Field measurement of survival rates of juvenile *Acanthaster planci* (L.): techniques and preliminary results *

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Abstract

Techniques are described which enable measurement of mortality rates of post-metamorphic Acanthaster planci as small as 0.5 mm in the field. Results indicate that mortality rates at this time are significant, viz. 6.49% day^{-1} for 1-month-old starfish (mean size = 1.1 mm) decreasing to 1.24% and 0.45% day^{-1} for 4 (2.7 mm) and 7-month-old (5.5 mm) starfish respectively. Experiments involving exclusion of predators indicated that the major source of mortality was predation by epibenthic fauna. Results also show that in the presence of adequate food supply juvenile A. planci move very little. This suggests that survival will be enhanced by settling in areas where predation will be minimal.

Introduction

Population outbreaks of the crown-of-thorns starfish, *Acanthaster planci* (L.) remain the most important management issue on coral reefs in the Indo-Pacific region (Birkeland and Lucas 1990). In common with most marine invertebrates, a major barrier to understanding the population dynamics of *A. planci* is that very little is known about the juvenile stage of life and the factors that affect survival at this stage. Variability in post-settlement survivorship has been demonstrated for a number of taxa and is dependent on a number of factors including habitat type, settlement density and

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predation (Cameron and Schroeter 1980, Young and Chia 1982,1984, Luckenbach 1984, Keough 1986, Keough and Downes 1982, Rowley 1989,1990, McShane 1991).

Acanthaster planci have a 2 week planktonic period after which larvae settle and metamorphose into 5-armed starfish about 0.5 mm in diameter (Yamaguchi 1974). They live in a complex habitat of dead coral rubble (Yokochi and Ogura 1987) and feed on coralline algae. After about 4-6 months at a size of about 10 mm the starfish change their diet and begin to feed on corals and grow much more rapidly (Yamaguchi 1974). Little is known of the mortality patterns of small *A. planci*, but that they are readily consumed by crabs (Lucas 1973) and under some circumstances field populations may be greatly affected by disease (Zann *et al.* 1987).

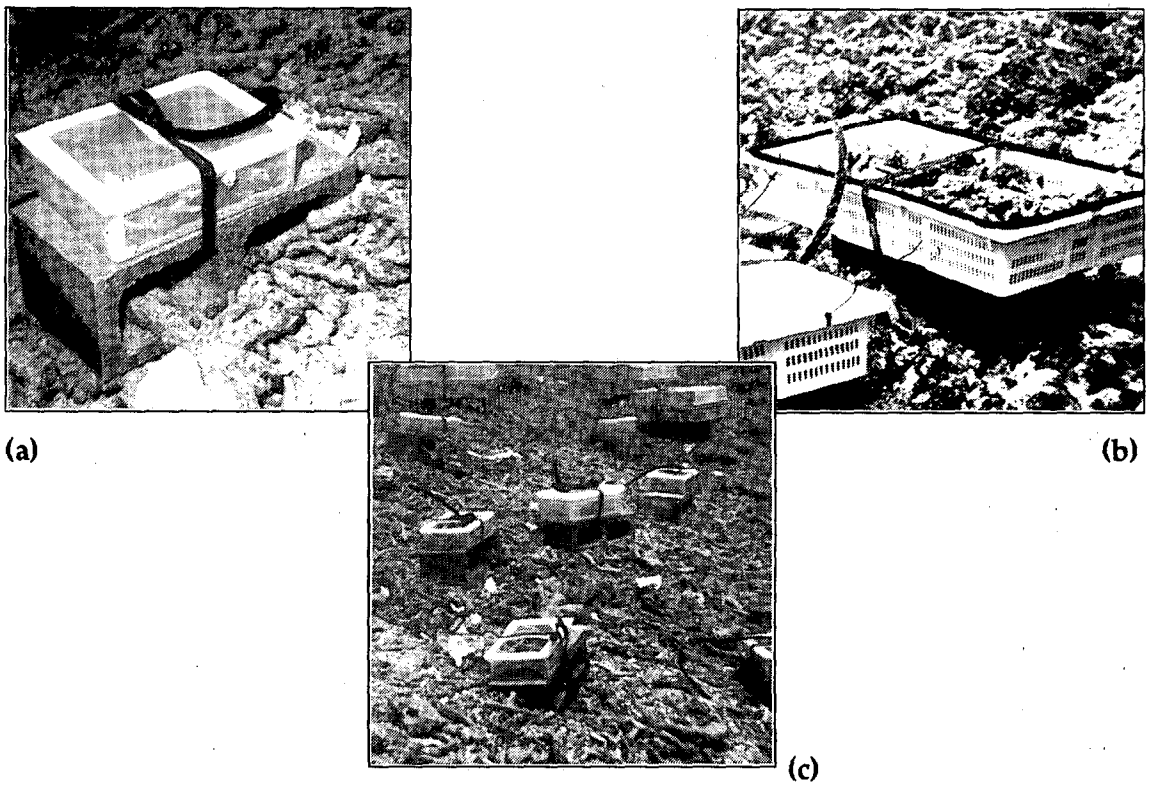
It is known that starfish outbreaks on reefs can be initiated by large recruitment events (Zann *et al.* 1987, Doherty and Davidson 1988). Some recruitment rates of *A. planci* have been measured and have been found to vary both on a temporal scale and spatially both between reefs (Doherty and Davidson 1988) and within reefs (Zann *et al.* 1987, Yokochi *et al.* 1988). However just how recruitment rates are related to rates of larval settlement is not known and whether any significant reduction in starfish numbers or some sort of regulatory control acts on starfish populations prior to them reaching a size at which recruitment can be measured with any accuracy (eg. about 20 mm or 8 months, Zann *et al.* 1987) is not known (Keesing and Halford 1992). In order to establish what levels of starfish settlement and/or recruitment on reefs are necessary to precipitate outbreak numbers of adults 3 years later, it is necessary to have an understanding of the mortality rates of small starfish and the factors which influence them.

