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AN INVESTIGATION INTO WHAT CONSTITUTES LETHAL WOUNDING IN *ACANTHASTER PLANCI* AND AN ASSESSMENT OF THE EFFECTS OF REGENERATION ON GONAD PRODUCTION.

Thesis submitted by

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in December 1994

for the research Degree of Master of Biology
in the Department of Marine Biology
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Abstract

The population outbreaks of the corallivorous asteroid *Acanthaster planci* have been of great interest to scientists, reef managers and the public for the last 25 years. As of yet, the causes of these outbreaks can only be surmised. A suite of hypotheses put forward to explain these outbreaks concerns the effect of predators on *A. planci* populations. One of these hypotheses suggests that predators, which have been reduced in number, previously controlled the starfish populations through mortal or sublethal predation.

This study focuses on what constitutes mortal damage to *A. planci* and, if damage is sublethal, whether subsequent healing and regeneration reduce gonad production.

Starfish in the population on Davies Reef, central Great Barrier Reef, were collected and subjected to a series of damage treatments (which included severed arms and bi- or trisection of the oral disc), then placed in cages to examine short term survival. Four separate experiments took place during 1991-1992, including both winter and summer. Some starfish were starved prior to the damage treatments. Additional small scale experiments were conducted to assess whether sex or density of the starfish in cages affected survival after damage.

Logistic regression models were produced to describe the variation in survival through the various levels of treatment. The level of damage consistently producing 100% mortality could not be determined. Mortality was highly variable among experiments and occurred in all treatments including controls. Increased damage generally resulted in increased proportions of starfish mortality, though this effect was reduced to differences between damaged and undamaged starfish when experiments were combined. Starvation was found to affect survival inconsistently. No seasonal effects on survival were detected. There were no significant effects of either density or sex on survival of treated starfish in cages. Size related effects could not be assessed due to insufficient numbers of significantly different sized starfish.

To assess the possible influences of regeneration on gonad production, starfish were collected from Davies Reef and adjacent Lynchs Reef. These starfish were examined for regeneration or damage. Their gonads were dissected out and weighed.

Wet gonad weight versus somatic weight was examined by regression analysis. The results for males and females differed. Total gonad weights were significantly reduced in regenerating males, showing that regeneration affects gonad production in males. Regenerating females, by contrast, did not show a reduction in total gonad weight.

When individual arms of the same sample of starfish were considered, regression models indicated a significant difference in gonad weight between regenerating arms and non-regenerating arms in males, but not in females. Relationships between the lengths of the regenerating arms and gonad weights within those arms were found in both sexes. The gonad weights of the arms adjacent to those regenerating were not detectably influenced by the regenerating arms in either sex. Short arms (arms not showing regeneration but obviously different in length) did not show reduced gonad weight when compared to normal length arms.

Gonad weights in lateral halves of arms were also compared. No effects of regeneration on gonad weight could be detected in the arms damaged nor in the normal arms adjacent to those damaged, for either sex. The gonad weights at this level were quite small and the variability was great.

The results of these studies provide important information about *Acanthaster planci*. It is clear that *A. planci* are susceptible to damage and that damage may result in either reduced gonad production or mortality. However, the variability in the results suggest that *A. planci* has a complex, perhaps plastic, life history which is influenced by many factors.

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Chapter 1

Hypotheses for the Causes of *Acanthaster planci* Outbreaks and Introduction to Experiments

Many hypotheses have been put forward to explain the recent outbreaks of the corallivorous asteroid *Acanthaster planci* (L.). Historically, these hypotheses can be divided into two distinct categories: natural causes and human related causes. These categories are now considered to be inter-related in many ways.

One of the earlier hypotheses describing natural causes for *A. planci* outbreaks was put forward by Dana *et al.* (1972). This hypothesis has been termed the Adult Aggregation Hypothesis. It suggests that adult starfish aggregate for a number of reasons (eg. limited areas of food). As a result of their close proximity, fertilisation becomes increasingly successful and the number of recruits increases. Eventually, the aggregations of starfish reach outbreak proportions. Originally it was proposed that natural disturbances to coral populations such as cyclones (Dana *et al.*, 1972) may cause aggregation. El Nino events (Glynn, 1985) and larval retention over small spatial scales (Moran, 1988) have more recently been suggested as potential causes of aggregation. This hypothesis is supported by studies that show the tendency of *A. planci* to aggregate (Vine, 1971) and showing that these aggregations are capable of remarkable fertilisation success (Babcock and Mundy, 1992). However, historical support for gradual increases in populations leading to an outbreak is lacking (Birkeland and Lucas, 1990).

Moore (1978) proposed that, given its large reproductive effort and fast growth, *A. planci* was naturally an r-strategist and that marked population fluctuations were normal. In support, both short term (Flanigan and Lamberts, 1981; Birkeland, 1981) and long term (Henderson and Walbran, 1992; Frankel, 1978) historical and geological evidence has been put forward to show that outbreaks have been occurring in *A. planci* populations for thousands of years. More recently, the geological evidence has been disputed (Keesing *et al*, 1992; Pandolfi, 1992; Fabricius and Fabricius, 1992).

Given the long life, large size, and iteroparous nature of this starfish (Birkeland and Lucas, 1990; Cameron and Endean, 1982), *A. planci* could equally be called a K-strategist. Wild fluctuations in population size (i.e. outbreaks) are not normal for this type of life history.

The Larval Recruitment Hypothesis is a more recent hypothesis involving natural causes. It was proposed by Lucas (1975) and Pearson (1975) and suggests that times of lowered salinity, high water temperature, and high organic content favour larval survival and therefore affect eventual recruitment. More recently (Birkeland, 1982), it was proposed that runoff from high islands and continental land masses provides nutrients that favour phytoplankton blooms, which in turn, favour zooplankton survival. This type of hypothesis has been supported by data which show that outbreaks occur more frequently near high island reefs and continental reefs, as opposed to isolated reefs or coral atolls (Zann, 1992).

There are two main hypotheses implicating humans as a cause of *A. planci* outbreaks. Pollution has long been considered as a possible cause of outbreaks of *A. planci*. Some of the earlier papers (Fischer, 1969; Randall, 1972) suggested that pollution killed predators of *A. planci*. Current thought, however, is that runoff contains elevated levels of nutrients which promote phytoplankton blooms, which in turn promote the survival of *Acanthaster* larvae (Brodie, 1992). As mentioned previously, these types of hypotheses have been supported by the incidence of outbreaks on high island reefs and continental reefs (Zann, 1992) where runoff is likely to influence the nearshore waters. In addition, research by Ayukai (1992) has suggested that natural levels of nutrients are probably limiting and that increases of nutrients may improve larval survival. There is, however, conflicting evidence (Brodie, 1992; Okaji, 1992; Olson, 1987) as to whether natural populations of *A. planci* larvae are food limited and so greater phytoplankton levels may not make a difference.

The second hypothesis indicating a human cause for *A.*

planci outbreaks is the "Overfishing Hypothesis". This hypothesis was proposed by Endean (1969) who suggested that over-collecting of the Pacific Triton (*Charonia tritonis*), an *A. planci* predator, has enabled *A. planci* populations to increase uncontrolled. The hypothesis has since been developed to incorporate overfishing of commercially exploited fish predators (Ormond *et al.*, 1990; McCallum *et al.*, 1989), though there is still little evidence in support of this.

That predation actually occurs on *A. planci* is not a matter of debate. There are many research papers, summarised by Birkeland and Lucas (1990), McCallum *et al.* (1989), Moran (1988) and Keesing and Halford (1992), which document feeding on adult *A. planci* by various organisms, including fish. A survey of divers by Marine Bio Logic (1990) documented 46 anecdotal sightings of fish predation on *A. planci*. Furthermore, McCallum *et al.* (1989) have found that up to ten arms on any starfish are damaged and up to 60% of the adult population have parts being regenerated. While it can be concluded that partial predation on adult *A. planci* is a common occurrence, how this predation effects the starfish population dynamics needs investigation.

There are basically two ways a predator can influence the numbers of adult *A. planci* on the Great Barrier Reef. The first way is through mortal predation. This may result from being completely ingested during the initial attack. Alternatively, mortality may occur indirectly as a result of partial predation with subsequent mortality resulting from infection or inability to regenerate sufficiently to function. McCallum (1988) proposed that a mortal predation rate of 0.015 per starfish per day would be sufficient to prevent outbreaks. However, although this rate of general mortality (i.e. not just predation) has been seen in juveniles (Zann *et al.*, 1987), an outbreak was not prevented. Such rates of predation have not been reported in adults.

The second way in which numbers of *A. planci* on a reef may be affected by predators is through sub-lethal predation, whereby subsequent healing and regenerative growth reduce an individual's allocation of energy to gonad production (Harris, 1989). This in turn may reduce the number of fertilized eggs which eventually develop into adults. The degree to which such partial predation may affect the fecundity of an individual is based on the regenerative process and how this interacts with the metabolism and life history of the injured individual.

This thesis will assess how partial predation affects *A. planci* populations. In particular, the experiments aim to determine: 1) how much damage *A. planci* can sustain and survive, and; 2) if *A. planci* survives, is its sexual reproduction reduced due to the cost of healing and regeneration? The study is very relevant to the importance of the predator "Overfishing Hypothesis" as a potential mechanism for initiating *A. planci* population outbreaks.

Chapter 2

Experiments Simulating Partial Predation

2.1 Introduction

Very little is known about what it takes to mortally wound an *A. planci*. *A. planci* survives small amounts of damage, such as lost arms, quite easily (Birkeland and Lucas, 1990). Research on survival after large amounts of damage has so far been relatively inconclusive. Indications are that *A. planci* is not as resilient as some other asteroids, such as *Linckia spp.*, which are able to survive and regenerate from small pieces (Lawrence, 1987). Data from *A. planci* mutilation studies are mostly anecdotal: Pearson and Endean (1969) cut several adult specimens of *A. planci* in half and both halves died. Research in the Red Sea, which suggested that any damage to the body disc of *A. planci* resulted in its death (Roads, 1973), supports the results found by Pearson and Endean. Owens (1971), however, cut one adult *A. planci* in half and both halves survived. Finally, in Okinawa, ninety-two *A. planci* were cut into quarters. One month later, two of the quarters were found still surviving (Birkeland and Lucas, 1990). Overall, it appears that *A. planci* can survive extensive damage but the relationship between mortality and the extent of damage is inconsistent.

The following experiments examined how much damage an *A. planci* can sustain without dying. They were completed systematically with uniform sample sizes that were large enough to detect reasonable differences in survival, given the likely inter-individual variation. The experiments were also replicated both in winter and summer to test for seasonal variation, with additional considerations of starvation, sex, and density of starfish in the experimental cages.

These experiments should provide useful information to reef managers, such as the Great Barrier Reef Marine Park Authority, about the resilience of starfish to damage. In particular, it was sought to understand what damage is required to kill *A. planci* reliably. This information should give an indication of the types of predators that are capable of inflicting mortal damage to *A. planci*. In addition, this information should provide some indication of the degree of mutilation required for outbreak control.

2.2 Methods

2.2.1 Collection of *A. planci*

A. planci were collected by snorkellers from Davies Reef, about 100 kilometres east north-east of Townsville (Lat. 18°50′, Long. 147°40′) on the Great Barrier Reef. To avoid handling the starfish and possible contact with toxic spines, the starfish were dislodged from the bottom using a perspex right angled stick. They were then taken to the surface to a waiting rubber dinghy where they were placed in plastic 60 litre bins full of sea water. Over the course of collection the water was frequently replenished.

The length of time needed to collect the starfish depended on how many starfish were present at the collection site. The times varied from one to three hours. Up to 30 starfish were collected before returning to the main vessel. There the starfish were placed in a 1000 litre tank with running sea water.

Unless otherwise stated, all starfish in this study were treated in a similar manner prior to processing.

2.2.2 Damage Treatments

The process of treating a starfish for damage involved placing the starfish on a flat surface out of the water and inflicting one of the following treatments with a dive knife:

Control - control for handling effects. Starfish taken out of water, placed on a flat surface and then put back into the water with no damage deliberately inflicted. The time spent out of the water was approximately the same time as required for the other damage treatments (approximately 30 seconds). This treatment is labelled as "Ctrl" in many places in this thesis.

-2 arms - two adjacent arms cut off along the edge of the disc, always with the inter-arm septa present. This level of damage was not used after the first experiment.

-4 arms - same as for above, except with 4 arms.

-8 arms - same as above except with eight arms. No consideration of arm number was made so the remaining number of arms was variable.

-1/2 - arms counted and starfish cut in half with equal numbers of arms on either side. Only one half was used. If there were unequal numbers of arms, the chosen half was random. This category was subsequently combined with the "-2/3" category in analyses.

-2/3 - arms counted and rounded to the nearest number divisible by three. A third constituted a pie section with one third of the number of arms. This category was used only after the pilot study and was merged with the "-1/2" category in analyses.

These damage treatments were chosen after considering data produced by McCallum *et al* (1989) and Kettle (unpublished) which showed that few starfish in natural populations had more than 8 arms missing (Figure 2.1).

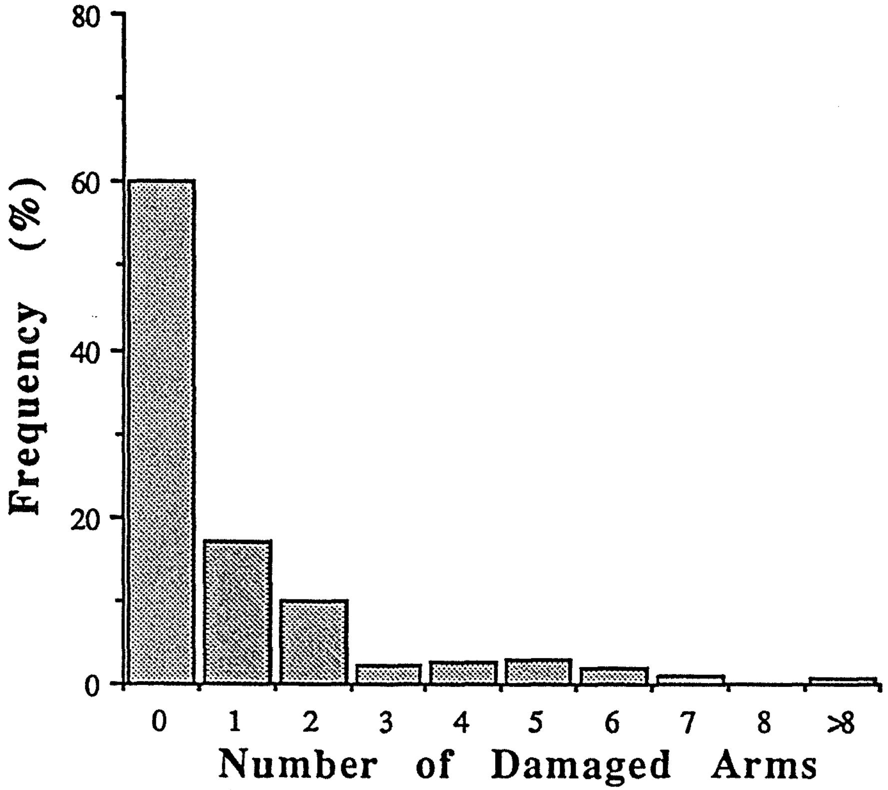
The starfish were treated in groups of five, with one individual allocated randomly to each treatment. Previous damage to the starfish was assumed to be irrelevant to survival and arms shortened by damage were as equally likely to be removed as normal length arms. Generally, 25 to 50 starfish were processed at any one time and these were placed in 60 litre bins to be transported into one of the cages. The time from removal from the 1000L tank to placement in the cage varied from 10 to 30 minutes. Left-over portions of the starfish were released or were used in feeding experiments, which were not part of this thesis.

2.2.3 Sample Size

The numbers of starfish required for these experiments were determined by power analysis. The method used is described in Cohen (1988) for comparing two proportions. The particular method chosen was not directly applicable to the data to be collected, which contained several proportions, but was used purely as an indicator of the numbers needed to detect a 20% change with 80% confidence with only a 0.05 chance of making a Type I error (i.e. that a difference is detected when there actually is no difference).

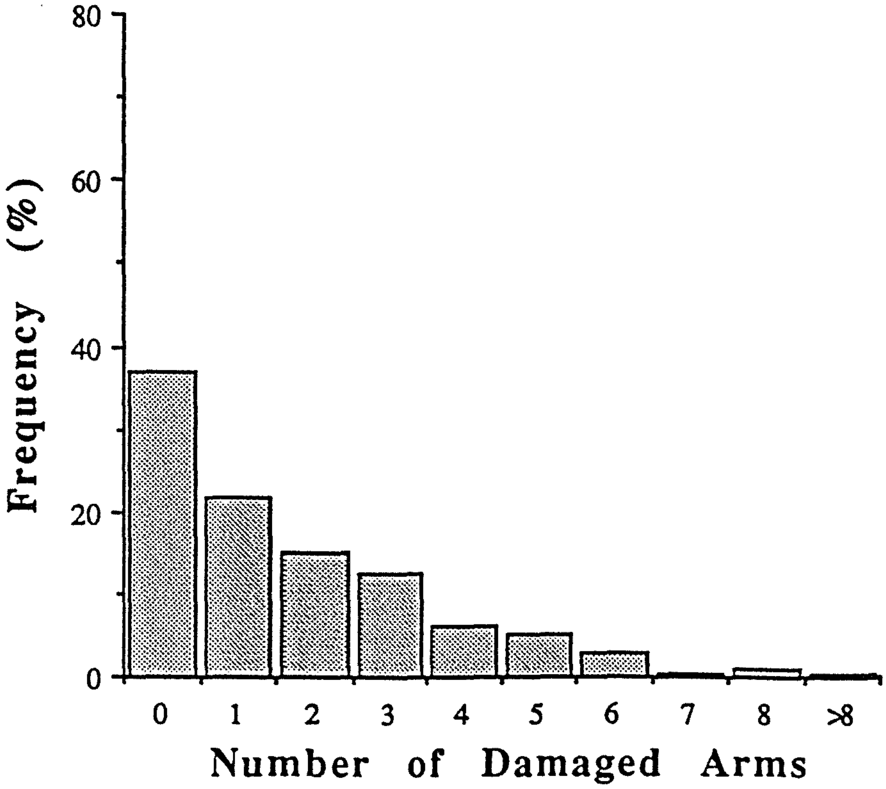
Figure 2.1 Frequency distribution of the number of damaged arms on *A. planci*. Data from: (A) McCallum (1989) on Holbourne Island (n=263) and (B) Kettle (unpublished) from Keeper and Helix Reefs (n=638).

A.



(From McCallum, 1989)

B.



(From Kettle, unpublished)

2.3 Description of Individual Experiments

The general methods of the four experiments are summarised in Table 2.1.

2.3.1 Experiment #1: Winter 1991

2.3.1.1 Damage Treatment

The first study group of starfish was collected between May 1st and May 4th, 1991. One hundred starfish were damaged and put into an open-topped pen on a coral rubble bottom. There were 20 starfish in each of five damage groups (controls, -2 arms, -4 arms, -8 arms and -1/2). Prior to treatment, the starfish were measured for diameter (measured from arm tip to opposite arm tip, excluding spines). These measurements were made to see if starfish size influenced survival after damage. It has been suggested that *A. planci* may survive damage better when they are at smaller body sizes (Birkeland and Lucas, 1990). Unfortunately, all the starfish fell into a narrow size range which precluded size related experiments (Figure 2.2).

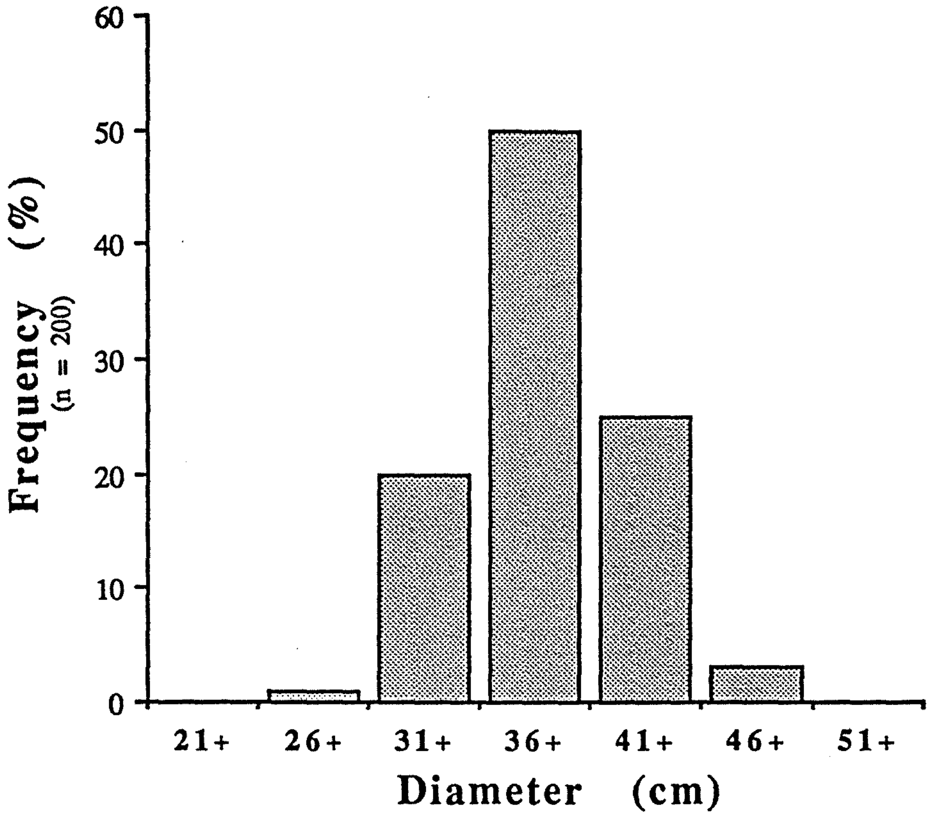
The starfish were checked daily for general healthy appearance, but were not counted. After a week, the starfish were all counted. While a small number of starfish were missing, it was not clear why this had occurred. Some possible explanations include: (1)

Table 2.1 Summary of the methods of the four experiments which look at what constitutes lethal damage to *A. planci*.

Experiment	1	2	3	4
Season	Winter	Summer	Winter	Summer
Enclosure	Open pen, sealed later	Dual sealed cages	Individual sealed cages	Individual sealed cages
Damage Treatment	Ctrl, -2,-4, -8 and 1/2	Ctrl, -4,-8, -1/2 and -2/3	Ctrl, -4,-8, -1/2 and -2/3	Ctrl, -8 and -1/2
Starvation Treatment	N.A.	Ctrl, -4,-8, -1/2 and -2/3	Ctrl and -8	Ctrl, -8 and -1/2
No. Starfish	100	223	125	181

N.A. = Not applicable

Figure 2.2 Size - frequency distribution of *A. planci* collected from Davies Reef during Experiment #1.



miscounting, (2) escape, which happened at least once - the starfish was returned to the cage, (3) predation or (4) mortality, though no remains could be found.

After 7 days the top of the pen was sealed with mesh. A total count of total remaining starfish was made 14 days later, that is 21 days after they were damaged. The starfish were then released.

2.3.2 Experiment #2: Summer 1991/92

2.3.2.1 Starvation Treatment

During the recent *A. planci* outbreak on the Great Barrier Reef Davies Reef maintained medium densities of starfish with no clear starfish "front" progressing across the reef. However, a starvation treatment would reflect the situation on other reefs where there had been a "front" of starfish that moved along eating most of the coral. Unless emigration from these reefs took place, starvation would probably pose a problem for the starfish during the later stages of an outbreak.

Kettle (1991) starved a number of *A. planci* to death and followed the decrease in caloric value of their pyloric caeca, the energy storage organs. He found that half of the total decrease in caloric value of the caeca occurred during the first six weeks of the starvation period. Given the loss of energy reserves shown in Kettle's experiments and the projected duration of 2-3 weeks for these damage experiments (in which no food would be provided), six weeks was chosen as a significant starvation period prior to the damage experiments.

Starfish were collected for starvation between the 31st October and the 4th of November, 1991. When the total number of starfish collected and placed in the 1000 litre tank reached fifty, the starfish were transported to a site where they were placed in a sealed cage. The 102 starfish accumulated in this way were left in these cages unfed until the damage experiments six weeks later.

2.3.2.2 Damage Treatment

A second of group starfish (125) was collected six weeks later between December 5th and December 13th, 1991. Both the newly collected starfish and the starved ones were stored in 60 litre bins prior to treatment because the roughness of the seas prevented the use of the 1000L tank. These bins were provided with running water. The confined nature of these bins did not appear to affect the health of the starfish as long as the flow-through of water was maintained. The time that the starfish spent in these 60 litre bins on the main vessel varied from around 20 minutes to around 18 hours.

The starfish in this experiment were processed as previously described and placed in the cages in groups of 25 or 50. These groupings represented the number of starfish needed to fill or half-fill a cage. Due to the low mortality in the winter experiment (Experiment #1) the treatment consisting of a loss of two arms was omitted and a more severe treatment, in which two-thirds of the starfish were removed, was added.

Initially, a total of 197 starfish were caged for this experiment - there were three less starfish in the starvation treatment due to mortality during the starvation period. Further collection of starfish was required due to unexpectedly high mortality in two cages. The two cages were attacked and holed during the first three days of the experiment and presumably this was the source of high mortality of both starved and unstarved starfish. A pufferfish, *Arothron stellatus*, a species known to attack cages containing *A. planci* in an attempt to eat them (Babcock pers. com.), was seen hovering over the cages at this time and was apparently the cause of the damage. The remaining starfish from cage 1 were placed into the appropriate halves of cage 2. The density of the remaining starfish in cage 2 was no higher than in any of the other cages. A further 25 newly processed starfish were placed in cage 1. No further attacks on the cages occurred. Collection and processing of all the starfish took five days.

The cages were checked and the starfish counted at least once a day to keep track of the mortality. Monitoring of the cages continued until day seven of the experiment. The starfish were then counted and were left until a final count two weeks later. They were then released.

2.3.3 Experiment #3: Winter 1992

2.3.3.1 Starvation Treatment

In total, 110 starfish were collected for starvation in early April, 1992. By the time of the damage experiment six weeks later, 85 of the 110 starfish (77%) had died. As a result, the starvation treatments of this experiment were limited to one cage with two damage categories: 12 controls and 13 starfish with -8 arms (i.e. 25 starfish in total).

