Factors influencing the timing and frequency of spawning and fecundity of the goldlined seabream (*Rhabdosargus sarba*) (Sparidae) in the lower reaches of an estuary

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Abstract—We have studied the reproductive biology of the goldlined seabream (*Rhabdosargus sarba*) in the lower Swan River Estuary in Western Australia, focusing particularly on elucidating the factors influencing the duration, timing, and frequency of spawning and on determining potential annual fecundity. Our results demonstrate that 1) *Rhabdosargus sarba* has indeterminate fecundity, 2) oocyte hydration commences soon after dusk (ca. 18:30 h) and is complete by ca. 01:30–04:30 h and 3) fish with ovaries containing migratory nucleus oocytes, hydrated oocytes, or postovulatory follicles were caught between July and November. However, in July and August, their prevalence was low, whereas that of fish with ovaries containing substantial numbers of atretic yolk granule oocytes was high. Thus, spawning activity did not start to peak until September (early spring), when salinities were rising markedly from their winter minima. The prevalence of spawning was positively correlated with tidal height and was greatest on days when the tide changed from flood to ebb at ca. 06:00 h, i.e., just after spawning had ceased. Because our estimate of the average daily prevalence of spawning by females during the spawning season (July to November) was 36.5%, individual females were estimated to spawn, on average, at intervals of about 2.7 days and thus about 45 times during that period. Therefore, because female *R. sarba* with total lengths of 180, 220, and 260 mm were estimated to have batch fecundities of about 4500, 7700, and 12,400 eggs, respectively, they had potential annual fecundities of about 204,300, 346,100 and 557,500 eggs, respectively. Because spawning occurs just prior to strong ebb tides, the eggs of *R. sarba* are likely to be transported out of the estuary into coastal waters where salinities remain at ca. 35‰. Such downstream transport would account for the fact that, although *R. sarba* exhibits substantial spawning activity in the lower Swan River Estuary, few of its early juveniles are recruited into the nearshore shallow waters of this estuary.

The goldlined seabream (*Rhabdosargus sarba*) is an important recreational and commercial fish species in numerous regions throughout the Indo-west Pacific (van der Elst, 1988; El-Agamy, 1989; Kuiter, 1993). Although this species is a protandrous hermaphrodite in certain regions, e.g., the waters of Hong Kong and South Africa (Yeung and Chan, 1987; Garratt, 1993), it is a rudimentary hermaphrodite in a range of environments in Western Australia (Hesp and Potter, 2003). Rudimentary hermaphrodites are those species in which the juveniles possess gonads consisting of both immature testicular and ovarian tissues that, in adults, develop permanently into either functional testes with rudimentary ovarian tissue or functional ovaries with rudimentary testicular tissue (Buxton and Garratt, 1990). In Western Australia, *R. sarba* attains similar maximum lengths, i.e., 346–370 mm, in temperate marine coastal waters and the lower reaches of the Swan River Estuary on the lower west coast of Australia and in a large subtropical embayment ca. 800 km farther north (Hesp et al., 2004). However, the maximum age recorded for this species in the estuary, 7 years, was far less than that for the other two environments: temperate marine coastal waters (11

years) and a large subtropical embayment (13 years) (Hesp et al., 2004).

Although *R. sarba* is typically regarded as a marine species that frequently uses estuaries as a nursery area (e.g., Wallace, 1975; Potter and Hyndes, 1999; Smith and Suthers, 2000), it spawns in the lower Swan River Estuary as well as in coastal waters outside this estuary (Hesp and Potter, 2003). However, this sparid attains maturity later in the estuary than in those nearby coastal marine waters. If this indication that the onset of spawning for *R. sarba* in the Swan River Estuary is related to the attainment of higher salinities in the spring, it would parallel the situation recorded for the spotted seatrout (*Cynoscion nebulosus*) in the estuaries of the Gulf of Mexico where this species completes its entire life cycle (Brown-Peterson et al., 2002).

Despite the importance and widespread occurrence of *R. sarba*, and the great value of fecundity data for stock assessments (Hunter et al., 1992; Nichol and Acuna, 2001), only one attempt has apparently been made to estimate the annual fecundity of wild populations of this sparid (El-Agamy, 1989). Although El-Agamy (1989) recognized that *R. sarba* is a "fractional" spawner and has a

protracted spawning season, he recorded the fecundity of this species as the number of larger eggs (diameter >180 μm) estimated to be present in the ovaries of mature females just prior to the commencement of the spawning period. Thus, the very strong possibility that some eggs with diameters <180 μm would have been destined to have become fully mature and released at some stage during the protracted spawning season, i.e., the species has indeterminate fecundity, was not taken into account.

In species with indeterminate fecundity, the distribution of oocyte diameters essentially forms a continuum, reflecting the continuous maturation of oocytes throughout the spawning season and thus the progression through to maturity of some of the small and previtellogenic oocytes that were present at the beginning of the spawning period. Consequently, counts of the standing stock of larger oocytes found just prior to the onset of spawning will result in an underestimate of the potential annual fecundity of such species (Hunter et al., 1985, 1992; Lisovenko and Andrianov, 1991). Estimation of the annual fecundity of species with indeterminate fecundity thus requires a combination of data on batch fecundity and spawning frequency (Hunter et al., 1985). Batch fecundity, i.e., the number of oocytes released during a single spawning event, can be estimated by counting the number of hydrated oocytes present in ovaries immediately prior to that spawning (Hunter et al., 1985). The frequency with which a fish spawns during the spawning period can be determined from the frequency of mature female fish possessing ovaries with either hydrated oocytes or postovulatory follicles (POFs) of a known age (Hunter and Macewicz, 1985).

The spawning of many marine species of teleosts and 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 or new moon (or both) (e.g., Johannes, 1978; Taylor and DiMichele, 1980; Greeley et al., 1986). Many fish and invertebrates with pelagic eggs spawn on high or ebb tides that enable eggs and the subsequent larval stages to be transported away from spawning areas, in which planktivorous predators are concentrated. This process thus reduces the likelihood of those early life cycle stages being subjected to predation (Taylor, 1984; Johnson et al., 1990; Morgan, 1990). The fact that there is very little recruitment of the early 0+ individuals of *R. sarba* into the lower Swan River Estuary, where extensive spawning occurs, indicates that tides transport the eggs of this species from spawning areas in the estuary into coastal marine waters (Hesp and Potter, 2003; Hesp et al., 2004).

