Biology of two species of sparid on the west coast of Australia

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Submitted by
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I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any university.

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Abstract

Various aspects of the biology of the tarwhine *Rhabdosargus sarba* and western yellowfin bream *Acanthopagrus latus* were studied. The studies on *R. sarba* have focused on populations in temperate coastal marine waters at ca 32°S and the lower reaches of an estuary (Swan River Estuary) located at the same latitude and in a subtropical embayment (Shark Bay) at ca 26°S, while those on *A. latus* were conducted on the population in the latter embayment.

A combination of a macroscopic and histological examination of the gonads demonstrated that *R. sarba* is typically a rudimentary hermaphrodite in Western Australian waters, *i.e.* the juveniles develop into either a male or female in which the ovarian and testicular zones of the gonads, respectively, are macroscopically undetectable. This contrasts with the situation in the waters off Hong Kong and South Africa, in which *R. sarba* is reported to be a protandrous hermaphrodite. However, it is possible that a few of the fish that are above the size at first maturity and possess, during the spawning period, ovotestes with relatively substantial amounts of both mature testicular and immature ovarian tissue, could function as males early in adult life and then change to females. Although *R. sarba* spawns at some time between late winter and late spring in Western Australia, spawning peaks later in the Swan River Estuary than in coastal, marine waters at the same latitude and Shark Bay, in which salinities are always close to or above that of full strength sea water, *i.e.* 35 ‰. While the males and females attain sexual maturity at very similar lengths in the Swan River Estuary and Shark Bay, *i.e.* *L*<sub>50</sub>s all between 170 and 177 mm, they typically reach maturity at an earlier age in the former environment, *i.e.* 2 vs 3 years old. Thus, length and consequently growth rate influence the timing of maturity rather than age. During the spawning period, only 9 % of the fish caught between 180 and 260 mm in nearshore, shallow marine waters had become mature, whereas 91 % of those in this length range over reefs were mature, indicating that *R. sarba* tends to move offshore only when it has become “physiologically ready” to mature. The *L*<sub>50</sub>s at first maturity indicate that the current minimum legal length in Western Australia (230 mm) is appropriate for managing this species.

Oocyte diameter frequency distributions, stages in oocyte development, duration of oocyte hydration and time of formation of post-ovulatory follicles in mature ovaries of *Rhabdosargus sarba* in the lower Swan River Estuary (32° 03′S,
115° 44’E) were used, in conjunction with data on tidal cycles, to elucidate specific aspects of the reproductive biology of this sparid in an estuarine environment. The results demonstrated the following. (i) *Rhabdosargus sarba* has indeterminate fecundity *sensu* Hunter *et al.* (1985). (ii) Oocyte hydration commences at about dusk (18:30 h) and is completed by ca 01:30-04:30 h, at which time ovulation, as revealed by the presence of hydrated oocytes in the ovarian duct and appearance of newly-formed post-ovulatory follicles, commences. (iii) The prevalence of spawning was positively correlated with tidal strength and was greatest on days when the tide changed from flood to ebb at ca 06:00 h, *i.e.* approximately when spawning ceases. Spawning just prior to strong ebb tides would lead to the transport of eggs out of the estuary and thus into salinities that remain at ca 35 ‰. The likelihood of eggs being transported downstream is further enhanced by *R. sarba* spawning in deeper waters in the estuary, where the flow is greatest. (iv) Although mature ovaries were found in *R. sarba* in the estuary between early July and December, the prevalence of atretic oocytes was high until September, when salinities started rising markedly from their winter minima. Batch fecundities ranged from 2,416 for a 188 mm fish to 53,707 for a 266 mm fish. The average daily prevalence of spawning amongst mature females during the spawning period of *R. sarba* caught in the lower estuary, *i.e.* July to end of October, was 36.5%. Thus, individual female *R. sarba* spawned, on average, at intervals of ca 2.7 days in each spawning season. Female *R. sarba* with total lengths of 200, 250 and 300 mm were estimated to have a batch fecundity of 7,400, 20,100 and 54,800 eggs, respectively and annual fecundities of 332,000, 903,000 and 2,461,000 eggs, respectively.

*Rhabdosargus sarba* is shown to undergo size-related movements in each of the three very different environments in which it was studied. In temperate coastal waters, *R. sarba* settles in unvegetated nearshore areas and then moves progressively firstly to nearby seagrass beds and then to exposed unvegetated nearshore areas and finally to areas around reefs where spawning occurs. Although *R. sarba* spawns in the lower Swan River Estuary, relatively few of its early 0+ recruits remain in the estuary and substantial numbers of this species do not start reappearing in the estuary until they are ca 140 mm. In Shark Bay, *R. sarba* uses nearshore mangroves as a nursery area and later moves into areas around reefs. The maximum ages recorded for *R. sarba* in coastal marine waters (11 years) and Shark Bay (13 years) were far greater than in the lower Swan River Estuary (6 years). However, the maximum lengths recorded in these three environments were all ca 350 mm. Due to the
production by size-related movements of differences amongst the lengths of *R. sarba* at given ages in different habitats in coastal marine waters, the composite suite of lengths at age was not fully representative of the population of this species as a whole in this environment. A von Bertalanffy growth curve, which was adjusted to take into account size-related changes in habitat type, significantly improved the fit to the lengths at age of individuals in the composite samples for the population beyond that provided by the unadjusted von Bertalanffy growth curve. This resulted in the maximum difference between the estimates of length at age from the two growth curves, relative to the $L_\infty$ derived from the unadjusted von Bertalanffy curve, reaching a value equivalent to 8 %. However, the maximum differences for the corresponding curves for populations in the lower Swan River Estuary and Shark Bay were far less, *i.e.* 1.7 and 3.2 %, respectively, and thus not considered biologically significant. *Rhabdosargus sarba* grew slightly faster in the lower Swan River Estuary than in either coastal marine waters or Shark Bay, possibly reflecting the greater productivity of estuarine environments.

*Acanthopagrus latus* is a protandrous hermaphrodite. Detailed macroscopic and histological examination of the gonads of a wide size range of fish, together with a quantification of how the prevalences of the different categories of gonad change with size and age and during the year, were used to elucidate the sequence of changes that occur in the ovotestes of *A. latus* during life. The scheme proposed in the present study for the protandrous changes in *A. latus* differed from those proposed for this species elsewhere, but was similar to that of Pollock (1985) for the congeneric *Acanthopagrus australis*. The ovotestes of functional males develop from gonads which, as in older juveniles, contain substantial amounts of testicular and ovarian tissue. Such ovotestes, and particularly their testicular component, regress markedly after spawning and then, during the next spawning season, either again become ovotestes in which the testicular zone predominates and contains spermatids and spermatozoa (functional males), or become ovotestes in which the ovarian zone predominates and contains vitellogenic oocytes (functional females). Once a fish has become a functional female, it remains a female throughout the rest of its life. The trends exhibited during the year by reproductive variables demonstrate that *A. latus* in Shark Bay typically spawns on a very limited number of occasions during a short period in August and September and has determinate fecundity. The potential annual fecundities of 24 *A. latus* ranged from 764,000 in a 600 g fish to 7,910,000 in a 2,050 g fish and produced a mean $\pm$1SE of 1,935,000 $\pm$ 281,000. The total length at
which 50 % of *A. latus* become identifiable as males (245 mm) is very similar to the current minimum legal length (MLL) of 250 mm, which corresponds to an age of 2.5 years less than the age at which 50 % of males become females. Current spawning potential ratios calculated over a range of alternative values for natural mortality (*M*) for *A. latus* in Shark Bay suggests that the present fishing pressure is sustainable, but that the current MLL should be reviewed if recreational fishing pressure continues to increase.

The age composition and von Bertalanffy growth parameters for *Acanthopagrus latus* have been determined. The relevant parameters were inserted into the empirical equations of Pauly (1980) and Ralston (1987) for estimating natural mortality (*M*). Total mortality (*Z*) was calculated using Hoenig’s (1983) equations, relative abundance analysis and a simulation based on maximum age and sample size. The two point estimates for *M* for *A. latus*, which were both 0.70 year⁻¹, greatly exceeded all estimates for *Z* (range 0.18 to 0.30 year⁻¹), which is clearly an erroneous result. To resolve this problem of inconsistent estimates, a Bayesian approach was developed, which, through combining the likelihood distributions of the various mortality estimates, produced integrated estimates for *M* and *Z* that are more consistent and precise than those produced for these two variables using the above methods individually. This approach now yielded lower values for *M* than *Z* and a measure of fishing mortality that appears to be consistent with the current status of the fishery. This approach is equally applicable to other fish species.
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Chapter 1: General Introduction

1.1 Habitats of marine teleosts in south-western Australia

Many marine species of teleosts live in shallow, nearshore marine and estuarine waters as juveniles and then migrate into deeper, offshore marine waters as they increase in size and/or become mature (e.g. Modde & Ross, 1981; Lenanton, 1982; Ayvazian & Hyndes, 1995; Clark et al., 1996a; Hyndes et al., 1996a; Ruiz et al., 1993). These shallow highly productive habitats, often termed nursery areas, enables young fish to grow rapidly and thereby become less susceptible to predation (Lenanton, 1977; Chubb et al., 1981; Kennish, 1990; Potter & Hyndes, 1999). Furthermore, since large predatory fish are not abundant in these habitats, the chances of predation are reduced (Blaber, 1980).

One of the main factors influencing the composition of the fish fauna in shallow, nearshore habitats is the degree to which they are exposed to wind and wave activity (Romer, 1990; Ayvazian & Hyndes, 1995, Clark et al., 1996b; Clark, 1997). Although the lower west coast of Australia is protected to a considerable extent from oceanic waves by the chains of limestone reefs and islands that occur along parts of that coastline, marked spatial variation occurs in the degree to which the nearshore waters in this region are exposed to wave activity (Hegge et al., 1996, Western Australia. Department of Environmental Protection 1996, Lemm et al., 1999, Masselink & Pattiaratchi, 2001a). These differences in exposure are reflected in differences in the morphology of beaches, their wave dynamics and the extent to which the nearshore waters contain seagrass beds. Thus, the nearshore bathymetry of sheltered beaches along the lower west coast of Australia is typified by gently sloping profiles and those nearshore waters often contain dense beds of seagrass.
(Hyndes et al., 1996a). Seagrass beds, in particular, provide an important nursery environment for many marine fish species, because they are generally more productive, structurally heterogeneous and offer greater protection to juvenile fishes from predators than unvegetated habitats (Orth & Heck 1980; Bell & Pollard, 1989; Middleton et al., 1984; Jenkins et al., 1997). For similar reasons, mangroves also act as important nursery habitats for juvenile fish (Robertson & Duke, 1987, 1989).

In comparison to sheltered beaches, the exposed beaches on the lower west coast of Australia typically have a steeper nearshore slope and contain little or no seagrass. These physically dynamic environments experience both seasonal and abrupt short-term changes in beach morphology and wave dynamics and associated water conditions, such as turbulence and sediment load (Masselink, 1996, Masselink & Pattiaratchi, 2001b). As a consequence, the fish communities of exposed beaches are often characterised by a more restricted suite of fish species which is often dominated numerically by a few species (Modde & Ross 1981; Lasiak, 1984; Romer, 1990; Ayvazian & Hyndes, 1995; Clark et al., 1996b). However, exposed surf zones do provide important nursery areas for some marine fish species and, in some cases, act as their sole nursery environment (Lenanton, 1982; Bennett 1989; Wright, 1988).

The estuaries of south-western Australia typically comprise a narrow entrance channel, one or two large basins and the saline lower reaches of tributary rivers (Potter & Hyndes, 1999). While some of these estuaries sometimes become landlocked through the formation of sand bars at their mouths, others such as the Swan River Estuary remain permanently open (Potter & Hyndes, 1999).

The limestone reef chains found along the coast of south-western Australia constitute important habitats for fish (Howard, 1989). Some fish species, e.g. the King George whiting *Sillaginodes punctata* and the tarwhine *Rhabdosargus sarba*,
inhabit nearshore areas as juveniles and then migrate outwards into these reef habitats as adults (Hyndes et al., 1996a).

1.2 Family: Sparidae

The Sparidae, which constitute those species commonly known as snapper and bream, is a relatively large family, with 22 genera and 41 species (Kuiter, 1993). Although most of these species occur in southern African waters, only a few members of this family are found in southern Australian waters (Kuiter, 1993). In southern Australia, the Sparidae is represented by a single species of Pagrus, namely P. auratus (previously Chrysophrys auratus), a species of Rhabdosargus, namely R. sarba, and five species of Acanthopagrus, namely A. latus, A. berda, A. palmaris, A. australis and A. butcheri (Munro, 1949; Hutchins & Thomson, 1983; Kuiter, 1993; Gomon et al., 1994)

1.3 Rhabdosargus sarba

In comparison with other sparids, the tarwhine (or silver bream) Rhabdosargus sarba has a more rounded head, slightly larger eye and, most notably, a golden spot or blotch on each scale (Starling, 1988). This species occurs in nearshore marine waters, including surf zones, within and near the mouths of estuaries and over coastal reefs (Munro, 1949; Wallace, 1975; Grant, 1993; Potter & Hyndes, 1999; Smith & Suthers, 2000) and is widely distributed throughout the Indo-Pacific (Kuiter, 1993). However, the species termed R. sarba may not always be the same species (Kuiter, 1993; Dr Barry Hutchins, pers. comm., Museum of Western Australia). 

Rhabdosargus sarba is found on both the east and west coasts of Australia. In eastern Australia, it occurs as far north as Townsville in Queensland and as far south as Mallacoota Inlet and very occasionally also to the Gippsland Lakes and Briadrib
River in eastern Victoria (Munro, 1949; Hutchins & Swainston, 1986). In Western Australia, tarwhine occur southwards from Coral Bay and eastwards as far as Albany (Munro, 1949; Hutchins & Swainston, 1986) (Fig. 1.1).

*Rhabdosargus sarba* is commonly taken as by-catch by Western Australian recreational fishers, who often catch this species when fishing using small rigs aimed at attracting species such as the Australian herring *Arripis georgianus* and various whiting species (Sillaginidae), and also occasionally on larger rigs aimed at catching species such as the tailor *Pomatomus saltatrix* (Cusack & Roennfeldt, 1988).

*Rhabdosargus sarba* is, however, a relatively large species, which attains a maximum weight of about 1.4 kg in Australian waters (Kuiter, 1993) and is targeted by numerous fishermen in some locations such as, for example, the lower Swan River Estuary and Cockburn Sound (pers. obs). Details of these localities are given in Chapter 2. The reluctance of many recreational fishers to target *R. sarba* specifically is due to their view that it is not a very edible species, complaining in particular that the texture of its flesh is too soft (Cusack & Roennfeldt, 1988).

However, tarwhine is considered by others to be a good table fish, but since it has delicate flesh, it needs to be iced down immediately after capture, to preserve its flavour (Grant, 1993; Yearsley *et al*., 1999).

### 1.4 Acanthopagrus latus (and congeneric species in Australian waters)

The black bream *Acanthopagrus butcheri* is an estuarine species which occurs in south-eastern Australia, including Tasmania, westwards along the south coast of Australia and northwards to Shark Bay, Western Australia (Kuiter, 1993). *Acanthopagrus australis*, which is commonly known as yellowfin bream and occurs from central Queensland to eastern Victoria on the east coast of Australia, lives around coastal reefs and in estuaries (Hutchins & Swainston, 1986; Kuiter, 1993;
Western yellowfin bream (*Acanthopagrus latus*)

Tarwhine / silver bream (*Rhabdosargus sarba*)

**Figure 1.1.** Distribution of *Rhabdosargus sarba* and *Acanthopagrus latus* in Australian waters.
Kuiter, 1996). *Acanthopagrus berda*, which is widespread in the tropical Indo-Pacific and commonly known as black bream or pikey bream in Queensland, occurs in coastal waters and usually over silty muddy substrates near jetties and river mouths (Allen, 1997). *Acanthopagrus palmaris*, also commonly known as the black bream, and the western yellowfin bream *Acanthopagrus latus*, which are widely distributed throughout the Indo-Pacific regions, occur northwards of Shark Bay in Western Australia (Fig.1). *Acanthopagrus latus* and *A. palmaris* both inhabit coastal reefs, and the latter sometimes enters estuaries (Allen, 1997). *Acanthopagrus latus* is distinguished from *A. palmaris* by its lighter colour and the yellow colouration of its fins. Western yellowfin bream reach a total length of about 45cm and attain a maximum weight of approximately 2 kg (Allen, 1997).

In Western Australia, commercial fishing for *A. latus* is concentrated in Shark Bay (Niel Sumner, pers. comm. Department of Fisheries, Western Australia) where it is caught by the commercial seine net and mesh net fishery located in that embayment, together with various species of whiting (Sillaginidae) and mullet (Mugilidae), the tailor (*Pomatomus salatrix*) and pink snapper *Pagrus auratus*, (Francesconi & Clayton, 1996). The main recreational fishery for *A. latus*, which has recently been estimated to be approximately half the size of the commercial catch, is concentrated in Exmouth Gulf at a distance of ca 400 km to the north of Shark Bay (Neil Sumner, pers. comm.). *Acanthopagrus latus* is highly-regarded for its eating qualities, and thus currently has the good retail price of $10 per kg per whole fish.

1.5 Determination of age growth

Since reliable data on the age structure, growth and mortality rates of an exploited fish species are essential for developing management plans for that species, it is
crucial that the ageing method employed is accurate and reliable (Hall, 1993). Sound data on the age composition and growth rate of a species facilitates reliable stock assessments, which can then, in turn, be used to develop appropriate management plans for the conservation and sustainable use of the stocks of that species (Beamish & McFarlane, 1987).

The age of a fish is usually determined by counting the number of annually-formed growth zones in hard tissues, such as scales, otoliths and less frequently certain other cartilaginous or bony structures (Bagenal & Tesch, 1978; Campana & Neilson, 1985; Campana, 2001). The zones are formed in response to seasonal variations in environmental conditions, which result in changes in the proportions of protein and calcium deposited during alternating slow and fast phases of growth (Campana & Neilson, 1985). As numerous studies have shown that the use of scales for ageing teleosts often yields unreliable results (Beamish & McFarlane, 1987, Casselman, 1987), otoliths are now almost exclusively used for ageing bony fishes. In the otoliths of temperate fishes, thin and thick zones are typically formed over the cooler “winter” months and warmer “summer” months, respectively (Johnson, 1983; Francis et al., 1992; Booth & Buxton, 1997; Sarre & Potter, 2000).

When using whole otoliths to age fish, the ages of older fish can be underestimated because of the difficulties in detecting the outer growth rings as a result of the allometric growth of the otolith (Beamish & McFarlane, 1987; Casselman, 1987; Hyndes et al., 1992a). Thus, it is often necessary to section otoliths in order to reveal all of their growth zones. As the sectioning of otoliths is time consuming, workers often make preliminary comparisons between the results obtained by counting growth zones on both whole and sectioned otoliths of a wide size range of fish to ascertain whether it is necessary to section all of the otoliths or just those with the most annuli (e.g. Hyndes et al., 1992a; Hyndes et al., 1998; Sarre
& Potter, 2000; Hesp et al., 2002). If there are discrepancies between the number of zones on whole and sectioned otoliths, such comparisons enable the number of zones at which such discrepancies start to occur to be determined. The ages of small and young fish can often be determined accurately by using the number of annuli on their whole otoliths, whereas those of larger and older fish have to be determined using those on sectioned otoliths (Campana, 1984).

A major requirement of all ageing studies is that the growth zones used for ageing are validated as having been formed annually (Beamish & McFarlane, 1983; Casselman, 1987; Francis et al., 1992; Hyndes et al., 1992a; Campana, 2001). The failure to carry out age validation has led on many occasions in the past to inaccurate ageing of fish and thus to such a gross misunderstanding of the biology of some species that fisheries managers did not establish appropriate plans to prevent serious overexploitation of fish populations or species (Campana, 2001). Two examples of fish stocks which became seriously overexploited, as a result of ageing errors, include orange roughy Hoplostethus atlanticus in New Zealand and Sebastes spp. in Canada (see Campana, 2001).

One method of validating that growth zones are formed annually in hard structures is by labelling the calcified tissue, which involves the capture, tagging and then injection of a label, e.g., tetracycline, and the subsequent recapture of those fish. Since the labelling compound leaves a mark in the calcareous structure, the use of the number of zones as annuli in those structures for ageing a particular species is valid if the number of zones in the otolith peripheral to the mark corresponds to the number of years between release and recapture (Casselman, 1983; Casselman, 1987). Another technique for validating that the growth zones on the calcified tissue of fish are formed annually is marginal increment analysis (Campana, 2001; Hyndes et al., 1992a). The marginal increment is the distance outside the outermost (opaque)
zone. If the opaque and translucent zones are formed annually, the marginal increment should undergo a marked decline at one time of the year, when a newly-formed opaque zone first becomes delineated and a new translucent zone begins to form at the otolith’s edge, and then increase progressively as that translucent zone widens as it continues to form (Hyndes et al., 1992a). Due to its modest sampling requirements and relatively low cost, this is the most commonly used method for age validation. However, in his review of age validation methods, Campana (2001) states that marginal increment analysis is also one of the most difficult ageing validation methods to carry out properly. This is because of the difficulties associated with viewing a partial increment affected by variable light refraction, through an edge which becomes increasingly thin as the margin is approached, as well as by light reflection off the curved surface of the edge (Campana, 2001). However, this approach is valid if carried out with sufficient rigour (Campana, 2001).

1.6 Reproductive biology

Information on the reproductive biology of a species, such as the length and age at first maturity, spawning period and fecundity are crucial for the production of stock assessment models (Hall, 1993; Hill, 1990). The length at which a fish species first reaches maturity is also often used to set minimum legal lengths for particular species, which has been a major tool for fisheries management in Australia for over a century. Managers in recent times have frequently noted that the foresight of the early managers in their selection of sizes has proved to be a vital and effective method for conserving Australia’s fish resources (Hill, 1990; Winstanley, 1990). Knowledge of the spawning period of a species enables a realistic birth date to be assigned to fish of all ages, a requirement for the construction of reliable growth curves. The spawning period of a fish species is often referred to the period
between the date of the first observed active female *i.e.* a female fish with ovaries containing yolked oocytes (that may not necessarily be spawning), until the date when the last active female was caught (Karlou-Riga & Economidis, 1997). The methods commonly used to determine the reproductive cycles of fish are reviewed by West (1990). These include the use of gonadosomatic indices (GSIs), which represents a direct comparison of gonad weight to body weight, the macroscopic staging of gonads in which the gonads are assigned a numerical maturation stage according to their visual appearance, and the sizes and stages of oocytes in histological sections of ovaries. The trends shown by GSIs provide an indication of the trends exhibited by gonadal maturation during the year, and the spawning strategy exhibited by a species. However, GSI trends do not provide a precise indication of when spawning occurs. The main criteria used for the macroscopical staging of gonads are the relative size and colour of the gonads and, in the case of ovaries, whether or not oocytes are visible through the ovarian wall and are opaque (yolked) or translucent (hydrated). However, since macroscopic staging relies heavily on the experience of the observer, this technique should be supported by other measures of gonadal development. Oocyte measurements, together with the stage of those oocytes, are a proven technique for assessing the stage of ovarian maturation. Histology is the most detailed and accurate method for assessing gonadal stage, but it is also time consuming and expensive. Ideally, several methods should be used together to improve the reliability of results (West, 1990). These methods, when used in combination, can provide sound data on the spawning period and also on the size and age at which sexual maturity is first reached by each sex (Bagenal & Braun, 1978; Pitcher & Hart, 1982).

In general, the term “fecundity” in fisheries biology is used to describe the number of eggs spawned by an individual female fish over a particular period
e.g. annually, or on a single spawning occasion (Hunter et al., 1992). As fecundity assessments are incorporated into production models for fish stock assessment, such studies are particularly important for the sustainable management of commercial and recreational species (Nichol & Acuna, 2001). Furthermore, fecundity estimates can also be used in conjunction with estimates of the abundance of eggs in the sea to estimate the biomass of a stock (Hunter et al., 1992). Indeed, because of the importance of estimates of fecundity and size at sexual maturity to species management, Hunter et al. (1992) suggested that inevitably, these parameters will be estimated for every species of economic consequence.

To determine the fecundity of a fish species, it is essential to know whether that species has determinate or indeterminate fecundity. Species with determinate fecundity are those in which the number of large oocytes present in their ovaries immediately prior to the onset of the spawning period corresponds to their potential annual fecundity, i.e. the number of oocytes potentially released by individual females during a spawning period (Hunter et al., 1985; Lisovenko & Andrianov, 1991). During the period immediately prior to and during spawning, the oocyte diameter distributions for ovaries of such species are characterized by a distinct gap between the small previtellogenic oocytes and the larger oocytes present within those ovaries (Hunter & Macewicz, 1985). In contrast, the distribution of oocyte diameters in species with indeterminate fecundity, during this same period, essentially form a continuum, reflecting the continuous maturation of oocytes throughout the spawning season. Consequently, estimates of the “standing stock” of large oocytes present in the ovaries of species with indeterminate fecundity just prior to the onset of spawning will almost inevitably result in an underestimate of their annual fecundity (Hunter et al., 1985, 1992; Lisovenko & Andrianov, 1991). Thus, estimation of the annual fecundity of species with indeterminate fecundity requires a combination of
data on batch fecundity and spawning frequency (Hunter & Macewicz, 1985; Hunter et al., 1985).

### 1.7 Hermaphroditism

Hermaphroditism may be defined as the presence of both sex functions at some time during the life of an individual (Ross, 1990). In most fish species, reproduction follows the normal vertebrate pattern, *i.e.* separate sexes, with hermaphroditism being considered abnormal (Chan & Yeung, 1983). However, there are several families in which hermaphroditism is common (Buxton & Garratt, 1990). Hermaphroditism finds its most complex expression in the Sparidae (Atz, 1964). In their review of the literature on reproductive styles in the Sparidae, Buxton & Garratt (1990) identified the following four types of hermaphroditism; (i) protogyny, the change of sex from functional females to males; (ii) protandry, the change of sex from functional males to females, *i.e.* the reverse of protogyny; (iii) rudimentary hermaphroditism (or late gonochorism), where young individuals possess an immature bisexual gonad, but mature as either female or male fish with no evidence of sex reversal and (iv) simultaneous hermaphroditism, whereby an individual functions as both a female and male at the same time.

As originally described by D’Ancona (1949), the bisexual gonads of sparids consist of a medio-dorsal ovarian zone and a latero-ventral testicular zone separated by a wall of connective tissue (Besseau & Bruslé-Sicard, 1995). However, the structure of the ovotestis effectively limits the number of methods available for providing conclusive evidence of sex change in sparid fishes (Buxton & Garratt, 1990). While sex inversion is relatively easy to identify if all individuals function initially as one sex and then later change to the other, it is difficult to identify when
both sexes are present at a particular length and only a proportion subsequently change sex (Reinboth, 1970).

There are several criteria that have been used to establish that a fish species is a hermaphrodite, some of which are more reliable than others. The reliability of these criteria were assessed in an extensive review by Sadovy & Shapiro (1987). Experimental evidence, whereby certain non-hormonal stimuli, i.e. those which occur in natural populations, is used to trigger sex reversal or, in the case of simultaneous hermaphrodites, self-fertilization of individuals, can provide strong evidence of hermaphroditism. Simultaneous hermaphrodites can also be determined by the presence, within a single gonad, of mature tissue of both genders (Sadovy & Shapiro, 1987).

Strong indicators of protogyny include the presence of membrane-lined cavities in the testes, transitional individuals and certain atretic bodies in atretic oocytes within testes and sperm sinuses in the gonadal wall. According to Sadovy & Shapiro (1987), strong indicators of protandry are transitional individuals whose gonads contain degenerating testicular tissue and developing ovarian tissue. The occurrence of bimodality in length distributions of females vs males is commonly considered to indicate either protogyny or protandry, but, as pointed out by Sadovy & Shapiro (1987), this is not a reliable indicator. The attainment of a large size by either males or females, differential growth rates, mortality, migration and spatial segregation of the sexes and selective sampling techniques can lead to the production of bimodal size-frequency distributions that reflect differences between the sexes that are not related to hermaphroditism. In contrast, bimodality in the distributions of fish lengths by age is a far more reliable indicator of hermaphroditism (Sadovy & Shapiro, 1987). When determining which particular type of hermaphroditism is exhibited by a species, the sampling procedure should ideally be random and a large
sample size should be collected, which includes representatives of all size classes in all months of the year and from a single location. As several methods may be used to identify each type of hermaphroditism, efforts should be made to exclude alternative explanations for each piece of evidence. In addition, histological examination of gonads must be used to confirm all macroscopic observations (Sadovy & Shapiro, 1987).

1.8 Main objectives of the study

1. Determine the age compositions and growth rates of *R. sarba* and *A. latus* and, in the case of *A. latus*, also natural and total mortality.

2. Determine the location and period of spawning of *R. sarba* and *A. latus*.

3. Determine the lengths and ages at which *R. sarba* and *A. latus* reach maturity.

4. Determine the habitats occupied by *R. sarba* and *A. latus* and whether these change with the size and age of fish and when sexual maturity has been reached.

5. Determine whether *R. sarba* and *A. latus* are hermaphroditic and, if so, what type of hermaphroditism they exhibit, and investigate its implications for the management of the fisheries for these species.

6. Estimate the annual fecundity of *R. sarba* and *A. latus* and, in the case of *R. sarba*, determine the time of day, period and frequency of spawning.

7. Determine whether the growth rate, timing and duration of spawning and the length and age at first maturity of *R. sarba* vary among assemblages found in different environments along the west coast of Australia.
Chapter 2: General materials and methods

2.1 Study areas

*Rhabdosargus sarba* was collected from coastal marine waters at *ca* 32°S, the lower reaches of the Swan River Estuary at the same latitude and from Shark Bay at *ca* 26°S (*Fig. 2.1*). *Acanthopagrus latus* was caught in Shark Bay (*Fig. 2.1*).

