Stump, Richard J. W. (1994) ***Age determination and life-history characteristics of Acanthaster planci(L.)(Echinodermata:Asteroidea).*** **PhD thesis, James Cook University.**

CHAPTER 1

GENERAL INTRODUCTION

Frequently the debate surrounding the occurrence of outbreaks has been reduced to ...whether they are natural or man induced, ...it is more realistic to suggest that outbreaks are a problem not because they may be natural or unnatural but because so little is known about them (Moran, 1986).

1.1. **Introduction**

Periodic, massive depletion of hard corals from large feeding aggregations of *Acanthaster planci* (L.) has been observed throughout the Indo-Pacific region. When abundant, the species is a major disruptive factor of coral communities; for its absence from the Caribbean brings about some fundamental differences in the dynamics of Atlantic and Pacific reefs extending through several trophic levels (Birkeland, 1989). Although the potential has been well recognised, the development of long-term effects on coral reefs from outbreaks of *A. planci* can not be determined from our present knowledge of coral reef ecosystems and the biology of this species.

Therefore, repeated episodes of outbreaks must remain a primary cause of concern for environmental management authorities and scientists. Moran (1986) focussed attention on our poor understanding when he coined the phrase "the *Acanthaster* phenomenon" to describe his review of this ænigmatic species. He found there was a lack of information on growth, longevity, mortality, movement and feeding; data which were essential to achieve a basic understanding of the dynamics of *A. planci* populations.

Birkeland and Lucas (1990) shared Moran's opinion about the need for life history data on *A. planci*, pointing out that attention had been focussed on ecological processes at the expense of species-specific phenomena. They suggested that the characteristics of the natural life history were essential to take into account when analysing theories concerning outbreaks. The unique morphology of *A. planci*, its ability to vary its behaviour and effects on its environment with circumstances, and our inadequate understanding of its biology and life history are the principal reasons why we are unable to explain the extraordinary influences that *A. planci* has on the coral community (Birkeland and Lucas, 1990). To comprehend the demographic processes involved in outbreaking events, dedicated field studies supported by carefully controlled experiments are needed, including detailed analyses of population dynamics and adequate description of life-history characteristics of the species. So far, progress in these areas of research on *A. planci* has been substantially restricted by the need to develop specific methods for studying populations and monitoring individuals in the field.

The difficulty in studying populations of *A. planci* is clearly evident when, despite more than 25 years of investigations, outbreaks remain unpredictable and efforts to protect live corals in many regions through implementation of large-scale control schemes have been futile (see Yamaguchi, 1986; Zann and Weaver, 1988; and Birkeland and Lucas, 1990). It is surprising that very few studies have actually described an outbreak episode through its course and, therefore, there is little rigorous evidence available to support theories on the cause of their initiation or decline. Outbreaks on the Great Barrier Reef (GBR) typically form where populations have been observed to increase over six orders of magnitude within a single year or two, and decline just as rapidly (Birkeland and Lucas, 1990; Kettle, 1990; Bell, Kettle and Stump, unpublished). However, long term studies from Fiji by Zann et al. (1990) found no invariate patterns involved in the population dynamics of *A. planci* and they concluded that outbreaks may build up gradually, or suddenly.

Most theories on the development of outbreaks have been derived from ecological models based on non-specific theory or by using case histories of interactions between various other taxa, i.e. where the population dynamics result from postulated interactions between predator, prey and the environment. These approaches were undertaken because of the inadequate data available on *A. planci* populations. Therefore, the characteristics of the phenomenon have been explained more often in terms of hypotheses using a range of eclectic factors, subject to the particular approach made by scientists, rather than being based on evidence from long-term studies on *A. planci* populations over a wide geographic range. The principal hypotheses include: aggregation of adults (Dana et al., 1972), variation in predation on adult starfish (Endean, 1969), variation in survival of early developmental stages (Birkeland 1982, Lucas, 1984), feeding preferences, aggregative tendency and their fecundity (Antonelli and Kazarinoff, 1984), the life history parameters of the prey and predator (Bradbury et al., 1985), differences between rates of coral consumption by starfish and the recovery of reefs (McCallum, 1990; Ormond et al., 1990; Parslow, 1990), and particular life-history characteristics not found in other asteroid species (Moore, 1978).

Several studies have described variable responses to the interaction between *A. planci* and its environment, and offer a source of potential clues as to how outbreaks might occur. The first study to report differences between high and low density populations, by Chesher (1969a), concluded that if population explosions were due to changes in the life history of *A. planci* then successful controls would probably not be possible. Ormond and Campbell (1974) argued that certain features of the biology of *A. planci* (the "special instability" hypothesis) predispose it to undergo large fluctuations in population size depending on the chance conditions surrounding spawning and recruitment. These attributes potentially make this starfish more susceptible than most other species to the changes in environmental conditions caused by human populations (Ormond and Campbell, 1974).

Moore (1990) suggested that the ability to alternate life histories between endemic and epidemic characters in different habitats is a common feature of "outbreaking organisms" and the potential to alternate life-history characteristics may explain their ability to cause intermittent outbreaks. In summary of a number of theoretical studies, Antonelli et al. (1990) decided that, apart from the life-history characteristics, the only process capable of suppressing outbreaks is increased predator pressure on starfish. Since most information on life-history characteristics of *A. planci* have been derived from laboratory rearing experiments (i.e. Yamaguchi, 1975; Lucas, 1984) and have yet to be confirmed from populations in the field, theories on outbreaks involving population characteristics must remain speculative.

Information on both the life history and physiological characteristics of a species are necessary before an understanding of their way of life (i.e. their ecology and population dynamics) is possible (Lawrence 1990), for progress in population dynamics and production biology depends on the accurate measurement of growth (Nauen and Böhm, 1979). The characteristics of life histories (e.g. growth, reproduction and longevity) on population dynamics in species which undergo periodic population explosions is clearly of primary importance, as has been shown in studies of the locust, *Schistocerca gregaria* (Cheke, 1978) as well as in starfish, i.e. *Asterias rubens* (Moore and Campbell, 1984). Phenotype plasticity and the ability to respond to altered environments has been implicated as the primary cause of the rapid colonisation of the Tasmanian coastline around the Derwent estuary by the introduced Japanese asteroid, *Asterias amurensis* (Davenport and McLoughlin, 1993). Therefore, it is only through an understanding of the population dynamics of *A. planci* that the issues of natural or unnatural causes and the prediction of outbreaks be properly addressed.

The present study was undertaken to build an understanding of the ecology and biology of *A. planci* and, by using information obtained from field populations and laboratory experiments, describe its life history in the field. The thesis presents accrued evidence for the validity of a novel method of age determination, a principal tool of demography and the key to understanding life history strategies. Core field research involved a mark/release/recapture exercise on Davies Reef (Central GBR) over 38 months from October 1988 to December 1991. The results of age estimation and skeletomorphometric analyses are also presented from studies of several other populations within the GBR region. Application of these results have formed the basis for a comparison of life history and physiological traits in several populations from the Western Pacific region. Traits are identified and analysed using allometric relationships, linear regression analyses and ANOVA techniques on a range of morphometric variables. This approach was undertaken because most traits are affected both by broad trends, common to a whole lineage, and by local forces specific to a single population. One way to separate these influences is to first identify the broad trends then analyse the deviations which characterise local conditions by identifying traits whose values lie outside their size-predicted scaling (Stearns, 1984). Finally, within the limitations of available data, I have contributed to the overall debate by suggesting a life-history strategy for *A. planci* which offers potential insights into its success in both high and low density populations.

1.2. Ecology of *A. planci* populations in the Indo-Pacific

With little or no evidence of local origin, most outbreak populations are referred to as being secondary. Secondary infestations are defined as high density populations developing from significant larval recruitment from centres of infestation or migration of large numbers of adult starfish from devastated reefs to other reefs (Endean, 1977). Evidence for the role of occasional large recruitment episodes from larval dispersal, in the occurrence of outbreaks, has accumulated with time, whereas no specific evidence for adult migration has emerged (Moore, 1990). Theories on causes of population outbreaks are discussed thoroughly in Moran (1986) and Birkeland and Lucas (1990). Although implicated, human influences on reefs have not yet been specifically linked with particular *A. planci* outbreaks. Population outbreaks of *A. planci* have occurred in natural environments without anthropogenic influences as well as in those exposed to various stresses linked to environmental modifications caused by human populations (Thomassin, 1984).

It is apparent that no one particular hypothesis can be used to predict outbreaks, however with existing information, their potential timing and location results largely from larval survival, strongly influenced by environmental conditions, and their distribution by current patterns (Birkeland and Lucas, 1990). Kettle (1990) studied a high-density population on Helix Reef (Central GBR) which appeared and then crashed between May 1984 and May 1986. His results were consistent with the hypothesis that recruitment occurred by the appearance of individuals as they switched from their cryptic juvenile phase; a consequence of massive larval settlement. From his physiological studies, Kettle (1990) observed that the starfish were weakened by food limitation and suggested they were not able to complete a journey between mid-shelf patch reefs on the GBR, even if they "knew" which direction to take. High-density outbreaks with limited resources experience short habitat duration and subsequent lifespan is inevitably attenuated due to starvation (Kettle, 1990). Therefore, unless reefs are very close or connected (i.e. by shallow banks), migration of *A. planci* populations between reefs is most unlikely.

A large-scale pattern of southerly spread has been observed twice on the GBR, one beginning around 1962 (Talbot and Talbot, 1971) and again after 1979 (Reichelt, 1990). This recruitment pattern resulted from larval dispersal in the southerly mainstream shelf current which occurs in the warmer months (Williams et al. 1984). Although there is virtually no information about *A. planci* on the GBR prior to 1960, Vine (1973) provided anecdotal evidence of an outbreak on Lodestone Reef, off Townsville, in 1954, suggesting outbreaking events may be periodic. Similar outbreaking events have been reported in other tropical regions of the Pacific. *A. planci* infestations in the Ryukyu archipelago, Japan, may have developed from "seed populations" which produced larval swarms that were carried by currents in widely scattered areas over many years (Yamaguchi, 1986). Annual fluctuations in catch records from control programs conducted at coastal locations reported by Yamaguchi (1986) reflected population densities and their persistence. Yamaguchi (1987) found that these populations along the Pacific Coast of Japan were likely to be sustained by successive generations until there was insufficient food remaining.

Although not inferred by Yamaguchi, peak catches for each location were likely to reflect the timing of greatest recruitment to the adult population; i.e. these populations would then be dominated by 2-3+ year recruits, where massive recruitment occurred two to three years prior to the recorded peak catch. Subsequent persistence of these populations (demonstrated by consistently attenuating catch records) can be interpreted as limited subsequent recruitment, which offers a means of obtaining an estimate of longevity for those individuals from the mass settlement cohort. Those populations where estimates could be obtained with some confidence showed a stable adult size-frequency distribution (i.e. demonstrating subsequent young adult recruitment levels were low). In Yamaguchi's results, the Kushimoto locality appears to best satisfy the proposed conditions for estimating longevity, assuming there were sufficient food resources for the starfish to achieve their maximum lifespans. They show the Kushimoto population persisted for five years after the peak catch record and therefore, suggests an estimated longevity of up to eight years.

Observations on outbreak populations have also been obtained from field studies in Micronesia. Chesher (1969a) first reported an outbreak around Guam early in 1967. Cheney (1974) surveyed three distinct populations around Guam and showed a positive relationship between coral abundance and starfish body size. He also reported that individuals from low density populations were cryptic by day, and variable in gonad state and body size, while high-density groups fed continuously, and had uniform gonad state and body size. Cheney's (1974) study provided the first evidence of intrinsic differences between populations of varying density and food resources.

An objective evaluation of characteristics of outbreak populations was attempted by Dana et al. (1972) using the data collected by the 1969 Westinghouse *Acanthaster* survey of coral reefs in the Pacific Ocean. They found abundances were highly variable with few of the reported infestations meeting the published criteria for distinguishing outbreaks from normal populations. They concluded that all population types had developed unimodal size-frequency distributions which required no abrupt change in population dynamics and therefore, it appeared that outbreaks had grown in a manner little different from those considered normal. Furthermore, Dana et al. (1972) rejected the interpretation offered by Ormond and Campbell (1971) that age-differences were the cause of the observed bimodality in starfish body size from different populations in the Red Sea. The observed size distributions might well represent the mean size of starfish of several year classes occupying habitats where differences in relative food supply had influenced growth rates (Dana et al., 1972).

Although not found in the Westinghouse survey, a number of studies since then have described multimodal size-frequency distributions within populations (Branham et al., 1971, Laxton, 1974; Endean and Stablum, 1973b; Kenchington, 1977; Conand, 1983; Yamaguchi, 1987). Despite finding determinate growth patterns in *A. planci* (where body growth ceases soon after maturity, i.e. asymptotic) from laboratory studies (Yamaguchi, 1974; Lucas, 1984), and also interpreted from field study results (Branham et al., 1971; Wilson and Marsh, 1974; and Zann et al. 1990), multiple size-frequency modes in adult populations suggesting age-structure (i.e. indeterminate growth) have been reported as a characteristic of many adult *A. planci* populations. Only a clear description of the growth patterns occurring in the field will resolve differences in interpretations of previously published data, and this is critical to understanding the population dynamics of *A. planci*. To achieve this goal, long-term field studies are required using slightly or non-invasive techniques for identifying individuals (so that growth and movement are not impeded), to follow individual cohorts in populations through their development, persistence and decline.

1.3. Characteristics of growth in Asteroidea

Most population studies of starfish have been limited by an inability to measure growth in the field because age determination and size-at-age assessment have not been possible. Smith (1940) stated that there does not seem to be any way in which the age of an individual starfish can be estimated unless the conditions under which it had grown were well defined. The primary factors influencing growth are seasonal changes in temperature, reproduction, and food supply (Feder, 1970; Lawrence and Lane, 1982) and these have been investigated for a number of asteroid species. Intraspecific variability in adult body size of many asteroids, reflecting variability in growth rate, is due to food type (e.g. Ormond and Campbell, 1971), food availability, reproduction, and resource partitioning (e.g. Scheibling, 1982b). The variability in growth processes under the influence of these factors casts significant doubt on the ability to determine age from body size measurements in this Class.

The energetics of growth and maintenance of somatic functions depend on the rate of metabolism and respiration. A review of respiration and growth of species in the Asteroidea by Nauen (1982) found that respiration was proportional to the surface area of the individual due to the absence of any specialised respiratory organs (i.e. growth must be asymptotic) with the relationship in the form;

$$Q = a \times W^b$$

where
Q = oxygen consumption per unit of time
W = weight of starfish
b = the weight dependent measure of increasing oxygen consumption (= 0.667)
a = species-specific coefficient

The interrelations between metabolism and growth were investigated by Pauly (1979), who showed that respiration limited by body surface area is an important assumption for the application of the von Bertalanffy growth (VBG) equation in poikilothermic species. The fact that starfish do not develop any specialised structures for respiration, except potentially by exposing peritoneal folds which penetrate the body wall over the aboral surfaces (the papulae), supports the assumption of VBG over the late juvenile and adult phases of the life cycle (see Nauen, 1982; Lucas, 1984; Birkeland and Lucas, 1990). The exponent of the relationship between oxygen consumption and body mass in *A. planci* was found to be 0.87 by Kettle and Lucas

(1987), significantly higher than the exponent (0.67) for a direct relationship between metabolic rate (MR) and body surface area, but significantly less than 1.0, where (MR) α body mass. This shows *A. planci* has some capacity to actively obtain oxygen rather than passively absorb in relation to surface area. Thus, the major part of growth of starfish may be adequately described by the VBG formula, apart from the early stages, where growth is exponential but slows towards maturity.

A brief review of the literature on growth characteristics in echinoderms was undertaken by Lawrence (1987), who concluded that the general post-settlement pattern is sigmoid. A determinate mode of growth was proposed for *A. planci* by Lucas (1984) having determined an asymptotic, sigmoid growth curve from his laboratory studies. His data reflected either a genetic basis for maximum size resulting from channelling energy to reproduction at maturity or simply decreasing physiological efficiencies with increasing size for a particular body form. On the other hand, Kenchington (1977) used an indeterminate growth curve (non-asymptotic) as the basis for using body size to determine age classes among populations in the Central GBR. Kenchington argued for indeterminate growth in view of: the large size range observed, the fact that no large immature specimens had been identified from the field, and that a large proportion of individuals represented modes with a mean diameter greater than 25cm in size-frequency distributions of data from GBR populations. He stated that these observations showed that determinate growth in *A. planci* was not usual in populations from the GBR region.

Lawrence and Lane (1982) suggested that higher density populations of echinoderms would reduce the amount of food available to each individual and an optimal size of individuals, i.e. an asymptotic size inversely related to density, would result. To support their argument, they cited studies on several asteroid species, including *Asterias vulgaris* (Smith, 1940), *Oreaster reticulatus* (Scheibling, 1980b), and *Pisaster ochraceus* (Paine, 1976a). Variability in growth and ultimate body size in many starfish shows that the growth characteristics of this Class are dependent on local conditions and possibly remain subject to changes throughout life. Lawrence and Lane (1982) argued for constrained maximum body size in echinoderms due to decreasing physiological efficiencies with increasing size and channelling of resources away from somatic growth to gametogenesis (at maturity). Lucas (1984) stated that these two factors are evident in the determinate growth exhibited by *A. planci*, and that maximum size and growth rate were highly susceptible to diet.

The determinate growth pattern, as defined by Sebens (1987), is moderated by energetics, where the environment acting through ontogeny determines the growth rate, final body size and other life-history characteristics. He stated that growth trajectory and asymptotic size are genetically determined with minor environmental modification and, therefore, individuals grow to an asymptotic size that maximises reproduction under particular habitat conditions. Indeterminate growth refers to a plastic condition where the potential for continuous size change throughout life is retained as environmental conditions change (Sebens, 1987). In his extensive study, Sebens (1987) found difficulty in applying the asymptote/non-asymptote growth definitions to many species and showed several major patterns in animals do not fit either definition exclusively. Instead, he categorised seven growth patterns based on the degree of influence from ontogeny, genetic and environmental constraints affecting growth. Therefore, the simple determinate/indeterminate (asymptote/non-asymptote) division previously used for characterising growth in *A. planci* may not be appropriate.

