PARASITES IN THE CLEANING INTERACTIONS BETWEEN *LABROIDES DIMIDIATUS* AND FISH

by

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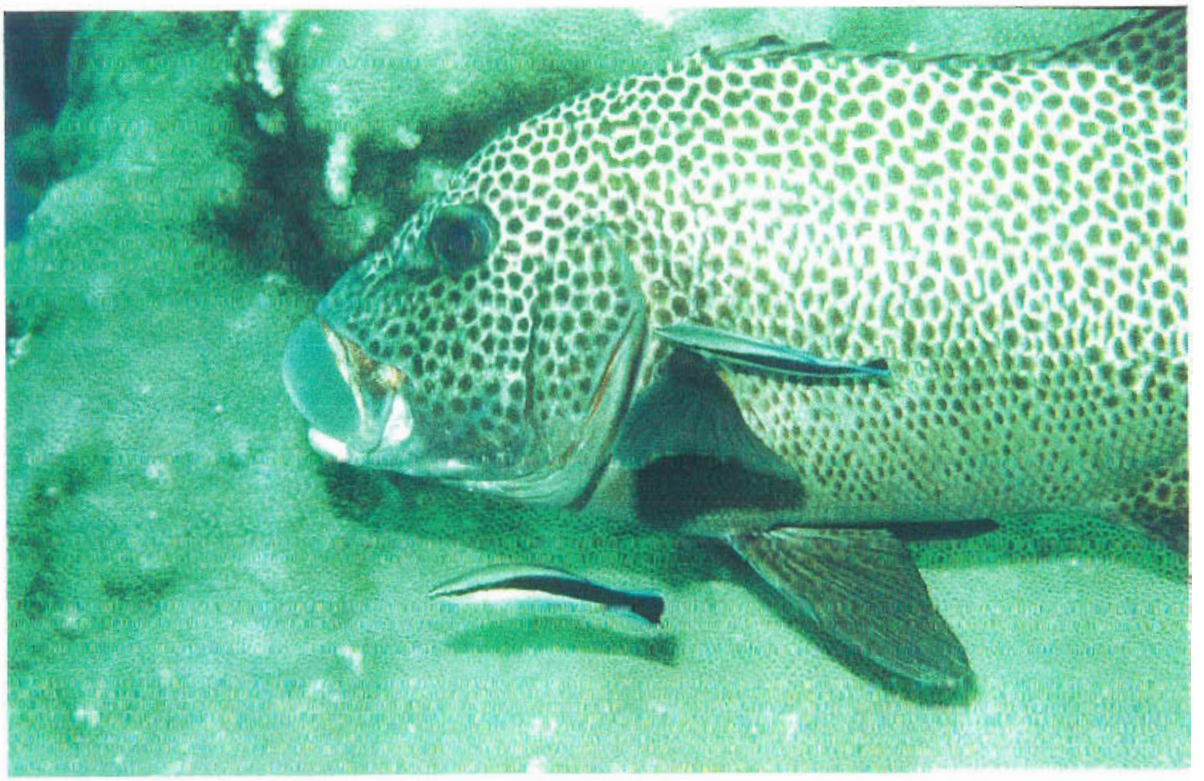
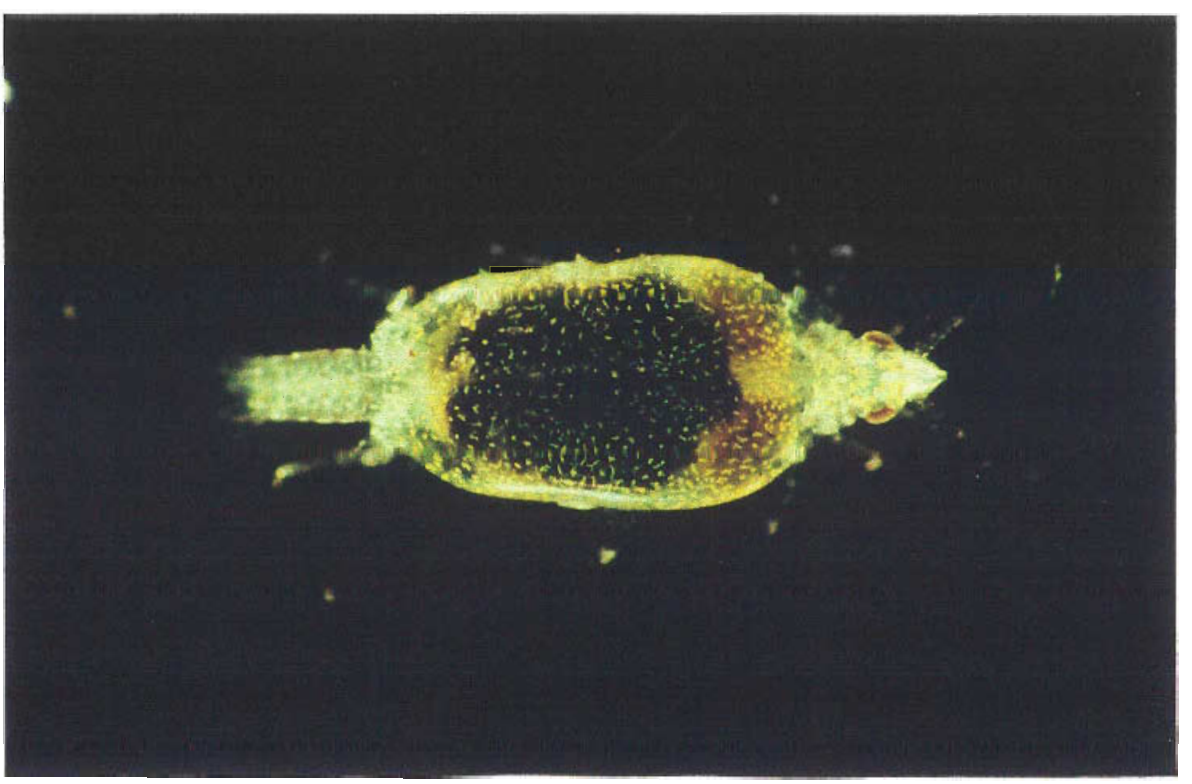


The cleaner wrasse *Labroides dimidiatus* inspecting the gill opening of a *Plectrorhinchus chaetodontoides*. Another *L. dimidiatus* is below *P. chaetodontoides*. (Photo: Mark A. Johnson)



Unidentified *Gnathia* sp. larva with developed stomach (TL=1.47mm)

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2/11/94

Abstract

Parasites are removed from host fish by cleaner fish but their significance in cleaning interactions is still not well understood. This study investigated the significance of parasites in cleaning interactions between the cleaner wrasse *Labroides dimidiatus* and its host fish at Lizard Island and Heron Island, Great Barrier Reef, Australia. Detailed information on the external parasite assemblages of several fish species, *L. dimidiatus* diet analyses and parasite removal rates, host cleaning rates (how often individual host fish are cleaned by *L. dimidiatus*), and an experiment involving the removal of all cleaner fish from reefs were used.

Host fish had species-specific parasite assemblages which were consitent among localities and time. These parasite assemblages were diverse and included copepods, isopods, monogeneans, digeneans, turbellaria, and unidentified platyhelminths. Host species identity explained most of the variation in the composition of these parasites, while host size was of secondary importance. Patterns of parasite abundance among fish species were similar between widely separated locations although the northern location (Lizard Island) had more species of parasites.

Labroides dimidiatus fed largely on crustaceans, similar to the feeding behaviour of most tropical labrids. However, they selectively fed on parasitic crustaceans rather than benthic crustaceans. Gnathiid isopod larvae were the most abundant crustaceans in the diet. At Lizard Island they selectively fed on larger gnathiids while at Heron Island their diet included fewer gnathiids but more mucus and benthic copepods. The number, size, and biomass of gnathiids at Lizard Island varied temporally with a greater proportion of small gnathiids and less biomass during the austral summer. Variability in the diet suggests both spatial and temporal flexibility in the foraging habits of *L. dimidiatus*.

Host cleaning rates were estimated for 11 fish species by following individuals and recording the number of times and duration that they were inspected by *Labroides dimidiatus*. Individuals of *Siganus doliatus* were cleaned the most and spent an estimated 32 minutes per day being cleaned.

Host cleaning rates were positively correlated with the parasite load and surface area of the host. However, surface area explained slightly more of the variation in cleaning rates. This may be because cleaner fish use size of fish as an indicator of food availability. In the fish species *Hemigymnus melapterus*, larger fish had more parasites and were cleaned more often and for a longer duration. The finding that larger fish with more parasites are cleaned more suggests that size and parasites play an important part in the cleaning behaviour of host fish.

The rate at which parasites (mainly gnathiids) were removed from host fish by *Labroides dimidiatus* was investigated. To examine the effect of parasite removal on parasites, the number of parasites removed per individual *Hemigymnus melapterus* per day was estimated and compared to the infection rate and abundnace of gnathiids on *H. melapterus*. Observations of cleaner fish feeding rates, estimates of host cleaning rates, stomach content analyses, and an experimental manipulation of gnathiid abundances on fish were used. *Labroides dimidiatus* inspected an estimated 2297 (±SE 83) fish per day and ate large numbers of parasites (mainly gnathiid isopods) each day (1218 ±SE 118). The estimated predation rate by *L. dimidiatus* was 4.8 (±SE 0.4) parasites per minute of inspection or 0.5 (±SE 0.05) parasites per fish inspected. However, the infection rate of gnathiids onto fish was high with reduced gnathiid loads (about 50%) on fish returning to levels similar to control fish within 1-6 days. These infection rates suggest that a significant proportion of gnathiids removed by cleaner fish are quickly replaced. However, the estimated number of gnathiids removed per *H. melapterus* per day by *L. dimidiatus* was 61 (±SE 5) which was over 5 times the standing crop of gnathiids on *H. melapterus* (11 ±SE 3). Such a high predation rate relative to the number of gnathiids on fish and their infection rates onto fish, implies that cleaner fish may have an effect on the abundance of gnathiids on fish. However to what extent gnathiid abundances are suppresed is unclear.

An experimental evaluation of the effect of *Labroides dimidiatus* on the fish *Pomacentrus moluccensis* was done by removing all *L. dimidiatus* from several reefs for 6 months. The subsequent effect on parasites (total number, number per taxonomic category of parasite, and size of parasite) and host fish abundance was estimated and compared to control reefs with *L. dimidiatus*.

This was the first time this experiment has been conducted in an area where *L. dimidiatus* has high densities of parasites in its diet. The absence of *L. dimidiatus* had no effect on total parasite abundance per fish, number per category of parasite per fish, and size of the most abundant copepod per fish. The abundance of *P. moluccensis* also did not differ among reefs with and without *L. dimidiatus*. Thus *P. moluccensis* did not leave reefs to seek cleaning elsewhere nor suffer increased mortality in the absence of *L. dimidiatus*. The absence of an effect of *L. dimidiatus* on the parasites of *P. moluccensis* is compatible with *L. dimidiatus* foraging behaviour as *L. dimidiatus* selectively fed on larger gnathiids not present on *P. moluccensis*. Why fish, such as *P. moluccensis,* which do not benefit from cleaning, seek cleaning may be due to factors other than ectoparasite removal, such as tactile stimuli provided by cleaners.

This study suggests that cleaner fish foraging patterns determine the effect cleaner fish have on parasites. Thus, although cleaning behaviour may be driven by tactile stimuli provided by cleaners, the effect of cleaners on hosts may vary according to the foraging patterns of cleaner fish and the parasite loads of hosts.

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CHAPTER I

GENERAL INTRODUCTION

1.1 OVERVIEW

Cleaning behaviour is an interaction between cleaners and hosts in which parasites and other material are removed from hosts by the cleaner. The behaviour is widespread in aquatic environments. Cleaners are generally fish or crustaceans while hosts are mainly fish. Cleaner fish have been recorded in freshwater systems (Spall 1970, Abel 1971, Wyman and Ward 1962) and in marine temperate (McCutcheon and McCutcheon 1964, Ayling and Grace 1971, Hobson 1971, Potts 1973a) and tropical systems (Feder 1966, Potts 1973b). Fish of the genus *Labroides*, of which there are five species, are the most common cleaner fish in tropical waters (Randall 1958, Randall *et al.* 1990). Although it is generally assumed that parasites are the targets for cleaning associations, the evidence is conflicting and the extent to which their abundance motivates cleaning is still not understood. This study examines the parasite assemblages on tropical reef fishes and whether cleaning by *Labroides dimidiatus* influences this assemblage.

A study of the importance of 'cleaning' to the diet of cleaners, the fish being cleaned, and its parasite assemblage requires an approach involving information on parasite assemblages of host fish and the rates and processes involved in their removal. By comparing cleaner fish diet analyses to host parasite assemblages, information on cleaner fish feeding selectivity is obtained. Analyses of the diet of cleaner fish also provide information on the contribution of parasites to the total diet. Examining the relationship between host cleaning behaviour and parasites provides insight into the significance of parasites in host cleaning behaviour. The cleaner fish feeding rates combined with diet analyses provide an estimate of the rate at which parasites are

removed from fish by cleaner fish. When these parasite removal rates are combined with parasite infection rates an estimate of the impact of cleaner fish on parasites is obtained. Finally, the effect of cleaner fish on parasites can also be evaluated by removing all cleaner fish from reefs and examining the subsequent effect on the parasites of fish. This study therefore used a combination of analyses of parasites on fish, cleaner fish diet analyses, host cleaning rates, cleaner fish feeding behaviour rates, a parasite manipulation experiment, and a cleaner fish removal experiment to investigate the significance of parasites in cleaning interactions.

1.2 ECTOPARASITES OF FISH

Parasitological information in cleaning studies is sparse. Behavioural studies on the effect of parasites on the feeding behaviour of the cleaner fish have shown that cleaner fish prefer fish with ectoparasites (Gorlick *et al.* 1984). The effect of parasites loads on host cleaning behaviour is conflicting (Losey 1971, 1979). While ectoparasite densities amplified the response of one host fish species towards cleaner fish and had no effect on the other species, tactile stimuli provided by cleaners had a very strong effect on host responses towards cleaners. Chikasue (1990) showed that host responses towards cleaners were stronger when parasite loads were higher. Cleaner fish removal experiments have also used parasite information ranging from "approximations of numbers of parasites" (Youngbluth 1968) to progressively more detailed counts of parasites (Losey 1972, Gorlick *et al.* 1987). Due to the paucity of studies of the cleaning procedure that have quantified parasites, the importance of parasite numbers and taxonomic composition remains unclear.

One of the reasons that parasitological information has rarely been collected in studies of cleaning interactions is because of the problems associated with sampling ectoparasites and identifying parasites. In addition, confined (laboratory) conditions of host fish often exasperate parasite

infections, producing a marked increase in parasite loads. Sampling procedures which are quick and reliable as well as studies involving natural parasite loads are needed for the study of parasites in cleaning interactions.

1.3 THE DIET OF CLEANER FISH

Cleaner fish are well documented to eat ectoparasites (Randall 1958, Strasburg 1959, Cressey and Lachner 1970, Böhlke and McCosker 1973, Potts 1973b). However, the contribution of parasites to the total diet of cleaner fish is still unclear. Although the diet of the genus *Labroides* has been examined (Randall 1958), there are only two detailed quantitative studies, one on *L. phthirophagus* (Youngbluth 1968), and one *L. dimidiatus* (Chikasue 1990). These, however, are not of sufficient detail to determine whether parasites are the most important items in the diet of these cleaner fish. There is also little information on how the diet varies temporally and spatially and how this relates to parasite loads of host fishes.

Work in Hawaii has suggested that mucus may be an important part of the diet of cleaner fish and that cleaner fish may feed on mucus when parasite loads are low (Gorlick 1980). The implication of these findings is that cleaner fish may, at times, be parasitic on their hosts (Gorlick 1980). The quantity and caloric value of mucus on hosts also influences what hosts cleaner fish prefer (Gorlick 1984). However, despite the importance of mucus in the diet of cleaner fish, the proportion of mucus in the diet of cleaner fish is unknown, as no diet analyses have quantified mucus. Diet analyses which quantify mucus, in addition to parasites and other materials, are needed to resolve this problem.

1.4 PARASITES AND HOST CLEANING

Cleaning interactions involve a series of behaviours which can be

influenced by the cleaner or host fish (Losey 1971). Host fish either approach cleaner fish or are approached by cleaners, hosts then respond to cleaners usually by "posing" with their fins extended and mouth open. Cleaners then "inspect" hosts by slowly swimming around the fish, often contacting it with its body, and using the body surfaces of fish as a substrate to feed from (Losey 1971). Information on how parasites influence the outcomes of these interactions is needed to understand the procedures involved in cleaning behaviour.

To address the question of the significance of parasites in cleaning interactions, the relationship between how often host fish are cleaned by *Labroides dimidiatus* and how many parasites hosts have has been examined. It has been suggested that larger fish are cleaned more often than smaller fish (Poulin 1993). However Poulin's study was based on the feeding rates of cleaners, rather than on how much host fish were cleaned, and no evidence on parasite numbers was supplied. The effect of parasite load on the responses of hosts towards cleaners is conflicting. Losey (1979) found that a host fish species showed more response to cleaners when it had parasites than when it had no parasites, however for another host fish species its response toward cleaners was the same regardless of parasite load. A study that correlates how often individual fish are cleaned (host cleaning rates) to their parasite loads will provide information on the importance of parasites in cleaning interactions.

Although removal of ectoparasites is largely assumed to be the ultimate cause of the behaviour, there is little evidence to support this. Several quantitative tests have been made to determine the effect of the absence of cleaner fish on the parasites and abundance of hosts (Youngbluth 1968, Losey 1972, Gorlick *et al.* 1987). Only Gorlick *et al.* (1987) found an effect in the form of larger parasites on fish without cleaners. However, whether hosts benefited from having a reduced biomass of parasites could not be determined. These experiments were done in Hawaii and at Enewetak Atoll where the

abundance of parasites in the diet of cleaner fish is relatively low (Youngbluth 1968, Losey pers. comm.). Losey (1987) suggested a removal experiment is needed in a different ecological setting where the removal of parasites may be more important to host fish. Such an experimental approach may provide insight into whether the ultimate cause of cleaning for hosts is ectoparasite removal or whether other factors, such as tactile stimuli provided by cleaners (Losey and Margules 1974, Losey 1977, 1979) are the cause of cleaning in hosts.

1.5 THESIS OUTLINE

This study examined the dynamics of the interactions among cleaner fish, host fish, and parasites using a quantitative approach. The cleaner wrasse *Labroides dimidiatus* was used in this study as its social behaviour and life history is well documented (Robertson 1974) and it is widely distributed (Randall 1958). Information on the parasite loads of fish serves as the foundation from which to interpret the significance of parasites in cleaning interactions. Because there is some evidence that the ecological role of cleaning varies geographically (Losey 1974), this study was conducted at two locations separated by 1000 km (Lizard Island and Heron Island, Great Barrier Reef, Australia).

This thesis is organised so that each data chapter is a complete work united by a common question. A summary is given at the start of each data chapter. The thesis consists of 8 chapters, 6 of these chapters (2-6) represent submitted papers, four of which are in press (Appendix I).

Chapter 2. A comparison of methods for sampling ectoparasites from coral reef fishes

This chapter addresses problems in sampling external parasites and develops a general method to reduce biases.

Chapter 3. Spatial and temporal variations of the ectoparasites of seven reef fish species from Lizard Island and Heron Island

The number and species composition of external parasites of seven fish species was examined at Lizard Island and Heron Island. This parasite information is used in interpreting interactions between cleaner fish and host fish.

Chapter 4. The diet of Labroides dimidiatus

Spatial variation in the diet of *Labroides dimidiatus* was examined among sites at two locations (Lizard Island and Heron Island) and between the two locations. Temporal variation in the diet was investigated at several month intervals. To determine whether cleaner fish selectively feed on larger parasites, the size of the most abundant parasite in the diet of *L. dimidiatus* was compared to the size of that parasite on the host fish species *Hemigymnus melapterus*.

Chapter 5. The relationship between cleaning rates and ectoparasite loads in coral reef fishes

The rate at which individual fish were cleaned by *Labroides dimidiatus* was quantified for 11 species. The duration and frequency of these cleaning events were compared to the mean parasite load and mean surface area of each species. To examine whether these cleaning rates varied within a fish species, the cleaning rates of a range of sizes of the host fish species *Hemigymnus melapterus* were estimated.

Chapter 6. Parasite removal rates by Labroides dimidiatus

In order to determine whether *Labroides dimidiatus* influence parasite abundance, the number of parasites removed (mainly gnathiids) by cleaner fish per unit time of cleaning was estimated and compared to gnathiid infection

rates.

Chapter 7. An experimental evaluation of the effect of Labroides dimidiatus on the fish Pomacentrus moluccensis

The effect of *Labroides dimidiatus* on the fish *Pomacentrus moluccensis* was examined by excluding all cleaner fish from several reefs for 6 months. The subsequent effect on the total number and species composition of external parasites, the size of the most abundant copepod, and the abundance *Pomacentrus moluccensis* among reefs with and without cleaners was examined.

CHAPTER II

A COMPARISON OF METHODS FOR SAMPLING ECTOPARASITES FROM CORAL REEF FISHES

2.1 SUMMARY

Methods for sampling ectoparasite assemblages were compared using 7 species of coral reef fishes (*Acanthochromis polyacanthus*, *Thalassoma lunare*, *Ctenochaetus striatus*, *Chlorurus sordidus*, *Scolopsis bilineatus*, *Hemigymnus melapterus*, and *Siganus doliatus*). Estimates of total numbers and composition of ectoparasites were dependent on post-collection handling techniques, and the method of ectoparasite removal. The following steps were used to obtain accurate estimates of parasite loads. Fish were enclosed within plastic bags underwater at the point of capture. Filtration of water from the plastic bags revealed a large number of parasites (mainly gnathiid isopods) which had detached from the host on capture. A subsequent seawater rinse removed a large number of ectoparasites but further treatment with the anaesthetic chloretone released additional individuals. A small number of parasites remained on fish after all treatments and were removed by visual inspection. A chloretone bath was more effective than a saltwater bath at removing parasites. The species composition of parasites recovered by a chloretone bath plus a visual survey was different to that recovered with a seawater bath and visual survey which suggests traditional scanning techniques may not detect all parasites.

2.2 INTRODUCTION

Accurate estimates of assemblages of parasites on fish are crucial to understanding the role of parasites in cleaning interactions. Parasites of fishes are also increasingly being used as tools for analysing host biogeography (Byrnes and Rohde 1992), host evolutionary relationships (Brooks and McLennon 1993), for fish stock discrimination (MacKenzie 1983, Lester *et al.* 1988, Lester 1990), and to validate host demography studies (Lester *et al.* 1985). However, recent work has cast doubt on the reliability of traditional fish parasite surveys

(Williams *et al.* 1991). There is also much variation in the protocols for sampling parasites which presents a problem for comparative studies as differences due to sampling methods can confound real biological differences in parasite loads.

Parasite sampling programs generally follow three steps which vary widely: host collection (Rhode and Roubal 1980, Yeo and Spieler 1980, Nagasawa 1985, Silan and Maillard 1989, Koskivaara *et al.* 1991, Whittington and Kearn 1993), post-collection handling (Collins 1984 , Silan and Maillard 1989, Byrnes and Rohde 1992) and parasite removal and quantification (Byrnes and Rohde 1992, Cowell *et al.* 1993, Whittington and Kearn 1993). With such a large number of different methods, the potential for methodological bias is high.

A few recent studies suggest that traditional methods used to quantify parasites may underestimate internal and external parasite loads with parasite losses occurring during the collection of host (Nagasawa 1985, Williams *et al.* 1991), in post-collection handling (Möller 1976, Hine 1980a, 1980b), and in the detection of parasites (Gaida and Frost 1991). That losses of internal parasites due to handling can occur (Möller 1976, Hine 1980a, 1980b, Williams *et al.* 1991) implies that external parasites are even more likely to be influenced by handling as they are more exposed than internal parasites. However, to my knowledge, only two studies have examined the effect of methods on ectoparasite losses, one which compares gillnets with longlines (Nagasawa 1985) and the other which compares visual censuses of parasites with and without a chemical agent which makes parasites opaque (Gaida and Frost 1991). Furthermore, only one parasite species was involved in each of these studies. Despite increasing evidence for sampling biases in the parasite quantification process the issue is seldom addressed in parasitological studies.

A program which identifies the sources of biases in parasite sampling is the first step in developing a protocol for the collection of parasites from host fishes. The second step is to examine other sources of sampling error such as spatial and temporal variation which may confound results (Chapter III). This study examines sampling biases during post-collection handling and parasite

Table 2.1. An outline of the methods used to examine the influence of methodology on the numbers and species composition of ectoparasites. Comparisons are numbered 1-6.

Method	Transport		Parasite recovery	
	container vs fish (alive) (1)	bag vs fish (dead) (2)	bag+rinse vs chloretone (3) 200μm vs 57μm filter(4) removed vs remaining (5)	chloretone vs seawater (6)
Species	*H. melapterus*	*H.melapterus* *T. lunare*	7 species[a]	*S. bilineatus*
Fish collection	net	net	net	net
Plastic bag size	20 L bag	2 L	2 L	2 L
Fish transport	live, container	dead, 2 L bag	dead, 2 L bag	dead, 2 L bag
Post transport	dead, 2 L bag	"	"	"
Pre-rinse	no	no	yes	no
Chloretone	yes	yes	yes	yes/no
Rinse	yes	yes	yes	yes
Filter size (μm)	200	200	200 then 57	200 plus 57
Scanned fish	no	no	yes	yes

[a] See methods and materials.

removal and quantification and develops a procedure, using comparisons among six methods to recover parasites, for measuring parasite loads. The study uses seven common coral reef fishes of varying morphology and ecology which are used in the study on host-cleaner fish interactions.

2.3 METHODS AND MATERIALS

2.3.1 SAMPLING DESIGN

The fish species used are *Acanthochromis polyacanthus* (Pomacentridae), *Ctenochaetus striatus* (Acanthuridae), *Scolopsis bilineatus* (Nemipteridae), *Siganus doliatus* (Siganidae), *Chlorurus sordidus* (Scaridae), *Thalassoma lunare* (Labridae), and *Hemigymnus melapterus* (Labridae). They were selected because they coexist in similar habitats on the reef and are abundant and relatively easy to capture. A total of 90 fish were collected from 4 sites on Lizard Island(North Point, Granite Bluff, Lagoon,Casuarina Beach), Great Barrier Reef, Australia (Fig. 2.1). The sites are in shallow fringing coral reefs which are located around the island and have different levels of wave exposure. The fish were used in 6 comparisons of methods. Due to logistical constraints, only the comparisons 3-5 used all 7 species (Table 2.1). For the remaining comparisons, 1-2 readily available species were selected. In August 1992, specimens of *H. melapterus* for comparison 1 and 2 and specimens of *T. lunare* for comparison 2 were collected. In January 1993, *A. polyacanthus*, *C. striatus*, *S. bilineatus*, *S. doliatus*, *C. sordidus*, *T. lunare*, and *H. melapterus* were collected for comparisons 3-5. In November 1993, *S. bilineatus* were collected for comparison 6.

2.3.2 METHODOLOGICAL COMPARISONS

The 6 comparisons (1-6) are summarised in Table 2.1. To examine post-collection handling, fish were either transported alive in large (20L) containers (1) or dead in plastic bags (2) and the fluids of the container or plastic bag and the fish examined for parasites. Parasite removal and quantification was

examined using comparisons 3-6 with the same specimens used in comparisons 3-5. Fish were removed from plastic bags and rinsed with seawater then soaked in the anaesthetic chloretone to determine if enclosure in a plastic bag and a rinse was enough to remove parasites and whether additional parasites were removed with the anaesthetic (3). To select a filter size for use in recovering parasites from liquids, all liquids (plastic bag contents, rinse, and chloretone) were filtered first with a 200 μm then a 57 μm filter and the parasites recovered with each filter compared (4). To examine how effective these methods were in removing all parasites, fish were scanned under a microscope for remaining parasites which were compared with the recovered parasites (plastic bag contents, rinse, and chloretone soak) (5). Comparison 6 measured the efficiency of chloretone at recovering parasites by comparing the parasites recovered when fish were soaked in either chloretone (dissolved in seawater) or seawater.

2.3.3 COLLECTION AND HANDLING OF FISH

Fish were captured using a 15 m X 1.6 m barrier net with a 20 mm mesh. Fish were herded into the net one at a time and captured with a hand net. All fish, except those to be transported alive in containers (see fish transport comparisons), were placed in a quick-seal 2 L plastic bag as quickly as possible (15-60 s). Fish were then enclosed in a second plastic bag, and kept underwater in a mesh bag for up to 1 h. Fish died quickly from lack of oxygen. Fish in bags were placed in a shaded 40 L plastic container of seawater for up to 1 hour and taken to the laboratory. Ice was added to the water supporting the bags, and the water and fish refrigerated for 2-10 hrs.

2.3.4 PARASITE RECOVERY FROM FISH

All fish, except those collected for the parasite recovery comparisons (3-5), were removed from plastic bags and the contents of the plastic bags set aside for filtration. The whole fish, with each operculum slit at the base and pried open, was covered in a solution of 0.4% chloretone (BDH Chemicals, Poole, England) in 57 μm filtered seawater for 30-60 min following Hargis (1953). Fish

Table 2.2. Codes of categories used for classifying ectoparasites from 7 coral reef fishes. Bold headings are broad categories.

Copepoda

HatH=*Hatschekia hemigymni*

HatA=*Hatschekia* sp. a

Orbi=*Orbitacolax* sp. nov.

Acan=*Acanthocolax* sp. nov.

Bomo=*Acanthocolax* sp. nov. males and/ or *Orbitacolax* sp. nov. males

Cali=*Pseudocaligus* sp. nov.

CopL=Caligidae larvae

Naup=Nauplii

UCop=Other unidentified spp.

Isopoda

Gnat=*Gnathia* spp. larvae

Monogenea

Anop=*Anoplodiscus* sp.

Bene=Benedininae spp.

Digenea

TraL=*Transversotrema licinum*

UDig= Unidentified larvae and/ or *Gyliauchea* sp.

Turbellaria

Tub=*Ichthyophaga* and/ or *Paravortex* spp.

Platyhelminthes

UFla=Unidentified flatworms

were then rinsed thoroughly with filtered seawater. During rinsing, the body surface, fins, gills, buccal cavity, lips, eyes, and nares were gently scraped with the squirt bottle nozzle. All plastic bags, filters, and containers were rinsed 3 times. The rinses, anaesthetic bath, and plastic bag contents were filtered (nylon plankton mesh). Parasites were removed from the filter and placed in vials containing 10% formalin in 57 μm filtered seawater. The gills of *Hemigymnus melapterus* contain many *Hatschekia hemigymni* (Copepoda) after the chloretone bath (pers. obs.) therefore the gills from all *H. melapterus* were removed before the chloretone bath and fixed for parasite counts.

2.3.5 QUANTIFYING PARASITES

The contents of the vials containing fixed parasites were allowed to settle for a minimum of 30 min and the excess liquid decanted. An inspection of the decanted material revealed no parasites. Before sorting parasites from *H. melapterus* gills, blood cells associated with the gills were removed by shaking the vials, allowing parasites to settle for 30 min, and suspended blood cells decanted. Less than 1% of the total parasites were present in decanted material. All remaining material was examined with a sorting tray under a stereo microscope (35X) and sorted into several categories (Table 2.2).

Only some parasites could be identified to genus or species with some of the few keys for Australian parasites (Bruce 1986, Lester and Cannon 1988, Kabata 1991, Cribb *et al.* 1992). The remaining parasites were placed in as narrow as possible categories and were examined by other workers (G. Boxshall, L. Cannon, B. Cohen, T. Cribb, Z. Kabata, I. Whittington). Some copepods were larvae or males and could therefore not be identified to species (Z. Kabata pers. comm.). Gnathiids can only be identified from adult males (Holdich and Harrison 1980), therefore adult males were reared from larvae found on *Siganus doliatus* and *Hemigymnus melapterus*. Juvenile larvae in the last larval stage were placed in vials containing filtered seawater for 1-2 weeks, after which they molted to adults. These reared adults were a new species of *Gnathia* (Gnathidae:) (B. Cohen pers. comm.). Fixed larvae either belonged to

this new species or to at least one other species of *Gnathia* (B. Cohen pers. comm.), therefore all gnathiids were combined under the category *Gnathia* spp. Little is known about parasitic turbellaria of fish with only one study in Australia which describes turbellarians to genera only (Cannon and Lester 1988). These two genera were found and combined.

To quantify the parasites in the preserved gills of *Hemigymnus melapterus*, gills were cut into individual arches, fixative added, and the contents shaken and rinsed 3 times. Parasite numbers were initially expressed as a function of weight of host and surface area of host. The relative difference among species was the same in both cases as surface area is curvilinear to weight (ln area = 0.665 x 2.198(weight)). Weight is a more easily estimated measure of body size therefore weight was used to adjust for differences in body size among species.

2.3.6 FISH TRANSPORT COMPARISONS (1&2)

To quantify parasite losses during the transport of live fish, *Hemigymnus melapterus* (n=7) were captured and placed in 20 L plastic bags to reduce handling stress. Fish were taken directly to the boat and placed in separate covered plastic containers (10-20 L seawater) for 2 h during transport to laboratory. Fish were then removed from containers, killed with a blow to the head, and placed in plastic bags and refrigerated. Parasites on the fish were recovered and quantified as above using a 200 μm filter. The contents of containers were filtered with a 200 μm filter and fixed. The parasites in the container were compared to those recovered from fish (comparison 1). To determine how many parasites are lost when fish are transported dead in plastic bags, *H. melapterus* (n=8) and *Thalassoma lunare* (n=13) were collected as described above, placed in 2L plastic bags where they died, and the parasites in the bag contents and on the fish quantified 2-10 h afterwards as described above (comparison 2).

2.3.7 PARASITE RECOVERY COMPARISONS (3-5)

All seven species were used to investigate the parasite recovery process (n=4-8 per species). Fish were removed from the 2L plastic bags and the contents of the plastic bag set aside. Fish were rinsed thoroughly and the rinse added to plastic bag contents (bag+rinse in comparison 3). All fish, except for *Chlorurus sordidus* (see below), were soaked in chloretone and rinsed (chloretone in comparison 3). The chloretone and rinse solutions were filtered with a 200 μm then 57 μm filter and the filtrates kept separately (comparison 4). To quantify how many parasites remain on the fish after the anaesthetic bath, the entire body surface of the above specimens, including the fins, eyes, and nares were inspected under a stereo microscope (16-20X). Gills were removed, cut into individual arches, and inspected. The operculum was removed to inspect the gill and buccal cavity. Any disfigurements of the skin or scales were examined further for turbellarians and other flatworms. Specimens of *C. sordidus* were scanned under a microscope rather than soaked in chloretone to recover parasites, as the thick mucus in their gills and body surface blocks filters. The parasites recovered (bag, rinse, and chloretone) were compared to those remaining on fish (comparison 5). The parasites recovered and those remaining on fish were summed to investigate differences in parasite numbers between species.

2.3.8 THE EFFECTIVENESS OF CHLORETONE (COMPARISON 6)

To measure the effectiveness of chloretone in recovering parasites, specimens of *Scolopsis bilineatus* were soaked in either chloretone (n=11) or in sea water (n=11) for 30 min and the parasites recovered compared (comparison 6). Fish were also scanned for remaining parasites. The parasites from the soak and the scan were summed and compared among treatments. Fish were collected, placed in bags, and soaked as described above. Both filtrates (200 and 57μm) were combined.

2.3.9 STATISTICAL ANALYSES

Differences in total numbers of parasites among species was tested with analysis of variance (ANOVA). The proportion of total parasites that fell off *Hemigymnus melapterus* during transport in containers or in bags were tested for differences with a nonparametric ANOVA (Kruskal-Wallis test) as was the proportion of the two most abundant parasite categories (*Hatschekia hemigymni* and *Gnathia* spp.). The proportion of parasites that were removed with a rinse, the 200 μm filter (arcsine transformed), and by all treatments (vs scan) was tested for differences among species (Kruskal-Wallis test). Fish with no parasites were omitted from the above analyses. The same test was used to investigate whether the proportion of parasites removed by a rinse or by the 200 μm filter differed among parasite categories. For the latter, only fish species with relatively large numbers of parasites were tested (*Scolopsis bilineatus*, *Siganus doliatus*, and *H. melapterus*), using 3-4 of the most abundant parasite categories (see Fig. 2.5e-g, and Fig. 2.7e-g for parasite categories), and only individuals with the parasite category present. To examine the overall efficiency of chloretone, the proportion of total parasites (arcsine transformed) recovered with seawater and chloretone were tested for differences with a t-test analysis. To determine whether the species composition of parasites recovered by the soak differed among the two baths, the number of parasites per category were tested for differences using a multivariate analysis of variance (MANOVA). To test if the parasites recovered with a bath plus a scan were the same among baths, the total numbers of parasites per category from bath plus scan were tested for differences with a MANOVA. A canonical discriminant analysis (CDA) was used to discriminate among treatments when MANOVAs were significant. Data were natural log (x+1) transformed in the ANOVA and MANOVAs. The multivariate test statistic, Pillai's Trace, was used in the MANOVAs because it is more robust to heterogeneity of variance and is less likely to involve Type-I error than comparable tests (Green 1979). When the assumption of homogeneity of variance in the Kruskal-Wallis test (Maxwell and Delaney 1990) was violated, data were arcsine transformed.

2.4 RESULTS

The total numbers of ectoparasites per fish differed among the fish species (ANOVA $F=10.12$, $df=6,27$, $p<0.001$). *Hemigymnus melapterus* and *Siganus doliatus* had the most parasites when expressed as numbers of parasites per fish and when adjusted for weight (Fig. 2.2). Two categories of parasite infestation were apparent, species such as *Scolopsis bilineatus* and especially *H. melapterus* and *S. doliatus* with relatively high parasite loads; and 4 species, *Chlorurus sordidus*, *Thalassoma lunare*, *Ctenochaetus striatus*, and *Acanthochromis polyacanthus* with few parasites (Fig. 2.2).

2.4.1 FISH TRANSPORT

Losses of parasites, mainly gnathiids, occurred in all transport comparisons (Fig. 2.3). The percent of total parasites that dropped off *Hemigymnus melapterus* transported in containers (22.4% ±SE 11.4) and bags (20.5% ±SE 6.3) was not significantly different (Kruskal-Wallis test=0.121, $p=0.728$). The mean percent of parasites that dropped off *Thalassoma lunare* transported in bags was 57.7% (±SE 11.8)(Fig. 2.3c). The proportion of parasites that dropped off fish was significantly different among transport methods for *Hatschekia hemigymni* (Kruskal-Wallis test=4.364, $p=0.037$) but not for *Gnathia* spp. (Kruskal-Wallis test=0.013, $p=0.908$).

