THE REPRODUCTIVE PHYSIOLOGY OF THE SOUTH AFRICAN PILCHARD,

Sardinops sagax.

by

Joy Jacqueline Leaner

Submitted in partial fulfilment of the requirements for the degree of Master of Science in the Department of Zoology, University of the Western Cape.

Promoter: Prof. W.J. Veith

Co-promoter: Dr. Y.C. Melo

April 1995

DECLARATION

I, _____, declare that **"The reproductive physiology of the South African pilchard, *Sardinops sagax*"** is my own work and that all the sources I have used or quoted, have been indicated and acknowledged by means of complete references.

Date: _____

DEDICATION

This thesis is dedicated to my family.

ACKNOWLEDGEMENTS

LIST OF ABBREVIATIONS

SARP   -   Sardine and Anchovy Recruitment Programme

RaFoS   -   Reproduction and Feeding of Sardine

CF   -   condition factor

SCF   -   somatic condition factor

GSI   -   gonadosomatic index

HSI   -   hepatosomatic index

Imm   -   immature ovarian maturation stage

Previt   -   previtellogenic ovarian maturation stage

Vit   -   vitellogenic ovarian maturation stage

P-Spent   -   partially spent ovarian maturation stage

T-Spent   -   totally spent ovarian maturation stage

n   -   sample size

E_2 -   oestradiol-17-ß hormone

TABLE OF CONTENTS

CHAPTER 3 - PHYSIOLOGICAL CHANGES ASSOCIATED WITH VITELLOGENESIS IN THE PILCHARD, *Sardinops sagax*

CHAPTER 4 - ENDOCRINE FLUCTUATIONS AND THE EFFECTS ON THE
REPRODUCTIVE PROCESSES IN PILCHARD, *Sardinops sagax*

CHAPTER ONE

AN OVERVIEW OF THE ORDER: CLUPEIFORMES, WITH PARTICULAR REFERENCE TO THE SOUTH AFRICAN PILCHARD, *Sardinops sagax*

CHAPTER 1: AN OVERVIEW OF THE ORDER: CLUPEIFORMES, WITH PARTICULAR REFERENCE TO THE SOUTH AFRICAN PILCHARD, *Sardinops sagax*

1.1 INTRODUCTION

Epipelagic fish species form an intergral component of the worlds' supply of protein. The most abundant of these fish belong to the order Clupeiformes (clupeoids) which include sardine, pilchard, sardinella, sprat and herring. This multi-species group has been estimated to have contributed up to 20% of the worlds' marine catch in the 1980's (Armstrong and Thomas, 1989).

The economic importance of the clupeoids was not only realized by the worlds' commercial pelagic fishing industry, but energy was soon directed into experimental research on the multi-species group, both locally and internationally. A major concern was the incredible susceptibility of these species to increased fishing pressure and fluctuating environmental parameters (O'Toole, 1977). This became evident when a decline was observed in the Californian sardine, *Sardinops caerulea* (Murphy, 1966; Sette, 1969), the Japanese sardine, *Sardinops melanosticta* (Nakai, 1960) and the South African pilchard, *Sardinops ocellatus* (Stander and Le Roux, 1968). The South African pilchard, *Sardinops ocellatus* is hereafter referred to as *Sardinops sagax*.

Extensive research on the general and reproductive biology, as well as modes of egg production in Clupeids has been undertaken. Generally, Clupeids are serial (batch) spawners, releasing several batches of eggs during one spawning season (Alheit, 1989). This mode of reproduction is evident in, amongst others, Pacific sprat (*Spratelloides delicatalus*), herring (*Herklotsichthys quadrimaculatus*) and sardine (*Amblygaster sirm*) (Milton *et al*, 1994), Northwest herring (*Clupea harengus harengus*) (Bradford, 1993), Brazilian sardine (*Sardinella brasiliensis*) (Isaac-Nahum *et al*, 1988), sardine (*Sardinops sagax*) (Retamales and Gonzàlez, 1985), gold spot herring (*Herklotsichthys quadrimaculatus*) (Williams and Clarke, 1983), scaled sardine (*Harengula jaguana* P.) (Martinez and Houde, 1975) and *S. ocellatus* (Davies, 1956; Le Clus, 1979; 1987). Similar studies on the Engrualids such as northern anchovy (*Engraulis mordax*) (Hunter and Goldberg, 1980; Hunter and Leong, 1981), Hawaiian anchovy (*Encrasicholina purpurea*) (Clarke, 1987; 1989), Cape anchovy (*Engraulis capensis*) (Melo, 1992; 1994a; 1994b), also showed that these fish are serial spawners.

In other studies extensive research was conducted on genetic and morphometric variation in Pacific sardine (*S. sagax caerulea*) (Hedgecock *et al*, 1989); the utilization of storage lipids and proteins by Northwest Atlantic herring (*C. harengus harengus*) (Bradford, 1993), egg mortality rates of *S. sagax* (Smith *et al*, 1989), the relationship between food, fat and spawning in Baltic herring (*Clupea harengus mambras*) (Rajasilta, 1992) and batch fecundity in *S. ocellatus* (Le Clus, 1988).

The present study examines parameters associated with the successful spawning of the pilchard *S. sagax* and attempts to clarify the events preceding the spawning activity of the species.

1.2   DISTRIBUTION OF PILCHARD, *Sardinops sagax*

The pilchard *S. sagax* is a cool-water species widely distributed along the Atlantic coast of Southern Africa, from Southern Angola (Baia dos Tigres), south to Cape Town and stretches northeast to Delagoa Bay (Fig. 1) (Smith and Heemstra, 1989).   The nought-year-old pilchard in South African waters, i.e. the "recruits", are abundant in the shelf waters between St. Helena Bay and the Orange River (Armstrong and Thomas, 1989).

1.3   HISTORY AND CURRENT STATUS OF *S. sagax* IN SOUTH AFRICA

The pelagic fishing industry forms an integral component of South Africa's welfare, both socially and economically.   The industry provides a valuable source of protein and provides employment opportunities in areas where unemployment is rife (Cochrane, 1993).   Three species contribute to the opulence of the pelagic fishing industry, viz. anchovy *E. capensis*, round herring *Etrumus whiteheadi* and pilchard *S. sagax* (Armstrong and Thomas, 1989; Boyd, 1993).   It has been estimated that these clupeoids contribute about 60% to the total annual catch of fish in South African waters (Payne and Crawford, 1989).   Since these species form an important component of the pelagic industry, proper management is essential to ensure a maximum sustainable yield.

The pilchard *S. sagax* in South African waters became economically important to the commercial purse-seine industry in 1943 in the St. Helena Bay area when a demand for canned fish arose during war-time conditions (Crawford, 1981a).   Pilchard was fished voluminously during that period and contributed substantially to the pelagic catches off the
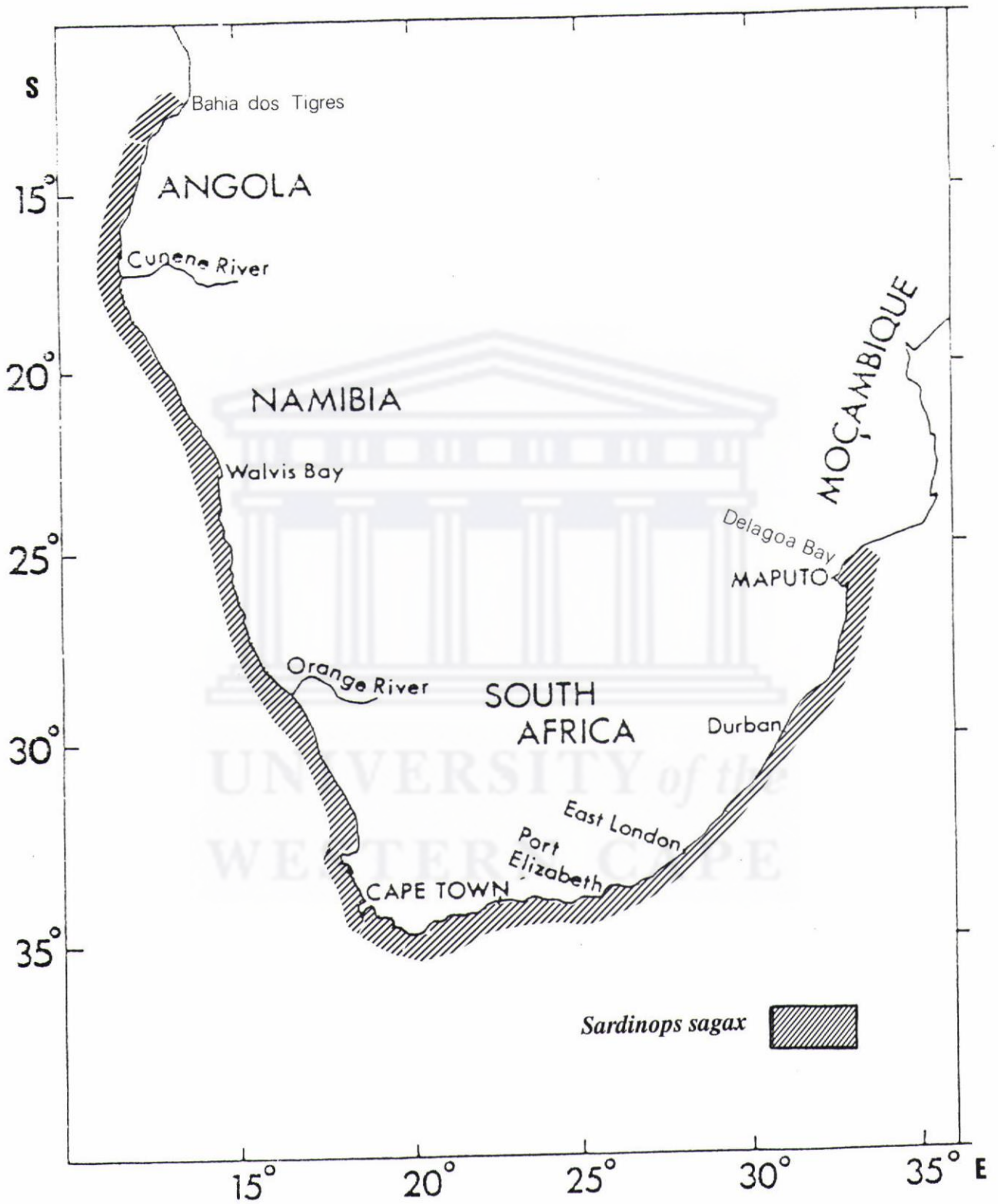
Figure 1: The distribution of the pilchard *Sardinops sagax* in Southern African waters.

west coast of South Africa. One could say that these were the "hey-days" of the pilchard industry, or simply "the silver harvest" as Armstrong and Thomas (1989) stated. It was suggested that strong pilchard recruitment during 1956 and 1959 probably contributed to the increase in the pilchard population observed (Crawford, 1981a). Landings of pilchard in South African waters during the early sixties approximated 402 192 and 410 159 metric tons in 1961 and 1962, respectively.

During later years, however, the pilchard fishing industry experienced problems when a steady decline in the harvest was experienced and by 1974 the pilchard landings had declined sharply to 561 558 metric tons (O'Toole, 1977). Increased fishing pressure and recruitment failure probably contributed to the marked decline in the pilchard population and hence the collapse of the fishing industry. Fluctuations in environmental parameters (Armstrong and Thomas, 1989) and heavy predation by birds, larger fish and marine mammals (Branch *et al*, 1994) and uncontrolled fishing, may influence the existence of the species. The decreased pilchard population, however, was associated with an increase in the anchovy population, *E. capensis*, thereby diverting much of the fishing effort to the latter species (Crawford, 1981a). The increased availability of anchovy is a common phenomenon in the pelagic fishing industry, when pilchard populations diminish (O'Toole, 1977). A possible reason for this phenomenon could be that food availability increases for the dominant species thereby causing flourishing populations, while the stocks of the opposing species declines as a result of disadvantageous environmental factors controlling the population numbers.

The South African pelagic fishing industry yielded minimal harvests until 1988, after which a steady incline in the pilchard population was observed. This became evident when the

biomass of the pilchard population, undertaken by the Department of Environmental Affairs, showed an increase during the last decade from 117 000 ton in 1988 to 460 000 ton in 1993 (Payne, pers. comm). The increased biomass of *S. sagax* suggests that pilchard may once again become the major contributor to the pelagic fishing industry.

The economic status of pilchard has prompted considerable research effort by the Department of Environmental Affairs and Tourism - Chief Directorate: Sea Fisheries in South Africa (Smith and Heemstra, 1989) and the Administration of South West Africa (now Namibia) (Nawratil, 1962; Davies, 1956). Much of this research was directed towards understanding age and growth (Baird, 1970), seasonal occurrence (Baird, 1971), catch compositions (Crawford, 1981a), distribution, availability and movements (Crawford, 1981b), batch fecundity (Le Clus, 1988) and the reproductive dynamics of *S. ocellatus* (Le Clus, 1987). These studies attempted to explain the population dynamics governing the reproductive output of the species.

Davies (1956) determined pilchard gonad activity and observed two definite peaks of spawning activity during spring (September and October) and summer (February) for pilchard spawning in Namibian waters, while Le Clus (1979; 1987) also reported two spawning peaks, except that it was during the winter-spring and summer-autumn seasons. O'Toole (1977) found that South African pilchard spawn continuously from late winter to autumn, showing peaks in late winter and summer / autumn. The latter study was, however, based on pilchard larvae obtained by bongo netting. Nevertheless, *S. sagax* has an extended spawning season (Matthews, 1964; Le Clus, 1987) and it is therefore important to assess pilchard spawning activity annually since it has been found that the intensity of spawning can change from month to month and season to season (O'Toole, 1977).

An understanding of the reproductive physiology and biochemistry could be used to determine the relationship between reproductive output and environmental parameters like food availabilty, temperature and photoperiod which are proximate controlling factors within a spawning season (Bye, 1990). The information gained through physiological studies may also improve the accuracy of the predictions regarding the reproductive success during the spawning season.

1.4 BACKGROUND TO THE RESEARCH QUESTION

The study on the reproductive biology of the pilchard *S. sagax* was initiated with the Department of Environmental Affairs and Tourism, Chief Directorate: Sea Fisheries Research Institute. The initial objective of the study was to determine the seasonality and duration of the non-synchronous mode of spawning of pilchard. In order to accomplish this objective, regular sampling during the year would have been desirable, however, constraints in terms of ship time and absence of pilchard catches during certain months limited the scope of this investigation. As pilchard are serial spawners (Le Clus, 1979; 1987; Akkers, pers. comm.), it was however possible to compare monthly samples, regardless of spatial or temporal differences. Moreover, the biochemical and physiological changes occurring during successive spawning cycles of pilchard *S. sagax*, could be correlated.

A key question of the study was to determine whether physiological parameters such as varying steroid hormone (viz. oestradiol-17-ß) levels or phosphoprotein fractions (viz. vitellogenin) as well as changes in general body parameters could be used as predictors of spawning activity.

1.5 REFERENCES

Alheit, J. (1989) - Comparative spawning biology of anchovies, sardines, and sprats. *Rapp. P.-v. Reun. Cons. int. Explor. Mer.* **191:** 7 - 14.

Armstrong, M.J. and Thomas, R.M. (1989) - Clupeoids. In *Oceans of Life off Southern Africa*. Payne, A.I.L. and Crawford, R.J.M. (Eds). Vlaeberg Publishers CC. Vlaeberg, South Africa: 105 - 121.

Baird, D. (1970) - Age and growth of the South African pilchard, *Sardinops ocellata* (1965 - 1967). *Investl. Rep. Div. Sea Fish. S. Afr.* **91:** 1 - 16.

Baird, D. (1971) - Seasonal occurrence of the pilchard, *Sardinops ocellata* on the East Coast of South Africa. *Investl. Rep. Div. Sea Fish. S. Afr.* **96:** 1 - 19.

Bradford, R.G. (1993) - Differential utilization of storage lipids and storage proteins by Northwest Atlantic herring (*Clupea harengus harengus*). *J. Fish Biol.* **43:** 811 - 824.

Boyd, A. (1993) - Where the shimmering fishes play. *Conserva.* **8:** #6, 4 - 6.

Branch, G.M., Griffiths, C.L., Branch, M.L. and Beckley, L.E. (1994) - *Two Oceans - A guide to the marine life of Southern Africa.* pp 220.

Bye, V.J. (1990) - Temperate marine teleosts. In: *Reproductive seasonality in Teleosts: Environmental Influences*. Munro, A.D.; Scott, A.P. and Lam, T.J. (Eds). CRC Press, Inc. Boca Raton, Florida: 126 - 143.

Clarke, T.A. (1987) - Fecundity and spawning frequency of the Hawaiian anchovy or nehu, *Engrasicholina purpurea*. *Fish. Bull.* **85:** # 1, 127 - 137.

