Age specific patterns of growth and reproduction in tropical herbivorous fishes

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ABSTRACT

Research of growth and reproduction was undertaken for scarids and acanthurids in coral reefs around Lizard Island, the Northern Great Barrier Reef, Australia. The study species were mainly the scarids *Scarus rivulatus* and *Scarus schlegeli*, and the acanthurids *Ctenochaetus binotatus* and *Ctenochaetus striatus*. The study focussed on the establishment of validated aging information for both scarids and acanthurids, and the age-specific patterns of reproduction of scarids.

Age and growth parameters were determined by enumerating growth increments within otolith microstructure for each species. Various mounting and grinding/polishing techniques were employed to reveal both fine lapillus growth rings in juveniles and sagitta growth bands in adults. Daily periodicity in otolith increments was demonstrated in 55 juvenile individuals in four of the main study species: *S.rivulatus* (20), *S.schlegeli* (21), *C.binotatus* (12) and *C.striatus* (2), and 28 individuals of other species within the two families. Ring periodicity was determined by staining the otoliths *in situ* with tetracycline, and maintaining the individuals in captivity to compare the rings laid down with the number of elapsed days. Double staining techniques were also employed to determine the rings laid down between stainings.

Annual periodicity in otolith bands was demonstrated by tag-recapture experiments in the both the aquarium and the field, and by otolith marginal increment analyses for the four study species. All recaptured specimens, including four *S.schlegeli* and four *C.striatus*, showed annual otolith bands. The otolith marginal development on regular samples over the year for *S.rivulatus* and *S.schlegeli* also

indicated that a single otolith band was formed during December to May.

By enumerating otolith increments and bands, age of the field captured individuals of each study species was estimated. The age of scarids ranged up to 8 years with the majority being younger than 5 years. The growth rate was increasing with age in days during the juvenile phase, and gradually decreased after that. The acanthurids lived for relatively long period in excess of 16 years, and the growth rate decreased with age after settlement. In addition, the age of settlement was estimated to be from 28 to 47 days for scarids and from 47 to 74 days for acanthurids.

Reproductive biology of scarids was studied by seasonal examination of gonads. The gonads were examined histologically to determine the sexual identity and maturity state of individuals. By using validated aging information, the dynamics of sexual transition was observed.

Mature gonads of the two species were found throughout the year. However, a pronounced spawning peak occurred between May and September in *S.schlegeli* while a relatively less pronounced spawning peak took place from September to January in *S.rivulatus*. These patterns were indicated by seasonal development of gonadosomatic index, seasonal distribution of mature gonads, oocyte length, and the proportion of mature stage oocytes within the gonads. The proportion of mature stage oocytes within mature ovaries of two species also suggested that these species were serial spawners. Enumerating mature oocytes within the subsamples of 20 individuals in each species showed positive relationships between fecundity and body length or age.

Both females and primary males of the two species reached sexual maturity at 2 years. Females started to change sex at 3 years, and the sexual transition of the

population lasted for approximately another 3 years. Similarly, the primary males started changing color phase at 3 years. Growth rates appeared to be different between the initial phase and the terminal phase individuals, and the terminal phase individuals had a higher growth rate than that of the initial phase individuals of the same age.

The proportional liver weight in *S.rivulatus* and *S.schlegeli* changed over time, and this reflected the compositional states. Larger livers had high levels of lipids, which fact was indicated by the colour and lipid droplets. High proportional liver weight occurred immediately before spawning for both species, suggesting that the liver is an important energy storage organ providing lipids for the gonadal development. For the two species of scarids studied in similar microhabitats and similar physical environments both showed seasonal patterns of liver weight and gonadal development, but it varied in timing and magnitude.

This study suggests that scarids, which have relatively fast growth rates and short lifespans, are more suitable candidates for intensive fisheries than the low-growth and long-lifespan acanthurids. However, as the population dynamics of scarids is complicated by the protogynous hermaphroditism, comprehensive management is required in scarid fisheries.

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DECLARATION

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

D.C. Lou
30 June 1992

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CHAPTER 1. GENERAL INTRODUCTION

This thesis focuses on growth and reproduction of an important group of herbivorous coral reef fish, the Family Scaridae. Two areas of research are critical to the understanding of the dynamics of coral reef fishes. Firstly, it is essential to establish growth rates and age structures by validated age information. Secondly, as the majority of coral reef fishes are protogynous hermaphrodites the patterns of different growth and mortality for each sexual group must be clarified. This thesis addresses these problem by undertaking a detailed analysis of two species of the Family Scaridae.

In addition this thesis also examines the age structure and growth rate of two species of the Family Acanthuridae, another important group of coral reef fishes. Unlike the majority of coral reef fishes, acanthurids are not protogynous hermaphrodites, and thus have a different life history pattern from scarids. By comparing growth between scarids and acanthurids, this study investigates variations on demography between protogynous hermaphroditic and non-protogynous hermaphroditic species of herbivorous reef fishes.

Scarids and acanthurids are two dominant components of the reef fish fauna on coral reefs, in terms of both biomass and species richness (Williams and Hatcher, 1983; Russ 1984a & b; Horn, 1989). Research into the age and demography of these herbivorous reef fish is because of the important role these fishes play in trophodynamics of coral reefs (Hatcher, 1981, 1983; Klumpp *et al.*, 1987; Klumpp and Polunin, 1989), bioerosion (Bardach, 1961; Gygi, 1969, 1975; Frydi and Stearn, 1978; Hutchings, 1986; Horn, 1989, Bellwood and Choat, 1990), and fisheries in the

Asia and Pacific region (Alcala and Luchavez, 1981; Johannes, 1981; Munro and Williams, 1985; Russ and St. John, 1988; Russ, 1991).

There is a strong need for the study of age determination in coral reef fishes, especially for adult individuals based on seasonal and annual banding patterns (Russ, 1991). Research into the dynamics of tropical fish populations has been hampered by the absence of long term age composition data (Longhurst and Pauly, 1987). Previous publications have contained a considerable diversity of opinions with regard to the methodology for the direct aging of tropical fish from hard anatomical structures (Weatherley and Gill, 1987). Data presented in the literature has suggested conflicting views for both the existence of annuli (Fagade, 1974; Blake and Blake 1978, 1981; Fowler, 1990; Lou, 1992) and non-existence of annuli (Holden, 1955; Pannella, 1974) within the hard anatomical structures. Moreover, Weatherley and Gill (1987), Dee and Radtke (1989) and Radtke (1987) considered that annual age determination was too difficult to carry out on tropical fishes. One factor contributing to this problem has been the use of inappropriate methodologies to describe or interpret calcified structures. In many instances there has been a lack of appropriate validation for justifying the use of a method for annual age determination.

Validation of the daily and annual periodicity in otolith growth increments has been a major breakthrough in the study of growth dynamics in tropical herbivorous reef fishes. Without the ability to age individuals, it is difficult to obtain estimates of cohort age from population samples. Early attempts to age scarids and acanthurids have involved the enumeration of marks on the cleithra (Warner and Downs, 1977), checks on the scale (Papaconstantinou and Petrakis, 1986) and supposed daily otolith

increments (Itano, 1988). However, none of these studies reached satisfactory conclusions with regard to using these structures to determine individual age.

In tropical reef fishes, daily otolith growth increments were first documented by Ralston (1976), and the majority of research has been carried out on larvae and juveniles within the last decade (Victor, 1982, 1983,1986a&b,1987; Brothers *et al.*, 1983; Pitcher, 1988; Fowler, 1989; Lou and Moltschaniwskyj, 1992). Annual otolith bands were recently reported on the small, tropical coral reef fish, *Pomancentrus mollucensis*, by Fowler (1990).

This growing pool of evidence regarding aging in tropical reef fish suggests that accurate size-at-age information could be obtained for tropical species and used to construct age-specific growth and maturity schedules. The majority of tropical herbivorous fish ageing research has been preliminary in scope and focused on larvae and juveniles. There was therefore a need to undertake a more detailed demographic analysis on the application of otolith age data for scarids and acanthurids.

The dynamics of some reef fish populations is complicated by sequential hermaphroditism, which is a phenomenon characterised by an individual changing from one sex to another at some point in its life history, and widespread in teleost fishes (Reinboth 1970; Pollock, 1985; Sadovy and Shapiro, 1987; Ebisawa, 1990). There are two types of such phenomenon, one of which is protandrous in that animals change from male to female and the other is the reverse (protogyny). Previous studies on the population dynamics of sequential hermaphroditic reef teleosts show that demographic parameters generally vary in different sexual groups within the population (Warner, 1975; Ross, 1984; Cowen, 1990).

Members of the Scaridae are predominantly protogynous hermaphrodites

(Choat and Robertson, 1975; Robertson and Warner, 1978; Randall and Choat, 1980; Randall and Bruce, 1983; Choat and Randall, 1986). Adult scarids possess two main colour phases, a drab color phase and a gaudy color phase. Drab color phase individuals may be either male or female, whilst gaudy color phase individuals are invariable male. Some individuals are male throughout their life, i.e. during both drab and gaudy color phases. Most males, however, spend the drab color phase period as a female before developing testes and exhibiting gaudy phase colouration. This complex life history in scarids has drawn the attention of many scientists. Consequently, a number of studies have been carried out on their life histories in regard to sexual patterns (Choat, 1969; Choat and Robertson, 1975; Robertson and Warner, 1978; Yogo *et al.*, 1980). However, there has been no study which reliably describes the sexual pattern in age specific terms even though such a study is the basis for estimating the demographic parameters for different sexual phases.

By using validated aging and growth data and histological examination of the gonads, this study investigates the detailed life pattern of different sexual groups within the scarid population. Furthermore, as the pattern and magnitude of seasonal change in reproduction and condition in coral reef fishes is virtually unknown, this will also be examined by regularly seasonal sampling.

Fish condition generally reflects the storage of energy within the body. The liver is an important organ of energy storage in teleosts (Weatherley and Gill, 1987), and it plays a significant role in the gonadal development of some species (Crupkin *et al.*, 1988; Smith *et al.*, 1990). Previous studies indicate that scarids have large and oily livers (Al Hussaini, 1945, 1947; Bellwood, 1985), but the function of these has not been described. Therefore, it is worthwhile to examine the seasonal and age

and reproduction.

The research results are organised into five chapters (numbered 3-7) along with a chapter dealing with the general background biology and biogeography of each main study species (Chapter 2). Chapter three and four deal with the establishment of techniques for the validation and enumeration of otolith growth increments in both juveniles and adults, while the next three chapters (5 - 7) deal with population biology, reproduction and liver function focusing primarily on the species *Scarus rivulatus* and *S.schlegeli*. The primary aims and topics addressed in each of the chapters are as follows:

Chapter 3. To examine whether juvenile individuals of the respective species possess growth rings within the otolith microstructure, whether experimental methods can be employed to determine the periodicity of these rings, whether any techniques can be employed to visualise and enumerate these growth rings and therefore to estimate the age of individual juveniles.

Chapter 4. To investigate if adult individuals of the respective species possess annual growth bands within the otolith microstructure, if experimental methods can be employed to determine the periodicity of these bands, if any techniques can be employed to visualise and enumerate these growth bands and therefore to estimate the age of individual adults.

Chapter 5. To estimate the age specific growth pattern of *Scarus rivulatus*, *S.schlegeli*, *Ctenchaetus binotatus* and *C.striatus* and how fast these species are

growing. To examine their settlement age and how large the variation in growth is between different species and different locations.

Chapter 6. To assist whether individual age analysis can reveal about the timing of maturity processes, sexual and colour phase change in scarids. By using gonadal analysis age-specific schedules of maturity and sexual transition were determined. The seasonal pattern of gonadal activities and the variation of demographic parameters between different sexual groups among color phases were also examined.

Chapter 7. Preliminary liver analysis was carried out to determine age-specific and seasonal variations of liver condition with regard to sexual maturity schedules.

Finally, a general discussion reviews the main results, and analyses these results in relation to the population dynamics of tropical scarids and acanthurids. Suggested directions for future study are also outlined. In addition, a summary of the main conclusions is listed.

CHAPTER 2. GENERAL METHODS

This chapter provides background information with regard to study species and the marine environment at localities sampled during this research program. General collection and preservation techniques are also provided. In addition, general terminology is described. More detailed specific methods are given in each of the respective chapters.

2.1 Study species

Ctenochaetus striatus (Quoy & Gaimard 1825) and *Ctenochaetus binotatus* (Randall 1955) are both belonged to the family Acanthuridae (common name: surgeonfish). This family is characterised by a small terminal mouth, a single row of teeth, a compressed body, thick skin with tiny scales and a pair of sharp spines on the caudal peduncle (Myers, 1989). Both species occur on coral reefs in the Indo-Pacific region (Randall *et al.* 1990), and are dominant components of the reef fish fauna on coral reefs (Williams and Hatcher, 1983; Russ, 1984a,b; Horn, 1989). They feed on fine detrital and soft algal material by sucking their sediments (Russ, 1984a; Randall *et al.*, 1990).

C.striatus inhabits over coral rock, pavement, or rubble substrate of reef flats, lagoon and seaward reefs to a depth of over 30 m. Similarly, *C. binotatus* inhabits in coral and rubble areas of deep lagoons and seaward reefs to a depth of up to 53 m (Myers, 1989). These two species do not change sex.

Scarus rivulatus (Valenciennes 1840) and *Scarus schlegeli* (Bleeker 1861) are grouped in the family Scaridae (common name: parrotfish). This family is

characterised by their bright colours and the fusion of their teeth forming beak-like plates in the jaws (Randall *et al.*, 1990). The former is widespread in the western Pacific but does not extend into the Indian Ocean (Choat and Randall, 1986; Randall *et al.*, 1990). While the latter is confined to the islands of Oceania (except the easternmost) and the western Pacific (Randall *et al.*, 1990). In the Great Barrier Reef both species are abundant on mid-shelf reefs while *S.rivulatus* is also abundant on inshore reefs (Russ, 1984a,b; Choat and Randall, 1986). They mainly feed on benthic algae which they scrape from dead coral rock. In this way they usually remove some of the surface layer of limestone, and are a major producer of sediment in tropical and subtropical seas (Randall *et al.*, 1990). Like most members of the family, *S. rivulatus* and *S.schlegeli* display distinct color phases at different stages of their life, and are protogynous hermaphrodites (Choat and Robertson, 1975; Randall and Choat, 1980; Choat and Randall, 1986).

2.2 Study area

The majority of sampling was undertaken at Lizard Island and neighbouring reefs while a small number of specimens was collected at Arlington & Thetford Reefs and Magnetic Island.

Lizard Island (Fig. 2.1) is a continental island in the northern Great Barrier Reef (GBR), and is situated 36 km off the mainland coast and 16 km from the outer-shelf reefs (14°45'S, 145°28'E). The prevailing wind direction at Lizard Island is from the southeast, particularly during the trade-wind months from March to September. From October to February, winds become lighter and more variable. Sea surface temperatures range from 22°C to 32°C. The wet season generally occurs

from December to May, and the dry season lasts from June to November. Sampling was carried out at Lizard Island between September 1987 and January 1991, and these specimens were used throughout this thesis. All sampling sites around Lizard Island mentioned in the text are depicted in Figure 2.1.

Arlington and Thetford Reefs are mid-shelf reefs (16°45'S 146°10'E), and located approximately 17 nautical miles northeast of Cairns in the northernmost of the central GBR (Fig. 2.2a). Magnetic Island is a continental island off Townsville (19°00'S, 146°10'E). The sample was taken at Nelly Bay and Geoffrey Bay, on the south-east coast of the Island (Fig. 2.2b). Sampling was conducted in these locations between April and October 1988. The specimens (*Scarus rivulatus*) were only used for growth comparison in Chapter 5.

2.3 Sampling fixative and preserving methods

All individuals were collected by netting and spearing (Tables 2.1,2). Large individuals (predominantly standard length (SL) > 135 mm) were generally collected with a 40-45 mm mesh square mono-gill net (30 m length, 1.5 m width) and a Horsepower spear gun. The post-juveniles (generally SL ranged from 80 to 135 mm) were usually sampled using a hand spear gun, and the juveniles (SL < 80 mm) were collected using 7 mm mesh square mono-filament barrier net (1.5 m length, 1 m width) and a hand net. All collection was carried out using SCUBA.

Most of the large acanthurids and scarids were fresh processed while a few specimens were immediately frozen upon collection for up to one day. All material for histological analysis was fixed immediately after capture (see Chapter 6 and 7). The small specimens (juveniles and post-juveniles) were either frozen or fixed

Fig. 2.1 The map of Lizard Island showing all sampling sites (arrowed).

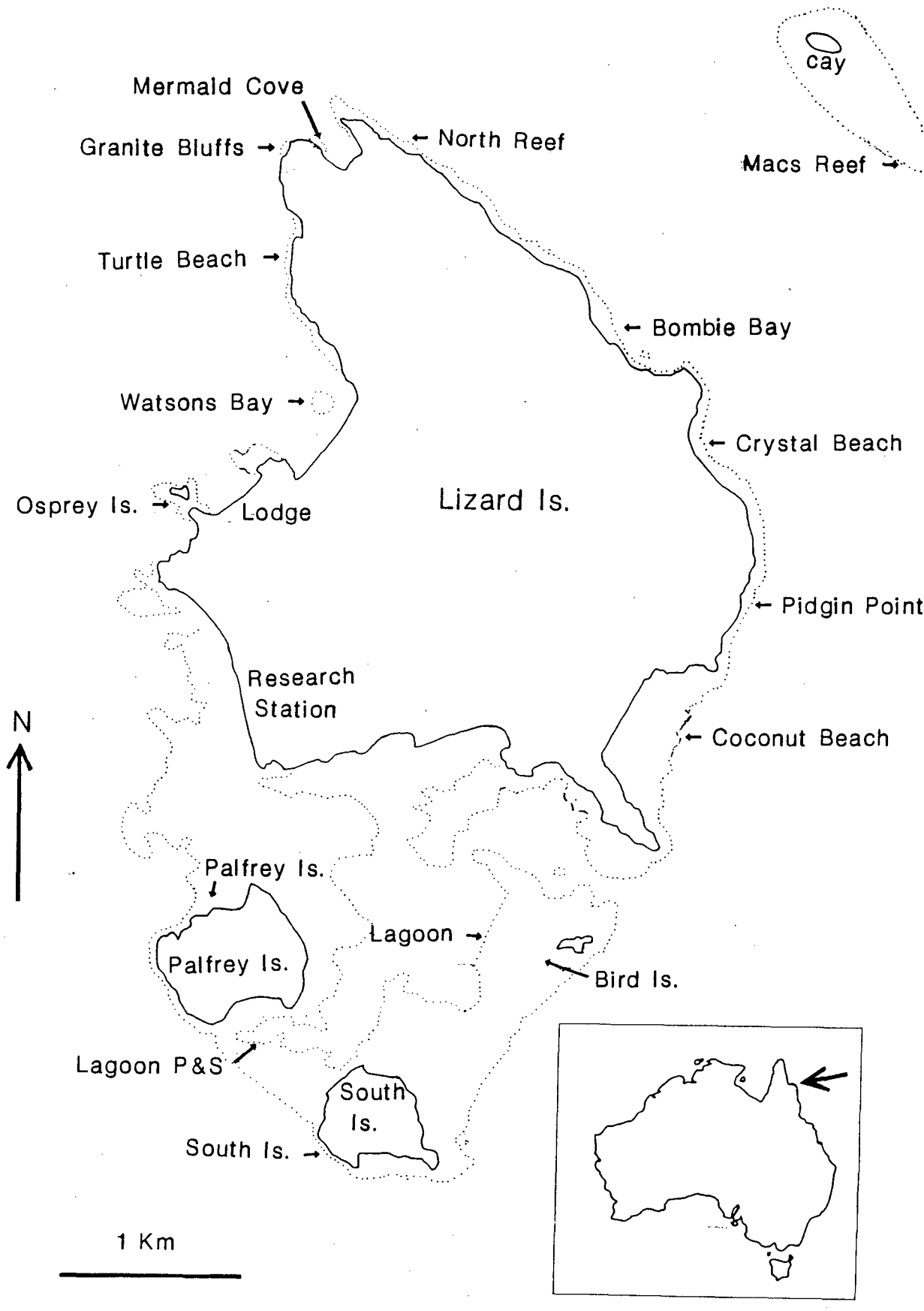

cay
Mermaid Cove
Granite Bluffs
North Reef
Macs Reef
Turtle Beach
Bombie Bay
Watsons Bay
Crystal Beach
Osprey Is.
Lodge
Lizard Is.
Pidgin Point
Research Station
N
Coconut Beach
Palfrey Is.
Palfrey Is.
Lagoon
Bird Is.
Lagoon P&S
South Is.
South Is.
1 Km

Fig. 2.2 Maps of Arlington & Thetford Reefs and Magnetic Island.

A. The map of Arlington and Thetford Reefs; **B**. The map of Magnetic Island showing Nelly Bay and Geoffrey Bay (arrowed); **C**. The map of the Great Barrier Reef showing relative position of **A** and **B**.

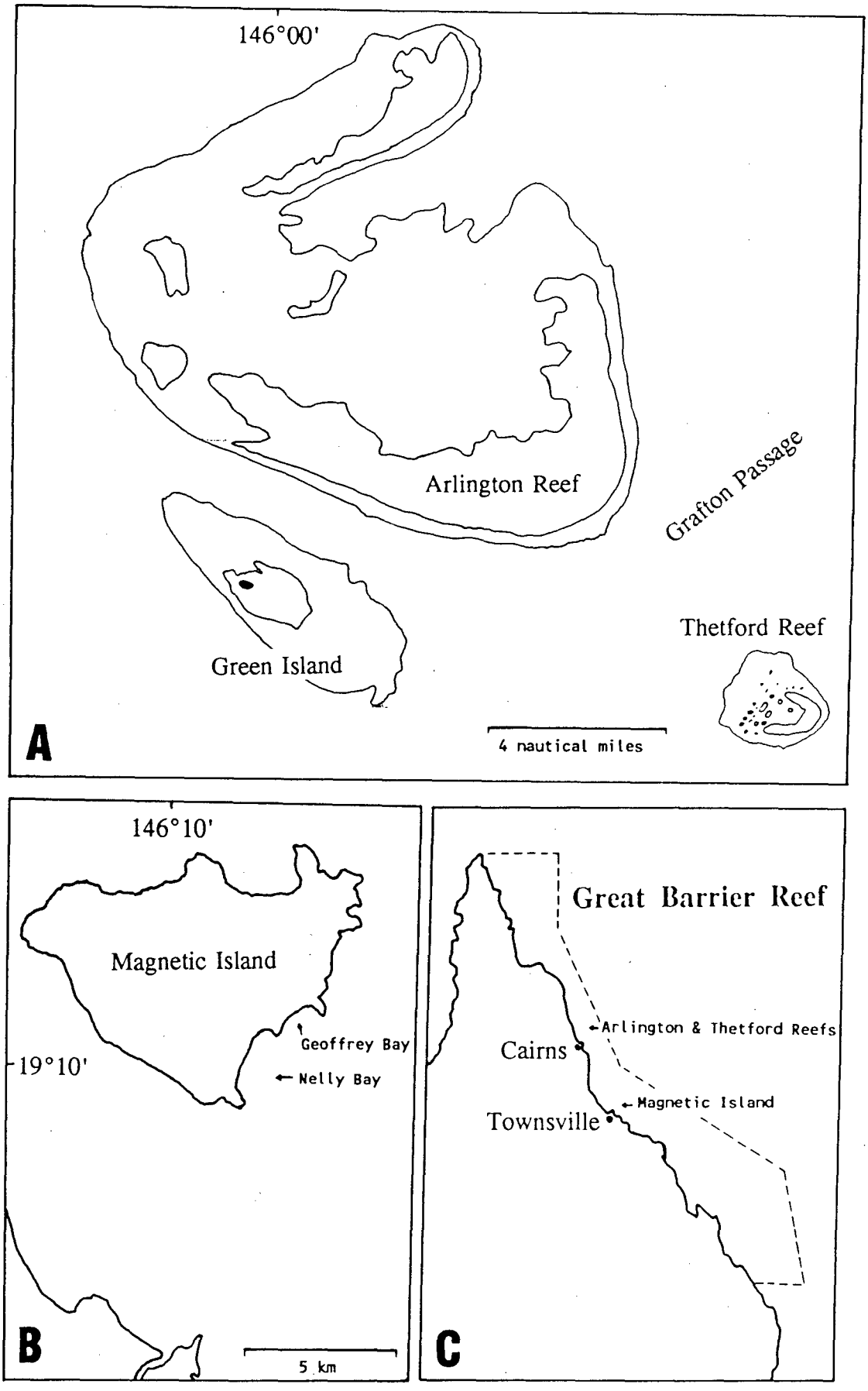

146°00'
Arlington Reef
Grafton Passage
Thetford Reef
Green Island
A
4 nautical miles
146°10'
Magnetic Island
Geoffrey Bay
Nelly Bay
19°10'
B
5 km
Great Barrier Reef
Arlington & Thetford Reefs
Cairns
Magnetic Island
Townsville
C

Table 2.1 Summary of samples from various sites around Lizard Island.

Sr - Scarus rivulatus; *Ss - Scarus schlegeli*; *Cb - Ctenochaetus binotatus*; *Cs - Ctenochaetus striatus*.

Sampling sites	Number of individuals	
	Netting	Spearing
Mermaid Cove	36 *Sr*, 74 *Ss*, 18 *Cb*, 5 *Cs*	19 *Sr*, 50 *Ss*, 5 *Cb*, 1 *Cs*
Granite Bluffs	14 *Sr*, 31 *Ss*, 1 *Cb*, 1 *Cs*	1 *Sr*, 4 *Ss*, 10 *Cb*, 15 *Cb*
Turtle Beach	19 *Sr*, 20 *Ss*, 7 *Cb*, 4 *Cs*	30 *Sr*, 54 *Ss*, 1 *Cb*
Watsons Bay	26 *Sr*, 41 *Ss*	5 *Sr*, 7 *Ss*
Osprey Island		10 *Sr*, 9 *Ss*
Palfrey Island	33 *Sr*, 16 *Ss*	4 *Sr*
Lagoon P&S	10 *Sr*, 1 *Ss*, 1 *Cb*, 1 *Cs*	
South Island	94 *Sr*, 15 *Ss*, 1 *Cb*, 1 *Cs*	5 *Sr*
Lagoon	39 *Sr*, 14 *Ss*, 4 *Cb*,	
Bird Island	7 *Sr*, 12 *Ss*,	2 *Sr*, 1 *Ss*, 7 *Cb*, 10 *Cs*
Coconut Beach		4 *Sr*, 12 *Ss*
Pidgin Point		2 *Sr*, 3 *Ss*
Crystal Beach	6 *Ss*	9 *Sr*, 7 *Ss*,
Bombie Bay	2 *Sr*, 2 *Cb*	3 *Sr*
Macs Reef	1 *Sr*, 9 *Ss*	6 *Sr*
North Reef	7 *Sr*, 19 *Ss*, 23 *Cb*, 2 *Cs*	7 *Sr*, 2 *Ss*, 10 *Cb*, 11 *Cs*
Total	288 *Sr*, 258 *Ss*, 60 *Cb*, 17 *Cs*	107 *Sr*, 149 *Ss*, 33 *Cb*, 40 *Cs*

Table 2.2 *Scarus rivulatus* **collected from the other sites.**

Sampling sites	Number of individuals	
	Netting	Spearing
Arlington Reef	10	
Thetford Reef	16	
Nelly Bay	9	5
Geoffrey Bay		19

directly in 70% ethanol.

2.4 Terminology

Acanthurids and scarids are generally referred to family *Acanthuridae* and *Scaridae*. However, these are in reference to the genera *Ctenochaetus* and *Scarus* in this thesis.

General fisheries measurements were used in this thesis, i.e. standard length (SL) - the length of fish body, taken from the middle of the tip of the upper jaw to the base of the caudal fin; total length (TL) - the greatest possible length of a fish, from the tip of the longest jaw to the longest caudal ray; body weight (WT) - wet body weight of fish with guts.

Standard scientific abbreviations are used throughout this thesis; μm=micrometre, mm=millimetre, m=metre, km=kilometre, °C=degree celsius, h=hour, d=day, mg=milligram, g=gram, ml=millilitre and l=litre.

In otoliths, the fine periodic laminae are referred to as increments, and the large periodic rings are referred to as bands throughout this thesis. The thin periodic rings on scales are defined as checks.

An age group in this thesis is defined as follows:

All fish of the same age are grouped together in a single age group which is designated by an arabic numeral indicating the number of years of life completed. Thus fish in their first year of life belong to the 0 group, in the second year to the 1 group, and so on. The arabic numeral may be followed by a + sign, which indicates that fish concerned have already passed through a portion of the next year of life.

Juveniles in this thesis refer to as young acanthurids and scarids which have settled on reefs, and whose SL is generally less than 100 mm. For purpose of this thesis adults are considered fishes whose SL exceeds 100 mm.

2.5 Color phase and sexual identity of scarids

Within many scarid species there exist three distinct, relatively permanent color patterns. In this study, the terminology used by Warner and Robertson (1978) is followed, i.e juvenile, initial and terminal phases. The juvenile phase is a color pattern characteristic of immature individuals. The initial phase is a drab color phase characteristic of small adult individuals while the terminal phase is a colourful phase characteristic of the largest males.

Work on protogyny in the scarids (Choat and Robertson, 1975; Robertson and Warner, 1978; Randall and Bruce, 1983) indicate that ontogenetically, there are two types of males, those born as male (primary males) and males which were originally females (secondary males). In addition, a further distinction is made between hermaphroditic males that functioned as females before changing sex (post-maturational secondary males) and those that change sex before they have matured as females (pre-maturational secondary males). If a species contains both primary and secondary males, it is termed diandric. When the only males present are secondary, it is monandric (Reinboth, 1970; Sadovy and Shapiro, 1987). The scarids in this study (*Scarus rivulatus* and *S.schlegeli*) are both diandric (Choat and Randall, 1986). In the growth part of this thesis unless stated otherwise (Chapter 5), males included primary and secondary males.

CHAPTER 3. VALIDATION OF AGING TECHNIQUES FOR JUVENILE ACANTHURIDS AND SCARIDS

3.1 INTRODUCTION

The use of otolith increments for aging larval and juvenile fish has become increasingly popular for establishing growth parameters in wild populations (Jones, 1986). This technique is based upon the assumption that otolith increments are formed at a rate of one per day and the degree of precision in increment counts reflects the accuracy in estimating age. A prerequisite of otolith age determination in each species is the validation of the periodicity of otolith increments (Beamish and McFarlane, 1983). The presence of daily increments has been validated in a number of juvenile coral-reef fish species on the Great Barrier Reef (GBR), Australia (eg. six species of pomacentrids, Pitcher, 1988; three species of chaetodonts, Fowler, 1989). In contrast, juvenile acanthurids and scarids have received little attention in regard to their age and growth from scientists although they are two dominant groups of herbivorous fishes in the region (Lou and Moltschaniwskyj, 1992).

On the assumption that growth increments in otoliths are deposited on a regular daily basis, Brothers *et al.* (1983) estimated the age of larval and juvenile acanthurids and scarids on GBR. However, this assumption has yet to be validated for the Scaridae and the Acanthuridae.

The reliability and precision in increment counts have yet to be assessed in tropical coral-reef fish. Such an assessment of the precision of daily increment counts will improve the aging methodology for tropical coral-reef fish.

Methods of validation that are widely used include otolith increment counts

of known-age fish hatched and raised in the laboratory (Brothers *et al.*, 1976; Neilson and Geen, 1982; Moksness and Wespestad, 1989), sequential sampling of a population with discrete cohorts (Struhsaker and Uchiyama, 1976), and chemically marking the otoliths to produce a time mark (Schmidt, 1984; Thorrold, 1989; D'Amours *et al.*, 1990). Chemical marking techniques commonly employ oxytetracycline-hydrochloride, which is incorporated into growing calcified tissue such as the margins of otoliths, and can be detected as it fluoresces under ultraviolet light (Wild and Foremen, 1980; Campana and Neilson, 1982; Beamish *et al.*, 1983; Hetter, 1984; Pitcher, 1988; Fowler, 1989; Schultz, 1990). Other methods for placing time marks on otolith involve stressing the fish at known dates (eg. subjecting fish to total darkness without food for ~4 days-Victor, 1982) or producing abrupt changes in increment width by providing larger quantities of food to fish over several days (eg. Struhsaker and Uchiyama, 1976; Victor, 1982). However, neither of these two techniques are unambiguous because the history of natural stresses on the fish is usually unknown (Campana and Neilson, 1985), and a lag time may exist between changes in food intake and changes in increment width (Neilson and Geen, 1985). In some cases, increased food intake apparently does not induce wider otolith increments (Milicich, 1986).

In the surgeonfishes and parrotfishes, sequential sampling seems to be unsatisfactory due to protracted spawning seasons (Thresher, 1984). While culturing fish from hatching (thereby obtaining known age of individuals) is often difficult and time consuming, artificially placing a chemical time mark on otoliths is perhaps one of the most convenient methods for validating the periodicity of otolith increments.

This chapter was emphasised on establishing an aging method for tropical

juvenile acanthurids and scarids. The detailed purposes included:

(1) to examine the otolith microstructure;

(2) to determine the periodicity of the otolith increments; and

(3) to test the consistency and reliability of increment counts in age estimates.

3.2 MATERIALS AND METHODS

3.2.1 Juvenile collection

Samples of juveniles were collected from the study sites around Lizard Island from December 1987 to February 1990 (see Chapter 2). A total of 69 juvenile *C.binotatus*, 30 juvenile *C.striatus*, 60 juvenile *S.rivulatus* and 34 juvenile *S.schlegeli* were sampled. Among them 12 *C.binotatus*, 2 *C.striatus*, 21 *S.rivulatus* and 21 *S.schlegeli* were collected alive, and used in the validation experiments. Ten pairs of otoliths from each species were also examined for the consistency test in increment counts in this chapter. In addition to the above species, a number of juvenile surgeonfishes and parrotfishes including several other species (see Table 3.1 for details) were also collected for the validation experiments.

3.2.2 Validation experiments

Validation experiments over short periods were conducted in aquarium facilities at the Lizard Island Research Station. A longer experiment was carried out at James Cook University of North Queensland. Otoliths were marked by immersing fish in a solution of tetracycline-hydrochloride and seawater. Prior to these experiments a pilot study had been done to determine concentrations and immersion times. Three concentrations (250, 300 and 400 mg/l) were tested from 10 to 20 hrs

using *Scarus rivulatus* of 37 to 61 mm SL. From each concentration and time period combination, otoliths were examined to assess the intensity of fluorescence. A minimum of 300 mg/l for 12 hrs was found to be necessary for tetracycline to be incorporated into otolith microstructure.

Details of validation experiments at Lizard Island are shown in Table 3.1. In the experiment carried out at the University, 20 *Scarus schlegeli* and 7 *S.rivulatus* were kept in outdoor aquaria after being marked with tetracycline (300 mg/l, 17 hrs). Several individuals were killed at regular intervals over a period of 56 days.

Following validation experiments, both SL (mm) and body weight (WT) to 0.001 g were recorded. Three pairs of otoliths (sagittae, lapilli and asterisci) were extracted, using the technique described by Schneidervin and Hubert (1986), and stored dry, away from light.

3.2.3 Otolith preparation

Otoliths were prepared in two ways depending on their size. For fish less than 20 mm SL, the lapilli were small and translucent enough that increment definition could be discerned without grinding and polishing. However, the lapilli from the fish greater than 20 mm SL generally required grinding and polishing to reveal the lapillus microstructure.

Initially, whole, unground lapilli were mounted in the dibutyl-polystyrene-xylene (D.P.X.). The lapillus was placed on a microscope slide, rinsed with ethanol and allowed to dry. The xylene assisted in decreasing the viscosity of the D.P.X. and facilitating the penetration of the mountant into the lapillus. A coverslip was then gently lowered on to the D.P.X. to spread the mountant out evenly. This mounting

Table 3.1. The details of validation experiments at Lizard Island.

Species	N	1st Staining	Immersion Period	2nd Staining	Immersion Period	Date of Sacrifice
Acanthurus nigrofuscus	2	13/12/87	12 hrs	-	-	25/12/87
Ctenochaetus binotatus	2	13/12/87	12 hrs	-	-	25/12/87
Naso annulatus	2	13/12/87	12 hrs	-	-	25/12/87
Scarus rivulatus	13	04/06/88	14.75 hrs	10/06/88	14 hrs	17/06/88
Scarus globiceps	11	04/06/88	14.75 hrs	10/06/88	14 hrs	17/06/88
Ctenochaetus binotatus	4	04/06/88	14.75 hrs	10/06/88	14 hrs	17/06/88
Scarus psittacus	3	04/06/88	14.75 hrs	10/06/88	14 hrs	17/06/88
Scarus sordidus	3	04/06/88	14.75 hrs	10/06/88	14 hrs	17/06/88
Scarus schlegeli	1	04/06/88	14.75 hrs	10/06/88	14 hrs	17/06/88
Zebrasoma scopas	1	04/06/88	14.75 hrs	10/06/88	14 hrs	17/06/88
Zobrasoma verliferum	1	04/06/88	14.75 hrs	10/06/88	14 hrs	17/06/88
Ctenochaetus binotatus	6	05/06/88	15 hrs	10/06/88	14 hrs	17/06/88
Ctenochaetus striatus	2	05/06/88	15 hrs	10/06/88	14 hrs	17/06/88
Scarus sordidus	2	05/06/88	15 hrs	10/06/88	14 hrs	17/06/88
Scarus niger	1	05/06/88	15 hrs	10/06/88	14 hrs	17/06/88
Scarus frenatus	1	06/06/88	13.75 hrs	10/06/88	14 hrs	17/06/88
Scarus oviceps	1	06/06/88	13.75 hrs	10/06/88	14 hrs	17/06/88

* All immersion was undertaken over night.

technique produced a high degree of increment resolution within the otolith microstructure of small individuals. This technique had the disadvantage, in that lapilli could not be manipulated or repositioned after mounting.

Subsequently, the thermoplastic cement, Crystal Bond, was also found to have excellent optical qualities as a mounting medium. After heating a microscope slide on a hot plate, a small piece of Crystal Bond was then placed on slide. After the cement has melted, a dry lapillus was placed within the cement. Moreover, while the cement was still warm and pliable, the lapillus could be manipulated before the glue hardened. The cement could also be remelted for further manipulation of the otoliths. This technique had the advantage, in that the orientation of the lapilli could be changed within the cement.

Crystal Bond was subsequently used as a mountant for otoliths of the larger specimens. Due to its convenience of being able to be remelted, the larger otoliths which required grinding and polishing could be easily manipulated. Furthermore, hardened Crystal Bond was relatively soft which facilitated grinding of the larger otoliths. Larger lapilli were embedded in the thermoplastic cement on a glass and ground by hand with wet 1200 grade carborundum paper. Scratches from the grinding were removed by hand polishing on wet suede with 0.05 μm alumina powder or by using a modified gem polishing machine equipped with a 16 cm rotating disc to which was attached a wet felt (Leco Lecloth) impregnated with alumina powder. Ground lapilli were either held by hand against the rotating polishing disc or lowered onto the disc with a special made microscope slide holding arm.

3.2.4 Otolith increment counting and its consistency test

Otolith increments between the characteristic yellow tetracycline fluorescence and the otolith margin were identified and counted under a Leitz microscope (400×-1000×) equipped with a combination of normal and fluorescent illumination. Fluorescent light was generated from a Ploemopak illuminator with a 50 watt mercury lamp. For individuals with two fluorescent bands, increments were counted from the innermost edge of the first fluorescent band to the innermost edge of the second fluorescent band. Increment counts were only considered valid if identical counts were obtained in two separate readings. Comparisons between the number of days post staining or between the number between the two stainings and actual increment counts were subjected to chi-square test, using the null hypothesis that the number of increments was equal to the number of days elapsed. For the extended University experiment, a linear regression was used to examine the relationship between the days elapsed and the number of otolith increments subsequent to the tetracycline band. Estimated age for each individual fish was taken as the mean of two independent counts of increments in lapilli. Increments were counted using an Olympus compound microscope mounted with an Ikegami ICD-290 high resolution black and white video camera connected to a Commodore-1084 monitor. Counting was facilitated by the use of a movable cursor superimposed over the image of the lapillus.

Although no systematic difference in increment number between the left and right sagittae has been reported (Campana and Neilson, 1982; Neilson and Geen, 1982; Geffen, 1982), such an examination has never been applied to lapilli. Consequently, the consistency of increment counts between left and right otoliths was

examined for ten randomly selected pairs of lapilli from the four species, *C.binotatus*, *C.striatus*, *S.rivulatus* and *S.schlegeli*.

In assessing the similarity in age estimates from left and right otoliths, the difference between two counts for each lapillus was calculated and an average difference calculated for all ten otoliths. A difference in the estimated age for a fish from the left and right lapilli was then calculated and averaged. The average difference in estimated age obtained between the lapilli was compared with the average difference from multiple counts of the left or right lapilli. If the average difference between multiple lapillus increment counts was similar to (using 95% confidence limits) or larger than the average difference between left and right lapilli then the estimating age of an individual from either lapilli was considered acceptable.

It is possible to obtain an indication of observer bias in age estimates by utilising several observers (Kimura and Lyons, 1990). To examine the precision of age estimates the left lapilli were counted by two experienced independent readers. The similarity of age estimates from different readers was treated as described for the left and right otoliths.

3.3 RESULTS

3.3.1 General structures

Sagittae of parrotfishes and surgeonfishes were thick and had a complex three-dimensional shape. Due to the limited depth of field for one plane of focus, some increments were not discernible from the nucleus to margin even though the otolith was ground and polished. The asterisci had distinct fine growth increments which could be seen under a light microscope without grinding. However, due to its

relatively small size, extraction and mounting was difficult. In contrast, the lapilli not only had the most distinct growth increments but were also relatively easy to extract (Plate 3.1a,b). Therefore, the lapillus was selected to use for aging juvenile parrotfishes and surgeonfishes.

Lapilli generally have similar morphological features within the same families. lapilli of surgeonfishes are bell-shaped with the nucleus located at the centre of dorsal dome (Plate 3.1a). Growth increments were most visible in the sulcal region. In parrotfishes, lapilli are usually round (Plate 3,1b) , with the nucleus located close to the centre near the dorsal surface. Growth increments were clearly seen in all regions except for the dorsal area.

Fine growth increments on the lapilli were bipartite in structure, consisting of incremental and discontinuous zones. Under the transmitted light, the incremental zone appeared as a broad, translucent band, while the discontinuous zone was relatively narrow and opaque (Plate 3.1c). lapillus increments in surgeonfishes were wider near the nucleus and narrow near the margin while the increment width was relatively consistent from the nucleus throughout the margin in parrotfishes.

3.3.2 Validation of daily otolith increments

Otoliths of fish immersed in tetracycline showed a bright fluorescent band medial to the edge. This band appeared to cover approximately three growth increments. The double marked otoliths displayed two bright fluorescent bands (Plate 3.2a,c), the outer band was close to the otolith edge, and overlapped by autofluorescence around the otolith margin. Such autofluorescence has been noted by Campana and Neilson (1982). The lapillus increments between tetracycline bands

Plate 3.1 Microstructure of lapilli of the juvenile surgeonfish and parrotfish.

D - discontinuous zone; I - incremental zone; N - nucleus. **a.** The lapillus of *Ctenochaetus binotatus* (SL = 28 mm, scale bar=100 μm). **b.** The lapillus of *Scarus schlegeli* (SL = 21 mm, scale bar=100 μm). **c.** The structure of daily increments in lapillus of *C.binotatus* (SL= 28 mm, scale bar=20 μm).

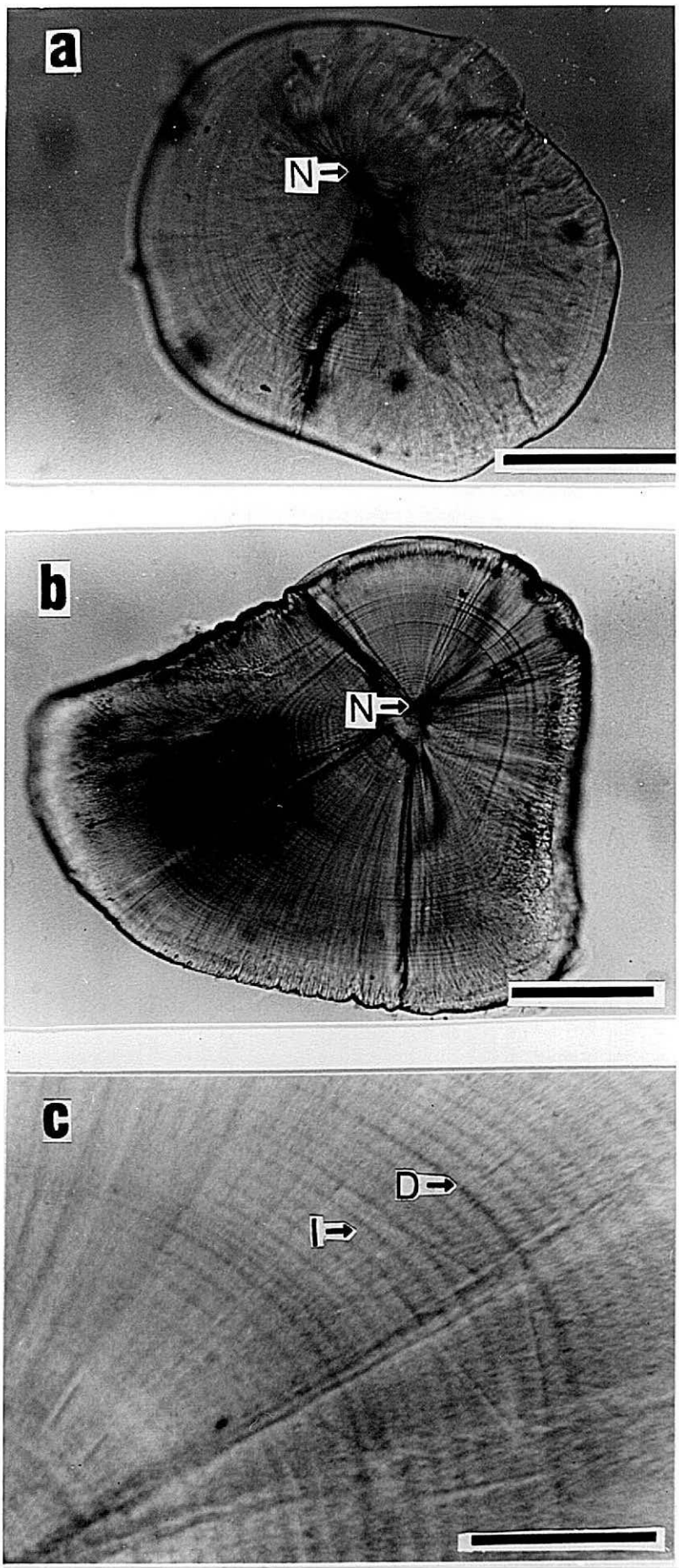
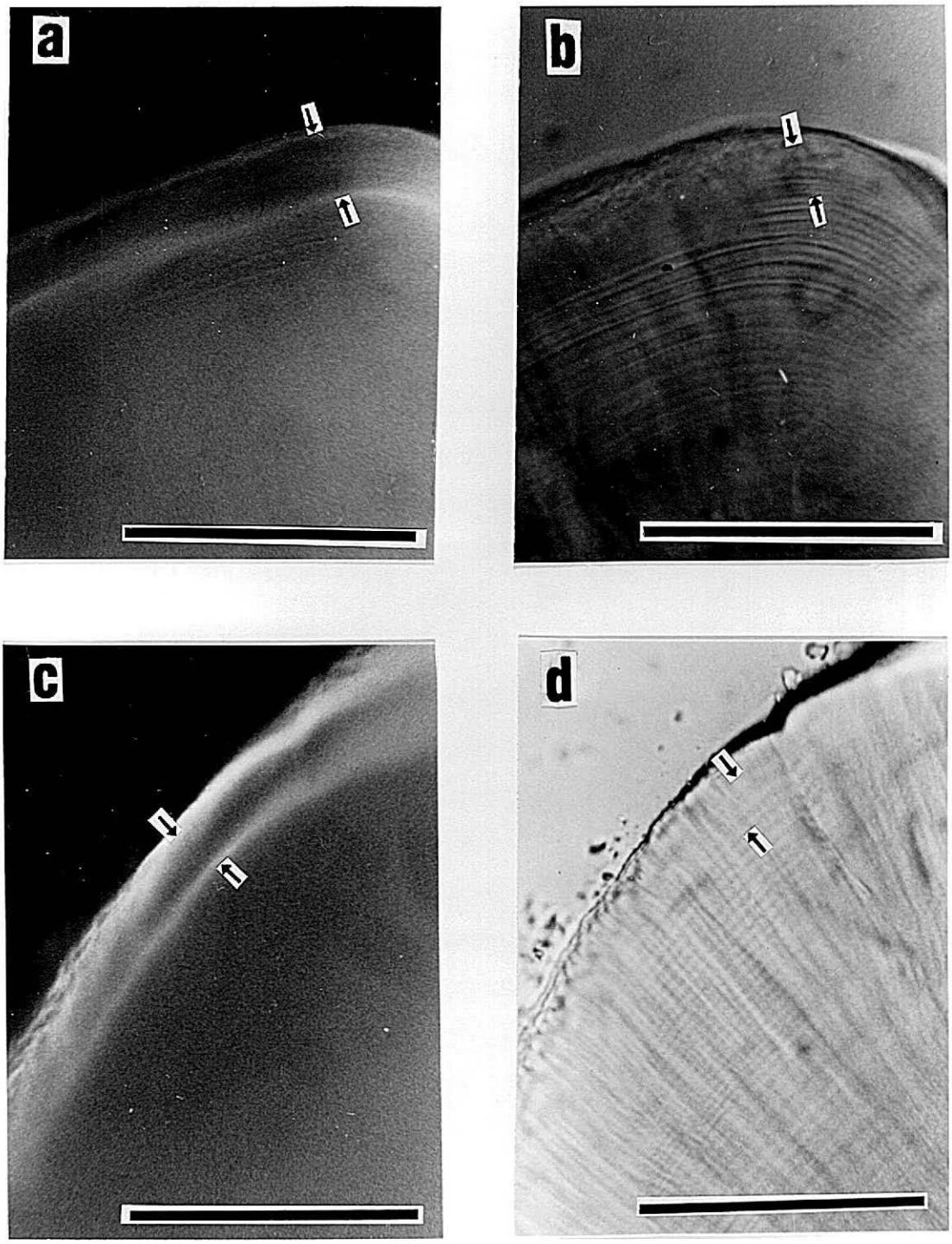

a
N
b
N
c
D
I

Plate 3.2 Validated daily increments on the lapilli of surgeonfishes.

a,c Fluorescent micrographs of lapilli from a 30 mm SL *Ctenochaetus binotatus* (**a**) and a 36 mm SL *C.striatus* (**c**) marked twice with tetracycline 6 days apart. **b,d** light micrographs of the same lapilli. Arrows indicate tetracycline bands (scale bar = 50 μm).


a
b
c
d

were visible under transmitted light (Plate 3.2b,d).

In the validation experiments at Lizard Island, Chi-square tests indicated that there was no significant difference between the number of chronological days subsequent to or between treatments and the number of increments deposited for *Scarus rivulatus*, *S.globiceps*, *S.sordidus*, *S.psittacus*, *Ctenochaetus binotatus*, *C.striatus*, *Acanthurus nigrofuscus* and *Naso annulatus* (Table 3.2). No test was carried out for *S.frenatus*, *S.niger*, *S.oviceps*, *S.schlegeli*, *Zebrasoma scopas* and *Z.veliferum* because only one specimen was examined for each species. However, the number of increments was equal to the number of chronological days for these six species.

Of 50 individuals marked twice with tetracycline, forty-three deposited daily otolith increments. The other seven individuals had one less or one more increment compared to the chronological days between treatments. In the single tetracycline marking experiment (n=6), however, three specimens deposited one less increments than the chronological days subsequent to treatments.

Fish stained twice with tetracycline had a high percentage of agreement between the number of increments produced and the number of chronological days. In contrast, fish stained once had a relatively low percentage in agreement between the increment number and number of chronological days because increment counting became increasingly difficult at the margin of the lapillus.

The validation experiment carried out in the University aquarium system demonstrated that parrotfishes from two species consistently deposited daily increments over a period of 11 to 56 days (Plate 3.3; Fig. 3.1). The slopes of the regressions were not significantly different from one, since the 95% confidence limits

Table 3.2. Results of Chi-square tests of the validation experiments at Lizard Island.

Days - chronological days subsequent to or between treatments. Counts - otolith increments between fluorescent bands or subsequent to fluoresent band. None were significantly different at $p<0.05$.

Species	N	Range SL (mm)	Mean of counts (±s.d.)	No. days	Chi-square	*p*
S.rivulatus	13	37 - 63	6.07 (0.26)	6	0.0705	1
C.binotatus	12	26 - 48	11.5 (0.5), 6 (0.7), 5.16 (0.27)	12,6,5	0.3022	1
S.globiceps	11	44 - 52	6.09 (0.51)	6	0.2372	1
S.sordidus	5	25 - 53	6 (0), 5 (0)	6,5	0.0000	1
S.psittacus	3	50 - 52	6 (0)	6	0.0000	1
C.striatus	2	36 - 47	5 (0)	5	0.0000	1
A.nigrofuscus	2	35 - 43	11.5 (0.5)	12	0.0221	0.881
N.annulatus	2	29 - 36	11.5 (0.5)	12	0.0221	0.881

Fig. 3.1 Regressions of otolith increments counted against number of days for individuals maintained after tetracycline staining.

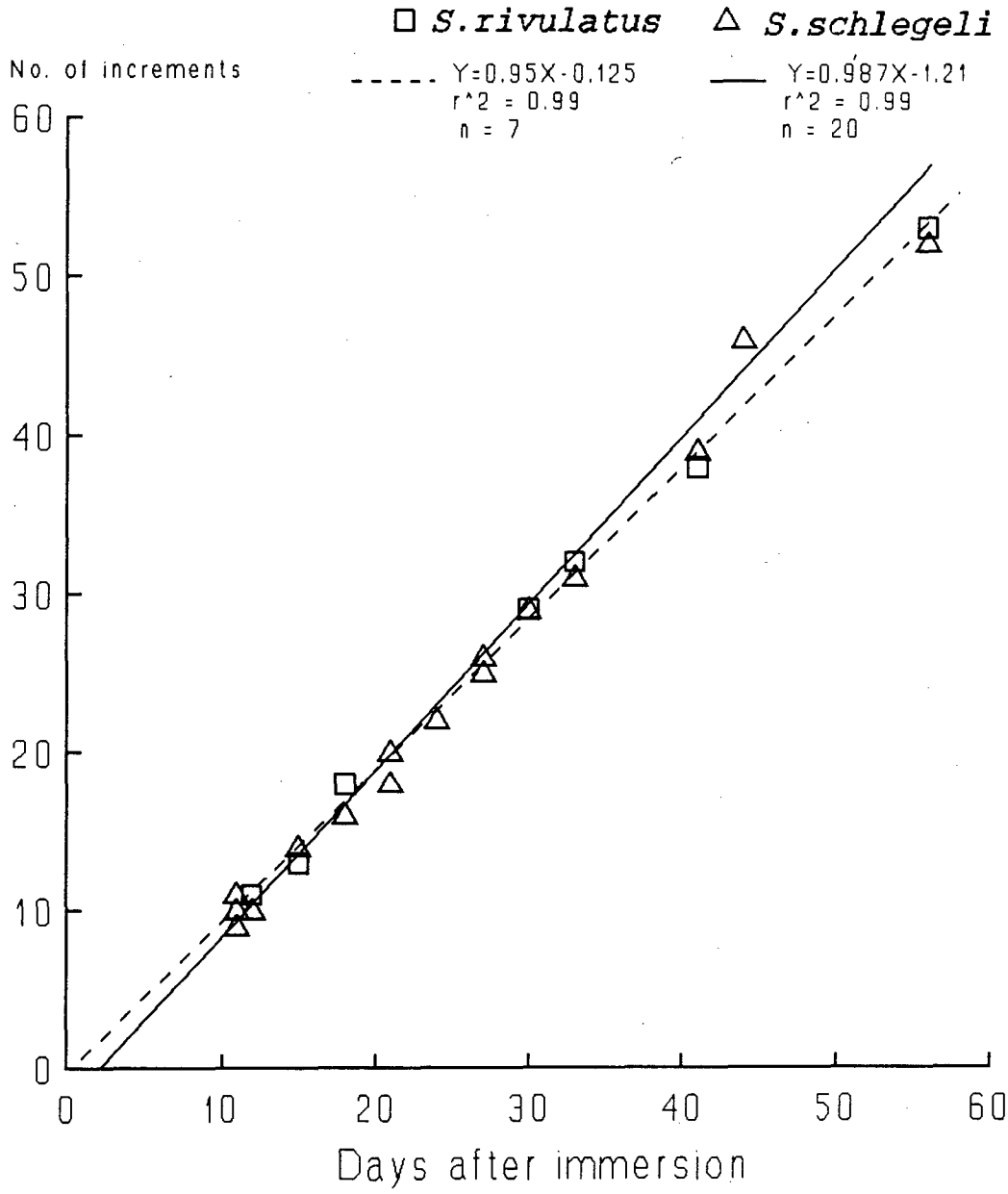
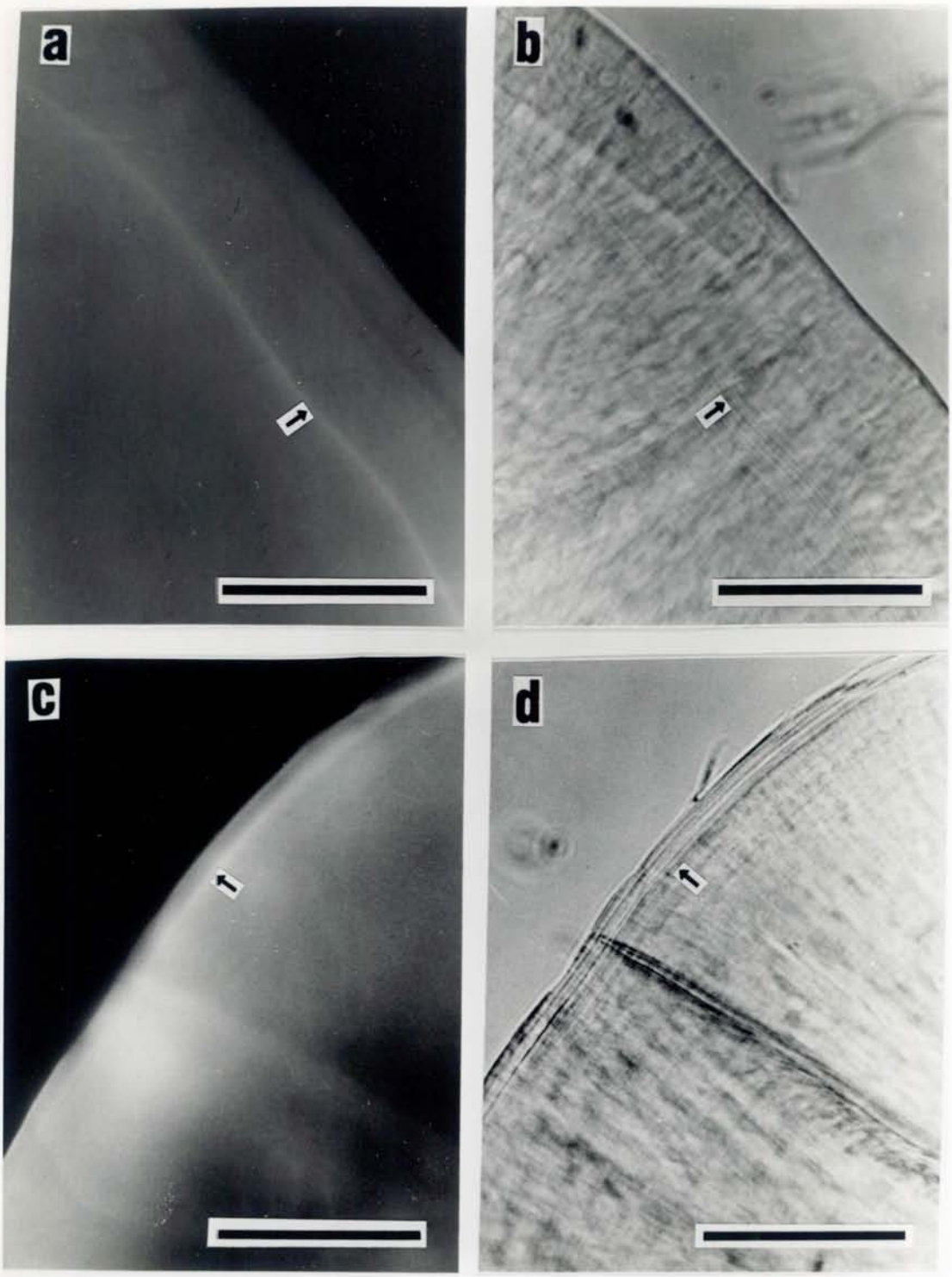

□ S.rivulatus
△ S.schlegeli
No. of increments
----- Y=0.95X-0.125
r^2 = 0.99
n = 7
—— Y=0.987X-1.21
r^2 = 0.99
n = 20
60
50
40
30
20
10
0
0
10
20
30
40
50
60
Days after immersion

Plate 3.3 Validated daily increments on the lapilli of parrotfishes.

a,c Fluorescent micrographs of lapilli from a 41 mm SL *Scarus schlegeli* (**a**) and 21 mm SL *S.rivulatus* (**b**), which were sacrificed 41 and 11 days respectively after tetracycline staining. **b,d** Light micrographs of the same lapilli. Arrows indicate tetracycline bands (scale bar = 50 μm).


a
b
c
d

for both *Scarus rivulatus* (0.942-1.03) and *S.schlegeli* (0.89-1.01) included one. Further confirmation of this trend would require a larger sample size.

3.3.3 Consistency test in increment counting

The average difference between multiple counts of each pair of left and right lapilli was found to be less than that difference between multiple readings of each lapillus for all four species (Table 3.3). It was therefore considered that the difference in estimating age obtained from the two lapilli was acceptable. Either the left or the right lapilli can be used to estimate age.

In assessing observer differences in estimating age it was found that for *C.striatus*, *S.rivulatus* and *S.schlegeli* the average difference in replicate counts by both readers was greater than the average difference between the two readers (Table 3.4). For *Ctenochaetus binotatus* reader 2 had a greater mean difference than between the two readers (Table 3.4). It was therefore concluded that the differences between the readers for the four species were acceptable.

The variation in counts by an observer was predominantly due to lapilli which were difficult to read. In addition, the difficulties in changing focus and in counting increments near the nucleus and close to the margin would have contributed to the variation.

3.4 DISCUSSION

Lapilli have been used in many studies of age determination for coral reef fishes (e.g. Victor, 1982; Pitcher, 1988; Fowler, 1989). Agreement in the number of daily increments between sagittae and lapilli has been found in many tropical

Table 3.3 Results of ring counts between left and right otoliths.

MD - mean difference; CL - confidence limits.

Species	Otolith	N	Range age (day)	MD	Range MD%	95% CL
S.rivulatus	Left	10	100 - 227	9.6	2.1 - 8.3	+/-8.4
	Right	10	93 - 217	8.5	2.0 - 8.2	+/-7.0
	Between	10	93 - 227	5.3	1.0 - 5.3	+/-6.0
S.schlegeli	Left	10	83 - 165	8.2	1.3 - 5.0	+/-4.8
	Right	10	85 - 170	7.0	1.4 - 4.0	+/-4.8
	Between	10	83 - 170	2.4	0.0 - 3.9	+/-3.6
C.binotatus	Left	10	69 - 121	7.6	1.4 - 5.2	+/-5.5
	Right	10	72 - 119	6.9	1.6 - 4.8	+/-3.7
	Between	10	69 - 121	2.5	0.8 - 4.8	+/-2.5
C.striatus	Left	10	81 - 202	7.2	3.4 - 7.9	+/-6.1
	Right	10	87 - 206	7.5	4.0 - 8.0	+/-5.0
	Between	10	81 - 206	5.4	0.5 - 9.1	+/-5.5

Table 3.4 Results of otolith ring counts between readers.

MD - mean difference; CL - confidence limits.

Species	Reader	N	Range age (day)	MD	Range MD%	95% CL
S.rivulatus	Reader 1	10	95 - 200	7.1	1.8 - 10.8	+/-6.0
	Reader 2	10	98 - 227	9.6	2.1 - 8.3	+/-8.4
	Between	10	95 - 227	6.3	0.9 - 9.0	+/-12.4
S.schlegeli	Reader 1	10	72 - 166	7.4	0.0 - 12.9	+/-10.2
	Reader 2	10	83 - 165	8.2	1.3 - 5.0	+/-4.8
	Between	10	72 - 166	7.3	0.6 - 14.9	+/-11.7
C.binotatus	Reader 1	10	73 - 125	2.9	1.2 - 5.4	+/-2.7
	Reader 2	10	69 - 121	7.6	1.4 - 4.9	+/-5.5
	Between	10	69 - 125	5.4	2.0 - 11.7	+/-5.9
C.striatus	Reader 1	10	87 - 189	7.1	3.6 - 8.7	+/-3.7
	Reader 2	10	82 - 202	7.2	3.4 - 7.9	+/-6.1
	Between	10	82 - 202	7.0	2.5 - 8.6	+/-5.0

species, for example, *Mugil cephalus* (Radtke, 1984) and *Plectroglyphidodon lacrymatus* (Polunin and Brothers, 1989). Furthermore, lapilli have been preferentially used in larvae and juveniles of some species (Brothers *et al.*, 1983; Fowler, 1989). These studies suggested that lapilli should be useful aging structures.

3.4.1 Daily otolith increments

Generally, the deposition rates published in the literature for juvenile coral reef fishes tend to be close to one-ring-per-day (Brothers and McFarland, 1981; Victor, 1982; Pitcher, 1988; Fowler, 1989). When viewed under transmitted light, lapilli of surgeonfishes and parrotfishes display clear bipartite structures, which are similar to that described by Pannella (1971, 1974), Campana and Neilson (1985) and Fowler (1989).

The lack of daily increment periodicity within the lapilli of few individuals in this study has also been observed in other validation studies of juvenile fish in the G.B.R. (Schmidt, 1984). This apparent difference may be partially due to the time for tetracycline to be incorporated into the growing otoliths. It is not known how long tetracycline apply to the otoliths of the parrotfishes and surgeonfishes. Campana and Neilson (1982) found that after injection, 50% of fish showed fluorescent otoliths after 10 hrs and 100% after 24 hrs. If this assumption is applicable to species in the present study, then a fluorescent band probably started to form 12 to 24 hrs after immersion. Since the discontinuous zone is thought to be formed daily from about 07:00 to 10:00 am in *Hypoatherina tropicalis* and *Spratelloides dellicatulus* caught near Lizard Island (Schmidt, 1984), increments counted on some individuals stained once were probably deposited one day after immersion.

