# Gut microorganisms of surgeonfishes (Family Acanthuridae).

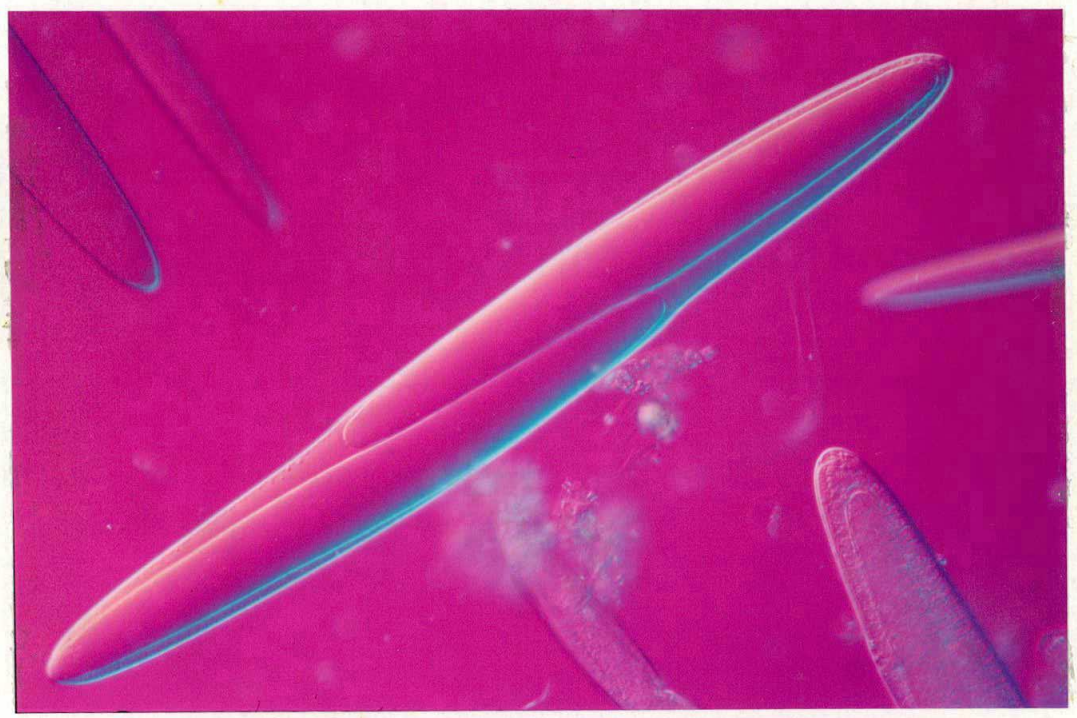
Thesis submitted by
Kendall David Clements MSc Hons (Auckland)
in March 1991

for the degree of Doctor of Philosophy in
the Department of Marine Biology at
James Cook University of North Queensland

## FRONTISPIECE

"Without exception, no individual prokaryote cell can be seen with the unaided human eye, the resolving power of which is about 0.2mm under optimal conditions."

Starr et al. (1981)



A 560$\mu$m long cell of the prokaryote *Epulopiscium fishelsoni*, from the gut of the surgeonfish *Acanthurus nigrofuscus*. The cell contains two daughter-cells.

## ABSTRACT

The main aim of this thesis was to describe the intestinal endosymbiotic communities of a range of marine herbivorous fishes, with particular emphasis on the surgeonfishes (Family Acanthuridae). There were three main components to this objective: (a) an examination of the structural features and systematic position of endosymbiotic microorganisms in herbivorous fishes; (b) an examination of the relationship between endosymbiont occurrence patterns and host distribution and feeding; and (c) an investigation of the mode of symbiont transmission between generations of host acanthurids. The study of endosymbiont transmission involved four additional elements: (a) a study of the distribution pattern of juvenile acanthurids relative to adult distribution at Lizard Island; (b) a behavioural study of the juveniles of two species of acanthurids; (c) an examination of the microbiota of juvenile acanthurids; and (d) an aquarium experiment to directly investigate epulo transmission.

The most characteristic element of the acanthurid microbiota was an assemblage of large protists, referred to as epulos. These epulo forms, or types, were characterized by differences in shape, size and mode of reproduction. One of the epulo forms appeared identical to microorganisms previously reported from acanthurids in the Red Sea. Electron microscope sections of epulos from Great Barrier Reef acanthurids revealed that the symbionts are prokaryotes, and thus together with *Epulopiscium fishelsoni* from the Red Sea represent the largest known forms of this cell type. Features identifying the epulos as prokaryotes include the presence of

## ABSTRACT

The main aim of this thesis was to describe the intestinal endosymbiotic communities of a range of marine herbivorous fishes, with particular emphasis on the surgeonfishes (Family Acanthuridae). There were three main components to this objective: (a) an examination of the structural features and systematic position of endosymbiotic microorganisms in herbivorous fishes; (b) an examination of the relationship between endosymbiont occurrence patterns and host distribution and feeding; and (c) an investigation of the mode of symbiont transmission between generations of host acanthurids. The study of endosymbiont transmission involved four additional elements: (a) a study of the distribution pattern of juvenile acanthurids relative to adult distribution at Lizard Island; (b) a behavioural study of the juveniles of two species of acanthurids; (c) an examination of the microbiota of juvenile acanthurids; and (d) an aquarium experiment to directly investigate epulo transmission.

The most characteristic element of the acanthurid microbiota was an assemblage of large protists, referred to as epulos. These epulo forms, or types, were characterized by differences in shape, size and mode of reproduction. One of the epulo forms appeared identical to microorganisms previously reported from acanthurids in the Red Sea. Electron microscope sections of epulos from Great Barrier Reef acanthurids revealed that the symbionts are prokaryotes, and thus together with *Epulopiscium fishelsoni* from the Red Sea represent the largest known forms of this cell type. Features identifying the epulos as prokaryotes include the presence of

bacterial-type flagella, a bacterial nucleoid, and the absence of a nucleus or any other membrane-bound organelle. A number of the different epulo types examined were found to share similarities in ultrastructure. The structural similarities and patterns of occurrence of these epulo types suggest that they may represent a suite of ecomorphotypes. A variety of protozoan taxa, including trichomonad, diplomonad, and opalinid flagellates and vestibuliferan and nyctotheran ciliates, were also found to inhabit the guts of herbivorous acanthurids.

Endosymbiotic communities were a characteristic feature of most species of herbivorous acanthurids and the pomacanthid *Centropyge bicolor*. Although some siganids and pomacentrids sometimes harboured endosymbiont populations, the inconsistency of microbial populations amongst these taxa suggested that the symbioses represented facultative associations. Endosymbionts were not detected in scarids. A range of epulo forms was observed in most herbivorous and detritivorous acanthurids; epulos were not found in planktivorous acanthurids. Epulos were also absent from the herbivorous species *Acanthurus achilles*, *A. leucosternon*, *A. nigricans*, and *A. xanthopterus*. The ubiquitous occurrence of epulos in several species of herbivorous acanthurids collected from a number of geographical regions suggests the possibility of an obligate relationship. The host/microorganism associations of the protozoan symbionts were found to be more variable than those of epulos, suggesting that the acanthurid/protozoan symbioses may be facultative relationships.

Most species of juvenile acanthurids were found to settle in habitats where adults

were common, suggesting that juveniles had access to adult endosymbionts at an early stage. Most acanthurids settled in very low numbers each season relative to adult populations. Juvenile *Acanthurus nigrofuscus* and *Ctenochaetus striatus* were found to resemble adult conspecifics in terms of daily feeding pattern, and appeared to be responsive to small-scale variation in habitat structure and possibly to the density of interacting species. Juvenile *A. nigrofuscus* practise conspecific coprophagy, a behaviour which appears to be a mechanism for the transfer or retention of endosymbionts. This behaviour was not observed in juvenile *C. striatus*, suggesting that these species may differ with respect to their mode of epulo retention. Newly settled acanthurids did not harbour endosymbionts, but intestinal populations of epulos were rapidly established following settlement. Populations of other endosymbiont taxa, such as flagellates and spirilla, took longer to become established in the host gut. The epulo types found in juveniles differed from those characteristic of adult conspecifics; in general the smaller epulo types predominated in juvenile acanthurids. The results of the aquarium experiment on endosymbiont transmission strongly suggested that newly settled acanthurids may be infected with epulos by exposure to the faeces of infected hosts.

## ACKNOWLEDGEMENTS

I am very grateful to my supervisor, Prof. Howard Choat, for his constant guidance, enthusiasm and encouragement; also for his perserverance as a sensible study of acanthurid recruitment was transmogrified into a discourse on bizarre microorganisms. I would also like to make special mention of two other people: my ex-office mate, Dr David Bellwood, whose sound advice influenced many aspects of my work; and Dr David Sutton, who made a great contribution to my work on the epulos.

I am very thankful to my Lizard Island research assistants Shelley Trueman, Julie Murdoch, Dorothée Teboul, Susan Barrie, Lin Schwarzkopf, and Lynn McIlwain.

I would also like to thank:

The Australian Museum for supporting my research at Lizard Island with a Lizard Island Postgraduate Fellowship;

the staff of the Lizard Island Research Station, especially Lance and Marianne Pearce and Paul and Margaret Watts;

James Cook University, the Great Barrier Reef Marine Park Authority and the Commonwealth Department of Education for financial support;

the technical staff of the School of Biosciences, James Cook University;

Alison Green, Dr Geoff Jones, Dr Uschi Kaly, Brigid Kerrigan, Dr Mike Kingsford, D. Lou, Mark McCormick, Mark Meekan, and Peter Parker for additional help in the field;

Orpha Bellwood, Richard Birdsey, Leanne Fernandes, Brigid Kerrigan, Dr Bruce Mapstone, Dr Brian McArdle, Mark McCormick, Maria Milicich, Natalie Moltschaniwskyj, Dr Richard Rowe, Ann Sharp, Dr Hugh Sweatman, Simon Thorrold, Dani Tikel, Dave Trenery, and Dr Tony Underwood for help with production, computing, sample design and analysis;

Prof. Stan Bullivant, Jim Darley, and Heather Winsor for help with electron microscopy;

Jim Darley, Zolly Florian, Terry Gruijters and Heather Winsor for help with photography;

Dr Jack Randall and Dr Garry Russ for help with fish taxonomy;

Prof. Norman Grim for help with protozoan taxonomy;

Mark Meekan, Dr John Paxton, and Dr Dave Williams for donating acanthurid specimens;

Esther Angert, Dr John Berriman, Prof. Stan Bullivant, Dr Peter Doherty, Dr Bill Foley, Prof. Norman Grim, Dr Judy Hansen, Dr Ros Hinde, Dr Geoff Jones, Dr Uschi Kaly, Dr Ralph Lewin, Prof. Dick Matthews, Prof. Linn Montgomery, Prof. Norman Pace, Dr David Patterson, Dr Peggy Pollak, Dr Richard Rowe, Dr Garry Russ, Prof. Michael Slaytor, Pam Veivers, Dr Dave Williams, and Prof Carl Woese for helpful comments on the study and the resulting manuscript.

Finally, I am grateful to my family: my parents, Anton and Miria Clements, my grandparents, Sid and Dorothy Moses, and my uncle and aunt, Roger and Bub Moses, for their support throughout this study.

## DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

K.D. Clements
22 March 1991

## STATEMENT OF ACCESS

I, the undersigned, the author of this thesis, understand that James Cook University of North Queensland will make it available for use within the University Library and, by microfilm or other photographic means, allow access to users in other approved libraries. All users consulting this thesis will have to sign the following statement:

"In consulting this thesis I agree not to copy or closely paraphrase it in whole or in part without the written consent of the author; and to make proper written acknowledgement for any assistance which I have obtained from it."

Beyond this, I do not wish to place any restriction on access to this thesis.

..... ....25/3/91....

(signature) (date)

TABLE OF CONTENTS

**Chapter 3: Aspects of juvenile acanthurid behaviour**



**Chapter 4: Characteristics and distribution of gut endosymbionts**

## LIST OF FIGURES

**List of figures cont.**

**List of figures cont.**

**List of figures cont.**

**List of figures cont.**

**List of figures cont.**



**Chapter three**

**List of figures cont.**

**Chapter four**

## LIST OF TABLES

## LIST OF PLATES

# CHAPTER 1: GENERAL INTRODUCTION

## 1.1 ENDOSYMBIOSIS IN MARINE HERBIVOROUS FISHES

Herbivory is the process by which photosynthetically derived energy is made available to animals. Most herbivores have access to an abundant food supply. Thus the problem in herbivory lies not in the procuring of food but in the subsequent processing of plant material necessary to exploit the energy and nutrients stored in plant structures. Without exception vertebrate herbivores lack the ability to produce endogenous enzymes that hydrolyze components of the plant cell wall (Zimmerman and Tracy 1989). All terrestrial herbivores which utilise the plant cell wall fraction of their diet have been found to rely on a gut microbiota (Bjorndal 1987).

Coral reefs support a great diversity of teleost fishes, including many species of herbivores (Ogden and Lobel 1978, Lobel 1981, Russ 1984a, b). In contrast to terrestrial herbivores, the digestive mechanisms of herbivorous fishes are poorly understood (Horn 1989). In particular, it is unclear how plant cell contents are made accessible to herbivorous fishes lacking specialised mechanical (e.g. pharyngeal apparatus) or chemical (e.g. highly acidic stomach) means to disrupt plant cell walls. It has been suggested that the ingestion of cellulolytic bacteria in detritus may provide the mechanism by which an herbivorous girellid digests plant cell walls (Anderson 1987). Recent studies have raised the possibility that endosymbionts in some herbivorous fishes may play a role in the digestive process (Rimmer and Wiebe 1987, Sutton and Clements 1989). The term symbiosis is used here to refer

to "the living together of two organisms in close association" (Boucher et al. 1982), and thus includes mutualism, commensalism and parasitism. To date there have been few systematic attempts to establish the nature and role of the endosymbiotic microbiota of herbivorous fishes. The lack of information available on this subject is indicated by a recent review of the bacterial flora of fishes (Cahill 1990), which did not contain a single reference to marine herbivorous fishes.

A number of very recent studies have identified diverse endosymbiotic communities in some marine herbivorous fishes (Fishelson et al. 1985, Rimmer 1986, Rimmer and Wiebe 1987, Montgomery and Pollak 1988a, Sutton and Clements 1989, Clements In Press). These include tropical surgeonfishes (Acanthuridae), subtropical rudder fishes (Kyphosidae), and exclusively temperate water groups such as the Odacidae. The endosymbiotic communities found in these fishes show similarities to those found in terrestrial herbivores such as termites (e.g. To et al. 1980, Czolij et al. 1985) and ruminants (e.g. Hungate 1966, Hungate 1975). There is a possibility that the well studied symbiotic relationships between gut microorganisms and terrestrial herbivores will serve as useful models for the investigation of herbivory in marine fishes. Before this can be accomplished more information is required on the gut microorganisms of herbivorous fishes and their distribution amongst fish taxa. This study therefore seeks to provide information which may serve as a framework for the investigation of symbioses between marine herbivorous fishes and gut microorganisms.

This thesis has two primary objectives. The first is to examine endosymbiosis in

tropical marine fishes. To do this, the study will focus on an abundant and widespread group of tropical herbivorous fishes, the Acanthuridae. The identity and distribution of gut microorganisms will be described and related to host identity and distribution patterns. The important questions in this part of the study will concern (i) the structural features and systematic position of the microorganisms, (ii) the relationship between acanthurid feeding behaviour and the microorganism assemblage, and (iii) the mode of symbiont transmission between generations of host acanthurids. The second aim of this study is to provide information relevant to more general questions concerning herbivory in marine fishes as a whole. These include the digestive physiology of herbivorous fishes and the basis of biogeographical trends in herbivorous fish distribution. Throughout this study, the well studied symbiotic relationships between gut microorganisms and terrestrial herbivores, such as termites and ruminants, will serve as useful models for the development of ideas in herbivorous fish/microorganism symbioses.

Microbial communities have to date been identified and described from the alimentary tracts of a small number of species from three families. A prerequisite to establishing the functional significance of the endosymbiont/host relationships in herbivorous fishes is more detailed descriptive information on the gut microorganisms themselves. Of particular interest are the unusual cigar-shaped protists found in Red Sea specimens of *Acanthurus nigrofuscus* (Fishelson et al. 1985). These organisms were described by Montgomery and Pollak (1988a) as *Epulopiscium fishelsoni,* and hence 'epulo' is used subsequently as a general term to refer to these symbionts and others of similar appearance. These organisms are

worthy of study in their own right, for many features of their structure and ecology are unique and poorly understood (Fishelson et al. 1985, Montgomery and Pollak 1988a, Montgomery and Pollak 1988b).

The distribution of epulos and other gut endosymbionts amongst potential host taxa has received little attention thus far, and is clearly of great importance to an assessment of endosymbiosis in herbivorous fishes. In the context of the endosymbiont/acanthurid association, several important questions remain unanswered: (i) does the symbiosis described by Fishelson et al. (1985) from *Acanthurus nigrofuscus* in the Red Sea also occur in other geographical locations? (ii) do other species of acanthurids harbour similar endosymbionts? and (iii) is this symbiosis restricted to members of the family Acanthuridae? These questions, which necessitate the sampling of a wide variety of taxa, form a main focus of this study. Detailed site-specific distributional information is essential to an interpretation of the patterns of endosymbiont occurrence amongst host taxa, and thus also forms an integral part of this work. Of equal significance to herbivory are the ontogenetic aspects of this symbiosis, in particular the mode of symbiont transmission between adult and juvenile acanthurids.

Several studies of terrestrial herbivores have emphasised the importance of microbiota acquisition by neonates (e.g. Hungate 1966, Troyer 1982, Jones 1984, Troyer 1984). Troyer (1984) makes the point that mechanisms to ensure the transmission of gut microorganisms between host generations may have been an important factor in the evolution of social systems in herbivores. To date, only one

study has examined the development of gut microbiota in juvenile marine herbivorous fish. Rimmer (1986) found that below a certain size *Kyphosus cornelii* lacked a significant microbial population in the gut, and speculated upon mechanisms for the acquisition of endosymbionts. The microbiota of juvenile acanthurids has not been described, and thus provides another focus for this study. Of equal interest is an examination of the mechanisms by which juvenile acanthurids acquire their gut symbionts. This entails three separate research programs: (i) a description of the distribution pattern of juveniles and its relationship to that of adults; (ii) a detailed study of juvenile behaviour in the field; and (iii) a series of aquarium experiments which address the issue of symbiont transmission directly. An understanding of the development of gut microbiota, particularly with respect to the timing of infection, is crucial to establishing the role of gut microbiota in herbivorous fishes.

As previously mentioned, the descriptive information provided by this thesis is relevant to a number of general questions concerning fish herbivory as a whole. Several of these questions were raised as important areas for future research by Horn (1989) at the conclusion of his comprehensive review. Firstly, the distribution of endosymbionts amongst various taxa of herbivorous fishes will allow an assessment of whether these relationships are obligate or facultative. This information is necessary to establish the role of gut microorganisms in the digestive process. Clearly, if a particular host/microorganism relationship is obligate, one would predict uniform occurrence of the endosymbiont within host specimens. Alternatively, the absence of endosymbionts from a large proportion of host

individuals suggests that the symbiosis is not essential for the host species.

Secondly, information on gut microorganisms may also contribute towards an understanding of the biogeographical trends in marine herbivorous fishes. These fishes are not distributed uniformly within the oceans of the world, but decrease in abundance and species richness with an increase in latitude (Choat 1982). The resultant transitions between temperate and tropical faunas may be of an abrupt nature and define biogeographical boundaries (Gaines and Lubchenco 1982). Although scarids, siganids and acanthurids are largely restricted to coral reef areas (Nelson 1984), a number of species are known to occur in subtropical areas such as southern Queensland and northern New South Wales (Hutchins and Swainston 1986). A clear implication is that access to intestinal symbionts may influence host distributions. How does the distribution of gut endosymbionts correlate with the distribution of host acanthurids at the limits of their latitudinal range? This question is addressed by the censusing and collecting of acanthurids from subtropical locations, thus enabling a comparison between the microbiota of tropical and subtropical hosts.

In summary, this thesis has four general aims:

(i) to describe the characteristics and occurrence of gut endosymbionts in acanthurids and other herbivorous groups;

(ii) to relate these occurrence patterns to host distribution patterns, at both small and

large spatial scales;

(iii) to examine the mechanisms of endosymbiont transmission in acanthurids; and

(iv) to describe the ultrastructural features of epulos, an enigmatic group of microorganisms characteristic of acanthurid microbiota.

This thesis is divided into four major sections, which constitute chapters 2, 3, 4 and 5. These are followed by a General Discussion (Chapter 6).

Chapter 2 contains distribution and abundance information on adult and juvenile acanthurids.

Chapter 3 examines the behaviour of juvenile acanthurids, especially in relation to endosymbiont transmission, and includes an experimental investigation of this process.

Chapter 4 describes the characteristics and occurrence of gut endosymbionts amongst adult acanthurids, juvenile acanthurids, and other fishes at various locations.

Chapter 5 is a detailed ultrastructural examination of epulos, the large, cigar-shaped endosymbionts characteristic of many acanthurids.

Information from several sources is required to understand a symbiotic relationship: the host, the endosymbionts themselves, mechanisms of symbiont transmission and so on. This information will be synthesised in this study to provide a basic description of the endosymbiotic relationship between gut microorganisms and their acanthurid hosts.

## 1.2 THE STUDY SPECIES: AN INTRODUCTION TO THE ACANTHURIDAE

Acanthurids are characterised by a small terminal mouth with a single row of teeth, a compressed body, thick skin with tiny scales, and one or more pairs of sharp spines on the caudal peduncle (Myers 1989). The family Acanthuridae contains 72 species, which are distibuted amongst seven genera (Randall et al. 1990). Five species of acanthurids occur in the Atlantic Ocean, with the remainder found in the Indian Ocean and the Pacific Ocean (Randall et al. 1990). Some 44 species occur in the areas where sampling was conducted during the course of this study (Randall et al. 1990, Jones et al. 1991). Acanthurids are a dominant component of the reef fish fauna of coral reefs, in terms of both biomass and species richness (Williams and Hatcher 1983, Horn 1989). They have a long tenure in the fossil record, with specimens known from Eocene deposits (Blot 1980). Acanthurids have a characteristic larval stage, the acronurus, and typically have a long larval lifespan (Randall 1956, Myers 1989). Reproduction involves either pair or group spawning, often on a lunar cycle (Randall 1961, Robertson et al. 1979, Robertson 1983, Fishelson et al. 1987). Acanthurids are not sex-reversing.

Acanthurids are diurnally active, and rest in shelter sites at night (Myers 1989). Although most species are either grazing or browsing herbivores, the family also contains zooplanktivorous and detritivorous representatives (Hiatt and Strasburg 1960, Jones 1968, Hobson 1974, Robertson et al. 1979, Lobel 1981, Robertson and Polunin 1981, Robertson 1983, Russ 1984a, b, Robertson and Gaines 1986). Acanthurids are typically selective feeders (Horn 1989), and many species also utilise specific feeding substrata (Jones 1968). Thus species such as *Acanthurus nigricauda, A. olivaceus* and *A. xanthopterus* typically feed on the diatom and algal flora of sand surfaces, while others such as *A. achilles, A. lineatus* and *A. nigricans* feed on the turf algae covering hard reef substrata (Jones 1968, Russ 1984a, b). Some species (e.g. *A. auranticavus, A. blochii, A. dussumieri*) feed over both sand and rock substrata (Russ 1984a, b, pers. obs.). The feeding types of acanthurids examined during this study are presented in Table 1.1, with their distributions within the areas studied.

Acanthurids exhibit a wide range of social systems (Barlow 1974a). Thus the family Acanthuridae contains actively schooling species (e.g. *A. blochii, A. triostegus,* and *Naso unicornis*), species which typically associate in pairs or small schools (e.g. *Zebrasoma rostratum, Z. veliferum*), highly aggressive territorial species (e.g. *A. lineatus, A. nigricans*), and species which are sometimes territorial and sometimes schooling (e.g. *A. nigrofuscus*) (Randall 1961, Barlow 1974, Robertson et al. 1979, Robertson and Polunin 1981, Choat and Bellwood 1985, Robertson and Gaines 1986, Montgomery et al. 1989). This variability in social organization is reflected in the degree of site-attachment shown by different species. Some acanthurid species

are strongly site-attached (e.g. *A. lineatus, C. striatus*), while others are highly mobile (e.g. *Z. veliferum, N. unicornis*) (Jones 1968, Choat and Bellwood 1985).

Acanthurids display a great range of colour patterns (Myers 1989, Randall et al. 1990), and many species are capable of rapid colour changes (Barlow 1974a). In many species of acanthurids there is little difference in colour pattern between juveniles and adults (e.g. *A. lineatus, A. triostegus, Z. veliferum*), while in others the adults and juveniles are highly distinct (e.g. *A. olivaceus, A. pyroferus*).

## 1.3 AN INTRODUCTION TO THE STUDY AREAS

The majority of this study was conducted in two locations: (i) Lizard Island and neighbouring outer-shelf reefs, and (ii) the northern Tuvalu islands of Nui, Nanumea and Niutao. Lizard Island is a continental island of the northern Great Barrier Reef (GBR), and is situated 36km off the mainland coast and 16km from the outer-shelf reefs (14°40'S, 145°28'E). Work for this study was carried out at Lizard Island between March 1987 and October 1990. The prevailing wind direction at Lizard Island is from the southeast, particularly during the trade-wind months of March through September. From October to February winds become lighter and more variable. Sea surface temperatures range from 23°C to 29°C. Lizard Island and outer reef sites mentioned in the text are depicted in Figs. 1.1 and 1.2. Additional site information is given in Chapter 2.

Details of Tuvalu sites are given in Kaly and Jones (1990). A map of the Tuvalu

islands is presented in Jones et al. (1991) (see Appendix). Work in Tuvalu was carried out between August and October 1989. A number of sub-tropical sites in southern Queensland and northern New South Wales were also visited. Details of these sub-tropical sites are given in Chapter 2, and depicted in Fig. 1.3. The latitude of the subtropical sites surveyed varied from 26°7'S (Inner Gneerings) to 30°0'S (Northwest Solitary Island).

**Table 1.1** **Feeding Type and Distribution of Acanthurids Examined in this Study.**

Abbreviations: L = Lizard Island, O = GBR outer-shelf reefs, T = Tuvalu, S = subtropical, M = museum specimen from Indian Ocean

| SPECIES | FEEDING TYPE | DISTRIBUTION |
|---|---|---|
| *Acanthurus achilles* | turf grazer | T |
| *Acanthurus albipectoralis* | planktivore | O |
| *Acanthurus auranticavus* | mixed grazer | L O T |
| *Acanthurus bariene* | mixed grazer | L |
| *Acanthurus blochii* | mixed grazer | L O T S |
| *Acanthurus dussumieri* | mixed grazer | L O T S |
| *Acanthurus grammoptilus* | mixed grazer | L |
| *Acanthurus guttatus* | turf grazer | O T |
| *Acanthurus leucocheilus* | mixed grazer | T |
| *Acanthurus leucosternon* | turf grazer | M |
| *Acanthurus lineatus* | turf grazer | L O T S |
| *Acanthurus maculiceps* | mixed grazer | T |
| *Acanthurus mata* | planktivore | L O T |
| *Acanthurus nigricans* | turf grazer | O T |
| *Acanthurus nigricauda* | sand grazer | L O T |
| *Acanthurus nigrofuscus* | turf grazer | L O T S |
| *Acanthurus nigroris* | turf grazer | O T |
| *Acanthurus olivaceus* | sand grazer | L O T S |
| *Acanthurus pyroferus* | mixed grazer | L O T |
| *Acanthurus thompsoni* | planktivore | O T |
| *Acanthurus triostegus* | turf grazer | L O T S |
| *Acanthurus xanthopterus* | sand grazer | L O T S |
| *Ctenochaetus binotatus* | detritivore | L O T |
| *Ctenochaetus hawaiiensis* | detritivore | T |
| *Ctenochaetus marginatus* | detritivore | T |
| *Ctenochaetus striatus* | detritivore | L O T |
| *Ctenochaetus strigosus* | detritivore | O T |
| *Paracanthurus hepatus* | planktivore | L O |
| *Zebrasoma rostratum* | browser | T |
| *Zebrasoma scopas* | turf grazer | L O T |
| *Zebrasoma veliferum* | browser | L O T |
| *Naso annulatus* | browser->planktivore | L O T |
| *Naso brevirostris* | browser->planktivore | L O T |
| *Naso hexacanthus* | planktivore | L O T |
| *Naso lituratus* | browser | L O T |
| *Naso thynnoides* | planktivore | T |
| *Naso tuberosus* | browser | L O T S |
| *Naso unicornis* | browser | L O T S |
| *Naso vlamingii* | browser->planktivore | L O T |
| *Prionurus maculatus* | turf grazer | S |
| *Prionurus microlepidotus* | turf grazer | S |

Fig. 1.1 Map of Lizard Island showing principal study sites (arrowed)

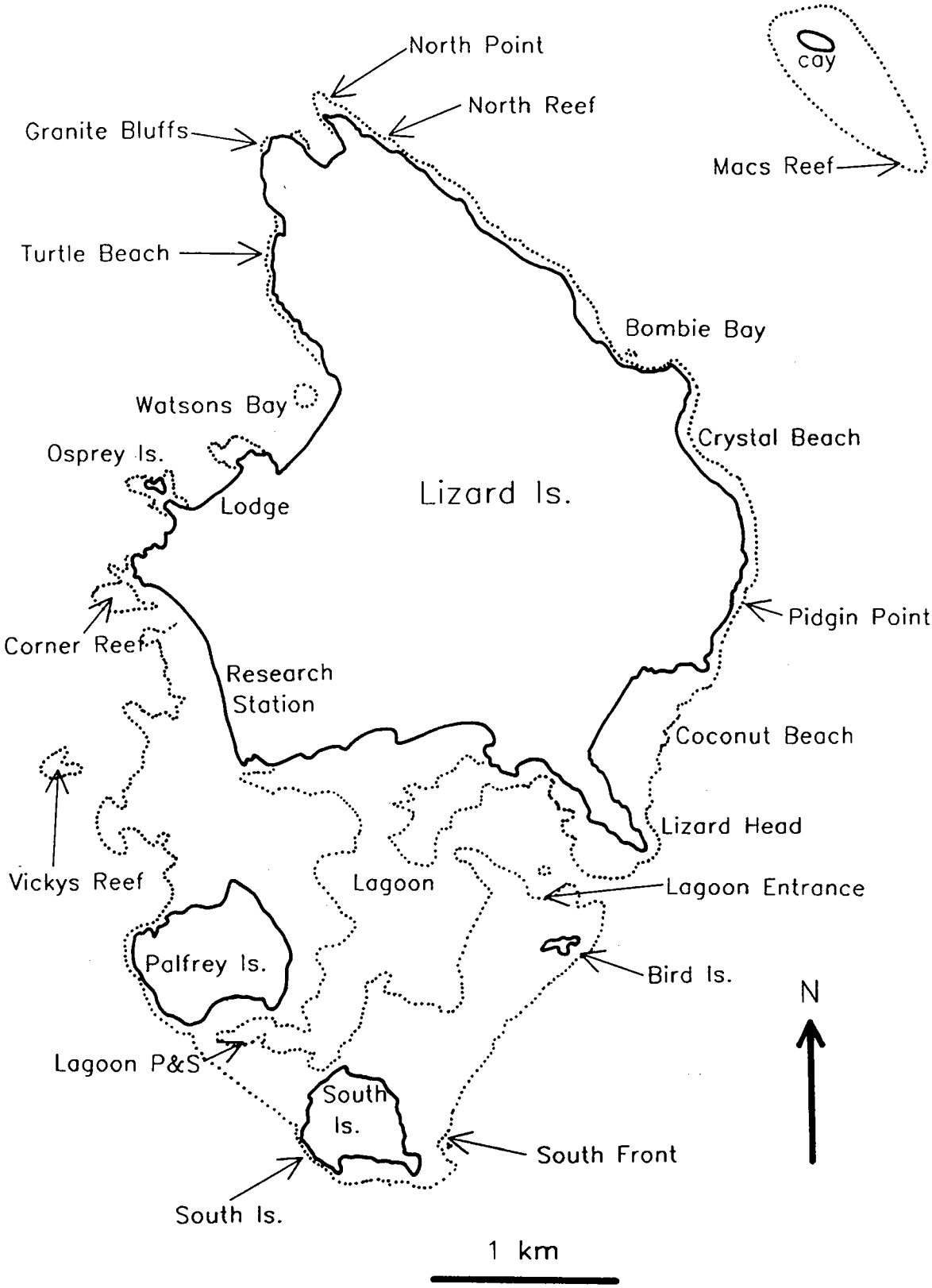


Fig. 1.2 Map of Lizard Island region showing outer reef sites (arrowed)

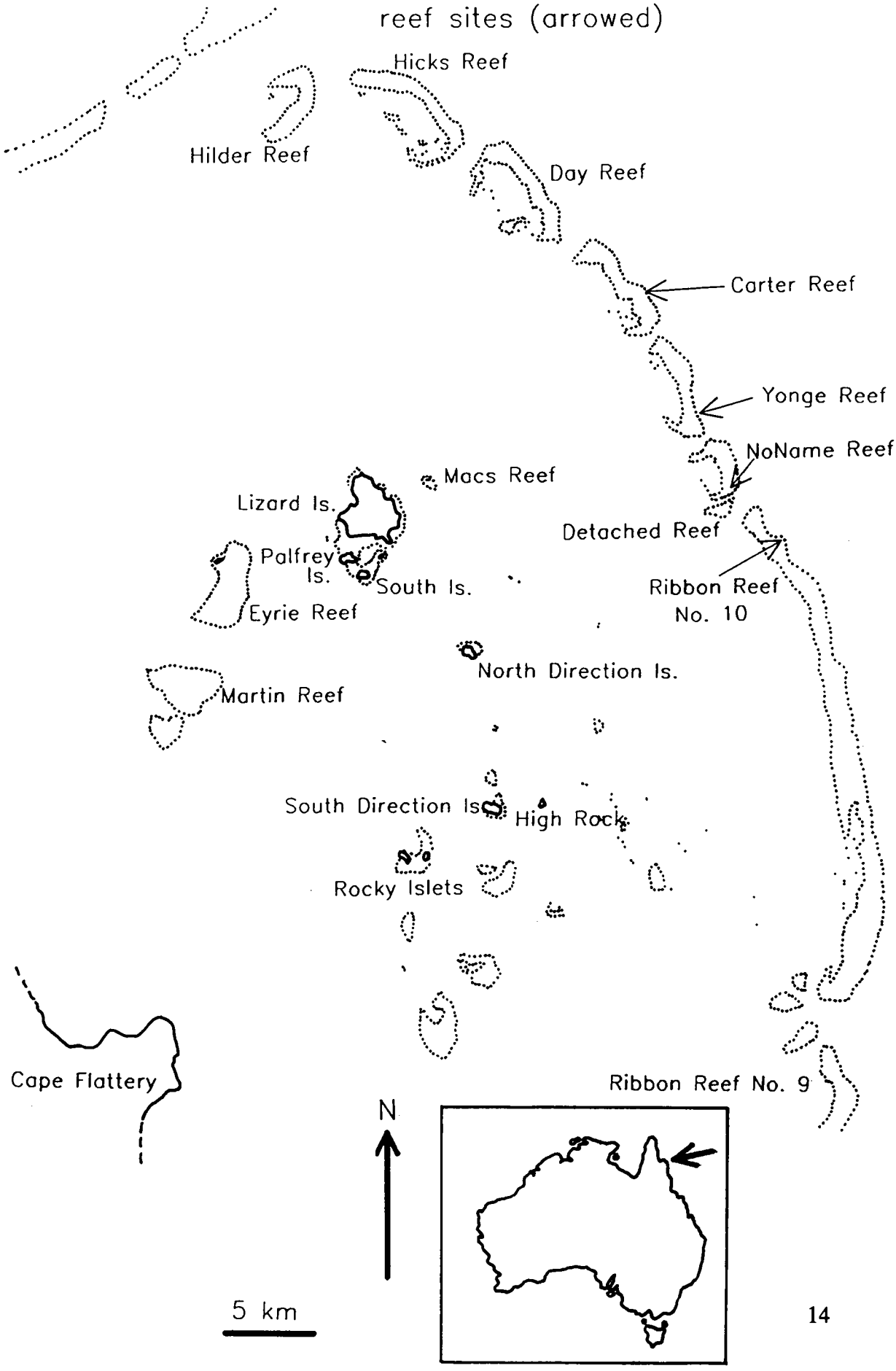
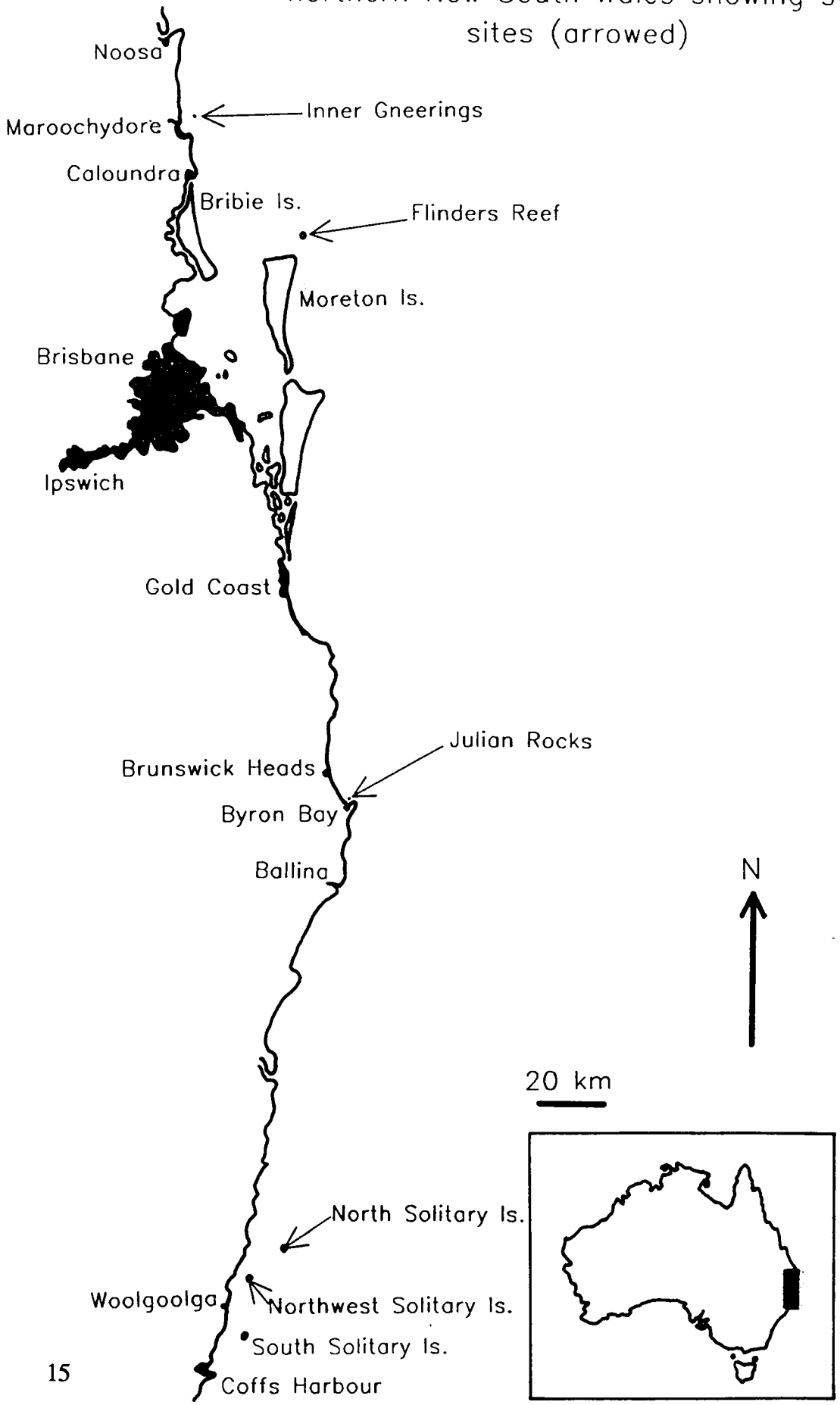


Fig. 1.3 Map of southeastern Queensland and northern New South Wales showing study sites (arrowed)



# CHAPTER 2: DISTRIBUTION OF ADULT AND JUVENILE ACANTHURIDS

## 2.1 INTRODUCTION

Previous studies have suggested that defecation by adult acanthurids spreads symbiont-laden faecal material over feeding substrata (Fishelson et al. 1985). Knowledge of the feeding behaviour and patterns of distribution of acanthurids is therefore essential to an understanding of the maintenance and transfer of symbionts in these fishes. The questions to be examined in this chapter therefore concern the distribution and abundance of adult and juvenile acanthurids. Of specific concern is the relationship between adult and juvenile distributions. A three-year study of acanthurid distribution at Lizard Island was designed to address the following questions: (1) where do adult acanthurids occur, and to a lesser extent which species co-occur (this latter information may be useful in comparing the microbiotas of different species), (2) where do juvenile acanthurids settle, and (3) is the pattern of settlement consistent between years?

Data on adult distribution patterns allow patterns of habitat-use to be assessed, and also add to the interpretation of microbiota samples taken from acanthurids collected in the vicinity of census sites. The distribution pattern of adult acanthurids was therefore assessed at sites where collections were made for endosymbiont examinations: outer GBR reefs, Tuvalu atolls, and subtropical sites in southern Queensland and northern New South Wales. Comparisons of distribution data collected at sites surveyed at different times makes the assumption that adult

acanthurids have stable year-round distributions. Such an assumption cannot be made for juvenile acanthurids. An adequate description of settlement patterns requires continuous monitoring of sites throughout the period during which settlement takes place. This was not possible for sites other than Lizard Island, thus data from these areas were restricted to adults.

Published data on the settlement behaviour of acanthurids are limited to a few species, notably *Acanthurus triostegus*. The studies of Randall (1961) and Sale (1968 and 1969) were carried out in Hawaii, where *A. triostegus* is an extremely abundant member of the shallow reef community. In a series of aquarium experiments, Sale (1969) found that presence of conspecifics was only of minor importance for the habitat selection of *A. triostegus*. Furthermore, the shallow reef-flat and tide-pool habitat of juvenile *A. triostegus* was not occupied by juveniles of other species of acanthurids (Sale 1969).

Although a few studies have examined the distribution of juvenile acanthurids on reefs (e.g. Robertson et al. 1979, Shulman 1985, Robertson 1988a, Godwin and Kosaki 1989), to date no study has quantitatively compared juvenile and adult distributions in different reef habitats. The distribution patterns of adult acanthurids on the other hand have been well documented by studies such as Robertson et al. (1979), Bouchon-Navaro and Harmelin-Vivien (1981), Robertson and Polunin (1981), Miller (1982), Russ (1984a, b), Choat and Bellwood (1985), Roberston and Gaines (1986), Fishelson et al. (1987), and Galzin (1987a, b) for a range of species and areas. It is apparent from these studies that the habitat-use and agonistic

relationships of acanthurid species may differ between areas on both large (e.g. Robertson and Gaines 1986) and small (e.g. Choat and Bellwood 1985) spatial scales. In the context of the present study therefore it was important to understand this variability as it related to my own study sites.

The results in this chapter are subdivided into four main sections:

(i) the distribution of adult acanthurids around Lizard Island;

(ii) the distribution of newly settled juvenile acanthurids around Lizard Island;

(iii) the distribution of adult acanthurids on outer reefs of the GBR and in Tuvalu; and

(iv) the distribution of adult acanthurids in southern Queensland and northern New South Wales.

## 2.2 MATERIALS AND METHODS

### (a) Visual transect technique

A standard census technique was used throughout this study to count both adults and juveniles. This technique involved replicate 5 minute timed swims, all of which were made using SCUBA. The decision to use timed swims rather than tape (i.e.

fixed area) transects was based on two factors: (a) the greater ease of operation of the former technique for a variety of weather and substrate conditions, and (b) the ability with timed swims to rapidly census a large number of sites with reasonable accuracy. Since the questions being posed concerned the habitat-use patterns of acanthurids, rather than highly accurate estimates of acanthurid densities, maximising the number of sites surveyed was more important than accuracy per se.

Pilot work conducted at Lizard Island in October 1987 established that 2 minute swims were of too short duration to census the uncommon acanthurid juveniles: most counts yielded zero values. Swims of 10 minute duration were tested, but the large distance covered during this time made adequate stratification and replication problematical. Therefore a compromise length of 5 minutes was employed. The actual technique was as follows. The observer swam at constant speed for the duration of the count, which was timed using the stopwatch function on a Casio water-resistant watch. Swimming speed was fixed to allow for the maximum time necessary to search the area of the transect, thus speed did not vary with the amount of cover present on the substratum. All fish observed in a belt 2m on either side of the diver (i.e. transect was 4m wide in total) were recorded on pre-marked plastic slates. The width of 2m was approximately the length of a diver wearing fins, so was easy to assess in the field. If the swim was interrupted in any way, for example by the need to pause to (a) identify a particular fish, or (b) to record a large number of species in a particular area, the stopwatch was stopped for the duration of the interruption. Spawning groups were not counted. All replicate counts were separated by a 2 minute swim to avoid recording the same individual in successive counts.

Throughout this entire study a replicate level of 4 was used. Thus each time a site was visited, each habitat category would be sampled by 4 independent timed swims. Timed swims were not conducted in underwater visibility conditions of <5m.

Consistency of swim distance was validated by conducting counts as normal, with the observer followed by another diver trailing a measuring tape fixed at the starting point of the count. In this way the actual length of the swim could be established without either (a) bias on the part of the observer, or (b) interfering with the swimming speed of the observer. Six swims were measured, 4 in one direction and 2 in the opposite direction to assess any effect of current (validation was performed at Granite Bluffs, consistently the site with the strongest currents at Lizard Island). The first 4 swims measured 100, 97, 98 and 92m, with the 2 swims in the opposite direction measuring 96 and 98m. The 6 counts yield a mean length of 96.83m, with an SD of 2.48m. Taking the width of the swim as a fixed 4m and the mean length of 96.83m, the calculated mean area of each timed swim is 387.33$m^2$.

(b) Specification of fish size for visual transects

Bellwood and Alcala (1988) discuss the importance of size-specification for visual censuses of fishes. With this in mind I will describe the features used to categorize juveniles and adults in this study. Visual transects of recently settled acanthurids are difficult for several reasons. Firstly, it is difficult to distinguish the juveniles of some species of acanthurids, especially in the field. This is particularly the case with a group of the large grazing species (*A. auranticavus*, *A. blochii*, *A. dussumieri*, *A.*

*grammoptilus*, *A. nigricauda* and *A. xanthopterus*). Adults of these species, which may be referred to as 'white-bar' species (since all are capable of displaying a white bar on the caudal peduncle), may be separated by subtle differences in colour pattern (Randall et al. 1990). However, since (a) the meristic characters of white-bar species overlap broadly, and (b) the adult colour patterns do not develop for some time after settlement, it was not possible to accurately distinguish the small juvenile stages. The smallest juveniles of these species are therefore not separated throughout this study, and are thus collectively referred to as *Acanthurus* 'white-bar' spp.

The second, and major difficulty encountered during the timed swim counts, was in determining which individuals were recently settled. The lack of pigmentation characteristic of the larval acronurus is only retained for a day or so following settlement. Therefore, if lack of pigment was used as a criterion of `newly-settled,' comparisons of settlement between sites censused on different days would be unreliable.

Size at settlement varies considerably both within and between species of acanthurid (Randall 1956, Pillai et al. 1983), and so the definition of a newly-settled juvenile based on size is somewhat subjective. Nevertheless, in the absence of any more reliable indicators, I treated the smallest recorded individuals of any species as newly-settled. Since the size of newly-settled individuals varies so much between species (for example the following transparent specimens were collected: *Acanthurus nigrofuscus* 32mmSL, *A. olivaceus* 22mmSL, *A. triostegus* 21mmSL, *Ctenochaetus binotatus* 28mmSL, *Naso annulatus/tuberosus* 29mmSL, *Zebrasoma scopas*

24mmSL and *Z. veliferum* 20mmSL), a fixed size specification could not be used for all species.

Because of the seasonality of settlement, year classes were easily separated on the basis of size. I was therefore able to detect newly-settled individuals as they appeared, without any danger of including juveniles that had settled the previous year. Thus by the time recurring settlement episodes occurred, I was able to differentiate the newly-settled juveniles on the basis of my earlier observations. The following maximum size classifications are those used for juveniles in this study:

(a) *Acanthurus* spp. other than *A. nigrofuscus* 35mmSL

(b) *A. nigrofuscus* 40mmSL

(c) *Ctenochaetus* spp. 35mmSL

(d) *Naso brevirostris*, *N. lituratus*, *N. unicornis* and *N. vlamingii* 60mmSL

(e) *N. tuberosus* and *N. hexacanthus* 40mmSL

(f) *Paracanthurus hepatus* 30mmSL

(g) *Zebrasoma* spp. 30mmSL.

Juveniles and adults were never counted simultaneously by the same diver. The juvenile and adult counts at Lizard Island over the summer of 1987/88 were performed simultaneously by twin observers, one counting adult acanthurids and the other juveniles. Overlap between these two categories was eliminated by the use of an intermediate size category, which was then discarded. This intermediate category included individuals larger than the juvenile category (described above) and smaller than 70mmSL. Individuals larger than 70mmSL were treated as adults. Although not

reproductively mature at 70mmSL, most acanthurids associate with conspecific adults by this size, and thus may be considered as adults in the context of distribution.

(c) Sites surveyed at Lizard Island

Throughout the first summer of this study (1987/88) 13 sites were surveyed at Lizard Island, each of which contained from 2 to 5 habitats. The habitats surveyed in each site are illustrated in Fig. 2.1. The locations of these sites around the island are indicated in Fig. 1.1. These 49 habitat/locality sites were designed to cover the range of exposures and habitats present around Lizard Island. Hence there were exposed (Pidgin Point, Bird Island and South Front), oblique (North Point, North Reef and South Island), leeward (Granite Bluffs and Turtle Beach), patch reef (Corner Reef and Vicky's Reef) and lagoon (Lagoon Entrance and Lagoon between Palfrey and South) sites. In addition, a site at the exposed south-eastern end of Mac's Reef (a sand cay situated off the north-eastern flank of Lizard Island) was surveyed.

The habitat stratification used at each site depended upon topography. Thus oblique and exposed sites had a defined flat, crest, drop-off, crest base and slope region. The leeward sites did not have a defined crest or crest base, and so required different habitat categories. The patch reef and lagoon sites were more simple topographically, hence were each only subdivided into two categories. The habitat

categories employed in this study were defined as follows:

Inner flat: proximal area of reef flat adjacent to emergent land. Characterised by extensive areas of rubble and turf-covered rock, and a relative lack of coral cover.

Outer flat: distal area of reef flat adjacent to crest. Characterised by moderate coral cover, although areas of rubble and turf-covered rock were sometimes present.

Crest: edge of reef flat adjacent to drop-off. Crest of exposed and oblique sites characterised by extensive coral cover, with little or no rubble. Crest of leeward sites little differentiated from flat in terms of coral cover.

Crest base: base of drop-off, or shallowest portion of slope.

Characterised by extensive areas of rubble, with relatively low coral cover.

Slope: sedimentary apron surrounding reef base. Slope of exposed and oblique sites characterised by rubble and often sand areas, with little coral cover. In leeward sites slope is usually turf- or sediment-covered rock.

Slope base: edge of sand area at base of leeward reefs. Often characterised by a small drop-off.

Patch reef: areas of shallow coral, turf-covered rock or rubble substrata surrounded by sand.

Patch edge: the periphery of patch reefs. Counts were made by following the patch reef/sand interface.

Lagoon flat: shallow lagoon reef areas, often with extensive coral cover.

Lagoon slope: edge of lagoon basin, often with extensive coral cover and/or sand.

Some sites lacked particular habitats because of topographical features. There was no inner flat at Mac's Reef or South Front because these sites had no emergent land. There was no outer flat at South Island because the flat was too narrow to accommodate three independent parallel transects (i.e. inner flat, outer flat and crest). Finally, Turtle Beach lacked a slope habitat category because the slope was too abbreviated to accommodate independent crest, slope and slope base transects. For most habitats, replicate swims were conducted in a linear fashion. For example, when the crest at Pidgin Point was surveyed, the 4 replicate swims would be conducted end-to-end, each separated by a swim of 2 minutes. For the patch reef habitat at each of the patch reef sites (where there was no defined contour to follow), the observer followed a random zig-zag pattern across the reef. Depths of habitat/locality sites, and details of substratum type and densities of herbivorous pomacentrids for sites where moderate to high acanthurid settlement occurred, are presented in Appendix 1.

The amount of time taken to survey all sites was weather-dependent, and in many cases the order in which sites were surveyed was determined by the prevailing wind conditions. Throughout this study therefore there was no fixed order in which sites were sampled. In the summer of 1987/88 all sites were surveyed 4 times for both adult and juvenile acanthurid abundance, thus 4 `rounds' of counts were completed during this summer. The dates spanning these `rounds' of counts in 1987/88 were as follows: (1) 31/10/87-13/11/87, (2) 16/11/87-25/11/87, (3) 28/11/87-4/12/87 (+ Mac's Reef on 15/12/87 - this delay was caused by weather), (4) 20/1/88-27/1/88.

Many of the habitats and sites surveyed in 1987/88 received little or no acanthurid settlement. I therefore decided to rationalise my sampling effort by reducing the habitats/sites to those likely to yield suitable results. For subsequent `rounds' of counts therefore the number of habitat/locality sites was reduced from 49 to 24. The following 25 habitat/locality sites were not surveyed after the summer of 1987/88: crest habitats at all sites, the two lagoon sites, the two patch reef sites, Mac's Reef, the slope base at Granite Bluffs, and the slope at South Island.

The remaining habitat/locality sites (24) were retained throughout the remainder of the study, during which juveniles only were surveyed (adults were thus only counted at Lizard Island in the summer of 1987/88). On the basis of the 1987/88 results it was apparent that many acanthurid species settled in late summer. Field work was planned accordingly for the 1988/89 and 1989/90 summers, when sites were surveyed on 6 occasions and 3 occasions respectively. The dates spanning these `rounds' of counts were as follows: (5) 22/11/88-25/11/88, (6) 7/12/88-9/12/88, (7) 17/12/88-20/12/88, (8) 26/1/89-30/1/89, (9) 13/2/89-16/2/89, (10) 24/2/89-28/2/89, (11) 21/12/89-26/12/89, (12) 4/2/90-7/2/90, and (13) 17/2/90-19/2/90.

Mean values for adult densities were generated by treating the 16 counts per habitat/locality site (i.e. 4 replicates were counted on each of the 4 times each site was surveyed) as replicates. This is possible since settling juveniles do not attain the size of adults within a single summer. Therefore estimates of the adult population would not be influenced by pulses of recruitment taking place in between successive `rounds' of adult counts. Mean values for juvenile densities were generated at a

replicate level of 4 only (i.e. one mean per `round' of counts), since settlement pulses continually added to the juvenile population.

A simple chi-square analysis was used to assess the similarity of juvenile and adult distributions. Species which settled in very low numbers (<25) throughout the 3 seasons of the study were not considered. To maximise numbers, juvenile counts were pooled across the 3 seasons, thus only the 24 habitat/locality sites surveyed throughout the study were considered. Numbers of juveniles during a season were taken to be the minimum consistent with the numbers of juveniles recorded in successive counts. The number of settlers for a given species for the 3 seasons were then summed to give a total value for each habitat/locality site. Based on the 1987/88 adult counts, habitat/locality sites were categorised for each species as either containing adults (mean number per transect $>1$, $n=16$) or lacking adults (mean number per transect $<1$, $n=16$). This then yielded proportions of sites with and without adults. The juvenile data were divided into numbers in habitat/locality sites with adults and numbers in habitat/locality sites without adults. These numbers were compared with the expectation of random settlement where proportion settling with adults would be the same as the proportion of sites with adults (Chi-square, 1 df).

The mean numbers of adults per habitat/locality site in Fig. 2.56 were calculated by obtaining a mean of each habitat between sites of a given exposure. Thus for example the value given for the inner flat of oblique reefs is the mean of the inner flat transect values for North Point, North Reef and South Island. Where a given

habitat was missing from a particular location (for example only 2 of the exposed locations have an inner flat habitat), the missing value was not included in the calculations (i.e. the mean was generated at n=2). The mean numbers of juveniles per habitat/locality site per season in Fig. 2.57 were calculated by obtaining a mean of the total settlement figures (i.e. n=3) as calculated for the chi-square analysis above.

(d) Outer Reef and Tuvalu sites

The structure of outer reefs on the GBR differs considerably from the structure of the fringing reefs at Lizard Island. It was therefore not possible to compare habitats directly between the two areas. To enable comparisons between outer reef sites and atoll sites, depth contours were used to stratify habitats, rather than habitat categories per se. For outer reef and atoll sites all counts were conducted on the outer slopes only, no counts were conducted in reef flat or backreef areas. At each site 4 replicate timed swims (described above) were conducted at 6, 9, 12, and 15m. Depth was continuously monitored on a depth guage during each timed swim. Replicate timed swims were conducted end-to-end as at Lizard Island, with each replicate separated by a 2 minute swim. Adult acanthurids only were surveyed on outer reef and atoll sites.

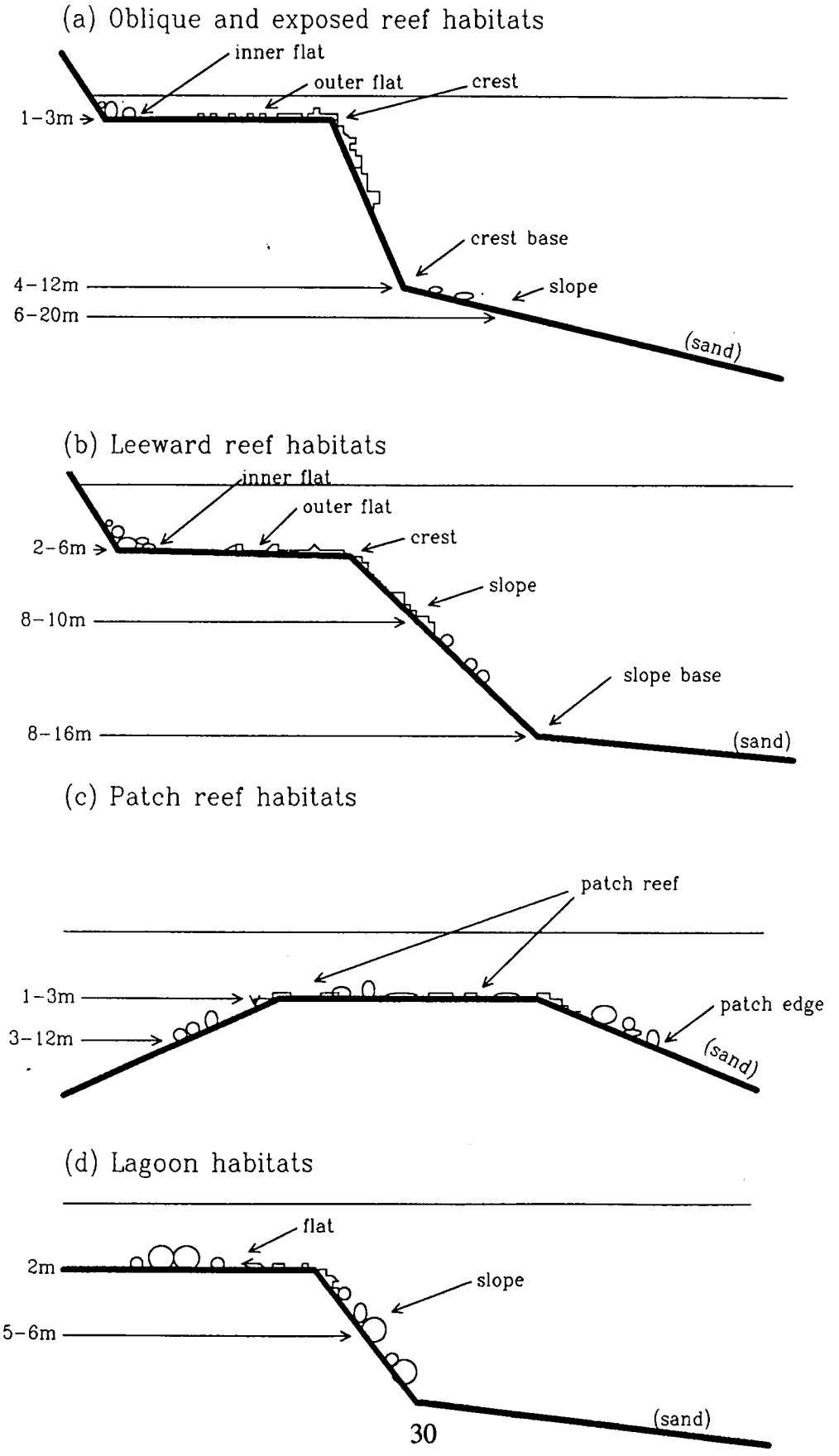
One site at each of four outer reefs was surveyed. Outer reefs and dates surveyed were as follows: Carter Reef (28/12/89), No. Ten Ribbon Reef (9/2/90), Yonge Reef (10/2/90), and NoName Reef (10/2/90). In addition two sites at the Coral Sea

atoll Osprey Reef were surveyed on 1/11/90 and 2/11/90. Three sites at each of the Tuvalu atolls Nui and Niutao were surveyed, from 4/9/89-7/9/89 and 29/9/89-3/10/89 respectively. Locations of outer reef sites are shown in Fig. 1.2. A map of the Tuvalu Islands is presented in Jones et al (1991) (see Appendix).

(e) Survey sites in southern Queensland and northern New South Wales

The sites and dates surveyed in sub-tropical localities were as follows: Northwest Solitary Island (23/5/89 and 25/5/89) - 2 sites, North Solitary Island (24/5/89) - 1 site, Flinders Reef (13/6/89) - 1 site, Inner Gneerings (15/6/89) - 2 sites, and Julian Rocks (20/6/89) - 2 sites. Adult acanthurids only were surveyed. Locations of study sites are presented in Fig. 1.3. The structure of the rocky reefs present in these sub-tropical localities was very different to that of coral reefs (for details of reef structure at Flinders Reef and the Solitary Islands see Veron (1986)). Limited time prohibited adequate site stratification by habitat. Therefore, to obtain an estimate of the species present in a given locality, I sampled the shallow subtidal habitat only. Timed swim transects at the Solitary Islands, Flinders Reef and Julian Rocks followed the base of emergent rock slopes, which constituted the shallowest reef area below the surge zone. At the two Gneerings sites, transects randomly traversed the shallowest portions of these totally submerged reefs. Depths surveyed throughout all sites varied between 2 and 7m.

Figure 2.1: Habitats surveyed at Lizard Island



## 2.3 RESULTS

### 2.3.1 Distribution of adult acanthurids at Lizard Island

Twenty-four species of acanthurids were recorded from Lizard Island in the course of the adult visual transects. The distribution of adult acanthurids at Lizard Island will be discussed below, with species treated separately and in alphabetical order. A summary of the distribution of adult acanthurids is presented in Fig. 2.56. Comparisons between adult and juvenile distributions will be made below in section 2.3.2.

*Acanthurus auranticavus/blochii* (Fig. 2.2): These two species were not differentiated (see section 2.2), thus the distribution pattern in Fig. 2.2 reflects the occurrence of both species. Details of the relative abundances of *A. auranticavus* and *A. blochii* are presented for 4 sites in Appendix 2. Taking these data into account, it is likely that *A. auranticavus* only occurred at a few habitat/locality sites, notably the inner and outer flat habitats of oblique and exposed reefs. Crest base and slope observations probably entirely represent *A. blochii* individuals, as do observations from leeward and patch reef sites. *A. blochii* was thus a widespread species, found in all depths at most sites around Lizard Island.

*Acanthurus bariene:* a single *Acanthurus bariene* was recorded at the crest base at Pidgin Point on 4/12/87.

*Acanthurus blochii:* See *A. auranticavus*.

*Acanthurus dussumieri* (Fig. 2.3): The habitat distribution of this species differed between sites. At the oblique sites (North Point, North Reef and South Island), *A. dussumieri* was found most commonly on the crest base. At the exposed sites (Pidgin Point, Bird Island and South Front) however, individuals were more common on the reef flat habitats. In general, however, *A. dussumieri* was found in most habitat/locality sites, with the notable exception of the lagoon.

*Acanthurus grammoptilus* (Fig. 2.4): This species was recorded occasionally from slope and crest base habitats right around the island, and on the flat at Granite Bluffs.

*Acanthurus lineatus* (Fig. 2.5): This species was very common on the crest of the oblique reefs sampled, with lower densities in the flat habitats of these sites. *A. lineatus* was also recorded from exposed sites, in the outer flat and crest habitats.

*Acanthurus mata* (Fig. 2.6): This planktivorous species was occasionally sighted in small schools in deeper areas around Lizard Island, and appeared to be highly mobile.

*Acanthurus nigricauda* (Fig. 2.7): This species was very widespread, and was recorded from most habitat/locality sites. *A. nigricauda* was however more common in deeper areas, particlarly those with a sand substratum (e.g. the lagoon slope).

*Acanthurus nigrofuscus* (Fig. 2.8): This ubiquitous species was recorded from every habitat/locality site. *A. nigrofuscus* was abundant in the flat habitats, but was also commonly recorded from crest base and slope habitats.

*Acanthurus olivaceus* (Fig. 2.9): This species was recorded from a number of habitat/locality sites, and showed no clear pattern in habitat distribution. *A. olivaceus* was most abundant at the South Front site.

*Acanthurus pyroferus* (Fig. 2.10): This species was rare at Lizard Island, and was recorded most often from crest base habitats.

*Acanthurus triostegus* (Fig. 2.11): This species was only recorded from inner and outer flat habitats, where it was often extremely abundant. *A. triostegus* was not recorded from sites without emergent land (i.e. Mac's Reef and South Front), and was rare or absent on leeward reefs.

*Acanthurus xanthopterus* (Fig. 2.12): This species was recorded from crest base and slope habitats around Lizard Island. It was most commonly observed over sand, particularly at the bases of leeward and patch reefs.

*Ctenochaetus binotatus* (Fig. 2.13): This species was moderately common in most crest base and slope habitats around Lizard Island. In leeward sites *C. binotatus* was also occasionally recorded from flat and crest habitats.

*Ctenochaetus striatus* (Fig. 2.14): This species was common to abundant at every site sampled. *C. striatus* were most common in inner flat, outer flat and crest habitats. However, individuals were also recorded from deeper crest base and slope habitats in some sites.

*Naso annulatus* (Fig. 2.15): This species was recorded as solitary individuals on rare occasions, but one school of approximately 70 fish was observed during a timed swim on the South Front slope on 25/1/88. This school consisted of large adult individuals which did not appear to be spawning.

*Naso brevirostris* (Fig. 2.16): This species was recorded at a number of sites, but showed no clear pattern of habitat distribution. Usually, *N. brevirostris* was recorded either adjacent to or over deep water (i.e. in crest, crest base or slope habitats).

*Naso hexacanthus* (Fig. 2.17): This species was uncommon at Lizard Island, where it was recorded from crest base and slope habitats.

*Naso lituratus* (Fig. 2.18): This species was uncommon at Lizard Island, where it did not display any obvious pattern of habitat distribution. This species was not observed on leeward reefs nor in the lagoon.

*Naso tuberosus* (Fig. 2.19): This species was moderately common in outer flat and crest habitats of the exposed sites. *N. tuberosus* was also recorded from flat habitats

at North Point and North Reef, and also from the lagoon flat habitat between Palfrey Island and South Island.

*Naso unicornis* (Fig. 2.20): This species was widespread around Lizard Island, and was recorded from all habitats. It was nonetheless most common in the outer flat and crest habitats of the 3 exposed sites.

*Naso vlamingii* (Fig. 2.21): This species was recorded from all sites with the exception of the two patch reefs, but was nowhere common. *N. vlamingii* was most commonly recorded from crest habitats of oblique and exposed reefs.

*Zebrasoma scopas* (Fig. 2.22): This species was recorded from all sites and habitat types. *Z. scopas* was most common on the crest of oblique and exposed reefs, and particularly at Mac's Reef (where it was also common on the crest base.

*Zebrasoma veliferum* (Fig. 2.23): Like *Z. scopas*, this species was recorded from all sites and habitat types, although it was much less common. *Z. veliferum* did not appear to show any clear patterns in habitat distribution.

### 2.3.2 Distribution of juvenile acanthurids at Lizard Island

Seventeen species of acanthurids were recorded from Lizard Island in the course of the juvenile visual transects, not including the clumped category *Acanthurus* `white-bar' spp. (see section 2.2). One species of acanthurid, *Paracanthurus hepatus*, was

recorded as newly settled juveniles in this study, although adults were never seen at Lizard Island. Most species settled in very low numbers each summer, and often a settlement `pulse' consisted of one or two individuals recorded from two or three habitat/locality sites.

The `patchy' nature of these data suggest that settlement distribution is best described by the choice of specific sampling episodes to illustrate the general pattern for each species. Since acanthurid species settled at different times throughout the sampling period, no single sampling episode could be chosen to represent them all. Thus I have chosen the sampling episode which includes the greatest number of habitat/locality site records to illustrate the settlement pattern for each species. Similarity of settlement patterns between years is illustrated using examples from the few species which settled in consistent numbers. To describe spatial patterns of settlement I have used two forms of presentation: (a) as a map showing all sites simultaneously for a single sampling episode (i.e. set of 4 replicate counts), and (b) as a series of repeated sampling episodes for one site. The distribution of juvenile acanthurids at Lizard Island will be discussed below, with species treated separately and in alphabetical order. The distribution of juvenile acanthurids at Lizard Island is summarised in Fig. 2.57.

*Acanthurus lineatus* (Fig. 2.24): This species settled in very low numbers throughout the three years of this study. In the summer of 1987/88 only 2 juveniles were recorded. Settlement chiefly occurred in sites occupied by adults, that is the oblique and exposed reefs. However juveniles of *A. lineatus* settled in inner and

outer flat habitats (Fig. 2.24), inshore of the adult distribution which was principally on the crest (Fig. 2.5).

*Acanthurus mata*: Eight juvenile *A. mata* were recorded in visual transects during the 3 year course of this study. Four of these records were from the slope habitat at Pidgin Point, 2 were from the slope at North Point, with 1 each from the crest bases at Bird Island and South Front. This distribution pattern is similar to that for the adults at Lizard Island (Fig. 2.6).

*Acanthurus nigrofuscus* (Figs. 2.25-2.30): This species settled in greater numbers than any other. Although the distribution of settlement was similar between years (Figs. 2.25-2.29), the magnitude of settlement varied considerably (Figs. 2.28-2.30). In fact, there was almost an order of magnitude difference between the numbers of juveniles settling in 1987/88 and 1988/89 (Figs. 2.28-2.30). The distribution of newly-settled juveniles and adults (Fig. 2.7) was very similar, with both groups most abundant in the outer flat habitats of oblique and exposed reefs. Therefore although the rankings of habitat/locality sites in terms of juvenile and adult abundance differed to some extent, it was clear that juveniles settled in habitat/locality sites occupied by adults.

*Acanthurus olivaceus* (Fig. 2.32): No juvenile *A. olivaceus* were recorded during the summer of 1987/88. It is likely that this species settled after the final survey for that summer, since large juveniles were collected at the island in August 1988. This species settled in low numbers, with a mean of more than 1 individual per replicate

transect recorded on only 15 occasions in 5 habitat/locality sites. The juvenile distribution pattern (Fig. 2.32) is similar to the adult pattern (Fig. 2.9), particularly if sites alone are ranked. The most consistent habitat/locality site for settlement was the slope at South Front. This area was characterised by a high percentage cover of rubble (see Appendix Fig. A1.2), and was shallower than most other slope rubble areas. Some juveniles were aggregated into small schools.

*Acanthurus pyroferus*: Only 4 juvenile *A. pyroferus* were recorded throughout the study. Three individuals were recorded from the crest base at Pidgin Point, and 1 was recorded from a rubble patch in the outer flat at the South Front site. All of these sightings took place in the summer of 1989/90. The predominance of juvenile sightings on the crest base at Pidgin Point is notable, since adult *A. pyroferus* were recorded most frequently there also (Fig. 2.10).

*Acanthurus triostegus* (Fig. 2.33-2.35): Almost all settlement of this species occurred in the inner flat habitats of 5 sites: Bird Island, North Point, North Reef, Pidgin Point and South Island. Throughout the entire study, only 5 *A. triostegus* settled in other habitat/locality sites. These were: Mac's Reef outer flat (1), North Point outer flat (1), North Reef outer flat (2), and Turtle Beach inner flat (1). The settlement pattern of juveniles thus corresponds almost exactly to the distribution pattern of adults (Fig. 2.11). *A. triostegus* settled earlier in the summer than most species (Figs. 2.34 and 2.35), and showed less variation in settlement densities between years than species such as *A. nigrofuscus* (Figs. 2.28-2.30). Juveniles of this species often occurred in small schools.

*Acanthurus* white-bar spp. (Fig. 2.36): Since these juveniles may represent individuals of at least 7 species (*A. auranticavus*, *A. bariene*, *A. blochii*, *A. dussumieri*, *A. grammoptilus*, *A. nigricauda* and *A. xanthopterus* were all recorded from Lizard island as adults - see section 2.2), little can be said concerning the similarity of juvenile and adult distributions. *A.* white-bar juveniles settled in a variety of habitat-locality sites, but were usually found in rubble areas. Many *A.* white-bar juveniles settled on artificial patch reefs sited in deeper sandy areas on the leeward and exposed sides of Lizard Island and in the lagoon (M. Meekan personal communication).

*Ctenochaetus binotatus* (Figs. 2.37-2.44): *C. binotatus* juveniles were characteristic of deeper areas, particularly the crest base habitat and in rubble patches on the slope. Thus juveniles were found in the same areas as adults (Fig. 2.13). Settlement was consistent between years in terms of pattern (Figs. 2.37-2.39), although some quantitative variation between years was apparent (Figs. 2.40-2.44). Several differences were apparent between the distributions of adults and juveniles. Settlement was consistently high at Granite Bluffs and South Front (Figs. 2.37-2.39), yet adults were not common there (Fig. 2.13). Conversely, settlement was proportionately low at Turtle Beach (data for 1987/88 only) and South Island, yet adults were common in both these sites.

*Ctenochaetus striatus* (Figs. 2.45-2.50): Juveniles of this species settled most commonly in the shallower inner and outer flat habitats, and thus had a similar distribution to the adults (Fig. 2.14). There were considerable differences between

years in the settlement of *C. striatus*, both quantitatively (Figs. 2.45-2.47) and in terms of distribution (Figs. 2.48-2.50). Very few *C. striatus* settled in the 1987/88 summer, particularly on the exposed reefs (Figs. 2.47 and 2.48). However in the subsequent 1988/89 summer the greatest numbers of settling juveniles were recorded at two of the exposed sites, Pidgin Point and South Front (Figs. 2.47 and 2.49). The ratio of newly settled to adult *C. striatus* was much lower than that for *C. binotatus* (Fig. 2.57).

*Naso brevirostris* (Fig. 2.51): This species settled in a wide variety of habitat/locality sites, and was found over both rubble and turf algal substrata. Juveniles were often aggregated into small schools, but were never common. The distribution of newly-settled juveniles (Fig. 2.51) did not overlap greatly with that of adults (Fig. 2.16).

*Naso hexacanthus* (Fig. 2.52): Juveniles of this species were recorded from the following habitat/locality sites: Granite Bluffs inner flat, outer flat and slope; North Point crest base and slope; North Reef inner flat, crest base and slope; Pidgin Point slope; and South Front slope. Like *N. brevirostris*, juveniles of this species also formed schools.

*Naso lituratus*: Only 4 juveniles of this species were recorded throughout this study. Two were recorded from the North Reef crest base, 1 from the North Point crest base, and 1 from the South Front slope. *N. lituratus* settled at a relatively large size (40 to 50mmSL).

*Naso tuberosus*: A total of 27 juveniles were recorded throughout the study from the following habitat/locality sites: North Reef inner flat and slope, North Point inner flat and slope, Bird Island inner flat and outer flat, South Island inner flat, and Granite Bluffs inner flat. These juveniles often formed small schools, and appeared to be highly mobile. It is possible that some of these juveniles were *A. annulatus*, since these 2 species are very difficult to differentiate at small sizes.

*Naso unicornis* (Fig. 2.53): Juveniles of this species were often observed in small schools, and were recorded from the following habitat/locality sites: Bird Island inner flat; Granite Bluffs inner flat, outer flat, and slope; North Point inner flat, crest base, and slope; North Reef inner flat, outer flat, crest base, and slope; Pidgin Point inner flat, outer flat, crest base, and slope; South Island inner flat and crest base; South Front outer flat and slope. Adult *N. unicornis* were most common in the exposed sites (Fig. 2.20), a distribution inconsistent with that of the typical settlement pattern (Fig. 2.53). *N. unicornis* settled at a large size (45 to 50mmSL).

*Naso vlamingii*: Seventeen newly settled *N. vlamingii* were recorded throughout the study, and unlike *N. unicornis* were usually observed as solitary individuals. Settlement was recorded in the following habitat/locality sites: Bird Island inner flat; Granite Bluffs outer flat and slope; North Point crest base and slope; North Reef inner flat, outer flat, crest base, and slope; Corner Reef patch reef; Pidgin Point outer flat; and South Island crest base. Like *N. unicornis*, there is little congruence between the patterns of settlement and adult distribution (Fig. 2.21).

*Paracanthurus hepatus*: Juveniles of this species settled at Granite Bluffs in both 1988/89 and 1989/90. One juvenile was recorded from the inner flat in 1988/89, while in February 1990 6 were recorded from the inner flat and 1 from the outer flat. All juveniles were found in live coral. Adult *P. hepatus* were not observed at Lizard Island during the study.

*Zebrasoma scopas* (Figs. 2.31 and 2.54): Newly settled individuals of this species were usually found in live coral, generally in areas with little wave action (i.e. leeward reefs or the deeper habitats of oblique and exposed sites). Few juveniles settled in 1987/88 (although 7 were recorded from the crest base at Mac's Reef in the January census), with moderate settlement occurring in the latter two sampling seasons (Fig. 2.31). Some similarity was evident between settlement (Fig. 2.54) and adult (Fig. 2.22) distributions, particularly in terms of sites. However *Z. scopas* juveniles were particularly characteristic of crest base areas (Fig. 2.54), while adults were more common on reef crests (Fig. 2.22).

*Zebrasoma veliferum* (Fig. 2.55): Like *Z. scopas*, juvenile of this species were frequently found sheltering in live coral. *Z. veliferum* settled in very low numbers during the study, with 8 juveniles recorded in 1987/88 and only 2 in the 1989/90 summer. *Z. veliferum*, like *Z. scopas*, tended to settle in areas with little wave action (Fig. 2.55). This species was one of the few that settled in the lagoon, with 3 juveniles recorded from the flat of these sites in 1987/88. Interestingly, throughout the study only one juvenile was recorded from Bird Island, where adults were relatively common (Fig. 2.23).

**Comparisons of juvenile and adult distribution**

The results of the chi-square analysis are presented in Table 2.1. The results for *Acanthurus lineatus*, *A. nigrofuscus*, *A. olivaceus*, *A. triostegus*, *Ctenochaetus binotatus* and *C. striatus* were all highly significant, indicating that many more recruits settle where there are adults than would occur by chance. The results for *Naso brevirostris* and *N. tuberosus* were also significant, but in the case of the former species only marginally so. The results for *N. unicornis* and *Zebrasoma scopas* were also highly significant, but an examination of the data indicates significant separation between adult and juvenile distributions. The result for *Z. veliferum* was non-significant, indicating no departure from random settlement.

A comparison of Fig. 2.56 with Fig. 2.57 indicates that (a) juveniles generally settled in habitats where conspecific adults were common, and (b) juveniles of most species settled in very low numbers relative to adult densities. This latter point was particularly so for *A. lineatus* and *C. striatus*, as is emphasized in the ranked abundance totals presented in Fig. 2.58. The opposite pattern was apparent for *C. binotatus* juveniles, which were much more common relative to adult abundance than other acanthurids at Lizard Island (Fig. 2.58).

### 2.3.3 Distribution of adult acanthurids on outer reefs and Tuvalu atolls

Species will be discussed separately and in alphabetical order.

*Acanthurus achilles*: This species was never recorded in transects, but was frequently observed in the shallow spur-and-groove zone of Tuvalu atolls.

*Acanthurus albipectoralis*: This species was recorded from 2 sites: No. 10 Ribbon Reef (6 and 9m) and Yonge Reef (12 and 15m). The No. 10 6m record was of two individuals, the remainder were solitary individuals.

*Acanthurus auranticavus*: Two individuals of this species were recorded from 6m at The Osprey Pass site.

*Acanthurus blochii* (Fig. 2.59): This species was very common at the Pass site at Osprey Reef, but only sporadic individuals were recorded elsewhere.

*Acanthurus dussumieri*: One or two individuals of this species were recorded from the following sites: Kulia North (Niutao) 15m; No. 10 6m; NoName Reef 6 and 9m; Osprey North 9m; and Osprey Pass 6m.

*Acanthurus guttatus*: This species was common in the shallow spur-and-groove zone of Tuvalu atolls (personal observation), but was rarely observed in the deeper areas where transects were conducted. *A. guttatus* were recorded in the 6m transects at

Kulia North (Niutao), at a mean density of 23.25±11.58.

*Acanthurus leucocheilus*: One or two individuals of this species were recorded from the North Control site at Niutao, at 6, 12 and 15m.

*Acanthurus lineatus* (Fig. 2.60): This species was recorded from all sites surveyed, and was usually most abundant in the shallow 6m transects. *A. lineatus* was particularly abundant in the Kulia North and Canoe City sites at Niutao.

*Acanthurus maculiceps*: Two individuals were recorded in a single 12m transect at the Nui Channel site.

*Acanthurus mata*: A school of 20 individuals were recorded in a single 9m transect at NoName Reef.

*Acanthurus nigricans* (Fig. 2.61): This species was recorded from all sites surveyed. *A. nigricans* were common to abundant at all depths surveyed in Tuvalu, but on the outer reefs they tended to be more common in the shallow 6m transects.

*Acanthurus nigricansxachilles*: Two individuals of this hybrid were recorded during the study: from a 12m transect at the Nui North Control site and from a 6m transect at the Nui South Control site.

*Acanthurus nigricauda* (Fig. 2.62): This species was recorded from 5 sites in Tuvalu

and 1 site on the outer reefs. *A. nigricauda* tended to occur over or nearby areas of sand substratum, and was usually observed in schools.

*Acanthurus nigrofuscus* (Fig. 2.63): This species was recorded from all sites surveyed, and showed no consistent distribution pattern with depth. *A. nigrofuscus* was generally observed in small groups.

*Acanthurus nigroris* (Fig. 2.64): This species was recorded from all Tuvalu sites, yet was only observed at No. 10 Reef on the GBR. Like *A. nigrofuscus*, *A. nigroris* generally occurred in small groups, and was recorded from most depths.

*Acanthurus olivaceus* (Fig. 2.65): Like *A. nigricauda*, this species occurred in schools over areas of sand substratum. *A. olivaceus* was abundant at the Muli North site at Niutao, where there were extensive sand patches (Kaly and Jones 1990 - see Niutao North Control site).

*Acanthurus pyroferus* (Fig. 2.66): This species was recorded at all sites except Carter Reef, and was generally more abundant in deeper areas in both the Tuvalu and outer reef sites.

*Acanthurus thompsoni* (Fig. 2.67): This planktivorous species was patchily distributed in the deeper areas of Tuvalu and outer reef sites.

*Acanthurus triostegus* (Fig. 2.68): Like *A. achilles* and *A. guttatus*, this species

generally occurred at depths shallower than those sampled. *A. triostegus* characteristically occurred in large schools.

*Acanthurus xanthopterus*: Two individuals of this species were recorded in transects: at 9m at the North Control Nui site and at 6m at the Osprey Pass site.

*Ctenochaetus binotatus* (Fig. 2.69): This species was common to abundant in outer reef sites, but only a few individuals were recorded in Tuvalu. In both regions, *C. binotatus* was most common in the deepest areas sampled. *C. binotatus* were recorded in 6m transects at only one site, NoName Reef.

*Ctenochaetus hawaiiensis* (Fig. 2.70): This species was only recorded from Tuvalu sites, and tended to occur in small groups in deeper areas.

*Ctenochaetus marginatus* (Fig. 2.71): This species was only recorded from Tuvalu sites, and like *C. binotatus* and *C. hawaiiensis* tended to occur in deeper areas.

*Ctenochaetus striatus* (Fig. 2.72): This species was abundant at the Nui sites in Tuvalu and on the outer reefs, but was uncommon at Niutao. *C. striatus* showed marked differences in depth distribution between sites. At the 3 Nui sites, NoName Reef and the two Osprey sites, *C. striatus* showed a clear trend of decreased abundance with depth. This trend was less obvious at No. 10 Reef, and not apparent at all at Yonge Reef. At Carter Reef *C. striatus* showed the opposite trend, being more abundant in the deeper transects.

*Ctenochaetus strigosus* (Fig. 2.73): This species was recorded from all sites except No. 10 Reef, yet was considerably more abundant in the Tuvalu sites. In all 6 Tuvalu sites, *C. strigosus* showed a trend of increasing abundance with depth. Indeed, at the 3 Nui sites a negative relationship between the abundance of this species and *C. striatus* was apparent. However in the GBR sites the opposite depth distribution was suggested, with *C. strigosus* more common in the shallow 6 and 9m transects.

*Naso brevirostris* (Fig. 2.74): This species was recorded at all sites except Osprey North. *N. brevirostris* showed no clear pattern of depth distribution, with schools frequently occurring adjacent to drop-offs in areas exposed to current.

*Naso hexacanthus* (Fig. 2.75): This species was moderately common at most of the Tuvalu sites, where it occurred predominantly in the deeper transects. However at the outer reef sites *N. hexacanthus* showed little preference for any particular depth, and like *N. brevirostris* tended to aggregate in areas exposed to strong water movement.

*Naso lituratus* (Fig. 2.76): This species recorded from all sites surveyed, but was only common at the Kulia North and Canoe City sites at Niutao. In the Tuvalu sites this species was more common in the deeper transects, whereas at the two Osprey sites *N. lituratus* were more common in the shallow transects. No depth trends were apparent at the 4 remaining outer reef sites. In Tuvalu this species often aggregated into large schools, while on the outer reefs *N. lituratus* were most commonly seen

in pairs.

*Naso thynnoides*: One individual of this species was recorded from a 12m transect at the Nui South Control site.

*Naso tuberosus* (Fig. 2.77): This species was not recorded from Tuvalu, but was very common at No. 10 Reef. *N. tuberosus* typically occurred in large schools on the crest of outer reefs, but often moved down the slope into deeper water.

*Naso unicornis* (Fig. 2.78): This species was recorded from 3 Tuvalu sites and 3 outer reef sites but was only common at NoName Reef. *N. unicornis* were generally observed in small schools in the shallow transects.

*Naso vlamingii* (Fig. 2.79): This species was recorded from the 3 Nui sites and all the outer reef sites. *N. vlamingii* consistently occurred in low numbers in the deeper transects, although it was relatively common in the 6m transects at the Osprey Pas site. Unlike the other planktivorous *Naso* species, *N. vlamingii* did not aggregate into large schools, and generally occurred as solitary individuals or in small groups.

*Zebrasoma rostratum* (Fig. 2.80): This species occurred in 5 of the 6 Tuvalu sites. No obvious trend in depth distribution was apparent.

*Zebrasoma scopas* (Fig. 2.81): This species was common to abundant in all 12 sites surveyed. In the 6 Tuvalu sites a strong trend of increasing abundance with depth

was evident. This trend was apparent at No. 10 Reef and NoName Reef, but not at the other 4 outer reefs surveyed. At the 2 Osprey sites, *Z. scopas* were most abundant in the shallow 6 and 12m transects.

*Zebrasoma veliferum* (Fig. 2.82): This species, which was not recorded from Niutao, showed no consistent trends in depth distribution. This species was almost always sighted in pairs.

### 2.3.4 Distribution of adult acanthurids in southern Queensland and northern New South Wales

In this section results will be presented in terms of sites.

Inner Gneerings: Only 4 species were recorded in transects at the 2 sites surveyed (Figs. 2.83 and 2.84): *Acanthurus dussumieri*, *A. nigrofuscus*, *N. unicornis* and *Prionurus microlepidotus*. In addition, *A. blochii*, *A. lineatus*, *A. xanthopterus* and *P. maculatus* (a school of approximately 50 individuals) were observed but did not occur in transects. *A. nigrofuscus* was the only species of acanthurid which was common at the Inner Gneerings.

Flinder's Reef: Fifteen species of acanthurids were observed at this site, of which 11 were recorded in transects (Figs. 2.83 and 2.84). Despite the relatively high species richness of this site, only 2 species were common: *Acanthurus nigrofuscus* and *Zebrasoma scopas* (Fig. 2.83). The 4 species observed but not recorded in

transects were *A. dussumieri*, *N. lituratus*, *N. tuberosus* and *Z. veliferum*.

Julian Rocks: Nine species were recorded from the 2 sites surveyed (a solitary *Naso annulatus* was observed outside the transects). *Prionurus microlepidotus* was the only the species of acanthurid which was common at Julian Rocks (Figs. 2.83 and 2.84).

North Solitary Island: Four species of acanthurids were recorded in transects at this site (Figs. 2.83 and 2.84), while several juvenile and a solitary adult *Acanthurus triostegus* were observed outside transects. *A. nigrofuscus* and *P. microlepidotus* were common at North Solitary Island.

Northwest Solitary Island: Only 4 species of acanthurids were recorded in the 2 sites surveyed (Figs. 2.83 and 2.84). The 2 *Prionurus* species were extremely abundant, while *Acanthurus dussumieri* and *A. nigrofuscus* occurred in low numbers.

**Fig. 2.2 Distribution of adult *Acanthurus auranticavus/blochii* at Lizard Island.**

Mean number of individuals per transect (n=16) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CR = crest, CB = crest base, SL = slope, SB = slope base, PR = patch reef, PE = patch edge, FL = flat.

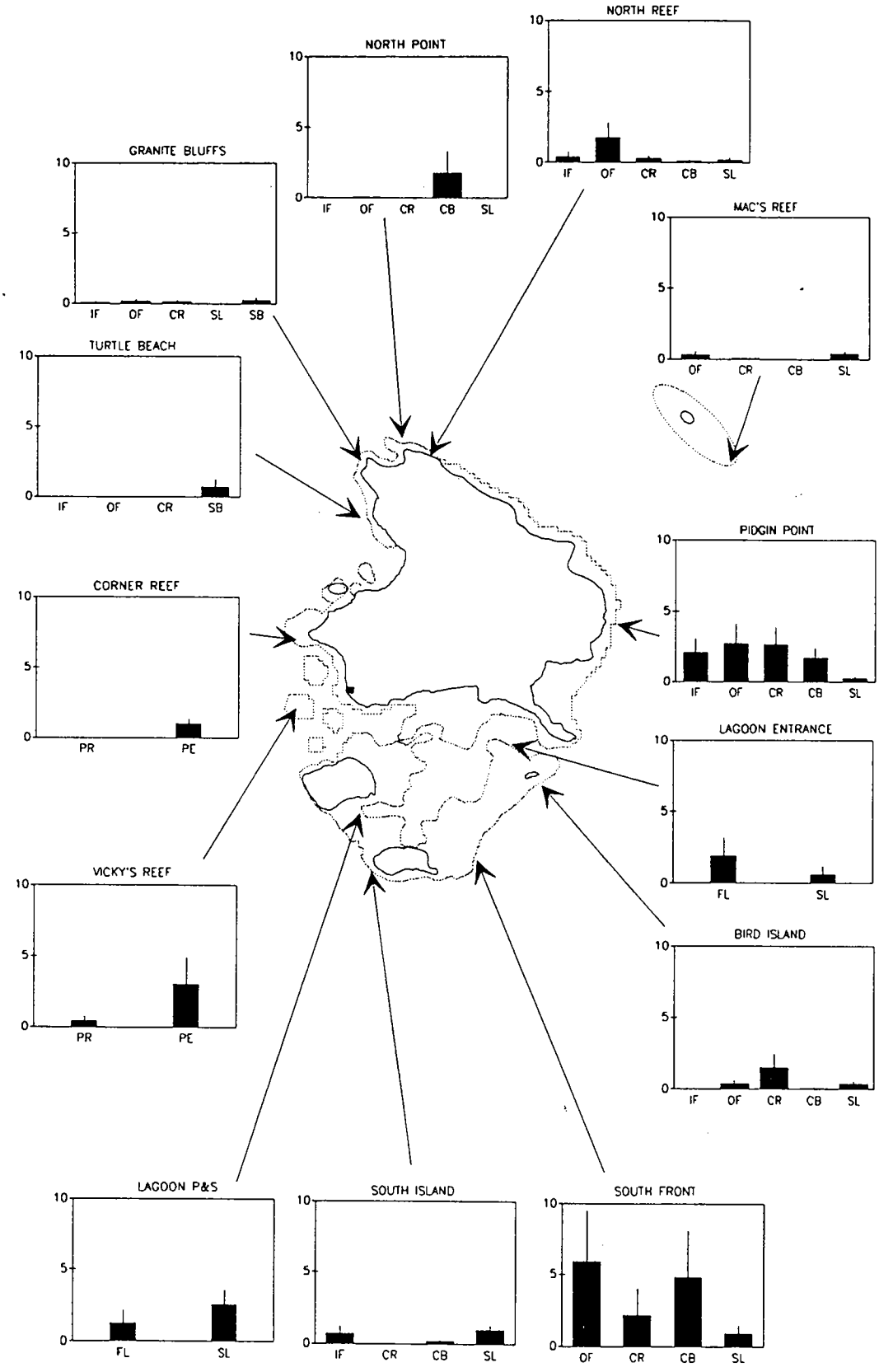

NORTH REEF
NORTH POINT
GRANITE BLUFFS
MAC'S REEF
TURTLE BEACH
PIDGIN POINT
CORNER REEF
LAGOON ENTRANCE
VICKY'S REEF
BIRD ISLAND
LAGOON P&S
SOUTH ISLAND
SOUTH FRONT
IF OF CR CB SL
IF OF CR SL SB
OF CR CB SL
IF OF CR SB
PR PE
FL SL
IF CR CB SL
OF CR CB SL

**Fig. 2.3 Distribution of adult *Acanthurus dussumieri* at Lizard Island.**

Mean number of individuals per transect (n=16) ± standard error.
Abbreviations: IF = inner flat, OF = outer flat, CR = crest, CB = crest base, SL = slope, SB = slope base, PR = patch reef, PE = patch edge, FL = flat.

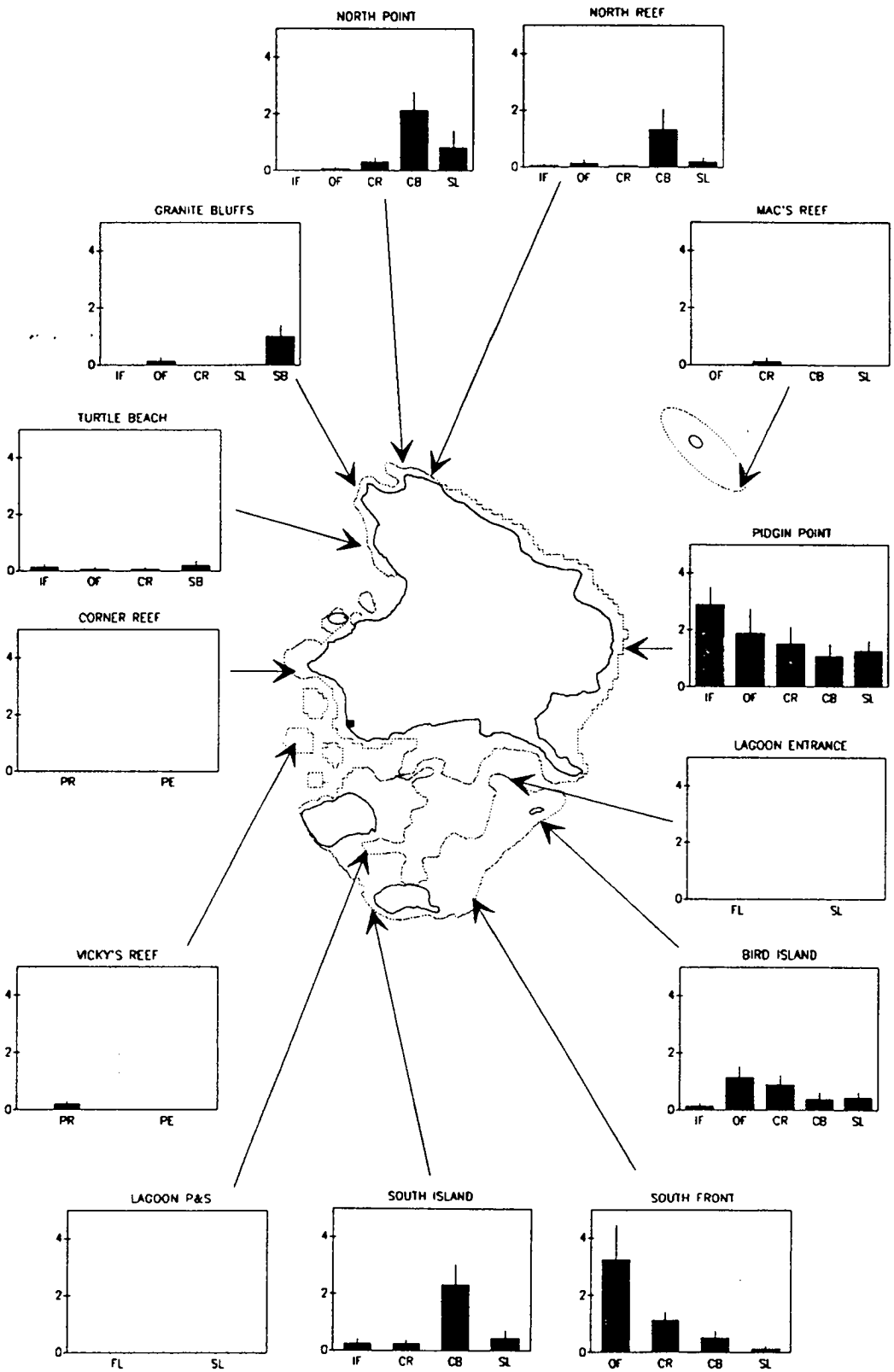

NORTH POINT
IF OF CR CB SL
NORTH REEF
IF OF CR CB SL
GRANITE BLUFFS
IF OF CR SL SB
MAC'S REEF
OF CR CB SL
TURTLE BEACH
IF OF CR SB
PIDGIN POINT
IF OF CR CB SL
CORNER REEF
PR PE
LAGOON ENTRANCE
FL SL
VICKY'S REEF
PR PE
BIRD ISLAND
IF OF CR CB SL
LAGOON P&S
FL SL
SOUTH ISLAND
IF CR CB SL
SOUTH FRONT
OF CR CB SL

**Fig. 2.4 Distribution of adult *Acanthurus grammoptilus* at Lizard Island.**

Mean number of individuals per transect (n=16) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CR = crest, CB = crest base, SL = slope, SB = slope base, PR = patch reef, PE = patch edge, FL = flat.

