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**GROWTH AND MORTALITY RATES OF  
SARDINE (*Sardinops sagax*) PRE-RECRUITS  
IN THE SOUTHERN BENGUELA REGION  
DURING EARLY 2001**

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**Thesis submitted for the degree of  
Master of Science  
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**May 2006**

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## DECLARATION

I hereby declare that I did the work presented in this dissertation under the supervision of Prof. J. G. Field (MBRI, UCT) and Dr. D. Durholtz (Marine and Coastal Management). My supervisors and colleagues made valuable comments on the drafts, but all the age readings, calculations, data analysis and the ideas included in this study are largely my own. I participated in the MCM cruises to collect the samples, although the scientific and technical staff of MCM collected the length-frequency and acoustic data. Dr. Carl van der Lingen (MCM) made the clupeoids data (especially sardine) available. Miranda Waldron (University of Cape Town Electron Microscope Unit) assisted in otolith preparation and scanning electron microscopy. Dr. Durholtz gathered the available survey data from the MCM staff.

No portion of this thesis has been submitted in support of an application for any other degree or qualification at this or any other university.

Elliot...Weni.....

...25 May 2006.....

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## ABSTRACT

Larvae and juveniles from the 2000/2001 year class of the South African sardine *Sardinops sagax* were collected during two consecutive surveys in March and May 2001 in the southern Benguela region off South Africa. The main objectives of this study were: (1) to develop a method of accurately and consistently counting daily increments; (2) to use the increments in otoliths counts to estimate age of the individuals; (3) to back-calculate hatch-dates to identify cohorts, and, (4) to analyze growth and mortality of sardine larvae and juveniles in the Southern Benguela region during early 2001.

Sagittal otoliths were extracted from sub-samples of larvae and juveniles in the laboratory and prepared for microscopic examination of daily increments. Sampled larvae (n=111) ranged from 10 to 39 mm, while the juveniles sub-sampled (n=82) ranged from 65 to 127 mm. These samples were successfully prepared for light and scanning electron microscopy. Three replicate counts of the number of daily increments in each otolith were conducted. Precision was relatively low, with larval otolith daily counts deviating by a mean of 20 days (as much as 48 days), while the juvenile daily increments deviated by a mean of 26 days (as much as 58 days). Mean ages calculated from these data were used to set up age-length keys and back-calculate the hatch-date distributions of the fish sampled during each survey.

Most larvae sampled during the March survey hatched from 17 January 2001 to 26 February 2001, whereas the juveniles sampled in May hatched during early November 2000 to mid-January 2001. The growth rates of larvae and juveniles were estimated to be  $0.104 \text{ mm.day}^{-1}$  and  $0.127 \text{ mm.day}^{-1}$ , respectively, (both lower than in other studies e.g. Kerstan & Waldron 1995 found the growth rate of  $0.45 \text{ mm.day}^{-1}$ ) for the larvae and juveniles, while the larval and juvenile mortality rates were estimated to be  $0.09 \text{ larvae.day}^{-1}$  and  $0.043 \text{ juveniles.day}^{-1}$ .

It is for the very first time that larvae and juvenile mortality rates are measured in the Benguela region. The differences between the hatch date distributions of larvae and juveniles suggest increased survival of the sardine spawned in mid-summer. Density dependent competition for food may have led to the sardine slow growth rates. Also, the difficulty that was often experienced in counting the innermost rings that became less clear towards the otolith centre may have caused under-estimation of daily growth marks.

## **CHAPTER 1 - INTRODUCTION**

### **1.1 Distribution and Biology**

Sardines are small, schooling fish occurring in most of the world's oceans and seas except in the Antarctic. Sardines are planktivorous and they are mostly confined to the shallower waters of continental shelves where there is a high production rate of plankton. These fishes are most abundant in the four major upwelling regions in the world (California, Peru, North West Africa and in the Benguela system in Southern Africa). In these regions, upwelling of cold nutrient-rich bottom water enriches the upper sea layers resulting in fast planktonic growth that benefits clupeoid populations, generally dominated by sardine and anchovy.

There appear to be two self-sustaining populations of sardine. The first extends northwards along the Namibian coast from the Lüderitz upwelling cell (27°S) to the warm-water front off southern Angola (~15°S). The second population occurs to the south, extending from the Orange River around the Cape to KwaZulu/Natal (27°S) (Armstrong and Thomas 1989, Cloete *et al.* 1997). The area of cold up-welled water present at Lüderitz for most of the year appears to prevent the large-scale mixing of the two sardine stocks. King (1977a) suggested the possible existence of separate sub-stocks off central and northern Namibia and southern Angola on the basis of the distribution patterns of eggs and larvae.

Grant (1985), however, found that there was no significant genetic difference between sardines collected in northern and central Namibia, and the Namibian sardine is now considered to consist of a single stock (Cochrane and Boyer, 1997). Poorly controlled increases in effort combined with fluctuating recruitment (Newman *et al* 1979; Crawford 1981b; Armstrong and Thomas 1989) in Namibia, after the catch peaked at 1.4 million tons in the early 1960s, resulted in a collapse of the stock in 1960. There was a slight recovering of the Namibian stock in the early 1970s although it had completely collapsed in late 1970s and has yet to show any signs of recovery.

Sardine form dense shoals in favorable conditions. Baia dos Tigres in Angola, Walvis Bay in Namibia and St. Helena Bay in South Africa are the three major areas where dense shoaling is known to occur forming the basis of lucrative pelagic fisheries. The dynamics of sardine shoals have been studied off both Namibia (Kruger and Cruikshank 1982) and South Africa (Barange

and Hampton 1997). In all cases, sardines were generally found in dense shoals in the upper 50m of the water column.

The South African sardine, or pilchard, *Sardinops sagax*, inhabits a temperate ocean system extending from northern Namibia to KwaZulu/Natal (Armstrong and Thomas, 1989). Crawford (1979, 1980 and 1981) and Crawford *et al.* (1980) examined the distribution of sardine in South African waters and, on the basis of commercial catch information collected from 1964 to 1976, concluded that sardine exhibit age-specific patterns of availability and distribution. An investigation of sardine distribution over the period from 1983 to 1985 using data from several sources (Armstrong *et al.* 1987) concluded that sardines are distributed in many size classes.

Either recruits of the year or large, mature adult fish dominate West Coast commercial catches while the catches on the South and East Coasts are dominated by intermediate-sized fish of 14-20 cm (standard length, SL). Seasonal hydrology appears to act in conjunction with age- or size-related behavior to produce these size-distribution patterns.

Spawning adults are generally concentrated on the Agulhas Bank in summer although some spawning has been recorded off St Helena Bay (Beckley and van der Lingen, 1999). The adults then appear to migrate eastwards past Cape Agulhas with the advent of autumn. The regular winter occurrence of shoals of similar-sized sardines close inshore off the Transkei and KwaZulu/Natal coasts suggests that part of the 2-year-old (and older) cohort is located on the Eastern Cape. Many sardines that are older than 2 years are in fishing grounds, giving rise to the phenomenon known locally as the “sardine run”.

The upwelling-favorable south-easterly and southerly winds on the West Coast cause a drift of surface water from the Agulhas Bank towards Cape Point and northwards. This helps in the transportation of eggs and larvae to the nursery areas on the west coast where feeding conditions favor rapid growth. Nursery areas, where the youngest fish are abundant, extend from St. Helena Bay to Orange River and to the north of the spawning grounds off South Africa in autumn. Recruits, 0 to 1-year-old fish, (the two words, recruits and pre-recruits will be used interchangeably with juveniles and larvae, respectively, since they mean the same thing) are then distributed along the entire West Coast from May onwards, their numbers peaking in September. Slightly older fish (1 year olds) appear to favour the western areas (i.e. offshore along the edge of the continental shelf) (Beckley and van der Lingen 1999). Schools

of these juvenile fish move gradually southwards down the West Coast during autumn and winter. South-flowing inshore currents that become more prevalent during winter (Armstrong and Thomas 1989) may aid the southward movement of these fish.

No clear patterns of diel vertical migration were apparent. Densities were lower at night than during the day. Geo-statistic analysis of acoustic data (Barange and Hampton 1997) from the 1994 spawner biomass survey indicated that sardine aggregations remained patchily distributed at night.

Clupeoids play an important role in the world economy and have both economic and social value. The estimated wholesale value of the pelagic catch in South Africa was almost R860 million in 2001 and just over R1 billion in 2002 (MCM, unpublished data).

### 1.1.1 Feeding

Sardines are commercially important and they are also an important component of the pelagic food web, being the major prey item for several species of fish, birds and marine mammals. In turn, they feed on phytoplankton and zooplankton (Beckley and van der Lingen 1999). In doing so, they transfer energy from lower to upper trophic levels. Upwelling systems are commonly characterized by the term “wasp-waist ecosystems” (Cury *et al.* 2000), meaning the occupancy of mid-trophic level position by a small number of species.

Early studies on sardine diet in the Benguela region showed that the species feeds primarily on phytoplankton, particularly diatoms (Davies 1957, King and Macleod 1976). The ability of sardine to capture considerable quantities of phytoplankton through filter-feeding was confirmed by van der Lingen (1994). Sardine can also assimilate diatom carbon and nitrogen efficiently (van der Lingen 1998a).

Based on studies in North and South America, Durbin (1979) suggested that filter feeding in sardine is the dominant feeding mode, in its natural environment. In South Africa, it was noted that adult sardine displayed no feeding periodicity and appeared to feed continuously through the diel cycle (van der Lingen 1998b). *S. sagax* is an omnivorous clupeoid, feeding on both phytoplankton and zooplankton. This was shown by the dominance of small prey items, principally dinoflagellates, cyclopoid and calanoid copepods and crustacean and anchovy eggs in the sardine stomachs that were examined by van der Lingen (2002). In contrast, the co-

existing anchovy mainly employs the particulate-feeding mode of nutrition, preferring larger prey particles, mostly meso-zooplankton (van der Lingen 2002).

## **1.2 The Fishery for *Sardinops sagax* in Southern Africa.**

Sardine is a valuable resource in South Africa, the annual catch in 2002 valued at R330 million (MCM, unpublished data). The sector provides employment to approximately 4000 full-time personnel, 1500 seasonal workers and 600 fishermen. The estimation is that a further 2400 indirect jobs are created in service industries associated with the sector (Beckley and van der Lingen 1999). This fishery commenced in the 1930s although the major development of the pelagic fishery only occurred after World War II. The bulk of sardine catches in Southern Africa is landed by the pelagic fishery, which also targets anchovy and juvenile horse mackerel. The fishery began on the West Coast in the St Helena Bay area (Du Plessis 1959; Stander 1967), after which it expanded from south of Doring Bay on the West Coast to Cape Agulhas on the south coast. Sardines are also harvested in the Eastern Cape in the region of Port Elizabeth, and a small seasonal beach-seine fishery in southern KwaZulu-Natal takes advantage of the annual sardine run.

Sardines are usually harvested using purse-seine nets. A fleet of 70 South African purse-seiners operates out of the harbors at Lambert's Bay, St Helena Bay, Saldanha Bay, Hout Bay, Gans Bay and Port Elizabeth, and there are currently eight processing factories where pelagic catches are canned or reduced to fish-meal and oil. Sardine biomass was high during the 1950s and early 1960s, decreasing to low levels thereafter off both South Africa and Namibia. In the early 1960s, sardine were the primary target of the purse-seine fishery and a peak catch of about 410 200 tons was reached in 1962 in South Africa.

In 1964, the pelagic fishery began to target anchovy rather than sardine, and 13 mm mesh-size nets (compared to 32 mm) were initiated in the Western Cape. This resulted in a change in the size composition of the sardine catch from 20-24 cm fish to 16-20 cm fish (Stander and le Roux 1998). Hutchings (1996) reported that subsequently, most 0+ sardines (8-13 cm) were caught as a by-catch to the anchovy-directed fishery. The annual sardine catch during the 1980s was low, generally less than 50 000t, while anchovy catches of up to 600 000 tons were landed. From the mid-1980s, sardine biomass off South Africa has shown a steady increase while the sardine biomass off Namibia has remained low since the collapse in the mid 1960s.

In South Africa, sardine catches have improved in the last 12 years as a result of effective management procedures. de Oliveira *et al* (1986b) reported that from 1990 the annual sardine catch increased, exceeding 100 000 t since 1996. Catches on the western Agulhas Bank have been comparable with those on the west coast, despite the fact that most of the factories are located on the west coast since 1991 (Roel *et al.* 1994). The pelagic industry in 2000 enjoyed the benefits of an extremely favorable year. A total catch of 266 000 tons of anchovy together with 135 000 tons of sardine *S. sagax* (including both directed and by-catch) were landed and these landings boosted total pelagic landings in 2000 to over 400 000 tons.

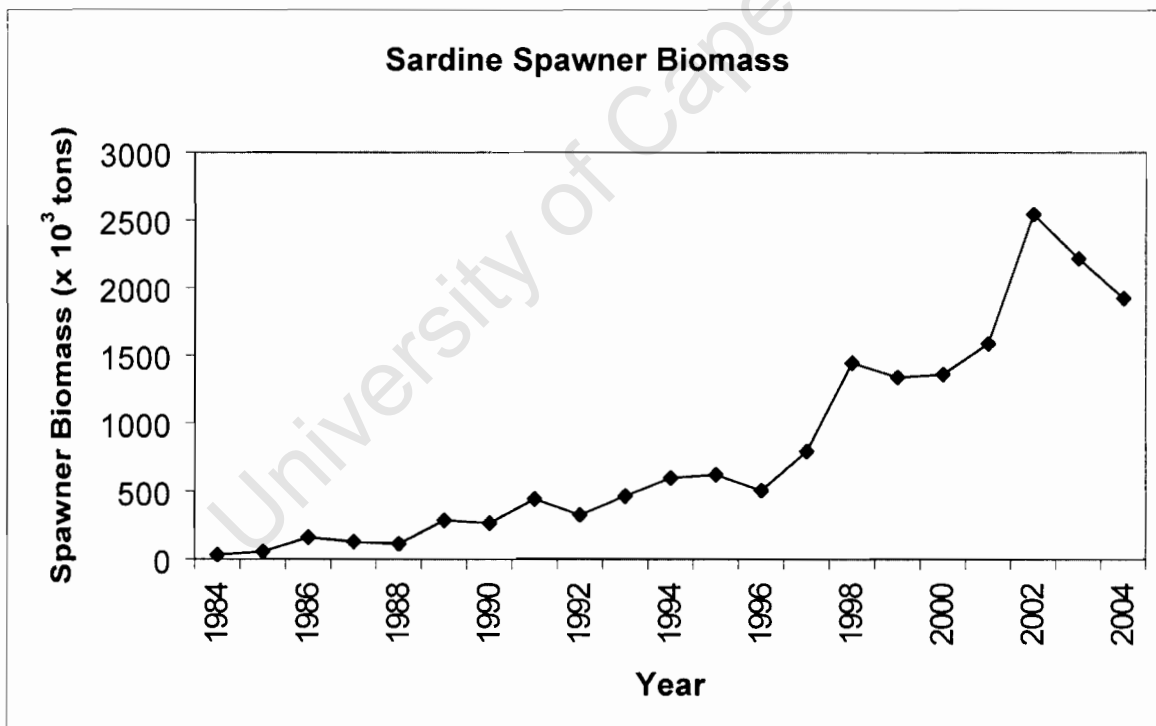
The value of the sardine resource, coupled with the variability inherent in sardine biomass and hence catches described above, emphasizes the importance of effectively managing the pelagic stocks to ensure their sustainable utilization. While a significant proportion of this variability in the past few decades can be attributed to fishing pressure, recent research indicates that such variability is natural for clupeoid populations, even in the absence of exploitation. Clupeoid populations, including sardine, are sensitive to environmental variability that can be reflected in large inter-annual variations in abundance. This sensitivity is particularly profound at the level of the younger life stages, and is manifested in the magnitude of the recruitment in any given year.