2.2 Coastal marine waters at *ca* 32°S

*Cockburn Sound*

Cockburn Sound (32°08′S, 115°45′E to 32°16′S, 115°41′E), which is located just to the south of the mouth of the Swan River Estuary (*Fig. 2.1*), is part of a depression between the Spearwood Ridge to the east and the Garden Island Ridge to the west (*Fig. 2.1*). The waters of Cockburn Sound occupy an area of *ca* 10,050 hectares (Hutchinson & Moore, 1979). This embayment consists of a basin, the main part of which is 17 to 22 m in depth, and a shallow sill of 2 to 3 m in depth at its southern end. Two banks, the Success and Parmelia banks in the north of the sound, formed by erosion and accretion processes associated with changes in sea levels over the last 10,000 years, together with Garden Island to the west, protect the sound from storm waves (Hutchinson & Moore, 1979). Large expanses of seagrass beds, which provide important habitats for fish, stabilize the banks in Cockburn Sound. Since there is limited interaction between the waters in the sound and the ocean, it acts for much of the time as a “tidal lake” (Hutchinson & Moore, 1979) and has thus been susceptible to pollution and eutrophication as a result of a range of industrial and human activities. Approximately half of its seagrass beds were lost due to the effects of eutrophication between the early 1970s and 1990s (Lord & Associates, 2001;
Figure 2.1. Sampling locations for *Rhabdosargus sarba* and *Acanthopagrus latus* in coastal marine waters and the lower Swan River Estuary and Shark Bay. ● denotes sampling sites. C, channel of the lower Swan River Estuary; E, exposed nearshore sites; M, mangrove sites; R, reef sites; S, sheltered nearshore sites; *, site also contains areas of seagrass (*Posidonia sinuosa*) as well as bare sand, both of which were sampled.
Kendrick et al., 2002). However, recent studies indicate that the water quality of Cockburn Sound has improved over the past decade, and there have been no further significant losses of seagrass beds due to poor water quality during this period (Lord & Associates, 2001).

**Shallow, nearshore marine waters south of Cockburn Sound**

Shoalwater Bay, which lies immediately to the south of Cockburn Sound (Fig. 2.1), is relatively well protected by a series of islands and reefs. However, two substantial bays that lie further south, namely Warnbro Sound and Comet Bay (Fig. 2.1), vary considerably in the degree to which their beaches are exposed to wave and swell activity (Valesini et al., 1998).

**Coastal reefs**

The substratum of coastal waters between Cape Naturaliste (34°S, 115°E’) and the Northwest Cape (22°S, 115°E), which covers a distance of ca 1,500 km of coastline, is dominated by limestone reefs. The coral reefs in coastal waters of south-western Australia consist of isolated coral colonies that rest on submerged limestone platforms (Hatcher, 1991). The reef fish fauna that inhabits the west and south coasts of Western Australia is highly diverse (Hutchins, 1994). However, the fish faunas in different regions are reasonably discrete, largely reflecting the varying influences of ocean currents, and particularly of the Leeuwin Current (Hutchins, 1994). The latter eastern boundary current, which flows southward along the Western Australian coastline, transports eggs and larvae southward and thereby disperses tropical fishes into temperate areas, which then influences the composition of the fish fauna in those areas (Hutchins, 1991).
2.3 Lower Swan River Estuary

The Swan River Estuary, which covers a surface area of approximately 53 km², comprises the saline lower reaches of two main tributaries, the Avon and Canning rivers, the two large central basins into which those rivers discharge and a 7.5 km long and narrow entrance channel that opens into the lower of the two basins (Loneragan et al., 1989). These three regions are referred to as the upper, middle and lower estuary (Chalmer et al., 1976).

The mouth of the Swan River estuary is located at 32° 04’ S, 115° 44’E (Fig. 2.1). The construction of Fremantle harbour just inside this mouth radically changed the environment near the entrance of the Swan River estuary. The conclusion by the early explorer Captain Stirling R. N. that the mouth of the Swan River would constitute an excellent all-weather harbour was one of the major factors that led the British Government’s decision in 1829 to establish a colony in Western Australia. However, these waters were exposed to winter storms and, although the river possessed wide reaches, it was shallow and possessed a rock bar at its entrance (Hasluck, 1965). One of Australia’s most famous engineers, C. Y. O’Connor, first submitted the plans for the construction of a harbour in 1891 and these were eventually adopted (Fremantle Port Authority, 1966). Moles were constructed on either side of the estuary mouth to protect the entrance to the estuary from the effects of storms and ocean swell, and the rock bar in that region was removed by blasting and the water dredged to a depth of 11 m. Land was reclaimed and wharves and jetties were constructed (Tull, 1997). Several bridges were also constructed close to the mouth of the estuary, three of which remain. One of these, the Fremantle Traffic Bridge, which was completed in 1940 (Ewers, 1971; Edmonds, 1997), is a popular recreational fishing location, particularly for tarwhine.
The most successful and experienced recreational fishers have developed a specialized approach to catching *R. sarba* in the entrance channel of the Swan River Estuary. They obtain mussels for bait by scraping the bridge pilons using long-handled scoops. Some of the mussels are crushed and small portions of those are continuously thrown into the water as berley. Other mussels are cracked open and the flesh is used to bait the hooks. Fishers typically use “bait-holding” hooks, which have small barbs on the shank of the hook, so that this “soft” bait is retained on the hook. Furthermore, experienced fishers fish only on either a slack or moderate ebb tide as they consider that these conditions are optimal for fishing, since it facilitates a slow dispersion of the berley trail, attracting the large tarwhine that reside amongst the many pilons within the harbour, downstream from the traffic bridge.

Upstream of Fremantle Harbour is a shallow stretch of water which, due to the presence of a sill, is less than 5 m deep, and this leads into a deep (10-16 m) trough known as Blackwall Reach (Gaughan *et al.*, 1990; Stephens & Imberger, 1996). Although, due to their proximity to the ocean, the waters of Fremantle Harbour and adjacent sill remain essentially “marine” for much of the year, they do decline markedly in winter and early spring when freshwater discharge is greatest (Stephens & Imberger, 1996; Gaughan *et al.*, 1990). Therefore, for much of the year, the lower Swan River Estuary is essentially a marine environment.

2.4 Shark Bay

Shark Bay, which is one of only 14 World Heritage areas listed for Australia (Francesconi & Clayton, 1996; http://www.unesco.org/whc/heritage.htm) is located approximately 800 km north of the Swan River Estuary (Fig. 2.1). Seventy one percent of its total area, *i.e.* 2.2 million hectares, consists of marine habitat, and constitutes the largest marine embayment in Australia (Francesconi & Clayton,
The embayment extends southward from the northern tip of Bernier Island (24°45'S, 113°10'E) to the southernmost point of Freycinet Harbour (26°36’S, 113°41’E) and longitudinally lies between ca 113° and 114°30’E (Marsh, 1989).

Shark Bay is one of only a few natural heritage areas to meet all of the four following criteria for World Heritage Listing (Francesconi & Clayton, 1996):

- an outstanding example representing the major stages of the earth’s evolutionary history
- an outstanding example representing significant ongoing geological processes, biological evolution and human interaction with the natural environment
- contains unique, rare or superlative natural phenomena, formations or features of exceptional natural beauty
- contains important and significant habitats where threatened species of plants and animals of outstanding universal value from the point of view of science and conservation still survive.

The flora and fauna of Shark Bay is very diverse. This is mainly due to the fact that it is located transitionally between tropical and temperate regions and thus contains both tropical and temperate species. Furthermore, Shark Bay contains the largest and most diverse suite of seagrass meadows in the world (Walker, 1989) and these provide not only habitats for many species, but also influence the hydrology of the bay. This ultimately leads to highly elevated salinities in the inner reaches of the bay, which, in turn, have greatly affected the distribution and diversity of its flora and fauna (Walker, 1989). A total of 323 fish species have been recorded in the bay, 83 % of which are tropical, 11 % subtropical and 6 % warm temperate (Hutchins, 1991).

2.5 Sampling methods

Seine nets

Nearshore, shallow waters at sites in coastal marine and estuarine waters at ca 32°S and in Shark Bay were sampled using a 21.5 m long seine net with 3 mm mesh
in the bunt and, with the exception of Shark Bay, also frequently with a 5.5 m long
seine net made entirely of 1 mm mesh. The 5.5 m seine net was dragged parallel to
the shore for a distance of 50 m, covering an area of \textit{ca} 250 m$^2$. The 21.5 m seine net
was stretched parallel to the beach in a water depth of \textit{ca} 1-1.5 m and its ends then
drawn inwards in a circle and on to the beach. The area covered by this seine net was
\textit{ca} 116 m$^2$.

Rod and line fishing
\textit{Rhabdosargus sarba} was sampled by rod and line fishing over reefs in marine waters
near Perth, which were typically 20-30 m deep and \textit{ca} 5-7 km from the shore, and in
the channel of the lower Swan River Estuary using a platform beneath the Fremantle
Traffic Bridge. It was also sampled at various locations over reefs and rocky
intertidal areas in Shark Bay. The sampling areas were located using a Global
Positioning Satellite (GPS) system and echo sounder. To determine the type of
habitat occupied by \textit{R. sarba} in offshore marine waters, a colour video camera,
attached by cable to a television monitor and video recorder, was lowered over the
substrata whilst fishing.

Gill nets
Monofilament gillnets were used in preliminary trials to assess whether they were
suitable for catching \textit{R. sarba} adjacent to shallow reefs in waters near Perth. Three
nets were constructed, each containing four 20 m long panels. The panels were
constructed of either \textit{ca} 2, 3, 4 or 5 inch mesh. The gill nets were set at dusk
immediately adjacent to reefs on the northern tip of Garden Island (\textbf{Fig. 2.1}) and also
nearby at Stragglers Rocks and were retrieved three hours later. However, since these
gill nets were largely unsuccessful in catching *R. sarba*, their use was discontinued after those preliminary trials.

Samples from commercial fishers
When available, *A. latus* were collected from local fish processors who had obtained them from commercial haul net fishers in Shark Bay. A relatively small number of *R. sarba* that had been caught opportunistically by commercial wetline fishers were also collected from these local fish markets.

2.6 Study sites
The environment(s), habitat types, methods of capture and sampling dates on which the juveniles and adults of *R. sarba* and *A. latus* were collected are listed in Table 2.1a,b. To assist the reader, various aspects of the sampling regime have been repeated in some of the chapters and, where applicable, additional details on specific sampling sites or aspects of sampling are given. Some of the sites that were regularly sampled in marine waters at ca 32°S, the lower Swan River Estuary and Shark Bay, are shown in Figs 2.2 to 2.4.
Table 2.1a. Sampling regime for tarwhine, *Rhabdosargus sarba*. Note that sampling was not always continuous, and that the data for samples for the corresponding months of each year were pooled, unless otherwise stated.

<table>
<thead>
<tr>
<th>Environment</th>
<th>Life stage</th>
<th>Habitats</th>
<th>Method</th>
<th>Dates</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Marine waters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at ca 32oS</td>
<td>Juvenile</td>
<td>Sheltered beaches</td>
<td>5.5 m and 21.5 m seine</td>
<td>Feb 1999 to May 2001</td>
</tr>
<tr>
<td></td>
<td>Juvenile</td>
<td>Exposed beaches</td>
<td>21 m seine</td>
<td>Feb 1999 to April 2001</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>Waters around reefs</td>
<td>Rod and line</td>
<td>Feb 1999 to July 2002</td>
</tr>
<tr>
<td><strong>Lower Swan River Estuary</strong></td>
<td>Juvenile and adult</td>
<td>Sheltered beaches</td>
<td>21.5 m seine</td>
<td>May 2000 to Aug 2002</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>Channel dredged to 11 m</td>
<td>Rod and line</td>
<td>July 1999 to Oct 2002</td>
</tr>
<tr>
<td><strong>Shark Bay</strong></td>
<td>Juvenile</td>
<td>Mangroves</td>
<td>21.5 m seine</td>
<td>Oct 1999 to Apr 2002</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>Shallow, nearshore sandstone rocky areas and reefs</td>
<td>Rod and line</td>
<td>Apr 1999 to Apr 2002</td>
</tr>
</tbody>
</table>

Table 2.1b. Sampling regime for western yellowfin bream, *Acanthopagrus latus*.

<table>
<thead>
<tr>
<th>Environment</th>
<th>Life stage</th>
<th>Habitats</th>
<th>Method</th>
<th>Dates</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Shark Bay</strong></td>
<td>Juvenile</td>
<td>Mangroves</td>
<td>21.5 m seine</td>
<td>Dec 1999 to April 2002</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>Shallow, nearshore sandstone rocky areas and reefs</td>
<td>Rod and line</td>
<td>Dec 1999 to June 2002</td>
</tr>
</tbody>
</table>
Figure 2.2. Two of the sites at which *Rhabdosargus sarba* was sampled in marine waters at *ca* 32°S. (a) Mangles Bay, located at the southern end of Cockburn Sound (see Fig. 2.1), where 0+ juveniles were caught by seine netting and (b) a moderately exposed beach, where many older juveniles were caught.
Figure 2.3. Two of the sampling sites in the lower Swan River Estuary. (a) A seine netting site over a sill *ca* 4 km from the mouth of the estuary and (b) the Fremantle Traffic Bridge, a popular fishing location and important sampling site for *R. sarba*. The bridge is located at the upstream end of the Fremantle harbour, at a distance of *ca* 3 km from the mouth of the estuary.
Figure 2.4. Two of the sampling sites in Shark Bay, which were typical of habitats occupied by *Rhabdosargus sarba* and *Acanthopagrus latus* in this embayment. (a) A small intertidal mangrove creek, which was inhabited by juveniles and was sampled by seine netting and (b) a rocky intertidal area, which was inhabited mainly by adults, and was sampled by (rod and) line fishing.
Chapter 3: Reproductive biology of the tarwhine 
*Rhabdosargus sarba* (Sparidae) in three different 
environments on the west coast of Australia

3.1 Introduction

Previous studies have yielded conflicting results on the hermaphroditic characteristics of *Rhabdosarugs sarba*, a species which, like other sparids, possesses a type of gonad termed an ovotestis (D’Ancona, 1949; Besseau & Bruslé-Sicard, 1995). For example, Kinoshita (1939) regarded *R. sarba*, which he referred to as *Sparus aries*, as essentially a gonochoristic species, but which exhibits a protandric feature during early life, with the testicular tissue in the gonads of juveniles developing early and before the ovarian tissue. In contrast, Yeung and Chan (1987) and Garratt (1993) considered *R. sarba* a *bona fide* protandrous hermaphrodite.

Past studies have demonstrated that *R. sarba* has a protracted spawning season in the Arabian Gulf, India and South Africa, and that the spawning period varies markedly with geographical region (El-Agamy, 1989; Patnaik, 1973; Wallace, 1975). Although the reproductive biology of this species has not been studied in Australia, the capture of its post-larvae between autumn and early summer as they were entering nearshore coastal waters and estuaries of eastern Australia, strongly suggests that this species has a very protracted spawning period in this part of the continent (McNeill *et al*, 1992; Miskiewicz, 1986; Munro, 1949; Smith & Suthers, 2000; Trnski, 2001). In Western Australia, small numbers of post-larval *R. sarba* were caught entering an estuary on the south coast in December and January (Neira & Potter, 1992). Although *R. sarba*, like the congeneric *Rhabdosargus holubi*, is known to spawn in marine waters (Blaber, 1974; Blaber, 1984; Potter & Hyndes,
1999) and sometimes near the mouths of large rivers (Wallace, 1975), preliminary sampling in the lower reaches of an estuary in Western Australia yielded fish with mature gonads, which raised the possibility that this sparid also spawns in this type of environment.

*Rhabdosargus sarba* attains maturity in South African coastal waters at ca 260 mm TL (Wallace, 1975), whereas 75% of the individuals of this species in the Arabian Gulf are mature at 180 cm TL (El-Agamy, 1989) and most of these in Chilka Lake, India, are mature at ca 190 mm TL (Patnaik, 1973). These differences may be related to the fact that the maximum length attained by *R. sarba* is far greater in South African waters, *i.e.* 750 mm (van der Elst, 1988; Oceanographic Research Institute, 2000) than in the Arabian Gulf, *i.e.* 300 mm (El-Agamy, 1989) and in India, *i.e.* 390 mm (Patnaik, 1973). *Rhabdosargus sarba* is reported to attain 450-500 mm TL in Australian waters (Kuiter, 1993; Yearsley *et al.*, 1999). Since the size of *R. sarba* at maturity in Western Australia is not known, it is not possible to determine whether the current minimum legal length (MLL) of 230 mm TL, designated for *R. sarba* by the Western Australian Department of Fisheries, is appropriate for managing this species in this state.

The first aim of the present study was to examine macroscopically and histologically the gonads of a wide size range of *R. sarba* from different environments in Western Australia in order to determine with certainty the type(s) of hermaphroditism exhibited by this sparid in this region. Particular emphasis has been placed on elucidating whether the same type of hermaphroditism is found in the very different environments selected for study, *i.e.* an estuary and marine waters in a temperate region and a large marine embayment (Shark Bay) in the subtropics ca 800 km further to the north. The second aim was to determine whether *R. sarba* spawns in nearshore as well as offshore coastal waters and whether it spawns in estuaries.
and, if so, whether this is dependent on the presence of “marine” salinities, \( i.e. \ ca \ 35 \% \). The third aim was to test the hypothesis that the spawning period of \( R. \ sarba \) in the southern and more northern water bodies will differ as a result of differences in water temperatures. The fourth aim was to determine the size and age of \( R. \ sarba \) at first maturity in the different water bodies to elucidate whether these variables differ between diverse environments in the same broad geographical region and whether they indicate that the current MLL of 230 mm is appropriate for managing this species of sparid in Western Australian waters. Finally, the data collected during the present study for \( R. \ sarba \) in Western Australian waters were compared with those recorded for this important commercial and recreational fish species elsewhere.

3.2 Materials and methods

3.2.1 Sampling and measurements

\( Rhabdosargus \ sarba \) was collected from the following three localities in Western Australia (see Fig. 2.1). (1) Marine waters at \( ca \ 32^\circ \ 00^\prime \ S, \ 115^\circ \ 45^\prime \ E, \) including both nearshore waters (< 1.5 m deep) and offshore waters over reefs (20-30 m deep). (2) The lower reaches of the Swan River Estuary, which is situated at a similar latitude, including both nearshore waters (< 1.5 m) and a deep region of the entrance channel (\( ca \ 11 \) m). (3) Shark Bay, which is located at \( ca \ 25^\circ \ 55^\prime \ S, \ 113^\circ \ 30^\prime \ E, \) including both intertidal mangrove areas and waters over reefs (1-5 m deep). Nearshore and intertidal waters were sampled using a 21.5 m long seine net with a 3 mm mesh in the bunt and, in the former environment, also with a 5.5 m long seine net made of 3 mm mesh. The waters over reefs were sampled by rod and line fishing. The water temperature and salinity at the bottom of the water column at each sampling site were measured on each sampling occasion. The total length (TL) and
wet weight of each fish were recorded to the nearest 1 mm and 0.1 g, respectively, and its gonads removed and weighed to the nearest 0.01 g. Each fish was aged using the number of annuli on their otoliths, which had been validated as being formed each year (see Chapter 5).

As with other sparids, the ovotestes of *R. sarba* comprise paired bisexual gonads which each consist of a medio-dorsal ovarian zone and a latero-ventral testicular zone that are separated by a wall of connective tissue (D’Ancona, 1949; Besseau & Bruslé-Sicard, 1995). Examination of the gonads of a large number of *R. sarba*, covering a wide size range, demonstrated that each gonad could be assigned macroscopically to one of the following categories. (1) Very thin, strand-like and sexually indeterminate. (2) Ovotestes containing relatively substantial amounts of both immature testicular and ovarian material. (3) Ovotestes in which only a testicular zone was detectable macroscopically and (4) ovotestes in which only an ovarian zone could usually be seen macroscopically. Note however that a small testicular and ovarian zone could usually be observed in histological sections of ovotestes belonging to categories 3 and 4, respectively. Fish with gonads at categories 3 and 4 were considered to be males and females, respectively.

Each gonad belonging to categories 3 and 4 was allocated, on the basis of its macroscopic appearance, to one of the following eight maturity stages, based on the scheme of Laevastu (1965), *i.e.* I = virgin, II = immature/resting, III = developing, IV = maturing, V = mature, VI = spawning, VII = spent, VIII = spent recovering.

Each month, the gonads of at least 20 individuals were removed from fish, that covered a wide length range of fish and contained the range of gonad stages present in the samples. The gonads were placed in Bouin’s fixative for 48 h and dehydrated in a series of increasing concentrations of ethanol. The mid-regions of each gonad, and in some cases also their anterior and posterior regions, were
embedded in paraffin wax, cut into 6 µm thick transverse sections and stained with either Mallory’s trichrome or Ehrlich’s haematoxylin and eosin. The sections were used to determine which stages in spermatogenesis and oocyte development were present in the testicular and ovarian zones, respectively, and to validate that the predominant zone of each ovotestis had been assigned to the appropriate stage. The importance of using an histological approach to support macroscopic studies when investigating hermaphroditism in fishes has been emphasised by Sadovy & Shapiro (1987).

3.2.2 Reproductive variables

The percentage contributions made to each length by those males and females with gonads at stages III to VIII in samples collected from marine waters at ca 32°S and Shark Bay were subjected to logistic regression analysis to determine the length at which 50 % of *R. sarba* first reached sexual maturity in each sampling locality. The data for each assemblage were randomly resampled and analyzed to create 1000 sets of bootstrap estimates for the parameters of the logistic regression and estimates of the probability of maturity within the range of recorded lengths. The 95 % confidence limits of the $L_{50}$s were derived using this resampling technique, taken as the 2.5 and 97.5 percentiles of the corresponding predicted values resulting from this resampling analysis. The point estimates of each parameter and of each probability of maturity at the specified length were taken as the medians of the bootstrap estimates.

The form of the logistic equation is:

$$P = \frac{1}{1 + \exp[-\ln(19)(L - L_{50})/(L_{95} - L_{50})]}$$

where $P =$ proportion mature, $L =$ total length, $L_{50}$ and $L_{95} =$ the length at which 50 % and 95 % of fish reach sexual maturity, respectively, and $\ln =$ the natural logarithm.

The same resampling technique was used to determine the $L_{50}$s and $L_{95}$s and associated 95 % confidence limits for male and female *R. sarba* in the lower Swan...
River Estuary. However, the above logistic curve could not be fitted to the prevalence of female mature fish in the Swan River Estuary because the prevalences of such fish in this environment never reach 100% in the larger size classes. Thus, the additional parameter $P_{\text{max}}$ was incorporated, as follows, into the logistic regression equation, $P = \frac{P_{\text{max}}}{1 + \exp[-\ln(19)(L - L_{50})/(L_{95} - L_{50})]}$. The logistic regressions describing the length at maturity relationships for data from each water body were compared using a likelihood ratio test (e.g. Cerrato, 1990) and employing a Bonferroni correction.

Gonadosomatic indices of each male and female *R. sarba* were determined from the equation, $W_1/(W_2-W_1) \times 100$, where $W_1 = \text{wet weight of the gonad}$ and $W_2 = \text{wet weight of the whole fish}$. The indices were calculated using data for fish $\geq L_{50}$ at first maturity in Shark Bay and the lower Swan River Estuary, and for all fish caught over reefs in coastal marine waters at ca 32°S since, during the spawning period, the vast majority of fish in that habitat was mature. The monthly proportions amongst the above three groups of fish of mature and spawning fish, *i.e.* individuals possessing gonads at stages V and VI, were also calculated.

3.3 Results

3.3.1 *Water temperature and salinity*

Mean monthly water temperatures in each of the sampling regions followed similar trends, declining markedly from their maxima in summer to their minima in winter and then rising sharply in spring (Fig. 3.1). The mean temperatures for Shark Bay exceeded by up to *ca* 3°C those in the more southern sampling localities in each month except August. The mean monthly salinities in marine waters at *ca* 32°S and
in Shark Bay at ca 26°S both remained relatively constant throughout the year (Fig. 3.1). However, they were typically 3 to 5 ‰ greater in the latter than former region, where they were always close to full strength sea water, i.e. 35 ‰. Although mean monthly salinities in the lower Swan River Estuary in late spring to early winter were similar to those in nearshore marine waters outside the estuary, they fell precipitously to a minimum of 23 ‰ in mid to late winter, and then rose sharply in early to mid-spring (Fig. 3.1).

3.3.2 Histology of gonads

The gonads of *R. sarba* < 80 mm in length contained predominantly connective tissue. Gonial cells were first found in a fish of ca 80 mm in length, and oocytes were first observed in an individual with a length of 91 mm (Fig. 3.2a). The gonad of the latter fish also contained clusters of the highly basophilic granulocytes that were often present in the gonads of fish with lengths between 80 and 100 mm. The gonads of *R. sarba* with lengths of ca 120 to 160 mm, i.e. < length at first maturity (see later), were either small and strand-like and consisted almost exclusively of immature testicular tissue (Fig. 3.2b) or were larger and consisted either of relatively substantial amounts of both immature testicular and ovarian tissue (Fig. 3.2c) or of predominantly ovarian tissue (Fig. 3.2d). In the first and second of these three categories of ovotestis, the testicular zone contained spermatogonia and spermatocytes and occasionally spermatids, whereas that of the third category only contained spermatogonia. All of the oocytes in the ovarian zone of each of the three categories were at a previtellogenic stage of development. It should be noted that each of the above three categories of ovotestis could clearly be distinguished macroscopically by the time *R. sarba* had reached a length of 140 mm.
Figure 3.1 Mean water temperatures and salinities at the bottom of the water column in marine waters at ca 32°S (black triangles), the lower Swan River Estuary (white squares) and Shark Bay (grey circles). Closed rectangles on the horizontal axis refer to summer and winter months and the open rectangles to autumn and spring months.
Figure 3.2 Histological sections of the gonads of juvenile (a-91 mm, b-151 mm, c-146 mm, d-145 mm) and large Rhabdosargus sarba (e,f-192 mm). Note that the gonad contains oocytes in (a), almost exclusively testicular tissue in (b), substantial amounts of both testicular and ovarian tissue in (c), and predominantly ovarian tissue in (d). Ovotestis of (e) from a mature-sized fish containing relatively substantial amounts of both testicular and ovarian tissue and (f) part of the testicular zone of that ovotestis. gc, granulocytes; o, ovarian zone; oc, oocytes; t, testicular zone; sc, spermatocytes; sp, spermatozoa. Scale bars. a, 25 µm; b, 500 µm; c, 250 µm; d, 1000 µm; e, 2000 µm; f, 25 µm.
Section 3.2a stained with Ehlrich’s haematoxylin and eosin and sections 3.2b-f with Mallory’s trichrome.
A small proportion of larger (mature-sized) *R. sarba*, *i.e.* > 180 mm, contained ovotestes consisting of substantial proportions of both testicular and ovarian tissue (Fig. 3.2e). During the spawning period, the ovarian zone of such ovotestes was always immature, whereas the testicular zone was mature, *i.e.* contained some spermatozoa in addition to spermatocytes and spermatids (Fig. 3.2f).

Just prior to and during the spawning period, many of the gonads of *R. sarba* > 180 mm consisted of developing or mature testicular tissue, *i.e.* contained predominantly spermatids and spermatozoa, and a very small amount of ovarian tissue in which the oocytes were arrested at the chromatin nucleolar stage (Fig. 3.3a) or occasionally the cortical alveoli stage (Fig. 3.3b). The chromatin nucleolar oocytes were occasionally undergoing atresia, while the cortical alveoli oocytes were almost invariably atretic. Such gonads are considered to be functional testes and thus belong to functional males. Comparisons of the gonads of larger fish throughout the year provided overwhelming evidence that, while the testicular zone of such ovotestes regressed to an immature state during the non-spawning period, that zone always remained dominant in terms of size. Fish with this type of ovotestis are clearly males.

In contrast to the above type of ovotestis, most of the other mature-sized *R. sarba*, that were obtained either just prior to or during the spawning period, contained a large ovarian zone and a testicular zone that was always very small. When the ovarian zone of these ovotestes were at stage III-IV of maturity, they contained oocytes at various stages of maturation (Fig. 3.3c), while the testicular zone in the same ovotestis possessed only spermatogonia (Fig. 3.3d). Later in its development, the ovarian zones of this type of ovotestis contained large numbers of yolk granule oocytes (Fig. 3.3e) and, in the most advanced stages, also hydrated...
Figure 3.3 Regions of the ovotestes of two fish in which the testicular tissue predominated and was full of spermatids and the oocytes in the ovarian zone were either previtellogenic or undergoing atresia (a,b). Ovotestes in which the ovarian zone was at maturity stage III (c) and stage V (e) and the corresponding testicular zones (d & f, respectively). a, atretic oocyte; ca, cortical alveolar oocyte; cn, chromatin nucleolar oocyte; dt, degenerating testicular tissue; pn, perinucleolar oocyte; sg, spermatogonia; st, spermatids; t, testicular tissue; yg, yolk granule oocyte. Scale bars. a,b,d,f 25 µm; c, 400 µm, e, 250 µm. All sections were stained with Mallory’s trichrome.
oocytes and/or post-ovulatory follicles. The associated testicular zone has become very small (Fig. 3.3f) and cannot be detected macroscopically. Fish containing the above type of ovotestis are clearly females.

3.3.3 Sex ratios

The following quantitative account of the changes in the prevalences of fish with different “types” of gonads, including those fish that were clearly males or females, is based on a macroscopic examination of the gonads of R. sarba. However, it should be recognized that each of these “types” can clearly be matched with a particular “type” in histological sections. None of the gonads of individuals that were caught in coastal marine waters at ca 32°S and measured < 120 mm contained either testicular or ovarian tissue that could be identified macroscopically (Fig. 3.4).

Gonads in which both testicular and ovarian zones could be detected macroscopically, and thus corresponded to the gonads that were designated histologically as category 2 in juvenile fish (see previous section), were first found in the 120-139 mm length class. The percentage contribution made by fish containing macroscopically identifiable testicular and ovarian tissue declined sequentially from 12 % in this size class to < 5 % in the 180-199 to 240-259 mm length classes and remained at 0 % in all subsequent length classes. Juvenile fish with gonads in which only testicular tissue could be detected macroscopically were first recorded in the 120-139 mm length class. At this stage, these gonads correspond to those designated histologically as category 1 in juveniles (see previous section). The contribution made by fish in which the gonads consist almost exclusively of testicular tissue and are thus males remained at between 42 and 67 % until the 280-299 mm length class.

Although the percentages of male R. sarba were as high as 79 and 70 % in the two size classes of largest fish, respectively, the numbers of fish collected for
Figure 3.4 Prevalence of fish with different gonadal categories in sequential 20 mm length classes of *Rhabdosargus sarba* caught in marine waters at ca 32°S, the lower Swan River Estuary and Shark Bay. The categories are (1) indeterminate, *i.e.* small juveniles (dark grey), (2) ovotestes containing relatively substantial amounts of both immature testicular and ovarian tissue (white), (3) ovotestes in which the testicular zone clearly predominates, *i.e.* definitive males (white and diagonal lines), and (4) ovotestes in which the ovarian zone clearly predominates, *i.e.* definitive females (black). Sample sizes for each 20 mm length class are shown.
these size classes were only 14 and 10, respectively. Fish with ovotestes in which only ovarian tissue could be detected macroscopically were also first recorded in the 120-139 mm length class.