The effect of tetracycline on otoliths may also influence the increment depositing rates. Tetracycline may inhibit mineralisation in scales and bone (Harris, 1960; Kobayashi *et al.*, 1964). Since other studies have recorded no adverse effects of tetracycline on growth or mineralisation (Weber and Ridgway, 1967), it was not considered to be of importance in this study.

Evidence from this research for daily periodicity of otolith growth increments, from chemical marking experiments, now exists for 83 individuals out of 14 species of tropical coral reef fishes (6 acanthurids and 8 scarids). Similar results were found in other tropical coral reef fish on the GBR (Pitcher, 1988; Fowler 1989). The juveniles of tropical species in this study provide further evidence for the one-ring : one day hypothesis, suggesting that daily otolith growth increments are a widespread phenomenon among young tropical teleost species.

Obtaining accurate age estimates from young coral reef fishes has proven to be valuable to the understanding of their earlier biology. Important biological and demographic parameters can be ascertained by using otolith growth increments. However, the results obtained are only tentative until the periodicity of the increments can be validated.

Since these validation experiments were carried out in artificial laboratory conditions, it can only be assumed that otolith increment periodicity which has been observed in the species of this study are similar to what occurs in the natural environment. Furthermore, the size range of each species validated was limited. Therefore, it is necessary to increase the sample size of validated individuals to extend age validation work beyond the scope of preliminary findings.

3.4.2 Counting procedure

Gjøsaeter *et al.* (1985) pointed out that if a reader is accurately reading the otolith, but there are larger differences in estimates between readers, the number of counters should be increased. For these species examined in this study, the difference in otolith increment counts between observers was smaller than or within 95% confidence limits of the difference between multiple counts by the same observer. Therefore, counts involving several persons may in principle appear unnecessary. However, increasing multiple counts of each otolith may be considered necessary in order to increase precision of the age estimate.

Otolith growth increment analysis promises to be the most useful method for estimating juvenile age. However, this technique is only of value when it can be assured that there is a high level of accuracy in the increment counts. My test on increment count systematically addressed the degree of variation in age estimates between both otoliths and observers. To avoid such counting bias, replicate counts should be made for each otolith to provide estimates of variance in increment number. In addition, only a limited deviation between replicate counts should be accepted if age estimates are to be considered valid.

In conclusion, daily otolith growth increments can be found in juvenile surgeonfishes and parrotfishes. These increments can be used in age estimates for juveniles. However, the accuracy of such estimates is largely dependent upon counting procedure. It is recommend that the degree of variation in increment counting should systematically be addressed before using this aging method, and that replicate counts with a standard deviation should be made for each otolith to improve the precision of age estimates for tropical coral-reef fish.

CHAPTER 4. VALIDATION OF AGEING TECHNIQUES FOR ADULT ACANTHURIDS AND SCARIDS

4.1 INTRODUCTION

Length frequency analysis, tag-recapture and direct aging from the hard anatomical structures are three basic techniques for providing information on the age and growth of fishes (Ricker, 1979). Of these, the later is the preferred approach as it is least prone to subjective interpretation and tagging artefacts (Brothers, 1982). In temperate waters, fisheries management largely relies on this technique for aging, and the otoliths have emerged as the anatomical structure most-often used (Pentilla and Dery, 1988).

Aging tropical fishes based on their hard anatomical structures has not shared the same success as that experienced in temperate regions (Pannella, 1980; Longhurst and Pauly, 1987). This has been attributed to a lack of a discernible or interpretable pattern in structures such as the otoliths, which has been related to equitable growth in the supposed seasonal environment of the tropics (Pannella, 1980; Brothers, 1982). However, observations for some species of the families Lutjanidae (Johnson, 1983; Manooch, 1987), Sciaenidae (Poinsard and Troadec, 1966), Pomacentridae (Fowler, 1990), Serranidae and Lethrinidae (Loubens, 1978; McPherson *et al.*, 1988) have indicated that annuli may occur more frequently in tropical reef taxa than was previously thought (Longhurst and Pauly, 1987).

Aging and growth works up-to-date have mainly focused on large, carnivorous commercially important species (Radtke, 1987; McPherson *et al.*, 1988; Dee and Radtke, 1989). However, many groups of herbivorous reef fishes are

important as food and intensively harvested through Asia and the tropical Pacific. Despite this importance in this context there has only been few research in regarding to their growth rates or age structures (Lou, in press). Parrotfishes (Family Scaridae) and surgeonfishes (Family Acanthuridae) are the two dominant groups of herbivorous fishes inhabiting coral reefs throughout the Indo-Pacific region. They are abundant on the Great Barrier Reef (GBR), Australia. As herbivores, they play an important role in the flow of energy through the reef ecosystem, and can modify the distinctive pattern of sessile reef organisms. They are also important food fishes throughout much of their range in Asia and the Pacific.

However, little is known of their population dynamics, and there is no reliable information on age structure, growth and mortality, which is fundamental for establishing important population dynamic parameters.

The emphasis of this chapter was to establish methods for estimating ages of adult scarids and acanthuids on the northern GBR. The objectives include:

(1) to examine the microstructure of sagittae;

(2) to validate periodicity of the otolith bands;

(3) to assess the accuracy of age estimates based on the otolith bands; and

(4) to examine scale checks in parrotfishes with comparison of the otolith bands.

4.2 MATERIALS AND METHODS

4.2.1 Specimen collection

Sagittae were extracted from individuals collected from the Lizard Island study sites (see Chapter 2) between August, 1987 and January, 1991. A total of 384,

403, 90 and 57 pairs of sagittae were examined in *S.rivulatus*, *S.schlegeli*, *C.binotatus* and *C.striatus* respectively in this chapter. The otoliths from the rest individuals were damaged during sampling by the spear.

4.2.2 Analysis of otoliths

4.2.2.1 Whole otoliths

Sagittae were weighted to the nearest 0.01 mg on a Sartorius 2004 MP microbalance and measured along 2 axes (see Fig. 4.1a) to the nearest 10 μm using a dissecting microscope (40×). These axes were otolith length (OL) and otolith width (OW). All measurements were made as close as possible through the otolith nucleus. The length and weight of each sagitta were related to the size of fish by fitting the best regression curves using a Cricket Graphic computer package. The relative growth rates (GR) of sagitta dimensions (size, weight) were calculated by the following equation:

$$GR = f'(SL)/f(SL)$$

Where f(SL) - the relationship of sagitta dimension and fish SL;

f'(SL) - the first derivative of f(SL).

4.2.2.2 Transverse section

At the beginning of this study, whole sagittae, which were ground and polished, were examined. As no clear band or increment was observed on the whole otoliths for the scarids and acanthurids, consequently, transverse otolith sections (TS) were used in all aging work in this study.

The sagitta from each fish was ground and polished to produce a thin section

as close as possible to the nucleus. The sagittae were embedded in Spurr's histological resin. One face of the resin block was then ground to a level near the nucleus, perpendicular to the long axis of the otolith. This was achieved by grinding on several grades of ebony paper (180, 600, 1200). Polishing was carried out using a wet (LECO) felt and 0.02 μm alumina powder which was attached to a rotating metal plate. The polished surface was glued to a microscope slide with crystal bond, and the block was cut near the nucleus with a low speed saw. It was then ground and polished from the opposite side to produce a polished, thin TS section between 150 to 300 μm thick.

All measurements were made using a leitz compound microscope with an eyepiece micrometer under transmitted light. Otolith radius (OR) was taken as the distance from the nucleus to the dorsal side of the sulcus for the parrotfishes (*S.rivulatus* and *S.schlegeli*), and the distance from the nucleus to the ventral side of the sulcus for the surgeonfishes (*C.binotatus* and *C.striatus*). Age in years was estimated by counting opaque bands from the nucleus to the edge along the OR (Fig. 4.1b).

All TS were read twice within at least a two month interval to examine any variation between two readings. Furthermore, fifty otoliths were randomly selected from *S.rivulatus* and *S.schlegeli* and counted by a second experienced otolith reader to examine any difference on otolith reading between readers. The Student T-pair test was used to analyse the reading results. The counting test by two readers was not applied to the otolith of surgeonfishes due to the smaller sample sizes of adult individuals.

4.2.3 Validation of periodicity of otolith growth bands

4.2.3.1 Tagging experiment

The tagging experiment was conducted between November 1989 and June 1991. Tagging sites were Granite Bluff and North Reef off Lizard Island (see Chapter 2). Forty-eight *S.schlegeli*, 18 *S.rivulatus*, 22 *C.striatus* and one *C.binotatus* were collected using fence nets and antillean fish traps. Standard and total length were measured to the nearest millimetre. Each fish was injected with tetracycline into the body cavity by means of a continuous pipetting syringe , with a dosage of 50 mg/kg. Before releasing, each fish was tagged with a yellow double barb dart tag in the back by a Monark tagging gun. Each tag was individually coded. The average time from capture to release was approximate one minute.

Due to the low recapture rate for tagged reef fishes (Randall, 1962), 8 injected individuals of *S.schlegeli* were collected and maintained at the University aquarium. The aquarium was a large concrete tank, approximately 10 m long, 5 m wide and 3 to 4 m in depth, giving a total water capacity of 150,000 litres. Water quality was maintained by pumping the water over eight algal turf panels. The tank was equipped with a hydraulic wave-making machine to simulate wave motion. The floor of the aquarium was covered with coral sand, and there were piles of coral rock for shelter.

Four *C.striatus* and 1 *S.schlegeli* were recaptured from the field by spearing between December 1990 and June 1991. Three of the 8 *S.schlegeli* in the aquarium were also sacrificed approximate one year later. The three pairs of otoliths (sagitta, lapillus and asteriscus) were removed. The characteristic yellow tetracycline fluorescence on the otoliths was identified with a Leitz microscope (40×) equipped

with fluorescent Ploemopak illuminator with a 50 watt mercury lamp. Age in year of the tetracycline marked fish was estimated by counting opaque bands from the nucleus to the outer sagittal edge on the TS. The fine increments were also counted from the innermost fluorescent band to the margin on the TS of sagittae and lapilli using the method described in Chapter 3.

4.2.3.2 Analysis of otolith marginal increments

Among the total specimens, 317 *S.schlegeli*, 286 *S.rivulatus*, 41 *C.striatus* and 76 *C.binotatus* were collected at approximate regular intervals (Tables 4.1,2). Sagittae of these fishes were used for the analysis of otolith marginal increments.

Otolith sections were examined with a binocular microscope under transmitted light (×40). Time of opaque increment formation was determined by the method described by Barger (1985). In this method, the section edge on the OR was examined to determine whether it was opaque or transparent, and the percentage of opaque otolith margin was then calculated at every sampling occasion.

Age composition was also estimated for each sample at every sampling occasion for both *S.rivulatus* and *S.schlegeli*. Due to the small number of individuals, a similar estimate was not carried out for *C.binotatus* and *C.striatus*.

4.2.4 Scale analysis

Parrotfishes have relatively largest scales. The scale checks can generally be observed macroscopically without any treatment. As no study has been carried out on the scales of parrotfishes, the scale checks were also examined from the fish aged in the otolith studies.

Table 4.1 Details of sampling for the otolith marginal analysis in surgeonfishes.

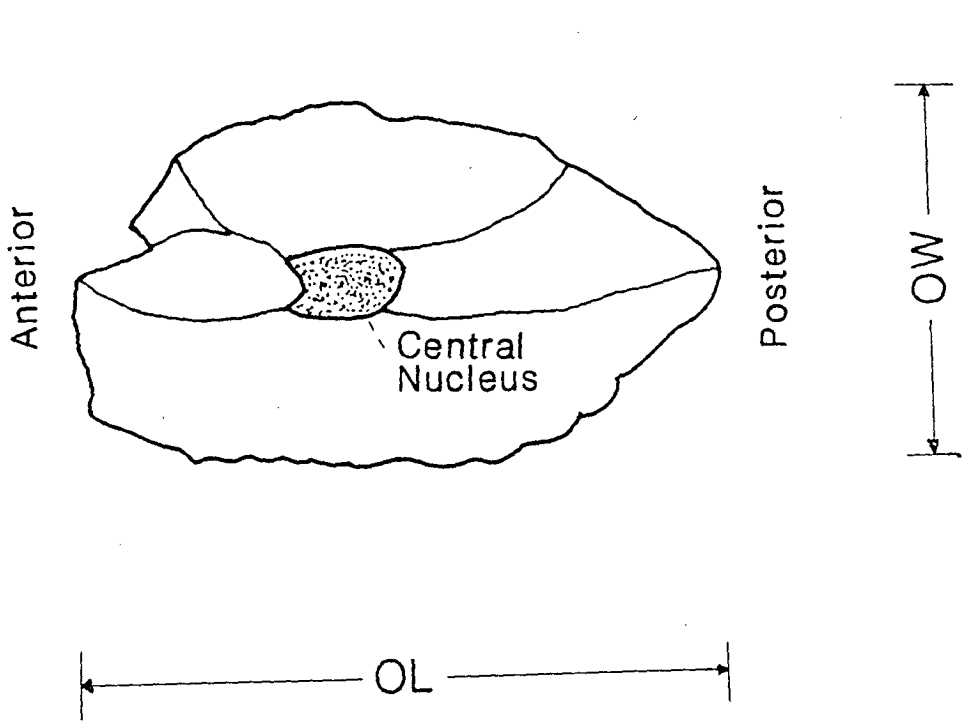
Species	Sampling time	08/87	09/87	12/87	01/88	06/88	08/88
Ctenochaetus binotatus	Sample size	9	16	27	14	10	
Ctenochaetus striatus	Sample size		26	10		7	5

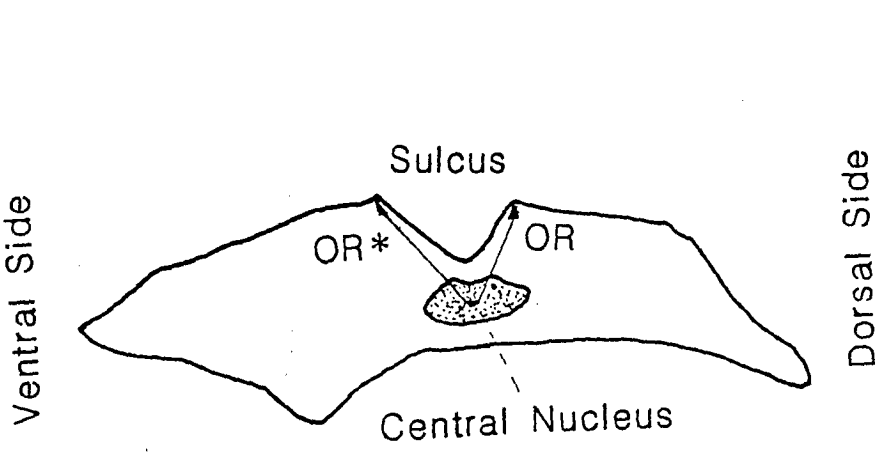
Table 4.2 Details of sampling for the otolith marginal analysis in parrotfishes.

Species	Sampling time	11/89	12/89	02/90	03/90	05/90	07/90	09/90	11/90	01/91
Scarus schlegeli	Sample size	14	67	29	38	32	42	34	39	22
Scarus rivulatus	Sample size		74	32	34	28	39	30	37	12

Fig. 4.1 Sagitta diagrams.

(a) Whole sagitta. (b) Transverse section. Schematic diagrams showing axis along which the various measurements were made. Central, dense opaque regions are stippled. OR* - Otolith radius for surgeonfishes.


a
Anterior
Central
Nucleus
Posterior
OW
OL


b
Sulcus
Ventral Side
OR*
OR
Dorsal Side
Central Nucleus

Ten homologous scales were taken from an area beneath the lateral line and either above, or directly beneath, the posterior half of the pectoral fin where possible. This method helps to limit the variation in size and shape that is encountered if scales are taken from different areas of a fish's body (Paul, 1968). Scales were stored in labelled plastic bags.

Before examination, scales were washed in freshwater, and all tissue was teased away with a brush. Scales were then placed between two microscope slides, and observed with a Nikon-6C projector (10×) under transmitted light. For some unclear scales, a drop of glycerin was added to improve the clarity (Munro, 1983). The radius of each scale was measured from the nucleus to the anterior margin. The clearest scale was chosen for each individual fish. Age in years was estimated as the number of checks which extended most of the way around the scale from the nucleus to the margin along the scale radius (SR). Age estimates from scales were then compared with the age estimates from the sagittae used in the analysis of otolith marginal increments.

4.3 RESULTS

4.3.1 Analysis of otoliths

4.3.1.1 Whole sagittae

The relationship between OL (otolith length) and OW (otolith width), and SL could be best described by a second-degree polynomial for *C.binotatus*, *C.striatus*, *S.rivulatus* and *S.schlegeli*, although a linear regression fitted the data (OL and OW) well. The weight of sagittae (OWT) increased exponentially with SL for all four species (Tables 4.3, 4.4, 4.5, 4.6; Fig.s 4.2, 4.3, 4.4, 4.5). The relative growth rate

(GR) of OL and OW decreased with increasing SL while the GR of OWT was consistent with increasing SL for the all four species (Fig. 4.6).

4.3.1.2 Transverse sections

The OR increased exponentially with SL for *C.binotatus*, *C.striatus*, *S.rivulatus* and *S.schlegeli* (Tables 4.3, 4.4, 4.5, 4.6; Fig.s 4.2, 4.3, 4.4, 4.5). The RG of OR appeared to be consistently related to SL for all four species (Fig. 4.6). This relationship indicated that the otolith radius grew at a regular rate even if the growth of fish slowed down.

When TS was viewed under transmitted light each had a central opaque region surrounded by a translucent region. Following this translucent region was alternating sequences of thin opaque bands separated by wider translucent area. These opaque bands were present in all TS of large specimens (SL > 60 mm in the surgeonfishes; SL > 102 mm in the parrotfishes). In *C.binotatus*, some had no band while other had up to 10 (Plate 4.1). A maximum of sixteen bands were found in *C.striatus* (Plate 4.2). Both species of parrotfishes were found to have a maximum of eight opaque bands (Plates 4.3, 4.4). Within each species, the number of opaque bands generally increased with SL.

Otolith sections of 150 to 300 μm thickness were the clearest and least ambiguous to interpret using transmitted light. In such sections, opaque bands were thin relative to the clear regions between them and occasionally a band could be followed from the ventral to the dorsal tips. Generally, however, the bands were easier to discern only in the dorsal areas side of the sulcus in parrotfishes, and the ventral side of the sulcus in surgeonfishes. Therefore, band count was primarily

Table 4.3 *Ctenochaetus striatus*. **Comparison between otolith dimensions and fish length (SL).**

For regression analysis, SL was used as independent variable. All regressions were significant at $p < 0.001$. Correlation coefficient of the linear regressions between otolith dimensions and SL (r_l) were also provided for comparing to the other regressions.

Otolith dimension	df	F	p	Equation	r^2	r_l^2
OWT (mg)	55	617	<0.001	$OWT = 2.012 \times 10^{0.00774SL}$	0.918	0.760
OL (mm)	54	532	<0.001	$OL = 0.732 + 0.0448SL - 8.66 \times 10^{-5}SL^2$	0.951	0.938
OW (mm)	54	525	<0.001	$OW = 0.664 + 0.0225SL - 2.16 \times 10^{-5}SL^2$	0.951	0.949
OR (μm)	55	1021	<0.001	$OR = 129.122 \times 10^{0.00522SL}$	0.948	0.883

Table 4.4 *Ctenochaetus binotatus*. **Comparison between otolith dimensions and fish length (SL).**

For regression analysis, SL was used as independent variable. All regressions were significant at $p<0.001$. Correlation coefficient of the linear regressions between otolith dimensions and SL (r_l) were also provided for comparing to the other regressions.

Otolith dimension	df	F	p	Equation	r^2	r_l^2
OWT (mg)	88	2206	<0.001	$OWT=1.532\times10^{0.0098SL}$	0.962	0.856
OL (mm)	87	1229	<0.001	$OL=0.590+0.0516SL-1.18\times10^{-4}SL^2$	0.966	0.957
OW (mm)	87	1624	<0.001	$OW=0.6385+0.0227SL-7.24\times10^{-6}SL^2$	0.974	0.972
OR (μm)	88	1590	<0.001	$OR=144.012\times10^{0.00575SL}$	0.947	0.862

Table 4.5 *Scarus rivulatus*. **Comparison between otolith dimensions and fish length (SL).**

For regression analysis, SL was used as independent variable. All regressions were significant at $p<0.001$. Correlation coefficient of the linear regressions between otolith dimensions and SL (r_l) were also provided for comparing to the other regressions.

Otolith dimension	df	F	p	Equation	r^2	r_l^2
OWT (mg)	382	3101	<0.001	$OWT=0.8918\times10^{0.00609SL}$	0.890	0.780
OL (mm)	381	2059	<0.001	$OL=0.657+0.0313SL-3.1\times10^{-5}SL^2$	0.915	0.904
OW (mm)	381	974	<0.001	$OW=0.474+0.0170SL-1.7\times10^{-5}SL^2$	0.837	0.826
OR (μm)	382	1648	<0.001	$OR=104.328\times10^{0.00296SL}$	0.812	0.727

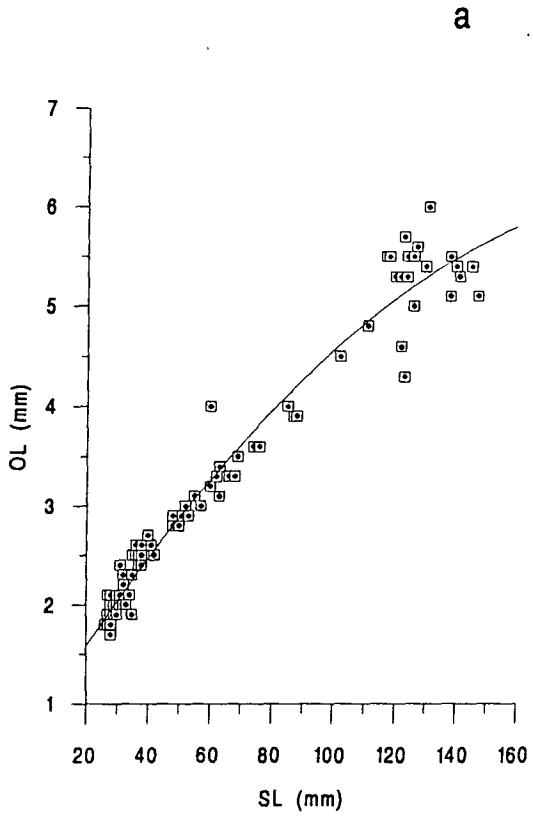
Table 4.6 *Scarus schlegeli*. **Comparison between otolith dimensions and fish length (SL).**

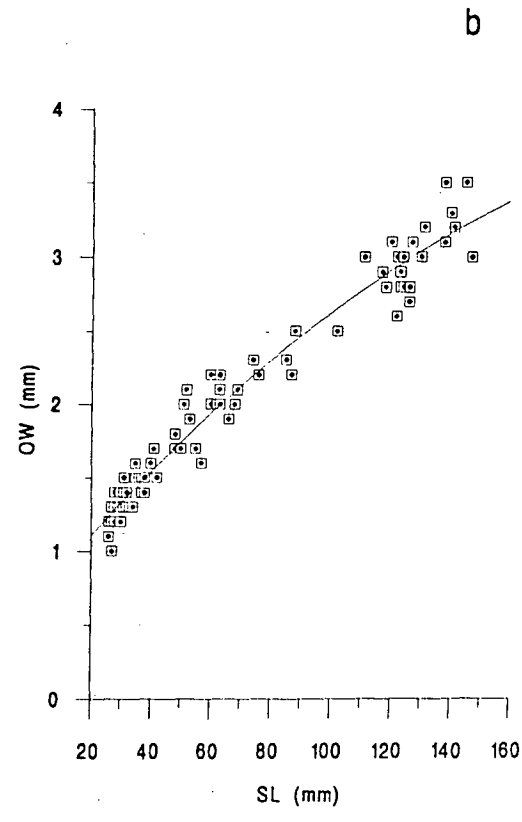
For regression analysis, SL was used as independent variable. All regressions were significant at $p < 0.001$. Correlation coefficient of the linear regressions between otolith dimensions and SL (r_l) were also provided for comparing to the other regressions.

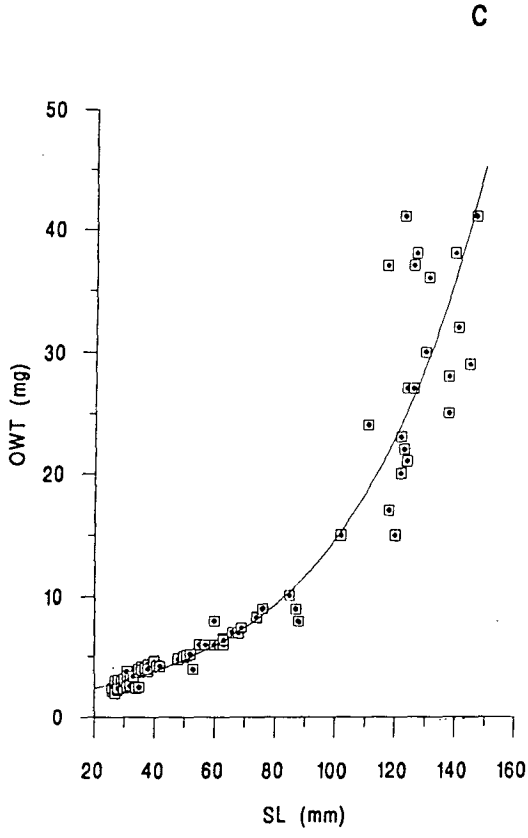
Otolith dimension	df	F	p	Equation	r^2	r_l^2
OWT (mg)	401	3999	<0.001	$OWT = 1.078 \times 10^{0.00613SL}$	0.909	0.747
OL (mm)	400	1748	<0.001	$OL = 0.373 + 0.0377SL - 4.92 \times 10^{-5}SL^2$	0.897	0.880
OW (mm)	400	1120	<0.001	$OW = 0.3864 + 0.0184SL - 1.77 \times 10^{-5}SL^2$	0.848	0.841
OR (μm)	401	2194	<0.001	$OR = 92.768 \times 10^{0.00321SL}$	0.845	0.741

Fig. 4.2 *Ctenochaetus binotatus*. **Relationships between otolith dimensions and fish length (SL).**

(a) Sagitta length (OL); (b) Sagitta width (OW); (c) Sagitta weight (OWT) and (d) Sagitta radius (OR).


a
OL (mm)
SL (mm)
1
2
3
4
5
6
7
20
40
60
80
100
120
140
160


b
OW (mm)
SL (mm)
0
1
2
3
4
20
40
60
80
100
120
140
160


c
OWT (mg)
SL (mm)
0
10
20
30
40
50
20
40
60
80
100
120
140
160

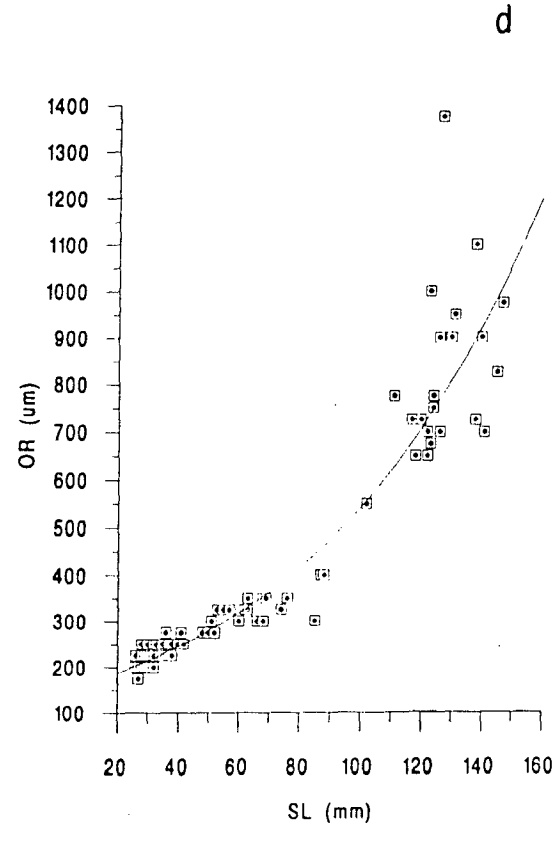
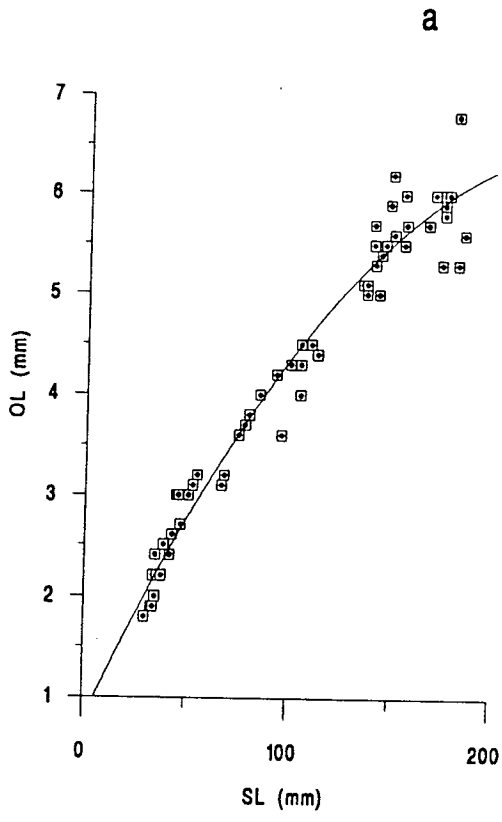
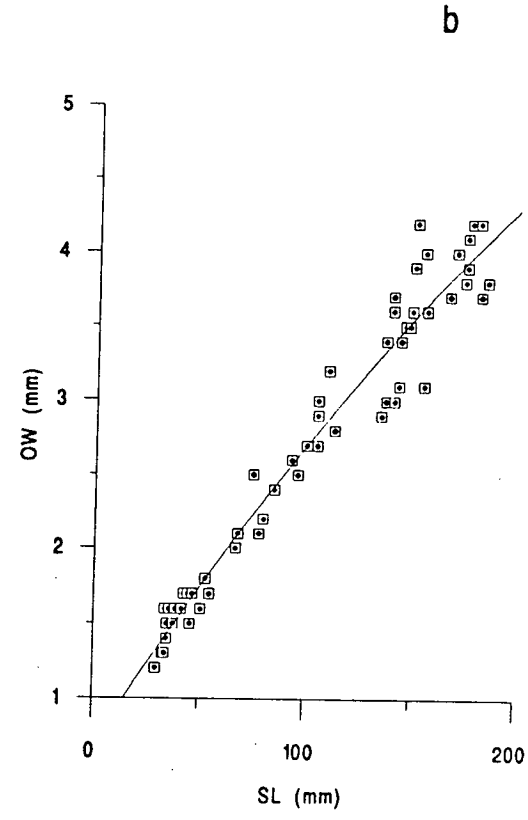
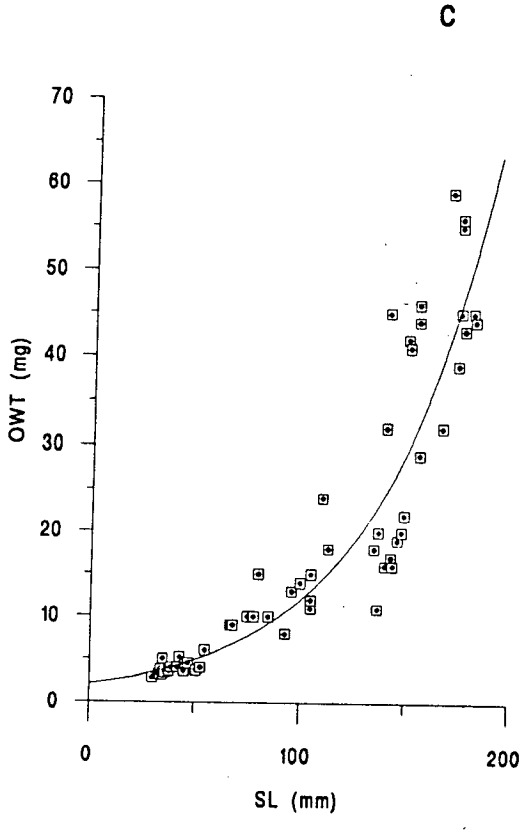

d
OR (um)
SL (mm)
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
20
40
60
80
100
120
140
160

Fig. 4.3 *Ctenochaetus striatus*. **Relationships between otolith dimensions and fish length (SL).**

(a) Sagitta length (OL); (b) Sagitta width (OW); (c) Sagitta weight (OWT) and (d) Sagitta radius (OR).


a
OL (mm)
SL (mm)
1
2
3
4
5
6
7
0
100
200


b
OW (mm)
SL (mm)
1
2
3
4
5
0
100
200


c
OWT (mg)
SL (mm)
0
10
20
30
40
50
60
70
0
100
200

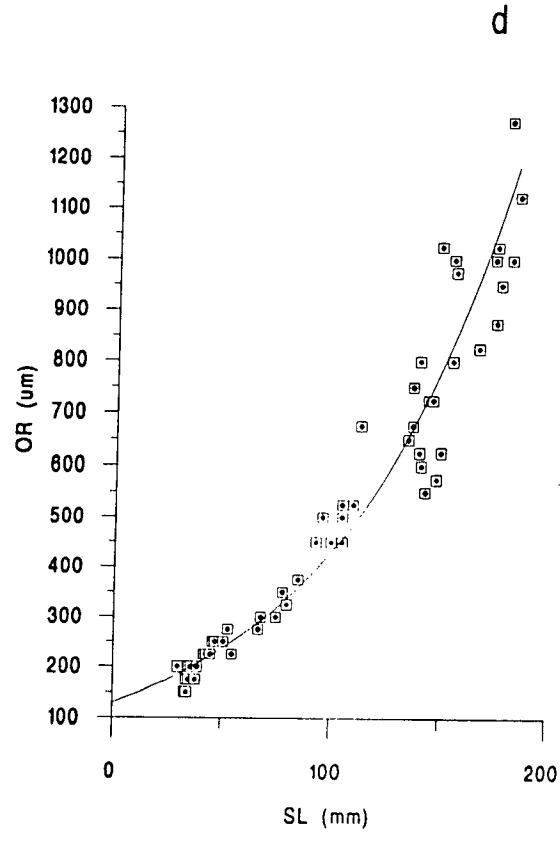
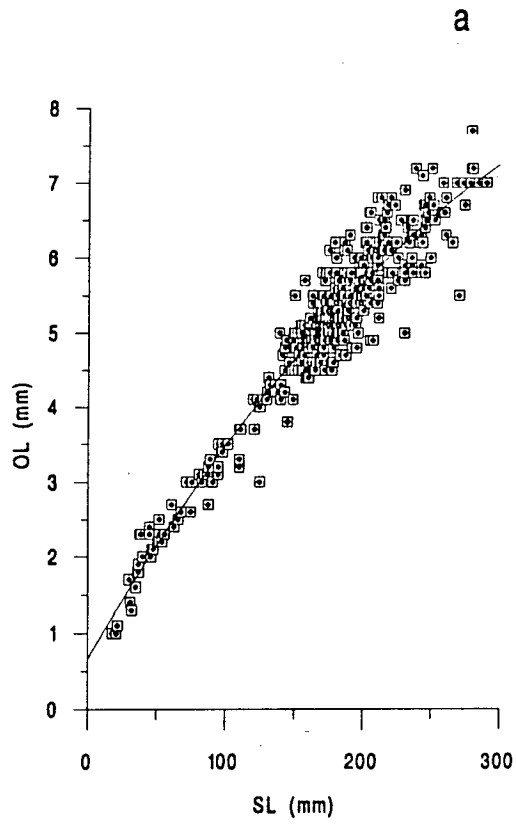
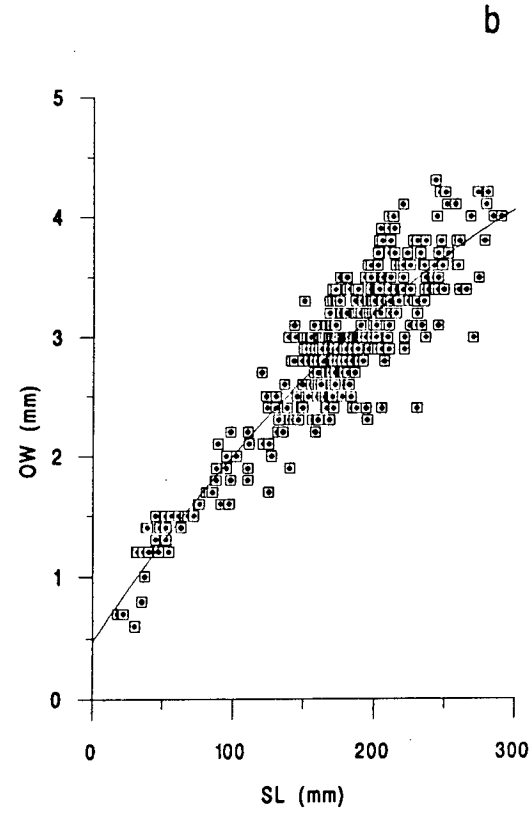
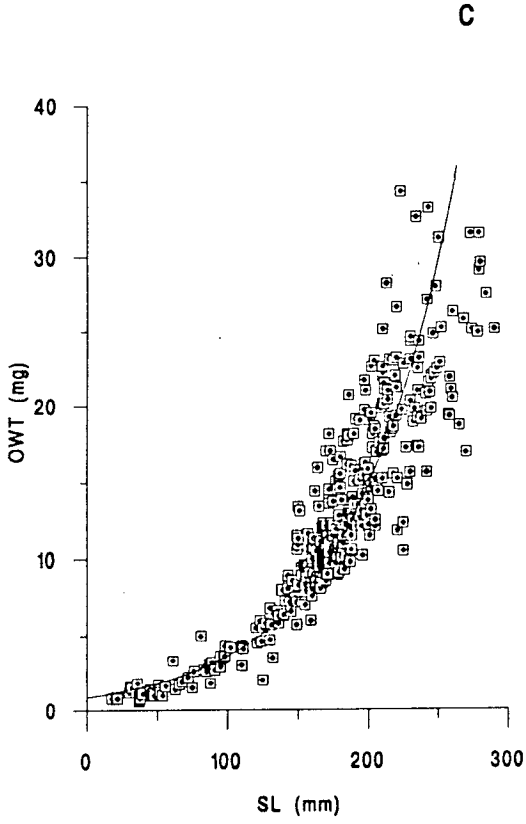

d
OR (um)
SL (mm)
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
0
100
200

Fig. 4.4 *Scarus rivulatus*. **Relationships between otolith dimensions and fish length (SL).** (a) Sagitta length (OL); (b) Sagitta width (OW); (c) Sagitta weight(OWT) and (d) Sagitta radius sagitta (OR).


a
OL (mm)
SL (mm)
0
1
2
3
4
5
6
7
8
0
100
200
300


b
OW (mm)
SL (mm)
0
1
2
3
4
5
0
100
200
300


c
OWT (mg)
SL (mm)
0
10
20
30
40
0
100
200
300

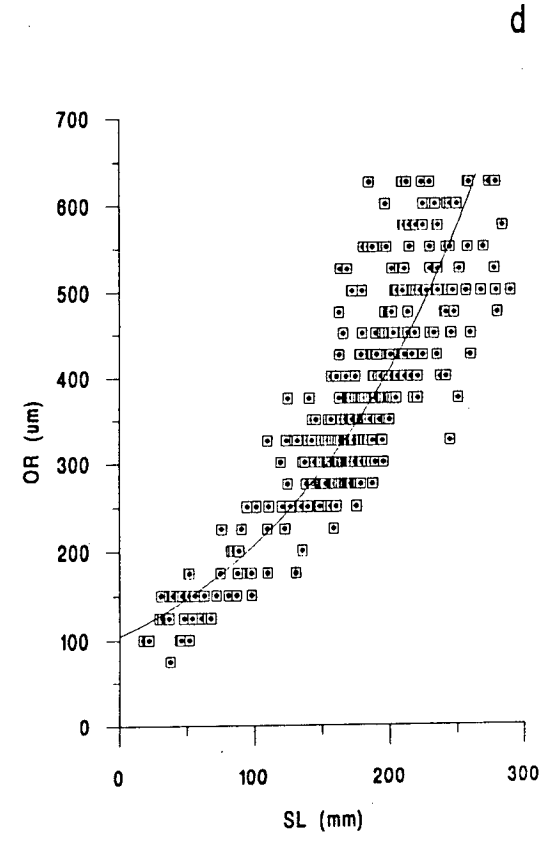

d
OR (um)
SL (mm)
0
100
200
300
400
500
600
700
0
100
200
300

Fig. 4.5 *Scarus schlegeli*. **Relationships between otolith dimensions and fish length (SL).**

(a) Sagitta length (OL); (b) Sagitta width (OW); (c) Sagitta weight (OWT) and (d) Sagitta radius (OR).

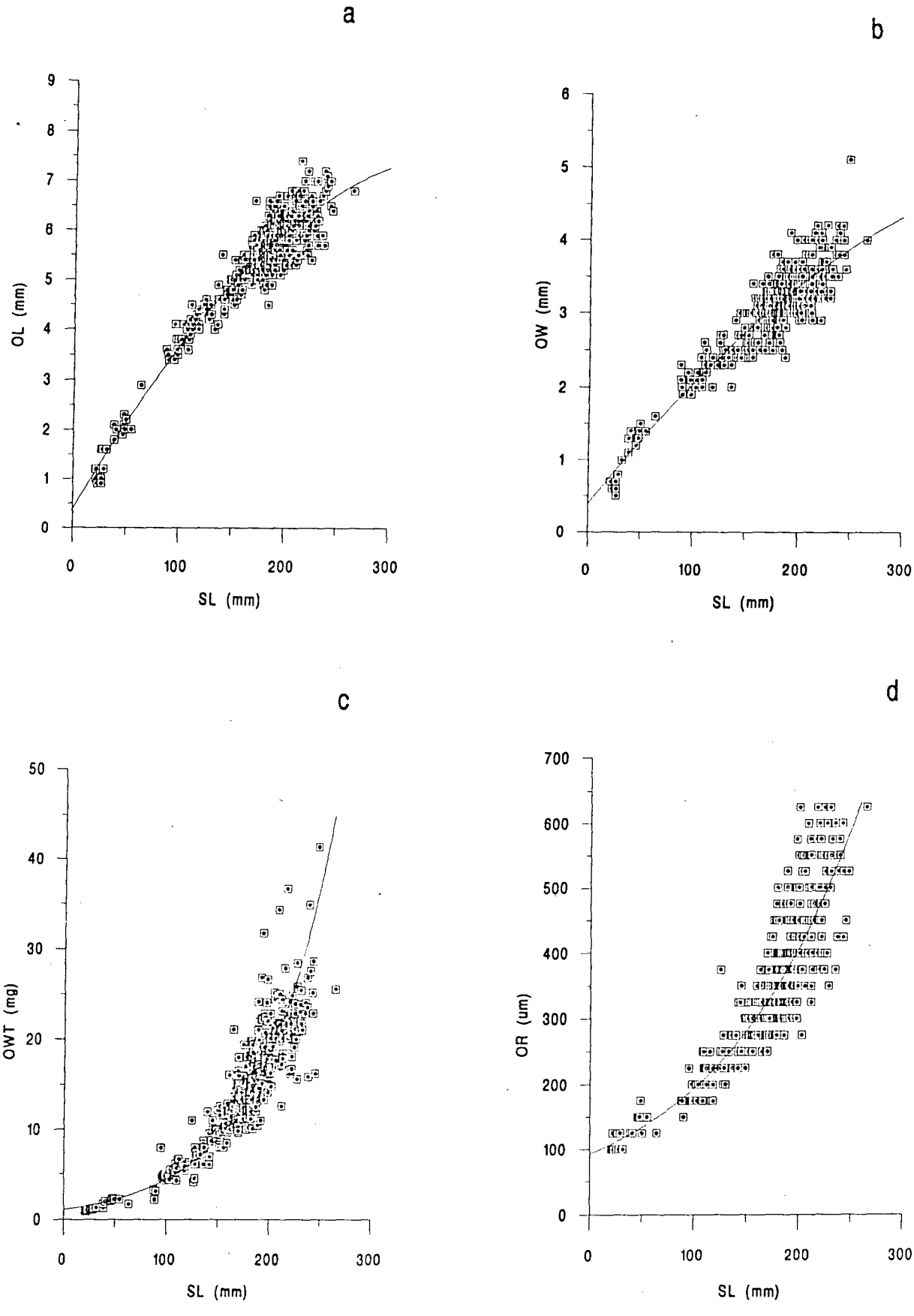
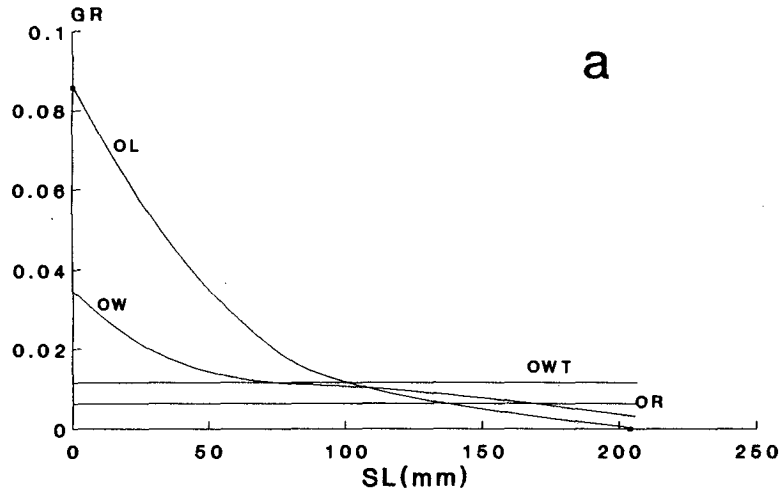
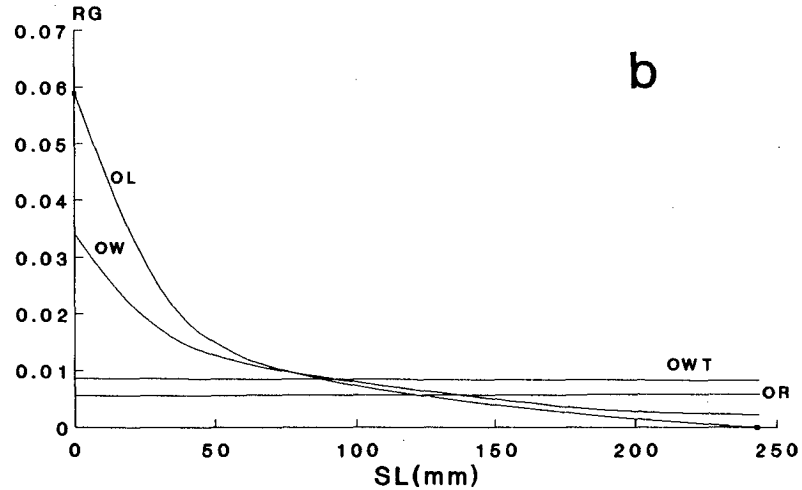
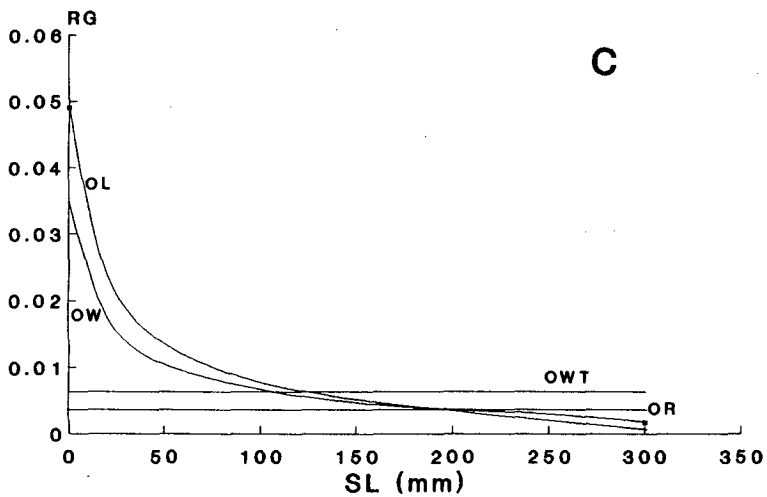

a
OL (mm)
SL (mm)
b
OW (mm)
SL (mm)
c
OWT (mg)
SL (mm)
d
OR (um)
SL (mm)

Fig. 4.6 Relationships between fish length (SL) and relative growth rates of otolith dimensions. OL - sagitta length, OW - sagitta width, OWT - sagitta weight and OR - sagitta radius. (a) *Ctenochaetus binotatus*. (b) *Ctenochaetus striatus*. (c) *Scarus rivulatus*. (d) *Scarus schlegeli*.


GR
a
OL
OW
OWT
OR
0.1
0.08
0.06
0.04
0.02
0
0
50
100
150
200
250
SL(mm)


RG
b
OL
OW
OWT
OR
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
50
100
150
200
250
SL(mm)


RG
c
OL
OW
OWT
OR
0.06
0.05
0.04
0.03
0.02
0.01
0
0
50
100
150
200
250
300
350
SL (mm)

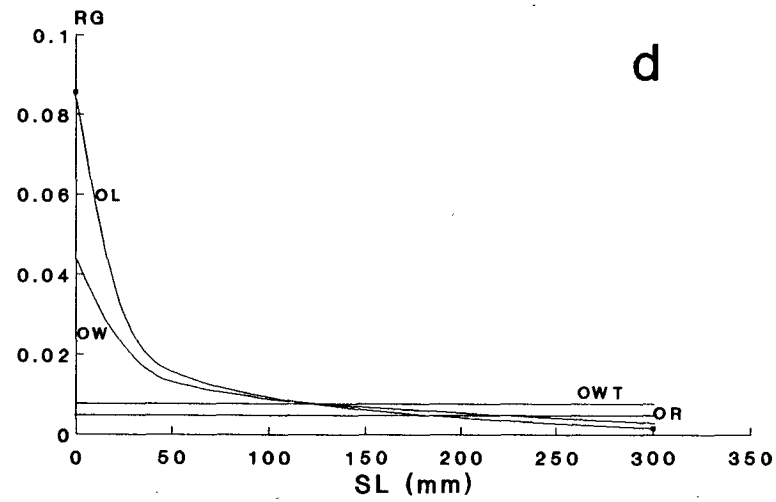

RG
d
OL
OW
OWT
OR
0.1
0.08
0.06
0.04
0.02
0
0
50
100
150
200
250
300
350
SL (mm)

Plate 4.1 *Ctenochaetus binotatus*. **Otolith transverse sections with the various number of bands.**

Bands are indicated by white triangles. All scale bar = 1 mm. (a) One-band-fish (SL=63 mm). (b) Two-band-fish (SL=88 mm). (c) Five-band-fish (SL=138 mm). (d) Nine-band-fish (SL=140 mm). (e) Ten-band-fish (SL=145 mm).

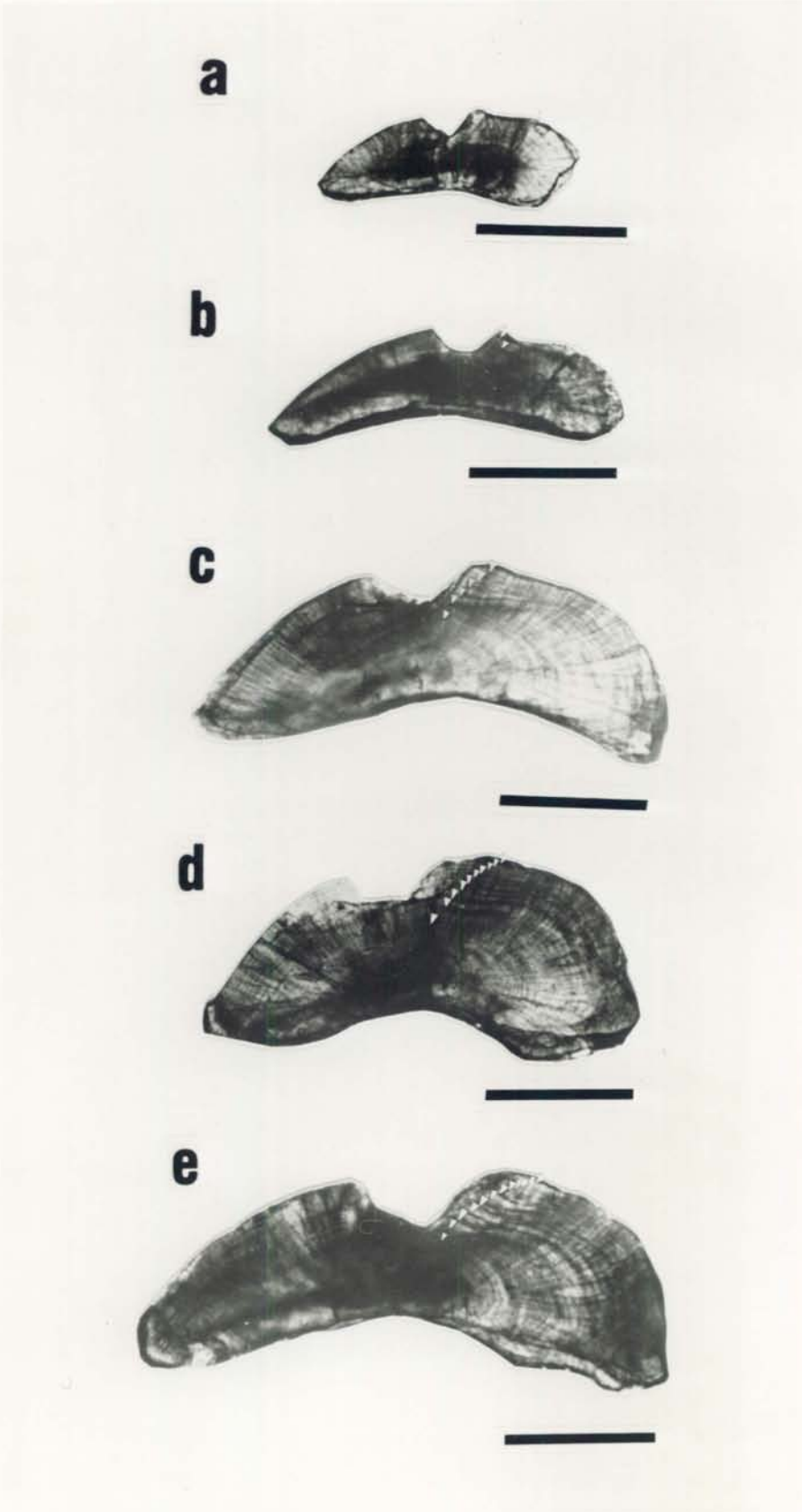

a
b
c
d
e

Plate 4.2 *Ctenochaetus striatus*. **Otolith transverse sections with the various number of bands.**

Bands are indicated by white triangles. All scale bar = 1 mm. (a) One-band-fish (SL=90 mm). (b) Two-band-fish (SL=125 mm). (c) Three-band-fish (SL=143 mm). (d) Four-band-fish (SL=135 mm). (e) Eight-band-fish (SL=154 mm).

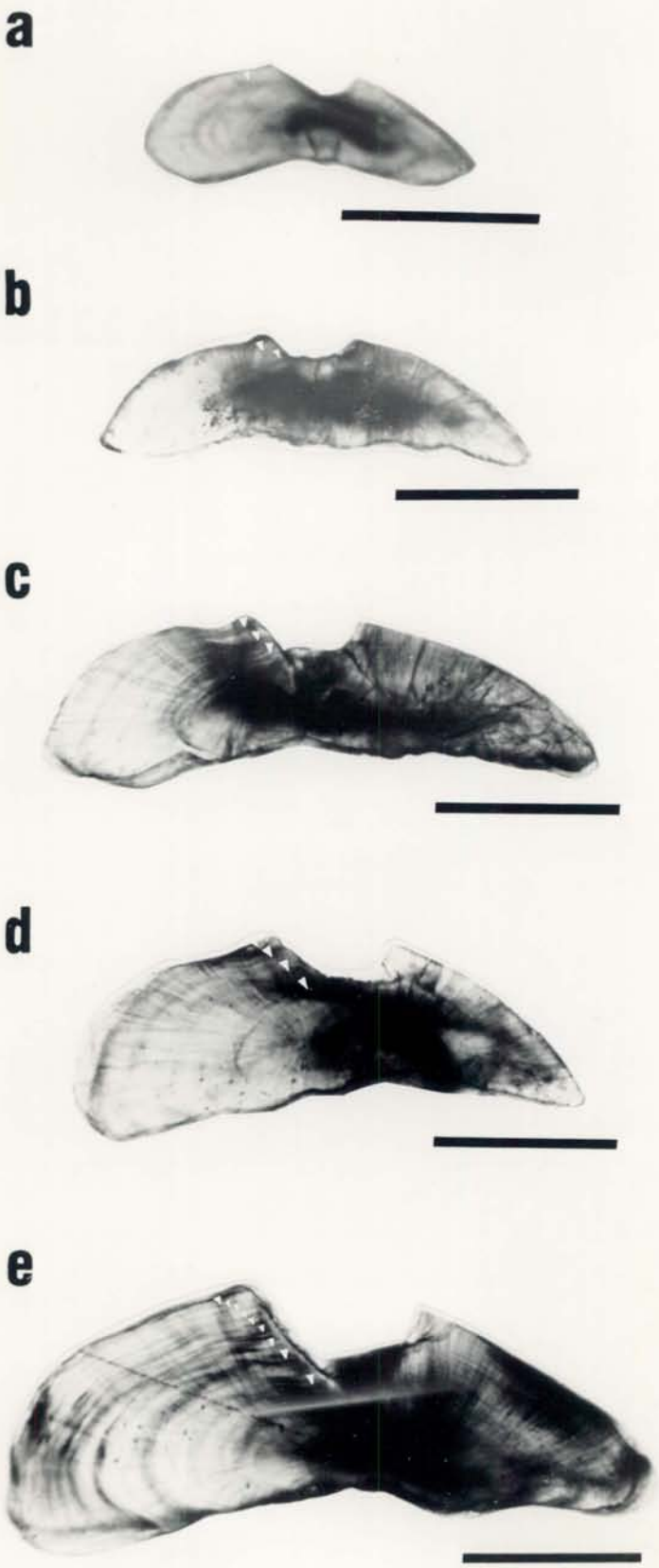

a
b
c
d
e

Plate 4.3 *Scarus rivulatus*. **Otolith transverse sections with the various number of bands.**

Bands are indicated by white triangles. All scale bar = 1 mm. (a) Zero-band-fish (SL=98 mm). (b) One-band-fish (SL=150 mm). (c) Two-band-fish (SL=166 mm). (d) Three-band-fish (SL=185 mm). (e) Four-band-fish (SL=247 mm).

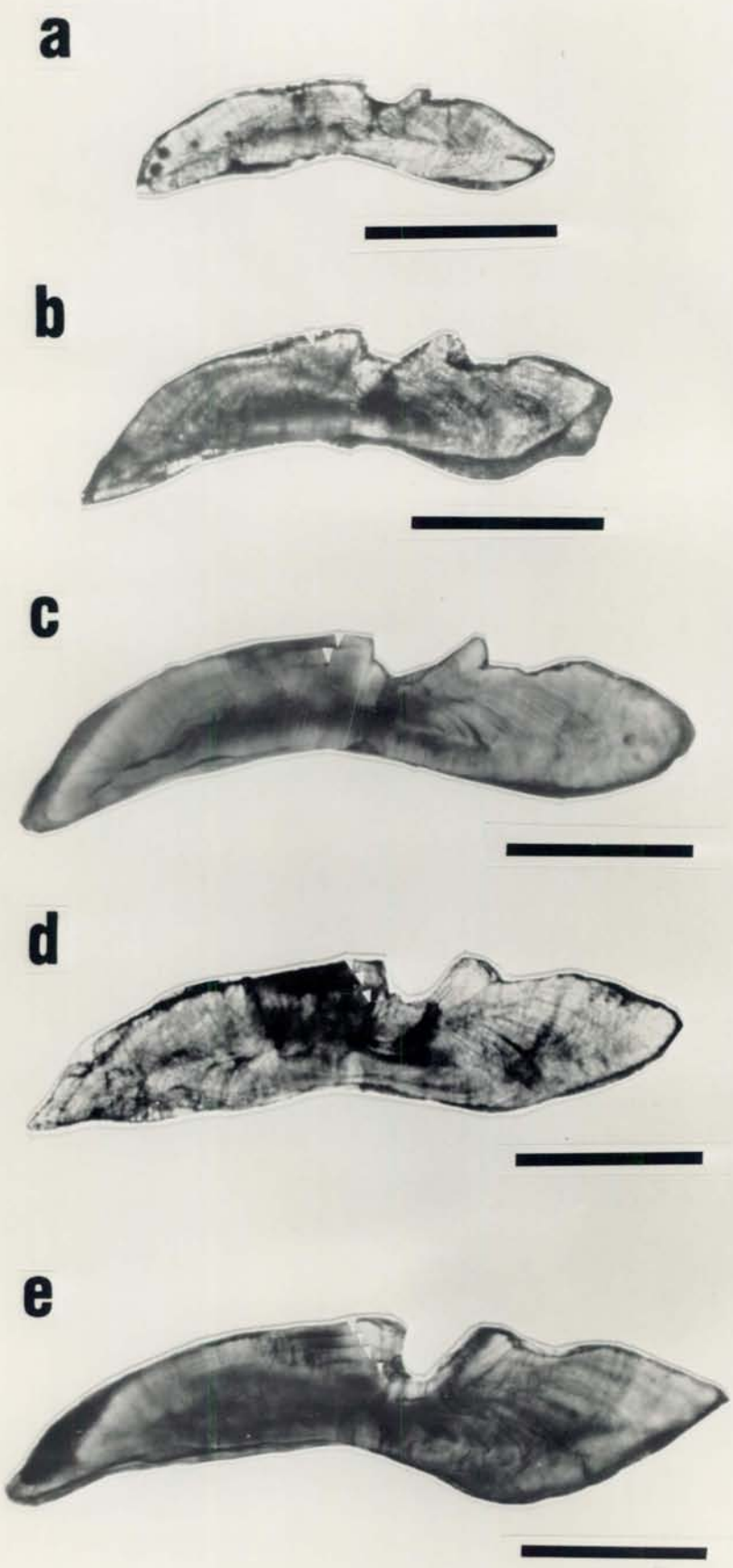

a
b
c
d
e

Plate 4.3 Cont.

(f) Five-band-fish (SL=249 mm). (g) Six-band-fish (SL=214 mm). (h). Seven-band-fish (SL=243 mm). (i) Eight-band-fish (SL=245 mm).

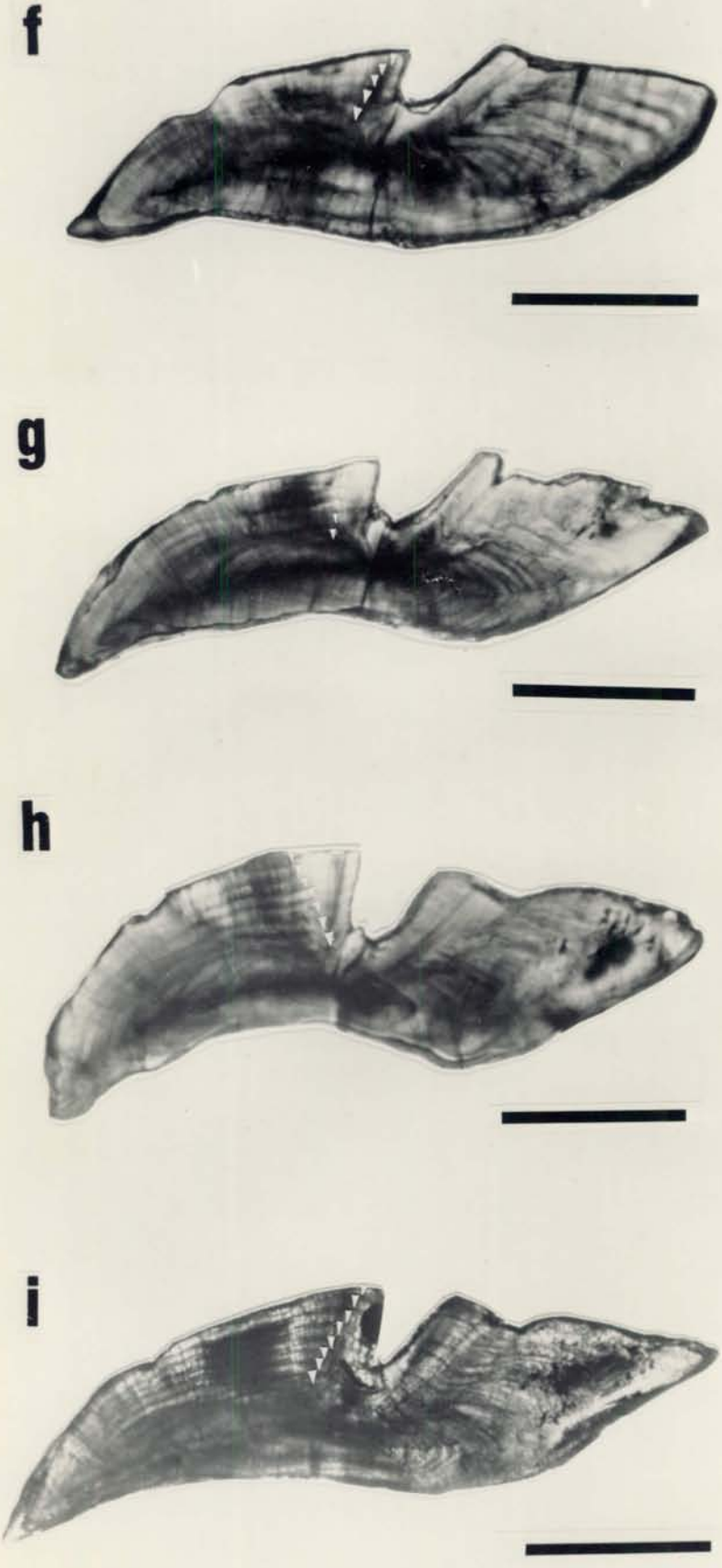

f
g
h
i

Plate 4.4 *Scarus schlegeli*. **Otolith transverse sections with the various number of bands.**

Bands are indicated by white triangles. All scale bar = 1 mm. (a) Zero-band-fish (SL=118 mm). (b) One-band-fish (SL=125 mm). (c) Two-band-fish (SL=166 mm). (d) Three-band-fish (SL=195 mm). (e) Four-band-fish (SL=210 mm).