2.4.2 RINSE VS. CHLORETONE

A thorough rinse of the body surface and gills removed a large number of parasites from all 7 species, however additional parasites were recovered when fish were subsequently soaked in chloretone (Fig. 2.4). The number removed with the rinse differed due to differences in the total number of parasites among species, however the proportion of parasites removed with the rinse was not significantly different among fish species (Kruskal-Wallis test=10.22, $p=0.116$). Many different parasite categories were removed by the rinse (Fig. 2.5). Among abundant categories, the proportion of parasites

removed with a rinse was not significant different for *Siganus doliatus* (Kruskal-Wallis test=3.91, p=0.141), nor for *Scolopsis bilineatus* (Kruskal-Wallis Test=1.42, p=0.701), but was significantly different for *Hemigymnus melapterus* (Kruskal-Wallis Test=6.71, p=0.035) (See Fig. 2.5e-g for parasite categories tested). The parasites which were almost always completely removed with the rinse were all copepods. These consisted of unidentified Copepoda spp. (Fig. 2.5b, d-f), Caliginae spp. (Fig. 2.5b), Caligidae larvae (Fig. 2.5c, e, & f), nauplii (Fig. 2.5e-g), *Acanthocolax* sp. nov. and or *Orbitacolax* sp. nov. males (Fig. 2.5c, f, & g), and *Orbitacolax* sp. nov. (Fig. 2.5c, d, f, & g).

Parasites removed with chloretone were often species found in gills such as unidentified flatworms (Fig. 2.5a, b, & e), Turbellaria (Fig. 2.5b, d, g), and Dactylogyridea spp. (Fig. 2.5e & f), or under scales (*Transversotrema licinum*) (Fig. 2.5b, f) and in epidermal pockets (Turbellaria) or were possibly internal parasites released post-mortem (unidentified Digenea spp., T.H. Cribb pers. comm.)(Fig. 2.5c, e, f, & g). Finally, a proportion of *Gnathia* spp. remained after the rinse on most species, which was recovered only with the chloretone soak (Fig. 2.5a, c-g).

2.4.3 FILTER SIZE

The smaller filter was easily blocked with mucus and debris and often required cleaning. However, additional filtering at 57 μm revealed additional parasites for all species except *Chlorurus sordidus* (only the plastic bag contents were filtered for this species) (Fig. 2.6). Although some turbellarians (small parasites) were recovered by scanning on *C. sordidus* (Fig. 2.7d). The numbers removed by the large filter differed among fish species, however the proportion of total parasites removed by the large filter was not significantly different among fish species (Kruskal-Wallis Test=6.31, p=0.390). The proportion of parasites removed by the large filter was not significantly different among parasite categories for *Siganus doliatus* (Kruskal-Wallis Test=4.02, p=0.134), but was significantly different among parasite categories for *Scolopsis bilineatus* (Kruskal-Wallis Test=15.58, p=<0.001) and for *Hemigymnus melapterus*

Table 2.3. The mean number of parasites remaining on fish (SE), found by scanning fish under a stereo microscope, after fish were soaked in chloretone[a]. See Table 2.2 for definitions of parasite categories.

Fish Species [sample size]	UCop		Dact		TraL		UDig		Turb		UFla	
C. striatus [5]	0	-	0	-	0	-	1.20	(1.20)	0.20	(0.20)	0	-
A.polyacanthus[5]	1.20	(1.20)	0	-	0	-	0	-	0	-	0.60	(0.60)
T. lunare [5]	0	-	0	-	0	-	0.20	(0.20)	0	-	0	-
C. sordidus[a] [5]	0	-	0	-	0	-	0	-	0.20	(0.20)	0.20	(0.20)
S. doliatus [5]	0	-	0.80	(0.80)	0	-	1.00	(0.77)	0.20	(0.20)	0.60	(0.40)
S. bilineatus [4]	0	-	0	-	0.50	(0.29)	0	-	0.75	(0.48)	1.25	(1.25)
H. melapterus [5]	0	-	0	-	0	-	12.60	(9.28)	2.80	(2.33)	0	-

[a] *Chlorurus sordidus* was not soaked in chloretone but scanned under microscope for parasites.

(Kruskal-Wallis Test=7.52, p=0.023)(See Fig. 2.7e-g for parasite categories tested). The smallest parasites, copepod nauplii, 140-200 μm in length, were mostly recovered with the small filter (Fig. 2.7e-g). A proportion of Turbellaria, usually less than 300 μm in length with a diameter of 100 μm, passed through the 200 μm filter in all species (Fig. 2.7). The only categories that were always fully recovered with the 200 μm filter were *Orbitacolax* sp. nov. (Fig. 6c-f), *Acanthocolax* sp. nov. (Fig. 2.7f), and Caliginae spp. (Fig. 2.7b). These are relatively large parasites 710 μm-2.5 mm in length. Many gnathiid isopods, which range from 280 μm to 2.7 mm in length, were also recovered by the large filter (Fig. 2.7a, c-g).

2.4.4 REMOVED VS. REMAINING

Although many parasites were removed by the process of rinsing and soaking the fish in anaesthetic, and by filtering all liquids with the 200 plus 57 μm filters, a number of parasites remained on fish which were found by visual survey (Fig. 2.8). The proportion of parasites removed, however, was not significantly different among fish species (Kruskal-Wallis test=6.06, p=0.416). The parasites most commonly found remaining on the fish were unidentified Digenea spp., Turbellaria, and unidentified flatworms (Table 2.3). Copepoda were found only on *Acanthochromis polyacanthus* (Table 2.3) and these were gill copepoda from one fish.

2.4.5 CHLORETONE EFFICIENCY

A chloretone bath removes many more parasites than does a seawater bath (Fig. 2.9). The percentage of total parasites recovered with a chloretone bath (88% ±SE 2.8) and with a seawater bath (37% ±SE 8.7) was significantly different (t=-5.52, df=20, p <0.001). The species composition of recovered parasites among treatments were different with the chloretone treatment characterised by many *Transversotrema licinum* and *Anoplodiscus* sp. and to a lesser degree by Dactylogyridea spp. (MANOVA Pillai's Trace=0.868, F=5.996, df=11,10, p=0.004). If all parasites not removed by a bath were recovered by

a scan then the species composition of all parasites collected (bath plus scan) should be the same. This was not the case as the species composition of all parasites (bath plus scan) was significantly different among treatments and was mainly due to more *Anoplodiscus* sp. being removed by the chloretone treatment and scan (MANOVA Pillai's Trace=0.872, F=6.193, df=11,10, p=0.004).

2.5 DISCUSSION

The study demonstrates that the method used to transport fish and remove ectoparasites can have a large influence on the numbers and species composition of recovered parasites. Two general categories of parasites are apparent: mobile crustaceans, which vacated their host on disturbance, and cryptic parasites which remained on host through most protocols but were recovered by the anaesthetic. Mobile parasites, particularly gnathiid isopods, which dropped off fish during transport of live and dead fish were recovered by retaining and filtering all transport liquids. It is likely that the stress of transport resulted in this loss. Several studies have shown that transporting (Aldrin *et al.* 1979, Specker and Schreck 1980, Pankhurst *et al.* 1991), handling (Pickering and Macey 1977, Pickering *et al.* 1982), and capture (Mazeaud *et al.* 1977, Perrier and Perrier 1978) of fish results in biochemical changes which are likely to be detected by parasites. Davies and Johnston (1976) found that the capture of a blenny with a handnet disturbed ectoparasites, including a gnathiid, and used anaesthetics to capture the fish and decrease gnathiid loss. Thus methods that maintain a low stress level during capture and handling of fish will likely result in lower parasite losses.

The method described here lowers loss of parasites during capture by using a net with a small mesh which reduces entanglement of fish and thus abrasion of parasites. Handling time is decreased by using SCUBA, by capturing one fish at a time, and by placing fish into plastic bags as quickly as possible. Surprisingly, plastic bags have only occasionally been used to reduce parasite

losses (Hobson 1971, Losey 1974, Gorlick *et al.* 1987).

Enclosure of fish in a bag and rinsing may be a useful method for recovering some types of mobile copepods as this method mainly removed copepods which have retained the ability to swim (Yamaguti 1963). However, if all parasites are sought, especially flatworms, an anaesthetic soak is more effective. Not only were more parasites recovered by the chloretone bath compared to the seawater bath, most importantly, a different species composition was obtained when the combination of anaesthetic bath and visual scan was used. The difference was due to recovery of fewer *Anoplodiscus* sp. in the seawater bath. However, *Anoplodiscus* sp. not recovered by the seawater bath were not detected in the subsequent scan. This suggests that some parasites may remain undetected when the traditional method of visual scanning is the only method used to recover parasites.

Although there were no significant differences among fish species in the proportion of total parasites removed with the rinse, large filter, and by all treatments vs scan, there was some variation in the proportion removed in some parasite categories. In addition, many parasites categories were missed completely by the rinse and large filter. Thus parasite composition may be influenced during the stages of the parasite recovery process .

Initially, a standard method which could be used for all species was sought. However, the general protocol was constrained by differences among species so the methods were modified slightly for some species. For one species with a high mucus load which blocked filters, filtration was minimised by scanning the whole fish and gills and filtering only the transport liquids. Chloretone did not recover all the gill copepods on one species so the gills were removed and examined separately.

Using the comparisons as a guideline, the most efficient method for obtaining reliable estimates of the ectoparasites of the species investigated appears to be the following: fish are placed in plastic bags as quickly as possible, preferably underwater, and all liquids retained; for species with many gill-inhabiting copepods, the gills are removed and fixed separately; after a soak in

anaesthetic, a subsample of fish should be scanned to check for accuracy; finally, although time consuming, all liquids should be filtered with a 57 μm filter.

This study differs from most other studies of this nature because it includes a wide range of ectoparasites and fish species, examines parasite losses at more than one sampling stage, and involves parasites of tropical fish. The benefit of this method over other methods is that it reduces field laboratory time because all parasites are removed from the fish and fixed; parasites can therefore be sorted and identified at a later date. The need for modifications suggests that either the method of parasite quantification must be extremely rigorous, or an optimal method must be identified for each species of interest. It is only then that reliable estimates of parasite loads will be obtained. The importance of using appropriate sampling techniques in the study of cleaning interactions is illustrated by the effect of methodology on gnathiids-parasites extremely important in cleaning interactions.

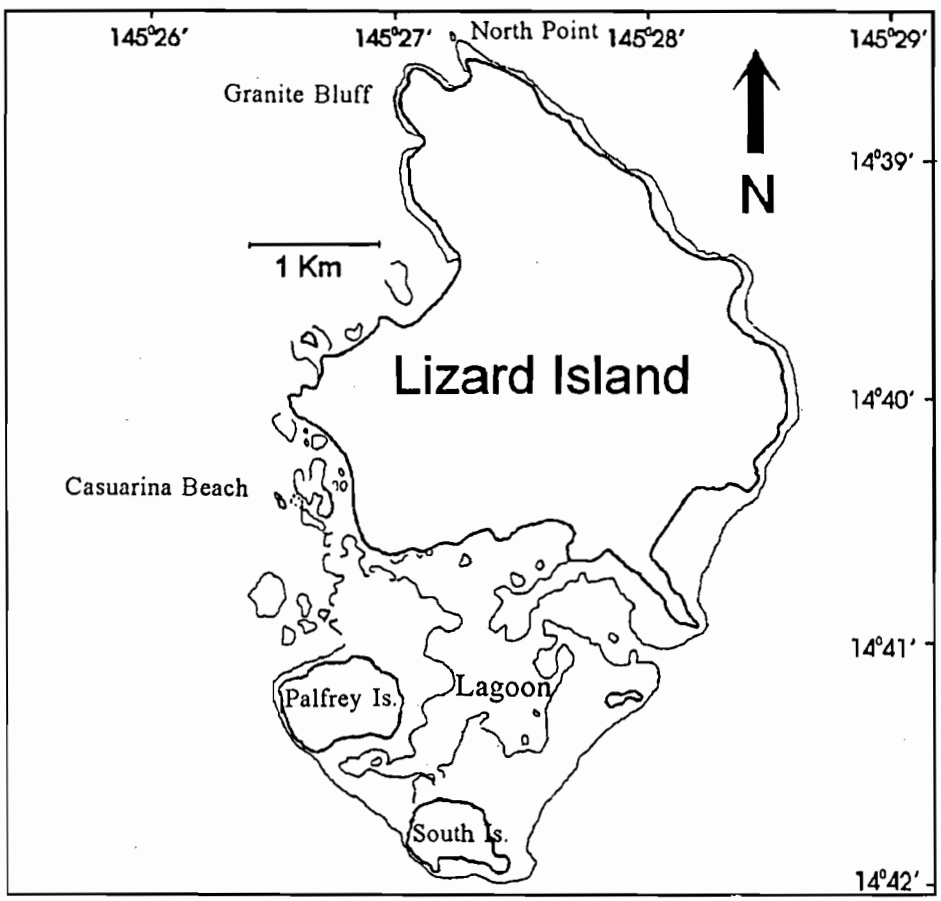

Figure 2.1. Map of Lizard Island showing study sites.

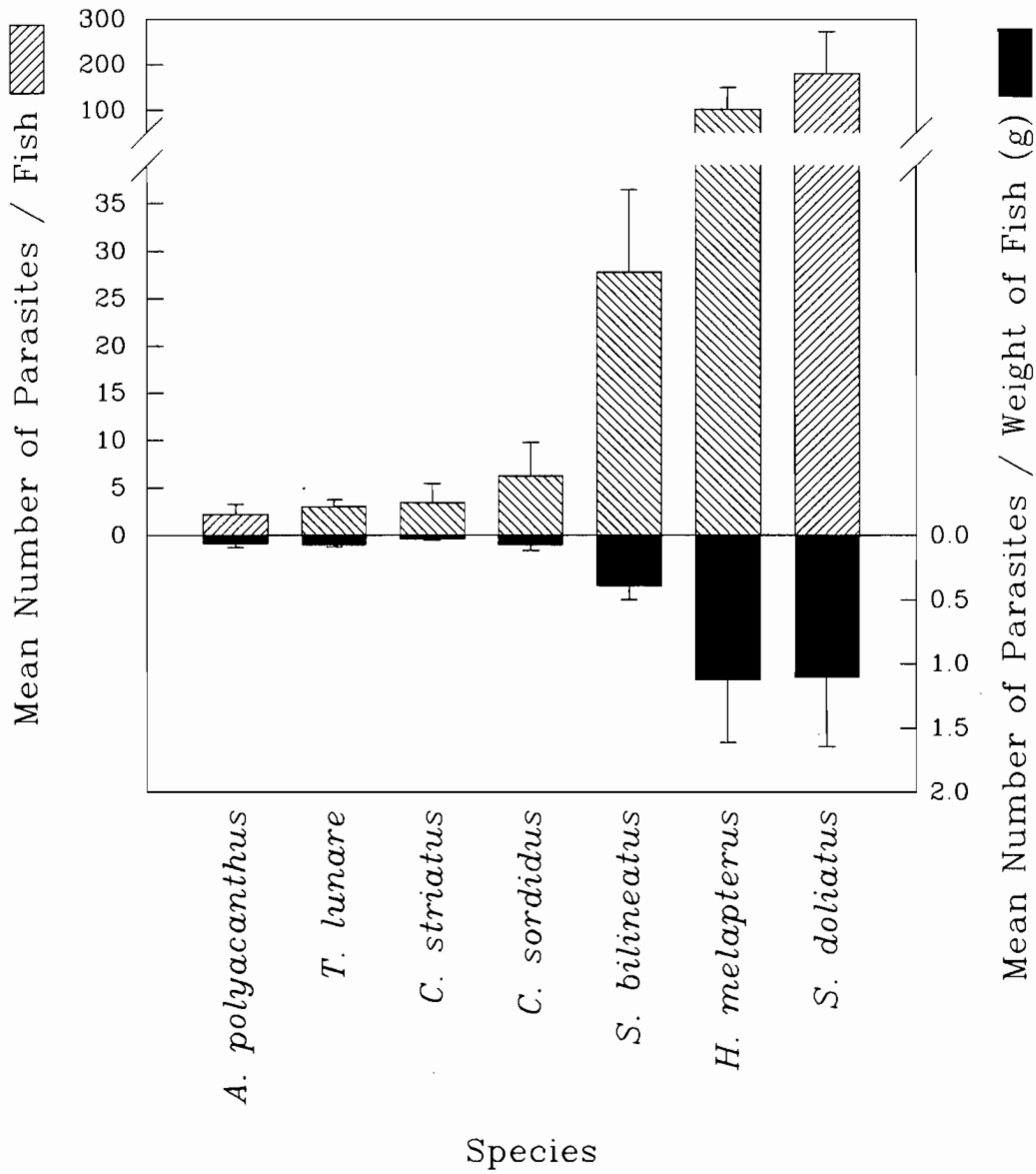

Figure 2.2. The mean sum of the parasites removed with a chloretone bath plus parasites remaining on fish (±SE). The range in standard length (cm) of each species from left to right followed by the sample size is (8.9-9.2,5), (10.3-15.0,5), (9.4-15.5,5), (11.5-17.5,5), (11.0-15.5,4), (9.2-20.3,5), (14.6-18.3,5).

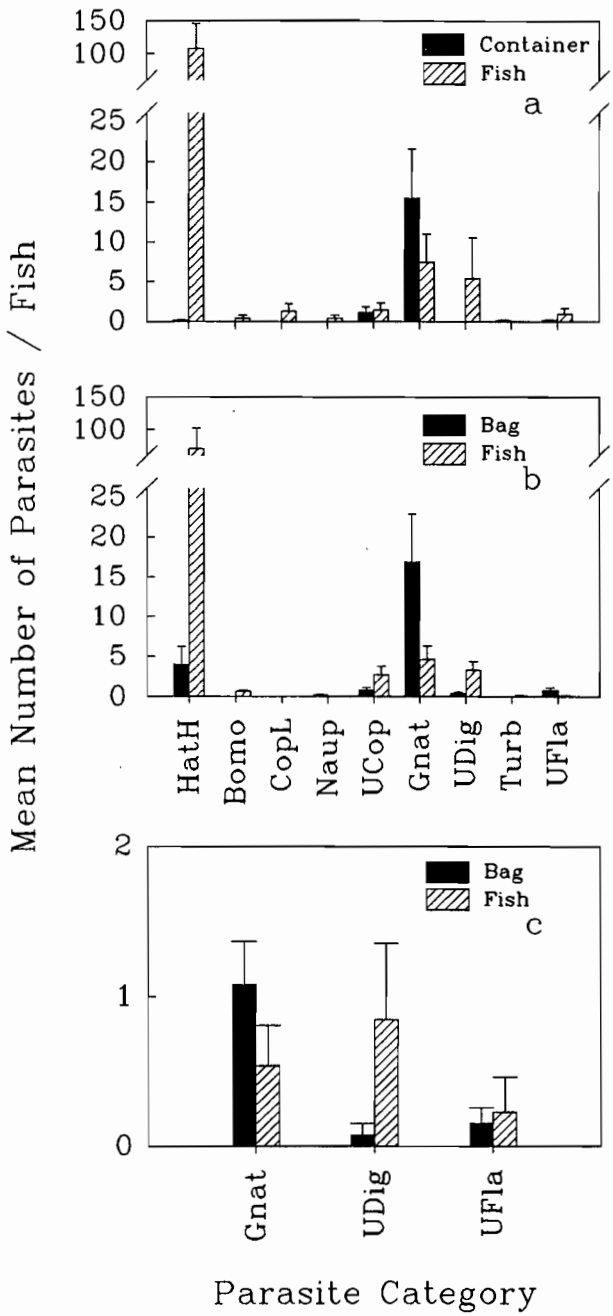

Figure 2.3. The mean number of parasites (±SE) found in the containment liquid (container or bag) compared to those on fish after transport. **a.** After *Hemigymnus melapterus* were held alive in container for 2 h, **b.** After *H. melapterus* were held dead in bag 2-10 h, **c.** After *Thalassoma lunare* were held dead in bag 2-10 h. Note *Gnathia* spp. were the most commonly lost parasites. See Table 2.2 for definitions of parasite categories.

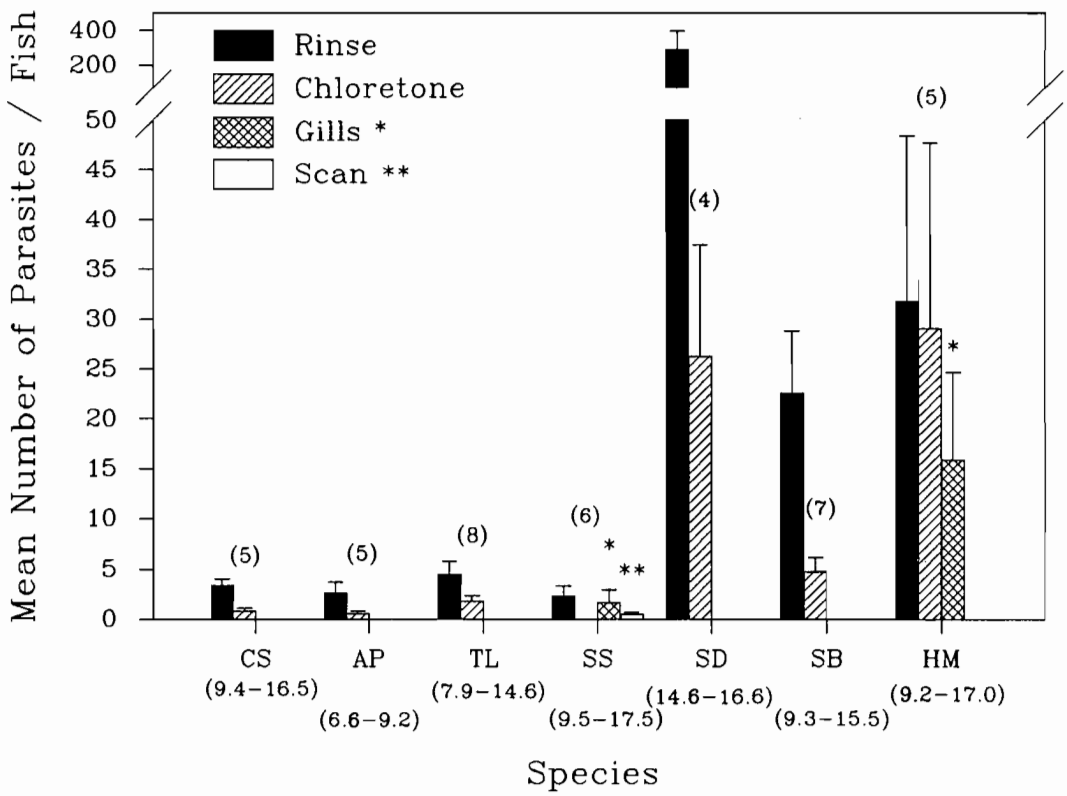

Figure 2.4. The mean total number of parasites (±SE) removed with a seawater rinse compared with additional parasites recovered after a chloretone bath. Rinse includes contents of plastic bag. Sample size is above the mean. Parasites recovered separately from gills and by scanning are labelled separately. Legend for "gills" and "scan" apply only to species labelled with * or **. Fish species: CS= *Ctenochaetus striatus*, AP= *Acanthochromis polyacanthus*, TL= *Thalassoma lunare*, SS= *Chlorurus sordidus*, SD= *Siganus doliatus*, SB= *Scolopsis bilineatus*, HM= *Hemigymnus melapterus*. The standard length range (cm) is below the fish species code, sample sizes are above means.

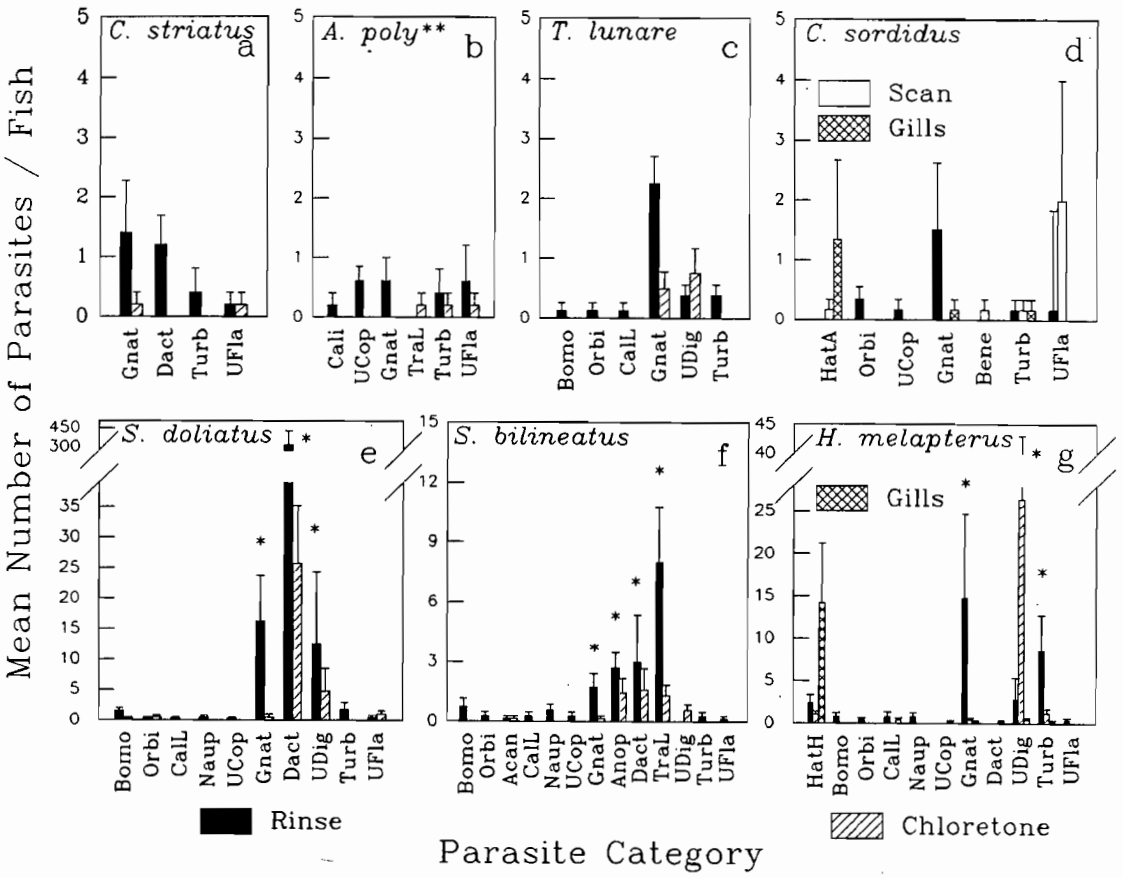

Figure 2.5. The mean number of parasites (±SE) in each category removed with a seawater rinse compared with additional parasites recovered after a chloretone bath. Parasites recovered separately in gills and by scanning are labelled separately. ***Acanthochromis polyacanthus*. See Table 2.2 for definitions of parasite categories. *Parasite categories tested for differences in the proportion removed with a rinse.

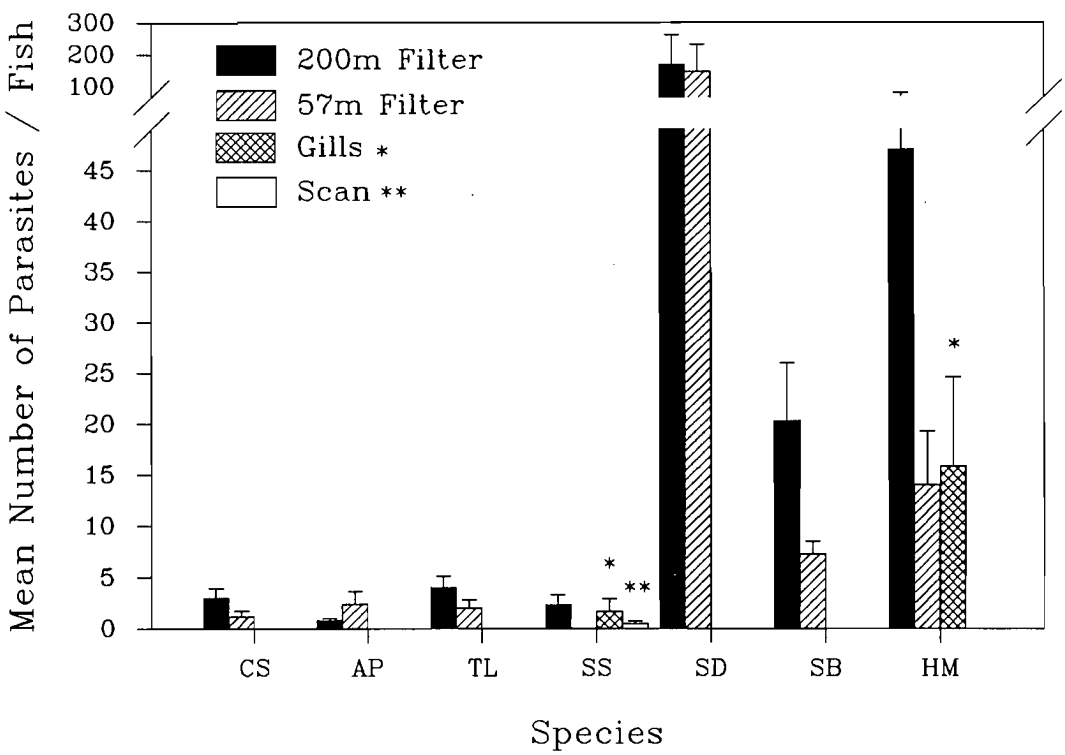

Figure 2.6. The mean total number of parasites (±SE) removed first with a 200 μm filter then an additional 57 μm filter. Parasites recovered separately in gills and by scanning are labelled "scan" and "gills" and apply only to species labelled with * or **. CS= *Ctenochaetus striatus*, AP= *Acanthochromis polyacanthus*, TL= *Thalassoma lunare*, SS= *Chlorurus sordidus*, SS= *Siganus doliatus*, SB= *Scolopsis bilineatus*, HM= *Hemigymnus melapterus*.

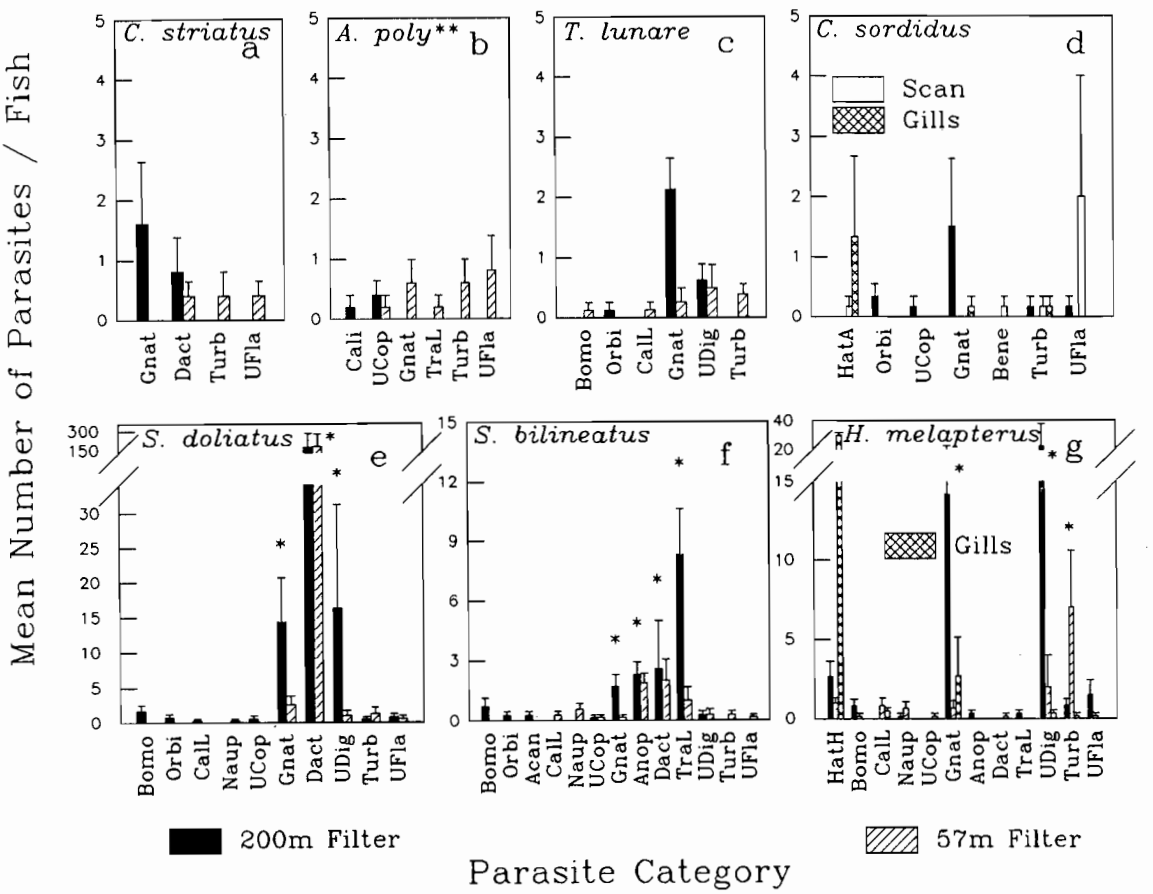

Figure 2.7. The mean number of parasites (±SE) in each category removed first with a 200 μm filter then an additional 57 μm filter. Parasites recovered separately in gills and by scanning are labelled separately. ***Acanthochromis polyacanthus***. See Table 2.2 for definitions of parasite categories. *Parasite categories tested for differences in the proportion recovered with the large filter.

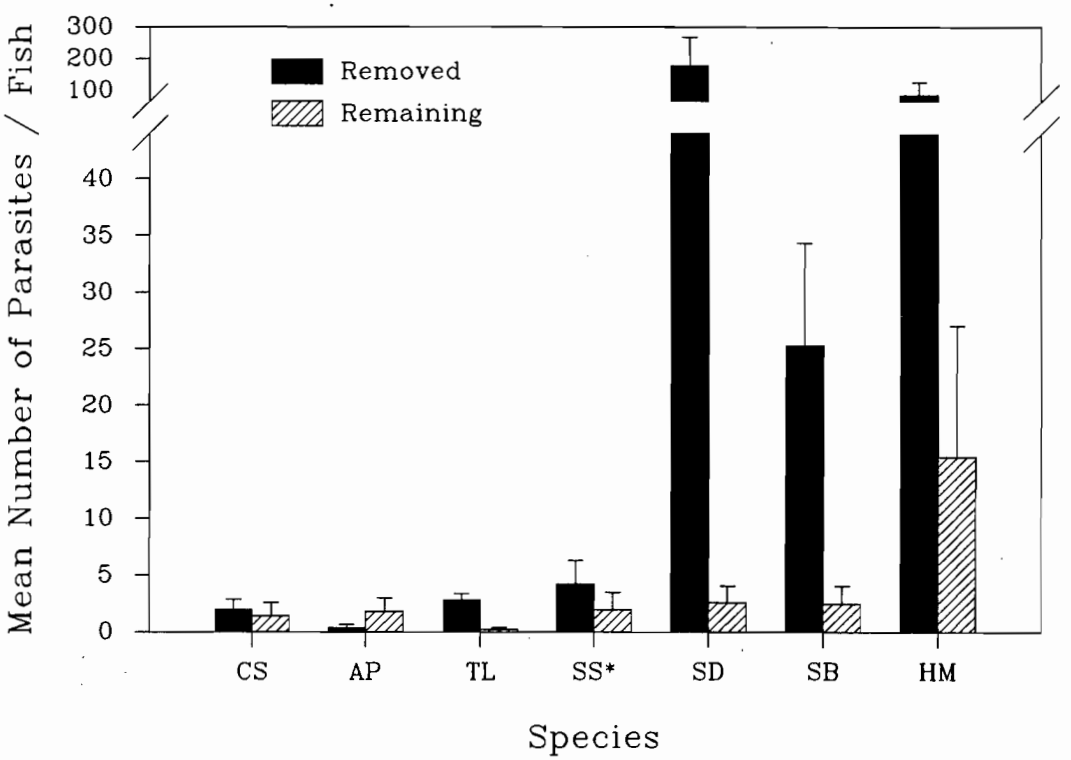

Figure 2.8. The mean number of parasites (±SE) removed with a chloretone bath compared with parasites remaining on fish after the chloretone bath. Remaining parasites were found by scanning fish under a microscope. CS= *Ctenochaetus striatus*, AP= *Acanthochromis polyacanthus*, TL= *Thalassoma lunare*, SS= *Chlorurus sordidus*, SS= *Siganus doliatus*, SB= *Scolopsis bilineatus*, HM= *Hemigymnus melapterus*. *Scanned under microscope rather than soaked in chloretone.

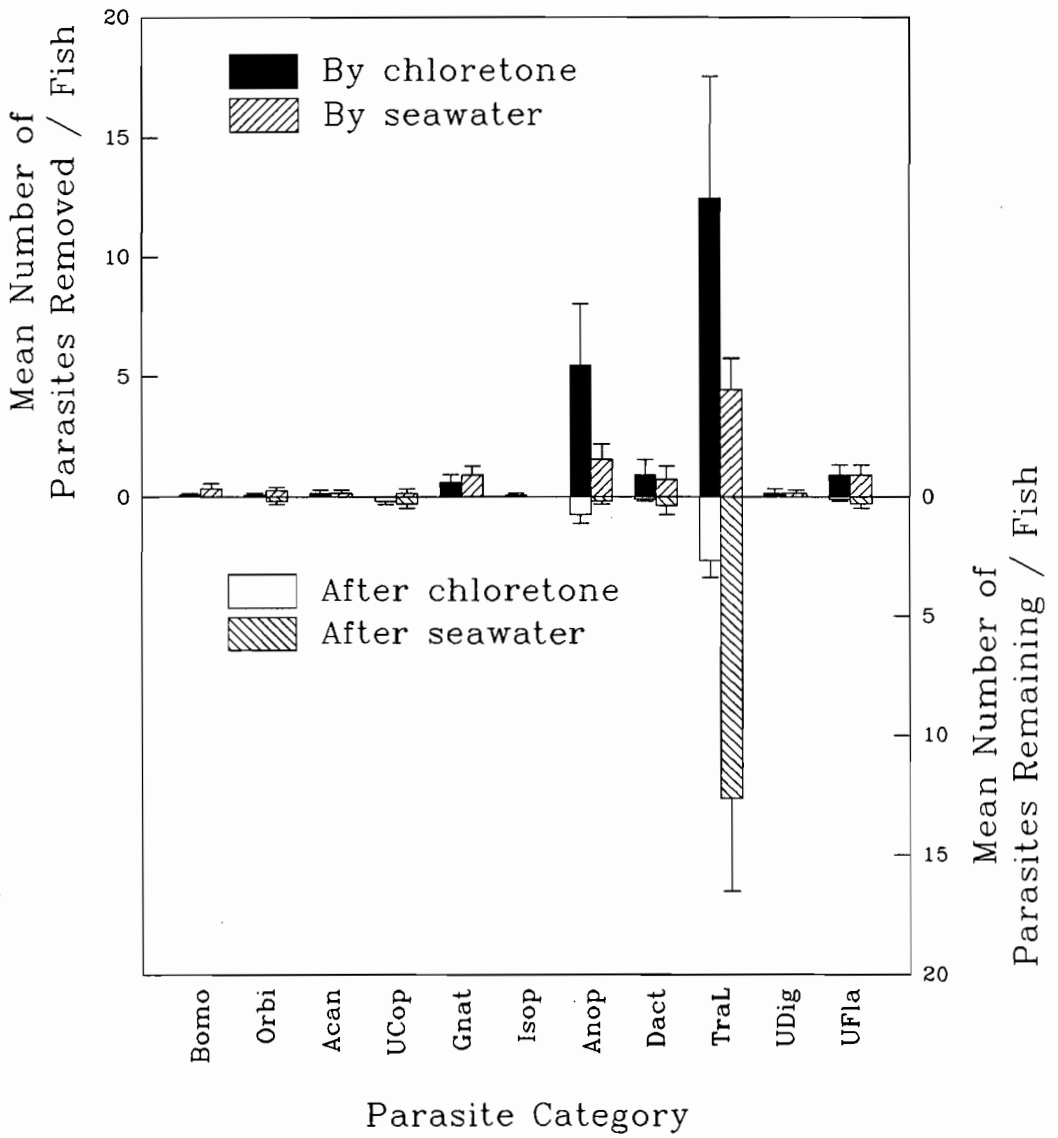

Figure 2.9. The mean number of parasites (±SE) removed from and remaining on *Scolopsis bilineatus* soaked in either 0.4% chloretone or seawater only. The mean standard length (cm) (SE) of chloretone soaked fish is 12.3 (0.7) and seawater soaked fish is 11.7 (0.7). See Table 2.2 for definitions of parasite categories.