At this stage, these gonads correspond to those designated histologically as category 3 in juveniles and were thus destined to become functional ovaries in adults (see previous section). The percentage of these females in each successive 20 mm length class between 160-179 and 260-279 mm, each of which was particularly well represented and contained < 9 % of fish possessing gonads with substantial amounts of testicular and ovarian tissue, lay between 33 and 57 % (Fig. 3.4).

The trends exhibited in Shark Bay by the percentage of fish with ovotestes containing both testicular and ovarian material, and of the males and females, were similar in the size classes between 160 to 279 mm to those just described for the same and likewise well represented length classes in marine waters (Fig. 3.4). Although no *R. sarba* of 80 to 139 mm were caught in the Swan River Estuary, the data for the other size classes are entirely consistent with those recorded for marine waters at *ca 32°*S and Shark Bay (Fig. 3.4).

The gonads of all 0+ fish were strand like and did not contain clearly identifiable testicular or ovarian zones. The percentage of *R. sarba* with gonads containing macroscopically identifiable testicular and ovarian zones declined from 7 % in the 1+ age class to 2 % in the 4+ age class and 0 % in all older age classes while the percentage of males and females increased from 33 and 21 %, respectively, in the 1+ age class to reach, in the case of both sexes, between 40 and 60 % in older age classes. Similar trends were exhibited by *R. sarba* in Shark Bay and the lower reaches of the Swan River Estuary (Fig. 3.5).
Figure 3.5 Occurrence of fish with different gonadal categories in sequential age classes of *Rhabdosargus sarba* in marine waters at ca 32°S, the lower Swan River Estuary and Shark Bay. The categories are (1) indeterminate, *i.e.* small juveniles (dark grey), (2) ovotestes containing relatively substantial amounts of both immature testicular and ovarian tissue (white), (3) ovotestes in which the testicular zone clearly predominates, *i.e.* definitive males (white and diagonal lines), and (4) ovotestes in which the ovarian zone clearly predominates, *i.e.* definitive females (black). Sample size for each age class are shown.
3.3.4 Length and age at maturity

The $L_{50}$ of males at first maturity in coastal marine waters at $ca$ 32°S, derived from samples collected in both nearshore waters and offshore over reefs, was 206 mm, which was far greater than the 173 mm estimated for males in Shark Bay, and the 171 mm estimated for the lower Swan River Estuary (Fig. 3.6). Similar differences were observed for females, with the $L_{50}$ of 218 mm in marine waters at $ca$ 32°S being greater than the 170 and 177 mm recorded for Shark Bay and the lower Swan River Estuary, respectively. The logistic curves for both males and females were far steeper in the lower Swan River Estuary than in either marine waters at the same latitude or in Shark Bay (Fig. 3.6). The $L_{50}$s for the two sexes at first maturity were not significantly different ($p > 0.05$) in any of the three water bodies. While the $L_{50}$s for neither females nor males in the Swan River Estuary and Shark Bay were significantly different ($p > 0.05$), they were each significantly different from those of the corresponding sex in marine waters at $ca$ 32°S ($p < 0.001$).

Maturity was first attained at the end of their first year of life by very few males in marine coastal waters at ca 32°S and by some males in the Swan River Estuary, and by some males at the end of their second year of life in Shark Bay (Fig. 3.7). Some females had attained maturity by the end of their second year of life in marine coastal waters and Shark Bay and by most females of that age in the Swan River Estuary.

During the spawning period of $R. sarba$, 92 % of the females caught in nearshore shallow marine waters were immature, whereas 94 % of the females caught over reefs were mature (Fig. 3.8). The trends exhibited by males (data not shown) in these waters were essentially the same as those just described for females.
Figure 3.6 Logistic regressions and their 95% confidence limits fitted to the percentage contributions made by mature individual male and female Rhabdosargus sarba at each length during the spawning season in marine waters at ca 32°S, the lower Swan River Estuary and Shark Bay. Months refer to spawning season.
Figure 3.7 Percentage frequency of occurrence of gonads at stages III-VIII in sequential ages of male and female *Rhabdosargus sarba* during the spawning season in marine waters at ca 32°S, the lower Swan River Estuary and Shark Bay. Sample size for each age category are shown.
Figure 3.8 Percentage frequency of immature (white) and mature (grey) female *Rhabdosargus sarba*, i.e. with gonads at stages I-II and III-VIII, respectively, in each successive 20 mm length class in shallow, nearshore and deeper, offshore marine waters over reefs at ca 32°S and in shallow, nearshore and the deeper channel waters of the lower Swan River Estuary. Sample size for each length class is shown.
This parallels the situation in Shark Bay where, during the spawning period, no mature male or female *R. sarba* were caught in shallow mangrove areas, whereas 76 and 66 % of the males and females, respectively, caught over reefs and in intertidal rocky areas were mature. Although the majority of female *R. sarba* that were caught at lengths < 180 mm in nearshore waters of the lower Swan River Estuary were immature, the majority of females above this length were mature, as was also the case with the females in the deep waters of the channel of this estuary (Fig. 3.8). The same trends were exhibited by males in the estuary (data not shown).

### 3.3.5 Trends in reproductive variables

The mean monthly GSIs of female *R. sarba* in the Swan River Estuary declined from 1.2 and 2.1 in January and February to 0.9 in March and then increased progressively to between 4.0 and 4.4 in September to November, before declining precipitously to 1.8 in December (Fig. 3.9). The mean monthly GSIs of males followed a similar trend (Fig. 3.9). The mean monthly GSIs of female *R. sarba* over reefs in marine offshore waters at ca 32°S remained low, *i.e.* < 1.0, from February to April and then increased progressively to 2.6 in June and then to a peak of 3.2 in July. No fish were caught in August and September, due largely to the difficulties posed to fishing over reefs during the inclement weather that prevails in those months. The mean monthly GSIs in October and December, *i.e.* 1.5 and 1.2, respectively, were far lower than in June and July (Fig. 3.9). The trends exhibited by the mean monthly GSIs of male *R. sarba* from the same location were similar to those just described for females. The mean monthly GSIs for females and males in Shark Bay formed sharp peaks in July/August and August, respectively (Fig. 3.9). The trends exhibited in each month by the percentages of female and male fish with gonads at stages V/VI, *i.e.* mature/spawning, followed closely the mean monthly trends in GSIs, *i.e.* the months
Figure 3.9 Mean monthly gonadosomatic indices ±1SE and monthly prevalences (%) of gonad stages V and VI of *Rhabdosargus sarba* collected from marine waters over reefs, the lower Swan River Estuary and Shark Bay, using data for fish ≥ $L_{50}$ at first maturity. Sample size for each month is shown. Closed rectangles on the horizontal axis refer to summer and winter months and the open rectangles to autumn and spring months.
in which those percentages were low and high corresponded to those in which the GSIs were low and high, respectively (Fig. 3.9).

Histological sections demonstrated that many female *R. sarba* caught over reefs at ca 32°S in June and July were in spawning condition, *i.e.* contained either migratory nucleus or hydrated stage oocytes and/or contained post ovulatory follicles (Fig. 3.10a) and that oocytes were rarely undergoing atresia during this period. However, in contrast to marine waters over reefs at ca 32°S, most of the advanced-stage oocytes in ovaries of *R. sarba* caught in the lower reaches of the Swan River Estuary in June and July were atretic (Fig. 3.10b).

The 5.5 m larval seine net first caught early post-settlement *R. sarba*, *i.e.* < 15 mm, in nearshore coastal waters at ca 32°S in September of 1999 and in August of 2000 (Fig. 3.11). Juveniles < 15 mm were caught in these waters until December and November, respectively. In 1999, the modal length class of 0+ *R. sarba* in those waters increased from 10-14 mm in September to 15-19 mm in November and then to 55-59 mm in January 2000. In 2000, the modal length classes of 0+ *R. sarba* remained at 10-14 mm between August and September, with fish with lengths < 10 mm being caught in the first two of these months. The minimum lengths of *R. sarba* in November, December and January were 11, 12 and 15 mm, respectively (Fig. 3.11).
**Figure 3.10** Histological section of ovarian zones of female *Rhabdosargus sarba* caught (a) during the spawning period in offshore marine waters over reefs at ca 32°S and (b) during July in the lower Swan River Estuary. a, atretic oocyte; ca, corticle alveolar oocyte; mn, migratory nucleus oocyte; pof, post-ovulatory follicle; yg, yolk granule oocyte. Scale bars = 250 µm. Both sections were stained with Mallory’s trichrome.
Figure 3.11 Length-frequency histograms for early 0+ juvenile *R. sarba* in successive 20 mm length classes caught in sheltered marine waters at ca 32°S in each month between August and January in 1999/2000 (black) and 2000/2001 (white).
3.4 Discussion

3.4.1 Is Rhabdosargus sarba a protandrous hermaphrodite?

The results of this study provide the following evidence that, in Western Australian waters, *Rhabdosargus sarba* is typically a rudimentary hermaphrodite (late gonochorist) *sensu* Buxton & Garratt (1990). Namely, it is a species in which the juveniles possess gonads with both testicular and ovarian tissue and eventually develop into either males with functional testes and rudimentary ovarian tissue or females with functional ovaries and rudimentary testicular tissue (*Fig. 3.12*). (1) Within each water body, the males and females of *R. sarba* attain maturity at a similar length and age. (2) The ratio of males to females was typically close to parity in all length and age classes that were above the length and age at which maturity is typically first attained and which were well represented in the samples in terms of their numbers. In other words, there was no tendency for the proportions of one sex to rise consistently with increasing length or age and for those of the other sex to exhibit the reverse trend. (3) Fish that contained gonads with testicular and ovarian zones that were both macroscopically conspicuous were relatively rare above the length and age at first maturity. Although there can be little doubt that most *R. sarba* develop rapidly into either a male or a female during juvenile life, there is still the question of the fate of the relatively small number of individuals of this species that are greater than the length at maturity and possess gonads in which both testicular and ovarian zones can be clearly detected macroscopically. This question is explored in section 3.4.2.

The overwhelming evidence that *R. sarba* is typically a rudimentary hermaphrodite in a range of Western Australian environments, contrasts with the conclusions drawn by Yeung and Chan (1987) and Garratt (1993) that this species is
Figure 3.12 Diagrammatic representation of gonadal changes undergone by Rhabdosargus sarba in Western Australian waters. Bold lines denote main avenues of change. Lighter lines denote the possible lines of development of the least common category of ovotestis, *i.e.* that in which both testicular and ovarian tissues are relatively abundant. Note that the term mature refers to the condition during the spawning period.
a protandrous hermaphrodite in waters off Hong Kong and South Africa, respectively. The macroscopic and histological data provided by Yeung & Chan (1987) clearly demonstrated that the relative abundance of males in the assemblage they studied declined progressively with increasing body size, whereas that of females pursued the opposite trend, thus providing very strong evidence of protandry. The trends were not as clearly pronounced in Garratt’s study. Furthermore, the results of the present study are not consistent with the conclusion of Kinoshita (1939) for a population in Japanese waters that the testicular tissue develops first in juvenile *R. sarba*. However, his results were based on a very small sample size and must therefore be treated with caution (see also Garratt, 1993).

The contrast between the results of this study and those produced by Yeung & Chan (1987) and Garratt (1993) suggests that whether or not *R. sarba* undergoes sex reversal might depend on environmental conditions. Certainly, the co-existence of male and female tissues in an individual gonad provides the structural basis for natural sex reversal (Yeung & Chan, 1987). However, it has been demonstrated in this study that *R. sarba* is typically a rudimentary hermaphrodite in three very different environments in Western Australia, namely in both marine and estuarine temperate waters at *ca* 32°S and a subtropical embayment *ca* 800 km to the north. Alternatively, it is possible that the differences in the type of hermaphroditism exhibited by *R. sarba* in different regions may be due to what is now regarded as a single species comprising either subspecies or even separate species (Kuiter, 1993). Such a conclusion would be consistent with the fact that *R. sarba* attains a far greater size in the waters off South Africa (van der Elst, 1988; Garratt, 1993; Radebe et al., 2002) than those recorded for this species in India (Patnaik, 1973), the Arabian Gulf (El-Agamy, 1989) and Australian waters (Kuiter, 1993; Yearsley et al., 1999). Although the morphological characteristics of the specimens of *R. sarba* from South
Africa, that were deposited in the Museum of Western Australia, and also of those of *R. sarba* from Western Australia all fall within the details given in the key for this species, the specimens from the two regions differed in appearance (B. J. Hutchins, pers. comm.).

### 3.4.2 Scheme for gonadal changes in *Rhabdosargus sarba*

The results of this study demonstrate that, in three very different environments in Western Australia, the gonads of juvenile *R. sarba* develop from a thin strand-like structure, consisting predominantly of connective tissue, into gonads that contain gonial cells and then into ovotestes that possess either predominantly testicular tissue, or into larger structures that contain either relatively substantial amounts of both testicular and ovarian tissue or predominantly ovarian tissue (Fig. 3.12). The change, in the majority of fish, from a gonad with just gonial cells into one in which the main component is almost exclusively testicular tissue or ovarian tissue occurs over the relatively short period that it takes the fish to grow from *ca* 90 to 120 mm. Since the differences are already present in fish of 120-160 mm, they have developed prior to the length at which the first mature males and females were found. It is thus assumed that the juvenile fish in which the testicular zone predominates become functional males, whereas those in which the ovarian zone predominates become functional females. This conclusion is based on the fact that most of the fish caught above the size at first maturity contained gonads in which either only testicular or ovarian tissue could be detected macroscopically and that this was often the case with these ovotestes even in histological sections. Thus, once a fish develops into either a definitive male or definitive female, it remains so for the rest of its life.

Fish with the least prevalent type of gonad, *i.e.* that in which both testicular and ovarian zones could be detected macroscopically, declined progressively as the
lengths of those fish increased from 120 to 200 mm and were generally absent in the larger size classes. While such fish must thus presumably develop into either definitive males or females during late juvenile or early adult life, it is not clear which is the predominant or exclusive direction of such change. Although, during the spawning period, the testicular zone of the ovotestes of these fish contain spermatozoa, it is relatively far smaller than that in the ovotestes of definitive males, in which the testicular zone is very large and contains mature tissue and the adjacent ovarian zone is so small that it cannot be detected macroscopically and often cannot be detected even in histological sections. Furthermore, whereas during the spawning period, the spermatogenic cells in the ovotestes of fish possessing conspicuous testicular and ovarian zones were at various stages of development and included substantial numbers of spermatocytes, those in the testicular zone of definitive males were almost invariably spermatids or spermatozoa. While the possibility cannot be excluded that the small number of mature-sized fish with the former characteristics could function as males, they are clearly not as functionally well adapted to do so as are definitive males.

The gonadal development in *R. sarba* is similar to that of the black bream *Acanthopagrus butcheri*, that occurs in the middle and upper reaches of the Swan River Estuary in Western Australia and is a rudimentary hermaphrodite (Sarre & Potter, 1999; Sarre, 1999).

### 3.4.3 Spawning period

The sharp rise in the mean monthly GSIs and prevalence of stage V and VI ovaries in the females of *R. sarba* in Shark Bay between June and July and their precipitous decline in September provides strong evidence that this species spawns in this subtropical embayment between July and September. Such a view is consistent
with the presence of hydrated oocytes in the ovaries of several fish in July, and the
capture with the 21.5 m seine net of a few post-settlement juveniles, with total
lengths as small as 17 mm as early as August and of large numbers of small juveniles
< 25 mm between September and November. It is also broadly consistent with the
trends exhibited by the mean monthly GSIs and prevalences of stage V and VI testes
among males.

Unfortunately, *R. sarba* was not obtained from over reefs in offshore marine
waters at **ca 32°S** in either August or September. This was due to a combination of
the effects of the inclement weather that prevails on the lower west coast of Australia
during this time, which frequently made fishing in these waters difficult or
impossible, and also, as revealed by underwater video footage, to the disappearance
of *R. sarba* from the same areas of the reefs that they had occupied in the
immediately preceding months. Yet, it is highly relevant that the trends exhibited by
the mean monthly GSIs and/or prevalences of stage V and VI gonads in females
during the preceding months and in October were similar to those exhibited by
females in Shark Bay. However, the marked rise that occurs in these two
reproductive variables in both males and females took place one month earlier in
marine waters at 32°S than in Shark Bay, *i.e.* between May and June. Furthermore,
the mean monthly GSIs and prevalence of gonads at stages V and VI during July in
marine waters over reefs at **ca 32°S** reached very similar levels in that month to that
of the mean monthly maximum in Shark Bay, which was recorded in August. Thus,
although *R. sarba* spawns at a similar time of the year in Shark Bay and in offshore
marine waters at **ca 32°S**, it apparently commences one month earlier in the latter
waters. Since temperature is typically a major stimulus for spawning activity in fish
(Lam, 1983), this regional difference could be related to the fact that the recovery of
water temperatures from their winter minima commences in August in marine waters
at ca 32°S and in September in Shark Bay. The indications that spawning occurs between June and August on the lower west coast is consistent with the collection of substantial numbers of post-settlement *R. sarba* with lengths < 15 mm between August and November.

The overall progressive rise in the mean monthly GSIs and prevalences of stage V and VI gonads of females occurred *ca* two months later in the lower Swan River Estuary than in offshore coastal waters at 32°S. Thus, while in June, for example, the mean monthly GSI was still only 1.7 in the estuary, it had already reached 2.6 in marine waters and the mean monthly GSIs recorded for females in the estuary in August and September had been attained in marine waters by July. Furthermore, in the estuary the mean monthly GSIs did not reach their maxima until November and the prevalences of stage V and VI ovaries were still high in this month, whereas both of these variables had already undergone a pronounced decline in marine waters by October. It is also noteworthy that the GSIs and prevalence of stage V and VI ovaries of fish in the estuary were high for a relatively long period.

The above comparisons suggest that spawning commences appreciably later and extends for a longer period in the Swan River Estuary than over offshore coastal reefs in marine waters at the same latitude. Furthermore, in contrast to the situation during the spawning period in marine waters, the yolk granule oocytes of many fish caught in the lower Swan River Estuary between June and August were undergoing atresia (Fig. 3.10), which implies that spawning success would have been less in the estuary. The later onset of spawning and apparently reduced spawning success of *R. sarba* within rather than outside the estuary in winter could reflect a detrimental influence of the low salinities found during that period in the estuary on this essentially marine species. Thus, in August, when spawning would presumably have been occurring in marine waters, mean monthly salinities in the lower Swan River
Estuary had fallen to their minima of just over 20 ‰ and individual salinities had, at times, declined below this level. Thus, the delay in spawning and high prevalence of atretic oocytes in females of *R. sarba* in the lower Swan River Estuary may be due to the reduced salinities found at critical periods in that system. It is also relevant that the laboratory studies of Mihelkakis & Kitajima (1994) showed that the production of live larvae with no abnormalities was the greatest by far in salinities of 20 to 30 ‰. Since *R. sarba* spawns just before the ebb tides in the lower Swan River Estuary (Chapter 4), the larvae are swept out to sea and into waters that will remain at ca 35 ‰, a movement that accounts for the low number of juveniles < 120 mm that were caught in the estuary. My data shows that *R. sarba* starts to reappear in the estuary when they have reached ca 120 mm.

The time of spawning and recruitment of post-settlement juveniles of *R. sarba* in waters over reefs and in nearshore waters, respectively, were very similar to that of the King George whiting *Sillaginodes punctata* in the same waters (Hyndes *et al*., 1998; A. Whitehead, unpublished data, Murdoch University). The fact that the timing of spawning and recruitment of the early post-settlement juveniles of these two species coincides with the time at which water temperatures begin to rise after the cold winter period, provides these species with a long first growing season, which therefore increases the chances of their juveniles surviving (see Conover, 1992; Cushing, 1990).

**3.4.4 Length and age at maturity**

The lengths at which the males and females of *R. sarba* attain maturity in the Swan River Estuary, *i.e.* 171 and 177 mm, respectively, were very similar to the lengths which the corresponding sexes reach maturity in Shark Bay, *i.e.* 173 and 170 mm, respectively. However, *R. sarba* tended to reach maturity earlier in the
Swan River Estuary than in Shark Bay, which implies that the attainment of maturity by this sparid is related more to length and thus to growth rate than to age.

Few of the fish caught in nearshore marine waters at 32°S during the spawning period were mature, even at lengths well above the 170–177 mm at which 50% reached maturity in the Swan River Estuary and Shark Bay, whereas the vast majority of the fish of this size in offshore waters were mature. This implies that *R. sarba* tends to remain in nearshore marine waters until it is physiologically ‘ready’ to become mature. Since the number of fish caught in nearshore waters was greater than in offshore waters, and fish did not tend to become mature in those waters at the same age as in offshore waters, the logistic curves for the prevalence of mature male and female fish in nearshore and offshore waters collectively were shifted to the right. Thus, the *L*₅₀s for male and female *R. sarba* at first maturity in coastal marine waters (*i.e.* 206 and 218 mm, respectively) were almost certainly anomalously high for the population in temperate marine waters as a whole.

The finding that the maturity curves for females and males of *R. sarba* in the lower Swan River Estuary were steeper, *i.e.* all fish reach maturity at a similar length, than in marine waters at ca 32°S or Shark Bay, may reflect the fact that the sampling sites in the lower Swan River Estuary were all in relatively close proximity (< 2 km apart) and covered only one type of habitat. In contrast, in the later two environments, fish were caught over a relatively large area and in a number of different habitat types.

The implications of the size at first maturity for management are far less severe for rudimentary hermaphrodites than for protandrous and protogynous hermaphrodites, where the direction of fishing effort towards large individuals can lead to a deleterious reduction in females and males, respectively (Buxton, 1992). Since *R. sarba* generally reaches first maturity at a length well below the current
minimum legal length (MLL) for the retention of this species in Western Australia, *i.e.* 230 mm TL, the majority of the individuals of this species have the opportunity to spawn at least once before they are legally allowed to be fished. However, the results of this and other studies have emphasized the importance of protected nearshore marine waters as nursery areas for *R. sarba*, as well as for other species such as the King George whiting *Sillaginodes punctata*, which have a very similar life history (Hyndes *et al.*, 1996a; Hyndes *et al.*, 1998).
4.1 Introduction

Although *R. sarba* is typically regarded as a marine species that frequently uses estuaries as a nursery area (*e.g.* Wallace, 1975; Potter & Hydnes, 1999; Smith & Suthers, 2000), it spawns in both coastal marine waters and the Swan River Estuary on the lower west coast of Australia (Chapter 3). However, in comparison with populations of *R. sarba* in nearby coastal marine waters, the individuals of this species in the lower Swan River Estuary attain maturity later in the year, *i.e.* spring vs winter, and therefore after salinities have risen from their winter minima. If this indication that the onset of spawning of *R. sarba* in the Swan River Estuary is influenced by salinity was proved to be correct, it would parallel that exhibited by another teleost species, *Cynoscion nebulosus*, in estuaries entering the Gulf of Mexico (Brown-Peterson *et al.*, 2002).

The spawning of many species of teleost and marine invertebrates is correlated with lunar periodicity and the associated tidal cycles (*e.g.* Schwassmann, 1971; Taylor, 1984; Greeley *et al.*, 1986; Hoque *et al.*, 1999), with the spawning of such fish species typically peaking around the full and/or new moons (*e.g.* Johannes, 1978; Taylor & DiMichele, 1980; Greeley *et al.*, 1986). Many fish and invertebrates with pelagic eggs spawn on high or ebb tides, which enables those eggs and the subsequent larval stages to be transported away from the spawning areas, where planktivorous predators are concentrated, and thereby reduce the likelihood of predation on those early life cycle stages (Taylor, 1984; Johnson *et al.*, 1990; Morgan, 1990). The possibility that the eggs of *R. sarba* may be transported away
from spawning areas in the lower Swan River Estuary by tides is raised by the fact that the recruitment of the early 0+ individuals of this species into the lower estuary where extensive spawning occurs is very poor (Chapters 3 and 5).

For determining the fecundity of a fish species, it is essential to know whether that species has determinate or indeterminate fecundity. Species with determinate fecundity are those in which the number of vitellogenic oocytes present in their ovaries immediately prior to the onset of the spawning period corresponds to their potential annual fecundity (Hunter et al., 1985; Lisovenko & Andrianov, 1991). In other words, none of the previtellogenic oocytes will become mature once spawning has commenced and thus be able to contribute to the annual fecundity of that species. During the period immediately prior to and during spawning, such species are characterized by the presence of a distinct gap between the upper limit of the diameters of the previtellogenic oocytes and the lower limit of those of the vitellogenic oocytes (Hunter et al., 1985; Kjesbu et al., 1991).

In contrast, in species with indeterminate fecundity, the distribution of oocyte diameters essentially forms a continuum, reflecting the continuous maturation of oocytes throughout the spawning season, with the result that a number of previtellogenic oocytes will progress through to maturity during that period. Consequently, counts of the standing stock of vitellogenic oocytes present just prior to the onset of spawning will almost inevitably result in an underestimate of the annual fecundity of such species (Hunter et al., 1985, 1992; Lisovenko & Andrianov, 1991; Osborne et al., 1999). Thus, estimation of the annual fecundity of species with indeterminate fecundity requires a combination of data on batch fecundity and spawning frequency (Hunter et al., 1985). For this purpose, batch fecundity, which refers to the number of oocytes released during a single spawning event, can be estimated by counting the number of hydrated oocytes in ovaries immediately prior
to that spawning (Hunter et al., 1985). However, to determine batch fecundity, it is essential to know the time of day when oocytes become hydrated and released (Lisovenko & Andrianov, 1991). The frequency with which a fish spawns during the spawning period can be determined from the frequency either of mature female fish with ovaries that contain post-ovulatory follicles (POFs) of known age or of mature fish possessing ovaries with hydrated oocytes (Hunter & Macewicz, 1985).

Despite the importance and widespread occurrence of *R. sarba*, and the great value of fecundity data for stock assessments (Hunter et al., 1992; Nichol & Acuna, 2001), there has been only one attempt to estimate the annual fecundity of wild populations of this sparid (El-Agamy, 1989). Although El-Agamy (1989) considered that *R. sarba* is a “fractional” spawner and recognised that it has a protracted spawning season, his fecundity estimates represented the number of mature eggs present in the ovaries of mature females just prior to the commencement of the spawning period. Thus, the possibility that this species has indeterminate fecundity was not taken into account and his counts may therefore have represented a gross underestimate of the annual fecundity of this species. Although *R. sarba* is known to spawn during the winter and spring in Western Australia (Chapter 3), there are no data on the time of day at which this species spawns nor its frequency of spawning.

The aims of this study were as follows. (i) Determine whether *R. sarba* has determinate or indeterminate fecundity. (ii) Establish the time of day when the oocytes of *R. sarba* in the lower reaches of the Swan River Estuary become hydrated and when ovulation and spawning occur. (iii) Determine the relationship between batch fecundity and fish length. (iv) Estimate the average frequency with which *R. sarba* spawns in the lower Swan River Estuary, and use this to calculate the annual fecundity of this species in this estuary. (v) Establish if the spawning of *R. sarba* is correlated with the strength and type (ebb vs flood) of tide in the lower
reaches of the Swan River Estuary and whether it occurs mainly when salinities are high and thus approach those of the marine waters in which this species typically spawns.

4.2 Materials and methods

4.2.1 Tides

The maximum daily tidal heights at the mouth of the Swan River Estuary were calculated using the tidal prediction data of the Coastal Data Centre at the Department of Planning and Infrastructure, Government of Western Australia (http://www.coastaldata.transport.wa.gov.au). The maximum tidal range at the mouth of the Swan River Estuary is small, *i.e.* ≤ 0.8 m, and tides can be diurnal or semi-diurnal, depending on the time of year (Spencer, 1956).

4.2.2 Sampling and measurements

To obtain material for determining the period and frequency of spawning and batch fecundity of *R. sarba*, and also whether spawning is related to tidal cycle, 1098 fish were collected in 2001 and 2002 by seine netting in nearshore, shallow waters of the Swan River Estuary at a distance of *ca* 3 km from its mouth. Since a previous study had demonstrated that, in the lower Swan River Estuary, *R. sarba* reaches maturity between July and November (Chapter 3), sampling, which was undertaken at least once weekly, was restricted to these months. It was also restricted to between 22:00 and 24:00 h since a preliminary examination of ovaries removed from mature fish caught at intervals during the day showed that, through their possession of macroscopically visible hydrated oocytes, it became possible at this time to identify those female fish that were about to spawn (for further details, see results). It should
be noted that during this and a previous study, seine netting in nearshore, shallow waters yielded very few *R. sarba* between 03:00 h and the dusk of the following evening. However, 693 *R. sarba* were caught in deeper waters (11 m) further offshore within the estuary channel by rod and line angling at regular intervals up to 04:30 h at night.

*Rhabdosargus sarba* was collected at regular intervals on both 1-2 September and 13 September of 2001 in order specifically to determine the precise timing of oocyte hydration on a given day. On the first occasion, *R. sarba* was sampled for up to ca 2 h at intervals commencing at 18:30, 21:30, 00:30 and 03:30 h and on the second occasion for a comparable period at intervals commencing at 18:30 and 22:30 h. Fish caught at night on other occasions during the spawning period by seine netting in nearshore, shallow waters and by rod and line angling in deeper waters were used both to validate that the trends in oocyte hydration on the above dates were typical and to estimate the time when ovulation and spawning occurred.

The sex, total length (to the nearest 1 mm) and total weight and gonad weight (to the nearest 0.01 g) of each fish caught between July and November of 2001 and 2002 were recorded.

### 4.2.3 Gonadal staging and histology of ovaries

On the basis of its macroscopic appearance, each gonad was assigned to one of the following stages in maturation, based on the scheme of Laevastu (1965), *i.e.* I = virgin, II = immature/resting, III = developing, IV = maturing, V = mature, VI = spawning, VII = spent, VIII = spent/recovering. When hydrated oocytes could be seen through the ovarian wall, a record was kept as to whether they were still distributed throughout the ovary or were located in the ovarian duct, *i.e.* whether or not the fish were ovulating (see later).
Both ovaries were removed from typically five fish caught during each 3 h sampling interval on 1-2 September 2001 and 13 September 2001. One of the ovaries of each of these fish, and of a large subsample of stage V and VI ovaries from other catches of *R. sarba*, were placed in Bouin’s fixative for ca 48 h. Part of the mid region of each of these ovaries, and also part of the anterior and posterior regions of a subsample of these ovaries, were dehydrated in a series of ethanols, embedded in paraffin wax, cut into 6 µm sections and stained with Mallory’s trichrome. The oocyte diameters in sections of the mature ovaries (stage V) of individuals caught during the spawning period were measured to the nearest 10 µm using an eyepiece graticule in a compound microscope. The resultant oocyte diameter distributions, in conjunction with the stages of development of the oocytes, were used to determine whether *R. sarba* has determinate or indeterminate fecundity *sensu* Hunter *et al.* (1985). The other sections were employed to examine the characteristics of the ovaries at different times, *e.g.* stages in development of oocytes and whether post-ovulatory follicles (POFs) were present and, if so, their approximate age, and whether any oocytes were undergoing atresia.