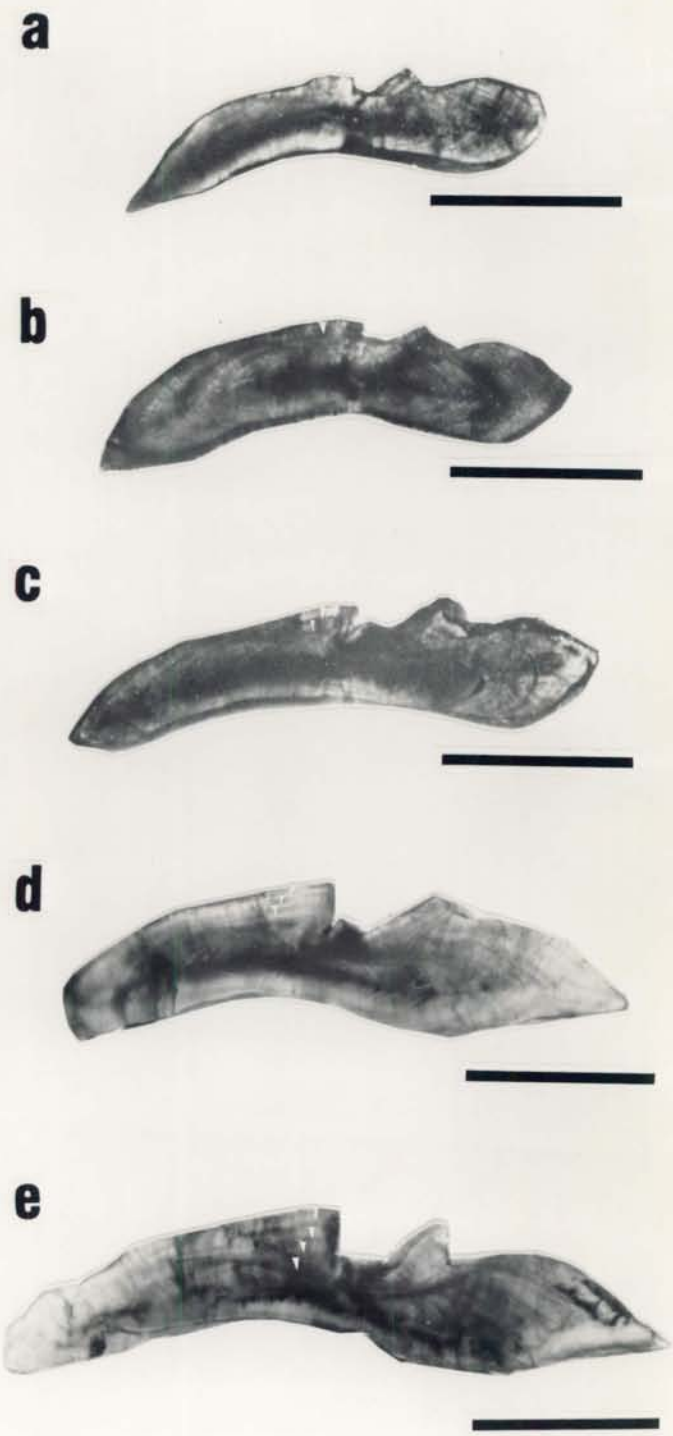
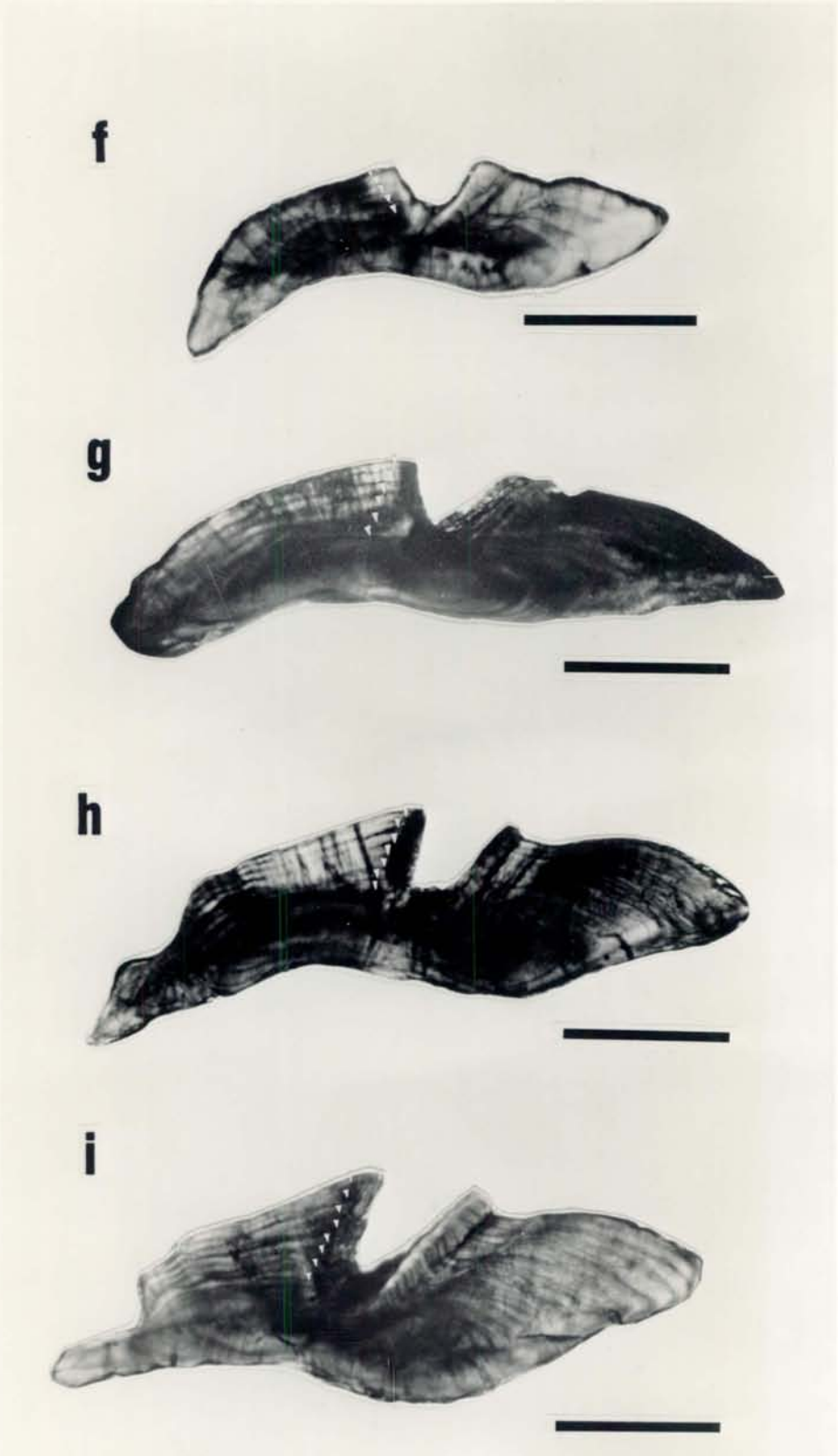

a
b
c
d
e

Plate 4.4 Cont.

(f) Five-band-fish (SL=211 mm). (g) Six-band-fish (SL=227 mm). (h) Seven-band-fish (SL=209 mm). (i) Eight-band-fish (SL=248 mm).


f
g
h
i

made along the OR.

In surgeonfishes, 2 of 90 *C.binotatus* sagittae counted on two occasions were considered unreadable on one or both occasions. For the remainder, counts ranged between 0 and 12, while the differences between counts of the same otolith ranged between 0 to 2. For 85% of the otoliths the 2 counts were the same, for 8.9% they differed by 1 and for 2.2% they differed by >1. In *C.binotatus*, one of 57 sagittae counted twice was unreadable. Counts of the remainder ranged from 0 to 16 while the differences between counts of the same otolith ranged between 0 and 2. The 86% of the otoliths showed the same number of bands, 7% had different number of the bands by 1 and the remainder had different number of the bands by 2 (Table 4.7).

In parrotfishes, 5 of 384 *S.rivulatus* sagittae were considered unreadable on one or both occasions. For the remainder, the differences between counts of the same otolith ranged from 0 to 2. For 88% of the otoliths the 2 counts were the same, for 9% they differed by 1 and for 1% they differed by >1. Of 403 sagittae in *S.schlegeli*, 3 were considered unreadable on both occasions, 373 (92%) showed the same number of bands on both occasions, 27 (7%) had different number of bands by 1 and the rest had different number of bands by 2 (Table 4.8).

In the counting test by two readers, counts ranged between 0 and 8, while the differences between readers ranged between 0 and 2 for both *S.rivulatus* and *S.schlegeli* (Tables 4.9, 4.10). In *S.rivulatus*, 74% of the otolith counts were the same, 22% differed by 1 and the rest differed by >1. The overall difference on the counts between two readers was no significant (t_{49}=-0.50 p=0.62). For 70% of the otolith in *S.schlegeli* the counts of two readers are same, for 26% they differed by 1 and for 4% they differed by 2. The overall difference on counts between two

readers was also no significant ($t_{49}=1.09$, $p=0.28$).

4.3.2 Validation of the periodicity of band formation

4.3.2.1 Tagging recapture

The TS of tag-recaptured *C.striatus* showed a strong fluorescent band medial to the edge, in addition to the weak fluorescence at the edge (Plate 4.5a). On the transverse sections of sagittae of *C.striatus* recaptured in December 1990 there was a narrow opaque band following by a wider translucent band between the fluorescent mark and the edge under transmitted light (Plate 4.5b). Two opaque bands were found beyond the fluorescent mark on the sagitta of one *S.schlegeli* and *C.striatus*, which were tagged in November, 1989 and recaptured in February and June, 1991 respectively. The opaque band between fluorescent mark and the margin, therefore, appeared to form between November to February the following year. Sagittae of the three *S.schlegeli* maintained in the University aquarium also had a similar opaque band between tetracycline fluorescence and the otolith margin (Plate 4.5c,d).

Growth rate and length-at-age data were determined for the recaptured specimens based on the otolith bands (Table 4.11). Among the recaptured specimens, parrotfishes grew fastest with 47 mm over 453 days in a 3-band *S.schlegeli*. The surgeonfish had the slowest growth, with 3 mm over 574 days in an 12-band, 1 mm over 398 days in an 8-band *C.striatus*, no growth over 396 and 398 days in a 12 and 13-band *C.striatus*. *S.schlegeli* kept in the aquarium showed approximately similar growth to that in the field with possible up to 36, 40 and 69 mm over 297 and 431 days in three 2-band *S.schlegeli*.

Apparent daily increments were observed in the otoliths (sagittae, lapilli and

asterisci) of all recaptured specimens. However, the fine increments in the acanthurids were too thin and crowded to count in the otolith margin. Consequently, only the otoliths of *S.schlegeli* were counted. The periodicity of these fine increments was much less than daily when compared with the days at liberty (Table 4.12).

4.3.2.2 Analysis of otolith marginal increments

The plots of marginal appearance indicated that a single peak of opaque margins occurred each year for *S.rivulatus* and *S.schlegeli*. Although weakened by the limited samples, the results in *C.binotatus* and *C.striatus* were also close to that of the scarids in terms of the overall trend.

In *S.rivulatus* only 2.56% of otoliths had opaque margins in July, but 93.75% and 85.29% of them did in February and March. The percentage with opaque margins gradually increased from September, reached a peak around January and February and then gradually decreased until May (Fig. 4.7). A similar pattern was found in the otoliths of *S.schlegeli*. No otolith had opaque margins in July, but 96.55% and 75.67% of them did in February and March (Fig. 4.8). These patterns suggest that a single annulus forms each year though it may occur any time from November to May for individual fish.

No otolith in *C.binotatus* had opaque margins in August, but 92.85% of them did in January. The percentage of opaque margins appeared to increase gradually from September throughout January, and it was uncertain in the following months. However, the percentage in June was low (Fig. 4.9). There was a small number of samples available for *C.striatus*. But the overall trend of otolith marginal appearance appeared to be close to that of the other three pecies (Fig. 4.10). For the specimens

Table 4.7 Comparison of two counts on otolith bands from surgeonfishes.

Difference refers to the deviation of the two counts (-ve indicates the second count lower than the first count, +ve indicates the second count higher than the first count).

Species	*C.binotatus*					*C.striatus*					
First Count	N	Difference -2	-1	0	1	N	Difference -2	-1	0	1	2
0	60			56	4	15			14	1	
1	6		1	5		8		2	6		
2	1			1		4			4		
3	3			3		4			3	1	
4	6			5	1	4			4		
5	2			1	1	3			3		
6	1			1		3			3		
7						1			1		
8	2			2		3			3		
9	3		1	2		1			1		
10	3	1		2		1			1		
11						1			1		
12	1	1				1			1		
13						3			2		1
15						1	1				
16						3			2		1

Table 4.8 Comparison of two counts of the otolith bands from parrotfishes.

Difference refers to the deviation of the two counts (-ve indicates the second count lower than the first count, +ve indicates the second count higher than the first count).

Species	*S.rivulatus*							*S.schlegeli*						
First count	N	Difference						N	Difference					
		-2	-1	0	1	2			-2	-1	0	1	2	
0	35			24	11			22			22			
1	19		9	7	3			41		9	31	1		
2	107		4	102		1		75			71	4		
3	78	1	1	75	1			143		3	137	3		
4	64		2	62				74		4	68	2		
5	41	2	2	37				33			33			
6	24		2	22				9	1	1	7			
7	9	1		8				2			2			
8	2		1	1				1			1			

Table 4.9 Results of otolith band counting between two readers in *Scarus rivulatus*.

N	Author count	2nd Reader Mean count (±s.d.)	Difference from author				
			-2	-1	0	+1	+2
1	0	0.0 (0.00)			1		
5	1	1.0 (0.63)		1	3	1	
9	2	2.0 (0.47)		1	7	1	
18	3	3.2 (0.60)		1	14	2	1
8	4	3.9 (0.59)		2	5	1	
4	5	4.8 (0.43)		1	3		
3	6	6.3 (0.47)			2	1	
1	7	7.0 (0.00)			1		
1	8	8.0 (0.00)			1		

Table 4.10 Results of otolith band counting between two readers in *Scarus schlegeli*.

N	Author count	2nd Reader Mean count (±s.d.)	Difference from author				
			-2	-1	0	+1	+2
1	0	0.0 (0.00)			1		
5	1	1.0 (0.63)		1	3	1	
8	2	2.2 (0.82)		1	5	1	1
24	3	3.1 (0.59)		2	18	3	1
4	4	4.0 (0.70)		1	2	1	
4	5	5.2 (0.43)			3	1	
2	6	5.5 (0.50)		1	1		
1	7	7.0 (0.00)			1		
1	8	8.0 (0.00)			1		

Table 4.11 Results of tag-recapture experiments.

AQ - Fish kept in the University aquarium.

Species	TL (mm)	Tag No	Days at liberty	Growth at liberty	Bands formed at liberty	No. of Bands
Ctenochaetus striatus	215	1567	398	1 (mm)	1	8
Ctenochaetus striatus	249	1565	398	0	1	13
Ctenochaetus striatus	214	1480	574	3 (mm)	2	12
Ctenochaetus striatus	240	1588	396	0	1	12
Scarus schlegeli	220	1386	453	47 (mm)	2	3
Scarus schlegeli	160	AQ	297	7 - 40 (mm)	1	2
Scarus schlegeli	156	AQ	297	3 - 36 (mm)	1	2
Scarus schlegeli	182	AQ	431	29 - 69 (mm)	1	2

Table 4.12 The results of counts on the fine increments in otoliths of tag-recaptured *Scarus schlegeli*.

AQ - fish kept in the aquarium of the University.

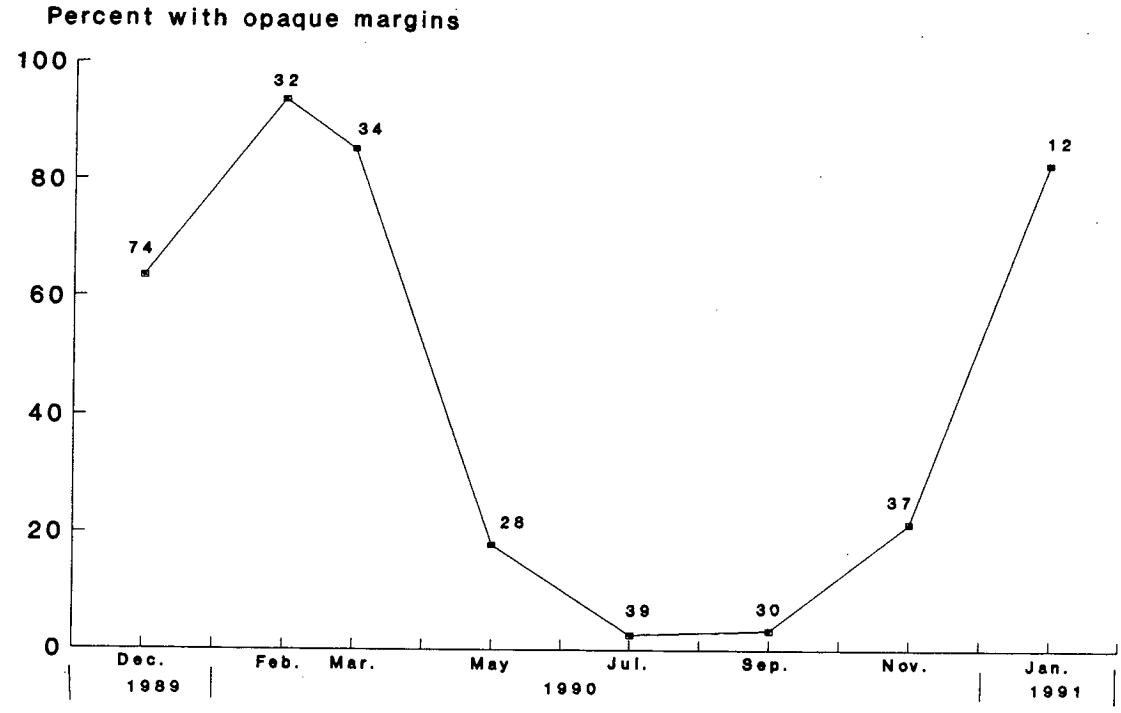
TL (mm)	Tag No	Days at liberty	Increments formed at liberty in sagittae	Increments formed at liberty in lapilli
220	1386	453	242	199
160	AQ	297	129	128
150	AQ	297	132	125
182	AQ	431	197	161

Fig. 4.7 *Scarus rivulatus*. **Percent of otoliths with opaque margins by each sampling occasion.**

Numbers of otoliths are indicated next to points.

Fig. 4.8 *Scarus schlegeli*. **Percent of otoliths with opaque margins by each sampling occasion.**

Numbers of otoliths are indicated next to points.


Percent with opaque margins
100
80
60
40
20
0
74
32
34
28
39
30
37
12
Dec.
1989
Feb.
Mar.
May
Jul.
Sep.
Nov.
Jan.
1990
1991

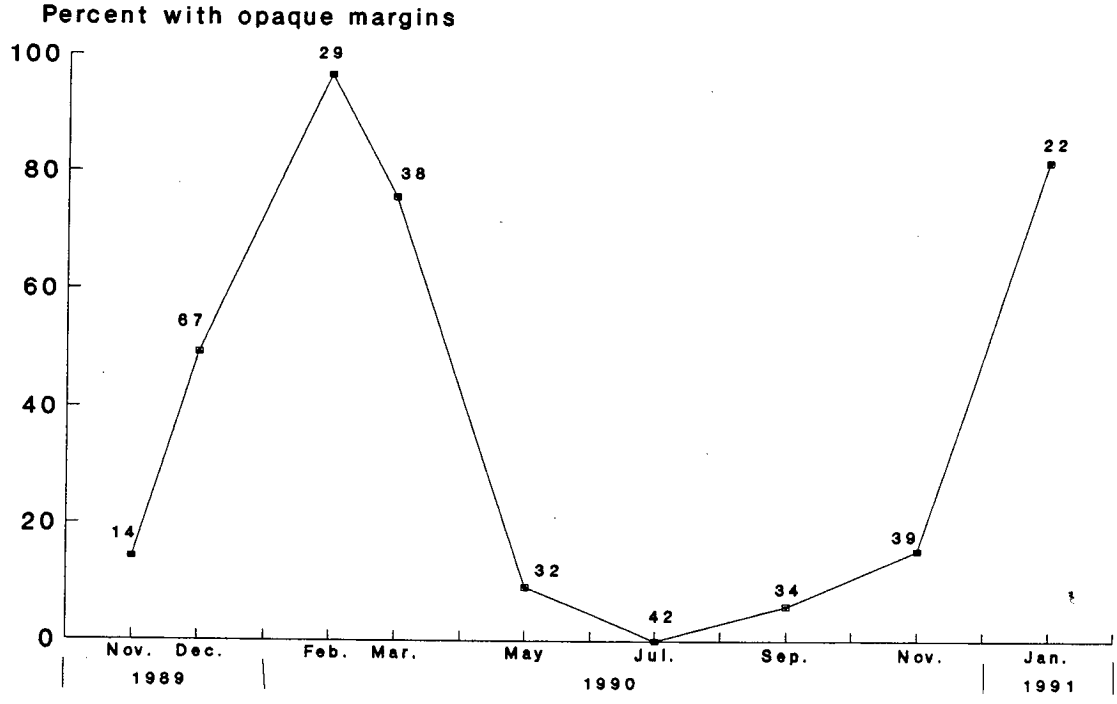
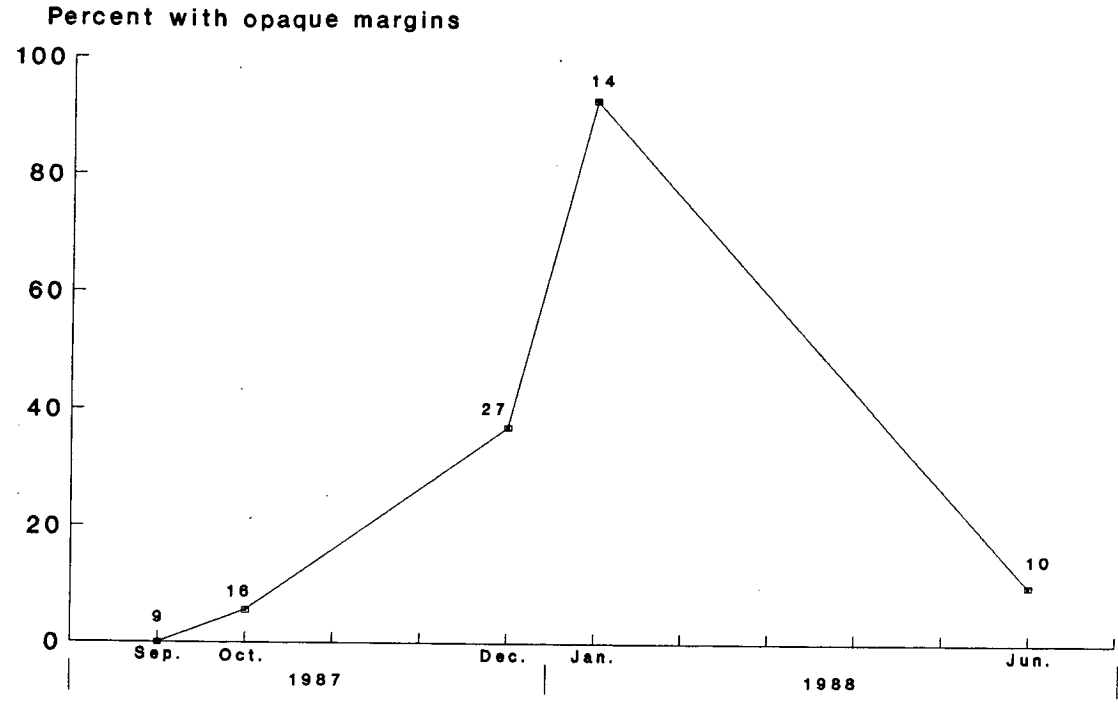

Percent with opaque margins
100
80
60
40
20
0
14
67
29
38
32
42
34
39
22
Nov.
Dec.
1989
Feb.
Mar.
May
Jul.
Sep.
Nov.
Jan.
1990
1991

Fig. 4.9 *Ctenochaetus binotatus*. **Percent of otoliths with opaque margins by each sampling occasion.**

Numbers of otoliths are indicated next to points.

Fig. 4.10 *Ctenochaetus striatus*. **Percent of otoliths with opaque margins by each sampling occasion.**

Numbers of otoliths are indicated next to points.


Percent with opaque margins
100
80
60
40
20
0
9
16
27
14
10
Sep.
Oct.
Dec.
Jan.
Jun.
1987
1988

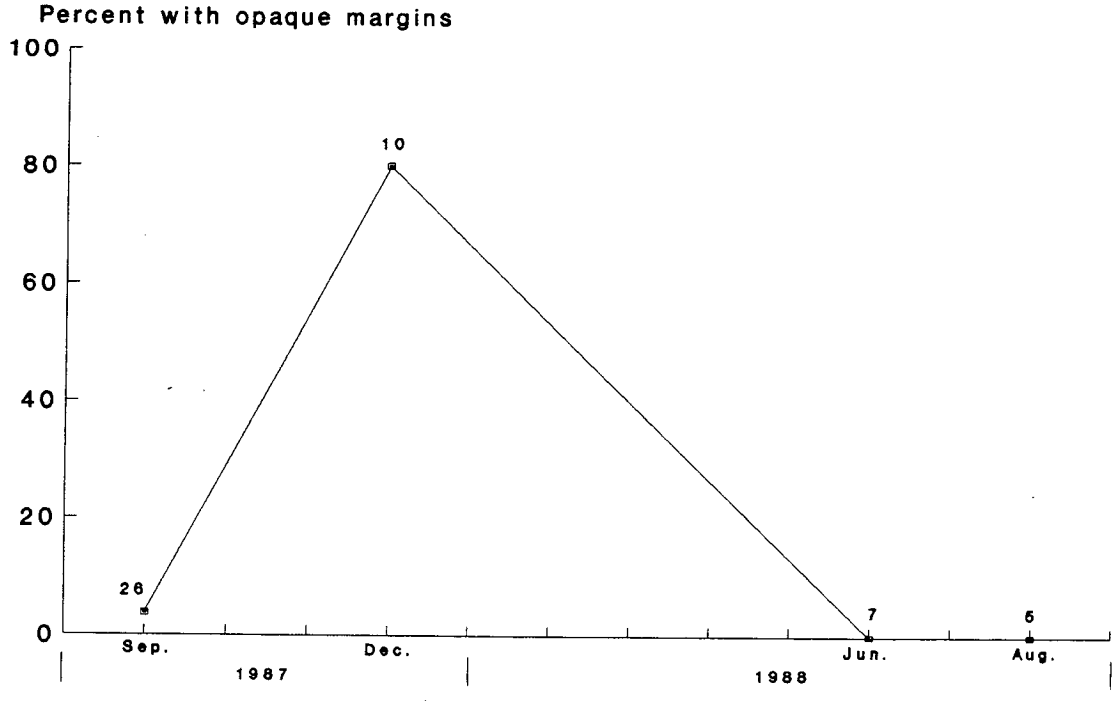
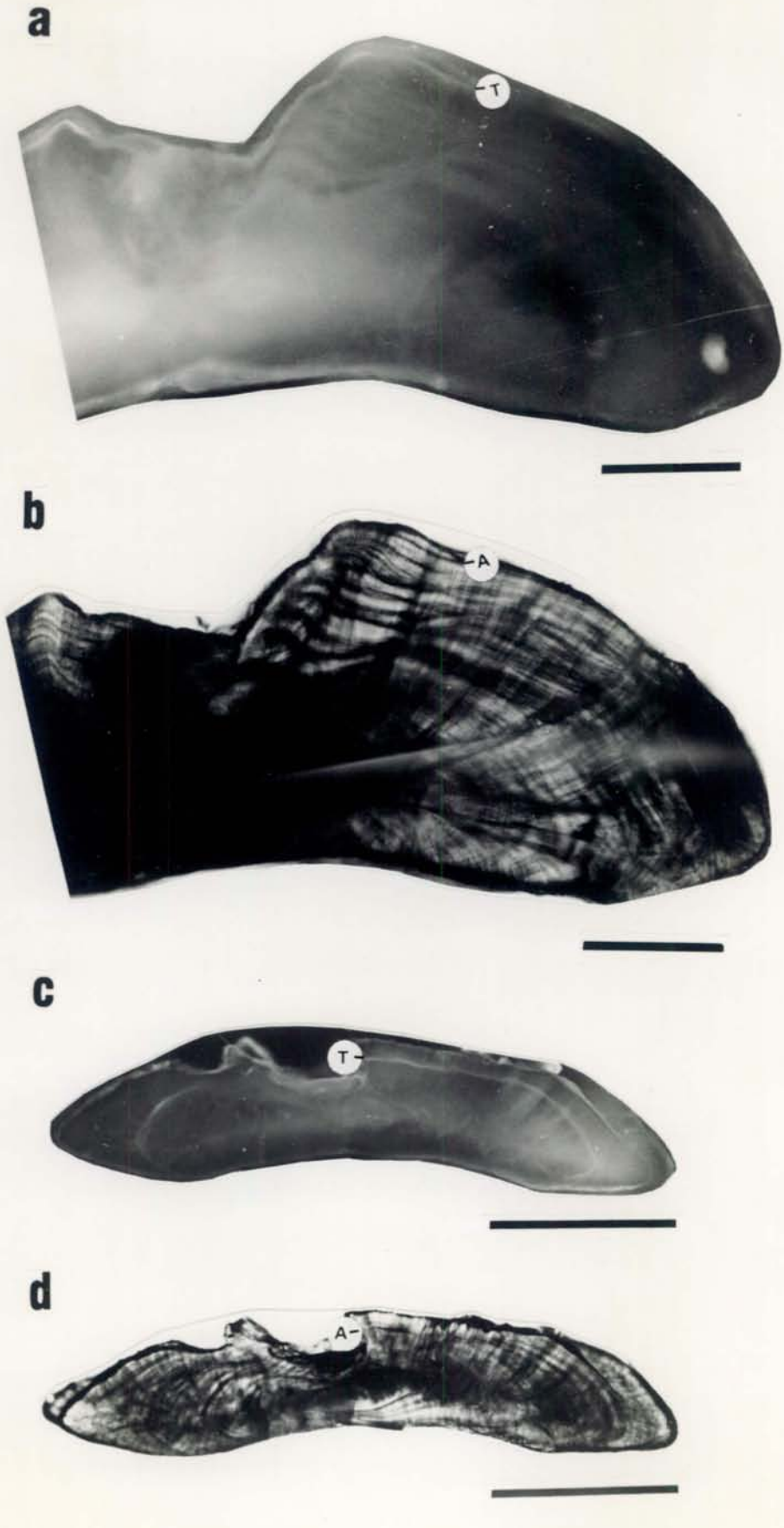

Percent with opaque margins
100
80
60
40
20
0
26
10
7
6
Sep.
Dec.
Jun.
Aug.
1987
1988

Plate 4.5 Otolith transverse sections of tag-recapture specimens from both field and the aquarium.

(a) *Ctenochaetus striatus* from field. Micrograph under fluorescent light and showing tetracycline band (T). (b). The same section of (a) under transmitted light showing one opaque band (A) between the tetracycline band and the margin. (c) *Scarus schlegeli* from the aquarium. Micrograph under fluorescent light and showing tetracycline band (T). (d). The same section of (c) under transmitted light showing one opaque band (A) between the tetracycline band and the margin. All scale bar = 750 μm.


a
T
b
A
c
T
d
A

collected at three occasions, otoliths from the summer showed the highest percentage of opaque margins.

The distribution of ages in each sample mainly contained 2, 3, 4 and 5 age classes in both *S.rivulatus* and *S.schlegeli* (Tables 4.13, 4.14). For the overall samples from the Lizard Island, the most abundant age-classes of *S.rivulatus* and *S.schlegeli* were also 2, 3, 4 and 5 year.

4.3.3 Analysis of scales

The scale radius of *S.rivulatus* and *S.schlegeli* increased with increasing SL (Fig.s 4.11a,b). The relationship for both species was linear (Table 4.15). It was not possible to sample the specific scales from the same area for all samples of both species as older individuals showed a large number of regenerated scales that characterized by opaque nuclei (Plate 4.5a). For these older specimens scales were taken from the nearby area. The proportion of regenerated scales generally increased with increasing SL.

The surface area of a non-regenerated scale in *S.rivulatus* and *S.schlegeli* has two distinct regions. The overlapped region is deeply embedded within the dermis and covered by anterior and lateral scales. It is composed of concentric circuli, spreading around the nucleus. In the anterior field these circuli are interrupted by radial grooves, or radii (Fig. 4.12). The radial grooves ranged from 20 to 45 in *S.schlegeli*, and 14 to 48 in *S.rivulatus*. Generally, numbers of the radii increased with increasing SL, but varied considerably among individuals. The exposed region of scale is covered only by a thin layer of loose dermis and epidermis, and contains only tubercles and ridges.

As the scale grows, the circuli are laid down in the anterior field at irregularly spaced intervals. the rate of scale growth varied between species, as well as between individuals. When viewed under transmitted light, larger scales displayed clear thin checks in the anterior field, which were characterised by an increase in the distance between the circuli and broken circuli (Fig. 4.12). There was an increase in circuli density just before these checks. The number of these checks ranged from zero to seven in *S.rivulatus* and zero to six in *S.schlegeli*, and generally increased with SL (Plate 4.5).

4.3.4 Comparison of otolith and scale methods

In general, age estimates from scale checks were relatively close to the age estimates from otolith annual bands for younger fishes up to 5 years in both *S.rivulatus* and *S.schlegeli* (Tables 4.16, 4.17). However, variation between age estimates from the two methods became increasing when the fishes got older. It indicates that the scale aging method underestimates the age of old fish. The overall agreement on age estimates for the specimens examined by the both methods was 80.4% and 68.1% in *S.rivulatus* and *S.schlegeli* respectively. In another 18.5% in *S.rivulatus* and 30.6% in *S.schlegeli* there was a difference of one year.

4.4 DISCUSSION

4.4.1 Otolith aging method

For otoliths to be used as an aging tool they must grow throughout the lives of fish. For *Ctenochaetus striatus*, *C.binotatus*, *Scarus rivulatus* and *S.schlegeli* OL and OW increased isometrically with SL, but growth around the lateral edges slowed

Table 4.13 *Scarus rivulatus*. **The age composition of samples used in the otolith marginal analysis.**

Age estimate was based on annual band counting.

Sampling month	N	Range of SL (mm)	Age (year)								
			0	1	2	3	4	5	6	7	8
Dec. 89	74	21 - 249	3		44	12	11	3			1
Feb. 90	32	18 - 290	5	7	5	4	4	5	2		
Mar. 90	34	31 - 279	3		6	9	7	5	3	1	
May 90	28	123 - 260		1	7	5	2	5	4	4	
Jul. 90	39	135 - 280		1	7	11	9	6	4	1	
Sep. 90	30	149 - 252			9	8	8	1	3	1	
Nov. 90	37	141 - 247			13	11	9	2	2		
Jan. 91	12	150 - 242			6	3	2	1			

Table 4.14 *Scarus schlegeli*. **The age composition of samples used in the otolith marginal analysis.**

Age estimate was based on annual band counting.

Sampling month	N	Range of SL (mm)	Age (year)								
			0	1	2	3	4	5	6	7	8
Nov. 89	14	39 - 238	1	2	5	2	2	1	1		
Dec. 89	67	22 - 265	10	11	14	22	8	1	1		
Feb. 90	29	32 - 248	2		9	13	3	1			1
Mar. 90	38	39 - 243	6	2	6	13	8	3			
May 90	32	89 - 237		4	1	15	6	4	1	1	
Jul. 90	42	99 - 242		3	6	19	7	6	1		
Sep. 90	34	97 - 243		5	3	18	5	3			
Nov. 90	39	90 - 227	2	10	5	12	7	3			
Jan. 91	22	108 - 237		1	3	6	5	6	1		

Table 4.15 Comparison between scale radius (SR) and fish length (SL).

The initial relationship refers to the nature of relationship between SR and SL. For regression analysis, SL was used as independent variable. All regressions were significant at $p<0.001$.

Species	Initial relationship	df	F	p	Equation	r^2
S.rivulatus	Linear	384	4011	<0.001	SR(mm)=0.9772+0.0493SL	0.913
S.schlegeli	Linear	386	3301	<0.001	SR(mm)=0.7192+0.0491SL	0.895

Table 4.16 *Scarus rivulatus*. **Comparison of age estimates from otoliths and scales.**

Difference refers to the deviation of age determined by otoliths from the age determined by scales (-ve indicates the scale age lower than the otolith age, +ve indicates the scale age higher than the otolith age. Variation refers to the percentage of individuals whose otolith age varied by the amounts indicated.

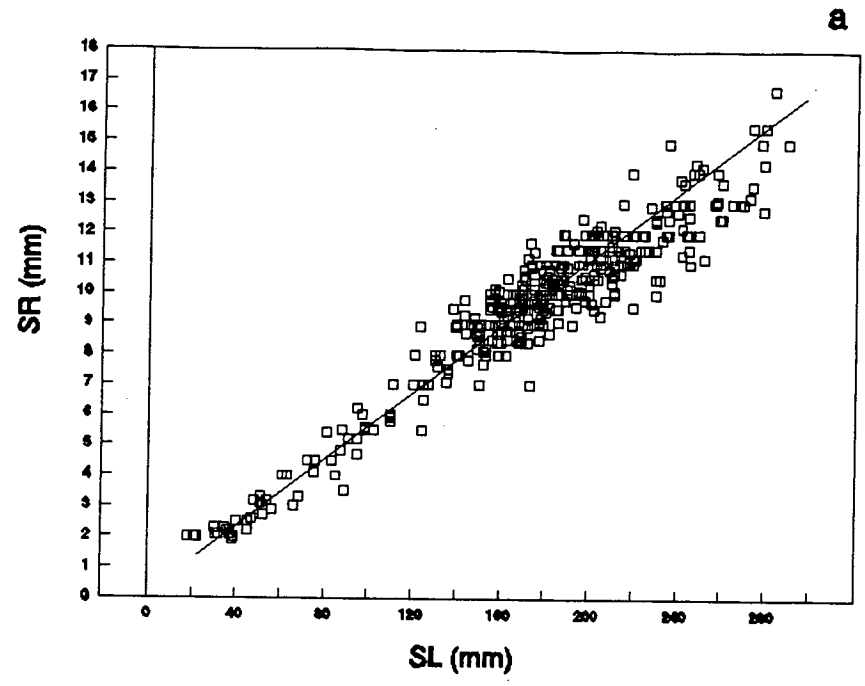
Otolith age	Difference						Total	Variation			
	-3	-2	-1	0	1	2		0	±1	±2	-3
0				11			11	100			
1			1	7	1		9	77.8	22.2		
2			1	76	20		97	78.4	21.6		
3			2	55	6		63	87.3	12.7		
4			3	47	2		52	90.4	9.6		
5			7	18	3		28	64.3	35.7		
6		1	2	15			18	83.3	11.1	5.6	
7		1	5	1			7	14.3	71.4	14.3	
8	1						1				100

Table 4.17 *Scarus schlegeli*. **Comparison of age estimates from otoliths and scales.**

Difference refers to the deviation of age determined by otoliths from the age determined by scales (-ve indicates the scale age lower than the otolith age, +ve indicates the scale age higher than the otolith age. Variation refers to the percentage of individuals whose otolith age varied by the amounts indicated.

Otolith	Difference								Variation				
age	-4	-3	-2	-1	0	1	2	Total	0	±1	±2	-3	-4
0					21			21	100				
1				5	26	7		38	68.4	31.6			
2					38	14		52	73.0	27.0			
3			1	7	96	16		120	80.0	19.2	0.8		
4				8	37	6		51	72.5	27.5			
5			1	6	19	2		28	67.8	28.6	3.6		
6					3	2		5	60.0	40.0			
7		1						1				100	
8	1							1					100

Fig. 4.11 Relationships between scale radius (SR) and fish length (SL). (a) *Scarus rivulatus*. (b) *Scarus schlegeli*.


a
SR (mm)
SL (mm)

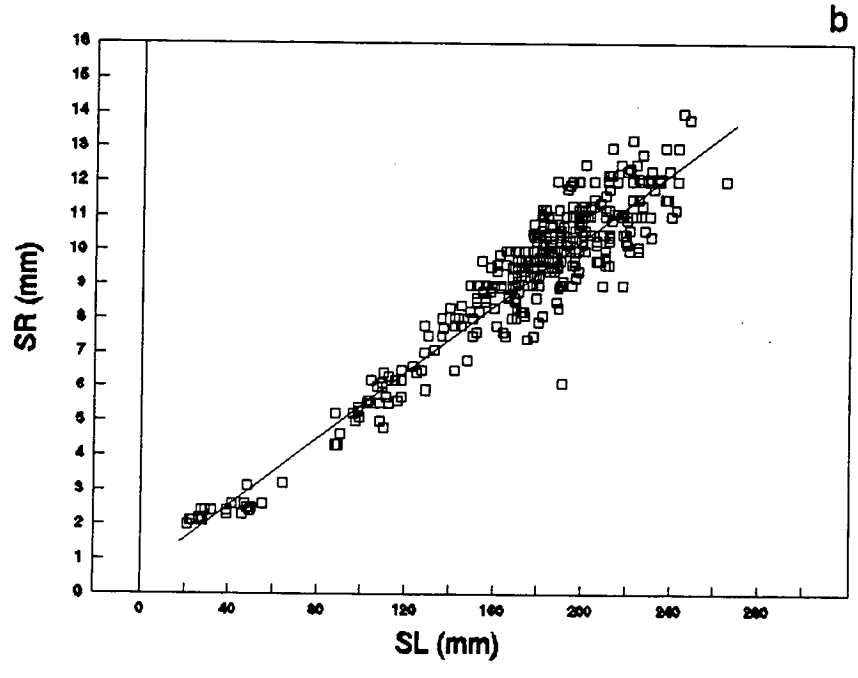

b
SR (mm)
SL (mm)

Fig. 4.12 Schematic diagram of parrotfish scale.

The diagram shows general surface structure and the structure of scale check.

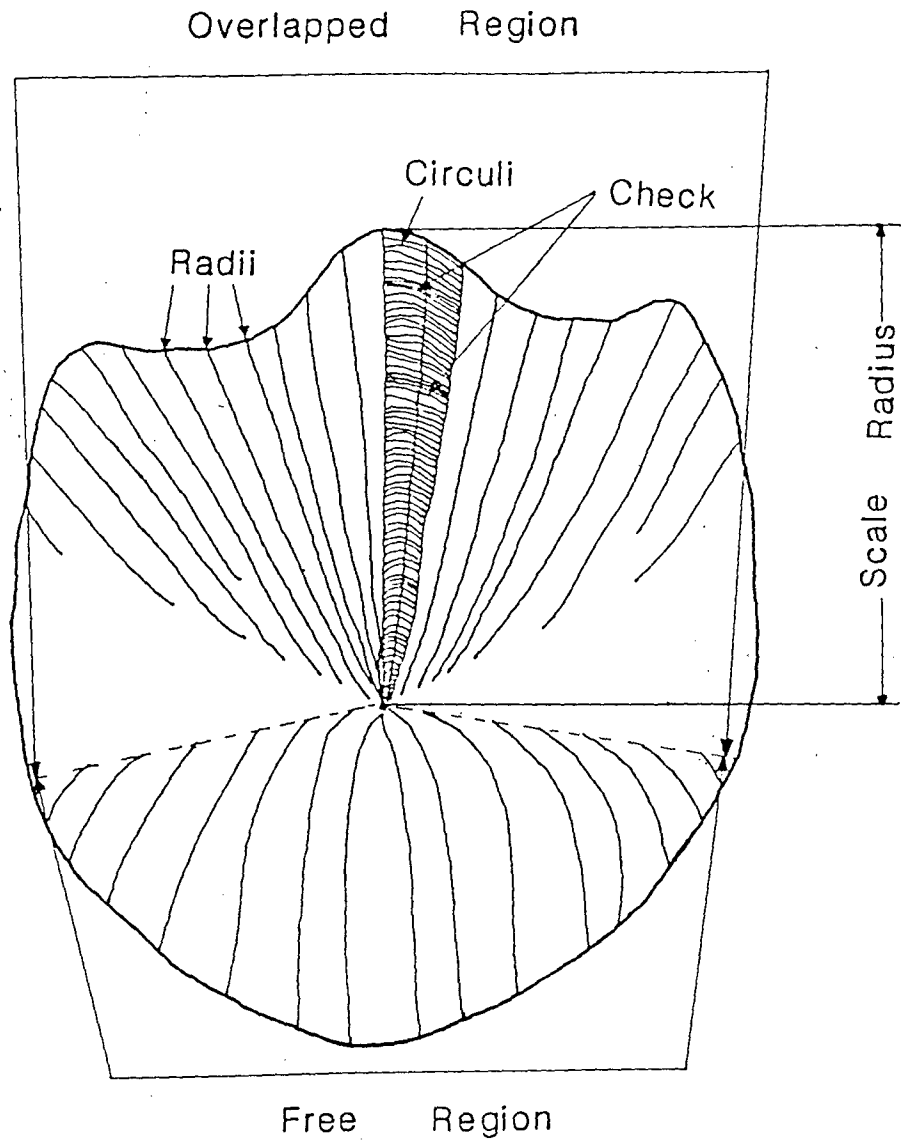
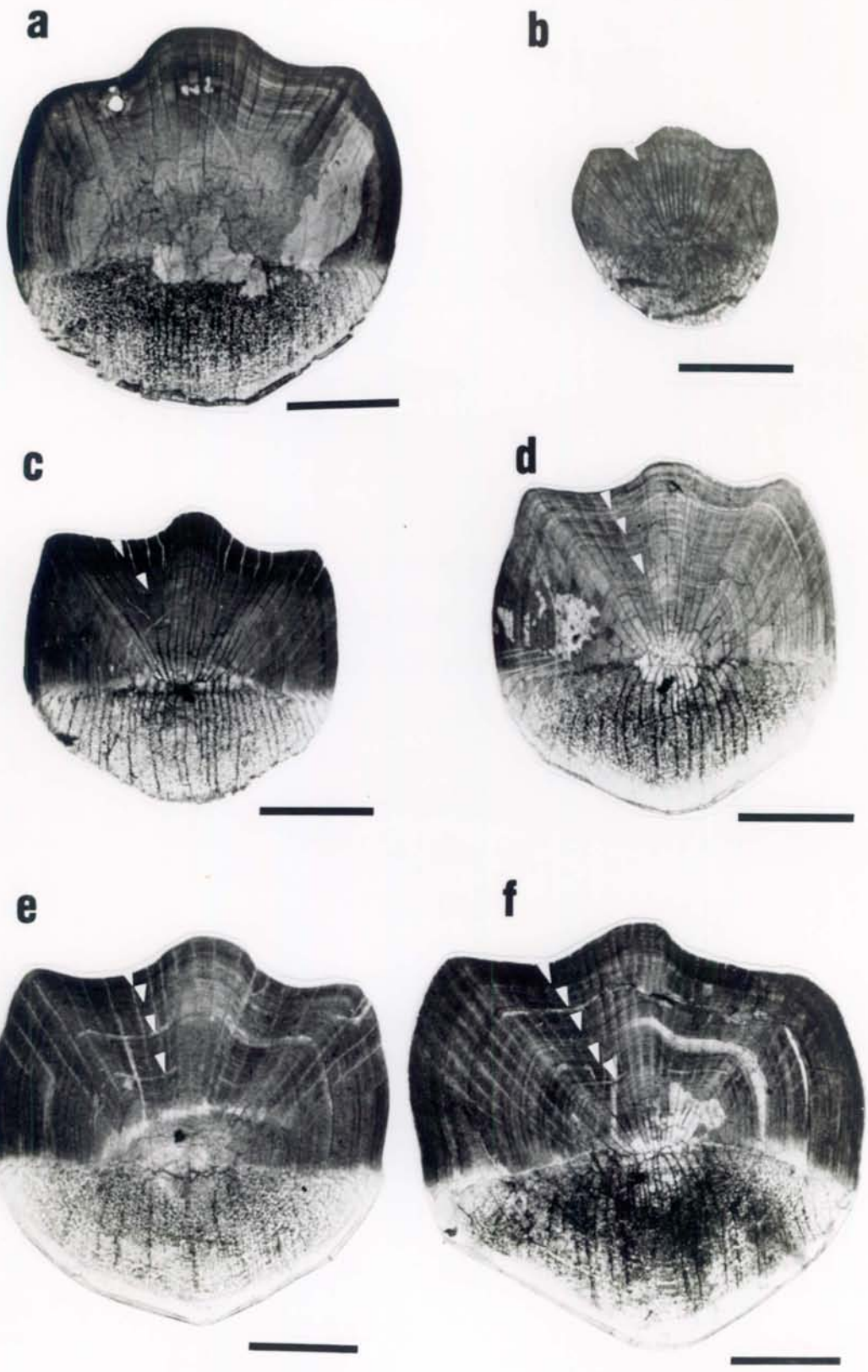

Overlapped Region
Circuli
Check
Radii
Scale Radius
Free Region

Plate 4.6 Parrotfish scales.

White triangles indicate checks. All scale bar = 5 mm. (a) A regenerated scale from *Scarus rivulatus* (SL=230 mm). (b) One-check-fish (*Scarus schlegeli* SL=128 mm). (c) Two-check-fish (*Scarus rivulatus* SL=163 mm). (d) Three-check-fish (*Scarus schlegeli* SL=184 mm). (e) Four-check-fish (*Scarus schlegeli* SL=212 mm). (f) Five-check-fish (*Scarus rivulatus* SL=234 mm).


a
b
c
d
e
f

down, indicating asymmetrical growth of the otolith through time, resulting in an ontogenetic change in shape. Initially, sagittae grew consistently in length, width and thickness (OR), but later new layers were added to the medial surface which thickened the structure (increasing OR) while growth in OL and OW decreased. Therefore, otolith mass (OWT) accumulated at a rate that was not directly proportional to the growth of the fish in SL. For all four species examined otoliths continued to grow in thickness and mass even though the growth of old fish was slowed down.

To be useful for aging, otoliths must also have a discernible internal increment structure. The narrow opaque sagitta bands alternating with wider translucent bands in *C.striatus*, *C.binotatus*, *S.rivulatus* and *S.schlegeli* were similar to patterns described in other tropical coral reef fish (Hill and Radtke, 1988; Fowler, 1990). The clarity of these bands was dependant upon the quality of the preparation (predominantly the orientation and thickness of the sections). For a few otoliths the sections did not show clear bands. This phenomenon has been commonly observed in many studies of age determination (Withell and Wankowski, 1988).

The chronological meanings of these bands in this study was assessed using two independent methods. Both methods resulted in similar conclusions. The marginal increment analysis was performed with specimens collected at regular intervals in *S.rivulatus* and *S.schlegeli*. Even though a small number of fish was sampled for each occasion, the range of SL did cover the most size range of the population. As with other studies of marginal increment analysis (Barger, 1985), these results suggested that one opaque band was deposited every year from November to May.

The tagging experiment provided the direct evidence for annuli periodicity in *S.schlegeli* and *C.striatus*. All recaptured specimens from both natural and artificial environment (ages between 2-13 yr), which were injected with tetracycline, consistently added one new band in the period between staining and capture (297-431 days), and two new bands over 453 to 574 days. Although the number of the recaptured fish was small, the periodicity of annuli was consistent. This result, in addition to the marginal increment analysis, strongly supports the hypothesis that the opaque bands on sagittae were deposited annually. In addition, by similar mark-recapture experiments annual bands were also observed in the otoliths of other three species of parrotfishes in genus *Scarus* on the GBR (Table 4.18). Several recent studies on the microstructure of otoliths of other tropical coral reef fish also support the above results. For the demersal fish *Dascyllus albisella* from Hawaii, there was a good correspondence between assumed daily increments and annual bands (Hill and Radke, 1988). Furthermore, annual otolith bands have been demonstrated to be exist in *Pomacentrus mollucensis* from the central GBR. by Fowler (1990) using a tag-recapture experiment.

Descriptions of annulus formation in otoliths have sometimes been confused, particularly concerning timing of formation of opaque and translucent bands (Casselman, 1982, Beckman *et al.*, 1990), in part due to ambiguous terminology (Casselman, 1987). For example, the type of light source (transmitted versus reflected) affects the appearance of growth bands. Discrepancies in annulus appearance could also reflect inconsistent formation of annuli through the otolith. For example, I observed opaque and translucent bands being deposited concurrently in different regions.

The physiological cause of opaque bands may not be related to spawning as spawning of the scarids occurs year round at Lizard Island (see Chapter 6), and identical annuli were also observed in immature individuals. By a mark-recapture study seasonal growth has been demonstrated for one tetradontidid species at Lizard Island (Gladstone and Westoby, 1988). It is likely that the fish studied here had experienced considerable environmental seasonality though their lives. Shallow habitats on reefs around Lizard Island such as reef flats, crests and lagoons have an annual variation of water temperature of up to 10°C (Gladstone and Westoby, 1988) with the seasonal range of temperature being similar to the southern ends of the GBR, although the thermal regime shifts downwards with increasing latitude. Since the specimens in this study were all taken from these shallow habitats, the opaque otolith bands were more likely to be the production of seasonal increments (Longhurst and Pauly, 1987). However, timing of formation of the opaque otoliths band was not clear in this study. It appeared to vary among individuals from September to May. Further work is required in order to determined the formation time.

The intra and inter-observer reliability of annual band counting suggests that annuli were not only exist in tropical parrotfishes (*S.rivulatus* and *S.schlegeli*) and surgeonfishes (*C.binotatus* and *C.striatus*) but can also be relatively precise to use for age estimates.

The application of daily growth increments to age determination has been widely used to age tropical larval and juvenile fish (Victor, 1982; Fowler, 1989). However, daily increments have been of little use in aging older fishes (Beamish and McFarlane, 1987). Juvenile parrotfishes (*S.schlegeli*), like other tropical coral reef

fish (Brothers *et al.*, 1983, Thresher, 1988), appear to consistently lay down daily increments at least up to one year. Apparent daily increments can also be detected on the sagittae and lapilli of adult fish. However, these increments in older fish were inconsistent with the chronological days based on the tagging experiments.

These findings appear to be different from the results obtained by Dee and Radtke (1989) on the brick soldierfish *Myripristis amaena* from Hawaii. They validated daily otolith increments over a period of 21 days for fish up to 112 mm SL (approximate 3 years old). Such a difference is probably due to biological variation between the two species (eg. soldierfish mature at about 6 years of age while parrotfish reach maturation at 2 years). Furthermore, the different techniques used (eg. scanning electron microscopy in their study versus light microscopy used in this study). However, it is unlikely that using scanning electron microscopy with otoliths of the species of this study would result in considerably different results. Both the appearance and width of the observed otolith increments were similar to daily increments described by Pannella (1971, 1974 and 1980). Furthermore the observed increments in this study and other studies by Pannella were distinct and regular from the nucleus to the edge. Older fish apparently stop depositing daily increments on their otoliths.

This phenomenon has been well documented in the literature. When Pannella (1971) first discovered daily increments in fish otoliths, he found that the number of daily increments in adult and older fishes decreased with age. In largemouth bass *Micropterus salmoides*, there were only approximately 150 daily increments between two annuli (Taubert and Tranquilli, 1982), and Miller and Storck (1982) showed that daily increments counted from older young-of-the-year fish tend to underestimate true

age. Therefore, age estimates based on daily otolith increments of adult and older fish should be interpreted cautiously as most age estimates using this technique will probably underestimate actual age.

4.4.2 Scale aging method

Scale radius of parrotfishes (*S.rivulatus* and *S.schlegeli*) increased consistently with SL, indicating a directly proportional relationship. When viewed under transmitted light scales had thin checks characterised by increased distances between circuli and broken circuli, similar to the pattern described in other tropical fish on the GBR (Walker, 1975). The clarity of these checks appeared to be uniform within the non-regenerating scales from an individual fish.

For the majority of the population (age 0^+ - 5^+), fish had a close number of bands or checks from both otoliths and scales. Even though some older individuals (age 6^+, 7^+ and 8^+) had less scale checks than otolith bands, scales from parrotfishes could generally provide a fast, relatively reliable age-estimation for most of the population. However, the scale method does not release the longevity of a population. Therefore, it is limited to use for general age estimates for individual fishes, and can not apply for demographic studies.

Scale technique promises to provide useful and easily obtainable age information in future reef fish ageing studies. However, the technique requires further more rigorous testing to determine how accurate and robust scale ageing technique are across a variety of reef species. Future studies should be focused on comparing annual scale checks to annuli on other bony structures in other reef species like having been done here and in some temperate species (Boxrucker, 1986;

Chilton and Stocker, 1987).

In conclusion, tropical parrotfishes (*S.rivulatus* and *S.schlegeli*) formed bands on their otoliths on an annual basis, which can be used to age individual fish. In addition, similar otolith annuli were also observed in tropical surgeonfishes (*C.binotatus* and *C.striatus*), the further validation of which may need larger samples. Apparent daily increments on the otolith of adult scarids and acanthurids could underestimate the actual age.

Scales in the scarids provide a fast, relatively reliable estimate of age for younger fishes up to 5 years, which will be most suitable to use in field.

Table 4.18 Otolith annual bands of the other tetracycline mark-recapture parrotfishes from the Great Barrier Reef (GBR).

JBR - John Brewer Reef in the central GBR; Aquarium - The aquarium of James Cook University of North Queensland.

Species	SL (mm)	Collecting location	Maintaining condition	Liberty period	Growth at liberty	Bands formed at liberty	Total bands
Scarus niger	247	Lizard Is.	Field	10/11/89 - 17/12/90	22 mm	1	6
Scarus frenatus	250	JBR.	Aquarium	20/05/88 - 20/04/91	17 mm	3	7
Scarus sordidus	208	JBR.	Aquarium	20/05/88 - 23/11/90	15 mm	2	5

CHAPTER 5. AGE AND GROWTH OF SCARIDS AND ACANTHURIDS

5.1 INTRODUCTION

With the validation of aging techniques for both juvenile (daily otolith increment) and adult (annual otolith band) scarids and acanthurids, it is now possible to provide a comprehensive description of growth in representatives of each group. From the juvenile studies information is obtained on growth during the periods of settlement and recruitment. For adults annual bands provide estimates not only a growth but also longevity. Taken together this information provides estimates of age-specific growth and mortality patterns, and of the turnover of populations.

Due to previous difficulties in direct aging of coral reef fish, there is a little information available on growth and age structures of scarid and acanthurid populations (Russ and St. John, 1988; Lou, 1993). Randall (1962) examined growth of coral reef fishes in the Virgin Islands based on tagging experiments. He suggested that parrotfishes grew fastest among all coral reef herbivorous fishes, but the effect of tagging on individual growth was not assessed on the species examined. Recently, daily otolith growth increments have been used to estimate growth of adult *Ctenochaetus striatus* (Itano, 1988). However, this technique can underestimate the age of adult individuals of studying species as already indicated in the previous chapter.

Recent studies of scarids and acanthurids have shown distinct differences in the early settlement patterns (Leis and Rennis, 1984) and post settlement feeding biology (Bellwood, 1988) between these two families. Furthermore, Russ and St. John (1988) suggested that acanthurids had slow growth among all species of

herbivorous reef fishes. There is an increasing interest in growth differences between these two families, as these differences may reflect biological differences between them.

This chapter will examine growth and age structures of scarids and acanthurids at Lizard Island. The detailed objectives of this chapter were:

(1) the growth of juveniles;

(2) age at settlement;

(3) the growth and age structure of adults; and

(4) the comparison on growth between the scarids and acanthurids. In addition, the geographic differences on growth of *Scarus rivulatus* will also be examined.

5.2 MATERIALS AND METHODS

5.2.1 Estimates of age and growth for juveniles

The samples of juveniles collected from the study sites around Lizard Island (see Chapter 3) were processed for the otolith analysis. The identification of juvenile scarids was according to the methods described by Bellwood and Choat (1989). A total of 69 juvenile *C. binotatus*, 30 juvenile *C.striatus*, 60 juvenile *S.rivulatus* and 34 juvenile *S.schlegeli* were examined in this chapter.

Standard length, TL in mm and WT to 0.001 g were recorded in the laboratory. Lapilli of the specimens were removed and processed according to the method described in 3.2.3. Specimen age in days (i.e otolith increment number) was established by taking the mean of at least two counts that deviated less than 10% of the mean. Specimen age was rounded to the nearest day.

The form of juvenile growth was described by fitting various curves to the SL-at-age data, and determining the best fit using a Cricket Graphic computer software. The SL-at-age data was then divided into several age groups (approximate 10 d for each group), and compared with predicted SL within each age group. The predicated SL was calculated by fitting the growth curve to the mean of observed age. The growth rates were estimated by fitting the first differentiation from each growth curve to the mean of observed age.

5.2.2 Settlement checks

The lapilli of all juveniles were examined microscopically for a transition check similar to that reported in other coral reef fishes (e.g Pitcher, 1988). As the fishes were collected after settlement on reefs, the first otolith transition check from the nucleus was considered as the settlement check, and the date of settlement for any individual was estimated by counting daily increments from the nucleus to the innermost edge of the transition along the longest axis of the lapillus.

5.2.3 Estimates of age and growth for adults

The adult individuals collected from the study sites around Lizard Island (see Chapter 4) were used in the growth analysis. In addition, some juveniles of *C.binotatus* and *C.striatus* which were older than one-year of age were also included in this analysis. A total of 314 *S.rivulatus*, 350 *S.schlegeli*, 22 *C.binotatus* and 35 *C.striatus* sagittae, which showed the identical number of otolith bands between counts on the two occasions in Chapter 4, was examined in this chapter.

Standard length, TL in mm and WT to 0.1 g were recorded for all specimens.

Sexual identifications were simply determined as male and female by observing gonads macroscopically for the 314 *S.rivulatus* and 343 *S.schlegeli*. Macroscopical examination of the gonad was sufficient to distinguish male and female, but not to identify the different sexual categories (see Chapter 6). The initial estimates of growth were carried out on males and females. The transverse sections of sagittae were microscopically examined according to the method described in 4.2.2. In addition, the distance from the nucleus to the innermost edge of each annulus was measured along the sagitta radius using an eyepiece micrometer. Individual age in years, which had been estimated in Chapter 4, was adopted in this chapter.

5.2.3.1 Growth functions and curves

Since the growth in fish length and weight varies in relative magnitude, it is desirable to obtain the length-weight relationship. The SL-WT relationship was, therefore, described by fitting to the following power curve:

$$WT = aSL^b$$

Where a is a constant, and b is an exponent. For most fish species the exponent value is approximal 3.

The overall SL-WT relationship was generated for all four species. By using the analysis of covariance (Jones *et al.*, 1988), the SL-WT relationship between male and female parrotfishes was compared while the SL-age relationship between sexual groups was examined in Chapter 6. A similar comparison was not carried out for surgeonfishes as there were few mature specimens available.

The pattern of growth was described by three types of functional curves, e.g. an empirical, back-calculated and theoretical curves. The back-calculated length was

estimated according to the method described by Bagenal and Tesch (1978). As an exponential relationship was found between otolith radius and SL in all four species (see Chapter 4), the formula for back-calculated length therefore was:

$$SL_n = SL \times Log(OR_n/a)/Log(OR/a)$$

where SL_n = Standard length of fish when 'n' annuli were formed;

OR_n = Otolith radius of annulus 'n'(at fish length SL_n);

a = A constant of OR-SL regression.

The theoretical growth curves were selected by fitting the common growth curves (Kaufmann, 1981) to length-at-age data, determining the best fit . The von Bertalanffy growth equation (von Bertalanffy 1938, 1957) was subsequently found to fit the data best, and the formula of which was:

$$L_t = L_\infty(1-e^{-k(t-to)})$$

Where L_t = Standard length at age t;

L_∞ = The asymptotic standard length;

K = The growth coefficient;

to = Age when length theoretically would be zero.

In the calculation the von Bertalanffy theoretical growth curve was generated based on the length-at-age data using ETAL computer program (Gaschutz *et al.*, 1980).

For the overall growth pattern, juveniles' age in day was transfered into the age in year, and the data weighted according to the sample sizes.

5.2.4 Growth comparisons

5.2.4.1 Between species

All juvenile growth curves were pooled together to compare any variation on growth. In addition, otolith increment widths were compared among the four species. 10 lapilli from each species were randomly chosen and the width of daily increments was measured to the nearest 0.1 μm using a custom computer program. In the measurement a movable cursor was superimposed over the image of lapilli on the Commodore-1084 monitor, which was connected to an Ikegami ICD-290 high resolution black and white video camera mounted on an Olympus compound microscope (400×-1000×). An increment width was determined by grouping the increments into 5 day blocks and taking the mean of each 5 day block along the longest axis of the lapillus.

In order to compare the overall growth rates between different species, a growth performance index (Munro and Pauly, 1983) was calculated according to the following equation:

$$\emptyset = \text{Log}_{10}K + 0.67\text{Log}_{10}W_{\infty}$$

Where W_{∞} = The asymptotic weight.

W_{∞} was obtained by fitting SL-WT relationship to the L_{∞}.

5.2.4.2 Between locations

Otoliths of *S.rivulatus* collected from Magnetic Island, Arlington and Thetford Reefs (see Chapter 2) were processed for age determination using the method described in Chapter 3 and 4.

The means of length-at-age from different locations including Lizard Island were compared with each other using the analysis of variance (ANOVA). Von Bertalanffy growth curves were also generated for each location, and the growth

performance indexes were compared.

5.3 RESULTS

5.3.1 Age and Growth in scarids

5.3.1.1 SL-WT relationship

For 110 male and 204 female *S.rivulatus*, the analysis of covariance on the SL-WT regressions from Log transformed data between 110 and 290 mm SL showed no significant difference on both the slopes and the intercepts at $p<0.05$ between both sexes. No significant difference was also found in the SL-WT relationships between 135 male and 208 female *S.schlegeli* with SL range from 100 to 248 mm (Table 5.1).

An overall SL-WT relationship, including juveniles, males and females, was calculated for both species (Fig.s 5.1, 5.2). Based on 374 *S.rivulatus* with SL-range from 18 to 290 mm, the least square regression of Log transformed data gave the expression:

$$WT = 1.728\times10^{-5}SL^{3.14} \qquad (r^2=0.9815 \quad p<0.001)$$

Based on the 384 *S.schlegeli* with SL-range of 22 to 248 mm, an overall SL-WT relationship was:

$$WT = 1.862\times10^{-5}SL^{3.12} \qquad (r^2=0.9924 \quad p<0.001)$$

5.3.1.2 Juvenile growth

Two of the 60 lapilli in *S.rivulatus* and two of the 34 lapilli in *S.schlegeli* were discarded from the aging analysis due to a larger deviation between the replicate counts. A plot of length against age in days was exponential for both species (Fig.s

5.3, 5.4). The growth equation for *S.rivulatus* was:

$$L_t = 9.326e^{0.0088t} \qquad (r^2 = 0.9460 \quad P < 0.001)$$

and for *S.schlegeli* was:

$$L_t = 11.916e^{0.0075t} \qquad (r^2 = 0.9644 \quad P < 0.001)$$

Based on these growth equations the predicated SL-at-age was calculated for both species (Tables 5.2, 5.3). Within the SL-range examined differences between the means of observed SL and the predicated SL were from 0.2 to 3.8 mm in *S.rivulatus*, and up to 4.2 mm for most age-groups in *S.schlegeli*. For one age group (140-149) in *S.schlegeli* there was a difference of 8.5 mm. The growth rate estimated from the growth equation increased with age over the age-range examined for both species.

