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PROTANDROUS SEX CHANGE IN THE TROPICAL SPARID *ACANTHOPAGRUS BERDA* AND THE IMPLICATIONS OF GEOGRAPHICAL VARIABILITY.

Thesis submitted by Andrew James TOBIN BSc (*UQ*) in May 1998

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

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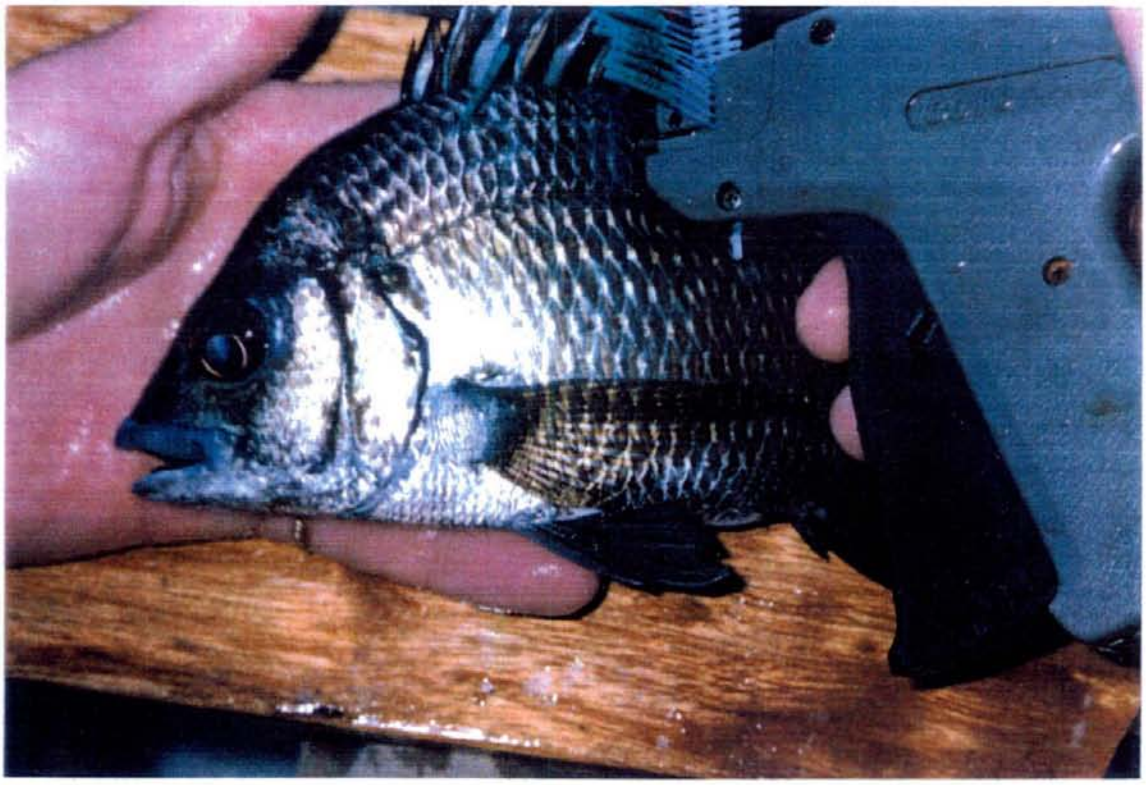

FRONTISPIECE. Tagging an *Acanthopagrus berda* as part of a tag-release study detailed in this dissertation.

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ABSTRACT

This study investigated the sex change of the tropical sparid, *Acanthopagrus berda*. A hierarchical approach incorporating multiple diagnostics was applied. Fish were collected from seven tropical estuaries (Blacksoil, Cocoa, Alligator, Cattle, Mendel and Meunga Creeks as well as Deluge Inlet) in North Queensland, Australia. Both length- and age-frequency distributions showed strong bimodality indicative of protandrous sex change. The smaller length-classes and younger age-classes (years) of all samples were dominated by male fish. Female fish dominated larger length-classes and older age-classes. Adult sex ratios for five of the seven estuaries were largely male biased, presenting further evidence that protandrous sex change may occur in *A. berda*.

The gonad structure of *A. berda* was investigated both macroscopically and microscopically through the complete annual reproductive cycle. In all gonads examined, the characteristic sparid ovotestis structure was observed. During the spawning period (June to September), male and female ovotestes were strongly dimorphic in structure. Ovotestis structures that may represent a transitional stage in a protandrous sex change process were observed during the three month post-spawning period (October to December). The location of sectioning position was found to influence the interpretation of sexual function of some gonad types. Sections need to be taken along the length of the gonad before sexuality can be determined conclusively.

Definitive evidence of protandrous sex change was provided by an in-field tag-release experiment conducted in two estuaries. Fish were sexed in the field by a validated and non-intrusive technique before being tagged and released. The subsequent recapture of fish allowed the sexuality of individual fish to be monitored through time. Protandrous sex change was detected in 3 (6.1%) and 4 (7.8%) fish from Blacksoil Creek and Deluge Inlet respectively. Each of these fish were sexed as male at initial capture, and possessed functional female ovotestes at the time of recapture. Further evidence of protandrous sex change was provided by aquarium experiments in which a total of ten *A. berda* underwent protandrous sex change.

There was considerable geographical variation in the growth of *A. berda* among locations. The growth of *A. berda* in Deluge Inlet and Mendel Creek was considerably slower than the other 3 estuaries sampled (Blacksoil, Cocoa and Cattle Creeks). The comparison of male and female growth for each estuary suggested in most cases, differences between male and female growth were negligible.

The rate at which protandrous sex change occurs was modelled for each estuary. The rate of sex change for Cocoa and Cattle Creeks was modelled by logistic regression. For Blacksoil Creek, Mendel Creek and Deluge Inlet modelling required a combination of the logistic and linear regression models. Following the geographical variation detected in the age and growth of *A. berda,* the modelled rates of sex change also showed considerable variation between estuaries. While sex change was modelled to occur across 5 age-classes for Cocoa Creek, sex change in all other estuaries was modelled to occur across at least 8 age-classes.

Further research into the apparent plasticity of the protandrous sex change of *A. berda* is required. The estuaries sampled in this study represent a very small area of the geographical range of *A. berda* in Australian waters. Furthermore, the extensive international range of *A. berda* may also be characterised by more extensive variability in the protandrous sex change of *A. berda* than detected by this study.

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DECLARATION

I declare that this thesis is my own work and has not been submitted in any other 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.

A. J. Tobin
11 May, 1998.

CHAPTER 1.

GENERAL INTRODUCTION - SEX CHANGE IN TELEOSTS.

In addition to the normal single sex development of gonochores, teleost fishes display an extraordinary array and diversity of sexual patterns (Warner, 1988a). Hermaphroditism (defined here as either the simultaneous or sequential existence of male and female reproductive tissues within an individual fish) was once believed to be rare in teleosts (Bullough, 1947). However, many studies have now shown hermaphroditism is common (Atz, 1964; Lodi, 1967; Smith, 1967; Buslé & Buslé, 1975; Choat & Robertson, 1975; Fricke & Fricke, 1977; Reinboth, 1980; Alekseev, 1982; Sadovy & Shapiro, 1987), and may take either a functional or non-functional form.

Functional hermaphroditism includes simultaneous and sequential hermaphroditism. Simultaneous hermaphrodites have the ability to produce both viable eggs and sperm concomitantly during a single spawning event [e.g. *Serranus fasciatus* (Hastings & Petersen, 1986); *Rivulus marmoratus* (Harrington, 1971); *Hypoplectrus nigricans* (Fischer, 1980); *Lythrypnus dalli* and *L. zebra* (St. Mary, 1993)]. However, individual fish are usually not capable of self-fertilisation (Oliver, 1997). In contrast, sequential hermaphrodites reproduce initially as one sex before later in life functioning as the opposite sex (Sadovy & Shapiro, 1987). Consequently, sex changing fish produce only one type of gamete during a spawning event, with the process of changing sex usually taking place immediately following spawning (Sadovy & Shapiro, 1987; Cody & Bortone, 1992).

Non-functional hermaphroditism, also known as rudimentary hermaphroditism, has been identified in sparid fishes. Fish of this type display hermaphroditism in the sense that both male and female reproductive tissues are simultaneously present in the gonad, and therefore an individual apparently has the potential to sex change (Buxton & Garratt, 1990). However, this type of hermaphroditism is non-functional in that sex change does not occur and only one of the sexual tissues will mature and produce viable gametes. That is, normal gonochoristic development occurs with one of the sexual tissues functioning throughout life while the other remains dormant.

Some reports have suggested rudimentary or non-functional hermaphroditism represents a stage in the evolutionary process of sequential hermaphroditism (sex change)(Buxton & Garratt, 1990). That is, species of fish that are rudimentary hermaphrodites have not yet encountered those conditions that may select sex change as a more viable reproductive strategy over the 'normal' gonochoristic reproductive development. In opposition to this, Mehl (1973) suggests the rudimentary hermaphroditism of the sparid *Lithognathus lithognathus* may be a transitional stage towards the evolutionary development of gonochorism.

1.1 TELEOST SEX CHANGE.

Sequential hermaphroditism occurs as a natural reproductive strategy in many teleosts (Sadovy & Shapiro, 1987) and may be defined as the sequential maturation of male and female sex tissues at different stages of an individual's life (Yeung & Chan, 1987). Sex change is suggested to occur in a species when an individual reproduces more successfully as one sex when small or young, but as the opposite sex when large or old (Ghiselin, 1969; Warner, 1988a). If females increase in reproductive ability (e.g. fecundity) with length or age faster than do males, then sex change from male to female will be favoured (Warner, 1988b). This type of sex change, in the male to female direction, is known as protandrous sex change. The reverse pattern of sex change, functioning initially as female before becoming male at a later stage, is known as protogynous sex change. Protogyny is generally accepted as occurring more commonly than protandry in fishes (Davis, 1982; Policansky, 1982; Francis, 1992; Shapiro, 1996).

In addition to protandrous and protogynous sex change, some fish have evolved a more labile form of the sex change strategy. Where most species of fish undertake sex change in only one direction (either protandry or protogyny), with each fish changing sex only once, a few species of fish have the ability to change sex in either direction and undertake sex change on more than one occasion. Such plasticity in sex change has been reported for the gobiid *Trimma okinawae* (Sunobe & Nakazono, 1993) and the cirrhitid *Cirrhitichthys aureus* (Kobayashi & Suzuki, 1992). Under particular social manipulations, the primary direction of sex change can be reversed so that a sex changed fish may revert back to its original functional sex.

In a further isolated case, Rowland & Snape (1994) report a unique variation in the reproductive strategy of the sparid *Acanthopagrus butcheri*. In their study, Rowland & Snape (1994) suggest environmental factors combined with fishing pressures has seen protogynous sex change evolve in populations of *A. butcheri* of some lake systems, while 'normal' gonochoristic development is expressed in others. Though sex change is well documented in the Sparidae (Buxton & Garratt, 1990), a reproductive strategy of this lability has not been described for any other species of sparid.

1.2 RECOGNITION OF SEX CHANGE.

As sex change involves a switch of functional sex, the strongest available evidence of sex change must come from the observation of an identified individual functioning initially as one sex before later in life being observed functioning as the opposite sex (Shapiro, 1987). The tag and release of fish of known sex that are subsequently recaptured functioning as the opposite sex (e.g. Moore, 1979; Tobin *et al.*, 1997) constitutes such evidence. Biopsy experiments, where portions of gonad material are removed from identified individuals over time, may also identify sex change (e.g. Zohar *et al.*, 1979; Yeung & Chan, 1987). However, the results of such experimentation may be considered questionable due to the intrusive manipulation required for gonad biopsies. The stress associated with biopsy procedures may generate changes within gonad structure not related to the process of sex change.

Alternatively, where species are sexually dimorphic in external morphology or colouration, following the sex of individual fish may become a non-intrusive exercise. Provided any external morphological differences are proven to be reliable indicators of the sexuality of individual fish, monitoring changes in external morphology and colouration of individuals through time may provide evidence of sex change (Shapiro, 1987).

Though not as reliable as observing individual fish change sex, observing fish in transitional states of sex change may also be considered strong evidence. The gonads of fish undergoing sex change may be expected to show degeneration of the germinal tissue of the primary sex accompanied by proliferation of the tissue of the opposite sex (Sadovy & Shapiro, 1987). The transitional gonad of a protogynous sex changing fish may be characterised by the presence of an ovarian lumen in the newly forming testis, and the presence of ovarian remnants such as oocytes and yellow bodies (Shapiro, 1987). On the other hand, a transitional gonad of a protandrous fish may show degenerating testicular tissue intermingled with early stages of oogenic activity.

Though transitional fish detected in the field offer stronger evidence than captive fish, transitional individuals are usually infrequent in field samples (Shapiro, 1987), and captive experiments may be necessary. Further, the period of sex change may vary widely (Sadovy & Shapiro, 1987). Some sparids (e.g. *Pagellus acarne*) take a little less than a year for the last remnants of testis to resorb (Reinboth, 1980), while in some protogynous wrasse spp., ovarian tissue may be completely replaced by the testis and the production of mature spermatozoa may occur within a matter of a few weeks (Reinboth, 1980).

A number of aspects of population structure, such as length-frequency and age-frequency distributions and adult sex ratios, are commonly used as evidence in the diagnosis of sex change (Sadovy & Shapiro, 1987; Shapiro, 1987). Where utilised as evidence of sex change, trends such as bimodal length- or age-frequency distributions are usually presented (e.g. Robinson, 1976; Waltz *et al.*, 1982; Alekseev, 1982; Pollock, 1985; Pajuelo & Lorenzo, 1995). In the case of a protandrous sex changing species, the smaller length-classes and younger age-classes are predominated by male fish, while larger length-classes and older age-classes are dominated by female fish. For protogynous sex changing fish, the converse would be true.

However, although widely used in the diagnosis of sex change, Sadovy & Shapiro (1987) warn against claiming bimodal length- and age-frequency distributions as definitive evidence of sex change. Biological mechanisms other than sex change may produce bimodality in length- and/or age-frequencies. In some non-sex changing fish bimodality in length-frequency distributions may be produced by size dimorphism and differential growth rates between sexes (Mackay, 1973; Clarke, 1983). Differential mortality rates and migratory patterns (i.e. spatial segregation of the sexes) between males and females may also produce bimodal length-distributions (Sadovy & Shapiro, 1987).

In comparison to the use of length-frequencies, age-frequency distributions may present fewer interpretational problems. For example, differential growth rates between male and female fish may produce bimodal length-frequencies in a gonochoristic population, however age-frequencies may not be expected to show a bimodal pattern. Though many sex change studies on sparid fish have investigated only length-frequencies (e.g. Dubovitsky, 1976; Pollock, 1985; Buxton, 1989; Buxton & Clarke, 1989; Krug, 1990), at every opportunity age-frequencies should also be investigated.

Adult sex ratios are another aspect of population structure that are occasionally used for indicating sex change may occur in fish (Sadovy & Shapiro, 1987). Protandrous sex changing species tend to display male-biased sex ratios while protogynous sex changing species will be female-biased. Again however, exceptions do occur and interpretations of adult sex ratios should be made cautiously (Pulfrich & Griffiths, 1988). Some sex changing species do not show biased sex ratios (Lodi, 1967; Erickson &

Grossman, 1986). Further, biological mechanisms such as differential mortality rates between the sexes or differential habitat preferences of male and female fish may result in some gonochoristic species displaying biased sex ratios. For example, Dooley (1978) detected biased sex ratios in the gonochoristic tilefish, *Lopholatilus chamaeleonticeps*.

Sadovy & Shapiro (1987) suggest the collection of large samples covering all size classes and taken from a single location will help identify sex change if it does in fact occur. Certainly, the criteria available for the diagnosis of sex change in fish are highly variable in their relative strengths and weaknesses. Ideally, any attempts at diagnosing sex change in fish should include a number of criteria rather than be based solely upon a single diagnostic.

1.3 SEX CHANGE IN FISH OF THE SPARIDAE.

The fish of the Sparidae possess a particularly diverse array of reproductive strategies (Atz, 1964). Apart from normal gonochoristic development, all forms of hermaphroditism (protogyny, protandry, simultaneous and rudimentary) have been recorded (Buxton & Garratt, 1990). The diversity of reproductive strategies may be attributed to the characteristic delimited structure of the sparid gonad (Besseau & Brusle-Sicard, 1995), a gonad structure unique among the teleost fishes. Termed an ovotestis, both male and female tissues occur simultaneously in the gonad and are separated into zones by collagenous connective tissue (Fig. 1.1). Buxton and Garratt (1990) concluded that the bipotentiality of the sparid gonad is a pre-adaptation for the development of sequential hermaphroditism (sex change) in species in which reproductive success is size related. However, apart from providing the structural basis for the development of the diverse array of reproductive strategies found in the Sparidae, the structure of the ovotestis also effectively limits the number of methods available that provide conclusive evidence of sex change in sparid fishes (Sadovy & Shapiro, 1987; Garratt, 1993).

Although a popular and strong diagnostic of sex change in fish, the nature of the sparid ovotestis structure effectively complicates attempts at identifying transitional gonad structure (Tobin *et al.*, 1997). Observing testicular and ovarian tissues concomitantly within the gonad of fish from families other than the Sparidae may be treated as a fairly sound indicator that sex change may occur. In the sparid fish however, the simultaneous presence of testicular and ovarian tissue does not necessarily indicate that sex change occurs. Rudimentary hermaphroditism is common in the sparids and ovotestes may be detected throughout the lifetime of a fish even though sex change does not occur. As such, the identification and description of transitional gonad structures for sparid fish should be made cautiously.

In reviewing the reproductive strategies of the Sparidae, Buxton & Garratt (1990) stated that many of the reports lack clarity of terminology and in some cases are based upon superficial observation. Interestingly, many sparid studies have conclusions based on sparse data and diagnostics that are at best recognised as weak. Further, although describing transitional gonad structure by microscopic observation has been reported as difficult (Sadovy & Shapiro, 1987; Garratt, 1993; Tobin *et al.*, 1997), a surprising number of studies have chosen to describe transitional gonad structures by macroscopic examination only (Table 1.1). It is suggested that, without further diagnostics supporting the transitional gonad structure, the results and conclusions of these studies should be treated with scepticism. Further to this, in reporting the sparid *Chrysoblephus laticeps* as a protogynous hermaphrodite, Penrith (1972) also claimed "normal bisexual development (ie: gonochorism) definitely occurs in the fellow sparids - *Boopsoidea inornata*, *Spondyliosoma emarginatum*, *Petrus rupestris*, *Pterogymnus laniarius*, *Pachymetopon grande*, *Diplodus sargus*, *Polysteganus undulosus* and *Rhabdosargus globiceps*" without presenting any supporting evidence. In fact, *P. laniarius* was later reported to undertake protogynous sex change (Hecht & Baird, 1977), and *D. sargus* reported as a protandrous sex changer (Coetzee, 1986; Micale *et*

Figure 1.1 : Schematic representation of the sparid ovotestis (transverse view) showing the delimited nature of the testis and ovarian lobe separated by a layer of collagenous connective tissue.

Figure 1.1

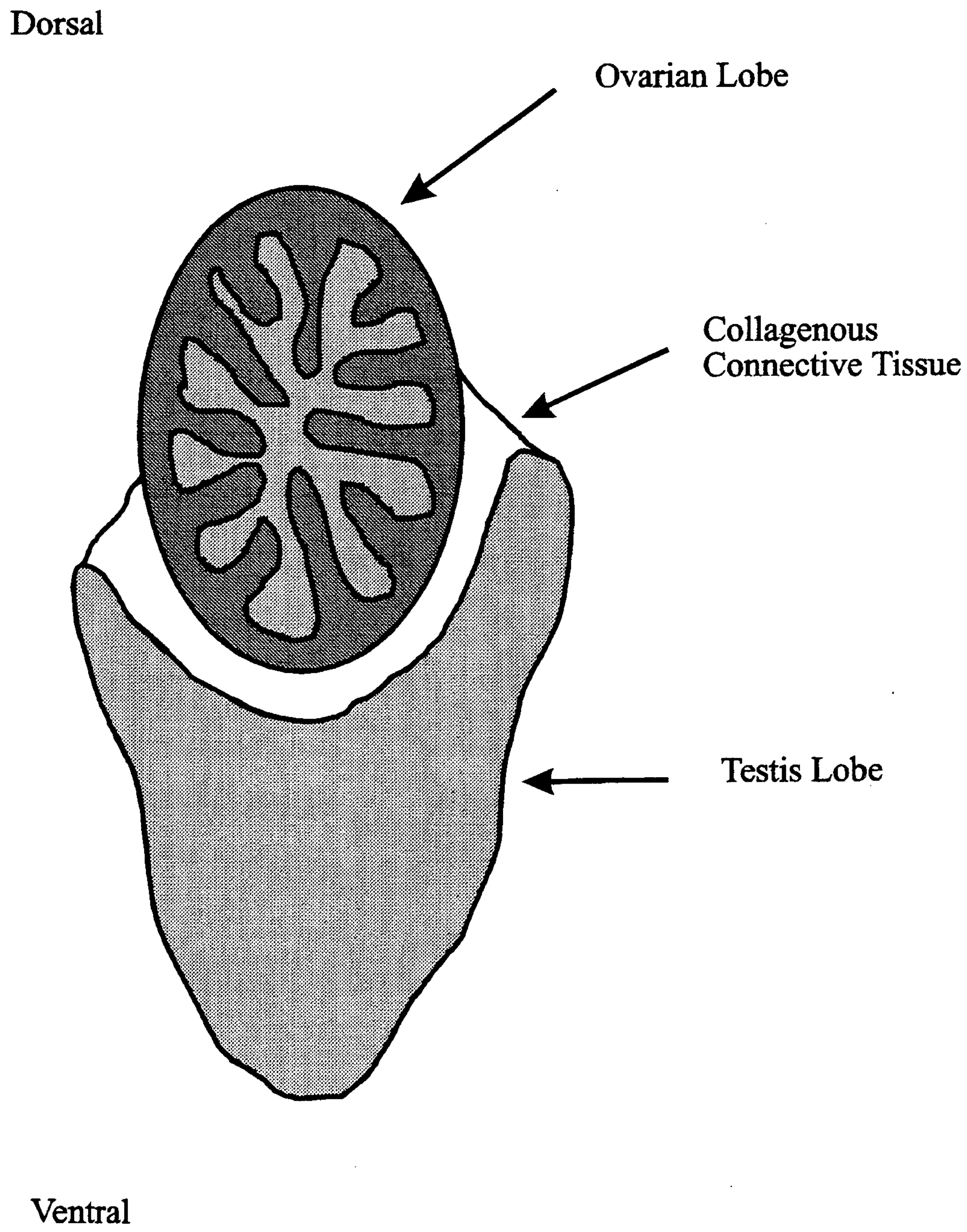

Dorsal
Ovarian Lobe
Collagenous Connective Tissue
Testis Lobe
Ventral

Table 1.1 : A summary of some of the reports detailing sparid fish reproductive strategies, detailing the types of diagnostic criteria used by each study. Only primary references were included. The diversity of reproductive strategies within the Sparidae is evident. The shading highlights those reports that offer contradictory conclusions for the reproductive strategies of particular species.(✔ indicates those diagnostic criteria used to define a *sex change* reproductive strategy; ✖ indicates the diagnostic criteria used to define *rudimentary hermaphroditism* as a reproductive strategy).

Table 1.1

SPECIES	AUTHOR (Date)	Sexuality	Sex Ratio	Length-frequency	Age-frequency	Macroscopic Intersex	Microscopic Intersex	Biopsy Experiment
Acanthopagrus australis	Pollock (1985)	Protandrous		✔			✔	
Acanthopagrus bifasiatus	Etessami (1983)	Protandrous					✔	
Acanthopagrus butcheri	Rowland & Snape (1994)	Protogynous (Labile)	✔				✔	
Acanthopagrus schlegeli	Chang & Yueh (1990)	Protandrous					✔	
Archosargus probatocephalus	Render & Wilson (1992)	Rudimentary					✖	
Boopsoidea inornata	Penrith (1972)	Gonochore						
Calamus leucosteus	Waltz *et al.* (1982)	Protogynous		✔	✔		✔	
Calamus proridens	Dubovitsky (1976)	Protogynous	✔	✔			✔	
Cheimerius nufar	Coetzee (1983)	Rudimentary	✖	✖			✖	
Chrysoblephus cristiceps	Robinson (1976)	Protogynous		✔		✔		
	Buxton (1989)	Protogynous	✔	✔			✔	
Chrysoblephus gibbiceps	Penrith (1972)	Gonochore						
Chrysoblephus laticeps	Penrith (1972)	Protogynous	✔	✔		✔		
	Buxton (1989)	Protogynous	✔	✔			✔	
Chrysoblephus puniceus	Garratt (1986)	Protogynous	✔	✔			✔	
Cymatoceps nasutus	Buxton & Clarke (1989)	Protogynous		✔		✔		
Dentex dentex	Glamuzina *et al.* (1989)	Protandrous		✔				
Dentex gibbosus	Pajuelo & Lorenzo (1995)	Protogynous	✔	✔				
Diplodus sargus	Penrith (1972)	Gonochore						
	Joubert (1981)	Rudimentary	✖	✖		✖		
	Coetzee (1986)	Protandrous		✔			✔	
	Micale *et al.* (1987)	Protandrous		✔			✔	
Lagodon rhomboides	Cody & Bortone (1992)	Rudimentary	✖	✖			✖	
Lithognathus lithognathus	Mehl (1974)	Rudimentary	✖				✖	

Table 1.1 (cont)

SPECIES	**AUTHOR (Date)**	**Sexuality**	Sex Ratio	Length-frequency	Age-frequency	Macroscopic Intersex	Microscopic Intersex	Biopsy Experiment
Pachymetopon aeneum	Buxton & Clarke (1986)	Protogynous		✔		✔	✔	
Pachymetopon grande	Penrith (1972)	Gonochore						
	Buxton & Clarke (1992)	Rudimentary	✖		✖		✖	
Pagellus bogaraveo	Krug (1990)	Protandrous	✔	✔			✔	
Pagrus auriga	Alekseev (1982)	Protogynous				✔	✔	
	Alekseev (1983)	Protogynous	✔	✔				
Pagrus ehrenbergi	Alekseev (1982)	Protogynous				✔	✔	
	Alekseev (1983)	Protogynous	✔	✔				
Pagrus orphus	Alekseev (1982)	Protogynous				✔	✔	
	Alekseev (1983)	Protogynous	✔	✔				
Pagrus pagrus	Alekseev (1982)	Protogynous				✔	✔	
	Alekseev (1983)	Protogynous	✔	✔				
	Pajuelo & Lorenzo (1996)	Protogynous	✔	✔		✔		
Petrus rupestris	Penrith (1972)	Gonochore						
	Smale (1988)	Rudimentary	✖	✖		✖	✖	
Polysteganus undulosus	Penrith (1972)	Gonochore						
Pterogymnus laniarius	Penrith (1972)	Gonochore						
	Hecht & Baird (1977)	Protogynous	✔	✔		✔		
	Booth & Buxton (1997)	Rudimentary		✖		✖	✖	
Rhabdosargus globiceps	Penrith (1972)	Gonochore						
Rhabdosargus sarba	Yeung & Chan (1987)	Protandrous		✔			✔	✔
Sarpa salpa	Joubert (1981)	Rudimentary	✖	✖				
Sparodon durbanensis	Buxton & Clarke (1991)	Rudimentary		✖			✖	
Sparus aurata	Zohar *et al.* (1978)	Protandry					✔	✔
	Brusle-Sicard & Fourcault (1997)	Protandry					✔	
Spondyliosoma emarginatum	Penrith (1972)	Gonochore						

al., 1987). In a further example, Bonnet (1969) described *D. gibbosus* as a protandrous hermaphrodite, however a later report by Pajuelo & Lorenzo (1995) reported *D. gibbosus* to be protogynous. Considering these points, it is not surprising that contradictory reports can be found in the sparid sexuality literature (see Table 1.1).

In order to confidently demonstrate sex change in a sparid fish, an individual fish needs to be observed functioning initially as one sex before later in life functioning as the opposite sex (Tobin *et al.*, 1997). With species that are relatively sedentary in nature, a field tag and release study similar to that of Moore (1979) or Garratt (1993) may be implemented. The sexual status of individual fish may be monitored over time and sex change detected if in fact it does occur. If logistical problems prevent an in-field assessment or the species is particularly itinerant, the sexual status of individuals held in a captive environment may be monitored over time. However, if sex change is detected in a captive situation it may be argued to have occurred as a result of the captive environment (Garratt, 1993). Alternatively, where sex change may be observed in fish in the field, holding fish in captive environments may impede sex change.

Further to monitoring the sexual status of individual fish, other diagnostic criteria (such as length- and age-distributions, transitional gonad structure and adult sex ratios) may also be investigated. While a number of sparid studies have reported and described reproductive strategies on the basis of a single diagnostic (e.g. Etessami, 1983; Render & Wilson, 1992; Brusle-Sicard & Fourcault, 1997), ideally a study reporting a reproductive strategy should utilise as many of the available diagnostics as possible.

1.4 THE IMPORTANCE OF RECOGNISING SEX CHANGE.

Aside from the general biological interest, identification of sex change in fish is important as the sex change process may have serious implications for the management and general survival of a species (Garratt, 1986a; Buxton, 1992). The managerial aspects are particularly important for the sparid fish where many species are not only sex changers, but also contribute largely to recreationally and commercially important fisheries (Druzhinin, 1975; Smale & Buxton, 1985; Garratt, 1986a; Garratt, 1988; Al-Hassan, 1990). Further to this, many sparids are important aquaculture species (Kitajima & Tsukashima, 1983; Reina *et al.*, 1994; Garrido-Ramos *et al.*, 1995; Jug-Dujaković *et al.*, 1995), with the Sparidae contributing to a large proportion of world fin-fish culture (New, 1991). A comprehensive understanding of the reproductive biology of sparids is therefore paramount.

Further, the sexual succession of sex change may cause differential influence on males and females by exploitation as fishing pressures often selectively target and remove the larger fish from a population (Alekseev, 1982; Buxton, 1993). Fisheries exploitation of protandrous and protogynous fishes may be expected to selectively remove the larger female and male fish respectively. As such, a species that is of both commercial and recreational importance requires adequate management to ensure the population will sustain fishing pressures from both the recreational and commercial sectors.

For some sex changing species that show strong size dimorphism and support commercial and recreational fisheries, maximum as well as minimum size limits have been introduced (Hilborn & Walters, 1992). The Barramundi, *Lates calcarifer* (Pisces: Centropomidae) is one such example and is a protandrous hermaphrodite undertaking sex change across a broad age and size range (Moore, 1979). Within Australia, minimum size limits were introduced for *L. calcarifer* to ensure fish were able to reach maturity and spawn as males before recruitment into the fisheries. However, as a protandrous hermaphrodite it was recognised that only the smaller male fish were protected by the minimum size limit. Without offering protection to the larger females the reproductive potential of *L. calcarifer* stocks may be severely impeded. Consequently, a maximum size limit over which no fish is allowed to be taken was introduced to ensure the survival and successful reproduction of large females. Similar managerial steps may need to be taken with some sex changing sparid species to ensure long-term sustainability.

Already fishing pressures have been identified as having undesirable effects on some sparid fishes. Garratt (1985) reports fishing pressure to have significantly altered sex ratios in populations of *Chrysoblephus puniceus* and suggests the reproductive potential of the species may be limited. Similarly, Buxton (1993) found negative consequences of fishing exploitation on the sex change process of *C. cristiceps*. Sex ratios in exploited stocks of *C. cristiceps* were skewed towards the females significantly more than unexploited stocks, and the size at sex change was significantly reduced. The smaller size at sex change may be recognised as a compensatory mechanism where efforts to restore some desirable sex ratio are being made (Smith, 1982). Informed management strategies such as those in place for *L. calcarifer* are required, and need to be made on the basis of a comprehensive understanding of the reproductive biology of the species in question.

1.5 THE OBJECTIVES OF THIS THESIS.

As a member of the Sparidae, the pikey or river bream *Acanthopagrus berda* (Forskål, 1775) has an extensive distribution extending from South Africa to India and the western Pacific, including Japan and northern Australia (Fischer & Bianchi, 1984). *A. berda* is a common component of the fish fauna of tropical Australian estuaries (Beumer, 1978; Blaber, 1980: Robertson & Duke, 1987; Blaber *et al.*, 1989; Sheaves, 1992) and has a wide distribution extending from Shark Bay (26^0 07'S, 113^0 25'E) in the west across the northern coasts to Rockhampton (23^0 22'S, 150^0 32'E) in the east (Fig. 1.2)(Grant, 1987). Throughout its Australian distribution, *A. berda* represents a significant proportion of recreational anglers catches (Sheaves, 1992; Palmer *et al.*, 1994), and although not yet exploited by a commercial fishery, stock numbers are sufficient to warrant future interest. Considering the wide international distribution of *A. berda* it is surprising that little research has been conducted on the biology of this sparid.

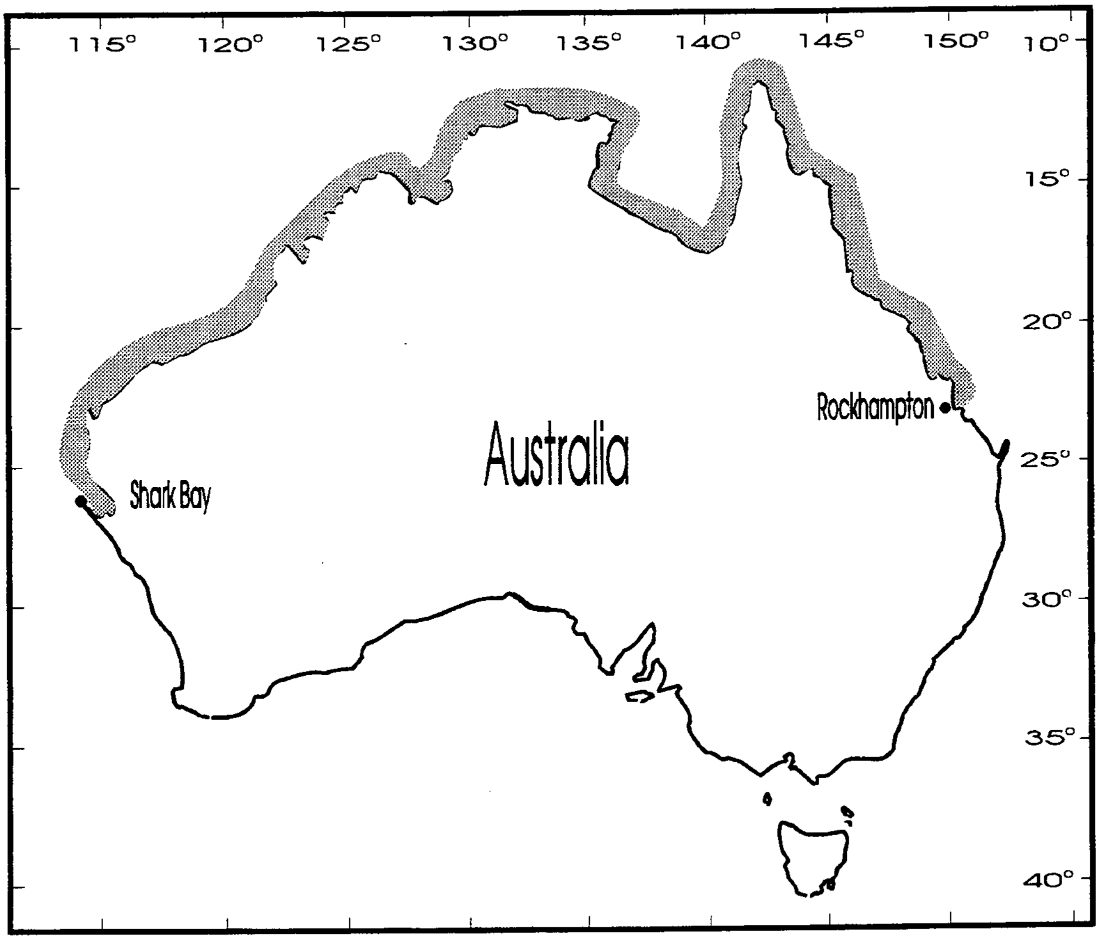
The reproductive biology of *A. berda* is of obvious interest. Although Garratt (1993) attempted to produce evidence of protandrous sex change in *A. berda* sampled from a South African estuary, Garratt was unable to collect sufficient data. As yet, the reproductive strategy of *A. berda* is still to be defined.

The objectives of this thesis are firstly to determine and define the reproductive strategy of *A. berda*. Rather than relying on one or two criteria for the diagnosis of reproductive strategy, a hierarchical approach utilising length-frequencies, age-frequencies, adult sex ratios, gonad structure, and the monitoring of individual fish's sexuality both in aquaria and the field will be used. By addressing a range of diagnostic criteria this thesis aimed to define the reproductive strategy of *A. berda* with certainty. Further, to provide a spatial perspective, replicate samples of *A. berda* from seven estuaries in northeastern tropical Australia will be investigated.

Secondly, the age and growth of *A. berda* will be investigated and compared across relatively small geographic ranges. Age and growth of *A. berda* sampled from six estuaries (spanning approximately 250 km of coastline) will be investigated and compared. Spatial variability in the growth of a number of sparids has been recorded (e.g. Longhurst, 1958; Paul, 1976; Sanders & Powell, 1979; Francis & Winstanley, 1989; Krug, 1990; Paul, 1992; Buxton, 1993), however growth variation has only been detected across relatively large geographical ranges. Considering the reasonably sedentary nature suggested for *A. berda* in estuaries of northeastern Australia (Sheaves, 1993; Sheaves *et al.*, in review), it may be possible that significant variation in the growth of *A. berda* occurs between estuaries separated by only a few kilometres of coastline.

Finally, the rate of sex change will be modelled for each of six estuaries by evaluating the change in sex ratios through the age classes. The probability of sex change of fish in a given age class will then be estimated for each estuary, and comparisons of the rate of sex change between estuaries made possible. Although differential rates of sex

Figure 1.2


115°
120°
125°
130°
135°
140°
145°
150°
10°
15°
20°
25°
30°
35°
40°
Australia
Shark Bay
Rockhampton

change have been suggested to occur in some sex changing sparids (e.g. Alekseev, 1982; Micale *et al.*, 1987 c.f. Abou-Seedo *et al.*, 1990; Krug,1990), as yet no study has attempted to model and compare the rates of sex change for any sparid species.

1.6 SPECIFIC AIMS.

The specific objectives of this thesis are to -

Firstly, determine and define the reproductive strategy of *Acanthopagrus berda*.

Secondly, geographical variability in the age and growth of *A. berda* will be investigated. Variation of growth between the sexes will also be examined.

Thirdly, the rate of sex change for each age-class will be estimated. The effect of geographical variation on the rate of sex change will be assessed.

CHAPTER 2.

TRADITIONAL INDICATORS OF PROTANDROUS SEX CHANGE APPLIED TO *ACANTHOPAGRUS BERDA*.

2.1 INTRODUCTION.

Two aspects of population structure, length- and age-frequency distributions, are frequently investigated in the diagnosis of sex change in teleost fish (Sadovy & Shapiro, 1987). The comparison of length- and age-frequency distributions of male and female fish is often an investigators first line of inquiry when assessing the possibility of sex change (e.g. Penrith, 1972; Robinson, 1976; Horvath *et al.*, 1990), because the necessary data are simple to collect and analyse. The basis for this approach is that protogynous sex changing fish should display female dominance of smaller length- and/or younger age-classes, and male fish predominate in the larger length- and/or older age-classes. A converse pattern should be seen in the length- and age-distributions of protandrous fishes.

Although many sparid sex change studies have presented bimodal length-distributions as evidence of sex change (e.g. Yeung & Chan, 1987; Buxton, 1989; Glamuzina *et al.*, 1989; Krug, 1990; Pajuelo & Lorenzo, 1995), age-frequency distributions have rarely been investigated. This is surprising considering bimodal age-frequencies are considered a stronger diagnostic of sex change than length-frequencies (Sadovy & Shapiro, 1987). In comparison to length-frequencies, age-frequencies are not biased by such biological mechanisms as sex-specific differential growth or mortality rates. In two isolated cases, Waltz *et al.* (1982) presented a bimodal age-frequency for the protogynous *Calamus leucosteus*, and Buxton & Clarke (1992) found no significant difference between the mean length-at-age of males and females of the rudimentary hermaphrodite *Pachymetopon grande*.

Adult sex ratios have also been used as indicators of sex change. Sex ratios are tested for departure from the 1 : 1 "theoretically expected" ratio of gonochorists, with a female or male bias in sex ratios interpreted as suggesting protogynous or protandrous sex change respectively may occur (Sadovy & Shapiro, 1987). Cautious interpretation is required however, as some studies have reported gonochoristic species with sex ratios differing substantially from the 1 : 1 "theoretically expected" ratio (e.g. Dooley, 1978; Erickson & Grossman, 1986).

In this study *Acanthopagrus berda* collected from seven tropical estuaries of north-eastern Australia were investigated for evidence of sex change using bimodal length- and age-frequency distributions, and adult sex-ratios.

