Screening of the white margined sole, *Synaptura marginata* (Soleidae), as a candidate for aquaculture in South Africa

THESIS

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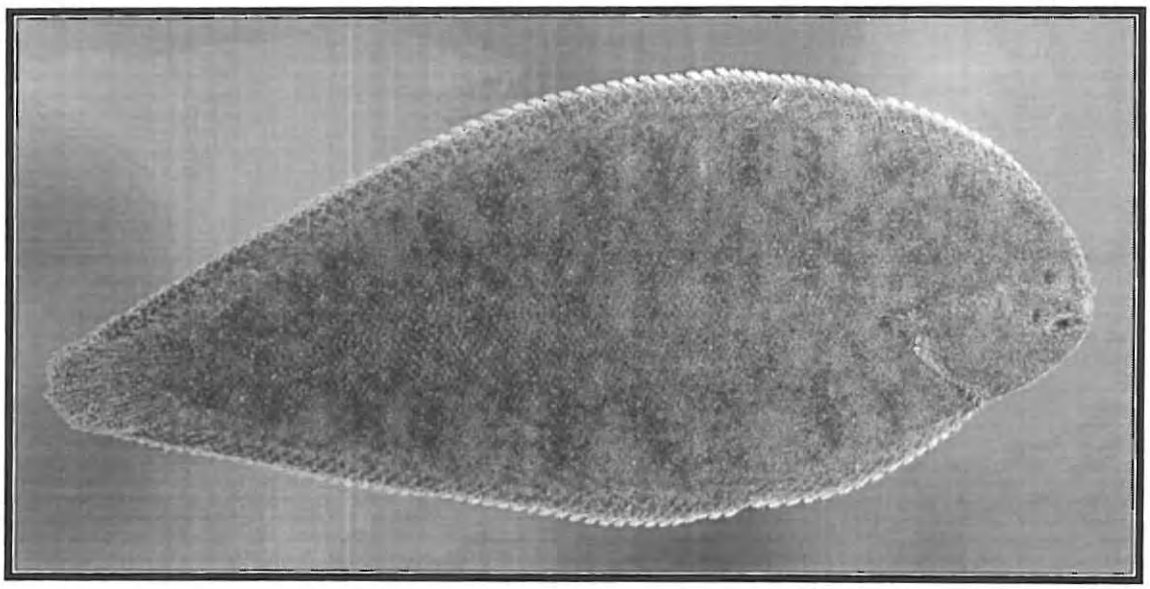

The white-margined sole, *Synaptura marginata* (Boulenger, 1900)(Soleidae), 300 mm TL (Kleinemonde). *Photograph: James Stapley*

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Screening of the white margined sole, *Synaptura marginata* (Soleidae), as a candidate for aquaculture in South Africa

The white margined sole *Synaptura marginata* (Soleidae) was isolated as the most likely candidate for flatfish aquaculture in South Africa. The aim of the study was to screen the sole as a candidate aquaculture species by way of a comprehensive study of its biology and life history strategy and to identify possible "bottlenecks". The study was undertaken on the assumption that the biological data would provide valuable information for developing specific technologies that might be required for the farming of this species. Specimens were collected monthly by spearing along the Eastern Cape coast of South Africa between December 2000 and March 2002. Length-at-age data required for modeling the growth of *S. marginata* was obtained from sectioned otoliths. A Von Bertalanffy growth model with an absolute error structure best describes the growth for this species. The model parameters were: L_∞ = 429.5 mm TL, K = 0.24 and t_0 = -1.79 years. Analysis of gut contents showed that *S. marginata* feed exclusively on polychaete worms, mainly of the genus *Morphysa*. *S. marginata* shows a protracted summer spawning season of six months, from October to April. This was determined by the calculation of a monthly gonadosomatic index and a macroscopic maturity scale. Histological examination of the ovaries revealed five ovarian developmental stages. Size at 50% and 100% sexual maturity for females was calculated to be 235 mm TL and 300 mm TL (ca. 1.5 – 2.5 years of age) and all males > 154 mm TL were mature. *S. marginata* is a batch spawner, releasing a minimum of 3 batches of eggs per year. Relative fecundity is high (34000 eggs per year / kg) and this coupled with the protracted spawning season would make it possible to obtain adequate numbers of juveniles (for farming) for approximately five to six months of the year. Comparative analysis of the biological characteristics in relation to other soles farmed elsewhere in the world suggests that *S. marginata* is a suitable candidate for marine fish culture in South Africa.

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Chapter 1: Introduction

The need for diversification in aquaculture has led to a marked increase in the number of studies focussing on new fish species (Quéméner *et al.* 2002). Selecting and studying new fish species suitable for aquaculture is pivotal for the successful and rapid development of the industry (Paquotte 1998, New 1999, Quéméner *et al.* 2002).

The high cost of developing specific technologies and protocols for the farming of a particular species and the cost of production, requires careful consideration with regards to the selection of a species from a biological and zootechnical perspective (Quéméner *et al.* 2002). It is of course also crucial that there be a consumer demand for the selected species and that factors affecting the supply and demand of the specific market are understood (Reig *et al.* 2000). Aquaculture ventures involving marine finfish usually target species with a high market value that are relatively easy to domesticate and rear (Paquotte 1998, Reig *et al.* 2000, Tait & Hickman 2001, Le François *et al.* 2002).

Flatfish (Pleuronectiformes) have consistently attracted high prices on the European market (Howell 1997, Brown 2002) and are generally considered to be well adapted for intensive culture conditions (Slaski 1999 a & b). This makes it a favourable group for aquaculture, as is evident in the strong representation of flatfish species on the list of "new species for aquaculture" (Brown 2002).

Currently flatfish aquaculture is dominated by the production of around 14 127 MT / year and 7 075 MT / year of Bastard halibut (*Paralichthys olivaceus*)[1] in Korea and Japan respectively, and 5 000 MT / year of turbot (*Scophthalmus maximus*), mainly in Spain, Portugal and France (FAO 2002). Other farmed flatfish species of commercial importance include Atlantic halibut (*Hippoglossus hippoglossus*) in Iceland (35 MT / year), sole (*Solea solea, S. senegalensis*) in Spain and Portugal (23 MT / year), and various flounder species, e.g.

1) See appendix A for Scientific Authorities

Paralichthys dentatus in North America (Brown 2002, FAO 2002). Chile is also rapidly becoming one of the important producer countries. Currently, some 200 MT of flatfish, predominantly turbot (*Scophthalmus maximus)*, is produced there per year (Alvial & Manríquez 1999, FAO 2002). A small experimental turbot (*Scophthalmus maximus*) farm exists in South Africa that produces around 12 MT / year (C. Viljoen, Jacobsbaai Sea Products, *pers. comm.*). According to Reig *et al.* (2000) the largest European market for turbot and sole is in Spain.

Various other flatfish species with aquaculture potential are being investigated worldwide. Many of these species have been successfully reared through their larval stages, and include the winter flounder, *Pleuronectes americanus* (Litvak 1994, 1996) in Canada, the southern flounder, *Paralichthys lethostigma* (Luckenbach *et al.* 2003) in North America, the Caribbean flounder, *Paralichthys tropicus* (Rosas *et al.* 1999), the Pacific halibut, *Hippoglossus stenolepis* (Stickney & Liu 1999) in British Columbia, the New Zealand turbot, *Colistium nudipinnis* and brill, *C. guntheri* (Tait & Hickman 2001) and the greenback flounder, *Rhombosolea tapirina* (Barnett & Pankhurst 1998) in Tasmania.

There are several biological factors that currently restrict the production of noteworthy quantities of soleid species worldwide (e.g. *Solea solea* and *Solea senegalensis*) (Reig *et al.* 2000, Gavaia *et al.* 2002). These include malpigmentation, skeletal deformities, incomplete eye migration and long grow-out periods. Furthermore, these problems usually only manifest themselves during and after metamorphosis (Pittman *et al.* 1998, Gavaia *et al.* 2002). The causes are often related to diet during early larval development, although it appears that these can be mitigated against by proper diet formulation (Solbakken *et al.* 1999, Bolker & Hill 2002, Sæle *et al.* 2002). Similar problems have been experienced with Atlantic halibut, *Hippoglossus hippoglossus* (Pittman *et al.* 1998, Sæle *et al.* 2002), olive flounder, *Paralichthys olivaceus* (Seikai 1985) and turbot, *Scophthalmus maximus* (Shields 2001). Gavaia *et al.* (2002) summarise other possible causes of the above problems, the principal one of

which appears to be unfavourable abiotic conditions (Faustino & Power 1999). Extensive research has shown that these problems can be reduced to a point where they no longer restrict the production of some flatfish species (Seikai 1985, Pittman *et al.* 1998, Shields 2001, Gavaia *et al.* 2002, Sæle *et al.* 2002).

Pigmentation problems are divided into two categories, namely hypomelanosis on the ocular side and hypermelanosis on the blind side (Bolker & Hill 2000). Flatfish typically show an asymmetrical external pigmentation pattern (Norman 1934). The blind side is usually white in colour, while the ocular surface is generally brown. Pigmentation problems also occur in the wild. Koshiishi *et al.* (1991) suggest that such individuals would be selected against and would probably be removed from the population through predation. Commercially, abnormal pigmentation reduces the value of the fish for aesthetic reasons (Venizelos & Benetti 1999, Bolker & Hill 2002). Hypomelanosis can sometimes occur over the entire ocular side of the fish. Hypermelanosis causes dark spots on the blind side but can also manifest itself over the entire side (Venizelos & Benetti 1999, Bolker & Hill 2000). The Japanese flounder, *Paralichthys olivaceus* (Seikai 1985), Senegal sole, *Solea senegalensis* (Dinis *et al.* 1999), Dover sole, *Solea solea* (Reig et al. 2000), plaice, *Pleuronectes platessa* (Dickey-Collas 1993), Atlantic Halibut, *Hippoglossus hippoglossus* (Pittman *et al.* 1998) and turbot, *Scophthalmus maximus* (Shields 2001) all show pigmentation abnormalities under culture conditions.

Common skeletal deformities in cultured pleuronectiform fishes include unattached dorsal fins and jaw deformities. These have been noted in Senegal sole, *Solea senegalensis* (Gavaia *et al.* 2002), Atlantic halibut, *Hippoglossus hippoglossus* (Pittman *et al.* 1998) and turbot, *Scophthalmus maximus* (Ellis *et al.* 1997, Shields 2001). Sæle *et al.* (2002) found that the causes of such deformities were also principally due to nutritional deficiencies during early larval development.

Growth rate, as well as stocking density, is important since it determines the productivity under culture conditions. Nothing is known about the optimal stocking density for *S. marginata*, but based on other flatfish species this should be reasonable high as previously stated. Grow out periods are highly variable in flatfish species. For example, *Solea solea* reaches a size of 0.2 kg in one year (Howell 1997), *Rhombosolea tapirina* reaches 0.5 kg in two years (Hart 1994), *Paralichthys adspersus* reaches 0.8 kg in two years (Silva & Velez 1998) and *Scophthalmus maximus* reaches 2 kg in two years (Anon 1999). Growth rate under culture conditions can however be enhanced through adequate nutrition (Tacon 1995), growing the fish under optimal environmental conditions (Bartley 1998, Le François *et al.* 2002, Quéméner *et al.* 2002) and through genetic techniques (De Silva & Anderson 1995, Basavaraju *et al.* 2002). It is never the less advisable to select a new flatfish species on the basis of comparable growth rates to other farmed species.

Many fish species show sexual dimorphism in terms of growth (Bruton 1989) and for some, monosex cultures are preferred (Carrillo *et al.* 1995, Howell *et al.* 1995, Saillant *et al.* 2001, 2002). Several flatfish species also show sex-related differences in growth rate. For example, female southern flounder (*Paralichthys lethostigma*) grow faster and reach a size three times larger than the males (Monaghan & Armstrong 2000, King *et al.* 2001). Females are therefore favoured for aquaculture to reduce grow out periods (King *et al.* 2001). Similarly, *Paralichthys dentatus* (King *et al.* 2001) and *Paralichthys olivaceus* (Yamamoto 1995) females are also favoured in aquaculture for their faster growth. Temperature, salinity and stocking density appear to be the principle abiotic factors affecting the sex ratio of bothids, pleuronectids and soleids (Baroiller *et al.* 1999, Baynes & Hallam 1999) under culture conditions. Elevated temperature during larval development has been shown to result in a sex ratio in favour of males for several flounder species. Species for which this phenomenon has been documented include *Paralichthys olivaceus*, *Verasper moseri* and *Paralichthys lethostigma* (Yamamoto 1995, 1999, Luckenbach *et al.* 2003). More recently,

1) See appendix A for Scientific Authorities

work has been reported on the establishment of all female *H. hippoglossus* populations to improve the growth rate under culture conditions by way of endocrine manipulation (Hendry *et al.* 2002).

In South Africa, like many other countries, there is a drive towards indigenous aquaculture (Hecht & Endemann 1998, Hecht 2000). The potential for, as well as the limitations to aquaculture development in South Africa are well documented (Hecht & Britz 1990, Hecht 1992, Hecht 2000) and various marine and freshwater species are currently successfully farmed (Hecht & Britz 1990, FAO 2002, Hecht 2000).

Research on marine finfish aquaculture has, until recently, not been prioritized because of wild catches meeting the local demand (T. Hecht *pers. comm.*). However, declines in wild stocks and sharp increases in fish price over the past five years have made aquaculture of marine finfish a viable business proposition. As a consequence, various projects investigating the potential of marine finfish species for aquaculture are currently underway, with a particular focus on the sciaenid, *Argyrosomus japonicus*.

Only two sole species are targeted by trawlers along the South African coast. These are *Austroglossus pectoralis*, which is caught mainly on the south coast from Port Elizabeth (33°57'S; 25°38'E) to Mossel Bay (34°08'S; 22°08'E) and *A. microlepis*, which is found mainly on the west coast, as well as in Namibia. The *A. pectoralis* fishery is managed on a precautionary basis with a Total Allowable Catch of 800 MT per annum. It appears that this level of exploitation is sustainable, although the average size of sole is decreasing (Badenhorst 1987, Britz *et al.* 2001). Nothing is known about the state of the *A. microlepis* stock, except that the industry is reporting a much reduced catch rate (Anon. 2002). It is reasonable to conclude that there is little scope (or hope) to increase the commercial catches of these two species. A third species, *Cynoglossus*

zanzibarensis forms part of the by-catch of the shallow water hake trawl fishery on the south east coast (Booth & Walmsley-Hart 2000).

The current demand for flatfish in South Africa is estimated, by industry, to be in excess of 1 500 MT. This cannot be met by local trawlers, which land around 785 MT / year (Anon. 2002). The shortfall is currently imported from Namibia (*A. microlepis*), Pakistan and India (*Cynoglossid spp.*). Very little is known about the state of the stocks in Namibia, Pakistan and India. This is mainly because these species are part of the by-catch, and are not directly targeted. Industry is of the opinion that the current supply of flatfish for the South African market will not be sustained. This makes the culture of flatfish in South Africa an attractive alternative, and the main reason why industry is interested in investing in research and development.

In recent decades, marine finfish culture in developed countries has been driven largely by the demand for high value species and operational profitability (Avault 1993, Reig *et al.* 2000, Tait & Hickman 2001, Le François *et al.* 2002). The increasing demand for high quality fish is also the principal motivation behind the interest in marine fish culture in South Africa. To reduce investment risk it is critically important that the most suitable species is chosen and to then screen that species to determine its biological suitability and performance capabilities under culture conditions. Species are selected for aquaculture on the basis of several biological and ecological criteria (Avault 1993, Le François *et al.* 2002, Quéméner *et al.* 2002). These include growth rate, ease of rearing, optimal temperature for growth, size of the larvae and market price (Le François *et al.* 2002, Quéméner *et al.* 2002). It is also important to select a species on the basis of aesthetic appearance, organoleptic properties and demand for the product (Quéméner *et al.* 2002).

A total of 56 flatfish (Pleuronectiformes) species have been recorded along the South African coast (Smith & Heemstra 1986). Knowledge of their biology and

ecology is restricted to *Austroglossus pectoralis* (Zoutendyk 1973 a & b, 1974, Hecht 1976, Payne 1986, Le Clus *et al.* 1996), *Austroglossus microlepis* (Payne 1979), *Solea bleekeri* (Cyrus 1991), *Heteromycteris capensis* (Cyrus & Martin 1991, Whitfield 1998) and *Cynoglossus zanzibarensis* (Booth & Walmsely-Hart 2000). Very little is known about the others.

As a first step in the selection procedure, a few simple exclusion criteria were applied. All species that did not attain a maximum size of at least 250 mm TL were excluded. Le François *et al.* (2002) regarded this as the minimum size for a suitable candidate species. Those species considered to be rare by Smith and Heemstra (1986) were also excluded due to the possible difficulty in establishing broodstock populations. Le François *et al.* (2002) and Quéméner *et al.* (2002) also suggested that species with a high abundance in an area should be favoured for aquaculture studies because they would theoretically adapt to the local rearing environment much quicker than others. Species occurring at depths greater than 50 m were also excluded. Quéméner *et al.* (2002) used a depth of 200 m as an exclusion criterion. In the present study, it was decided that the 50 m depth limit would be used to avoid logistic problems in obtaining broodstock and possible broodstock performance problems. Capture and handling stress has profound effects on circulation levels of gonadal steroids (Pankhurst & Van Der Kaak 1997, Cleary *et al.* 2000). It has been shown for snapper (*Pagrus auratus*) that the stress associated with baratrauma has a significant impact in acclimatising broodstock and on future spawning events. Moreover, the high stress levels during broodstock capture of *Pagrus auratus* has been found to affect egg quality (Battaglene & Talbot 1992, Battaglene 1995).

Using a minimum cut off size of 250 mm TL, a total of eight of the 56 species remained as possible candidates for aquaculture. These were *Pseudorhombus arsius* and *Paralichthodes algoensis* (Bothidae), *Cynoglossus capensis* and *Cynoglossus zanzibarensis* (Cynoglossidae), *Austroglossus pectoralis*, *Solea fulvomarginata*, *Synaptura marginata* and *Synapturichthys kleini* (Soleidae). Using the rarity criterion (Smith and Heemstra, 1986) three of the remaining eight

species were further eliminated. These were *Cynoglossus capensis, Solea fulvomarginata* and *Paralichthodes algoensis. Pseudorhombus arsius* was also excluded due to its rarity (A.K. Whitfield, South African Institute of Aquatic Biodiversity - SAIAB *pers. comm.*). *Austroglossus pectoralis* and *C. zanzibarensis* were also excluded because of their very rare occurrence in waters less than 50 m (T. Hecht, unpublished trawl data sheets, pers. comm.), leaving two species from which to select a candidate species for biological screening, namely *Synaptura marginata* and *Synapturichthys kleini* (Soleidae). Due to the wide distribution along the South African coast, *S. marginata* was chosen as its theoretically wider environmental tolerances (e.g. temperature) would be less limiting on future aquaculture ventures.

The white-margined sole, *Synaptura marginata* (Soleidae) has an Indo-west Pacific distribution, and in southern Africa occurs from the Mozambique Channel southward to Knysna on the south east coast of South Africa (Smith & Heemstra 1986, Robins *et al.* 1991) and is also known from Japan and the Philippines (Masuda *et al.* 1984, Robins *et al.* 1991). In South Africa, it is targeted exclusively by recreational fishermen (Smith & Heemstra 1986) (Chapter 2). Among coastal communities, it is regarded as one of the best tasting flatfish species in South Africa. According to FAO (1978), *S. marginata* is elsewhere targeted commercially.

Previous studies dealing with the selection of suitable aquaculture fish species have used biological characteristics as separate and discrete entities (Webber & Riordon 1976, Avault 1993, Le François *et al.* 2002, Quéméner *et al.* 2002). It is however questionable whether this approach is correct because species do not show random combinations of life history characters, but rather have suites of characters that are typically associated with one another (Begon & Mortimer 1986, Bruton 1989). It may therefore be more useful to consider the suitability of a species for aquaculture in the light of life history theory.

The most commonly accepted theories include the theory of r-and K- selection (MacArthur & Wilson 1967, Pianka 1970) and altricial / precocial homeorhetic states (Bruton 1989).

A typical *r*-selected species maximizes its fitness by reproducing rapidly in an unpredictable environment. To do this, they need to have a high reproductive effort. This reduces the energy allocated to somatic growth and maintenance and causes them to have shorter life spans and generation times (Begon & Mortimer 1986). There is thus a selection pressure acting on the expression of life history characteristics, favouring large numbers of small eggs, smaller offspring (Bromage 1995), large reproductive effort, early maturity and a shorter life span (Begon & Mortimer 1986, Balon 1984, Bruton 1989).

A typical K- selected species usually inhabits stable and predictable environments. Such environments are characterized by a high level of density-dependent competition for space and resources. Therefore, the production of a few large young and higher levels of parental care will maximize fitness by providing the juveniles with a competitive advantage in such an environment (Begon & Mortimer 1986). The need for parental care and the need to reduce density-dependent effects on the young will lead to reproduction being extended over time (Begon & Mortimer 1986). Thus selection pressure acts to produce a species that is characterized by smaller reproductive efforts, fewer and larger eggs, parental care, maturity at a later stage and longevity (Begon & Mortimer 1986, Balon 1984, Bruton 1989). Determining the relative position of a species on the r and K continuum may provide us with better clues upon which to decide on its suitability for aquaculture.

The aim of this project was to investigate certain life history parameters and characteristics and to use these to firstly, assess the biological suitability of *S. marginata* as a candidate species for aquaculture and secondly, to use the data

to make some preliminary performance predictions for the species under culture conditions.

This study examined the following aspects of the biology of *S. marginata*. Age and growth of *S. marginata* is described in Chapter 3. Length-at-age data are essential to quantify certain life history parameters such as longevity, growth rate in length and weight, and age at maturity (Coetzee & Baird 1981, Griffiths & Hecht 1995, Booth & Buxton 1997), and are important parameters for the ultimate selection of a species for aquaculture. Faster growing species have shorter grow-out periods and should be favoured. Growth rate is also directly related to longevity and age at maturity (Roff 1981, Bruton 1989). Longer living species normally reach sexual maturity at a later age and have a longer reproductive life span (Roff 1981). Delayed sexual maturation has distinct advantages for aquaculture. In particular, the period prior to the onset of gonad maturation is characterised by rapid growth and high food conversion efficiency (Halver 1989, De Silva & Anderson 1995, Berill *et al.* 2003). The natural growth rate (coupled to other life history parameters) may also be used to predict the theoretical growth rate of *S. marginata* under culture conditions.

Chapter 4 describes the feeding biology of the species. The study was undertaken to establish what constitutes the diet of the species, what quantities are consumed, and how feeding behaviour is affected by environmental factors such as temperature, season, habitat and prey size (Windell 1968). Proximate analysis and amino acid profiles of *S. marginata* as well as prey items were also undertaken. A first attempt was made to use this data to predict the theoretical nutritional requirements of *S. marginata* (Cowey & Tacon 1982, Wilson 1985, Wilson 1989).

Understanding and controlling reproduction is central to the efficient propagation of a species (Devlin & Nagahama 2002). Aspects of the reproductive biology of *S. marginata* are presented and described in Chapter 5 and included

investigations on reproductive seasonality, sex ratio, size at sexual maturity and fecundity, as well as a detailed description of ovarian development and female gametogenesis. Some preliminary observations on keeping adult fish in captivity, feeding, induction and egg size is also provided.

An analysis of the biological and life history characteristics that are important for aquaculture is presented in Chapter 6. By comparison with other farmed marine fish species and those under investigation for their farming potential, an attempt was made to evaluate the suitability of *S. marginata* for aquaculture and to predict performance under culture conditions.

Chapter 2: General Materials and Methods

Sampling area

Specimens of *Synaptura marginata* were collected between Port Elizabeth (33°57'S; 25°38'E) and Great Fish River Point (33°31'S; 27°06'E) on the south east coast of South Africa (Figure 2.1). The topography of this coastline is highly variable. The shores of Algoa Bay are generally sandy with rocky ledges at Woody Cape and Cape Padrone. The area from Cape Padrone to Great Fish Point consists of rocky outcrops alternating with sandy beaches (Hydrographer S.A. Navy 1985). The intertidal and subtidal rocky outcrops are temporal and are often buried under sand and then washed open again by currents. These currents are mainly determined by the prevailing winds (Lutjeharms 1998).