Clarke, T.A. (1989) - Seasonal difference in spawning, egg size, and early development time of the Hawaiian anchovy or nehu, *Encrasicholina purpurea*. *Fish. Bull.* **87:** # 3, 593 - 600.

Cochrane, K. (1993) - Managing a valuable marine resource. Marine Day Issue - *Conserva*. **8:** 7 - 9.

Crawford, R.J.M. (1981a) - Catch per standard-boat-day and deployment of effort in the South African purse-seine fishery, 1964 - 1976. *Investl. Rep. Div. Sea Fish. S. Afr.* **122:** 1 - 24.

Crawford, R.J.M. (1981b) - Distribution, availability and movements of pilchard, *Sardinops ocellata* of South Africa, 1964 - 1976. *Fish. Bull. S. Afr.* **14:** 1 - 46.

Davies, D.H. (1956) - The South African pilchard, *Sardinops ocellata*. Sexual maturity and reproduction, 1950 - 1954. *Investl. Rep. Div. Fish. S. Afr.* **22:** 1 - 155.

Hedgecock, D., Hutchinson, E.S., Gang Li, Sly, F.L. and Nelson,K. (1989) - Genetic and Morphometric variation in the Pacific sardine, *Sardinops sagax caerulea*: Comparisons and contrasts with historical data and with variability in the Northern anchovy, *Engraulis mordax*. *Fish. Bull.* **87:** 653 - 671.

Hunter, J.R. and Goldberg, S.R. (1980) - Spawning incidence and batch fecundity in northern anchovy *Engraulis mordax*. *Fish. Bull.* **77:** 641 - 652.

Hunter, J.R. and Leong, R. (1981) - The spawning energetics of female northern anchovy *Engraulis mordax*. *Fish. Bull.* **79:** 215 - 230.

Isaac-Nahum, V.J., Cardoso, R. DE D., Servo, G. and Rossi - Wongtschowski, C.L. DEL B. (1988) - Aspects of the spawning biology of the Brazilian sardine, *Sardinella brasiliensis* (Steindachner, 1879), Clupeidae. *J. Fish. Biol.* **32:** 383 - 396.

Le Clus, F. (1979) - Oocyte development and spawning frequency in the South West African pilchard *Sardinops ocellata*. *Fish. Bull. S. Afr.* **12:** 53 - 68.

Le Clus, F. (1987) - Reproductive dynamics of female pilchard *Sardinops ocellatus* in the northern Benguela system, with particular reference to seasonality, the environment and fish condition. Ph.D. thesis, University of Port Elizabeth.

Le Clus, F. (1988) - Batch fecundity of pilchard, *Sardinops ocellatus* in the Northern Benguela System estimated from hydrated oocytes. *S. Afr. J. mar. Sci.* **7:** 243 - 254.

Martinez, S. and Houde, E.D. (1975) - Fecundity, sexual maturation, and spawning of scaled sardine (*Harengula jaguana* Poey). *Bull. Mar. Sci.* **25:** #1, 35 - 45.

Matthews, J.P. (1964) - The pilchard of South West Africa (*Sardinops ocellata*). Sexual development, condition factor and reproduction, 1957 - 1960. *Investl. Rep. mar. Res. Lab. S.W.Afr.* **10:** 96 pp.

Melo, Y.C. (1992) - The reproductive biology of the anchovy, *Engraulis capensis* in the Benguela Current Region. Ph.D. thesis, University of Stellenbosch.

Melo, Y.C. (1994a) - Multiple spawning of the anchovy *Engraulis capensis. S. Afr. J. mar. Sci.* **14:** 313 - 320.

Melo, Y.C. (1994b) - Spawning frequency of the anchovy *Engraulis capensis. S. Afr. J. mar. Sci.* **14:** 321 - 332.

Milton, D.A.; Blaber, S.J. and Rawlinson, N.J.F. (1994) - Reproductive biology and egg production of three species of Clupeidae from Kiribati, tropical central Pacific. *Fish. Bull.* **92:** 102 - 121.

Murphy, G.I. (1966) - Population biology of the Pacific sardine (*Sardinops caerulea*). *Proc. Calif. Acad. Sci.* **34:** #1, 1 - 88.

Nakai, Z. (1960) - Changes in the populations and catches of the Far East sardine area. Proceedings of the world scientific meeting on the biology of sardines and related species. *Food and Agricultural Organization of the United Nations, Rome.* **3:** 807 - 853.

Nawratil, O. (1962) - The pilchard of South West Africa, (*Sardinops ocellata*). Age composition of *Sardinops ocellata* in the commercial catches 1958 and 1959 with reference to a change in rate of growth. *Investl. Rep. mar.Res. Lab. S.W. Afr.* **6:** 1 - 33.

O'Toole, M.J. (1977) - Investigations into some important fish larvae in the South East Atlantic in relation to the hydrological environment. Ph.D. Thesis, University of Cape Town.

Payne, A.I.L. and Crawford, R.J.M. (1989) - The major fisheries and their management. In *Oceans of Life off Southern Africa.* Payne, A.I.L. and Crawford, R.J.M. (Eds). Cape Town; Vlaeberg, South Africa. pp. 50 - 61.

Rajasilta, M. (1992) - Relationship between food, fat, sexual maturation, and spawning time of Baltic herring (*Clupea harengus mambras*) in the Archipelago Sea. *Can. J. Fish. Aquat. Sci.* **49:** 644 - 654.

Retamales, R.G. and Gonzàlez, M. (1985) - Incidence of El Niño 1982 - 1983 phenomenon on the spawning of the Chilean pilchard, (*Sardinops sagax*). *Invest. Pesq.* (Chile) **32:** 161 - 165.

Schulein, F.H. (1971) - Rekruteringstudies oor die Suidwes - Afrikaanse sardyn, *Sardinops ocellata*. 1954-1968. M.Sc.thesis, University of Stellenbosch: 62pp.

Sette, O.E. (1969) - A perspective of a multispecies fishery. *Rep. Calif. Oceanic Fish. Invest.* **13:** 81 - 87.

Smith, M.M. and Heemstra, P.C., *eds.*, (1989) - In Smith's sea fishes. Macmillan South Africa (Publishers) (P.T.Y.) Ltd. Braamfontein, Johannesburg: 1 - 1047.

Smith, P.E., Santander, H. and Alheit, J. (1989) - Comparison of the mortality rates of Pacific sardine, *Sardinops sagax*, and Peruvian anchovy, *Engraulis ringens*, Eggs off Peru. *Fish. Bull.* **87:** 497 - 508.

Stander, G.H. and Le Roux, P.J. (1968) - Notes on fluctuations of the commercial catches of the South African pilchard (*Sardinops ocellata*) 1950 - 1965. *Investl. Rep. Div. Sea Fish. S. Afr.* **65:** 1 - 14.

Williams, V.R. and Clarke, T.A. (1983) - Reproduction, growth, and other aspects of the biology of the gold spot herring, *Herklotsichthys quadrimaculatus* (Clupeidae), a recent introduction to Hawaii. *Fish. Bull.* **81:** 587 - 597.

CHAPTER TWO

WITHIN-SEASON FLUCTUATIONS IN THE GENERAL BODY PARAMETERS OF REPRODUCTIVELY ACTIVE PILCHARD,

Sardinops sagax

CHAPTER 2: WITHIN-SEASON FLUCTUATIONS IN THE GENERAL PARAMETERS OF REPRODUCTIVELY ACTIVE PILCHARD, *Sardinops sagax*

ABSTRACT

Changes in the gonadosomatic index (*GSI*), condition factor (*CF*) and somatic condition factor (*SCF*) were determined for female pilchard *Sardinops sagax* for different ovarian maturation stages during a monthly and 48 h sampling period. The *GSI* value was lowest in immature pilchard until maximal values were reached in vitellogenic females and declined in partially spent and totally spent females. Maximal *GSI* values were recorded in January and declined dramatically in February, an indication that spawning took place during the latter month. Histological staging verified the serial mode of spawning in pilchard and that peak spawning took place in February. The *GSI* was estimated to be at a peak at 14h02 and at 07h46 during the 48 h sampling period, but evidence of spawning was not observed.

2.1 INTRODUCTION

Reproduction in marine teleosts, has been studied through macroscopic and histological assessment of gonads as well as the determination of maturity indexes (viz. gonadosomatic index) and changes in the biochemical composition of blood and tissues of the animals. It has been suggested that these parameters can be used as indicators of sexual maturity and hence spawning in the reproductively active animal (Martinez and Houde, 1975). The

spawning success of an animal is dependent on numerous environmental parameters such as temperature and photoperiod, which are proximal controlling factors (Bye, 1990) and food availability, which is particularly important for energy invested during the gonadal maturation process (Wooton *et al*, 1978).

The general life history, reproductive biology and economical importance of *S. sagax* has been described previously (Davies, 1956; Nawratil, 1962; Baird, 1970; 1971; Le Clus, 1979; 1987; 1988; Crawford, 1981a; 1981b and O'Toole, 1977). It has been suggested that serial (batch) spawning in pilchard takes place during late winter and autumn (O'Toole, 1977) since larvae obtained by bongo netting, were abundant during these seasons, as well as in the transition phase from summer to autumn. Furthermore, Le Clus (1979; 1987) suggested that peak spawning in *S. sagax* takes place during winter-spring and summer-autumn, while Matthews (1964) reported that spawning takes place over an extended period during August and March.

The purpose of this study was to characterize the maturation cycle of the pilchard *S. sagax*, as well as to identify the peak of spawning during the reproductive season as inferred from the gonadosomatic index of the species. This index is a good indicator of fluctuations in the breeding acitivity of a fish species throughout the year (Stoumboudi *et al*, 1993), especially if used in association with histological determinations of ovarian maturation stages (Hunter and Macewicz, 1985b). The ovarian developmental pattern was also examined and environmental parameters such as temperature were measured since it has been shown that reproduction in teleosts is influenced by the latter (Bye, 1990).

2.2   MATERIALS AND METHODS

2.2.1   Animals and sampling procedures

Sexually mature and immature female pilchard, *S. sagax* (fish length: 180 - 210 mm) were collected at monthly intervals from September 1993 to March 1994 from the area indicated in Figure 2.1.a.   Complications in sampling strategies precluded the capture of pilchard during November and December 1993.   Samples of sexually mature and immature female pilchard were collected over a 48 h period in September 1994 from the area indicated in Figure 2.1.b.   The samples collected during the study formed part of the monthly SARP cruises on the *R.S. Algoa*; the RaFoS cruises on the *R.S. Algoa* and *F.R.S. Africana*, as well as the experimental catches on the *R.S. Sardinops*.

Pilchard were collected by means of an Engels midwater trawl net and transferred immediately to aerated 100 *l* holding tanks with a continuous sea water flow system aboard the research vessel.   Live pilchard were sexed and macroscopically staged according to the classification of Davies (1956).   Blood was collected from the caudal vein of individually tagged fish for further physiological and biochemical analyses.   Immediately thereafter, fish were sacrificed by severing the spinal cord.   The total and eviscerated body masses were determined to nearest 0.1 g and standard body length (caudal length, mm) was measured in order to calculate the condition factor - *CF* (Wooton *et al*, 1978).   The somatic condition factor (*SCF*) was also determined, (Wooton *et al*, 1978).   The ovary was excised and weighed to the nearest 0.1 g for calculation of the gonadosomatic index (*GSI*) for each stage of ovarian maturity (Melo, 1994a and Johnson, *et al* 1991).
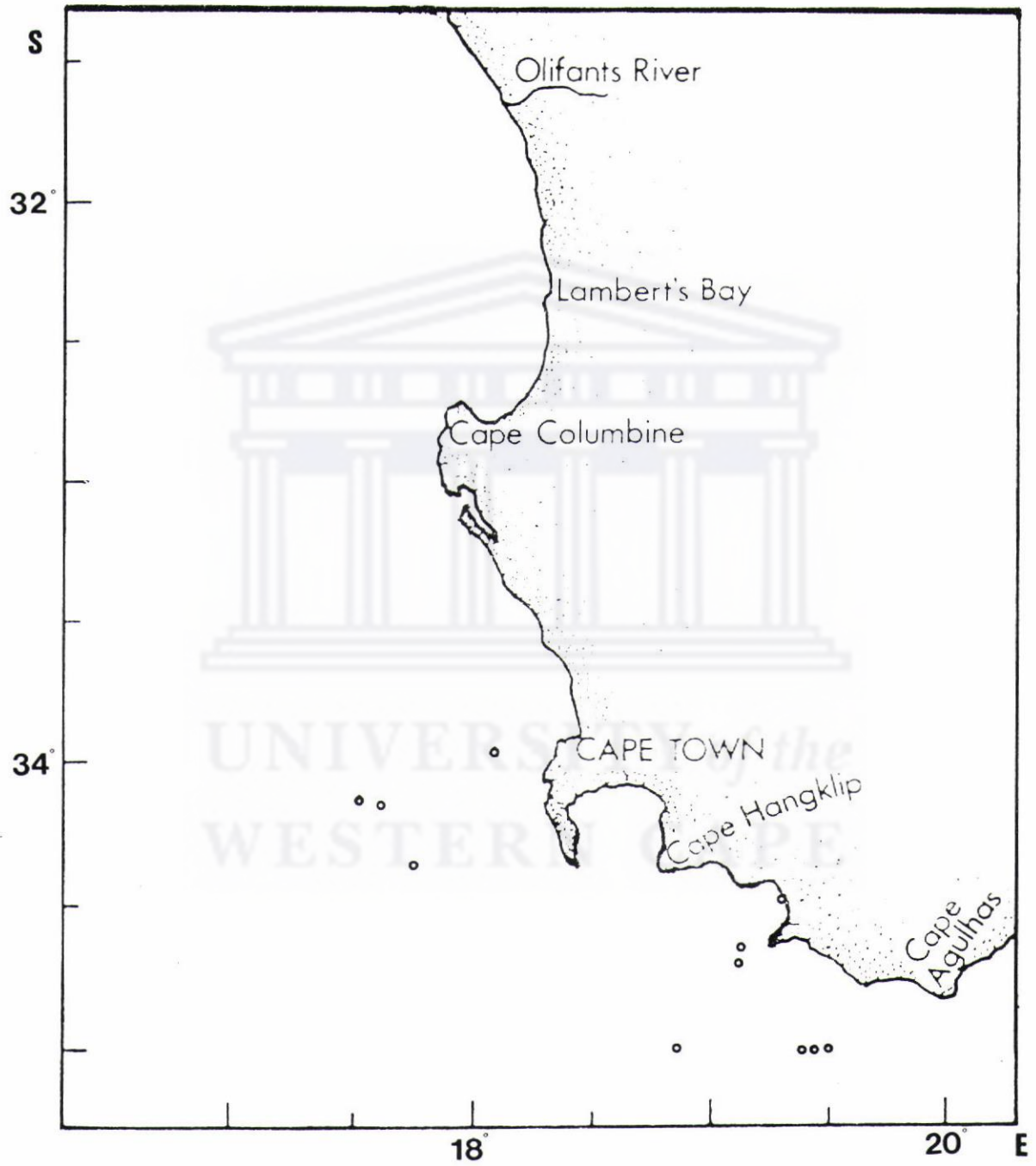
Figure 2.1.a: **The location of experimental catches (○) during the monthly sampling collection of the SARP cruises.**
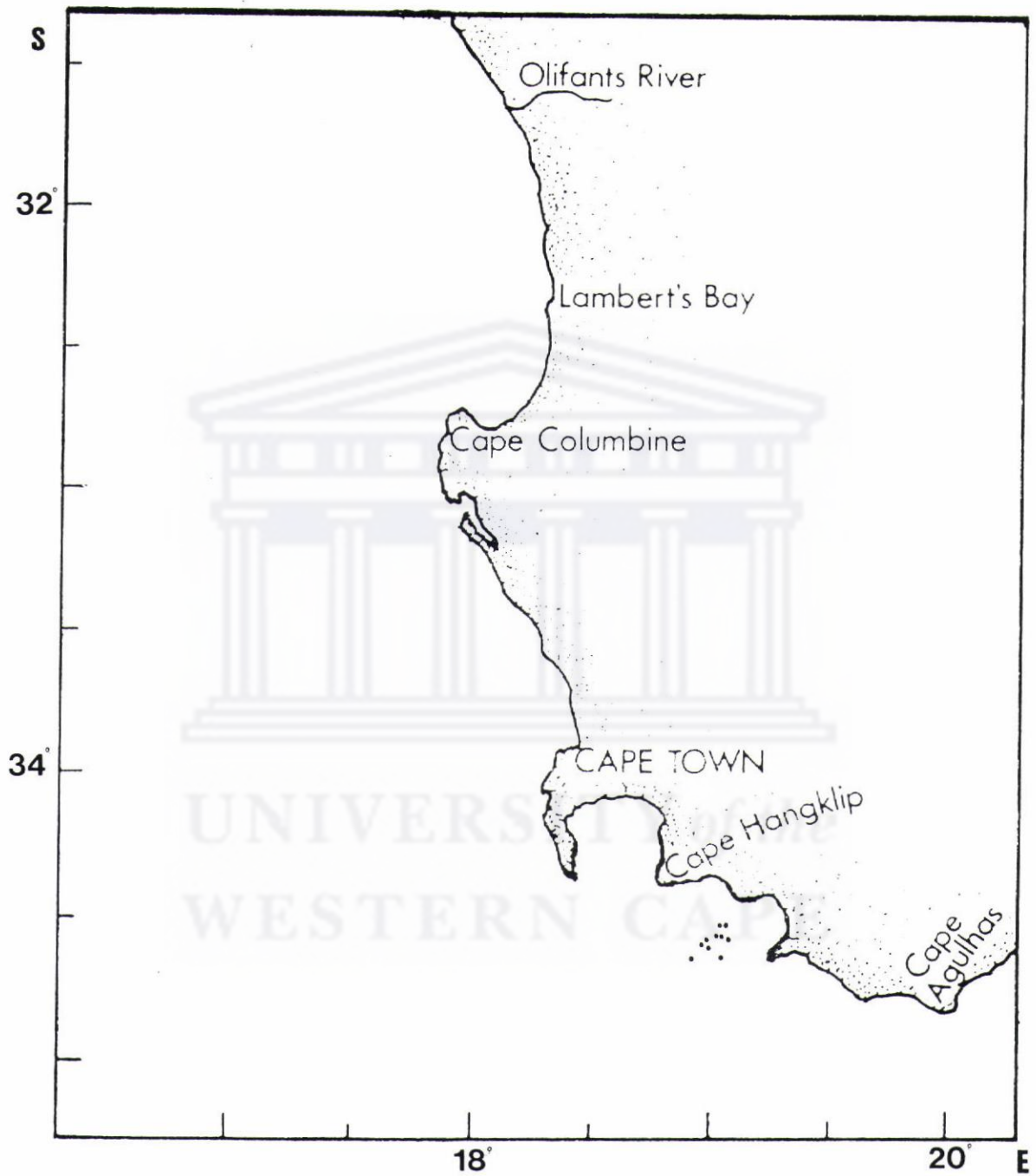
Figure 2.1.b: The location of experimental catches (•) during the sampling collection in September 1994.