Recruitment is generally considered to be the numbers of younger fish that survive the egg, larval and juvenile stage and grow to reach a size at which they are vulnerable to fishing gear and can be sampled by the fishery. Considering that a significant proportion of pelagic catches comprises recruits, it is clear that an understanding of the factors influencing recruitment strength (specifically the growth and mortality of eggs, larvae and pre-recruits) is fundamental to effective management of the sardine resource.

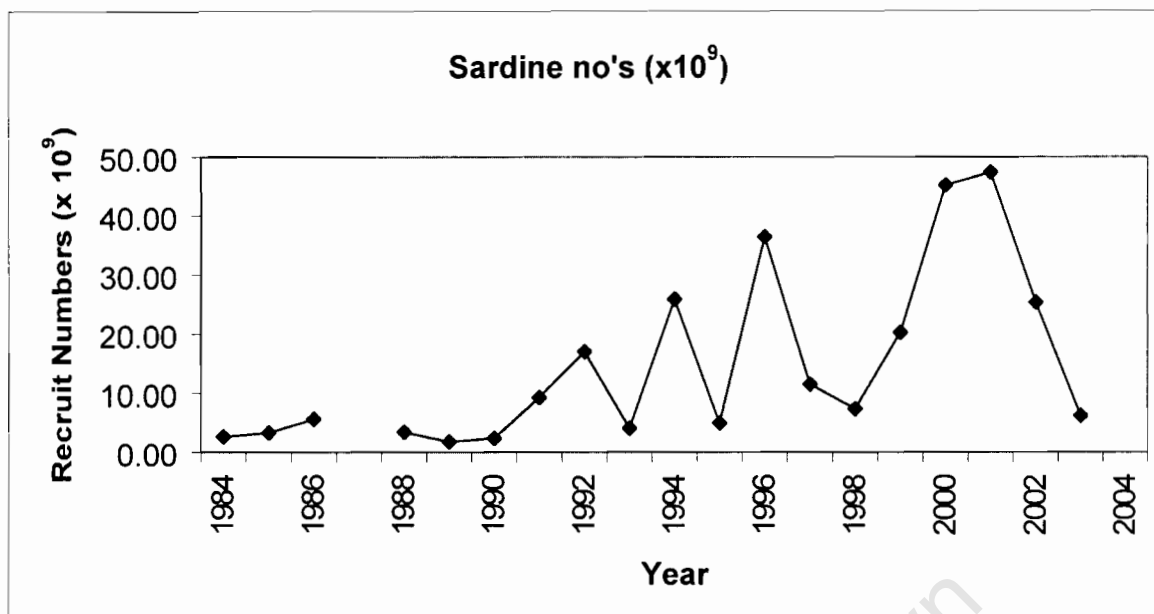
Recruitment has a pivotal position in regulating fish abundance. In spite of a considerable amount of research devoted to investigating factors influencing recruitment (and hence stock abundance), there are few reliable predictors of year-class success prior to recruitment. Lasker (1981) noted that both environmental variables and biological processes might influence recruitment although it is unlikely that either acts in isolation. The causal processes had been assessed although it had been difficult; numerous parameters have been correlated with cohort survival, yet there are few that provide any predictive power (Sissenwine 1984). Although the study focused on anchovy in the southern Benguela region, Painting *et. al* (1998) indicated that

environmental and biological variables may be used in a structured way to forecast the pelagic recruitment.

Acoustic estimates of biomass show that the sardine population has grown steadily over the last decade (Fig. 1.1), and this is reflected in a general increase in landings. Recruitment, however, has displayed considerably more variability. See section 1.5 for the description of the approach of recruitment collected during these surveys (Fig. 1.2) which illustrate the variability mentioned above. The highest recruit abundances of the time series (more than 40 billion individuals) were recorded in 2000 and 2001, following relatively low estimates of recruitment in 1997 - 1999 (Fig. 1.2). Sardine recruitment then decreased dramatically over the period 2002 to 2003, approaching the low levels previously observed in the 1980's. This variability in recruitment is attributed primarily to environmental variability impacting on the early life stages of sardine.



**Figure 1.1.** Acoustic estimates of sardine spawner biomass since 1984 (South African Marine and Coastal Management).



**Figure 1.2.** The relative abundance of sardine recruits from 1984 to 2003 (South African Marine and Coastal Management).

Factors that are of importance in terms of physical environmental influences on sardine recruitment include unfavourable temperatures that may result in poor survival and growth; winds and water currents that may transport sardine eggs and larvae out of environmentally favourable habitats, thereby contributing to increased mortality.

### 1.3 Recruitment

Poor correlation between recruitment and stock size of pelagic fish led Hjort (1914) to propose a link between feeding, larval survival and recruitment. He hypothesized that the strength of the year-class is established by the early post yolk-sac stage of the larval fish. He further stated that the number of living larvae was directly proportional to food abundance i.e. less food will result in reduced larval survival (or more larval death due to starvation). In 1926, Hjort put forward the Advection hypothesis that suggests that eggs and larvae could be carried out of productive areas by advection, leading to early larval mortality (Hjort 1926, Parish *et al.* 1981).

Cushing (1975, 1982, 1990) extended Hjort's (1914) hypothesis and introduced the term "match-mismatch", which is used when the phytoplankton production does not coincide with zooplankton production. This leads to poor survival of the fish and hence, poor recruitment (the match-mismatch hypothesis). For pelagic species, both seasonal and annual food production limit recruitment by regulating the annual carrying capacity of a system (Parrish and MacCall 1978).

The stability hypothesis of Lasker (1975) proposes that the chlorophyll maximum layers that develop when the water column is stable are vital for the survival of fish larvae, providing high concentrations of phytoplankton and other food for fish. Competition with other planktivores, the small-scale turbulence effect that would increase encounter rates between larvae and their prey and concentrations of food patches available to fish larvae on a micro scale are all factors that are considered to affect feeding and hence survival.

The sardine recruitment study, by Hardman-Mountford (2001), in the Benguela system stated that Northerm Benguela sardine spawn between September and April with peaks in September-November and February-April (Le Clus 1990, Kreiner *et al.* 2001). The sardine planktonic egg and larval stages last for 50-100 days before metamorphosis (Shannon 1998). It is only after this period that fish are able to swim against currents and actively forage. During this planktonic period, environmental conditions can strongly influence larval survival. Successful recruitment requires 3 main factors (Bakun 1996), which are nutrient enrichment; food particles concentration and retention, and the upwelling Benguela system produces these favourable conditions.

#### **1.4 Age, growth and mortality**

Recruitment is a direct function of the growth and mortality of the youngest year class in the population. In order to identify and investigate the factors influencing recruitment, it is necessary to be able to measure the growth and mortality of these younger life stages. Both growth and mortality are time-dependent processes, and it is therefore critical that the time component can be measured. In the case of living organisms, this component is best measured as the age of the individuals. In the case of fish, the analysis of calcified tissues is the generally accepted technique of age estimation. Examples of the structures that are commonly used for age and growth inferences of fish are scales, otoliths, opercular bones, finrays and vertebrae.

The discovery that many species of fishes deposit daily increments in their otoliths (Pannella, 1971, 1974, 1980) had a profound impact on studies of fish ecology. The ability to measure the age of fish on a scale of days is a powerful tool with many applications in the study of fish biology and population dynamics. Apart from a mere estimate of age, for example, individual increments can also be interpreted in terms of the date of formation, through knowledge of the date of sampling i.e. date of formation of the marginal, or last-formed increment. A typical

sequence of daily growth increments lends itself to most applications more than those at the yearly level, the reason being they have a longer and temporally more exact sequence (Campana and Jones 1992). Dated increments were found to have increasing value to analyses of cross-relating environmental factors to the otolith growth sequence (e.g. Methot 1981; Campana and Hurley 1989). Campana and Neilson (1985), and Campana and Jones (1992) have provided detailed descriptions of other applications of the daily increment technique.

To study relative growth rates, many workers plot fish size against the number of increments. These fishes were obtained from different environmental conditions (Kendell and Gordon 1981; Volk *et al* 1984). Methot (1981) measured the outer three daily increments of larval fishes as indicators of recent growth. Methot (1981); Laroche *et al.* (1982); Lough *et al.* (1982); and Campana (1984) all noted that a curvilinear (or allometric) otolith length-fish relationship is characteristic of many species during the larval phase whereas the juvenile phase is characterized by a linear (isometric) relationship (Messieh 1975; Rosenberg 1982; Campana 1984b).

In order for the back-calculations of growth from juvenile to larvae to be done, the size at metamorphosis must be known. Campana (1984b) states that in starry flounder larvae, increasing increment widths were better correlated with increasing age rather than increased growth rate, at least before the larvae reached metamorphosis. In another study, the growth of larval American shad (*Alosa sapidissima*) as inferred from increment counts and length data, decreased as they approached metamorphosis (Crecco *et al.* 1983).

The other major parameter affecting recruitment strength is mortality, and the daily increment technique is being increasingly applied to studies of mortality and survivorship of young fish. Measuring age-specific abundance and survival is a significant improvement over mortality estimation focusing only on size, primarily because size in young fish is not a good measure of age (Campana and Jones, 1992). Use of the daily increment technique permits identification of cohorts (a limited group of individuals that once hatched can only decline in abundance). Monitoring of the decrease in abundance of these cohorts over time can then be used to estimate age-specific mortality rates.

A temperature, food abundance and predator abundance are the most of the commonly noted correlates of a recruitment and they have direct influences upon fish growth, which in turn are reflected in the incremental growth sequence in the otolith. It is of importance that the growth

sequence spans both the larval and juvenile stages of the life history. Determined size at age and growth rate at age from the otolith microstructure enables the collection of the age at which class strength is determined. This can be accomplished through collection of independent estimates of cohort size over a range of years, which in turn can be correlated with the growth at age data in the development of a predictive model of recruitment.

Campana and Jones (1992) stated that removal of information concerning the observed variance in size at age is the common model and because of that they suggested that the useful starting point for any growth analysis would be to make a simple scatter plot of fish size against age.

Measuring age-specific abundance and survival is a significant improvement over mortality estimation focusing only on size, but in young fish size is not a good measure of age since the size of the fish does not reflect the age of the fish. Houde (1989), Owen *et al.* (1989), Alhossaini *et al.* (1989), Fortier and Gagne (1990) and Pepin (1991) are a few recent papers relying on daily ageing for measuring mortality.

Sardines (and clupeoids in general) are fast-growing and short-lived species. Baird (1970b) used otoliths to determine the age and growth of the sardines caught off South-Western Cape. His findings were that the von Bertalanffy growth function ( $l_t = l_\infty [1 - e^{-K(t-t_0)}]$ , where  $l_t$  = length at age  $t$ ,  $l_\infty$  = asymptotic length (theoretical maximum),  $t$  = age in days and  $K$  = a growth coefficient) was :  $l_t = 30.6[1 - e^{-0.2247(t+1.5046)}]$  having 13.2 cm and 16.6 cm as the average total lengths of one- and two-year-old fish, respectively.

More recent research on ageing of South African sardine includes routine ageing of otoliths from commercial catches (Kerstan and Waldron 1995), validation of annuli by daily ring counts using scanning electron microscopy (Waldron 1998; Taubert and Coble, 1976; Brothers, Matthews and Lasker, 1976, Geffen, 1982) and construction of birth-date distributions of juvenile sardine (Kerstan and Waldron 1995). The results of Kerstan and Waldron (1995) suggest that the average growth rates of sardine recruits (less than 6 months old) ranges between 0.55 and 0.6 mm per day.

## **1.5 Objectives and approach**

The general objective of this study is to understand the fishery of sardine, especially the recruitment since it is highly variable. Age and growth studies will aid in understanding the growth and mortality rates of the sardine. The overall objective of the research described in this thesis was to measure sardine pre-recruit growth and mortality in the Southern Benguela region in early 2001 with the intention of gaining an understanding of factors influencing recruitment.

The years 1999 to 2001 have been an interesting period in the Southern Benguela region. Although sardine adult spawner biomass has been increasing steadily since the mid-1980s, sardine recruitment has been highly variable. The second highest recruit density on record (0.73 individuals  $1\ 000\ m^{-3}$ ) was recorded during 2001, following the lowest ever estimate of recruitment in 2000 (Crawford 1980, de Oliveira *et al.* 1998b; Marine and Coastal Management unpublished). Anchovy population abundance has increased significantly in subsequent years, following an apparent decline towards the end of the 1990s. This period is consequently of interest in terms of recruitment studies on pelagic fish.

The main objectives of this study are to accurately and consistently count daily increments in otoliths of sardine pre-recruits sampled in the Southern Benguela during early 2001, to use the increment counts to estimate age of these individuals and hence calculate growth rates and to back-calculate hatch-dates to identify cohorts for analysis of mortality.