The rocky outcrops in some areas are covered by the green algae, *Caulerpa filiformis* (Branch *et al.* 1994), which is often the dominate seaweed. *C. filiformis* beds are often the only evidence of rocks that are buried beneath the sand (*pers. obs.*). It is mainly around these *C. filiformis* beds where *S. marginata* is abundant. Possible reasons for this are presented in Chapter 4.

The main collection sites along this stretch of coast included Cape Recife, Cannon Rocks, Port Alfred, Kleinemonde and Great Fish River Point (Figure 2.1).

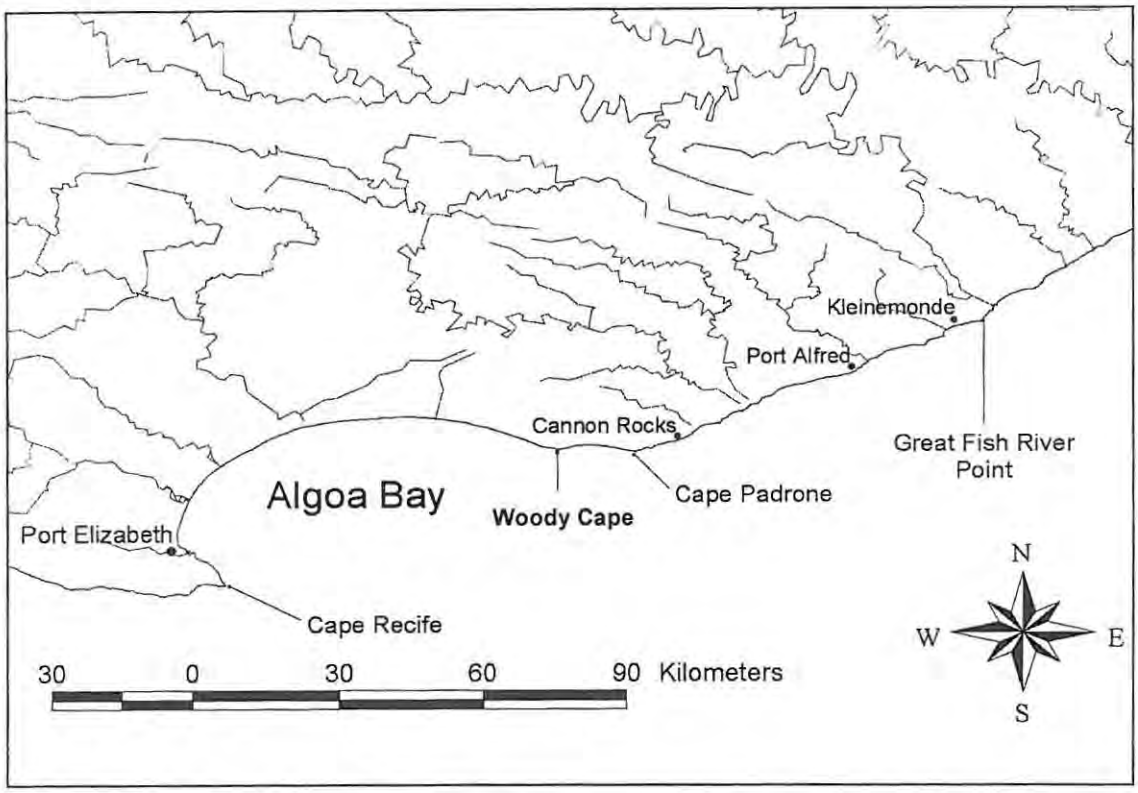

Figure 2.1: Map of the study area showing the main sampling sites along the South African south east coast.

Sampling techniques

S. marginata were caught by wading on shallow sandbanks and spearing (blindly) with a four-pronged pitch fork (locally called graining) (Figure 2.2). This technique is also used by all recreational sole fishermen in the area.

These forks come in a wide range of shapes and sizes, but all forks have three basic parts to them; the head, prongs and handle (Figure 2.3). The prongs are barbed and are made of steel or steel alloy, and are attached to the head. The head is attached to the handle, usually a wooden broom stick.

Figure 2.2: Graining on shallow sandbanks at Kleinemonde on the south east coast of South Africa.

S. marginata are generally more abundant on newly formed sandbanks (0 to 1.5 meters deep in the water) with dispersed rocks covered by *C. filiformis*. Sampling usually took place two days before and after spring tide (five days in total) for a period of about two hours before and after low tide during the day (four hours sampling), when sandbanks were easily accessible.

Other flatfish capture techniques were also tested. These included the use of seine nets, fyke nets, baited traps and diving. Shallow sand banks were seined using two different seine nets. The first was a 15 x 1.5 m deep net with a stretched mesh size of 25 mm, and the second a 25 x 1.5 m net with stretched mesh size of 40 mm. A tickle chain was attached to the bottom line of the seine nets to assist in keeping the net on the bottom, and as a means to get the sole to swim up from underneath the sand. Ten hauls with each net, in areas where soles were known to occur, were undertaken under ideal sea conditions without any success.

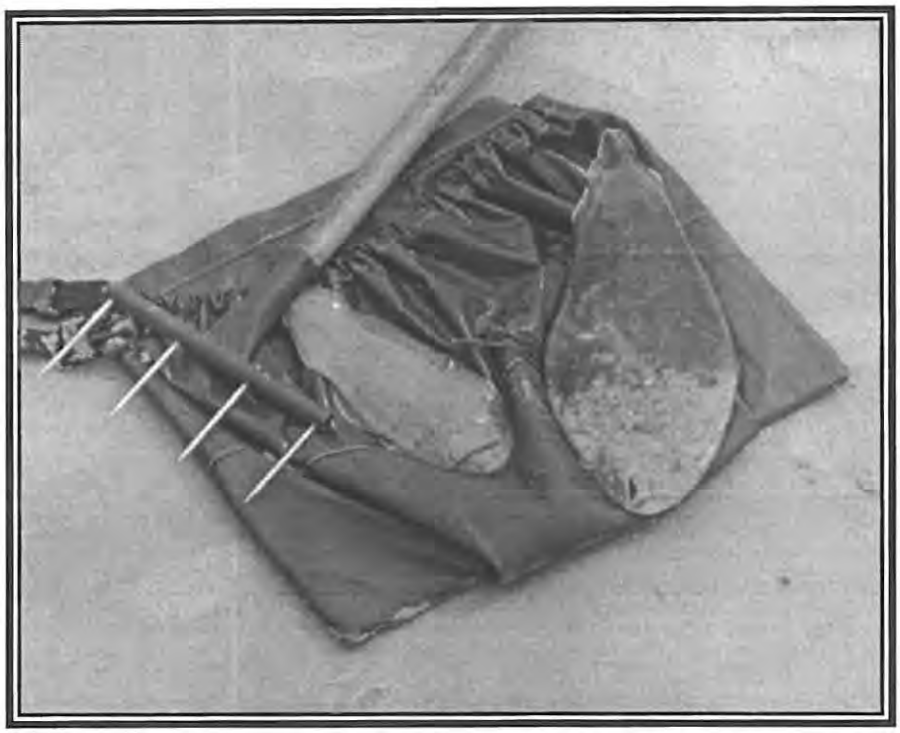

Figure 2.3: Typical pronged pitch fork used to capture *S. marginata* on the south east coast of South Africa.

Fyke nets with 3 m guide nets were set on shallow sandbanks (0.5 – 1.5 m depth) with and without dispersed rocks covered with *C. filiformis*. Due to wave conditions, the nets could only be left in the water for a maximum of one hour. A total of 12 sets were undertaken with no success. This might be due to the short soak times.

Baited traps were also used, and these had the following specifications. The frame of the trap (1 x 1 x 0.3m) was constructed with 20 mm poly-ethylene pipe and covered with plastic mesh (25 mm). Two entrance funnels were provided on two opposing sides, gradually narrowing to slits of about 10 cm wide. The trap was baited with crushed sand mussel (*Donax serra*), fish and polychaetes in a small-mesh bag. Twenty-five traps were set with an average soak time of 1.5 hours on sand banks with and without *C. filiformis* covered rocks. Though there was a high catch of other small fish such as mullet (*Liza richardsonii*), evileye blaasop (*Amblyrhynchotes honckenii*), blacktail (*Diplodus sargus capensis*) and zebra (*Diplodus cervinus hottentotus*), no soles were captured.

A total of 32 diving excursions were also undertaken by snorkeling at mid tide. However, these were also unsuccessful due to rough water conditions and the very cryptic nature of the sole when buried under the sand.

S. marginata may be a nocturnal species, as is the case with many other sole species (De Groot 1971) and this might explain the lack of catches in the two passive fishing gears (fyke nets and baited traps), since sampling took place during the day.

Every effort was made to obtain a reasonable sample size per month. To increase the monthly samples obtained by graining, a poster was designed and displayed in 23 shops in the study area. These included beach cafes, grocery shops, angling and tackle shops. Several sole fishermen responded, which helped to increase the monthly sample size. Biological data were also collected from fish caught by fishermen on the beach.

Sampling period, effort and sample sizes

Sampling took place over a 16 month period, from December 2000 to March 2002, during which a total of 148 soles were speared. The average monthly effort consisted of 80 hours of graining (two persons for four hours per day for ten days a month over the two spring low tides per month). Sample sizes for different months are presented in Table 2.1. This table clearly shows higher catches between December and April. Biological data from an additional 23 soles were obtained from recreational fishermen during the sampling period.

Since the effort throughout the year remained relatively constant for each month, the CPUE data may reflect a seasonal inshore / offshore spawning migration (see Chapter 5). Similar observations have been made for ten commercially important flatfish species inhabiting the Yellow Sea, China (Dagang *et al.* 1992).

Table 2.1: Catches of *Synaptura marginata* over the period of December 2000 to March 2002.

	Dec. 2000	Jan. 2001	Feb. 2001	March. 2001	Apr. 2001	May 2001	Jun. 2001	Jul. 2001	Aug. 2001	Sep. 2001	Oct. 2001	Nov. 2001	Dec. 2001	Jan. 2002	Feb. 2002	March 2002	Total
Number of females	10	11	2	15	18	4	4	1	7	6	3	3	4	21	11	6	126
Number of males	2	2	0	2	5	1	0	0	0	0	1	0	1	4	4	0	22
Combined sexes	12	13	19	17	23	5	4	1	7	6	4	3	5	25	15	6	148

Size frequency of catches

Where possible, all sole speared were measured (total length to the nearest mm) and weighed (nearest g) immediately after capture. Although other small soleid species like *Heteromycteris capensis*, *Solea bleekeri* and *Solea fulvomarginata* ranging in size from 60 to 100 mm TL were speared, no *S. marginata* smaller than 102 mm were caught during the entire sampling period (Figure 2.4). The absence of juvenile *S. marginata* was therefore not a consequence of the sampling method, but may rather indicate their absence from the intertidal area.

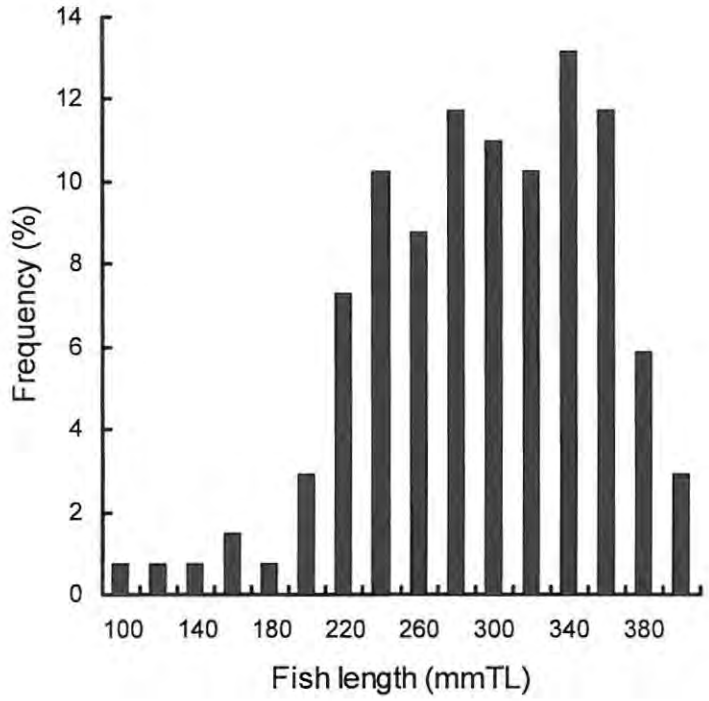

Figure 2.4: Length-frequency distribution of *Synaptura marginata* captured on the south east coast of South Africa during the period December 2000 to March 2002.

Detailed descriptions of the processing of samples are provided in the appropriate chapters.

Chapter 3: Age and Growth

Introduction

Growth rate is an important biological factor to consider when choosing a species for aquaculture (Webber & Riordan 1976). Faster growing species have reduced grow-out periods, and it stands to reason that this allows for greater turnover, which will result in greater profit. The ability to determine the age of fishes is also an important tool in fisheries biology (Bagenal 1974, Fossen *et al.* 2003). Accurate age and growth studies often provide the fundamental basis for management as these are the foundation on which many management models are based (Ricker 1975).

A wide variety of techniques are used for age determination in fishes (Campana 2001). These methods include direct and indirect age estimation techniques, both of which have been used for ageing of flatfish. Direct age estimation involves the use of hard tissues of fishes such as otoliths, scales, vertebrae, spines, fin rays and operculi. Hard tissues have in most instances been shown to exhibit rings that indicate various growth stages in the life of a fish. These techniques have been used in both temperate as well as tropical regions worldwide (Laevastu 1965, Weatherly & Rogers 1978, Suzuki & Kimura 1990, Newman & Dunk 2002). The deposition of growth zones on hard tissue is affected by internal factors such as spawning and stress, and external factors such as temperature, food availability and photoperiod (Weatherly 1990).

Scales are the easiest to collect and prepare for age estimation, but are less reliable than otoliths (Weatherly & Rogers 1978, Royce 1984). Zoutendyk (1974) examined the value of scales for estimating age in the Agulhas sole, *Austroglossus pectoralis,* but found no conclusive correlation between scale markings and age. In general, otoliths are the most frequently used hard tissue for ageing fish (Fossen *et al.* 2003). Once removed and cleaned, otoliths can be stored dry and are either read whole, or sectioned through the nucleus.

Two ways of indirect age estimation are commonly used. The Petersen method involves the analysis of length frequency data (Laevastu 1965, Tesch 1968, Pauly 1983) and the second technique involves tagging studies (Royce 1984). The Petersen method is best used for temperate species, where spawning is distinctly seasonal and is based on the assumption that length frequencies have modes representing single cohorts of recruitment events (Hilborn & Walters 1992), as well as for tropical species where rings on hard parts are often not very distinct (Pauly 1983). Tagging studies are generally used over extended study periods, allowing for adequate tag returns (Beamish & McFarlane 1983).

Both direct and indirect ageing techniques have been used for growth studies in flatfish. McCaughran (1981) used the mark-recapture method to estimate growth parameters for the Pacific halibut (*Hippoglossus stenolepis*), while Martínez-Munoz and Ortega-Salas (2001) used length frequency analysis to determine the growth of the bigmouth sole (*Hippoglossina stomata*). Most other growth studies on flatfish species have made use of otoliths, e.g. the soleid, *A. pectoralis* in South Africa (Zoutendyk 1974), the paralichthyd, *Etropus crossotus* in South Carolina (Reichert *et al.* 2000), the cynoglossid, *Cynoglossus zanzibarensis* in South Africa (Booth & Walmsley-Hart 2000), the pleuronectids, *Parophrys vetulus* in Canada (Fargo & Kronlund 2000) and *Pleuronectus platessa* in the Irish Sea (Nash *et al.* 2000), the scophthalmids, *Psetta maxima* and *Scophthalmus rhombus* in the Adriatic Sea (Arneri *et al.* 2001) and *Lepidorhombus boscii* in the Atlantic (Landa *et al.* 2002). Deniel (1990) used otoliths to age 10 flatfish species on the west coast of Brittany, which included five soleids, two bothids, two pleuronectids and one scophthalmid.

The sagittae, in comparison to the lapilli and asterici, are the most commonly used otoliths for age studies, due to their large size and relative ease of extraction (Gauldie & Nelson 1990). Otoliths are composed of calcium carbonate crystals that radiate outwards on an otolin (organic protein) matrix in three dimensions from the nucleus (Williams & Bedford 1974, Gauldie & Nelson 1990). Differential deposition rates of these two components are visible as zones with different optical densities (McEachran & Davis 1970, Williams & Bedford 1974, Fossen *et al.* 2003).

Adjacent translucent and opaque zones are collectively referred to as an annulus on the assumption that they are laid down annually (Griffiths 1988). Validation of the annuli is therefore of pivotal importance (Staples 1971, Payne 1977, Beamish & McFarlane 1983, Pulfrich & Griffiths 1988, Schwartz 1990, Griffiths 1996).

Numerous models have been developed to describe growth rates (Ricker 1975, Schnute 1981, Tsoularis & Wallace 2002). The simplest, with the least number of variables is the Verhulst logistic growth model, which forms the basis of more complex models such as the Gompertz, von Bertalanffy and Schnute models (Tsoularis & Wallace 2002). The Von Bertalanffy and the Schnute models are the most commonly used in fisheries biology. Model parameters allow for comparisons with other species or between sexes of the same species (Isarev 1976). This is particularly important for many species of flatfish, in which sexual dimorphism is a common feature (Colman 1994).

No previous age and growth investigations have been conducted on *Synaptura marginata*. The aims of this study were twofold. Firstly, to obtain accurate length-at-age estimates to calculate age at sexual maturity and secondly, to calculate the growth rate of the species under natural conditions, which can be used as a means of predicting the growth under culture conditions.

Materials and methods

Fish samples were obtained during monthly collecting trips along the Eastern Cape coast of South Africa (Figure 2.1). The fish were weighed (to the nearest g) and the total length and greatest width (fins folded) was measured to the nearest mm. Sagittal otoliths were removed, cleaned and stored dry in cross referenced brown paper envelopes.

A total of 111 otoliths were removed and sectioned successfully. The length of a sub-sample of 40 left and right otoliths was measured to the nearest 0.05 mm. Otolith length was then plotted against fish length (mm TL) and modeled using regression analysis. Left and right otoliths were tested for differences in size using a pared t – test. Zoutendyk (1974) noted for the Agulhus sole, *Austroglossus pectoralis* that the nucleus of the right otolith was off-centre. Since there was no significant difference in the size of

left and right otoliths and because the nucleus of the right otolith was in an off centre position (Figure 3.1), only the left sagittae were used for age estimation

Otoliths were embedded in clear casting resin rods, and then cut using a double bladed diamond-edge saw. Sections with an average thickness of 0.4 mm were cut through the nucleus. These sections were mounted on microscope slides using DPX slide mountant. Otolith sections were read on a black background using a stereo dissecting microscope under reflected light.

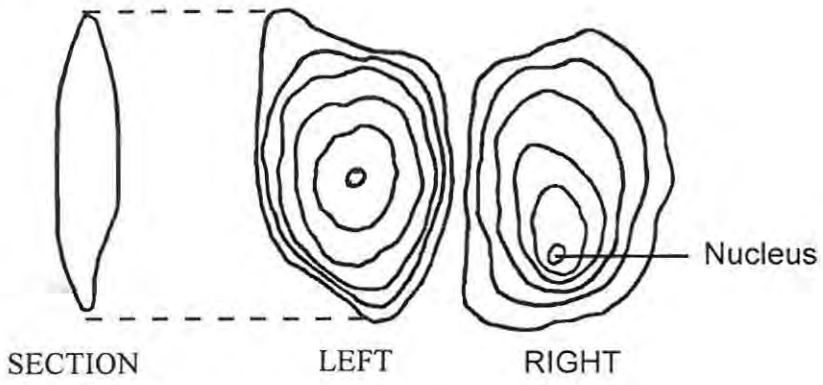

Figure 3.1: Left and right sagittal otoliths of *Synaptura marginata* viewed from the ventral surface, showing growth rings and the position of the nucleus.

Marginal zone analysis was used to determine the time of zone formation (Hecht & Baird 1977, Buxton & Clarke 1992, Booth & Buxton 1997, Booth & Walmsley-Hart 2000). This was achieved by noting the optical appearance of the otolith margin (either opaque or translucent) and expressing it as a percent of the monthly otolith sample.

Otolith readings were made without reference to fish size. Each section was read twice by independent readers on different occasions. If two readings were not equal, a third was made. An age estimate was accepted from the two most similar readings. If the age range was not more than two years, a mean age estimate was taken, otherwise the otolith was rejected.

In many fish species, a juvenile ring is deposited on the otolith before the end of its first year of life (Wohler 1997, Hicks & Gilbert 2002). If a juvenile ring is indeed present and

not identified, then this may result in the overestimation of age. This would affect the prediction of growth under culture. To test whether the first visible growth check was a juvenile ring or the first annulus, the otoliths of 12 fish, less than 240 mm TL, were polished down to a thin section (0.3 mm) using Buehler Alpha micropolish (Alumina C) and Canada balsam as a mountant. The polished surface was viewed under a compound microscope at 100 x magnification, and daily growth rings were counted to the end of the first translucent zone, on the same axis used for counting annuli (Figure 3.7).

Length, width and weight data were tested for normality with a normal probability plot and Shapiro-Wilk test, while homoscedacity was checked with a Levene's test. If data was normal and variances equal, a t-test for independent sample groups was used, otherwise a Kolmogorov-Smirnov test was used to test for differences in length, width and weight for different sexes.

The length-width, length-weight and width-weight relationships were used to test for sexual dimorphism in growth patterns. The length-weight and width-weight data were log-transformed to obtain a linear function.

The slopes of the length-width relationships and the natural log linearised length-weight and width-weight relationships were compared using ANCOVA (Zar 1996). If the differences between sexes were not significant, morphometric data for both sexes were combined and relationships calculated using regression analyses.

The growth rate of *S. marginata* was modeled using the three-parameter von Bertalanffy equation with a relative as well as an absolute error structure (Ricker 1975, Punt 1989).

$$L_t = L_\infty \left(1 - e^{-K(t-t_0)}\right)$$

where L_∞ is the predicted asymptotic length, L_t is the length-at-age t, t_0 is the age at "zero" length, K is the Brody growth constant, and the four-parameter Schnute growth model,

$$L_t = \left[L_1^b + \left(L_2^b - L_1^b\right)\left\{\frac{1-e^{-a(t-t_1)}}{1-e^{-a(t_2-t_1)}}\right\}\right]^{1/b},$$

where t_1 is the smallest age in the sample, t_2 is the largest age in the sample, L_t is the length-at-age, L_1 is the estimated mean length of t_1 year old fish, L_2 is the estimate mean length of t_2 year old fish (Schnute 1981).

The parameters for each model were estimated using a non-linear, downhill search to minimize the sum of the squared absolute and relative residuals (Nelder & Mead 1965). An absolute error structure assumes normal residuals (E_t),

$$\hat{L}_t = L_t + E_t \qquad E_t \sim N(0, \delta^2) \qquad \text{(Schnute 1981)}$$

where L is the model predicted length-at-age, t is the relative error structure which assumes that error increases with age such that:

$$\hat{L}_t = L_t e^{E_t} \qquad E_t \sim N(0, \delta^2) \qquad \text{(Schnute 1981).}$$

The quantity to be minimized for the absolute error structure of the model was

$$SS = \sum\left(L_t - \hat{L}\right)^2 \qquad \text{(Schnute 1981)}$$

and for the relative error structure of the model

$$SS = \sum \ln\left(\frac{L_t}{\hat{L}}\right)^2 \qquad \sum\left[t \ln\left(\frac{L_t}{\hat{L}_t}\right)\right]^2 \qquad \text{(Schnute 1981)}$$

A non-parametric one sample runs tests was applied to test randomness of the residuals and a Bartletts's test used to test for homoscedacity (Booth & Buxton 1997). The simplest model with the least number of variables was chosen if the residuals were both random and homoscedastic. Variance estimates were calculated using the (conditioned) parametric bootstrap resampling technique with 500 bootstraps (Efron 1981). Standard errors and confidence intervals were constructed from the sorted bootstrap data using the percentile method (Buckland 1984).

The length-weight relationship was modeled in the form of $W = aL^b$, where W is weight, L is length (mm TL) and a and b are constants (Ricker 1975). This exponent is needed to calculate the weight-based von Bertalanffy growth equation (Sparre & Venema 1998). The equation for growth in weight is as follows:

$$W=W_{\infty}(1-e^{-K(t-t_0)})^b$$

where W is weight at time t, W_{∞} is the maximum theoretical weight, t_0 is the age at "zero" length and b the exponent of the length-weight relationship.