2.2 MATERIALS AND METHODS.

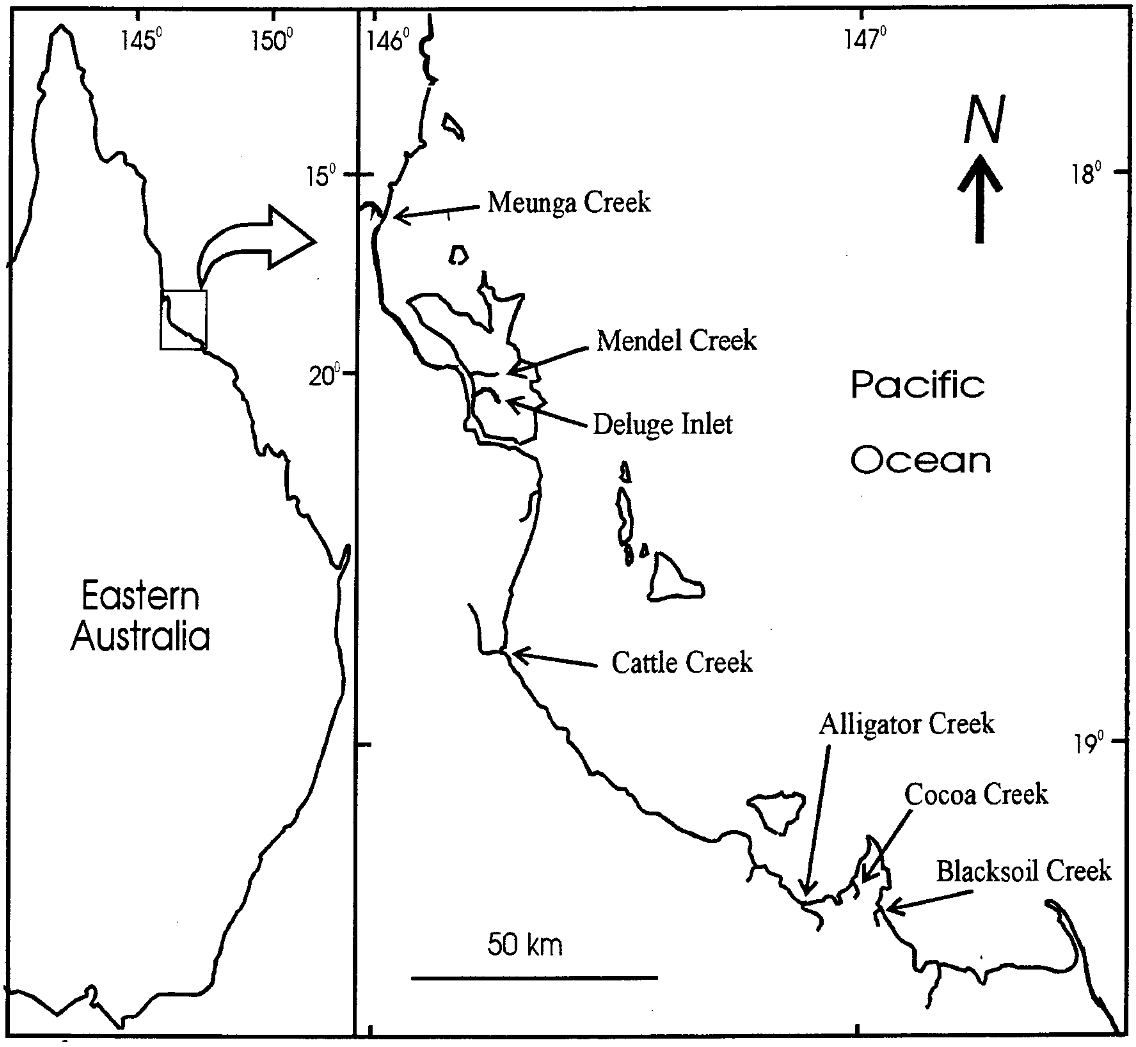
2.2.1 COLLECTION OF SAMPLES

Between August 1993 and July 1997, samples of *Acanthopagrus berda* were collected from Blacksoil Creek, Cocoa Creek, Alligator Creek, Cattle Creek, Deluge Inlet, Mendel Creek and Meunga Creek (Fig. 2.1), seven tropical mangrove estuaries on the north-east coast of Australia. To allow the sex of fish to be determined unambiguously, the majority of sampling trips were conducted during the winter spawning period of *A. berda* [extending from June through to September (Tobin *et al.*, 1997)]. The majority of sampling was conducted by hook and line fishing, as *A. berda* shows a preference for habitats of structural heterogeneity such as the fallen timber and mangrove prop root "snags" common in the mangrove lined estuaries of tropical Australia (Sheaves, 1996). Hook and line fishing is the most efficient and cost effective sampling method when targeting *A. berda* in this type of habitat.

The hook and line sampling was supplemented with some fish trapped using Antillean Z-traps of similar design to those used by Sheaves (1996).

Figure 2.1 : Location map showing the seven estuaries sampled in northeastern tropical Australia during the current study.

Figure 2.1


145°
150°
15°
20°
Eastern Australia
146°
147°
18°
19°
N
Meunga Creek
Mendel Creek
Deluge Inlet
Pacific Ocean
Cattle Creek
Alligator Creek
Cocoa Creek
Blacksoil Creek
50 km

2.2.2 COMPARISON OF LENGTH AND AGE STRUCTURES BY SEX

Fork length of each fish was measured to the nearest millimetre. Fish captured during the spawning period, were sexed by a non-intrusive milking method described by Tobin *et al.* (1997). As part of an ongoing study (see Chapter 4), many of these spawning fish were then tagged and released. Fish captured during the non-spawning period (October to May) were sacrificed because internal examination was required to determine sex unambiguously. The sagittal otoliths were removed, cleaned and stored dry in envelopes. The otoliths were prepared by taking thin transverse sections through the nucleus and read by viewing under a high powered microscope. Aging *A. berda* by sectioned sagittal otoliths has been validated [Tobin (in review) - see Appendix 1]. Age data was available for all creeks except Alligator Creek.

2.3 RESULTS.

2.3.1 LENGTH-FREQUENCY ANALYSIS

Although there was considerable variation among samples from different creeks, length-frequency analysis showed a common trend. In all samples, male *A. berda* dominated the smaller length-classes and females the larger length-classes for all creek samples (Fig. 2.2). Fish up to 150mm FL for Deluge Inlet and Mendel Creek, 160mm FL for Blacksoil Creek, 170mm FL for Alligator Creek, 180mm FL for Cocoa Creek, and 190mm FL for Cattle and Meunga Creeks were exclusively male. Further, the number of male fish below these lengths represented substantial proportions of their respective samples (12%, 15%, 28%, 36%, 51%, 29% and 71% of the samples respectively).

2.3.2 AGE-FREQUENCY ANALYSIS

Similar to the length-frequency distributions, the age-frequency distributions for *A. berda* also showed strong bimodal patterns (Fig. 2.3). Fish in younger age-classes

Figure 2.2 : Length-frequency graphs showing the absolute frequencies of male (light shade) and female (dark shade) *A. berda* sampled during the study.

Figure 2.2

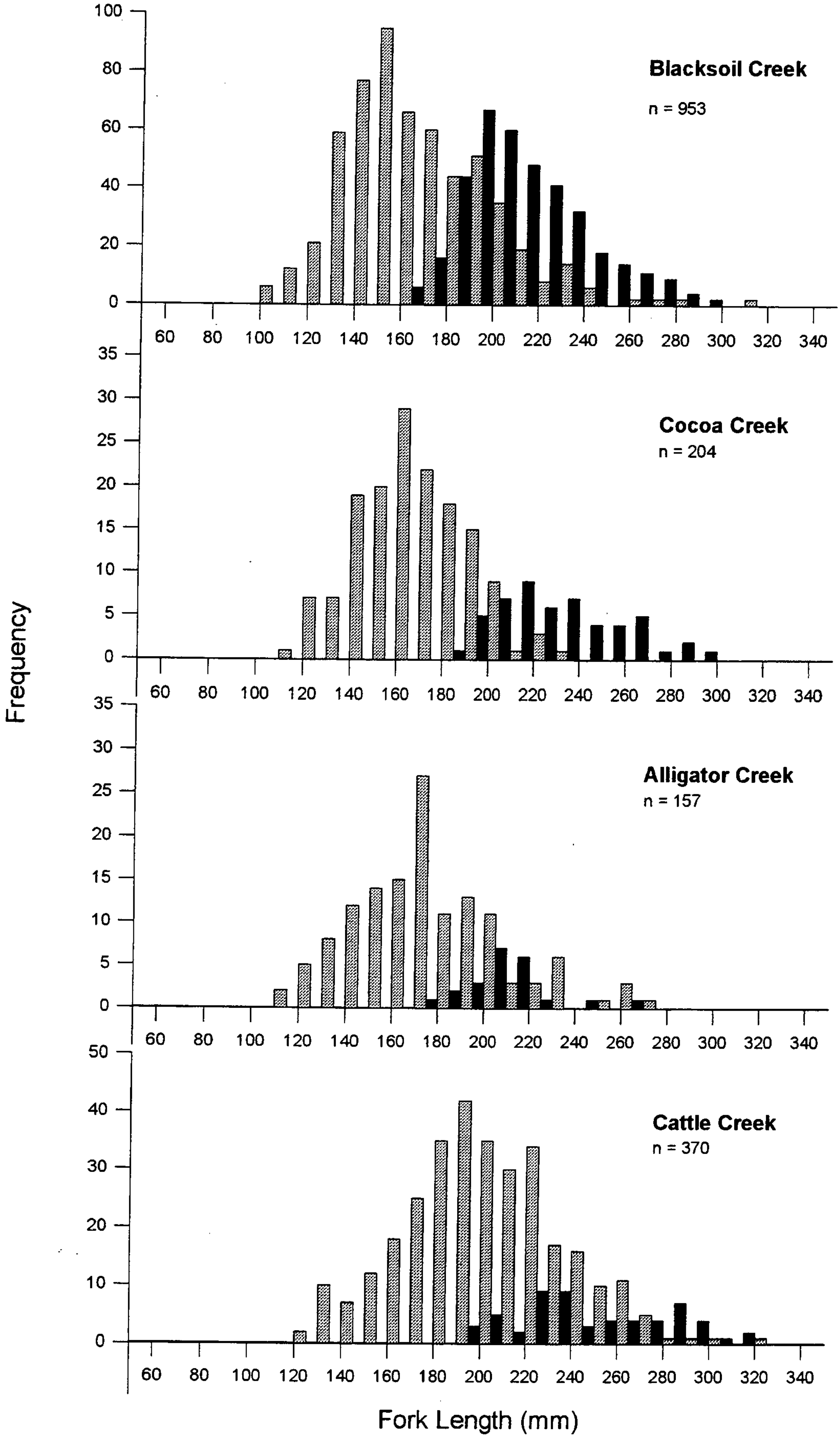

Blacksoil Creek
n = 953
Cocoa Creek
n = 204
Alligator Creek
n = 157
Cattle Creek
n = 370
Frequency
Fork Length (mm)
0
20
40
60
80
100
0
5
10
15
20
25
30
35
0
10
20
30
40
50
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340

Figure 2.2 (cont.)

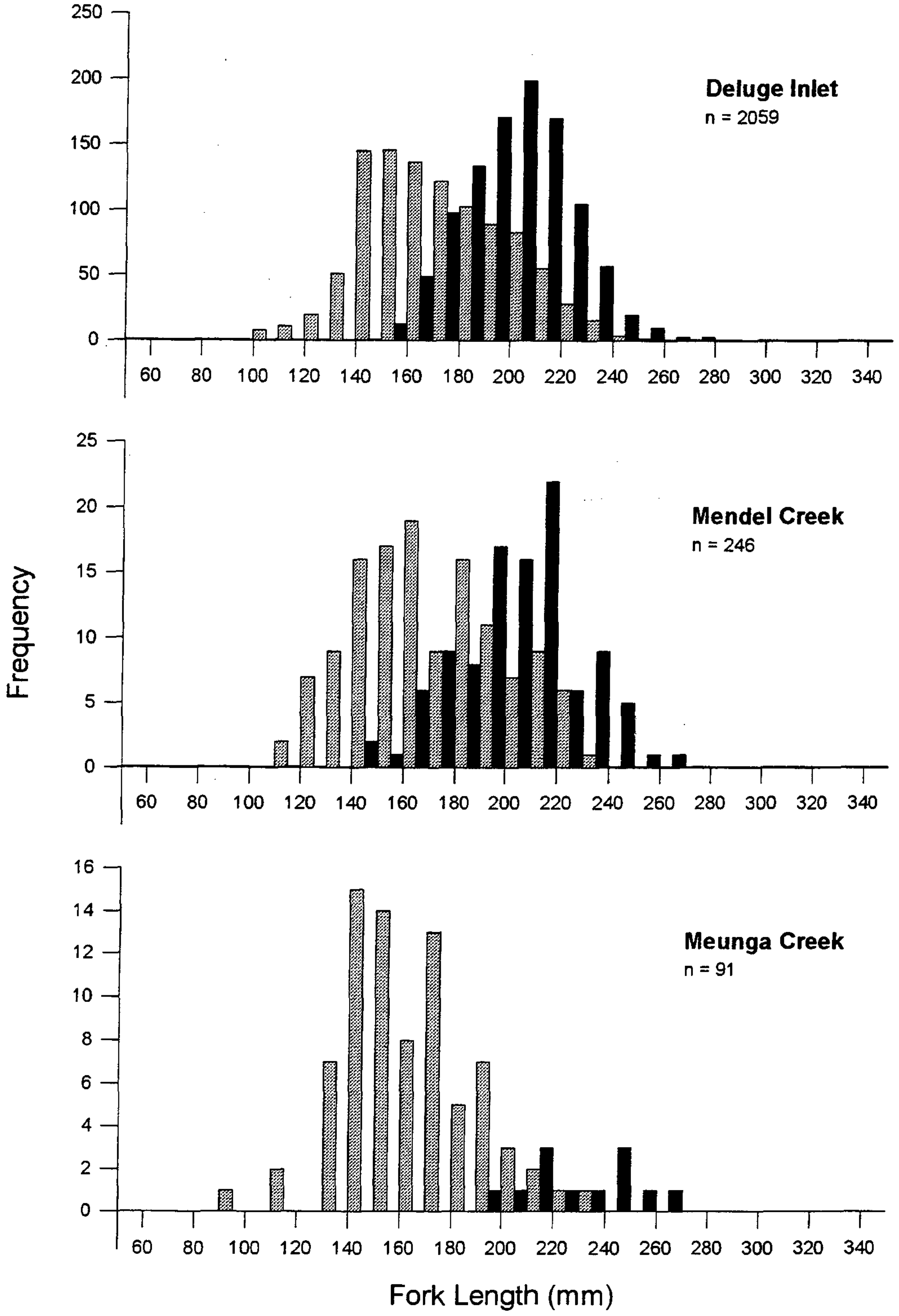


Figure 2.3 : Age-frequency graphs showing the absolute frequencies of male (light shade) and female (dark shade) *A. berda* sampled during the study.

Figure 2.3

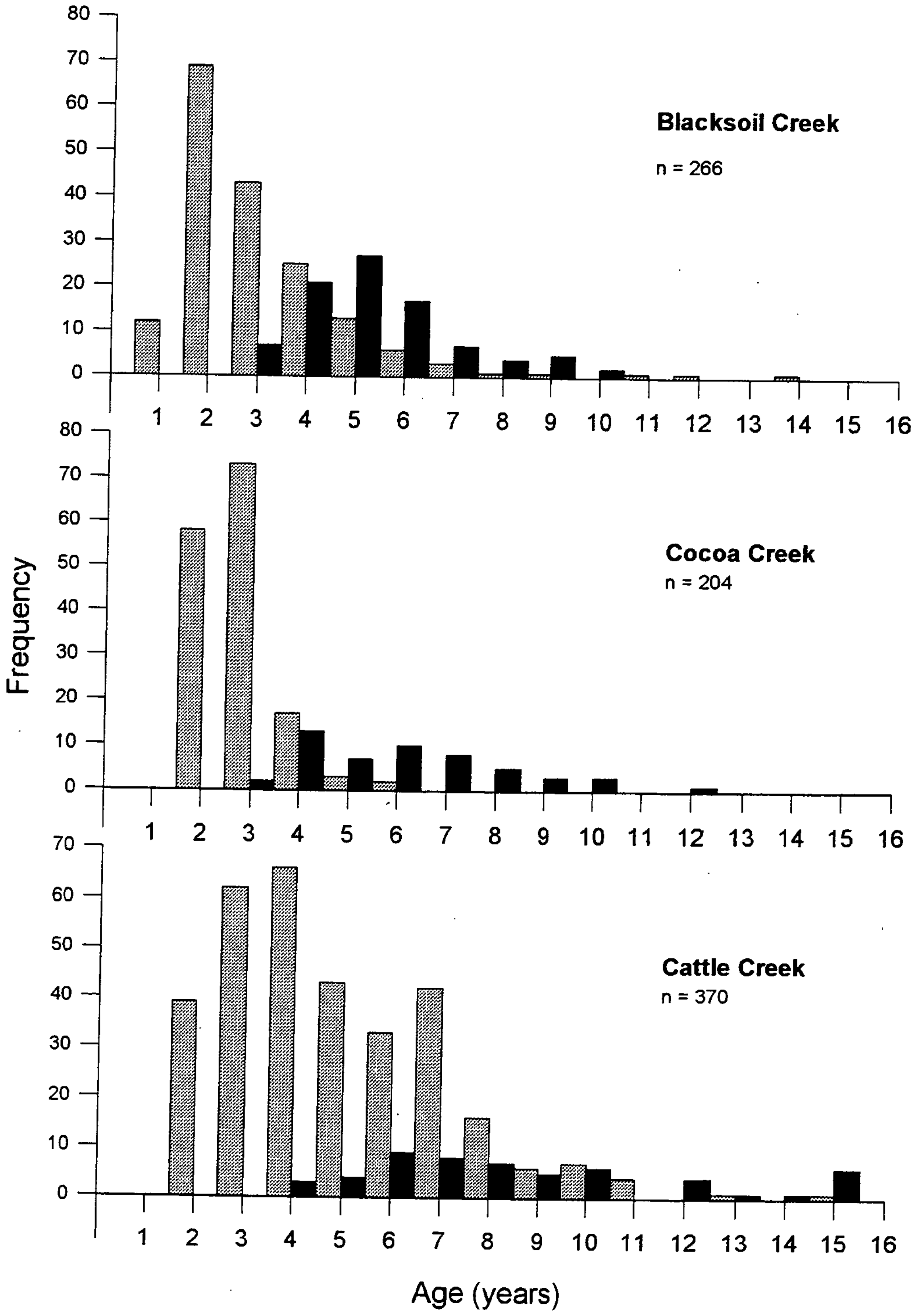
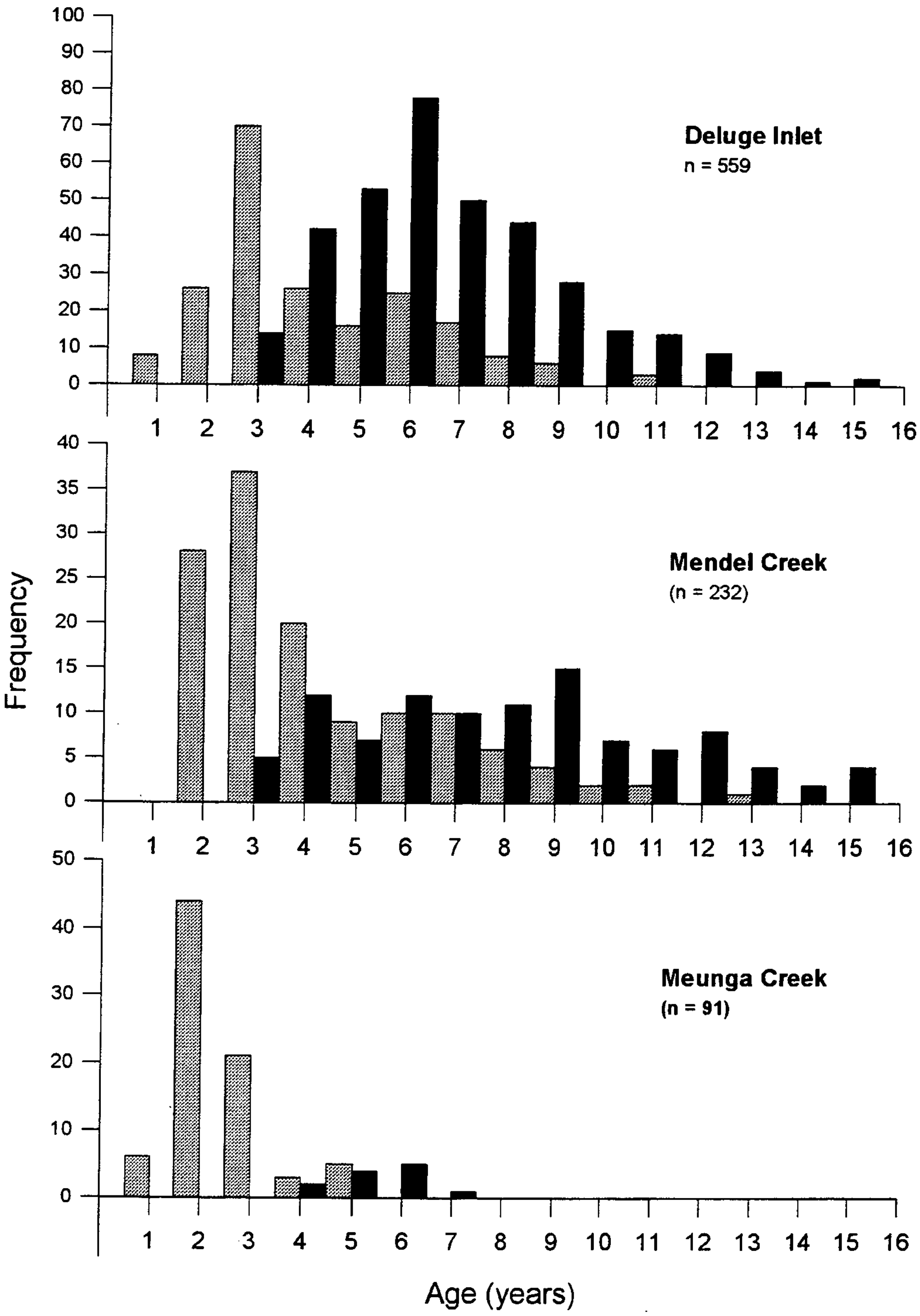

Blacksoil Creek
n = 266
Cocoa Creek
n = 204
Cattle Creek
n = 370
Frequency
0
10
20
30
40
50
60
70
80
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Age (years)

Figure 2.3 (cont.)



of all samples were exclusively male, while female fish predominated in the older age classes. Only some of the sampled fish were mature as males at one year of age, however by the second year all fish sampled were mature males. Females were not present in any sample until at least the third year. In all samples, the modal age-class for males was younger than the modal age-class for females.

2.3.3 SEX RATIOS

Except for Deluge Inlet and Mendel Creek, the sex ratios from all other locations displayed obvious male bias (Table 2.1). The sex ratios of both Deluge Inlet and Mendel Creek were not significantly different from the "theoretically expected" 1 : 1 ($\chi^2 = 0.06$, df = 1, p = 0.83 and $\chi^2 = 2.70$, df = 1, p = 0.12 respectively). At all other sites, sex ratios were significantly different.

Table 2.1: The overall male : female sex ratios for the samples of *Acanthopagrus berda* collected in the present study.

Location	Sex Ratio
Deluge Inlet	1.01 : 1
Mendel Creek	1.25 : 1
Cocoa Creek	2.92 : 1
Blacksoil Creek	3.40 : 1
Cattle Creek	5.52 : 1
Alligator Creek	6.14 : 1
Meunga Creek	6.58 : 1

2.4 DISCUSSION.

The strong bimodality observed in the length- and age-frequency distributions collected in this study, suggests protandrous sex change may occur in *Acanthopagrus berda* in tropical northern Australia. The length-frequency distributions of all samples showed a striking pattern of exclusive male dominance of smaller length-classes. Female fish were present only in larger length-classes. Further, the exclusive male dominance of the smaller length-classes represented a considerable proportion of the sample sizes collected for each location. Age-frequency distributions also suggest protandrous sex change may occur in *A. berda*. The bimodal patterning of the age-frequency distributions mirrored the trend observed in the length-frequency distributions.

Although these patterns strongly suggest sex change, it is possible that biological mechanisms other than sex change may be responsible for the bimodal age- and length-frequency distributions. Bimodal length-frequencies may be caused by differential growth or maturity rates, differential mortality rates or differential migratory patterns between males and females (Sadovy & Shapiro, 1987; Matsuyama *et al.*, 1988). Further, it is also possible for gonochoristic species to display bimodal distribution of the sexes (Sadovy & Shapiro, 1987). For example, the rudimentary hermaphrodite *Cheimerius nufar* may display a significant female dominance of smaller length-frequency classes (Garratt, 1985).

Age-frequency distributions present fewer problems than length-frequency distributions (Sadovy & Shapiro, 1987), however, they do not provide conclusive evidence of sex change. For example, it is possible that young female *A. berda* were present in the sampled creeks though were either not susceptible to the sampling gears utilised or were present only in parts of the creeks not covered by sampling.

If protandrous sex change does occur in *A.berda*, the length- and age-distributions suggest sex change appears to occur across a broad length / age range. Similar broad length and/or age ranges of sex change have been reported for the sparids

Pagellus acarne (Pajuelo & Nespereia, 1994), *Calamus leucosteus* (Waltz *et al.*, 1982), *Pagrus pagrus* (Pajuelo & Lorenzo, 1996) and the congeneric *A. australis* (Pollock, 1985). Further, the presence of large and old males in some of the samples suggests a more complex or plastic process may operate. Again this plasticity of sex change is common in the sparid fish (eg: Kinoshita, 1936; Huang *et al.*, 1974; Abu-Hakima, 1984; Yeung & Chan, 1987; Abou-Seedo *et al.*, 1990; Horvath *et al.*, 1990). The presence of large and old males in populations of protandrous species has a number of possible explanations. It could be that these fish lack the genetic ability to change sex (Buslé & Buslé, 1975), or that the appropriate environmental cue for sex change was lacking (Sadovy & Shapiro, 1987). However, another possibility is that if sex change does occur it may be a more labile process where some fish that have undergone protandrous sex change revert later to their original sex (Shapiro, 1992). Such plasticity of sex change has been demonstrated in the gobiid *Trimma okinawae* (Sunobe & Nakazono, 1993) and the cirrhitid *Cirrhitichthys aureus* (Kobayashi & Suzuki, 1992). Though this type of plasticity in sex change has not been described for any sparid, the possibility cannot be discounted.

With the exception of the Deluge Inlet and Mendel Creek samples, the sex ratios of the samples of *A. berda* were substantially male biased. Garratt (1993) reported a similarly male biased sex ratio (8.8 : 1) for *A. berda* collected from a South African estuary. The male bias of the sex ratios may also present evidence of protandrous sex change in *A. berda*. However, sex ratio data is certainly not conclusive evidence (Sadovy & Shapiro, 1987), and demands cautious interpretation (Pulfrich & Griffiths, 1988). The unreliability of sex ratios is evident in numerous studies. Pajuelo & Nespereia (1994) reported a female biased sex ratio for the protandrous sparid *Pagellus acarne*; Nakazono (1979) reported a male biased sex ratio for the protogynous labrid *Halichoeres tenuispinis*; and more recently Adams (1998) reported a male biased sex ratio in the protogynous serranid *Plectropomus leopardus*. Sex ratio data has been shown to be similarly misleading in studies on the tilefish, *Lopholatilus chamaeleonticeps*. Dooley (1978) reported skewed sex ratios indicative of protogynous sex change. However, Erickson & Grossman (1986) suggest that sex-specific differential growth rates were responsible rather than protogynous sex change.

Of interest in this study however, is the variation of sex ratios between locations. Although both the Deluge Inlet and Mendel Creek samples showed strong bimodal patterns in both length- and age-frequency distributions, in neither did the sex ratios differ significantly from unity. In comparison, the sex ratios for all other locations displayed pronounced male biases. If sex ratios alone were investigated as a diagnostic for sex change in *A. berda*, samples collected from Deluge Inlet and Mendel Creek would have indicated a gonochoristic reproductive style rather than the protandrous sex change suggested by the heavily male biased sex ratios detected in all other locations.

A trend is apparent that suggests Mendel Creek and Deluge Inlet may have stocks of *A. berda* that differ substantially from the *A. berda* sampled from the other locations in this study. Unlike Garratt (1993) who used a variety of sampling techniques, the same types of sampling gears were used at each location and sampling bias is unlikely to be responsible for the high variation observed in the sex ratios between locations.

Similarly, where variation in the sex ratios of some other sparids may be attributable to exploited and unexploited stocks (Garratt, 1985), it is unlikely that variable fishing pressure on *A. berda* is responsible for the pattern of sex ratios observed in this study, as the species receives little exploitation in the study area (Sheaves, pers. comm.).

Although the length- and age-frequency distributions, together with the male biased sex ratio data, may be considered to strongly suggest protandrous sex change occurs, more conclusive data is required before the reproductive strategy of *A. berda* can be defined with certainty.

CHAPTER 3.

GONAD STRUCTURE : FURTHER INDICATIONS OF PROTANDROUS SEX CHANGE IN *ACANTHOPAGRUS BERDA*.

3.1 INTRODUCTION.

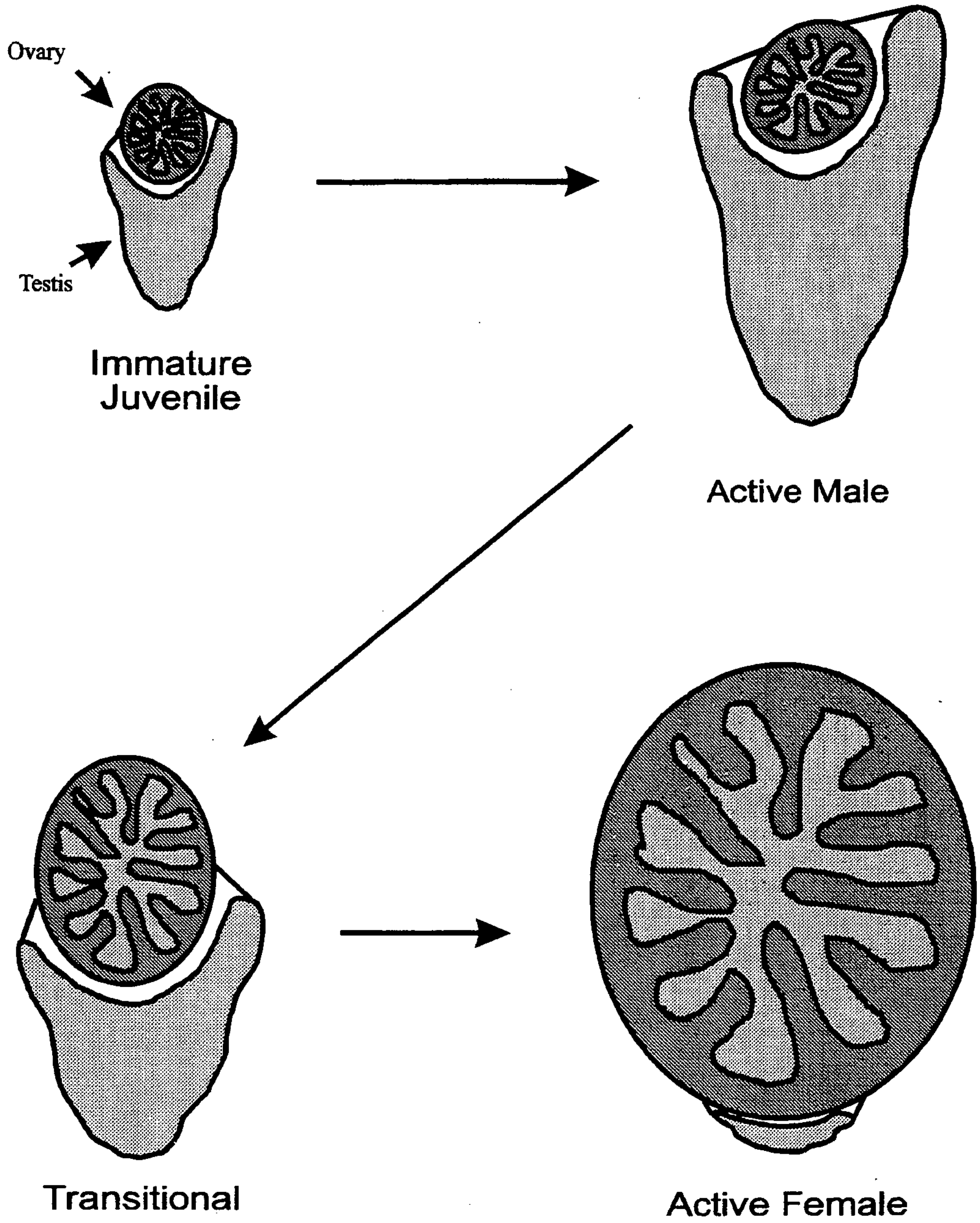
Both the prevalence of hermaphroditism and the variety of reproductive styles present within the Sparidae (Atz, 1964), may be attributed to the characteristic structure of the sparid gonad (Besseau & Bruslé-Sicard, 1995). Termed an ovotestis, both male and female tissues occur simultaneously within a sparid gonad and are separated clearly into zones by collagenous connective tissue (see Fig. 1.1). This "bi-potentiality" of the sparid gonad has been suggested by Buxton & Garratt (1990) "to be a pre-adaptation for the development of sequential hermaphroditism in species in which reproductive success is size related".

Many sparid studies have investigated and used the structure of the sparid ovotestis as a diagnostic of sex change (see Table 1.1). Such studies include the description of an ovotestis structure suggested to represent the transitional stages of a sex change process. Descriptions of transitional stage(s) of ovotestes have been presented by both macroscopic and microscopic examination, with the basis for claiming evidence of sexual transition being the observation of the concomitant degeneration of one of the sexual tissues accompanied by the development of the opposite sexual tissue. However, the structure of the sparid ovotestis may actually complicate attempts to identify transitional ovotestis structure (Yeung & Chan, 1987). The novel structure of the sparid ovotestis may allow sex change to be a simple and rapid process involving the atrophy of the tissue of one sex together with the hypertrophy of the other (Fig. 3.1)(Garratt, 1986; Sadovy & Shapiro, 1987).

Interestingly, in a review of the reproductive styles of the Sparidae, Buxton & Garratt (1990) state that many of the reports detailing sex change lack clarity of

Figure 3.1 : Schematic representation of the possible protandrous sex change process of sparid fish, where atrophy of the testicular tissue is accompanied by hypertrophy of the ovarian tissue.

Figure 3.1


Ovary
Testis
Immature Juvenile
Active Male
Transitional
Active Female

terminology and in some cases are based on superficial observation. Considering a number of sparid reproductive studies have conducted only macroscopic investigation of ovotestis structure, it may be possible that some of these studies incorrectly interpreted ovotestis structure resulting in conflicting reports of the sexual strategies of a number of sparids (see Table 1.1). In light of this, the suggestion by Sadovy & Shapiro (1987) that macroscopic gonad evaluations should be accompanied by detailed histological investigation before the reproductive strategy of a species is defined, should be heeded.

Cody & Bortone (1992) state that sex change in fishes is most likely to occur in the months immediately following spawning when the gonad is undergoing the greatest amount of structural and physiological change. Further, Sadovy & Shapiro (1987) suggest sex change is usually completed within a period of 9 to 180 days after the completion of spawning. Therefore, it is expected species with a defined spawning period should display an annual pattern of transitional gonad presence and absence (Shapiro *et al.*, 1993). Numerous studies of sparid reproduction have reported the period immediately post-spawning is the time when sex change is undertaken (e.g. Waltz *et al.*, 1982; Kadmon *et al.*, 1985; Pollock, 1985; Micale & Perdichizzi, 1994; Buslé-Sicard & Fourcault, 1997).

The primary aim of the present study is to investigate the structure of the ovotestis of *A. berda* throughout a complete annual reproductive cycle. If protandrous sex change does occur, ovotestis structure(s) representing the transitional stage(s) of a sex change process may be identified. When investigating the presence of transitional individuals of *A. berda*, particular attention will be focused on post-spawning samples.

As *A. berda* reproduces between June and September (Tobin *et al.*, 1997), if protandrous sex change does occur, it may be likely that the male to female transition is undertaken during the post-spawning period (October onwards) and may be detected. Further, if *A. berda* is a protandrous sex changer, it may be possible that an ovotestis of transitional structure similar to that described for the protandrous sparids *Diplodus* *sargus* (Micale & Perdichizzi, 1994) and *Sparus aurata* (Brusle-Sicard & Fourcault, 1997) may be observed and described. Both Micale & Perdichizzi (1994) and Brusle-Sicard & Fourcault (1997) describe the transitional ovotestis as characterised by atrophy of the testis (lack of spermatogenic activity), accompanied by the presence of yellow(ish) pigment, and proliferation of connective cells and fibres; the ovarian tissue also begins to develop.

The secondary aim of this study, is to address the validity of describing transitional ovotestis structure(s) by macroscopic observations alone (as many previous sparid studies have done [eg: Penrith, 1972; Robinson, 1976; Joubert, 1981; Buxton & Clarke, 1989; Pajuelo & Lorenzo, 1996]). This study will compare the detail of ovotestis structure available from macroscopic and microscopic examination.

Finally, this study will also address the effect of sectioning location on the interpretation of ovotestis structure. Although some sparid studies have described histological techniques where transverse sections are taken along the length of the ovotestis (eg: Waltz *et al.*, 1982; Buxton & Clarke, 1991; Buxton & Clarke 1992; Cody & Bortone, 1992; Bennett, 1993), no study has discussed the effect sectioning location has on the structural interpretation of the ovotestis.

3.2 MATERIALS AND METHODS.

3.2.1 SAMPLE COLLECTION

Fish for this study (n = 722) were collected in monthly samples from Deluge Inlet (see Fig. 2.1) between the period of June 1994 to June 1995. Immediately following capture, fish were euthanased and held on ice until dissection. All gonads were dissected within 12 hours of capture. Gonads were dissected from 502 fish collected during the spawning period (June - September), 126 fish collected during the 3 month post-spawning period (October - December), and 94 fish collected from the January - May reproductively inactive period.

3.2.2. MACROSCOPIC DESCRIPTION

Following dissection, each gonad was sexed macroscopically and classified into one of five ovotestis types - active male, active female, inactive male, inactive female or possible transitional. Though transitional ovotestis structures have been described macroscopically for a number of sex changing sparids (Robinson, 1976; Hecht & Baird, 1977; Buxton & Clarke, 1989; Pajuelo & Lorenzo, 1996), the author included the tentative "possible transitional" ovotestis category in order to avoid the potential for superficial observation that is suggested to occur by Buxton & Garratt (1990).

3.2.3. HISTOLOGY AND MICROSCOPIC DESCRIPTION

Following macroscopic investigation, a subsample of the spawning gonads (n = 30), together with all post-spawning samples (n = 126), and a subsample of the gonads collected during the inactive-period (n = 23) were preserved in FAAC fixative [formaldehyde 4%, acetic acid 5%, calcium chloride 1.3%; (McCormick and Molony, 1992)]. Transverse sections (at a thickness of 6µm) were taken at four locations approximately equidistant along the length of each gonad. Sections were stained with haemotoxylin and eosin before the sex and stage of each gonad was determined by microscopic examination.

The stage of development of the ovarian lobes were assigned according to the scheme described by West (1990):

Stage 1 - Chromatin nucleolar stage,
Stage 2 - Perinucleolar stage,
Stage 3 - Yolk vesicle formation,
Stage 4 - Vitellogenic stage,
Stage 5 - Ripe.

The germ cells of the testes were staged by modified criteria used by Gwo and Gwo (1993) for the congeneric *Acanthopagrus schlegeli*. The stages were: Spermatogonia, spermatocytes, spermatids and spermatozoa. After microscopic observation, each gonad was classified into one of six ovotestis types - active male, inactive male, active female, inactive female, transitional and indeterminate.

3.2.4 MACROSCOPIC 'VS' MICROSCOPIC

In order to evaluate the respective strengths and weaknesses of macroscopic versus microscopic examination, the proportions of each ovotestis type identified by macroscopic and microscopic examination were compared graphically.

3.3 RESULTS.

3.3.1 MACROSCOPIC GONAD DESCRIPTION

The structure of *A. berda* gonads have the typical arrangement of an sparid ovotestis. In all specimens dissected during the spawning period (149 males and 353 females) the demarcation of the male and female lobes was clearly discernible. During the spawning period, male and female ovotestes of *A. berda* showed strong structural dimorphism and no "possible transitional" type ovotestes were sampled.

Active Males

Semen ran freely from all specimens upon the application of slight abdominal pressure (Fig. 3.2). The ovotestes of active males were dominated by the large ivory-white testicular lobes that dominated the inactive ovarian lobes, which lay within grooves in the dorso-medial surface of the testes (Fig. 3.3A). In very ripe specimens the ovarian lobes were almost completely encircled by the extensively expanded testicular tissue.

Inactive Males

The ovotestes of inactive males had a thin, strap-like appearance (Fig. 3.3B). The volume and size of the ovotestis was substantially reduced from spawning condition size, representing approximately 1/10th of the volume of mature male ovotestes. The testis lobe was usually dominant, though the testis and ovarian lobes were occasionally of similar size. The ovarian lobe still occupied a groove on the dorsal surface of the testicular lobe.

Active Females

Active female gonads were usually characterised by pink to orange colouration and a cylindrical shape (Fig. 3.4A). Ova were visible macroscopically in most specimens. The reduced and inactive testicular lobes lay ventro-laterally to the turgid ovaries, appearing as thin bands of tissue that extended less than two-thirds along the posterior portions of the ovaries. Identifying the testicular tissue was difficult on the distended ovarian lobes of ripe females.

Inactive Females

All females sampled in October (first month post-spawning period) were spent. A small number of spent females were also encountered late during the spawning period (late - September). The ovotestes of the spent and inactive females were still dominated (volumetrically) by the ovarian lobes, however the ovarian lobes were flaccid (Fig. 3.4B). The testicular lobes of these specimens again appeared as thin bands of tissue along the ventrolateral surface of the ovarian lobes.

Possible Transitional Gonads

A substantial proportion of the ovotestes sampled during the post-spawning months (October to December) were difficult to classify as either male or female. The recent male function of these ovotestes was evident by the usually well developed though regressing testis lobe (Fig. 3.5). However, from macroscopic observation only it was impossible to classify any of these ovotestis structures as definite sex changers (transitional individuals). Some of these

Figure 3.2 : Semen running freely from a male fish captured during the spawning season. The fish is being tagged and released as part of a separate study (see Chapter 4).

Figure 3.2

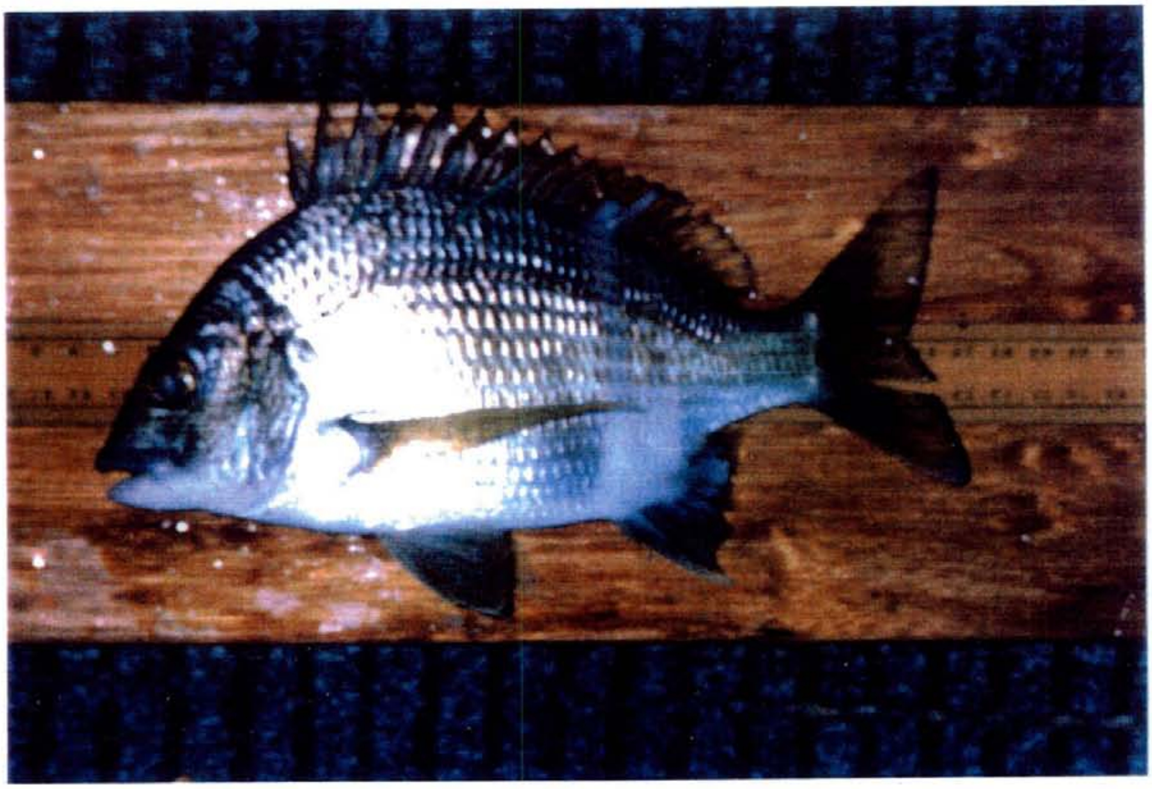


Figure 3.3 : Macroscopic representation of the active and inactive male ovotestes of *A. berda* - **A.** The medial surface of the right-hand lobe of an active male ovotestis. The testis lobe (TS) is dominant with the dormant ovarian lobe (OV) lying within a groove on the dorsal surface of the testis. **B.** The medial surface of an inactive male ovotestis. The testis (TS) and ovarian (OV) lobes of the ovotestis are of similar size. Scale bar = 5 mm.