The approach employed in this research was to sample the 2000/2001 sardine year class at two separate points during early 2001 to monitor changes in growth and abundance. The two sampling points comprised the routine pelagic pre-recruit and recruit surveys conducted by Marine and Coastal Management in March and May of each year, respectively. The pre-recruit survey conducted in March is designed to provide a “first-look” at recruitment strength of anchovy and sardine for the coming year. A standard survey area extending from the Orange River mouth to Cape Columbine is sampled using a Methot frame trawl.

The recruit survey, conducted in May-June each year covers a survey area extending from the Orange River mouth to Port Alfred on the east coast of South Africa. This survey uses hydroacoustic technology supplemented by mid-water trawling to obtain an estimate of sardine and anchovy recruit biomass and hence recruitment strength for that year. Sardine larvae and

juveniles sampled during these two surveys were kept for analyses of otolith microstructure and age estimation.

Recruitment can be understood by looking at the growth and mortality of young life stages. Both growth and mortality are time-dependent processes, and the time component has to be measured in order estimate growth and mortality. In a study such as the one documented in this thesis, the time component is measured as the age of the individuals. In the current study, otoliths increment rings are used to estimate the age.

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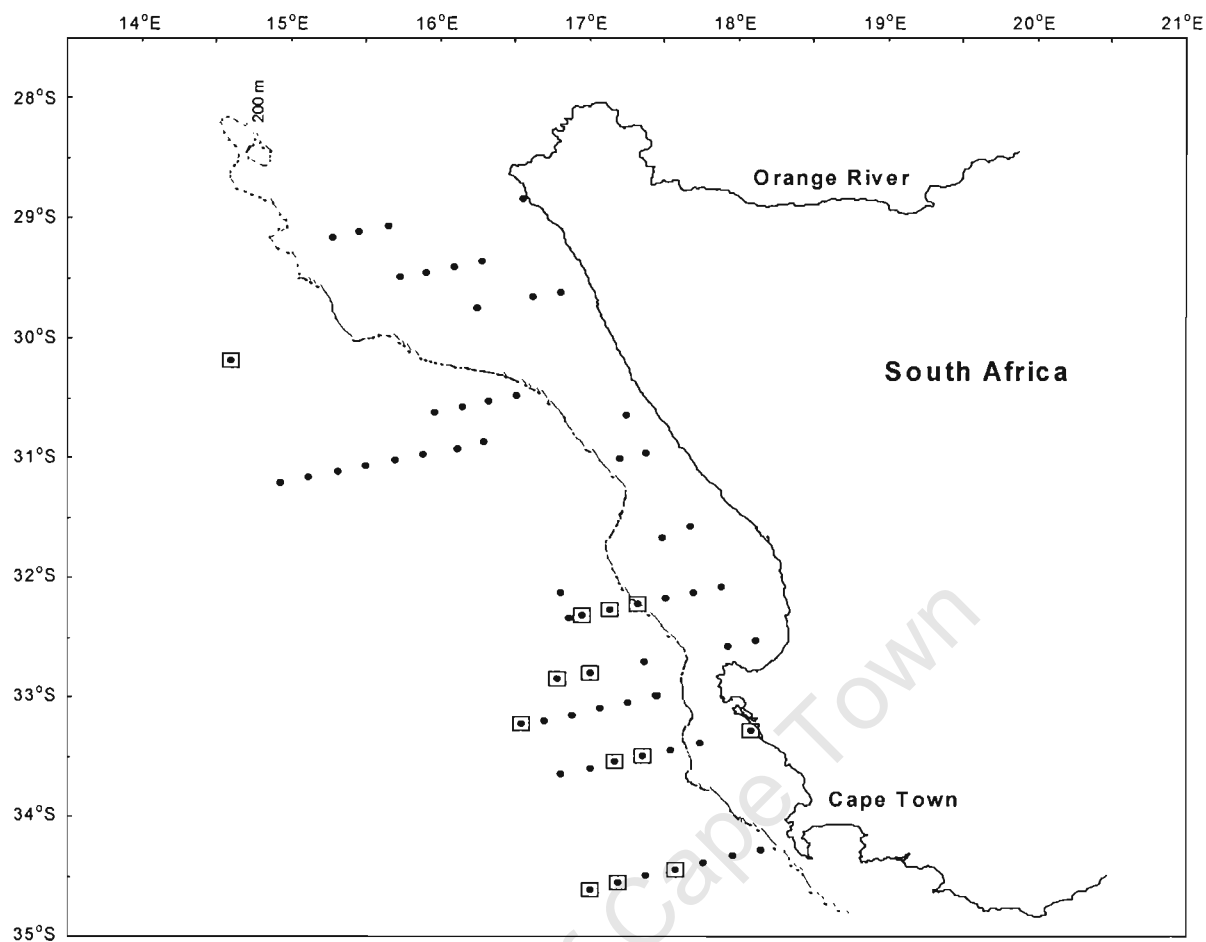
## **CHAPTER 2 – MATERIALS AND METHODS**

### **2.1 Sampling**

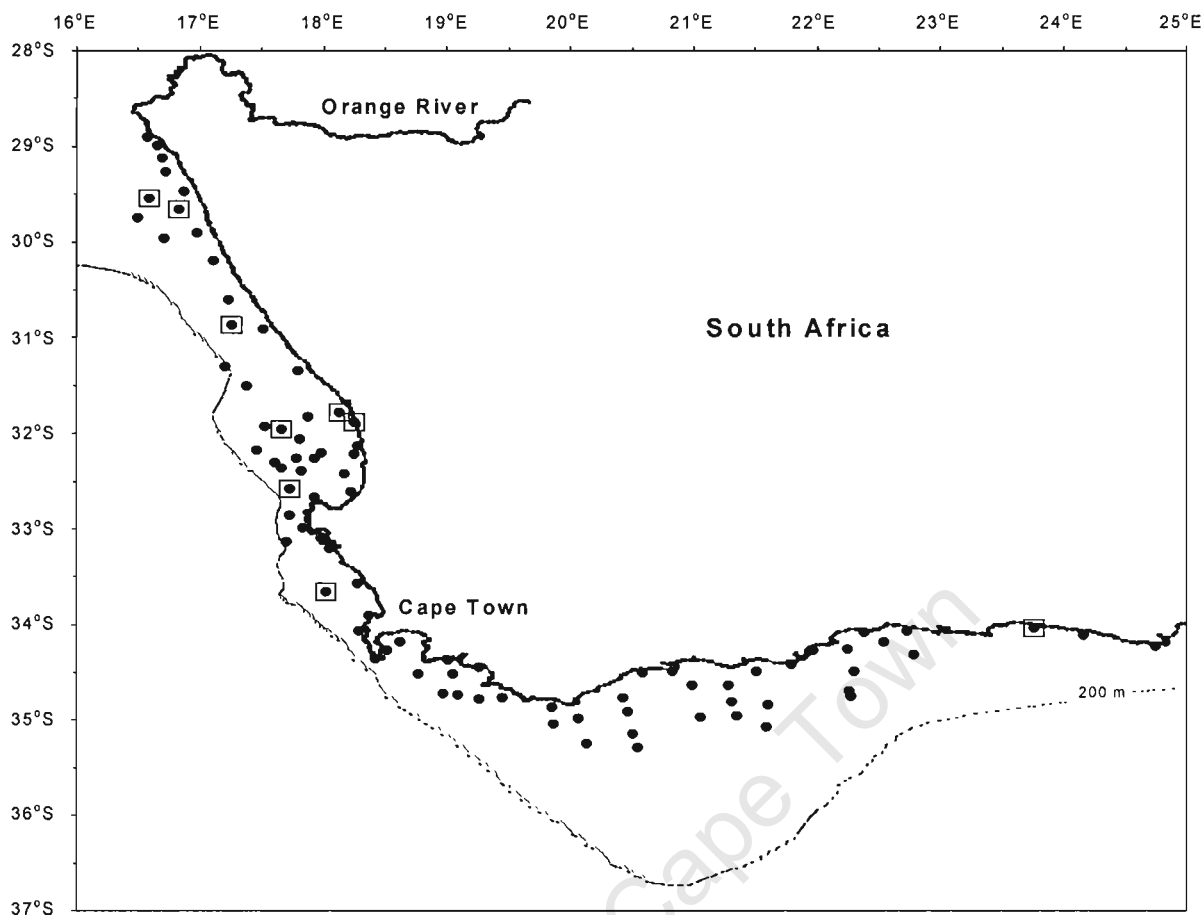
#### **2.1.1 March 2001 Pelagic Pre-Recruit Survey:**

The Pelagic Pre-Recruit Survey was conducted from 9-25 March 2001 aboard the Marine and Coastal Management (MCM) vessel, *RS Algoa*. The survey extended from the Orange River mouth to Cape Point (Fig. 2.1), with sampling occurring at periodic intervals along randomly selected transects extending from the coast offshore to the edge of the shelf. During the survey, a Methot net was employed to sample pelagic fish larvae (Beckley and van der Lingen, 1999). The net was deployed from the ship and was towed following a standard step-wise, oblique protocol. The net was deployed to a depth of 35 m where it was fished for 2 minutes. It was subsequently retrieved at depth intervals of 5 m, fishing for 2 minutes at each depth interval. All sampling was conducted nocturnally. There were 111 hauls, with more than 2420 sardine larvae captured. At the completion of each tow, the cod-end contents were placed in a tray and the *S. sagax* larvae were separated from other clupeoid larvae, such as anchovy and round herring. At each sampling site (station), sardine larvae captured in the Methot net were counted and measured. The larvae were then stored in sealed, labelled plastic bags and frozen for later processing in the laboratory.

A flow meter attached to the Methot net allowed the calculation of the volume of water filtered by the net, which, together with the number of larvae caught provided an estimate of the density (numbers per m<sup>3</sup>) within each size class.



**Fig 2.1** March 2001 Pre-recruit Survey. Stations positive for sardine (i.e. sardine larvae present in the Methot net) are indicated with dots, while the stations sub-sampled for otolith analyses are shown with squares. The position of the 200m isobath is indicated with a dotted line. Although one sardine pre-recruit was measured far away from the others, due to bad weather conditions that led to difficulty to sample inbetween, it followed the same relationships as other sardine larvae.



**Fig 2.2.** May 2001 Recruit Survey. Stations positive for sardine are indicated with dots, while the stations subsampled for otolith analyses are shown with squares. The position of the 200m isobath is indicated with a dotted line.

### 2.1.2 May 2001 Pelagic Recruit Survey:

This survey was conducted aboard R/V ALGOA (Voyage 095), between 3-30 May 2001 to estimate the biomass of pelagic recruits (primarily anchovy and sardine). The survey area extended from the Orange River to Port Alfred (Fig. 2.2) and the survey grid was designed using the stratified random transect principles of Jolly and Hampton (1990). Acoustic measurements were recorded continuously during the survey, and the abundance of anchovy and sardine were computed from these measurements using standard echo-integration methods (MacLennan and Simmonds, 1992).

The abundances were averaged over each survey line and weighted over the size of the strata (Hampton 1987). Trawls to identify acoustically detected targets were conducted periodically using an Engels 308 mid-water trawl, and it was from the fish collected during these trawls that

the sub-samples for age estimation were obtained. There were 111 hauls, with 7012 sardine juveniles captured. The subsamples were frozen on the vessel for later analysis in the laboratory.

## **2.2 Otolith processing and analysis**

### **2.2.1 Processing of larval otoliths**

In the laboratory, the larvae were measured (in mm) and thawed. The method used for dissecting, mounting and observing the otoliths was that described by Ré (1983). The otoliths were extracted from the larvae with the use of sharp wooden tooth-picks to avoid scratching or breaking the otoliths, as may result from metal needles. Dissection was performed under a dissecting microscope to which a polarizing filter was attached. The extracted otoliths were fixed to the microscope slides with the use of the DPX mountant. Both otoliths were mounted on the same slide, with their plane face (internal face) downwards. When one of the otoliths was broken, only one was mounted.

Four replicate counts of the number of daily increments in each otolith were conducted without reference to the prior counts. Intervals of three weeks to a month elapsed between replicate counts in order to avoid possible bias. Occasionally, daily increments were difficult to resolve within short segments of the otoliths. In such cases, the widths of the increments, (and their number), were interpolated by using linear approximation based on the widths of adjacent, clearly visible daily increments. If the data of otolith reading from the interpolation process affected more than 5% of the age estimate, it was rejected as unreliable (Alvarez and Alemany 1996).

The otoliths were separated from the resin, re-polished and mounted on the stubs after which they were dried, gold-coated and then vacuum-cleaned for the SEM analysis. A montage of micrographs of each otolith from the nucleus to the periphery was used to identify and count daily increments in the otolith. The interpolation process described above was also used here in cases where daily increments could not be clearly seen.

### 2.2.2 Processing of juvenile otoliths

The juveniles were thawed and their lengths measured. The cranium of the fish was sectioned to reach the chambers of the inner ear. This allowed the easy removal of the otoliths. After the exposure of the saccule, the otoliths were drawn out carefully with forceps, and dried on a microscope slide.

The otoliths were embedded in laboratory-prepared plastic resin blocks in order to facilitate handling and obtain precise sections. A small amount of plastic resin was poured into a latex mould that had been pre-sprayed with silicon (to facilitate extraction of the resin block) and allowed to partially cure. Otoliths were carefully positioned on the resin, and covered with more of the resin to provide support during cutting. The resin blocks were allowed to cure for approximately 24 hours in a dust-free area before any further treatment.

A precision saw with diamond coated blades was used to cut the otolith-containing block along the transverse plane through the otolith. The two halves of each block were mounted on either end of a microscope slide with DPX mounting medium and ground using rough sandpaper to expose the inner core of the otolith (Struhsaker and Uchiyama 1976). After grinding, the otoliths were further hand-ground with very fine sandpaper (Castonguay 1987) and then polished with a 0.3  $\mu\text{m}$  alumina polish. For viewing, the sections were coated with immersion oil to make them clearer for light microscope analysis. The otoliths were viewed using a video camera mounted on a compound microscope interfaced to a personal computer, and increments counted off the monitor of the computer. The otoliths were separated from the resin, re-polished and mounted on the stubs after which they were dried, gold-coated and then vacuum-cleaned for the SEM analysis. A sub-sample of otoliths was examined using scanning electron microscope (SEM) in an attempt to establish whether or not light microscopy (LM ) underestimates age due to lower resolution. A montage of micrographs of each otolith from the nucleus to the periphery used to identify and count daily increments in the otolith. The same interpolation process was used here in cases where the counts could not be clearly seen.