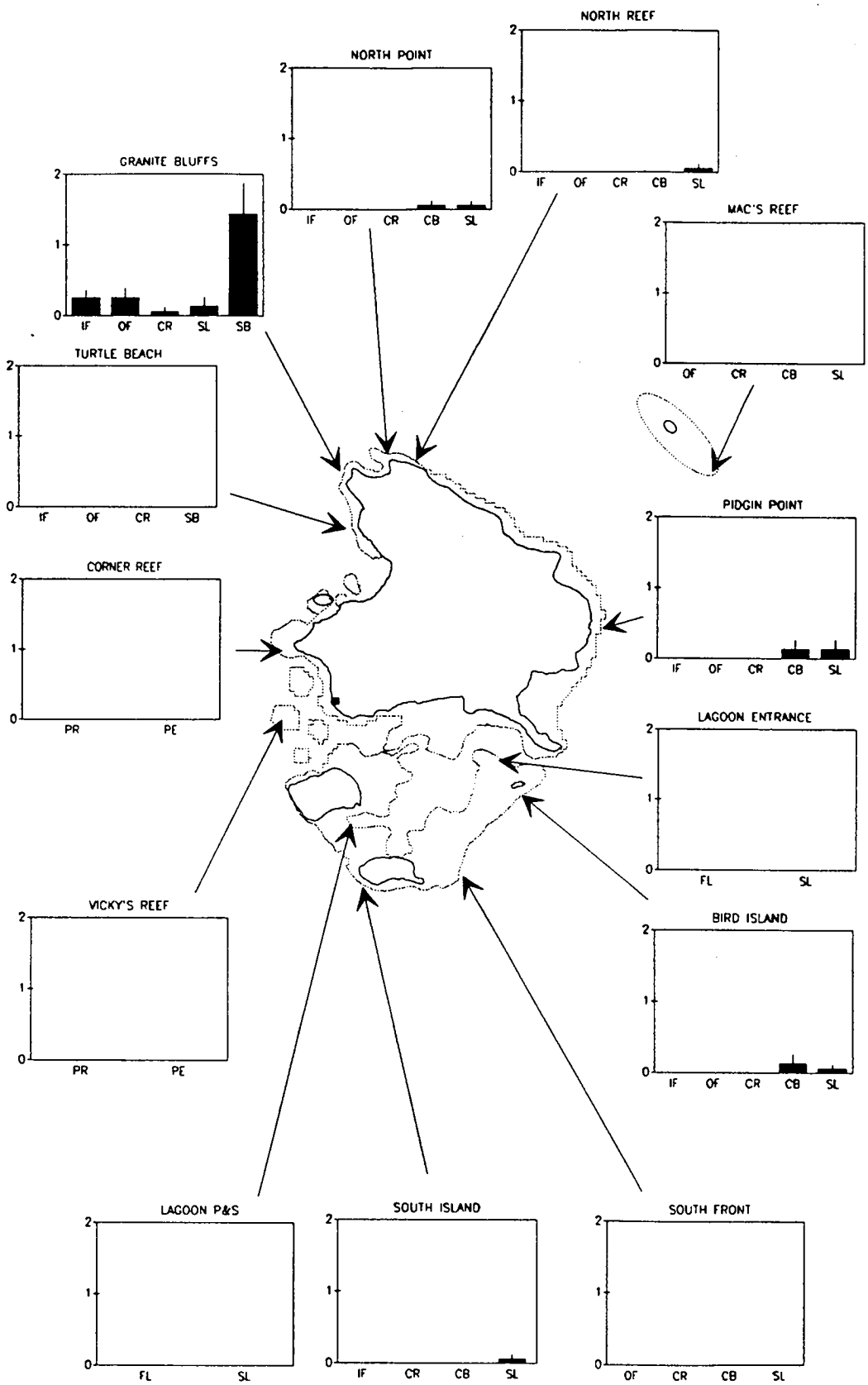

NORTH REEF
NORTH POINT
GRANITE BLUFFS
MAC'S REEF
TURTLE BEACH
PIDGIN POINT
CORNER REEF
LAGOON ENTRANCE
VICKY'S REEF
BIRD ISLAND
LAGOON P&S
SOUTH ISLAND
SOUTH FRONT
IF OF CR CB SL
IF OF CR SL SB
OF CR CB SL
IF OF CR SB
PR PE
FL SL
IF CR CB SL
OF CR CB SL

**Fig. 2.5 Distribution of adult *Acanthurus lineatus* at Lizard Island.**

Mean number of individuals per transect (n=16) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CR = crest, CB = crest base, SL = slope, SB = slope base, PR = patch reef, PE = patch edge, FL = flat.

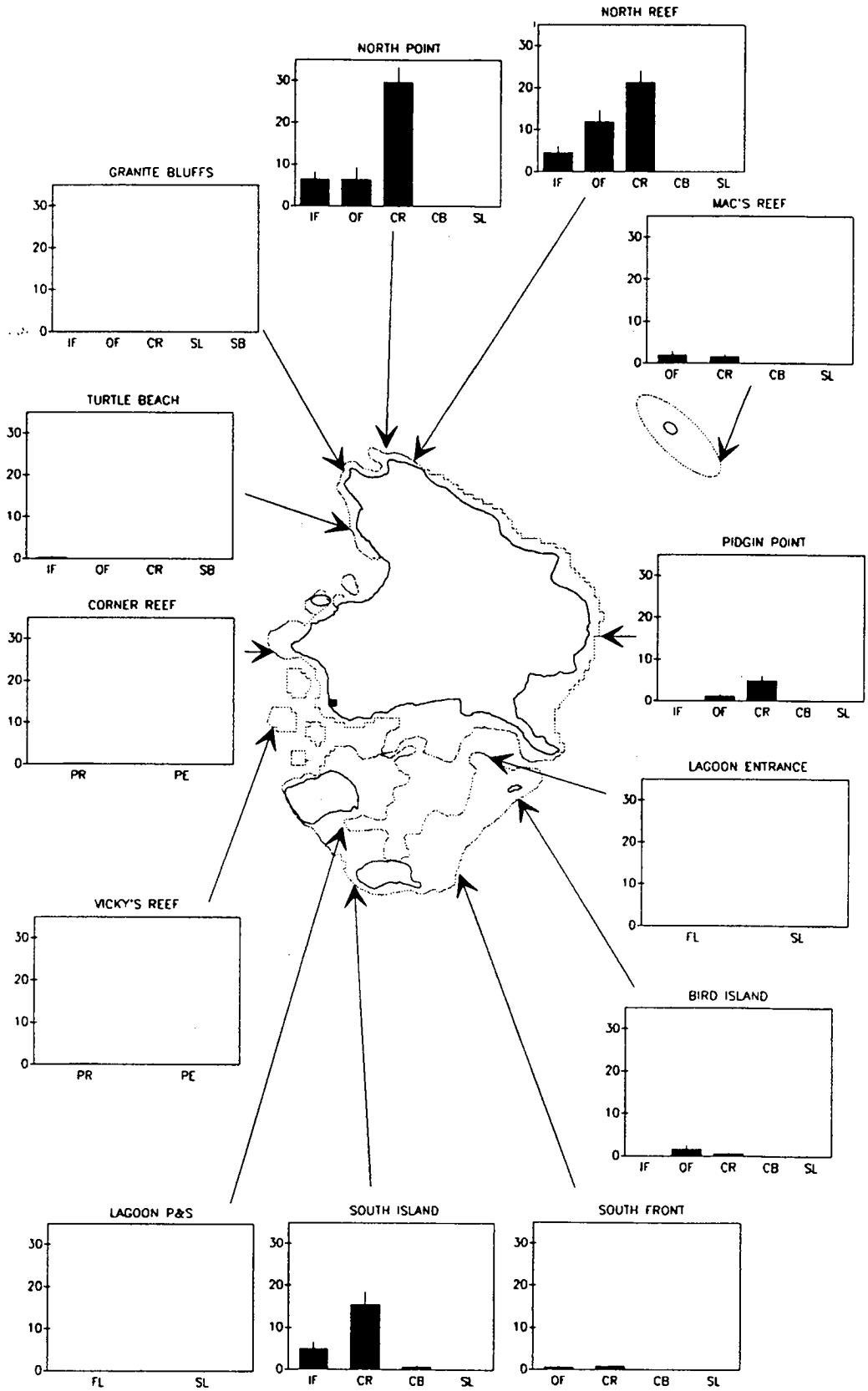

NORTH REEF
NORTH POINT
GRANITE BLUFFS
MAC'S REEF
TURTLE BEACH
PIDGIN POINT
CORNER REEF
LAGOON ENTRANCE
VICKY'S REEF
BIRD ISLAND
LAGOON P&S
SOUTH ISLAND
SOUTH FRONT
IF OF CR CB SL
IF OF CR SL SB
OF CR CB SL
IF OF CR SB
PR PE
FL SL
0 10 20 30

**Fig. 2.6 Distribution of adult *Acanthurus mata* at Lizard Island.**

Mean number of individuals per transect (n=16) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CR = crest, CB = crest base, SL = slope, SB = slope base, PR = patch reef, PE = patch edge, FL = flat.

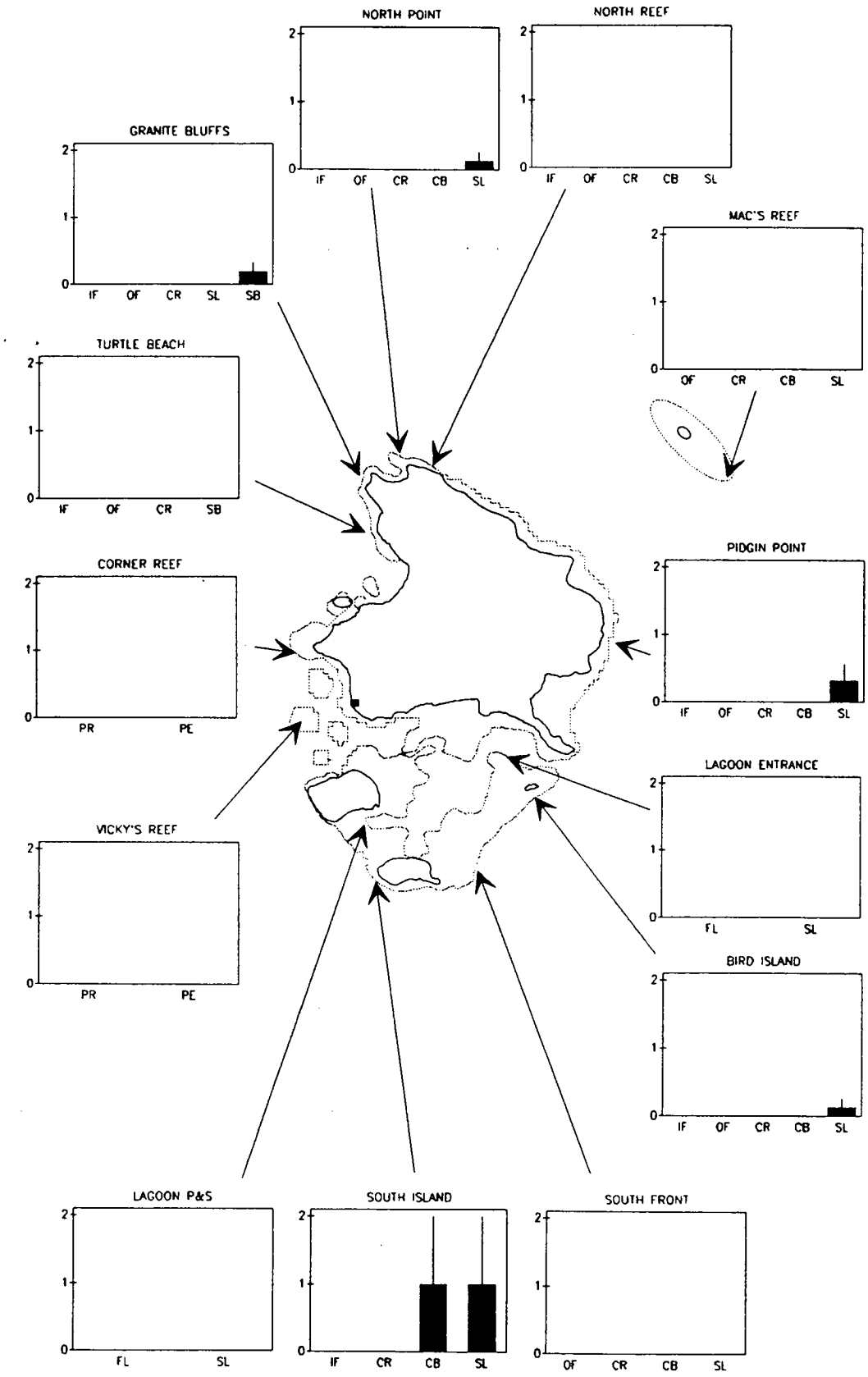

NORTH POINT
IF OF CR CB SL
NORTH REEF
IF OF CR CB SL
GRANITE BLUFFS
IF OF CR SL SB
MAC'S REEF
OF CR CB SL
TURTLE BEACH
IF OF CR SB
CORNER REEF
PR PE
PIDGIN POINT
IF OF CR CB SL
LAGOON ENTRANCE
FL SL
VICKY'S REEF
PR PE
BIRD ISLAND
IF OF CR CB SL
LAGOON P&S
FL SL
SOUTH ISLAND
IF CR CB SL
SOUTH FRONT
OF CR CB SL

**Fig. 2.7 Distribution of adult *Acanthurus nigricauda* at Lizard Island.**

Mean number of individuals per transect (n=16) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CR = crest, CB = crest base, SL = slope, SB = slope base, PR = patch reef, PE = patch edge, FL = flat.

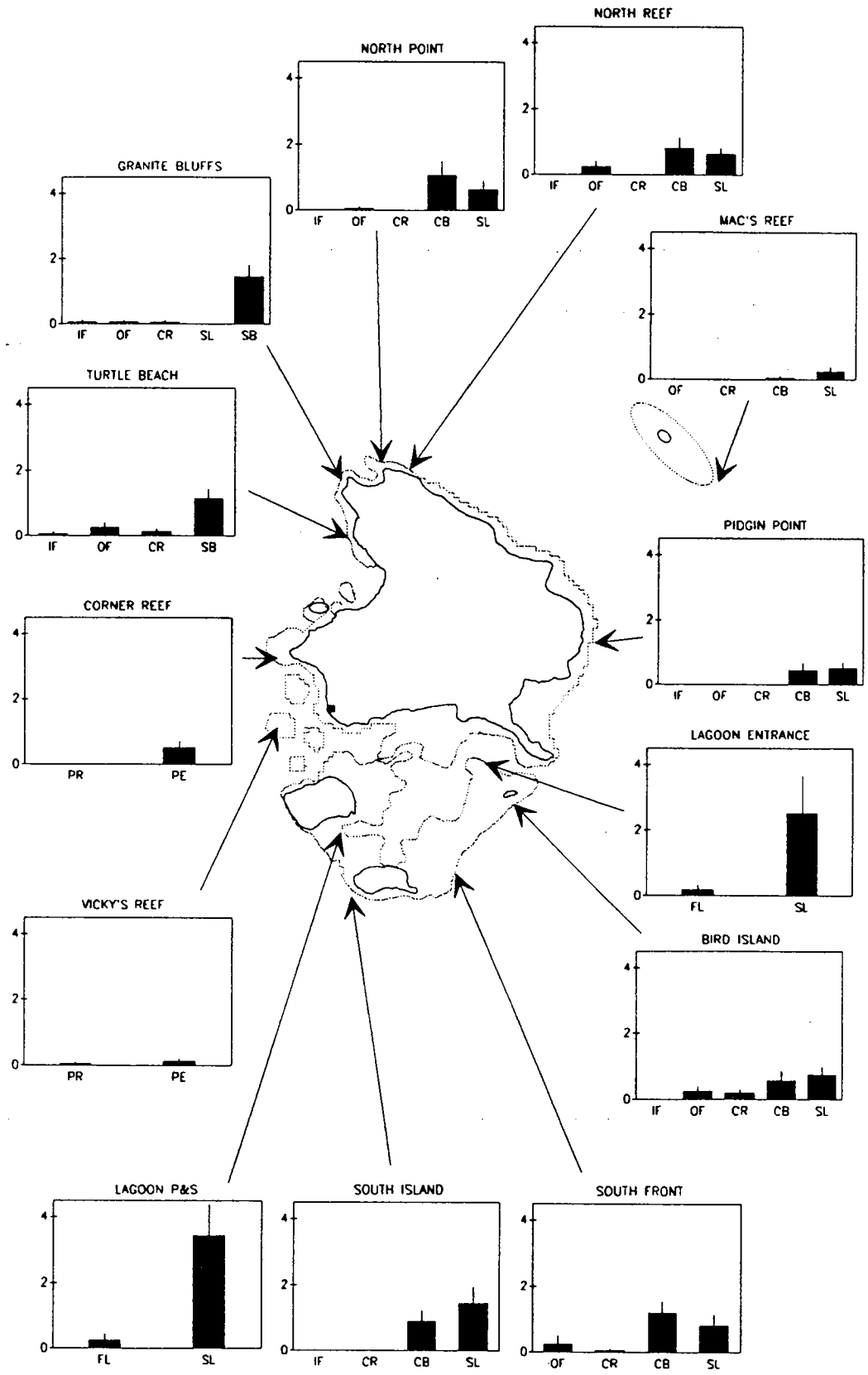

NORTH REEF
IF OF CR CB SL
NORTH POINT
IF OF CR CB SL
GRANITE BLUFFS
IF OF CR SL SB
MAC'S REEF
OF CR CB SL
TURTLE BEACH
IF OF CR SB
PIDGIN POINT
IF OF CR CB SL
CORNER REEF
PR PE
LAGOON ENTRANCE
FL SL
VICKY'S REEF
PR PE
BIRD ISLAND
IF OF CR CB SL
LAGOON P&S
FL SL
SOUTH ISLAND
IF CR CB SL
SOUTH FRONT
OF CR CB SL

**Fig. 2.8 Distribution of adult *Acanthurus nigrofuscus* at Lizard Island.**

Mean number of individuals per transect (n=16) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CR = crest, CB = crest base, SL = slope, SB = slope base, PR = patch reef, PE = patch edge, FL = flat.

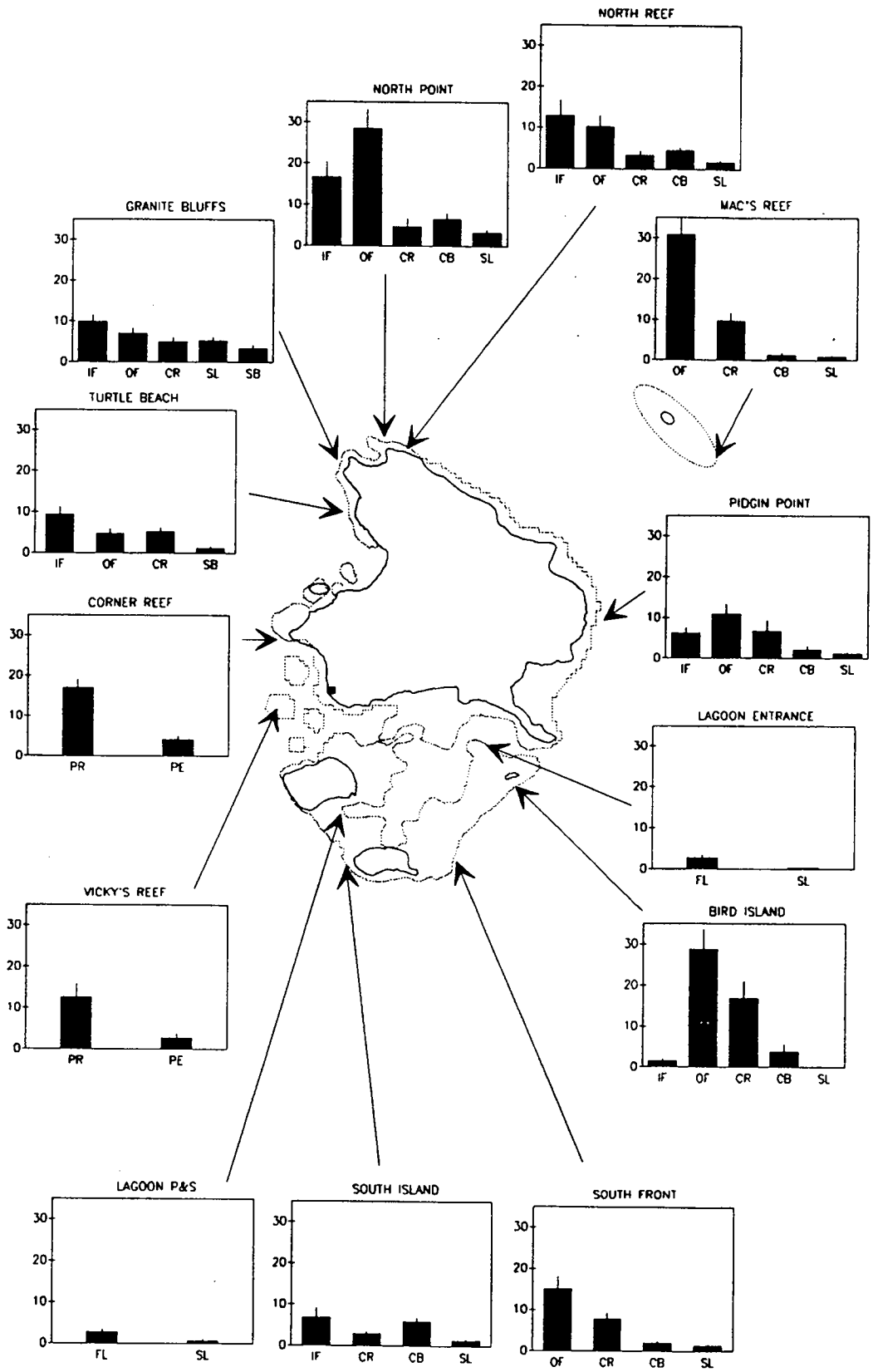

NORTH REEF
NORTH POINT
GRANITE BLUFFS
MAC'S REEF
TURTLE BEACH
PIDGIN POINT
CORNER REEF
LAGOON ENTRANCE
VICKY'S REEF
BIRD ISLAND
LAGOON P&S
SOUTH ISLAND
SOUTH FRONT
IF
OF
CR
CB
SL
SB
PR
PE
FL

**Fig. 2.9 Distribution of adult *Acanthurus olivaceus* at Lizard Island.**

Mean number of individuals per transect (n=16) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CR = crest, CB = crest base, SL = slope, SB = slope base, PR = patch reef, PE = patch edge, FL = flat.

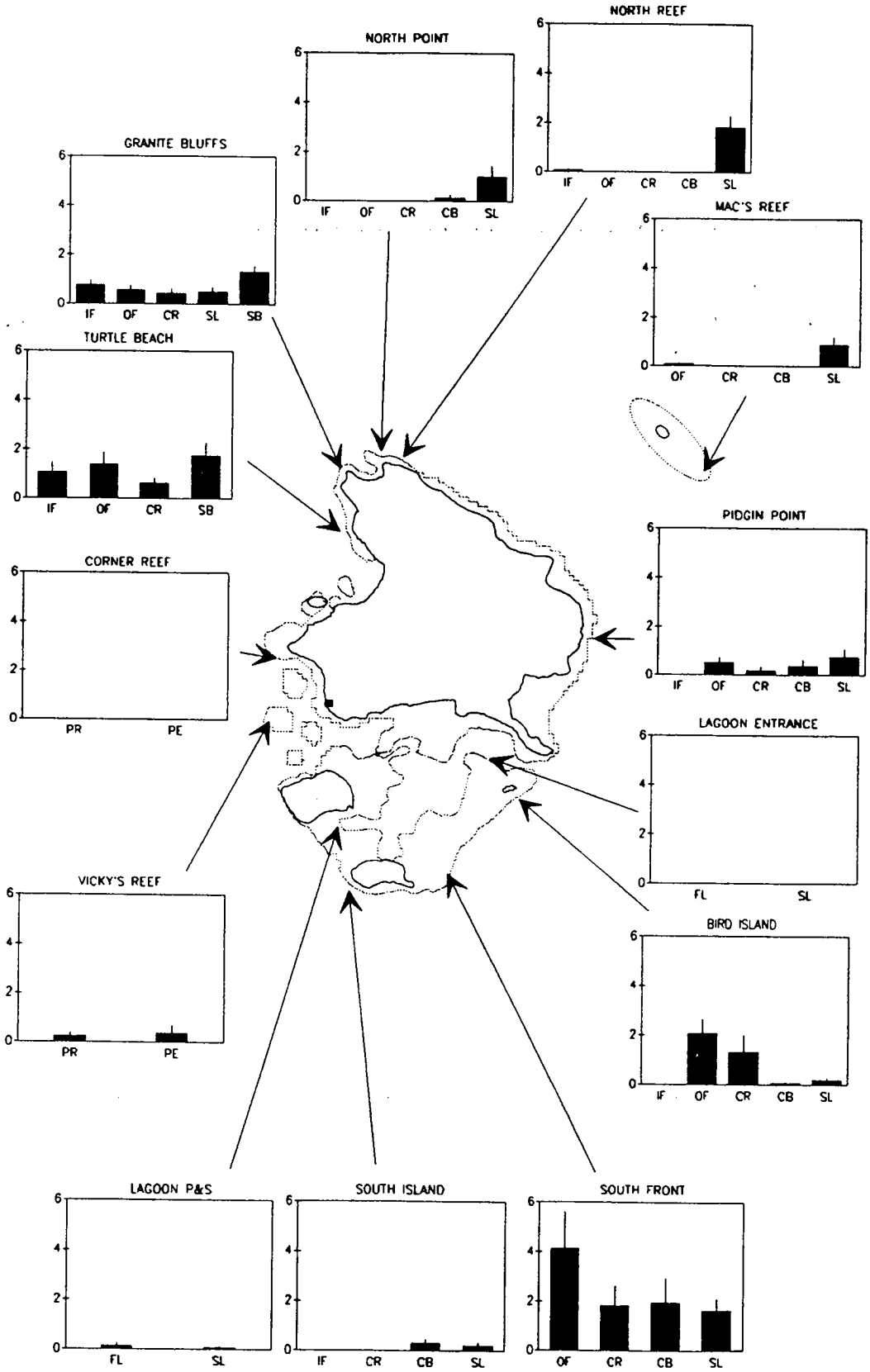

NORTH REEF
NORTH POINT
GRANITE BLUFFS
MAC'S REEF
TURTLE BEACH
PIDGIN POINT
CORNER REEF
LAGOON ENTRANCE
VICKY'S REEF
BIRD ISLAND
LAGOON P&S
SOUTH ISLAND
SOUTH FRONT
IF OF CR CB SL
IF OF CR SL SB
OF CR CB SL
IF OF CR SB
PR PE
FL SL
IF CR CB SL
OF CR CB SL

**Fig. 2.10 Distribution of adult *Acanthurus pyroferus* at Lizard Island.**

Mean number of individuals per transect (n=16) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CR = crest, CB = crest base, SL = slope, SB = slope base, PR = patch reef, PE = patch edge, FL = flat.

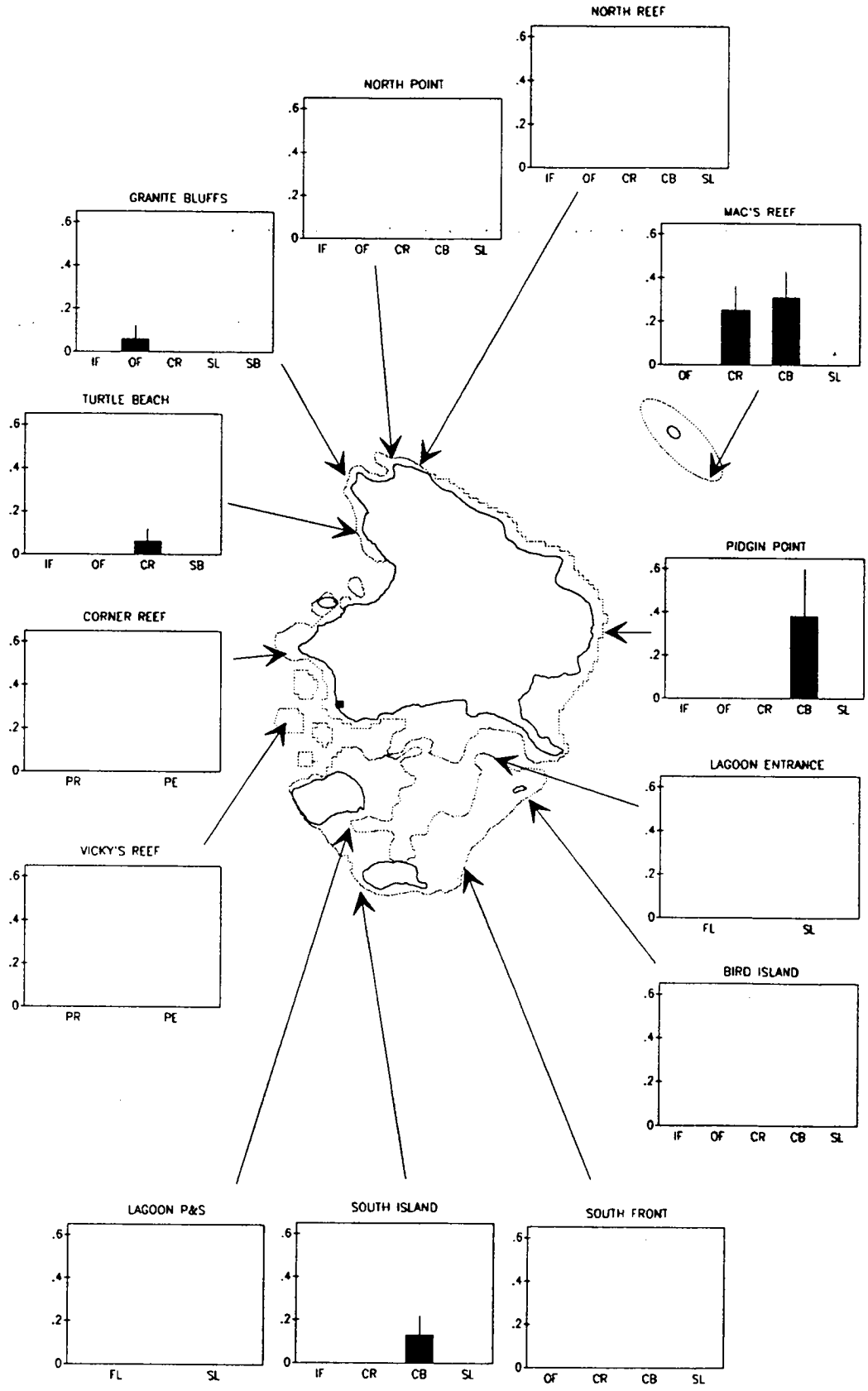

NORTH REEF
NORTH POINT
GRANITE BLUFFS
MAC'S REEF
TURTLE BEACH
PIDGIN POINT
CORNER REEF
LAGOON ENTRANCE
VICKY'S REEF
BIRD ISLAND
LAGOON P&S
SOUTH ISLAND
SOUTH FRONT
IF OF CR CB SL
IF OF CR SL SB
OF CR CB SL
IF OF CR SB
PR PE
FL SL
IF CR CB SL
OF CR CB SL
0 .2 .4 .6

**Fig. 2.11 Distribution of adult *Acanthurus triostegus* at Lizard Island.**

Mean number of individuals per transect (n=16) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CR = crest, CB = crest base, SL = slope, SB = slope base, PR = patch reef, PE = patch edge, FL = flat.

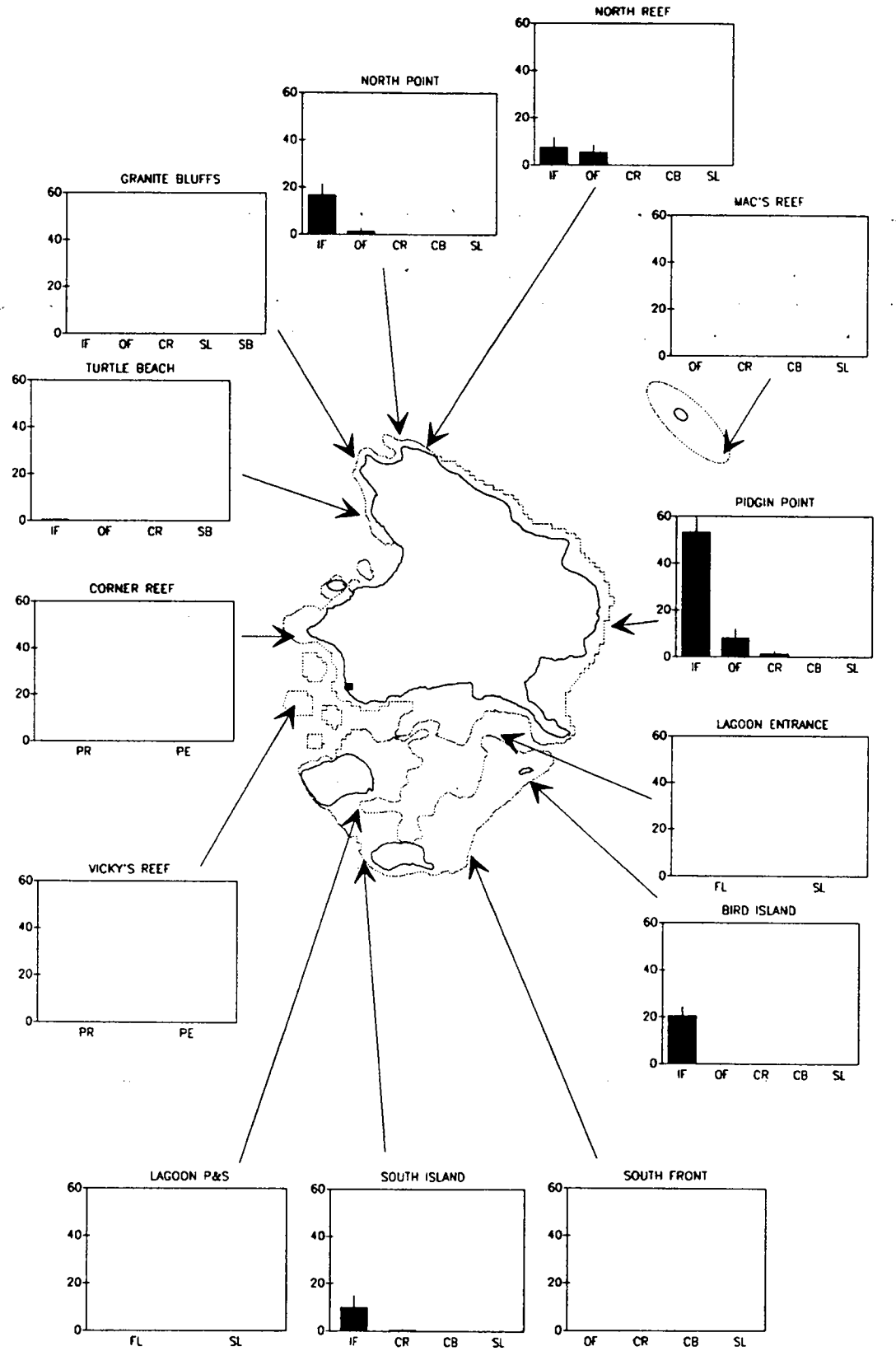

NORTH REEF
NORTH POINT
GRANITE BLUFFS
MAC'S REEF
TURTLE BEACH
PIDGIN POINT
CORNER REEF
LAGOON ENTRANCE
VICKY'S REEF
BIRD ISLAND
LAGOON P&S
SOUTH ISLAND
SOUTH FRONT
IF OF CR CB SL
IF OF CR SL SB
OF CR CB SL
IF OF CR SB
PR PE
FL SL
OF CR CB SL
0 20 40 60

**Fig. 2.12 Distribution of adult *Acanthurus xanthopterus* at Lizard Island.**

Mean number of individuals per transect (n=16) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CR = crest, CB = crest base, SL = slope, SB = slope base, PR = patch reef, PE = patch edge, FL = flat.

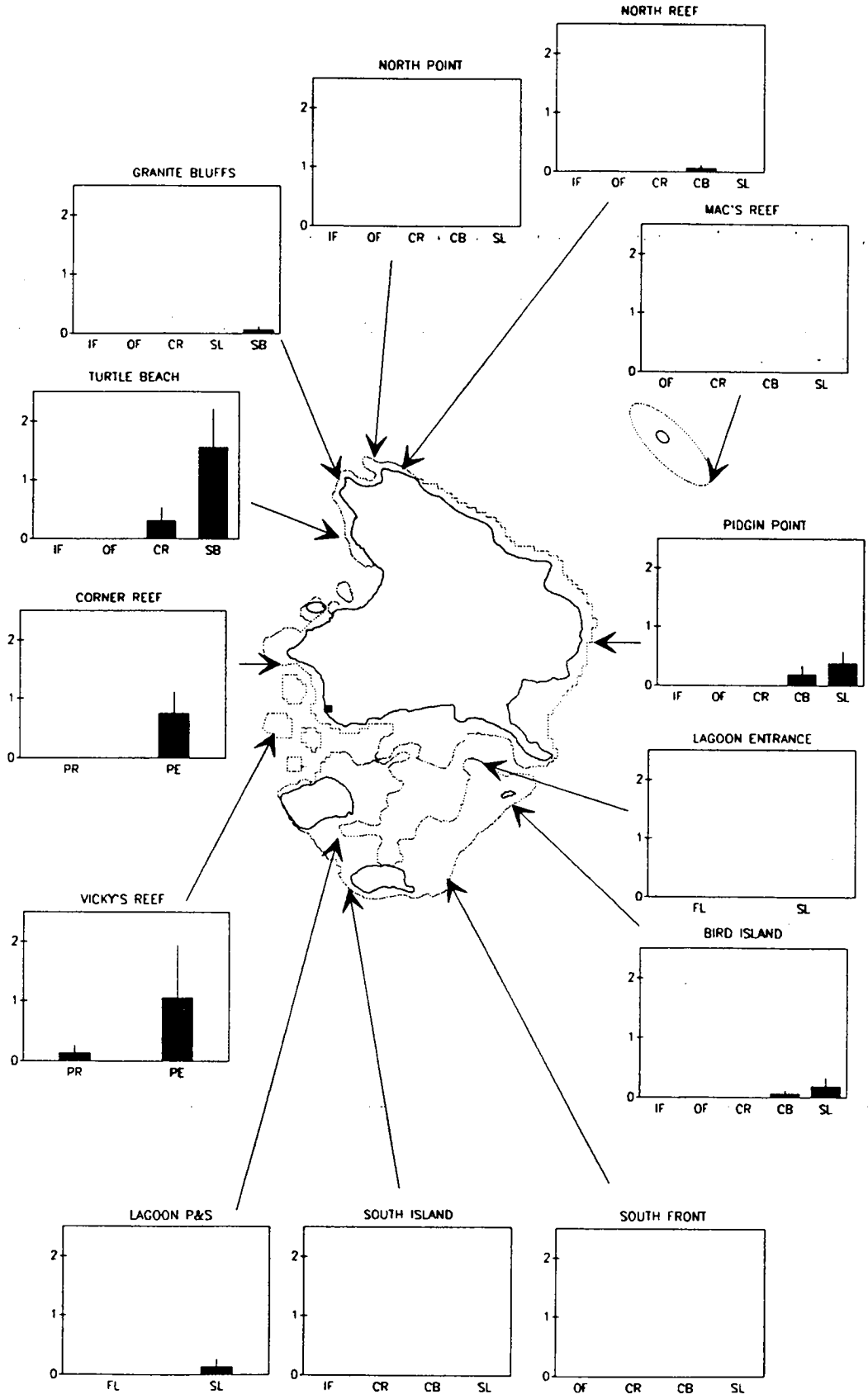

NORTH REEF
IF OF CR CB SL
NORTH POINT
IF OF CR CB SL
GRANITE BLUFFS
IF OF CR SL SB
MAC'S REEF
OF CR CB SL
TURTLE BEACH
IF OF CR SB
PIDGIN POINT
IF OF CR CB SL
CORNER REEF
PR PE
LAGOON ENTRANCE
FL SL
VICKY'S REEF
PR PE
BIRD ISLAND
IF OF CR CB SL
LAGOON P&S
FL SL
SOUTH ISLAND
IF CR CB SL
SOUTH FRONT
OF CR CB SL

**Fig. 2.13 Distribution of adult *Ctenochaetus binotatus* at Lizard Island.**

Mean number of individuals per transect (n=16) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CR = crest, CB = crest base, SL = slope, SB = slope base, PR = patch reef, PE = patch edge, FL = flat.

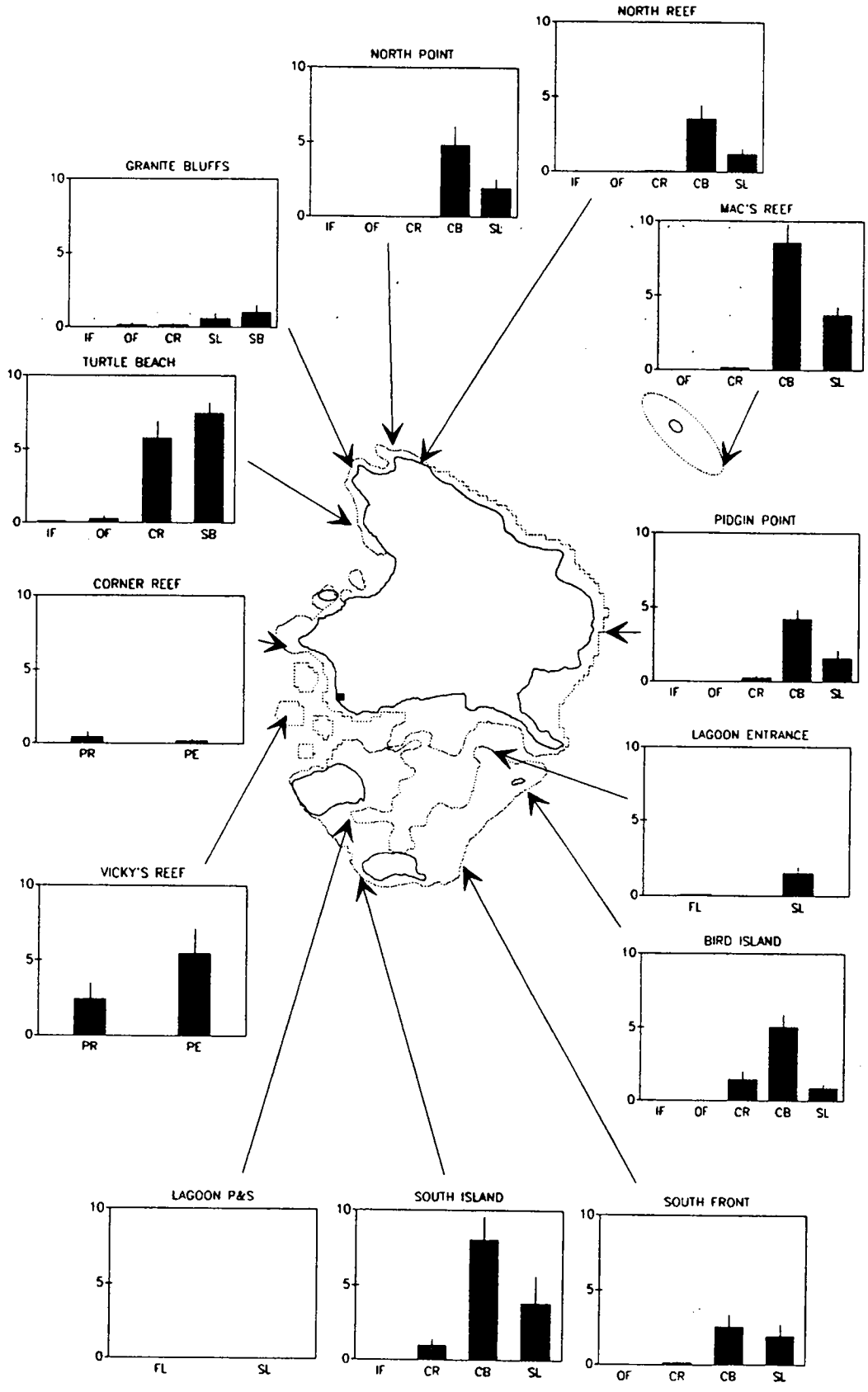

NORTH REEF
NORTH POINT
GRANITE BLUFFS
MAC'S REEF
TURTLE BEACH
PIDGIN POINT
CORNER REEF
LAGOON ENTRANCE
VICKY'S REEF
BIRD ISLAND
LAGOON P&S
SOUTH ISLAND
SOUTH FRONT
IF OF CR CB SL
IF OF CR SL SB
OF CR CB SL
IF OF CR SB
PR PE
FL SL
0 5 10

**Fig. 2.14 Distribution of adult *Ctenochaetus striatus* at Lizard Island.**

Mean number of individuals per transect (n=16) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CR = crest, CB = crest base, SL = slope, SB = slope base, PR = patch reef, PE = patch edge, FL = flat.

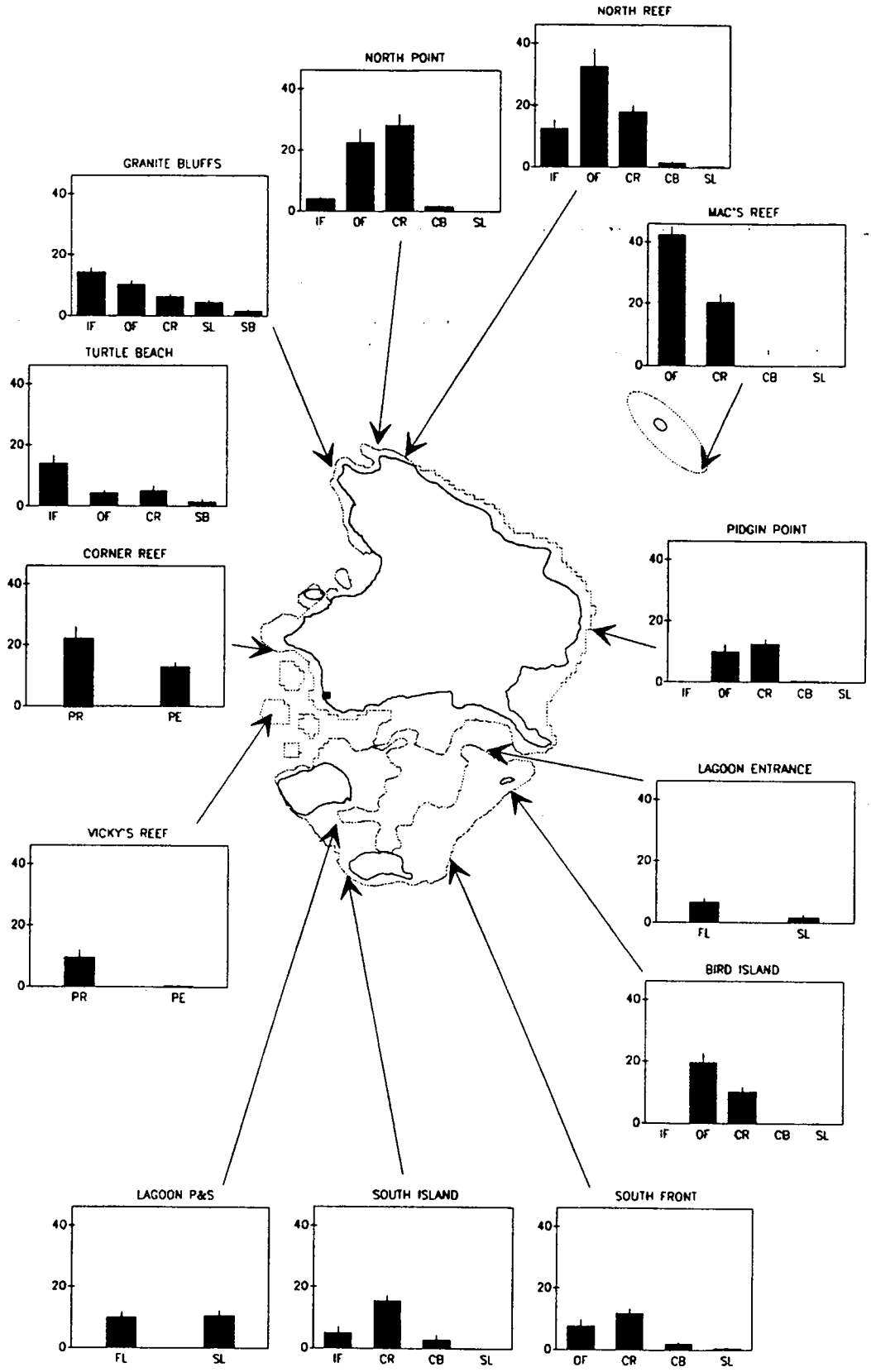

NORTH REEF
NORTH POINT
GRANITE BLUFFS
MAC'S REEF
TURTLE BEACH
CORNER REEF
PIDGIN POINT
LAGOON ENTRANCE
VICKY'S REEF
BIRD ISLAND
LAGOON P&S
SOUTH ISLAND
SOUTH FRONT
IF OF CR CB SL
IF OF CR SL SB
OF CR CB SL
IF OF CR SB
PR PE
FL SL
0 20 40

**Fig. 2.15 Distribution of adult *Naso annulatus* at Lizard Island.**

Mean number of individuals per transect (n=16) ± standard error.
Abbreviations: IF = inner flat, OF = outer flat, CR = crest, CB = crest base, SL = slope, SB = slope base, PR = patch reef, PE = patch edge, FL = flat.

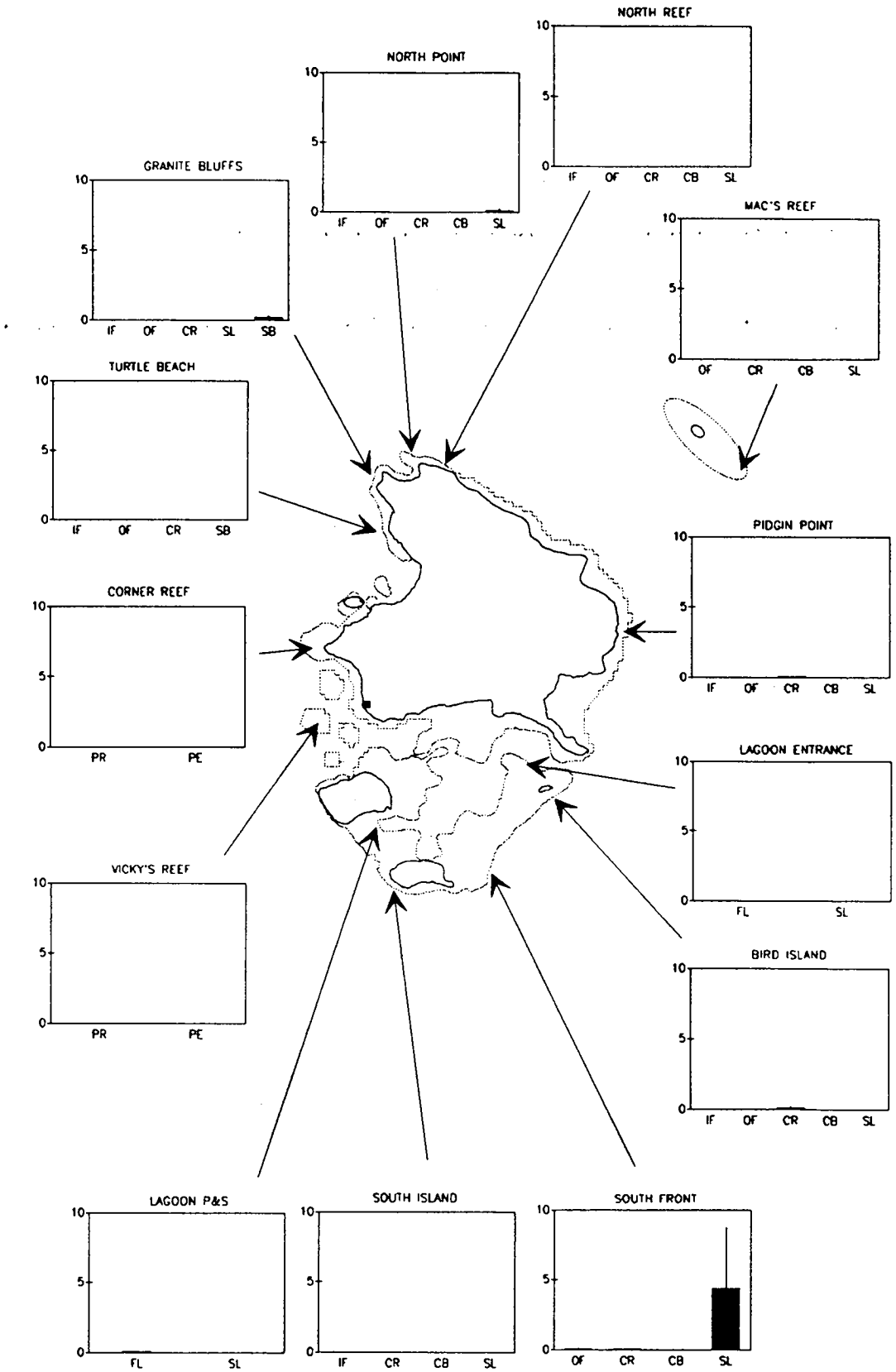

NORTH REEF
NORTH POINT
GRANITE BLUFFS
MAC'S REEF
TURTLE BEACH
PIDGIN POINT
CORNER REEF
LAGOON ENTRANCE
VICKY'S REEF
BIRD ISLAND
LAGOON P&S
SOUTH ISLAND
SOUTH FRONT
IF OF CR CB SL
IF OF CR SL SB
OF CR CB SL
IF OF CR SB
PR PE
FL SL
IF CR CB SL
OF CR CB SL
0 5 10

**Fig. 2.16 Distribution of adult *Naso brevirostris* at Lizard Island.**

Mean number of individuals per transect (n=16) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CR = crest, CB = crest base, SL = slope, SB = slope base, PR = patch reef, PE = patch edge, FL = flat.

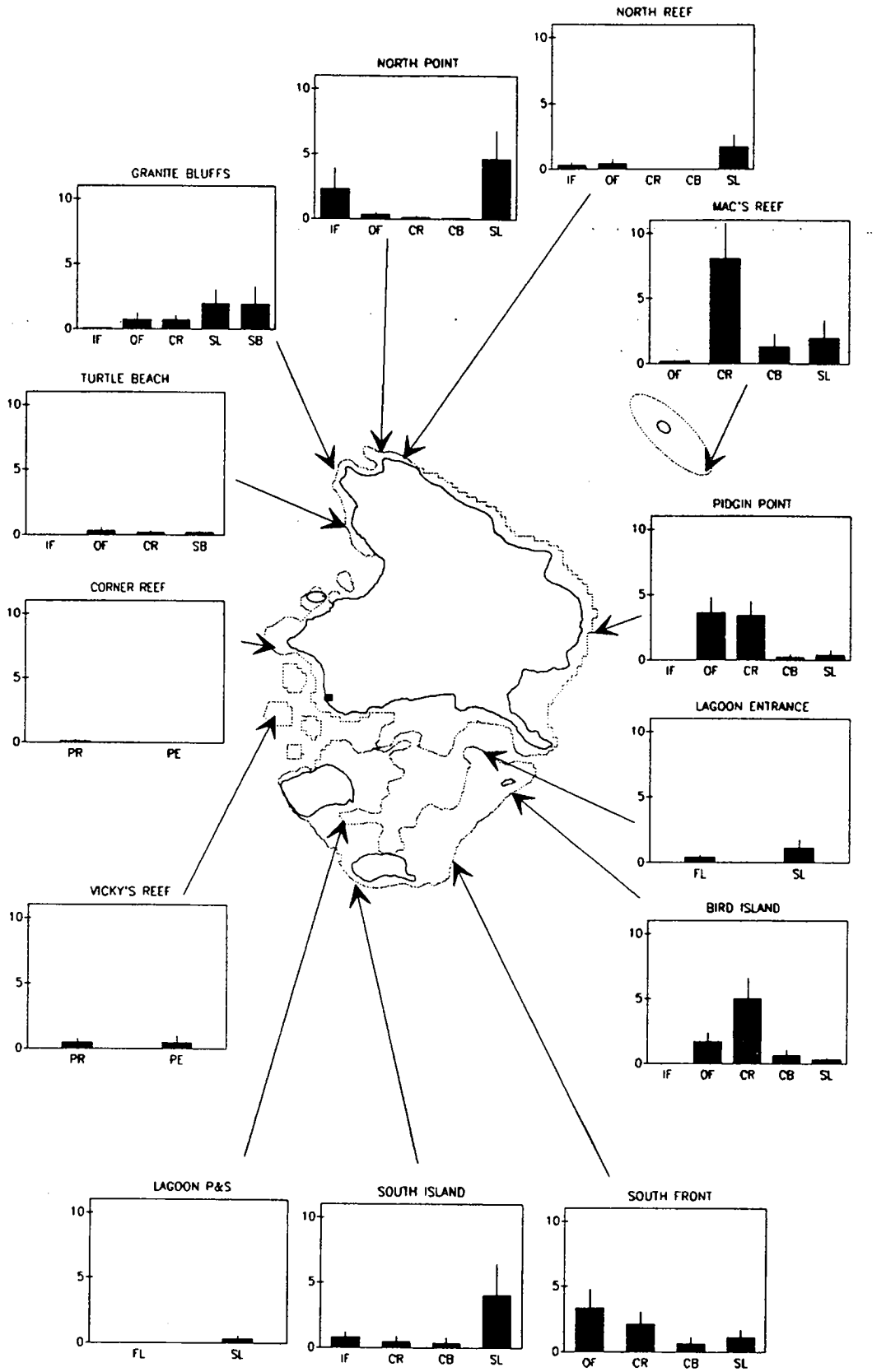

NORTH REEF
NORTH POINT
GRANITE BLUFFS
MAC'S REEF
TURTLE BEACH
PIDGIN POINT
CORNER REEF
LAGOON ENTRANCE
VICKY'S REEF
BIRD ISLAND
LAGOON P&S
SOUTH ISLAND
SOUTH FRONT
IF OF CR CB SL
IF OF CR SL SB
OF CR CB SL
IF OF CR SB
PR PE
FL SL
IF CR CB SL
OF CR CB SL

**Fig. 2.17 Distribution of adult *Naso hexacanthus* at Lizard Island.**

Mean number of individuals per transect (n=16) ± standard error.
Abbreviations: IF = inner flat, OF = outer flat, CR = crest, CB = crest base, SL = slope, SB = slope base, PR = patch reef, PE = patch edge, FL = flat.

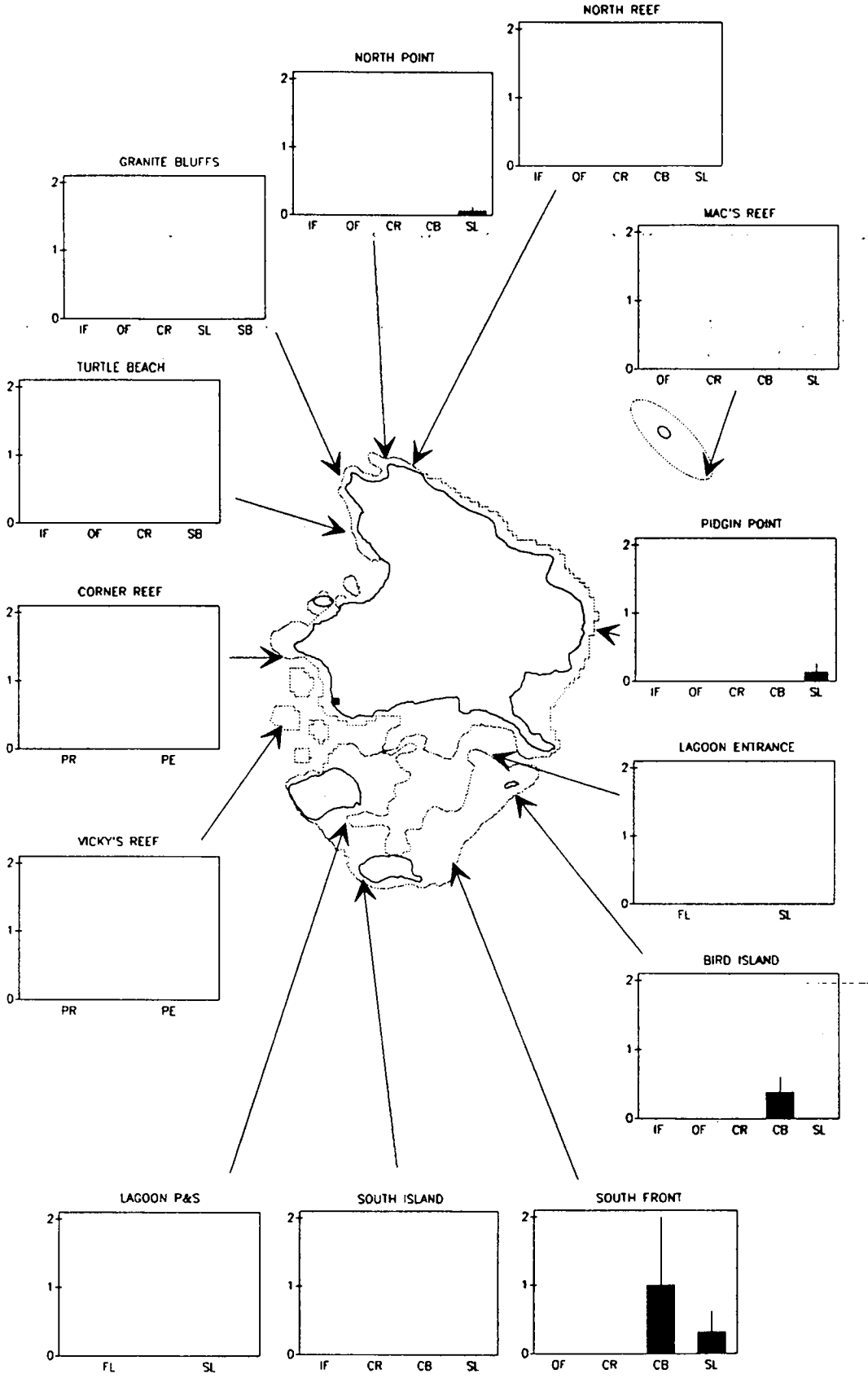

NORTH REEF
NORTH POINT
GRANITE BLUFFS
MAC'S REEF
TURTLE BEACH
PIDGIN POINT
CORNER REEF
LAGOON ENTRANCE
VICKY'S REEF
BIRD ISLAND
LAGOON P&S
SOUTH ISLAND
SOUTH FRONT
IF OF CR CB SL
IF OF CR SL SB
OF CR CB SL
IF OF CR SB
PR PE
FL SL
IF CR CB SL
OF CR CB SL

**Fig. 2.18 Distribution of adult *Naso lituratus* at Lizard Island.**

Mean number of individuals per transect (n=16) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CR = crest, CB = crest base, SL = slope, SB = slope base, PR = patch reef, PE = patch edge, FL = flat.

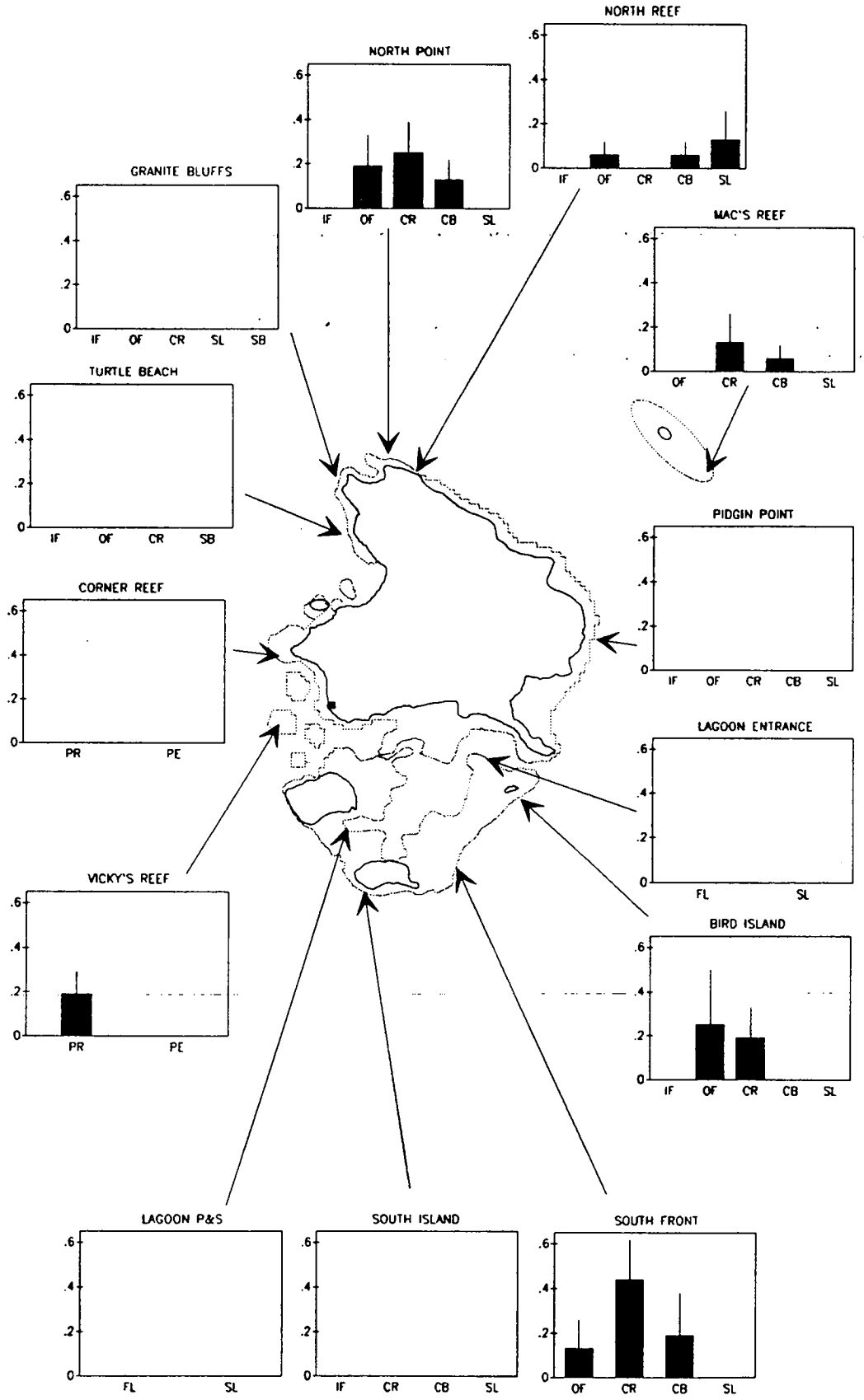

NORTH REEF
NORTH POINT
GRANITE BLUFFS
MAC'S REEF
TURTLE BEACH
PIDGIN POINT
CORNER REEF
LAGOON ENTRANCE
VICKY'S REEF
BIRD ISLAND
LAGOON P&S
SOUTH ISLAND
SOUTH FRONT
IF OF CR CB SL
IF OF CR SL SB
OF CR CB SL
IF OF CR SB
PR PE
FL SL
0 .2 .4 .6

**Fig. 2.19 Distribution of adult *Naso tuberosus* at Lizard Island.**

Mean number of individuals per transect (n=16) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CR = crest, CB = crest base, SL = slope, SB = slope base, PR = patch reef, PE = patch edge, FL = flat.

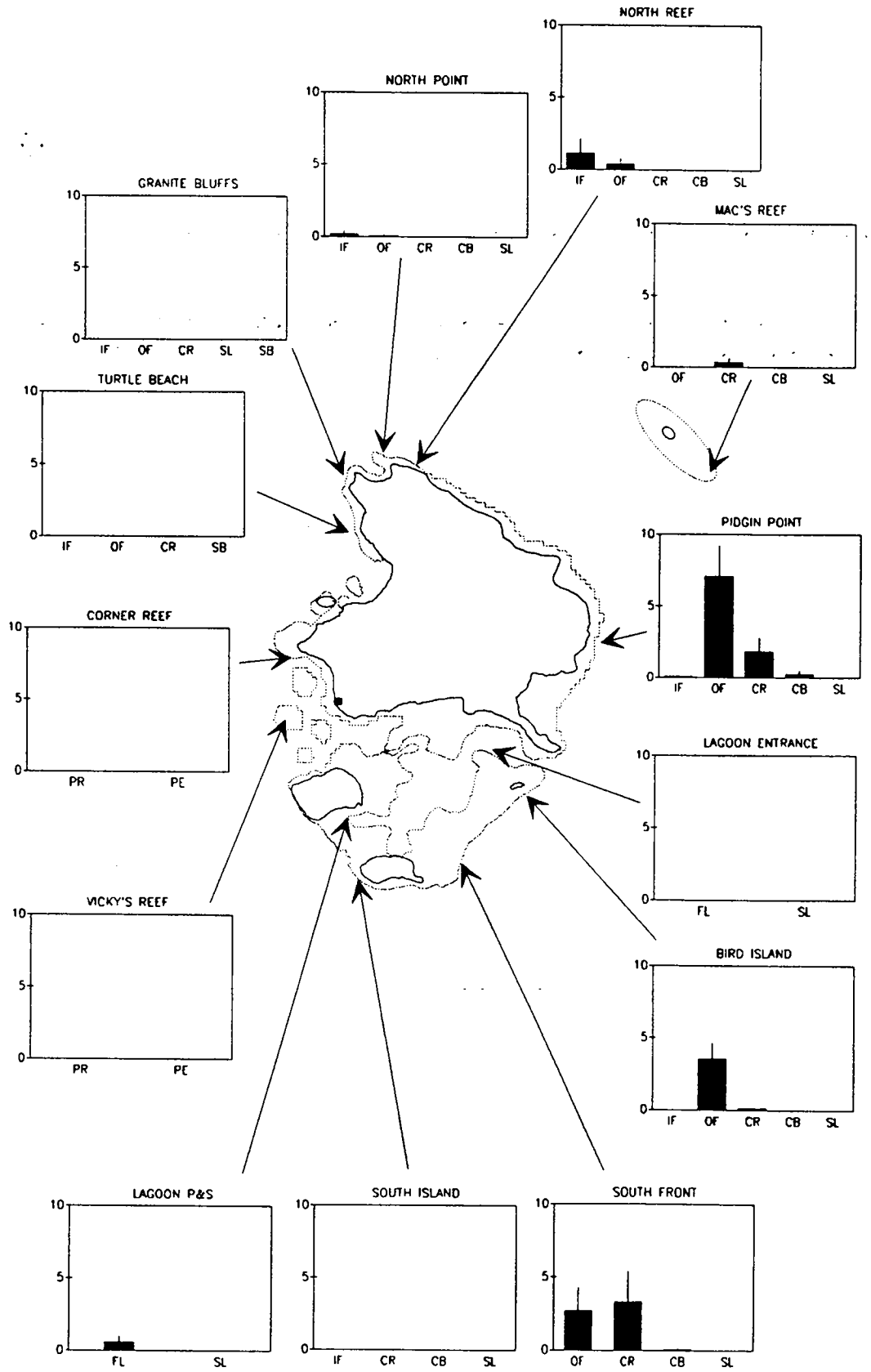

NORTH REEF
NORTH POINT
GRANITE BLUFFS
MAC'S REEF
TURTLE BEACH
PIDGIN POINT
CORNER REEF
LAGOON ENTRANCE
VICKY'S REEF
BIRD ISLAND
LAGOON P&S
SOUTH ISLAND
SOUTH FRONT
IF OF CR CB SL
IF OF CR SL SB
OF CR CB SL
IF OF CR SB
PR PE
FL SL
IF CR CB SL
OF CR CB SL
0 5 10

**Fig. 2.20 Distribution of adult *Naso unicornis* at Lizard Island.**

Mean number of individuals per transect (n=16) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CR = crest, CB = crest base, SL = slope, SB = slope base, PR = patch reef, PE = patch edge, FL = flat.

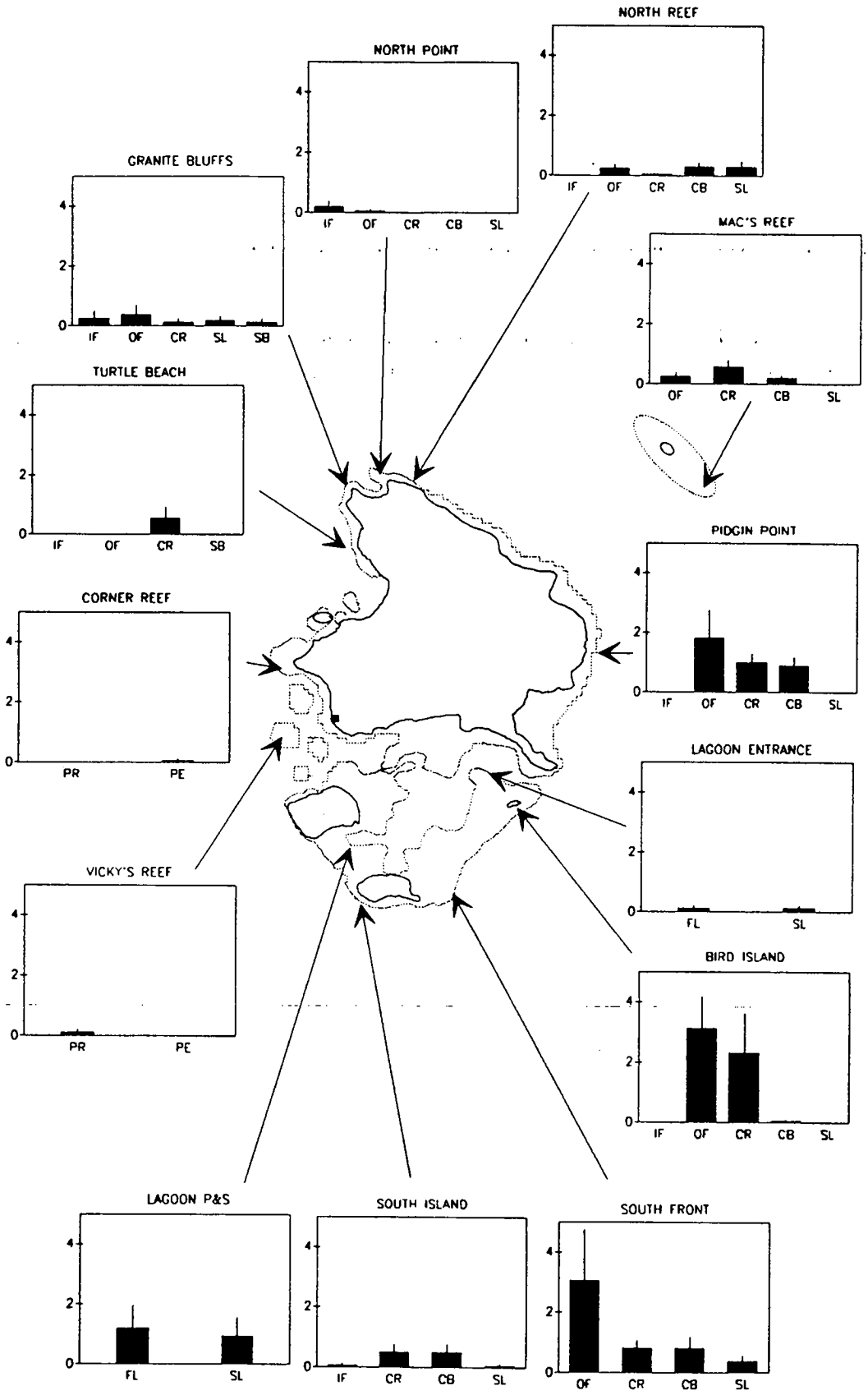

NORTH REEF
IF OF CR CB SL
NORTH POINT
IF OF CR CB SL
GRANITE BLUFFS
IF OF CR SL SB
MAC'S REEF
OF CR CB SL
TURTLE BEACH
IF OF CR SB
PIDGIN POINT
IF OF CR CB SL
CORNER REEF
PR PE
LAGOON ENTRANCE
FL SL
VICKY'S REEF
PR PE
BIRD ISLAND
IF OF CR CB SL
LAGOON P&S
FL SL
SOUTH ISLAND
IF CR CB SL
SOUTH FRONT
OF CR CB SL

**Fig. 2.21 Distribution of adult *Naso vlamingii* at Lizard Island.**

Mean number of individuals per transect (n=16) ± standard error.
Abbreviations: IF = inner flat, OF = outer flat, CR = crest, CB = crest base, SL = slope, SB = slope base, PR = patch reef, PE = patch edge, FL = flat.

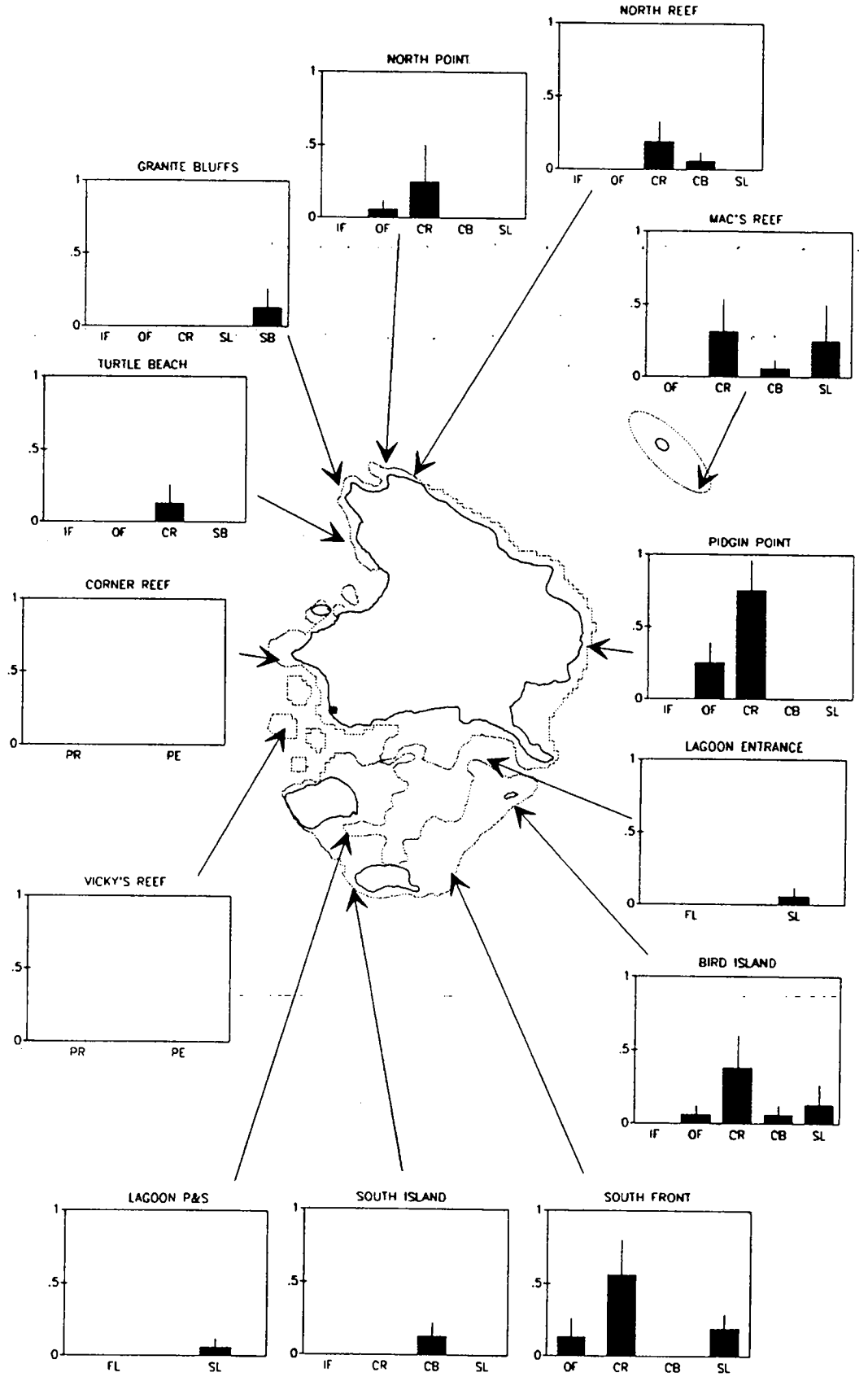

NORTH REEF
1
.5
0
IF OF CR CB SL
NORTH POINT
1
.5
0
IF OF CR CB SL
GRANITE BLUFFS
1
.5
0
IF OF CR SL SB
MAC'S REEF
1
.5
0
OF CR CB SL
TURTLE BEACH
1
.5
0
IF OF CR SB
PIDGIN POINT
1
.5
0
IF OF CR CB SL
CORNER REEF
1
.5
0
PR PE
LAGOON ENTRANCE
1
.5
0
FL SL
VICKY'S REEF
1
.5
0
PR PE
BIRD ISLAND
1
.5
0
IF OF CR CB SL
LAGOON P&S
1
.5
0
FL SL
SOUTH ISLAND
1
.5
0
IF CR CB SL
SOUTH FRONT
1
.5
0
OF CR CB SL

**Fig. 2.22 Distribution of adult *Zebrasoma scopas* at Lizard Island.**

Mean number of individuals per transect (n=16) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CR = crest, CB = crest base, SL = slope, SB = slope base, PR = patch reef, PE = patch edge, FL = flat.

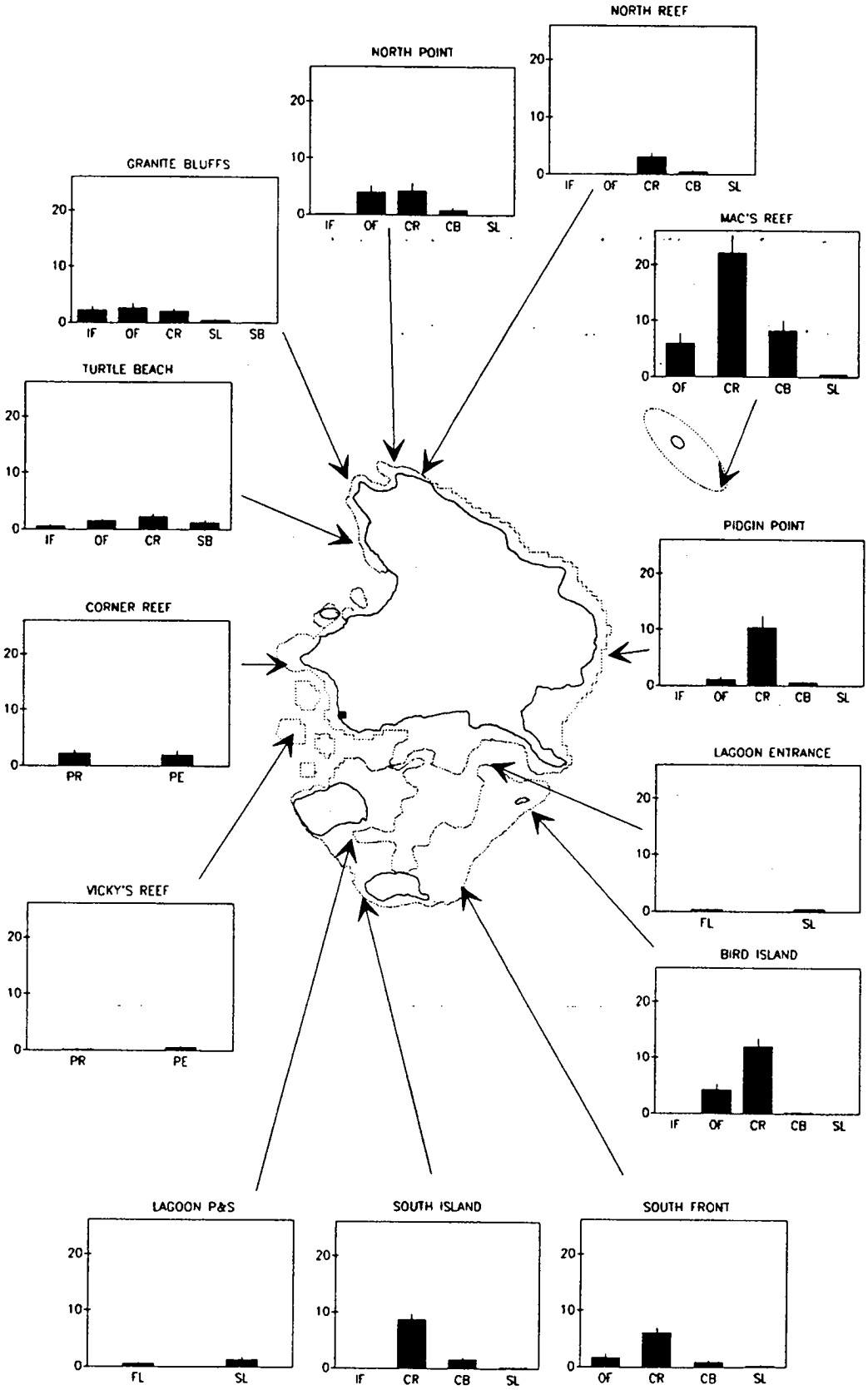

NORTH REEF
NORTH POINT
GRANITE BLUFFS
MAC'S REEF
TURTLE BEACH
PIDGIN POINT
CORNER REEF
LAGOON ENTRANCE
VICKY'S REEF
BIRD ISLAND
LAGOON P&S
SOUTH ISLAND
SOUTH FRONT
IF OF CR CB SL SB
PR PE
FL SL

**Fig. 2.23 Distribution of adult *Zebrasoma veliferum* at Lizard Island.**

Mean number of individuals per transect (n=16) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CR = crest, CB = crest base, SL = slope, SB = slope base, PR = patch reef, PE = patch edge, FL = flat.

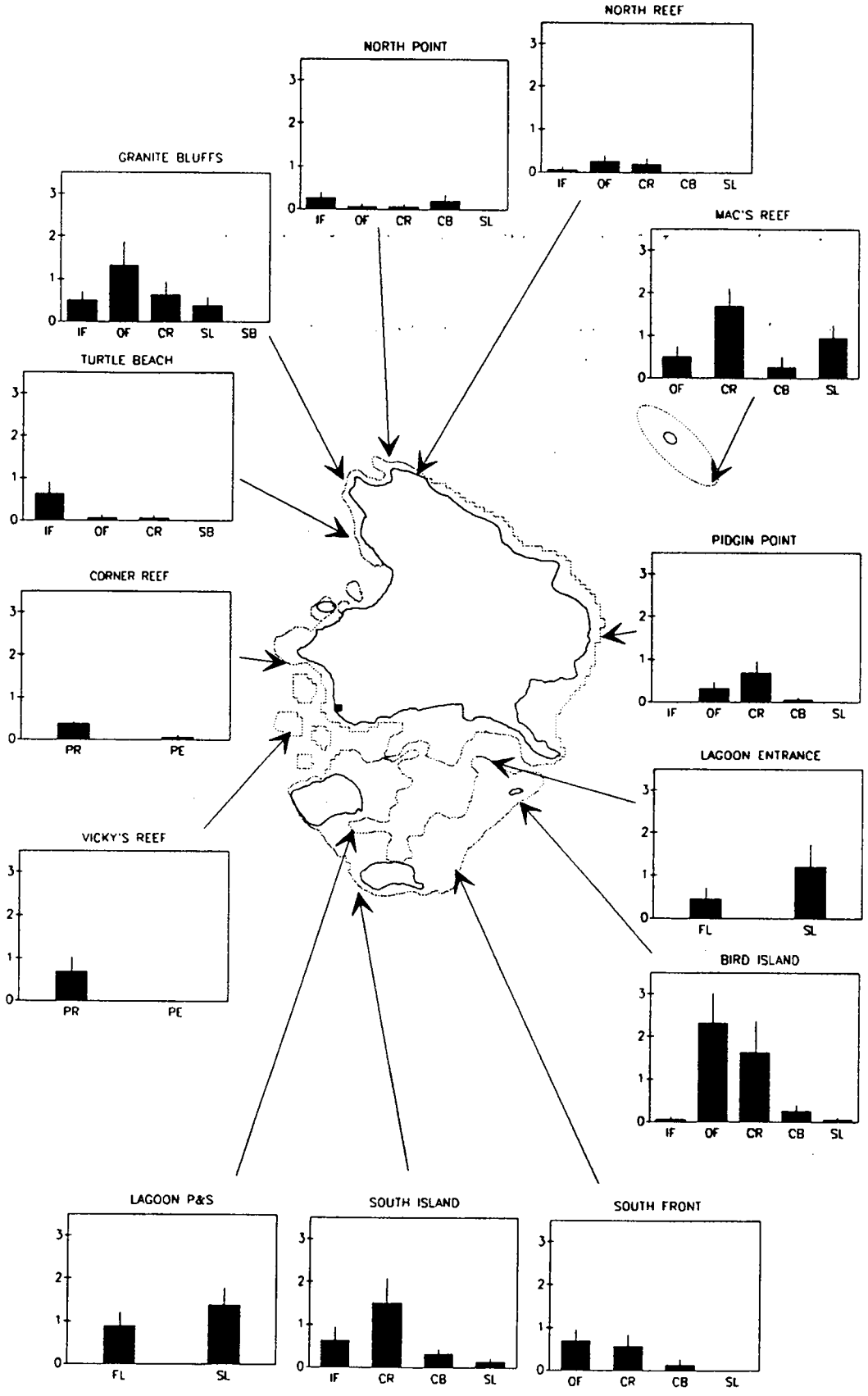

NORTH REEF
NORTH POINT
GRANITE BLUFFS
MAC'S REEF
TURTLE BEACH
PIDGIN POINT
CORNER REEF
LAGOON ENTRANCE
VICKY'S REEF
BIRD ISLAND
LAGOON P&S
SOUTH ISLAND
SOUTH FRONT
IF OF CR CB SL
IF OF CR SL SB
OF CR CB SL
IF OF CR SB
PR PE
FL SL

**Fig. 2.24 Distribution of juvenile *Acanthurus lineatus* at Lizard Island, 21/12/89.**

Mean number of individuals per transect (n=4) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CB = crest base, SL = slope.

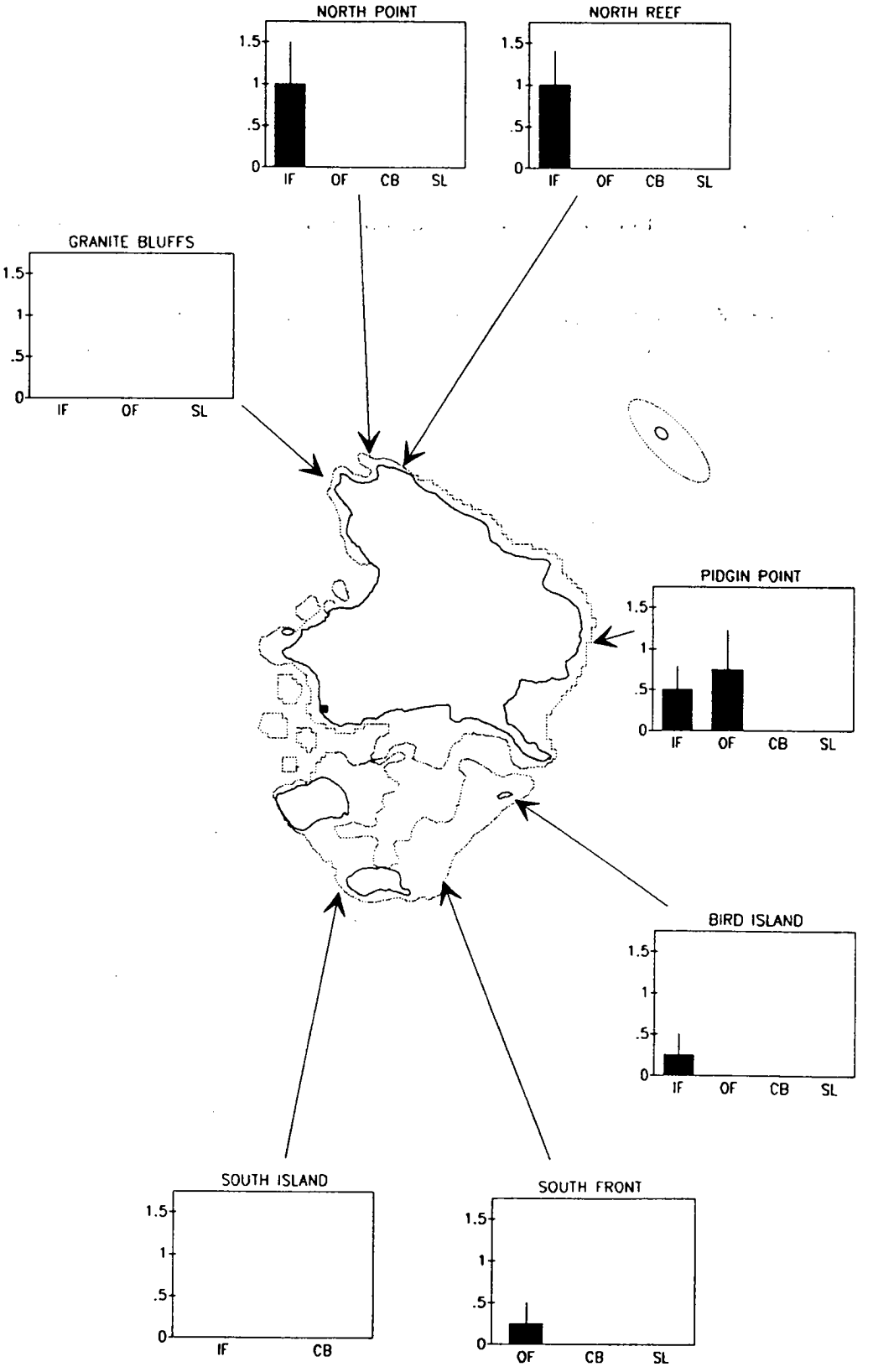

NORTH POINT
1.5
1
.5
0
IF
OF
CB
SL
NORTH REEF
1.5
1
.5
0
IF
OF
CB
SL
GRANITE BLUFFS
1.5
1
.5
0
IF
OF
SL
PIDGIN POINT
1.5
1
.5
0
IF
OF
CB
SL
BIRD ISLAND
1.5
1
.5
0
IF
OF
CB
SL
SOUTH ISLAND
1.5
1
.5
0
IF
CB
SOUTH FRONT
1.5
1
.5
0
OF
CB
SL

**Fig. 2.25 Distribution of juvenile *Acanthurus nigrofuscus* at Lizard Island, 20/1/88.**

Mean number of individuals per transect (n=4) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CR = crest, CB = crest base, SL = slope, SB = slope base, PR = patch reef, PE = patch edge, FL = flat.

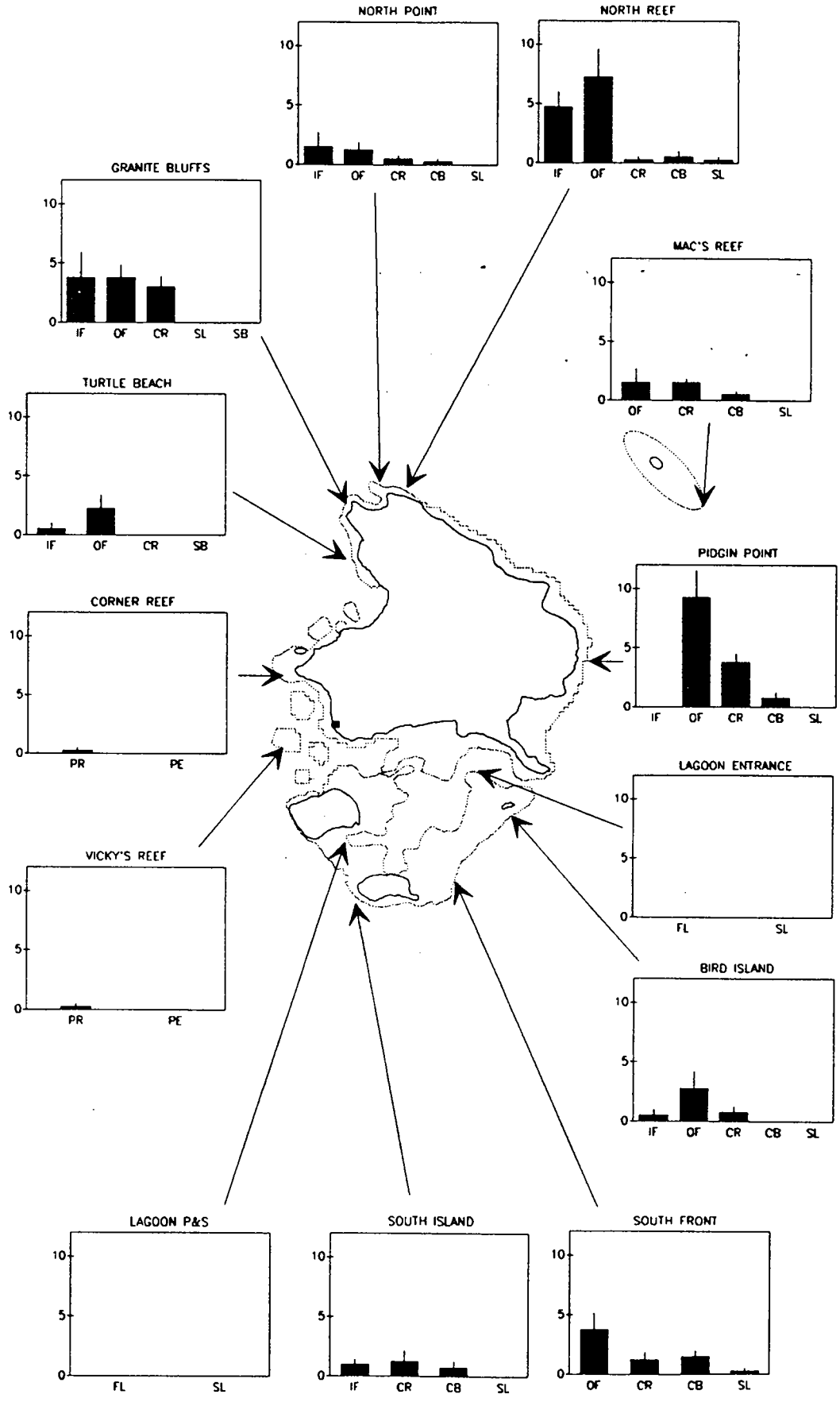

NORTH POINT
10
5
0
IF OF CR CB SL
NORTH REEF
10
5
0
IF OF CR CB SL
GRANITE BLUFFS
10
5
0
IF OF CR SL SB
MAC'S REEF
10
5
0
OF CR CB SL
TURTLE BEACH
10
5
0
IF OF CR SB
PIDGIN POINT
10
5
0
IF OF CR CB SL
CORNER REEF
10
5
0
PR PE
LAGOON ENTRANCE
10
5
0
FL SL
VICKY'S REEF
10
5
0
PR PE
BIRD ISLAND
10
5
0
IF OF CR CB SL
LAGOON P&S
10
5
0
FL SL
SOUTH ISLAND
10
5
0
IF CR CB SL
SOUTH FRONT
10
5
0
OF CR CB SL

**Fig. 2.26 Distribution of juvenile *Acanthurus nigrofuscus* at Lizard Island, 26/1/89.**

Mean number of individuals per transect (n=4) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CB = crest base, SL = slope.