The other lobe of each pair of ovaries belonging to those fish that had been caught at intervals on 1-2 September 2001 and 13 September 2001 was preserved in 10 % neutrally-buffered formalin solution and used for determining the distribution of the diameters of its oocytes at those different intervals and, in those cases when that ovary contained hydrated oocytes, also the batch fecundity of the fish (see later). For determining the distribution of oocyte diameters, each of these ovarian lobes were cut into several pieces and placed in containers containing 10 % neutrally-buffered formalin, which were vigorously shaken until their oocytes had become evenly suspended in the solution. The solution was then passed through a 125 µm sieve to remove the smaller and non-vitellogenic oocytes. The diameters of a
representative subsample of the remaining and very largely vitellogenic oocytes were measured under a dissecting microscope using an eyepiece graticule.

On the basis of their histological characteristics, atretic oocytes were separated into one of four sequential stages according to the extent to which they had been resorbed, *i.e.* α, β, γ, and δ-stages (Hunter & Macewicz, 1985). However, since the first two stages are far more readily identified in ovaries, attention was focused on these stages, as is typically the case in this type of study (*e.g.* Hunter *et al.*, 1986; Karlou-Riga & Economidis, 1997; Nichol & Acuna, 2001).

Mature ovaries have been categorized according to their proportions of α and β atretic oocytes (Hunter & Macewicz, 1985). Thus, atretic state 0 = ovaries with yolked oocytes but no α atretic oocytes; atretic state 1 = ovaries in which < 50 % of the yolked oocytes are in the α stage of atresia; atretic state 2 = ovaries in which > 50 % of the yolked oocytes are α atretic and atretic state 3 = ovaries which contain no yolked oocytes but do possess β atretic oocytes. During the present study, atretic state 1 ovaries were further divided into three categories on the basis of the percentage of α atretic yolk granule oocytes in histological sections, namely early (< 10 %) mid (10-35 %) and late (35-50 %) atretic state 1, an approach similar to that adopted by Farley & Davis (1998).

An age was assigned to the POFs found in ovaries of fish caught at different times of the day, based on the timing of ovulation and the degree to which those POFs had degenerated (Hunter & Goldberg, 1980; Hunter & Macewicz, 1985). Ages were only assigned to POFs < 48 hours old, since older POFs are difficult to distinguish from late-stage atretic oocytes, *i.e.* γ- and δ-stage atretic oocytes (Hunter & Macewicz, 1985; Macchi, 1998).

Ovaries in atretic state 0 or early state 1, *i.e.* < 10 % of yolk granule oocytes were atretic, and which contained hydrated oocytes, were further examined.
histologically to confirm that they did not contain newly-formed POFs, i.e. those < 12 hours old, or migratory nucleus stage oocytes (Hunter et al., 1992; Nichol & Acuna, 2001). The other member of the pair of each ovary that fulfilled the above criteria, and which had been preserved in 10 % neutrally-buffered formalin (see earlier), was used for estimating the batch fecundity of individual fish (Hunter et al., 1985). For this purpose, this ovarian lobe was dried with blotting paper and ca 180-200 mg of tissue was removed from each of its anterior, middle and posterior regions and weighed to the nearest 1mg. These pieces of tissue were placed on separate slides, covered with 30 % glycerol and examined under a dissecting microscope. The oocytes were then teased apart using a blunt needle and the number of hydrated oocytes recorded. The number of hydrated oocytes in each of the three pieces of ovarian tissue of known weight were then used, in conjunction with the weight of both ovaries, to estimate the total number of hydrated oocytes (= batch fecundity) that would have been present in the pair of ovaries of each fish.

4.3 Results

Although mean monthly salinities in the lower Swan River Estuary in late spring to early winter were close to that of full strength sea water (35 ‰), they fell precipitously to a minimum of 23 ‰ (minimum individual value = 14 ‰) in August, and then rose sharply in early to mid-spring (Fig. 4.1).

4.3.1 Oocyte diameter frequency distributions in ovaries of mature females

The oocyte diameter frequency distributions in the ovaries of two individual mature
female *R. sarba* collected during the spawning period each produced a prominent modal class at 40 to 79 µm (Fig. 4.2). The oocyte diameters in both ovaries, which
were typical of those of *R. sarba* examined during the spawning period, formed an essentially continuous distribution, reflecting the fact that these ovaries contained oocytes at different stages in development, *i.e.* small previtellogenic, cortical alveolar and yolk granule stages.

4.3.2 Period of hydration and spawning

The diameter frequency distributions of vitellogenic oocytes in ovaries of *R. sarba* collected at each sampling interval on 1-2 September 2001 and 13 September 2001 produced a modal class interval which always fell within the range of 420-600 µm (Fig. 4.3). At *ca* 18:30 h on 1 September 2001, the oocyte diameters formed a single mode, with the vast majority of oocytes being less than 720 µm and producing a modal class at 420-539 µm (Fig. 4.3). However, by *ca* 21:30 h on the same evening, the maximum diameter of the oocytes had increased markedly and the distribution of the oocyte diameters was starting to become bimodal, with modal classes at 480-539 µm and 780-839 µm. The oocyte diameter frequencies at similar times on 13 September were essentially the same as those on 1 September, with the distributions being unimodal at 18:30 h and bimodal at 22:30 h (Fig. 4.3). By 00:30 h on 2 September, the oocyte diameter distributions had become markedly bimodal, with the modal diameter class of the largest oocytes at this time, and also at 03:30 h, lying between 840 and 959 µm (Fig. 4.3).

Histological sections showed that, at 18:30 h on 1 September 2001, most of the mature ovaries contained migratory nucleus stage oocytes, *i.e.* oocytes in which
Figure 4.2. Oocyte diameter frequency distributions for stage V ovaries of two female *Rhabdosargus sarba.*
Figure 4.3. Frequency for oocyte diameters in mature ovaries of Rhabdosargus sarba caught on 1-2 and 13 September 2001. The time of commencement of each sampling interval is shown. n = number of fish used for oocyte diameter measurements.

the nucleus was migrating towards the edge of the cytoplasm and a conspicuous lipid droplet was present in the cytoplasm (Fig. 4.4a). However, it was difficult to
distinguish between oocytes at the migratory nucleus and yolk granule stage under a dissecting microscope (Fig. 4.4b). By 21:30 h, the yolk and lipid of the larger oocytes had begun to coalesce and the nucleus could sometimes be seen near the edge of the cytoplasm (Fig. 4.4c).

The relatively larger size, translucent appearance and ability to detect their lipid droplet enabled these hydrating oocytes to be far more readily distinguished from yolk granule oocytes under a dissecting microscope than was the case with earlier stages (cf Figs 4.4b and d). By 00:30 h, the largest oocytes had increased further in size and all of their lipid and yolk material had coalesced (Fig. 4.4e). Under the dissecting microscope, these hydrated oocytes were of similar appearance to the corresponding oocytes at 21:30 h (Fig. 4.4f). Although mature fish with ovaries containing the above stages in oocyte hydration were frequently found in nearshore, shallow waters, the number of such fish in these waters declined markedly after about 00:30 h and none of the few fish caught there after this time contained recently-formed POFs. However, as described below, fish with ovaries containing newly-formed POFs were found in deeper waters.

4.3.3 Post-ovulatory follicles

When hydrated oocytes could be seen macroscopically through the ovarian wall, they were either distributed throughout the ovary or concentrated in the ovarian duct and had thus been ovulated and were about to be released. Histological examination demonstrated that, when hydrated oocytes were present in the ovarian duct, the ovary contained recently-formed POFs. These post-ovulatory structures are formed by the
Figure 4.4. Histological sections of ovaries of individual *Rhabdosargus sarba* caught on 1 and 2 September 2001 at *ca* 18:30 h (a), 21:30 h (c) and 00:30 h (e) and photographs of the oocytes in the other lobe of the ovary of the same three fish (b,d,f). *c*, coalescing yolk and lipid; *ho*, hydrating oocyte; *l*, lipid droplet; *mn*, migratory nucleus oocyte; *n*, nucleus; *yg*, yolk granule oocyte. Scale bars = 200 µm.

**Figure 4.4.** Histological sections of ovaries of individual *Rhabdosargus sarba* caught on 1 and 2 September 2001 at *ca* 18:30 h (a), 21:30 h (c) and 00:30 h (e) and photographs of the oocytes in the other lobe of the ovary of the same three fish (b,d,f). *c*, coalescing yolk and lipid; *ho*, hydrating oocyte; *l*, lipid droplet; *mn*, migratory nucleus oocyte; *n*, nucleus; *yg*, yolk granule oocyte. Scale bars = 200 µm.

thecal and granulosa layers of the oocytes, which surround the zona radiata externa (Fig. 4.5a). The thecal and granulosa layers of newly-formed POFs form convoluted folds in which the darkly staining nuclei of their cells are arranged in lines (Fig. 5b).
However, ovaries often contained POFs which, in comparison with newly-formed POFs, were more compact and less structured and comprised cells in which the nuclei were degenerating (Figs 4.5c-f).

Newly-formed POFs, with the characteristics described above, were first observed in the ovaries of females caught at ca 01:30 h and were present in the ovaries of several fish caught in the ensuing four hours. In contrast, no newly-formed POFs were found in the ovaries of *R. sarba* at dusk, *i.e.* ca 18:30 h. At this time, the POFs comprised one or other of two morphological forms. Although the first and least degenerate form is still relatively well organised, it is slightly smaller in size than newly-formed POFs and its nuclei are becoming pycnotic (Fig. 4.5c). At the same time of day, the second form of POF is highly degenerate, smaller and the nuclei of its degenerating cells are barely visible or absent (Fig. 4.5f). As was the case at ca 18:30 h, POFs in ovaries of *R. sarba* caught at ca 22:00 and 01:00 h comprised one or other of two morphological forms. The least degenerate form of POFs in ovaries of fish caught at ca 22:00 and 01:00 h (Fig. 4.5d-e) represent intermediate stages in degeneracy between the two different morphological forms described above for the ovaries of fish caught at 18:30 h. They were therefore compact and, while some of their nuclei were still detectable, they were highly pycnotic. POFs of the other form at ca 22:00 and 01:00 h were highly degenerated and becoming increasingly difficult to distinguish from oocytes in the late stages of atresia (see Hunter & Macewicz, 1985).
Figure 4.5. Histological sections through ovaries of *Rhabdosargus sarba* showing (a) the outer layers of a yolk granule oocyte, and POFs in the ovaries of fish collected at *ca* (b) 02:00, (c) 18:30, (d) 22:00, (e) 01:00 and (f) 18:30 h. g, granulosa layer; t, thecal layer; yg, yolk granule; yv, yolk vesicle; zre, zona radiata externa. Scale bars. a, 25 µm, b-f, 50 µm.

4.3.4 Atresia

Two of the four types of atretic oocytes (α and β) were commonly observed in the
ovaries of *R. sarba*. Although the zona radiata of early α atretic oocytes was distorted in places, yolk granules and yolk vesicles were still present in these oocytes and showed no conspicuous signs of resorption (Fig. 4.6a). In late α atretic stage oocytes, the zona radiata had become resorbed and vacuoles and also, in some cases, amorphous particle(s), which probably represented the remnants of yolk and lipids, were present (Fig. 4.6b). The thecal layer could be distinguished in this and the β stages of atresia (Figs 4.6b-d). By the early β atretic stage, the oocyte had begun to shrink and all of its yolk and lipid material had been resorbed, while its vacuoles had become larger (Fig. 4.6c). During the β stage in atresia, the oocyte continued to shrink and the proportion of its volume occupied by vacuoles had increased (Fig. 4.6d).

Sixty two percent (n = 13) and 72 % (n = 44) of the stage V ovaries (i.e. mature ovaries that did not contain migratory nucleus stage or hydrating oocytes or POFs) that were sectioned in July and August, respectively, were at mid-late atretic state 1, i.e. 11-50 % of their yolk granule oocytes were α atretic (Fig. 4.1a). However, the prevalence of such ovaries declined precipitously to 28-29 % in September and October, as salinities rose markedly, and remained at a similar level in November, when the number of mature spawning females in samples (collected in 2000 and 2001) had declined markedly (Fig. 4.1).

In July and August, only 39 % of the 57 pairs of ovaries of *R. sarba* that were macroscopically assigned as stage V/VI ovaries, contained migratory nucleus or hydrated stage oocytes or POFs, i.e. on the basis of their histology, they were at stage VI, and thus belonged to fish that were about to spawn, were spawning or had spawned recently. However, in the following two months, 76 % of the 88 pairs of
Figure 4.6. Histological sections of ovaries of *Rhabdosargus sarba* showing an oocyte at (a) the early α, (b) late α, (c) early β, (d) late β stage of atresia. ap, amorphous particle; v, vacuole; t, thecal layer; zr, zona radiata. All scale bars = 50 µm.

Ovaries of *R. sarba* that were macroscopically assigned as stage V/VI were shown by histological sections to be at stage VI.
4.3.5 Batch fecundity

The relationship between batch fecundity ($BF$) and total length ($TL$) shown in Fig. 4.7, and between batch fecundity and somatic body weight ($SW$) are described by the following equations:

$$\ln BF = 4.305\ln TL - 13.867 \quad (p < 0.001; \ R^2 = 0.38, \ n = 31)$$

$$BF = 4519.5e^{0.0791SW} \quad (p < 0.001) \ R^2 = 0.33, \ n = 31).$$

The batch fecundities of $R.\ sarba$, predicted by the above equations for fish with lengths of 200, 250 and 300 mm, were ca 7,420, 20,180 and 54,850, respectively, and for fish with somatic weights of 100, 200 and 300 g, were ca 6,010, 14,050 and 32,880, respectively.

4.3.6 Daily proportions of spawning females

Between early September and late October, the percentage of fish with mature ovaries ($PS$), i.e. those macroscopically assigned to either stage V or VI that, between 22:00 and 24:00 h, contained hydrated oocytes, was positively correlated ($p < 0.05$) with maximum daily tidal height ($T$). $PS = 72.76T + 17.67 \ (R^2 = 0.23$, number of sampling occasions = 16) (Fig. 4.8a). Note that September and October were chosen for exploring this relationship as these were the months in which the prevalence of fish with ovaries containing hydrated oocytes was highest and spawning activity was therefore greatest.

In the period between 22:00 and 24:00 h, the prevalence on any day of mature females with ovaries containing hydrated oocytes ($PS$), i.e. of females about to
Figure 4.7. Relationship between batch fecundity (= number of hydrated oocytes) and total length (mm).
Figure 4.8. Relationship between prevalence of spawning and firstly (a) the predicted maximum change in daily tidal level and secondly (b) the difference between the time of high tide and the time at which spawning in *R. sarba* is estimated to be completed in the Swan River Estuary (06:00 h).
spawn, and the difference between the times in hours when spawning is believed to cease (06:00 h, see later) and high tide is inversely correlated. $PS = -9.247(T) + 72.97$ ($R^2 = 0.52, n = 16$) (Fig. 4.8b). Hence, the prevalence of these “spawning” females was greatest on those days when the time that the tide was about to change from flood to ebb coincided with the time at which R. sarba is considered to cease spawning.

4.3.7 **Spawning frequency and annual fecundity**

The percentage of mature females with ovaries that, between 22:00 and 24:00 h, contained hydrated oocytes during the spawning period of R. sarba in the lower estuary was 36.5. Therefore, during this period, individual females spawned, on average, once every 2.7 days and thus about 45 times during the spawning season. The potential annual fecundities of female R. sarba with lengths of 200, 250 and 300 mm were therefore estimated to be ca 292,000, 796,000 and 2,163,000, respectively.

4.4 Discussion

4.4.1 **Mode of spawning**

Since the ovaries of mature females collected during the spawning season contained oocytes with diameters exhibiting an essentially continuous distribution, reflecting the presence of oocytes at several stages of development, including small previtellogenic, cortical alveolar and yolk granule oocytes, R. sarba is an indeterminate spawner sensu Hunter et al. (1985). Thus, since the potential annual fecundity is not fixed prior to the commencement of the spawning period, the annual
fecundity of *R. sarba* has to be estimated using a combination of batch fecundities and spawning frequency.

### 4.4.2 Hydration period and time of spawning

Since the ovaries of mature fish caught between 18:30 and 20:30 h on both 1 and 13 September never contained recently-formed POFs, those fish had not spawned in the previous few hours. However, the ovaries of most of these fish did contain oocytes at the migratory nucleus stage and which were thus about to become hydrated.

Although the frequency distributions of the oocyte diameters were still unimodal at this time, they had become bimodal by 21:30 to 23:30 h, reflecting the fact that numerous oocytes had become enlarged through hydration. These oocytes had the translucent appearance that develops during hydration as they become filled with fluid secreted by the granulose cells that form the granulosa layer surrounding the oocyte (Hunter *et al.*, 1985). However, none of these ovaries yet possessed newly-formed POFs.

The ovaries of neither of the two mature female *R. sarba* caught in nearshore, shallow waters between 00:30 and 05:30 h on 2 September contained newly-formed POFs. However, it is important to recognise that, despite extensive seine netting, it became increasingly difficult to catch mature fish during this period on any night. Yet, several mature females with ovaries containing both newly-formed POFs and concentrations of hydrated oocytes in their oviducts were caught by rod and line angling in deeper water between 01:30 and 04:30 h. It is thus concluded that *R. sarba* move out from nearshore, shallow waters into deeper waters just prior to ovulation.

The spawning of several other species of marine teleost with indeterminate fecundity has been shown typically to be completed within 10-14 hours of the time when their oocytes commence hydration, *e.g.* the northern anchovy *Engraulis*
mordax (Hunter & Macewicz, 1985), the spotted seatrout Cynoscion nebulosus (Brown-Peterson et al. 1988) and the horse mackerel, Trachurus trachurus (Karlou-Riga & Economidis, 1997) and, on average, about 2-5 hours after the commencement of ovulation, e.g. the spotted seatrout Cynoscion nebulosus (Brown-Peterson et al. 1988), the Black Sea anchovy Engraulis encrasicolus (Lisovenko & Andrianov, 1991) and the weakfish Cynoscion regalis (Taylor & Villoso, 1994). From the above data on the timing of hydration and ovulation of R. sarba and other species, it is concluded that, in the lower Swan River Estuary, R. sarba spawns mainly between 02:00 and 06:00 h. This conclusion is consistent with the fact that the histological characteristics of the newest of the POFs in the ovaries of those R. sarba that were caught at dusk, i.e. 18:30 h, were similar in appearance to those of POFs of about the same age, i.e. 10-14 h, in skipjack tuna Katsuwonus pelamis (Hunter et al., 1986).

4.4.3 Spawning frequency

As demonstrated in a previous study, the gonadosomatic indices and prevalence of mature females of R. sarba peaked later in the Swan River Estuary than in coastal marine waters at the same latitude (Chapter 3). Since salinities in the estuary were still low in August, i.e. < 23 ‰, when the above two reproductive variables were attaining high levels in coastal marine waters, those low salinities may have been retarding the final maturation of oocytes in this essentially marine species. This conclusion is also supported by the following findings. (1) The prevalence of fish with ovaries at mid to late atretic state 1 (i.e. between 10 and 50 % of all yolk granule stage oocytes are atretic) is far higher during the early part of the spawning period (July and August), when salinities are lower, than in other months of the year when they are close to 35 ‰. (2) Spawning activity by female R. sarba, as reflected in the presence of migratory nucleus or hydrated stage oocytes or POFs in
histological sections of their ovaries, increased markedly after August when salinities rose sharply and soon approached ca 35 ‰. This apparent influence of salinity on the timing of gonadal maturation parallels that proposed for Cynoscion nebulosus in estuaries entering the Gulf of Mexico (Brown-Peterson et al., 2002). The resorption of yolk granule oocytes by R. sarba in July and August would help conserve energy at a time when, if they progressed through to final maturation and were released, would be exposed to greatly reduced salinities, which are known not to be conducive to their optimal development (Mihelkakis & Kitajima, 1994).

During the main part of the spawning period, i.e. September and October, the mature ovaries of several fish caught in deeper waters by rod and line between ca 01:30 and 04:30 h which were undergoing ovulation, i.e. contained concentrations of hydrated oocytes in their ovarian ducts, contained up to three categories of POFs. These three types were (1) those with no detectable signs of degeneration and estimated to be < 3 hours old, (2) those that were at an intermediate stage of degeneration and presumed to be ca 24 h old, and (3) those that were so highly degenerated that they were sometimes difficult to distinguish from late atretic stage oocytes and were presumably ca 48 h old. Hence, individual R. sarba are capable of spawning on at least three successive days, during that part of the month when spawning activity is greatest.

### 4.4.4 Estimates of batch and annual fecundity

As pointed out by Brown-Peterson et al. (1988), it is very difficult to obtain accurate estimates of the annual fecundity of multiple spawning fishes that have prolonged spawning seasons. For such estimates, it is particularly important to take into account the fact that spawning frequency will be likely to vary during the spawning period. However, since the spawning frequency estimated for R. sarba represents the
average for samples collected at regular intervals over the duration of the spawning period, variations in the spawning frequency of *R. sarba* over that period have, at least partly, been taken into account. A further consideration is the influence of atresia. The influence of atresia on the relationships between batch fecundity and fish length (and weight) have been minimized by rejecting all ovaries other than those at state 0 (no atresia) and early state 1 (< 10% of their yolk granules stage oocytes are atretic) for estimation of batch fecundity. It is also recognized that the variable salinities within the estuary exert a major influence on the timing of the spawning period of *R. sarba* and that low salinities may retard final oocyte maturation (see earlier). Therefore, the estimates for the average spawning frequency and estimates for annual fecundity may differ from those which are typical of this species, *i.e.* for *R. sarba* in the marine environment.

The estimates of annual fecundity obtained for *R. sarba* during the present study, (*i.e.* ranging from 109,000 to 2,417,000 for fishes of 188 and 266 mm total length, respectively) exceed those of El-Agamy (1989), (*i.e.* ranging from 23,000 to 99,000 for fishes of 170 and 260 mm total length, respectively). Since El-Agamy (1989) did not take into account that fact that this species has indeterminate fecundity, the values he obtained for the annual fecundity of *R. sarba* in the Arabian Gulf are likely to represent large underestimates of the true annual fecundity of this species in that region.

### 4.4.5 Relationship between spawning time and tidal cycle

Since *R. sarba* typically spawns just prior to the commencement of a relatively strong ebb tide, the fertilised eggs would be likely to be transported downstream and out of the estuary. Emigration of eggs from the estuary would enhance the chances of the survival of the eggs of this essentially marine species by ensuring that they will
be likely to develop in a marine environment in which salinity constantly remains at ca 35‰, rather than in one in which seasonal rainfall can result in sudden marked declines in salinity. However, it has also been suggested that the possession of spawning cycles linked to lunar periodicity can reduce the likelihood of predation (Taylor, 1984). For example, Johannes (1978) pointed out that, since spawning in many reef-dwelling fishes is synchronized with the lunar cycle and occurs on high or ebbing tides, their eggs would be transported away from reefs where the concentration of predators is high, and that this will thereby reduce the likelihood of predation during the early stages of life. Since planktivorous fishes are known to be abundant in estuaries (Johnson et al., 1990; Morgan, 1990), a movement of the eggs of *R. sarba* out of the estuary may therefore enhance their chances of avoiding predation. In this context, it is relevant that large numbers of the planktivorous and thus potential predator *Spratelloides robustus* were caught whilst seine netting. Finally, it is worth noting that transport of eggs out of the estuary would account for the fact that relatively few young 0+ juveniles are recruited into nearshore, shallow waters in the region of the estuary where spawning occurs (Chapters 3 and 5). Indeed, substantial recruitment into these nearshore waters does not occur until *R. sarba* is about one year old (Chapter 5).
Chapter 5: Comparisons between the movements, age compositions and growth rates of *Rhabdosargus sarba* (Sparidae) in three different environments

5.1 Introduction

The size attained by *R. sarba* varies greatly among regions. For example, the maximum total length recorded by Radebe *et al.* (2001) for South African waters, *i.e.* ca 782 mm (derived from a fork length of 680 mm), is far greater than those of 300 mm for the Arabian Gulf (El-Agamy, 1989), 390 mm for Chilka Lake in India (Patnaik, 1973) and 450 mm for Australian waters (Kuiter, 1993). These differences in maximum size are paralleled by differences in maximum age, with, for example, the greatest age of 16 years reported by Radebe *et al.* (2001) for *R. sarba* in South African waters far exceeding the eight years estimated by El-Agamy (1989) for this species in the Arabian Gulf. Indeed, Kuiter (1993) has even queried whether all of the populations attributed to *R. sarba* represent a single species.

The first aim of this study was to determine how the types of habitat occupied by *R. sarba* in three very different environments, namely temperate coastal marine and estuarine waters and a large subtropical embayment on the west coast of Australia, change during the life cycle of this important sparid species. The second aim was to test the hypothesis that, since estuaries are highly productive ecosystems (Schleske & Odum, 1961), the growth of *R. sarba* will be more rapid in the Swan River Estuary than in either coastal marine waters or Shark Bay. However, preliminary examination of the lengths at age of *R. sarba* caught in different habitat types in coastal marine waters at ca 32°S indicated that, as a whole, those data were not adequately described by the von Bertalanffy (Ricker, 1975), the Schnute (1981)
or Schnute & Richards (1990) growth curves. An examination of the residuals for the lengths at age indicated that this inadequacy was attributable to habitat variations in the mean lengths at a given age. Thus, separate length-at-age curves for *R. sarba* in each habitat type, derived from a traditional, unadjusted von Bertalanffy equation have been used in combination with two functions that describe the probabilities of fish of different lengths moving between habitats, to construct a single adjusted von Bertalanffy growth curve for the population as a whole. This adjusted von Bertalanffy growth curve is shown to provide a better description of the lengths at age of the population as a whole than that produced by the unadjusted von Bertalanffy growth curve that was fitted in the traditional manner. The same approach was applied to the lengths at age of fish in the Swan River Estuary and Shark Bay to ascertain whether it was advisable to adopt a similar modified approach to describing the growth of *R. sarba* in these two water bodies.

5.2 Materials and methods

5.2.1 Sampling and measurements

*Rhabdosargus sarba* was collected from the following three localities in Western Australia (Fig. 2.1). (1) Marine waters at *ca* 32° S, including sheltered and exposed waters (<1.5 m deep) and offshore waters over reefs (20-30 m deep). (2) The lower reaches of the Swan River Estuary, including both its nearshore waters (<1.5 m deep) along a protracted stretch of this part of the estuary and an adjacent deeper region of this entrance channel (*ca* 11 m deep). (3) Shark Bay, including nearshore sandy areas amongst mangroves (<1.5 m deep) and waters over reefs (1-5 m deep). Details of the habitat(s) that were sampled at each site and the period and method of sampling at each site are given in Table 5.1. Note that Mangles Bay, at the southern
Table 5.1. Details of the dates and methods of sampling for *Rhabdosargus sarba* in the different habitat types in coastal marine waters at ca 32°S, the lower Swan River Estuary and Shark Bay.

<table>
<thead>
<tr>
<th>Environment</th>
<th>Habitat</th>
<th>Sites</th>
<th>Method</th>
<th>Dates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coastal marine waters at ca 32°S</td>
<td>Sheltered nearshore</td>
<td>Mangles Bay</td>
<td>5.5 m seine (sand) 21.5 m seine (sand and seagrass)</td>
<td>June 1999 to May 2000</td>
</tr>
<tr>
<td></td>
<td>Sheltered nearshore</td>
<td>Mangles Bay and four other sites in and just to the south of Cockburn Sound</td>
<td>5.5 m seine (sand) 21.5 m seine (sand and seagrass)</td>
<td>June 2000 to May 2001</td>
</tr>
<tr>
<td>Exposed nearshore</td>
<td>One site</td>
<td>21 m seine (sand)</td>
<td></td>
<td>February 1999 to April 2001</td>
</tr>
<tr>
<td>Reefs</td>
<td>Three main sites</td>
<td>Rod and line</td>
<td></td>
<td>February 1999 to July 2002</td>
</tr>
<tr>
<td>Lower Swan River Estuary</td>
<td>Sheltered nearshore</td>
<td>Sites over a protracted length on the northern side of the estuary</td>
<td>5.5 m seine (sand)</td>
<td>June 2000 to May 2001</td>
</tr>
<tr>
<td></td>
<td>Sheltered nearshore</td>
<td>Sites over a protracted length on both sides of the estuary</td>
<td>21.5 m seine (sand)</td>
<td>May 2000 to August 2002</td>
</tr>
<tr>
<td></td>
<td>Deeper channel</td>
<td>Fremantle Traffic Bridge</td>
<td>Rod and line</td>
<td>July 1999 to November 2001</td>
</tr>
<tr>
<td>Shark Bay</td>
<td>Mangrove</td>
<td>Two sites</td>
<td>21.5 m seine</td>
<td>October 1999 to April 2002</td>
</tr>
<tr>
<td></td>
<td>Reefs</td>
<td>Six sites</td>
<td>Rod and line</td>
<td>April 1999 to April 2002</td>
</tr>
</tbody>
</table>
end of Cockburn Sound (Fig. 2.1), was chosen specifically because earlier extensive sampling had demonstrated that relatively large numbers of *R. sarba* settled in its nearshore waters (Valesini *et al.*, 1998). Likewise, the exposed site, at the extreme southern tip of Warnbro Sound (Fig. 2.1), was selected because the catches of *R. sarba* at this site had been shown to be substantial and typical of exposed sites in the region (F. Valesini, in prep).

Nearshore, shallow waters at sites in each of the three localities were sampled using a 21.5 m long seine net with 3 mm mesh in the bunt and also frequently with a 5.5 m long seine net made entirely of 1 mm mesh. The 5.5 m seine net was dragged parallel to the shore for a distance of 50 m, during which it covered an area of *ca* 250 m². The 21.5 m seine net was stretched parallel to the beach in a water depth of *ca* 1 m and its ends then drawn inwards in a circle and on to the beach. The area covered by this seine net was *ca* 116 m². On each sampling occasion at each site, three replicate samples were collected using the 21.5 m long seine net and also the 5.5 m long seine net when that net was employed. When required, further opportunistic seining was undertaken at the sites shown in Fig. 2.1 to obtain sufficient samples of fish for use in growth analyses.