## **2.3 Data Analysis**

### 2.3.1 Nature of the age data

Four and three replicate counts of the number of daily increments were conducted for each otolith from the larvae and juveniles, respectively. The mean of the replicate counts was calculated to arrive at an estimate of the age of each fish in days.

### 2.3.2 Ageing precision and bias

Age estimates are most valuable when they are both accurate and precise. Precision refers to the reproducibility of the individual measurement while accuracy refers to the proximity of the estimate to the “true” value. Campana and Moksness (1991) cited that accurate results need not be precise, and vice versa. The mean count can be accurate (close to the truth) while the individual observations vary.

An index of precision calculated from the results of the replicate readings was used to assess the quality of the data. Although the percent agreement statistic has been used in the past, Beamish and Fournier (1981) have pointed out the deficiencies of this index of precision. Less than 95% agreement to within one year between two readers of Pacific cod (*Gadus macrocephalus*, 38 years longevity) constituted poor precision, given the few year classes in the fishery, whereas 95% agreement to within 5 years would constitute good precision for spiny dogfish (*Squalus acanthias*), given its 60 year longevity (Beamish and Fournier, 1981).

Beamish and Fournier (1981) consequently recommended the use of the average percent error (APE) (to check the precision of the readings) which compares the percent of determinations that are in agreement (Beamish and Fournier, 1981), and this statistic was adopted for the purpose of this study. The APE equation is as follows:

$$APE_j = 100\% \times \frac{1}{R} \sum_{i=1}^R \frac{|x_{ij} - x_j|}{x_j}$$

where  $x_{ij}$  = the  $i$ th age determination of the  $j$ th fish

$x_j$  = the mean age estimate of the  $j$ th fish

$R$  = the number of times each fish is aged.

Together with accuracy and precision, an assessment of the bias inherent in the age data increases the value of the data. To determine bias, the otoliths rings were read and the mean was taken as the true or reference value. Each mean value of the 4 (in case of larvae) or 3 (in case of juveniles) replicate readings was then compared against the average residual values to assess bias.

### 2.3.3 Estimating growth rates

Growth models vary in complexity from simple linear regressions of fish size on age to sophisticated maximum likelihood estimates of size at age. Model preparation allows the prediction of an expected mean size or growth rate at some age and to facilitate comparison of estimated growth rates with other published estimates.

Removal of information concerning the observed variance in size at age has been noted as being common to many models (Campana and Jones 1992). A simple scatter-plot of fish size versus age is therefore regarded as being a useful starting point for any analysis of growth (Campana and Jones 1992). To calculate the growth rate of sardine in this study, measurements of fish length were plotted against the estimated age of the fish. Simple linear regression models were fitted to the data, the slope of the models providing an estimate of the average growth rates of the sardine sampled during surveys. Linear regression was used to estimate the growth rate (slope of the curve), since it is, (a) most commonly applied of what are generally termed growth models, (b) easily fitted, (c) easily interpreted and (d) amenable to confidence interval calculations both around the slope (growth rate) and around the point values (Campana and Jones 1992).

### 2.3.4 Adjustment of age and length

The size at-age data were used to draw up Age Length Keys (ALKs) that show the number of fish of each age class within each size class. With these ALKs, 5mm size-class intervals have been set along with 5 day age-class intervals.

In order to set up the ALKs and calculate mortality, the size and age of the fishes sampled during both surveys were adjusted to the mid-dates of the respective surveys to account for the time differences inherent in the sampling. Fish sampled before the mid-date will be larger and

older at the mid-date than they were at sampling, while fish sampled after the mid-dates will be smaller and younger at the mid-dates than at sampling. Individual fish age was adjusted by computing the time difference between the date of the sampling and the mid-date of each survey, and, either subtracting (in the case of fish sampled after the mid-dates), or adding (fish sampled before the mid-date) this value from the age at sampling estimated from the otoliths.

Adjusting the length of individual fish to what it was at the mid-date was done using the growth rate estimates obtained from the linear regressions described in section 2.3.3 above. The duration between sampling and mid-date was multiplied by the growth rate (slope of the regression line) and the resulting change in length was then either added to (fish sampled before the mid-date) or subtracted from (fish sampled after the mid-date) the size at sampling. The mid-dates of the two surveys were fortuitously exactly 60 days apart, and consequently 5-day intervals were used to pool the age data. The values obtained were then raised to the population frequencies by multiplying each percentage by the number of fish in the population that fall into each size class.

In order to compute the size and age structure of the sardine pre-recruit population at the time of sampling, each station sampled with the Methot net was taken to be the centre of a rectangle extending from the mid-point between adjacent stations and adjacent lines. The area of each rectangle was multiplied by 35m (the depth of sampling) to generate an overall volume for each station. The total number of sardine corresponding to each station was then calculated by multiplying the density of sardine measured at the station by the overall volume corresponding to the station. This total was then split up into the appropriate size classes by assuming simple proportionality. The size of the sardine in each size class was adjusted to the mid-point of the survey (17 March) as described above. The sum of sardine numbers per size class over all the stations was then considered to be the total number of sardine (per size class) in the Southern Benguela on 17 March 2001.

The same procedure as above was followed for the juveniles. The size of the sardine in each size class was adjusted to the mid-point of the survey (16 May) as described above. The sum of sardine numbers per size class over all the stations was then considered to be the total number of sardine (per size class) in the Southern Benguela on 16 May 2001.

### 2.3.5 Estimates of mortality

Campana and Jones (1992) describe two methods that can be used to calculate mortality rates :

(1) Mark and recapture method,

(2) Catch curve analysis.

The latter approach will be used in this study. It plots the log-transformed frequency of fish grouped by age or size, with the use of the following equation (negative exponential model of decay) :

$$\ln N_t = \ln N_0 - Zt$$

where ,

Z = the instantaneous daily mortality rate

$N_t$  = the number of individuals at age-class t

$N_0$  = the number of individuals at age-class 0

t = age in days.

Using this approach, mortality is estimated by measuring the decline in the abundance of a cohort over a known period of time. This decline is caused by a number of factors, including starvation, predation, disease, and advection to areas where survival is diminished.

The assumptions involved in this approach to the estimation of mortality are (a) the fish had a continuous spawning period (Melo 1994b) and mortality, together with advection, are the only causes of decrease in abundance, (b) the whole range of the fish population is covered by the survey area, as fish may migrate or be advected into or out of the area where the survey was done (Vetter 1998), and, (c) the fish counted and measured on the surveys are counted in proportion to their true abundance, since the fish can escape the net, get out through the net or swim away from sampling areas (Lo *et al.* 1989 and Smith 1981).

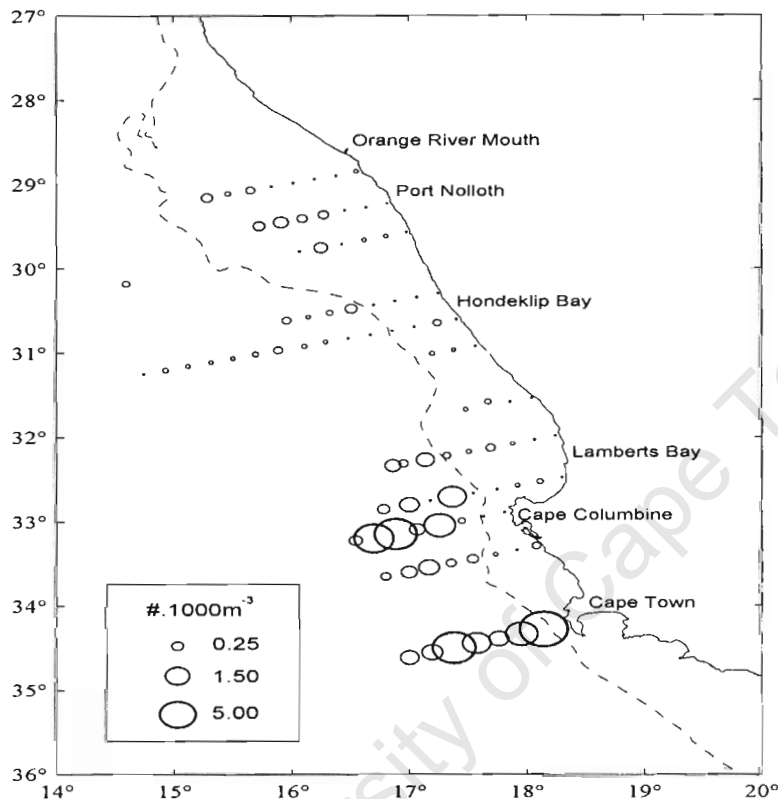
### 2.3.6 Hatch-date estimation

Hatch date analysis plays a pivotal role in the study of the recruitment process. In order to set up a hatch date distribution, one must have a random sample of fish caught on a known date, and estimate the age of each fish. The frequency distribution of hatch dates for the survivors in the population can be calculated (Campana and Jones 1992). The resulting hatch date distribution is a mirror image of the age-frequency distribution. To get the hatch dates, the estimated age of the fish (through otolith microstructure examination) was subtracted from the date of capture of each fish.

University of Cape Town

### CHAPTER 3 – RESULTS OF THE ANALYSES OF LARVAL OTOLITHS

Although bad weather restricted this survey (March 2001), 111 Methot hauls were performed and more than 2420 sardine larvae were collected. The distribution and densities of the sardine larvae sampled during the survey are illustrated in Figure 3.1 below.



**Fig. 3.1** Distribution and abundance of sardine larvae (numbers per 1000 m<sup>3</sup>) measured during the March 2001 Pre-recruit survey. The size of the circles indicates the density of sardine larvae at each station.

Sardine pre-recruits were very abundant in March 2001, being found in 58% of the Methot hauls and having a mean pre-recruit density of 0.73 individuals. 1000 m<sup>-3</sup> (MCM Research Highlights 2000/2001). This is the second highest sardine pre-recruit density on record, representing a marked increase in abundance from the low abundance observed in 2000.



### 3.1 Size distribution of larvae

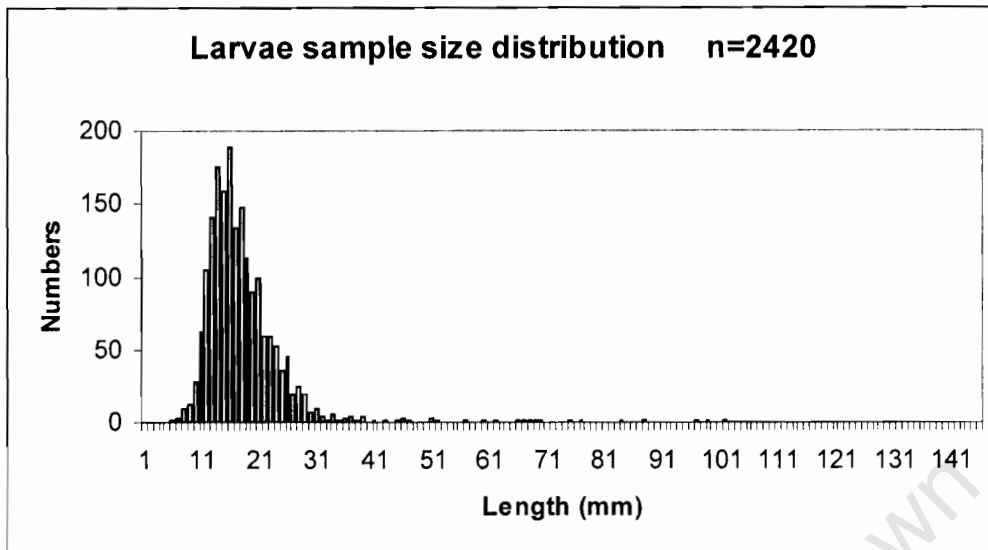


Fig 3.2 Size distribution of the larvae samples caught in March 2001.

The range of the size distribution of the larvae caught in March 2001 was between 4 and 101, 11 – 26 mm being the dominant size (Fig. 3.2). The size distribution, raised to the population level, is illustrated in Fig. 3.3

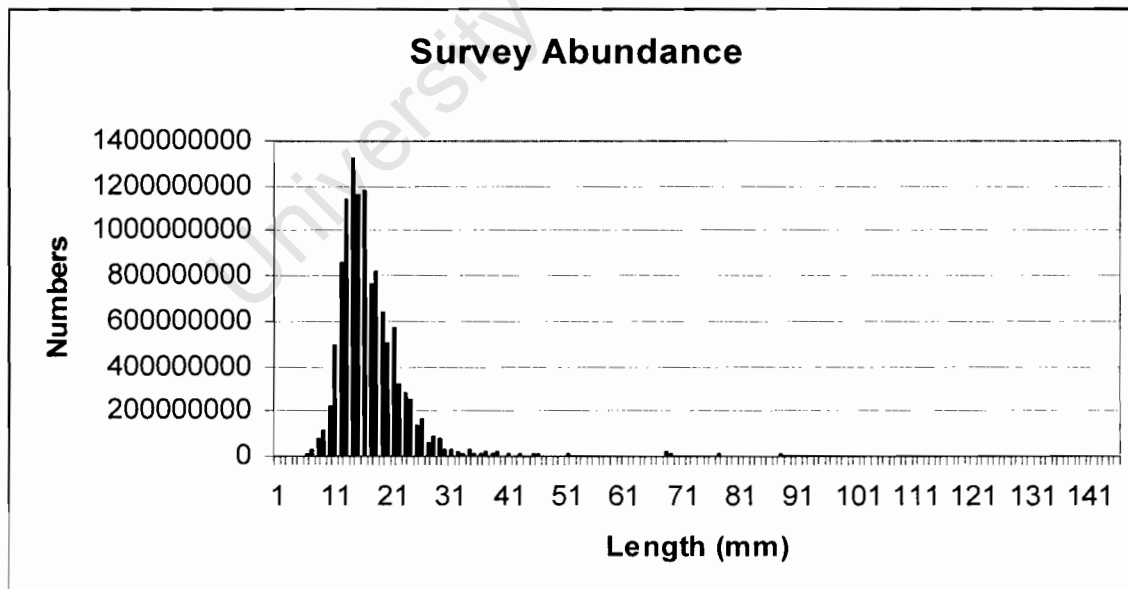


Fig 3.3 Survey size distribution of the larvae caught on the 17<sup>th</sup> of March 2001.