CHAPTER III

SPATIAL AND TEMPORAL VARIATIONS OF THE ECTOPARASITES OF SEVEN REEF FISH SPECIES FROM LIZARD ISLAND AND HERON ISLAND

3.1 SUMMARY

The spatial and temporal variations in abundance of ectoparasites from seven coral reef fish species *Hemigymnus melapterus*, *Siganus doliatus*, *Scolopsis bilineatus*, *Thalassoma lunare*, *Chlorurus sordidus*, *Ctenochaetus striatus*, and *Acanthochromis polyacanthus* at two locations, Lizard Island and Heron Island, were investigated. The study demonstrates that there is a significant species-specific parasite fauna which is conserved over space and time. Host identity explained most of the variation in parasite composition and abundance while host size explained a smaller proportion of the variation. For each species the parasite assemblage showed little variation among local, but physically varied, sites. Species specific patterns of parasite abundance were similar between widely separated locations although there were more categories of parasites at the northern location, Lizard Island. The abundance and species composition of parasites of seven fish species at Lizard Island did not vary among collection times except for *S. doliatus* which had a 7 fold increase between May 1992 and January 1993, mainly due to variation in the abundance of dactylogyridean monogeneans. Parasite abundance was positively correlated with fish standard length for 3 fish species.

3.2 INTRODUCTION

The numbers and species composition of marine ectoparasites vary both among fish species and within a fish species. Variation in parasites within a species can occur on a small spatial scale (Yeo and Spieler 1980) or on a large spatial scale latitudinally (Dogiel 1961, Polyanski 1961b, Rohde 1993). Parasites also vary seasonally (Kennedy 1975) and as a function of host size (Bortone *et al.* 1978). These sources of variation can confound results and therefore must be considered in parasitological studies.

Estimates of spatial variation are needed when designing sampling

programs in order to obtain reliable estimates of parasites. Before large scale spatial comparisons among locations can be made, estimates of small scale spatial variation among sites are needed to avoid confounding effects (Hurlbert 1984). Estimates of small scale variation are also important if fish collections are logistically constrained. For example, if some fish species are more easily collected at some sites than others, knowledge of the spatial variability among sites will determine whether sampling can be reduced to particular sites or combined among sites.

Information on the temporal variability of parasites is needed to establish whether the parasite fauna observed is representative of the overall parasite fauna. Temporal variability in parasites can also affect factors influenced by parasites. For instance, the diet of cleaner fish or the cleaning behaviour of fish hosts could change if the parasite loads of fish change over time. Studies of temporal variation in fish parasites have been largely confined to cold temperate seas (Llewellyn 1959, Noble 1963, Kennedy 1975, Rawson 1976) with the few studies in the tropics restricted mostly to the parasites of snails (Rohde and Sandland 1973, Rohde 1981, Cannon 1978, 1979).

The diversity of coral reef fishes found in the Great Barrier Reef is high (Randall *et al.* 1990). The diversity of monogenean parasites in the Great Barrier Reef is almost certainly greater than that of fish species (Rohde 1977) and the number of parasite species at Heron Island alone have been estimated at 20,000 (Rohde 1977). If both parasite fauna and fish are diverse the potential for variability in the interactions between fish and parasites is high. Such questions require estimates of how parasites vary amongst species and the degree to which they are constant over space and time. Not only is this information relevant for this type of study but also for other parasitological work such as in fish stock discrimination (Lester *et al.* 1988) and evolution (Brooks and McLennon 1993).

Much parasitological work is observational or descriptive and thus tends to be non-quantitative (Sindermann 1986). This study is quantitative rather than qualitative with the emphasis placed on measuring the variability in numbers of parasites using broad categories of ectoparasites. The complete ectoparasite

faunas of seven relatively small fish species which are common on coral reefs of the Great Barrier Reef are quantified. The variations in ectoparasite numbers and species composition among fish species, sites, locations, and times of collection as well as the relationship between host size and parasite abundance were investigated. The species span a range of taxonomic and ecological relationships but are all common in shallow coral reef waters.

3.3 METHODS AND MATERIALS

The fish species investigated are *Hemigymnus melapterus*, *Siganus doliatus*, *Scolopsis bilineatus*, *Thalassoma lunare*, *Chlorurus sordidus*, *Ctenochaetus striatus*, and *Acanthochromis polyacanthus*. All species have different feeding habits and three (*H. melapterus, T. lunare, C. sordidus*) are taxonomically related and belong to the order Labroidei (*sensu* Greenwood *et al.* 1966). A total of 304 fish were collected.

3.3.1 SAMPLING DESIGN

Spatial variation was examined at two scales, within a reef system (Lizard Island) incorporating different habitats and between reef systems separated by 1000 km (Lizard Island and Heron Island)(Fig. 3. 1). Small scale variation was examined at 3 sites (1-5 km apart) located around Lizard Island which are in shallow coral reefs (2-7 m) and have different levels of wave exposure. Site 1 (North Point) is the most exposed, site 2 (Granite Bluff) is less exposed, and site 3 (Lagoon) is in a protected lagoon behind a small island and has little wave exposure. The differences among sites are reflected in the fish fauna (Choat and Bellwood 1985).

Lizard Island is a continental island with fringing reefs while Heron Island is a coral cay with a large platform reef. The locations were selected because they represent reef systems at the extremes of the Great Barrier Reef, yet the species investigated are present at both reefs. Fish were collected at three times (seasons) from Lizard Island (May 1992, August 1992, and January 1993) and

once from Heron Island (June 1993).

3.3.2 SITES

To investigate the number and composition of parasites among fish species collected from different sites, 5-9 fish per site from 3 sites were collected from each species in May 1992. Specimens of approximately similar size were collected from within a species to reduce variation due to host size.

3.3.3 LOCATIONS

The abundance and composition of assemblages of parasites on different fish collected at two locations was examined. The collections at Lizard Island (January 1993) were from the above 3 sites. The collections at Heron Island (June 1993) were from 2 sites on the reef slope (2-10 m in depth) located on opposite sides of the island (2 km apart). Between 5 and 9 specimens were collected from the above seven species at each location. Sites at each location were combined as the sample sizes of fish from each location were too small and unbalanced to test for differences among sites.

3.3.4 TIME

The number of parasites per fish on *Hemigymnus melapterus* and *Thalassoma lunare* were analysed for differences among 3 collection times (seasons) at Lizard Island (May 1992, August 1992, January 1993). The remaining 5 species were tested for differences in parasite numbers among two collection times (May 1992, January 1993). Only *Hemigymnus melapterus* and *Thalassoma lunare* had sufficient sample sizes at each time to test for differences in parasite composition among times. To obtain sufficient degrees of freedom for a multivariate analysis of variance, the number of variables were reduced by selecting parasite categories (Table 3.1) that were present in 30% or more of the fish. This reduced the parasite categories from 7 to 2 for *T. lunare* (gnathiids and unidentified Digenea spp.) and from 12 to 7 in *H. melapterus* (gnathiids, *Hatschekia hemigymni*, unidentified Digenea spp., *Acanthocolax* sp.

nov. and or *Homobomolochus* sp. nov. males, Caligidae larvae, *Orbitacolax* sp. nov., and Turbellaria. The reduced variables constituted 90.1% and 98.4% of the total parasites respectively.

3.3.5 FISH SIZE

The relationship between total parasite numbers per fish and standard length was investigated for all species collected at Lizard Island using linear correlation. The sample size of fish was increased by using fish collected at different times. So that time did not confound results, only fish species that had no significant differences in total number of parasites among times were used. Outliers (total parasites of an individual fish) were tested (t-statistic) and omitted when $p<0.05$ (p corrected with Bonferroni's inequality).

3.3.6 STATISTICAL ANALYSES

Separate multifactor analyses of covariance (ANCOVA) were used to test for differences in the total number of parasites among species and sites, and among species and locations with fish surface area as the covariable. For each species, a single factor ANCOVA was used to test for differences in the total number of parasites per fish among times using fish standard length as the covariable. The slopes were not significantly different ($p<0.05$) in all ANCOVAs so the interaction term was dropped.

Separate multifactorial multivariate analyses of variance (MANOVA) were used to test for differences in the number of parasites per category among species and sites, and among species and locations; separate single factor MANOVAs was used to test for differences in the number of parasites per parasite category among times for each of the species *Hemigymnus melapterus* and *Thalassoma lunare*. All *Transversotrema* spp. at Heron Island were pooled to increase the degrees of freedom in the MANOVAs. The multivariate test statistic, Pillai's Trace, was used in all MANOVAs because it is more robust to heterogeneity of variance and is less likely to involve Type-I error than comparable tests (Green 1979). To discriminate among species and sites, and

among species and locations a canonical discriminant analysis was used. To satisfy the assumptions of the statistical analyses performed, all data were natural log (x+1) transformed to achieve homogeneity of variance or linearity. Surface area was used as covariable when making comparisons among species while standard length was used as a covariable when making comparisons within a species. Surface area of all species was natural log transformed to achieve linearity as was the standard length of *Hemigymnus melapterus*.

3.3.7 CAPTURE OF FISH AND PARASITE COLLECTION

Fish were collected and parasites quantified as described in Chapter II. All fish were immediately placed in a plastic bag upon capture, then soaked in anaesthetic, and all liquids filtered to remove parasites. Because the thick mucus produced by *Chlorurus sordidus* blocks filters, for *C. sordidus* collected in January 1993 and June 1993, the plastic bag contents were filtered, and the whole fish scanned under a stereo microscope (20X) for parasites.

Although some parasites pass through the 200 μm filter, which are recovered with the 57 μm filter (Chapter II), the use of the 57 μm filter is very time consuming as it quickly blocks with fish mucus. Therefore due to time constraints imposed by the large sample size the 200 μm filter was used for the spatial (sites), temporal, and host size comparisons. An additional 57 μm filter was used in January 1993 (Lizard Island) and June 1993 (Heron Island) therefore the location comparisons are based on parasites removed by both filters.

Parasites were identified and placed into categories as described in Chapter II. Not all parasites were identified to species as the study was designed as a quantitative study rather than a qualitative study with the emphasis placed on estimating the spatial and temporal variability of ectoparasites. A large sample size was required, thus it was beyond the scope of this study to identify parasites that were found only 1-2 times during the study. In addition, the multivariate tests could not be carried out with numerous variables (parasite species) due to insufficient degrees of freedom nor with variables which

TABLE 3.1. Parasite categories and their codes used for classifying ectoparasites from 7 coral reef fishes. Bold headings are broad descriptions of categories. Size ranges of parasites are in brackets.

Copepoda	**Isopoda**	**Digenea**
HatH=*Hatschekia bemigymni* (280μm-1mm)	Gnat=gnathiid larvae (280μm-2.7mm)	TraL=*Transversotrema licinum* (370μm-1.7mm)
HatA=*Hatschekia* sp. a (1.9-2.3mm)	Isop=*Anilocra nemipteri* and *Anilocra* sp. juv. (4.2-23.0mm)	TraH=*Transversotrema haasi* (1.1-3.3mm)
Bomo=*Acanthocolax* sp. nov. & *Orbitacolax sp. nov.* males (540-710μm)	**Monogenea**	Tran=*Transversotrema* spp. (600μm)
Orbi=*Orbitacolax* sp. sp. nov. females (710μm-1.4mm)	Anop=*Anoplodiscus* spp. (310μm-1.6mm)	UDig=Other unidentified spp. (220μm-1.4mm)
Acan=*Acanthocolax* sp. nov. females (1.9-2.3mm)	Bene=Benedininae spp. (340μm-2.0mm)	**Turbellaria**
Cali=Caligidae spp. (1.4-2.5mm) See methods for species	Dact=Dactylogyridea spp. (170-600μm)	Turb=*Ichthyophaga* and or *Paravortex* spp. (110μm-1.3mm)
CalL=Caligidae larvae (220-600μm)		**Platyhelminthes**
Naup=Nauplii (140-200μm)		UFla=Unidentified flatworms (200μm-1.4mm)
UCop=Unidentified spp. (280μm-1.6mm)		

consisted mainly of zeros. Therefore as a consequence of the above constraints it was necessary to combine some parasites species into broader categories (Table 3.1).

The category Caliginae are *Pseudocaligus* sp. nov. (on *Acanthochromis polyacanthus*, *Siganus doliatus*, and *Scolopsis bilineatus*), male *Lepeophtheirus* sp. (*S. doliatus*), male *Caligus* sp. (*S. doliatus*, *Chlorurus sordidus*), and a male caligid on *C. sordidus* (Z. Kabata pers. comm.). Due to difficulties in separating *Orbitacolax* sp. nov. and *Acanthocolax* sp. nov. males, these were combined. Because adult caligids were relatively rare on all species with a prevalence of only 0-16% among all fish species, all were combined for the statistical analyses under the category Caliginae spp. Caligidae larvae and copepod nauplii were unidentifiable (Z. Kabata pers. comm.). The isopods are *Anilocra* sp. found on one individual of *Hemigymnus melapterus*, and *Anilocra nemipteri* (on *Scolopsis bilineatus*). Some of the dactylogyrideans were identified as ancyrocephaline (I. Whittington pers. comm.). Unidentified digenea consisted of *Gyliauchea* sp. and other larvae which could not be identified (T. Cribb pers. comm.). All reared gnathiids from Lizard Island were *Gnathia* spp. (B. Cohen pers. comm.) and the only reared gnathiid specimen from Heron Island belonged to *Caecognathia* sp. (G. Poore pers. comm.). These two genera plus *Elaphognathia* have been identified in the Great Barrier Reef (Cohen and Poore 1994), thus all were combined under gnathiids.

Fish surface area was used as a covariable when making comparisons across species to standardise for differences in fish body size among species. Surface area was measured by removing paired fins, pinning all fins and body onto waterproof paper, and drawing an outline of the fish. The area of the outline was measured using the software Framegrabber 3.2 and Image 1.4. For some specimens, surface area was estimated from standard length with linear regressions as they were highly correlated ($r>=0.95$). To estimate the error associated with using an outline of the fish rather than the actual surface area, the surface area of the least laterally compressed species, *Hemigymnus melapterus*, was measured with aluminium foil. The outline area was 28.9% (SE

Table 3.2. Two factor analysis of covariance of total number of parasites per fish from 7 fish species collected at 3 sites at Lizard Island with fish surface area (natural log transformed for linearity) as the covariable. Data are ln(x+1) transformed.

Source	df	F	p
Covariable: Area	1	14.69	<0.001
Species	6	53.53	<0.001
Site	2	1.23	0.295
S x S	12	0.80	0.654
Residual	141		

1.3) of the foil area. The species *Acanthochromis polyacanthus, Ctenochaetus striatus*, and *Siganus doliatus* are all very laterally compressed so the error is probably less, while the shapes of the remaining 3 species are similar to *H. melapterus*.

3.4 RESULTS

3.4.1 SPECIES AND SITES

The total number of parasites per fish was different among species (Table 3.2). The covariable, surface area, was significant indicating that it explained some of the variation, however after accounting for size, the differences among species remained (Table 3.2). The total number of parasites per fish was not affected by the local area of collection (Table 3.2), so the total numbers of parasites for each site were pooled for each species for graphical display (Fig. 3.2). The mean total number of parasites per fish differed markedly among species (Fig. 3. 2a). *Hemigymnus melapterus* had the most parasites, with a mean of 110 parasites per fish, *Siganus doliatus* and *Scolopsis bilineatus* had fewer, with slightly over 20 parasites per fish, and for *Thalassoma lunare*, *Chlorurus sordidus*, *Ctenochaetus striatus*, and *Acanthochromis polyacanthus* the numbers were lower with 1-5 parasites per fish (Fig. 3.2a). Although there was a trend for larger species to have more parasites than smaller species, the differences among species in parasite numbers were not entirely due to differences in standard length (Fig. 3.2a). Some larger species, such as *Chlorurus sordidus* and *Ctenochaetus striatus*, had few parasites compared to the somewhat smaller species *Scolopsis bilineatus* which had 5 times as many parasites (Fig. 3.2a).

The above relationship was preserved when numbers of parasites were adjusted for size with some minor variations for *Siganus doliatus* (Fig. 3.2b). Due to the large surface area of the laterally compressed *S. doliatus*, the relative numbers of parasites on *S. doliatus* were less with respect to the other species when they were expressed as parasites per surface area than as parasites per fish

Table 3.3. Multivariate factorial analysis of variance of numbers of parasites in all categories (except nauplii) from 7 fish species (*Hemigymnus melapterus*, *Siganus doliatus*, *Scolopsis bilineatus*, *Thalassoma lunare*, *Chlorurus sordidus*, *Ctenochaetus striatus* and *Acanthochromis polyacanthus*) collected at 3 sites. Data are ln(x+1) transformed. See Table 3.1 for parasite categories.

Source	Pillai's Trace	F	df	p
Site	0.345	1.56	34, 254	0.030
Species	3.157	8.56	102, 786	<0.001
S x S	1.694	1.33	204, 1644	<0.001

(Fig. 3.2). Thus the shape of the fish can influence the relative difference in parasite abundance among species when it is expressed as surface area. Surface area is not easily measured, however it is curvilinear to weight and can be easily estimated (ln area=2.19+0.67ln weight) (r=0.98).

There were differences in the types of parasites found on the seven species of fish (Table 3.3). The species by site interaction in the MANOVA was significant which shows that the differences among species were not consistent among sites (Table 3.3).

The differences among species and sites were discriminated using a canonical discriminant analysis (Fig. 3.3). Examination of the first three canonical discriminants, which described 89% of the variation, revealed that the parasite assemblages of *Hemigymnus melapterus*, *Scolopsis bilineatus*, and *Siganus doliatus* were very different from the remaining species. *Hemigymnus melapterus* was best characterised by *Hatschekia hemigymni* and gnathiids; *S. bilineatus* by *Transversotrema licinum*, *Anoplodiscus* sp., and *Acanthocolax* sp. nov.; and *S. doliatus* by Dactylogyridea spp. The variation due to sites was minor and was mainly attributable to relatively rare species present at some sites and not others (Unidentified flatworms, *Hatschekia* sp. a, and Caliginae spp.) or to unidentified digenea which are probably internal parasites released post mortem (T. H. Cribb pers. comm.).

Because the differences among sites within a species were small compared with the species differences, the number of parasites per category were combined across the 3 sites to summarise graphically the overall abundance of the different parasites on each species (Fig. 3.4). The number of parasites in each category varied among species, with usually one or two parasite types dominating the parasite assemblage of a species (Fig. 3.4). Several types of parasites were common to most fish species at Lizard Island. Gnathiid isopods were found on all species and were often one of the most abundant parasites (Fig. 3.4). Turbellaria, although not very abundant, were found on *Hemigymnus melapterus*, *Siganus doliatus*, *Scolopsis bilineatus*, and *Acanthochromis polyacanthus* (Fig. 3.4a-c, g); unidentified Digenea spp. and

Table 3.4. Two factor analysis of covariance of total numbers of parasites from 7 fish species collected at Lizard Island (January 1993) and Heron Island (June 1993) with fish surface area as the covariable. Parasite numbers were ln(x+1) transformed, surface area was natural log transformed.

Source	df	F	p
Covariable: Area	1	29.24	<0.001
Location	1	11.13	<0.001
Species	6	42.06	<0.001
L x S	6	6.83	<0.001
Residual	83		

Table 3.5. Multivariate factorial analysis of variance of numbers of parasites in all categories (*Transversotrema* spp. were pooled) from 7 fish species (*Hemigymnus melapterus*, *Siganus doliatus*, *Scolopsis bilineatus*, *Thalassoma lunare*, *Chlorurus sordidus*, *Ctenochaetus striatus*, and *Acanthochromis polyacanthus*) collected at Lizard Island and Heron Island. Data are ln(x+1) transformed.

Source	Pillai's Trace	F	df	p
Location	0.695	8.48	18, 67	<0.001
Species	3.523	5.69	108, 432	<0.001
L x S	2.409	2.68	108, 432	<0.001

unidentified flatworms were found on all species except *Ctenochaetus striatus* (Fig. 3.4a-e,g).

The number of parasite categories per species varied among the fish species at Lizard Island. Fish species with more total parasites per fish (*Hemigymnus melapterus*, *Siganus doliatus*, and *Scolopsis bilineatus*) had more parasite categories (10-13) per species (Fig. 3.4a-c). The fish species with few parasites (*Thalassoma lunare*, *Ctenochaetus striatus*, and *Acanthochromis polyacanthus*) had few parasite categories (5-6)(Fig. 3.4d, f, g), except for *Chlorurus sordidus* which had few parasites but had many (10) types of parasites (Fig. 3.4e).

3.4.2 LOCATIONS

The total number of parasites per fish was different among locations and species (Table 3.4). The covariable, surface area, was significant which indicates that it was responsible for some of the variation among locations and species (Table 3.4). The significant interaction term in the ANOVA was due to *Hemigymnus melapterus* which had many more parasites at Heron Island than at Lizard Island and to *Siganus doliatus* and *Scolopsis bilineatus* which had more parasites at Lizard Island than Heron Island (Fig. 3.5).

The number of parasites per taxonomic category was also significantly different between locations and among species (Table 3.5). The significant interaction term indicates that the differences in parasite categories were not consistent over locations (Table 3.5). A canonical discriminant analysis was used to discriminate between species and locations (Fig. 3.6). The first three canonical discriminants, which explained 87% of the variation, revealed that the parasite assemblages of *Scolopsis bilineatus*, and especially *Hemigymnus melapterus* and *Siganus doliatus* from Lizard Island were different from the parasite assemblages of the same species from Heron Island. Most of the centroid means on the 3 canonical discriminants were the same sign, within a species, for the above 3 species. This indicates that the differences between locations for each species were due more to differences in number of parasites

in a category than to differences in overall species composition. This is also demonstrated by comparing the numbers of parasites in each category between locations in Figure 3.7. There were more *Hatschekia hemigymni* and copepod nauplii on *Hemigymnus melapterus* collected from Heron Island than Lizard Island (Fig. 3.7a) and fewer dactylogyrideans and gnathiids on *Siganus doliatus* collected from Heron Island than Lizard Island (Fig. 3.7b). For *Scolopsis bilineatus*, the differences are not as obvious but fish from Lizard Island had more categories and more parasites in the categories found at both places (Fig. 3.7c).

All fish species from Lizard Island had more parasite categories than those at Heron Island (Fig. 3.7). It is unlikely that this is a result of placing parasites from Heron Island into broader categories, such as unidentified Digenea spp., unidentified Copepoda spp., and unidentified flatworms, as these categories rarely had more parasites at Heron Island (Fig. 3.7). Most of the parasite categories found on fish from Heron Island were the same as those at Lizard Island. However one obvious exception was that *Transversotrema haasi* were found on most *Chlorurus sordidus* at Heron Island but never on any collected at Lizard Island (Fig. 3.7e & 3.4e).

3.4.3 TIME

Only *Siganus doliatus* had a significant difference in the total parasites per fish among collection times (ANCOVA $F=13.87$, $df=1,26$, $p<0.001$). The mean parasites per fish (SE) increased in May 1992 from 27.2 (3.7) to 177.2 (90.6) in January 1993, mainly due to an increase in Dactylogyridea spp. Therefore, for *S. doliatus*, only the data from May was used to investigate the relationship between parasite numbers and host length. The total number of parasites per fish for the other species was not different among collection times (ANCOVA *Acanthochromis polyacanthus*: $F=0.46$, $df=1,27$, $p=0.052$; *Ctenochaetus striatus*: $F=0.10$, $df=1,23$, $p=0.759$; *Scolopsis bilineatus*: $F=0.02$, $df=1,28$, $p=0.892$; *Chlorurus sordidus:* $F=0.09$, $df=1,24$, $p=0.772$; *Thalassoma lunare*: $F=0.10$, $df=2,43$, $p=0.907$; *Hemigymnus melapterus:* $F=0.19$, $df=2,55$,

p=0.825) and thus were pooled over times to investigate the parasite number/host size relationships of each species. The number of parasites per parasite category were not significantly different among collection times for *H. melapterus* (MANOVA Pillai's Trace=0.483, F=1.42, df=14,62, p=0.175) and *T. lunare* (MANOVA Pillai's Trace=0.163, F=1.95, df=4,88, p=0.109).

3.4.4 FISH SIZE

There was a positive correlation between the total number of parasites per fish and standard length for the species *Hemigymnus melapterus*, *Scolopsis bilineatus*, and *Ctenochaetus striatus* but not for *Acanthochromis polyacanthus*, *Siganus doliatus*, *Chlorurus sordidus*, and *Thalassoma lunare* (Fig. 3.8).

3.5 DISCUSSION

The study demonstrates that most of the fish species investigated have a species specific parasitic fauna whose abundance and composition is conserved over space and time. Although these fish species coexist in similar habitats their parasitic fauna varies greatly among species and mirrors the high diversity of coral reef fish species. Even taxonomically related species such as *Hemigymnus melapterus*, *Thalassoma lunare*, and *Chlorurus sordidus* had very different parasite assemblages. The differences observed in the parasitic faunas may be influenced by differences among species in the life span, mobility, gregarious habits, and the size of the host (Polyanski 1961a).

A large fluctuation in the parasite population of *Siganus doliatus* occurred at Lizard Island. Dactylogyridean monogeneans on *S. doliatus* increased 7 fold from May 1992 to January 1993. It is well known that monogenean populations in cold waters are seasonal (Llewellyn 1959, Kennedy 1975). This study shows temporal fluctuations of monogeneans can also occur in the tropics. Such temporal variability in parasites may have important implications for host fish. For example, if host cleaning is influenced by parasite

load then it is possible that changes in parasites will be reflected in how often host fish are cleaned. It may also be representative of punctuated selection that may occur for host fish to respond to cleaner fish. Finally, it emphasises the need for repeated sampling at different times.

Gnathiids were common among all the fish species investigated, and has also been found on 12 out of 25 other coral reef fish species examined (n=218)(pers. obs.). Their mobility (Davies and Johnston 1976, Chapter 1) and their life history, which involves leaving the host to moult 3 times (Wägele 1988), may explain their low host specificity.

Although Lizard Island and Heron Island are over 1000 km apart and fish were collected at different times, the relative patterns of parasite abundance among fish species were similar between the two locations. The same fish species had low parasite loads at both locations. The largest differences were due to 1-2 parasite types being more abundant at one location than the other. For *Siganus doliatus* the difference between locations was probably an effect of time rather than location. Dactylogyridea spp. on *S. doliatus* from Lizard Island increased between May 1992 and January 1993 at Lizard Island while the numbers of Dactylogyridea spp. on fish from Heron Island in June 1993 are similar to those collected at Lizard Island in May 1992.

The fact that the numbers of parasite categories per fish species are lower at Heron Island than at Lizard Island may be due to latitudinal and or temperature differences, as it has been shown that fish from high latitudes have fewer types of parasites (Dogiel 1961, Polyanski 1961b, Rohde 1994). Most of the parasites found on fish at Heron Island were found on fish from Lizard Island except for some monogenea. Byrnes and Rohde (1992) also found that the geographical distributions of copepods and isopods among *Acanthopagrus* spp. (Sparidae) were very wide while monogeneans were more restricted. Because the comparison presented here is confounded by time no definite conclusions can be made about a location effect on the parasite assemblages. However, the comparison is still useful as it shows how similar the parasite assemblages are between these two distant locations.

The relationship between parasite load and host size varied between fish species with *Hemigymnus melapterus* showing the strongest positive correlation, mainly due to the gill-inhabiting copepod *Hatschekia hemigymni*. Gill surface area also increases with size of fish (Hughes 1966), therefore the parasite load of *H. melapterus* species is more specifically a function of gill size. Larger fishes often have higher numbers of parasites (Noble *et al.* 1963, Bortone 1971, Cressey and Collete 1971, Buchmann 1989).

The fish species that had no clear correlation of parasite load with host size had either few parasites or were collected from a narrow size range which may have obscured the relationship between host size and parasite abundance. The absence of a positive correlation between parasite abundance and host size may also be a result of older hosts developing immunity to infestation (Noble *et al.* 1963) or due to the specialist/generalist nature of parasites (Cressey & Collette 1971). The wide variety of parasites included in this analysis may also have obscured relationships between specific parasitic types and host size. The large differences in parasite load and species composition among species raise the questions of why such large differences occur in co-existing species, how they are reflected in cleaning behaviour, and how the patterns are conserved over space and time.

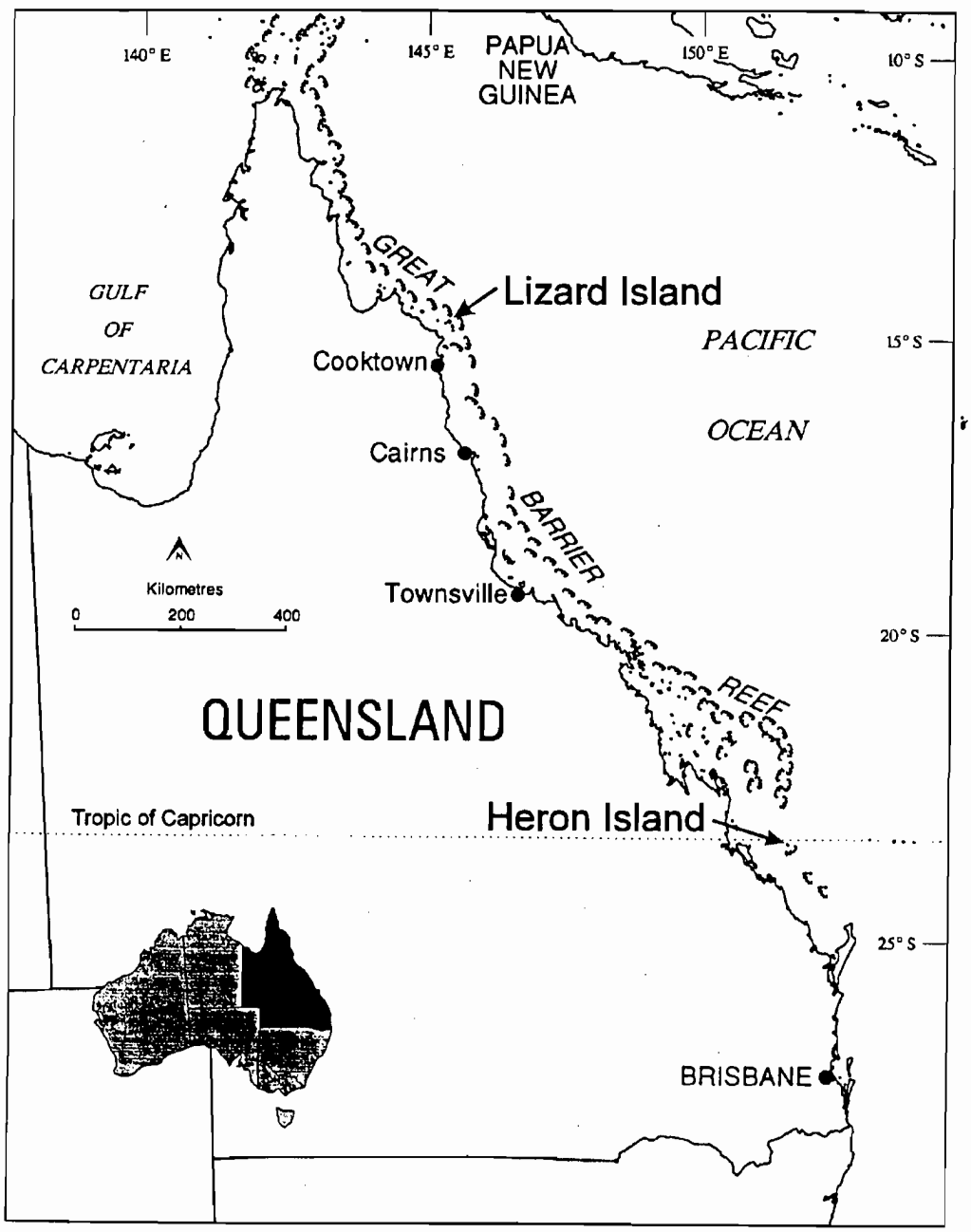

Figure 3.1. Map of Queensland, Australia showing the locations of Lizard Island and Heron Island.

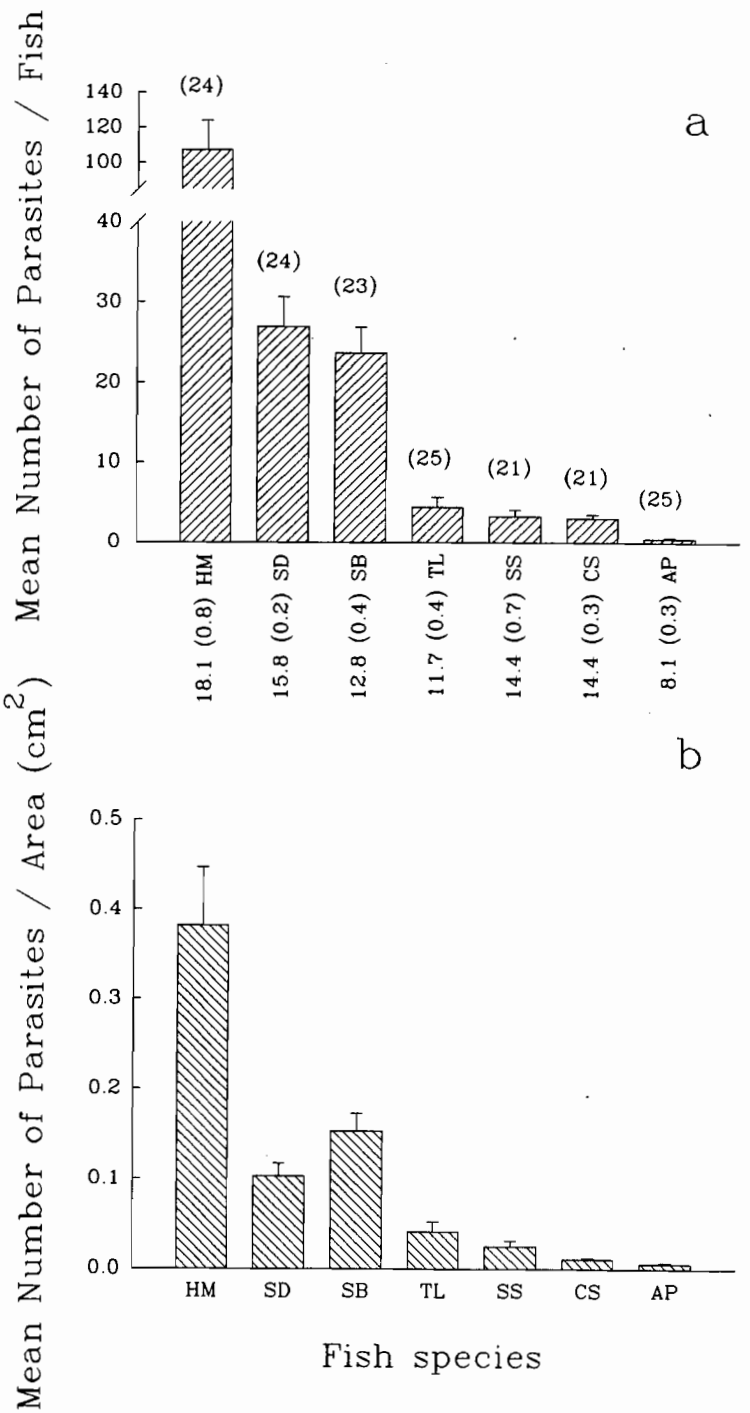

Figure 3.2. The mean number of parasites (±SE) per fish species combined from fish collected at 3 sites at Lizard Island in May 1992. **a**. The mean number of parasites per species. The sample size of each species are in brackets above the means. The mean standard length (cm) (SE) of each fish species is on the x-axis. **b**. The mean number of parasites per estimated unit surface area. AP=*Acanthochromis polyacanthus*, CS=*Ctenochaetus striatus*, SS=*Chlorurus sordidus*, TL=*Thalassoma lunare*, SB=*Scolopsis bilineatus*, HM=*Hemigymnus melapterus*, SD=*Siganus doliatus*.

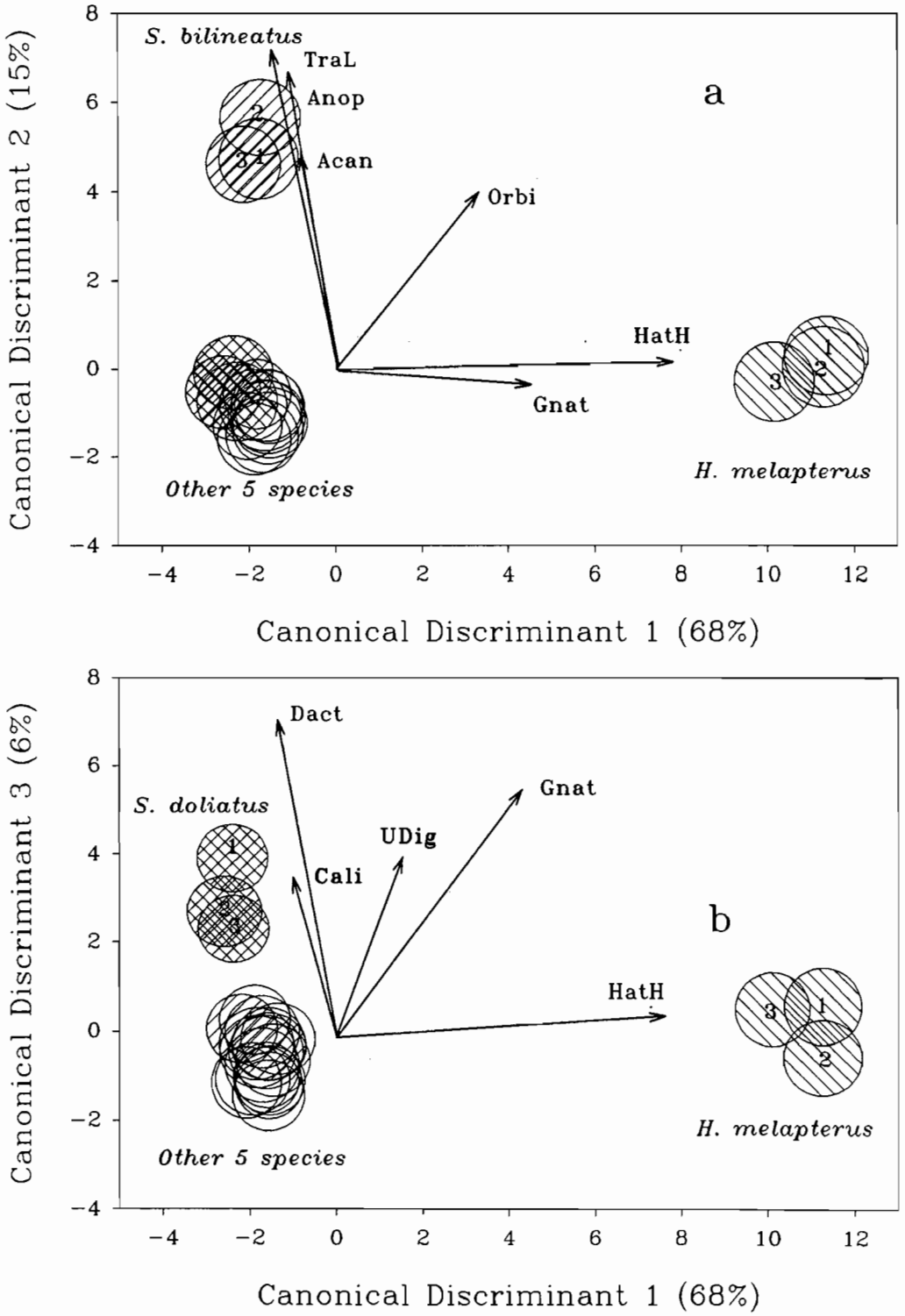

Figure 3.3. Biplot of centroid means with associated 95% confidence intervals (circles) from the canonical discriminant analysis of parasite categories from seven fish species collected at 3 sites at Lizard Island. Vectors represent parasites which discriminate the centroid means (See Table 3.1 for definitions of parasite codes). The variables were composed of all the parasite categories (Table 3.1) except nauplii, all *Transversotrema* spp. were pooled. **a.** Canonical discriminants 1 and 2. **b.** Canonical discriminants 1 and 3. Data are ln(x+1) transformed. Sites are only labelled (1-3) for some species.

