.3). Mean survival of trochus was not statistically different between the two intertidal levels at any of the two censuses, but survival decreased significantly with time (Table 8.1).

Trochus were lost at a greater rate in the first day of release than between days one and three: 42% (mid level) and 36% (high level) trochus were lost within the first day of release, whereas respective values were 7% (mid level) and 4% (high level) trochus per day between days one and three (Fig. 8.3).

Signs of trochus mortality were few, with only five sites (24%) in the mid intertidal level and two sites (9%) in the high level showing evidence of predator activity (Table 8.2). The most common sign of predation was crushing (Table 8.2). Potential predators of trochus were observed in 86% sites in the mid level and in

Fig. 8.3 Release Exps. 2 and 3 using small (4-8 mm SW) juvenile *Trochus niloticus*. Plots on the left show estimates of survival after 1-3 days at two levels on the intertidal reef flat at Orpheus Is., high (○, solid line) at 20 m from the boulder beach, and mid level (△, dashed line) at 70-80 m from the boulder beach. Plots on the right show corresponding estimates of loss rate.

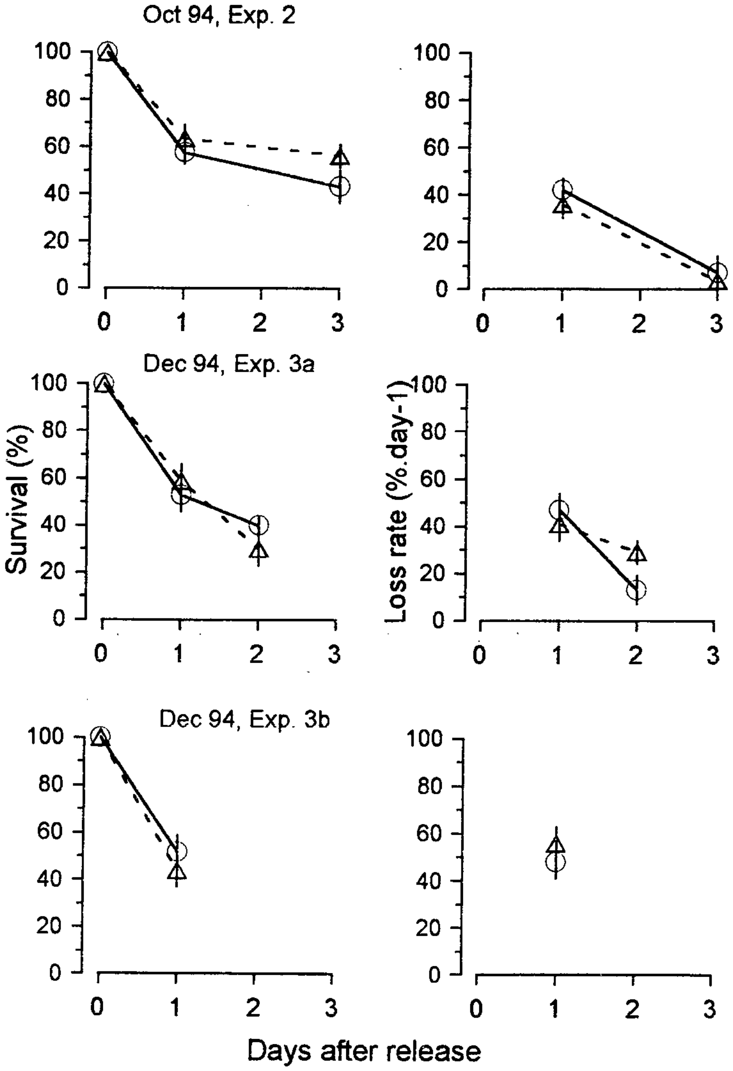


Table 8.1 Results of the repeated measures analysis of variance for Release Exp. 2 to examine effect of tidal level and time on the number of juveniles surviving. *Trochus* 4-8 mm SW were released at two levels on the intertidal reef flat at Orpheus Is. and censused at one and three days.

Tests of Between-Subjects Effects.

Source	**DF**	**MS**	**F**	**p**
Tidal Level	1	19.55	1.74	0.195
Error	41	11.25		

Tests involving 'TIME' Within-Subject Effect.

Source	**DF**	**MS**	**F**	**p**
Time	1	25.53	10.65	0.002
Time*Tidal Level	1	2.69	1.12	0.296
Error	41	2.40		

Table 8.2 Release Exp. 2. Number of sites with signs of trochus mortality and presence of potential invertebrate predators. Numbers in parenthesis represent percentages. Ten *Trochus niloticus* 4-8 mm SW were released at each site (N= total number of sites).

TIDAL LEVEL	N	CRUSHED SHELLS	WHOLE SHELLS	POTENTIAL PREDATORS	
				Molluscs	Crustaceans
MID	21	4 (19%)	1 (5%)	10 (47%)	7 (33%)
HIGH	22	2 (9%)	0	14 (64%)	3 (14%)

64% of the sites in the high level (Table 8.2). The most common carnivorous gastropods were two muricid gastropods, *Morula fiscella* and *M. biconica*, and one fasciolariid *Peristernia australiensis*. Among crustacean predators, portunid crabs, *Thalamita admete* and *T. stimpsoni*, and stomatopods, *Gonodactilus* spp. were the most common. The absence of potential predators while sampling was not indicative of high survival: in all those sites where potential predators where not observed nearby, trochus were also lost.

Experiment 3

The number of trochus found at three sites (13%) on day two was greater than the number previously found on day one. Based on these data the probability of sighting a trochus was estimated as 0.97 (SE 0.02).

As in the previous experiment, mean survival of trochus was not significantly different between the two intertidal levels in any of the two experiments (Table 8.3). In the first release, the percentage of trochus estimated to be alive after one day was 59% in the high intertidal level, and 53% in the mid intertidal level (Fig. 8.3). By the second day, the opposite pattern was observed, with survival decreasing to 30% in the high level and 40% in the mid level (Fig. 8.3). The decrease between days 1 and 2 was statistically significant (Table 8.3). For the second group released, mean survival after one day was 52% in the mid level and 44% in the high level (Fig. 8.3).

Trochus were lost at a rate between 13 - 56% individuals.day^{-1} (Fig. 8.3). Similar to the previous experiment (Exp. 2), there was indication of a decrease in loss rate within the first two days of release.

Evidence of predation on released trochus was also very low (Table 8.4). Carnivorous gastropods were most common in the high intertidal level. During the census, I observed two trochus in the process of being drilled by these gastropods (*Morula biconica* and *Peristernia australiensis*).

8.4.2 Tether Experiments

Tether Exp. 1

Trochus survival differed significantly among the three intertidal levels (Table 8.5). Fifty five percent of the trochus survived after eight days in the low intertidal level compared to 40% in the high and 40% in the mid levels.

Table 8.3 Release Exp. 3. Results of the analysis of variance to examine the effect of intertidal level and time on the number of juvenile *Trochus niloticus* 4-8 mm SW, surviving after released on the intertidal reef flat at Orpheus Is. In Exp. 3a release sites were censused after one and two days, in Exp. 3b there was only one census after one day.

Exp. 3a

Tests of Between-Subjects Effects.

Source	**DF**	**MS**	**F**	**p**
Tidal Level	1	0.55	0.08	0.784
Error	22	7.18		

Tests involving 'TIME' Within-Subject Effect.

Source	**DF**	**MS**	**F**	**p**
Time	1	53.07	18.67	<0.001
Time*Tidal Level	1	7.98	2.81	0.108
Error	22	2.84		

Exp. 3b

Source	**DF**	**MS**	**F**	**p**
Tidal Level	1	3.09	0.57	0.459
Error	20	5.42		

Table 8.4 Number of sites with signs of trochus mortality and presence of potential invertebrate predators in Release Exp. 3(a and b), at Orpheus Is. Number in parenthesis represent percentages. Ten juvenile *Trochus niloticus* 4-8 mm SW were released at each site (N= total number of sites). [1] all dead trochus found in this category were drilled.

TIDAL LEVEL	N	CRUSHED SHELLS	WHOLE SHELLS[1]	POTENTIAL PREDATORS	
				Mollusc	Crustacean
Exp. 3a					
MID	12	1 (8%)	0	1 (8%)	2 (17%)
HIGH	12	1 (8%)	1 (8%)	10 (83%)	1 (8%)
Exp. 3b MID	11	1 (9%)	1 (9%)	3 (27%)	2 (18%)
HIGH	11	1 (9%)	1 (9%)	5 (45%)	1 (9%)

Table 8.5 Tether Exp. 1. Analysis of variance table to test for effect of intertidal level and trochus size (small vs. medium) on the number of juvenile *Trochus niloticus* found alive after eight days, on the reef flat at Orpheus Is.