1.4. Longevity in Asteroidea

There is scarce information on the longevity of asteroid species and an imbalance in data for their populations compared with the quantity of literature available on echinoid species. The early review by Feder and Christiansen (1966) reported there was little information on the age reached by starfish, but may well be long-lived, for once they reach adult size they have few predators and can be expected to die a "natural" death. Since then, population studies of asteroids have progressed slowly without the ability to accurately determine age, apart from the few experiments using cultured specimens. The meagre data concerning longevity is due principally to the fact that large test plates, or spines, used by skeletochronometric methods in echinoids, are generally not found in asteroids or are very much reduced in relative size, such that skeletal growth increments do not develop or are not readily discernible. The ossicles within the body walls of asteroids generally increase in number with overall body growth preventing the development of consistent growth lines (Lawrence, personal communication). A skeleton composed of small ossicles is characteristic of the Asteroidea and allows for a wide range of flexible body movements, including locomotion, climbing and food handling. The application of an alternative, problematic bioassay method of determining age through quantification of the accumulation of age pigments has been developed with mixed success using other taxa (eg: Eldred et al., 1982; Nicol, 1987; Crossland et al., 1987; Clarke et al., 1990; Hill and Radke, 1988; Bruslé et al., 1990; Hill and Wommersley, 1991). This method has also met inherent difficulties with application to *A. planci* (Stump, unpublished) and the approach has been deemed unsuccessful without; (a) the additional input of a significant biochemical study to isolate, characterise and quantify the specific fluorophore compounds involved, (b) an alternative means of identification and quantification, i.e. transmission electron microscopy (TEM), and (c) the availability of known-age, adult specimens for reference material (Stump, unpublished).

Most previous attempts to age adult *A. planci* from field collections have used whole body measurements (Endean, 1969; Branham et al. 1971; Ormond and Campbell, 1971; Nishihira and Yamazato, 1972; Ebert, 1973; Endean and Stablum 1973b; Laxton, 1974; Ormond and Campbell, 1974; Kenchington, 1977; Conand, 1983; Nakamura, 1986; Zann et al. 1987; Yamaguchi, 1987), although none were able to test the inherent assumptions associated with the method. Kettle (1990) found that larger starfish are only likely to be older over a very large size range and, therefore, acknowledged that this method of age determination is probably unreliable. At best, size is only loosely related to age in asteroids (Crump and Emson, 1978) because it is more often related to the availability of resources.

There has been broad speculation on the longevity of *A. planci*; from short-lived (Kenchington, 1977) to long-lived (Cameron and Endean, 1981). Ebert (1983) presented an hypothesis which suggested that environmental factors associated with latitude may affect life-span and recruitment so that faster growth and more rapid build up of populations should occur at lower latitudes. In doing so, populations close to the equator would experience rapid decline through shorter longevities, higher mortality or migration. Therefore, in higher tropical latitudes the starfish are predicted to be longer lived and possibly slower growing, so that *A. planci* in the southern GBR should achieve the maximum longevities for the region. However, no consistent analyses of populations over a wide range of latitudes has yet been undertaken to test this theory.

1.5. The study of life history in *A. planci*

If it is to survive, every species must possess reproductive capacities sufficient to replace the existing species' population by the time it has disappeared (Cole, 1954) and so, life history strategies develop out of combinations of age-specific survival probabilities and fecundities which can be displayed in the natural environment (Partridge and Sibly, 1991). Therefore, the physiological and life-history characteristics of *A. planci* are ultimately determined by reproduction and mortality schedules influencing growth rate and maintenance allocation, and are constrained proximally by resource limitation in high densities or low resource habitats. One way to make informed predictions concerning life history strategies is to compare relative data from populations which inhabit different environments or demographic regimes (Charlesworth, 1980; Horn et al. 1982). This approach has been used in the present study to compare with the previously published life-history characteristics, outlined below.

1.5.1. Evaluation of life-history theory

How species go about growth, survival and reproduction through their life-cycle is "the general life history problem" (Schaffer, 1983) and recent studies have attempted to understand the optimising processes involved in the allocation of resources among these life-cycle functions (Stearns, 1992). Most taxa experience physiological constraints, imposed by habits and habitat, such that the number of life cycle patterns is limited in particular environmental conditions. The underlying mechanism which allows for the variety of patterns of life histories displayed by natural populations is not well understood, however it is generally held that they are regulated by abiotic (density-independent) and biotic (density-dependent) factors which interact under genetic control and operate through maximising (or optimising) some measure of population growth (Gross, 1986). Life history theorists have attempted to classify these patterns and their proposed methods have been reviewed extensively (e.g. Stearns, 1976; Charlesworth, 1980; Maynard Smith, 1989; Stearns, 1989b; Roff, 1992; Stearns, 1992). These theories have been generally developed from analyses of how variation in life-history traits leads to variation in fitness among individuals (assuming that the overall strategy results from a process of fitness optimisation), where the traits and their plasticities determine the population dynamics of species (Roff, 1992; Stearns, 1992). Life-history strategy studies, therefore, are of primary importance to understanding the *Acanthaster* phenomenon.

Life cycles are moulded by the way resources derived from food are allocated between various metabolic demands, because the allocation of resources is fundamental for the form, function and fitness of organisms (Calow, 1984). The three major processes implicated as selective forces in the development of life-history theory (Stearns, 1976; 1977) are: juvenile and adult mortality (Cole, 1954; Murphy, 1968), the extremes of population density (MacArthur, 1962; MacArthur and Wilson, 1967), and survival and fertility in relation to age (Medawar, 1957; Williams, 1957; Istock, 1983). However, often the simplifying assumptions incorporated in life history theories (see Sibly and Calow, 1983; Charnov, 1993) have led to disagreement concerning the influence of these processes and their resolution in real life situations (Calow, 1984).

Early predictions derived from life-history theory were based on the premise that populations have evolved by selective forces which promoted adaptations that either maximise ***r*** (the maximal intrinsic rate of natural increase) or produced longer lived competitors with ***K*** characteristics (organisms which exploit the carrying capacity of their environment), termed ***r*** and ***K*** selection (see MacArthur and Wilson, 1967; Pianka, 1970). The concept has received much attention and there is general agreement that the theory involves density-regulated population selection for maximising population size, although problems have arisen in its application to natural populations particularly when defining life-history traits (Roff, 1992).

An alternative concept is that in conditions of uncertainty in relation to fluctuating or unpredictable habitat quality, a bet-hedging strategy (Stearns, 1976) will adjust life histories with changes to mortality and fecundity in the opposite direction to those predicted from ***r*** and ***K*** selection. An adequate definition of fitness for all life-history theory has not been possible, for it must take into account the existence of different environments and explicitly model the interactions of particular environments and organisms (Stearns, 1976). However, Charlesworth (1980) used modelling analyses to show that the products of evolution can be expected to maximise ***r*** and therefore considered it was an adequate measure of fitness in most organisms.

The classification of life histories based on the ***r*** - ***K*** dichotomy has been criticised for being too superficial. Life histories are generally more complex and there is a lack of theory on how ***r*** and ***K*** characteristics develop under density dependent compensation, i.e. changes in environmental parameters whose effects on ***r*** are related to population density (Sibly and Calow, 1986, 1987; Stearns, 1992) or what parts of the life history are affected by density dependence (Charnov, 1993). However, Boyce (1984) was critical of Stearns' (1976; 1977) ideas, which argued against ***r*** and ***K*** selection, stating he (Stearns) misinterpreted the theory as originally defined. Since density is only one of many environmental forces shaping life histories, we cannot expect life history patterns to reflect the consequences of ***r*** - ***K*** selection, except in rare cases (Boyce, 1984). The notion of the ***r*** - ***K*** continuum has been developed in terms of the classical logistic theory and its assumptions, and is therefore incompatible with complex life history considerations (Mertz, 1975) such as predicting life histories from models other than those based on density-dependent population growth (Mueller, 1988a).

In reviewing the characteristics of asteroid biology, Nauen (1982) concluded that there is generally a high degree of flexibility in growth response to variable environments, such that many asteroid species exhibit a low basic metabolism but occasionally high feeding rates and fast growth characterises them as ***r*** strategists. The ***r* - *K*** continuum (Pianka, 1970) represents two opposing kinds of selection: from short-lived ***r*** organisms which allocate all available energy into reproduction, to produce the greatest number of offspring for the least possible amount invested in each one; to ***K*** organisms which live in high density populations, survive high levels of competition for resources, and allocate energy to maintenance for a long life and produce low numbers of offspring. This classification was derived primarily from observations on terrestrial species and aquatic poikilotherms do not appear to obey the generalisations of ***r* - *K*** selection determined from terrestrial species (Pianka, 1970).

A. planci, compared with many other coral reef echinoderms, exhibit characteristics which indicate it is a strongly ***r*** - selected species with high larval mortality, variable population sizes, few competitors, and high growth rates; i.e. they are opportunistic, tending to exploit short-lived habitats by devoting energy and resources to maximise reproductive potential (see Moore, 1978, Kettle, 1990). However, other reported attributes suggest a tendency to ***K*** - selection, including variable recruitment rates, being potentially long-lived, with large body sizes, and a well-developed defence battery of spines (see Cameron, 1977). Larval survival is mostly unpredictable and therefore, variable recruitment of juveniles must be counteracted by a shift towards ***K*** - selection in the adult phase, reflected by increased longevity (Moore, 1978). This apparent dichotomy in characteristics within a species shows that *A. planci* may not fit easily into the ***r* - *K*** selection scheme and, in response to widespread criticisms (see Boyce, 1984; Roff, 1992; and Stearns, 1992), other schemata may be more useful in describing this species.

Whether ***r*** and ***K*** characteristics can be useful in determining the life-history strategy in *A. planci* has not yet been demonstrated. There is overwhelming agreement in recent reviews about a lack of comprehensive life-history theory, although there has been some success with age-specific models, and with rejection of ***r* - *K*** selection there is now a need for new density-dependent life history theories (Stearns, 1992).

The use of particular environmental conditions to classify life history strategies has had some success by developing mechanisms which link habitats to life histories, i.e. fecundity and mortality schedules, where habitat determines the mortality rate which in turn forms the reproductive life history (Stearns, 1992). Extrinsic influences associated with patterns in mortality rates have been shown to be much the same for taxa which have a similar "design" (Charnov and Berrigan, 1990), supporting the habitat-dependence approach. In this way, the response to external conditions was first invoked as the primary force which determines a range of ecological strategies in plant species (Grime, 1977; Table 6.1) allowing the development of a common life history framework among species. Grime used these general responses to predict that species which have adapted to different habitats develop particular life history strategies. The three basic functional strategies structuring the habitat-dependent framework are described in Table 1.1.

Table 1.1. The primary life history strategies in species, *sensu stricto* Grime (1977), determined by factors limiting biomass, i.e. environmentally determined combinations of characteristics for habitat-determined strategies.

Strategy	Definition
disturbance	caused by damage or loss of biomass
stress	caused by limiting production
competition	low disturbance and stress in habitats of high productivity and long duration

Grime's strategies were developed out of the influence of various levels of intensity of these two factors, where low levels of both promoted the development of *competitive* characteristics. Although, Boyce (1984) found that Grime's (1977) classification was overly simplified and warned that it condensed all types of stress related processes into a single factor which can only obscure the actual selective forces that operate in different habitats under a wide variety of stress phenomena.

However, Lawrence (1990; 1991) interpreted Grimes' three strategy classification through the availability of energy in biological systems, which is limited intrinsically or extrinsically, and by external factors (both abiotic, i.e. temperature, salinity and light, etc. and biotic, i.e. food availability) which can cause *stress* or *disturbance* affecting the retention of energy by organisms. Given the importance of energy to biological systems, organisms should respond to external factors in an adaptive way, through variation in the life-history characteristics of longevity (or mortality) and reproduction which are the basic components of fitness (Lawrence, 1991).

Lawrence (1990; 1991) applied Grimes' triangular model to construct a two-tiered classification of echinoderm species which rationalised and grouped this highly diverse Phylum according to their life history and physiological characteristics. Habitat-dependent characteristics in echinoderms can be divided into primary (life history factors: reproduction and mortality) and secondary (physiological factors: feeding, growth and maintenance) strategies where the criteria for separating species involve; size at first reproduction, reproductive effort, reproductive output (fecundity), mode of feeding, potential growth rate, and susceptibility to predation (Lawrence, 1990). Through this approach he identified high resource availability and long duration of habitat as the principal factors which shape the life history of *A. planci*. He noted the important characteristics of the species included a high potential for resource acquisition (being an active forager), long lifespan, iteroparity with high fecundity and relatively ineffective protection against *disturbance* (i.e. predation). Therefore, he concluded *A. planci* is a *competitive* echinoderm with low reproductive effort but high fecundity and a rapid growth rate.

1.5.2. Age and life histories

The incorporation of age structure in theoretical models has been used to investigate variation in fecundities and probability of future survival of individuals in populations (Charlesworth, 1980). The methods used to generate these models have been based on the simplifying assumptions, i.e. a stable age distribution, that may not always be legitimate. Ebert (1984) found this requirement forms the foundation of population analyses, with little basis of validity in echinoid populations which are usually declining, but experience occasional increases. This description would also apply to *A. planci* populations where recruitment involves a number of stochastic processes (see Zann et al., 1990) resulting in high recruitment variation. It is apparent at this time in the development of life-history theory, that there are few generalisations which are applicable to wide ranging taxa, and most now require more comprehensive testing on field populations over their geographic and environmental ranges.

1.5.3. Assessment of life histories

The use of constants calculated from combinations of values derived from analyses of life-history characteristics of species or taxonomic groups has been reintroduced by Charnov and coworkers (Vøllestad et al., 1993) for estimates of mortality $\boldsymbol{M}$, maximum size $\mathbf{L}_{\infty}$, size at maturity $\mathbf{L}_{\alpha}$, and the growth constant from the von Bertalanffy equation $\boldsymbol{K}$. The most difficult parameter to assess is $\boldsymbol{M}$, although it has been linked to the parameters $\mathbf{L}_{\infty}$ and $\boldsymbol{K}$ through the von Bertalanffy growth equation, i.e. while there appears to be a relationship between longevity and $\boldsymbol{K}$, it is not straightforward, and relies on at least three variables (Pauly, 1991);

(a) body size.
(b) growth rate as expressed by $\boldsymbol{K}$.
(c) the mean environmental temperature.

Life-history constants have been advocated as a means of understanding life-history variation and evolution because they show different characteristic values in the major animal groups, i.e. fish, reptiles, birds and mammals (Beverton and Holt, 1959; Beverton, 1963; Charnov and Berrigan, 1991; Charnov, 1993; Vøllestad et al., 1993). These numbers are used to express patterns in the relations of life-history characteristics (growth, mortality, maturity and fecundity) with the same units of measure, i.e. they are dimensionless and therefore, describe magnitudes of these relations in an absolute sense, independent of survival times or reproductive values (Charnov and Berrigan, 1991). The use of this approach to the study of life histories is attractive because it offers the potential to obtain broad empirical rules (Charnov and Berrigan, 1991) which may be useful for modelling and predicting the dynamics of populations, the cornerstone to understanding *A. planci* outbreaks.

The three principal Beverton-Holt constants nominated by Charnov (1993) are:

(a) $K/M \approx$ constant
(b) $M.\alpha \approx$ constant
(c) $L_\alpha/L_\infty \approx$ constant, where $L_\alpha/L_\infty = (1 - e^{-K.\alpha})$

where
K = von Bertalanffy growth constant (month^{-1})
M = mortality constant (month^{-1})
α = age at maturity (month)
L_α = size at maturity*
L_∞ = asymptotic size*

* in this study both spine ossicle length and whole body diameter are used as size determinants

Although the life-history constants have been shown to be consistent in various groups of animals (Charnov, 1993), Vøllestad et al. (1993) found they varied according to the particular population and habitat in brown trout, showing that the complexity of life histories and the variability of habitat type may be the main determinants of life-history constants.

The concept of determining a life-history strategy through optimisation of trade offs between characteristics of populations may well be optimistic, but the models do offer an experimental starting point by exploring potential interactions, based on resource use, under a number of simplifying assumptions (Calow, 1984). Therefore, to understand the functional biology and life-history strategy of *A. planci*, data from a wide range of populations must be obtained to evaluate the potential trade offs in life-history traits and the predictions that follow, as well as examining the environmental influences on their life history.

Given the many difficulties in maintaining numbers of *A. planci* in controlled experimental conditions (personal observation; see Yamaguchi, 1975; Lucas, 1984) the best approach to study the life history, at least in the short-term, is through investigations of life-history characteristics from population studies. This project has attempted to develop a life-history strategy from population studies on *A. planci*, however, there is still a great deal of information that is needed to produce a satisfactory description of the characteristics of this species.

CHAPTER 2

VALIDATION STUDY OF THE SPINE PIGMENT BAND COUNT (SPBC) METHOD OF AGE DETERMINATION FOR *A. planci*.

2.1. **Introduction**

2.1.1. Morphological characteristics, growth and age

A. planci is a relatively soft-bodied asteroid that can achieve very large body sizes up to 100cm (GBRMPA COT database). With a characteristic multiarmed, highly flexible morphology, it has the unique ability amongst corallivorous asteroids to exploit dense stands of branching coral species (Lawrence, 1988; Birkeland, 1989; Lawrence and Moran, 1992), involving a high quality, large food resource biomass, particularly on GBR reefs. No doubt it is the quantity of resources which allow *A. planci* to attain such large body sizes in the field, although the mode under which very large sizes develop is not known. From laboratory studies of growth in *A. planci*, Lucas (1984) found little relationship between size and age since adults ceased growing for a number of years, having achieved relatively small asymptotic sizes, and then underwent a period of negative growth prior to death. The review by Birkeland

and Lucas (1990) concluded that there may be little relationship between body size and age in *A. planci* in populations and therefore the size-determining age technique, at best, depends on a number of assumptions related to the population density and habitat characteristics with many inherent limitations. Seasonal variation in body size of *A. planci* has also been demonstrated in a population near Hawaii by Branham et al. (1971) where an initial increase followed by a decrease in the size distribution correlated with the gametogenic cycle. They also found that mean body diameter was significantly larger in non-aggregated individuals than in aggregated populations within a similar habitat, reflecting the influence of food availability on size.

An alternative method used to age echinoderms is to measure growth bands in skeletal elements (skeletochronometry) if such structures occur and to validate the periodicity in each growth band class (see Beamish and MacFarlane 1983). Echinoderm skeletons are composed of many calcite ossicles secreted by mesodermal tissue, termed stroma. A three-dimensional latticework of calcite trabeculae form the skeletal stereom of each ossicle, with stroma occupying the pore spaces (Smith 1980). Sclerocytic cells involved with stereom formation near the edges of the ossicle preserve a locally continuous cytoplasmic sheath separating them from the tissue within the ossicle involved with ossicle maintenance (stroma) (Dubois and Chen, 1989). Stereom growth is both intracellular (intrasyncytially) in early stages of stereom formation, and extracellular around the growing edges and in regenerating zones following damage, although the relationships between sclerocytes and the stereom during mineralisation remain poorly understood (Dubois and Chen, 1989).