The calculations were done as follows:

$$CF = (\text{Total Body Mass, g } / \text{ Total Length}^3, \text{ mm }) \times 10^6 \; ;$$

$$SCF = (\text{Eviscerated Body Mass, g } / \text{ Total Length}^3, \text{ mm }) \times 10^6 \; ;$$

$$GSI = \frac{\text{Mass of Ovary, g}}{\text{Mass of fish, g } - \text{ Mass of ovary and viscera, g}} \times 100\%$$

2.2.2 Histological characterization of the ovaries

The larger of the two ovaries of each fish was preserved in Bouins' fixative (Drury and Wallington, 1967) for further histological examination to validate the macroscopical staging of each pilchard ovary. The ovary was embedded in paraffin wax, sectioned at 7 μm (Melo, 1992) and stained with Harris' haemotoxylin and eosin (Drury and Wallington, 1967). Each histological section was examined by light microscopy and classified into specific developmental stages (Table 2.1), modified from the classification of Melo (1992) and Retamales and Gonzàlez (1985). Each developmental stage was characterized by the most advanced stage of oocyte present on the histological section. Post-ovulatory follicles were classified according to the characteristics described for *Engraulis capensis* (Melo, 1994b).

2.2.3 Photography

Histological sections were photographed under a Zeiss compound microscope attached to a Zeiss MC63 Photographic Integrator.

2.2.4 Statistical analysis

Tests for significance were performed by Kruskal - Wallis Nonparametric Analysis of Variance (ANOVA), followed by Dunns' Multiple Comparisons test, using the INSTAT computer package.

2.3 RESULTS

2.3.1 Patterns of oocyte development

Histological sections of pilchard ovaries, verified the serial mode of spawning of this species, as reported earlier (Le Clus, 1979; 1987). Prior to the onset of gonadal maturation, the ovaries of pilchard were arrested in an immature state and showed mainly oocytes in the early perinucleolar state which are characterized by numerous small nucleoli on the periphery of the germinal vesicle (Plate 2.1). As gonadal maturation proceeded, the ovary showed perinucleolar and yolk vesicle stage oocytes characterized by lipid vacuoles adjacent to the membrane (Plate 2.2) that would later fill the ooplasm as yolk globules are formed (Plate 2.3). At this stage during the development, the vitellogenic ovary was dominated by yolked and vacuolated oocytes. Prior to spawning, ovaries contained hydrated oocytes (Plate 2.4) which developed as a result of increased water uptake. The occurrence of such ovaries were minimal and were therefore not considered in the present study. Numerous post-ovulatory follicles were observed in the ovaries of pilchard in the partially spent condition (Plate 2.5). Totally spent ovaries were characterized by massive atresia of all yolked oocytes and the presence of perinucleolar oocytes (Plate 2.6). Ovaries in the previtellogenic (resting - inactive) ovarian maturation stage were not encountered.

22
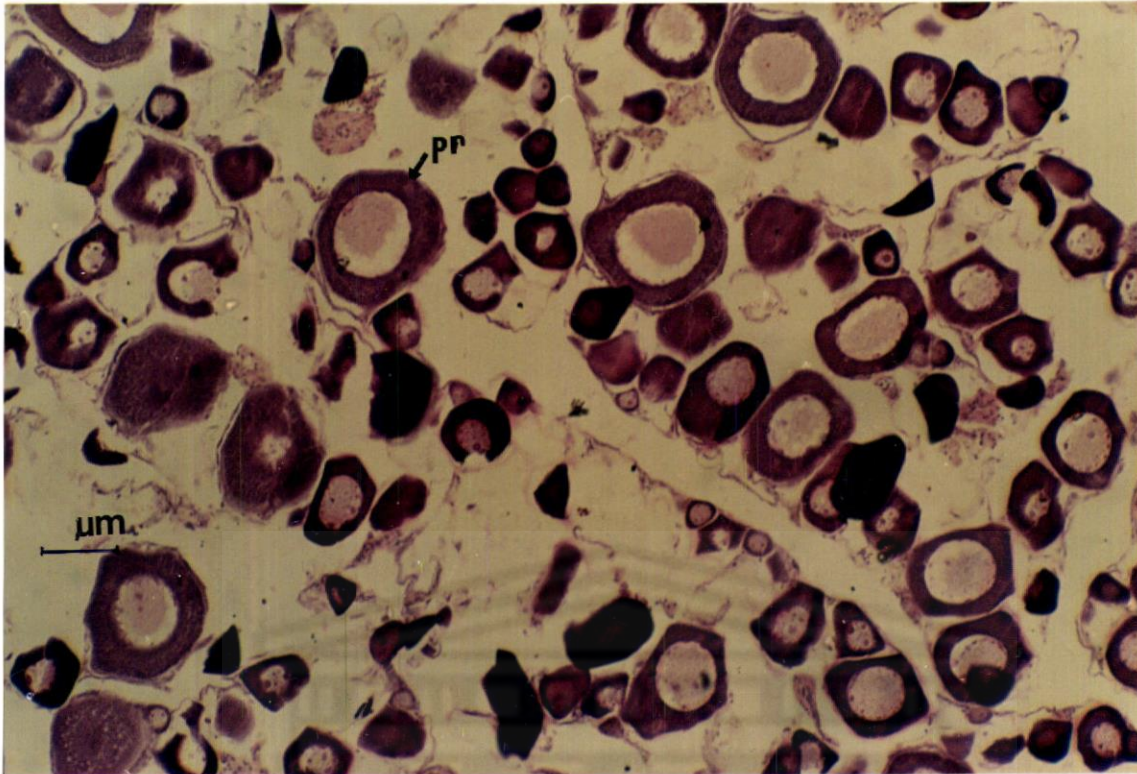
Plate 2.1: Section of an immature ovary showing perinucleolar oocytes (pr).
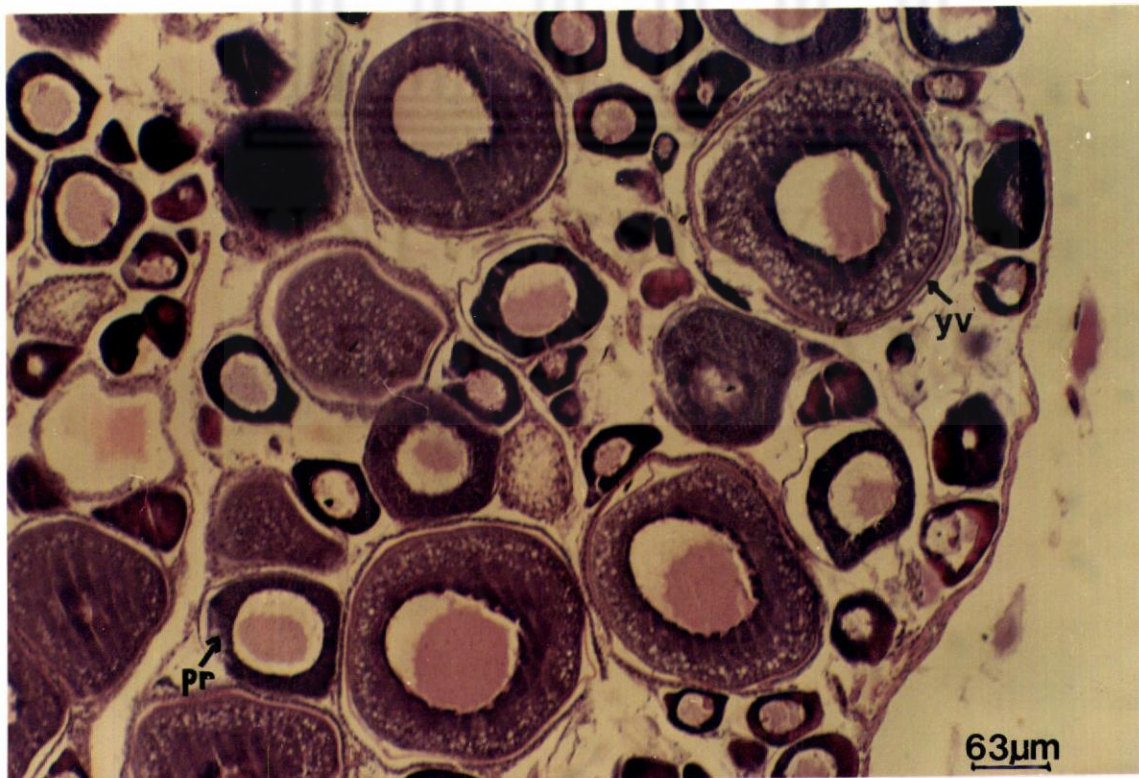
Plate 2.2: Section of a previtellogenic (*maturing virgin*) ovary showing perinucleolar (pr) and yolk vesicle stage (yv) oocytes.
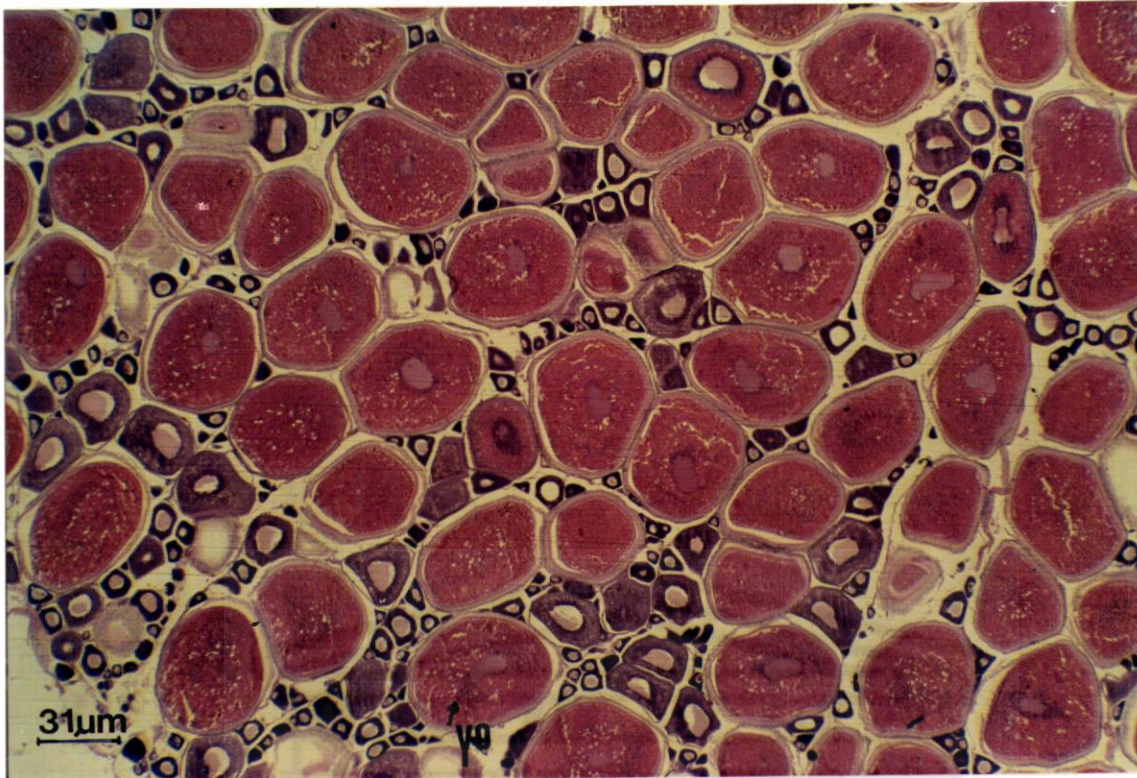
Plate 2.3: Section of a vitellogenic ovary showing yolked oocytes (yo).
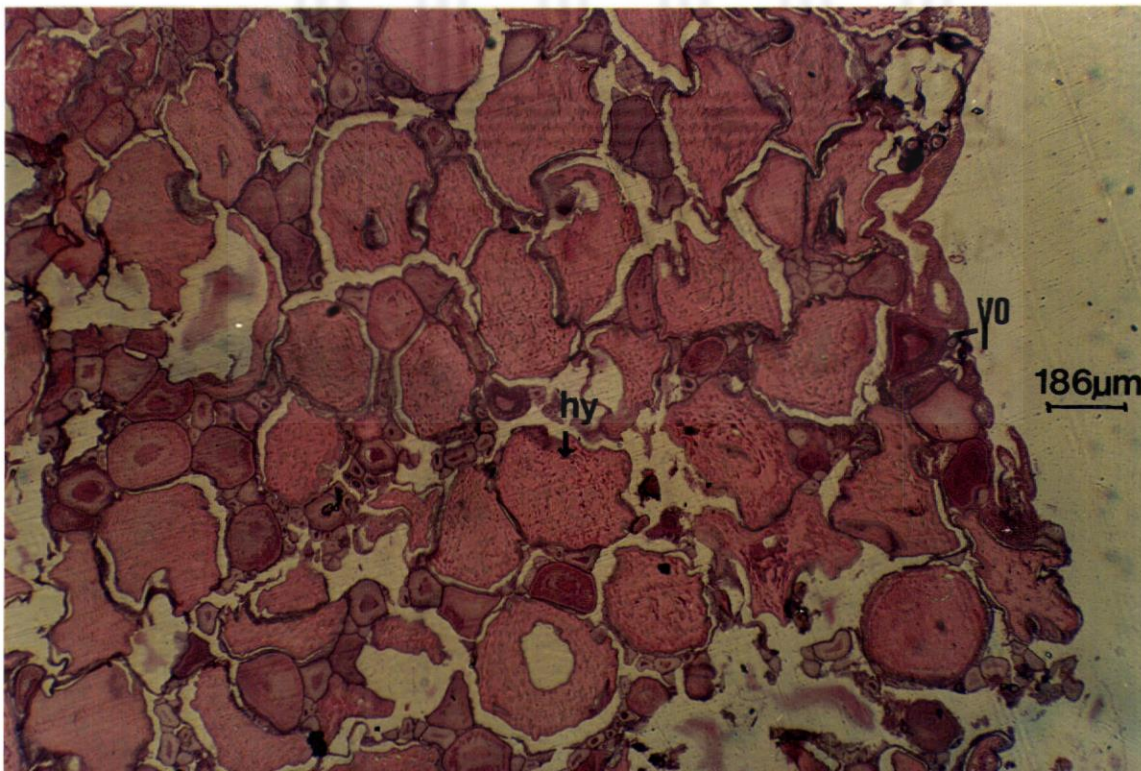
Plate 2.4: Section of a spawning ovary showing yolked (yo) and hydrated (hy) oocytes, partly collapsed in sectioning.
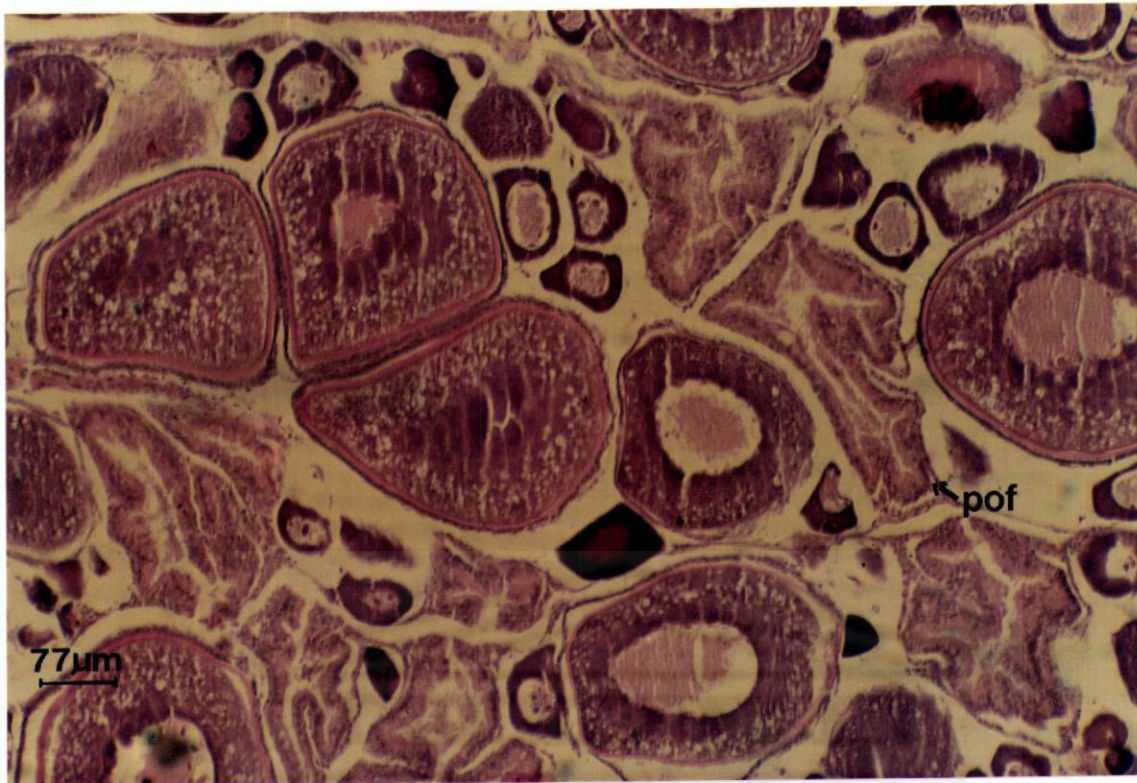
Plate 2.5: Section of a partially spent ovary showing post - ovulatory follicles (pof).