Source	**DF**	**MS**	**F**	**p**
Trochus size	1	8.533	32.00	<0.001
Intertidal level	2	0.933	5.60	0.019
Site(level)	12	0.167	0.62	0.790
Size* Level	2	0.133	0.50	0.619
Error	12	0.267		

Trochus size also had a significant effect on survival: survival of medium sized-trochus was 3 - 7 times greater than small trochus (Fig. 8.4, Table 8.5). When pooling the results for all three levels, overall survival after eight days was estimated as 16.5 % for small trochus and 73.5 % for medium sized trochus. Mortality explained 63% of loss in the small size class, whereas 20% where categorised as missing (Fig. 8.4). For medium size trochus, mortality explained 10% of their loss, leaving 17% missing (Fig. 8.4).

Survival was quite variable among individual sites within a single level, with some sites losing all trochus while others having all or most of them alive (Fig. 8.5). Loss rate was 3 - 3.6 times greater for small than for medium-sized trochus (Table 8.6). Average loss rate was 10% of individuals.day^{-1} (SE 1) for small trochus and 3.3% of individuals.day^{-1} (SE 0.4) for medium-sized trochus.

Crushing and non-crushing sources of mortality were evident among the dead shells (Table 8.7).

Tether Exp. 2

Mean survival of trochus after four days was very similar among the three intertidal levels, with the lowest survival of 54% in the mid level, followed by 59% in the high level and 63% in the low level (Fig. 8.6). Analysis of variance indicated that the depth on the intertidal reef flat where trochus were released had no significant effect on their survival (Table 8.8).

Survival of large trochus was 1.2 to 1.8 times greater than medium-sized trochus (Fig. 8.6) and the effect of size on survival was statistically significant (Table 8.8). Overall survival after four days was 49% for medium-sized trochus and 68% for large trochus. Mortality explained 33% of loss in the medium size class, with 17.5% missing; whereas the proportion of large juveniles lost to certain mortality was 24%, with 8% missing (Fig. 8.7)

Seven tagged trochus (3%) were found unattached but next to their tethers. In one case the thread was shredded and the trochus had chipped lips, indicating that it had been attacked by a predator. One trochus was observed initially retracted and it was found dead on the fourth day with decomposing flesh inside the shell. This trochus was most likely to be sick since the beginning of the experiment. These observations represent 3% of tethers becoming undone and 0.4% of mortality possibly due to handling.

Fig. 8.4 Tether Exp. 1. Estimates of survival (open bars), missing (filled bars) and mortality (bars with vertical hatching) of small (12 mm) and medium (23 mm) size juvenile *Trochus niloticus*, at three levels on the intertidal reef flat at Orpheus Is., high (20 m from beach), mid (70 m) and low (120 m), after 8 days.

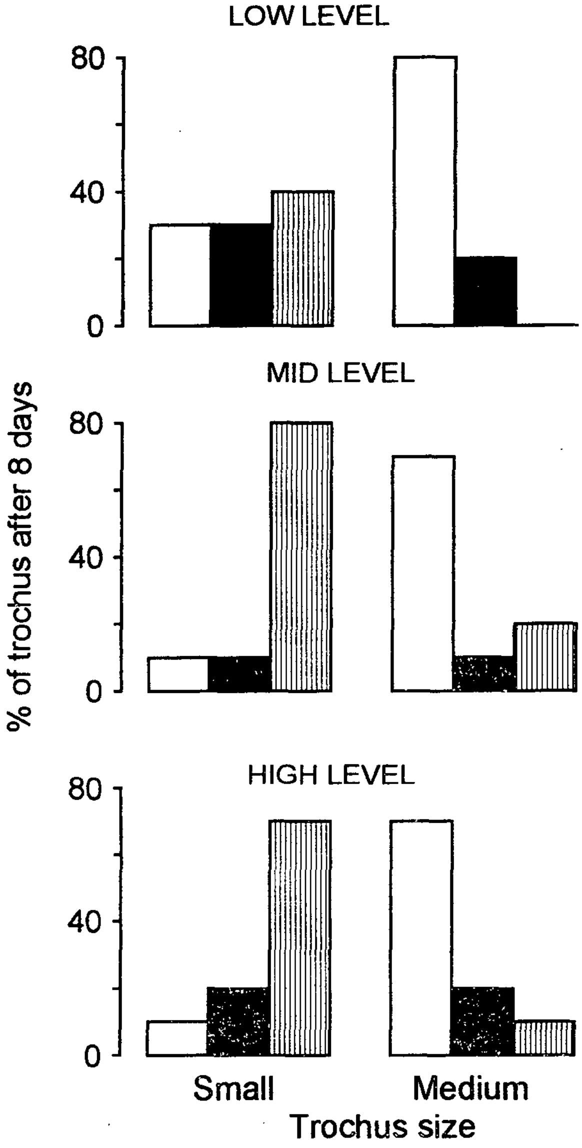


Fig. 8.5 Tether Exp. 1. Variability in survival of juvenile *Trochus niloticus* after 8 days, among replicate sites at three intertidal levels on the reef flat at Orpheus Is. Four trochus were tethered to a rod in each site.

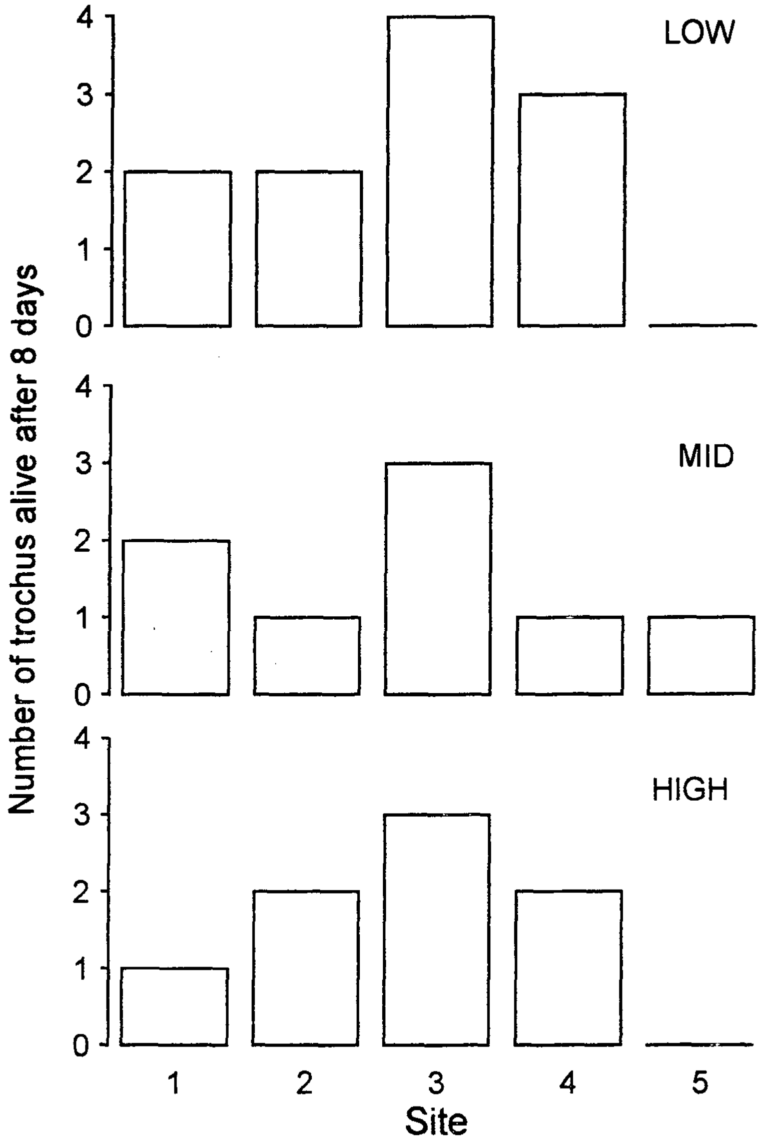


Table 8.6 Tether Exp. 1. Trochus loss rate (% individuals.day^{-1}) of small (12 mm SW, SD 2) and medium (23 mm SW, SD 2) size juvenile *Trochus niloticus* at each of three intertidal levels on the reef flat at Orpheus Is.

INTERTIDAL LEVEL	TROCHUS SIZE (mm) SMALL	 MEDIUM
LOW	9	2.5
MID	11.3	3.8
HIGH	11.3	3.8

Table 8.7 Tether Exp. 1. Number of shells within each category of mortality for small (S) (12 mm SW, SE 2) and medium (M) (23 mm SW, SE 2) size *Trochus niloticus* after 8 days. Ten trochus of each size class were released within each level on the intertidal reef flat at Orpheus Is. N= total number of dead trochus.

Level	**Size**	**CRUSHED**	**WHOLE SHELLS** **Damaged**	 **Undamaged**	**N**
LOW	**S**	2	1	1	4
	M	0	0	0	0
MID	**S**	5	0	3	8
	M	0	2	0	2
HIGH	**S**	1	6	0	7
	M	1	0	0	1

Fig. 8.6 Tether Exp. 2. Changes in survival of medium (20 mm)(○, solid line) and large (27 mm)(□, dashed line) juvenile *Trochus niloticus* during the first four days of release, at three levels on the intertidal reef flat at Orpheus Is.