Heavily calcified species are more likely candidates for the skeletochronometry techniques and this is why echinoid populations have been used almost exclusively, i.e. Yonge, 1949; McRae, 1959; Moore, 1966; Weber, 1969; Heatfield, 1971; Taki, 1972b; Pearse and Pearse; 1975; Timko, 1975. Smith (1980) reported that skeletal growth lines formed in relation to the growing edges are found in most echinoids. Pearse and Pearse (1975) reviewed a number of early studies on growth zones in echinoid skeletons and concluded that seasonal changes in growth rates may result in seasonal patterns in growth zones which shows they can be reliable indicators of age. These studies have estimated age classes using growth rings or pigment bands in test plates and spines. They have either attempted to show or have assumed that the growth rings are formed in response to seasonal or lunar and tidal periodicity.

No success with skeletochronometric techniques for asteroid species has been documented, principally because most asteroids do not develop skeletal elements large enough to reveal distinct growth zones. Lawrence (1979) however, reported concentric growth rings similar to those of echinoids in the heavy, terminal ossicles and the large ossicles bordering the edge of the body wall of the intertidal asteroid *Nidorellia armata* (Oreasteridae). The basis for the deposition of growth rings in *N. armata* was not determined. Similar growth rings in ossicles were also reported by Dubois and Jangoux (1984) from *Asterias rubens*.

The aboral body surface of *A. planci* is covered with long sharp spines. The spine ossicle is mounted on a prominence of the body wall called a pedicel (Motokawa, 1986). Normally the spines are held stiff to deter predators, but they can bend at the joint with the pedicel to facilitate their often cryptic behaviour (Watanabe, 1983). Motokawa (1986) described the histology of the primary aboral spine joint; both the spine and the pedicel have shallow depressions at their centres and the joint is surrounded by epidermis and filled by an underlying dermis of collagen fibres and scattered muscle fibres. This and the presence of neurosecretory cells suggests that the connective tissue has catch properties, becoming soft to allow passive bending of the spine appendage (Motokawa, 1986). This attribute allows *A. planci* to negotiate dense branching stands of coral while foraging and to occupy cracks and crevices during quiescent periods to escape predators.

2.1.2. Marking and identification in the field

The principal techniques used to obtain data on population dynamics involve:

(a) accurate marking and identifying individuals in the field.

(b) a valid method of age determination.

The problem with identifying individuals in the field and following them for long periods of time is because it is not possible to tag or mark starfish (O'Gower et al., 1973). Tagging or marking *A. planci* has met with consistent failure as staining does not last, while tags are shed within one to two weeks (Glynn, 1982b; Keesing and Lucas, 1992). However, there are a number of morphological features which can be used as a means of identification, including: multiarmed morphology for arm and spine amputation (Owens, 1971; Cheney, 1972b; Ormond and Campbell, 1974); natural external features, i.e., colour patterns, body size, number of arms, and position of disfigurements from partial predation attack or mutation (Aziz and Sukarno, 1978); and, the arrangement of madreporites on the aboral disc surface, number of anuses, and number of spines (Glynn, 1982b).

Although the madreporite coding descriptor was considered to be the preferred means for identification by Glynn (1982b), he found the method to be relatively time-consuming and probably unsuitable for large population studies. Since then, no population studies have attempted to employ the madreporite pattern method but two studies have successfully used it to identify individuals in aquaria over long periods (see Lucas, 1984; Kettle, 1990). Therefore, development of an efficient method of identifying individuals in the field was undertaken involving the use of the madreporite coding method for identifying individuals, after Glynn (1982b). The method was trialled on a large population on Davies Reef to assess the potential for validation of the proposed method of age determination.

2.1.3. The SPBC method of age determination

A novel method for determining age in adult *A. planci* from the Great Barrier Reef (GBR) has been developed from skeletochronometric techniques (Stump and Lucas, 1990). The method was based on pigment band counts on the shaft of aboral spine ossicles sampled from arms of individuals collected from Davies Reef, Central GBR. Growth in aboral spine ossicles of *A. planci* is essentially by elongation with addition of stereom primarily at the base. Thus, the spine's growth history is preserved along its length. The spine pigment band count (SPBC) hypothesis for age determination is that pigment bands alternate with light coloured bands and form an annual band pair where light bands correlate with the warmer months of the year and dark bands over winter.

Post-larval development in *A. planci* encompasses an increase in linear dimensions of approximately three orders of magnitude in the two years to maturity (Yamaguchi, 1974). Stump and Lucas (1990) proposed that spine ossicle growth occurred by expansion both in length and diameter during the juvenile phase when body size increase is most rapid. Continued spine growth after maturity occurs by elongation alone, to maintain the needle-like shape, which offers increased protection and allows for continuous repair of damage that may occur to spines. The pattern of spine ossicle growth maintains an allometric relationship with body size, under selective pressure for optimal protection with available energy from the overall allocation to somatic development, which decreases significantly at the onset of maturity under the determinate growth pattern previously described for *A. planci*.

The principal objective of this Chapter is to present accrued evidence for a valid method of age determination from a mark and recapture exercise. Other objectives related to the development of a valid method of age determination involving the methods employed in this study, included the assessment of; reliable individual identification, skeletal marking using tetracycline, sample sizes for skeletal ossicle morphometry, the reliability of spine pigment band counts between observers, and the assessment of whole body size morphometry in unfed starfish.

2.2. **Methods**

2.2.1. Davies Reef recapture study

The position of Davies Reef in relation to the coastline and the surrounding GBR region is described in Figure 2.1. Davies Reef is an inner mid-shelf patch reef approximately 80 kilometres north east of Townsville. The field study was divided into two programs involving:

(a) searches for recaptured marked starfish for SPBC method validation.

(b) population subsampling exercises for time-series analyses of morphometric characteristics at approximately twice yearly over 38 months.

The results in this Chapter involve aspects of (a) only, (b) is treated in Chapter 3.

2.2.2. Site selection

The mark and recapture exercise was conducted on Davies Reef (Central GBR). The study was commenced in October 1988, over 12 months after the reef had been declared to be under the influence of an outbreak (see Johnson et al., 1988). The site was selected for several reasons, including accessibility through sharing shiptime with benevolent AIMS staff. However, the most important factor involved was that at the commencement of the study *A. planci* recruitment did not appear to have been on the scale that had occurred on many of the reefs in the Central Section. Massive recruitment usually leads to the early collapse of populations (see Kettle, 1990) and would have severely limited assessment of the field methods proposed in this study.

The outbreak cohort was estimated to be aged 2+ years when first sighted during the surveys in 1987 (size at age, under normal conditions, when they become apparent during daylight hours) and therefore would have been aged 3+ years in October 1988 at the time the present field study commenced. The early sighting of the outbreak cohort (and therefore its accurate age prediction) are assumed to be reliable due to the large numbers of AIMS scientists and divers regularly visiting this reef.

2.2.3. Morphometry in other populations

Collections of spine ossicle samples and whole body morphometric data was undertaken in a comparative study of eight populations from a wide geographic range in the Western Pacific region. Analysis of the relationship between the development of spine ossicles (and spine pigment bands) and body size was carried out as a preliminary assessment of the potential for application of the spine pigment band count (SPBC) method to populations outside the GBR.

2.2.4. Experimental methods

2.2.4.1. Use of tetracycline as a permanent skeletal marker

A preliminary test found that oxytetracycline hydrochloride, an antibiotic in the tetracycline class, was incorporated into the spine and pedicel ossicles one month after administering approximately 200µg in solid form directly into the oral cavity (cardiac stomach) of adult *A. planci*. Tetracycline has been used to mark growth zones in echinoid species with a dosage of approximately 100mg/kg wet weight (after Taki, 1972a). Therefore an experiment was conducted to test the effect of variable concentrations of tetracycline on *A. planci* and the optimum dosage for incorporation into ossicles as a skeletal marker to identify zones of skeletal growth. The dosages were varied by approximately 1 order of magnitude above and below the recommended dosage, after Taki (1972a).

A. planci were collected from Davies Reef (April, 1989) and transported to the AIMS outdoor aquaria facilities. The starfish were maintained in 40cm x 60cm x 35cm plastic bins (open circuit) and were fed on frozen scallop meat for 1 month due to the difficulty in maintaining a sufficient supply of hard corals. The scallops were presented on the half shell, which facilitated the cleaning maintenance of the aquaria and were replaced every 2 to 3 days. Oxytetracycline was mixed with filtered seawater and administered into the coelomic cavity by syringe fitted with a hypodermic needle. The intracoelomic administration of tetracycline was used to ensure the accuracy of the dosage as there is a potential for the antibiotic to be lost from the oral cavity during ingestion and complete absorption may take several days due to a slow dissolution rate in seawater. Tetracycline dissolution was found to be incomplete after 4 days in a vial containing seawater at approximately 22°C (personal observation).

The experiment was divided into 6 dosage treatments ($\mu g.kg^{-1}$ whole wet weight) using four adult starfish per treatment:

(a) dosage = $25\mu g.kg^{-1}$
(b) dosage = $75\mu g.kg^{-1}$
(c) dosage = $100\mu g.kg^{-1}$
(d) dosage = $250\mu g.kg^{-1}$
(e) dosage = $750\mu g.kg^{-1}$
(f) dosage = $1500\mu g.kg^{-1}$

2.2.4.2. Effects of starvation on morphometric variables

Six starfish, identified by their madreporite patterns, were maintained unfed in a 2000L aquarium for approximately six months. Oxytetracycline was administered by intracoelomic injection with a dosage of approximately $100\mu g.kg^{-1}$ to mark spines for growth assessment. To minimise disturbance from handling, whole wet weight and whole body diameter were measured at the start, middle and termination of the experiment, while underwater weight was measured at the start and end only (due to the greater degree of handling involved). Whole body diameter was obtained by placing the starfish on a flat surface and measuring the greatest length arm-tip to arm-tip across the aboral surface. Whole body weights were obtained using a portable digital balance (Murakami Co.) accurate to ± 1g.

Spine ossicle samples were obtained from all surviving individuals (5) at the termination of the experiment and prepared for analysis by fluorescent microscopy (see morphometry section) to identify the persistence of the skeletal marker in starved specimens. Changes in underwater weight were analysed with a paired t test. Changes in whole wet weight and whole body diameter were analysed using paired t tests and survival rates were estimated from rates of body shrinkage determined from curves fitted using the equation for exponential decay in the form:

$$y = a^{(-b \, . \, x)}$$

where a = y-axis intercept

(0.69/b) = estimated time taken to halve weight (an arbitrary estimate for time of survival). The rate of weight loss was assumed to be related to survival time in *A. planci* according to Chossat's rule (Kleiber, 1961), when total weight loss approaches half initial body weight, death is assumed to result.

The SigmaPlot curve fitting program (Revision SPD-1.1; Jandel Scientific, 1992) was used to estimate the parameters of equations used to describe curves. The program fits curves iteratively using least squares to minimise the sums of the squares of the differences between the equation value and the sample data under the assumption that the standard deviations of each sample are equal. The significance of the curve parameters were tested using a t test (where t = parameter coefficient/standard error of the parameter).

2.2.5. Field Collections

A. planci were collected during daylight hours and therefore only adult specimens were obtained as juvenile *A. planci* are usually cryptic by day. Collections were undertaken at random points around the reef, although were focussed on areas of highest densities, whenever aggregations were encountered, to reduce the time spent diving under limited allocation of field time. Sampling for the skeletochronometric study on Davies Reef, Central GBR, occurred in: October 1988 (n = 142, 80 marked and released); April 1989 (n = 142, 81 marked and released; October 1989 (n = 300, 200 marked and released); March 1990 (n = 400, 300 marked and released); October 1990 (n = 200); December 1990 (n = 102), May 1991 (n = 202) and December, 1991 (n = 198). Those starfish not involved in the mark and release exercise were sampled for the morphometric study (Chapter 3).

All marked individuals on Davies Reef (n=661) were released within the same area on the north-west side of the reef. After the initial recapture rate was found to be low (c.a. 5%) the remaining sample sizes were increased according to the field time available on each trip. The marking procedure included three steps;

(a) the madreporite pattern was obtained by the method after Glynn (1982b),

(b) two fully-grown adjacent arms were removed and their position recorded in relation to the madreporite pattern, the arms were then processed for a spine reference collection,

(c) tetracycline was administered into the body cavity either through the wounds created by the amputation procedure or by injection through the aboral body wall directly into the coelomic cavity.

The method of describing the madreporite pattern (after Glynn (1982b)) is achieved by developing a string of numbers where each number in the sequence represents string counts of madreporites or absence of madreporites in consecutive interarm regions, beginning with the largest number of consecutive madreporites as the first number (see Table 2.3). Therefore, the 1st, 3rd, 5th, etc. (odd) numbers in the sequence involve madreporite counts and the 2nd, 4th, 6th, etc. (even) numbers in the sequence involve absences in the interarm zones (i.e. 4 1 3 6 equals 4 mads. + 1 absence, + 3 mads. + 6 absences). The string always begins with madreporites counts and ends with an absences count and will always contain an even number of counts.

Oxytetracycline was used in the form of commercially manufactured beads, with an enteric coating to slow the rate of breakdown, and administered in prepared capsules (Doxycycline © Faulding Pharmaceuticals, an antibiotic in the tetracycline class) for facility in marking the starfish. A dosage of approximately 50 - 100mg.kg^{-1} was administered by capsule directly into the body cavity using a modified teflon vaginal applicator. The madreporite coding method for identifying individuals (Glynn, 1982b) was modified to streamline the procedure so that increased numbers of individuals could be processed aboard research vessels on site. Potential recaptured starfish were screened through three levels of identification before acceptance;

(a) initial discrimination *in situ* during field collections, through the slow and equivalent regrowth of the two amputated, adjacent arms sampled prior to their release.

(b) provisional identification on site (visual inspection of emersed starfish on board the mother ship using madreporite coding and other morphological characteristics).

(c) use of laboratory prepared thin-sections of spine ossicles for fluorescent microscopy to identify the tetracycline trace, a characteristic bright yellow fluorescent marker, for unequivocal identification as a recaptured individual.

Spine samples from recaptured individuals were then matched to the pattern of pigmentation in spines from the original sample to determine spine and pigment band growth during the interim release period.

2.2.6. Morphometry and age determination procedures

Spine samples were preserved in 5% formalin-sea water on collection. In the laboratory soft tissues were dissolved in 10% sodium hypochlorite. The ossicles were then rinsed thoroughly in tap water and oven dried at 60°C. Care was taken with spine preparation and measurement as the tips of spines were easily broken. Spine ossicle lengths were measured with vernier callipers (to 0.1mm) with ca. 10 spine ossicles per individual. Pigment band counts on spine samples from all individuals were conducted on three separate occasions to ensure consistency with counts.

Spine sections were also prepared from preserved, known-age specimens of *A. planci* which had been reared in the laboratory study of Lucas (1984). In that study, juveniles of *A. planci* were obtained through in vitro larval development and then reared in a recirculating sea-water system at 24 ±3°C under artificial lighting conditions. After the phase of feeding on coralline algae, the juvenile starfish were regularly provided with corals with some periods of several weeks without food. Spines taken from these laboratory specimens (aged 20 months) were compared with those from the Davies Reef population as a guide to the relationship between stages in spine growth.

Body diameter (BD) and mean spine (S) and pedicel ossicle length (P) (c.a. 10 ossicles) were recorded for all individuals. The longest spines and pedicels were selected from the two arms removed for marking or from more arms from the samples sacrificed for internal ossicle morphometric analyses, excluding any distinctly short ossicles which may have been regenerated. The aboral arm spines are usually of similar length except for those near the arm-tip and those from regenerating arms.

2.2.7. Population size and density

The Davies Reef population size was determined from the mark/recapture results. The equation was developed by Chapman (1951) for a closed population with an approximately unbiased estimate of variance developed by Seber (1982).

$$N_c = \frac{(n_1 + 1)(n_2 + 1)}{(m_2 + 1)} - 1 \qquad (2.1)$$

an approximate variance estimate:

$$\mathrm{var}N_c = \frac{(n_1 + 1)(n_2 + 1)(n_1 - m_2)(n_2 - m_2)}{(m_2 + 1)^2(m_2 + 2)} \qquad (2.2)$$

and approximate 95% confidence interval

$$= \quad \pm 1.96 \times (\mathrm{var}N_c)^{0.5} \qquad (2.3)$$

where:
N_c = Chapman estimate for population size
n_1 = number caught and released
n_2 = number sampled for recaptures
m_2 = number recaptured

2.2.8. Spine growth in the Western Pacific populations

Samples were collected from 8 locations from a wide range of latitudes in the Western Pacific region (Figure 2.1).

Table 2.1 Location and dates for the collections of *A. planci* from populations in the Western Pacific region.

Location	Site(s)	^{0}lat.	T(^{0}C)	(n)	Collection dates
GBR region	Davies Reef (Central Section).	19	24.5	293	10/88; 4/89
	Butterfly Bay, Hook Island (Whitsunday Group).	20	24	68	10/89; 11/90
	Lady Musgrave Reef (Southern Section).	24	22.5	11	3/86; 4/90
NSW	Lord Howe Island.	31	19	39	2/90 to 2/91
Fiji	Suva Reef.	18	25.5	74	7/90; 10/91
Tonga	Ha'atafu Beach Reserve	21	24	43	10/92
Kiribati	Onotoa Lagoon and Abemama	0.5	26.5	51	6/90; 7/92
Guam	Hospital Point, Tumon Bay and Double Reef	13.5	28.5	120	7/92

where
^{0}lat. = latitude
T(^{0}C) = approximate mean annual seawater temperature

The Guam data were combined from three locations to maximise the ranges of whole body diameter and spine ossicle length for these analyses (see Chapter 4 for further analyses). Linear regression analyses were used to relate spine ossicle size with body size (whole body diameter) to determine differences among the populations which might influence the assessment of the SPBC method.

2.3. **Results**

2.3.1. The aboral spine appendage

Initial study of the skeletal ossicles of *A. planci* for evidence of periodic growth patterns showed that these were best developed in the aboral spine ossicles. Aboral arm spine ossicles are the longest ossicles in *A. planci* and they give the best resolution of pigment bands and growth lines. Hence this study concentrated on these ossicles. The structure of each spine appendage consists of a spine ossicle articulating on a basal ossicle, the pedicel (Fig. 2.2a).