Results

Morphometrics

The length, weight and width frequency of males and females are shown in Figure 3.2. Females attained a significantly larger size and weight than the males. The largest female measured 410 mm TL and weighed 940 g, compared to 320 mm TL and 370 g for the largest male. The mean length and weight for females were 314.2 ± 47.6 mm SL and 415.5 ± 181.2 g respectively, while males measured 255.9 ± 48.1 mm and weighed 178.3 ± 88.0 g (Table 3.1).

Table 3.1: Comparison of length, weight and width for male and female *Synaptura marginata.*

	Males (x ± SD)	Females (x ± SD)	t-stat	Df	P-value
Length	255.9 ± 48.1 (mm)	293.9 ± 61.8	-3.1	165	0.002
Width	97.2 ± 21.8 (mm)	119.0 ± 30.5	-2.6	77	0.011
Weight	178.3 ± 88.0 (g)	349.6 ± 203.1	n/a	95	<0.005

There were no significant differences between the length-weight, width-weight and length-width relationships for males and females (Table 3.2). Regression analyses could therefore be undertaken on the combined sex data to obtain the length-weight, length-width and width-weight relationships for *S. marginata*. The equations describing these relationships are presented in Table 3.3 and illustrated in Figure 3.3, 3.4 and 3.5.

Table 3.2: ANCOVA results from a comparison of morphometric relationships between male and female *Synaptura marginata.*

	F - stat	df	p
Length – Width	1.52	1;73	0.22
Length – Weight	0.11	1;93	0.73
Width – Weight	1.10	1;73	0.30

Table 3.3: Equations describing the morphometric relationships of *Synaptura marginata.*

Equation	R^2	n
Weight (g) = $(6 \times 10^{-6}) \times$ Length (mm TL) $^{3.117}$	0.971	95
Weight (g) = $(1.1 \times 10^{-3}) \times$ Width (mm) $^{2.631}$	0.950	77
Width (mm) = 0.471 x Length (mm TL) – 18.219	0.922	77

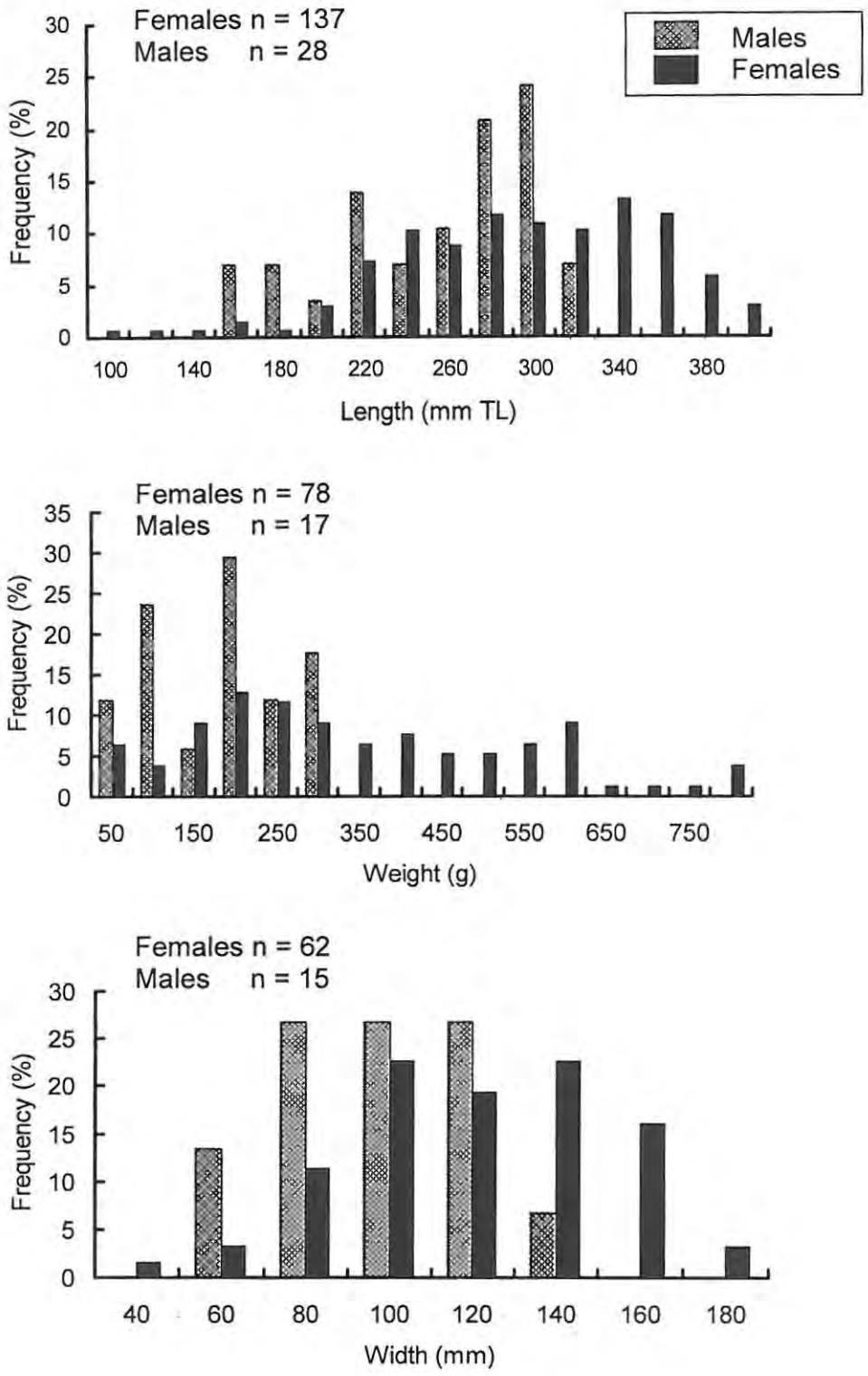

Figure 3.2: Total length, weight and width percent frequency histograms of male and female *Synaptura marginata* collectedon the south east coast of South Africa between December 2000 and March 2002.

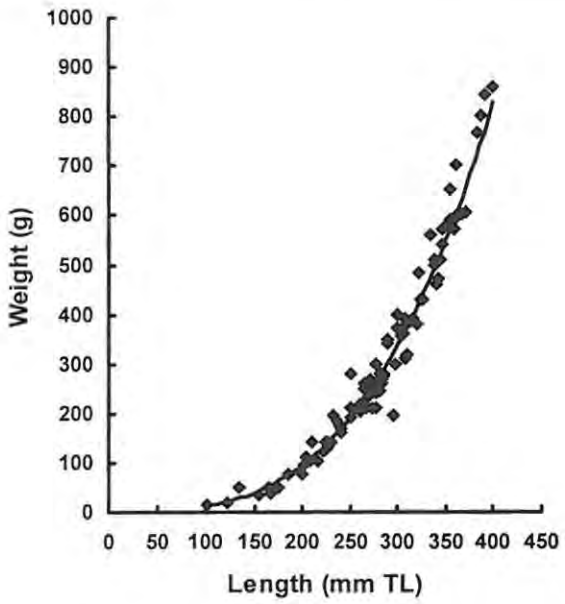

Figure 3.3: Relationship between total length and total weight for *Synaptura marginata*.

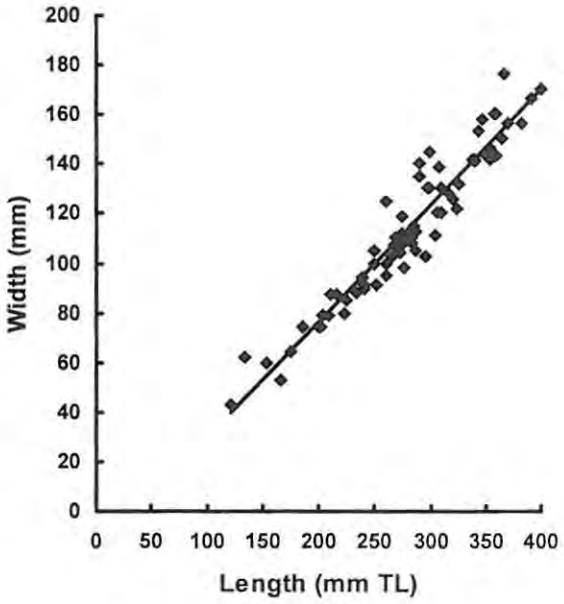

Figure 3.4: Relationship between total length and width for *Synaptura marginata*.

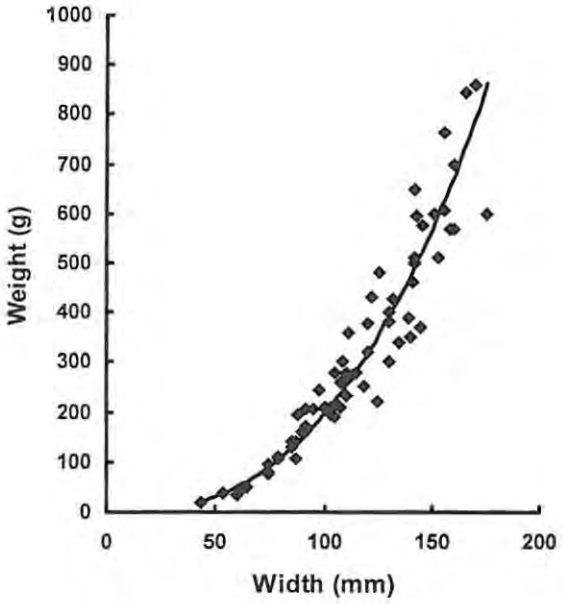

Figure 3.5: Relationship between width and total weight for *Synaptura marginata*.

There was a linear relationship between fish length (mm TL) and otolith length (mm) for both right and left otoliths (Figure 3.6).

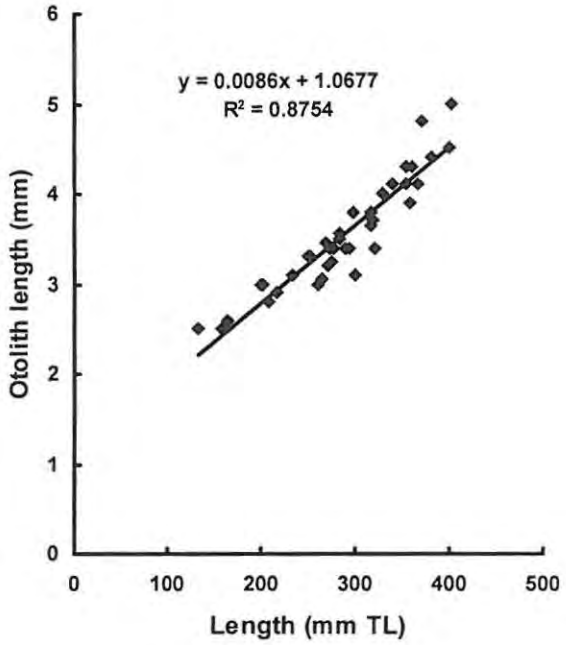

Figure 3.6: Relationship between fish length (mm TL) and otolith length (mm) for *Synaptura marginata*

Alternating opaque and hyaline zones were clearly distinguishable on sectioned otoliths. The oldest fish in the sample was 7 years of age.

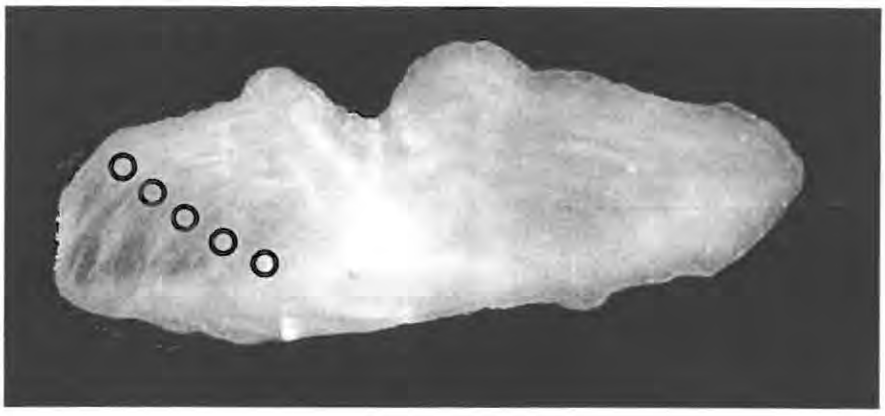

Figure 3.7: Sectioned sagittal otolith of *Synaptura marginata*. Reading axis is indicated.

Marginal zone analysis confirmed that one opaque and one hyaline zone were laid down each year, with the optically dense opaque zone laid down between March and September (Figure 3.8).

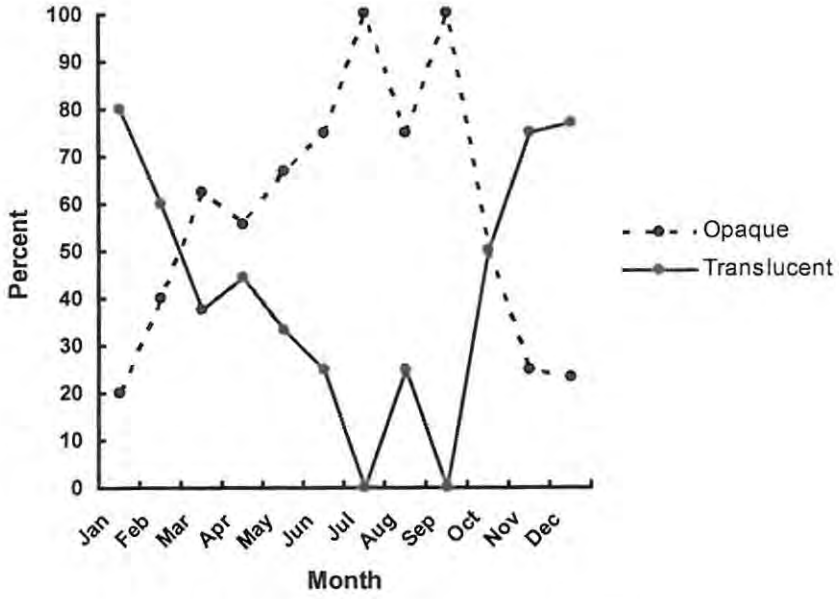

Figure 3.8: Monthly percent of opaque and translucent otolith margins for *Synaptura marginata*.

Daily growth ring analyses of 12 fish ranging in length from 102 to 240 mm TL are shown in Table 3.4. The mean length of one year old fish was calculated to be 210.18 ± 15.54 mm TL. All fish below this length did not show an opaque zone after the translucent zone following the opaque nucleus. Therefore, of the fish that were less than one year old, the number of daily growth rings would be less than the expected 365. By contrast, all otoliths of fish greater than 210 mm TL were expected to show in the region of ≥ 365 rings. The results show that the mean number of daily rings on otoliths of fish < 164 mm TL was 236 ± 36, whereas the otoliths of fish >200 mm TL had 353 ± 18 daily growth zones. From this it was concluded that the first growth check (opaque nucleus and first translucent zone) is the first annulus and not a juvenile ring. The mean distance from the centre of the nucleus to the end of the first growth check was 0.54 ± 0.02 mm

(Table 3.4). This measurement was used to identify the end of the first year on those otoliths in which the first annulus was not clearly visible.

Consequently, one opaque and one translucent zone represents one year growth. Of the 111 otoliths analysed, 14 (12.6%) were rejected as unreadable. The age-length key for *S. marginata* is presented in Table 3.5.

Table 3.4: Daily otolith growth ring count and analysis for *Synaptura marginata*.

Fish number	**Total length (mm)**	**No. of daily growth zones**	**Age calculated as a fraction of a year**
1	102	186	0.51
2	123	204	0.56
3	134	256	0.70
4	154	234	0.64
5	156	280	0.77
6	164	257	0.70
Fish number	**Total length (mm)**	**No. of daily growth zones to the first growth check**	**Distance from centre of nucleus to end of first check**
7	220	345	0.55
8	212	328	0.51
9	200	340	0.54
10	240	376	0.52
11	234	367	0.53
12	227	362	0.57
		353 ± 18	0.54 ± 0.02

The combined sex and the female length-at-age data satisfied the assumptions of equal variances and random residuals for the von Bertalanffy and Schnute Absolute Error Structure models (Schnute 1981). No significant differences were found between the two growth models fitted to the combined sex data using an F-test ($F = 0.043$, $df = 1;96$, $p > 0.05$). The special three-parameter von Bertalanffy model was therefore used to

describe the growth of *S. marginata.* Figure 3.9 shows the growth curve of *S. marginata* (males and females) and the daily growth ring counts. Figure 3.10 shows the associated residuals plot. The estimated growth parameters and the associated variance for females and the combined sex data are summarized in Tables 3.6 and 3.7.

The weight-based von Bertalanffy growth equation is as follows,

$W_t=966.27(1-e^{-0.24(t+1.79)})^{3.117}$

and is illustrated in Figure 3.11.

Table 3.5: Age-length key for *Synaptura marginata* (combined sexes).

Total length (mm)	*Number of fish in each age-class*						
	1	**2**	**3**	**4**	**5**	**6**	**7**
170	1						
180							
190							
200	4						
210	2						
220	3		1				
230	1	3	1				
240		2					
250		3					
260		4	3				
270		5	5				
280		1	4	2			
290			3	1			
300			3	3	1		
310			1	2	1		
320			1	3	1	1	
330				2	3		
340				3	3	1	
350			1	2	3		
360			1	1	2		1
370						1	1
380				2	1		1
390							
400							1
n	*11*	*18*	*25*	*21*	*15*	*3*	*4*

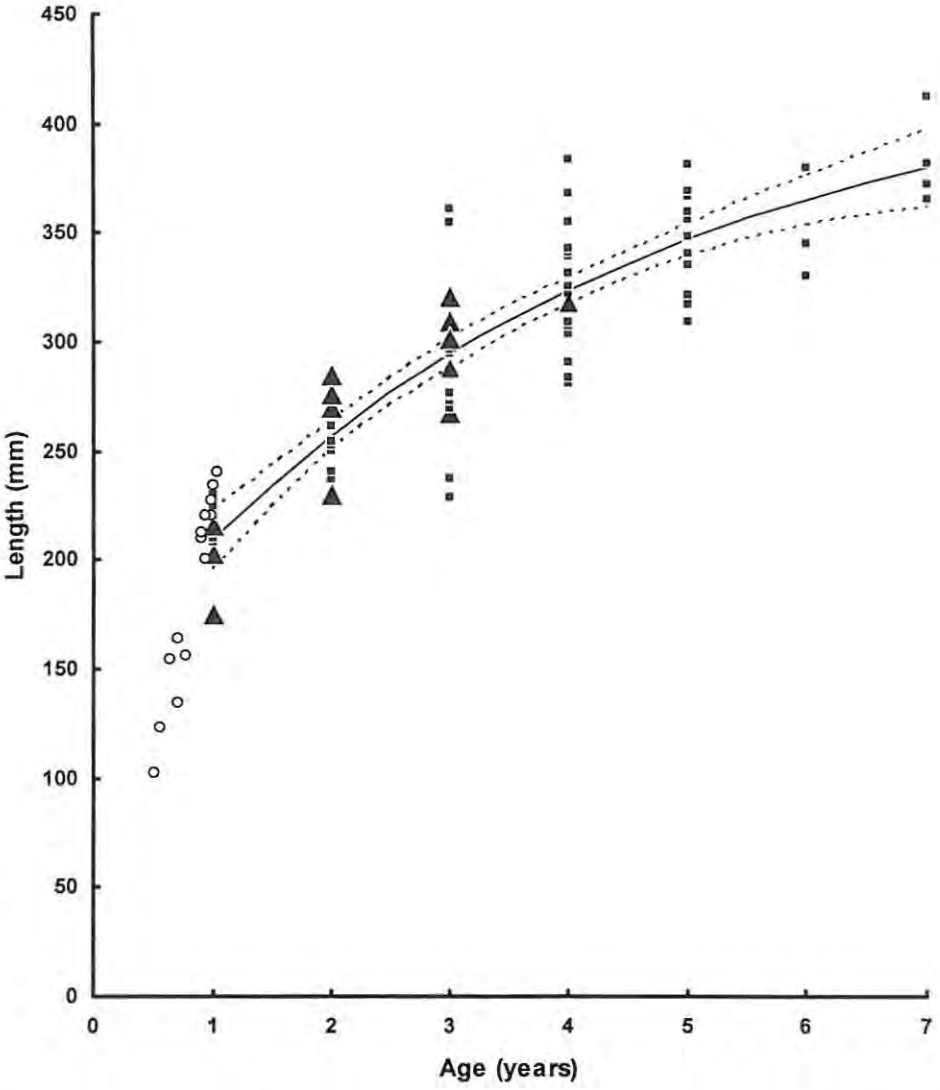

Figure 3.9: Observed length-at-age of male (large triangles) and female (small squares) *Synaptura marginata* and the specialized von Bertalanffy growth curve for combined sex data. Dotted lines are 95% confidence intervals. The circles indicate the length-at-age in days of the 12 fish less than 240 mm TL calculated as a fraction of a year.

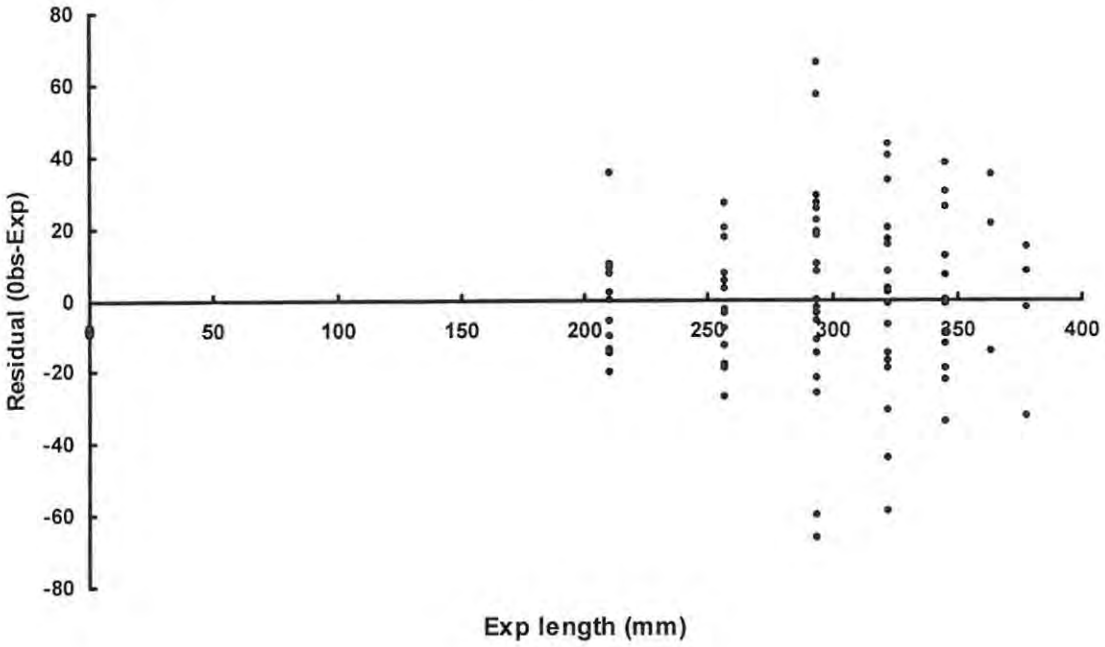

Figure 3.10: Residual plot of observed length minus expected length for *Synaptura marginata*.

Table 3.7: Point estimates, associated standard errors (SE) and 95% confidence intervals (CI) for female and combined sex data for *Synaptura marginata* from the specialized Von Bertalanffy model.

Parameter	Point estimate	SE	95% CI	
			Lower	Upper
Both Sexes (n = 97)				
L_∞	429.50 mm TL	55.47	370.25	578.03
K	0.24·year^{-1}	0.07	0.11	0.38
t_0	-1.79·year	0.63	-3.40	-0.93
Females (n = 79)				
L_∞	426.14 mm TL	45.60	378.93	537.16
K	0.26·year^{-1}	0.07	0.14	0.40
t_0	-1.56·year	0.56	-3.01	-0.78

Table 3.6: Observed (± *SE*) and expected lengths-at-age for female *Synaptura marginata* and for combined sexes.