This investigation, which involved a detailed study of the females of *R. sarba* in the lower Swan River Estuary, had the following aims: 1) to test the hypothesis that *R. sarba* has indeterminate fecundity; 2) to establish the period during the day when the oocytes of *R. sarba* become hydrated and when ovulation and spawning occur; 3) to establish whether *R. sarba* spawns mainly when salinities are high and thus approach those of the marine waters in which this species typically breeds and whether spawning is correlated with the strength and type (ebb vs flood) of tide in the lower reaches of the Swan River Estuary; 4) to estimate the average frequency of spawning for *R. sarba* during the spawning period; 5) and to determine the relationship between batch fecundity and fish length, and to use this relationship, in combination with the average spawning frequency, to calculate the potential annual fecundity of *R. sarba* of different sizes.

Materials and methods

Tide, lunar phase, and salinity

The maximum daily tidal heights at the mouth of the Swan River Estuary were calculated by 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 semidiurnal, depending on the time of year (Spencer, 1956). Salinity was measured on each sampling occasion by using a Yellow Springs Instruments salinity meter (YSI model number 30, Yellow Springs Instrument Co., Inc., Yellow Springs, OH).

Sampling

During 2001 and 2002, female *Rhabdosargus sarba* were collected by seine netting in nearshore shallow waters at distances of ca. 2.5 to 5 km from the mouth of the Swan River Estuary, and by rod and line fishing in water depths of 10–12 m at a distance of ca. 150 m from the shore (for details of sampling region and seine net, see Hesp and Potter, 2003). Sampling was undertaken at least once weekly between July and November, the period when *R. sarba* reach maturity in the lower Swan River Estuary (Hesp and Potter, 2003). It was restricted to the hours between dusk (ca. 18:00 h) and dawn (ca 06:00 h) because extensive seine netting and angling during the day in our earlier study failed to yield any *R. sarba*. The failure to capture *R. sarba* by these methods during daylight reflected the offshore movement of this species from the shallows prior to dawn and a far stronger targeting of bait by the large numbers of the banded toadfish (*Torquigener pleurogramma*) that feed in the offshore waters of the lower estuary during the day. Because the lower reaches of the Swan River Estuary act as a shipping harbor, alternative sampling methods, such as gill netting and spearing, could not be used to catch *R. sarba* during the day. The data for 2000 and 2001 were augmented by those derived from fish collected from the same location by using the same methods in 1998 and 1999 (Hesp and Potter, 2003). In total, the results of the present study are based on an examination of over 2000 *R. sarba*, of which 510 were

Table 1

Characteristics of each macroscopic stage in the development of the ovaries of *Rhabdosargus sarba*, and its corresponding histological characteristics. Adapted from Laevastu (1965). Terminology for oocyte stages follows Wallace and Selman (1989).

Stage	Macroscopic characteristics	Histological characteristics
I Virgin	Ovary is very small and strand-like.	*Rhabdosargus sarba* is a rudimentary hermaphrodite, *sensu* Hesp and Potter (2003). Thus, the gonads of small juveniles contain only connective tissue. Larger juveniles possess gonads (ovotestes) in which each ovarian lobe consists of an immature ovarian and testicular zone, separated by connective tissue. The ovotestes develop later into gonads containing almost entirely ovarian tissue (functional ovaries) or, in the case of males, gonads containing almost entirely testicular tissue (functional testes).
II Immature and resting	Small and transparent. Yellowish-orange in color. Oocytes not visible through ovarian wall.	Ovigerous lamellae highly organized. Chromatin nucleolar and perinucleolar oocytes dominate the complement of oocytes. Oogonia sometimes present. Chromatin nucleolar oocytes present in all subsequent ovarian stages.
III Developing	Slightly larger than stage II. Reddish color. Oocytes visible through ovarian wall.	Chromatin nucleolar, perinucleolar and cortical alveolar oocytes present.
IV Maturing	Larger than stage III. Reddish-orange in color. Yolk granule oocytes visible through ovarian wall.	Cortical alveolar and yolk granule oocytes abundant.
V Mature	Larger than stage IV, occupying half to two thirds of body cavity. Extensive capillaries visible in ovarian wall.	Yolk granule oocytes predominant.
VI Spawning	Hydrated oocytes visible through ovarian wall. Note that fish with ovaries in "spawning condition" can only be detected macroscopically when caught during the hydration period.	Migratory nucleus oocytes, hydrated oocytes, or postovulatory follicles present.
VII Spent	Smaller than V and VI and flaccid. Some yolk granule oocytes visible through ovarian wall.	Some remnant yolk granule oocytes present, all or almost all of which are typically undergoing atresia.
VIII Spent and recovering	Small and dark red.	Extensive scar tissue present. Ovarian lamellae becoming reorganized. No yolk granule oocytes present.

females with stage-V (mature) or stage-VI (spawning) ovaries (see Table 1 for definitions of these stages).

During the above sampling, *R. sarba* was collected for up to 2 hours at intervals commencing at 18:30, 21:30, 00:30, and 03:30 h on 1–2 September 2001 and for up to 2 hours at intervals commencing at 18:30 and 22:30 h on 13 September 2001. One of the ovarian lobes of up to five fish caught during each of these above time intervals was cut into several pieces, preserved in 10% neutrally buffered formalin solution and used for determining the distributions of oocyte diameters at the above different times. The other lobe was used for histology to determine the oocyte stages present in that lobe, and thus, by extrapolation, also the stages of the oocytes in the lobe that had been preserved in formalin. The resultant comparisons were used, in conjunction with data from other times, to elucidate the pattern of oocyte development during hydration and the duration of hydration and timing of ovulation.

Gonadal staging and histology of ovaries

The sex, total length (to the nearest 1 mm), and total weight and gonad weight (to the nearest 0.01 g) of each fish were recorded. From 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 and resting, III = developing, IV = maturing, V = mature, VI = spawning, VII = spent, VIII = spent and recovering. The corresponding histological characteristics of each macroscopic stage are shown in Table 1. When hydrated oocytes could be seen through the ovarian wall of a fish, a note was made as to whether they were distributed throughout the ovary or were in

the ovarian duct and thus whether or not ovulation had commenced at the time of capture of that fish.

For all histological studies of the gonads, part of the mid region of one of the ovarian lobes was placed in Bouin's fixative for ca. 48 hours, dehydrated in a series of ethanols, embedded in paraffin wax, cut into 6-μm sections, and stained with Mallory's trichrome. The ovaries were fixed within 1–3 hours of capture of the fish.

To test the hypothesis that *R. sarba* has indeterminate fecundity, the diameters of 100 oocytes in histological sections of stage-VI ovaries of two fish caught during the spawning period were measured to the nearest 10 μm by using an eyepiece graticule in a compound microscope and the stage of each of those oocytes was recorded. Measurements were restricted to oocytes in which a nucleus was visible in their center to ensure that the oocytes had been sectioned through their center and that the diameters were thus measured accurately. This approach could not be used to measure the oocyte diameters of hydrated oocytes in histological sections because the nucleus of these oocytes undergoes germinal vesicle breakdown.