5.3.1.3 Age structure and growth in adults

Maximum ages of *S.rivulatus* sampled were 8 years for a 245 mm SL female, 7 years for 6 TP males (243-279 mm SL) and 5 years for a 230 mm SL IP males. In *S.schlegeli* the maximum ages were 8 years for a 248 mm SL TP male, 7 years of a 209 mm SL female and 5 years of a 220 mm SL IP male.

Samples of both species had very similar age structures, with mainly age 2^+, 3^+ and 4^+. In *S.rivulatus* 32% of samples was in age 2^+, 23% in age 3^+ and 20% in age 4^+. While in *S.schlegeli* 20% of samples was in age 2^+, 39% in age 3^+ and 19% in age 4^+ (Tables 5.4, 5.5). The similar age structure in the both species may be due to the similar sampling program used. The age distribution in the samples of older ages may be considered representatives of the populations of *S.rivulatus* and *S.schlegeli*. However, smaller individuals appeared not to be sampled well as some

gear selectivities were inevitable.

Tables 5.4 and 5.5 also showed the back-calculated SL for 314 *S.rivulatus* and 350 *S.schlegeli* from Lizard Island over eight age groups, derived from the sagitta radius measurements. The means of back-calculated SL are also given for comparison with the means of observed SL. The observed length generally increased with age for both species. However, such increase was not smooth, and some fluctuation in growth did exist, which might reflect the growth variation between different years. The back-calculated SL, on the other hand, smoothly increased with age in both species. Generally, there was a similar trend in both empirical and back-calculated growth curves although the means of back-calculated SL were generally smaller than the means of observed SL, especially in young ages (Fig.s 5.5, 5.6). Such age-dependent differences between the observed and back-calculated length indicated that Rosa Lee's phenomenon (Ricker, 1979) exists in otolith aging method in the scarids.

Based on SL-at-age data from juveniles and adults, the 5 common growth curves (Kaufmann, 1981) were generated for both species. Since poor fits were obtained for exponential, Gompertz and logistic models, they were, therefore, not presented. Von Bertalanffy and power growth curves had best fitting to the data.

Von Bertalanffy equation fitted to weighted data in *S.rivulatus* was:

$$L_t = 267.75(1-e^{-0.3929(t+0.11)}) \qquad (r^2=0.9644 \;\; p<0.001)$$

and in *S.schlegeli* was,

$$L_t = 248.48(1-e^{-0.4407(t+0.23)}) \qquad (r^2=0.9605 \;\; p<0.001)$$

Von Bertalanffy curves appeared to fit to the most age groups, but not to age 1 and some youngest groups for both species (Fig.s 5.7, 5.8).

Table 5.1 Length-weight relationships for *Scarus rivulatus* and *S.schlegeli* between males (M) and females (F).

F-test indicated there were no significant difference between both sexes at $p<0.05$ for both species. F_b - *F* value for the slops; *F*a - *F* value for the intercepts.

Species	Sex	N	Equation	r^2	F_b	d.f.	*p*	F_a	d.f.	*p*
S.rivulatus	F	204	$WT=4.93\times10^{-5}SL^{2.94}$	0.944						
	M	110	$WT=3.68\times10^{-5}SL^{3.01}$	0.976	0.702	1/310	>0.05	3.39	1/313	>0.05
S.schlegeli	F	208	$WT=5.30\times10^{-5}SL^{2.92}$	0.951						
	M	135	$WT=4.35\times10^{-5}SL^{2.96}$	0.978	0.171	1/339	>0.05	1.149	1/342	>0.05

Table 5.2. *Scarus rivulatus*. **Comparison between the mean of observed SL and the predicted SL.**

Predicted values were estimated by fitting the exponential growth curve to the observed age. GR. - growth rates.

Age group	N	Observed		Predicted	
		Mean age (day) ± s.d	Mean SL (mm) ± s.d	SL (mm)	GR. (mm/day)
100 - 109	3	103.0 ± 1.6	20.3 ± 1.7	23.1	0.2031
120 - 129	1	128.0	30.0	28.8	0.2532
140 - 149	2	140.5 ± 0.5	35.5 ± 0.5	32.1	0.2825
150 - 159	4	153.0 ± 3.5	36.0 ± 2.8	35.8	0.3154
160 - 169	8	164.2 ± 3.5	41.1 ± 3.2	39.6	0.3481
170 - 179	5	172.0 ± 2.3	46.2 ± 4.9	42.4	0.3728
180 - 189	5	182.6 ± 3.3	43.4 ± 7.8	46.5	0.4092
190 - 199	5	191.4 ± 1.0	50.8 ± 2.5	50.2	0.4422
200 - 209	3	206.7 ± 1.2	57.3 ± 3.3	57.5	0.5060
210 - 219	4	214.0 ± 3.3	59.0 ± 6.8	61.3	0.5395
230 - 239	2	230.0 ± 0.5	67.0 ± 1.0	70.6	0.6211
240 - 249	3	243.7 ± 2.0	78.3 ± 6.9	79.6	0.7007
250 - 259	7	254.3 ± 3.6	87.4 ± 4.5	87.4	0.7692
260 - 269	5	263.6 ± 3.0	94.4 ± 3.9	94.9	0.8347
270 - 279	1	271.1	98.0	101.2	0.8910

Table 5.3 *Scarus schlegeli*. **Comparison between the mean of observed SL and the predicted SL.**

Predicted values were estimated by fitting the exponential growth curve to the observed age. GR. - growth rate.

Age group	N	Observed		Predicted	
		Mean age (day) ± s.d	Mean SL (mm) ± s.d	SL (mm)	GR. (mm/day)
80 - 89	2	84.5 ± 2.5	22.5 ± 0.5	22.4	0.1684
90 - 99	2	98.5 ± 0.5	23.5 ± 2.5	24.9	0.1871
100 - 109	1	102.0	22.0	25.6	0.1920
120 - 129	5	123.4 ± 2.8	28.6 ± 1.8	30.1	0.2253
130 - 139	2	132.0 ± 2.0	33.0 ± 6.0	32.1	0.2405
140 - 149	2	143.5 ± 3.5	43.5 ± 2.5	35.0	0.2622
160 - 169	2	166.0 ± 2.0	43.5 ± 4.5	41.4	0.3104
170 - 179	1	170.0	48.0	42.6	0.3198
180 - 189	2	189.5 ± 0.5	48.5 ± 1.5	49.3	0.3702
200 - 209	3	200.7 ± 0.5	56.1 ± 6.1	53.7	0.4025
260 - 269	1	260.0	88.0	83.7	0.6281
270 - 279	4	275.0 ± 1.2	91.0 ± 3.5	93.7	0.7029
280 - 289	5	283.2 ± 3.5	97.2 ± 1.5	99.7	0.7475

Table 5.4 *Scarus rivulatus*. **Back-calculated lengths for each age group.**

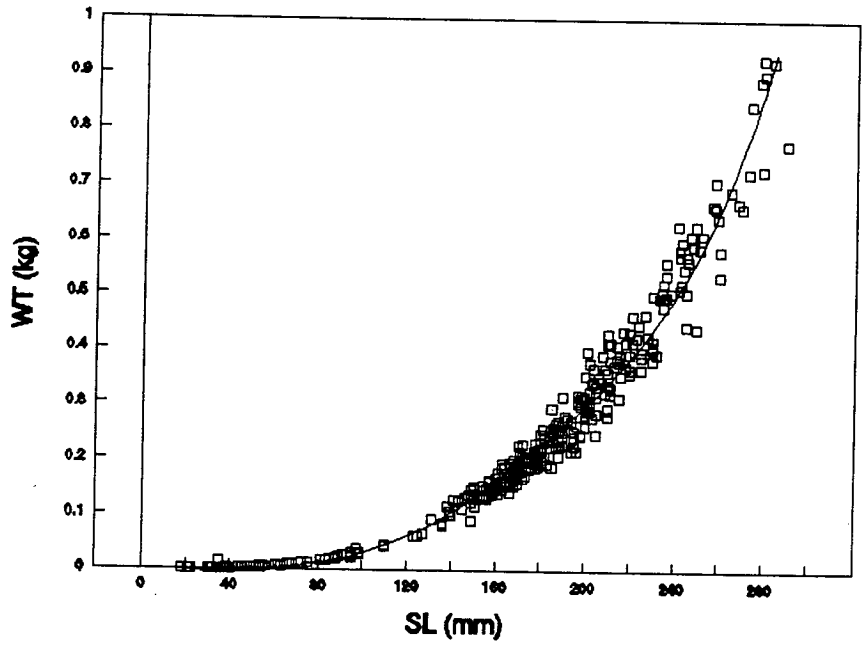
Age Group	N	Mean SL (mm)	Back-calculated SL (mm) at ages							
			1	2	3	4	5	6	7	8
1^+	7	123	92							
2^+	102	163	53	131						
3^+	75	181	85	118	162					
4^+	62	204	78	119	159	184				
5^+	37	230	57	126	169	195	219			
6^+	22	252	51	124	167	196	224	242		
7^+	8	252	53	122	157	186	210	230	243	
8^+	1	245	72	122	148	179	198	219	233	239
Mean			68	123	160	188	213	230	238	239
N			314	307	205	130	68	31	9	1

Table 5.5 *Scarus schlegeli*. **Back-calculated length (mm) for each age group.**

Age Group	N	Mean SL (mm)	Back-calculated SL (mm) at ages							
			1	2	3	4	5	6	7	8
1^+	31	121	78							
2^+	71	165	69	136						
3^+	137	185	58	126	166					
4^+	68	206	65	128	164	192				
5^+	33	225	65	127	164	194	214			
6^+	7	236	64	127	168	190	214	229		
7^+	2	227	63	109	142	174	191	207	221	
8^+	1	248	67	138	175	204	220	235	242	248
Mean			66	127	163	191	210	224	232	248
N			350	319	248	111	43	10	3	1

Fig. 5.1 *Scarus rivulatus*. **The relationship between standard length and body weight.**

Fig. 5.2 *Scarus schlegeli*. **The relationship between standard length and body weight.**


WT (kg)
SL (mm)

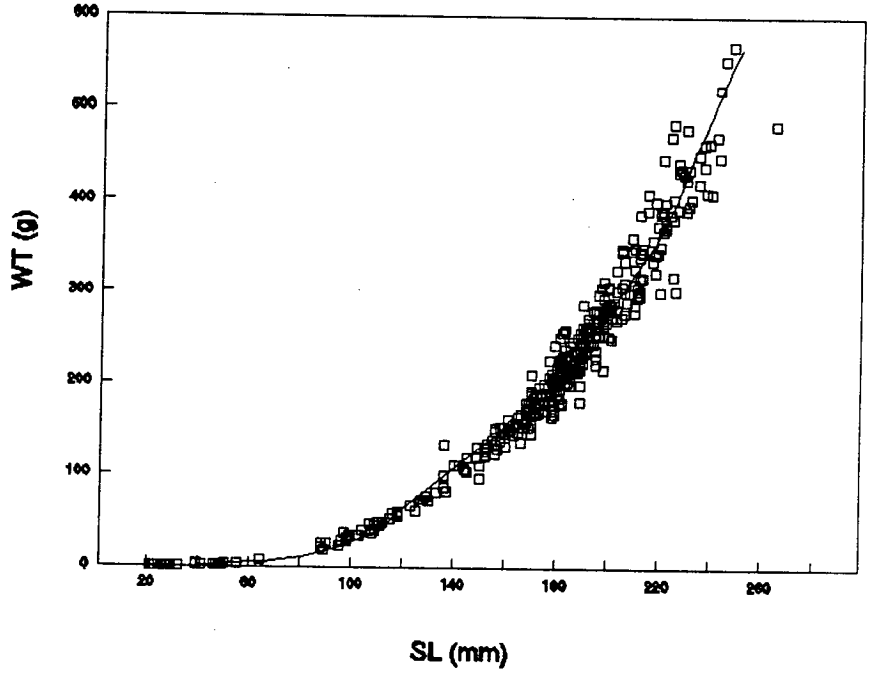
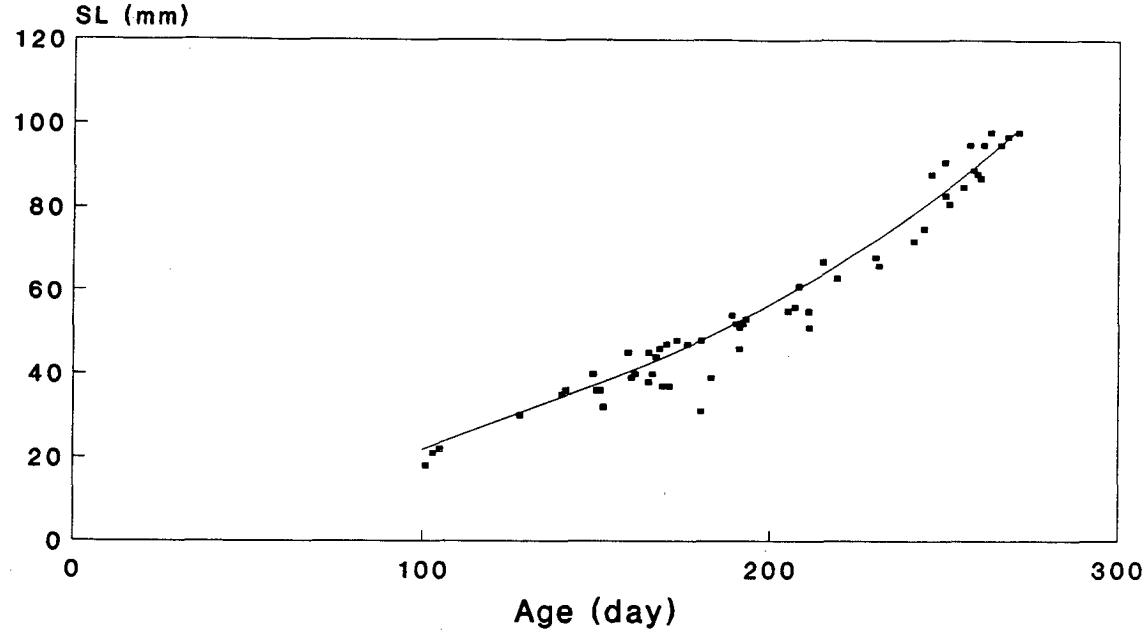

WT (g)
SL (mm)

Fig. 5.3 *Scarus rivulatus*. **The growth curve of juveniles.**

Fig. 5.4 *Scarus schlegeli*. **The growth curve of juveniles.**


SL (mm)
120
100
80
60
40
20
0
0
100
200
300
Age (day)

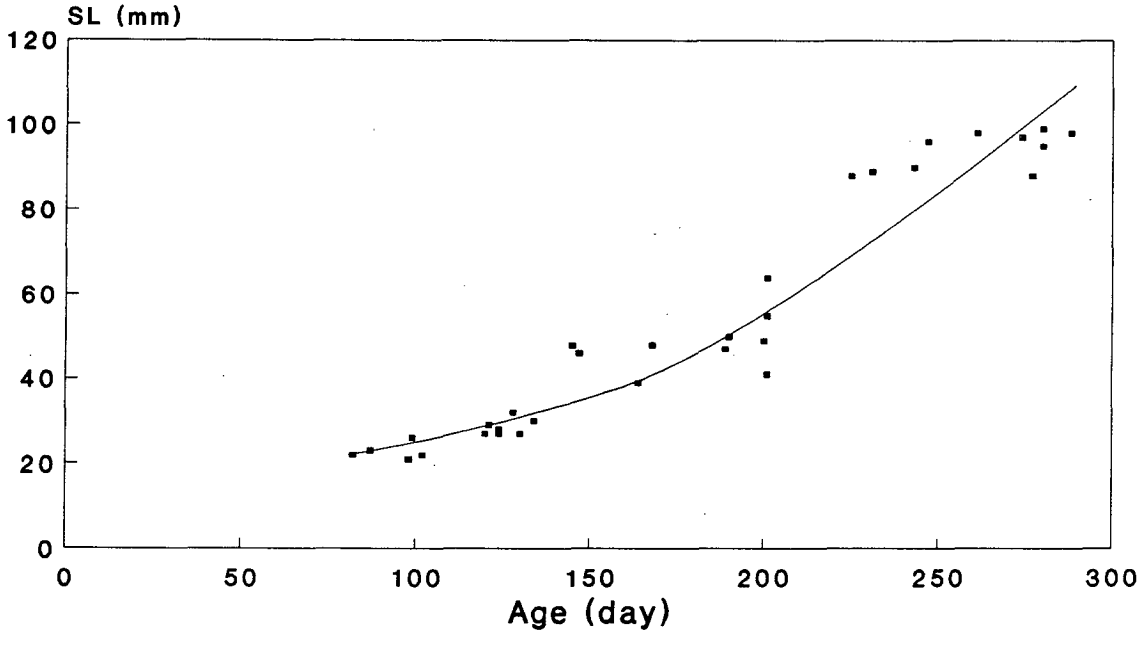
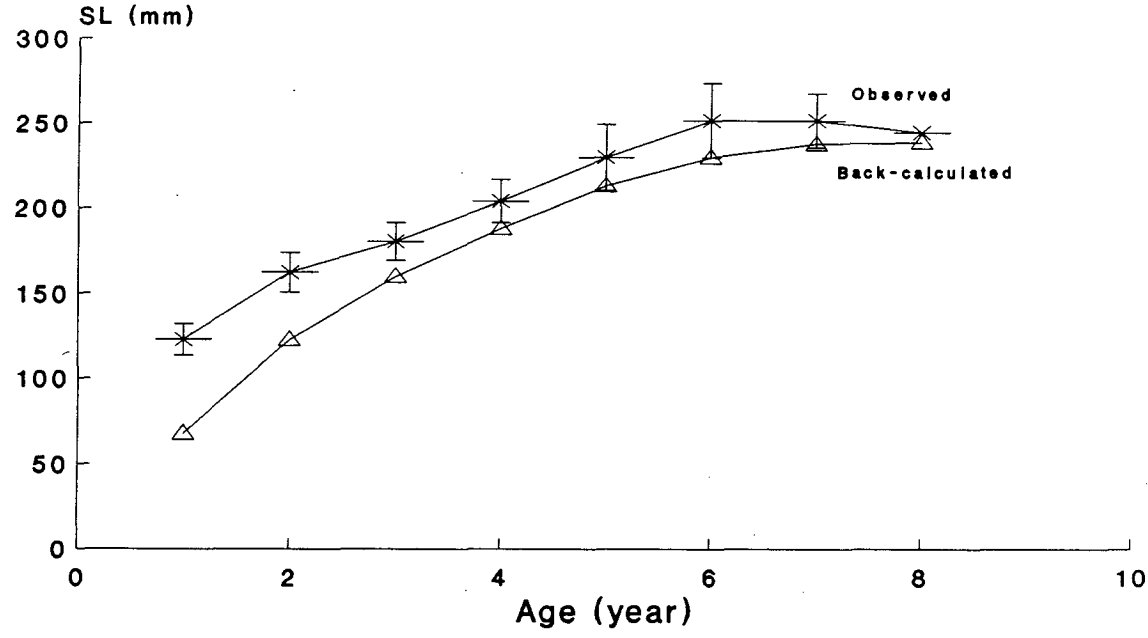

SL (mm)
120
100
80
60
40
20
0
0
50
100
150
200
250
300
Age (day)

Fig. 5.5 *Scarus rivulatus*. **Means of observed SL versus age and means of back-calculated SL.**

Vertical bars indicate the standard deviation of observed SL.

Fig. 5.6 *Scarus schlegeli*. **Means of observed SL versus age and means of back-calculated SL.**

Vertical bars indicate the standard deviation of observed SL.


SL (mm)
300
250
200
150
100
50
0
0
2
4
6
8
10
Age (year)
Observed
Back-calculated

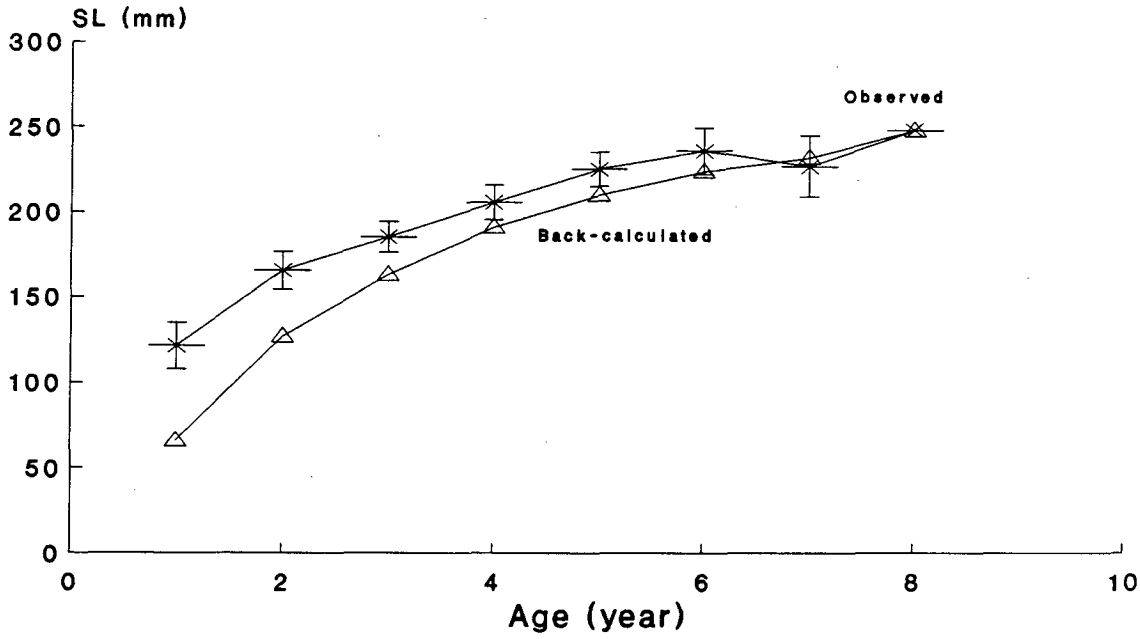
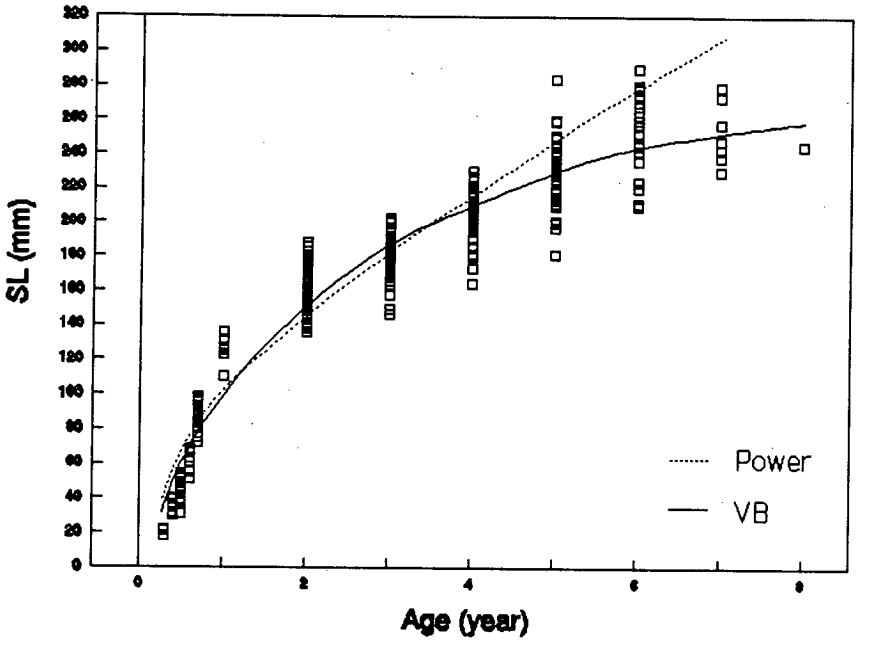

SL (mm)
300
250
200
150
100
50
0
0
2
4
6
8
10
Age (year)
Observed
Back-calculated

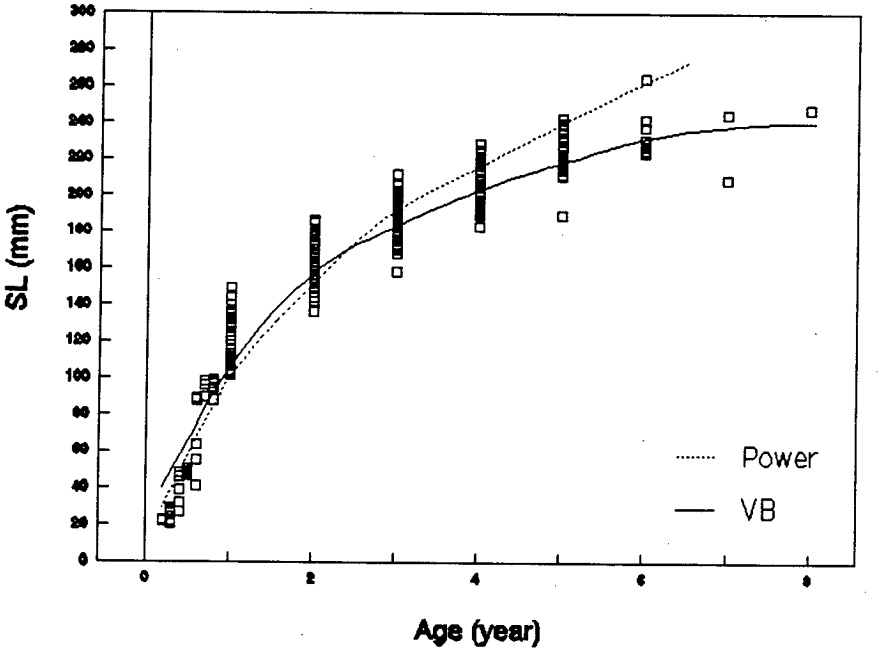
Fig. 5.7 *Scarus rivulatus*. **The overall theoretical growth curves.**

VB - von Bertalanffy growth curve; Power - power growth curve.

Fig. 5.8 *Scarus schlegeli*. **The overall theoretical growth curves.**

VB - von Bertalanffy growth curve; Power - power growth curve.


SL (mm)
Age (year)
Power
VB


SL (mm)
Age (year)
Power
VB

Power curves appeared to fit the data well for younger age classes (not including age 1) in both species (Fig.s 5.7, 5.8). However, these models did not fit the growth of the scarids more than 4 years of age. Fitted equations were, for *S.rivulatus*:

$$L_t = 88.84t^{0.6423} \qquad (r^2=0.8879 \;\; p<0.01)$$

and for *S.schlegeli*:

$$L_t = 94.38t^{0.6097} \qquad (r^2=0.8608 \;\; p<0.01)$$

5.3.2 Age and growth in acanthurids

5.3.2.1 SL-WT relationship

The SL-WT relationship for combined sexes in *C.striatus* was determined from 57 individuals which ranged from 30 to 188 mm SL. The least square regression was:

$$WT=2.15\times10^{-5}SL^{3.167} \qquad (r^2=0.9896 \quad p<0.001)$$

The SL-WT relationship was also obtained for combined sexes in *C.binotatus*. The expression, determined from 90 individuals ranged from 26 to 147 mm in SL, was:

$$WT=1.84\times10^{-5}SL^{3.213} \qquad (r^2=0.9893 \quad p<0.001)$$

The acanthurids appeared to have heavier body weight than the scarids of the same sizes (Fig.s 5.9, 5.10).

5.3.2.2 Juvenile growth

Four of the 30 lapilli in *C.striatus* and 3 of the 69 lapilli in *C.binotatus* were discarded due to the larger deviation between the replicate counts. The juvenile age

in days was curvelinearly related to SL. Second degree polynomial curves were best fitted to the SL-at-age data for both species (Fig.s 5.11, 5.12). The growth equation for *C.striatus* was:

$$L_t = 13.411 + 0.2271t - 0.00004t^2 \quad (r^2 = 0.9788 \quad P < 0.001)$$

for *C.binotatus* was:

$$L_t = 6.894 + 0.2967t - 0.00019t^2 \quad (r^2 = 0.966 \quad P < 0.001)$$

Based on the above two growth equations, the predicted SL and the growth rates were calculated for both species (Tables 5.6, 5.7). Within the SL-range examined, differences between the mean of observed SL and predicted SL were from 0.6 to 5.9 mm for *C.striatus* and from 0.4 to 2.7 mm for *C.binotatus*. Unlike in the scarids, the growth rates in both species decreased stably with age.

5.3.2.3 Age and growth in adults

Maximum ages of the acanthurids sampled were 16 years of two *C.striatus* individuals from 181 to 182 mm SL, and 10 years of two *C.binotatus* individuals with SL of 145 to 147 mm. Although the samples were collected by the unselected methods, their age distribution could not be considered to be representatives of the populations for both species due to the small sampling sizes.

The back-calculated SL were listed for 35 *C.striatus* and 22 *C.binotatus* from Lizard Island over 16 age groups, which was determined from the otolith radius measurements. In addition, means of both back-calculated and observed SL were also listed for comparison (Tables 5.8, 5.9). Like in the scarids, a variable growth was displayed on the observed length for both species, especially in *C.striatus*. The back-calculated SL in both species smoothly increased with age. Rosa Lee's phenomenon

(Ricker, 1979) was also found to exist in the acanthurid otolith aging method as indicated by large variations between the observed and back-calculated SL at younger ages (Fig.s 5.13, 5.14).

Exponential, Gompertz and logistic models, not presented, were also found to have poor fitting to the data in the acanthurids. Von Bertalanffy and power growth curves appeared to fit the data best. The overall growth curves were based on the SL-at-age data from juvenile and adult individuals. In *C.striatus*, von Bertalanffy growth equation was:

$$Lt=184.07(1-e^{-0.2533(t+0.22)}) \quad (r^2=0.9851 \quad p<0.001)$$

and in *C.binotatus* was:

$$L_t=146.28(1-e^{-0.4289(t+0.39)}) \quad (r^2=0.9736 \quad p<0.001)$$

Both curves generally appeared to fit to the most age groups, but not to age 1 and 2 (Fig.s 5.15, 5.16).

Power curves appeared to fit the data best for younger ages (less than 3) in *C.striatus*. However, these models did not fit the growth of the acanthurids more than 5 and 10 of age in *C.binotatus* and *C.striatus* respectively (Fig.s 5.15, 5.16). Fitted equations were, for *C.striatus*:

$$L_t=68.655t^{0.4166} \quad (r^2=0.9711 \quad p<0.001)$$

and for *C.binotatus*:

$$L_t=63.956t^{0.4465} \quad (r^2=0.9384 \quad p<0.001)$$

The higher value of correlation coefficient in the power curves was due to the large proportion of younger-age specimens in the samples of both species.

Table 5.6 *Ctenochaetus striatus*. **Comparison between the mean of observed SL and the predicated SL.**

Predicted values were estimating by fitting the growth curve to the observed age. GR.- growth rates. Standard deviations are indicated in parenthesises.

Age group	N	Observed		Predicted	
		Mean age (day) ± s.d	Mean SL (mm) ± s.d	SL (mm)	G.R. (mm/day)
80 - 89	4	83.5 ± 1.6	33.3 ± 1.9	32.1	0.2204
90 - 99	2	95.0 ± 4.0	34.0 ± 1.0	34.6	0.2195
100 - 109	1	108.0	39.0	37.5	0.2185
110 - 120	4	114.5 ± 4.1	39.8 ± 2.8	38.9	0.2179
150 - 159	2	156.0 ± 0.0	46.0 ± 1.0	47.8	0.2146
170 - 179	1	175.0	46.0	51.9	0.2131
190 - 199	2	197.5 ± 0.5	53.0 ± 2.0	56.7	0.2113
200 - 209	1	201.0	53.0	57.4	0.2111
231 - 239	1	231.0	68.0	63.7	0.2087
255 - 265	2	260.0 ± 5.0	71.0 ± 4.0	69.7	0.2063
280 - 289	2	287.0 ± 1.0	79.0 ± 1.0	75.3	0.2042
300 - 309	1	306.0	85.0	79.1	0.2027
380 - 389	1	381.0	93.0	94.1	0.1967
400 - 420	2	408.5 ± 6.5	98.0 ± 2.0	99.5	0.1945

Table 5.7 *Ctenochaetus binotatus*. **Comparison between the mean of observed SL and the predicted SL.** Predicted values were estimated by fitting the growth curves to the observed age. GR. - growth rates, Standard deviations are indicated in parenthesises.

Age group	N	Observed		Predicated	
		Mean age (day) ± s.d	Mean SL (mm) ± s.d	SL (mm)	GR. (mm/day)
60 - 69	3	65.7 ± 2.5	28.3 ± 2.6	25.6	0.2717
70 - 79	7	74.2 ± 2.8	28.6 ± 1.5	27.9	0.2685
80 - 89	14	83.6 ± 2.6	29.7 ± 1.8	30.4	0.2649
90 - 99	10	92.2 ± 2.2	33.1 ± 3.5	32.6	0.2616
100 - 109	6	104.0 ± 3.6	35.0 ± 2.4	35.7	0.2572
110 - 119	2	113.5 ± 1.5	37.5 ± 0.5	38.1	0.2515
125 - 134	3	131.0 ± 2.2	41.0 ± 0.8	42.5	0.2469
165 - 174	2	171.0 ± 2.0	52.5 ± 4.5	52.1	0.2317
180 - 189	5	185.6 ± 4.2	54.8 ± 5.8	55.4	0.2261
190 - 199	2	193.0 ± 1.0	59.5 ± 8.5	57.1	0.2233
200 - 209	3	200.3 ± 0.5	58.0 ± 4.3	58.7	0.2205
210 - 219	1	218.0	63.0	62.5	0.2138
220 - 229	2	223.0 ± 2.0	64.5 ± 1.5	63.6	0.2119
240 - 280	3	262.3 ± 16.6	73.0 ± 2.9	71.6	0.1970
340 - 370	3	352.0 ± 8.8	86.7 ± 1.2	87.8	0.1629

Table 5.8 *Ctenochaetus striatus*. **Back-calculated length for each age group.**

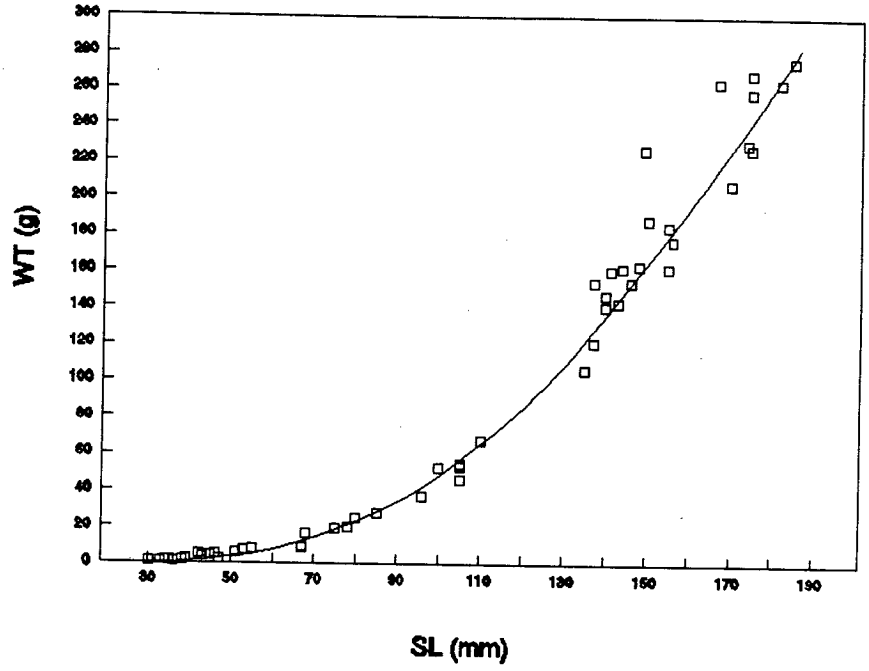
Age Group	N	Mean SL (mm)	Back-calculated SL (mm) at ages															
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1^+	6	76	61															
2^+	4	102	55	87														
3^+	3	117	50	89	106													
4^+	4	140	57	97	116	133												
5^+	3	140	50	83	106	123	135											
6^+	3	147	61	85	102	118	132	141										
7^+	1	155	47	85	101	123	134	144	150									
8^+	3	153	59	85	101	114	127	143	144	150								
9^+	1	167	59	83	102	117	126	138	145	152	161							
10^+	1	170	50	76	95	111	129	144	152	160	165	168						
11^+	1	174	56	78	106	119	131	141	150	158	163	167	172					
12^+	1	175	56	78	96	114	130	143	149	154	159	164	169	173				
13^+	2	175	46	73	94	112	126	135	142	150	156	161	165	170	172			
16^+	2	183	48	73	93	108	118	126	133	140	145	151	157	162	169	173	178	182
Mean			54	83	102	117	129	139	145	152	158	162	166	168	171	173	178	182
N			35	29	25	22	18	15	12	11	8	7	6	5	4	2	2	2

Table 5.9 *Ctenochaetus binotatus*. **Back-calculated lengths for each age group.**

Age Group	N	Mean SL (mm)	Back-calculated SL (mm) at ages									
			1	2	3	4	5	6	7	8	9	10
1+	5	74	57									
2+	1	102	42	78								
3+	3	117	41	77	107							
4+	5	121	38	76	99	113						
5+	1	124	48	75	92	105	119					
6+	1	126	23	56	72	86	101	116				
8+	2	135	42	65	83	95	106	114	123	130		
9+	2	141	41	63	78	93	104	113	122	130	138	
10+	2	146	40	58	81	93	105	114	122	129	138	144
Mean			41	70	87	98	107	114	122	130	138	144
N			22	17	16	13	11	8	7	6	4	2

Fig. 5.9 *Ctenochaetus striatus*. **The relationship between standard length and body weight.**

Fig. 5.10 *Ctenochaetus binotatus*. **The relationship between standard length and body weight.**


WT (g)
SL (mm)

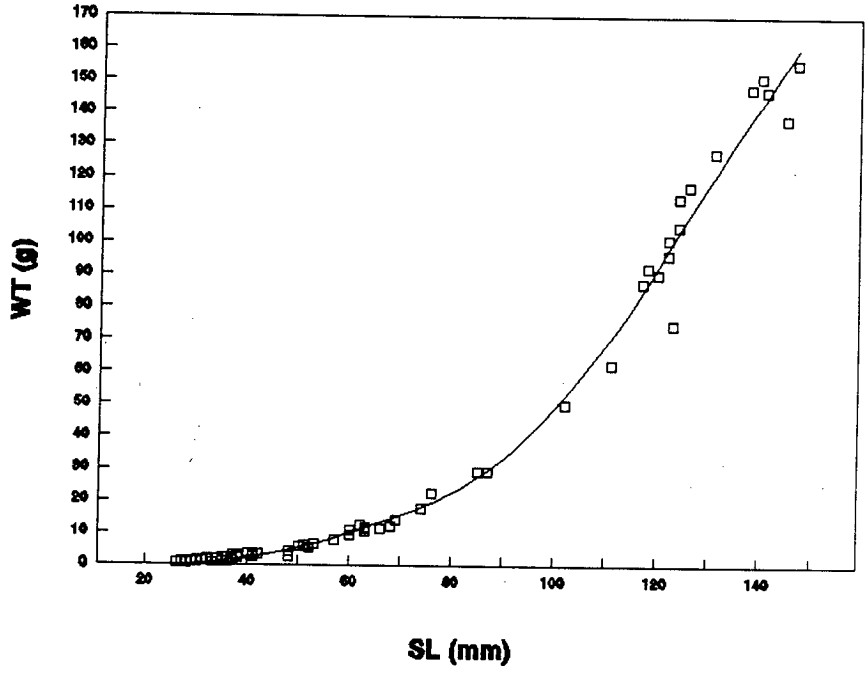
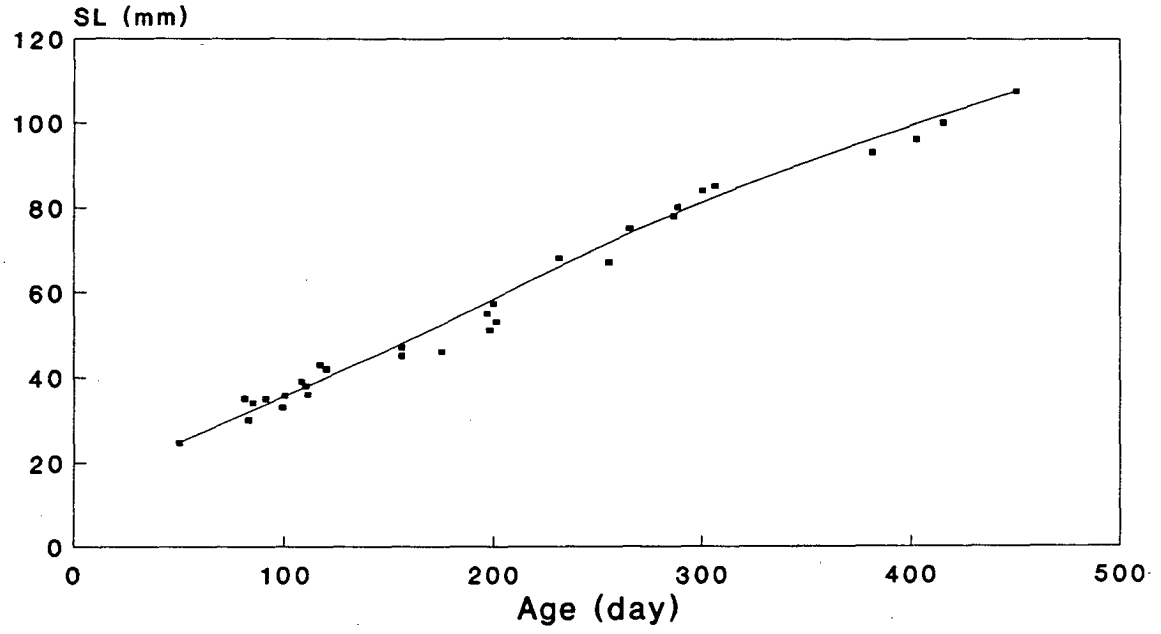

WT (g)
SL (mm)

Fig. 5.11 *Ctenochaetus striatus.* **The growth curve of juveniles.**

Fig. 5.12 *Ctenochaetus binotatus.* **The growth curve of juveniles.**


SL (mm)
120
100
80
60
40
20
0
0
100
200
300
400
500
Age (day)

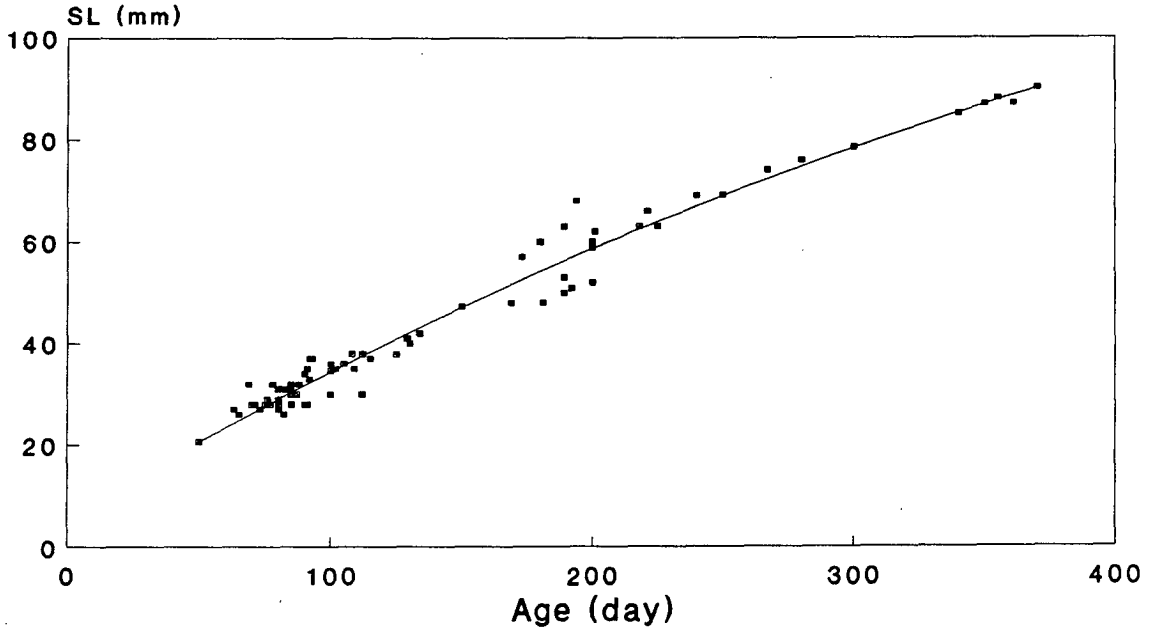
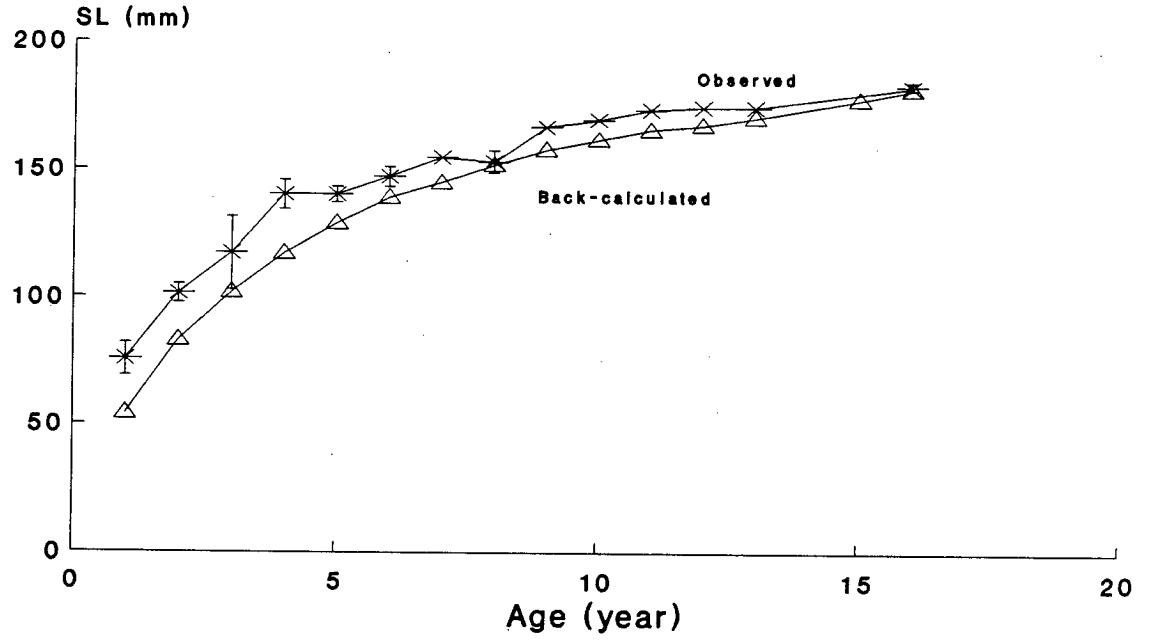

SL (mm)
100
80
60
40
20
0
0
100
200
300
400
Age (day)

Fig. 5.13 *Ctenochaetus striatus*. **Means of the observed SL versus age and means for back-calculated SL.**

Vertical bars indicate the standard deviation of observed SL.

Fig. 5.14 *Ctenochaetus binotatus*. **Means of the observed SL versus age and means of back-calculated SL.**

Vertical bars indicate the standard deviation for the observed SL.


SL (mm)
200
150
100
50
0
Observed
Back-calculated
0
5
10
15
20
Age (year)

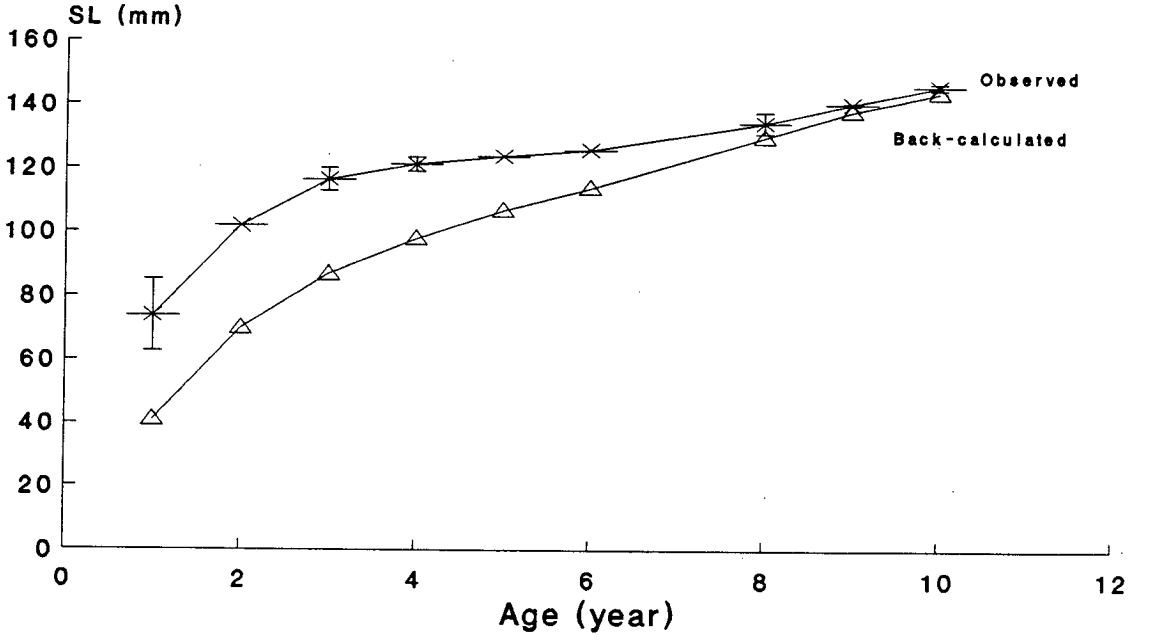
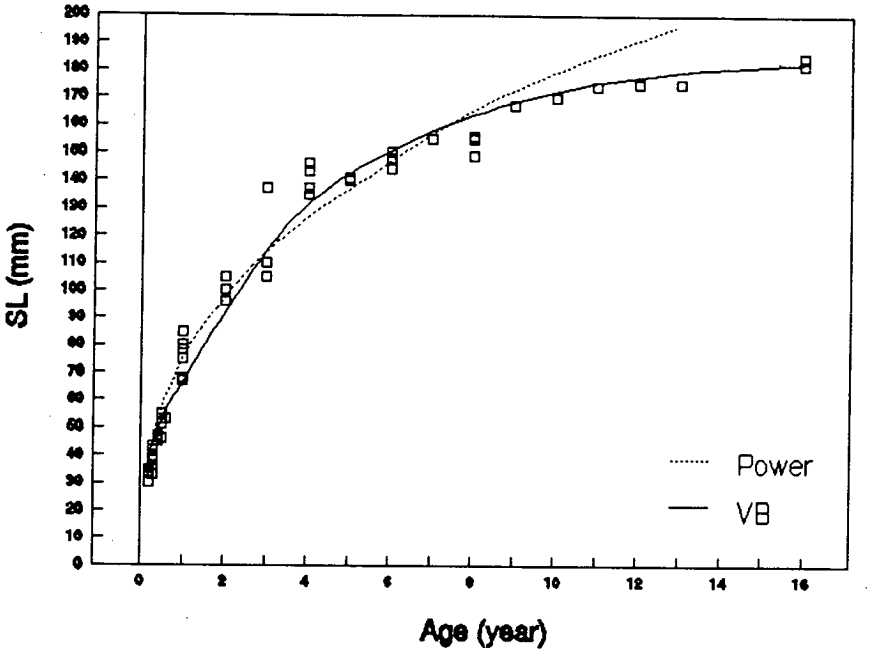

SL (mm)
160
140
120
100
80
60
40
20
0
Observed
Back-calculated
0
2
4
6
8
10
12
Age (year)

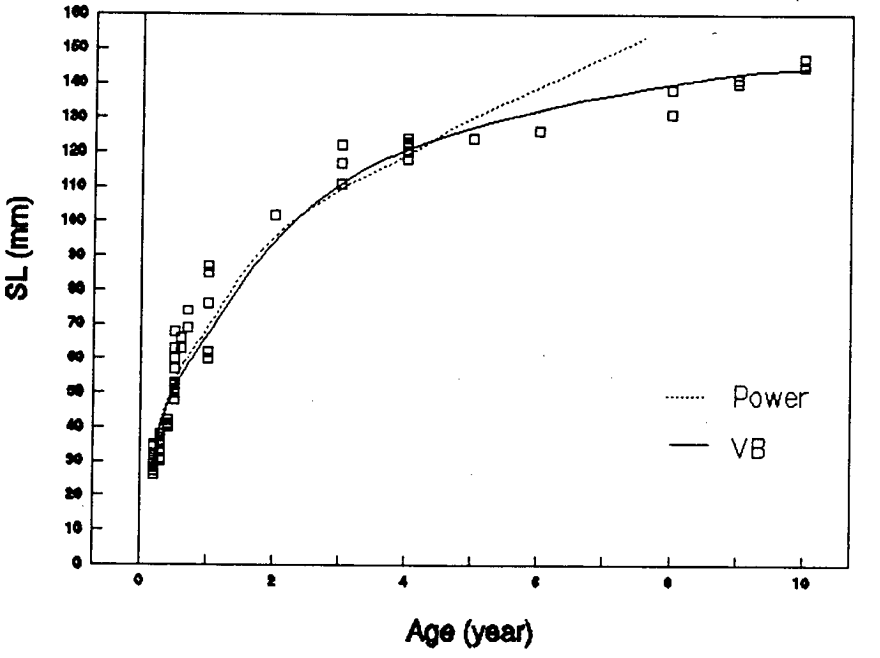
Fig. 5.15 *Ctenochaetus striatus*. **The overall theoretical growth curves.**

VB - von Bertalanffy growth curve; Power - power growth curve.

Fig. 5.16 *Ctenochaetus binotatus*. **The overall theoretical growth curves.**

VB - von Bertalanffy growth curve; Power - power growth curve.


SL (mm)
Age (year)
Power
VB


SL (mm)
Age (year)
Power
VB

5.3.3 Settlement age

Lapilli from *C.binotatus* and *C.striatus* were found to have a conspicuous check resulting from a change in the structure of growth increments (Plate 5.1a,b). At this transition, the initial prominent dark lines delineating each increment abruptly disappeared. Regular increments only reappeared after a band without discrete increments was formed. The number of visible otolith increments prior to the settlement check ranged from 47 to 74 in the sample of the 66 juvenile *C.binotatus*, and from 53 to 70 in 26 juveniles of *C.striatus* (Table 5.10).

A similar otolith check was also observed on lapilli of *S.rivulatus* and *S.schlegeli* (Plate 5.1c,d). The number of daily increments from the nucleus to this check ranged from 28 to 47 in 58 juveniles of *S.rivulatus*, and from 35 to 45 in 32 juveniles of *S.schlegeli* (Table 5.10).

5.3.4 Comparison on growth between acanthurids and scarids

5.3.4.1 Juveniles

All growth curves of the four species studied were compared (Fig. 5.17). Within the same family, species exhibited quite similar growth patterns. However, different growth patterns became evident when comparing species from different families. In the early growth phase (less than approximate 200 d), *C.binotatus* and *C.striatus* had larger length than *S.rivulatus* and *S.schlegeli* of the same age. In contrast, *S.rivulatus* and *S.schlegeli* were longer than *C.binotatus* and *C.striatus* of the same age after about 200 days.

The above phenomenon was further demonstrated in the otolith increment widths from the four species. Otolith increment widths of *C.binotatus* and *C.striatus*

initially increased rapidly and reached a peak of 5 to 9 μm at the age of 30 to 40 days. Increment width then declined gradually to less than 2 μm per day from approximately 90 d (Fig. 5.18a, b). In contrast, the variation in increment width was not so marked in *S.rivulatus* and *S.schlegeli*. The increment widths were almost constant between 3 to 4.5 μm from the nucleus to the 50th increment followed by a slow decline leading to a fairly constant increment width of 2 μm per day from approximately 100 d (Fig. 5.18c, d).

5.3.4.2 Adults

Table 5.11 provides the asymptotic standard length (L_∞), the growth coefficient (K) of the von Bertalanffy growth curves and the growth performance indexes (Ø) of the four species studied. Within the same family, Ø-values of each species were close, although there were larger differences between L_∞ and K. However, Ø of *S.rivulatus* and *S.schlegeli* was higher than that of *C.binotatus* and *C.striatus*. Student T-test indicated there was a significant difference between the means of Ø-values from the acanthurids and scarids (t_2=28.21, $p<0.002$). This confirms that in overall scarids may grow more quickly than acanthurids of similar sizes or ages (Fig. 5.19).

5.3.5 Growth differences between locations in *Scarus rivulatus*

5.3.5.1 Magnetic Island

A significant correlation between SL and WT was obtained for *S.rivulatus* in Magnetic Island ($WT=2.79\times10^{-5}SL^{3.029}$ $r^2=0.9957$ $p<0.001$). The age of specimens with the SL-range of 37-240 mm was estimated up to 5 years. Von Bertalanffy

Table 5.10 Summary of settlement patterns for the species studied.

Species	N	Mean age of settlement (day)	SL (mm) Range	s.d.
Ctenochaetus striatus	26	59	53 - 70	4.5
Ctenochaetus binotatus	66	58	47 - 74	5.0
Scarus rivulatus	58	39	28 - 47	4.6
Scarus schlegeli	32	39	35 - 45	2.5

Table 5.11 Growth performance indexes (Ø) of the species studied.

Species	K	L_∞ (mm)	Ø
Ctenochaetus binotatus	0.4289	146.28	1.1164
Ctenochaetus striatus	0.2533	184.57	1.0894
Scarus rivulatus	0.3929	267.75	1.5109
Scarus schlegeli	0.4407	248.48	1.4821

Plate 5.1 Settlement check (SC) on the lapillus.

Bar = 75 μm. (a) *Ctenochaetus binotatus*, (b) *Ctenochaetus striatus*, (c) *Scarus rivulatus* and (d) *Scarus schlegeli*.

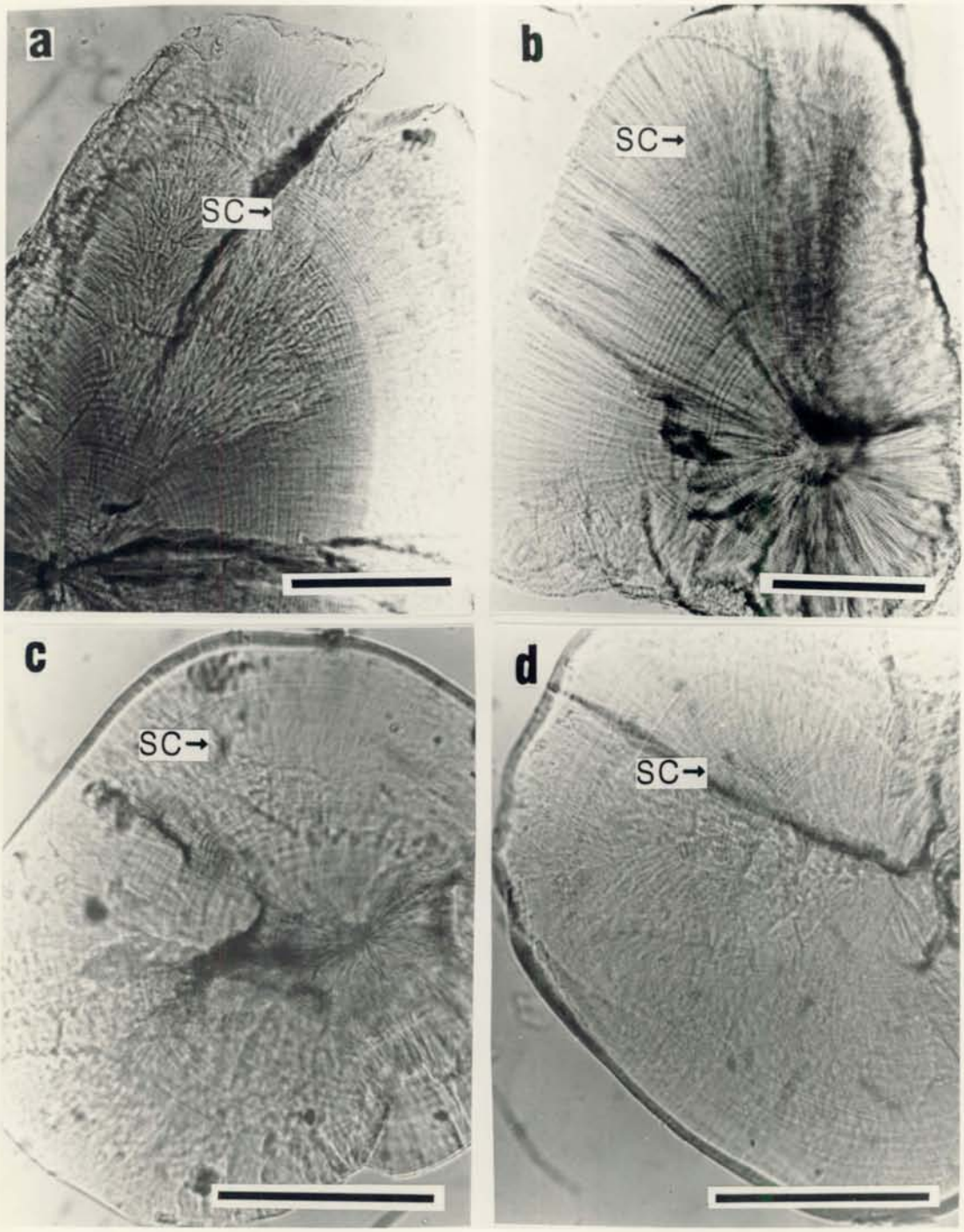

a
SC→
b
SC→
c
SC→
d
SC→

Fig. 5.17 The juvenile growth curves of *Ctenochaetus binotatus*, *C.striatus*, *Scarus rivulatus* **and** *S.schlegeli*.

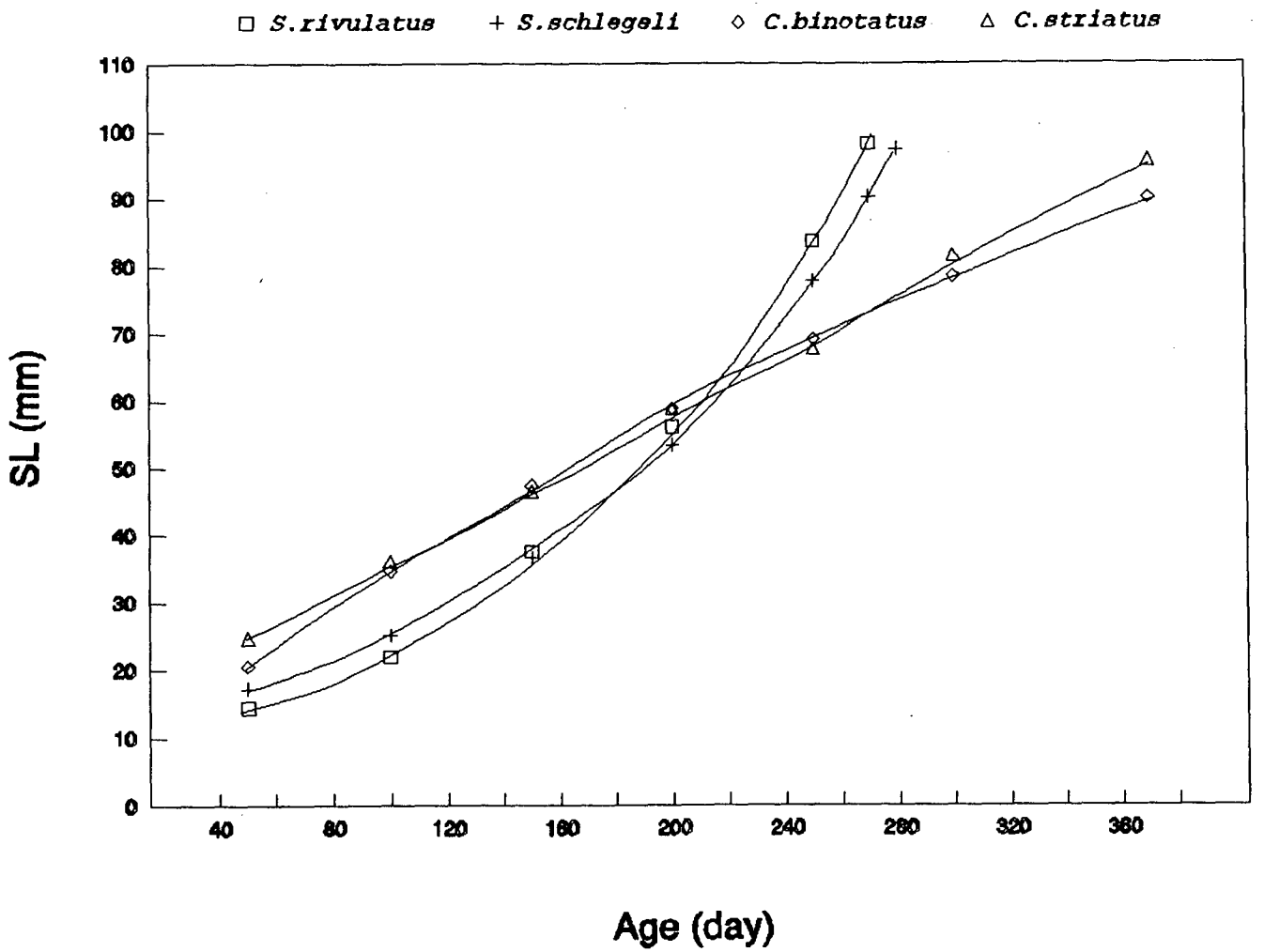
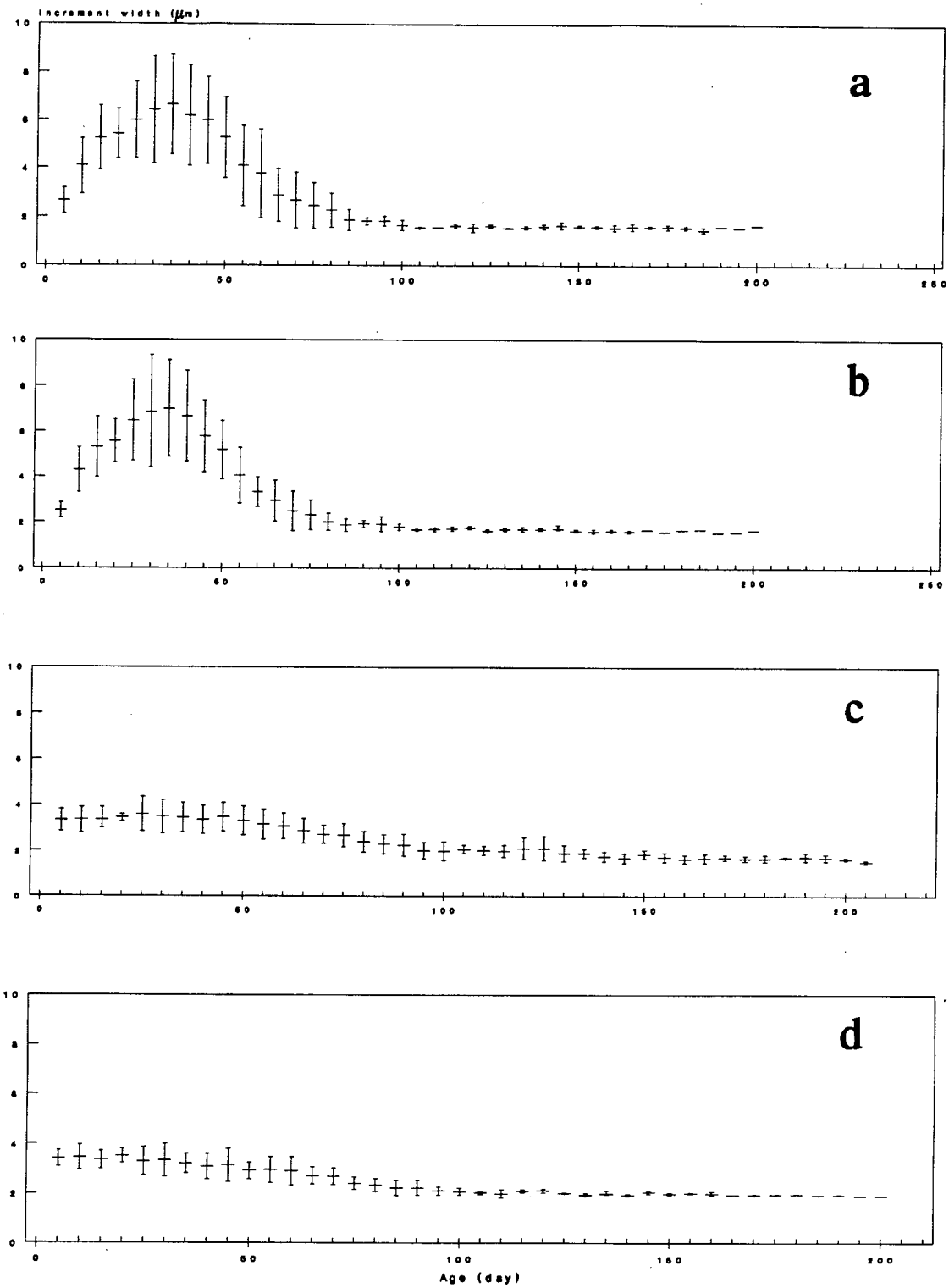

□ S.rivulatus
+ S.schlegeli
◇ C.binotatus
△ C.striatus
SL (mm)
110
100
90
80
70
60
50
40
30
20
10
0
40
80
120
160
200
240
280
320
360
Age (day)

Fig. 5.18 Otolith increment width series from ten juveniles.