Age (years)	Length-at-age (mmTL)					
	Both Sexes			Females		
	n	Observed (±SE)	Expected	n	Observed (± SE)	Expected
1	11	210.18±15.54	209.91	7	213.57±11.25	207.66
2	18	259.94±16.73	256.91	13	254.33±17.16	257.91
3	24	289.04±30.77	293.84	18	291.06±30.41	296.60
4	21	328.29±29.19	322.87	20	337.61±23.14	326.39
5	15	345.27±20.24	345.69	15	345.27±20.24	349.33
6	3	350.00±25.24	363.63	3	350.00±25.24	366.99
7	4	380.75±20.71	377.73	4	380.75±20.71	380.60

The male age-length data did not meet the assumption of random residuals for either the von Bertalanffy or the Schnute (absolute and relative error structure) models. The growth parameters for males could thus not be calculated. This is probably a result of the small sample size (n = 18). Table 3.8 shows the mean length-at-age (± SD) for the first three age classes of males and the corresponding data for females. A Mann-Whitney U test was used to compare the length-at-age for males and females. The results clearly show that there was no significant difference in growth rate between the sexes for the first three years. It was not possible to do any statistical analysis on four year old fish, since only one male of this age was collected.

Table 3.8: Length-at-age for males and females for ages one to three. The Z-statistic and the associated probability value (p) from the Mann-Whitney U test are also given.

Age (years)	Females x ± SD (mm TL)	Males x ± SD (mm TL)	Z-stat	p
1	213.6±11.3	197.3±20.7	1.25	0.21
2	254.3±17.2	266.4±22.0	-1.38	0.17
3	291.1±30.4	297.1±16.5	-1.17	0.24

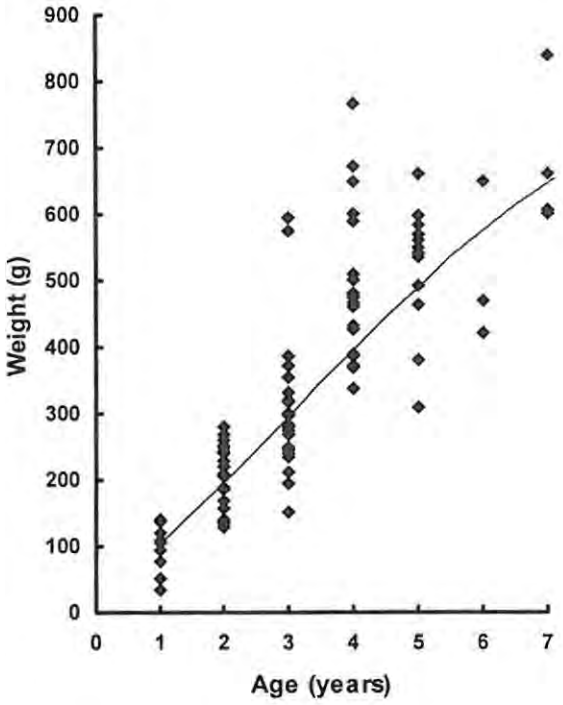

Figure 3.11: Growth in weight for male and female *Synaptura marginata.*

Discussion

Sexual dimorphism is a common feature among many species of flatfish (Colman 1994), with females reaching a larger size than males. In flatfish this is a result of the onset of sexual maturity at an earlier age in males (Roff 1981). This is also evident in *Synaptura marginata*. There were no differences in the length-weight, width-weight and length-width relationships between the sexes, which corresponds with what has been found for other commercially important South African flatfish species, e.g. *Austroglossus pectoralis* (Hecht 1976) and *Cynoglossus zanzibarensis* (Booth & Walmsley-Hart 2000).

Marginal zone analysis of otoliths confirmed the annual formation of growth zones. The first annulus was also validated by counting daily growth increments within the first annulus. The physiological processes governing zone formation in otoliths are not well understood (Blacker 1974). Otolith growth occurs as a result of differential deposition of calcium carbonate and protein, due to changes in the metabolic rate of the fish (Casselman 1974, Simkiss 1974, Griffiths 1988, Buxton & Clarke 1989, Lang 1992).

Exogenous factors including temperature, food availability and photoperiod, as well as endogenous factors such as spawning and stress have been shown to affect the metabolic rate of fishes and thus zone formation in the otoliths (Campana & Neilson 1985, Weatherly 1990, Beckman & Wilson 1995).

The optically dense opaque zone was found to be laid down during the period March to September (late summer to early spring) and the wider translucent zone from October to February (summer), coinciding with the breeding season (see Chapter 5). This pattern of growth zone deposition appears to be a common feature in flatfish (sole, plaice, turbot and brill) from temperate areas (Arneri *et al.* 2001) and has also been noted for South African species like *A. pectoralis* (Hecht 1976) and *C. zanzibarensis* (Booth & Walmsley-Hart 2000). Many other South African marine teleosts (Geldenhuys 1973, Hecht & Baird 1977, Coetzee & Baird 1981, Buxton 1987, Booth & Buxton 1997) show the opposite pattern of growth zone deposition. In these species, the opaque zone is laid down during the breeding season as a consequence of a reduction in growth rate due to the metabolic drain of spawning (Wallace & Schleyer 1979).

The deposition of the opaque zone during the winter months in flatfish is suggested to be a consequence of lower temperatures or a reduction in food availability (Arneri *et al.* 2001). Clarke (1988) however, found that food availability on sub-tidal reefs on the South African east coast does not change during the year. Given that the main prey item of *S. marginata* (Chapter 4) is associated with reefs, it is highly unlikely that food availability would have affected growth zone formation. There is thus a strong possibility that temperature was the main factor affecting seasonal zone formation, with the lower winter temperatures resulting in a reduced metabolic rate, thus causing the formation of the opaque zone.

The length-at-age data and the growth model estimates suggest that *S. marginata* in South Africa is a relatively fast growing species with a lifespan of approximately seven years. The mean maximum theoretical length (429.5 mm TL) predicted from the Von Bertalanffy growth model was slightly higher than the maximum observed length of 410 mm TL recorded during this study. Smith and Heemstra (1986), however, recorded a maximum length of 500 mm TL for this species, which falls within the upper 95%

confidence interval of 578 mm. Reports from anglers (although unsubstantiated) suggest that the species may grow to 550 mm TL. There are no reports on the maximum length attained by *S. marginata* in other areas in its Indo-west Pacific distribution.

The growth model predicts a fast growth rate for the first two years. This corresponds to the onset of 50% sexual maturity, predicted to be at 235 mm (Chapter 5). A similar pattern of growth has been observed for other soleids and cynoglossids (Zoutendyk 1974, Hecht 1976, Ramos 1982, Payne 1986, Rajaguru 1992, Terwilliger & Munroe 1999, Booth & Walmsley-Hart 2000). This change in growth rate after the onset of sexual maturity is attributed to the utilization of available energy for reproduction instead of somatic growth (Howell *et al.* 1995, Booth & Walmsley-Hart 2000, Berill *et al.* 2003).

In many marine teleost species, males grow faster than females (Pauly 1994). This is also the case in various Pleuronectiformes, like the cynoglossid, *C. zanzibarensis* (Booth & Walmsley-Hart 2000), the bothid, *Paralichthys olivaceus* (Shouzeng 1995) and the pleuronectid, *Pleuronichthys cornutus* (Shouzeng 1995). However, in some species such as the bothid, *Paralichthys lethostigma* (Luckenbach *et al.* 2003), the pleuronectids, *Hippoglossus hippoglossus* (Bjørnsson 1994) and *Limanda limanda* (Lozán 1992) and two soleids, *Solea solea* and *Solea lascaris* (Deniel 1990), females have a faster growth rate than males. There seems to be no pattern with respect to the various flatfish families. Comparison between the male and female length-at-age for the first three years for *S. marginata*, showed no appreciable difference in the growth rate between the sexes, but more male specimens are required to substantiate this assumption.

Chapter 4: Feeding

Introduction

The growth and production of all farmed fish is dependent on the intake of essential nutrients. Under intensive, commercial scale aquaculture conditions fish are normally provided with formulated feeds as a complete source of dietary nutrients. These feeds (pellets) have an overall nutrient profile which approximates as far as economically viable the known dietary nutrient requirements of the culture species (Tacon 1995).

Food represents a large part of the production costs for intensive aquaculture and is normally in the region of 42 – 52 % of the total operating costs (Shepherd 1988). Of all the nutrients, protein is probably the most important as its high price affects the costs of formulated diets (Watanabe 1988, Benitez 1989). Energy in the diet is supplied by protein, carbohydrates and lipids. Fish have a limited ability to digest carbohydrates and therefore utilize lipids and proteins as the main energy source. The Dover sole, *Solea solea*, however, shows an unusually high ability to digest and metabolise carbohydrates, partly due to strong amylase and maltase activities in the digestive tract (Cowey *et al.* 1975, Clark *et al.* 1984). The primary concern in fish nutrition is to find a compromise between the minimum dietary protein content, a high growth rate and satisfactory feed utilisation (Steffens 1989).

The amino acid profile of the dietary protein is also of particular importance, since this determines the nutritional value of a protein source. This is especially true with respect to the essential amino acids (EAA). EAA are those that a fish cannot synthesize itself and are obtained entirely from the dietary protein (Cowey *et al.* 1970). A diet deficient in any one of the EAA will result in poor growth and a decreased food conversion efficiency, even if the diet has a high protein level (Piper *et al.* 1982). While animals can synthesize the non-essential amino acids at a rate sufficient enough to satisfy normal somatic

growth, they may not be able to synthesize them at a sufficient rate to meet other physiological requirements such as reproduction (Cowey *et al.* 1970).

It is suggested that fish, in common with other vertebrates, require the same 10 EAA (Ketola 1982). These amino acids are: arginine (arg), histidine (his), isoleucine (ile), leucine (leu), lysine (lys), methionine (met), threonine (thr), phenylalanine (phe), tryptophane (trp) and valine (val), although cystine (cys) and tyrosine (tyr) are sometimes considered to be semi-essential amino acids (Steffens 1989). Cystine spares part of the methionine requirement in fish, and although not fully substantiated, it is thought that tyrosine probably has a similar sparing effect on phenylalanine in fish diets (Ketola 1982). Sole and plaice show the same EAA requirements as other fish (Cowey *et al.* 1970, Guillaume *et al.* 1991). Proline was, however, synthesised very slowly and may indicate that flatfish have an extra requirement for this amino acid, although it is not essential (Cowey *et al.* 1970). If there is a lack of knowledge on the EAA requirements of a particular species, the EAA pattern of the entire body tissue can provide a first approximation of the required amino acid composition of the dietary protein (Cowey & Tacon 1982, Wilson 1985).

It is equally important to optimise broodstock nutrition. Broodstock nutrition is one of the most poorly understood areas of finfish nutrition (Izquierdo *et al.* 2001). Unpredictable and variable reproductive performance is an important limiting factor for successful mass production of juveniles, and can often be directly linked to broodstock nutrition (Bromage 1995, Izquierdo *et al.* 2001). This is especially true for the total lipid and fatty acid contents of the broodstock diet, which have been shown to affect egg and larval quality (Watanabe *et al.* 1984a, b). Due to the lack of information of their nutritional requirements, broodstock are most often fed on their known natural diet, or a combination of artificial food and natural prey, as is the case for *Solea senegalensis* (Dinis *et al.* 1999) and *Hippoglossus hippoglossus* (Olsen *et al.* 1999). This is practised to ensure that all the essential nutrients needed for gonad development are provided. A sound knowledge of the nutritional requirements of a potential culture species is thus of utmost importance for aquaculture (Cowey *et al.* 1970, Windell & Bowen 1978, Royce 1984).

Much of our understanding of the ecological role of fish populations has been derived from dietary studies (Windell & Bowen 1978). De Groot (1971) clarified the interrelationships between the morphology of the alimentary tract, the type of food, the feeding behaviour and diurnal activity patterns for pleuronectiform fishes. Based on these interrelationships he was able to categorise flatfish species into three feeding groups:

i) Fish feeders – Psettodidae, Bothidae, Pleuronectidae
ii) Crustacean feeders – Pleuronectidae, Cynoglossidae
iii) Polychaete-mollusc feeders – Soleidae, Pleuronectidae

Fish from the same feeding group showed remarkable taxonomic affinities and also physiological similarities. Although no feeding study has been conducted on *S. marginata*, De Groot (1971) investigated the morphology of the alimentary tract and predicted that it belongs to the Class iii feeding group, together with other species of this genus, *Synaptura nigra* (Roughley 1951), *S. kleini* (De Groot 1971) and *S. zebra* (Ochiai 1966).

The buccal and pharyngeal cavities, the oesophagus and the stomach form about 20 % of the alimentary tract in *S. marginata* and pyloric appendices are absent (Ochia 1966, De Groot 1971). The significance of these morphological characteristics is clear if one considers the size of the prey (De Groot 1971). There is no need for a large buccal cavity, a pharyngeal cavity, oesophagus or stomach when the dominant prey is small (De Groot 1971).

Soleids find their food mainly by olfactory cues, but visual stimuli can also play a role. Soleids also show very low activity during the day. At night they actively search for prey on the bottom, especially on sandy and/or muddy substrata. Feeding continues throughout the night till early morning, after which the stomach gradually empties (De Groot 1971).

The main objective of this feeding study was to obtain some preliminary information on the dietary requirements of *S. marginata*. In particular, since

most of the fish caught were sexually mature, the data would provide a first approximation of the diet requirements of broodstock.

Materials and methods

The alimentary tract was removed from all fish that were captured by cutting through the oesophagus just before the stomach, and the small intestine just before the anus. The contents of the small intestine could not be identified due to the high degree of digestion of prey items. Therefore only the stomachs were dissected and the contents, if present, removed and preserved in a 10% buffered formalin solution in cross-referenced sample bottles. After preservation, prey items were identified to the lowest possible taxonomic group under a dissecting microscope (Day 1969, Branch *et al.* 1994) and the number of individuals of each prey type counted and weighed.

A number of gut content analysis methods are available, none of which can be used alone due to bias created by number, mass or weight of prey items (Hynes 1950, Windell 1968, Hyslop 1980, Cortés 1997). Cortés (1997) summarises a number of new methods for study of stomach contents and concluded that the index of relative importance (IRI) is the most appropriate with least amount of bias. The IRI is described by the equation;

$$IRI = (\%N + \%W) \times \%O$$ (Pinkas *et al.* 1971, Hobson 1974)

i) frequency of occurrence (%O) is the number of stomachs containing each taxonomic group and expressed as a percent of the total number of stomachs examined

ii) contribution by number (%N) is the number of individual in each prey species group and expressed as a percent of the total of all the items in all species groups in all the stomachs examined

iii) wet weight: each taxonomic group was individually weighed for each stomach. The values were then expressed as a percent (%W) of the total of all the items in all species groups in all the stomachs examined

Proximate composition and Amino acid analysis

The proximate composition of the fish and the dominant prey item was determined. Two fish were used for proximate analysis. Both specimens (267 & 324 mm TL) were caught in April 2002 (after the spawning season) and frozen fresh as soon as possible after capture. The gonads of the fish were removed. Specimens of the dominant prey species, *Morphysa sanguine* (see results) were collected from their natural habitat and also frozen fresh. The fish and the prey were freeze dried and the samples where milled in a mortar to pass through a 0.5 mm screen.

Crude protein content was determined using the Micro-Kjeldahl method (AOAC 1984). Five samples of fish and five samples of prey of approximately 100 mg each were weighed into digestion flasks. 2.5 g Selenium catalyst and 2.5 ml of concentrated sulphuric acid was added to each flask, including the blanks. These flasks were placed on the preheated block and washed with 1 ml hydrogen peroxide every 10 minutes for 60 minutes. On completion, the digestion tubes were left for a further 20 minutes, then removed from the heating block to cool. Whilst the flasks cooled, an equivalent number of 200 ml Erhlenmeyer flasks were set up. Approximately 10 ml distilled water was added to each digestion flask and the contents transferred to the clean steam-distillation flask. The digestion flask was rinsed with 10 ml caustic/hypo mixture and then transferred to the distillation flask. This was then positioned in the Steam-distillation apparatus and distilled for seven minutes. A prepared 200 ml Erhlenmeyer flask was set up in the collecting position so the tip delivery tube was submersed in the boric acid solution. The resultant solution in the Erhlenmeyer flask was titrated with the standard 0.015 Molar HCl solution to the grey end-point. The percentage nitrogen was calculated from the volume of HCL titrated according to the formula:

$N = [M\ Cl \times 14.007 \times 100] / \text{weight of sample (mg)}$,
where M Cl is the volume of HCl (litre) used for the titration multiplied by the concentration of 0.015.

Percent crude protein was calculated as N x 6.25 (AOAC 1984). The procedures for formulating chemical solutions are according to Steyn (1957).

Total crude fat content was determined gravimetrically after extraction, according to the method used by Knauer *et al.* (1994), modified from Folch *et al.* (1957). Five 0.2 g sub-samples of powdered fish as well as five 0.2 g sub-samples of prey were re-hydrated with 3ml distilled water. Methanol (6.25 ml) and chloroform (6.25 ml) were added to each of the re-hydrated samples to form solutions. These solutions were homogenized for 2 minutes, after which 6.25 ml distilled water was added. The solutions were then homogenized for another minute. The homogenized solutions were then centrifuged at 3000 g for 10 minutes. A total of 0.75 ml of the bottom layer of the supernatant, containing the crude fat, from each of the solutions was pipetted into clean, dry crucibles of known weight and evaporated to dryness on a hot plate. The crucibles were cooled in a desiccator and weighed to give the weight of the crude fat in each sub-sample. Percentage fat was calculated according to the formula:

% Fat = [(mass of fat (g) x 1.67) / mass of sample (g)] x 100

(Knauer *et al.* 1994)

The percent moisture was determined by weighing 3 x 1.0 g fresh fish samples and 3 x 0.1 g fresh prey samples before and after drying at 70 °C until a constant weight was achieved.

Ash was determined by burning 3 x 0.5 g dried (at 70 °C) powdered sample in open crucibles in a muffle furnace at 550 °C for 7 h (Montgomery & Gerking 1980) and cooled in a desiccator. Following that, the final weight was determined.

A sample of the protein was hydrolysed and the constituent amino acids isolated by ion-exchange chromatography and finally purified by partition

chromatography (Cowey *et al.* 1970). Tryptophane, destroyed during acid hydrolysis (Cowey *et al.* 1970), was determined using ion-exchange chromatography (AOAC 1990). The analyses were conducted by Marianne Hundley of the Department of Animal Science, University of Natal.

The essential amino acid (A/E) ratio (Arai 1981 in Ogata *et al.* 1983) is commonly used to express the EAA balance of a protein source (Ogata *et al.* 1983, Cowey *et al.* 1985, Halver 1989, Moon & Gatlin 1991). Since all amino acids, excluding those that are essential, can be synthesized from compounds normally available in the body, it is the A/E ratio of a protein source that determines the nutritional value of that source. Although the A/E ratio is commonly used, it only considers the ratio at a specific time, not contributing any information on the rate a particular EAA is used. The true EAA requirements can only be determined through feeding studies. A/E ratios are thus only a guideline for feeding studies.

Using the amino acid profiles of the sole, *S. marginata* and *Morphysa sanguine*, the theoretical essential amino acid ratio was calculated. This ratio, as defined by Ogata *et al.* (1983) and is expressed as follows:

A/E ratio = (EAA / Total EAA) x 1000

Where EAA = essential amino acid value in g / 100 g dry matter and the total EAA includes tyrosine and cystine (combined with phenylalanine and methionine respectively). The A/E ratio of *M. sanguine* can then be compared to that of the sole, and the limiting EAA identified. The A/E ratio for low temperature Danish fishmeal (A grade) was also calculated and compared to *S. marginata*, since fishmeal is the main protein source in formulated diets (Hertrampf & Piedad-Pascual 2000).

Results

Only 25 of the 148 sole (16.9%) caught during the sampling period contained food in their stomachs. While some sand and pieces of the green seaweed *Caulerpa filiformis* were sometimes present in the stomachs, *S. marginata* feeds exclusively on polychaetes. Sand and *Caulerpa filiformis* fronds are assumed to be taken up accidentally while actively feeding for polychaetes on sand covered reefs. Three polychaete species were positively identified. These were *Arabella iricolor*, *Morphysa sanguine* and *Lumbrineris tetraura.* Four *Orbinia* species (Polychaetes) were also present in the stomachs. The *Orbinia* species could not be identified to species level due to the high degree of digestion.

In terms of frequency of occurrence, percent number, percent weight and IRI, *Morphysa sanguine* was the most dominant prey item contributing 95.5% to IRI. The second most important polychaete prey species was *Arabella iricolor.* The others each contributed less than 1% to the diet (Table 4.1).

Table 4.1: Relative importance of prey items by frequency of occurrence (% O), number (% N), weight (% W) and IRI for *S. marginata* (n=25).

Prey Species	% N	% W	% O	IRI	% IRI
Arabella iricolor	14.9	4.4	20	385.9	3.2
Lumbrineris tetraura	4.5	1.3	12	69.8	0.6
Orbinia spp.	6.0	1.2	12	86.6	0.7
Morphysa sanguinea	74.6	93.0	68	11401.9	95.5

The results of the proximate analysis for *S. marginata* and *Morphysa sanguine* are presented in Table 4.2. The disparity in the sum of the percentages of crude protein, fat and ash for especially *M. sanguine* in the proximate analysis, can be contributed to mainly carbohydrates.

Table 4.2: Proximate analysis (g / 100 g dry weight) of *Synaptura marginata* and the dominant polychaete prey item, *Morphysa sanguine*.

	S. marginata	*Morphysa sanguine*
Moisture	78.6	84.9
Crude Protein	74.1	57.6
Fat	0.7	4.7
Ash	20.0	19.5

The amino acid profiles of *S. marginata* and *M. sanguine* are presented in Table 4.3 and the A/E ratio in Table 4.4.

Table 4.3: Amino acid composition (g / 100 g dry weight) of *Synaptura marginata* and the dominant prey item. Essential amino acids are in bold.

Amino Acid	*S. marginata*	*M. sanguine*
Aspartic	8.18	5.80
Threonine	**3.03**	**1.77**
Serine	2.71	1.30
Glutamic	11.27	8.28
Proline	4.08	1.91
Glycine	3.93	3.47
Alanine	5.59	3.38
Valine	**3.90**	**2.61**
Methionine	**2.06**	**1.05**
Isoleucine	**3.50**	**2.40**
Leucine	**5.82**	**4.07**
Tyrosine	2.15	1.42
Phenylalanine	**3.01**	**2.20**
Histidine	**1.60**	**1.12**
Lysine	**6.50**	**3.87**
Arginine	**5.27**	**3.87**
Ammonia	4.63	4.22
Cystine	0.76	0.98
Tryptophane	**0.68**	**0.71**

Table 4.4: Essential amino acid ratio of *Synaptura marginata*, *Morphysa sanguine* and low temperature Danish fishmeal.

EAA	*S. marginata*	*M. sanguine*	Fishmeal
Arginine	140	152	**127***
Histidine	42	44	55
Isoleucine	93	95	102
Leucine	155	161	165
Lysine	173	**153***	174
Phe + Tyr	137	143	**90***
Cys + Met	75	80	**69***
Threonine	81	**70***	95
Valine	104	103	123

* denotes limiting amino acids relative to the EAA ratio of *S. marginata*.

Table 4.4 clearly shows that the EAA ratio of fishmeal, the main constituent of artificial food, lack some EAA like Arginine, Phenylalanine and Methionine that may be required by *S. marginata*. The dominant prey, *M. sanguine*, lacks only two EAA required by *S. marginata*. These are Lysine and Threonine.

Discussion

The low number (25) of fish with prey in their stomachs could be due to various factors. Firstly, it could be due to the nocturnal feeding habit of flatfish (De Groot 1971), who suggested that flatfish are mainly nocturnal feeders, which digest food very quickly after feeding. Since all of the sampling took place during the day (mainly between 9 and 11 am), it may be hypothesised that only a few fish would still have any prey items in the stomach. It is also known that fish regurgitate their stomach contents after capture (Marais 1984) and this was observed on numerous occasions. The lack of stomach contents makes any complex gut analysis tentative. It was therefore not possible to determine with any degree of confidence whether there were any seasonal changes in diet, or ontogenetic dietary shifts.

Stomach content analyses confirmed that *S. marginata* was in the polychaete-mollusc feeding group, as suggested by De Groot (1971). The only other feeding study of sole in South Africa was that of Hecht (1976) who examined the gut contents of *Austroglossus pectoralis*. This species also fed mainly on polychaetes and amphipods.