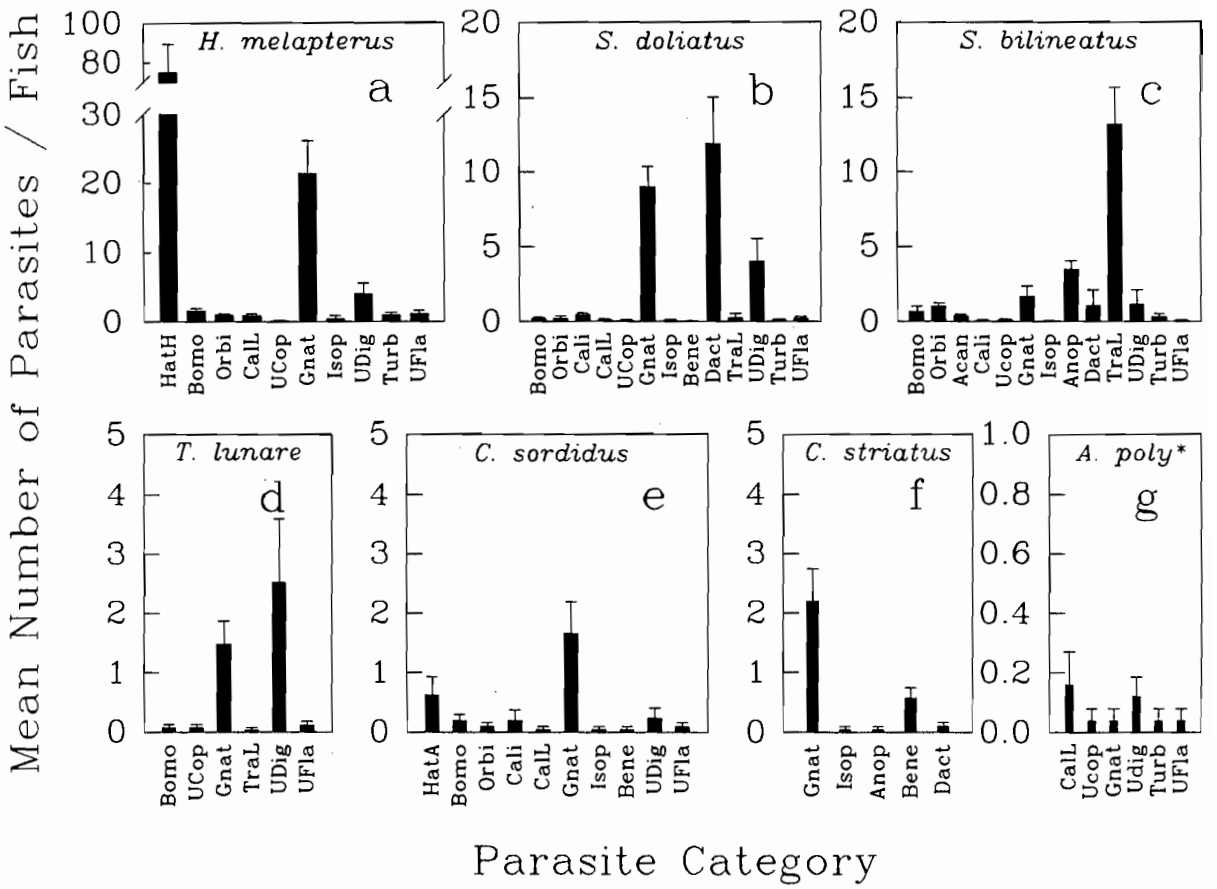

Figure 3.4. The mean number of parasites (±SE) per taxonomic category from fish collected at 3 sites (pooled) at Lizard Island in May 1992. **Acanthochromis polyacanthus*. See Table 3.1 for definitions of parasite categories.

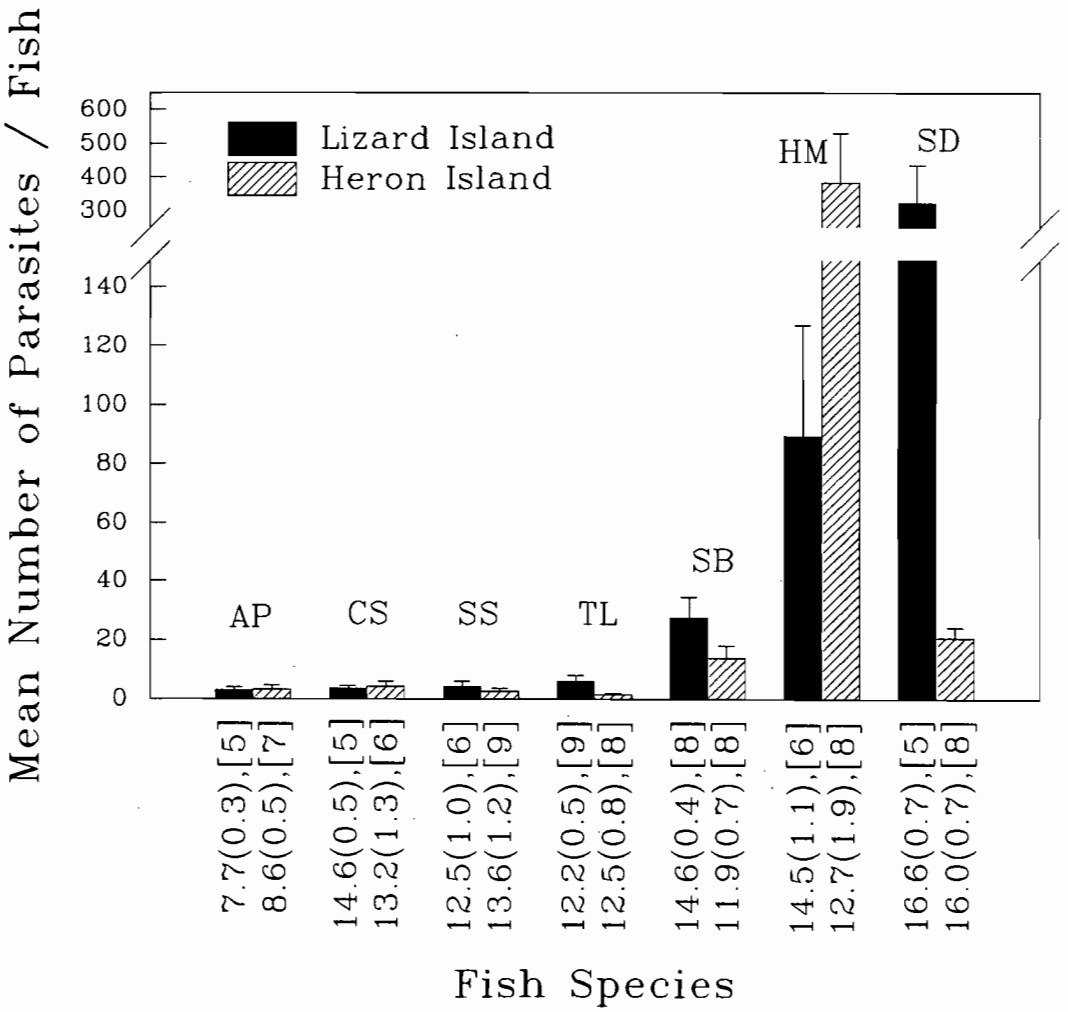

Figure 3.5. The mean number of parasites (±SE) per fish collected at Lizard Island and Heron Island. The mean standard length (cm) (SE), [sample size] of each species is given. AP=*Acanthochromis polyacanthus*, CS=*Ctenochaetus striatus*, SS=*Chlorurus sordidus*, TL=*Thalassoma lunare*, SB=*Scolopsis bilineatus*, HM=*Hemigymnus melapterus*, SD=*Siganus doliatus*.

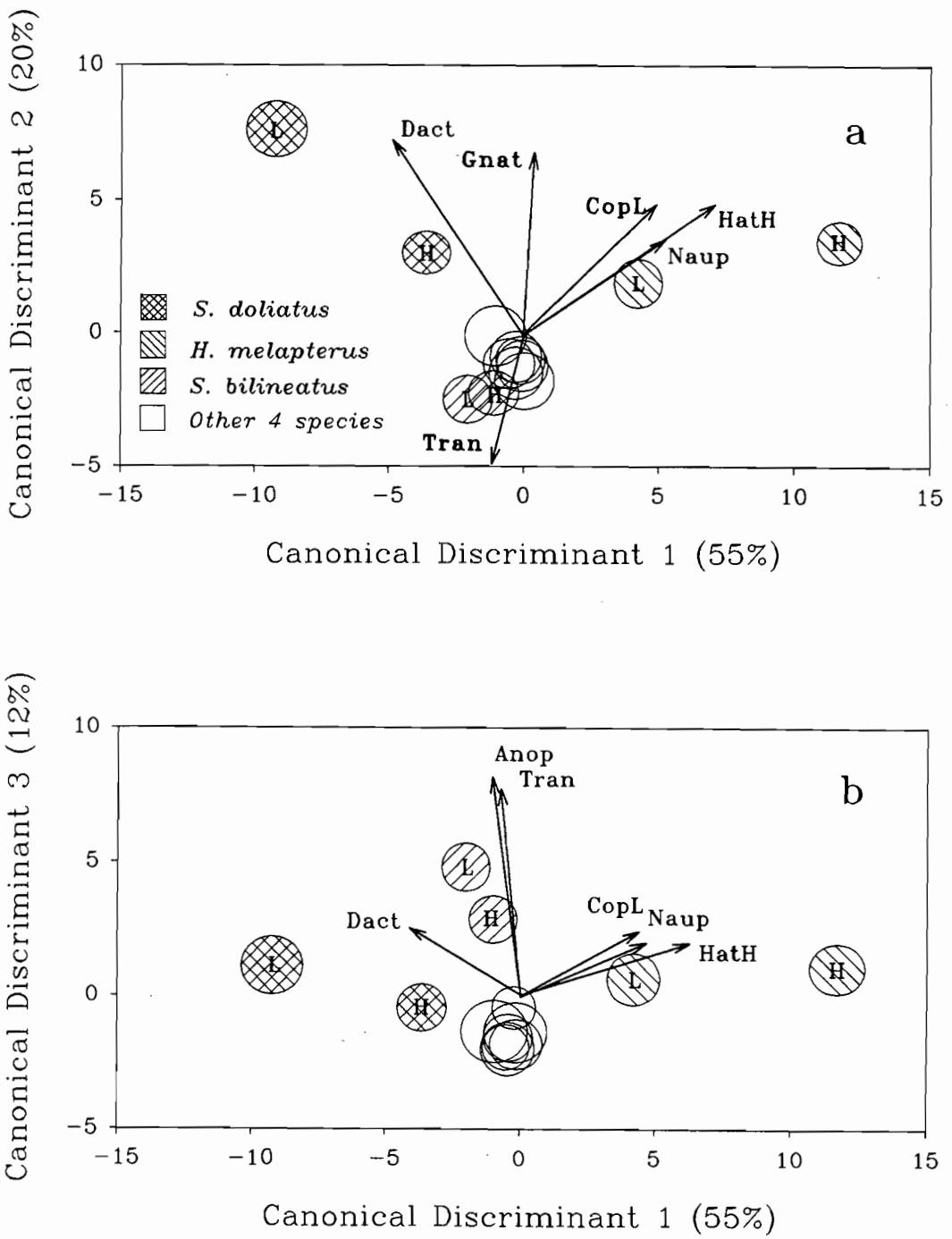

Figure 3.6. Biplot of centroid means with 95% confidence intervals (circles) from the canonical discriminant analysis of parasite categories on seven fish species collected at Lizard Island and Heron Island. Variables which discriminate the centroid means are represented by vectors (See Table 3.1 for definition of variables). The variables were composed of all parasite categories (Table 3.1), all *Transversotrema* spp. were pooled. a. Canonical discriminants 1 and 2. b. Canonical discriminants 1 and 3. Data are ln(x+1) transformed. L=Lizard Island, H=Heron Island.

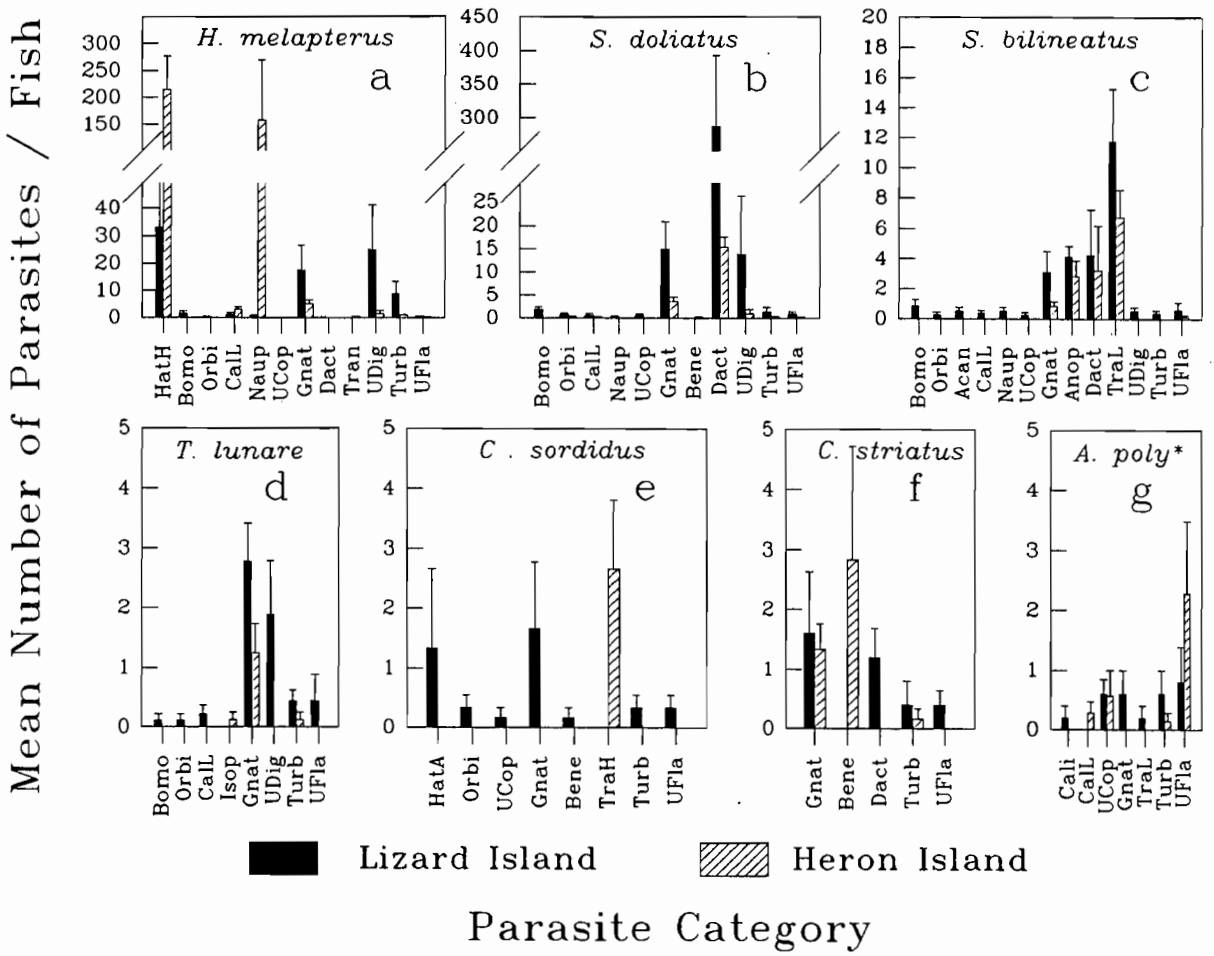

Figure 3.7. The mean number of parasites (±SE) per parasite category from fish collected at Lizard Island and Heron Island. **Acanthochromis polyacanthus*. See Table 3.1 for definitions of parasite categories.

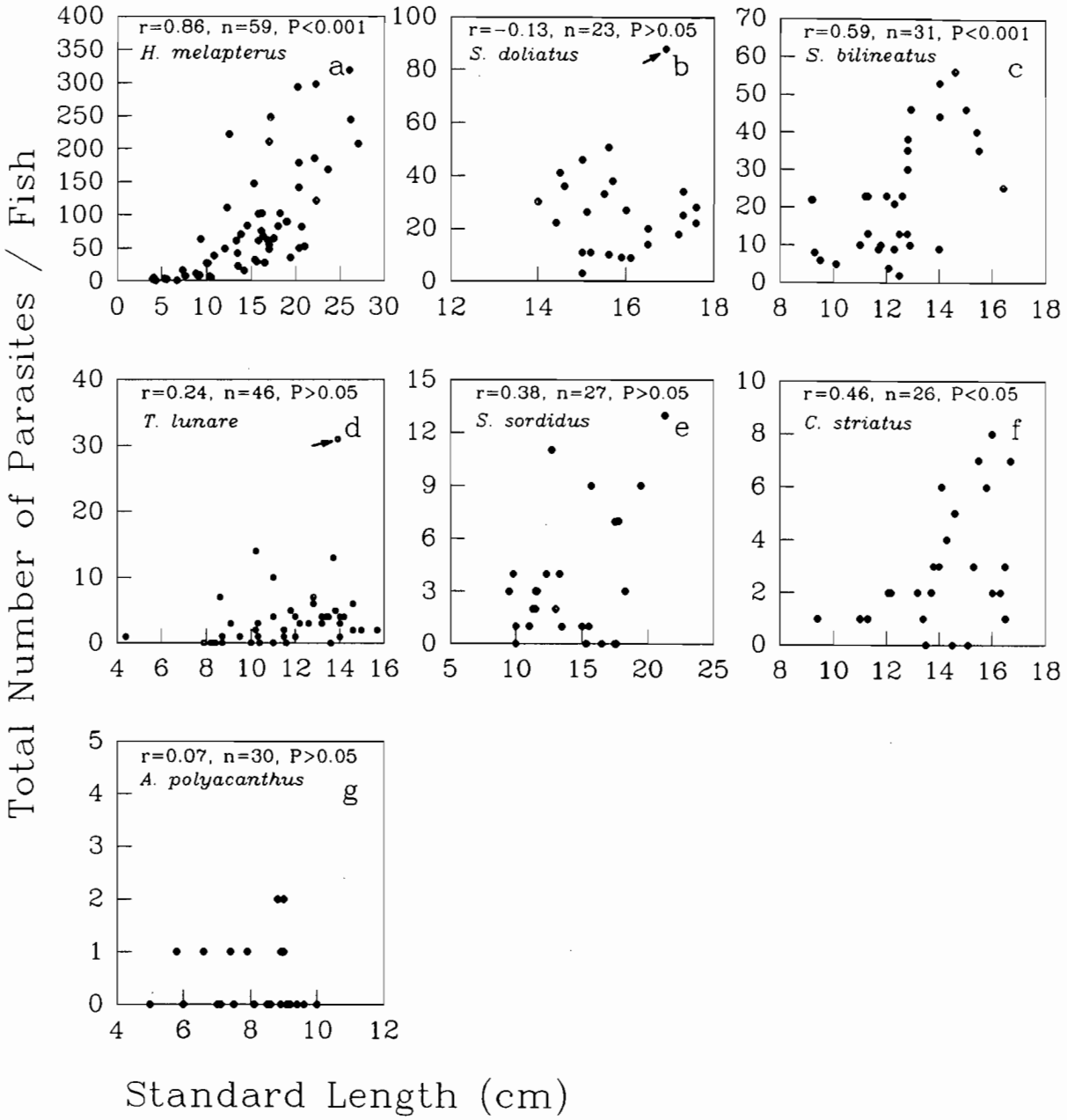

Figure 3.8. The total number of parasites per fish against standard length of fish, with the correlation coefficient (r), the sample size (n), and significance level of the correlation for each species. The parasites of *Hemigymnus melapterus* were (ln(x+1)) transformed and the standard length natural log transformed to satisfy the assumption of linearity in the correlation analysis. Outliers ($p<0.05$) were not included in the analysis (labelled with an arrow).

CHAPTER IV

THE DIET OF *LABROIDES DIMIDIATUS*

4.1 SUMMARY

The diet of the cleaner wrasse *Labroides dimidiatus* was examined by analysing alimentary tract contents of individuals collected from 2-3 sites at Lizard Island and Heron Island. Diets consisted of mixtures of individual prey species and their parts, and unidentified organic matter. Dietary trends were evaluated by counting the number of prey individuals and by estimating the total amount of each dietary category present (the amount of each food category was estimated by measuring its two-dimensional cover in sorting trays). Parasitic gnathiid isopods made up the greatest amount of food (50% cover) and were the most numerous prey items (95% of all identified individuals). The remaining 50% of the food material was mainly unidentified organic matter. The greatest difference in the diet occurred between the two locations, with only small differences among sites at Lizard Island. When converted to biomass, the total amount of gnathiid isopods in the diet was four times higher at Lizard Island than Heron Island. Fish from Lizard Island also contained more caligid larvae and other parasitic copepods. In contrast, fish from Heron Island contained more non-parasitic copepods and mucus. Temporal dietary trends were followed at Lizard Island. The number and estimated biomass of gnathiids more than doubled from May to January while the size of gnathiids decreased during this time. The size of gnathiids on the body of a host fish species also decreased during this time. However the difference in size of gnathiids on the fish and in the diet suggests that *L. dimidiatus* may select larger gnathiids at Lizard Island but not at Heron Island. Variability in the diet implies both spatial and temporal flexibility in the foraging patterns of *L. dimidiatus*.

4.2 INTRODUCTION

Information on the diet of cleaner fish is essential to understanding cleaner fish-host fish interactions with the importance of parasites in the diet playing a significant role in interpretations of cleaning interactions (Losey 1974).

There is variability in the abundance of parasites in the diet among cleaner fish species (Youngbluth 1968, Losey 1974, Chikasue 1990). In addition, cleaner fish feed both on ectoparasites and non-parasitic sources such as host tissues, scales (Youngbluth 1968), fish eggs, zooplankton (Losey 1979), and mucus (Gorlick 1980). In Hawaii, where parasite loads are low (Losey 1972, 1974), mucus is important in the feeding behaviour and diet of cleaner fish (Gorlick 1980). Such variation in the abundance of parasites in the diet among cleaner fish species, in combination with other factors such as interactions between cleaner fish and host fish, has been used to make conclusions about the nature of cleaning relationships (Losey 1972, 1974). Thus, cleaning interactions which involve high predation rates on parasites may be mutualistic (Losey 1974) while cleaner fish which largely feed on mucus may have a commensal or even parasitic relationship with their hosts (Gorlick 1980). These comparisons however have not been made using the same cleaner fish species.

The species composition of parasites on hosts, when compared to those in the diet of cleaner fish, provide a measure of the feeding selectivity of the cleaner wrasse *Labroides dimidiatus* (Labridae). Variation in the number and species composition of parasites has been examined for several fish species among sites at Lizard Island and between Heron Island and Lizard Island (Chapter III). By sampling the diet of *Labroides dimidiatus* at the same sites and locations as the host fish a comparison of the composition of the diet with the parasites on fish can be made.

Temporal and small scale spatial variation can confound comparisons at larger spatial scales. Temporal variation is important in studies involving parasites as they undergo temporal fluctuations (Rawson 1976, Chapter III). These fluctuations are often related to their life history (Rawson 1976), therefore, the size of a common parasite in the diet of cleaner fish (gnathiid isopods) is measured over time. The size of gnathiids on a host fish species is also measured to determine whether cleaner fish select parasites based on their size.

A method that combines estimates of the number and the bulk of a food

category provide the best measure of dietary importance (Hyslop 1980). Analyses of the diet of cleaner fish to date have only used the number of individuals in each food category (Youngbluth 1968, Chikasue 1990). However, mucus, which is amorphous and cannot be counted in discrete units, can be an important food source for cleaner fish (Gorlick 1980). Therefore, in addition to numerical counts, the abundance of each food category is estimated using the two dimensional cover of items on sampling trays. In addition, numerical estimates of gnathiids in the diet are converted to biomass (weight). Finally, the extent of digestion of parasites is quantified to determine whether digestion rates are constant over space and time.

The cleaner wrasse *Labroides dimidiatus* is the most widespread of the genus ranging from Africa to the tropical Pacific (Randall 1958). Information on its diet in Japan is available (Chikasue 1990). Furthermore, it is found at both extremes of the Great Barrier Reef. All these factors make it ideal for regional comparisons.

This study investigates variation in the diet of *Labroides dimidiatus*. The questions asked are: What is the composition of the diet? Is there variation in the diet among sites within each reef? Does the diet vary between Lizard Island and Heron Island? Is there temporal change in the diet throughout the year? Finally, is the variation in the diet related to the size of parasites found in the diet and on host fish or is it due to variation in digestion rates?

4.3 METHODS AND MATERIALS

4.3.1 SPATIAL AND TEMPORAL VARIATION IN THE DIET

Spatial variation in the diet of *Labroides dimidiatus* was examined at two scales, within a reef system (Lizard Island and Heron Island) and between reef systems (Lizard Island and Heron Island). Small scale variation was measured at 3 sites (North Point, Granite Reef, Lagoon Fig. 2.1) at Lizard Island in August 1992 and at 2 sites (Shallow Lagoon and Petra's Bommie) at Heron Island in June 1993. The sites at both locations are 1-5 km apart in shallow coral reefs (2-

7 m) and have different levels of wave exposure. The differences among sites at Lizard Island are reflected in the fish fauna (Choat and Bellwood 1985). Temporal variation in the diet of *L. dimidiatus* at Lizard Island was investigated at several month intervals. As there was evidence that variation in the diet among sites was small, fish for the temporal and large spatial scale comparisons were collected only at North Point.

4.3.2 FISH COLLECTION

All *Labroides dimidiatus* were collected using a 1.5 x 1 m barrier net and handnet (10 mm mesh) and placed in a small quick-seal plastic bag. Fish were immediately fixed underwater by filling the gut cavity with 20% formalin in filtered saltwater (57 μm) using a 25G x 16 mm needle and 1 ml syringe. The whole fish was fixed 1-2 hrs later in 10% formalin in saltwater. All collections were made between the hours 15:00-17:00, except those in October, which were made between the hours 08:30-09:30. The number of items in the diet at these two times has been found to be similar (Chapter VI). *Hemigymnus melapterus* (Labridae) were collected following Chapter III.

4.3.3 DIET ANALYSES

Both the number and two dimensional cover of items in sampling trays were quantified. Gut contents were placed in the following categories: gnathiid isopod larvae, Caligidae copepoda, caligid larvae, other parasitic copepoda spp., non-parasitic copepoda (benthic and planktonic), scales, other items, mucus, and digested material. The number of items in all the above categories (except mucus and digested material) were counted using a sorting tray. Gnathiids were easily identified as they have a distinctly shaped head. Thus gnathiid heads, with or without an attached body, were used to estimate the number of gnathiids in the diet. The amount of different food items in the diet was estimated by line transects and expressed as two dimensional food cover, providing an unbiased estimator of the fraction of the total area covered by these food items. Areal density (cover) was estimated by placing a straight line, the transect, at random

under the area to be surveyed and the intersections of gut contents measured. A pilot study was used to select the area of dish and the number and length of transects needed to detect all food categories. Cover was estimated with 20 1cm transects on a 2.7 cm diameter sampling dish and the 20 transects summed. To prevent clumping of items, the contents of the dish were shaken regularly. The absolute values of cover were used in the statistical analyses in order to detect any differences in the total amount of gut content.

4.3.4 GNATHIID SIZES

As most gnathiids were partially digested, the length of gnathiids in the diet was estimated from the heads which remained intact. The widths of ten randomly selected gnathiid heads were measured. For comparison, the length (not including uropods) of gnathiids collected from the fish species *Hemigymnus melapterus* was measured under a stereo microscope at 35X . *Hemigymnus melapterus* were collected from all the above sites, however sites were pooled to increase the sample size for the statistical analyses. All gnathiids were placed in 4 size classes (<1.10mm, 1.10-1.39mm, 1.40-1.69mm, >1.7mm) for statistical analyses.

4.3.5 GNATHIID BIOMASS

Total gnathiid weight was used to estimate gnathiid biomass in the diet of *Labroides dimidiatus* among locations and times. The number of gnathiids per size class was estimated by multiplying the proportion of gnathiids per size class (from the head width measurements) by the mean total number of gnathiids counted in the diet. This number was then multiplied by the median weight of gnathiids in each size class to obtain biomass. The weight of gnathiids was estimated from their length. Gnathiids collected from the body of *Hemigymnus melapterus* were placed into 5 size classes and weighed to the nearest 0.01 mg. Individual specimens (fixed in 10% formalin in seawater) were too light to weigh separately and were weighed together for each size class. A linear regression was calculated using the mean weight and mean length

(natural log transformed) of gnathiids.

4.3.6 EXTENT OF DIGESTION OF GNATHIIDS

Variation in the extent of digestion of gnathiids was examined among locations and months in order to asses whether it contributed to changes in the diet. The category gnathiid was subdivided into whole gnathiid, head plus part of body still attached, and head only and the subcategories summed and tested using chi-square analysis of homogeneity.

4.3.7 STATISTICAL ANALYSES

The total number and cover of items were tested for differences among sites, locations, and months with separate one way analyses of variance (ANOVA) as not all sites were sampled at each time. To test for differences in the composition of the diet among sites, locations, and months the cover and number of items per food category were tested with separate multivariate analyses of variance (MANOVA). Significant MANOVAs were examined and interpreted with canonical discriminant analysis. Data were transformed [ln (x+1)] to satisfy the assumptions of the multivariate analyses. The category mucus was not included as a variable in the tests among sites nor time as its presence in the diet was only 12.5% and 18.7% respectively. The lengths of gnathiids in the diet and on the body of *Hemigymnus melapterus* were placed into 4 size classes and tested for differences among locations with chi-square analysis of homogeneity. Similarly, to test for differences in size distributions of gnathiids among times and source (in diet or on body) a multiway frequency (loglinear) analysis was used (Tabachnick and Fidell 1989). See previous section for statistical analyses of the extent of digestion of gnathiids among locations and times.

4.4 RESULTS

Gnathiid isopod larvae were the most numerically abundant items in the

diet of *L. dimidiatus* at Lizard Island (76-99% among months) and Heron Island (76%). These parasites (0.7-2.9mm in length), which are parasitic only as larvae, can only be identified from adult males and were therefore only identified to family (Holdich and Harrison 1980). However, it is likely that they belong to *Elaphognathia*, *Gnathia* or *Caecognathia* as only these genera have been found in the Great Barrier Reef (Cohen and Poore 1994). The latter two genera have been identified from adult males reared from larvae collected from host fish (Chapter III). The copepoda included one individual *Hatschekia* sp., several bomolochids and penellids, and other partially digested unidentified copepods which ranged from 0.2-4.8mm. Different types of scales were found from a wide size range (0.2-3.1mm). Other items in the gut included copepod egg cases, unidentified white hard material (coral?), algae, a larval fish, a tanaid crustacean, and skin (0.3-3.7mm in length). Non-parasitic copepods were almost all benthic harpactacoid copepods which ranged in size from 0.3-0.7mm. Digested material was an aggregation of small brown particles. The standard length of *Labroides dimidiatus* ranged from 43-65 mm at Lizard Island and from 43-77mm at Heron Island.

4.4.1 COMPARISON OF DIET AMONG SITES

At Lizard Island, there were no differences in the total number of items ($F=0.789$, $df=2,21$, $p=0.467$) and the total cover of items ($F=0.502$, $df=2,21$, $p=0.613$) in the diet among sites (Fig. 4.1). Thus *Labroides dimidiatus* had similar total quantities of food in their gut at each site. The number of items per food category differed among sites at Lizard Island, which when examined with a canonical discriminant analysis (not displayed), was found to be mainly due to more caligid larvae and other copepods at the Granite Reef site and more scales at the Lagoon site (Pillai's Trace=0.974, $F=2.171$, $df=14,32$, $p=0.034$) (Fig. 4.2a). There were no differences in the cover per category among sites (Pillai's Trace=0.976, $F=1.754$, $df=16,30$, $p=0.090$) (Fig. 4.2b).

The total number of items in the diet at Heron Island did not differ among sites ($F=0.179$, $df=1,15$, $p=0.683$), neither did the total cover of items

(F=0.793, df=1,15, p=0.397) which was similar to the pattern observed at Lizard Island. The number of items per food category (Pillai's Trace=0.527, F=1.557, df=7,9, p=0.263) and the cover of each food category (Pillai's Trace=0.497, F=0.991, df=8,8, p=0.505) also did not differ among sites. Therefore, all sites at Heron Island were pooled for the comparisons among locations.

4.4.2 COMPARISON OF DIET BETWEEN LOCATIONS

Although fish were collected during the same season (May at Lizard Island and June at Heron Island) they were collected 1 year apart, therefore the comparison is confounded by year. The total number of items in the diet was not different among locations (F=1.406, df=1,23, p=0.248) nor was the total cover of items (F=1.837, df=1,23, p=0.188). Thus fish had similar quantities of food in their gut at both locations (Fig. 4.3). There were no differences in the number of items per food category among locations (Pillai's Trace=0.309, F=1.087, df=7,17, p=0.413). The cover per food category, however, differed among locations with more gnathiids, caligid larvae, and other parasitic copepods at Lizard Island and more non-parasitic copepods and mucus at Heron Island (Pillai's Trace=0.646, F=3.856, df=9,19, p=0.006)(Fig. 4.4).

4.4.3 COMPARISON OF DIET AMONG TIMES

The total number of items in the diet differed markedly among months (F=4.073, df=3,28, p=0.016) and more than doubled between May and January (Fig. 4.5). This increase was largely due to an increase in gnathiids (Fig. 4.6). The total cover of items differed among months (F=4.932, df=3,28, p=0.007) with an increase in October (Fig. 4.5). This increase was a result of more digested material in the diet (Fig. 4.6b) (fish in October were collected in the morning rather than in the afternoon). The number of items per category differed among times (Pillai's Trace=1.089, F=1.956, df=21,72, p=0.019). When examined with canonical discriminant analysis (not displayed), most of the variation was found to occur in May which was characterised by fewer gnathiid

Table 4.1. Tests of significance (partial likelihood ratio X^2) for a three-way frequency (log linear) analysis testing for differences among the size of gnathiids in the diet of *Labroides dimidiatus* and on the body of *Hemigymnus melapterus* collected at three times (total n=1046).

Source	df	Chi-Square	p
Time	2	155.41	<0.001
Source of gnathiid	1	81.39	<0.001
Time x Source of gnathiid	2	15.68	<0.001
Size class	3	23.26	<0.001
Time x Size class	6	60.26	<0.001
Source of gnathiid x Size class	3	91.71	<0.001
Likelihood ratio	6	12.32	0.055

isopods and slightly more other parasitic copepods and scales (Fig. 4.6a). The composition of the cover of items was not significantly different among months (Pillai's Trace=0.772, F=0.995, df=24,69, p=0.484) (Fig. 4.6b).

4.4.4 GNATHIID SIZES

The length of gnathiids in the diet was estimated from the head width of gnathiids using the equation length (mm)=-0.232+6.245(head width μm) (r=0.843). Differences in the size of gnathiids in the diet at Lizard Island and on *Hemigymnus melapterus* among times were tested with a three way loglinear analysis with time, source of gnathiid, and size class of gnathiid as predictors. Only 4% of the expected frequencies were under 5. All first order effects and two-way associations were significant, and there was no three-way interaction (Table 4.1). The interaction between source of gnathiid and size class indicates that there are more large gnathiids in the diet than on the body of *Hemigymnus melapterus* at all times which shows that *Labroides dimidiatus* selectively feeds on larger gnathiids (Fig. 4.7). Regardless of whether gnathiids were from the diet or on the body of *H. melapterus*, there was an increase in small gnathiids from May to January (time x size class) (Fig. 4.7). The remaining interaction term (time x source of gnathiid) reflects differences in the sample size (Fig. 4.7). In contrast, the size frequency distribution of gnathiids in the diet and on *Hemigymnus melapterus* at Heron Island were not different (χ^2=2.52, df=3, p=0.472) and consisted mainly of many small gnathiids (Fig. 4.8). A comparison of the size frequency distribution of gnathiids at Heron Island (Fig. 4.8) with the size of gnathiids at Lizard Island at a similar time of year (May) (Fig. 4.7) reveals that the patterns in the diet and on the fish differ among locations. First, there are more large gnathiids, both in the diet and on the host, at Lizard Island than at Heron Island. And second, the size selectivity of gnathiids by *L. dimidiatus* at Lizard Island does not appear to occur at Heron Island.

4.4.5 GNATHIID BIOMASS

Gnathiid weight was estimated using the linear regression ln wt =-

2.501+2.058(ln L) (r=0.999). The estimated biomass of gnathiids in the diet at Lizard Island was 24.07 μg in May 1992, 34.02 μg in August 1992, 23.51 μg in October 1992, and 46.55 μg in January 1993 which indicates a doubling in biomass from May to January. This increase is almost as high as the increase in number of gnathiids over time (Fig. 4.6a) but it is not consistent with the cover of gnathiids which did not change during this time (Fig. 4.6b). At Heron Island, the estimated biomass was 6.27 μm in June 1993. Thus the biomass found at Lizard Island at a similar time of year (May 1992) was almost four times higher than that at Heron Island. This is in contrast to the cover of gnathiids in the diet which was about three times higher at Lizard Island than Heron Island (Fig. 4.4b) and to the number of gnathiids in the diet at Lizard Island which did not differ from the number at Heron Island (Fig. 4.4a).