Dark mauve pigment bands are visible on the surface of aboral spines and pedicels from adult specimens of *A. planci.* They are also apparent but are less evident in longitudinal thin-sections of spines. The bands alternate with lighter, less pigmented or non-pigmented areas, perpendicular to the vertical axes of the spine and pedicel (Figure 2.2a). Therefore, they occur parallel to but have a longer periodicity than the growth lines observed in thin sections.

Spine pigment bands vary in size and intensity between individual starfish. Pigment bands were routinely counted along the spine shaft from the apex toward the base with each dark and light zone constituting a band pair. Counting bands was facilitated by using a number of similar sized arm spines from a specimen and lining them up next to each other. The pattern is virtually identical for all undamaged spines although there is some variation in distance between the bands on spines from the same individual. There are 6 pigment bands on the spine shaft in Figure 2.2a.

2.3.1.1. Structure and growth of aboral spines

Spine ossicles from the aboral surface of arms of adult *A. planci* have a relatively constant shaft diameter with a tapering apex and spear-tip (Figure 2.2a). Variation in spine shape between adult individuals is most evident in different lengths of spine shafts. Shaft growth was confirmed by the characteristic tetracycline staining of the trabeculae in the spines of 20 individuals treated from the dosage experiment (dosages above 25μg.kg^{-1} whole wet weight; Section 2.3.2.1.). One month following tetracycline treatment the stain was detected with UV microscopy as a characteristic bright yellow fluorescent line parallel to and near the spine base (Figure 2.2b). This demonstrates that primary spine growth in adult *A. planci* occurs at the ossicle base from a more or less planar surface perpendicular to the vertical axis. Stereom growth also occurs from the spine tip and the spine tissues are able to repair the tips of broken spines into adulthood (Lucas, unpublished observations).

Pedicel ossicles, on which the spine ossicles rest, also have a relatively constant diameter. Their growth occurs predominantly at the tip, i.e. adjacent to the principal zone of spine ossicle growth at the spine articulation (Figure 2.2c). This was confirmed by tetracycline staining. Pedicel growth, however, also occurs in the flanged root at the pedicel base. Secretion of skeletal material at the pedicel base serves to strengthen the anchoring point of the developing spine on the reticulate endoskeleton of the body wall.

The stereom of aboral spines of adult *A. planci* consists of a central medulla and an outer cortex zone (Figure 2.2d). Within the spine shaft the medulla is defined by linear rows of trabeculae parallel to the long axis while the cortex has a more closely-packed trabecular construction. Sequential growth lines parallel to the basal growth surface lie perpendicular to the spine ossicle axis. They are clearly evident in longitudinal section of the spine shaft (Figure 2.2d). There is considerable variability in the definition of growth lines which makes them difficult to count in any consistent manner.

Juvenile aboral spines of laboratory-reared starfish (aged 20 months) reveal the ossicle shape formed during pre-adult development of the spine ossicle (Figure 2.2e). The base of the juvenile spine stereom shows the initial development of the medulla. The base of the spine reveals the linear arrangement of the trabeculae which is peculiar to the trabeculae in the medulla. Longitudinal section of the adult spine apex confirms the origin of the medulla is in the core of the spine apex (Figure 2.2f).

Therefore, the medulla extends from the base of the remanent juvenile spine and the entire history of stereom growth in spines is preserved in the skeletal structure of undamaged spines.

Spine growth continues at the spine apex during the development of the spine shaft forming the spear tip of the spine. Several individuals collected from Davies Reef had spine ossicles at the sub-adult stage of growth prior to the development of the spine shaft. Figure 2.2g compares a whole spine and pedicel from the juvenile laboratory specimen and the sub-adult stage of spine development from a field specimen (BD = 12 cm).

2.3.2. Skeletal ossicle and whole body morphometry

2.3.2.1. Effect of concentrations of tetracycline marker

Preliminary analyses found no significant differences in whole wet weight (ANOVA: $F = 1.09$; $P = 0.40$; Bartlett's test of equal variances, $P = 0.82$) and underwater weight (ANOVA: $F = 1.92$; $P = 0.14$; Bartlett's test of equal variances, $P = 0.38$) among the six groups of four starfish prior to treatment.

All four starfish given the highest concentration of tetracycline dosage ($1500\mu g.L^{-1}$) died on the second day after the experiment began. One of the starfish given the second highest dosage died on the third day. Analyses by fluorescent microscopy of spine ossicle samples collected at the termination of experiment are presented in Table 2.2.

Table 2.2. Effect of varying concentration of oxytetracycline on spine ossicle marking.

Dosage	$(WET)_{mean}$	$(UW)_{mean}$	$(\%)_{survival}$	Marker
$25\mu g.kg^{-1}$	1654	93	100	not observed
$75\mu g.kg^{-1}$	1495	108	100	very weak
$100\mu g.kg^{-1}$	1325	88	100	weak
$250\mu g.kg^{-1}$	1282	69	100	strong
$750\mu g.kg^{-1}$	1188	68	75	strong
$1500\mu g.kg^{-1}$	1647	89	0	NA

There were similar, low levels of new spine growth found in spine ossicles analyses from all treatments, including the zone of trabeculae which incorporated the fluorescent stain. Therefore a dosage between 100 and $250\mu g.kg^{-1}$ was estimated to be the optimum range for marking.

2.3.3. Validation of the age determination method

All recaptured starfish were checked through the three level schedule for individual identification. Various combinations of secondary characteristics, which were observed at the time of marking, were used as well as the madreporite pattern where necessary (i.e. mutation/partial predation features, colour, number of anuses, and the particular position of arm amputation in relation to the madreporite pattern). This additional information was necessary because of the number of replicated madreporite patterns encountered during the marking procedure (Table 2.3).

Table 2.3. Replicated madreporite patterns from the Davies Reef *A. planci* population.

	Pattern	Arms	Repeats	(%)
1	8 4 1 1	14	3	0.45
2	9 4 1 1	15	3	0.45
3	1 2 1 5 1 4	14	2	0.3
4	4 1 4 4 1 1	15	2	0.3
5	5 1 3 4 1 2	16	2	0.3
6	5 4 1 1 4 1	16	4	0.61
7	7 1 1 3 1 2	15	2	0.3
8	7 1 1 4 1 1	15	2	0.3
9	7 4 1 1 1 1	15	2	0.3
10	8 1 1 4 1 1	16	4	0.61
11	9 1 1 4 1 1	17	3	0.45
12	3 1 1 4 1 2 3 1	16	2	0.3
13	3 1 2 1 2 3 1 2	15	2	0.3
14	3 1 2 2 2 1 1 4	16	2	0.3
15	3 1 2 4 1 2 1 1	15	2	0.3
16	3 1 3 1 1 4 1 2	16	4	0.61
17	3 3 1 1 1 4 1 2	16	2	0.3
18	4 1 1 1 3 4 1 1	16	2	0.3
Overall patterns total			45	7 %

A total of seven percent of the patterns obtained in the mark recapture exercise (n = 661) were not unique.

The recapture results for validation of the method are presented in Table 2.4. Each of ten recaptured individuals developed a dark and light band over a period of 13 or 14 months (October 1989 - October 1990 and March 1990 - May, 1991. In addition the dark band (representing the winter season in 1991) had begun to develop near the base of the spine ossicles. Three individuals produced two light bands and one dark band over a period of 20 months (May, 1990 to September, 1991). Spine samples from three individuals illustrating spine growth and banding prior to release and following recapture are presented in Figure 2.3a to c. An example of the regeneration of arms amputated for spine samples is shown in Figure 2.3d. The arms are approximately two thirds the length of adjacent arms and spines are commensurately short.

One individual with an initial pigment band count of five (estimated age 7+ years) did not produce a clearly discernible banding pattern over a period of 18 months. The recapture involved had many damaged arms and was observed to be in relatively poor condition at the time of recapture (very limp body and extensive body wall damage). Both old age and the health of the individual may have contributed to its poor spine growth. The spine samples collected from this individual contained a high proportion of regenerating ossicles, indicating substantial damage, which would have affected growth and its assessment in this individual.

Table 2.4a. Summary of results from the mark and recapture exercise for *A. planci* on Davies Reef (October, 1988 to December, 1991). Recapture results (mm) for validation of the method where individuals were released and recaptured after more than 12 months.

ID	$(BD)_m$	$(BD)_r$	$(S)_m$	$(S)_r$	$(B)_m$	$(B)_r$	SGR
R11	39	39	30.0	30.9	2	3	0.913
1404	42	40	27.2	29.5	0+	1+	2.314
R103	37	33	30.3	32.2	1	2	1.914
1283	37	34	29.1	31.9	1+	2+	2.814
1402	36	37	29.7	31.1	1+	2+	1.414
1331	38	33	29.7	30.8	1+	2+	1.114
1239	37	34	29.7	32.2	1+	2+	2.514
1335	40	38	26.2	29.0	1+	2+	2.814
1241	36	35	28.7	29.7	2+	3+	1.014
1224	37	33	30.7	32.5	2+	3+	1.814
1284	35	34	26.6	31.9	1	2+	5.320
1208	43	44	30.2	33.5	1	2+	3.320
R12	41	35	38.5	~38.8	5+	ND	~0.318
R302	42	39	31.9	34.7	2+	4+	0.233

Table 2.4b. Recapture results (mm) for validation of the method where individuals were released and recaptured within 12 months.

ID	$(BD)_m$	$(BD)_r$	$(S)_m$	$(S)_r$	$(B)_m$	$(B)_r$	SGR
413	36	39	28.8	32.7	1+	1+	3.96
247	37	37	24.4	30.2	0+	1+	5.86
212	38	36	22.6	26.1	0+	1+	3.56
231	43	43	23.8	25.7	0+	1+	1.96
246	36	35	23.8	26.6	0+	1+	2.86
R3	41	36	27.2	31.2	1+	2+	4.06
G165	45	43	29.1	30.6	1+	2+	1.51
R48	38	36	28.0	33.4	0+	1+	5.41
R18	39	35	31.2	33.3	1+	2+	2.11

where ID = sample identification code
$(BD)_{(m)}$; $(BD)_r$ = whole body diameter (cm) (mark; recapture)
$(S)_{(m)}$; $(S)_r$ = spine ossicle length (mm) (mark; recapture)
$(B)_{(m)}$; $(B)_r$ = spine pigment band count (mark; recapture)
SGR = spine growth rate (mm.year.$^{-1}$)
N/D = pigment band growth was equivocal

2.3.4. Assessment of the SPBC method

2.3.4.1. Recapture analyses

The results from 13 of the 14 recaptured individuals (Table 2.4a) were used as evidence for validation of the SPBC method in band count classes 1, 2 and 3, which represents the year classes 3+, 4+ and 5+, respectively. Individuals were not recovered in the other band count classes due to the relatively low numbers of individuals representing these classes in the population combined with the low recapture rate (3.5% recovered following release). Mean adult body growth in the recaptured starfish, using mean whole body diameter was -2.0cm.yr^{-1} (SE = 0.6; range = -7.5 to 6.0cm.yr^{-1}; n = 23), i.e. net shrinkage. Mean spine ossicle growth for the same recaptured starfish was +3.4mm.yr^{-1} (SE = 0.61; range = 0.3 to 5.9mm.yr^{-1}; n

= 23). Therefore, while there was significant growth in spine ossicle length (paired t test for spine growth adjusted for a standard release period of 12 months: $t = 5.50$; $t_{(0.01(1)22)} = 2.51$; $P < 0.01$) there was significant shrinkage in whole body diameter (paired t test for whole body diameter growth adjusted for a standard interim of 12 months: $t = -3.24$; $t_{(0.01(1)22)} = 2.51$; $P < 0.01$). Analyses of spine ossicle growth rates in relation to spine length assessed from growth increment data were analysed according to the methods of Gulland and Holt (1959) to estimate the ***K*** value for spine growth (from the von Bertalanffy equation) from the slope of this relationship (Figure 2.4). The linear relationship for the change in rate of spine growth with spine length was:

$$(SGR) = 17.34 - (0.464 \times (S)_m$$

$$r^2 = 0.47;\ P < 0.01;\ n = 23;\ MSE = 7.35$$

where (SGR) = spine growth rate ($mm.yr^{-1}$)

$(S)_m$ = estimated spine length halfway between the time of release and recapture (mm).

A polynomial curve was tested against the linear regression but was found not to significantly improve the fit of the data ($\mathbf{F}_{(ratio)} = 0.14 < \mathbf{F}_{(\alpha 1;0.01;1,21)} = 8.03$; $P > 0.25$). Therefore the linear regression was preferred to describe this relationship and is assumed for the Gulland and Holt plot analyses.

Using the methods of Gulland and Holt (1959), and assuming no significant environmental differences in spine growth among cohorts, estimates of $(S)_\infty$ (asymptotic length of spine ossicles in the population) and ***K*** (the growth constant from the von Bertalanffy growth equation) were derived from x axis intercept (SGR = 0) and the regression coefficient of the Gulland and Holt plot.

where $(S)_\infty = 37.4$mm

$K = 0.039$ month^{-1}

2.3.4.2. Spine ossicle length and whole body diameter

Analyses of spine ossicle length (S) and estimated age (AGE) for the combined October 1988 and April 1989 collections showed that the use of a quadratic equation significantly improved the fit of the data over a linear regression (ANOVA; $F = 13.61 > \mathbf{F}_{(\alpha1;0.01;1,281)} \approx 6.72$) (Figure 2.5a). The quadratic equation determined is:

$$(S) = 5.708 + (0.5417 \times (AGE) - (0.00219 \times (AGE)^2)$$

$r^2 = 0.68$; $P < 0.01$; $n = 283$; $MSE = 5.795$

A least squares regression of (BD) and estimated age (AGE) was also obtained from the same collections of starfish. A quadratic equation significantly improved the fit of the data over a linear regression (ANOVA; $F = 7.22 > \mathbf{F}_{(\alpha1;0.01;1,281)} \approx 6.72$) (Figure 2.5b). The quadratic equation determined is:

$$BD = 19.2452 + 0.5368\,(AGE) - 0.0031\,(AGE)^2$$

$r^2 = 0.18$; $P < 0.01$; $n = 283$; $MSE = 22.22$

The least squares linear regression analysis using (AGE) explains only 18% of the variation in (BD) compared with 68% of the variation in (S). Both mean (BD) and (S) increase with increasing number of spine pigment bands, but for each pigment band number the range of (BD) measurements is relatively greater than for (S) ranges. Therefore, the (S) ranges are more discrete than (BD) ranges for adjacent pigment band counts.

An asymptote (S_∞) of 39.15mm was determined from the spine ossicle length and (AGE) from the spine pigment band count data using the von Bertalanffy equation in the form:

$$(S) = (S)_\infty \cdot (1 - e^{(-K(t-t0))}) \qquad (3.6)$$

where (S) = spine ossicle length (mm)

$\mathbf{(S)_\infty}$ = asymptotic spine ossicle length (mm)

K = von Bertalanffy growth constant t = age (month)

Therefore, the maximum estimated longevity using the combined samples from October, 1988 and April, 1989 is 8.3 years. This asymptotic age concurs with the oldest estimated age, established using the SPBC method, found in these samples (maximum determined (AGE) = 6 SPBC ≈ 8+ years)

2.3.4.3. Estimated population size and density

The Chapman estimate of population size (PS) estimated at the time of the second sampling trip (April 1989) i.e. by combining the recapture results into a single sample beginning at the time of the second field trip. The estimate was then determined from the results of the rate of recapture:

PS = 47 464

CI = ± 19 845

where CI = Confidence Interval (95%)

Therefore, during the height of the outbreak, the estimated population density of starfish (PD) in the Davies Reef population was;

$PD = 420\ ha^{-1}\ (CI = \pm\ 176\ ha^{-1})$

The proportion of the starfish population sampled during the study was approximately 3.5% of the estimated population size.

2.3.5. Analyses of the morphometric variables

2.3.5.1. Assessment of pigment band readability

The consistency of repeated pigment band counts in results from two independent "readers" was compared to test for pigment band readability.

For each of three repeated tests on the same 100 samples given to each individual, an ANOVA was used to test for consistency of counts in Reader "A" and Reader "B".

Table 2.5 Results of assessment of readability of spine pigment band counts between and within readers.

Reader	Analysis
A	$F = 1.26 < \mathbf{F}_{(\alpha 2;0.01;2,297)} \approx 5.44; P > 0.25$
B	$F = 1.35 < \mathbf{F}_{(\alpha 2;0.01;2,297)} \approx 5.44; P > 0.25$
and $\mathbf{F}_{(ratio)}$ test between readers	
A - B	$F = 1.14 < \mathbf{F}_{(\alpha 1;0.01;1,294)} \approx 6.72; P > 0.25$

Therefore, there was no significant difference between repeated counts using the same reader, and between repeated counts between readers of spine pigment bands in 100 spine ossicle samples.

2.3.5.2. Sample sizes of variables within individuals

The sample size required to obtain reliable estimates of skeletal ossicle variables from individuals was determined using plots of the coefficient of variation (CV %) for: spine ossicle length (S); whole spine appendage length (WS) which is spine ossicle length (S) + pedicel ossicle length (P); primary oral ossicle weight (PO); and secondary oral ossicle weight (SO).

The CV values plotted in (S) and (WS) (Figure 2.6a, b) were determined from individuals selected from the Davies Reef study. The trends in both variables were not significant ((S), $r^2 < 0.01$; $P = 0.77$; $n = 252$; $MSE = 2.02$) and ((WS), $r^2 < 0.01$; $P = 0.80$; $n = 192$; $MSE = 1.12$) with increasing sample size, however, the standard error decreased over the range of sample size from two up to approximately six measurements (Figures 2.4a, b). Therefore, samples of approximately five to ten spines give a consistent CV < 3%. The combined lengths of pedicel and spine ossicle showed a lower CV with samples of approximately 10 giving a consistent CV < 2.4%. Less variation in the length of whole spine appendages within individual starfish is an important attribute for an effective defence system covering the aboral surface.

Analyses of CV (%) using ossicle samples from the oral ossicle group were determined from 11 individuals selected from the Davies Reef study (the oral ossicle group are analysed in the Davies Reef allometric study, Chapter 3). The CV for sample sizes in primary and secondary oral ossicles were more variable than for spines and pedicel lengths (Figure 2.7a, b). There was a weakly significant increasing trend with increasing sample size for primary oral ossicles ($r^2 = 0.09$; $P = 0.01$; $n = 88$; $MSE = 0.86$). The sample size of < 10 ossicles showed consistent CV values of less than 3%. The analyses of secondary oral ossicles showed a non significant trend with increasing sample size ($r^2 = 0.02$; $P = 0.24$; $n = 88$; $MSE = 4.64$). There is a greater variability in CV of around 5% in sample size < 10 secondary oral ossicles.