Plate 2.6: Section of a totally spent ovary, showing perinucleolar oocytes (pr) and massive atresia of all remaining oocytes (a).

2.3.2 Maturity determined by the gonadosomatic index (*GSI*)

A steady increase in the gonadosomatic index (*GSI*) was observed up to the vitellogenic stage, declining towards the spent stage (Fig. 2.2). Females in the immature (1.27%) and previtellogenic (1.34%) conditions had significantly lower ($p \leq 0.001$) *GSI* values than females in the vitellogenic condition (3.77%). The *GSI* decreased in partially spent females, declining further to 1.29% in totally spent females. Accordingly, the *GSI* of females in the partially spent condition was significantly higher than that of females in the immature and totally spent condition, $p \leq 0.05$ and $p \leq 0.001$, respectively.

Monthly changes in *GSI* status were recorded during the spawning season (Fig. 2.3). Similar *GSI* values were recorded for females caught in September and October. No data was available for the months of November and December, but by January a peak *GSI* value of 4.5% was observed. Thereafter, a gradual decline in the *GSI* value was observed from February through to March. The *GSI* value of fish sampled in January was significantly higher ($p \leq 0.01$) than that of fish sampled in other months (Fig. 2.3).

Daily changes in the *GSI* values of fish sampled over a 48 h period during September 1994 are presented in Fig. 2.4. The *GSI* values remained relatively constant throughout the sampling period, except for the occassional peaks at 14h02 (4.88%) and 07h46 (4.79%), approximately 38 and 53 h after sampling had commenced. Both *GSI* values were significantly higher ($p \leq 0.01$) than fish sampled at 13h59, 16h45 and 23h12. Furthermore, the fish sampled at 08h26 had a significantly higher *GSI* ($p \leq 0.01$) than that of fish sampled at 23h12.
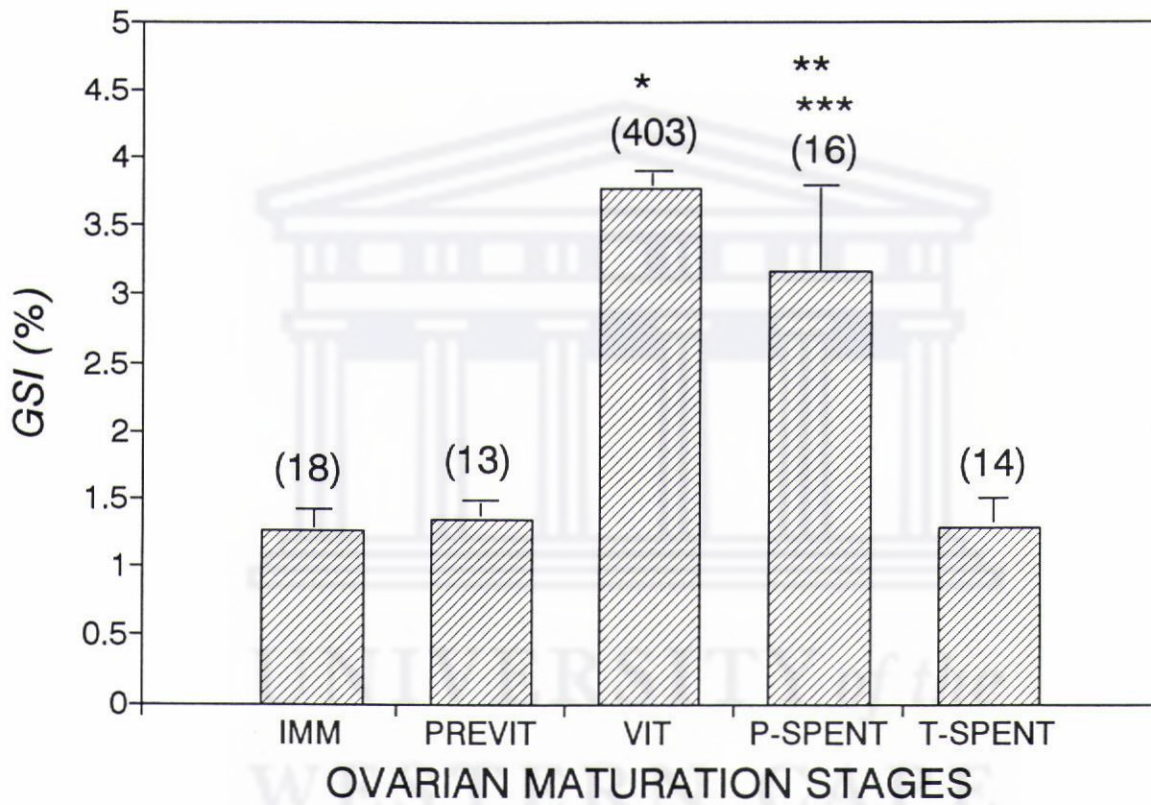
Figure 2.2: Gonadosomatic index (*GSI*) values measured in different ovarian maturation stages in the pilchard, *S. sagax*. Vertical bars represent Mean $\pm$ SEM; values in brackets = n; * = p $\leq$ 0.001 (Vit vs Imm, Previt); ** = p $\leq$ 0.05 (P-Spent vs Imm); *** = p $\leq$ 0.001 (P-Spent vs T-Spent).
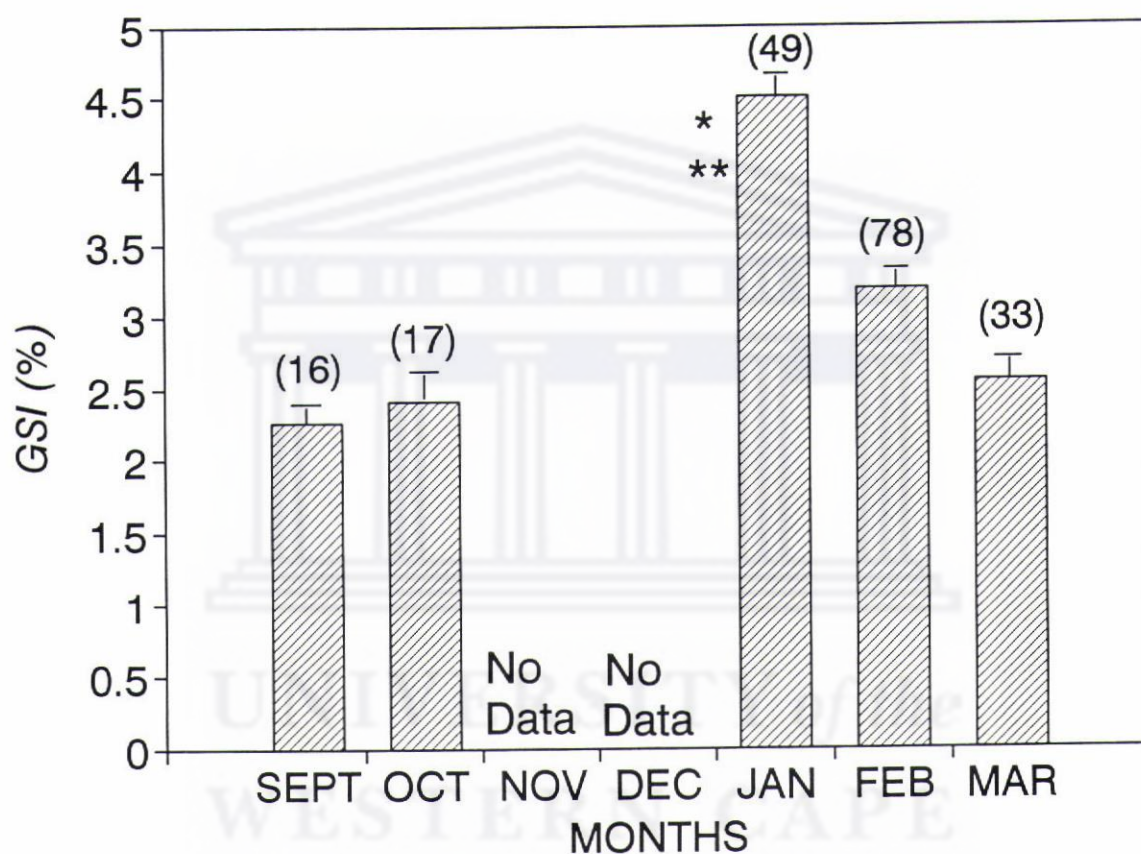
Figure 2.3: Monthly gonadosomatic index (*GSI*) values in the pilchard *S. sagax*. Vertical bars represent Mean $\pm$ SEM; values in brackets = n; * = p $\leq$ 0.001 (Jan vs Sept, Oct, Mar); ** = p $\leq$ 0.01 (Jan vs Feb).
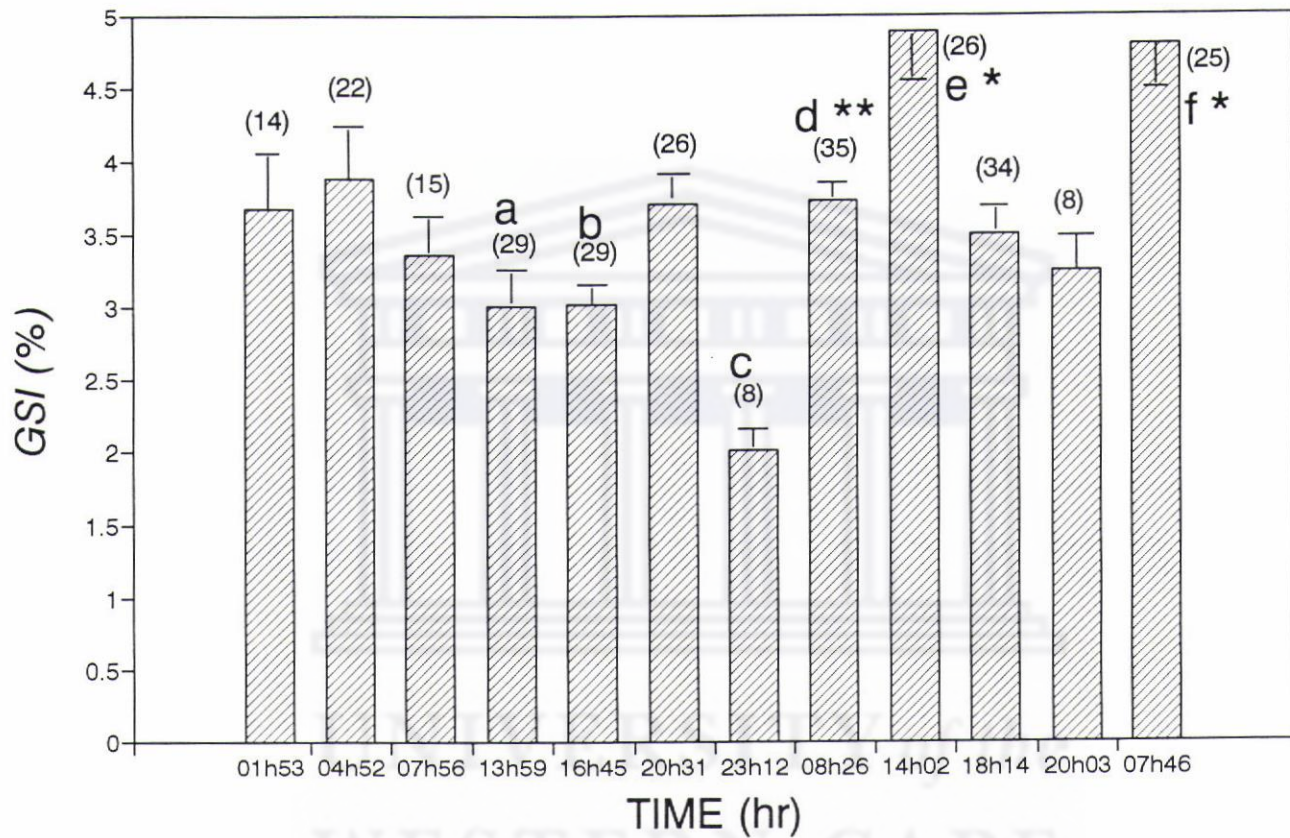
Figure 2.4: Gonadosomatic index (*GSI*) values measured over a 48 h period in the pilchard *S. sagax*. Vertical bars represent Mean ± SEM; values in brackets = n; * = $p \leq 0.01$ (e, f vs a, b, c); ** = $p \leq 0.01$ (d vs e).

2.3.3 Timing of gonadal maturation and spawning

Proportions of female ovarian maturation stages over a monthly period, are represented in Fig. 2.5. Female pilchard showing immature ovaries were obtained in February, while females with ovaries in the previtellogenic condition were obtained from September through to January. In January, however, the percentage females in the previtellogenic condition was minimal. Vitellogenic ovaries were present in all months studied, the highest recorded in January. Ovaries in which post-ovulatory follicles (i.e. partially spent stage) occurred, were observed in January through to March, the proportion being highest in February. Totally spent females appeared in January and increased in proportion through to March.

Proportions of the ovarian maturation stages during the 48 h sampling period are presented in Fig. 2.6. Females with new post - ovulatory follicles (Day 0: time elapsed from spawning about 2 to 6 h) appeared at the start of the sampling period at 01h53 and 04h42, the greatest proportion found in the former, while Day 2 post-ovulatory follicles (time elapsed from spawning about 38 to 42 h) were observed at 14h02. Pilchard in the totally spent condition were observed at 20h31, after the first reported spawning. At 20h03 the entire catch comprised of females in the vitellogenic condition. The greatest proportion of female pilchard during the sampling period, was in the vitellogenic condition.