2.3.3.2 Damage Treatment

A total of 100 starfish were collected for the unstarved portion of these experiments. The damage experiment commenced on the 25th of May, 1992. The damage treatments used were -4 arms, -8 arms, -1/2 and -2/3.

The possibility that confining large numbers of damaged individuals may lead to high transmission of bacterial infection and therefore increased mortality was considered at this time. An experiment was designed to explore this possibility. Forty starfish were collected for this additional experiment. Ten unstarved starfish (five controls and five with -8 arms) were placed in each of four cages as a comparison to the rest of the damage experiment cages, which had 25 starfish per cage.

2.3.4 Experiment #4: Summer 1992/93

2.3.4.1 Starvation Treatment

One hundred and two starfish were collected in early October. These starfish were starved for six weeks. This time only 7 starfish (6.9%) died during the starvation period.

2.3.4.2 Damage Treatment

The damage experiment commenced on the 23rd of November, 1992. Over 150 starfish were collected for the unstarved treatments. For this experiment the number of damage categories was reduced to: undamaged controls; -4 arms; and halves. The treatment consisting of tri-section of starfish was eliminated because previous experiments showed that the starfish in this group either died or could not be distinguished reliably from the halves. This was particularly the case because half starfish tended to lose arms adjacent to the cut edge through necrosis. To make up for the reduced number of damage treatments so that the density of starfish was consistent throughout the experiments, the number of starfish per treatment was increased. In all, there were three cages of 27 starved starfish (nine per damage treatment) and four cages of between 24 and 27 unstarved starfish (8 or 9 starfish per damage treatment).

As a result of the findings of other experiments outlined in Chapter 3, attempts were made to see if there were differences between gender in terms of their ability to survive damage. The starfish were sexed via examination of samples of the gonad obtained with a syringe. Unfortunately, the scarcity of starfish and a high ratio of males to females (2:1) prevented the collection of adequate numbers of females (only 16 found) in the time available. These were divided into two treatment groups (eight controls and eight with -4 arms) and put into a cage with eight males (identified by two separate pairs of missing groups of -2 arms) to make the density comparable to other experiments. A second cage was produced consisting of 24 males (eight controls, eight with -4 arms, and eight halves).

2.3.5 Changes to Caging Design

For a variety of practical reasons the design of the starfish holding cages changed through the course of these experiments. In Experiment #1 the holding area for the treated starfish consisted of a circular pen 12 metres in circumference in five metres of water. The open-topped design of the pen enabled easy access of divers, as well as predators, to the starfish. The walls of the pen were made with 0.8mm gauge 12mm x 12mm galvanised welded mesh and were 1.1 metres high with an additional 600mm horizontal overhang. The mesh size and the overhang were designed according to the recommendations of Bell *et al.* (1987) for preventing the escape of *A. planci*. The bottom of the pen was the sea floor which consisted of sand and small coral rubble. Larger pieces of dead coral rubble were placed in the pen to provide a natural surface for the starfish to hold on to. This rubble ended up providing too good a hiding place for the starfish and made the starfish difficult to identify with respect to treatment.

In Experiment #2 the cage design was changed to a completely sealed rectangular box. This prevented escape of the starfish and also prevented access to the starfish by predators. The cage was made of galvanised steel mesh (size: 12mm x 12mm with 0.8mm gauge) with dimensions 3.5m x 1.5m x 0.45m. So that it could accommodate both starved and unstarved starfish during the damage experiments, this cage was split into two halves by placing a divider made of welded mesh across the 1.5m width.

The cages were changed again for Experiment #3. As described, the cages in Experiment #2 were damaged by some predator (presumed to be the pufferfish *Arothron stellatus*), so the gauge of the mesh was increased to 1.2mm. Aluminium frames were added to support the greater weight of the cage. This frame also enabled the use of lift-up lids for easy access by divers to the inside of the cages for examining and counting starfish. The size of the cages was changed to 2.4m x 1.2m x 0.4m for easier transportation of the otherwise cumbersome cages. These same cages were used for Experiment #4.

Various forms of cover were provided in the cages to reduce stressing the starfish. Coral rubble was provided in Experiment #1, however the rubble made it difficult for divers to remove the starfish for examination without damaging them. In Experiment #2, layered fibro sheets separated by coral rubble were provided to the starfish to reduce the incidental damage. These became easily deformed with the moisture and with the weight of the starfish and eventually afforded no cover for the starfish. Finally, full cage covers were trialled. Black woven plastic sheets ("weedmat") and blue plastic tarpaulins were used to cover all but one of the side of the cages. The exposed side allowed water circulation. These covers worked satisfactorily, although they may have facilitated the shifting of the cages along the bottom during strong currents.

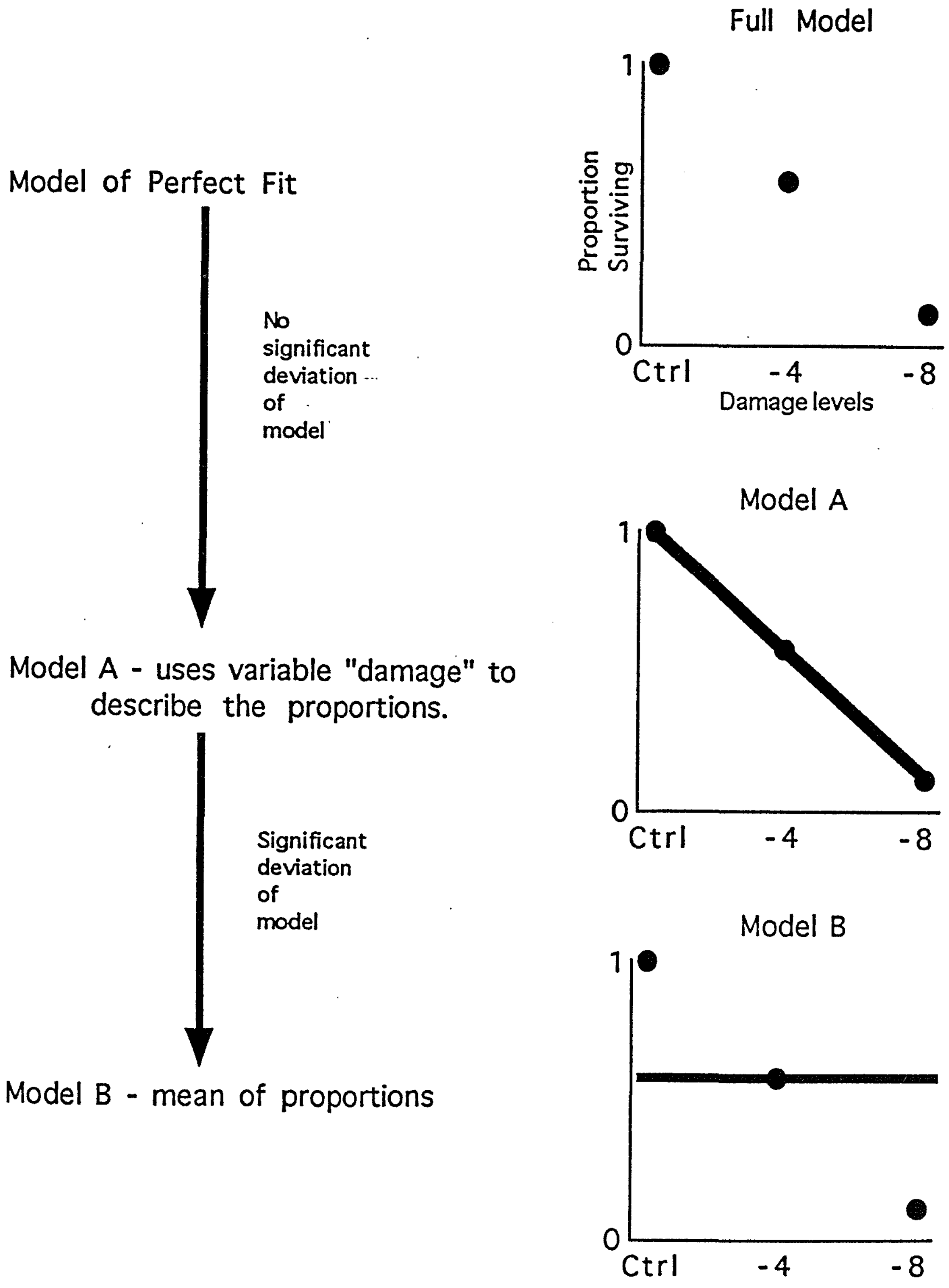
2.3.6 Analysis of the Data

The proportions of survival resulting from the four groups of experiments were examined using logistic regression. For these data, the term used to describe damage was in some instances quantitative and it was assumed that the damage treatments represented a linear increase in damage. This assumption appeared to be supported by the data in most of the individual experiments.

Fitting logistic regression models proceeded as follows. First, a model was fitted iteratively by the statistical program to explain all the variation in the data. Essentially this included a term for every data point (in this case, proportion of survival). The model was then simplified by reducing the number of descriptive terms. This is known as backward elimination. This simplification continued until removal of another factor resulted in a significant increase in deviance from that associated with the previous model. The previous model was then considered to provide the most parsimonious description of the data.

The process of backward elimination using a simple hypothetical example is shown in Figure 2.3. In this example starfish are divided equally into each of three damage treatments (e.g. Controls, -4 arms, and -8 arms).

Figure 2.3 A simple hypothetical example of backward elimination of a term from a model to produce a more simplified model that describes proportions of survival.



The three treatment groups give three proportions of survival. These three proportions represent the initial model used by the statistical program for comparisons - essentially a perfect fit.

In Figure 2.3, Model A is the first reduced model to be compared to the perfect fit, or full, model. This model describes the proportions as having a linear relationship described by the quantitative term "damage". The model is represented by a line. As in the actual experiments, the relationship is based on the amount of damage and how this should affect survival. In Figure 2.3 (Model A), damage is negatively related to survival.

As can be seen in this figure, the relationship between Model A (the line) and the perfect fit model (the three data points) is close, suggesting that damage does affect survival. Therefore, the value of the deviance would be low and the model would be considered as not significantly different from the perfect fit model and a good simplified way of describing the data.

Model B in Figure 2.3 is a further reduced model which describes the proportions in the individual treatments as being no different from the overall mean value. As can be seen from the graph, the model (the line) no longer describes the data well and the deviance would likely be very high. As a result of this poor fit, this model would not be considered a good simplified way of describing the data. Model A would be considered the most parsimonious model that best describes the data.

Figure 2.4 is a more complex example of a backward elimination. This is typical of the models used in these experiments where both damage and starvation are considered. Table 2.2 contains a logistic regression table for the hypothetical example in Figure 2.4. This table is provided as an example of how the results are presented later in the actual experiments. Descriptive words are used in this table in place of values. In this hypothetical example, two quantitative terms are used to describe proportions of survival. Model 1 suggests that each of the terms directly affects the data and that the terms interact to affect the data. Model 1 differs from the perfect fit model, but not enough to warrant a significant deviance (see Table 2.2). Model 2 suggests no interaction between the terms, but indicates effects by each of the terms. Model 2 fits the data quite well so there is little deviance. Following the decision tree down further simplifies the model to where either term is eliminated and then finally to where the data may be simply described by their overall mean value. These further simplifications substantially differ from the data and that is reflected in the high deviance (Table 2.2). Therefore, Model 2 is the most parsimonious model that best fits the data. In the actual results of the experiments, only the models which

Figure 2.4 A complex hypothetical example of backward elimination of two terms to produce increasingly simpler models that describe proportions of survival.

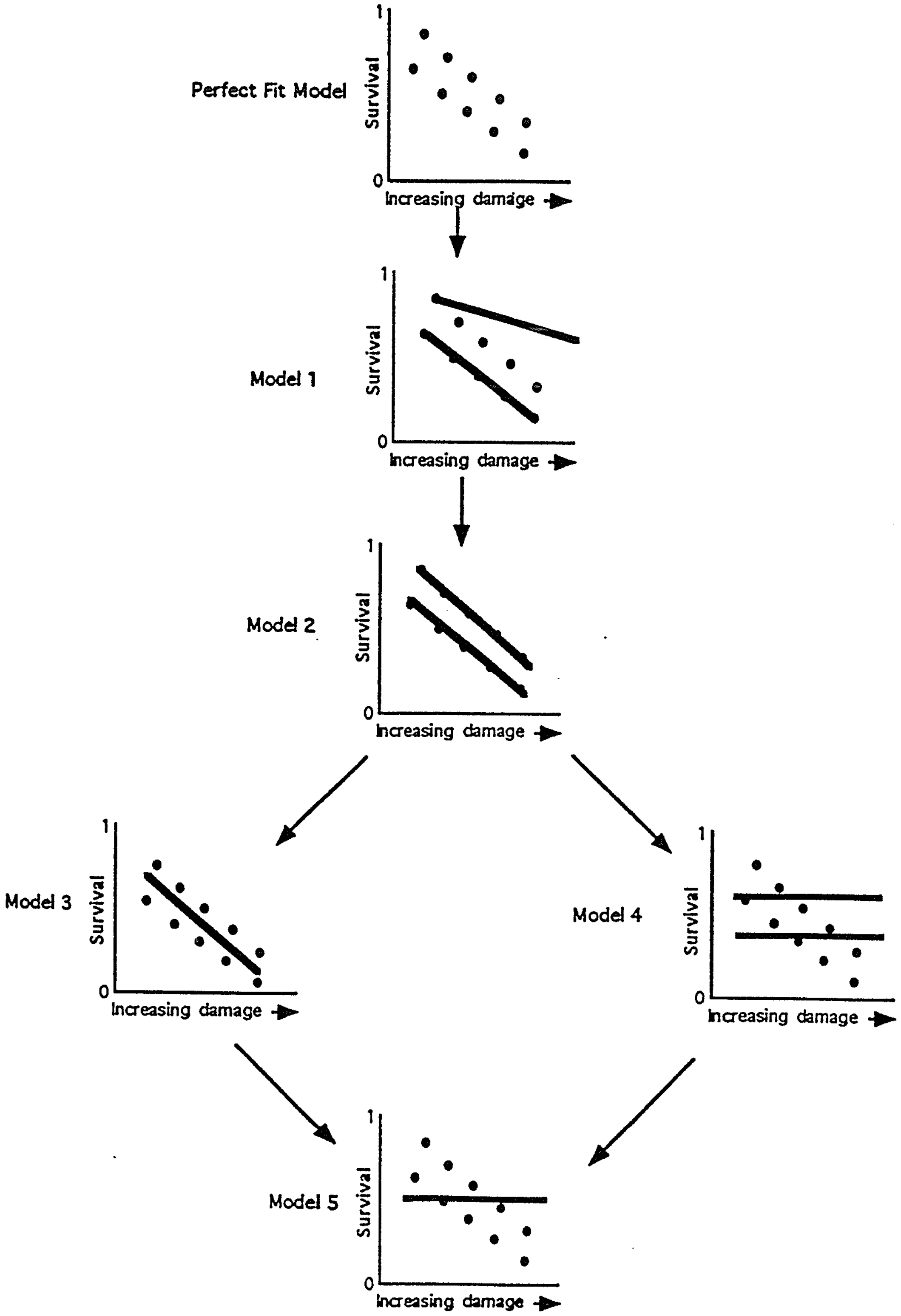


Table 2.2 Logistic regression table showing the backward elimination of terms from the hypothetical model in Figure 2.4.

T1 = Term 1
T2 = Term 2
T1T2 = Interaction of T1 and T2

Terms Present	Deviance	Change of Deviance	Component Tested	P
T1+T2+ T1T2	low	low	variance	N.S
T1 + T2	low	low	T1T2	N.S.
T1	high	high	T2	S.
T2	high	high	T1	S.
Mean	v. high	v. high	T1 or T2	V.S.

have been tested will be displayed in the regression table. For example, in this hypothetical example, Model 5 results would not be displayed since this model is even more simplified than Models 2 or 3, which were rejected as being inadequate.

This process of simplifying the model is usually straight forward and only the deviance (comparable to chi square) from one model to the next needs to be analysed (i.e. deviance of simpler model - deviance of more complex model with (d.f.(simple) - d.f.(complex) degrees of freedom). However, if the data are extremely variable, any model simpler than the perfect fit model will not adequately explain the variability in the data (indicating perhaps that there are other factors involved). This is called over-dispersion. If over-dispersion is present, the relative contributions to the variability in the data by individual or groups of terms can be assessed via nested F tests (Collett, 1991). In these cases, the significance of the deviance can be determined by examining F tables where F is calculated by:

$$F_{((df_a-df_b),df_b)} = \frac{(\text{Deviance of a} - \text{Deviance of b}) / (df_a-df_b)}{\text{Deviance of b} / df_b}$$

where: a = simpler model
b = more complex model
df = degrees of freedom

The nested F-test is essentially an analysis of variance comparing the two different models. Generally, the P value for the test is two tailed. When damage terms are being removed, however, the test becomes one tailed because of the assumption that damage results in a greater chance of mortality. This change to a one tailed test also increases the power of the test to detect the influence of damage.

The use of logistic regression for the analyses in this chapter enabled the calculation of odds of mortality of the change between treatments. In the damage experiments, odds could not be calculated for the change between two levels of damage within an individual trip because the sample sizes were too small (G. De'Ath, pers. com.). When all the individual experiments were combined, the analyses had a large enough sample size to calculate odds of mortality between damage levels.

2.4 Results

2.4.1 Combined Results

All four experiments were combined for analysis. The overall combined proportions of survival within damage treatments are shown in Figure 2.5. Only treatments common to all experiments were included (i.e. starvation and -2 arm damage treatments were left out). The damage treatment of -8 arms was included despite not being used in Experiment #4. Figure 2.6 shows the variability in the results through the four experiments. Note the high survival rate of the -1/2,-2/3 starfish in experiments 1 and 4 as compared to their survival in experiments 2 and 3.

The data for this grouped analysis were treated in two different ways. Firstly, damage levels were treated as quantitative terms and it was assumed that the increase in damage level was linear and would result in a linear change in mortality (Table 2.3). Secondly, the damage levels were treated as qualitative terms and the relative importance of each damage level could be tested independently (Table 2.4). This second method enabled the easy grouping of the treatment levels.

Table 2.3 shows that the quantitative method did not produce a model that adequately described the data. This

Figure 2.5 Proportions of survival of *A. planci* versus amount of damage for the combined experiments. Only the treatments common to all experiments are included.

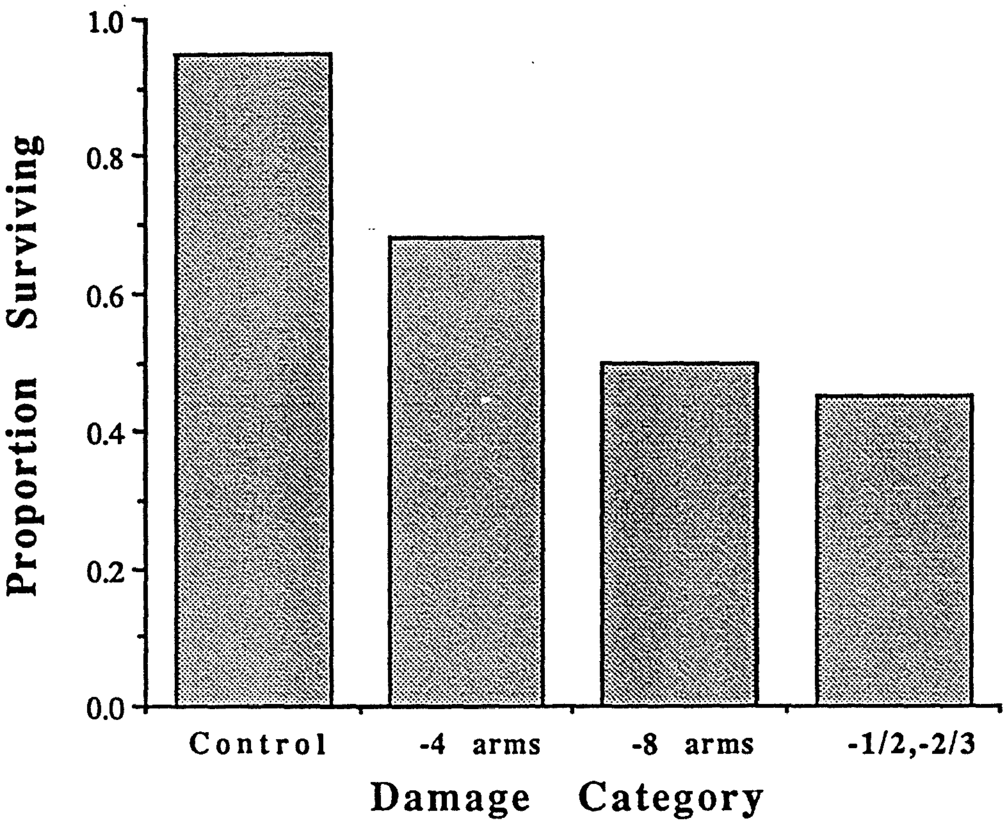


Figure 2.6 Proportions of survival of *A. planci* versus damage treatment through the four experiments (Data for starved starfish not included).

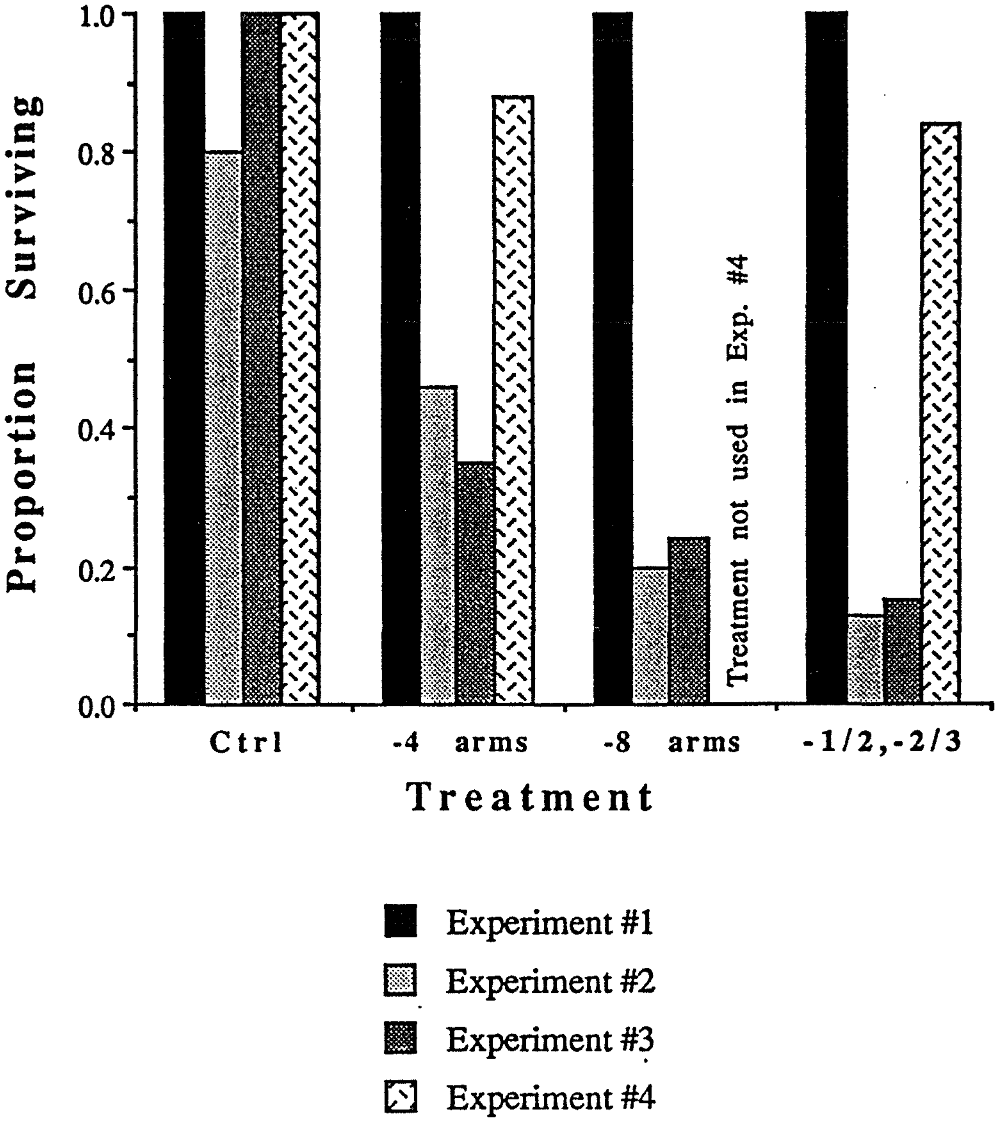


Table 2.3 Logistic regression table showing the backward elimination of terms from a model relating mortality of *A. planci* to extent of damage, to experiment and to their interactions. The term for effects of damage is quantitative.

E1, E2, E3, E4 = Experiments 1, 2, 3, and 4
Season = Experiments grouped by season
D = Effect of damage
DE1, DE2, DE3, DE4 = Interaction between damage and experiment #

Terms Present	Deviance	Change of Deviance (Nested F*)	Component Tested	D.F.	P
D+E1+E2+E3+E4+DE1+DE2+DE3+DE4	21.91	21.91	Variance	7	0.0026
D+E1+E2+E3+E4	29.28	0.786*	DE	9	0.5387
E1+E2+E3+E4	84.98	19.0232*	D	11	0.0007
D + Season	165.55	23.27*	Variance of E	12	0.0002

Table 2.4 Logistic regression table showing the backward elimination of terms from a model relating mortality of *A. planci* to extent of damage and to experiment. The terms for effects of damage are qualitative.

E1, E2, E3, E4 = Experiments 2,3, and 4
Season = Experiments grouped by season
D1, D2, D3, D4 = Damage levels Ctrl,-4arms,-8arms, and halves/thirds
D234 = Damage levels grouped together versus controls

Terms Present	Deviance	Change of Deviance	Component Tested	D.F.	P
D1+D2+D3+D4+E1+E2+E3+E4	14.17	14.17	Variance	8	0.0775
D1+D2+D3+D4	152.69	138.52	E	11	0.0000
E1+E2+E3+E4	84.98	70.81	D	11	0.0000
D234+E1+E2+E3+E4	19.11	4.94	Variance in D	10	0.0846
D234 + Season	162.90	143.79	Variance in E	12	0.0000

meant that the data were too variable and that there were factors other than just physical damage which were influencing the results. This over-dispersion in the data meant that nested F-tests were required to determine the relative contributions of the terms to the variance in the data.