Since the use of a video camera, which was attached by cable to a television monitor and video recorder and lowered over the substrata in offshore waters, demonstrated that, in offshore waters, *R. sarba* occupy areas near and over reefs, those waters were sampled using rod and line angling. The reefs in offshore deeper waters were thus targeted for fishing by using a Global Positioning Satellite (GPS) system and echo sounder. Additional samples of *R. sarba*, caught by commercial fishers over reefs in Shark Bay, were purchased from commercial fish markets to provide further lengths at age of fish for use in determining the growth of this sparid. The total length to the nearest 1 mm and sex of each fish were recorded.
5.2.2 Age determination

The two sagittal otoliths of each fish were removed, cleaned, dried and stored in paper envelopes. They were later placed in methyl salicylate and examined under reflected light against a black background using a dissecting microscope. Since the opaque zones in the whole otoliths of large fish were so closely spaced that they were often difficult to distinguish from one another, the otoliths were sectioned to ascertain whether this provided a better resolution of these opaque zones. For sectioning, one of the otoliths of each fish was mounted in clear epoxy resin and cut along the same plane into ca 500 μm thick sections using a low speed diamond saw (Buehler). The sections were cleaned and mounted on slides using DePX mounting medium and examined under reflected light, employing a dissecting microscope attached to a video camera (Panasonic WV-CD20). The image was analysed using the computer imaging package OPTIMAS 5 (Optimas Corporation). The number of opaque zones on each of 100 sectioned otoliths, containing a range of numbers of opaque zones, was compared with those recorded for the same otoliths prior to sectioning. Note that the number of opaque zones on each otolith was counted without knowledge of the size or time of capture of the fish from which the otolith had been removed. Since sectioning improved the resolution of the opaque zones in the otoliths of individuals of *R. sarba* in which there were > 5 opaque zones (see results), it was decided to be conservative and section all otoliths with ≥ 4 opaque zones.

Marginal increment analysis was employed to determine whether the opaque zones detectable on whole and sectioned otoliths of *R. sarba* with < 4 and ≥ 4 opaque zones, respectively, were formed annually. For this purpose, measurements were made of the distance between the primordium and the outer edge of both the otolith and the single opaque zone, when only one such zone was present, and of the
distances between the outer edge of the otolith and each of the two outermost opaque zones, when two or more opaque zones were present. These measurements, which were made perpendicular to the opaque zones and without knowledge of the date of capture of the fish from which that otolith had been removed, were recorded to the nearest 0.01 mm using the computer imaging package OPTIMAS 5. The marginal increment on each otolith, i.e. the distance between the single or outermost opaque zone and the edge of that otolith, was expressed as a proportion of the distance between the primordium and the outer edge of the opaque zone, when only one opaque zone was present, and as a proportion of the distance between the outer edges of the two outermost opaque zones, when two or more opaque zones were present. The marginal increments on otoliths with the same number of opaque zones in the corresponding calendar months of different years were grouped together. Images of both whole and sectioned otoliths of *R. sarba* are presented in the Appendix.

5.2.3 The unadjusted von Bertalanffy growth equation

The approximate time of peak spawning was estimated from the trends shown throughout the year by the gonadosomatic indices, gonadal maturity stages and pattern of oocyte development (Chapter 3). This was considered to correspond to the birth date, which was then used, in conjunction with the time of year when the opaque zones (annuli) on the otoliths become delineated, to determine the age of each fish at its date of capture.

Unadjusted von Bertalanffy growth curves were fitted in the traditional manner to the lengths at age of all male and female *R. sarba* in coastal marine waters, the lower Swan River Estuary and Shark Bay, by maximising the log-likelihood using AD Model Builder (Fournier, 1994). The von Bertalanffy growth equation is \( \hat{L}_t = L_\infty (1 - e^{-k(t-t_0)}) \), where \( \hat{L}_t \) is the predicted total length-at-age \( t \) years, \( L_\infty \)
is the mean asymptotic length predicted by the equation, \( k \) is the growth coefficient (year\(^{-1} \)) and \( t_o \) is the hypothetical age at which fish would have zero length, if growth had followed that predicted by the equation.

A likelihood ratio test was used to compare the growth curves derived for each sex in coastal marine waters, the Swan River Estuary and Shark Bay. The null hypothesis, \( \omega \), that the growth curve could be described by a common growth curve for the two sexes was compared with the alternative hypothesis, \( \Omega \), that the data would be better described by a separate growth curve for each sex. The log-likelihood was determined for the null hypothesis and for the alternative hypothesis as \( \lambda_\omega \) and \( \lambda_\Omega \), respectively, where each was calculated from the product of the values of the probability density function for the observed lengths at age. Next, the test statistic for the likelihood ratio test was calculated as \( 2(\lambda_\Omega - \lambda_\omega) \). The null hypothesis was rejected at the \( \alpha = 0.05 \) level of significance if the test statistic exceeded \( \chi^2(q) \), where \( q \) is the difference between the numbers of parameters in the two growth curves (e.g. Cerrato, 1990).

The residuals from each of the analyses were calculated, standardized by dividing by their standard deviation, and plotted against age to ascertain whether the resulting growth curves deviated systematically from the observed lengths at age.

### 5.2.4 The adjusted von Bertalanffy growth equation

The lengths at age of male and female fish in each population of \( R. \ sarba \) were assumed to be normally distributed around von Bertalanffy growth curves, \( i.e. \ L \sim N(\hat{L}, \sigma^2) \). Therefore, the probability density function for the lengths at age is

\[
\phi(L) = \frac{1}{\sqrt{2\pi\sigma^2}} \exp\left(-\frac{(L - \hat{L})^2}{2\sigma^2}\right).
\]

However, if movement is size-related, this normal distribution will comprise separate component distributions for the fish found within
each habitat type. Thus, in the case of coastal marine waters at ca 32°S, the lengths at age of *R. sarba* in sheltered inshore, exposed inshore and offshore waters over reefs were described by separate probability distributions. These distributions were determined by multiplying, for each length at age, the probability of a fish having that specified length at age, as calculated from the normal probability distribution of lengths at age around the von Bertalanffy growth curve, by the proportion of fish at that length within each habitat type. The latter proportion was determined from the separate logistic equations that describe the movement of fish between each of the different habitat types. The likelihood of each observed length at age within each habitat type was thus calculated from the probability distribution of lengths at age for the corresponding habitat type. The combined likelihood was then estimated and used to fit an adjusted von Bertalanffy growth curve which, through accounting for the different length at age distributions within each of the habitat types, describes the growth of fish in the population as a whole. Details of the equations employed to undertake the above procedures for *R. sarba* in coastal marine waters, where this species occupies three main habitat types, now follow.

The probability that a fish of length $L_t$ was located in habitat type $h$ ($h = h_1, h_2$ or $h_3$) and caught with the sampling methods employed in this habitat type was determined by

$$p_h(L_t) = \begin{cases} 1 - \psi(L_t, h_1 \rightarrow h_2) & \text{if } h = h_1 \\ \psi(L_t, h_1 \rightarrow h_2)[1 - \psi(L_t, h_2 \rightarrow h_3)] & \text{if } h = h_2 \\ \psi(L_t, h_1 \rightarrow h_2)\psi(L_t, h_2 \rightarrow h_3) & \text{if } h = h_3 \end{cases}$$

where $\psi(L, h_1 \rightarrow h_2) = \frac{1}{1 + \exp\left[-\ln(19)\frac{L - L_{50}^{h_1 \rightarrow h_2}}{L_{95}^{h_1 \rightarrow h_2} - L_{50}^{h_1 \rightarrow h_2}}\right]}$ is the probability that a fish of length $L$ will have moved from habitat $h_1$ to $h_2$, and $L_{50}^{h_1 \rightarrow h_2}$ and $L_{95}^{h_1 \rightarrow h_2}$ are the
lengths at which 50 and 95 % of the fish will have moved. $L_{95}^{h_1 \rightarrow h_2}$ was constrained to exceed $L_{95}^{h_1 \rightarrow h_2}$ by at least 1 mm.

The probability density function of the lengths of fish of age $t$ in habitat type $h$ has been approximated by

$$f(L_t, h) = \frac{p_h(L_t) \phi(L_t)}{\int_{L_{t_1} - t_5}^{L_{t_1} + t_5} p_h(L) \phi(L) dL}$$

where $s$ is the estimated value of $\sigma$. The mean length at age within each habitat is then calculated as $L_{t,h} = \int_{L_{t_1} - t_5}^{L_{t_1} + t_5} f(L, h) L dL$. The integrals were evaluated using numerical quadrature. The likelihood of the observed lengths at age was calculated as

$$\lambda = \prod_{j=1}^{n} f(L_{t,j}, k).$$

AD Model Builder (Fournier, 1994) was used to maximize the likelihood and estimate the parameters $L_{\infty}$, $k$, $t_0$, $\sigma$, $L_{95}^{h_1 \rightarrow h_2}$, $L_{95}^{h_1 \rightarrow h_2}$, $L_{50}^{h_2 \rightarrow h_3}$ and $L_{95}^{h_2 \rightarrow h_3}$.

These formulae were modified slightly for Shark Bay and the Swan River Estuary, as $R. sarba$ only occupied two habitat types in these systems. The probability that a fish of length $L_t$ was located in habitat type $h$ ($h = h_1$, or $h_2$) was then calculated as

$$p_h(L_t) = \begin{cases} 1 - \psi(L_t, h_1 \rightarrow h_2) & \text{if } h = h_1 \\ \psi(L_t, h_1 \rightarrow h_2) & \text{if } h = h_2 \end{cases}$$

The standardized residuals for the estimated lengths at age of the population of $R. sarba$ in each of the three water bodies, derived from the use of the adjusted von Bertalanffy growth equation, were plotted and compared with those derived from the traditional, unadjusted von Bertalanffy growth curve.

The likelihood ratio test, as described earlier, was used to determine whether the adjusted von Bertalanffy growth curves for males and females in coastal marine waters, the lower Swan River Estuary and Shark Bay were significantly different.
The same test was also used to determine whether the adjusted von Bertalanffy growth curve improved the predicted lengths at age of fish in each of these three environments from those that were produced using the traditional, unadjusted von Bertalanffy growth curve. It was also used to test for differences between the growth curves for the populations in the three different environments.

5.3 Results

5.3.1 Comparisons between numbers of opaque zones on whole and sectioned otoliths

Although the numbers of opaque zones observed on whole otoliths of *Rhabdosargus sarba*, prior to sectioning, were the same as those in sections of the corresponding otoliths when the number of opaque zones was five or less, this was frequently not the case when otoliths contained a greater number of opaque zones. In the latter cases, the number of opaque zones that could be detected on each whole otolith was less than that which was visible on the corresponding sectioned otolith. Furthermore, as the number of opaque zones increased, the extent of the discrepancies increased from one, when six zones were visible after the otolith had been sectioned, to a maximum of five, for an otolith for which 12 zones were counted after the otolith had been sectioned.

5.3.2 Validation that opaque zones are formed annually

The mean marginal increments on the otoliths of *R. sarba* caught in coastal marine waters and estuarine waters at *ca* 32°S and on which there was one opaque zone declined precipitously from *ca* 0.2 in July to September to a minimum of 0.03 in
November and then rose progressively to reach a maximum of 0.21 in June (Fig. 5.1). The same trend was followed by the mean monthly marginal increments on otoliths with a greater number of opaque zones (Fig. 5.1) as well as on those from *R. sarba* caught in Shark Bay (data not shown), except that, in the latter environment, the new opaque zone typically became delineated slightly later, *i.e.* November and December rather than October and November. Since, irrespective of the number of opaque zones in the otolith, the mean monthly marginal increment rose and declined only once during the year, a single opaque zone is laid down in the otoliths of *R. sarba* annually. Thus, the number of opaque zones can be used to facilitate the ageing of *R. sarba*.

5.3.3 Length composition and relationship to habitat type of each age class

In coastal marine waters, the 0+ age class was caught predominantly in sheltered, nearshore waters (Fig. 5.2). Some 1+ fish and a very small number of 2+ fish were also found in these sheltered waters. A few 0+ fish and substantial numbers of the 1+ to 4+ age classes were caught in exposed, nearshore waters (Fig. 5.2). The relative contributions made by the *R. sarba* caught in offshore waters over reefs to the samples of the different age classes increased progressively between the 1+ to to 5+ age classes, and all fish >5+ years were found exclusively in that habitat type. Similar trends were observed in the lower Swan River Estuary, with the 0+ age class being found predominantly in nearshore waters and all fish greater than 2+ years of age being caught only in deeper waters.

Examination of the size distributions of fish from different habitat types in the samples of each age class from marine coastal waters demonstrated that, within any age class, the smaller fish tended to have been caught mainly in the exposed
Figure 5.1. Mean monthly marginal increments ±1SE on sagittal otoliths of *Rhabdosargus sarba* in marine and estuarine waters at ca 32°S. Sample sizes are shown above each mean. Closed rectangles on horizontal axis refer to winter and summer months and open rectangles to spring and autumn months.
Figure 5.2. Length-frequency histograms for the different age classes of *Rhabdosargus sarba* in coastal marine waters and the lower Swan River Estuary. Grey shading, sheltered nearshore waters; black shading, exposed nearshore waters; white, reefs in coastal marine waters and channel in estuary.
nearshore habitat, whereas the larger fish tended to have been collected from over reefs further offshore (Fig. 5.2). This trend is exhibited particularly well by the size distributions of 3+ and 4+ *R. sarba* within the above two habitat types. It is further illustrated by the fact that the mean lengths of 3+ and 4+ fish in the exposed nearshore habitat were 224 and 244 mm, respectively, compared with 251 and 266 mm, respectively, for fish of the same age classes over reefs.

### 5.3.4 Monthly length-frequency distributions of age classes in coastal marine waters

In coastal marine waters, the 0+ age class was caught first in August and in large numbers in September (Fig. 5.3). These early post-settlement fish, which were all caught in sheltered, nearshore waters, ranged in length from 8 to 23 mm and produced a modal length class of 0-19 mm. The modal length class of the 0+ cohort increased to 40-59 mm in January and 60-79 mm in March, after which month this age class was no longer caught in sheltered, nearshore waters and had not yet appeared in the samples collected from exposed, nearshore waters (Fig. 5.3). The modal length class of the 1+ age class increased from 140-159 in August to 160-179 mm in December and then to 180-199 mm in June (Fig. 5.3). The increase in length of the 1+ age class throughout the year is also reflected in the fact that the mean length increased from 158 mm in August to 187 mm in July.

### 5.3.5 Changes in habitat type occupied by 0+ fish in coastal marine waters

During the recruitment period in 2000/2001, 0+ *R. sarba* were caught at only two of the five sheltered, nearshore sites that were sampled monthly using the 5.5 m seine net and 94 % of all of those small individuals were caught at the Mangles Bay site, which is located at the southern end of Cockburn Sound (Fig. 2.1). The 5.5 m long
Figure 5.3. Monthly length-frequency distributions of different age classes of *Rhabdosargus sarba* in coastal marine waters.
seine net caught 0+ *R. sarba* over bare sand in sheltered, nearshore waters in each month between August and December (*Fig. 5.4*). The 21.5 m seine net caught 0+ *R. sarba* in the same habitat first in November and then in declining numbers in each subsequent month until February. The use of the same net over seagrass yielded 0+ *R. sarba* between December and March (*Fig. 5.4*).

The lengths of *R. sarba* caught over sand in sheltered, nearshore waters using the 5.5 m long seine net ranged from 8 to 49 mm (\( \bar{x} = 15 \) mm), while those caught in the same habitat using the 21.5 m seine, which also sampled waters slightly further offshore than the 5.5 m long seine net, ranged from 17 to 85 mm (\( \bar{x} = 39 \) mm) (*Fig. 5.5*). The fish caught using the 21.5 m long seine net in seagrass meadows, which were located just beyond the narrow band of bare sand along the water’s edge, ranged in length from 28 to 89 mm (\( \bar{x} = 62 \) mm).

### 5.3.6 Comparisons between growth curves

Initial analyses demonstrated that the growth curves of the males and females of *R. sarba* did not differ significantly in either coastal marine waters, the Swan River Estuary or Shark Bay, irrespective of whether the unadjusted or subsequently the adjusted von Bertalanffy growth equations were employed (\( p > 0.05 \)). Thus, for subsequent analyses and comparisons, the lengths at age of the two sexes of this species in each of these three water bodies have been pooled to produce each of these forms of the von Bertalanffy growth curve.

Examination of the standardized residuals, derived from fitting the unadjusted von Bertalanffy growth curve to the observed lengths at age for *R. sarba* in coastal marine waters at ca 32°S, strongly indicated that this curve did not describe accurately the distribution of the lengths at age of fish in these waters (*Fig. 5.6*).
Figure 5.4. Mean monthly densities of 0+ *Rhabdosargus sarba* ±1 SE between June 2000 and May 2001, derived from catches using the 5.5 m long seine net (grey) and the 21.5 m long seine net (black) over bare sand and the latter net (white) in vegetated areas at a sheltered beach in coastal marine waters at ca 32°S. Closed rectangles on horizontal axis refer to winter and summer months and open rectangles to spring and autumn months.
Figure 5.5. Number of 0+ *Rhabdosargus sarba* in each 5 mm length class that were caught using the 5.5 m long seine net over sand (grey) and the 21.5 m long seine net over sand (black) and in seagrass (white).
Figure 5.6. Standardized residuals derived from fitting the traditional, unadjusted and adjusted von Bertalanffy growth curves to the lengths at age of *Rhabdosargus sarba* collected from coastal marine waters, the lower Swan River Estuary and Shark Bay. Grey circles, sheltered nearshore waters; black triangles, exposed nearshore waters; white squares, reefs; white circles, estuarine channel.
Examination of the separate groups of residuals for the lengths at age of fish in sheltered nearshore, exposed nearshore and offshore waters over reefs indicated that the inadequacies in the fit of the unadjusted von Bertalanffy growth curve to the full suite of lengths at age of *R. sarba* in that coastal region were due to a bias produced in the overall size composition at age data by size-related movements between habitats and possibly also to the use of different types of sampling gear.

The small gap between the distributions of fish lengths in the sheltered and exposed, nearshore waters (Fig. 5.7) reflected my inability to find fish during the brief period when young fish had reached a certain size and were moving from the first to the second of the above two habitat types. The fact that the lengths at age of fish in exposed, nearshore waters lie well below those for fish in offshore waters over reefs presumably reflects a tendency for fish to move offshore when, in turn, they have also reached a certain size (Fig. 5.7).

Comparisons between the distribution of the standardized residuals, derived from the adjusted von Bertalanffy growth curve and which allowed for differences in the size distribution of fish among different habitat types, and those of the unadjusted von Bertalanffy growth curve, indicated that the former curve provided the best fit to the lengths at age of *R. sarba* in coastal marine waters (Fig. 5.6). The likelihood ratio test confirmed that the adjusted von Bertalanffy growth curve provided a significantly better description for the distribution of the lengths at age of *R. sarba* (*p* < 0.001, Table 5.2, Fig. 5.7). The differences between the adjusted and unadjusted von Bertalanffy growth curves are reflected in a greater asymptotic length (*L*∞), *i.e.* 290 vs 266 mm, which is equivalent to a 9% difference (Table 5.2; Fig. 5.8). A comparison of the curves for the population in this environment (Fig. 5.8) demonstrated that the difference between the predicted lengths at age reached a maximum at 11 years in age, which was estimated from the equations to be 21 mm.
Figure 5.7. Curves fitted to lengths at age of *Rhabdosargus sarba* in sheltered nearshore (white squares), exposed nearshore (grey triangles) and offshore waters over reefs (white circles) in a coastal marine area at ca $32^\circ$S. Curves were derived using the modified approach to fitting the von Bertalanffy growth curve.
Table 5.2. Parameters, including their SEs, for the non-adjusted and modified von Bertalanffy growth curves for *Rhabdosargus sarba* in coastal marine waters and the lower Swan River Estuary at *ca* 32°S and Shark Bay. LL = log-likelihood.

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Figure 5.8. (a) Adjusted von Bertalanffy growth curves for the lengths at age of *Rhabdosargus sarba* (a) in coastal marine waters, (b) the lower Swan River Estuary and (c) Shark Bay. (d-f) Comparisons between the unadjusted (dotted line) and adjusted (solid line) von Bertalanffy growth curves derived from the lengths at age of fish in each of the above three environments. Grey circles, sheltered nearshore waters; black triangles, exposed nearshore waters; white squares, reefs; white circles, estuarine channel.
This difference in length is equivalent to 7.7 % of the $L_\infty$ predicted using the traditional, unadjusted von Bertalanffy growth equation.

The distributions of the residuals for the unadjusted and adjusted von Bertalanffy growth curves, derived from the lengths at age for $R. sarba$ in both the Swan River Estuary and Shark Bay, indicated that the use of the latter growth curve did not lead to a conspicuous improvement in the description of the lengths at age for the populations in either of those environments (Fig. 5.6). Although the likelihood ratio test demonstrated that the adjusted growth curve provided a significantly better fit to the lengths at age of fish in both populations ($p < 0.001$), the values derived for the growth parameters $L_\infty, k$ and $t_0$ for each population by the two types of growth curve were very similar. Thus, for example, the $L_\infty$s for $R. sarba$ derived using the two equations for the populations in the Swan River Estuary and Shark Bay differed by only 10 mm (3 %) and 11 mm (3 %), respectively (Table 5.2; Fig. 5.8). The maximum differences between the predicted lengths at age occurred at ca 3 years in the lower Swan River Estuary and 13 years in Shark Bay (Fig. 5.8). At these ages, the difference between the predicted lengths of the two curves, expressed as a percentage of the corresponding $L_\infty$ for the traditional, unadjusted von Bertalanffy growth equation, was only 1.7 % for the population in the Swan River Estuary and only 3.2 % for that in Shark Bay.

The adjusted growth curves for $R. sarba$ in coastal marine waters, the Swan River Estuary and Shark Bay were significantly different from each other (all $p < 0.001$). The lengths at age, predicted from the equations for ages up to 6 years, were always greater for the population in the Swan River Estuary than for those in either coastal marine waters or Shark Bay (Fig. 5.9).
Figure 5.9. Comparisons between the three adjusted von Bertalanffy growth curves derived from lengths at age of *Rhabdosargus sarba* in coastal marine waters (solid line), the lower Swan River Estuary (dotted line) and Shark Bay (dashed line).
5.4 Discussion

5.4.1 Movements between habitats

The use, at regular intervals, of a 5.5 m long seine net made of 1 mm mesh and a 21.5 m long seine net with 3 mm mesh in the pocket demonstrated that, in marine waters on the lower west coast of Australia, the new 0+ recruits of *Rhabdosargus sarba* started to settle over unvegetated sand in sheltered, nearshore marine waters during September and October in 1999 and in August in 2000. Their small size at this time, *i.e.* \(< 15\) mm, implies that they had been transported relatively rapidly from the waters around reefs in which they had presumably been spawned (Chapter 3). This conclusion is consistent with the fact that *R. sarba* spawns between June and August on this coastline (Chapter 3).

It is highly relevant that small juveniles of *R. sarba*, *i.e.* \(< \text{ca} 40\) mm, were caught in large numbers in the nearshore waters of only one of five similarly sheltered and unvegetated sites in and just to the south of Cockburn Sound and were rarely caught in seagrass in that area. It is proposed that the concentration of post-settlement juveniles at a single site at the southern end of Cockburn Sound (Fig. 2.1) occurs as a result of the southwards transport of larvae from the areas over reefs to the north of this embayment where *R. sarba* is known to spawn (Chapter 3). Such a conclusion would be consistent with the fact that the prevailing current in winter is southerly (Hutchinson & Moore, 1979) and the causeway constructed at the southern end of Cockburn Sound restricts the further passage of water out of the Sound and further southwards (Western Australia. Environmental Protection Authority, 1996). Since the southern region of Cockburn Sound is highly productive (Hutchinson & Moore, 1979), it provides an excellent environment for the rapid growth of the juveniles of *R. sarba*, as well as those of *Sillaginodes punctata* and *Pelates sexlineatus* which likewise settle at the same location at the same time of the year.
The tendency for *R. sarba* to settle in nearshore waters in a particularly restricted region in Cockburn Sound parallels the situation with this species in Botany Bay at a similar latitude in eastern Australia and which has likewise been attributed to the characteristics of the currents in that embayment (McNeill et al., 1992).

The fact that *R. sarba* settles over sand in Cockburn Sound contrasts with the situation found with this species in eastern Australia, where it settles in seagrass (McNeill et al., 1992; Smith & Suthers, 2000). This probably reflects differences in the characteristics of the seagrass species found in the two regions, with the long and thick blades of the *Posidonia sinuosa* present in Cockburn Sound forming a far denser and less penetrable canopy than the species of *Zostera* and *Heterozostera* that occur in embayments in eastern Australia.

Although *R. sarba* does not settle in seagrass in Cockburn Sound, the sequential data obtained during this study for the sizes of this species in different habitats demonstrate that it starts to move into *Posidonia* meadows when it has reached lengths of *ca* 40 mm and is abundant in those seagrass beds until it is *ca* 90 mm. Juveniles were caught in these sheltered waters until March, when they had reached *ca* 80-90 mm in length and were approximately seven months old. The appearance of late 0+ fish in samples collected in June and July at lengths of *ca* 140 mm in more exposed nearshore waters implies that *R. sarba* starts migrating from sheltered to exposed nearshore waters when they attain a length at age of *ca* 90 mm. However, despite extensive sampling, 0+ *R. sarba* were not caught for a short transitional period of two to three months between the times when the juveniles were last caught in sheltered, nearshore habitats and reappeared in more exposed,
nearshore habitats. When the fish reach a certain size and become adults, they move offshore to areas around reefs (Chapter 3).

The data collected in Shark Bay imply that the habitat of *R. sarba* also changes in this environment as the individuals of this species increase in size, with the juveniles occupying nearshore mangrove areas, while the adults live in waters around reefs. The conclusion that *R. sarba* occupies the latter type of habitat when it is an adult is consistent with the fact that this species was not recorded during regular otter trawl sampling of offshore seagrass meadows and unvegetated areas at several sites in different water depths in Shark Bay and which were located in the same general region of this large embayment as those sampled during the present study (Travers & Potter, 2002).

*Rhabdosargus sarba* is typically a marine species that is transported into estuaries *sensu stricto* as post-larvae or young juveniles and spends a period in estuaries before returning to coastal marine waters (e.g. Wallace, 1975; Potter & Hyndes, 1999; Smith & Suthers, 2000). However, *R. sarba* behaves in a quite different manner in the Swan River Estuary. Thus, unlike the situation in other estuaries on the lower west coast of Australia and elsewhere, *R. sarba* spawns in the lower reaches of the Swan River Estuary. Since spawning occurs close to the commencement of the ebb tide and only a few small *R. sarba* were caught in the estuary, many of the eggs produced by this species in the lower Swan River Estuary are presumably swept out to sea (Chapter 4). However, *R. sarba* does become recruited back into the Swan River Estuary when this species is about one year old and has reached a length of ca 140 mm. The unusual behaviour of *R. sarba* in the lower Swan River Estuary may be related to the presence of numerous underwater structures associated with bridges, jetties and wharfs, as these could provide an
environment that “mimics” the complex structure of reefs where this species typically spawns on the lower west coast of Australia.

5.4.2 Age and growth of *Rhabdosargus sarba*

The trends exhibited during the year by the mean monthly marginal increments on the otoliths of *R. sarba* demonstrated that, as is typically the case with other teleosts in south-western Australia (e.g. Hyndes et al., 1992a, 1998; Fairclough et al., 2000; Sarre & Potter, 2000; Hesp et al., 2002), a single annulus is formed in the otoliths of this sparid each year and that, as a consequence, the number of annuli could be used to age individual fish.

The fact that the individuals of *R. sarba* in marine waters on the lower west coast of Australia change habitat progressively as they increase in size made it difficult to obtain a composite collection of samples that represented the distribution of the lengths at age of the population as a whole. Indeed, as Ricker (1969, 1979) has emphasized, it is rare to be able to obtain samples that are truly representative of a population. When considering the problems posed by sampling bias and how they can influence estimates of growth, Ricker pointed out that any attempt to obtain representative samples of all size classes is likely to require the use of different sampling methods. However, while different methods usually have to be employed to obtain samples of fish of different sizes and in different habitats, the efficiency of those methods will differ and thus yield variable sample sizes. In the case of my study, these points are illustrated by the fact that, although different sampling methods were used to collect *R. sarba* in coastal marine waters, this did not fully overcome sampling bias. Thus, although the traditional, unadjusted von Bertalanffy growth curve provided a reasonable fit to the lengths at age of all but the oldest *R. sarba* in coastal marine waters, it was considered necessary to explore other
methods of analyzing growth in order to obtain a growth curve that was appropriate for the full range of lengths at age.

The use of the adjusted von Bertalanffy growth curve developed during the present study clearly provided a significantly better fit to the lengths at age of *R. sarba* in coastal marine waters than that obtained employing the traditional, unadjusted form of the von Bertalanffy model. The improvement in fit was reflected in the fact that the maximum difference between the predicted lengths at age, relative to the $L_\infty$ of the unadjusted von Bertalanffy growth curve, was as high as 7.7%. However, although the use of the adjusted growth equation for *R. sarba* in the lower Swan River Estuary and Shark Bay also resulted in a statistically significant improvement in the fit of the growth curves for both of those populations, the shift in the maximum difference between the lengths at age using the traditional, unadjusted and adjusted growth curves (see Fig. 5.7) relative to the corresponding $L_\infty$ of the traditional, unadjusted von Bertalanffy growth curve, was only 1.7 % and 3.2 % for these two populations, respectively. It is thus considered that the improvement brought about by the use of the adjusted curve to describe the growth of these two populations is of limited biological significance. The far smaller influence produced by using the adjusted as opposed to traditional, unadjusted von Bertalanffy growth curve for the populations in the lower Swan River Estuary and Shark Bay, than was the case for the population in coastal marine waters, presumably reflects, at least in part, the fact that, because the individuals of *R. sarba* in these two environments only move between two habitat types and those habitat types are in relatively close proximity, samples that were more representative of the population as a whole were able to be obtained.

It is proposed that it would be appropriate to consider the use of an alternative, statistically significant growth curve only when that curve results in a
shift exceeding 5% in the predicted lengths, relative to the $L_\infty$ for the traditional, unadjusted von Bertalanffy growth equation. This type of approach parallels that adopted for determining, in another case, when it might be appropriate to shift from using a von Bertalanffy growth curve to a more complex growth curve (White et al., 2002).