2.3.4 Changes in the condition of *S. sagax*

The overall condition of female pilchard collected monthly is presented in Fig. 2.7. The condition factor (*CF*) and somatic condition factor (*SCF*) were relatively low at the start of the sampling season in September and October. No samples were obtained in November and
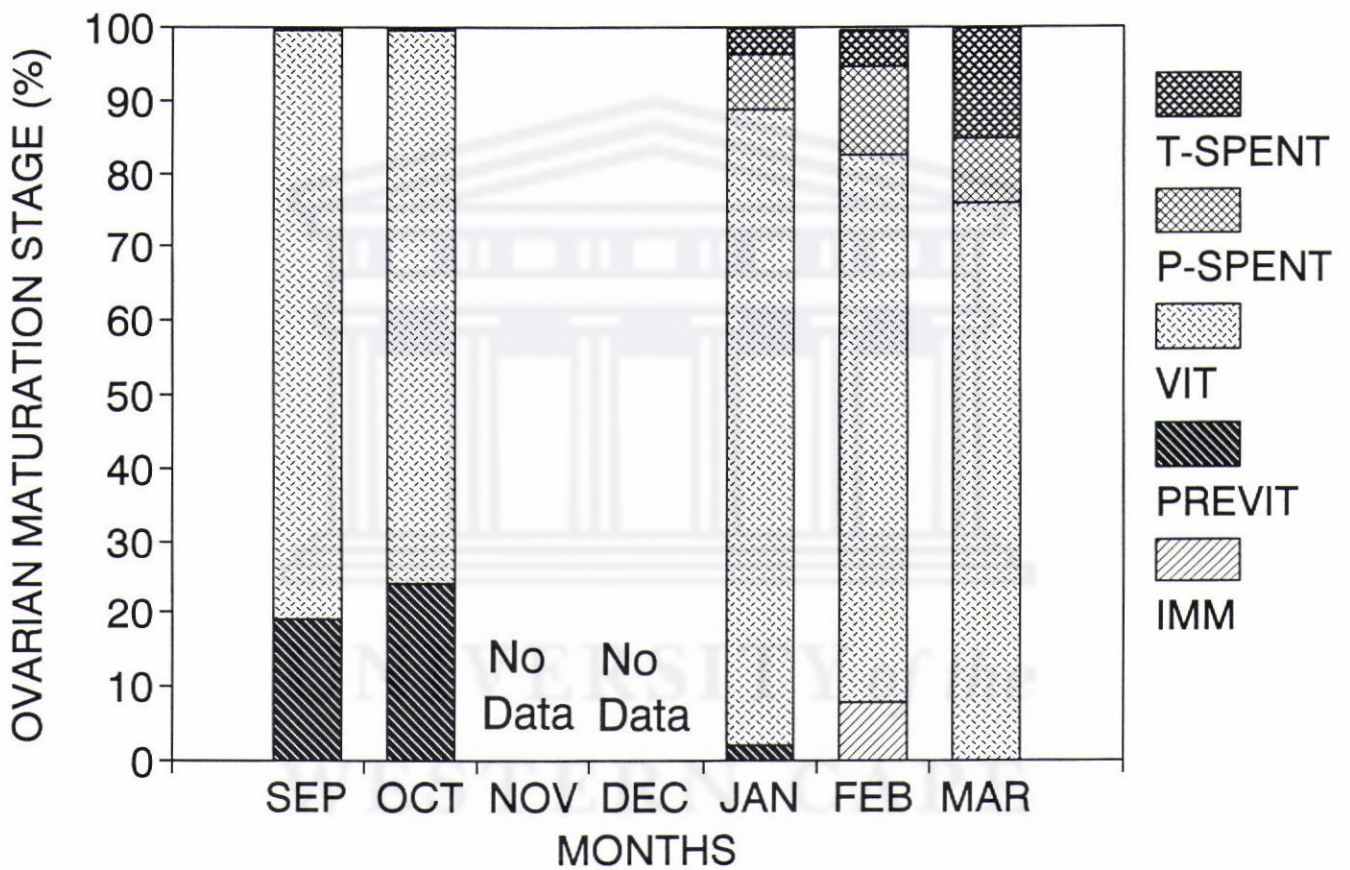
Figure 2.5: Monthly proportions of gonad condition in the pilchard *S. sagax*, based on histological examination.
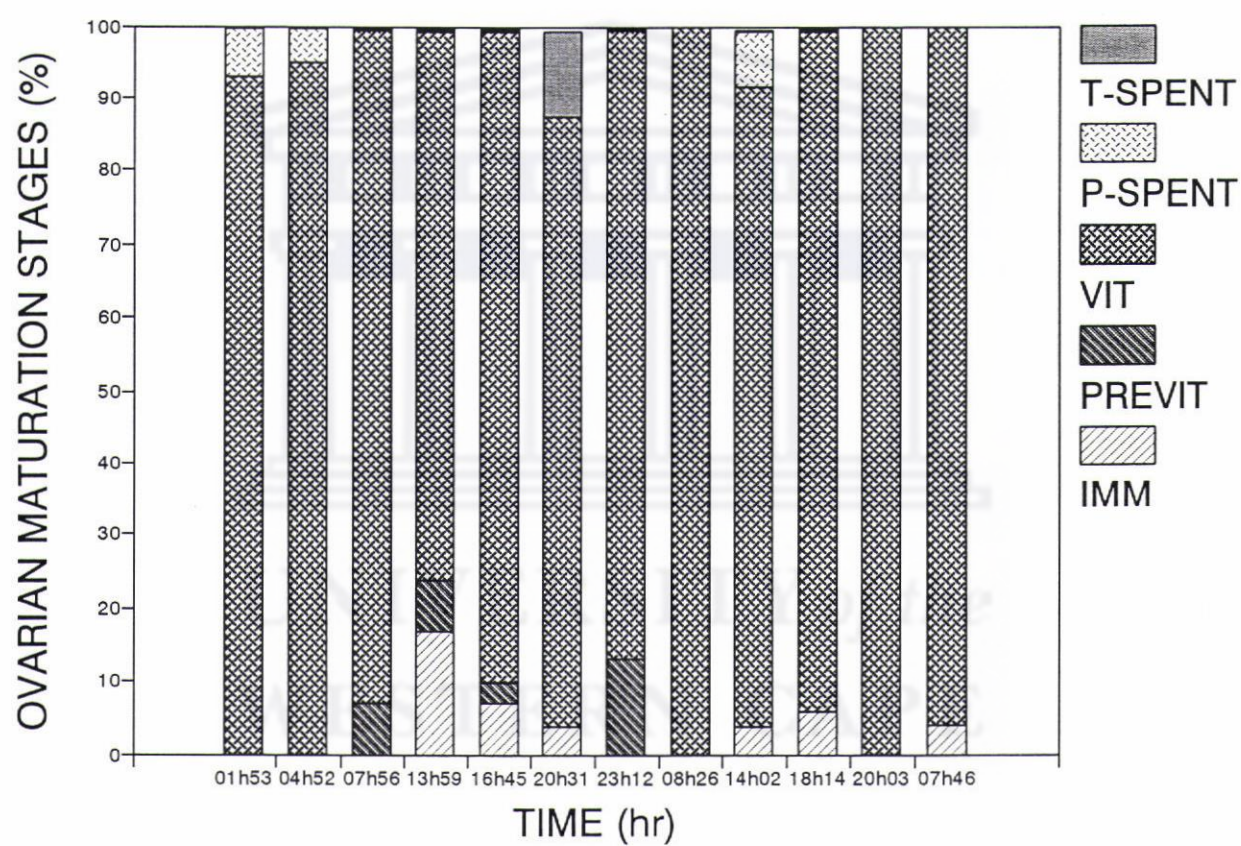
Figure 2.6: Proportions of gonad condition in the pilchard *S. sagax* over a 48 h period during September 1994.
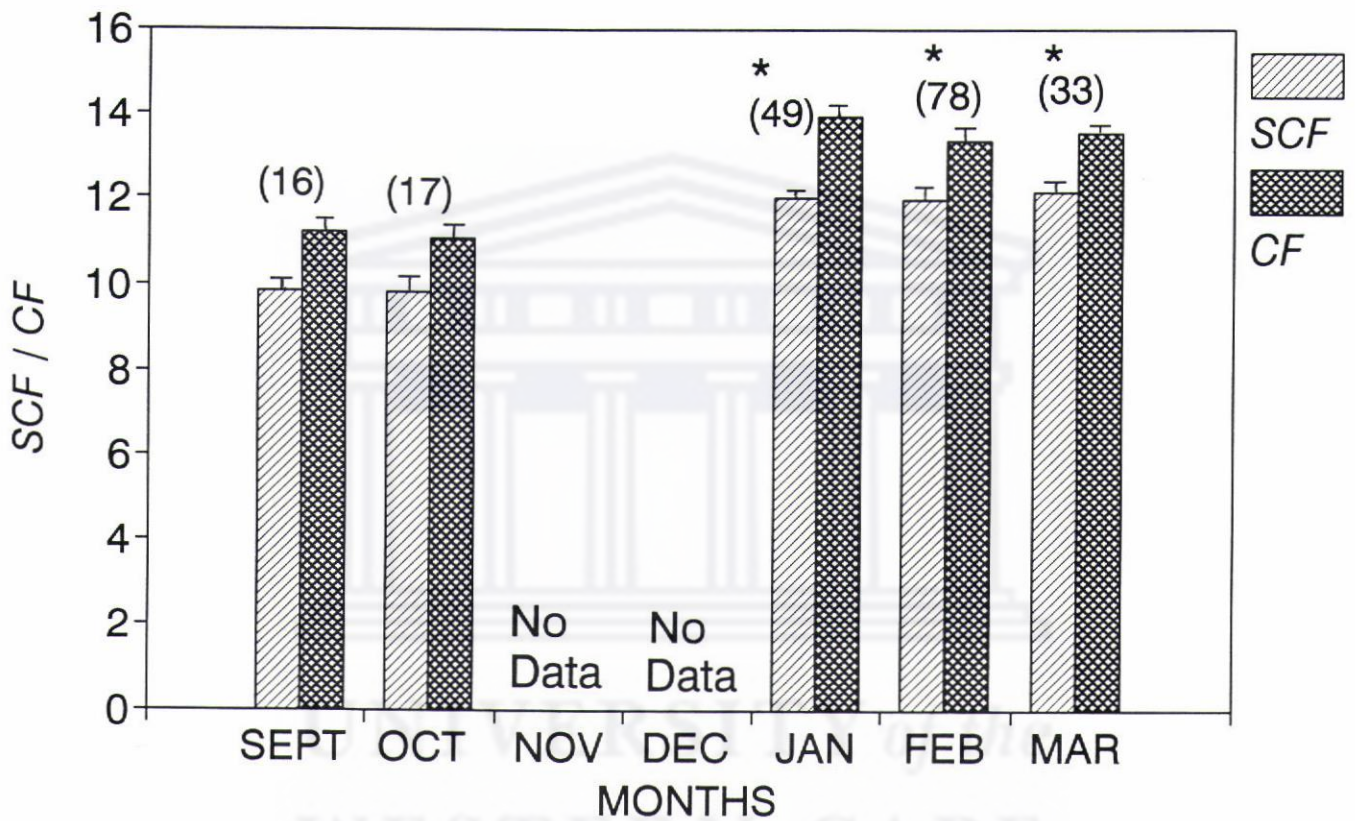
Figure 2.7: Somatic condition factor (*SCF*) and condition factor (*CF*) of the pilchard *S. sagax*, measured at monthly intervals. Vertical bars represent Mean $\pm$ SEM; values in brackets = n; * = $p \leq 0.001$ (Jan, Feb, Mar vs Sept, Oct).

December. The *CF* and *SCF* increased by approximately 20% in January, February and March, relative to that observed in September and October (p ≤ 0.001).

The overall condition of pilchard measured during the 48 hour period is represented in Fig. 2.8. Both the *CF* and *SCF* remained relatively constant throughout the entire sampling period. However, samples collected at 20h03 had *SCF* and *CF* values approximately 10% higher than samples obtained at 04h42, 13h59 and 07h46 (p ≤ 0.05). The *SCF* of pilchard collected at 20h03, was also significantly higher (p ≤ 0.05) than that of fish sampled at 20h31.

2.4   DISCUSSION

2.4.1   Oocyte development

Ovarian development in the pilchard *S. sagax* was characterized by the presence of two distinct clutches of oocytes, viz: one consisting of non-yolked primary oocytes, while the other displayed an advanced condition undergoing vitellogenesis. During successive spawnings, follicles with completed vitellogenesis, undergo maturation and ovulation. Consequently, vitellogenic growth in the younger follicles continues and the ovary contains oocytes in different maturation stages. This pattern of oocyte development verifies the serial spawning nature of *S. sagax* (Le Clus, 1979; 1987; Akkers, unpublished data). Most clupeoids are serial (multiple) spawners (Alheit, 1989), including species such as the Northwest Atlantic herring *Clupea harengus harengus* (Bradford, 1993); the herring *Herklotsichthys quadrimaculatus*, the sprat *Spratelloides delicatulus* and sardine *Amblygaster*
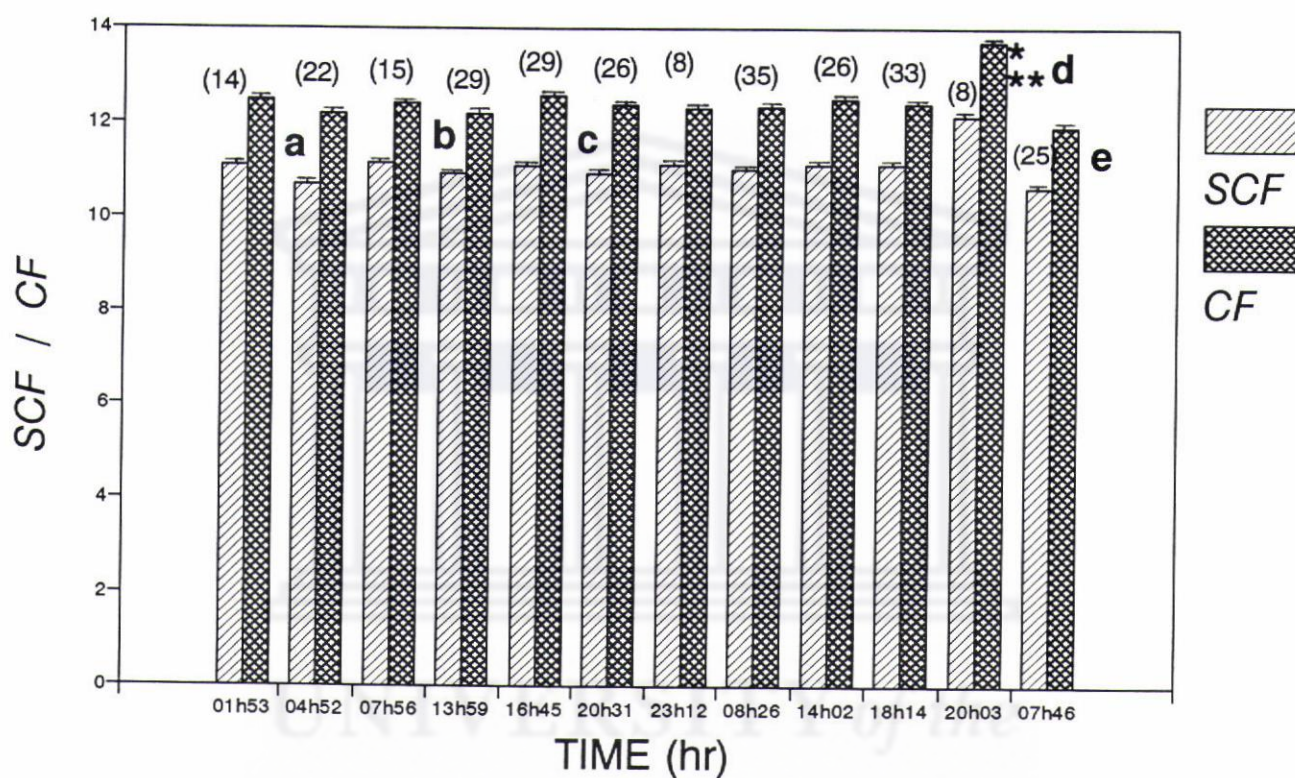
Figure 2.8: Somatic condition factor (*SCF*) and condition factor (*CF*) of the pilchard *S. sagax*, measured over a 48 h period during September 1994. Vertical bars represent Mean ± SEM; values in brackets = n; * = $p \leq 0.05$ (*SCF* & *CF*: d vs a, b, e); ** = $p \leq 0.05$ (*SCF*: d vs c).

sirm (Milton *et al*, 1994). Similarly, species such as goldfish *Carassius auratus* (Kagawa *et al*, 1983), giltheaded seabream *Sparus aurata* (Zohar *et al*, 1986) and Cape anchovy *Engraulis capensis* (Melo, 1994a) have ovaries containing younger vitellogenic oocytes as well as follicles undergoing maturation and ovulation. The phenomenon of fish containing gonads with oocytes in different ovarian maturation stages is characteristic of teleosts exhibiting a serial mode of spawning (Alheit, 1989). Similarly, Hunter and Macewicz (1985a) reported a continuous recruitment of oocytes throughout the spawning season of the serial spawning northern anchovy, *Engraulis mordax*. It is possible that multiple spawning may be advantageous since relatively stable populations can be maintained in unpredictable environments, i.e. the fish would have a better chance of survival if eggs are released in batches, than when all eggs are released simultaneously (Le Clus, 1987).