To determine what factors are important to *A. planci* population dynamics we have developed techniques to work with juvenile *A. planci* as small as 0.5 mm in the field. These techniques enable rates of mortality to be measured under near natural conditions. The purpose of this paper is to describe these techniques and present the results of field deployment of 1, 4, 7 and 16-month-old laboratory reared *A. planci* juveniles.

Materials and Methods

Large numbers of small juvenile *A. planci* were reared in the December-January period of 1989/90 and 1990/91 using techniques similar to that described by Birkeland and Lucas (1990). Full details of the rearing operation are to be described elsewhere. Field deployment experiments were carried out in February 1991 (1 month post-settlement), May 1991 (4 and 16 months) and August 1991 (7-month-old). Starfish were deployed in the field in plastic boxes with tight fitting lids. Three sizes of commercially available plastic boxes were used. Boxes 26 cm x 18 cm x 8 cm deep were used to deploy 1 and 4-month-old starfish. Larger boxes were used to deploy 16-month-old starfish (35 cm x 25 cm x 12 cm) and 7-month-old starfish (57 cm x 32 cm x 9 cm). The lids and sides of the two smaller box types were covered in 0.2-mm mesh (*Fig. 1a*) and 0.5 mm mesh was used to cover all surfaces of the larger boxes (*Fig. 1b*). The lids of the largest of the boxes did not fit as well as those on the smaller boxes and some problems with starfish escape were subsequently encountered (see later). The boxes containing starfish were deployed on the leeward side of Davies Reef (18°50'S, 147°39'E) in about 12 - 15 m depth in an area of dead coral rubble (Fig. 1c).

The basic aim of the experiments was to compare the survival of small starfish living in their natural habitat (among dead coral coated in crustose coralline algae and other organisms, see Yokochi and Ogura 1987) with that of starfish living among a similar substrate which provided a food source and shelter, but in the absence of potential predators or other hazards. For the natural rubble treatment (hereafter TREATMENT), pieces of unconsolidated dead coral rubble complete with algal and motile epifaunal assemblages were collected directly from the seabed at the time of deployment. For the control rubble treatment (hereafter CONTROL), sun-bleached or beach-collected coral rubble was used after conditioning in flow-through-aquaria for several weeks to allow a biological coating of bacteria, diatoms, filamentous and coralline algae to grow on the rubble surface. Benthic diatoms provide an adequate food source for young juvenile starfish (Keesing and Halford, *unpublished*).