2.3.5.3. Morphometry of unfed *A. planci*

The six month study of unfed individuals between 25 June and 6 December 1991 included the seasonal increase in water temperatures from 22 to 27°C. Although the starfish were unfed, small amounts of nutrients were probably gained from an algal film and fouling which developed on the sides of the aquaria and circular white patches were observed regularly on the walls after individuals had remained for periods with their stomachs everted. The influence of starvation on whole body size during the experiment (whole body diameter (BD), whole wet weight (WET) and underwater weight (UW)) is described in Figures 2.8a to c, where all morphometric variables decreased significantly over the period of the experiment (Table 2.6).

Table 2.6 Analyses of changes in whole body morphometry in unfed *A. planci* over six months (where a = start; b = 3 months; c = 6 months): (i) Paired t test; (ii) ANOVA; (iii) Curve fitting analyses, using $y = a \cdot e^{-bx}$.

(i)

Variable	Paired t test (0 to 6mo.)	Mean difference
(BD)	$t = 10.96$; $P < 0.01$; $n = 6$	-7.2cm SE = 0.70
(WET)	$t = 5.56$; $P < 0.01$; $n = 6$	-676g SE = 123
(UW)	$t = 13.17$; $P < 0.01$; $n = 6$	-18g SE = 1.4

(ii)

Variable	ANOVA	Tukey (HSD)
(BD)	$F = 36.6$; $P < 0.01$; $n = 18$; MSE = 2.1; $P_B = 0.44$	a > b > c
	mean a = 35.3; mean b = 31.5; mean c = 28.2	**Q** = 4.8; $V_c = 2.85$ (SE = 0.84)
(WET)	$F = 6.71$; $P \approx 0.01$; $n = 18$; MSE = 10430; $P_B = 0.62$	a ≈ b; b ≈ c; a > c
	mean a = 1342; mean b = 1092; mean c = 666	**Q** = 4.8; $V_c = 634$ (SE = 187)

(iii)

Variable	Parameter analyses (critical $t_{(0.01(1)16)} = 2.58$)	Survival
(BD)	a = 36.5cm; $t = 54.9$; $P < 0.01$	
	b = 0.0357; $t = 8.71$; $P < 0.01$	
	$r^2 = 0.82$; $P < 0.01$; $n = 18$; MSE = 2.02	(0.69/b) = 19.3 months
(WET)	a = 1459g; $t = 10.4$; $P < 0.01$	
	b = 0.0795; $t = 3.26$; $P < 0.01$	
	$r^2 = 42$; $P < 0.01$; $n = 18$; MSE = 1679480	(0.69/b) = 8.7 months

where MSE = mean error squared

P_B = Bartlett's test of equal variances

Q = critical values for the Tukey (HSD) test

V_c = critical value for comparison (with SE, standard error)

At the termination of the experiment the starfish were dissected for samples of the major ossicles. The gonads of all starfish were small with small sized lobules but were developed enough to have contained a significant amount of energy. Assuming that the gonads developed within the experimental period, it demonstrates that gametogenesis had priority over somatic maintenance, even under severe nutritional stress. The estimated survival of the starfish under these conditions was determined by curve analyses using the equation for exponential decay. The analyses incorporated Chossat's rule which was initially determined for mammal species but was applied to this species to obtain an approximate estimate and because of the observed state of the starved individuals at the termination of the experiment (very thin body walls and an inability to adhere to the aquarium walls) suggesting the onset of morbidity and death. Therefore, significant fitted curves were obtained for (BD), estimated survival under starvation was 19 months, and (WET) estimated survival of nine months. As the internal body changes (ie. pyloric caeca) are better reflected by whole wet weight, the estimate of nine months survival, unfed, is preferred.

The floor of the aquarium was cleaned approximately twice weekly and, following the commencement of the experiment, a number of spine ossicles were removed. Low numbers of spines were removed occasionally from the aquaria throughout the experiment. These spines were either damaged during handling in the initial morphometric procedures despite due care or were lost as part of the effects of starvation as organic material is reallocated from the body wall to maintenance. This loss of spine ossicles would have contributed to the significance of the analyses for (WET) and (UW) but it was not considered to be a large factor in the results. Although the starfish had little body wall resilience when removed from the aquarium at the end of the experiment, and despte their poor appearance were otherwise normal and did not exhibit any gross signs of having shed spines.

2.3.5.4. Population allometry in the Western Pacific region

Linear regression analyses were undertaken using morphometric data from the populations; Davies Reef (DA), Hook Island (HI), Lady Musgrave Island (LM) GBR, Lord Howe Island (LH) NSW, Suva Reef (SU) Fiji, Ha'atafu Beach Reserve (TO) Tonga, and combined Hospital Point, Tumon Bay and Double Reef (GU) Guam

(Figure 2.9) using spine ossicle length (S) and whole body diameter (BD). The data were first log transformed after preliminary inspection of residuals demonstrating heteroscedasticity. Higher variances of spine ossicle length in larger adult body size show there is an allometric growth relationship between these two variables in at least some of the populations. Linear regression analyses describing the minimal model determined for (S) and (BD) in seven populations in relation to the regression for the Davies Reef population (October, 1988 and April, 1989 only) are:

Dependent Variable = ln (S)

Ind. V.	Coefficient	Std. Error	t-stat.	P
Constant	1.4629	0.0925	15.81	< 0.01
ln (BD)	0.5103	0.0256	19.96	< 0.01
$(GU)_{elev.}$	-0.1379	0.0165	-8.36	< 0.01
$(TO)_{slope}$	-0.0688	0.0063	-10.85	< 0.01
$(LH)_{elev.}$	-0.3849	0.0218	-17.67	< 0.01
$(LM)_{elev.}$	-1.4656	0.4774	-3.07	< 0.01
$(LM)_{slope}$	0.3858	0.1230	3.14	< 0.01
$(SU)_{elev.}$	-2.1255	0.1717	-12.38	< 0.01
$(SU)_{slope}$	0.5776	0.0523	11.04	< 0.01

AOV of regression model

	SS	df	MS	$\mathbf{F_{(ratio)}}$	**P**
model	47.9959	8	5.9995	340.6	< 0.01
error	11.9787	680	0.0176		
total	59.9746	688			

minimal model:

$r^2 = 0.80$; $n = 689$; $P < 0.01$; MSE = 0.0176

ANOVA between full model and minimal model:

$\mathbf{F_{(ratio)}} = 2.33 < F_{(\alpha 1;0.01;7,682)} \approx 2.64$; $0.025 > P > 0.02$

A plot of the standardised residuals from the minimal model analyses showed no apparent residual trends after application of the model to the data and therefore, is considered to be an adequate description of the data (Figure 2.9a). The analyses show that all populations form significantly different regression lines in either slope or elevation or both. This includes a small but significant difference in the slope of the relationships for the Lady Musgrave Reef (LM) population and the Suva Reef (SU) populations. There are small but significant differences in slope but no difference in elevation between the regressions for Kiribati (KI), Davies Reef (DA), Hook Island (HI) and Lady Musgrave Island (LM) (Figure 2.9b). The regression slopes were also tested for conformity to the hypothetical isometric relationship where spine ossicle growth remains consistent throughout the range of body size (Table 2.7).

Table 2.7 Summary of regression analyses of the relationship between spine ossicle length and whole body diameter in eight populations from the Western Pacific region (DA Davies Reef; HI Hook Island; KI Kiribati; GU Guam; TO Tonga; LH Lord Howe Island; SU Suva Reef; LM Lady Musgrave Reef).

Pop.	Regression equation	Regression and isometry analyses
DA	$\ln(S) = (0.4434 \times \ln BD) + 1.7054$	$r^2 = 0.17$; $n = 284$; $P < 0.01$; $MSE = 0.019$
	t test for isometry	$t = 9.44 > t_{(0.01(1)282)} \approx 2.34$; $P < 0.01$
HI	$\ln(S) = (0.5529 \times \ln BD) + 1.3117$	$r^2 = 0.24$; $n = 68$; $P < 0.01$; $MSE = 0.017$
	t test for isometry	$t = 3.69 > t_{(0.01(1)66)} \approx 2.38$; $P < 0.01$
KI	$\ln(S) = (0.3078 \times \ln BD) + 2.1092$	$r^2 = 0.36$; $n = 51$; $P < 0.01$; $MSE = 0.011$
	t test for isometry	$t = 11.86 > t_{(0.01(1)49)} = 2.41$; $P < 0.01$
GU	$\ln(S) = (0.6532 \times \ln BD) + 0.8591$	$r^2 = 0.41$; $n = 119$; $P < 0.01$; $MSE = 0.017$
	t test for isometry	$t = 4.78 > t_{(0.01(1)117)} \approx 2.36$; $P < 0.01$
TO	$\ln(S) = (0.3608 \times \ln BD) + 1.7433$	$r^2 = 0.17$; $n = 41$; $P < 0.01$; $MSE = 0.008$
	t test for isometry	$t = 4.98 > t_{(0.01(1)39)} = 2.43$; $P < 0.01$
LH	$\ln(S) = (0.6259 \times \ln BD) + 0.6816$	$r^2 = 0.59$; $n = 43$; $P < 0.01$; $MSE = 0.023$
	t test for isometry	$t = 4.50 > t_{(0.01(1)41)} = 2.42$; $P < 0.01$
SU	$\ln(S) = (1.0879 \times \ln BD) - 0.6627$	$r^2 = 0.89$; $n = 72$; $P < 0.01$; $MSE = 0.017$
	t test for isometry	$t = 1.95 < t_{(0.01(1)70)} = 2.38$; $0.025 < P < 0.05$
LM	$\ln(S) = (0.8961 \times \ln BD) - 0.0027$	$r^2 = 0.81$; $n = 11$; $P < 0.01$; $MSE = 0.026$
	t test for isometry	$t = 0.72 < t_{(0.01(1)9)} = 2.82$; $0.10 < P < 0.25$

where (BD) = whole body diameter (cm)
(S) = spine ossicle length (mm)

The pattern of population regressions presented in Figure 2.9 shows there are regional differences in the relationship between spine ossicle length and whole body diameter as indicated by the variation in elevations of regressions. These differences were investigated using approximate mean annual seawater temperatures and latitude for each location. A multiple regression equation was developed to investigate the significance of both water temperature and latitude on the spine ossicle length and whole body diameter relationship.

Dependent Variable = ln (S)

Ind. V.	Coefficient	Std. Error	t-stat.	P
Constant	-1.8053	0.2690	-6.71	< 0.01
ln (BD)	0.9243	0.0225	41.11	< 0.01
ln (TEMP.)	0.5797	0.0729	7.96	< 0.01
ln (LAT.)	-0.0498	0.0069	-7.18	< 0.01

where (TEMP) = approximate mean annual surface seawater temperature
(LAT) = latitude (^{0}N or ^{0}S)

AOV of regression model

	SS	df	MS	$F_{(ratio)}$	**P**
model	42.7695	3	14.2565	567.61	< 0.01
error	17.2051	685	0.0251		
total	59.9746	688			

minimal model:

$r^2 = 0.71$; $n = 689$; $P < 0.01$; $MSE = 0.0251$

ANOVA for the variable representing latitude:

$\mathbf{F}_{(ratio)} = 51.6 < F_{(\alpha 1;0.01;1,685)} \approx 6.64$; $P < 0.01$

ANOVA for the variable representing temperature:

$F_{(ratio)} = 63.3 < F_{(\alpha 1;0.01;1,685)} \approx 6.64; P < 0.01$

The analyses show that both mean annual surface seawater temperature and latitude are significant factors contributing to the development of spine ossicle length in the regions where the spines were sampled. However, the combination of factors used to determine spine ossicle length, body size, seawater temperature and latitude, explains only 71% of the variability in spine ossicle length among populations. Further multiple regression analyses were undertaken to determine which populations do not comply with the body size, geographic location and seawater temperature determinants of spine ossicle length in relation to the Davies Reef population.

Dependent Variable = ln (S)

Ind. V.	Coefficient	Std. Error	t-stat.	P
Constant	-34.3831	3.1666	-10.86	< 0.01
ln (BD)	0.5531	0.0297	18.59	< 0.01
ln (TEMP.)	11.1573	0.9939	11.23	< 0.01
$(GU)_{elev.}$	-1.8072	0.1550	-11.66	< 0.01
$(LH/TO)_{elev.}$	2.4624	0.2505	9.83	< 0.01
$(SU)_{elev.}$	-2.4145	0.1875	-12.88	< 0.01
$(KI)_{slope}$	-0.2675	0.0263	-10.18	< 0.01
$(SU)_{slope}$	0.5348	0.0543	9.85	< 0.01
$(LM)_{slope}$	0.2514	0.0238	10.58	< 0.01
$(HI)_{slope}$	0.0626	0.0069	9.12	< 0.01

AOV of regression model

	SS	df	MS	$F_{(ratio)}$	P
model	48.1288	9	5.3476	306.5	< 0.01
error	11.8458	679	0.0174		
total	59.9746	688			

minimal model:

$r^2 = 0.80; n = 689; P < 0.01; MSE = 0.0174$

ANOVA between full model and minimal model:

$$\mathbf{F}_{(\mathrm{ratio})} = 1.44 < \mathbf{F}_{(\alpha 1;0.01;6,673)} \approx 2.80;\ 0.25 > \mathrm{P} > 0.10$$

The regressions for populations outside of the GBR Region (excluding Kiribati, Figure 2.9) developed shorter spine ossicle lengths over similar body size ranges than those sampled from the three populations within the GBR. Spine ossicle length in relation to body size and mean annual seawater temperature, were similar among Davies Reef, Hook Island, Lady Musgrave Reef and Kiribati populations and smaller in the Guam and Suva Reef populations. Spine ossicle length in relation to body size increased at a significantly greater rate in the Hook Island (small but significant), Suva Reef, and Lady Musgrave Island populations than the other populations (t test results were not significantly different from isometry), therefore, spine growth in relation to body size in those populations differed significantly from the remaining populations. The analysis including the variable representing mean seawater temperatures for each population showed that regional differences in spine length in relation to body size were less well defined after accounting for this environmental effect.

2.4. **Discussion**

2.4.1. Validity of the method for age determination

Evidence for the validation of the spine pigment band count (SPBC) method of age determination was obtained in the 1, 2 and 3 pigment band classes (Table 2.4a), which correspond to ages 3+, 4+ and 5+ years. Therefore, estimation of ages beyond these classes was by extrapolation of the results and therefore, carried the assumption of validity. Error in age estimates can occur unless all age classes in a population have been validated from mark/recapture studies or known age individuals (Beamish and MacFarlane, 1983). For this reason further studies employing the SPBC method should include a mark and recapture exercise in their field studies to validate the method in the particular population under investigation.

The pigment band method offers a simple procedure to determine age under conditions when the assumption that pigment bands represent annual increments is valid. Physiological delays, involving the inhibition of pigment band development for 12 months or more (i.e. through starvation) are required to falsify this assumption and invalidate the method. Although the hypothesis has not been tested there is circumstantial evidence which shows that such delays may not occur in the field, or would be rare. Kettle (1990) tested the ability of *A. planci* to withstand starvation and found that large adults could last only 6 months. In this project, the rate of weight loss in six unfed starfish was applied to Chossat's general rule concerning weight loss and survival (Kleiber, 1961) to show that starvation for more than 9 months was unlikely (using the estimate obtained from changes in whole wet weight). However, the lack of clear development of pigment banding over an 18 month release period in one recaptured individual (apparently unhealthy with extensive arm and spine damage) indicates that there is potential for temporal variation in pigment band formation. This variation may be due to the energetic demands of tissue repair and regeneration affecting the health of starfish and influencing spine growth. Therefore, the extent of injury (scars) and regeneration should be assessed in individuals which are involved in this method of age determination.

2.4.2. Growth lines and pigment bands

Growth lines have now also been found in the large spines of the aboral wall in *Nidorellia armata* collected from the Pacific coast of Panama which supports the notion that the techniques developed in the present study may have broad usage among asteroids (Lawrence, personal communication). The numerous growth lines along the shaft of the spine ossicle in *A. planci* probably result from short term fluctuations in feeding rate or physiological functioning involving seasonal changes, for the coast of Panama also has distinct seasonal changes. The spine growth and pigment banding observed from recaptured individuals, together with known seasonality of physiological processes in the life history of *A. planci* from the GBR (Lucas, 1973, 1984), show that these pigment bands have a strong case for representing seasonal variation and therefore, individual age.

Spine pigment band counts from the Davies Reef samples obtained in various months of the year has allowed an estimation of the timing of seasonal pigment band initiation. It occurs in the late summer or early winter months. Therefore, light band formation correlates with the warmer period of the year and dark bands with the cooler months. The exact timing of the development of the bands appears to vary among individuals (but not within individuals). Therefore, it would appear that banding is caused by an endogenous factor which may in turn be stimulated by exogenous influences. In GBR waters the period when the non-pigmented zones are formed in spine ossicles encompasses the major phase of gametogenesis and spawning in early to mid summer (December/January) (Lucas, 1973), and the period of highest annual water temperatures (range: approximately 22°C to 29°C). The transition from pigment to non-pigment banding during spine growth conforms with the timing of reproductive events or to seasonal changes in metabolic rate from variation in seawater temperature influencing growth.

Pigment zones are formed by the incorporation of spinochromes in the trabeculae of echinoderm species (Pearse and Pearse, 1975). Echinochrome is a general term for the napthoquinone group of biochromes (Pearse and Pearse, 1975) and it has been isolated in various forms (red, green, violet and black) from the ovary, spines and test plates in a number of urchin species; *Arbacia pustulosa*, *Arbacia lixula*, *Strongylocentrotus purpuratus*, *Paracentrotus lividus*, *Echinus esculentus*, *Echinarachnius miabilis*, and *Diadema setosum* (Nishibori, 1959; Anderson et al., 1965; Anderson and Thomson, 1966; Salaque et al., 1967) as well as from the spines of *A. planci* (Singh et al., 1967). Smith (1980) reviewed studies where pigmented stereom was found to be deposited during periods of either rapid or slow growth, depending on the species. This showed that the deposition of spinochromes is controlled by seasonal factors rather than the rate of growth *per se*.

A study on the possible functions of napthoquinone pigments in echinoid larvae (Koltsova et al., 1981) has established:

(a) quinonoid pigments from developing sea urchin embryos display strong antioxidative activity.

(b) concomitant complex mutual effects take place between quinones and other components contained in the embryo pigments and lipid extracts.