Figure 3.3

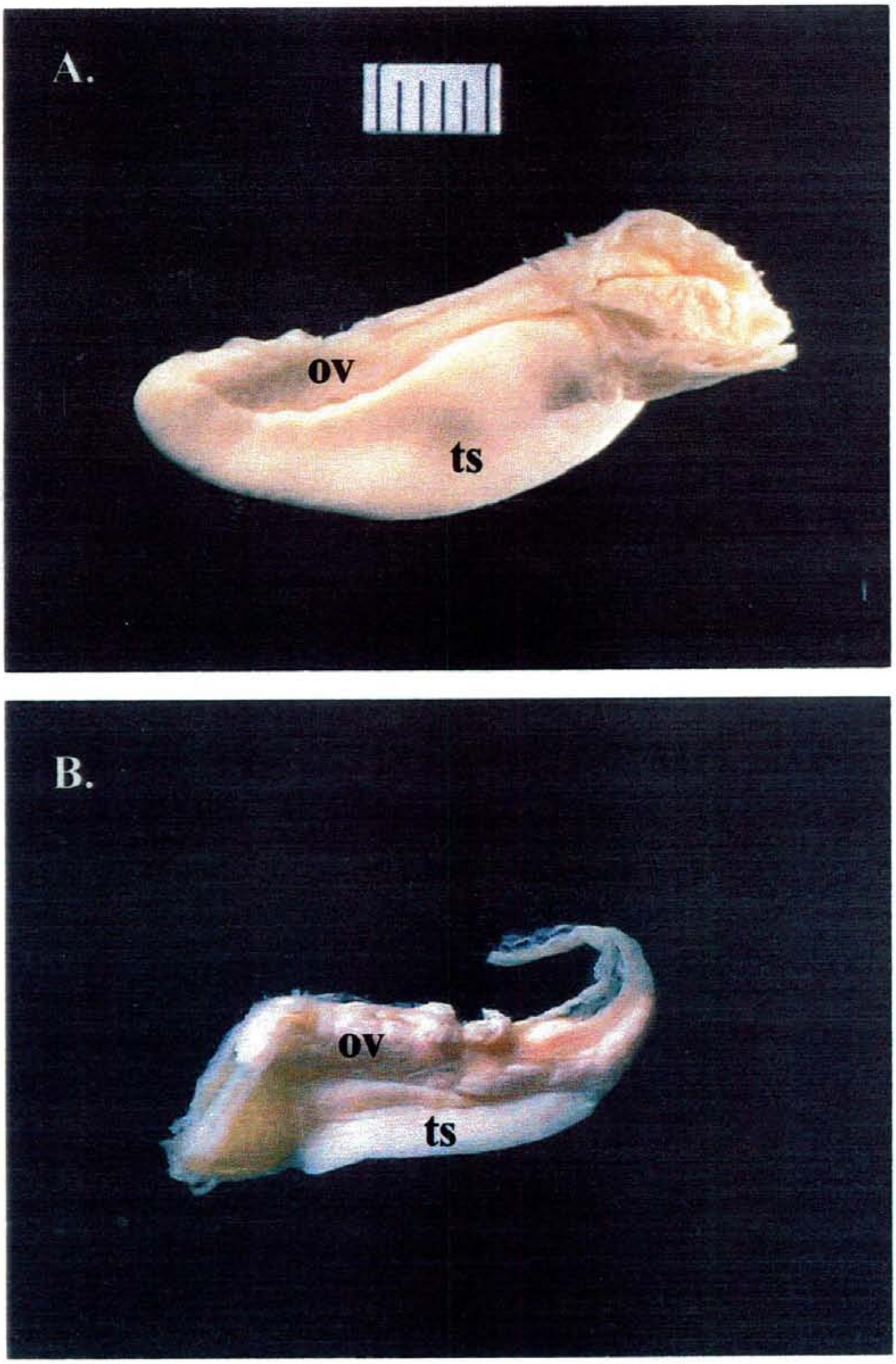


Figure 3.4 : Macroscopic representation of the active and inactive female ovotestes of *A. berda* - **A.** The ventral surface of the left-hand lobe of an active female ovotestis. The large cylindrical ovarian lobe (OV) is dominant with the inactive testis lobe (TS) present as a thin band of tissue on the ventro-lateral surface. **B.** The ventral surface of the inactive female ovotestis showing the flaccid ovarian lobe (OV) and thin band of testis tissue (TS) on the ventro-lateral surface. Scale bar = 5 mm.

Figure 3.4

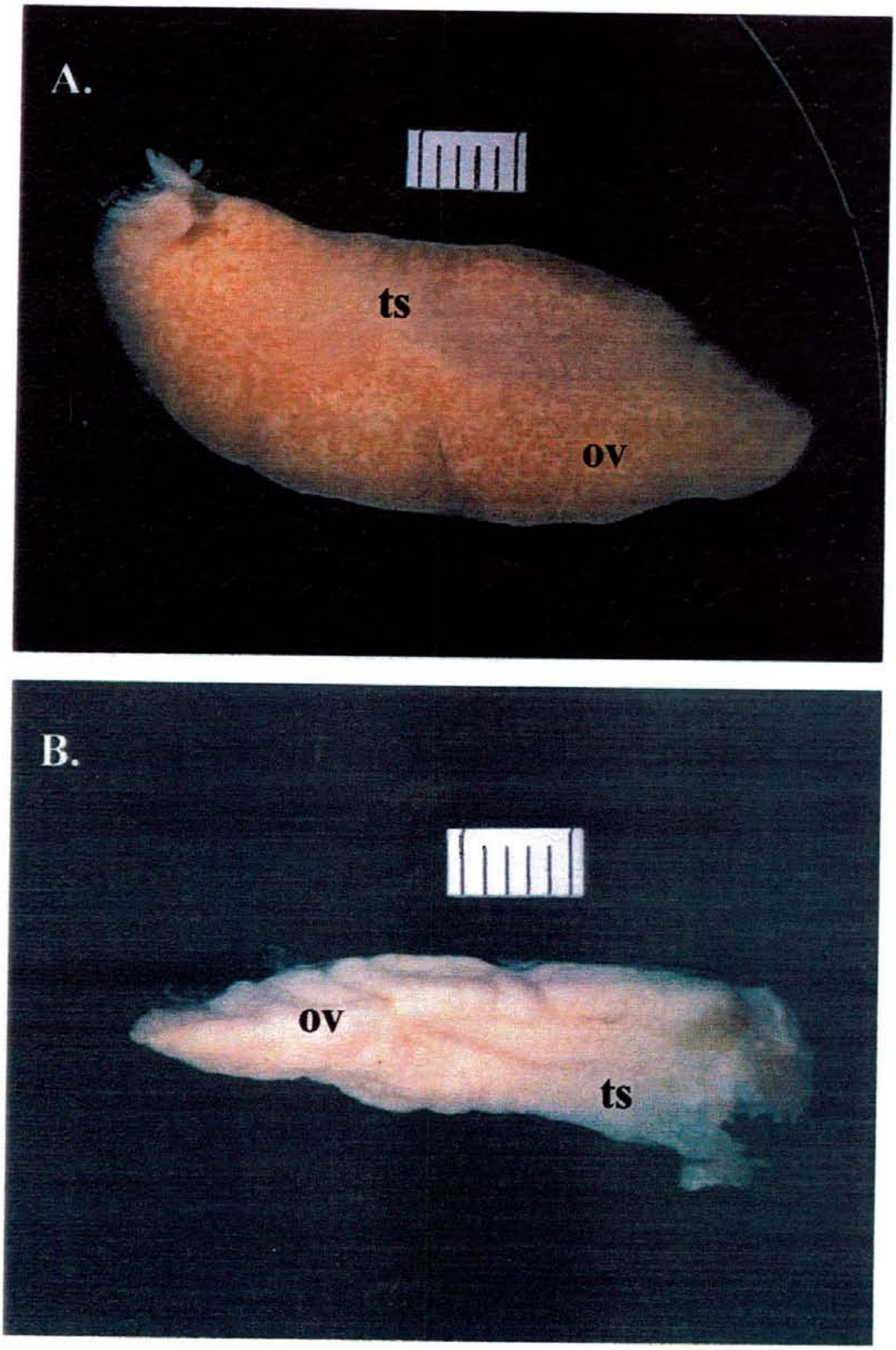
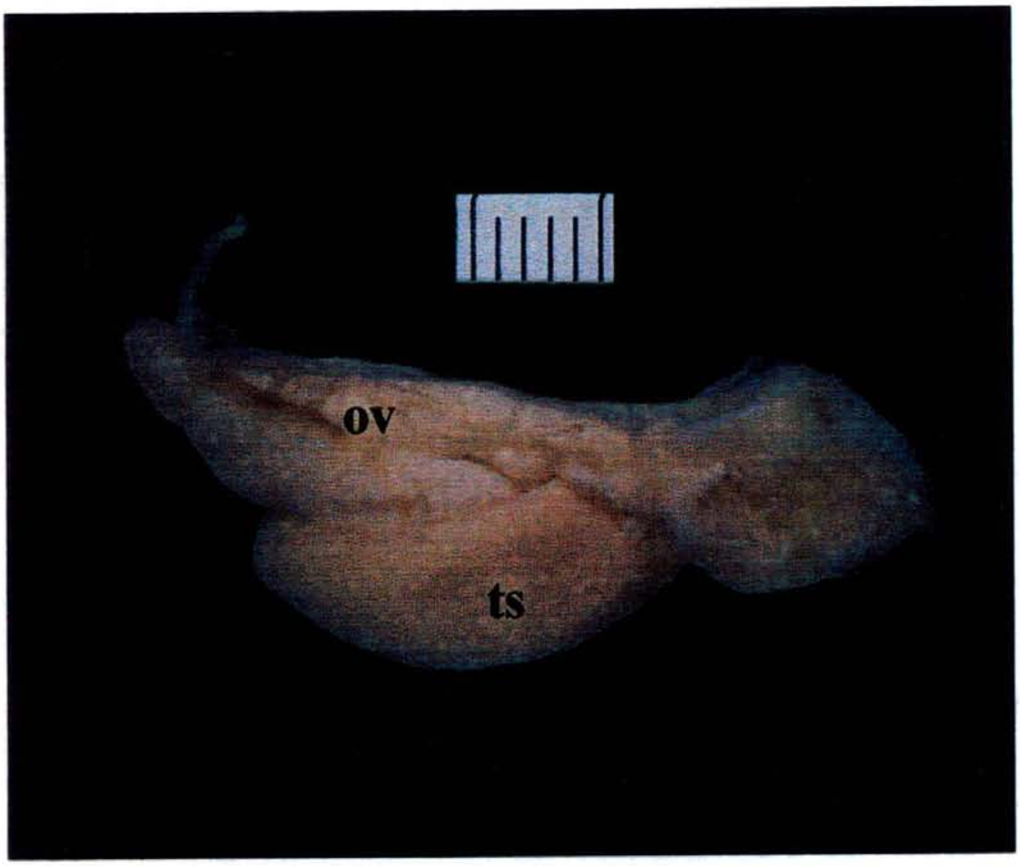


Figure 3.5 : Macroscopic representation of the medial surface of a possible transitional ovotestis. Regression of the testis lobe (TS) along the dormant ovarian lobe (OV) is evident. Scale bar = 5 mm.

Figure 3.5



gonads may have been undergoing sex change, however a substantial proportion may have simply been undertaking natural testicular regression following the recent spawning period.

3.3.2 TEMPORAL PATTERN OF MACROSCOPIC GONAD TYPES

Active male and female ovotestes were observed during the June - September spawning period only. Inactive (recently spawned) females were observed mostly during the non-spawning period (October - May), though some spent females were present during the latter stages of the spawning period (late September). Though some slight regression in testis size was observed in some males during late September, inactive males were only sampled during the October - May period. Possible transitional gonads were observed only during the 3 months post-spawning (October - December) (Table 3.1).

Table 3.1 : The numbers of active and inactive male, active and inactive female, and possible transitional ovotestes of *A. berda* sampled throughout a complete annual reproductive cycle, as determined by macroscopic investigation of ovotestis structure.

Time	**Males**		**Females**		**Possible**
	Active	**Inactive**	**Active**	**Inactive**	**Transitional**
June - September *(Spawning period)*	149 (29.7%)	0	340 (67.7%)	13 (2.6%)	0
October	0	14 (34.1%)	0	19 (46.3%)	*8 (19.6%)*
November	0	17 (37.0%)	0	16 (34.8%)	*13 (28.2%)*
December	0	11 (28.2%)	0	19 (48.7%)	*9 (23.1%)*
January - May *(Resting period)*	0	57 (60.6%)	0	37 (39.4%)	0

3.3.3 MICROSCOPIC GONAD DESCRIPTION

All fish examined had the hermaphroditic ovotestis structure characteristic of sparid fish. Testicular and ovarian tissues were present in all ovotestes, with the heterosexual tissues clearly separated by a layer of collagenous connective tissue. Six types of ovotestis structure were observed and described. The relative proportions of testicular and ovarian tissue varied considerably between the different ovotestis types.

Active Males.

The testes of active male *A. berda* were of the typical unrestricted or lobular type (Grier, 1981) in which spermatogonial cells are distributed along the length of the tubule in crypts (Fig. 3.6A). Each crypt contained germ cells of a single developmental stage. Spermatogonia dominated crypts towards the periphery of the testis, with the more advanced gametogenic stages (spermatocytes, spermatids and spermatozoa) dominating the interior lumen. Spermatogonia were uncommon in the ovotestes of males of more advanced spermatogenesis. Crypts of spermatocytes, spermatids and spermatozoa dominated available tubule space.

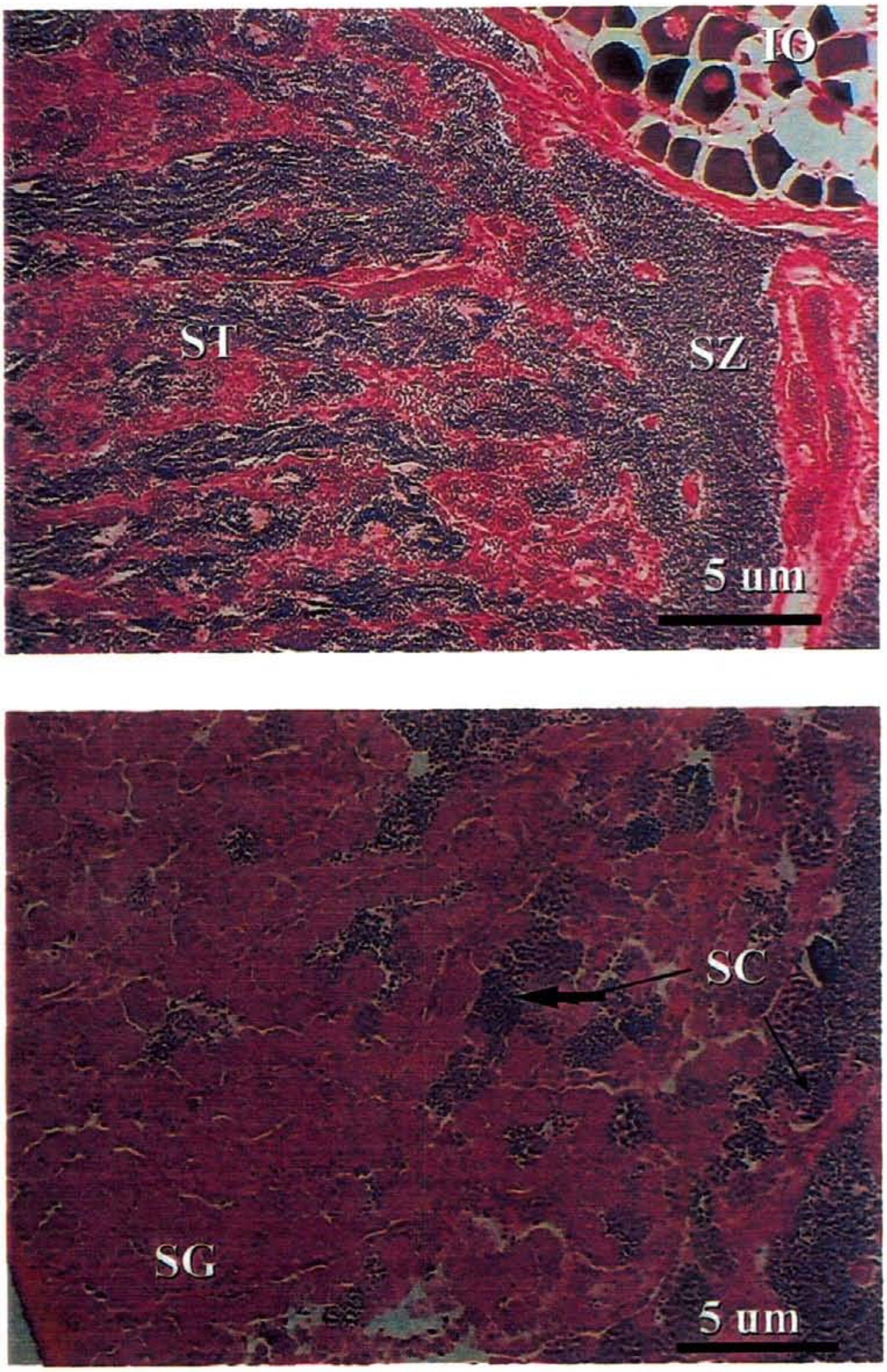
The small and dormant ovarian lobes were characterised by oocytes in chromatin nucleolar and perinucleolar stages of development. No active oogenesis was observed.

Inactive Males.

Transverse sections of inactive male ovotestes showed that the ovarian lobe was mostly larger than the testicular lobe (Fig. 3.6B). However, ovarian development appeared arrested at the chromatin nucleolar and perinucleolar stages. The testis is characterised by spermatogonia, and some occasional spermatogenic activity.

Figure 3.6 : Microscopic representation of the active and inactive male ovotestes of *A. berda* - **A.** Transverse section taken through an active male ovotestis. Within the testis lobe crypts of spermatids (ST) dominant with spermatozoa (SZ) abundant in the inner tubules - the dormant ovarian lobe is characterised by inactive oocytes (IO) in the chromatin nucleolar and perinucleolar stages. **B.** Transverse section taken through an inactive male ovotestis. The testis is dominated by spermatogonia (SG) though some spermatocytes (SC) are present.

Figure 3.6



Active Females.

In the ovotestes of female fish the ovarian lobes were volumetrically dominant. Ovarian lamellae were numerous and well developed, filling all available space within the ovarian cavity. In the ovaries of females at more advanced stages of oogenesis, the ovarian lamellae became indistinguishable. All oocytes in these specimens were generally in advanced stages of development (i.e. active vitellogenesis), with some previtellogenic oocytes present (Fig. 3.7A).

The remnants of the testis lobes appeared as thin bands of tissue lying on the ventro-lateral surface of the ovaries and no spermatogenic cells (spermatogonia, spermatocytes, spermatids or spermatozoa) were observed. The testes represented only a very small proportion of the volume of the ovotestes.

Inactive Females.

The ovotestis structure of inactive females was almost identical to that of active females. However, the numerous and well developed ovarian lamellae of inactive females only contained chromatin nucleolar and perinucleolar stage oocytes (Fig 3.7B). Oocytes at more mature stages of development were observed only in female fish sampled during the spawning period.

Remnants of the testis lobe were still observable on the ventro-lateral surface and again the testes represented only a very small proportion of the volume of the ovotestes.

Transitional ovotestes.

Ovotestes classified as transitional gonads were characterised by testicular and ovarian lobes of similar size, though sometimes the testicular lobe was considerably reduced in size and proportionally very much smaller than the accompanying ovarian lobe. Signs of degeneration in the testicular lobe included increased vascularisation, the proliferation of connective tissue and empty sperm ducts (Fig 3.8A). Isolated crypts of spermatocytes were occasionally present.

No obvious development was observed in the ovarian lobes which were characterised by inactive oocytes of the chromatin nucleolar and perinucleolar stages only (Fig. 3.8B).

Indeterminate ovotestes.

Ovotestes classed as being of indeterminate active sex were similar to the transitional type ovotestes, but lacked any evidence of testicular degeneration. Indeterminate ovotestes were characterised by an apparent functional testicular lobe containing most stages of spermatogenic cells, from spermatogonia to spermatozoa. No obvious testicular degeneration was evident, and in some specimens spermatozoa were still observed in the duct system (Fig 3.9A). Similarly to the transitional ovotestes, the ovarian lobe was characterised by inactive oocytes of the chromatin nucleolar and perinucleolar stages only (Fig 3.9B).

3.3.5 TEMPORAL PATTERN OF MICROSCOPIC GONAD TYPES

Active male and female ovotestes were observed only during the spawning period (June - September). Inactive males and females were observed throughout the remaining months (October - May) of the annual reproductive cycle (Table 3.2).

Indeterminate and transitional sex change ovotestes were observed only in the post-spawning months of October - December (Table 3.2). If protandrous sex change does occur, the transitional ovotestes represent an estimated sex change rate of 6.9%. The rate of sex change was derived by expressing the 5 identified transitional ovotestes as a porportion of the total number of ovotestes that may have undertaken sex change (that is, a summation of the numbers of transitional, indeterminant and male ovtestes identified).

Figure 3.7 : Microscopic representation of the active and inactive female ovotestes of *A. berda* - **A.** Transverse section taken through an active female ovotestis showing the dominance of oocytes in yolk vesicle (YV) and vitellogenic (VT) stages. The testis (TS) is present only as a thin band of tissue of the ventro-lateral surface of the ovary. **B**. Transverse section through inactive female ovotestis showing inactive oocytes (IO) of chromatin nucleolar and perinucleolar stages. The testis (TS) is dormant and present as a thin band of tissue on the ventrolateral surface of the ovary.

Figure 3.7

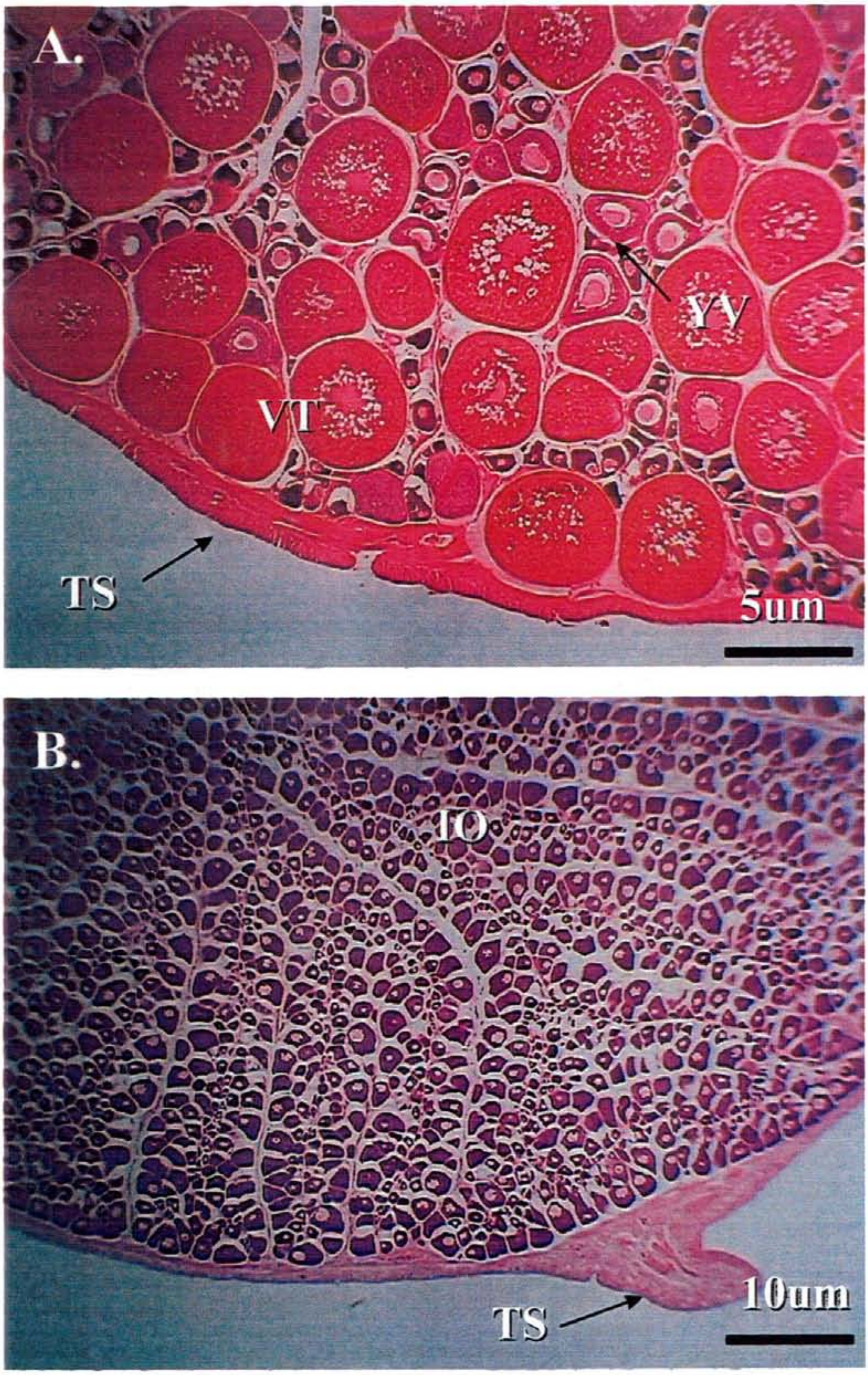


Figure 3.8 : A transverse section taken through the mid-region of a transitional ovotestis. **A**. The testicular lobe shows signs of degeneration characterised by increased vascularisation (VA), proliferation of connective tissue (CT) and empty sperm ducts (SD). Some occasional crypts of spermatocytes (SC) are observable; **B.** The ovarian lobe is characterised by inactive oocytes (IO) of chromatin nucleolar and perinucleolar stages only.

Figure 3.8

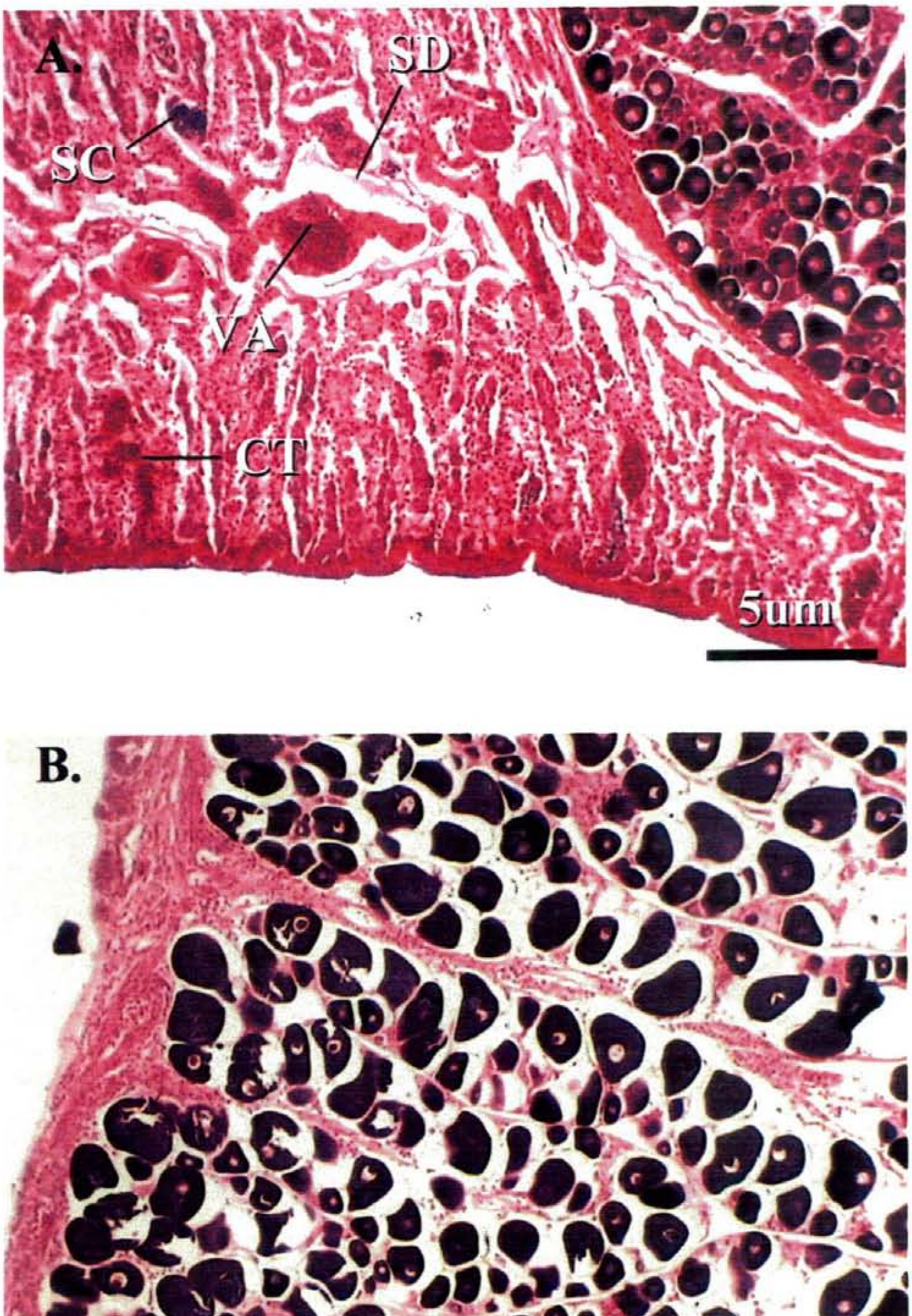


Figure 3.9 : Transverse section through an indeterminate type ovotestis. **A.** The testis lobe contains spermatocytes (SC) and spermatids (ST). Spermatozoa are occasionally observed in the sperm duct (SD). No sign of testicular degeneration is evident. **B.** The ovarian lobe is characterised by inactive oocytes (IO) of chromatin nucleolar and perinucleolar stages only.

Figure 3.9

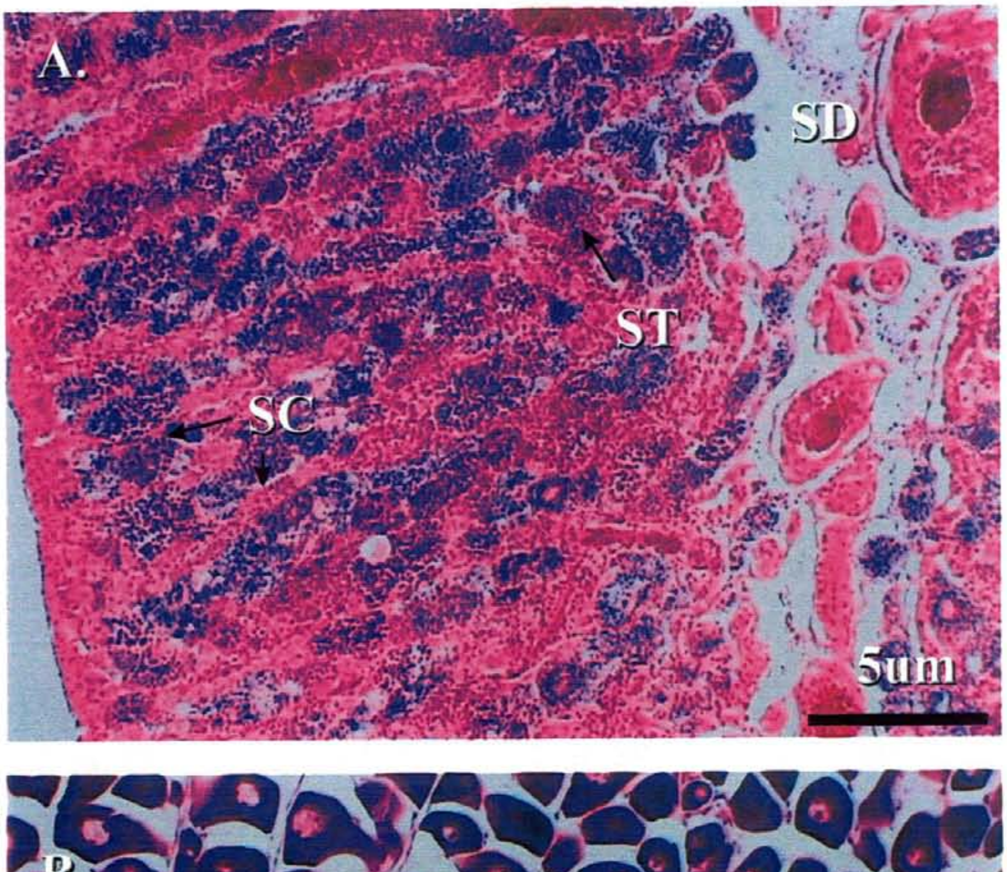


Table 3.2 : The numbers of male, female (both active and inactive), indeterminate (Ind.) and transitional ovotestes of *A. berda* sampled throughout a complete annual reproductive cycle, as determined by microscopic investigation of ovotestis structure.

	MALES		FEMALES			
Time	**Active**	**Inactive**	**Active**	**Inactive**	**Ind.**	**Transitional**
June - September (*Spawning period*)	18 (60%)	0	12 (40%)	0	0	0
October	0	8 (19.5%)	0	19 (46.3%)	12 (29.3%)	2 (4.9%)
November	0	11 (23.9%)	0	16 (34.8%)	17 (37.0%)	2 (4.3%)
December	0	15 (38.5%)	0	19 (48.7%)	4 (10.3%)	1 (2.6%)
January - May (*Resting period*)	0	14 (61.0%)	0	9 (39.0%)	0	0

3.3.6 MACROSCOPIC 'VS' MICROSCOPIC EXAMINATION

For nine months of the annual reproductive cycle (January to September), macroscopic examination was sufficient for classifying *A. berda* ovotestes. During both the inactive or resting period (January to May) and the June to September spawning period, the ovotestes of male and female *A. berda* were sufficiently dimorphic that identification by macroscopic investigation was uncomplicated (Fig 3.10).

However, during the three month post-spawning period (October to December), classification of ovotestes by macroscopic examination became difficult. In many ovotestes, the testicular lobe was observed to be regressing posteriorly along the ovarian lobe. Whether these ovotestis were undertaking sex change or alternatively undertaking natural testicular regression following spawning was difficult to interpret by macroscopic examination alone. As such, these ovotestes were tentatively identified as possible transitional ovotestes. These potential transitional ovotestes were most frequently observed between October and December (Fig 3.10), and suggests sex change is most likely to occur immediately following spawning.

In comparison, microscopic examination was able to identify a transitional ovotestis structure with more confidence. Proportionally less ovotestes were identified as transitional by microscopic examination than macroscopically (Fig 3.10). Furthermore, an indeterminate ovotestis category was required for microscopic classification as in many cases it was often difficult to interpret whether ovotestes were undertaking natural male regression or were in the initial stages of sex change.

3.3.5 THE EFFECT OF SECTIONING POSITION ON STRUCTURAL INTERPRETATION

The location of sectioning position had a notable effect on the interpretation of ovotestis structure for some of the sampled fish. For both active and inactive males, sections along the length of the ovotestes did not vary in development or relative proportions of testicular and ovarian lobes. The ovotestes of active and inactive females were similarly unaffected by section location, although sometimes the testicular lobe was not visible in anterior sections.

Ovotestes categorised as either transitional or indeterminate were affected by section location. Sections taken from the posterior end of the ovotestis were dominated by the testicular lobe, with sections from the anterior end dominated by the ovarian lobe (Fig. 3.11). Sections taken from the mid-regions of the gonads showed similar proportions of testicular and ovarian tissue. Taking sections from the posterior or anterior regions only may have resulted in the incorrect classification of ovotestes as male or female respectively.

Figure 3.10 : A comparison of the ovotestis types identified by macroscopic and microscopic examination.

Figure 3.10

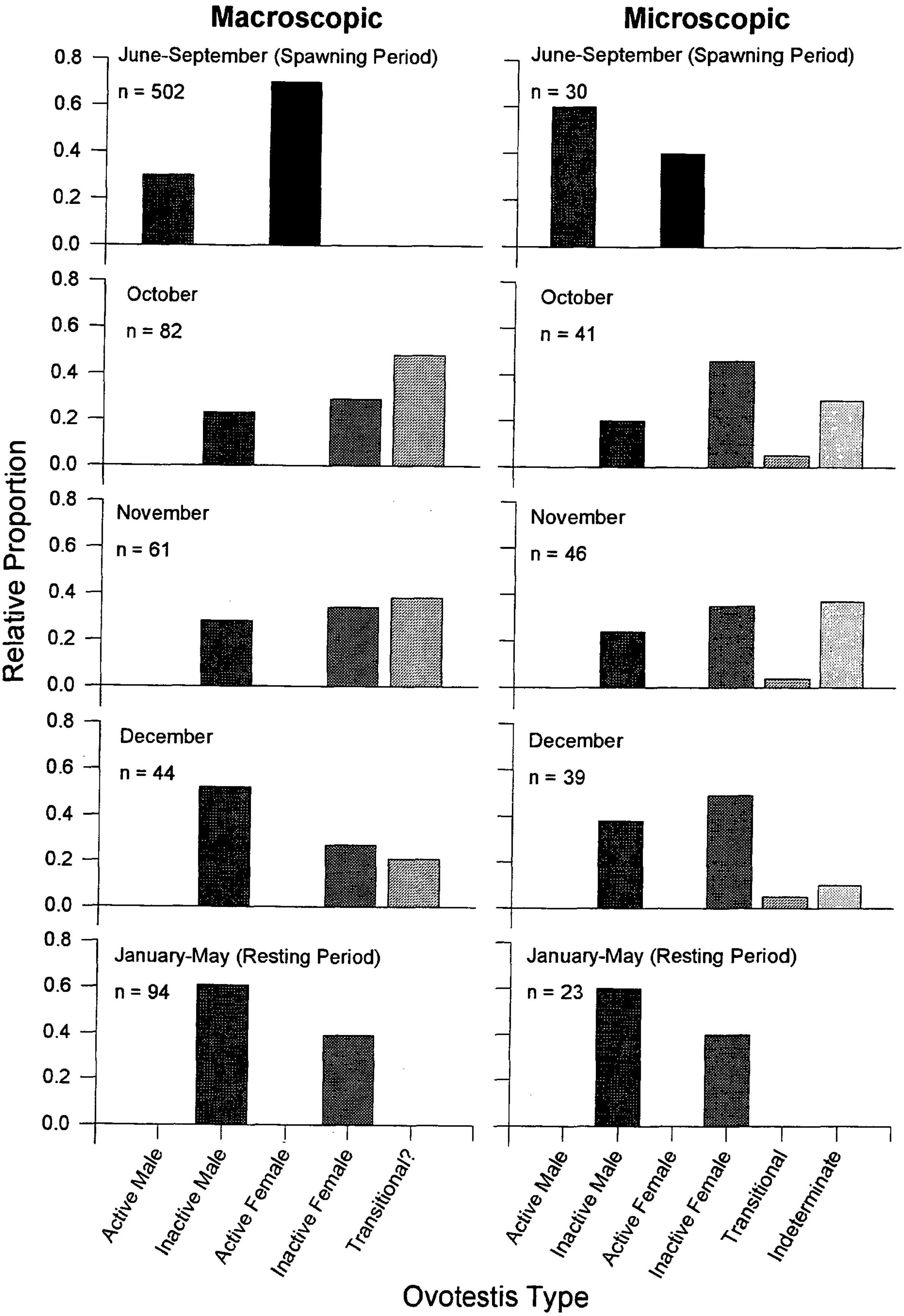
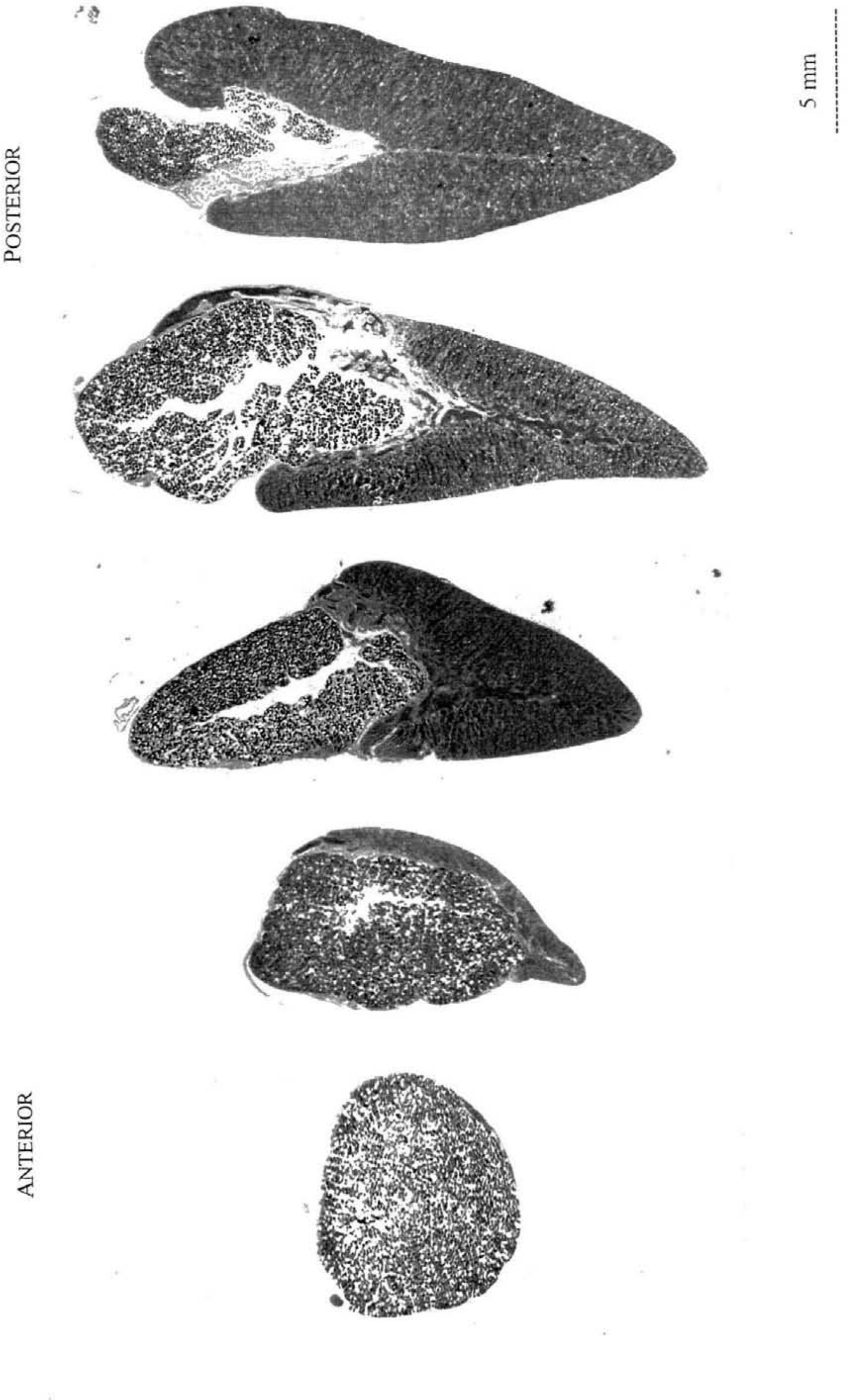

Macroscopic
Microscopic
June-September (Spawning Period)
n = 502
n = 30
October
n = 82
n = 41
November
n = 61
n = 46
December
n = 44
n = 39
January-May (Resting Period)
n = 94
n = 23
0.8
0.6
0.4
0.2
0.0
Relative Proportion
Active Male
Inactive Male
Active Female
Inactive Female
Transitional?
Transitional
Indeterminate
Ovotestis Type

Figure 3.11 : The effect of sectioning position on the interpretation of gonad structure. Sections taken from the anterior end of the ovotestis are dominated by the ovarian lobe (OV), while sections taken from the posterior end are dominated by the testis lobe (TS).