4.4.6 EXTENT OF DIGESTION OF GNATHIIDS

The extent of digestion of gnathiids in the diet was significantly different between locations (χ^2=51.12, df=2, p<0.001). The proportion of whole gnathiids was higher at Heron Island indicating gnathiids in the diet were less digested at this location (Fig. 4.9). There was a marked change in the digestive state of gnathiids among months at Lizard Island (χ^2=414.47, df=6, p<0.001). The proportion of whole gnathiids in the diet was smallest in January, while the proportion was greatest which demonstrates that gnathiids in the diet at this time were more digested (Fig. 4.10).

4.4 DISCUSSION

Labroides dimidiatus at Lizard Island and Heron Island has a specialised diet which consists largely of gnathiid isopod larvae. Gnathiid abundance in the diet is disproportionately high compared with the wide diversity and abundance of other parasites found on fish on the Great Barrier Reef (Rohde 1977, Roubal 1981, Lester and Sewell 1989, Byrnes and Rohde 1992, Chapter III). Few copepods were found in the diet, while monogenean and digenean trematodes,

which are relatively common (Rohde 1977, Roubal 1981, Lester and Sewell 1989, Byrnes and Rohde 1992) and often abundant on fish (Chapter III) were completely absent from the diet. Laboratory studies indicate that other fish species feed on monogeneans (Kearn 1978, Cowell *et al.* 1993). But many parasites, including copepods and monogeneans, are cryptic, living in the gills, buccal cavity, nares, and under scales and skin which reduces their likelihood of predation by cleaner fish. Some monogeneans are also pigmented which may serve as camouflage (Kearn 1979). Although monogeneans have soft bodies, it is unlikely that they were lost by digestion as guts were fixed within seconds of capture. The diet reflects the obvious external crustacean element of the parasite fauna. Gnathiids are often found on external surfaces (Monod 1926, Wägele 1988, pers. obs.), and most of the other parasites in the diet were mainly caligid adults and larvae (Fig. 4.2 & 4.6) which also live on the surfaces of fish (Kabata 1979). The fact that *L. dimidiatus* mainly feeds on crustaceans is not surprising as most tropical labrids feed on crustaceans (Hiatt and Strasburg 1960, Hobson 1974).

Several factors may explain why gnathiids were the most abundant crustacean in the diet of *Labroides dimidiatus*. Some of these may also explain why gnathiids are also found, although in lower numbers, in the diet of other cleaner fish species (Youngbluth 1968, Böhlke and McCosker 1973, Hobson 1971). Gnathiids have low host specificity as they are found on many fish species (Davies and Johnston 1976, Paperna and Por 1977). At Lizard Island they have been found on 21 out of 32 fish species examined (Chapter III, pers. obs.). Thus there is a high probability that *L. dimidiatus* will encounter gnathiids. Gnathiids are not firmly attached. They readily leave the host when disturbed (Davies and Johnston 1976) and leave the host to moult three times (Wägele 1988). Gnathiids are also relatively large in size compared with other abundant parasites at Lizard Island and Heron Island (Chapter III). Finally, their extremely large stomachs, which fill with blood or lymphatic fluid during feeding (Wägele 1988), probably make them a rich food source.

Spatial and temporal variation in the diet of the *Labroides dimidiatus*

indicates flexibility in its foraging patterns. Although gnathiids made up most of the identifiable items at all times and places, there were differences in the composition of the remaining items in the diet. The most striking difference was that between Lizard Island and Heron Island. The diet of *L. dimidiatus* at Heron Island had about one fourth less biomass of gnathiids and about one third less cover of gnathiids than that at Lizard Island and was characterised by more mucus and non-parasitic copepods. It has been suggested that cleaner fish may feed on mucus when parasite loads are low (Gorlick 1980). Cleaner fish may also include other food items, such as non-parasitic copepods, when parasites are not as abundant. The nature of the dietary differences among the localities suggest that relationships between cleaner fish and hosts vary between the two locations. Temporal variation in the number and biomass of gnathiids in the diet suggests that these relationships may also vary over time.

Changes in the diet of fish over space and time may be a function of absolute and relative abundances of food items (Stoner 1979, Cowen 1986). Thus, the abundance of gnathiids in the diet of cleaner fish may reflect the abundance on hosts. Both the cover and biomass of gnathiids in the diet were less at Heron Island than at Lizard Island. Similarly there was a trend for some fish species to have fewer or no gnathiid parasites at Heron Island (Chapter III). Finally, overall patterns of spatial variation in the diet of *L. dimidiatus* are similar to the spatial variation of parasites on several host fish species collected at the same sites and locations (Chapter III).

Labroides dimidiatus at Lizard Island select larger parasites. The size frequency distribution of gnathiids in the diet at Lizard Island had more large individuals compared to those on the host fish *Hemigymnus melapterus*. This was not the case at Heron Island, as gnathiid size frequencies in the diet and on the fish were similar. The size frequency distributions at Heron Island were skewed towards small gnathiids so it is possible that *L. dimidiatus* had little choice but to feed on small gnathiids. The above comparisons are based on the assumption that the size frequency distribution of gnathiids on *H. melapterus* is representative of all fish species cleaned which is likely as gnathiids have very

low host specificity (Chapter III). There is other evidence for size selectivity in *L. dimidiatus*. Adult caligid copepods, which are relatively large ectoparasites (Kabata 1979, Chapter III), were also common in the diet of *L. dimidiatus* at Lizard Island and Heron Island. They are also one of the most numerous ectoparasites in the diet of *Labroides* spp. in other studies (Youngbluth 1968, Chikasue 1990).

Higher temperature and smaller food items can result in higher digestion rates (Fänge and Grove 1979, dos Santos and Jobling 1991) and may explain spatial and temporal differences in the digestion rate of *Labroides dimidiatus*. Digestion rates were higher at Lizard Island which also has warmer water compared to Heron Island (Fig. 4.9). The rate of digestion was also higher during the austral summer when waters are warmer (Fig. 4.10). Finally, gnathiids were smaller during the summer (Fig. 4.7, 4.8) which may have contributed to the higher digestion rate at this time. The implications of these results are that the dietary differences among locations are real and not merely a result of different digestion rates. Because digestion rates are lower at Heron Island, and not higher as would be expected with lower amounts of food, dietary differences among locations may be greater than those observed.

The diet of *Labroides dimidiatus* at Lizard Island and Heron Island has the highest recorded numbers of parasites for cleaner fish of similar size. The numbers of parasites in the diet of *L. dimidiatus* at Lizard Island (May-January) are 6-13 times higher than for *L. dimidiatus* in Japan (Chikasue 1990) and 16-36 times higher than for *L. phthirophagus* in Hawaii (Youngbluth 1968). Parasite loads of some fish species at Lizard Island and Heron Island (Chapter III) and Japan (Chikasue 1990) appear to be higher than the parasite loads of similar sized fish in Hawaii (Losey 1972). However, these comparisons involve relatively few fish species. They are also confounded by the method of collection of parasites which can influence parasite loads (Nagasawa 1985, Williams *et al.* 1991). Comparisons of the parasite loads of more species using similar methods are needed to measure the variation in parasite loads among these locations.

The large number of gnathiids found in the diet of cleaners may have

important implications for the health of host fish. Damage caused by gnathiids is variable and ranges from slight blemishes (Davies 1981), lesions (Monod 1926), to heavy inflammation and hypertrophy of tissues (Honma *et al.* 1991) and death of fish in cages and in nets (Paperna and Por 1977). However, the effects of gnathiids on host fish in the Great Barrier Reef is unknown.

Dietary specialisation is often correlated with increasing food abundance. This conclusion is supported by models of predator-prey relationships (see review by Pyke *et al.* 1977, 1984) and empirical studies with fish (Zaret and Rand 1971, Werner and Hall 1974). Gnathiids, although not the most abundant parasite on host fish at Lizard Island (Chapter III), are relatively common (pers. obs.). Dietary selectivity has important implications for the ecological significance of cleaning. One of these is that if cleaner fish have an effect on parasite loads the effect will not be equal across all parasite species.

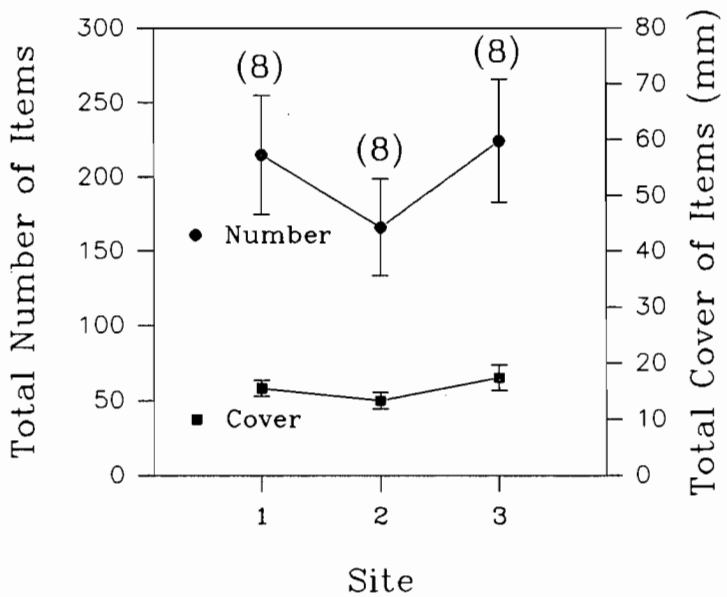

Figure 4.1. The mean total number of items (±SE) and the mean total cover (±SE) of items in the diet of *Labroides dimidiatus* at three sites on Lizard Island (1=North Point, 2=Granite Reef, 3=Lagoon). Cover is expressed as line intercept length. Sample sizes are in brackets.

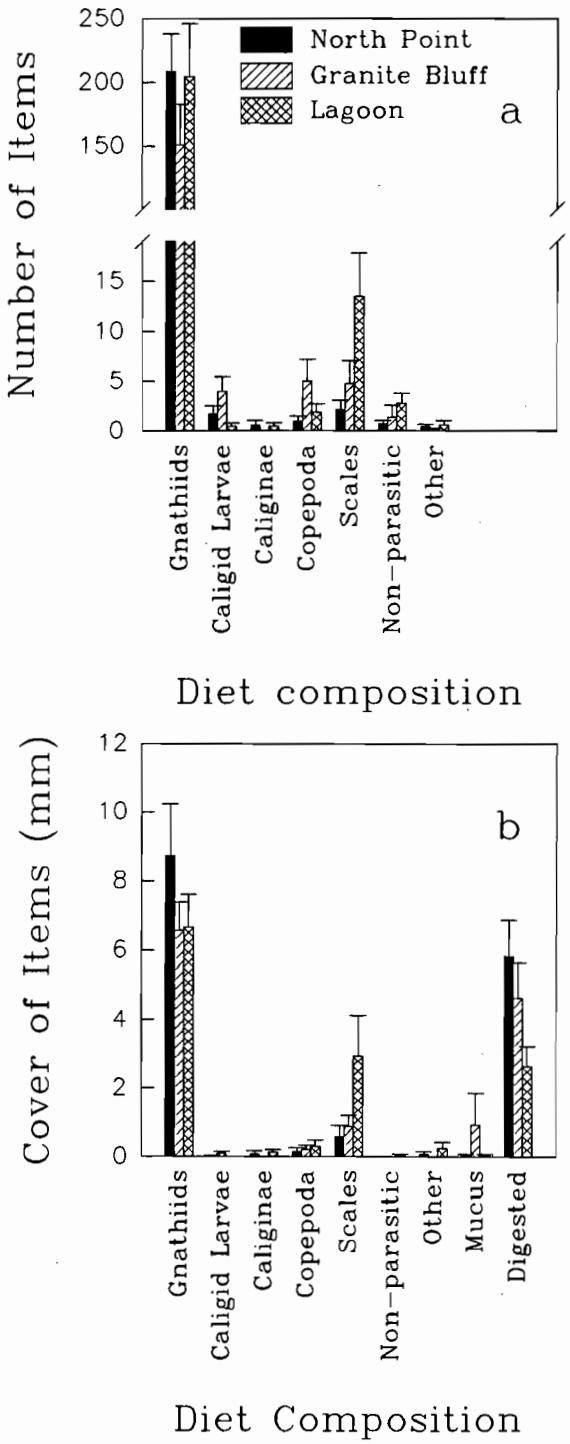

Figure 4.2. The composition of the diet of *Labroides dimidiatus* at three sites on Lizard Island. **a.** The mean number of items (±SE) per category. **b.** The mean cover of items (±SE) per category. Cover is expressed as line transect intercept length. Non-parasitic refers to non-parasitic copepods.

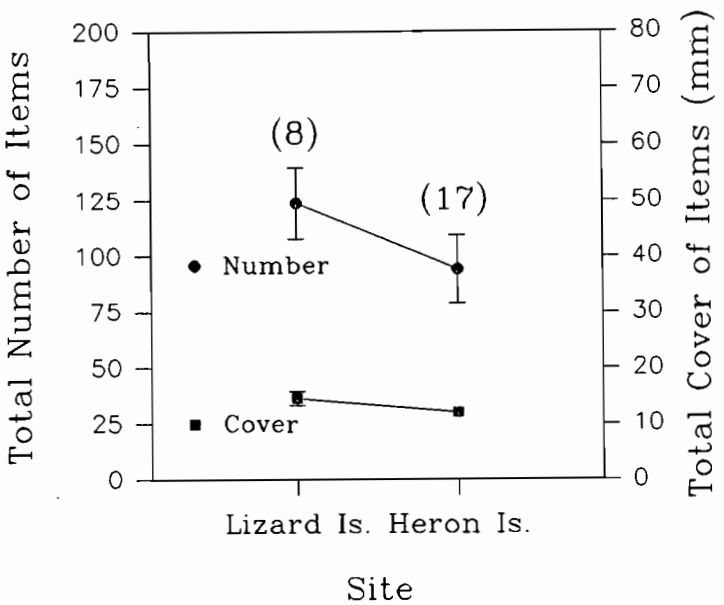

Figure 4.3. The mean total number of items (±SE) and the mean total cover (±SE) of items in the diet of *Labroides dimidiatus* at two locations. Cover is expressed as line transect intercept length. Sample sizes are in brackets.

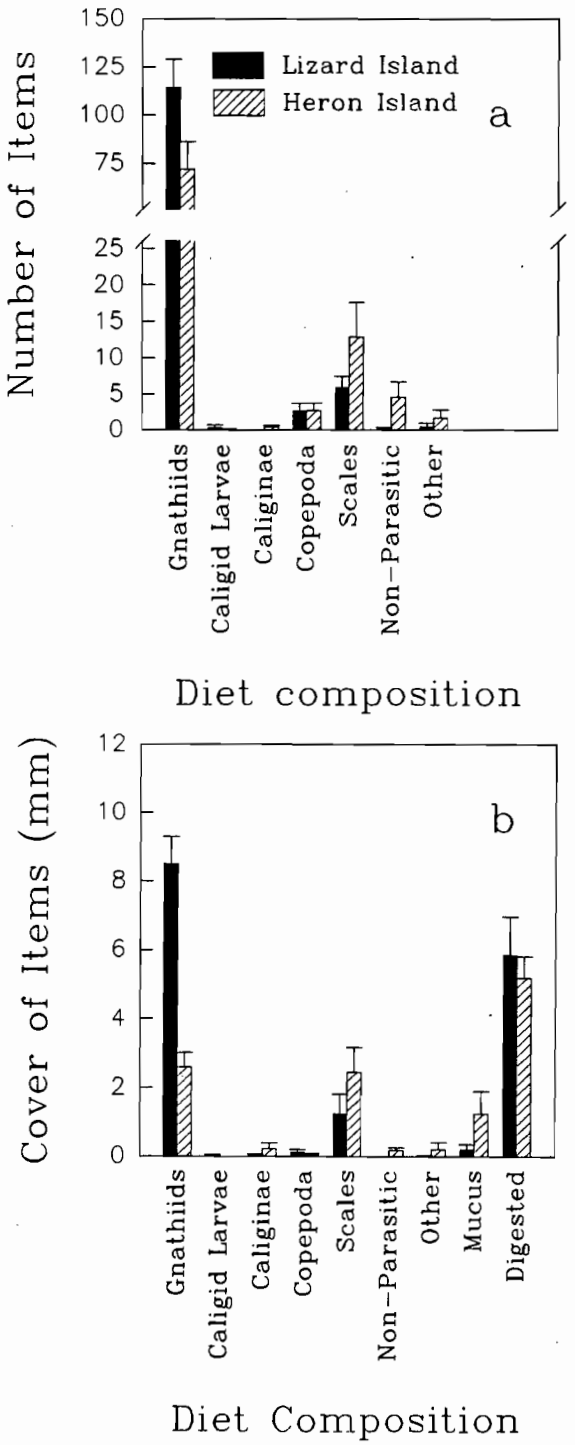

Figure 4.4. The composition of the diet of *Labroides dimidiatus* at Lizard Island and Heron Island. a. The mean number of items (±SE) per category. b. The mean cover of items (±SE) per category at two locations. Cover is expressed as line transect intercept length.

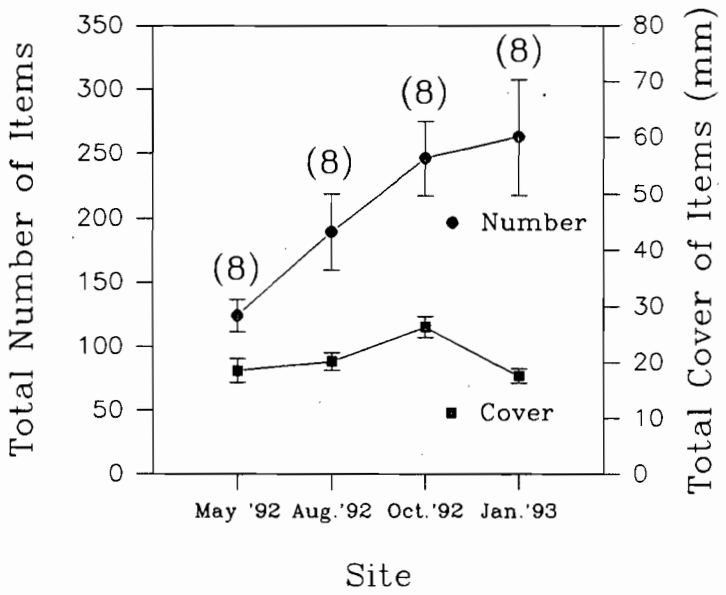

Figure 4.5. The mean total number of items (±SE) and the mean total cover (±SE) of items in the diet of *Labroides dimidiatus* at different times. Cover expressed as line transect intercept length. Sample sizes are in brackets.

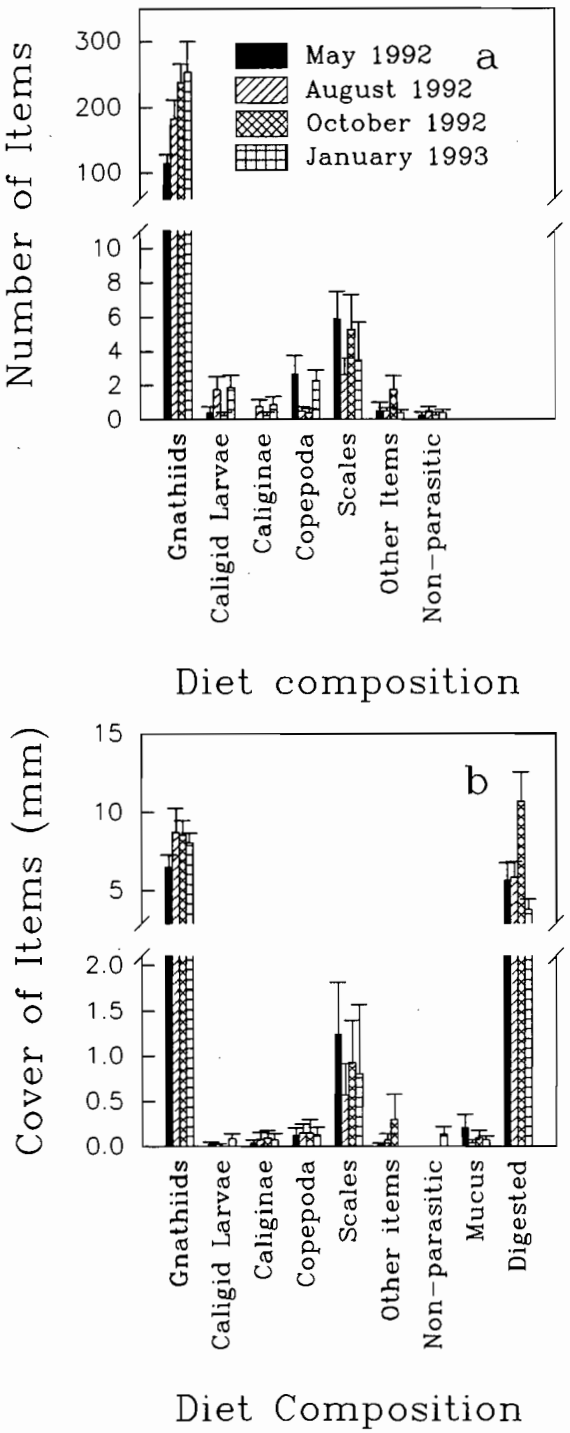

Figure 4.6. The composition of the diet of *Labroides dimidiatus* at different times. **a.** The mean number of items (±SE) per category. **b.** The mean cover of items (±SE) per category. Cover is expressed as line transect intercept length.

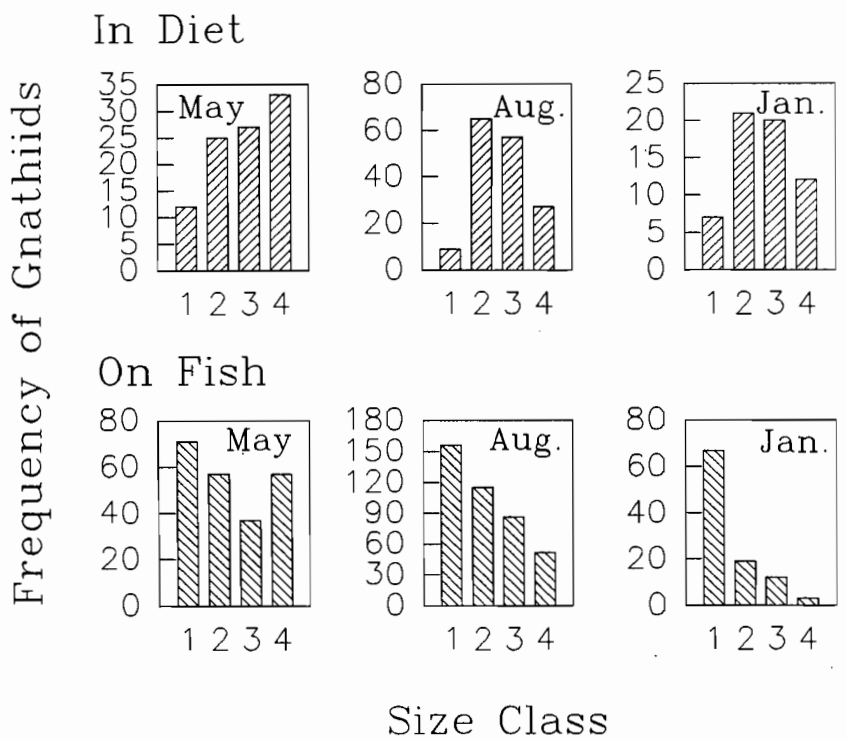

Figure 4.7. The size frequency distributions of gnathiid isopod larvae found in the diet of *Labroides dimidiatus* and on the body of the fish *Hemigymnus melapterus* collected at different times at Lizard Island. Size classes: 1=<1.10mm, 2=1.10-1.39mm, 3=1.40-1.69mm, 4=>1.7mm.

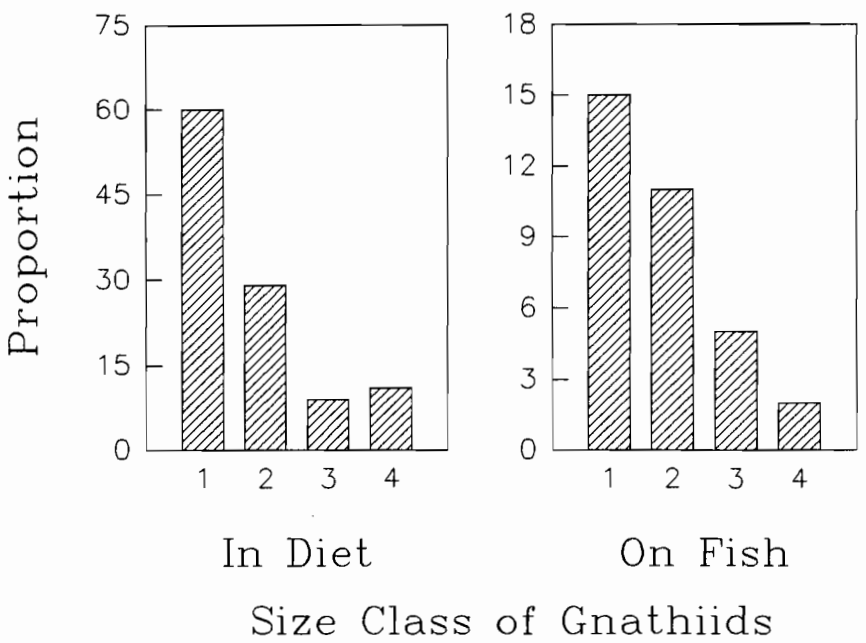

Figure 4.8. The size frequency distributions of gnathiid isopod larvae found in the diet of *Labroides dimidiatus* and on the body of the host fish *Hemigymnus melapterus* collected at Heron Island. Size classes: 1=<1.10mm, 2=1.10-1.39mm, 3=1.40-1.69mm, 4=>1.7mm.

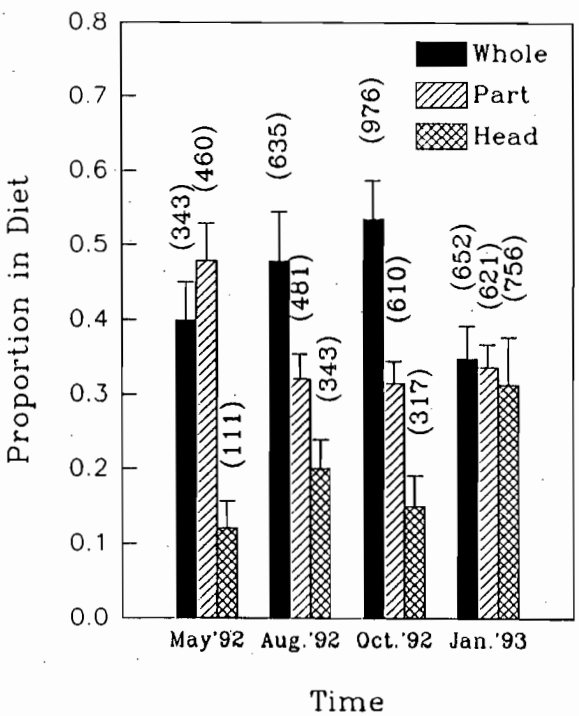

Figure 4.10. The extent of digestion of gnathiids in the diet of *Labroides dimidiatus* at different times at Lizard Island. Whole=complete gnathiid, Part=gnathiid head with a part of the body still attached, Head=gnathiid head only. Sample sizes are in brackets.

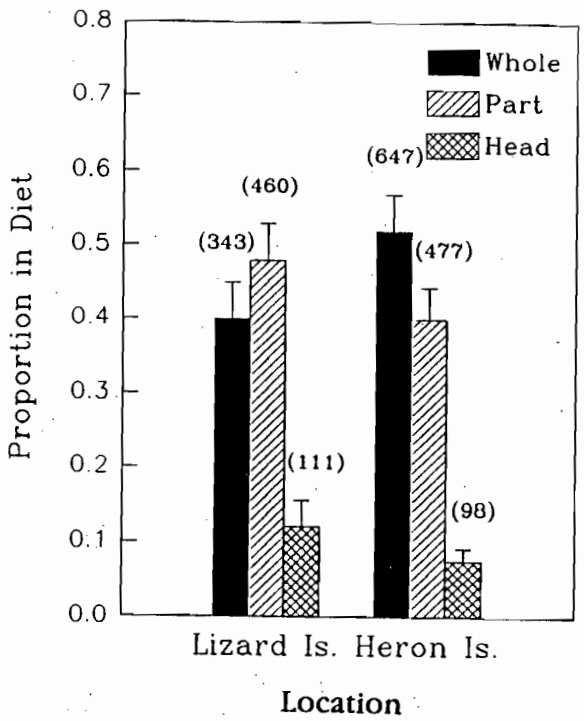

Figure 4.9. The extent of digestion of gnathiids in the diet of *Labroides dimidiatus* at two locations. Whole=complete gnathiid, Part=gnathiid head with a part of the body still attached, Head=gnathiid head only. Sample sizes are in brackets.

CHAPTER V

THE RELATIONSHIP BETWEEN CLEANING RATES AND ECTOPARASITE LOADS IN CORAL REEF FISHES

5.1 SUMMARY

Individuals from 11 fish species were followed and the number of times and duration that fish were inspected by the cleaner wrasse *Labroides dimidiatus* recorded around Lizard Island, Great Barrier Reef. The frequency and duration of inspection were positively correlated with the mean parasite load and mean surface area of the 11 fish species. Surface area, however, explained slightly more of the variation in inspection frequency and duration among species than did ectoparasite load. This suggests surface area may be useful for predicting the cleaning rates of fish species. When the frequency and duration of inspection were corrected for mean surface area and mean ectoparasite load, differences among fish species disappeared. Observations of three size classes from one fish species, *Hemigymnus melapterus*, revealed that larger fish, which have more parasites, were inspected more often and for longer periods than smaller fish with fewer parasites. This study indicates that parasites and surface area play an important role in cleaning behaviour.

5.2 INTRODUCTION

Understanding of the stimuli that motivate a fish to seek cleaning is important to the study of cleaning behaviour (Losey 1987, 1993, Poulin 1993) as is the need for new approaches to this phenomenon (Losey 1987, Poulin 1993). Studies using models of cleaner fish suggest that tactile stimuli drive host cleaning (Losey and Margules 1974, Losey 1977, 1979). The influence of ectoparasites on the response of fish toward cleaners is conflicting and remains unresolved (Losey 1971, 1979). Parasites were found to have little effect on the response of one host species towards cleaner fish models while they only increased the response to tactile stimuli in another host species (Losey 1979).

An examination of the relationship between cleaning rates and parasite load is needed to understand the role of parasites in cleaning interactions.

Most studies that have measured fish cleaning rates have quantified cleaning from the perspective of the cleaner (Okuno 1969, Hobson 1971, Potts 1973a, 1973b). The motivation for cleaner fish to clean is food, thus this sampling method provides information on the foraging and feeding behaviour of cleaner fish. However, it can create confounding problems if used to estimate the cleaning rates of hosts. Cleaner fish prefer some fish over others (Gorlick 1978, 1984) and often clean some fish in proportion to their abundance (pers. obs.).

Observations that focus on the host, rather than on the cleaner fish, measure how often individuals are cleaned (host cleaning rates). How host cleaning rates vary among species and within species can provide a measure of the relative importance or potential effect of cleaning. Host attributes that may influence cleaning behaviour can also be correlated with these cleaning rates. The relationship between these characteristics and cleaning may be useful for predicting cleaning behaviour and may also provide insight into what drives the behaviour.

The study of cleaning behaviour is complicated by several factors. Fish abundance, fish size, and ectoparasite loads all vary among host species. Most importantly, cleaning rates can be influenced as much by actions of the cleaner fish as by the actions of the host (Losey 1971). Therefore, when measuring cleaning behaviour, it is difficult to separate completely the effect of the cleaner on rates of cleaning from the effect of the host. When exploring factors that may explain variation in cleaning, factors that may influence host behaviour should be considered, as well as those that may influence cleaner fish feeding. Cleaner fish prefer host species with more ectoparasites (Gorlick 1984) or with more mucus (Gorlick 1980). Another factor that may influence cleaner fish behaviour is host size. Ectoparasite load is often correlated with host size in fish (Noble *et al.* 1963, Bortone 1978, Cressey and Collette 1971, Buchmann 1989, Chapter III). Larger fish may also represent a richer source of food for cleaners in the form of mucus and other surface materials.

For host fish, the reason they respond to a cleaner fish may be ectoparasite removal, which can be either the proximate or ultimate cause of the behaviour or both (Gorlick *et al.* 1978). The average ectoparasite load of some hosts has been shown to be species-specific (Chapter III). Thus different parasite loads may result in different cleaning rates among species. There is also intraspecific variation in parasite loads (Chapter III), which may affect responses to hosts. Despite several studies (Gorlick *et al.* 1978, Gorlick 1984, Losey 1979), the role of parasites in cleaning interactions, particularly in host fish cleaning behaviour, is still not fully understood.

It is likely that cleaning rates are, in part, influenced by an interaction of host size and parasite load but there is little information on the parasite loads of fish and their relationship to size and cleaning rate. The objectives of this study are divided into two parts. The first part was designed to: (a) test whether inspection by the cleaner fish *Labroides dimidiatus* was correlated with parasite load and size (surface area) of host fish species; (b) apportion variation in inspection rates due to parasite load and host size; and (c) test whether there were any true species differences in cleaning rates once inspection was adjusted for parasite load and surface area. A second general aim was to test whether inspection rates within a host species differed among three size classes of fish which have different parasite loads (Chapter III).

5.3 METHODS AND MATERIALS

The fish species (family) investigated are *Ctenochaetus striatus* (Acanthuridae), *Scolopsis bilineatus* (Nemipteridae), *Siganus doliatus* (Siganidae), *Chlorurus sordidus* (Scaridae), *Thalassoma lunare*, *Hemigymnus melapterus* (Labridae), *Acanthochromis polyacanthus*, *Neopomacentrus azysron*, *N. cyanomos*, *Ambliglyphidodon curacao*, and *Pomacentrus moluccensis* (Pomacentridae). The species were selected because they live in similar habitats, they differ ecologically but are all reef associated, they are relatively abundant, and are all cleaned by the cleaner wrasse *Labroides dimidiatus*. The body sizes of the 11 fish species investigated range from 33 to

250 mm in standard length (estimated from fish collected for parasites). The study was carried out at several sites (North Point, Granite Bluff, Lagoon, and Casuarina Beach) around Lizard Island (Fig. 2.1).

5.3.1 HOST CLEANING BEHAVIOUR

Focal-animal sampling (Altmann 1974) was used to estimate host cleaning rates. This method records actions that are directed to or received by the observed animal and over a fixed length of time this record provides an estimated rate of the behaviour recorded (Altmann 1974). During sampling, a host fish was selected haphazardly and observed from a distance of 2-5 m. The abundance of each fish species was relatively high which reduced the likelihood that the same fish were accidently selected more than once (pseudoreplication *sensu* Hurlbert 1984). Inspection time by the cleaner fish was used as a measure of cleaning behaviour because it could be measured more precisely than other feeding behaviours of *Labroides dimidiatus*. Inspection was defined as any event that involved visual examination of the body surfaces and gills of the host. The length of an inspection event was determined from the time when a cleaner fish approached a host fish until it departed the host. The duration of inspection is positively correlated with number of bites (Youngbluth 1968, Losey 1971, Chapter VI) taken by cleaner fish and thus estimates amount of feeding. The duration of each inspection of a host by *L. dimidiatus* was recorded, and the frequency of inspections per sampling period calculated. These were summed over the 30 min sample period to obtain the total number of times a fish was inspected and the total duration of inspection received by cleaner fish. The length of each sampling period was sufficient to record at least one cleaning event per period for most fish species, yet sufficiently short to allow at least two sample periods per dive. Sample periods that had no inspections were recorded as zeros. All observations were made by a SCUBA diver so that mobile species could easily be followed and were made between the hours 07:00 and 18:00.

5.3.2 INSPECTION RATES AMONG SPECIES

The fish species *Acanthochromis polyacanthus*, *Ctenochaetus striatus*,

Scolopsis bilineatus, *Siganus doliatus*, *Chlorurus sordidus*, *Thalassoma lunare*, and *Hemigymnus melapterus* were sampled at North Point during January 1993 (n=16-18 per species). Sampling was divided equally into 4 time periods (06:00-08:59, 09:00-11:59, 12:00-14:59, 15:00-18:59 hrs); and an initial two factor analysis of variance (ANOVA) was used to test whether there was an effect of time of day on the frequency and duration of inspection. The factor time and the interaction term (time x species) were not significant ($0.50>p>0.15$) so both the frequency and duration of inspection for these seven species were pooled across times.

The remaining fish species, *Neopomacentrus azysron*, *N. cyanomos*, *Ambliglyphidodon curacao*, and *Pomacentrus moluccensis*, were sampled 10-11 times in November 1993. These samples were taken at 8 small patch reefs in the Lagoon and at Casuarina Beach (Fig. 2.1). It was assumed that the relationships between cleaning rates and parasites or surface area were not influenced by time or site (a preliminary analysis of the cleaning rates of *Hemigymnus melapterus* at two different times and at two sites revealed no significant effect of time nor site). Based on this assumption, these observations were combined with those of the above seven species to increase the sample size. Within a species, fish were of a narrow range of sizes and all were adults except for *Chlorurus sordidus* which likely were immature initial phase females.

To test how species differed in their cleaning rates when parasite load and surface area were used as covariates an analysis of covariance (ANCOVA) was conducted. Covariates, estimated as mean number of parasites per fish species and mean surface area of each species, were calculated from fish collected after the observations. This meant that fish used for estimating the parasite load and surface area were not the same as those observed, thus a mean was used for each species in the analyses. To determine whether one covariate explained more of the variation in the frequency of inspections or whether both explained the variation equally, covariates were added to the ANCOVA sequentially. The analysis was then repeated with the order of the covariables reversed. This resulted in two models which were compared to determine whether one or both covariables best explained the variation in

inspection frequency. Type 1 sums of squares, also called sequential sums of squares, were used because the effects of each factor are sequentially removed from the model (S.A.S. Institute Inc. 1991). Thus covariables are added one at a time and are cumulative. The same analyses were used to test for differences in the duration of inspection among species. For all the above analyses, frequency and duration of inspection were log10 (x+1) transformed, and parasite load and surface area were log10 transformed to satisfy the assumption of homogeneity of variance and linearity.

5.3.3 INSPECTION RATES WITHIN *HEMIGYMNUS MELAPTERUS*

In the second set of observations, inspection rates were recorded across a size range of *Hemigymnus melapterus*. This species was selected because it shows a strong correlation between ectoparasite load and host size (Chapter III). To determine whether inspection rates varied with size of fish, three size classes of fish were distinguished (< 8 cm, 10-15 cm, and > 20 cm in standard length), and fish were selected haphazardly from these size classes. Thirty sampling sessions were done at Granite Bluff (Fig. 2.1) in August 1992. The duration and frequency of inspections among size classes was tested with an analysis of variance (ANOVA). Data were log10 (x+1) transformed to satisfy the assumption of homogeneity of variance.

5.3.4 PARASITE LOAD ESTIMATES

The mean parasite load of each fish species was estimated from fish collected several days after the behavioural observations. The species *Acanthochromis polyacanthus*, *Ctenochaetus striatus*, *Scolopsis bilineatus*, *Siganus doliatus*, *Chlorurus sordidus*, *Thalassoma lunare*, and *Hemigymnus melapterus* (a combined total of 34 fish) were collected with a barrier net and handnet and placed in a plastic bag underwater. Collections were made from North Reef, Granite Bluff, and the Lagoon (Fig. 2.1). The parasite loads of these fish species show very little variation among these sites (Chapter III); so all parasite samples from fish were pooled across sites. Parasites were collected as in chapter II and involved rinsing fish with saltwater, soaking the fish in the

anaesthetic chloretone for 30-60 min, filtering all liquids at 200μm then 57μm, and then scanning the whole fish under a stereo microscope (35x) to recover any remaining parasites. The parasite assemblages of the seven species are described in Chapter III.