(c) high increases in the amount of pigment occurred in the pluteal stage when larvae entered an active phase of metabolism and formed a calcareous skeleton, thus an antioxidant depot is formed in the skeleton which can be mobilised in order to suppress excess lipid peroxidation.

These observations permit speculation that the appearance of light coloured bands (i.e. an absence of pigment) observed in *A. planci* which coincides with the annual gametogenic phase are caused by the mobilisation of the antioxidant pigment away from skeletal elements during gametogenesis for the purpose of antioxidative action within the developing, lipid-rich gonads. The discovery of coelomic microcanaliculae in the stroma of spines and pedicels of the body wall in a number of asteroids, connect the coelomic side of the body wall with the spine tissues and are apparently involved in the calcification/resorption process of skeletal ossicles (Jangoux et al., 1984). These spine tissue components offer a possible pathway for pigment movement through the body wall should they also be found in *A. planci*. Seasonal advection of pigment from spine ossicles to the gonads via these microcanaliculae for lipid peroxidation activities during gametogenesis provides a plausible mechanism for the annual production of light and dark band pairs in growing spine ossicles.

2.4.3. Spine growth in echinoderms

The linear mode of spine ossicle growth reported here for *A. planci* has not been reported previously for an echinoderm. There is no evidence of concentric ring formation of trabeculae in the aboral spines of *A. planci*, as reported for spines in several echinoid species (Moore, 1966; Weber, 1969; Heatfield, 1971; Ebert, 1986) since spine growth occurs principally by elongation in *A. planci*. Nor is there evidence of growth-related resorption of the spine stereom in both the experimentally starved starfish or in field specimens, as proposed by Ebert (1968) for *Strongylocentrotus purpuratus* (Echinoidea). Resorption would destroy the record of ossicle growth revealed in longitudinal thin-section of spines.

Ebert (1984) reviewed the skeletochronometric studies by Moore (1966), Ebert (1967, 1968) and Weber (1969) and rejected the hypothesis that growth rings in echinoid test plates are periodic. He argued that if test length increased linearly the test mass would have to increase exponentially, a rate which cannot be maintained. Ebert (1986) then proposed a theory of ring formation in sea urchin spines as a "growth event" when the spine no longer fits the growing tubercle on which it sits. He concluded that spine growth occurs during short episodes and cited the poor success rate of marking individuals with tetracycline as an indication of discontinuous skeletal growth. However, these points concerning the non-periodic nature of ring formation in echinoids do not apply to *A. planci*. Microscopic observations of *A. planci* spines in thin section demonstrate that growth and growth line formation are very different to those processes reported for echinoid spines, and tetracycline is incorporated into spine ossicle stereom in a consistent manner. There is no need for "growth events" to explain spine growth because the spine shaft in *A. planci* has a relatively constant diameter along its length and remains on a similar diameter pedicel throughout adult life. The primary direction of growth in asteroid spines is apparently by elongation and enlargement, since Dubois and Jangoux (1984) showed that concentric prismatic layers run parallel to the longitudinal axis of the trabeculae in the spine ossicles of *Asterias rubens*.

2.4.4. Growth and age estimation of *A. planci*

Nash (1983) first suggested that spine length in *A. planci* may be a better criterion of growth and age than body diameter, being less affected by nutritional status. He used the ratio of total length of aboral arm spines to body diameter as an index of growth rate, i.e. the ratio is lower in faster-growing individuals. Data obtained here from the Davies Reef population support Nash's suggestion, in that mean spine ossicle length, not mean body diameter of the population, increased during the 38 month period. In addition, there was a relatively high coefficient of determination for the relationship between spine ossicle length and estimated age ($r^2 = 0.68$) (Figure 2.5a), but not between whole body diameter and estimated age ($r^2 = 0.18$) (Figure 2.5b), using the data obtained in the first two sampling trips prior to the influence of rapid population decline, spine ossicle growth attenuation and increased food limitation. Further analyses of growth are presented in Chapter 3.

2.4.5. Regeneration of amputated arms

The initial field identification of recaptured individuals was achieved by observing two short, adjacent regenerating arms of similar size. The arms were removed at the base of the arm on the perimeter of the disc area and therefore, regeneration from amputation was often distinguished from predation attacks because the damage caused by predators generally involves only the distal part of the arm. Regeneration from this kind of predator injury often occurs from a fully developed arm stump which often retains fully grown spines. Arm regeneration in adult starfish was observed to be initially slow which concurs with observations made by Owens (1971) who found regrowth in arms was approximately 10mm in 116 days, suggesting that regeneration is physiologically, not highly prioritised. Therefore, the recognition of this kind of marking procedure should be possible for at least 2 years and possibly longer, as regenerated spine ossicles did not achieve a similar length to the original spines from undamaged arms over this time period.

2.4.6. Optimum dosage of tetracycline

The approximate minimum dosage of oxytetracycline ($100\mu g.kg^{-1}$) was determined using fluorescence microscopy to assess the strength of the observed fluorescence emitted from the tetracycline stain in the base of the spine ossicles sampled. It is possible that a high dosage of tetracycline may inhibit skeletal ossicle growth, at least temporarily, and therefore, a lower dosage in the range where it can be identified is preferable. High concentrations of tetracycline dissolved in seawater were observed to cause a reduction or failure of the formation of skeletal elements in larvae of the sand dollar *Echinarachnius parma* and hypomineralisation of regenerated shell in the mollusc *Pinna* sp. (Bevelander, 1963). To avoid this problem an optimum dosage of tetracycline close to the minimum for observing under low power is recommended for skeletal marking in *A. planci*, i.e. within a range of approximately 100 to 200μg.kg^{-1}. Analyses of very faint staining observed in thin sections of spine ossicles can be improved by micrographic techniques through increased periods of exposure of high speed film.

2.4.7. Spine growth and life history of *A. planci*

The validity of the SPBC method also relies on the assumption of the initiation of pigment banding at the same age (2+ years; i.e. 1st pigment band is developed at 3+ years) throughout the population. This implies that reproduction diverts energy from continuous growth to temporal separation of somatic growth and reproduction such that total production may relatively constant throughout life. The initial stages in growth of the spine ossicle of *A. planci* are tentatively matched to growth and reproductive stages in the life history of *A. planci* described from the laboratory study by Lucas (1984), using spine samples from individuals reared in that study and therefore of known age.

The juvenile spine is probably fully developed between 12 and 18 months, when the stereom begins to differentiate into medulla and cortex, and to expand in diameter until the spine apex is formed. The sub-adult spine ossicle develops into an elongate, conical shape, which forms the adult spine apex, including a spear-like tip on some spines (Fig. 1F). During this period, the juvenile spine margins are enveloped by expansion of the cortex region and secondary growth of the apical tip. This phase of increased calcite secretion coincides with the rapid growth phase between 12 and 30 months (see Lucas, 1984). *A. planci* generally mature at 2 years, but their fecundity is low. The first major commitment to reproduction is at 3 years of age and body growth is relatively slower thereafter due to channelling of resources to reproduction (Lucas, 1984; Kettle and Lucas, 1987). At 3 years the spine apex is well developed and the spine shaft begins to elongate with a constant diameter by parallel growth layers forming from the base.

2.4.8. Population allometry of body size and spine ossicle length

Analyses of spine ossicle length and whole body diameter revealed substantial differences between the populations sampled within the GBR and those outside the region (Figure 2.9). The populations grouped by the initial regression analyses were Davies Reef, Hook Island and Kiribati showing that geographic separation was not an important factor in this relationship. Other factors potentially involved in this relationship are genetic differences, resource availability, temperature and age structure of each population. Water temperature (i.e. latitude) also appears to have some influence on the regressions since Lord Howe Island has the highest latitude (being sub-tropical) of all the study sites and its population had the lowest regression elevation (Figure 2.9). Therefore, the differences in elevation but not slope in the Davies Reef, Hook Island, Kiribati, Guam and Lord Howe Island populations reflects, primarily the location (latitude) and possibly, resource availability to each population. Prior to outbreaks, the habitats of *A. planci* on the GBR were generally higher in coral resources compared with other regions; however, individual body growth among populations also depends on any genetic differences developed out of geographic isolation.

Kettle (1990) suggested the changes in the arm spine length to diameter ratio (ASL/D) during a two year sampling period at Helix Reef (Central GBR) were caused by either preferential growth of spines as body growth slowed (which apparently contradicts the argument for a size refuge from predation) or body shrinkage from tissue resorption due to starvation, with spines maintaining a fixed length. There were also several possible explanations for a stabilised ASL/D ratio in the final months of sampling as the population crashed. Kettle (1990), hypothesised that selective mortality of larger individuals or sub-adult recruitment averaged the increasing ASL/D ratio of older starfish giving the impression of stability in the ratio analyses. The results of the present study show that spine length continues to grow throughout life and, therefore, the influence of recruitment was the most likely explanation for this apparent stability under conditions where individuals in the population were shrinking.

Populations of species which are geographically separated by large distances can differ in morphology, physiology and behaviour reflecting genetic differences or environmentally-induced phenotypic plasticity (see Mayr, 1963; Marcus, 1980), which are not easily distinguishable. A genetic basis was found to be at least partially responsible for interpopulation differences in the relationship between spine length and whole body diameter in the echinoid, *Arbacia punctulata* (Marcus, 1980). Adult specimens were collected from sites near Woods Hole (WH) and the northeastern Gulf of Mexico (GM), regions separated by the Florida Peninsula, and crossed to produce offspring from both populations which were reared under similar conditions. The results showed; 47 (GM) produced the longest spines, 71 (WH) produced the shortest spines, and 41 (GM x WH, hybrids) produced intermediate sized spines. Although spines from the GM stock were longer than WH stock in similar sized urchins, spine development was faster at higher temperatures (Davies et al., 1972 in Marcus, 1980), which implicates both environmental and genetic factors influencing spine length in this species (Marcus, 1980).

The results of the analyses of spine ossicle length and whole body diameter among *A. planci* populations showed both conformity to isometry (Lady Musgrave and Suva Reef populations) and allometry in the relationships among the other populations. According to Sebens (1987) these types of variable results support the idea that growth patterns are based on the extent of influence exerted by a combination of ontogeny, genetics and habitat factors.

The regression relationship for the Lord Howe Island population was positioned closer to those from the Tonga and Suva Reef populations than the GBR samples which suggests affinities with Central West Pacific populations, despite the closer geographic proximity to the GBR (Figure 2.1). This conclusion is supported by the electrophoretic studies of Benzie and Stoddart (1992b) whose results showed that the average genetic distance between the Lord Howe Island and GBR populations was approximately four times (Nei's $D = 0.046$) that found over the whole western Pacific region (Nei's $D = 0.012$). McKnight (1978) reported a population of *A. planci* from the Kermadec Islands, north of New Zealand, and suggested they had arisen from reefs around Tonga (500km) or Raratonga (1200km) since they were the closest source of larvae with the prevailing currents at that time. Although there are no special adaptations for a teleplanic mode of life in the larval stage, the duration of larval development can be up to six weeks (Lucas, 1982) which suggests that they can occasionally cross oceanic distances (Moore, 1990). Considering the distance between Lord Howe Island and the Central West Pacific islands, there were probably intermediate populations involved in the supply of larvae if the hypothesised gene flow can be substantiated.

Figures

Figure 2.1. Map of the Western Pacific Region with location of seven populations of *A. planci* used in the comparative morphometric study between spine ossicle length and whole body diameter.

Figure 2.2a-h.

a. Aboral spine ossicle and pedicel from the aboral arm of *A. planci*. The spine ossicle shows 6 pigment bands on its shaft and a pigment capped spine apex (a). The pedicel has a flanged root (r) at its base and pigment bands occur toward the tip (t). The base of the spine ossicle (b) rests on the tip of the pedicel (t), where the whole spine articulates. (bar = 10mm)

b. Section of the base of an aboral spine ossicle of *A. planci*, showing stereom growth stained with tetracycline. The stained region lies parallel to the basal surface (b). (bar = 0.5mm)

c. Section of the tip of an aboral spine pedicel of *A. planci*, showing stereom growth stained with tetracycline. The stained region lies parallel to the surface of the pedicel tip. (bar = 0.7mm)

d. Longitudinal thin-section of an aboral spine ossicle of *A. planci*, showing numerous growth lines parallel to the spine base (b) in both the medulla (m) and outer cortex (c). (bar = 0.3mm)

e. Longitudinal thin-section of aboral spine ossicle from *A. planci* raised in the laboratory (20 months). The developing structure of the medulla with linear arrangement of trabeculae (lt) is distinct from the rest of the juvenile spine stereom. (bar = 0.5mm)

f. Aboral spine ossicle of an adult *A. planci* in longitudinal section showing detail of the intersection between the spine apex and shaft. The medulla (m) develops from the spine apex. The cortex (c) develops and expands from the remanent juvenile spine margins. (bar = 0.5mm)

g. Spine and pedicel ossicles from a juvenile *A. planci* reared in the laboratory (Whole Body Diameter BD = 12cm) and an adult *A. planci* (BD = 34cm) collected from Davies Reef, Central GBR. The spear-like spine apex has developed prior to the spine shaft in the older specimen. (bar = 10mm)

h. Aboral spines from one *A. planci* recaptured after 6 months on Davies Reef, Central GBR (October, 1988 to April, 1989 (A)). Pigment bands are matched between the two sets of spines revealing the new spine growth and banding in the lower group of 3 spines. (bar = 10mm)

Figure 2.3a-c Spine ossicle samples from 3 marked and recaptured *A. planci* individuals which demonstrate the growth and banding pattern developed over 12 to 14 months; arrowheads indicate light bands (L) developed between dark bands (D) on the shaft after spine apex (scale approx. 3:1): (a) released 10/89, 14 months growth D + L; (b) released 10/89, 18 months growth D + L + D; (c) released 3/90, 14 months growth L + D. Figure 2.3d. Recapture (body diameter = 37cm) after identification in the field using two adjacent regenerated arms (arrows) which are smaller than neighbouring arms and the spines are commensurately short.

Figure 2.4 Plot of linear regression for spine ossicle growth increment and spine ossicle length (at 0.5 x duration of the interim period) in 23 recaptured *A. planci* from Davies Reef (October, 1988 to December, 1991). Where the interim period is the time between release and recapture.

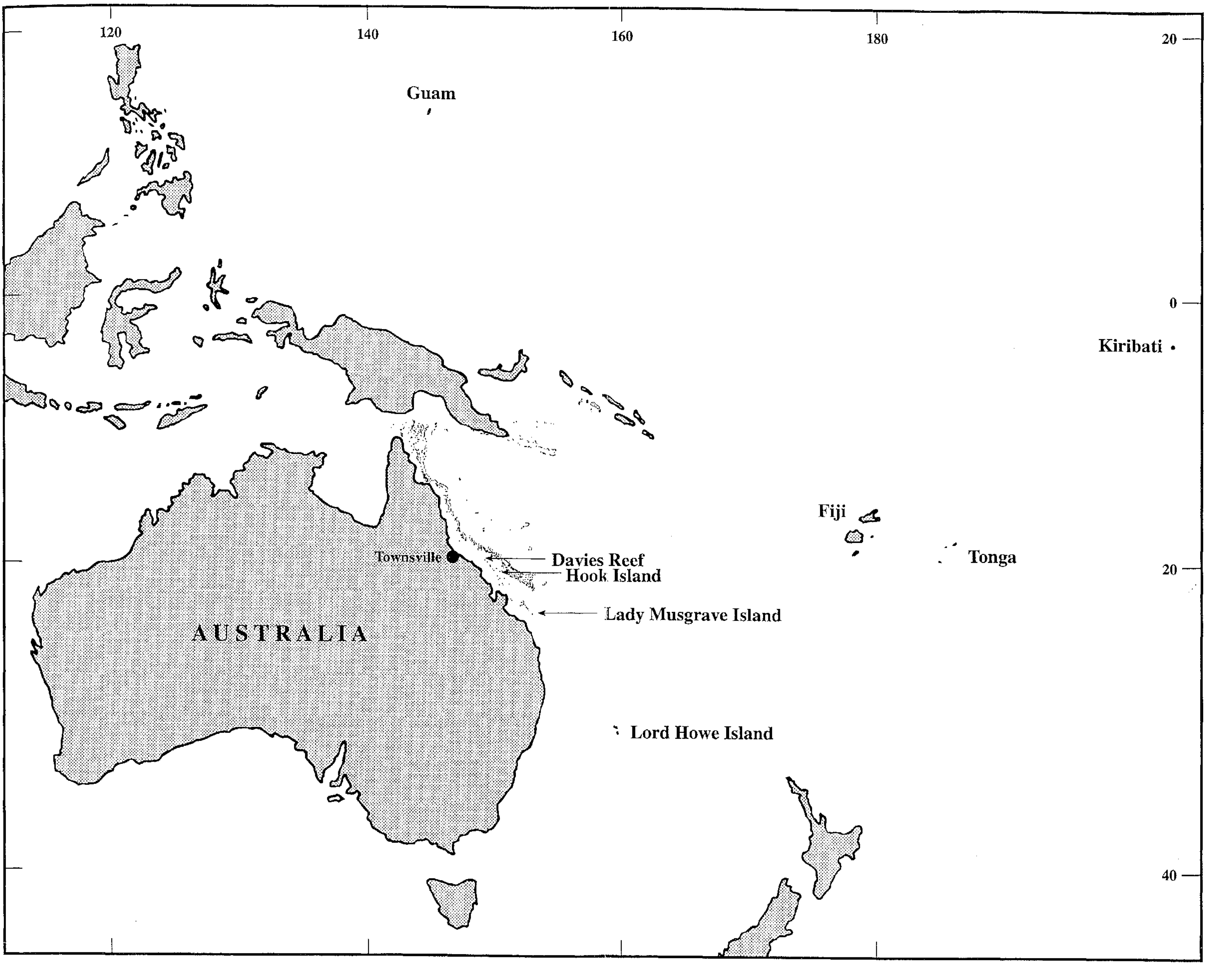
Figure 2.5 (a) Relationship between spine ossicle length and estimated age (month) from the Davies Reef *A. planci* population, using samples October, 1988 and April, 1989; (b) Relationship between whole body diameter and estimated age (month) from the Davies Reef population, using samples October, 1988 and April, 1989.