Table 2.3 also shows, using nested F tests, that increased damage increased mortality. The degree to which damage increased the likelihood of mortality is discussed in the section below on odds ratios. The differences between experiments also influenced mortality, but this did not interact with damage treatments. Combining the experiments into seasons caused a significant increase in deviance, indicating that this simplification could not be made. Thus, increasing damage reduces survival, but the variability of survival between experiments indicates no statistically significant seasonal effect.

The qualitative model (Table 2.4) differed from the quantitative model (Table 2.3) in that the qualitative model did not deviate significantly from the full model. As with the quantitative method, damage was found to reduce survival. Thus there was a clear effect of damage on survival but the effects were not linear (i.e. proportionate increases in damage did not result in proportionate decreases in survival), as presumed in the quantitative model. There was a significant effect of damage among experiments, but there was no evidence of seasonal pattern in this variation.

Since this analysis was completed using qualitative damage terms it was possible to group the damage levels which caused similar effects on mortality. A variety of combinations were analysed. All the damage levels could be grouped together versus the controls and the model still did not deviate significantly from the full model. The simplified proportions of survival are 0.96 for controls and 0.5 for the damaged starfish. Overall, for the combined data, damage reduces survival, but there are no significant differences among damage treatments.

Odds ratios show that with an increase of one damage category (e.g. from Ctrl to -4 arms) a starfish is between 2 and 3.7 times more likely to die (95% confidence intervals). As a further example, this means that a starfish with eight arms removed is between 4 and 13.7 times more likely to die than a control. If the damage categories are grouped together versus the controls, as suggested by the qualitative model, the chance of mortality with damage is increased by between 11 and 100 times, with 95% confidence.

2.4.2 Results - Experiment #1: Winter 1991

Ninety-eight of the 100 starfish survived. A few starfish, primarily those which had been halved, had necrotic spines and lesions at the end of the test period, but they were still considered healthy. The general good health of these starfish was suggested by sealed wounds, erect spines and general rigidity of their bodies. Two starfish were missing from the -2 arm treatment and could not be accounted for. There were no skeletal remnants to indicate death and decay though such remnants were clearly present in other experiments. Even though it is probable they had escaped, they were considered to have died.

2.4.3 Results - Experiment #2: Summer 1991/92

The results of the summer experiment were markedly different from the winter before. Two cages of starfish were eliminated from these analyses because of predator attacks on the cages which appeared to cause high mortality. The proportions of survival for Experiment #2 are shown in Figure 2.7. These ranged from no survival at all in the two-thirds damaged group, to 0.8 survival in the control groups.

Employing the logistic regression, the model that best described the data contained only the quantitative term damage as a parameter (Table 2.5). Increased damage

Figure 2.7 Proportions of survival of *A. planci* versus damage treatment in Experiment #2: Summer 1991/92.

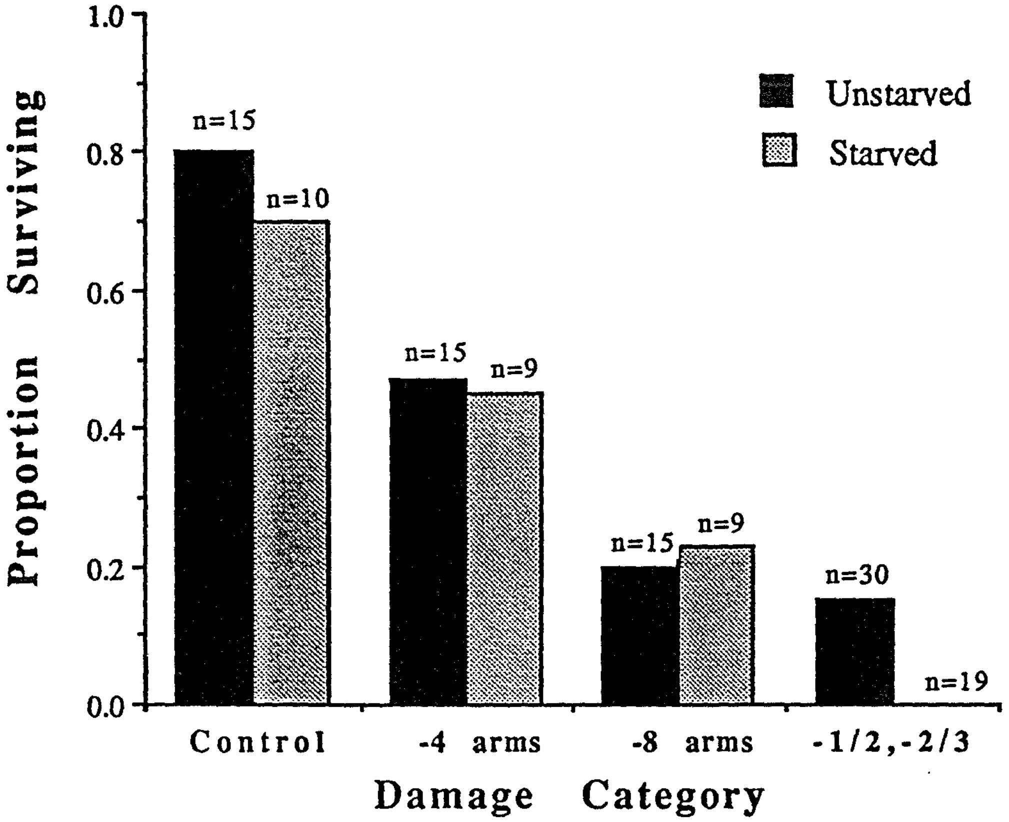


Table 2.5 Logistic regression table showing the backward elimination of terms from a model relating mortality of *A. planci* to extent of damage and to starvation in Experiment #2: Summer 1991/92.

D = Quantitative damage effect
S = Effect of starvation
D*S = Interaction of damage and starvation

Terms present	Deviance	Change of Deviance	Component Tested	D.F.	P
D + S + D*S	2.82	2.82	Vari-ability	4	0.2945
D + S	3.49	0.67	D*S	1	0.2065
D	4.57	1.08	S	1	0.1493
S	43.81	40.32	D	1	0.0000

reduces survival but the effect of starvation was not significant, although there appeared to be an effect in the greatest damage category. The detection of starvation effects was potentially reduced by the small final sample size.

2.4.4 Results - Experiment #3: Winter 1992.

The mortality in this experiment varied from 0% to 85% among treatments (Figures 2.6 and 2.8). Figure 2.6, as previously considered, does not include the proportions of survival from the starvation treatments. Only the data for which there are starvation and non-starvation treatments are included in Figure 2.8. As described before, only two starvation groups were produced as a result of 77% mortality during the six week starvation period. Consequently, two damage treatment groups (-4 arms and the combined -1/2 and -2/3 treatments) were left out of the single experiment analysis.

The resulting proportions of survival were sufficiently variable to prevent simplifying any logistic model from a full model (Table 2.6). It appears from Figure 2.8 that this is probably a result of high mortality in the starved controls.

The proportions of survival in the density experiments are shown in Figure 2.9. There was greater mortality of

Figure 2.8 Proportions of survival of *A. planci* versus damage and starvation treatments in Experiment #3: Winter 1992.

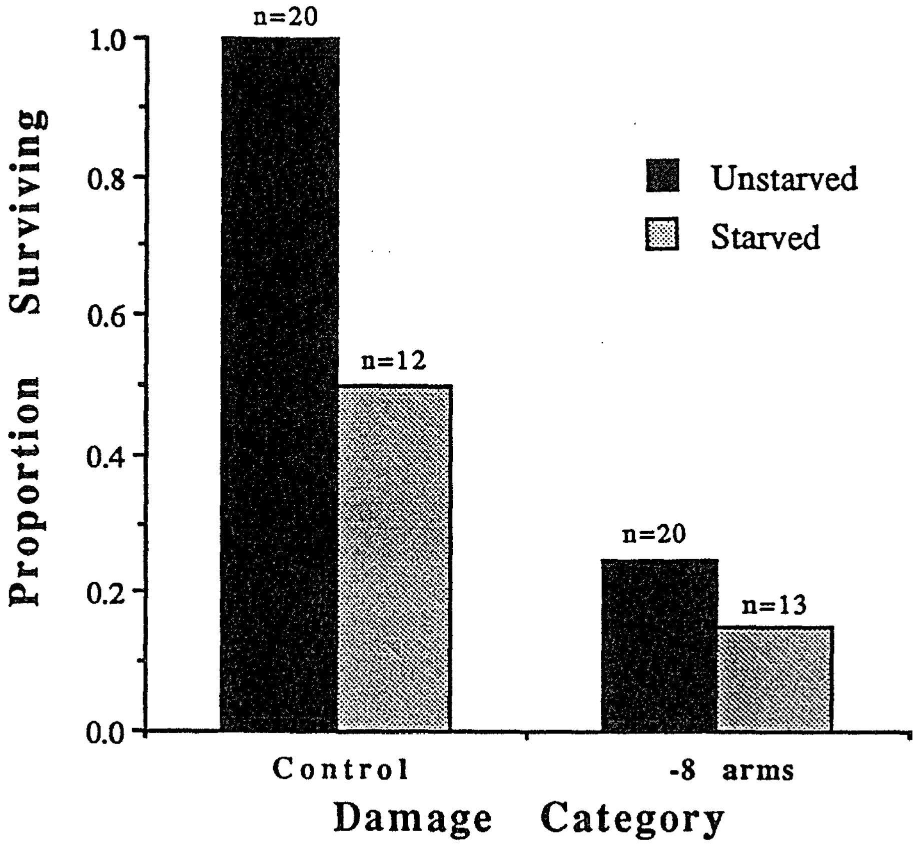
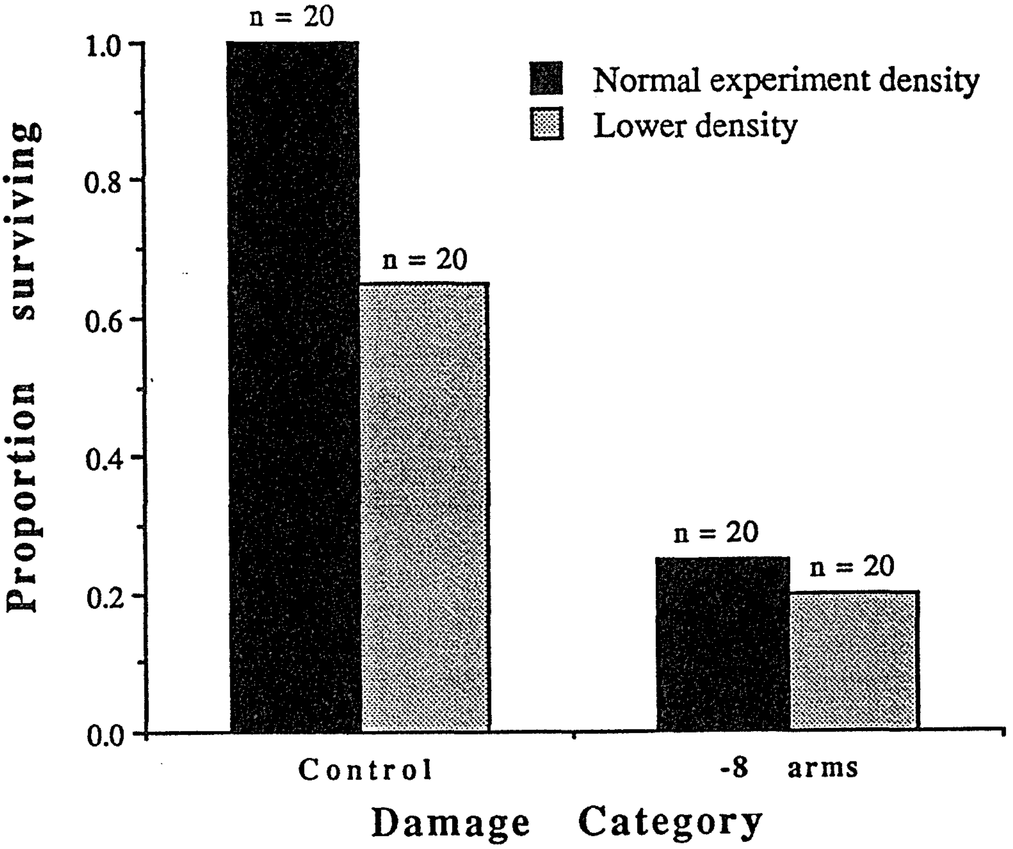


Table 2.6 Logistic regression table showing the backward elimination of terms from a model relating mortality of *A. planci* to extent of damage and to starvation in Experiment #3: Winter 1992.

D*S = Interaction of damage and starvation
(In this case D*S = full model)
D = Quantitative effect of damage
S = Effect of starvation

Terms Present	Deviance	Change of Deviance (Nested F^*)	Component tested	D.F.	P
D + S	5.78	5.78	D*S	1	0.0081
D	14.7	1.54^*	S	2	0.2657
S	33.98	4.88^*	D	2	0.1358

Figure 2.9 Proportions of survival of *A. planci* relating to damage and density treatments in experiments in Winter 1992. Normal density refers to the density of starfish normally placed in the experimental cages.



the starfish in the lower density cage, though the data are over-dispersed (Table 2.7). While this may indicate an interaction between density and damage, given the direction of the effects it seems more likely that there are other unconsidered factors involved.

2.4.5 Results - Experiment #4: Summer 1992/93.

The mortality in this experiment varied from 0% to 66% (Figure 2.10). The attempt to simplify the logistic model is shown in Table 2.8. Omitting the interaction term (D*S) produces significant deviance, indicating that the effect of damage on mortality depended on whether the starfish were starved or not. This interaction was probably the result of the high mortality in the starved half starfish which was quite different from the controls and less damaged starfish (Figure 2.10).

The odds of mortality were calculated from the model. Starvation in this experiment increased the odds of mortality by between 1.5 and 12 times compared to damage without starvation.

The influence of starfish gender was also tested in this experiment. The backward elimination of terms is shown in Table 2.9. The proportions of survival did not correspond with damage treatment (Figure 2.11). There were no statistically significant influences of either sex or damage on mortality.

Table 2.7 Logistic regression table showing the backward elimination of terms from a model relating mortality of *A. planci* to extent of damage and to densities of starfish in cages in the Winter 1992 set of experiments.

D = Quantitative effect of damage
Den = Effect of density

Terms Present	Deviance	Change of Deviance	Component Tested	D.F.	P
D + Den	6.13	0.0133	Variance	1	0.0133

Figure 2.10 Proportions of survival of *A. planci* versus damage and starvation treatments in Experiment #4: Summer 1992/93.

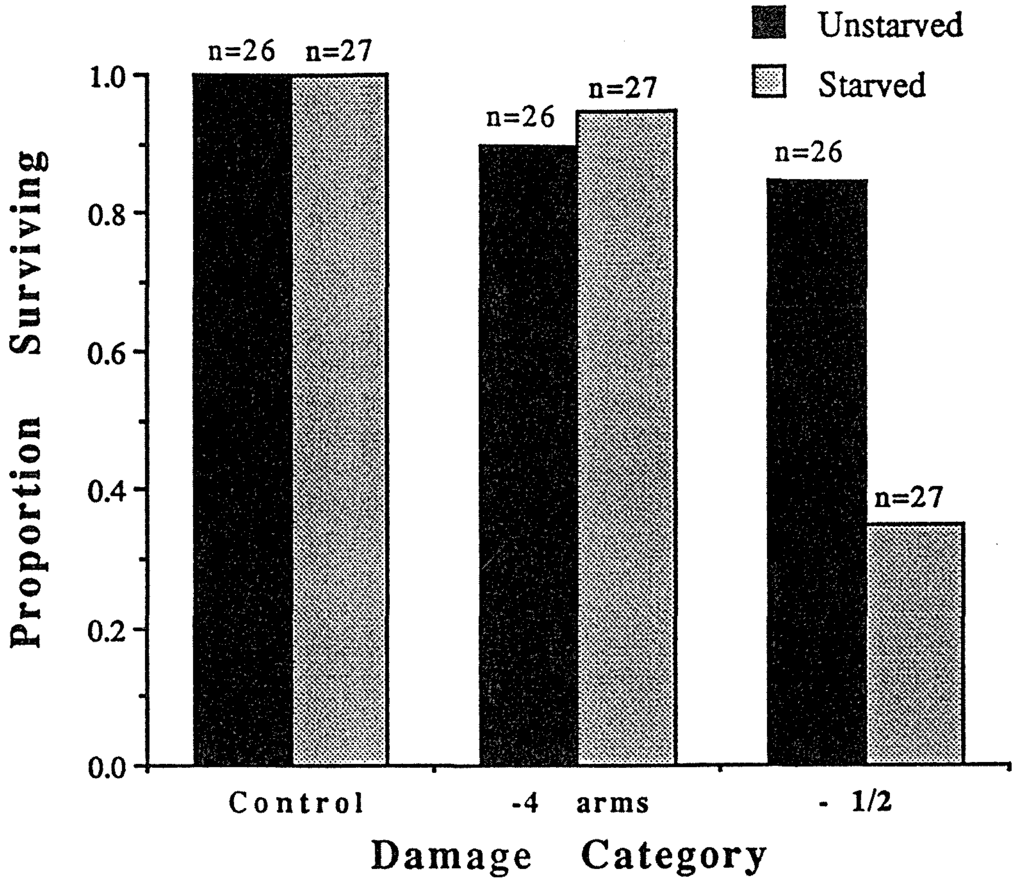


Table 2.8 Logistic regression table showing the backward elimination of terms from a model relating mortality of *A. planci* to extent of damage and to starvation in Experiment #4: Summer 1992/93.

D = Quantitative effect of damage
S = Effect of starvation
D*S = Interaction of damage and starvation

Terms Present	Deviance	Change of Deviance	Component Tested	D.F.	P
D + S + D*S	2.18	2.18	Variance	2	0.168
D + S	8.86	6.68	D*S	3	0.0049
D	17.25	8.39	D	4	0.0018
S	51.5	42.64	S	4	0.0000

Figure 2.11 Proportions of survival of *A. planci* versus sex and damage treatments in the Summer 1992/93 set of experiments.

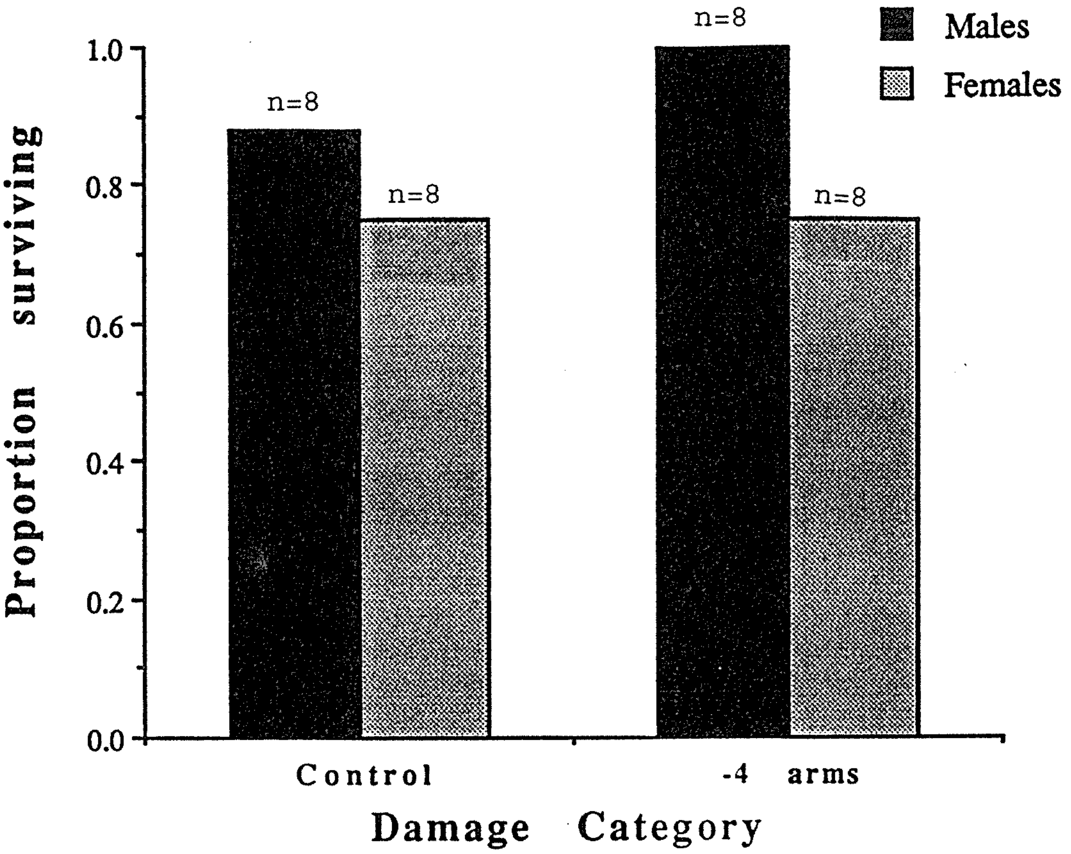


Table 2.9 Logistic regression table showing the backward elimination of terms from a model relating mortality of *A. planci* to extent of damage and to gender in the Summer 1992/93 experiments.

D = Quantitative effect of damage
Sex = Effect of sex
Mean = The mean of all the proportions

Terms Present	Deviance	Change of Deviance	Component Tested	D.F.	P
D + Sex	1.2	1.2	Variance	1	0.2738
D	3.48	2.28	Sex	2	0.3198
Sex	1.45	0.25	D	2	0.4417
Mean	3.71	2.51	D, Sex	3	0.2352

2.5 Discussion

2.5.1 Lethal Level of Damage

A general conclusion to be drawn from these experiments is that there are aspects of the life history and biology of *Acanthaster planci* which are complex. The purpose of these experiments was to find an amount of damage to *A. planci* that was consistently lethal. No simple answer was forthcoming. In experiments 2 and 3, mortality occurred in all treatments, including those with no deliberate damage inflicted. In experiments 1 and 4 the resistance to even severe damage was quite high and mortality was low. While it was clear that mortality was generally greatest in the bi- and tri-sectioned starfish, this was inconsistent among the experiments and appeared to be affected by starvation in some cases.

The inability to clearly determine a consistently lethal level of damage in these studies is consistent with the variability found between past damage experiments. Pearson and Endean (1969) found that less damage (cut in half) was required to mortally wound an *A. planci* individual, though only one starfish was examined. Roads (1973) found that individuals simply required damage to the body disk for mortality. On the other hand, damage experiments in Okinawa found that a small proportion of quarter-starfish remained alive after a month (Birkeland

and Lucas, 1990). Finally, recent starfish control experiments on Bait reef by the Great Barrier Reef Marine Park Authority (U. Englehardt - pers. com.) found that greater than 70% of caged pieces of starfish cut into quarters remained alive after 16 days.

It is clear that damage affects the odds of survival of starfish. An increase of damage from one level to the next (e.g. from -4 arms to -8 arms) was found to increase the chance of mortality by up to 3.7 times. If all the damage categories are grouped, as was suggested by the qualitative model, chance of mortality with any type of damage increases by up to 100 times.

2.5.2 Variability

The variability in mortality described above is not surprising given the unexplained inconsistency between results in many aspects of Crown of Thorns Starfish research. This variability is exemplified by related control experiments completed by the Great Barrier Reef Marine Park Authority (U. Englehardt, pers. com). In these experiments, a large number of individuals were injected with varying amounts of copper sulphate. The minimum lethal dose of copper sulphate (ca. 15mL) from an initial experiment differed markedly from the minimum dose required in a subsequent experiment (45mL).

The variability of the results found in this thesis suggests that there must be other factors that affected the survival of the starfish. Some of the possible factors which were not accounted for and which may have affected survival in the experiments, and therefore contributed to variability, are: water temperature, age, size, nutritional status, post-reproductive morbidity, local abundance of predators, removal from water for treatment and differences in starfish handling. These factors were not incorporated into these experiments since huge numbers of starfish would have been required to set up satisfactory multifactorial experiments, not to mention the concomitant logistical problems.

2.5.3 Long Term Effects

No prior experiments have followed through to examine if heavily damaged starfish are able to feed, regenerate and eventually survive in the long term. While it was not in the scope of this study to follow the long term effects of damage, the starfish in these sets of experiments were released so that there was chance of finding them at a later date. Through haphazard searching subsequent to the completion of the experiments, four starfish were recaptured which were presumed to be from these experiments. Two were from the -4 arms category, one from the -8 arms category, and one from the half category. All were found feeding on coral and all were regenerating their lost portions. Thus, it appears that some of the starfish from these experiments were able to survive the damage inflicted and regenerate. However, the fact that so few starfish were recovered when hundreds were released suggests that: 1) the starfish were very mobile and moved away from the area, 2) they had become cryptic and difficult to find or 3) only a few eventually survived the inflicted damage. This should be examined in the future.

2.5.4 Starvation

The influence of starvation on mortality was not clear. Two of the three experiments which incorporated starvation showed significant effects of starvation. In Experiment #2, starvation alone increased the chance of mortality, by between 1.5 and 12 times (with 95% confidence intervals) (Note: interaction prevented calculation of odds for Experiment #3). Although the effects of starvation on mortality were not clear in the damage experiments, the great, albeit inconsistent, mortality experienced during starvation prior to the experiments suggests that starvation was a significant factor.

Any discussion of the results of the current experiment needs to be viewed with an understanding of the natural environment of the *A. planci* used in these experiments. A significant starfish population has been present on Davies Reef for many years. Percent coral cover is relatively low compared to other reefs (Baker et al., 1992). During the collection of starfish, many were found to be feeding on algae, soft corals or not feeding at all, which indicates low food supplies. It would not be surprising if the starfish were generally in poor condition as a result of lack of food. Also noted during collection were the occasional patches of relatively high coral cover in which a group of starfish would be found. These starfish would probably be in a relatively healthier state than starfish found in low coral cover areas. This variability may have contributed to the lack of clarity of the starvation study results.

2.5.5 Seasonal Effects

Some seasonal effects on survival of the starfish were expected. *A. planci* tend to spawn from December to January on Davies Reef (Babcock and Mundy, 1992). The energy committed to spawning could have reduced the chances of survival after damage compared to the winter non-spawning time. Post-reproductive morbidity does not appear to have been documented in asteroids, but has been suggested for other invertebrates such as the fruit fly *Drosophila melangaster* (Partridge,1987).

Differences in water temperatures between seasons may also have affected survival. Warmer water temperatures can lead to an increased incidence of disease. Previous studies have shown that diseases have been implicated in the death of *A. planci* on a number of occasions (Sutton *et al*, 1988; Lucas, 1984).