Using a 1.5 x 1 m barrier net with 10 mm mesh, specimens (n=8 per species) from the species *Ambliglyphidodon curacao*, *Neopomacentrus azysron*, *N. cyanomos*, and *Pomacentrus moluccensis* were collected in a similar way from areas of observations. The parasites of *A. curacao* were collected as above, but fish were not scanned under a microscope after the soak. Their gills were removed in the same way as the gills of *Hemigymnus melapterus*. The remaining species, *N. azysron*, *N. cyanomos*, and *P. moluccensis*, are relatively small (33-53.6 mm) so the whole fish and contents of the plastic bag were fixed and the fish surface, gills, and fixative examined for parasites under a stereo microscope (25x). The parasites of these four species were mainly copepods with a few digeneans, monogeneans, and turbellarians.

Fish collected for estimates of parasite loads were similar in size to the fish used during behavioural observations, except for *Hemigymnus melapterus*. Individuals of the size observed could not be captured in sufficient numbers. The parasite load of *H. melapterus* is positively correlated with standard length (Chapter III), thus its parasite load was estimated from the mean standard length observed (23.8 cm).

5.3.5 SURFACE AREA ESTIMATES

Surface area is an appropriate measure of host size for studies that involve cleaner fish which feed on mucus, skin, scales, and ectoparasites all which are found on the surfaces of fish. Surface areas were estimated as in Chapter III. The surface areas of *Neopomacentrus azysron*, *N. cyanomos*, and *Pomacentrus moluccensis* were measured directly using the fish collected for measurement of parasite loads. Specimens of the remaining eight species were not available for surface area measurements. Therefore their area was estimated from their standard length using other specimens measured as described above. The surface areas of *Ambliglyphidodon curacao* were estimated using

Table 5.1. Analysis of covariance used to test for differences in the frequency of inspection among 11 fish species with mean surface area per fish species and mean total parasites per fish species as covariates. Tests of significance use Type I sequential sums of squares. **a.** Model 1. The frequency of inspection is first related to parasite load, the residual variation is then related to surface area, finally the remaining variation is examined for an effect of species. **b.** Model 2. The frequency of inspection is tested as above but with the order of the covariables reversed. CD=coefficient of determination.

a.

Source	df	MS	F	p	CD
Parasites	1	3.795	42.72	<0.001	0.185
Area	1	2.499	28.12	<0.001	0.122
Species	8	0.151	1.69	0.104	0.059

b.

Source	df	MS	F	p	CD
Area	1	5.997	67.50	0.001	0.293
Parasites	1	0.297	3.34	0.070	0.015
Species	8	0.151	1.69	0.104	0.059

specimens collected as above (n=11, r>0.95). The surface areas of the remaining seven species were estimated similarly, following Chapter III (n=16-26 per species, all r>0.95).

5.4 RESULTS

The mean parasite load of each species increased exponentially as the mean surface area of the fish species increased (Fig. 5.1). The number of times fish were inspected increased as parasite load increased (Fig. 5.2a) and as the surface area of the fish species increased (Fig. 5.2b). The species with the highest inspection rate was the large *Siganus doliatus*, which had about 110 parasites per fish and which was inspected about 6 times per 30 min (species 4 in Fig. 5.2). This species's cleaning rate does not differ throughout the day (see methods), which means that on average, individuals of this species were cleaned about 144 times per day (based on 12 daylight hours). In contrast, some smaller species with few parasites were inspected less than once per 30 min (species 8, 9, 10 in Fig. 5.2). The duration of inspection for each fish species also increased with increasing parasite load (Fig. 5.3a) and increasing surface area (Fig. 5.3b). *Siganus doliatus* (species 4 in Fig. 5.3) had the longest duration of inspection (about 80 sec per 30 min), which means individuals spend about 32 minutes per day being inspected by cleaner fish (product of 80 sec/30 min and 12 daylight hours). In comparison, small fish were often inspected for less than 1 sec per half hour (species 8, 9, 10 in Fig. 5.3).

The frequency of inspection among species covaried with both parasite load and surface area (Table 5.1). In the first model of the ANCOVA, with the covariate parasite load introduced to the model first, parasite load was a significant covariate (Table 5.1a). However, surface area was still a significant covariate when it was adjusted for numbers of parasites (Table 5.1a). The second model, in which the order of covariates was reversed, shows area was a significant covariate (Table 5.1b). However, parasite load was no longer a significant covariable when adjusted for surface area (Table 5.1b). The results of the first and second models in Table 5.1 were not the same which indicated

Table 5.2. Analysis of covariance used to test for differences in the duration of inspection among 11 fish species with mean surface area per fish species and mean total parasites per fish species as covariates. Tests of significance use Type I sequential sums of squares. **a.** Model 1. The duration of inspection is first related to the parasite load, the residual variation is then related to the surface area, and finally the remaining variation is examined for an effect of species. **b.** Model 2. The duration of inspection is tested as above but with the order of the covariables reversed. CD=coefficient of determination.

a.

Source	df	MS	F	p	CD
Parasites	1	23.597	67.21	<0.001	0.252
Area	1	13.358	38.05	<0.001	0.142
Species	8	0.698	1.99	0.052	0.007

b.

Source	df	MS	F	p	CD
Area	1	34.593	98.53	<0.001	0.369
Parasites	1	2.361	6.73	0.011	0.025
Species	8	0.698	1.99	0.052	0.007

that the covariables did not explain the variation equally. A comparison of the two models, using the values of F ratio and coefficient of determination from each sequential analysis, show that surface area explained slightly more of the variation in the frequency of inspection (29.3%) than did parasite load (18.5%). This can also be seen in Fig. 5.2 which shows that the frequency of inspection has a higher correlation with surface area than with parasite load. Finally, the frequency of inspection was not significantly different among species when the effects of parasite load and surface area were removed (Table 5.1).

Parasite load and surface area were also significant covariates in the ANCOVA of the duration of inspection among species (Table 5.2 a & b). The results of the first and second models were not the same, which indicated that the covariates did not explain the variation equally. Again, surface area appeared to explain slightly more of the variation in the duration of inspection (36.9%) than parasite load (25.2%). However, parasite load was still a significant covariate when adjusted for area but explained only 2.5% of the variation. Although the effect of species, when the effects of the covariates were removed, was nearly significant it accounted for little of the total variation (0.7 % Table 5.2 a & b). These analyses are supported by Figure 5.3, which shows that the duration of inspection has a higher correlation with surface area than with parasite load (Fig. 5.3).

Within a species, the number of times *Hemigymnus melapterus* was inspected by *Labroides dimidiatus* was significantly different among size classes (ANOVA df=2,26, F=21.0, $p<0.001$, CD=0.618) (Fig. 5.4a). Larger fish were cleaned more often per 30 min than smaller fish, with the largest fish being cleaned about 5 times, medium sized fish about 4 times and small sized fish being cleaned less than once per 30 min (Fig. 5.4a). The duration of inspection per sample period was also significantly different among size classes (ANOVA df=2,26, F=35.69, $p<0.001$, CD=0.733). On average, larger fish were cleaned for more time, with large fish being cleaned for about 45 seconds per sample period, medium sized fish for 30 sec, and small fish for 1 sec (Fig. 5.4b).

5.5 DISCUSSION

Fish which were larger and had more parasites were inspected by cleaner fish more often than smaller fish that had fewer parasites. This pattern occurred both among and within a species. However, surface area, rather than parasite load, best explained the variation in cleaning rates among host species. This suggests that surface area may be useful for predicting host inspection rates. The link of host size with cleaning has already been suggested (Poulin 1993).

Because both the cleaner fish and host can determine the outcome of a cleaning event (Losey 1971), the above patterns are probably a result of the behaviour of both cleaner fish feeding and host. If ectoparasite removal is the cause of the behaviour for the host, one would expect parasite load to have a stronger effect on cleaning than host size. However because surface area is so important in the analyses described here, ectoparasite removal does not appear to be the primary cause of the behaviour. It has been demonstrated that tactile stimuli has a large influence on the hosts' responses towards cleaners (Losey 1979). However, the relationship between host size and tactile stimuli is unclear. Manipulation of parasite loads on fish and the subsequent effects on cleaning behaviour are needed to examine whether parasite load influences the behaviour of hosts seeking cleaning.

Cleaner fish can also influence cleaning rates by initiating and or terminating an interaction (Losey 1979). If search time is a function of fish surface area, cleaner fish may influence the duration of inspection by spending more time on larger fish. The longer inspection time required to find food on a larger host may also result in more tactile stimulation for hosts. How frequently host fish are cleaned may also be influenced by cleaner fish. Cleaner fish can reliably estimate the surface area of a fish from a distance but cannot estimate the parasite load of a fish until they have scanned the body for a few seconds. Because size and parasite load are so closely related (Noble *et al.* 1963, Cressey and Collette 1971, Bortone et al. 1978, Buchmann 1989, Chapter III) and because cleaner fish also feed on the surface mucus of hosts (Gorlick 1980) and other surface materials (Randall 1958, Youngbluth 1968) cleaner fish may use size as an indicator of food availability.

Parasites could therefore still drive the association but their proximate role would be somewhat masked by perceptual constraints acting on cleaners.

Surface area may be a better predictor of host cleaning rates simply because, within a species, surface may be less variable than parasite abundance. Within a species, parasite loads are variable (Chapter III). Therefore the variation introduced into the study by using estimated mean parasite loads for each species, rather than using the parasite load of each fish observed, may have introduced error.

Once the effects of surface area and parasite load were removed the differences in the inspection rates among species were not significant. This is surprising as some other host species display high levels of aggression towards cleaner fish during cleaning interactions, which may affect cleaner preference (Gorlick 1978, 1984). Some species also appear to seek cleaners more often while others often ignore cleaner fish. Fish species also have species-specific assemblages of parasites (Chapter III), which could influence cleaner fish feeding behaviour. It is likely that fish size has a stronger effect on cleaning than species identity, thus species differences may be more apparent among similar sized species.

This study did not control for phylogenetic relationships among species which can introduce bias if closely related species share characteristics (e.g. size, parasite load)(Harvey and Pagel 1991). Phylogenetic effects in the relationship between the tendency of hosts to seek cleaning (measured as the number of times fish species were observed with *Labroides dimidiatus* compared to the number of fish observed elsewhere) and the species' size (Poulin 1993) have been controlled using the independent comparisons method (Harvey and Pagel 1991). Five of the eleven species in this study belong to the family Pomacentridae and are therefore more closely related to one another than the other six species. They are also all small and have few parasites. Thus the possibility arises that they were cleaned less than the other species because their lineage never developed a close association with cleaners. However, the relationship among cleaning, size, and parasite load still appears to hold among these five species. Thus, if phylogeny is important it may just influence the intensity of the relationship. Studies using phylogenetically-independent contrasts of a range of species from a range of sizes are needed to resolve this issue. It should be noted that for the comparison across fish species, cleaning rates were estimated from a narrow range of adult fish sizes (except for

Chlorurus sordidus which were likely immature initial phase females) and therefore apply only to fish in that size range only.

Interestingly, from the cleaner fish's point of view, the fish species *Acanthochromis polyacanthus*, is one of the most frequently cleaned species at Lizard Island (pers. obs.). Yet, as this study has shown, on an individual basis they are cleaned relatively infrequently. This species is relatively abundant (pers. obs) which may explain why it was cleaned so frequently by *Labroides dimidiatus*. In general, the larger species in this study were more abundant, however there is much variation in the relationship between size and abundance of reef fish. Host abundance may play a role in cleaning rates, but it is only one of the many factors involved. The mobility of the species examined varies, with the larger species, in general, covering more area than the smaller species. However, it is unlikely that this had a large affect on their cleaning rates as all fish observed had a cleaner fish in their home ranges or territories. Finally, it has been shown that the mucus load of fish influences the cleaner fish host preference (Gorlick 1984). However, mucus forms a small part of the diet of cleaner fish at Lizard Island (Chapter IV), thus its role in determining cleaning rates is probably small.

The cleaning rates obtained may be useful for measuring how many parasites cleaner fish remove from fish. Individuals of the species *Siganus doliatus*, for example, are cleaned about 144 times per day and for 32 minutes per day. This information, combined with rates of feeding by cleaner fish and parasite removal rates can be used to estimate the number of parasites that a cleaner fish removes from a *S. doliatus* per day (see Chapter V).

Studies on factors influencing host cleaning behaviour have suggested that parasite loads do not greatly affect cleaning behaviour (Losey 1971, 1979). However, this study shows that some species that do have many parasites, spend a relatively large proportion of their waking hours being cleaned. Furthermore, both among and within species, fish with more parasites were cleaned more often and for a longer time. Finally, parasite load still explained some of the variation in the duration of inspection when adjusted for area. These findings indicate that both parasites and surface area play an important role in host cleaning.

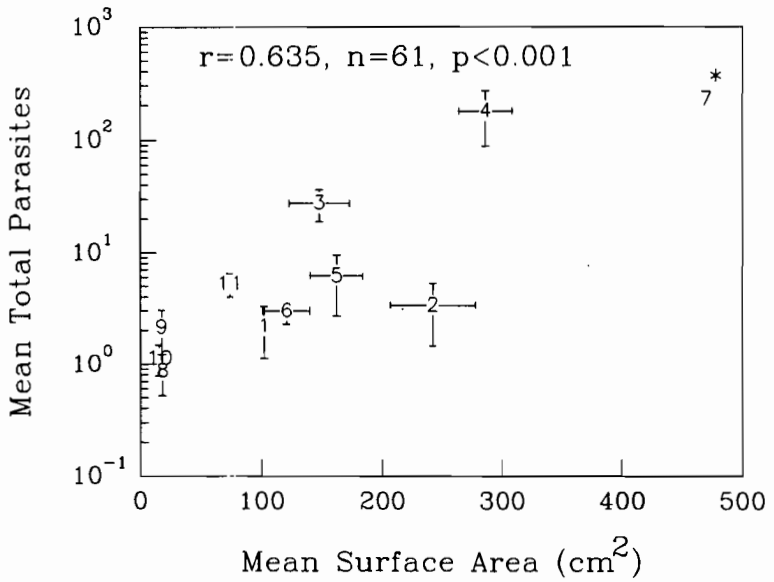

Figure 5.1. The mean number of parasites (± SE) per fish species as a function of mean surface area (± SE) of the fish species. The number of parasites were log10 transformed to satisfy the assumption of linearity in the correlation analysis. 1=*Acanthochromis polyacanthus*, 2=*Ctenochaetus striatus*, 3=*Scolopsis bilineatus*, 4=*Siganus doliatus*, 5=*Chlorurus sordidus*, 6=*Thalassoma lunare*, 7=*Hemigymnus melapterus*, 8=*Neopomacentrus azysron*, 9=*N. cyanomos*, 10=*Pomacentrus moluccensis*, 11=*Ambliglyphidodon curacao*. *No SE available as parasite load was estimated from the mean standard length of fish observed.

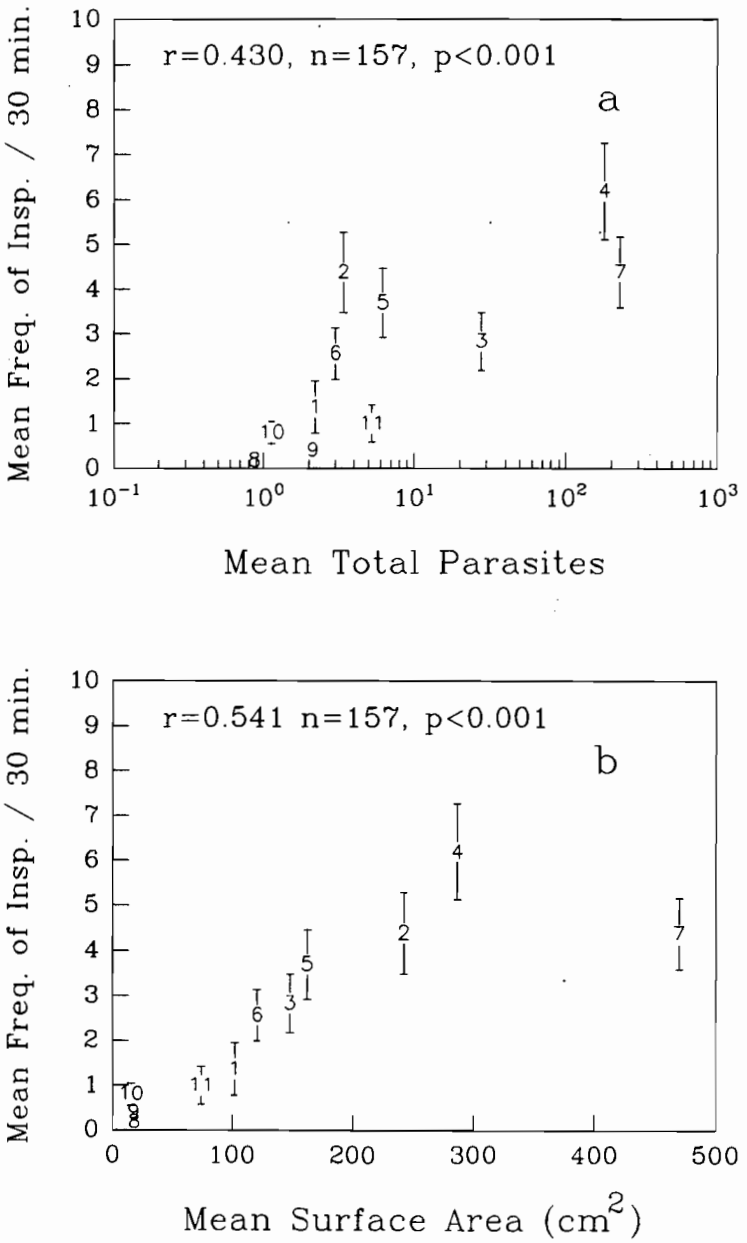

Figure 5.2. The mean number of inspections (± SE) by *Labroides dimidiatus*, per 30 min observation period, as a function of: **a.** The mean number of parasites per fish species and **b.** The mean surface area per fish species. The frequency of inspection was log10 (x+1) transformed while both mean number of parasites and mean surface area were log10 transformed to obtain linearity and homogeneity of variance for the correlation analyses. See Figure 5.1 for definitions of species.

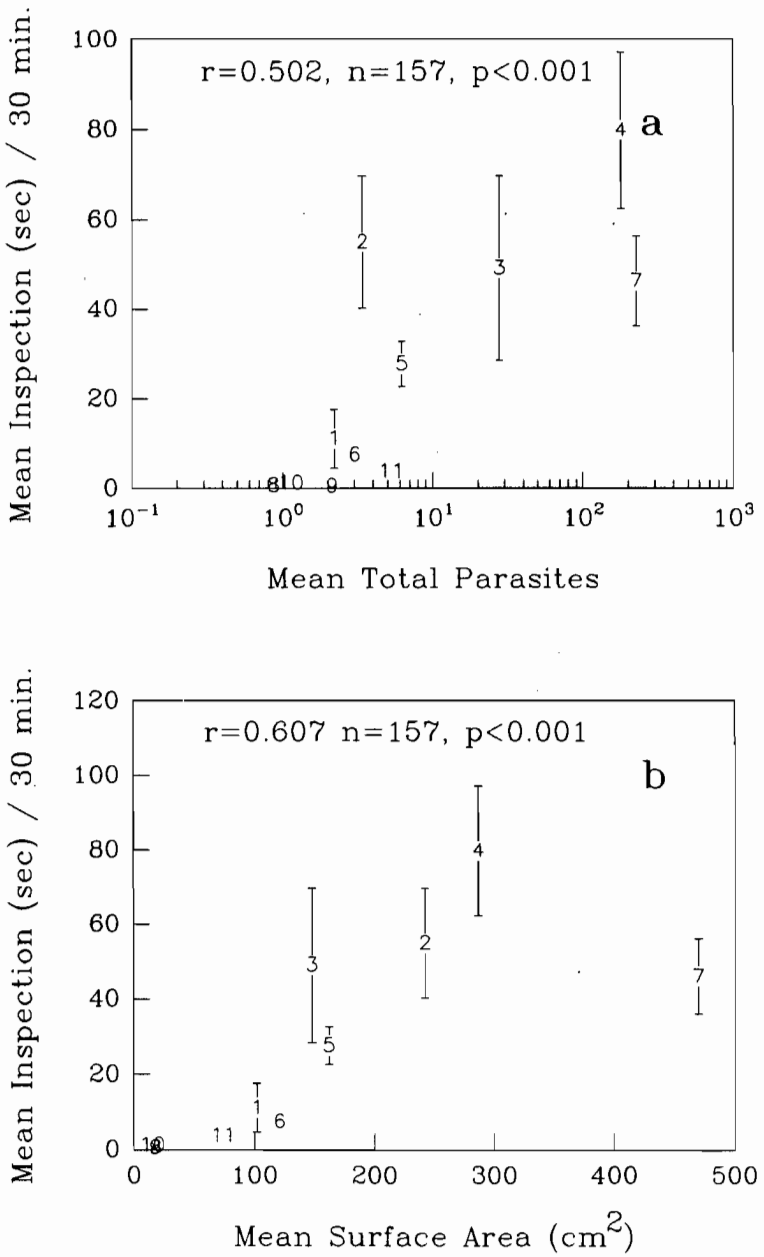

Figure 5.3. The mean sum of the duration of inspections (± SE) by *Labroides dimidiatus*, per 30 min observation period, as a function of: **a.** The mean number of parasites per fish species and **b.** The mean surface area per fish species. The duration of inspection was log10 (x+1) transformed while both parasites and surface area were log10 transformed to obtain linearity and homogeneity of variance for the correlation analyses. See Figure 1 for definitions of species.

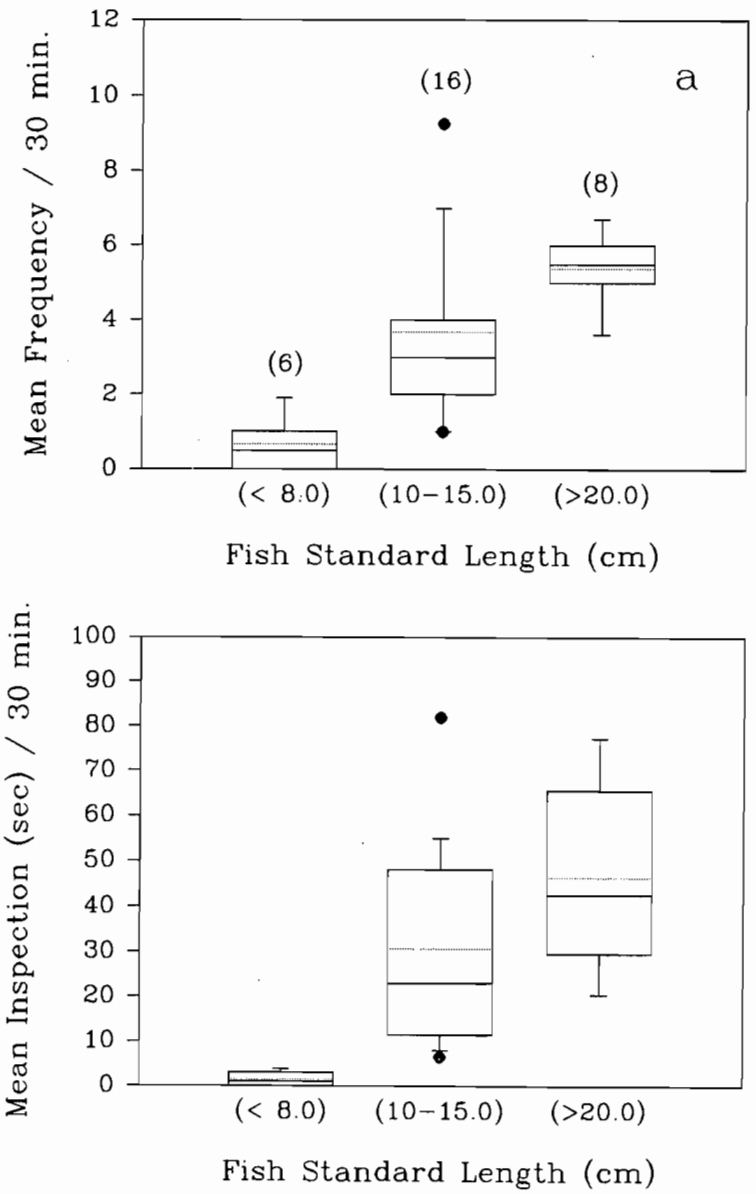

Figure 5.4. Box plots of the inspections by *Labroides dimidiatus* of *Hemigymnus melapterus* from three size ranges. **a.** The mean number of inspections per 30 min (sample size) (dotted line). **b.** The mean sum of the duration of all inspections per 30 min observation (dotted line). Error bars indicate the upper 95% and lower 5% quartile. Solid lines indicate the median, circles represent outliers.

CHAPTER VI

PARASITE REMOVAL RATES BY *LABROIDES DIMIDIATUS*

6.1 SUMMARY

The rate at which parasites (mainly gnathiid isopod larvae) were removed from fish by the cleaner wrasse *Labroides dimidiatus* was investigated. To examine the effect of this parasite removal on the parasites of fish, the number of parasites removed per individual host fish *Hemigymnus melapterus* per day was estimated and compared to the infection rate and abundance of gnathiids on *H. melapterus*. The study was conducted at Lizard Island, Great Barrier Reef, using a combination of observations of the feeding rates of cleaners, estimates of how much time individual hosts spend being cleaned, cleaner fish stomach content analyses, and a gnathiid manipulation experiment. The frequency and duration of inspection by *L. dimidiatus* were measured to provide an estimate of the feeding rate. Individual *Labroides dimidiatus* spent on average 256 (±SE 11) min per day inspecting 2297 (±SE 83) fish. *Labroides dimidiatus* consumed a large number of parasites (1218 ±SE 118) (mainly gnathiid isopods) each day. The estimated predation rate by *L. dimidiatus* was 4.8 (±SE 0.4) parasites per minute of inspection or 0.5 (±SE 0.05) parasites per inspection. The infection rate of gnathiids onto fish was high, with reduced gnathiid loads (by about 50%) on fish returning to levels similar to control fish within 1-6 days. These high infection rates suggest that a significant proportion of gnathiids removed by cleaner fish are quickly replaced. The high predation rate relative to the number of gnathiids on fish and their infection rate shows that cleaner fish have an effect on the abundance of gnathiids on fish.

6.2 INTRODUCTION

To understand the ecological significance of cleaning behavior in reef fish, information on the effect of cleaner fish on parasites is needed. How

cleaner fish affect parasites is dependant on the rates at which parasites are removed by cleaner fish and added through colonization. Examining the rates and processes involved in parasite removal and infection provides insight into the mechanisms involved in cleaner fish-parasite interactions; however few workers have taken this approach.

The effect of cleaners on host fish has been measured by removing all cleaner fish from reefs and measuring the subsequent effect on host fish (Limbaugh 1961, Youngbluth 1968, Losey 1972, Gorlick *et al.* 1987, Grutter in review a). Of these, only Gorlick *et al.* (1987) have been able to demonstrate quantitatively, in the field, an effect of a cleaner fish on an ectoparasite. They found that cleaner fish influenced the size frequency distribution of a parasite species on a fish species, with fish having larger parasites in the absence of cleaner fish. Such studies provide information on the long-term effects of cleaners on fish. However, to understand what produces these effects, information on processes involved in the removal of parasites is needed.

The rate at which parasites are removed from fish (predation rate) provides information on the short-term dynamics of parasites in cleaning interactions. Two approaches have been used to measure rates of predation on parasites by cleaner fish. Gut content has been used as a measure of the amount of food eaten daily in conjunction with estimates of densities of cleaner fish and host fish (to calculate predation rates per m^2 of reef and per host fish per day) (Losey 1974). Estimates of daily consumption by temperate cleaner fish have been obtained by estimating the number of parasites on fish before and after adding cleaner fish to caged salmon (Treasurer 1994). However, both these estimates are probably conservative because the former does not account for the movement of food through the gut and the latter does not account for the potential turnover of parasites on fish.

Rates of predation on parasites by cleaner fish can also be obtained using a combination of diet analyses and cleaner fish feeding rates. Such an approach also provides insight into the feeding biology of cleaner fish. Diet analyses made throughout the day and corroborated with an estimate of the time required for

food to pass through the digestive tract provide a more reliable estimate of the food eaten per unit time. Feeding rates of cleaner fish can be estimated using the duration of inspection of hosts as this is correlated with the number of bites taken by cleaner fish (Youngbluth 1968, Losey 1971). The predation rate is, thus, the number of parasites eaten per unit time of inspection by cleaners. This is calculated by dividing the number of parasites eaten per unit time by the feeding rate (duration of inspection) of cleaners per unit time. Coral reef fish behavior often varies throughout the day (Hobson 1991, Polunin and Klumpp 1989, Choat and Clements 1993). By repeating observations of inspection behavior by cleaner fish throughout the day, temporal variation in feeding rates is accounted for, resulting in a more accurate estimate of predation rates by cleaner fish.

The number of parasites removed per individual fish per day can be estimated using predation rates and estimates of the amount of time individual hosts spend being inspected by *Labroides dimidiatus* per unit time (Grutter 1995a). Information on the infection rate provides an estimate of the rate at which parasites removed by cleaner fish are replaced through colonization. The rate of infection of parasites onto host fish can be estimated by reducing the number of parasites on fish and assessing the time required for parasite levels to return to normal. These estimates of predation rates by cleaner fish and infection rates of parasites, when combined with information on the gnathiid loads of fish, provide insight to the potential impact of cleaner fish on gnathiid abundance on host fish. This method is particularly useful for examining the effect of cleaner fish on mobile fish species as these species cannot be used in traditional cleaner fish removal experiments.

This study estimates the rate at which parasites (mainly gnathiids) are removed by the cleaner wrasse *Labroides dimidiatus* from host fish using observations of the feeding rates of cleaner fish, diet analyses, and estimates of the amount of time individual host fish spend being cleaned. The effect of parasite removal on the abundance of parasites on fish is explored using estimates of a) the total number of parasites removed per individual fish per

day, b) the number of gnathiids on fish, and c) the rate of infection of gnathiids onto fish. The parasite predation rate by *L. dimidiatus* is calibrated for changes in feeding rates throughout the day and is corroborated with an estimate of the rate of passage of food through the digestive tract. The fish species *Hemigymnus melapterus* is used in this study because its parasite assemblage is well known and includes gnathiid isopods (Grutter 1994) and because information on procedures for manipulating the abundance of gnathiids on this species is available (Grutter 1995b).

6.3 METHODS AND MATERIALS

6.3.1 FEEDING RATES

The duration of cleaner fish inspection of host fish, which involved visual examination of the body surfaces and or gills of host fish, was used as an estimate of amount of feeding by *Labroides dimidiatus*. The duration and frequency of inspection were tested for temporal differences within and among days. This information was also used to estimate the total duration of inspection and the total frequency of inspections per day per *L. dimidiatus* and for calculating the rate of predation on parasites per unit time of inspection. The length of an inspection event was determined from the time when a cleaner fish approached a host fish until it departed the host.

Eight adult *Labroides dimidiatus* were selected haphazardly at North Point on Lizard Island (14° 40' S, 145°26' E), along the reef crest and slope (2-7 m) and their locations marked on a map. Cleaner fish and host fish were given 1-2 minutes to habituate to diver presence prior to commencing observations. A total of 50 hours of observations were made by two observers. Each *L. dimidiatus* was observed for 15 minutes from a distance of 3-5 m. The duration of each inspection of a host fish by *L. dimidiatus* was recorded and the frequency of inspections calculated from these. The observations were made during 5 time periods (05:45-8:00, 08:15-10:45, 11:30-13:15, 15:00-17:10, 17:10-19:00) to account for temporal variation in inspection rates.

Observations were made in December-January on 30/12/1992, 2/1/1993, 10/1/1993. To determine whether these estimates were representative of cleaning rates throughout the year, two additional days of observations were made on 24/7/1993 and 27/10/1993. To examine the relationship between the duration of inspection and the number of bites taken by *Labroides dimidiatus*, the number of bites taken by *L. dimidiatus* was also recorded by one observer on 27/10/1993.

6.3.2 PARASITE INGESTION RATE

To calculate the number of parasites eaten by adult *Labroides dimidiatus* per unit time, cleaner fish were collected throughout the day. Collections of fish began and ended when fish left and entered their sleeping holes. Fish (n=7-9) were collected during six time periods (06:00-06:59,09:00-9:59, 10:00-11:59, 13:00-13:59, 14:00-15:59, 16:00-17:59) on 5 days in November 1993. The number of parasites in their digestive tract was quantified following Grutter (in review b). The heads of gnathiid isopods were used to estimate gnathiid abundance as heads remain intact throughout the gut.

6.3.3 GUT CLEARANCE RATE

To estimate the rate of food passage through the digestive tract of adult *Labroides dimidiatus*, the gut was labelled with empty *Artemia* cysts mixed with live *Artemia* to ensure ingestion. This feeding experiment was conducted in the field. *Artemia* and cysts were held in a 2L quick-seal plastic bag which was opened briefly, 50-150cm from *L. dimidiatus*, releasing several hundred live *Artemia* and cysts which were immediately eaten by *L. dimidiatus*. Fish (n=6-9 per time interval) were then collected at subsequent time intervals for diet analyses (0, 1-2hrs, 2-3hrs, 3-4hrs, and 4-7hrs). The whole gut was divided into 5 equal segments and the number of cysts in each segment was quantified. The proportion of cysts in each segment were plotted against time to estimate the time required for cysts to pass through the gut. Fish were collected from North Point in January 1993.

6.3.4 THE NUMBER OF PARASITES REMOVED PER INDIVIDUAL FISH PER DAY

The number of parasites removed per individual *Hemigymnus melapterus* per day was estimated with the product of the predation rate (number of parasites eaten per unit time of inspection, see statistical analyses section) and the amount of time individual hosts spent being inspected by cleaner fish per day. The amount of time individual fish (10-15 cm) were inspected per day was estimated by multiplying the average duration that *H. melapterus* were inspected per 30 min (30.6 sec ±SE 0.625 which was not significantly different throughout the day, Grutter 1995a), by the average number of waking hours of *Labroides dimidiatus* (12. 65 ±SE 0.04 hrs).

6.3.5 PARASITE INFECTION RATE

The rate of infection of gnathiids onto the host fish *Hemigymnus melapterus* was estimated by measuring the time required for reduced levels of gnathiid isopods on treated fish to return to normal and comparing these with control fish. Treated fish (n=7) were captured with a net, immediately placed in separate plastic bags, and taken to the boat (following Grutter 1995b). The number of gnathiids was reduced by placing each fish in a shaded container with seawater (10-20 L) for two hours (Grutter 1995b). This method has been shown to reduce the number of gnathiids on this fish species by 73% ±SE 7.6 (Grutter 1995b). Fish were tagged (see below) and recaptured 1-16 days later.

Control fish (n=20) were captured as above but not released. Nine of the control fish were left in plastic bags and all their parasites quantified. To estimate the proportion of gnathiids removed above, the parasite loads of the remaining control fish (n=11) were reduced as above. However, these fish were not released but were retained to quantify the parasites remaining on their body. This experiment was conducted between December 1992 and January 1993 on two large patch reefs (approximately 180x100 m and 75x100 m).

6.3.6 TAGGING

In order to recapture treated individuals, *Hemigymnus melapterus* were

tagged by a dermal injection of acrylic paint (Vynol-Derivan, Alexandria, Australia) on the operculum. Two 5-8 mm stripes of different colored paint were used on each operculum to aid in the identification of individual fish. During tagging, fish were held in 2 L quick-seal plastic bags with some seawater to reduce handling stress and to contain parasites.

6.3.7 STATISTICAL ANALYSES

The sum of the duration of all inspections per observation (15 min) was used in all analyses. Separate univariate repeated measures analyses were used to test for differences in the duration and in the frequency of inspection per observation period among times periods of day and among the 5 days. The above analyses were repeated with only the 3 days in December-January in order to avoid any potential seasonal confounding effects when calculating predation rates for December-January and to determine whether or not to average across times of day and days when calculating the mean duration and frequency inspection. Outliers had a marked effect on the homogeneity of variance among treatments (Mauchly's sphericity test applied to orthogonal components had significant χ^2 values ($p<0.05$)), but these could not be omitted because univariate repeated-measures analyses cannot be run with missing values (S.A.S. 1991). The study also required estimates of the normal feeding rates of cleaner fish which could be biased by outliers. Therefore, outliers (values 2 to 6 times higher than the mean) were replaced with means and these data were used in all analyses. Spericity tests conducted without outliers indicated that the assumption of homogeneity of variance was satisfied. Two outliers in the duration of inspection (1533 and 1664 sec) were replaced with the overall mean (280 sec) and one outlier for the frequency of inspection (127) was replaced with the overall mean (52.5). Because unadjusted tests in univariate repeated-measures analyses are extremely sensitive to the assumption of sphericity, the Greenhouse-Geisser adjusted procedure for the significance test was used as it maintains Type 1 error rate at or below the nominal value (Maxwell and Delaney 1990). The beginning and end of the day were defined as the mean

time fish were observed leaving and entering their sleeping holes respectively.

The predation rate of *Labroides dimidiatus* (number of parasites eaten per unit time of inspection) was estimated by dividing the number of parasites in a full gut by the inspection time required to achieve a full gut. It was assumed that the gut was full when parasite numbers in the diet reached a maximum (the time required to achieve a full gut was compared with the estimate of the food passage rate to determine whether gut evacuation occurred before the gut was full). To find the maxima, the total number of parasites per gut were plotted against time of collection and a line fitted using locally-weighted regression scatter-plot smoothing (LOWESS regression) (Trexler and Travis 1993). The peak in the above plot was assumed to represent a full gut. The standard error of this value was calculated using the number of parasites in the diet of fish collected around this time (n=10). This study assumes that the efficiency of cleaner fish predation is the same throughout the day.

To estimate the total number of parasites eaten per individual cleaner fish per day the predation rate was multiplied by the mean total duration of inspection per day (see results for calculation of latter). The number of parasites removed per fish inspected was calculated by dividing the mean total number of parasites eaten per day by the mean total frequency of inspections per day (see results for calculation of latter). Appropriate standard errors were calculated for the above estimations (Parratt 1966).

An analysis of covariance (ANCOVA) was used to test whether the number of parasites on recaptured fish was the same as on control fish with standard length of fish as the covariate. The same analysis was used to test for differences in the cumulative number of gnathiids recovered from treated fish (parasites in container plus on recaptured fish) and the number of gnathiids on controls. The slopes were not significantly different in both ANCOVAs ($p=0.342$ and $p=0.293$ respectively) so the interaction term was dropped (S.A.S. 1991). For the ANCOVAs, data were natural log transformed to satisfy the assumption of linearity.

Table 6.1. Univariate repeated measures analysis testing for differences in the duration of inspection (summed over 15 min) by *Labroides dimidiatus* among time periods of day and among days. **a.** The duration of inspection on 5 days. **b.** The duration of inspection on 3 of the 5 days (December-January).

a.