5.4.3 Comparisons between the growth of Rhabdosargus sarba in different environments

It was argued above that it would be appropriate to shift to the use of the adjusted von Bertalanffy growth curve for describing the growth of $R. sarba$ in coastal marine waters and to retain the traditional, unadjusted von Bertalanffy growth curve to describe the growth of this species in the Swan River Estuary and Shark Bay. However, when comparing growth amongst populations in different environments, it would also clearly be appropriate to make such comparisons using data that had been derived in precisely the same way. Thus, the comparisons made in the results between the growth curves of the populations in coastal marine waters, the lower Swan River Estuary and Shark Bay employed adjusted von Bertalanffy growth curves in each case, because it was considered important to use the adjusted form of the growth curve for the first of those populations. The use of the likelihood ratio test demonstrated that the adjusted growth curves of the populations in the three different environments were significantly different.

Any comparisons made between the growth curves of the different populations of a species using the likelihood ratio test must take into account the fact that this test is highly sensitive, especially when the curves are based on a substantial number of data points. Furthermore, due to a paucity of lengths at age for older fish, caution must be exercised in drawing conclusions from the trajectory of the growth
curve for *R. sarba* > 5 years in the lower Swan River Estuary, 7 years in coastal marine waters and 9 years in Shark Bay. However, the relative positions of the growth curves for the three populations in Fig. 5.9 strongly indicate that, from a biological perspective, the growth rate of *R. sarba* during the early years of life is genuinely greater in the lower Swan River Estuary than in either coastal marine waters at ca 32°S or Shark Bay. This point is illustrated by the fact that the lengths derived from the adjusted von Bertalanffy growth curve for ages 3 and 4 in the lower Swan River Estuary, *i.e.* about 2 and 3 years after *R. sarba* has reappeared in substantial numbers in that region, each exceeded by more than 5% the lengths at the same corresponding ages in both coastal marine waters and Shark Bay. In contrast, the percentage differences between the lengths at the same ages of *R. sarba* in both coastal marine waters and Shark Bay were less than 5%. The faster growth in the lower Swan River Estuary is consistent with the fact that, in comparison with coastal marine waters, estuarine environments are particularly productive and thus more conducive to rapid growth (Schelske & Odum, 1969; Kennish, 1990; Potter & Hyndes, 1999).

Although *R. sarba* grew at a faster rate in the lower Swan River Estuary than in either coastal marine waters or Shark Bay, the predicted lengths of fish in the three populations at, for example, ages 3 and 4 still differed by only 28 and 19 mm, respectively (Fig. 5.9). The absence of a very pronounced difference in growth amongst the three populations is remarkable in view of the marked differences in the environments in which they live, and contrasts with the very considerable variations exhibited by the growth of another sparid, *Acanthopagrus butcheri*, in four estuaries and a coastal lake in which variables such as potential prey and salinity differed markedly (Sarre & Potter, 2000). This implies that, in comparison with *A. butcheri*,...
the pattern of growth of *R. sarba* is, to a greater extent, fixed and thus has only a limited potential to respond to environmental influences.

Despite the considerable recreational and, in many areas of the world, commercial importance of *R. sarba*, there have apparently been only three studies of the growth of the populations of this species. In one of these studies, the growth of *R. sarba* in Chilka Lake in India was merely described by fitting a curve by eye to the modal lengths of fish in monthly samples (Patnaik, 1973). The lengths at ages 1, 2 and 3 years in that population were 175, 250 and 315 mm and thus far greater than the 142, 216 and 254 mm recorded even in the lower Swan River Estuary during the present study. The von Bertalanffy growth curve calculated by El-Agamy (1989) for *R. sarba* in the Arabian Gulf, based on ages obtained from counts of annuli on scales, yielded a $L_\infty$ of 375 mm, which is *ca* 80 mm greater than the largest fish collected in that study. Furthermore, the $t_0$ of -1.32 years was relatively large for a population in which the maximum age was just over 8 years and the growth curve was shown as passing through the origin on the figure for that curve. Comparisons with the growth curve for a South African population of *R. sarba* by Radebe *et al.* (2002) are made difficult by the fact that the ages given in that paper appear to refer to the number of annuli rather than the true age and the $t_0$ has a negative value of *ca* –1. However, it is highly relevant that the maximum total length of 782 mm, derived from the fork length of 680 mm, recorded by Radebe *et al.* (2002), was far greater than the 390 and 300 mm recorded for the populations in Chilka Lake and the Arabian Gulf, respectively, and the 370 mm for any of the populations in the present study and the 450 mm for anywhere in Australia (Kuiter, 1993). Such a very marked difference would be consistent with the suggestion by Kuiter (1993) that the populations currently reported to be *R. sarba* may consist of more than one species.
Chapter 6: Sequence of gonadal changes, duration and mode of spawning, fecundity and spawning potential ratio of the protandrous hermaphrodite *Acanthopagrus latus*

6.1 Introduction

Previous studies on the reproductive biology of *A. latus*, which have all been undertaken on populations in the northern hemisphere, have shown that the spawning period of *A. latus* in different regions varies markedly (Hussain & Abdullah, 1977; Abu-Hakima, 1984; Abol-Munafi & Umeda, 1994; Chang *et al*., 2002). This species, like other sparids, possesses ovotestes, *i.e.* gonads which comprise paired bisexual gonads consisting of a medio-dorsal ovarian zone and a latero-ventral testicular zone, separated by a wall of connective tissue (D’Ancona, 1949; Besseau & Bruslé-Sicard 1995). However, there is a divergence of opinion as to whether all of the individuals of *A. latus* are protandrous hermaphrodites or whether all or some individuals are essentially gonochorists (*cf* Kinoshita, 1939; Abu-Hakima, 1984; Abol-MunafÎ and Umeda, 1994; Abou-Seedo *et al*., 2003). Abol-MunafÎ and Umeda (1994) based their conclusion that *A. latus* was a protandrous hermaphrodite on the fact that the individuals in the population they studied shifted from exclusively males to very largely females as they increased in size. However, as sex-related bimodal size-frequency distributions can be produced by factors other than sequential hermaphroditism (Sadovy & Shapiro, 1987), this finding does not constitute definitive evidence of protandry. Indeed, Buxton & Garrett (1990) considered many of the reports that certain sparid species are protandrous to be questionable and that at least some of those species are rudimentary hermaphrodites, *i.e.* an individual progresses from a juvenile possessing gonads with both immature testicular and
ovarian tissue into either a functional male with an ovotestis containing ovarian rudiments or a functional female with an ovotestis containing testicular rudiments.

Successful fisheries management requires that the reproductive capacity of a stock is sustained above a threshold level that is sufficient to ensure that a high level of egg production is maintained and that recruitment is not jeopardised (Goodyear, 1993; Mace & Sissenwine, 1993; Mace, 2001). Since exploitation is often size selective, species that undergo an irreversible sex change, i.e. protandrous and protogynous hermaphrodites, may be particularly susceptible to recruitment overfishing unless specific measures, such as the introduction of appropriate minimum legal lengths for capture, are undertaken to ensure that sufficient numbers of both sexes are maintained (Buxton, 1992; Milton et al., 1998). The development of reliable estimates for the optimal levels of exploitation for hermaphroditic species, such as those derived from per recruit models, requires a thorough understanding of the pattern of sex change exhibited by these species as individuals increase in size and/or age. Data on changes in sex were thus required for producing the type of per recruit analyses that were undertaken by Bannerot et al. (1987), Buxton (1992), and Punt et al. (1993) for protogynous species and by Milton et al. (1998), in essence, for a protandrous species.

The first aim of the present study was to examine, both macroscopically and histologically, the gonads of a wide size and age range of A. latus from Shark Bay in order to determine the changes undergone by the ovotestes during the life of this hermaphroditic sparid. Particular emphasis has been placed on ascertaining quantitatively how the prevalence of fish with different types of ovotestes in the different size classes changes throughout the year and in both sequential size and age classes during the spawning period. The latter data have then been used to ascertain the lengths and ages over which this species changes sex and to provide reliable data
on sex ratios for use in stock assessment. The second aim was to determine the
duration of the spawning period and whether _A. latus_ has determinate or
indeterminate fecundity _sensu_ Hunter _et al._ (1985), _i.e._ whether or not the number of
eggs released by individual females within a spawning period is determined prior to
that period. The third aim was to determine the relationships between potential
annual fecundity and both the total length and somatic weight of _A. latus_. The fourth
aim was to determine the spawning biomass per recruit of male and female _A. latus_
and egg production per recruit for the females by using an estimate derived for total
mortality (Chapter 7) and a range of natural mortalities. Finally, the management
implications of the results for _A. latus_ are discussed.

### 6.2 Materials and methods

The gonads of a large number of _A. latus_ that covered a wide size range were
assigned, on the basis of a macroscopic investigation, to one of the following
categories. (1) Very thin, strand-like and of indeterminate sex. These are found only
in juveniles < _ca_ 160 mm in total length, (2) ovotestes containing substantial
amounts of both immature testicular and ovarian material, (3) ovotestes in which the
testicular zone clearly predominates and (4) ovotestes in which the ovarian zone
clearly predominates. Fish with gonads at categories 3 and 4 during the spawning
period were considered to be functional males and functional females, respectively.

Each gonad that contained almost exclusively either ovarian or testicular
tissue was allocated, on the basis of the macroscopic characteristics of the dominant
tissue, to one of the following eight maturity stages, derived from the scheme of
Laevastu (1965), _i.e._ I = virgin, II = immature/resting, III = developing, IV =
maturing, V = mature, VI = spawning, VII = spent, VIII = spent recovering. Since it
was often not possible to distinguish macroscopically between stages V and VI, the
data for these two stages have been pooled.

Each month, gonads were removed from at least 20 individuals covering a
wide length range of fish and encompassing the range of gonadal stages present in
that month. These gonads were used to produce histological sections for determining
which stages in spermatogenesis and oocyte development were present in the
testicular and/or ovarian zone in each ovotestis and to validate that the predominant
zone of each ovotestis had been assigned to an appropriate stage of gonadal maturity.
The gonads were placed in Bouin’s fixative for 48 h and dehydrated in a series of
increasing concentrations of ethanol. The mid-regions of each gonad, and in some
cases also of their anterior and posterior portions, were embedded in paraffin wax,
cut into 6 µm thick transverse sections and stained with Mallory’s trichrome.

The patterns of change in prevalence of juveniles to functional males and
then to functional females as *A. latus* increased in size, based on data collected just
prior to and during the spawning period, were described using the following
approach. The proportion of *A. latus* that were immature, *i.e.* juveniles, at the length
$L_a$ corresponding to age $a$, was calculated using the following equation:

$$P_{a,juvenile} = 1 - \frac{1}{1 + \exp\left(-\log(19)\frac{L_a - L_{50,\text{male}}}{L_{95,\text{male}} - L_{50,\text{male}}}ight)}.$$ 

The proportion of functional female fish at this length (and age) was calculated as

$$P_{a,female} = \left(1 - P_{a,juvenile}\right) - \frac{1}{1 + \exp\left(-\log(19)\frac{L_a - L_{50,\text{female}}}{L_{95,\text{female}} - L_{50,\text{female}}}ight)}$$

and the proportion of functional males was described as

$$P_{a,male} = 1 - P_{a,juvenile} - P_{a,female}.$$ 

The parameters $L_{50,\text{male}}$, $L_{95,\text{male}}$, $L_{50,\text{female}}$ and
$L_{95,\text{female}}$ represent the lengths at which 50 and 95 % of juveniles and functional males
become functional males and functional females, respectively. Natural logarithms are used in these equations. Similar relationships were used to represent these proportions as functions of age (see Chapter 7 for details on ageing of *A. latus*), where

\[
P_{a, \text{juvenile}} = 1 - \frac{1}{1 + \exp\left[-\log(19) \frac{\exp(-K_{\text{male}} a) - \exp(-K_{\text{male}} a_{50, \text{male}})}{\exp(-K_{\text{male}} a_{95, \text{male}}) - \exp(-K_{\text{male}} a_{50, \text{male}})}\right]},
\]

\[
P_{a, \text{female}} = \frac{(1 - P_{a, \text{juvenile}})}{1 + \exp\left[-\log(19) \frac{\exp(-K_{\text{female}} a) - \exp(-K_{\text{female}} a_{50, \text{female}})}{\exp(-K_{\text{female}} a_{95, \text{female}}) - \exp(-K_{\text{female}} a_{50, \text{female}})}\right]},
\]

and \( P_{a, \text{male}} = 1 - P_{a, \text{juvenile}} - P_{a, \text{female}} \). The parameters \( a_{50, \text{male}} \), \( a_{95, \text{male}} \), \( a_{50, \text{female}} \), and \( a_{95, \text{female}} \) represent the ages at which 50 and 95 % of the juveniles and functional males become functional males and functional females, respectively, while the parameters \( K_{\text{male}} \) and \( K_{\text{female}} \) determine the shapes of the curves that relate proportions to age. Estimates of these parameters were obtained by fitting these models to the number of fish within each category for each length or age class, using the maximum likelihood technique. Because relatively few fish were older than 6 years, the values for age classes 7-9, 10-12 and > 12 are shown later in figure 6.7 as pooled values.

The gonadosomatic index (GSI) of each female was calculated using the following equation: \( W1/(W2-W1) \times 100 \), where \( W1 = \) wet weight of the gonad and \( W2 = \) wet weight of the whole fish, *i.e.* \( W2-W1 = \) somatic weight. Both of the mature (stage V) ovotestes were removed from 24 *A. latus* that were caught at the beginning of the spawning season (mid-July - see results) in 2001 and placed in Gilson’s fluid. Note that the amount of testicular material in these gonads was negligible. The ovotestes were shaken once weekly for approximately 6 months to facilitate the breakdown of ovarian connective tissue. The contents of the two ovotestes were then sieved through firstly a 600 \( \mu \)m mesh sieve to remove any remaining undissolved
tissue and then through a 125 μm mesh sieve. This essentially facilitated the retention of just cortical alveolar and yolk granule oocytes, which were then weighed to the nearest 0.001 g. Three random subsamples of these oocytes were weighed (mean = ca 0.05 g) and a count made of the oocytes they each contained. The numbers of eggs in the three ovarian subsamples of known weight were then used, in conjunction with the total weight of all of the oocytes, to estimate the total number of eggs in the ovaries of each fish. The relationships between the total number of eggs and both the total length and somatic weight of these 24 fish were determined by fitting linear regressions to the natural logarithms of the variables.

The spawning stock biomasses per recruit (SSB/R) for female and male _A. latus_ were calculated assuming knife-edge recruitment at 3 years (Chapter 7), constant total mortality for fully-recruited fish and a maximum age of 40 years, as

\[
SSB/R_{\text{female}} = \sum_{a=3}^{40} p_{a,\text{female}} W_a \exp(-Z a) \quad \text{and} \quad SSB/R_{\text{male}} = \sum_{a=3}^{40} p_{a,\text{male}} W_a \exp(-Z a),
\]

respectively, where the proportions of females and males at age _a_ during the spawning period, \( p_{a,\text{female}} \) and \( p_{a,\text{male}} \), were determined from the functions that relate the proportions directly to age. The total body weight, \( W_a \), at age _a_ for each sex was determined from the length at age that was calculated from the von Bertalanffy growth curve (Chapter 7, \( L_\infty = 419 \text{ mm}, k = 0.320 \text{ year}^{-1} \) and \( t_0 = 0.081 \text{ years} \)), employing the total body weight (g) to length (mm TL) relationship for _A. latus_ in Shark Bay, which is \( W = 0.0000174L^{2.997} \). Eggs per recruit, \( E/R \), were determined from the equation

\[
E/R = \sum_{a=3}^{40} p_{a,\text{female}} F_a \exp(-Z a),
\]

using the fecundity \( F_a \) calculated from the length at age _a_, that was derived from the von Bertalanffy growth curve. Using an estimate of total mortality, \( Z \), of 0.23 year\(^{-1}\), which was determined from catch curve analysis (Chapter 7) and a range of alternative values for the
 instantaneous rate of natural mortality, the spawning potential ratio (SPR) was calculated by dividing the estimated value of the current level of $E/R$ by the value of $E/R$ calculated for the unfished stock (Goodyear, 1993).

6.3 Results

6.3.1 Macroscopic and histological observations of gonad tissues

The gonads of *A. latus* < 90 mm TL are thin and strand like and consist almost entirely of connective tissue, the stroma cells of which are *ca* 50 µm in diameter and possess deeply-staining nuclei (Figs 6.1a, 6.2). Gonial cells were first detected in the gonads of fish of 80 to 90 mm, (Fig. 6.2), while gonads with both identifiable testicular and ovarian tissue were first observed in a fish of 95 mm and could almost invariably be detected in fish > 110 mm (Fig. 2). The testicular zone of fish of 110 to 190 mm, *i.e.* < the length at which functional males were first found during the spawning period (see later), contains spermatogonia, spermatocytes and occasionally spermatids, which are located in crypts, while the ovarian zone contains immature oocytes, *i.e.* oogonia and chromatin nucleolar oocytes (Figs 6.1b, 6.2). During the middle of the non-spawning period, the gonads of fish > *ca* 190 mm in length, possess either substantial amounts of ovarian and testicular tissue or contain predominantly or exclusively ovarian tissue (Fig. 6.2). The testicular zone of the first type of ovotestis is flattened and reddish-grey to greyish-white, while its ovarian zone is rounded and translucent-orange. Macroscopically, such gonads are slightly larger but otherwise indistinguishable from those of juveniles of 110-190 mm (Fig. 6.2). The testicular zone contains either spermatogonia, spermatocytes and some spermatids in crypts (Fig. 6.1c,d), or substantial amounts of connective tissue and brown bodies and no crypts or evidence
Figure 6.1. Histological sections of gonads of *Acanthopagrus latus*. Gonads of juvenile fish measuring (a) 84 mm and (b) 137 mm. Ovotestes of fish of (c) 380 mm and (e) 400 mm caught in the middle of the non-spawning period and which contained substantial amounts of testicular and ovarian tissue. (d) Spermatogenesis in the testicular zone of the ovotestis shown in (c). (f) Degeneration of the testicular zone of the ovotestis shown in (e). b, brown bodies; cn, chromatin nucleolar oocytes; ct, connective tissue; e, erythrocytes; o, ovarian zone; s, stroma cells; sc, spermatocytes; sg, spermatogonia; st, spermatids; t, testicular zone. Scale bars. a,d,f = 50 μm; b = 100 μm; c,e = 2000 μm.
Figure 6.2. Schematic representation of sequence of changes that occur in the ovotestes of *Acanthopagrus latus* during life.
of spermatogenesis (Fig. 6.1e,f). In both types of ovotestis, the oocytes in the ovarian zone are still at an early previtellogenic stage (Fig. 6.2).

In the one to two months prior to the commencement of the spawning period, the testicular zones of some ovotestes are white, lobular and far larger than their ovarian zones (Fig. 6.3a) and contain spermatocytes, spermatids and spermatozoa (Figs 6.2, 6.3b). Fish with this type of gonad, which are destined to become functional males, are assumed to have developed from fish, which, during the non-spawning period, possessed ovotestes containing substantial amounts of immature testicular tissue with gametes in the early stages of spermatogenesis and immature ovarian tissue with previtellogenic oocytes (Fig. 6.2).

During the spawning period, the crypts in the testicular zone of functional males break down and release their spermatozoa. Although a small amount of ovarian tissue is present in the gonads of functional males, the oocytes in this tissue never mature beyond the chromatin nucleolar stage. After spawning, the testicular zone of such gonads decreases in size and becomes dark red-grey and similar in size to the ovarian region. These gonads thus revert to a form of ovotestes that is similar to, but of larger size than that of juveniles of 110-190 mm (Fig. 6.2). The periphery of the testicular zone possesses crypts, some of which contain residual spermatids or spermatozoa (Fig. 6.3c). During the middle part of the non-spawning period, the testicular zones of these ovotestes contain substantial amounts of degenerating tissue and, as a consequence of their degeneration, have become far smaller than the ovarian zone (Figs 6.2, 6.3d). Shortly prior to the commencement of the spawning period, the ovarian zone of all ovotestes with these signs of degenerating testicular tissue contain several stages in oocyte development, i.e. chromatin nucleolar, perinucleolar and some cortical alveolar oocytes (Figs 6.2, 6.4a). Although the very small testicular zone consists mainly of connective tissue, some residual germ cells
Figure 6.3. Histological sections of (a) an ovotestis of a 307 mm fish caught just prior to the commencement of the spawning period in which the testicular zone predominates, (b) the testicular zone of the ovotestes shown in (a) with extensive spermatogenesis, (c) the testicular zone of an ovotestis of a 441 mm functional male at the end of the spawning period and (d) an ovotestis of a 257 mm fish caught during the non-spawning period. ct, connective tissue; o, ovarian zone; sc, spermatocytes; sp, spermatatozoa; st, spermatids; t, testicular zone. Scale bars. a = 2000 µm; b,c = 100 µm; d = 1000 µm.
Figure 6.4. Histological sections of part of the ovotestes of a fish of 242 mm (a and b) and 283 mm (c and d). The ovarian zones of the ovotestes shown in (a) and (c) were at stages III and IV, respectively, and the testicular zone shown in (d) had regressed further than that in (b). b, brown body; ca, cortical alveolar oocyte; ct, connective tissue; o, ovarian zone; pv, previtellogenic oocyte; rg, residual germ cell; t, testicular zone; yg, yolk granule oocyte. Scale bars. a,c, 100 µm; b,d, 50 µm.
are present near its periphery (Fig. 6.4b). Fish with this type of ovotestis are clearly destined to become functional females in the next spawning season (Fig. 6.2). In functional ovaries, *i.e.* those with large numbers of yolk granule stage oocytes, the testicular zone is very small and no longer contains detectable germ cells (Fig. 6.4 c,d).

### 6.3.2 Seasonal changes in gonadal categories with increasing length and age

During summer (December to February), the gonads of *A. latus* possess either no macroscopically identifiable ovarian or testicular tissue, a condition found in fish < 160 mm (Fig. 6.5), or contain relatively substantial amounts of both immature ovarian and testicular tissue or consist predominantly of ovarian tissue. The contributions made by fish with gonads containing both immature ovarian and testicular tissue declined from 100 % in fish of 160-240 mm to 48 % in those of 280-299 mm and 12 % in those of 400-419 mm (Fig. 6.5). In contrast, the contributions made by fish with gonads containing predominantly ovarian tissue increased from 7 % in fish of 240-259 mm to 54 % in those of 360-379 mm and 100 % in those of 420-439 mm.

In autumn (March to May), a fourth type of gonad was detected, in which the testicular zone is far larger than the ovarian zone and which, on the basis of the histological study reported earlier, belongs to fish presumably destined to become functional males (Fig. 6.2). The lengths of all of these fish lay between 200 and 319 mm (Fig. 6.5). The contributions made by the number of males increased greatly in winter, and rose sharply from 29 % in the 200-219 mm length class to 100 % in the 240-259 mm length class, before declining to 56 % in the 320-339 mm length class and 10 % in the 420-439 mm length class. In contrast, the number of fish with
Figure 6.5. Seasonal frequencies of occurrence of different gonadal categories (1-4) in sequential 20 mm length classes of *Acanthopagrus latus* in each season. (1) indeterminate gonads of small juveniles (dark grey), (2) ovotestes containing substantial amounts of both immature testicular and ovarian material (light grey and horizontal lines), (3) ovotestes in which the testicular zone predominates (white and diagonal lines) and (4) ovotestes in which the ovarian zone predominates (black).
ovotestes containing substantial amounts of both immature testicular and ovarian tissue declined during winter (June to August), due to the intervening conversion of such fish into males (Fig. 6.2). The remainder of the fish with gonads with immature testicular and ovarian zones correspond to juveniles, a conclusion consistent with the fact that their lengths lay almost exclusively between 60 and 219 mm and thus largely less than all but the smallest functional males (Fig. 6.2). The overall contribution made by functional males decreased markedly in spring (September to November) (Fig. 6.5). The continued and similar contribution made by fish with ovotestes containing predominantly ovarian tissue throughout the year implies that once a fish has become a functional female during the spawning period, it remains a female for the rest of its life (Fig. 6.2).

6.3.3 Proportions of juveniles, males and females immediately prior to and during the spawning period

In the weeks prior to and during the spawning period, the gonads of all A. latus < 190 mm were either undifferentiated or contained both immature testicular and ovarian tissue. The contributions made by fish with such gonads subsequently declined precipitously to zero at just above 300 mm (Fig. 6.6). In contrast, the contributions of males rose from zero at ca 190 mm to a maximum of ca 85 % at ca 270 mm and then fell to zero at ca 470 mm, trends that are reflected in the ‘parabolic curve’ that describes these changes in contribution. Females were first found at lengths of ca 290 mm, after which their contribution increased in a logistic manner, eventually reaching over 95 % in the largest fish (Fig. 6.6). The parameters describing the transition of fish from indeterminate to male and subsequently to female, i.e. \( L_{50,\text{juvenile}} \), \( L_{95,\text{juvenile}} \), \( L_{50,\text{female}} \) and \( L_{95,\text{female}} \), were 245, 310, 348 and 494 mm TL, respectively.
Figure 6.6. Models fitted to the observed frequencies of juveniles (black circles), functional males (white squares) and functional females (grey circles) in sequential 20 mm length classes of *Acanthopagrus latus* caught just prior to and during the spawning season. Sample sizes of fish < 200, 200-299, 300-399 and > 400 were 75, 99, 199 and 40, respectively.
The above trends in the percentage contributions of juveniles, functional males and functional females just prior to and during the spawning season are paralleled by those they exhibit with the age of fish (Fig. 6.7). Thus, the functional male category dominated the 2+, 3+ and 4+ age classes, but then declined markedly in the 5+ and older age classes. In contrast, the proportion of functional females increased from relatively low values in the 2+, 3+ and 4+ age classes to become the dominant category in the 5+ and older age classes (Fig. 6.7). The parameters of the logistic curves relating the proportions of each sex to age, i.e.

\[ a_{50,\text{male}}, a_{95,\text{male}}, a_{50,\text{female}}, a_{95,\text{female}}, K_{\text{male}} \text{ and } K_{\text{female}} \] were 1.75, 3.70, 4.78 and 15.45 years and 0.658 and 0.160 year\(^{-1}\), respectively. Note that, since the older age classes were not well represented and thus carry a reduced weight, the curves do not pass through the points for those age classes.

### 6.3.4 Reproductive variables

The mean monthly GSIs of females, i.e. fish with ovotestes containing almost exclusively ovarian tissue (Fig. 6.2), rose sharply between June and August and then fell precipitously during the ensuing two months (Fig. 6.8). The mean monthly GSIs of fish that did not possess ovotestes with the above characteristics and were > 245 mm, the length at which 50 % of juveniles became males, and were thus considered to be presumptive or functional males, followed an essentially identical trend to that of females. The sharp rise and then marked decline in the mean monthly GSIs indicate that *A. latus* has a short spawning period.

The macroscopic characteristics of the different stages in the development of the ovarian tissue of females, together with the cytological characteristics of each of those stages, are presented in Table 6.1. The ovarian tissue of all females in March and April were at stage I/II, i.e. virgin or immature/resting (Fig. 6.9). Fish with
Figure 6.7. Models fitted to the observed frequencies of juveniles (black circles), functional males (white squares) and functional females (grey circles) in sequential age classes of *Acanthopagrus latus* caught just prior to and during the spawning season. Because relatively few fish were older than six years, the data for age classes 7-9 (n = 38), 10-12 (n = 17) and > 12 (n = 14) have been pooled.
Figure 6.8. Mean monthly gonadosomatic indices +1SE of (a) females and (b) collectively for males and fish with gonads that contained relatively substantial amounts of female and male tissue and were > 245 mm. Closed rectangles on the horizontal axis refer to summer and winter months and the open rectangles to autumn and spring months. Number of fish used to derived each mean is shown.
Table 6.1. Characteristics of the macroscopic stages, and each corresponding histological stage, in the development of functional ovaries of *Acanthopagrus latus*. Adapted from Laevastu (1965).

<table>
<thead>
<tr>
<th>Stage</th>
<th>Macroscopic characteristics</th>
<th>Histological characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>III - Developing</td>
<td>Slightly larger than at stage II. Reddish colour. Oocytes visible through ovarian wall.</td>
<td>Chromatin nucleolar, perinucleolar and cortical alveolar oocytes present.</td>
</tr>
<tr>
<td>IV - Maturing</td>
<td>Larger than stage III, occupying about half of body cavity. Reddish-orange in colour. Yolk granules visible through ovarian wall.</td>
<td>Cortical alveolar and yolk granule oocytes abundant.</td>
</tr>
<tr>
<td>V/VI -Mature/spawning</td>
<td>Large, occupying about two thirds of body cavity. Extensive capillaries visible in ovarian wall. Hydrated oocytes sometimes visible through ovarian wall in stage VI ovaries.</td>
<td>Yolk granule oocytes predominant. Migratory nucleus oocytes, hydrated oocytes or post ovulatory follicles present in stage VI ovaries.</td>
</tr>
<tr>
<td>VII - Spent</td>
<td>Smaller than V/VI and flaccid. Some yolk granules visible through ovarian wall.</td>
<td>Remnant yolk granule oocytes present, typically undergoing atresia.</td>
</tr>
<tr>
<td>VIII- Spent/Recovering</td>
<td>Small and dark red.</td>
<td>Extensive scar tissue present. Ovarian lamellae becoming reorganised. No yolk granule oocytes present.</td>
</tr>
</tbody>
</table>

* Stage I – Virgins of larger juveniles contain substantial amounts of both testicular and ovarian tissue.
Figure 6.9. Monthly percentage frequency histograms for gonadal maturity stages of female *Acanthopagrus latus*. n = sample size.
ovaries at stages III (developing) and IV (maturing) were first found in May and those with ovaries at stages V-VI (mature or spawning) were first recorded in June and were collectively the most prevalent category in July and the sole category in August. Although the above trends suggest that spawning could commence as early as June, the fact that the mean monthly GSIs were still continuing to increase markedly between July and August implies that, by July, the gonads of many fish had still not reached full maturity, *i.e.* were not producing and releasing hydrated oocytes. This conclusion is consistent with the observation that, in contrast with the situation in August, the ovaries of very few fish in July contained hydrated oocytes or post-ovulatory follicles. Fish with stage VII (spent) ovaries were first found in September and, together with stage VIII (recovering spent) ovaries, collectively dominated the complement of ovaries recorded in that month. Furthermore, very few stage V/VI ovaries were found in fish caught during October (*Fig.* 6.9). The above trends imply that very little spawning occurs after September. Thus, spawning takes place predominantly during a relatively short period in late winter and early spring. Furthermore, since *A. latus* has determinate fecundity (see later), the large contribution (*i.e.* sometimes up to *ca* 50 %) made by hydrated oocytes to the suite of vitellogenic oocytes in ovaries with this category of oocyte, suggests that *A. latus* spawns on a limited number of occasions within this short period.