2.4.2 Fluctuations in the gonadosomatic index (*GSI*) of *S. sagax*

2.4.2.1 *Fluctuations in the GSI in different ovarian maturation stages*

GSI values of pilchard peaked during the vitellogenic period. This corresponds well with *GSI* patterns observed in other teleosts (eg: English sole *Parophrys vetulus*, Johnson *et al*, 1991 and Cape anchovy *E. capensis*, Melo, 1992), when yolk deposition and hence the number of oocytes in advanced stages become evident. The considerable variation observed in the *GSI* of pilchard in the partially spent condition could be ascribed to the serial mode of spawning of the species since different degrees of oocyte stages were observed in the partially spent ovaries. Nonetheless, in the present study, the *GSI* of females in the partially spent condition was lower than that of vitellogenic females. This is to be expected since hydrated oocytes had been spawned and therefore contributed to the decrease observed in the gonadal weight and hence the *GSI*. Similarly, decreased *GSI* values were reported for Cape

anchovy *E. capensis* (Melo, 1992) and the group-synchronous spawning English sole *P. vetulus* (Johnson *et al*, 1991), once the fish had spawned.

In the present study, however, the number of spawning female pilchard was minimal and is thus not accounted for. The high *GSI* found in spawning *P. vetulus* (Johnson *et al*, 1991) and *E. capensis* (Melo, 1992) was therefore not observed in the present study.

2.4.2.2 *Monthly fluctuations in the GSI*

It is normally assumed that the high *GSI* values during the reproductive period are indicative of spawning (Stoumboudi *et al*, 1993). This peak, however, represents the storage of reproductively mature oocytes (Stoumboudi *et al*, 1993). In the present study, monthly changes in the *GSI* values indicated that peak gametogenesis took place in January. A small proportion of the fish obtained in the latter month had commenced spawning while the greater proportion showed ovaries in the late vitellogenic condition. It has been suggested that spawning be inferred from the *GSI* value when a decrease in the ovarian mass is observed once oocytes have been released during ovulation (Stoumboudi *et al*, 1993). The decrease in the *GSI* and the high proportion of pilchard in the partially spent condition in February suggests that maximum spawning activity took place during this month, in the present study. It is, however, possible that this may not be a true reflection of maximum reproductive output, since Akkers (unpublished data) observed spawning peaks in *S. sagax* in August and December. The spawning peaks observed in August and December (Akkers, unpublished data) were not observed in the present study, due to the absence of samples during those months. Nevertheless, Akkers (unpublished data) also found a high proportion of females in the partially spent condition during February, as was observed in the present study.

Favourable environmental parameters like abundant food availability and temperature, generally trigger spawning behaviour in teleosts (Lam, 1983). Measurements of integrated chlorophyll - A (Richardson, unpublished data) and environmental temperature (16.5 ± 1 °C) at the specific sampling location in February indicated that these parameters were suitable for spawning to take place. It is therefore possible that maximal use of the optimal conditions resulted in spawning during February as was observed in the present study. According to Bye (1990), the effects of increasing or decreasing temperature tend to be inhibitory rather than regulatory. Furthermore, gonad maturation continues to an advanced stage under natural environmental conditions, becoming arrested in this state until temperatures conducive for spawning occurs.

The first signs of ovarian maturation in turbot, *Scophthalmus maximus* and Dover sole, *Solea solea* has been shown to occur shortly after an increase in the condition factor was observed (Bye, 1990). The overall condition factor value of pilchard during the summer-autumn season was higher than in spring and this suggests that ovarian growth was favoured during the season when food was abundant.

In this study, spawning in pilchard corresponded well with that of the summer-autumn peak described by Le Clus (1987) and Matthews (1964). Most teleosts are, however, either spring (eg: right-eye flounder *Pleuronectes herzensteini*, Tominaga *et al*, 1991 and three-spined stickleback *Gasterosteus aculeatus* L., Wooton *et al*, 1978) or spring-summer (eg: sweep *Scorpis lineolatus* K., Dedual and Pankhurst, 1992 and pike *Esox lucius* L., Lenhardt, 1992) spawners, taking advantage of the abundance of zooplankton during this period. Another clupeoid species utilizing the abundant zooplankton in spring-summer is the scaled sardine

Harengula jaguana P. (Martinez and Houde, 1975). Studies on temperate clupeoids, however, suggest that they do not spawn during periods of high food abundance, but store energy as fat (Hunter and Leong, 1981), while Rajasilta (1992) showed that gonad maturation in temperate herring *Clupea harengus*, is linked to food availability.

In this study, pilchard were caught only during SARP and RaFoS cruises and consequently certain months are not presented. The spawning activity from spring to summer (Davies, 1956) and the two spawning peaks in winter-spring and summer-autumn (Le Clus, 1987) could thus not be verified. Akkers (unpublished data) showed that peak spawning in pilchard took place in August and December and suggested that February represented the time when females in the spawning condition was high, as was found in this study.

The presence of totally spent females in January and the proportional increase thereafter, could indicate the end of the spawning season since a greater proportion of females in the totally spent condition was observed in March. In goosefish, *Lophius americanus*, it was concluded that spawning had terminated, once females in the spent condition appeared (Armstrong *et al*, 1992). Similarly, in the serial spawning Cape anchovy *E. capensis* the end of the spawning season was indicated by a marked increase in females in the totally spent condition (Melo 1992). In the present study, therefore it could be deduced that spawning activity took place in February and declined in March. No data for the months, April to August was available and therefore no definite end to the spawning activity in pilchard can be concluded from this study.

2.4.2.3 *Daily fluctuations in the GSI*

In the present study, sampling was undertaken over a 48 h period commencing after midnight and ending at daybreak. It is known that pilchard in Namibian waters spawn off-shore (Le Clus, 1987) and thus the absence of pilchard in the spawning condition (ripe / ripe running) in this study could be attributed to inshore sampling at all times. Nevertheless, changes in the *GSI* values and proportions of ovarian maturation stages of *S. sagax* indicate that spawning occurs during the early part of the night. New post - ovulatory follicles (Day 0: time elapsed from spawning about 2 to 6 h) were evident in the first two batches of samples caught (after midnight), indicating that spawning had commenced prior to midnight. The presence of Day 2 post-ovulatory follicles at 14h02 confirmed spawning activity prior to midnight since these follicles were about 38 to 42 h old. Similarly, Melo (1994b), Hunter and Macewicz (1980) and Alheit *et al*. (1984) found that spawning occurs prior to or near midnight for *E. capensis, Engraulis mordax* and *Engraulis ringens*, respectively. In contrast, Milton *et al* (1994) found that the greatest proportion of post ovulatory follicles in the sardine *Amblygaster sirm,* the herring *Hefklotsichthys quadrimaculatus* and sprat *Spratelloides delicatulus* was found prior to midnight.

The peak *GSI* values at 14h02 and 07h46 indicates that the gonad was preparing for spawning. Evidence of spawning activity was not observed in subsequent samples and thus it cannot be concluded that spawning took place during the second sampling cycle, irrespective of the lowered *GSI* observed after 14h02.

It has been suggested that the nutritional status of the species influences gametogenesis in Dover sole *S. solea* and turbot *S. maximus* (Bye, 1990). If this is correct, then the peak *GSI* values observed during the 48 h sampling period in this study should have been preceded by a high condition factor. The condition factor was high (at 20h03) only once prior to a peak *GSI* value and therefore it cannot be concluded that gametogenesis is influenced by condition factor, since two *GSI* peaks were observed in this study. The peak in condition factor at 20h03 could possibly be attributed to the favourable environmental conditions which prevailed at this sampling time, since the stomach of most pilchard were food-laden.

The use of *GSI* values as an indicator of annual gonadal activity in fish, has been questioned (de Vlaming *et al*, 1982 and Stoumboudi *et al*, 1993). Moreover, Delahunty and de Vlaming (1980) reported that *GSI* may vary to a large degree in serial spawners. In the present study, the *GSI* in combination with histological examination of ovarian maturation stages (Hunter and Macewicz, 1985b) and environmental parameters like temperature were used to verify the accuracy of using the *GSI* value as an indicator of spawning in pilchard. The combined use of the *GSI* value and histological examination of ovarian maturation stages is important in serial spawners since gonadal activity occurs throughout the spawning season.

2.5   CONCLUSION

The present study showed that the gonadosomatic index (*GSI*) can be used as an indicator of spawning activity, only if used in addition to histological examination of ovarian maturation stages to verify gonadal activities in *S. sagax*.   Monthly changes in the *GSI* indicated that maximum spawning took place in February, possibly commencing prior to midnight, as evidenced in the 48 h sampling period, in this study.   It seems probable that spawning during January and March was favoured by suitable environmental conditions, since the *CF* and *SCF* values were highest at that time.

The presence of vitellogenic oocytes throughout the sampling period, verified the continuous mode of spawning in pilchard, *S. sagax* (Le Clus, 1987).   The consistency of spawning, however, depends on the environmental conditions prevailing in the area where spawning is to occur.

2.6 REFERENCES

Alheit, J.; Alarcon, V.H. and Macewicz (1984) - Spawning frequency and sex ratio in the Peruvian anchovy, *Engraulis ringens. Rep. Calif. coop. oceanic Fish. Investl.* **25:** 43 - 52.

Alheit, J. (1989) - Comparative spawning biology of anchovies, sardines, and sprats. *Rapp. P.-v. Reun. Cons. int. Explor. Mer.* **191:** 7 - 14.

Armstrong, M.J.; Musick, J.A. and Colvocoresses (1992) - Age, growth, and reproduction of the goosefish *Lophius americanus* (Pisces: Lophiiformes). *Fish. Bull.* **90:** 217 - 230.

Baird, D. (1970) - Age and growth of the South African pilchard, *Sardinops ocellata* (1965 - 1967). *Investl. Rep. Div. Sea Fish. S. Afr.* **91:** 1 - 16.

Baird, D. (1971) - Seasonal occurrence of the pilchard, *Sardinops ocellata* on the East Coast of South Africa. *Investl. Rep. Div. Sea Fish. S. Afr.* **96:** 1 - 19.

Bradford, R.G. (1993) - Differential utilization of storage lipids and storage proteins by Northwest Atlantic herring (*Clupea harengus harengus*). *J. Fish Biol.* **43:** 811 - 824.

Bye, V.J. (1990) - Temperate marine teleosts. In: *Reproductive seasonality in Teleosts: Environmental Influences.* Munro, A.D.; Scott, A.P. and Lam, T.J. (Eds). CRC Press, Inc. Boca Raton, Florida. pp. 126 - 143.

Crawford, R.J.M. (1981a) - Catch per standard-boat-day and deployment of effort in the South African purse-seine fishery, 1964 - 1976. *Investl. Rep. Div. Sea Fish. S. Afr.* **122:** 1 - 24.

Crawford, R.J.M. (1981b) - Distribution, availability and movements of pilchard, *Sardinops cellata* of South Africa, 1964 - 1976. *Fish. Bull. S. Afr.* **14:** 1 - 46.

Davies, D.H. (1956) - The South African pilchard, *Sardinops ocellata*. Sexual maturity and reproduction, 1950 - 1954. *Investl. Rep. Div. Fish. S. Afr.* **22:** 1 - 155.

Dedual, M. and Pankhurst, N.W. (1992) - Plasma steroid hormone concentrations in relation to reproductive cycle of the Sweep *Scorpis lineolatus* (Kyphosidae) caught from the wild. *Aust. J. Mar. Freshwater Res.* **43:** 753 - 763.

Delahunty, G. and de Vlaming, V.L. (1980). Seasonal relationships of ovary weight, liver weight and fat stores with body weight in the goldfish, *Carassius auratus* (L.). *J. Fish Biol.* **16:** 5 - 13.

de Vlaming, V.L.; Grossman, G. and Chapman, F. (1982). On the use of the gonadosomatic index. *Comp. Biochem. Physiol.* **73 A:** 31 - 39.

Drury, R.A.B. and Wallington, E.A. (1967) - General Staining Procedures. In *Carleton's Histological Techniques*. Oxford University Press, New York, Toronto. pp. 114 - 137.

Hunter, J.R. and Leong, R. (1981) - The spawning energetics of female northern anchovy *Engraulis mordax. Fish. Bull.* **79:** 215 - 230.

Hunter, J.R. and Macewicz, B.J. (1980) - Sexual maturity, batch fecundity, spawning frequency and temporal pattern of spanwing for the northern anchovy, *Engraulis mordax*, during the 1979 spawning season. *Rep. Calif. coop. oceanic Fish. Invest.* **21:** 139 - 149.

Hunter, R.J. and Macewicz, B.J. (1985a) - Rates of atresia in the ovary of captive and wild northern anchovy, *Engraulis mordax. Fish. Bull.* **83:** 119 - 136.

Hunter, R.J. and Macewicz, B.J. (1985b) - Measurement of spawning frequency in multiple spawning fishes. In: *An egg production method for estimating spawning biomass of pelagic fish: Application to the northern anchovy, Engraulis mordax. NOAA Technical Report NMFS 36.* p. 79 - 94.

Johnson, L.L.; Casillas, E.; Myers, M.S.; Rhodes, L.D. and Olson, O.P. (1991) - Patterns of oocyte development and related changes in plasma 17-ß estradiol, vitellogenin, and plasma chemistry in English sole *Parophrys vetulus* Girard. *J. Exp. Mar. Biol. Ecol.,* **152:** 161 - 185.

Kagawa, H.; Young, G. and Nagahama, Y. (1983) - Changes in plasma steroid hormone levels during gonadal maturation in female goldfish *Carassius auratus. Bull. Jap. Soc. Sci. Fish.* **49:** # 12, 1783 - 1787.

Lam, T.J. (1983) - Environmental influences on gonadal activity in fish. In: *Fish Physiology*. Hoar, W.S.; Randall, D.J. and Donaldson, E.M. (Eds). Academic Press, New York, London. **Vol. IX Part B**: 65 - 101.

Le Clus, F. (1979) - Oocyte development and spawning frequency in the South West African pilchard *Sardinops ocellata. Fish. Bull. S. Afr.* **12:** 53 - 68.

Le Clus, F. (1987) - Reproductive dynamics of female pilchard *Sardinops ocellatus* in the northern Benguela system, with particular reference to seasonality, the environment and fish condition. Ph.D. thesis, University of Port Elizabeth.

Le Clus, F. (1988) - Batch fecundity of pilchard, *Sardinops ocellatus* in the Northern Benguela System estimated from hydrated oocytes. *S. Afr. J. mar. Sci.* **7:** 243 - 254.

Lenhardt, M. (1992) - Seasonal changes in some blood chemistry parameters and in relative liver and gonad weights of pike (*Esox lucius* L.) from the River Danube. *J. Fish. Biol.* **40:** 709 - 718.

Martinez, S. and Houde, E.D. (1975) - Fecundity, sexual maturation, and spawning of scaled sardine (*Harengula jaguana* Poey). *Bull. Mar. Sci.* **25:** #1, 35 - 45.

Melo, Y.C. (1992) - The reproductive biology of the anchovy, *Engraulis capensis* in the Benguela Current Region. Ph.D. thesis, University of Stellenbosch: 1 - 180.

Melo, Y.C. (1994a) - Multiple spawning of the anchovy *Engraulis capensis*. *S. Afr. J. mar. Sci*. **14:** 313 - 320.

Melo, Y.C. (1994b) - Spawning frequency of the anchovy *Engraulis capensis*. *S. Afr. J. mar. Sci*. **14:** 321 - 332.

Matthews, J.P. (1964) - The pilchard of South West Africa (*Sardinops ocellata*). Sexual development, condition factor and reproduction, 1957 - 1960. *Investl. Rep. mar. Res. Lab. S.W.Afr.* **10:** 96 pp.

Milton, D.A.; Blaber, S.J. and Rawlinson, N.J.F. (1994) - Reproductive biology and egg production of three species of Clupeidae from Kiribati, tropical central Pacific. *Fish. Bull.* **92:** 102 - 121.

Nawratil, O. (1962) - The pilchard of South West Africa, (*Sardinops ocellata*). Age composition of *Sardinops ocellata* in the commercial catches 1958 and 1959 with reference to a change in rate of growth. *Investl. Rep. mar.Res. Lab. S.W. Afr.* **6:** 1 - 33.

O'Toole, M.J. (1977) - Investigations into some important fish larvae in the South East Atlantic in relation to the hydrological environment. Ph.D. Thesis, University of Cape Town.