Vertical bars indicate the standard deviation. (a) *Ctenochaetus binotatus*, (b) *Ctenochaetus striatus*, (c) *Scarus rivulatus* and (d) *Scarus schlegeli*.


Increment width (μm)
a
b
c
d
Age (day)

growth curve based on SL-at-age data from 15 juvenile and 18 adult individuals was:

$$L_t = 247.63(1-e^{-0.4847(t+0.02)}) \quad (r^2=0.9775 \quad p<0.001)$$

This curve appeared to fit the most age groups including the youngest groups (Fig. 5.20).

5.3.5.2 Arlington & Thetford Reef

The SL-WT relationship ($WT=1.48\times10^{-5}SL^{3.174}$ $r^2=0.9966$ $p<0.001$) was significant correlated for *S.rivulatus* in Arlington and Thetford Reefs. The size of specimens examined ranged from 33 to 257 mm in SL, and the age estimated was up to 5 years. The von Bertalanffy growth curve based on SL-at-age data from 8 juveniles and 18 adults was:

$$L_t = 279.37(1-e^{-0.3676(t+0.10)}) \quad (r^2=0.9710 \quad p<0.001)$$

This curve appeared only to fit to the growth of age 3, 4 and 5 (Fig. 5.20).

5.3.5.3 Growth comparison

Means of SL for each age group were listed for samples from Lizard Island, Arlington & Thetford Reef and Magnetic Island (Table 5.12). The growth in few young age groups (less than 2 years) at Arlington & Thetford Reef appeared to be slower than the growth in the other two locations as the SL was short comparing to the SL of the same age in the other two locations. However, for the most of age groups there was no large difference between the means of SL, especially in early ages (Fig. 5.20). This was also indicated by the close growth performance indexes from the three locations (1.5109, 1.4945 and 1.5311 for Lizard Island, Magnetic Island and Arlington & Thetford Reef respectively).

Table 5.12 Mean SL of *Scarus rivulatus* from different parts of the GBR.

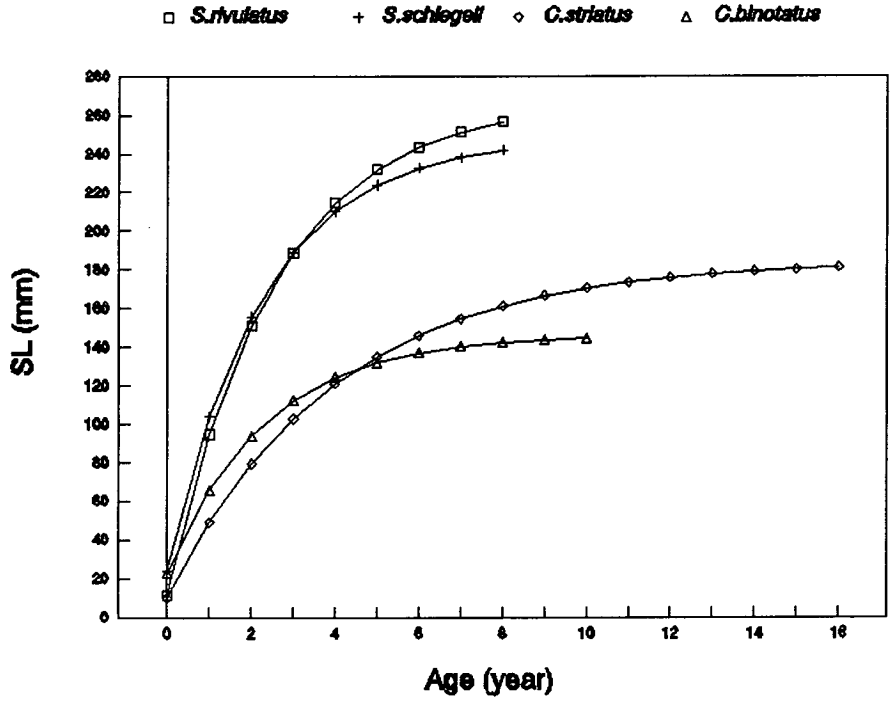
Sample size indicated in parenthesises. - No data available.

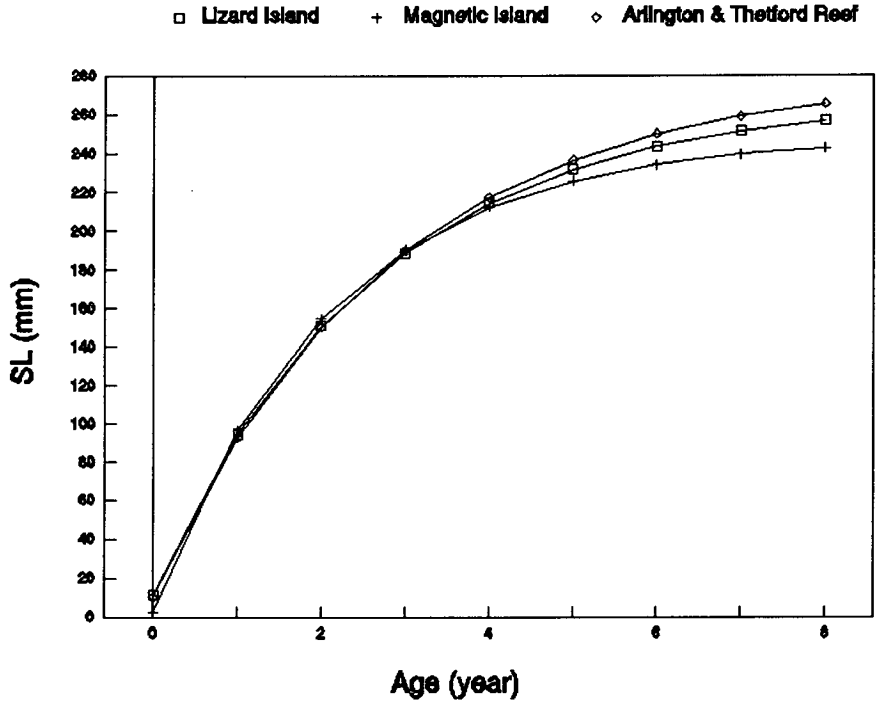
Age	Mean SL±s.d. (mm)		
	Lizard Island	Magnetic Island	AT. & TF. Reefs*
0.3	20.3±2.1 (3)	37 (1)	-
0.4	36.4±3.3 (10)	42.7±0.1 (3)	33 (1)
0.5	45.6±6.2 (20)	51.8±8.8 (6)	41.5±5.5 (2)
0.6	60.2±6.0 (9)	70.0±2.2 (3)	49.8±3.8 (4)
0.7	88.6±7.2 (16)	72.0±4.0 (2)	57 (1)
0.8	-	70.0 (1)	-
1	123.1±9.3 (7)	128.0±1.0 (2)	118.0±6.4 (3)
2	162.5±11.7 (102)	159.8±9.5 (5)	131.5±2.4 (2)
3	180.6±11.0 (75)	180.2±12.0 (6)	185.7±7.0 (6)
4	204.2±12.7 (62)	211.0±3.0 (2)	209.0±8.0 (4)
5	229.5±19.6 (37)	231.0±9.0 (2)	240.0±16.3 (3)

* Arlington and Thetford Reefs.

Fig. 5.19 von Bertalanffy growth curves for *Ctenochaetus biontatus*, *C.striatus*, *Scarus rivulatus* and *S.schlegeli*.

Fig. 5.20 von Bertalanffy growth curves for *Scarus rivulatus* **collected from Lizard Island, Magnetic Island and Arlington & Thetford Reef.**


S.rivulatus
S.schlegeli
C.striatus
C.binotatus
SL (mm)
Age (year)


Lizard Island
Magnetic Island
Arlington & Thetford Reef
SL (mm)
Age (year)

5.4 Discussion

5.4.1 Juvenile growth

The accuracy of the estimated age of juvenile acanthurids and scarids was dependent on when daily increment formation commenced within the otolith microstructure, and on the accuracy of delineating these increments. The age at which daily increments commence in the otoliths is not known in the species studied. Formation of increments may, however, start from as early as the pre-hatching "embryonic" phase, or as late as yolk absorption, depending upon the species. For fishes with pelagic eggs like *C.binotatus*, *C.striatus*, *S.rivulatus* and *S.schlegeli* distinct growth increments form at hatching or just thereafter (Brothers *et al.*, 1983). Therefore, juvenile age estimates in this study are most likely age estimates from hatching.

Estimates of the size at age of juveniles in the acanthurids could be checked against independent estimates made by Brothers *et al.* (1983) on members of the same family collected at the One Tree Reef of the southern GBR. Brothers *et al.* (1983) estimated the age of an acanthurid *Naso* sp. of 30.6 mm SL to be 84 days. This is similar to the estimates for *Ctenochaetus* in this study. As indicated in Tables 5.6 and 5.7, a mean of 83.6 ($\pm$2.6) daily increments were counted on 14 juvenile *C.binotatus* with a mean of 29.7 mm SL, and 83.5 ($\pm$1.6) of 4 *C.striatus* with 33.3 mm SL. Furthermore, Itano's (1988) estimate of 128 mm in fork length for one year old *Ctenochaetus striatus* is close to the estimate of 415 days for 124 mm in fork length (100 mm in SL) of the same species in this study (Fig. 5.11).

Brothers *et al.* (1983) also estimated the mean age of collection of unidentified individuals of the genus *Scarus* (mean length of 8.4 mm to be 42 to 58 days). No

scarid of this size was checked in this study. However, an individual of *S.rivulatus* 18 mm SL was 101 days and an individual of *S.schlegeli* 22 mm SL was 82 days. Until small individual of scarids of known identity can be collected and checked, it is not possible to directly compare the finding of Brothers *et al.* (1983) with this study.

Field observations of settlement of individuals were not carried out for the species in the present study. However, estimates of the numbers of days prior to settlement from otolith microstructure were in agreement with estimates of larval life by other authors. By comparing time of the spawning and the recruitment season, Randall (1961) estimated a larval life of about 8 weeks in the acanthurid *Acanthurus triostegus*. Counts of otolith increments in the acanthurids *C.binotatus* and *C.striatus* (Table 5.10) suggested a larval period from 47 to 74 d (about 7 to 10 weeks). Furthermore, by using pre-transition counts on otolith increments, Brothers *et al.* (1983) estimated a larval life of 74 d for another acanthurid (*Naso* sp), and a larval life of 30 to 50 d for two unidentified scarids. Estimates of larval life for the scarids (*S.rivulatus* and *S.schlegeli*) in this study ranged from 28 to 47 d (Table 5.10). Similar otolith checks to those observed in this study have been confirmed to be settlement checks in some species of parrotfishes and surgeonfishes (Brothers *et al.*, 1983). This study confirms that the acanthurids have relatively longer pelagic life than that of the scarids.

5.4.2 Adult growth

The growth estimates obtained from the size at age calculated from sagitta annuli of the acanthurids and scarids, were very close to the direct measurement of

growth based on the tag-recapture data (Table 4.11). The tagged *C.striatus* of 8^+ to 13^+ years old grew up to 3 mm TL over a period of 396 to 574 days while the mean of annual growth was estimated as 4.8 mm TL (3.3 mm SL) for the individuals of the same species within the same range of ages. A tagged scarid (*S.schlegeli*) at liberty for 453 days increased in TL from 173 mm to 220 mm (47 mm). This represents an annual growth increment of 38 mm TL. Counts of annual bands demonstrated that this individual was 3 years old at the time of recapture. The mean of annual growth from the size at age data (Table 5.5) for individuals of this species was 39.2 mm TL (32 mm SL) between age 1^+ and 3^+. For tagging work it is more appropriate to use TL due to difficulties in field measurements of SL.

Growth of the study species was described by three types of estimates: empirical methods which represent the mean size at age estimates for each species; back-calculation which is an estimate of the growth history of each individual; and theoretical growth curves obtained by fitting data to a model of growth curves.

Empirical growth curves provide size and age data. The reliability of this depends on a large sample size and size range. The second curve (back-calculated) was based on a relationship between the otolith radius and fish length, and may reveal the previous growth history of individuals. This curve generally had a close trend to that of the empirical curve, but provided an underestimate of length in comparison with size at age data in younger ages. In addition to Rosa Lee's phenomenon (Ricker, 1979), the tropical water environment could probably contribute the partial variation between empirical and back-calculated length. In the tropical water of the northern GBR, the time of reef fish spawning is more difficult to estimate since reproductive activity occurs throughout year (Thresher, 1984).

Mature gonads were detected in scarids during every sampling occasion (see details in Chapter 6). Unlike in other aging studies in temperate or cold water (Webb and Grand, 1979; Powell, 1982), an arbitrary brithdate was not estimated for the study species in this program. Therefore, an age group in this study included the individuals with the same number of otolith annuli but a range of birthdates. Consequently, the observed SL in the all four species studied is generally larger than the back-calculated SL, especially in the young ages due to faster growth in the fish earlier life. The backcalculation appares of little use.

In general, growth in SL in fish decreases exponentially with age (Jones, 1976). This was true in this study, and growth of the study species was best described by von Bertalanffy growth equation. Based on von Bertalanffy growth model, the four study species *C.binotatus*, *C.striatus*, *S.rivulatus* and *S.schlegeli* achieved 45%, 36%, 35% and 42% of the L_{∞} in the first year of their life respectively. Buesa (1987) presented similar percentage estimates of growth rate in tropical demersal fishes. The results in this study are close to that obtained by Buesa (1987), and suggest that many tropical demersal species have rapid growth during the early stage of life.

The estimated growth performance indices (Ø) for the all four study species appear to be slightly lower than that of members of the same genus calculated by Russ and St. John (1988) based on data obtained using the other techniques, which include tag-recapture (Randall, 1962), modal progression (Galzin, 1977) and direct observation (Bellwood, in prep.). In particular, the Ø value of *C.striatus* from Moorea (Galzin, 1977) is approximate 20% higher than that of the same species in the current study. While the Ø value of *Scarus frenatus* and *S.niger* from GBR

(Bellwood, in prep.) is relatively close to that of *S.rivulatus* and *S.schlegeli* in present study. These differences in the Ø value may be due to different locations or different methods of obtaining growth. More work is required.

The theoretical growth models in this study do not describe well the early growth of the scarids and acanthurids, and estimates of t_0 (von Bertalanffy model) and intercepts (power model) are biologically unrealistic. Power models only described the growth of the younger scarids and acanthurids, however, none of the models could be used to predict age accurately during the early life stage.

5.4.3 Growth comparison

The distinct differences in the growth pattern between acanthurids and scarids have been suggested by other authors (see review by Russ and St.John, 1988). Based on the validated aging methods for both juveniles and adults, this study confirm these growth difference between the scarids (*S.rivulatus* and *S.schlegeli*) and the acanthurids (*C.binotatus* and *C.striatus)*. As indicated by the growth performance indices, the adult parrotfishes grew significantly faster than the adult surgeonfishes (Table 5.11). In contrast, growth rates of the early-stage juvenile parrotfishes was relatively lower than that of the surgeonfishes of the same age (Tables 5.2, 5.3, 5.6 and 5.7). This phenomenon seems to partially agree with Randall's (1962) conclusion that the parrotfishes grew fastest among the coral reef herbivorous fishes. However, further detailed otolith microstructural analysis of other coral reef herbivorous species is required before Randall's (1962) conclusion can be confirmed.

The mechanisms of these observed familial differences in growth rates are unknown. Bellwood (1988) found significant differences in ontogenetic changes in

(Bellwood, in prep.) is relatively close to that of *S.rivulatus* and *S.schlegeli* in present study. These differences in the Ø value are probably due to the different method used in obtaining the growth data.

The theoretical growth models in this study do not describe well the early growth of the scarids and acanthurids, and estimates of t_0 (von Bertalanffy model) and intercepts (power model) are biological unrealistic. Power models only described the growth of the younger scarids and acanthurids, however, none of the models could be used to predict age accurately during the early life stage.

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The mechanisms of these observed familial differences in growth rates are unknown. Bellwood (1988) found significant differences in ontogenetic changes in

the diet between early post-settlement *Scarus* species and Acanthuridae. Based on analysis of intestinal contents, he found a marked change-over from carnivory to herbivory for early post-settlement scarids while acanthurids were found to be almost exclusively herbivorous. Moreover, Brothers *et al.* (1983) and Leis and Rennis (1984) demonstrated a different duration in the larval period of the two families, which was also observed in this study. It is presumed that this familial difference in settlement patterns (in terms of the age at settlement) may be attributed to the differences in the early growth, possibly influenced by dietary differences. To have a better understanding of the mechanism of this familial difference in growth rates, more comprehensive research should be carried out.

The comparison of growth rates of *S.rivulatus* from the different parts of the GBR in this study should be considered as a preliminary result since the sample sizes from Magnetic Island and Arlington & Thetford Reef were too small to be representatives of the populations. However, the limited length-at-age data did indicate that in general the growth of the parrotfishes from these places were similar. Further confirmation of this result will need larger samples.

In conclusion, the age of parrotfish population (*S.rivulatus* and *S.schlegeli*) at Lizard Island ranged up to 8 years with the majority being younger than 5 years. The growth rate in scarids increased with age in days during juvenile phase, and gradually decreased after that. The acanthurids (*C.striatus* and *C.binotatus*) lived longer than the scarids, and their growth rate decreased with age from as early as the juvenile phase. The acanthurids had a longer larval period from 47 to 74 days while the scarids have a relative shorter larval life of 28 to 47 days.

CHAPTER 6. REPRODUCTIVE BIOLOGY OF SCARIDS

6.1 INTRODUCTION

Most of the previous research on reproductive biology of sequentially hermaphroditic scarids has dealt with the distribution of sexes with size, sometimes correlated with a histological investigation of gonads (Choat, 1969; Choat and Robertson, 1975; Robertson and Warner, 1978; Yogo *et al.*, 1980). Very few studies have examined protogynous hermaphroditism in the context of age-specific events. Studies on scarids to date have used unverified aging methods (Warner and Downs, 1977). Validated age estimates and growth rates provide the basis for estimating the duration of different sexual phases, their growth and survivorship rates, times and rates of sexual transformation. Furthermore, other studies of reproductive biology in the closely relevant family Labridae show that individual age and growth play an important role in the process of sex change (Warner, 1975; Ross, 1984; Cowen, 1990). To understand the dynamics of sex-specific growth and mortality rates in scarids similar information has been gathered on the two studying species *S.rivulatus* and *S.rivulatus*.

Scarus rivulatus and *S.schlegeli* are both dichromatic and diandric (Choat and Robertson, 1975; Choat and Randall, 1986). The reproductive biology of both species has been studied at Heron Island in the southern GBR by Choat (1969). Based on field observation and histological examination, he examined the seasonal and ontogenetical features of reproductive cycles, where again he lacked information on demography.

This chapter will examine the following aspects of *Scarus rivulatus* and

S.schlegeli at Lizard Island:

(1) general sexual structure;

(2) the sexual patterns in relation to colour phase, size, and age;

(3) spawning schedule;

(4) spawning patterns and fecundity; and

(5) the impact of protogynous hermaphroditism and dichromation on age and growth.

6.2 MATERIALS AND METHODS

6.2.1 Specimen collection and processing

The analysis of reproductive biology was carried out with aged adults of *S.rivulatus* (n=314) and *S.schlegeli* (n=343) captured from June 1988 to January 1991 from Lizard Island (see Chapter 2).

At the laboratory at Lizard Island, gonads were dissected out from fresh specimens, and fat, mesentery and free fluid were removed from gonads before the gonads were weighed to the nearest 0.001 g on a Sartorius 2004 MP balance. Sexual identification of individual fishes was initially determined by examining the colour and general appearance of gonads (Ntiba and Jaccarini, 1990), and later confirmed by the histological examination. Gonads then were preserved with formaldehyde-CaCl2-Acetic acid (F.C.A.) fixative.

6.2.2 Histological examination of gonads

Gonads of the both species were prepared for histological examination. In the histological process, the middle part of left lobe from each gonad was cut, and

embedded in paraffin wax. Histological sections were cut at 6 μm thickness and stained with Mayer's Haematoxylin followed by a counterstaining with Young's Eosin-Erythrosin. More details of the histological process were described by Winsor (1983).

6.2.2.1 Classification of gonads

Each gonad was classified according to its sex and state of development. Assignment of a developmental class depended upon the predominant stage of gametogenesis seen in the gonad. The division of gametogenic stage was described as following:

Oogenesis was divided into five stages, following mainly the criteria described by West (1990) with some minor modification in regarding to the characteristic of scarid oocytes (Table 6.1).

Spermatogenesis occurred in small crypts. in which all the cells were at the same stage. The development and appearance of the spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, and mature sperm followed very closely the description in histological structure by Moe (1969), and illustrated in Table 6.2.

6.2.2.1.1 Female and secondary male

The gonadal developmental classes were based on gametogenesis states. The classification for females and secondary males (2♀♂) was described as following:

Class 1. Immature female; Plates 6.1a,2a.

Stage 1 and 2 oocytes were present, atretic or brown bodies were absent. The

ovarian lamellae were pressed closely together and the lumen was small.

Class 2. Mature resting female; Plates 6.1b,2b.

Stage 1, 2 and 3 oocytes present, with stage 2 predominating. Atretic bodies were usually present.

Class 3. Mature active female; Plates 6.1c,2c.

Stage 3, 4 oocytes were predominated in the lamellae. In late class 3, stage 5 oocytes were also present.

Class 4. Postspawning female; Plates 6.1d,2d.

Ovary was distended, with many empty follicles and degenerating stage 3 and 4 oocytes. Stages 1 and 2 were proliferating.

Class 5. Transitional; Plates 6.4a.

Both stage 2 oocytes and early stages of spermatogonia were predominated the gonad.

Class 6. Mature inactive male; Plate 6.3b.

Crypts containing primary and secondary spermatocytes were predominated; few spermatids and mature sperm were seen.

Class 7. Active mature male; Plate 6.3c.

Spermatids and spermatozoa were predominated the gonad. Collection of tailed sperm were common. Crypts of spermatogonia and primary spermatocytes were rare.

Class 8. Postspawning male; Plate 6.3d.

Ducks were still expanded, but few sperm could be seen in them. Many new crypts containing spermatogonia were present. Testes was dominated by stormal tissue and rapidly cryptos of spermatogonia.

Table 6.1 Stages of scarids' oogenesis.

See West (1990) for histological description. Oogenesis length was measured on the histological sections

Stage	Description	Oogenesis length (μm)	
		S.rivulatus	*S.schlegeli*
1	Chromatin nucleolar stage	10-30	15-30
2	Perinucleolar stage	30-170	30-140
3	Yolk vesicle formation	150-410	80-390
4	Vitellogenic stage	350-810	200-550
5	Mature (ripe) stage	up to 1080	up to 670

Table 6.2 Stages of scarids' spermatogenesis.

See Moe (1969) for histological description. Spermatogenesis diameter was measured on the histological sections.

Stage	Description	Spermatogenesis diameter (μm)	
		S.rivulatus	*S.schlegeli*
1	Spermatogonia	7-9	7-9
2	Primary spermatocytes	3-4	3-4
3	Secondary spermatocytes	2-3	2-3
4	Spermatids	1	1
5	Spermatozoa	<1	<1

Plate 6.1 *Scarus rivulatus*. **Photomicrographs of ovary development classes 1 through 4.**

S1, 2, 3 and 4 indicate the oocytes at stage 1, 2, 3 and 4. Scale bar = 200 μm. **a.** Class 1, immature female; age 2, 167 mm SL. **b.** Class 2, mature resting female; age 3, 187 mm SL. **c.** Class 3, mature active female; age 2, 166 mm SL. **d.** Class 4, postspawning female; age 5, 234 mm SL.

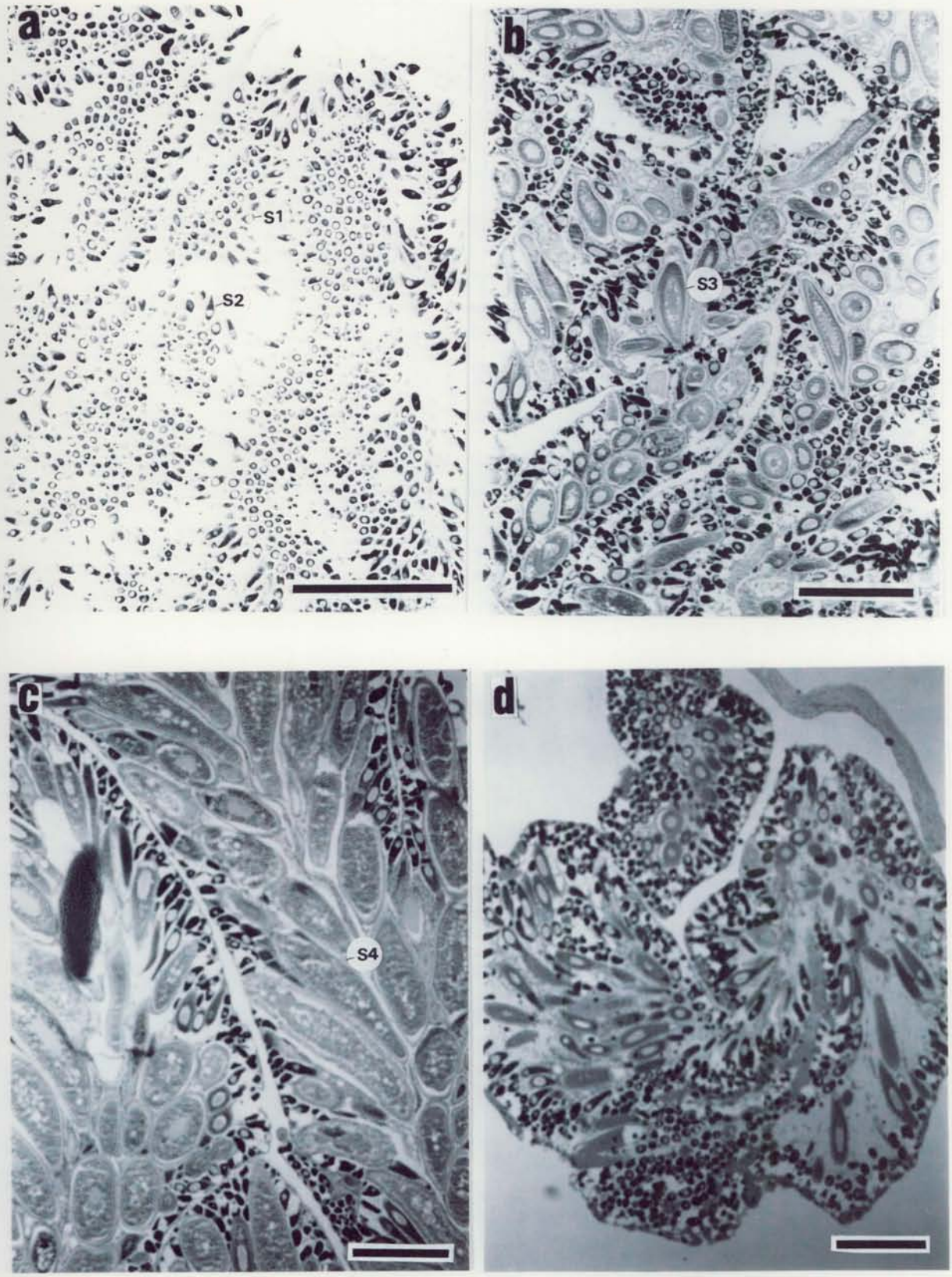

a
S1
S2
b
S3
c
S4
d

Plate 6.2 *Scarus schlegeli*. **Photomicrographs of ovary development classes 1 through 4.**

S1, 2, 3 and 4 indicate the oocytes at stage 1, 2, 3 and 4. Scale bar = 200 μm. **a.** Class 1, immature female; age 3, 158 mm SL. **b.** Class 2, mature resting female; age 2, 169 mm SL. **c.** Class 3, age 4, 200 mm SL. **d.** Class 4, postspawning female; age 5, 217 mm SL.

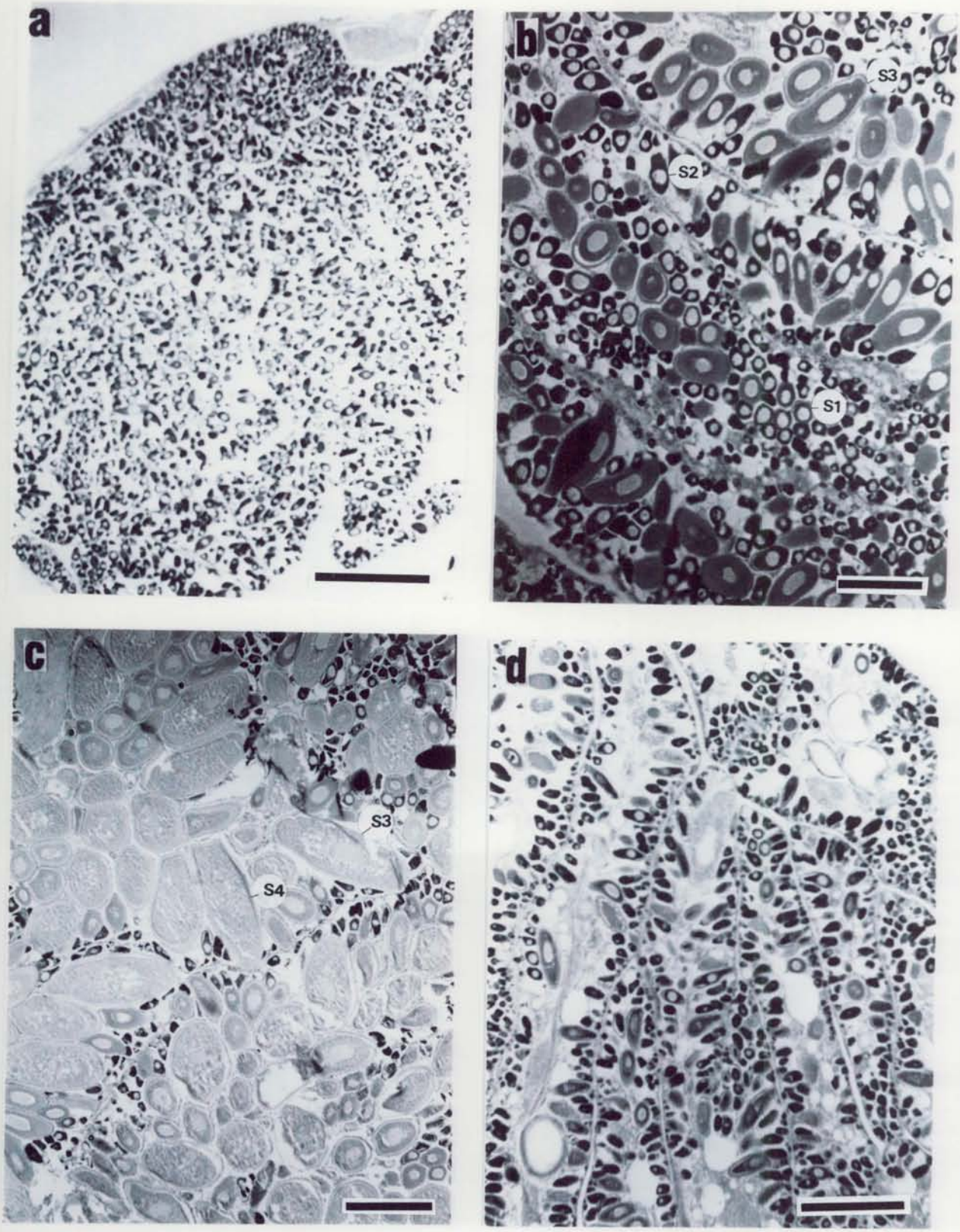

a
b
S3
S2
S1
c
S3
S4
d

Plate 6.3 Photomicrographs of gonad development for male *Scarus rivulatus* and *S.schlegeli*.

Sg - spermtogonia; PSc - primary spermatocyte; SSc - secondary spermatocyte; Sd - spermatids; Sz - spermatozoa; TS - tailed sperm. Scale bar = 20 μm. **a.** Class 1, immature male; age 1, 127 mm SL. **b.** Class 2 (Class 6), mature resting male; age 3, 185 mm SL. **c.** Class 3 (Class 7), mature active male; age 3, 184 mm SL. **d.** Class 4 (Class 8), postspawning male; age 3, 187 mm SL.

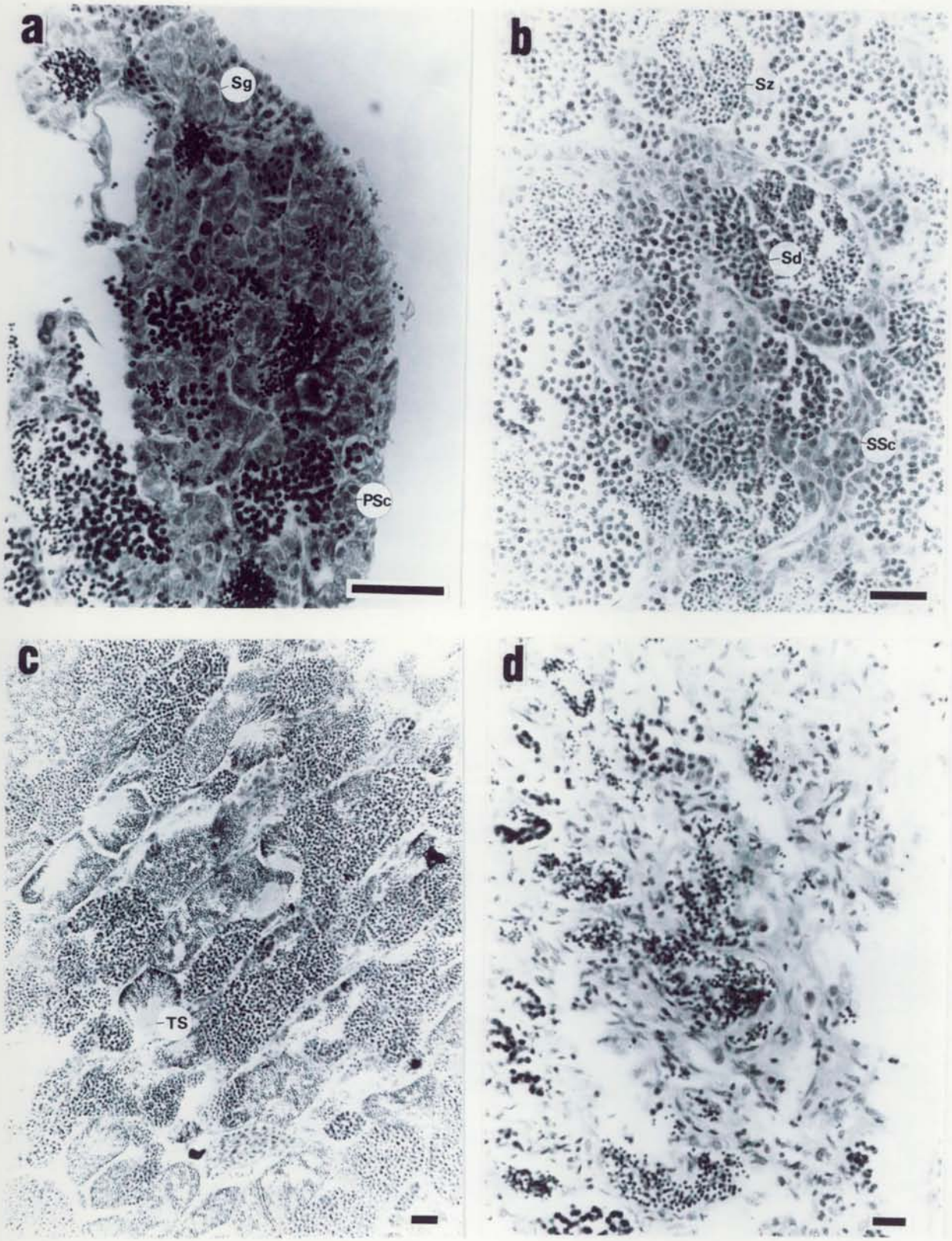
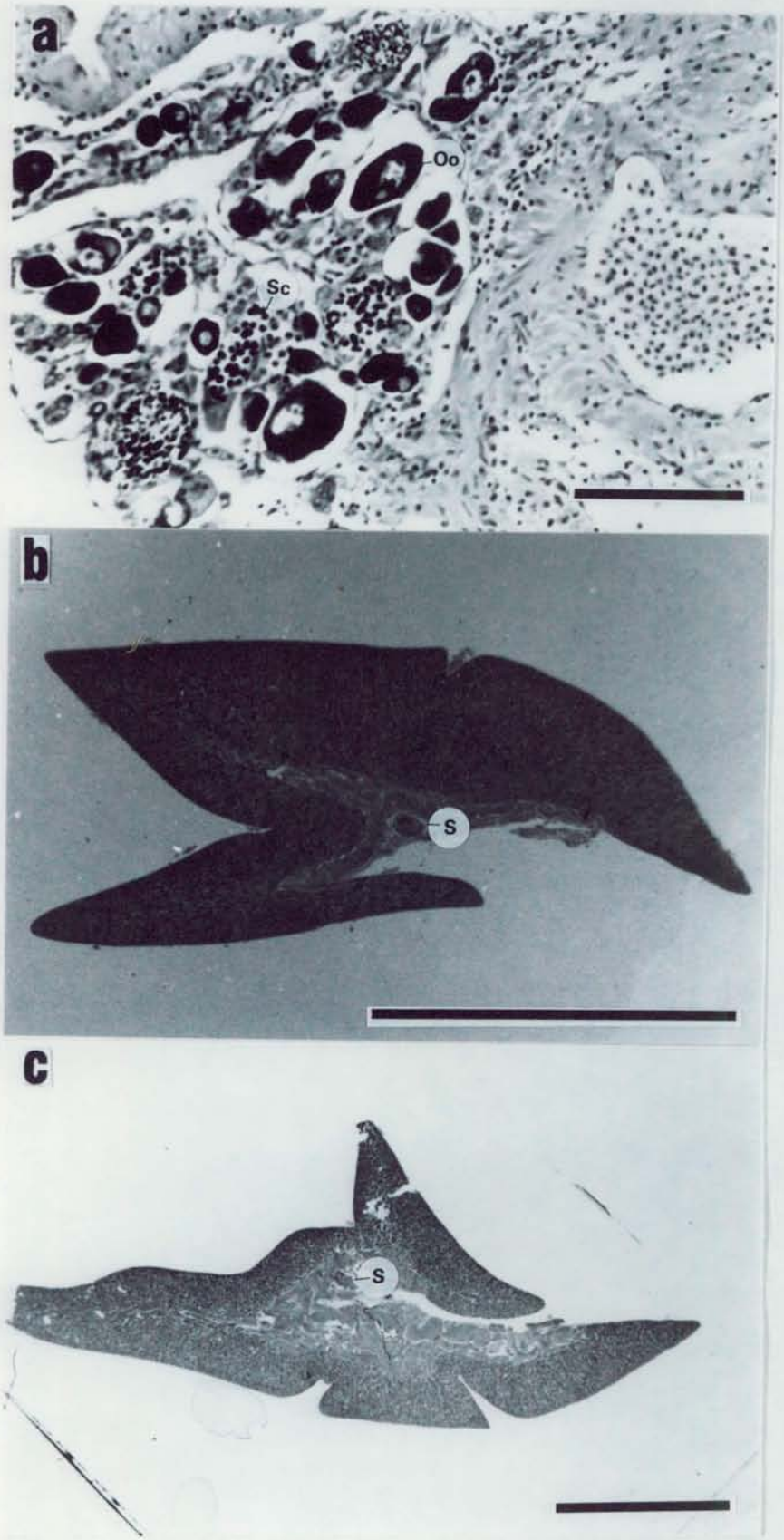

a
Sg
PSc
b
Sz
Sd
SSc
c
TS
d

Plate 6.4 The gonads of transitional and 1°♂♂ individuals.

a. The transitional gonad of *Scarus schlegeli*; age 3, 190 mm SL. Oo - oocyte; Sc - spermatocyte. Scale bar = 25 μm. **b.** The testes of 1°♂♂ *S.rivulatus*; age 4, 201 mm. **c.** The testes of 1°♂♂ *S.schlegeli*; age 4, 228 mm SL. In **b** and **c** S - sperm duct. Scale bar = 5 mm.


a
Oo
Sc
b
S
c
S

6.2.2.1.2 Primary male

The state of testes development was divided into four classes for primary males (1°♂♂). The later three classes were similar to the above Class 6, Class 7 and Class 8 respectively while the class 1 was immature male (Plate 6.3a). In this class spermatogonia were dominated in most crypts with sometimes primary spermatocytes present.

6.2.2.1.3 Distinction between 1°♂♂ and 2°♂♂

There was no morphological difference between 1°♂♂ and 2°♂♂ in scarids. However, the structure of testis in both types of males generally reflected their sexual origins, which can microscopically be told on the histological sections. The criteria described by Sadovy and Shapiro (1987) was followed in this study to distinguish the testises of 1°♂♂ and 2°♂♂.

6.2.2.2 Pilot study on oocyte sampling

A pilot study was carried out to establish the most efficient sampling program for the estimation of oocyte size over different seasons. The sources of variation checked in this pilot program were as follows: times (months) with each season; individuals with the sample taken each month; the region of the gonad from which oocytes were measured. This provided the basis for comparing oocyte size in mature scarids collected at different seasons, taking all the above sources of variation into account.

In this pilot study three mature active ovaries (Class 3) were randomly selected from the samples collected in each sampling month for summer (December

and March) and winter (May and July) for the both species. As the pervious tests for differences in average oocyte size or oocyte size-frequency distribution along the length of the ovary generally showed no significant differences (see review by West 1990), the histological sections used were taken from the middle of each left lobe. Each section was divided into left (ML), central (MC) and right (MR) three regions, and two sampling parts were selected within each region (Fig. 6.1a,b). The area of each part was determined by a Weibel eyepiece as 0.81 mm^2 at 100×. Within each sampling part, the maximal lengths of 5 biggest oocytes were measured using an eyepiece micrometer due to a banana-shape of scarids' eggs, and the area fraction of later stage oocytes was also estimated by the stereological technique (Weibel and Elias, 1967; Briavty, 1975). In this technique each part was evenly divided into 42 points under a Weibel eyepiece, and points were counted for every stage oocytes which were covered by the points using a multi-counter. The analysis of variance (ANOVA) was used to examine results of the measurements in oocyte length using a mixed model, in which the factors of season and region were fixed while the rest factors were random. The calculation was made using the computer package of Statistic Analysis System (SAS). The sampling design for this pilot study was shown in Figure 6.1a.

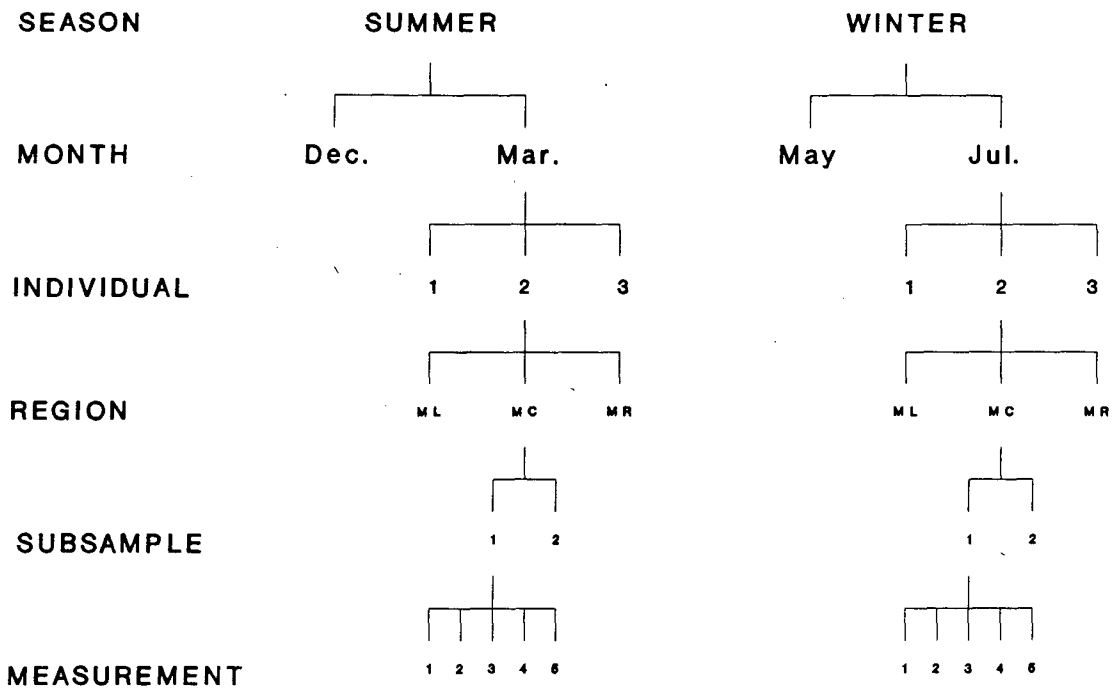
6.2.2.3 Ovary examination

Following the pilot study the length of oocytes in each mature ovary (Class 2 - 4) was then determined as the mean length of the five biggest oocytes from the central area (MC) of each cross-section, and measured for the specimens caught from December 1989 to January 1991. Furthermore, area fractions of the oocytes in later

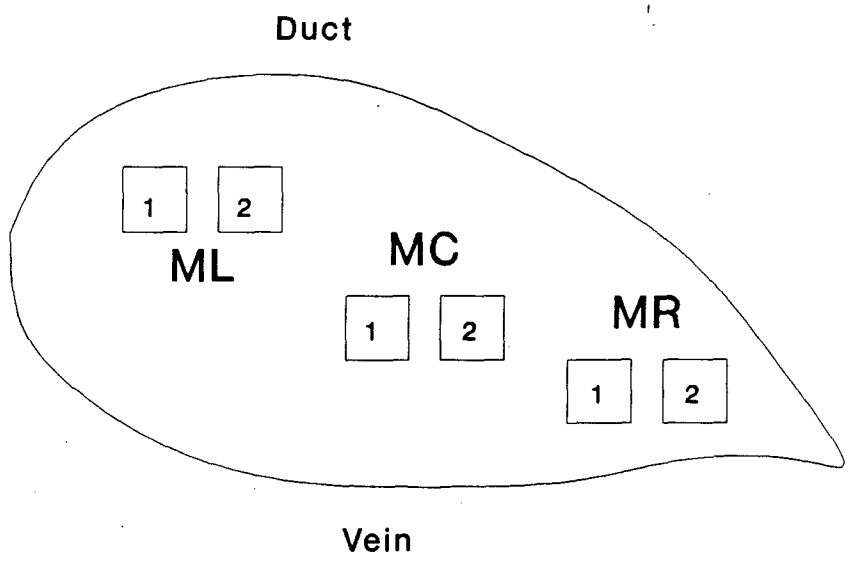
Fig. 6.1 Summary of the pilot study on oocyte measurements.

(a). Sampling design; (b) Sampling locations on a transverse section of the ripe ovary.

a



b



A transverse section of ovary

stages (stage 3,4 and 5) were also estimated from the same area in each section.

6.2.3 Gonadosomatic Index

To examine trends in gonad development and size, a gonadosomatic index (GSI) was calculated as:

GSI=gonad weight(g)×100/WT(g)

As the mature stage in TP males was generally not relative to the size or weight of gonads in scarids (Choat,1969), the GSI of TP males was not examined in this study. Specify size limits for while GSI were calculated.

6.2.4 Fecundity

Fecundity estimates were made using egg samples from the posterior portion of the left lobes in 20 mature ovaries from each species. The size and age ranges of these ripe females were selected as wider as possible. The gravimetric subsampling method (MacCregor, 1957; Hunter *et al.*, 1985) was used to estimate the total number of eggs in the ovaries (individual fecundity). In this method, a thick cross-section was cut from the posterior part of each lobe, weighed, and then agitated to dislodge as many oocytes as possible from the ovarian lamellae. Oocytes remaining in the lamellae were teased out so that a complete count could be made. The minimal weight of cross-section was one-tenth of the ovary. An estimate of the number of yolky oocytes per gram of ovary could then be made directly from the sample. The total number of eggs in the ovary was then approximated by multiplying by the total weight of the ovary. The relationships between fecundity and size as well as age were then examined for both species.

6.2.5 The relationship between sex and growth rate

To determine the relationship of sex and individual growth, mean lengths of females, IP 1○♂♂, TP 1○♂♂ and 2○♂♂ in each age group were compared. In addition, means of gonad weights between these sexual types were also compared. All these comparisons were made by using ANOVA.

6.3 RESULTS

6.3.1 Anatomical features of the gonads

Primary males in the both species generally had large testes with centrally located sperm ducts (Plate 6.4b,c). In contrast, the testes of 2○♂♂ were relatively small with peripheral sperm sinuses. Furthermore, the testes of 2○♂♂ clearly indicated their ovarian origin by being lobate, with a central lumen and atretic bodies (Plate 6.5a,b). Viewed under higher magnification, the atretic bodies appeared compact, containing granular yellow-brown materials intersperated with often basophilic, nuclei (Plate 6.5c). However, a few atretic bodies were also occasionally presented in testes of 1°♂♂ in *S.schlegeli*. This made distinction of 2°♂♂ testes difficult by only observing these atretic. Therefore, a combination of all main criteria were followed to distinguish testes of both 1°♂♂ and 2°♂♂.

The above features in the testis of 1°♂♂ and 2°♂♂, however, seemed to change with increasing the time individuals spent after sexual transformation or color phase change. The testis of 2°♂♂ were split open along the weaker ventral seam and the lamellar were evaginated soon after sexual transformation. Consequently, the ovarian lumen gradually disappeared, and the marginal sperm sinuses gradually became central located. The testis of 1°♂♂ generally reduced its size after changing

into the terminal color phase (Fig. 6.2). Eventually, the testis of 1°♂♂ may loss lobes and coalece, changing into the lobe shape like 2°♂♂ testes. It would, therefore, seem to be difficult to distinguish testes between the oldest 1°♂♂ and 2°♂♂ as the both types of testes could be similar in appearance.

The transitional gonads in *S.schlegeli* showed that male and female germinal tissues were not separated by connective tissue, and the two tissue types were intermixed during the course of sex reversal (Plate 6.4a). This type of the configuration of germinal tissues was classed as the undelimited type 2 (Sadovy and Shapiro, 1987).

6.3.2 General sexual structure

Of 314 *S.rivulatus* examined, 204 individuals were female and the rest 110 were both IP (n=57) and TP (n=53) male. Histological examination indicated that all 57 IP male were 1○♂♂ while in the 53 TP males 34 were 2○♂♂, and the rest were 1○♂♂.

In 343 *S.schlegeli*, 6 specimens were found to be sexually transitional, 208 individuals were female and the rest 129 were are either IP (n=71) or TP male (n=58). In the 58 TP males there was a large number of 2○♂♂ (n=53).

6.3.3 Distribution of sexual and colour patterns

To illuminate the life history patterns of parrotfish population at Lizard Island, the gonad development classes were grouped in the following categories:

(1). immature (IM): Class 1 female and male;

(2). female: Classes 2,3 and 4;

Plate 6.5 The testes of 2°♂♂ individuals.

OL - ovarian lumen; ss - sperm sinus; AB - atretic body. In **a** and **b** scale bar = 1 mm. **a.** The gonad of 2○♂♂ *Scarus rivulatus*; age 3, 194 mm SL. **b.** The gonad of 2○♂♂ *Scarus schlegeli*; age 5, 225 mm SL. **c.** The similar gonad of **b** viewed under high power. Scale bar = 25 μm.

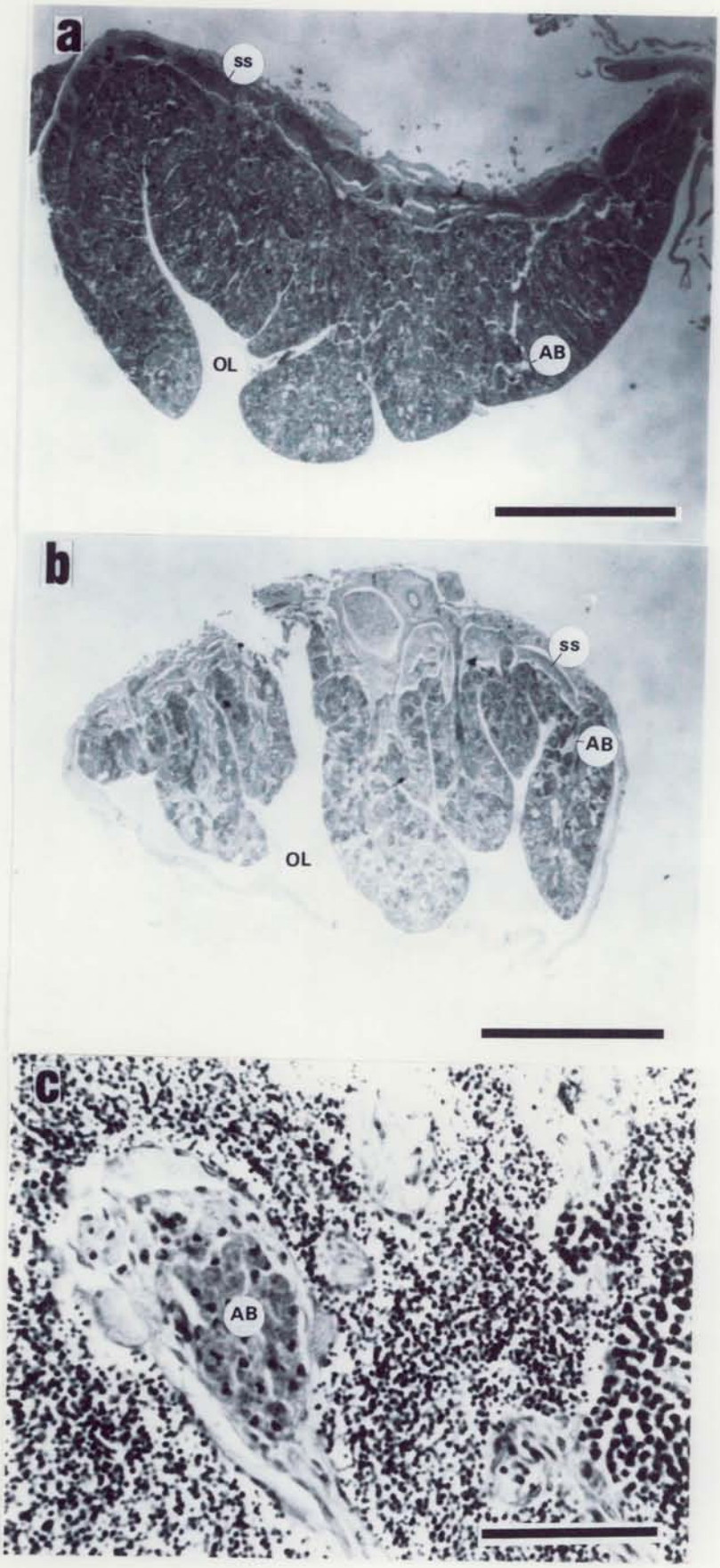

a
SS
OL
AB
b
SS
AB
OL
c
AB

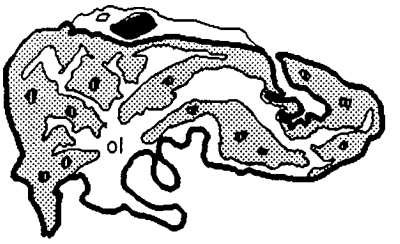
Fig. 6.2 The schematic diagrams showing morphological change of testes in 1°♂♂ and 2°♂♂ with age.

ol - ovarian lumen; ▨ - spermtagenetic tissue; ▥ - oogenetic tissue and ■ - sperm ducts.

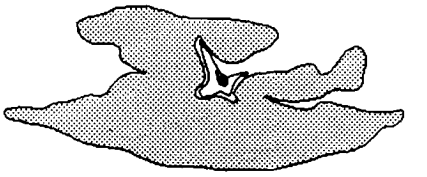
2° ♂♂ TESTIS

1° ♂♂ TESTIS

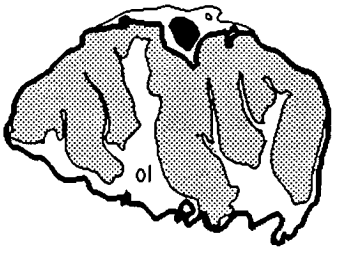
Transitional



IP 1° ♂♂

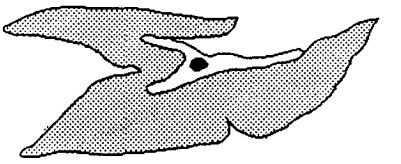


Young 2° ♂♂

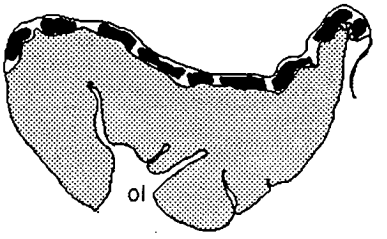


Increasing Age

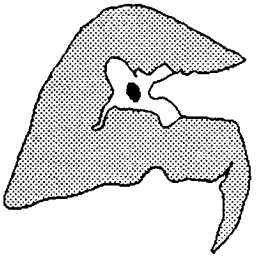
Young TP 1° ♂♂



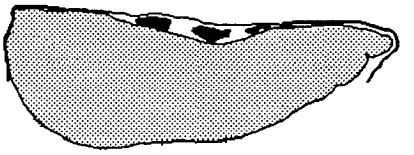
2° ♂♂



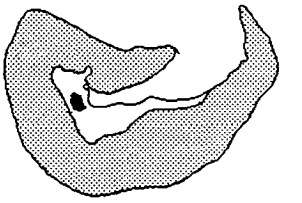
TP 1° ♂♂



Older 2° ♂♂



Older TP 1° ♂♂



(3). transitional: Class 5 only;

(4). 1○♂♂: Male Classes 2,3,4; and

(5). 2○♂♂: Classes 6,7 and 8.

6.3.3.1 *Scarus rivulatus*

The distribution of sexual and colour patterns by SL (Table 6.3) as well as the relative frequencies for each size grouping (Fig. 6.3) indicated that the small size class (100 -119 mm) was made up exclusively of IM female individuals. Mature females gradually became abundant in the next size classes (140 -239 mm), and then became less numerous as TP 1○ and 2○♂♂ began to predominate in the largest sizes (240 - 299 mm).

Within IM and IP individuals males (1○♂♂) shared the small proportion (29.72% in IM; 20.53% in IP). Within TP individuals 2○♂♂ occupied a relative large proportion (64%), the size range of which was close to that of 1○♂♂.

At Lizard Island most females and 1○♂♂ matured at SL between 140 and 180 mm with the minimal SL at sexual maturation was recorded 141 mm for female, 138 mm for 1○♂♂. Although no individual at sexual transformation was collected, it appeared that sexual transformation began from approximate 200 mm SL as 2○♂♂ started to present from this size. Moreover, IP 1°♂♂ seemed to change colour phase slightly earlier than females.

The actual time courses for all these events became evident when the relative frequency of the sexual types in each age group were graphed (Fig. 6.4). The curves were based on the frequency distribution listed in Table 6.4. Sexual maturity began in the second year of life for most females and males. females were abundant at age

Class 2,3 and 4 while males increased at age class 5 and thereafter. Although one female was found to be the oldest (8^+ year) the proportion of male was high in age class 6^+ and 7^+. Majority of *S.rivulatus* spent no more than 3 or 4 years as functional females. However, the age estimate data suggested that some females retained thire sexual identity throughout life.

6.3.3.2 *Scarus schlegeli*

The distribution of sexual and colour types by SL (Table 6.5) as well as the relative frequencies for each size group (Fig. 6.5) showed that the IM male and female individuals were exclusively predominated in the smallest size class (100 - 119 mm). Both the mature female and 1♀♂ were gradually abundant in the next size classes (160 - 199 mm), and then became less numerous as TP 2♀♂ began to dominate in the largest size classes. Sexual transformation was found in intermediate sizes ranging from 160 to 239 mm while the colour phase of IP 1°♂ also started from 160 mm SL.

In the IM and IP 1♀♂ individuals shared 40.81% and 22.17% respectively, and no 2♀♂ individual was found. Within the terminal phase 2♀♂ occupied a larger proportion with a wider size range (81%) while a few 1♀♂ were observed within the relative smaller size range (200 - 239 mm).

Most of female and 1♀♂ matured at SL between 140 and 180 mm with the minimal mature SL was measured as 138 mm for female and 152 mm for 1♀♂. Sexual transformation occurred over a broader size range from 160 to 239 mm SL.

The relative frequencies of sexual types in each age group (Fig. 6.6) based on data in Table 6.6 indicated the actual time courses of these life events. Sexual

Table 6.3 Frequency of sexual types, color phase in each 20-mm size class for *Scarus rivulatus*.

Colour	phase	IP				TP	
SL (mm)	N	IM ♀♀	IM 1°♂	♀♀	1○♂♂	1○♂♂	2○♂♂
100-119	2	2					
120-139	8	4	3		1		
140-159	36	10	5	12	9		
160-179	94	10	3	59	22		
180-199	59			49	9	1	
200-219	53			40	4	7	2
220-239	28			15	1	5	7
240-259	21			3		4	14
260-279	10					2	8
280-299	3						3
Total	314	26	11	178	46	19	34

Table 6.4 Frequency of sexual types and color phase in each age group of *Scarus rivulatus*.

Colour phase		IP				TP	
Age (year)	N	IM ♀♀	IM 1°♂♂	♀♀	1○♂♂	1○♂♂	2○♂♂
1+	7	4	3				
2+	103	18	8	52	25		
3+	74	4		54	14	2	
4+	62			45	6	8	3
5+	37			19	1	4	13
6+	22			5		4	13
7+	8			2		1	5
8+	1			1			
Total	314	26	11	178	46	19	34

Table 6.5 Frequency of sexual types and color phase in each 20-mm size class for *Scarus schlegeli*.

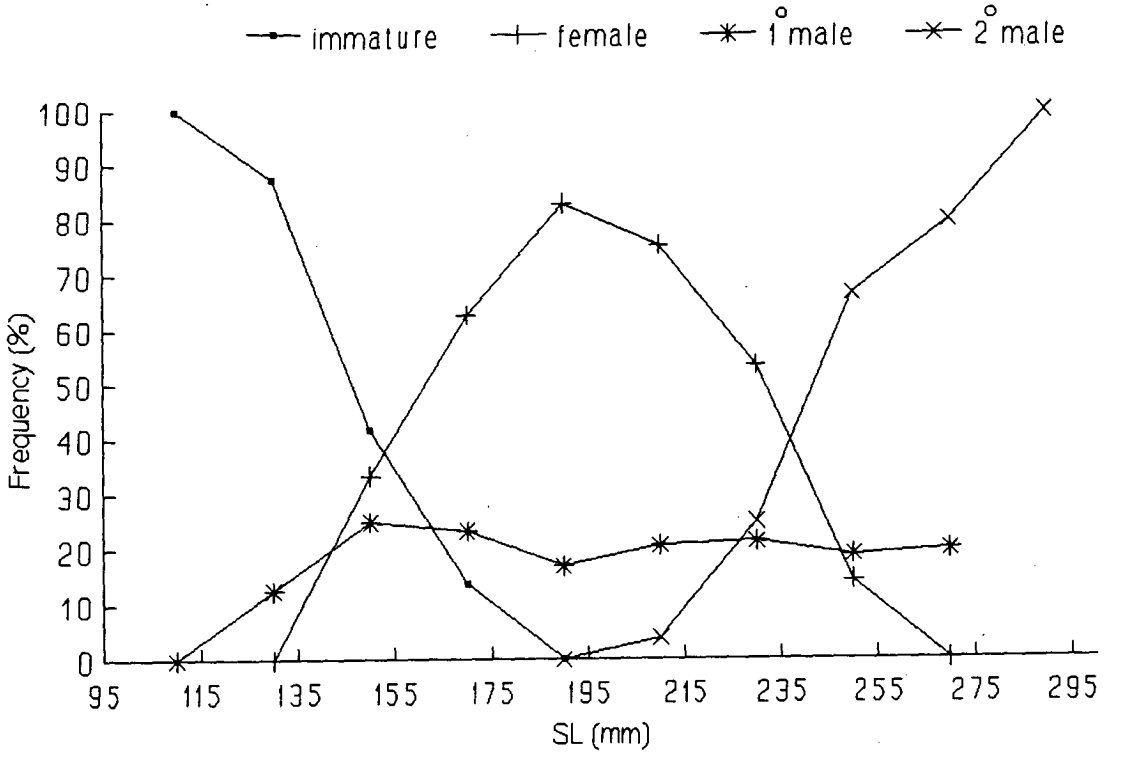
Colure	phase	IM		IP		Trans		TP	
SL (mm)	N	♀♀	1°♂♂	♀♀	1°♂♂	♀♀→2°♂♂	1°♂♂→1°♂♂	1°♂♂	2°♂♂
100-119	14	6	8						
120-139	8	4	3	1					
140-159	24	9	6	6	3				
160-179	74	10	3	45	14	1	1		
180-199	126			94	28	1			3
200-219	54			30	4	1		2	17
220-239	36			3	2	2		3	26
240-259	6								6
260-279	1								1
Total	343	29	20	179	51	5	1	5	53

Table 6.6 Frequency of sexual types and color phase in each age group of *Scarus schlegeli*.