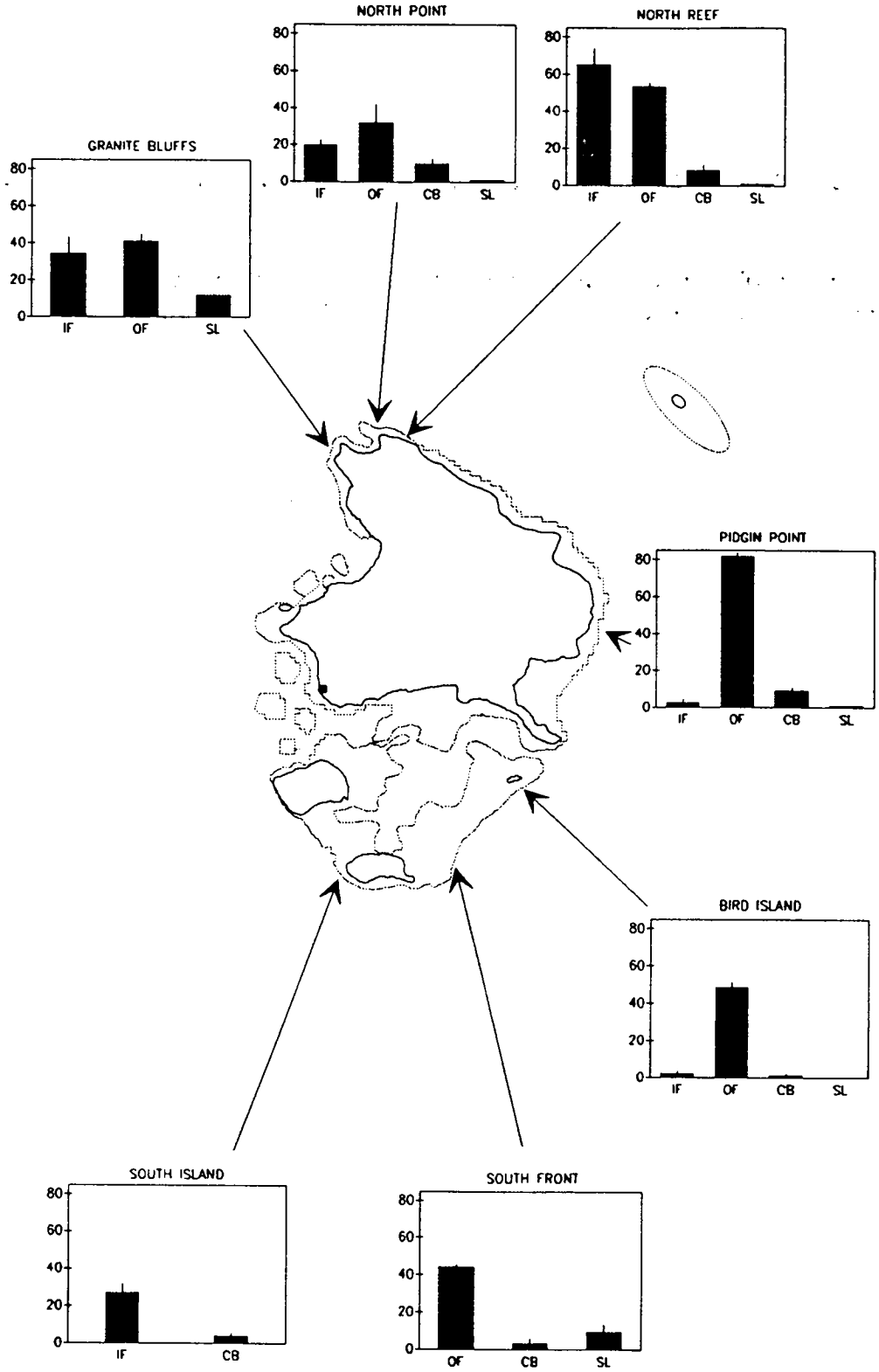

NORTH POINT
80
60
40
20
0
IF
OF
CB
SL
NORTH REEF
80
60
40
20
0
IF
OF
CB
SL
GRANITE BLUFFS
80
60
40
20
0
IF
OF
SL
PIDGIN POINT
80
60
40
20
0
IF
OF
CB
SL
BIRD ISLAND
80
60
40
20
0
IF
OF
CB
SL
SOUTH ISLAND
80
60
40
20
0
IF
CB
SOUTH FRONT
80
60
40
20
0
OF
CB
SL

**Fig. 2.27 Distribution of juvenile *Acanthurus nigrofuscus* at Lizard Island, 4/2/90.**

Mean number of individuals per transect (n=4) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CB = crest base, SL = slope.

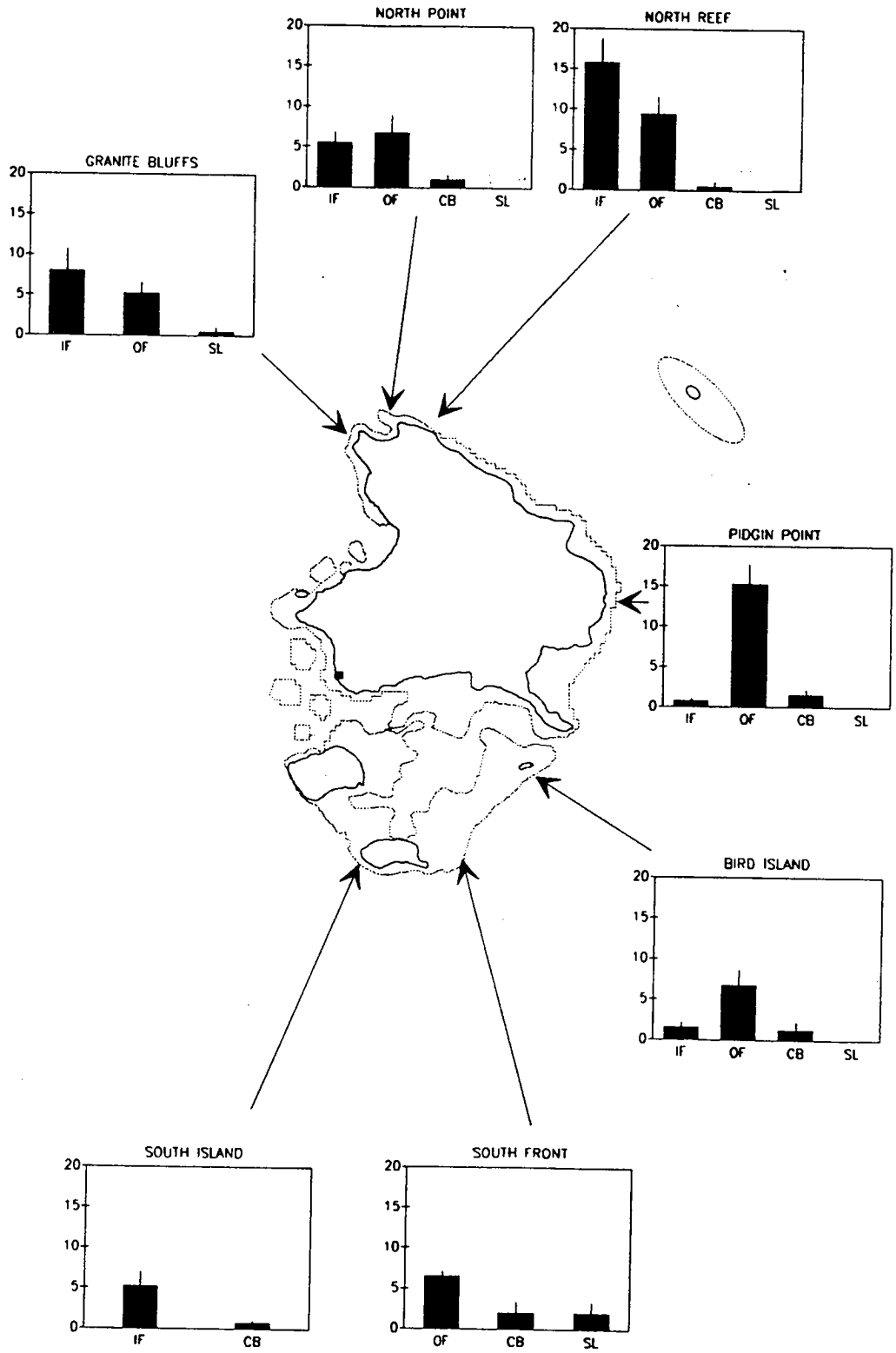
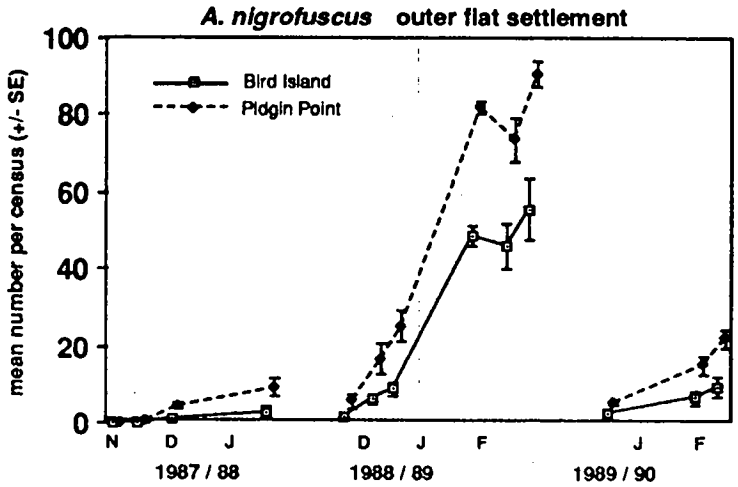

NORTH POINT
NORTH REEF
GRANITE BLUFFS
PIDGIN POINT
BIRD ISLAND
SOUTH ISLAND
SOUTH FRONT
IF
OF
CB
SL
0
5
10
15
20

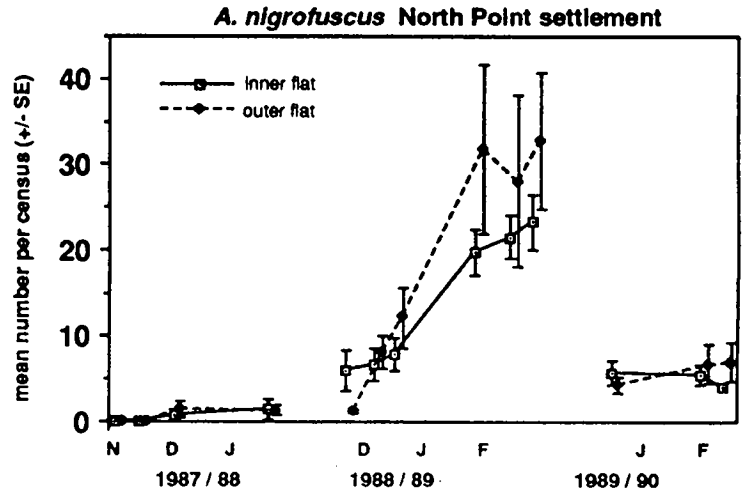
**Fig. 2.28 Density of juvenile *Acanthurus nigrofuscus* in the outer flat habitat at Bird Island and Pidgin Point throughout the study.**
Mean number of individuals per transect (n=4) ± standard error.

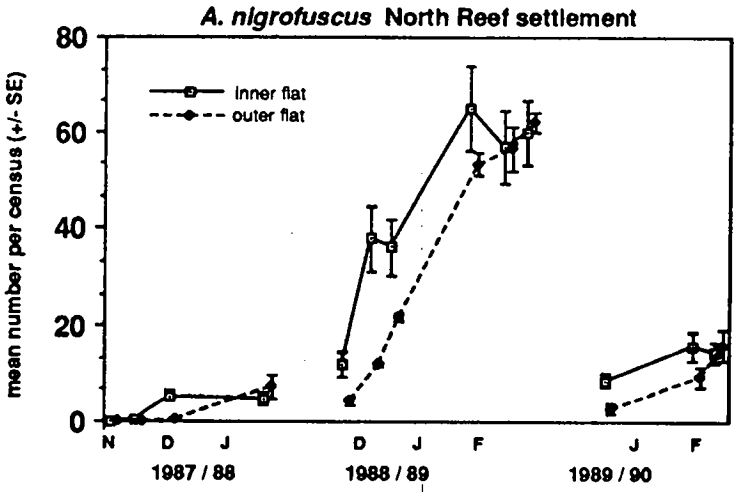
**Fig. 2.29 Density of juvenile *A. nigrofuscus* in the inner and outer flat habitats at North Point throughout the study.**
Mean number of individuals per transect (n=4) ± standard error.

**Fig. 2.30 Density of juvenile *A. nigrofuscus* in the inner and outer flat habitats at North Reef throughout the study.**
Mean number of individuals per transect (n=4) ± standard error.

**Fig. 2.31 Density of juvenile *Zebrasoma scopas* in the crest base habitat at Pidgin Point and South Front throughout the study.**
Mean number of individuals per transect (n=4) ± standard error.


A. nigrofuscus outer flat settlement
mean number per census (+/- SE)
Bird Island
Pidgin Point
100
80
60
40
20
0
N
D
J
D
J
F
J
F
1987 / 88
1988 / 89
1989 / 90


A. nigrofuscus North Point settlement
mean number per census (+/- SE)
Inner flat
outer flat
40
30
20
10
0
N
D
J
D
J
F
J
F
1987 / 88
1988 / 89
1989 / 90


A. nigrofuscus North Reef settlement
mean number per census (+/- SE)
Inner flat
outer flat
80
60
40
20
0
N
D
J
D
J
F
J
F
1987 / 88
1988 / 89
1989 / 90

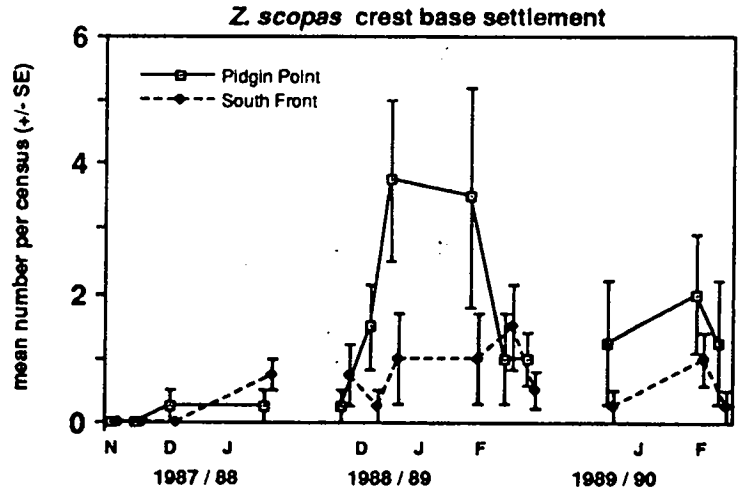

Z. scopas crest base settlement
mean number per census (+/- SE)
Pidgin Point
South Front
6
4
2
0
N
D
J
D
J
F
J
F
1987 / 88
1988 / 89
1989 / 90

**Fig. 2.32 Distribution of juvenile *Acanthurus olivaceus* at Lizard Island, 4/2/90.**

Mean number of individuals per transect (n=4) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CB = crest base, SL = slope.

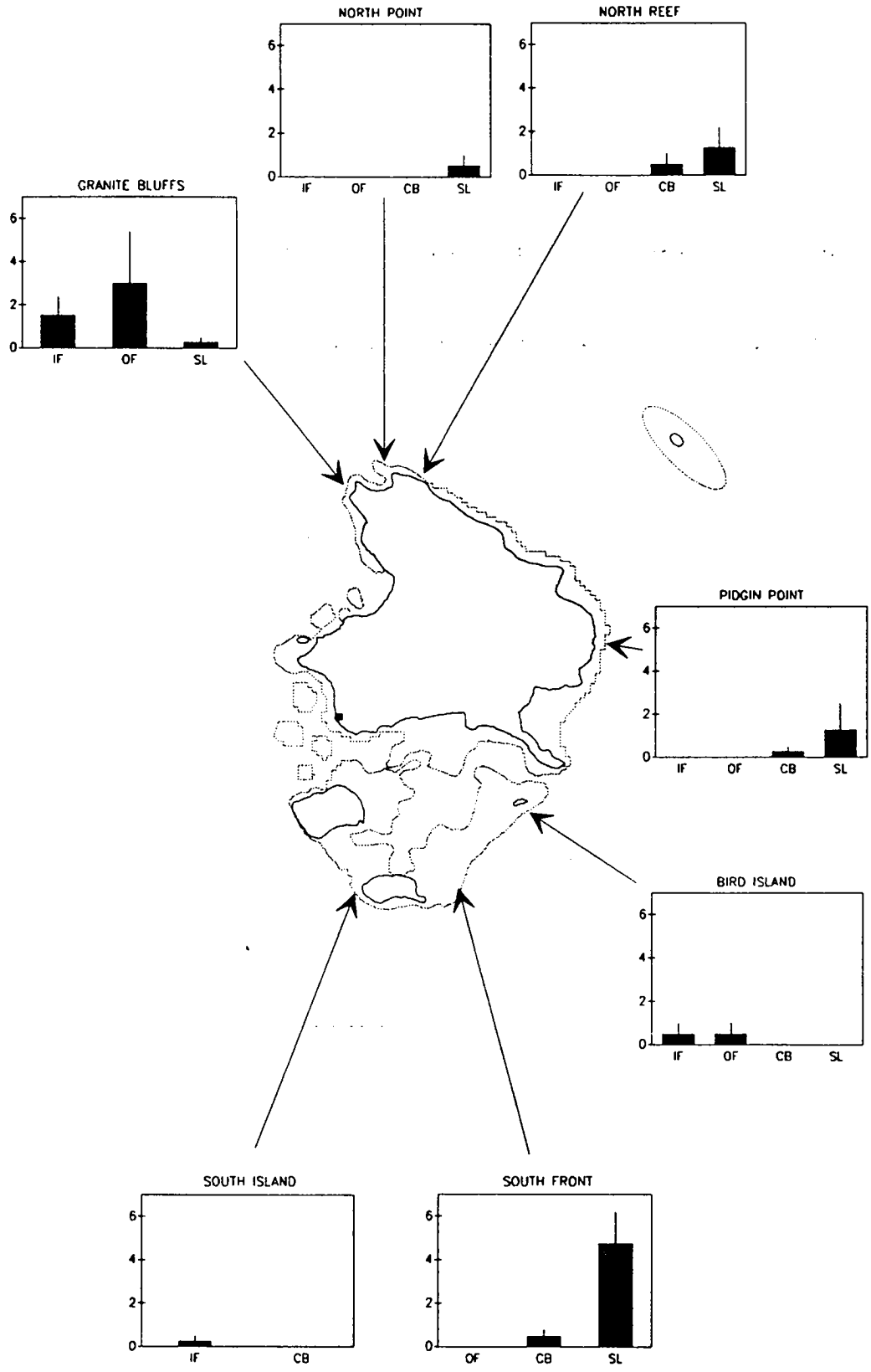

NORTH POINT
NORTH REEF
GRANITE BLUFFS
PIDGIN POINT
BIRD ISLAND
SOUTH ISLAND
SOUTH FRONT
IF
OF
CB
SL
0
2
4
6

**Fig. 2.33 Distribution of juvenile *Acanthurus triostegus* at Lizard Island, 17/12/88.**

Mean number of individuals per transect (n=4) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CB = crest base, SL = slope.

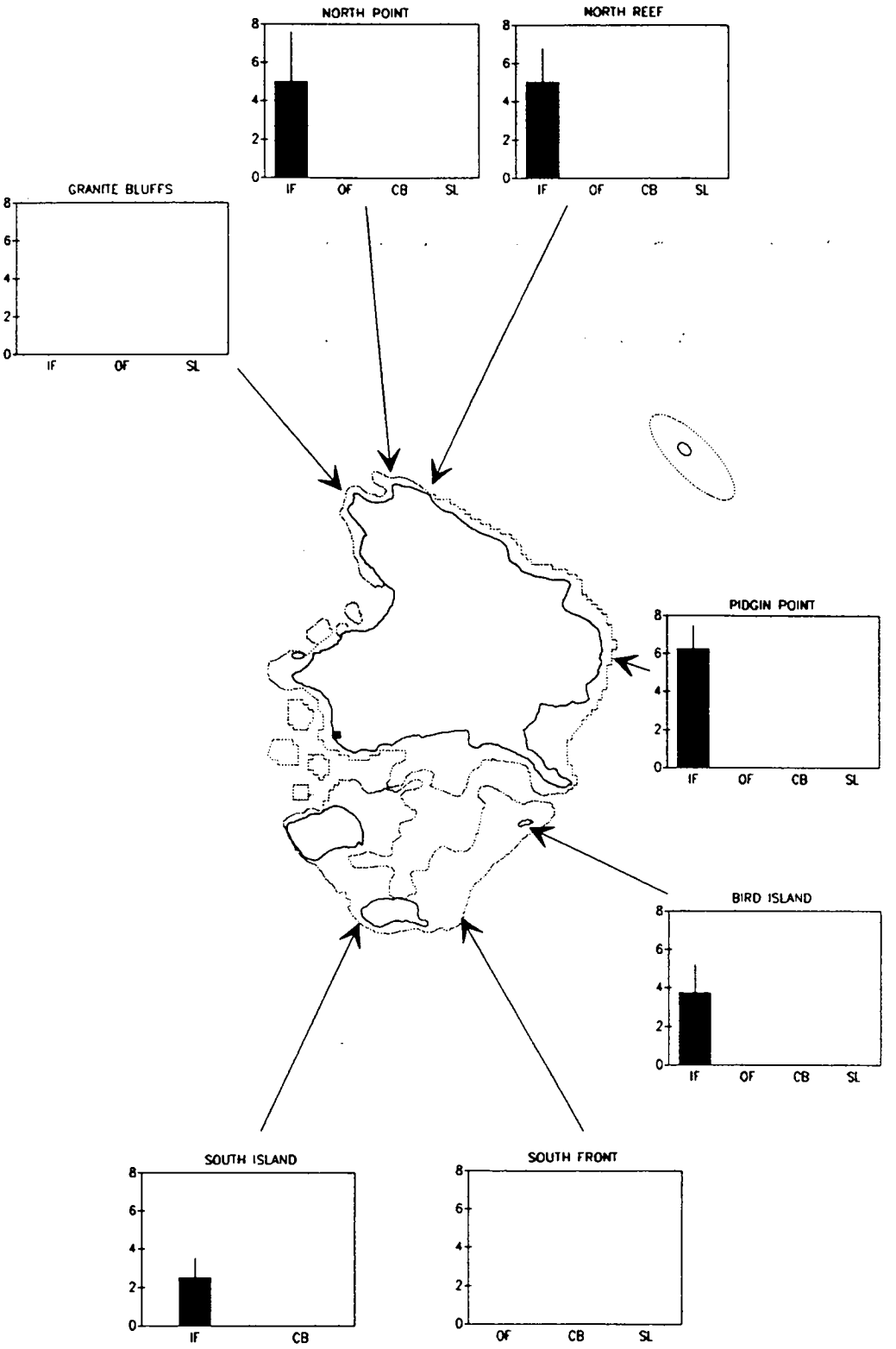
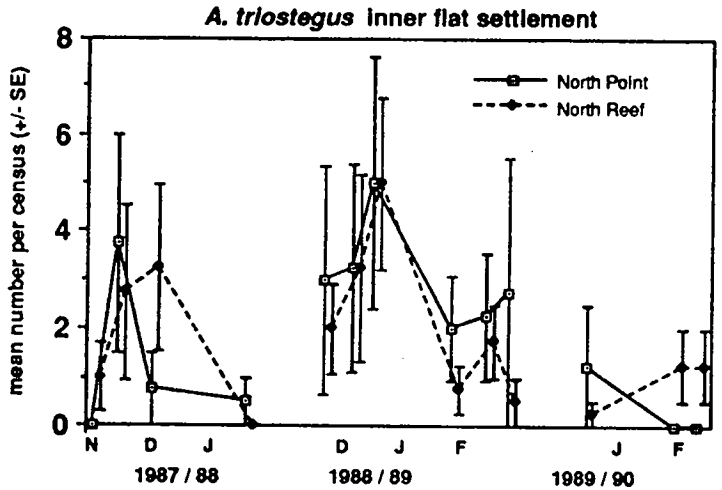

NORTH POINT
NORTH REEF
GRANITE BLUFFS
PIDGIN POINT
BIRD ISLAND
SOUTH ISLAND
SOUTH FRONT
IF
OF
CB
SL
0
2
4
6
8

**Fig. 2.34 Density of juvenile *Acanthurus triostegus* in the inner flat habitat at North Point and North Reef throughout the study.**
Mean number of individuals per transect (n=4) ± standard error.

**Fig. 2.35 Density of juvenile *A. triostegus* in the inner flat habitat at Pidgin Point and South Island throughout the study.**
Mean number of individuals per transect (n=4) ± standard error.


A. triostegus inner flat settlement
mean number per census (+/- SE)
0
2
4
6
8
North Point
North Reef
N
D
J
D
J
F
J
F
1987 / 88
1988 / 89
1989 / 90

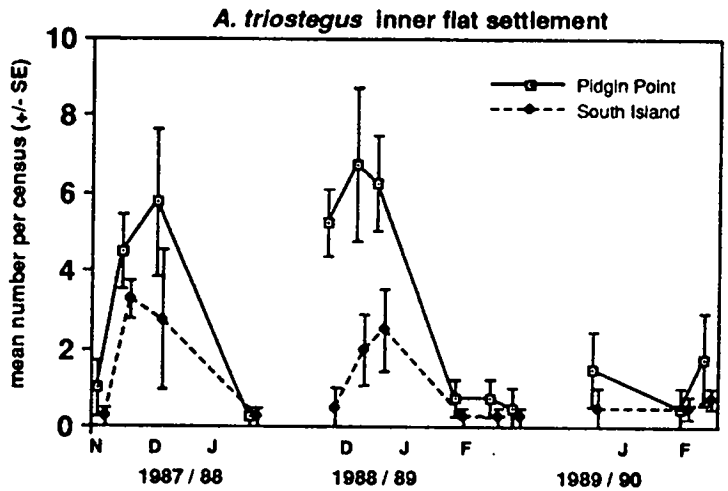

A. triostegus inner flat settlement
mean number per census (+/- SE)
0
2
4
6
8
10
Pidgin Point
South Island
N
D
J
D
J
F
J
F
1987 / 88
1988 / 89
1989 / 90

**Fig. 2.36 Distribution of juvenile *Acanthurus* white-bar spp. at Lizard Island, 21/12/89.**

Mean number of individuals per transect (n=4) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CB = crest base, SL = slope.

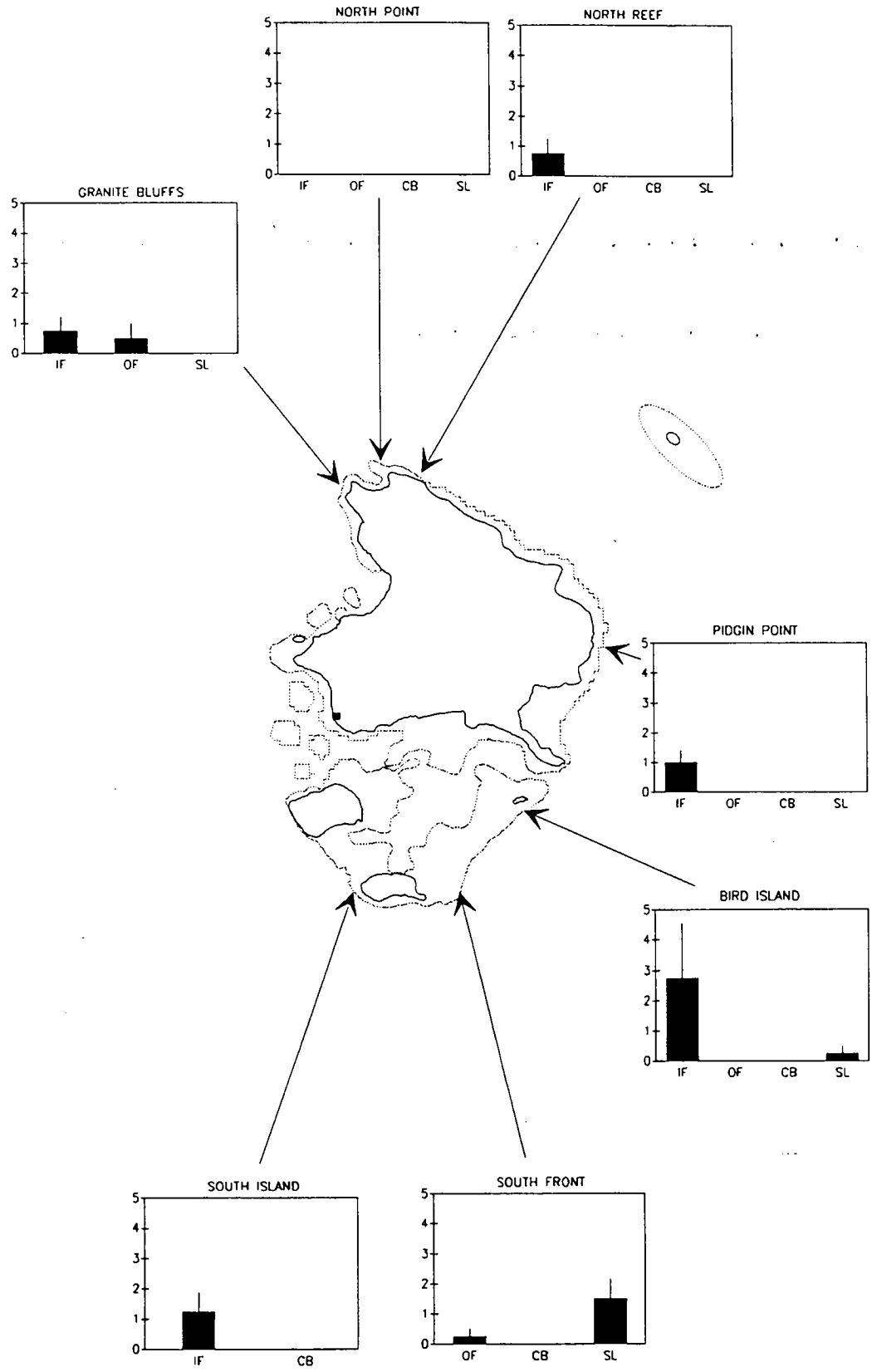

NORTH POINT
NORTH REEF
GRANITE BLUFFS
PIDGIN POINT
BIRD ISLAND
SOUTH ISLAND
SOUTH FRONT
IF
OF
CB
SL

**Fig. 2.37 Distribution of juvenile *Ctenochaetus binotatus* at Lizard Island, 20/1/88.**

Mean number of individuals per transect (n=4) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CR = crest, CB = crest base, SL = slope, SB = slope base, PR = patch reef, PE = patch edge, FL = flat.

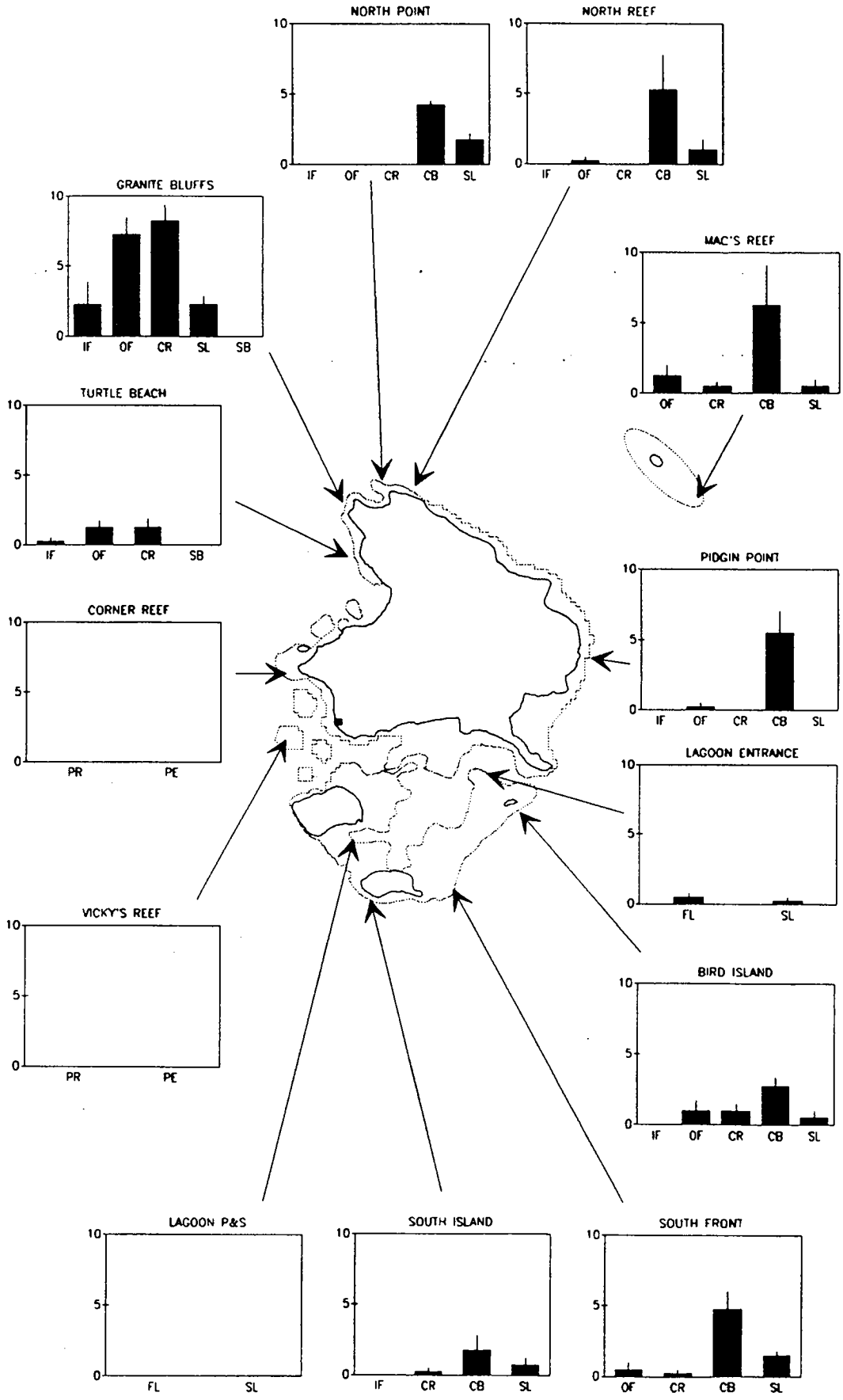

NORTH POINT
10
5
0
IF OF CR CB SL
NORTH REEF
10
5
0
IF OF CR CB SL
GRANITE BLUFFS
10
5
0
IF OF CR SL SB
MAC'S REEF
10
5
0
OF CR CB SL
TURTLE BEACH
10
5
0
IF OF CR SB
PIDGIN POINT
10
5
0
IF OF CR CB SL
CORNER REEF
10
5
0
PR PE
LAGOON ENTRANCE
10
5
0
FL SL
VICKY'S REEF
10
5
0
PR PE
BIRD ISLAND
10
5
0
IF OF CR CB SL
LAGOON P&S
10
5
0
FL SL
SOUTH ISLAND
10
5
0
IF CR CB SL
SOUTH FRONT
10
5
0
OF CR CB SL

**Fig. 2.38 Distribution of juvenile *Ctenochaetus binotatus* at Lizard Island, 26/1/89.**

Mean number of individuals per transect (n=4) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CB = crest base, SL = slope.

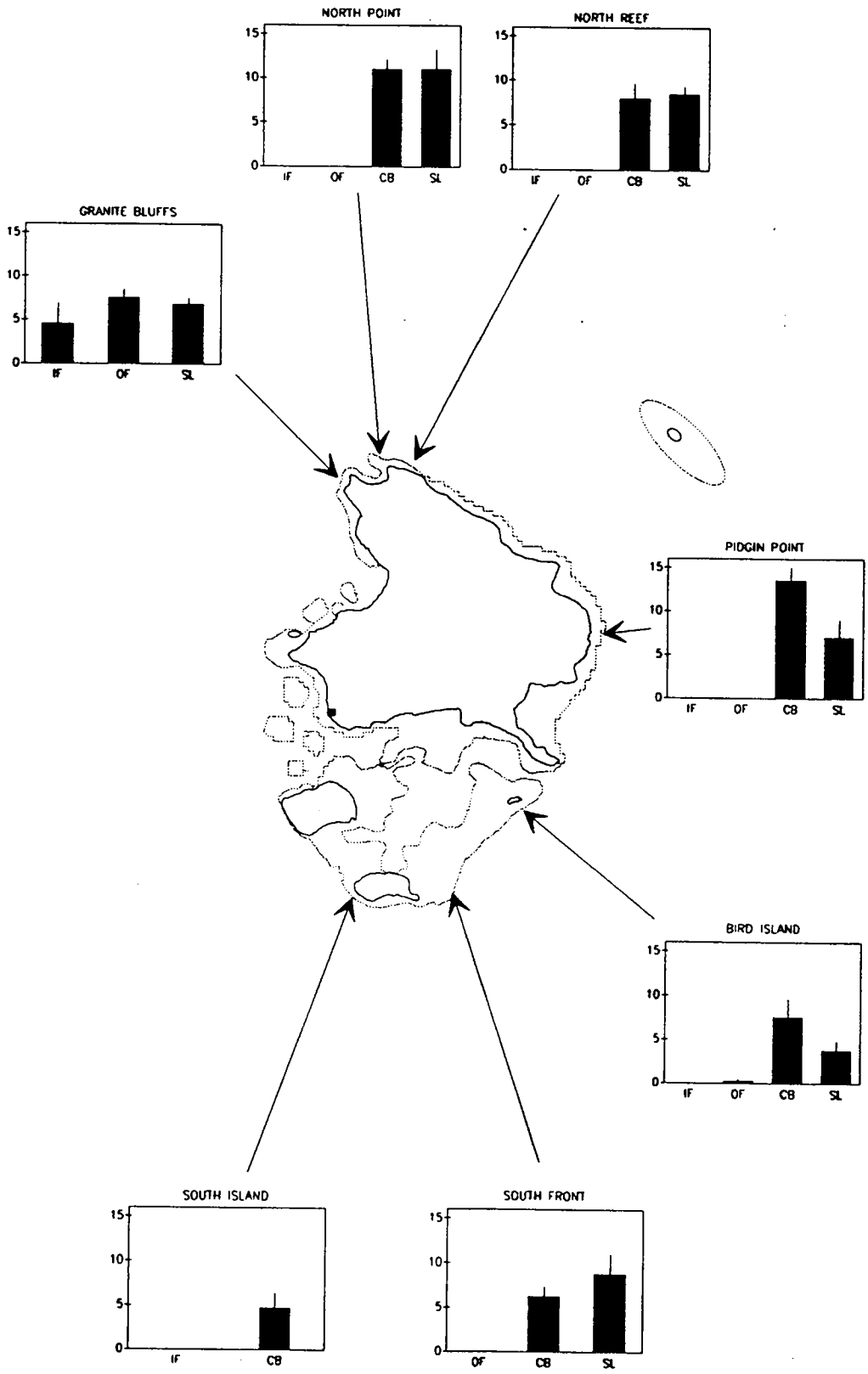

NORTH POINT
NORTH REEF
GRANITE BLUFFS
PIDGIN POINT
BIRD ISLAND
SOUTH ISLAND
SOUTH FRONT
IF
OF
CB
SL
0
5
10
15

**Fig. 2.39 Distribution of juvenile *Ctenochaetus binotatus* at Lizard Island, 4/2/90.**

Mean number of individuals per transect (n=4) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CB = crest base, SL = slope.

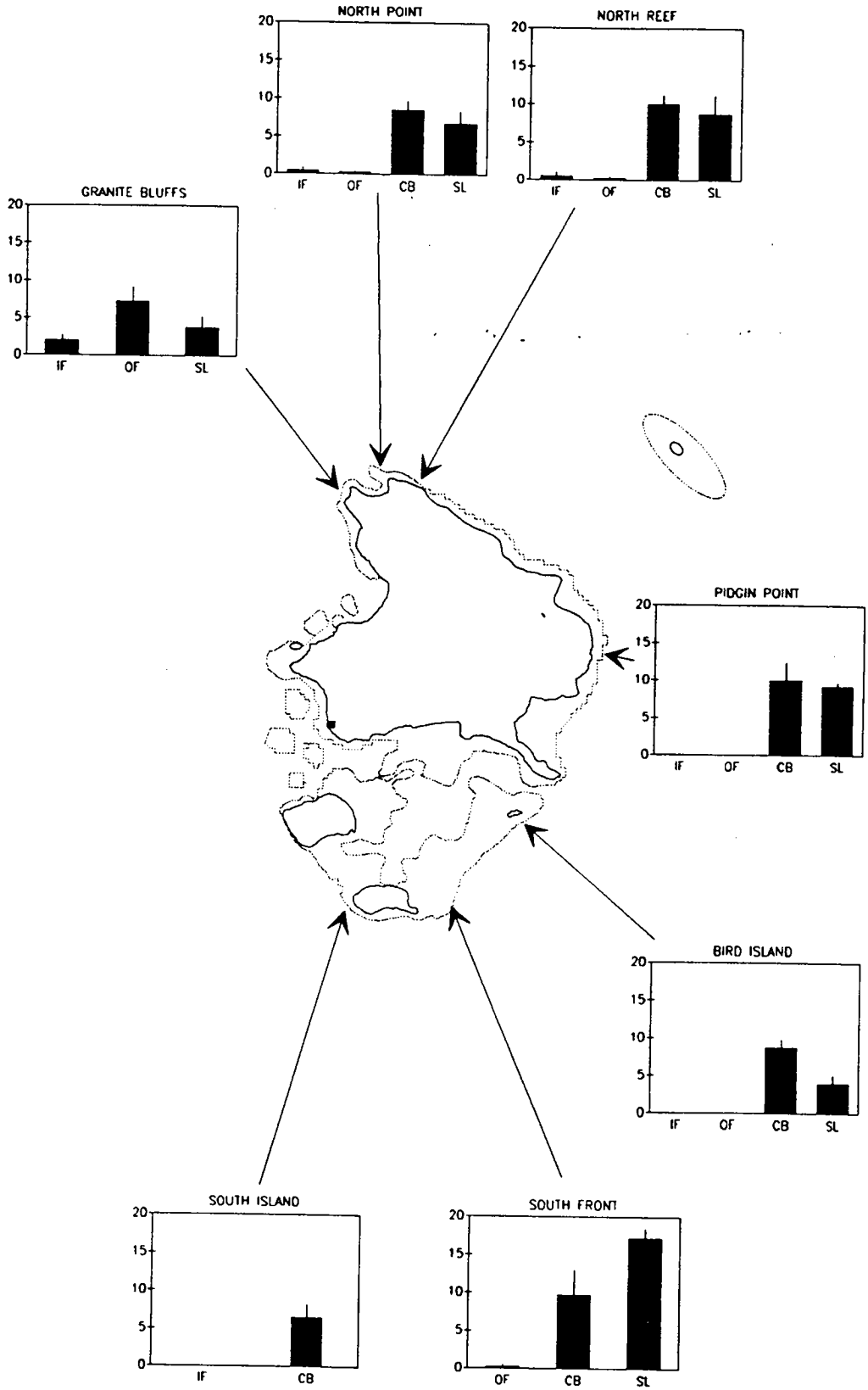
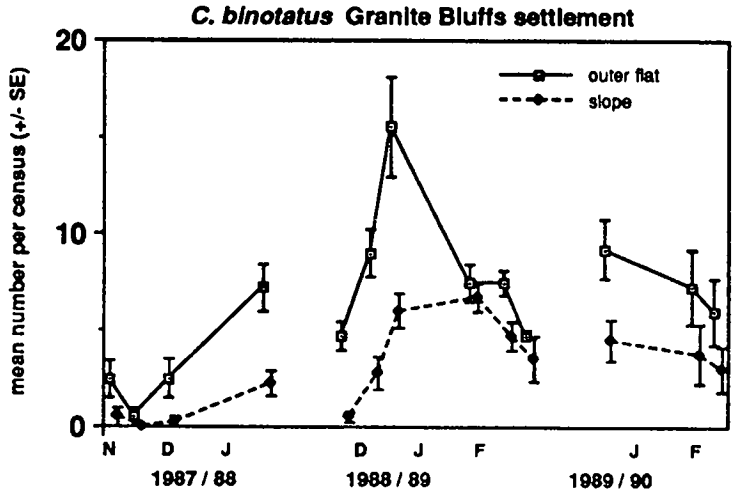

NORTH POINT
NORTH REEF
GRANITE BLUFFS
PIDGIN POINT
BIRD ISLAND
SOUTH ISLAND
SOUTH FRONT
IF
OF
CB
SL
0
5
10
15
20

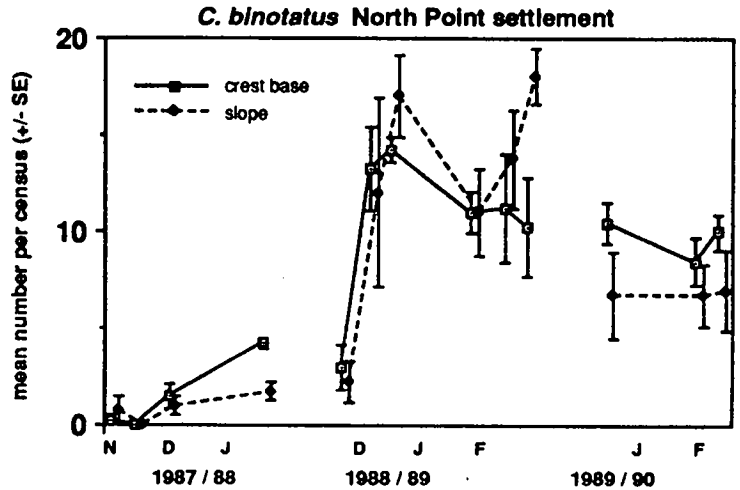
**Fig. 2.40 Density of juvenile *Ctenochaetus binotatus* in the outer flat and slope habitat at Granite Bluffs throughout the study.**
Mean number of individuals per transect (n=4) ± standard error.

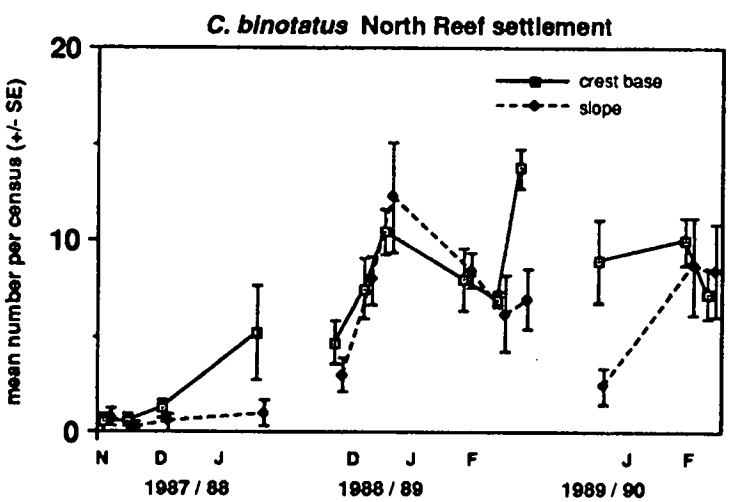
**Fig. 2.41 Density of juvenile *C. binotatus* in the crest base and slope habitats at North Point throughout the study.**
Mean number of individuals per transect (n=4) ± standard error.

**Fig. 2.42 Density of juvenile *C. binotatus* in the crest base and slope habitats at North Reef throughout the study.**
Mean number of individuals per transect (n=4) ± standard error.

**Fig. 2.43 Density of juvenile *C. binotatus* in the crest base and slope habitats at Pidgin Point throughout the study.**
Mean number of individuals per transect (n=4) ± standard error.


C. binotatus Granite Bluffs settlement
mean number per census (+/- SE)
20
10
0
outer flat
slope
N
D
J
D
J
F
J
F
1987 / 88
1988 / 89
1989 / 90


C. binotatus North Point settlement
mean number per census (+/- SE)
20
10
0
crest base
slope
N
D
J
D
J
F
J
F
1987 / 88
1988 / 89
1989 / 90


C. binotatus North Reef settlement
mean number per census (+/- SE)
20
10
0
crest base
slope
N
D
J
D
J
F
J
F
1987 / 88
1988 / 89
1989 / 90

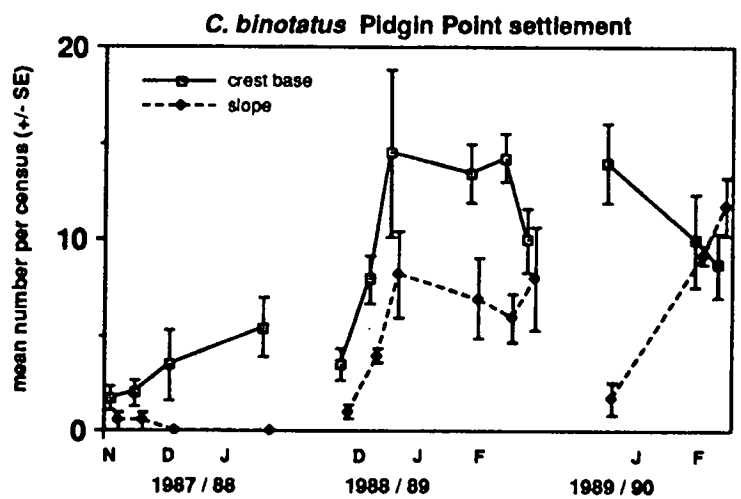
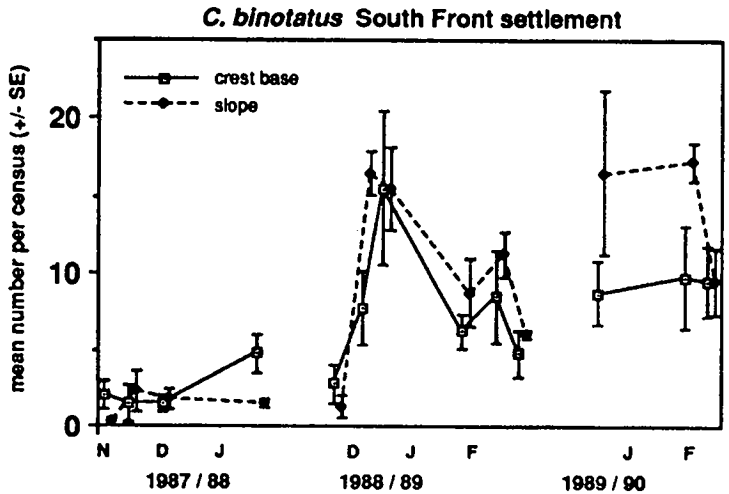

C. binotatus Pidgin Point settlement
mean number per census (+/- SE)
20
10
0
crest base
slope
N
D
J
D
J
F
J
F
1987 / 88
1988 / 89
1989 / 90

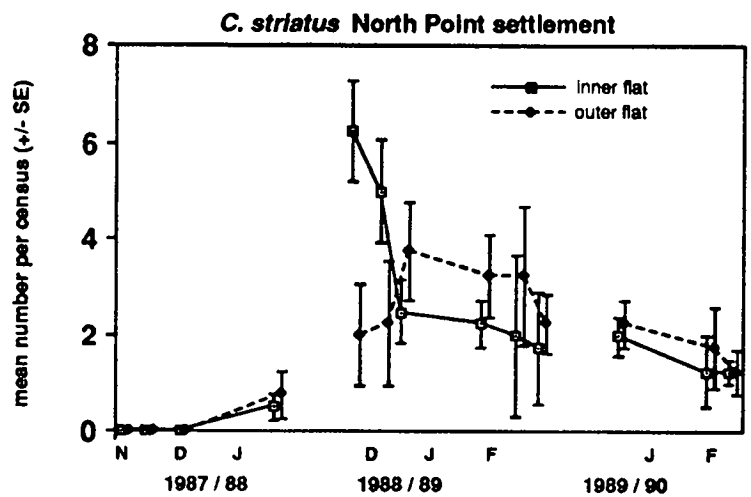
**Fig. 2.44 Density of juvenile *Ctenochaetus binotatus* in the crest base and slope habitats at South Front throughout the study.**
Mean number of individuals per transect (n=4) ± standard error.

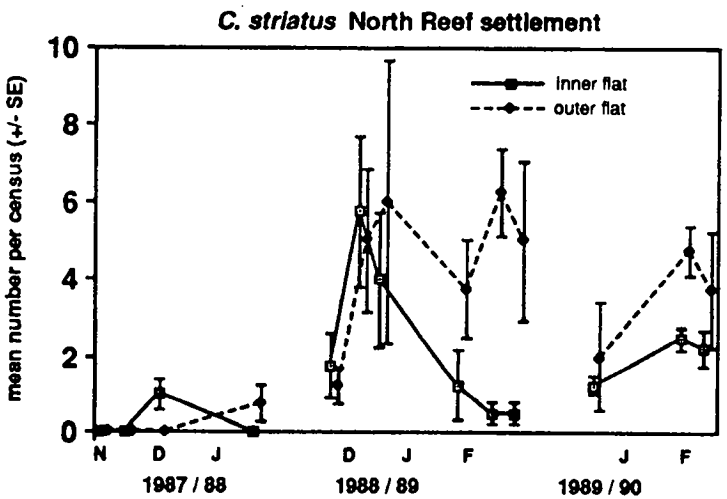
**Fig. 2.45 Density of juvenile *C. striatus* in the inner flat and outer habitats at North Point throughout the study.**
Mean number of individuals per transect (n=4) ± standard error.

**Fig. 2.46 Density of juvenile *C. striatus* in the inner flat and outer flat habitats at North Reef throughout the study.**
Mean number of individuals per transect (n=4) ± standard error.

**Fig. 2.47 Density of juvenile *C. striatus* in the outer flat habitat at Pidgin Point and South Front throughout the study.**
Mean number of individuals per transect (n=4) ± standard error.


C. binotatus South Front settlement
crest base
slope
mean number per census (+/- SE)
0
10
20
N
D
J
D
J
F
J
F
1987 / 88
1988 / 89
1989 / 90


C. striatus North Point settlement
inner flat
outer flat
mean number per census (+/- SE)
0
2
4
6
8
N
D
J
D
J
F
J
F
1987 / 88
1988 / 89
1989 / 90


C. striatus North Reef settlement
inner flat
outer flat
mean number per census (+/- SE)
0
2
4
6
8
10
N
D
J
D
J
F
J
F
1987 / 88
1988 / 89
1989 / 90

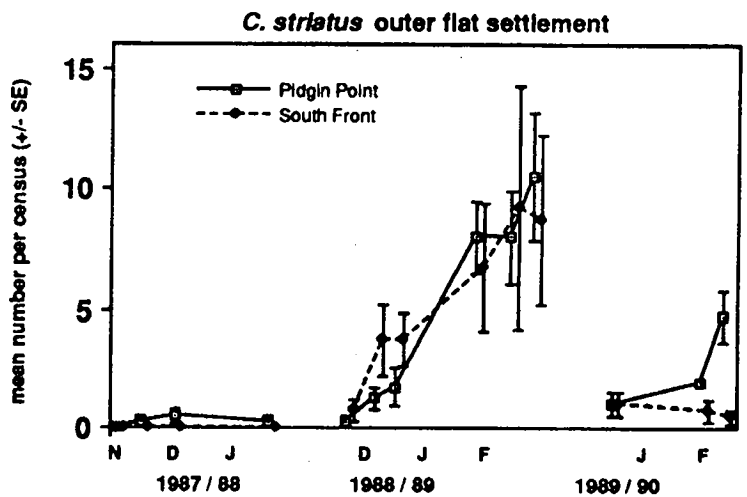

C. striatus outer flat settlement
Pidgin Point
South Front
mean number per census (+/- SE)
0
5
10
15
N
D
J
D
J
F
J
F
1987 / 88
1988 / 89
1989 / 90

**Fig. 2.48 Distribution of juvenile *Ctenochaetus striatus* at Lizard Island, 20/1/88.**

Mean number of individuals per transect (n=4) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CR = crest, CB = crest base, SL = slope, SB = slope base, PR = patch reef, PE = patch edge, FL = flat.

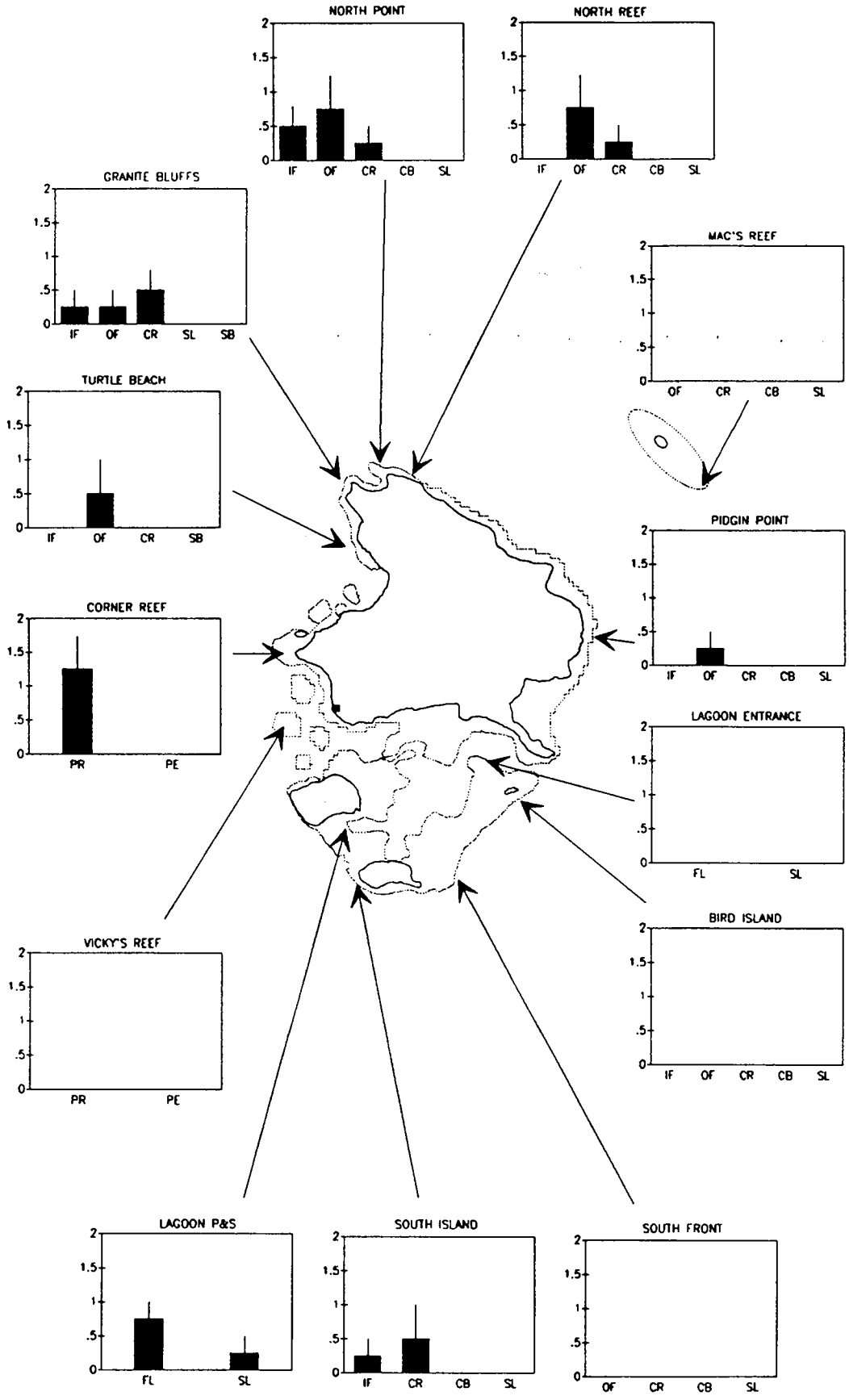

NORTH POINT
IF OF CR CB SL
NORTH REEF
IF OF CR CB SL
GRANITE BLUFFS
IF OF CR SL SB
MAC'S REEF
OF CR CB SL
TURTLE BEACH
IF OF CR SB
PIDGIN POINT
IF OF CR CB SL
CORNER REEF
PR PE
LAGOON ENTRANCE
FL SL
BIRD ISLAND
IF OF CR CB SL
VICKY'S REEF
PR PE
LAGOON P&S
FL SL
SOUTH ISLAND
IF CR CB SL
SOUTH FRONT
OF CR CB SL

**Fig. 2.49 Distribution of juvenile *Ctenochaetus striatus* at Lizard Island, 26/1/89.**

Mean number of individuals per transect (n=4) ± standard error.
Abbreviations: IF = inner flat, OF = outer flat, CB = crest base, SL = slope.

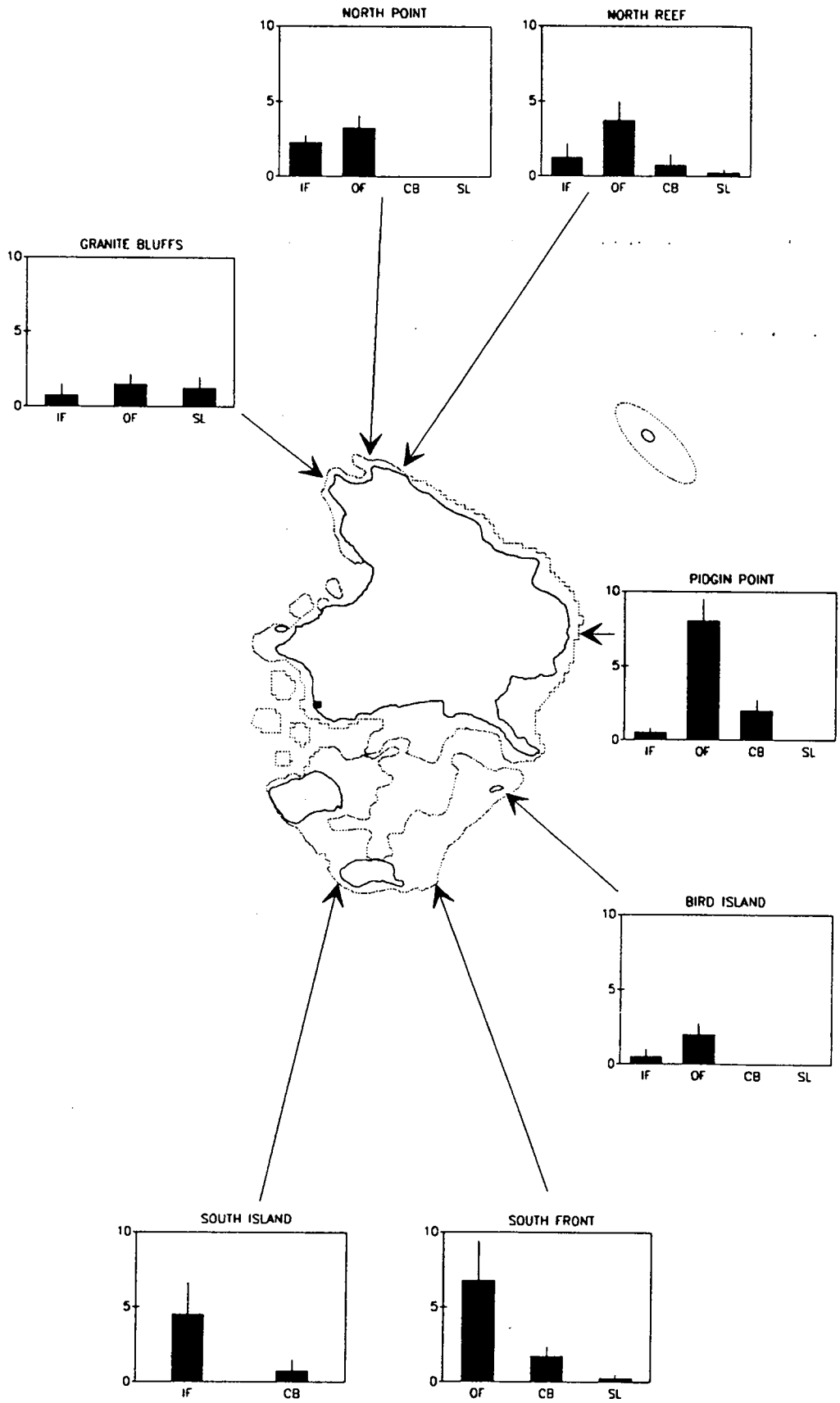

NORTH POINT
10
5
0
IF
OF
CB
SL
NORTH REEF
10
5
0
IF
OF
CB
SL
GRANITE BLUFFS
10
5
0
IF
OF
SL
PIDGIN POINT
10
5
0
IF
OF
CB
SL
BIRD ISLAND
10
5
0
IF
OF
CB
SL
SOUTH ISLAND
10
5
0
IF
CB
SOUTH FRONT
10
5
0
OF
CB
SL

**Fig. 2.50 Distribution of juvenile *Ctenochaetus striatus* at Lizard Island, 4/2/90.**

Mean number of individuals per transect (n=4) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CB = crest base, SL = slope.

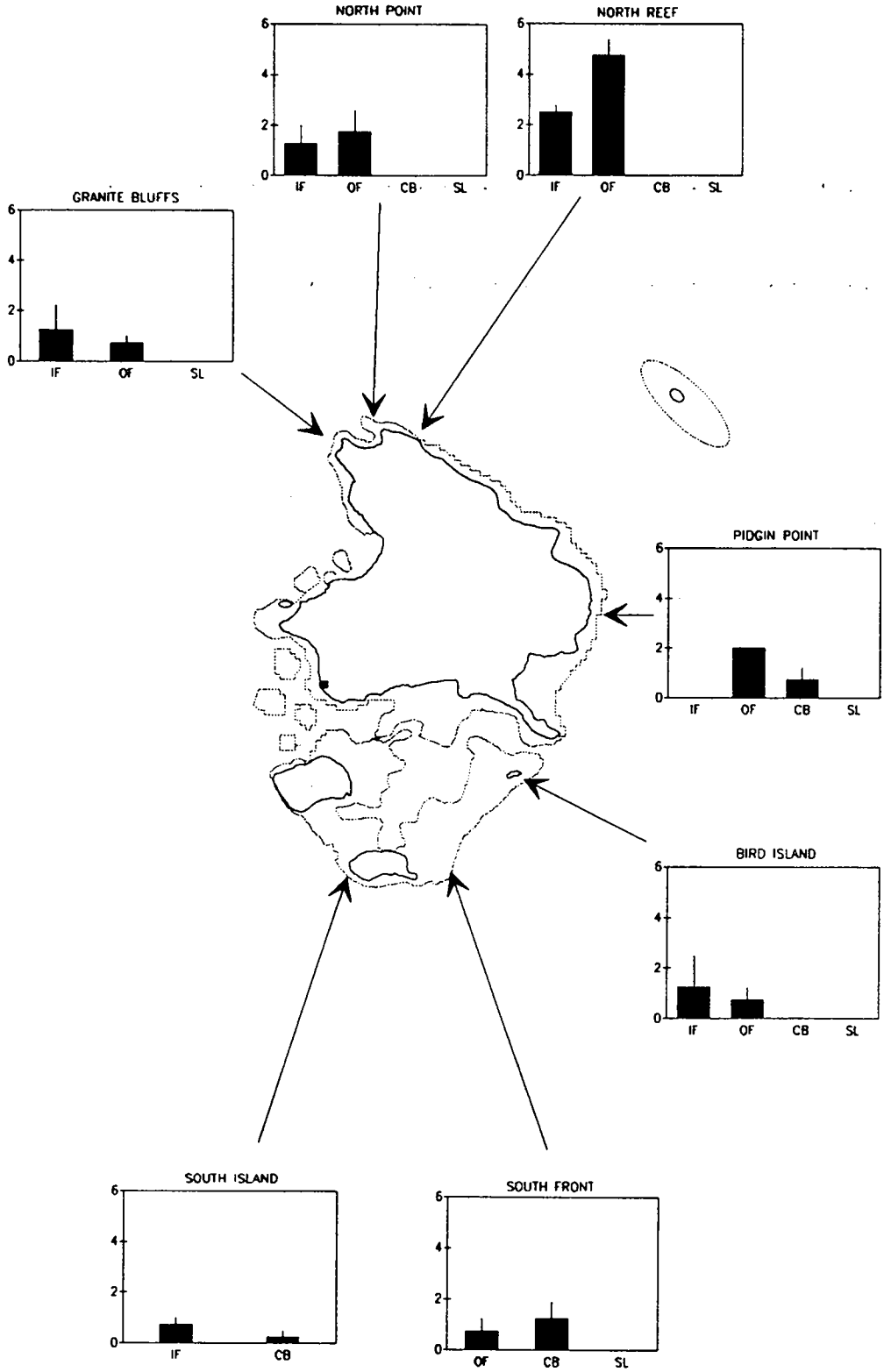

NORTH POINT
NORTH REEF
GRANITE BLUFFS
PIDGIN POINT
BIRD ISLAND
SOUTH ISLAND
SOUTH FRONT
IF
OF
CB
SL

**Fig. 2.51 Distribution of juvenile *Naso brevirostris* at Lizard Island, 13/2/89.**

Mean number of individuals per transect (n=4) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CB = crest base, SL = slope.

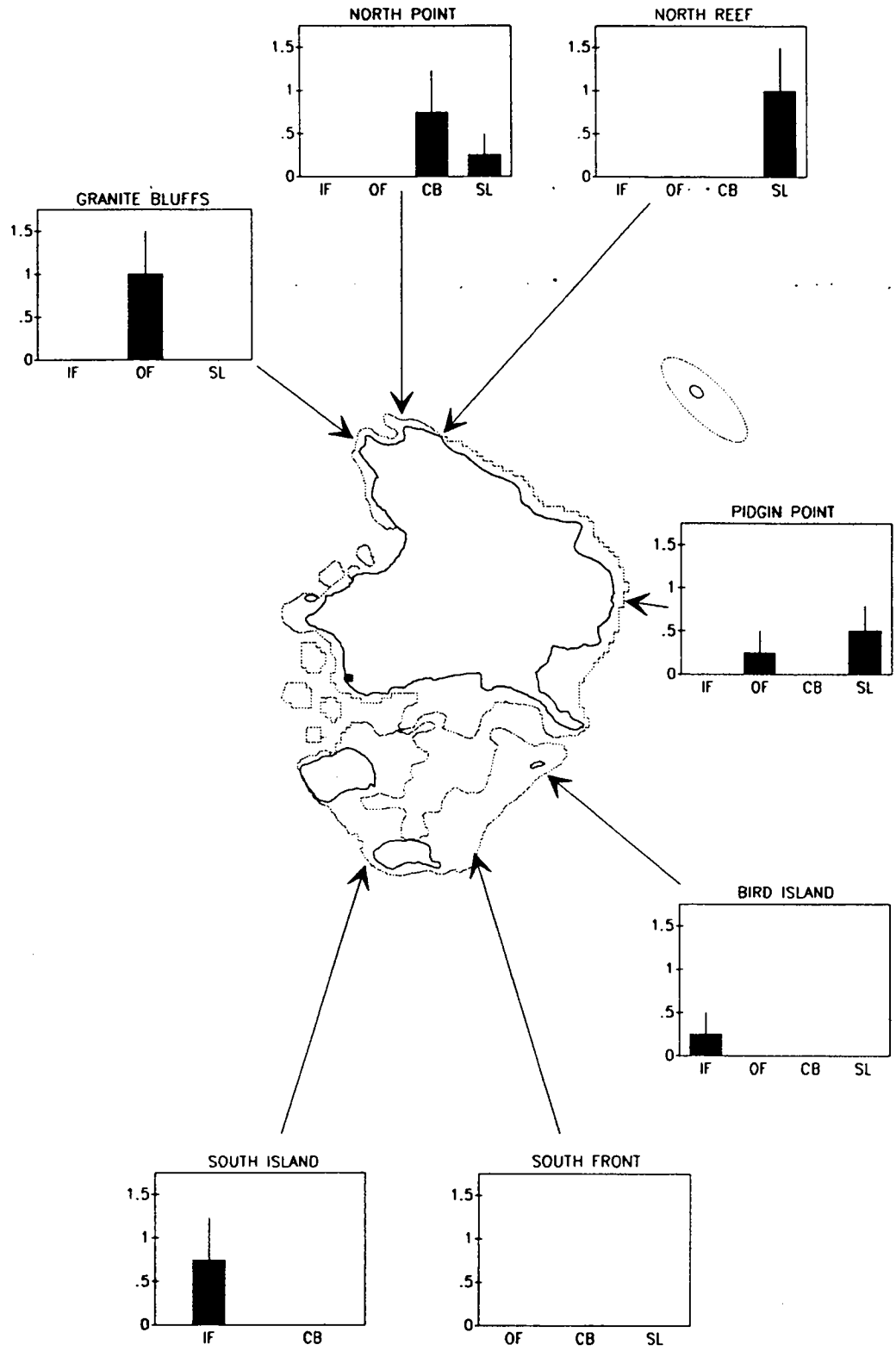

NORTH POINT
1.5
1
.5
0
IF
OF
CB
SL
NORTH REEF
1.5
1
.5
0
IF
OF
CB
SL
GRANITE BLUFFS
1.5
1
.5
0
IF
OF
SL
PIDGIN POINT
1.5
1
.5
0
IF
OF
CB
SL
BIRD ISLAND
1.5
1
.5
0
IF
OF
CB
SL
SOUTH ISLAND
1.5
1
.5
0
IF
CB
SOUTH FRONT
1.5
1
.5
0
OF
CB
SL

**Fig. 2.52 Distribution of juvenile *Naso hexacanthus* at Lizard Island, 13/2/89.**

Mean number of individuals per transect (n=4) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CB = crest base, SL = slope.

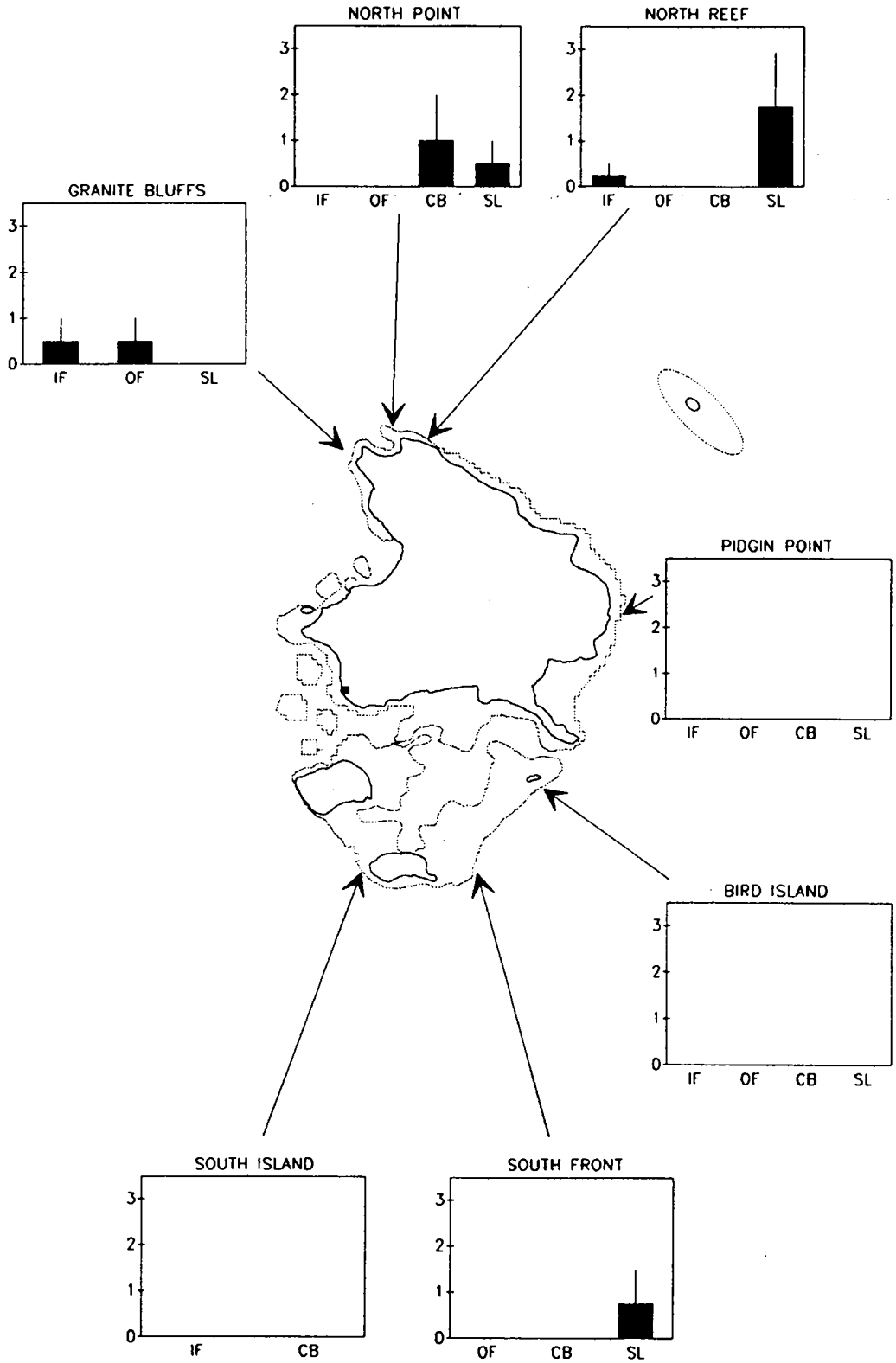

NORTH POINT
NORTH REEF
GRANITE BLUFFS
PIDGIN POINT
BIRD ISLAND
SOUTH ISLAND
SOUTH FRONT
IF
OF
CB
SL
0
1
2
3

**Fig. 2.53 Distribution of juvenile *Naso unicornis* at Lizard Island, 13/2/89.**

Mean number of individuals per transect (n=4) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CB = crest base, SL = slope.

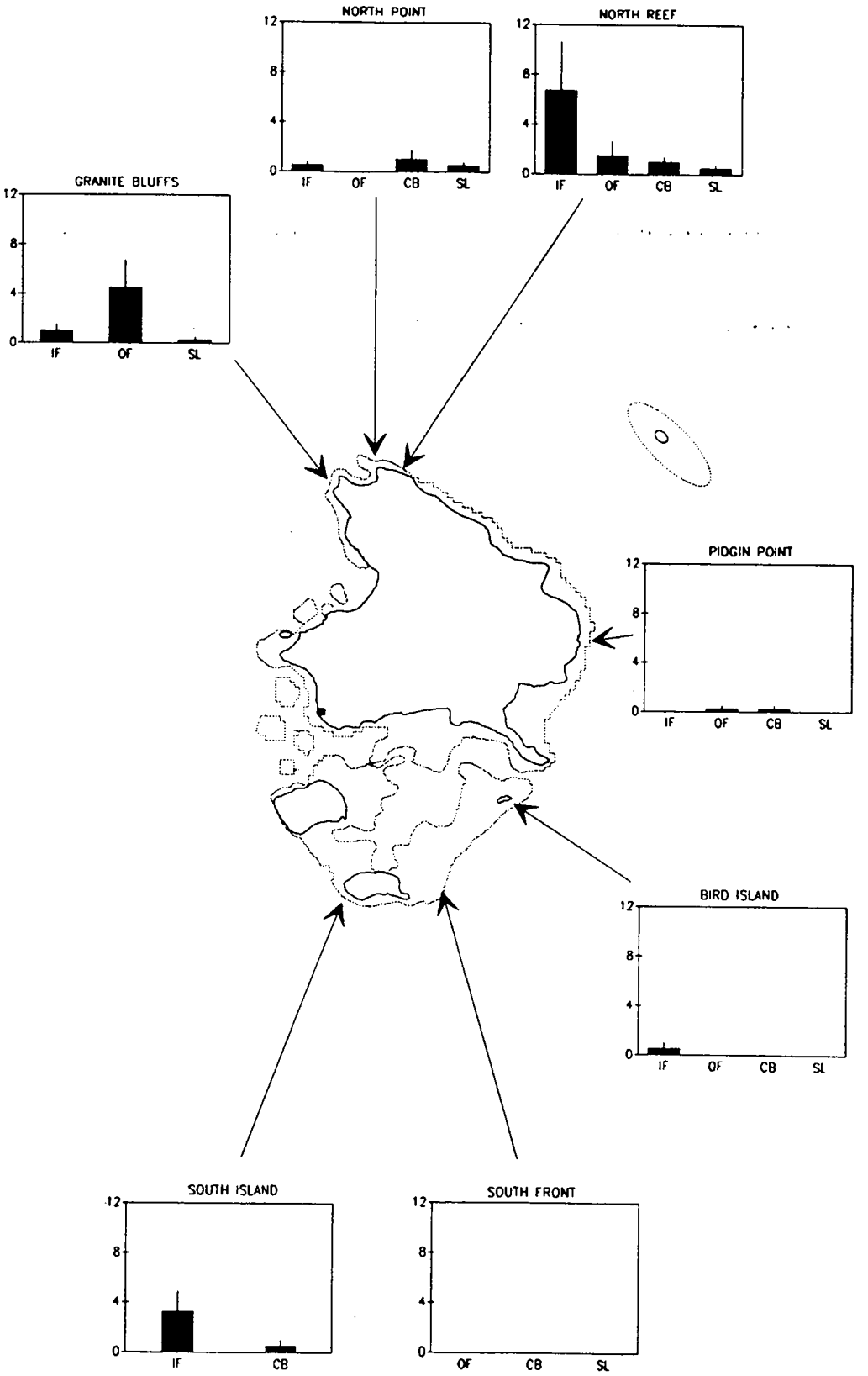

NORTH POINT
NORTH REEF
GRANITE BLUFFS
PIDGIN POINT
BIRD ISLAND
SOUTH ISLAND
SOUTH FRONT
12
8
4
0
IF
OF
CB
SL

**Fig. 2.54 Distribution of juvenile *Zebrasoma scopas* at Lizard Island, 17/12/88.**

Mean number of individuals per transect (n=4) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CB = crest base, SL = slope.

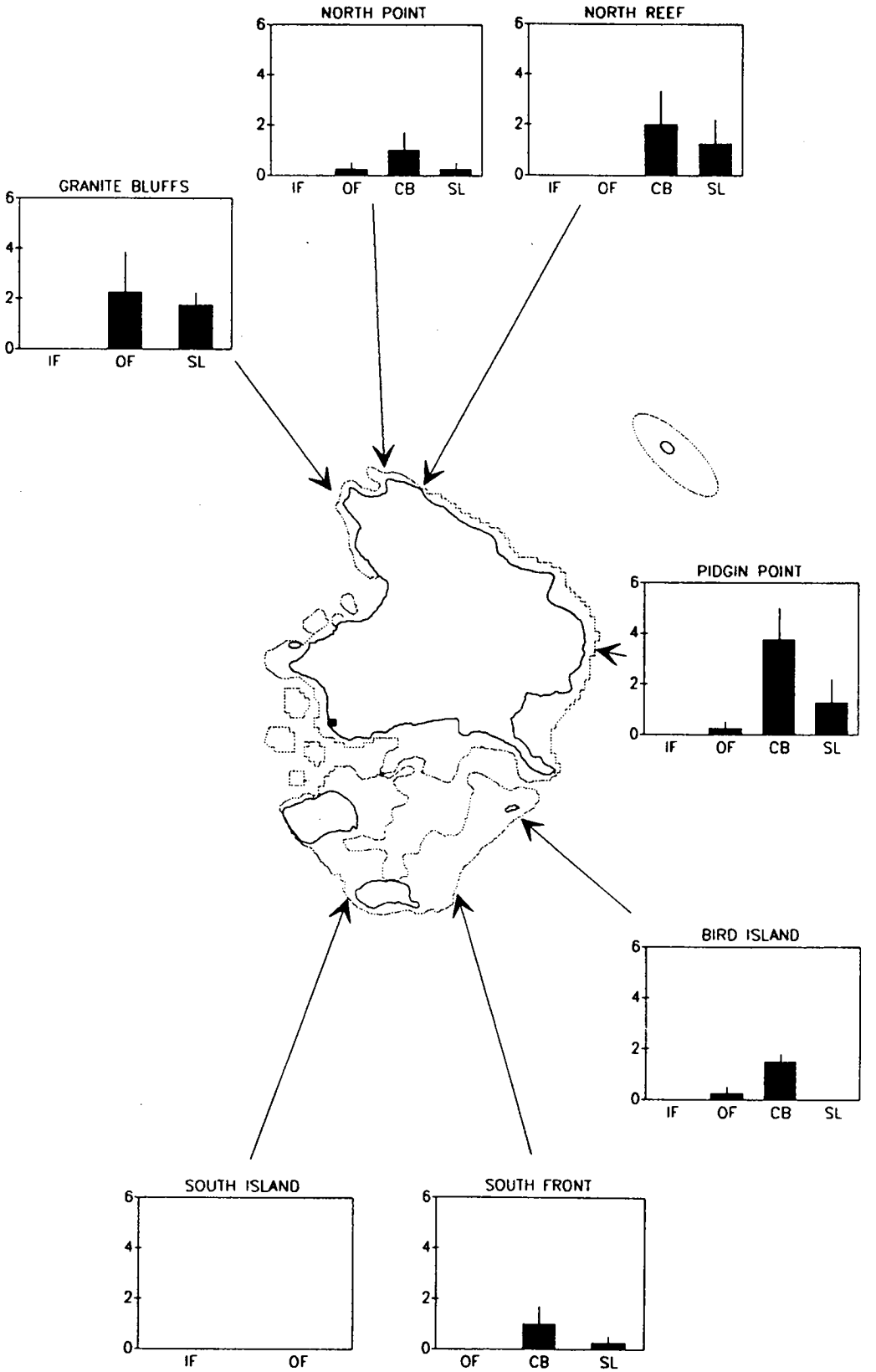

NORTH POINT
NORTH REEF
GRANITE BLUFFS
PIDGIN POINT
BIRD ISLAND
SOUTH ISLAND
SOUTH FRONT
IF
OF
CB
SL
0
2
4
6

**Fig. 2.55 Distribution of juvenile *Zebrasoma veliferum* at Lizard Island, 13/2/89.**

Mean number of individuals per transect (n=4) $\pm$ standard error.
Abbreviations: IF = inner flat, OF = outer flat, CB = crest base, SL = slope.

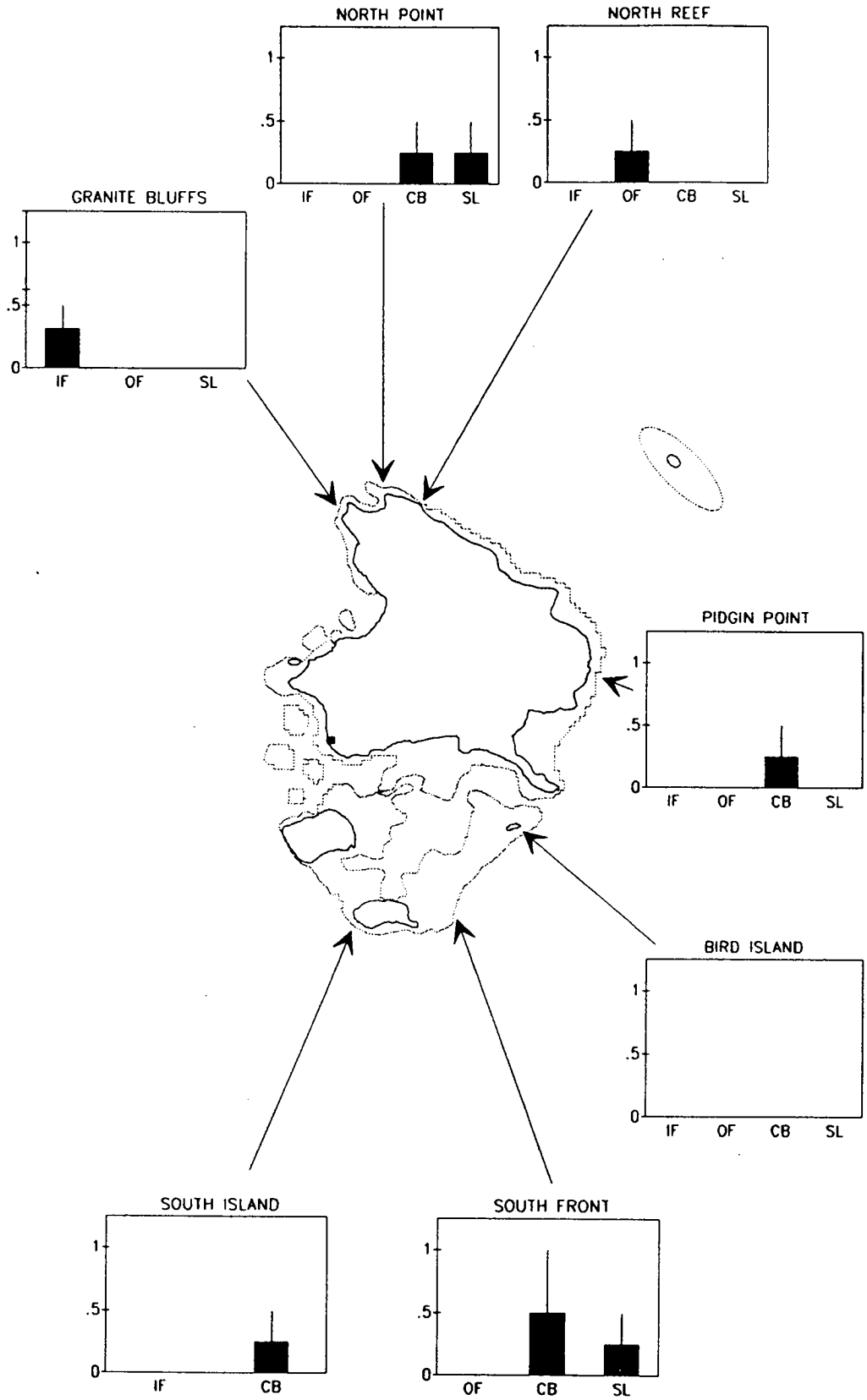

NORTH POINT
1
.5
0
IF
OF
CB
SL
NORTH REEF
1
.5
0
IF
OF
CB
SL
GRANITE BLUFFS
1
.5
0
IF
OF
SL
PIDGIN POINT
1
.5
0
IF
OF
CB
SL
BIRD ISLAND
1
.5
0
IF
OF
CB
SL
SOUTH ISLAND
1
.5
0
IF
CB
SOUTH FRONT
1
.5
0
OF
CB
SL

**Table 2.1** **Chi-Square Comparison of Adult and Juvenile Distribution.**

| Species | #Habitat x locality sites | | Total juveniles | | $\chi^2$ | $P > \chi^2$ |
|---|---|---|---|---|---|---|
| | + adults | - adults | + adults | - adults | | |
| *A. lineatus* | 7 | 17 | 25 | 6 | 39.78 | 0.001 |
| *A. nigrofuscus* | 23 | 1 | 3036 | 0 | 132 | 0.001 |
| *A. olivaceus* | 6 | 18 | 46 | 55 | 22.73 | 0.001 |
| *A. triostegus* | 8 | 16 | 210 | 0 | 420 | 0.001 |
| *C. binotatus* | 10 | 14 | 1165 | 309 | 846.90 | 0.001 |
| *C. striatus* | 15 | 9 | 365 | 37 | 137.33 | 0.001 |
| *N. brevirostris* | 8 | 16 | 20 | 21 | 4.40 | 0.05 |
| *N. tuberosus* | 4 | 20 | 10 | 16 | 8.90 | 0.005 |
| *N. unicornis* | 3 | 21 | 2 | 131 | 14.71 | *0.001 |
| *Z. scopas* | 7 | 17 | 20 | 106 | 10.77 | *0.005 |
| *Z. voliferum* | 2 | 22 | 2 | 27 | 0.08 | 0.95 |

* = Juvenile and adult distribution significantly different.

**Fig. 2.56 Summary of the distribution of adult acanthurids at Lizard Island.**

Mean number of individuals per transect for each habitat (mean of locations with a given exposure).
Abbreviations: IF = inner flat, OF = outer flat, CR = crest, CB = crest base, SL = slope, SB = slope base, FL = flat, PR = patch reef, PE = patch edge.

Summary of the distribution of adult acanthurids at Lizard Island

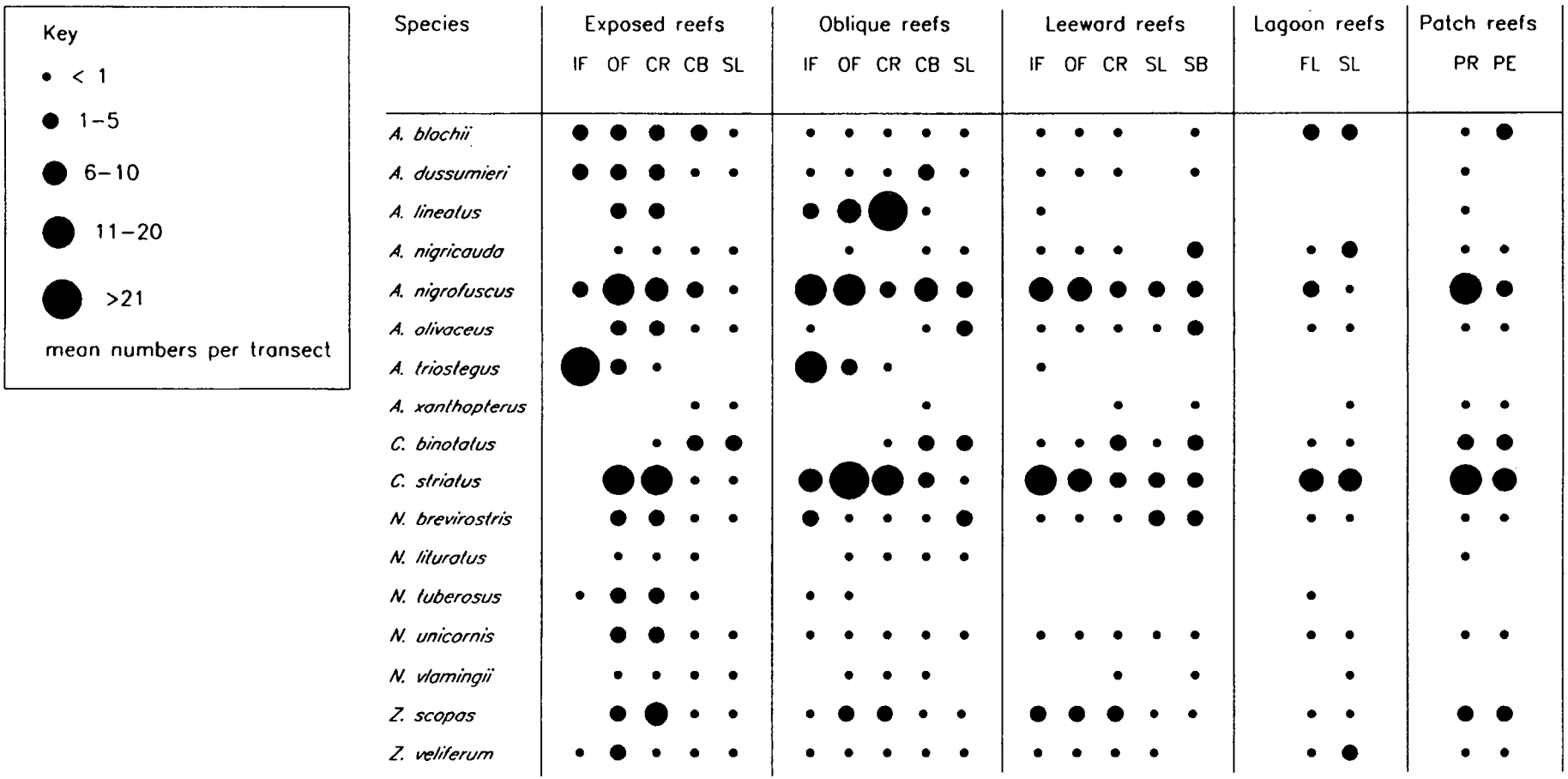


**Fig. 2.57 Summary of the distribution of juvenile acanthurids at Lizard Island.**

Total juvenile numbers per habitat/locality site (mean values across seasons, i.e. n=3). Total numbers of juveniles per habitat/locality site were taken to be the minimum consistent with the numbers of juveniles recorded in successive counts throughout each season. Abbreviations: IF = inner flat, OF = outer flat, CB = crest base, SL = slope.