Rajasilta, M. (1992) - Relationship between food, fat, sexual maturation, and spawning time of Baltic herring (*Clupea Harengus mambras*) in the Archipelago Sea. *Can. J. Fish. Aquat. Sci.* **49:** 644 - 654.

Retamales, R.G. and Gonzàlez, M. (1985) - Incidence of El Niño 1982 - 1983 phenomenon on the spawning of the Chilean pilchard, (*Sardinops sagax*). *Invest. Pesq.* (Chile) **32:** 161 - 165.

Stoumboudi, M.Th.; Villwock, W.; Sela, J. and Abraham, M. (1993) - Gonadosomatic index in *Barbus longiceps, Capoeta damscina* and their natural hybrid (Pisces, Cyprinidae), *versus* spermatozoan index in the parental males. *J. Fish. Biol.* **43:** 865 - 875.

Wooton, R.J.; Evans, G.W. and Mills, L. (1978) - Annual cycle in female three-spined sticklebacks (*Gasterosteus aculeatus* L.) from an upland and lowland population. *J. Fish Biol.* **12:** 331 - 343.

Zohar, Y.; Pagelson, G. and Tosky, M. (1986) - Daily changes in reproductive hormone levels in the female gilthead seabream *Sparus aurata* at the spawning period. *Reproduction in fish - Basic and applied aspects in endocrinology and genetics.* pp. 119 - 125.

CHAPTER THREE

PHYSIOLOGICAL CHANGES ASSOCIATED WITH

VITELLOGENESIS IN THE PILCHARD,

Sardinops sagax

CHAPTER 3: PHYSIOLOGICAL CHANGES ASSOCIATED WITH VITELLOGENESIS IN THE PILCHARD, *Sardinops sagax*

ABSTRACT

Ovarian development in response to liver activity was monitored in female pilchard, *Sardinops sagax* in different ovarian maturation stages during a monthly and over a 48 h sampling period. The hepatosomatic-index value (*HSI*) remained relatively constant throughout the sampling period and reached maximal values in partially spent females in February - March and at 14h02 during the 48 h sampling period, indicative of the active role of the liver during final maturation and ovulation of *S. sagax*. The lipophosphoprotein complex, vitellogenin, appeared in vitellogenic and partially spent female *S. sagax*, but not in males. This is indicative of the sex-specificity of vitellogenin and the liver's activity during the vitellogenic period.

3.1 INTRODUCTION

The period preceding the spawning season has been aptly termed vitellogenesis by Pan *et al* (1969) and can be described as the period when fish "enter a phase of maturation of their oocytes in preparation for spawning" (Mommsen and Walsh, 1988). During the vitellogenic period, environmental cues trigger the release of gonadotropic hormone (GtH1) from the pituitary, which stimulates the biosynthesis of estradiol-17-ß hormone (E_2) in the ovarian follicle. The E_2 taken up by the liver stimulates the hepatic synthesis and secretion of Zr-

proteins (Zr-P) as well as a lipophosphoprotein called vitellogenin (Vg), which is highly phosphorylated and complexed with variable amounts of lipid (Fig. 3.1). The hepatically derived vitellogenin is then sequestered by the ovary for yolk deposition during exogenous vitellogenesis (van Bohemen *et al*, 1981; Ng and Idler, 1983). The most notable change in the blood is therefore, the appearance of the yolk-precursor protein, vitellogenin (Craik, 1978; Emmersen and Petersen, 1976; Craik and Harvey, 1984). Measurements of vitellogenin levels in teleost blood have been determined previously by various methods involving radioimmunossay (Copeland and Thomas, 1988; So *et al*, 1985; Copeland *et al*, 1988 and Benfey *et al*, 1989), enzyme-linked immunosorbent assay (ELISA) (Pacoli *et al*, 1990), rocket electrophoresis (Le Guellec, 1988), inorganic phosphate determination (Martin and Doty, 1949) and sodium-dodecyl-sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Bradley and Grizzle, 1989). Emmersen and Petersen (1976) and Korsgaard and Petersen (1979) reported that phosphoprotein determination in the plasma of European flounder *Platichthys flesus* and blenny *Zoarces vivipara*, respectively, can be used to identify vitellogenin presence in fish.

The pilchard, *S. sagax*, has been described previously as a serial spawner, spawning several times during one spawning season, (Le Clus, 1979; 1987). Information on the presence of vitellogenin in pilchard, *S. sagax*, is not available. As a result of the serial mode of spawning, one would expect varying levels of vitellogenin in the blood plasma at different times during the reproductive period. The purpose of this study was to monitor vitellogenin presence in the blood plasma of *S. sagax* during the reproductive period and to relate the observations to oocyte development. Non-denaturing polyacrylamide gradient gel electrophoresis (Gambert *et al*, 1982) and methyl green staining (Cutting and Roth, 1973)
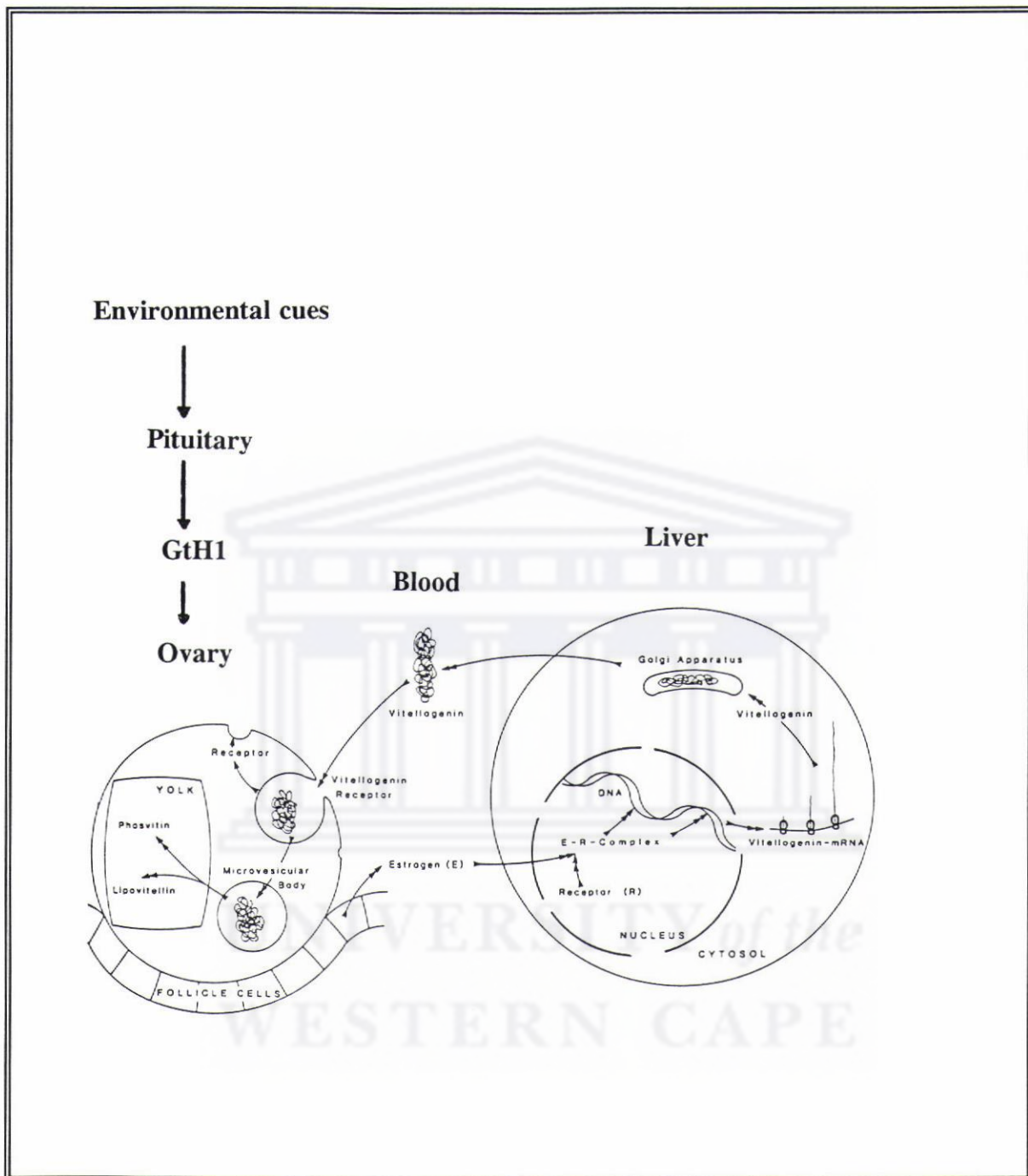
Figure 3.1: Environmental cues trigger the release of gonadotropic hormone (GtH1) from the pituitary, which stimulates the release of estradiol-17-ß (E2) from the ovary. A feedback system exist between the ovary and the liver during vitellogenesis when vitellogenin (Vg) is released. Adapted from Mommsen and Walsh (1988).

were used to determine the presence of phosphoprotein and hence the vitellogenin complex in the blood plasma of *S. sagax*. In addition, analysis of the hepatosomatic index (*HSI*) in relation to vitellogenin synthesis is reported for pilchard obtained throughout the sampling period.

3.2   MATERIALS AND METHODS

3.2.1   Animals and sampling procedures

Sexually mature and immature female *S. sagax*, (fish length: 180 mm and 210 mm) were collected at monthly intervals between September 1993 and March 1994 on the SARP cruises. Samples of mature and immature female pilchard were also collected during a 48 h period in September 1994 on the RaFoS cruises. Complications in sampling strategies precluded the capture of pilchard during November and December 1993.

Pilchard were collected by means of an Engels midwater trawl net and transferred immediately to aerated 100 *l* holding tanks with a continuous sea water flow system aboard the research vessel. Live pilchard were sexed and macroscopically staged according to the classification of Davies (1956). Approximately 1.5 ml blood was collected from the caudal vein of individually tagged fish for further physiological and biochemical analyses. Blood samples were stored on ice for no longer than 1 h and centrifuged on site in a SIGMA 112 centrifuge at 3000 xg for 10 min. The supernatant was aliquoted and stored frozen at - 20 °C until further laboratory analysis. Thereafter, all fish were sacrificed by severing the spinal cord. The liver was excised and weighed to the nearest 0.01 g for hepatosomatic index (*HSI*) determination of individual fish (Melo, 1994; Retamales and Gonzàlez, 1985).

Calculations were done as follows:

$$HSI = \frac{\text{Liver weight (g)}}{\text{Body Mass (g) - Liver mass (g)}} \times 100$$

3.2.2 Quantitative analysis of serum vitellogenin

3.2.2.1 *Electrophoretic techniques*

Non-denaturing polyacrylamide gradient gel electrophoresis was performed as described by Nichols *et al* (1986). A 4 - 18% gradient polyacrylamide and 3% stacking gel (Laemmli, 1970) was cast using the BIO-RAD MINI PROTEAN II™ minigel (70 x 80 mm) system. The plasma samples for vitellogenin determination were prepared as follows: 10 μl saturated sucrose / bromophenol blue solution (tracking dye) and 10 μl Sudan black stain were added to 40 μl fish plasma. 20 μl of the mixture was loaded per lane (in duplicate). The gels were run at 60 mA for 30 min, then for 2 hours at 130 mV (constant voltage), all performed at 4 °C. At termination of the run, the tracking dye was approximately 1 cm from the end of the gel.

3.2.2.2 *Staining of gels and vitellogenin determination*

After electrophoresis, gels were prefixed in 10% sulphosalicic acid, followed by phosphoprotein staining in 0.5% methyl green and destaining in 10% sulphosalicic acid according to Cutting and Roth (1973). The gels were scanned on a Hoeffer GS 300 Transmittance / Reflectance scanning densitometer and dried on a Hoeffer Scientific drygel jr. SE 540 (Nichols *et al*, 1986).

3.2.3   Photography

Methyl green stained gels were positioned onto a lightbox and photographed (with a blue light filter attached) and photocopied, immediately after densitometric scanning.

3.2.4   Statistical analysis

Tests for significance were performed by Kruskal - Wallis Nonparametric Analysis of Variance (ANOVA), followed by Dunns' Multiple Comparisons test, using the INSTAT computer programme. Correlations were performed by Regression Analysis using the STATISTICA computer programme (Statsoft™, Inc.).

3.3   RESULTS

3.3.1   Hepatic changes associated with vitellogenesis and spawning

The hepatosomatic index (*HSI*) values at different ovarian maturation stages are presented in Fig. 3.2. The *HSI* generally remained constant in the different ovarian maturation stages, except for the higher *HSI* value observed in partially spent females. The *HSI* value in the latter stage was approximately 30% higher than that of pilchard with ovaries in the immature and totally spent condition and was 40% higher than females in the previtellogenic condition. Immediately thereafter, the *HSI* of totally spent pilchard declined to the initial values observed prior to spawning.
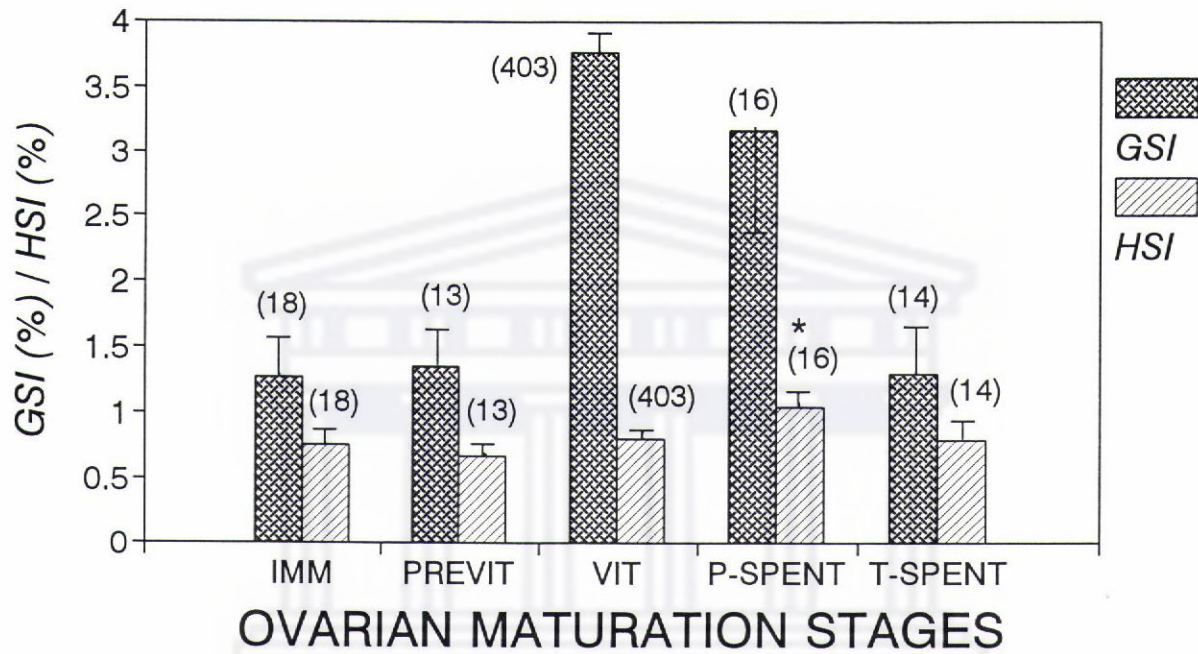
Figure 3.2: Hepatosomatic index (*HSI*) and gonadosomatic index (*GSI*) values measured at different ovarian maturation stages in *S. sagax*. Vertical bars represent Mean $\pm$ SEM; values in brackets = n; * = $p \leq 0.05$ (P-Spent vs Previt); p values for *GSI* are given in Fig. 2.2.

Monthly changes in the *HSI* of female pilchard during the spawning season are presented in Fig. 3.3. *HSI* values averaged 0.74% in September, declining thereafter in October and January. Data for November and December are not available, however. A 30% and 40% increase (p ≤ 0.001) in the *HSI* of fish sampled in February and March, relative to October and January was observed (Fig. 3.3).

The *HSI* of different pilchard, sampled over a 48 h period during September 1994, was noted (Fig. 3.4). The *HSI* of fish sampled at 04h42, 08h26, 07h46, 13h59, 14h02 and 20h31 was significantly higher (p ≤ 0.01) than fish sampled during the late afternoon at 16h45 and 18h14. The *HSI* value at 14h02 was significantly higher (p ≤ 0.01) than that of fish sampled at 07h56. An increase of approximately 45% (p ≤ 0.05) was observed in the *HSI* value of pilchard sampled at 07h46 and 14h02, relative to fish sampled at 23h12.