Colour	phase	IM		IP		Trans		TP	
Age (year)	N	♀♀	1°♂♂	♀♀	1○♂♂	♀♀→2°♂♂	1°♂♂→1°♂♂	1○♂♂	2○♂♂
1+	25	10	14	1					
2+	71	15	6	37	11	1	1		
3+	136	4		92	35	1		1	3
4+	69			42	4	1		1	21
5+	32			5	1	2		1	23
6+	7			1				2	4
7+	2			1					1
8+	1								1
Total	343	29	20	179	51	5	1	5	53

Fig. 6.3 Proportions of each sexual type in successive 20 mm SL groupings of *Scarus rivulatus* from Lizard Island.

Fig. 6.4 Proportions of each sexual types in each age group of *Scarus rivulatus* from Lizard Island.


immature
female
1°male
2°male
Frequency (%)
100
90
80
70
60
50
40
30
20
10
0
95
115
135
155
175
195
215
235
255
275
295
SL (mm)

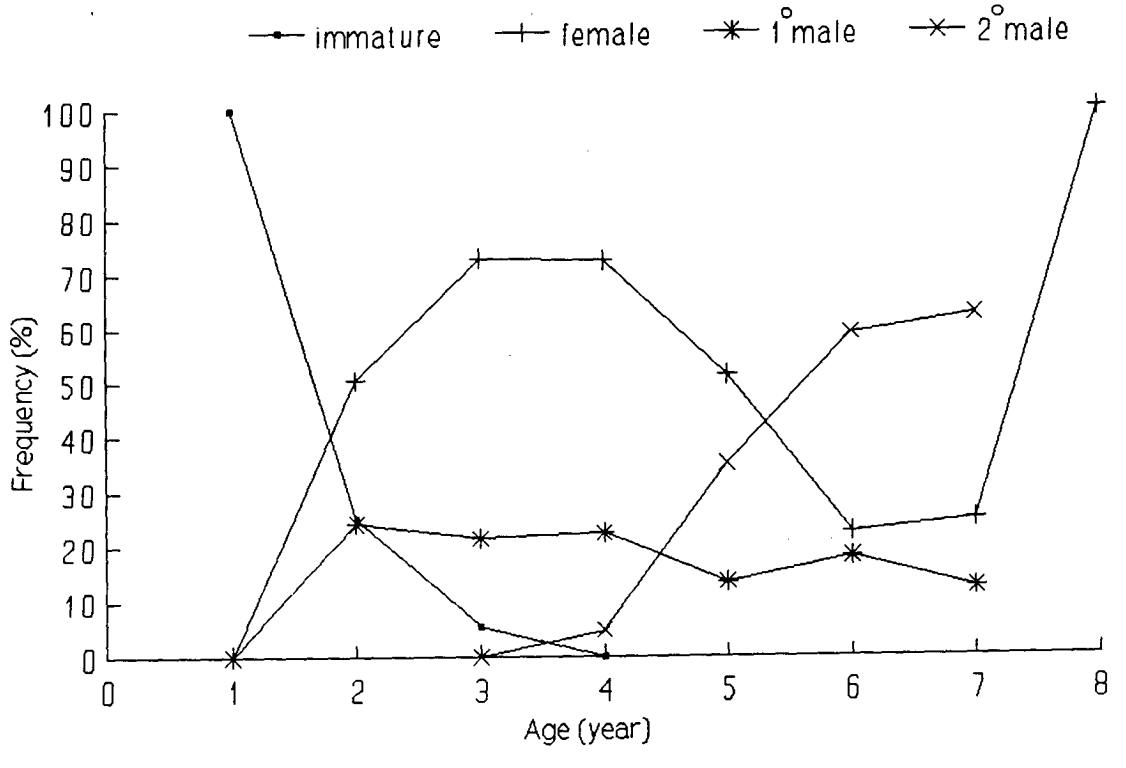
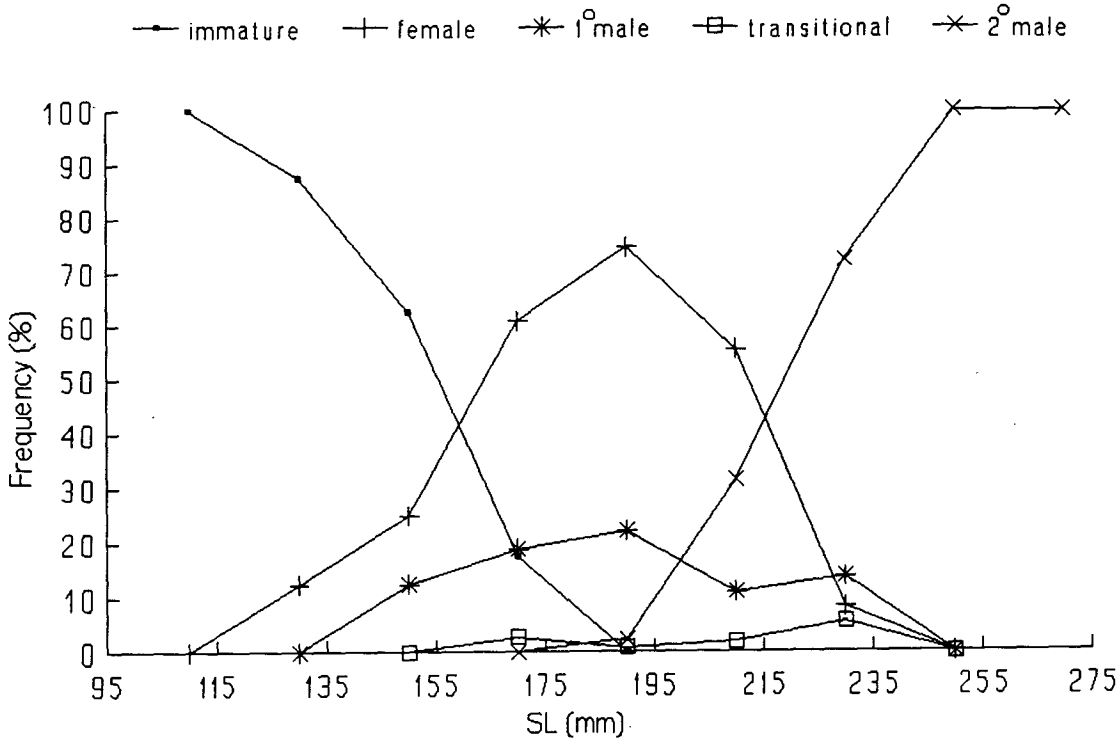

immature
female
1°male
2°male
Frequency (%)
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
8
Age (year)

Fig. 6.5 Proportions of each sexual type in successive 20 mm SL groupings of *Scarus schlegeli* from Lizard Island.

Fig. 6.6 Proportions of each sexual type in each age group of *Scarus schlegeli* from Lizard Island.


immature
female
1°male
transitional
2°male
Frequency (%)
100
90
80
70
60
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40
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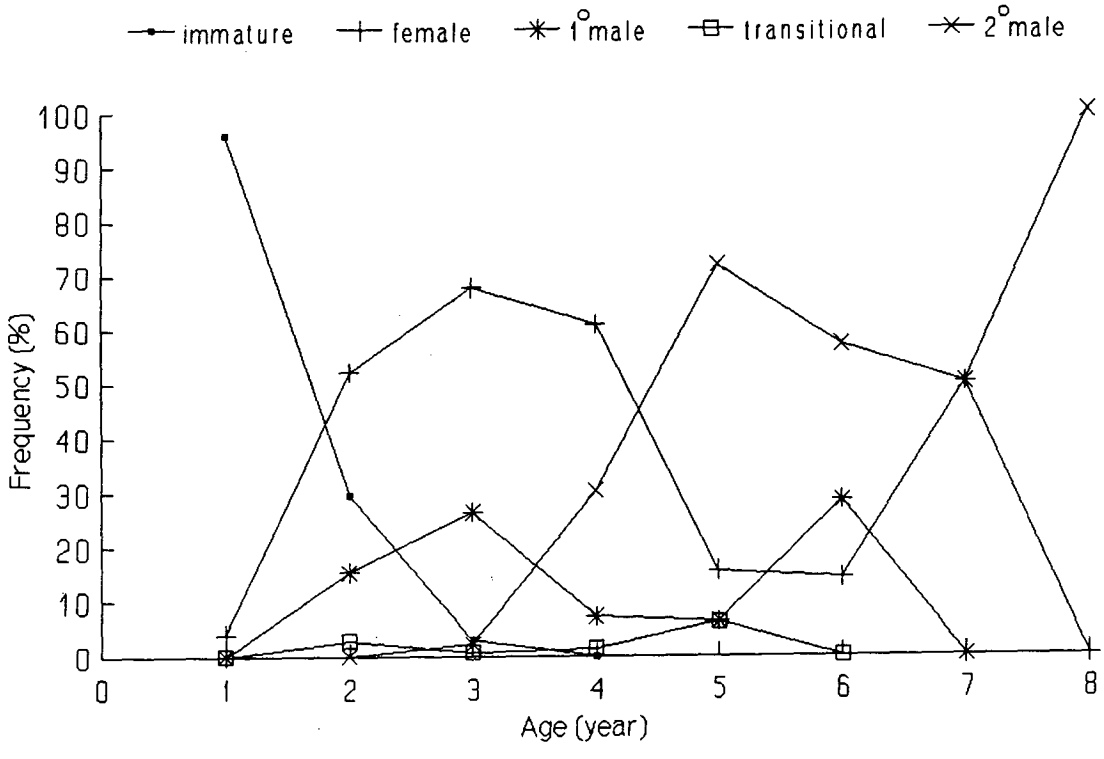
10
0
95
115
135
155
175
195
215
235
255
275
SL (mm)


immature
female
1°male
transitional
2°male
Frequency (%)
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
8
Age (year)

maturity started in the secondary year of life for most of females and males. In age groups 2, 3 and 4 females occupied the largest proportion in the population while males became to predominate at age group 5 and thereafter even though one individual female lived up to age 7^{+}. Like *S.rivulatus*, most of *S.schlegeli* spent 2 or 3 years as functional female while few individuals lived longer as female.

6.3.4 Seasonal pattern of gonad activities

6.3.4.1 Temporal distribution of gonad development classes

6.3.4.1.1 *Scarus rivulatus*

The seasonal distribution of ovary development classes for 178 *S.rivulatus* collected between December 1989 and January 1991 was shown in Figure 6.7. As expected in tropical water, mature active females (Class 3) and resting females (Class 2) occurred throughout the year. However, their relative proportions varied through the year. The active females (Class 3) occupied the largest proportion in September (75%), November (87%), December (81%) and January (87%) respectively while they were the lowest in proportion in February (33%). This pattern suggested that spawning peak took place from September throughout January.

Further support for designating this period as the spawning peak came from the GSI distribution (Fig. 6.8) of mature females caught in the different months. This reflected a close pattern seen in the analysis of gonad development states (Fig. 6.7). After a quiescent period from February through May, the ovaries began to increase in size until a maximum was reached in November and December. The GSI showed a trend of gradual reduction from December.

The seasonal distribution of testes development classes indicated that active

1°♂♂ (Class 3) and 2°♂♂ (Class 7) occurred almost year round (Fig. 6.9). A trend in proportion of active testes in 1°♂♂ was close to that of the ovaries while no such trend was found in proportion of active testes in 2°♂♂. The active testes in 2°♂♂ appeared to be abundant in most of months through the year even though a small number of specimens was collected in each sampling occasion. Moreover, most of TP samples caught during January, February and March were found as the resting males of either Class 6 or Class 8.

The GSI distribution of mature IP males was generally similar to that of females although the standard deviations were found to be wider for specimens in the some months (Fig. 6.10).

6.3.4.1.2 *Scarus schlegeli*

A different pattern of the seasonal distribution in ovary development states from *S.rivulatus* was found in *S.schlegeli* (Fig. 6.11). Even though active females were present in all sampling months throughout the year, the largest proportion occurred in July (90%) and September (86%) while 10% was estimated in February. This pattern strongly suggested that spawning peak took place from May through the end of September.

This pattern in spawning was further supported by the seasonal distribution of mature ovary indices from 139 *S.schlegeli* caught from December 1989 to January 1991 (Fig. 6.12). The distribution reflected a similar pattern to that of ovary development states. After a quiescent period from November through February, the ovaries started to increase in size until a maximum was reached in July. The average index reduced steadily from then.

The distribution of testes development states based on the limited specimens showed an approximate similar trend to that of the ovaries (Fig. 6.13). Furthermore, unlike in *S.rivulatus* the distribution of active testes in both 1°♂♂ and 2°♂♂ apperaed to be close. All transitional species was sampled in September, November and January after the spawning peak.

A similar distribution of the GSI to that of the ovaries was found in the mature IP male (Fig. 6.14). The means of GSI in May, July and September were much higher than that in the other months although the sample sizes were small.

In *S.rivulatus* the relative ovary weight as percentage of body weight (GSI) generally appeared to be larger than that in *S.schlegeli*. In contrast, GSI of testes was found to be larger in *S.schlegeli* than that in *S.rivulatus*.

6.3.4.2 Seasonal variation in oocyte size and relative abundance of mature stages in the gonad

6.3.4.2.1 Pilot sampling program

The design for the pilot study program was described in the materials and methods (Fig. 6.1). The variable measured was the length of the five largest mature oocytes in each field. The factors tested were seasons, months within seasons, individuals within months and regions of the gonads.

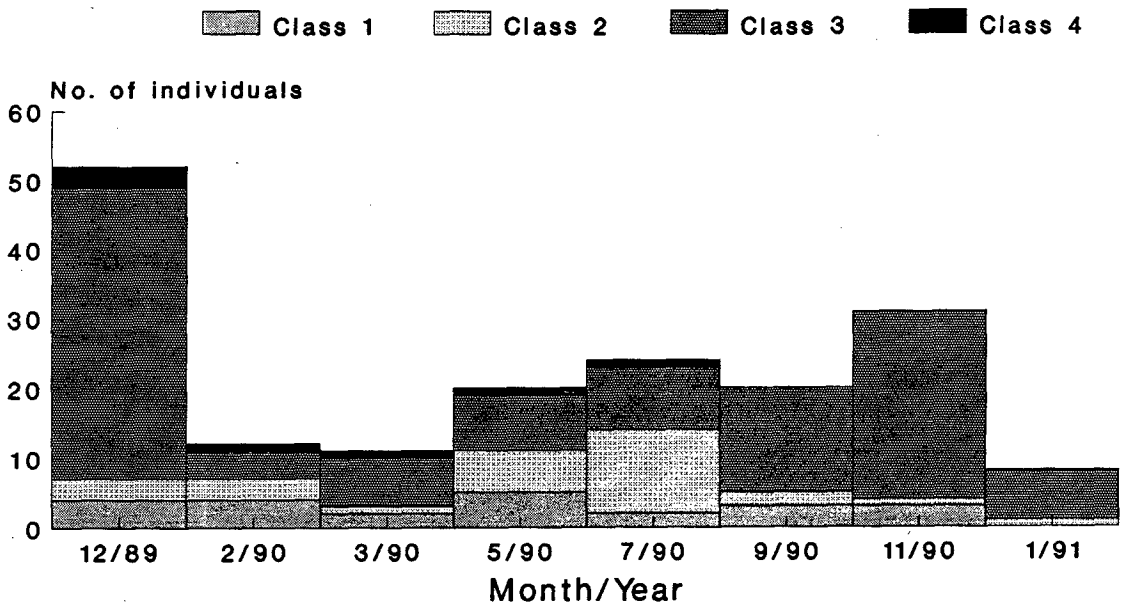
For female *S.schlegeli* there was a significant difference in mature oocyte size between seasons. This reflects the spawning seasonal pattern in GSI (Fig. 6.12) for ovaries in this species. No other factors were significant although the significant level of $p=0.0643$ between months within seasons suggested localizably temporal differences in oocyte size which may reflect local spawning activities. All other terms

Fig. 6.7 Number of individuals of *Scarus rivulatus* in each ovary development class.

The samples were taken at Lizard Island between December 1989 and January 1991.

Fig. 6.8 Distribution of average mature ovary indices (GSI) from *Scarus rivulatus*.

The specimens were taken at Lizard Island between December 1989 and January 1991. Vertical Bar indicates standard deviations. Sample sizes are shown beside the vertical bar.


Class 1
Class 2
Class 3
Class 4
No. of individuals
60
50
40
30
20
10
0
12/89
2/90
3/90
5/90
7/90
9/90
11/90
1/91
Month/Year

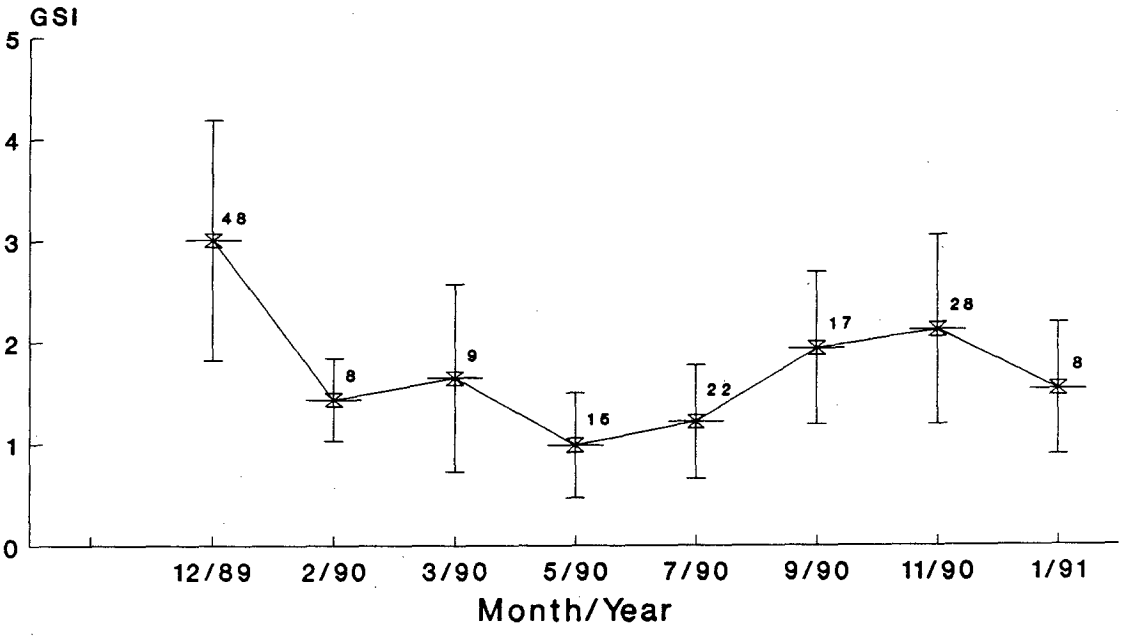
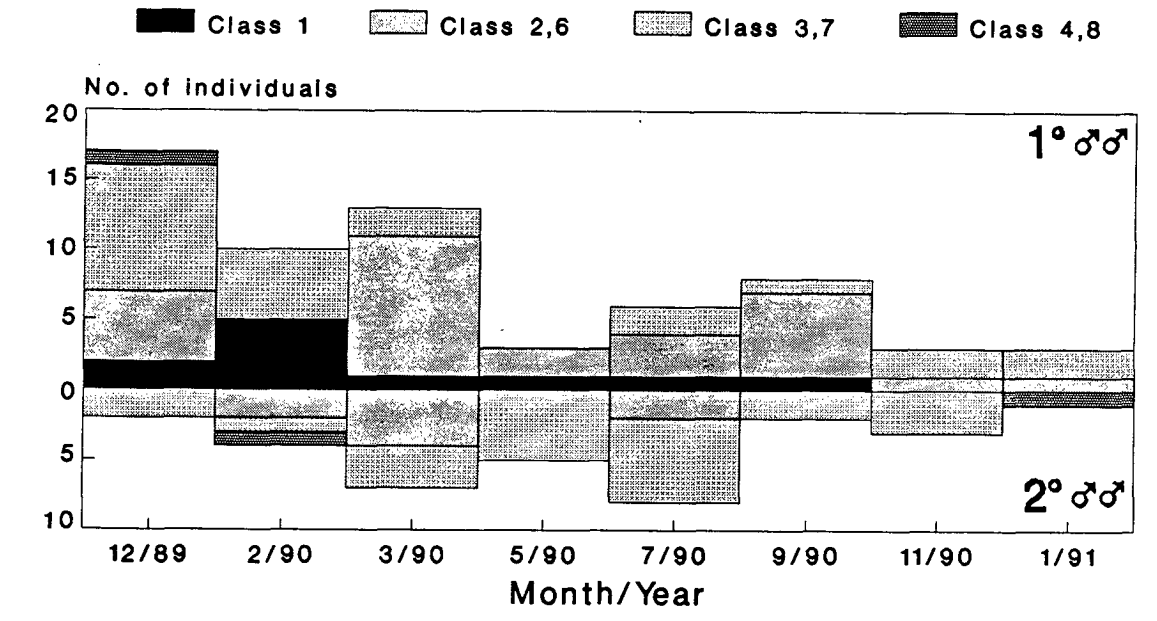

GSI
5
4
3
2
1
0
48
8
9
15
22
17
28
8
12/89
2/90
3/90
5/90
7/90
9/90
11/90
1/91
Month/Year

Fig. 6.9 Number of individuals of *Scarus rivulatus* in each testes development class.

The samples were taken at Lizard Island between December 1989 and January 1991.

Fig. 6.10 Average mature testes indices (GSI) from IP male *Scarus rivulatus*.

The specimens were taken at Lizard Island between December 1989 and January 1991. Vertical Bar indicates standard deviations. Sample size are shown beside the vertical bar.


Class 1
Class 2,6
Class 3,7
Class 4,8
No. of individuals
20
15
10
5
0
5
10
1° ♂♂
2° ♂♂
12/89
2/90
3/90
5/90
7/90
9/90
11/90
1/91
Month/Year

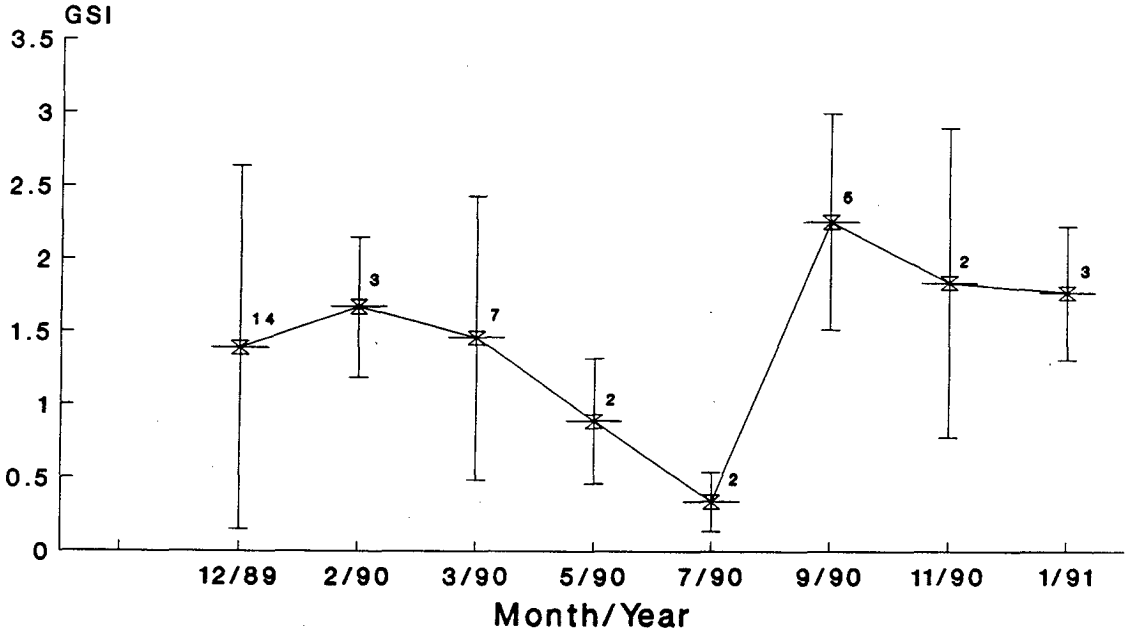
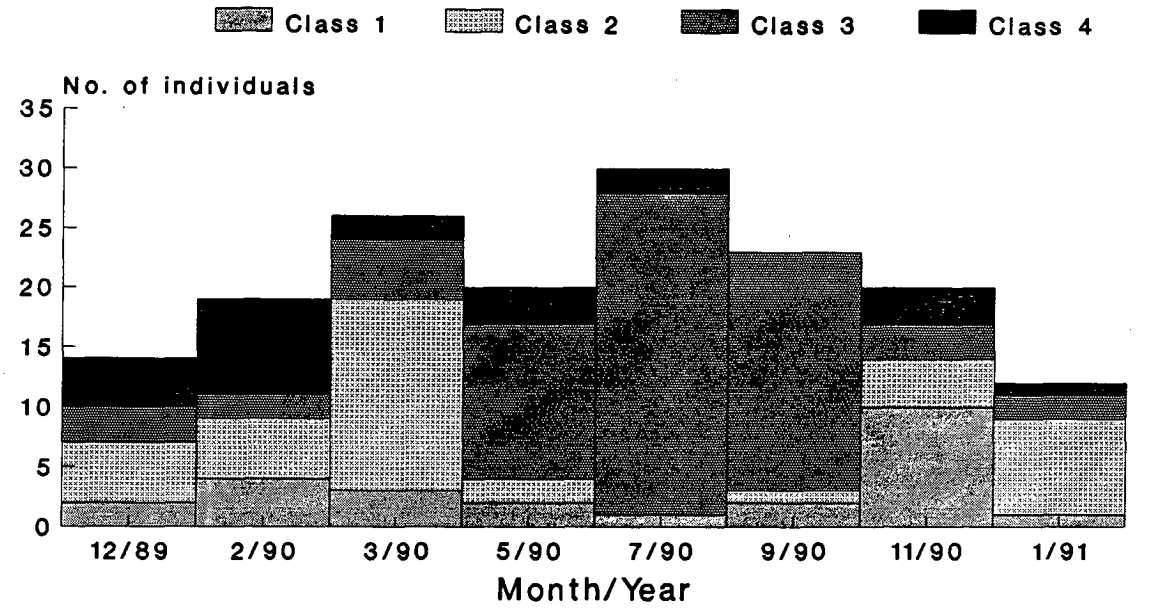

GSI
3.5
3
2.5
2
1.5
1
0.5
0
14
3
7
2
2
6
2
3
12/89
2/90
3/90
5/90
7/90
9/90
11/90
1/91
Month/Year

Fig. 6.11 Number of individuals of *Scarus schlegeli* in each ovary development class.

The samples were taken at Lizard Island between December 1989 and January 1991.

Fig. 6.12 Average mature ovary indices (GSI) from *Scarus schlegeli*.

The specimens were taken at Lizard Island between December 1989 and January 1991. Vertical bar indicates standard deviations. Sample sizes are shown beside the vertical bar.


Class 1
Class 2
Class 3
Class 4
No. of individuals
35
30
25
20
15
10
5
0
12/89
2/90
3/90
5/90
7/90
9/90
11/90
1/91
Month/Year

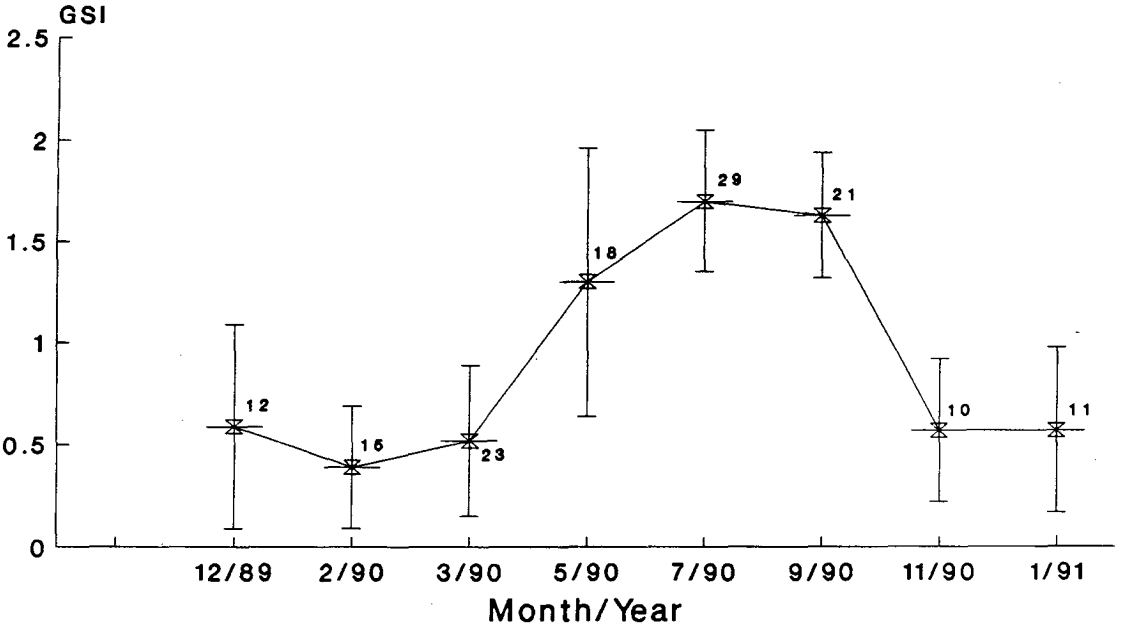
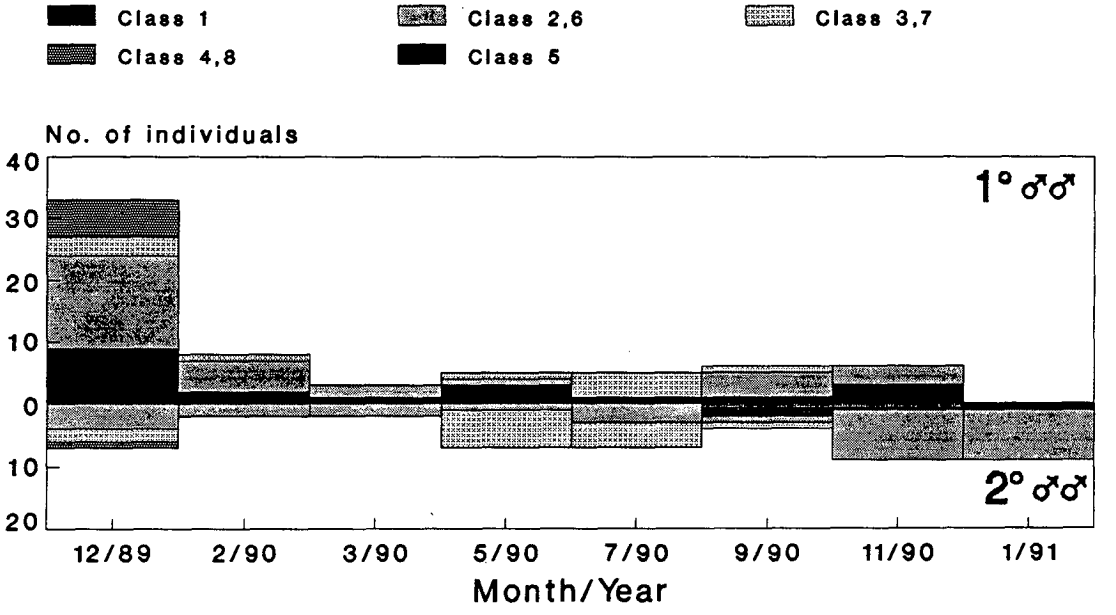

GSI
2.5
2
1.5
1
0.5
0
12
15
23
18
29
21
10
11
12/89
2/90
3/90
5/90
7/90
9/90
11/90
1/91
Month/Year

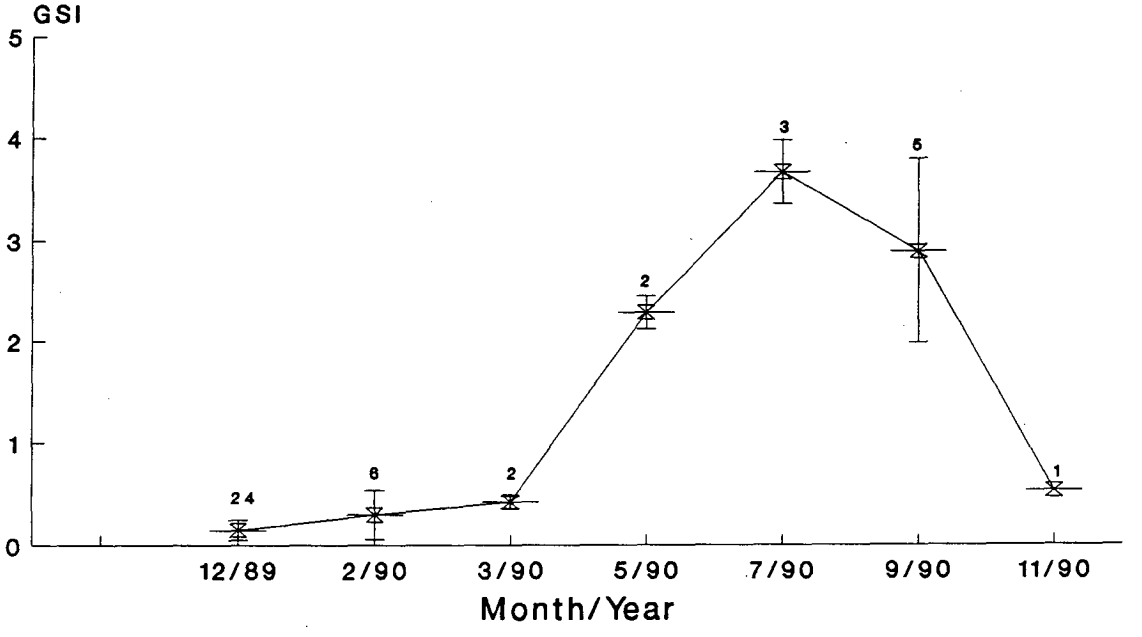
Fig. 6.13 Number of individuals of *Scarus schlegeli* in each testes development class.

The sample taken at Lizard Island between December 1989 and January 1991.

Fig. 6.14 Average mature testes indices (GSI) from IP male *Scarus schlegeli*.

The samples were taken at Lizard Island between December 1989 and January 1991. Vertical bar indicates standard deviations. Sample sizes are shown beside the vertical bar.


Class 1
Class 2,6
Class 3,7
Class 4,8
Class 5
No. of individuals
40
30
20
10
0
10
20
1° ♂♂
2° ♂♂
12/89
2/90
3/90
5/90
7/90
9/90
11/90
1/91
Month/Year


GSI
5
4
3
2
1
0
24
6
2
2
3
5
1
12/89
2/90
3/90
5/90
7/90
9/90
11/90
Month/Year

had p level greater than 0.25. Oocyte size tended to be fairly uniform among individuals and parts at the gonad (Table 6.7).

For female *S.rivulatus* the results were more complicated. Although not significant there was a trend (p=0.065) for a seasonal effect. There were however marked differences in mature oocyte sizes between months and between individuals in the sampling design (Table 6.8). This reflected the absence of a clear spawning pattern and short term change in ovary size over time in this species (Fig. 6.8). For this species care must be taken in interpreting reproductive events. For the samples of individual gonads of both species the results indicated the regions of the gonad from which the sampling was taken, and the area where the measuring field was located were not critical. Therefore, all subsequent measurement in the oocyte length would make in the central region (MC) of each cross section.

One other variable was measured in this program. This was the percentage covered by different oocyte stages in each sample field of view. These data were proportional and strongly non-normal distributed. No ANOVA was carried out. However, the major trend in this data could be identified. In this data set (percentage cover of oocyte stages), stage 5 was not included due to their rarity and sporadic appearance.

The proportion of different oocyte stages varied over time, and among individuals. In *S.schlegeli* the proportion of stage 4 oocyte showed little variation between individuals or months within seasons, and varied considerably between seasons (Fig. 6.15). There was a trend for a greater percentage cover of stage 4 oocytes in winter than in summer; mean = 44% $\pm$ 10.7 in winter as compared to summer mean = 27% $\pm$ 7.3. This reflected the differences seen in oocyte length

(Table 6.7). In contrast, there was a greater variation of the stage 4 oocyte area between individuals within seasons for *S.rivulatus* (Fig. 6.16). For both species there was little variation in the area covered by oocyte stages between the regions of the gonad. This was similar to the results of the measurements for oocyte length (Table 6.7, 6.8). The central region (MC) was consequently chosen for measuring the area fractions of stage 3, 4 and 5 in all mature ovaries for both species.

6.3.4.2.2 Distribution of mature oocytes and their sizes

6.3.4.2.2.1 *Scarus rivulatus*

The stage 5 oocytes were rarely seen in histological sections of ripe ovaries due to shrinking and distortion of these cells during histological process (West 1990), and the rate at which these were spawned. The area covered by vitellogenic oocytes (stage 4) was the highest in September and November, and decreased gradually from then reaching the lowest in July (Fig. 6.17). This trend was similar to that seen in change of GSI over time (Fig. 6.8). The area cover of the early vitellogenic oocytes (stage 3) was highest in February, and gradually decreased after then reaching the lowest in May. The trends seen in stage 3 and 4 oocytes were close.

The length of later stage oocytes within the mature ovary showed a similar pattern of change over time to that in the area cover of later stage oocytes (Fig. 6.17) and GSI (Fig. 6.8). The largest oocytes were found in September, and the smallest in May and July. The oocyte length did not change much from December to March, but decreased afterthere (Fig. 6.18).

The temporal pattern in reproduction of female *S.rivulatus* could be assessed by ovary weights, area cover of latter stage oocytes and oocyte length. All three

methods showed a similar result that spawning occurred year round, and was relative vigorous from September to Decmber.

6.3.4.2.2.2 *Scarus schlegeli*

Like in *S.rivulatus*, stage 5 oocytes for *S.schlegeli* were rare in the specimens examined, and thus these were not measured. The area covered by stage 4 oocytes decreased from December, and reached the lowest in February followed by a fast increase throughout May. After reaching a peak in July, it started decreasing gradually throughout January (Fig. 6.19). This trend was also close to that seen in change of GSI over time (Fig. 6.12). The stage 3 oocytes covered a quite similar area to that of stage 4 oocytes in the ovaries in different months.

The overall change in the oocyte length was vary much similar to that of ovary GSI, and reflected a strong seasonal pattern. Like the area cover of stage 4 oocytes, the mean of oocyte length decreased from December, and reached the minimum in February followed by a rapid increase throughout May. From May to September the mean was almost steady at the peak (Fig.6.20).

The similar result obtained from measurements on GSI, oocyte lengths and area cover of latter stage oocytes showed a strong seasonal pattern of reproduction in *S.schlegeli*. Although mature active ovaries oocurred year round like that in *S.rivulatus*, *S.schlegeli* spawned much more vigorous in winter and early spring (May to September).

The measurements of oocyte length from histological sections suggested that oocytes of *S.rivulatus* were greater that those of *S.schlegeli* (Fig.s 6.18, 6.20). However, as the eggs of scarids were elongate the true lengths were difficult to

Table 6.7 Results of ANOVA on the data of oocyte length for *Scarus schlegeli* in the pilot study.

SS = type I sums of squares estimates.

Source of variation	Df	SS	*F*	*P>F*
Between seasons	1	27562	5.66	0.0180
Between months within season	2	27002	2.77	0.0643
Between regions	2	5943	0.61	0.5441
Between interactions of seasons and regions	2	485	0.05	0.9515
Between interactions of month and regions within season	4	4555	0.23	0.9192
Between individuals within month and season	8	41884	1.07	0.3809
Between interactions of individual and region within month and season	16	27968	0.36	0.9899
Between subsamples within season, month, individual and region	36	48810	0.28	1.0000

Table 6.8 Results of ANOVA on the data of oocyte length for *Scarus rivulatus* in the pilot study.

SS = type I sums of squares estimates.

Source of variation	DF	SS	*F*-value	$p>F$
Between seasons	1	44104	3.42	0.0653
Between months within season	2	472929	17.56	0.0001
Between regions	2	18935	0.70	0.4959
Between interactions of season and region	2	5174	0.19	0.8253
Between interactions of month and region within season	4	2234	0.04	0.9967
Between individuals within month and season	8	677718	6.29	0.0001
Between interaction of individual and region within month and season	16	116228	0.54	0.9249
Between subsamples within season, month, individual and region	36	264071	0.54	0.9249

Fig. 6.15 Distribution of the different stage oocytes from testing specimens of *Scarus schlegeli*.

L - ML; C - MC; R - MR.

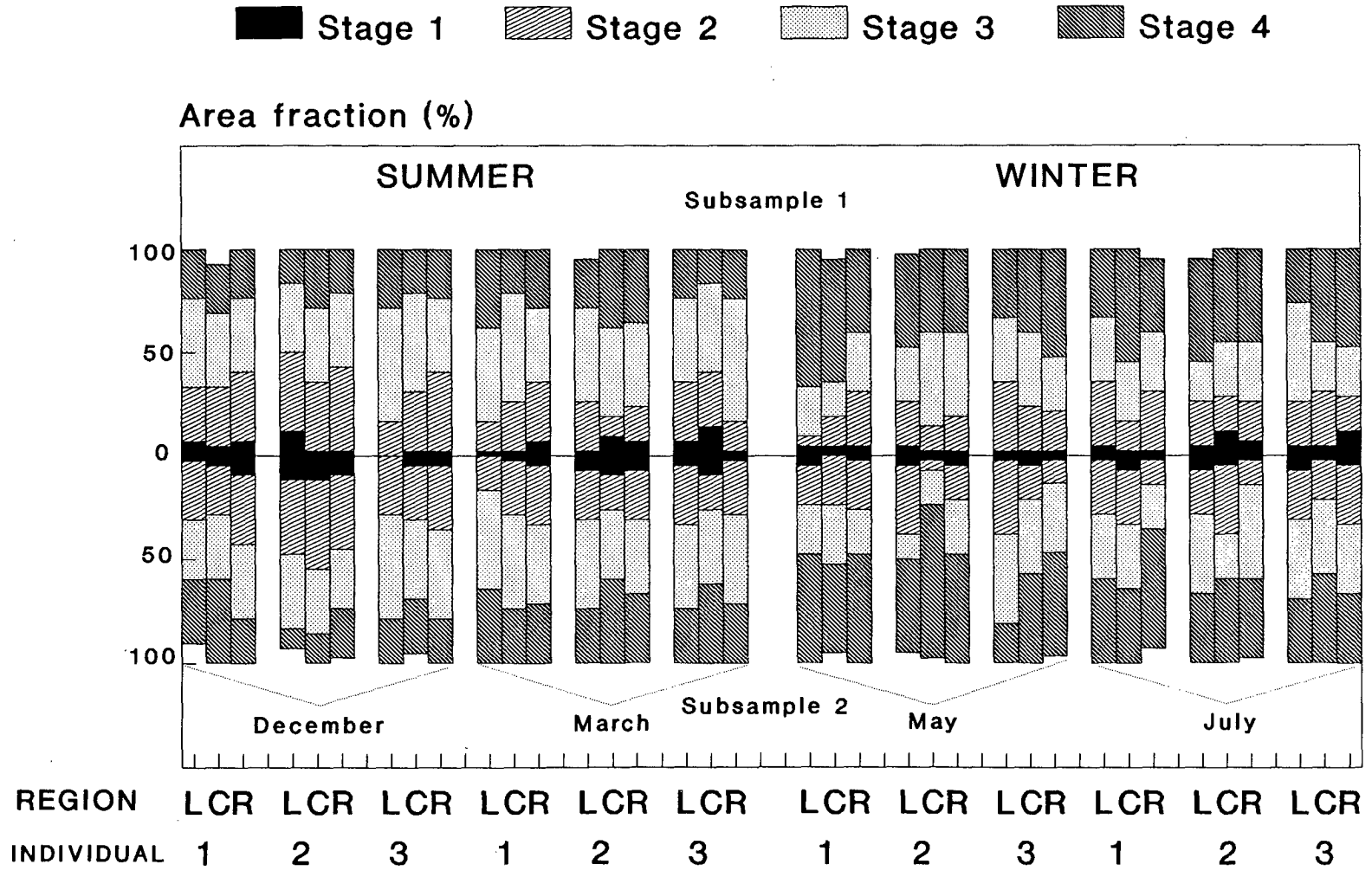

Stage 1
Stage 2
Stage 3
Stage 4
Area fraction (%)
SUMMER
WINTER
Subsample 1
100
50
0
50
100
December
March
Subsample 2
May
July
REGION
LCR 1
LCR 2
LCR 3
LCR 1
LCR 2
LCR 3
LCR 1
LCR 2
LCR 3
LCR 1
LCR 2
LCR 3
INDIVIDUAL

Fig. 6.16 Distribution of the different stage oocytes from the testing specimens of *Scarus rivulatus*.

L - ML; C - MC; R - MR.

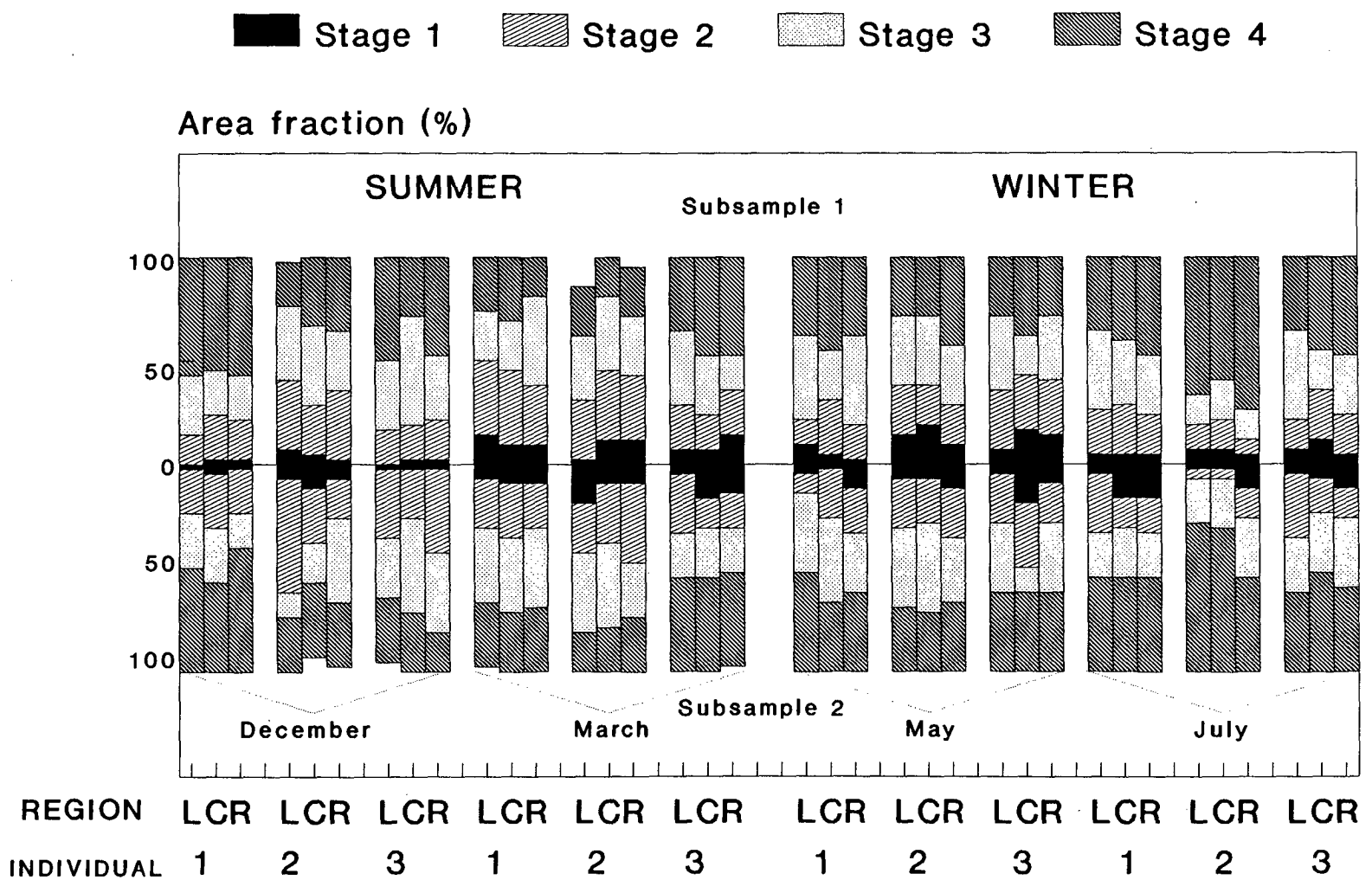
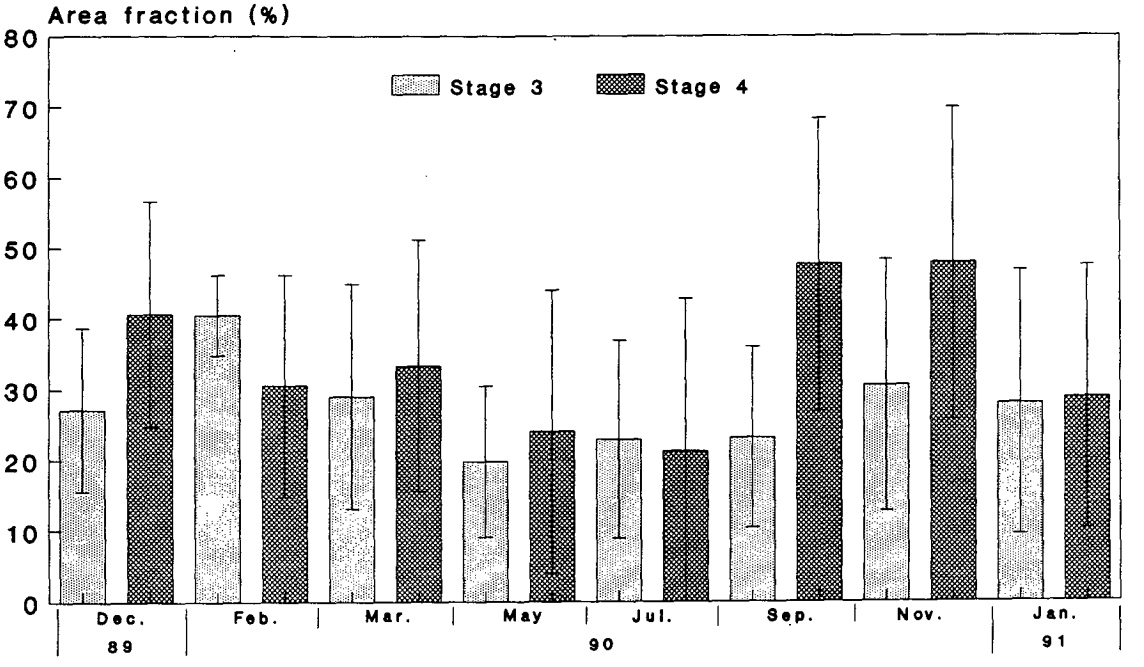

Stage 1
Stage 2
Stage 3
Stage 4
Area fraction (%)
SUMMER
WINTER
Subsample 1
Subsample 2
100
50
0
50
100
December
March
May
July
REGION LCR LCR LCR LCR LCR LCR LCR LCR LCR LCR LCR LCR
INDIVIDUAL 1 2 3 1 2 3 1 2 3 1 2 3

Fig. 6.17 Average proportion of stage 4 and 3 oocytes for mature females of *Scarus rivulatus*.

The specimens were taken at Lizard Island between December 1989 and January 1991. Vertical bar indicates standard deviations. Sample sizes are similar to that in Fig. 6.8.

Fig. 6.18 Average oocyte length for mature females of *Scarus rivulatus*.

The specimens were taken at Lizard Island between December 1989 and January 1991. Vertical Bar indicates standard deviations. Sample sizes are shown beside the vertical bar.


Area fraction (%)
80
70
60
50
40
30
20
10
0
Stage 3
Stage 4
Dec.
89
Feb.
Mar.
May
Jul.
90
Sep.
Nov.
Jan.
91

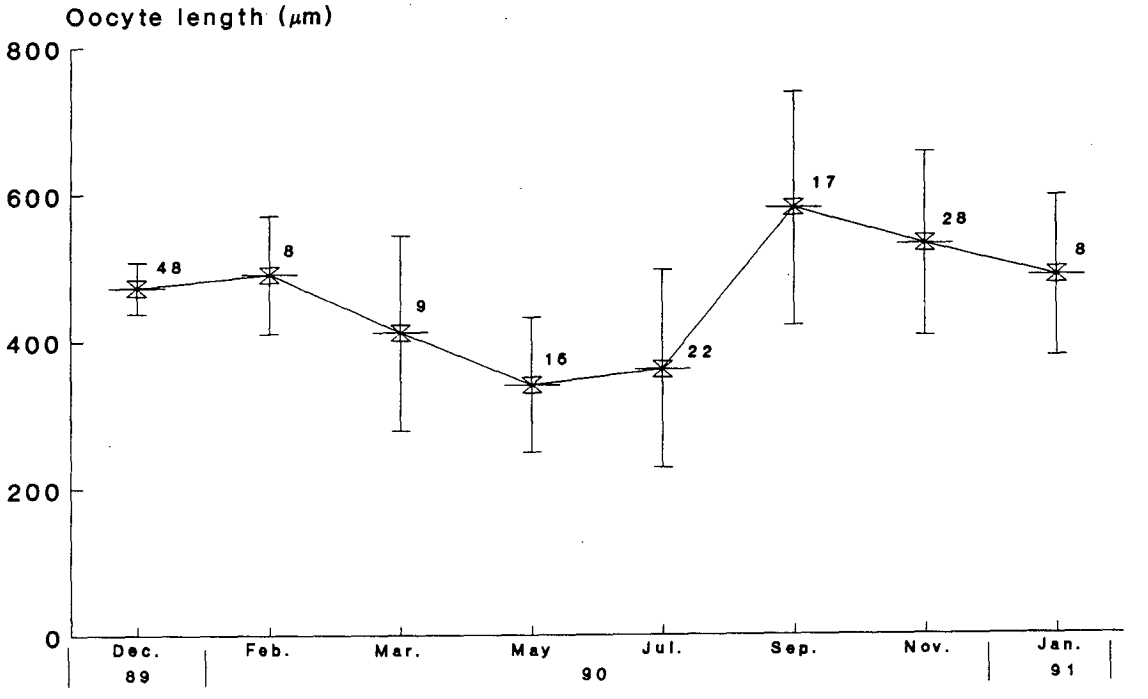
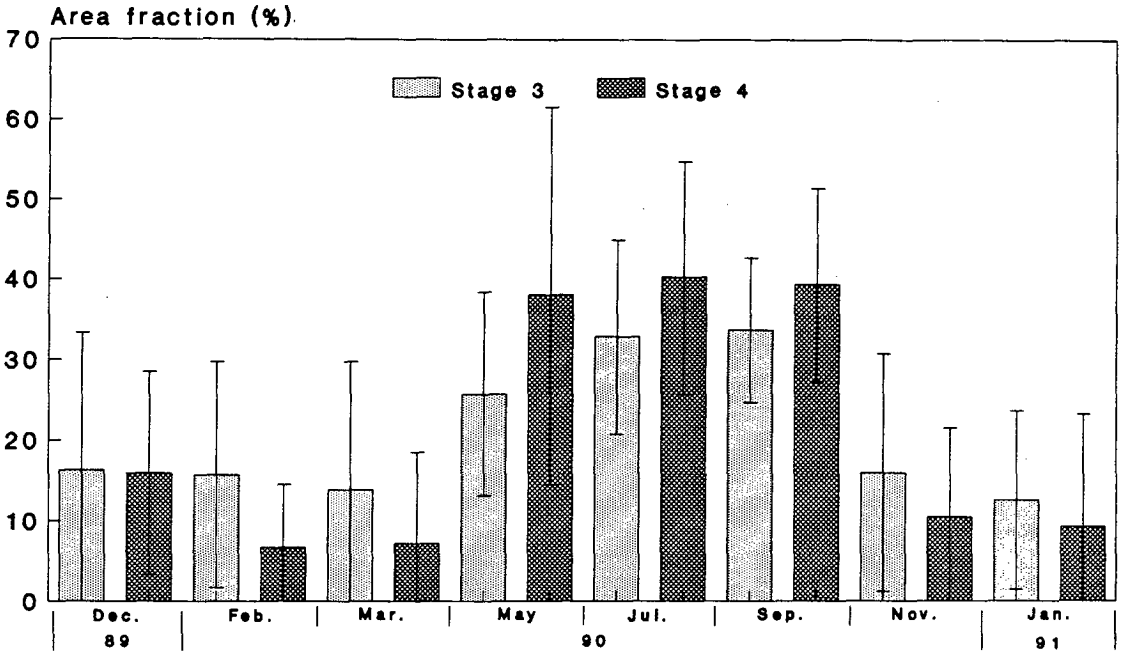

Oocyte length (μm)
800
600
400
200
0
48
8
9
16
22
17
28
8
Dec.
89
Feb.
Mar.
May
Jul.
90
Sep.
Nov.
Jan.
91

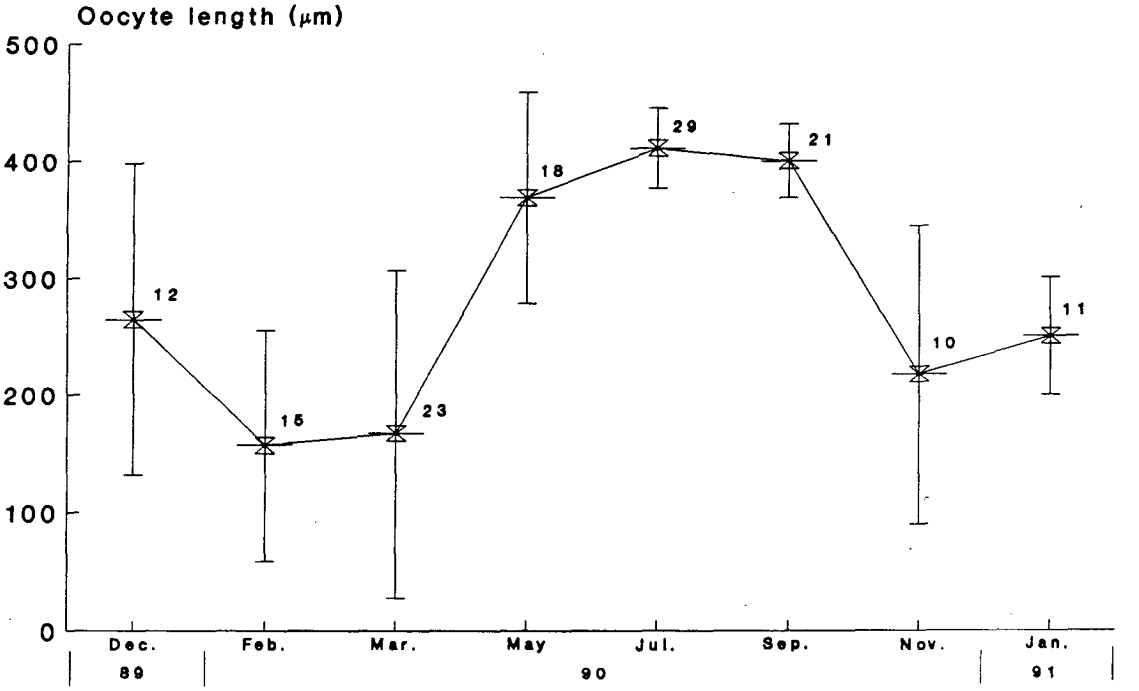
Fig. 6.19 Average proportion of stage 4 and 3 oocytes for mature females of *Scarus schlegeli.*

The specimen were taken at Lizard Island between December 1989 and January 1991. Vertical bar indicates standard deviations. Sample sizes are similar to that in Fig. 6.12.

Fig. 6.20 Average oocyte length for mature females of *Scarus schlegeli.*

The specimens were taken at Lizard Island Between December 1989 and January 1991. Vertical bar indicates standard deviations. Sample sizes are shown beside the vertical bar.


Area fraction (%)
70
60
50
40
30
20
10
0
Stage 3
Stage 4
Dec.
89
Feb.
Mar.
May
Jul.
90
Sep.
Nov.
Jan.
91


Oocyte length (μm)
500
400
300
200
100
0
12
15
23
18
29
21
10
11
Dec.
89
Feb.
Mar.
May
Jul.
90
Sep.
Nov.
Jan.
91

obtain as the orientation of oocytes within the ovary varied. As a check, the whole oocytes from mature ovaries of *S.rivulatus* and *S.schlegeli* were measured individually after being teased out of fixed ovaries. The measurements on 30 whole eggs at vitellogenic stage gave a mean length of 1245 μm (sd. = 34.4) for *S.rivulatus* (SL = 214 mm), and 838 μm (sd. = 31.9) for *S.schlegeli* (SL = 209 mm).

6.3.4.3 Multiple spawning and fecundity

From the area cover of different stage oocytes (Fig.s 6.17, 6.19) it was apparent that two distinct groups of ripening oocytes usually presented in the ovaries of *S.rivulatus* and *S.schlegeli* examined. One group of eggs (stage 4) was ready to be spawning, and other distinct group (stage 3) was undergoing vitellogensis. This type of successive maturation of several groups of oocytes was termed asynchrony. With the additional evidence of presence of ripe eggs (Plates 6.2, 6.3), such type of ovaries indicated that the both species exhibited the multiple spawning pattern within each season (West, 1990).

In *S.rivulatus* the estimates of the yolky oocytes in subsamples of 20 ovaries were exponentially correlated to SL (Fig. 6.21), and linearly correlated to age (Fig. 6.22). The relationship between fecundity and SL was:

$$\text{Fecundity} = 0.0279SL^{2.7974} \quad (r^2 = 0.8579 \quad p < 0.001)$$

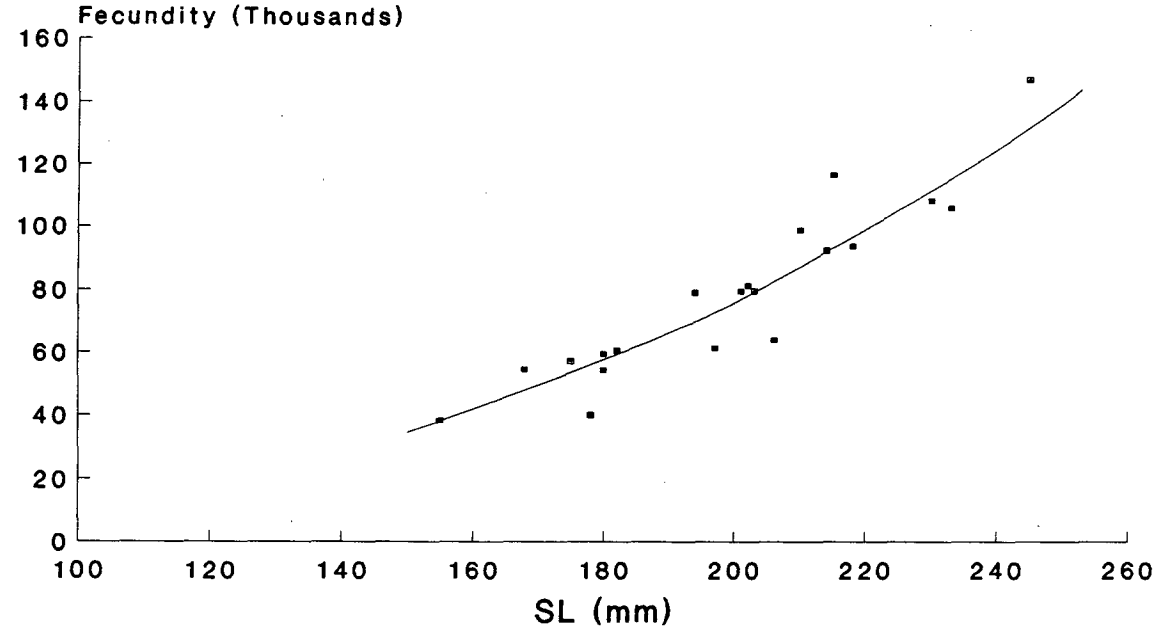
and between fecundity and age (t) was:

$$\text{Fecundity} = 8375 + 18509t \quad (r^2 = 0.8221 \quad P < 0.001)$$

In *S.schlegeli* the number of the yolky oocytes from the subsamples was also exponentially relative to SL (Fig. 6.23). There was no close mathematical relationship between fecundity and age for all age specimens. However, a positive

Fig. 6.21 Distribution of the total number of vitellogenic oocytes of *Scarus rivulatus* taken at Lizard Island by SL.

Fig 6.22 Distribution of the total number of vitellogenic oocytes of *Scarus rivulatus* taken at Lizard Island by age.