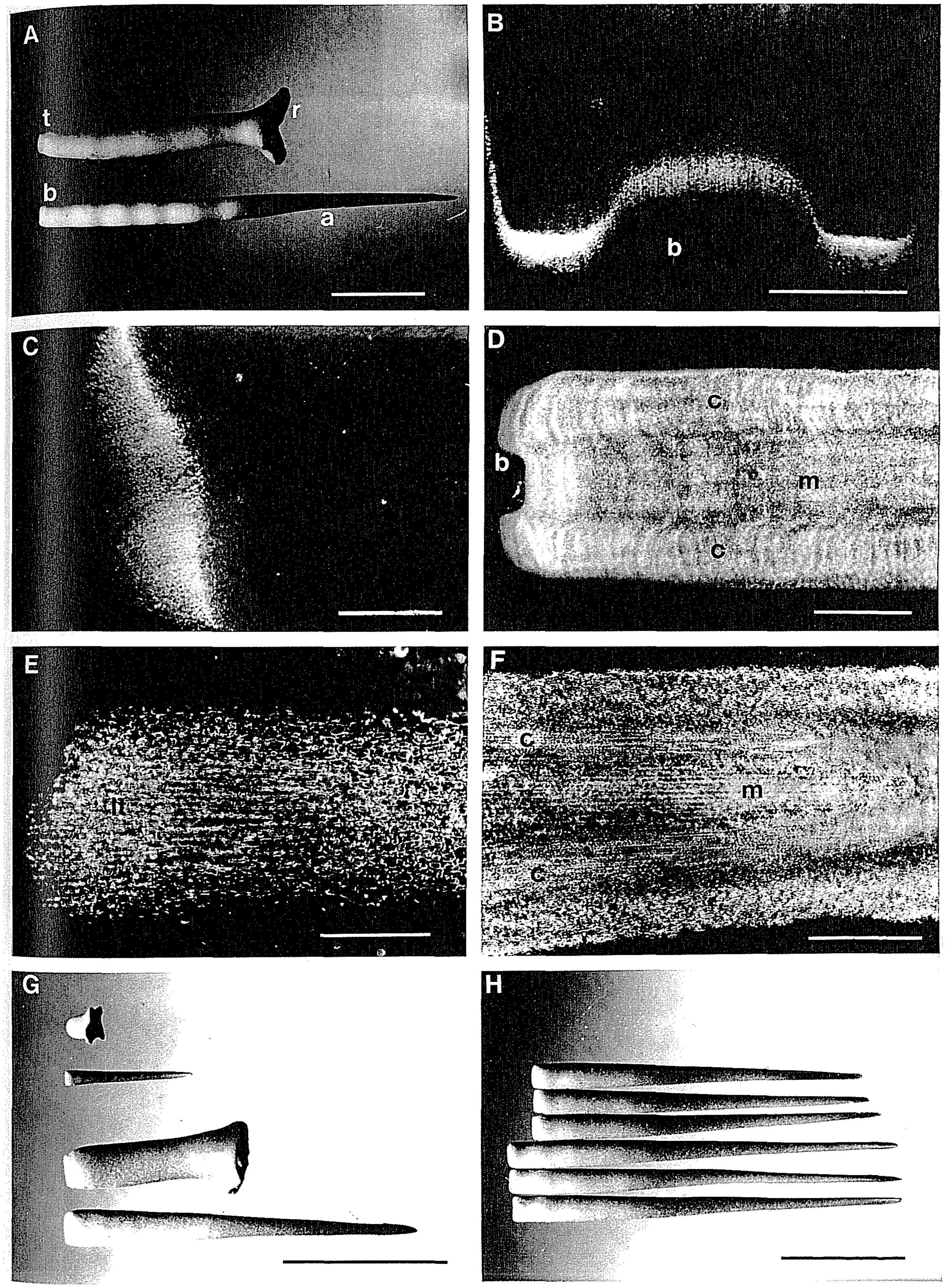
Figure 2.6 The effect of sample size on the coefficient of variation (CV %) for: (a) Spine ossicle length (mm); (b) Whole spine appendage (mm) in *A. planci* from the Davies Reef population.

Figure 2.7 The effect of sample size on the coefficient of variation (CV %) for: (a) Primary oral ossicle weight (g); (b) Secondary oral ossicle weight (g) in *A. planci* from the Davies Reef population.

Figure 2.8. The influence of starvation in *A. planci* over six months, for three morphometric variables: (a) Whole wet weight (g); (b) Whole body diameter (cm); (c) Underwater weight (g).

Figure 2.9 (a) Plot of standardised residuals for multiple regression model demonstrating a lack of trend in plotted data; (b) Plot of log transformed spine ossicle length (mm) and whole body diameter (cm) for seven *A. planci* populations sampled from the Western Pacific Region: Davies Reef, Central GBR (DA); Hook Island, Whitsunday Group GBR (HI), Lady Musgrave Reef, Southern GBR (LM), Tonga, Central West Pacific (TO), North West Pacific Guam (GU), Lord Howe Island, NSW (LH) and Suva Reef, Fiji Central West Pacific (SU).


120
140
160
180
20
Guam
0
Kiribati
Fiji
Tonga
Townsville
Davies Reef
Hook Island
Lady Musgrave Island
20
AUSTRALIA
Lord Howe Island
40


A
t
r
b
a
B
b
C
D
c
b
m
c
E
lt
F
c
m
c
G
H

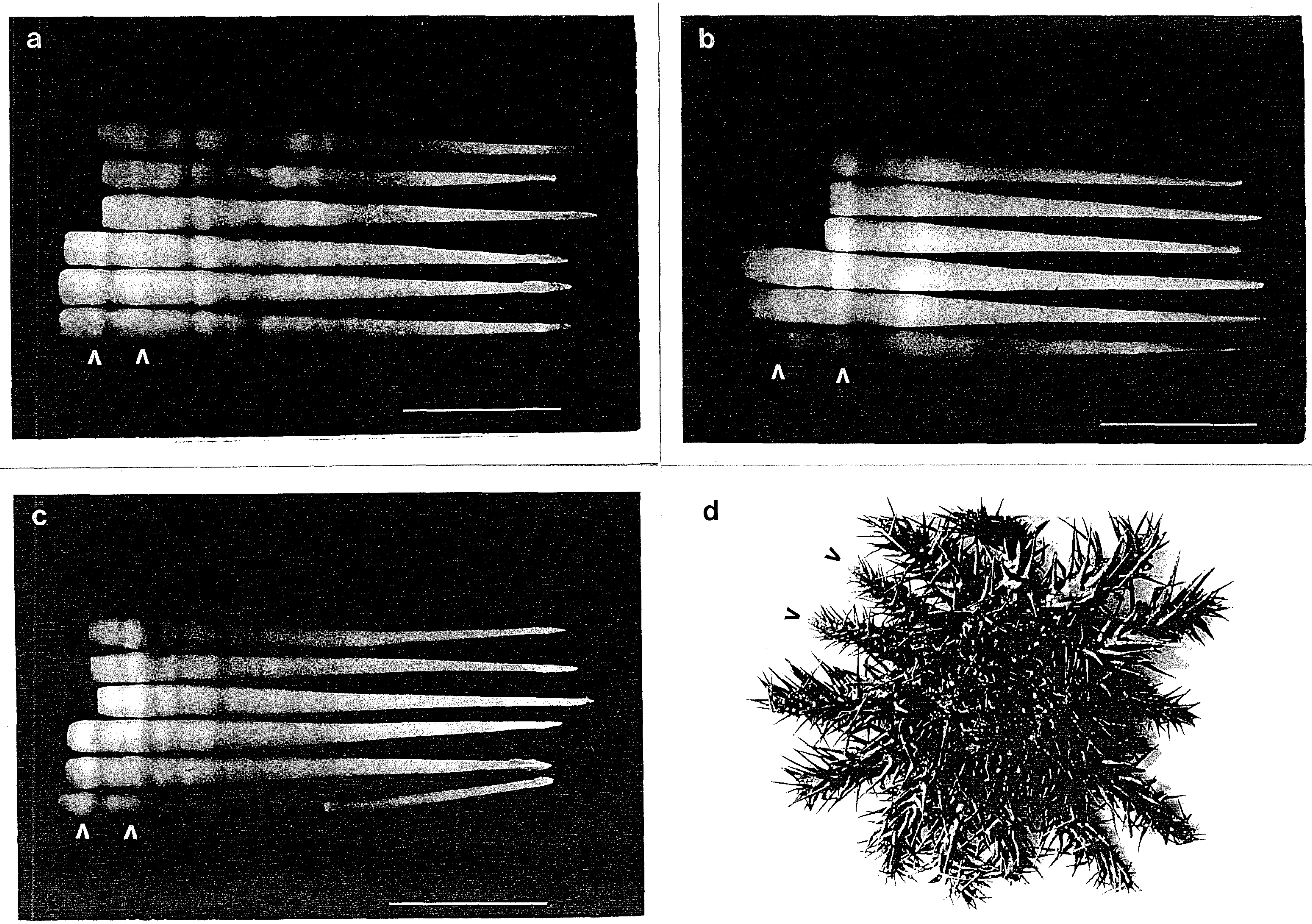

a
b
c
d

FIGURE 2.5

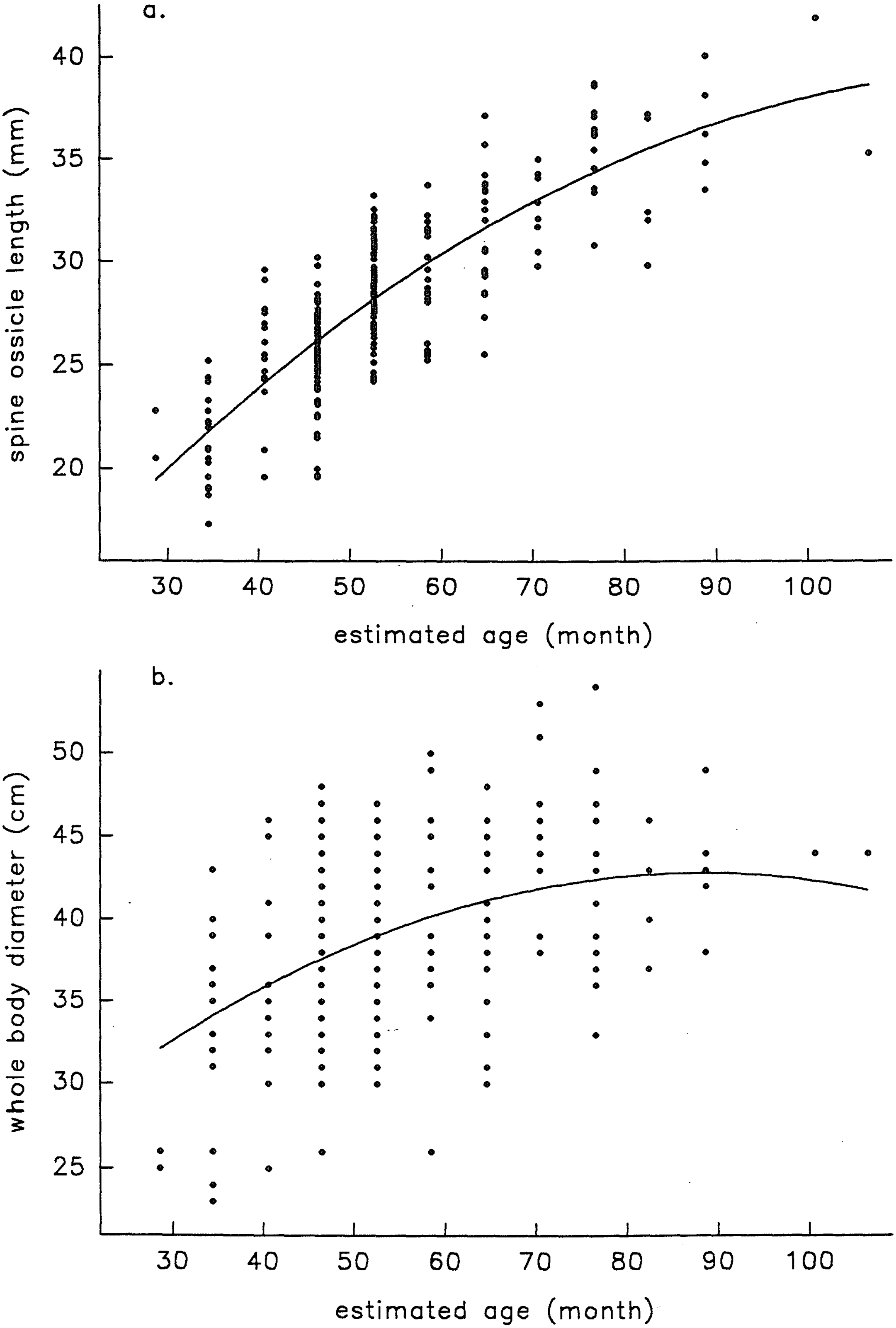

a.
spine ossicle length (mm)
20
25
30
35
40
30
40
50
60
70
80
90
100
estimated age (month)
b.
whole body diameter (cm)
25
30
35
40
45
50
30
40
50
60
70
80
90
100
estimated age (month)

FIGURE 2.6

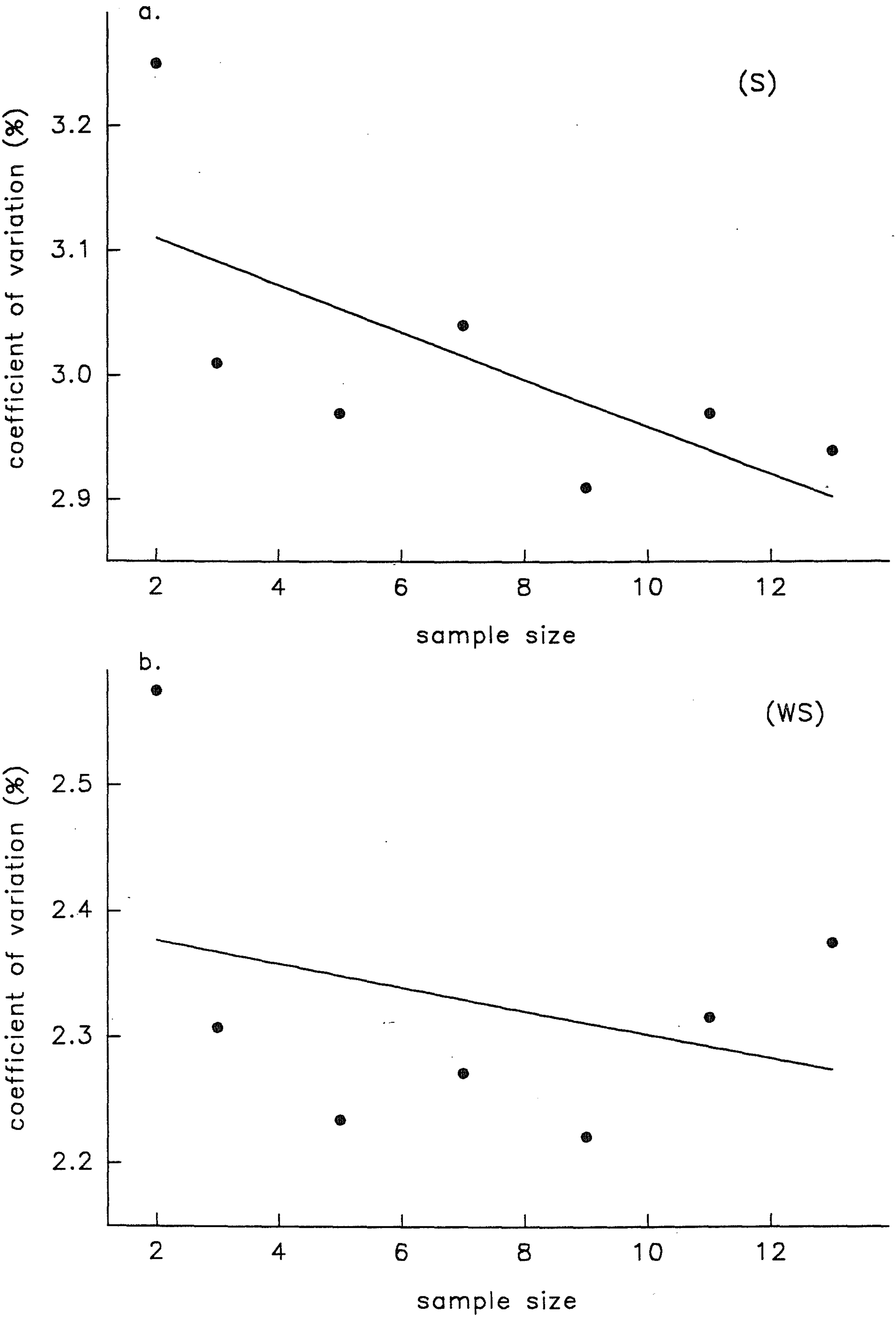

a.
(S)
coefficient of variation (%)
3.2
3.1
3.0
2.9
2
4
6
8
10
12
sample size
b.
(WS)
coefficient of variation (%)
2.5
2.4
2.3
2.2
2
4
6
8
10
12
sample size