Given the results of the initial winter 1991 (low mortality) and summer 1991/92 (high mortality) experiments (Sweatman and Butler, 1993), it appeared that seasonal differences were affecting starfish mortality. As it turned out, however, the second winter (1992) resulted in high mortality and the second summer (1992/93) resulted in low mortality. No explanations can be given for these differences in mortality. After all the experiments were completed and grouped, no seasonal effects could be detected.

2.5.6 Density Effects

Given the high mortality in the first summer experiment, it was suspected that the high density of starfish in the cages, in combination with the warm summer temperatures, might have increased transmission of infectious bacteria between starfish. High transmission rates of infections have been found to occur in *A. planci* (Lucas, 1984), although under aquarium conditions. The results of the damage/density experiments showed little difference in mortality between normal and reduced density cages. The cage with the reduced density of starfish, if anything, showed higher mortality than the higher density cage. This is an unexpected and unexplainable effect and is a another good example of the unpredictable variability found throughout the experiments.

The information to be gained from the density experiments is limited as a result of the limited scope of these density experiments. Firstly, the reduced density cages did not contain bi- or tri-sectioned starfish. Inclusion of these more damaged starfish could have increased the likelihood of bacterial infection as a result of reduced health stemming from the greater damage. Secondly, it was hoped that the difference in densities between cages would be adequate but there is still a question of whether a density of 10 or 25 starfish in a cage really makes a difference. Both are unnaturally high densities in confined spaces and the actual transmission rates of infection may not differ enough to be detected. Thirdly, these density experiments were carried out in winter. Water temperatures tend to be around 8°C colder at this time and bacterial infections may not play such an important part in mortality. Finally, the overall sample size for the density experiment was small which would make it difficult to detect small differences if they existed. Further investigation is clearly required.

2.5.7 Sex Effects

The possible influences of gender became of interest towards the end of these damage experiments. Previous studies (see Chapter 3) have shown that males and females respond differently when confronted with a need to heal and regenerate. Regenerating males showed reduced gonad production, while this was not true for females. This suggests that males invest more energy in somatic maintenance than females. If this is the case, then perhaps a greater proportion of males should survive damage.

An attempt was made to test differences in gender with respect to ability to survive damage. Unfortunately, few females could be found and the resultant experiment was on a very small scale and consequently inconclusive.

As discussed in Section 2.3.4.2, twice as many males than females were found. This finding is unlikely given the sample size (200+ starfish) and the 1:1 sex ratio usually found for *A. planci* (Birkeland and Lucas, 1990). Therefore, there is still the question of whether sexual differences influenced the survival of the starfish on Davies Reef. While it is possible that females are more cryptic than males just prior to spawning, as suggested by Babcock (Pers. com.), the mortality found in the Experiment #4 may be related to there being a higher proportion of maintenance-dominated males. These relatively healthy males may have been more capable of surviving damage than females. The effects of gender should be considered in any future experiments of this sort.

2.5.8 Handling Effects

While every effort was made to minimise the potential adverse effects on the starfish caused by handling (and to standardise this methodology), there is little doubt that the techniques employed may have contributed to the variation in mortality found in these experiments. For example, some starfish were more difficult to extricate from the coral than others and as a result may have suffered greater stress or damage. Handling time also varied between starfish. Some starfish were collected, treated and placed in the underwater cage within a space of 30 minutes, while others took up to 19 hours if they had to remain overnight in the holding bins on the boat. Finally, the removal of the starfish from the water for treatment could have placed considerable stress on the starfish (eg. loss of coelomic fluid) and may have affected mortality. This effect may explain the occasional mortality of undamaged control starfish. The degree of effect may have varied between starfish depending on, for example, the amount of coelomic fluid lost.

2.5.9 Control of *A. planci*

Mutilation may serve as a means of local *A. planci* control, though careful consideration of its limitations is required. *Acanthaster planci* shows reasonable resistance to damage but is not as capable of surviving damage as are some other starfish such as *Nepanthia belcheri* and *Linckia spp.* (Emson and Wilkie, 1980). This study has shown that severe mutilation does not guarantee mortality since the effect of mutilation appears to vary with time between individuals, populations and locations. Even chopping starfish into quarters is no guarantee that 100% of the portions will die, as shown by experiments in Okinowa (Birkeland and Lucas, 1990) and more recently the Great Barrier Reef (U. Englehardt- pers. com.). Chopping them into less than four pieces therefore has the potential to increase the population if the pieces are capable of surviving. Trials should be conducted prior to any campaign of *A. planci* control via mutilation.

A. planci control programs by the Great Barrier Reef Marine Park Authority have previously attempted mutilation for controlling starfish numbers and it is not considered a preferred option (B. Lassig, pers. com.). They have found this method to be very labour intensive, taking 30-50 times longer than copper sulphate injection (i.e. 60 - 120 seconds to remove and mutilate, versus 2 seconds to inject *in situ*). This makes mutilation less economical in terms of person-hours (n.b. Note that this assumes searching is negligible time in outbreak conditions). In addition, the increased amount of handling the starfish leads to an increase in numbers of painful spinings.

Overall, if local control is considered to be needed in the future, the toxic environmental problems posed by methods such as copper sulphate injection will need to be weighed against the variably successful and more labour intensive technique of mutilation.

2.5.10 Future Experiments

These experiments have provided a wealth of practical experience with *A. planci*. In particular, the variability in the results has indicated the complexity of *A. planci* biology. Given the lessons learned in these experiments, there are a number of ways that future experiments can be improved to account for the potential variation.

2.5.10.1 Caging

In these experiments, cage and site effects were assumed to be non-existent since the cages were similar and in each experiment spatially fairly close together (within 30 metre radius). However, some of the cages were closer to coral outcrops/bommies than others, which may have facilitated attacks by the various benthic predators known to prey on *A. planci* (though none were seen). The positions of cages should in future be standardised in their proximity to such underwater features.

2.5.10.2 Starfish Size

It was hoped prior to the commencement of these experiments that the starfish collected would have sufficient diversity in size to enable size related experiments. Unfortunately, the starfish were all of a similar size and size related experiments could not be attempted. Size has been strongly suggested by Birkeland and Lucas (1990) as a potentially important source of variation in *A. planci*. As starfish increase in age, they tend to spend more on reproduction and use up energy stored in the form of test (Kettle,1991). This would potentially affect the ability of *A. planci* to seal and heal wounds. Therefore, if a large range of sizes is available, size treatments should be incorporated into any future experimental design.

2.5.10.3 Damage Treatments

Slight variations in damage treatments were ignored in these experiments since there was no simple way of making all the starfish within a damage group the same. While every effort was made to standardise the handling and treatment (e.g. controls were left out of the water for generally the same length of time as those starfish being mutilated), it was unavoidable that some differences in handling and treatment occurred. Some examples of these were: different lengths of time out of water, slight injury to adjacent arms, variable loss of coelomic fluid in controls, difficulty in removing starfish from water tanks, incidental damage during collection, and differing transport times from collection sites. Attempts should be made in future experiments to standardise or eliminate all of these potential sources of variability (e.g. transport and cut the starfish underwater if possible).

2.5.10.4 Long Term Effects

As discussed earlier, long term ability to survive damage was not in the scope of these experiments. In order to assess this, there must be a way to maintain contact with the starfish and yet allow them to feed naturally. The problem of long term tagging of *A. planci* persists and must be surmounted if successful long term studies are to take place.

Chapter 3

An Investigation Into the Effects of Sub-lethal Predation on Gonad Weight in *A. planci*

3.1 Introduction

In Chapter 2, the first of two ways in which a predator can control population of *A. planci* - mortal predation - was examined. In this chapter, the second method will be examined - whether partial predation can influence populations through reduced fecundity.

A. planci clearly experiences predation throughout its life history. While not a preferred food item, the eggs and sperm are known to be eaten by fish (Keesing and Halford, 1992), as are the larvae (Moran, 1988). Juvenile starfish suffer heavy predation from small benthic organisms such as crabs (Keesing and Halford, 1992). Finally, adults are preyed upon by a variety of invertebrates and fish (Birkeland and Lucas, 1990; Pers. observ.), though the number of sightings are low (e.g. Marine Bio Logic, 1990). While the natural limitation of adult populations has been suggested to occur naturally in the eastern Pacific as a result of predation by the amphinomid worm *Pherecardia striata* (Glynn,1984) and the shrimp *Hymenocerca picta* (Glynn, 1981), there is still very little evidence that *A. planci* populations are limited, or even eaten in great numbers, by fish.

Any organism may be affected by predators through partial sub-lethal predation. In the case of those with regenerative ability, subsequent healing and regenerative growth may reduce the allocation of energy to reproduction (Harris, 1989). The degree to which such partial predation may affect the fecundity of an individual is based on the regenerative process and how this may interact with the life history and the environment of the injured individual.

The process of regenerative healing has been shown in many organisms to be energetically expensive. This is also true for echinoderms. For example, reefal ophiuroids show over an order of magnitude increase in rates of respiration and excretion when regenerating arms (Sullivan, 1988). This regenerative growth can result in up to 14 times more energy usage than for normal growth (Sullivan, 1988). If partial predation occurs repeatedly, large proportions of energy may be diverted to healing and regenerative processes. For instance, the ophiuroid *Amphiura filiformis*, which has its arms cropped by fishes, has been shown to have 76% of its tissue growth in the form of regenerating tissue (O'Conner *et al*, 1986). Given these high energy costs, it is not surprising that regenerative growth may divert energy from other important physiological activities.

Regenerative growth has been shown to be correlated with reduced fecundity in many organisms such as lizards (Dial and Fitzpatrick, 1981), polychaetes (Zajac, 1985), and plants (Crawley, 1983). This has also been found in the Echinodermata. The asteroids *Pisaster giganteus* (Harrold and Pearse, 1980) and *Coscinasterias calamaria* (Johnson and Threlfall, 1987) both show reductions in gonad development or fecundity apparently due to the cost of regeneration. The asteroid *Nepanthia belcheri* has been shown not to reproduce sexually and display gonad regression during post-fission regeneration (Ottesen and Lucas, 1982).

There is no set life history pattern in echinoderms for allocation of energy to either regeneration or reproduction. Regeneration may not always be dominant to sexual reproduction in terms of energy allocation. For example, in the asteroids *Luidia clathrata* and *Echinaster sp.*, reproduction may take precedence over regeneration (Lawrence, in press; Lawrence *et al.*, 1986). If *L. clathrata* sustains an injury during times of spawning and food levels are low, the wound is healed but there is no regenerative growth. Gonad production, however, does occur. In these same experiments, *L. clathrata*, if provided with ample food, regenerates lost tissue and continues normal gonad production (Lawrence and Ellwood, 1990). This demonstrated the importance of food supply to allocation of energy.

The studies in this chapter examined differences in gonad weights between starfish with and without regenerating arms in a natural population. Differences in gonad weights were considered at three levels in order to establish effects on gonad weight by regeneration.

First, regenerating and non-regenerating starfish were examined over the whole sample to see if there were significant differences in total gonad weight. This was the level of primary interest since it is at this level that differences would indicate the potential for substantial effects on fecundity. It is also at this level that reduced gonad weights could realistically be expected to affect future recruitment. It was also expected that this would be the only level at which a reduction in gonad could be detected, given the open structure of the coelom which allows for the general sharing of nutrients equally among arms.

Second, regenerating and non-regenerating arms within a regenerating starfish were compared. This, in combination with the analyses at the other levels would indicate whether effects of regeneration are localised around the site of regeneration or whether the effects are generalised all over the starfish.

Finally, differences between regenerating and non-regenerating lateral halves of gonads within adjacent arms (explained later) were examined for differences in gonad weight. This would provide more information on the physiological connectivity between the gonad lobes in adjacent arms.

There was also an assessment of gonad weights in short arms as compared with gonad weights in regenerating and normal length non-regenerating arms.

3.2 Methods

3.2.1 Pilot Study

A pilot study was carried out to gain insight into the physiology of *A. planci*. Unpublished data consisting of body wet weights, total gonad weights, and sexes for starfish from Davies Reef and other nearby reefs in the central section of the Great Barrier Reef were obtained from Dr. Russ Babcock from the Australian Institute of Marine Science.

Models were fitted via multiple regression to try to identify relationships in the data. This kind of analysis is described in detail in Section 3.2.6. Male *A. planci* tended to be 5% smaller in body wet weight than females and they produced 6-10% less gonad, even after adjustment for differences in body weight.

Using the methods of Cohen (1988), power analyses were conducted on the pilot data to estimate the number of male and female starfish that would be required for the main study. In order to obtain meaningful results at power = 0.8, around 170 females (85 regenerating and 85 not regenerating) and 100 males (50 regenerating and 50 not regenerating) were required. It was anticipated that such numbers would be difficult to obtain, but the study was attempted with these numbers as a goal.

3.2.2 Main Study, Method of Collection

Acanthaster planci were collected between the 15th and 25th of November 1991, about two weeks prior to the commencement of their spawning period. A total of 149 starfish were collected by the same methods used for the survival experiment in Chapter 2. Ninety-seven starfish were collected from Lynchs Reef, adjacent to Davies Reef (Latitude 18°50′, Longitude 147°40′). These were of a uniform small size of about 30cm diameter. Due to the difficulty in finding enough starfish to reach the desired sample sizes and an interest in obtaining larger-sized specimens, the collection site was moved to Davies reef where the remaining 52 starfish were collected.

Up to 30 starfish were collected and placed in the 1000 litre tank prior to commencing dissection. The total time spent in the tank, essentially the time needed to complete the dissections of 29 starfish, was up to 48 hours.

3.2.3 Dissection Method

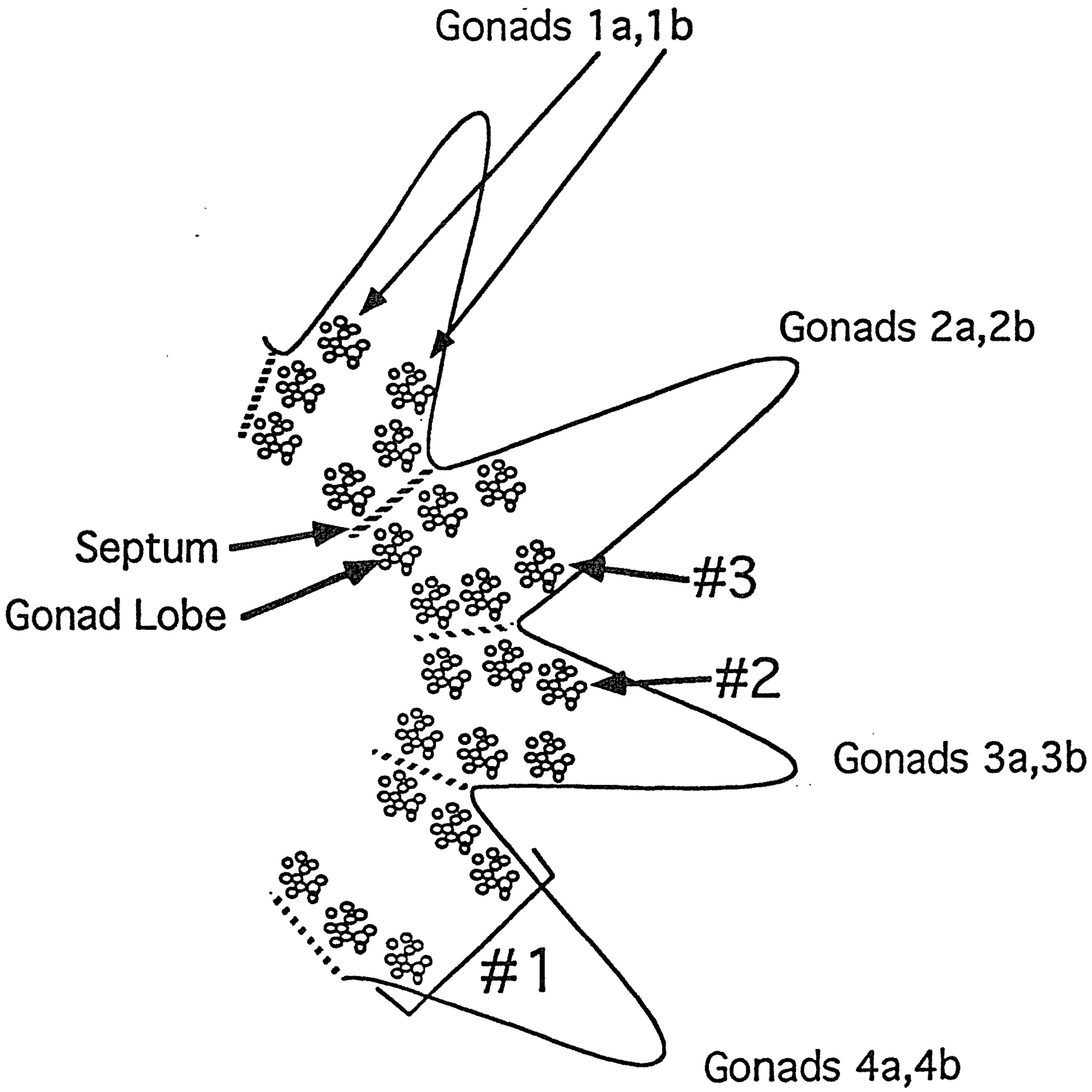
The steps for processing the starfish were as follows. First, a starfish was removed from the 1000L tank. This starfish was held up for about three seconds and shaken slightly to drain excess water, then placed into a plastic tray. The time taken for this initial step was minimised because the body wall of the starfish bursts easily and a substantial portion of the wet weight of the starfish is lost with this coelomic fluid.

Next, the starfish was wet weighed. The starfish was checked for regenerating arms. Categorisation of whether or not a starfish was regenerating was based on whether there was obvious new tissue being formed. Regenerating arms are distinguishable by their lighter colour, their slenderness, and shortness of their spines. The range of starfish described as regenerating included those with small new growth buds on obviously damaged arms to those with long new arms. All other starfish arms, including those deformed or short, if not obviously regenerating, were categorised as not regenerating.

Before dissection, a starting point was chosen and the starfish arms were numbered consecutively from this starting point (See Figure 3.1). All regenerating arms were identified using the arm number. The length of the regenerating arm was estimated by comparing it to the same starfish's normal length arms (e.g. 80% normal length). The amount of regeneration actually taking place on that arm was also estimated. This was described as the percentage of the whole regenerating arm taken up by the regenerating portion. A range of abnormalities in the physical appearance of the starfish was noted, such as

Figure 3.1 Anatomical diagram of some arms of A. planci with gonads and septa identified. Method of numbering is also shown.

#1 = one arm of gonads between septa
#2,#3 = physiologically connected gonad lobes on either side of a septum



short undamaged arms, miniature arms, or arms bifurcating midway.

To aid explanation, Figure 3.1 shows a diagram of a number of arms of *A. planci* and the relative position of gonads and septa. Initially, dissection involved the removal of all the gonad material from each arm as delineated by septa (i.e. all the gonads between septa and in area labelled #1 (Fig. 3.1)). A slit was made with a long handled scalpel on the aboral side of the arm. The sex was noted. The gonad material was placed in a freezer bag and the bag given the number of the arm. This procedure was completed for all the arms of the starfish. The gonads were then frozen.

It was decided during the course of the experiment to try and separate the two halves of the gonads within the arms to gain a better picture of the distribution of gonad weights. Anatomically, the gonads are arranged in lobes attached to the septa on either side of the arm within the oral disk. Each septum supports gonad lobes on both its sides, so the gonad lobes on contiguous sides of adjacent arms may be physiologically and anatomically related (i.e. gonads labelled #2 and #3 in Figure 3.1 may be related). In forty of the starfish, the sides of each arm were labelled A and B and the direction of dissection around the starfish was noted.

Finally, a somatic weight was obtained after the dissection was complete. Somatic weight represents the weight of the left-over material after dissection. It consisted of all material except the gonads and coelomic fluid. This weight was taken in addition to the wet weight to avoid the probable variance associated with wet weights and the likelihood of coelom rupture. Total dissection time was up to one hour per starfish.

3.2.4 Laboratory Processing

The frozen gonads were weighed to 0.0001 g and then placed in a 50^0 C. oven. The drying time for the gonad samples was found to vary with gonad size. Completely dry weights were found by letting a series of samples dry until no more weight change was seen in the fourth decimal place. This required thirteen days for the largest sample (Figure 3.2). To dry all the 3000 arm samples for this length of time was too time consuming so it was decided to dry the samples to where they were within 5% of the asymptotic dry weight. This was estimated from Figure 3.2. After half of the starfish arms had been weighed, the relationship between the wet and dry weights was examined (Figure 3.3). Ninety-eight percent of the variation in the dry weight was explained by the variation in wet weight. Since the relationship was linear and wet weight explained 98% of the variation in dry weight, the relationship seemed good enough to discontinue the drying

Figure 3.2 Rate of drying of *A. planci* gonads of three sizes at 55°C. Weight is expressed as the percentage deviation from final dry weight.

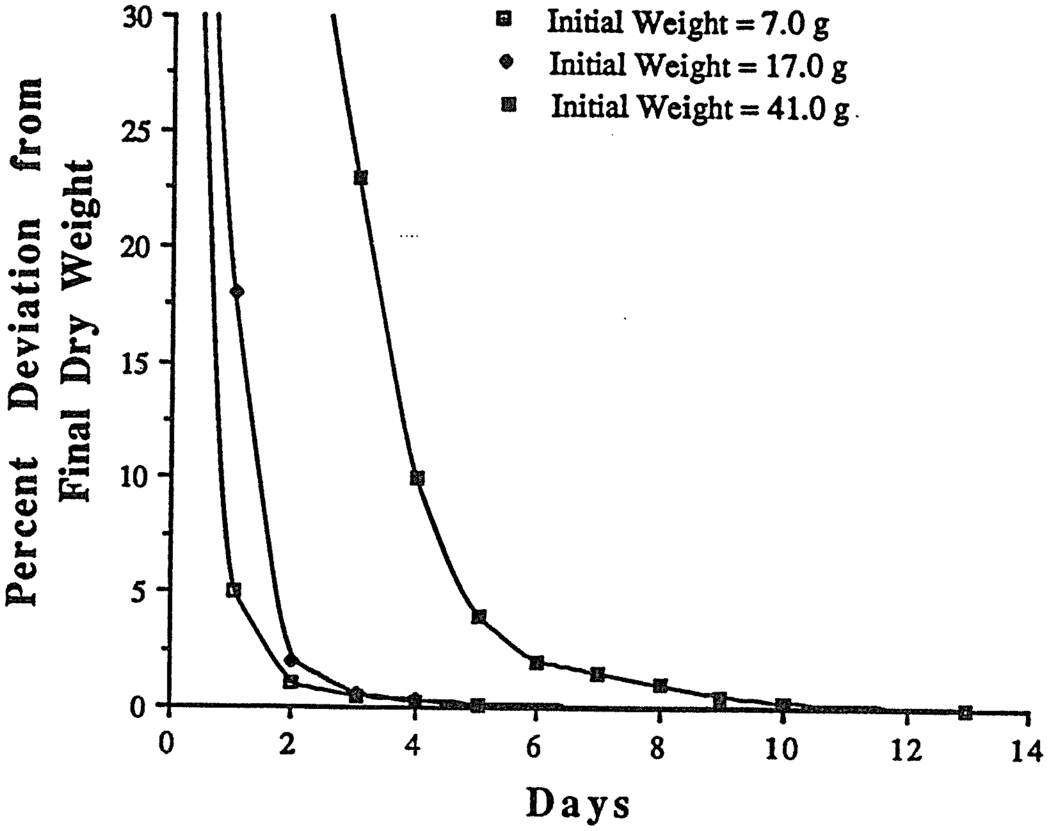
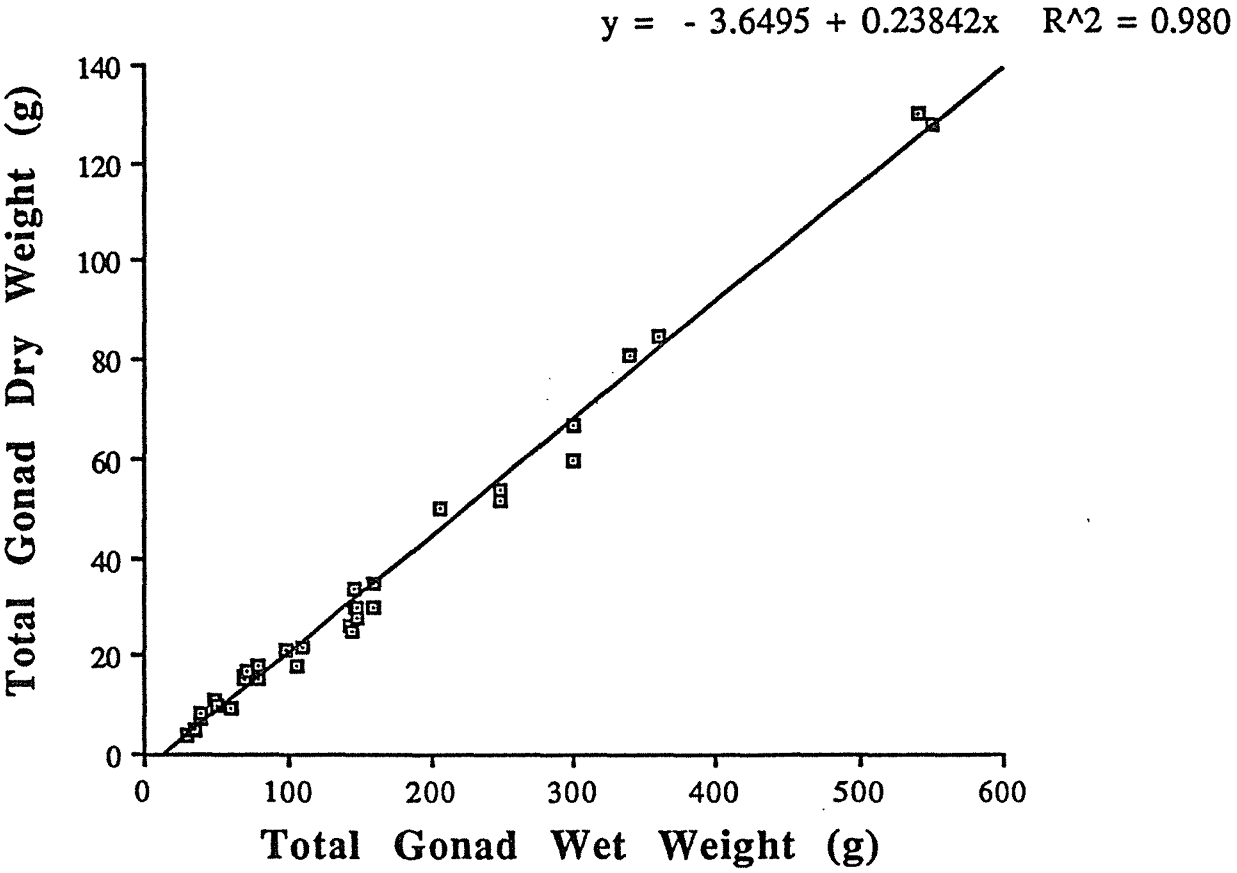


Figure 3.3 Relationship of total wet gonad weights to total dry gonad weights in *A. planci*.



process. For individual arms, the variation in dry weight explained 97.6% of the variation in wet weight.