Summary of the distribution of juvenile acanthurids at Lizard Island: mean of 3 seasons

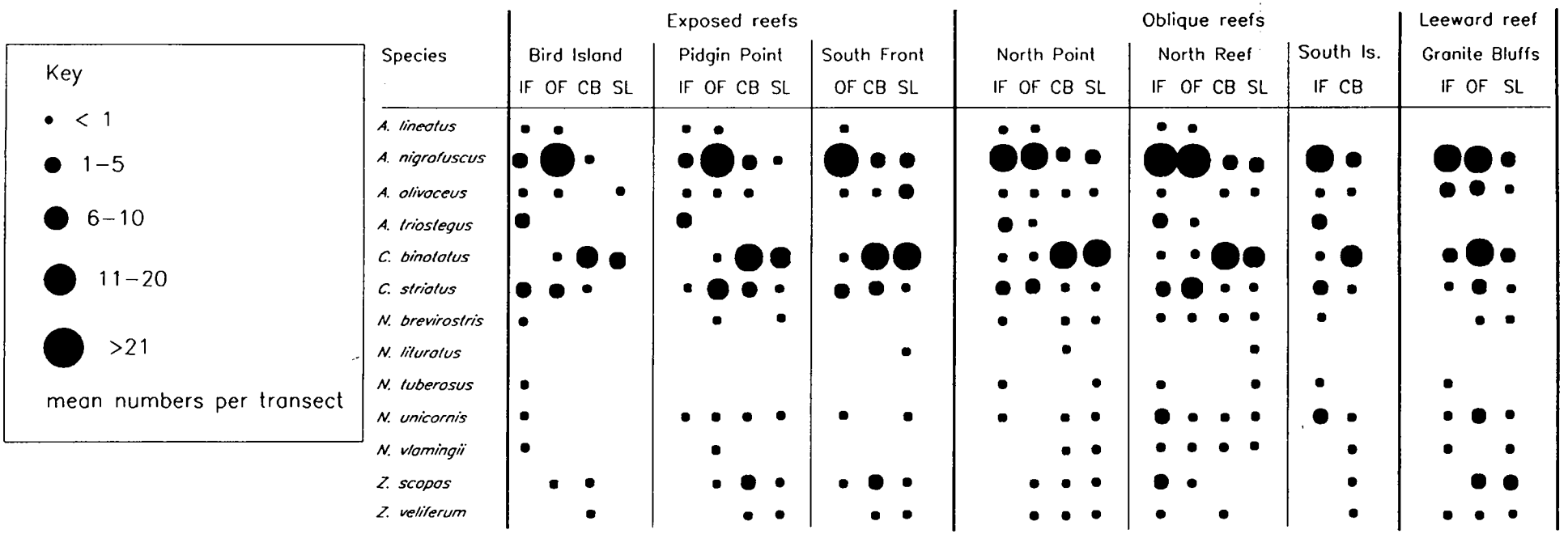


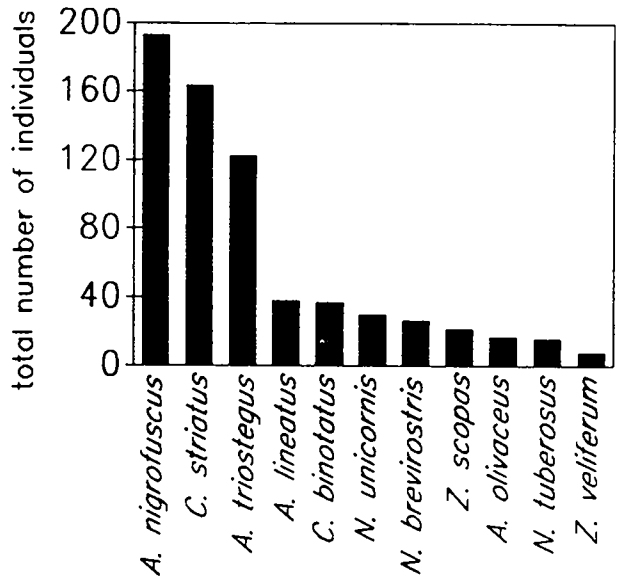
**Fig. 2.58 Ranked abundance of acanthurids at Lizard Island.**

Adult numbers are totals (sum of 24 habitat/locality sites) of 1987/88 census means. Juvenile numbers are mean values across seasons (i.e. n=3) of total juvenile numbers (sum of 24 habitat/locality sites).

# Ranked abundance of acanthurids at Lizard Island

sum of the 24 habitat/locality sites surveyed throughout the study

Adults: total of 1987/88 census




Juveniles: mean numbers settling per year

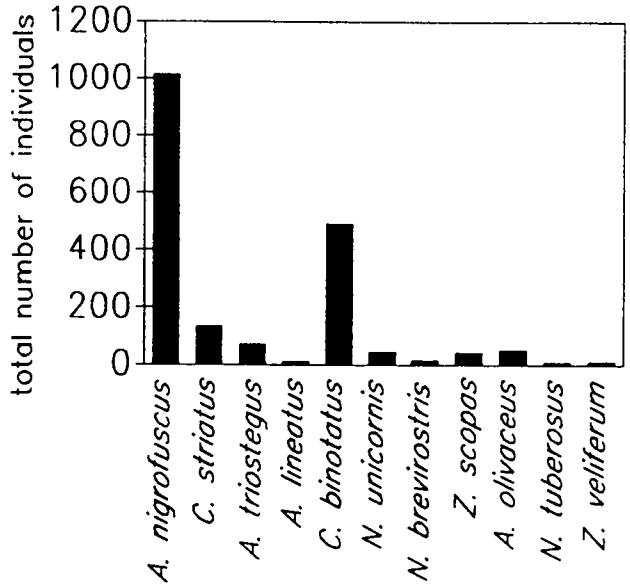


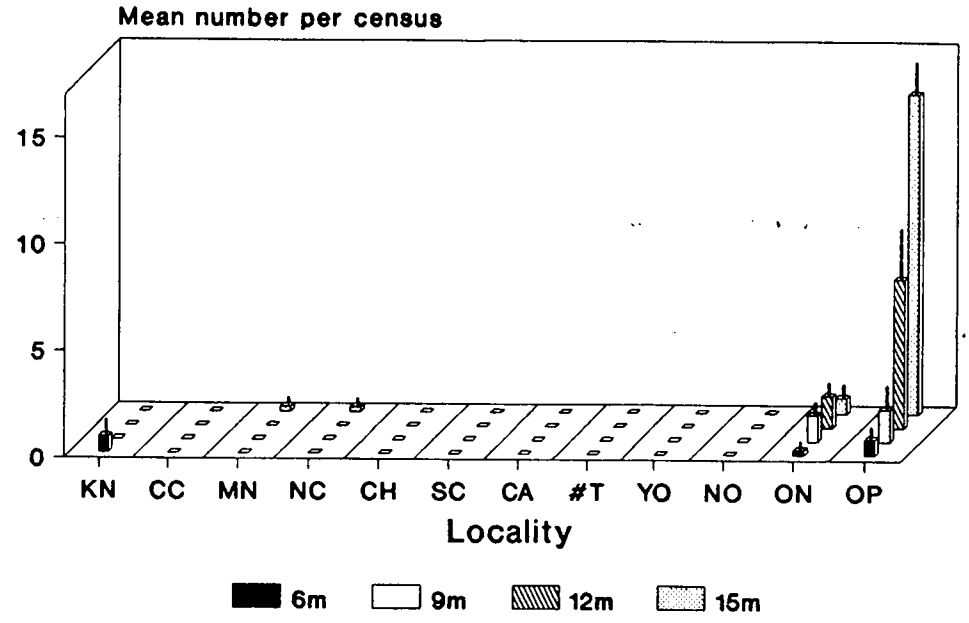
**Fig. 2.59 Depth distribution of *Acanthurus blochii* on GBR outer reefs and Tuvalu atolls.**

Mean number of individuals per transect (n=4) ± standard error.
Abbreviations:
Tuvalu sites: KN = Kulia North (Niutao), CC = Canoe City (Niutao), MN = Muli North (Niutao), NC = North Control (Nui), CH = Channel (Nui), SC = South Control (Nui).
GBR outer reef sites: CA = Carter Reef, #T = No. 10 Ribbon Reef, YO = Yonge Reef, NO = NoName Reef, ON = North site on Osprey Reef, OP = Pass site on Osprey Reef.

**Fig. 2.60 Depth distribution of *Acanthurus lineatus* on GBR outer reefs and Tuvalu atolls.**

Mean number of individuals per transect (n=4) ± standard error.
Abbreviations:
Tuvalu sites: KN = Kulia North (Niutao), CC = Canoe City (Niutao), MN = Muli North (Niutao), NC = North Control (Nui), CH = Channel (Nui), SC = South Control (Nui).
GBR outer reef sites: CA = Carter Reef, #T = No. 10 Ribbon Reef, YO = Yonge Reef, NO = NoName Reef, ON = North site on Osprey Reef, OP = Pass site on Osprey Reef.

# *A. blochii*




# *A. lineatus*

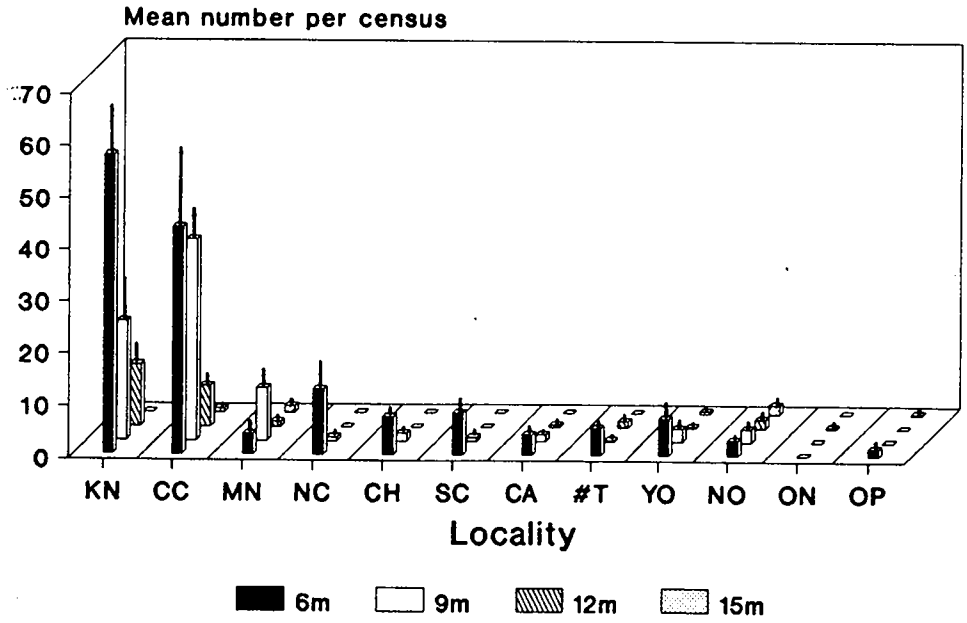


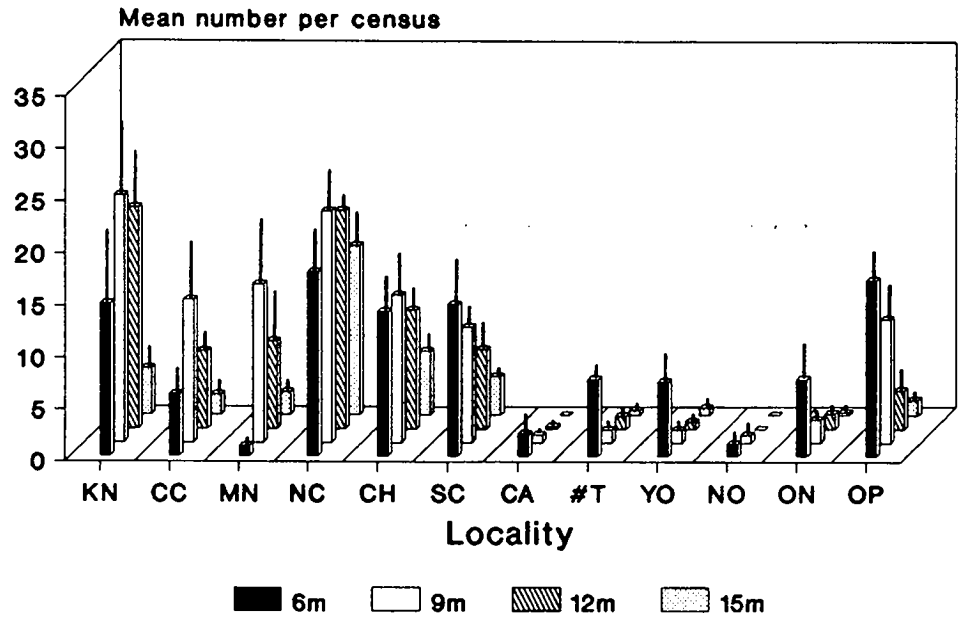
**Fig. 2.61 Depth distribution of *Acanthurus nigricans* on GBR outer reefs and Tuvalu atolls.**

Mean number of individuals per transect (n=4) ± standard error.
Abbreviations:
Tuvalu sites: KN = Kulia North (Niutao), CC = Canoe City (Niutao), MN = Muli North (Niutao), NC = North Control (Nui), CH = Channel (Nui), SC = South Control (Nui).
GBR outer reef sites: CA = Carter Reef, #T = No. 10 Ribbon Reef, YO = Yonge Reef, NO = NoName Reef, ON = North site on Osprey Reef, OP = Pass site on Osprey Reef.

**Fig. 2.62 Depth distribution of *Acanthurus nigricauda* on GBR outer reefs and Tuvalu atolls.**

Mean number of individuals per transect (n=4) ± standard error.
Abbreviations:
Tuvalu sites: KN = Kulia North (Niutao), CC = Canoe City (Niutao), MN = Muli North (Niutao), NC = North Control (Nui), CH = Channel (Nui), SC = South Control (Nui).
GBR outer reef sites: CA = Carter Reef, #T = No. 10 Ribbon Reef, YO = Yonge Reef, NO = NoName Reef, ON = North site on Osprey Reef, OP = Pass site on Osprey Reef.

# *A. nigricans*




# *A. nigricauda*

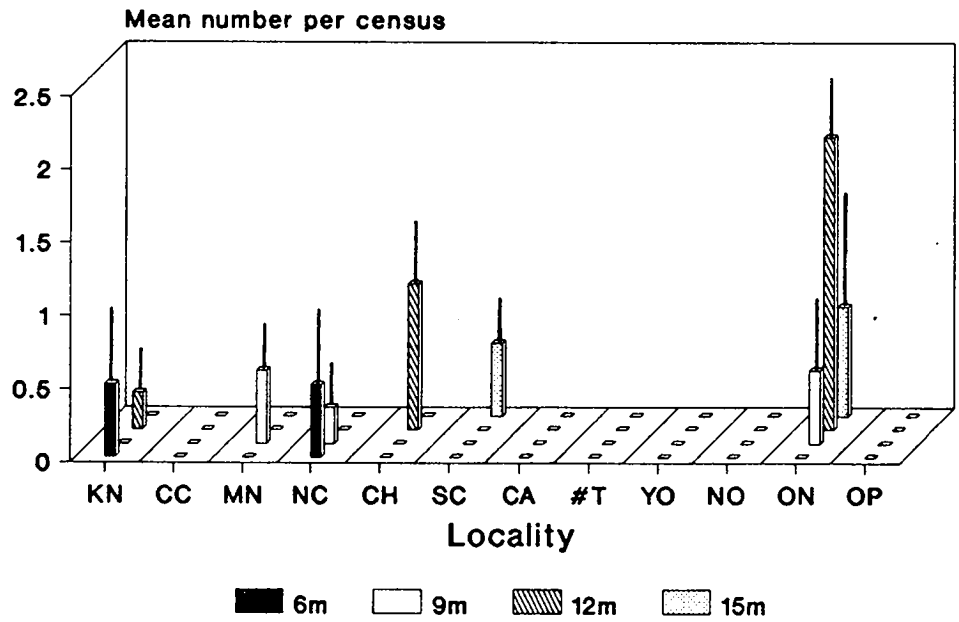


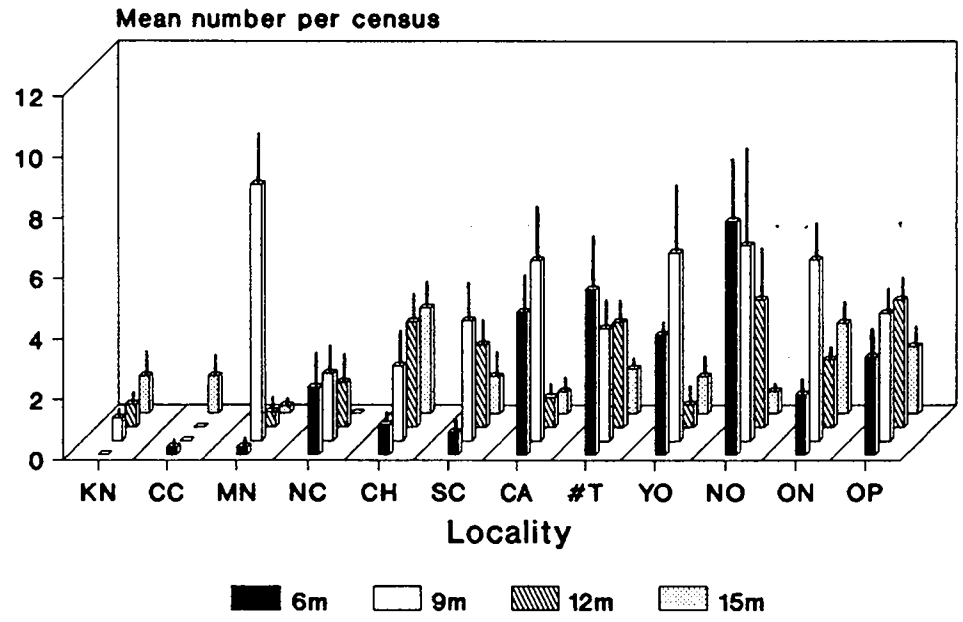
**Fig. 2.63 Depth distribution of *Acanthurus nigrofuscus* on GBR outer reefs and Tuvalu atolls.**

Mean number of individuals per transect (n=4) ± standard error.
Abbreviations:
Tuvalu sites: KN = Kulia North (Niutao), CC = Canoe City (Niutao), MN = Muli North (Niutao), NC = North Control (Nui), CH = Channel (Nui), SC = South Control (Nui).
GBR outer reef sites: CA = Carter Reef, #T = No. 10 Ribbon Reef, YO = Yonge Reef, NO = NoName Reef, ON = North site on Osprey Reef, OP = Pass site on Osprey Reef.

**Fig. 2.64 Depth distribution of *Acanthurus nigroris* on GBR outer reefs and Tuvalu atolls.**

Mean number of individuals per transect (n=4) ± standard error.
Abbreviations:
Tuvalu sites: KN = Kulia North (Niutao), CC = Canoe City (Niutao), MN = Muli North (Niutao), NC = North Control (Nui), CH = Channel (Nui), SC = South Control (Nui).
GBR outer reef sites: CA = Carter Reef, #T = No. 10 Ribbon Reef, YO = Yonge Reef, NO = NoName Reef, ON = North site on Osprey Reef, OP = Pass site on Osprey Reef.

# *A. nigrofuscus*




# *A. nigroris*

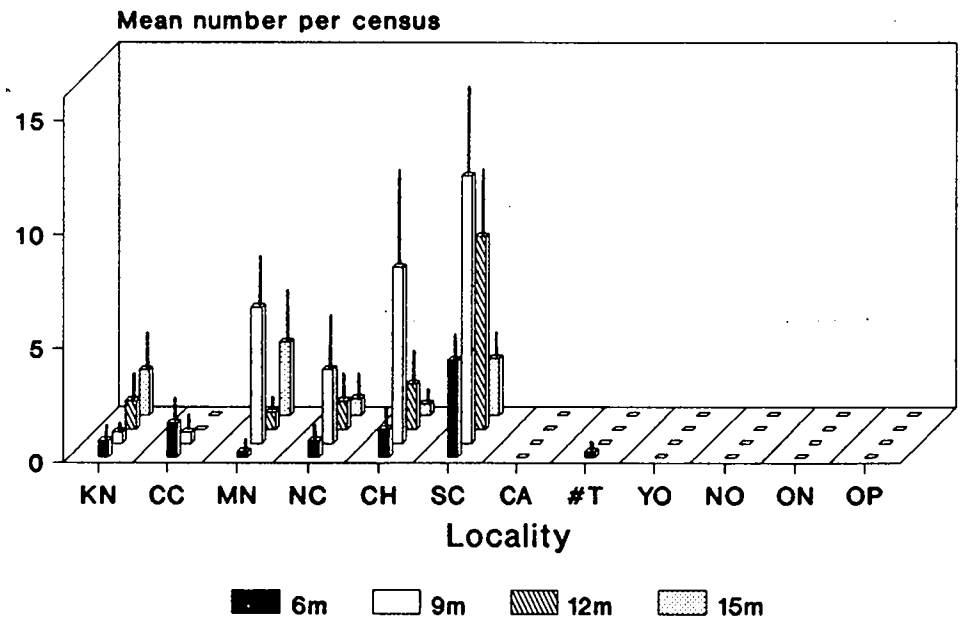


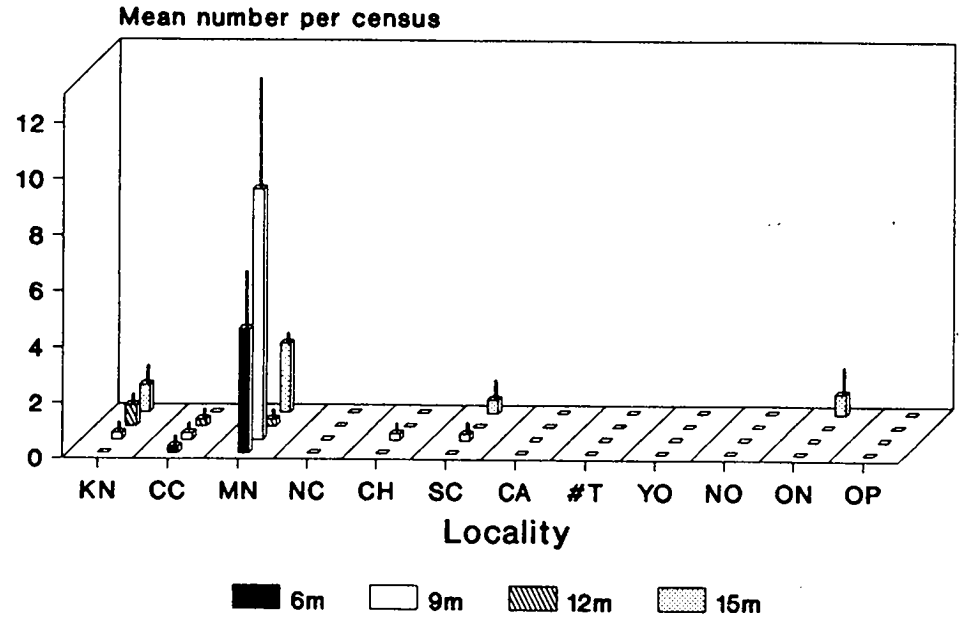
**Fig. 2.65 Depth distribution of *Acanthurus olivaceus* on GBR outer reefs and Tuvalu atolls.**

Mean number of individuals per transect (n=4) ± standard error.
Abbreviations:
Tuvalu sites: KN = Kulia North (Niutao), CC = Canoe City (Niutao), MN = Muli North (Niutao), NC = North Control (Nui), CH = Channel (Nui), SC = South Control (Nui).
GBR outer reef sites: CA = Carter Reef, #T = No. 10 Ribbon Reef, YO = Yonge Reef, NO = NoName Reef, ON = North site on Osprey Reef, OP = Pass site on Osprey Reef.

**Fig. 2.66 Depth distribution of *Acanthurus pyroferus* on GBR outer reefs and Tuvalu atolls.**

Mean number of individuals per transect (n=4) ± standard error.
Abbreviations:
Tuvalu sites: KN = Kulia North (Niutao), CC = Canoe City (Niutao), MN = Muli North (Niutao), NC = North Control (Nui), CH = Channel (Nui), SC = South Control (Nui).
GBR outer reef sites: CA = Carter Reef, #T = No. 10 Ribbon Reef, YO = Yonge Reef, NO = NoName Reef, ON = North site on Osprey Reef, OP = Pass site on Osprey Reef.

# *A. olivaceus*




# *A. pyroferus*

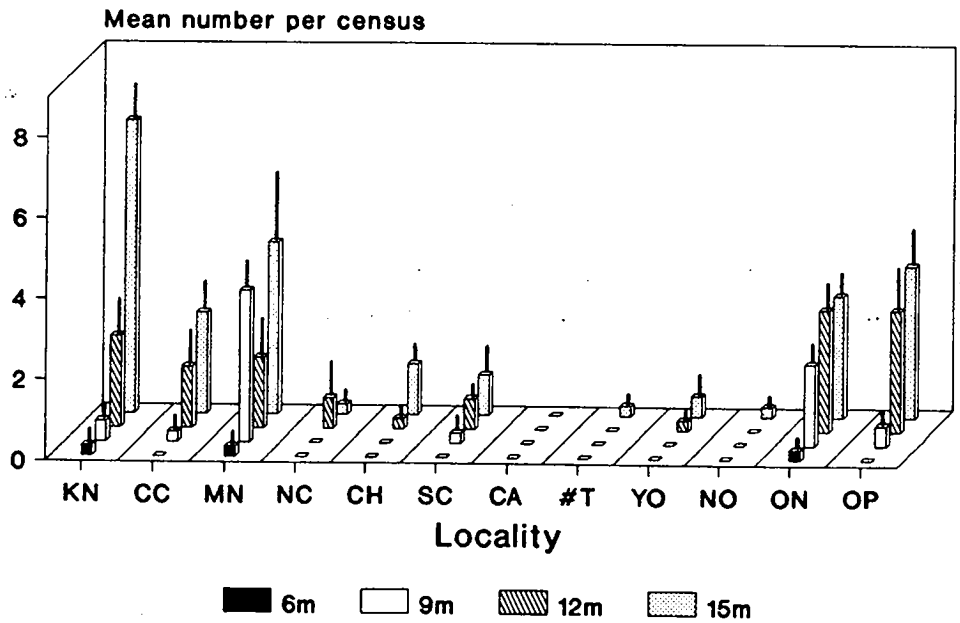


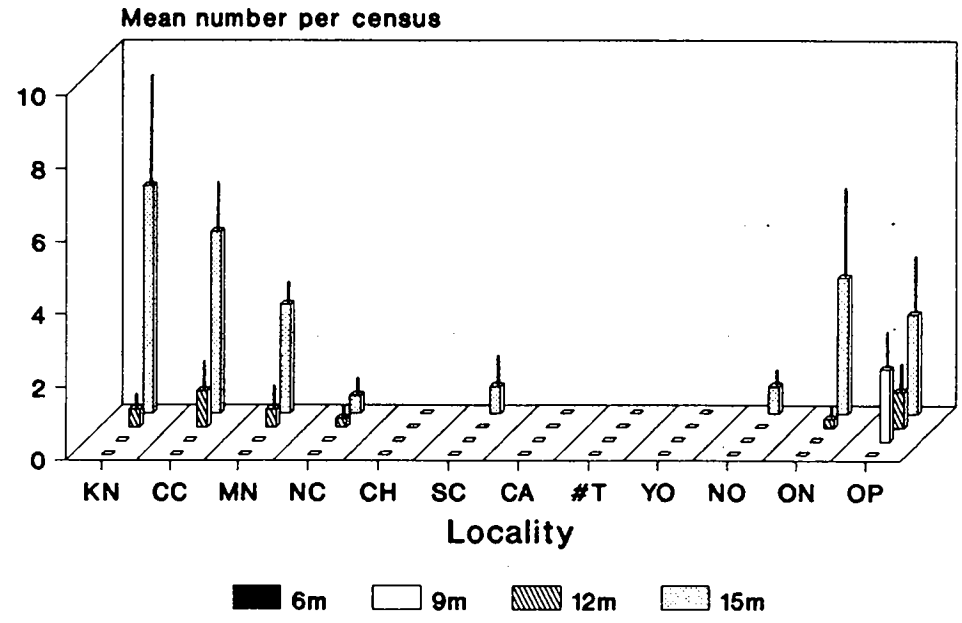
**Fig. 2.67 Depth distribution of *Acanthurus thompsoni* on GBR outer reefs and Tuvalu atolls.**

Mean number of individuals per transect (n=4) ± standard error.
Abbreviations:
Tuvalu sites: KN = Kulia North (Niutao), CC = Canoe City (Niutao), MN = Muli North (Niutao), NC = North Control (Nui), CH = Channel (Nui), SC = South Control (Nui).
GBR outer reef sites: CA = Carter Reef, #T = No. 10 Ribbon Reef, YO = Yonge Reef, NO = NoName Reef, ON = North site on Osprey Reef, OP = Pass site on Osprey Reef.

**Fig. 2.68 Depth distribution of *Acanthurus triostegus* on GBR outer reefs and Tuvalu atolls.**

Mean number of individuals per transect (n=4) ± standard error.
Abbreviations:
Tuvalu sites: KN = Kulia North (Niutao), CC = Canoe City (Niutao), MN = Muli North (Niutao), NC = North Control (Nui), CH = Channel (Nui), SC = South Control (Nui).
GBR outer reef sites: CA = Carter Reef, #T = No. 10 Ribbon Reef, YO = Yonge Reef, NO = NoName Reef, ON = North site on Osprey Reef, OP = Pass site on Osprey Reef.

# *A. thompsoni*




# *A. triostegus*

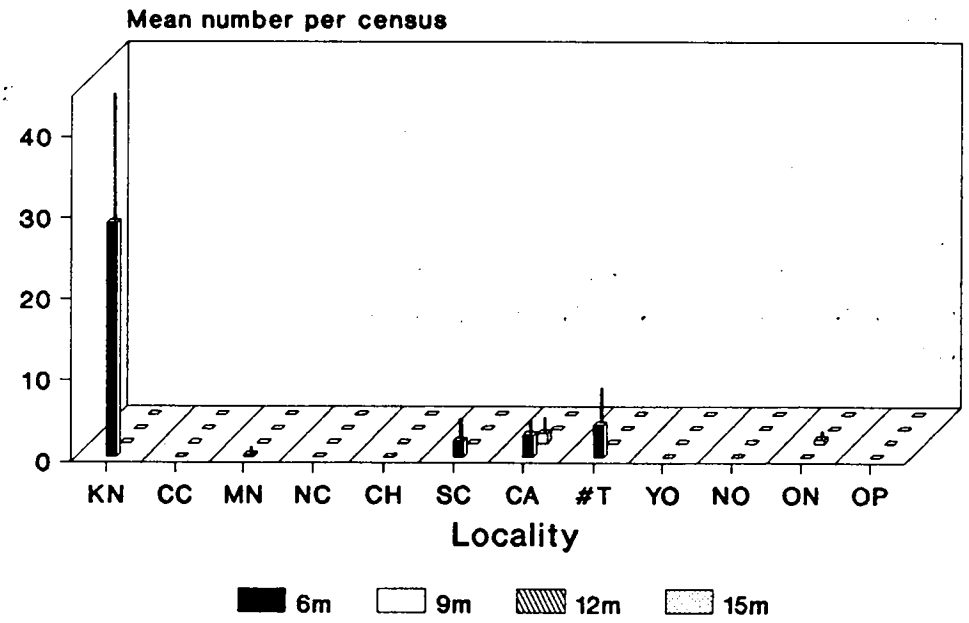


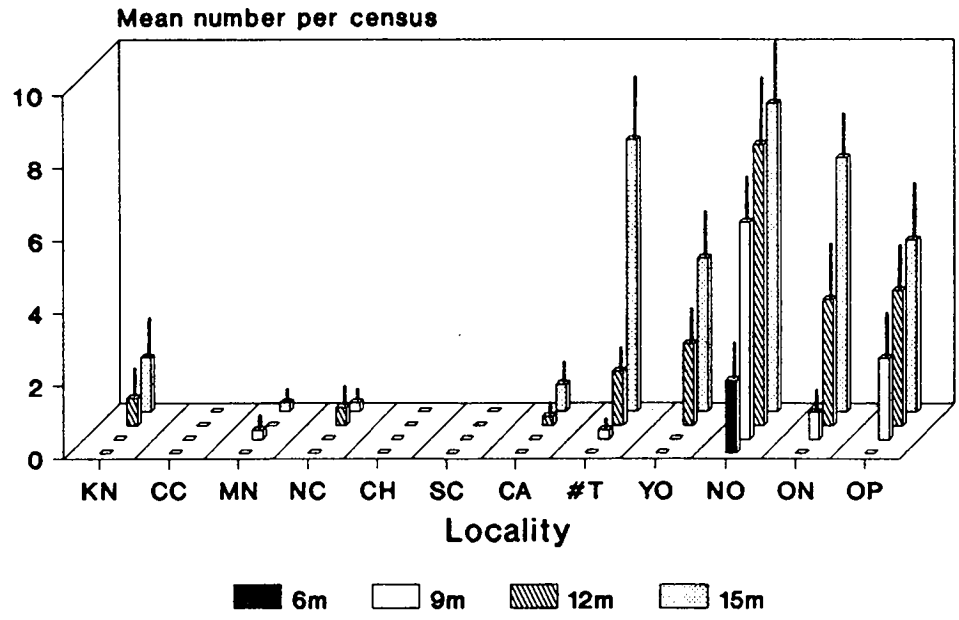
**Fig. 2.69 Depth distribution of *Ctenochaetus binotatus* on GBR outer reefs and Tuvalu atolls.**

Mean number of individuals per transect (n=4) ± standard error.
Abbreviations:
Tuvalu sites: KN = Kulia North (Niutao), CC = Canoe City (Niutao), MN = Muli North (Niutao), NC = North Control (Nui), CH = Channel (Nui), SC = South Control (Nui).
GBR outer reef sites: CA = Carter Reef, #T = No. 10 Ribbon Reef, YO = Yonge Reef, NO = NoName Reef, ON = North site on Osprey Reef, OP = Pass site on Osprey Reef.

**Fig. 2.70 Depth distribution of *Ctenochaetus hawaiiensis* on GBR outer reefs and Tuvalu atolls.**

Mean number of individuals per transect (n=4) ± standard error.
Abbreviations:
Tuvalu sites: KN = Kulia North (Niutao), CC = Canoe City (Niutao), MN = Muli North (Niutao), NC = North Control (Nui), CH = Channel (Nui), SC = South Control (Nui).
GBR outer reef sites: CA = Carter Reef, #T = No. 10 Ribbon Reef, YO = Yonge Reef, NO = NoName Reef, ON = North site on Osprey Reef, OP = Pass site on Osprey Reef.

# *C. binotatus*




# *C. hawaiiensis*

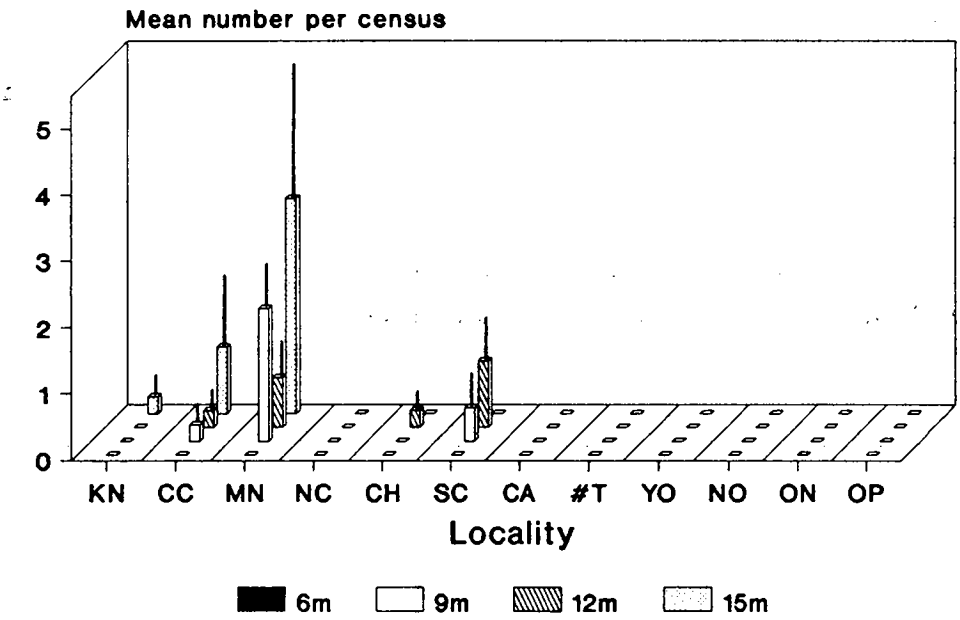


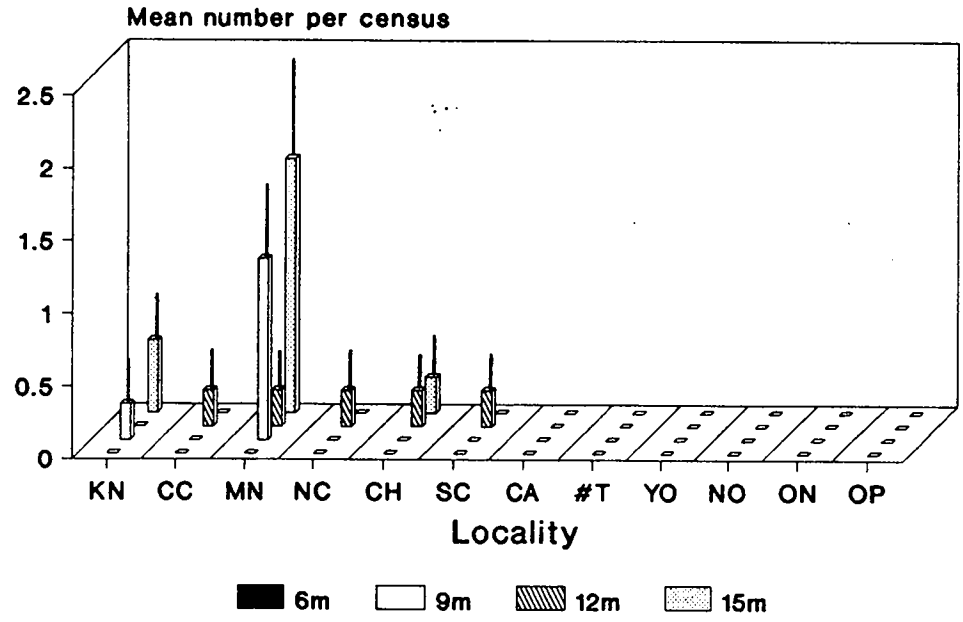
**Fig. 2.71 Depth distribution of *Ctenochaetus marginatus* on GBR outer reefs and Tuvalu atolls.**

Mean number of individuals per transect (n=4) ± standard error.
Abbreviations:
Tuvalu sites: KN = Kulia North (Niutao), CC = Canoe City (Niutao), MN = Muli North (Niutao), NC = North Control (Nui), CH = Channel (Nui), SC = South Control (Nui).
GBR outer reef sites: CA = Carter Reef, #T = No. 10 Ribbon Reef, YO = Yonge Reef, NO = NoName Reef, ON = North site on Osprey Reef, OP = Pass site on Osprey Reef.

**Fig. 2.72 Depth distribution of *Ctenochaetus striatus* on GBR outer reefs and Tuvalu atolls.**

Mean number of individuals per transect (n=4) ± standard error.
Abbreviations:
Tuvalu sites: KN = Kulia North (Niutao), CC = Canoe City (Niutao), MN = Muli North (Niutao), NC = North Control (Nui), CH = Channel (Nui), SC = South Control (Nui).
GBR outer reef sites: CA = Carter Reef, #T = No. 10 Ribbon Reef, YO = Yonge Reef, NO = NoName Reef, ON = North site on Osprey Reef, OP = Pass site on Osprey Reef.

# *C. marginatus*




# *C. striatus*

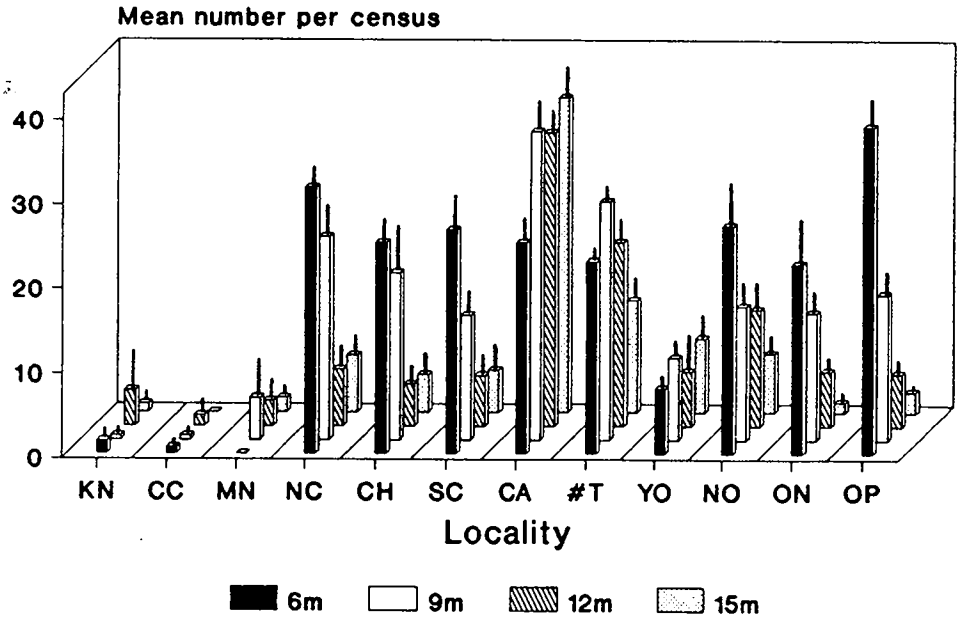


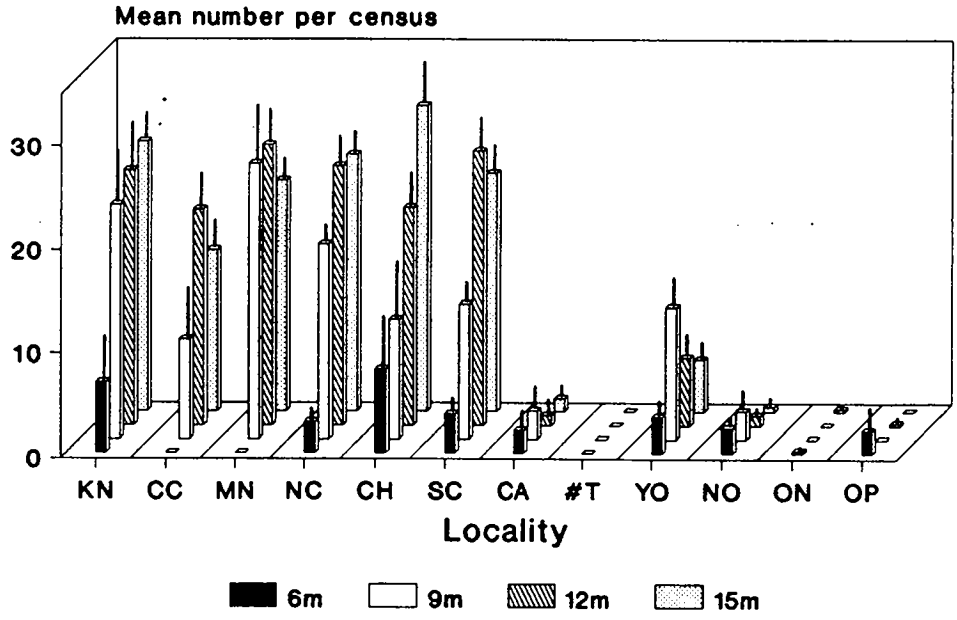
**Fig. 2.73 Depth distribution of *Ctenochaetus strigosus* on GBR outer reefs and Tuvalu atolls.**

Mean number of individuals per transect (n=4) ± standard error.
Abbreviations:
Tuvalu sites: KN = Kulia North (Niutao), CC = Canoe City (Niutao), MN = Muli North (Niutao), NC = North Control (Nui), CH = Channel (Nui), SC = South Control (Nui).
GBR outer reef sites: CA = Carter Reef, #T = No. 10 Ribbon Reef, YO = Yonge Reef, NO = NoName Reef, ON = North site on Osprey Reef, OP = Pass site on Osprey Reef.

**Fig. 2.74 Depth distribution of *Naso brevirostris* on GBR outer reefs and Tuvalu atolls.**

Mean number of individuals per transect (n=4) ± standard error.
Abbreviations:
Tuvalu sites: KN = Kulia North (Niutao), CC = Canoe City (Niutao), MN = Muli North (Niutao), NC = North Control (Nui), CH = Channel (Nui), SC = South Control (Nui).
GBR outer reef sites: CA = Carter Reef, #T = No. 10 Ribbon Reef, YO = Yonge Reef, NO = NoName Reef, ON = North site on Osprey Reef, OP = Pass site on Osprey Reef.

# *C. strigosus*




# *N. brevirostris*

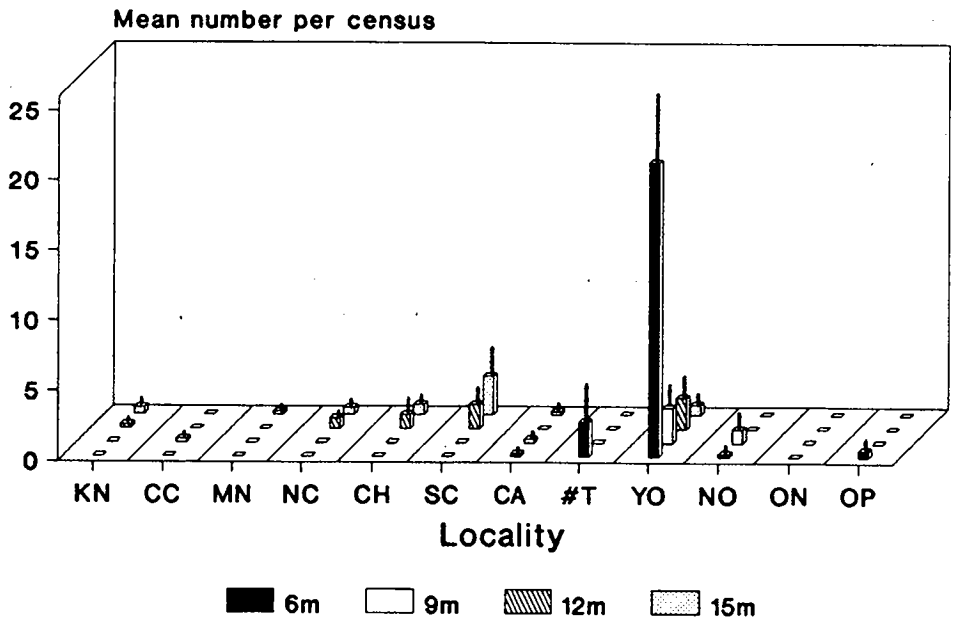


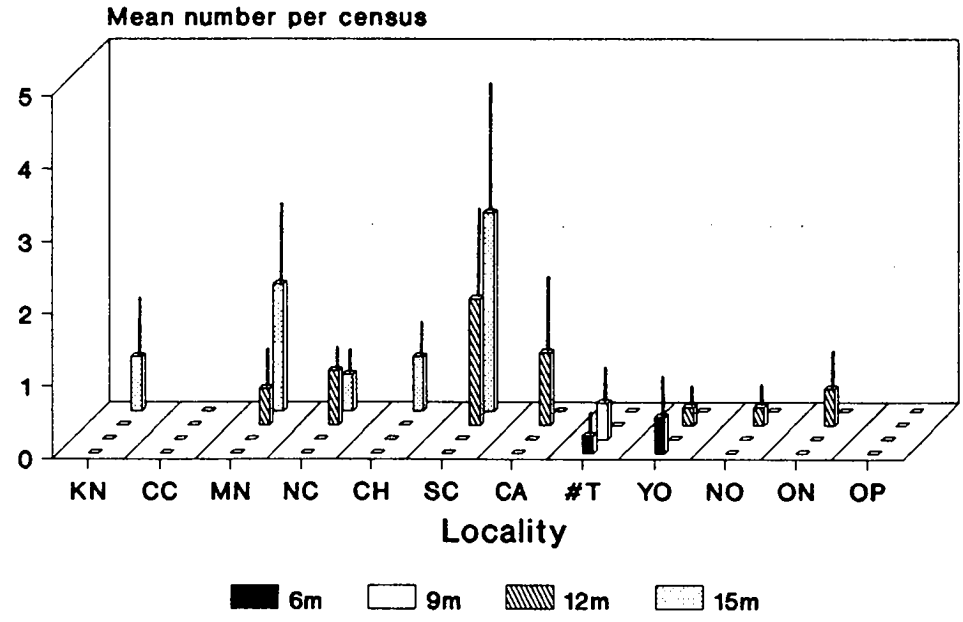
**Fig. 2.75 Depth distribution of *Naso hexacanthus* on GBR outer reefs and Tuvalu atolls.**

Mean number of individuals per transect (n=4) ± standard error.
Abbreviations:
Tuvalu sites: KN = Kulia North (Niutao), CC = Canoe City (Niutao), MN = Muli North (Niutao), NC = North Control (Nui), CH = Channel (Nui), SC = South Control (Nui).
GBR outer reef sites: CA = Carter Reef, #T = No. 10 Ribbon Reef, YO = Yonge Reef, NO = NoName Reef, ON = North site on Osprey Reef, OP = Pass site on Osprey Reef.

**Fig. 2.76 Depth distribution of *Naso lituratus* on GBR outer reefs and Tuvalu atolls.**

Mean number of individuals per transect (n=4) ± standard error.
Abbreviations:
Tuvalu sites: KN = Kulia North (Niutao), CC = Canoe City (Niutao), MN = Muli North (Niutao), NC = North Control (Nui), CH = Channel (Nui), SC = South Control (Nui).
GBR outer reef sites: CA = Carter Reef, #T = No. 10 Ribbon Reef, YO = Yonge Reef, NO = NoName Reef, ON = North site on Osprey Reef, OP = Pass site on Osprey Reef.

# *N. hexacanthus*




# *N. lituratus*

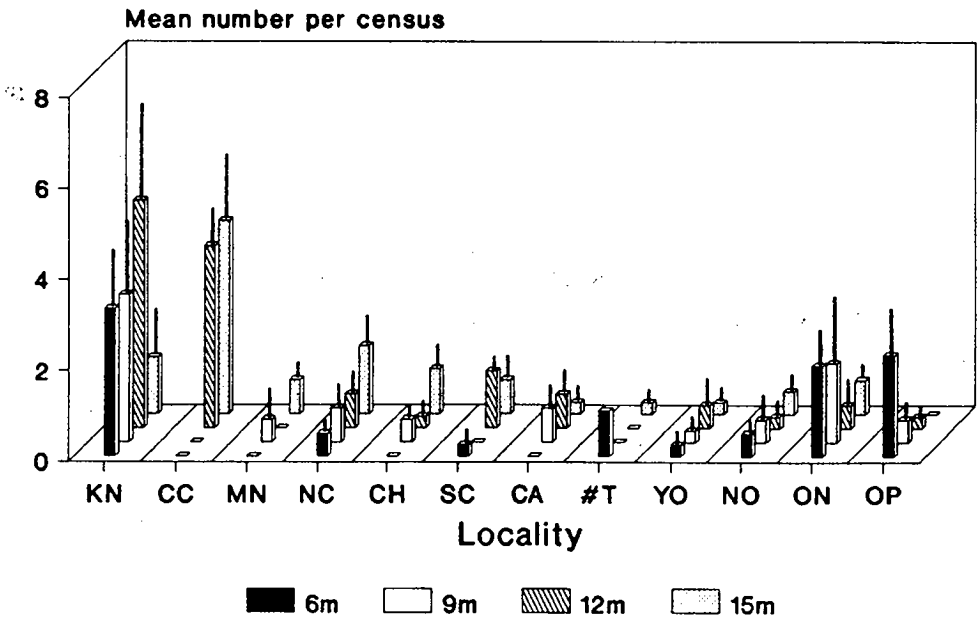


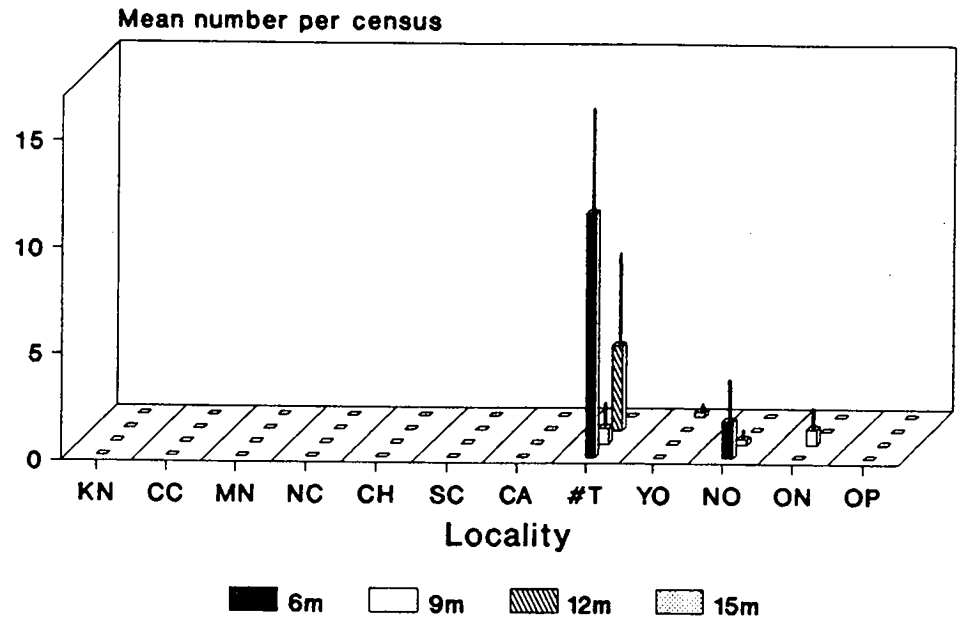
**Fig. 2.77 Depth distribution of *Naso tuberosus* on GBR outer reefs and Tuvalu atolls.**

Mean number of individuals per transect (n=4) ± standard error.
Abbreviations:
Tuvalu sites: KN = Kulia North (Niutao), CC = Canoe City (Niutao), MN = Muli North (Niutao), NC = North Control (Nui), CH = Channel (Nui), SC = South Control (Nui).
GBR outer reef sites: CA = Carter Reef, #T = No. 10 Ribbon Reef, YO = Yonge Reef, NO = NoName Reef, ON = North site on Osprey Reef, OP = Pass site on Osprey Reef.

**Fig. 2.78 Depth distribution of *Naso unicornis* on GBR outer reefs and Tuvalu atolls.**

Mean number of individuals per transect (n=4) ± standard error.
Abbreviations:
Tuvalu sites: KN = Kulia North (Niutao), CC = Canoe City (Niutao), MN = Muli North (Niutao), NC = North Control (Nui), CH = Channel (Nui), SC = South Control (Nui).
GBR outer reef sites: CA = Carter Reef, #T = No. 10 Ribbon Reef, YO = Yonge Reef, NO = NoName Reef, ON = North site on Osprey Reef, OP = Pass site on Osprey Reef.

# *N. tuberosus*




# *N. unicornis*

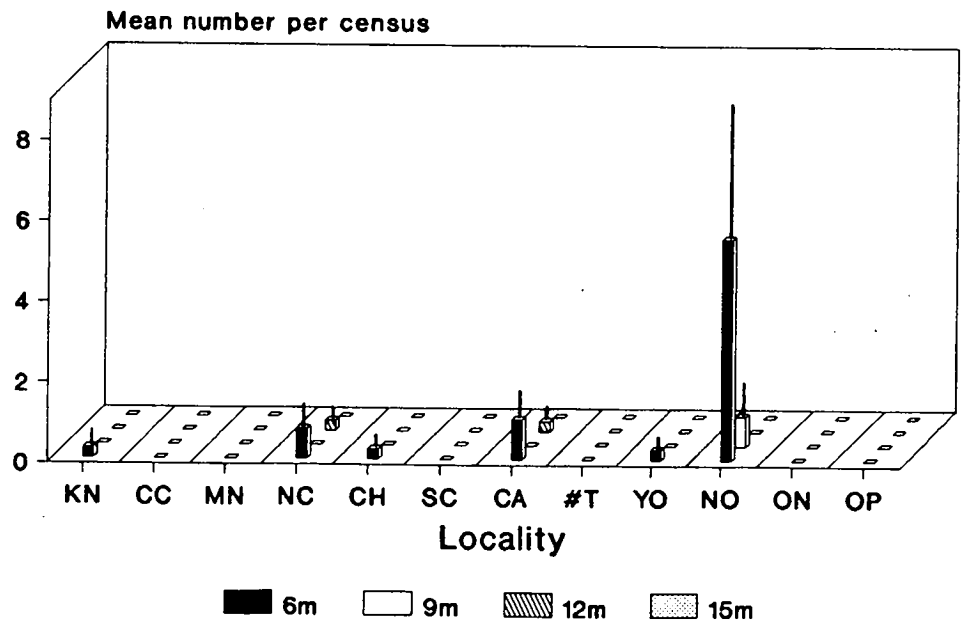


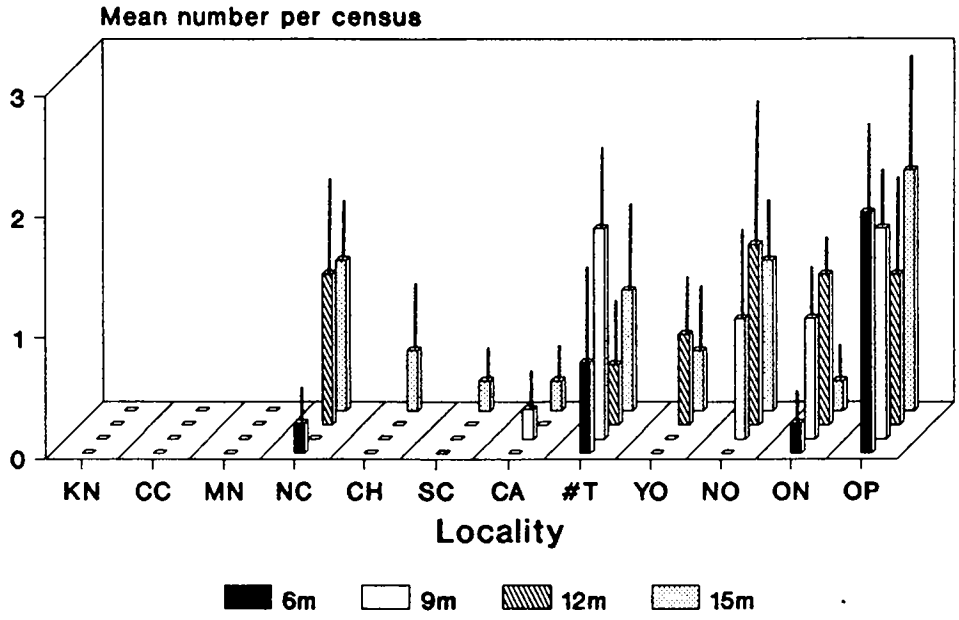
**Fig. 2.79 Depth distribution of *Naso vlamingii* on GBR outer reefs and Tuvalu atolls.**

Mean number of individuals per transect (n=4) ± standard error.
Abbreviations:
Tuvalu sites: KN = Kulia North (Niutao), CC = Canoe City (Niutao), MN = Muli North (Niutao), NC = North Control (Nui), CH = Channel (Nui), SC = South Control (Nui).
GBR outer reef sites: CA = Carter Reef, #T = No. 10 Ribbon Reef, YO = Yonge Reef, NO = NoName Reef, ON = North site on Osprey Reef, OP = Pass site on Osprey Reef.

**Fig. 2.80 Depth distribution of *Zebrasoma rostratum* on GBR outer reefs and Tuvalu atolls.**

Mean number of individuals per transect (n=4) ± standard error.
Abbreviations:
Tuvalu sites: KN = Kulia North (Niutao), CC = Canoe City (Niutao), MN = Muli North (Niutao), NC = North Control (Nui), CH = Channel (Nui), SC = South Control (Nui).
GBR outer reef sites: CA = Carter Reef, #T = No. 10 Ribbon Reef, YO = Yonge Reef, NO = NoName Reef, ON = North site on Osprey Reef, OP = Pass site on Osprey Reef.

# *N. vlamingii*




# *Z. rostratum*

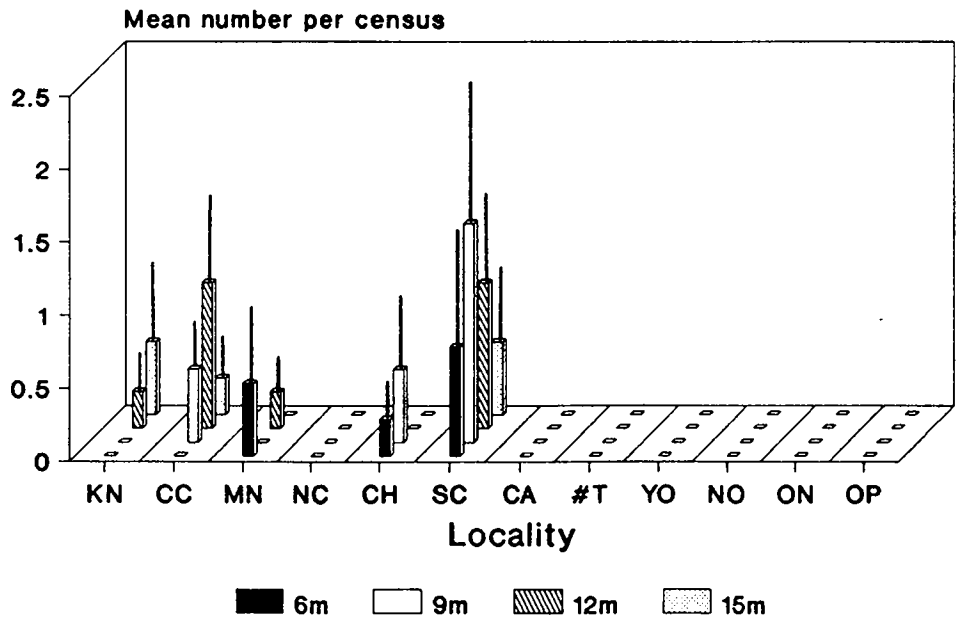


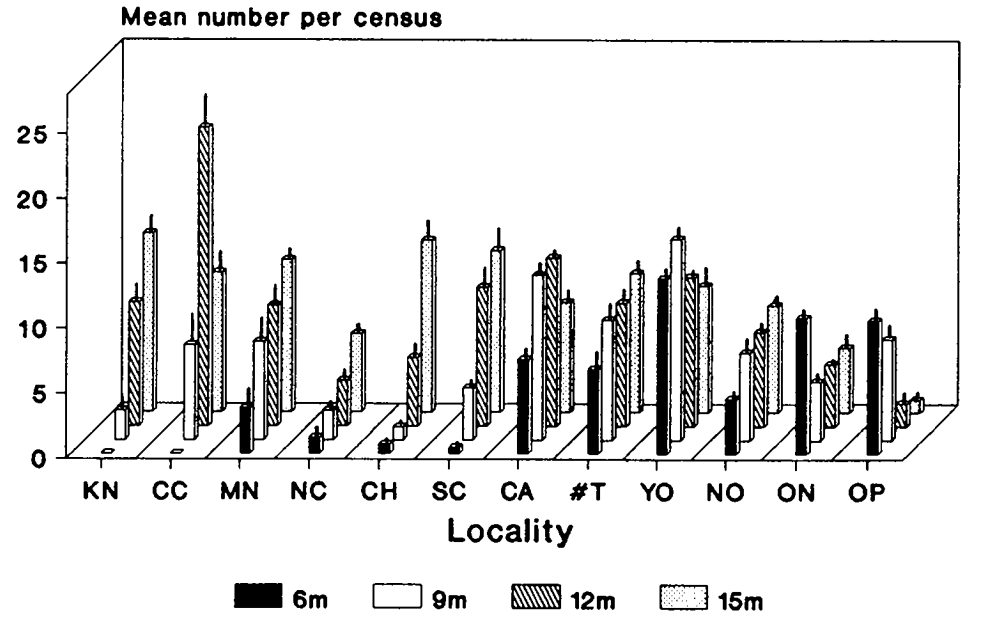
**Fig. 2.81 Depth distribution of *Zebrasoma scopas* on GBR outer reefs and Tuvalu atolls.**

Mean number of individuals per transect (n=4) ± standard error.
Abbreviations:
Tuvalu sites: KN = Kulia North (Niutao), CC = Canoe City (Niutao), MN = Muli North (Niutao), NC = North Control (Nui), CH = Channel (Nui), SC = South Control (Nui).
GBR outer reef sites: CA = Carter Reef, #T = No. 10 Ribbon Reef, YO = Yonge Reef, NO = NoName Reef, ON = North site on Osprey Reef, OP = Pass site on Osprey Reef.

**Fig. 2.82 Depth distribution of *Zebrasoma veliferum* on GBR outer reefs and Tuvalu atolls.**

Mean number of individuals per transect (n=4) ± standard error.
Abbreviations:
Tuvalu sites: KN = Kulia North (Niutao), CC = Canoe City (Niutao), MN = Muli North (Niutao), NC = North Control (Nui), CH = Channel (Nui), SC = South Control (Nui).
GBR outer reef sites: CA = Carter Reef, #T = No. 10 Ribbon Reef, YO = Yonge Reef, NO = NoName Reef, ON = North site on Osprey Reef, OP = Pass site on Osprey Reef.

# *Z. scopas*




# *Z. veliferum*

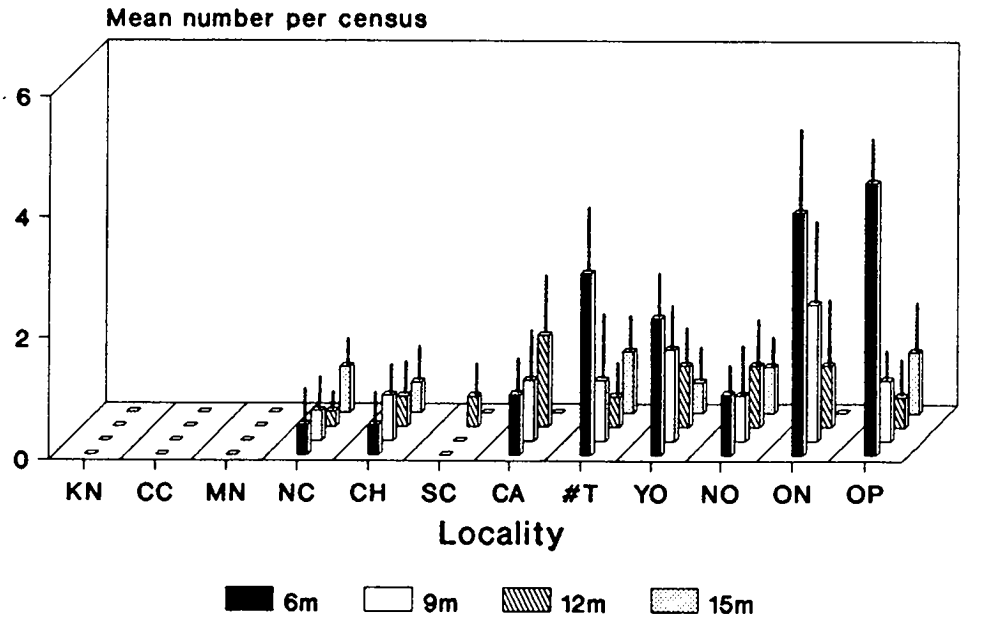


**Fig. 2.83 Abundance of 'tropical' acanthurid taxa in southern Queensland and northern New South Wales.**

Mean number of individuals per transect (n=4) $\pm$ standard error.
Abbreviations: A.d = *Acanthurus dussumieri*, A.n = *A. nigrofuscus*, A.o = *A. olivaceus*, A.t = *A. triostegus*, C.b = *Ctenochaetus binotatus*, C.s = *C. striatus*, N.a = *Naso annulatus*, N.t = *N. tuberosus*, N.u = *N. unicornis*, P.h = *Paracanthurus hepatus*, Z.s = *Zebrasoma scopas*.

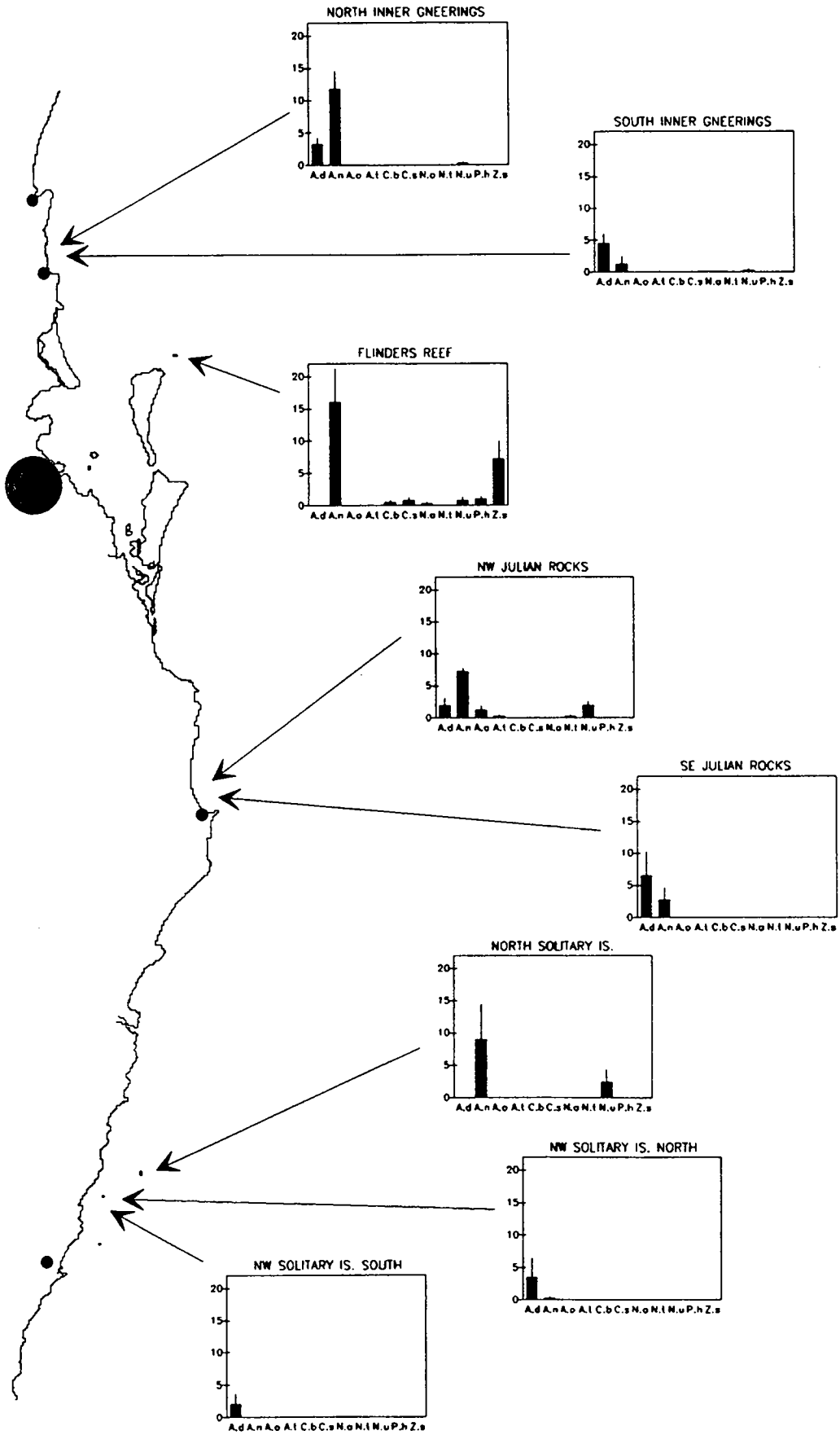
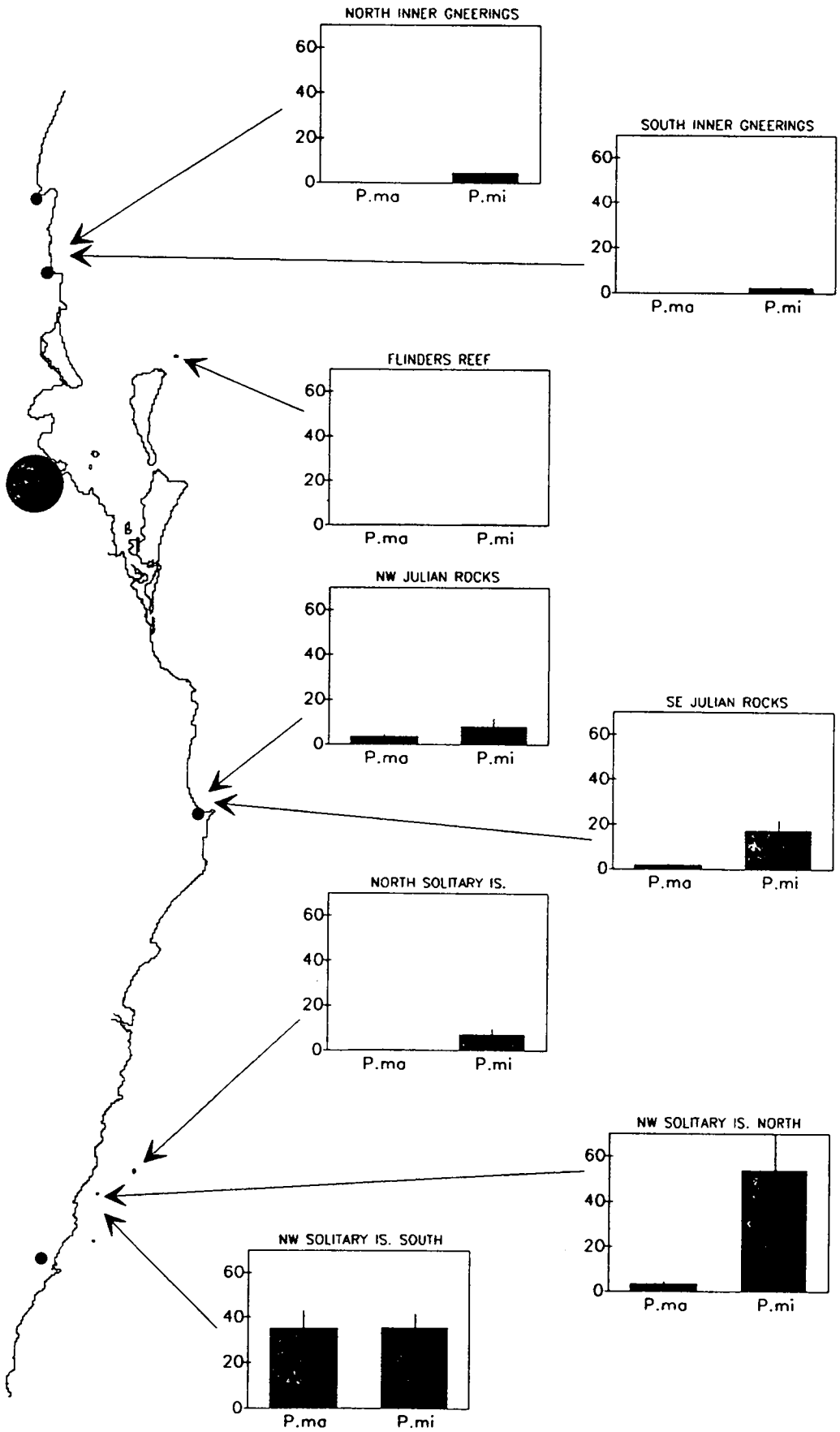

NORTH INNER GNEERINGS
SOUTH INNER GNEERINGS
FLINDERS REEF
NW JULIAN ROCKS
SE JULIAN ROCKS
NORTH SOLITARY IS.
NW SOLITARY IS. NORTH
NW SOLITARY IS. SOUTH
A.d A.n A.o A.t C.b C.s N.o N.t N.u P.h Z.s

**Fig. 2.84 Abundance of *Prionurus* spp. in southern Queensland and northern New South Wales.**

Mean number of individuals per transect (n=4) $\pm$ standard error.
Abbreviations: P.ma = *Prionurus maculatus*, P.mi = *P. microlepidotus*.


NORTH INNER GNEERINGS
60
40
20
0
P.ma
P.mi
SOUTH INNER GNEERINGS
60
40
20
0
P.ma
P.mi
FLINDERS REEF
60
40
20
0
P.ma
P.mi
NW JULIAN ROCKS
60
40
20
0
P.ma
P.mi
SE JULIAN ROCKS
60
40
20
0
P.ma
P.mi
NORTH SOLITARY IS.
60
40
20
0
P.ma
P.mi
NW SOLITARY IS. NORTH
60
40
20
0
P.ma
P.mi
NW SOLITARY IS. SOUTH
60
40
20
0
P.ma
P.mi

## 2.4 DISCUSSION

The detailed distribution and abundance data presented in this chapter provide two different sets of information relevant to the study of endosymbiosis in acanthurids. The first of these is adult distribution. The patterns of space utilization of adult acanthurids, combined with published data on feeding behaviour, allow an assessment of the ecological similarity of the species of acanthurids examined in this study. Clearly, an understanding of ecological similarity is an essential factor in interpreting patterns of endosymbiont occurrence amongst species. Thus while the patterns of adult distribution will be discussed briefly below, their significance in the context of endosymbiosis will be developed further in later chapters. The second set of information in this chapter relevant to endosymbiosis is juvenile distribution, and in particular the way this relates to the distribution of adults. The discussion below will thus firstly compare the distribution of adult acanthurids amongst all sites investigated, and secondly focus on the relationship between juvenile and adult distributions at Lizard Island.

At the spatial scale investigated in this study, it was evident that there was considerable overlap in the adult distribution of acanthurid species. Adults of species such as *A. blochii*, *A. dussumieri*, *A. nigricauda*, *A. nigrofuscus*, *A. olivaceus*, *Naso lituratus*, and *N. unicornis* occurred at all depths surveyed, and appeared to be habitat-generalists. Several non-schooling species, such as *Ctenochaetus striatus* and the two *Zebrasoma* species, were also widespread in terms of depth distribution. Only two species appeared to be habitat-specialists: *A. lineatus* and *A. triostegus*.

Both species were only common in shallow sites, and the latter species was only recorded adjacent to emergent land at Lizard Island. How do these distribution patterns compare with those of published studies?

Russ (1984b) found a wide range of patterns of within-reef abundance in his study of the herbivorous fish fauna of midshelf and outershelf reefs of the GBR. The midshelf reefs censused by Russ were not fringing reefs like those around Lizard Island, being more topographically similar to the outer reefs of the present study. Nevertheless, the similarity between Russ' work and the present study in terms of habitat stratification and census technique makes a detailed comparison worthwhile. Russ detected 3 generalized patterns of abundance: (i) species abundant in only 1 or 2 reef zones (analogous to habitats in the present study), (ii) species peaking in abundance in 1 zone but occurring in many other zones, and (iii) species that were almost equally abundant in all zones. Three acanthurid species were characteristic of the first pattern: *Acanthurus lineatus* (reef crest), *A. triostegus* (reef crest, reef flat), and *Ctenochaetus binotatus* (reef slope, back reef). *A. blochii* (referred to as *A. 'mata'* by Russ) and *A. nigrofuscus* were most abundant in 1 or 2 zones, but were also commonly present elsewhere. Finally, *Naso unicornis* was equally abundant in all zones. These results are very similar to those generated by the present study at GBR sites.

Detailed comparisons between the results of the present study and other published data are made difficult by differences in sampling methodology, habitat stratification, and means of data presentation. It is thus only possible to compare

patterns of adult abundance derived from different studies in general terms. Some species vary considerably in habitat distribution between localities. For example, the depth distribution of *Acanthurus triostegus* varied considerably between Hawaii (Barlow 1974b), Aldabra (Robertson and Gaines 1986), Moorea (Galzin 1987a), and Nanumea, Tuvalu (Kaly and Jones 1990). These differences may in part be explicable in terms of environmental differences, such as exposure, between these localities.

Differences in habitat distribution between localities may also be due to the presence of an agonistically dominant species in a preferred habitat. Thus the relatively high abundance of *Acanthurus nigrofuscus* on the crest at Bird Island may be due to the very low numbers of *A. lineatus* at this habitat/locality site. *A. nigrofuscus* was relatively uncommon on the crest of oblique reefs at Lizard island, where *A. lineatus* were abundant. *A. lineatus* is highly aggressive towards schooling herbivores such as *A. nigrofuscus* (Choat and Bellwood 1985). Similarly, a negative correlation between the abundance of *A. triostegus* and *A. nigrofuscus* on Hawaiian reefs was reported by Barlow (1974b). Whatever the factors influencing habitat distribution may be, it is evident that detailed site-specific knowledge is required to assess the distribution of adult acanthurids within a given area. This detailed information will be used in later chapters to interpret the distributions of endosymbionts among different host species.

The census data collected at the subtropical sites show that a number of acanthurid species survive and grow to adult size in environments very different from true

coral reefs. *Prionurus maculatus* and *P. microlepidotus* were very abundant at some of these sites. The distribution of these two species suggests that they are most common in the intermediate environments between coral-dominated tropical reefs and macroalgae-dominated temperate reefs. Only 3 `tropical' species were frequently recorded in these subtropical areas however: *Acanthurus dussumieri*, *A. nigrofuscus* and *Naso unicornis*. All of these species were recorded in most coral reef habitats censused in this study, and thus do not appear to have precise habitat requirements. Interestingly, the only subtropical site at which the detritivorous *Ctenochaetus* species were recorded was Flinders Reef. Although it is not a true coral reef (being composed of sandstone rather than limestone), Flinders Reef nevertheless has a dense coral cover and a high coral diversity (Veron 1986). The absence of *Ctenochaetus* species from subtropical sites other than Flinders Reef, plus the fact that these species are not present on inshore reefs of the GBR (Russ 1984a), suggests that the distribution of these fishes may not be solely due to larval supply. It is possible that *Ctenochaetus* species, at least as juveniles, may have more specific habitat requirements than many herbivorous acanthurids.

The distribution and abundance results for juvenile acanthurids make two major points relevant to endosymbiont transmission: (a) most acanthurid species settled in very low numbers relative to adult densities; and (b) most acanthurid species settled in areas where adult conspecifics were common. It is thus likely that juveniles of the majority of acanthurid species encountered conspecific adults with reasonable frequency after settlement. The following discussion examines the above two points in turn.

Many acanthurid species which were moderately common as adults at Lizard Island (e.g. *Acanthurus lineatus*, *A. olivaceus*, *Naso brevirostris*, *N. unicornis*, and *Z. veliferum*) showed a recurrent pattern over 3 seasons of very limited settlement. Even abundant species such as *Ctenochaetus striatus* and *Zebrasoma scopas* settled in relatively low numbers throughout the 3 years of the study. Juveniles of only 2 species were common during the study, *A. nigrofuscus* and *C. binotatus*. Juveniles of the former species were abundant at several sites during the summer of 1988/89. Both *A. nigrofuscus* and *C. binotatus* showed marked variation in the magnitude of settlement between years.

Variability in the magnitude of settlement between years is characteristic of many coral reef fishes (e.g. Williams 1980, Williams and Sale 1981, Williams 1983, Eckert 1984, Doherty and Williams 1988, Mapstone and Fowler 1988, Robertson 1988a), including acanthurids (Pillai et al. 1983). In a study of the settlement of Caribbean acanthurids, Robertson (1988a) found a 4- to 7-fold variation in the total numbers of settlers of *Acanthurus bahianus*, *A. chirurgus* and *A. coeruleus* over an 8 year period. The abundance of newly settled juveniles of *A. nigrofuscus* and *Ctenochaetus striatus* varied by approximately an order of magnitude within the 3 years of the present study.

One of the Lizard Island sites appeared exceptional in terms of the number of juveniles which settled there throughout the three seasons of the study. Pidgin Point was a disproportionately important settlement site for several acanthurid species, notably *Acanthurus lineatus*, *A. mata*, *A. nigrofuscus*, *A. triostegus*, *A. pyroferus*

and *Zebrasoma scopas*. The fact that this settlement was distributed amongst all of the habitats within this site raises the possibility that the predominance of this site was due to larval delivery rather than any particular habitat characteristics of the site. This hypothesis was invoked by Robertson (1988b), who found that spatial patterns of settlement of *Balistes vetula* were consistent with delivery by water currents.

At Lizard Island, the majority of acanthurid species displayed a strong tendency to settle most abundantly in areas where adults were also common. This was particularly true for *Acanthurus lineatus*, *A. nigrofuscus*, *A. olivaceus*, *A. triostegus*, *Ctenochaetus binotatus* and *C. striatus*, and to a lesser extent for *Naso brevirostris* and *N. tuberosus*. Although *C. striatus* settled in sites where adults were common, the low numbers of juveniles of this species contrasted strongly with the abundance of the adults. The reverse was true of *C. binotatus*, which were more common as juveniles than adults. If the 3 seasons of this study are representative of settlement patterns as a whole, there must be major differences between the demographic processes of the two *Ctenochaetus* species.

The settlement distributions of some species did not correspond well to adult distributions. This was particularly true for *Naso unicornis*, *N. vlamingii*, *Zebrasoma scopas* and *Z. veliferum*. Indeed, for *N. unicornis* and *Z. scopas* the results indicated that juveniles tended to settle in areas where adults **did not** occur, while the distribution of *Z. veliferum* juveniles appeared independent of that of adults. Adults of *Naso* species form schools and are highly mobile (Hiatt and

Strasburg 1960, Jones 1968), and indeed this mobility is also characteristic of juveniles. Juveniles of *Naso* species settled into areas of low coral cover on the reef flat or slope, and were noticeably more vagile than the more site-attached *Acanthurus* or *Ctenochaetus* juveniles.

The separation of the juvenile and adult distributions of *Zebrasoma scopas* detected in the chi-square analysis requires some examination. Juveniles of this species settled predominantly in the crest base habitats, where adults did occur but were relatively uncommon. Adult *Z. scopas* were most abundant in the shallower crest habitats around Lizard Island. Thus in many cases juveniles of this species occupied sites almost directly beneath adult conspecifics. One possible hypothesis to account for the difference in juvenile and adult depth distribution is that the newly settled juveniles, which are deep-bodied and highly-compressed (Randall 1955a), are more sensitive to turbulent water conditions than the more robust adults. This hypothesis may also account for the distribution of juvenile *Z. veliferum*, which are similar in shape to juvenile *Z. scopas* (Randall 1955a) and also settle predominantly in sheltered areas.

In summary, at Lizard Island the majority of acanthurid species settled in areas where conspecific adults were present, if not common. Thus it is likely that most juveniles forage in areas visited by adult conspecifics, even if adults feed in slightly different microhabitats (see Chapter 3). Juveniles of most species would therefore be exposed to adult faecal material, the presumed vector for endosymbiont transmission (Fishelson et al. 1985), soon after settlement. This may not be the case for several

*Naso* species and the two *Zebrasoma* species however, since juveniles of these species occurred in areas where adults were not common.

# CHAPTER 3: ASPECTS OF JUVENILE ACANTHURID BEHAVIOUR

## 3.1 INTRODUCTION

Intergenerational transfer of microbes is a critical element in the maintenance of mutualistic symbioses (Troyer 1982, Troyer 1984, Jones 1984, Smith and Douglas 1987). Troyer (1984) argued that herbivores must evolve specialized behaviours to assure the transmission of microbes to their offspring. Mechanisms ensuring intergenerational continuity in insects include retention of part of the peritrophic membrane, cyst and spore production into the habitat, social stomatodoel and proctodoel feeding, and transport and transfer of mutualist inoculum (references in Jones 1984). Since the fermentative microbes of vertebrates typically have narrow environmental tolerances (Troyer 1984), and thus are unable to survive outside the host environment for long, the mechanism of intergenerational transfer must in general involve direct contact between generations. Herbivorous mammals acquire fermentative microbes through contact with the mother, usually by consuming faecal material (Hungate 1966). Juvenile herbivorous iguanas acquire microflora by actively consuming the faeces of adult conspecifics (Troyer 1982). However, very little is known about the mechanisms of intergenerational transfer employed by herbivorous fishes.

Rimmer (1986) investigated the development of microbial digestion in a subtropical herbivorous fish, *Kyphosus cornelii*. He found that juveniles of less than 36mmFL lacked a significant microbial population in their gut. While Rimmer found no direct

evidence of how the fish acquired endosymbionts, his observations on the feeding behaviour of juveniles suggested a likely mechanism. Juvenile and adult *K. cornelii* utilised the same feeding substrata, and the water in the vicinity of these areas was often clouded with faecal material (Rimmer 1986). Since juveniles were observed to feed on material drifting in the water column, it was likely that they acquired endosymbionts by ingesting adult faecal fragments. The ingestion of adult faecal material was also proposed by Fishelson et al. (1985) as the means by which *Acanthurus nigrofuscus* became infected by their endosymbionts.

The results presented in Chapter 2 demonstrated that at Lizard Island most species of acanthurids settled into areas where adult conspecifics were common. This finding suggested that newly settled juveniles were exposed to adult faecal material in a spatial sense. However, an understanding of a number of more specific aspects of juvenile ecology is required before the mechanisms of symbiont transmission can be determined. The main focus of this chapter therefore is an examination of the ecology of juvenile acanthurids, particularly with respect to factors potentially influencing symbiont transmission such as feeding behaviour and movement patterns.

Published studies on the feeding behaviour of juvenile acanthurids are few (Randall 1961, Wolf 1985, Robertson et al. 1979, Bellwood 1988), but suggest that the diets of juvenile and adult acanthurids are very similar. With the exception of this dietary information, little is known about the behaviour of juvenile acanthurids. This is perhaps surprising, when one considers the number of published studies dealing with

aspects of adult acanthurid behaviour (e.g. Jones 1968, Robertson and Polunin 1981, Choat and Bellwood 1985, Robertson and Gaines 1986, Montgomery et al. 1989, Polunin and Klumpp 1989).

Two complementary research programs were undertaken to address the more specific aspects of symbiont transmission in acanthurids. The first involved a study to describe the daily behaviour pattern of juvenile acanthurids. Since the activities of pomacentrids and other potentially interacting species are thought to influence feeding and distribution in acanthurids (Low 1971, Robertson and Polunin 1981, Choat and Bellwood 1985, Hourigan 1986, Reinthal and Lewis 1986, Roberts 1987), agonistic interactions were also investigated. A locality component was incorporated into the design, since it has been suggested that small-scale differences in habitat structure and the density of interacting species may influence behaviour in herbivorous fish (Choat and Bellwood 1985, Montgomery et al. 1989).

The second research program involved an experimental investigation of microbiota transmission between juveniles maintained in captivity. The aims of this experiment were threefold: (a) to assess whether the presence of endosymbionts (in this case epulos) within host individuals could be monitored over time by examinations of host faeces, (b) to assess whether juvenile acanthurids maintained in captivity over a period of time would retain endosymbionts, and (c) to attempt to infect newly settled acanthurids with endosymbionts by exposing them to the faeces of known host individuals. A positive result for this experiment would indicate that, as suggested by Fishelson et al. (1985), faecal material is a potential vector for microbial

transmission in acanthurids.

The results in this chapter are divided into two main sections:

(i) the juvenile behaviour study, and

(ii) the aquarium experiment on epulo transmission.

## 3.2 MATERIALS AND METHODS

### 3.2.1 Study of juvenile behaviour

Three locations were selected for the behaviour study: Granite Bluffs, North Reef, and Pidgin Point. These represent leeward, oblique and exposed reefs respectively. Each location was subdivided into 2 sites, referred to subsequently as site I and site II. Sites were separated by a distance of approximately 30m (a distance exceeding the home range size of juvenile acanthurids as established by pilot studies). All sites lay within the outer flat habitat as defined in Chapter 2 (NB - the outer flat at Granite Bluffs was deeper than the outer flat at the other 2 locations). Both the Granite Bluffs and the Pidgin Point sites lay within the count sites of the same name described in Chapter 2. North Reef site I lay within the west end of the North Reef count site, while North Reef site II lay within the east end of the North Point count site.

Behavioural observations were divided between 4 time periods: 0600-0900, 0900-1200, 1200-1500, 1500-1800. During the period of the behaviour study (31/1/89-15/2/89), sunrise occurred between 0605 and 0612hrs, and sunset occurred between 1856 and 1851hrs. At each site 10 15-minute observation periods were conducted on juvenile *Acanthurus nigrofuscus*, during which the number of bites and agonistic interactions were recorded on a plastic slate. An optimization procedure based on pilot work using 20 minute observation periods established that 15 minute observation periods were most cost-effective. Agonistic interactions were subdivided into positive (subject fish was aggressor) and negative (subject fish was target of aggression). In addition, a visual estimate of the area ($m^2$) covered by the fish during each observation period was recorded. This estimate is referred to as 'activity range.' All observations were made while using SCUBA. An effort was made to avoid observing individual juveniles more than once during the course of the study, although the possibility that this occurred cannot be completely discounted. Each juvenile was followed for approximately 1 minute prior to recording to accustom the fish to the presence of the observer.

On each day observations were made in 2 time periods: 1 and 3 or 2 and 4. The pair of time periods to be surveyed on any given day, and the locations visited in each of these time periods, were chosen randomly. Once at a location however, both sites were sampled (i.e. 5 separate juveniles at each site were followed for a period of 15 minutes). Since each location was visited on 2 days for each time period, the order in which sites were surveyed was reversed between the 2 days. This avoided complete confounding of day, site, and intra-time period effects.

The same data as above were recorded concurrently for juvenile *Ctenochaetus striatus* by another observer (S. Barrie). Due to the lower abundance of juvenile *C. striatus*, it was possible to record only 10 15-minute observations per time period per location. Locations were not subdivided into sites for *C. striatus*, and the study area for the *C. striatus* study encompassed both of the sites described above for each locality.

The SAS General Linear Models ANOVA procedure was used for all analyses of variance. Homogeneity of variance was tested using Cochran's C test. Both the *A. nigrofuscus* and *C. striatus* activity range data displayed significant heteroscedasticity (Cochran's C test, $P<0.05$), and were thus $\log_{10}$ transformed prior to analysis. Ryan's Q tests were used for *a posteriori* comparison of means (Day and Quinn 1989).

The *A. nigrofuscus* bite rate data were initially analysed separately for each time period at each locality to examine the possibility of site*day interactions. There were two reasons for this procedure: (a) to examine the extent of variation among days, and (b) to examine the interaction between days and sites. The latter was suggestive of an intra-time period effect, while $day_1site_1 = day_1site_2$ and $day_2site_1 = day_2site_2$ suggested a day effect. A pattern was consistent with an intra-time period effect if bite rates were similar between locations for a given intra-time period on separate days. The separate analysis of the time/locality cells demonstrated that day effects were consistently non-significant ($P>0.25$) and variance among days was consistently trivial or zero. Days were therefore unlikely to cause spurious

effects since: (a) days were randomised with respect to the main effects of interest (location and time); (b) variation among days was generally trivial; and (c) because both sites at each location were sampled on each of 2 days, days were partially crossed with, and never systematically confounded with, location and time. It was thus reasonable to assume that day-to-day variation was contributing little or nothing to overall variation. Consequently days were not considered in the overall analyses, with the 5 replicates from each day being pooled to give n=10. The results for time period 1 were consistent with intra-time period effects, which would be expected to increase residual variation. The *Acanthurus nigrofuscus* bite rate data were therefore analysed with and without time period 1. Exclusion and inclusion of time period 1 made no difference to the significance of the results, thus only the latter analysis is presented.

Three replicate 30x4m transects were surveyed within each site to determine the density of territorial pomacentrids and juvenile acanthurids. Transects were randomly placed within each site, and measured using a 30m underwater tape. Transect width was measured using 2m aluminium rods. Transects were surveyed as a series of adjacent 2x2m$^2$ quadrats, which were then pooled to give the total result for 120m$^2$. Transects were done on 4/2/89 and 18/2/89.

### 3.2.2 Aquarium experiment on epulo transmission

The aquarium study on epulo transmission was conducted between 19/11/88 and 22/12/88 at the Lizard Island Research Station. Experimental aquaria were all of

equal size and were maintained throughout on a constant sea-water flow through system. Large pieces of algae-covered dead coral were provided for shelter. These were collected from natural reef substrata. The algal cover on the dead coral served as food throughout the experiment. Juvenile *Acanthurus nigrofuscus* were collected using a combination of a small drive net and the anaesthetic quinaldine from shallow reef areas in Mermaid Cove. Twelve newly settled (i.e. transparent) *A.* 'white-bar' spp. (see section 2.2) were donated by M. Meekan. Five of these fish were used in the aquarium experiment, the remaining 7 (21-25mmSL) were preserved immediately following capture. These fish were collected from artificial patch reefs using the same methods described above for *A. nigrofuscus*. Four experimental aquaria were set up as follows:

#1: contained 5 juvenile *A. nigrofuscus* collected on 24/11/88

#2: contained 5 juvenile *A. nigrofuscus* collected on 20/11/88

#3: contained 5 juvenile *A. nigrofuscus* collected on 20/11/88

#4: contained 5 newly settled *A.* white-bar spp. collected on 19/11/88

On 5/12/88 faeces from all four aquaria were collected using a siphon and wet mounted on numbered glass slides. The slides were then examined for the presence of epulos using a light microscope at high power. Following this, faeces were siphoned from aquaria #1-3 and introduced to aquarium #4. On 7/12/88 air was sucked into the seawater system, causing the death of 1 *A.* spp. from aquarium #4 and 4 *A. nigrofuscus* from aquarium #2. The remaining *A. nigrofuscus* in aquarium #2 was transferred to aquarium #1. On 12/12/88 3 further newly settled *A.* white-bar spp. juveniles were placed in aquarium #2 to serve as controls. On 13/12/88

aquarium #4 was siphoned thoroughly, and all faecal material removed. On 15/12/88 faeces were collected from aquarium #4 and examined as above. Also on 15/12/88, the 3 *A.* white-bar spp. juveniles in aquarium #2 died, probably again due to problems with the seawater intake. On 19/12/88 4 of the *A. nigrofuscus* were preserved for the examination of endosymbionts. On 22/12/88 the 4 remaining *A.* white-bar spp. were preserved for the examination of endosymbionts. The design and results of the entire experiment are summarised in Fig. 3.1.

## 3.3 RESULTS

### 3.3.1 Juvenile behaviour study

**Bite rate:** Bite rates for juvenile *Acanthurus nigrofuscus* and *Ctenochaetus striatus* are presented in Figs. 3.2 and 3.5 respectively (means values are tabulated in Appendix 3). To allow an assessment of day-to-day variation in bite rate, intra-time period means (n=5) are plotted for *A. nigrofuscus* in Fig. 3.3. The consistent difference between the 0600-0730 and 0730-0900 intra-time periods was due to the commencement of feeding during the first of these intervals. This is demonstrated by a plot of the 0600-0900 data, (Fig. 3.4).

The analysis of variance results for *A. nigrofuscus* and *C. striatus* bite rate are presented in Tables 3.1 and 3.2 respectively. Location did not significantly influence bite rate in either species, although there was a significant location*time interaction for *A. nigrofuscus* ($F=7.116$, $P<0.0050$). The source of this interaction is evident

from the mean comparisons for location, where the ranking of location means differs between time periods.

Time significantly influenced bite rate in both *A. nigrofuscus* and *C. striatus* ($F=195.285$, $P<0.0001$ and $F=74.30$, $P<0.0001$ respectively), although the effect was location dependent for *A. nigrofuscus*. A similar pattern was evident for both species. Time period 1 (0600-0900) was significantly less than the other 3 time periods for all locations. Time period 2 (0900-1200) was significantly less than time period 3 (1200-1500) at all 3 locations for *A. nigrofuscus*, but only at Granite Bluffs for *C. striatus*. There was no consistent trend between locations in mean comparisons of time period 3 (1200-1500) and time period 4 (1500-1800).

**Agonistic interactions:** Interaction data are presented for *A. nigrofuscus* and *C. striatus* in Figs. 3.6 and 3.5 respectively. No consistent trend between time periods was apparent in either positive or negative interactions for *A. nigrofuscus*. Mean rates of negative interactions for *A. nigrofuscus* appear higher at North Reef than at the other 2 locations, although this difference is probably not significant (Fig. 3.6). The positive interaction results for *C. striatus* also lack a pattern between locations that is consistent across times (Fig. 3.5). *C. striatus* negative interactions were highest for time period 1 at all 3 locations, but only markedly so at North Reef. There was little suggestion of any difference between locations in the *C. striatus* interaction data.