FIGURE 2.7

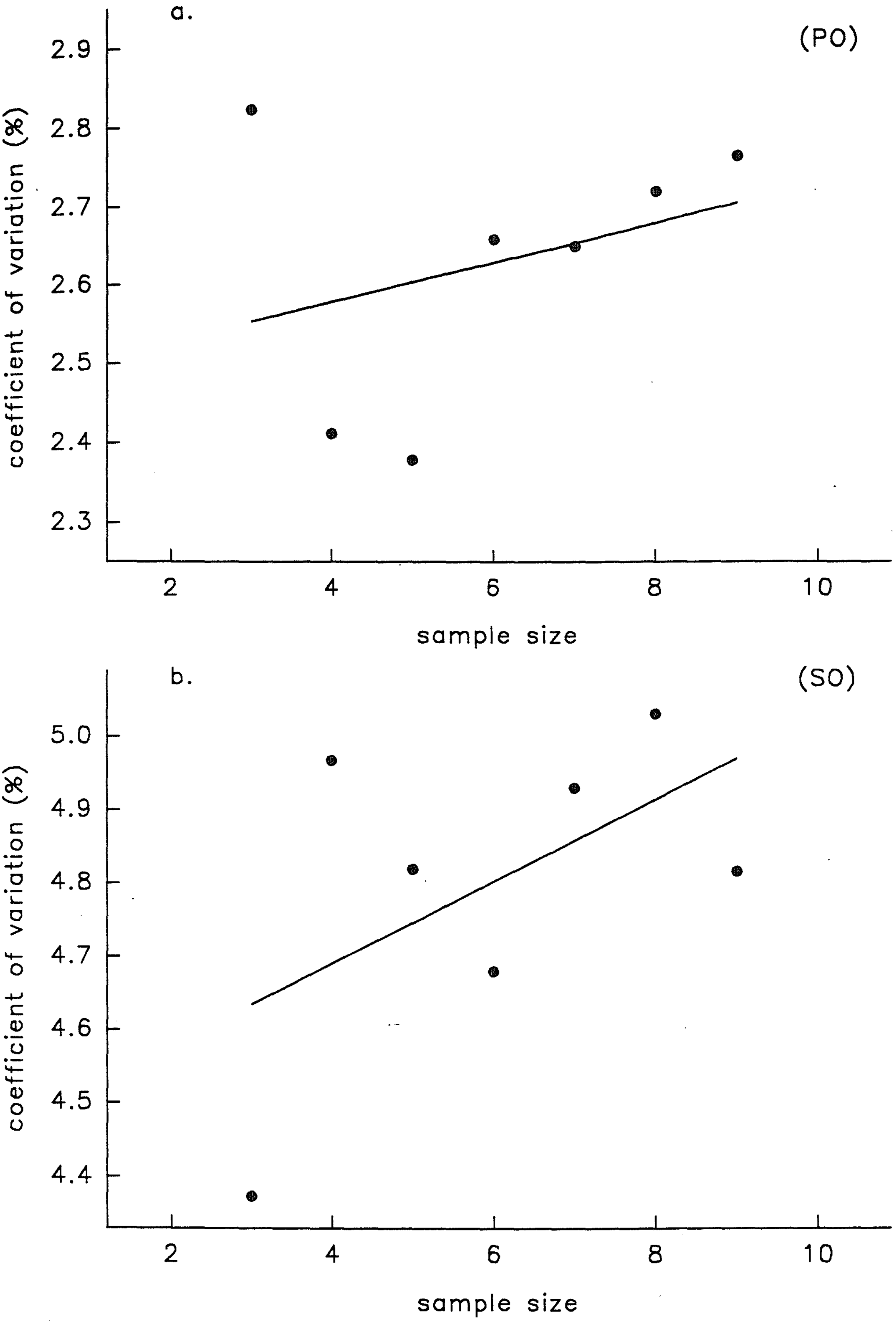

a.
(PO)
coefficient of variation (%)
2.9
2.8
2.7
2.6
2.5
2.4
2.3
2
4
6
8
10
sample size
b.
(SO)
coefficient of variation (%)
5.0
4.9
4.8
4.7
4.6
4.5
4.4
2
4
6
8
10
sample size

FIGURE 2.8

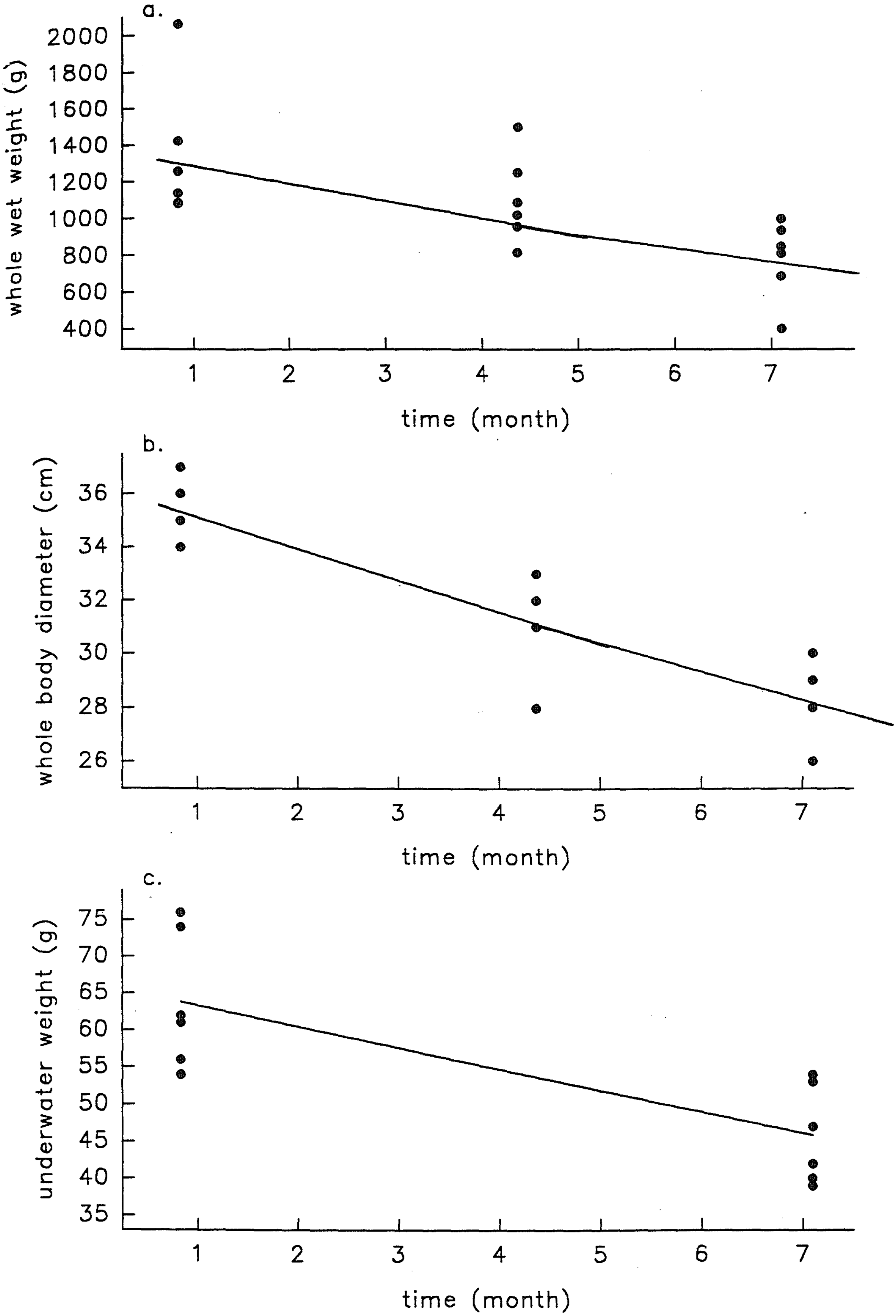

a.
whole wet weight (g)
2000
1800
1600
1400
1200
1000
800
600
400
1
2
3
4
5
6
7
time (month)
b.
whole body diameter (cm)
36
34
32
30
28
26
1
2
3
4
5
6
7
time (month)
c.
underwater weight (g)
75
70
65
60
55
50
45
40
35
1
2
3
4
5
6
7
time (month)

FIGURE 2.9

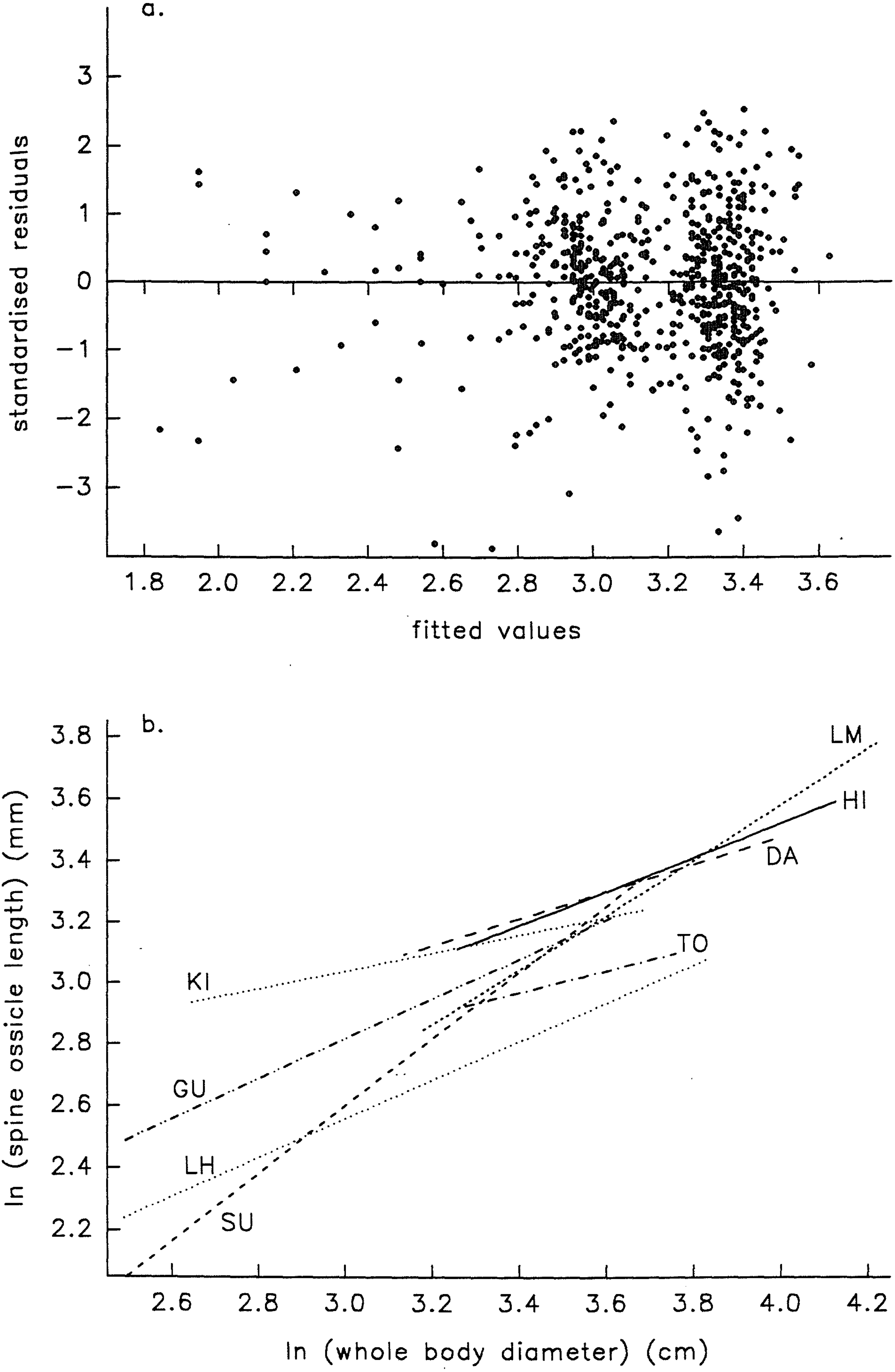
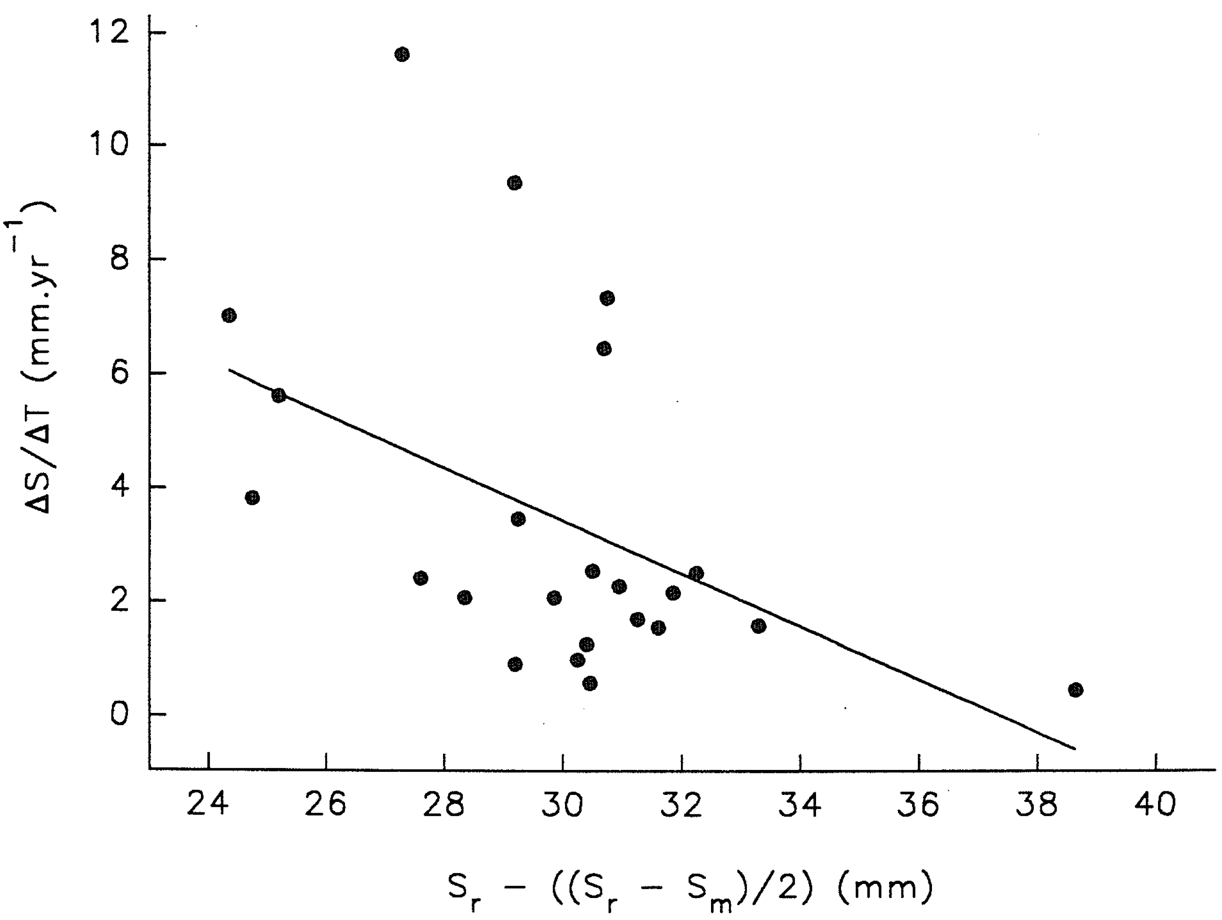
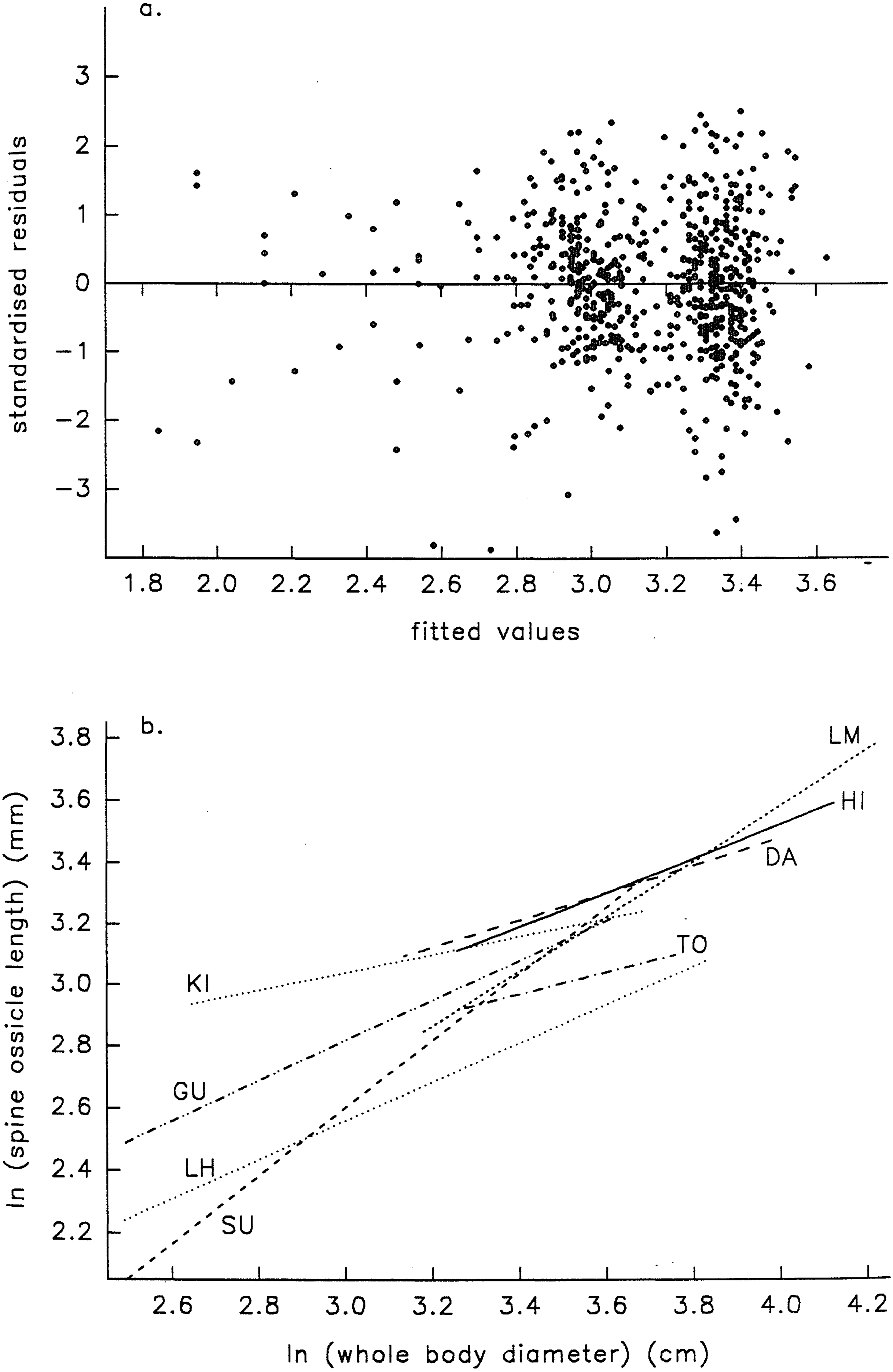

a.
standardised residuals
3
2
1
0
−1
−2
−3
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
3.4
3.6
fitted values
b.
ln (spine ossicle length) (mm)
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
LM
HI
DA
TO
KI
GU
LH
SU
2.6
2.8
3.0
3.2
3.4
3.6
3.8
4.0
4.2
ln (whole body diameter) (cm)

FIGURE 2.4



12
10
8
6
4
2
0
$\Delta S/\Delta T$ (mm.yr^{-1})
24
26
28
30
32
34
36
38
40
$S_r - ((S_r - S_m)/2)$ (mm)

FIGURE 2.9


a.
standardised residuals
3
2
1
0
−1
−2
−3
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
3.4
3.6
fitted values
b.
ln (spine ossicle length) (mm)
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.6
2.8
3.0
3.2
3.4
3.6
3.8
4.0
4.2
ln (whole body diameter) (cm)
LM
HI
DA
TO
KI
GU
LH
SU