3.2.5 Arm Selection Methods

Selection of individual arms for the whole-arm and half-arm studies was as follows. Only starfish that were regenerating were used for these studies. The number of arms to be used in the statistical analyses depended on the number of regenerating arms present in the starfish. This will be best explained through an example.

If a starfish had three arms regenerating, then the three gonads weights for all three arms were entered into the "regeneration" category. For each one of the regenerating arms, one of two adjacent arm's gonads were randomly chosen and were entered into the "adjacent" category and one normal arm was randomly selected and its gonads entered into the "normal" category. No adjacent arms were considered as normal. Therefore, in this example, there would be three different sets of gonads in each of the three categories. Several other conditions applied. In some cases where there were several adjacent regenerating arms, it was not possible to enter data for an adjacent arm. Starfish with more than four regenerating arms were left out of the analyses because of the difficulty in obtaining normal arms. In the half-arm studies, only one half of a regenerating arm was used and the adjacent half was necessarily the one physiologically connected.

3.2.6 Statistical Methods

3.2.6.1 Multiple Regression Modelling

The statistical methods for testing hypotheses via multiple regression are similar to analyses of covariance. Multiple regression models, which are actually equations describing trends in the data, were produced. The models are initially of a large size containing all the possible variables or terms. For example, the first model to describe the male population included terms for: a constant; somatic weight; presence or absence of regeneration; and interaction between regeneration and somatic weight (i.e. the regressions describing regenerating and non-regenerating starfish have different slopes). If one of the terms was not significant (assessed by t-test) to the overall model, then that term was removed. This method of removing terms is called "Backward elimination". Once all the terms have been examined for significance, the remaining model is considered to be the simplest model that describes the data. Whichever terms remained in the model were considered to make a significant contribution to describing the data and were likely to be biologically important. For example, if the term describing the presence/absence of regeneration remains in a model, then regeneration is considered to be important to the model and is considered to be biologically important in determining the gonad weights of the starfish.

Generally, the first set of explanatory terms removed were differences attributable to slope. For this study, differences of slope would have meant some interaction between body size and the various explanatory terms. In some cases, terms describing measurements were removed along with slope differences to simplify the description of the backward elimination. In either case, if more than one of the descriptor terms is removed in a single analysis, a Nested F-test is required in place of the t-test (Collett, 1991). Calculation of Nested F is via the following equation:

$$F((df_a-df_b),df_b) = \frac{(SS_a - SS_b) / (df_a-df_b)}{MSE_b}$$

where: a = simpler model
b = the previous and more complex model
SS = sum of squares
MSE = residual mean square
df = degrees of freedom

Nested F-tests can also be used to remove a single term. In the statistics package used, however, a t-test is provided which determines the significance of each term individually. The t value provided is directly comparable to the F from the Nested F-test for single terms. The more complicated Nested F-test is therefore not required for each single term elimination.

3.2.6.2 Power Analyses

Power analyses were performed on the analyses of total gonad levels where no significant differences were found. The methods suggested by Cohen (1988) for power analyses of Analysis of Covariance were employed. The minimum desired power was set at 0.8, as suggested by Cohen (1988) and Fairweather (1991). The alpha level was held at 0.05. An effect size of 25% was used to calculate power. Where required, assessments of recommended sample sizes at power = 0.8 are provided.

3.3 Results

3.3.1 General Results

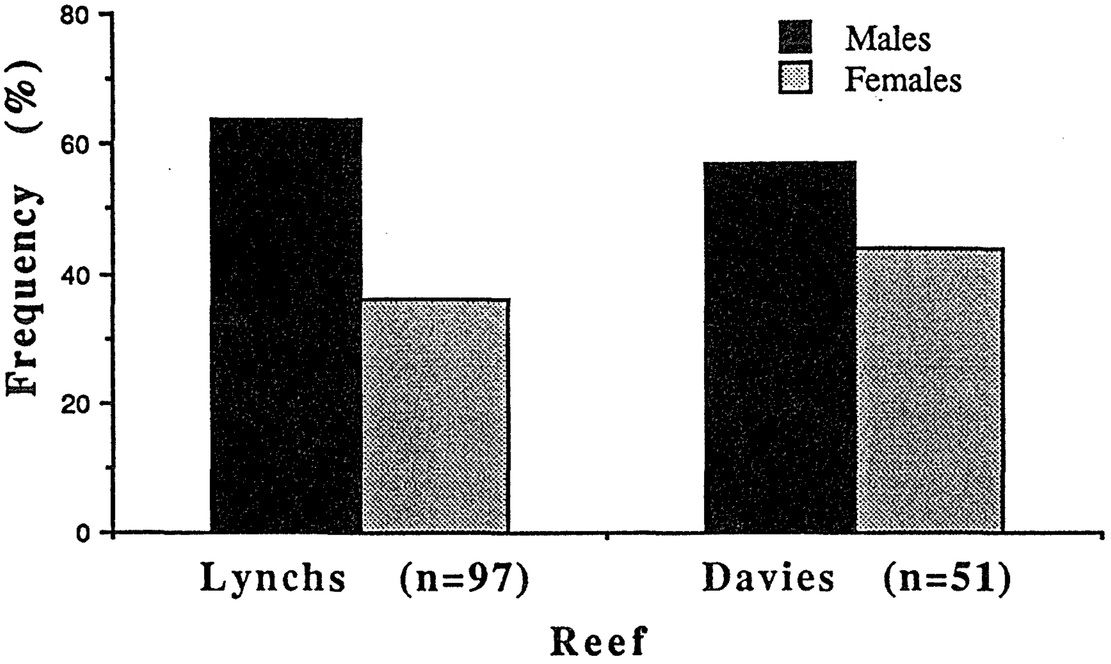
A total of 148 starfish were dissected. There were almost twice as many males(91) as females(57), a result of the high proportion of males on both reefs (Figure 3.4).

Five starfish were omitted from the analyses. One was omitted due to the extreme outlying value of its gonad weight. The gonads of this starfish were brownish in colour which suggested that it had spawned. The remaining four starfish were omitted because of missing data.

Seven male and three female starfish had deformities that were assumed to be insignificant for the analyses and they were left in the data set. Four starfish (2 males and 2 females) had arms bifurcated beyond the body disc; these two were considered as one arm. Five starfish (all male) had fewer than normal numbers of arms (around 11) and a large "dent" in the side of the body disc. On either side of this "dent" were miniature arms. One female had a miniature arm that was found on the oral side of the body disc. These miniature arms were included in the data set as normal arms.

Average male somatic weight for both reefs was 887g, while the average female somatic weight was 934g, but this

Figure 3.4 Frequency of male and female *A. planci* collected from Davies and Lynchs Reefs.



was not statistically significant (t = -0.84 P= 0.4325). The total range of somatic weights was 348 to 1878g, the range for each sex is shown in Figure 3.5.

The population size structures for Lynchs and Davies Reefs are shown in Figure 3.6. Sizes have been expressed as wet weights for ease of comparison with other studies. As is evident from this figure, the two populations were very different in size structure. Lynchs Reef had a dominant cohort of starfish around 1200g while starfish on Davies Reef were spread widely between 800g and 3000g.

Relative average somatic weights of males and females on Lynchs Reef are 767g and 724g (not statistically significant t = 0.98, P= 0.3285). On Davies Reef, average male somatic weight was 1155g while average female somatic weight was 1232g (not statistically significant t = -0.81, P = 0.4325).

Figure 3.7 shows diameter versus somatic weight for the starfish of the two reefs. The starfish on the two reefs clearly have the same relationship, as shown by the line of best fit, though the starfish of Davies Reef are larger.

The number of arms per starfish ranged from 13 to 19 with 90% or more having 15 to 17 arms. The frequency distribution for the number of arms per starfish in the

Figure 3.5 Size frequency of male and female *A. planci* collected from Lynchs and Davies Reefs combined.

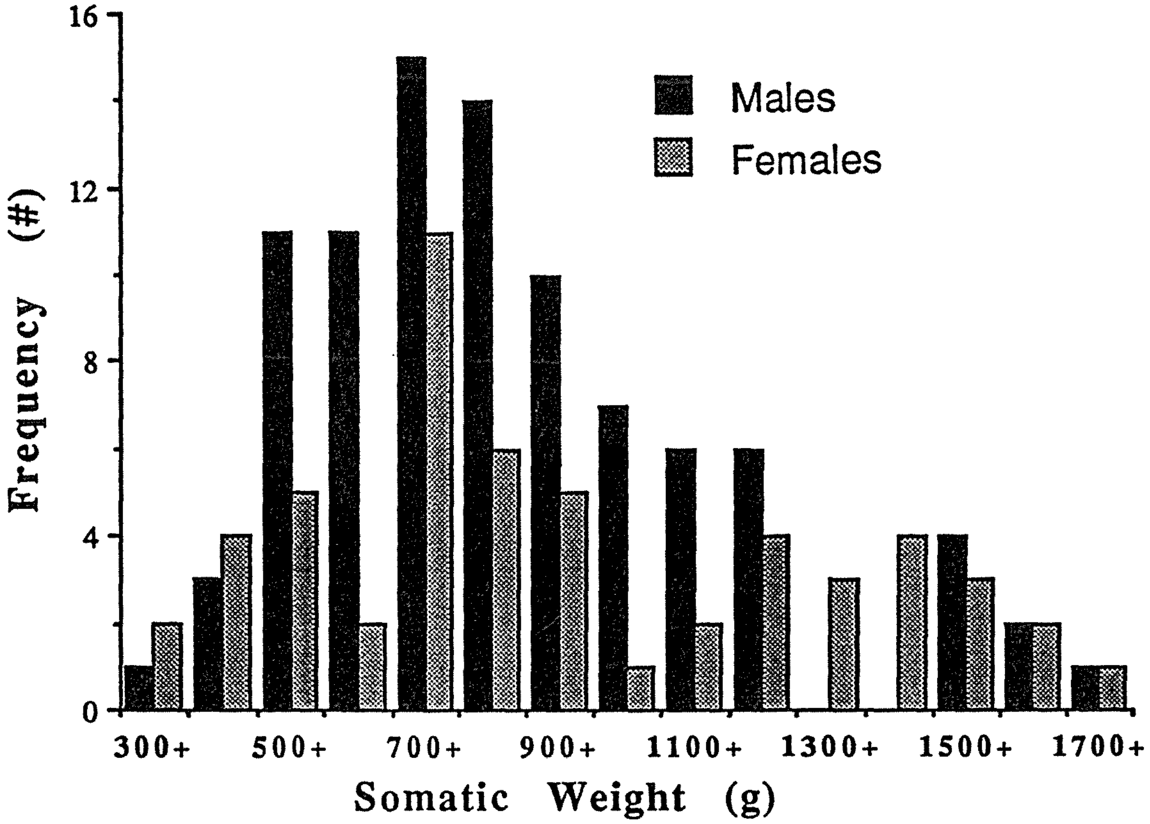


Figure 3.6 Frequency distribution of wet weights of *A. planci* collected from Davies and Lynchs reefs (sexes combined).

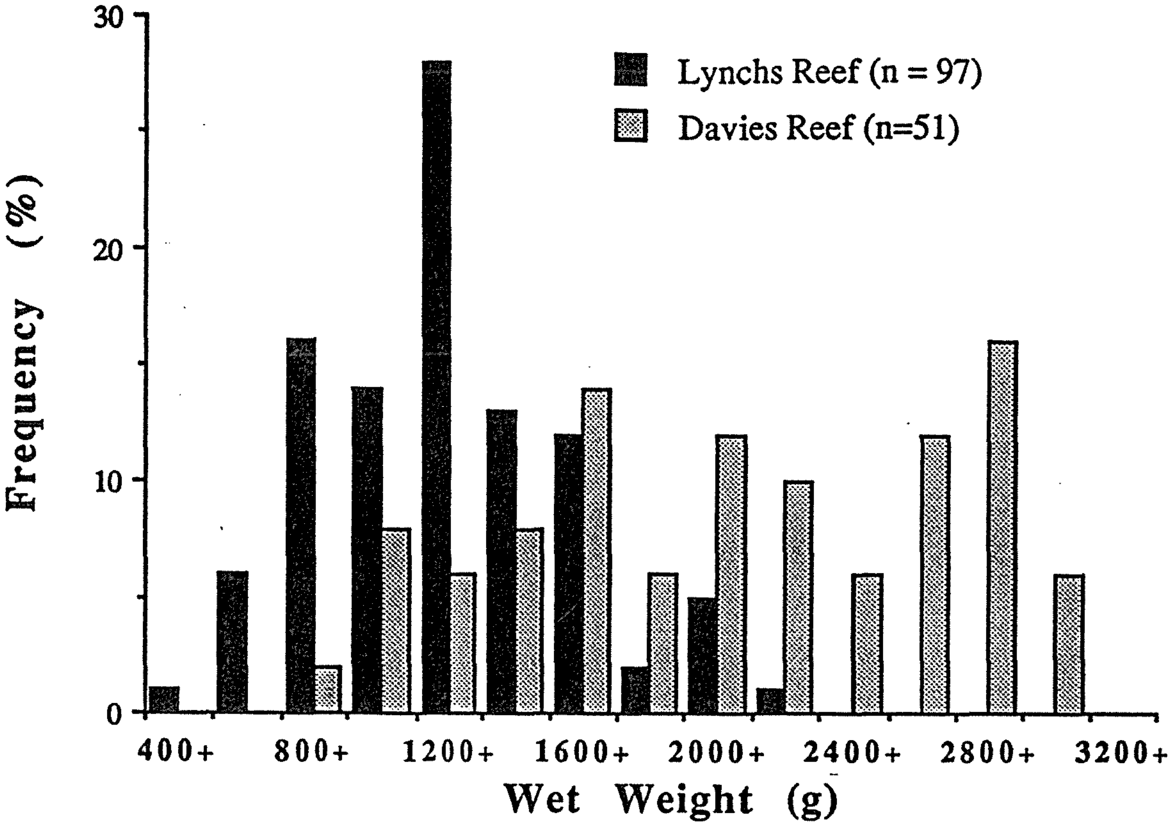
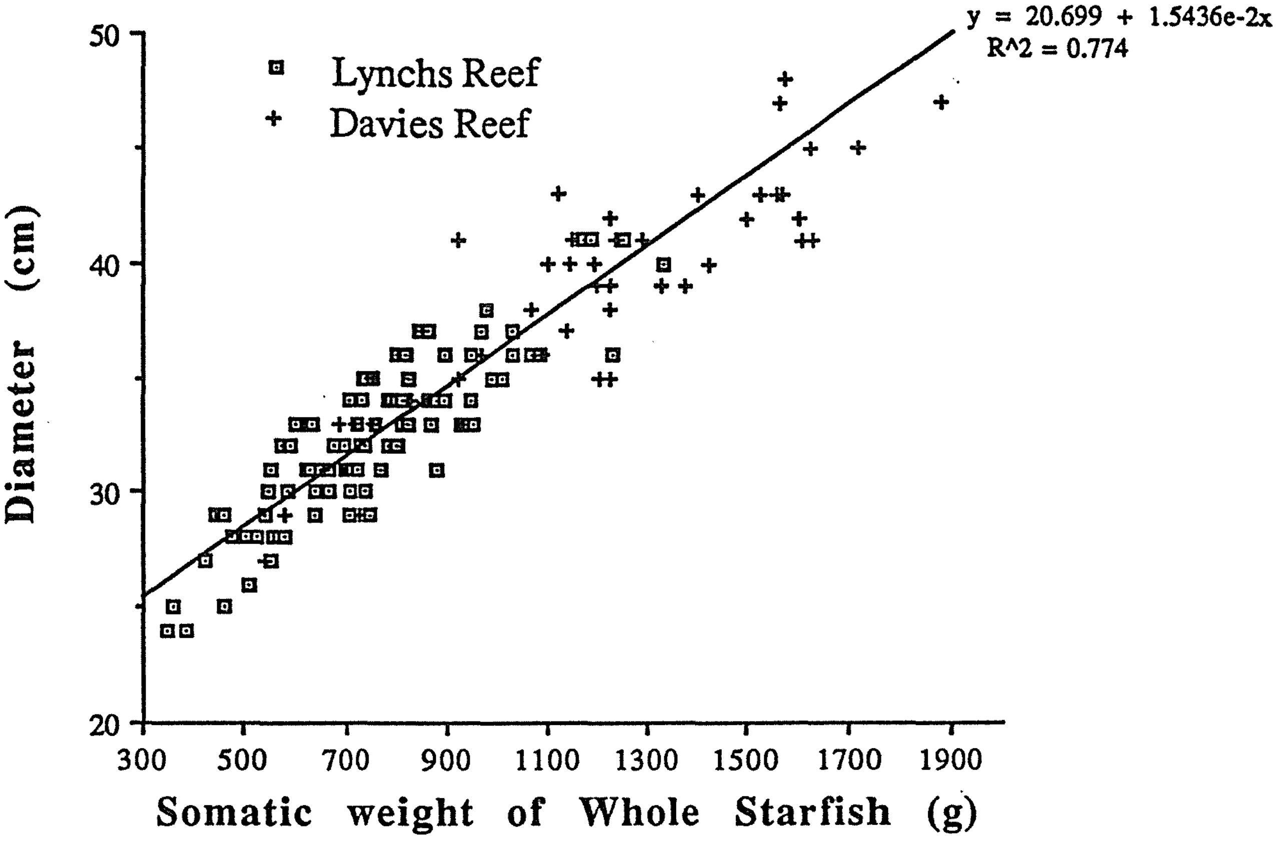


Figure 3.7 Diameter versus somatic weight for *A. planci* from Davies and Lynchs Reefs.



two sexes is nearly identical, as shown in Figure 3.8. Figure 3.9 shows arm number versus somatic weight. There is a positive relationship between increasing numbers of arms and increasing somatic weight but there is also great variation, as is evident from the 95% confidence limits.

Up to seven regenerating arms were found on starfish in this study. The mean number of damaged arms did not differ between the sexes (Figure 3.10; t=0.63, p=0.5195). There was also no difference between sexes in the frequency distribution of the damage ($X^2_{(d.f.=3)}$ = 3.86, p>0.25).

The change in Gonad Index (percentage of somatic weight represented by gonad weight) with size is shown for both sexes in Figure 3.11 a,b,c. Gonad index ranges from a mean of 3% at 200g somatic weight to a mean of 12% at 2000g for males and between 4% to 17% for females over the same weight range. These data are shown in terms of diameter in Figure 3.12 for comparison to other studies. Males vary from 3% at 20 cm to 10% at 50 cm and females vary from 3% at 20 cm to 17% at 50 cm. These results are compared to Kettle and Lucas (1987) in Figure 3.13.

3.3.2 Results Applicable to Statistical Analysis

As in the pilot data, the relationship between body size and total gonad size was found to be a power curve.

Figure 3.8 Frequency of male and female *A. planci* versus number of arms.

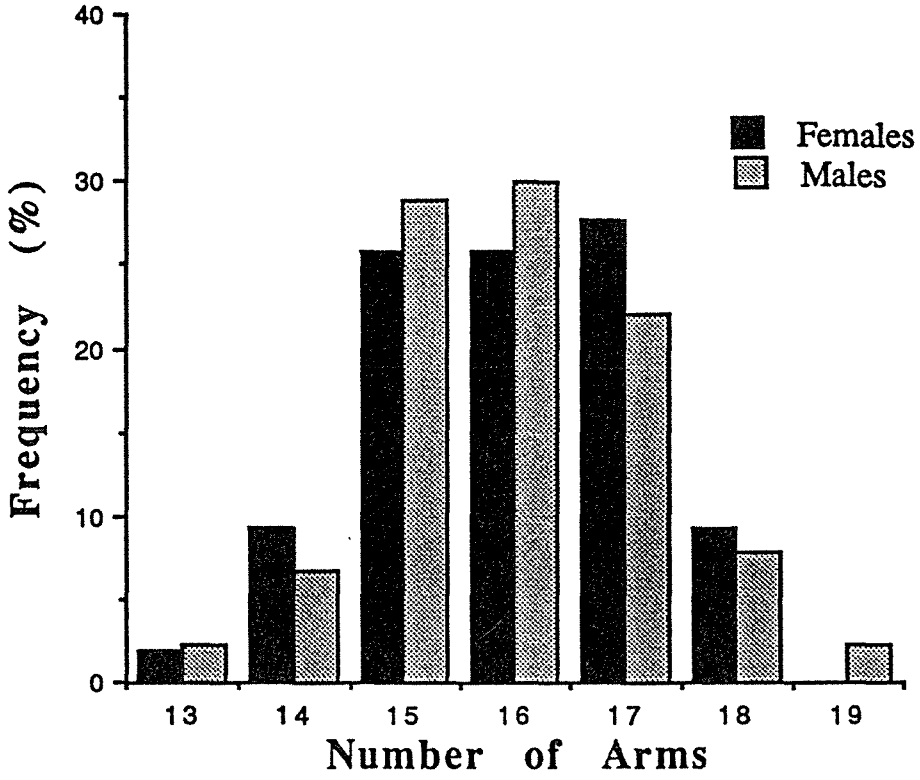


Figure 3.9 Mean somatic weight (with 95% confidence limits) of *A. planci* versus number of arms.

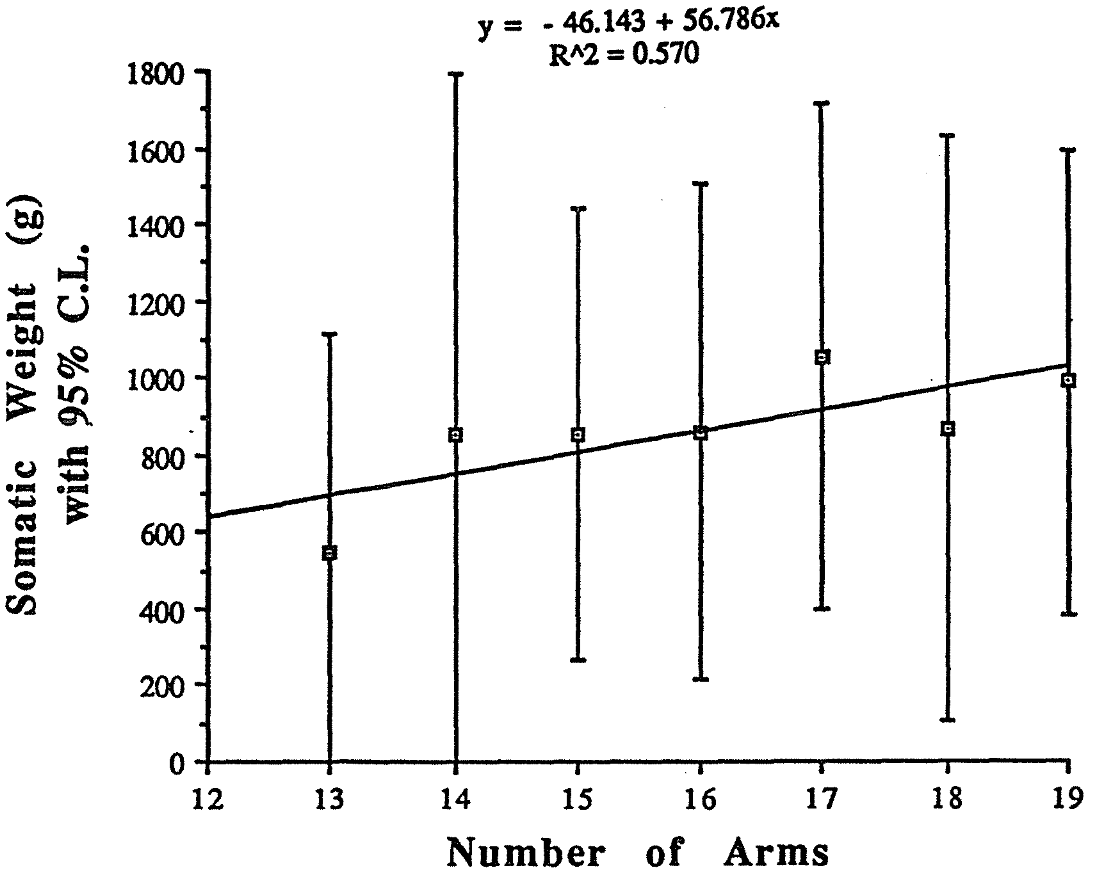


Figure 3.10 Frequency of regenerating arms in male and female *A. planci* from Davies and Lynchs Reefs.

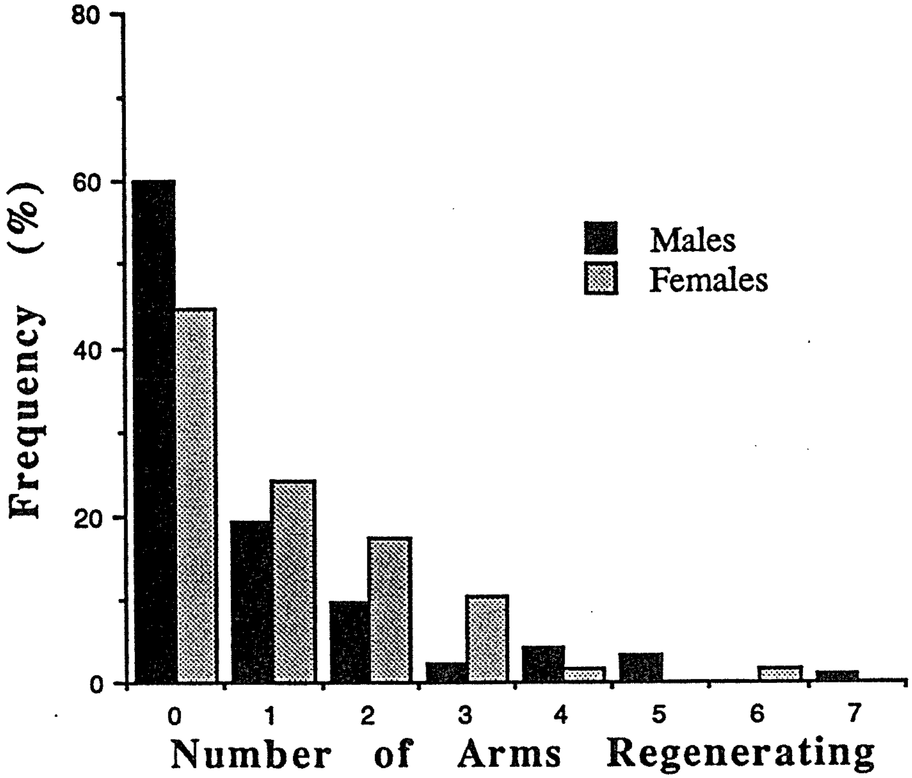


Figure 3.11 Total gonad weight as proportion of total body wet weight (Gonad Index) for *A. planci* plotted against somatic weight. Sexes are separate.