Morphysa sanguine, the dominant prey item of *S. marginata*, is not commonly available to the sole, since it inhabits tubes found in deep crevices among intertidal and shallow sub-tidal reefs (Branch *et al.* 1994). It is hypothesised that moving sand banks interfere with the normal water flow within these tubes. This may create anoxic conditions within the tubes, forcing the polychaetes out, making them available to the sole as prey (Chapter 2). The other prey items, including *Arabella iricolor*, *Lumbrineris tetraura* and *Orbinia* spp. inhabit sand and muddy banks (Branch *et al.* 1994), where they are easily accessible to the sole when exposed through wave action.

Proximate analyses, the AA profile and the A/E ratios can give useful indicators for the formulation of diets under aquaculture conditions (Cowey & Tacon 1982, Cowey *et al.* 1985, Wilson 1985, De Silva & Anderson 1995, Kaushik 1998). Since most of the fish were captured during the spawning season and were sexually mature (Chapter 2), the information obtained from the analyses is only relevant in the design of broodstock diets, and may not be appropriate for other ontogenetic stages.

The protein content of *S. marginata* (74.1 %) falls within the range of crude protein contents of marine finfish, which varies between 65 and 75 % (Wilson 1989). Crude protein requirements under culture conditions are influenced by species, age and temperature (Cowey *et al.* 1970, Cowey *et al.* 1972). The crude protein level of *M. sanguine* (58%) suggests that broodstock *S. marginata* may have a high protein requirement. In general, nutritional studies on flatfish indicate that they have a higher crude protein requirement in comparison to other finfish species. For example, the best performance for the Dover sole, *Solea solea* was achieved with a dietary crude protein level of 57 – 58 % (Cadena-Roa 1983 in Guillaume *et al.* 1991). Similarly,

Pleuronectes platessa (Pleuronectidae) showed an optimal crude protein requirement level of 57 % (Cowey *et al.* 1972). The proximate composition of *M. sanguine* falls within the range of other polychaetes or vermi meal products (Hertrampf & Piedad-Pascual 2000).

Although there is a big difference in the crude protein level between *S. marginata* and *M. sanguine* (74.1 and 57.6 %, respectively), a comparison in the A/E ratio between the two (Table 4.4) shows that the protein content of *M. sanguine* meets the EAA requirements of sole, except for Lysine and Threonine. Low temperature Danish fishmeal lacks Arginine, Phe + Tyr and Cys + Met. A comparison of the A/E ratio between *S. marginata* and low temperature Danish fishmeal indicates a high requirement for Arginine, Phe + Tyr and Cys + Met. In turbot, *Scophthalmus maximus*, Phe + Tyr and Cys + Met were also found to be the limiting essential amino acids (Burel *et al.* 2000). Any artificial diet, made from fishmeal should take this into consideration to ensure a balanced protein for optimal growth and food utilisation. Table 4.3 suggests that broodstock diet, consisting mainly of fishmeal, would have to be supplemented with vermi meal to ensure the right combination of EAA. According to Hertrampf & Piedad-Pascual (2000) vermi meal protein usually has a digestibility of above 95 %. *Solea solea* show a 96 % digestibility of vermi meal made from different oligochaetes (Hepher 1988).

The total fat level obtained for *S. marginata* of 0.7 % is much lower than the level obtained for a host of other fish species, which ranges between 2.4 and 17.9 % (Hertrampf & Piedad-Pascual 2000). This casts some doubt on the result. Never the less, a possible reason for this may be that the fish were obtained just after the spawning season, when fat reserves could have been at a lower point. This pattern of resource allocation is common among many marine teleosts (Wallace & Schleyer 1979).

To summarise, *S. marginata* feeds principally on polychaetes of which *M. sanguine* is the preferred prey item. Given the high protein content of its preferred prey, suggests that broodstock *S. marginata* may have a high

protein demand. The A/E ratios would also suggest that a fishmeal based diet for broodstock may have to be supplemented with vermi meal.

Chapter 5: Reproduction

Introduction

An understanding of the reproductive biology is fundamental for the process of selecting suitable species for aquaculture. Egg size, size at 50% maturity, fecundity, sex ratio and reproductive seasonality all play an important role in selecting a species for aquaculture (Chapter 1) and provide valuable information for the development of specific technologies that might be appropriate for that species under culture conditions. For example, the natural sex ratio might be important to determine how many females are required per male in establishing broodstock populations. The information gained from a study of the reproductive biology of a species is also fundamental in order to effectively manage and promote the artificial maturation of captive fish (Bromage 1995).

Most marine teleosts are broadcast spawners that release many, small pelagic eggs into the water column (Bruton 1989). This is also generally true for flatfish (Pleuronectiformes), and has been noted for various species, e.g. *Solea solea* (Baynes *et al.* 1993), *Paralichthys olivaceus*, *Cynoglossus semilaevis*, *Zebrias zebra* (Shuozeng 1995), *Solea senegalensis* (Dinis *et al.* 1999), *Paralichthys dentatus* (Merson *et al.* 2000) and *Limanda aspera* (Nichol & Acuna 2001).

Broadcast spawners should only breed when and where particular environmental factors (the ultimate factors) are likely to be optimal for the survival of the progeny, since reproduction is associated with a considerable energy investment at the expense of somatic growth (Roff 1981 & 1982, Munro *et al.* 1990, Krebs & Davies 1997). These ultimate factors include predation, food availability and water quality, and have been shown to have major effects on larval survival (Munro *et al.* 1990), and especially in flatfish (Roff 1981).

The macroscopic development of ovaries has been well documented in many flatfish species (Hecht 1976, Cyrus 1991, Booth & Walmsley-Hart 2000, Caputo *et al.* 2001). Although the utility of macroscopic examination for determining the breeding season of a species is apparent in the field, the criteria used for macroscopic staging do not provide information on patterns and processes of oocyte development (Merson *et al.* 2000), and these are pivotal for developing captive breeding protocols (Smith *et al.* 1999). It is also evident that distinguishing immature fish from mature fish in the 'resting' phase can be difficult using macroscopic staging techniques (Ramsay & Witthames 1996) and are generally considered to be subjective (West 1990, Ramsay & Witthames 1996).

Generally, pleuronectiformes are gonochorists (Dinis *et al.* 1999, Devlin & Nagahama 2002), with a 1 : 1 sex ratio (Nikolsky 1978, Dagang *et al.* 1992). They usually have pelagic eggs, with the exception of *Pseudopleuronectes yokohamae,* which has demersal eggs (Shuozeng 1995). They also have long reproductive life spans and are iteroparous batch spawners, to compensate for the variable survival of eggs and larvae (Roff 1981).

Despite its very wide distribution throughout the Indo-west Pacific, the reproductive biology of *Synaptura marginata* has not previously been investigated. The aims of this study were to describe the reproductive biology of *S. marginata* by providing a description of the seasonal ontogenetic development of gonads, determining size at sexual maturity, sex ratio, reproductive seasonality and fecundity. A thorough knowledge of the reproductive biology will help to assess the suitability of this species for aquaculture.

Materials and Methods

Biological data were collected from all soles captured during the sampling period (see Chapter 2).

Sex ratio

The sex ratio of *Synaptura marginata* was determined using all adult fish. A Chi-square test was used to test for unity.

Reproductive seasonality

Reproductive seasonality was determined by two methods. The ovaries of all soles caught during the sampling period were visually assessed and assigned a maturation index stage. A seven stage macroscopic maturity scale was used (Nikolsky 1963) to differentiate between gonad stages. A similar scale was used by Hecht (1976) for the Agulhas sole, *Austroglossus pectoralis* and by Cyrus (1991) for *Solea bleekeri.* It was impractical to assign macroscopic maturity stages to the testes, due to their small size.

The stages were assigned to ovaries on the basis of the following descriptions.
Stage 1: Immature – Ovaries are pinkish-white in colour, and are thin, elongated, thread-like organs.
Stage 2: Quiescent or inactive/recovery – Ovaries are a little bigger than in the previous stage and appear orange to pink in colour. No eggs are visible with the naked eye. It is very difficult to distinguish between stages 1 and 2.
Stage 3: Developing or active – Ovaries become light orange in colour and occupy about a third of the body cavity. Eggs can be seen with the naked eye after an incision is made through the ovi-sac.
Stage 4: Active/Ripe – Ovaries become yellow-orange in colour and occupy almost half of the body cavity.

Stage 5: Ripe – Ovaries occupy almost the whole body cavity. Oocytes are translucent and bright yellow and can be extruded by applying light pressure on the abdomen.
Stage 6: Spawning – Ovaries are in a similar state as above, but oocytes are extruded by simply handling the fish.
Stage 7: Spent – Ovaries are deflated and bloodshot after spawning.

The macroscopic maturity index was validated by a histological study of the ovaries, and adapted accordingly. The monthly percent distribution of the adapted maturation stages where then plotted and used as a predictor of spawning seasonality.

Reproductive seasonality was verified by calculating a monthly gonadosomatic index (GSI). To achieve this, the gonads of all fish from both sexes were removed and weighed (to the nearest 0.01 g) and expressed as a percent of eviscerated body mass on a monthly basis, using the following equation:

GSI = Gonad mass (g) / Eviscerated body mass (g) x 100

The monthly or seasonal relationship between length and weight (Fulton's condition factor, K) was used to assess the effect of spawning on the condition of the fish and also to determine the timing and duration of the spawning season. The exponent "b" of the length-weight relationship $W=aL^b$, was calculated from the entire sample of soles and Fulton's condition factor was calculated monthly using the equation:

$K = (W / L^b) \times 10^6$ (Ricker 1975, Cone 1989),

where W and L is the eviscerated weight (g) and the total length (mm) of a fish, respectively, and b is the exponent of the length-weight relationship for all the fish sampled. Seasonal differences were then tested using ANOVA.

Gonad histology

Monthly gonad samples from both males and females (n = 139) were fixed in a 10 % buffered formalin solution. After seven days of fixation, the gonad tissue was stored in 70% ethanol. Methods described by Sumner & Sumner (1969), Bernard & Hodgson (1988), and Hinton (1990) were employed to prepare and section tissue, and are briefly described here.

Tissue was trimmed to blocks of about 5 x 5 x 5 mm and dehydrated by passage through a series of ethanol solutions of increasing concentrations (80 %, 90 % and 2 x absolute ethanol) each for an hour. Dehydration is essential prior to embedding the tissue in paraffin wax, as the wax will not penetrate tissue in the presence of water (Hinton 1990). To reduce and prevent cell shrinkage, the ethanol was then removed from the tissue by immersion in two solutions of 100% xylene, each for an hour. Impregnation of tissue with paraffin wax took place over night under a vacuum of 460 mm / Hg at 57 °C (Townsen & Mercer Vacuum Oven). The samples were then imbedded in paraffin wax in small molds with pre-cast wax beds and left overnight to harden. These blocked samples were trimmed, mounted and sectioned with a microtome at 5 – 8 µm. The resulting ribbons were floated onto slides in a warm water bath (40 °C) and attached using Haupt's adhesive. The slides were dried over night at 37 °C. The basophilic cellular constituents were stained using haematoxylin and acidophilic constituents with eosin. The final sections were covered with DPX slide mountant and a coverslip. The slides were then examined and photographed under 40 - 400 x magnification.

Slides were read in a random order to prevent possible interpretational bias associated with prior knowledge of collection time and fish size. Gonads were assigned to a developmental stage based on the most mature cell(s) present, regardless of how many there were (West 1990, Sadovy *et al.* 1994), to validate the macroscopic staging. The classification of oocyte development and degeneration was based on criteria used by Buxton (1990) and Samoilys &

Roelofs (2000). The different oocyte stages were measured with an ocular graticule under a compound microscope.

Size at maturity

Female and male length-at-maturity was calculated by determining the proportion of reproductively active fish in each 2-cm size-class. Maturity was estimated by fitting a logistic ogive to the observed data using a non-linear minimization of squared residuals (Booth & Walmsley-Hart 2000), using the equation;

$$P_l = \frac{1}{1 - e^{(L - L_{50})/\delta}}$$

where P_l is the percentage of fish matured at length L, L_{50} the length at which 50 % of the fish in the size-/age class are sexually mature and δ the steepness of the ogive. The length at sexual maturity was taken as the size at which 50 % and 100 % of the population was mature, as defined by several authors (e.g. Beverton & Holt 1957, Booth & Walmsley-Hart 2000). The length at 100% maturity was estimated to ensure that all fish used as broodstock are sexually mature.

Fecundity

Because of the small size of the left ovary in comparison to the right ovary (see results), the following assumptions were made in all fecundity estimates. Firstly, that the oocytes in each of the two ovaries where at the same stage of development and secondly, that there was no significant difference between the number of vitellogenic oocytes per gram of ovary between the left and the right ovaries. Based on these assumptions, the fecundity estimates for the right ovary were extrapolated to total gonad weight.

Batch fecundity as well as relative fecundity were determined. Batch fecundity is defined as the potential number of eggs released during one spawning event in

multiple spawners based on oocyte counts (Hunter *et al.* 1985). Relative fecundity is defined as the total potential number of eggs spawned per gram of female per year based on oocyte counts (Hunter *et al.* 1985, Samoilys & Roelofs 2000). The gravimetric fecundity assessment method as described by Samoilys & Roelofs (2000) was used to determine relative and batch fecundity.

All preserved ovaries of known weight with hydrated eggs (n = 11) were used for oocyte counts. The oocytes in these ovaries were sufficiently developed to distinguish between hydrated and other advanced stage oocytes, especially secondary and tertiary yolk vesicle oocytes. Hydrated oocytes where easily distinguished from other vitellogenic oocytes due to their large size (x ± SD, 0.74 ± 0.09 mm in diameter). Secondary and tertiary vitellogenic oocytes ranged in size from 0.28 ± 0.06 mm and 0.51 ± 0.09 mm, respectively.

To estimate batch fecundity, one transverse tissue block of approximately 3 mm in width was cut from the proximal, medial and distal sections from the larger right (dorsal) ovary (n = 11). Each sub-sample was weighed to the nearest 0.01 g and these had a mean weight of 1.14 ± 0.23 g. Oocytes in each sample were teased apart with a dissecting needle and counted under a dissecting microscope at 10 x magnification. Hydrated oocytes, identified by their size as measured in the histological sections, where isolated and counted. The average number of hydrated oocytes for the three sub-samples from each ovary was extrapolated to whole gonad weight (left and right ovary) (Samoilys & Roelofs 2000).

The same method was also used to determine relative fecundity. All vitellogenic oocytes, including hydrated oocytes were counted in the sub-samples and extrapolated to whole gonad weight (left and right ovary) to give an estimated

total fecundity. Relative fecundity was then calculated by dividing the total number of vitellogenic oocytes in both ovaries (Total fecundity) by eviscerated fish mass (g). Total fecundity was plotted against fish size and weight, and modeled using regression analysis.

Results

Gonad structure

In *Synaptura marginata*, the gonads are situated posterior to the urogenital pore. The testes and ovaries are paired structures, and it is easy to distinguish between the sexes. The right ovary is in a secondary extension of the visceral cavity, while the left ovary is situated between the epipleural ribs and muscle tissue on the blind (left) side of the fish. Males have very small testes that do not exceed 0.4 g, in comparison with the ovaries that reach a mass in excess of 47 g. The testes, which are flat, lobe-like structures, did not vary in shape, size or colour, during the sampling period. Testes have a creamy-white colour. The ovaries are elongate tube-like structures. The ovaries varied in size and colour over the sampling period. The left (ventral) and right (dorsal) ovaries in *S. marginata* are not equally developed. The right ovary is approximately 96.4 % larger than the left ovary (Mann-Whitney U-test: $U = 119.5$, $p < 0.005$, $n = 70$) (Figure 5.1).

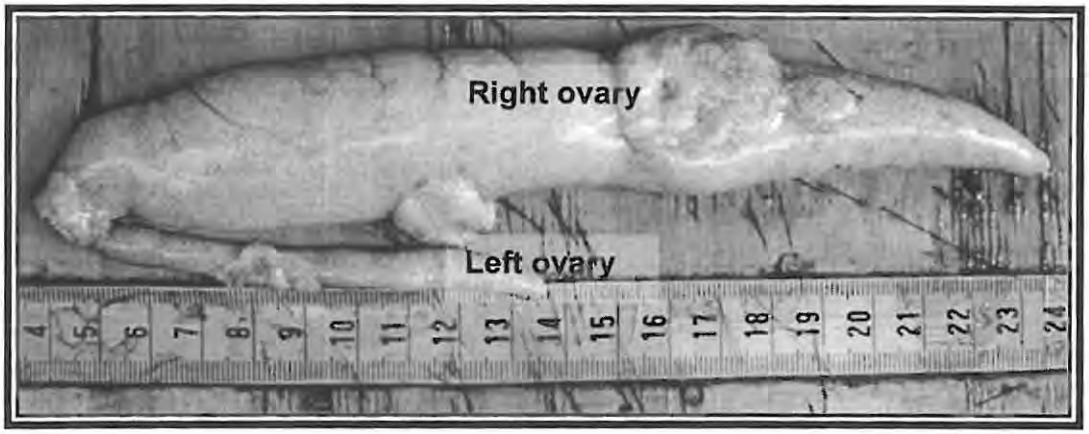

Figure 5.1: The size difference between the right and the left ovaries in of a 380 mm TL female *Synaptura marginata*.

Sex ratio

The total sample of fish obtained from December 2000 to March 2002 (n = 171) was dominated by females, with a ratio of 1 male: 5.1 females. This was significantly different from unity (1:1) (chi-square = 77.3, $p < 0.05$).

Macroscopic stages and seasonal oocyte development

Five ovarian developmental stages were identified from the histological examination. It was thus not possible to match the seven stage macroscopic maturity scale with the histological changes in ovary development. Using the histological data, the seven stage scale was corrected to a five stage scale. This was achieved by combining stage 3 (active) and 4 (active ripe) as well as 5 (ripe) and 6 (spawning) of the seven stage scale into stage 3 (maturing) and stage 4 (ripe), respectively, of the five stage scale (Table 5.1). The five revised macroscopic stages are described below, together with the corresponding histological developmental stages (Figure 5.2).

Stage 1 or **immature** ovaries (Plate 1) only have pre-vitellogenic oocytes, including oogonia, chromatin nucleolus oocytes, and early and late perinucleolus oocytes. The oocytes are uniformly and strongly basophilic. There is also no sign

Table 5.1: Female gonadal development stages and the equivalent macroscopic characteristics for *Synaptura marginata*. Defining characteristics are shown in bold italics. Other criteria are useful aids in assigning gonads to a developmental stage. Abbreviations refer to the annotations in the plates.

Developmental Stage	Oocyte Stages	Other Criteria	Macroscopic Characteristics
Immature, IM **Stage 1**	***Pre-vitellogenic oocytes:*** • oogonia, **oo** • chromatin nucleolus, **cns** • early perinucleolus, **eps** • late perinucleolus, **lps**	No sign of prior spawning: • ***thin gonad wall*** • lamellae not compact, often vaculated	The ovary is pinkish-white in colour, and is a thin, elongated, thread-like organ.
Resting, RE **Stage 2**	***Pre-vitellogenic oocytes:*** (as above)	Compact , lamellae well packed ***thick gonad wall***	Ovary is a little bigger than in the previous stage and appears orange to pink in colour. No eggs are notable with the naked eye.
Maturing, MA **Stage 3**	***Vitellogenic oocytes:*** • primary yolk vesicle, **pyv** • secondary and tertiary yolk vesicle, **syv, tyv**	May have atretic oocytes (**ao**), post-ovulatory follicles (**pof**)	Ovaries become yellow-orange in colour, and occupy almost half of the body cavity. Eggs can be seen with the naked eye after an incision is made through the ovi-sac.
Running Ripe, RR **Stage 4**	• migratory nucleus stage, **mns** ***Hydrated oocytes,*** **hy**	Post-ovulatory follicles and atretic oocytes may be present	Ovaries occupy almost the whole body cavity. Oocytes are translucent and bright yellow and can be extruded by simply handling the fish or applying a light pressure on the abdominal wall.
Spent, SP **Stage 5**	***Atretic vitellogenic oocytes,*** **ao** ***Pre-vitellogenic oocytes,*** **eps, lps**	Lamellae disrupted and disorganised	Ovaries deflated and bloodshot after spawning.

of prior spawning, i.e. having a very thin gonad wall with the lamellae often vaculated.
During this stage the ovaries are pinkish-white in colour and are thin, elongated, thread-like organs.

Stage 2 or **resting** ovaries (Plate 2) also contain only pre-vitellogenic oocytes like in stage 1, but there are proportionally more (up to 90%) late perinucleolus oocytes. The higher prevalence of late perinucleolus oocytes makes the sections less basophilic. The start of the zona radiata is visible just beneath the follicle layer in late perinucleolus oocytes. Lipid droplets and cortical alveoli also start to appear in the cytoplasm. The lamellae look compact, while the gonad wall is very thick. The presence of the thick gonad wall and compact lamellae indicates prior spawning activity.
During this stage the ovaries are slightly larger than immature ovaries and appears orange to pink in colour. No eggs are visible with the naked eye.

Stage 3 or **maturing** ovaries (Plate 3) have a preponderance of vitellogenic oocytes, including primary, secondary and tertiary yolk vesicle oocytes. Atretic oocytes and post-ovulatory follicles may be present and indicate previous spawning activity. During the early stages of maturation, many small lipid droplets and cortical alveoli are dispersed in the cytoplasm. The lipid droplets coalesce centrally and the cortical alveoli at the follicular envelope. This process occurs progressively throughout vitellogenesis.
During this stage the ovaries are yellow-orange in colour and occupy almost half the body cavity. Eggs can be seen with the naked eye after an incision is made through the ovi-sac.

Stage 4 or **ripe** ovaries (Plate 4) contain migratory nucleolus oocytes and hydrated oocytes. Sections become acidophilic, with the yolk globules staining red. The nucleus starts to migrate to one side of the cell during the migratory nucleus phase. Events taking place include the breakdown of the germinal

vesicle, coalescence of lipid droplets into a few larger oil droplets and hydration of oocytes.
During this stage the ovaries are translucent and bright yellow in colour and occupy almost the whole body cavity. Eggs can be extruded by simply handling the fish or applying light pressure on the abdominal wall.

Stage 5 or **spent** ovaries (Plate 5) are characterized by the presence of post-ovulatory follicles and atretic oocytes. Pre-vitellogenic oocytes, including early and late perinucleolus oocytes, and early vitellogenic oocytes, especially primary and secondary yolk vesicle oocytes can also be present, depending on the time of the last spawning event.
During this stage the ovaries are deflated and bloodshot. Some eggs might still be seen, giving it a slight orange appearance.

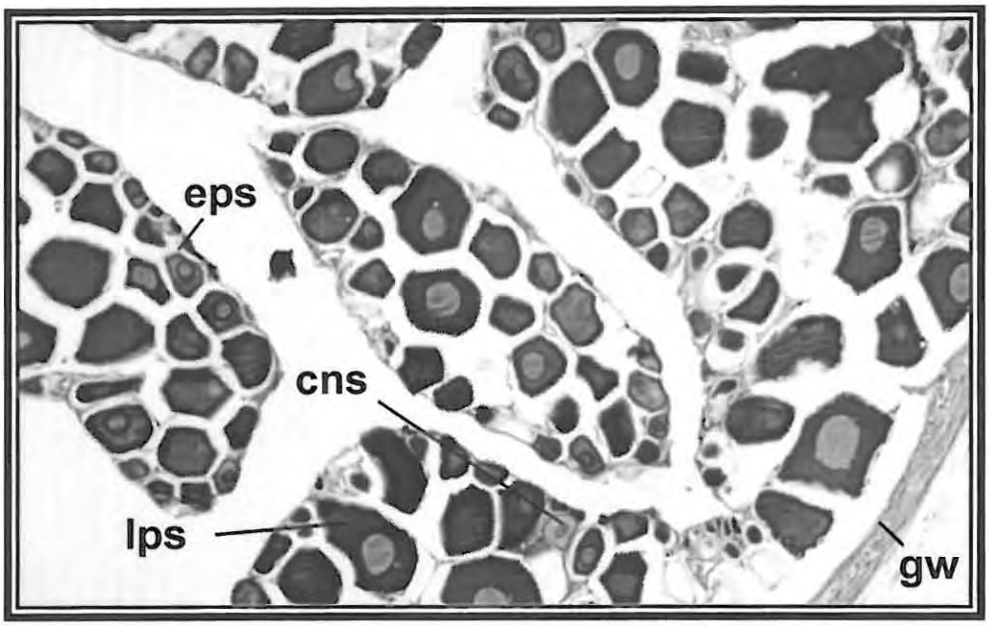

Plate 1. Immature female (see Table 5.1). cns = chromatin nucleolus stage oocyte, eps = early perinucleolus stage oocyte, lps = late perinucleolus stage oocyte, gw = gonad wall.