### 3.2 Age distribution of larvae

Otoliths from sardine collected at 13 of the stations (see Fig. 2.1) were extracted for age estimation, and of these, 111 otoliths were successfully aged. The size distribution of the successfully aged larvae (Fig 3.4) is considered to be representative of the sardine pre-recruit population at the time of sampling. Due to the difficulty in extracting otoliths without breaking them, most of the smaller size classes (< 20 mm) are missing in this study.

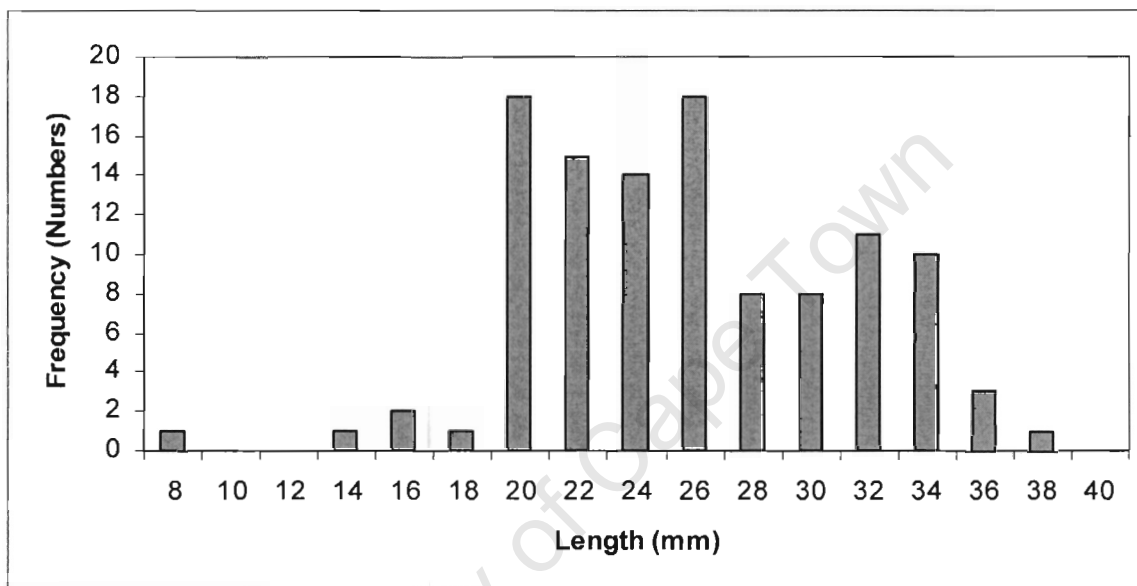


Fig. 3.4 Size frequency distribution of the successfully aged larvae.

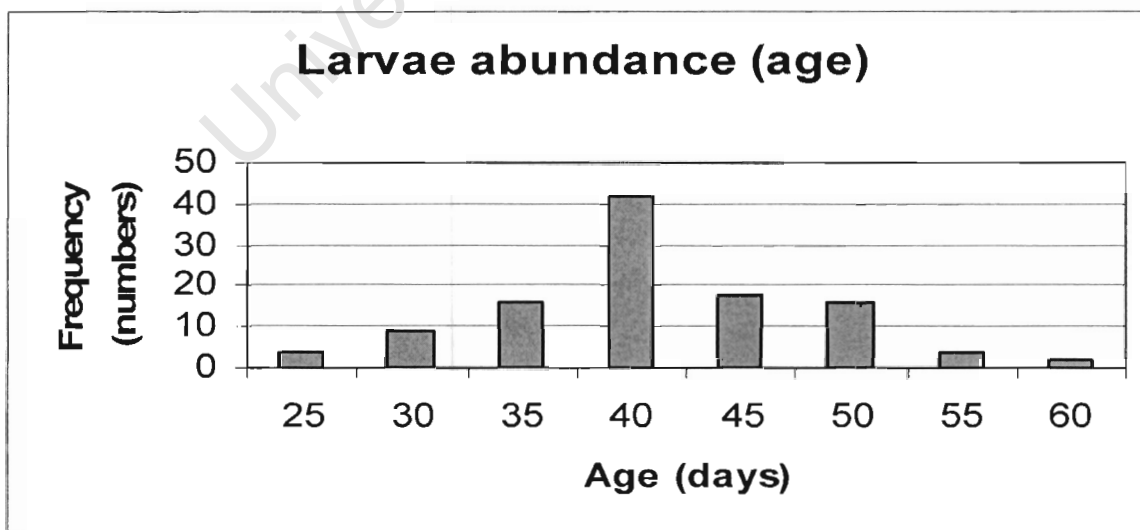


Fig 3.5 Numbers of sardine larvae distributed according to their age (in 5-day interval).

The growth ring counting results indicate that most larvae fell within the age range of 35 to 50 days, a few individuals reaching ages of up to 60 days (Fig. 3.5). The age-length key

constructed from these data (Table 3.1 above), when applied to the population size distribution illustrated in Fig 3.3, generated the population age distribution illustrated in Fig 3.6.

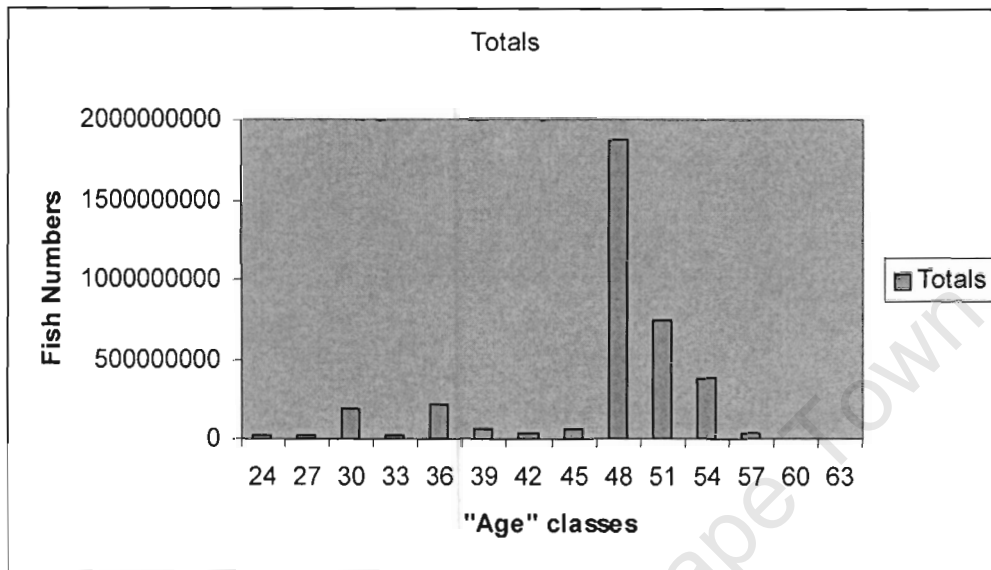
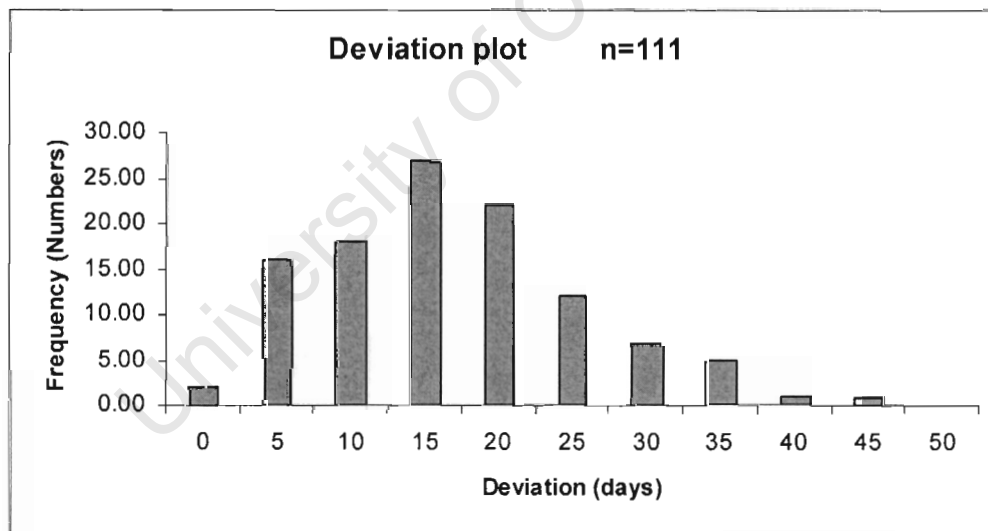


Fig 3.6 Histogram illustrating the abundance of fish larvae according to their age classes.

### 1.3 Precision and Bias

Measurements were made four times for each otolith, to see how consistent the counts were. The range of the total lengths of larvae was between 8 and 38mm. The minimum calculated average percent error (APE), was 3.5 %, corresponding to a coefficient of variation (CV) of 4.8 %. The maximum APE was 41.9 % (corresponding to a CV of 57.8 %). The APE averaged over all otoliths was 17.7 %, corresponding to an average CV of 23.3 %. Campana (2001) reported that many studies can be carried out with  $CV < 7.6\%$  and corresponding APE of 5 % which serves as a reference point for many fishes of moderate longevity and reading complexity. The precision (reproducibility of the readings) is relatively low. The counts deviated by as much as 48 days, with a mean of 20 days (see Fig. 3.7). The reason for this relatively low level of precision is illustrated in Figure 3.8, where a clear indication of bias is apparent as a tendency to increasingly overestimate the age of fish with each successive replicate reading. The last two replicates, however, indicated that the difference between successive counts was decreasing, suggesting that the interpretation criteria employed by the reader were stabilising.



**Fig. 3.7** Histogram illustrating counting deviations. Age deviations give the maximum difference in age assigned to each otolith during 4 replicate counts. The counts deviated by as much as 48 days.

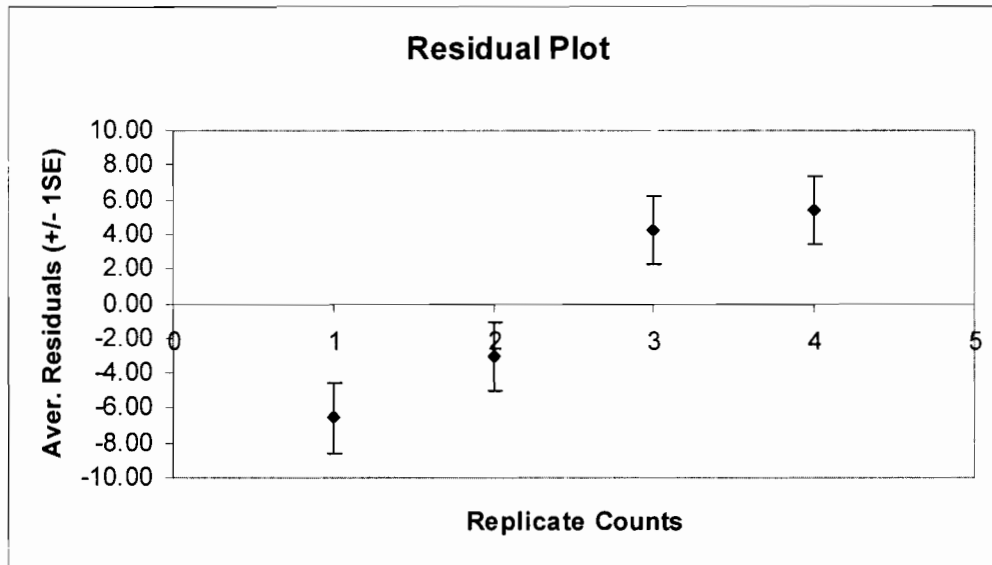


Fig. 3.8 Residual plot illustrating bias in the reading of the growth increments of the larvae.

There was a bias in counting of the growth rings, especially at the first two counts but the consistency was achieved as more counts were conducted.

### 3.4 Growth estimates

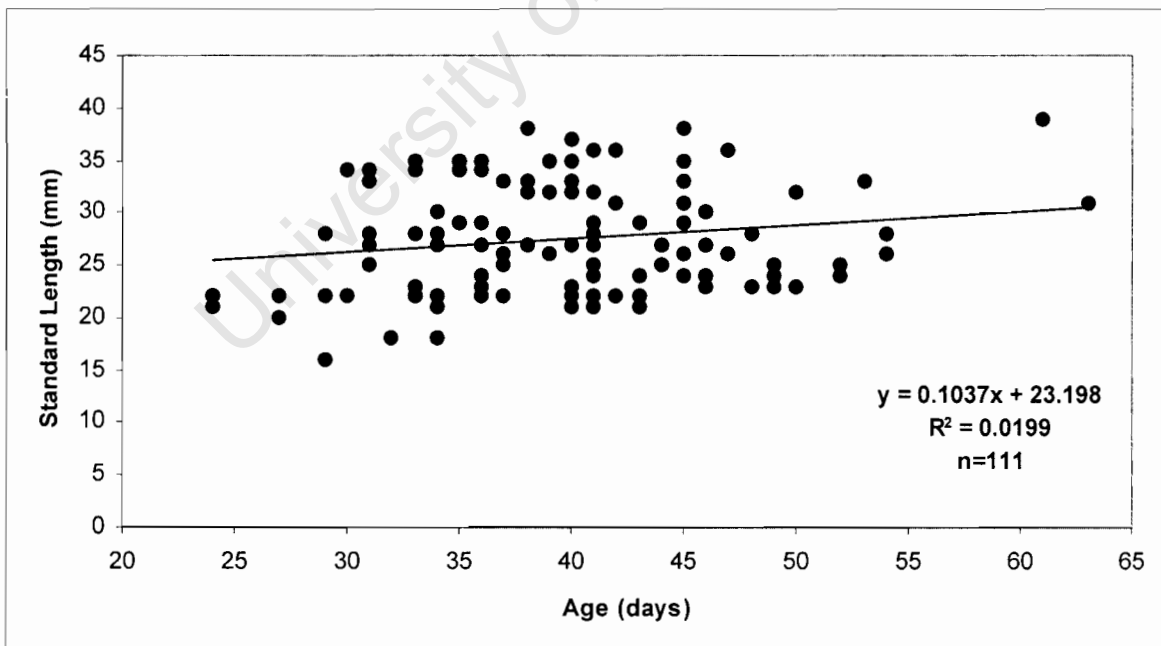


Fig. 3.9 Growth curve of the sardine larvae caught in March 2001. A linear regression line was fitted to the points.