(a) (b) (c)

Figure 1: (a): Small mesh boxes (26 x 18 x 8 cm) fixed to concrete block. This size box was used to deploy 1 and 4-month-old *A. planci;* (b): Large mesh boxes (57 x 32 x 9 cm) used to deploy 7-month-old *A. planci*. Lid removed from right box to show rubble inside; (c): Dead coral rubble habitat on Davies Reef where experimental deployments were carried out. Large boxes in background are ESCAPE CONTROLS and the left most box in the foreground is a DOUBLE-MESH type box (see text). The starfish reared in the laboratory and the conditioned rubble were taken to the field in 100-litre tanks aboard a research vessel. They were then transferred to the deployment site in a small inflatable boat. Conditioned rubble was placed into the CONTROL boxes on the surface at the deployment site while rubble from the seabed at the deployment site was collected by divers and placed in the TREATMENT boxes and then brought to the surface. Batches of starfish which had been counted out previously were then washed into the CONTROL and TREATMENT boxes and the lids were fitted. The boxes were then gently lowered into the water to allow any trapped air to escape and taken to the bottom by divers where the boxes were tied to concrete blocks using rubber straps.

Initial densities varied for each experiment depending on the availability of starfish at different ages. For the first experiment, 100 of the 1-month-old starfish were stocked to each of the boxes (equivalent to 2137 m^{-2}), 35 per box at 4 months (748 m^{-2}), 20 per box at 7 months (110 m^{-2}) and 10 per box at 16 months (114 m^{-2}). Starfish sizes could not be measured prior to deployment (but see *Table 1).*

Water exchange in boxes

The mesh on the boxes was cleaned each day or every second day by lightly brushing the mesh to ensure water flow was not occluded by silt. To assess the adequacy of water flow, water samples were syringed from the boxes periodically over the course of one experiment and the dissolved oxygen content was measured using an ICI Dissolved Oxygen Meter. Water samples were also taken from CLOSED boxes (no possible water flow) and boxes within boxes (DOUBLE-MESH, *Fig. 1c*) which had been deployed with natural coral rubble. Fluorocene dye was also injected into some extra boxes to further examine the effect of the caging on water flow.

Escape controls

Ten boxes for each of the CONTROL and TREATMENT type were used for the 1, 4 and 7-month-old starfish whilst five natural rubble TREATMENT boxes only were used for the 16-month-old starfish. Two variations of the boxes were used for deployment of the 1, 7 and 16-month-old *A. planci*. Five replicate OPEN treatment boxes (the same as TREATMENT boxes but without lids) were deployed with 7-month old *A. planci*. These OPEN boxes allow free movement of starfish and predators in and out of the boxes. In order to examine movement rates of the small starfish and their propensity to escape from the OPEN boxes we used small boxes without lids inside larger boxes. These are termed ESCAPE controls and were deployed for the 1, 7 and 16-month-old starfish. Upon recovery of the boxes we counted the number of starfish that had moved out of the smaller inner box into the larger outer box.

Recovery of deployed starfish

After the boxes had been in place for 6, 13, 16 and 13 days for the 1, 4, 7 and 16-month-old starfish respectively, they were collected by divers and brought back to the research vessel where the contents were carefully washed into bottles and the entire contents fixed in buffered 10% formalin in seawater. The fixative also contained Rose-Bengal stain to make subsequent sorting of animals from rubble and debris easier. Prior to fixing, any starfish which could be recovered alive were removed, counted and measured (greatest diameter).

The fixed contents of the boxes were sorted by washing the rubble over 6, 1 and 0.1 mm mesh screens. In this way all rubble was retained in the 6 mm mesh and starfish and other epifauna were retained by the smaller meshes. Following this, each piece of rubble was individually washed over the mesh. All starfish were then recovered from the mesh screens and counted and measured. The other epifauna in the 1 mm screen were also counted and categorised with the aid of a dissecting microscope.