Fecundity (Thousands)
160
140
120
100
80
60
40
20
0
100
120
140
160
180
200
220
240
260
SL (mm)

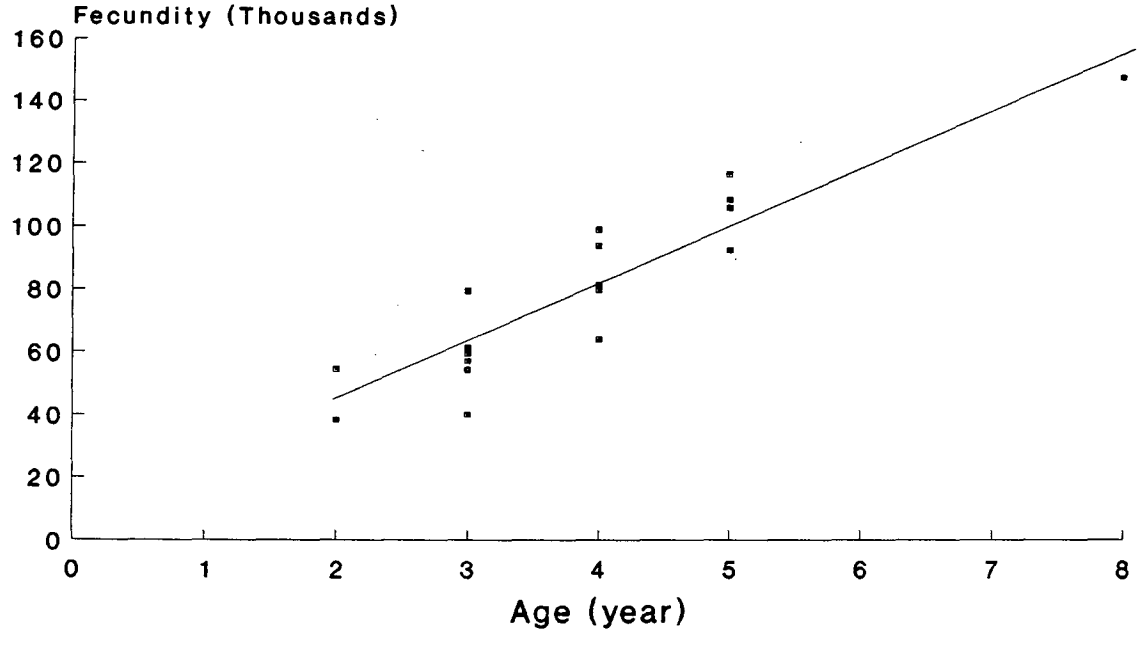
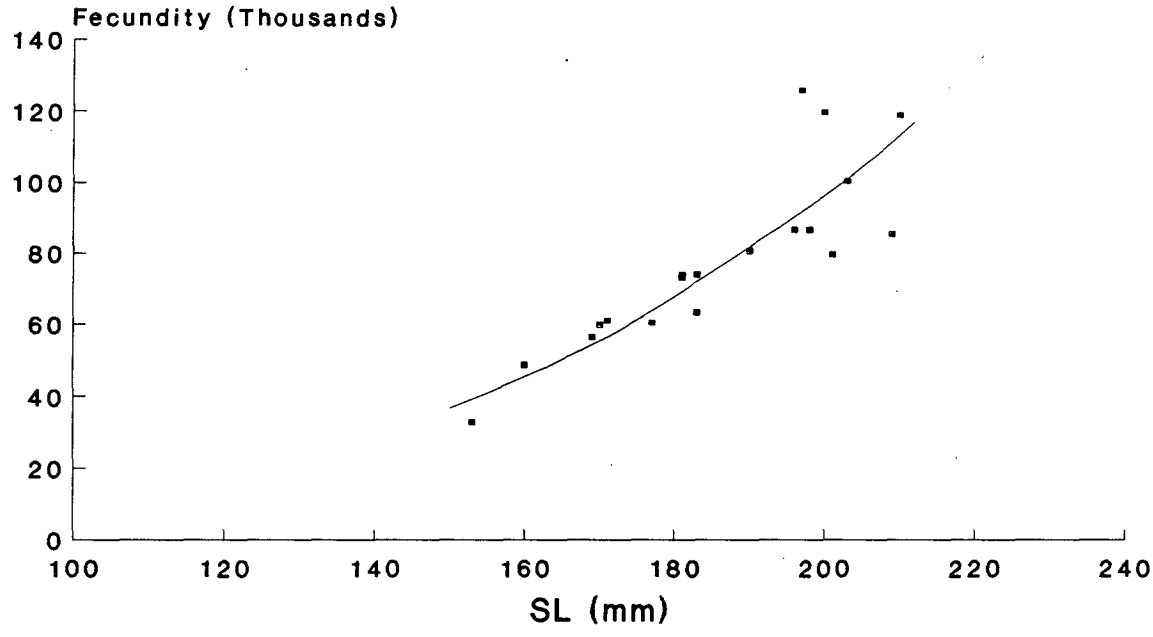
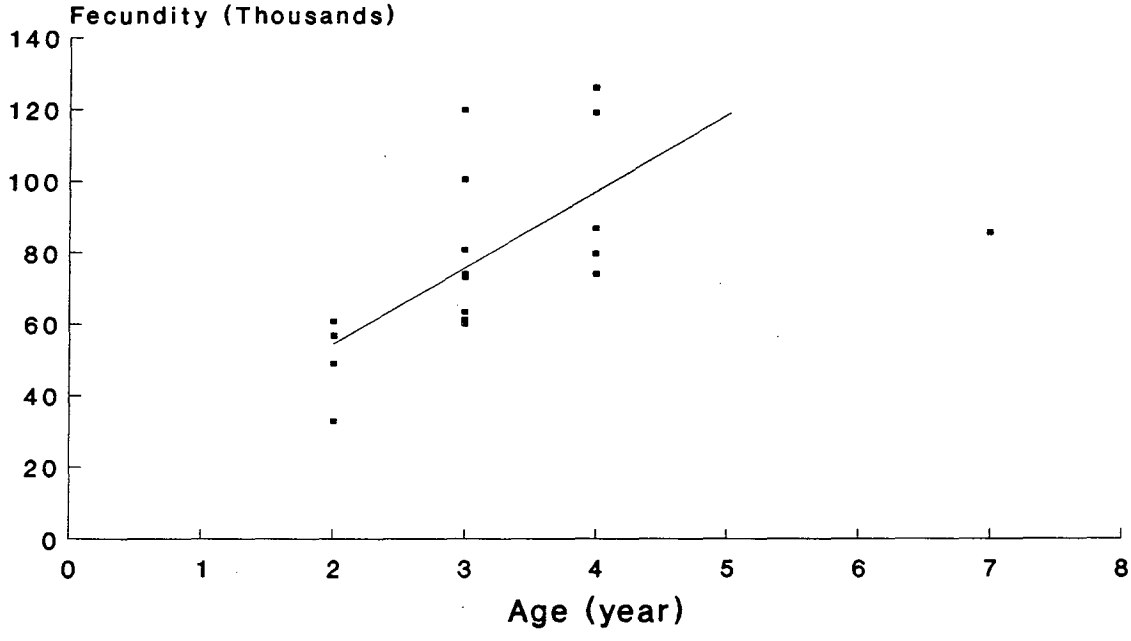

Fecundity (Thousands)
160
140
120
100
80
60
40
20
0
0
1
2
3
4
5
6
7
8
Age (year)

Fig 6.23 Distribution of the total number of vitellogenic oocytes of *Scarus schlegeli* taken at Lizard Island by SL.

Fig. 6.24 Distribution of the total number of vitellogenic oocytes of *Scarus schlegeli* taken at Lizard Island by age.


Fecundity (Thousands)
140
120
100
80
60
40
20
0
100
120
140
160
180
200
220
240
SL (mm)


Fecundity (Thousands)
140
120
100
80
60
40
20
0
0
1
2
3
4
5
6
7
8
Age (year)

linear correlation could be estimated between fecundity and the ages young than 5 years (Fig. 6.24). Fecundity-SL relationship was:

$$\text{Fecundity} = 0.0024SL^{3.3038} \quad (r^2 = 0.8297 \quad p < 0.001)$$

Fecundity-age (t) relationship was:

$$\text{Fecundity} = 21918t + 10022 \quad (r^2 = 0.4625 \quad P = 0.0014)$$

As growth rates in SL generally decreased with age in both species (see Chapter 5), the time in which an increment in SL could be reached in young individuals was less than that in older individuals. Consequently, an exponential relationship between fecundity and SL resulted in a linear relationship between fecundity and age.

6.3.5 Size at age among sexes and color phases

Mean length at age was compared among the different combination of color phase and sexual identity in each species. Because of the size and age related distribution in the color phase and sexual identity not all comparison could be achieved. For example, there were no TP individuals less than 3 years old or IP 1°♂♂ older than 5 years in the samples. The important comparisons were between all fishes in the 3^+ - 5^+ age groups and between IP females and TP males in the 4^+ to 7^+ age groups.

For both species almost no IP 1°♂♂ greater than 4^+ year were collected. Again in both species not TP males were young than 3^+ years. This suggested that most IP 1°♂♂ either do not live past 4 years or recruit to TP population by color change. As has been established for most another protogynous species both color phase (IP → TP) and sex (♀ → ♂) change occurs only after sexual maturity has been

reached. In both species this was 2 years.

Comparison of TP individuals (both 1° and 2° ♂♂) with IP individuals showed that for the same age TP fish were larger than IP fish (Tables 6.9, 6.10). Size at age in 1° and 2°♂♂ tended to similar. In addition, for IP fish 1°♂♂ and females had similar sizes at age. These data (Tables 6.9, 6.10) suggested that growth rate in TP individuals was greater than in IP individuals regardless of sexual identity. This was illustrated in the growth curves constructed from size at age data for each species (Fig.s 6.25, 6.26). The data suggested a higher growth rate in TP individuals although the mechanism was not clear.

Estimates of gonad weight for each color phase and sex identity in each age class were shown in Tables 6.11 and 6.12. For both species the gonad weight (expressed in gram) was fairly similar in IP females and 1°♂♂. Both had relative larger ovaries or testes. The gonad weight of TP individuals was predicatively less. Both 1°♂♂ and 2°♂♂ tented to have smaller gonads than IP individuals of similar age, even though TP ♂♂ were larger than IP fish at a given age. However, within the TP color phase 1°♂♂ tended to have larger testes than 2°♂♂ for a given age. This may reflect their sexual origin.

6.4 DISCUSSION

6.4.1 Anatomical features of the gonads and sexual transformation schedule

The testes of 1°♂♂ and 2°♂♂ in young TP individuals can be readily distinguished (Plate 6.5) for *S.rivulatus* and *S.schlegeli* at Lizard Island because the ovarian origin of the 2°♂♂ testis can be clearly seen. These testes were essentially identical with that of the same species at Heron Island in the southern GBR, which

Table 6.9 Mean SL ± s.d. (sample size) of *Scarus rivulatus*.

The results of analysis of variance of mean SL between sexes with different colour phases were also provided. n.s. - not significant difference at $p<0.05$; - no data available

Age	IP		TP		Comparison		
	♀♀	1○♂♂	1○♂♂	2○♂♂	*F*	d.f	*p*
1^+	120±10.9(4)	127±3.3(3)	-	-	0.742	1/5	n.s.
2^+	163±11.3(70)	161±10.4(33)	-	-	0.573	1/101	n.s.
3^+	182±10.5(58)	174±12.4(14)	195±7.0(2)	-	4.260	2/71	<0.05
4^+	203±12.1(45)	198±11.0(6)	209±9.1(8)	221±3.3(3)	2.879	3/58	<0.05
5^+	217±15.6(19)	230 (1)	243±9.6(4)	243±15.9(13)	11.65	2/33	<0.01
6^+	220±9.5(5)	-	254±15.7(4)	264±14.2(13)	16.12	2/19	<0.01
7^+	236±6.5(2)	-	258 (1)	256±16.6(5)	1.898	1/5	n.s.
8^+	245 (1)	-	-	-	-	-	-

Table 6.10 Mean SL ± s.d. (sample size) of *Scarus schlegeli*.

The results of analysis of variance of mean SL between sexes with different colour phases were also provided. n.s. - not significant difference at $p<0.05$; - no data available.

	IP		TP		Comparison		
Age	♀♀	1○♂♂	1○♂♂	2○♂♂	*F*	d.f	*p*
1^+	120±10.7(11)	123±15.6(14)	-	-	0.236	1/23	n.s.
2^+	165±13.5(52)	165±13.2(17)	-	-	0.000	1/67	n.s.
3^+	185±7.2(96)	185±7.9(35)	206 (1)	198±5.9(3)	3.420	2/131	<0.05
4^+	201±8.2(42)	211±6.1(4)	209 (1)	215±9.8(21)	19.916	2/64	<0.01
5^+	210±10.0(5)	220 (1)	228 (1)	229±8.8(23)	15.810	1/26	<0.01
6^+	225 (1)	-	227±3.5(2)	243±13.8(4)	1.621	1/4	n.s.
7^+	209 (1)	-	-	245 (1)	-	-	-
8^+	-	-	-	248 (1)	-	-	-

Table 6.11 Means of gonad weight (g) ± s.d. (sample size) of *Scarus rivulatus*.

The results of anaalysis of variance of the means between sexes with different colour phases were also provided. n.s. - not significant difference at $p<0.05$; - no data available.

	IP		TP		Comparison		
Age	♀♀	1○♂♂	1○♂♂	2○♂♂	*F*	d.f	*p*
1^+	0.05±0.05(4)	0.08±0.02(3)	-	-	0.85	1/5	n.s.
2^+	2.70±2.24(70)	1.61±1.67(33)	-	-	6.02	1/101	<0.05
3^+	4.63±3.06(58)	3.45±1.77(14)	2.34±0.66(2)	-	1.33	2/71	n.s.
4^+	5.76±3.30(45)	6.12±3.89(6)	2.45±0.99(8)	1.25±0.80(3)	4.10	3/58	<0.05
5^+	6.01±3.86(19)	5.78 (1)	2.29±0.48(4)	1.70±1.64(13)	8.08	2/33	<0.01
6^+	5.23±2.03(5)	-	7.97±5.88(4)	1.41±0.80(13)	8.60	2/19	<0.01
7^+	2.49±0.58(2)	-	15.43 (1)	1.70±1.25(5)	0.53	1/5	n.s.
8^+	15.69 (1)	-	-	-	-	-	-

Table 6.12 Means of gonad weights (g) ± s.d. (Sample size) of *Scarus schlegeli*.

The results of analysis of variance of the means between sexes with different colour phase were also provided. n.s.- not significant difference; - no data available.

	IP		TP		Comparison		
Age	♀♀	1○♂♂	1○♂♂	2○♂♂	*F*	d.f	*p*
1^+	0.14±0.16(11)	0.13±0.25(14)	-	-	0.02	1/23	n.s.
2^+	1.53±1.28(52)	0.98±0.21(17)	-	-	1.97	1/67	n.s.
3^+	2.42±1.78(96)	1.99±2.88(35)	0.30 (1)	0.17±0.11(3)	1.99	2/131	n.s.
4^+	3.20±2.01(42)	5.02±3.70(4)	0.29 (1)	0.39±0.37(21)	19.50	2/64	<0.001
5^+	3.32±1.55(5)	0.91 (1)	4.00 (1)	0.46±0.41(23)	54.77	1/26	<0.001
6^+	0.98 (1)	-	1.78±0.06(2)	0.33±0.13(4)	158.20	1/4	<0.001
7^+	4.91 (1)	-	-	0.55 (1)	-	-	-
8^+	-	-	-	0.23 (1)	-	-	-

Fig. 6.25 Mean lengths for successive age groups of females, IP 1○♂♂, TP 1○♂♂ and 2○♂♂ of *Scarus rivulatus*.

The specimens were sampled at Lizard Island. Vertical bar indicates standard deviations.

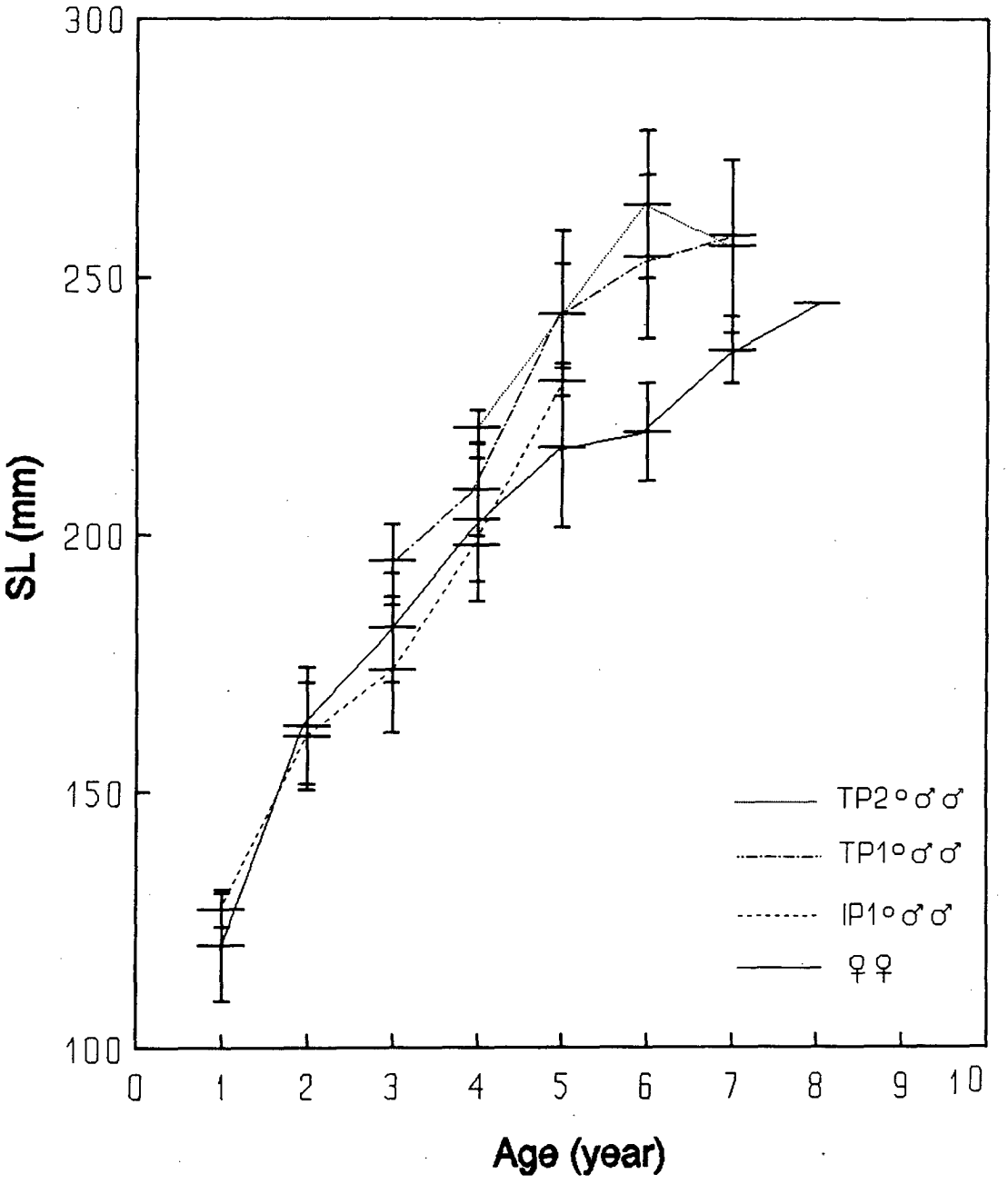
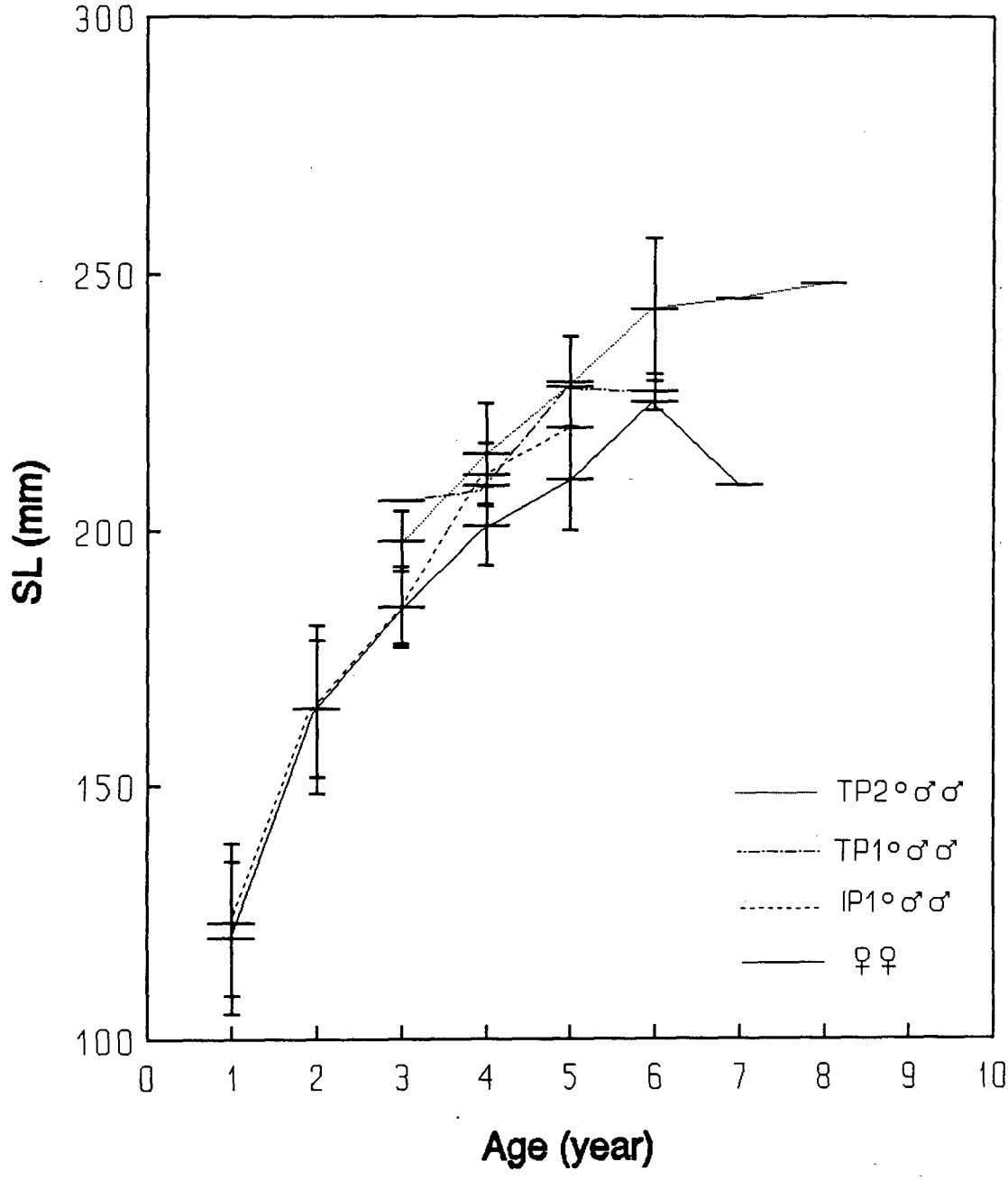

300
250
200
150
100
SL (mm)
0
1
2
3
4
5
6
7
8
9
10
Age (year)
TP2♀♂♂
TP1♀♂♂
IP1♀♂♂
♀♀

Fig. 6.26 Mean lengths for successive age groups of females, IP 1○♂♂, TP 1○♂♂ and 2○♂♂ of *Scarus schlegeli*.

The specimens were sampled at Lizard Island. Vertical bar indicates standard deviations.


300
250
200
150
100
SL (mm)
0
1
2
3
4
5
6
7
8
9
10
Age (year)
TP2♀♂♂
TP1♀♂♂
IP1♀♂♂
♀♀

were studied in detail by Choat and Robertson (1975). However, it has been demonstrated that the testes of both 1°♂♂ and 2°♂♂ change morphologically with increasing age in the terminal phase (Fig. 6.2). Distinction between these two types of testes becomes more difficult with age if using the criteria described in the literature (see review by Sadovy and Shapiro, 1987). Therefore, the sexual identities of some of the older TP males in this study should be considered as a preliminary result. There was a potential bias towards 1°♂♂ as evagination occurs in the testis of older 2°♂♂ (Jones, 1980). However, it is difficult to assess such a bias without a new technique, which can histochemically distinguish between these testes.

Parrotfishes (*S.rivulatus* and *S.schlegeli*) around Lizard Island fit the general scarid pattern of size, colour and sex distribution (Choat and Robertson, 1975; Robertson and Warner, 1978). In *S.rivulatus*, both immature 1○♂♂ and females were found in the smallest sizes (100 - 119 mm SL) examined while Choat and Robertson (1975) found these immature individuals in the small size (81 - 120 mm SL) at Heron Island. The minimal SL of TP 1○♂♂ and 2○♂♂ observed in the present study was also close to that observed by Choat and Robertson (1975) as well as the proportion of IP and TP 1○♂♂.

Scarus schlegeli from both Lizard island and Heron Island had generally a common sexual transition schedule in terms of SL of transitional individuals. However, a larger proportion of IP and TP 1○♂♂ was observed in this study than was observed by Choat and Robertson (1975). This difference is unlikely to result from histological interpretation as the criteria used by Choat and Robertson (1975) were very similar to those used in the current study (Sadovy and Shapiro, 1987). Geographical locations generally affect the distribution and abundance of herbivorous

reef fishes on the GBR (Russ, 1984a & b). Heron Island is over 2000 km away from Lizard Island, and in subtropical environment. These variations were likely to be caused by the two locations. In addition, sampling sizes and the density of fishes on reefs may have partially contributed to the differences observed (e.g. 343 in this study vs 73 in Choat and Robertson, 1975). Therefore, unlike being a weakly diandric species at Heron Island (Choat and Randall, 1986), *S.schlegeli* showed a high proportion of IP and TP 1○♂♂ at Lizard Island (Table 6.5). This phenomenon suggests that *S.schlegeli* at Lizard Island is a strongly diandric species.

The overall observed sizes of both species at Heron Island were 40 to 60 mm larger than the overall observed sizes at Lizard Island. Consequently, the maximal observed SL and sexual maturity SL in the present study were smaller than that observed by Choat and Robertson (1975). The warmer environment at the northern GBR may cause earlier sexual maturity, and relative shorter lifespan.

The age-specific description of sexual patterns reveals the actual time schedule of sexual transition. Other protogynous teleosts which have been investigated regarding age of transformation show a variety of patterns in the distribution of sex reversal over life-span. Moe (1969) demonstrated that sexual transformation in the serranoid *Epinephelus morio* occurred over at least 5 years (ages 5 to 10). A similar transition period was also found in the labrid (*Pimelometopon pilchrum*) by Warner (1975). However, Cowen (1990) estimated a longer period of sexual transformation (5-14 yrs) for the same labrid at different locations. The sexual transition period in Lizard Island parrotfishes (*S.rivulatus* and *S.schlegeli*) generally appears to be shorter than the above species. The relative younger age for starting the sexual transformation was probably due to warmer water in the tropics.

6.4.2 The breeding season, multiple spawning, and fecundity

Unlike the other studies involved in scarid reproductive biology (Choat, 1969; Yogo *et al.*, 1980; Dubin, 1981; Clavijo, 1983; Gladstone, 1986), this study only concentrated on analysis of sexual organs (gonads), and field observations on the spawning activities were not carried out for both species examined. Spawning schedules were assessed by several methods including the GSI, maturity stage, oocyte analysis, and all approaches gave similar results that spawning occurred year round with a low peak from September to January for *S.rivulatus* and a high peak from May to September for *S.schlegeli*.

Thresher (1984) has summarised the information available on the spawning seasons of tropical parrotfishes, and points out that spawning appears to occur year-round, though it is more frequent and more vigorous in the summer. Investigation of Lizard Island *S.rivulatus* and *S.schlegeli* generally appeared to agree with this since they do not display discrete patterns of reproductive events whose timing consistently coincides with some seasonal manifestation. Instead they have extended spawning seasons which renders it difficult to estimate the duration of reproductive activity. The peak in winter spawning in *S.schlegeli* at Lizard Island is also found in the various species of coral reef fishes (see the summary by Robertson, 1991), and observed on the same species at Heron Island by Choat (1969).

The protracted spawning season of the parrotfishes at Lizard Island was also indicated by the asynchronous ovaries and the multiple spawning patterns (West, 1990). Ovaries containing several modes of developing oocytes are commonly seen in reef taxa (Warner, 1975; Ebisawa, 1990).

The egg counts in this study were probably overestimates of the number of

eggs spawning during a spawning season. This was because the relationship between the number of these oocytes and the number actually spawned depends on the survival rate of the oocytes to maturity and the proportion actually ejected from the body. The relative close area covered by the latter stage oocytes (stage 4, 3) indicated a smaller number of the later stage oocytes (stage 4) relative to the earlier stages (Fig.s 6.17, 6.19). This showed a loss during oocyte development. There were certainly some eggs left in the lumen of postspawning females. These degenerate and were presumably absorbed during the resting phase. However, these estimates did show an overall relationship between fecundity and SL, and between fecundity and age. The size-specific increases in fecundity seen in Lizard Island parrotfishes were also observed in the other protogynous hermaphroditic species (Warner, 1975; Waltz *et al.*, 1982; Ebisawa, 1990).

6.4.3 Growth among sexes and color phases

In *Scarus croicensus* the IP male investment in reproduction in terms of gonadal weight is lower than it is in females (Warner and Downs, 1977). They were estimated to grow faster and adopt the terminal phase at a younger age, although the aging method could not be validated. It seems not to be the case for *S.rivulatus* and *S.schlegeli* at Lizard Island. The IP males of *S.rivulatus* and *S.schlegeli* generally have similar investment in reproduction to that of females (Tables 6.11, 6.12). Furthermore, growth in both IP males and females is not significantly different (Tables 6.9, 6.10). However, the duration, in which IP males change color phase, appears shorter than that of the hermaphrodites.

With the age information available, an assessment of the effect of sex reversal

and dichromatism can be made. Other studies involved with both reproductive biology and age determination on the protogynous teleosts generally show a positive effect of the transformation on individual growth (Warner, 1975; Ross, 1984; Cowen, 1990). Warner (1975) found the sexually transformed males had longer SL than the females of the same age, and he pointed out that the females that did not change sex after the "critical" length appeared to be those individuals with relatively low rates of growth. A similar growth pattern was also observed on the same species at different locations by Cowen (1990). My results appear to agree with these findings. In addition, a positive effect of dichromatism on growth was demonstrated in the present study.

The physiological cause of this effect was not studied in this project. However, the relative distribution of fish energy was probably attributed to partial differences in individual growth. As indicated in Tables 6.11 and 6.12, female and IP male individuals seemed to spend relatively more energy in reproductive activities (larger gonads) than TP 1♂ and 2♂ individuals. This phenomenon is common in protogynous hermaphroditic teleosts (Choat and Robertson, 1975; Ross, 1984). A detailed schedule of these energy transformations is unknown in scarids, and it would require further study.

In conclusion, parrotfishes (*S.rivulatus* and *S.schlegeli*) at Lizard Island generally started sexual transformation (for females) and color phase change from IP to TP (for both females and 1♂) at age 3^{+}, and the process lasted approximately three years for the populations. Multiple spawning occurred year round for both species, with a low spawning peak of September to January for *S.rivulatus* and a high

spawning peak of May to September for *S.schlegeli*. Sexual transformation had a positive effect on individual growth rates for both species, as had dichromatism.

CHAPTER 7. LIVER AND HEPATIC LIPIDS OF TROPICAL SCARIDS

7.1 INTRODUCTION

Both somatic growth and gonadal development require energy resources. The energy is derived from the food an animal digests. The energy may accumulate within the animal body as different stored products (Weatherley and Gill, 1987). In fishes, these are generally lipid, glycogen and protein, and lipid is a main energy source (Shul'man, 1974). The pattern of energy storage appears to vary considerably among various organs and tissues within the fish body, and between different species. In the benthic species of low mobility, the liver is an important organ of storage (Shul'man, 1974).

Patterns of energy storage within the bodies of fish may vary with age, sexual identity and seasons (Weatherley and Gill, 1987). Little is known of these features of herbivorous reef fish biology. Although scarids have large livers which may be an important source of stored energy (Bellwood, 1985) there has been no study which provides estimates of liver size and condition over an annual cycle.

In this chapter, the following aspects of livers in *Scarus rivulatus* and *S.schlegeli* at Lizard Island are investigated:

(1) general structure;

(2) the relationship between liver size and fish length and age;

(3) seasonal patterns of liver size and hepatic lipids; and

(4) the relationships between liver size and reproductive state.

7.2 MATERIALS AND METHODS

7.2.1 Liver collection

Livers were dissected free of mesenteries in fresh specimens of 276 *S.rivulatus* and 289 *S.schlegeli* collected from Lizard Island from November 1989 to January 1991 (for details see Chapter 2). These fish were aged in Chapter 5, and their sexual identities were confirmed in Chapter 6. The livers were weighed to the nearest 0.01 g on an electron balance before their colour was recorded. A middle section of the right liver lobe was then cut and preserved in formal-acetic acid-calcium chloride (FAACC) fixative for histological examination as a similar section from the left liver lobe was generally too large to fit on a microscope slide. Hepatosomatic index (HSI) was expressed as the wet liver weight as a percentage of wet body weight, and calculated for all specimens.

7.2.2 Histological examination

7.2.2.1 Process

Samples of the liver tissue were embedded in paraffin wax. Histological sections were cut at 6 μm thickness and stained with Mayer's haematoxylin followed by a counterstaining with Young's Eosin-Erythrosin. The section then dried in a histological oven at 65°c for several days.

7.2.2.2 Hepatic lipid measurement

The sections of liver tissue were initially examined and the general structure noted, which mainly included the distribution of blood vessels and pancreatic tissue. Liver sections were similar in terms of these characteristics. However, a more detailed examination of the liver parenchyma tissue revealed the presence of

vacuoles, which varied among individuals and over time.

The pattern of variation and the biochemical nature of these vacuoles was examined by histochemical methods. For this a pilot study was performed to determine if the vacuoles were sites of lipid storage. By visual inspection three types of liver with different proportions of vacuolate tissue were selected for histochemical analysis. Samples with low, medium and high proportion of the vacuolate liver parenchyma were sectioned and infiltrated with gum sucrose solution overnight at 4°c (Bancroft, 1975). The sections were then cut at 8-10 μm at -16°c on a Damon IEC cryotome, and mounted directly on gelatinized slides. Lipids were demonstrated using the Bromine-Sudan B method (Bayliss-High, 1984). These sections were then compared to the sections processed by the normal histological procedure (see 7.2.2.1) from the same liver tissue.

Following this pilot study four areas were selected on the each histological section, which were dorsal (DO), central (CE), ventral (VE) and proximal (PR) areas (Fig.7.1). The size of each sample area was estimated using a Weibel eyepiece as 0.05 mm^2 at 400×. Within this area the proportion of the vacuolate liver parenchyma was estimated by the stereological technique described in 6.2.2.2. A hepatic lipid index (HLI) was determined as a total area fractions of vacuolate tissue as a percentage of the total measuring areas (168 points), and calculated for each individual specimen.

7.3 RESULTS

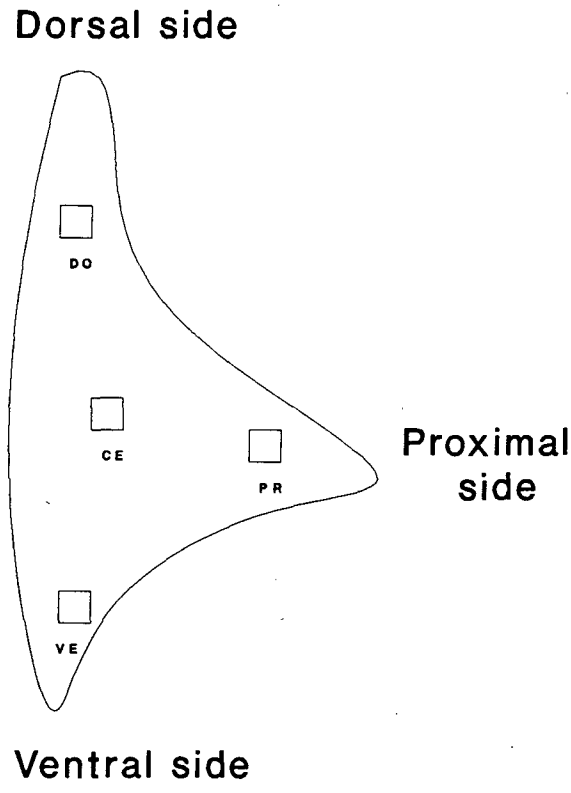
7.3.1 General structure

Livers of *S.rivulatus* and *S.schlegeli* consisted of two main lobes located in

Fig. 7.1 A schematic diagram of histological liver section.

The diagram shows areas in which lipid droplets were estimated. DO - dorsal area; CE - central area; VE - ventral area and PR - proximal area.

A transverse section of the right liver lobe



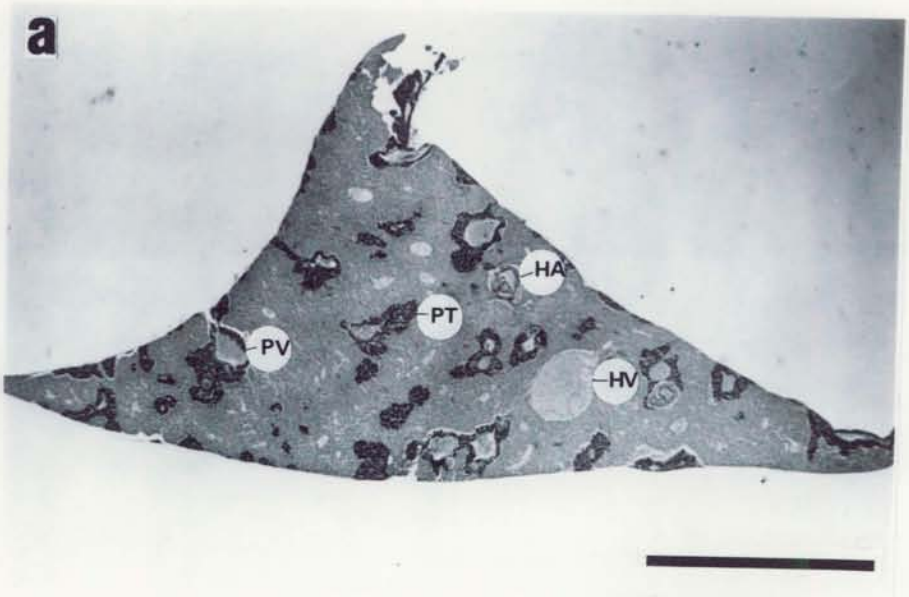
the gut cavity below the swim-bladder. The left lobe (sinistral) was typically larger than the right (dextral) lobe. Both lobes connected each other anteriorly, and attached proximally to the intestinal tract by the connective tissue. The surface of liver was covered with serous membrane, and some connective tissue from this capsule extended inward into the parenchyma. The size of liver was large ranging from 0.74% to 8.92% of body weight ($\overline{X}$ = 3.55% $\pm$ 1.65) in *S.schlegeli*, and from 0.56% to 5.45% ($\overline{X}$ = 2.16% $\pm$ 0.81) in *S.rivulatus*.

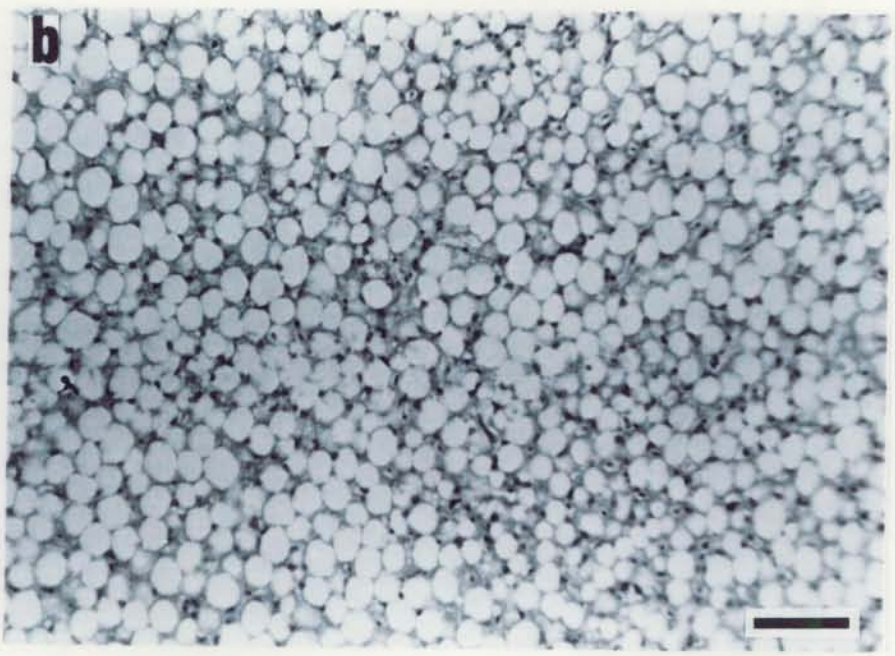
Liver colour in both species showed the same range from brown to light yellow, and could generally be grouped into 4 categories as brown, light brown, putty grey and light yellow. As liver colour generally changed after freezing for a long period (approximately more than 10 hours), liver colour was only recorded for the specimens dissected within a half day from the collection. For 116 individuals examined in *S.schlegeli*, the distribution of liver colour was not uniform. Most individuals (90%) had either putty grey or light yellow liver. Only a minority (10%) were brown. For *S.rivulatus* (n=109), the colour distribution was different. Most individuals had light brown or grey livers (18% and 66% respectively). There were relatively few with brown or light yellow livers. This variation in liver colour provided a basis for correlating liver colour with other properties such as lipid content.

Viewed under a microscope (40×), a liver transverse section generally exhibited hepatic artery, hepatic vein, pancreatic tissue and partial vein branches (Plate 7.1a). Some liver sections showed a large amount of the vacuolate liver parenchyma (Plate 7.1b) under the higher magnification (>=400×), and these vacuoles varied considerably in amount among individuals while the other features

Plate 7.1 General structure of a transverse section of scarid liver from 230 mm SL *Scarus schlegeli*.

a. General structure. HA - hepatic artery; HV - hepatic vein; PT - pancreatic tissue and PV -portal vein branch. Scale bar = 250 μm. **b**. The same section viewed under higher power (400×), showing large accumulation of liver vacuoles. Scale bar = 20 μm.


a
HA
PT
PV
HV


b

generally remained consistent.

7.3.2 Hepatosomatic index

7.3.2.1 Distribution by SL and age

The plots of HSI against SL showed a great deal of variation in the relationship between sexual types and two color phases for *S.rivulatus* (Fig. 7.2). It was not possible to draw any conclution about the relationship between HSI and SL. However, it appeared that mature females had higher HSI than that of mature males within the initial phase based on the mean HSI value (Table 7.1), and that some individuals of mature females had the highest HSI. A distribution of HSI by age (Fig. 7.3; Table 7.2) was also highly variable, but again showed that IP mature females had higher HSI than that in IP mature males.

For *S.schlegeli* the plots of HSI against SL in all sexual identities with different color phases (Fig. 7.4) showed a very similar relationship to that of *S.rivulatus*. The overall distribution was also highly variable, and there was not any evident trend existed. However, the transitional individuals showed remarkably low values of HSI among the all specimens (Table 7.3). A distribution of HSI by age appeared to be similar to that by SL (Fig. 7.5; Table 7.4). Overall, this was also a highly variable distribution without any evident trend.

From the above results it was clear that there was no any correlation between HSI and fish length or age for both species. However, as indicated in the other studies involved with fish livers (Pulliainen and Korhonen, 1990), HSI generally tends to vary over different seasons. It was, therefore, necessary to further examine a seasonal HSI pattern seperated by sexual identity.

Table 7.1 Means±s.d. of HSI between sexual types, color phases in each 20-mm size class for *Scarus rivulatus*.

Sampling sizes show in parenthesises.

SL (mm)	Overall	IP		TP	
		♀♀	1○♂♂	1○♂♂	2○♂♂
100-119	2.56±1.23(2)	2.56±1.23(2)			
120-139	1.81±0.41(8)	1.63±0.82(4)	2.00±0.31(4)		
140-159	2.00±0.51(34)	2.09±0.54(21)	1.87±0.43(13)		
160-179	2.00±0.74(85)	2.08±0.79(62)	1.80±0.56(23)		
180-199	2.25±0.94(52)	2.38±0.90(44)	1.64±0.41(7)	1.11 (1)	
200-219	2.30±0.82(47)	2.32±0.84(36)	2.16±0.09(2)	2.32±0.87(7)	1.92±0.69(2)
220-239	2.27±0.90(21)	2.12±1.13(11)		2.32±0.60(3)	2.49±0.44(7)
240-259	2.61±0.95(19)	1.85±0.72(2)		3.29±0.93(3)	2.57±0.79(14)
260-279	1.90±0.64(6)				1.90±0.64(6)
280-299	1.77±0.46(2)				1.77±0.46(2)
Overall		2.19±0.84(182)	1.83±0.49(49)	2.44±0.97(14)	2.33±0.79(31)

Table 7.2 Means±s.d. of HSI between sexual types, color phases in each age class for *Scarus rivulatus*.

Sampling sizes show in parenthesises.

Age Group	Overall	IP		TP	
		♀♀	1○♂♂	1○♂♂	2○♂♂
1+	1.97±0.83(7)	1.97±1.05(4)	1.97±0.36(3)		
2+	1.92±0.61(100)	1.97±0.62(68)	1.82±0.56(32)		
3+	2.22±0.80(63)	2.33±0.85(51)	1.72±0.33(10)	1.99±0.88(2)	
4+	2.32±0.92(52)	2.38±1.01(37)	2.06±0.18(4)	2.14±0.81(8)	2.41±0.19(3)
5+	2.32±0.82(29)	2.24±0.78(15)		3.89±0.49(2)	2.15±0.65(12)
6+	2.59±1.08(17)	3.03±1.25(4)		2.64±0.53(2)	2.42±1.05(11)
7+	2.40±0.55(7)	2.10±0.46(2)			2.52±0.54(5)
8+	1.13 (1)	1.13 (1)			
Total	276	182	39	14	31

Table 7.3 Means±s.d. of HSI between sexual types, color phases in each 20-mm size class for *Scarus schlegeli*.

Sampling sizes show in parenthesises.

SL (mm)	Overall	IP		Trans.		TP	
		♀♀	1○♂♂	♀→♂	♂→♂	1○♂♂	2○♂♂
100-119	1.73±0.74(14)	1.75±0.66(6)	1.72±0.80(8)				
120-139	2.89±1.28(6)	3.44±1.40(3)	2.34±0.85(3)				
140-159	2.86±1.31(19)	2.40±0.61(11)	3.50±1.69(8)				
160-179	3.12±1.63(59)	3.11±1.33(44)	3.19±1.15(13)	2.21 (1)	3.89 (1)		
180-199	3.76±1.28(110)	3.88±1.76(80)	3.47±1.10(27)	2.10 (1)			3.70±1.70(2)
200-219	4.43±1.77(47)	4.98±1.73(23)	4.49±2.33(4)	2.06 (1)		3.72±0.77(2)	3.89±1.48(17)
220-239	3.64±1.65(28)	2.95±0.59(2)	3.35±1.93(2)	1.46 (1)		5.29 (1)	3.80±1.67(22)
240-259	3.68±1.76(5)						3.68±1.76(5)
260-279	3.45(1)						3.45 (1)
Overall		3.63±1.73(169)	3.21±1.46(65)	1.96±0.29 (4)	3.89 (1)	3.92±0.69(3)	3.81±1.60(47)

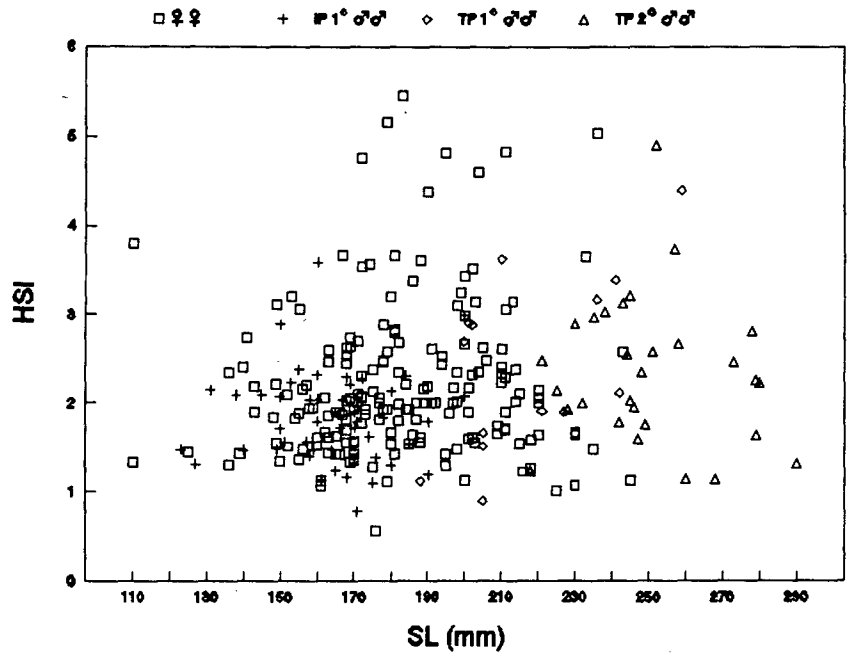
Table 7.4 Means±s.d. of HSI between sexual types, color phases in each age class for *Scarus schlegeli*.

Sampling sizes show in parenthesises.

Age Group	Overall	IP		Trans.		TP	
		♀♀	1○♂♂	♀→♂	♂→♂	1○♂♂	2○♂♂
1+	2.19±1.03(23)	2.31±1.26(9)	2.11±0.84(14)				
2+	3.12±1.31(52)	3.09±1.26(36)	3.23±1.45(14)	2.10 (1)	3.89 (1)		
3+	3.58±1.53(124)	3.63±1.66(87)	3.52±1.17(32)	2.21 (1)		2.95 (1)	3.62±1.39(3)
4+	4.23±1.88(54)	4.65±1.96(30)	4.01±2.69(4)	2.06 (1)		4.49 (1)	3.69±1.32(18)
5+	4.08±1.75(29)	3.65±1.29(5)	5.29 (1)	1.46 (1)		4.33 (1)	4.25±1.80(21)
6+	2.50±0.58(5)	2.36 (1)					2.54±0.64(4)
7+	7.08 (1)	7.08 (1)					
8+	2.30 (1)						2.30 (1)
Total	289	169	65	4	1	3	47

Fig. 7.2 *Scarus rivulatus*. **The distribution of hepatosomatic index (HSI) by SL in different sexual types and color phases.**

Fig. 7.3 *Scarus rivulatus*. **HSI distribution by age classes in different sexual types and color phases.**


♀♀
IP 1° ♂♂
TP 1° ♂♂
TP 2° ♂♂
HSI
SL (mm)

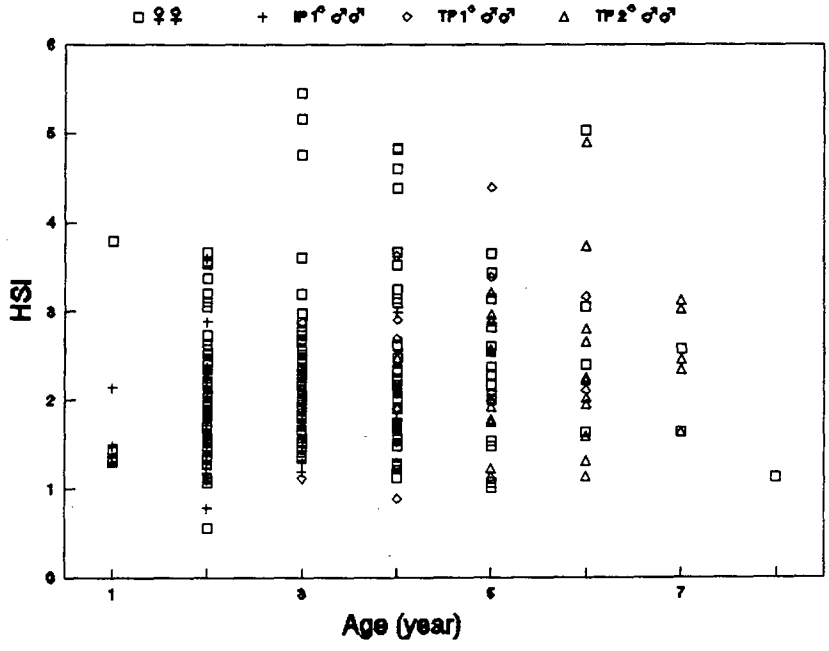
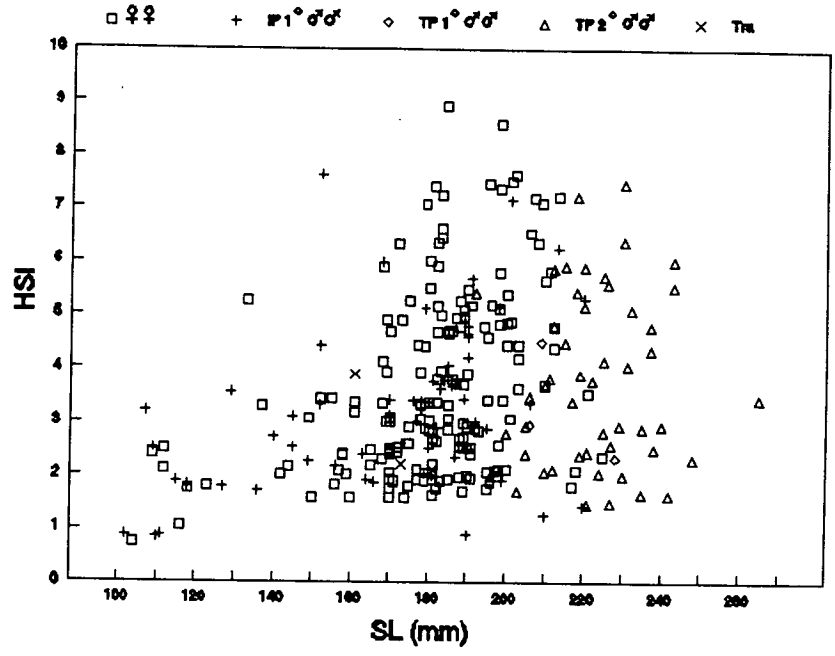
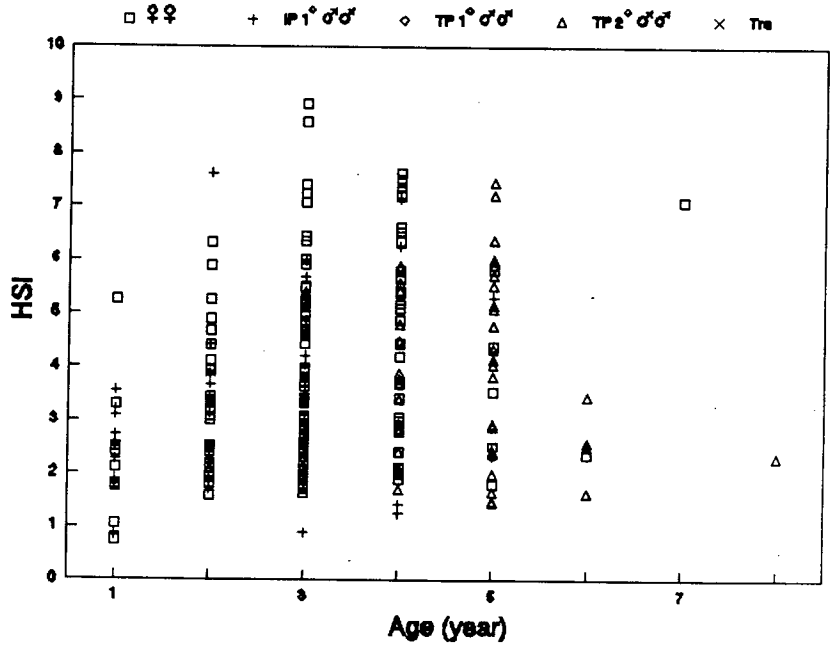

♀♀
IP 1° ♂♂
TP 1° ♂♂
TP 2° ♂♂
HSI
Age (year)

Fig. 7.4 *Scarus schlegeli*. **HSI distribution by SL in different sexual types and color phases.**

Fig. 7.5 *Scarus schlegeli*. **HSI distribution by age class in different sexual types and color phases.**


♀♀
IP1° ♂♂
TP1° ♂♂
TP2° ♂♂
Tns
HSI
SL (mm)


♀♀
IP1° ♂♂
TP1° ♂♂
TP2° ♂♂
Tns
HSI
Age (year)

7.3.2.2 Seasonal distribution

The analysis of seasonal distribution on livers was only carried out on the specimens collected from December 1989 to January 1991 since only few livers were sampled in November 1989. Due to a small number of specimens collected in each sampling occasion, individuals with all sexual identities in different colour phases were not presented in some months. Consequently, three sexual types were designed as immature, female and male. The first two types were similar to that described in 6.3.3 while the third one included mature 1♀♂, 2♀♂ and transitional individuals.

7.3.2.2.1 *Scarus schlegeli*

The temporal distribution of HSI showed a strong seasonal pattern in mature males and females (Fig 7.6). The mean HSI of males and females was the lowest in September and gradually increased from November, reaching the peak around March to May. After May the mean HSI decreased rapidly throughout September. A comparison of HSI between the high value period (March - May) and the low value period (September - November) showed significant differences in males ($F=9.88$, $p<0.01$) and females ($F=43.17$, $p<0.001$). The mean HSI of immature fishes appeared to remain relatively constant over time.

Figure 7.6 showed that the seasonal distribution of HSI peaked in March and May in mature males and females prior to the main peak of gonad development expressed as GSI.

7.3.2.2.2 *Scarus rivulatus*

A different pattern of the seasonal distribution in HSI from *S.schlegeli* was

found in *S.rivulatus* (Fig. 7.7). Even though the mean HSI in both mature males and females varied over time, the lowest value was found in February for males and March for females while the highest value appeared from May to September for both sexes. Furthermore, large deviations were obtained for both sexes in most months. A comparison of HSI between the highest value period (May - September) and the lowest value period (February in males; March in females) showed significant differences in males ($F=3.05$, $p<0.05$) and females ($F=3.44$, $p<0.05$). Like in *S.schlegeli*, the mean HSI of immature fish in *S.rivulatus* was also relatively constant.

The seasonal pattern in HSI in *S.rivulatus* also appeared to be related to the spawning activities. As indicated in Figure 7.7, the peak of seasonal HSI distribution for both mature sexes appeared to occur soon before that of seasonal GSI distribution in females.

It was clear that HSI in sexually mature individuals of both species tended to vary in different months over the year. These seasonal variations in HSI also varied in pattern and magnitude between two species. However, for both species such seasonal variations all appeared to couple with the reproductive activities such as spawning.

7.3.3 Hepatic lipids

7.3.3.1 Lipid droplets

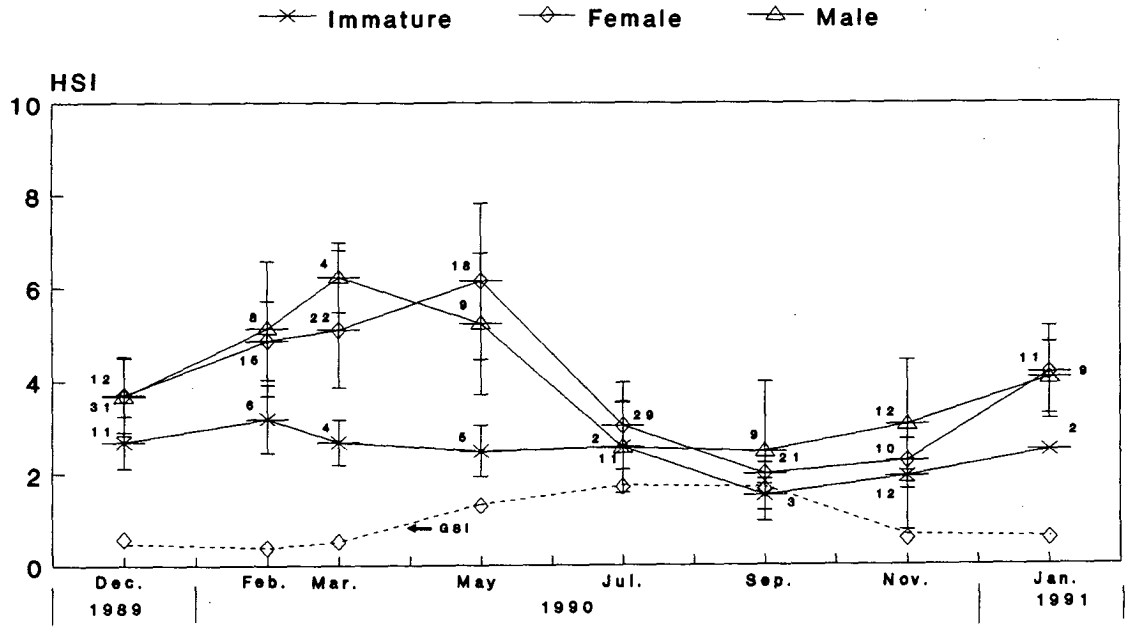
The pilot study showed that the liver tissue with high proportion of vacuolate liver parenchyma contained the highest accumulation of lipids (Plates 7.2a,b), followed by the liver tissue with medium proportion of the vacuoles (Plates 7.2c,d),

Fig. 7.6 Average HSI from *Scarus schlegeli*.

The specimens were taken at Lizard Island between December 1989 to January 1991. Average GSI of females was also given for comparison. Vertical bar indicates standard deviations. Sample sizes are shown beside the vertical bar.

Fig. 7.7 Average HSI from *Scarus rivulatus*.

The specimens were taken at Lizard Island between December 1989 to January 1991. Mean GSI of females was also given for comparison. Vertical bar indicates standard deviations. Sample sizes are shown beside the vertical bar.


Immature
Female
Male
HSI
10
8
6
4
2
0
GSI
Dec.
1989
Feb.
Mar.
May
Jul.
Sep.
Nov.
Jan.
1990
1991

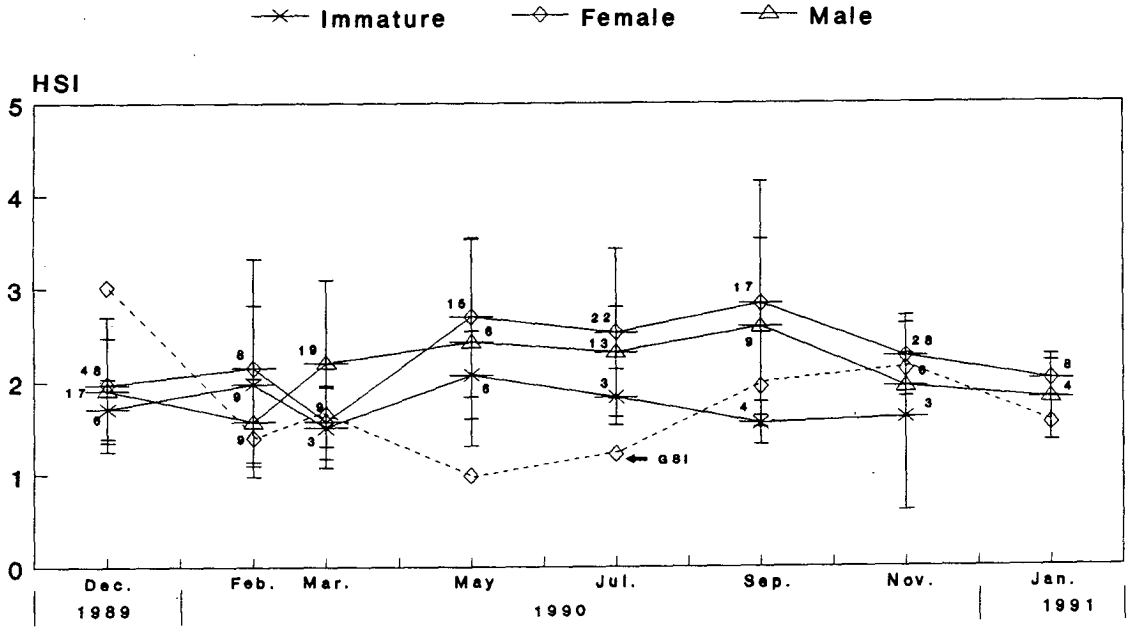
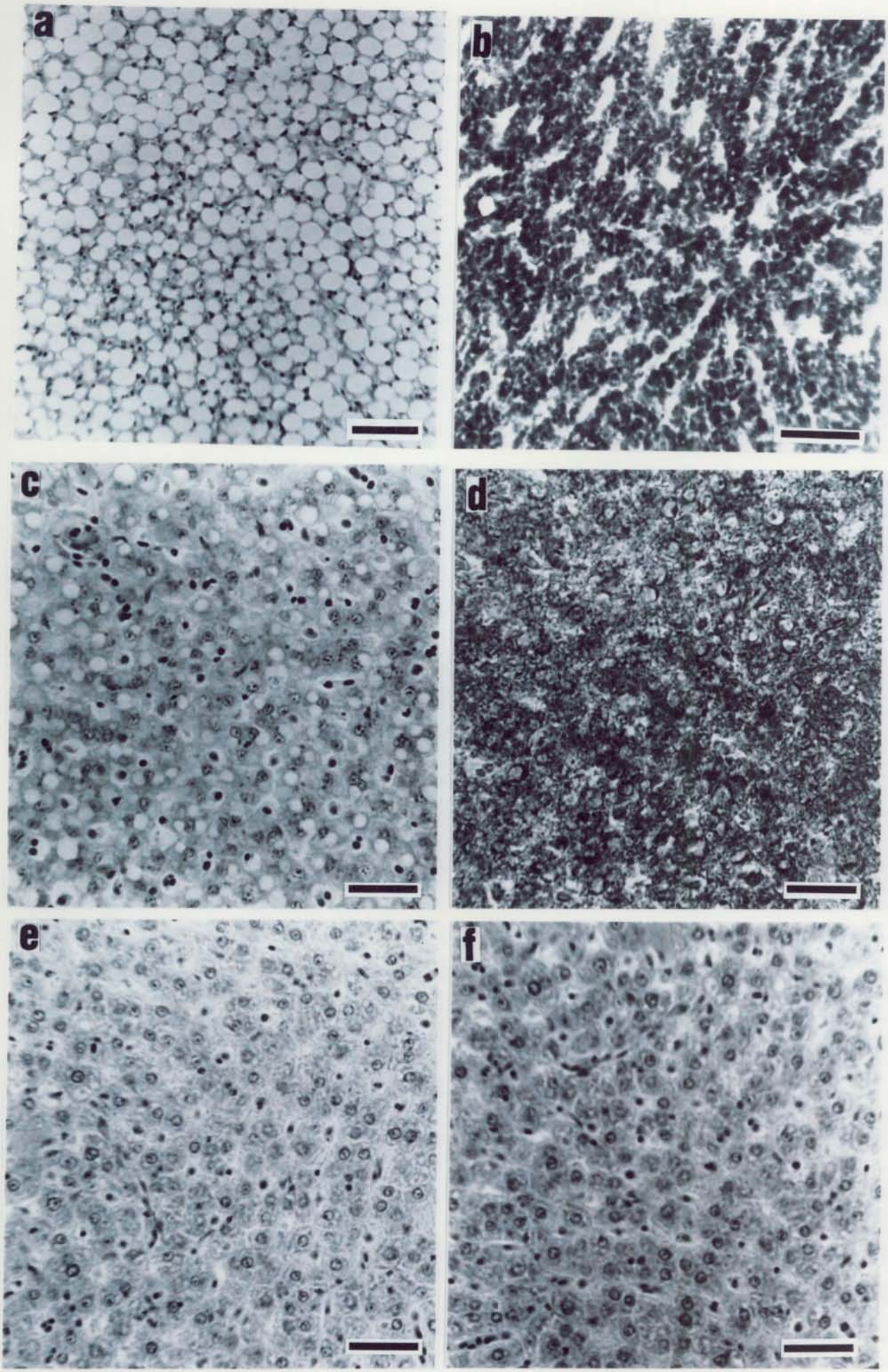

Immature
Female
Male
HSI
5
4
3
2
1
0
GSI
Dec.
1989
Feb.
Mar.
May
Jul.
Sep.
Nov.
Jan.
1990
1991

Plate 7.2 Comparison between the normal histological liver sections and the liver-extracted sections in the scarids.