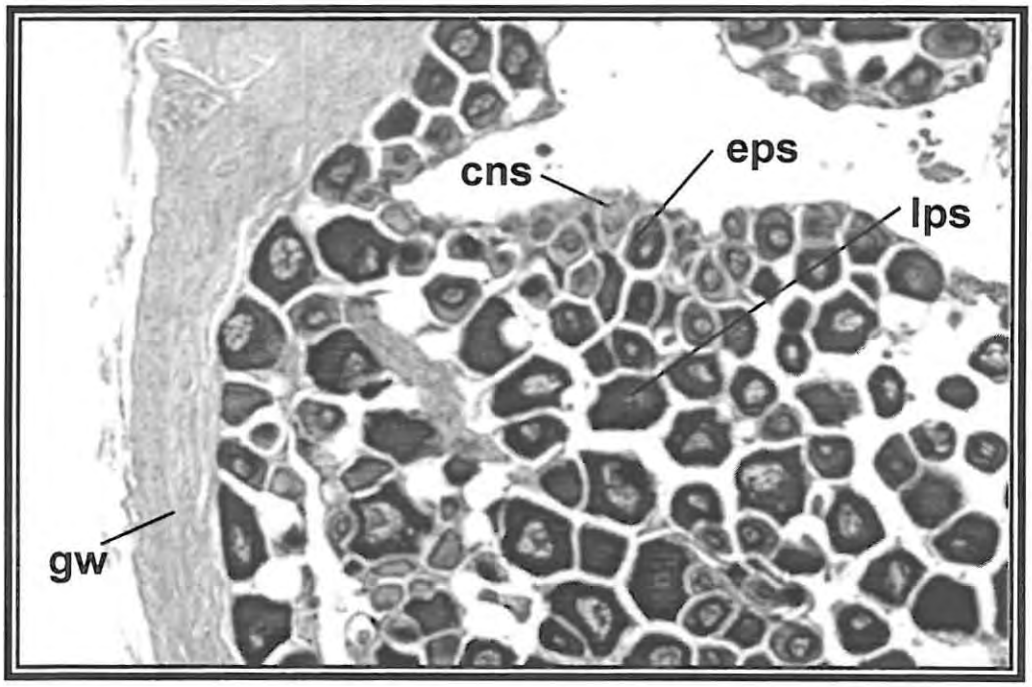

Plate 2. Resting mature female (see Table 5.1). cns = chromatin nucleolus stage oocyte, eps = early perinucleolus stage oocyte, lps = late perinucleolus stage oocyte, gw = gonad wall.

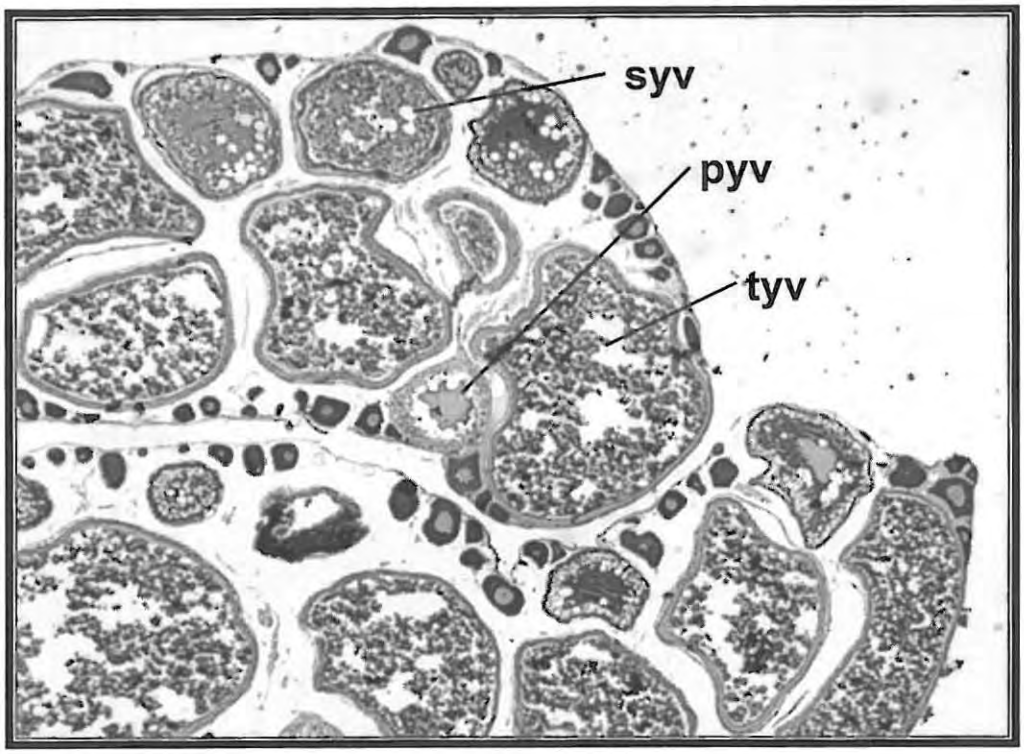

Plate 3. Vitellogenic or maturing female (see Table 5.1). pyv = primary yolk vesicle oocyte, syv = secondary yolk vesicle oocyte, tyv = tertiary yolk vesicle oocyte.

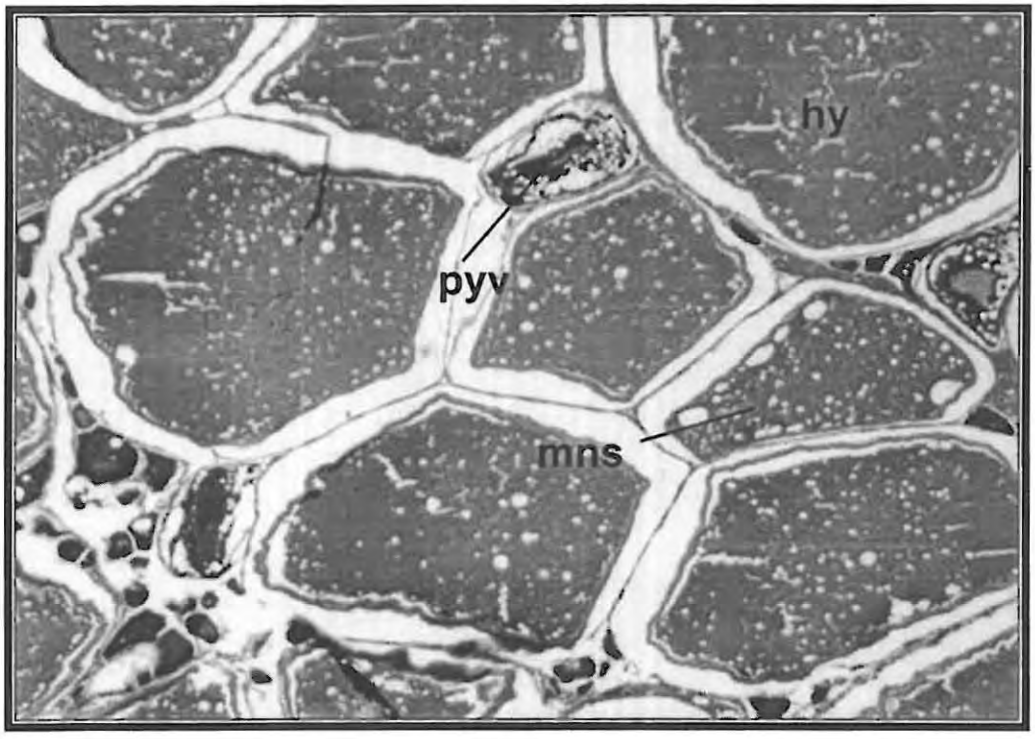

Plate 4. Hydrated or ripe females (see Table 5.1). mns = Migratory nucleus stage oocyte, hy = hydrated oocytes. Other abbreviations as in Plate 3.

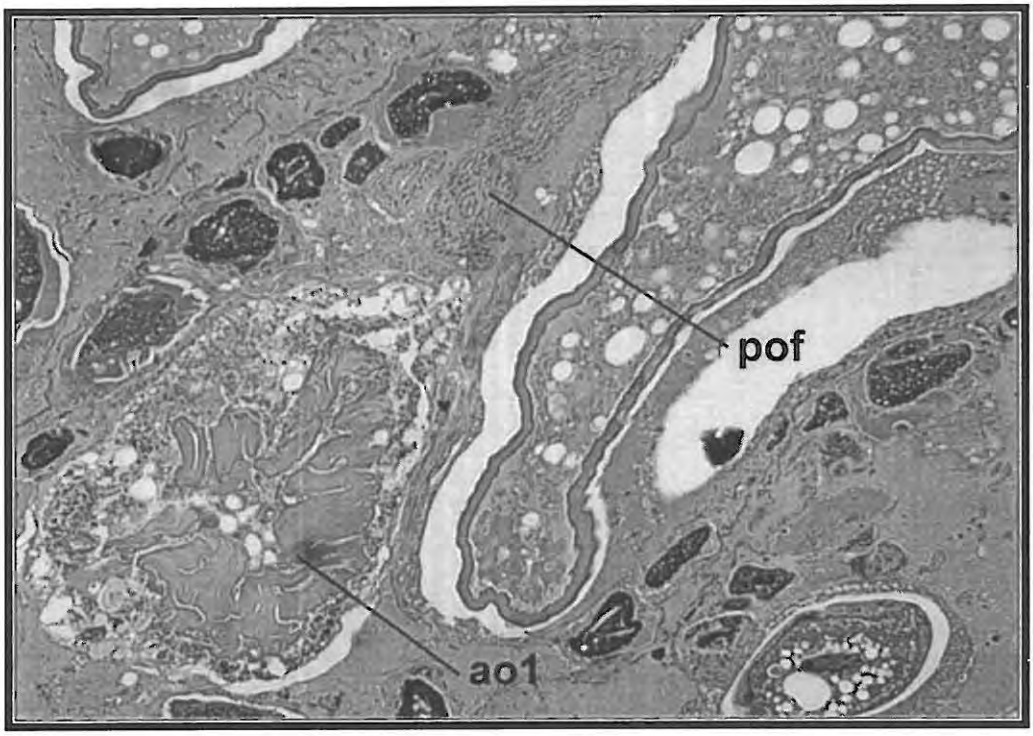

Plate 5. Spent female (see table 5.1).pof = post-ovulatory follicle, ao1 = early stage atretic oocyte.

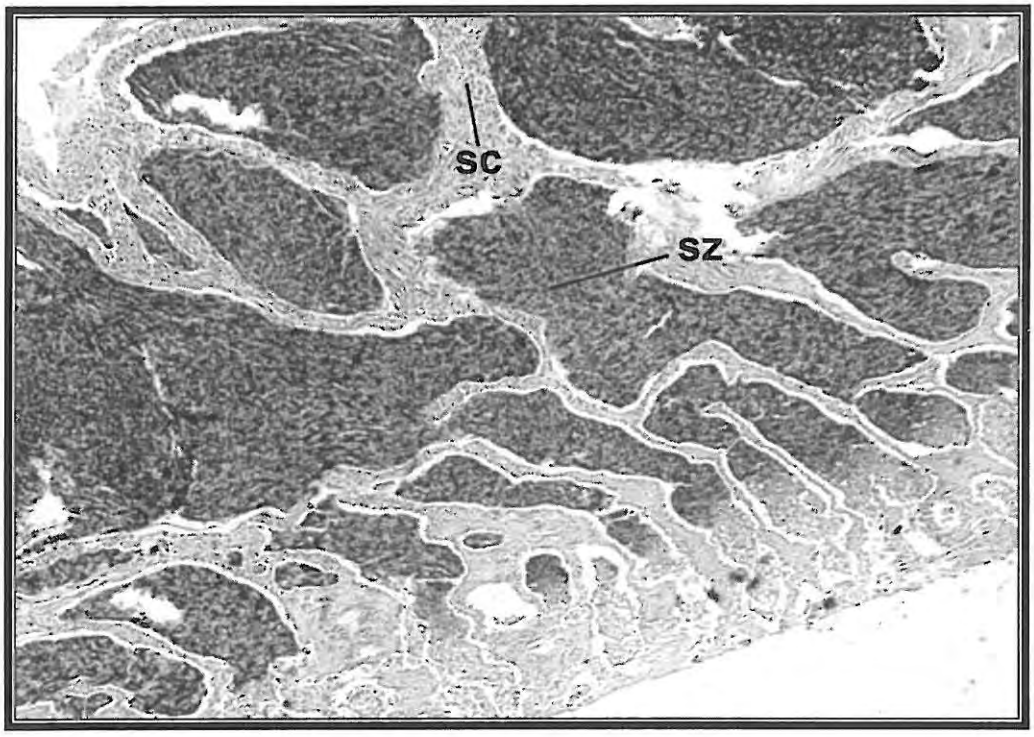

Plate 6. Mature male. sz = spermatozoa, sc = spermatocytes.

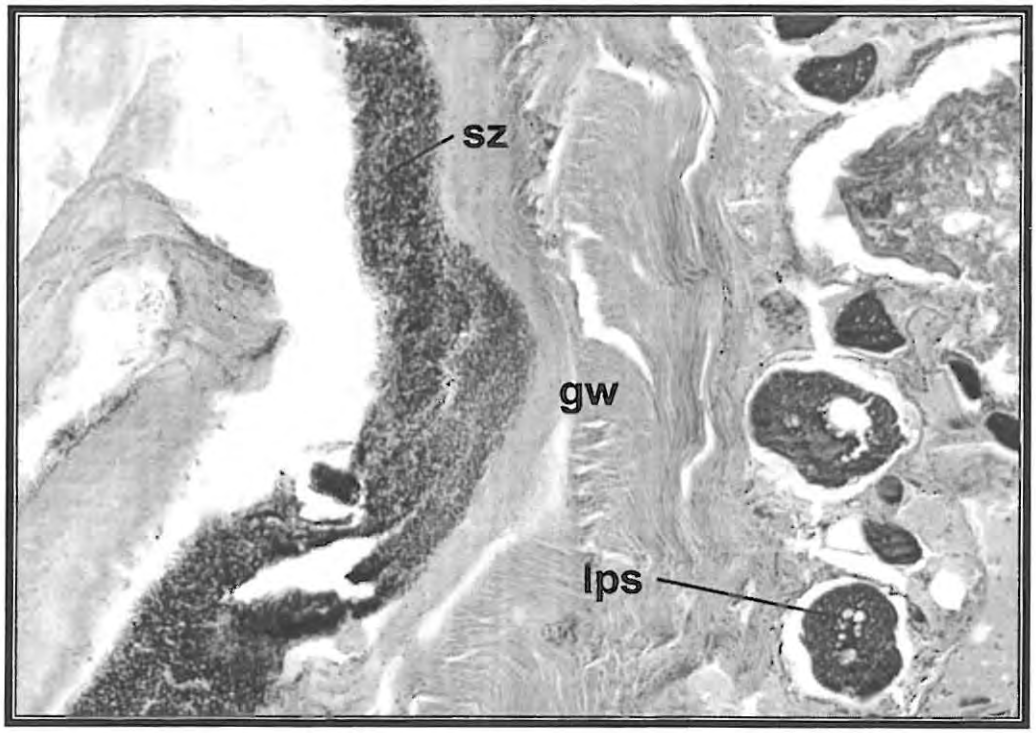

Plate 7. Rudimentary Hermaphrodite. gw = gonad wall, sz = spermatozoa, lps = late perinucleolus stage oocyte.

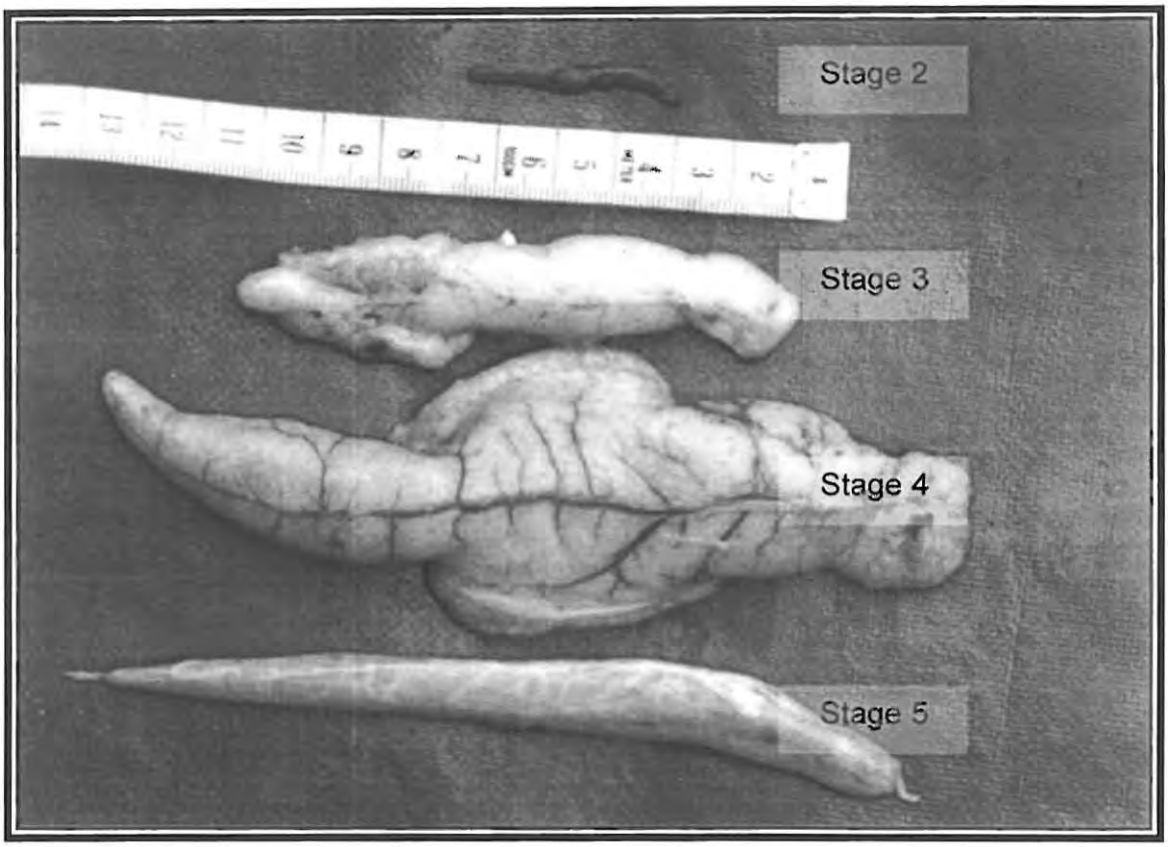

Figure 5.2: *Synaptura marginata* ovaries in four different maturity stages (five stage scale). Stage 2 = resting, Stage 3 = maturing, Stage 4 = running ripe and Stage 5 = spent.

The histological examination revealed that 16.2 % of ovaries were incorrectly assigned to macroscopic developmental stages. Classifying immature ovaries as resting ovaries constituted 83.3 % of the incorrect classification (Table 5.2).

Table 5.2: The classification of ovaries into different macroscopic developmental stages for *Synaptura marginata*. The italicized numbers were the ovaries that were incorrectly assigned.

Histological stages	Key indicator defining histological stage	Macroscopic developmental stages				
		1	2	3	4	5
1	Pre-vitellogenic oocytes, thin gonad wall	**10**	*16*			
2	Pre-vitellogenic oocytes, thick gonad wall		**28**			
3	Vitellogenic oocytes			**11**		
4	Hydrated oocytes			*1*	**20**	
5	Atretic oocytes			*2*		**29**

The low number of males caught during the 16 month sampling period (n = 22) did not allow for a similar detailed description of spermatogenesis. Although no males were captured in August, September and November, sperm was present in the testes throughout the other months. Plates 6 shows sectioned testis. The spermatozoa are very close together in large numbers, and have small nuclei, which stain dark blue with haematoxylin. The tails stain pink with eosin, but are difficult to recognize except under high magnification.

Testicular tissue was observed in 19 (17.8 %) of the 107 mature females gonads sectioned. There was, however, no pattern in this phenomenon with respect to fish size (Figure 5.3). The male tissue is separated from the female tissue by a thick layer of connective tissue (Plate 7).

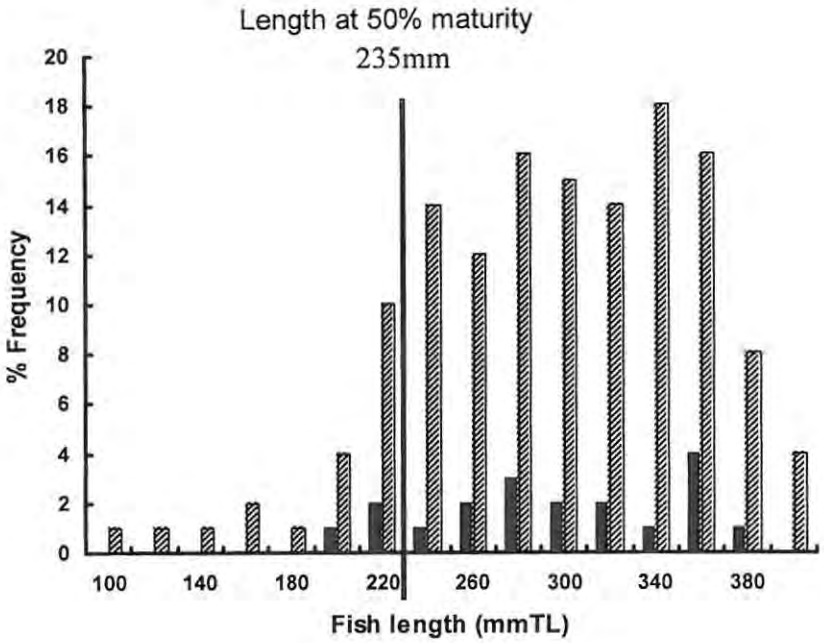

Figure 5.3: Size - frequency distribution of females and rudimentary hermaphrodites. Solid bars indicate rudimentary hermaphrodites.

Reproductive seasonality

The monthly percent distribution of macroscopic maturity stages for *S. marginata* is presented in Figure 5.4, suggesting a spawning season of about six months, from November to March. This is clear from the high percent of late maturity stages (ripe and spent ovaries) during this time. The female gonadosomatic index (GSI) shows maximum ovarian activity between October and April (Figure 5.5), which confirms the spawning period suggested by the gonad maturation indices.

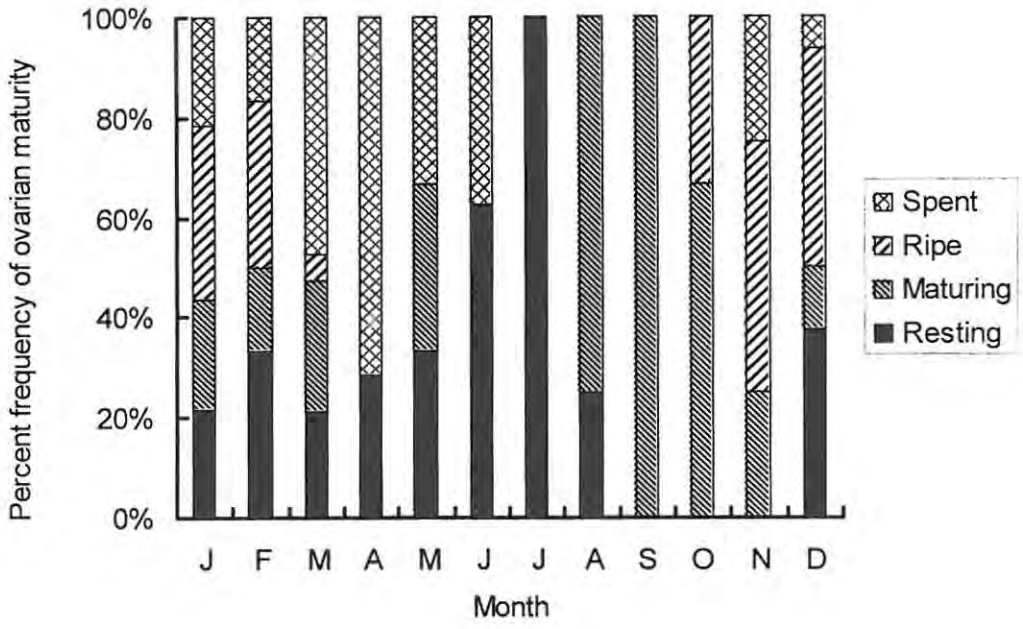

Figure 5.4: Monthly variation in the percent of macroscopic gonad stages in the ovaries of *Synaptura marginata* (n = 107).