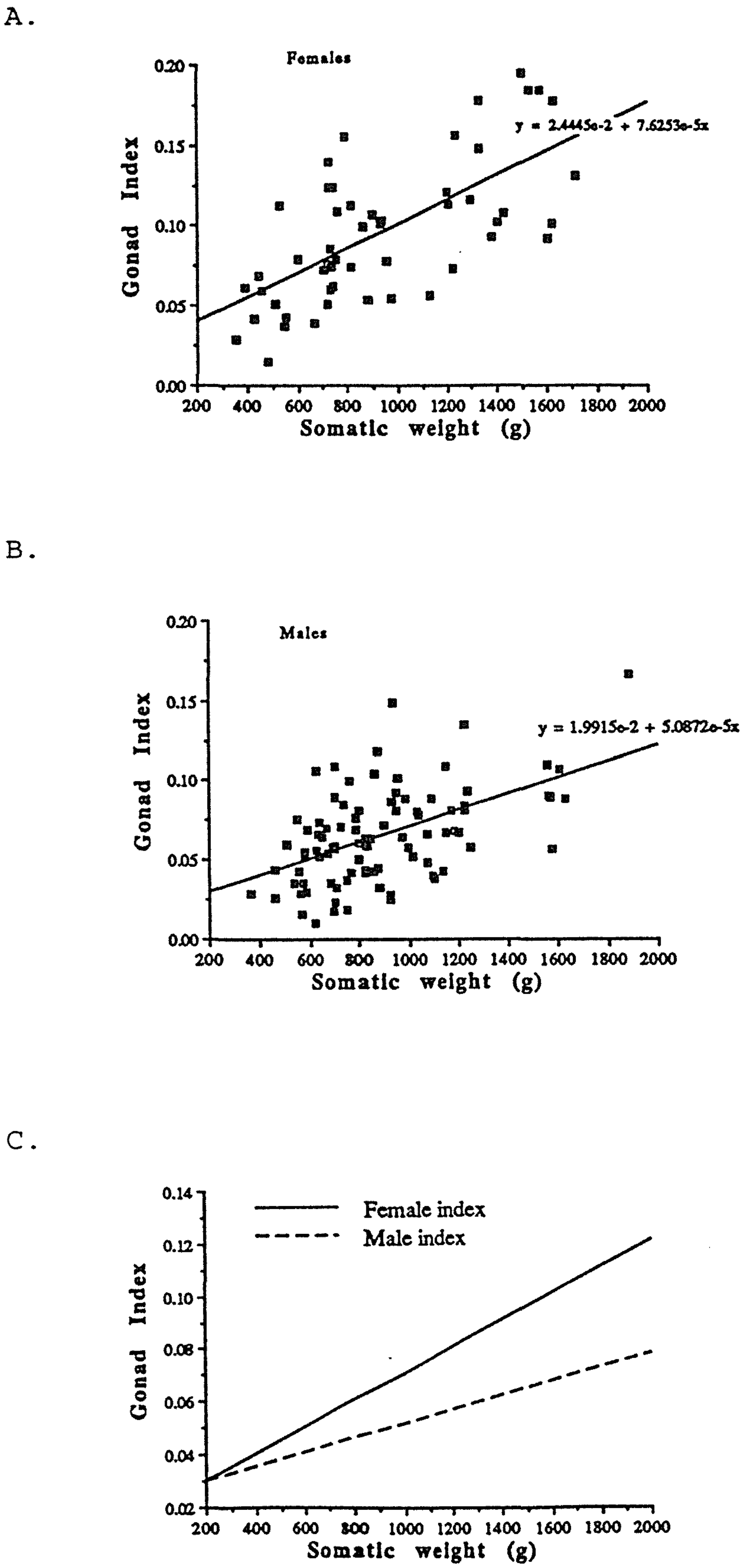


Figure 3.12 Total gonad weight as proportion of total body wet weight (Gonad Index) for *A. planci* plotted against starfish diameter.

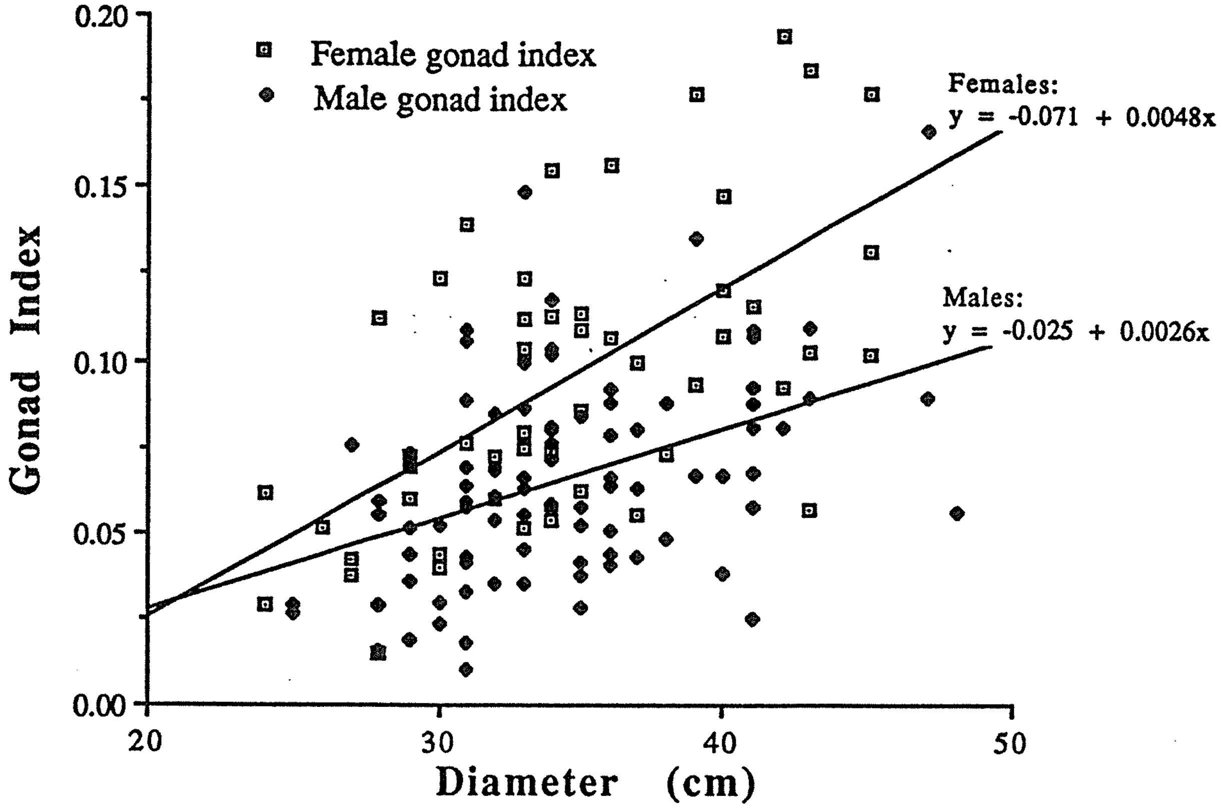
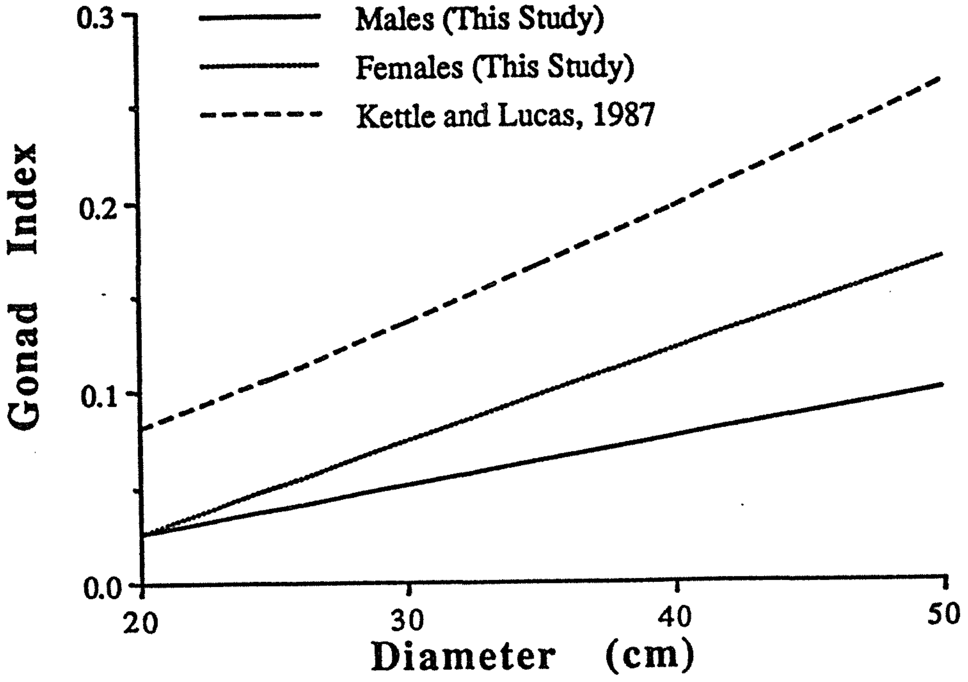


Figure 3.13 Total gonad weight as proportion of total body wet weight (Gonad Index) for *A. planci* versus starfish diameter. For comparison, the results of Kettle and Lucas,(1987) from Helix Reef (sexes combined) have also been included.



The data were found to be non-normal due to an increased variability of gonad size with increased body size. The data were made normal and more linear by square root transformation of the gonad weights. This was confirmed by inspection of the residuals. A log transformation is a commonly employed transformation for this type of data since it provides convenient back transformation. The square root transformation was used because it explained the variance in the data better than log transformation and because it provided data that were more normal and more linear in appearance, as determined by analysis of residuals and rankit plots.

The equation describing the relationship between somatic weight and gonad weight for both sexes is:

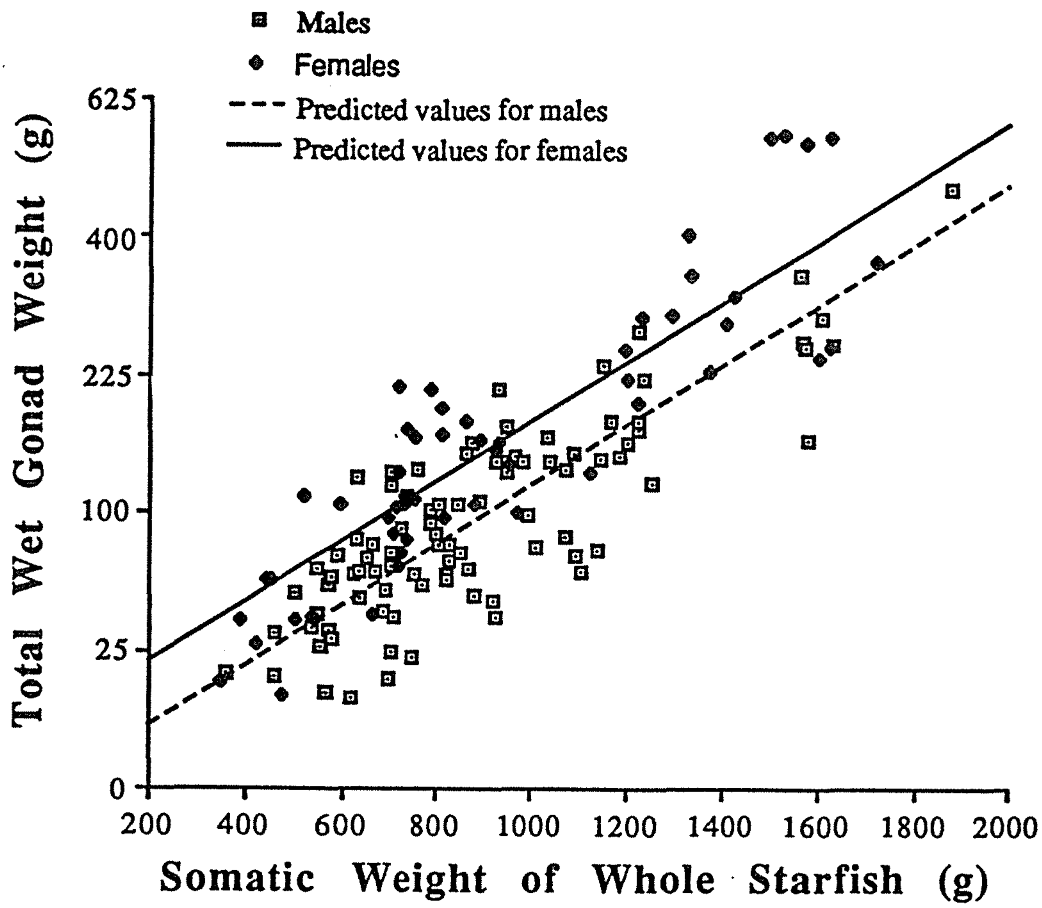
$$TotalGonadWeight=(0.10761+0.01087\,wght+2.30130\,sex)^2$$

where: wght = somatic weight
sex : male = 0, female = 1

There is a significant difference between the sexes ($p=0.0000$) with regards to total gonad weight. Male total gonad weights, where male and female somatic weights were adjusted to be the same, were around 26% smaller than the female total gonad weights (Figure 3.14).

Wet gonad weights and somatic body weights (= wet body weights less gonads and coelomic fluid) were used for

Figure 3.14 Total gonad weight versus somatic weight for *A. planci*, sexes separate. Regression lines indicate the values predicted by the equation on the previous page for the total gonad weights of males and females. Y axis is square root scale.



all of the tests. The somatic body weights were used because they explained 10% more of the variance as compared to wet body weights less gonads. This was probably because the wet weights suffered from variability as a result of lost coelomic fluid during handling and prior to weighing. The relationship between somatic and wet body weights for the starfish in this study are shown in Figure 3.15.

3.3.3 Results at Whole Starfish Level

Male starfish show a general reduction in total gonad weight when one or more arms are regenerating. There was a 26% decrease in gonad weight in the smaller starfish and a 10% decrease in gonad weight in the larger starfish (Figure 3.16).

The relationship between regeneration and gonad weight does not vary significantly with body size. The 10% to 26% range described above is simply a result of the absolute differences in the gonad weight number and how they are affected by the back transformation from square root.

Table 3.1 shows the backward elimination of factors to give the simplest model. The interaction term ("slope") was the first term to be removed. Its removal exposed the significance of regeneration (Model B).

Figure 3.15 Relationship between somatic weight and wet body weight for *A. planci* sampled in these experiments.

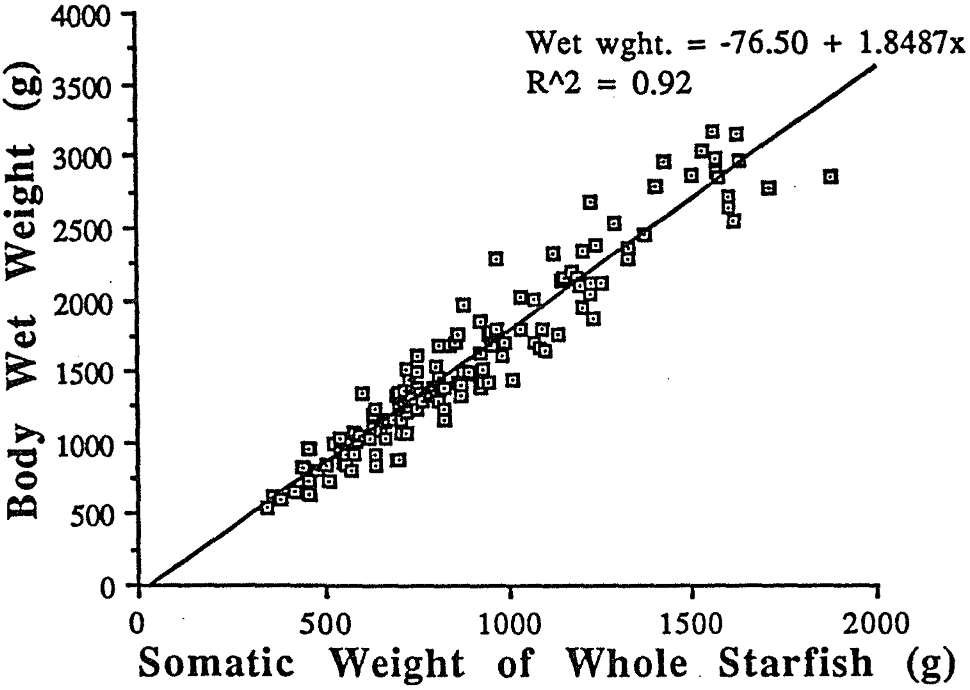


Figure 3.16 Total gonad weights plotted versus somatic weight of male *A. planci*. Lines represent a regression model that includes a term for the presence of regeneration (Model B from Table 3.1). In this case, it is the most parsimonious model that describes the data. Y axis is square root scale.

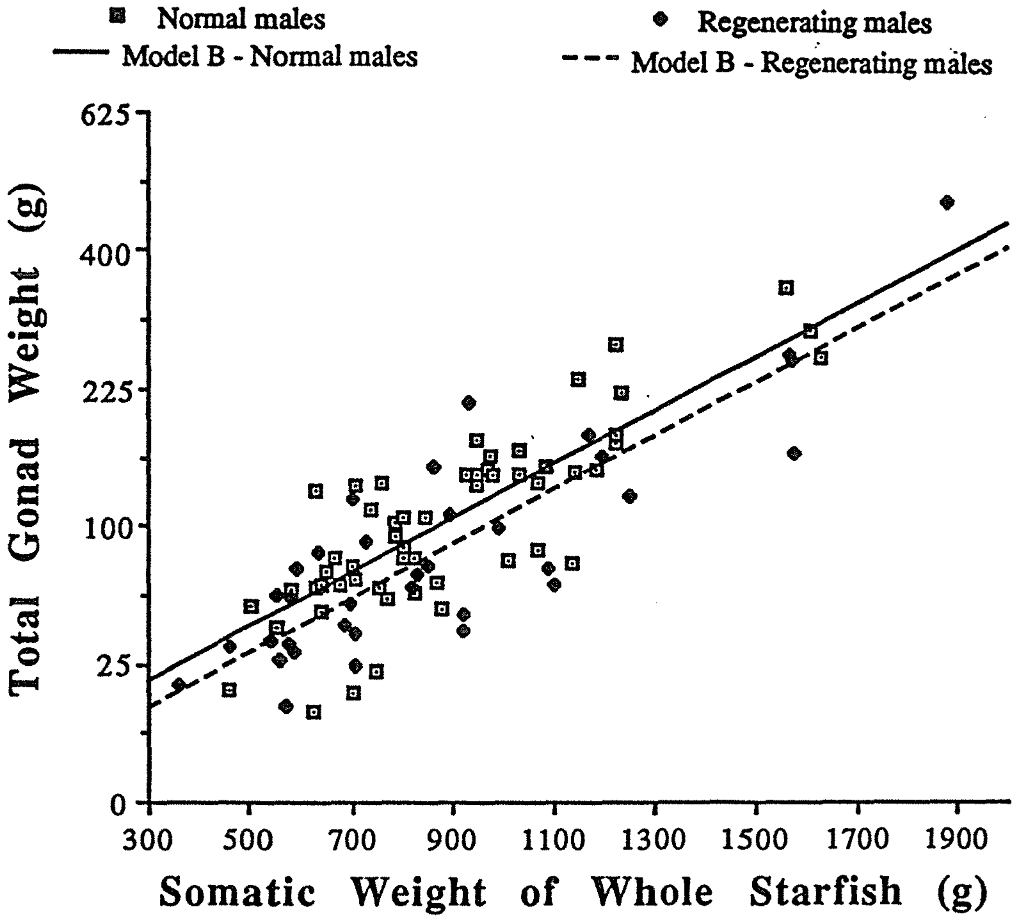


Table 3.1 Multiple regression tables showing the backward elimination of terms from a model relating gonad weight to body weight and regeneration for male *A. planci*.

A : Full model including terms for amount of regeneration (= number of arms regenerating) and interaction (somatic weight X presence of regeneration)

B : The most parsimonious model

A.

Predictor Terms	Coefficient	T	P
Constant	1.00769	1.06	0.2906
Somatic Weight	0.01025	10.09	0.0000
Regeneration	0.57139	0.40	0.6867
Amount of Regeneration	-0.28721	-1.36	0.1789
Slope	-0.00099	-0.72	0.4749

B.

Predictor Terms	Coefficient	T	P
Constant	1.4037	2.08	0.0404
Somatic weight	0.00981	14.17	0.0000
Regeneration	-0.9034	-2.12	0.0369

n(regen.) = 35, n(not regen.) = 55
Adjusted R-squared = 0.6978

Male starfish do not show any significant relationship between the number of regenerating arms and total gonad weight. This is shown by the lack of significance of the term "Amount of regeneration" (Table 3.1, Model A) and its subsequent removal from the model. Model B is shown in Figure 3.16 in relation to the data.

Female starfish do not show any general reduction of gonad weight when regenerating. There is also no evidence to suggest that the number of regenerating arms has any effect on total gonad weights nor that there is any interaction between body weight and regeneration.

The derivation of the simplest model for females at the whole starfish level is shown in Table 3.2. The simplest model which best describes the data (Model C) does not contain terms for either presence of regeneration or amount of regeneration. Model B, the model that tests the significance of regeneration, is shown in Figure 3.17.

The power of the analysis to detect a 25% difference in gonad weights between regenerating and non-regenerating female starfish is 0.74.

Table 3.2 Multiple regression tables showing the backward elimination of terms from a model relating gonad weight to body weight and regeneration for female *A. planci*.

A : Full model including terms for amount of regeneration (= number of arms regenerating) and interaction (somatic weight X presence of regeneration)

B : The model which tests the significance of regeneration

C : The most parsimonious model

A.

Predictor Terms	Coefficient	T	P
Constant	1.21330	1.00	0.2906
Somatic Weight	0.01199	10.04	0.0000
Regeneration	1.16599	0.57	0.6867
Amount of Regeneration	-0.34795	-0.89	0.1789
Slope	-0.00019	-0.11	0.4749

B.

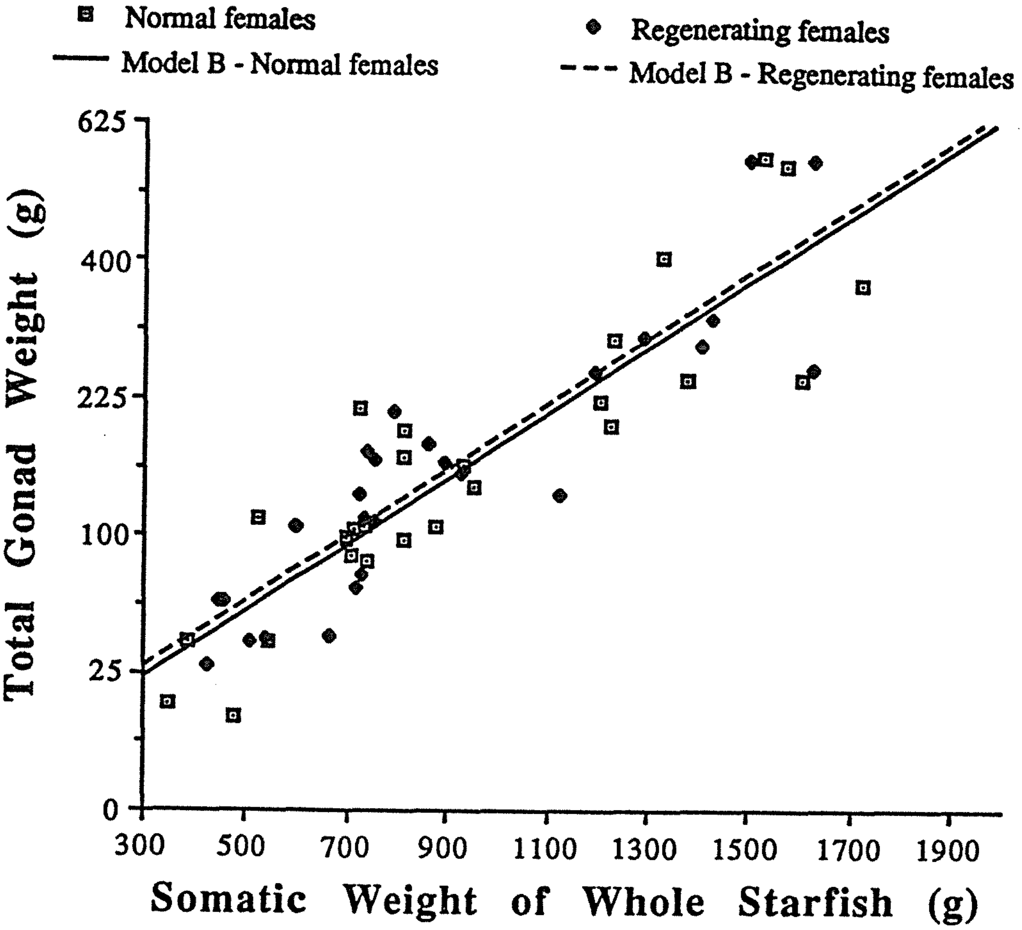
Predictor Terms	Coefficient	T	P
Constant	1.2003	1.32	0.1943
Somatic weight	0.01198	14.22	0.0000
Regeneration	0.35395	0.56	0.5768

C.