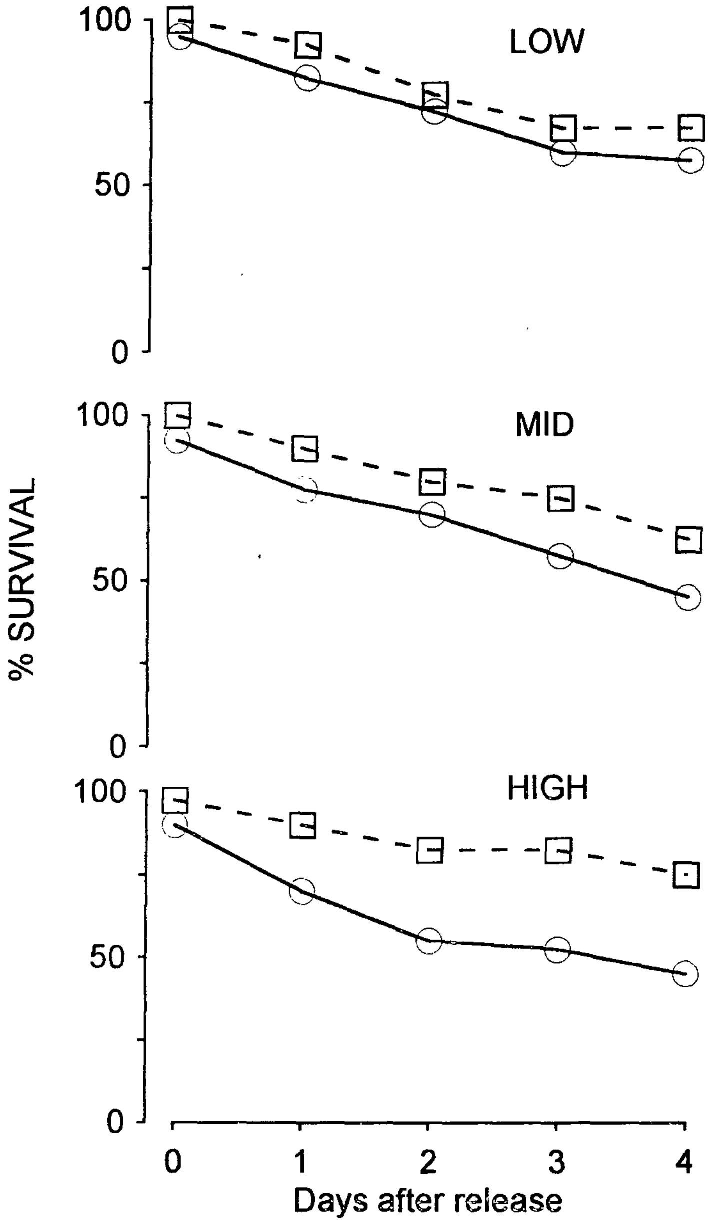
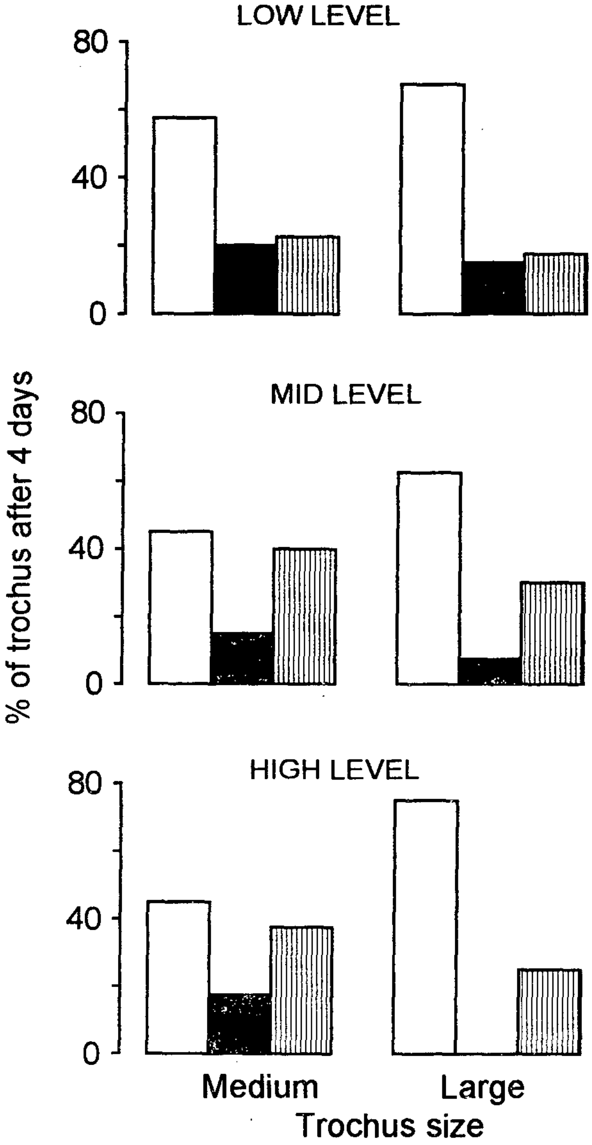


Table 8.8 Tether Exp. 2. Results of the analysis of variance to test for the effect of intertidal level and trochus size on the number of tethered trochus found alive after four days of release on the reef flat at Orpheus Is.

Source	**DF**	**MS**	**F**	**p**
Tidal Level	2	0.308	0.47	0.625
Size	1	4.800	11.82	0.001
Site (level)	57	0.650	1.60	0.039
Level*Size	2	0.525	1.29	0.283
Error	57	0.406		

Fig. 8.7 Tether Exp. 2. Estimates of survival (open bars), missing (filled bars) and mortality (hatched bars) of medium (20 mm) and large (27 mm) size juvenile *Trochus niloticus*, at three levels on the intertidal reef flat, four days after released on the reef flat at Orpheus Is.



The mean rate of trochus loss was 8% day^{-1}.intertidal $level^{-1}$ for medium-sized trochus and 6% for large trochus (Fig. 8.8). Pooling the data for all three intertidal levels, trochus loss was 8.3% (SE 1) day^{-1} for medium-sized and 6.1% (SE 0.6) .day^{-1} for large trochus. Within the four days that this experiment lasted, there was no indication that loss rate decreased with time (Figs. 8.6 and 8.8).

Predation on tethered trochus occurred even after only 1.5 hours of setting up the experiment, when nine individuals (4% of the population released) had already been or were being attacked by predators: seven were crushed (in two cases there was a stomatopod *Gonodactylus* sp. eating the trochus), one had survived an attack (i.e. it was upside down with chipped apertural lips, but alive), and another one had a gastropod *Morula biconica* on top, although the attack was eventually unsuccessful.

As in Tether Exp.1, the number of trochus lost varied markedly among sites (Fig. 8.9). However, in only two cases, both in the mid intertidal level, were all tethered trochus lost (Fig. 8.9). Trochus loss occurred gradually, generally about one individual per day (Fig. 8.9). Only in two cases (mid level, row 1) were three trochus lost within one day and never were all four trochus lost within the same day. Crushed and whole shells were both found frequently. Crushing was most common among the medium-sized trochus in the low and mid levels, but whole shells were as frequent as crushed shells in the high level (Table 8.9). The results for the large trochus were slightly different, with an overall trend for higher frequency of whole dead shells except for the mid level were crushed shells were most common (Table 8.9)

Trochus survival was negatively correlated with pool depth and positively correlated with the presence of rubble (Table 8.10). Fig. 8.10 shows the relationship between survival and habitat characteristics when data from all three habitat levels were pooled. There was a trend for survival to be greater in areas that were in only a few centimetres of water at low tide, compared to deeper pools. Areas that had no or very little rubble were associated with lower survival. Habitat characteristics (pool, rubble and sand), however, explained only up to 22% of the variation in juvenile survival (Table 8.10).

Fig. 8.8 Tether Exp. 2. Loss rate of medium (□, dashed line) and large (○, solid line) juvenile *Trochus niloticus*, at three levels on the intertidal reef flat at Orpheus Is., during the first four days after release.