Histological sections of numerous ovaries were used to determine the timing of the formation and degeneration of postovulatory follicles (POFs). 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 and Goldberg, 1980; Hunter and Macewicz, 1985). Histological sections were also used to determine the relative abundance of the different stages of atresia in ovaries at different times during the spawning period.

The jars containing the ovarian lobes that had been preserved in formalin at the different time intervals on 1, 2, and 13 September 2001 (see earlier) were shaken until the oocytes of each ovary had become evenly suspended in the solution. The resultant solution from each ovary was then passed through a 125-μm sieve to remove the smallest oocytes, and we were able thus to focus our study more specifically on the vitellogenic oocytes. Comparisons of the appearance of the larger oocytes under a dissecting microscope with those of the different oocyte stages in histological sections of the other ovarian lobe of the same fish were used to allocate the oocytes observed under the dissecting microscope to a specific stage in oocyte development. Each oocyte in a representative subsample of 100 oocytes from each formalin-preserved ovarian lobe was measured under a dissecting microscope with an eyepiece graticule. This approach enabled the diameters of hydrated oocytes to be measured accurately, which was not possible with histological sections (see earlier).

Categorization of stages in atresia, fecundity estimates, and spawning frequency

On the basis of their histological characteristics, atretic oocytes were allocated to either the α or β stages, by using the criteria of Hunter and Macewicz (1985). Mature

ovaries were categorized according to the proportions of their α and β atretic oocytes (Hunter and Macewicz, 1985). Thus, atretic state 0 = ovaries with yolked oocytes but no α atretic oocytes; atretic state 1 = ovaries in which less than 50% of the yolked oocytes are in the α stage of atresia; atretic state 2 = ovaries in which less than 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 (36–50%) atretic state 1, an approach similar to that adopted by Farley and Davis (1998).
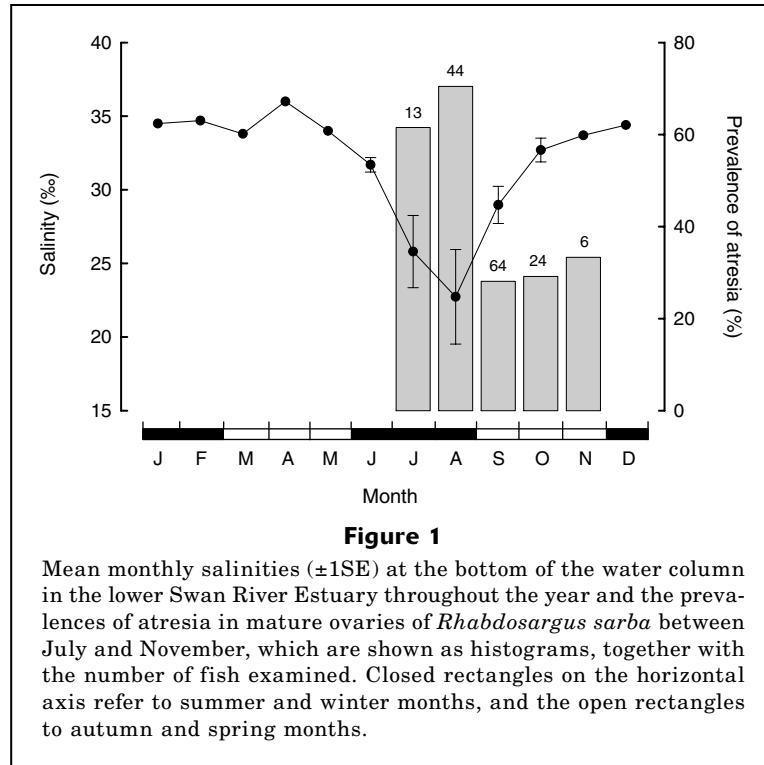
The batch fecundities of 31 *R. sarba* were estimated from the number of hydrated oocytes in one of the ovarian lobes of fish that had been preserved in 10% neutrally buffered formalin. These fish were chosen because histological examination of their other ovarian lobe demonstrated that the ovaries were in atretic state 0 or early state 1, i.e., less than 10% of their yolk granule oocytes were atretic and newly formed POFs were not present (Hunter et al., 1992; Nichol and Acuna, 2001). The formalin-preserved 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 1 mg. 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 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 ovarian lobes, to estimate the total number of hydrated oocytes (=batch fecundity) that would have been present in the pair of ovarian lobes of each fish. The prevalence of spawning on any given night is expressed as the percentage of female fish with hydrated eggs (ovarian stage VI) among all female fish with stage-V (mature) and stage-VI (spawning) ovaries. These estimates were based on an examination of samples collected between 22:00 and 01:30 h, when it was possible to determine which female fish were going to spawn in the ensuing few hours (see Hunter et al., 1985, for further details of this method).

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. 1).

Staging of the ovaries and confirmation of indeterminate fecundity

The characteristics of each macroscopic stage of the ovaries of *R. sarba* and the corresponding histologi-

Figure 1

Mean monthly salinities (±1SE) at the bottom of the water column in the lower Swan River Estuary throughout the year and the prevalences of atresia in mature ovaries of *Rhabdosargus sarba* between July and November, which are shown as histograms, together with the number of fish examined. Closed rectangles on the horizontal axis refer to summer and winter months, and the open rectangles to autumn and spring months.

cal characteristics are presented in Table 1. Because stages V and VI could be distinguished macroscopically only during the period of oocyte hydration, the macroscopic data for these two stages had to be combined for other times. The diameters of the oocytes in histological sections of an ovarian lobe from each of two mature female *R. sarba* caught during the spawning season—oocyte diameters that were typical of those from mature *R. sarba* during this period—formed an essentially continuous distribution (Fig. 2). This distribution reflected the presence of oocytes at all stages in development from chromatin nucleolar oocytes to yolk granule oocytes and demonstrated that *R. sarba* has indeterminate fecundity *sensu* Hunter et al. (1985). Thus, the potential annual fecundity is not fixed prior to the commencement of the spawning period and consequently the potential annual fecundity of *R. sarba* has to be estimated by using a combination of batch fecundity and spawning frequency.