Source	F	df	p
Time of day	0.47	4, 28	0.685
Day	1.66	4, 28	0.233
T x D	14.26	16, 112	0.001

b.

Source	F	df	p
Time of day	2.51	4, 28	0.124
Day	3.84	2,14	0.054
T x D	0.73	8, 56	0.585

6.4 RESULTS

6.4.1 FEEDING RATES

The duration of inspection by cleaner fish did not differ among times of day nor among the 5 days (Table 1a). However, the interaction term (Time of day x Day) was significant (Table 1a) which indicates the duration of inspection among times of day was not the same over all days. This is likely due to inspections on 24 July which were higher during the midday hours compared with other days (Fig. 1a). Among the three days in December-January, there was no significant difference in the duration of inspection among times of day nor days (Table 1b), therefore the mean duration of inspection was calculated by averaging across times of day and days (303±SE 13 sec/15 min).

The estimated mean total duration of inspection by cleaner fish per day was 256 (±SE 11) min and includes all host fish species inspected in a day. This estimate is based on a daily activity of 12.65 ±SE 0.04 hrs, determined from the mean time that cleaners left and entered their sleeping holes (06.03 ±SE 0.02 hrs and 18.68 ±SE 0.04 hrs respectively).

The frequency of inspection among the 5 days varied significantly during the day (Table 2a) and was highest during the early morning (Fig. 1b). There were no differences in the frequency of inspection among the 5 days (Table 2a). The frequency of inspection in the 3 days in December-January did not differ among times of day and among days (Table 2b)(Fig. 1b), therefore the mean frequency of inspection was calculated by averaging across times of day and days (45 inspections/15 min ±SE 1). The estimated number of fish inspected by cleaner fish per day was 2297 fish (±83) (the product of the mean frequency of inspection and the mean number of waking hours of *L. dimidiatus*).

The frequency of bites taken by *Labroides dimidiatus* was positively correlated with the duration of each inspection (Fig. 2) which shows that duration of inspection is a measure of feeding in *L. dimidiatus*. The total number of bites, the sum of the duration of inspection, and the frequency of inspection per observation follow a similar pattern of activity to each other

Table 6.2. Univariate repeated measures analysis testing for differences in the frequency of inspection (per 15 min) by *Labroides dimidiatus* among time periods of day and among days. **a.** The frequency of inspection on 5 days. **b.** The frequency of inspection on 3 of the 5 days (December-January).

a.

Source	F	df	p
Time of day	4.78	4, 28	0.025
Day	0.55	4, 28	0.606
T x D	2.02	16, 112	0.102

b.

Source	F	df	p
Time of day	2.46	4, 28	0.112
Day	0.15	2, 14	0.752
T x D	2.89	8, 56	0.058

throughout the day (Fig. 3). This shows that when the frequency of inspection and the duration of inspection were low, *L. dimidiatus* was also taking fewer bites and therefore eating less.

6.4.2 PARASITE INGESTION RATE

Most of the parasites in the diet were gnathiid isopod larvae (99.7% ±SE 0.06). The remainder consisted of a few caligid copepods and other parasitic copepods. The number of parasites in the diet throughout the day showed two peaks, one at 09:36 hrs and another at 16:00 hrs (LOWESS regression f=0.35)(Fig. 4). The average number of parasites ingested by the time of the first peak was 343 (±SE 26) min. The amount of time *L. dimidiatus* spent inspecting fish from 06:18 hrs to 9:36 hrs (at which time its gut was filled), was 72 ±SE 3 min (product of 303 ±SE 13 sec of inspection/ 15 min and 3.57 hrs). This gave an estimated predation rate of 4.8 ±SE 0.4 parasites eaten per minute of inspection (343 ±SE 26 parasites/day divided by 72 ±SE 3 min of inspection/day). When the predation rate was multiplied by the total duration of inspection per day (256 ±SE 11 min), the estimated total number of parasites eaten per cleaner fish per day was 1218 ±SE 118. Therefore, the number of parasites removed per inspection is 0.5 ±SE 0.05 (the number of parasites eaten per day divided by the number of fish inspected per day).

By the time cleaner fish had a full gut, roughly 6 bites were taken for every parasite eaten which shows that not all bites involved the removal of parasites. This was estimated by dividing the total number of bites taken during the time interval (the product of the mean bite rate during this time interval and the time interval) by the number of parasites in a full gut.

6.4.3 GUT CLEARANCE RATE

The maximal time required for food to pass through the gut was slightly over 3.7 hrs (Fig. 5). Cysts were found in the fifth segment 1.47-3.7 hrs after they were eaten while none of the 9 fish collected after this time had any cysts in their gut. Such a rate of digestion is consistent with the estimate of the total

number of parasites eaten per day. Empty shells of *Artemia* cysts were easily distinguished throughout the gut. Although not all fish collected contained cysts, the number of fish which did was sufficient to estimate the time required for food to pass through the gut. Many of the guts without cysts contained *Artemia* nauplii which indicates they were involved in the feeding experiment. However they were not used for estimating gut clearance rates as *Artemia* were only identifiable in the gut when in large quantities and only in the foregut.

6.4.4 NUMBER OF PARASITES REMOVED PER FISH PER DAY

An estimated 61 (±SE 3) parasites were removed per individual *Hemigymnus melapterus* (10-15 cm) per day. These fish have, on average, 11 (±SE 3) gnathiids per individual.

6.4.5 PARASITE INFECTION RATE

The rate of infection of gnathiids onto fish was high. The number of gnathiids on recaptured *Hemigymnus melapterus* was not significantly different from that on control fish (ANCOVA Treatment: $F=1.26$ $df=1,34$ $p=0.269$; covariate (SL): $F=19.45$, $df=1,34$ $p<0.001$) which indicates that gnathiid abundance had returned to control levels during an interval of 1-16 days (88% of fish were collected within the first 6 days of releasing fish)(Fig. 6a). Because of the difficulties associated with finding and then collecting tagged fish it was not possible to recapture fish at regular intervals. The total cumulative number of gnathiids recovered from treated fish (gnathiids in container plus on recaptured fish) was significantly higher than on control fish (ANCOVA Treatment: $F=14.97$ $df=1,34$ $p<0.001$; covariate (SL): $F=25.51$, $df=1,34$ $p<0.001$)). Thus gnathiids quickly recolonized treated fish after they were released (Fig. 6b). Placing control fish in a container lowered gnathiid numbers by 50% (±SE 7). Parasite manipulations and tagging did not appear to alter the behavior of the fish in the field as observations after their release indicated that they fed and behaved normally.

6.5 DISCUSSION

Labroides dimidiatus inspect a large number of fish and in the process feed on large numbers of parasites (mainly gnathiid isopods). The movement of gnathiids onto fish (infection) and off fish (mortality by predation from cleaner fish or emigration) is highly dynamic. The large number of gnathiids removed per individual fish on a daily basis, relative to the number of gnathiids on fish at a given time, shows that cleaner fish have an effect on the abundance of gnathiids on fish. However, more information on the carrying capacity of gnathiids on hosts and the processes involved in the infection of gnathiids onto fish is needed to determine to what extent gnathiid abundance is suppressed.

Labroides dimidiatus is an effective predator and removes, on average, 4.8 parasites (mainly gnathiids) per minute of inspection or 0.5 parasites for each fish inspection event. Individuals of some fish species are cleaned, on average, about 3 to 6 times per 30 min (e.g. *Hemigymnus melapterus* 10-15 cm in SL are inspected 3.7 times/30 min)(Grutter 1995a). The number of cleaning events individual fish experience may therefore be as high as 144 inspections per day (Grutter 1995). It is therefore likely that some fish have many parasites removed on a daily basis.

The number of parasites removed per fish inspected is similar to that of Losey (1974) who estimated that cleaner gobies in Puerto Rico ate 0.5 gnathiids per host fish. Losey also estimated that cleaner gobies in Puerto Rico ate 1.6 parasites per m^2 while *L. phthirophagus* in Hawaii ate only between 0.003 and 0.03 parasites per day per m^2. Based on these large differences in predation rates, Losey (1974) concluded that cleaning in Puerto Rico may be mutualistic. The predation pressure per unit area at Lizard Island appears to be much higher than that of Puerto Rico and Hawaii with an estimated 17.9 parasites eaten per m^2 per day (based on 1.47 *L.dimidiatus* per 100 m^2, Green 1994). However, caution must be taken when making comparisons between these studies, as Losey's (1974) predation rates are based on gut contents and the abundance of cleaner fish and host fish, while this study was based on cumulative gut contents and the feeding behavior of cleaner fish.

The rate of infection by gnathiids onto *Hemigymnus melapterus* was relatively high. The abundance of gnathiids (which had been reduced by about 50%) on treated fish quickly returned to levels of gnathiids found on control fish. Thus, on

average, gnathiid abundance on fish doubled from the time fish were initially treated to when they were recaptured. The majority of treated fish (88%) were recaptured within 6 days (Fig. 6). However, many of the total cumulative parasite loads on treated fish recaptured in less than 6 days appear to be higher than control fish (Fig. 6b). This suggests that the doubling of gnathiid abundance occurred in less than 6 days. Other studies have found that gnathiids, which are only temporary parasites, remain on hosts for 'several hours' (Stoll 1962), 2-4 hours or 1 or more days depending on site of attachment (Paperna and Por 1977), and 2-24 hrs (Davies 1981). Thus complete reinfection of *H. melapterus* by gnathiids likely occurred in less than 6 days.

By multiplying the predation rate by an estimate of the time that individual *Hemigymnus melapterus* spend being inspected by *L. dimidiatus* in a day, the number of parasites (mainly gnathiids) that were removed from an individual *H. melapterus* was estimated (61 ±SE 5). This number is about five times higher than the number of gnathiids found on individual hosts at a given time (11 ±SE 3). The removal of such a high number of gnathiids on a daily basis, relative to the low standing crop of gnathiids, is possible if the turnover rate of gnathiids on fish is high enough that gnathiids removed by cleaner fish are quickly replaced by other gnathiids. This study and other studies (Stoll 1062, Paperna and Por 1977, Davies 1981) suggest that the infection rate of gnathiids can be relatively high.

Whether the predation rate has an impact on gnathiid abundance on *Hemigymnus melapterus* depends on the infection rate. The estimated infection rate in this study predicts a doubling in gnathiid abundance in 1-6 days. This infection rate of gnathiids onto fish is little compared to the daily predation rate (61 ±SE 5) which is 6 times the standing crop of gnathiids. This suggests that gnathiid abundance on *H. melapterus* is suppressed by *Labroides dimidiatus*. Individuals of other fish species are cleaned as often as or even more than *H. melapterus* (Grutter 1995a) and also have gnathiids (Grutter 1994). Thus a similar effect of cleaner fish predation on gnathiids is to be expected on these fish species.

There is evidence that gnathiids mainly infect fish during the night (Potts 1973, Paperna and Por 1977). It is therefore highly likely that a proportion of gnathiids removed by cleaner fish during the day may be replaced at night. If much infection occurs at night, the effect of cleaner fish on gnathiid abundance may be

temporary and occur only during the day.

Despite heavy predation on gnathiids by cleaner fish, hosts still have gnathiids. Whether this abundance is lower than the maximum carrying capacity of gnathiids on hosts is crucial to understanding the extent to which cleaner fish suppress gnathiid abundances. High gnathiid densities on fish have been reported but these have been on captive fish (Paperna and Por 1977, Mugridge and Stallybrass 1983). Whether such high densities occur in the wild are unknown. The number of gnathiids on fish increases with the size of *Hemigymnus melapterus* (Fig. 6) and with the size of the fish species (Grutter 1994) suggesting an effect of space on the abundance of gnathiids. Studies on the effect of the absence of cleaner fish on gnathiid abundances are needed to resolve this question.

Possibly, the standing crop of gnathiids is related to the number of cryptic sites on fish. The time gnathiids remain on fish after engorgement varies according to the site of attachment, with gnathiids attached to the gills and pharyngeal chamber remaining on the fish much longer than those on the skin (Paperna and Por 1977). This behavior may be related to risk of predation, and may explain why reduced levels of gnathiids on treated fish did not keep increasing but 'stabilized' at numbers similar to those on controls.

The predation estimates assume that parasites are evenly distributed among fish. However, this is unlikely as parasite loads and species composition are often host-specific (Grutter 1994). *Labroides dimidiatus* also feeds selectively on gnathiids (Grutter in review b) whose abundance often varies according to the size of fish (Fig. 6, Grutter 1994). Furthermore, *L. dimidiatus* foraging efficiency probably varies among fish species due to differences in host fish morphology and behavioral responses among species. Therefore, the number of parasites *L. dimidiatus* obtains per unit time probably varies among fish sizes and species.

Higher rates of feeding by cleaner fish in the morning may be due to several factors. *Labroides dimidiatus* may become satiated after feeding at a rapid rate and respond by lowering its feeding rate. This has been shown for skipjack tuna which reduces its responsiveness to food when its stomach is half full (Magnusen 1969). Alternatively, since host fish have some control over the outcome of a cleaning interaction (Losey 1971), the possibility arises that the behavior of hosts may influence the feeding behavior of *L. dimidiatus*. Potts (1973) suggested that hosts

were more available in the morning as a result of increased infection of gnathiids at night. Although a preliminary investigation has revealed no significant effect of time of day on individual host cleaning rates (Grutter 1995a) only seven host fish species were examined. Finally, coral reef fish often behave differently in the morning and/or at nightfall compared to the midday (Hobson 1991, Polunin and Klumpp 1989, Choat and Clements 1993). The fact that the frequency of inspection varied more among times of day than the duration of inspection suggests that factors which influence host fish abundance influence how many fish cleaners inspect.

Although the duration of inspection among days and the interaction term (Time of Day and Day) for the frequency of inspection in December-January were almost significant (Table 1b and 2b) both were averaged across times of day and days. This may have introduced some error into the estimates of daily duration and frequency of inspection. More studies are needed to examine temporal variation in the inspection variation of *Labroides dimidiatus*.

The longer duration of inspection by *Labroides dimidiatus* in July (Fig. 1a) suggests there may be seasonal variation in feeding rates. There is considerable temporal variation among months in the abundance of gnathiids in the diet of *L. dimidiatus*, and in the size frequency distribution of gnathiids in the diet and on a host fish *Hemigymnus melapterus* (Grutter in review b). Such temporal changes in the diet probably coincide with changes in foraging patterns and feeding rates. Although there was little variation in the duration of inspection among days in December-January, the feeding rates in July were only recorded on one day and must therefore be interpreted cautiously. More observations at different times are needed to determine whether there is seasonal variation in feeding rates of *L. dimidiatus*.

The number of gnathiids consumed daily per cleaner fish, when converted to biomass (223 μg) (following Grutter in review b) is 7% of the body weight of *L. dimidiatus*. This estimate agrees with the estimates of the daily requirements of similar sized fish (Daan 1973, Ruggerone 1989). Organisms similar to gnathiids, crustacean zooplankton, are high in protein (Parsons and Takahashi 1973). These suggest gnathiids probably provide the bulk of the food requirements of *L. dimidiatus*.

The bimodal pattern of parasite abundance in the diet throughout the day

may be due to changes in feeding rates throughout the day and to a delay in gastric emptying. If feeding rates (and defecation rates) are constant then parasite abundance in the gut should reach an asymptote. Instead, parasite abundance increased rapidly in the morning while feeding rates were high, and then, although the numbers of parasites in the diet did appear to reach a maximum (at 09:36 hrs), the number of parasites in the gut dropped. This drop is probably partly due to reduced feeding rates at this time and to the gut clearance rate. Assuming that gastric emptying continued at a constant rate, the continued decline in parasite abundance in the gut during midday suggests that the intake of parasites was reduced as a result of depressed feeding rates. This is reinforced by the increase in parasite abundance in the gut which coincided with an increase in feeding rates. Such variable patterns in gut contents have important implications for the timing of sampling of gut contents.

The time required for food to pass through the digestive tract (about 3.7 hrs) agrees with a similar gut clearance study which also used labelled food, fish that fed continuously and were of a similar size to *L. dimidiatus*, and a similar water temperature (Noble 1973). The rate is consistent with and therefore corroborates the estimate of the total number of parasites eaten per cleaner fish per day.

This study suggests that cleaner fish suppress gnathiid abundances on fish. Whether hosts benefit from the removal of gnathiids depends on their effect on hosts. The effects of gnathiids on hosts vary, ranging from slight blemishes (Davies 1981) and lesions (Monod 1926), to heavy inflammation and hypertrophy of tissues (Honma *et al.* 1991), and death (Paperna and Por 1977, Mugridge and Stallybrass 1983). The latter deleterious effects, however, occurred in captivity (Paperna and Por 1977, Mugridge and Stallybrass 1983) and involved large gnathiids (Paperna and Por 1977, Honma *et al.* 1991). Gnathiids at Lizard Island are relatively small (Grutter 1994) and therefore may not be as damaging to the host. There is circumstantial evidence that gnathiids may be a vector for the blood parasite *Haemogregarina bigemina* but this has not been refuted nor substantiated (Davies and Johnston 1976). Whether gnathiid parasites have any lasting effect on the health of host fish is therefore unclear.

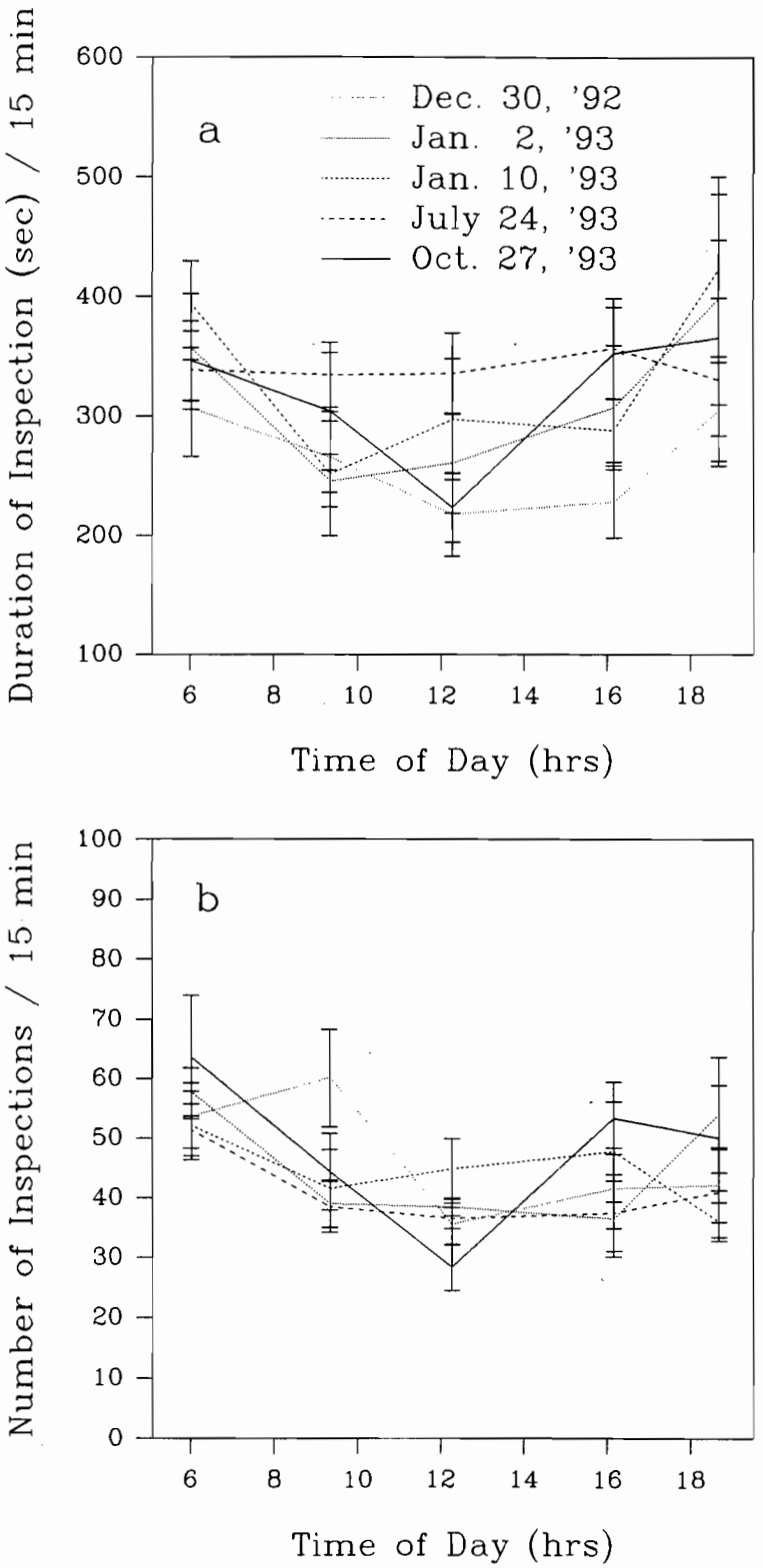

Figure 6.1. Inspection rates by *Labroides dimidiatus* during several time periods of day and on several days. **a.** The sum of the duration of all inspections per 15 min observation period. **b.** The number of inspections per 15 min observation period.

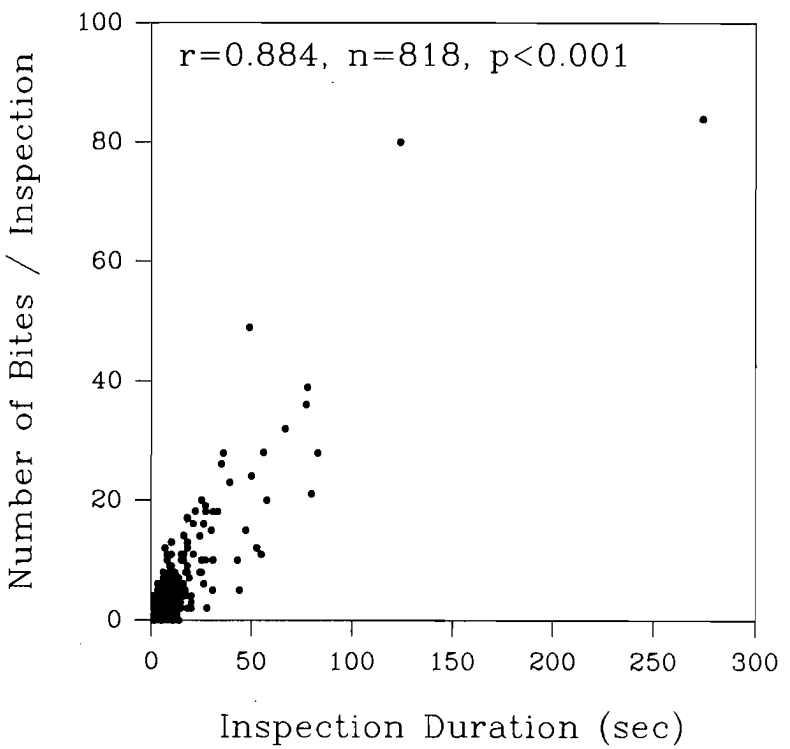

Figure 6.2. The number of bites taken by *Labroides dimidiatus* per inspection event compared with the duration of the inspection.

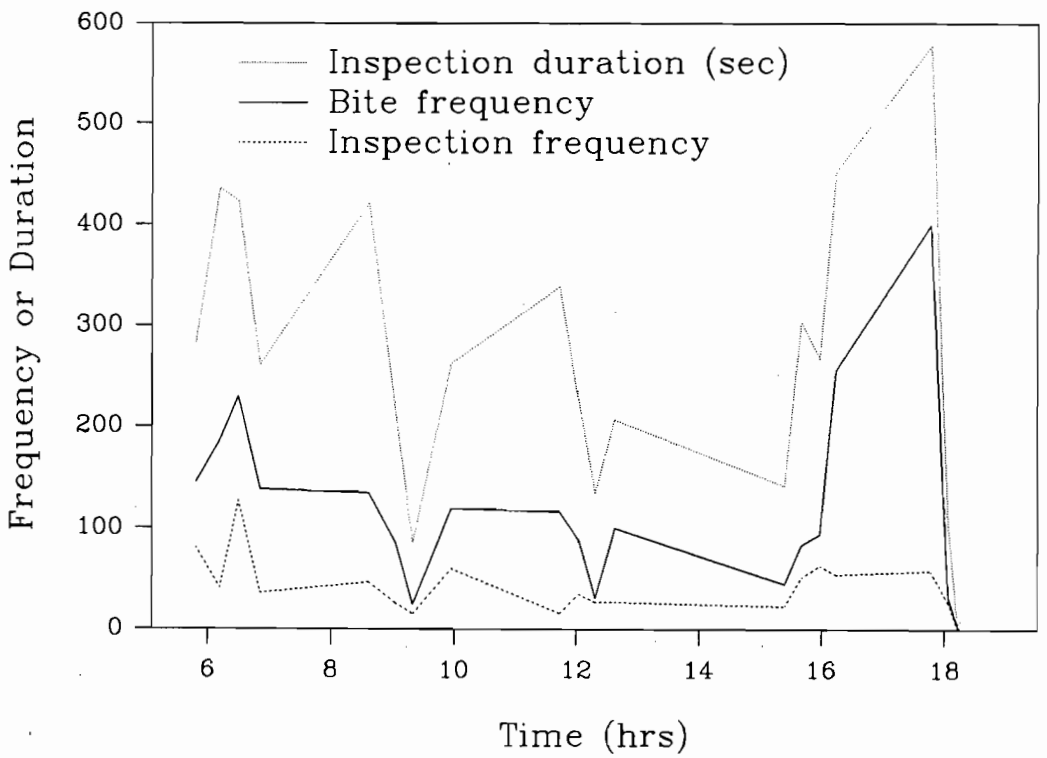

Figure 6.3. Simultaneous measurements of the feeding behaviour of *Labroides dimidiatus* throughout the day (Oct. 27, 1993).

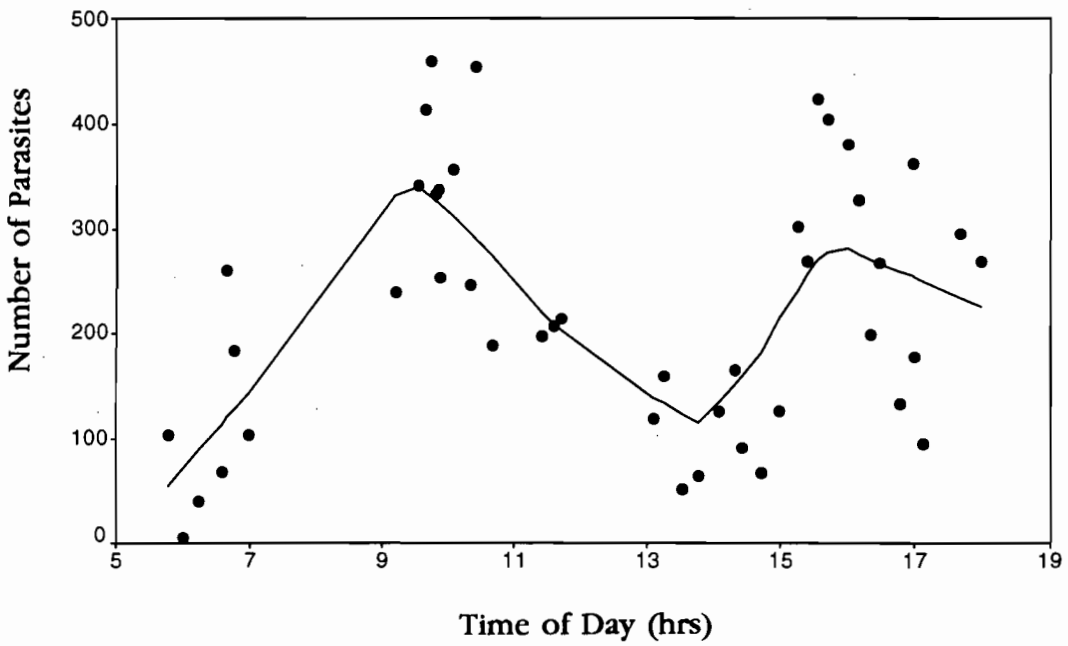

Figure 6.4. The total number of parasites in the digestive tract of *Labroides dimidiatus* used to calculate their parasite predation rate and total daily intake of parasites per day. The mean standard length of *L. dimidiatus* was 5.5 (±SE 0.1) cm.

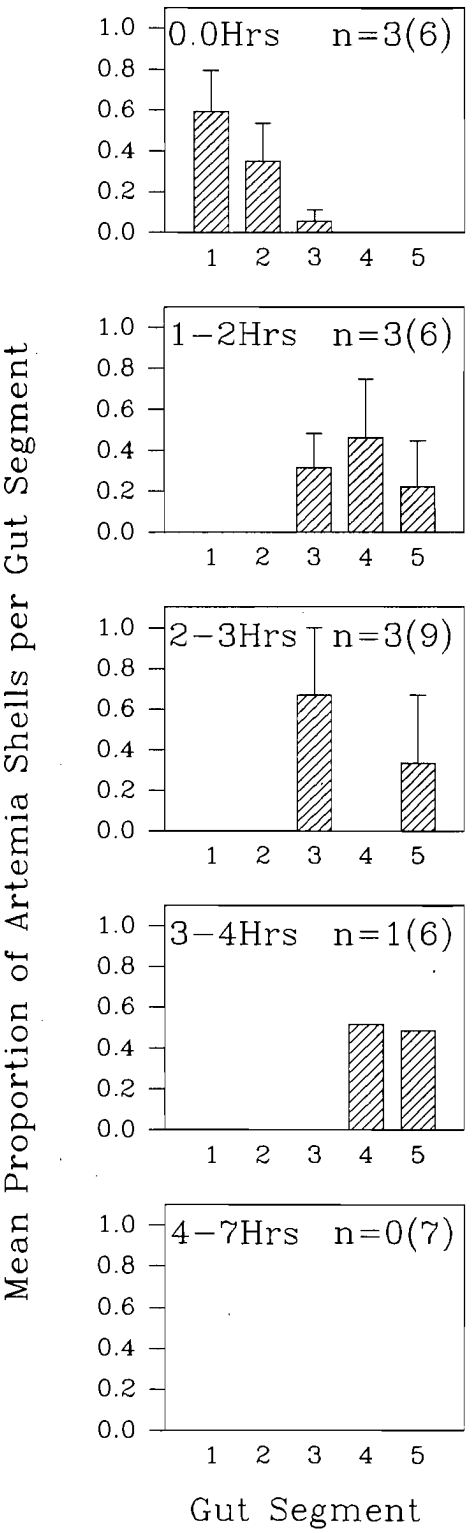

Figure 6.5. The mean proportion of empty shells of *Artemia* cysts (± SE) per gut segment of *Labroides dimidiatus* at different times. The sample sizes of guts containing cysts are provided; the numbers in brackets are the total number of fish fed the *Artemia* mixture. Note: no fish (n=9) recaptured more than 3.7 hours after feeding on *Artemia* contained cysts. The mean standard length of *L. dimidiatus* was 5.4 (±SE 0.1) cm.

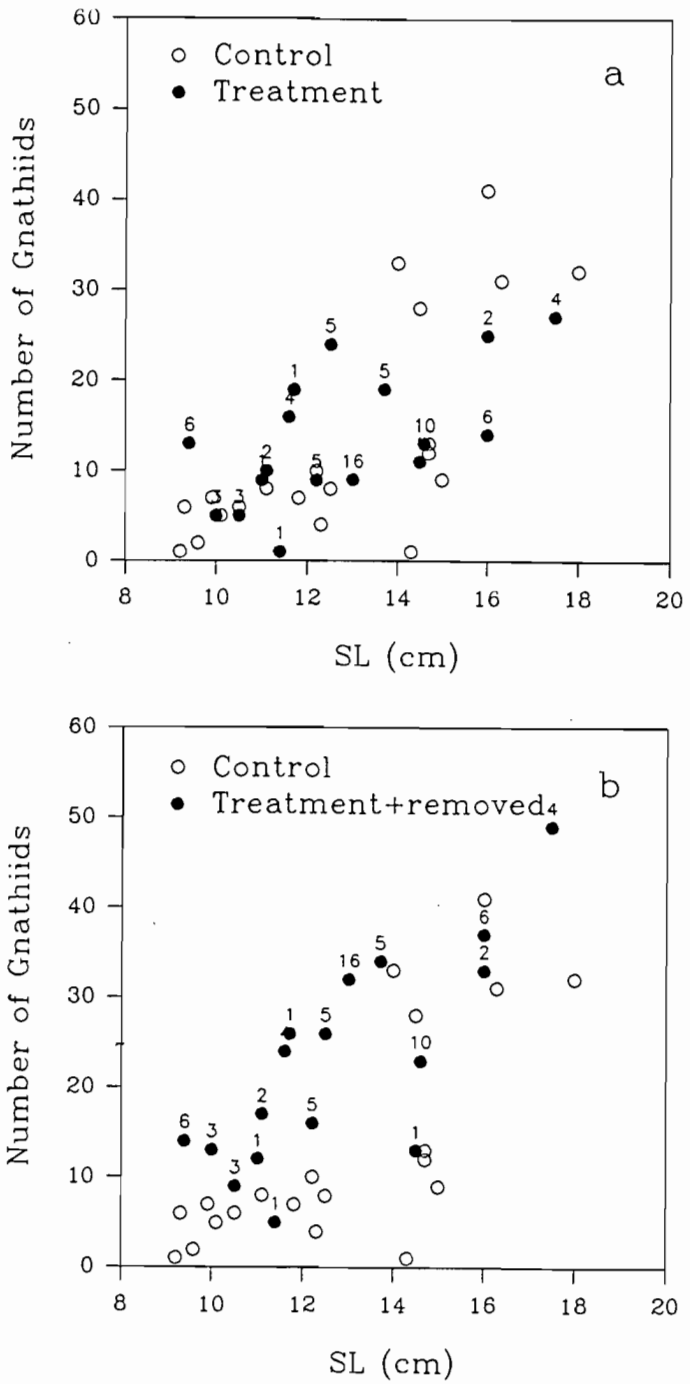

Figure 6.6. Gnathiid isopod abundance on *Hemigymnus melapterus* used for estimating gnathiid infection rates. **a.** The number of gnathiids on control fish and on treated recaptured fish (1-16 days after their gnathiid loads were reduced by 50%). **b.** The number of gnathiids on control fish compared with the cumulative number of gnathiids recovered from treated fish (the sum of the number of gnathiids on fish after 1-16 days plus the gnathiids removed on day zero). Fish are labelled with the number of days from parasite manipulation to recapture.

CHAPTER VII

AN EXPERIMENTAL EVALUATION OF THE EFFECT OF *LABROIDES DIMIDIATUS* ON THE FISH *POMACENTRUS MOLUCCENSIS*

7.1 SUMMARY

To date, the benefits of cleaner fish to the host have not been clearly identified. This study investigates the effect of the cleaner wrasse *Labroides dimidiatus* on the damselfish *Pomacentrus moluccensis* by excluding all cleaner fish from several reefs for 6 months at Lizard Island. The subsequent effect on parasites (total number, number per category of parasite, and size of parasite) and host abundance was estimated and compared to control reefs with *L. dimidiatus*. Parasite loads of *P. moluccensis* were low (usually 0-3 per fish) and were dominated by small copepod larvae (260-1370 μm). The absence of *L. dimidiatus* had no effect on total parasite abundance, number of parasites per category, and size of the most abundant copepod species. There was, however, a significant difference in the total number of parasites per fish among reefs. The abundance of *P. moluccensis* declined during the experiment (7-33%) but the decline did not differ among reefs with and without *L. dimidiatus*. This indicates that *P. moluccensis* did not leave reefs to seek cleaning elsewhere nor suffer increased mortality in the absence of *L. dimidiatus*. The fact that *L. dimidiatus* had no effect on the parasites of *P. moluccensis* is compatible with studies of *L. dimidiatus* foraging behaviour since *L. dimidiatus* feed primarily on parasitic gnathiid isopods. It is likely that factors other than ectoparasite removal motivate hosts to seek cleaning.

7.2 INTRODUCTION

The ecological significance of cleaning behaviour in the marine environment is still poorly understood, despite many studies (see review by Losey 1987). Although there is no doubt that cleaner fish benefit from cleaning, which provides them with food (Randall 1958, Youngbluth 1968, Gorlick 1980), the benefit of cleaning for client fish remains unresolved (Youngbluth 1968,

Losey 1972, Gorlick *et al.* 1987). Recent work has shown that individuals of some diurnal species spend a significant proportion of their time (up to 32 min per day) being cleaned (Chapter V). Thus it appears that cleaning is important, but as yet there is little evidence which suggests a particular cause.

In considering the ecological effects that drive cleaning interactions, the ultimate and proximate causation must be separated (Gorlick *et al.* 1978, Losey 1987, 1993). Ultimate causation or adaptive value refers to factors which result in the evolution of the association such as increased reproductive success as a result of ectoparasite removal by cleaner fish; proximate causation refers to factors that maintain the behaviour such as the attraction of fish to cleaners (Gorlick *et al.* 1978). An obvious candidate for the ultimate cause of cleaning in fish is ectoparasite removal. A direct test of this ultimate cause would be to remove all cleaners and measure the effect on parasites and or hosts (Losey 1972, 1987). This effect can be measured as variation in parasite infection, changes in host condition and or abundance, or by an increase in cleaning by other organisms (Losey 1972, 1987).

This experiment has been done several times with different results. Only one experiment has shown that the removal of cleaners results in increased infection or emigration of host fish (Limbaugh 1961). Limbaugh's study, conducted in the Bahamas, removed 'all the known cleaning organisms' but no quantitative data and controls were used. Youngbluth (1968) found no increase in infection of host fish nor change in density of fish when he removed all cleaner fish, *Labroides phthirophagus* (Labridae), from a reef in Hawaii. However only 'approximations of numbers of parasites' were used and fish abundance was not directly quantified. A more quantitative study by Losey (1972), at the same sites used by Youngbluth (1968), found no increase in parasite abundance nor changes in fish abundance in the absence of cleaner fish.

In a detailed quantitative removal study, Gorlick *et al.* (1987) found that the number of parasites was not affected but that parasites were larger in the absence of cleaner fish. This study was made at Enewetak Atoll and involved the

cleaner fish *Labroides dimidiatus* and one host fish species (*Pomacentrus vaiuli*) which only had one parasite species. This was the first quantitative demonstration of an effect of cleaner fish on an ectoparasite. Whether hosts benefited as a result of the decrease in parasite size could not be determined (Gorlick *et al.* 1987).

Examination of the diet of cleaner fish provides information on the potential effect its feeding habits may have on hosts. The number of parasites in the diet of *Labroides dimidiatus* at Lizard Island are much higher than in Japan (Chikasue 1990), Enewetak Atoll (Losey pers. comm.), and at Heron Island (Chapter IV). The diet of cleaner fish at Heron Island contains more mucus and non parasitic copepods than at Lizard Island. These suggests that *L. dimidiatus* may be targeting parasites more at Lizard Island. *Labroides dimidiatus* at Lizard Island are also selective feeders (Chapter IV) and therefore may influence the species composition of parasites. The only *L. dimidiatus* removal experiment to date (Gorlick *et al.* 1987) was made in an area where cleaner fish have few parasites in their diet (Losey pers. comm.) and which at times eat zooplankton (Losey 1979). It also involved a fish species which only had one parasite species. A study in a an area where cleaner fish eat more parasites and fish have a range of parasites provides a more appropriate ecological setting to examine the effect of cleaner fish on hosts.