Figure 3.11



3.4 DISCUSSION.

The gonad of *A. berda* has the typical structure of the sparid ovotestis, with both testicular and ovarian tissue simultaneously present within the gonad separated by a layer of collagenous connective tissue. In all specimens observed in this study, either macroscopically or microscopically, the delimited nature of the male and female tissues was clear, during both the active (spawning) and inactive (resting) periods of the annual reproductive cycle.

Macroscopically, the ovotestes of active males were dominated by a large and mature testicular lobe but were also characterised by well developed, though inactive, ovarian lobes. The testicular lobe of the more mature males almost completely encircled the inactive ovarian lobe which lay in grooves on the dorsal surface of the testes. Conversely, small non-functional testicular lobes were observed with the large mature ovarian lobes of the female ovotestes. Ovotestes of similar structure have been described for a number of both protandrous (Abu-Hakima, 1984; Pollock, 1985; Yeung & Chan, 1987; Chang & Yueh, 1990), as well as protogynous sparids (Garratt, 1986; Buxton & Clarke, 1989; Buxton, 1990). The co-existence of the spatially separated male and female tissues in the ovotestes of many sparid fishes provides the structural basis for the occurrence of sex change (Yeung & Chan, 1987; Render & Wilson, 1992). Therefore, based upon macroscopic observation alone, the hermaphroditic structure of the *A. berda* gonad suggests the potential for sex change.

Difficulty in categorising some *A. berda* ovotestes macroscopically was experienced during the post-spawning period. During this period it was often difficult to determine whether a previously active male ovotestis was undergoing natural regression post-spawning, or alternatively undertaking sex change. As such, these ovotestes were classified as "possible transitional" ovotestes. Yeung & Chan (1987) and Brusle-Sicard & Fourcault (1997) reported similar problems with the macroscopic examination of the ovotestes of the protandrous sparids *Rhabdosargus sarba* and *Sparus aurata* respectively. In the present study, macroscopic investigation identified "possible transitional" gonads in which the testicular and ovarian lobes of the ovotestis were of similar size, with macroscopic appearance suggesting the testicular lobe was regressing posteriorly along the ovarian lobe. However, which sex would be active during the following reproductive season was impossible to predict.

In order to determine which sex would be active during the following spawning season, a histological investigation of the ovotestis is often suggested (Waltz *et al.*, 1982; Brusle-Sicard & Fourcault, 1997; Booth & Buxton, 1997). The detail available from histological examination may reveal a concomitant degeneration of the testicular lobe accompanied by development and maturation of the ovarian lobe, indicating a change in sexual function is occurring.

In the present study, transitional ovotestes were observed and described for *A. berda* by microscopic examination. The ovotestes categorised as transitional were characterised by degenerating testicular lobes and dormant ovarian lobes. The degenerating testicular lobes were classified on the basis of a reduction in proportional (relative to the ovarian lobe) size, proliferation of connective tissue and blood vessels, and a distinct lack of spermatogenic activity. None of the ovotestes classified as transitional (or indeed any of the ovotestes examined outside the June to September spawning period), showed signs of active oogenesis.

The transitional ovotestis structure described for *A. berda* by the current study is in contrast to other protandrous sparids. Micale & Perdichizzi (1994) describe the transitional ovotestis of the protandrous *Diplodus sargus* as characterised by "the presence of a degenerating testis, beside *advanced vitellogenic oocytes*". Similarly, Brusle-Sicard & Fourcault (1997) also stated oogenic activity as a characteristic of the transitional ovotestis of the protandrous *Sparus aurata*. Furthermore, Brusle-Sicard & Fourcault (1997) suggest the development of the ovary of *S. aurata* may be initiated progressively each year during the post-spawning period, until presumably one year sex change is finally completed.

In the present study, no oogenic activity was observed in the transitional ovotestes of *A. berda*. Only oocytes of the chromatin-nucleolar and perinucleolar stages of development were observed. More advanced stages of oogenic activity (i.e. yolk vesicle and vitellogenic stage oocytes) were not observed in any ovarian lobe until the commencement of the recognised spawning period (June). A similar pattern was describe for the protandrous sparids *A. australis* (Pollock, 1985) and *Rhabdosargus sarba* (Yeung & Chan, 1987).

If *A. berda* is in fact a protandrous hermaphrodite, the rate of sex change detected appears to be relatively low. In this study, the identified transitional ovotestes represented 6.9% (5 of 72) of those fish having the potential to sex change (i.e. fish classified as either male or indeterminate), and only 4.0% of the post-spawning sample (October to December, n = 126). Although the proportion of transitional ovotestes detected by this study may be considered low, some researchers have warned that transitional individuals may be poorly represented in field samples (Sadovy & Shapiro, 1987; Nakamura *et al.*, 1989). If sex change is a rapid process, transitional individuals may be present for only a relatively short period each year. If the protandrous sex change of *A. berda* is a relatively cryptic process (i.e. a rapid process involving the atrophy of the testicular lobe accompanied by hypertrophy of the ovarian lobe), it may be possible that some of those ovotestes classified as indeterminate sex were in fact in the process of sex change. Considering this, the true rate of sex change in the population sampled by this study may be substantially higher than the rate (6.9%) estimated.

In contrast, some sparid sex change studies have reported transitional individuals accounting for more substantial proportions of the samples collected. Waltz *et al.* (1982) recorded transitional *Calamus leucosteus* as occurring in 11.7% of August samples and 13.6% of September samples. Micale & Perdichizzi (1994) recorded transitional individuals of *Diplodus sargus* as representing 26.7% of collected samples, while Brusle-Sicard & Fourcault (1997) report 45% of 2 year old and 100% of 3 year old *Sparus* *aurata* show signs of sex change. However, some of the high proportions of transitional individuals reported by these studies may be artefacts of the superficial observation and poor definition of terminology referred to by Buxton & Garratt (1990).

Although many histological studies of sparids have stated gonad sectioning was conducted along the length of the ovotestis (e.g. Waltz *et al.*, 1982; Buxton & Clarke, 1991; Buxton & Clarke 1992; Cody & Bortone, 1992; Bennett, 1993), the effect of different sectioning positions on the interpretation of ovotestis sexual status has not been discussed for any species. In this study, the position of gonad sectioning was found to effect the interpretation of ovotestis structure for fish collected during the post-spawning period (October to December). Ovotestes categorised as indeterminate sex displayed dominance of the ovarian lobe at the anterior end of the ovotestis and dominance of the testicular lobe at the posterior end of the ovotestis; the mid section of the gonad usually had similar areas of testicular and ovarian tissue. Sectioning these gonads at the anterior end could result in the incorrect classification of these fish as functional females, while sections taken from the posterior end may indicate male function.

The knowledge that sectioning position can affect the interpretation of ovotestis structure may confound the interpretations of studies that have been less critical with their definitions of transitional ovotestis structure. Often the relative areas of the heterosexual tissues of the ovotestis have been used as indicators of sexuality and transitional individuals in sparid sexuality studies (Buslé-Sicard, 1997). If, as in *A. berda*, the relative size of the heterosexual lobes varies along the length of the ovotestis in other sparid species, the position at which sectioning is conducted is critical in the structural interpretation of the ovotestis. That is, in some studies transitional gonad structure may be incorrectly described and erroneously used as evidence of sex change. Further, biopsy experiments may be similarly effected. Yeung & Chan (1987), who biopsied portions of the gonads of *Rhabdosargus sarba* did not elaborate on which portion of the gonad was biopsied, or on the possible effect of position on the interpretation of gonad structure.

The heterosexual nature of the ovotestis identified in *A. berda* in this study is similar to the ovotestis structures described for a number of other protandrous sparids (Pollock, 1985; Yeung & Chan, 1987; Chang & Yueh, 1990), and suggests that protandrous sex change may occur in *A. berda*. Further, ovotestis structure that may represent individuals in transitional stages of protandrous sex change were recorded. Considering these observations with the bimodal length- and age-frequency distributions already presented (Chapter 2), protandrous sex change seems likely to occur in *A. berda*. However, though many sparid sexuality studies have reported sex change with less convincing evidence, the strongest evidence of sex change in sparid fishes requires individual fish to be observed functioning initially as one sex before functioning as the opposite sex later in life. Before stating protandry as the reproductive strategy of *A. berda*, individual sexuality will be monitored in order to gather evidence to support or refute the evidence presented in this chapter.

CHAPTER 4.

PROTANDROUS SEX CHANGE DETECTED IN INDIVIDUALLY MONITORED *ACANTHOPAGRUS BERDA*.

4.1 INTRODUCTION.

Definitive evidence of sex change requires producing transitional or sex changed individuals experimentally using non-hormonal techniques (Sadovy & Shapiro, 1987). Altering the social environment of the subject species (such as altering sex ratios, or removal of dominant group members) has been used successfully to demonstrate sex change in a number of fish species (Fishelson, 1970; Robertson, 1972; Shapiro & Lubbock, 1980; Fricke, 1983; Ross *et al.*, 1983; Shapiro, 1984; Sunobe & Nakazono, 1993). Even more substantial proof of sex change may come from the observation of sex change in individually identified fish in the field, without experimental manipulation of the social environment. Passive observation was utilised by Moore (1979) to show protandrous sex change in the centropomid, *Lates calcarifer*.

In order to confidently demonstrate sex change, an individual needs to be observed functioning initially as one sex before later in life functioning as the opposite sex (Tobin *et al.*, 1997). However, despite many reports of sex change in sparid fish, the direct detection of sex change has not yet been achieved for any sparid species. In a South African estuary, Garratt (1993) attempted to produce evidence of protandrous sex change in *A. berda* by a tag, release, recapture technique. Unfortunately, Garratt (1993) was unable to collect sufficient data to determine the reproductive strategy of *A. berda*.

The aim of the present study, was to determine if sex change does occur in *A. berda* in aquarium and field experiments by utilising techniques similar to those of Garratt (1993).

4.2 MATERIALS AND METHODS.

4.2.1. IN-FIELD SEXING TECHNIQUE AND VALIDATION

All fish used in this study were initially captured in the June to September spawning period of *A. berda* (Tobin *et al.*, 1997) during the years 1993, 1994 and 1995. The sex of all fish was determined immediately following capture by milking the abdominal regions in an anterior-posterior motion, a technique validated by Tobin *et al.* (1997). Fish that extruded semen from their urogenital pores were recorded as male (see Fig. 3.2), those that did not as probable females.

A sample of 502 *A. berda* collected from Deluge Inlet (see Fig. 2.1) during the 1994 spawning period was used to validate the in-field sex identification technique. Immediately following capture, the sex of each of these fish was determined by abdominal milking and recorded. Each fish was then sacrificed and their gonads dissected and examined macroscopically to determine the true sex of each fish.

4.2.2 AQUARIUM EXPERIMENTS

Two aquarium experiments were conducted in a large 150,000 litre aquarium. Samples of *A. berda* for both experiments were collected from Alligator Creek (see Fig. 2.1). The first experiment was initiated in August 1994. Three female and 18 male *A. berda* were sex identified (see above) and tagged, before being released into the aquarium. The experiment was terminated during February 1995 when all fish were sacrificed. The second experiment was initiated in July 1995 with 24 males and 2 females sex identified, tagged and released into the aquarium. The second experiment was terminated with the sacrifice of fish during June 1996. These time periods included at least one post-reproductive period; the period when sex change is likely to occur (Sadovy & Shapiro, 1987; Cody & Bortone, 1992). The fork lengths of all fish were recorded at initial capture and again at the termination of the experiments. Gonads (for histological examination) and otoliths (for ageing) were dissected from all fish at their time of sacrifice.

4.2.3 IN-FIELD EXPERIMENTS

Blacksoil Creek

During the 1993 spawning period 307 *A. berda* were captured, tagged and released in Blacksoil Creek (see Fig. 2.1). The sex of each fish was identified (see above). This procedure was repeated during the spawning period of 1994 for a further sample of 298 fish. The subsequent recapture of tagged fish during 1994 (n = 25), 1995 (n = 28) and 1996 (n = 15) allowed for the direct assessment of protandrous sex change in *A. berda*. At final recapture the sex of each sacrificed fish was determined macroscopically, and for those that had changed sex, checked by histological examination. Each sacrificed fish was aged by sectioning the sagittal otoliths, and age at initial capture determined by back-calculation.

Deluge Inlet

Fish of known sex were also tagged and released in Deluge Inlet (see Fig 2.1). Samples of 616 and 522 *A. berda* were sexed, tagged and released during the 1994 and 1995 spawning periods, respectively. The subsequent recapture of tagged fish during 1995 (n = 51), 1996 (n = 13) and 1997 (n = 4) again allowed for the direct assessment of protandrous sex change in *A. berda*. The sex of each fish at final recapture was again determined macroscopically, and for those that had changed sex, checked by histological examination. Again, each sacrificed fish was aged by sectioning the sagittal otoliths, and age at initial capture determined by back-calculation.

4.3 RESULTS.

4.3.1 VALIDATION OF IN-FIELD SEXING

Examination of the gonads of the 502 fish collected from Deluge Inlet showed that all 132 individuals classified as males were identified correctly (Table 4.1). However, the sex of some fish recorded as female were incorrectly identified (17 of 370). Macroscopic observation of the gonads of these fish showed them to be spent males. Thus, the majority of male fish (88.6%) could be confidently identified by the milking technique. The small error rate (4.6%) in the sex identification of female fish was unimportant as no female to male sex changes were detected. The overall accuracy of the in-field sexing was high (96.6%).

Table 4.1 : The accuracy of the in-field sexing technique (Deluge Inlet).

	Sex Determination		**Misidentified**	
	In-field Milking	Macroscopic Examination	Males as Females	Females as Males
Male	132	149	17 (11.4%)	
Female	370	353		17 (4.6%)

4.3.2. AQUARIA SEX CHANGE

Of the 21 fish (18 males and 3 females) introduced into the aquarium in July 1994, five male fish had changed sex and were female when retrieved from the tank in February 1995 (Table 4.2). All three females introduced into the tank in July 1994 remained female.

Table 4.2: Protandrous sex change detected in *A. berda* in the first aquarium experiment, July 1994 to February 1995.

	Males	**Females**	***Protandrous Sex Change***
Introduced July 1994	18	3	
Retrieved February 1995	13	8	5 (27.8%)

Histology conducted on the gonads of the five fish that had sex changed showed a structural similarity to the gonads of those fish that were introduced into the tank as female (Fig. 4.1); the ovarian lobe of the sex changed fish was the dominant lobe of the ovotestes. Oocytes within the ovary were in the resting or perinucleolar stage. The testis region of each gonad had reduced significantly in size and volume and no spermatogenic activity was apparent.

In the second experiment, 24 male and 2 female *A. berda* were introduced into the aquarium during July 1995. On retrieval from the aquarium during June 1996, sex change had occurred in 5 of the male fish (Table 4.3).

Table 4.3: Protandrous sex change detected in *A. berda* in the second aquarium experiment, July 1995 to June 1996.

	Males	**Females**	***Protandrous Sex Change***
Introduced July 1995	24	2	
Retrieved June 1996	19	7	5 (20.8%)

The gonads of the five female fish that had changed sex were in an advanced reproductive state. The gonads of all sex changed fish were dominated by oocytes at the yolk vesicle formation stage (Fig. 4.2). The testis lobes of each of these gonads were considerably reduced, present as thin bands of tissue on the ventro-lateral surface of the ovaries. No spermatogenic activity was present.

Figure 4.1 : Transverse sections through the ovotestis of a sex changed fish from the first aquarium experiment. The ovarian lobes (OV) of the sex changed fish were dominant and characterised by chromatin nucleolar and perinucleolar stage oocytes. The degenerated testis (TS) is present as a thin band of tissue on the ventro-lateral surface of the ovarian lobe.

Figure 4.1

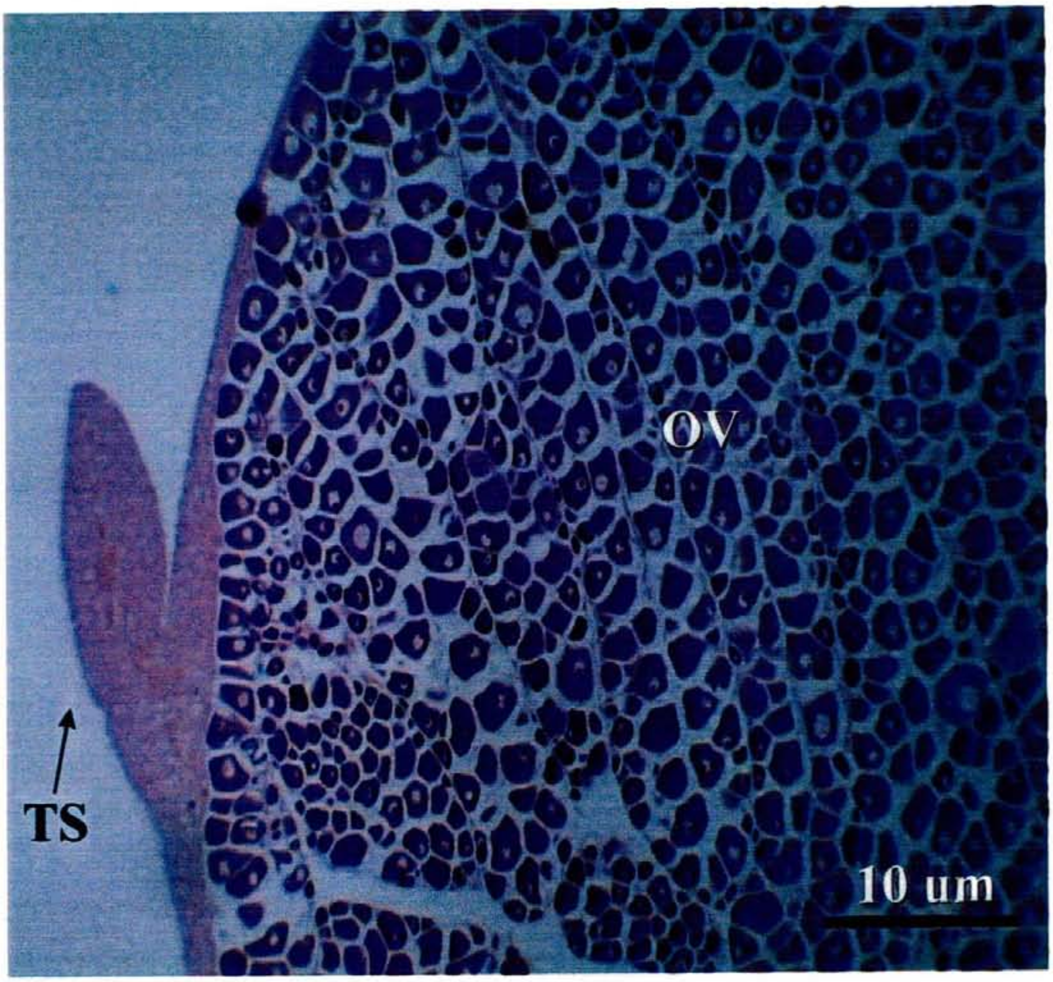
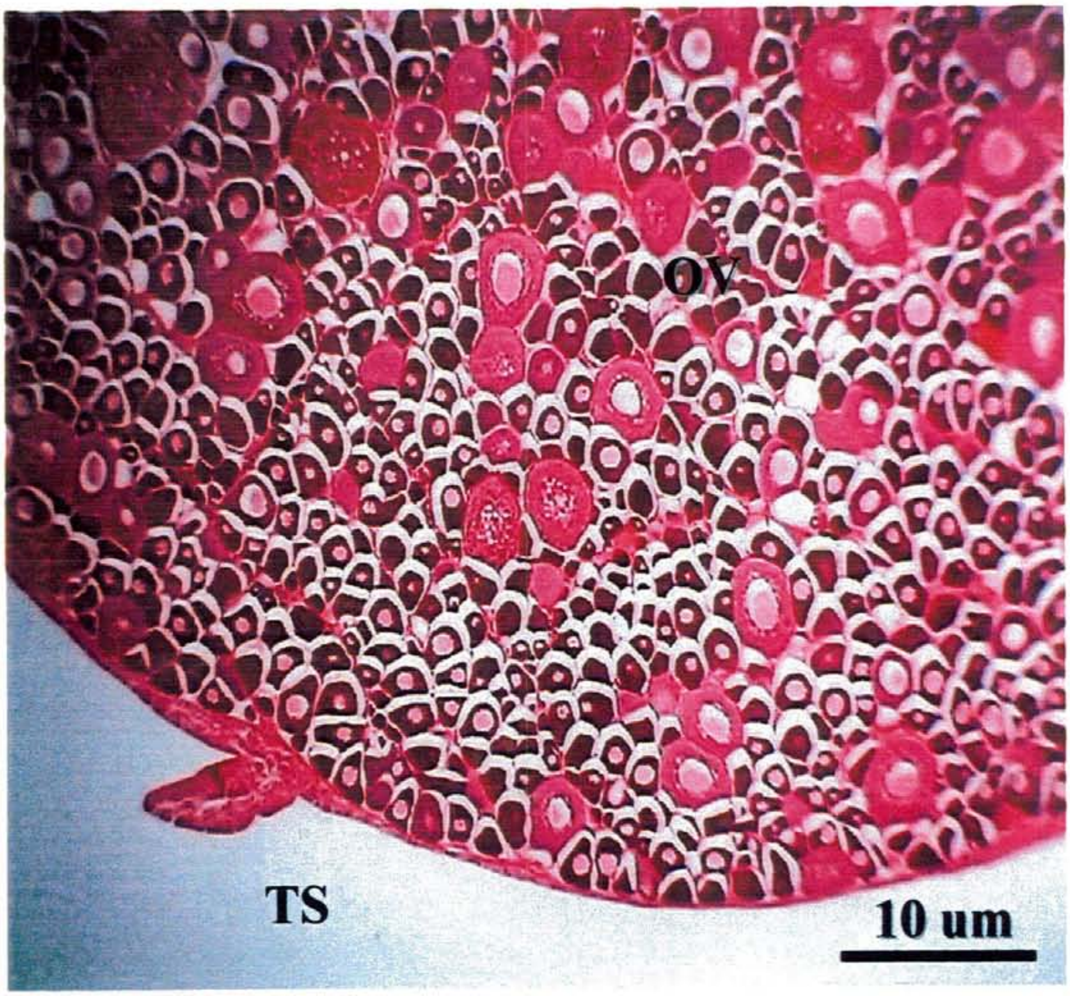


Figure 4.2 : All sex changed fish from the second aquarium experiment showed active development with the ovarian lobes (OV) characterised by oocytes in the yolk vesicle stage. The dormant testis (TS) was present as a thin band of tissue on the ventrolateral surface of the ovary.

Figure 4.2



4.3.3 In-field Sex Change

Blacksoil Creek

Of the 307 and 245 *A. berda* tagged and released in Blacksoil Creek during 1993 and 1994 respectively, a total of 68 fish were recaptured over the 1994 to 1996 period. Three of the 49 recaptured fish that were sexed as male when released, had undergone protandrous sex change and were females at their time of recapture (Table 4.4).

Table 4.4: Sex change detected in *A. berda* sampled from Blacksoil Creek.

	Males	Females	*Protandrous Sex Change*
Tagged 1993	157	150	
Tagged 1994	166	79	
Recaptured 1994	14	11	2 (12.5%)
Recaptured 1995	21	7	0 (0%)
Recaptured 1996	11	4	1 (8.3%)
	46	22	3 (6.1%)

Deluge Inlet

Sampling conducted at Deluge Inlet from 1995 to 1997, resulted in the recapture of 72 of the 616 and 522 fish of known sex were tagged and released during 1994 and 1995 respectively. Four of the fifty-one fish released as male, had undergone protandrous sex change and were females at their time of recapture (Table 4.5). Each fish had well developed female ovotestes (Fig 4.3).

Table 4.5: Sex change detected in *A. berda* sampled from Deluge Inlet.

	Males	Females	***Protandrous Sex Change***
Tagged 1994	330	286	
Tagged 1995	396	126	
Recaptured 1995	34	17	2 (5.6%)
Recaptured 1996	11	2	1 (8.3%)
Recaptured 1997	2	2	1 (33.3%)
	47	21	4 (7.8%)

4.3.4. Age and length at sex change

Protandrous sex change was detected in a total of 17 fish - 10 in aquarium studies and 7 from field studies. The length and age at the time of sex change varied considerably between fish (Table 4.6). The sex changes detected occurred across a length range of 177 - 273mm FL, and an age range of 3 - 9 years.

Figure 4.3 : The well developed female ovotestis dissected from a Deluge Inlet *A. berda* that had changed sex.

Figure 4.3

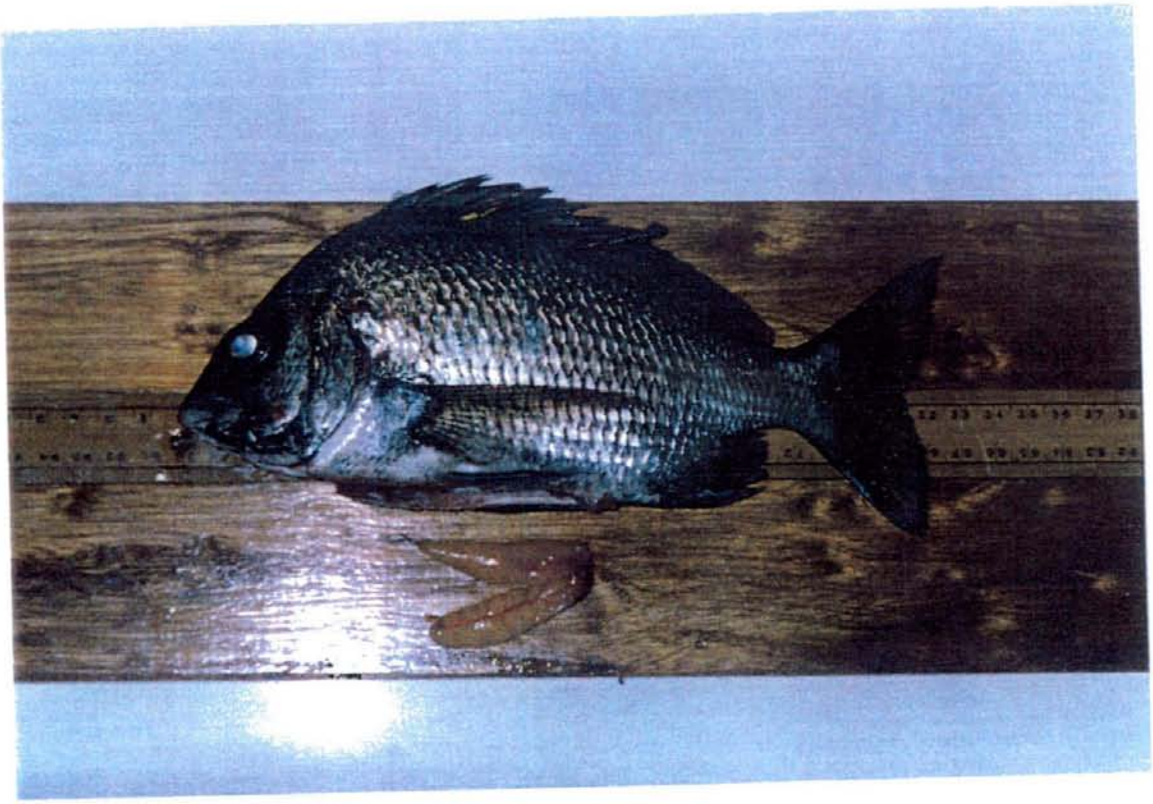


Table 4.6: Summary information of those fish detected to sex change during the current study, showing the variability of length and age at which sex change occurred (BS = Blacksoil Creek; DI = Deluge Inlet; AQ = Aquarium).

	RELEASE DATA				RECAPTURE DATA			
Fish ID.	Age	FL	Sex	Date	Age	FL	Sex	Date
BS - 5076	3	182	M	3-Sep-93	4	194	F	31-Jul-94
BS - 14664	4	191	M	16-Sep-93	5	216	F	13-Oct-94
BS - 5147	5	193	M	10-Sep-93	8	228	F	7-Sep-96
DI - 216	4	201	M	6-Jun-94	5	205	F	25-Jul-95
DI - 613	5	201	M	26-Jul-94	6	210	F	20-Aug-95
DI - 1151	6	202	M	1-Oct-94	8	216	F	12-Aug-96
DI - 1015	3	170	M	7-Sep-94	6	203	F	15-Jul-97
AQ - 10416	3	194	M	29-Jul-94	4	196	F	16-Feb-95
AQ - 10404	4	196	M	29-Jul-94	5	195	F	16-Feb-95
AQ - 3862	4	180	M	29-Jul-94	5	185	F	21-Feb-95
AQ - 3015	5	208	M	29-Jul-94	6	213	F	15-Feb-95
AQ - 3036	9	273	M	29-Jul-94	10	273	F	15-Feb-95
AQ - 913	3	190	M	17-Jul-95	4	205	F	6-Jun-96
AQ - 997	4	194	M	17-Jul-95	5	211	F	6-Jun-96
AQ - 909	5	177	M	17-Jul-95	6	200	F	6-Jun-96
AQ - 933	5	193	M	17-Jul-95	6	202	F	7-Jun-96
AQ - 907	6	211	M	17-Jul-95	7	239	F	6-Jun-96

No relationship between length of fish and occurrence of sex change was discernible. Those fish detected to change sex in the aquarium experiments did not show any tendency to be the largest individuals in their respective age-classes (Fig. 4.4). Fish that changed sex in field experiments at both Blacksoil Creek (Fig. 4.5) and Deluge Inlet (Fig 4.6) also showed a lack of a size or age pattern.

Figure 4.4 : Length increment data from the aquarium experiments - A. 1994-95, B. 1995-96. Dashed and solid lines represent fish that remained males and females respectively, throughout the experiments. Fish that were detected to change sex are indicated by dark lines and ▲ symbols.

Figure 4.4

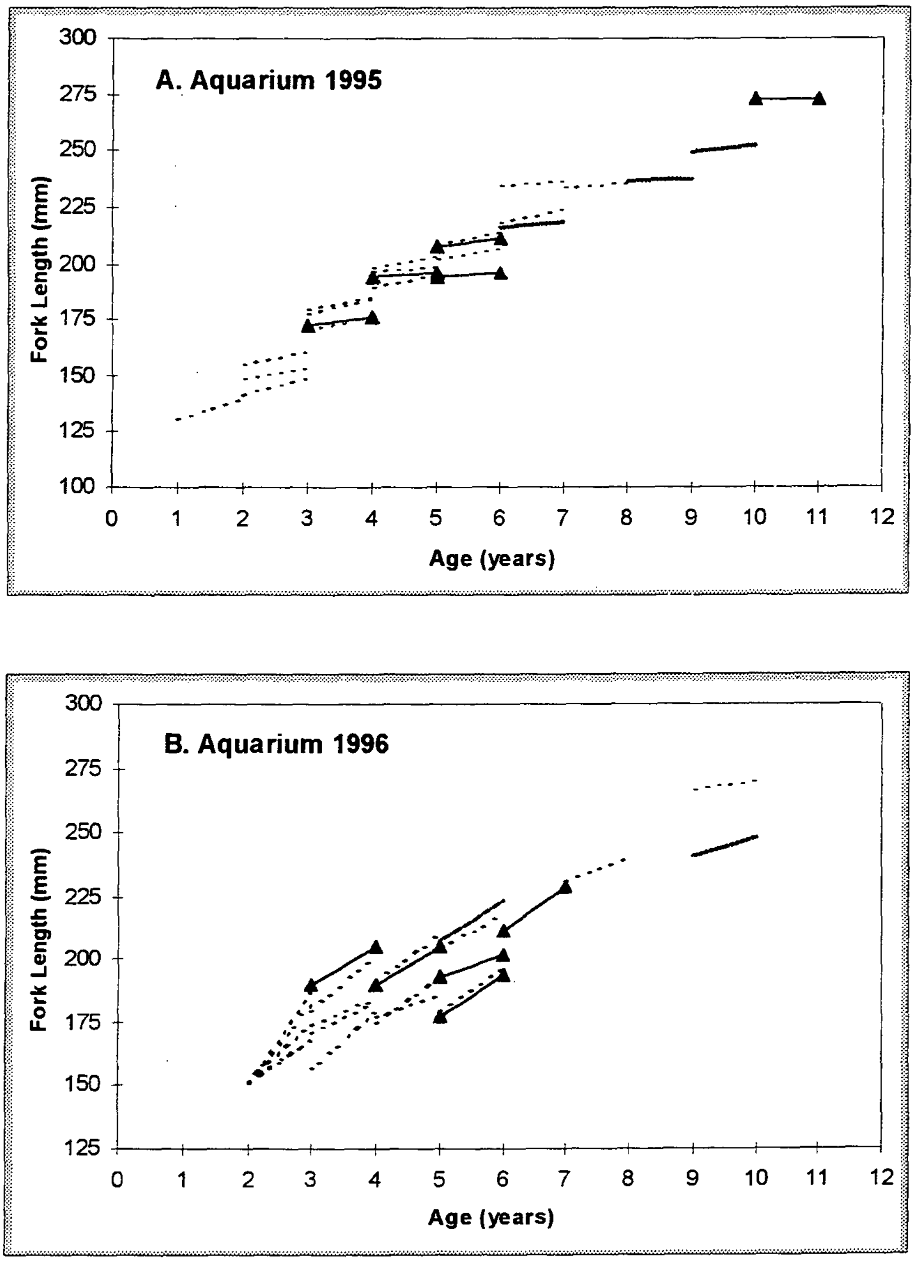

A. Aquarium 1995
Fork Length (mm)
300
275
250
225
200
175
150
125
100
0
1
2
3
4
5
6
7
8
9
10
11
12
Age (years)
B. Aquarium 1996
Fork Length (mm)
300
275
250
225
200
175
150
125
0
1
2
3
4
5
6
7
8
9
10
11
12
Age (years)

Figure 4.5 : Length increment data for fish recaptured from the Blacksoil Creek in-field experiment. The A and B graphs represent fish recaptured during 1994 and 1995 respectively. Dashed and solid lines represent fish that remained males and females respectively, throughout the experiments. Fish that were detected to change sex are indicated by dark lines and ▲ symbols.

Figure 4.5

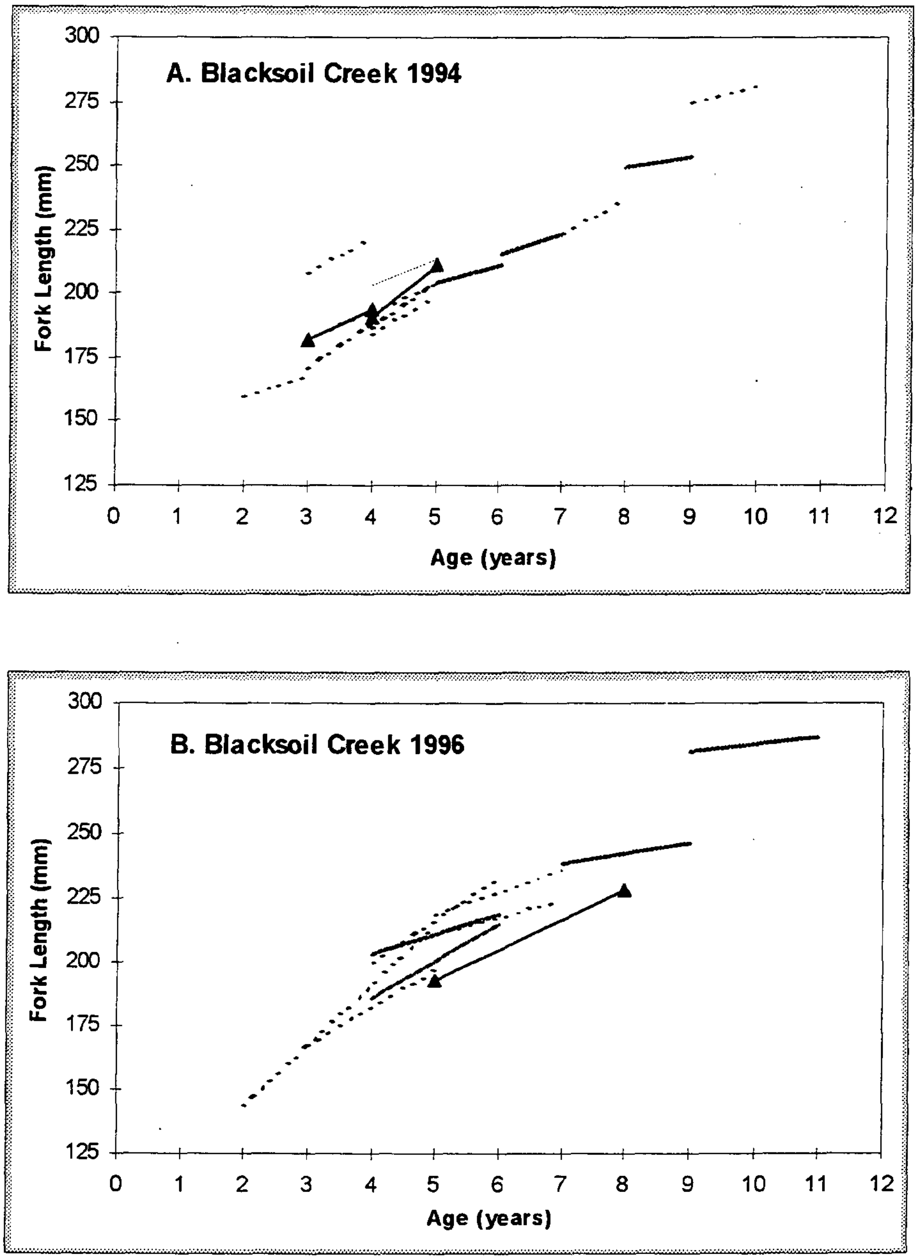
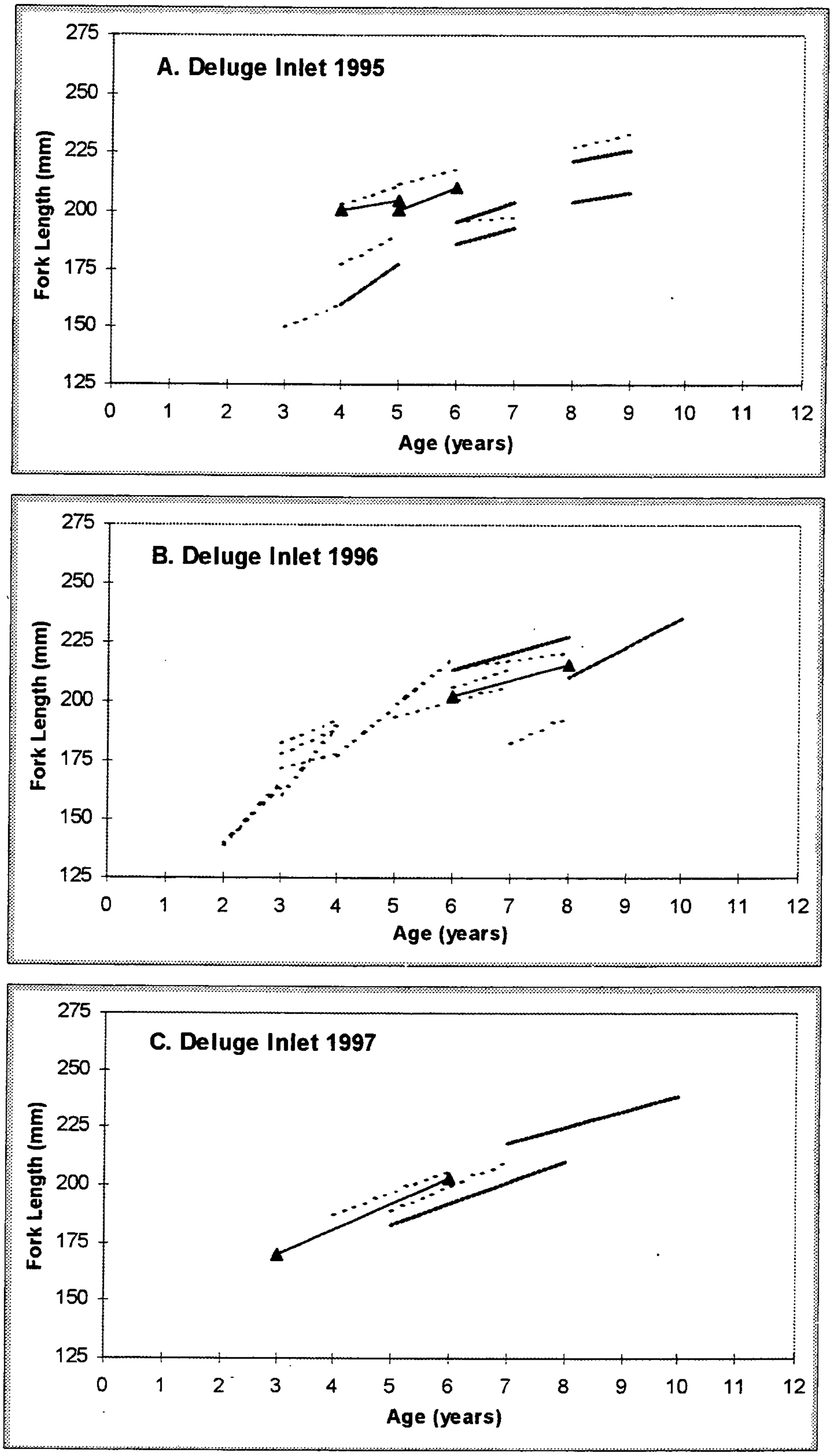

A. Blacksoil Creek 1994
Fork Length (mm)
300
275
250
225
200
175
150
125
0
1
2
3
4
5
6
7
8
9
10
11
12
Age (years)
B. Blacksoil Creek 1996
Fork Length (mm)
300
275
250
225
200
175
150
125
0
1
2
3
4
5
6
7
8
9
10
11
12
Age (years)

Figure 4.6 : Length increment data for fish recaptured from the Deluge Inlet in-field experiment. The A, B and C graphs represent fish recaptured during 1995, 1996 and 1997 respectively. Dashed and solid lines represent fish that remained males and females respectively, throughout the experiments. Fish that were detected to change sex are indicated by dark lines and ▲ symbols.