The scatter plot shown in Figure 3.9 shows the relationship between the age (in days) and the length (in mm) of the larvae that were sampled for this study. The growth rate (slope) of the

larvae data, from this graph is  $0.1037 \text{ mm.d}^{-1}$ . It was this growth rate estimate that was used to adjust the survey length frequency data (and the size at age data) to the midpoint of the survey. The adjusted (see chapter 2 methods) sample data were then used to construct the Age-Length Key (Table 3.1).

### 3.5 Mortality and Hatchdates

The results obtained were then used to set up catch curves from which an estimate of the instantaneous mortality rate ( $M=Z$ ) was calculated by fitting a linear regression to the decreasing part of the catch curve (fig 3.10).

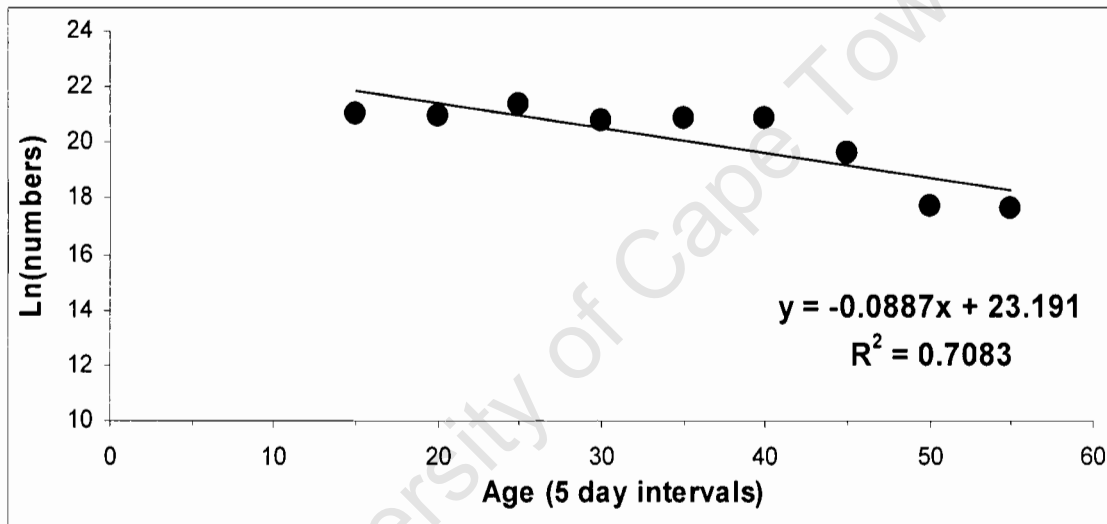
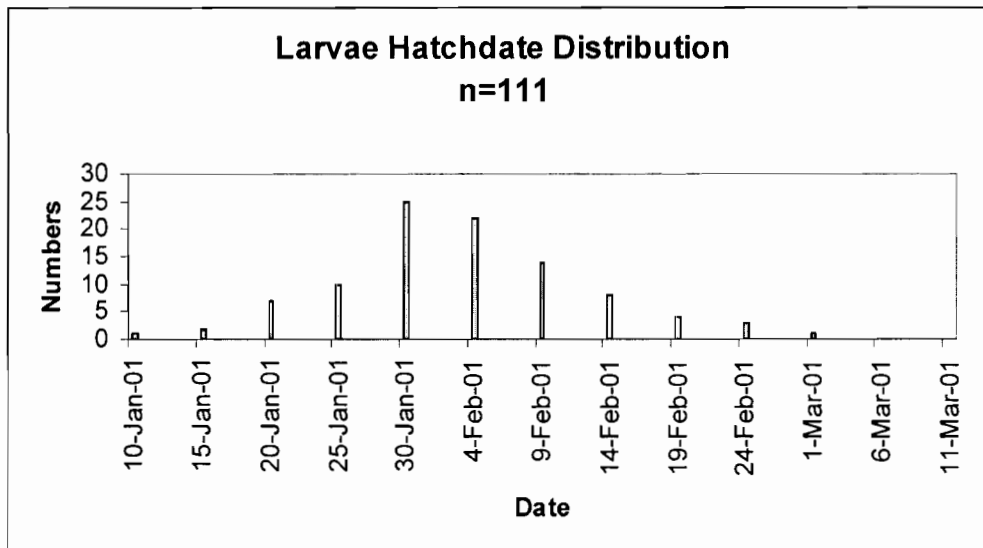


Fig 3.10 The mortality rate of the sardine larvae caught in March 2001.

Figure 3.10 is the sardine larvae catch curve and the regression line was fitted with the resulting estimate of mortality ( $M=Z$ ) = 0.0887 larvae per day, and it is this estimate of mortality that was used to correct for the effect of cumulative mortality on the estimate of population age structure that was presented earlier.



**Fig. 3.11.** Hatch-dates of the larvae caught in March 2001 in the Southern Benguela, in five-day intervals.

The hatch dates range from mid- January to early March 2001 (Fig. 3.11). If the assumption that the increments in sardine otoliths are deposited daily, back-calculated hatch-dates indicate that the majority of the larvae sampled hatched during the period from 30<sup>th</sup> of January 2001 to the 4<sup>th</sup> of February 2001. The abundances have been corrected to account for cumulative natural mortality.

## CHAPTER 4 – RESULTS OF THE ANALYSES OF JUVENILE OTOLITHS

During this survey, there were 111 hauls with 7072 sardine juveniles captured. 88 stations which were positive for sardine (Fig 2.2) were sampled, of which 9 (encircled stations in Fig. 2.2) were sub-sampled for otolith analysis. The otoliths were chosen based on the abundance proportion. 84 otoliths were prepared and 82 were successfully analysed, of which 20 were further prepared and successfully analysed using SEM.

**Table 4.1:** Age length key of sardine juveniles sampled during May 2001. Data are adjusted to reflect a sampling date of 16 May 2001.

	Age (days)																	
Lt (mm)	100	105	110	115	120	125	130	135	140	145	150	155	160	165	170	175	180	Total
30																		0
40																		0
50																		0
60					1													1
70		1						1	1									3
80					3	1	1	2		2		1						10
90					5	3	5		1	3	1	1						19
100				1	5	4	4	3	3		3		1			1		25
110					2	1	1	4	5	2	1	1	2					19
120				1	1	2	2		1									7
Tot	0	1	0	2	17	11	13	10	11	7	5	3	3	0	0	1	0	84

### 4.1 Size distribution of juveniles

There are two modes in the juvenile size distribution (Fig. 4.1). A mode at 85-100mm dominated the population, while the second one was at 155 mm fish size. These modes presumably corresponded to two peaks in spawning or survivorship. Two additional peaks at 155mm and 185 mm (perhaps corresponding to 1 year olds and older fish) were also apparent.

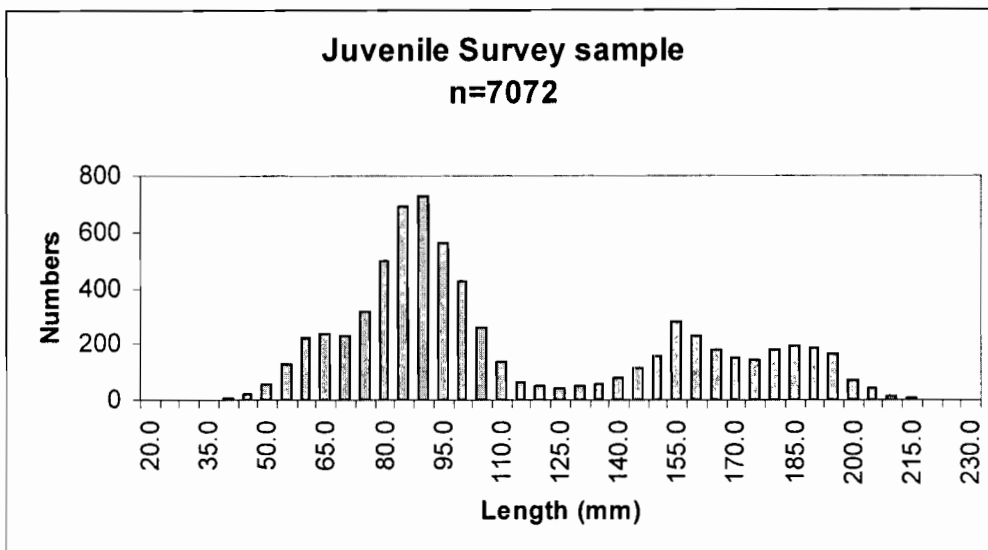


Fig 4.1 Size distribution of the juveniles caught on 16<sup>th</sup> of May 2001

The juvenile sample was further sub-sampled for otoliths processing. It was imperative that this sub-sampling size distribution (from 65 mm – 127 mm) represent the survey sample size distribution (first peak from the survey catch). This was not the case, as the subsampling was randomly conducted, i.e. the juveniles were never measured before subsampled hence the two graphs (Fig 4.1 and 4.2) do not exactly resemble each other. One of the aims of this study is to see the shift/change in recruitment between March and May, hence the first peak (smaller scale) will be considered. The juveniles were randomly sub-sampled, hence there were few fish in the length range between 90 mm and 100 mm. 82 otoliths were successfully aged, the corresponding size distribution of these samples is illustrated in Fig. 4.2.

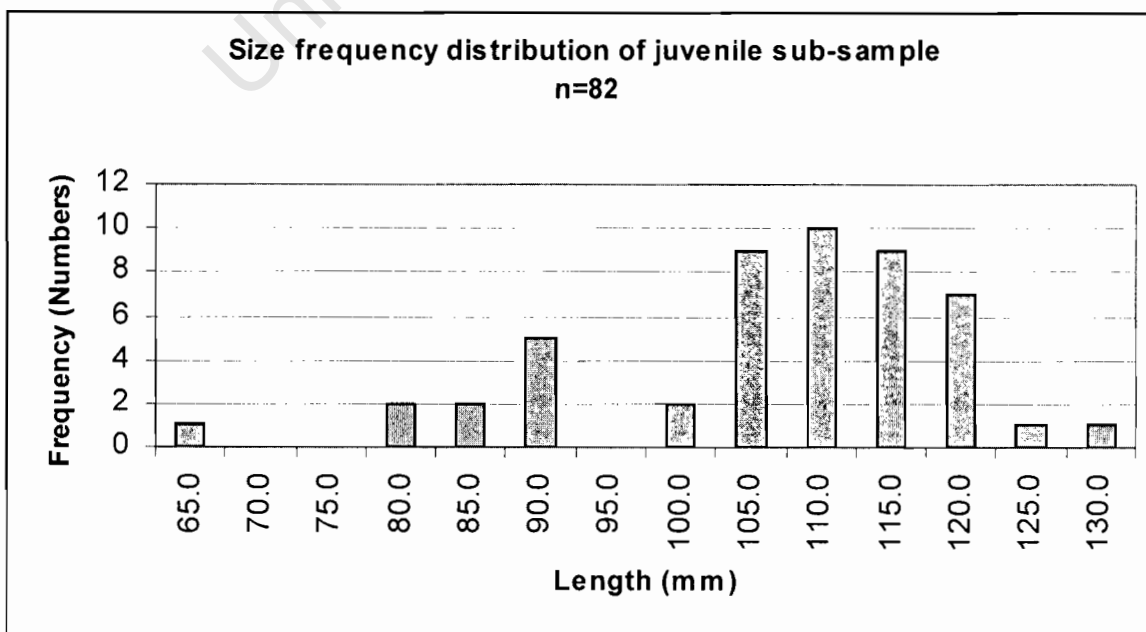
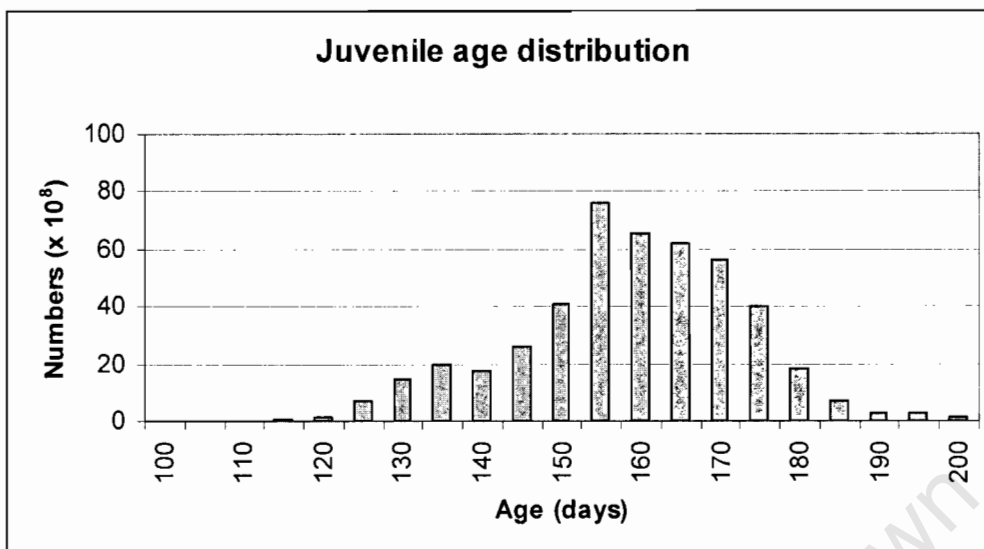


Fig 4.2 Size distribution of the juvenile sub-sample caught in on 16 May 2001.

## **4.2 Age distribution**



**Fig 4.3** Histogram illustrating the abundance of juveniles according to their age classes.

The age data collected from the 82 juvenile otoliths, when raised to the population level using the age length key provided in Table 4.2, yielded the juvenile population age structure illustrated in Fig. 4.3. The age distribution shows that there are two modes of the age, 135 and 155 days which correspond to the fish size 65 mm and 85 mm, respectively (see size distribution Fig. 4.1).

## **4.3 Precision and bias**

The overall average percent error calculated according to Beamish and Fournier (1981) was 7.6 %, corresponding to an overall mean CV of 10.3 %. The minimum calculated APE was 0.4 % (corresponding to a CV of 0.6 %) while the maximum APE was 18.1 % (CV = 23.5 %). Campana (2001), as in chapter 3 above, reported that many studies can be carried out with  $CV < 7.6\%$  and corresponding APE of 5 % which serves as a reference point for many fishes of moderate longevity and reading complexity. The precision (reproducibility of the readings) is relatively low. The counts deviated by as much as 58 days, with a mean of 26 days (Fig. 4.4).