Controls for handling and sorting efficiency

To measure the efficiency of the recovery (sorting) technique, small starfish (N=50 for 1-month-old and N=30 for 4-month-old) were seeded into each of four TREATMENT

boxes and the contents were fixed after a few minutes and sorted as for the experimental samples. To ensure that the degree of handling of the starfish during the counting and deployment procedure did not contribute to starfish mortality, handling controls were also carried out. These consisted of 2 or 4 replicate batches of starfish, which had been subject to the same amount of handling as those placed in the boxes were kept in dishes overnight and examined the morning following the deployment of the boxes.

Analysis

The proportions of surviving starfish in the CONTROLS and TREATMENTS were compared using a t-test. Where required an *arcsin* transformation was used to correct variance structure. Mortality rate was determined to be the daily rate of loss in the TREATMENT boxes. Predation rate was determined to be the difference between the mortality rates in the TREATMENT and CONTROL boxes where a significant difference in survival was detected between the two.

Results

Starfish size

The sizes of the different age classes of starfish recovered after each experiment are indicated in *Table 1*. The mean size of starfish remaining in the TREATMENT and CONTROL boxes following recovery was determined to examine for evidence of size-dependent mortality. *Table 1* indicates that for 1 and 4-month-old *A. planci*, on which significant effects of predation were detected, that no differences in starfish size existed at the end of the deployment period (t-test, $p > 0.05$). This indicates no size- selective predation was occurring over the limited size range of starfish present. Seven-month old *A. planci* recovered from the TREATMENT boxes were significantly larger (on average 0.6 mm larger) than in the CONTROLS (t-test, $p = 0.011$). This is most likely the result of faster growth in the boxes containing natural rubble, but may indicate the larger starfish were more likely to escape (see later). Note that the 7 and 16-month-old starfish are stunted compared to what could be expected on average for starfish of that age in a natural population [eg. 21 mm (range 12-28) and 77 mm (range 34-156) respectively (Zann *et al.* 1990)]. The stunting in our laboratory reared animals is due to an absence of coral food but does not appear to affect the health of starfish for up to two years of age (Lucas 1984, Keesing and Halford 1992).

Table 1: Mean size $\pm$ 1SD of juvenile *Acanthaster planci* recovered after field experiments. The 1, 4, 7 and 16-month-old starfish were deployed for 6, 13, 16 and 13 days respectively; nd: no data.

Date deployed	*Starfish age (month)*	*Controls mean size (mm)*	*Treatments mean size (mm)*	*Size range (mm)*
Feb 1991	1 month	1.0 ± 0.1	1.1 ± 0.1	0.6 - 1.7
May 1991	4 months	2.8 ± 0.1	2.7 ± 0.2	0.7 - 4.7
Aug 1991	7 months	4.9 ± 0.4	5.5 ± 0.4	1.4 - 6.0
May 1991	16 months	nd	16.2 ± 0.3	9.0 - 23

Handling and sorting efficiency controls

No starfish in the handling controls died. The mean number of 1-month old starfish recovered from the sorting efficiency controls was 45.25 ± 1.71, n = 4, indicating a 9.5% loss. In subsequent analyses, this correction factor was added to the number of starfish recovered from each of the natural rubble TREATMENT boxes. For 4-month-old starfish, all individuals were recovered from the sorting efficiency controls, so no correction factor was necessary.

Survival rates of starfish

1-month-old starfish

After deployment in the field for 6 days (actual time 5.75 days including 5 full night periods), a mean of 62% of starfish were recovered from the TREATMENT boxes and 92% were recovered from the CONTROLS (*Table* 2). This indicates a significant rate of mortality (t-test on transformed data, p = 0.0005) equivalent to about 6.49% day^{-1} in the TREATMENT boxes compared to 1.44% in the CONTROLS. This constitutes a predation rate of 5.05% day^{-1} in the TREATMENT boxes.