All scale bar = 20 μm. **a.** A liver-section (HSI=5.66) treated in the normal histological process, showing a large amount of vacuoles. **b.** A lipid-extracted section of the same liver (**a**), showing tremendous accumulation of lipids. **c.** A liver-section (HSI=2.29) treated in the normal histological process, showing a mid amount of vacuoles. **d.** A lipid-extracted section of the same liver (**c**), showing some accumulation of lipids. **e.** A liver-section (HSI=1.47) treated in the normal histological process, showing no vacuole. **f.** A lipid-extracted section of the same liver (**f**), showing almost no accumulation of lipids.


a
b
c
d
e
f

and that the liver tissue with low proportion of the vacuoles had almost no lipid (Plates 7.2e,f). These results demonstrated that the vacuolate liver parenchyma presented in ordinary histological sections were sites of lipid storage in consequence of disappearing lipid droplets after the ordinary histological procedure involving the use of fat solvent. No attempt was made to characterize the nature of these lipids as it was beyond the scope of this study.

7.3.3.2 The relationship between liver colour and HLI

The results of correlating liver colour with HLI were shown in Tables 7.5 and 7.6. It can be seen in *S.schlegeli* that for light yellow livers there was a high HLI suggesting that the light yellow colour was indicative of a high lipid contained. A similar but less consistent relation was seen in livers with a grey colour. For both brown and light brown livers the lipid content appeared to be more variable ranging from very low (<20%) to very high (>80%) values.

For *S.rivulatus* the relationship between liver colour and lipids was more variable (Table 7.6). Although HLI values were higher (>40%) in most putty grey livers and lower (<40%) in most light brown livers the both colour livers had a wide range of HLI values (<20% - >80%).

7.3.3.3 Distribution of HLI

Area fractions of lipid droplets were close to each other between 4 measuring areas within the same liver section for both species (Fig.s 7.8, 7.9). This indicated approximately an even distribution of hepatic lipids across a liver section.

HLI value generally increased with increasing HSI value for both species

Table 7.5 Relationship between liver colour and hepatic lipids in *Scarus schlegeli*.

HLI	N	Number of livers with colour of			
		Brown	Light brown	Putty grey	Light yellow
< 20%	5	3	2		
20% - 39%	0				
40% - 59%	5		1	4	
60% - 80%	18		1	13	4
>80%	88	2	2	42	42

Table 7.6 Relationship between liver colour and hepatic lipids in *Scarus rivulatus*.

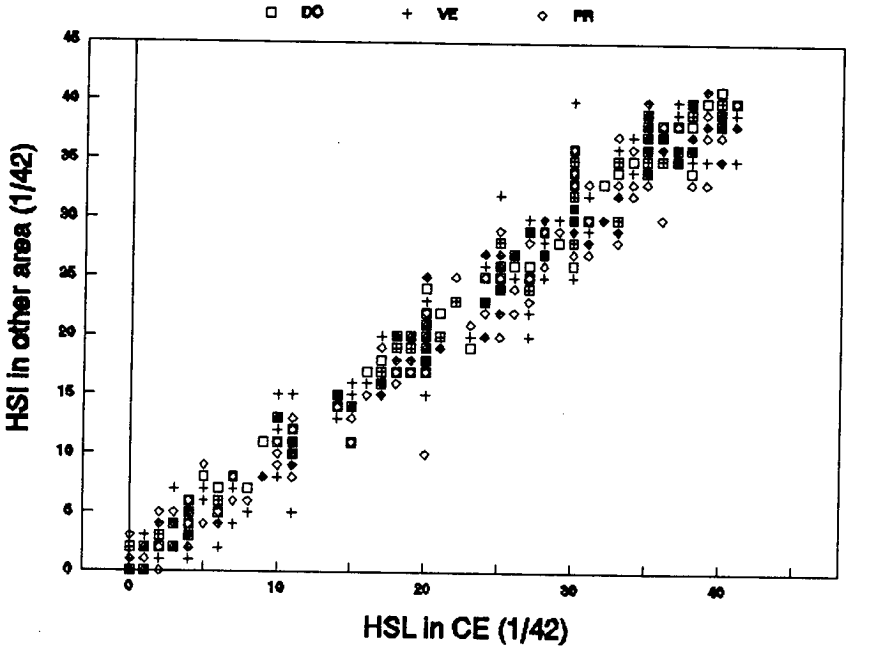
HLI	N	Number of livers with colour of			
		Brown	Light brown	Putty grey	Light yellow
< 20%	28	10	9	9	
20% - 39%	9		3	5	1
40% - 59%	28	4	4	20	
60% - 79%	23		3	19	1
> 80%	21		1	20	

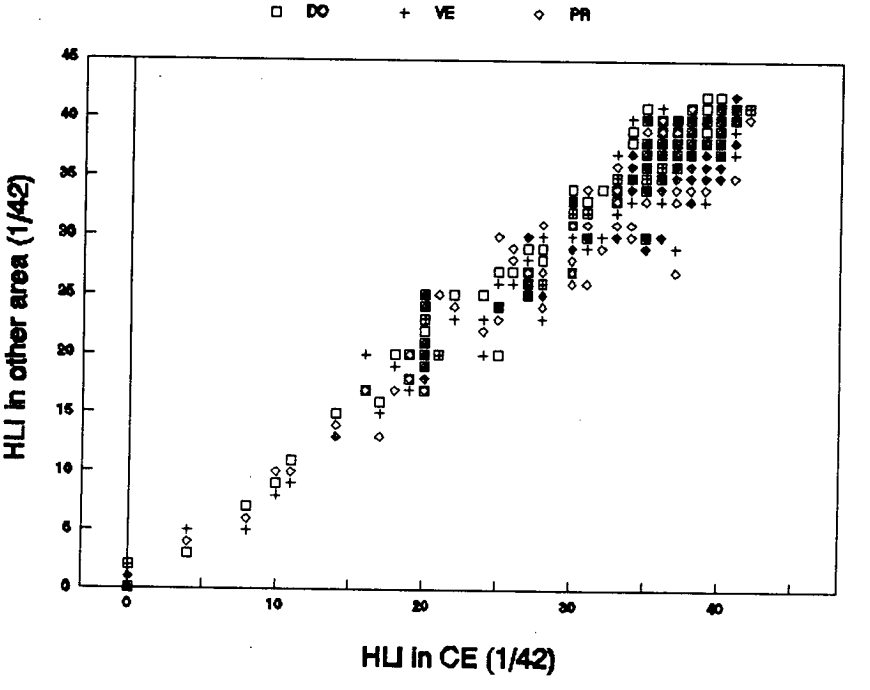
Fig. 7.8 Distribution of hepatic lipid droplets in four measuring areas (DO, VE, PR and CE) in 276 *Scarus rivulatus*.

The speciemens were collected between November 1989 and January 1991 at Lizard Island, indicating approximately even distributions of lipid droplets between the 4 areas.

Fig. 7.9 Distribution of hepatic lipid droplets in four measuring areas (DO, VE, PR and CE) in 289 *Scarus schlegeli*.

The specimens were collected between November 1989 and January 1991 at Lizard Island, indicating approximately even distributions of lipid droplets between the 4 areas.


DO
VE
PR
HSI in other area (1/42)
HSL in CE (1/42)
0
5
10
15
20
25
30
35
40
45
0
10
20
30
40


DO
VE
PR
HLI in other area (1/42)
HLI in CE (1/42)
0
5
10
15
20
25
30
35
40
45
0
10
20
30
40

(Fig.s 7.10, 7.11). The individuals with HSI value larger than 3 consistently showed a higher HLI value while a wide range of HLI values from 0 to 100 was observed in the individuals with HSI less than 3. Only one individual in *S.rivulatus*, that had HSI value larger than 3, showed a low value of HLI.

7.3.3.3.1 *Scarus rivulatus*

The distribution in HLI varied in different seasons over the year in both females and males (Fig. 7.12). The livers of males and females, that collected from May to September, generally contained higher level of lipids (HLI > 40%) while the livers had lower level of lipids (HLI < 40%) in March for females and in November and January for males. The mean HLI in females decreased gradually from December, and reached the lowest in March, increasing after that until July. A close trend in the distribution of mean HLI was found in males. Although a small fluctuation was observed during May to September, mean HLI generally increased from December throughout September followed by the lowest in November. The sample sizes were too small to make any satisfactory statement in the immature fish.

The overall trend of seasonal HLI distribution showed a very similar pattern to that of seasonal HSI distribution (Fig. 7.7), indicating that hepatic lipids predominated the size and weight of liver in *S.rivulatus*.

7.3.3.3.2 *Scarus schlegeli*

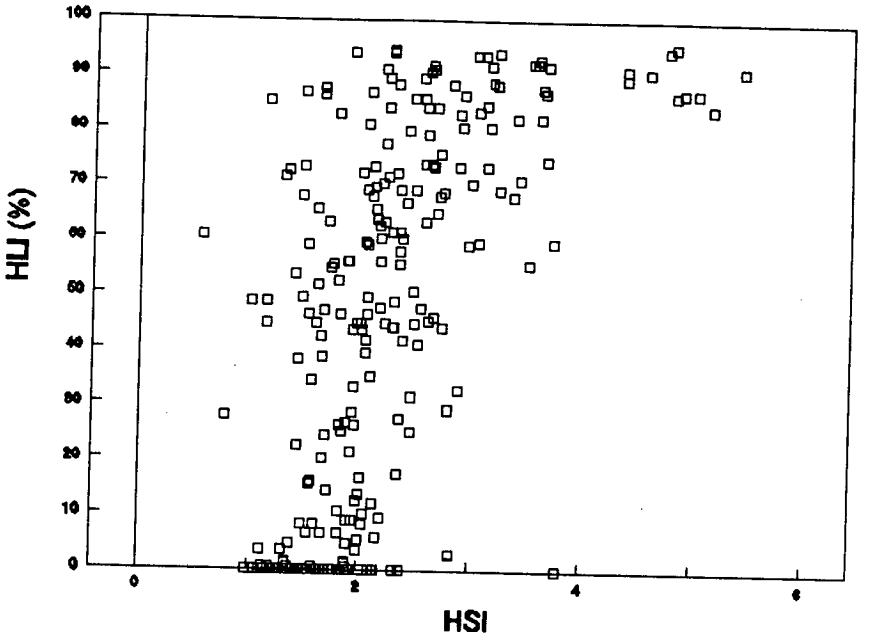
The season variation in HLI was larger in *S.schlegeli* than that in *S.rivulatus* for both males and females (Fig. 7.13). For both sexes, the livers collected in December, Febuary and March generally contained a higher level of lipids

Fig. 7.10 An overall distribution of hepatic lipid index (HLI) by HSI for *Scarus rivulatus*.

The samples were taken between November 1989 and January 1991 at Lizard Island.

Fig. 7.11 An overall distribution of hepatic lipid index (HLI) by HSI for *Scarus schlegeli*.

The samples were taken between November 1989 and January 1991 at Lizard Island.


HLI (%)
HSI

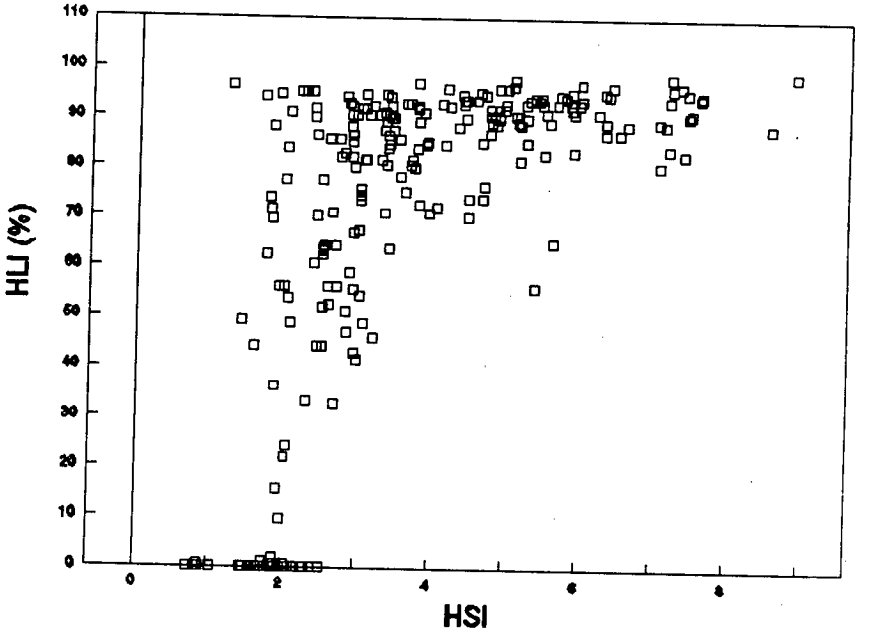
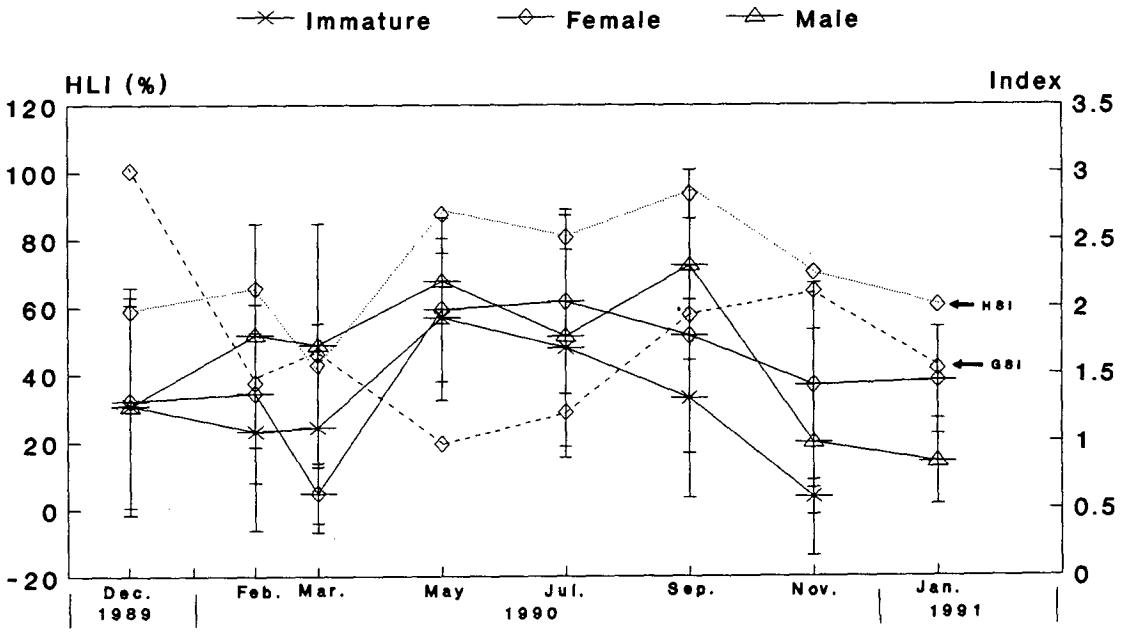

HLI (%)
HSI

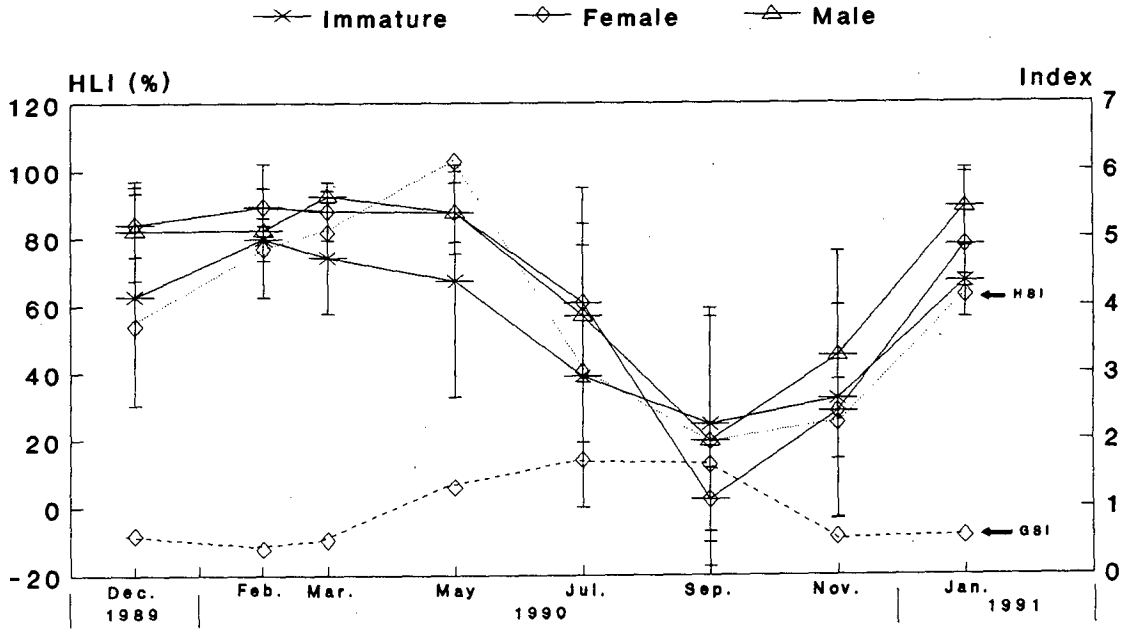
Fig. 7.12 Seasonal distributions of hepatic lipid index (HLI) for female, male and immature *Scarus rivulatus*.

The samples were taken between December 1989 and January 1991. Sample sizes were shown in Figure 7.7.

Fig. 7.13 Seasonal distributions of hepatic lipid index (HLI) for female, male and immature *Scarus schlegeli*.

The samples were taken between December 1989 and January 1991. Sample sizes were shown in Figure 7.6.


Immature
Female
Male
HLI (%)
Index
120
100
80
60
40
20
0
-20
3.5
3
2.5
2
1.5
1
0.5
0
HSI
GSI
Dec.
1989
Feb.
Mar.
May
Jul.
Sep.
Nov.
Jan.
1991
1990


Immature
Female
Male
HLI (%)
Index
120
100
80
60
40
20
0
-20
7
6
5
4
3
2
1
0
HSI
GSI
Dec.
1989
Feb.
Mar.
May
Jul.
Sep.
Nov.
Jan.
1991
1990

(HLI > 60%) while 95% (females) and 78% (males) of the livers collected in September had a lower level of lipids (HLI < 20%). The mean HLI in females was the highest in Febuary and March, gradually decreased from March, and reached the lowest in September. The seasonal distribution of HLI in the immature fish appeared to be close to that of the females and males. However, this trend could not be confirmed with few specimens in most months.

Like in *S.rivulatus*, the seasonal distribution of HLI in both sexes was also similar to that of HSI in *S.schlegeli* (Fig. 7.6). This similarity again conformed the predominating role of hepatic lipids in determining liver size and weight.

For both species livers in the sexually mature individuals were lipid storage, and these storage varied in quantity with seasons, and between species. As reflected by a strong seasonal pattern in spawning activities *S.schlegeli* also had a strong seasonal pattern in HSI and HLI. On the other hand, the seasonal pattern in HSI and HLI was not very pronounced in *S.rivulatus*, which was due to the protracted spawning season in this species.

7.4 DISCUSSION

The general structure of liver in *S.rivulatus* and *S.schlegeli* appears to be similar to that of other members of the same genus (Bellwood, 1985). However, the relative liver weight (HSI) in *S.schlegeli* tends to be beyond the range of 1.5 to 7.4%, which was estimated in scarids by AL Hussaini (1945, 1947), Gohar and Latif (1959) in the Red Sea. This suggests that liver weight in scarids is largely variable between species in different locations.

Rossouw (1987) found that liver colour was an index of hepatic lipids in the

lesser sand shark, *Rhinobatos annulatus* (Müller & Henle). Colour of scarid liver (at least in *S.schlegeli*) seems similarly to reflect lipid content, indicating that liver colour can be used to measure hepatic lipids macroscopically. As liver colour generally changes after a long period of preservation, it will be most suitable to estimate hepatic lipids for fresh specimens in field.

In teleosts, liver proportional weights generally do not change rapidly or dramatically with increasing fish size or even very much at all (Weatherley and Gill, 1987). The results obtained in this study seem to agree with this as indicated by the highly variable distribution of HSI vs SL or age. As HSI in adult fishes tends to be related to the metabolic condition (Shul'man, 1974; Crupkin *et al.*, 1988) and feeding state (Heiding and Crawford, 1977, Rosenlund *et al.*, 1984), it is indeed difficult to obtain a relationship between HSI and SL or age for adult fishes without any other influence. In the tropical scarids HSI in adult individuals was more related to the gonadal development, and therefore it is more seasonally variable. The remarkably low value of HSI obtained from the sexual transitional individuals in *S.schlegeli* (Tables 7.3, 7.4) seems to be caused by spawning activities rather than the sexual state as some individuals collected in the same time had even lower value of HSI than that of these transitional individuals.

Smith *et al.* (1990) found that in the Pacific cod *Gadus macrocephalus* liver energy stores were mobilized differently by the two sexes. He suggested the possibility that liver energy in males was more directly tied to gonad development than females. The relatively low values of HSI in IP 1°♂♂ in *S.rivulatus* (Tables 7.1,2) seem unrelated to differences in energy storage between both sexes. Both mature male and female *S.rivulatus* showed not only a very similar trend in seasonal

patterns of HSI (Fig. 7.7) but also similar seasonal fluctuations of hepatic lipids (Fig. 7.12). Moreover, it appears that seasonal variation in HSI is also not attributable to this difference since both female and IP 1°♂♂ individuals were collected in every sampling month over the year. Therefore, this low value of HSI in 1°♂♂ is more likely caused by an other mechanism, such as ontogenesis in this species.

The histochemical nature of liver vacuoles has been demonstrated as lipid droplets in other studies involved in fish livers. Oguri (1978a) found that vacuoles in hepatic cytoplasm appeared in consequence of disappearing fat droplets after ordinary histological method involving the use of fat solvent in ratfish, *Hydrologus colliei*, and rabbitfish, *Chimaera monstrosa*. Similar results were also obtained in European spotted dogfish *Scyliorhinus canicula* (Oguri, 1978b). In addition, Oguri (1978b) observed a positive relationship between HSI and the hepatic lipids contained. My results in the tropical scarids appeared to be similar to the above findings, suggesting that a positive relationship between HSI and hepatic lipids exists universally in teleosts and that HSI can generally indicate levels at which lipids are stored in individual livers.

Lipids as a main energy source in fish affect growth in a complex way, quantitatively and qualitatively. Shul'man (1974) found that the lipid accumulation in Black Sea anchovy tended to increase with increasing body weight. In contrast, Thongrod *et al.* (1989) observed that high levels of total hepatic lipids were not related to improvement in growth of fingerling white fish *Coregonus lavaretus maraena*. As the nature of hepatic lipids is not determined in the current study, a similar assessment of the relationship between hepatic lipids and growth was not carried out for the study species. Further research regarding the nature of hepatic

lipids is required before such an assessment can be made.

In conclusion liver weight in *S.rivulatus* and *S.schlegeli* at Lizard Island changed over time, and this reflected the compositional states. High HSI occurred when liver had high levels of lipids. HSI was also closely related to GSI. High HSI occurred immediately before spawning, which suggested that the liver is an important energy storage organ and that lipids provides the material for gonad development. The two study species in similar microhabitats and similar physical environment showed seasonal patterns, but varying in timing and magnitude.

CHAPTER 8. GENERAL DISCUSSION AND CONCLUSIONS

8.1 GENERAL DISCUSSION

Scarids and acanthurids are two abundant groups of herbivorous reef fish. They play significant roles in the trophodynamics of coral reefs (Choat, 1991) and bioerosion (Hutchings, 1986; Horn, 1989; Bellwood and Choat, 1990). They are also important in some fisheries in the Asia and Pacific region (Russ, 1991). The age structure and growth rates of these two groups are thus of interest. As suggested by previous studies (see review by Russ and St. John, 1988), scarids, although relatively large fish, appear to have rapid growth rates and relatively short lifespans while acanthurids including small species of *Acanthurus* and *Ctenochaetus* have slow growth rates and extended lifespans. This can only be confirmed by reliable aging information in tropical reef fishes.

By using the validated aging methods for both juveniles and adults, this study has confirmed distinct differences in age structure and growth rates between the representatives of acanthurids (*C.binotatus* and *C.striatus*) and scarids (*S.rivulatus* and *S.schlegeli*). The acanthurids may have lifespans in excess of 16 years. They have a slow and decreasing growth rate after settlement. In contrast, the scarids have a relatively short lifespan to 8 years, and have a rapid early growth rate. Although the growth rate in the scarids decreases after the juvenile phase, the overall rate is higher than that in the acanthurids. Furthermore, the otolith microstructures suggest that scarids have a short pelagic life from 28 to 47 days, while acanthurids have a long larval period from 47 to 74 days. These results have confirmed the above suggestion as far as the four species studied.

There are further important differences in life patterns and demography between the two groups. Individuals of acanthurids do not change sex (Clements, 1991), and they have relatively simple patterns of sexual development compared with the protogynous hermaphroditic scarids. The demographic information from this study also suggests differences in recruitment rates and longevity. Differences in longevity imply different mortality rates in acanthurids and scarids. However, until age-specific patterns of survivorship are determined we can only note differences in longevity.

Although scarids and acanthurids are both herbivores, grazing on reef substrate they have different developmental histories and methods of processing algae. Bellwood (1988) found a change-over from carnivory to herbivory for early post-settlement scarids while acanthurids were found to be almost exclusively herbivorous. Scarids feed by grinding algal materials and carbonates in pharyngeal mill, and had a relatively short sacculated gut (Choat, 1966; Bellwood, 1985). On the other hand, acanthurids have a longer gut often with a gizzard and caeca, and appear to have more complex endoflora (Clements, 1991). Consequently, scarids have large amounts of carbonate materials and sediments in guts (Russ and St. John, 1988). Furthermore, scarids display a wide range of feeding strategies (Bellwood, 1985). Although some of these strategies overlap with some species of acanthurids, no acanthurid possesses all these feeding strategies (Bellwood, 1985). There are marked differences between these two families. However, there are relatively few studies which have attempted to identify differences between species with the same family.

The key to understanding the dynamics of scarid and acanthurid populations

is age-specific descriptions of growth, reproductive events and mortality. The validated age and growth estimates obtained in this study open the door to the population dynamics of tropical scarids and acanthurids. The age at settlement and observations of early growth rates will help in the understanding of the recruitment pattern, while the age structure reveals the longevity of the population. Moreover, the lifespan suggests the rates of recruitment, growth and turnover of individuals in a population. Furthermore, the age composition data can be used to estimate the rate of mortality and survivorship. All these demographic parameters are impossible to estimate for the tropical scarid and acanthurid populations without the validated aging information. However, as the sample size in this study is limited, especially in the acanthurids, and the age distribution of the sample may not be representative of the population, further studies are required before accurate estimates of demographic parameters can be made for tropical parrotfishes and surgeonfishes.

In this preliminary comparison of the demography between scarids and acanthurids, it suggests that scarids may be suitable candidates for intensive fisheries. This is because scarids not only have relatively large size but also have a high growth rate, and the populations turnover quickly. Therefore, it is necessary that other aspects of scarid population are examined. The reproductive biology is of particular interest. Like the majority of coral reef fishes, scarids are predominantly protogynous hermaphrodites (Choat and Robertson, 1975; Randall and Bruce, 1983; Choat and Randall, 1986). The dynamics of sexual transition needs to be understood before a comprehensive management can be established for scarid fishery.

Scarids have complex patterns of sexual development which include a number of different sexual identities (females, primary males and secondary males) and color

phases. Previous studies have identified the sequences of sex and color change in a number of species. However, in the absence of a reliable method of aging it has not been possible to estimate the duration of the different sexual and color phases, and the rate at which individuals may transfer from one state to another.

The validated age estimates and growth rates in this study allow us to illustrate the dynamics of the different sexual history for each group in the scarid population. These scarids appear to have a shorter sexual transition period than that of some species of the labrids (Warner, 1975; Cowen, 1990). Some of the female scarids studied (*S.rivulatus* and *S.schlegeli*) started changing sex to secondary males as early as 3 years old, and had undergone this sexual transformation by approximately 6 years of age (Warner, 1988; Shapiro, 1989). Similarly, some individuals of the primary males started changing their color phase into the terminal phase also at age of 3 years. The primary males seem to have a relatively shorter lifespan than females. Furthermore, it is demonstrated by the comparison on growth between different sexual groups among color phases that the terminal phase males have faster growth rates than the initial phase individuals of the same age. These results indicate that protogynous hermaphroditism and dichromatism in the tropical scarids complicate population dynamics. It is necessary to understand the patterns of growth and maturity in the different identities before we can develop a picture of a whole scarid population.

By seasonal examination of gonads, this study also demonstrated a seasonal pattern in reproduction for tropical scarids. Although spawning occurs year round for both species, the proportion of reproductively active individuals within the population varies over time. In *S.schlegeli*, there is a strong seasonal pattern of gonad activities,

and most individuals spawn during the austral winter (May - September). For the other species (*S.rivulatus*), such a seasonal pattern tends to be less pronounced, and spawning is more extended covering the period from September to January. This seasonal pattern in reproduction is further supported by a corresponding seasonal pattern in the condition of the liver expressed as the proportional liver weight and liver composition. In both species, seasonal change in hepatic condition were observed and in association with gonadal development. The highest value of the hepatic condition occurs immediately before spawning.

It is noteworthy that the two study species have different annual patterns of reproduction and hepatic condition in terms of timing and magnitude. Both species not only have similar age structure and growth pattern but also were collected from the similar microhabitats in a similar physical environment. This phenomenon indicates that patterns of spawning and somatic condition can not be solely attributed to the physical environment. The history of each species and their patterns of development must also play a part.

As a distinct group of herbivorous reef fishes, the population of scarids exhibits fast growth. By rapid growth in the early life stage, scarids reach sexual maturity at the relatively young age of 2 years. After that, the majority of individuals in the population start to change sex and color phase as this alternative life pattern increases the fecundity of the population. As indicated by the faster growth rates and the relatively short lifespan (compared with the acanthurids), the turnover rate of scarid population is relatively high. Furthermore, the populations of individual scarid species may have a pronounced seasonal spawning pattern although the timing and magnitude may be species-specific. Scarids are intensively fished in a number of

areas, and appear to be able to maintain good numbers in the face of this. Such intensive fishing is usually size-specific, and this may cause problems in a protogynous population. The ability of smaller individuals to change sex in response to systematic removal of larger individuals is an area requiring further studies.

The results presented in this thesis are based on the examination of the specimens collected, and the size of samples were restricted by the limited funding and time. Validation of annual otolith bands in the acanthurids is weakened by the small number of specimens, which should be considered as a preliminary result. Sexual identities of the older terminal phase males (relative age within the terminal phase) are difficult to determine based on the up-to-date criteria for distinguishing testes between the primary and secondary males in the scarids. Therefore, the sexual identity of older terminal phase males examined may also be misinterpreted in this thesis.

Based on the findings as well as problems in this study, the following research should be carried out in the future:

1. A validation study with a large number of specimens will further confirm the annual periodicity of otolith bands for acanthurids and large scarids in tropical water.

2. With the otolith microstructure (daily increments), a more detailed study should be carried out on the recruitment pattern and earlier mortality for tropical herbivorous reef fishes.

3. A histochemical study on tissue of the testes in the terminal phase males could be used to distinguish the testes of older primary and secondary males.

4. A biochemical study to determine the nature of hepatic lipids, and the

implication of hepatic lipids on reproduction and individual growth can then be assessed. In addition, more seasonal sampling for livers and gonads will further reveal diverse seasonal patterns of reproduction and hepatic conditions for the tropical scarids.

8.2 CONCLUSIONS

1. Fine increments on lapilli of juvenile scarids and acanthurids are deposited on a daily basis. These increments can be used in age estimates for individual juveniles.

2. There is generally no difference in the increment number between left and right lapillus in scarids and acanthurids.

3. The accuracy of juvenile age estimates based on daily otolith increments is largely depended on the increment counting procedure. Replicate counts with a limited deviation are required when counting daily otolith increments.

4. The tropical scarids (*S.rivulatus* and *S.schlegeli*) form annual bands on their sagittae, which can be used to estimate age of adult individuals. Similar otolith bands also exist in the tropical acanthurids (*C.binotatus* and *C.striatus*) although the validation of annual periodicity of these bands may need a large number of specimens.

5. Fine otolith increments in adult individuals of *S.schlegeli* can underestimate the true age if these increments are counted as daily rings.

6. Scales in the scarids are relatively reliable estimates of age for younger individuals up to 5 years old. The scales in older individuals (>5 years) generally underestimate the actual age.

7. The young scarids grow relatively fast, and their growth rates increase with age in days during the juvenile phase.

8. The young acanthurids grow relatively slowly, and their growth rates decrease with age in days after settlement.

9. The acanthurids have a longer larval period from 47 to 74 days while the scarids have a shorter larval period of between 28 and 47 days.

10. Age distribution of the scarid population at Lizard Island ranges up to 8 years with the majority being younger than 5 years.

11. The acanthurids at Lizard Island live much longer than the scarids. The old individuals (>10 years) generally cease longitudinal growth.

12. The scarids at Lizard Island reach sexual maturity at the age of 2 years for both males and females.

13. The sexual transformation (for females) and color phase change (for males and females) start at the age of 3 years, and take place over approximately another three years in the scarids.

14. The multiple spawning occurs year round for scarids. *S.schlegeli* has a pronounced seasonal pattern of reproduction with a high spawning peak between May and September. In *S.rivulatus*, the seasonal pattern of reproduction is less evident with a low spawning peak around September and January.

15. The terminal phase males have higher growth rates than the initial phase individuals of the same age in the scarids.

16. Liver proportional weight in the scarids reflects hepatic lipid content. The liver colour is also indicative of lipid content.

17. Liver proportional weight of the scarids changes with the gonadal

development, being highest immediately before spawning.

18. In similar microhabitats and similar physical environments both species of scarids have quite different seasonal patterns in reproduction and hepatic condition in terms of timing and magnitude.

REFERENCES

Al Hussaini, A.H. (1945) The anatomy and histology of the alimentary tract of the coral feeding fish *Scarus sordidus* (Klunz.). *Bulletin de l'Institut D'Egypte* **27:** 349-377

Al Hussaini, A.H. (1947) The feeding habits and the morphology of the alimentary tract of some teleosts (living in the neighbourhood of the Marine Biological Station, Ghardaga, Red Sea). *Publications of the Marine Biological Station, Ghardaga (Red Sea)* NO. **5:** 61pp

Alcala, A.C. and Luchavez, T. (1981) Fish yield of the coral reef surrounding Apo Island, Negros Oriental, Central Visayas, Philippines. *Proceedings of the 4th International Coral Reef Symposium*, Manila. **1:** 69-73

Bagenal, T.B. and Tesch, F.W. (1978) Age and growth. In T.B. Bagenal (ed.) Methods for assessment of fish production in fresh water (3rd ed.). Blackwell Scientific Publication, London

Bancroft, J.D. (1975) Histological Techniques (2nd ed.). London, Butterworths Press. pp.348

Bardach, J.B. (1961) Transport of calcareous fragments by reef fishes. *Science* **133:** 96-99

Barger, L.E. (1985) Age and growth of Atlantic croakers in the northern Gulf of Mexico, based on otolith sections. *Transactions of the American Fisheries Society* **114:** 847-850

Bayliss-High, O.B. (1984) Lipid histochemistry. Royal Microscopical Society, *Microscopy Hand Book* **06**: pp.68. Oxford University Press, Oxford, U.K.

Beamish, R.J. and McFarlane, G.A.(1983) The forgotten requirement for age determination in fisheries biology. *Transactions of American Fisheries Society* **112:** 735-743

Beamish, R.J., McFarlane, G.A. and Chilton, D.E. (1983) Use of oxtetracycline and other methods of age determination for sablefish. *Proc. 2nd Lowell Wakefield Fish. Symp.* Anchorage Pp. 95-116

Beamish, R.J. and McFarlane, G.A. (1987) Current trends in age determination methodology. In: R.C. Summerfelt and G. Hall (eds.) Age and Growth of Fish. Iowa State University Press, U.S.A.

Beckman, D. W., Stanley, A. L., Render, J. H. (1990) Age and growth of black drum in Louisiana waters of the Gulf of Mexico. *Transactions of American Fisheries Society* **119:** 537-544

Bellwood, D.R. (1985) The functional morphology, systematics and behavioural ecology of parrotfishes (Scaridae). Unpubl. Ph.D. thesis, James Cook University of North Queensland, Townsville, Australia.

Bellwood, D.R. (1988) Ontogenetic changes in the diet of early post-settlement Scarus species (Pisces: Scaridae). *J. Fish. Biol.* **33:** 213-219

Bellwood, D.R. and Choat, J.H. (1989) A description of the juvenile phase colour patterns of 24 parrotfish species (family Scaridae) from the Great Barrier Reef, Australia. *Records of the Australian Museum* **41:** 1-42

Bellwood, D.R. and Choat, J.H. (1990) A functional analysis of grazing in parrotfishes (family Scaridae): the ecological implications. *Environ. Biol. Fishes.* **28:** 189-214

Blake, C. and Blake, B.F. (1978) The use of opercula bones in the study of age and growth in *Labeo senegalensis* from Lake Kainji, Nigeria. *J. Fish Biol.* **13:** 287-295

Blake, C. and Blake, B.F. (1981) Age determination in six species of fish from a Mexican Pacific coastal lagoon. *J. Fish. Biol.* **18:** 471-478

Boxrucker, J. (1986) A comparison of the otolith and scale methods for ageing white crappies in Oklahoma. *North American Journal of Fisheries Management* **6:** 122-125

Briarty, L.G. (1975) Stereology: Methods for quantitative light and electron microscopy. *Science Progress* **62:** 1-32

Brothers, E.B. (1982) Ageing reef fishes. Pp.3-22 In G.R. Huntsman, W.R. Nicholson, and W.W. Fox Jr. (Eds.) The biological bases for reef fishery management. NOAA Tech. Memo. NMFS-SEFC-80

Brothers, E.B. and McFarland, W.N. (1981) Correlation between otolith microstructure, growth and life history transitions in newly recruited French Grunts (*Haemulon Flavolineatum* (Desmarest). Haemulidae). *Rapp. P-V. REun. Cons. Int. Explor. Mer* **178:** 368-374

Brothers, E.B., Mathews, C.P. and Lasker, R. (1976) Daily growth increments in otoliths from larval and adult fishes. *Fish. Bull.* **74:** 1-8

Brothers,E.B., Williams, D.McB. and Sale, P.F (1983) Length of larval life in twelve families of fishes at "One Tree Lagoon", Great Barrier Reef, Australia. *Marine Biology* **76:** 319-324

Buesa, R.J. (1987). Growth rate of tropical demersal fishes. *Marine Ecology Progress Series* **36:** 191-199

Campana, S.E. and Neilson, J.D.(1982) Daily growth in otoliths of starry flounder *Platichthys stellatus* and the influence of some environmental variables in their production. *Can. J. Fish. Aquat. Sci.* **39:** 937-943

Campana, S.E. and Neilson, J.D. (1985) Microstructure of fish otolith. *Can. J. Fish. Aquat. Sci.* **42:** 1014-1032

Casselman, J.M. (1982) Age and growth assessment of fish from their calcified structures - techniques and tools. *NOAA (National Oceanic and Atmospheric Administration) Technique Report NMFS (National Marine Fisheries Service)* **8:** 1-17

Casselman, J.M. (1987) Determination of age and growth. Pages 209 -242 in A.H. Weatherly and H. S. Gill. The biology of fish growth. Academic Press, New York.

Chilton, D.E. and Stocker, M. (1987) A comparison of otolith and scale methods for ageing Pacific herring. *North American Journal of Fisheries management* **7:** 202-206

Choat, J.H. (1966) Parrotfish. *Australian Natural History.* **15(8):** 265-268

Choat, J.H. (1969) Studies on the biology of Labroid fishes (Labridae and Scaridae) at Heron Island, Great Barrier Reef. Part I. 294 pages Ph.D Thesis, University of Queensland.

Choat, J.H. (1991) The biology of herbivorous fishes on coral reefs. In P. Sale (ed.) The Ecology of Fishes on Coral Reefs. Academic Press, London.

Choat, J.H. and Robertson, D.R. (1975) Protogynous hermaphroditism in fishes of the family Scaridae. PP 263-283. In: Reinboth (ed.). Intersexuality in the Animal Kingdom. pp 263-283. Springer Verlag.

Choat, J.H. and Randall, J.E. (1986) A review of the parrotfishes (Family Scaridae) of the Great Barrier Reef of Australia with description of a new species. *Records of the Australian Museum* **38:** 175-228

Clavijo, I.E. (1983) Pair spawning and formation of a lek-like mating system in the parrotfish *Scarus vetula.* Copeia **1983(1):** 253-256

Clements, K.D. (1991) Gut microorganisms of surgeonfishes (Family Acanthuridae). Unpub. Ph.D Thesis, James Cook University of North Queensland, Townsville, Australia.

Cowen R.K. (1990) Sex change and life history patterns of the labrid *Semicossyphus pulcher*, across an environment gradient. *Copeia* **1990(3):** 787-795

Crupkin, M., Montecchia, C.L. and Trucco, R. (1988) Seasonal variations in gonadosomatic index, liver-somatic index and myosin/actin ratio in actomyosin of mature hake (*Merluccius hubbsi*). *Comp. Biochem. Physiol.* **89A(1)**: 7-10

D'Amours, D., Landry, J.G. and Lambert, T.C. (1990) growth of juvenile (0-group) Atlantic mackerel (*Scomber scombrus*) in the Gulf of St. Lawrence. *Can. J. Fish. Aquat. Sci.* **47**: 2212-2218

Dee, A.J. and Radtke, R.L.(1989) Age and growth of the brick soldierfish, *Myripristis amaena*. *Coral Reefs* **8:** 79-85

Dubin, R.E. (1981) Pair spawning in the princess parrotfish, *Scarus taeniopterus*. *Copeia* **1981 (2):** 475-477

Ebisawa, A. (1990) Reproductive biology of *Lethrinus nebulosus* (Pisces: Lethrinidae) around the Okinawan Waters. *Nippon Suisan Gakkaishi* **56(12):** 1941-1954

Fagade, S.O. (1974) Age determination in *Tilapia melanothern* (Ruppell) in the Lagos Lagoon, Lagos, Nigeria. In "Ageing of Fish-Proceedings of an international Symposium" (T.B.Bagenal, ed.). Unwin, London.

Fowler, A.J. (1989) Description, interpretation and use of the microstructure of otoliths from juvenile butterflyfishes (Family Chaetodontidae). *Marine Biology* **102:** 167-181

Fowler, A.J. (1990) Validation of annual growth increments in the otoliths of a small, tropical coral reef fish. *Mar. Eco. Pro. Ser.* **64:** 25-38

Frydi, G. and Stearn, C.W. (1978) Rate of bioersion by parrotfish in Barbados, West Indies, *Internationale Revue der gesamentary Hydrobiologie und Hydrographte.* **64:** 737-748.

Galzin, R. (1977) Ecologie des poissons recifaux de Ploynesie Francaise. These (Docteur de Specialiate) 1'Universite des Sciences et Techniques du Languedoc. 195 pp.

Gaschutz, G., Pauly, D. and David, N. (1980) A versatile BASIC program for fitting weight and seasonally oscillating length growth data. *I.C.E.S. Statistics Committee CM 1980/D* **6:** 23pp

Geffen, A.J. (1982) Otolith ring deposition in relation to growth rate in herring *Clupea harengus* and turbot *Scophthalmus maximus* larvae. *Mar. Biol.* **71:**317-322

Gjøsaeter, J., Dayaratne, P, Bergstad, O.A., Gjøsaeter, H., Sousa, M.I. and Beck,

I.M. (1985) Ageing tropical fish by growth rings in the otoliths. *FAO Fisheries Circular* **776:** Pp.54

Gladstone, W. (1986) Spawning behaviour of the bumphead parrotfish *Bolbometopon muricatum* at Yonge Reef, Great Barrier Reef. *Japanese J. Ichtyology* **33(3):** 326-328

Gladstone, W., Westoby, M. (1988) Growth and reproduction in the *Canthigaster valentini* (Pisces, Tetraodontidae): a comparison of a toxic reef fish with other reef fishes. *Environmental Biology of fishes* **21(3):** 207-221

Gohar, H.A.F. and Latif, A.F.A. (1959) Morphological studies on the gut of some scarid and labrid fishes. *Publications of the Marine Biological Station, Ghardaga (Red Sea)* NO. **10:** 145-189

Gygi, R.A. (1969) An estimate of the erosional effect of Sparisoma viride (Bonnaterre), the green parrotfish, on some Bermuda reefs. In: R. N. Ginsberg and P. Garrett (eds) Seminar on Organism-Sediment Relationships. Bermuda Biological Station Special Publication, **2:** 137-143

Gygi, R.A. (1975) *Sparisom viride* (Bannaterre), the stoplight parrotfish, a major sediment producer on coral reefs of Bermuda? *Ecloge Geologicae Helvetide.* **68:** 327-359

Harris, W. H. (1960). A microscopic method of determining rates of bone growth. *Nature (Lond.)* **188**, 1038-1039

Hastings, P.A. (1981) Gonad morphology and sex succession in the protogynous hermaphrodites *Hemanthias vivanus* (Jordan and Swain). *J. Fish Biol.* **18:** 443-454

Hatcher, B.G. (1981) The interaction between grazing organisms and the epilithic algal community of a coral reef: A quantitative assessment. *Proc. Int. Coral Reef Symp., 4th* **2:** 515-524.

Hatcher, B.G. (1983) Grazing in coral reef ecosystems in Barnes, D. J. (eds) Perspectives on Coral Reefs. pp.164-179 Aust. Inst. Mar. Sci., Townsville, Australia.

Heidinger, R.C. and Crawford S.D. (1977) Effect of temperature and feeding rate on the liver-somatic index of the largemonth bass, *Micropterus salmoides. J. Fish. Res. Bd. Can.* **34:** 633-638

Hetter, W.F. (1984) Fluctuation in the year classes of important food fishes. *J. cons. int. Explor. Mer.* **1:** 5-38

Hill, K.T. and Radtke, R.L. (1988) Gerontological studies of the damselfish

Dascyllus albisella. *Bull. Mar. Sci.* **42 (3):** 424-434

Holden, M.J. (1955) Ring formation in the scales of *Tilapia variabilis* Boulenger and *Tilapia esculentus* Graham from Lake Victoria. *East Afr. Fish. Res. Organ. Ann. Rep.* **1954-1955**, 36-40

Horn, M.H. (1989) Biology of marine herbivorous fishes. *Oceanohr. Mar. Biol. Ann. Rev.* **27:** 167-272

Hunter, J., Roe, N.C., Lo, H. and Leong, R.J.H. (1985) Batch fecundity in multiple spawning fishes. In R. Lasker (ed.), An egg production method for estimating spawning biomass of pelagic fish: application to the Northern anchovy, *Engraulis mordax* pp.67-78. U.S. Dept. Commer. NOAA tech. Rep. NMFS 36.

Hutchings, P.A. (1986) Biological destruction of coral reefs. A review. *Coral reefs* **4:** 239-252

Itano, D. (1988). American Samoa bottomfish assessment program. Proc. South Pacific Commission Inshore Fisheries Resources Workshop Proc, SPC, Noumea. Background Paper 68.

Johannes, R.E. (1981) Words of the Lagoon - fishing and marine lore in the Palau district of Micronesia. 245 pp. University of california Press.

Johnson, A.G. (1983) Age and growth of yellowtail snapper from South Florida. *Trans. Am. Fish. Soc.* **112:** 173-177

Jones, C. (1986) Determining age of larval fish with the otolith increment technique. *Fish. Bull.* **84:** 91-103

Jones, G.P. (1980) Growth and reproduction in the protogynous hermaphrodite *Pseudolabrus celidotus* (Pisces: Labridae) in New Zealand. *Copeia* **1980:** 660-675

Jones, R. (1976) Growth of fishes. In Cushing, D.H., Walsh, J.J. (eds.) *The ecology of the seas*. Blackwell Scientific Publications, Oxford. pp.251-279.

Jones, R.E., Marsh, H., Taylor, J.F. and Marsh, L.M. (1988) Biometrics lecture summary. James Cook University of North Queensland.

Kimura, D.K. and Lyons, J.J. (1990) Between-reader bias and variability in the age-determination process. *Fishery Bulletin, U.S.* **89:** 53-60

Kaufmann, K.W. (1981) Fitting and using growth curves. *Oecologia (Berl)* **49:** 293-299

Klumpp, D.W. and Polunin, N.V.C. (1989) Partitioning among graziers of food resources within damselfish territories on a coral reef. *J. Exp. Mar. Bio. Ecol.* **125:** 145-169

Klumpp, D.W., McKinnon, D. and Daniel, P. (1987) damselfish territories: Zones of high productivity on coral reefs. *Mar. Ecol. Prog. Ser.* **40:** 41-51

Kobayashi, S., Yuki, R., Furui, T., and Kosugiyama, T. (1964) Calcification in fish and shell-fish I. Tetracycline labelling patterns on scale, centrum and otolith in young goldfish. *Bulletin of the Japanese Society of Scientific Fisheries* **30,** 6-13

Leis, J.M. and Rennis, D.S. (1984). The larvae of Indo-Pacific coral reef fishes. N.S.W. University Press, Sydney. pp269

Longhurst, A.R. and Pauly, D. (1987) Ecology of tropical oceans. Academic Press Inc. London.

Lou, D.C. (1992) Validation of annual growth bands in the otolith of tropical parrotfishes (*Scarus schlegeli* Bleeker). *Journal of Fish Biology* **41:** 775-790

Lou, D.C. (1993) Growth in juvenile *Scarus rivulatus* and *Ctenochaetus binotatus*: a comparison of families Scaridae and Acanthuridae. *Journal of Fish Biology* **42:**15-23

Lou, D.C. and Moltschaniwskyj, N.A. (1992) Daily otolith increments in juvenile tropical parrotfishes and surgeonfishes. *Australian Journal of Marine and Freshwater Research* **43:** 973-981

Loubens, G. (1978) Biologie de quelque especes de poisson du lagon Neo-Caledonien I. Determination de I'age (otolithometrie). *Cah. ORSTOM Oceanogr.* **XVI:** 263-283

MacGregor, J.S. (1957) Fecundity of the Pacific sardine (*Sardinops caerulea*). *Fish. Bull. U.S.* **57:** 427-449

Manooch, C.S. III (1987) Age and growth of snapper and groupers. In: Polovina, J.J., Ralston, S. (eds) Tropical snappers and groupers. Biology and fisheries management. Westview Press, Boulder, p.329-373

McPherson, G., Squire, L. and O'Brien, J. (1988) Demersal reef fish project 1984-85. Age and growth of four important reef fish species. *Report to Great Barrier Reef Marine Park Authority, Townsville, Qld*

Milicich, M.J. (1986) Aspects of the early life history of *Parika saber* (Pisces: Monacanthidae). Unpub. MSc. Thesis, University of Aucland, New Zealand

Miller, S.J. and Storck, T. (1982) Daily rings in otoliths of young-of-the-year largemouth bass. *Trans. Amer. Fish. Soc.* **111:** 527-530

Moe, Martin A. Jr. (1969) Biology of the red grouper *Epinephelus morio* (Valenciennes) from the eastern Gulf of Mexico. Florida Department of Natural Resources Marine Research Laboratory, St. Petersburg Florida. Professional paper series number **10.**

Moksness, E. and Wespestad, V. (1989) Ageing and back-calculating growth rates of Pacific herring, *Clupea pallasil*, larvae by reading daily otolith increments. *Fishery Bulletin* **87:** 509-513

Munro, J.L. (1983) Caribbean coral reef fishery resources. Published by the international centre for living aquatic resources management, Mania. p.276

Munro, J.L. and Pauly, D. (1983) A simple method for comparing the growth of fishes and invertebrates. *Fishbyte* **1(1):** 5-6

Munro, J.L. and Williams, D.McB. (1985) Assessment and management of coral reef fisheries: biological, environmental and socio-economic aspects. *Proc. 5th Int. Coral Reef Congr.* **4:** 544-581

Myers, R.F. (1989) Micronesian reef fishes. Coral graphical, Guanm.

Neilson, J.D. and Geen, G.H. (1982) Otolith of chinook salmon Onchorhynchus tshawytscha: daily growth increments and factors influencing their production. *Can. J. Fish. Aquat. Sci.* **39:** 1340-1347

Neilson, J.D. and Geen, G.H. (1985) Effects of feeding regimes and diel temperature cycles on otolith increment formation in juvenile chinook salmon, *Onchoehynchus tshswytscha. Fish. Bull.* **83:** 91-101

Ntiba, M.J. and Jaccarini, V. (1990) Gonad maturation and spawning times of *Siganus sutor* off the Kenya coast: evidence for definite spawning seasons in tropical fish. *J. Fish Biol.* **37:** 315-325

Oguri, M. (1978a) On the hepatosomatic index of holocephalian fish. *Bulletin of the Japanese Society of Scientific Fisheries* **44(2):** 131-134

Oguri, M. (1978b) Histochemical observation on the interregnal gland and liver of European spotted dogfish. *Bulletin of the Japanese Society of Scientific Fisheries* **44(7):** 703-707

Pannella, G. (1971) Fish otoliths: Daily growth layers and periodical patterns. *Science* **173:**1124-1127.

Pannella, G. (1974) Otoliths growth patterns: An aid in age determination in

temperate and tropical fishes. Pp. 28-39 in A.B. Bagenal(Ed) The ageing of fish. Unwin Bros. U.K.

Pannella, G. (1980) Growth patterns in fish sagittae. Pp. 519- 560 In: D.C. Rhoades and R.A. Lutz (Eds.) Skeletal growth of aquatic organisms. New York: Plennum Press

Papaconstantinou, C. and Petrakis, G. (1986). Preliminary study on age, growth and reproduction of parrot fish, *Sparisoma cretense* (L.), from Kastellorizo Island, Greece. *F.A.O Fish. Rep.* No: **361** 113-118.

Paul, L.J. (1968) Early growth characteristics of the New Zealand snapper *Chrysophrys auratus* (Forster), with reference to selection of a scale-sampling site. *N.Z. Mar. Freshwater Res.* **2:** 273-292

Pentilla, J. and Dery, L.M. (eds) (1988) Age determination methods for Northwest Atlantic species. *NOAA Tech. Rep. NMFS72, U.S Dept. Commerce, Washington*, p.1-35

Pitcher, C.R. (1988) Validation of a technique for reconstructing daily patterns in the recruitment of coral reef damselfish. *Coral Reefs* **7:** 105-111

Poinsard, F. and Troadec, J. (1966) Determination de I'age par la lecture des otoliths chez deux especes de Sciaenides Ouest-Africains. (*Pseudolithes senegalensis* C. V. et. *Pseudolithes typus* Blkr). *J. Cons. perm. int. Explor. Mer.* **30:** 291-307

Pollock, B.R. (1985) The reproductive cycle of yellowfin bream, *Acanthopagrus australis* (Gunther), with particular reference to protandrous sex inversion. *J. Fish Biol.* **26:** 301-311

Polunin, N. V. C. and Brothers, E. B. (1989) Low efficiency of dietary carbon and nitrogen conversion to growth in an herbivorous coral reef fish in the wild. *Journal of Fish Biology* **35:** 869-879

Powell, A.B. (1982) Annulus formation and growth of young summer flounder from Pamlico Sound, North Carolina. *Transaction of the American Fisheries Society*. **111:** 288-693

Pulliainen, E. and Korhonen, K. (1990) Seasonal changes in condition indices in adult mature and non-maturing burbot, *Lota lota* (L.) in the north-eastern Bothnian Bay, northern Finland. *Journal of Fish Biology* **36:** 251-259

Radtke, R.L. (1984) Formation and structural composition of larval striped mullet otoliths. *Transactions of the American Fisheries Society* **113:** 186-191

Radtke, R.L. (1987) Age and growth information available from the otoliths of the

Hawaiian snapper, *Pristipomoides filamenetosus*. *Coral Reefs* **6:** 19-25

Ralston, S. (1976) Age determination of a tropical reef butterflyfish utilizing daily growth rings of otoliths. *Fish. Bull.* **74:** 991-994.

Randall, J.E. (1961). A contribution to the biology of the convict surgeonfish of the Hawaiian islands, *Acanthurus triostegus sandvicensis*. *Pacif. Sci.* **15:** 215-272

Randall, J.E. (1962) Tagging reef fishes in the Virgin Islands. *Proc. Gulf Caribb. Fish. Inst.* **14:** 201-241

Randall, J.E. and Choat, J.H (1980) Two new parrotfishes of the genus *Scarus* from the Central and South Pacific, with further examples of sexual dichromatism. *Zoological Journal of the Linnean Society* **70(4):** 383-419

Randall, J.E. and Bruce, R.W. (1983). The parrotfishes of the subfamily Scarinae of the western Indian Ocean with description of three new species. *Ichthyological Bulletin Rhodes University* **47:** 1-39

Randall, J.E., Allen, G.R. and Steene, R.C. (1990) Fishes of the Great Bariier Reef and Coral Sea. Crawford House Press, Bathurst, Australia

Reinboth, R. (1970) Intersexuality in fishes. *Mem. Soc. Endocrinal. (Hormones and environment)* **18:** 515-543

Ricker, W.E. (1979) Growth rates and models. In: Hoar, W.S. Randall, D.J., Brett, J.R. (eds). Fish physiology, Academic Press. New York, p. 677-742

Robertson, D.R. (1991) The role of adult biology in the timing of spawning of tropical reef fishes. In The Ecology of Fishes on Coral Reefs. Sale, P.F. (ed). Academic Press, London.

Robertson, D.R. and Warner, R.R. (1978) Sexual patterns in the Labroid fishes of the Western Caribbean, II: the Parrotfishes (Scaridae). *Smithsonian Contributions to Zoology* **255.**

Rosenlund, G., Lund, B., Sandnes, K., Braekkan, O.R. and VOn der Decken, A. (1984) Muscle protein synthesis in *vitro* of saithe (*Pollachius virens*) correlated to growth and daily energy intake. *Comp. Biochem. Physiol.* **77B:** 7-13.

Ross, R.M. (1984) Growth and sexual strategies in the fish *Thalassoma duperrey* (Labridae), a protogynous hermaphrodite. *Environmental Biology of Fishes* **10(4):** 253-259

Rossouw, G.J. (1987) Function of the liver and Hepatic lipids of the lesser sand shark, *Rhinobatos annulatus* (Müller & Henle). *Comp. Biochem. Physiol.*

86B(4): 785-790

Russ, G.R. (1984a) Distribution and abundance of herbivorous grazing fishes in the central Great Barrier Reef. I. level of variability across the entire continental shelf. *Marine Ecology-Progress Series* **20:** 23-34

Russ, G.R. (1984b) Distribution and abundance of herbivorous grazing fishes in the central Great Barrier Reef. II. Patterns of zonation of mid-shelf and outershelf reef. *Marine Ecology-Progress Series* **20:** 35-44

Russ, G.R. (1991) Coral reef fisheries: Effects and yields. In P. Sale (ed.) The Ecology of Fishes on Coral Reefs. Academic Press, London.

Russ, G.R. and St.John, J. (1988). Diets, growth rates and secondary production of herbivorous coral reef fishes. *Proc. 6th Int. Coral Reef Congr.* **2:** 37-43

Sadovy, Y. and Shapiro, D.Y. (1987) Criteria for the diagnosis of hermaphroditism in fishes. *Copeia* **1987(1)** pp.136-156

Schneidarvin, R.W. and Hubert, W.A. (1986) A rapid technique for otolith removal from salmonoids and catostomids. *North American Journal of Fisheries Management* **6:** 287

Schultz, E.T. (1990) Daily otolith increments and the early life history of a viviparous fish, *Micrometrus minimus* (Embiotocidae). *Copeia* **1990(1):** 59-67

Shapiro, D.Y. (1989) Sex change as an alternative life-history style. In "Alternative life-history styles of animals" (M.N. Bruton, ed.) Kluwer Academic, Dordrecht, The Netherlands. Pp. 177-195

Shul'man, G.E. (1974) Life Cycles of Fish. New York, Wiley Press.

Schmidt, P.D. (1984) Marking growth increments in otoliths of larval and juveniles fish by immersion in tetracycline to the rate of increment formation. *Fish. Bull.* **82:** 237-242

Smith, R.L., Paul, A.J. and Pual, J.M. (1990) Seasonal changes in energy and the energy cost of spawning in Gulf of Alaska Pacific cod. *J. Fish Biol.* **36:** 307-316

Struhsaker, P. and Uchiyama, J.H. (1976) Age and growth of the nehu, *Stolehporus purpureus* (Pisces: Engraulidae), from the Hawaiian Islands as indicated by daily growth increments of sagittae. *Fish. Bull.* **74:** 9-17

Taubert, B.D. and Tranquilli, J.A. (1982) Verification of the formation of annuli in otoliths of largemouth bass. *Trans. Amer. Fish. Soc.* **111:** 531-534

Thongrod, S., Takeuchi, T., Satoh, S. and Watanabe (1989) Requirement of fingerling white fish *Coregonus larvaretus maraena* for dietary n-3 fatty acids. *Nippon Suisan Gakkaishi* **55(11):** 1983-1987

Thorogood, J. (1987) Age and growth rate determination of southern bluefin tuna, *Thunnus maccoyii*, using otolith banding. *J. Fish. Biol.* **30:** 7-14.

Thorrold, S.R. (1989) Estimating some early life history parameters in a tropical clupeid, *Herklotsichys castelnaui*, from daily growth increments in otoliths. *Fishery Bulletin* **87:** 73-83

Thresher, R.E. (1984) Reproduction in reef fishes. TFH publ., Neptune City, New Jersey.

Thresher, R.E. (1988) Otolith microstructure and the demography of coral reef fishes. *TREE.* **3:** 78-80

Victor, B.C. (1982) Daily otolith increments and recruitment in two coral reef wrasses, *Thalassom bifasciatum & Halichoeres bivittatus. Marine Biology* **71:** 203-208

Victor, B.C. (1983) Settlement and larval metamorphosis produce distinct marks on the otoliths of the slippery dick, *Halichoeres bivttatus*. In "The Ecology of Deep and Shallow Coral Reefs" (M. L. Reaka, ed.), Symp. Ser. Undersea Res., Vol. 1, pp 47-51. Natl. Oceanic Atmos. Adm., Rockville, Maryland.

Victor, B.C. (1986a) Delayed metamorphosis with reduced larval growth in a coral reef fish (*Thalassoma bifaciatum*). *Can. J. Fish. Aquat. Sci.* **43:** 1208-1213

Victor, B.C. (1986b) Duration of planktonic stage of one hundred species of Pacific and Atlantic wrasses (family: Labridae). *Mar. Biol.* **90:** 317-326

Victor, B.C. (1987) Growth, dispersal, and identification of planktonic labrid and pomacentrid reef-fish larvae in the eastern Pacific Ocean. *Mar. Biol.* **95:** 145-152

von Bertalanffy, L. (1938) A quantitative theory of organic growth. II. Inquiries on growth laws. *Human Biol.* **10:** 181-213

von Bertalanffy, L. (1957) Quantitative laws in metabolism and growth. *Q. Rev. Biol.* **32:** 217-231

Walker, M.H. (1975) Aspects of biology of Emperor fishes, Family Lethrinidae, in North Queensland Barrier Reef waters. Ph.D Thesis, James Cook University of North Queensland, Australia.

Waltz, C.W., Roumillat, W.A. and Wenner, C.A. (1982) Biology of the whitebone porgy, *Calamus leucosteus*, in the South Atlantic Bight. *Fishery Bulletin* **80:** 863-874

Warburton, K. (1978) Age and growth determination in a marine catfish using an otolith check technique. *J. Fish. Biol.* **13:** 429-434

Warner, R.R. (1975) The reproductive biology of the protogynous hermaphrodite *Plmelometopon pulchrum* (Pisces: Labridae). *Fishery Bulletin* **73:** 262-283

Warner, R.R. (1988) Sex change and the size-advantage model. *Trends Ecol. Evol.* **3:** 133-136

Warner, R.R and Downs, D.R. (1977) Comparative life histories: Growth vs. Reproduction in normal males and sex-changing hermaphrodites in the striped parrotfish. *Scarus croicensis. Procedings of the 3rd International Symposium on Coral Reefs*, **I (Biology):** 275-282.

Warner, R.R. and Robertson, D.R. (1978) Sexual patterns in the Labroid fishes of the Western Caribbean, I: The Wrasses (Labriodae). *Smithsonian Contributions to Zoology* **254.**

Weatherley, A.H. and Gill, H.S. (1987) The biology of fish growth. Publ. by Academic Press, London. Pp. 443

Webb, B.F. and Grant, C.J. (1979) Age and Growth of Jack Mackerel, *Trachurus declivis* (Jenyns), from South-eastern Australian Waters. *Aust. J. Mar. Freshwater Res.* **30:** 1-9

Weber, D., and Ridgway, G. J. (1967). Marking Pacific salmon with tetracycline antibiotics. *Journal of Fisheries Research Board of Canada* **24**, 849-865

West, G. (1990) Methods of assessing ovarian development in fishes: a review. *Aust. J. Mar. Freshwat. Res.* **41:** 199-222

Weibel, E.R. and Elias, H. (1967) Quantitative methods in morphology. Publ. by Springer-Verlag. Pp. 267

Wild, A. and Foreman, T.J. (1980) The relationship between otolith increments and time for the yellowfin and skipjeck tuna marked with tetracycline. *Int. Am. Trop. Tuna. Comm. Bull.* **17:** 509-560

Williams, D.McB. (1983) Daily, monthly and yearly variability in recruitment of a guild of coral reef fishes. *Marine Biology Progress Series* **10:** 231-237

Williams, D.McB. and Hatcher, A.I. (1983) Structure of fish communities on outer slopes of inshore, mid-shelf and out shelf reefs of the Great Barrier Reef.

Mar. Ecol. Prog. Ser. **10:** 230-250

Winsor, L. (1983) Manual of basic zoological microtechniques for light microscopy. Publ. by James Cook University of North Queensland. Pp. 146

Withell, A.F. and Wankowski, J.W. (1988) Estimates of age and growth of ocean perch, Helicolenus percoides Richardson, in south-eastern Australian waters. *Aust. J. mar. Freshwat. Res.* **39:** 441-459

Yogo, Y., Nakazono, A. and Tsukahara, H. (1980) Ecology studies on the spawning of the parrotfish, *Scarus sordidus* Forsskal. *Sci. Bull. Fac. Agr. Kyushu Univ.* **34:** 105-114