The small sample size for males (n = 22) makes any conclusion difficult. Nevertheless, the monthly male GSI trends followed the same pattern as for the females with a peak between October and March (Figure 5.6).

There was no difference in the monthly condition factor for mature females between the reproductive season and the remainder of the year ($F_{3;52} = 0.92$, p = 4.36) (Figure 5.7).

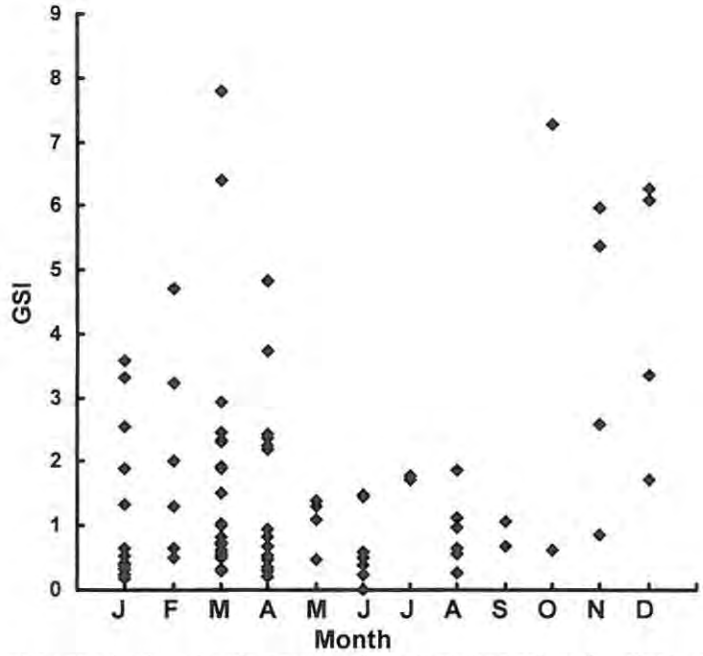

Figure 5.5: Monthly variation in the gonadosomatic index for female *Synaptura marginata* (n=107).

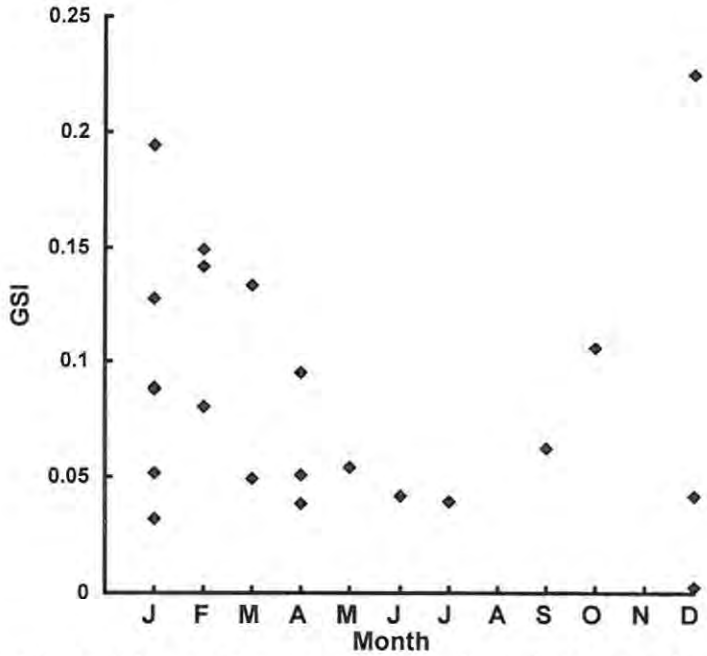

Figure 5.6: Monthly variation in the gonadosomatic index for male *Synaptura marginata*.

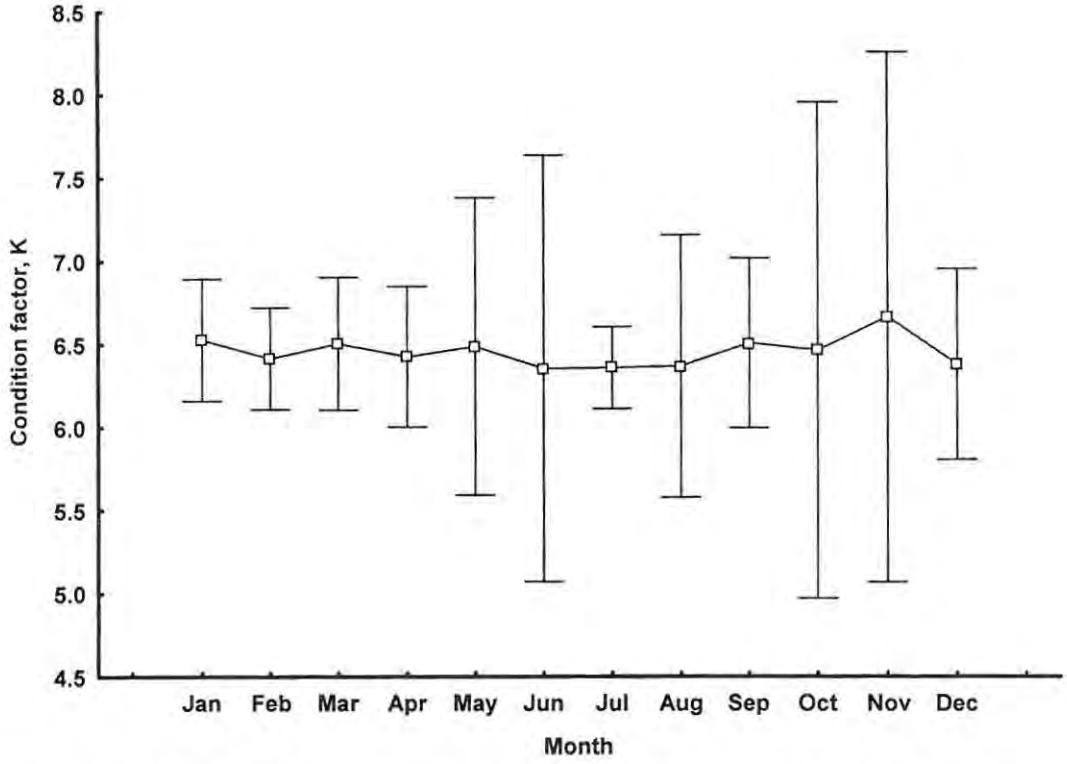

Figure 5.7: Seasonal variation in Fulton's condition factor (K) for *Synaptura marginata*. Error bars indicates 95% confidence intervals.

Size at maturity

Due to the high misidentification of immature females as resting females using macroscopic criteria, histological sections were used to confirm whether a fish was mature or not. The smallest mature male and female were 154 mm TL and 210 mm TL, respectively. No immature males were sampled during the study period. Percent maturity as a function of total length for females is presented in Figure 5.8. The best fit logistic ogive parameters were L_{50} = 234.96 mm and δ = 15.22. Females thus reach 50 % maturity at a length of 235 mm TL and 100 % maturity at 300 mm TL.

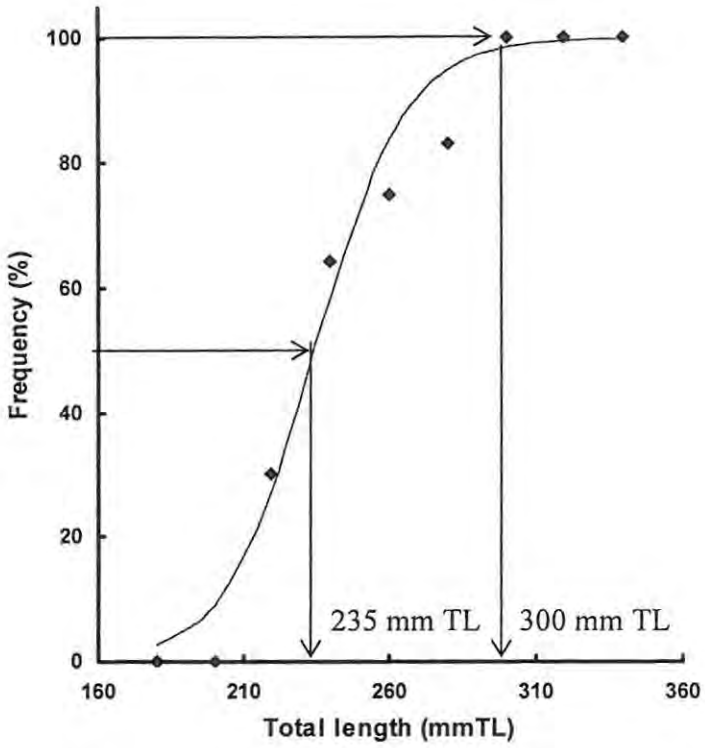

Figure 5.8: Percent frequency of mature female *Synaptura marginata* sampled between December 2000 to April 2002.

Figure 5.9 shows that the greater proportion (84 %) of the soles collected during the study period were mature females. Immature males were completely absent from the catches, as well as fish smaller than 102 mm TL.

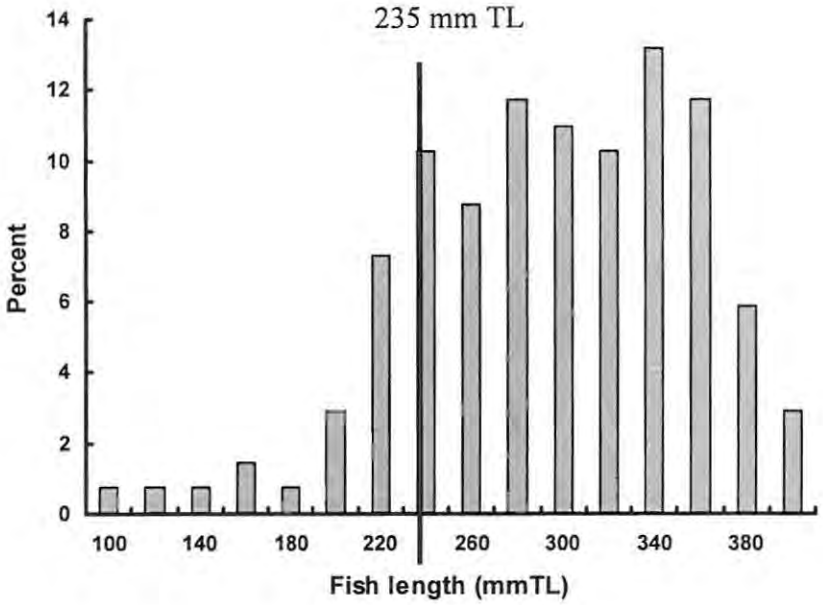

Figure 5.9: Size frequency distribution of females. The length at 50 % sexual maturity is shown by the line at 235 mm TL.

Fecundity

Hydrated oocyte counts ranged from 215 to 350 eggs / g of ovary, while vitellogenic oocyte counts ranged from 489 to 805 eggs / g of ovary (Table 5.3). This resulted in a calculated batch fecundity for *S. marginata* of 14 ± 5 eggs/g of fish and a relative fecundity of 34 ± 13 eggs / g of fish. Total fecundity shows a typical power relationship with fish size (mm TL) (Figure 5.10). The equation (r^2 = 0.72) describing the relationship between fecundity and fish size was:

Total fecundity (eggs) = 233.97 exp $^{[0.0123 \times \text{Total length (mm)}]}$

Table 5.3: Egg counts for the 11 gonads with well hydrated oocytes. The batch as well as relative fecundity is given for each fish.

Length (mm TL)	Weight (g)	Hydrated oocytes/ ovary	All vitellogenic oocytes/ ovary	Batch fecundity (hydrated oocytes/ g of fish)	Relative fecundity (all vitellogenic oocytes/ g of fish)
315	387	249	534	14	30
337	450	312	489	20	32
342	456	268	567	15	31
351	511	350	645	23	43
353	563	248	655	14	37
368	648	215	568	8	22
376	745	244	611	16	38
382	765	208	785	11	38
387	800	281	684	18	43
391	842	267	654	13	32
400	860	257	805	11	34
x ± SD		264 ± 41	636 ± 98	15 ± 4	34 ± 6

The histological examination suggested a minimum of three spawning events per female during the spawning period (see Plate 3), with up to three standing crops of vitellogenic oocytes.

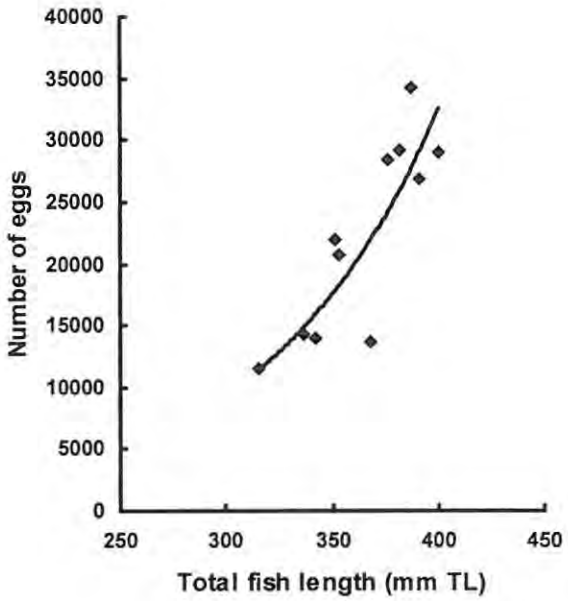

Figure 5.10: The relationship between total fish length (mm) and total fecundity for *Synaptura marginata*.

Discussion

In species where the sex ratio is determined genetically, there is little evidence that the sex ratio should vary from 1 : 1 (Krebs & Davies 1997). It has been assumed that this is the case in species like *Solea solea* and *Scophthalmus maximus*, although skewed sex ratios in these species suggest that the functional sex can be influenced by factors such as rearing temperature, stocking density, growth rate and water quality (Baynes & Hallam 1999). This is evident under experimental conditions, where the sex ratio has been altered in favour of males by extreme rearing temperatures (Chapter 1).

Synaptura marginata showed a sex ratio of 1 male to 5.1 females. Other cynoglossid and soleid species also show female dominated sex ratios, 1 : 2.4 for *Cynoglossus zanzibarensis* (Booth & Walmsley 2000) and 1 : 2.35 for *Austroglossus pectoralis* (Hecht 1976). Dagang *et al.* (1992) found that 14

species of flatfish in the subfamily Pleuronectinae had a sex ratio of 1 : 1. Highly skewed sex ratios can be an indication of very close courtship behaviour during spawning (Buxton & Garratt 1990) (see later). The skewed sex ratio in favour of females may also be an artifact of the sampling protocol and technique. Sampling was only done at spring low tides, in the intertidal zone during the day (Chapter 2) and the sex ratio could have been affected by differences in behaviour patterns between the sexes. This hypothesis would, however, have to be tested. The left ovary was much smaller than the right ovary in *S. marginata*. This might be due to the position of the right ovary between the epipleural ribs and muscles of the ventral body wall. No other reference to this could be found among flatfish. Other soleids and cynoglossids like *C. zanzibarensis* (Booth & Walmsley-Hart 2000) and *A. pectoralis* (Hecht 1976) have equal size ovaries, situated in the visceral cavity.

Comparison of the macroscopic and microscopic stages showed that 16.2 % of the ovaries were misidentified. This was mainly a consequence of misidentifying stage 1 or immature ovaries as stage 2 or resting ovaries, or visa versa. A similar problem was encountered by Merson *et al.* (2000) working on the summer flounder, *Paralichthys dentatus*. Assigning discrete stages to a continuous variable is always problematic. However, the ability to categorize gonads into maturity stages is clearly useful to determine the spawning period and spawning frequency under natural conditions (Merson *et al.* 2000). The difference between macroscopical and histological based descriptions of gonadal ontogeny stresses the need for a histological approach if an accurate description is to be achieved.

The macroscopic maturity index and the GSI data show that *S. marginata* has a protracted spawning season of five to six months, from October to March. This is slightly longer in comparison with other soleid species. For example, a spawning season of four to six months was found for both the Dover sole, *Solea solea*, and the Senegal sole, *Solea senegalensis* (Dinis *et al.* 1999), while *A. pectoralis*

(Hecht 1976) and *Solea bleekeri* (Cyrus 1991) have a four month spawning season.

The duration and timing of reproductive activity should be geared towards maximising survival of offspring according to basic life history theory (Krebs & Davies 1986). Spawning will theoretically thus only take place when and where environmental factors are favourable for the larvae. Most teleost species investigated on the Agulhas Bank exhibit protracted spawning periods over the spring and summer months (Booth & Walmsley-Hart 2000). The Agulhas Bank is a relatively stable and highly productive system (Largier *et al.* 1992, Schumann *et al.* 1992). Booth and Warmsley-Hart (2000) suggest that the oceanographic conditions and indirectly food availability, rather than taxonomic affinities, affect the length of the spawning season of various species on the Agulhas Bank. It has been shown that under favourable environmental conditions, an extended spawning season will reduce the risk of catastrophic mortality for the sparid, *Diplodus sargus* (Thresher 1984). This spawning strategy will also ensure that recruitment into inshore areas are spread over time, minimizing the possibility of inter and intraspecific competition for food and habitat space (Christensen 1978, Johannes 1978). The extended spawning period of *S. marginata* during the summer months conforms to the general trend of most other species in the region, except *A. pectoralis* which spawns in winter and early spring between May and September (Hecht 1976).

Fat reserves may be mobilised during periods of migration, reproduction or nutritional stress (Nikolsky 1963, Fishelson *et al.* 1985). Botha (1971) predicted that a condition factor will follow the expected trend of being low during spawning activity and vice-versa. This was found to be the case for the Agulhas sole, *A. pectoralis* (Hecht 1976), but not for *S. marginata*. This study clearly shows that the condition of the fish is relatively constant throughout the year. Condition factor is thus a poor indicator of the spawning season for *S. marginata*. The

relatively constant condition factor could be related to the high food availability throughout the year (Chapter 3).

The testes showed no appreciable increase in size during the reproductive season. Testes seem to be active year round, with sperm clearly present in all the histological preparations. This supports the lack of visual changes in the testes throughout the year. The male GSI however also showed increased activity during the period October through to April.

In most fishes, reproduction follows the normal vertebrate pattern of gonochorism (Buxton & Garratt 1990). Alternative reproductive styles are, however, common among some taxonomic groups (Bruton 1989), such as the Sparidae (Buxton & Garratt 1990), Serranidae (Shapiro 1981), Labridae (Shapiro & Rasotto 1993, Andrew *et al.* 1996) and Gobiidae (Cole 1990). These groups show various forms of hermaphroditism, such as simultaneous hermaphroditism, protogynous hermaphroditism, protandrous hermaphroditism and rudimentary hermaphroditism (Buxton & Garratt 1990).

Rudimentary hermaphrodites or 'late' gonochorists have only been observed in the Sparidae (Buxton & Garratt 1990). Fish belonging to this group possess immature inter-sexual gonads that ultimately function as either male or female, with no evidence of sex change. These are regarded as 'late' gonochorists and function, for most purposes, similarly to normal gonochorists. This phenomenon should mostly be present in smaller individuals, since functional ovaries and testes develop from immature inter-sexual ovotestis to function as either male or female (Booth & Buxton 1997). Rudimentary hermaphrodites show life history characteristics such as schooling, migration, equal sizes of the sexes, similar sex ratio and similar ovary/testis size (Booth & Buxton 1997). The equal sex size favours random mating behaviour between pairs or groups, within spawning aggregations (Buxton & Garratt 1990).

The data presented here seem to suggest that *S. marginata* might be a rudimentary hermaphrodite or 'late' gonochorist and not a true gonochorist (Dinis *et al.* 1999, Devlin & Nagahama 2002). This implies the presence of non-functional hermaphroditic tissue in mature individuals (Buxton & Garratt 1990). From a review of the literature, it appears that this phenomenon has hitherto not been reported for any pleuronectiform species. Male tissue was evident in 17.6 % of ovaries sectioned. There was however no pattern in the presence of male tissue in relation to fish size, as is the case with the sparid, *Pterogymnus laniarius* (Booth & Buxton 1997), a rudimentary hermaphrodite. Though it appears that the presence of male tissue in female gonads is completely random, the phenomenon is clearly worthy of future detailed study.

The small testis size in *S. marginata* requires comment. Large testes are selected for under conditions of intense sperm competition in group spawners, while small testes are selected for when sperm wastage is minimal with pair formation and spawning (Choat & Robertson 1975). Pair spawning is a common feature among many diverse groups of marine and freshwater fish. The relatively small testes to ovary size in *S. marginata* suggest that pair spawning may take place, as is the case in other flatfish (Baynes *et al.* 1994, Booth & Walmsley-Hart 2000, Hickman & Tait 2001). Pair formation and spawning has been observed in the Dover sole (*S. solea*), which also has small testes (Baynes *et al.* 1994). Pair spawning in soles takes place by the male swimming in under the female and lifting her off the substratum into the water column to spawn (Konstantinou & Shen 1995). Close contact during the spawning process would therefore ensure little sperm wastage and an increased fertilization rate (Buxton & Garratt 1990, Booth & Walmsley-Hart 2000).

A highly skewed sex ratio in favour of females, small testis to ovary ratio and sexual dimorphism all suggest pair spawning behaviour. From this it can be hypothesized that *S. marginata* is a pair spawner, with one male spawning with several females. Sexual dimorphism, with the males being much smaller than the

females, is often another important characteristic of fish that exhibit pair spawning (Buxton & Garratt 1990).

S. marginata females reach 50 % maturity at a length of 235 mm TL. This is a large proportion of its maximum length (57 %), but a small proportion (22 %) of its maximum age. Similar ratios have been documented for the cynoglossids, *C. zanzibarensis* (Booth & Walmsley-Hart 2000) and *Cynoglossus arel* (Rajaguru 1992), and the soleids, *A. pectoralis* (Zoutendyk 1974) and *Microchirus azevia* (Andrade 1998). Roff (1982) suggests that this growth pattern would help fish to attain a size large enough to avoid predators, compete with conspecifics to spawn, and to ensure a reasonably high fecundity at first spawning.

The relative fecundity and egg size of any fish is dependent on the reproductive strategy of that species (Begon & Mortimer 1986, Bruton 1989). Flatfish generally show large variations with respect to relative fecundity and egg size, and can range from 23 – 108 eggs / g and an egg size range of 1.2 – 1.6 mm for Dover sole, *Solea solea* (Baynes *et al.* 1993) to 200 – 580 eggs / g and an egg size range of 0.8 – 1.2 mm for *Paralichthys olivaceus* (Shuozeng 1995). *S. marginata* is thus very similar to *S. solea* with an relative fecundity of 34 ± 6 eggs / g (x ± SD) of fish and an egg size of 1.38 ± 0.18 mm (measured from captive spawned eggs).

S. marginata, like many other sole species, is a batch spawner (Nichol 1995, Dinis *et al.* 1999, Nichol & Acuna 2001). This is evident from the three crops of vitellogenic oocytes in the ovaries, suggesting three batches. There is a very large degree of uncertainty concerning the calculation of total fecundity in batch spawners (Nichol 1995, Nichol & Acuna 2001). Batch fecundity can vary throughout the spawning period, depending on species and environmental conditions (Walker *et al.* 1994, McFarlane & Saunders 1997). The number of batches per female during the spawning season are also not always clear (Nichol & Acuna 2001). The presence of residual carrion tissue and the readying of

another crop of vitellogenic oocytes in the same spawning season in the yellowfin sole, *Limanda aspera* (Nichol & Acuna 2001), suggested more than one series of batches during a spawning season. No such residual carrion tissue was present in ovarian sections of *S. marginata*. The total number of batches for *S. marginata* is thus not known, which casts some doubt on the estimate of total fecundity. One can, however, hypothesize that *S. marginata* does have more than the observed number of batches of eggs during the spawning season, due to the high percent of maturing, ripe and spent ovaries present throughout the long spawning period.