Period of hydration and spawning

The diameters of oocytes in ovaries of fish collected at intervals on 1–2 September 2001 and 13 September 2001 and which had been retained on the 125-μm sieve, produced a modal class that, for each time interval, fell between 420 and 600 μm (Fig. 3). At ca. 18:30 h on 1 September 2001, the oocyte diameters formed a single mode, and the vast majority of oocytes were less than 720 μm and produced a modal class at 420–539 μm (Fig. 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 beginning to become bimodal, with modal classes at 480–539 and 780–839 μm. By 00:30 h on 2 September, the oocyte diameter distributions had become markedly bimodal, and the modal diameter class of the largest oocytes at this time, and also at 03:30 h, lay between 840 and 959 μm (Fig. 3). The oocyte diameter frequencies on 13 September were essentially the same as those at similar times on 1 September; the distributions were unimodal at 18:30 h and bimodal at 22:30 h (Fig. 3). The oocyte diameters of each fish within a given time slot on 1, 2, and 13 September exhibited essentially the same distribution.

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 the nucleus was migrating towards the edge of the cytoplasm and a conspicuous lipid droplet was present in the cytoplasm (Fig. 4A). However, it was difficult at this time to distinguish migratory nucleus oocytes from yolk granule oocytes under a dissecting microscope (Fig. 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. 4C). Their relatively larger size, translucent appearance, and one's 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 earlier in the evening (cf. Fig. 4, B 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. 4E). Under the dissecting microscope, these hydrated oocytes were of similar appearance to the corresponding oocytes at 21:30 h (Fig. 4F). Although mature fish with ovaries containing the above stages in oocyte hydration were frequently found in nearshore shallow waters, the numbers 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, fish with ovaries containing newly formed POFs were caught in offshore deeper waters.

Histological examination demonstrated that, when hydrated oocytes were present in the ovarian duct, the ovary contained recently formed POFs, which are formed by the thecal and granulosa layers of the oocytes that surround the zona radiata externa (Fig. 5A). Newly formed POFs (0–6 h old) possess a conspicuous lumen and their granulosa cells contain prominent darkly stained nuclei (Fig. 5B). These newly formed postovulatory follicles 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 of two morphological forms. The first and least degenerate form was less well organized than newly formed POFs and its nuclei were becoming pycnotic (Fig. 5C); the second form was smaller and highly degenerate and its nuclei had become far less visible or undetectable (Fig. 5D). The least degenerate of the two forms of POFs in ovaries of fish caught at ca. 22:00 and 01:00 h (Fig. 5, D and E) represents stages in degeneracy that are intermediate between those of the two different forms described above for the ovaries of fish caught at 18:30 h. These POFs were thus compact and, although some of their nuclei were still detectable, they were markedly pycnotic.

Influence of salinity and tides on spawning

Both α and β atretic oocytes were frequently observed in the ovaries of *R. sarba*. The chorion (zona radiata) of the early α atretic vitellogenic oocyte was distorted, fragmented, and had moved inwards (Fig 6A). By the β atretic stage, the yolk and lipid had been resorbed and a large proportion of the oocyte volume was occupied by vacuoles (Fig. 6B).

Sixty-two percent and 72% of the stage-V and stage-VI ovaries sectioned in July and August, respectively, were at mid or late atretic state 1, i.e., 11–50% of their yolk granule oocytes were α atretic (Fig. 1). However, the prevalence of these mid-late state-1 ovaries declined precipitously to 28% in September, as salinities rose markedly, and remained at a similar level until the end of spawning in late November.

Histological sections showed that, in July and August, only 39% of the 57 pairs of ovarian lobes of *R. sarba* that were macroscopically assigned as stage V and stage VI contained migratory nucleus oocytes, hydrated
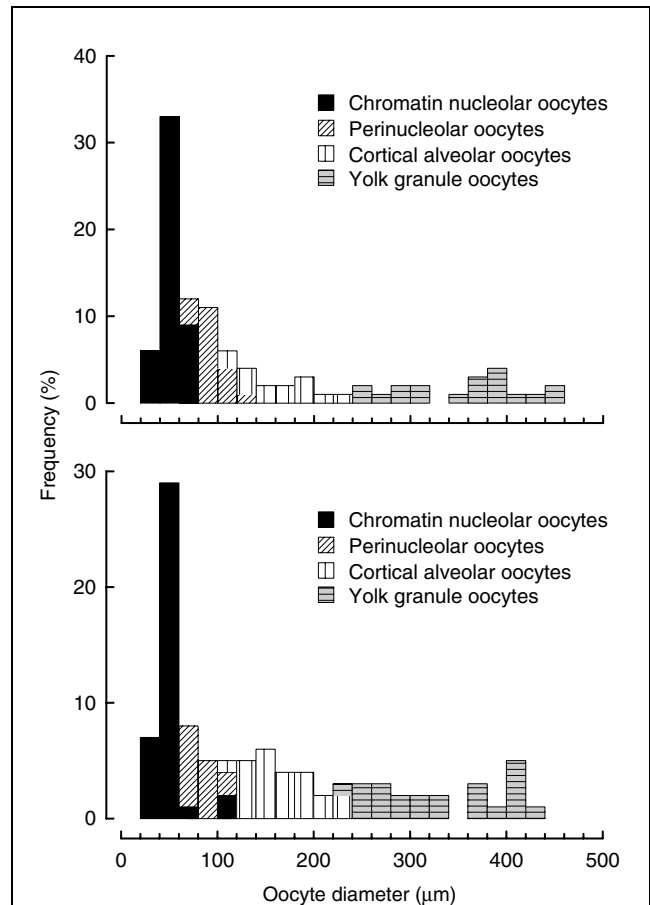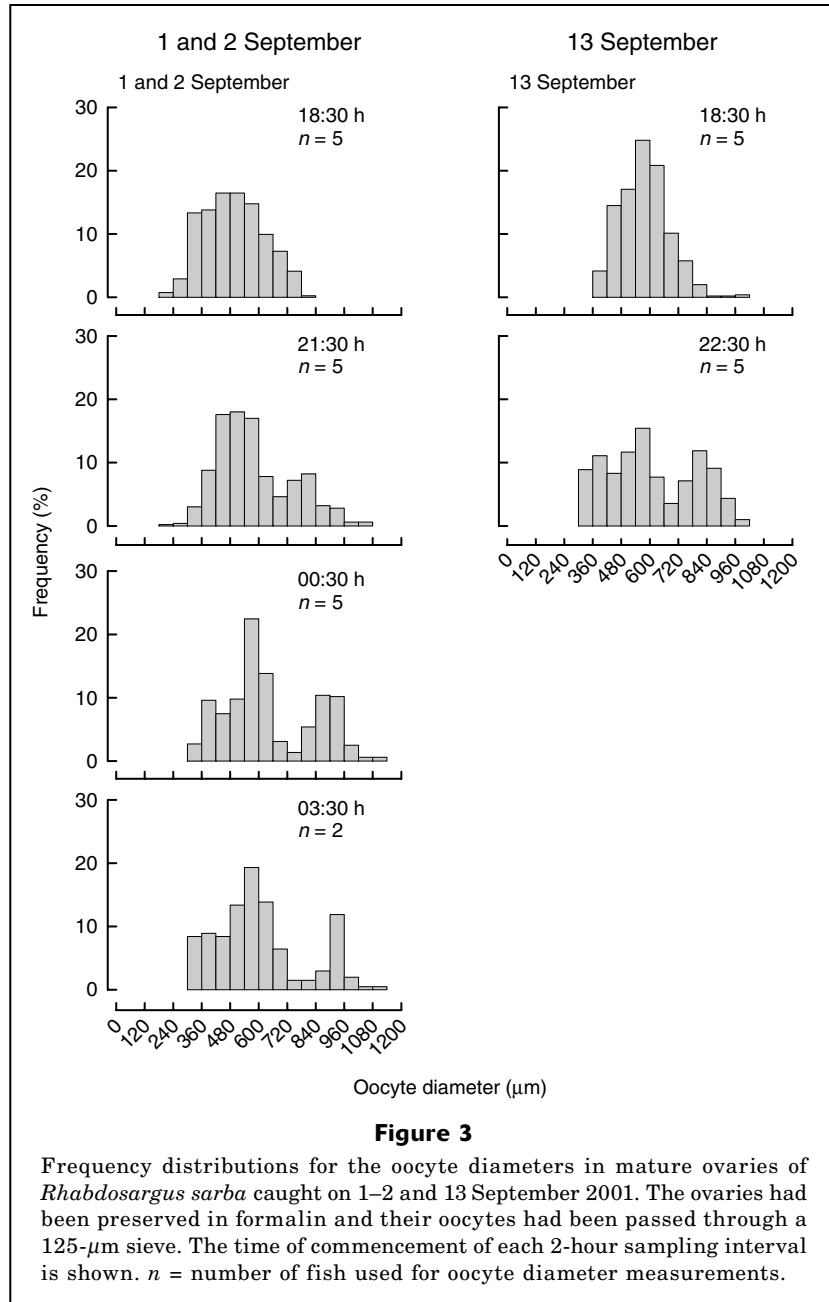