Figure 4.6


A. Deluge Inlet 1995
B. Deluge Inlet 1996
C. Deluge Inlet 1997
Fork Length (mm)
275
250
225
200
175
150
125
0
1
2
3
4
5
6
7
8
9
10
11
12
Age (years)

4.3.5 Rate of sex change

The rates of sex change detected in the two aquarium experiments were reasonably high. Sex change was detected in 27.8% of 18 (Table 4.2), and 20.8% of 24 male fish (Table 4.3) introduced into the aquarium for the 1994/95 and 1995/96 experiments respectively. Lower rates of sex change were detected in Blacksoil Creek and Deluge Inlet. Sex change was detected in 6.1% of 49 fish released as male that were recaptured from Blacksoil Creek (Table 4.4), and in 7.8% of 51 fish released as male that were recaptured from Deluge Inlet (Table 4.5).

4.4 DISCUSSION.

In this study, male to female sex change was detected in seven *A.berda* from field experiments and ten fish from aquaria experiments. At their initial capture, each of these fish was confirmed as male by validated observation - semen extruded from the urogenital pore of each fish upon abdominal milking. However, at subsequent recapture, each fish was found to possess an active female ovotestis. Though reports of sex change are common in the Sparidae (Buxton & Garratt, 1990), this type of direct assessment of individual sexual function has not been achieved for any other sparid.

The high rate of sex change in the aquarium experiments may be due to the controlled environment. It is possible that sex change events detected in the aquarium fish may have been initiated by the captive environment (Garratt, 1993). Nevertheless, the results of aquarium experiments clearly display the ability of *A. berda* to undergo protandrous sex change. The first aquarium experiment was run for a period of six months, and fish were sacrificed during a reproductively inactive period of the year (February). Those fish detected to change sex possessed inactive female gonads characterised by a large ovarian lobe containing chromatin nucleolar and perinucleolar stage oocytes. This structural organisation is characteristic of inactive females sampled from the field at this time of year. The degenerated testis tissue was present as a thin band of mainly connective tissue on the ventro-lateral surface of the ovary. Though considered unlikely, Micale & Perdichizzi (1994) suggest it may have been possible for these ovotestes to regain their original male function during the following spawning period. In order to address this possibility, the second aquarium experiment was conducted for a complete annual cycle with all fish sacrificed during the first month (June) of the recognised spawning period (Tobin *et al.*, 1997). Those male fish that had changed sex to female showed extensive oocyte maturation, characteristic of a female fish in spawning condition. Oocytes of the vitellogenic and ripe stages were common in the turgid ovaries of the sex changed fish. Two of the five sex changed fish had ripe oocytes extruding from their urogenital pores at their time of capture. It is likely these fish were in the processes of ovulation when removed from the aquarium. The results of the aquarium experiments clearly demonstrate the ability of *A. berda* to undertake protandrous sex change.

The non-manipulative field experiments of this study represent the strongest available evidence of protandrous sex change in *A. berda*. Further, protandrous sex change was detected in *A. berda* sampled from two spatially separated locations; Blacksoil Creek and Deluge Inlet which are separated by approximately 200kms of coastline. Three and four fish from Blacksoil Creek and Deluge Inlet respectively, were released as active male fish only to be later recaptured as females. These seven fish represent direct evidence of protandrous sex change - initial male function preceding latter female function.

Though many studies have detected sex change by altering the social environment (Shapiro, 1984; Cole & Robertson, 1988; Sunobe & Nakazono, 1993), few studies have detected sex change in the field by passive observation alone. Moore (1979) was able to show protandrous sex change occurred in the Centropomid *Lates calcarifer* by a similar in-field experiment to this study. Though Cole (1983) suggests experimental induction may be the only method capable of demonstrating sex change occurs in some species, certainly the passive observation utilised in this study and that of Moore (1979), to show an individual fish functioning initially as one sex before later in life functioning as the opposite sex must equate to the strongest available evidence of sex change.

In the present study, no reliable method for determining the sex of *A. berda* from external morphology was available. For species that lack external sexual dimorphism, finding a reliable sexing technique becomes particularly important (Sadovy & Shapiro, 1987). A catheterisation method was not attempted in order to reduce chances of stress and trauma that may have altered the normal reproductive mode or behaviour (Faranda *et al.*, 1985) of *A. berda*. Abdominal milking or stripping is a regularly used technique for sexing sparid fish in the aquaculture industry (eg: Glamuzina *et al.*, 1989; Kime *et al.*, 1991; Gwo & Gwo, 1993). Though both Garratt (1993) and Palmer *et al.* (1994) used similar techniques for sexing male *A. berda*, neither technique was validated. Identifying the sex of individual *A. berda* in this study relied on milking the abdominal cavities of fish captured during the spawning period. Fish extruding semen from their urogenital pores were recorded as male, while those that did not were recorded as probable female. The technique was validated with a high percentage of overall accuracy (96.6%).

In reporting protogynous sex change in the sparids *Pagrus auriga, P. ehrenbergi*, *P. orphus* and *P. pagrus*, Alekseev (1983) suggests that it is the largest fish in an age-class that are the first to change sex. The findings of the current study do not support this premise. Those fish detected to sex change in this study were not necessarily the largest fish recaptured in their respective age-classes for either the aquaria or field experiments. Similarly, Micale & Perdichizzi (1994) found no relationship between fish size and occurrence of sex change in the protandrous *Diplodus sargus*. The lengths and ages at which protandrous sex change was detected to occur in *A. berda* in this study support the earlier suggestion (see Chapter 2) that sex change may occur across broad length and age ranges. In this study, sex change was detected to occur across a range of ages (at least 3 to 6 years) and lengths (at least 177 to 273mm FL). Though length rather than age has been considered a more consistent correlate to sex change in some fish

(Warner, 1975; Jones. 1980; Shapiro, 1981), many sparid studies have reported sex change occurring across similarly broad length and age ranges (Huang *et al.*, 1974; Waltz *et al.*, 1982; Abu-Hakima, 1984; Pollock, 1985; Yeung & Chan, 1987; Horvath *et al.*, 1990).

The substantially higher rate of sex change detected in the aquarium experiments as opposed to the relatively low rates detected in the Blacksoil Creek and Deluge Inlet field experiments suggests that social demography may play a significant role in the mediation of sex change in *A. berda*. That is, the higher rate of sex change detected in aquarium experiments may have been due to the largely male biased sex ratios underwhich the experiments were conducted. However, the higher rate of sex change in the aquarium experiments may also be the result of the confined aquarium environment. Future experimentation using controls and replication may shed more light on this aspect of the sex change of *A. berda*.

The knowledge that *A. berda* undergoes protandrous sex change will be valuable information to the future management of this species in Australian waters. At present, *A. berda* is a very common component of recreational angler catches (Sheaves, 1992), and although it is not presently targeted by a commercial fishery, stock numbers are sufficient to attract commercial interests in the near future (Tobin *et al.*, 1997). Sex changing sparids that support important recreational and/or commercial resources may require more dramatic management measures than gonochoristic or rudimentary species (Booth & Buxton, 1997). Fishing effort is ultimately directed towards the larger fish of the population (eg: Horvath *et al.*, 1990), and in heavily exploited populations sex ratios may be dramatically altered (Buxton, 1993) which may impair natural spawning behaviour (Buxton, 1992). In *A. berda* high recreational fishing pressure may result in the removal of larger female fish, which may affect the fecundity and sustainability of stocks. If *A. berda* stocks are subject to high exploitation rates within the future, some measures of protecting the larger more fecund females may be warranted.

CHAPTER 5.

GEOGRAPHICAL VARIABILITY IN THE GROWTH OF *ACANTHOPAGRUS BERDA*.

5.1 INTRODUCTION.

Geographical variability in growth of fish is well documented and has been reported in numerous studies (e.g. Longhurst, 1958; Paul, 1976; Sanders & Powell, 1979; Francis & Winstanley, 1989; Krug, 1990; Paul, 1992; Buxton, 1993). Variation in growth (size-at-age) between locations has serious implications for fisheries management (Griffin, 1988; Buxton, 1993). If geographic variation of growth is combined with a reproductive strategy of sex change management of fisheries resources can become a challenging task (Shaklee & Salini, 1983; Garratt, 1986a; Garrett, 1987).

Sex change may promote diphasic growth, where growth of male and female fish is sufficiently different to warrant description by separate growth curves (Reynolds & Moore, 1982). Separate growth curves are needed because differences in the growth rates and size-at-age between the sexes are common for sex changing fishes (e.g. Garratt *et al.*, 1993; Pajuelo & Lorenzo, 1995; Pajuelo & Lorenzo, 1996). The larger sizes of protandrous and protogynous fishes are usually dominated by male and female fish respectively (Sadovy & Shapiro, 1987). Thus because fisheries usually target the largest fish in stocks, they can inadvertently promote a sex-specific harvest (Shaklee & Salini, 1983; Hilborn & Walters, 1992). Although in some crustacean fisheries sex-specific harvesting is practiced under the presumption that females make a disproportionate contribution to the successful reproduction of the stock (Hilborn & Walters, 1992), in most finfish fisheries sex-specific harvesting is recognised as a potential source of management problems (Shaklee & Salini, 1983; Buxton & Clarke, 1986; Garratt, 1986a; Buxton, 1993). Sex-specific harvesting has the potential to drastically alter sex ratios (Garratt, 1985; Booth & Buxton, 1997). This in turn may reduce the reproductive viability of a species (Smith, 1982; Shaklee & Salini, 1983; Garratt, 1985; Buxton & Smale, 1989; Buxton, 1992; Garratt *et al.* 1993).

An example of geographical variation in growth (size-at-age) and its subsequent effect on the sex change process of the protandrous centropomid *Lates calcarifer* has been recorded (Davis, 1984). Though geographical variation in size-at-age and growth between regions had been presented previously for *L. calcarifer* in Australian waters (Davis, 1982; Davis & Kirkwood, 1984), Davis (1984) identified a precocious population of *L. calcarifer* in the northeast region of the Gulf of Carpentaria (Australia) where size-at-maturity and size-at-sex change were found to be considerably smaller than previously reported. Davis (1984) suggested that the stunting in growth observed in the precocious population may have been due to environmental conditions and emphasised the importance of monitoring the size at which sex change occurs in different populations.

Although stocks of *A. berda* in tropical north Queensland, Australia represent relatively under-exploited resources (Sheaves, pers. com.), the species does support an extensive and economically important recreational fishery (Sheaves, 1992), and stock numbers appear sufficient to attract commercial interests in the near future. These factors make the examination of *A. berda* growth a unique prospect enabling the description of the biological characters of a population prior to commercial exploitation.

The aim of this study is firstly to investigate differences in the growth rates of male and female *A. berda*. Secondly, this study will compare the growth rates for *A. berda* collected from a number of geographically isolated locations.

5.2 MATERIALS AND METHODS.

5.2.1 MODELLING GROWTH CURVES

The von Bertalanffy growth equation has been used to successfully describe the growth of many sparids (e.g. Waltz *et al.*, 1982; Samuel & Mathews, 1987; Horvath *et* *al.*, 1990; Pajuelo & Lorenzo, 1995; Kraljevic *et al.*, 1996), and was the growth equation utilised by the current study for modelling the growth of *A. berda*. Length-at-age samples were collected from Blacksoil (n = 266), Cocoa (n = 204), Cattle (n = 370), and Mendel (n = 232) Creeks as well as Deluge Inlet (n = 559) - five estuaries spanning approximately 200 km of coastline on the tropical northeastern coast of Australia (see Fig 2.1). Upon capture, the fork lengths (FL) of all fish were recorded and sagittal otoliths removed. All fish were aged by sectioning the sagittal otoliths and counting the number of annuli, a successfully validated method for ageing *A. berda* (Tobin, in review [see Appendix 1]).

The von Bertalanffy growth equation :

$$Lt = L\infty \,.\, (1 - e^{(-k(t - to))})$$

was fitted to each length-at-age data set by non-linear regression using the method of least squares. The residuals obtained by fitting the von Bertalanffy growth equation were converted to standardised residuals, and data points excluded from analysis if the standard residuals were greater than 2.5.

5.2.2 COMPARISON OF MALE AND FEMALE GROWTH

For each location, growth was modelled separately for males and females and then again for the complete (male and female) data sets. That is, growth was modelled for three data sets from each location - one model describing male and female fish grouped, and two models describing male and female growth separately. The generation of three residual sums of squares (RSS) for each location allowed investigation by F-Test of the applicability of separate male and female growth curves or alternatively a single common growth curve for each location (Chen *et al.*, 1992). The value of the numerator (three) is derived from the fact that the three von Bertalanffy parameters are estimated for each model (Chen *et al.*, 1992). A cautious approach using the Bonferroni inequality for correction of significance level, was adopted. As a total of 5 F-Tests were conducted, the significance level used was p = 0.01.

5.2.3 COMPARISON OF GROWTH CURVES BETWEEN LOCATIONS

The residual sums of squares (RSS) of growth models for fish from two locations were similarly compared by F-Tests (Chen *et al.*, 1992). Each combination of locations was investigated for significant variation, a total of 10 such combinations. Again the Bonferroni correction was applied, and a significance value of p = 0.005 used.

5.3 RESULTS.

5.3.1 GROWTH OF *A. BERDA*

The modelled growth curves show *A. berda* to be a relatively long-lived and slow growing sparid. Though considerable variation was evident between growth curves modelled for each location, a consistent trend showed the growth of *A. berda* to be relatively rapid during the initial 4 to 5 years, before tapering off (Fig. 5.1).

5.3.2 COMPARISON OF MALE AND FEMALE GROWTH

A significant difference between the male and female growth was detected for Cocoa Creek only (Table 5.1), though the small (age) range of overlap between males and females relative to the other locations sampled (Fig. 5.2) make the Cocoa Creek comparison invalid. With the exception of Blacksoil Creek, the modelled length-at-age of female fish was mostly larger than that of male fish for all locations (Fig. 5.2).

Figure 5.1 : The modelled growth trajectories (sexes combined) - as determined by the von Bertalanffy growth equation - showing considerable variation for *A. berda* sampled from five estuaries in tropical Australia.

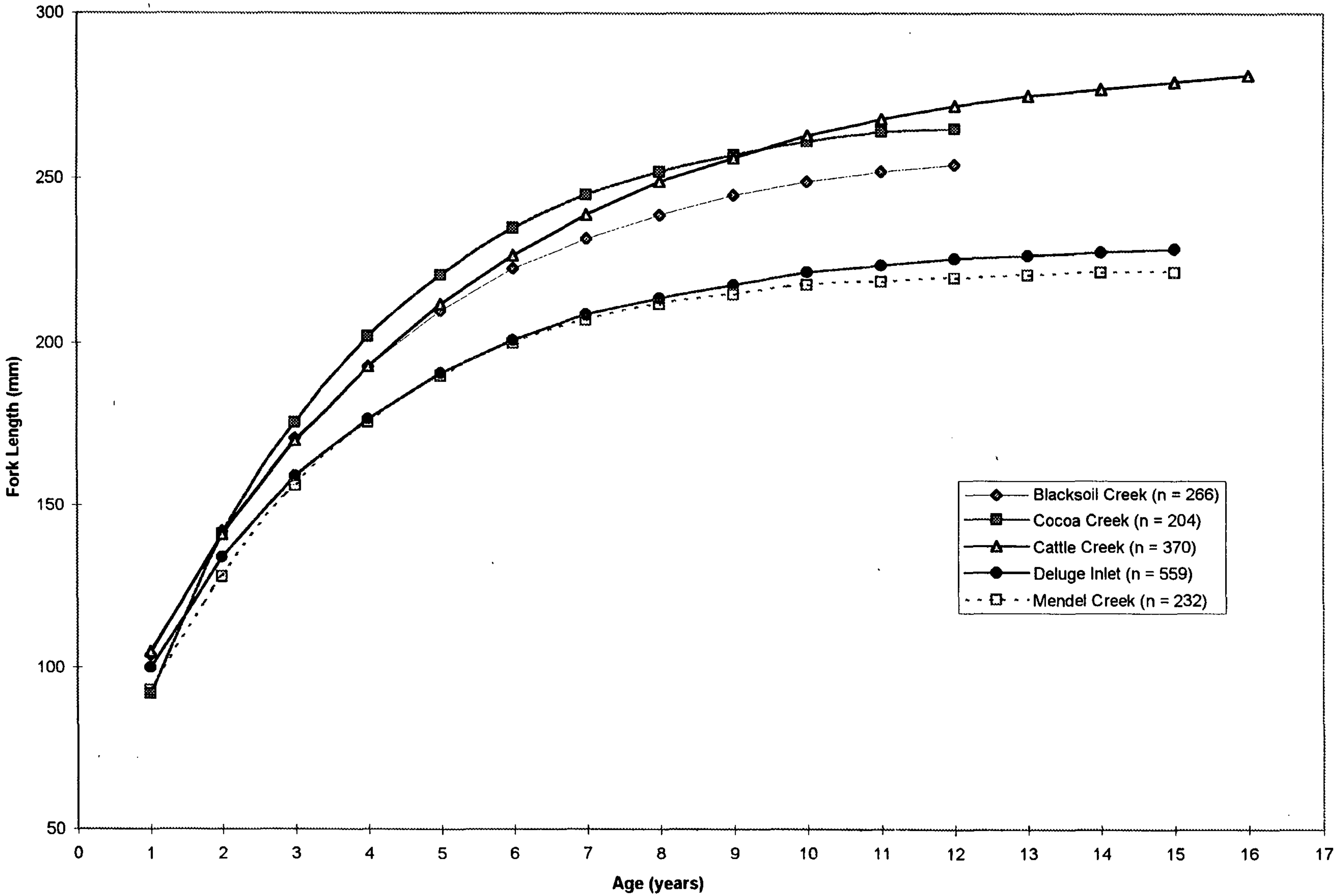

Fork Length (mm)
300
250
200
150
100
50
Age (years)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
Blacksoil Creek (n = 266)
Cocoa Creek (n = 204)
Cattle Creek (n = 370)
Deluge Inlet (n = 559)
Mendel Creek (n = 232)

Figure 5.1

Table 5.1 : A comparison of the parameters (and associated standard errors) estimated for male and female *A. berda* collected from Blacksoil, Cocoa, Cattle, Mendel Creeks and Deluge Inlet (using Bonferroni inequality, significance level p = 0.01).

		t0	k	L∞	Significance
Blacksoil Creek	**Male**	-0.89	0.26	268	$F_{(3.368)} = 0.782$
		(0.123)	(0.022)	(8.560)	
	Female	-0.67	0.31	256	p = 0.505
		(0.117)	(0.025)	(6.017)	
Cocoa Creek	**Male**	0.13	0.55	224	$F_{(3.282)} = 5.44$
		(0.093)	(0.058)	(7.050)	
	Female	-0.11	0.37	265	**p = 0.001**
		(0.106)	(0.029)	(4.931)	
Cattle Creek	**Male**	-0.53	0.30	262	$F_{(3.492)} = 2.86$
		(0.115)	(0.018)	(3.945)	
	Female	-0.60	0.26	285	p = 0.04
		(0.136)	(0.017)	(4.656)	
Deluge Inlet	**Male**	-0.46	0.40	215	$F_{(3.614)} = 1.58$
		(0.071)	(0.020)	(2.503)	
	Female	-0.94	0.28	234	p = 0.192
		(0.080)	(0.012)	(1.996)	
Mendel Creek	**Male**	-0.08	0.44	215	$F_{(3.270)} = 1.25$
		(0.086)	(0.025)	(2.693)	
	Female	-0.30	0.37	223	p = 0.29
		(0.115)	(0.023)	(2.265)	

Figure 5.2 : A comparison of the growth trajectories modelled for male and female *A. berda*.

Figure 5.2

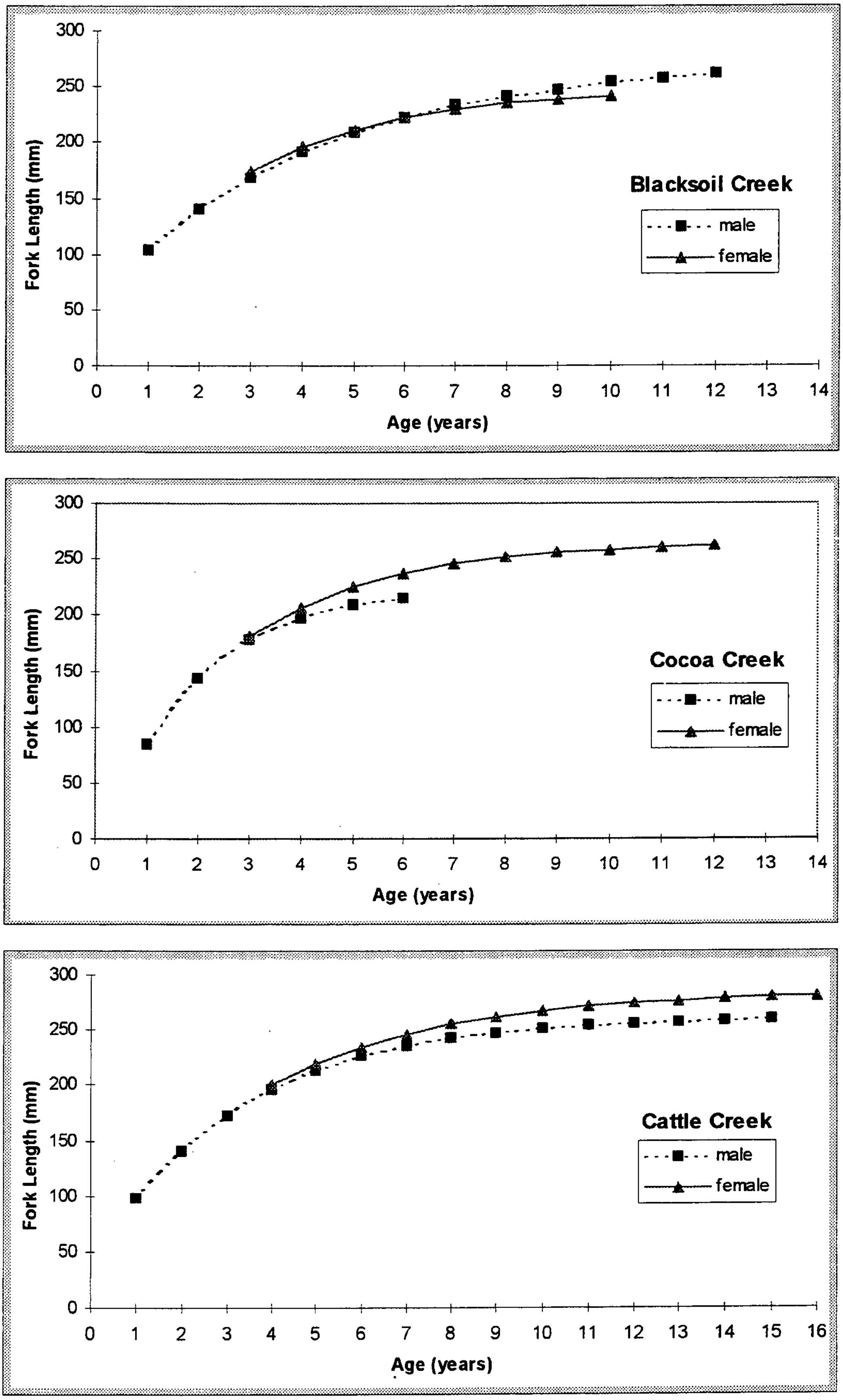
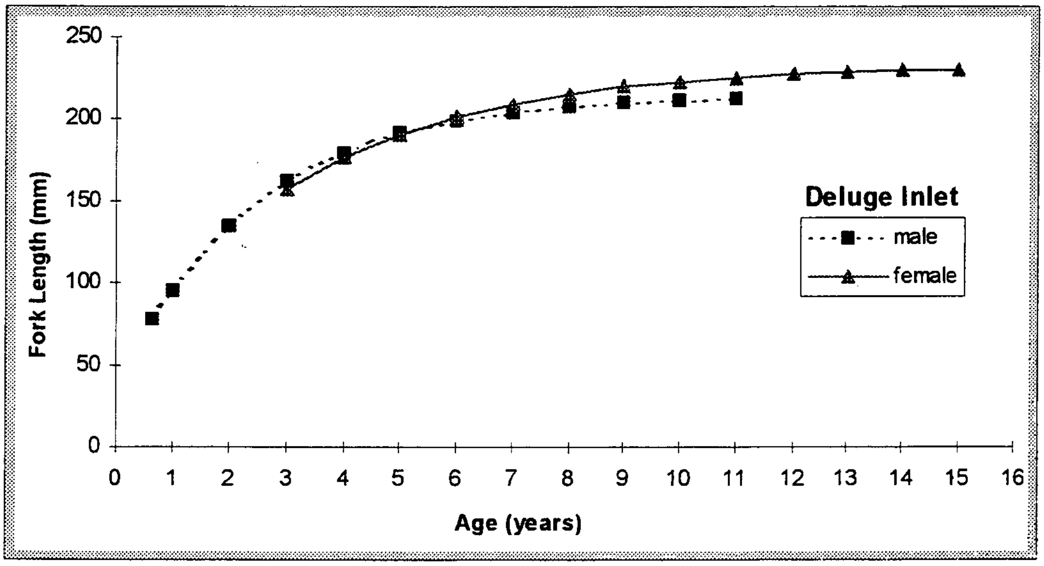
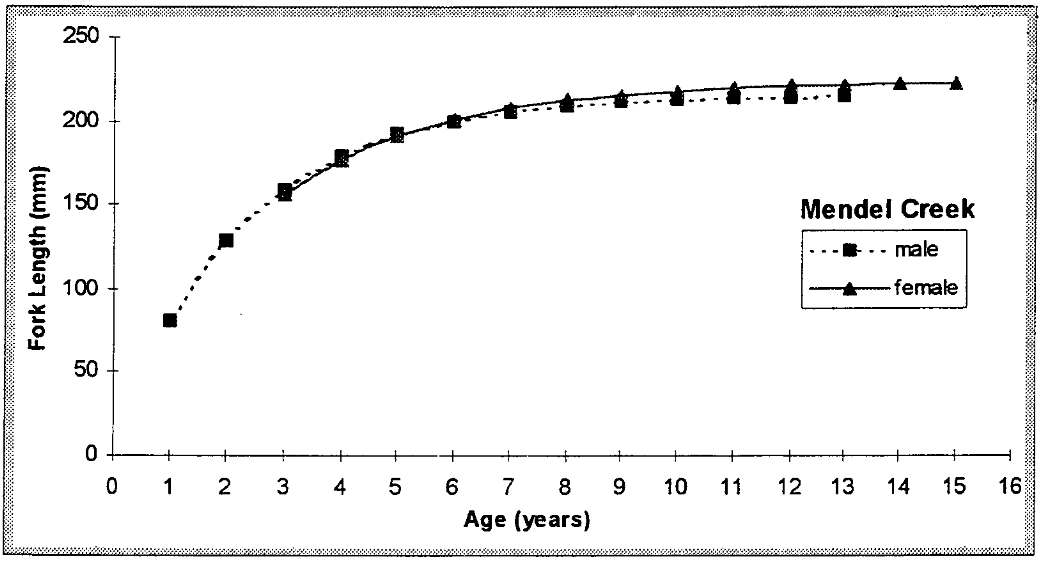

Blacksoil Creek
male
female
Fork Length (mm)
300
250
200
150
100
50
0
Age (years)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Cocoa Creek
male
female
Fork Length (mm)
Age (years)
Cattle Creek
male
female
Fork Length (mm)
Age (years)
15
16

Figure 5.2 (cont).





5.3.3 COMPARISON OF GROWTH ACROSS THE GEOGRAPHICAL SCALE

A comparison of the parameters of the fitted growth equations suggest variation exists between locations (Table 5.2). While the growth of *A. berda* from Mendel Creek and Deluge Inlet was not significantly different, these curves were significantly different from Blacksoil, Cocoa and Cattle Creek (Table 5.3). Further, though the combinations of Cattle and Blacksoil Creeks, and Cattle and Cocoa Creeks did not show significant differences, there were significant differences between all other combinations of growth curves (Table 5.3).

Comparison of the F values suggests the modelled growth curves may be separable into two groups (Fig. 5.1). Though a significant difference was detected between Blacksoil and Cocoa Creeks, the magnitude of difference (i.e. F value) was relatively small in comparison to the differences (i.e. F values) detected between Blacksoil, Cocoa, Cattle Creeks and Mendel Creek, Deluge Inlet (Table 5.3). The very large differences detected between the Cattle Creek - Deluge Inlet and Cattle Creek - Mendel Creek combinations (Table 5.3) may, in part, be attributed to the power of the analyses. The power of an F-Test increases as sample size (n) increases. Further, given substantial differences between both these combinations of growth trajectories were notable when graphed, the very small probabilities of these F-ratios are not entirely unexpected.

Further, though a similar maximum age was recorded by Samuel & Mathews (1987) for *A. berda* in Kuwait (i.e. 14 years), the von Bertalanffy growth parameters estimated by the present study suggest *A. berda* has a considerably slower growth rate in tropical northeastern Australia (Table 5.2). The magnitude of the difference is obvious when the growth curve modelled by Samuel & Mathews is plotted against the growth trajectories of *A. berda* modelled by this study (Fig 5.3).

Table 5.2 : Estimated parameters and associated standard errors of the modelled von Bertalanffy growth equations for each location. Maximum size and age is also shown for each location.

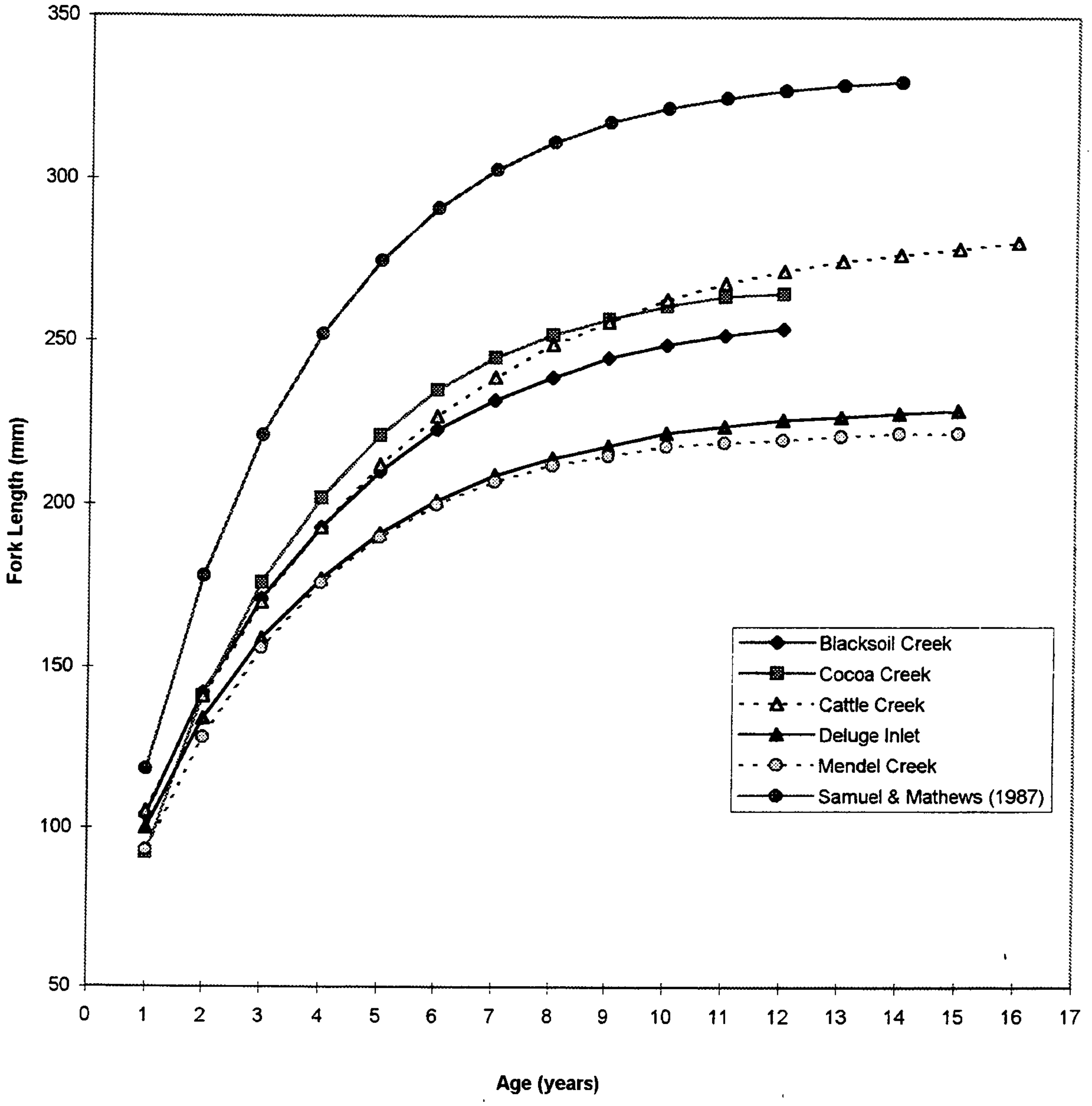
	Maximum Size	Maximum Age	t_0	k	L_∞
Blacksoil Creek	282	12	-0.80	0.28	261
			(0.13)	(0.02)	(4.73)
Cocoa Creek	286	12	-0.30	0.32	271
			(0.11)	(0.02)	(5.63)
Cattle Creek	313	16	-1.06	0.22	288
			(0.14)	(0.01)	(4.54)
Deluge Inlet	250	15	-0.92	0.30	231
			(0.10)	(0.01)	(2.19)
Mendel Creek	248	15	-0.37	0.36	223
			(0.10)	(0.02)	(1.90)
Samuel & Mathews (1987) - Kuwait	**N / A**	**14**	**-0.346**	**0.325**	**374**

Table 5.3 : Matrix showing the combinations of creeks where significant differences (indicated by bold figures) in growth rates were detected.

	Blacksoil Creek	**Cocoa Creek**	**Cattle Creek**	**Deluge Inlet**	**Mendel Creek**
Blacksoil Creek		**$F_{(3,483)}$ = 8.12** **p = 2.8E -05**	$F_{(3,654)}$ = 1.84 p = 0.138	**$F_{(3,828)}$ = 48.27** **p = 9.4E -29**	**$F_{(3,515)}$ = 78.46** **p = 8.2E -42**
Cocoa Creek	**$F_{(3,483)}$ = 10.24** **p = 2.8E -05**		$F_{(3,588)}$ = 1.97 p = 0.118	**$F_{(3,758)}$ = 108.7** **p = 1.5E -58**	**$F_{(3,439)}$ = 93.61** **p = 7.6E -47**
Cattle Creek	$F_{(3,654)}$ = 1.84 p = 0.138	$F_{(3,588)}$ = 1.97 p = 0.118		**$F_{(3,948)}$ = 167.9** **p = 2.8E -87**	**$F_{(3,610)}$ = 158.2** **p = 7.7E -76**
Deluge Inlet	**$F_{(3,828)}$ = 48.27** **p = 9.4E -29**	**$F_{(3,758)}$ = 108.7** **p = 1.5E -58**	**$F_{(3,948)}$ = 167.9** **p = 2.8E -87**		$F_{(3,799)}$ = 0.65 p = 0.586
Mendel Creek	**$F_{(3,515)}$ = 78.46** **p = 8.2E -42**	**$F_{(3,439)}$ = 93.61** **p = 7.6E -47**	**$F_{(3,610)}$ = 158.2** **p = 7.7E -76**	$F_{(3,799)}$ = 0.65 p = 0.586	

Figure 5.3 : Plotted growth curves indicating the magnitude of difference between the growth modelled by the current study and that by Samuels and Mathews (1987) for *A. berda* from Kuwait waters.

Figure 5.3


350
300
250
200
150
100
50
Fork Length (mm)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
Age (years)
Blacksoil Creek
Cocoa Creek
Cattle Creek
Deluge Inlet
Mendel Creek
Samuel & Mathews (1987)

5.4 DISCUSSION.

The modelled length-at-age data collected in this study shows *Acanthopagrus berda* in tropical northeastern Australia to be a relatively long-lived and slow growing sparid. Slow growth and longevity are considered normal of the sparid family (Buxton & Clarke, 1989; Buxton, 1993), and the growth of *A. berda* was modelled in this study as relatively rapid for the initial few years, before tapering off to a slow and steady rate of growth in the older age-classes. Similar growth trajectories have been described for a number of other sparids, including *Calamus leucosteus* (Waltz *et al.*, 1982) *Calamus nodosus* (Horvath *et* al., 1990), *Dentex gibbosus* (Pajuelo & Lorenzo, 1995) and *Lithognathus mormyrus* (Kraljevic *et al.*, 1996).

Interestingly, the results of this study show the growth of *A. berda* in tropical northeastern Australia may vary considerably between locations that are separated by only relatively small distances (10s of kilometres). The comparison of growth equations for each combination of locations suggested the application of a common growth equation for the description of the growth of *A. berda* in northern Australia would be invalid. Modelling growth for *A. berda* collected from Deluge Inlet and Mendel Creek revealed an initial rapid growth before showing a tendency to asymptote after the fifth or sixth year. In both locations the growth of *A. berda* in latter age-classes was minimal. In comparison, the growth of *A. berda* collected from Blacksoil, Cocoa and Cattle Creeks was significantly faster. In these locations, growth in the first 5 to 6 age classes was more rapid and the theoretical maximum lengths ($L\infty$) were much larger.

Geographical variation in growth is not uncommon in teleost fish (Paul, 1992), and has been reported for numerous sparids (e.g. Krug, 1989; Francis & Winstanley, 1989; Krug, 1990; Paul, 1992; Buxton, 1993). However reports of geographic variability in growth should be treated cautiously. For example, the contrasting growth rates reported for the sparid *Pterogymnus laniarius* (Hecht & Baird, 1977 c.f. Booth & Buxton, 1997), are suggested be due to a temporal rather than a spatial effect (Booth &

Buxton, 1997). Further, the geographical differences in growth reported for some sparids may be confounded by sampling or aging methodologies. Variation in the growth reported for *Lithognathus mormyrus*, may be the effect of different aging techniques (Kraljevic *et al.* 1996), while the growth (and age range) variations reported for *Dentex gibbosus*, may be confounded by the different sampling methodologies and gears used by earlier studies that may have effectively excluded larger fish from the samples collected (Pajuelo & Lorenzo, 1995).

No other sparid study has presented geographical variability of growth over the relatively small spatial scales that the current study has investigated. Further, the variations detected in the growth of *A. berda* by the current study are considered to reflect true growth variations between the locations sampled. Firstly, the sampling methods were consistent for each location; secondly, the aging methods were consistent for all fish; and thirdly, though some samples were collected across a three year period, the author considers this length of time to be insufficiently short for temporal variation to be responsible for the growth variations detected in *A. berda*.

Furthermore, the growth trajectories modelled for *A. berda* by the current study differ markedly from the growth reported for *A. berda* in Kuwait waters (Samuels & Mathews, 1987). This evidence suggests *A. berda* may display considerable variation in growth throughout its wide geographic range.

5.4.1 COMPARISON OF MALE AND FEMALE GROWTH

The current study suggests differences in the growth of male and female *A. berda* are negligible. The growth of male and female *A. berda* for four locations (Blacksoil Creek, Deluge Inlet and Mendel Creek) were found not to differ significantly, and may be described by a common growth equation. Only in Cocoa Creek was male and female growth found to be significantly different. However, the growth difference detected between male and female *A. berda* in Cocoa Creek is invalidated by the relatively small over-lapping age range of the male and female fish collected in this sample. While females were represented in all age-classes from the 3rd to the 12th, male fish were represented in the 1st to the 6th age-classes only. Further, few males were present in the fifth (n = 3) and sixth (n = 2) age-classes. It is therefore not surprising the growth of females was significantly faster reaching a larger asymptotic length than male fish. For all other locations, the overlapping ranges of male and female fish were much larger. This pattern may suggest that protandrous sex change occurs exclusively in the younger age-classes at Cocoa Creek.

With the exception of Blacksoil Creek, a consistent pattern was modelled for male and female growth in all samples. Female length-at-age was consistently larger than male length-at-age. This pattern may suggest that it is the larger males of each age-class that change sex. Alekseev (1983) suggested that the larger fish in each age-class would change sex before the smaller fish for the protogynous *Pagrus pagrus*, *P. orphus*, *P. ehrenbergi* and *P. auriga*. Although the sex change experiment did not detect such a pattern for *A. berda* (see Chapter 4), the sample of sex change fish may have been too small to detect such a pattern if it does in fact exist.

An alternative explanation is that the sex change process of *A. berda* may be followed by a growth spurt. A spurt of growth accompanying sex change has been modelled for the protogynous sparid *Chrysoblephus puniceus* (Garratt *et al.*, 1993; Punt *et al.*, 1993) and has been postulated for a number of other species (Warner, 1988; Hoffman *et al.*, 1985; Ross, 1987). If sex change in *A. berda* was followed by a similar growth spurt, such a rapid increase in growth may explain the observed trend of faster female growth.

Those sparid sex change studies that have investigated diphasic growth of the sexes have varying reports. The male and female growth of the protogynous sparid *Chrysoblephus puniceus* was found to vary significantly, with male fish modelled as having larger L_∞ (Garratt *et al.*, 1993). Similarly, Pajuelo & Lorenzo (1995) detected significantly faster growth in male fish of the protogynous sparid *Dentex gibbosus*, and Pajuelo & Lorenzo (1996) detected differences in growth of males and females of the protogynous *Pagrus pagrus*. In opposition to this trend, Kraljevic *et al.* (1996) reported no significant difference between male and female growth of the protandrous sex changing *Lithognathus mormyrus*.