6.3.5 Fecundity

The distributions of the oocyte diameters in the mature (stage V) ovaries of the two mature females shown in *Fig.* 6.10 were markedly bimodal, as was the case with other such ovaries during the spawning period. The distribution of the diameters of the group of smallest oocytes, *i.e.* typically < 120 µm, which were predominantly at the chromatin nucleolar or perinucleolar stages, was strongly skewed to the left, with
Figure 6.10. Oocyte diameter frequencies for two mature (stage V) female *Acanthopagrus latus.*
a modal class at 20-39 µm. In both ovaries, the largest oocytes, which were mainly yolk granule oocytes, were represented by a modal diameter class that lay between 380 and 419 µm. The presence of a gap between the diameters of the groups of small and large oocytes in mature females demonstrates that this species has determinate fecundity sensu Hunter et al. (1985). Thus, the number of large eggs in the ovaries of females caught immediately prior to the commencement of the spawning period was used to provide estimates of the fecundity of *A. latus*. Furthermore, the fact that the ovaries of females with stage VI ovaries contained substantial numbers of both yolk granule oocytes with no signs of hydration and oocytes in an advanced stage of hydration, strongly indicates that *A. latus* is a multiple spawner sensu de Vlaming (1983), i.e. individual females release oocytes on more than one occasion during a spawning season, even though such spawning may occur on only a few occasions within that season.

The relationships between the natural logarithms of potential annual fecundity and the total length (Fig. 6.11a) and somatic weight of *A. latus* (Fig. 6.11b) are described as follows:

(1) \( \log F = 4.080 \log L - 9.651 \) \( (R^2 = 0.54, n = 24) \)

(2) \( \log F = 1.328 \log W + 5.528 \) \( (R^2 = 0.66, n = 24) \),

where \( F \) = annual fecundity, \( L \) = total length (mm), \( W \) = somatic weight (g) and \( R^2 \) = the coefficient of determination.

The potential annual fecundities of *A. latus* predicted by the exponential equations for female *A. latus* with lengths of 300, 350, 400 and 450 mm are 959,410, 1,662,900, 2,882,220 and 4,995,620 eggs respectively, and for female *A. latus* with somatic weights of 500, 1,000, 1,500 and 2,000 g, are 1,076,000, 2,062,000, 3,958,000 and 7,566,000 eggs. The mean fecundity ±1SE for the 24 fish was 1,935,000 ± 281,000. The fecundity ranged between 764,000 for a fish of 331 mm
Figure 6.11. Relationship between potential annual fecundity and (a) total length and (b) somatic weight of *Acanthopagrus latus*.
and 600 g (total body weight), and 7,910,000 eggs for a fish of 450 mm and 2,050 g (total body weight), respectively.

### 6.3.6 Spawning biomass per recruit

The spawning biomass per recruit (SSB/R) analysis indicated that, with a total mortality ($Z$) of 0.23 year$^{-1}$, determined from catch curve analysis (Chapter 7), the spawning biomass of females currently exceeds that of males by 122 $\%$, i.e. 2,350 vs 1,060 g recruit$^{-1}$. If the levels of natural mortality were 0.1, 0.15 and 0.2 year$^{-1}$, the egg production per recruit by the females would correspond to SPRs of 0.29, 0.51 and 0.80, respectively.

### 6.4 Discussion

#### 6.4.1 Acanthopagrus latus is a protandrous hermaphrodite

Analysis of the trends exhibited by microscopic and macroscopic changes to the gonads over the full size and age range of *Acanthopagrus latus* in Shark Bay, and during both the non-spawning and spawning periods, clearly demonstrates that this sparid is a protandrous hermaphrodite in this large embayment. These trends have also enabled the precise sequence of changes that occur in the ovotestes of *A. latus* from early in life to be elucidated, when they consist almost exclusively of connective tissue, through to late in life when essentially all individuals are females (Fig. 6.2). Furthermore, the data on the changes in the percentages of the two sexes with increasing body size and age, using data for the period immediately prior to and during the spawning period, also demonstrate that all males are almost certainly destined to become females.
The problems inherent in using differences in the size distributions of males and females as evidence that a species is either a protandrous or protogynous hermaphrodite have been pointed out by Sadovy & Shapiro (1987), and were negated in the present study by the adoption of a rigorous and comprehensive sampling regime and methods for analysing the data. For example, (1) it is highly unlikely that the sex-related bimodality in the size distributions of *A. latus* in Shark Bay were due to the larger males of this species moving offshore since my fish were obtained from the full range of depths (0.5 m to 5 m) in which this species is caught by commercial and recreational fishers using a range of fishing methods. (2) It is also highly unlikely that the very conspicuous sex-related differences in length were due to selective fishing since substantial numbers of fish were obtained by each of three different sampling methods, *i.e.* seine netting, haul netting and rod and line fishing. (3) Furthermore, these differences were not due to differences in mortality rates between the sexes rather than to the influence of protandry, since only males were represented among the smaller and younger of the mature-sized fish during the spawning period. (4) Moreover, because the changes in the contributions made by each sex with increasing age parallel those that occur with increasing size, the differences in the lengths of the two sexes cannot be attributed to differences between the growth rates of males and females. Although *A. latus*, and also *A. australis* and *Acanthopagrus berda*, which are likewise found in Australia (Pollock, 1985; Tobin *et al.*, 1997), are protandrous hermaphrodites, another Australian congeneric, *i.e.* *A. butcheri*, is a rudimentary hermaphrodite (Sarre & Potter, 1999; Sarre, 1999).

My scheme for the sequential changes undergone during life by the gonads of *A. latus* in Shark Bay (*Fig. 6.2*) contrasts markedly with that of Abol-Munafi & Umeda (1994), who concluded that, as *A. latus* increases in size, the ovarian zone of
its ovotestes gradually enlarges at the expense of its testicular zone and that, as a consequence, the males become gradually transformed into females. Although the protandric changes undergone by *A. latus* may vary among populations, it seems far more likely that the conclusion drawn by Abol-Munafi & Umeda (1994) represents a failure to recognise that the ovotestes of males revert to a form similar to that of a large juvenile after spawning and thus, at that time, could not be distinguished as belonging to either a male or female. Furthermore, the fact that the prevalence of the females of *A. latus* in Shark Bay rose progressively to ca 100 % with increasing length and age also contrasts with the conclusions of Abu-Hakima (1984) and Abou-Seedo *et al.* (2003) that many individuals of this species do not change sex. It appears highly relevant that those latter workers reached this conclusion without analysing the ways in which the prevalence of females changed over the full size and age range of their populations.

My scheme for the pattern of gonadal changes in *A. latus* is similar to that of Pollock (1985) for the protandrous *Acanthopagrus australis* in eastern Australia. However, in contrast to Pollock (1985), I conclude that it is very unlikely that any fish develop directly from an immature juvenile into a female. This conclusion is based largely on the fact that, during the spawning period, the vast majority of the fish of ca 270 mm in length were males and no female fish were caught at this or lesser lengths. This conclusion is given further weight by the fact that, since a single smooth curve could be used to describe the growth of the individuals in the population of *A. latus* in Shark Bay (Chapter 7), the sex-related bimodality in the lengths of individuals in this population could not have been due to differences in the rate of growth of the two sexes. Moreover, in view of the very strong protandric trends exhibited overall by *A. latus*, it appears reasonable to propose that the few fish that still possessed immature juvenile gonads when they had reached lengths of
ca 290 mm, the size at which females were first found, would have been destined to become males (Fig. 6.6).

6.4.2 Aspects of spawning and estimates of fecundity
Since *A. latus* spawns in Shark Bay in late winter and early spring, the larvae are produced at a time when water temperatures in that embayment are starting to increase progressively from their winter minima (Travers & Potter, 2002). This therefore provides the juveniles of *A. latus* with a protracted first growing season and therefore increases their chances of survival (see Conover, 1992; Cushing, 1990). The spawning period of *A. latus* is similar to that of *R. sarba* (Chapter 3), with which it often co-occurs in Shark Bay.

Since *A. latus* has determinate fecundity, the standing stock of large oocytes in functional females with mature ovaries in July represents the potential annual fecundity of this species in Shark Bay. The fecundities estimated in the present study are consistent with those derived for five females in the Arabian Gulf by Abu-Hakima (1984) on the basis of the number of oocytes with a diameter ≥ 180 µm, an approach that would have essentially only included cortical alveolar and yolk granule oocytes and, if they were present in their fish, also hydrated oocytes. However, my estimates and those of Abu-Hakima (1984) are far lower than those of Abol-Munafi and Umeda (1994), differences which can be attributed to those latter workers having counted all of the oocytes in the ovary and having thus included oocytes in earlier stages of development. My conclusion that *A. latus* is a multiple spawner conflicts with that of Abu-Hakima (1984), but agrees with that of Abol-Munafi & Umeda (1994).
6.4.3 Implications for management

The current minimum legal total length for *A. latus* in Western Australia is 250 mm, which is very similar to the length at which 50% of the fish first become identifiable as functional males during the spawning season, *i.e.* 245 mm. Since this species requires a further 2½ years to reach 348 mm (Chapter 7), the length at which 50% of the males will become females, it can legally be fished during this protracted period. Following his consideration of the implications of the results of sex changes on yield-per-recruit models for two protogynous species, Buxton (1992) proposed that the minimum legal length (MLL) for these species should be greater than the length at which they change from female to male. Since mortality due to fishing will have a greater impact on the females than the males of a protandrous species, it is even more valid to impose a MLL that is greater than the typical length at which sex change occurs in protandrous species such as *A. latus*. Indeed, if the current MLL for *A. latus* is maintained, the pressure from an ever-increasing number of recreational fishers could have a severe impact on the spawning biomass of the females of this species. Such a view would be consistent with the conclusion of the Western Australian Department of Fisheries that, prior to the implementation of management changes that reduced the number of commercial fishing licenses, commercial fishers were apparently having a detrimental impact on the stock of this species in Shark Bay (Shaw, 2000).
Chapter 7: Age and size compositions, growth and mortality of *Acanthopagrus latus* in Shark Bay

7.1 Introduction

The age composition and growth of *A. latus* in the Arabian Gulf were determined by Samuel & Mathews (1987), using data derived from the number of opaque zones in otoliths, which had been cracked and burnt. However, no attempt was made during that study to validate that this method was appropriate for ageing this species, a procedure now regarded as a prerequisite before such counts can be used with confidence to estimate the ages of individual fish (Campana, 2001). Catch curve analyses by Samuel & Mathews (1987) strongly indicated that the total mortality of *A. latus* in the Arabian Gulf during the early 1980s was high. However, the estimate of the instantaneous coefficient of natural mortality, $M$, obtained by Samuel & Mathews (1987) using the equation of Pauly (1980), was far greater than their estimate of the instantaneous coefficient of total mortality, $Z$. Since the same anomalous results were recorded for three other *Acanthopagrus* species, Samuel & Mathews (1987) concluded that the Pauly equation was inappropriate for estimating the natural mortality of these four sparid species.

Estimates of natural mortality have often been derived from empirical equations, such as those of Pauly (1980) and Ralston (1987), which had been developed using data for a large number of species from several different regions. The data employed to develop the regression equations of Pauly (1980) and Ralston (1987) were water temperature, the growth coefficient, $k$, and asymptotic length, $L_\infty$, in the von Bertalanffy growth equation, in the case of the former equation, and solely the above growth coefficient in the latter equation. In contrast, Hoenig (1983)
derived equations for total mortality, based on the assumption that the estimated total mortalities are related to the maximum observed ages for the range of stocks that he used. Since Hoenig’s (1983) regression equation for total mortality for fish employed data for a range of lightly-fished stocks, it yielded values that would be similar to natural mortality in such stocks, but not in those that are heavily-exploited.

As pointed out by Vetter (1988), each of the above equations provides a single and very imprecise point estimate for mortality. Furthermore, the growth data employed by Pauly (1980) and Ralston (1987) and the age data used by Hoenig (1983) for constructing their equations were obtained prior to the time when it became mandatory to validate the procedure employed for ageing fish for growth studies (Beamish & McFarlane, 1983; Campana, 2001). Moreover, other studies have often found that the individual values derived for natural mortality using the equations of Pauly, Ralston and Hoenig, varied markedly (e.g. Burton, 2001). Since fishing mortality represents the difference between total and natural mortality, it is obviously important to obtain a reliable estimate of both of these types of mortality and, as has been stressed by Pascual & Iribarne (1993) and Cubillos et al. (1999), to assess the level of precision associated with these point estimates.

During the present study, lengths at age for individuals of *A. latus* in Shark Bay were obtained employing a validated ageing procedure, and used to determine the age compositions and growth of this species in this large marine embayment. The von Bertalanffy growth parameters derived from the present study were inserted into the empirical equations of Pauly (1980) and Ralston (1987) for determining natural mortality, *M*. Estimates for total mortality, *Z*, were then derived by inserting the maximum age of *A. latus* in commercial fish catches from Shark Bay into the equations of Hoenig (1983). Further estimates of *Z* were obtained by employing simulation to compute the approximate probability density function for *Z* associated
with this maximum age and by subjecting the age composition data to relative
abundance analysis. However, the resultant estimates of $M$ for *A. latus* in Shark Bay
were far greater than all of those for $Z$ (see results). Therefore, a Bayesian approach
was used, which, through incorporating all of the available information relating to
mortality, yields more realistic and consistent estimates for the very important
parameters $M$ and $Z$ than were produced by the empirical equations on their own.
This approach involved combining the likelihood distributions associated with each
of the separate estimates of $M$ and $Z$ to derive single likelihood distributions for each
of these two mortality variables. The Bayesian approach used in the present study is
equally applicable for determining natural and total mortality of other fish species for
which there are multiple and often inconsistent estimates of these parameters.

7.2 Materials and methods

*Acanthopagrus latus* was obtained from Shark Bay (22°50’S, 113°50’E) between
August 1999 and December 2001, both through line fishing and seine netting and by
obtaining samples from the haul net catches supplied to fish processing plants by
commercial fishers. The total length (TL) and wet weight of each fish were recorded
to the nearest 1 mm and 0.1 g, respectively. The gonads of each fish were removed
and examined macroscopically to ascertain whether they contained ovarian or
testicular material. Some of these gonads were also prepared for subsequent
histological examination to ensure that gonadal designations were appropriate.

The two sagittal otoliths of each fish were removed, cleaned, dried and stored
in paper envelopes. They were later placed in methyl salicylate and examined
microscopically under reflected light against a black background, employing a
dissecting microscope attached to a video camera (Panasonic WV-CD20). The image
was analysed using the computer imaging package OPTIMAS 5 (Optimas Corporation, Bothell). Since the otoliths of large fish contained opaque zones that were so numerous and closely spaced that they were often indistinguishable from one another, they were sectioned in order to increase the resolution of those zones. For sectioning, the otoliths were mounted in clear epoxy resin and cut into sections of \(ca\) 500 \(\mu \text{m}\) using a low speed diamond saw (Buehler). The sections were cut through the primordium at right angles to the longest axis of the otoliths. The resultant sections were cleaned and mounted on slides using DePX mounting medium, and examined in the same manner as whole otoliths. The number of opaque zones on each of 100 sectioned otoliths, containing a range of numbers of opaque zones, was compared with those recorded for the same otoliths prior to sectioning. Note that the number of opaque zones on each otolith was counted without knowledge of the size or time of capture of the fish from which the otolith had been removed. Since sectioning improved the resolution of the opaque zones in the otoliths of those \(A.\ latus\) in which there were > 8 opaque zones (see results), it was decided to be conservative and section all otoliths with \(\geq 5\) opaque zones.

Marginal increment analysis was employed to determine whether the opaque zones detectable on whole and sectioned otoliths of \(A.\ latus\) with < 5 and \(\geq 5\) opaque zones, respectively, were formed annually. For this purpose, measurements were made of the distance between the primordium and the outer edge of both the otolith and the single opaque zone, when only one such zone was present, and of the distances between the outer edge of the otolith and each of the two outermost opaque zones, when two or more opaque zones were present. These measurements, which were made perpendicular to the opaque zones and without knowledge of the date of capture of the fish from which that otolith had been removed, were recorded to the nearest 0.01 mm using the computer imaging package OPTIMAS 5. The marginal
increment on each otolith, *i.e.* the distance between the single or outermost opaque zone and the edge of that otolith, was expressed as a proportion of the distance between the primordium and the outer edge of the opaque zone, when only one opaque zone was present, and as a proportion of the distance between the outer edges of the two outermost opaque zones, when two or more opaque zones were present. The marginal increments on otoliths with the same number of opaque zones in the corresponding calendar months of different years were grouped together. Images of both whole and sectioned otoliths of *A. latus* are presented in the Appendix.

An independent reader (Dr G. A. Sarre, Murdoch University) counted the opaque zones that he observed in 100 sectioned otoliths removed from a wide size range of *A. latus*. Ninety percent of the counts were the same as those of the senior author. In six of the ten cases where there were discrepancies, it was mutually agreed, after re-examination of the otoliths, that the independent reader had not detected the outermost opaque zone on those otoliths. Both readers agreed that some of the opaque zones in the other four otoliths could not be readily distinguished. Hence, these otoliths, and the small number of other otoliths for which it was not possible to clearly detect each of the opaque zones, were not employed for ageing.

### 7.2.1 von Bertalanffy growth equations

The approximate time of peak spawning was estimated from the trends shown throughout the year by the gonadosomatic indices, gonadal maturity stages and pattern of oocyte development (Chapter 6). This was considered to correspond to the birth date, which was then used to determine the age of individual fish on their date of capture. A single von Bertalanffy growth curve was fitted to the lengths at age of all individuals of *A. latus*, using non-linear regression in SPSS (SPSS Inc., 1999).

The von Bertalanffy equation is \( L_t = L_\infty \left[ 1 - e^{-k(t-t_0)} \right] \), where \( L_t \) = the total length at age
t (years), \( L_\infty \) = the mean of the asymptotic length predicted by the equation, \( k \) = the growth coefficient (year\(^{-1}\)) and \( t_0 \) = the hypothetical age at which fish would have zero length, if the growth followed that predicted by the equation.

### 7.2.2 Natural mortality

Pauly’s (1980) regression model was refitted to the data for the 175 fish stocks used in his study in order to provide the additional information required for calculating the confidence limits for an estimate of natural mortality, \( M \), derived from that regression equation. This model took the following form,

\[
\log_e M = -0.0152 - 0.279 \log_e L_\infty + 0.6543 \log_e k + 0.4634 \log_e T,
\]

where \( T \) = mean annual surface water temperature (°C) and \( L_\infty \) and \( k \) are the von Bertalanffy growth parameters. Since the length measurements used by Pauly were recorded as total length in cm, this policy was adopted for this mortality equation. A point estimate of \( M \) was then determined for \( A. latus \) in Shark Bay by inserting into this regression model the estimates of \( L_\infty \) and \( k \), derived from the above growth equation, and the mean annual surface water temperature of 22.5°C at Denham in Shark Bay, which was derived from data obtained by the Australian Oceanographic Data Centre (http://www.AODC.gov.au).

The likelihood distribution of \( M \) for \( A. latus \) in Shark Bay was also estimated from the refitted regression equation and the associated variance-covariance matrix for the parameter estimates. For this calculation, it was assumed that the predicted value of \( \log_e M \) for \( A. latus \), which was determined from the regression equation, has a Student’s \( t \)-distribution with 171 degrees of freedom and a mean and standard error for the prediction, which were calculated from the values for the independent variables (for formulae see Sokal & Rohlf, 1995, p. 633). If \( f(\log_e M) \) is the
probability density function of $\log_e M$, then the probability density function of $M$ is 
\[ g(M) = f(\log_e M) / M. \]
Accordingly, the likelihood associated with each value of $M$ was calculated by dividing by $M$ the likelihood of $\log_e M$, as determined from the $t$-distribution derived from the regression equation. Estimates of the confidence limits for $M$ were calculated from the resulting likelihood distribution for $M$.

An estimate of $M$ and its confidence limits were also calculated by refitting Ralston’s (1987) data to his regression equation, i.e. $M = 0.0189 + 2.06k$, where $k$ is the von Bertalanffy growth coefficient.

### 7.2.3 Total mortality

Hoenig’s (1983) regression model was refitted to the data for the 82 fish stocks used in his study (provided in Hoenig, 1982) to provide the additional information required for calculating the confidence limits for the estimates of total mortality, $Z$. The model took the form, 
\[ \log_e Z = 1.46 - 1.01 \log_e t_{\text{max}}, \]
where $Z$ = total mortality (year$^{-1}$) and $t_{\text{max}}$ = the maximum observed age. An estimate of $Z$ for *A. latus* in Shark Bay was calculated by inserting the maximum age observed in the sample of 291 fish collected from the commercial catches of *A. latus* in Shark Bay into this equation. These catches were used for this purpose since commercial fishers operate throughout Shark Bay and were therefore likely to obtain a more representative sample than were collected by sampling using seine netting and rod and line angling in more restricted areas.

The values for $Z$, predicted using Hoenig’s regression equation for fish, are assumed to have a Student’s $t$-distribution, 80 degrees of freedom and a mean and standard error calculated from the independent variable, longevity. If $f(\log_e Z)$ is the probability density function of $\log_e Z$, the probability density function of $Z$ is
\[ g(Z) = \frac{f(\log_e Z)}{Z} \]. Thus, the likelihood associated with \( Z \) for \( A. latus \) in Shark Bay was calculated by dividing by \( Z \) the likelihood of \( \log_e Z \), as determined from the \( t \)-distribution derived from the refitted regression equation. Estimates of the confidence limits for \( Z \) were calculated from the resulting likelihood distribution for \( Z \).

A further point estimate of \( Z \), which adjusts for sample size, was obtained by substituting the observed maximum age for \( E(t_{\text{max}}) \) for the sample from the commercial catches of \( A. latus \) from Shark Bay in the following equation of Hoenig (1983), i.e.

\[
E(t_{\text{max}}) = \frac{1}{Z} \sum_{i=1}^{n} \frac{1}{i} + t_c,
\]

where \( n \) = sample size and \( t_c \) = first age fully represented in the catches.

An approximation to the probability density function for \( Z \), that is associated with the maximum age recorded in a random sample of a specified sample size, was obtained using simulation. For this purpose, it was assumed that the sample was taken from fish that had reached the age at which they became fully vulnerable to fishing gear, i.e. 3 years, and that total mortality, \( Z \), was constant above this age. The sample size, which was employed for this simulation, was the number of fish that were \( \geq 3 \) years old in the sample of 291 fish collected from the commercial catches of \( A. latus \) in Shark Bay. The simulation was run 100,000 times to develop an approximation to the probability density function for the maximum age recorded in a random sample of the specified sample size for each of a large number of discrete values of \( Z \), ranging from 0.05 to 10 year\(^{-1} \) at 0.01 intervals and covering the full range of feasible values for \( Z \). These results were then used to determine the likelihood distribution for \( Z \) for \( A. latus \) in Shark Bay, by applying Bayes’ theorem and assuming a uniform prior probability distribution function for \( Z \). That is,
\[ p(Z_j | t_{\text{max}}) = \frac{P(t_{\text{max}} | Z_j)P(Z_j)}{\sum_k P(t_{\text{max}} | Z_k)P(Z_k)}, \]

where \( P(Z) \) is the prior probability for \( Z \) and \( Z_j \) is the \( j \)'th value of the set of discrete values of \( Z \) that are considered in this analysis and which cover the full range of possible values for this variable.

An estimate of \( Z \) was also obtained by analysing the catch curves derived from the commercial samples of \( A. \ latus \) in Shark Bay. Since a major assumption of catch curve analysis is that the sample is taken randomly from the fully-recruited age classes (Ricker 1979), the data used to construct my catch curves for \( A. \ latus \) were restricted to those on the descending limbs of the catch curves.

Relative abundance analysis, developed by Deriso et al. (1985) as a natural extension of catch curve analysis, was used to analyse the age composition data. This approach to analysing catch curves avoids the assumption that recruitment is constant and overcomes the problem of applying a log transformation to the frequencies for older age classes with zero fish. For a fish stock that experiences a constant level of total mortality, \( Z \), from the age of full recruitment, \( a=t_c \) years, the estimated proportion, \( \hat{p}_{a,j} \), at age \( a \) in year \( t \) is

\[ \hat{p}_{a,j} = \frac{R_{a,t_c} \exp[-(a-t_c)Z]}{\sum_{j=t_c}^A R_{j,t_c} \exp[-(j-t_c)Z]}, \]

where \( A \) is the maximum observed age. \( R_y \) is the level of recruitment for year class \( y \), where recruitment represents the fish that reach the age of full recruitment \( t_c \), relative to the average level, i.e. \( \bar{R}_y = 1 \). It is assumed that the age composition for fish of ages \( t_c \leq a \leq A \) observed in year \( t \), represents a random sample from a multinomial distribution with uniform selectivity from the age of full recruitment. Thus, ignoring constants, the log-likelihood, \( \lambda \), of the age compositions observed in the various years may be calculated as

\[ \lambda = \sum_t \sum_{a=t_c}^A n_{a,j} \log[\hat{p}_{a,j}], \]

where \( n_{a,j} \) is the observed number of fish of age \( a \) in year \( t \). The parameters of the model were estimated for \( A. \ latus \) in
Shark Bay using AD Model Builder (Fournier, 1994) to maximise the log-likelihood, firstly with the relative levels of recruitment being constrained to the average level, \( \overline{R} \). The relative levels of recruitment were then, one by one, successively introduced as parameters to be estimated by the model. At each stage of this stepwise procedure, the parameter that was finally selected for inclusion in the model was the one that produced the greatest increase in the log-likelihood. Parameters were only added to the model if they resulted in a statistically significant improvement to the fit of the model to the data, as determined using a likelihood ratio test (e.g. Kimura, 1980), while the remaining levels of recruitment continued to be constrained to the average level. Estimates of the profile likelihood distributions of \( Z \) for \( A. latus \) in Shark Bay were also obtained for this analysis using AD Model Builder.

### 7.2.4 Integrating the separate mortality estimates

Three independent estimates of \( Z \) had been calculated for \( A. latus \) in Shark Bay, using the methods described above, namely those obtained from Hoenig’s (1983) equation, the simulation method and the relative abundance analysis which assumes that recruitment is variable. The two estimates of \( Z \) calculated for \( A. latus \), using the relative abundance analysis, are not independent as they are derived from the same data. Since the variable recruitment hypothesis imposes less stringent conditions on the data, it is likely to yield a more reliable estimate than that which assumes constant recruitment. Although Hoenig’s regression equation and the calculation of \( Z \) from the approximate probability density functions derived from simulation both use the longevity of \( A. latus \) in Shark Bay, the former method employs information contained in the set of data from the range of species and stocks to which Hoenig fitted his regression equation. Thus, the information used to develop these two estimates may be assumed to be independent. For the first set of data considered in
the analysis, $D_1$, i.e. the longevity of $A. latus$ in Shark Bay and the data to which Hoenig’s (1983) fish equation was fitted, it is assumed that the prior probabilities for $Z$ are uniformly distributed. Therefore, from Bayes’ (1763) theorem,

$$p(Z_j | D_1) = \frac{p(D_1 | Z_j)p(Z_j)}{\sum_k p(D_1 | Z_k)p(Z_k)}.$$  For the second set of data, $D_2$, i.e. the longevity of $A. latus$ in Shark Bay and the sample size from which this longevity was obtained (as used in the simulation study), the prior probabilities for $Z$ may now be taken as

$$p(Z_j | D_1).$$

Hence, from Bayes’ theorem,

$$p(Z_j | D_1, D_2) = \frac{p(D_2 | Z_j)p(Z_j | D_1)}{\sum_k p(D_2 | Z_k)p(Z_k | D_1)}.$$  Similarly, the same approach may be applied for the age composition data that were used in the relative abundance analysis, and subsequently any other data sets that are collected. Thus, for the $n$’th set of data, $D_n$,

$$p(Z_j | D_1, D_2, \ldots, D_n) = \frac{p(D_n | Z_j)p(Z_j | D_1, D_2, \ldots, D_{n-1})}{\sum_k p(D_n | Z_k)p(Z_k | D_1, D_2, \ldots, D_{n-1})}.$$  Accordingly, the information concerning $Z$ that is contained in the separate data sets is combined by forming the product of the likelihoods for each value of $Z$ derived from each data set and then normalizing the resulting products.

The likelihood of the natural mortality $M$ was calculated from the likelihood for $Z$ by assuming that, for each value of $Z$, there is a uniform probability that $M < Z$.

Thus, if $F(Z)$ represents the cumulative likelihood of $Z$ and $Z$ is the expected value of $Z$, the likelihood of $M$ may be calculated as $(1 - F(Z))/\bar{Z}$. The resulting likelihood distribution for $M$ was then combined with that for the estimate of $M$ derived from Pauly’s (1980) equation by forming their product at each corresponding value of $M$ and normalizing the resultant products. Because there was considerable overlap in the data used by Ralston (1987) to develop his regression equation and those used by Pauly (1980), the likelihood distribution calculated for $M$ using the Ralston equation was not used in the derivation of the combined likelihood distribution for $M$.  

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7.3 Results

7.3.1 Comparisons between the number of opaque zones on whole and sectioned otoliths

Although the same number of opaque zones were observed on the otoliths of *A. latus* prior to and after they had been sectioned when the number of such zones was seven or less, this was frequently not so when otoliths contained a greater number of opaque zones. In the latter cases, the number of opaque zones that could be observed on whole otoliths was less than those that were visible on sectioned otoliths. Furthermore, as the number of opaque zones increased, the frequency and extent of the discrepancies increased from 44 % and 1, respectively, when the number of zones visible on the otolith after sectioning lay between 8 and 11, to 92 % and 5, respectively, when their number lay between 12 and 15, respectively.