A breakdown of the interaction data is presented for *A. nigrofuscus* in Tables 3.3

and 3.4 and for *C. striatus* in Tables 3.5 and 3.6. Aggressive behaviour by juvenile *A. nigrofuscus* was mainly directed towards conspecific juveniles (Table 3.3). Pomacentrids were chased relatively infrequently. *Pomacentrus chrysurus* and conspecific juveniles were the main components of the juvenile *A. nigrofuscus* negative interactions (Table 3.4). *A. lineatus* were responsible for a large proportion of negative interactions at one site only, North Reef Site I.

The main objects of aggressive behaviour by juvenile *C. striatus* differed between locations (Table 3.5). At Granite Bluffs, most positive interactions were directed towards juvenile *A. nigrofuscus* and blennies. At North Reef, conspecifics (mostly juveniles), juvenile *A. nigrofuscus* and blennies were the dominant components. At Pidgin Point, blennies were the most frequent objects of aggression, followed by juvenile *A. nigrofuscus*. Pomacentrids were infrequent objects of aggression at all 3 sites. Most *C. striatus* negative interactions at both Granite Bluffs and Pidgin Point were caused by juvenile *A. nigrofuscus*, and to a lesser extent conspecifics (Table 3.6). At North Reef conspecifics were the predominant cause of aggression towards juvenile *C. striatus*, followed by juvenile *A. nigrofuscus* and *Pomacentrus chrysurus*. The latter species, along with *P. bankanensis*, was also responsible for a large proportion of the negative interactions at Pidgin Point.

**Activity range:** Activity range results for juvenile *Acanthurus nigrofuscus* and *Ctenochaetus striatus* are presented in Figs. 3.7 and 3.8 respectively (mean values of both transformed and untransformed data are tabulated in Appendix 3). No consistent time pattern is apparent for either species, although activity range was

greatest in time period 1 for *A. nigrofuscus* at each site at North Reef and Pidgin Point. The possibility of a location effect in both species is suggested by the comparatively high Granite Bluffs values for all 4 time periods. Juvenile *A. nigrofuscus* at North Reef Site I had consistently low activity range values.

The analysis of variance results for activity range of *A. nigrofuscus* and *C. striatus* are presented in Tables 3.7 and 3.8 respectively. Location and time did not significantly influence activity range in juvenile *A. nigrofuscus*, while site was highly significant ($F=7.45$, $P<0.0001$). Mean comparisons at the site level (pooled by time) show that both of the Granite Bluffs sites were significantly greater than the remaining 4 sites. Activity range at Site I at North Reef was significantly less than at the other 5 sites.

Both location ($F=14.15$, $P<0.0001$) and location*time ($F=2.91$, $P<0.0115$) were significant for juvenile *C. striatus*, while time was not. Comparisons of location means show that activity range at Granite Bluffs was significantly greater than North Reef in all time periods except time period 4, when activity range at the 2 locations was not significantly different. In all time periods Granite Bluffs ranked higher than Pidgin Point, but differences were significant in only 2 of the time periods (2 and 4).

**Herbivore densities at behaviour sites:** The densities of the most abundant territorial herbivorous and detritivorous fish species are presented in Fig. 3.9 and 3.10. The density of juvenile *A. nigrofuscus* was similar at all six sites, although it

was apparent that there were considerable differences between locations in the abundances of herbivorous pomacentrid species. The most abundant pomacentrid at Granite Bluffs was *Pomacentrus amboinensis,* while at North Reef it was *P. chrysurus*. The density of *P. chrysurus* at the 2 North Reef sites was approximately double that at the Granite Bluffs and Pidgin Point sites. Juvenile *Ctenochaetus binotatus* were only recorded from the Granite Bluffs sites, while juvenile *C. striatus* were generally less common at Granite Bluffs than at the other 2 locations (Fig. 3.10).

**Behavioural observations:** On 5 separate occasions during the behaviour study juvenile *A. nigrofuscus* were observed to ingest material defecated by juvenile conspecifics. All of these observations took place between 0615 and 0634 hrs. Two of these observations took place at Pidgin Point Site I, and one each at Pidgin Point Site II, Granite Bluffs Site I and Granite Bluffs Site II. All of the observations followed a similar pattern. The juvenile *A. nigrofuscus* formed small schools prior to the commencement of feeding, usually immediately after they emerged from night-time shelter sites. These schools consisted of between 4 and 20 individuals. Individual juveniles were observed to expel a bolus of undigested algae, which was fragmentary and quite unlike the typically consolidated faecal pellets of this species. Other juveniles in the school promptly rushed in and ingested this material before it reached the substratum. Juveniles were never observed to ingest their own bolus, as it was always ingested immediately by conspecifics. During one of the Pidgin Point observations, juveniles of a single school were seen to ingest material from 4 conspecific individuals between 0626 and 0634 hrs. Juvenile *A. nigrofuscus* were

observed to ingest labrid faeces on 2 occasions during the course of the behaviour study. These observations took place at 1046 and 1640 hrs, both at Pidgin Point.

Juvenile *C. striatus* were never observed to ingest conspecific faecal material. Indeed, juveniles of this species only defecated in characteristic 'toilet sites.' These 'toilet sites' usually consisted of a sand gutter on the periphery of the observed activity area, but sometimes juveniles would defecate in a hole in the substratum. Defecation behaviour was highly characteristic, and involved the cessation of feeding activity and a direct movement toward the 'toilet site'. Once over the 'toilet site,' the juvenile deposited a faecal pellet and then returned to the area in which it had been feeding. This process was usually repeated 3-5 times during a 15 minute observation period, but was not observed until after about 0800. Juvenile *C. striatus* were never observed to feed within a 'toilet site.' This behaviour was also characteristic of juvenile *C. binotatus*, which were observed at length during pilot studies. Juvenile *A. nigrofuscus* did not perform this behaviour, and appeared to defecate randomly over feeding areas.

### 3.3.2 Aquarium experiment on epulo transmission

The examination of faeces from all experimental aquaria on 5/12/88 revealed the following (summarised in Fig. 3.1). Type A, H and I epulos (Clements et al. 1989) were present in all of the *Acanthurus nigrofuscus* faeces from aquaria #1-3, while no epulos were found in any of the samples from aquarium #4 (which contained the newly settled *A.* white-bar spp.). Faeces collected from aquarium #4 on 15/12/88 (8

days after the introduction of *A. nigrofuscus* faeces containing epulos) did not contain epulos. Three of the 4 *A. nigrofuscus* preserved on 19/12/88 contained type H and I epulos in abundance, and 2 of these specimens also contained low numbers of type A epulos. The fourth *A. nigrofuscus* examined was the only fish which did not have a full gut, and did not contain epulos. Three of the 4 *A.* white-bar spp. preserved on 22/12/88 contained numerous type H epulos. The fourth (and smallest) fish had a fungal infection, an empty gut, and did not contain epulos. None of the 7 *A.* white-bar spp. collected at the same time as the 5 experimental fish contained epulos.

**Fig. 3.1 Epulo transmission experiment.**

Summary of experimental protocol and results. See text for details.

# Epulo transmission experiment

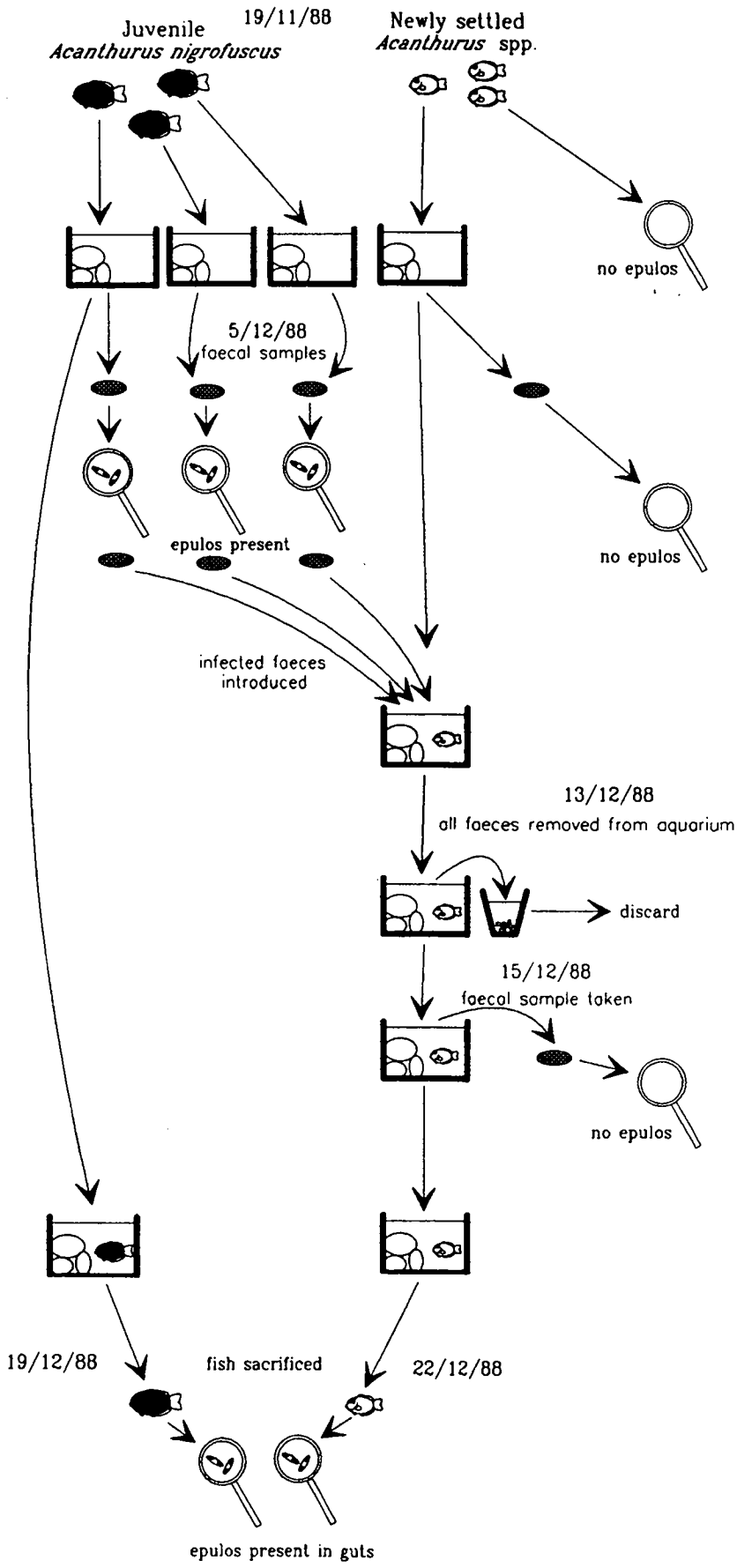
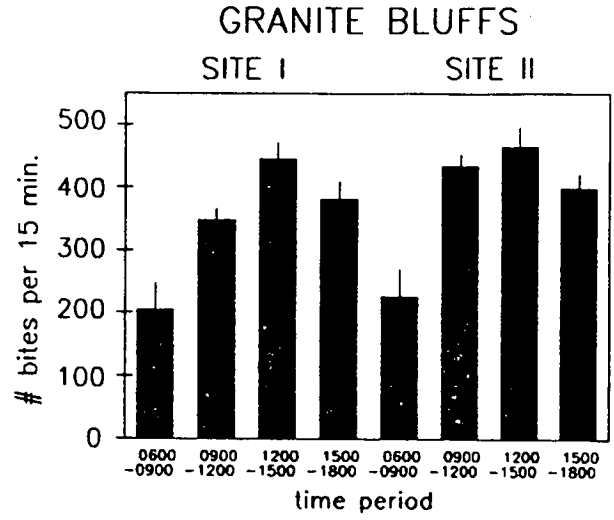
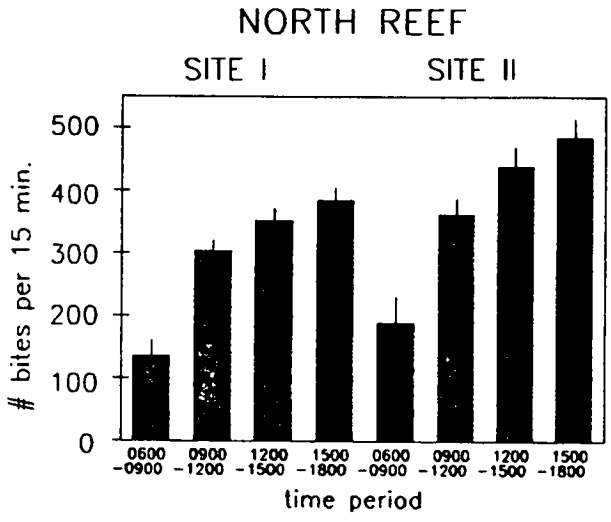


**Fig. 3.2 Juvenile *Acanthurus nigrofuscus* bite rate means.**

Mean (n=10) number of bites taken per 15 minute observation period $\pm$ standard error.

# Juvenile *Acanthurus nigrofuscus* bite rate



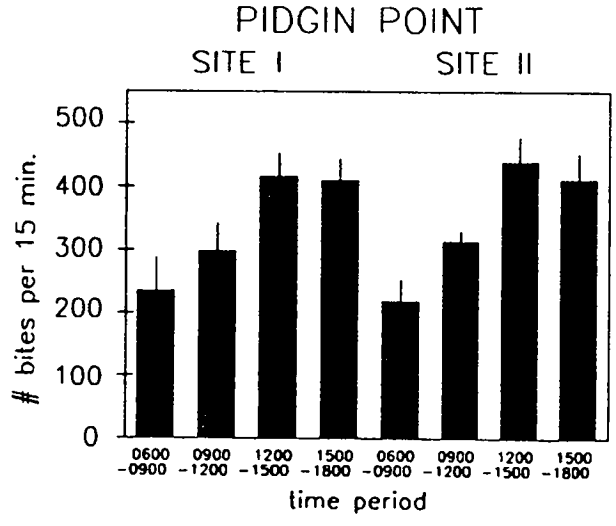



**Fig. 3.3 Intra-time period variation in juvenile *Acanthurus nigrofuscus* bite rate.**

Mean (n=5) number of bites taken per 15 minute observation period $\pm$ standard error.

# Intra–time period variation in *Acanthurus nigrofuscus* bite rate

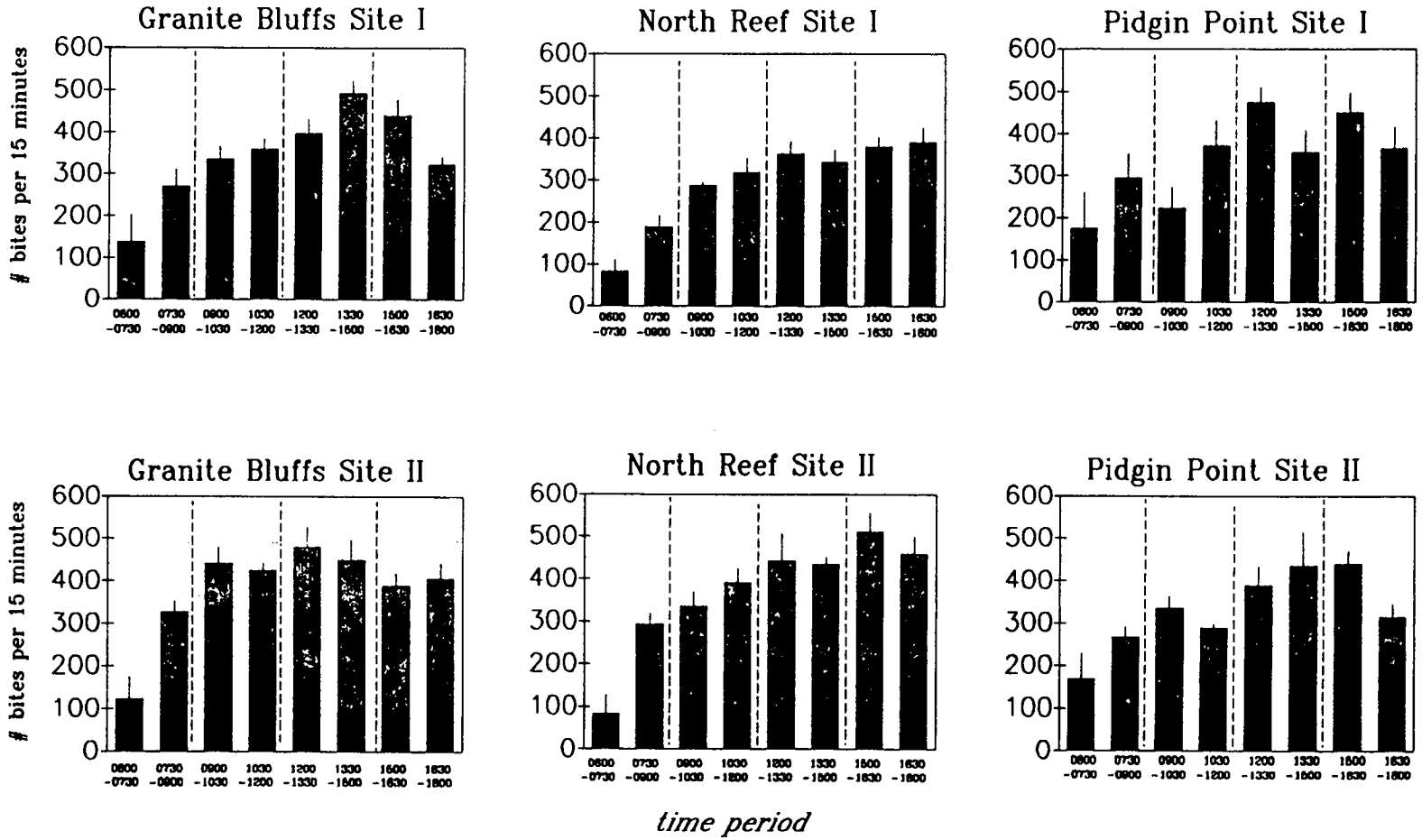


**Fig. 3.4 Plots of juvenile *Acanthurus nigrofuscus* bite rate between 0600 and 0900hrs.**

Points represent the number of bites taken by each individual per 15 minute observation period.

*Acanthurus nigrofuscus*
bite rate 0600–0900

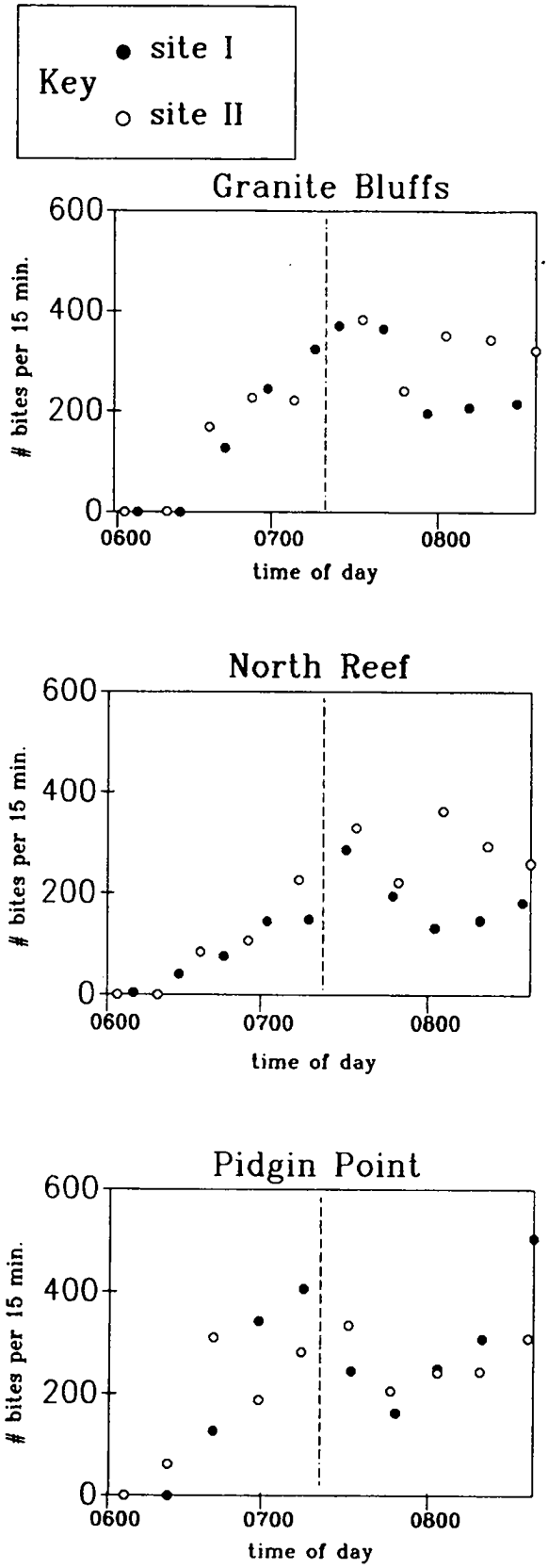
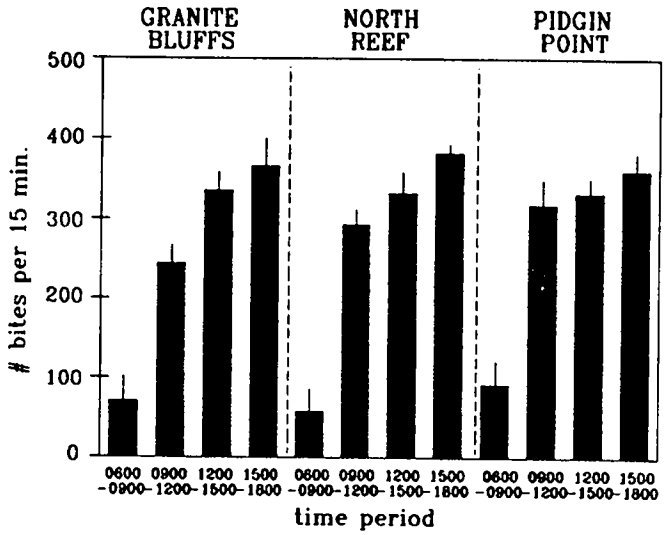


**Fig. 3.5 Juvenile *Ctenochaetus striatus* bite rate and agonistic interaction means.**

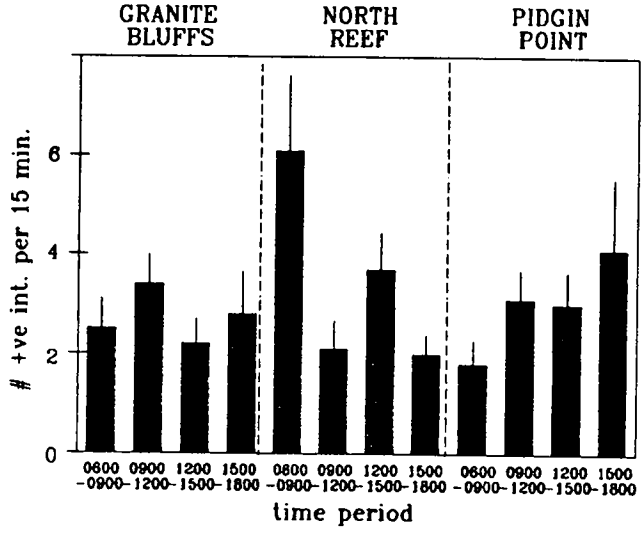
Mean (n=10) number of bites taken, mean (n=10) number of positive interactions and mean (n=10) number of negative interactions per 15 minute observation period $\pm$ standard error.

# Juvenile *Ctenochaetus striatus* behaviour

Juvenile *Ctenochaetus striatus* bite rate




Juvenile *Ctenochaetus striatus* positive interactions




Juvenile *Ctenochaetus striatus* negative interactions

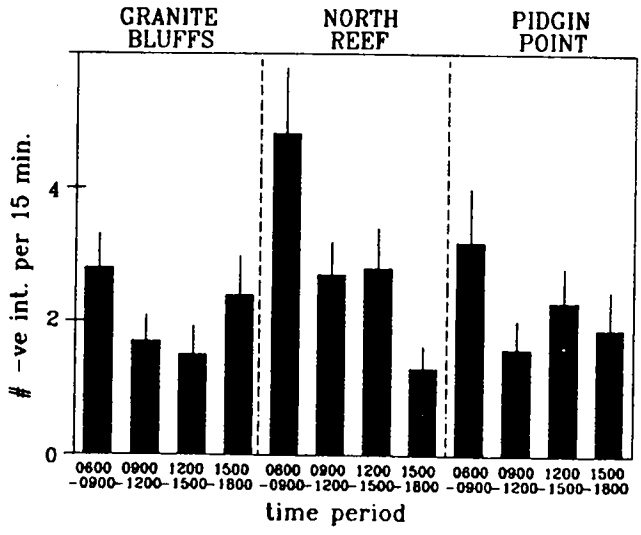
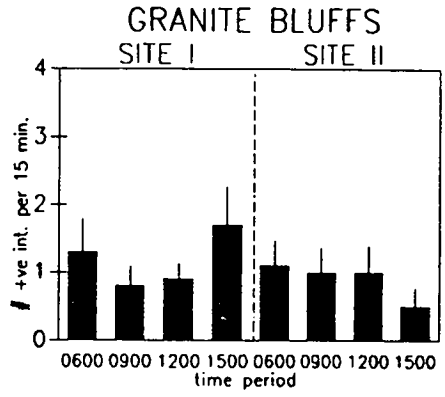


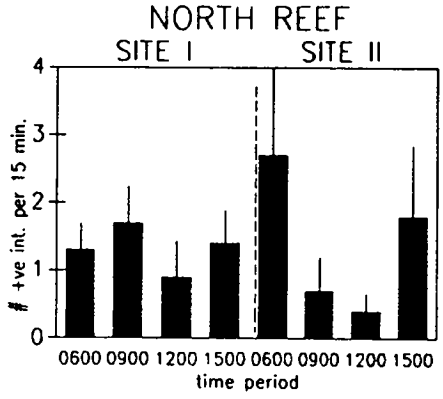
**Fig. 3.6 Juvenile *Acanthurus nigrofuscus* agonistic interaction means.**

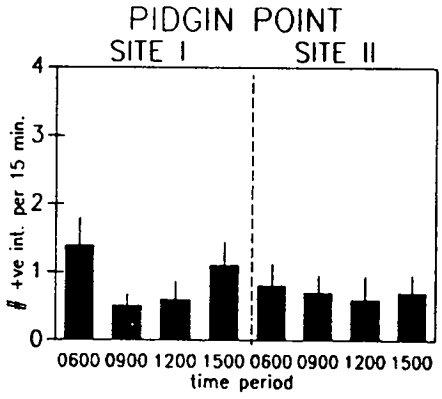
Mean (n=10) number of positive and negative interactions per 15 minute observation period $\pm$ standard error.

# *Acanthurus nigrofuscus* behaviour

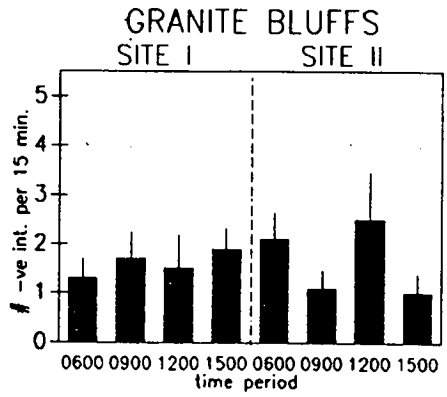
Positive interactions

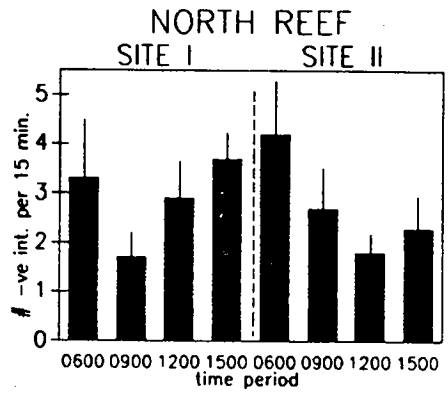



Negative interactions



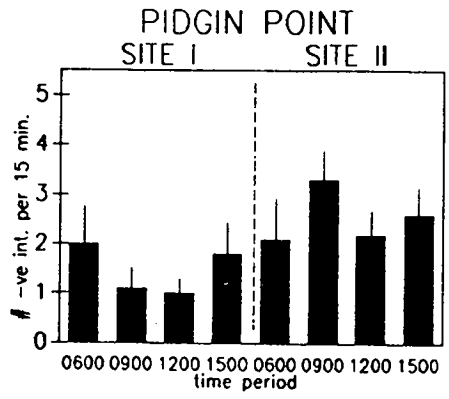
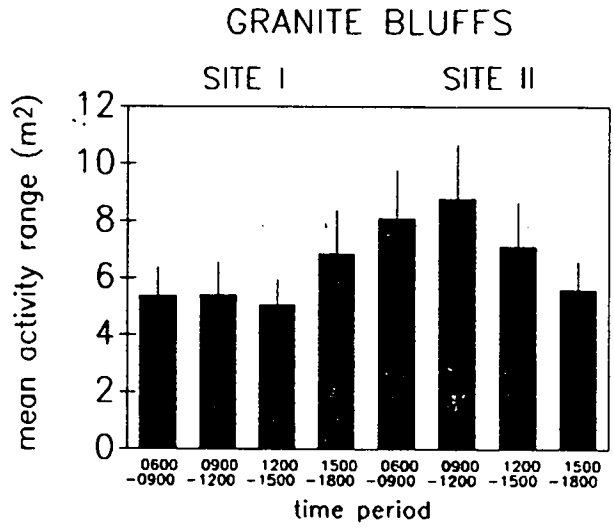
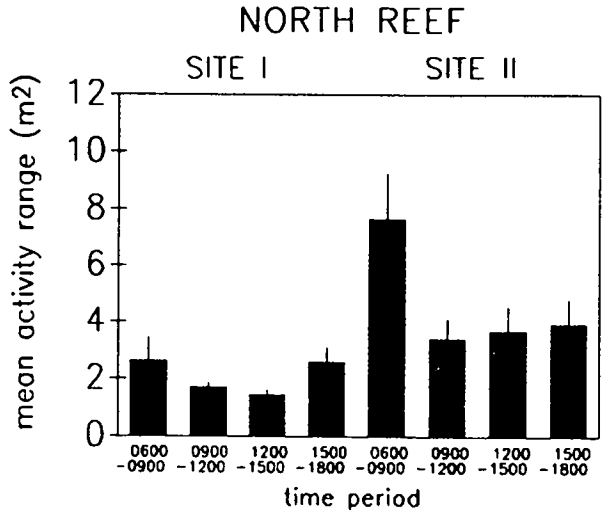



**Fig. 3.7 Juvenile *Acanthurus nigrofuscus* activity range means.**

Mean (n=10) area of activity range estimates per 15 minute observation period ± standard error.

# Juvenile *Acanthurus nigrofuscus* activity range



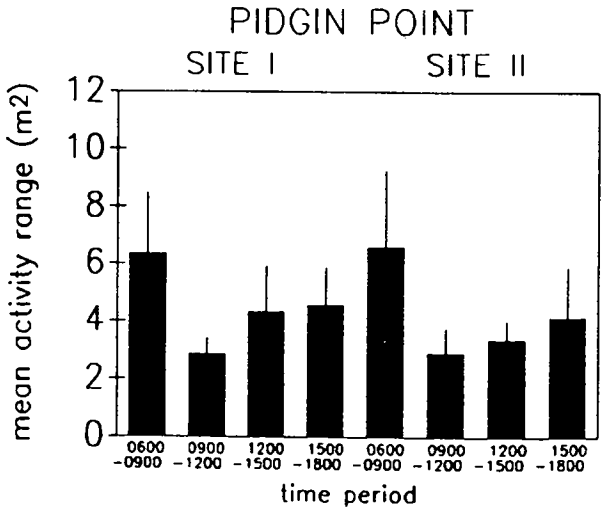



**Fig. 3.8 Juvenile *Ctenochaetus striatus* activity range means.**

Mean (n=10) area of activity range estimates per 15 minute observation period ± standard error.

Juvenile *Ctenochaetus striatus*
activity range

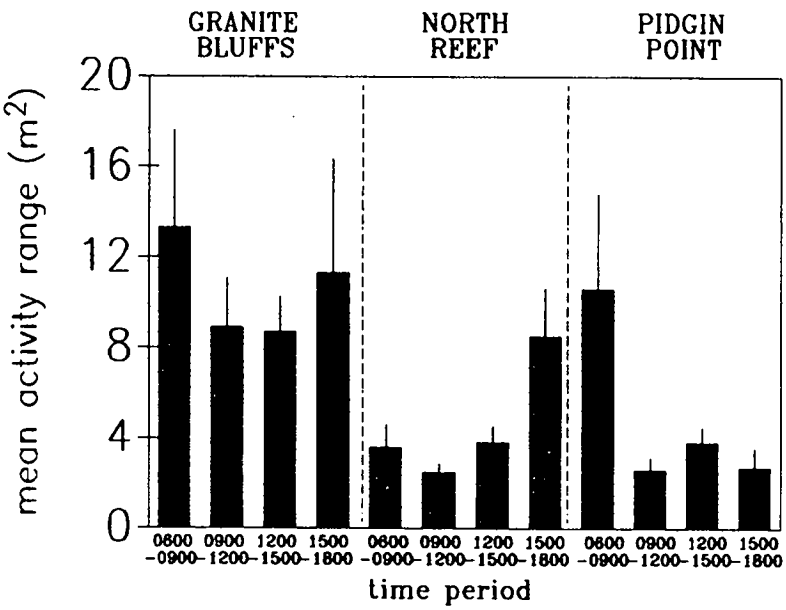
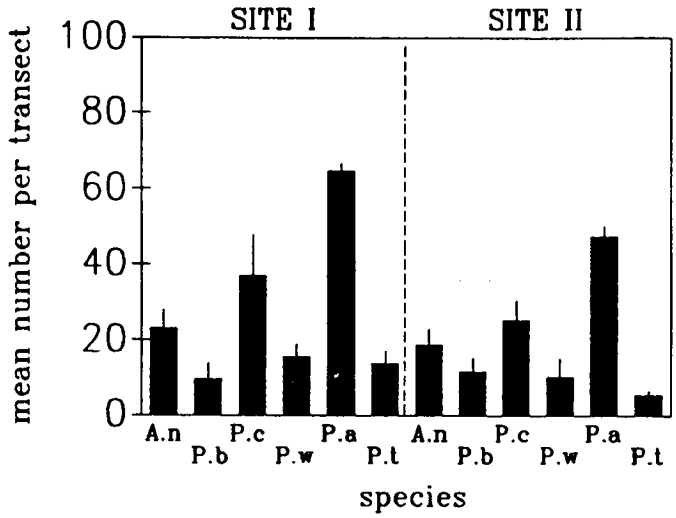


**Fig. 3.9 Herbivore densities at behaviour sites.**

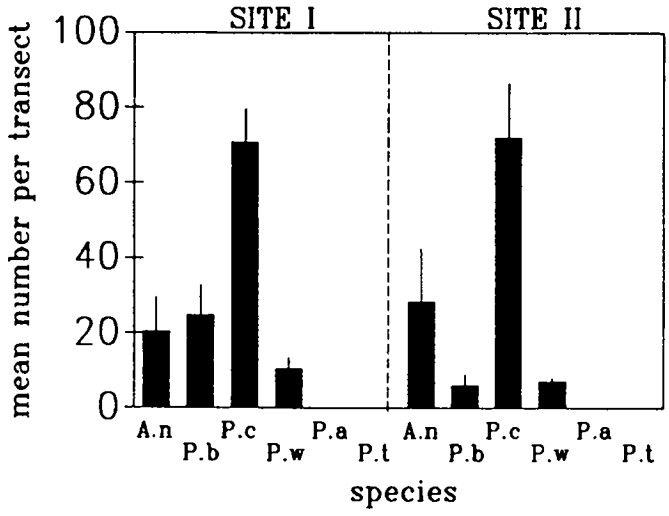
Mean (n=3) number of individuals per 30x4m transect ± standard error.
Abbreviations: A.n = juvenile *Acanthurus nigrofuscus*, P.b = *Pomacentrus bankanensis*, P.c = *P. chrysurus*, P.w = *P. wardi*, P.a = *P. amboinensis*, P.t = *P. taeniometopon*.

# Herbivore densities at behaviour sites

## Granite Bluffs




## North Reef




## Pidgin Point

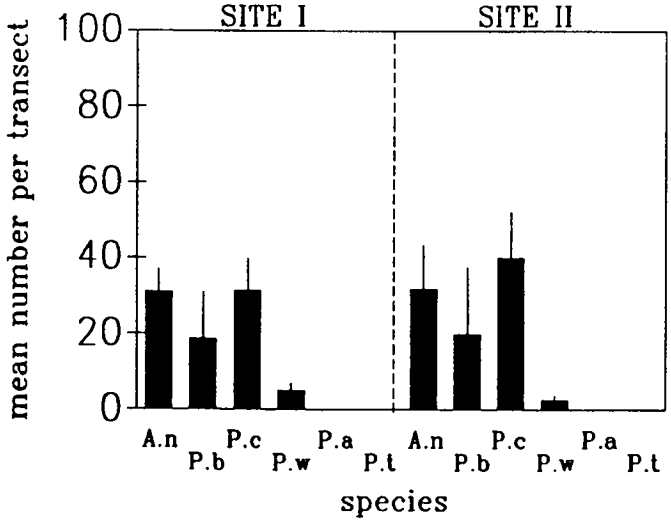
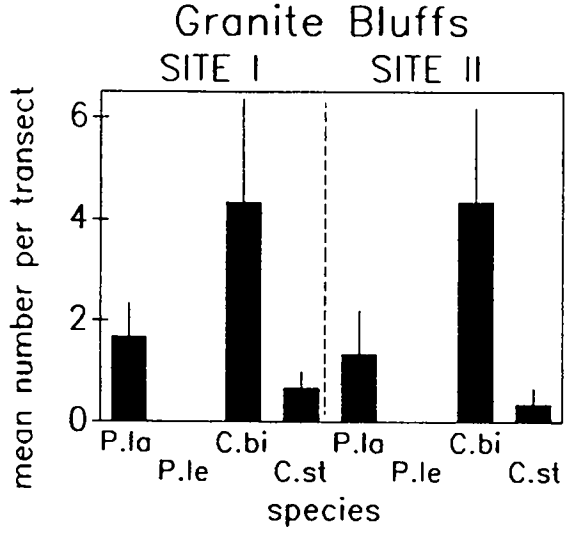
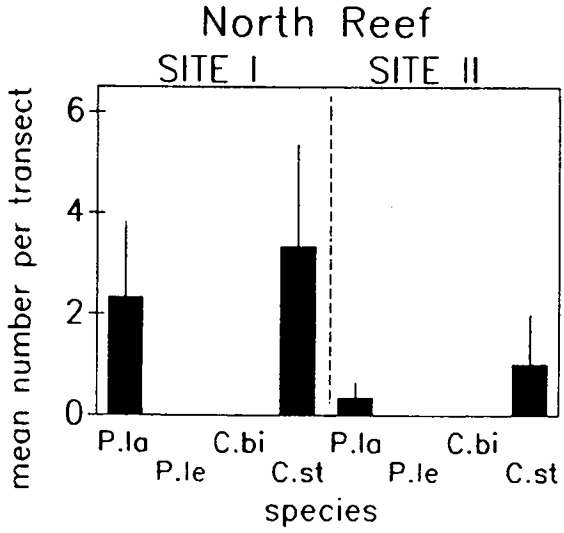


**Fig. 3.10 Densities of *Plectroglyphidodon* spp. and juvenile *Ctenochaetus* at behaviour sites.**

Mean (n=3) number of individuals per 30x4m transect $\pm$ standard error.
Abbreviations: P.la = *Plectroglyphidodon lachrymatus*, P.le = *P. leucopomus*, C.bi = juvenile *Ctenochaetus binotatus*, C.st = juvenile *C. striatus*.

# *Plectroglyphidodon* and juvenile *Ctenochaetus* densities at behaviour sites



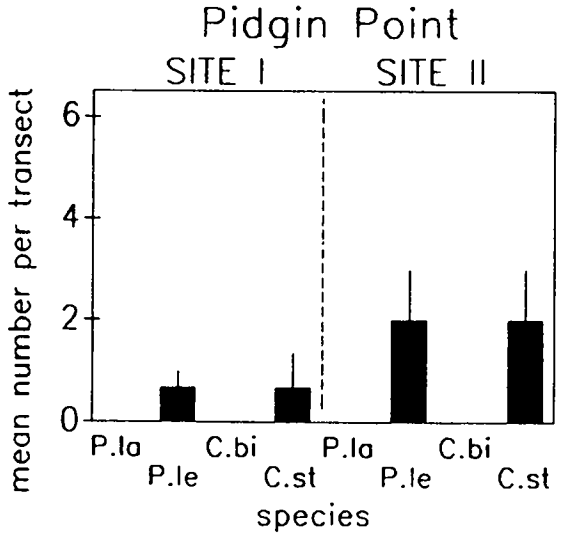



**Table 3.1** **Analysis of Variance Table for Juvenile *Acanthurus nigrofuscus* Bite Rate.**

The analysis is a mixed model with three factors: locations are fixed, sites random, and times fixed. DF = degrees of freedom, MS = mean square.

| SOURCE | DF | MS | F VALUE | Pr>F |
|---|---|---|---|---|
| LOC | 2 | 20173.363 | 0.437 | 0.6817 |
| SITE (LOC) | 3 | 46203.408 | 4.33 | 0.0055 |
| TIME | 3 | 630757.606 | 195.285 | 0.0001 |
| LOC*TIME | 6 | 22984.985 | 7.116 | 0.0050 |
| SITE*TIME (LOC) | 9 | 3229.931 | 0.30 | 0.9734 |
| ERROR | 216 | 10680.683 | | |
| TOTAL | 239 | | | |

COCHRAN'S C = 0.107 P > 0.05

| | LOCATION | | TIME |
|---|---|---|---|
| T1 | N < G P | G | T1 < T4 T2 < T3 |
| T2 | P N < G | N | T1 < T2 < T3 T4 |
| T3 | N P G | P | T1 < T2 < T4 T3 |
| T4 | G P N | | |

LINES JOIN MEANS NOT SIGNIFICANTLY DIFFERENT, P < 0.05

**Table 3.2** **Analysis of Variance Table for Juvenile *Ctenochaetus striatus* Bite Rate.**

The analysis is a two-way fixed model, factors are location and time.
DF = degrees of freedom, MS = mean square.

| SOURCE | DF | MS | F VALUE | Pr > F |
|---|---|---|---|---|
| LOC | 2 | 4236.058 | 0.63 | 0.5359 |
| TIME | 3 | 501633.142 | 74.30 | 0.0001 |
| LOC*TIME | 6 | 4416.558 | 0.65 | 0.6867 |
| ERROR | 108 | 6751.405 | | |
| TOTAL | 119 | | | |

COCHRAN'S C = 0.152 P > 0.05

TIME

| | |
|---|---|
| G | T1 < T2 < <u>T3 T4</u> |
| N | T1 < <u>T2 T3 T4</u> |
| P | T1 < <u>T2 T3 T4</u> |

LINES JOIN MEANS NOT SIGNIFICANTLY DIFFERENT, P < 0.05

**Table 3.3** **Juvenile *Acanthurus nigrofuscus* Positive Interactions.**

Abbreviations: GRA = Granite Bluffs etc., NOR = North Reef, PID = Pidgin Point, *Pom.* = *Pomacentrus*

| SPECIES | % INTERACTIONS BY SPECIES | | | | | |
|---|---|---|---|---|---|---|
| | GRA I | GRA II | NOR I | NOR II | PID I | PID II |
| juv. *A. nigrofuscus* | 42.6 | 74.3 | 88.2 | 80.7 | 91.9 | 80.0 |
| ad. *A. nigrofuscus* | - | 8.6 | - | - | - | - |
| *Pom. amboinensis* | 6.4 | 2.9 | - | - | - | - |
| *Pom. bankanensis* | 6.4 | - | 4.0 | - | 2.7 | 8.0 |
| *Pom. chrysurus* | 12.8 | 2.9 | 7.8 | 17.5 | - | - |
| *Pom. mollucensis* | 6.4 | - | - | - | - | - |
| juv. scarid | 12.8 | 8.6 | - | 1.8 | - | - |
| others | 12.6 | 2.7 | - | - | 5.4 | 12.0 |

**Table 3.4** **Juvenile *Acanthurus nigrofuscus* Negative Interactions.**

Abbreviations: GRA = Granite Bluffs etc., *Pom.* = *Pomacentrus*, *Ploc.* = *Plectroglyphidodon*

| SPECIES | % INTERACTIONS BY SPECIES | | | | | |
|---|---|---|---|---|---|---|
| | GRA I | GRA II | NOR I | NOR II | PID I | PID II |
| juv. *A. nigrofuscus* | 34.4 | 49.3 | 22.4 | 31.8 | 54.2 | 24.5 |
| *A. lineatus* | - | - | 18.1 | 3.6 | 5.1 | 1.0 |
| *Pom. amboinensis* | 6.3 | - | - | - | - | - |
| *Pom. bankanensis* | 4.7 | 6.0 | 3.4 | - | 10.2 | 4.9 |
| *Pom. chrysurus* | 35.9 | 32.8 | 51.7 | 63.6 | 23.7 | 55.9 |
| *Pom. wardi* | 7.8 | - | - | - | - | - |
| *Plec. lachrymatus* | 7.8 | 4.5 | - | - | - | - |
| *Plec. leucozona* | - | - | - | - | 3.4 | 7.8 |
| others | 3.1 | 7.4 | 4.4 | 1.0 | 3.4 | 5.9 |

**Table 3.5** **Juvenile *Ctenochaetus striatus* Positive Interactions.**

Abbreviations: GRA = Granite Bluffs etc. *Pom.* = *Pomacentrus*

% INTERACTIONS BY SPECIES

| SPECIES | GRANITE | NORTH | PIDGIN |
|---|---|---|---|
| juv. *A. nigrofuscus* | 31.4 | 27.1 | 32.0 |
| *C. binotatus* | 9.5 | - | - |
| *C. striatus* | 1.9 | 30.0 | 5.7 |
| *Pom. bankanensis* | 4.8 | 8.6 | 4.9 |
| *Pom. chrysurus* | 3.8 | 12.1 | 11.5 |
| juv. scarid | 7.6 | 2.9 | 0.8 |
| blenny | 27.6 | 17.9 | 43.4 |
| others | 13.4 | 1.4 | 1.7 |

**Table 3.6** **Juvenile *Ctenochaetus striatus* Negative Interactions.**

Abbreviations: GRA = Granite Bluffs etc. *Pom.* = *Pomacentrus*

% INTERACTIONS BY SPECIES

| SPECIES | GRANITE | NORTH | PIDGIN |
|---|---|---|---|
| juv. *A. nigrofuscus* | 41.0 | 24.8 | 49.5 |
| *A. lineatus* | - | 7.7 | - |
| *C. binotatus* | 12.0 | - | - |
| *C. striatus* | 12.0 | 31.6 | 12.1 |
| *Pom. bankanensis* | 8.4 | 6.0 | 16.5 |
| *Pom. chrysurus* | 7.2 | 23.9 | 17.6 |
| *Pom. wardi* | 7.2 | - | - |
| others | 12.2 | 6.0 | 4.3 |

**Table 3.7** **Analysis of Variance Table for Juvenile *Acanthurus nigrofuscus* Activity Range.**
***N.B. Results based on $LOG_{10}$ transformed data.**

The analysis is a mixed model with three factors: locations are fixed, sites random, and times fixed. DF = degrees of freedom, MS = mean square.

| SOURCE | DF | MS | F VALUE | Pr>F |
|---|---|---|---|---|
| LOC | 2 | 2.226 | 2.634 | 0.2186 |
| SITE (LOC) | 3 | 0.845 | 7.45 | 0.0001 |
| TIME | 3 | 0.220 | 2.242 | 0.1526 |
| LOC*TIME | 6 | 0.088 | 0.900 | 0.5342 |
| SITE*TIME (LOC) | 9 | 0.098 | 0.86 | 0.5581 |
| ERROR | 216 | 0.113 | | |
| TOTAL | 239 | | | |

COCHRAN'S C = 0.0900 P > 0.05

SITE

NI < PII PI NII < GI < GII

LINES JOIN MEANS NOT SIGNIFICANTLY DIFFERENT, P < 0.05

**Table 3.8** **Analysis of Variance Table for Juvenile *Ctenochaetus striatus* Activity Range.**
***N.B. Results based on $LOG_{10}$ transformed data.**

The analysis is a two-way fixed model, factors are location and time.
DF = degrees of freedom, MS = mean square.

| SOURCE | DF | MS | F VALUE | Pr>F |
|---|---|---|---|---|
| LOC | 2 | 1.771 | 14.15 | 0.0001 |
| TIME | 3 | 0.300 | 2.40 | 0.0720 |
| LOC*TIME | 6 | 0.364 | 2.91 | 0.0115 |
| ERROR | 108 | 0.125 | | |
| TOTAL | 119 | | | |

COCHRAN'S C = 0.1837 P > 0.05

| | LOCATION | | TIME |
|---|---|---|---|
| T1 | N < P G | G | T4 T2 T3 T1 |
| T2 | P N < G | N | T2 T1 T3 T4 |
| T3 | N P G | P | T4 T2 T3 T1 |
| T4 | P < G N | | |

LINES JOIN MEANS NOT SIGNIFICANTLY DIFFERENT, P < 0.05

## 3.4 DISCUSSION

A comparison of the results of the juvenile behaviour study with published information on adult acanthurid behaviour reveals many similarities. The pattern of feeding rate throughout the day for both juvenile *Acanthurus nigrofuscus* and *Ctenochaetus striatus* strongly resembles that of adults, with the exception of the late afternoon decline in rate detected in adults (Montgomery et al. 1989, Polunin and Klumpp 1989). The latter difference may be due to the fact that observations in the juvenile study were terminated at 1800hrs, approximately 50 minutes before sunset. Thus a pre-sunset decline in juvenile feeding rate may have gone undetected.

Daily feeding patterns of adult *A. nigrofuscus* and *C. striatus* from the Red Sea differ considerably, with the former species having a more rapid increase in rate throughout the morning and a higher maximal rate of feeding (Montgomery et al. 1989). A similar contrast between the two species is indicated by the results of the present juvenile study, although the difference in maximal feeding rate between the species as juveniles was not as marked as between the Red Sea adults. Montgomery et al. (1989) suggested that the lower feeding rate of *C. striatus* compared to *A. nigrofuscus* may be a consequence of the enhanced digestive efficiency of the former species, partly as a result of *C. striatus* grinding food in a muscular stomach. Another possibility is that *C. striatus* ingests more material per bite than *A. nigrofuscus*, and thus has a higher food intake relative to bite rate. This possibility is suggested by the much wider jaw gape of *C. striatus* relative to *A. nigrofuscus* (Jones 1968). It is likely that the difference between *A. nigrofuscus* and *C. striatus*

in gape dimension increases with fish size, which is consistent with the size-specific differences in feeding rates between the two species.

It has been suggested that daily feeding rates in herbivorous fish may be a response to diurnal variation in algal quality (Taborsky and Limberger 1980, Polunin and Klumpp 1989). This hypothesis fails to account for the daily foraging pattern of *C. striatus* however, for this species is a detritivore and ingests comparatively little algae (Randall 1955b, Hiatt and Strasburg 1960, Jones 1968, Robertson 1982, Robertson and Gaines 1986, Galzin 1987c, Nelson and Wilkins 1988). Another possibility is that the slow increase in feeding rate throughout the morning characteristic of *C. striatus* may be related to increased social or agonistic activity during this period (Montgomery et al. 1989). Support for this notion in the present study comes from the observation that negative interactions for this species were highest in the early morning at all 3 locations. This result was not however reflected in the activity range results, suggesting that interaction rate and movement range of *C. striatus* are to some extent independent.

The juvenile behaviour study failed to detect an influence of location on feeding rate in either species. The 3 locations in the study differed considerably in terms of the densities of adult acanthurids (see Chapter 2) and pomacentrids, yet this was not generally reflected in relative rates of either feeding or agonistic interactions. Juvenile *A. nigrofuscus* at North Reef did suffer relatively higher rates of negative interactions, and indeed a partial explanation for this pattern may be the relative abundance of *A. lineatus* at this location (see Chapter 2). This species is highly

aggressive towards *A. nigrofuscus* (Choat and Bellwood 1985), and was an important component of negative interactions at North Reef Site I. A major problem faced by studies of behavioural interactions between herbivorous fishes is that typically low encounter rates, combined with high variability, necessitates great sampling effort in order to achieve meaningful results. It is thus possible that there is a relationship between interaction rate and feeding rate in juvenile acanthurids, but that this was simply not detected in the present study.

Montgomery et al. (1989) suggested that the difference in *A. nigrofuscus* feeding rate between two study sites at Eilat was related to differences in the proximity of shelter. It is possible that the inequality of activity ranges between locations detected for juvenile *A. nigrofuscus* and *C. striatus* in this study may be related to this also. A comparison of the substratum composition data for the outer flat habitat at Pidgin Point, North Point, North Reef, and Granite Bluffs (Appendix 1) shows that the latter has the lowest proportion of coral cover amongst these locations (encrusting corals do not provide shelter, therefore are not considered). It is therefore possible that the higher activity range values for juvenile acanthurids at Granite Bluffs reflect the greater distance between adjacent shelter sites at this location. Some species of juvenile scarids are known to feed more intensively near to shelter sites (D.R. Bellwood pers. comm.), and it is possible that juvenile acanthurids move over a larger area in sites with less shelter to gain access to sufficient food adjacent to shelter.

There are at least two further testable hypotheses which account for the difference

between locations in *A. nigrofuscus* and *C. striatus* activity range: (i) the difference was a response to aggression from interacting species, thus activity range was reduced in North Reef where there was a high density of territorial pomacentrids; and (ii) the difference was the result of inferior food quality at Granite Bluffs, perhaps as a consequence of lower light levels (due to deeper water) and increased siltation (Granite Bluffs is a leeward site). Hypothesis (i) assumes that there is a strong relationship between density of territorial species and the rate *of* negative interactions, which as discussed above was not established by this study. This is perhaps not surprising, given that small-scale differences in interaction rates involving territorial herbivorous fishes have been reported by previous studies (e.g. Choat and Bellwood 1985, Reinthal and Lewis 1986). These studies have demonstrated that the relationships between species of herbivorous fish are complex and site-dependent, suggesting that simple quantitative comparisons of interaction rates between sites are unlikely to yield clear patterns.

In general, the results of the behaviour study suggest that juvenile acanthurids behave in a similar fashion to adult conspecifics, particularly with respect to feeding rates and diet. However, the conspecifc coprophagy by juvenile *A. nigrofuscus* observed in this study has not been reported previously for adults. Coprophagy by adult acanthurids has been investigated by Bailey and Robertson (1982) and Robertson (1982), who found that most coprophagic interactions were between members of different trophic groups. Many species were found to eat fish faeces, with faecal material moving through a trophic network (Robertson 1982). The direction of this network moved from carnivores to herbivores with low carbonate

diets of microalgae, to herbivores with low carbonate diets of macroalgae, to high carbonate diet herbivores and detritivores. The caloric value of faeces decreased through this network (Bailey and Robertson 1982), and thus coprophagy in this context is evidently of nutritional benefit. Robertson (1982) never observed conspecific (intraspecific) coprophagy in adult acanthurids.

Juvenile *A. nigrofuscus* were never observed to actively ingest faecal material from adult conspecifics in this study, although since adults frequently fed (and defecated) alongside juveniles it is most likely that adult faeces were ingested incidentally. However, juveniles were observed to eat labrid faeces on two occasions, a behaviour analogous to that reported by Robertson (1982). The nature of the conspecific coprophagy observed in juvenile *A. nigrofuscus* suggests that two distinct forms of coprophagy are practised: (i) ingestion of unconsolidated material retained overnight within the intestine of conspecifics (Fishelson et al. 1985, Montgomery et al. 1989), which occurs prior to the commencement of benthic feeding; and (ii) nutritional coprophagy, the ingestion of faecal material of other species (e.g. labrids), which occurs after the commencement of benthic feeding. The observation that this bolus of unconsolidated material is symbiont-laden (Fishelson et al. 1985), plus the fact that conspecific faeces would be of no nutritional benefit, suggests that conspecific coprophagy may be a mechanism for the transfer and/or retention of gut microbiota. Whether this behaviour is practised by adult acanthurids is unclear. Robertson (1982) does not specify the time at which his observations were recorded. Conspecific coprophagy was observed in juvenile *A. nigrofuscus* only at dawn, thus it is possible that Robertson did not detect it in his study. Because the relative

capacity of the intestinal tract decreases with a decrease in body size, retention of gut microorganisms is more critical to relatively small hosts (Stevens 1988). Conspecific coprophagy may thus be relatively more important in juvenile rather than adult *A. nigrofuscus*.

The separation of defecation and feeding areas observed in juvenile *C. striatus* in this study has also been reported for *Plectroglyphidodon lachrymatus* (Polunin and Koike 1987), *A. lineatus* and *A. nigricans* (Robertson 1982), and juvenile scarids (D.R. Bellwood pers. comm.). The fact that *A. nigrofuscus* does defecate over feeding substrata may simply be a reflection of the comparatively non-territorial nature of this species. The observation that *C. striatus* does not retain a bolus of food overnight in the posterior intestine (Montgomery et al. 1989) suggests that the failure to record conspecific coprophagy in juveniles of this species (or *C. binotatus*) does not merely reflect a failure to detect it. The apparent lack of a mechanism of microbiota retention by *C. striatus* may indicate that the relationship between this species and its intestinal endosymbionts (Clements et al. 1989) differs from that of *A. nigrofuscus*.

The results of the aquarium experiment strongly suggest that newly settled (i.e. non-infected) acanthurids may be infected with epulos by exposure to faecal material from infected hosts. The lack of a specific control means that it is not possible to completely discount the hypothesis that epulos were introduced into the aquarium during the course of the experiment through the water system or via food. This possibility is however extremely unlikely due to the scarcity of epulos outside the

host intestine. Epulos have not been found associated with foods or in free water collected from aquaria in which hosts had been held (Montgomery and Pollak 1988a), nor have they been detected in reef sediments (over which acanthurids are known to feed) during the course of microbiota surveys (Moriarty et al. 1985, Hansen et al. 1987, J.A. Hansen pers. comm.). The apparent restriction of epulos to the intestine of host fishes indicates a limited range of environmental tolerance. Epulos rapidly lose motility upon exposure to seawater (pers. obs.), suggesting that these microorganisms may be anaerobes. Attempts to maintain epulos in a variety of aerobic and anaerobic conditions (in collaboration with D. Sutton) were unsuccesful, suggesting that in the absence of an encysted phase (which is undetected thus far) the survival of epulos outside the environment of the host gut may be brief.

It is noteworthy that epulos were not detected in the faeces of the *A.* white-bar spp. 10 days after epulos were introduced into the aquarium. Clearly epulos must have been present in the intestine of the *A.* white-bar spp. by this time, for subsequent to this the fish no longer had access to *A. nigrofuscus* faeces. It is possible that epulos do not appear in the faeces of juvenile acanthurids for some time after infection, perhaps until the epulos have attained a certain density within the host intestine.

In summary, juvenile *A. nigrofuscus* and *C. striatus* resemble adult conspecifics in terms of feeding rate and diet, and appear responsive to small-scale variation in habitat structure and possibly to the density of interacting species. Juvenile *A. nigrofuscus* practice conspecific coprophagy, a behaviour which appears to be a mechanism for the transfer and/or retention of endosymbionts. This behaviour was

not observed in juvenile *C. striatus*, suggesting that these species may differ with respect to their mode of epulo retention. Finally, the results of the aquarium experiment strongly suggest that newly settled (i.e. non-infected) acanthurids may be infected with epulos by exposure to the faeces of infected hosts.

# CHAPTER 4: CHARACTERISTICS AND DISTRIBUTION OF GUT ENDOSYMBIONTS

## 4.1 INTRODUCTION

The endosymbiotic communities of tropical herbivorous fishes have received little attention until recently. Rimmer and Wiebe (1987) described the complex gut-resident microbiota of the sub-tropical herbivores *Kyphosus cornelii* and *K. sydneyanus*. This microbiota consisted of an abundant, diverse assemblage of bacteria, and a range of ciliated and flagellated protozoans. Clements (In Press) descibed the endosymbiotic community of two species of temperate, herbivorous odacid fishes. This microbiota was dominated by prokaryotic microbes, but also contained zooflagellates. Perhaps the most spectacular microbiota yet discovered in an herbivorous fish is the endosymbiotic community descibed from Red Sea specimens of *Acanthurus nigrofuscus* by Fishelson et al. (1985). The findings of Fishelson et al. (1985) will be briefly reviewed here, for they formed the basis on which this present study was designed.

Fishelson et al. (1985) found that every adult *A. nigrofuscus* they examined contained dense populations of unusual protists. These microorganisms were subsequently described as *Epulopiscium fishelsoni* by Montgomery and Pollak (1988a). Fishelson et al. (1985) reported that within *A. nigrofuscus* from the Red Sea the protists attained densities of 20,000 to 100,000 cells per millilitre of gut contents, and were much larger (ranging in length from 30 to 500 μm) than most

other gut microbes. These protists were also present in *A. sohal* from the Red Sea, but absent from other members of the Acanthuridae and the herbivorous *Siganus lividus*. Protists were not observed from specimens of two Gulf of California surgeonfish, *A. nigricans* and *Prionurus punctatus*. These data suggested that the protists exhibited some degree of host specificity. Fishelson et al. (1985) described the structural characteristics, mode of reproduction and within-gut distribution of these microorganisms, which are referred to in this study as epulos. Fishelson et al. (1985) concluded that epulos were a symbiotic feature of the gastrointestinal microflora of *A. nigrofuscus*, as they were present in every specimen collected from a number of Red Sea locations. The first objective of my study therefore was to investigate the geographical extent of this microorganism/host relationship, by determining whether the epulos were present in *A. nigrofuscus* from the GBR.

*A. nigrofuscus* collected at Lizard Island in 1987 did indeed harbour epulos, or at least organisms very similar to those reported from the Red Sea. This finding led to the second objective of my work, to determine the distribution of the epulos amongst potential host taxa on the GBR. The occurrence of epulos in herbivorous surgeonfish suggested that this family (Acanthuridae) should be targeted in particular. Approximately 37 species of surgeonfish occur on the GBR (see Chapter 1). These comprise herbivorous, planktivorous and detritivorous species (Hiatt and Strasburg 1960, Jones 1968, Hobson 1974, Myers 1989, Robertson et al. 1979, Lobel 1981, Robertson and Polunin 1981, Robertson 1983, Russ 1984a, Russ 1984b, Robertson and Gaines 1986), and include several of the species examined by Fishelson and coworkers in the Red Sea. The GBR also supports several other

families containing herbivorous fish, including the Kyphosidae, Pomacanthidae, Pomacentridae, Scaridae, Siganidae and Blenniidae. To obtain a more comprehensive picture of the occurrence of the epulos in GBR fish, I collected representatives of all these taxa, along with the single species of the family Zanclidae. It was thus hoped to obtain an understanding of the extent of potentially obligate symbioses amongst tropical herbivores in general.

My material therefore represented both phylogenetically related taxa and taxa characterized by similarities in diet and trophic morphology. This range of host material provided the opportunity for a third objective, to describe the distribution, diversity and structural and reproductive characteristics of epulos in terms of the phylogeny, ecology and digestive morphology of the host species. The results of these three objectives were presented in Clements et al. (1989), which describes the occurrence and characteristics of epulos amongst herbivorous fishes of the GBR. This chapter incorporates the results of Clements et al. (1989), but expands considerably upon both the range of endosymbionts investigated (to include protozoans and other prokaryote components in addition to epulos), and the number and geographical scope of host taxa examined (to include juvenile acanthurids and specimens from areas other than the GBR).

There were four main reasons for expanding the study in this direction. Firstly, the inclusion of protozoans enabled a comparison to be made with the epulo microflora. Thus data were available to investigate phenomena such as host-specificity and geographical variation using more than one group of endosymbionts. Secondly,

extending the geographic scope of the study not only increased the information available on the range of the symbiosis, but also provided a wide range of material to assess the morphological variability of the epulos. Thirdly, examination of juvenile acanthurids allowed an understanding of the development of the endosymbiotic community (i.e. infection - see Chapter 3). Fourthly, the examination of acanthurids from southern localities enabled an assessment of the pattern of endosymbiont occurrence at the limits of acanthurid distribution (see Chapter 2).

The results in this chapter are subdivided into four main sections:

(i) endosymbiont descriptions (morphological characteristics of the prokaryote and eukaryote microbiota encountered, as well as notes on cell division and motility; ultrastructure of the epulos will be presented in Chapter 5);

(ii) the occurrence of endosymbionts amongst fish families examined;

(iii) the occurrence of endosymbionts amongst acanthurid species: (a) from different sites within the GBR, (b) from the GBR and other tropical areas, and (c) from the subtropical coast of southeastern Australia; and

(iv) the microbiota of juvenile acanthurids.

## 4.2 MATERIALS AND METHODS

The fishes examined in this study were collected by spear or fence net from four main regions:

(a) the Great Barrier Reef (Day Reef, John Brewer Reef, Lizard Island, MacGillivray Reef, Magnetic Island, Myrmidon Reef, NoName Reef, North Direction Island, #10 Ribbon Reef, Thetford Reef and Yonge Reef),

(b) Tuvalu (the islands of Nui, Nanumea and Niutao),

(c) the Solitary Islands off Coff's Harbour, New South Wales (North Solitary Is., Northwest Solitary Is., and Split Solitary Is.), and

(d) a number of points along the coast of eastern Australia: Flinder's Reef (off Moreton Island), the Inner Gneerings (off Mooloolaba, the Sunshine Coast), Julian Rocks (Byron Bay, NSW), Arrawarra Headland (NSW), Muttonbird Island (Coff's Harbour, NSW), and Cape Banks (Sydney, NSW).

Since time of day is known to affect the distribution and abundance of epulos within the intestines of acanthurids (Fishelson et al. 1985, Montgomery and Pollak 1988a), fish for this study were collected between 1100 and 1800hrs. Only specimens with full guts were examined for endosymbionts. Additional samples were obtained from three sources: (a) juvenile acanthurids collected from artificial patch reefs at Lizard Island were donated by Mark Meekan (Griffiths University); (b) formalin-preserved fish collected at explosive stations from Rib and Myrmidon reefs during 1980 were donated by Dr David Williams (Australian Institute of Marine Science, Townsville); and (c) Dr John Paxton granted permission for me to examine the gut contents of

acanthurids in the collection of the Australian Museum, Sydney. Despite the age of the explosive station and museum specimens, the appearance of gut endosymbionts was indistinguishable from those of recently collected conspecifics. Details of all sample localities are given in Chapter 1. Sample sizes and size classes of the taxa examined are given in Tables 4.1 and 4.2.

The gut morphology of each acanthurid species was examined and assigned to a particular gut morphology category. A combination of gut content and field observations were used to assign fish species to feeding type categories. Gut morphology and feeding type categories are presented in Table 4.3.

All gut material was fixed within three hours of capture with 10% formalin in sea water. Sample guts were either removed and fixed whole, or aliquots of intestinal contents were taken from the gut and stored separately. Despite the fact that there are differences in stomach morphology and relative gut length between acanthurid species (Randall, 1956; Hiatt and Strasburg, 1960; Jones, 1968), the general arrangement of the alimentary canal in this family is remarkably consistent (Jones, 1968; Mok, 1977). As the coiling pattern is generally similar among species and genera, it is possible to sample gut contents from a similar relative location in species which have different gut lengths. Pilot work carried out on acanthurids established that the intestine at the base of the left hand ascending loop contained the most consistent populations of epulos. This region (indicated by Point A in Fig. 4.1) had the added advantage of being easy to locate for sampling. However, since some zooflagellates are known to inhabit the posterior regions of the gut (Clements, In

Press), I also took samples from the rectal loop (Fig. 4.1). Therefore, with the exception of material collected in Tuvalu (see below), the microbiota composition data presented below are based on the examination of two samples from separate points of the intestine. Logistic limitations in Tuvalu were such that I was only able to transport one gut sample per specimen. I therefore decided to take my samples from point A (Fig. 4.1), as this at least would give me a reliable indication of the occurrence of epulos. The lack of rectal samples from Tuvalu may therefore result in an underestimation of the percentage occurrence of some zooflagellates, while having little or no effect on the occurrence of epulos (which are rare or absent in the rectal loop - Fishelson et al. 1985 and pers. obs.).

For determination of the presence or absence of epulos in non-acanthurid species, samples were taken from at least five regularly-spaced points along the intestine. All samples in this study were mounted in sterile sea water on glass slides and examined microscopically (light, phase-contrast, Nomarski interference-contrast or Hoffman interference-contrast). Microbiotic components (described below) were recorded on a presence/absence basis. To ensure that the microbiota recorded as present were endosymbiotic (i.e. gut resident), I wished to exclude from consideration microorganisms which may have been incidentally ingested by the host. Since epulo populations are characterized by high densities (Fishelson et al. 1985, Mongomery and Pollak 1988a), epulo categories were only scored as present if at least ten individuals were observed. A different strategy was employed for the eukaryote components. Some of these organisms may be parasitic endosymbionts, in which case low infestations could still be indicative of gut-resident status. Furthermore,

several of the zooflagellate categories (see below) are known to be obligate anaerobes, which makes incidental ingestion by the host unlikely. Therefore, the eukaryote components were scored as present once one individual was observed. Endosymbiont occurrence data are presented in terms of percentage occurrence, i.e. the proportion of specimens of each host species which contain a particular endosymbiont category. Although proportional data cannot be associated with an error term, sample size (i.e. the number of host specimens examined) and the frequency of occurrence of a particular endosymbiont category yield an idea of rigor.

Three methods were used to measure endosymbiont size. In most cases a calibrated optical micrometer was employed. However in the early stages of my work many epulos (almost 3000) were measured by superimposing the microscope image on a computer screen, and using an Amiga 2000 computer to calculate lengths. Zooflagellates were measured by comparison to calibrated scale bars on scanning electron micrographs. Because of the size range of the prokaryote and eukaryote microorganisms investigated (5-570μm), each sample was examined using both low (10X) and high (40X) power objectives. An oil-immersion objective (100X) was used to examine zooflagellates and ciliates. The classification of these organisms requires special staining techniques in order to reveal internal structure. Gut samples containing ciliates were sent to Prof. Norman Grim (Northern Arizona University) for preliminary identification. Gut samples containing zooflagellates were initially examined using scanning electron microscopy. While this technique is unsuitable for the proper identification of zooflagellate taxa (because it does not reveal internal

structure), it provided an adequate means of categorising zooflagellates on the basis of external morphology. In this manner a catalogue of voucher scanning electron micrographs was built up, enabling the diversity of zooflagellate morphotypes to be assessed. Subsequent to this, zooflagellates could be recognised in unstained preparations (by oil immersion light microscopy) by comparing size, shape and the location and number of flagella with the voucher photographs. Samples containing zooflagellates which could not be confidently placed within existing categories were also examined by scanning electron microscopy.

Samples of gut contents for scanning electron microscopy were prepared from the same formalin-fixed material examined using the light microscope. Aliquots of gut contents were allowed to settle and the bacteria/protozoan layer pipetted off (0.5 - 1.0ml). This was then diluted 5 to 10 times with 2% ammonium acetate ($NH_4C_2H_3O_2$) and allowed to settle. The supernatant was removed and fresh ammonium acetate added. This procedure was repeated 4-5 times. After settling, the supernatant was removed again and the remaining material suspended and placed on freshly cleaved mica. The mica plates were then air dried (at room temperature), gold splatter-coated and examined using a JEOL JXA 840A scanning electron microscope at 15KV. Whilst some shrinkage occurred as a result of this procedure (as compared to wet preparations), this technique proved very suitable for the examination of external structures.

For description of epulo internal structure I use the terminology of Fishelson et al. (1985) and Montgomery and Pollak (1988a). These authors used the term daughter-

cell to refer to oblong structures within the epulos (= maternal cells), and from observations of many cells identified a sequence of development that culminated in the emergence of these structures through a split in the cell envelope. This process was interpreted as a mode of reproduction, although in the absence of culturing techniques this cannot be experimentally verified. Kunstyr et al. (1988) made the same interpretation for the sequence they observed in *Metabacterium* spp., but termed the structures endospores. Until the terminology applicable to these microorganisms is resolved, I prefer to retain the more general term daughter-cell. In addition to the sequence of daughter-cell production, I observed a sequence of internal wall formation in many individual epulos. This sequence included stages from slight inclusions of adjacent outer cell walls to the point at which wall formation was complete. I interpret this sequence as stages in cell division by binary fission. Ultrastructural evidence for this process in the epulos will be presented in Chapter 5.

For assessment of epulo motility, fish were initially returned live to the laboratory and sacrificed immediately prior to examination. However, epulo motility was found to be retained in the intestines of fish examined within three hours of death. Most investigations of motility were therefore made on material speared and returned immediately to the laboratory (Lizard Island Research Station). Gut content samples were examined undiluted on glass microscope slides, since the addition of sea water was found to cause cessation of motility within a few minutes (purging of sea water with nitrogen prior to use produced no apparent prolongation of motility).

**Table 4.1** **Sample Sizes and Collection Localities of Acanthurids Examined for Endosymbionts in this Study.**

Abbreviations: GBR = Great Barrier Reef, TUV = Tuvalu, SEA = Subtropical Eastern Australia, NS = newly settled, <70 = <70mmSL, >70 = >70mmSL

| Species | Sample location | | | | | | |
|---|---|---|---|---|---|---|---|
| | GBR NS | GBR <70 | GBR >70 | TUV >70 | SEA <70 | SEA >70 | Aust. Museum |
| *A. achilles* | | | | 6 | | | 2 |
| *A. achilles x nigricans* | | | | 3 | | | |
| *A. auranticavus* | | | 8 | | | | |
| *A. blochii* | | 2 | 12 | | | 2 | |
| *A. dussumieri* | | 2 | 8 | | | 14 | 3 |
| *A. grammoptilus* | | | 5 | | | | |
| *A. guttatus* | | | | 6 | | | 2 |
| *A. leucosternon* | | | | | | | 6 |
| *A. lineatus* | | 7 | 9 | 10 | | 1 | 2 |
| *A. maculiceps* | | | | 1 | | | 1 |
| *A. mata* | | 7 | 5 | | | | |
| *A. nigricans* | | | 13 | 5 | | | 1 |
| *A. nigricauda* | | 1 | 6 | 5 | | | |
| *A. nigrofuscus* | 1 | 46 | 20 | 5 | 10 | 21 | 5 |
| *A. nigroris* | | | 1 | 6 | | | 1 |
| *A. olivaceus* | 1 | 10 | 14 | 5 | | 1 | |
| *A. pyroferus* | | 2 | 7 | 5 | | | |
| *A. thompsoni* | | | 6 | | | | |
| *A. triostegus* | 2 | 21 | 8 | 6 | 2 | | 4 |
| *A. xanthopterus* | | 6 | 6 | | | 1 | |
| *A.* white-bar spp. | 51 | 113 | | | | | 1 |
| *C. binotatus* | 3 | 45 | 37 | 1 | | | 1 |
| *C. hawaiiensis* | | | | 5 | | | |
| *C. marginatus* | | | | 6 | | | 1 |
| *C. striatus* | | 23 | 39 | 7 | | | 1 |
| *C. strigosus* | | 1 | 6 | 5 | | | |
| *N. brevirostris* | | 12 | 19 | | | | |
| *N. hexacanthus* | | 5 | 2 | | | | |
| *N. lituratus* | | 5 | 7 | 5 | 1 | | |
| *N. tuberosus* | 9 | 20 | 8 | | | 1 | |
| *N. unicornis* | | 10 | 6 | | | | |
| *N. vlamingii* | | 3 | 5 | | | | |
| *P. hepatus* | | | | | | | 3 |
| *P. maculatus* | | | | | | 3 | 1 |
| *P. microlepidotus* | | | | | 6 | 6 | 1 |
| *Z. rostratum* | | | | 7 | | | |
| *Z. scopas* | 2 | 20 | 27 | 4 | | | |
| *Z. veliferum* | | 13 | 6 | 5 | | | |

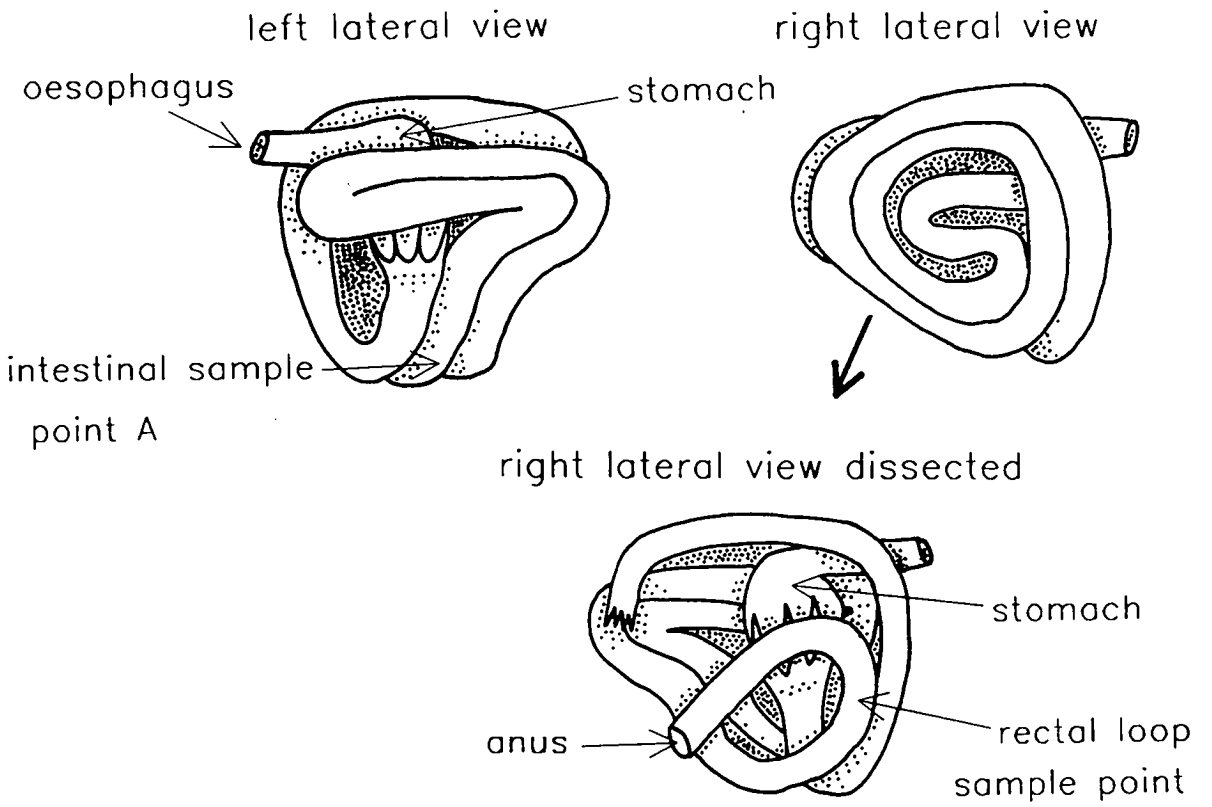
**Table 4.2** **Sample Sizes of Non-Acanthurid Taxa Examined for Endosymbionts in this Study**

| Species | (N) | Species | (N) |
|---|---|---|---|
| Blenniidae | | Siganidae | |
| *Atrosalarias fuscus* | 5 | *Siganus corallinus* | 4 |
| *Salarias fasciatus* | 5 | *S. doliatus* | 5 |
| | | *S. guttatus* | 1 |
| Kyphosidae | | *S. puellus* | 1 |
| *Kyphosus cinerascens* | 1 | *S. punctatissimus* | 2 |
| | | *S. punctatus* | 5 |
| Pomacanthidae | | *S. spinus* | 4 |
| *Centropyge bicolor* | 5 | *S. vulpinus* | 5 |
| *C. bispinosus* | 5 | | |
| *C. vrolicki* | 1 | Zanclidae | |
| | | *Zanclus cornutus* | 6 |
| Pomacentridae | | | |
| *Dischistodus perspicillatus* | 8 | | |
| *D. prosopotaenia* | 5 | | |
| *Plectroglyphidodon dickii* | 2 | | |
| *P. lachrymatus* | 6 | | |
| *Pomacentrus amboinensis* | 4 | | |
| *P. bankanensis* | 4 | | |
| *P. grammorhynchus* | 6 | | |
| *P. wardi* | 1 | | |
| *Stegastes apicalis* | 9 | | |
| *S. nigricans* | 6 | | |
| Scaridae | | | |
| *Cetoscarus bicolor* | 2 | | |
| *Scarus chameleon* | 1 | | |
| *S. flavipectoralis* | 2 | | |
| *S. frenatus* | 4 | | |
| *S. ghobban* | 1 | | |
| *S. gibbus* | 5 | | |
| *S. globiceps* | 2 | | |
| *S. niger* | 5 | | |
| *S. psittacus* | 4 | | |
| *S. rivulatus* | 4 | | |
| *S. rubroviolaceus* | 1 | | |
| *S. schlegeli* | 5 | | |
| *S. sordidus* | 5 | | |

**Table 4.3 Feeding Type and Stomach Morphology of Acanthurids Examined for Endosymbionts in this Study.**

| Species | Feeding type | Stomach type |
|---|---|---|
| *A. achilles* | turf grazer | thin-walled |
| *A. achillesxnigricans* | turf grazer | thin-walled |
| *A. auranticavus* | mixed grazer | muscular |
| *A. blochii* | mixed grazer | muscular |
| *A. dussumieri* | mixed grazer | muscular |
| *A. grammoptilus* | mixed grazer | muscular |
| *A. guttatus* | turf grazer | muscular |
| *A. leucosternon* | turf grazer | thin-walled |
| *A. lineatus* | turf grazer | thin-walled |
| *A. maculiceps* | mixed grazer | muscular |
| *A. mata* | planktivore | thin-walled |
| *A. nigricans* | turf grazer | thin-walled |
| *A. nigricauda* | sand grazer | muscular |
| *A. nigrofuscus* | turf grazer | thin-walled |
| *A. nigroris* | mixed grazer | thin-walled |
| *A. olivaceus* | sand grazer | muscular |
| *A. pyroferus* | mixed grazer | muscular |
| *A. thompsoni* | planktivore | thin-walled |
| *A. triostegus* | turf grazer | thin-walled |
| *A. xanthopterus* | sand grazer | muscular |
| *A.* white-bar spp. | sand or mixed grazer | muscular |
| | | |
| *C. binotatus* | detritivore | muscular |
| *C. hawaiiensis* | detritivore | muscualr |
| *C. marginatus* | detritivore | muscular |
| *C. striatus* | detritivore | muscular |
| *C. strigosus* | detritivore | muscular |
| | | |
| *N. brevirostris* | browser->planktivore | thin-walled |
| *N. hexacanthus* | planktivore | thin-walled |
| *N. lituratus* | browser | thin-walled |
| *N. tuberosus* | browser | thin-walled |
| *N. unicornis* | browser | thin-walled |
| *N. vlamingii* | browser->planktivore | thin-walled |
| | | |
| *P. hepatus* | planktivore | thin-walled |
| | | |
| *P. maculatus* | turf grazer | thin-walled |
| *P. microlepidotus* | turf grazer | thin-walled |
| | | |
| *Z. rostratum* | browser | thin-walled |
| *Z. scopas* | turf grazer | thin-walled |
| *Z. veliferum* | browser | thin-walled |

Fig. 4.1 Diagram of acanthurid alimentary tract showing points at which endosymbiont samples were taken.



## 4.3 RESULTS

### 4.3.1 Endosymbiont descriptions

(i) Epulos and other prokaryotes

The examination of the intestinal microbiota of GBR acanthurids revealed a diverse assemblage of cigar-shaped microorganisms. Some of these organisms closely resembled *Epulopiscium fishelsoni* (as described in Fishelson et al. 1985 and Montgomery and Pollak 1988a), while others varied in size, shape and mode of cell division. Since the relationships between these similar organisms were unclear, I subdivided this diversity into several morphotypes, henceforth referred to simply as `types.' Because of the superficial similarity of these organisms, I used the general term 'epulo' to describe the whole assemblage. This term, coined by W.L. Montgomery (pers. comm.), is to be understood as a convenient label only. It is not meant to imply that all 'epulos' are necessarily related. The characteristics of the epulo morphotypes as defined in this study are presented in Table 4.4. Most epulo types (described below) were observed in more than one host species. In addition to the ten epulo categories, two categories of 'normal-sized' bacteria were recognised. Epulos were found throughout the intestinal contents, although their distribution across the intestinal lumen was not quantitatively assessed.

**Type A** (Plate 4.1): This epulo category is very similar to the protists originally reported from Red Sea specimens of *Acanthurus nigrofuscus* by Fishelson et al.

(1985), and subsequently described by Montgomery and Pollak (1988a) as *Epulopiscium fishelsoni*. It is typically cigar-shaped, with rounded ends, although a continuum exists between this shape and individuals which are more oval or elongate (Plate 4.1A-D). This form varies greatly in size (Plate 4.1A&B), and includes the largest epulos measured. Type A epulos are typically 100-200μm in length, although individuals 300-400μm are not uncommon. One 136mmSL specimen of *A. nigrofuscus*, collected at Lizard Island in June 1987, contained the largest epulos recorded so far (Plate 4.1A-C). The largest of these `microorganisms' was 576μm in length, and many individuals were in excess of 500μm. This epulo type seemed to be larger in some host species than others, although it retained certain distinguishing characteristics.

Reproduction in this form typically involved the formation of daughter-cells within the maternal cell. Early stages of daughter-cell formation were visible as distinct areas in the cytoplasm, later as miniature adults within the maternal cell. In the latter case, the typical pattern was two daughter-cells side-by-side or at least partially overlapping (Plate 4.1B&C). Rarely however, individuals were found with widely-separated daughter cells. Plate 4.1D illustrates such a case, where the developing daughter-cells (visible at the poles of the cell) were separated by uncharacteristically granular cytoplasm. One 177mmSL *A. blochii* collected from Lizard Island harboured large epulos (largest measured was 201μm) which contained from two to four daughter-cells (Plate 4.1E). This was the only sample I examined in which type A epulos contained more than two daughter-cells. An equally puzzling exception is illustrated in Plate 4.1F, which shows type A epulos dividing by binary

fission. Conspecific specimens collected at the same time contained typical type A epulos, with two daughter-cells. These two exceptions notwithstanding, type A epulos may be characterized on the basis of their large size and elongated shape.

**Type B** (Plate 4.2A-C): This category was distinguished by its size (>60 μm in length), shape, and the presence of individuals with more than two daughter cells (Plate 4.2A&B). Some individuals were similar to type A above in having two daughter-cells, but differed in being more rounded and were invariably associated with similar individuals having up to five daughter-cells.

**Type C** (Plate 4.2D-F): This form did not attain the size of the two forms described above. It never had more than two daughter-cells, and these were usually very distinct within the maternal cell (Plate 4.2D). This form characteristically occured in high densities, often with cells joined together in clumps.

**Type D** (Plate 4.3A): This form appeared very similar to type C above, but was consistently smaller. Like type C it too had very distinct daughter-cells, which were either separated (as in Plate 4.2A), adjacent, or overlapping. Unlike types A, B and C, the daughter-cells of type D appeared bright by phase-contrast microscopy.

**Type E** (Plate 4.3B): This form was very variable in size. It was characterized by its shape, which was tubular and longitudinally-compressed (i.e side walls are essentially parallel), and by the presence of similarly elongate daughter cells. As with type D, the daughter-cells of this form appeared phase-bright. Daughter-cells

were widely-separated (as in Plate 4.3B), or overlapping. Individuals with one daughter-cell were not uncommon (Plate 4.3B).

**Type F** (Plate 4.3C&D): This form was characterized by the presence of individuals with more than two daughter-cells, which appeared phase-bright. Individuals similar to type D with two daughter-cells were common. These daughter-cells were widely-separated, or overlapping (as in Plate 4.3C). However these two daughter-cell individuals were usually larger than type D, and were always associated with similar individuals with up to seven daughter-cells.

**Type G** (Plate 4.3E&F and Plate 4.4A): Type G appeared to reproduce by binary fission, was not seen with daughter-cells and often had well-defined cell walls (Plate 4.3E). This type appeared uniform or had distinct bodies at the extreme ends of the cell (as in Plate 4.3F). Where such bodies were present, they were circular and unlike the elongate daughter-cells of other forms. Note that binary fission occured both in the presence (Plate 4.4A) and absence (Plate 4.3E) of these polar bodies. The mode of cell division and cell wall structure of these epulos (Plate 4.3E) is characteristic of gram-positive eubacteria (Koch 1990).

**Type H** (Plate 4.4A&B): This form was probably not homogenous, as it contained a wide variety of epulos which may have been the non-reproductive stages of other forms. Nevertheless, this form had certain distinguishing features. It was uniform in appearance or possessed two distinct internal structures (Plate 4.4A). Rarely, three internal structures were present (Plate 4.4B). These structures may have been

incipient daughter-cells, but did not appear bright by phase-contrast microscopy. This form included those larger epulos for which no distinctive reproductive stage had been detected. It is likely that this form contained ontogenetic stages of both daughter-cell and binary-fission forms, and contained those epulos which could not unequivocally be assigned to one of the other categories.

**Type I** (Plate 4.4C&D): This form occured in the gut of the juveniles of several *Acanthurus* species, but it also occured in some adults. This form was characterized by its mode of reproduction, which appeared to represent simultaneous daughter-cell formation and binary fission. Daughter-cells were either located at the poles of the cell (as in Plate 4.4D), or were overlapping (as in Plate 4.4C). A comparison of Plates 4.4C and 4.4D shows that internal wall formation was not always associated with full development of daughter-cells (i.e. the two forms of reproduction were not necessarily synchronous).

**Type J** (Plate 4.4E): This form was similar in size and shape to type E. However, individuals were more tubular and elongate, and divided by binary fission (see Chapter 5). It was also similar to types I and G above, but lacked their characteristic cigar-shape and never had any apparent internal structures.

Epulos of types A, B, C, D, E, F, G, H and J were motile. Epulos of type I were never observed as fresh specimens. In all cases motility involved a spiralling motion and an ability to reverse direction rapidly. In most cases, protist shape did not change during locomotion. However the elongate types E and J were observed to

flex in a sinuous snake-like manner, although they still clearly rotated about their long axis while moving.

All the motile epulos observed were capable of generating powerful currents, as evidenced by the movements of particulate matter adjacent to the epulo surface. Many of the epulos examined using dark field lighting had elongate filaments emanating from the outer membrane and sometimes occurring in clusters at the poles of the cells. However these filaments were not apparently involved in locomotion, and not all motile epulos possessed them.

**Spirilla**: Spirilla are a polyphyletic assemblage of prokaryotes characterized by their helically coiled shape (Starr et al. 1981). The spirilla examined in this study ranged in length from 5 to 20μm. Although the size of these organisms varied between some of the host species examined, no attempt was made to subdivide this category.

**Small rod-shaped bacteria**: This category included elongate bacterial cells of less than 8μm in length. While it is possible that some small representatives of epulo types D and E were included in this category, small rods were characterized by the absence of any internal structures and their small size.

(ii) Protozoans

Ciliates (Plates 4.5 & 4.6)

Ciliates (Phylum Ciliophora) were found in two species of siganids and eight species of acanthurids. Several taxa were present, some of which are probably undescribed. A large ciliated organism from *Acanthurus nigrofuscus* which was at first thought to be a ciliate is in fact a zooflagellate of the genus *Protoopalina* (J.N. Grim pers. comm.). Due to its distinctive size and characteristics it is included here rather than in the zooflagellate section below. Proper identification of ciliates requires the use of cytological stains and/or electron microscopy (Lee et al. 1985, J.N. Grim pers. comm.), which precludes the possibility of directly comparing untreated samples under the light microscope. Since I am unable to resolve the specific status of most of these organisms, I have clumped them into one category for the purposes of species and geographical comparisons. A range of the ciliate forms observed during this study are illustrated in plates 4.5 and 4.6. Some of these taxa have been provisionally identified by Prof. Norman Grim, on the basis of fixed material and photographs. Since many of the ciliates appeared to be host-specific, they will be discussed in the context of individual host species (section 4.3.3).

Zooflagellates (Plates 4.7-4.10)

Zooflagellates (Class Zoomastigophorea, Subphylum Mastigophorea, Phylum Sarcomastigophorea) were extremely common in many of the taxa examined. As

with the ciliates, proper identification (which requires staining and mounting) was precluded by the need to process many samples. For the purposes of comparing the eukaryote endofaunas of different host species, therefore, zooflagellates were placed in several categories on the basis of basic morphology. Features such as size, shape, presence of an axostyle or undulating membrane, and number and position of flagella were used to delimit categories. While these categories may lack taxonomic validity, they serve as a useful means of comparing the microbiota of host species at a basic level. Almost all of the zooflagellates encountered fell into one of five categories. Lengths were measured as maximum body dimension minus axostyle (where present). The length range, mean *(+SE) and* the number of individuals measured of each zooflagellate category are given.

**Type U zooflagellates** (Plate 4.7A-D): These distinctive organisms had two anterior flagella bundles, each of which was comprised of 4 or 5 flagella. The posterior end was variable in appearance. Usually, it appeared as though two flagella and an axostyle were present (Plate 4.7A, C & D). Often, however, the posterior end diverged into a series of rootlike appendages (Plate 4.7B). Structures resembling therecurrent flagella of trichomonads (Plate 4.7C&D) were present on some individuals examined. These zooflagellates ranged in length from 9.3-19μm ($13.01 \pm 0.47$, n=26). Movement involved a relatively slow, spiralling motion. This form does not resemble any of the zooflagellate taxa presented in Lee et al. (1985).

**Type K zooflagellates** (Plate 4.7E & F): This form had an elongate, tear-shaped body with two flagella. Both flagella appeared to originate from the anterior end,

with one recurrent and running in a groove for most of the length of the body. These zooflagellates ranged in length from 12-17.5μm (14.61±0.63, n=9), and bear similarity to some members of the Order Kinetoplastida.

**Type D zooflagellates** (Plate 4.8A-C): This form was distinguished by the number and arrangement of flagella. Six flagella originat.ed from the anterior (rounded) end, while two originated from the posterior (pointed) end. The anterior flagella emerged in two distinct clusters. This arrangement is characteristic of members of the Order Diplomonadida, which have paired karyomastigont systems and are thus symmetrical (Lee et al. 1985). Forms observed in this study resembled the genera *Hexamita* and *Spironucleus*, which have four flagella associated with each karyomastigont system three anterior locomotory flagella and one recurrent trailing flagellum (Kulda and Lom 1964, Lee et al. 1985). This form varied from elongate (Plate 4.8A) to truncate (Plate 4.8C), and ranged in length from 6-11.5μm (8.97±0.40, n=14).

**Type M zooflagellates** (Plate 4.8D-F): This form was distinguished by the presence of an axostyle, three anterior flagella, and a recurrent flagellum greater in length than the anterior flagella. This form lacked an undulating membrane. These zooflagellates bear similarity to members of the Subfamily Monocercomonadinae (Family Monocercomonadidae, Order Trichmonadida), particularly the genus *Tricercomitus* (Lee et al. 1985). This form ranged in length from 5.2-8.4μm (6.90±0.19, n=23). The body shape of this form varied from pear-shaped (Plate 4.8F) to elongate with a rounded anterior end (Plate 4.8D). In some organisms two of the anterior flagella appeared to be attached along part of their length (Plate

4.8E).

**Type T zooflagellates** (Plate 4.9A, B & D): This form was characterised by the presence of an axostyle and three to four anterior flagella, all of approximately equal length. On some individuals an undulating membrane associated with a recurrent flagellum was present. A posterior free flagellum was also sometimes present. This form appears referable to the Order Trichomomadida, which is characterised by 4-6 flagella (one recurrent) per mastigont system, an axostyle, and an undulating membrane, if present, associated with the recurrent flagellum (Lee et al. 1985). Several taxa are almost certainly present. One form, which may represent the genus *Tetratrichomonas* Parisi (Lee et al. 1985), had four anterior flagella, an undulating membrane extending most of the length of the body, and a free posterior flagellum (Plate 4.9A & B). Another form with three anterior flagella is illustrated in Plate 4.9D. Type T zooflagellates were distinguished from type M zooflagellates (to which they are probably related) on the basis of the number and dimension of flagella. Type T zooflagellates ranged in length from 7.6-13.5μm ($11.05 \pm 0.55$, $n=10$).

Miscellaneous **flagellates** (Plate 4.9 and 4.10): This group contained a small number of zooflagellate forms of which only a few individuals were seen, or which were only encountered in a small number of host specimens. A biflagellated zooflagellate with a spheroid body (Plate 4.9C) was encountered in two specimens of *Acanthurus nigrofuscus*. These organisms had a body diameter of approximately 4μm. They may represent a reproductive stage of another zooflagellate form.

Two zooflagellate forms are illustrated in Plate 4.10. The first of these (Plate 4.10A & B) had a similar flagella arrangement to diplomonads (with two clusters of three flagella and two posterior flagella), yet differed in body shape (oval rather than elongate as in the diplomonads) and in the origin of the flagella clusters (lateral rather than anterior as in the diplomonads). These organisms were found in three specimens of *Acanthurus nigrofuscus* (from the GBR) and one specimen of *A. nigricansxachilles* (from Tuvalu). Three factors suggest that this form may represent a reproductive stage of diplomonads: (a) the small size of these organisms (<5μm in maximum body dimension); (b) the similarity in number and arrangement of flagella; and (c) each of the four host specimens also contained diplomonad (type D) zooflagellates.

The form illustrated in Plate 4.10C & D was found in 5 specimens of *Naso brevirostris*, 2 specimens of *Acanthurus nigrofuscus*, and 1 specimen of *Ctenochaetus binotatus*. This form was particularly abundant in the *N. brevirostris* specimens. Like the type K zooflagellates, it had two flagella, one of which was recurrent and attached along its length. However this form differed in both body shape and size. The body was truncate and characterised by longitudinal striations, and was thus quite distinct from the elongate type K. The size ranged from 6-10μm ($7.93\pm0.51$, n=7), which was considerably smaller than the 12-17.5μm range of type K. These flagellates bear some similarity to members of the Phytomastigophorean order Euglenida (J.N. Grim pers. comm.).