Predictor Terms	Coefficient	T	P
Constant	1.3943	1.66	0.1026
Somatic Weight	0.01196	14.31	0.0000

n(regen.) = 37, n(not regen.) = 37
Adjusted R-squared of final model (C) = 0.7935

Figure 3.17 Total gonad weights plotted versus somatic weight of female *A. planci*. Lines represent a regression model that includes a term for the presence of regeneration (Model B from Table 3.2). It is not the most parsimonious model that describes the data. Y axis is square root scale.



3.3.4 Results at Individual Arm Level

3.3.4.1 Regenerating and Adjacent Arms

Gonad weights are reduced in the regenerating arms of male starfish. Small starfish show a reduction of 27% in the regenerating arm while large starfish show a reduction of 13% relative to non-regenerating arms. The graphical description of the effect of regeneration (along with adjacency) in the model is shown in Figure 3.18.

As at the whole starfish level, there was no interaction between regeneration and body size affecting gonad weight. The range of effects of regeneration on gonad weight are a result of back transformation from square root.

Arms adjacent to regenerating arms do not have significantly reduced gonad weights compared to normal arms. This is graphically presented in Figure 3.18, along with regeneration. There was also no interaction between body size and the size of the gonads in adjacent arms. The statistical presentation of the results of backward elimination for males are shown in Table 3.3. The simplest model (Model C) describing the individual arm data contains only the term for the effect of regeneration. Interaction terms for both regeneration and adjacency were eliminated from the model as was the term for the effect of regeneration on adjacent arm gonad

Figure 3.18 Gonad weights from individual arms plotted versus somatic weight of male *A. planci*. Lines represent a regression model that includes terms for regeneration and adjacency (Model B from Table 3.3). It is not the most parsimonious model that describes the data. Y axis is square root scale.

A : Distribution of gonad weights of normal arms

B : Distribution of gonad weights of regenerating arms

C : Distribution of gonad weights of adjacent arms

D : Comparison of regression lines plotted on same axes to compare elevation

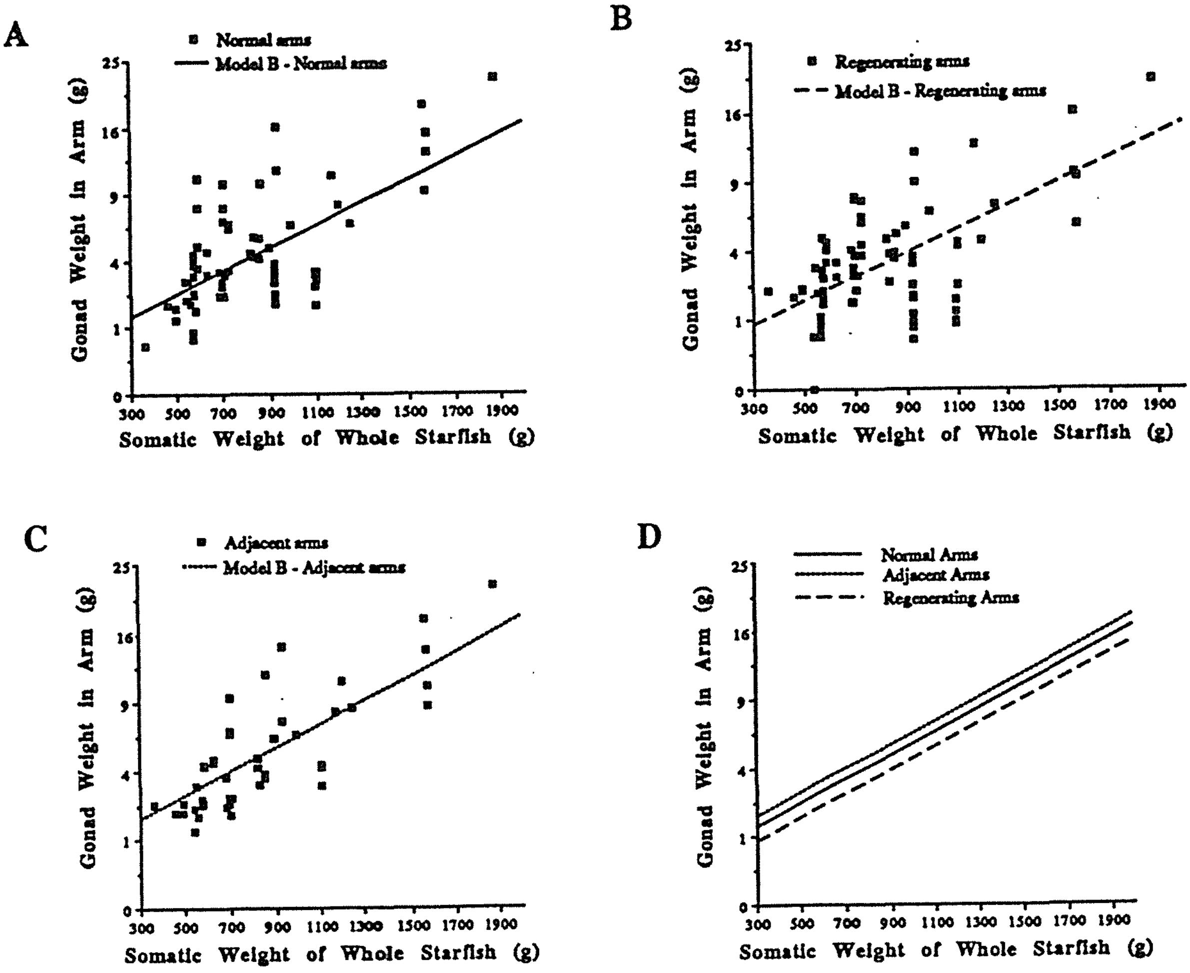


Table 3.3 Multiple regression tables showing the backward elimination of terms from a model relating individual arm gonad weight to body weight and regeneration for male *A. planci*.

A : Full model including interaction terms for somatic weight X regeneration and somatic weight X adjacency

B : The model which tests the significance of regeneration and adjacency

C : The most parsimonious model

A.

Predictor Terms	Coefficient	T	P
Constant	0.57835	2.80	0.0057
Somatic Weight	0.00179	7.57	0.0000
Regeneration	0.03562	0.12	0.9032
Adjacency	0.02467	0.08	0.9382
Slopes of Regeneration vs. normal	-0.00031	-0.93	0.3537
Slopes of Adjacent vs. normal	0.00014	0.39	0.6943

B.

Predictor Terms	Coefficients	T	P
Constant	0.63614	4.70	0.0000
Somatic weight	0.00172	12.21	0.0000
Regenerating	-0.22054	-2.24	0.0260
Adjacent	0.14513	1.27	0.2066

C.

Predictor Terms	Coefficient	T	P
Constant	0.68435	5.26	0.0000
Somatic Weight	0.00172	12.24	0.0000
Regenerating	-0.27400	-3.08	0.0024

n(not regen) = 77, n(regen) = 77, n(adjacent) = 45
Adjusted R-squared for simplest model = 0.4448

weights.

Females do not show gonad weight reduction in either regenerating or adjacent arms. Nor do they show any interaction with body size affecting gonad weight. A graph displaying Model B, the model that describes the different terms' affects on gonad weights, is shown in Figure 3.19 and shows that there is no significant difference between normal arms, adjacent arms and regenerating arms.

The statistical presentation of the results of backward elimination for females is shown in Table 3.4. Model C is the simplest model that describes the data for individual arms. The interactions of regeneration and adjacency with body weight, as well as the simple effects of regeneration and adjacency have been eliminated.

3.3.4.2 Length and Regeneration

The weights of gonads in individual arms of both males and females appear to be influenced by the length of the regenerating arm (Tables 3.5 and 3.6). In each case increased length is related to greater gonad weight. From the model, a 10% increase (e.g. from 70% to 80%) in length of the regenerating arm corresponded to a 4% increase in gonad weight in the larger sized starfish and a 10% increase in the gonad weight in the smaller sized

Figure 3.19 Gonad weights from individual arms plotted versus somatic weight of female *A. planci*. Lines represent a regression model that includes terms for regeneration and adjacency (Model B from Table 3.4). It is not the most parsimonious model that describes the data. Y axis is square root scale.

A : Distribution of gonad weights of normal arms

B : Distribution of gonad weights of regenerating arms

C : Distribution of gonad weights of adjacent arms

D : Comparison of regression lines plotted on same axes to compare elevation

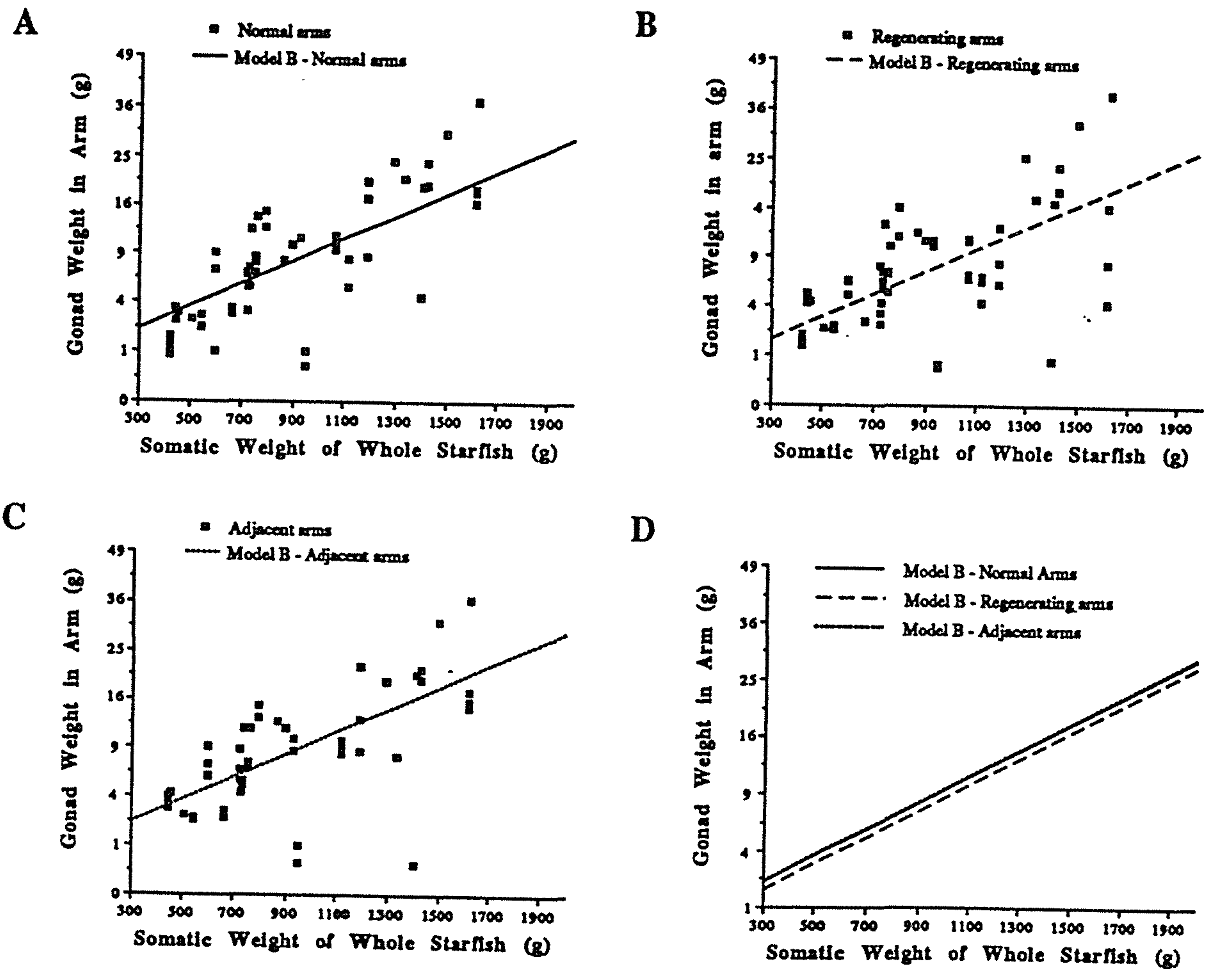


Table 3.4 Multiple regression tables showing the backward elimination of terms from a model relating individual arm gonad weight to body weight and regeneration for female *A. planci*.

A : Full model including interaction terms for somatic weight X regeneration and somatic weight X adjacency

B : The model which tests the significance of regeneration and adjacency

C : The most parsimonious model

A.

Predictor Terms	Coefficient	T	P
Constant	0.40832	1.35	0.1792
Somatic Weight	0.00269	8.53	0.0000
Regeneration	0.44352	1.04	0.3017
Adjacency	0.51080	1.10	0.2744
Slopes of Regeneration vs. normal	-0.00066	-1.48	0.1412
Slopes of Adjacent vs. normal	-0.00054	-1.14	0.2548

B.

Predictor Terms	Coefficients	T	P
Constant	0.75518	3.72	0.0003
Somatic weight	0.00230	12.18	0.0000
Regenerating	-0.14302	-0.89	0.3763
Adjacent	0.02240	0.13	0.8956

C.

Predictor Terms	Coefficient	T	P
Constant	0.70460	3.85	0.0002
Somatic Weight	0.00230	12.27	0.0000

n(not regen) = 59, n(regen) = 59, n(adjacent) = 48
Adjusted R-squared of simplest model = 0.4756

Table 3.5 Multiple regression table showing a model that relates individual arm gonad weight with both regeneration and length (= length of arm as percentage of normal arm length) for male *A. planci*.

Predictor Terms	Coefficients	T	P
Constant	0.61905	3.97	0.0001
Somatic weight	0.00174	10.27	0.0000
Regenerating	-0.58725	-3.29	0.0013
Length	0.00701	2.68	0.0081

Adjusted R-squared = 0.4208

Table 3.6 Multiple regression table showing a model that relates individual arm gonad weight with both regeneration and length (= length of arm as percentage of normal arm length) for female *A. planci*.

Predictor Terms	Coefficients	T	P
Constant	0.65781	2.97	0.0036
Somatic weight	0.00241	11.17	0.0000
Regenerating	-0.72596	-2.52	0.0131
Length	0.01065	2.40	0.0179

Adjusted R-squared = 0.5152

starfish.

3.3.4.3 Short Arms

Short arms, arms that are obviously shorter than normal length but are not regenerating, do not show reduced gonad weights relative to normal length arms in either male or female starfish (Figures 3.20 and 3.21). The lengths of the short arms (as percentage length of normal arm) in both sexes are also not related to the gonad weights within the short arms and do not aid with the models description of the data.

The backward elimination of terms (length not considered) for males is shown in Table 3.7. The best fit model is Model C in which short arm gonad weights are not significantly different from normal arm gonad weights. This is also the case for females as shown in Table 3.8. The backward elimination of terms where length is considered are shown in Table 3.9 for males and Table 3.10 for females. In both sexes Model B is the model of best fit and does not include the length of short arms as a significant term.

3.3.5 Results at Half Arm Level

3.3.5.1 Regenerating and Adjacent Arms

Regenerating and adjacent arms do not show

Figure 3.20 Gonad weights from individual arms plotted versus somatic weight of male *A. planci*. Lines represent a regression model that includes a term for whether the arm is short (Model B from Table 3.7). It is not the most parsimonious model that describes the data. Y axis is square root scale.

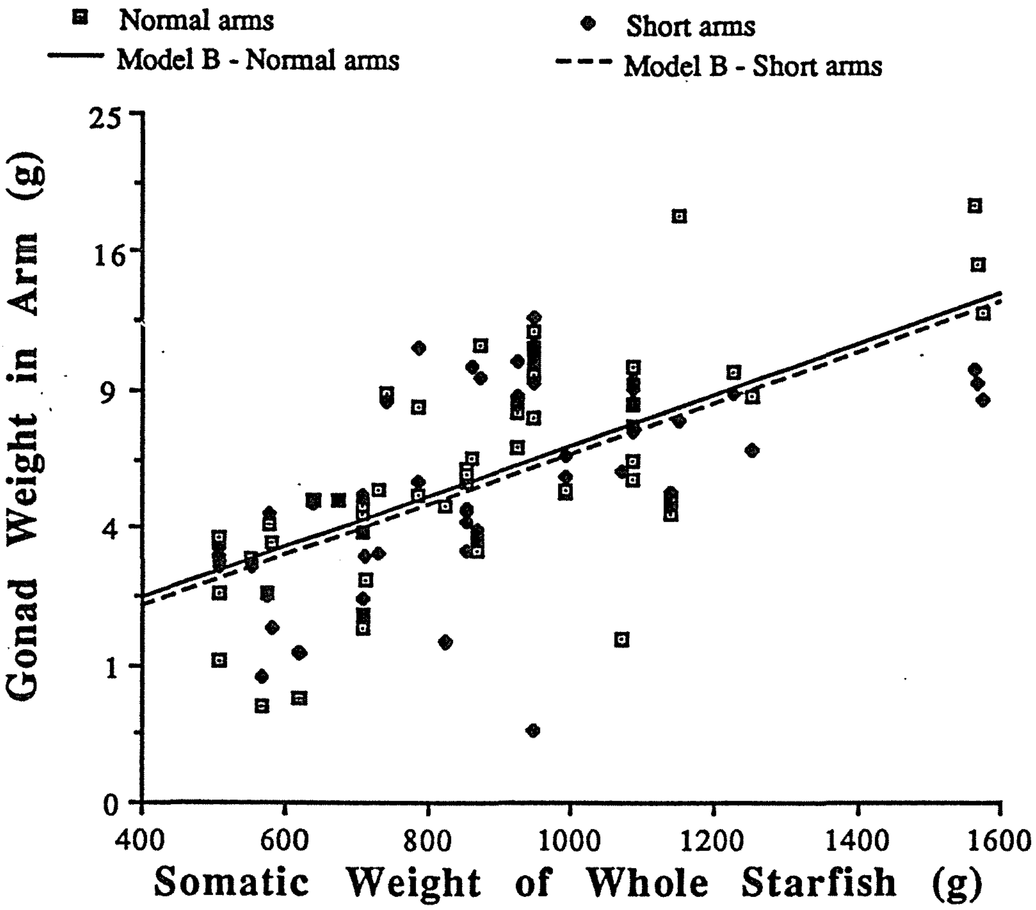


Figure 3.21 Gonad weights from individual arms plotted versus somatic weight of female *A. planci*. Lines represent a regression model that includes a term for whether the arm is short (Model B from Table 3.8). It is not the most parsimonious model that describes the data. Y axis is square root scale.

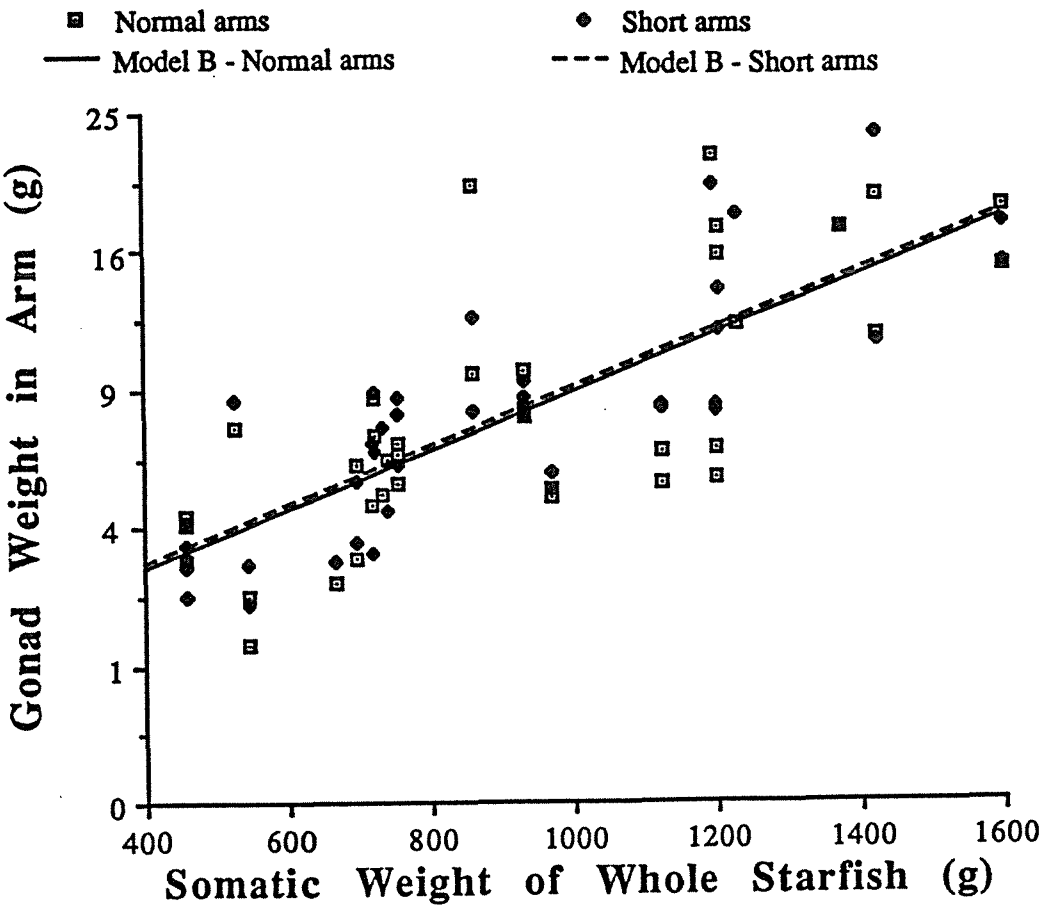


Table 3.7 Multiple regression tables showing the backward elimination of terms from a model which relates individual arm gonad weight to somatic weight and to presence of short arms in male *A. planci*.

A : Full model including a term for the somatic weight X short arm interaction

B : The model which tests the significance of short arms

C : The most parsimonious model

A.

Predictor Terms	Coefficient	T	P
Constant	0.46353	1.71	0.0908
Somatic Weight	0.00219	7.41	0.0000
Short	0.46441	1.21	0.2293
Slope	0.00061	-1.46	0.1475

B.

Predictor Terms	Coefficient	T	P
Constant	0.73245	3.65	0.0004
Somatic weight	0.00188	8.97	0.0000
Short	-0.07342	-0.68	0.5005

C.

Predictor Terms	Coefficient	T	P
Constant	0.69574	3.61	0.0005
Somatic Weight	0.00188	8.99	0.0000

n(normal) = 55, n(short) = 55
Adjusted R-squared = 0.4227

Table 3.8 Multiple regression tables showing the backward elimination of terms from a model which relates individual arm gonad weight to somatic weight and to presence of short arms in female *A. planci*.

A : Full model including a term for the somatic weight X short arm interaction

B : The model which tests the significance of short arms

C : The most parsimonious model

A.

Predictor Terms	Coefficient	T	P
Constant	0.91380	3.74	0.0908
Somatic Weight	0.00205	7.94	0.0000
Short	-0.09061	-0.26	0.2293
Slope	0.00014	0.38	0.1475

B.

Predictor Terms	Coefficient	T	P
Constant	0.85230	4.69	0.0000
Somatic weight	0.00212	11.67	0.0000
Short	0.03238	0.27	0.7862

C.

Predictor Terms	Coefficient	T	P
Constant	0.86849	5.08	0.0000
Somatic Weight	0.00212	11.74	0.0000

n(normal) = 41, n(short) = 41
Adjusted R-squared = 0.6282

Table 3.9 Multiple regression tables showing the backward elimination of terms from a model which relates individual arm gonad weight to somatic weight and to length (= percentage of normal length) of short arms in male *A. planci*.

A : The model which tests the significance of the length of short arms

B : The most parsimonious model

A.

Predictor Terms	Coefficient	T	P
Constant	0.71277	3.02	0.0032
Somatic weight	0.00189	8.90	0.0000
Length	-0.00025	-0.13	0.9001

B.

Predictor Terms	Coefficient	T	P
Constant	0.69574	3.61	0.0005
Somatic Weight	0.00188	8.99	0.0000

n(normal) = 55, n(short) = 55
Adjusted R-squared = 0.4227

Table 3.10 Multiple regression tables showing the backward elimination of terms from a model which relates individual arm gonad weight to somatic weight and to length (= percentage of length of normal arm) of short arms in female *A. planci*.

A : The model which tests the significance of the length of short arms

B : The most parsimonious model

A.

Predictor Terms	Coefficient	T	P
Constant	0.84343	4.58	0.0000
Somatic weight	0.00212	11.67	0.0000
Length	0.00063	0.38	0.7055

B.

Predictor Terms	Coefficient	T	P
Constant	0.86849	5.08	0.0000
Somatic Weight	0.00212	11.74	0.0000

n(normal) = 41, n(short) = 41
Adjusted R-squared = 0.6282

significantly reduced gonad weights in either the male or female starfish. This is despite the apparent reductions of gonad weights in regenerating half arms (15% to 25% in males, 11% to 28% in females). The low R-squared values suggest that there is a great deal of variability in the data explained by other factors. The relationships between regeneration or adjacency and gonad weight does not vary significantly with body size.

The graphical presentations of Model B, the model that tests whether adjacent or regenerating arm halves have reduced gonad weights compared to normal half arms, is shown in Figure 3.22 (males) and Figure 3.23 (females). The difference between the parallel lines shows the differences in means. The individual data points display the variability of the data set.

The backward elimination of terms to give the simplest best fit models are shown in Table 3.11 (males) and Table 3.12 (females). Model C is the simplest model that best describes the data and does not contain terms for either adjacent or regenerating arms; nor does it contain terms for describing interactions between adjacent or regenerating arms with somatic weights.

Figure 3.22 Gonad weights from half arms plotted versus somatic weight of male *A. planci*. Lines represent a regression model that includes terms for regeneration and adjacency (Model B from Table 3.11). It is not the most parsimonious model that describes the data. Y axis is square root scale.