7.3.2 Validation that opaque zones are formed annually

The mean monthly marginal increments on otoliths of *A. latus* with two opaque zones rose from *ca* 0.5 in July to reach a maximum of *ca* 0.6 in October, before declining to *ca* 0.4 in November and a minimum of *ca* 0.2 in December and then rising in the ensuing months (**Fig. 7.1**). Essentially the same trend was followed by the mean monthly marginal increments on otoliths with a greater number of opaque zones. Although none of the fish caught in January to April contained otoliths with a single opaque zone, the trends exhibited by the mean monthly marginal increments for the other months of the year were consistent with those exhibited by otoliths with a greater number of opaque zones (**Fig. 7.1**).
Figure 7.1. Mean monthly marginal increments ± 1SE for sagittal otoliths of *Acanthopagrus latus*. Sample sizes are shown above each mean. On the x axis, black rectangles refer to winter and summer months and open rectangles to spring and autumn months.
Since, irrespective of the number of opaque zones in the otolith, the mean monthly marginal increment rose and declined only once during the year, a single opaque zone is laid down in the otoliths of *A. latus* each year. As the mean monthly marginal increment typically peaks in October or November and subsequently declines to its lowest level in December or January, the new opaque zone usually becomes delineated between the middle of spring and early summer.

### 7.3.3 Length-frequency distributions of the different age classes

As the trends exhibited during the year by the GSIs, stages in gonadal maturation and pattern of oocyte development of *A. latus* demonstrated that the spawning of *A. latus* in Shark Bay peaked in late August/early September (Chapter 6), this species was assigned a mean birth date of 1 September. 0+ *A. latus* were first caught in November, when their lengths ranged from 23 to 40 mm (Fig. 7.2). The modal length class reached 60-79 mm by March and 100-119 mm by June. The length distributions of the 0+ age class overlapped that of the 1+ age class in May, and the same situation applied to each pair of successive age classes. However, the length distributions of each of the older age classes produced a distinct modal class in some months. This applied, for example, to the 1+ age class in October, December and July, to the 2+ age class in October and January and to the 3+ age class in July (Fig. 7.2).

### 7.3.4 Growth of Acanthopagrus latus

Since the coefficient of determination for the composite curve was as high as 0.921 and the age at zero length (*t*₀) was close to zero, *i.e.* 0.081 years, the lengths at age of all individuals of *A. latus*, irrespective of whether fish are of indeterminate sex or
Figure 7.2. Length-frequency distributions for different age classes of *Acanthopagrus latus*. n = number of fish aged.
males or females, are described well by a single von Bertalanffy growth curve (Fig. 7.3, Table 7.1). The von Bertalanffy curve demonstrates that, by ages 1 to 5, the individuals of *A. latus* had, on average, reached lengths of 107, 192, 254, 299 and 332 mm, respectively, and that by 10 and 15 years, they had attained lengths of 401 and 415 mm, respectively. The maximum recorded length and age for *A. latus* was 466 mm and 24.9 years, respectively. The age at which *A. latus* reached the minimum legal length for capture (MLL) of 250 mm was ca 2.9 years.

The regression equation relating the total length in mm (*L*) and weight in g (*W*) of *A. latus* was \( \log_e W = 2.997 \log_e L - 10.972 \) (n = 942, \( R^2 = 0.997 \)).

### 7.3.5 Mortality estimates and year class strengths

The point estimates for the instantaneous coefficient of natural mortality, *M*, for *A. latus*, calculated using the equations of Pauly (1980) and Ralston (1987), were both 0.70 year\(^{-1}\) (Table 7.2). The confidence intervals derived from these regression equations were very broad, particularly in the case of the Pauly equation (Table 7.2). The value of 0.18 year\(^{-1}\) derived for total mortality, *Z*, using the regression equation refitted to Hoenig’s (1983) fish data, which related the expected maximum age to *Z* for lightly-exploited fish stocks, was far lower than the point estimates for *M* that were calculated from the Pauly and Ralston equations (Table 7.2). The same generalisation was true for the estimate of 0.30 year\(^{-1}\) for *Z* obtained using Hoenig’s (1983) equation, which contained an adjustment for sample size. The estimate of *Z* = 0.30 year\(^{-1}\), obtained from the simulation study, was identical to that calculated using this last equation, as calculated using the relative abundance analysis (Table 7.2). The confidence intervals for all of these estimates of *Z* were much tighter than those determined for *M* using the refitted Pauly or Ralston equations (Table 7.2).
Figure 7.3. von Bertalanffy growth curve for *Acanthopagrus latus* determined using the lengths-at-age of 922 fish.
Table 7.1. von Bertalanffy growth parameters derived from lengths at age for *Acanthopagrus latus*, including 95% confidence limits, the coefficient of determination ($R^2$) and number of fish aged ($n$).

<table>
<thead>
<tr>
<th>von Bertalanffy growth parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L_\infty$ (mm)</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Estimate</td>
</tr>
<tr>
<td>Lower</td>
</tr>
<tr>
<td>Upper</td>
</tr>
</tbody>
</table>

Table 7.2. Mortality (year$^{-1}$) of *Acanthopagrus latus* in Shark Bay calculated using different life history models, estimation of longevity based on simulation or relative abundance analyses. N = no value could be obtained, $M$ = natural mortality, $Z$ = total mortality.

<table>
<thead>
<tr>
<th>Method of analysis</th>
<th>$M$ or $Z$</th>
<th>Estimate</th>
<th>Lower 95%</th>
<th>Upper 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refitted Pauly (1980)</td>
<td>$M$</td>
<td>0.70</td>
<td>0.16</td>
<td>1.54</td>
</tr>
<tr>
<td>Refitted Ralston (1987)</td>
<td>$M$</td>
<td>0.70</td>
<td>0.38</td>
<td>0.96</td>
</tr>
<tr>
<td>Revised Hoenig (1983), fish equation</td>
<td>$Z$</td>
<td>0.18</td>
<td>0.05</td>
<td>0.38</td>
</tr>
<tr>
<td>Hoenig (1983), adjusted for sample size</td>
<td>$Z$</td>
<td>0.30</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Estimation of longevity based on simulation</td>
<td>$Z$</td>
<td>0.30</td>
<td>0.21</td>
<td>0.46</td>
</tr>
<tr>
<td>Relative abundance analysis, constant recruitment</td>
<td>$Z$</td>
<td>0.20</td>
<td>0.17</td>
<td>0.23</td>
</tr>
<tr>
<td>Relative abundance analysis, variable recruitment</td>
<td>$Z$</td>
<td>0.23</td>
<td>0.18</td>
<td>0.27</td>
</tr>
<tr>
<td>Mortality estimate from Bayesian approach</td>
<td>$M$</td>
<td>0.19</td>
<td>0.10</td>
<td>0.25</td>
</tr>
<tr>
<td>Mortality estimate from Bayesian approach</td>
<td>$Z$</td>
<td>0.23</td>
<td>0.21</td>
<td>0.26</td>
</tr>
</tbody>
</table>
The strength of the different age classes in the fishery in 1999 and 2000, varied markedly (Fig. 7.4). Therefore, while the 1985 and 1990 year classes were strong in both 1999 and 2000, the recruitment in those years were as much as 2.6 (SE ± 0.8) and 3.2 (SE ± 0.5) times greater than the average, respectively. The 1989 and 1995 year classes were also strong, with recruitment being 1.8 (SE ± 0.4) and 1.8 (SE ± 0.3) times the average, respectively.

The application of a relative abundance analysis, which assumed that recruitment is constant, yielded an estimate for Z for *A. latus* of 0.20 year\(^{-1}\), which was thus approximately the same as that produced by the refitted version of Hoenig’s (1983) equation for fish, whilst a relative abundance analysis, which did not require that assumption, yielded a slightly higher estimate for Z, *i.e.* 0.23 year\(^{-1}\) (Table 7.2). However, the confidence intervals for the estimates of Z obtained by both of these analyses were far narrower than those obtained using the refitted Hoenig (1983) equation.

The likelihood distribution for the estimates of Z, derived using the equation that was refitted to Hoenig’s (1983) data, provided more conservative estimates of total mortality than those that were derived from the relative abundance analyses, while the distribution derived from the simulation study produced much higher estimates of Z (Table 7.2; Fig. 7.5a). The overall likelihood distribution for Z was determined by combining the separate likelihood distributions for the various estimates of Z (Fig. 7.5b), but excluding that for the relative abundance estimate assuming constant recruitment. This resultant likelihood distribution was dominated by the relative abundance estimate assuming variable recruitment (*cf* Figs 7.5a,b). The resulting likelihood distribution for M, which was determined from this combined likelihood distribution for Z and the requirement that \(M \leq Z\), is shown in
Figure 7.4. Numbers of individuals of the 1975 to 1997 year classes of *Acanthopagrus latus* in samples collected by commercial fishers in 1999 and 2000. Relative abundance analyses were used to fit lines to the observed frequency of abundance of fish in each year class, assuming that recruitment is either constant (dashed line) or variable (dotted line).
Figure 7.5. (a) Estimated likelihood distributions for total mortality, $Z$, of *Acanthopagrus latus*, derived using Hoenig’s (1983) regression equation for fish (dashed line), relative abundance analysis assuming variable recruitment (solid line) and a simulation method based on maximum age and sample size (dotted line). (b) The combined likelihood distribution for $Z$ for *Acanthopagrus latus* derived from the separate likelihood distributions shown in (a).
**Fig. 7.6a**, together with the broad likelihood distribution for $M$ that was calculated from the refitted Pauly equation. The resultant likelihood distribution for $M$, which combines the likelihood distributions of the various estimates for $Z$ and $M$, but excluding that for the relative abundance estimate assuming constant recruitment, is shown in **Fig. 7.6b**. Based on these resultant likelihood distributions, the values for $M$ and $Z$ of *A. latus* in Shark Bay and their confidence limits are estimated to be 0.19 (0.10 to 0.25) year$^{-1}$ and 0.23 (0.21 to 0.26) year$^{-1}$, respectively.

7.4 Discussion

**7.4.1 Age and growth of Acanthopagrus latus**

The trends exhibited during the year by the mean monthly marginal increments on the otoliths of *A. latus* demonstrated that, as is typically the case with other teleosts in south-western Australia (*e.g.* Hyndes *et al.*, 1992b, 1996b, 1998; Fairclough *et al.*, 2000; Sarre & Potter 2000; Hesp *et al.*, 2002), a single annulus is formed in the otoliths of this sparid each year and that their number could therefore be used for ageing individual fish. This suggests that the corresponding growth zones in the otoliths of *A. latus* in Kuwait, (Samuel & Mathews, 1987) are probably formed annually and that the estimates of age derived by those workers from their counts of these zones were also valid.

The $L_\infty$ in the von Bertalanffy growth equation for *A. latus* in Shark Bay at 22°50’ S, *i.e.* 419 mm TL, was only slightly greater than that recorded by Samuel & Mathews (1987), *i.e.* 405 mm, for the same species in the very different environment of the Arabian Gulf, which is located at 29° N. Although the value obtained in the present study for $k$ was also slightly greater, *i.e.* 0.32 vs 0.26, that for $t_0$ was far
Figure 7.6. (a) Likelihood distributions derived for $M$ for *Acanthopagrus latus* from Pauly’s (1980) equation (dotted line) and the likelihood distribution for $M$ (solid line), assuming that it is less than the combined estimate for $Z$ that was derived from Hoenig’s (1983) regression equation, simulation-derived estimates of the likelihood distributions of the maximum age and the relative abundance analysis. (b) Combined likelihood distributions for $Z$ (solid line) and $M$ (dotted line) for *Acanthopagrus latus*, representing the combined likelihood distribution for estimates of $Z$ determined from Hoenig’s (1983) regression equation, simulation-derived estimates of the likelihood distributions of the maximum age and the relative abundance analysis, and the likelihood distribution of $M$ derived from Pauly’s (1980) method, combined with the likelihood distribution of $M$ based on the combined likelihood distribution for $Z$. 
closer to zero, i.e. 0.08 vs −0.96. The fact that the $t_0$ obtained by Samuel & Mathews (1987) approached −1 can be attributed, at least in part, to the paucity of fish < 100 mm total length and thus of a length that could constrain the bottom end of the growth curve.

The values for the $L_\infty$ of $A. latus$ in Shark Bay and the Arabian Gulf are similar to those recorded for $Acanthopagrus bifasciatus$ and $Acanthopagrus berda$ in the latter water body (Samuel & Mathews, 1987) and of $Acanthopagrus butcheri$ in four estuaries and a coastal lagoon at distances in excess of 800 km further south of Shark Bay and thus in a far more temperate and very different environment (Sarre & Potter, 2000). Although the value for $k$ for $A. bifasciatus$ in the Arabian Gulf, i.e. 0.19, was lower than those recorded for the above populations, this can be attributed, at least in part, to the highly negative and thus anomalous value of −2.24 for $t_0$ for this species (Samuel & Mathews, 1987). While the value for $k$ for $A. butcheri$ in another south-western Australian estuary, the Moore River Estuary, was far lower than those recorded in the above populations, i.e. 0.11, this estuary is highly atypical in that salinities remain at very low levels throughout the year (Young et al., 1997) and such conditions have been shown, in aquaculture studies, not to be conducive to a rapid growth of juvenile black bream (Partridge & Jenkins, 2002). Furthermore, the maximum age recorded for all but one of the above ten populations of $Acanthopagrus$ species was at least 14 years. From the above, the values for $L_\infty$, $k$ and $t_{\max}$ for $Acanthopagrus$ species each apparently typically lie within a relatively narrow range, even when the species live in very different regions.

### 7.4.2 Estimates of mortality

The estimates of $M$ calculated for $A. latus$ during the present study using the refitted equations of Pauly (1980) and Ralston (1987) were both far greater than those
calculated for Z for this species using the two Hoenig (1983) equations, the simulation study and the two relative abundance analyses. This type of inconsistency parallels that found by Samuel and Mathews (1987), in that the estimates they derived for M for A. latus and three other Acanthopagrus species in the Arabian Gulf using the Pauly (1980) equation were also considerably greater than those derived for Z using catch curve analysis. However, natural mortality cannot, of course, exceed total mortality. Since the estimates obtained for Z in the present study derived using both of the Hoenig (1983) equations, simulation and relative abundance analyses are consistent and based on sound data, the high point estimates obtained for the natural mortality of A. latus using the refitted equations of Pauly (1980) and Ralston (1983) are erroneously elevated. Although the value for t_max for a species is strongly influenced by sample size and can therefore affect the resulting value of Z derived from Hoenig’s (1983) fish equation, this influence has been taken into account through using the simulation method described in this chapter.

The substitution of the estimate of 0.70 year\(^{-1}\) for M for A. latus, derived from Pauly’s (1980) equation into Hoenig’s (1983) equation, which relates the expected maximum age to sample size, yields an expected age of 12.0 years for an unfished population with an hypothetical sample of 291 fish, i.e. the number of fish in the sample from commercial catches of A. latus in Shark Bay. However, the maximum recorded age for this population in Shark Bay was 24 years, with many fish exceeding 15 years of age. Thus, the mortality of A. latus lies well outside the norm for the mortalities of the species used by Pauly (1980) for estimating mortalities.

By using a Bayesian approach to combine the likelihood distributions associated with each of the various estimates of Z and M, the inconsistencies between those estimates (see Table 6.2) can be reconciled through the production of single integrated estimates of both of these mortality variables. The likelihood distribution
that has been calculated from each of the empirical equations is derived from the uncertainty associated with the parameter estimates from the regression equation, as was used by Cubillos et al. (1999), but also takes into account the correlations between the parameter estimates and the variability of values of the dependant variable about the regression line. The resultant likelihood distribution for Z was dominated by the likelihood distribution of the relative abundance analysis assuming variable recruitment, which produced far more precise estimates of Z than were obtained using either the refitted Hoenig equation or the simulation study (cf Figs 5a,b). Thus, assuming the resulting estimates of Z are correct, the only feasible values of M for A. latus are clearly those that fall within the lower tail of the likelihood distribution derived from the refitted Pauly equation (Fig. 6a).

The estimate obtained in the present study of 0.23 year\(^{-1}\) for Z for A. latus in Shark Bay, derived from the combination of likelihood distributions, was far lower than the 0.60 year\(^{-1}\) calculated by Samuel & Mathews (1987) for the same species in the Arabian Gulf. This implies that the fishing pressure on A. latus is currently far lower in Shark Bay than it was in the Kuwaiti waters of the Arabian Gulf during the 1970s and 1980s, when that species was known to have been heavily fished. In the case of the population of A. latus in Shark Bay, the estimate of 0.19 year\(^{-1}\) calculated for M from the combination of likelihood distributions is close to the current estimate of 0.23 year\(^{-1}\) for Z. Indeed, the value of only 0.04 year\(^{-1}\) for fishing mortality, F, calculated as \(F = Z - M\), implies that just under 4 % of the fish over three years of age are taken by the fishery each year. Thus, while the population of A. latus used to be heavily targeted by commercial fishers in Shark Bay to the point of apparently being over-exploited, the position has now apparently been improved by the reduction in the number of fishing units from 17 to 9 (Shaw, 2000).
This study has demonstrated that the use of a range of traditional techniques to estimate mortality in *A. latus*, such as empirical equations and relative abundance analysis, as well as simulation, invariably yielded far lower values for total mortality than for natural mortality, which clearly cannot be the case. However, the analyses also showed that, as a result of a lack of precision and variability in the underlying data, the probability distributions for most of the various estimates of *M* and *Z* for *A. latus* are so broad that they overlap. Therefore, during the present study, a Bayesian approach was adopted to develop a method in which the information content used to develop the life history based equations for estimating mortality in fish species is integrated and combined with data that was obtained during the present study for *A. latus*. This approach has produced far more precise and consistent estimates of both *Z* and *M*. Precisely the same approach can be used to improve the quality of the estimates for *Z* and *M* in other fish species.
Chapter 8: Discussion

8.1 *Comparisons between the life histories of Rhabdosargus sarba and Acanthopagrus latus*

Acanthopagrus latus

Although *Rhabdosargus sarba* and *Acanthopagrus latus* belong to the same family, *i.e.* Sparidae, have similar distributions and sometimes co-occur in the same habitats, *e.g.* in shallow water habitats in Shark Bay, the work conducted for this thesis has shown that aspects of their reproductive biology differ markedly. For example, in Western Australian waters, *R. sarba* is a rudimentary hermaphrodite (Chapter 3), whereas *A. latus* is a protandrous hermaphrodite (Chapter 6). Protandry results in the majority of the smaller fish being males and most of the larger fish being females. Thus, as fecundity is directly related to body size, protandry optimizes the production of eggs by those species that exhibit this form of hermaphroditism. Furthermore, since the small males of a species can typically produce sufficient sperm to fertilize the eggs of even the largest females (Ghiselin, 1969), it will not be disadvantageous for the males of a species to be relatively small, providing that mating is not size dependant. The above features thus increase the reproductive success of individual fish over their life time (Warner, 1974; 1988).

It is relevant that, at a single location on any particular day, the samples of *A. latus* typically contained fish of a wide size range, whereas those of *R. sarba* caught using the same sampling method (rod and line angling), typically comprised fish covering a smaller size range. This point is illustrated by the fact that, in catches obtained over reefs during the spawning periods of these two species, the difference between the minimum and maximum lengths of each species, expressed as a percentage of their $L_{\infty}s$, never exceeded 24 % in the case of *R. sarba*, whereas they
always exceeded 21 % and sometimes reached up to 50 % with *A. latus*.

Furthermore, unlike the situation with *R. sarba*, the samples of *A. latus* caught on several of those occasions contained essentially the full size range of the mature individuals of this species. This indicated that, during the spawning period, the groups of mature *A. latus* contain fish of a wide size range, whilst those of *R. sarba* contain fish of a far narrower size range. These results demonstrate that, as would be expected of a protandrous species (and in contrast to the rudimentary hermaphrodite *R. sarba*), *A. latus* does not tend to “school” according to size during the spawning period.

Another major difference between the two sparid species resides in the fact that *R. sarba* has indeterminate fecundity, a relatively protracted spawning period and a low maximum mean monthly GSI (*ca* 5), whereas *A. latus* has determinate fecundity, a restricted spawning period and a high maximum mean monthly GSI (*ca* 15). Hence, *R. sarba* exhibits a reproductive “bet-hedging” strategy *sensu* Rinchard & Kestemont (1996), *i.e.* individuals release eggs over a protracted period. This reduces the likelihood of all of the eggs being released at a time when climatic or hydrographic conditions are unfavourable and of the eggs and larvae being subjected to heavy predation by a large school of fish (Lambert & Ware, 1984; Weddle & Burr, 1991; McEvoy & McEvoy; 1992). Thus, at least some of the eggs and larvae of *R. sarba* will be likely to survive and eventually develop into adults. In contrast, *A. latus* tends to have an “all at once” spawning strategy, *i.e.* spawning is concentrated in a restricted period that coincides with the time (environmental window) when conditions for egg and larval survival are optimal (Cury & Roy, 1989). Spawning over a short period could also have the beneficial effects of leading to “predator swamping”, *i.e.* the production of more eggs and larvae than can be consumed by predators in the period soon after spawning (Lambert & Ware, 1984).
However, the marked interannual variation in the recruitment of *A. latus*, as demonstrated by the relative abundance analyses, demonstrates that the production and/or survival of the eggs and larvae does vary markedly from year to year, which is to be expected for a species that spawns over such a restricted period. From the above, it follows that the spawning by *A. latus* over a short period is a more “risky” strategy than that exhibited by *R. sarba*. It therefore appears relevant that the maximum ages recorded for *A. latus* and *R. sarba* during the present study, *i.e.* 24 vs 13, respectively, as well as differences in the overall age compositions (Chapters 5 and 7), implies that the former species has greater longevity. Such longevity would help compensate for the detrimental effects of high inter-annual recruitment variability on population size. Indeed, it is noteworthy that the natural mortality of *A. latus* lies at the extreme lower end of the range for those species that grow to a similar size and which were used by Pauly (1980) to construct his equation for estimating natural mortality (Chapter 7). In contrast to the above conclusions drawn for *A. latus*, the age composition data for *R. sarba* in three different environments strongly indicate that the recruitment of this species in these environments each year is relatively strong (Chapter 5). Thus, selection pressures for increased longevity would not be as strong in *R. sarba* as *A. latus*.

### 8.2 The spawning times of *Rhabdosargus sarba* and *Acanthopagrus latus*

It is generally accepted that mortality in fishes is greatest at the larval stage and that the two major causes of larval mortality are starvation and predation (*e.g.* Kane, 1984; Houde, 1987; Cury & Roy, 1989; Wootton, 1990). In temperate environments, where there are predictable periods of high water temperature and food abundance and low water temperature and food scarcity, fish typically reproduce during periods when water temperatures and thus food availability are greatest (Shuter & Post,
This generality is borne out by the fact that, in temperate Western Australia, many teleost species, which exhibit a range of different life cycles, spawn at this time. These include (1) yellowfin whiting *Sillago schomburgkii* and Ogilby’s hardyhead *Atherinomorus* (previously *Pranesus*) *ogilbyi*, which spend their lives in nearshore environments (Prince & Potter, 1982; Hyndes & Potter 1997), (2) the stout whiting *Sillago robusta* and West Australian Dhufish *Glaucosoma hebraicum*, which live in offshore marine waters (Hyndes & Potter, 1996; Hesp et al., 2002), (3) the trumpeter whiting *Sillago burrus*, banded whiting *Sillago vittata* and western school whiting *Sillago bassensis*, which use shallow, nearshore coastal waters as nursery areas and then migrate into deeper waters as they increase in size and become mature (Hyndes & Potter, 1996; Hyndes et al., 1996b), (4) the black bream *Acanthopagrus butcheri*, the catfish *Cnidoglanis macrocephalus* and several species of the Atherinidae, which spend their entire life cycle in estuaries (Prince & Potter, 1982; Nel et al., 1985; Sarre & Potter, 1999) and (5) the flathead *Platycephalus speculator*, cardinalfish *Apogon rueppellii* and the atherinid *Atherinosoma presbyteroides*, which spawn in estuaries and also in the marine environment (Prince & Potter, 1982; Chrystal et al., 1985; Hyndes et al., 1992b).

There are, however, a limited number of species of teleost in that Western Australia do not spawn in the warmer summer months. These include the King George Whiting *Sillaginodes punctata* (Hyndes et al., 1998), which spawns in winter, the sea mullet *Mugil cephalus*, which spawns in autumn and winter and the yellow-eye mullet *Aldrichetta forsteri* which spawns from late summer to early winter (Chubb et al., 1981; Orr, 2000). As with *R. sarba*, these species typically spawn at sea (Chapter 3) and often use estuaries as nursery areas (Potter & Hyndes, 1999; Smith & Suthers, 2000). The timing of the spawning periods of the three former species enable the larvae and small juveniles to enter Western Australian...
estuaries from the marine environment during winter and spring, when the sand bars at their mouths have been breached by seasonal freshwater discharge and thus allow exchange of water between the ocean and the estuary. Since estuaries, such as the Peel-Harvey Estuary (Potter et al., 1983) and the Blackwood Estuary (Valesini et al., 1997) on the lower west coast of Australia, are also used as nursery areas by R. sarba, the winter spawning of this species would enable the larvae of this species to enter these estuaries at a time when the estuary mouth is widest and deepest. However, despite the fact that R. sarba spawns on reefs in coastal marine waters in the vicinity of the Swan River Estuary, very few small juveniles of this species, i.e. < 140 mm, are recruited into this estuary from the marine environment. Furthermore, extensive sampling of sheltered, nearshore coastal waters within relatively close proximity of the Swan River Estuary only yielded substantial numbers of 0+ juveniles at one site. Since this site is located at the southern end of Cockburn Sound, it is proposed that most of the juveniles produced on the reefs outside the mouth of the Swan River Estuary are swept southward by the prevailing current and settle in the calm nearshore waters of that part of the sound.

The spawning of A. latus during the winter parallels the situation recorded for Acanthopagrus australis at similar (subtropical) latitudes in eastern Australia (Pollock, 1982) and A. berda in northern Australia (Tobin et al., 1997). While such a spawning time thus appears to be a characteristic of marine representatives of Acanthopagrus in Australian waters, Acanthopagrus butcheri spawns between mid-spring and early summer in estuaries on the lower (temperate) west coast of Australia (Sarre & Potter, 1999). However, as freshwater discharge reaches a maximum in winter, a winter spawning period by any fish species in the estuaries of this region would be likely to lead to the eggs and larvae of that species being flushed out the estuary. It is therefore hypothesized that selection pressures led to the spawning
period being delayed in *A. butcheri* until a time when conditions in the estuary were much more stable and would thus facilitate the successful retention and development of the larvae within the estuary.

**8.3 Hermaphroditism of fishes**

The ability to determine with certainty whether a species undergoes sequential hermaphroditism requires the acquisition of sound quantitative data on the pattern of sex change as individuals increase in size and age. Although previous workers have concluded that *A. latus* is a protandrous hermaphrodite, their schemes for the gonadal changes undergone by this species during its life were either inadequate or incorrect due mainly to the absence of crucial information (Chapter 6).

As pointed out by Sadovy & Shapiro (1987), studies of hermaphroditism in fish should be based on an analysis of large samples of all size and age classes throughout the year. For *A. latus*, this type of comprehensive sampling proved critical because the process of sex change in this species is linked to the annual reproductive cycle. Thus, the males could only be identified just prior to, during and immediately after the spawning period. Furthermore, the analysis of the “sex ratios” in age composition data at different times of the year was crucial for validating that the inference from size-frequency data that *A. latus* was protandrous was correct. These data were also crucial for determining the precise pattern of protandrous changes in *A. latus*.

Sadovy & Shapiro (1987) suggest that the presence of gonads containing both degenerating testicular tissue and proliferating ovarian tissue provides strong evidence of protandry. However, some individuals of *R. sarba*, which is a rudimentary hermaphrodite in Western Australian waters (Chapter 3), contained gonads consisting predominantly of ovarian tissue and a small amount of
degenerating testicular tissue. In sparids in general, including those that are only rudimentary hermaphrodites, the juveniles possess gonads with both testicular and ovarian tissue (*sensu* Buxton & Garratt, 1990). Therefore, when the juveniles of sparid species develop into either males or females, one type of gonadal tissue will proliferate, while the other type of gonadal tissue will either remain small or degenerate, as occurs with *R. sarba* in Western Australian waters. Hence, the presence of a sparid species with gonads that simultaneously contain degenerating testicular tissue and proliferating ovarian tissue does not necessarily indicate that this species is a protandrous hermaphrodite. Therefore, as most [if not all] indicators of hermaphroditism may be produced by more than one means, convincing diagnoses of hermaphroditism must consist of several lines of evidence and efforts should always be made to exclude alternative explanations for each piece of evidence (Sadovy & Shapiro, 1987).
References


Bennett, B. A. (1989). The fish community of a moderately exposed beach on the southwestern cape coast of South Africa and an assessment of this habitat as a nursery for juvenile fish. Estuarine Coastal and Shelf Science 28, 293-305.


Appendix: Ageing of *Rhabdosargus sarba* and *Acanthopagrus latus*

![Otoliths](image)

**Figure 1 of Appendix.** Whole otoliths of *Rhabdosargus sarba* with (a) one and (b) two opaque zones. The opaque zone(s) in these otoliths are indicated by white circles.

**Marginal increment analysis**

The marginal increment on the otolith, *i.e.* the distance between the outermost opaque zone and the edge of the otoliths, is represented by the green line. For otoliths with one opaque zone, the marginal increment was expressed as a proportion of the distance between the outer edge of the outermost opaque zone and the centre of the nucleus (*i.e.* length of green line divided by length of red line, shown in a). For otoliths with ≥2 opaque zones, the marginal increment was expressed as a proportion of the distance between the outer edge of the two outermost opaque zones (*i.e.* length of green line divided by length of red line, shown in b). The same method for marginal increment analysis was used for *Acanthopagrus latus.*
Figure 2 of Appendix. Otoliths of a large *Rhabdosargus sarba* viewed (a) whole and (b) after sectioning.

*Sectioning of otoliths*
Although all of the opaque zones in otoliths of small *R. sarba* can be detected relatively easily when viewed whole (see Figure 1 of Appendix), this is not the case for otoliths of large individuals of this species, in which the outermost opaque zones, in particular, are difficult to detect. However, all of the opaque zones (indicated by white circles) become far easier to detect once the otoliths have been sectioned.
Figure 3 of Appendix. Whole otoliths of *Acanthopagrus latus* with (a) one and (b) four opaque zones. The opaque zone(s) in these otoliths are indicated by white circles. An opaque zone has just become delineated from the edge of the otolith shown in b.
Figure 4 of Appendix. Otoliths of large *Acanthopagrus latus* viewed (a) whole and (b) after sectioning. The opaque zones in these sectioned otoliths are indicated by white circles. Although it became increasingly difficult to detect the outermost opaque zones on whole otoliths of *A. latus* as the number of such zones increased, this was as serious a problem as with the whole otoliths of *R. sarba*. However, the definition of the opaque zones was improved by sectioning the otoliths. The opaque zones in the sectioned otolith are indicated by white circles.