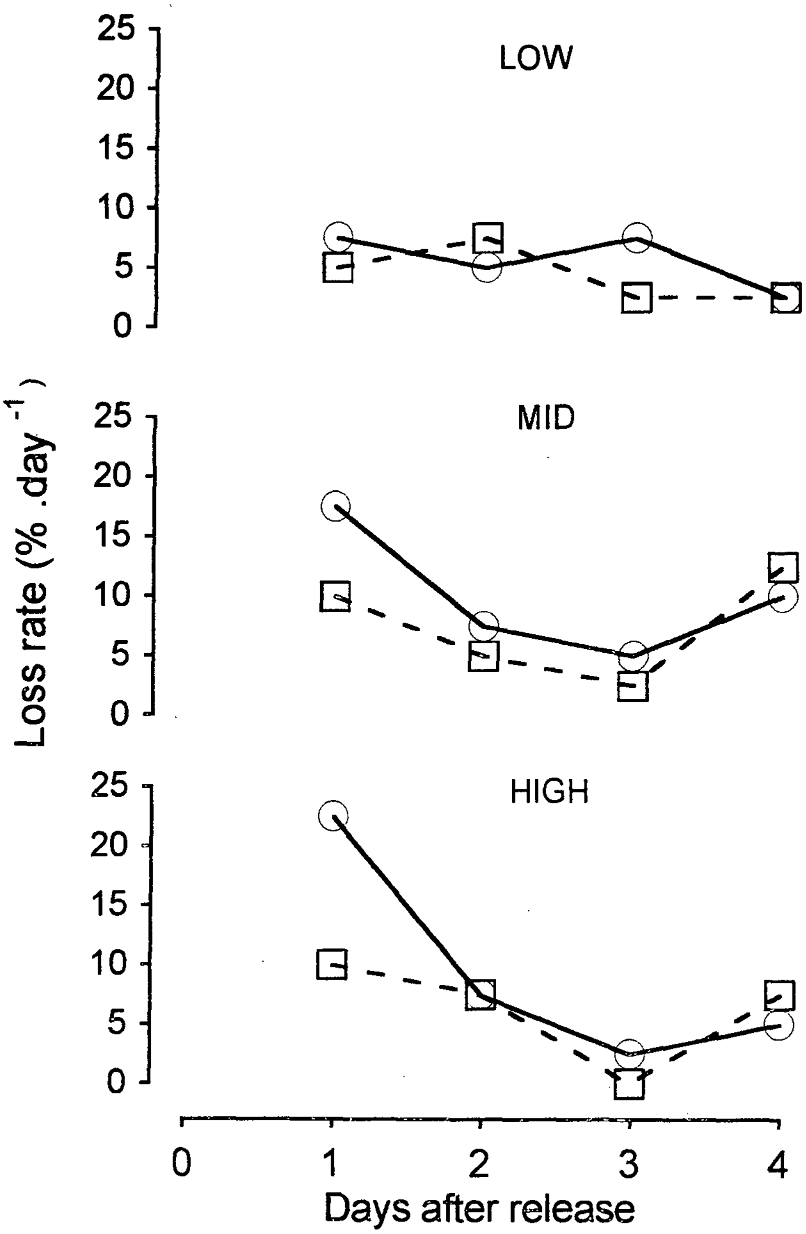


Fig. 8.9 Tether Exp. 2. Variability in survival of juvenile *Trochus niloticus* among replicate sites at three intertidal levels at Orpheus Is., during the first four days of release. Four trochus were tethered to a rod in each site. Distance between adjacent sites was 5 m. There are five bars for each rod, with each bar representing days from 0 - 4 after the release. Absence of a bar means that no trochus were lost at that site on that day.

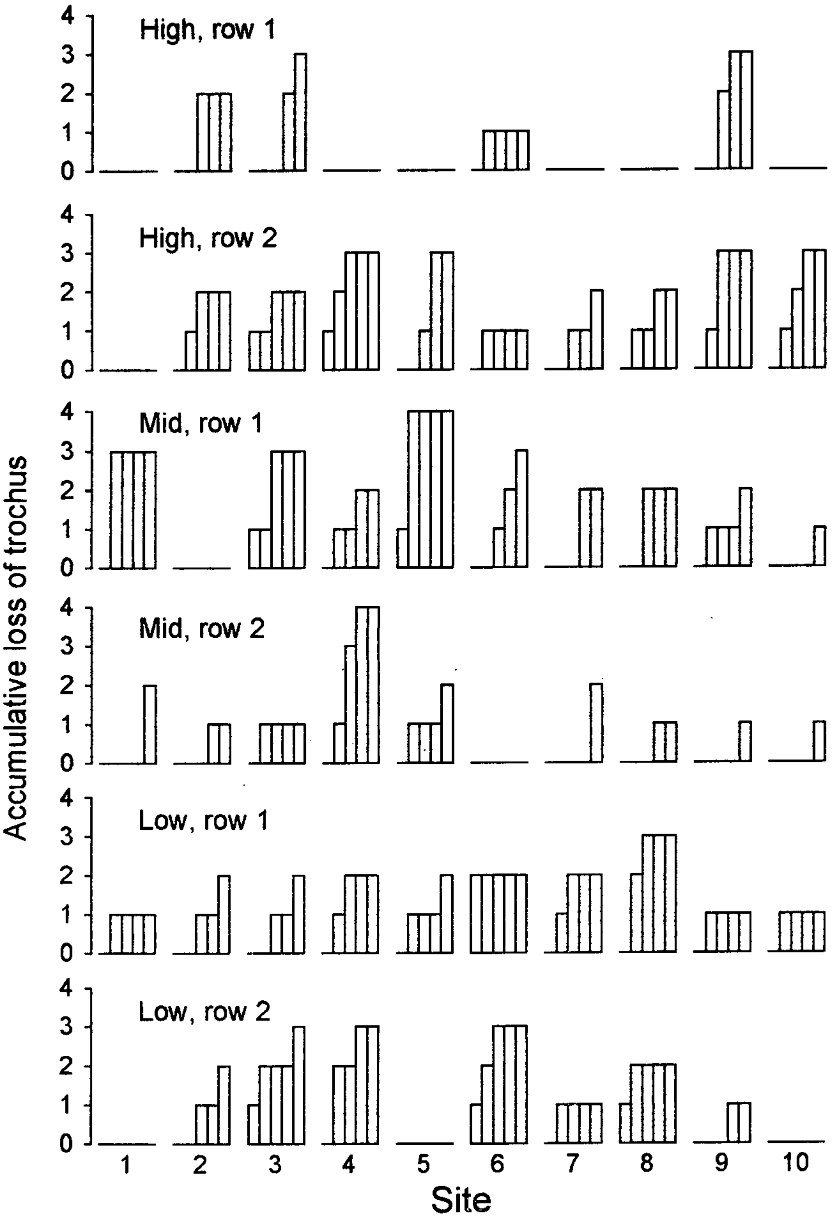


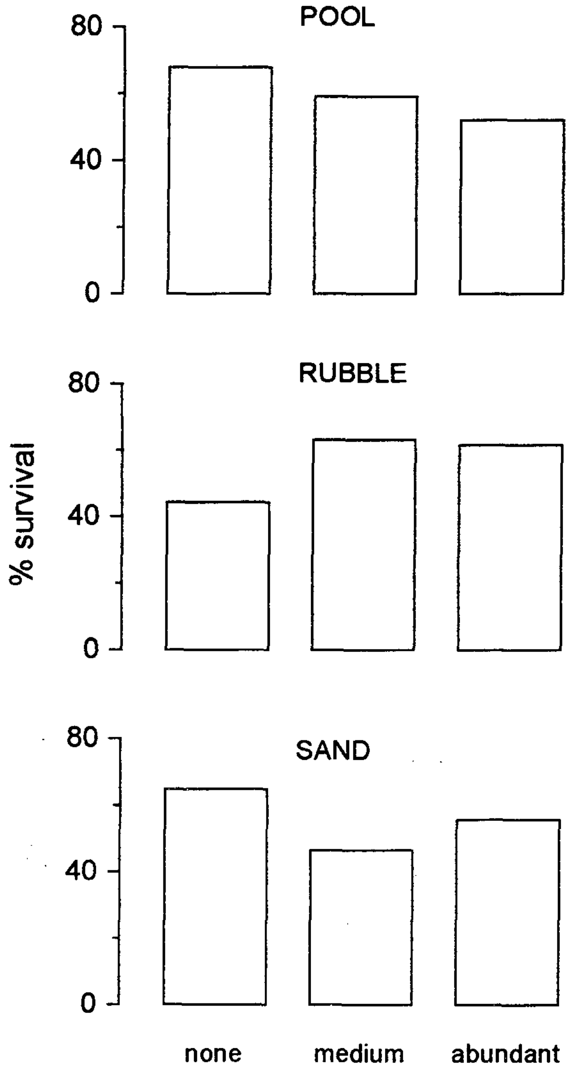
Table 8.9 Tether Exp. 2. Number of shells within each category of mortality of medium (M) (20 mm SW, SD 0.2) and large (L) (27 mm SW, SD 0.2) juvenile *Trochus niloticus*, after four days of release at Orpheus Is. Forty trochus of each size class were released within each of three levels on the intertidal reef flat. N= total number of dead shells.

Level	**Size**	**CRUSHED**	**WHOLE SHELLS** **Damaged**	 **Undamaged**	**N**
LOW	M	6	0	3	9
	L	2	1	3	6
MID	M	11	2	1	14
	L	7	1	4	12
HIGH	M	9	1	7	17
	L	2	2	5	9

Table 8.10 Exp. Tether 2 at Orpheus Is. Correlation analysis to examine the relationship between substrate characteristics and the number of tethered juvenile *Trochus niloticus* surviving after four days. The data for all intertidal levels were pooled (N=60).

	Survival	Pool	Sand	Rubble
Survival	1			
Pool	-0.2166	1		
Sand	-0.1784	-0.2347	1	
Rubble	0.2113	0.3266	-0.4825	1

Fig. 8.10 Tether Exp. 2. Relationship between survival of juvenile *Trochus niloticus* and habitat characteristics four days after their release on the intertidal reef flat at Orpheus Is. None= 0-10%, medium= 20-60%, abundant= 60-100%).