Table 2: Contents of CONTROL and TREATMENT boxes 6 days after deployment at Davies Reef. Each box was deployed with 100 juvenile *A. planci* (mean size ca. 1 mm, range 0.7 - 1.6 mm) initially. Unequal sample sizes resulted from 3 of the samples being lost when the barge carrying them accidentally submerged. Units are mean numbers ± 1SD; n: no. of replicates.

Fauna recovered from rubble	*Natural rubble treatment (n = 9)*	*Aquarium rubble control (n = 8)*
small *A. planci*	62.0 ± 16.1	92.0 ± 7.8
Worms		
mostly polychaetes	70.8 ± 32.7	18.0 ± 6.3
turbellarians	0.7 ±1.3	-
Crustaceans		
crabs	1.1 ± 1.0	-
shrimps	3.2 ± 2.8	-
Galathiads	2.2 ± 1.2	-
isopods	2.7 ± 1.2	3.0 ± 2.7
others (mostly amphipods)	33.0 ± 12.9	2.2 ± 1.4
Molluscs		
gastropods	7.5 ± 7.4	-
bivalves	3.0 ± 1.4	0.5 ± 1.4
chitons	1.1 ± 0.9	-
opistobranchs	0.9 ± 0.6	-
Echinoderms		
echinoids	1.4 ± 1.3	-
ophiuroids	0.7 ± 0.7	0.6 ± 0.7
Fish	0.1 ± 0.3	-

4-month-old starfish

Starfish of this age were deployed for 13 days and after that time a mean of 85% of starfish had survived in the TREATMENT boxes compared with 95% in the CONTROLS (*Table* 3). This 10% difference was significant (t-test, $p = 0.002$) and amounts to a mortality rate of 1.24% day^{-1} in the TREATMENTS and 0.39% in the CONTROLS (predation rate=0.85% day^{1}).

Table 3: Contents of CONTROL and TREATMENT boxes 13 days after deployment at Davies Reef. Each box was deployed with 35 juvenile *A. planci* (mean size ca. 3 mm, range 1.5 - 4.5 mm) initially. Units are mean numbers ± 1SD; n: number of replicates.

Fauna recovered from rubble	*Natural rubble treatment (n = 10)*	*Aquarium rubble control (n = 10)*
small *A. planci*	30.1 ± 2.4	33.4 ± 1.7
Worms		
mostly polychaetes	47.4 ± 13.1	16.1 ± 6.9
turbellarians	0.5 ± 1.1	0.1 ± 0.3
Crustaceans		
crabs	1.8 ± 0.9	-
shrimps	4.2 ± 3.0	0.1 ± 0.3
isopods	6.9 ± 6.5	1.4 ± 1.9
others (mostly amphipods)	19.0 ± 11.6	0.1 ± 0.3
Molluscs		
gastropods	45.5 ± 13.8	-
bivalves	9.2 ± 2.3	
chitons	1.1 ± 0.9	0.1 ± 0.3
Echinoderms		
echinoids	1.0 ± 0.9	-
ophiuroids	0.9 ± 1.3	-
Fish	0.3 ± 0.5	-

7-month-old starfish

After 16 days deployment a mean of 94% of starfish were recovered from the TREATMENT boxes and 88% from the CONTROLS. This difference was not significant (t-test, $p = 0.184$). As all starfish recovered from the CONTROLS appeared healthy and no potential predators were encountered when sorting through the contents of the boxes it was feared that the majority of starfish missing from the CONTROLS had either somehow escaped between the box and the lid in response to lack of food or had not been sorted and counted efficiently (rubble from CONTROLS was not fixed in formalin and sieved prior to sorting). A trial was carried out *a posteriori* in large aquaria to examine whether starfish would escape from the boxes in the absence of food and to measure the sorting efficiency. The trial, carried out over five days, showed that while sorting efficiency was 100%, that 30% of the starfish escaped from the boxes that had no substratum and 5% escaped from the boxes with

CONTROL rubble. Thus we expect that the lower rate of recovery of starfish from the CONTROL boxes in the field experiment was due largely to escape.