Figure 2

Percent frequency distributions for the oocyte diameters of different oocyte stages in histological sections of stage-VI ovaries of two female *Rhabdosargus sarba*.

oocytes, or POFs, i.e., were at stage VI. However, in the following two months, 76% of the 88 pairs of ovarian lobes of *R. sarba*, that were macroscopically assigned as stage V or stage VI, were shown by histology to be at stage VI.

During September, when spawning activity was greatest, the prevalence of spawning (*PS*) was positively correlated (*P*<0.05) with maximum daily tidal height (*T*). $PS = 91.72T + 20.73$ (r^2=0.46, number of sampling occasions=10) (Fig. 7A).

Data for the same days as those used to provide the points shown in Fig. 7A demonstrated that the prevalence of spawning (*PS*) is inversely correlated with the difference in hours between the time when spawning is believed to cease (ca. 06:00 h, see later) and the time of high tide. $PS = -8.26(T) + 78.22$ (r^2=0.49, number of sampling occasions=10) (Fig. 7B). Thus, 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 when *R. sarba* is considered to cease spawning.

Figure 3

Frequency distributions for the oocyte diameters in mature ovaries of *Rhabdosargus sarba* caught on 1–2 and 13 September 2001. The ovaries had been preserved in formalin and their oocytes had been passed through a 125-μm sieve. The time of commencement of each 2-hour sampling interval is shown. n = number of fish used for oocyte diameter measurements.

Batch fecundity, spawning frequency, and potential annual fecundity

The relationship between batch fecundity (BF) and total length (TL) shown in Figure 8, and between batch fecundity and somatic weight (W) are described by the following equations:
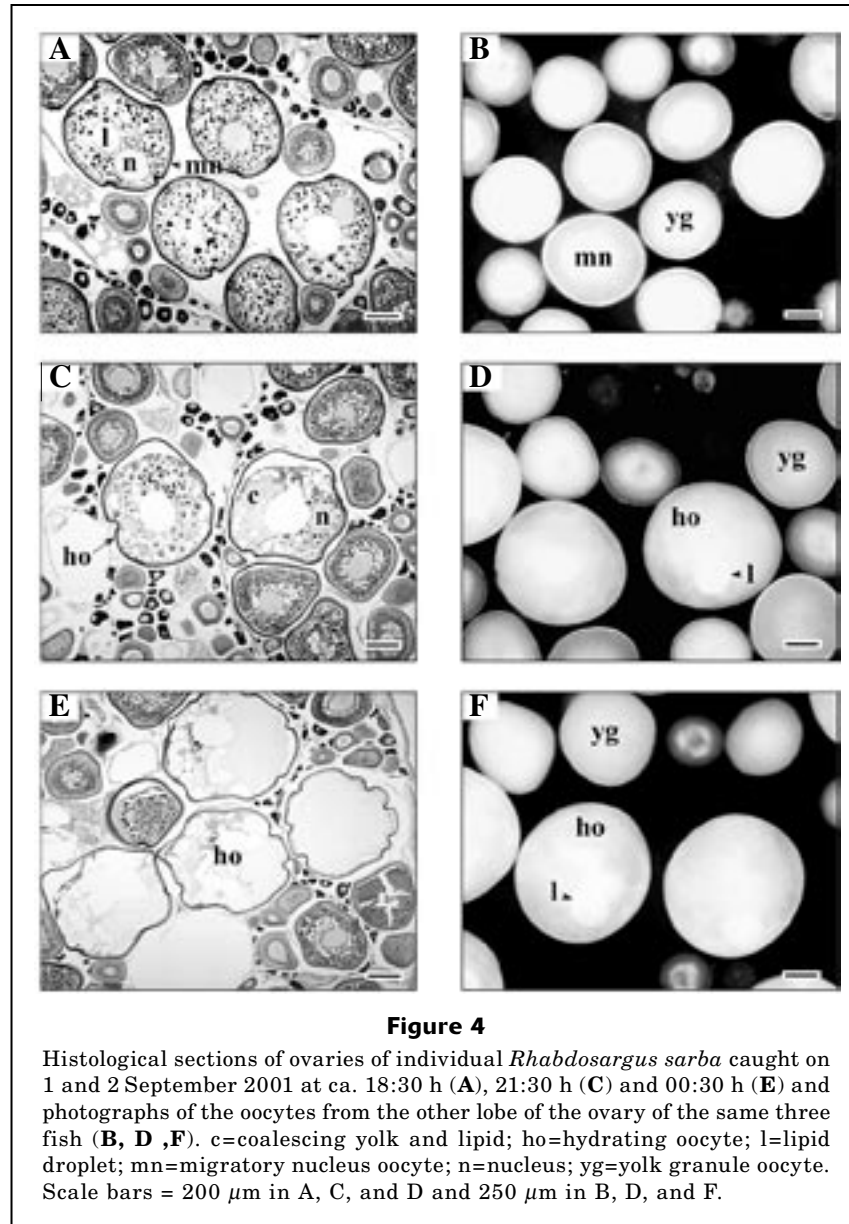
$$\ln BF = 5.0025\ln TL - 17.557$$
$$(P<0.001; r^2=0.52, n=30),$$
$$BF = 1997e^{0.0105W}$$
$$(P<0.001; r^2=0.55, n=30).$$