**Table 4.4 Characteristics of Epulo Morphotypes.**

Abbreviations: ? = unclear, - = absent

| Epulo type | Size range observed (µm) | Shape | Number of daughter-cells | Binary fission |
|---|---|---|---|---|
| A | 100-576 | cigar-oval | 0-5 | Rare |
| B | 60-322 | oval | 0-5 | No |
| C | 50-100 | cigar | 0-2 | No |
| D | 8-50 | cigar | 0-2 | No |
| E | 8-120 | elongate | 0-2 | No |
| F | 15-60 | cigar | 0-7 | No |
| G | 9-70 | cigar | - | Yes |
| H | 10-100 | cigar | ? | ? |
| I | 20-120 | cigar | 4+ | Yes |
| J | 10-240 | elongate | - | Yes |

**Plate 4.1 Type A epulos**

All light microscope photographs taken with Nomarski interference contrast. All scale bars = 100μm.

**A**: Type A epulos from 136mmSL *Acanthurus nigrofuscus* collected at Lizard Island. The ciliated organism at the right is the flagellate *Protoopalina*.

**B**: Type A epulos from 136mmSL *A. nigrofuscus* collected at Lizard Island. Daughter-cells are clearly visible within the maternal cortex.

**C**: Oval-shaped type A epulo from 136mmSL *A. nigrofuscus* collected at Lizard Island.

**D**: Type A epulo with unusual granulated cytoplasm from 187mm *A. lineatus* collected at Lizard Island.

**E**: Type A epulo containing 3 daughter-cells from 177mmSL *A. blochii* collected at Lizard Island.

**F**: Binary fission type A epulos from 170mmSL *A. lineatus* collected at Niutao, Tuvalu.

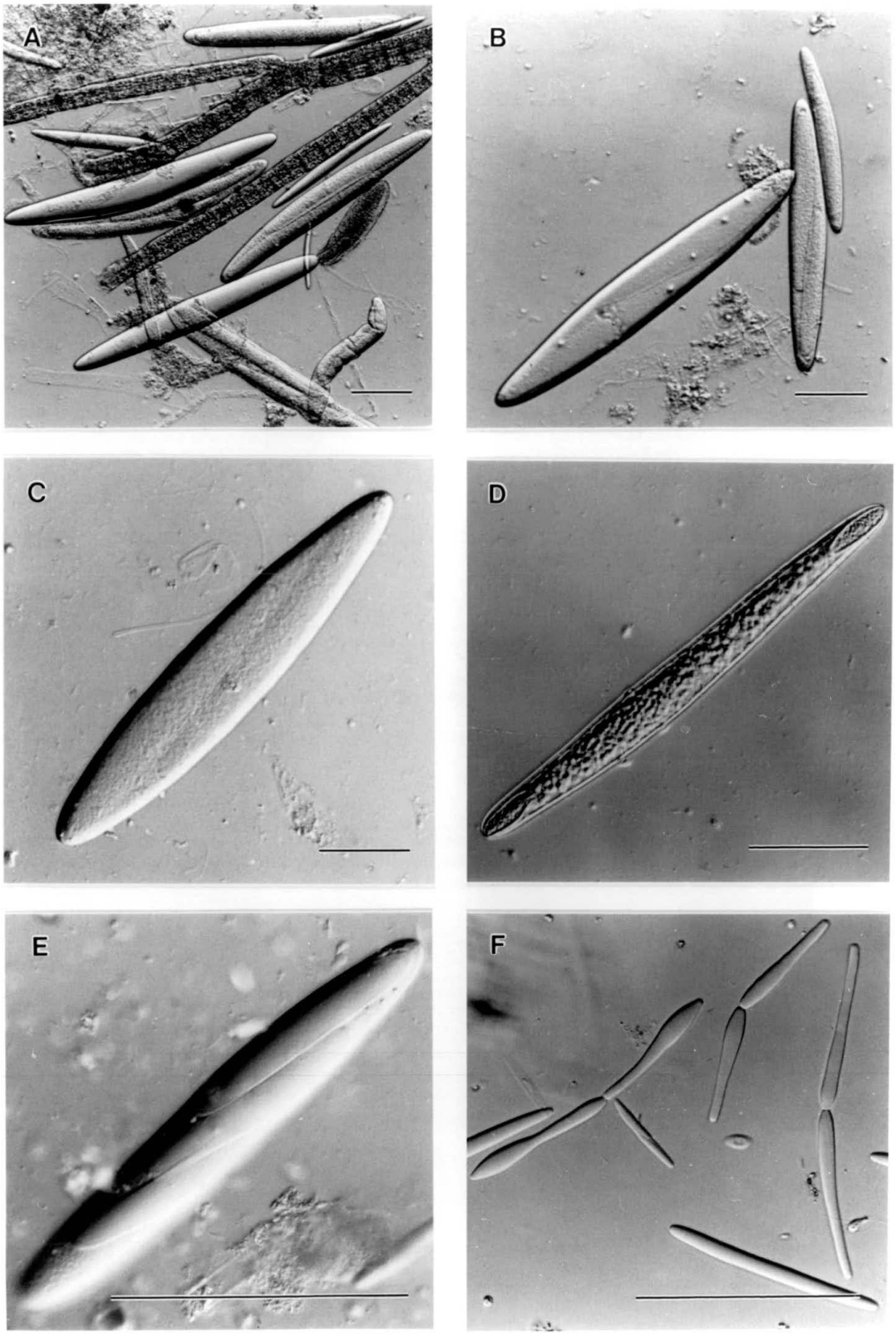

A
B
C
D
E
F

**Plate 4.2 Type B and C epulos.**

All light microscope photographs taken with Nomarski interference contrast. All scale bars = 20μm.

**A**: Type B epulos from 155mmSL *Acanthurus olivaceus* collected from Lizard Island.

**B**: Type B epulo containing 5 daughter-cells from 155mmSL *A. olivaceus* collected from Lizard Island.

**C**: Type B epulo containing 2 daughter-cells from 255mmSL *Naso tuberosus* collected from Lizard Island.

**D**: Type C epulo containing 2 daughter-cells from 117mmSL *N. unicornis* collected from Lizard Island.

**E**: Type C epulo containing 2 daughter-cells from 185mmSL *N. unicornis* collected from Lizard Island.

**F**: Type C epulos from 207mmSL *N. lituratus* collected from Lizard Island.

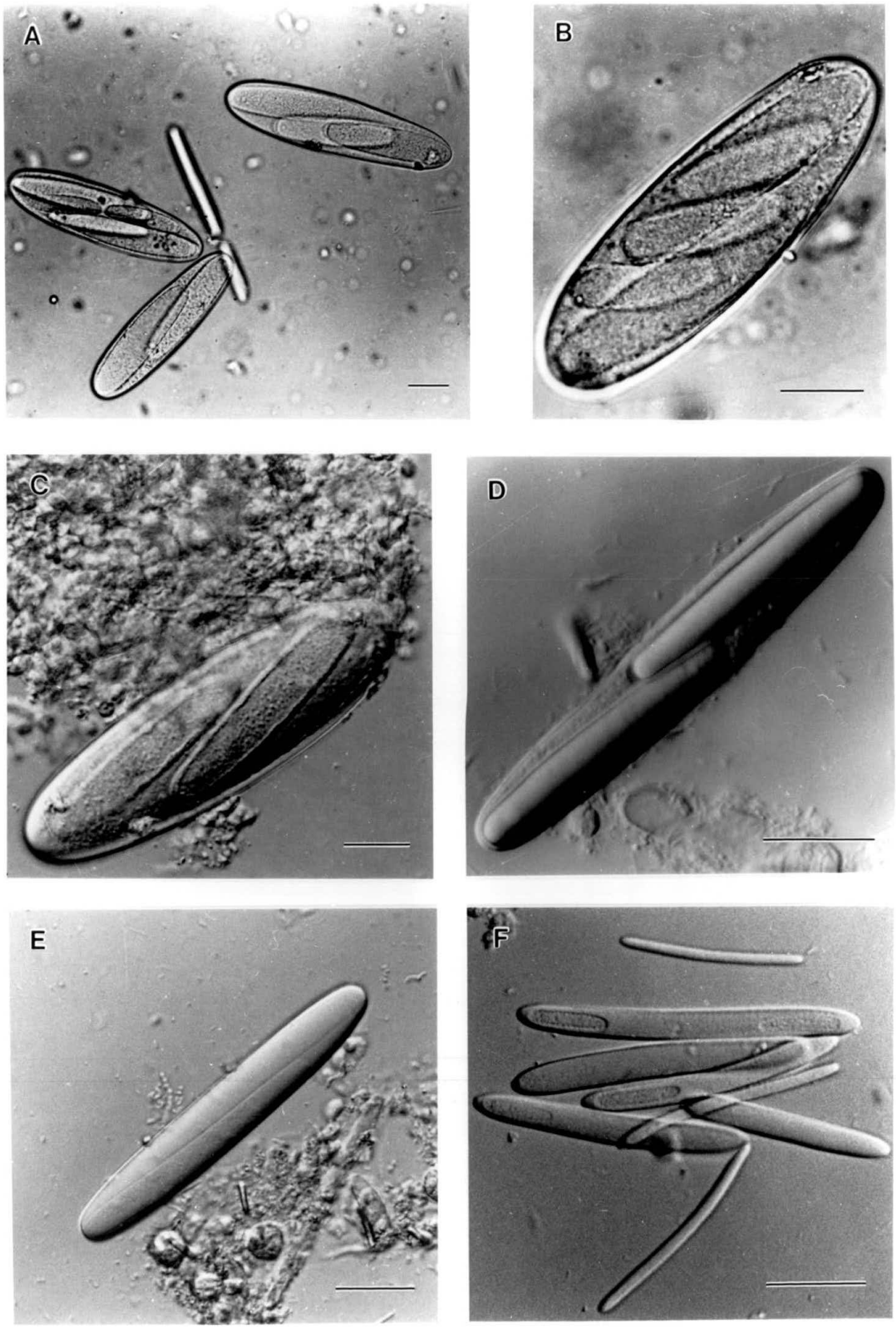

A
B
C
D
E
F

**Plate 4.3 Type D, E, F and G epulos**

All light microscope photographs taken with Nomarski interference contrast. All scale bars = 20μm.

**A**: Type D epulos from 208mmSL *Zebrasoma veliferum* collected from Lizard Island.

**B**: Type E epulos from 166mmSL *Z. scopas* collected from John Brewer Reef.

**C**: Type F epulos containing 2-5 daughter-cells from 190mmSL *Acanthurus olivaceus* collected from Nanumea, Tuvalu.

**D**: Type F epulos containing 6 daughter-cells from 115mmSL *Ctenochaetus strigosus* collected from NoName Reef.

**E**: Type G epulo from 130mmSL *A. pyroferus* collected from Lizard Island. The mode of cell division is characteristic of gram-positive eubacteria (Koch 1990).

**F**: Type G epulo with distinct polar bodies from 130mmSL *A. pyroferus* collected from Lizard Island.

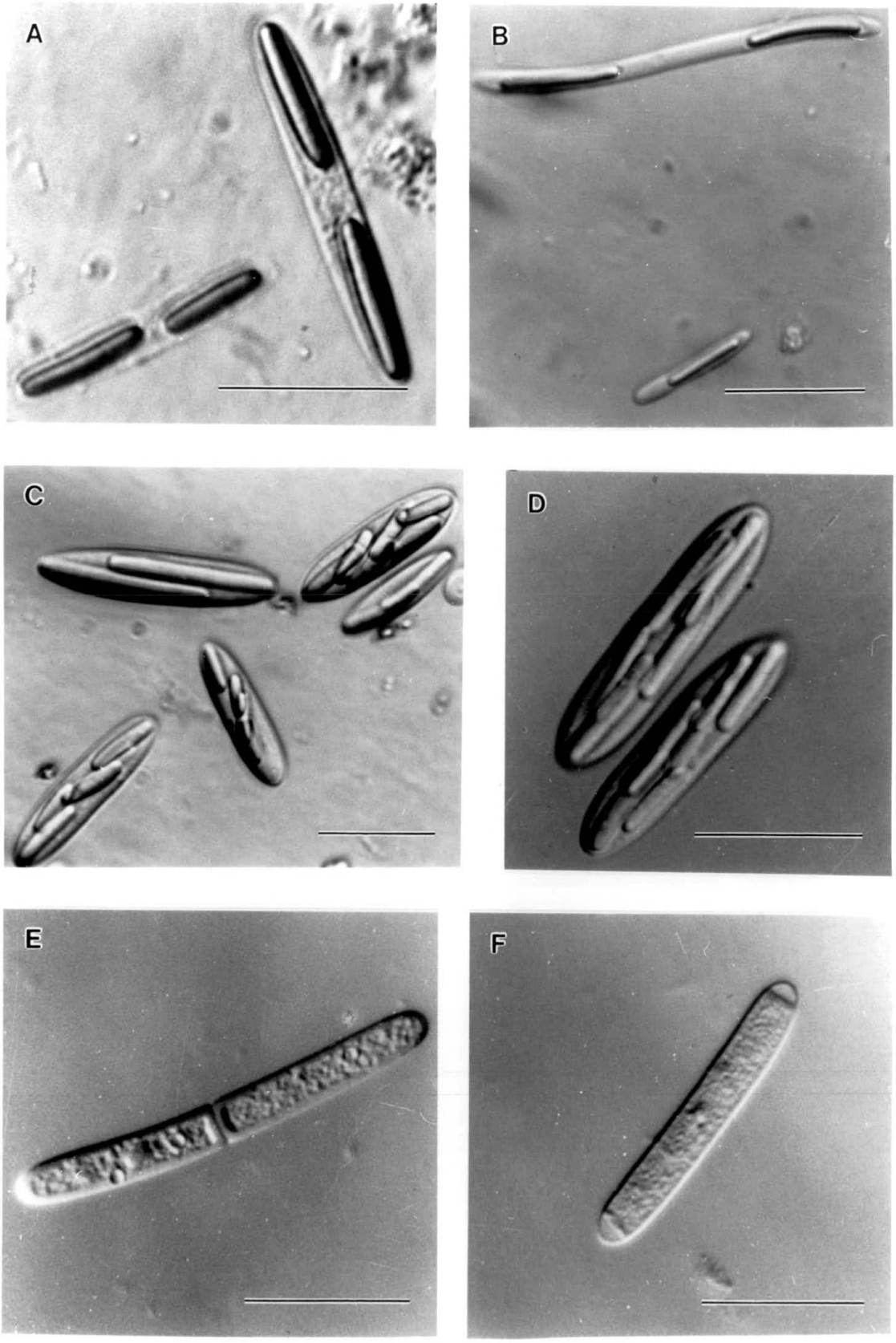

A
B
C
D
E
F

**Plate 4.4 Type G, H, I and J epulos.**

All light microscope photographs taken with Nomarski interference contrast. All scale bars = 20μm.

**A**: Type G and H epulos from 97mmSL *Acanthurus nigroris* collected from Myrmidon Reef.

**B**: Type H epulo with 3 internal structures, possibly incipient daughter-cells, from 124mmSL *Ctenochaetus binotatus* collected from John Brewer Reef.

**C**: Type I epulos from 67mmSL *A. olivaceus* collected from Lizard Island, showing simultaneous division by daughter-cell formation and binary fission.

**D**: Type I epulos from 40mmSL *A. triostegus* collected from Lizard Island.

**E**: Type J epulos from 90mmSL *Naso unicornis* collected from Lizard Island.

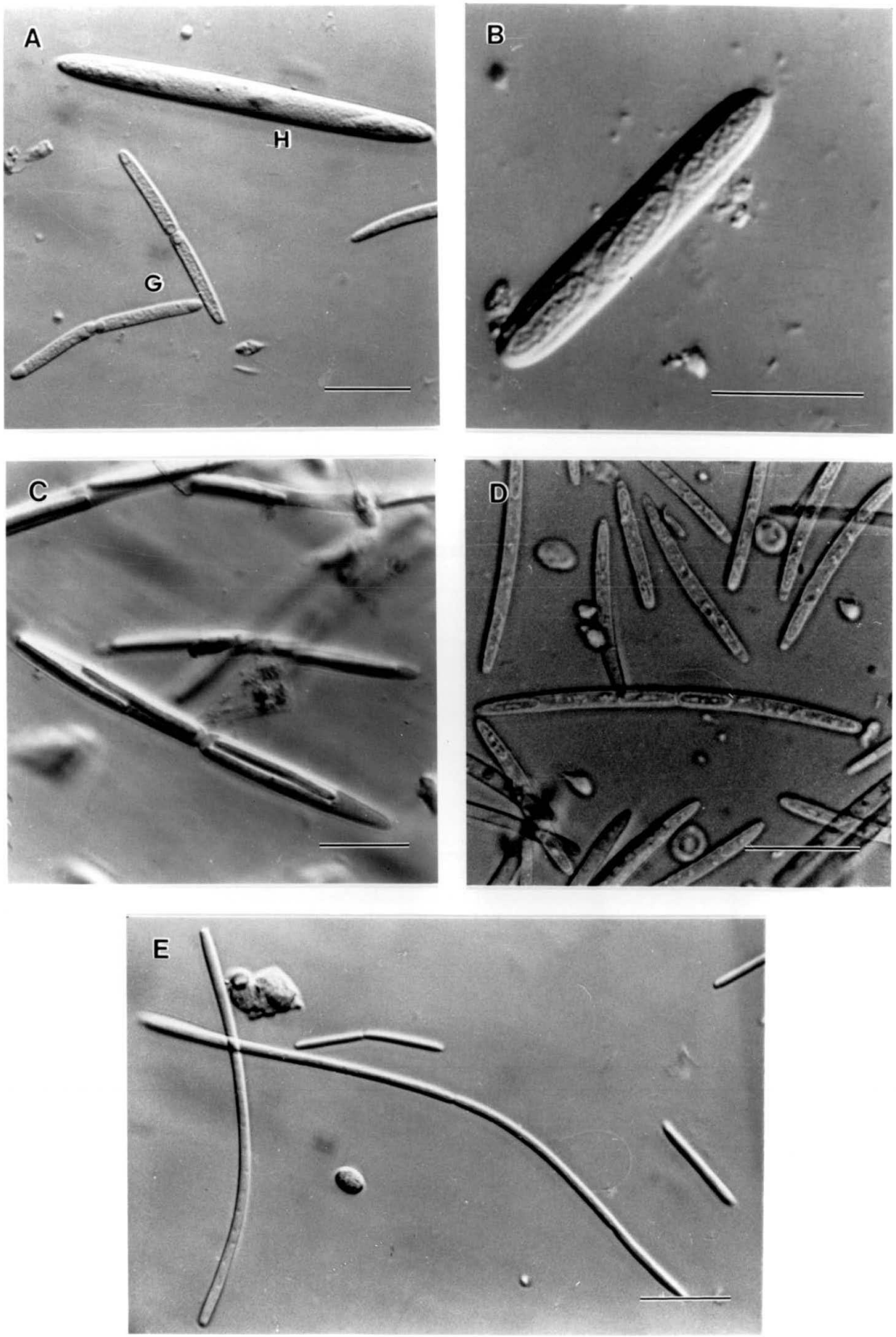

A
H
G
B
C
D
E

**Plate 4.5 Ciliated protozoans**

All light microscope photographs taken with Nomarski interference contrast. All scale bars = 10μm.

**A**: Unidentified ciliate from 127mmSL *Acanthurus achilles* collected from Nui, Tuvalu.

**B**: Unidentified ciliate, possibly a vestibuliferan, from 127mmSL *A. achilles* collected from Nui, Tuvalu.

**C**: Unidentified ciliate, possibly a vestibuliferan, from 125mmSL *A. guttatus* collected from Nui, Tuvalu.

**D**: Ciliate, possibly a nyctotheran, from 114mmSL *A. nigricans* collected from Myrmidon Reef.

**E**: Opalinid zooflagellate of the genus *Protoopalina* from 130mmSL *A. nigrofuscus* collected from Lizard Island.

**F**: *Balantidium* sp. from 255mmSL *Naso tuberosus* collected from Lizard Island.

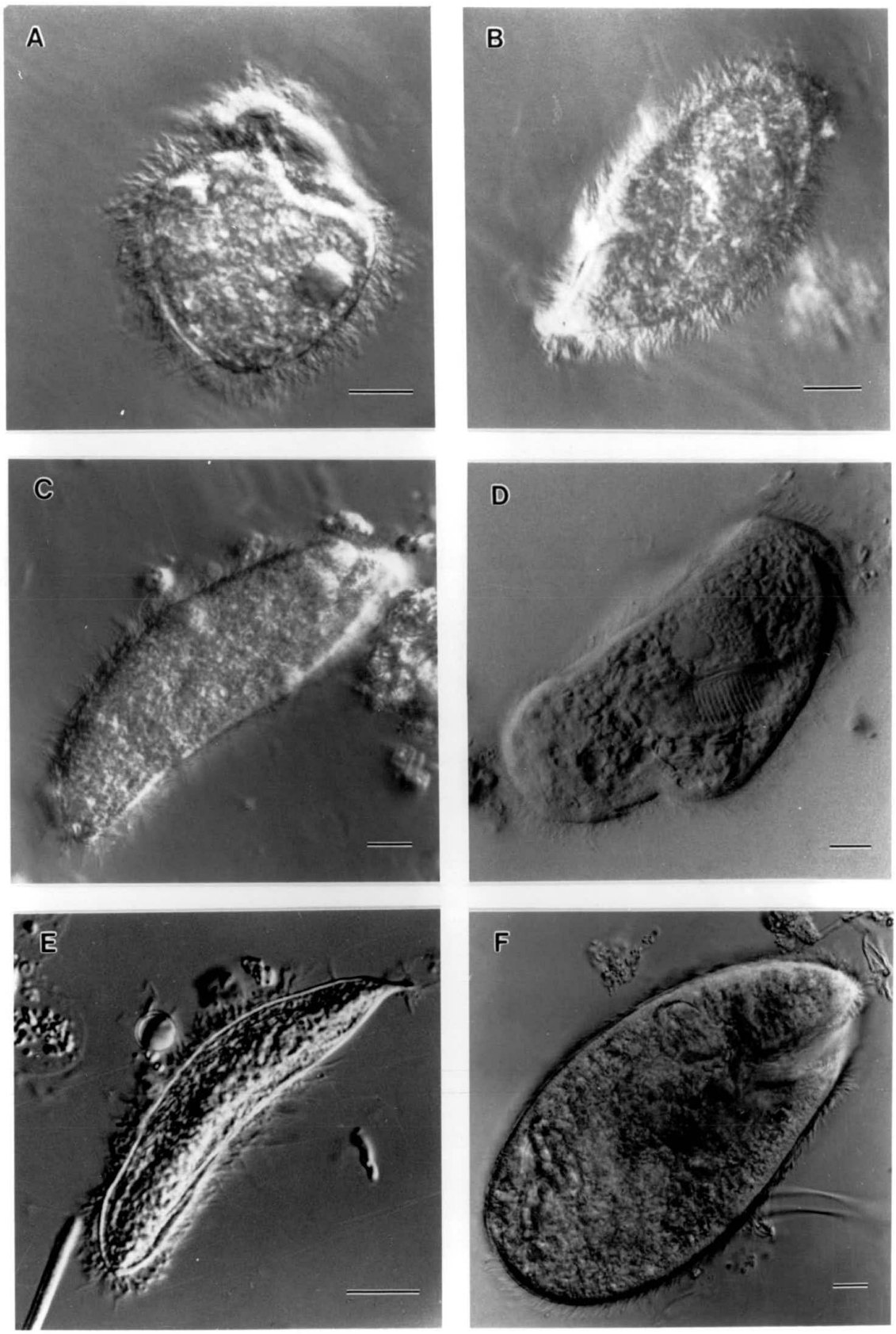

A
B
C
D
E
F

**Plate 4.6 Ciliated protozoans cont.**

All light microscope photographs taken with Nomarski interference contrast. All scale bars = 20μm.

**A**: Unidentified ciliate, possibly a vestibuliferan, from 145mmSL *Zebrasoma rostratum* collected from Nui, Tuvalu.

**B**: Unidentified ciliate from 121mmSL *Z. scopas* collected from Rib Reef.

**C**: Unidentified ciliate, possibly a nyctotheran, from 185mmSL *Z. veliferum* collected from Nanumea, Tuvalu.

**D**: Unidentified ciliate, possibly a nyctotheran, from 170mmSL *Z. veliferum* collected from Nanumea, Tuvalu.

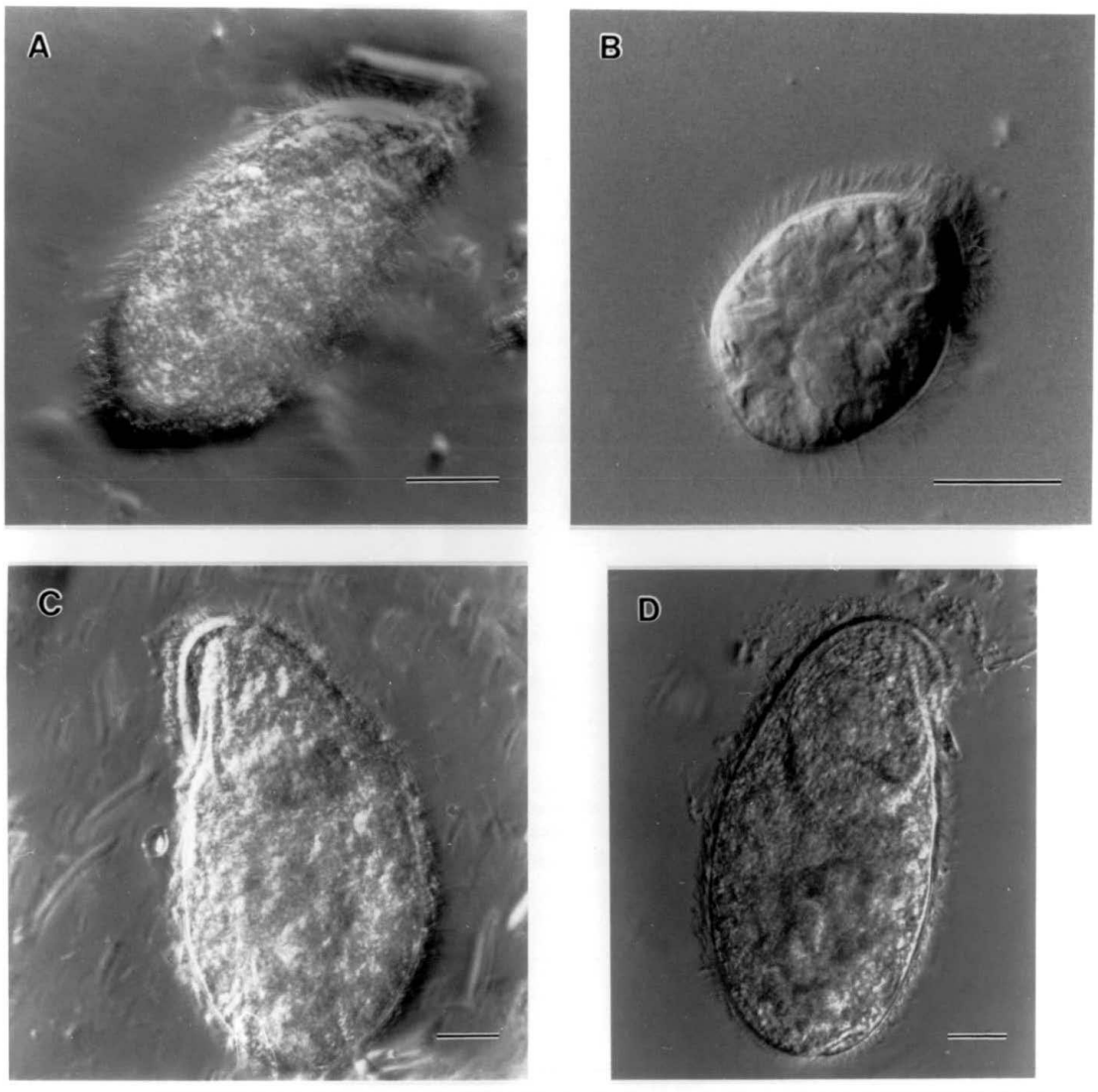

A
B
C
D

**Plate 4.7 Type U and K zooflagellates**

All photos are scanning electron micrographs. All scale bars = 5μm.

**A**: Type U zooflagellate from 80mmSL *Acanthurus nigrofuscus* collected from Julian Rocks, Byron Bay. The oval object lying between the posterior flagella is an artefact.

**B**: Type U zooflagellate from 80mmSL *A. nigrofuscus* collected from Julian Rocks, Byron Bay. This individual has a series of rootlike appendages projecting from the posterior end.

**C**: Type U zooflagellate from 97mmSL *A. nigroris* collected from Myrmidon Reef. Structures possibly representing recurrent flagella are arrowed.

**D**: Type U zooflagellate from 75mmSL *A. nigrofuscus* collected from Julian Rocks, Byron Bay. Structures possibly representing recurrent flagella are arrowed.

**E**: Type K zooflagellate from 125mmSL *A. nigrofuscus* collected from Julian Rocks, Byron Bay.

**F**: Type K zooflagellate from 75mmSL *A. nigrofuscus* collected from Julian Rocks, Byron Bay.

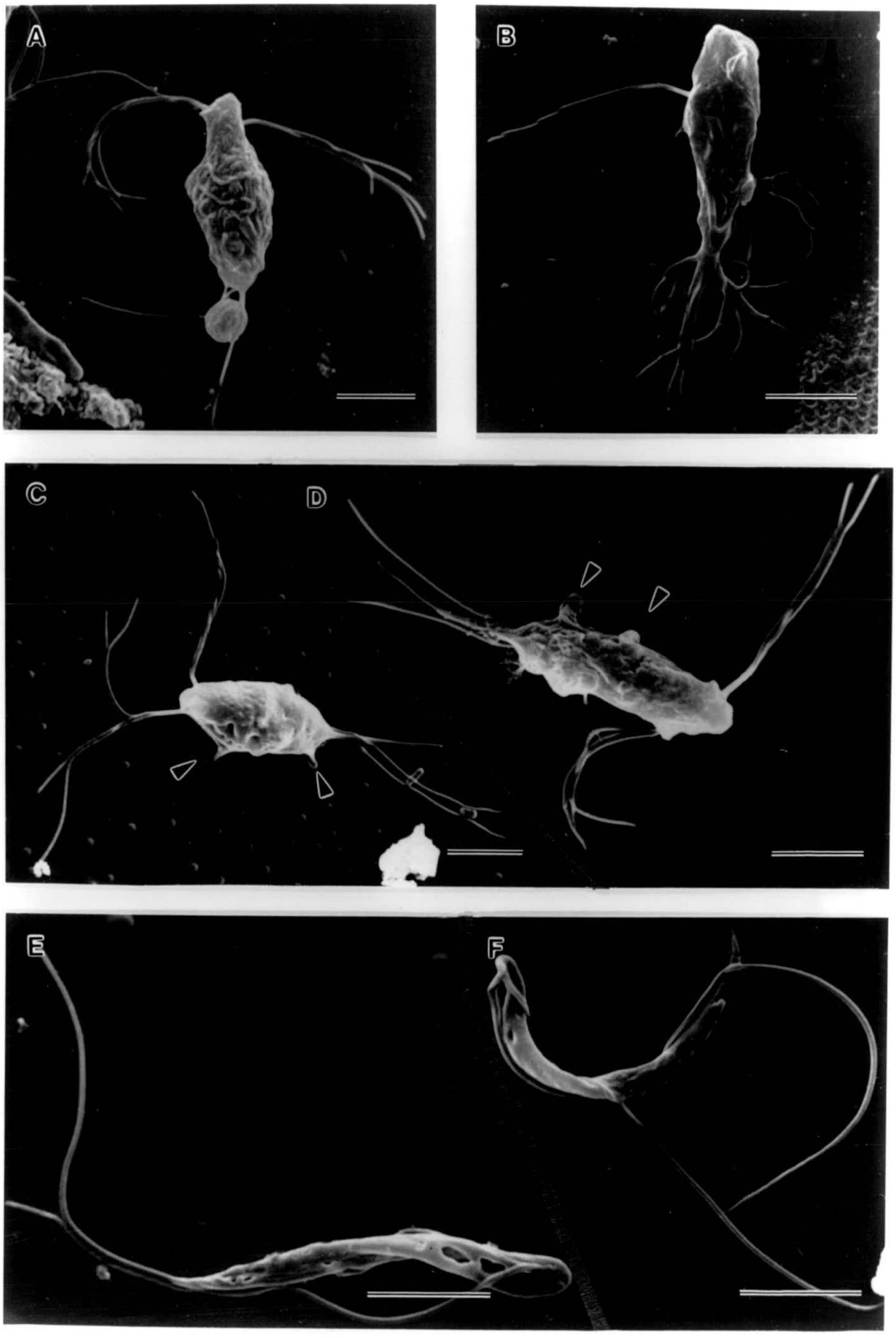

A
B
C
D
E
F

**Plate 4.8 Type D and M zooflagellates**

All photos are scanning electron micrographs. All scale bars = 5μm.

**A**: Type D zooflagellate from 80mmSL *Acanthurus nigrofuscus* collected from Julian Rocks, Byron Bay.

**B**: Type D zooflagellate from 186mmSL *Siganus spinus* collected from Lizard Island.

**C**: Type D zooflagellate from 39mmSL *A. nigrofuscus* collected from Lizard Island.

**D**: Type M zooflagellate from 205mmSL *A. dussumieri* collected from Lizard Island. Axostyle is arrowed.

**E**: Type M zooflagellate from 70mmSL *A. nigrofuscus* collected from Lizard Island. Axostyle is arrowed.

**F**: Type M zooflagellate from 80mmSL *A. nigrofuscus* collected from Julian Rocks, Byron Bay. Axostyle is arrowed.

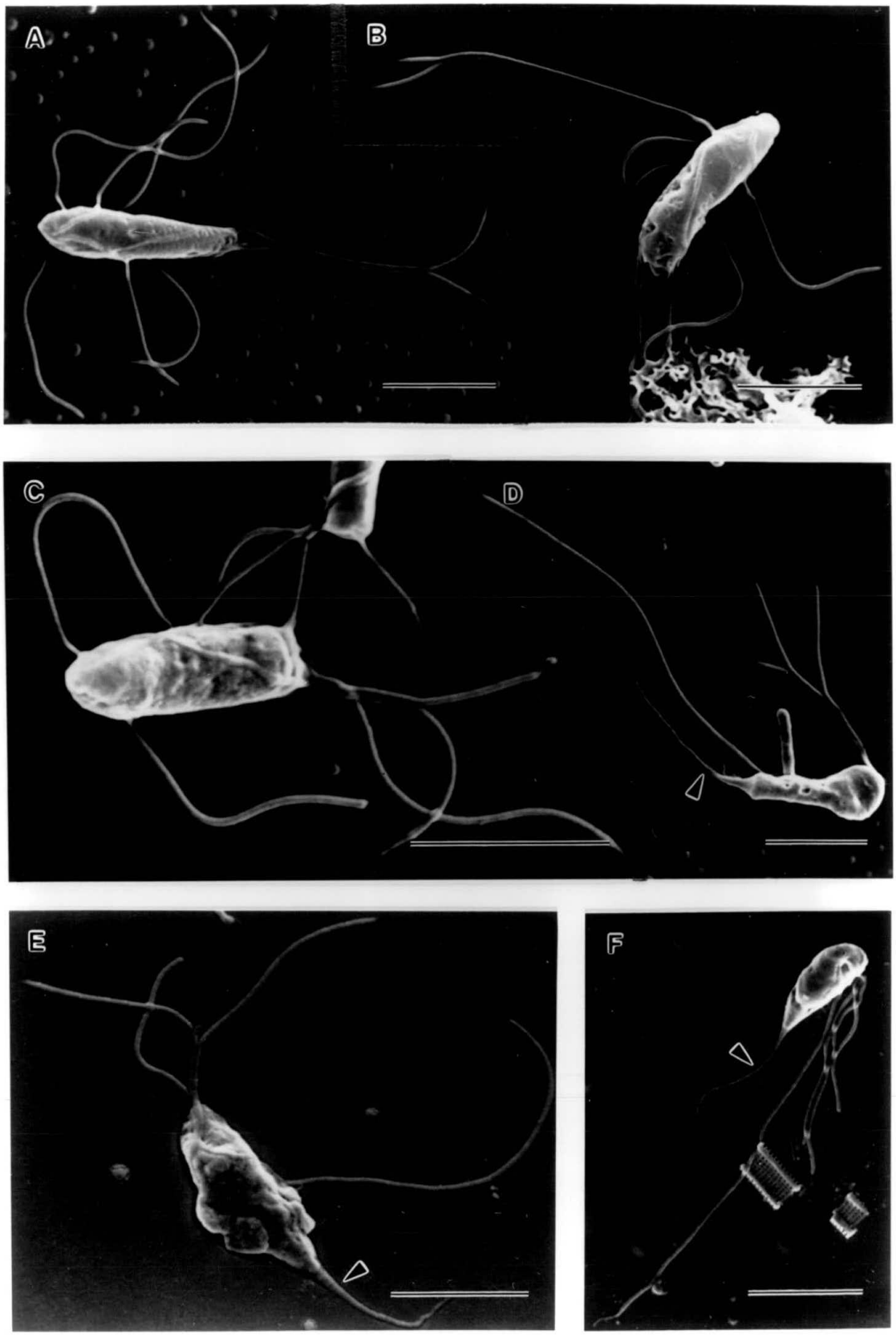

A
B
C
D
E
F

**Plate 4.9 Type T and miscellaneous zooflagellates**

All photos are scanning electron micrographs. All scale bars = 5μm.

**A**: Type T zooflagellate, possibly of the genus *Tetratrichomonas*, from 150mmSL *Acanthurus mata* collected from North Direction Island. Axostyle is arrowed.

**B**: Type T zooflagellate, possibly of the genus *Tetratrichomonas*, from 150mmSL *A. mata* collected from North Direction Island. Recurrent flagellum associated with undulating membrane is arrowed.

**C**: Biflagellated zooflagellate from 100mmSL *A. nigrofuscus* collected from the Inner Gneerings, Maroochydore.

**D**: Type T zooflagellate from 153mmSL *Naso brevirostris* collected from Lizard Island. Axostyle is arrowed.

**Plate 4.7 Type U and K zooflagellates**

All photos are scanning electron micrographs. All scale bars = 5μm.

**A**: Type U zooflagellate from 80mmSL *Acanthurus nigrofuscus* collected from Julian Rocks, Byron Bay. The oval object lying between the posterior flagella is an artefact.

**B**: Type U zooflagellate from 80mmSL *A. nigrofuscus* collected from Julian Rocks, Byron Bay. This individual has a series of rootlike appendages projecting from the posterior end.

**C**: Type U zooflagellate from 97mmSL *A. nigroris* collected from Myrmidon Reef. Structures possibly representing recurrent flagella are arrowed.

**D**: Type U zooflagellate from 75mmSL *A. nigrofuscus* collected from Julian Rocks, Byron Bay. Structures possibly representing recurrent flagella are arrowed.

**E**: Type K zooflagellate from 125mmSL *A. nigrofuscus* collected from Julian Rocks, Byron Bay.

**F**: Type K zooflagellate from 75mmSL *A. nigrofuscus* collected from Julian Rocks, Byron Bay.

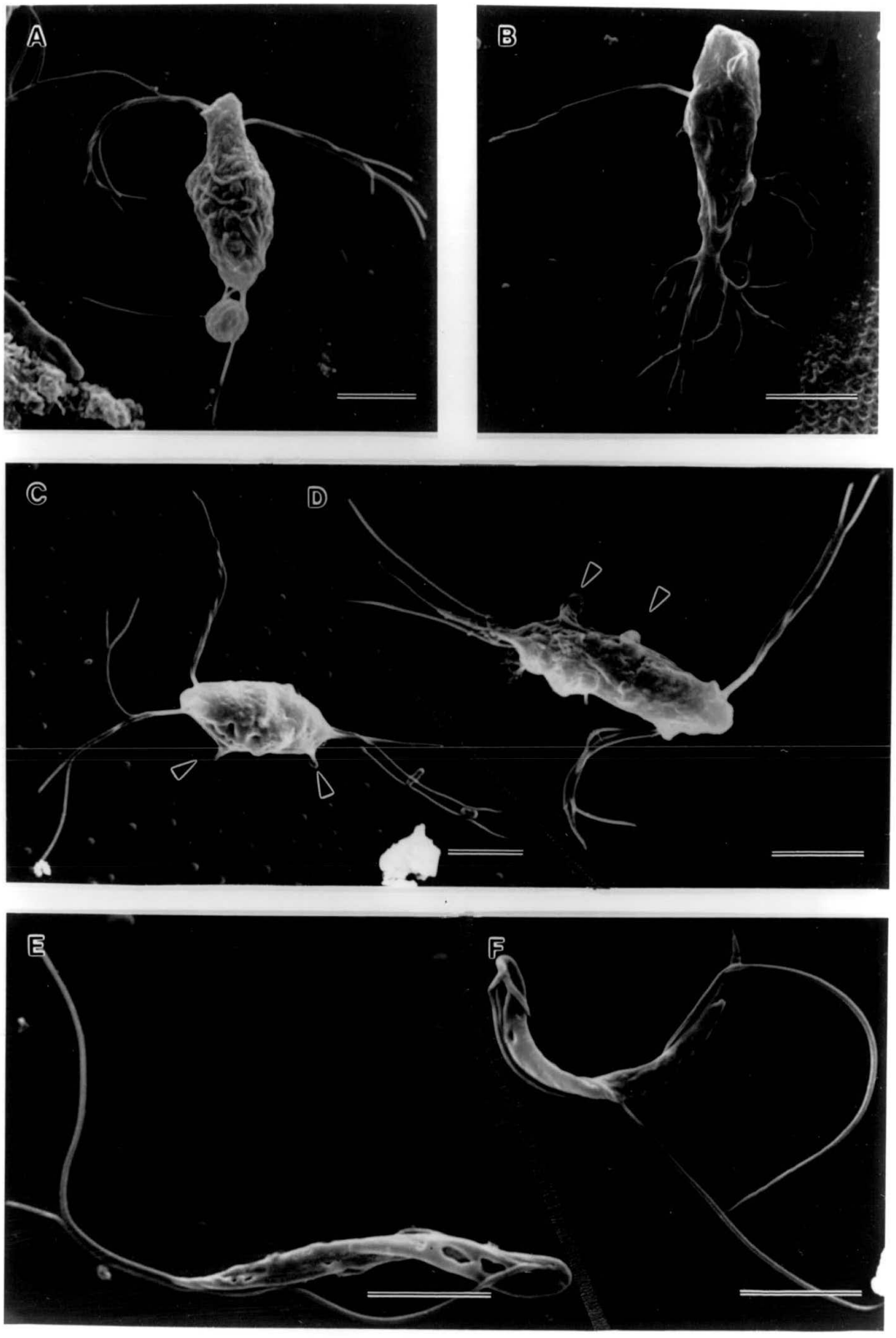

A
B
C
D
E
F

**Plate 4.8 Type D and M zooflagellates**

All photos are scanning electron micrographs. All scale bars = 5μm.

**A**: Type D zooflagellate from 80mmSL *Acanthurus nigrofuscus* collected from Julian Rocks, Byron Bay.

**B**: Type D zooflagellate from 186mmSL *Siganus spinus* collected from Lizard Island.

**C**: Type D zooflagellate from 39mmSL *A. nigrofuscus* collected from Lizard Island.

**D**: Type M zooflagellate from 205mmSL *A. dussumieri* collected from Lizard Island. Axostyle is arrowed.

**E**: Type M zooflagellate from 70mmSL *A. nigrofuscus* collected from Lizard Island. Axostyle is arrowed.

**F**: Type M zooflagellate from 80mmSL *A. nigrofuscus* collected from Julian Rocks, Byron Bay. Axostyle is arrowed.

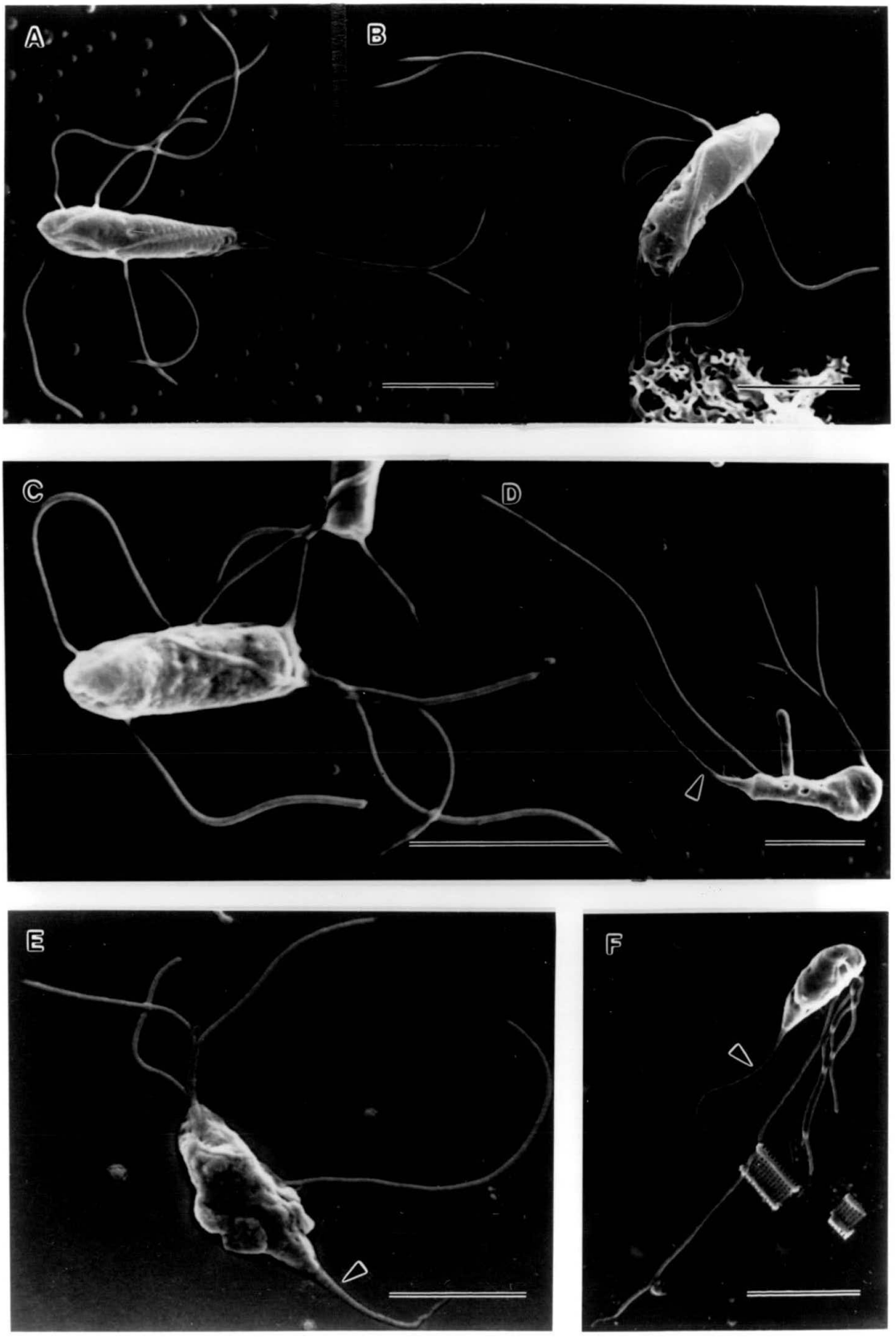

A
B
C
D
E
F

**Plate 4.9 Type T and miscellaneous zooflagellates**

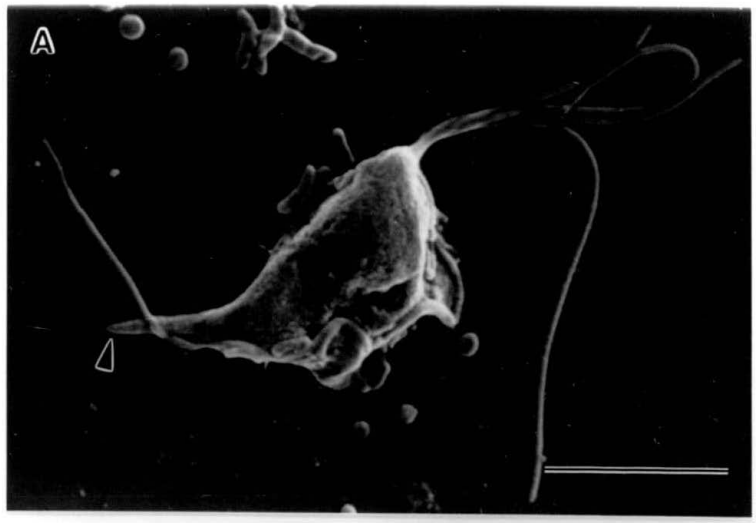
All photos are scanning electron micrographs. All scale bars = 5μm.

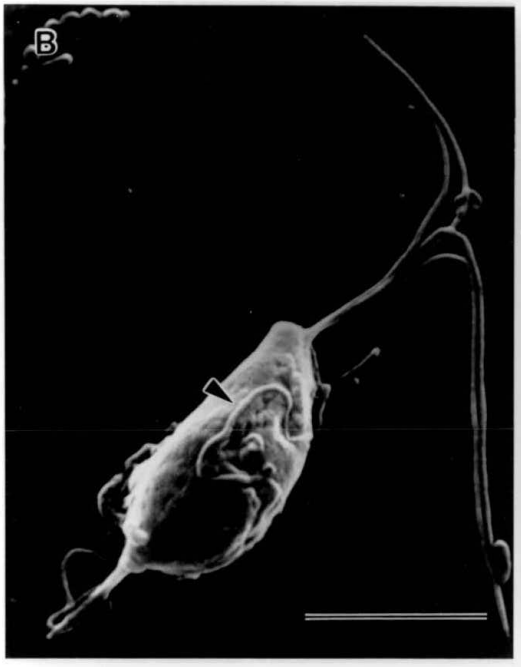
**A**: Type T zooflagellate, possibly of the genus *Tetratrichomonas*, from 150mmSL *Acanthurus mata* collected from North Direction Island. Axostyle is arrowed.

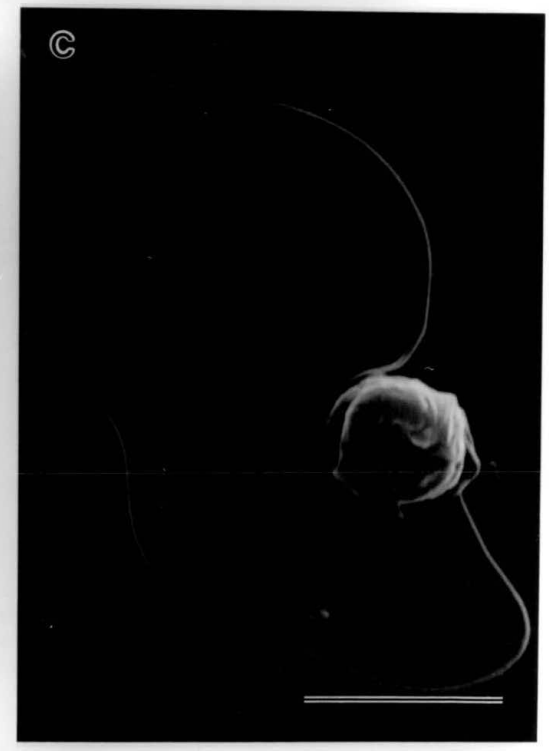
**B**: Type T zooflagellate, possibly of the genus *Tetratrichomonas*, from 150mmSL *A. mata* collected from North Direction Island. Recurrent flagellum associated with undulating membrane is arrowed.

**C**: Biflagellated zooflagellate from 100mmSL *A. nigrofuscus* collected from the Inner Gneerings, Maroochydore.

**D**: Type T zooflagellate from 153mmSL *Naso brevirostris* collected from Lizard Island. Axostyle is arrowed.


A


B


C

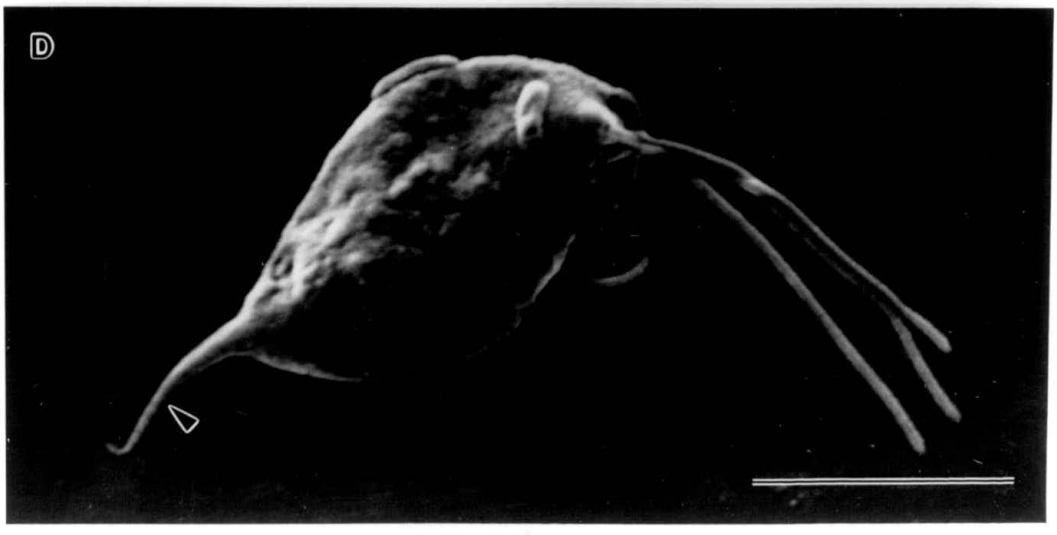

D

**Plate 4.10 Miscellaneous zooflagellates**

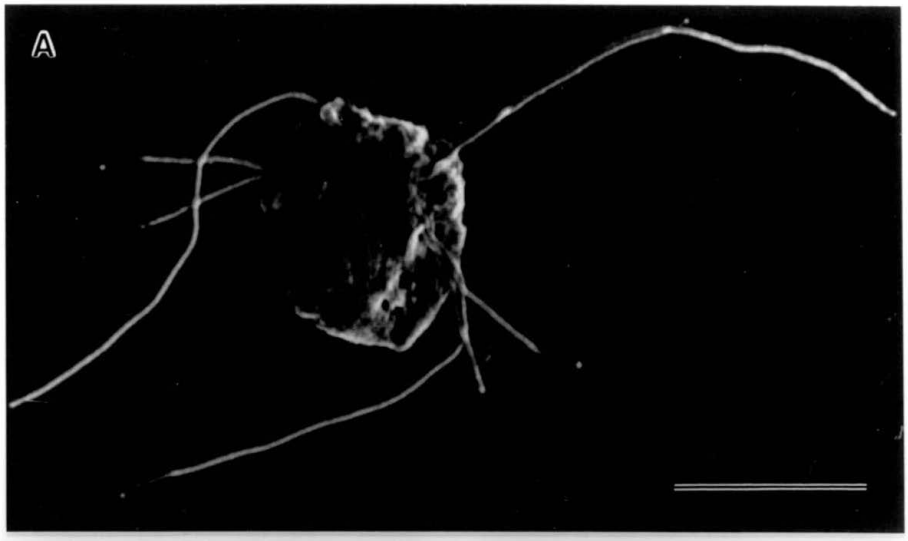
All photos are scanning electron micrographs. All scale bars = 5μm.

**A**: Possible type D zooflagellate from 39mmSL *Acanthurus nigrofuscus* collected from Lizard Island.

**B**: Possible type D zooflagellate from 37mmSL *A. nigrofuscus* collected from Lizard Island.

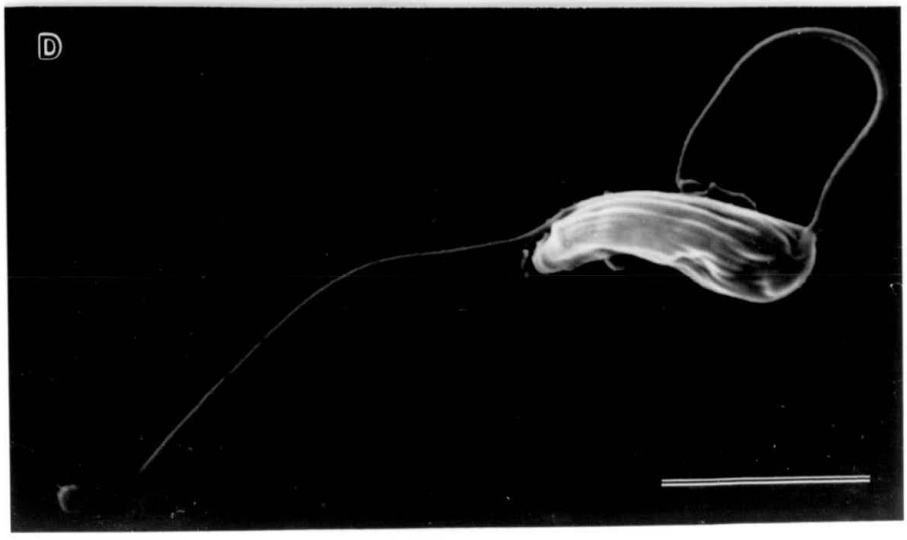
**C**: Unidentified zooflagellates from 72mmSL *Naso brevirostris* collected from Lizard Island.

**D**: Unidentified zooflagellate from 72mmSL *N. brevirostris* collected from Lizard Island.


A


B


C


D

### 4.3.2 Occurrence of endosymbionts amongst fish families examined

A total of 74 species from 8 families were examined in this study. Details of these taxa and sample sizes are presented in Tables 4.1 and 4.2. In this section I will discuss the distribution of intestinal endosymbionts (described above in section 4.3.1) amongst the fish families examined. Families will be presented in alphabetical order.

Family Acanthuridae

The Acanthuridae were found to have the most diverse microbiota of any family examined in this study. All adults of herbivorous species harboured one or more types of epulos, with four exceptions: (a) the two sub-tropical *Prionurus* species, (b) *Acanthurus xanthopterus*, (c) the three species of the "*A. achilles*" group (*A. achilles*, *A. leucosternon*, *A. nigricans* and the hybrid *A. achillesxnigricans*), and (d) *Zebrasoma rostratum* and 2 Tuvalu specimens of *Z. veliferum*. Planktivorous acanthurids, at least as adults, did not harbour epulos. Most species of acanthurids also harboured one or more categories of protozoan. The microbiota of acanthurid species, with the exception of *Prionurus maculatus* and *P. microlepidotus* (discussed below), will be discussed in section 4.3.3.

The microbiota of adult *Prionurus maculatus* and *P. microlepidotus* is summarised in Fig. 4.2. Small bacterial rods, plus spirilla in *P. microlepidotus*, were the only endosymbionts common in the prionurid surgeonfish examined. A few type G

epulos were found in a single 180mmSL *P. microlepidotus* from Northwest Solitary Island. Zooflagellates were present in a few individuals only.

Family Blenniidae

Epulos were not found in either of the two species of this family examined. This family was not examined in detail for the presence of small bacterial rods, spirilla or protozoans.

Family Kyphosidae

Epulos were not found in the single specimen of *Kyphosus cinerascens* examined. The microbiota of two species of temperate and sub-tropical kyphosids has been described by Rimmer and Wiebe (1987).

Family Pomacanthidae

The microbiota of adult *Centropyge bicolor* and *C. bispinosus* is summarised in Fig. 4.2. Extremely elongate bacteria with polar daughter-cells were abundant in the posterior intestine of each of the *C. bicolor* examined. These microorganisms attained a length of 200-240μm, yet were only 3-4μm wide, and may be unrelated to the type E epulos of acanthurids (although they are presented as such in Fig. 4.2). A few organisms resembling type G epulos were found in a single *C. bicolor*. A few elongate rods, some of which were undergoing binary fission and thus

resembled type J epulos, were found in a single *C. bispinosus*. Apart from this, no organisms resembling epulos were found in either the 5 *C. bispinosus* or the single *C. vrolicki* examined. All *Centropyge* examined contained dense populations of spirilla and small bacterial rods, particularly towards the posterior of the intestine and in the rectal swelling. Type T zooflagellates were found in a few individuals.

Family Pomacentridae

The microbiota of the pomacentrids examined in this study is summarised in Fig. 4.3. Many species contained small bacterial rods and spirilla. Low numbers of epulos were found in some individuals of a few species, notably *Pomacentrus amboinensis*, *P. bankanensis* and *Plectroglyphidodon lachrymatus*. No endosymbionts other than small bacterial rods were found in any of the *Pomacentrus wardi*, *Dischistodus perspicillatus*, *D. prosopotaenia* and *Plectroglyphidodon dickii* examined.

Family Scaridae

Endosymbionts were not detected in any of the scarid taxa examined. The gut contents consisted of a slurry of calcareous sediment, algal fragments, diatoms, and in some cases (notably *Scarus gibbus*) sand.

Family Siganidae

The microbiota of the siganids examined in this study is summarised in Fig. 4.2. Epulos were not detected in any of the taxa examined. Almost all siganids examined contained abundant populations of spirilla and small bacterial rods, particularly towards the posterior of the intestine. Type D, type M and type T zooflagellates were found in many individuals, although only in *Siganus punctatus* did they occur in the majority of individuals in a species. Type U and type K zooflagellates were not found in this family. Small (50-60μm) unidentified ciliates were found in both specimens of *S. punctatissimus* (Lizard Island) examined and in a single individual of *S. doliatus* (Rib Reef).

Family Zanclidae

The microbiota of *Zanclus cornutus* examined in this study is summarised in Fig. 4.2. Epulos were not detected in any of the six (three from Yonge Reef, three from Myrmidon Reef) adult individuals examined. All individuals contained large numbers of small bacterial rods. All but one individual contained spirilla, which were more abundant towards the rear of the intestine. Type T zooflagellates were found in the three specimens from Yonge Reef.

### 4.3.3 Occurrence of endosymbionts amongst acanthurid species

This section is subdivided into three parts. The first describes the variation in the

gut microbiota of acanthurid species between different localities **within** the GBR. An assessment of this sort is a necessary prerequisite to comparisons across a broader geographical range. The second part of this section describes the occurrence of endosymbionts amongst the species of tropical acanthurids examined in this study, and includes information from the GBR and other coral reef areas. The third part of this section descibed the occurrence of endosymbionts amongst acanthurids collected from the sub-tropical Australian coast south of the GBR.

(i) Comparison of sites within the Great Barrier Reef

The microbiota of samples from four acanthurid species from several GBR sites is summarised in Fig. 4.4. Samples from each site contained at least five individuals of each species. The microbiota of samples of *Acanthurus nigrofuscus* taken from three sites (one leeward Lizard Island, one exposed Lizard Island and an outer-shelf reef in the central GBR) is presented in Fig. 4.4. It is apparent that there is broad similarity between sites, at least for the common organisms (i.e. those occurring in the majority of host individuals). Thus the proportional occurrence of type A epulos, spirilla, *Protoopalina*, and to a lesser extent several of the zooflagellate categories is very similar between sites. Differences that exist between sites tend to involve the less common microbiota categories, such as type G and J epulos, and type D zooflagellates.

The microbiota of samples of *Zebrasoma scopas* taken from two sites (a mid-shelf reef and an outer-shelf reef in the central GBR) is presented in Fig. 4.4. As with

*Acanthurus nigrofuscus*, the predominant microbiota categories (in this case type E and J epulos, spirilla and ciliates) are similar between the two sites. Two exceptions to this are apparent for this species: type D and type M zooflagellates. Type D zooflagellates predominated amongst fish taken at the outer-shelf reef, while type M zooflagellates predominated amongst fish from the mid-shelf reef.

A comparison of the microbiota of *Ctenochaetus binotatus* and *C. striatus* from several sites on the GBR (two mid-shelf reefs and an outer-shelf reef in the central GBR, and the channel entrance at Lizard Island for *C. binotatus*; and a mid-shelf reef and an outer-shelf reef in the central GBR, and two Lizard Island sites for *C. striatus* - Fig. 4.4) shows a more confused picture. In these species, both detritivores (see Table 4.3), few microbiota categories occurred in a majority of individuals. This makes a comparison between sites more difficult. Nevertheless, it is apparent that apart from the zooflagellates (which also displayed some site variation in *Acanthurus nigrofuscus* and *Zebrasoma scopas* above) there are more similarities between sites than differences. For example, type G epulos and spirilla were found in at least some individuals at each site. Type A, B, C, I, and J epulos were not found in any individuals, nor were ciliates or type U and K zooflagellates. The main variation between sites seems to relate to differences in the proportions of a few endosymbiont categories, rather than to large differences in microbiota composition.

A comparison between the four species shows that there are major differences in the microbiota composition of *Acanthurus nigrofuscus*, *Zebrasoma scopas* and the two

*Ctenochaetus* species. *A. nigrofuscus* is characterised by the presence of type A epulos and spirilla (which were present in every individual examined at each site). *Z. scopas* is characterised by high occurrences of type E and J epulos, the former of which was absent and the latter rare in *A. nigrofuscus*. The *Ctenochaetus* species often contained type F epulos, which were not found in either *A. nigrofuscus* and *Z. scopas*. The differences in microbiota composition between species thus seem to be of greater magnitude than the differences between the sites examined. It should be noted that while these samples encompass potentially important axes of variation (e.g. exposed vs. leeward, mid-shelf vs. outer shelf, northern GBR vs. central GBR), they do not represent a balanced design. I am therefore unable to exclude the possibility that site differences were present but not detected. I decided to clump GBR samples on the basis of: (a) the small magnitude of site variation suggested by the above observations, (b) the need to make broad-scale geographical comparisons (in which case samples representing the variation within the GBR region as a whole were required), and (c) the desirability to maximise sample sizes for each host species.

(ii) Comparison of the GBR and other coral reefs

In this section acanthurid species will be discussed separately and in alphabetical order. Geographical comparisons will be made within the context of individual species, where possible. The information in this section relates to adult (>70mmSL) specimens only. The microbiota of juvenile acanthurids is discussed in section 4.3.4.

*Acanthurus achilles* (Fig. 4.5): Epulos were not found in the 6 Tuvalu specimens, nor in the two Australian Museum specimens (from Oahu, Hawaii and Tarawa, Kiribati) examined. Ciliates were found in three of the Tuvalu *A. achilles* specimens examined (Plate 4.5 A & B). Two forms were present: a small *Balantidium* species (Family Balantidiidae, Order Vestibuliferida, Subclass Trichostomatia, Class Litostomatea, Subphylum Rhabdophora), and a larger nyctotheran (Family Nyctotheridae, Order Clevelandellida, Subclass Heterotrichia, Class Spirotrichea, Subphylum Postciliodesmatophora).

*Acanthurus auranticavus* (Fig. 4.5): This species characteristically harboured type A epulos, which ranged in length up to 222μm. The one individual examined which lacked type A epulos contained type H epulos. No protozoans were observed in this species.

*Acanthurus blochii* (Fig. 4.5): Type A epulos (up to 266μm in length) were found in every individual examined. No other endosymbiont category was found in more than a few individuals of this species.

*Acanthurus dussumieri* (Fig. 4.5): Type A epulos (up to 222μm in length) were found in every individual examined. Spirilla and type M zooflagellates were found in most individuals examined. An Australian Museum specimen from Tulear Barrier Reef, Madagascar, did not contain type A epulos, but did contain large numbers of type G and H.

*Acanthurus grammoptilus* (Fig. 4.6): Type A epulos (up to 168μm in length) were found in four of the five individuals examined. The individual lacking type A epulos contained type G epulos. Apart from type A epulos, no endosymbiont category was found in more than two specimens.

*Acanthurus guttatus* (Fig. 4.6): Only one of the 6 Tuvalu specimens contained type A epulos, the remaining 5 specimens contained either type G, type H, or both. Ciliates of the genus *Balantidium* were found in 4 of the 6 Tuvalu specimens (Plate 4.5C). Australian Museum specimens from the Marquesas Islands and Tubuai Island (South Pacific) contained type E and H epulos.

*Acanthurus leucosternon:* The six Australian Museum specimens examined were collected from four localities: Christmas Island, Indian Ocean; Tahiti (2 specimens); Koh Koa, Thailand (2 specimens); and Salomon Atoll, Indian Ocean. No epulos were found in any of these specimens, which were not examined for zooflagellates.

*Acanthurus lineatus* (Fig. 4.6): Type A epulos (up to 333μm in length) were found in every individual examined from both GBR and Tuvalu samples. Type M zooflagellates were found in the majority of specimens from both localities. Spirilla and type K zooflagellates (which were more common in anterior rather than posterior intestine samples) were found in most GBR specimens, but were absent from the Tuvalu specimens. An Australian Museum specimen from Savo Island, Solomon Islands, contained large numbers of type A epulos.

*Acanthurus maculiceps*: The single specimen collected in Tuvalu had an empty gut, yet two type H epulos were found in gut fluid. An Australian Museum specimen from Lihou Reef in the Coral Sea contained type A epulos.

*Acanthurus mata* (Fig. 4.6): Two small specimens (125 and 150mmSL) collected from North Direction Island both contained small (25-31μm) type G epulos, and one contained a few type T zooflagellates. No epulos were found in 3 large (290-360mmSL) specimens collected from Yonge Reef front.

*Acanthurus nigricans* (Fig. 4.7): No epulos were found in any of the GBR or Tuvalu specimens examined, nor in an Australian Museum specimen from Osprey Reef in the Coral Sea. Nyctotheran ciliates were found in 5 of the 13 GBR specimens examined (Plate 4.5D), but were absent from the Tuvalu material.

*Acanthurus nigricauda* (Fig. 4.7): Type A epulos were only found in a few specimens of this species. The type C epulos found in the Tuvalu specimens ranged in length from 70-92μm. Type F epulos were found in all of the Tuvalu specimens, but in only 2 of 6 GBR specimens. Spirilla occurred in half of the GBR specimens, and were entirely absent from Tuvalu specimens. Type M zooflagellates were found in all of the GBR and Tuvalu specimens examined. Type D zooflagellates were found in half of the GBR specimens, but were absent from the Tuvalu specimens.

*Acanthurus nigrofuscus* (Fig. 4.7): Every specimen examined contained type A epulos, with the exception of one 94mmSL specimen from Tuvalu, which contained

type G epulos. The type A epulos in this species include the largest epulos measured, up to 576μm in length. GBR specimens also always contained spirilla, and usually harboured opalinid zooflagellates, and type U, type K, and type M zooflagellates. Specimens from Flinder's Reef (to the south of the GBR) differed little in microbiota composition from the GBR specimens. The Tuvalu material differed from the two Australian samples in lacking opalinid zooflagellates and type U and type K flagellates. Opalinid zooflagellates were found in an Australian Museum specimen from Scott Reef (off northwestern Australia), but were absent from museum specimens collected in the Solomon Islands, in the Capricorn-Bunker Group in the southern GBR and at Middleton Reef (near Lord Howe Island).

The opalinid zooflagellates found in *A. nigrofuscus* (Plate 4.5E) may be tentatively assigned to the genus *Protoopalina* (J.N. Grim pers. comm.). The subphylum Opalinata is a discrete group of zooflagellates covered in cilia (Lee et al. 1985), although they bear a strong resemblance to some ciliate taxa. Opalinids differ from ciliates in several respects, including (i) the fibrillar associates of the kinetosomes (basal bodies) in opalinids are unlike those of ciliate kinetosomes; and (ii) unlike ciliates, opalinids add new kinetosomes and files of cilia at the anterior margin of the cell (Lee et al. 1985). Opalinids lack a cytostome or cytopharynx and ingestion occurs by a modified pinocytosis (Lee et al. 1985).

*Acanthurus nigroris* (Fig. 4.7): The single specimen of this species taken from the GBR (Myrmidon Reef) contained type A epulos, spirilla, and type U and type M zooflagellates. The latter 3 endosymbiont categories were also present in the 6

Tuvalu specimens examined, yet these contained type G and H epulos.

*Acanthurus olivaceus* (Fig. 4.8): The GBR and Tuvalu samples both contained type F epulos, spirilla, and type D and type M zooflagellates. However the GBR specimens also contained type B epulos (up to 112μm in length), spirilla, and type U zooflagellates, which were not found in the Tuvalu material.

*Acanthurus pyroferus* (Fig. 4.8): Both the GBR and Tuvalu specimens contained type G and type H epulos and type M zooflagellates. Four of the 7 GBR specimens examined also contained type F epulos, absent from the Tuvalu material. Similarly, spirilla were present in GBR specimens but not in the Tuvalu specimens.

*Acanthurus thompsoni* (Fig. 4.8): No epulos or zooflagellates were found in the six specimens examined.

*Acanthurus triostegus* (Fig. 4.8): Type A epulos (ranging in length up to 433μm) and type M zooflagellates were found in every GBR specimen examined, while spirilla and type U and type K zooflagellates occurred in the majority of individuals. In the Tuvalu material type M zooflagellates were present in every specimen, while no type U or type K zooflagellates were found. A single Tuvalu specimen did not contain type A epulos, but harboured type C epulos ranging up to 84μm in length. No spirilla were found in the Tuvalu specimens. The four Australian Museum specimens examined (two specimens each from Mauritius and Abaiang Atoll, Kiribati) all contained type A epulos. The two specimens from Abaiang Atoll also

contained type G epulos, which were also present in half of the Tuvalu specimens.

*Acanthurus xanthopterus* (Fig. 4.9): None of the specimens examined contained epulos. All but the smallest (92mmSL) specimen contained the ciliate *Vestibulongum corlissi* (Family Pychnotrichidae, Class Litostomatea, Subphylum Rhabdophora). *V. corlissi* had previously been recorded from *A. xanthopterus* collected in South Africa (Grim 1988).

*Ctenochaetus binotatus* (Fig. 4.9): This species contained a variety of the smaller epulo types, the most common of which was type G. Spirilla occurred in the majority of the GBR specimens but not in the single individual collected in Tuvalu. An Australian Museum specimen from Osprey Reef, Coral Sea, contained numerous type F epulos.

*Ctenochaetus hawaiiensis* (Fig. 4.9): The only endosymbionts found in this species were unidentified ciliates, which were up to 117µm in length.

*Ctenochaetus marginatus* (Fig. 4.9): One individual of the six specimens collected in Tuvalu contained a few small (up to 50µm in length) type H epulos. An Australian Museum specimen from the Marquesas Islands also contained low numbers of small type H epulos.

*Ctenochaetus striatus* (Fig. 4.10): This species often contained type F epulos and type D and type M zooflagellates. Type G epulos and spirilla were found in the

majority of GBR specimens, but were absent from the Tuvalu material. An Australian Museum specimen from Osprey Reef, Coral Sea, contained numerous type F epulos.

*Ctenochaetus strigosus* (Fig. 4.10): Type F epulos were found in all of the GBR specimens examined, and in all but one of the Tuvalu specimens. Type G epulos were also common in the Tuvalu samples. Type D and type M zooflagellates were present in two and one of the Tuvalu specimens respectively.

*Naso brevirostris* (Fig. 4.10): Only subadults up to 190mmSL of this species were examined. The planktivorous adults, which attain a length of 450mmSL (Myers 1989), occupy the upper water column and were not collected. Type E and G epulos and spirilla occurred in the majority of specimens. A variety of protozoans were found in this species, but none occurred in many individuals. Five individuals of this species also contained large numbers of the zooflagellate illustrated in Plate 4.10C & D.

*Naso hexacanthus*: Epulos were absent from the two adult specimens of the species examined. Both contained spirilla and small rods, while the smaller of the two (160mmSL) also contained small ciliates (50-64μm in length) and type T zooflagellates.

*Naso lituratus* (Fig. 4.10): Type C epulos and spirilla occurred in the majority of individuals from both the GBR and Tuvalu samples. There was a difference between

the two samples in the relative proportions of type E and type G epulos. Type M zooflagellates, absent from GBR samples, were found in 4 out of 5 Tuvalu specimens.

*Naso tuberosus* (Fig. 4.11): All but one individual of this species contained large type B epulos (up to 322μm in length), spirilla and the ciliate *Balantidium* sp. (Grim In Press). *B.* sp. is illustrated in Plate 4.5F. The single individual lacking type B epulos and ciliates was the smallest specimen examined (138mmSL), and contained type F epulos.

*Naso unicornis* (Fig. 4.11): This species contained a large variety of epulo types, with often 2 or 3 epulo types present in one specimen. Type C epulos were the most prevalent, and type A epulos (up to 112μm in length) were present in 2 of the 6 specimens examined.

*Naso vlamingii* (Fig. 4.11): Only subadults (up to 175mmSL) of this species, which attains a length of 500mmFL (Myers 1989), were examined. Small epulos (type E or type G) were present in every specimen.

*Paracanthurus hepatus*: No epulos were found in the 3 Australian Museum specimens (from the Philippines, Vanuatu and Stradbroke Island, Queensland) examined. All 3 specimens of this predominantly zooplanktivorous species (Myers 1989) contained filamentous algae in addition to crustaceans.

*Zebrasoma rostratum* (Fig. 4.11): Very low numbers of type E epulos, which may have been incidentally ingested, were found in 3 of the 7 specimens examined. Ciliates of the genus *Balantidium* (Plate 4.6A) were found in one 145mmSL specimen from Nui.

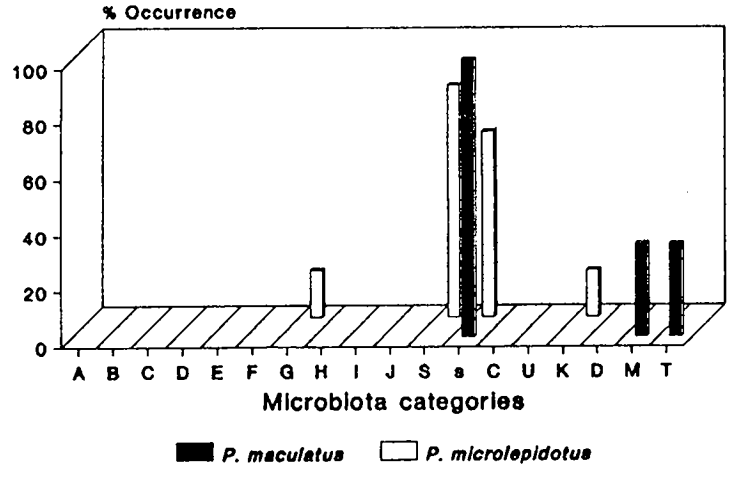
*Zebrasoma scopas* (Fig. 4.12): Type E and J epulos and spirilla occurred in most of the GBR specimens examined. A single specimen from Myrmidon Reef contained a few type A epulos (largest measured 104μm in length). Two forms of ciliates (one of which is illustrated in Plate 4.6B), a *Balantidium* sp. and a nyctotheran, were found in GBR specimens, along with type D and type M zooflagellates. Type J epulos, spirilla and type D epulos were not found in the Tuvalu specimens, and ciliates only occurred in one individual.

*Zebrasoma veliferum* (Fig. 4.12): All specimens from the GBR and 3 of the Tuvalu specimens contained a range of small (D, E, G and H) epulo types. However 2 of the 5 Tuvalu specimens did not contain epulos. Spirilla were found in all of the GBR specimens. Two forms of ciliates, a *Balantidium* sp. (Plate 4.6C) and a nyctotheran (Plate 4.6D), were found in the Tuvalu specimens. No ciliates were found in GBR material. Type M zooflagellates were found in most of the GBR specimens, yet were absent from the Tuvalu samples.

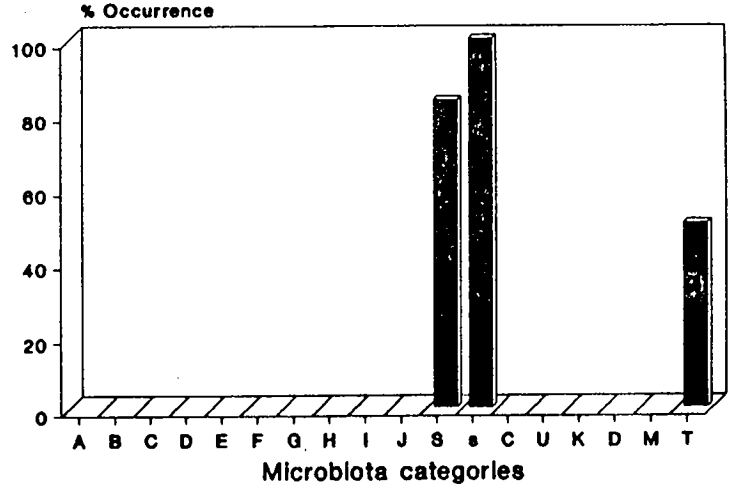
**Fig. 4.2 Microbiota of *Prionurus* species, *Zanclus cornutus*, siganids and *Centropyge* species.**

Percent occurrence of microbiota categories amongst individuals examined. See Tables 4.1 and 4.2 for sample sizes of taxa examined.
Abbreviations:
Epulo types: A = type A epulos, B = type B epulos, C = type C epulos, D = type D epulos, E = type E epulos, F = type F epulos, G = type G epulos, H = type H epulos, I = type I epulos, J = type J epulos.
Other prokaryotes: S = spirilla, s = small rod-shaped bacteria.
Protozoans: C = ciliates, U = type U zooflagellates, K = type K zooflagellates, D = type D zooflagellates, M = type M zooflagellates, T = type T zooflagellates.

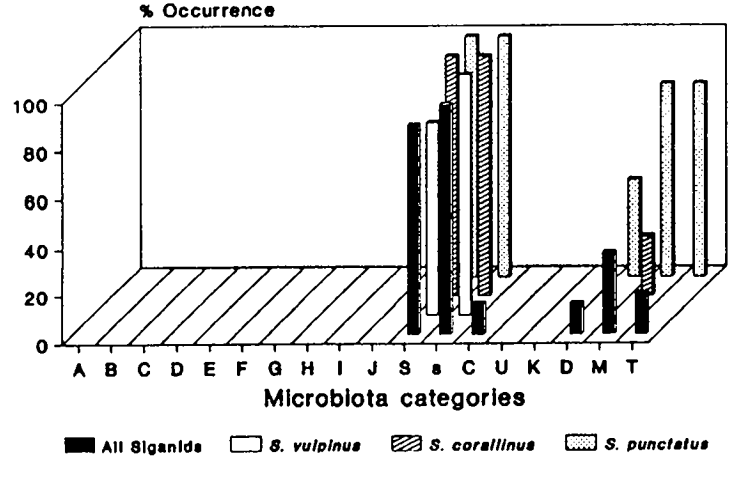
## *Prionurus*




## *Zanclus cornutus*




## Siganids




## *Centropyge*

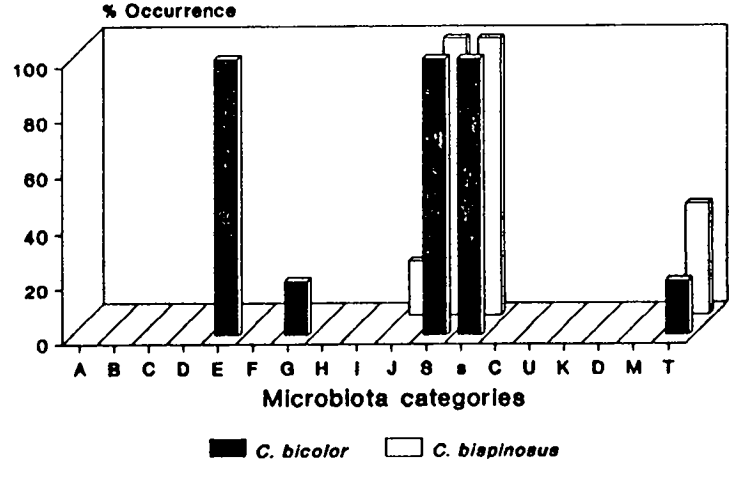
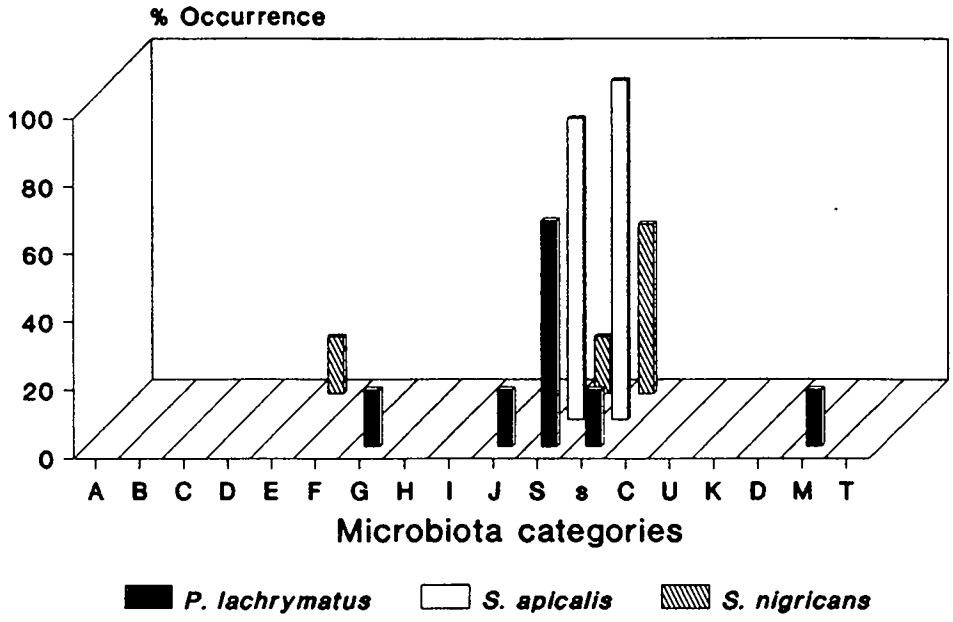


**Fig. 4.3 Microbiota of Pomacentrids.**

Percent occurrence of microbiota categories amongst individuals examined. See Table 4.2 for sample sizes of taxa examined.
Abbreviations:
Epulo types: A = type A epulos, B = type B epulos, C = type C epulos, D = type D epulos, E = type E epulos, F = type F epulos, G = type G epulos, H = type H epulos, I = type I epulos, J = type J epulos.
Other prokaryotes: S = spirilla, s = small rod-shaped bacteria.
Protozoans: C = ciliates, U = type U zooflagellates, K = type K zooflagellates, D = type D zooflagellates, M = type M zooflagellates, T = type T zooflagellates.

# Pomacentrids I




# Pomacentrids II

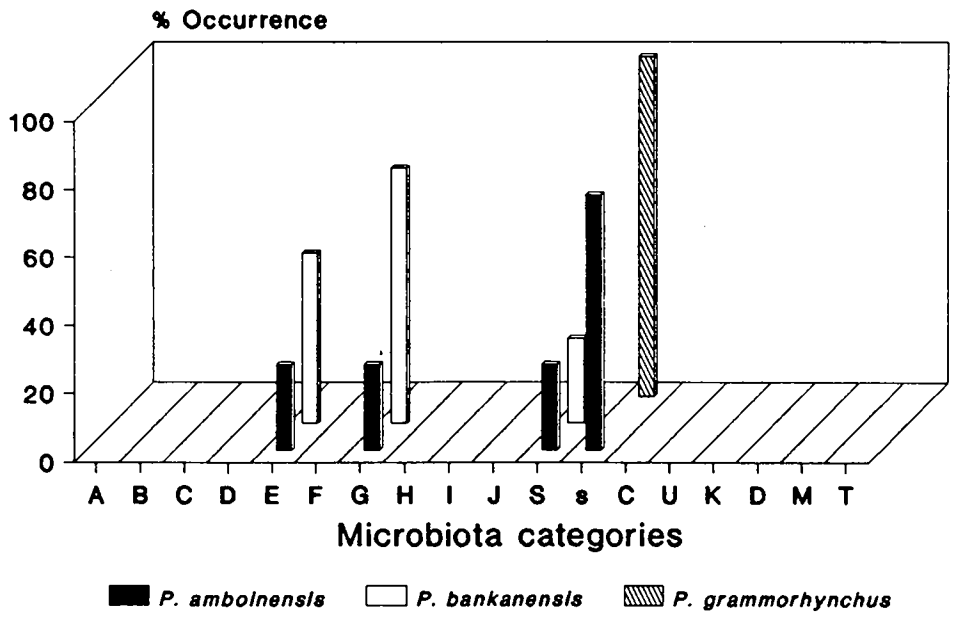
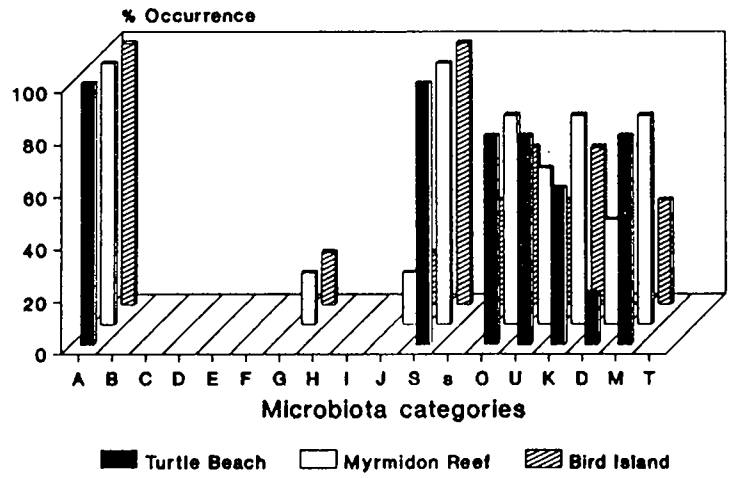


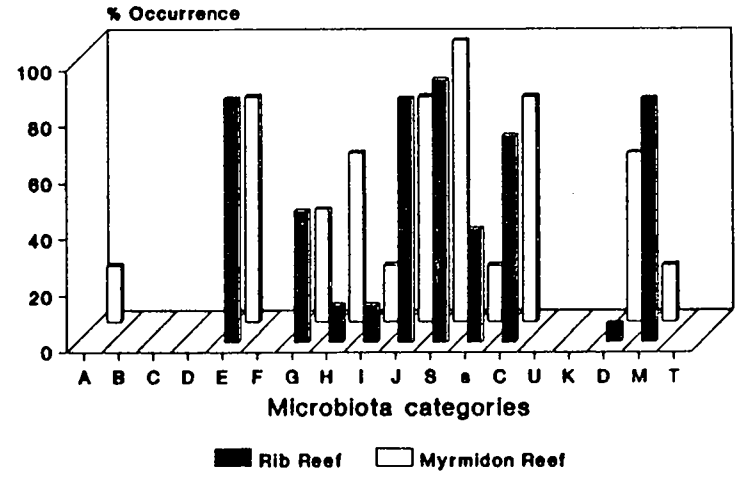
**Fig. 4.4 Within GBR variation in microbiota of *Acanthurus nigrofuscus*, *Zebrasoma scopas*, *Ctenochaetus binotatus* and *C. striatus*.**

Percent occurrence of microbiota categories amongst individuals examined. At least five individuals of each species examined from each location.
Abbreviations:
Epulo types: A = type A epulos, B = type B epulos, C = type C epulos, D = type D epulos, E = type E epulos, F = type F epulos, G = type G epulos, H = type H epulos, I = type I epulos, J = type J epulos.
Other prokaryotes: S = spirilla, s = small rod-shaped bacteria.
Protozoans: C = ciliates, U = type U zooflagellates, K = type K zooflagellates, D = type D zooflagellates, M = type M zooflagellates, T = type T zooflagellates, O = opalinids.

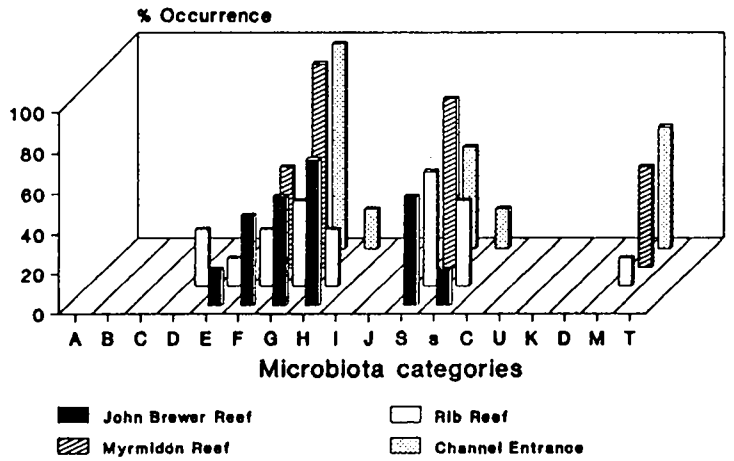
## *A. nigrofuscus*




## *Z. scopas*




## *C. binotatus*




## *C. striatus*

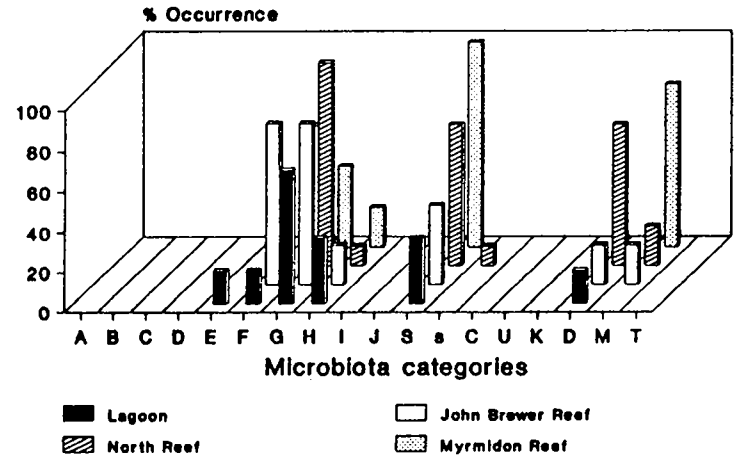
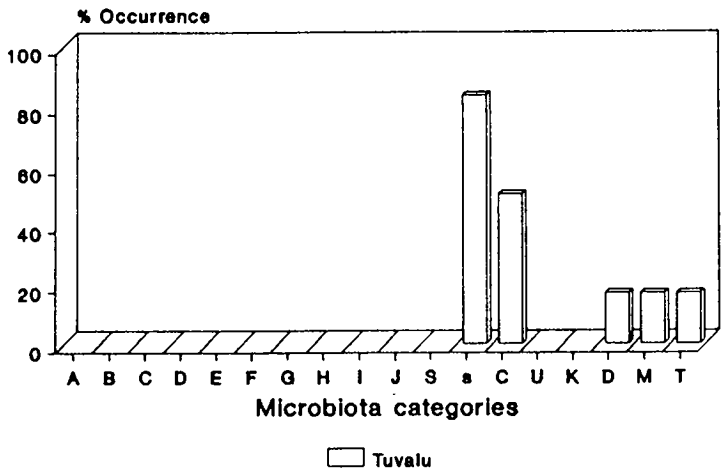


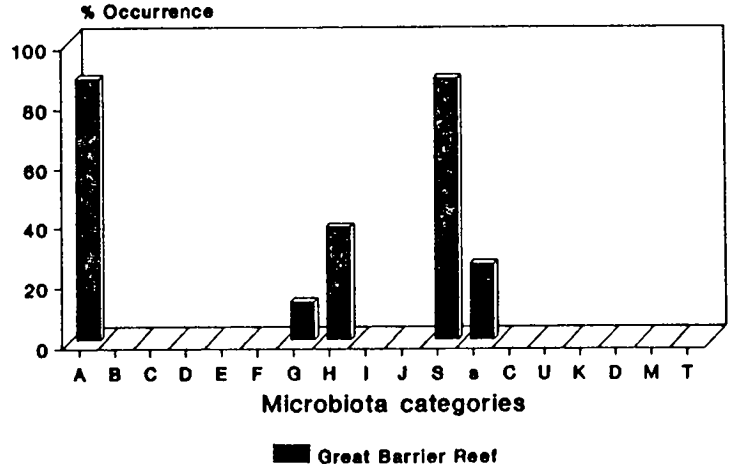
**Fig. 4.5 Microbiota of *Acanthurus achilles*, *A. auranticavus*, *A. blochii* and *A. dussumieri*.**

Percent occurrence of microbiota categories amongst individuals examined. See Table 4.1 for sample sizes of taxa examined.
Abbreviations:
Epulo types: A = type A epulos, B = type B epulos, C = type C epulos, D = type D epulos, E = type E epulos, F = type F epulos, G = type G epulos, H = type H epulos, I = type I epulos, J = type J epulos.
Other prokaryotes: S = spirilla, s = small rod-shaped bacteria.
Protozoans: C = ciliates, U = type U zooflagellates, K = type K zooflagellates, D = type D zooflagellates, M = type M zooflagellates, T = type T zooflagellates.

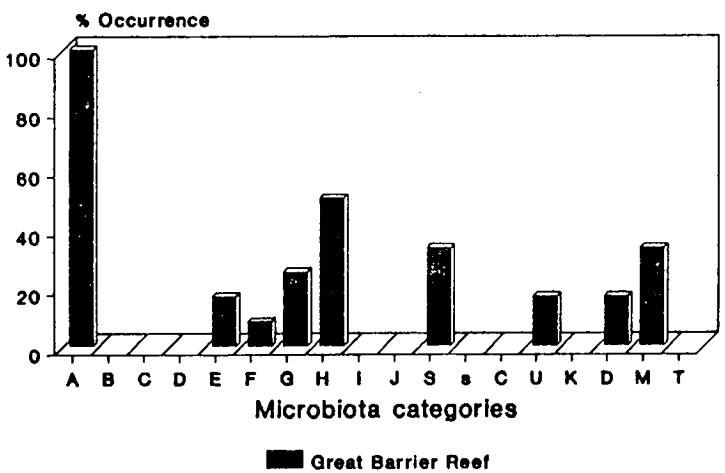
## *A. achilles*




## *A. auranticavus*




## *A. blochii*




## *A. dussumieri*

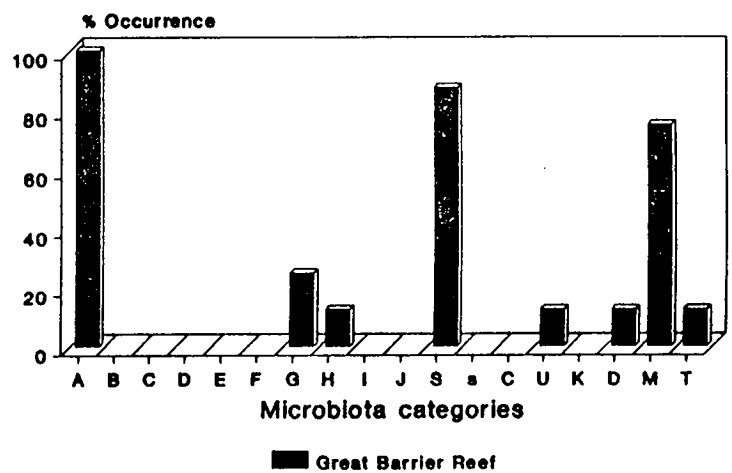
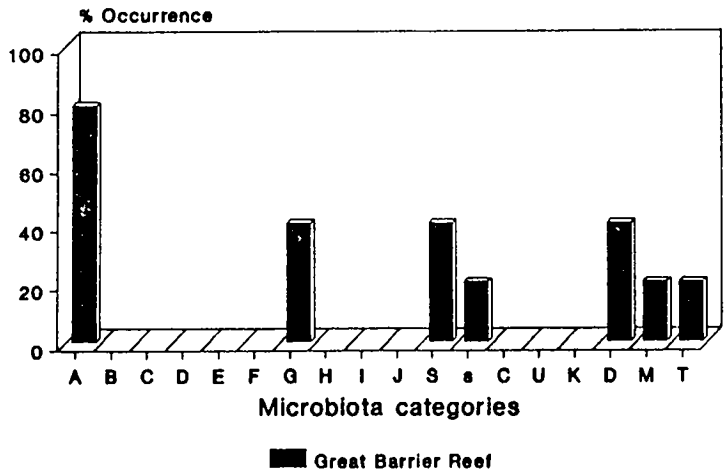


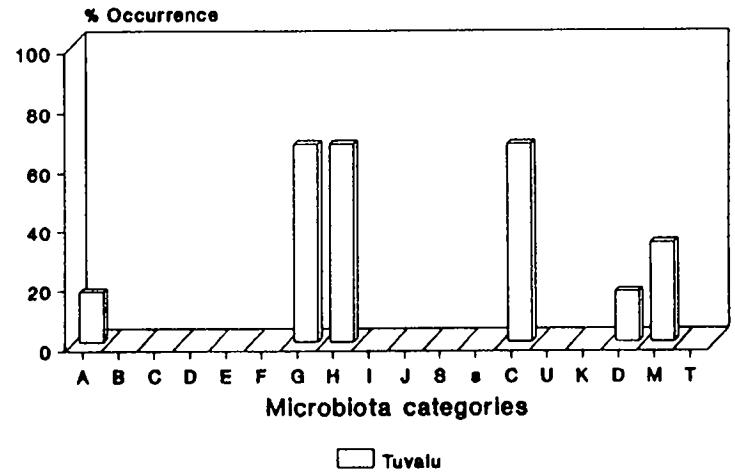
**Fig. 4.6 Microbiota of *Acanthurus grammoptilus*, *A. guttatus*, *A. lineatus* and *A. mata*.**

Percent occurrence of microbiota categories amongst individuals examined. See Table 4.1 for sample sizes of taxa examined.
Abbreviations:
Epulo types: A = type A epulos, B = type B epulos, C = type C epulos, D = type D epulos, E = type E epulos, F = type F epulos, G = type G epulos, H = type H epulos, I = type I epulos, J = type J epulos.
Other prokaryotes: S = spirilla, s = small rod-shaped bacteria.
Protozoans: C = ciliates, U = type U zooflagellates, K = type K zooflagellates, D = type D zooflagellates, M = type M zooflagellates, T = type T zooflagellates.