The mean percentage of *A. planci* recovered from the OPEN boxes was 93%. There was no significant difference between this and the percentage recovered from the TREATMENT boxes (t-test, $p = 0.816$). These data indicate firstly, that exposing the animals to mobile predators outside the boxes did not contribute significantly to the mortality rate over the period of the deployment. Secondly, they indicate that few starfish escaped from the OPEN boxes and that leaving the lids off the boxes did not contribute significantly to the escape rate. So although escape from the TREATMENT boxes cannot be ruled out we suggest the measured mortality rate of 0.45% day^{-1} is reasonable.

16-month-old starfish

No starfish were lost from the experimental deployment of this age class over 13 days. Longer deployments using larger enclosures will be required to assess whether mortality rates of these larger starfish are high enough in field populations to be detected.

Rubble epifauna

Assessment of the motile epifauna present among the natural rubble indicated that worms, crustaceans and molluscs were common amongst the rubble (*Tables 2/3*). Polychaetes, amphipods and gastropods made up most of the mobile epifauna, although crustaceans such as crabs and shrimps were also found in the samples. It is likely that some of these animals would be predators of the small starfish. Non-mobile epifauna were not quantified but included corals, bryozoa, zoanthids, sponges and oysters.

Escape Controls

Results from the recovery of starfish from the ESCAPE controls (*Table 4*) indicate that starfish move very little initially and become more mobile as they grow. None of the 1-month-old starfish moved from the inner to the outer section of the boxes. The low rates of movement in the larger starfish suggests that in the future these can be deployed in larger OPEN boxes for longer periods which may provide greater information on predation rates.

Table 4: Proportion of juvenile *Acanthaster planci* recovered from ESCAPE control boxes after field experiments. All starfish were initially placed in the inner boxes; 50 starfish per box at age 1 month, 20 at 7 months and 10 at 16 months. Calculation of percentage escaped assumes that starfish unaccounted for died in the inner and outer boxes in proportion to the living ones recovered from those boxes; n: number of replicates.

Deployment date (days)	*Starfish age (months)*	*Inner box (X ± 1SD)*	*Outer box (X ± 1SD)*	*Percent escaped (%)*	*n*
Feb 1991 (6)	1	44.5 ± 0.7	0.0 ± 0.0	0	2
Aug 1991 (16)	7	16.6 ± 1.5	2.0 ± 1.0	11	5
May 1991 (13)	16	7.8 ± 2.2	2.2 ± 2.2	22	5

Water exchange in boxes

Fluctuations in dissolved oxygen content of water in the CLOSED box demonstrate the effects of obstructing water flow to the epibenthic community living on dead coral rubble (*Table 5*). Deprived of any water exchange, oxygen levels in the CLOSED box rose rapidly to more than double that of normal by the second day and then plummeted to only 20% of normal after 3 days. Slight elevation followed by depression of oxygen levels in the DOUBLE-MESH boxes indicates that some significant reduction in water flow was occurring. Oxygen levels in the TREATMENT boxes were unaffected over the four days examined. All visible fluorocene had cleared from the TREATMENT boxes within 10 minutes. Substantial amounts of the dye remained in the DOUBLE-MESH box after 30 minutes but had cleared completely after three hours. These results made us confident that sufficient water exchange was occurring in the TREATMENT boxes and not contributing to survival rates of starfish.

Table 5: Dissolved oxygen content of water (mean ± 1SD) sampled from TREATMENT boxes 0 to 4 days after deployment; n: number of replicates; nd: no data.