Not surprisingly, the reporting of variation in male and female growth of rudimentary hermaphroditic sparids is uncommon. No variation between male and female growth was detected for the rudimentary *Pachymetopon grande* (Buxton & Clarke, 1992), and though reported as a protogynous sex changer and a rudimentary hermaphrodite by Hecht & Baird (1977) and Booth & Buxton (1997) respectively, neither study found differences in the growth of male and female *Pterogymnus laniarius*. Male and female growth of the fellow rudimentary hermaphrodite, *Sparodon durbanensis* (Buxton & Clarke, 1991) was also found to be similar.

5.4.2 EVIDENCE SUGGESTING GENETIC SUB-STOCKS OF *A. BERDA*

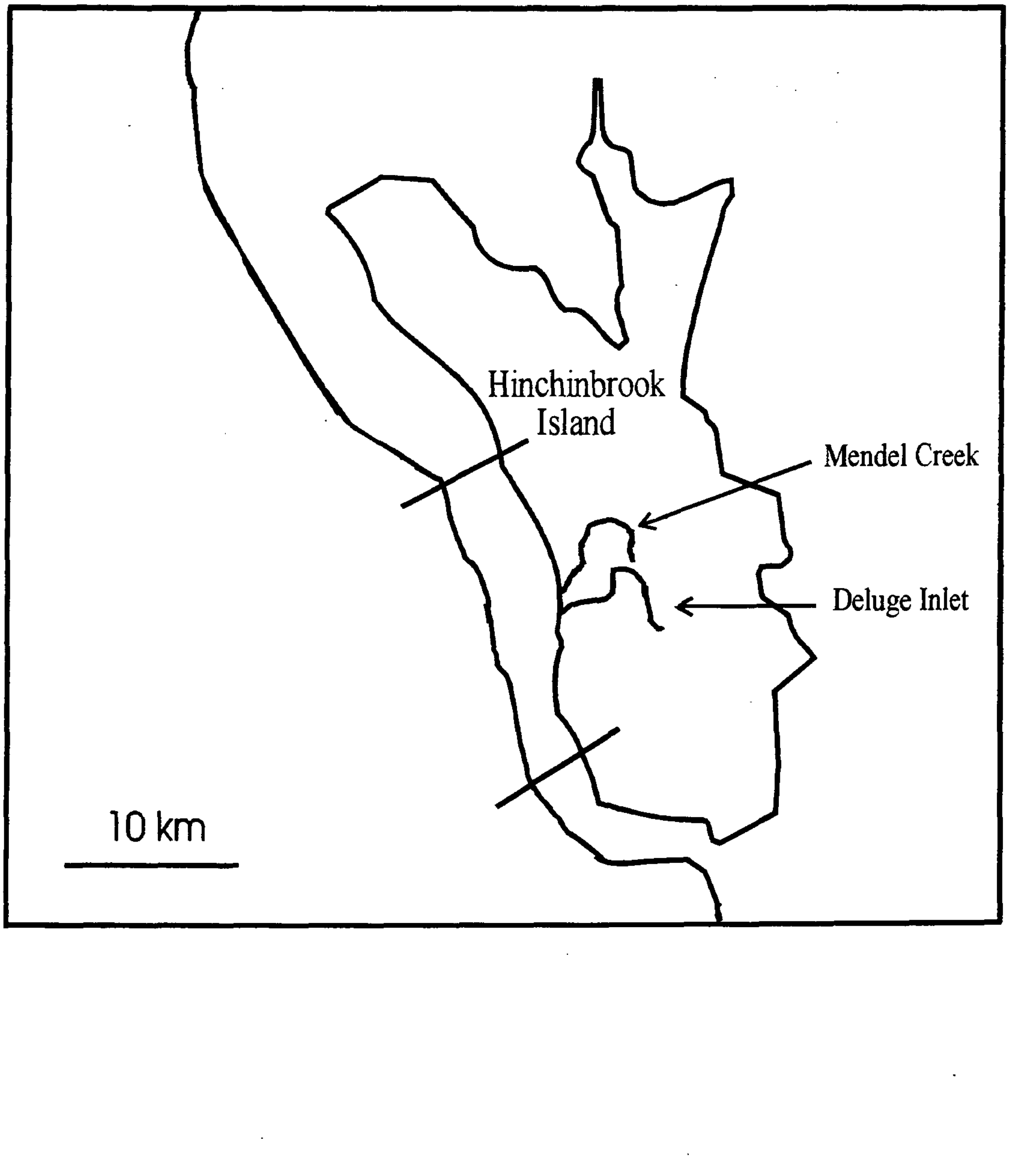
The growth curves modelled for the five locations sampled in this study present an interesting pattern that, though may be a result of simple natural spatio-temporal variability (Paul, 1992), may alternatively be the result of genetic sub-stock structuring. The growth of *A. berda* from Mendel Creek and Deluge Inlet was not significantly different, though was much slower than the growth of *A. berda* collected from the other three locations (Blacksoil, Cocoa and Cattle Creeks). This was not surprising as it was noticed that the *A. berda* collected from Deluge Inlet and Mendel Creek were generally smaller than fish sampled from other locations. Large fish were relatively infrequent in the Deluge Inlet and Mendel Creek samples and though higher mortality rates of *A. berda* in Deluge Inlet and Mendel Creek was considered as a possible biological explanation for the absence of large fish, the presence of many old fish in both age samples discounted this possibility.

The estuarine systems of Mendel Creek and Deluge Inlet are in very close proximity to each other (their respective entrances separated by less than 1 kilometre of coastline)(see Fig 2.1), and are located within the central region of the Hinchinbrook Channel. The upper reaches of Deluge Inlet and Mendel Creek are often joined when the mangrove forests separating the two estuaries are flooded by hightides, and it may be possible the *A. berda* in Mendel Creek and Deluge Inlet represent a "common stock". Though a tagging study did not detect any movement of fish between Deluge Inlet and Mendel Creek (Sheaves *et al.*, in review), it is possible *A. berda* move freely between the estuaries on hightides.

A possible explanation of the stunted growth of *A. berda* detected in this region, is that a genetic sub-stock of *A. berda* exists in the central Hinchinbrook Channel. Studying the hydrodynamic characteristics of the Hinchinbrook Channel, Wolanski *et al.* (1990) reported that water in the middle third of the Hinchinbrook Channel may be trapped in the channel system for periods in excess of 2 weeks. Consequently, the positioning of Mendel Creek and Deluge Inlet in the central region of the Hinchinbrook Channel (Fig. 5.4), may severely impede the natural genetic dispersal of the eggs and larvae spawned by *A. berda* in this area. Spawning *A. berda* are known to aggregate at that mouths of estuaries and coincide spawning activities with large ebb tides (pers. obs.; Garratt, 1993) to promote the wide dispersion of planktonic embryos and larvae. However, the hydrodynamic nature of the Hinchinbrook Channel may limit the genetic dispersal of *A. berda* spawning in the central region of the Hinchinbrook Channel. Further, *A. berda* spawn during the winter dry season in tropical Australia, a period recognised as further enhancing the trapping of water in tropical estuarine systems of northern Australia (Wolanski, 1988). Considering this, though genetic dispersal is considered to be wide ranging from estuaries that drain directly into coastal waters because of longshore and/or tidal currents (Shaklee & Salini, 1985), the hydrodynamic

Figure 5.4 : The location of Deluge Inlet and Mendel Creek within the central region of the Hinchinbrook Channel. Bars represent the approximate middle third of the channel system where water is reported to remain trapped for periods in excess of 2 weeks (see Wolanski *et al.*, 1990).

Figure 5.4



characteristics of the Hinchinbrook Channel may provide a unique situation that has resulted in a genetic sub-stock of *A. berda* inhabiting this area.

An investigation of this possibility should be of high priority. The commercially and recreationally important centropomid *Lates calcarifer*, is known to consist of at least seven distinct genetic stocks in both Queensland (Shaklee *et al.*, 1993) and the Northern Territory (Salini & Shaklee, 1988) of Australia. Not surprisingly, variation in age and growth between regions has been detected for *L. calcarifer* (Davis & Kirkwood, 1984). Further, a population of *L. calcarifer* with a particularly stunted growth trajectory has been identified and described in the north-western region of the Gulf of Carpentaria, northern Australia (Davis, 1984). The particularly stunted growth of *L. calcarifer* in this region is reflected by considerably smaller lengths-at-maturity and lengths-at-sex change (Davis, 1984).

To determine whether the slower growth of *A. berda* detected in Mendel Creek and Deluge Inlet is the result of stock structuring a more comprehensive sampling regime would be required. Sampling numerous locations along the complete length of Hinchinbrook Channel may reveal a cline in the growth of *A. berda* where slow growth occurs in the central region of the channel with fish at the southern and northern extremes of the channel growing faster.

5.4.3 MANAGEMENT IMPLICATIONS

If a population consists of a number of genetically isolated stocks, any localised fishing effort will effect only that component stock of the population targeted (Shaklee & Salini, 1983). Consequently, it may be possible to effectively fish out particular component stocks. Management strategies must be implemented to prevent this possibility (Shaklee & Salini, 1985; Jean *et al.*, 1996), otherwise the natural genetic diversity of species may be threatened. The centropomid *Lates calcarifer* presents a classic example where the existence of at least 14 genetic stocks throughout the

Australian range of the species (Salini & Shaklee, 1988; Shaklee *et al.*, 1993) demands management strategies be implemented that provide protection for each component stock of the population to ensure the viability of the total resource (Shaklee *et al.*, 1993).

Under high levels of exploitation, the natural demographics of a sex changing population may be altered. A consequence of high exploitation may be significantly altered sex ratios. High exploitation of the protogynous sparids *Chrysoblephus laticeps* and *C. cristiceps* has resulted in the skewing of sex ratios towards females because of the size selective removal of males which grow larger than females in these species (Buxton, 1993). A similar situation has also been reported for the protogynous *C. puniceus* (Garratt, 1985). Future monitoring of the *A. berda* populations sampled in this study will be conducted. Future monitoring may determine whether recreational exploitation is high enough to alter sex ratios and possibly growth rates, as the fisheries exploitation of any stock may be reflected with faster growth rates and larger sizes at maturity and sex change (Griffin, 1988).

Further, the largely male biased sex ratios detected at Alligator and Cattle Creeks (see Chapter 2) may also be the result of higher recreational fishing pressures at these locations. Though no data on recreational fishing effort is available, these two estuaries are preferred angling locations of many recreational fishers in north Queensland (Sheaves, pers. comm.). Continued monitoring may determine whether the exploitation of *A. berda* by recreational fishers is high enough to alter the demographics of the *A. berda* populations.

CHAPTER 6.

THE RATE OF PROTANDROUS SEX CHANGE IN *ACANTHOPAGRUS BERDA*.

6.1 INTRODUCTION.

Although sex change has been well documented in sparid fishes, no study has attempted to model the rate at which sex change occurs at the population or stock level. Most reports of sparid sex change have identified a range of ages over which sex change is suggested to occur (e.g. Waltz *et al.*, 1982; Buxton & Clarke, 1986; Kralevic *et al.*, 1996), however the rate of sex change has not been investigated. As a consequence it is unclear if sex change occurs across a small age range, or if it occurs across a wide range of ages with equal probability. If the rate of sex change can be modelled successfully, geographic variability in the rate of sex change can be studied. Extensive geographical variability in the protandrous sex change process of the centropomid, *Lates calcarifer* has been documented across northern Australia (Davis, 1987). Variation in the pattern of sex change of the temperate sparid *Acanthopagrus butcheri*, has also been recorded in several lake systems of south-eastern Australia (Rowland & Snape, 1994), with variant forms of protogynous sex change detected between lake systems. Similarly, Alekseev (1982) has suggested the sex change processes of some sparids may differ in different populations.

The objective of this study is to construct a model that could be used to predict the probability of sex change of *A. berda* by age-class. Geographical variation in the rate of sex change will be investigated.

6.2 MATERIALS AND METHODS.

6.2.1 COLLECTION OF SAMPLES

From August 1993 to September 1997, samples of *A. berda* were collected from Blacksoil (n = 266), Cocoa (n = 204), Cattle (n = 370), Mendel (n = 232) Creeks and Deluge Inlet (n = 408). All fish were collected during the spawning period to minimise problems of sex identification. Samples were collected by hook and line angling and were assumed to be random and representative selections taken from the populations of *A. berda* at each location. As far as possible, the same fishing methods (such as hook size and bait type) were applied at each location, and at each time.

With the exception of Deluge Inlet, the age samples collected from all other locations represent the *total* samples collected. Further, these samples were collected across relatively short time scales (2 to 4 months). Because of the tag-release study conducted at Deluge Inlet, the sample collected for aging (205♂, 354♀) was not representative of the overall male to female ratio (1.01 : 1) detected. To be representative of the overall sex ratio, 203 females were chosen randomly from the total of 354 females sampled. To test the validity of the random sampling, the length-frequency distributions of the 205 males and 203 females age samples were compared against the overall male (n = 1036) and female (n = 1023) length-frequency distributions sampled from Deluge Inlet by chi-squared homogeneity tests. No significant difference was found for either the male ($\chi^2 = 1.06$, $p > 0.95$, df = 5) or female ($\chi^2 = 0.42$, $p > 0.99$, df = 5) subsamples collected for aging. On this basis, the age sub-sample for Deluge Inlet was then assumed to be representative of the population sampled.

6.2.2 DATA ANALYSIS

6.2.2.1 ASSUMPTIONS

Before any data analysis or interpretation was initiated, a number of assumptions were made.

1. The sex ratios of each sample were representative of the sex ratios of the populations of *A. berda* in those locations sampled.

2. The proportion (or sex ratio) of male and female fish in each age-class were representative of the sex ratios of those age classes in each location sampled.

3. The change in the sex ratios between each age-classes was a direct reflection of the rate at which sex change occurs.

6.2.2.2 GRAPHICAL INVESTIGATION

To investigate trends in the sex ratios through the age-classes, the proportions of male fish in each age class were calculated. Only age-classes with at least 5 fish were used in analyses. Sample sizes of 5 fish or less were considered to be insufficiently robust in terms of representing the sex ratios of the age-classes sampled.

For each scatterplot, major trends were identified using LOWESS, a locally-weighted scatterplot smoother (Cleveland, 1979). The fitting of the LOWESS was done with the smoothness parameter f set as 50% (where f = the fraction of the data set used for the estimation of y_i for each x_i) (Trexler & Travis, 1993). Using an f value of 50% is suggested to smooth the data sufficiently without distorting any underlying pattern that may be present in the data (Cleveland, 1985).

6.2.2.3 REGRESSION ANALYSIS

Where appropriate, logistic regression was used to model the change in proportion of males through the age-classes. The logistic regression is appropriate when the dependent variable is binary type data (Shanubhogue & Gore, 1987; Collett, 1991), and is best fitted using the maximum likelihood method (Trexler & Travis, 1993), and was the method used by this study.

Where required, a combination of logistic and linear regressions were used.

6.2.2.4 COMPARING THE MODELLED RATES OF SEX CHANGE

Following the fitting of appropriate rate-of-sex-change models for each location, the respective models were compared by dummy variable analysis (Dobson, 1990) allowing similarities and/or differences between the individual models of each location or groups of locations to be identified.

6.2.2.5 ESTIMATING THE RATE OF SEX CHANGE

After the appropriate models were fitted, the rate of sex change for each age-class of each location was able to be estimated. The fitted models allowed the proportion of males in each age-class to be estimated, and the change in proportion of males of successive age-classes was utilised as an estimate of the rate of sex change. For example, the rate of sex change in the second age-class was estimated by expressing the difference in the proportion males between the second and third age-classes as a percentage of the original (second year age-class) proportion of males. The resulting probability value was considered as an estimate of the probability of a male fish of the initial age-class undergoing sex change.

6.3 RESULTS.

6.3.1 LOWESS TRENDS

The overall trend for all locations was for the proportion of males to decrease with increasing age. The fitted LOWESS lines indicated a similar trend in the Blacksoil Creek, Deluge Inlet and Mendel Creek samples (Fig. 6.1). In the younger age-classes, the proportion of males showed an initial rapid decrease before showing a tendency to level off in the older age-classes. These trends suggest a two stage or piece-wise model may be suitable for modelling the relationship between age-class and proportion of males.

In contrast, the proportional data of Cocoa showed a tendency for rapid transition between male and female dominance (Fig 6.1) suggesting a single model would be sufficient for modelling the decrease in proportion of males.

The Cattle Creek sample displayed a different trend. Though the proportion of male fish decreased with increasing age, the decrease was more gradual than detected at the other locations (Fig. 6.1).

6.3.2 REGRESSION MODELLING

Logistic regression was found to fit and adequately model the data sets of Cocoa and Cattle Creeks (Fig 6.2). Logistic regression was also found to appropriately model the initial decreases in the proportions of males for Blacksoil and Mendel Creeks as well as Deluge Inlet (Fig. 6.3). Each logistic fit was found to appropriately model the decrease in proportion of males with increasing age (Table 6.1).

Table 6.1 : The parameter estimates and significance of the LOGISTIC model fits.

	a	b	Deviance	Prob.	df.
Cocoa Creek	-1.94	8.68	7.72	0.11	4
Cattle Creek	-0.57	5.38	4.97	0.31	7
Blacksoil Creek	-2.10	8.47	2.77	0.25	2
Deluge Inlet	-2.20	8.87	0.68	0.71	2
Mendel Creek	-1.80	7.63	1.25	0.26	2

Figure 6.1 : Fitted LOWESS regressions identifying trends and pivotal data points in the proportional scatterplots.

Figure 6.1

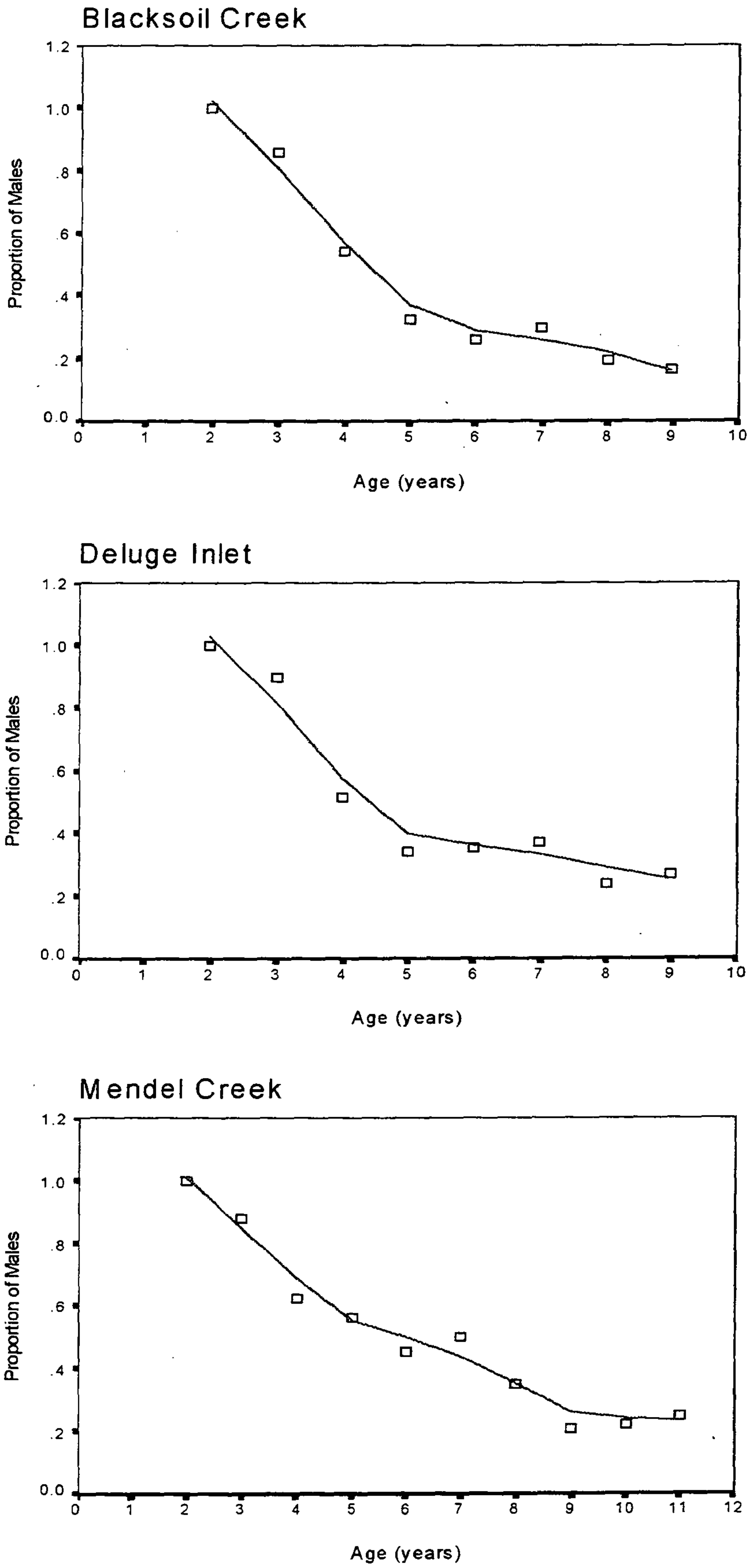
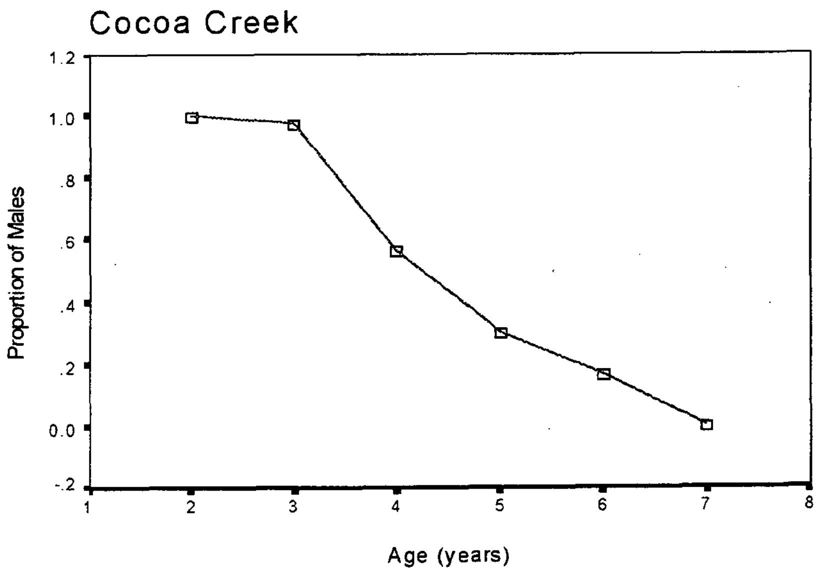

Blacksoil Creek
Proportion of Males
1.2
1.0
.8
.6
.4
.2
0.0
0
1
2
3
4
5
6
7
8
9
10
Age (years)
Deluge Inlet
Proportion of Males
1.2
1.0
.8
.6
.4
.2
0.0
0
1
2
3
4
5
6
7
8
9
10
Age (years)
Mendel Creek
Proportion of Males
1.2
1.0
.8
.6
.4
.2
0.0
0
1
2
3
4
5
6
7
8
9
10
11
12
Age (years)

Figure 6.1 (cont.)



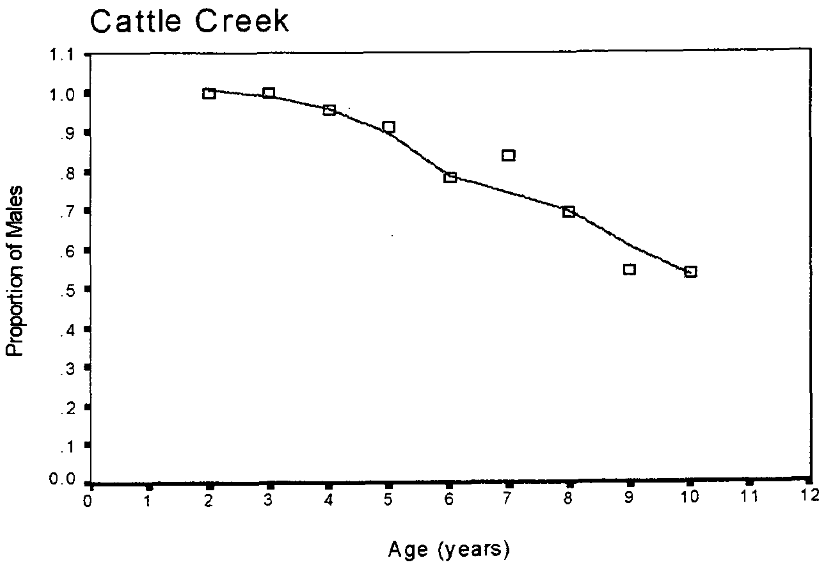
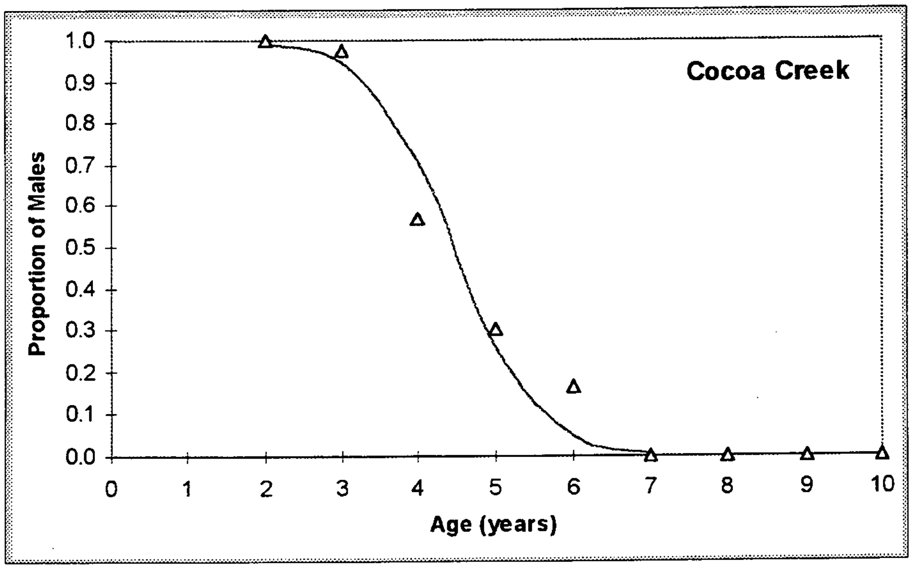


Figure 6.2 : Logistic regressions fitted to Cocoa and Cattle Creeks.

Figure 6.2


Cocoa Creek
Proportion of Males
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
1
2
3
4
5
6
7
8
9
10
Age (years)

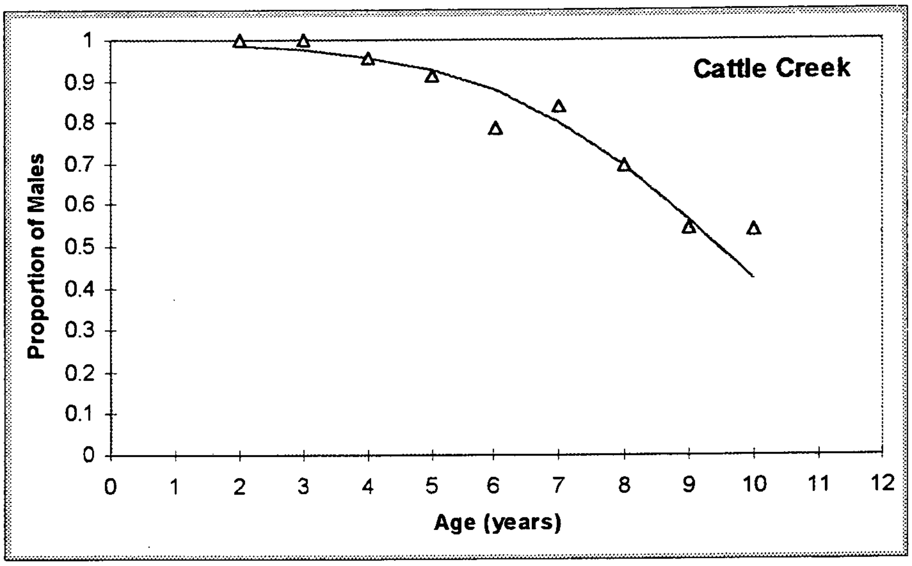
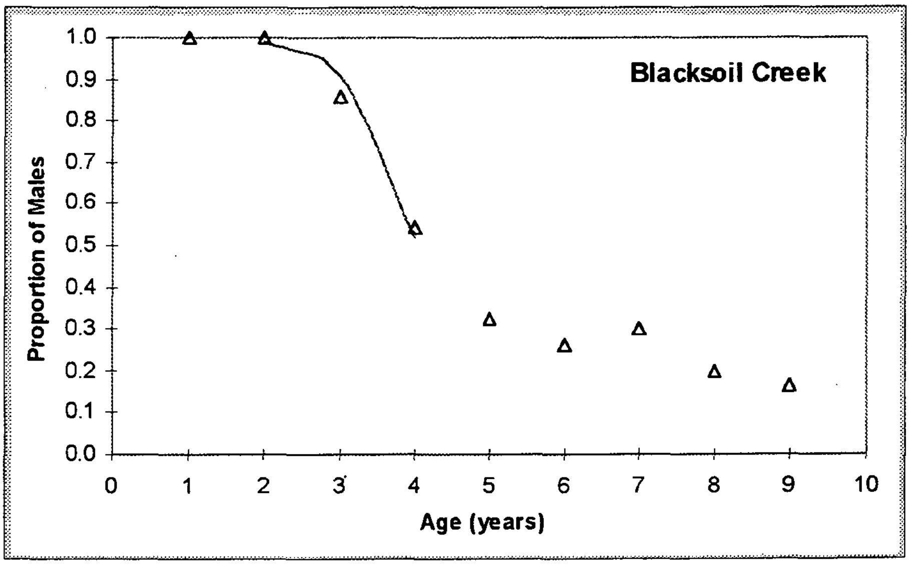
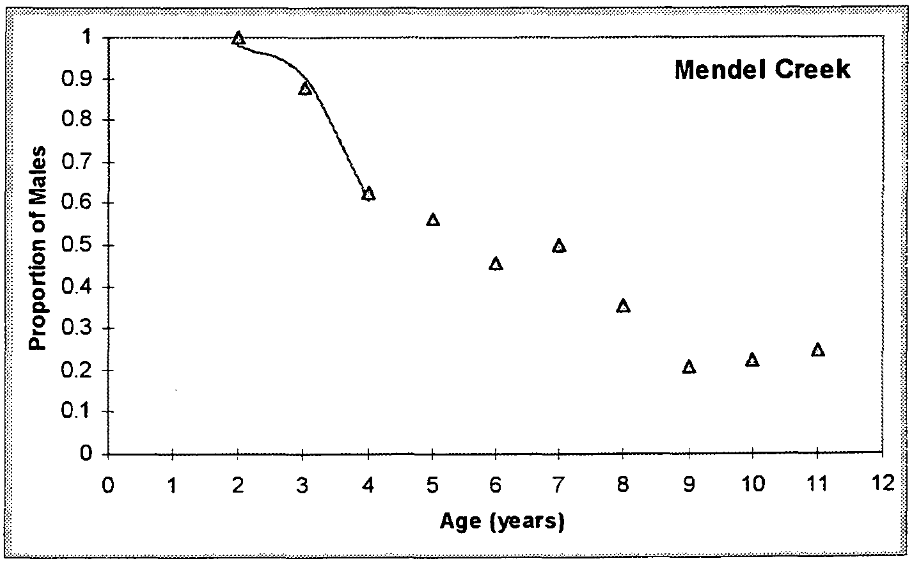
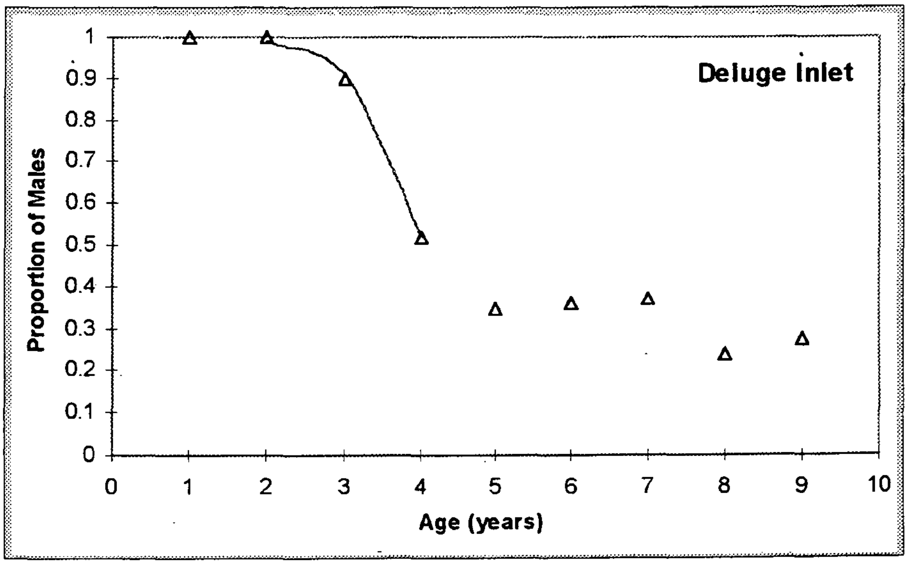

Cattle Creek
Proportion of Males
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
4
5
6
7
8
9
10
11
12
Age (years)

Figure 6.3 : Logistic regressions fitted to the initial Blacksoil Creek, Mendel Creek and Deluge Inlet data.

Figure 6.3


Blacksoil Creek
Proportion of Males
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
1
2
3
4
5
6
7
8
9
10
Age (years)


Mendel Creek
Proportion of Males
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
4
5
6
7
8
9
10
11
12
Age (years)


Deluge Inlet
Proportion of Males
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
4
5
6
7
8
9
10
Age (years)

Above the 5th age-classes of Blacksoil Creek, Deluge Inlet and Mendel Creek the proportion of male fish showed a tendency to decrease more slowly and with a linear trend. Linear regressions were used to model the decrease in proportion of males in these latter age-classes (Fig 6.4). The position (or breakpoint age-class) between the logistic and linear modelling was checked for appropriateness by altering the age-class of the breakpoint and monitoring residual sum of squares (RSS). The most appropriate model will minimise the RSS. For each sample, the position of the breakpoints (between the fourth and fifth age-classes) were found to minimise the RSS (Fig 6.5).

6.3.3 COMPARISON OF THE MODELLED RATES OF SEX CHANGE

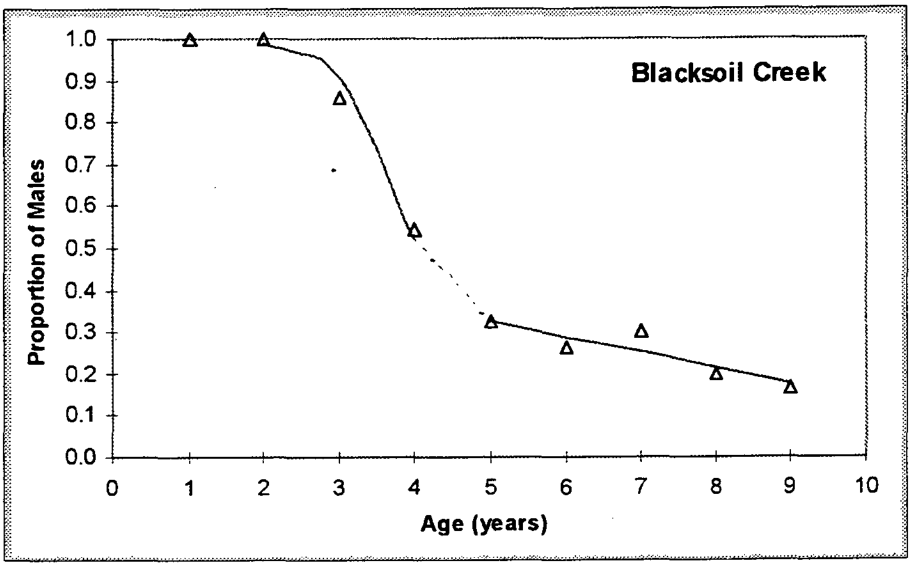
6.3.3.1 COCOA AND CATTLE CREEKS

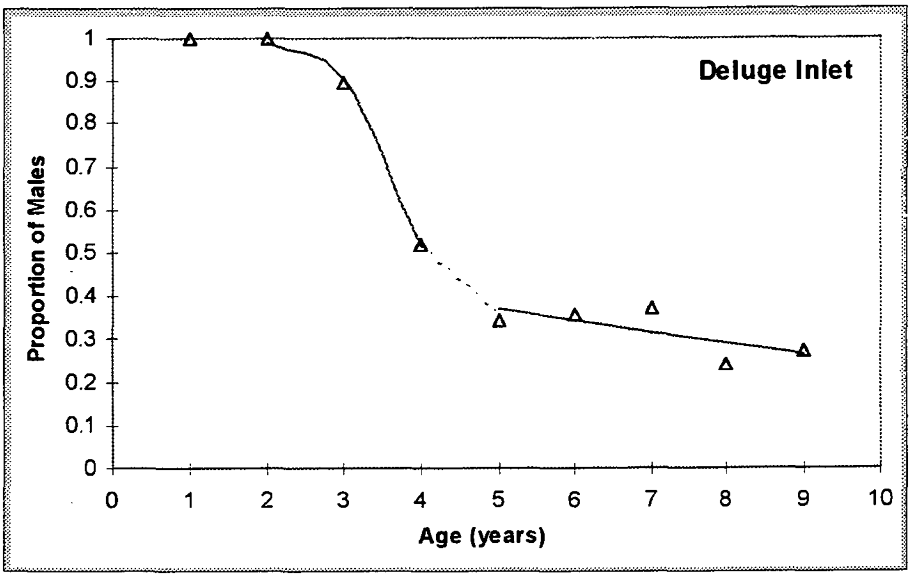
Considerable variation between the modelled rates of sex change was evident between locations (Fig 6.6). The extent of variation(s) between the logistic models fitted to Cocoa and Cattle Creeks were compared by dummy variable analysis. The analysis identified that a separate slope term was required for Cattle Creek (Table 6.2).

The change in deviance ($\Delta D = 6.08$) and degrees of freedom ($\Delta df = 3$) can be used to judge the importance of additional terms as deviance has an asymptotic χ^2 distribution (Nelder & Wedderburn, 1972). The dummy variable analysis conducted for the logistic modelling of Cocoa and Cattle Creeks resulted in less terms required in the model, and importantly the changes made to modelling did not result in a significantly worse fit for the description of the rates of sex change ($\Delta D = 6.08$, $\Delta df = 3$, $p > 0.10$).

Figure 6.4 : Linear regressions fitted to the latter age data of Blacksoil Creek, Deluge Inlet and Mendel Creek.

Figure 6.4


Blacksoil Creek
Proportion of Males
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
1
2
3
4
5
6
7
8
9
10
Age (years)


Deluge Inlet
Proportion of Males
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
4
5
6
7
8
9
10
Age (years)

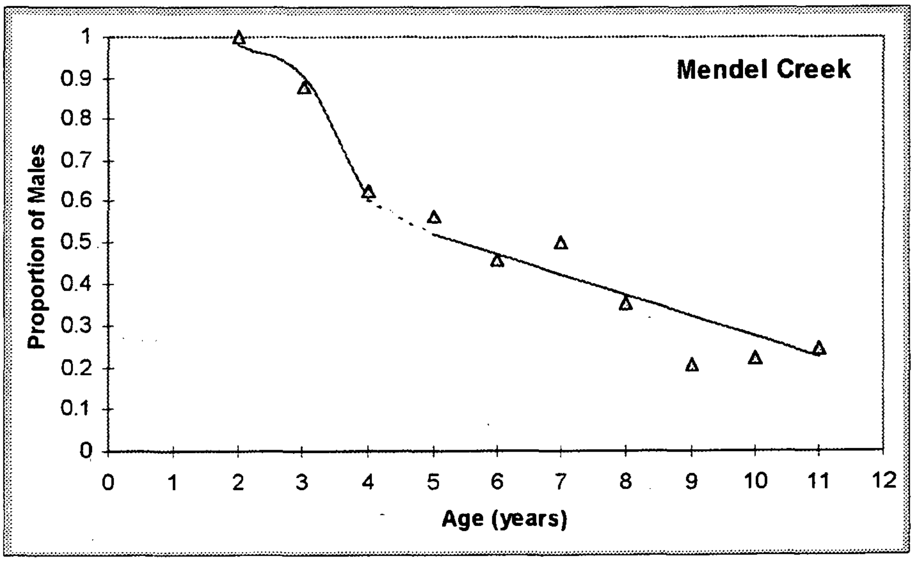

Mendel Creek
Proportion of Males
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
4
5
6
7
8
9
10
11
12
Age (years)

Figure 6.5 : Scree-plots identifying those breakpoint age-classes minimising the residual sum of squares of the complete models.

Figure 6.5

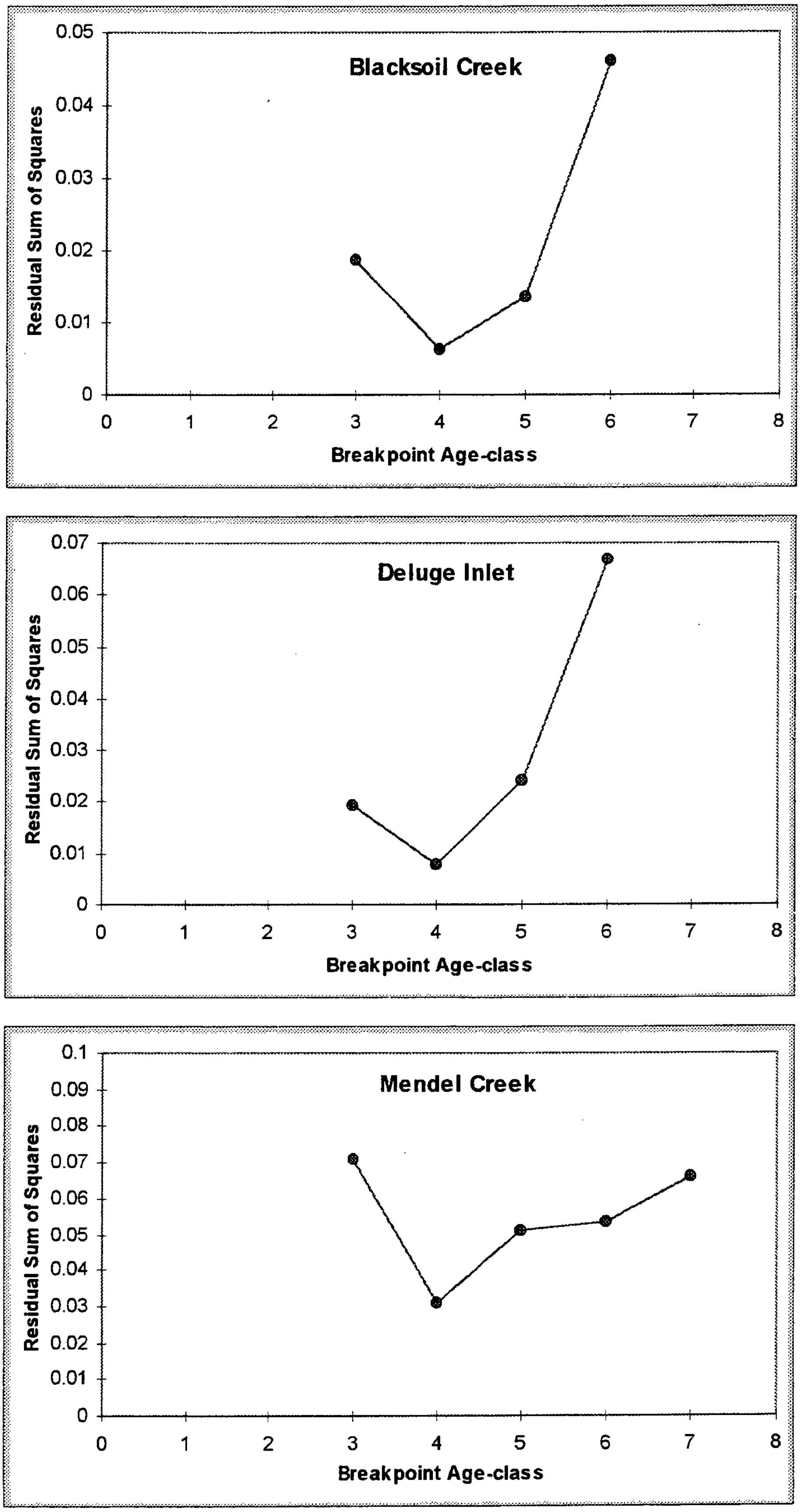

Blacksoil Creek
Residual Sum of Squares
0.05
0.04
0.03
0.02
0.01
0
0
1
2
3
4
5
6
7
8
Breakpoint Age-class
Deluge Inlet
Residual Sum of Squares
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
1
2
3
4
5
6
7
8
Breakpoint Age-class
Mendel Creek
Residual Sum of Squares
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
1
2
3
4
5
6
7
8
Breakpoint Age-class

Figure 6.6 : The variation present between the individual models before comparison.