The batch fecundities of *R. sarba*, predicted by the above equations for fish with lengths of 180, 220, and 260 mm, were ca. 4500, 7700, and 12,400, eggs, respectively, and for fish with somatic weights of 100, 150, and 200 g were ca. 5700, 9600, and 16,300 eggs, respectively. The average daily prevalence of spawning during the spawning period was 36.5%. Thus, during this period, individual females spawned, on average, once every 2.7 days and therefore about 45 times during the spawning season. The potential annual fecundities of female *R. sarba* with lengths of 180, 220, and 260 mm were thus estimated to be ca. 204,300, 346,100, and 557,500 eggs, respectively.
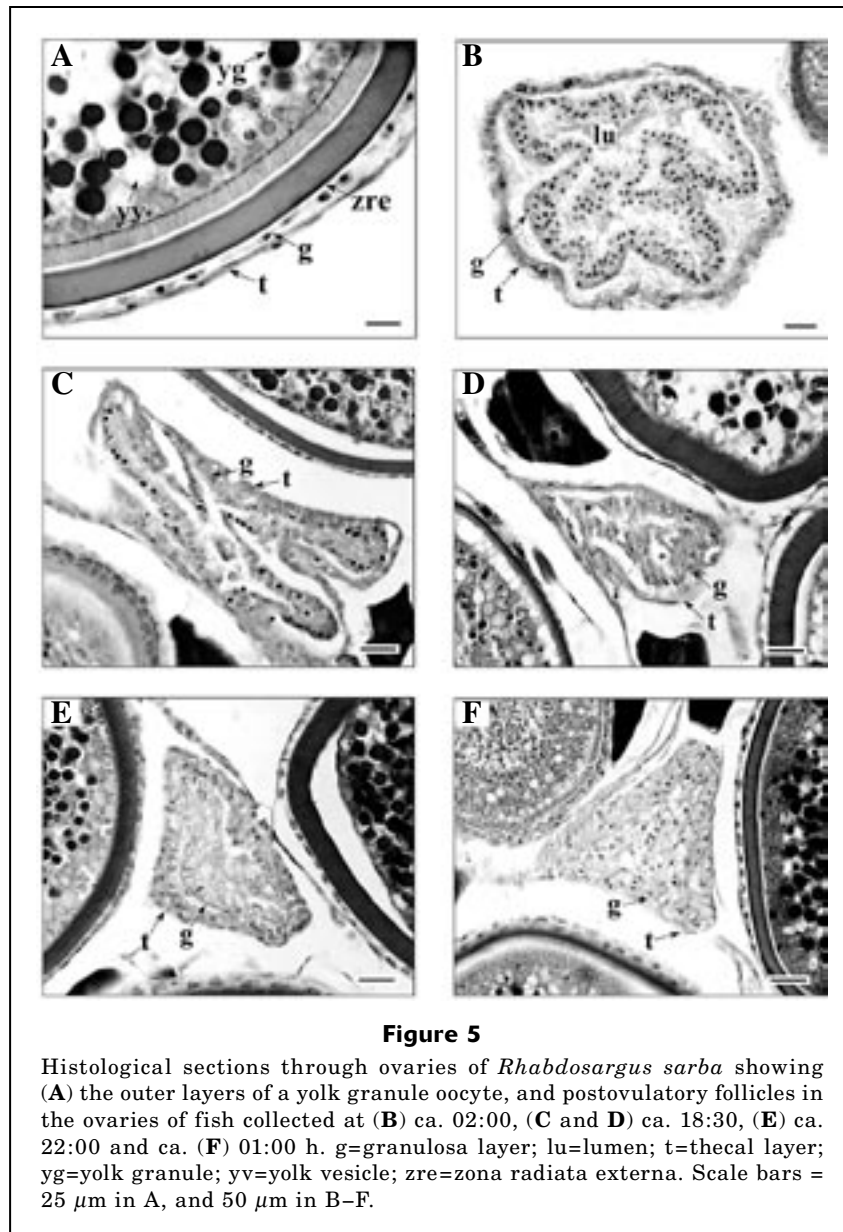
Figure 4

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 from 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 in A, C, and D and 250 μm in B, D, and F.

Discussion

Oocyte hydration, ovulation, and spawning periods

Because histological studies showed that the ovaries of numerous fish caught on different occasions between 18:30 and 20:30 h did not contain recently formed POFs, we deduced that these fish had not spawned in the previous few hours. However, at this time, the ovaries of many fish, that were designated macroscopically as at stage V and stage VI, often contained numerous migratory nucleus-stage oocytes and, towards the end of this period, often a few oocytes in the early stages of hydration. Although the frequency distributions of the oocyte diameters of fish examined on both 1 and 13 Sep-

tember were still unimodal at 18:30 to 20:30 h, they had become bimodal by 21:30 to 23:30 h (Fig. 3), reflecting the fact that, by this time, numerous oocytes had become markedly enlarged through hydration. The above data demonstrate that hydration typically commences soon after dusk. Furthermore, because a number of *R. sarba* caught between 01:30 and 04:30 h, and particularly towards the end of this time interval, contained ovaries undergoing ovulation and had newly formed POFs, the period between the onset of hydration and commencement of ovulation typically lasts about 7–10 hours, which is very similar to the duration estimated for species such as the black sea anchovy (*Engraulis encrasicholus*) (Lisovenko and Andrianov, 1991) and ballyhoo (*Hemiramphus brasiliensis*) (McBride et al., 2003). Although we
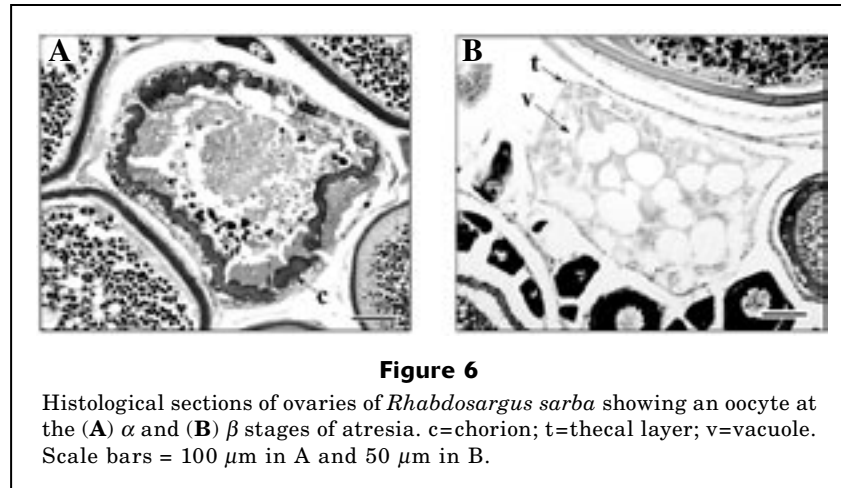
Figure 5