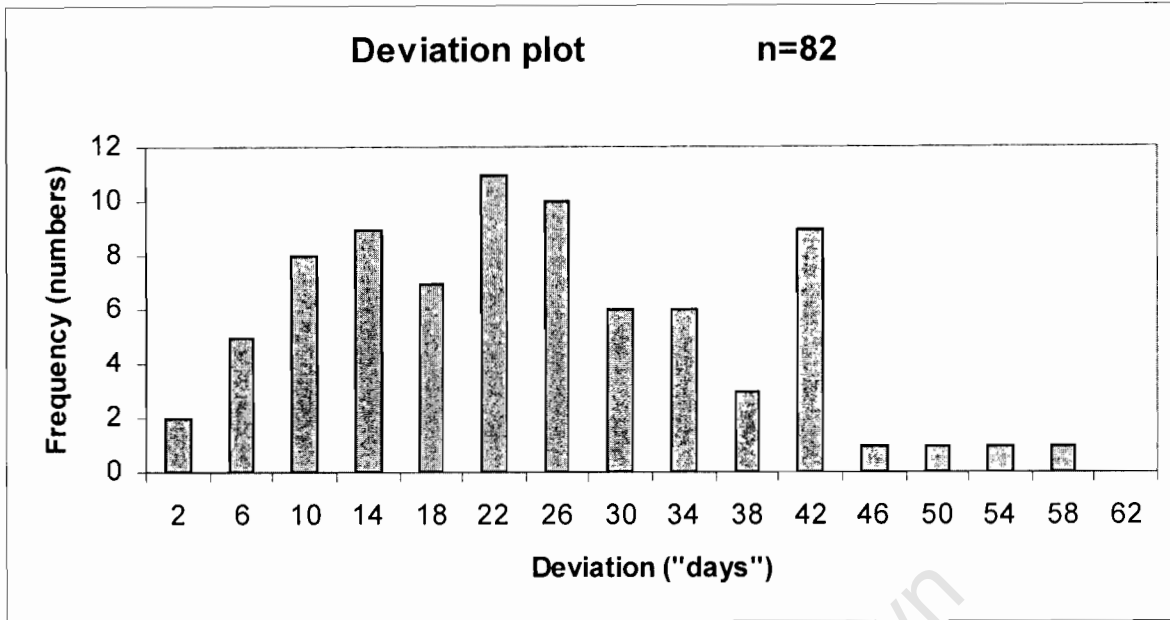


Fig. 4.4. Histogram illustrating counting deviations. The deviations are the maximum difference in the age assigned to each otolith during three replicate counts.

Figure 4.5 shows that there was an element of bias in the counting of daily increments. The first and the last counts tended to be similar to each other, while the second count tended to overestimate the age of juvenile sardine. The mean of the three replicate age estimates was then used as the age of each fish.

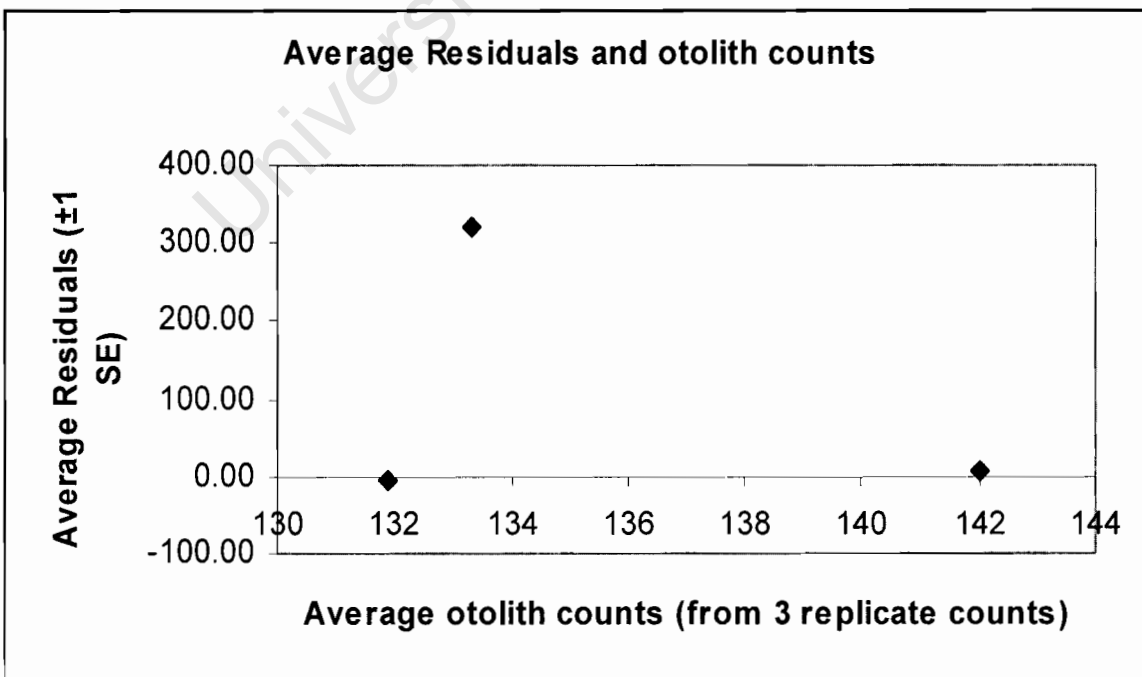


Fig. 4.5. Residual plot illustrating bias in the reading of increments of juvenile sardine on successive counts.

## 4.4 Ageing Results

### 4.3.1 Comparison of results obtained using light and scanning electron microscopy

Age data collected using light microscopy were used in the analyses described in this chapter, but it was first needed to ensure that this method would not underestimate age. The light microscope readings of a sub-sample of 20 otoliths were therefore compared with age estimates obtained from the same otoliths using scanning electron microscopy (SEM). The latter technique has a substantially higher resolving power than the former, and it would therefore be expected to generate more accurate counts of the total number of increments. The size range of the fish in this sub-sample was from 65 -130 cm. A paired t-test (Table 4.2) was performed to assess whether the light microscope readings differed significantly from the SEM readings (i.e., to see if there is any significant difference between the two methods).

**Table 4.2.** Results of the matched pairs t-test comparing light microscopy (LM) with scanning electron microscopy (SEM) (n = 20).

Variable	SEM	LM	p-value
Counts (mean±SD)	114.8(±24.7)	132.0(±13.1)	0.0082

Table 4.2 shows that the p-value is 0.008, which suggests that age estimates obtained using SEM are significantly lower than those obtained using a light microscope. Furthermore, the SEM estimates are more variable than the LM estimates, justifying the decision to use the LM age data in subsequent analyses.

### 4.3.2. Growth and age structure

The sample of 82 juvenile sardine analysed during this study yielded ages between 98 and 176 days. A linear regression model fitted to the size at age data (Fig. 4.3) yielded a positive growth rate of approximately 0.13 mm.d<sup>-1</sup>.

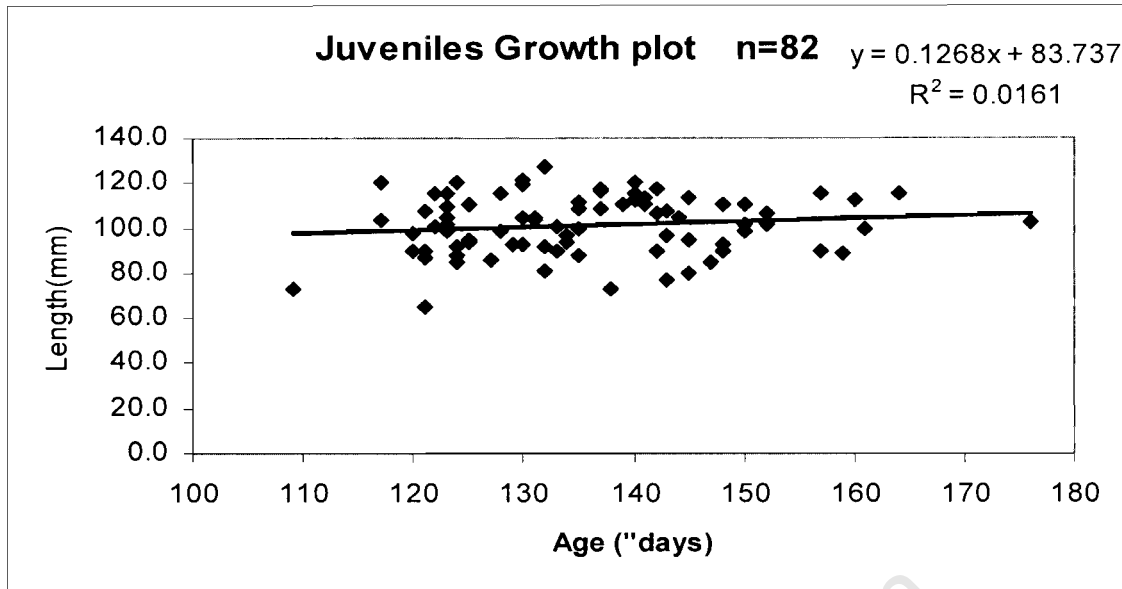


Fig 4.6. Growth curve of juvenile sardine sampled in May 2001. The solid line is the linear regression model fitted to the size at age data.

This growth rate estimate was then used to adjust the survey length frequency data (and the size at age data) to the midpoint of the survey. The adjusted data were then used to construct the Age-Length Key presented in Table 4.1, which, when applied to the population data, generated the population size and age structure (Fig. 4.1 and 4.3) at the midpoint of the survey (16 May 2001).

#### 4.4 Mortality and hatch dates

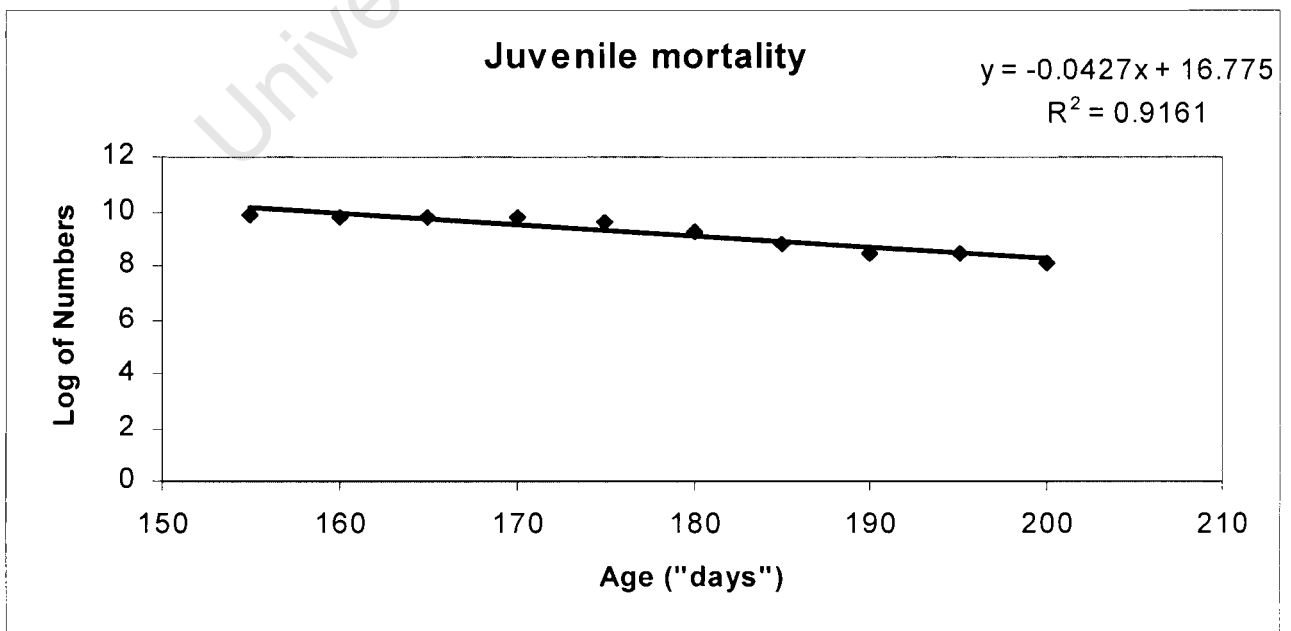


Fig. 4.7. Scatter plot showing the mortality rate of sardine juveniles sampled in May 2001. The solid line is a linear regression model that was fitted to the data.

As in the larval data (Fig. 3.10), the natural logarithm of the population numbers was plotted against the corresponding age (5 day age intervals) of the fish. From the age of 155 days, the juvenile numbers decreased. The slope of the curve in Figure 4.7 implies a mortality rate of  $0.0427 \text{ fish.day}^{-1}$ . These are the first documented estimates of mortality in young South African sardine, but considering that the aging data on which they are based are rather imprecise, the estimates should be considered to be tentative.

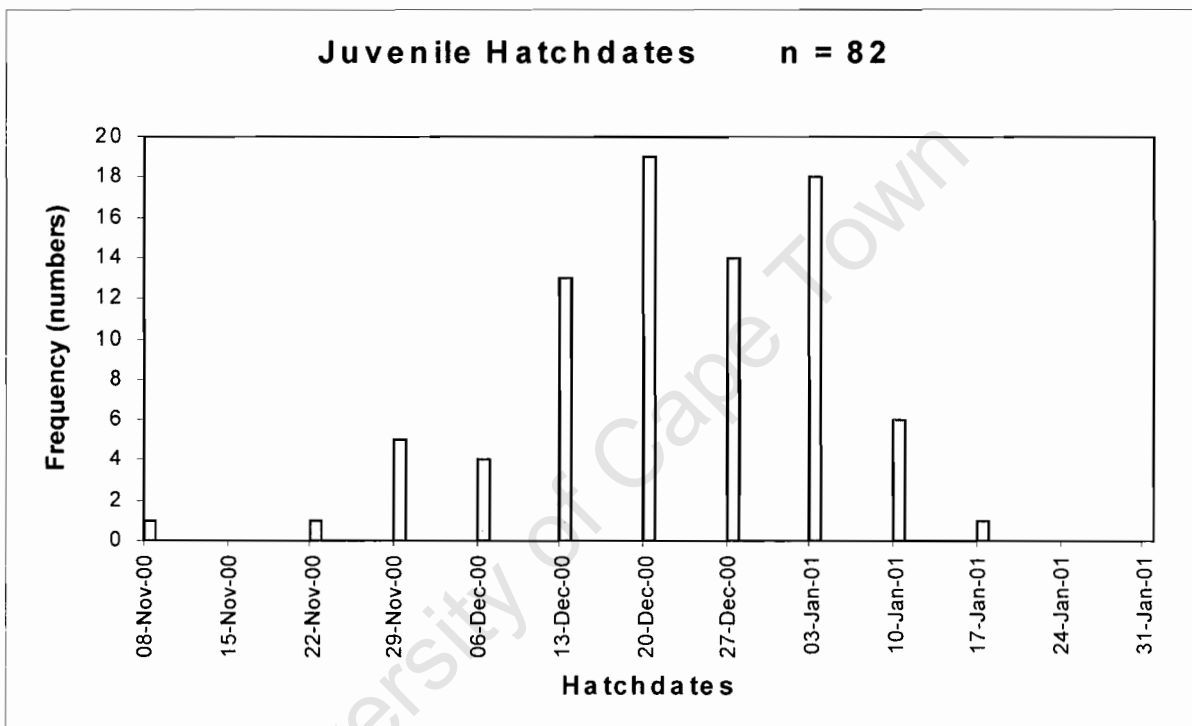


Fig 4.8 Hatch-dates of the sub-sampled sardine juveniles.