Figure 6.6

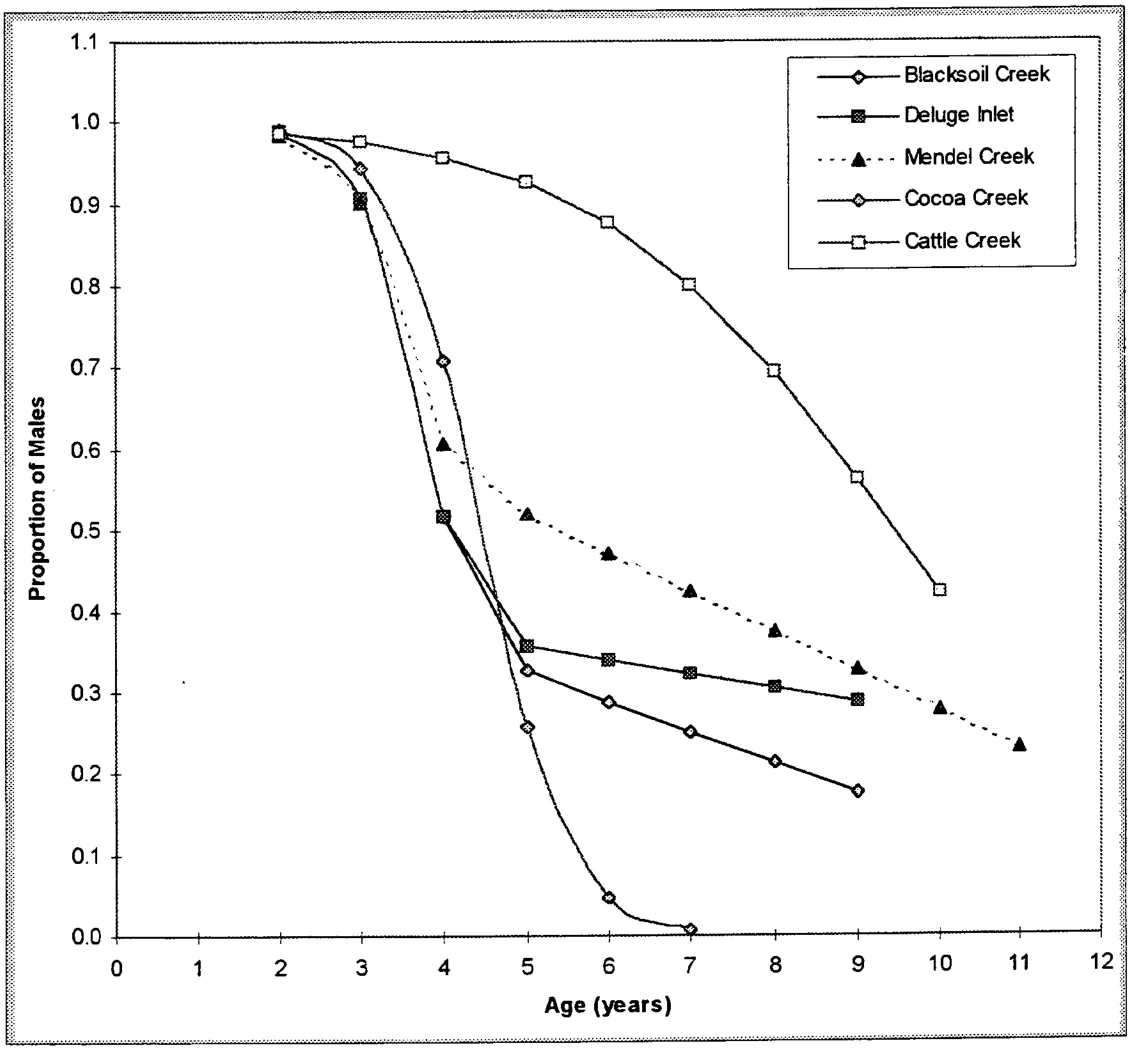

Blacksoil Creek
Deluge Inlet
Mendel Creek
Cocoa Creek
Cattle Creek
Proportion of Males
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
1
2
3
4
5
6
7
8
9
10
11
12
Age (years)

Table 6.2 : Dummy variable analysis was used to compare the logistic models fitted to Cocoa and Cattle Creeks. The analysis identified those parameters not significant to the model (high-lighted in bold). Discarding the intercept term for Cattle Creek allowed for a more descriptive model to be defined. Parameter estimates are shown with standard errors.

Location	Parameters	Parameter Estimates	Probability
Cocoa Creek	intercept (a)	8.70 (1.24)	0.0000
	slope (b)	-1.95 (0.31)	0.0000
Cattle Creek	**intercept (a)**	**6.16 (1.50)**	**0.0912**
	slope (b)	-0.66 (0.33)	0.0001
	Dev. = 16.16	**Prob. = 0.3715**	**df = 15**

The Final Model :

Cocoa Creek	intercept (a)	7.53 (0.67)	0.0000
	slope (b)	-1.62 (0.17)	0.0000
Cattle Creek	slope (b)	-0.85 (0.09)	0.0000
	Dev. = 22.24	**Prob. = 0.2213**	**df = 18**

6.3.3.2 BLACKSOIL CREEK, DELUGE INLET AND MENDEL CREEK

The logistic and linear portions of the models were compared separately. Dummy variable analysis indicated variation between the logistic models was minimal and may be described by a single model equation (Table 6.3). Similarly, dummy variable analysis indicated that the linear portions of the modelled rates of sex change for Blacksoil and Mendel Creeks and Deluge Inlet may also be described by a single linear regression model (Table 6.4).

Table 6.3 : Dummy variable analysis comparing the fitted *logistic models* of Blacksoil Creek, Deluge Inlet and Mendel Creek. The analysis identified those parameters not significant to the model (high-lighted in bold). A common logistic model was found to model the initial rate of sex change for Blacksoil Creek, Deluge Inlet and Mendel Creek combined. Parameter estimates are shown with standard errors.

Location	**Parameters**	**Parameter Estimates**	**Probability**
Deluge Inlet	intercept (a)	7.63 (1.89)	0.0001
	slope (b)	-1.80 (0.52)	0.0005
Blacksoil Creek	**intercept (a)**	**8.47 (2.41)**	**0.7267**
	slope (b)	**-2.10 (0.66)**	**0.6487**
Mendel Creek	**intercept (a)**	**8.87 (2.48)**	**0.6164**
	slope (b)	**-2.20 (0.68)**	**0.5468**
	Dev. = 4.70	Prob. = 0.4532	df = 5

Final Model :

Deluge, Blacksoil	intercept (a)	8.41 (0.95)	0.0000
& Mendel	slope (b)	-2.06 (0.26)	0.0000
	Dev. = 5.59	Prob. = 0.78	df = 9

Again dummy variable analysis identified terms not useful in the logistic modelling of the rate of sex change for Blacksoil and Mendel Creeks and Deluge Inlet. Importantly the discarding of the non-significant parameters of the model did not result in a significantly worse fit for the description of the rates of sex change ($\Delta D = 0.89$, $\Delta df = 4$, $p > 0.90$).

Table 6.4 : Dummy variable analysis used to compare the *linear models* of the modelled rates of sex change for Blacksoil Creek, Deluge Inlet and Mendel Creek. The analysis identified those parameters not significant to the model (high-lighted in bold). A common linear model was found for Deluge Inlet and Blacksoil and Mendel Creeks. Standard errors are shown in parentheses.

Location	Parameter	Estimate	Probability
Mendel Creek	intercept (a)	0.761 (0.071)	0.0000
	slope (b)	-0.048 (0.007)	0.0000
Deluge Inlet	intercept (a)	0.447 (0.116)	0.0177
	slope (b)	-0.018 (0.004)	0.0481
Blacksoil Creek	**intercept (a)**	**0.515 (0.145)**	**0.1123**
	slope (b)	**0.038 (0.019)**	**0.5927**
	F = 10.61	df = 5,13	p = 0.0003

Re-run model discarding Blacksoil terms :

Mendel Creek	intercept (a)	0.581 (0.090)	0.0000
	slope (b)	-0.034 (0.011)	0.0065
Deluge Inlet	**intercept (a)**	**0.447 (0.171)**	**0.4458**
	slope (b)	**-0.018 (0.012)**	**0.4568**
	F = 3.64	df = 3,15	p = 0.0300

Final Model : (discarding Deluge Inlet terms)

Mendel,	intercept (a)	0.545 (0.073)	0.0000
Blacksoil,			
Deluge	slope (b)	-0.030 (0.009)	0.0038
	F = 11.22	df = 1,17	p = 0.004

6.3.4 FINAL MODELS

The comparison of the modelled rates of sex change has identified 3 models that are able to sufficiently describe the rate of sex change for the five locations sampled (Fig 6.7). Model 1, a combination of logistic and linear modelling, was found to sufficiently describe the rate of sex change for Blacksoil Creek, Deluge Inlet and Mendel Creek. Model 2, a single logistic model was appropriate for describing the rate of sex change for Cocoa Creek, while a further logistic model (Model 3) was required to describe the rate of sex change for Cattle Creek.

Interestingly, the same model (Model 1) described the rate of sex change for Deluge Inlet and Mendel Creek (Fig 6.8). This is in agreement with the similarities in the growth of *A. berda* detected earlier (see Chapter 5).

6.3.5 ESTIMATING RATES OF SEX CHANGE

The differences observed in the modelled curves were reflected in differences in the estimated probabilities of sex change for each age-class (Table 6.5). Model 1 predicted sex change in Blacksoil Creek, Mendel Creek and Deluge Inlet was most likely to occur in the 3rd and 4th age-classes. Model 2 indicated the probability of sex change increased with age in Cocoa Creek, reflecting the absence of male *A. berda* in the older age-classes. Model 3 predicted that the probability of a young male changing sex at Cattle Creek was considerably less than that for any other location. After the 6th age-class, the probability of a Cattle Creek male changing sex begins to increase.

Figure 6.7 : The final three models after comparison.

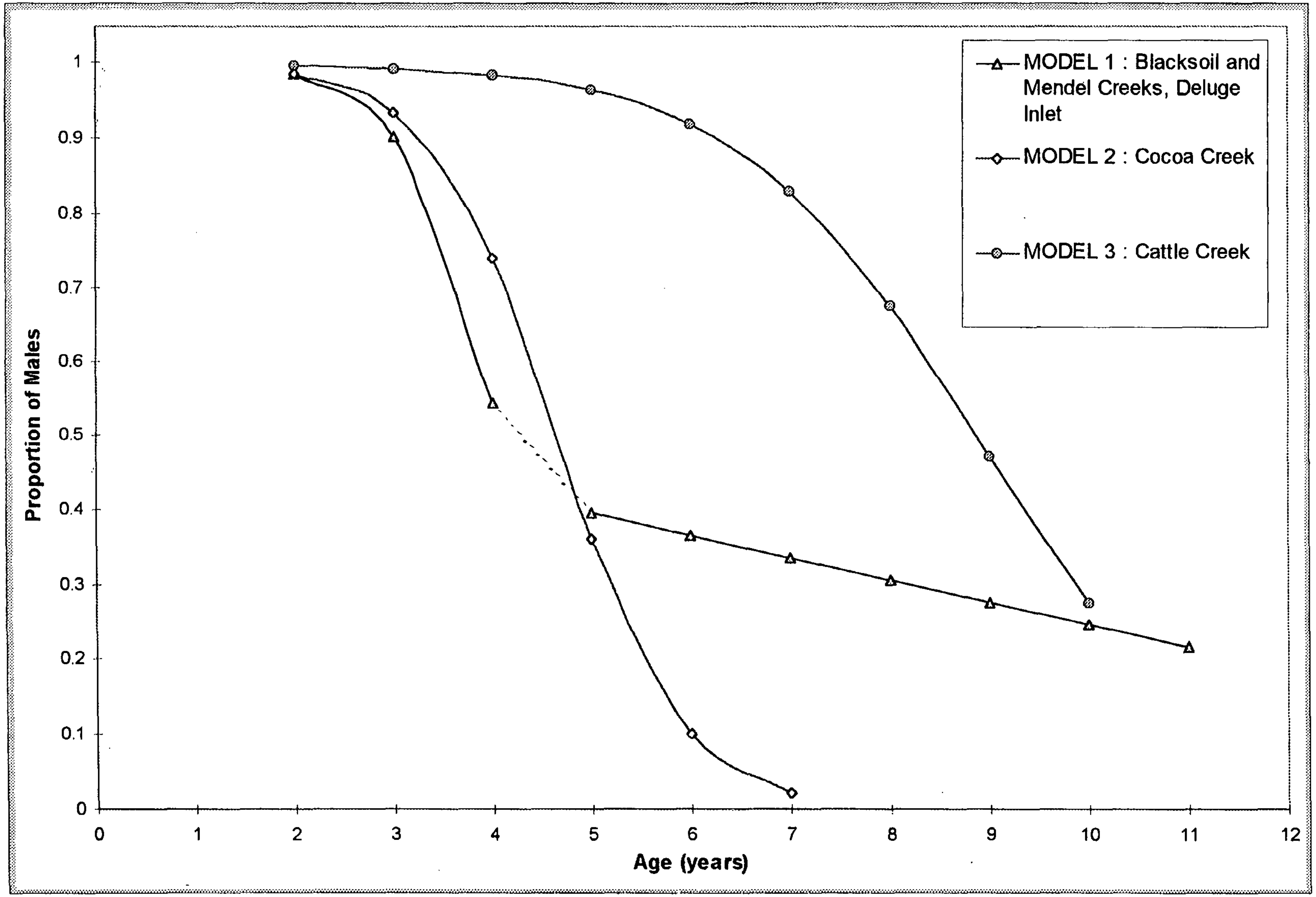

MODEL 1 : Blacksoil and Mendel Creeks, Deluge Inlet
MODEL 2 : Cocoa Creek
MODEL 3 : Cattle Creek
Proportion of Males
Age (years)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
4
5
6
7
8
9
10
11
12

Figure 6.7

Figure 6.8 : Location map showing the relationship between estuary location and model type.

Figure 6.8

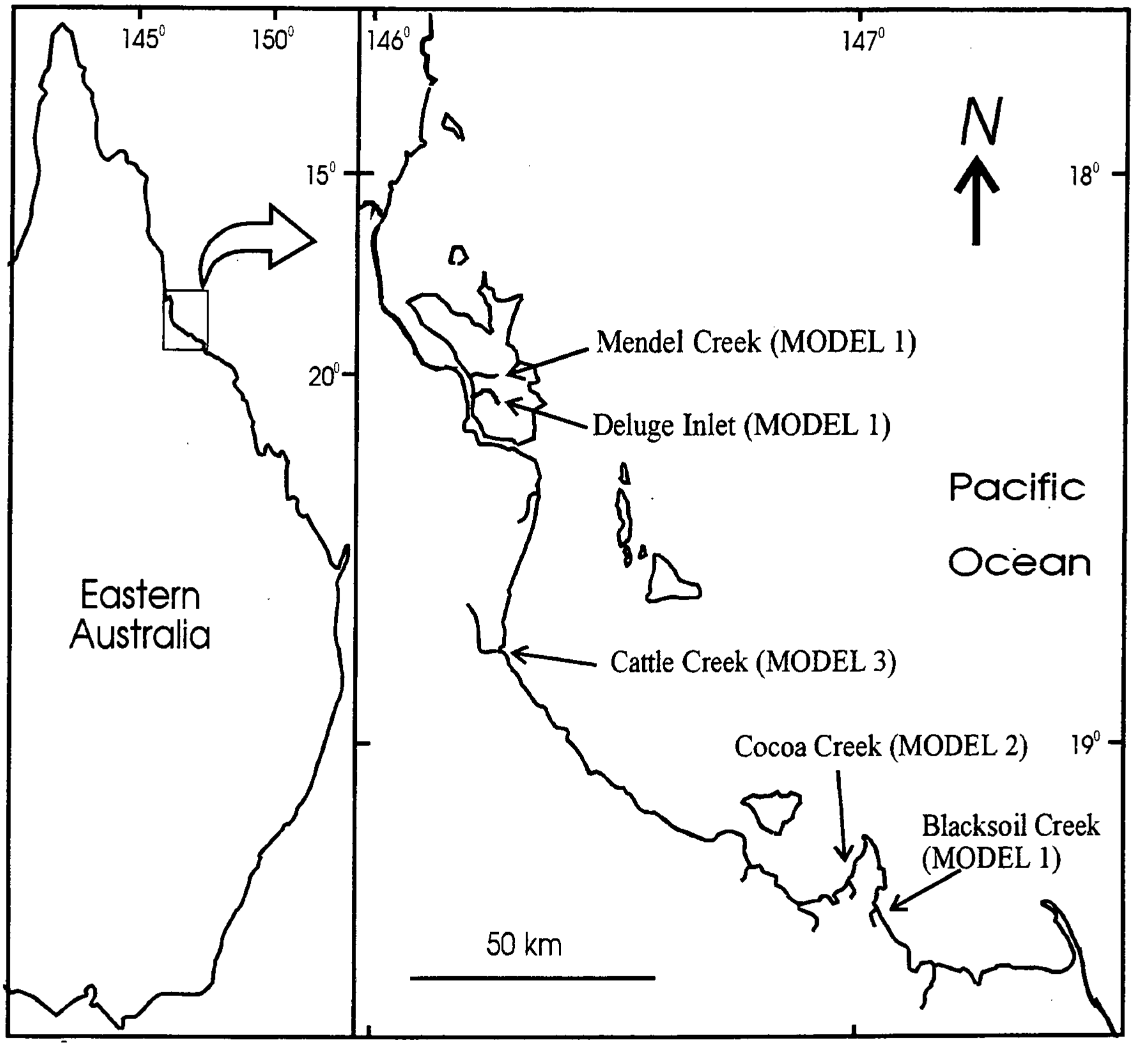

145°
150°
15°
20°
Eastern Australia
146°
147°
18°
19°
N
Mendel Creek (MODEL 1)
Deluge Inlet (MODEL 1)
Pacific Ocean
Cattle Creek (MODEL 3)
Cocoa Creek (MODEL 2)
Blacksoil Creek (MODEL 1)
50 km

Table 6.5 : The estimated probabilities of a male fish changing sex in each age-class.

PROBABILITY OF SEX CHANGE BY AGE					
	Model 1			**Model 2**	**Model 3**
Age	Blacksoil Creek	Mendel Creek	Deluge Inlet	Cocoa Creek	Cattle Creek
1					
2	0.08	0.08	0.08	0.05	
3	0.40	0.40	0.40	0.21	0.01
4	0.27	0.27	0.27	0.51	0.02
5	0.08	0.08	0.08	0.72	0.05
6	0.08	0.08	0.08	0.78	0.10
7	0.09	0.09	0.09		0.19
8	0.10	0.10	0.10		0.30
9			0.11		0.42
10			0.12		

6.4 DISCUSSION.

6.4.1 MODELLING THE RATE OF SEX CHANGE

The relative abundance of male fish decreased with increasing age at all locations. Variation in the rate of decrease with age was evident between locations. The LOWESS regression model fitted to the proportional data sets, identified some common trends in the data sets. The LOWESS trends suggested two stage models may be required for Blacksoil Creek, Mendel Creek and Deluge Inlet data sets; separating an initial rapid decrease in proportion of males in the younger age-classes from a more gradual decrease in the older age-classes. In contrast, the LOWESS trends indicated single models would be adequate to model the decrease in proportion of males for Cocoa and Cattle Creeks. Cocoa Creek displayed a rapid transition from male to female dominance, while the Cattle Creek trend suggested a more gradual decrease in proportion of males with increasing age.

While the decrease in proportion of males with age for Cocoa and Cattle Creeks was able to be modelled by logistic regression alone, a combination of logistic and linear regression models were required to model the rate of sex change for Blacksoil Creek, Mendel Creek and Deluge Inlet. Though logistic regression adequately modelled the initial decrease in the proportion of males by age-class, the decrease in proportion of males by age-class showed a tendency to linearise after the 5th age-classes for Blacksoil Creek, Mendel Creek and Deluge Inlet. Linear regression models were required to model the rate of sex change in these older age-classes.

The logistic regression was used in the modelling of this study as the logistic regression allows the relationship between a binomial dependent variable and an independent (either continuous or discrete) variable to be described (Shanubhogue & Gore, 1987; Collett, 1991; Trexler & Travis, 1993). Though high order polynomials may be used to mimic almost any pattern in a data set, the use of polynomial regression for modelling the rate of sex change of *A. berda* was avoided for two reasons. Firstly, polynomials can be very sensitive to outliers and are therefore not robust; and secondly, polynomials are not flexible because they do not fit values locally; the shape of data at large values of the independent variable will determine the fitted values at smaller values of the independent variable (Trexler & Travis, 1993).

Dummy variable analysis used to compare the logistic models of Cocoa and Cattle Creeks and the logistic and linear model portions of Blacksoil Creek, Deluge Inlet and Mendel Creek indicated that 3 models may be used to describe the rate of sex change of *A. berda* in the five locations sampled. Model 1, a combination of logistic and linear regressions described the rate of sex change for Blacksoil and Mendel Creeks and Deluge Inlet. Model 2 and Model 3, both logistic models represented the rate of sex change for Cocoa Creek and Cattle Creek respectively.

6.4.2 THE RATE OF SEX CHANGE MODELLED FOR *A. BERDA*

The modelling conducted in this study has suggested the protandrous sex change of *Acanthopagrus berda* is a plastic process that varies between age-classes and between locations in northern Queensland, Australia. Sex change in Blacksoil and Mendel Creeks as well as Deluge Inlet was predicted to be most likely undertaken by 3 and 4 year old fish, while sex change in Cocoa Creek was predicted as more likely in the older age-classes. Similarly, the likelihood of sex change was higher in the older age-classes of *A. berda* from Cattle Creek.

The high rate of sex change in the older age-classes of Cocoa Creek reflects the absence of male fish in the older age-classes (see Chapter 2). The high probabilities of sex change in the older age-classes suggest that all male *A. berda* in Cocoa Creek change sex. Similarly, sex change was predicted as more likely in the older fish of Cattle Creek. However, rather than an absence of males in the older age-classes, the higher rate of sex change estimated for the older fish of Cattle Creek probably reflects the paucity of younger female fish collected in the sample taken from Cattle Creek.

In contrast to the pattern modelled for Cocoa Creek, male fish are known to persist into the older age-classes of both the Blacksoil Creek, Mendel Creek and Deluge Inlet samples (see Chapter 2). As a consequence, the estimated probabilities of sex change in the older age-classes were considerably lower, and suggests that some male fish in these populations may reproduce only as males throughout life and not undertake sex change. Being an old male of a large size may be advantageous even in a protandrous sex changing species. If only relatively few males persist in the older age-classes, these large males may be able to better monopolise spawning events and successfully spawn with more females than smaller males. Subsequently, if large males were able to better monopolise spawning events, the smaller less successful spawning males may be more likely to undertake sex change.

Though in some locations (Blacksoil, Mendel and Cocoa Creeks as well as Deluge Inlet) some sex change was estimated to occur in two-year old fish, the probability or proportion of males predicted to change sex was low. The low proportion of two year old males estimated to change sex may not be surprising. Fish of two years of age are generally small (130 to 150 mm fork length), and may not have the required energy stores to be able to undertake the sex change process and develop a mature and functional ovary. Further, it has often been stated that being a large rather than a small female is more advantageous it terms of reproductive output (Fricke & Fricke, 1977; Warner, 1988; Mann & Mann, 1995). Larger females may have the ability to produce more eggs each spawning season. A strong and positive correlation between body weight and ovary weight exists for *A. berda* (unpub. data), and suggests larger female *A. berda* spawn more eggs each spawning season relative to smaller females. Considering this, it may be an advantage for male *A. berda* to delay change sex until achieving a reasonable size.

Considering sex change has been widely documented in many sparids (Buxton & Garratt, 1990), it is surprising that relatively few studies have reported at what age or age range sex change is likely to occur. Of those studies that have investigated age at sex change most report sex change is most likely to occur in the younger age-classes. Waltz *et al.* (1982), reported sex change occurred predominantly between the 2nd and 4th age-classes for the protogynous *Calamus leucosteus* while Kraljevic *et al.* (1996) and Buxton and Clarke (1986) reported the protogynous sex change of *Lithognathus mormyrus* and *Pachymetopon aeneum* respectively, was most likely to occur between the 4th and 8th age-classes. Similarly, Abou-Seedo *et al.* (1990) suggested the protandrous sex change of *Diplodus sargus kotschyi* in the Arabian Gulf occurred largely in the second year of life.

Evidence of geographical variability in the protandrous sex change of *Diplodus sargus kotschyi* is presented with Micale *et al.* (1987) reporting sex change in *D. sargus kotschyi* in the Mediterranean is not undertaken until at least the third year of life. However, the differences in the sex change process of *D. sargus kotschyi* reported by Micale *et al.* (1987) and Abou-Seedo *et al.* (1990) may represent differences in sampling methodologies between the studies rather than natural geographical variation in the sex change process of *D. sargus kotschyi*. The sampling methodology of the current study was standardised between locations so as not to bias the samples collected.

The variation in the rates of sex change for *A. berda* between locations should not be surprising considering the variability in growth previously detected (Chapter 5). Further, a number of sparid studies have suggested geographical variability in the sex change process. As previously noted, the nature of the protandrous sex change of *Diplodus sargus kotschyi* has been suggested to vary geographically [(Micale *et al.*, 1990) c.f. (Abou-Seedo *et al.*, 1990)]. Further, in reporting protandrous sex change in the sparid *Pagellus bogaraveo*, Krug (1990) suggested variation in the sex change process may occur between geographically isolated stocks as an adaptive response to population changes. In support of this statement, Alekseev (1982) has also suggested the nature of sexuality of sparids may differ in different populations.

The present study has shown that the likelihood of male fish of particular age-classes changing sex varies considerably between locations. Further, the age range over which sex change occurs was also found to vary between locations. These findings present interesting information that further supports the possibility that genetic sub-stocks of *A. berda* may occur in the estuaries of tropical Australia. The current study found that the rate of sex change for *A. berda* collected from Deluge Inlet and Mendel Creek may be described by a common model. In conjunction with the similarity detected in the growth of *A. berda* from Deluge Inlet and Mendel Creek (Chapter 5), this finding presents further evidence that sub-stock structuring of *A. berda* may exist in the central Hinchinbrook Channel region. The future sampling of other locations within and outside this region (i.e. the central Hinchinbrook Channel) may confirm or refute this suggestion.

Certainly further more extensive research is required. However, it seems likely the variability detected here may be due to differences in the population size, structure (sex ratios) and densities of *A. berda* between the locations sampled. Longevity and maximum size of male and female *A. berda* has been shown to vary between locations (Chapter 5), and these differences are certainly reflected in the variation detected in the modelled rates of sex change. The locations sampled vary markedly in the physical characteristics of size/volume, habitat structure and spawning site availability. Although no data are available, it seems likely that the carrying capacity (total number of *A. berda*) of the estuaries sampled varied considerably. Since many sex change studies have reported demographic influences on the rate / occurrence of sex change (e.g. Fishelson, 1970; Shapiro, 1981; Fricke, 1983; Cole & Robertson, 1988; Shapiro *et al.*, 1993), estimations of population sizes and densities (especially within the winter spawning aggregations) may provide data on population demographics of *A. berda* that may allow the relevance of the variability in the rates of sex change modelled in this study to be investigated in more detail.

Future continued monitoring combined with a genetic study may identify whether discrete sub-populations of *A. berda* do in fact exist in tropical Australia. Allozyme electrophoretic techniques have been used successfully to identify genetically isolated stocks of the temperate sparid *Pagrus auratus* (Smith *et al.*, 1978; MacDonald, 1980; Johnson *et al.*, 1986), and for the successful stock structure analysis of *Acanthopagrus latus* in Iraq waters (Al-Hassan, 1990), and may be useful for determining the extent of genetic variability or isolation of stocks of *A. berda* in tropical Australia.

CHAPTER 7.

GENERAL DISCUSSION

7.1 DETERMINING THE REPRODUCTIVE STRATEGY OF *ACANTHOPAGRUS BERDA*

The tropical sparid *Acanthopagrus berda* has been shown to undergo protandrous sex change. The methodology of this study incorporated multiple diagnostics including length- and age-frequency distributions, adult sex ratios, both macroscopic and microscopic gonad descriptions, and monitoring the sexuality of individual fish through time. However, though length- and age-frequency distributions, adult sex ratios and gonad structural descriptions all indicated protandrous sex change as the likely reproductive strategy of *A. berda*, monitoring the sexual status of individual fish in the field and in aquaria produced the only unequivocal evidence of sex change. Previously, no other sparid study had observed an individual fish function initially as one sex before later in life functioning as the opposite sex.

Though this type of direct assessment of reproductive strategy has not previously been achieved for any other sparid, the use of similar in-field and captive experiments should be encouraged in the future. Many sparid studies reporting sex change as a reproductive strategy have based their conclusions on the indications of only a few diagnostics (see Table 1.1). Further, none of the diagnostics listed in Table 1.1 provide definitive evidence of sex change. Not surprisingly, a number of contradicting reports may be found in the sparid sex change literature (see Table 1.1). It has been suggested that these conflicting reports may be the result of inconsistencies in the diagnostic criteria (Cody & Bortone, 1992) and/or terminologies (Buxton & Garratt, 1990) utilised by the studies. Alternatively, those reports that do offer contradictory reports of reproductive strategy may reflect an as yet undetected degree of plasticity in the reproductive strategy of some sparids. It may be possible that spatially separated populations of the same species display markedly different reproductive strategies in response to localised differences in population dynamics or environmental factors (e.g. Rowland & Snape, 1994). In order to investigate this potential, every effort should be made to standardise the procedures for determining the reproductive strategies of sparid fish to allow intra-specific studies to be better compared.

Future sparid studies aiming to define a reproductive strategy should be based upon the collection of large replicate samples, ideally from a number of spatially isolated locations, and the use of multiple diagnostic criteria. The collection of replicate samples assessed for common patterns should be encouraged, because as evident in the adult sex ratio samples collected by this study, a common pattern may not be displayed in all samples collected. Replicate samples may allow anomalous locations or populations to be identified, and importantly may provide more confident indications of reproductive strategies. It is suggested that because large adult sex ratio, and length- and age-frequency samples are relatively easy to collect and analyse, these diagnostics should form the basis of future sparid reproductive studies.

Though many sparid reproductive studies have presented sound evidence of ovotestes in transitional stages of sex change, the identification of transitional ovotestes in *A. berda* was difficult and certainly could not be claimed a conclusive diagnostic of protandrous sex change. The transitional ovotestes of *A. berda* were classified on the basis of degeneration of the testis lobe alone as no development of the accompanying ovarian lobe was observed. Without actually observing the future function of the ovotestis as the opposite sex (as female, in the case of *A. berda*), doubt has to remain as to whether sex change would have occurred at all.

The sequence of gonadal events has been investigated in detail for a limited number of sparids, and suggests that a considerable degree of plasticity exists in developmental pathways of the ovotestis (e.g. Shapiro, 1992; Besseau & Brusle-Sicard, 1995). In order to confidently describe a transitional ovotestis structure and the accompanying sex change pathway, it may be possible to conduct serial biopsies on the ovotestes of *A. berda* specimens throughout the duration of the sex change process. Unfortunately though, the intrusive and disruptive process of biopsying the ovotestis may interfere with normal gonad development.

In an effort to produce definitive evidence of sex change, monitoring the sexuality of individual fish in the field should be attempted. If monitoring fish in the field is logistically difficult (as would be the case with particularly vagrant species), an aquarium experiment may need to be implemented. Though the results of aquarium experiments may be limited by the captive environment (Garratt, 1993), the results will at the very least demonstrate that the capacity for sex change does in fact exist.

Future sparid studies should consider it paramount that reproductive strategies are defined with certainty, as not only do many sparids support important recreational (e.g. Pollock & Williams, 1983; Rowland, 1984; Smale & Buxton, 1985; Buxton & Clarke, 1989; Smale & Punt, 1991; Scott *et al.*, 1993) and commercial fisheries (e.g. Pollock, 1980; Francis & Pankhurst, 1988; Vaughan *et al.*, 1992; Bennett, 1993; Chang *et al.*, 1995), but many sparids also form the basis of numerous aquaculture ventures (e.g. Garratt *et al.*, 1989; Kime *et al.*, 1991; New, 1991; Cavari *et al.*, 1993; Gwo, 1994; Reina *et al.*, 1994). Considering the world-wide economic importance of these industries, any future study should strive to provide a comprehensive description and understanding of the reproductive biology of sparid species.

7.2 GEOGRAPHICAL VARIATION IN GROWTH AND RATE OF SEX CHANGE

Considerable variation in the growth of *A. berda* was detected between the locations sampled by this study. Though reports of large scale geographical variations in growth are not uncommon in sparid studies (e.g. Longhurst, 1958; Paul, 1976; Sanders & Powell, 1979; Francis & Winstanley, 1989; Krug, 1990; Paul, 1992; Buxton, 1993), the current study identified growth variations in *A. berda* over relatively small spatial scales (10s of kilometres).

Interestingly, the findings of this study suggest the growth of *A. berda* in the central Hinchinbrook Channel region may be stunted and a possible explanation is a pattern of unique hydrodynamic forces operating within the channel system. Trapping of water within the central region of the Hinchinbrook Channel (where Deluge Inlet and Mendel Creek are located) has been demonstrated by Wolanski *et al.* (1990), and may act to limit the natural genetic dispersal of *A. berda* spawning within this region. A distinct stock structuring of *A. berda* may have occurred within this region and may explain the pattern of growth modelled. Large *A. berda* have been sampled from locations at both the southern and northern ends of the channel system (pers. obs.), and though small sample sizes prevented any further growth rate analysis and comparison by this study, indications were present that *A. berda* at the southern and northern extremes of the channel have faster growth rates and achieve larger sizes than fish collected from within the central channel. An interesting direction of future research would model the growth for *A. berda* at an even finer scale by sampling from a number of locations along the length of the channel system. If the hydrodynamics of the channel system are responsible for the stunted growth in the mid-channel region, it may be possible to detect a clinal pattern in *A. berda* growth along the length of Hinchinbrook Channel. Alternatively, if the slower growth of *A. berda* within the central channel region is the result of natural spatio-temporal variability, this may also be identified.

Further, though considerable variation was detected between locations separated by only relatively small distances in this study, it may be possible that larger variations in growth occur throughout the extensive Australian and international range of *A. berda*. In fact, the growth rate reported for *A. berda* in Kuwait waters by Samuel & Mathews (1987) indicates that although *A. berda* in the Kuwait region do not live longer, they do achieve much larger sizes. Anecdotal evidence received from recreational anglers suggests that *A. berda* inhabiting more northern Australian waters (in particular the waters of the Gulf of Carpentaria) may achieve considerably larger sizes than those sampled during the current study. Further investigation of the growth characteristics of *A. berda* throughout its extensive distributional range is warranted.

Following the geographical variability detected in the growth of *A. berda*, the modelled rates of sex change also varied considerably between locations. Both the rate of sex change and the age range over which sex change occurred varied between locations. While the proportion of male fish decreased from 1 to 0 over only 5 age-classes for Cocoa Creek, the proportion of males at all other locations approached 0 over at least 8 age-classes. Similar to other sparid studies (Waltz *et al.* 1982; Buxton & Clarke, 1986; Abou-Seedo *et al.* 1990; Kraljevic *et al.* 1996), most sex change was estimated to occur in the younger (3rd to 5th age-class) rather than the older age-classes for *A. berda*. However, considerable variation was detected by this study with the likelihood of sex change increasing with age for male fish within Cocoa and Cattle Creeks.

The variability detected in the rates of sex change for *A. berda* may also be explained by the existence of spatially separated stocks. Alekseev (1982) suggests the nature of sex change of some sparids may vary between spatially separated stocks of the same species. Though variation was detected between locations separated by only relatively small distances in this study, it is possible that larger variations in the sex change process may occur throughout the extensive Australian and international range of *A. berda*.

A high degree of plasticity in the sexuality of sparids has been briefly discussed (e.g. Shapiro, 1992; Besseau & Brusle-Sicard, 1995). After investigating the ovotestis ontogeny of the protandrous *Lithognathus mormyrus*, Besseau & Brusle-Sicard (1995) suggested considerable intra-specific plasticity in sexuality may occur due to the potentialities of the sparid ovotestis. Further interesting questions not considered by this thesis arise. Though not investigated by the present study, is it possible for female fish to develop and mature directly from the juvenile form? Further, is the ovotestis ontogeny of

A. berda plastic enough that if a particular stock is severely depleted of male fish, it may be possible for a sex changed female to revert back to its original male function? Future studies may wish to examine the possibility of bidirectional sex change which has been recorded in a few teleosts (e.g. Kobayashi & Suzuki, 1992; Sunobe & Nakazono, 1993), and would have considerable implications for the recovery of heavily fished sparid populations.

7.3 IMPLICATIONS FOR MANAGEMENT AND FUTURE DIRECTIONS OF RESEARCH

Worldwide many sparids are exploited by both recreational and commercial fishing industries (e.g. Pollock & Williams, 1983; Smale & Buxton, 1985; Garratt *et al.*, 1989), and in a disturbing trend many sparid stocks have shown notable declines in recent times as a result of fisheries exploitation (Smale, 1988; Garratt, 1985; Buxton, 1993; Garratt, 1996). As many sparid fish have reproductive strategies that include sex change (Buxton & Garratt, 1990; Mann & Mann, 1995), some serious implications for successful fisheries management are encountered. A sex changing reproductive strategy may promote sex-selective fisheries if fishing effort is directed towards the larger individuals (Shaklee & Salini, 1983; Buxton & Clarke, 1986; Garratt, 1986a; Buxton, 1993). This has the potential to significantly alter the normal demographics of populations, which may in turn impair spawning behaviour (Buxton, 1992).

In addition to the fishing pressures exerted on sparids, habitat destruction has also been identified as a contributing factor to the decline of sparid stocks. Jean *et al.* (1990) noted that the abundance of wild sparid populations have decreased in recent times due to a combination of habitat destruction and illegal fishing practices. In fact, the destruction of estuarine habitats world-wide has been recognised as a major problem (Saenger *et al.*, 1983), and may have serious implications for the estuarine dependent *A. berda*. Unfortunately, habitat destruction has already been identified as a major concern for *A. berda* populations in South Africa (Begg, 1978; van der Elst, 1988).

This study has defined the reproductive strategy of *A. berda* and demonstrated considerable plasticity exists in the growth rates and sex change schedules over small spatial scales. The mechanisms for these spatial differences are currently unknown and will require further research to implicate genetic or environmental factors as being responsible. An initial focus of future research should include a temporal analysis of the persistence of these spatial trends over a number of years. Continued monitoring of the population parameters estimated by this study would identify temporal shifts in the protandrous sex change behaviour of *A. berda* if they do occur. Coupled with methods for assessing population demographics and quantifying potentially important environmental variables, may allow a more detailed account of the spatial and temporal dynamics of the protandrous nature of *A. berda* to be made.

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APPENDIX I

Age and growth of the tropical sparid, *Acanthopagrus berda* - with evidence of geographical variability.

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Abstract.

In northeastern Australia *Acanthopagrus berda* is a relatively long-lived and slow-growing sparid rarely attaining lengths greater than 250 mm (FL) or ages in excess of 12 years. The age of *A. berda* sampled from two tropical estuaries (Blacksoil Creek and Deluge Inlet) were determined from sectioned sagittal otoliths. Oxytetracycline marking and marginal increment analysis indicated that one opaque and one translucent band were laid down annually. von Bertalanffy growth curves were fitted to both the Blacksoil Creek and Deluge Inlet data sets separately. Growth increment data collected from tag-recapture experiments did not significantly deviate from the estimated growth curves. Evidence of geographical variation in the growth of *A. berda* between estuaries in tropical Australia is presented.

Introduction.

The pikey or river bream, *Acanthopagrus berda* (Forskål, 1775), is a relatively small sparid with a wide distribution throughout the tropical Indo-Pacific (Munro, 1949; Smith & Heemstra, 1986). *A. berda* is abundant throughout tropical Australia where it inhabits estuarine and shallow coastal waters (Robertson and Duke, 1987; Blaber *et al.*, 1989; Sheaves, 1992). Although widely distributed, little biological information is available for *A. berda*. Garratt (1993) discussed the spawning of *A. berda*, and suggested that on the southeastern coast of Africa *A. berda* underwent protandrous sex change. In a tag-release-recapture study, Tobin *et al.* (1997) were able to demonstrate protandrous sex change occurs in *A. berda* in tropical northeastern Australia. Age and growth of *A. berda* collected from Kuwait waters has been examined by Samuel & Mathews (1987), who suggest potential aquaculture applications because of relatively fast growth rate. However, the aging technique of Samuel and Mathews (1987) was only informally validated "by examination of the general appearance, structure of periodicity of marks" in broken and burnt otoliths.