Histological sections through ovaries of *Rhabdosargus sarba* showing (**A**) the outer layers of a yolk granule oocyte, and postovulatory follicles in the ovaries of fish collected at (**B**) ca. 02:00, (**C** and **D**) ca. 18:30, (**E**) ca. 22:00 and ca. (**F**) 01:00 h. g=granulosa layer; lu=lumen; t=thecal layer; yg=yolk granule; yv=yolk vesicle; zre=zona radiata externa. Scale bars = 25 μm in A, and 50 μm in B–F.

caught several *R. sarba* with new POFs in their ovaries and hydrated oocytes in their oviducts, we were able to catch only one individual of this species in which the ovaries possessed new POFs and no hydrated oocytes. The latter fish, which had clearly just completed spawning, was caught between 05:00 and 06:00 h.

Several species are known typically to complete spawning in the 10–14 hours after the time when their oocytes commence hydration, e.g., the northern anchovy (*Engraulis mordax*) (Hunter and Macewicz, 1985), the spotted seatrout (*Cynoscion nebulosus*) (Brown-Peterson et al., 1988) and the horse mackerel (*Trachurus trachurus*) (Karlou-Riga and Economidis, 1997). Furthermore, spawning is typically completed 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 encrasicholus*) (Lisovenko and Andrianov, 1991) and the weakfish (*Cynoscion regalis*) (Taylor and Villoso, 1994). These consistent data, when considered in conjunction with the similar duration of hydration of *R. sarba*, and the capture of a very recently spawned fish between 05:00 and 06:00 h, provide very strong circumstantial evidence that, in the lower Swan River Estuary, *R. sarba* spawns mainly between 02:00 and 06:00 h.

The newest POFs in the ovaries of *R. sarba* caught at dusk, i.e., at 18:30 h, had degenerated to an extent similar to those of ca. 12-h-old POFs in the ovaries of other species, e.g. the skipjack tuna (*Katsuwonus pelamis*) (Hunter et al., 1986) and the whitemouth croaker

Figure 6

Histological sections of ovaries of *Rhabdosargus sarba* showing an oocyte at the (**A**) α and (**B**) β stages of atresia. c=chorion; t=thecal layer; v=vacuole. Scale bars = 100 μm in A and 50 μm in B.

(*Micropogonias furnieri*) (Macchi et al., 2003). This finding provides further evidence that *R. sarba* spawns close to dawn. Certainly, the state of degeneration of the newest POFs in mature ovaries of *R. sarba* at dusk provides very strong circumstantial evidence that spawning could not have occurred during at least most of the previous daylight hours.

Our results demonstrate that the prevalence of fish with ovaries at mid to late atretic state 1 declined precipitously as salinities increased from their winter minima in July and August and that this decrease was accompanied by an increase in the prevalence of migratory nucleus oocytes, hydrated oocytes or POFs. The implication that the oocytes of *R. sarba* are often inhibited from undergoing final oocyte maturation when salinities are low parallels the conclusions drawn for the influence of salinity on the gonadal development of *Cynoscion nebulosus* in estuaries entering the Gulf of Mexico (Brown-Peterson et al., 2002). The resorption of yolk granule oocytes by the ovaries of *R. sarba* in July and August would help conserve energy at a time when, if those oocytes progressed through to final maturation and were released, they would be exposed to salinities that are known to be lower than those required for optimal development (Mihelkakis and Kitajima, 1994).
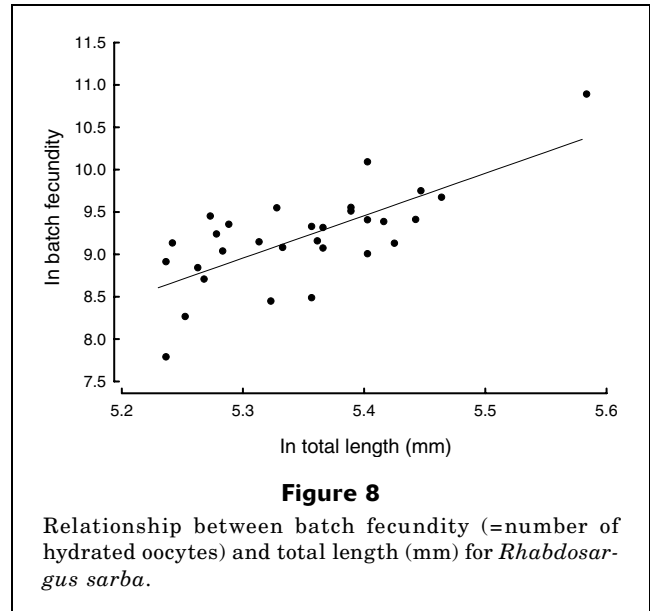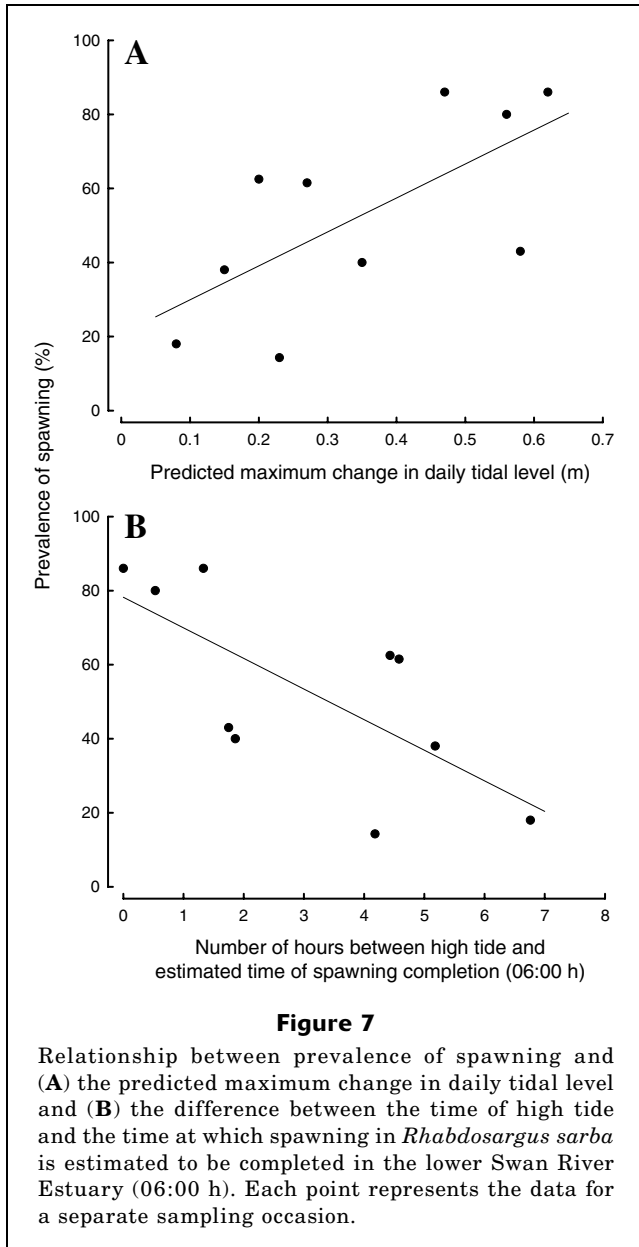
Frequency of spawning