8.5 Discussion

A summary of the results of all the experiments described is given in Table 8.11 and Fig. 8.11. Two main patterns emerged with high consistency in the results. Firstly, when trochus of different sizes were released in the same place and at the same time, survival of larger trochus was always greater than survival of the smaller trochus (Release Exp. 1, Tether Exps. 1 and 2). Secondly, trochus survival at different depth levels on the intertidal reef did not vary significantly in any but one experiment (Release Exps. 2 and 3, Tether Exps. 1 and 2), indicating that distance from the beach (the criteria used to define intertidal levels) was a poor predictor of trochus survival.

Various aspects of the results provide an insight into the complexity of the processes that affect survival of trochus on the reef. The two most notable are the variability in survival between experiments, and the variability in survival on a scale of metres on the reef. I will refer to them in more detail while discussing the effects of trochus size and habitat on survival, the two main factors examined in this study.

8.5.1 Trochus Size

Estimates of survival varied considerably among experiments (Fig. 8.11, Table 8.11). As a result, there were instances where survival of a larger size class in one experiment was lower than survival of a smaller size class in another experiment. This could have been partly due to differences in the two methods involved (free release and tethering), but the same phenomenon was observed among experiments using the same methods. For example, 74% of medium-sized trochus survived after 8 days in Tether Exp. 1, whereas survival of medium and large trochus were, respectively, 69% and 68% in only half of that period in Tether Exp. 2 (Table 8.11). The conclusion that larger trochus survive better than smaller trochus applies to simultaneous releases, but not always to cases where trochus are released at different times in different locations, even on the same reef. This result has important relevance to the study of *Trochus* seeding and seeding in general. Firstly, it stresses the importance of including all levels of a particular parameter (i.e. juvenile size, density or depth) within a single experiment, to minimise the potential for confounding factors affecting the results (e.g. time and location). Trochus of various size classes have been released in other studies (e.g. Hoffschir et al., 1989; Kubo, 1991, 1992). Based on releases at various sites, Kubo

Fig. 8.11 Summary of results of release and tether experiments at Orpheus Is., (Release Exps. 2 and 3, Tether Exps. 1 and 2). Estimates of survival for juvenile *Trochus niloticus* of different size classes, within the first days of release.

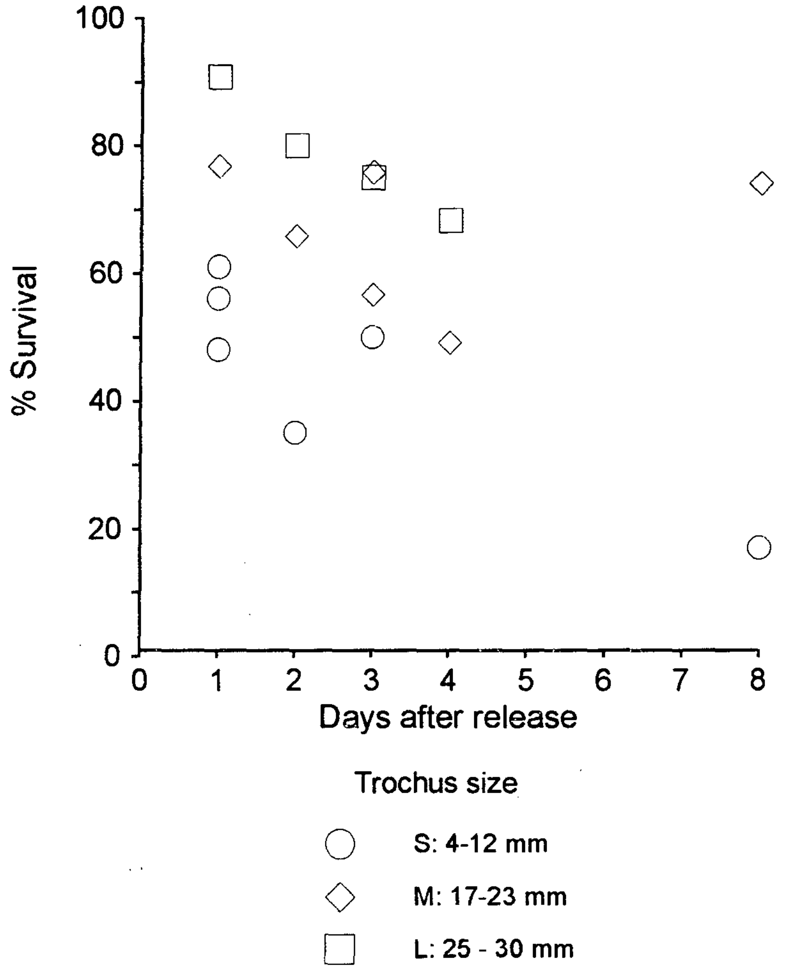


Table 8.11 Summary of field experiments at Orpheus Is. Trochus size S= small, M= medium, L=large

EXP.	DATE	N	Trochus SIZE (mm SW)	RESULTS	TIME (days)	SURVIVAL %	Source
RELEASE 1	Nov 93-Feb 94	943	13 - 44	Greater survival of larger trochus	3 30 111	51 16 24	Chapter 8 (Exp.1)
RELEASE 2	October 94	430	S 4.0 - 8.0	No effect of intertidal level	1 3	61 50	Chapter 8 (Exp. 2)
RELEASE 3	December 94	460	S 4.0 - 8.0	No effect of intertidal level	a) 1 2 b) 1	56 35 48	Chapter 8 (Exp. 3)
TETHER 1	April 94	60	S 12 ± 2 M 23 ± 2	Greater survival of larger trochus	8	S - 17 M - 74	Chapter 8 (Exp. 1)
TETHER 2	June 94	240	M 20 ± 0.2 L 27 ± 0.2	Greater survival of larger trochus	4	M - 49 L - 68	Chapter 8 (Exp. 2)
RELEASE 4	June-July 94	360	M 23 ± 0.1	No effect of intertidal level	2-3 30	76 9	Chapter 6 and unp.

(1991) concluded that survival rate was not related to trochus size within the range of 8 - 16 mm SW. His result of no difference in survival between the two size classes could have been partly due to the different characteristics of the release sites. In another study where large (27-32 mm SW) and medium-sized (20 mm SW) trochus were released simultaneously, survival of large trochus after the first day was 55-76% whereas that of the medium-sized trochus was 34-47% (Kubo, 1992). Hoffschir et al. (1989) released trochus of either 18 or 23 mm mean SW at 20 different stations. Their results after 15 days show high variability with estimates of survival ranging between 0 - 70%. Since size groups were released at different stations (presumably not overlapping), any difference in survival between these two similar size classes could have been masked by habitat differences. Appeldoorn and Ballantine (1983) pointed out the difficulty in comparing seeding experiments with juvenile *Strombus gigas* because of highly variable mortality.

The smallest trochus used in these experiments (4-12 mm SW) were lost at a rate of 13-56% per day (Figs. 8.3, 8.4 and 8.8). Medium and large trochus (20-27 mm SW) were lost at a rate of 3-8% per day (Figs. 8.4 and 8.8). These results correspond to the first few days after the release. There is not enough information at present to predict if loss rates will decrease with time, though such pattern was observed in Exps. 2 and 3 (Fig. 8.3). If loss rate changes in time, one would predict that it will decrease rather than increase. Vulnerability is likely to be higher after release because of handling and adaptation to new conditions (Schiel, 1993). In addition, trochus will grow and attain a size refuge from some predators (Kubo, 1991; Chapter 3). In such case, using initial loss rates would provide conservative estimates of juvenile loss with time.

A second implication of variability among experiments is that the time of release may be an important factor that needs to be further examined. For example, survival in Tether Exp. 1, conducted in February 94, was greater than survival in Tether Exp. 2 which was conducted in June 94 (Table 8.11). Variability in time could be related to seasonal effects and habitat differences between areas of release, even if releases were on the same reef, as in this study. February and June correspond respectively to mid-late summer and winter at Orpheus Is. The difference in water temperature between the warmest and coolest months is on average 8°C (Chapter 2). The most obvious habitat difference between February and June was the greater abundance of macroscopic green algae during February.

Survival of wild and hatchery-reared juvenile conch *Strombus gigas* in tether experiments was higher during summer compared to autumn (Stoner and Davis, 1994). Similarly, larger juvenile *Strombus costatus* survived better in the first release than in the second conducted two months later (Appeldoorn, 1984). In this study, the same experimental method using trochus 4-8 mm SW produced survival estimates of 50% after three days in October 94, but 35% after two days in December 94 (Release Exps. 2 and 3, Table 8.11). Analysis of the variation in abundance of alternative prey and potential predators were beyond the scope of this study, but my results suggest that these deserve further attention to determine the magnitude of such changes and their potential effect on survival of *Trochus* seed. My impression throughout the study was that invertebrate predators on the reef flat were sometimes more abundant than others, but this was more related to a particular day than to a particular month or season. Only a quantitative study would provide the required information.