In this paper the age and growth of *A. berda* collected from two spatially separated locations on the northeastern coast of tropical Australia is presented. The sectioning of sagittal otoliths has been successfully used to age many sparids [eg: *Chrysoblephus puniceus* (Garratt *et al.* 1993), *Dentex gibbosus* (Pajuelo & Lorenzo, 1995), *Pagrus pagrus* (Pajuelo & Lorenzo, 1996) and *Pagrus auratus* (Francis *et al.* 1992)] and was the ageing method adopted in this study. The importance of validating ageing techniques has been stressed repeatedly (eg: Beamish and McFarlane, 1983). Successful age validation studies address the periodicity of otolith band formation, allow confident identification of bands, and clear edge interpretation (Francis *et al.*, 1992). Lang and Buxton (1993) suggest that oxytetracycline (OTC) marking of the otolith is a relatively simple way of validating age estimates in sparid fish. As an adjunct to this, marginal increment analysis allows the pattern and cyclic nature of band formation to be assessed, and has been used successfully in many sparid ageing studies (e.g. Bennett, 1993; Pajuelo & Lorenzo, 1995; Booth & Buxton, 1997).

This study incorporates three methods of validation: OTC marking, marginal increment analysis and tag-release-recapture. OTC marking was conducted in an effort to validate the periodicity of otolith band formation. However, as it is often difficult to validate every band observed in fish otoliths by OTC marking, marginal increment analysis was also conducted. Marginal increment analysis may supplement OTC marking by validating the time of formation of otolith bands not validated by OTC marking. Finally, tag-release-recapture experiments provided growth increment data that was comparable to the growth curves estimated from the von Bertalanffy growth equation. Although a less direct method of validation, growth increment data of individual fish can be compared to the "template" growth trajectory estimated by the von Bertalanffy equation.

Materials and Methods.

Sampling Sites.

A. berda were collected from Blacksoil Creek and Deluge Inlet (Tobin *et al.*, 1997), two estuaries separated by approximately 150 kms of coastline on the north-eastern coast of tropical Australia (Fig. 1). The sagittal otoliths of 562 fish (19 juvenile, 205 males, 341 females) from Deluge Inlet and 269 fish (17 juveniles, 174 males, 88 females) from Blacksoil Creek were removed and cleaned. All adult fish were sampled during the height of the winter spawning period (Tobin *et al.*, 1997) during the period June 1994 to September 1996. At the time of capture the fork lengths (FL) of all fish were measured to the nearest millimetre.

The juvenile fish were collected from Blacksoil Creek and Deluge Inlet by seine netting during March 1996. Assuming a birth date of 1 July 1995 [as the spawning period of *A. berda* extends from June-September with a peak during July-August (unpub. data)], these fish were assigned the age of 0.67 years. The lengths of these new recruits are in agreement with those recorded by Harrison (1991), who followed the growth of juvenile *A. berda* throughout the first year of life.

Otolith preparation and reading.

One otolith from each pair was chosen randomly and mounted in a epoxy resin block before a transverse section was taken through the nucleus. Sections were then polished on 800-grade emerypaper before reading under a low power microscope with a transmitting light source.

A pilot study indicated an opaque and translucent banding pattern was most easily observed along the edge of the sulcus and that clear edge interpretation was also possible on this axis. All band counts were then made along this axis. Each sectioned otolith was observed on at least 2 separate occasions by the author and the number of opaque bands recorded. If the two counts did not agree, an independent observer was asked to count and the author also conducted a third count. If agreement between the author and the independent observer was not achieved the section was rejected as unreadable.

As most otoliths were collected during the June-September spawning period (the assumed time of the winter opaque band deposition), the author considered that the opaque band from the current winter may not yet be visible on the otolith margin. It is suggested these otoliths may be distinguished by the presence of a broad translucent band at the otolith margin, with no evidence of the formation of the winter opaque band. The author considers that inferring an age to these fish by counting the number of opaque bands may underestimate the age of the fish by one year, and add considerable variation to the length-at-age data. Accordingly, although the number of opaque bands counted may have been x, the age recorded was $x+1$. Marginal increment analysis was conducted in order to validate this assumption.

Otolith band validation.

MARGINAL INCREMENT ANALYSIS.

A minimum of 20 otoliths collected from each calender month were examined and the type of band, either opaque or translucent, present on the margin of the otolith noted. The proportion of the type of marginal band throughout the year was examined to determine: A. the time of the year of opaque and translucent band deposition; B. if there was an annual cycle of marginal bands; and C. to substantiate the practice of adding an extra count onto those otoliths sampled early within the spawning period where the opaque band was not yet visible.

OXYTETRACYCLINE MARKING.

During the September 1993 sampling at Blacksoil Creek, 307 fish captured were given intraperitoneal injections of OTC at a dosage of 0.1ml/100gm before being tagged and released. The subsequent recapture of 21 of these fish (over time periods ranging from 123 to 711 days) allowed the assessment of otolith checks for annual periodicity.

A methodology similar to that of Francis *et al.* (1992) was employed for the assessment of annual periodicity of otolith band marks. Before the location of the OTC mark was revealed under an ultraviolet (UV) light source, the expected position of the OTC mark within the otolith section was inferred from a knowledge of time of fish recapture and time at liberty. The true position of the OTC mark was then revealed under UV light and compared to the inferred location.

Growth curves.

FITTING AND COMPARISON OF GROWTH CURVES.

The von Bertalanffy growth equation was fitted to the collected length-at-age data for both Blacksoil Creek and Deluge Inlet fish separately, by non-linear regression using the method of least squares. The residuals obtained by fitting the von Bertalanffy growth curve model were converted to standardised residuals. Data points were classified as unnecessarily large influential points and excluded from analysis if - standardised residuals were greater than 2.5 and their removal resulted in an significant change in the modelled growth curve.

The growth curves for Blacksoil Creek and Deluge Inlet were compared for significant difference by an F-Test. The residual sum of squares (RSS) of two growth models were compared. Model 1 consisted of 6 parameters ($t_{0(1)}$, $k_{(1)}$, $L_{\infty(1)}$ and $t_{0(2)}$, $k_{(2)}$, $L_{\infty(2)}$ for Blacksoil Creek and Deluge Inlet respectively) and was run as a non-linear regression by the method of least squares. The simpler Model 2 was a combination of both Blacksoil Creek and Deluge Inlet length-at-age data sets and was run as a normal three parameter von Bertalanffy equation, again as a non-linear regression using the method of least squares.

GROWTH CURVE VALIDATION.

The recapture of tagged fish provided growth increment data for both Blacksoil Creek ($n = 19$) and Deluge Inlet ($n = 23$). The FL of fish at initial capture and subsequent recapture were known. Fish age at sacrifice was determined by otolith sectioning, and age at initial capture back-calculated from the knowledge of time at liberty.

Utilising the von Bertalanffy growth curve as a template for the trajectory of fish growth, the expected FL of fish at recapture was estimated from a knowledge of initial age and FL. From this, the expected FL at recapture was estimated for each fish by the equation:

$$\mathbf{FL_{exp} = FL_{init} + vBGF_{inc}}$$

where, FL_{exp} = expected FL at recapture; FL_{init} = FL at the time of initial release; and $vBGF_{inc}$ = the predicted increase in FL by the time of recapture assuming fish growth follows the growth trajectory estimated by the von Bertalanffy growth equations. The vBGF $_{inc}$ was calculated for each fish individually by assessing the difference between initial FL and the FL predicted by the von Bertalanffy equation for a fish of that age.

The expected FLs at recapture as predicted by the von Bertalanffy growth equation were compared to the true (observed) FLs at recapture by paired t-tests for departure from the von Bertalanffy growth trajectory.

Results.

A distinct pattern of alternating translucent and opaque bands was discernible on the sectioned sagittae of *A. berda* (Fig. 2) with only 7 and 12 otoliths rejected as unreadable from Blacksoil Creek and Deluge Inlet respectively. Opaque bands, assumed to coincide with slower winter growth, appeared narrow and dark when viewed under transmitted light. The contrasting lighter translucent bands, assumed to be deposited on the otolith margin during the months of faster summer growth, separated the opaque bands.

Otolith check validation.

MARGINAL INCREMENT ANALYSIS.

The pattern of edge increments observed within the sectioned sagittal otoliths of *A. berda* indicated an annual pattern with a pair of opaque and translucent bands laid down each year. Marginal increment analysis showed translucent bands present on the otolith margin from January through to June, while opaque bands were observed from July through to December (Fig 3). This suggests opaque bands are deposited during the winter - spring period, while translucent bands are deposited during summer - autumn. This observation suggests the practice of adding an additional year to some otolith counts is valid.

FLUORESCENT MARKING.

Of the 307 fish injected with OTC and released in Blacksoil Creek during September 1993, 21 fish were recaptured. These fish were at liberty between 0.23 and 3.05 years. Although the OTC fluorescent marker failed to 'take up' in the otoliths of 4 fish, the otoliths of the remaining 17 fish displayed fluorescent marks when observed under ultra-violet light.

The position of the OTC mark on each of these otoliths was correctly inferred from a knowledge of time of OTC injection, and time at liberty. All fish were injected with OTC during September 1993, coinciding with the time of opaque band deposition. As expected, the tetracycline markers were observed to coincide with opaque bands of all otoliths (Fig. 4). The opaque and translucent banding pattern observed on the sagittal otoliths of *A. berda* was then assumed to conform to a yearly cycle with each opaque band counted taken to represent 1 year of growth.

Age and Growth.

In the study area, *A. berda* are relatively long-lived and slow growing, with few fish attaining lengths greater than 300mm FL, or ages in excess of 12 years. Growth of *A. berda* was relatively rapid during the initial 4 to 5 years of growth, before tapering off after about 6 years of age (Fig. 5).

For Blacksoil Creek and Deluge Inlet, 6 and 15 data points respectively were omitted from analysis because of having standardised residuals greater than 2.5. These fish were omitted from the analyses because they were likely to have undue influence on the line fitting procedure.

Growth comparison between locations.

A significant difference between the growth curves of Blacksoil Creek and Deluge Inlet fish was detected ($F_{(3, 817)} = 116.18$, $p < 0.001$)(Fig. 6). Only 0.7% (4 / 565) of the

Deluge fish sampled were larger than 240mm FL as compared to 5.1% (15 / 277) of the Blacksoil sample. Interestingly, this situation was reversed in the age data. Only 1.1% (3 / 277) of fish from Blacksoil Creek were aged at 10 or greater, compared to 7.6% (43 / 565) from Deluge Inlet. These differences were reflected in the L_∞ parameters of the von Bertalanffy growth curves (Table 1).

Table 1 : Comparison of the von Bertalanffy growth curve parameters for Blacksoil Creek and Deluge Inlet estimated by the current study, against those of Samuels & Mathews (1987).

Location	Max. Size	Max. Age	t_0	k	L_∞
Blacksoil Creek	282	12	-0.80	0.28	261
Deluge Inlet	250	15	-0.92	0.30	231
Kuwait (Samuel & Mathews, 1987)	n/a	14	-0.346	0.325	374

GROWTH INCREMENT DATA.

The growth increment data collected from both Blacksoil Creek and Deluge Inlet did not significantly deviate from the growth trajectories predicted by the von Bertalanffy growth equations. Paired t-tests showed no significant differences existed between the observed and the expected FLs at recapture for both Blacksoil Creek ($t_{(df,\ 20)} = 1.72$, $p = 0.10$) and Deluge Inlet ($t_{(df,\ 22)} = 2.08$, $p = 0.91$) fish. The increment data plotted over the growth curves showed individual fish growth did not differ appreciably from the average growth for either Blacksoil Creek or Deluge Inlet (Fig. 7).

Discussion.

Otolith validation.

In validating the ageing of *Pagrus auratus* by sectioned otoliths, Francis *et al.* (1992) recommended that successfully validated otolith studies clearly address three potentially confounding factors; the correct identification of otolith bands, assessment of the periodicity under which otolith bands are deposited, and the potential misinterpretation of the otolith margin and associated bands.

In the sectioned sagittal otoliths of *Acanthopagrus berda*, a clear and consistent alternating pattern of opaque and translucent bands was observed. Considering the spawning period of *A. berda* coincides with winter, the banding pattern in the otolith would be expected to be quite clear. Growth is generally considered to slow or cease during the colder months of the year, and coupled with energetically stressful spawning may be expected to produce a clear alternating pattern of opaque and translucent bands.

Marginal increment analysis indicated that the opaque band (coinciding with the slow-growth of winter and concomitant spawning), is deposited on the margin of the sagittal otoliths during the winter - spring (June to October) period, and the fast-growth translucent band during summer - autumn. However, although the opaque band may be forming on the otolith margin during the winter months, its presence may not be easily recognised on the otolith margin until some subsequent deposition of the following summer translucent band; a phenomenon also recorded in *Pagrus auratus* by Francis *et al.* (1992). Only translucent bands were observed on the otolith margin from January through to June; a reflection of the fast growth of *A. berda* during this period.

Validation of band periodicity was also achieved using OTC marking of sagittal otoliths. Although the fluorochrome marker failed to take up in the otoliths of four of the 21 fish recaptured, the position of the OTC mark in the other 17 sectioned otoliths was correctly inferred prior to the revealing of the true positions of the OTC marks. Unfortunately, not all of the maximum number of bands observed in this study (15 years being the maximum age detected) were validated by OTC marking. However, combined with the marginal increment data, information from OTC marking provides strong evidence that the opaque-translucent banding patterns in the sagittal otoliths of *A. berda* conform to an annual cycle throughout the lifetime of *A. berda*.

The final requirement of successful validation is that of correct edge interpretation. In their study, Francis *et al.* (1992) encountered difficulty in edge interpretation of fish sampled during spring and summer. Some subjective judgement was involved in determining whether the previous winters' opaque band was yet visible on the otolith margin (Francis *et al.*, 1992). Fish sampled for this study were collected largely within the winter spawning period (June-Sept) of *A. berda*. Although opaque bands were observed on the margins of relatively few otoliths during June and July, the broad translucent band on the remaining otoliths indicated that the opaque band was probably forming and would have become visible soon. This assumption was supported by the large proportion of otoliths observed with opaque bands on their margins during the September - December period.

Age & Growth.

This study has shown *A. berda* sampled from tropical estuaries in North Queensland, Australia to be a relatively short lived and slow growing sparid. Although the maximum age recorded for *A. berda* in this study was 15 years, most fish were aged at less than 12 years. Similar age ranges have been recorded for a number of other sparids; namely *Acanthopagrus australis* (Dredge, 1976), *Lithognathus mormyrus* (Kraljević *et al.*, 1996), *Dentex gibbosus* (Pajuelo & Lorenzo, 1996), *Pagrus pagrus* (Manooch & Huntsman, 1977; Pajuelo & Lorenzo, 1996), and *Pterogymnus laniarias* (Booth & Buxton, 1997).

The size and age ranges of the samples collected from Blacksoil Creek and Deluge Inlet were 64-322 mm / 0.67-14 years and 59-322 mm / 0.67-15 years respectively. Although the L∞ parameters for both Blacksoil Creek (261 mm FL) and Deluge Inlet (231 mm FL) were considerably less than the maximum sizes recorded, fish with FLs greater than 250 mm were uncommon in both samples. The size of *A. berda* sampled from tropical Australia appears to be similar to populations of *A. berda* in South Africa, where although lengths of 450-750 mm have been recorded (van der Elst, 1988) fish rarely exceed 300 mm (Garratt, 1993). Although the data is unavailable, it would be interesting to compare the age and growth of *A. berda* between Australia and other countries throughout its distribution.

Geographical Variation.

Of considerable interest in this study, is a marked difference evident in the age and growth of *A. berda* from the two locations sampled. Blacksoil Creek and Deluge Inlet are separated by approximately 150kms of coastline and *A. berda* is abundant in both systems. At the time of sampling, it was noticed that relatively fewer large fish were sampled from Deluge Inlet as opposed to Blacksoil Creek. This field observation was confirmed by the fitting of von Bertalanffy growth curves and their respective parameters. The parameter for maximum body size L∞, was considerably larger for Blacksoil Creek (259.0) than Deluge Inlet (231.0). This was the case even though substantially more fish of at least 10 years of age were sampled from Deluge Inlet (n = 46) than Blacksoil Creek (n = 5). Not surprisingly, a significant difference between the Blacksoil Creek and Deluge Inlet growth curves was detected.

Geographical differences in age and growth have been suggested for a number of other sparids. Krug (1989) showed marked differences between the age and growth reported for *Pagellus bogaraveo* by four independent studies. Sanders & Powell (1979) detected a significant difference in the growth rates between eastern and western stocks of *Chrysophyrus* (≅*Pagrus*) *auratus* in Victoria, Australia. Paul (1976) reports there may be significant growth variations in the same species sampled from Hauraki Gulf, New Zealand. Some obvious differences in the size distribution of *Petrus rupestris* from various depths and geographical ranges were noted by Smale (1988), however Smale and Punt (1991) consider it unlikely that the species consists of more than one stock. Garratt (1985) reported distinct differences present in the population structure of *Chrysoblephus puniceus* sampled from Natal (South Africa) and Mozambique waters. Although growth was not investigated, the length-frequency distributions and sex-ratios of the samples were significantly different. Garratt (1985) suggests the observed differences between the Natal and Mozambique "populations" may represent exploited and unexploited fisheries respectively.

The evidence presented of geographical variation in the growth of *A. berda* sampled from tropical Australia, warrants a further more thorough investigation. The question is raised as to whether these perceived differences between Blacksoil Creek and Deluge Inlet are the result of genetic stock structuring, or alternatively simple natural spatio-temporal variability. If in fact the differences are the result of genetic stock structuring, such a situation should be identified. Further, if genetic differences between Blacksoil Creek and Deluge Inlet do exist, are these genetic differences as pronounced across smaller spatial scales? Such information is vital to implementing suitable management strategies, and to further the understanding of the dynamics of tropical fish biology in their ever threatened coastal mangrove environment.

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CAPTIONS LIST.

Figure 1. Locality map showing the location of Blacksoil Creek and Deluge Inlet on the tropical northeastern coast of Australia.

Figure 2. Transverse section of a sagittal otolith showing alternating translucent and opaque banding. This fish was sampled from Deluge Inlet on August 17th. Though only eight bands are clearly visible within the section, a ninth band can be seen forming on the otolith margin. Accordingly, age was recorded as 9 years.

Figure 3. Proportions of the type of band on the otolith margin throughout the year - opaque (dashed line) and translucent (solid line) bands. Sample sizes for each month are shown.

Figure 4. Photographs of otolith section under (A.) transmitted light - showing the inferred position of OTC mark (arrows), and (B.) ultraviolet light - showing the true position of the OTC mark.

Figure 5. The von Bertalanffy modelled growth for *Acanthopagrus berda* sampled from (A.) Blacksoil Creek and (B.) Deluge Inlet. Data points excluded as outliers are represented by the symbol □ .

Figure 6. The growth curves modelled for Blacksoil Creek and Deluge Inlet by this study, plotted with that of Samuels and Mathews (1987).

Figure 7. Growth increment data plotted over the von Bertalanffy growth curves for (A.) Blacksoil Creek and (B.) Deluge Inlet. Paired t-tests indicated the growth increment data did not significantly deviate from the average fish growth.

Figure 1.

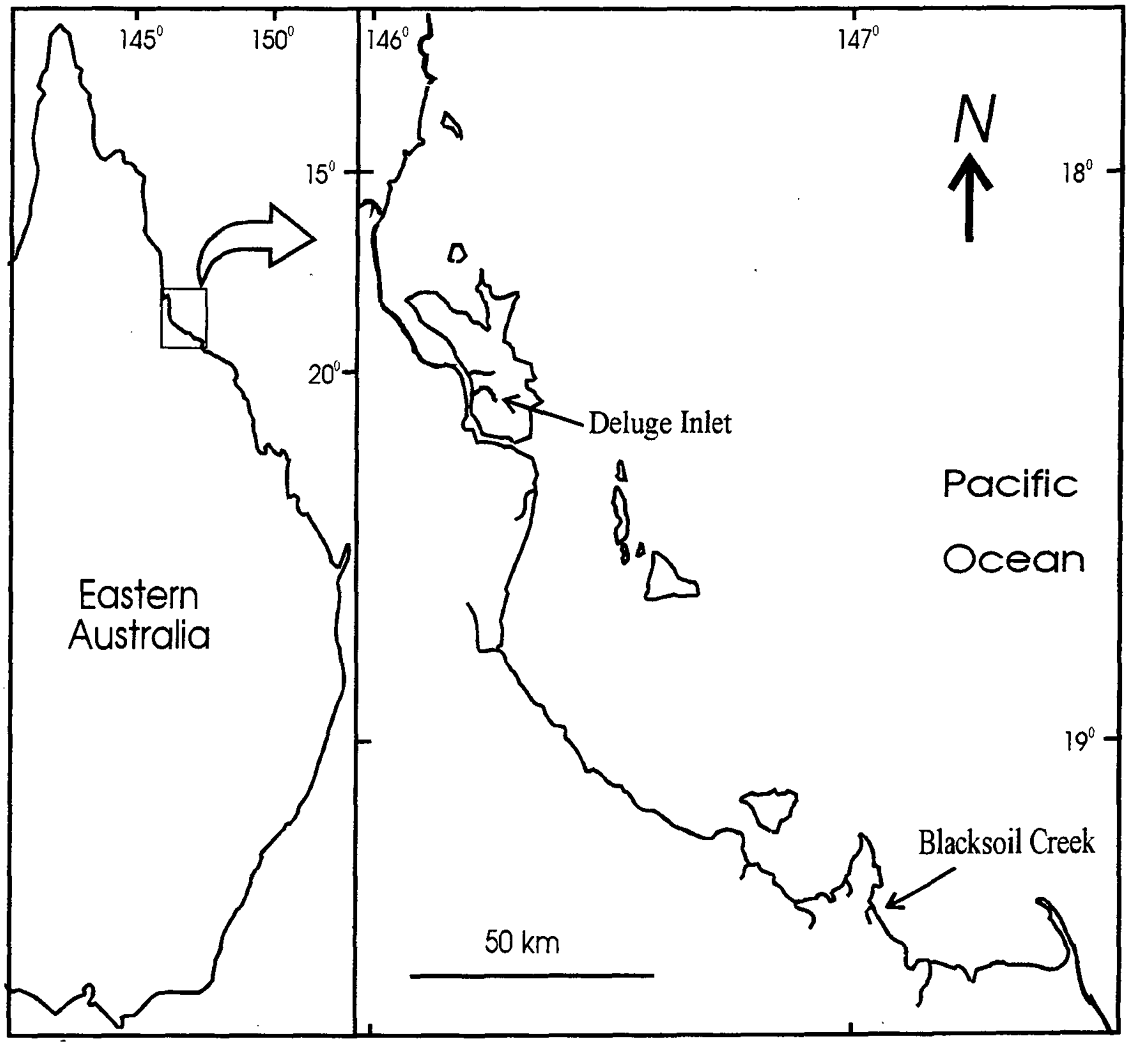

145°
150°
15°
20°
Eastern Australia
146°
147°
18°
19°
N
Deluge Inlet
Pacific Ocean
Blacksoil Creek
50 km

Figure 2.

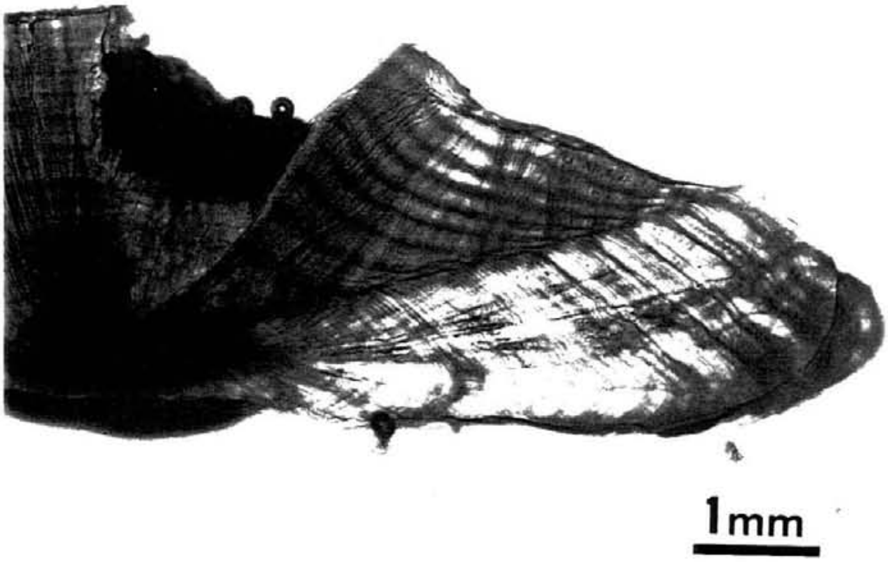
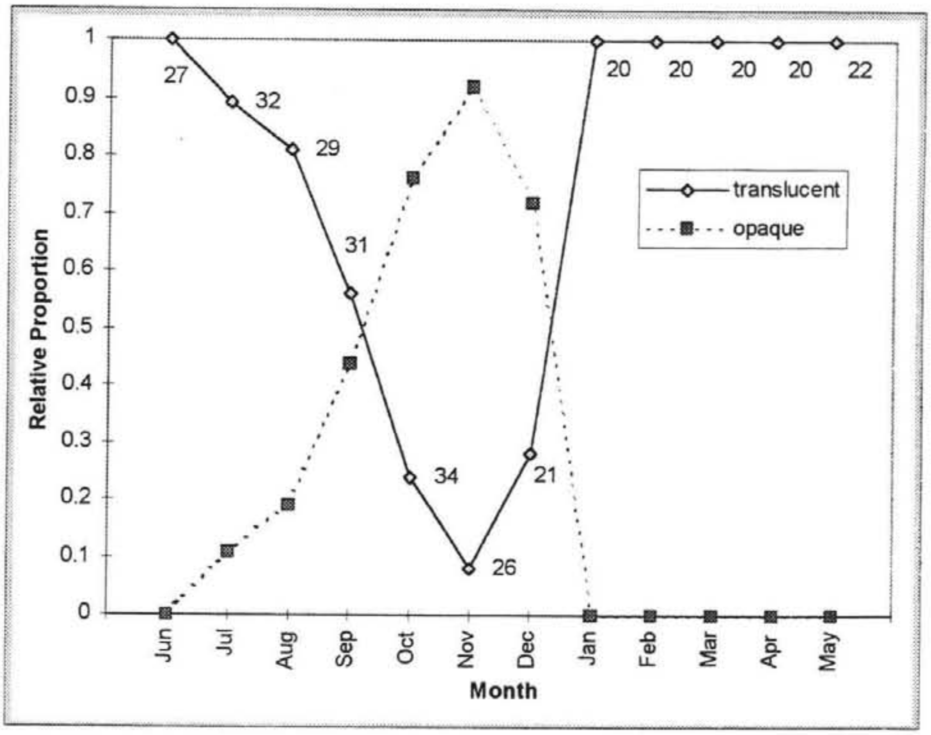


Figure 3.


1
0.9
0.8
0.7
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Relative Proportion
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opaque
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Month

Figure 4.

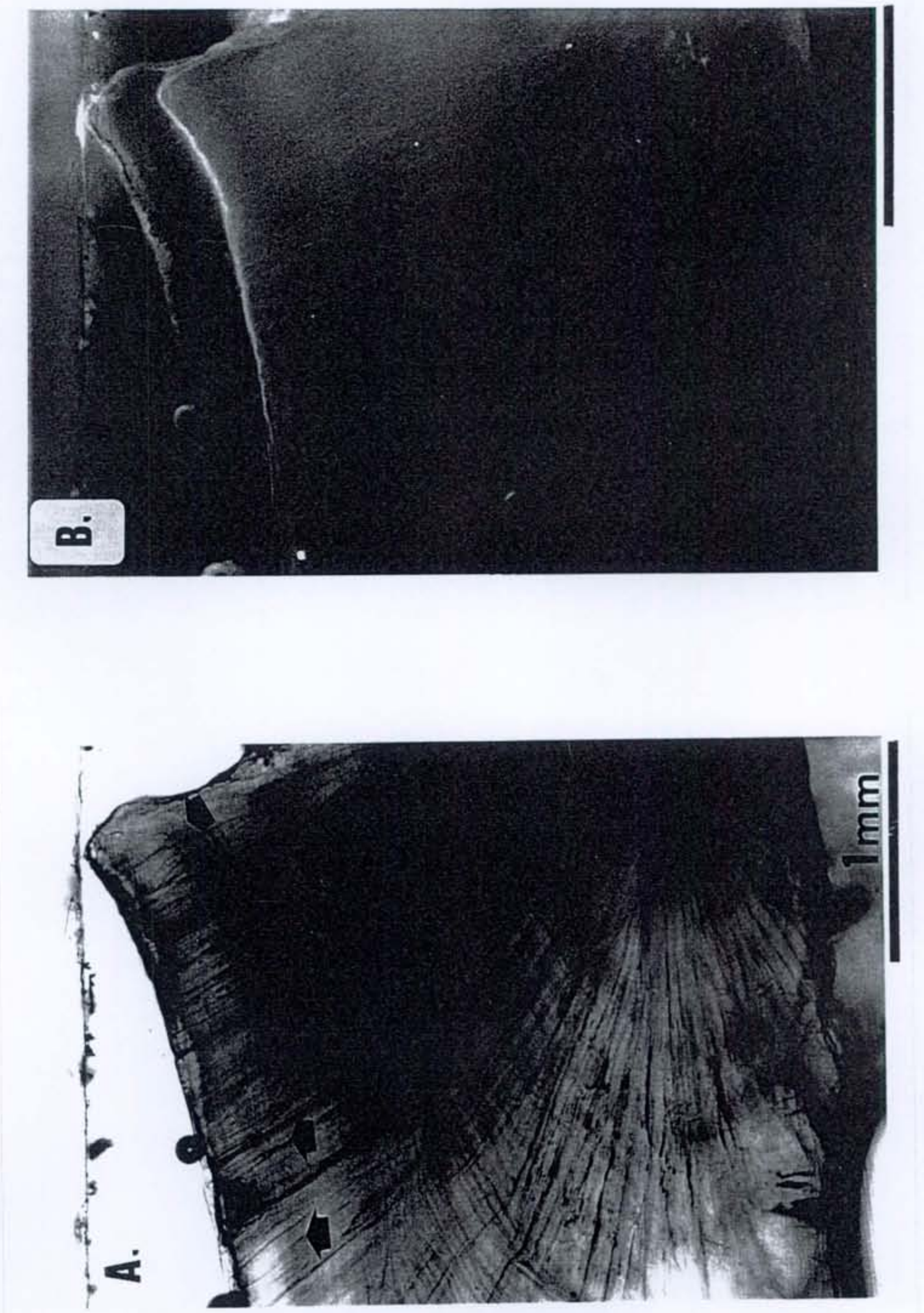
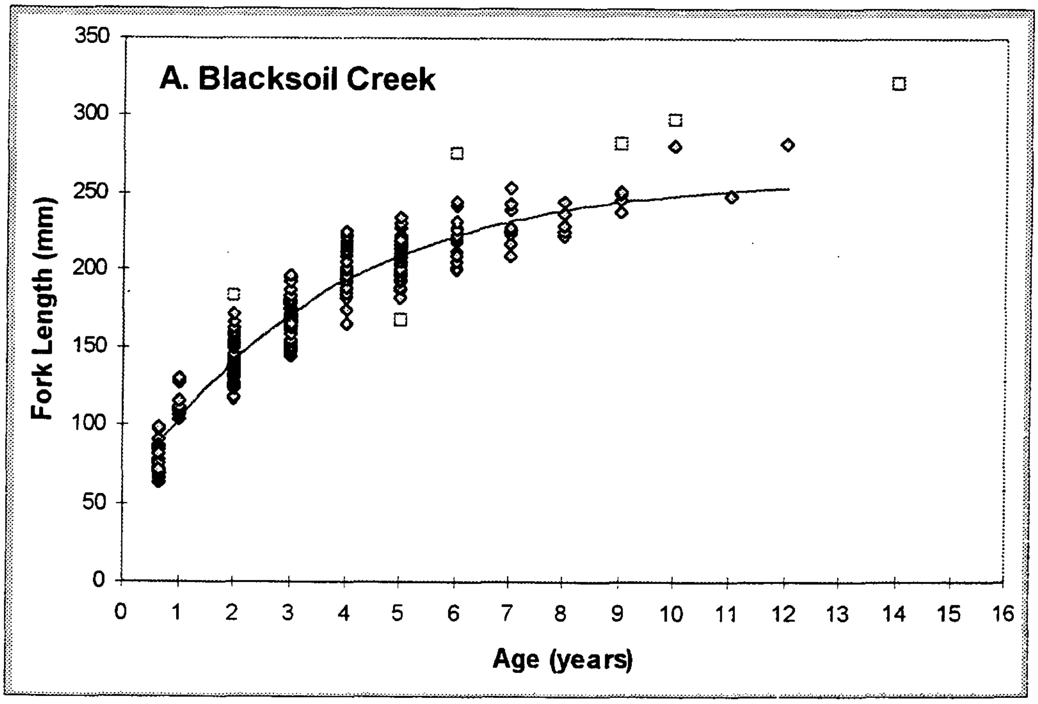


Figure 5.


A. Blacksoil Creek
Fork Length (mm)
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50
100
150
200
250
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350
Age (years)
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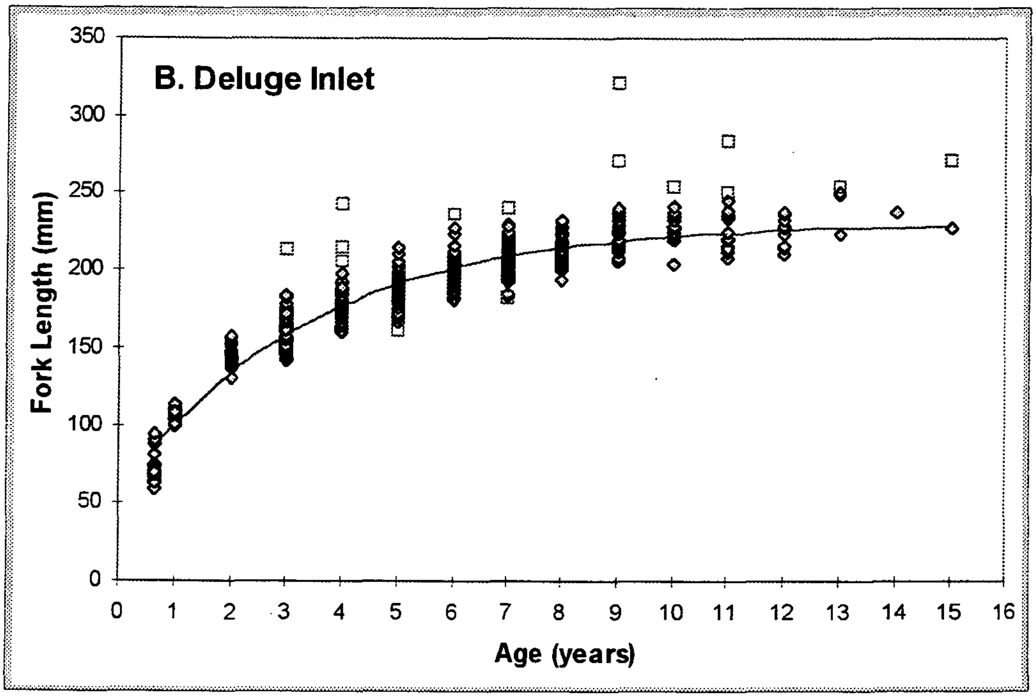
B. Deluge Inlet
Fork Length (mm)
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Age (years)
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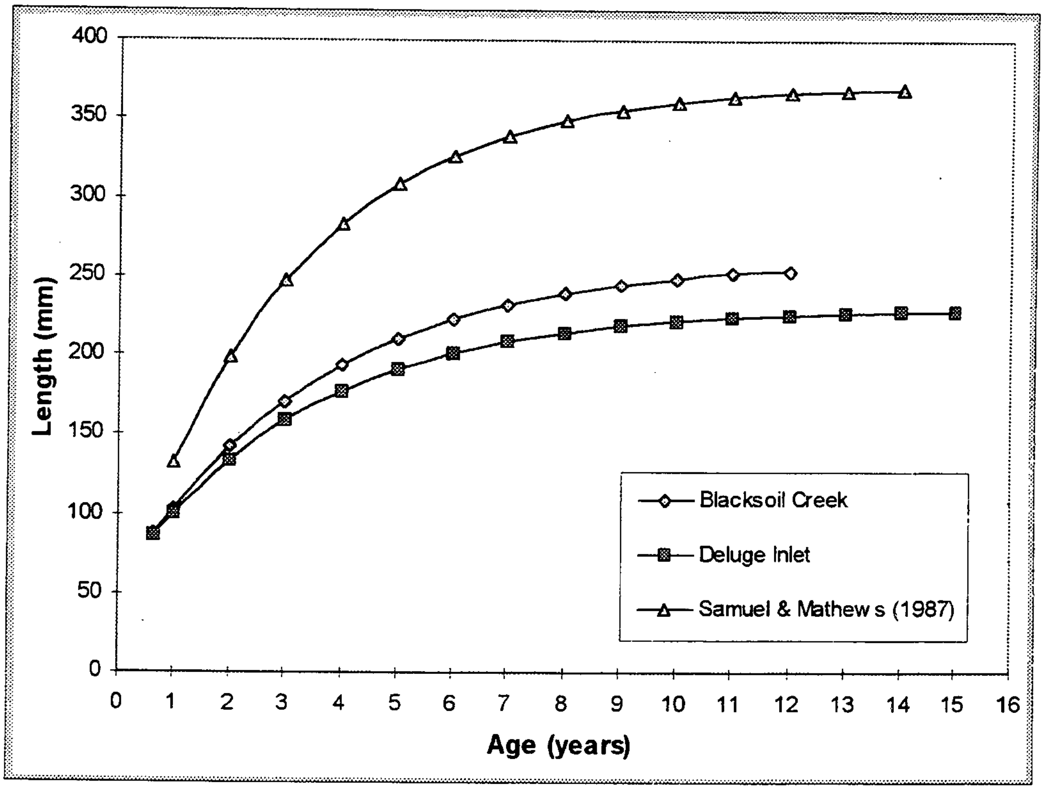
Figure 6.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Length (mm)
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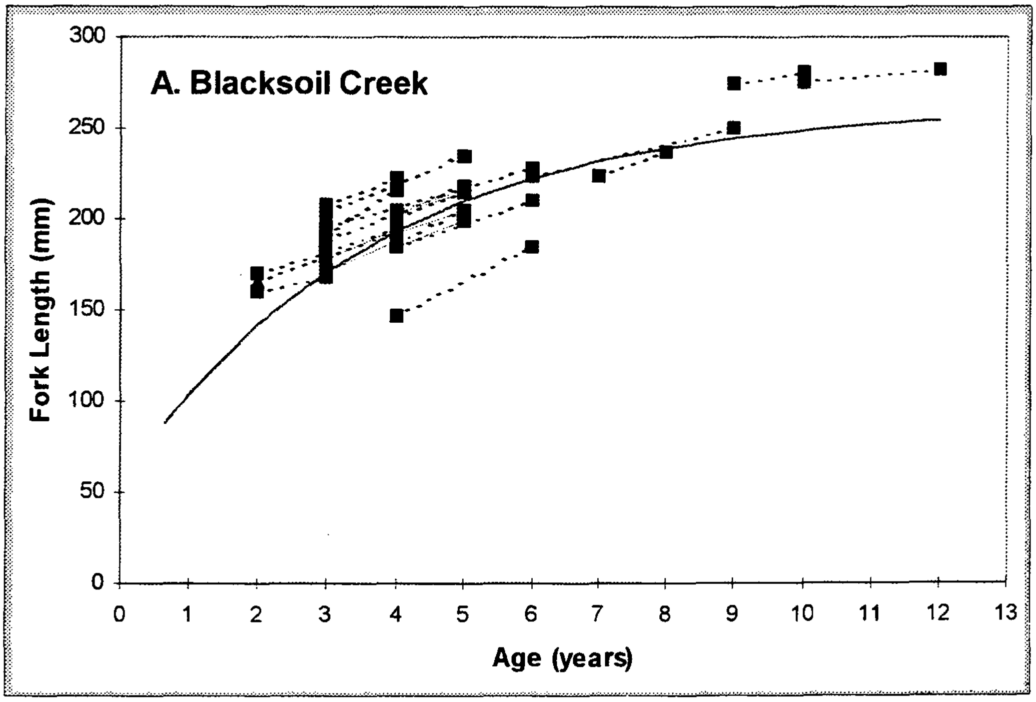
Age (years)
Blacksoil Creek
Deluge Inlet
Samuel & Mathews (1987)

Figure 7.


A. Blacksoil Creek
Fork Length (mm)
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Age (years)
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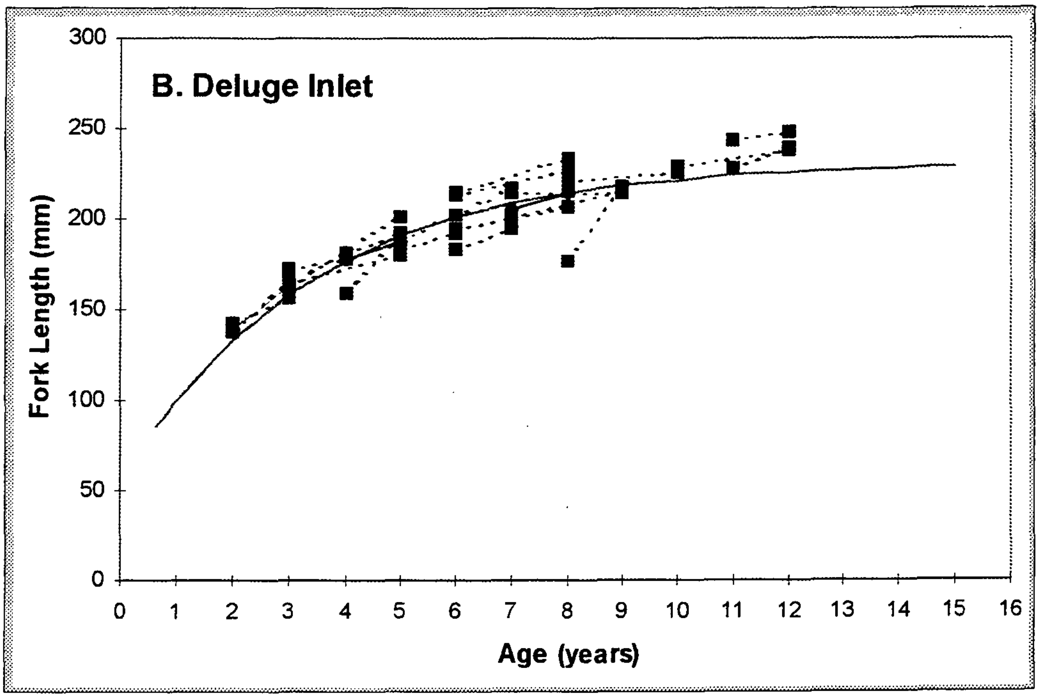
B. Deluge Inlet
Fork Length (mm)
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Age (years)
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