Because ovulation lasts for ca. 2–5 hours, the POFs that were present in ovulating ovaries and that showed no detectable signs of degeneration were presumably <3 hours old. It then follows that, when POFs at intermediate and advanced stages of degeneration were present in those same ovaries, those POFs were presumably ca. 24 and ca. 48 hours old, respectively. Thus, the presence of these three very distinct forms of POFs in the same ovary of a fish implies that individual *R. sarba* are capable of spawning on at least three successive days during that part of the month when spawning activity is greatest.

The estimated average frequency of spawning by *R. sarba*, i.e., once every 2.7 days, is essentially the same as that recorded for several other species, including e.g., spotted seatrout (*Cynoscion nebulosus*) (Brown-Peterson et al., 1988, 2002), red drum (*Sciaenops ocellatus*) (Wilson and Nieland, 1994), and common snook (*Centropomus undecimalis*) (Taylor et al., 1998). The resultant conclusion that *R. sarba* spawns about 45 times during a spawning period is comparable to that estimated for black sea anchovy (*Engraulis encrasicholus*) (Lisovenko and Andrionov, 1991) and cobia (*Rachycentron canadum*) (Brown-Peterson et al., 2001). However, spawning frequency does vary markedly among species.

Relationship between spawning time and tidal cycle

Although seine netting between 00:30 and 05:30 h on a number of days yielded no female *R. sarba* with newly formed POFs, rod-and-line angling in deeper water between 01:30 and 04:30 h yielded several females in which the ovaries contained both newly formed POFs and concentrations of hydrated oocytes in their oviducts, and also running ripe males. This finding provides strong circumstantial evidence that, just prior to ovulation, *R. sarba* moves from nearshore shallows to offshore deeper waters.

Because *R. sarba* typically spawns just prior to the commencement of a relatively strong ebb tide, the fertilized eggs would likely be transported downstream and out of the estuary. The conclusion that eggs are swept out of the estuary is supported by the fact that only 15 larvae of *R. sarba* were caught during extensive sampling of the lower Swan River Estuary and that virtually all of these larvae were caught at its mouth (Gaughan et al., 1990). A downstream movement of eggs would be further facilitated by *R. sarba* spawning in deeper waters, where the current is greatest. Emigration of eggs from the estuary would enhance the chances of survival of the eggs of this essentially marine species by ensuring that they would develop in

Figure 7

Relationship between prevalence of spawning and (**A**) the predicted maximum change in daily tidal level and (**B**) the difference between the time of high tide and the time at which spawning in *Rhabdosargus sarba* is estimated to be completed in the lower Swan River Estuary (06:00 h). Each point represents the data for a separate sampling occasion.

Figure 8

Relationship between batch fecundity (=number of hydrated oocytes) and total length (mm) for *Rhabdosargus sarba*.

Spratelloides robustus was particularly numerous in some of our seine-net catches, a movement of the eggs of *R. sarba* out of the estuary would also enhance their chances of avoiding predation by that species.

A downstream transport of eggs would account for the relatively few young 0+ juveniles that are recruited into the nearshore shallow waters of the estuary (Hesp et al., 2004). Indeed, substantial recruitment into these nearshore waters, presumably as a result of immigration from coastal marine waters, does not occur until *R. sarba* is about one year old and about 140 mm in length (Hesp et al., 2004). Because *R. sarba* settles at a length of ca. 12 mm (Hesp et al., 2004) and ca. 30 days of age (Neira[1]), this immigration back into the estuary does not occur until 11 months after settlement. In contrast to the situation in the Swan River Estuary, *R. sarba* elsewhere typically spawns in marine waters and their larvae often enter estuaries on flood tides (e.g., Miskiewicz, 1986; Neira and Potter, 1992).

Potential annual fecundity

The estimates of potential annual fecundity derived for *R. sarba* during the present study, which ranged from 109,000 to 2,417,000 eggs for fishes of 188 and 266 mm total length, respectively, greatly exceed those of El-Agamy (1989), which ranged from 23,000 to 99,000 eggs for fishes of 170 and 260 mm total length, respectively. However, because El-Agamy (1989) based his estimates on the number of large oocytes present in the ovaries of individual *R. sarba*, he did not take into account the fact

a marine environment in which salinity remained constantly at ca. 35‰, rather than in one in which sudden rainfall could result in sudden marked declines in salinity. However, the possession of spawning cycles linked to lunar and tidal periodicities can reduce the likelihood of predation (Taylor, 1984). For example, Johannes (1978) pointed out that, because the spawning of 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 consequently the likelihood of predation during the early stages of life would be reduced. Because planktivorous fishes are abundant in estuaries (Johnson et al., 1990; Morgan, 1990), including the Swan River Estuary where the planktivorous

that this species has indeterminate fecundity. Thus, the values recorded for the annual fecundity of *R. sarba* in the Arabian Gulf almost certainly represent a marked underestimate of the true annual fecundity of this species in that region.

Acknowledgments

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