8.5.2 Habitat

Another aspect of the results was that variability in survival among replicate plots was high. This was best seen in the tether experiments, where some sites lost most trochus while adjacent ones 5 m away lost none or very few (Figs. 8.5 and 8.9). Given such variability, it was not unexpected that the effect of the broader scale classification of habitat used in this study was not significant in all but one experiment at Orpheus Is. In a series of releases at different levels on the reef flat, Kubo (1989) also found no significant relationship between tidal level and recovery of *Trochus* seed. The results suggest that small scale ($1m^2$) characteristics of the habitat may be of greater importance than previously thought. Various observations provide further evidence to support this. 1) There was a negative relationship between juvenile survival, intertidal pools and sandy substratum, but a positive relationship between juvenile survival and the amount of rubble (Fig. 8.10, Table 8.10). 2) For wild trochus, the smaller individuals were more frequently found in areas of small size rubble and very shallow intertidal pools (Chapter 2). 3) Wild trochus were found all along the reef flat although in greater numbers in the middle section away from the most inshore and offshore areas (Chapter 2).

The characteristics of the intertidal reef at Orpheus Is. (i.e. abundant coral rubble, tidal exposure and wave action), and the presence of a established population of juveniles and adults on the reef (Chapter 2), indicate that the reef flat offers a favourable habitat for juvenile *Trochus*. However, within a favourable habitat there are places of high and low mortality. These results and observations on wild juveniles (Chapter 2) suggest that characteristics such as the depth of intertidal pools and the size and abundance of coral rubble are related to juvenile survival. How they affect survival is not clear. Predators are likely to be distributed according to habitat characteristics. Patches that provide better refuge for predators may suffer higher predation intensity than patches with lower availability of refuges (Auster and Malatesta, 1991). Survival of transplanted mussel clumps on an intertidal reef was highly variable on scales of metres, partly due to nearby clumps being missed by the main predator, *Pisaster ochraceus* (Menge et al., 1994). Analysis of trochus loss in Tether Exp. 2 provides interesting information on the process of predation on *Trochus*. In 78% of events where the number of juveniles tethered to a rod decreased from one day to the next, only one juvenile was lost (in most cases it was certain that predation was the cause). After encountering and eating a trochus, predators did not remain in the area long enough to find another prey.

The relative importance of large versus small scale characteristics of the reef will obviously vary depending on the particular location. For example, at Moso Is. (Vanuatu) survival of released juvenile *Trochus* was significantly greater in the deeper zone. This pattern was evident after only 2-3 days and remained after 40 days of the release (Fig. 7.4, Chapter 7). Differences between the shallow and deeper zones were greater at Moso Is. compared to Orpheus Is. At Moso Is., the shallow zone had more coral bench, with holes and crevices difficult to reach by hand, whereas rocks were more sparse and had less holes in the deeper zone. Crab predators were observed hiding only in the shallow zone. Then, large scale characteristics are certainly important, and they must be considered to take a decision on whether to release trochus in a site. However, the results of this study indicate that microhabitat characteristics are also very important and the identification of those that favour trochus survival would benefit considerably the success of seeding.

8.5.3 Patterns of Mortality

Using the relative proportions of crushed vs. whole shells as an indication of the predation intensity by crushing and non-crushing organisms is subject to limitations. First, it is more likely that whole shells will be easier to find than shell fragments, and second, a number of crushing predators are likely to take the prey away, such as stomatopods, which generally take their prey back to the refuge (Cardwell, 1989), and fishes, which will spit the prey's fragments into the water column, where they could easily be carried away from the place of capture. Evidence of predation was very low in experiments where small trochus (4-8 mm) were released freely (Release Exps. 2 and 3). In Tether experiment 2, however, records of mortality were relatively high and showed some patterns, which, for the above mentioned reasons, should be interpreted with caution. These patterns were: 1) crushing was more frequently among the medium-sized trochus whereas non-crushing was more frequent in the large trochus (Table 8.9); 2) non-crushing mortality was observed more frequently in the high level. A decrease in crushing frequency with an increase in trochus size is not unexpected, since the number of predators that could successfully crush the shells is likely to decrease as juveniles grow (see for example Chapter 3). A similar effect would be predicted on predators that drill the shell, since shell thickness increases with shell size (e.g. Palmer, 1990). However, *Thais tuberosa* and *Morula biconica* were both observed eating prey through the aperture without drilling the shell (Chapter 5), although *M. biconica* was also observed drilling its prey. In addition, both of these species and *Peristernia australiensis* occasionally formed groups of 3-9 individuals feeding on the same prey. The effect of increased prey size on these predators may be different from the effect on predators that crush and use only drilling to kill their prey.

The relatively high frequency of whole dead shells in the high intertidal level (at 20 m from the boulder beach) coincides with the observation that *M. biconica* and *P. australiensis* are restricted to the first 50 metres of the reef flat and are found at high densities of 2-6 individuals .m^{-1}, although occasionally at densities as high as 25 per m^{-1} (Chapter 2). Even though these predators were more abundant in this zone and that non crushing mortality was also higher, overall survival did not differ significantly from the other two intertidal levels.

CHAPTER 9

GENERAL DISCUSSION

9.1 Limitations and assumptions

The conclusions reached throughout this study are based on short term experiments, ranging from few days to one month after release, with the exception of one experiment which lasted four months. Estimating the proportion of individuals surviving to maturity or marketable size is a key factor in assessing the viability of an enhancement program. For this, longer term monitoring is necessary. Knowledge of the factors that affect survival once individuals are released into their natural habitat is essential before attempting large scale seeding (Sandt and Stoner, 1991; Stoner, 1994). One of the objectives of this study was to identify some of those factors and determine how they affect survival. The choice of short-term over long-term studies was based on two reasons:

9.1.1 Highest mortality shortly after release

There is overwhelming evidence from seeding studies on most mollusc species (abalone, conch, scallops, clams) that mortality is greatest immediately after the release (Schiel, 1992; 1993; Hatcher et al., 1993; Stoner and Davis, 1994; Hatcher et al., 1996). Results for *Trochus* show the same pattern (Hoffschir et al., 1989; Amos, 1991; Kubo, 1991; 1992). This suggests that if a particular factor is shown to have an effect on juvenile survival immediately after release, such effect would be carried over on the longer term. Results from studies where different experimental treatments were censused more than once, generally show that those treatments with initial higher survival also have higher survival in the later censuses (Amos, 1991; Stoner and Davis, 1994). Thus, conditions that enhance survival immediately after release will most likely enhance survival in the longer term.

An assumption of short-term studies is that an effect of the factor (examined) will be evident within the first days. An effect of trochus size on survival was evident within the first day of release (Chapter 8). An effect of depth was evident at Moso Is. within the first two days (Chapter 6). The absence of an effect of large scale habitat characteristics (i.e. intertidal level) at Orpheus Is. was observed within the first days and also in the longer term (see Table 8.11, Chapter 8). This absence of an effect could be the result of low statistical power: in other words, biological differences too small to be detected as significant with the experimental design (Cohen, 1988). Variability in pattern of survival with intertidal level differed between experiments: for example, in Release Exp. 4, the high intertidal level had the highest survival, whereas in Tether Exps. 1 and 2 the low level had the highest survival (Chapters 6 and 8). Similar inconsistencies were observed in the experiments with small trochus (Release Exps. 2 and 3, Chapter 8). If the absence of an effect had been solely the consequence of low statistical power, a more consistent pattern of survival with intertidal level would be expected. I interpreted these results as indicative of the greater importance of small scale habitat characteristics. These were evident within the first few days (Chapter 8).

The lack of an effect in the short-term does not necessarily mean the same in the longer term. This may have been the case for seeding density. Both experiments examining seeding density (Chapter 7) found no effect on survival within the first 2-3 days. Nor was an effect on survival apparent in the longer term, but an effect on growth rate was detected. There was also a suggestion in the results of a trend for greater survival at low density. The density difference between treatments was four to sixfold (5 vs 20-30 trochus.m^{-2}). A greater difference could have resulted in a greater effect. Furthermore, these juveniles were released in small patches spread apart by 3-20 m. The way trochus are spread out on the reef may be important in their survival. If the area of release had been larger and greater densities had been used, an effect of density could have been observed. I discuss this possibility in more detail below. It highlights some potential limitations of short-term studies that must be considered when interpreting results.

9.1.2 The logistics of working with juvenile *Trochus* in their reef environment.

One aspect of this, their detectability, was addressed in detail in Chapter 6 where I showed its importance in obtaining reliable estimates of survival. Censusing areas to monitor survival is very time consuming because trochus juveniles are cryptic and the reef substrata is highly heterogeneous. Determining whether a factor has an effect on survival or not is best done by a proper experimental design and associated statistical tests. Pilot experiments indicated that recapture rates were highly variable and this was evident in all experiments described in Chapters 6,7 and 8. Thus, it was important to increase the number of replicate units that could be assigned to an experimental treatment. Because the work had to be done at low tide (wave exposure made diving impossible except on unusually calm days), it was only possible to work on the reef flat 4-7 days every month. One way to increase the number of replicates was to reduce their size and consequently reducing the number of individuals per replicate. This approach proved useful but only for short term studies due to the rapid decline in recapture rate. Rates of decline as measured in my experiments have been reported by other authors. For example, survival of 20mm trochus ranged between 76 - 98% after 4 days and between 39 - 72% after 12 days in Vanuatu (Amos, 1991). Kubo (1992) reported estimates of survival between 55 - 76% for 32 mm trochus and 34 - 47% for 20 mm trochus, after one day of release. However, as the number of individuals recaptured drops statistical tests quickly loose their power and experiments become inconclusive (Toft and Shea, 1983; Fairweather, 1991).