One of the main objectives of this study was to describe the life history of the species. Unfortunately this was not completely possible due to complete absence of fish smaller than 102 mm TL. Given that other small flatfish species like *Heteromycteris capensis*, *Solea bleekeri* and *Solea fulvomarginata* ranging in size from 60 – 100 mm TL were caught by the spearing method, we concluded that the absence of small *S. marginata* was not a consequence of the sampling method, but rather indicates their absence from the intertidal zone. In addition, no *S. marginata* specimens were obtained during intensive shallow inshore, small-mesh trawling surveys conducted in and around Algoa Bay (Wallace *et al.* 1984). In these surveys other flatfish species like *Cynoglossus capensis*, as small as 25 mm, were caught. During these surveys, demersal trawls were conducted between 400 m to 8 km offshore, ranging in depth from 4.5 to 100m. Lasiak (1983, 1984) also found no juvenile *S. marginata* during her survey of the intertidal area in Algoa Bay. Moreover, there is also no evidence of estuarine dependence, as shown by the very intensive estuarine surveys on the south and east coast of South Africa (Whitfield 1998). Based on the above, it may be possible to speculate that fish smaller than 102 mmTL might occur on subtidal sandbanks at depths of approximately 3 to 7 meters that range from 50 to 400 m offshore – known as the near-shore zone. No sampling has ever been attempted in this zone, mainly due to logistical problems (Dr. Strydom, South African Institute of Aquatic Biodiversity, *pers. comm.*).

To date we also have no information on the actual spawning habitat of *S. marginata*. However, given that larvae have been caught in the surfzone during the spawning season (Beckley 1986) and the higher CPUE during this period (Chapter 2), it can be hypothesized that *S. marginata* might undertake a seasonal, but depth restricted, inshore/offshore spawning migration, coming inshore to spawn. Larvae might undergo metamorphosis and settle behind the surf zone (50 – 400 meters off-shore) and only participate in this theoretical migration when they become sexually matured. This may explain the lack of juvenile *S. marginata* in catches. Inshore/offshore spawning migration patterns are not uncommon among flatfish. Dagang *et al.* (1992) and Shuozeng (1995) demonstrated this to be the case for most flatfish species in the Yellow Sea, China. Further work is however required to test this hypothesis.

S. marginata seems to be more *K*-selected, with respect to reproduction, in comparison to other flatfish, because of its lower fecundity and larger egg size. The Dover sole, *Solea solea* was found to be the most similar to *S. marginata*. Both species show relatively low fecundities, but bigger eggs. The implications of this in terms of aquaculture are discussed in Chapter 6.

Chapter 6: General discussion

The principal objective of this chapter is to synthesize the information presented in the preceding chapters and to reach a preliminary decision on the suitability of *Synaptura marginata* as a candidate species for marine fish farming in South Africa. This is achieved by comparing the biological parameters and characteristics of *S. marginata* to those of farmed pleuronectiform fishes. It is hypothesised that such a comparison would be the most suitable means of assessing and discussing the suitability of *S. marginata* for aquaculture in relation to other species. An attempt is also made to predict the growth rate of *S. marginata* under culture conditions. Finally, the chapter concludes by highlighting aspects that require further study to make an ultimate decision about whether *S. marginata* is a suitable candidate species for aquaculture or not.

The primary aim of fish farming is to maximize survival and growth while minimising costs (Knight 1985). Therefore, any biological characteristic of a species that affects growth and survival under culture conditions will play an important role in deciding whether the species is suitable for aquaculture. The most important traits for aquaculture are growth, size and age at sexual maturity, fecundity, egg size and reproductive life span. An attempt was made to provide a quantitative perspective on the life history characteristics of *S. marginata* as a means by which to assess and compare its potential for aquaculture relative to other pleuronectiform species. This was achieved by using *r* – K life history theory (MacArthur & Wilson 1967, Pianka 1970, Bruton 1989). The implications of certain life history traits for aquaculture have been well documented (Le François *et al.* 2002, Quéméner *et al.* 2002), providing some theoretical basis for assessing the suitability of a species.

Growth was compared using the growth performance index, phi prime (ø'), which has previously been used to compare growth between species (Moreau *et al.* 1986, Pauly *et al.* 1988, Moreau & Pauly 1999). The growth performance index is described by $(ø') = \log K + 2 \times \log L_\infty$. In this way the growth rate of *S.*

marginata can be compared to six other flatfish species that are currently farmed and for which there are good growth data.

To compare the size at which the fish attain sexual maturity, the total length at 50% maturity (L_{50}) was expressed as a ratio of L_{max}. Since the age – length relationship for most species reaches an asymptote at older ages, there are little to no differences in length between individuals at older ages (Quinn & Deriso 1999). L_{max} can thus be used with reasonable certainty that it closely approximates the average maximum size. The size at sexual maturity can also be expressed as a ratio of L_∞, predicted by the Von Bertalanffy growth equation. However, L_∞ is a value describing a model and has very little biological meaning (Quinn & Deriso 1999, He & Stewart 2002). The onset of sexual maturity is associated with reduced food conversion efficiency (Halver 1989, Berill *et al.* 2003) and can dramatically affect farming economics and profitability; thus fish that have a high L_{50} / L_{max} ratio should be favoured.

Successful rearing is mainly dependent on egg and larval quality and specific larval characteristics (Hickman & Tait 2001). Larger eggs usually yield larger larvae (Bromage 1995), which are easier to rear as they take larger prey items at first feeding (Dabrowski & Bardega 1984). Therefore, species that have larger eggs should be favoured for aquaculture and a direct comparison between the eggs of the various farmed flatfish was made. Aquaculture is, however, not limited to such species and zootechnical advances have lead to the development of successful industries on species previously thought to be difficult to rear (Dhert *et al.* 1998).

Following the work by Roff (1981), the natural log of total fecundity was calculated and used for comparative purposes. As indicated in Chapter 5, the total fecundity for *S. marginata* was considered to be underestimated and this would obviously affect its relative position on the *r* – K continuum for this characteristic. However, it was argued that total fecundity was probably underestimated for most species for which accurate data on batch spawning is not available.

The reproductive life span of a species is the number of years during which an individual contributes to reproduction (Roff 1981, Dagang *et al.* 1992). For the comparison between species this was calculated as the time, in years, from the age of 50% sexual maturity to the maximum recorded age for that species (Roff 1981). *S. marginata* reaches 50% maturity at an age of two years (Chapter 5) and attains a maximum estimated age of seven years (Chapter 3), and therefore has a reproductive life span of five years. There was also no evidence of senescence in older fish. Although not mentioned by Roff (1981), this is based on the assumption that an individual will spawn every year after the onset of sexual maturity and will produce viable gametes. This may not always be the case, for example Baynes *et al.* (1994) showed that female *Solea solea* may skip reproductive seasons after the onset of sexual maturity. As indicated in Chapter 3, the maximum age and size for *S. marginata* may have been underestimated, as larger individuals have been reported by anglers. It would, however be reasonable to assume that this limitation was also the case for the other species used here for comparison, although increased sample sizes in other studies might reduce this effect.

The parameters for each of the above defined life history traits were obtained for 26 pleuronectiform species (Table 6.1). The highest and lowest value for each characteristic was recorded (Table 6.2) and used to develop an r – K continuum (Table 6.2). For comparison, *S. marginata* was then placed on the continuum together with seven other farmed flatfish species (Figure 6.1). These were *Hippoglossus hippoglossus*, *Hippoglossus stenolepis*, *Paralichthys olivaceus*, *Pleuronectes platessa, Pleuronectes americanus*, *Scophthalmus maximus* and *Solea solea*. *S. solea* is the most important of these, since it is one of only two soleid species (the other is *S. senegalensis*) currently being farmed and since it is a well studied species and has a similar ecology and biology to *S. marginata*. It was broadly hypothesized that the farmed species may possibly be grouped and occupy a more K selected position on the continuum. For several reasons, care should however be taken not to over-interpret the data presented in Figure 6.1. It is a relative scale, which cuts across families and is constructed merely to explore if farmed flatfish species exhibit some commonalities.

Table 6.1: Selected life history traits of 26 pleuronectiform species. The family of each species is also given. (a) Pleuronectidae, (b) Cynoglossidae, (c) Paralichthyidae, (d) Scophthalmidae and (e) Soleidae.

Species	Phi prime, ø'	Reproductive life span	Log Fecundity	Egg size	Size at maturity	Reference
(a) *Cleisthenes herzensteini*	2.39	12	4.89	0.87-0.9	0.38	Dagang *et al.* 1992, Shouzeng 1995, FISHBASE
(b) *Cynoglossus abbreviatus*	2.62		5.49	1.19-1.23	0.60	Shouzeng 1995, FISHBASE
(b) *Cynoglossus joyneri*	2.19		4.58	0.76-0.9	0.58	Shouzeng 1995, FISHBASE
(b) *Cynoglossus semilaevis*	3.19		5.96	1-1.24	0.60	Shouzeng 1995, FISHBASE
(a) *Eopsetta grigorjewi*	2.54	4	4.72	1-1.1	0.30	Dagang *et al.* 1992, Shouzeng 1995, FISHBASE
(a) *Hippoglossoides platessoides*	2.56	17	4.8	3	0.24	Roff 1981, 1982, FISHBASE
(a) *Hippoglossus hippoglossus*	3.62	38	4.7	3.0-3.8	0.45	FISHBASE
(a) *Hippoglossus stenolepis*	3.40	16	4.98	3.27	0.47	Roff 1981, 1982, FISHBASE
(a) *Kareius bicoloratus*	2.81	8	6.01	0.9-1.18	0.42	Dagang *et al.* 1992, Shouzeng 1995, FISHBASE
(a) *Limanda aspera*	2.61	9.5	5.74		0.60	Roff 1981, 1982, FISHBASE
(a) *Limanda ferruginea*	2.92	7	5.8	0.4	0.50	Roff 1981, 1982, FISHBASE
(a) *Limanda limanda*	2.54	4	4.65	0.93	0.31	Roff 1981, 1982, FISHBASE
(a) *Microstomus kitt*	2.76	15	5	1.29	0.43	Roff 1981, 1982, FISHBASE
(a) *Microstomus pacificus*	2.74	14	6.65	2.33	0.50	Roff 1981, 1982, FISHBASE
(c) *Paralichthys olivaceus*	3.21		6.5	0.8-1.2	0.39	Shouzeng 1995, FISHBASE
(a) *Parophrys vetulus*	2.62	13	4.63		0.69	Roff 1981, 1982, FISHBASE
(a) *Pleuronectes americanus*	2.61	5.5	5.23	0.81	0.39	Roff 1981, 1982, FISHBASE
(a) *Pleuronectes platessa*	2.62	26.5	4.83	1.92	0.30	Roff 1981, 1982, FISHBASE
(a) *Pleuronichthys cornutus*	1.94	8	4.56	1.09-1.19	0.43	Dagang *et al.* 1992, Shouzeng 1995, FISHBASE
(a) *Pseudopleuronectes herzensteini*	2.64	2	5.18	0.85-1.05	0.42	Dagang *et al.* 1992, Shouzeng 1995, FISHBASE
(a) *Pseudopleuronectes yokohamae*	2.54	4.5	6.04	0.85-0.9	0.44	Dagang *et al.* 1992, Shouzeng 1995, FISHBASE
(d) *Scophthalmus maximus*	2.96	33.5	6.3	1.06	0.46	Roff 1981, 1982, FISHBASE
(e) *Solea solea*	2.62	13	5	1.2-1.6	0.46	Baynes et al. 1993, Dinis et al. 1999, FISHBASE
(a) *Verasper variegatus*	3.09		5.58	1.5-1.6		Shouzeng 1995, FISHBASE
(e) *Zebrias zebra*	2.15		4.71	1.42-1.69	0.63	Shouzeng 1995, FISHBASE
(e) *Synaptura marginata*	2.65	5	4.36	1.31-1.49	0.47	This study

Table 6.2: The highest and lowest recorded value for selected life history traits of 26 pleuronectiform species (Table 6.1).

Life history characteristic	*r* - selected	K - selected
Reproductive life span	Short 2 years *Pseudopleuronectes herzensteini*	Long 38 years *Hippoglossus hippoglossus*
Fecundity	High 6.65 (log total fecundity) *Microstomus pacificus*	Low 4.36 (log total fecundity) *Synaptura marginata*
Eggs	Small 0.4 mm *Limanda ferruginea*	Large 3.8 mm *Hippoglossus hippoglossus*
Maturity	Young 0.24 (ratio of L_{50}/L_{max}) *Hippoglossoides platessoides*	Old 0.69 (ratio of L_{50}/L_{max}) *Parophrys vetulus*
Phi prime, ø'	High 3.62 *Hippoglossus hippoglossus*	Low 1.94 *Pleuronichthys cornutus*

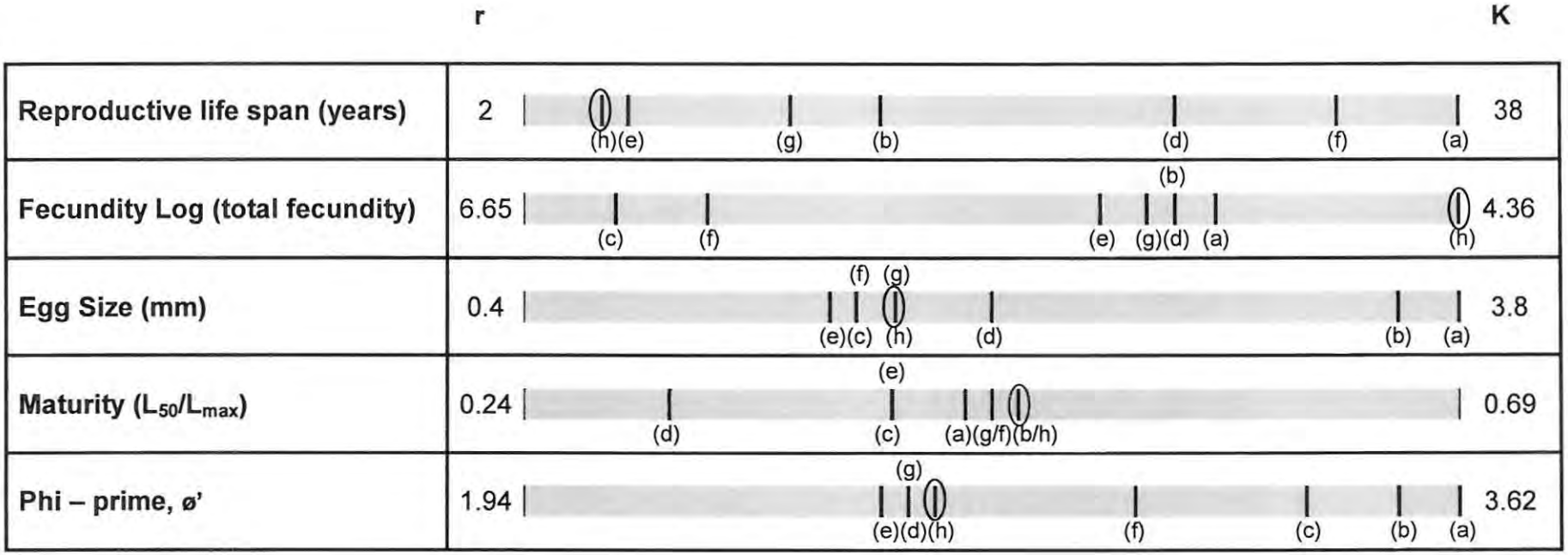

Figure 6.1: A tentative attempt to place several life history traits of *S. marginata* and other farmed flatfish species on a *r* – K continuum of pleuronectiform species. (a) *Hippoglossus hippoglossus*, (b) *Hippoglossus stenolepis*, (c) *Paralichthys olivaceus*, (d) *Pleuronectes platessa,* (e) *Pleuronectes americanus*, (f) *Scophthalmus maximus*, (g) *Solea solea* and (h) *Synaptura marginata*.

It was shown in Chapter 3 that *S. marginata* has a relatively high growth rate (K = 0.24) and reaches a length in excess of 43 cm in about 7 years. The calculated ø' value for *S. marginata* (2.65) was similar to *Solea solea* (2.62), *Pleuronectes americanus* (2.61) and *Pleuronectes platessa* (2.62) (Table 6.1 and Figure 6.1). The other four farmed pleuronectiform species namely *Hippoglossus hippoglossus*, *Hippoglossus stenolepis*, *Paralichthys olivaceus* and *Scophthalmus maximus* show a much higher growth rate ranging from 2.96 to 3.62. However, this was expected as these four species are piscivorous (De Groot 1971).

The size at sexual maturity (expressed as a ratio of L_{50}/L_{max}) is very similar for all farmed species, other than *P. platessa* (Figure 6.1). Since this comparison is a function of L_{max}, it is important to realise that this similarity does not imply a similar size of sexual maturity. For example, *S. solea* reaches sexual maturity at a size of 320 mm, while *S. marginata* reaches sexual maturity at 235 mm. The similarity between the L_{50}/L_{max} ratio is thus due to the fact that *S. solea* reach a larger maximum size than *S. marginata*.

Egg size and fecundity also show a degree of grouping of the species on this relative scale (Figure 6.1). However, it is worthwhile to note that the total fecundity of *S. marginata* does not conform to the general grouping of the farmed species for this trait. The reproductive life spans of the different farmed species are spread out over the whole theoretical range, without any grouping of species. Overall, the hypothesis that farmed pleuronectiform species would show some form of grouping on the K side of the continuum has to be rejected.

To assess the suitability of *S. marginata* for aquaculture it is also important to consider the life history traits collectively. The most important of these relationships is that between growth and size at sexual maturity. The relatively high natural growth of *S. marginata* should be viewed in relation to the small size and age at which it attains sexual maturity, which as previously explained, could be problematic.

Fish with large eggs usually also have a low relative fecundity (Bruton 1989) as both characteristics are regarded as K – selected traits (Balon 1984, Bruton 1989). Low fecundities can however be compensated for by increasing the number of broodstock fish, so it is of lesser importance as a selection criterion for aquaculture. The size range of eggs that the general grouping of farmed flatfish exhibits (1.2 to 3.8 mm – Figure 6.1) usually also have no associated problems with larval rearing to first feeding (Howell 1997, Pittman et al. 1998, Dinis *et al.* 1999).

From Figure 6.1 there is no information to suggest a general trend for farmed flatfish to be more K – selected for any life history trait over other flatfish. Characteristics such as growth, egg size, and size at sexual maturity suggest however that *S. marginata* falls within the general values obtained for currently farmed species. Although total fecundity does not conform to this pattern, it is of lesser importance as a selection criterion. The small size (235 mm) at which *S. marginata* reaches sexual maturity may present a problem under culture conditions. This potential problem will depend on the required market size (in comparison to size at 50 % maturity) and the potential profitability.

An attempt was made to predict the growth of *S. marginata* under culture conditions by modelling the growth of various farmed finfish species under natural and farmed conditions. However, this was not successful for several reasons. Firstly, natural growth parameters, obtained from von Bertalanffy growth models, are highly variable among different studies on the same species, especially concerning L_∞. Secondly, known growth parameters of species under culture conditions are limited to only a few species since scientists often only quote specific growth values and do not model the actual growth (Howell 1997, Silva & Velez 1998, Dinis et al. 1999, King et al 2001). Due to the usefulness of such a prediction in deciding if a species is suitable for aquaculture, it is suggested that more comprehensive growth studies be done under farming conditions.

There are various ways of increasing the growth rate of a fish species under culture conditions (Chapter 1). In flatfish, as in many other species, the main method of enhancing growth rate is through the establishment of monosex populations (King *et al.* 2001). In many flatfish, females grow faster and to a larger size than males (Yamamoto 1995, Monaghan & Armstrong 2000, King *et al.* 2001). Although no difference in the growth rate between males and females was detected for *S. marginata*, it was shown (Chapter 3) that females grow to a larger size than males, indicating that a female monosex population of *S. marginata* would be preferable under culture conditions.

S. marginata show many biological characteristics that would suggest natural pair formation, although this has not been observed. These characteristics include a highly skewed sex ratio in favour of females and the small testis relative to ovary size. Pair spawning has been observed in many other flatfish species (Baynes *et al.* 1994, Hickman & Tait 2001). On the assumption that pair formation does occur in *S. marginata* and that the natural sex ratio is not skewed as a consequence of sampling error, it is suggested that a natural ratio of one male to five females be used as a starting point. For *S. solea*, which has a natural sex ratio of one male to one female, the number of males stocked for every female has ranged from 0.5 to 3 under culture conditions (Baynes *et al.* 1993). At this stage, it is also suggested that the broodstock stocking density used for *S. solea* also be used for *S. marginata*, *viz* 1 – 1.5 kg / m^2 (Dinis *et al.* 1999).

S. marginata has a protracted spawning season of six months, which is longer than most other farmed flatfish species (Chapter 5). This implies that eggs could be obtained for half of the year. Though not part of the objectives of this study, some spawning success was achieved by hormonal induction with a single dose (0.5 ml / kg) of Aquaspawn (Millar's Laboratories, Touwsrivier, South Africa), an analogue of a Gonadotropin-Releasing Hormone. Fertilization of the eggs was however, not successful due to over-hydration. Egg quality is very variable among batches obtained from induced spawning (Bromage 1995). Natural spawning is the preferred method. It is clear that *S. marginata* is a batch spawner, with enough evidence to suggest that at least

three batches of eggs are spawned during the spawning season (Chapter 5). This information, together with fecundity, can be used very successfully to determine the number of broodstock fish that will be required for a farm. Broodstock will have to be collected from the wild. It is recommended that this be done during the breeding season when females are readily identified by the swollen ovary (Chapter 5).

Providing broodstock fish with a nutritionally optimal diet is a prerequisite for good egg quality and robust larvae (Watanabe *et al.* 1984a,b, Watanabe 1988, Izquierdo *et al.* 2001). Since most of the fish caught during this study were sexually mature, the proximate composition and amino acid profile of the diet of *S. marginata* (Chapter 4) provides valuable information for the design of broodstock diets (Cowey & Tacon 1982, Wilson 1985). This information suggests that broodstock *S. marginata* will require a minimum crude protein and crude fat in the diet of 57.6 % and 4.7 %, respectively.

In conclusion, this study has shown that *S. marginata* (relative to other flatfish species) appears to be biologically suitable for aquaculture in South Africa. The natural growth rate of *S. marginata* is comparable to other cultured flatfish and, although it is not among the highest, it shows acceptable natural growth potential for farming. The other life history traits investigated for *S. marginata* also seem to fall within the general grouping for farmed flatfish. Under ideal conditions the next step in developing the protocol for the farming of *S. marginata* would be to undertake a growth trial with wild caught juveniles. Unfortunately it was not possible to locate the nursery grounds to obtain juveniles for such an exercise. Consequently, to advance sole culture in South Africa it is suggested that the next step should focus on inducing mature females to spawn to obtain larvae and juveniles. The information provided in this thesis is a contribution towards achieving that goal. Ultimately, the economic parameters and market characteristics need be carefully considered to determine whether *S. marginata* will be a successful aquaculture species in South Africa.

Chapter 7: References

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Appendix A: Scientific Authorities of the various Flatfish species

Scientific name	Scientific authority
Paralichthys olivaceus	Temminck & Schlegel, 1846
Scophthalmus maximus	Linnaeus, 1758
Hippoglossus hippoglossus	Linnaeus, 1758
Solea solea	Linnaeus, 1758
S. senegalensis	Kaup, 1858
Paralichthys dentatus	Linnaeus, 1766
Pleuronectes americanus	Walbaum, 1792
Paralichthys lethostigma	Jordan & Gilbert, 1884
Paralichthys tropicus	Ginsburg, 1933
Hippoglossus stenolepis	Schmidt, 1904
Colistium nudipinnis	Waite, 1911
C. guntheri	Hutton, 1873
Rhombosolea tapirina	Günter, 1862
Pleuronectes platessa	Linnaeus, 1758
Paralichthys adspersus	Steindachner, 1867
Austroglossus pectoralis	Kaup, 1858
A. microlepis	Bleeker, 1863
Solea bleekeri	Boulenger, 1898
Heteromycteris capensis	Kaup. 1858
Cynoglossus zanzibarensis	Norman, 1939
Pseudorhombus arsius	Hamilton-Buchanan, 1822
Paralichthodes algoensis	Gilchrist, 1902
Cynoglossus capensis	Kaup, 1858
Solea fulvomarginata	Gilchrist, 1904
Synapturichthys kleini	Risso, 1833
Etropus crossotus	Jordan & Gilbert, 1882
Parophrys vetulus	Girard, 1854
Scophthalmus rhombus	Linnaeus, 1758