The above plot (Fig. 4.8) is the histogram of the hatch dates and frequencies of the juveniles that were caught in May 2001. If the assumption that the increments in sardine otoliths are deposited daily, back-calculated hatch-dates indicate that the majority of the juveniles sampled hatched between mid-December 2000 and early January 2001. Others were hatched from early November 2000 to mid- January 2001.

## **CHAPTER 5 – DISCUSSION AND CONCLUSIONS**

The research described in this thesis adopted a daily age determination approach to investigate growth and survival of sardine pre-recruits in early 2001. Sardine pre-recruits and recruits of the 2000/2001 year class were sampled during early 2001, their otoliths extracted and their ages estimated from counts of daily increments.

The minimum calculated average percent error (APE) for larval data was 3.5 %, corresponding to a coefficient of variation (CV) of 4.8 % while the maximum APE was 41.9 % (corresponding to a CV of 57.8 %). The APE averaged over all otoliths was 17.7 %, corresponding to an average CV of 23.3 %. The overall average percent error calculated for the juveniles data, according to Beamish and Fournier (1981), was 7.6 %, corresponding to an overall mean CV of 10.3 %. The minimum calculated APE was 0.4 % (corresponding to a CV of 0.6 %) while the maximum APE was 18.1 % (CV = 23.5 %).

Fish-age determination is fundamental to fisheries biology and management. Mortality and growth rates are age-dependent parameters and they underlie the population dynamics models used in fishery analyses. Age studies can furnish other basic data such as stock age structure, age at first maturity, age of spawning, individual and stock responses to changes in the habitat, recruitment success, etc.

### **5.1 Larvae**

The precision with which age estimates were obtained from larval sardine otoliths was low. This may have been caused by the lack of experience of the reader (being for the first time). The four replicate counts deviated by an average of 48 days (Fig. 3.6) and associated APE and CV values were substantially higher than what would be expected for work of this nature, but Campana (2001) reported that many studies can be carried out with a CV less than 7.6% and corresponding APE of 5 % which serves as a reference point for many fish species of moderate longevity and reading complexity.

There was a bias (i.e. a tendency to increasingly overestimate age with each replicate) but it appeared to achieve some consistency in the last two counts (Fig. 3.7). The first two counts tended to be lower than the subsequent counts. However, the interpretation criteria employed

to identify and count daily increments appeared to stabilize with successive readings, and it was felt that the precision with which age estimates were being obtained would consequently improve with further replicates. In spite of this, the mean of all four counts was used as the age of each fish. Most larvae that are less than 20 mm were not aged, although they were sampled, because their otoliths broke during processing. This may result to another bias of the study.

The age frequency distribution histogram (Fig 3.8) shows that most of the larvae fell into the age classes of 35 to 50 days with a peak at 40 days. The slope of the linear regression model fitted to the size at age data (Fig. 3.9) indicated an average larval growth rate of  $0.1037 \text{ mm.day}^{-1}$ . Worldwide, growth rates of *S. sagax* larvae that were 30 days old were observed to fall within the range of 0.40-0.80 mm per day (Gaughan, Fletcher and White 2001). Sepulveda (1994) reported growth estimates of 0.556 mm per day in European sardine at a mean temperature of  $17.8 \text{ }^{\circ}\text{C}$ .

Thomas (1985) estimated the average larval growth rate of 0.68 mm per day off Namibia, while Davies (1957, cited by Beckley and van der Lingen 1999) reported a larval growth rate of 0.8 cm per month (0.30 mm per day) in St Helena Bay (South Africa). Thus the average growth rate of the larvae measured in this study is considerably lower than those listed above (example is in Table 3.1 which shows the Age Length Keys of the sardine larvae). This sub-sample shows a slow growing individual aged 39 days and 9 mm long. This was found to be real since the size of the fish is not always directly proportional to its age. The factors that determine the laying on of daily growth rings are mentioned in this study.

The causes of these differences in larval growth rates are uncertain as the temperature on the spawning grounds (Richardson *et al.* 1998), food availability, successful transport of eggs and larvae to the nursery ground (Shelton *et al.* 1985; Huggett *et al.* 1998) and upwelling intensity were all in favour of good growth and recruitment (Roy *et al.* 2001). Van der Lingen *et al.* (2001) cited that the temperature of  $> 19^{\circ}\text{C}$  is ideal for spawning (Wilhelm *et al.* 2005). The difference in ageing error between small and large larvae may have also caused the growth rates to be low.

Although Owens *et al.* (1990) suggested that availability of food is not the limiting factor for some larval clupeoids, Oozeki and Zenitani (1996) stated that in *S. sagax* and other fish larvae, growth rate is dependent on the food supply (Jenkins *et al.* 1991; Cass-Calay 1997). Campana and Hurley (1989) concurred with this observation. Gallego *et al.* (1996) reported that increased food concentration increases the growth rate, if predator-prey and turbulence terms are ignored.

Sardine and anchovy larvae co-exist and feed predominantly on the same type of phytoplankton and zooplankton (crustacean eggs, calanoid copepods, nauplii larvae and diatoms (James and Findlay 1989). During the previous year (2000), anchovy experienced a very large recruitment, hence an increased final anchovy Total Allowable Catch was allocated (TAC) (MCM Research Highlights 2000-2001). The upwelling strength, that results in diatoms blooms, leads to the food availability in the west coast nursery (Verheye *et al.* 1992, Hutchings and Field 1997). The limited food availability following the massive anchovy recruitment the previous year – i.e. many more anchovy on the west coast that may have had a large impact on zooplankton (potential food for both sardine and anchovy larvae) abundance may explain the slow growth rate of sardine that was found in this study.

The estimate of larval mortality rate (obtained from the catch curve analysis) was 0.0887 larvae per day. This is a new result as no other estimates of the mortality of sardine larvae appear to have been documented in the Southern Benguela region.

The analysis of hatching date distribution is a promising tool for studying the process of recruitment (Campana and Jones 1992), and the back-calculated hatching date distribution of the sardine larvae collected in March 2001 shows two peaks. The first observed peak is around 01 February 2001 and the second one is on 16 February 2001 (Fig. 3.9). Hatching dates range from 17 January 2001 to 26 February 2001. These are important estimates

because they tell us when to look for likely environmental factors that might influence growth and recruitment. I don't know how much change there would be, had the larvae less than 20 mm been age.

## **5.2. Juveniles**

Errors in the SEM method can arise at three stages of daily ring counting method; during sampling, during otolith preparation, and during daily ring enumeration (Campana and Neilson 1985). In the last source of error, rings often become more difficult to read with increasing age and, in older fish, deep intersections could be seen on their otoliths enumeration (Campana and Neilson 1985). In this work, although it is generally unlikely to happen, a pilot study on a sample of juvenile otoliths showed that the SEM method tended to give lower counts of growth increments and was more variable. This can be attributed to inexperience of the reader (as it was his very first time to use SEM for research). Due to the extra work and time involved, this (together with re-running of analyses of the last 2 larval readings and the last juvenile reading at which there was confidence) was not pursued further.

In the juveniles, the readings for each otolith were taken three times. The time lapse between the readings was approximately one month. This repetition in reading was then used to check how precise the readings were (as in larvae). Only precise and accurate data can be deemed valuable. Checking the precision of the readings was accomplished by using the average percent error, APE, (Beamish and Fournier 1981). The average APE was calculated to be 7.6 % with a corresponding CV of 10.3%. Every age estimate that had an average percent error higher than 8 % (8% being the standard value that included all the juveniles smaller than 130 mm) should have been excluded from further analysis (Beamish and Fournier 1981). This was not done because more than 50% of the data would be discarded and I would then have had too few data for valid analysis. Thus, it was decided that all data would be analyzed in spite of the poor precision of my counts.

The data analyzed showed that the juvenile fish sampled during the second survey ranged in age from 115 days to 200 days (Fig 4.3), with a peak at 155 days and encompassed a size range of 45 to 130 mm total length. The growth rate of the recruits was  $0.1268 \text{ mm day}^{-1}$ . Kerstan (1996), recorded juveniles growth rate of 0.95 mm/day (from 21.4 cm long fish), whereas Couser *et al.* (2005) noted the sardine juvenile growth rate of 0.319 mm/day (from 24.4 cm long fish). Low counts of primary increments in juvenile fish otoliths have been

attributed to the low resolution of optical microscopes, preparation techniques and complicated otolith structure (Campana & Neilson, 1985; Sogard, 1991; Neilson, 1992). Difficulty was often experienced in counting the innermost rings that became less clear towards the otolith centre. This may have resulted in under-estimation of daily growth marks.

In estimating juvenile fish mortality, catch curve analysis was performed on age. The reason for preferring age to size is that the latter is a poor indicator of cohort membership (May 1974; Warlen 1981). Both logarithmic plots of abundance-at-age in this study (larvae and juvenile) decreased linearly (Figures 3.10 and 4.7). Ricker (1975) stated that the mortality rate ( $Z$ ) could be estimated with either nonlinear regression of the untransformed data or by converting the abundance to log of the abundance. The latter has been considered for this study and it resulted to a more or less straight line with a negative slope which has been fitted through ordinary least squares regression (Robson & Chapman 1961; Ricker 1975; Draper and Smith 1981).

In Figure 4.7 (mortality rate of the juveniles), the abundance in the youngest age categories is less than the peak abundance; this was probably caused by incomplete capture by the sampling gear (the smaller fish were not representatively sampled), resulting in the ascending left “limb” of the graph. Campana and Jones (1992) stated that in the case of unexploited early life stages, the total mortality rate ( $Z$ ) is equal to natural mortality ( $M$ ). Assuming that the growth increments in juveniles were deposited daily and that the rings were counted accurately, this study has shown that the mortality rate is 0.0887 juveniles per day. No other estimates of natural mortality of sardine juveniles have been made in South African waters, so this value should provide a useful figure for stock assessment models of sardine.

The biology and ecology of sardines have been investigated over several decades in Benguela region. This was done with studies on distribution, age and growth, reproduction, feeding and predator-prey relationships. Beckley and van der Lingen (1999) stated that since 1984, fishery-independent hydroacoustic surveys have greatly contributed to elucidation of age-related and seasonal distribution of the sardine stock. The laying down of multiple otolith rings causes the accuracy of age estimates to vary greatly.

Other pressing issues in contemporary fisheries science are fluctuations of the populations (regime shifts) of pelagic fish such as sardine, and regime shifts. In South Africa and Namibia the sardine fishery collapsed dramatically in the 1960s. The cause of that dramatic

decrease was poor control on increase in effort and catches, expansion of the fishing grounds and variable recruitment, probably caused by environmental fluctuations. But in the 1990s the sardine biomass increased again in South African waters with a harvest of 100 000 tons per year (Beckley and van der Lingen 1999).

### **5.3 Conclusion**

Sardine larvae and juveniles otoliths were aged successfully, although there was a low precision during repeated ring counting. The skill of the reader improved with time. Growth rates of both larvae and juveniles that have been measured are slow compared to previous growth estimates in SA and elsewhere: This may be due to inexperience in ring counting using the two different techniques. This, on the other hand, may be real and be due to strong competition among the young stages of sardine when the co-existing anchovy population was at a relatively high level. Thus strong competition for food between anchovy and sardine may result in slower growth rates. Although the proportion of variance explaining the length-age relationship is less than 2%, it is still a general approach to use it to adjust the survey length frequency data to the midpoint of the survey and for back calculation, hence it has been used in this study.

Most of the juveniles ranged between 115 and 200 days old, with a peak in the 155 day age class (Fig. 4.3) whereas the larval age frequency distribution histogram (Fig 3.8), shows that most of the larval samples ranged between 15 and 55 days old, with a peak in the 40 day age class (more than a month old). This can be attributed to the likelihood that larvae younger than 26 days and juveniles younger than 130 days, both escaped through the respective sampling nets, while the older ones avoided the nets.

Back-calculated hatching date distribution of the sardine larvae indicate the range from 17 January 2001 to 26 February 2001, with peaks on the 1<sup>st</sup> and 16<sup>th</sup> of February 2001 whereas those of juveniles range from 8<sup>th</sup> of November 2000 to 17<sup>th</sup> January 2001, with peaks on 20<sup>th</sup> of December 2000 and 3<sup>rd</sup> of January 2001. The peaks of the two surveys don't agree, maybe due to the absence of the < 20 mm larvae data. These results provide an indication of when to look for likely environmental factors that might influence growth and recruitment.

From the data analyses of both sardine larvae and juveniles, hatchdates differed by 1-2 month's period (i.e. were not in the same month). Thus, the fish that were hatched before 3<sup>rd</sup>

of January 2001 demonstrated a better survival between the two surveys (March and May) than those that were hatched after 3<sup>rd</sup> of January 2001. The implication of this observation is that the environmental conditions favored the growth and survival of the larvae hatched before the 3<sup>rd</sup> of January 2001, but some factors (perhaps environmental and natural) changed to result in reduced growth and survival of the larvae hatched after 3<sup>rd</sup> of January 2001. Sardine mortality rates were calculated for the first time by this study in South Africa and this information should be useful for stock assessment models and for management of the sardine population that is a more valuable resource than anchovy because it is used for direct human consumption. The sardine population still seems to be recovering, due to conservative management and some years of good recruitment. Hopefully, this study will contribute towards a better understanding of the factors influencing recruitment and to improved management in the future.

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