9.2 Applications of the results

9.2.1 Ways of increasing trochus survival

This study shows that it is possible to enhance survival of released trochus. The two most conclusive ways are: 1) to release large rather than small trochus (> 20 mm SW), and 2) provided the area looks favourable, to spread them over a large (hundreds of metres) rather than a small (metres to tens of metres) area of the intertidal reef flat. With respect to the third factor examined, seeding density, its effect on growth rate (i.e. lower at greater densities) indicates that it is probably better to release trochus at low ($5.m^{-2}$) rather than at high ($20\text{-}30.m^{-2}$) density.

It must be stressed that most of my experiments were conducted at two localities and they may or may not be generally representative of the processes that affect cultured trochus after release. Comparison of the results between Orpheus and Moso Is. are limited to two of the three factors examined in the field: seeding density and intertidal level. The results with respect to trochus survival and seeding density were the same in the two study sites, in the short and in the long term. The results concerning intertidal level were different: at Orpheus Is. this had no effect, whereas an effect was found at Moso Is. The recommendation of releasing trochus over large rather than small areas is still valid, because the risk of choosing inappropriate areas is reduced. There is the need for a better understanding of what is a favourable habitat for juvenile *Trochus*.

I did not experiment directly on how the spatial distribution of trochus at release may affect their survival. Predation patterns in field experiments (Chapter 8) and laboratory studies of foraging behaviour (Chapter 4) suggest that this may be an important factor. Some possible scenarios for releasing trochus are: 1) Uniform distribution (over tens of metres), 2) Patchy distribution (at low or high density, and 3) release in one large (tens of metres) clump at high density. I will use the results of this study to make some predictions on possible consequences on trochus survival.

1) **Uniform distribution**

An uniform distribution over a large area means that trochus will be spread over most of the predators' foraging areas and the probability of being encountered by a predator would be higher than, for example, if trochus occupy only a fraction of the area (i.e. patch). Based on laboratory studies, low density should be preferred over high density to minimise the possibility of predators increasing their predation rate at increased densities. As density increases to very high levels, it would become a clumpy distribution, which is discussed below. Observations on the natural distribution of trochus on the reef flat (Chapter 2) indicated that (a) trochus were over most of the reef flat, but not randomly spread: they formed groups of 2-4 individuals more frequently than expected by random; and (b) they were at low density. Results from field releases (Chapter 8) indicate that survival is greater in some areas. Both results suggest that small scale differences in habitat are important. With time, an uniform distribution would likely become patchier as areas of higher mortality get depleted.

2) **Patchy distribution**

There are two important aspects of a patchy distribution, the size of the patches and the distance between them. The probability that a patch will be visited is most likely related to patch size (the smaller the patch, the lower the probability of a visit) and to the distance from a predator's refuge. Auster and Malatesta (1991) found that clam mortality increased significantly as patch size increased, and that clams released two metres from a crab's refuge had significantly higher mortality than those released at four and six metres. A field example of one of these scenarios is the tethering experiment described in Chapter 8 where four trochus were tethered in each site and sites were separated by five metres (i.e. low density and small patches spread apart). As predicted from the hypothesis that predator's distribution is largely determined by refuge availability, mortality was highly variable among sites. Furthermore, of 80 cases where the number of trochus in a site decreased from one day to the next, 78% (62) were represented by the loss of only one individual. This indicates that, after a successful encounter with a trochus, the predator did not remain long enough to encounter another one.

With respect to whether to release trochus at low or high density, results from predation experiments with *Thalamita* spp. in raceways (Chapter 4) predict that predation by these predators would be directly proportional to density, so in principle it would make no difference. However, predation rates of *T. stimpsoni* and *T. admete* have not been measured in the field and it is possible that those measured in the laboratory were affected by the high prey density in the raceway, i.e. measured predation rates were higher than on the reef flat where such high densities would not normally be encountered. In this case, it would be better to use low densities. In an experiment where trochus were released in patches of 5 (low density) or 30 (high density) individuals.m^{-2} separated by 3-4 m and spread systematically within a 10x10m plot (plots were assigned to either low or high density), density had no significant effect on the proportion of trochus surviving three days after the release (Chapter 7, Release Exp. 1). No effect was detected (statistically) after 30 days but the difference between treatments increased with time towards better survival at low density. Longer term patch experiments with bivalves found that survival was significantly higher at lower densities (e.g. Auster and Malatesta, 1991, in one week; Boulding and Hay, 1984, in six months). The possibility that the effect of density increases over time should not be dismissed, in which case the option of low density would be the preferred.

3) **All trochus released clumped in one area.**

This is the method of release that requires least work, but logic and experimental results suggest that it is also likely to be the least appropriate. The potential detrimental effects of high prey density and small area of spread, mentioned above, would apply. In an experiment where 2,000 juveniles 8 mm SW were released in a 2x2m plot, survival was estimated as 43% after two days and 5% after four and a half months (Kubo, 1988). Two similar experiments conducted in other areas yielded 46-54% survival after 56 days for a group of larger trochus 17mm SW, and 13-23% after 19 days for a group of 8 mm SW (Kubo, 1988). These are examples of releasing trochus in high density clumps; however, since this was the only method used, no comparison was possible.

Based on the previous discussion, releasing trochus in small patches spread apart and at low density may yield the highest survival. This needs testing, and the best approach to test these predictions would be to release trochus under all these conditions in one large experiment.

9.2.2. Mortality rates and feasibility of seeding

The number of individuals surviving after a period of time provide estimates of instantaneous mortality rates (M) that can then be used to make predictions.

I used results from free releases and tethering field experiments. Tethering is generally used to compare mortality rates between experimental treatments (e.g. Shulman, 1985; Heck and Wilson, 1987; Eggleston et al., 1990; Barbeau et al., 1994; Ray et al., 1994; Stoner and Davis, 1994). This was the approach used in Chapter 8. It is generally stated that the technique provides relative rates of mortality among experimental treatments rather than absolute rates because tethered individuals may be more susceptible to predation than free living ones, in which case the technique could lead to overestimates of mortality rates (e.g. Heck and Thomman, 1981; Ray et al., 1994). Peterson and Black (1990) pointed out potential artefacts of the technique. One of these artefacts, different effect depending on predator type, was demonstrated in the work of Barbeau and Scheibling (1994). Barbeau and Scheibling (1994) found that tethering increased the probability of scallops being eaten by starfishes, but not by crabs. Scallops respond to attacks by slow moving predators such as starfishes by swimming (Peterson et al., 1982).

Tether experiments with juvenile *Trochus* may produce reliable estimates of loss rates. It is unlikely that tethering could have affected antipredator responses of *Trochus*. *Trochus* are crawlers and are too slow to escape predation by mobile predators such as crustaceans and fishes. I never observed an individual moving rapidly or leaving their patch of rubble in experiments with crabs. Their response to carnivorous gastropods such as *Thais tuberosa* and *Morula fiscella* was to secrete a white mucus but not to escape by flight (Chapter 5). Tethered trochus had a certain degree of mobility (0.5 m thread) and were on many occasions well hidden and difficult to spot in spite of the tether. It is possible that some visual predators could learn of an association between rods, threads and potential prey. I tried to minimise the probability of this occurring by using tethering only for a period of days. If such an effect was present, one would predict an increase in predation rate with time (as predators learn). Such increase was not observed (Chapter 8).

Whether predation rates of tethered and untethered trochus were the same was not tested in the field. This would be a difficult experiment which would require the use of enclosures, which themselves introduce potential artefacts. Marshall (1992) found that predation rate of tethered conch *Strombus gigas*, another slow moving gastropod, was not significantly different from that of free conch. My results suggest that estimates of survival from tethering experiments were not underestimated. Most obvious is the great similarity in estimates of survival in free releases vs. tether experiments conducted simultaneously (Table 9.1). If predation had been artificially induced one would predict survival to be lower in tether experiments. Obviously, free trochus had the opportunity to disperse whereas tethered trochus did not. For this reason the area of search was correspondingly increased in free releases. Furthermore, dispersion of trochus shortly after release was low at Orpheus Is. (see discussion in Chapter 7).

M was estimated using the basic equation for population change:

$$N_t = N_0 \, . \, e^{-M.t}$$

where N_0 = number of trochus released
N_t = number of trochus estimated alive at time t
t = time since released in days

Table 9.2 shows estimates of M from all field experiments conducted throughout the study. In calculating these values I used the number of individuals recorded alive; those unaccounted for were not included. This is likely to result in some overestimation of mortality rate and resulting predictions are conservative.