The aim of this study was to evaluate experimentally the influence of *Labroides dimidiatus* on the parasites and abundance of the fish *Pomacentrus moluccensis* (Pomacentridae). This host species was selected to test this hypothesis because of its sedentary habits and because it is abundant and ubiquitous on isolated reefs at Lizard Island. Although individual *P. moluccensis* are cleaned relatively infrequently by *Labroides dimidiatus* (0.89 ± SE 0.25 times / 30 min or 1.5 ± SE 0.27 sec / 30 min) (Chapter V), the cumulative amount of cleaning over a period of several months is significant. *Pomacentrus moluccensis* also has several species of parasites. This range of parasites is ideal for testing whether *L. dimidiatus* has an effect on the species composition of parasites. This study is the first quantitative removal experiment in an area

which has high cleaner fish parasite feeding rates (Chapter VI).

7.3 METHODS AND MATERIALS

The appropriate reefs and fish species for the removal experiment were selected in a pilot study (January 1993). A survey of reefs at Lizard Island was made to select reefs which had *Labroides dimidiatus* yet were sufficiently isolated from other reefs so that movement of *L. dimidiatus* among reefs was unlikely. To select a fish species that was relatively abundant and present on all selected reefs, the abundance of all fish species was quantified. *Pomacentrus moluccensis* was selected as an abundant and ubiquitous species on which a variety of parasites were found.

Recruitment of *Labroides dimidiatus* juveniles onto reefs at Lizard Island is seasonal and occurs largely during the austral summer with little recruitment during April to October (A. Green pers. comm.). Similar patterns of recruitment also occur in *Pomacentrus moluccensis* (B. Kerrigan pers. comm.). The study was therefore conducted during April-October to reduce the likelihood of *L. dimidiatus* recruiting onto experimental reefs and to reduce variation due to recruitment of juvenile *P. moluccensis*. All *L. dimidiatus* were removed from reefs in April 1993. Reefs were surveyed for *L. dimidiatus* in May, July, and October 1993 and any new *L. dimidiatus* on treatment reefs removed. The abundance of *P. moluccensis* was estimated in April, prior to *L. dimidiatus* removals, and repeated in July and at the end of the experiment in October. *Pomacentrus moluccensis* were collected for parasite analysis in October after their abundance was estimated.

7.3.1 DESCRIPTION OF SITES

The experiment was conducted at Lizard Island in lagoonal habitats with sandy bottoms which reduced the likelihood of movement of fish between reefs. All *Labroides dimidiatus* were removed from 8 reefs while a further 8 were used as undisturbed control reefs. Ten of the reefs (5 treatment, 5 control) were

situated at one site (Lagoon) and six (3 treatment, 3 control) were situated at another site (Casuarina Beach) (Fig. 7.1). The reefs at the Lagoon site were at a depth of 2.0-7.3 m, ranged 31-245 m^2, and were 8.0-26.5 m from the nearest reef (except for two control reefs which were 5.8 m apart). Reefs at the Casuarina Beach site were in 5.2-7.3 m, ranged 91-356 m^2, and were 9.2-15.6 m from the nearest reef.

7.3.2 REMOVAL OF *LABROIDES DIMIDIATUS*

All *Labroides dimidiatus* were removed during 24-29 April 1993. Subsequent checks, made at intervals of several days for a period of 2 weeks, revealed no additional *L. dimidiatus*. The number of *L. dimidiatus* removed ranged from 2-8 per reef and included individuals 27-67 mm in standard length (SL). Control reefs had between 2-6 *L. dimidiatus* per reef. During a survey on 23/5/93, one *L. dimidiatus* juvenile was found (about 23 mm in SL) and removed; on 20-21/7/93, five *L. dimidiatus* juveniles (14-42 mm in SL) were found and removed (1-2 per reef). On the final survey (21-25/10/93), 6 additional *L. dimidiatus* (14-48 mm in SL) were found and removed (1-2 per reef).

7.3.3 COLLECTION OF PARASITES

Pomacentrus moluccensis (n=10-13 per reef) were collected on 19-30 October 1993 and their parasites quantified as described in Chapter V. Parasites were measured using an eyepiece micrometer at 35X and identified to lowest taxonomic grouping (usually family).

7.3.4 FISH ABUNDANCE ESTIMATES

The total number of *Pomacentrus moluccensis* per reef was estimated by slowly swimming around each reef and counting each individual. Two replicate counts were made, one after the other, and the mean used in the analyses. Counts were made on 12 reefs (6 treatment, 6 control). Fish on the remaining 4 reefs were not counted as the large size of these reefs made counting *P.*

Table 7.1 The ectoparasites of *Pomacentrus moluccensis* and their size ranges. Bold headings are broad descriptions of parasites.

Parasite	size (μm)	Parasite	size (μm)
Copepoda		**Digenea**	
Hatschekia crenulatus	890-940	Transversotrematidae	1000-1170
Sp. a larvae (caligiform)	260-1370	Other Digenea	860-1000
Sp. b larvae (Pennellidae?)	370-540	**Platyhelminthes**	260-460
Sp. c larvae (Hatschekidae?)	370-830	**Gill cysts**	260-310
Turbellaria	90-710	**Skin cysts**	1140-1660
Other	170-1000		

moluccensis too time-consuming. Thus these reefs were not used in the analyses of the abundance of *P. moluccensis*. Counts were made at intervals of about 3 months (13-23/4/93, 18-23/7/93, and 13-17/10/93).

7.3.5 STATISTICAL ANALYSES

Differences among treatments and reefs in the total number of parasites were tested using a nested analysis of variance (ANOVA). The number of parasites in each category was tested for differences among treatments using a multivariate analysis of variance (MANOVA). Data were ln (x+1) transformed to satisfy the assumptions of the ANOVAs and the MANOVA.

The size frequency distribution of caligiform copepods sp. a was tested for differences among treatments using chi-square analysis of homogeneity. To test for differences in the abundance of *P. moluccensis* over time and among treatments, a multivariate repeated-measures analysis was used with treatment as a between-subjects factor and time as a within-subjects factor.

7.4 RESULTS

The parasites of *Pomacentrus moluccensis* were from a wide taxonomic range and were all relatively small (Table 7.1). The gill copepod, *Hatschekia crenulatus*, consisted of females (some with eggs) and was the only adult copepod found. All other copepods were larval stages and could therefore not be identified: caligiform Copepoda sp. a (1st copepodids to 1st-3rd chalimus stages), Copepoda sp. b (late chalimus stage of Pennellidae?), and Copepoda sp. c (1st copepodid of Hatschekidae?)(G. Boxshall pers. comm.). The remaining parasites consisted of gill and skin cysts, turbellaria, transversotrematid Digenea, other Digenea, platyhelminths, and other unidentified parasites (Table 7.1).

The total number of parasites on *Pomacentrus moluccensis* did not differ among treatments ($F<0.01$, $df=1,14$, $p=0.952$) and was relatively low, usually 0-3 parasites per fish (Fig. 7.2). However, there was a significant difference in the total number of parasites per fish among reefs ($F=2.22$, $df=14,148$,

$p<0.001$). Although there were insufficient degrees of freedom to test for an effect of site (Lagoon and Casuarina Beach), examination of Figure 7.2 suggests there are no differences among sites. The species composition of parasites did not differ among treatments (Pillai's Trace 0.073, $F=1.088$, $df=11, 152$, $p=0.374$)(Fig. 7.3). Although, copepod sp. b and other digenea were only found on reefs without *Labroides dimidiatus* their abundance was extremely low and probably reflects sampling effort. The size frequency distribution of the most abundant copepod, caligiform Copepoda sp. a (Fig. 7.3), also did not differ among groups ($X^2=2.35$, $df=4$, $p=0.672$)(Fig. 7.4).

There was a significant effect of time on the abundance of *Pomacentrus moluccensis* per reef (Pillai's Trace 0.768, $F=14.919$, $df=2, 9$, $p=0.001$)(Fig. 7.5), however, the interaction term (time x treatment) was not significant (Pillai's Trace 0.065, $F=0.314$, $df=2, 9$, $p<0.738$) which indicates the decline did not differ among treatments. Although the number of *P. moluccensis* increased on some reefs between April and July the majority of the reefs showed a decline from April to October (Fig. 7.5).

7.5 DISCUSSION

Despite the large proportion of parasites in the diet (Chapter IV) and the high removal rates by *L. dimidiatus* (Chapter VI), the experiment did not provide any conclusive evidence of a *L. dimidiatus* cleaning effect on *Pomacentrus moluccensis*. There were no differences in the total number and species composition of parasites nor in the abundance of *P. moluccensis* among treatments. These results are similar to all other quantitative removal experiments (Youngbluth 1968, Losey 1972, Gorlick *et al.* 1987). There was also no effect on the size frequency distribution of the most abundant copepod species. This is in contrast to Gorlick *et al.*'s (1987) study which found larger parasites in the absence of *L. dimidiatus*.

Labroides dimidiatus also had no effect on the abundance of *Pomacentrus moluccensis* which showed an overall decline between April and

October. The changes in abundance of *P. moluccensis* were the same on the control and removal reefs which indicates that *P. moluccensis* did not leave reefs to seek cleaning elsewhere. The role of cleaning in promoting the healing of injured fish has been suggested (Foster 1985), however there was no evidence of increased mortality in the absence of *L. dimidiatus*. Youngbluth (1968), Losey (1972), and Gorlick *et al.* (1987) also found no changes in host fish density after the removal of cleaner fish from reefs. This is in contrast to Limbaugh's (1961) qualitative study which suggested that host fish emigrated from reefs after the removal of cleaners.

There was no evidence that other cleaners replaced the role of *Labroides dimidiatus*. Although a single juvenile *Thalassoma lunare* and a juvenile *Bodianus axillaris* were observed picking at fish on reefs without cleaner fish, the inspection was brief and was followed by benthic feeding behaviour. No other cleaning events by fish were observed during the many hours spent on all reefs counting fish. Cleaner shrimp were found on half of the control and removal reefs but there was no evidence that their cleaning rates increased in the absence of *L. dimidiatus*. They also spend less time inspecting fish than cleaner fish (pers. obs.). Furthermore, the only fish species observed being cleaned by cleaner shrimp were large mobile species (e.g. *Pomacanthus sexstriatus, Diagramma pictum, Plectorhinchus celebicus, Plectropomus leopardus*). *Pomacentrus moluccensis* was never observed being cleaned by cleaner shrimp. It is unlikely that other cleaner species influenced the parasites or abundance of *P. moluccensis* on reefs without cleaner fish.

Other studies have excluded all cleaner fish for 2 weeks (Limbaugh 1961), 1 month (Youngbluth 1968), 7 months (Losey 1972), and 2 years (Gorlick *et al.* 1987). It is interesting to note that, of these, the longest removal study had a significant effect of cleaning on parasites (Gorlick *et al.* 1987). Thus it may be argued that a longer removal experiment may have produced an effect in this study. However, only 6 months was feasible in this study because of fish recruitment patterns.

This raises the question of why hosts retain the motivation to seek

cleaning if *Labroides dimidiatus* has no effect on their parasites. There is strong evidence that the proximate cause of host cleaning behaviour is the tactile stimuli that hosts receive from cleaner fish during cleaning interactions (Losey 1971, 1977, Losey and Margules 1974). There are two possible ways that such a mechanism for cooperation could have developed (Losey 1987). The first is as a result of a positive survival value of cleaning and the second is that cleaner fish may have taken advantage of an existing tactile reward system in hosts (Losey 1987).

The lack of an effect of *Labroides dimidiatus* on the parasites of *P. moluccensis* may be influenced by the diet selectivity of *L. dimidiatus* (Chapter IV). *Labroides dimidiatus* selectively feeds on large gnathiids while *P. moluccensis* has relatively small parasites and no gnathiids. Thus *P. moluccensis* probably does not represent an attractive food source for *L. dimidiatus*. This implies that species which have more attractive parasites may be more affected by cleaner fish.

Many species are cleaned more frequently than *Pomacentrus moluccensis* (Chapter V) and also have many gnathiid isopods (Chapter III) which are removed in large quantities from some species and whose abundance may be suppressed by *Labroides dimidiatus* (Chapter VI). However these fish species are generally mobile species and therefore not amenable to traditional removal experiments which require the containment of fish to particular reefs. New approaches are needed to test the effect of *L. dimidiatus* on these mobile hosts. This study, and all other quantitative experimental evidence to date (Youngbluth 1968, Losey 1972, Gorlick *et al.* 1987), suggests that hosts do not benefit from cleaning. It is therefore very likely that other factors, such as hosts' responses towards tactile stimuli (Losey 1971, 1977, Losey and Margules 1974), may be the cause of cleaning responses in fish.

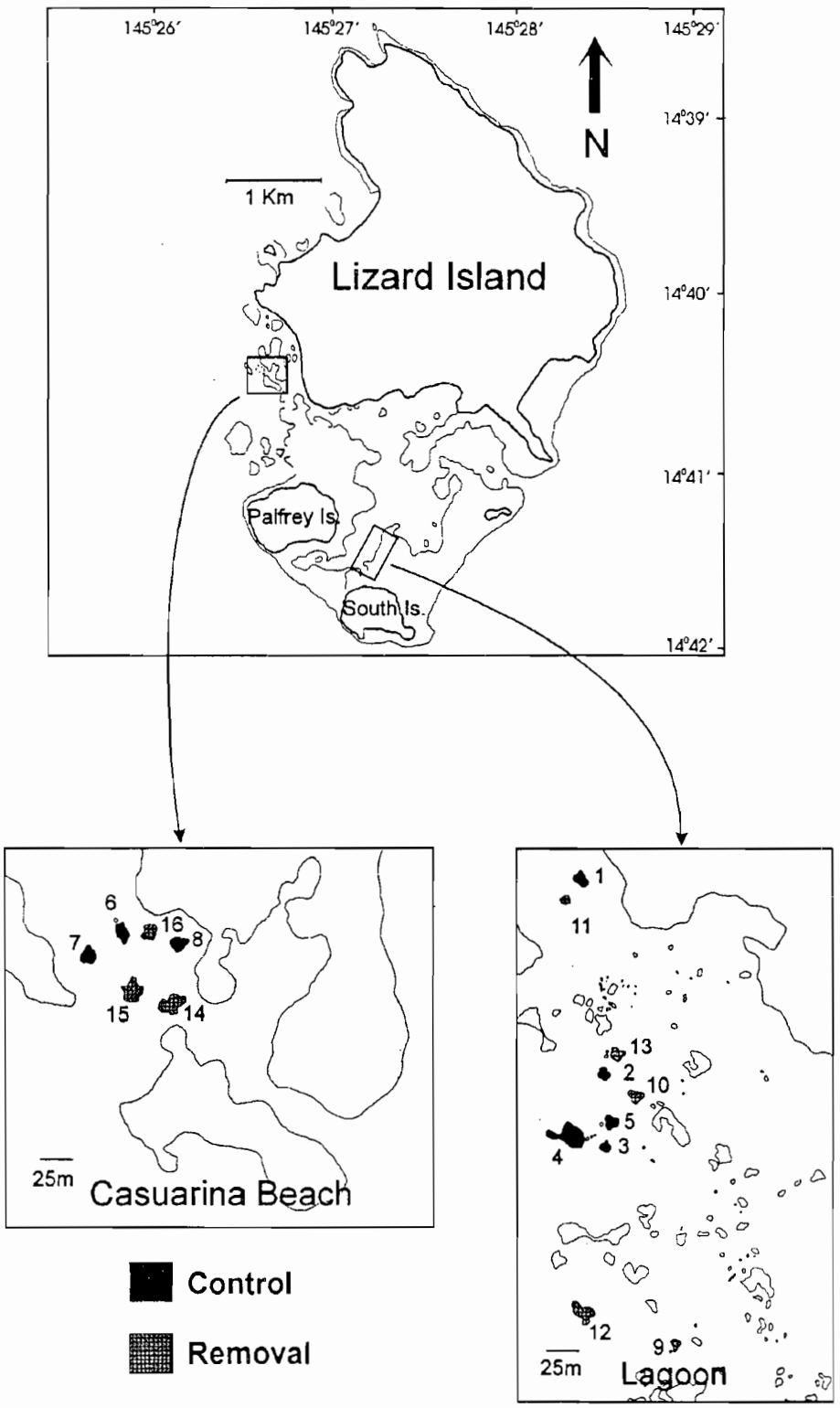

Figure 7.1. Map of Lizard Island showing the locations of control and treatment reefs used in an experimental removal of *Labroides dimidiatus*.

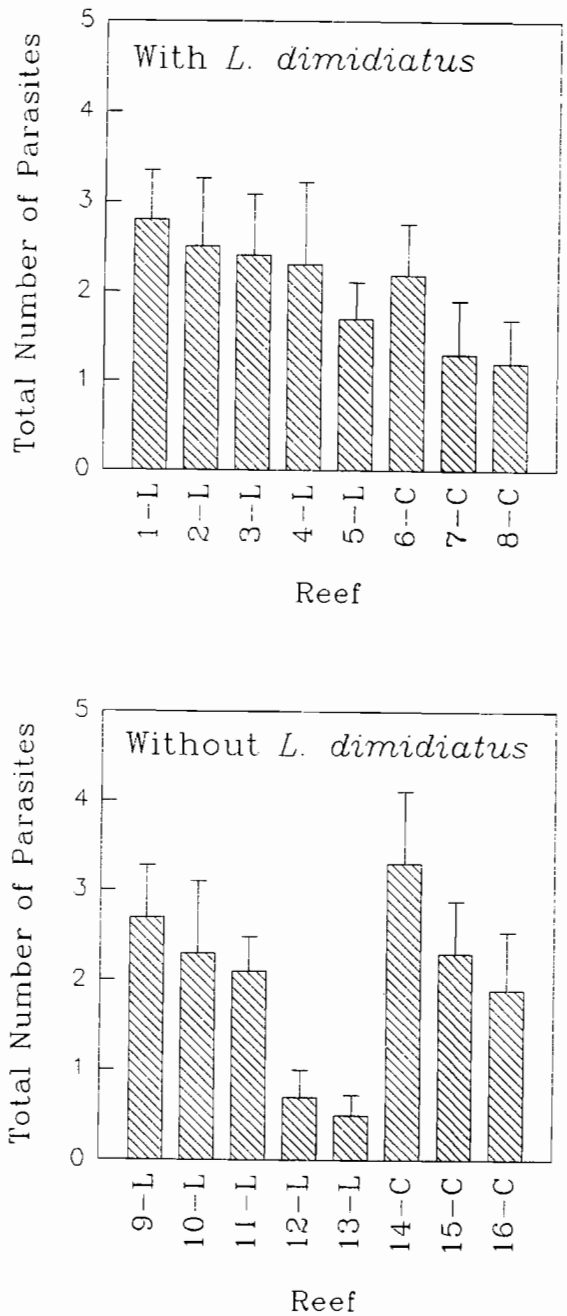

Figure 7.2. The mean total number of ectoparasites per fish (± SE) on *Pomacentrus moluccensis* from reefs with and without *Labroides dimidiatus*. L=Reefs located in the Lagoon. C=Reefs located at Casuarina Beach.

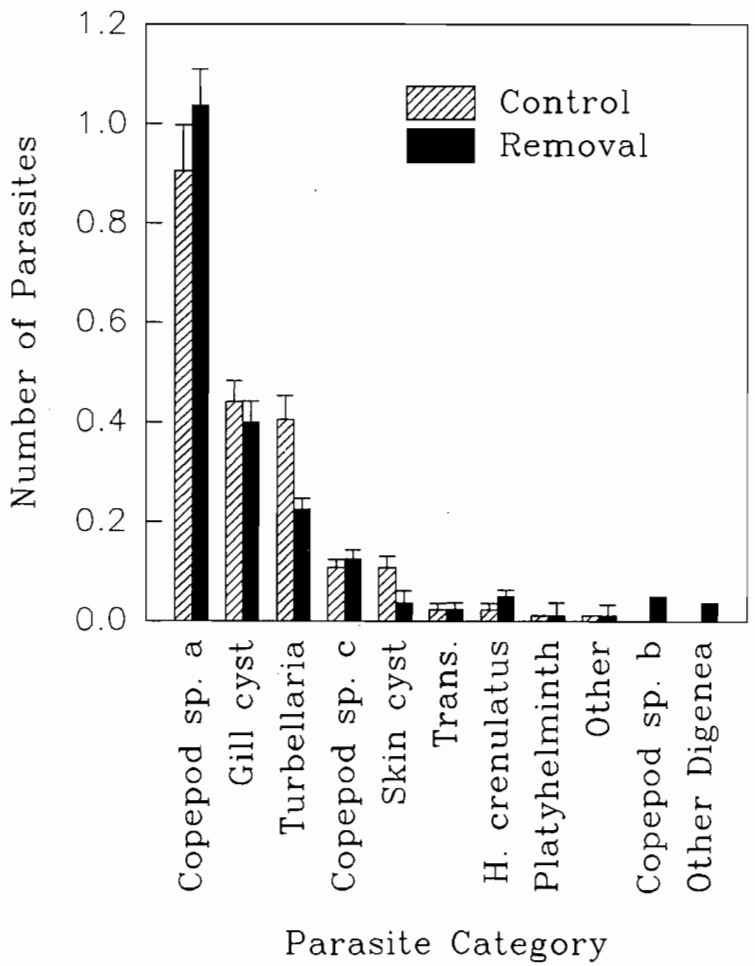

Figure 7.3. The average number of parasites (± SE) per category of parasite on *Pomacentrus moluccensis* from reefs with (control) and without (removal) *Labroides dimidiatus*. Trans=Transversotrematidae.

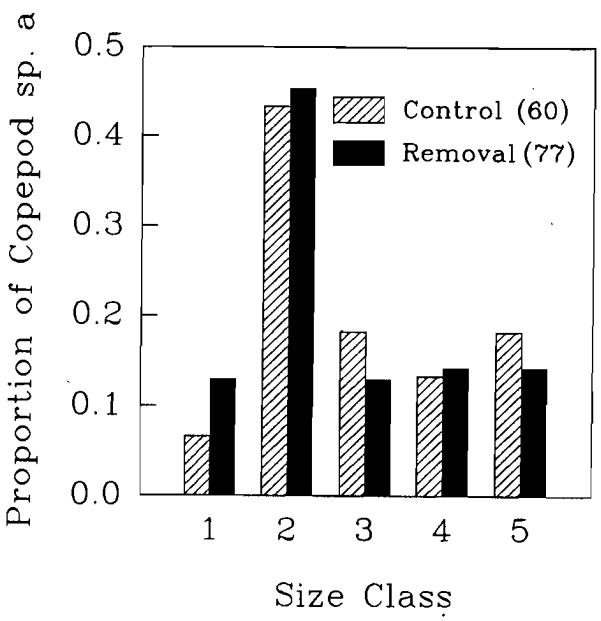

Figure 7.4. The proportion of Copepoda sp. a per size class on *Pomacentrus moluccensis* from reefs with (control) and without (removal) *Labroides dimidiatus*. 1=<0.30 mm, 2=0.30-0.49 mm, 3=0.50-0.69 mm, 4=0.70-0.89 mm, 5=>0.90 mm. Sample size is in brackets.

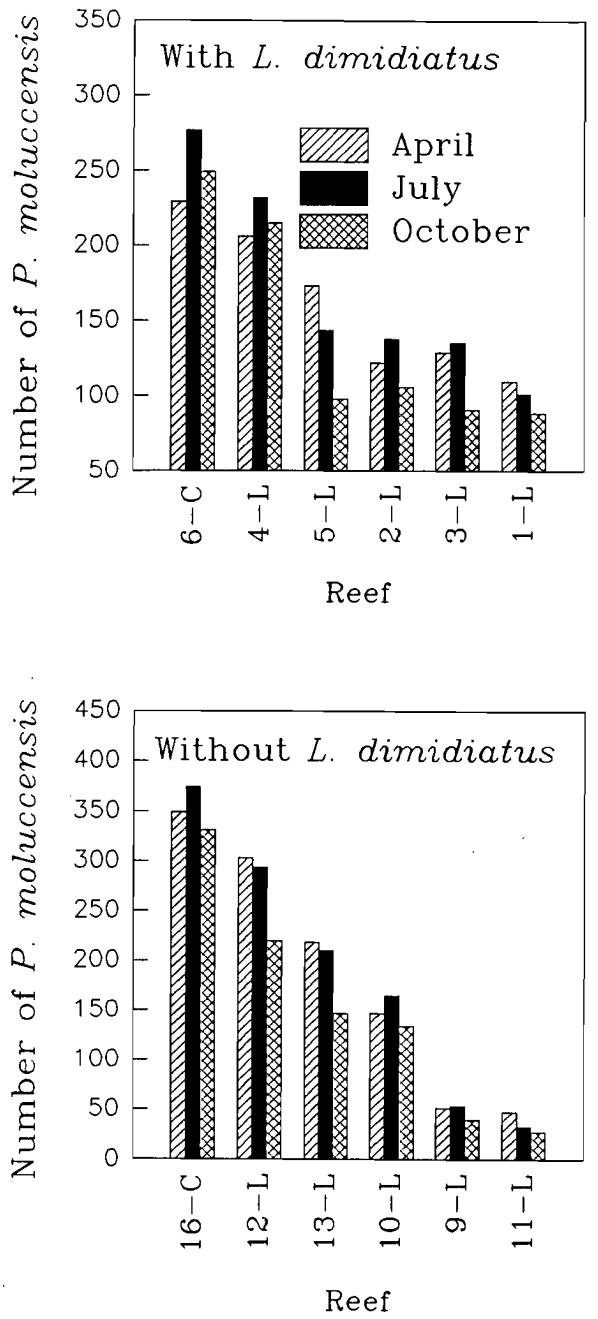

Figure 7.5. The abundance of *Pomacentrus moluccensis* on reefs with and without *Labroides dimidiatus*. Note: *L. dimidiatus* were removed from reefs after fish counts in April. L=Reefs located in the Lagoon. C=Reefs located at Casuarina Beach.

CHAPTER VIII

GENERAL DISCUSSION

8.1 THE ECTOPARASITES

The parasites of the fish cleaned by *Labroides dimidiatus* were taxonomically diverse and included copepods, isopods, monogeneans, digeneans, and turbellarians. Total abundance of parasites per fish was a function of species identity and size of fish. Parasite loads and assemblages were consistent at different localities and times. This information on the parasites of host fish was essential to several conclusions about cleaning interactions.

Parasitic gnathiid isopod larvae, which played a significant part in cleaning interactions, are unusual parasites. Their feeding mode, which involves short feeding bouts on hosts (Stoll 1962, Paperna and Por 1977, Davies 1981), and their life history, which involves several moulting stages in the benthos (Wägele 1988), make them a highly mobile temporary parasite. Gnathiids, in contrast to most parasites which use hosts as habitats and as sources of food, only use the host as a source of food and return to the benthos after feeding to digest and moult. Thus the relationship between gnathiids and host fish differs from that of most parasites-host associations.

The importance of appropriate methods for sampling parasites was highlighted by the fact that gnathiid isopods, in particular, were influenced by sampling methods. Gnathiids readily abandoned handled hosts. Without appropriate precautions to avoid this sampling bias, the abundance of gnathiids on fish would have been underestimated.

8.3 THE DIET OF *LABROIDES DIMIDIATUS*

This study showed that the diet of *Labroides dimidiatus* is consistent with that of other tropical labrids which have diets consisting mainly of crustaceans (Hiatt and Strasburg 1960, Hobson 1974). The main difference is

that *L. dimidiatus* target the external surfaces of fish, rather than the benthos, to obtain their crustacean diet. This agrees with the suggestion that one of the paths that led to the evolution of cleaners involved substrate picking species (Losey 1987).

Labroides dimidiatus has a very selective diet. Despite the wide variety of parasites on host fish, the diet of *L. dimidiatus* consisted mainly of parasitic gnathiid isopod larvae and an occasional copepod. When possible, cleaners also selectively fed on larger gnathiids. This diet selectivity may have important ecological consequences as host fish have many other parasites, such as monogeneans which can reach high numbers (Chapter III) and can have deleterious effects on host fish (Petrushevsky and Shulman 1961, Oliver 1977). Feeding selectivity by *L. dimidiatus* and the variation in the species composition and size of parasites on hosts means that some hosts may not have parasites removed. This implies that, if there is an effect of *L. dimidiatus* on fish, the effect may vary as a function of the identity and size of parasites.

The composition of the diet of *Labroides dimidiatus* suggests they are foraging so as to maximise their food intake per unit effort. The food value of gnathiids is likely very high as they have large alimentary tracts filled with the blood and lymphatic fluids of fish (Wägele 1988). At Lizard Island, where gnathiids are abundant, *L. dimidiatus* selectively feeds on larger gnathiids (Chapter V). In contrast, it does not selectively feed on large gnathiids at Heron Island where gnathiids appear to be less abundant (Chapter III). This may reflect the higher search costs associated with finding larger gnathiids at Heron Island. The search and handling time of gnathiids is probably relatively low, compared to other more cryptic permanent parasites, as gnathiids are common among fish species (Chapter III) and not firmly attached to fish.

Differences in the diet of fish over space and time may be related to food availability (Stoner 1979, Cowen 1986). This may explain spatial and temporal differences in the diet of *Labroides dimidiatus*. At Lizard Island, where there is a trend for more gnathiids on host fish than Heron Island, the diet of *L. dimidiatus* consisted mainly of gnathiids with very few other items in the diet.

In contrast, the diet at Heron Island contained fewer gnathiids (one fourth the biomass) and more mucus and non-parasitic copepods. That gnathiids in the diet at Heron Island appeared to be replaced by mucus and non-parasitic copepods (mainly harpactacoids) rather than other parasitic crustaceans is surprising, as parasitic crustaceans on hosts at Heron Island are abundant and in some cases even higher than at Lizard Island (Chapter III). It is highly likely that mucus and harpactacoids have a lower food value than gnathiids as fish mucus contains a large proportion of water (Gorlick 1980) and harpactacoids are generally smaller than gnathiids (Chapter III, V). However, mucus and harpactacoids may be more readily available compared to parasitic crustaceans which are often cryptic (Chapter III).

Spatial and temporal variation in the diet of *Labroides dimidiatus* may have significant consequences. It has been suggested that there may be a gradient in host-cleaner fish interactions ranging from mutualistic to commensal to parasitic according to parasite infection levels (Losey 1972, 1974). Mucus feeding by cleaner fish has also been suggested as a form of parasitism or commensalism (Gorlick 1980). Variation in the interactions of species is common and can occur along environmental gradients (Thompson 1988). If the diet is related to host parasite levels and if ectoparasite removal is important to cleaners then the above differences in the diet suggest that interactions between *Labroides dimidiatus* may vary among locations with host fish at Heron Island possibly benefiting less from ectoparasite removal than those at Lizard Island.

8.4 PARASITES IN INTERACTIONS BETWEEN CLEANERS AND HOSTS

This was the first study to examine how often individual fish are cleaned. Estimates of daily host cleaning rates ranged from less than one minute per day (*Neopomacentrus cyanomos*) to 32 minutes per day (*Siganus doliatus*). Thus some species spend a significant proportion of their time being cleaned. Host cleaning rates were positively related to host surface area and parasite load which shows that cleaning for the host is not a random event. Since surface

area, rather than parasite load, explained more of the variation in host cleaning rates it is likely that host cleaning rates were at least partly influenced by cleaner fish foraging behaviour. Because parasite loads are often correlated with the size of fish (Cressey and Collete 1971, Buchmann 1989, Chapter III) cleaners may use size of fish as an indicator of food availability and thus influence the cleaning rates of host fish. This occurs in oxpeckers (birds) which remove ticks from ungulates, with oxpeckers preferring larger host species with higher densities of ticks (Hart *et al.* 1990).

The movement of gnathiids onto fish (infection) and off fish (mortality or emigration) was highly dynamic. Cleaner fish inspected many fish and in the process removed many parasites (mainly gnathiids). Individuals of some species of host fish spend a long time being cleaned. With such high parasite removal rates by cleaner fish it is likely that these fish species have many gnathiids removed by cleaner fish.

The infection rate of gnathiids onto host fish was very high. Gnathiid loads reduced by 50% returned to normal within 1-6 days, possibly even sooner. Thus a significant proportion of gnathiids removed by cleaner fish are likely to be quickly replaced through colonisation. There is evidence that gnathiids only infect fish overnight (Potts 1973b, Paperna and Por 1977) thus it is possible that gnathiids removed by cleaner fish during the day are replaced by other gnathiids at night.

The estimated number of gnathiids removed per host fish *Hemigymnus melapterus* was 5 times higher than its standing crop of gnathiids. Such a high predation rate compared to the number of gnathiids on fish and their infection rate shows that cleaner fish have some effect on the abundance of gnathiids on fish.

How much of an effect cleaner fish have on the abundance of gnathiids depends on whether the standing crop of gnathiids found on cleaned fish is lower than their maximum carrying capacity of gnathiids. Although much greater gnathiid densities have been reported than those found at Lizard Island, they have been on captive fish (Paperna and Por 1977, Mugridge and Stallybrass

1983). Whether such great densities occur in the wild is unknown. Little is known about what influences the abundance of gnathiids on fish (other than predation). The number of gnathiids per fish increases with size within and among fish species (Chapter III). Very small fish species have no gnathiids (Chapter V) which suggests a minimal host size threshold for gnathiids. These imply an effect of space limitation on the abundance of gnathiids on fish. This may be related to the number of shelters from predation by cleaner fish as gnathiids attached to cryptic sites remain on fish for longer periods (Paperna and Por 1977). More information is needed on the carrying capacity of gnathiids on hosts and the processes involved in the infection of gnathiids onto fish to determine to what extent and for how long gnathiid abundance is suppressed by *Labroides dimidiatus*.

The absence of *Labroides dimidiatus* had no effect on the parasites of the fish *Pomacentrus moluccensis*. This is likely a result of the diet selectivity of *L. dimidiatus* as *P. moluccensis* has relatively small parasitic copepods and no gnathiids. *Labroides dimidiatus* may therefore not have had an effect on *P. moluccensis* because this fish does not represent an attractive food source.

Many studies have assumed or suggested that cleaning interactions are mutualistic (e. g. Randall 1958, Limbaugh 1961, Abel 1971, Losey 1974 but see critical reviews by Hobson 1969, Losey 1978, Gorlick *et al.* 1978, Losey 1987). The definition of mutualism states that both participants benefit from the association (Boucher 1982). In most studies of mutualism, however, interactions have failed to meet this condition (see Cushman and Beattie 1991 for review). Participants in mutualistic interactions have been defined as hosts that provide food or a domicile and visitors which provide beneficial services (Thompson 1982). Associations are usually viewed as mutualistic when just the hosts are shown to benefit from the services of the visitor. The results of this study indicate that the visitor (cleaner fish) benefits from the interactions but whether the host benefits is unclear. In the cleaning interactions investigated, cleaner fish clearly benefit from cleaning host fish as hosts provide cleaners with a reliable food source in the form of gnathiid isopod larvae. However, due to the

selectivity of the diet of *Labroides dimidiatus* on parasitic gnathiid isopods and its high rate of predation on gnathiids the effect of *L. dimidiatus* at Lizard Island on parasites is likely to be greatest on gnathiids. Deleterious effects of gnathiids vary with the most damage caused by large species (Paperna and Por 1977, Honma *et al.* 1991) or by gnathiids on captive fish (Paperna and Por 1977, Mugridge and Stallybrass 1983). Gnathiids at Lizard Island are relatively small (Chapter III) and therefore may not be as damaging to the host. Thus whether hosts benefit from their removal is unclear.

This study shows that the ecological importance of the interaction between cleaner fish and hosts may differ for the two participants, and that the impact on parasite loads may vary among hosts. Although hosts provide a meal for *Labroides dimidiatus*, cleaner fish in turn do not remove all potentially deleterious parasites to maintain the health of its hosts. Why hosts such as *P. moluccensis*, seek cleaning if *L. dimidiatus* has no effect on their parasites is not consistent with the notion that ectoparasite removal is the ultimate cause of cleaning. This suggests that factors other than parasite removal influence host cleaning behaviour.

An alternative cause for host cleaning behaviour has been suggested. This is that the tactile stimuli provided by cleaner fish may be the cause of cleaning in host fish (Losey and Margules 1974, Losey 1977, 1979). The responses to tactile stimuli do not seem to be adapted to cleaning as hosts pose for tactile stimuli much longer than they normally would for cleaning (Losey 1979). Cleaners may therefore exploit hosts' responses towards tactile stimuli in order to obtain a meal (Losey 1979). This study suggests that cleaner fish foraging patterns determine their subsequent effect on parasites. Thus, although host cleaning behaviour may be driven by tactile stimuli, the effect of cleaning on hosts may vary according to the diet of cleaner fish and the parasite load of hosts. Cleaning may therefore exists as a parasitic, commensal, or mutualistic association depending on the parasite assemblage of the host and the foraging behaviour of the cleaner.

8.5 FUTURE DIRECTIONS

One of the problems in determining the ecological significance of cleaning is that little is known about the effects of parasites on fish. Studies on the effects of parasites on the survival and or reproduction of fish are needed to determine whether fish suffer decreased fitness from infection. Although gnathiids can cause mortality (Paperna and Por 1977, Mugridge and Stallybrass 1983) the cause of mortality is unknown. Their effect on wild fish populations is also not known. More research on the effects of gnathiids on hosts in natural conditions is needed to determine whether hosts actually benefit from their removal.

Although this study showed that cleaner fish have some effect on the abundance of gnathiids the extent of this effect was unclear. Whether the standing crop of gnathiids on cleaned fish is lower than the maximum gnathiid carrying capacity of fish is essential to understanding to what extent cleaner fish effect their abundance. A study investigating the effect of the absence of *Labroides dimidiatus* on gnathiid abundance is needed to provide insight on the carrying capacity of gnathiids on fish. Information on what factors (other than cleaner fish) influence the abundance of gnathiids on hosts may also be useful for interpreting the dynamics of gnathiid abundance on fish.

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APPENDIX I

Publications resulting from this thesis

This is a list of the publications that have, to date, resulted from this study. Copies of papers in press are included in this section.

Published or in Press:

Grutter, A. S. (1994). Spatial and temporal variations of the ectoparasites of seven reef fish species from Lizard Island and Heron Island, Australia. Mar. Ecol. Prog. Ser. 115: 21-30

Grutter, A. S. (1995). Relationship between cleaning rates and ectoparasite loads in coral reef fishes. Mar. Ecol. Prog. Ser. 118: 51-58

Grutter, A. S. (1995). A comparison of methods for sampling ectoparasite from coral reef fishes. Mar. Freshwater Res. vol. 46 (in press)

Grutter, A. S. (1995) Parasite removal rates by the cleaner wrasse *Labroides dimidiatus*. Mar. Ecol. Prog. Ser. (in press)

Submitted:

Grutter, A. S. Feeding selectivity and spatio-temporal variation in the diet of the cleaner wrasse *Labroides dimidiatus*. J. Fish Biol.

Grutter, A. S. The effect of the cleaner wrasse *Labroides dimidiatus* on the fish *Pomacentrus moluccensis*. J. Exp. Mar. Biol. Ecol.

Grutter, A. S. (1994). Spatial and temporal variations of the ectoparasites of seven reef fish species from Lizard Island and Heron Island, Australia. Mar. Ecol. Prog. Ser. 115: 21-30.

Grutter, A. S. (1996) Parasite removal rates by the cleaner wrasse *Labroides dimidiatus*. Mar. Ecol. Prog. Ser. 130: 61-70.