A : Distribution of gonad weights of normal half arms

B : Distribution of gonad weights of regenerating half arms

C : Distribution of gonad weights of adjacent half arms

D : Comparison of regression lines plotted on same axes to compare elevation

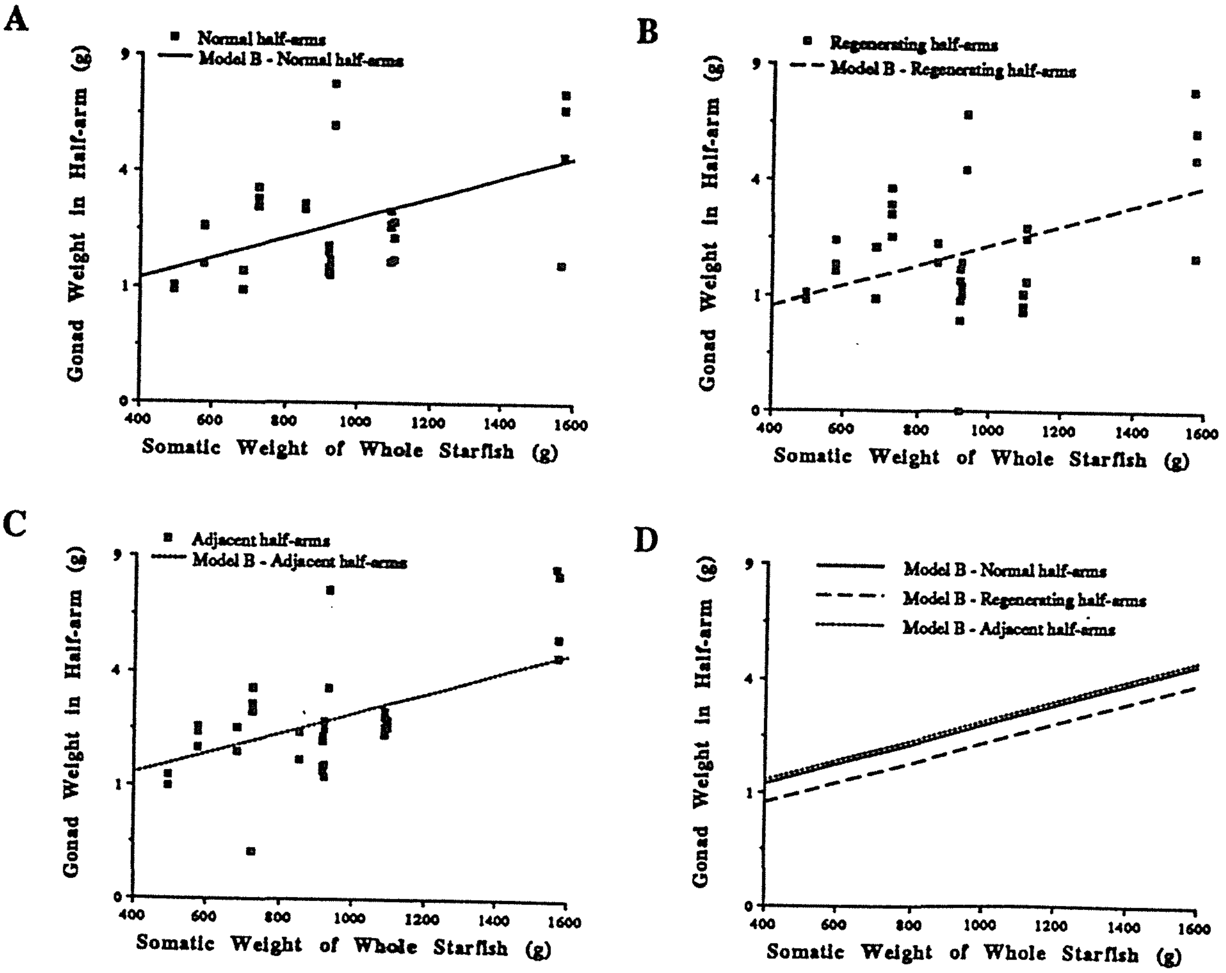


Figure 3.23 Gonad weights from half arms plotted versus somatic weight of female *A. planci*. Lines represent a regression model that includes terms for regeneration and adjacency (Model B from Table 3.12). It is not the most parsimonious model that describes the data. Y axis is square root scale.

A : Distribution of gonad weights of normal half arms

B : Distribution of gonad weights of regenerating half arms

C : Distribution of gonad weights of adjacent half arms

D : Comparison of regression lines plotted on same axes to compare elevation

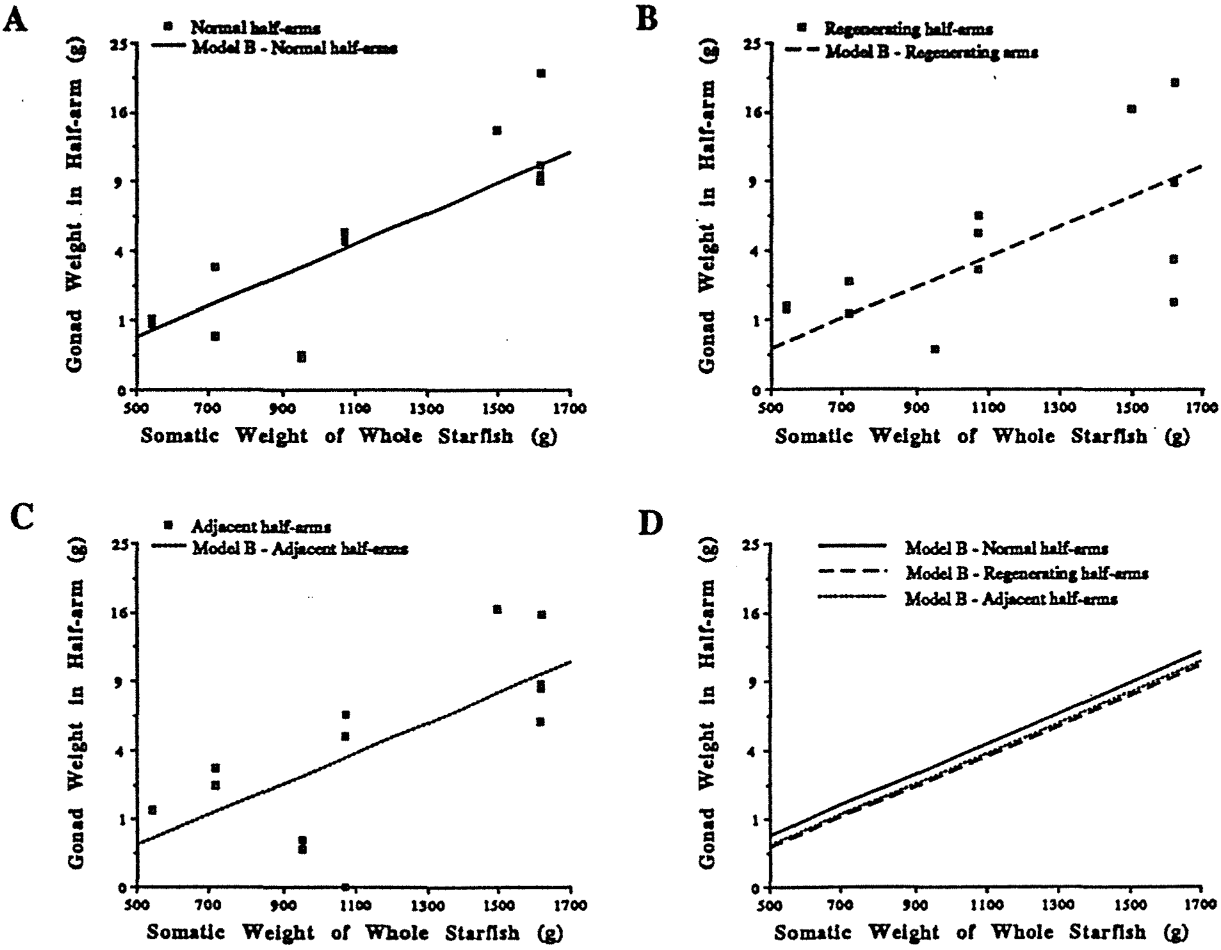


Table 3.11 Multiple regression tables showing the backward elimination of terms from a model relating gonad weight in half arms to body weight and to regeneration in male *A. planci*.

A : Full model including interaction terms for somatic weight X regeneration and somatic weight X adjacency

B : The model which tests the significance of regeneration and adjacency

C : The most parsimonious model

A.

Predictor Terms	Coefficient	T	P
Constant	0.84095	3.24	0.0017
Somatic Weight	0.00074	2.79	0.0063
Regeneration	-0.13543	-0.37	0.7133
Adjacency	-0.35179	-0.96	0.3409
Slopes of Regeneration vs. normal	-0.00003	-0.08	0.9356
Slopes of Adjacent vs. normal	0.00041	1.11	0.2685

B.

Predictor Terms	Coefficient	T	P
Constant	0.72008	4.44	0.0000
Somatic weight	0.00087	5.68	0.0000
Regenerating	-0.16391	-1.52	0.1309
Adjacent	0.03929	0.37	0.7158

C.

Predictor Terms	Coefficient	T	P
Constant	0.67854	4.48	0.0000
Somatic Weight	0.00087	5.63	0.0000

n(not regen) = 35, n(regen) = 35, n(adjacent) = 35
Adjusted R-squared = 0.2278

Table 3.12 Multiple regression tables showing the backward elimination of terms from a model relating gonad weight in half arms to body weight and to regeneration in female *A. planci*.

A : Full model including interaction terms for somatic weight X regeneration and somatic weight X adjacency

B : The model which tests the significance of regeneration and adjacency

C : The most parsimonious model

A.

Predictor Terms	Coefficient	T	P
Constant	-0.84867	-1.24	0.2231
Somatic Weight	0.00266	4.61	0.0000
Regeneration	0.75290	0.78	0.4418
Adjacency	0.42595	0.44	0.6599
Slopes of Regeneration vs. normal	-0.00084	-1.03	0.3116
Slopes of Adjacent vs. normal	-0.00051	-0.62	0.5382

B.

Predictor Terms	Coefficient	T	P
Constant	-0.34760	-0.80	0.4260
Somatic weight	0.00221	6.72	0.0000
Regenerating	-0.18342	-0.57	0.5701
Adjacent	-0.13729	-0.43	0.6705

C.

Predictor Terms	Coefficient	T	P
Constant	-0.45450	-1.19	0.2415
Somatic Weight	0.00221	6.86	0.0000

n(not regen) = 14, n(regen) = 14, n(adjacent) = 14
Adjusted R-squared = 0.5293

3.4 Discussion

3.4.1 General Differences Between Sexes

Nakamura (1986) examined male and female *Acanthaster planci* of the Ryukyus Islands, Japan, for various morphometric differences. He found little difference between the sexes except for size. Similarly, in this study, differences in mean size between the sexes were not found to be statistically significant. In contrast to Nakamura, some differences between the sexes were found. Females produced on average 26% more gonad by weight at any given somatic body weight than did males. The proportion of body weight occupied by the gonads also differed between the two sexes. Proportionately females produced around 50% more gonad than males. These proportions of body weight for *A. planci* agreed with Babcock and Mundy (1992) for Davies Reef but were well below those found by Kettle and Lucas (1987)(Figure 3.13) for Helix Reef and Conand (1975) in Noumea. While this may indicate that gonads were not at peak weight, the fact that the starfish spawned soon after these experiments on Davies Reef (Babcock and Mundy, 1992), suggests that the starfish on Davies Reef may have had relatively low fecundity.

3.4.2 Regeneration Effects on Gonad Weight

A clear relationship was found between the presence of regenerating arms and reduced gonad weight in male *A. planci* but not in the females. Although there was an association between regenerating arms and reduced gonad weight at the individual arm level in females, this was only when the model contained a length-of-arm covariable. This suggests that this is simply a result of shorter arms having less room for gonads.

This correlation of length with gonad weight was not found in short (not regenerating) arms, which may be for the following reason. The gonads within an arm generally occur in the area around the edge of the body disc and the proximal third of the arm. None of the arms categorised as short were less than half the length of a normal arm. Thus the short arms never really limited the space for gonad development. In contrast, some of the regenerating arms were found to be damaged up to the edge of the body disk and had no length at all - therefore reducing the space available for gonad production by at least a third.

Previous qualitative studies (Birkeland and Lucas, 1990) have indicated that regenerating arms have smaller distal gonad lobes. It was therefore expected that gonad weight would be reduced in individual regenerating arms in both sexes. It was a surprise, given the difference at the whole starfish level (i.e. 10%-26%), that the mean of the differences between regenerating and non-regenerating arms was so small (13% - 27%). This result, combined with the fact that adjacent arms were not statistically different from normal arms, shows that reduction in gonad weight is not concentrated around the site of regeneration but occurs over the whole starfish. This presumably reflects the presence of a continuous coelomic cavity throughout the disk and arms, such that reductions of nutrients for gonad production are not localised.

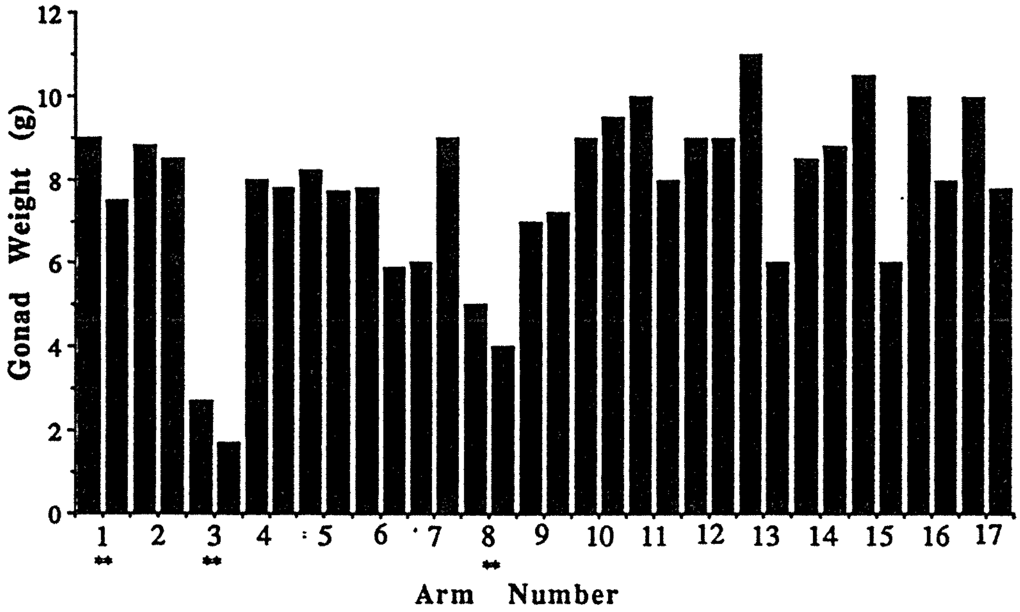
3.4.3 Adjacency and Gonad Weight

The lack of reduction in gonad weights in arms adjacent to regenerating arms compared to those arms that are regenerating was surprising. The physiological connection between adjacent arm gonads was expected to cause gonad lobes on both sides of the septum (i.e. portions #2 and #3 in Figure 3.1) to reduce gonad weight. This pattern was not found indicating that the two halves of the gonad are not physiologically closely linked.

3.4.4 Half Arms

The lack of regeneration effect at the half arm level was no surprise, given what was seen during dissection. As shown in Figure 3.24, which displays all the half arm gonad weights for all the arms of a typical female

Figure 3.24 Gonad weights for each of the half arms of a typical female *A. planci*. Regenerating arms are identified with **.



starfish, the variation of gonad weights between adjacent halves (both within an arm and either side of a septum) is great. During dissection, it was often difficult to distinguish which gonad clumps came from which side of the arm or septum. This would have caused at least part of the great variability. Natural variation between and within arms was also seen many times over the course of dissections, in contrast to the consistent weight between arms found by Conand (1975). It was occasionally noted that one half of an arm would have no gonads and the other half would have substantial amounts. In Figure 3.24, the two arms with the smallest gonads are regenerating arms. No statistically significant effect was found once the data from all the female starfish were combined.

3.4.5 Effect of Reef

There is a possibility that the effects of regeneration seen in the data are an artefact of where the starfish were collected. Two thirds of the starfish were collected from Lynchs Reef. These starfish appeared to be from a single cohort smaller in body weight than starfish from Davies Reef (Figure 3.6). Thus, the starfish from these two reefs are probably of different ages, which may cause them to react differently to regenerative needs. In addition, they have probably been subject to different nutritional regimes. Baker *et al* (1992) give the percentages of live coral cover for various reefs in the

Great Barrier Reef Park. In 1991, Lynchs Reef consistently showed low coral cover of 0-10% while the coral cover on Davies Reef varied between 0 an 50% with a median of 11-25%. Such a difference in coral cover would probably have implications for the relative health of starfish from these two reefs and could influence their relative nutritional status. This may in turn have influenced, to some degree, the results of the analyses. Figure 3.7 shows diameter versus somatic weight of the starfish from the two reefs. There appears to be no difference in the relationship between size and weight between the two reefs. If it is assumed that size to weight ratio indicates health status (e.g. if two starfish are of the same size but weigh significantly different, then the lower weight starfish is assumed to be relatively unhealthy, having used more of its body mass as an energy source), then there is no difference in health between the starfish of the two reefs.

The potential differences between reefs in terms of regeneration effects on total gonad weight were further investigated via multiple regression. This was only examined in the males since females showed no effect of regeneration. There was no significant difference between the reefs, as indicated by the lack of significance of a term describing which reef the starfish came from (t= -0.73, p=0.4680).

3.4.6 Mechanisms by Which Gonad Weights May Have Been Affected by Regeneration

The results for the overall study indicate that regeneration has an effect on male gametogenesis. Therefore, the process of regeneration in *A. planci* must in some way interfere with spermatogenesis. There are a number of papers that examine the concept of cost of reproduction in life history and some touch on the idea that maintenance (i.e. maintaining ones fitness as opposed to metabolic functions, which is often referred to as maintenance) and reproduction compete for energy resources (e.g. Kirkwood and Rose, 1991; Partridge, 1987). There appears, however, to be no treatment of the physiological mechanisms by which maintenance and reproduction interact. As a result, it is only possible to hypothesise about the mechanisms.

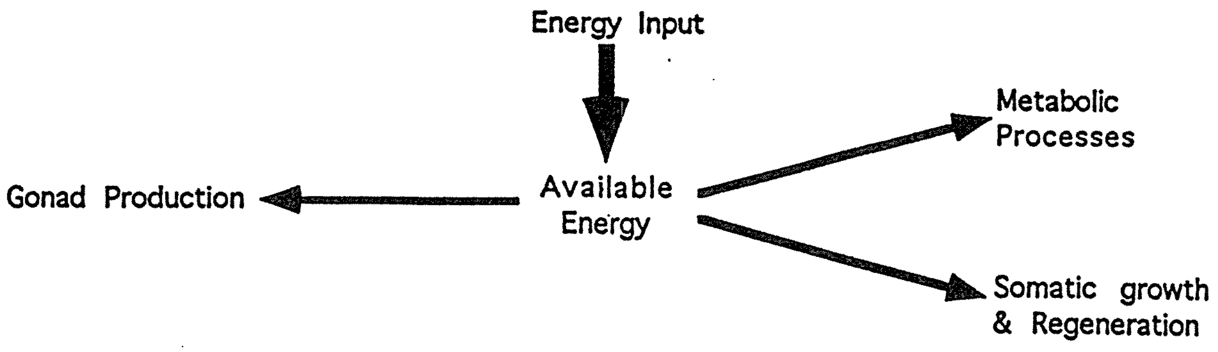
It seems likely that the "control" of gonad production and maintenance is through a direct nervous or hormonal means, but there appears to have been no attempts to follow these biochemical pathways. A likely and readily explainable method of gonad control with respect to regeneration is via simple energy budget constraints. This point was discussed in the introduction (section 3.1) and has been implicated in other echinoderms (e.g. Johnson and Threlfall, 1987; Otteson and Lucas, 1982; Harrold and Pearse, 1980; and Emson and Wilkie, 1980). This method of control can be explained with the aid of Figure 3.25. If it is assumed that nutrient supply and energy availability are limiting and that gonad production, somatic growth and metabolic processes have free access to energy supplies (i.e. from the widespread coelomic fluid), then the energy flow can be described as shown in Figure 3.25a. If a starfish requires healing and regeneration, then a greater amount of energy is required for this repair, as shown in reefal ophiuroids (Sullivan, 1988), and this energy would be taken up at a greater rate. Assuming that metabolic processes remain constant and that energy usage for gonad production is not increased as well, then this would reduce - by simple competition - the energy available to gonad production (Figure 3.25b).

3.4.7 Benefits of Reduced Gametogenesis

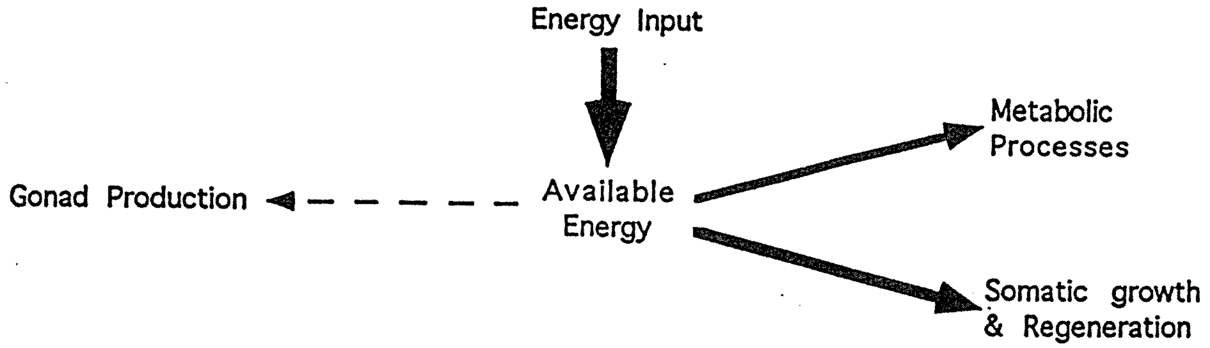
The benefits of reduced testis production to *A. planci* are not clear. If *A. planci* had only five arms and they were all necessary for mobility and food capture, then reduced gonad production would make sense. Reduction in gonad production would free up energy for healing and regeneration, enabling an individual to return to full mobility quickly and with fewer of the risks associated with reduced mobility (eg. predation). Such maintenance activity would lengthen the life of individual, enabling a greater number of years of reproduction. However, *A. planci* is not limited by its number of arms. In this

Figure 3.25 Energy flow model describing the possible partitioning of energy given the energy requirements of regeneration. A: normal situation. B: regenerating

A.



B.



population, *A. planci* had, on average, sixteen arms. The importance of any one arm was therefore far less than a starfish with only five arms. Since there were so many arms, it would have been expected that initial healing would commence to seal the body and once the wound was sealed, the regeneration would then stop. Reproduction would only be affected through the limitation of space in the shortened damaged arm. This is perhaps shown by the females which showed an effect of regeneration only in individual arms when the arm length was taken into account.

3.4.8 Effects on Recruitment

Given the reduction in gonad weight in regenerating males, it could reasonably be expected that regenerating males would fertilise 10% to 26% fewer eggs. This could in turn reduce the recruitment. Given that 40% of the males on these two reefs were in the process of regenerating arms, then numbers of recruits emanating from Davies and Lynchs Reefs to reefs downstream would be expected to be reduced between 4% and 10.4%. This, of course, ignores all the potential variation associated with fertilisation, larval and recruitment dynamics which generally causes very poor parent stock recruitment relationships (Sale, 1991; Longhurst and Pauly, 1987; Ebert, 1983). However, it does suggest that regeneration may play a part in the occurrence of outbreaks.

3.4.9 Variability

These findings have important implications for further studies of *A. planci* populations. Research into *A. planci* has consistently shown that both individuals and populations vary in many aspects of their biology and that there is great individual, temporal and spatial variation. Examples of this are shown in recent *A. planci* population control research by the Great Barrier Reef Marine Park Authority (Udo Englehardt, pers. com.) and damage studies by this author (Chapter 2) where survival after damage varied unpredictably.

A. planci appears to have a rather plastic life history, that is, it is able to adapt its biology or physiology to the habitat in which it lives. The significance of such plasticity was originally proposed for *A. planci* by Moore (1990) who suggested that life histories were variable depending on environment. More recently, support for this idea was provided by Stump (1992) who found *A. planci* to vary somatic weight and gonad production depending on food supply. The experiments in this chapter suggest that the differences found between the sexes are simply an additional difference to add to the differences already found within and between individuals and populations of *A. planci*. If the life history of *A. planci* is to be better understood, researchers should incorporate into future experiments as many aspects of biology, such as gender differences, as possible.

Chapter 4

Conclusion

The results of the research in this thesis indicate that *A. planci* populations can potentially be controlled through partial predation. The studies incorporating damage treatments have indicated that *A. planci* has reasonable resistance to damage but that, unlike some other species of echinoderm, its capacity for survival after extensive damage is limited. An exact measure of the damage required to consistently cause 100% mortality was not determined. Most of the starfish in these experiments died when cut in half or in thirds, however, many starfish also died as a result of lesser or no inflicted damage.

The great variability in mortality found through these experiments indicates that there were undetermined factors which substantially altered the starfishes' ability to survive damage. Starvation, for example, appeared to have an effect on starfish survival but this was not consistent through all the experiments. Other factors which may have led to this variability are: water temperature, age, size, nutritional status, post-reproductive morbidity and local abundance of small predators. Methodology, in particular the handling of the starfish and their removal from the water for treatment, may have also play a significant role in the variability. Further research is required on these other possible sources of variability as these factors could not be included in these experiments without unacceptably large increases in sample size and/or resources.

The above damage study indicates that, should the need arise, mutilation may serve as a chemical-free method of starfish elimination. If the starfish are cut into quarters then it is unlikely, given the results of the above research, that any of the pieces will survive. However, given the variability in mortality found in the above studies, trial mutilation should be conducted prior to large scale mutilation to ensure that mortality will consistently occur.

The studies of the effects of regeneration on gonad weight also indicate that *A. planci* populations can be limited through partial sub-lethal predation. Males were found to have a significant reduction in gonad weight when regenerating arms. Regeneration resulted in reduced gonad weights over the whole starfish, not just at the site of regrowth. It is suspected that this reduction in gonad weight was a result of competition for energy resources between regenerative needs, metabolic processes and gonad production.

Gonad weights were not reduced in regenerating female starfish. This result could not be explained but perhaps indicates that there are significant differences in biology and life history between male and female starfish which require further research.

Throughout these experiments, variability in gonad weight (independent of regeneration) was found to be quite high between: males and females, starfish of the same sex, arms within an individual starfish and between the clusters of gonads on either side of the individual arms. This variability and the variability found in the damage experiments suggest that *A. planci* is plastic in its biology and life history. This plasticity may help ensure *A. planci*'s success by enabling it to adapt to a wider range of habitats.

Overall, impacts of partial predation on survival of and gonad production by *A. planci* were significant. If *A. planci* suffers natural partial predation on a frequent basis then, given the results found in this thesis, this predation will lead to significant mortality or significant reduction in gonad production. In the latter case, fewer sperm may lead to reduced numbers of fertilised eggs. This in-turn could result in fewer numbers of adult starfish and maybe even prevent starfish outbreaks.

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