M decreases with time, a decrease which is not evident within days but on a longer time scale. Between 2-3 days and 30-40 days mortality rate decreased by a factor of 2-4 (Free release 1,4 and 5, Table 9.2). It decreased a further four times between 30 and 111 days (Free release 1, Table 9.2). Results of experiments where trochus were followed for several consecutive days, suggest that M does not decrease in a period of days (Free release 3, Tether 2, Table 9.2), although in one experiment M decreased from day one to day three (Free release 2). This indicates that mortality is highest at the moment of release and emphasises the importance of concentrating efforts to improve survival at that time.

Estimated M values are very high and although there are no data on natural M values for juvenile *Trochus*. Natural mortality rates must be much lower for populations to persist. Natural mortality rates of very small abalone, from settlement to 6 months, have been estimated as 3.5 - 4.6 for *Haliotis laevigata* and 6.6 - 10.2 year^{-1} for *H. rubra* (reviewed by Shepherd and Breen, 1992) (notice that units of M in Table 9.2 are days^{-1}). These values rapidly decrease as abalone grow. M values decreased to a mean of 0.64 for *H. laevigata* six months to two and a half years old (see review by Shepherd and Breen, 1992). Available data suggest that hatchery-reared individuals have a lower probability of surviving than wild individuals when released under the same conditions (Saito, 1984; Schiel, 1992; Stoner and Davis, 1994). Wild juvenile abalone *H. iris* had 40-72% greater survival than hatchery-reared abalone in the first 1-2 months after release (Schiel, 1992). Survival of wild juvenile conch *S. gigas* was 35-50% after two months compared to 20% for hatchery-reared conch (Stoner and Davis, 1994). Behavioural differences between wild and cultured individuals (Schiel and Welden, 1987; Stoner and Davis, 1994) and poorer quality of cultured seed (Schiel, 1992) are possible causes for the observed differences. Adding handling stress to these (Shield, 1993), it is not surprising that mortality rates from seed transplants are not representative of wild populations (Shepherd and Breen, 1992).

Also evident from Table 9.2 is that M values for small individuals were the highest. The initial mortality of trochus 4-12 mm is so high that their use in transplants is questionable. For example, based on the lowest M value for small trochus (0.233), of 10,000 individuals released only nine individuals are predicted to survive after one month. Although mortality possibly due to naivety would decrease with time of exposure to natural conditions, their small size means that they will remain susceptible to many predators for a longer period than large trochus.

The lowest short term estimate of M was 0.038, obtained for individuals 23 mm SW released in groups of four every five metres (Tether Exp. 1, Table 9.2). If we assume a constant M for the first month after release (a conservative assumption), a release of 10,000 trochus would yield 3,125 after one month (31% survival). If M decreases by a factor of two after one month, survival in the next month would be 17.5% (1,747 trochus), a decrease of only 13.5% compared to 70% in the first month. These values serve to illustrate that trochus enhancement may prove to be beneficial for population enhancement, but for this to be achieved, it is essential to start with the best possible survival. The results of this study showed how trochus size, seeding density and depth on the intertidal reef flat variously affect survival, and they suggest ways to improve it. They also suggest that the distribution of released trochus on the reef, an area that was not experimentally examined in this study, may affect their survival. Research in this area could provide further information on how to enhance survival of trochus shortly after their release.

Table 9.1 Results of free release and corresponding tether experiment, two days after trochus release on the reef flat. Estimates are expressed as % of the total population released after two days of the release. Survival estimates in release experiments have been corrected for sighting probability and mortality estimates are based on recovery of dead shells and fragments.

	RELEASE	TETHER
Orpheus Is.		
Survival	76	73
Mortality	8	19
Unaccounted	16	8
Moso Is.		
Survival	66	82
Mortality	3	15
Unaccounted	31	3

Table 9.2 Estimates of instantaneous mortality rate M (day^{-1}) for released cultured juvenile *Trochus niloticus* from field experiments described in previous chapters.

EXPERIMENT	MEAN SIZE	t (days)	M	Source
Free release 1	S, M, L (13-44mm)	3	- 0.224	Ch. 7
		30	- 0.055	
		111	- 0.013	
Free release 2	Small (4-8 mm)	1	- 0.500	Ch. 8
		3	- 0.233	
Free release 3	Small (4-8 mm)	a) 1	- 0.575	Ch. 8
		2	- 0.519	
		b) 1	- 0.740	
Free release 4	M (23 mm)	2	- 0.138	Ch. 6
		30	- 0.081	
Free release 5 (Moso Is.)	Large (30 mm)	2	- 0.183	Ch. 6,7
		40	-.0.070	
Tether 1	Small (12 mm)	8	- 0.224	Ch. 8
	Medium (23 mm)	8	- 0.038	
Tether 2	Medium (20 mm)	1	- 0.224	Ch. 8
		2	- 0.266	
		3	- 0.189	
		4	- 0.177	
	Large (27 mm)	1	- 0.096	
		2	- 0.112	
		3	- 0.096	
		4	- 0.095	

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APPENDIX 1.

Manuscripts derived from this thesis accepted for publication.

Castell, Laura L. and Sweatman, Hugh P.A. (In press). Predator - prey interactions among some intertidal gastropods on the Great Barrier Reef. **Journal of Zoology**.

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Abstract

On an intertidal reef flat at Orpheus Is. on the Great Barrier Reef, the gastropods *Turbo brunneus* and juvenile *Trochus niloticus* share the same habitat with the predatory gastropod *Thais tuberosa*. To determine if the two prey species differed in their antipredator behaviour and interactions with the predator, we examined: (1) the distributional pattern of the three species in the field; (2) the proportion of mortality attributable to non-crushing predators (i.e. *Thais tuberosa*) in *T. brunneus* and *T. niloticus*, determined by the frequency of freshly dead and undamaged shells, and (3) the response of *T. brunneus* and *T. niloticus* to *T. tuberosa* in laboratory and field experiments. We compared the responses of hatchery-reared and wild juvenile *T. niloticus* to determine if lack of previous exposure to the predator affected the behaviour of cultured juveniles. Finally, (4) we studied prey choice by *Thais tuberosa*.

We found that (1) the field distribution of all three species showed high overlap and prey and predator were often found in close proximity; (2) the proportion of recently killed undamaged shells was 28% for *T. brunneus* and 10% for *T. niloticus*; (3) *T. brunneus* and *T. niloticus* responded very differently to the predator: *Turbo brunneus* showed a conventional flight escape response, moving nine times faster than normal when close to *T. tuberosa*. The flight response was observed in all trials with *T. brunneus* in the laboratory but only in 52% of trials in the field. In contrast, *T. niloticus* did not change speed but instead released a white mucus in the presence of the predator. Response in the field was also less intense than in the laboratory. Cultured and wild *T. niloticus* showed the same response when exposed to *T. tuberosa* although cultured juveniles were on average slightly more active than wild juveniles. Lastly, (4) *Thais tuberosa* showed a strong preference for *T. brunneus* as prey. Food value, expressed as dry flesh weight, did not explain this preference. Capture rate of the preferred species *T. brunneus* fell to zero in water containing mucus released by *T. niloticus*.

The results indicate that predation by *T. tuberosa* is more intense for *T. brunneus* than for *T. niloticus* and that a likely cause for this difference lies in the antipredator responses of the two prey species. The mucus response of *T. niloticus* appeared to be more effective for avoiding predation by *T. tuberosa* than was the flight response of *T. brunneus*.

Castell, Laura L.; Naviti, William and Nguyen, Felix. 1996. Detectability of cryptic juvenile *Trochus niloticus* Linnaeus in stock enhancement experiments. **Aquaculture**, 144: 91-101.

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Abstract

Juvenile *Trochus niloticus* are very cryptic because of their small size and the complexity of the reef surface where they live. In this study we addressed the question of how the cryptic nature of juvenile *T. niloticus* can affect survival estimates in experiments to investigate seeding as a tool to enhance overfished stocks. We compared recapture rates of cultured trochus with only a numbered tag with that of flagged trochus, having both a tag and an additional bright coloured piece of tape glued to the shell. One experiment was done at Orpheus Is. in Australia and another at Moso Is. in Vanuatu. For each experiment the areas were censused two or three days after release. At Orpheus Is, 76% of the flagged trochus were recaptured compared to 52% of the unflagged trochus. Corresponding values for Moso Is. were 68% for flagged trochus and 56% for unflagged trochus. This pattern of higher recapture of flagged trochus was highly consistent among experimental plots, supporting the hypothesis that a significant proportion of the trochus are overlooked by the observer.

Mean sighting probability of trochus after two-three days at Orpheus Is. was 0.69 for an average size of 23 mm SW and 0.81 at Moso Is. for an average juvenile size of 30 mm SW. Without appropriate correction survival rates could be underestimated by 30% at Orpheus Is. and 19% at Moso Is. As indicated by our results, sighting probability will vary with locality and size. Thus, we recommend the estimation of sighting probability of juvenile *T. niloticus* as an essential component of seeding experiments.