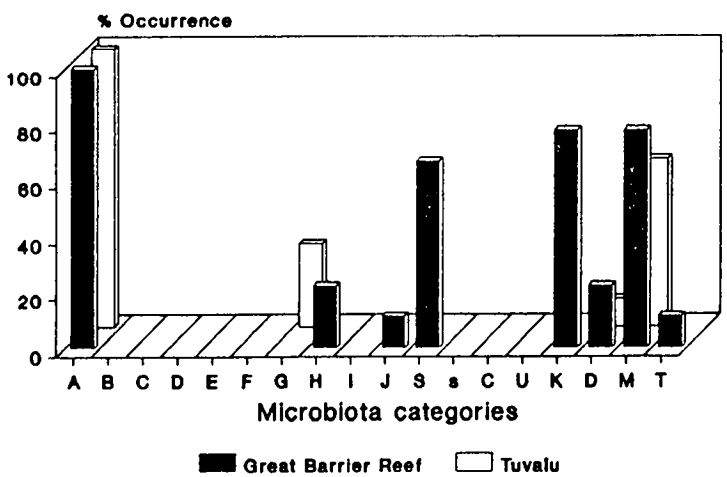
## *A. grammoptilus*




## *A. guttatus*




## *A. lineatus*




## *A. mata*

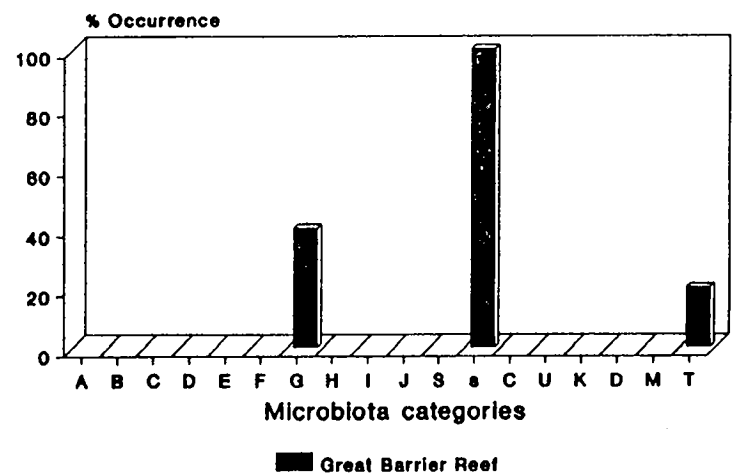
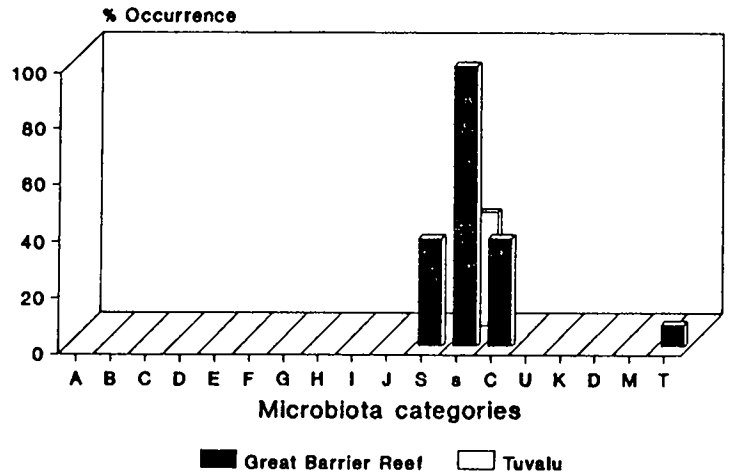


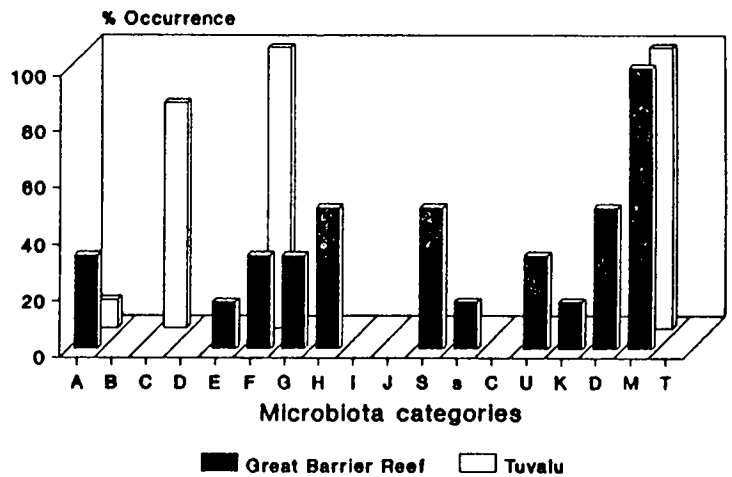
**Fig. 4.7 Microbiota of *Acanthurus nigricans*, *A. nigricauda*, *A. nigrofuscus* and *A. nigroris*.**

Percent occurrence of microbiota categories amongst individuals examined. See Table 4.1 for sample sizes of taxa examined.
Abbreviations:
Epulo types: A = type A epulos, B = type B epulos, C = type C epulos, D = type D epulos, E = type E epulos, F = type F epulos, G = type G epulos, H = type H epulos, I = type I epulos, J = type J epulos.
Other prokaryotes: S = spirilla, s = small rod-shaped bacteria.
Protozoans: C = ciliates, U = type U zooflagellates, K = type K zooflagellates, D = type D zooflagellates, M = type M zooflagellates, T = type T zooflagellates, O = opalinids.

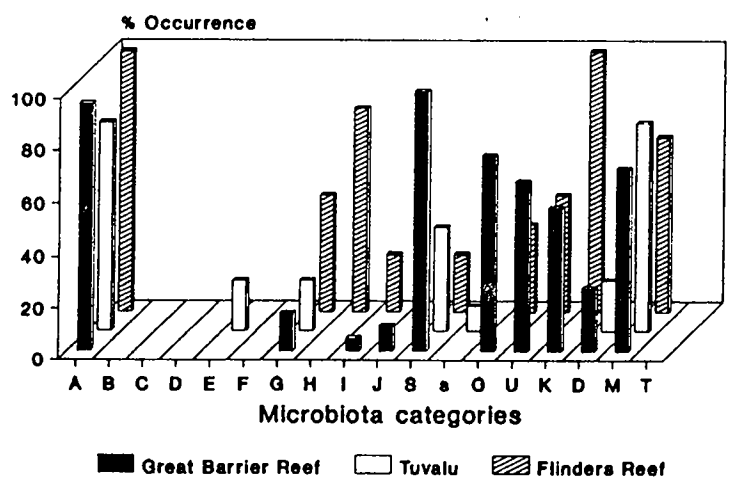
## *A. nigricans*




## *A. nigricauda*




## *A. nigrofuscus*




## *A. nigroris*

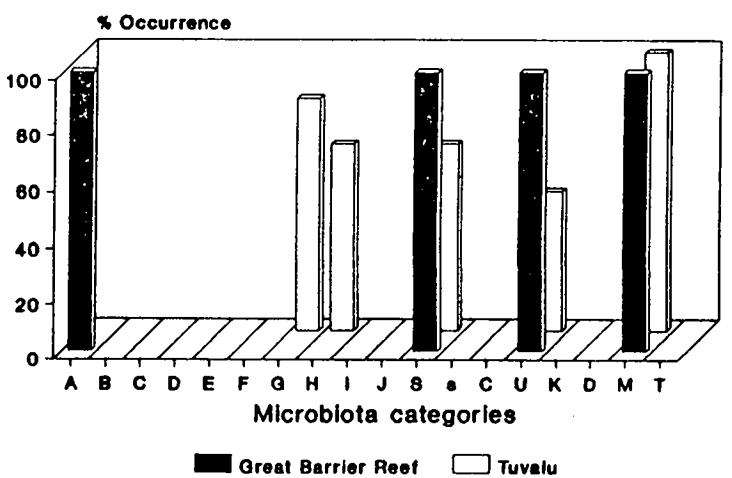
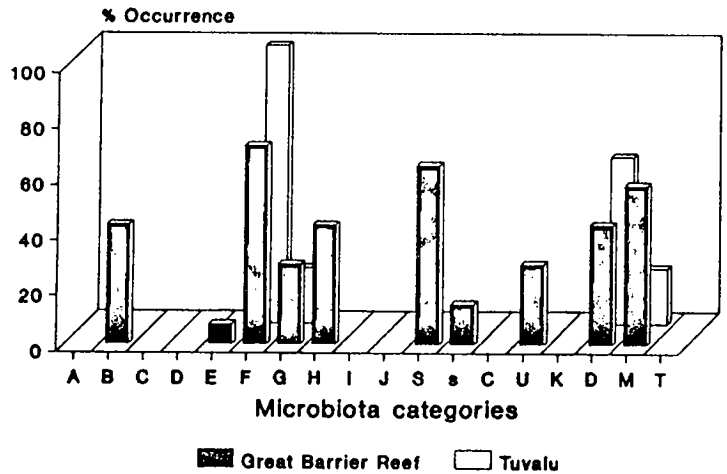


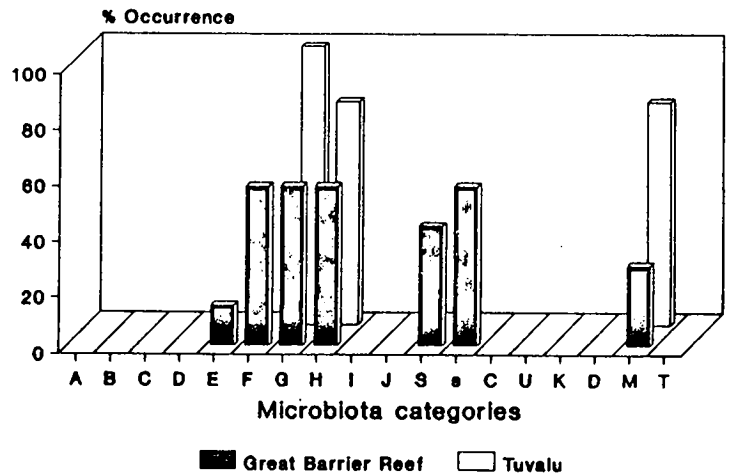
**Fig. 4.8 Microbiota of *Acanthurus olivaceus*, *A. pyroferus*, *A. thompsoni* and *A. triostegus*.**

Percent occurrence of microbiota categories amongst individuals examined. See Table 4.1 for sample sizes of taxa examined.
Abbreviations:
Epulo types: A = type A epulos, B = type B epulos, C = type C epulos, D = type D epulos, E = type E epulos, F = type F epulos, G = type G epulos, H = type H epulos, I = type I epulos, J = type J epulos.
Other prokaryotes: S = spirilla, s = small rod-shaped bacteria.
Protozoans: C = ciliates, U = type U zooflagellates, K = type K zooflagellates, D = type D zooflagellates, M = type M zooflagellates, T = type T zooflagellates.

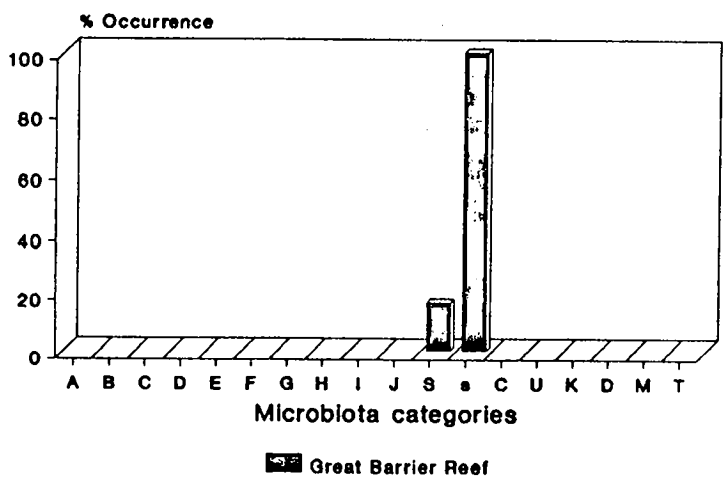
## *A. olivaceus*




## *A. pyroferus*




## *A. thompsoni*




## *A. triostegus*

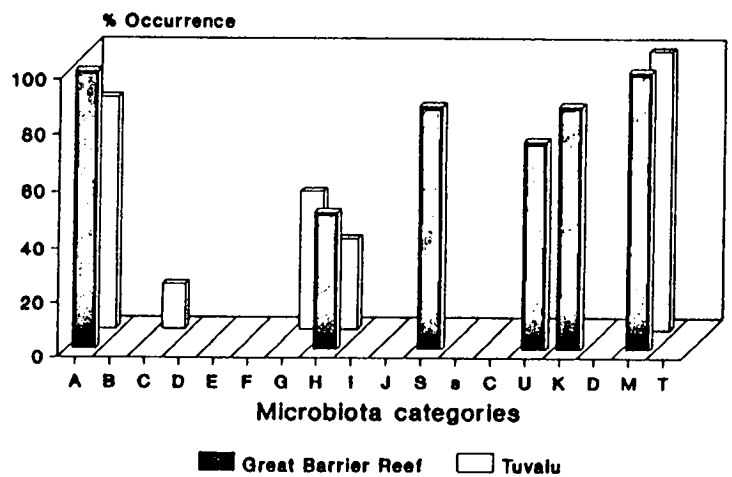
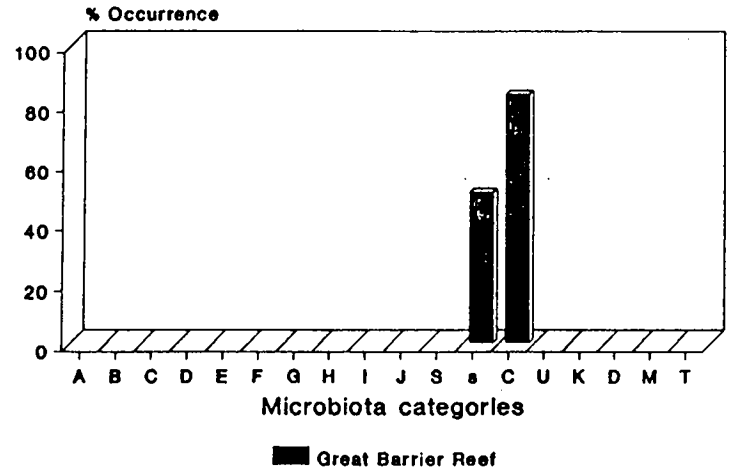


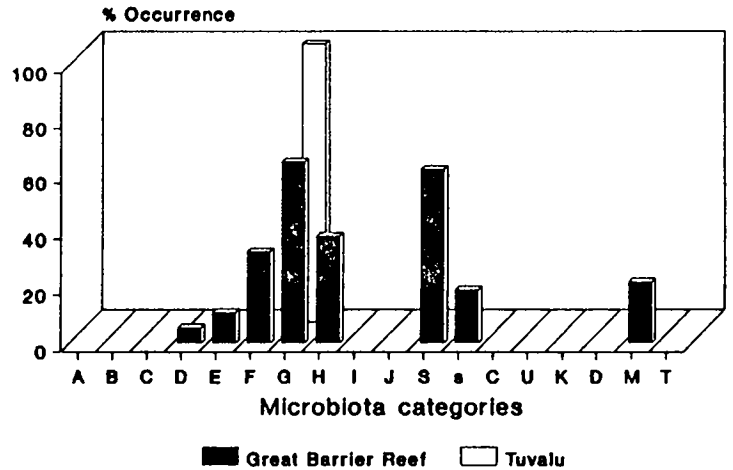
**Fig. 4.9 Microbiota of *Acanthurus xanthopterus*, *Ctenochaetus binotatus*, *C. hawaiiensis* and *C. marginatus*.**

Percent occurrence of microbiota categories amongst individuals examined. See Table 4.1 for sample sizes of taxa examined.
Abbreviations:
Epulo types: A = type A epulos, B = type B epulos, C = type C epulos, D = type D epulos, E = type E epulos, F = type F epulos, G = type G epulos, H = type H epulos, I = type I epulos, J = type J epulos.
Other prokaryotes: S = spirilla, s = small rod-shaped bacteria.
Protozoans: C = ciliates, U = type U zooflagellates, K = type K zooflagellates, D = type D zooflagellates, M = type M zooflagellates, T = type T zooflagellates.

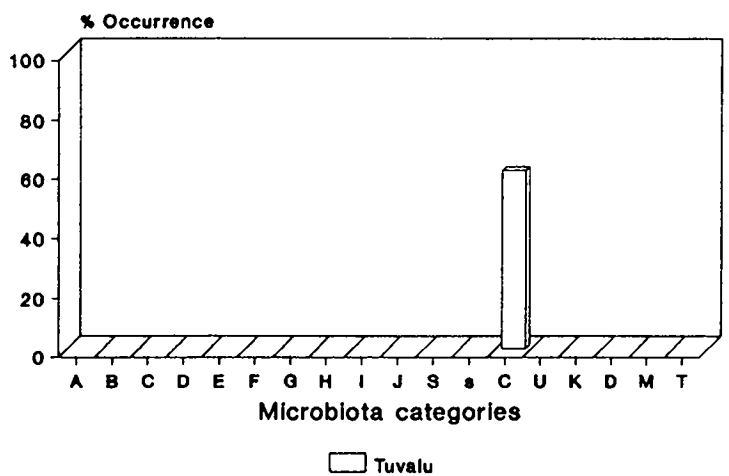
## *A. xanthopterus*




## *C. binotatus*




## *C. hawaiiensis*




## *C. marginatus*

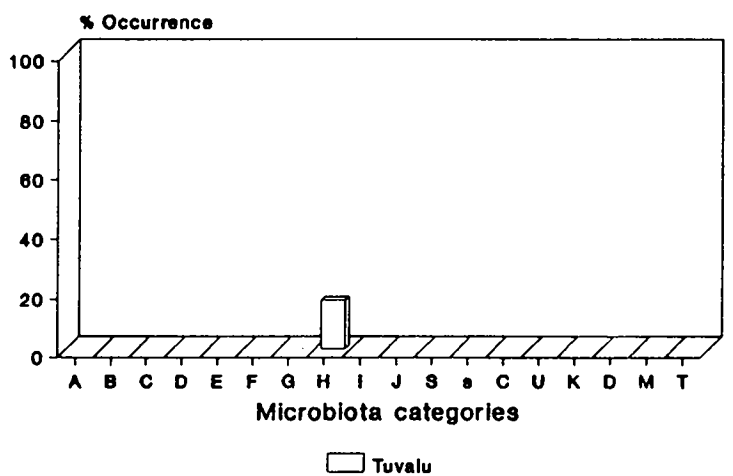
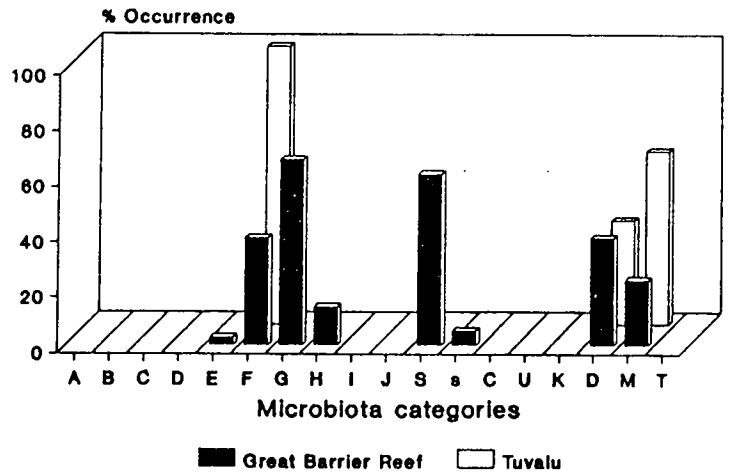


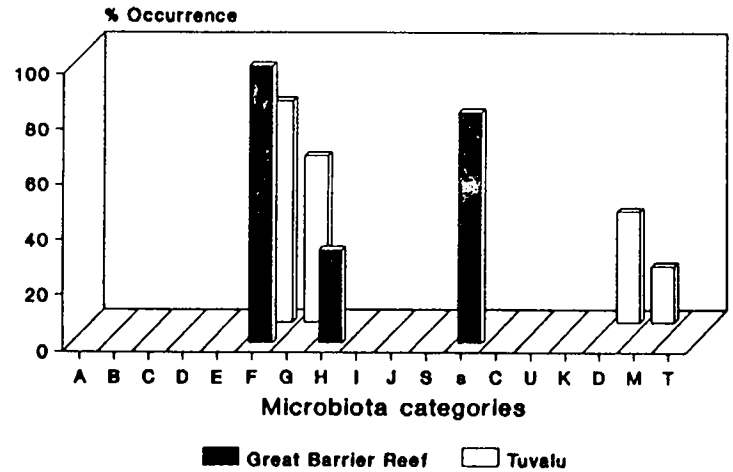
**Fig. 4.10 Microbiota of *Ctenochaetus striatus*, *C. strigosus*, *Naso brevirostris* and *N. lituratus*.**

Percent occurrence of microbiota categories amongst individuals examined. See Table 4.1 for sample sizes of taxa examined.
Abbreviations:
Epulo types: A = type A epulos, B = type B epulos, C = type C epulos, D = type D epulos, E = type E epulos, F = type F epulos, G = type G epulos, H = type H epulos, I = type I epulos, J = type J epulos.
Other prokaryotes: S = spirilla, s = small rod-shaped bacteria.
Protozoans: C = ciliates, U = type U zooflagellates, K = type K zooflagellates, D = type D zooflagellates, M = type M zooflagellates, T = type T zooflagellates.

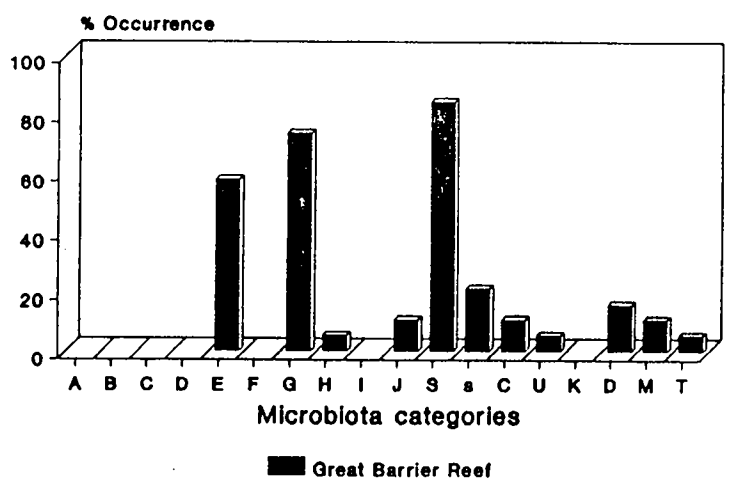
## *C. striatus*




## *C. strigosus*




## *N. brevirostris*




## *N. lituratus*

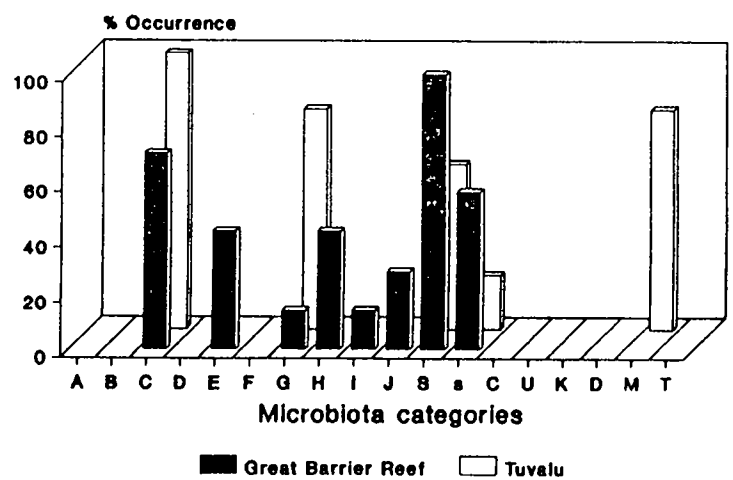
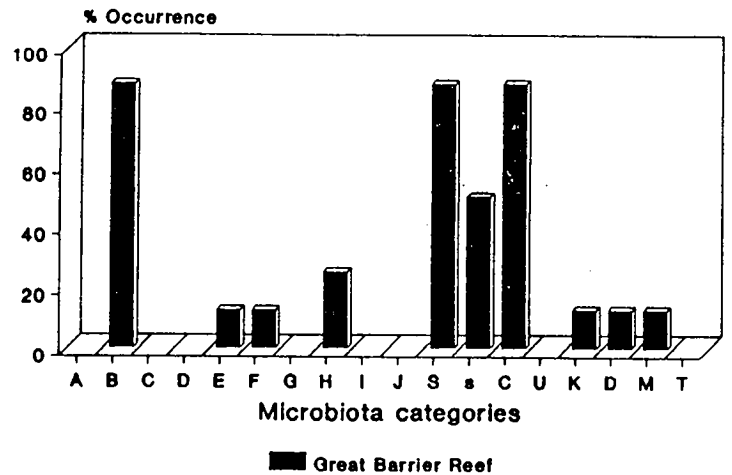


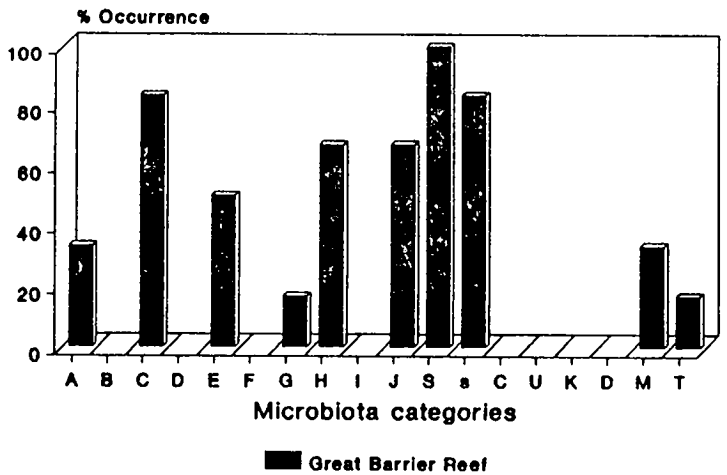
**Fig. 4.11 Microbiota of *Naso tuberosus*, *N. unicornis*, *N. vlamingii* and *Zebrasoma rostratum*.**

Percent occurrence of microbiota categories amongst individuals examined. See Table 4.1 for sample sizes of taxa examined.
Abbreviations:
Epulo types: A = type A epulos, B = type B epulos, C = type C epulos, D = type D epulos, E = type E epulos, F = type F epulos, G = type G epulos, H = type H epulos, I = type I epulos, J = type J epulos.
Other prokaryotes: S = spirilla, s = small rod-shaped bacteria.
Protozoans: C = ciliates, U = type U zooflagellates, K = type K zooflagellates, D = type D zooflagellates, M = type M zooflagellates, T = type T zooflagellates.

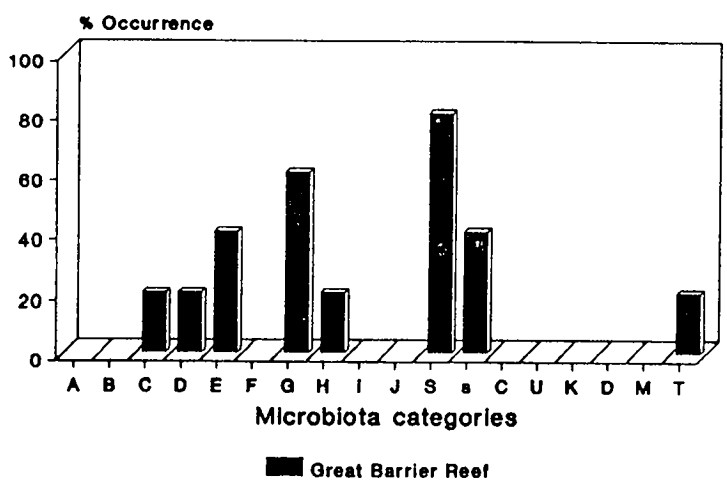
## *N. tuberosus*




## *N. unicornis*




## *N. vlamingii*




## *Z. rostratum*

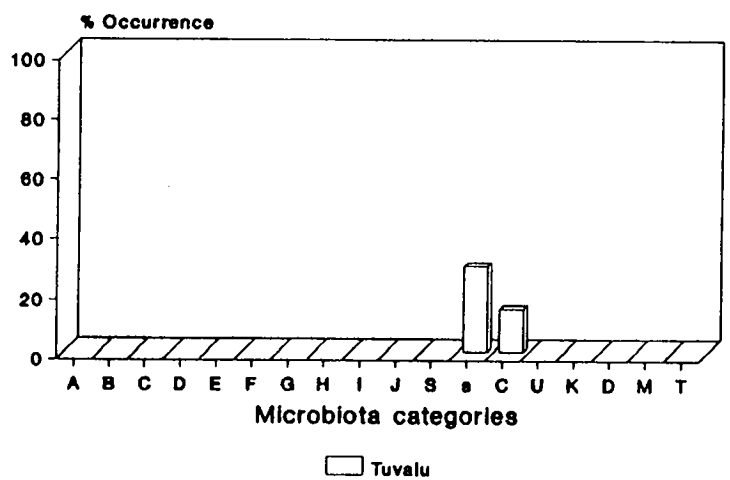
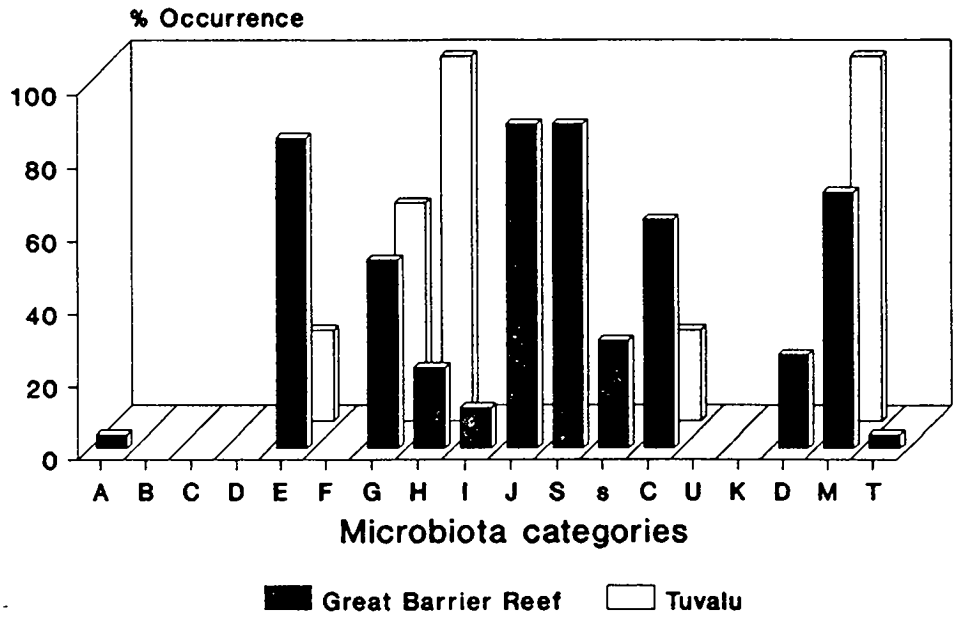


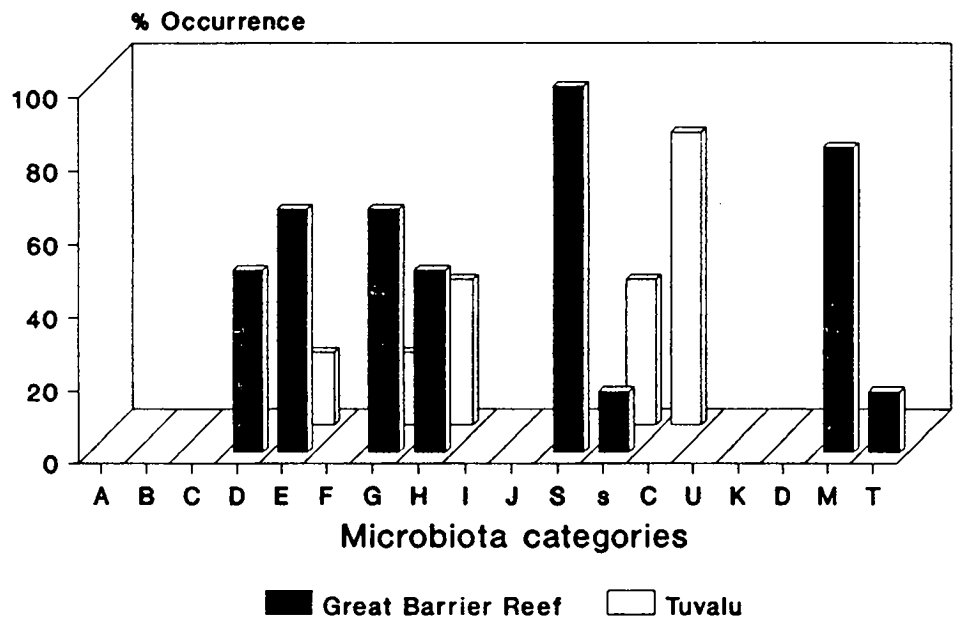
**Fig. 4.12 Microbiota of *Zebrasoma scopas* and *Z. veliferum*.**

Percent occurrence of microbiota categories amongst individuals examined. See Table 4.1 for sample sizes of taxa examined.
Abbreviations:
Epulo types: A = type A epulos, B = type B epulos, C = type C epulos, D = type D epulos, E = type E epulos, F = type F epulos, G = type G epulos, H = type H epulos, I = type I epulos, J = type J epulos.
Other prokaryotes: S = spirilla, s = small rod-shaped bacteria.
Protozoans: C = ciliates, U = type U zooflagellates, K = type K zooflagellates, D = type D zooflagellates, M = type M zooflagellates, T = type T zooflagellates.

# *Z. scopas*




# *Z. veliferum*



(iii) Occurrence of endosymbionts from acanthurids of southeastern Australia

As discussed in Chapter two, only a few few acanthurid species were common in the sub-tropical sites visited (Solitary Islands, Flinder's Reef, the Gneerings and Julian Rocks, Byron Bay - see Chapt. 2 for details). This meant that only two of the tropical acanthurid species (*Acanthurus dussumieri* and *A. nigrofuscus*) were adequately sampled (Table 4.1). Results will be presented in terms of sites sampled, from north to south.

Inner Gneerings, Mooloolaba: The 3 specimens of *Acanthurus nigrofuscus* collected contained type A epulos and type T, type K and type M zooflagellates, and thus closely resembled tropical samples. One of these specimens also contained opalinid zooflagellates. None of the 3 *A. dussumieri* collected contained type A epulos (characteristic of tropical specimens), but did contain type G and H epulos up to 70μm in length. One of the 2 *A. blochii* collected harboured type H epulos, while no endosymbionts were found in the other specimen. A single *A. lineatus* contained type G and H epulos (up to 64μm in length), but no type A epulos. No ciliates were found in the single specimen of *A. xanthopterus* collected. The microbiota of the single *Naso unicornis* specimen collected did not differ from tropical samples of this species.

Flinder's Reef: The microbiota of the nine *Acanthurus nigrofuscus* collected differed very little from the characteristic tropical assemblage of this host species (Fig. 4.7). However the type A epulos present were comparatively small compared to those

from tropical specimens, attaining a length of only 137μm. Opalinid zooflagellates were found in 2 of the 9 specimens examined. The single *A. dussumieri* collected did not harbour type A epulos, but contained type H epulos up to 87μm in length.

Solitary Islands: The microbiota of the *Acanthurus dussumieri* and *A. nigrofuscus* collected is summarised in Fig. 4.13. Every specimen of the former species contained type A epulos, and overall the microbiota did not differ from tropical samples. The *A. nigrofuscus* collected differed from tropical conspecifics in lacking type A epulos and opalinid zooflagellates, although the composition of the other endosymbiont categories closely resembled GBR samples. Each specimen of this species did however contain smaller type G or type H epulos.

Arrawarra Headland and Muttonbird Island: A single 85mmSL *Acanthurus dussumieri* collected from Arrawarra Headland contained neither epulos nor zooflagellates. One small (79mmSL) *A. nigrofuscus* collected from Muttonbird Island lacked type A epulos, but did contain type G and I epulos, spirilla and type D zooflagellates.

Julian Rocks, Byron Bay: Three *Acanthurus nigrofuscus* contained type H (up to 81μm in length) and I epulos, and type U, type E, type D and type M zooflagellates. Two *A. dussumieri* contained type H (up to 81μm in length) and type I epulos. One specimen of *A. olivaceus* contained many type I epulos. A single *Naso tuberosus* contained type G epulos, and lacked ciliates. A single *N. unicornis* contained many type H and type I epulos (up to 50μm in length), and was the only

specimen collected from Byron Bay which contained spirilla.

### 4.3.4 Microbiota of juvenile acanthurids

This section is subdivided into three parts. The first part examines the distribution of the major endosymbiont categories (epulos, spirilla and zooflagellates) amongst different size classes of six well-represented species. The purpose of this is to investigate the host size at which these endosymbionts are acquired. The second part of this section assesses the distribution of epulo categories amongst different size classes of two host species. Finally, the third part of this section will briefly discuss the microbiota of the juveniles collected in this study, in order of host species.

(i) Endosymbiont acquisition

The percentage occurrence of the major endosymbiont categories amongst different host size classes is presented in Fig. 4.14. It will be noted that the majority of pigmented (i.e. 1-2 days post-settlement) individuals of even the smallest size class of each species contain epulos. Epulos were present in 16 out of 50 newly-settled (i.e. transparent) *A.* white-bar spp. (see section 2.2 for a description of this species assemblage), most of which were collected from artificial patch-reefs. It is also apparent that most host acanthurids acquire epulos at smaller sizes than they acquire spirilla and zooflagellates.

(ii) Epulo category acquisition

The percentage occurrence of the predominant epulo categories amongst different size classes of *Acanthurus nigrofuscus* and *A.* white-bar spp. is presented in Fig. 4.15. It is apparent that the predominant epulo category differs between the different host size classes. Type A epulos are uncommon in the juvenile specimens examined, yet are the most common epulo category amongst adult specimens. In both *A. nigrofuscus* and *A.* white-bar spp. the predominant epulo category amongst the two smallest size classes are type G epulos. Type I epulos are common amongst late-juvenile and sub-adult *A. nigrofuscus*.

(iii) Microbiota of juvenile acanthurids - species accounts

*Acanthurus blochii*: Two sub-adult specimens (53 and 69mmSL) both contained type F (but no type A) epulos, spirilla and type D zooflagellates.

*Acanthurus dussumieri*: Two 47mmSL specimens were examined. One contained type G, H and J epulos, and type D zooflagellates. The other contained type E epulos, spirilla, and type D zooflagellates. Two Australian Museum specimens were examined. One 48mmSL specimen from Arrawarra Headland contained a few type G epulos. No epulos were found in a 44mmSL specimen from Manly, Sydney.

*Acanthurus lineatus*: Seven specimens ranging in length from 29-47mmSL were examined. Type A epulos were found in two of these specimens (38 and 47mmSL).

The remaining individuals contained a mixture of type E, G, H and I epulos. All but the two smallest individuals contained spirilla. Type D zooflagellates were found in two specimens (36 and 47mmSL).

*Acanthurus mata*: All of the seven specimens examined (31-58mmSL) were collected from artificial patch reefs. Type E epulos were found in one 42mmSL specimen, and a few type G epulos were found in a 35mmSL specimen. Other than this, no epulos, spirilla or zooflagellates were observed.

*Acanthurus nigricauda*: A single 52mmSL specimen contained type F and H epulos and type M zooflagellates.

*Acanthurus nigrofuscus*: Endosymbiont occurrence data for this species are presented in Figs. 4.14 and 4.15. The single newly-settled specimen collected (32mmSL) contained type A and type J epulos, spirilla and ciliates. Opalinid zooflagellates were found in 1 of 7 30-34mmSL specimens, 2 of 18 35-39mmSL specimens, and 7 of 11 40-69mmSL specimens. All but one of the 8 juvenile specimens collected from the Solitary Islands (36-68mmSL) contained epulos of type G, H or I. The 43mmSL specimen which did not contain epulos harboured type D zooflagellates. Zooflagellates (type K, D and M) were present in 7 of these 8 specimens. Two small (35 and 45mmSL) specimens collected from Cape Banks, Sydney, did not contain either epulos, spirilla, or zooflagellates.

*Acanthurus olivaceus*: All 11 juvenile specimens collected (22-68mmSL) contained

epulos of type E, G, H, I, or J. No type B or F epulos were found in these specimens. The 22mmSL specimen was newly settled and contained type G epulos, but no zooflagellates. Zooflagellates of type U, D or M were found in 6 of the specimens.

*Acanthurus pyroferus*: Two juvenile specimens (40 and 45mmSL) were collected from a backreef bommie at Yonge Reef. Both contained type F epulos, but no zooflagellates.

*Acanthurus triostegus*: Endosymbiont occurrence data for this species are presented in Fig. 4.14. Twenty-three juveniles were collected on the GBR (21-40mmSL), including two newly-setted specimens. All contained epulos. Type A epulos were found in 3 specimens (23-27mmSL), type E epulos were found in 3 specimens, type G epulos were found in 15 specimens, and type I epulos were found in 6 specimens. Both newly-settled individuals contained type G epulos, and one also contained type E epulos. Type K, D or M zooflagellates were found in 6 specimens (25-40mmSL). Two juveniles collected from North Solitary Island (33 and 65mmSL) both contained type G epulos and type D zooflagellates. The smaller of these two specimens also contained type I and J epulos.

*Acanthurus xanthopterus*: Six specimens which could unequivocally be referred to this species were collected (56-64mmSL) from the Lizard Island lagoon. All contained type G and H epulos, and two contained type A epulos. Ciliates were not found in any of the specimens. Type D and type M zooflagellates were each found

in 2 specimens.

*Acanthurus* white-bar spp.: Endosymbiont occurrence data for these species are presented in Figs. 4.14 and 4.15. Microbiota percentage occurrence data are presented in Fig. 4.16 for four artificial patch reef sites at Lizard Island. Two of these sites were in the lagoon (Lagoon and Palfrey), one was on the leeward side of the island (Resort), and one was on the exposed side of the island (Coconut). At least 7 specimens were collected from each of these sites. Some differences were detected between these sites in the occurrence of epulo types. The two lagoon samples were similar in containing predominantly type E and G epulos. The Resort sample also contained type E and G epulos, but in different proportions to the lagoon samples. The only epulo category found in the Coconut sample were type G epulos.

*Ctenochaetus binotatus*: The endosymbiont occurrence data presented in Fig. 4.14 suggest that some individuals of this species may acquire epulos at a larger size than the herbivorous species discussed above. None of the 3 newly-settled specimens collected (26-28mmSL) contained epulos. Type G was the only epulo category common in juveniles of this species, although a few individuals contained type F or H epulos.

*Ctenochaetus striatus*: Unlike *C. binotatus*, all 23 juveniles of *C. striatus* examined (30-68mmSL) contained epulos. Type E epulos were found in 3 specimens, type F epulos in 2 specimens, type G in 16 specimens and type H in 17 specimens. Type D

were the only zooflagellates found in the specimens examined, and these were present in 6 individuals.

*Ctenochaetus strigosus*: A single 39mmSL specimen, collected from a backreef bommie at Yonge Reef, contained type H epulos but no zooflagellates.

*Naso brevirostris*: Nine of the 12 specimens examined (51-70mmSL) contained either type E epulos, type G epulos, or both. No zooflagellates were found in any of the specimens.

*Naso hexacanthus*: Three of the 5 specimens examined (37-62mmSL) contained epulos (types E, G or J). The two specimens which did not not contain epulos (37 and 49mmSL) harboured spirilla. No zooflagellates were found in any of the specimens.

*Naso lituratus*: All of the 5 specimens examined (54-59mmSL) contained epulos and spirilla. Type A epulos (up to 112μm in length) were found in 3 specimens, type C in 1 specimen, type E in 4 specimens, type F in one specimen, and type J in 3 specimens. No zooflagellates were found in any of the specimens. A 60mmSL specimen collected at Flinder's Reef did not contain either epulos, spirilla or zooflagellates.

*Naso tuberosus*: Eight of the 29 specimens examined (22-61mmSL), which included 5 of the newly settled individuals, were collected from artificial patch reefs. The

remainder of specimens were collected from natural reef substrata. Two of the newly settled individuals contained epulos: a 29mmSL specimen from a lagoon patch reef contained type G epulos, and a 32mmSL specimen from Pidgin Point (Lizard Island - see Chapt. 2) contained a few type H epulos. Epulos were found in 17 of the 20 pigmented specimens: type E epulos were found in 2 specimens, type G epulos in 16 specimens, and type I epulos were found in one specimen. Ciliates were not found in any of the juvenile specimens examined. Type D zooflagellates were found in 2 specimens (32 and 36mmSL).

*Naso unicornis*: All but one (54mmSL) of the 10 specimens (47-66mmSL) examined contained epulos. Type E epulos were found in 4 specimens, type F epulos in 1 specimen, type G epulos in 6 specimens, and type J epulos in 5 specimens. No zooflagellates were found in any of the specimens.

*Naso vlamingii*: A 42mmSL specimen did not contain epulos, a 51mmSL specimen contained type G epulos and spirilla, and a 58mmSL specimen contained type E and G epulos and spirilla. Type M zooflagellates were found in the 51mmSL specimen.

*Zebrasoma scopas*: Endosymbiont occurrence data for this species are presented in Fig. 4.14. Two 24mmSL newly-settled specimens were collected: one contained type G epulos, the other did not contain any endosymbionts. Type E epulos were the predominant epulo type present, with type E, type I and type J epulos occurring in a few individuals. Ciliates were not present in any of the juvenile specimens examined. Type D zooflagellates were found in 2 individuals, and type M

zooflagellates were found in a single specimen.

*Zebrasoma veliferum*: All of the 13 specimens examined (20-59mmSL) contained epulos. Type G were the predominant epulo type present, with type D epulos present in a few individuals. No zooflagellates were found in any of the specimens examined.

**Fig. 4.13 Microbiota of *Acanthurus nigrofuscus* and *A. dussumieri* collected from the Solitary Islands.**

Percent occurrence of microbiota categories amongst individuals examined. See Table 4.1 for sample sizes of taxa examined.
Abbreviations:
Epulo types: A = type A epulos, B = type B epulos, C = type C epulos, D = type D epulos, E = type E epulos, F = type F epulos, G = type G epulos, H = type H epulos, I = type I epulos, J = type J epulos.
Other prokaryotes: S = spirilla, s = small rod-shaped bacteria.
Protozoans: C = ciliates, U = type U zooflagellates, K = type K zooflagellates, D = type D zooflagellates, M = type M zooflagellates, T = type T zooflagellates.

# Solitary Islands

## *A. nigrofuscus* & *A. dussumieri*

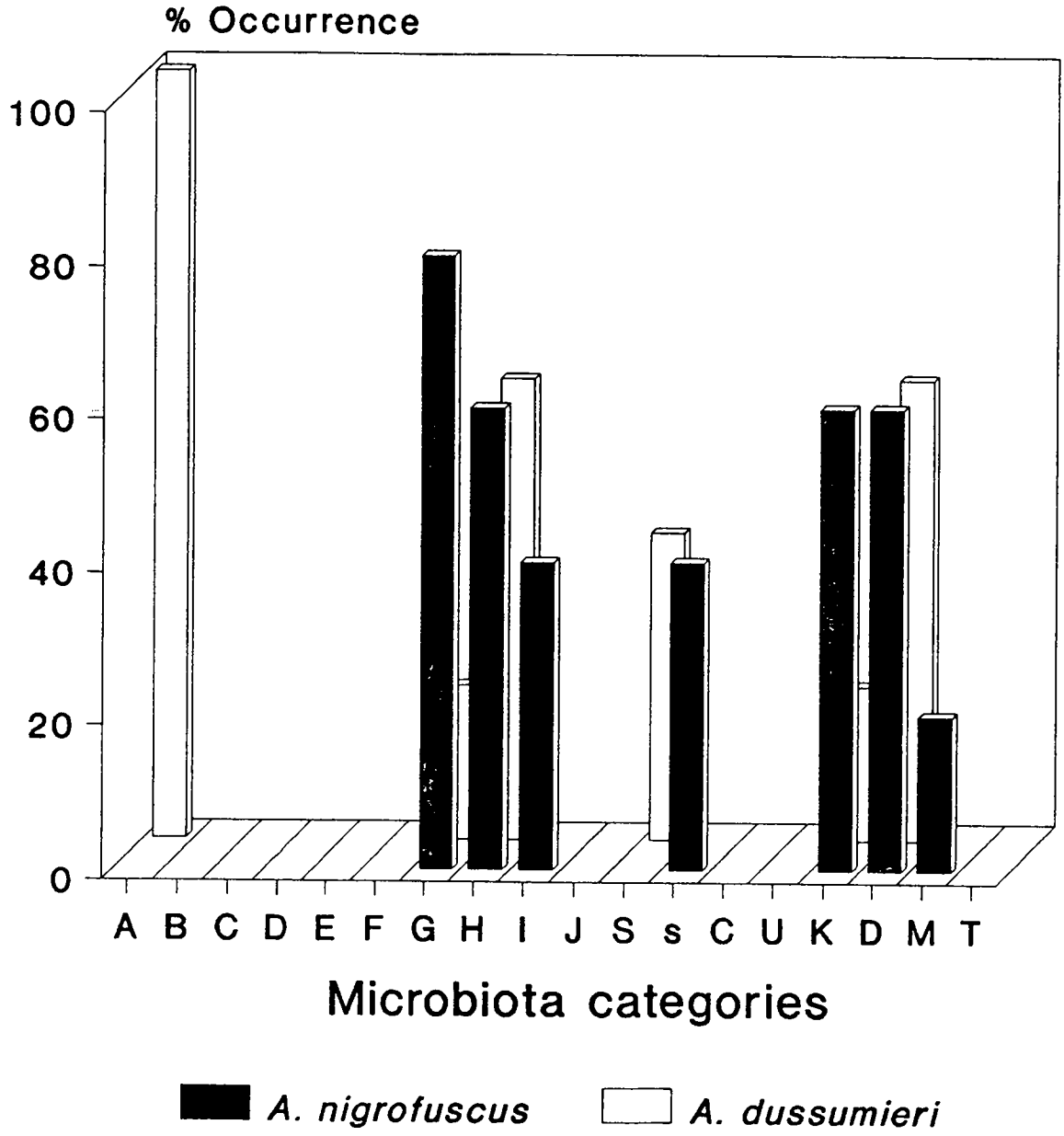


**Fig. 4.14 Acquisition of epulos, spirilla and zooflagellates by acanthurid species.**

Percent occurrence of microbiota categories amongst individuals of different sizes examined. Abbreviations: N = number of individuals of each size class examined.

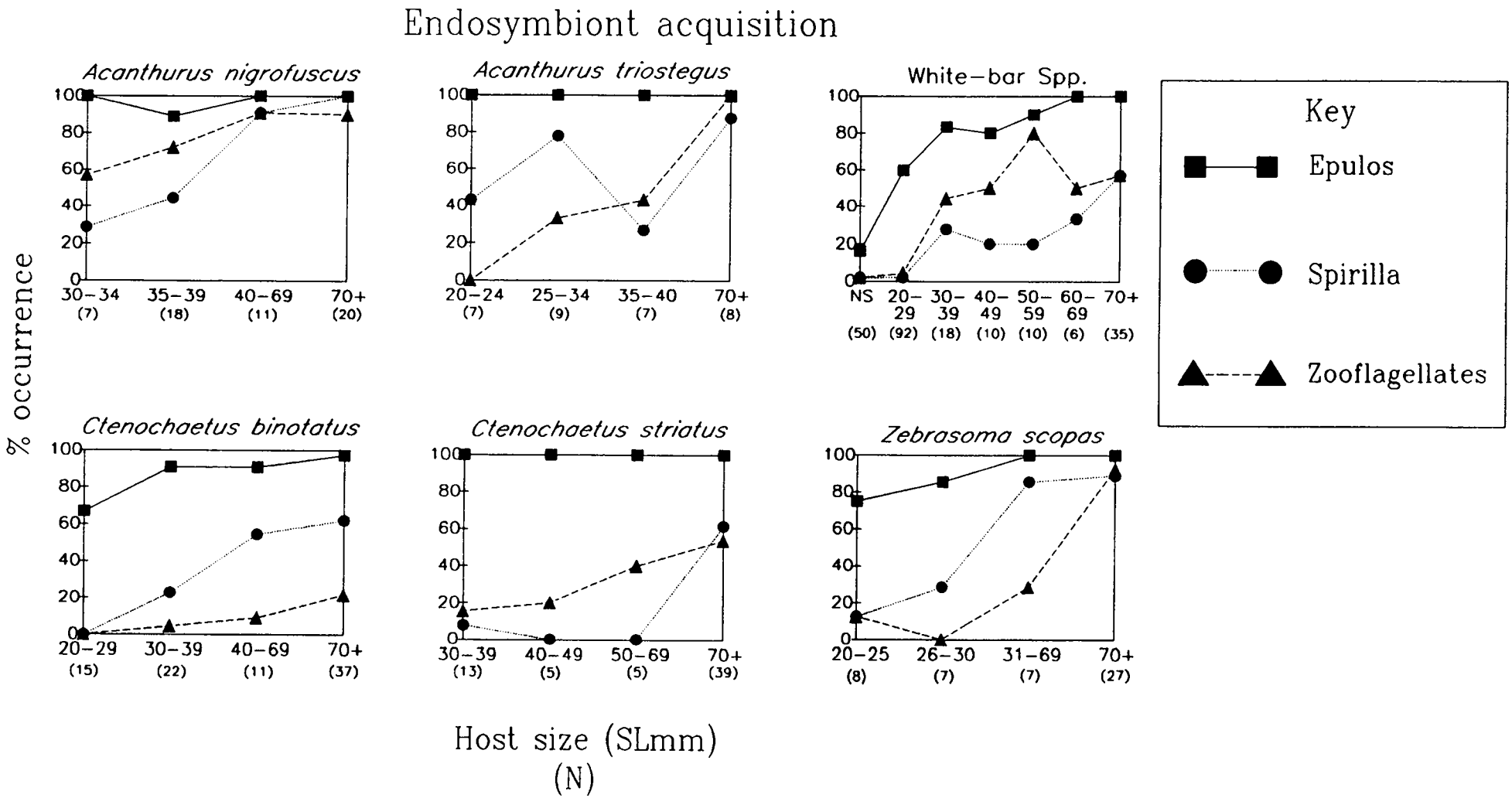

Endosymbiont acquisition
Acanthurus nigrofuscus
Acanthurus triostegus
White-bar Spp.
Ctenochaetus binotatus
Ctenochaetus striatus
Zebrasoma scopas
% occurrence
Host size (SLmm)
(N)
Key
Epulos
Spirilla
Zooflagellates
30-34 (7)
35-39 (18)
40-69 (11)
70+ (20)
20-24 (7)
25-34 (9)
35-40 (7)
70+ (8)
NS (50)
20-29 (92)
30-39 (18)
40-49 (10)
50-59 (10)
60-69 (6)
70+ (35)
20-29 (15)
30-39 (22)
40-69 (11)
70+ (37)
30-39 (13)
40-49 (5)
50-69 (5)
70+ (39)
20-25 (8)
26-30 (7)
31-69 (7)
70+ (27)

**Fig. 4.15 Occurrence of epulo types amongst *Acanthurus nigrofuscus* and *A.* white-bar spp. of different size classes.**

Percent occurrence of epulo types amongst individuals of different sizes examined.
Abbreviations: N = number of individuals of each size class examined.

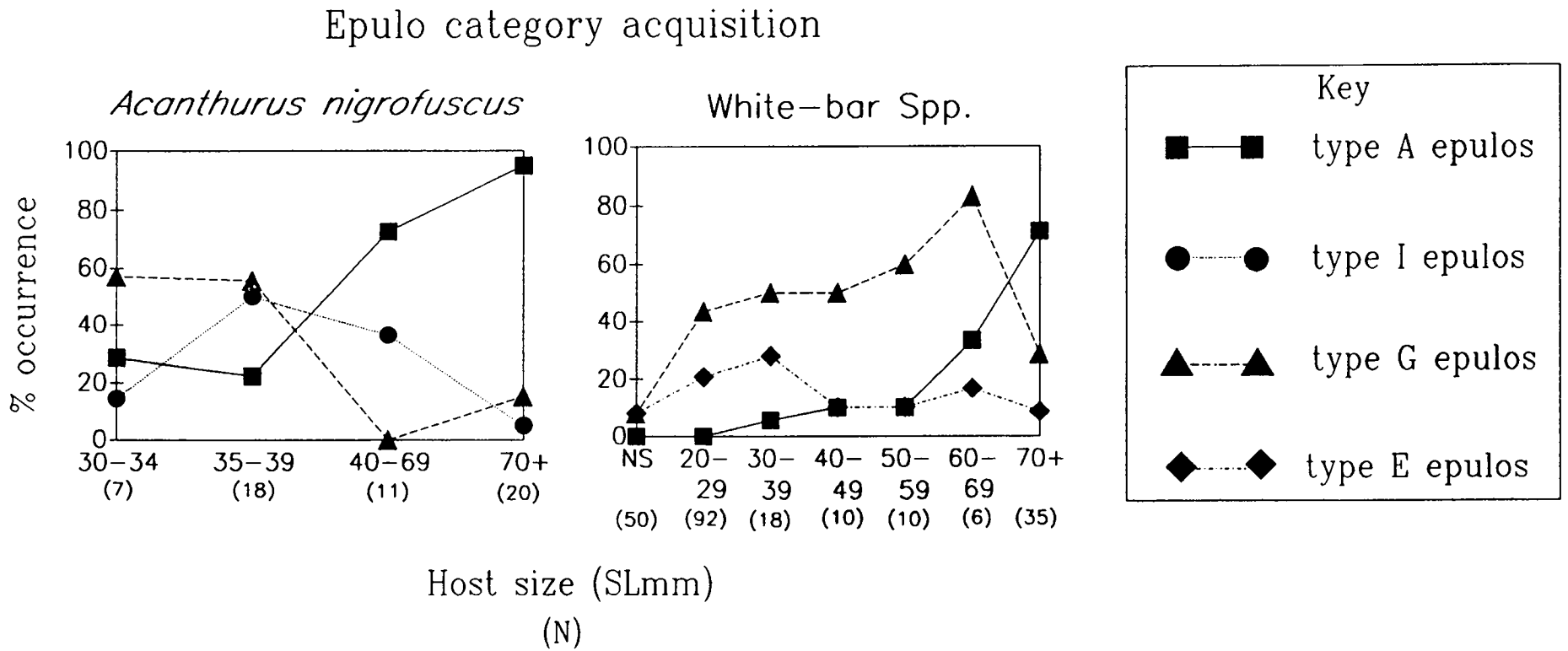
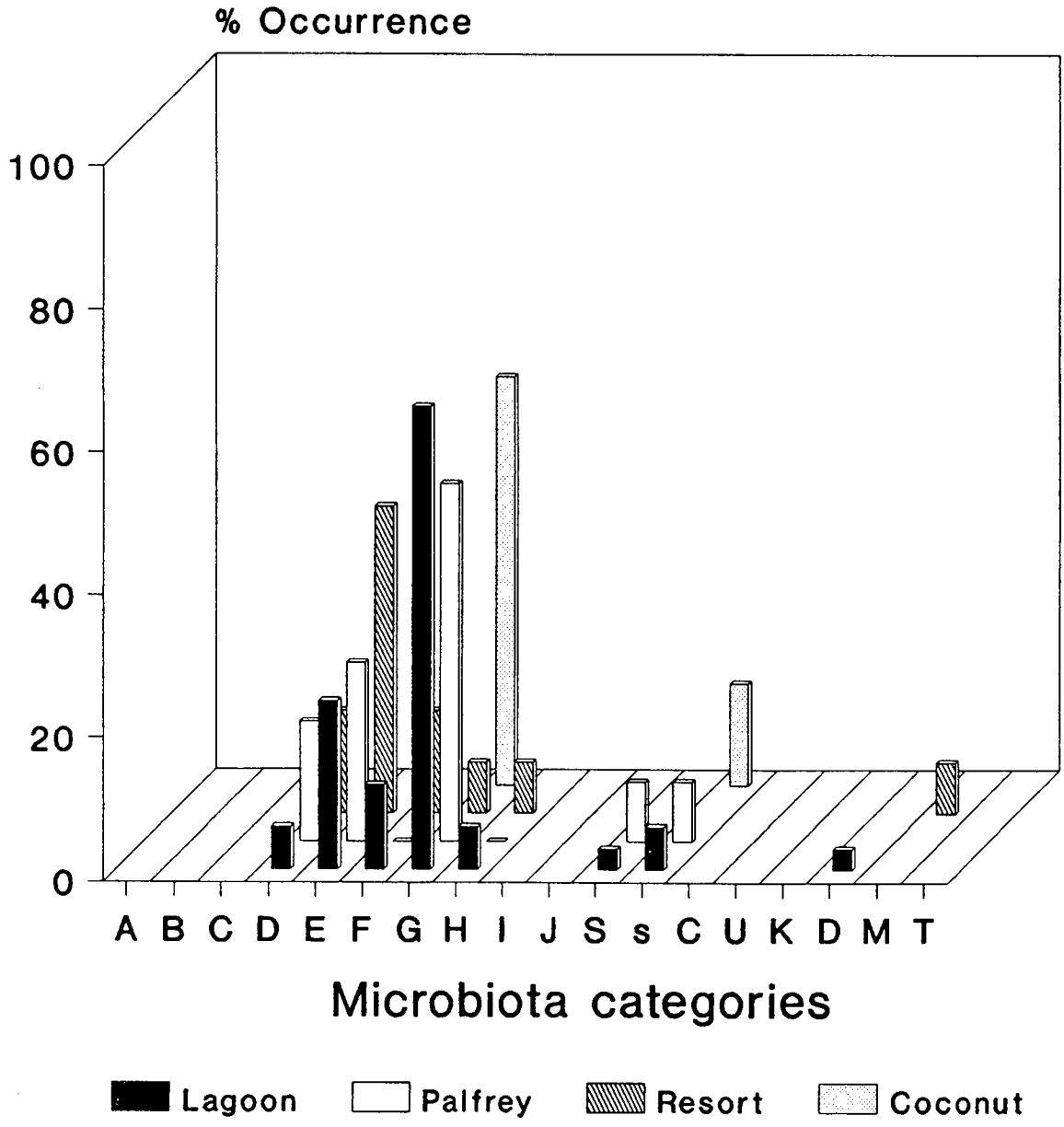

Epulo category acquisition
Acanthurus nigrofuscus
White-bar Spp.
% occurrence
100
80
60
40
20
0
30-34
(7)
35-39
(18)
40-69
(11)
70+
(20)
NS
(50)
20-29
(92)
30-39
(18)
40-49
(10)
50-59
(10)
60-69
(6)
70+
(35)
Host size (SLmm)
(N)
Key
type A epulos
type I epulos
type G epulos
type E epulos

**Fig. 4.16 Microbiota of juvenile *Acanthurus* white-bar spp.**

Percent occurrence of microbiota categories amongst individuals examined. At least five individuals examined from each location.
Abbreviations:
Epulo types: A = type A epulos, B = type B epulos, C = type C epulos, D = type D epulos, E = type E epulos, F = type F epulos, G = type G epulos, H = type H epulos, I = type I epulos, J = type J epulos.
Other prokaryotes: S = spirilla, s = small rod-shaped bacteria.
Protozoans: C = ciliates, U = type U zooflagellates, K = type K zooflagellates, D = type D zooflagellates, M = type M zooflagellates, T = type T zooflagellates.

# White-bar spp.
## 20-29mmSL



## 4.4 DISCUSSION

The results presented above demonstrate that herbivorous members of the Family Acanthuridae typically harbour a diverse endosymbiotic community within their alimentary tracts. The most characteristic element of this endofauna is an assemblage of large prokaryote (see Chapter 5) microorganisms, collectively termed epulos. These organisms were not found in any of the herbivorous members of the families Scaridae, Siganidae and Blenniidae examined in this study. Epulos were similarly absent from the specimens of *Zanclus cornutus* examined, although populations of other prokaryotic microbes were present. This species, which constitutes the monotypic family Zanclidae, is the closest relative of the Acanthuridae (Johnson and Washington 1987, Tyler et al. 1989).

Low numbers of epulos were detected in some individuals of four species of pomacentrids. This may be a result of the ingestion of acanthurid faeces containing the microorganisms. Epulos may then simply pass through the gut and be excreted, or alternatively the environment of the pomacentrid intestine may be sufficiently benign for some to survive and reproduce. The fact that epulos were present in some individual pomacentrids but not others suggests that the association is not obligate. The data therefore do not support a mutualistic relationship between epulos and pomacentrids.

Organisms resembling the elongate type E epulos of acanthurids were found in every specimen of *Centropyge bicolor* examined, suggesting the possibility of a

symbiotic relationship. The diet of this species has not been studied in detail, although literature information suggests that it is predominantly herbivorous (Myers 1989). Epulos were found in low numbers in only one specimen of *C. bispinosus*, and were absent from the single specimen of *C. vrolicki* examined. The dissimilarity in microbiota between the *Centropyge* species may be a result of dietary differences, with *C. bispinosus* and *C. vrolicki* taking more animal material than *C. bicolor* (D.R Bellwood Pers. Comm.).

The microbiota of *Centropyge bicolor* resembles the endosymbiotic communities of kyphosids (Rimmer and Wiebe 1987) and herbivorous odacids (Clements In Press) in being dominated by large bacterial rods and spirilla. Indeed, the elongate nature of the large bacterial rods in *C. bicolor* may indicate that these organisms are more closely related to kyphosid endosymbionts than epulos. Clearly, the diet and microbiota of *Centropyge* promise to be a rewarding area for future reseach.

Comparisons between the prokaryote endosymbionts of different host taxa (or even between conspecific hosts of different sizes) are made problematical by the difficulty of adequately differentiating the microorganisms. The epulos found in herbivorous acanthurids in this study consisted of a variety of morphotypes, differing in size, shape and/or mode of cell division. The relationships between these epulo morphotypes are unclear (see Chapt. 5). Several possibilities may be considered. Firstly, the different types observed, even within a single host, may represent quite different organisms. Alternatively, the different types may represent reproductive and intermediate or vegetative forms of the same microorganism. If the epulos from

different hosts are indeed related, then different morphology and mode of cell division may in part be determined by host physiology and/or the nature of the host diet (Clements et al. 1989).

Host diet certainly appears to be an important factor in explaining epulo occurrence patterns amongst acanthurid taxa. Epulos were not found in adult planktivorous acanthurids, although they did occur in some juveniles of the planktivorous species *Acanthurus mata* and *Naso hexacanthus*. An herbivorous phase appears to precede planktivorous feeding in several species of acanthurids, notably *N. brevirostris* and *N. vlamingii*. As mentioned in the results, the specimens of the two latter species collected for this study were subadults, which contained large amounts of algal material in their guts (in addition to epulos). It is probable that larger, planktivorous specimens, such as occur in open water off outer reefs (see Chapter 2), lose epulos when they cease benthic feeding.

Diet may also play a role in the absence of epulos from adult *Acanthurus xanthopterus*. Myers (1989) reports that *A. xanthopterus* is known to include animal material in its diet, so it is likely that this species is not a strict herbivore. Another factor in explaining the absence of epulos from adults of *A. xanthopterus* may be distribution. This species is characteristic of deeper water and may stray far from reefs (Randall 1956). At Lizard Island it was found in deeper water, around the edges of patch reefs and at the bases of reefs adjacent to sand slopes (see Chapter 2). This species is, therefore, similar to planktivorous acanthurids in not being closely associated with reefs.

The most incongruous finding of this part of the study was the apparently anomalous microbiota of the three species of the "*Acanthurus achilles*" group (*A. achilles*, *A. leucosternon* and *A. nigricans*). These are the only tropical, reef-dwelling, herbivorous *Acanthurus* species in which epulos were absent from all adult specimens. This result corroborates that of Fishelson et al. (1985), who did not find epulos in *A. nigricans* specimens collected in the Gulf of California. *A. achilles*, *A. leucosternon* and *A. nigricans* share morphological features which distinguish them from other members of the genus (Randall 1956), and so represent a distinct assemblage. These three species co-occur (see Chapter 2) and overlap in diet (Hiatt and Strasburg 1960, Jones 1968, Robertson et al. 1979, Robertson and Polunin 1981, Robertson and Gaines 1986) with other *Acanthurus* species containing epulos. It is possible therefore that this group differs from other species of the genus in some aspect of digestive physiology. Another possible reason for the absence of epulos from these species is that some aspect of their early post-settlement life differs from that of other *Acanthurus* species. This hypothesis is suggested by the observation that these species typically settle at a larger size than other *Acanthurus* recruits (Randall 1956). Perhaps a behaviour critical for the infection of *Acanthurus* juveniles by epulos is missing in *A. achilles*, *A. leucosternon* and *A. nigricans*.

Another interesting anomaly is the absence of epulos from *Zebrasoma rostratum* and two Tuvalu specimens of *Z. veliferum* (the other three specimens contained epulos). Three of the 7 *Z. rostratum* specimens collected in Tuvalu did contain a few type E epulo cells, but I could not discount the possibility that these had been incidentally ingested. The diet of *Z. rostratum* is undocumented, although it is most likely that it

shares a similar diet to *Z. scopas*. GBR specimens of *Z. veliferum* all contained epulos, so it is possible that the absence of epulos from the two Tuvalu specimens of this species was a temporary phenomenon.

Apart from the above exceptions however, epulos were always present in adults specimens of herbivorous acanthurid species. Type A epulos were largely restricted to *Acanthurus* species, although small individuals (of just over 100μm in length) were found in 2 specimens of *Naso unicornis* and 1 specimen of *Zebrasoma scopas*. Type A epulos were characteristic of both the large *Acanthurus* `white-bar' species which graze mixed-substrata (*A. auranticavus*, *A. blochii*, *A. dussumieri* and *A. grammoptilus*) and the smaller species which graze hard substrata (*A. lineatus*, *A. nigrofuscus* and *A. triostegus*). The two sand-feeding *Acanthurus* species, *A. nigricauda* and *A. olivaceus*, typically contained smaller epulos such as type G and F, although some GBR specimens contained type A and B epulos.

All specimens of three of the detritivorous *Ctenochaetus* species examined contained small type F and G epulos. Two species, *C. hawaiiensis* and *C. marginatus*, did not contain epulos. The reason for the difference in epulo occurrence between the five *Ctenochaetus* species is unclear. Both *C. hawaiiensis* and *C. marginatus* co-occur with species containing epulos, including *C. striatus* and *C. strigosus* (see Chapter 2). It is not known whether *C. hawaiiensis* and *C. marginatus* utilise the same food resource as the other 3 *Ctenochaetus* species. However, since these two species are larger than the other three species it is possible that some dietary difference exists. Since epulos were present in one specimen of *C. marginatus*, it would seem that this

species is at least a potential host. The lack of an obligate relationship in this species raises the question of the nature of the epulo/*Ctenochaetus* association as a whole.

*C. binotatus* and *C. striatus* move away from feeding substrata to defecate (see Chapter 3), a behaviour not observed in the well-studied host species *A. nigrofuscus*. Indeed, there is evidence that *A. nigrofuscus* deliberately voids undigested intestinal contents over feeding areas at the commencement of feeding (Fishelson et al. 1985, Chapter 3). This behaviour may be a mechanism for the retention of epulos by the host fish (Fishelson et al. 1985), a mechanism clearly lacking in *C. binotatus* and *C. striatus*. Indeed, the defecation behaviour of these two species would seem to hinder the prospect of intergenerational contact of microbes, a feature essential for obligate symbioses (Troyer 1984). The feeding behaviour of *Ctenochaetus* species suggests a likely vector for the transfer of endosymbionts. *Ctenochaetus* species feed by combing detritus and unicellular algae from reef surfaces (Randall 1955b, Jones 1968, Russ 1984a and b, Myers 1989), and are known to utilise the same substrata as herbivorous acanthurids (Choat and Bellwood 1985). It is therefore likely that *Ctenochaetus* species ingest a considerable amount of acanthurid faecal material, and thus may acquire epulos incidentally through their normal feeding activities. The above factors combine to suggest that the *Ctenochaetus*/epulo relationship may not be an obligate symbiosis.

Two of the browsing acanthurid species, *Naso lituratus* and *N. unicornis*, contained predominantly type C epulos. *N. tuberosus* contained the large distinctive type B epulos, and also typically harboured the ciliate *Balantidium jocularum*. The epulo

flora of the two *Zebrasoma* species differed in the occurrence of type J epulos (present in most *Z. scopas*, absent in *Z. veliferum*). This difference may be related to the diet of these species, with the latter species consuming macroalgae rather than filamentous algae (Robertson et al. 1979, Russ 1984a and b, Robertson and Gaines 1986). In general therefore, there appear to be similarities in epulo composition between ecologically and taxonomically distinct groups of species, such as the grazing *Acanthurus* species and the detritivorous *Ctenochaetus* species (Clements et al. 1989). How do these endosymbiont/host relationships compare between different geographical areas?

In general the GBR and Tuvalu samples were very similar in terms of epulo composition. Intraspecific comparisons show that the predominant epulo category was almost always the same in both areas. One major exception to this is *Zebrasoma scopas*, which typically contained type J epulos on the GBR yet lacked these endosymbionts in Tuvalu. The museum specimens examined from different areas all matched the GBR specimens in epulo composition, suggesting that host/epulo associations may be consistent throughout the tropical Indo-Pacific. Differences did occur in endosymbiont composition between the two areas in the occurrence of spirilla and protozoan categories. Spirilla were present in most GBR specimens of *Acanthurus lineatus*, *A. nigrofuscus*, *A. olivaceus*, *A. triostegus*, *Ctenochaetus striatus*, *Naso lituratus*, *Z. scopas* and *Z. veliferum*. Amongst these taxa, spirilla were only present in Tuvalu specimens of *A. nigrofuscus* and *N. lituratus*. Clearly spirilla were present in Tuvalu, and so the reasons for their decreased host range there are unclear.

Type K zooflagellates were present in most GBR specimens of *Acanthurus lineatus*, *A. nigrofuscus* and *A. triostegus*, yet these zooflagellates were not found in any Tuvalu specimens. It may be that these protozoans do not occur in Tuvalu (type K zooflagellates typically occurred in the anterior intestine sample, so it is unlikely that the lack of rectal samples have produced the negative result). All other zooflagellate types were represented in Tuvalu, although some showed a reduced host range. For example amongst the Tuvalu specimens type U zooflagellates were only recorded from *A. nigroris*, yet these protozoans were also found in GBR samples of *A. nigricauda*, *A. nigrofuscus*, *A. olivaceus* and *A. triostegus* (of species sampled in both areas).

A proper comparison of the ciliate fauna of the GBR and Tuvalu must await more detailed taxonomic study, although some differences between the two areas were apparent. Ciliates were present in some taxa in one area yet not the other. *A. nigricans* and *A. nigrofuscus* specimens from the GBR often contained ciliates and opalinids respectively, yet Tuvalu samples of these species did not. Conversely, 4 out of 5 Tuvalu specimens of *Zebrasoma veliferum* contained ciliates, while none were found in any of the 6 GBR specimens examined. *Z. scopas* specimens from both areas contained ciliates, yet at present it is not possible to say whether these represent the same taxa. Widespread ciliate/acanthurid symbioses have been recorded previously, *Vestibulongum corlissi* having been found in the guts of *A. xanthopterus* from both South Africa (Grim 1988) and the GBR (this study, N. Grim pers. comm.).

Some of the flagellate and ciliate taxa found in acanthurids during this study bear a strong similarity to those recorded from amphibians (Delvinquier 1988, Delvinquier and Jones 1988). The trichomonads *Trichomitus batrachorum* and *Tetratrichomonas prowazeki*, the retortamonad *Chilomastix caulleryi*, the monocercomonad *Monocercomonas batrachorum*, and the diplomonad *Spironucleus elegans* all occur in Australian anurans (Delvinquier and Freeland 1988a, Delvinquier and Jones 1988), and resemble forms encountered in acanthurids and siganids. Most of the zooflagellate species above have broad host ranges and occur in many amphibian and fish taxa (Delvinquier 1987, Delvinquier and Freeland 1988a), therefore their occurrence in acanthurids and siganids would not be surprising. However, the occurrence of the opalinid in *Acanthurus nigrofuscus* does appear paradoxical. The ultrastructure of this zooflagellate appears very similar to that described by Patterson and Delvinquier (1990) from a species of the genus *Protoopalina* from two species of Queensland frogs (J.N. Grim pers. comm.). *Protoopalina* species occur in many species of Australian anurans (Delvinquier 1987), and have been recorded from marine fish (D.J. Patterson pers. comm.). The genus thus has a broad host range. If this genus does occur in *A. nigrofuscus*, why does it not also occur in other species of acanthurids? Opalinids were only recorded from *A. nigrofuscus* collected from tropical and subtropical Australia, thus it is possible that these protozoans are restricted to the Australasian region.

The examination of samples from southeastern Australia suggested that the epulo/acanthurid symbiosis extends beyond coral reef environments to the limits of acanthurid distribution. Amongst these samples there was only a single result which

appeared anomalous in terms of tropical patterns. This was the absence of epulos from an 85mmSL *Acanthurus dussumieri* from Arrawarra Headland. Tropical specimens of this species always contained epulos. The specimen collected was the only *Acanthurus* individual observed at this locality, which was a coastal *Ecklonia* forest. In the absence of other conspecifics this fish may have had no contact with epulo populations, and thus remained uninfected. Some differences in the occurrence of epulo types were apparent between conspecifics from tropical and subtropical samples. For example, the *A. nigrofuscus* or *A. dussumieri* specimens collected at Julian Rocks contained type H and I epulos, rather than the type A epulos found in tropical specimens. The composition of these samples resembles the microbiota of tropical juvenile acanthurids. Indeed, comparison with the endosymbionts from juvenile acanthurids shows that some of the southern samples may represent a paedomorphic, rather than a necessarily distinct, assemblage.

Two points are immediately apparent from the juvenile microbiota results: (a) epulos are acquired after settlement, as they were absent fom the majority of transforming (i.e. newly-settled) specimens; and (b) juveniles usually contain different epulo types to adult conspecifics. An assessment of the microbiota of juvenile acanthurids raises a number of questions regarding the relationship of the epulo types found in adult specimens. In general, juvenile acanthurids harbour the smaller epulo categories, types E and G. Two hypotheses may account for the difference between adults and juveniles: (a) juveniles may initially become infected with small epulo varieties, and subsequently acquire larger epulos as they grow; or (b) the small epulo varieties may simply be morphotypes of the larger adult epulos, and transform into the larger

forms as the host grows. Both of these hypotheses will be examined in turn.

There would seem to be little support for the former hypothesis, that the epulo types are distinct and acquired at different host sizes (or ages). This hypothesis requires that juveniles initially become infected with epulos from adults other than conspecifics, an unlikely situation. However, until the precise relationships between epulo types are established, this hypothesis cannot be discounted.

The more likely explanation for the difference in adult and juvenile microbiota is that the epulos are variable in size, shape, and probably mode of cell division. Montgomery and Pollak (1988a) describe the daily cycle of reproduction and growth in *Epulopiscium fishelsoni* from Red Sea specimens of *Acanthurus nigrofuscus*. They demonstrate that the size distribution of *E. fishelsoni* changes throughout the day, with the smallest cell sizes predominating in the early morning (Montgomery and Pollak 1988a). This change in size distribution seems to be related to daughter-cell formation, which occurs without intervening periods of growth during the night. While Montgomery and Pollak (1988a) did not examine juvenile acanthurids, their results support the possibility that at least the large type A epulos vary considerably in size.

The variable-epulo hypothesis is also supported by the observation of Fishelson et al. (1985) that the small, immobile epulo cells found in the posterior intestine of adults may represent an encapsulated or encysted form. It is most likely that the epulos are anaerobes (Fishelson et al. 1985), and so such a resistant stage would

presumably be essential for intergenerational contact. It is therefore possible that infection of juvenile acanthurids occurs by the ingestion of faeces containing these small, possibly encapsulated epulos (unlike Montgomery and Pollak (1988a), I observed epulos in many faecal samples - see Chapter 3). The relationship between these small cells and type E or G epulos is unclear. However, the existence of type I epulos suggests that binary-fission and daughter-cell producing epulo forms may not be distinct. Indeed, type I epulos may represent an intermediate between the small, binary-fission type G epulos and the large, daughter-cell forming type A epulos. This possibility is also suggested by the distribution of these epulo types amongst *Acanthurus nigrofuscus* of different sizes. Type G epulos predominate in the smallest individuals, type I epulos are most common in large juveniles, while type A epulos predominate amongst the adults. This change in epulo form with host size may be due to changes in the intestinal environment as the host grows, or alternatively may represent a populational phenomenon of the epulos themselves. Perhaps daughter-cell formation is characteristic of mature, established epulo populations, and type G and type I epulos are more characteristic of rapidly growing or sub-equilibrium populations. If this is indeed the case, then the presence of the smaller type E and G epulos in adult acanthurids collected in southern localities may indicate that conditions are sub-optimal, perhaps due to low temperature.

In summary, the results of this chapter suggest that endosymbiotic communities are a characteristic feature of most species of herbivorous acanthurids and the pomacanthid *Centropyge bicolor*. Although other herbivorous groups are potentially capable of harbouring endosymbiont populations, the inconsistency of microbial

populations amongst taxa such as siganids and pomacentrids suggest that these represent facultative associations. A range of epulo forms was observed in herbivorous and detritivorous acanthurids, but epulos were not found in planktivorous acanthurids. These epulo forms, or types, were characterized by differences in shape, size and mode of reproduction. The occurrence of these epulo types amongst herbivorous acanthurids appeared to be consistent between the different geographical regions examined. A variety of flagellate and ciliate taxa were also found to inhabit the guts of herbivorous acanthurids. The host/microorganism associations of these protozoan symbionts were found to be more variable than those of epulos. Intestinal populations of epulos were rapidly established in juvenile acanthurids following settlement, however the epulo types found in juveniles differed from those characteristic of adult conspecifics. The structural similarities and patterns of occurrence of these epulo types suggest that they may represent a suite of ecomorphotypes.

# CHAPTER 5: EPULO ULTRASTRUCTURE

## 5.1 INTRODUCTION

An investigation of the gut contents of a Red Sea acanthurid, *Acanthurus nigrofuscus*, revealed the presence of a highly unusual microorganism (Fishelson et al. 1985). This endosymbiont was subsequently described as *Epulopiscium fishelsoni*, and tentatively assigned to the eukaryote Kingdom Protoctista (Montgomery and Pollak 1988a). Examination of the gut contents of herbivorous and detritivorous acanthurids from the GBR as part of this study revealed a range of similar microorganisms, described in Chapter 4 (see also Clements et al. 1989). Initial ultrastructural examination of this Great Barrier Reef material suggested that these organisms may in fact be prokaryotes, and not eukaryotes as previously thought (Montgomery and Pollak 1988a, 1988b). Furthermore, it was recently suggested that gram-negative bacteria of the genus *Metabacterium* from rodent intestines bore some similarity to the much larger epulos (Kunstyr et al. 1988).

A more detailed examination of epulo ultrastructure was therefore undertaken with two aims: (a) to resolve the uncertainty over the cellular nature of the epulos (i.e. prokaryotes vs. eukaryotes), and (b) to assess the relationships of the different epulo types described in Chapter 4. The first part of this work was done in collaboration with Prof. Stan Bullivant (Dept. Cellular and Molecular Biology, University of Auckland), a recognized authority on freeze-fracture techniques. The joint work concentrated on the largest of the GBR epulos, referred to as type A in Chapter 4.

Light photomicrographs of these organisms, which attain a length of 576μm on the GBR (Chapter 4), appear identical to the Red Sea form described as *Epulopiscium fishelsoni* (Montgomery and Pollak 1988a). The question of whether these large epulos were prokaryotes or eukaryotes was of particular interest because of the relatively huge size of these cells, and the implications that this may have for microbiology as a whole.

The second part of this work, an ultrastructural examination of different epulo types, enabled comparisons to be made at two levels. Firstly, a comparison between epulos of the 'same' morphotype from different host species sheds light on the putative host range of each morphotype. Secondly, a comparison between different epulo morphotypes allows an assessment of the structural diversity within this poorly understood, and possibly artificial, assemblage.

The results of this chapter are presented in two sections:

(i) Collaborative work with Prof. Bullivant on the ultrastructure of the large type A epulos, and

(ii) Ultrastructural comparisons of different epulo types.

## 5.2 MATERIALS AND METHODS

Epulo specimens were obtained from the host acanthurid species *Acanthurus dussumieri*, *A. lineatus*, *A. nigrofuscus*, *A. triostegus*, *Naso lituratus*, *N. unicornis*, *Ctenochaetus binotatus* and *Zebrasoma veliferum*. Acanthurids were collected by spear at Lizard Island, Great Barrier Reef, Australia, in 1987 and 1988. All material was processed within two hours of capture, during which time epulos retained motility. For thin section preparations, samples of gut contents were removed and fixed in 2.5% glutaraldehyde in 0.2M cacodylate buffer (pH 7.2) in 20% sea water for 30 minutes at 25°C. This material was then post-fixed in 1% osmium tetroxide in the same buffer as above, also for 30 minutes at 25°C. It was embedded in Spurr's resin, then sectioned and stained with uranyl acetate and lead citrate. The size of the large epulos examined with the electron microscope in the first part of this study varied between 200 and 400μm, making them visible through a dissecting microscope when embedded in the block. It was thus possible to select a particular specimen prior to sectioning.

For freeze-fracturing the material was fixed in 2.5% glutaraldehyde in 0.2M cacodylate buffered sea water, glycerinated and freeze-fractured following Bullivant and Ames (1966). Epulos were negatively-stained using 2% uranyl acetate at pH 7.2.

## 5.3 RESULTS

### 5.3.1 Ultrastructure of type A epulos

It is not possible to present an electron micrograph of a longitudinal section at a magnification showing both the complete organism (on the GBR the large type A epulos range from 70 to 576μm in length) and also sufficient internal detail. For a longitudinal view a light micrograph is therefore necessary (see Plate 4.1 in Chapter 4). A transverse section is used to show ultrastructural detail (Plate 5.1A). The organism has a mat of flagella on its surface. Light microscope video images of epulos swimming show a pulsatile layer of liquid movement in a narrow layer corresponding to this mat of flagella. Indeed, moving epulos appear to have waves passing over their surfaces and these waves change as swimming direction is reversed.

Beneath the outer surface is a peripheral layer of convoluted membranes, followed by a dense region with large lightly-stained inclusions. In the central region of the cell there is often a membrane-bound, dark-staining area (Plate 5.1B). This region has been previously referred to as a "daughter-cell" (Fishelson et al. 1985, Montgomery and Pollak 1988a, Clements et al. 1989). These structures are involved in the reproductive process, and upon completion of development emerge through a perforation in the maternal cortex (Montgomery and Pollak 1988a).

Type A epulos possess bacterial-type flagella (Stanier et al. 1987) rather than

eukaryote-type cilia. The epulo flagella have a diameter of 14 to 18nm in thin section (Plate 5.1B) and in freeze-fracture profile (Plate 5.1C), compared to 200 to 250nm for eukaryote cilia (Fawcett 1981). It was not possible to negatively stain the flagella of very large epulo specimens. When dried down in negative stain the flagella originating on the top surface of the organism adhered to that surface and did not reach down to the support film. Those flagella on the undersurface of the organism were obscured by the overhang. The flagella of similar but smaller type D epulos from *Zebrasoma veliferum* (Plate 5.1D) do show the characteristic helical subunit arrangement typical of bacterial flagella (Lowy and Hanson 1965). No eukaryote-type cilia with a 9+2 microtubule structure were observed in either this study or previous studies (Fishelson et al. 1985, Montgomery and Pollak 1988a).

Type A epulos possess bacterial-type nucleoids rather than eukaryote-type nuclei. The nucleoid DNA is found in circumscribed regions scattered throughout the mother-cell cytoplasm, whilst in the daughter-cells it is often found as a concentric peripheral region. It has a coagulated appearance (Plate 5.1E) typical of that seen in the bacterial nucleoid after standard fixation for electron microscopy (Hopwood and Glauert 1960, Schreil 1964). The nucleoids do not have a surrounding membrane. The limiting membranes of the daughter-cell do not show any structures resembling nuclear pores (Fawcett 1981), either by freeze-fracture (Plate 5.1F) or in thin section.

### 5.3.2 Ultrastructural comparisons of different epulo types

Type A epulos were examined from *Acanthurus triostegus* (Plate 5.2A) and *A. dussumieri* (Plate 5.2B). Type C epulos were examined from *Naso unicornis* (Plate 5.2C) and *N. lituratus* (Plate 5.2D). Type F epulos were examined from *Ctenochaetus binotatus* (Plate 5.2E and F), and type J epulos were examined from *N. unicornis* (Plate 5.3A-D). The type A, C and F epulos sectioned all appeared to have a similar general internal structure. All were characterised by the densely-stained central portion and lighter-stained outer layer described in 5.3.1 above. No membrane-bound organelles were apparent in any of the sections. The coagulated DNA structure seen in all sections is quite typical of the structure seen in the bacterial nucleoid after standard fixation (Hopwood and Glauert 1960, Schreil 1964), as described above in 5.3.1.

There were two minor differences in internal structure between the type A, C and F epulos sectioned. The type C epulos from the two *Naso* species both displayed a honeycomb-like network adjacent to the outer membrane (Plate 5.2C and D). It is possible that this structure is an artefact of fixation (K. Ryan, pers. comm.). Another difference between the type A, C and F epulos was the apparent lack of the outer convoluted membrane layer in the small type F epulos from *Ctenochaetus binotatus*. This convoluted layer was present in all type A and type C epulos examined in thin section.

Unlike sections of the epulo types containing daughter-cells, the sections of type J

epulos from *Naso unicornis* lacked the densely-stained central region (Plate 5.3A-D). Sections of type J epulos show a sequence of stages of internal wall, or septum, formation. This process begins with the formation of slight inclusions on adjacent outer cell walls (Plate 5.3B), through stages where an internal septum is complete (Plate 5.3A and D), to a stage where the resultant binary pair is almost separated (Plate 5.3C). The septum formed within the dividing cell in type J epulos is typical of the pattern seen in dividing gram-positive bacteria, rather than that seen in gram-negative bacteria where division results from a constriction of adjacent walls (Koch 1990).

**Plate 5.1 Ultrastructure of type A epulos**

**A**: Electron micrograph of thin transverse section of an epulo from *Acanthurus triostegus*. See text for description. In the daughter-cell (D) the concentric peripheral nucleoid region is arrowed. Scale bar = 10μm.

**B**: Electron micrograph of thin section of an epulo from *A. triostegus* showing peripheral convoluted membrane layer (top) within the cell and bacterial-type flagella projecting into the surrounding space (bottom). Scale bar = 0.5μm.

**C**: Electron micrograph of freeze-fracture replica of a region similar to that shown in B above, but from an epulo from *A. lineatus*, again showing convoluted membrane region (top) and flagella (bottom). Both cross (left arrow) and longitudinal (right arrow) fractures of flagella can be seen. Scale bar = 0.5μm.

**D**: Electron micrograph of negative-stain preparation of small epulo from *Zebrasoma veliferum*, showing bacterial-type flagella. Scale bar = 100nm.

**E**: Electron micrograph of thin section of nucleoid region in daughter-cell of epulo from *A. triostegus*. The coagulated appearance characteristic of bacterial DNA after standard fixation for electron microscopy is seen. Scale bar = 0.5μm.

**F**: Electron micrograph of freeze-fracture replica of epulo from *A. lineatus* showing mother-cell cytoplasm (CM) and the fracture faces of the plasma membrane (PM) and outer membrane (OM) of the daughter-cell. Scale bar = 0.5μm.

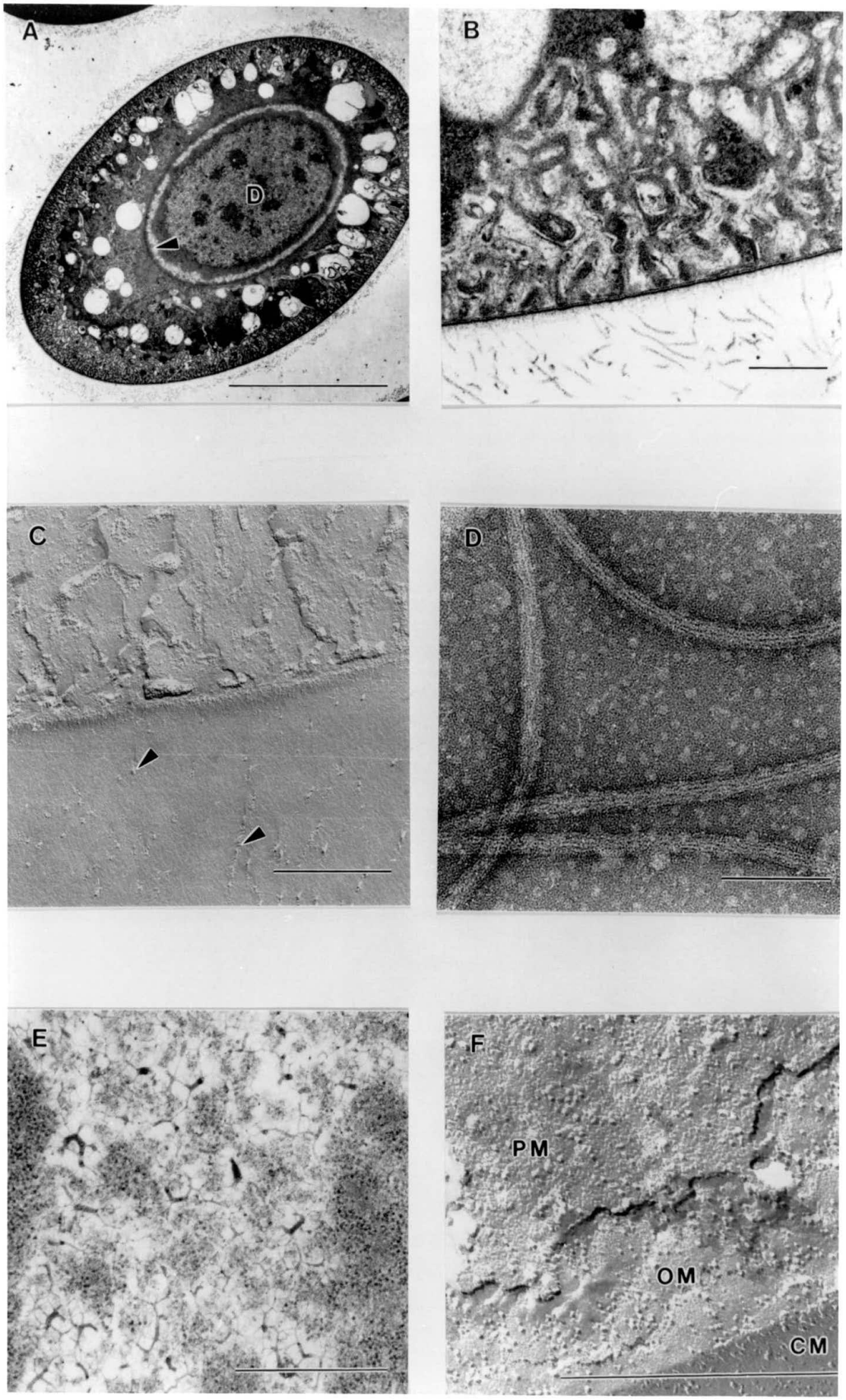

A
D
B
C
D
E
F
PM
OM
CM

**Plate 5.2 Ultrastructure of type A, C and F epulos**

All photos are transmission electron micrographs. All scale bars = 1μm.

**A**: Transverse thin section of type A epulo from *Acanthurus triostegus*, showing daughter-cell (D) at top, convoluted membrane region, and projecting flagella (bottom).

**B**: Transverse thin section of type A epulo from *A. dussumieri*, showing daughter-cell (D) at left and convoluted membrane region at right.

**C**: Transverse thin section of type C epulo from *Naso unicornis*, showing daughter-cell (D) and honeycomb-like outer region.

**D**: Transverse thin section of type C epulo from *N. lituratus*, showing daughter-cell (D) and honeycomb-like outer region.

**E**: Transverse thin section of type F epulo from *Ctenochaetus binotatus*, showing daughter-cell (D) and surrounding cytoplasmic layer.