Water sampled from		*Dissolved oxygen (mg/l^{-1})*			
	n	*Day 1*	*Day 2*	*Day 3*	*Day 4*
Water column	2	6.5 ± 0.1	nd	6.6 ± 0.0	6.6 ±0.0
Interstitial water	2	6.4 ± 0.1	nd	nd	6.7 ±0.1
Mesh box	2	6.4 ± 0.1	nd	6.6 ± 0.1	6.6 ±0.1
Double mesh box	2	6.3 ± 0.2	nd	6.8 ± 0.5	5.4 ±1.1
Closed box	2	6.5 ± 0.1	14.3 ± 0.1	1.4 ± 0.7	nd

Discussion

Mortality rates

Mortality rates for *A. planci* in field populations have been measured at 99.3% between 8 and 23 months of age (1.08% day^{-1}) (Zann *et al.* 1987) and 75% between 22 and 34 months of age (0.39% day^{-1}) (Doherty and Davidson 1988). Mortality rates for younger starfish have not previously been presented and our results confirm an expected age dependent decrease in mortality, based on the assumption that caging was restricting mortality of the larger starfish in the boxes. The high rates of mortality in 1-month-old starfish found in this study also indicate that mortality levels following settlement are very high and thus have the potential to influence the population dynamics of *A. planci*.

All measured rates of mortality for *A. planci* (ie. Zann *et al.* 1987, Doherty and Davidson 1988, this study) have come from populations with very high (ie. outbreak) densities. If mortality rates are density dependent then these rates may be lower than might be expected in low density populations. McCallum *et al.* (1989) have discussed how predators with a *Type II* functional response (*sensu* Holling 1959) could greatly affect population dynamics of *A. planci* at low densities. On the other hand if predators exhibit a *Type III* functional response (Ormond *et al.* 1990) then predation rates at low density will be disproportionately lower than at high density. Now that the techniques described here have been tested it is planned to repeat the experiments

described here examining the influence of starfish density on mortality rates. Density dependent mortality in newly settled non-sessile marine invertebrates (abalone) has been demonstrated by McShane (1991). He suggested that intraspecific competition for favourable living sites resulted in higher levels of predation and dislodgment by strong water movement at high densities.

A. planci have been observed in the laboratory to have strong preferences to settle on particular substrates, perhaps induced by some chemical cue provided by bacteria (Johnson *et al.* 1991). Just how important these settling preferences are in determining post-settlement survival rates is not known. However juvenile starfish in our experiments moved very little suggesting that settlement in areas where predators are few would enhance survival rates. This type of behaviour is known to enhance survival in echinoids (Highsmith 1982) and ascidians (Young 1989). However preferential settlement in areas of favourable food supply would also enhance survival as suitable food availability is known to greatly affect growth rates (Lucas 1984, Keesing and Halford 1992).

The only other published information on mortality rates of newly settled echinoderms in the field is that of Rowley (1990) who recorded change in density of newly settled sea urchins in two habitats and found that mortality over the first 40 days or so was 5.59% day^{-1} in kelp beds and 3.10% day^{-1} outside kelp beds. Such habitat differences may also be important for survival rates of *A. planci*. Yokochi *et al.* (1988) recorded recruitment rates of 0.82 m^{-2} on the slope and 0.06 m^{-2} on the reef flat of the same reef. Zann *et al.* (1987) also found within reef differences in recruitment rates. It is not known whether these differences result from differential levels of settlement or post-settlement mortality or a combination of both. Black and Moran (1991) have demonstrated how small scale hydrodynamics may influence settlement density of *A. planci* within reefs. To determine whether the most significant influences on *A. planci* population dynamics take place pre- or post-settlement, studies which seek to isolate settlement rates from recruitment rates will be vital.

Techniques

Whilst other studies have monitored the survival rates of newly settled marine invertebrates (eg. Rowley 1990, Luckenbach 1984) and carried out laboratory experiments on survival rates of reared juveniles (eg. Highsmith 1982), ours is the first study to our knowledge undertaking field deployment of large numbers of juveniles reared in the laboratory. This approach has been necessary because, despite large numbers of outbreaks of adults, juveniles are rarely encountered in the field (Johnson 1992) and certainly not in numbers required for manipulative ecological experimentation.

This study has demonstrated that the techniques outlined allow measurement of mortality rates of small non-sessile benthic animals in the field allowing for partitioning the effects of predator and non-predator induced mortality. The low rates of movement detected for the starfish suggests that in the future they can be deployed in OPEN boxes for long periods will provide greater information on predation rates and overcome the problem of artificially excluding the larger mobile predators such as fish which occurs in the boxes with lids.