**F**: Longitudinal thin section of type F epulo from *C. binotatus*, showing daughter-cell (D) and surrounding cytoplasmic layer.

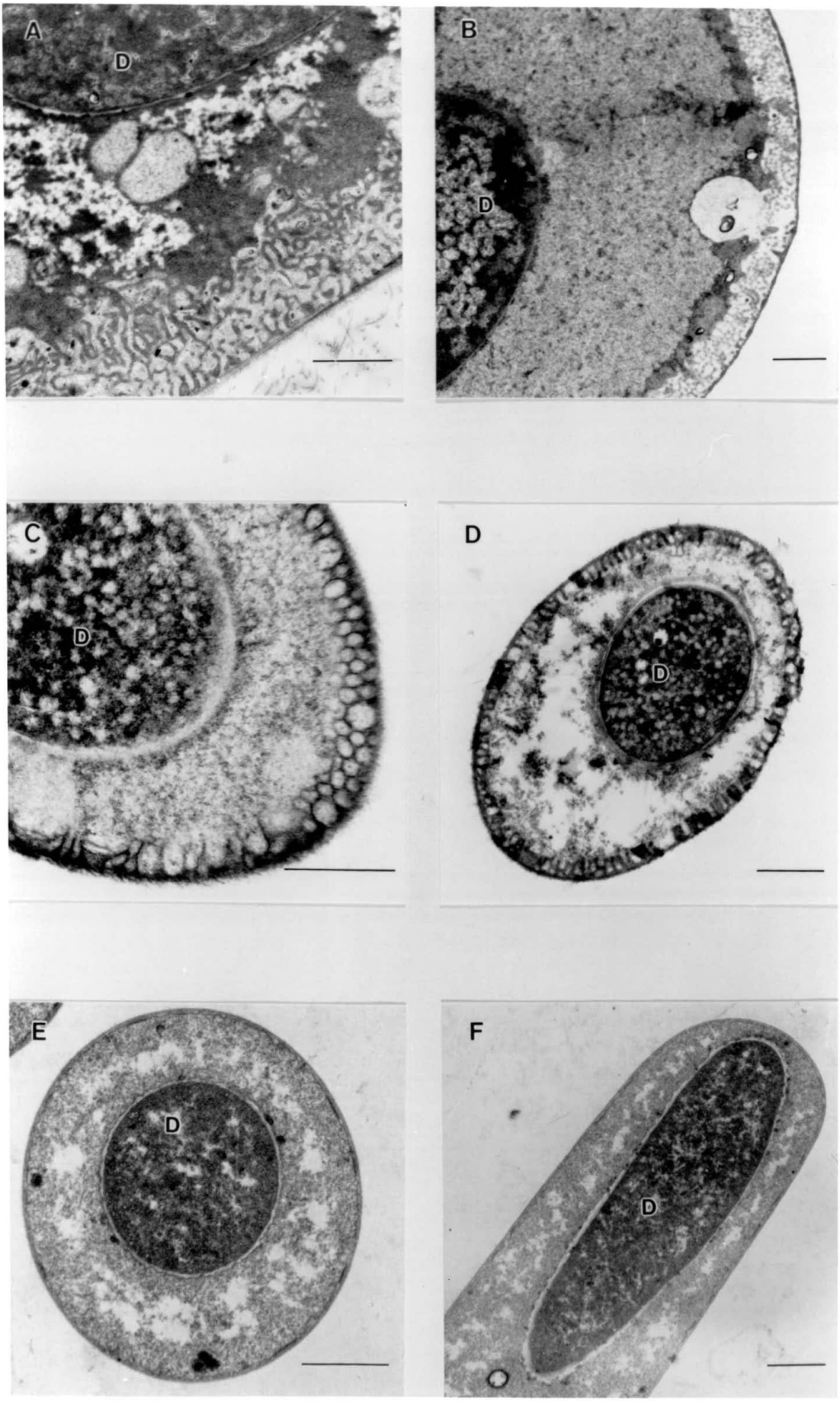

A
D
B
D
C
D
D
E
D
F
D

**Plate 5.3 Ultrastructure of type J epulos**

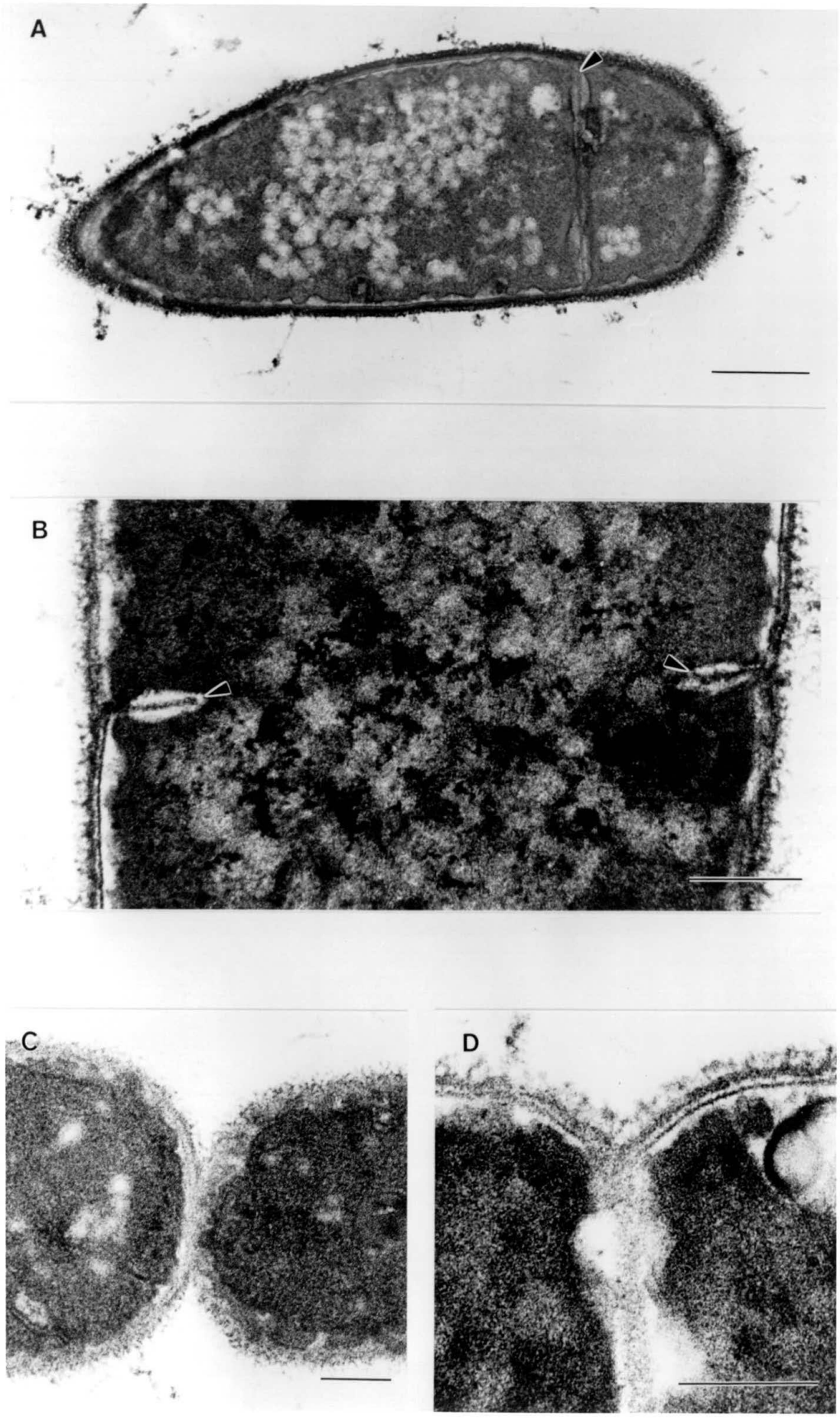
All photos are transmission electron micrographs.

**A**: Longitudinal thin section of type J epulo from *Naso unicornis*. Internal septum characteristic of binary fission in gram-positive eubacteria is arrowed. Scale bar = 0.5μm.

**B**: Longitudinal thin section of type J epulo from *Naso unicornis*. Inclusions adjacent to outer cell walls, which represent the early stages of internal septum formation, are arrowed. Scale bar = 0.2μm.

**C**: Longitudinal thin section of type J epulo from *Naso unicornis*. Binary pair of sibling cells are almost separated. Scale bar = 0.2μm.

**D**: Longitudinal thin section of type J epulo from *Naso unicornis*, showing detail of outer walls and complete internal septum. Scale bar = 0.2μm.


A
B
C
D

## 5.4 DISCUSSION

Definitive statements on the maximum size of prokaryote cells are scarce in the literature. Despite this, a maximum size of prokaryotes is generally assumed in discussions of the evolution of eukaryote cells (Schopf and Oehler 1976, Cavalier-Smith 1980). Amongst extant prokaryotes, *Spirochaeta plicatis* reaches maximum cell lengths of 250μm, but such cells are only 0.75μm in diameter (Blakemore and Canale-Parola 1973). *Lyngbya majuscula* cells are extremely flattened disks, and may be as large as 80 x 8μm (Demoulin and Janssen 1981). Individual cells of *Beggiatoa gigantea*, a disk-shaped chemo-autotrophic sulphur-oxidising eubacterium, may attain a diameter of 55μm and a width of 13μm (Starr et al. 1981). A recent report described unusually large *Beggiatoa* sp. from a hydrothermal deep-sea vent site (Jannasch et al. 1989). Filaments of these organisms attained 116 to 122μm in diameter. However these cells contained only a small amount of cytoplasm distributed around the outer cell wall, with the inner space of the cells filled by a large liquid vacuole (Jannasch et al. 1989). The largest spherical prokaryotes appear to be some members of the photosynthetic *Prochloron* species (Cox 1986), which are up to 30μm in diameter. Even a moderately sized epulo (200μm x 40μm) has a cell volume almost ten times that of the largest of the other prokaryotes listed above.

Two related factors are thought to be involved in constraining the size of the prokaryote cell: (a) the absence of intracellular transport mechanisms other than diffusion (Cavalier-Smith 1980, Starr et al. 1981); and (b) the organisation of DNA

replication and its control (Cavalier-Smith 1980, Demoulin and Janssen 1981). The ultrastructural observations in this study show that the large epulos possess features which may enable them to circumvent these limitations. The peripheral layer of highly convoluted membranes may represent infolding of the plasma membrane. Such infolding would vastly increase the surface area of the cell and enhance transport across the membrane into the interior. In addition, the peripheral convoluted layer may form the large compartment necessary to accumulate the proton pool (Manson et al. 1977) powering the numerous flagella needed to propel such a large organism. Finally, this convoluted membrane layer may also be involved in the coordination of flagellar action which results in the waves seen in the adjacent liquid.

The finding that epulos are giant prokaryotes raises the question of their phylogenetic status. The prokaryote-eukaryote dichotomy represents an organizational, rather than a phylogenetic, distinction (Woese and Fox 1977). The phylogenetic status of epulos may be resolved using rRNA sequence analysis, which has been widely used to assess the evolutionary relationships of microorganisms (Olsen et al. 1986, Pace et al. 1986, Patterson 1987, Woese 1987, Sogin 1989). A definitive rRNA analysis of Great Barrier Reef epulos is now underway in collaboration with Prof. N.R. Pace and Ms E. Angert of the University of Indiana. This analysis has however been complicated by the lack of epulos in pure culture, a problem which has required the development of new techniques to 'identify' the source of individual rRNA sequences (N.R. Pace and E. Angert, personal communication).

The finding that all epulos are prokaryotes suggests that the rRNA sequencing techniques described above will also be necessary to resolve the relationships among epulo morphotypes. This is because traditional techniques of bacterial classification using morphological or nutritional criteria may be uninformative in terms of phylogeny (Pace et al. 1986, Patterson 1987) and even species identity (Koch 1987). Thus conclusions based on ultrastructural features of the different epulo types must be made with caution. The type A epulos from different host species on the GBR (*Acanthurus dussumieri*, *A. lineatus*, *A. nigrofuscus* and *A. triostegus*) examined thus far in thin section appear to represent closely related, or possibly even the same, species (this study, Clements et al. 1989). Furthermore, the type A epulos from the GBR appear very similar to *Epulopiscium fishelsoni* from the Red Sea (Fishelson et al. 1985, Montgomery and Pollak 1988a), suggesting that these endosymbionts may represent a single widespread species.

While the data available indicate a similarity between epulos of the same morphotype from different host species, ultrastructural differences were evident between the different epulo morphotypes examined. Clearly, type J epulos appear quite unlike the other epulos examined, and instead resemble typical gram-positive rods found in termites (To et al 1980, Czolij et al. 1985) and other fishes (Rimmer and Wiebe 1987, Clements In Press). It is thus possible that the ten epulo morphotypes may represent an artificial assemblage of unrelated organisms. However, the general ultrastructural similarities between type A and C epulos, and to a lesser extent between these and type F epulos, suggest that at least some of the epulo morphotypes are closely related. In the absence of molecular data, little more

can be said concerning the interrelationships of these highly unusual prokaryotic endosymbionts.

# CHAPTER 6: GENERAL DISCUSSION

The format of this discussion follows the major aims of the study as stated in the General Introduction. Firstly, the occurrence patterns of endosymbiotic communities amongst taxa of herbivorous fish will be discussed. This will lead into a more specific treatment of the distribution patterns of endosymbionts amongst acanthurid species, and how these may indicate the possible roles of endosymbiont taxa. Secondly, the mechanisms of endosymbiont transmission described in this study will be discussed, and compared to those of similar gut microorganisms in amphibians. Information on endosymbiont transmission is highly germaine to an understanding of microorganism occurrence patterns, and so this will be briefly re-examined. Thirdly, the contribution of this study to the more general topics of (i) biogeography of herbivorous fishes and (ii) digestion in herbivorous fishes will be assessed. Finally, the discussion will close with some general conclusions and recommendations for future research.

## 6.1 Occurrence patterns of endosymbionts amongst herbivorous fishes

This study has demonstrated that diverse endosymbiotic communities are present in herbivorous representatives of two families of marine herbivorous fish: Acanthuridae and Pomacanthidae. The species of pomacentrids, scarids and blennies examined in this study did not consistently harbour large populations of endosymbionts, thus microorganisms are unlikely to be involved in obligate symbioses in these taxa. Some species of siganids contained large numbers of spirilla, but lacked the flora of

large prokaryote rods normally associated with herbivore/microorganism symbioses (e.g. To et al. 1980, Czolij et al. 1985, Margulis et al. 1986, Cruden and Markovetz 1987, Rimmer and Wiebe 1987, Grajal et al. 1989, Clements In Press).

Within the acanthurids, there was a strong relationship between diet and the occurrence of endosymbionts. Planktivorous acanthurids occasionally harboured zooflagellates, but lacked epulos. The detritivorous *Ctenochaetus* species often contained large epulo populations. However, epulos were absent from most individuals of two species (*C. hawaiiensis* and *C. marginatus*), and absent from a third species in some areas (*C. strigosus* specimens collected in Hawaii did not contain epulos, W.L. Montgomery pers. comm.). Thus it seems likely that the *Ctenochaetus*/epulo relationship may be facultative rather than obligate. For similar reasons, the *Zebrasoma*/epulo relationship may also represent a facultative association.

The distribution pattern of endosymbionts amongst herbivorous acanthurids suggests that differences exist between species in terms of their suitability as hosts of gut microorganisms. A large number of factors influence the likelihood that a particular host will be colonised by an endosymbiont. With relevance to intestinal symbioses, these include the following host characteristics: (i) antigen structure; (ii) gut volume; (iii) gut length; (iv) food retention time; (v) metabolic rate; (vi) nutrient levels in gut contents; (vii) particular gut microbiota; and (viii) composition of diet (Freeland 1983). In general, the more similar these factors are between two potential hosts, the greater the likelihood that both will be colonised (Freeland 1983). Thus the

systematic relationships of host species are generally strongly correlated with patterns of endosymbiont occurrence (e.g. Distel et al. 1988, Wren et al. 1989). Distel et al. (1988) found that the distribution of bacterial endosymbionts amongst six species of marine invertebrates did not reflect the geographical location or environment of the host, but rather reflected the phylogenetic relationship of the host species.

Consistency in patterns of endosymbiont occurrence between different geographical areas was also a feature of the epulo/acanthurid associations described in this study. Three different patterns of association were apparent: (a) consistent occurrence of epulos in a host species (e.g. *A. lineatus*, *A. nigrofuscus* and *A. triostegus*); (b) occurrence of epulos in some individuals of a host species (e.g. *Ctenochaetus* species and *Zebrasoma veliferum*); and (c) absence of epulos from almost all specimens of a species (e.g. *A. achilles*, *A. leucosternon* and *A. nigricans*). The first two of these patterns of epulo/host association are indicative of obligate and facultative relationships respectively. Thus while some species of acanthurids may be reliant upon an epulo flora for digestion (the possible role of these prokaryote endosymbionts in the digestive process will be discussed in section 6.3 below), others clearly are not.

A facultative epulo/host relationship in *Ctenochaetus* species does not mean that epulos do not play a role in digestion in these fishes. Facultative associations between herbivores and microorganisms frequently occur where the host is a generalist feeder (Jones 1984), in other words a species which utilises a wide variety

of dietary substrata. The variable occurrence of epulos in *Ctenochaetus* species may reflect the utilization of a variety of food resources by these fishes.

The absence of epulos from *A. achilles*, *A. leucosternon* and *A. nigricans* does not appear to be related to either dietary or habitat differences between these species and other herbivorous acanthurids. Rather, the absence of epulos from these three species indicates that they may be phylogenetically distinct from the other herbivorous members of the genus, as was suggested by Randall (1956). It may be that the superficial morphological and behavioural similarity of many acanthurid species is misleading, as has been found to be the case in the Scaridae (Bellwood and Choat 1990). A detailed systematic appraisal of the phylogenetic relationships amongst acanthurid species would greatly assist the interpretation of endosymbiont occurrence patterns.

The occurrence of protozoan endosymbionts within individual host species of acanthurids was less consistent than the pattern evident for epulos, suggesting a facultative rather than obligate relationship. Furthermore, the majority of the zooflagellate forms found in acanthurids appeared to have low host specificity, with the exception of the opalinid from *A. nigrofuscus*. This situation is very similar to that apparent in amphibian/protozoan symbioses, where some flagellates and ciliates have a single host species while others are ubiquitous (Delvinquier 1987, Delvinquier 1988, Delvinquier and Freeland 1988a). The presence of diplomonad and trichomonad zooflagellates in planktivorous, detritivorous and herbivorous acanthurids suggests that these protozoans are not associated with any particular

diet, and thus are unlikely to be directly involved in the digestive process.

Vertebrate intestinal flagellates, such as trichomonads and diplomonads, are commonly referred to as parasites although few are pathogenic (Honigberg 1963, Delvinquier and Freeland 1988a). Kinetoplastid and bodonid zooflagellates are known to feed as phagotrophic predators on bacteria (Haas and Webb 1979, Fenchel 1982). The ciliate *Balantidium jocularum* is also predatory, and has been found to ingest a range of prokaryotic and eukaryotic microorganisms in the gut of *Naso tuberosus* (J.N. Grim pers. comm.). Unlike many protozoan/termite associations, which are known to be mutualistic (Honigberg 1963, Honigberg 1970, Yamin and Trager 1979, Yamin 1980), the protozoan/acanthurid symbioses described in this study appear to represent either commensalism or parasitism.

### 6.2 Mechanisms of endosymbiont transmission in acanthurids

The information presented in this study strongly suggests that the epulo component of the gut microbiota, and probably also the protozoan component, is transferred between host generations by the ingestion of infected faecal material. The combination of three independent results lead to this conclusion: (i) the co-occurrence of adult and juvenile acanthurids at Lizard Island suggested that newly settled individuals in most cases were exposed to the faeces of adult conspecifics at an early stage; (ii) the aquarium experiment provided strong direct evidence that newly settled acanthurids became infected with epulos following exposure to the faeces of known hosts; and (iii) the ingestion of a bolus of undigested algae at the

commencement of feeding by juvenile *A. nigrofuscus* was highly indicative of a mechanism for the transfer or retention of endosymbionts. The finding that many acanthurid species share the same habitats, at least some of the time, also suggested the possibility that inter-specific infection of epulos may occur. This may be one explanation for the presence of type A epulos in large schooling species such as *A. auranticavus*, *A. blochii* and *A. dussumieri*. How does the mode of endosymbiont transmission in acanthurids compare to that found in amphibians?

Anurans acquire protozoan endosymbionts such as ciliates and opalinids by swallowing cysts released in the water with faeces of adults or tadpoles (Delvinquier 1987, Delvinquier 1988, Delvinquier and Freeland 1988b), and thus appear to have a similar mode of symbiont transmission to acanthurids. In many cases infection in anurans only occurs at the tadpole stage, and some microorganisms do not remain in the gut beyond metamorphosis into the adult frog (Delvinquier 1987, Delvinquier 1988, Delvinquier and Freeland 1988b). The resistance of some adult frogs to infection of microorganisms is related to ontogenetic changes in a number of parameters, including diet and the composition of the gut microbiota (Hazard 1941). It is possible that ontogenetic changes in some species of acanthurids may interfere with the mechanisms of endosymbiont transmission.

It has been suggested that the development of a muscular, gizzard-like stomach in some acanthurids may interfere with the establishment of epulo populations (Fishelson et al. 1985). Payne (1978) found that the gizzard-like stomach of grey mullet was capable of grinding ingested particles down to a size of 20μm. Nelson

and Wilkins (1988) have similarly shown that the acanthurid *Ctenochaetus striatus* is capable of significantly reducing the size of ingested particles. The majority of acanthurid species with gizzard-like stomachs do harbour epulo populations however. It is possible that epulos may circumvent passage through the harsh environment of a gizzard-like stomach by becoming established in the host intestine soon after settlement, when the stomach is not fully developed. It is also possible that other ontogenetic changes may inhibit the establishment of epulos in acanthurids above a certain size. The stomachs of adult *A. nigrofuscus* were found to be largely bacteriocidal (Sutton and Clements 1989), yet clearly epulo populations become established in juveniles of this species. It is possible that a 'window' for infection exists for a certain period during the ontogeny of acanthurids; this may be an explanation for the absence of epulos from *A. achilles*, *A. leucosternon* and *A. nigricans*, which all settle at relatively large sizes (Randall 1956).

### 6.3 Biogeography and digestion of herbivorous fishes: the role of gut microbiota

The study of endosymbionts in herbivorous marine fishes may contribute to an understanding of their biogeography in two ways: (i) if a fermentative microbiota is found to be essential to the digestive process in some species, then the availability of endosymbionts to juveniles may limit the distributions of host species; and (ii) the occurrence patterns of endosymbionts may be used as markers to indicate the phylogenetic and biogeographical affiliations of host taxa. The finding that epulos were present in the subtropical areas sampled, and our lack of information of the role that these organisms may play in digestion, precludes further comment on (i)

above. The distribution of some of the protozoan taxa found in acanthurids suggests that these endosymbionts have a more limited geographical range, and thus a more limited dispersal capability, than their hosts. For example, the viability of some opalinid cysts does not exceed three weeks (Delvinquier and Freeland 1988b), whereas acanthurids have a larval lifespan of 6 to 12 weeks (Brothers et al. 1983). Thus information on the distribution of these protozoans may be of potentially of great value to studies of fish distribution.

Several recent studies have suggested that gut microbiota may play a role in the process of digestion in herbivorous fishes. Rimmer and Wiebe (1987) described the gut-resident microbiota of two species of the Family Kyphosidae, and in addition detected the presence of volatile fatty acids (VFAs) in the posterior gut. Whilst this finding is very important in establishing that microbial fermentation does take place within the guts of fishes, it does not enable us to elaborate on the relationship between the endosymbionts and their fish host in the digestive process. Working on an herbivorous acanthurid, Montgomery and Pollak (1988b) identified a correlation between the presence of gut endosymbionts and a lowering of gut pH. This result demonstrates that endosymbionts may have an important influence on the intestinal environment, but does not allow us to speculate further on the importance of the microflora to the host. Finally, Sutton and Clements (1989) investigated the aerobic and facultatively anaerobic gastrointestinal bacterial flora of four Great Barrier Reef marine fishes. Amongst these, the herbivorous acanthurid *Acanthurus nigrofuscus* was found to have an intestinal microflora markedly different from the other fish examined, being dominated by agar-digesting non-*Vibrio* bacteria. The authors

suggested that these bacteria may have a role in the digestive breakdown of algal structural molecules, but whether these bacteria digest agar when actually in the intestinal environment was undetermined.

The lack of available information suggests that speculation on the role of the gut microbiota in herbivorous fishes should be limited to analogy with the well studied symbioses in terrestrial herbivores. Bjorndal (1987) lists several requirements that are necessary to maintain an efficient gut microbiota: constant body temperature, constant food supply, slow passage of digesta to allow sufficient time for microbial reproduction, anaerobic conditions, control of gut pH, and removal of fermentation waste products. The acanthurid/epulo relationship appears to conform to most of these requirements. However, differences in digestive physiology amongst herbivore taxa suggest that some caution is required to design models of fermentative digestion in herbivorous fishes based on terrestrial systems.

The lack of information on digestive parameters (e.g. gut transit time) in acanthurids makes direct comparisons with other herbivorous taxa difficult. Available data indicate that gut transit time is rapid in acanthurids (Randall 1961, Galzin 1987c), yet this does not appear to prevent the maintenance of endosymbiotic communities in many of these fishes. Many herbivores are known to retain some components of the digesta within the alimentary tract (Stevens 1988), therefore simple measurements of transit time do not allow interspecies comparisons (Guard 1980). Similarly, the importance of constant temperature to digestive processes may vary between herbivore groups; digestive efficiency is thermally stable in desert iguanas

(Zimmerman and Tracy 1989).

The consistent presence of dense populations of microorganisms in an anaerobic environment (the presence of trichomonad and diplomonad zooflagellates indicates that acanthurid intestines are largely anaerobic - Thong and Coombs 1987) suggests that it is possible that fermentative digestion is taking place within the guts of some acanthurids. Two observations reported by Fishelson et al. (1985) have to date led to the rejection of this possibility: (i) epulos were located near the gut lining and not in the food bolus; and (ii) starvation caused the loss of epulos, and starved fish did not become reinfected when released into the field (e.g. Horn 1989). Three observations cast doubt on these findings: (i) epulos were found throughout the intestinal lumen in this study, except in specimens with empty guts where they were found on the gut mucosa; (ii) work done on juvenile *A. nigrofuscus* in collaboration with Dr D. Sutton showed that starvation over a period of 2-3 days was an unreliable method of epulo elimination; and (iii) the observation that epulo-free *A. nigrofuscus* did not become reinfected when returned to the field was based on a single specimen (W.L. Montgomery pers. comm.). It is the conclusion of this study therefore that the possibility of microbial fermentation in acanthurids is worthy of future investigation.

## 6.4 General conclusions and recommendations for future research

The distribution pattern of gut microorganisms amongst taxa of herbivorous fishes suggests that these endosymbioses have evolved several times. This is indicated by

the presence of a gut microbiota in a number of unrelated teleost groups: kyphosids, odacids and some acanthurids. These taxa are however similar in one respect: they lack an obvious mechanical or chemical means of gaining access to plant cell contents. Taxa with obvious mechanisms for the degradation of plant cell walls (Lobel 1981), such as scarids or pomacentrids, do not contain endosymbiotic communities. Symbiotic associations with microorganisms may thus enable some groups of fishes to utilise plants as a food resource, as is the case in terrestrial plant/herbivore systems. If this is so, how may we explain the absence of gut microorganisms in herbivorous taxa such as *Prionurus*, *Acanthurus achilles*, *A. leucosternon*, *A. nigricans*, and siganids?

Reliance upon a gut microbiota may be a primitive feature in acanthuroid fishes. The situation may be analogous to patterns of symbioses between insects and microorganisms. Insects which lose symbionts over evolutionary time show either a shift in diet or evolve replacement metabolic capability; given the costs associated with mutualist loss it is likely that these evolutionary changes preceded symbiont loss (Jones 1984). As an example, the primitive lower termites are dependent upon obligate symbioses for cellulose digestion; higher termites frequently harbour gut microorganisms but can survive and grow without them (Eutick et al. 1978, O'Brien and Slaytor 1982, Veivers et al. 1983, Hogan et al. 1988). It is possible that the pattern of epulo occurrence in acanthurids represents a similar sequence of relationships: from obligate symbioses in species such as *A. nigrofuscus*; through facultative symbioses in species such as *Zebrasoma veliferum*; to species such as *A. achilles*, which have evolved endogenous mechanisms of digesting algae.

Viewing these arguments in a more general context, we see that the symbioses between herbivorous fishes and gut microorganisms may represent a number of different stages in the evolution of piscine herbivory. Variability in endosymbiont occurrence within a herbivorous taxon, such as within the Acanthuridae, may indicate a long history of herbivory in that group. One would predict the occurrence in representatives of these groups of both obligate and facultative symbioses, and possibly also the total loss of symbionts. The long tenure of acanthurids in the fossil record (Blot 1980), the widespread occurrence of epulos within the family, and the wide geographical extent of some acanthurid symbiotic relationships suggest that some acanthurid symbioses may indeed be ancient. Apparently obligate relationships, such as the *A. nigrofuscus*/epulo symbiosis, may represent the oldest form of association. This suggests that host species such as *A. lineatus* and *A. nigrofuscus* may represent the primitive condition of herbivory within the Acanthuridae. Species which have facultative associations with epulos, such as members of the genus *Zebrasoma*, and species which have lost the endosymbionts altogether, such as *A. achilles*, may represent more recently derived forms. On the other hand, the presence of a gut microbiota in all herbivorous representatives of some higher taxa, such as the Odacidae and the Kyphosidae, may indicate a relatively short history of herbivory in these groups. Clearly, these hypotheses are testable by detailed phylogenetic studies of the host fishes.

The most obvious requirement for an understanding of these symbioses between herbivorous fish and microorganisms is research into the field of digestive physiology, as was emphasized by Horn (1989). Almost nothing is understood of the

role that microorganisms play in the digestive process of these fishes, or indeed of the digestive physiology of the fishes themselves. Some important areas for consideration are: (i) what are the daily energy requirements of herbivorous fishes? (ii) is the daily energy requirement met from cell walls or cell contents? Allometric considerations suggest that cell contents are the major fuel (Stevens 1988). If so, how are cell contents released for digestion? (iii) how is gut pH controlled in herbivorous fishes? (iv) what is the redox state of the intestines of herbivorous fishes? (v) what is the rate of digesta transit in herbivorous fishes? Is this rate similar for all digesta components? (vi) which enzymes are characteristic of the guts of particular species of fishes, and are their sources endogenous or exogenous? (vii) what are the levels and rates of production of VFAs in the gut of these fishes, and are VFAs utilized by the fishes themselves? Information on these digestive processes, and thus the patterns of resource utilization by herbivorous fishes, is a prerequisite to understanding the role that these fishes play in the coral reef ecosystem.

Another area worthy of attention is more detailed study of the endosymbionts themselves. In particular, an understanding of the relationships of the epulo types described in this study would be very valuable to an interpretation of the host specificity of these organisms. As discussed in Chapter 5, rRNA sequence analysis of the epulo morphotypes is necessary to determine whether these microorganisms are distinct taxa or not. The results of this work could then be used to examine the symbiosis in an evolutionary context, as has been done elsewhere (e.g. Berry and Jensen 1988, Hafner and Nadler 1988, Wren et al. 1989). The elaboration of the

phylogenetic relationships of epulos and their host acanthurids would provide an insight into the evolution of herbivory in marine fishes.

## LITERATURE CITED


**Anderson, T.A.** 1987. Utilization of algal cell fractions by the marine herbivore the luderick, *Girella tricuspidata* (Quoy and Gaimard). J. Fish Biol. **31**: 221-228.

**Bailey, T.G., and D.R. Robertson.** 1982. Organic and caloric levels of fish feces relative to its consumption by coprophagous reef fishes. Mar. Biol. **69**: 45-50.

**Barlow, G.W.** 1974a. Contrasts in social behaviour between central American cichlid fishes and coral-reef surgeon fishes. Amer. Zool. **14**: 9-34.

**Barlow, G.W.** 1974b. Extraspecific imposition of social grouping among surgeonfishes (Pisces: Acanthuridae). J. Zool., Lond. **174**: 333-340.

**Bellwood, D.R.** 1988. Ontogenetic changes in the diet of early post-settlement *Scarus* species (Pisces: Scaridae). J. Fish Biol. **33**: 213-219.

**Bellwood, D.R., and A.C. Alcala.** 1988. The effect of a minimum length specification on visual estimates of density and biomass of coral reef fishes. Coral Reefs **7**: 23-27.

**Bellwood, D.R., and J.H. Choat.** 1990. A functional analysis of grazing in parrotfishes (family Scaridae): the ecological implications. Envir. Biol. Fish. **28**: 189-214.

**Berry, A., and R.A. Jensen.** 1988. Biochemical evidence for phylogenetic branching patterns. BioScience **38**: 99-103.

**Bjorndal, K.A.** 1987. Digestive efficiency in a temperate herbivorous reptile, *Gopherus polyphemus*. Copeia **1987**: 714-720.

**Blakemore, R.P., and E. Canale-Parola.** 1973. Morphological and ecological characteristics of *Spirochaeta plicatis*. Arch. Mikrobiol. **89**: 273-289.

**Blot, J.** 1980. La faune ichthyologique des gisements du Monte Bolca (Province de Verone, Italie): Catalogue systematique presentant l'etat actuel des recherches concernant cette faune. Bull. Mus. natn. Hist. nat., Paris, Series 4, **2**(C4): 339-396.

**Boucher, D.H., S. James and K.H. Keeler.** 1982. The ecology of mutualism. Annu. Rev. Ecol. Syst. **13**: 315-347.

**Bouchon-Navaro, Y., and M.L. Harmelin-Vivien.** 1981. Quantitative distribution of herbivorous reef fishes in the Gulf of Aqaba (Red Sea). Mar. Biol. **63**: 79-86.

**Brothers, E.B., D.McB. Williams and P.F. Sale.** 1983. Length of larval life in twelve families of fishes at "One Tree Lagoon," Great Barrier Reef, Australia. Mar. Biol. **76**: 319-324.

**Bullivant, S., and A. Ames III.** 1966. A simple freeze-fracture replication method for electron microscopy. J. Cell Biol. **29**: 435-447.

**Cahill, M.M.** 1990. Bacterial flora of fishes: a review. Microbial Ecol. **19**: 21-41.

**Cavalier-Smith, T.** 1980. Cell compartmentation and the origin of eukaryote membranous organelles. **In** W. Schwemmler and H.E.A. Schenk (ed.), Endocytobiology. Endosymbiosis and cell biology. Vol. I, p. 893-916. Walter de Gruyter and Co., Berlin.

**Choat, J.H.** 1982. Fish feeding and the structure of benthic communities in temperate waters. Annu. Rev. Ecol. Syst. **13**: 423-449.

**Choat, J.H., and D.R. Bellwood.** 1985. Interactions amongst herbivorous fishes on a coral reef: influence of spatial variation. Mar. Biol. **89**: 221-234.

**Clements, K.D.** In Press. The endosymbiotic communities of two herbivorous labroid fishes, *Odax cyanomelas* (Richardson) and *O. pullus* Schneider. Mar. Biol.

**Clements, K.D., D.C. Sutton and J.H. Choat.** 1989. Occurrence and characteristics of unusual protistan symbionts from surgeonfishes (Acanthuridae) of the Great Barrier Reef, Australia. Mar. Biol. **102**: 403-412.

**Cox, G.** 1986. Comparison of *Prochloron* from different hosts. I. Structural and ultrastructural characteristics. New Phytol. **104**: 429-445.

**Cruden, D.L., and A.J. Markovetz.** 1987. Microbial ecology of the cockroach gut. Ann. Rev. Microbiol. **41**: 617-643.

**Czolij, R., M. Slaytor and R.W. O'Brien.** 1985. Bacterial flora of the mixed segment and the hindgut of the higher termite *Nasutitermes exitiosus* Hill (Termitidae, Nasutitermitinae). Appl. Environ. Microbiol. **49**: 1226-1236.

**Day, R.W., and G.P. Quinn.** 1989. Comparisons of treatments after an analysis of variance in ecology. Ecol. Monogr. **59**: 433-463.

**Delvinquier, B.L.J.** 1987. Opalinidae in Australian Anura. Proc. R. Soc. Qld. **98**: 93-122.

**Delvinquier, B.L.J.** 1988. On some Nyctotheridae of the Australian Anura with a description of *Nyctotheroides raffae* n. sp.. Proc. R. Soc. Qld. **99**: 21-34.

**Delvinquier, B.L.J., and W.J. Freeland.** 1988a. Protozoan parasites of the Cane Toad, *Bufo marinus*, in Australia. Aust. J. Zool. **36**: 301-316.

**Delvinquier, B.L.J., and W.J. Freeland.** 1988b. Observations on *Zelleriella antilliensis* (Protozoa: Opalinata) from the Cane Toad *Bufo marinus* in Australia. Aust. J. Zool. **36**: 317-333.

**Delvinquier, B.L.J., and M.K. Jones.** 1988. A preliminary note on the intestinal flagellates of the Australian Anura. Mem. Qld. Mus. **25**: 333-334.

**Demoulin, V., and M.P. Janssen.** 1981. Relationship between diameter of the filament and cell shape in blue-gren algae. Br. phycol. J. **16**: 55-58.

**Distel, D.L., D.J. Lane, G.J. Olsen, S.J. Giovannoni, B. Pace, N.R. Pace, D.A. Stahl and H. Felbeck.** 1988. Sulfur-oxidising bacterial endosymbionts: analysis of phylogeny and specificity by 16S rRNA sequences. J. Bact. **170**: 2506-2510.

**Doherty, P.J., and D.McB. Williams.** 1988. The replenishment of coral reef fish populations. Oceanogr. Mar. Biol. Annu. Rev. **26**: 487-551.

**Eckert, G.J.** 1984. Annual and spatial variation in recruitment of labroid fishes among seven reefs in the Capricorn/Bunker Group, Great Barrier Reef. Mar. Biol. **78**: 123-127.

**Eutick, M.L., P. Veivers, R.W. O'Brien and M. Slaytor.** 1978. Dependence of the higher termite, *Nasutitermes exitiosus* and the lower termite, *Coptotermes lacteus* on their gut flora. J. Insect Physiol. **24**: 363-368.

**Fawcett, D.W.** 1981. The cell. 2nd ed. Saunders, Philadelphia.

**Fenchel, T.** 1982. Ecology of heterotrophic microflagellates. I. Some important forms and their functional morphology. Mar. Ecol. Prog. Ser. **8**: 211-223.

**Fishelson, L., W.L. Montgomery and A.A. Myrberg.** 1985. A unique symbiosis in the gut of tropical herbivorous surgeonfish (Acanthuridae: Teleostei) from the Red Sea. Science, N.Y. **229**: 49-51.

**Fishelson, L., W.L. Montgomery and A.A. Myrberg.** 1987. Biology of surgeonfish *Acanthurus nigrofuscus* with emphasis on changeover in diet and annual gonadal cycles. Mar. Ecol. Prog. Ser. **39**: 37-47.

**Freeland, W.J.** 1983. Parasites and the coexistence of animal host species. Amer. Nat. **121**: 223-236.

**Gaines, S.D., and J. Lubchenco.** 1982. A unified approach to marine plant-herbivore interactions. II. Biogeography. Annu. Rev. Ecol. Syst. **13**: 111-138.

**Galzin, R.** 1987a. Structure of fish communities of French Polynesian coral reefs. I. Spatial scales. Mar. Ecol. Prog. Ser. **41**: 129-136.

**Galzin, R.** 1987b. Structure of fish communities of French Polynesian coral reefs. II. Temporal scales. Mar. Ecol. Prog. Ser. **41**: 137-145.

**Galzin, R.** 1987c. Potential fisheries yield of a Moorea fringing reef (French Polynesia) by the analysis of three dominant fishes. Atoll Res. Bull. No. 305, Smithsonian Inst., Washington D.C.

**Godwin, J.R., and R.K. Kosaki.** 1989. Reef fish assemblages on submerged lava flows of three different ages. Pac. Sci. **43**: 289-301.

**Grajal, A., S.D. Strahl, R. Parra, M.G. Dominguez and A. Neher.** 1989. Foregut fermentation in the hoatzin, a neotropical leaf-eating bird. Science **245**: 1236-1238.

**Grim, J.N.** 1988. A somatic kinetid study of the pycnotrichid ciliate *Vestibulongum corlissi* N.G., N.Sp. (Class: Litostomatea), symbiont in the intestines of the surgeonfish, *Acanthurus xanthopterus*. J. Protozool. **35**: 227-230.

**Grim, J.N.** In Press. Description of four kinetid systems of *Balantidium jocularum*, n.sp., and possible taxonomic implications for the Class, Litostomatea, and the genus, *Balantidium*. J. Protozool.

**Guard, C.L.** 1980. The reptilian digestive system: general characteristics. **In**: Schmidt-Nielsen, K., L. Bolis, C.R. Taylor, P.J. Bentley and C.E. Stevens (eds.) Comparative physiology: primitive mammals, p. 43-51. Cambridge University Press, Cambridge.

**Haas, L.W., and K.L. Webb.** 1979. Nutritional mode of several non-pigmented microflagellates from the York River estuary, Virginia. J. exp. mar. Biol. Ecol. **39**: 125-134.

**Hafner, M.S., and S.A. Nadler.** 1988. Phylogenetic trees support the coevolution of parasites and their hosts. Nature **332**: 258-259.

**Hansen, J.A., D.M. Alongi, D.W. Moriarty and P.C. Pollard.** 1987. The dynamics of benthic microbial communities at Davies Reef, central Great Barrier Reef. Coral Reefs **6**: 63-70.

**Hazard, F.O.** 1941. The absence of opalinids from the adult Green Frog, *Rana clamitans*. J. Parasitol. **27**: 513-516.

**Hiatt, R.W., and D.W. Strasburg.** 1960. Ecological relationships of the fish fauna on coral reefs of the Marshall Islands. Ecol. Monogr. **30**: 65-127.

**Hobson, E.S.** 1974. Feeding relationships of teleostean fishes on coral reefs in Kona, Hawaii. Fish. Bull. **72**: 915-1031.

**Hogan, M., P.C. Veivers, M. Slaytor and R.T. Czolij.** 1988. The site of cellulose breakdown in higher termites (*Nasutitermes walkeri* and *Nasutitermes exitiosus*). J. Insect Physiol. **34**: 891-899.

**Honigberg, B.M.** 1963. Evolutionary and systematic relationships in the flagellate order Trichomonadida Kirby. J. Protozool. **10**: 20-63.

**Honigberg, B.M.** 1970. Protozoa associated with termites and their role in digestion. In: K. Krishna and F.M. Weesner (ed.), Biology of termites. Vol. 2, p. 1-36. Academic Press, New York.

**Hopwood, D.A., and A.M. Glauert.** 1960. The fine structure of *Streptomyces coelicolor*. II. The nuclear material. J. Biophys. Biochem. Cytol. **8**: 267-278.

**Horn, M.H.** 1989. Biology of marine herbivorous fishes. Oceanogr. Mar. Biol. Annu. Rev. **27**: 167-272.

**Hourigan, T.F.** 1986. An experimental removal of a territorial pomacentrid: effects on the occurrence and behaviour of competitors. Env. Biol. Fish. **15**: 161-169.

**Hungate, R.E.** 1966. The rumen and its microbes. Academic Press, New York.

**Hungate, R.E.** 1975. The rumen microbial ecosystem. Annu. Rev. Ecol. Syst. **6**: 39-66.

**Hutchins, B., and R. Swainston.** 1986. Sea fishes of southern Australia. Swainston Publishing, Perth.

**Jannasch, H.W., D.C. Nelson and C.O. Wirsen.** 1989. Massive natural occurrence of unusually large bacteria (*Beggiatoa* sp.) at a hydrothermal deep-sea vent site. Nature **342**: 834-836.

**Johnson, G.D., and B.B. Washington.** 1987. Larvae of the moorish idol, *Zanclus cornutus*, including a comparison with other larval acanthuroids. Bull. Mar. Sci. **40**: 494-511.

**Jones, C.G.** 1984. Microorganisms as mediators of plant resource exploitation by insect herbivores. In P.W. Price, C.N. Slobodchikoff and W.S. Gaud (ed.), A new ecology: novel aproaches to interactive systems, p. 53-99. John Wiley and Sons, New York.

**Jones, G.P., U.L. Kaly and K.D. Clements.** 1991. Preliminary records of the coral reef fishes of Tuvalu. S. Pac. J. Nat. Sci. **11**: 40-57.

**Jones, R.S.** 1968. Ecological relationships in Hawaiian and Johnston Island Acanthuridae (Surgeonfishes). Micronesica **4**: 309-361.

**Kaly, U.L., and G.P. Jones.** 1990. The construction of boat channels across coral reefs: an assessment of ecological impact. Unpublished Report Number 4 Prepared for: NZ Ministry of External Relations and Trade. 43pp.

**Koch, A.L.** 1987. Evolution from the viewpoint of *Escherichia coli*. In: P. Calow (ed.) Evolutionary physiological ecology, p. 85-103. Cambridge University Press, Cambridge.

**Koch, A.L.** 1990. Growth and form of the bacterial cell wall. Amer. Sci. **78**: 327-341.

**Kulda, J., and J. Lom.** 1964. Remarks on the diplomastigine flagellates from the intestine of fishes. Parasitology **54**: 753-762.

**Kunstyr, I., R. Schiel, F.J. Kaup, G. Uhr and H. Kirchhoff.** 1988. Giant gram-negative noncultivable endospore-forming bacteria in rodent intestines. Naturwissenschaften **75**: 525-527.

**Lee, J.J., S.H. Hutner and E.C. Bovee (ed.).** 1985. Illustrated guide to the Protozoa. Society of Protozoologists, Lawrence, Kansas.

**Lobel, P.S.** 1981. Trophic biology of herbivorous reef fishes: alimentary pH and digestive capabilities. J. Fish Biol. **19**: 365-397.

**Low, R.M.** 1971. Interspecific territoriality in a pomacentrid reef fish, *Pomacentrus flavicauda* Whitley. Ecology **52**: 648-654.

**Lowy, J., and J. Hanson.** 1965. Electron microscope studies of bacterial flagella. J. Molec. Biol. **11**: 293-313.

**Manson, M.D., P. Tedesco, H.C. Berg, F.M. Harold and C. Van der Drift.** 1977. A protonmotive force drives bacterial flagella. Proc. Natl. Acad. Sci. USA **74**: 3060-3064.

**Mapstone, B.D., and A.J. Fowler.** 1988. Recruitment and the structure of assemblages of fish on coral reefs. TREE **3**: 72-77.

**Margulis, L., D. Chase and R. Guerrero.** 1986. Microbial communities. BioScience **36**: 160-170.

**Miller, A.C.** 1982. Effects of differential fish grazing on the community structure of an intertidal reef flat at Enewetak Atoll, Marshall Islands. Pac. Sci. **36**: 467-482.

**Mok, H.-K.** 1977. Gut patterns of the Acanthuridae and Zanclidae. Jap. J. Ichthyol. **23**: 215-219.

**Montgomery, W.L., A.A. Myrberg and L. Fishelson.** 1989. Feeding ecology of surgeonfishes (Acanthuridae) in the northern Red Sea, with particular reference to *Acanthurus nigrofuscus* (Forsskål). J. Exp. Mar. Biol. Ecol. **132**: 179-207.

**Montgomery, W.L., and P.E. Pollak.** 1988a. *Epulopiscium fishelsoni* n. gen., n. sp., a protist of uncertain taxonomic affinities from the gut of an herbivorous reef fish. J. Protozool. **35**: 565-569.

**Montgomery, W.L., and P.E. Pollak.** 1988b. Gut anatomy and pH in a Red Sea surgeonfish, *Acanthurus nigrofuscus*. Mar. Ecol. Prog. Ser. **44**: 7-13.

**Moriarty, D.J.W., P.C. Pollard, D.M. Alongi, C.R. Wilkinson and J.S. Gray.** 1985. Bacterial productivity and trophic relationships with consumers on a coral reef (MECOR I). Proc. Fifth Int. Coral Reef Congress, Tahiti, Vol. 3, p. 457-462.

**Myers, R.F.** 1989. Micronesian reef fishes. Coral Graphics, Guam.

**Nelson, J.S.** 1984. Fishes of the world. John Wiley and Sons, New York.

**Nelson, S.G., and S.DeC. Wilkins.** 1988. Sediment processing by the surgeonfish *Ctenochaetus striatus* at Moorea, French Polynesia. J. Fish Biol. **32**: 817-824.

**O'Brien, R.W., and M. Slaytor.** 1982. Role of microorganisms in the metabolism of termites. Aust. J. Biol. Sci. **35**: 239-262.

**Ogden, J.C., and P.S. Lobel.** 1978. The role of herbivorous fishes and urchins in coral reef communities. Env. Biol. Fish. **3**: 49-63.

**Olsen, G.J., D.J. Lane, S.J. Giovannoni and N.R. Pace.** 1986. Microbial ecology and evolution: a ribosomal RNA approach. Annu. Rev. Microbiol. **40**: 337-365.

**Pace, N.R., G.J. Olsen and C.R. Woese.** 1986. Ribosomal RNA phylogeny and the primary lines of evolutionary descent. Cell **45**: 325-326.

**Patterson, C.** 1987. Introduction. **In**: Molecules and morphology in evolution: conflict or compromise? C. Patterson (ed.), p. 1-22. Cambridge University Press, Cambridge.

**Patterson, D.J., and B.L.J. Delvinquier.** 1990. The fine structure of the cortex of the protist *Protoopalina australis* (Slopalinida, Opalinidae) from *Litoria nasuta* and *Litoria inermis* (Amphibia, Anura, Hylidae) in Queensland, Australia. J. Protozool. **37**: 449-455.

**Payne, A.I.** 1978. Gut pH and digestive strategies in estuarine grey mullet (Mugilidae) and tilapia (Cichlidae). J. Fish Biol. **13**: 627-630.

**Pillai, C.S.G., M. Mohan and K.K. Kunhikoya.** 1983. On an unusual massive recruitment of the reef fish *Ctenochaetus strigosus* (Bennet) (Perciformes: Acanthuridae) to the Minicoy Atoll and its significance. Indian J. Fish. **30**: 261-268.

**Polunin, N.V.C., and D.W. Klumpp.** 1989. Ecological correlates of foraging periodicity in herbivorous reef fishes of the Coral Sea. J. Exp. Mar. Biol. Ecol. **126**: 1-20.

**Polunin, N.V.C., and I Koike.** 1987. Temporal focusing of nitrogen release by a periodically feeding herbivorous reef fish. J. Exp. Mar. Biol. Ecol. **111**: 285-296.

**Randall, J.E.** 1955a. A revision of the surgeon fish genera *Zebrasoma* and *Paracanthurus*. Pac. Sci. **9**: 396-412.

**Randall, J.E.** 1955b. A revision of the surgeon fish genus *Ctenochaetus*, Family Acanthuridae, with descriptions of five new species. Zoologica **40**: 149-168.

**Randall, J.E.** 1956. A revision of the surgeon fish genus *Acanthurus*. Pac. Sci. **10**: 159-235.

**Randall, J.E.** 1961. A contribution to the biology of the convict surgeonfish of the Hawaiian Islands, *Acanthurus triostegus sandvicensis*. Pac. Sci. **15**: 215-272.

**Randall, J.E., G.R. Allen and R.C. Steene.** 1990. Fishes of the Great Barrier Reef and Coral Sea. Crawford House Press, Bathurst.

**Reinthal, P.N., and S.M. Lewis.** 1986. Social behaviour, foraging efficiency and habitat utilization in a group of tropical herbivorous fish. Anim. Behav. **34**: 1687-1693.

**Rimmer, D.W.** 1986. Changes in diet and the development of microbial digestion in juvenile buffalo bream, *Kyphosus cornelii*. Mar. Biol. **92**: 443-448.

**Rimmer, D.W., and W.J. Wiebe.** 1987. Fermentative microbial digestion in herbivorous fishes. J. Fish Biol. **31**: 229-236.

**Roberts, C.M.** 1987. Experimental analysis of resource sharing between herbivorous damselfish and blennies on the Great Barrier Reef. J. Exp. Mar. Biol. Ecol. **111**: 61-75.

**Robertson, D.R.** 1982. Fish faeces as fish food on a Pacific coral reef. Mar. Ecol.

Prog. Ser. 7: 253-265.

**Robertson, D.R.** 1983. On the spawning behaviour and spawning cycles of eight surgeonfishes (Acanthuridae) from the Indo-Pacific. Envir. Biol. Fish. **9**: 192-223.

**Roberston, D.R.** 1988a. Abundances of surgeonfishes on patch-reefs in Caribbean Panama: due to settlement, or post-settlement events? Mar. Biol. **97**: 495-501.

**Robertson, D.R.** 1988b. Extreme variation in settlement of the Caribbean triggerfish *Balistes vetula* in Panama. Copeia **1988**: 698-703.

**Robertson, D.R., and S.D. Gaines.** 1986. Interference competition structures habitat use in a local assemblage of coral reef surgeonfishes. Ecology **67**: 1372-1383.

**Roberston, D.R., and N.V.C. Polunin.** 1981. Coexistence: symbiotic sharing of feeding territories and algal food by some coral reef fishes from the Western Indian Ocean. Mar. Biol. **62**: 185-195.

**Robertson, D.R., N.V.C. Polunin and K. Leighton.** 1979. The behavioural ecology of three Indian Ocean surgeonfishes (*Acanthurus lineatus*, *A. leucosternon* and *Zebrasoma scopas*): their feeding strategies, and social and mating systems. Envir. Biol. Fish. **4**: 125-170.

**Russ, G.** 1984a. Distribution and abundance of herbivorous grazing fishes in the central Great Barrier Reef. I. Levels of variability across the entire continental shelf. Mar. Ecol. Prog. Ser. **20**: 23-34.

**Russ, G.** 1984b. Distribution and abundance of herbivorous grazing fishes in the central Great Barrier Reef. II. Patterns of zonation of mid-shelf and outershelf reefs. Mar. Ecol. Prog. Ser. **20**: 35-44.

**Sale, P.F.** 1968. Influence of cover availability on depth preference of the juvenile manini, *Acanthurus triostegus sandvicensis*. Copeia **1968**: 802-807.

**Sale, P.F.** 1969. Pertinent stimuli for habitat selection by the juvenile manini, *Acanthurus triostegus sandvicensis*. Copeia **1969**: 616-623.

**Schopf, J.W., and D.Z. Oehler.** 1976. How old are the eukaryotes? Science **193**: 47-49.

**Schreil, W.H.** 1964. Studies on the fixation of artificial and bacterial DNA-plasms for the electron microscopy of thin sections. J. Cell Biol. **22**: 1-20.

**Shulman, M.J.** 1985. Recruitment of coral reef fishes: effects of distribution of predators and shelter. Ecology **66**: 1056-1066.

**Smith, D.C., and A.E. Douglas.** 1987. The biology of symbiosis. Edward Arnold, London.

**Sogin, M.L.** 1989. Evolution of eukaryotic microorganisms and their small subunit ribosomal RNAs. Amer. Zool. **29**: 487-499.

**Stanier, R.Y., J.L. Ingraham, M.L. Wheelis and P.R. Painter.** 1987. General microbiology. 5th ed.. Macmillan, London.

**Starr, M.P, H. Stolp, H.G. Truper, A. Balows and H.G. Schlegel (ed.).** 1981. The Prokaryotes. A handbook on habitats, isolation, and identification of bacteria, Springer-Verlag, Berlin.

**Stevens, C.E.** 1988. Comparative physiology of the vertebrate digestive system. Cambridge University Press, Cambridge.

**Sutton, D.C., and K.D. Clements.** 1989. Aerobic, heterotrophic gastrointestinal

microflora of tropical marine fishes. Proc. Sixth Int. Symp. Coral Reefs, J.H. Choat et al. (ed.), Vol. 3, p. 185-190. Townsville, Australia.

**Taborsky, M., and D. Limberger.** 1980. The activity rhythm of *Blennius sanguinolentus* Pallas, an adaptation to its food source? P.S.Z.N.I. Mar. Ecol. 1: 143-153.

**Thong, K.-W., and G.H. Coombs.** 1987. Comparative study of ferredoxin-linked and oxygen-metabolizing enzymes of trichomonads. Comp. Biochem. Physiol. **87B**: 637-641.

**To, L.P., L. Margulis, D. Chase and W.L. Nutting.** 1980. The symbiotic microbial community of the Sonoran Desert termite: *Pterotermes occidentis*. BioSystems **13**: 109-137.

**Troyer, K.** 1982. Transfer of fermentative microbes between generations in a herbivorous lizard. Science **216**: 540-542.

**Troyer, K.** 1984. Microbes, herbivory and the evolution of social behaviour. J. theor. Biol. **106**: 157-169.

**Tyler, J.C., G.D. Johnson, I. Nakamura and B.B. Collette.** 1989. Morphology of *Luvarus imperialis* (Luvaridae), with a phylogenetic analysis of the Acanthuroidei. Smithsonian Contr. Zool. #485, 78pp.

**Veivers, P.C., R.W. O'Brien and M. Slaytor.** 1983. Selective defaunation of *Mastotermes darwiniensis* and its effect on cellulose and starch metabolism. Insect Biochem. **13**: 95-101.

**Veron, J.E.N.** 1986. Corals of Australia and the Indo-Pacific. Angus and Robertson, Sydney.

**Williams, D.McB.** 1980. Dynamics of the pomacentrid community on small patch reefs in One Tree Lagoon (Great Barrier Reef). Bull. Mar. Sci. **30**: 159-170.

**Williams, D.McB.** 1983. Daily, monthly and yearly variability in recruitment of a guild of coral reef fishes. Mar. Ecol. Prog. Ser. **10**: 231-237.

**Williams, D.McB., and P.F. Sale.** 1981. Spatial and temporal patterns of recruitment of juvenile coral reef fishes to coral habitats within "One Tree Lagoon", Great Barrier Reef. Mar. Biol. **65**: 245-253.

**Williams, D.McB., and A.I. Hatcher.** 1983. Structure of fish communities on outer slopes of inshore, mid-shelf and outer shelf reefs of the Great Barrier Reef. Mar. Ecol. Prog. Ser. **10**: 239-250.

**Wolf, N.G.** 1985. Food selection and resources partitioning by herbivorous fishes in mixed-species groups. Proc. 5th Int. Coral Reef Congr., Tahiti. Vol. 4, p. 23-28.

**Wren, H.N., J.L. Johnson and D.G. Cochran.** 1989. Evolutionary inferences from a comparison of cockroach nuclear DNA and DNA from their fat-body and egg endosymbionts. Evolution **43**: 276-281.

**Woese, C.R.** 1987. Bacterial evolution. Microbiol. Rev. **51**: 221-271.

**Woese, C.R., and G.E. Fox.** 1977. The concept of cellular evolution. J. Mol. Evol. **10**: 1-6.

**Yamin, M.A.** 1980. Cellulose metabolism by the termite flagellate *Trichomitopsis termopsidis*. Appl. Environ. Microbiol. **39**: 859-863.

**Yamin, M.A., and W. Trager.** 1979. Cellulolytic activity of an axenically-cultivated termite flagellate, *Trichomitopsis termopsidis*. J. Gen. Microbiol. **113**: 417-420.

**Zimmerman, L.C., and C.R. Tracy.** 1989. Interactions between the environment and ectothermy and herbivory in reptiles. Physiol. Zool. 62: 374-409.

# APPENDIX 1: HABITAT DESCRIPTIONS OF LIZARD ISLAND SITES

Eighteen of the 49 habitat/locality sites described in Chapter 2 were selected on the basis of the results of the 1987 settlement data (i.e. after each site had been surveyed three times). Habitat/locality sites at which moderate to high acanthurid settlement occurred were examined in detail for percentage composition of substratum cover and the density of territorial pomacentrids and acanthurid juveniles. Both data sets were collected simultaneously between 17/12/87 and 2/1/88 using 30m tape transects.

The method for determining precentage substratum composition was as follows. A 30m tape was randomly placed on the substratum within the habitat area. A premarked 2m aluminium rod was placed perpendicular to the tape at each of ten randomly determined points, 5 on each side of the tape. This gave ten randomly situated (but not oriented) 2m transects within a 30x4m transect. At 20cm intervals along each of these random 2m transects (distances determined by marks on the aluminium rod) the substratum type was assessed and recorded. The point assessed was determined as that directly beneath the appropriate 20cm mark on the rod. Thirteen substratum categories were used:

Sand

Sand rubble - a mixture of sand and fine-grained coral rubble

Turf rubble - coarse-grained, turf-covered coral rubble

Turf algae - turf algae-covered rock

Coralline rock - rock covered with encrusting coralline algae

Sponge

Soft coral

Dead Coral

Encrusting coral - encrusting forms providing little or no shelter

Massive coral - large solid forms providing little cover

Plate coral

Columnar coral - upright forms with widely-spaced branching

Branching coral - finely-branched forms providing a high degree of shelter

Each 30m transect thus yielded 100 substratum points, which converted directly into percent cover. Four replicate 30m transects were surveyed in each of the 18 habitat/locality sites examined.

The densities of herbivorous pomacentrids and acanthurid juveniles were recorded within 2m either side of the same 30m tapes described above. Width of transect was determined using the same 2m rods described above. Transects were surveyed as a series of adjacent 2x2m quadrats, which were then pooled to give the total result for $120m^2$. Fish densities were assessed prior to recording substratum type to reduce diver effects on the fish.

The approximate depths of all 49 habitat/locality sites are as follows: Granite Bluffs inner flat 4-6m, outer flat 5-6m, crest 6-8m, slope 10m, slope base 14-16m; North Point inner flat 1-2m, outer flat 2m, crest 2m, crest base 4-6, slope 6-10m; North Reef inner flat 2m, outer flat 2m, crest 2m, crest base 4-8m, slope 8-17m; Pidgin

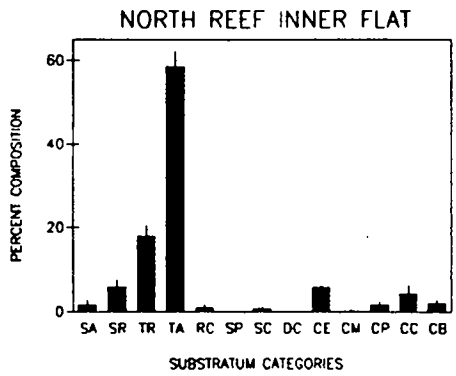
Point inner flat 1m, outer flat 2m, crest 2m, crest base 7-11m, slope 10-15m; Bird Island inner flat 1-2m, outer flat 1-2m, crest 2-3m, crest base 6-12m, slope 12-17m; South Front outer flat 2m, crest 2-3m, crest base 4-7m, slope 6-9m; South Island inner flat 2m, crest 2-3m, crest base 5-12m, slope 10-15m; Lagoon Entrance flat 2m, slope 6m; Lagoon between Palfrey and South flat 2m, slope 5m; Vicky's Reef patch reef 1-3m, patch edge 5-12m; Corner Reef patch reef 2-3m, patch edge 3-4m; Turtle Beach inner flat 2-4m, outer flat 3-4m, crest 4m, slope base 8-10m; Mac's Reef outer flat 2m, crest 2-3m, crest base 5-13m, slope 13-20m.

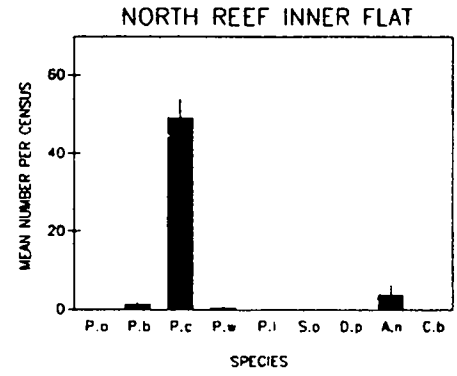
**Fig. A1.1 Substratum composition and density of common pomacentrids and juvenile acanthurids at North Reef census sites.**

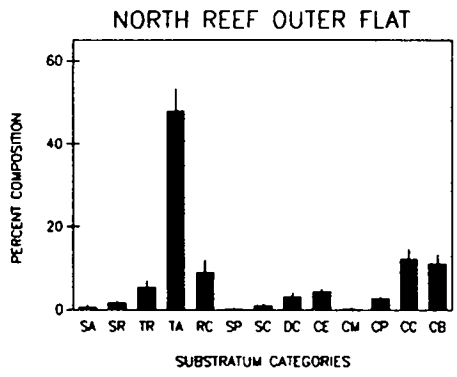
Mean (n=4) percent composition of substratum and mean (n=4) number of individuals per census $\pm$ standard error.
Abbreviations:
Substratum categories: SA = sand, SR = sand rubble, TR = turf rubble, TA = turf algae, RC = coralline rock, SP = sponge, SC = soft coral, DC = dead coral, CE = encrusting coral, CM = massive coral, CP = plate coral, CC = columnar coral, CB = branching coral.
Fish species: P.a = *Pomacentrus amboinensis*, P.b = *P. bankanensis*, P.c = *P. chrysurus*, P.w = *P. wardi*, P.l = *Plectroglyphidodon lachrymatus*, S.a = *Stegastes apicalis*, D.p = *Dischistodus prosopotaenia*, A.n = *Acanthurus nigrofuscus*, C.b = *Ctenochaetus binotatus*.

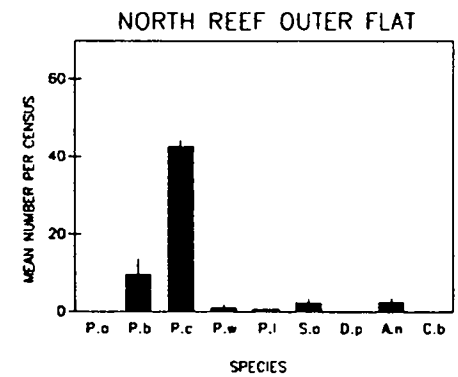
# SUBSTRATUM

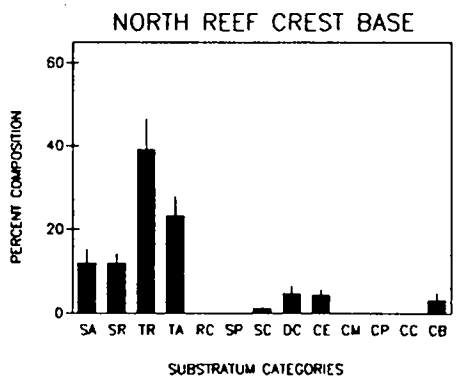
# FISH ABUNDANCE

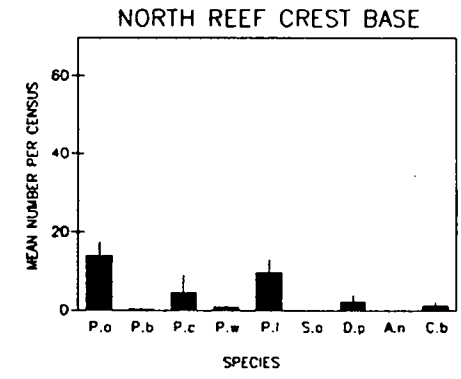


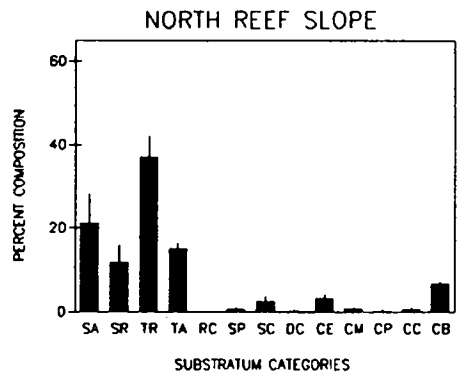



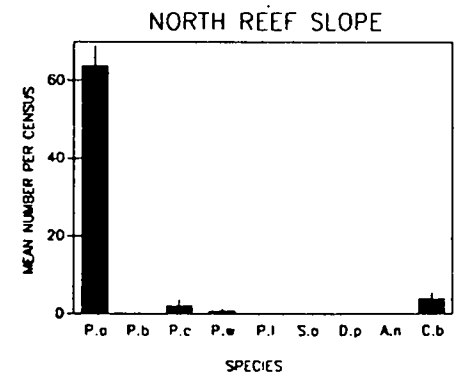
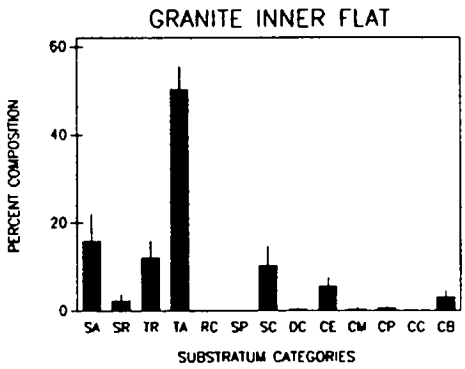
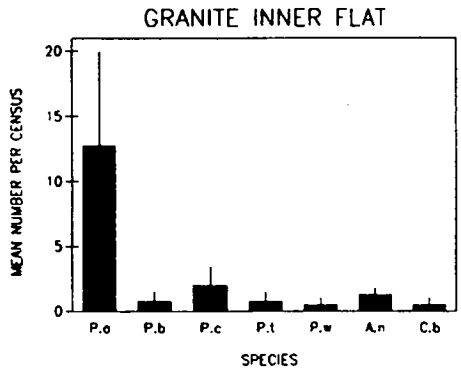



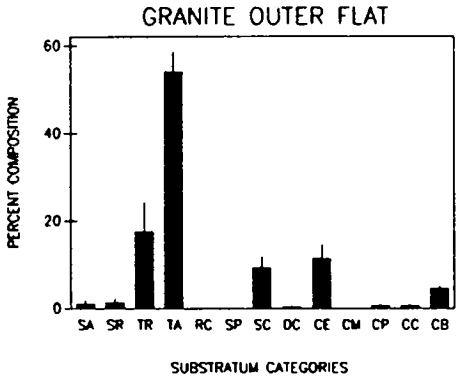
**Fig. A1.2 Substratum composition and density of common pomacentrids and juvenile acanthurids at Granite Bluffs and South Front census sites.**

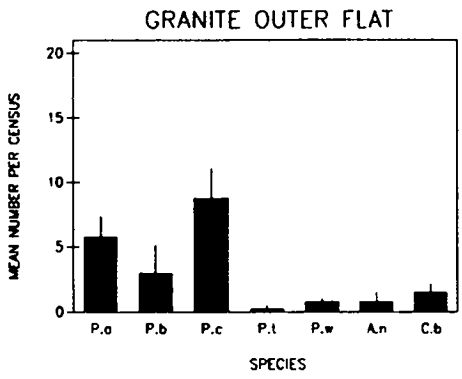
Mean (n=4) percent composition of substratum and mean (n=4) number of individuals per census ± standard error.
Abbreviations:
Substratum categories: SA = sand, SR = sand rubble, TR = turf rubble, TA = turf algae, RC = coralline rock, SP = sponge, SC = soft coral, DC = dead coral, CE = encrusting coral, CM = massive coral, CP = plate coral, CC = columnar coral, CB = branching coral.
Fish species: P.a = *Pomacentrus amboinensis*, P.b = *P. bankanensis*, P.c = *P. chrysurus*, P.t = *P. taeniometopon*, P.w = *P. wardi*, A.n = *Acanthurus nigrofuscus*, C.b = *Ctenochaetus binotatus*.

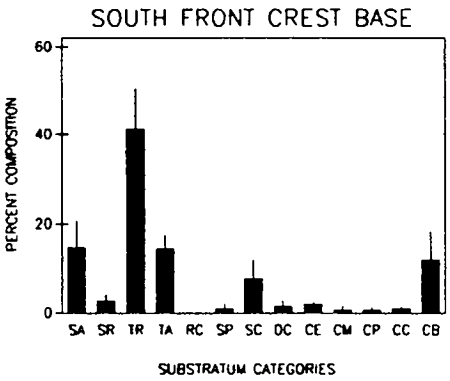
SUBSTRATUM FISH ABUNDANCE

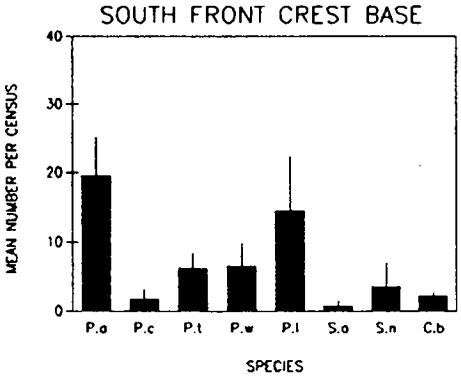


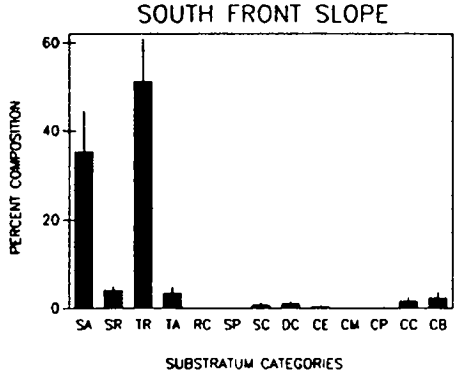



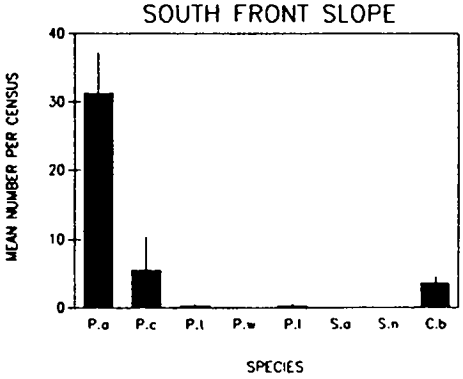
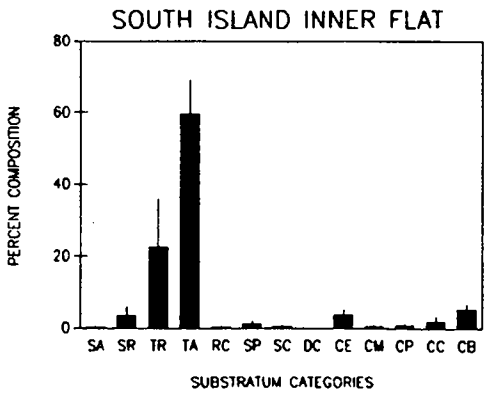
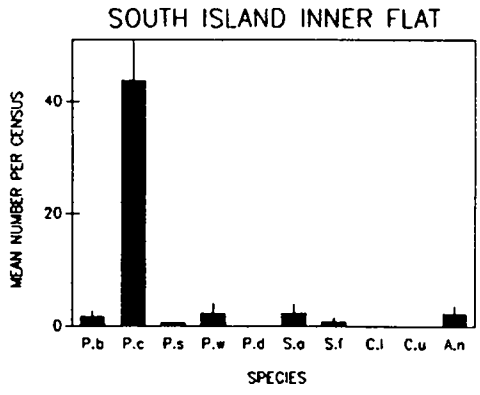



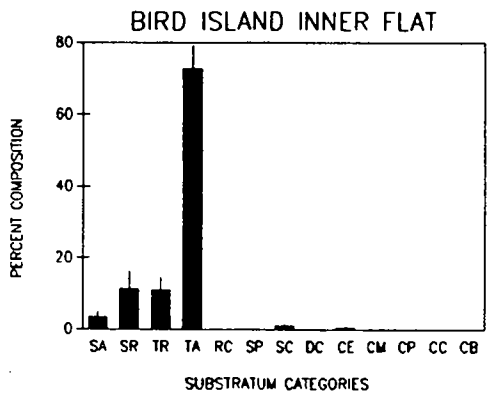
**Fig. A1.3 Substratum composition and density of common pomacentrids and juvenile acanthurids at South Island and Bird Island census sites.**

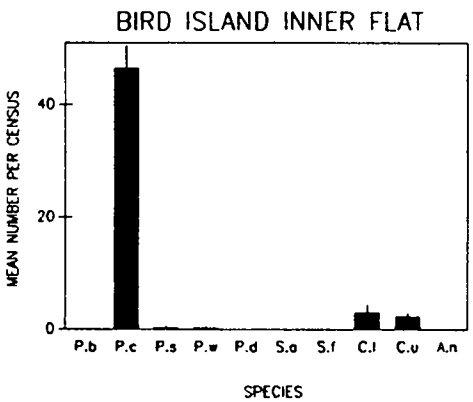
Mean (n=4) percent composition of substratum and mean (n=4) number of individuals per census $\pm$ standard error.
Abbreviations:
Substratum categories: SA = sand, SR = sand rubble, TR = turf rubble, TA = turf algae, RC = coralline rock, SP = sponge, SC = soft coral, DC = dead coral, CE = encrusting coral, CM = massive coral, CP = plate coral, CC = columnar coral, CB = branching coral.
Fish species: P.b = *Pomacentrus bankanensis*, P.c = *P. chrysurus*, P.s = *P. siamsiang*, P.w = *P. wardi*, P.d = *Plectroglyphidodon dickii*, S.a = *Stegastes apicalis*, S.f = *S. fasciolatus*, C.l = *Chrysiptera leucopoma*, C.u = *C. unimaculata*, A.n = *Acanthurus nigrofuscus*.

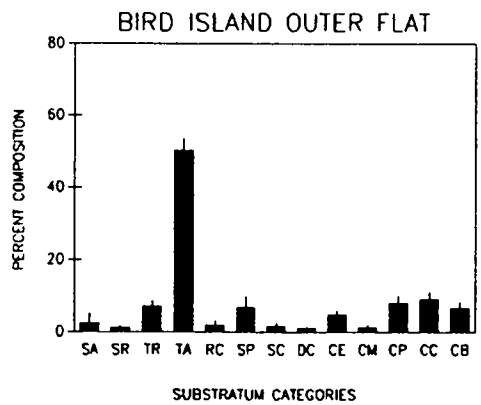
# SUBSTRATUM FISH ABUNDANCE



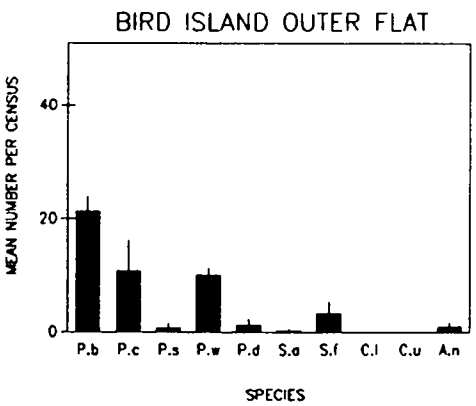
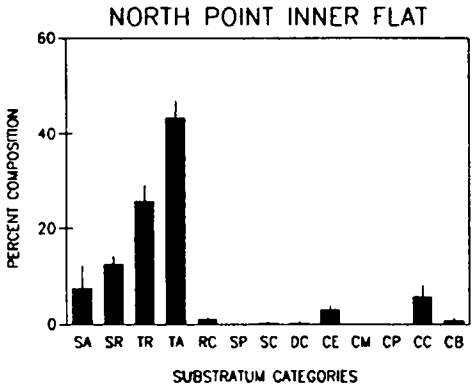
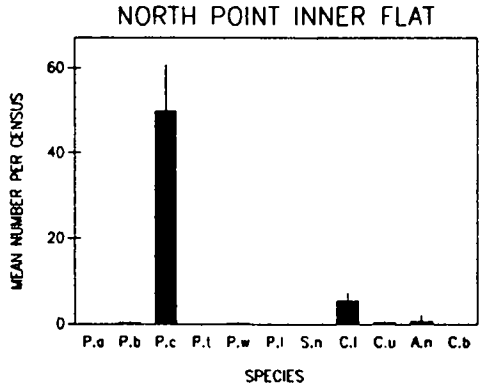



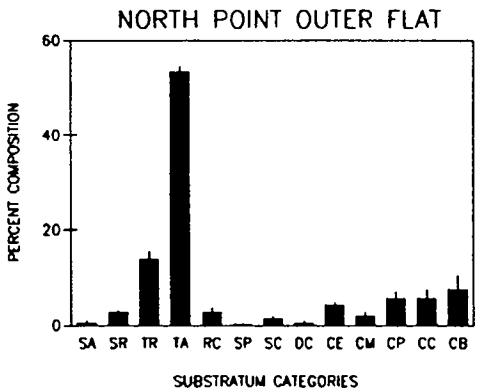
**Fig. A1.4 Substratum composition and density of common pomacentrids and juvenile acanthurids at North Point census sites.**

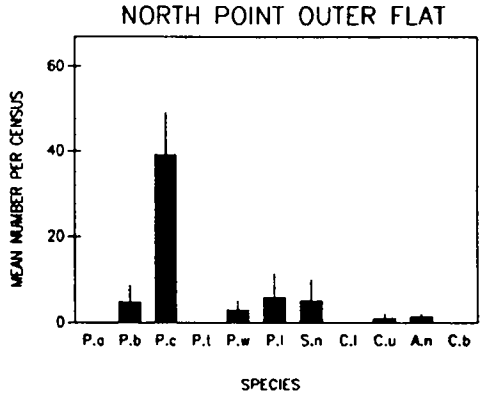
Mean (n=4) percent composition of substratum and mean (n=4) number of individuals per census $\pm$ standard error.
Abbreviations:
Substratum categories: SA = sand, SR = sand rubble, TR = turf rubble, TA = turf algae, RC = coralline rock, SP = sponge, SC = soft coral, DC = dead coral, CE = encrusting coral, CM = massive coral, CP = plate coral, CC = columnar coral, CB = branching coral.
Fish species: P.a = *Pomacentrus amboinensis*, P.b = *P. bankanensis*, P.c = *P. chrysurus*, P.t = *P. taeniometopon*, P.w = *P. wardi*, P.l = *Plectroglyphidodon lachrymatus*, S.n = *Stegastes nigricans*, C.l = *Chrysiptera leucopoma*, C.u = *C. unimaculata*, A.n = *Acanthurus nigrofuscus*, C.b = *Ctenochaetus binotatus*.

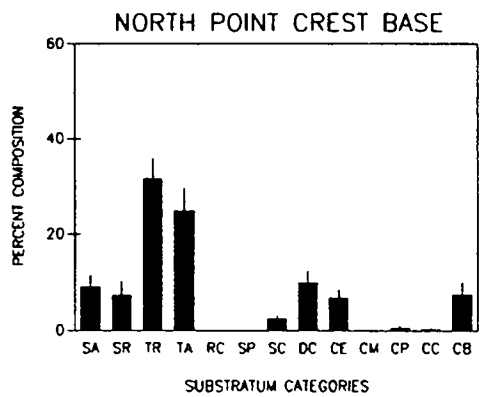
# SUBSTRATUM FISH ABUNDANCE

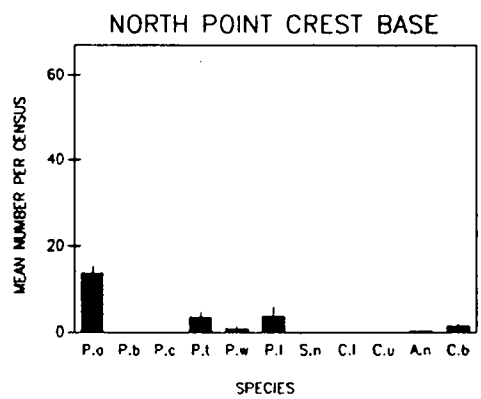


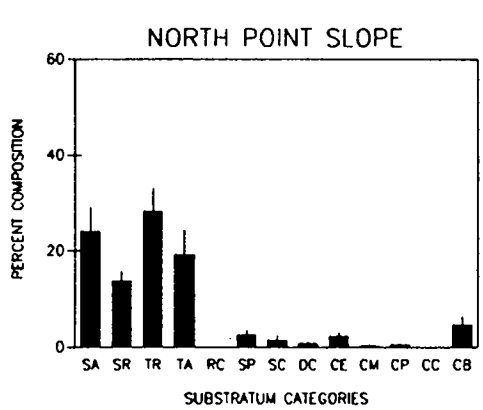



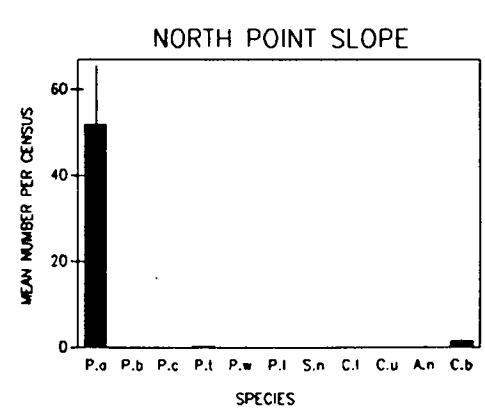
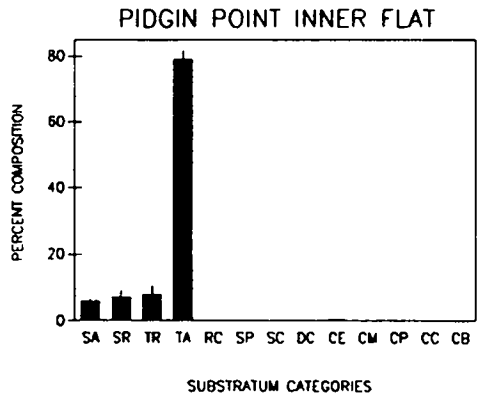
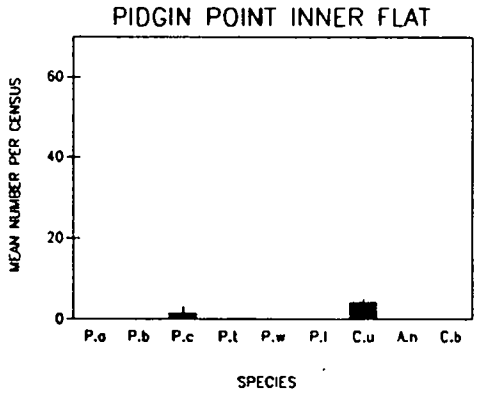



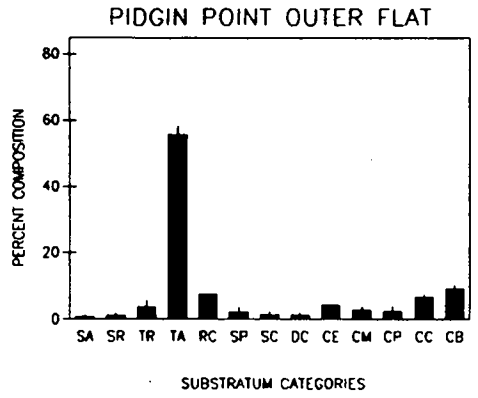
**Fig. A1.5 Substratum composition and density of common pomacentrids and juvenile acanthurids at Pidgin Point census sites.**

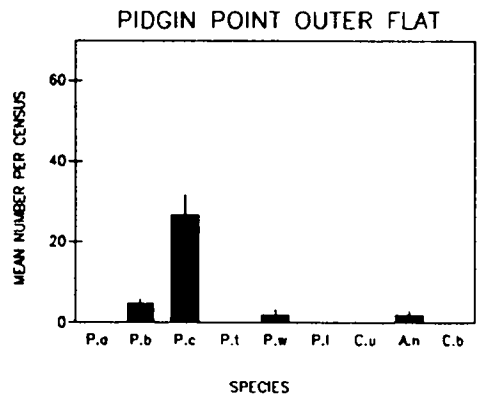
Mean (n=4) percent composition of substratum and mean (n=4) number of individuals per census ± standard error.
Abbreviations:
Substratum categories: SA = sand, SR = sand rubble, TR = turf rubble, TA = turf algae, RC = coralline rock, SP = sponge, SC = soft coral, DC = dead coral, CE = encrusting coral, CM = massive coral, CP = plate coral, CC = columnar coral, CB = branching coral.
Fish species: P.a = *Pomacentrus amboinensis*, P.b = *P. bankanensis*, P.c = *P. chrysurus*, P.t = *P. taeniometopon*, P.w = *P. wardi*, P.l = *Plectroglyphidodon lachrymatus*, C.u = *Chrysiptera unimaculata*, A.n = *Acanthurus nigrofuscus*, C.b = *Ctenochaetus binotatus*.

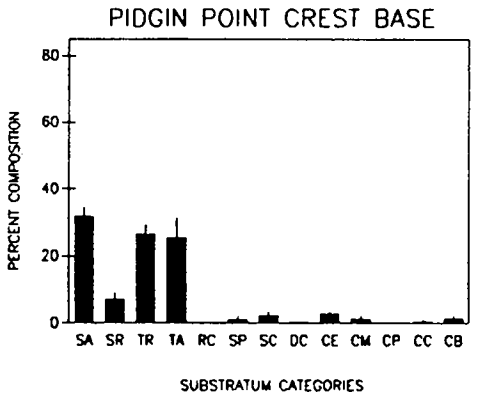
# SUBSTRATUM FISH ABUNDANCE

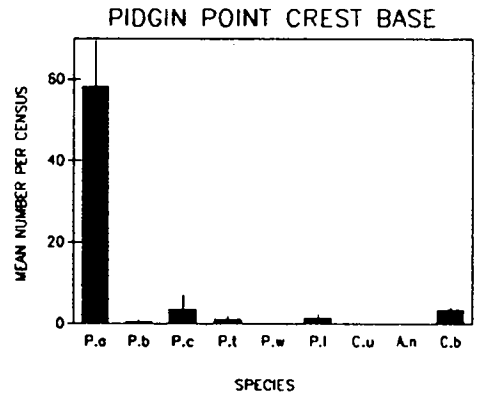


## APPENDIX 2: RELATIVE ABUNDANCE OF *Acanthurus auranticavus* AND *A. blochii*

*Acanthurus auranticavus* and *A. blochii* were not differentiated during the adult censuses conducted at Lizard Island in the 1987/88 summer (see Chapter 1). To determine the relative abundances of these species at Lizard Island, 4 dives were conducted at sites where both species had been observed. The procedure used was to swim for 30 minutes along the reef slope and then return to the start point along the reef flat. During this period all individuals of either species observed were positively identified and recorded. The 4 sites thus sampled were North Reef, Pidgin Point, Bird Island, and a site approximately 500m east of the usual North Reef site (see Chapter 1). The ratio of *A. auranticavus* to *A. blochii* at each of these sites was as follows (- indicates *A. auranticavus* not observed, *A. blochii* were present at all sites):

| | slope | flat |
|---|---|---|
| North Reef | - | 0.14 |
| Pidgin Point | - | 0.21 |
| Bird Island | - | 0.20 |
| east North Reef | - | - |

Clearly, *A. blochii* is by far the more common of the two species. *A. auranticavus* was never observed on the reef slope, and was more common on exposed rather than oblique reefs.

## APPENDIX 3: MEANS (± SE) OF JUVENILE BEHAVIOUR DATA

**Table A3.1** **Means (±) SE (N=10) of Juvenile *Acanthurus nigrofuscus* Bite Rate Study.**

| | TIME PERIOD | | | |
|---|---|---|---|---|
| LOCATION/SITE | 0600-0900 | 0900-1200 | 1200-1500 | 1500-1800 |
| GRANITE I | 205.0±42.02 | 347.4±18.45 | 445.1±26.87 | 381.5±28.02 |
| GRANITE II | 226.1±43.31 | 433.7±20.08 | 464.8±32.49 | 398.1±21.98 |
| NORTH I | 136.3±25.60 | 302.6±17.40 | 352.3±20.39 | 385.0±20.64 |
| NORTH II | 188.8±41.81 | 362.7±24.80 | 439.0±30.54 | 485.2±30.14 |
| PIDGIN I | 235.0±52.36 | 297.7±44.64 | 415.4±37.10 | 408.6±35.72 |
| PIDGIN II | 218.0±34.81 | 313.1±16.06 | 438.3±39.80 | 410.5±41.98 |

**Table A3.2** **Means (±) SE (N=10) of Juvenile *Ctenochaetus striatus* Bite Rate Study**

| | TIME PERIOD | | | |
|---|---|---|---|---|
| LOCATION | 0600-0900 | 0900-1200 | 1200-1500 | 1500-1800 |
| GRANITE | 70.8±30.94 | 244.6±23.33 | 336.2±22.49 | 365.8±35.04 |
| NORTH | 57.9±29.11 | 293.1±19.26 | 332.7±26.40 | 381.9±11.69 |
| PIDGIN | 92.2±29.67 | 318.1±31.50 | 332.5±17.91 | 360.4±21.70 |

APPENDIX 3 CONT.

Table A3.3 Means (±) SE (N=10) of Juvenile *Acanthurus nigrofuscus* Activity Range Study.

| | TIME PERIOD | | | |
|---|---|---|---|---|
| LOCATION/SITE | 0600-0900 | 0900-1200 | 1200-1500 | 1500-1800 |
| GRANITE I | 5.38±1.00 | 5.40±1.16 | 5.05±0.88 | 6.85±1.51 |
| GRANITE II | 8.08±1.69 | 8.78±1.89 | 7.10±1.56 | 5.58±1.00 |
| NORTH I | 2.63±0.81 | 1.68±0.20 | 1.43±0.16 | 2.58±0.51 |
| NORTH II | 7.65±1.60 | 3.40±0.71 | 3.68±0.87 | 3.93±0.87 |
| PIDGIN I | 6.33±2.13 | 2.85±0.56 | 4.33±1.58 | 4.55±1.32 |
| PIDGIN II | 6.58±2.67 | 2.90±0.86 | 3.38±0.66 | 4.15±1.76 |

Table A3.4 Means (±) SE (N=10) of Juvenile *Ctenochaetus striatus* Activity Range Study.

| | TIME PERIOD | | | |
|---|---|---|---|---|
| LOCATION | 0600-0900 | 0900-1200 | 1200-1500 | 1500-1800 |
| GRANITE | 13.30±4.33 | 8.90±2.22 | 8.70±1.57 | 11.30±5.07 |
| NORTH | 3.60±1.01 | 2.50±0.40 | 3.80±0.74 | 8.50±2.13 |
| PIDGIN | 10.60±4.20 | 2.60±0.54 | 3.80±0.71 | 2.70±0.90 |

**Table A3.5** **$LOG_{10}$ Transformed Means (±) SE (N=10) of Juvenile *Acanthurus nigrofuscus* Activity Range Study.**

| | TIME PERIOD | | | |
|---|---|---|---|---|
| LOCATION/SITE | 0600-0900 | 0900-1200 | 1200-1500 | 1500-1800 |
| GRANITE I | 1.38±0.34 | 1.53±0.19 | 1.44±0.22 | 1.71±0.22 |
| GRANITE II | 1.81±0.28 | 2.01±0.18 | 1.76±0.21 | 1.56±0.20 |
| NORTH I | 0.56±0.31 | 0.45±0.12 | 0.53±0.26 | 0.74±0.23 |
| NORTH II | 1.78±0.25 | 1.01±0.22 | 1.06±0.23 | 1.17±0.20 |
| PIDGIN I | 1.28±0.36 | 0.90±0.18 | 1.12±0.24 | 1.20±0.26 |
| PIDGIN II | 1.48±0.26 | 0.77±0.23 | 0.96±0.29 | 0.80±0.37 |

**Table A3.6** **$LOG_{10}$ Transformed Means (±) SE (N=10) of Juvenile *Ctenochaetus striatus* Activity Range Study.**

| | TIME PERIOD | | | |
|---|---|---|---|---|
| LOCATION | 0600-0900 | 0900-1200 | 1200-1500 | 1500-1800 |
| GRANITE | 2.29±0.24 | 1.90±0.26 | 2.00±0.20 | 1.71±0.38 |
| NORTH | 1.01±0.24 | 0.77±0.19 | 1.12±0.23 | 1.83±0.28 |
| PIDGIN | 1.81±0.33 | 0.75±0.22 | 1.16±0.20 | 0.65±0.26 |

# APPENDIX 4: RELEVANT PUBLICATIONS

Proceedings of the 6th International Coral Reef Symposium, Australia, 1988, Vol. 3

# AEROBIC, HETEROTROPHIC GASTROINTESTINAL MICROFLORA OF TROPICAL MARINE FISHES

D.C. Sutton[1] and K.D. Clements[2]

[1]Sir George Fisher Centre for Tropical Marine Studies, James Cook University, Townsville, Queensland 4811, Australia
[2]Dept of Marine Biology, James Cook University, Townsville, Queensland 4811, Australia

Content has been removed
due to copyright restrictions

Content has been removed
due to copyright restrictions

Content has been removed
due to copyright restrictions

Marine Biology 102, 403–412 (1989)

Marine Biology


# Occurrence and characteristics of unusual protistan symbionts from surgeonfishes (Acanthuridae) of the Great Barrier Reef, Australia

K. D. Clements [1], D. C. Sutton [2] and J. H. Choat [1]

[1] Department of Marine Biology, James Cook University of North Queensland, Townsville, Queensland 4811, Australia
[2] Sir George Fisher Centre for Tropical Marine Studies, James Cook University of North Queensland, Townsville, Queensland 4811, Australia

Content has been removed
due to copyright restrictions

Content has been removed
due to copyright restrictions

Content has been removed
due to copyright restrictions

Content has been removed
due to copyright restrictions

# PRELIMINARY RECORDS OF THE CORAL REEF FISHES OF TUVALU

G.P. Jones and U.L. Kaly

Department of Zoology
University of Auckland, Private Bag, Auckland
New Zealand.

and

K. Clements

Department of Marine Biology
James Cook University, Townsville 4811
Australia.



*S. Pac. J. Nat. Sci.*, 1991, 11:40-57

Content has been removed
due to copyright restrictions

Content has been removed
due to copyright restrictions

Content has been removed
due to copyright restrictions

Content has been removed
due to copyright restrictions

# The endosymbiotic communities of two herbivorous labroid fishes, *Odax cyanomelas* (Richardson) and *O. pullus* Schneider.

**K.D. Clements**
**Department of Marine Biology**
**James Cook University, Townsville,**
**Qld 4811, Australia.**

Marine Biology, In Press.

**Abstract**

Two herbivorous species of the temperate labroid fish family Odacidae were examined for the presence of gut endosymbionts. *Odax cyanomelas* from southern Australia and *O. pullus* from New Zealand both feed on macroalgae, yet appear to lack obvious morphological specializations for herbivory. Both species were found to harbour dense concentrations of prokaryotic and eukaryotic microbes in their lower intestines. The various cell types present were examined by light microscopy, scanning electron microscopy and transmission electron microscopy. Epifluoresence microscope counts were used to quantify the distribution and abundance of the microbiota along the gut of *O. cyanomelas*. Major differences were observed in the composition of microbiota between the two species. *O. cyanomelas* contained spirilla, large rod-shaped bacteria, filamentous bacteria and two forms of trichomonad flagellates. *O. pullus* also harboured dense large rod-shaped bacterial populations, but lacked the other two large prokaryote categories found in *O. cyanomelas*, and contained diplomonad flagellates. The large rod-shaped bacteria found in both species resembled prokaryotes described from other herbivorous fish and termites.