3.3.2 Correlation between the hepatosomatic index (*HSI*) and gonadosomatic index (*GSI*)

The correlation between the *HSI* and *GSI* was generally weak and not significant (p > 0.05) in the different ovarian maturation stages, except for a very small, but significant correlation (p ≤ 0.001) observed in females in the vitellogenic stage (Table 3.1). Similarly, the correlation between the *HSI* and *GSI* during the monthly sampling period was generally weak and not significant, except for the significant correlation (p ≤ 0.03) observed in March (Table 3.2). The correlation between the *HSI* and *GSI* during the daily sampling period was also generally weak and not significant, however a significant correlation (p ≤ 0.002) was observed in pilchard caught at 08h26 (Table 3.3).
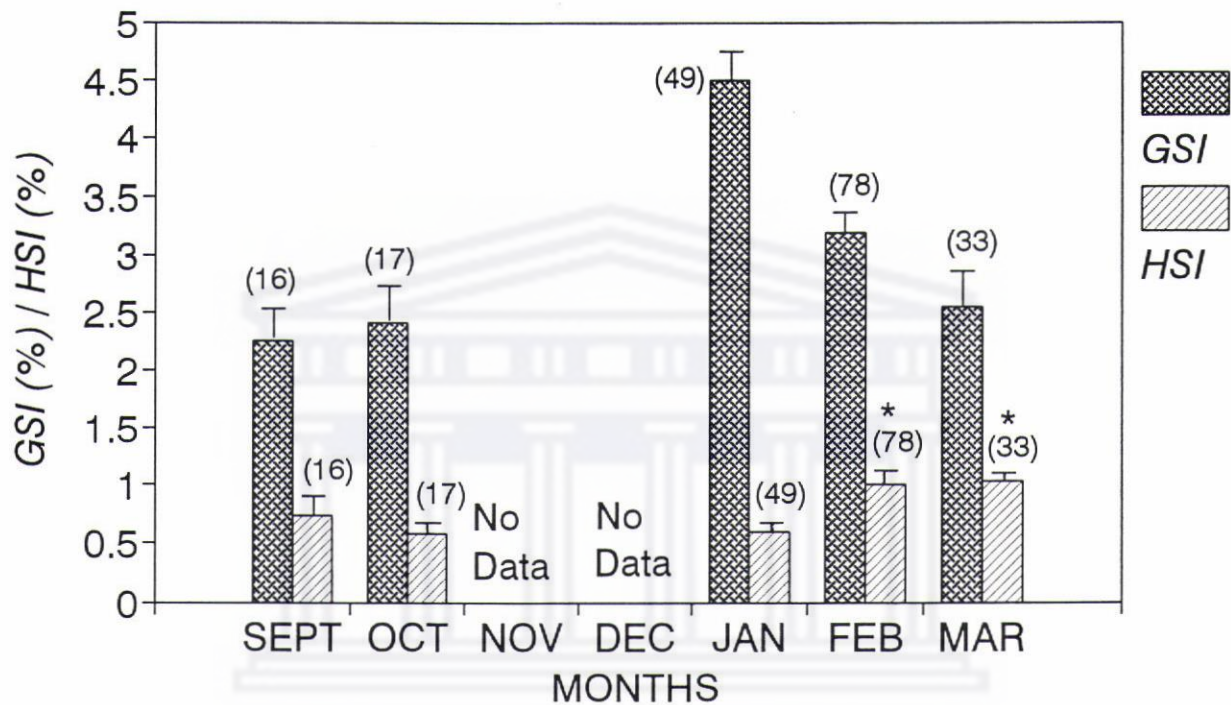
Figure 3.3: Monthly hepatosomatic index (*HSI*) and gonadosomatic index (*GSI*) values of *S. sagax*. Vertical bars represent Mean $\pm$ SEM; values in brackets = n; * = p $\leq$ 0.001 (Feb, Mar vs Jan, Oct); p values for *GSI* are presented in Figure 2.3.
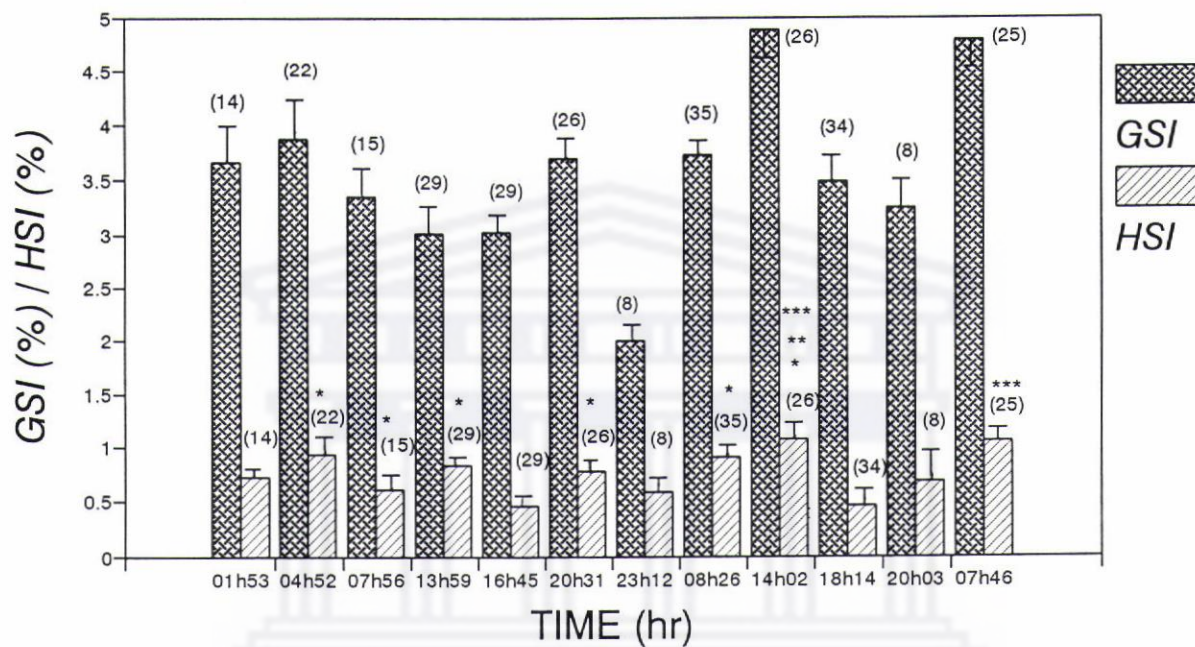
Figure 3.4.: Daily hepatosomatic index (*HSI*) and gonadosomatic index (*GSI*) values of the pilchard *S. sagax* measured over a 48 h period during September 1994. Vertical bars represent Mean ± SEM; values in brackets = n; * = p ≤ 0.01 (b, c, d, f, h, i vs e, j); ** = p ≤ 0.01 (i vs c); *** = p ≤ 0.05 (i, l vs g); p values for *GSI* are presented in Figure 2.4.

Table 3.1: **The relationship between hepatosomatic index (*HSI*) and gonadosomatic index (*GSI*) of *S. sagax* in different ovarian maturation stages.**

STAGE	n	HSI (%) vs GSI (%)	
		r	*p*
Immature	22	0.15	ns
Previtellogenic	14	-0.29	ns
Vitellogenic	411	0.17	sc
Partially-Spent	16	0.05	ns
Totally-Spent	14	-0.18	ns

n = sample size

r = correlation coefficient

ns = not significant

sc = significant correlation (p ≤ 0.001)

Table 3.2: The relationship between monthly changes in the hepatosomatic index (*HSI*) and gonadosomatic index (*GSI*) of *S. sagax*.

MONTH	n	HSI (%) vs GSI (%)	
		r	*p*
September	16	0.22	ns
October	17	-0.07	ns
January	49	0.06	ns
February	78	0.17	ns
March	33	0.38	sc

n = sample size

r = correlation coefficient

ns = not significant

sc = significant correlation (p $\leq$ 0.03)

Table 3.3: The relationship between the hepatosomatic index (*HSI*) and gonadosomatic index (*GSI*) of *S. sagax*, over a 48 h period during September 1994.

TIME	n	HSI (%) vs GSI (%)	
		r	*p*
01h53	14	-0.17	ns
04h52	22	-0.03	ns
07h56	15	0.25	ns
13h59	29	0.28	ns
16h45	30	0.03	ns
20h31	16	0.16	ns
23h12	16	0.16	ns
08h26	35	0.50	sc
14h02	26	0.32	ns
18h14	38	-0.18	ns
20h03	17	0.43	ns
07h46	25	0.21	ns

n   =   sample size

r   =   correlation coefficient

ns   =   not significant

sc   =   significant correlation (p $\leq$ 0.002)

3.3.3   Plasma vitellogenin (phosphoprotein) determination

The results obtained from gradient gel electrophoresis of plasma from female pilchard in different ovarian maturation stages and in males are summarized in Table 3.4, Fig. 3.5.a; b and Fig. 3.6.a; b. Plasma vitellogenin was not detected in males and in females in the immature, previtellogenic and totally spent condition as well as in 24% of pilchard in the vitellogenic condition. Vitellogenin was present in the plasma of the greater proportion of vitellogenic pilchard, while traces were observed in pilchard in the partially spent condition.

Three distinct lipoprotein bands were observed at the origin (or), mid (mr) and frontal (fr) region of the gel, prior to methyl green staining for phosphoprotein (vitellogenin) (Fig. 3.5.a and b). A faint lipoprotein band (fb) was observed between the mid and frontal regions of the gel in vitellogenic fish (Fig. 3.5.b).

After methyl green staining, a traces of phosphoprotein was observed in the region between the mid and frontal region of the gel in partially spent pilchard (not shown), but not in pilchard in the totally spent condition (not shown), immature nor previtellogenic condition (Fig. 3.6.a). The faint lipoprotein band between the mid and frontal region of the gel stained sharply for plasma phosphoprotein (vg) in a large proportion of vitellogenic pilchard (Fig. 3.6.b).

Three distinct protein bands were observed in the origin, mid and frontal regions of the gel of male pilchard, prior to methyl green staining (Fig. 3.5.a). Phosphoprotein bands were not detected in male pilchard after methyl green staining (Fig. 3.5.b).

Table 3.4: Presence of plasma vitellogenin in female pilchard at different ovarian maturation stages and in male pilchard, *S. sagax*.

SEX	STAGE	n	VITELLOGENIN
FEMALE	Immature	6	not detected
	Previtellogenic	5	not detected
	Vitellogenic	39	present
	Vitellogenic	12	not detected
	Partially-Spent	3	present
	Totally-Spent	3	not detected
MALE	-------------	5	not detected

n = sample size
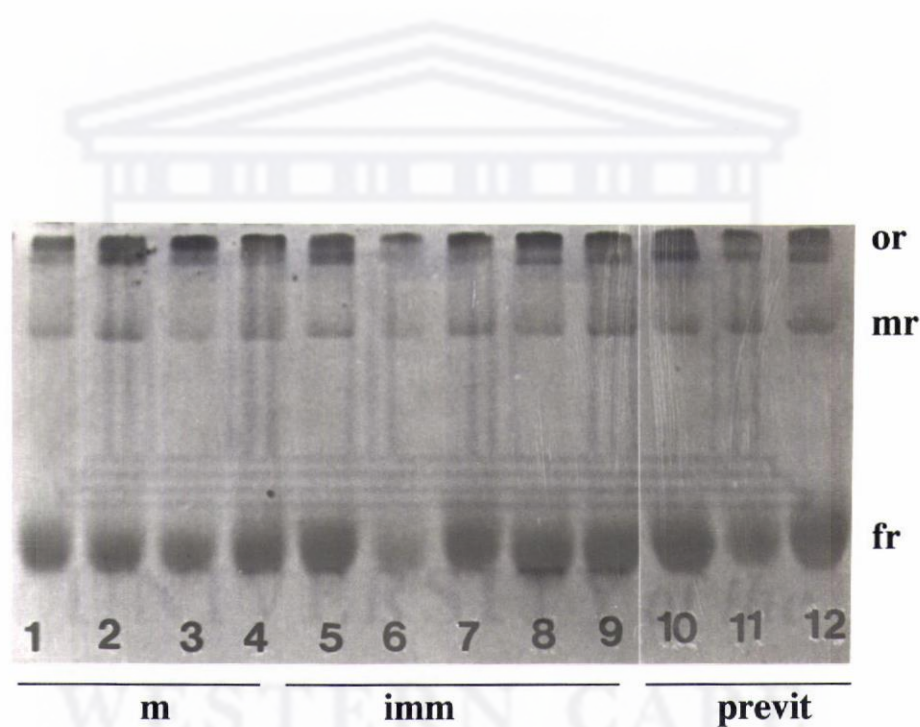
Figure 3.5.a: Lipoprotein bands observed in the plasma of male *S. sagax* (m) and female *S. sagax* in the immature (imm) and previtellogenic (previt) ovarian maturation stage, prior to methyl green staining. Sudan black stained lipoproteins are present at the origin (or), mid (mr) and frontal (fr) regions of the gel.
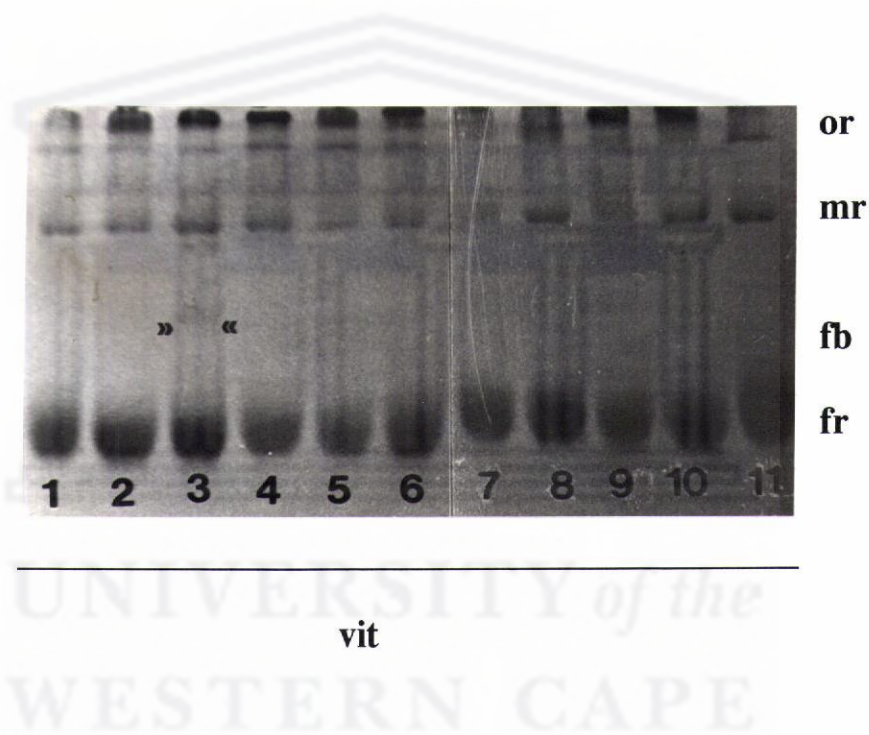
Figure 3.5.b: **Lipoprotein bands observed in the plasma of female pilchard, *S. sagax* in the vitellogenic stage (vit), prior to methyl green staining. Sudan black stained lipoprotein bands are present at the origin (or), mid (mr) and frontal (fr) regions of the gel. A faint lipoprotein band (fb) is present between the mid and frontal region of the gel.**
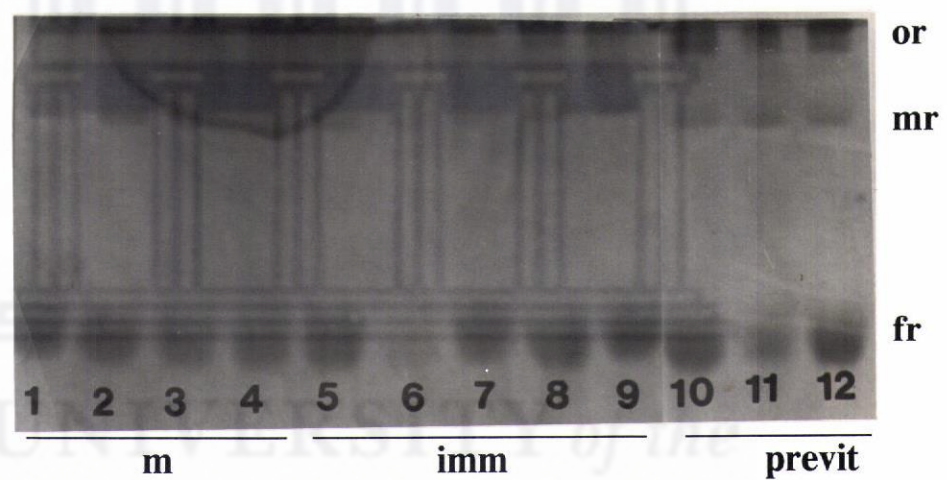
Figure 3.6.a: Phosphoprotein bands were not detected in male (m) *S. sagax* and in female *S. sagax* in the immature (imm) and previtellogenic (previt) ovarian maturation stages, following methyl green staining.
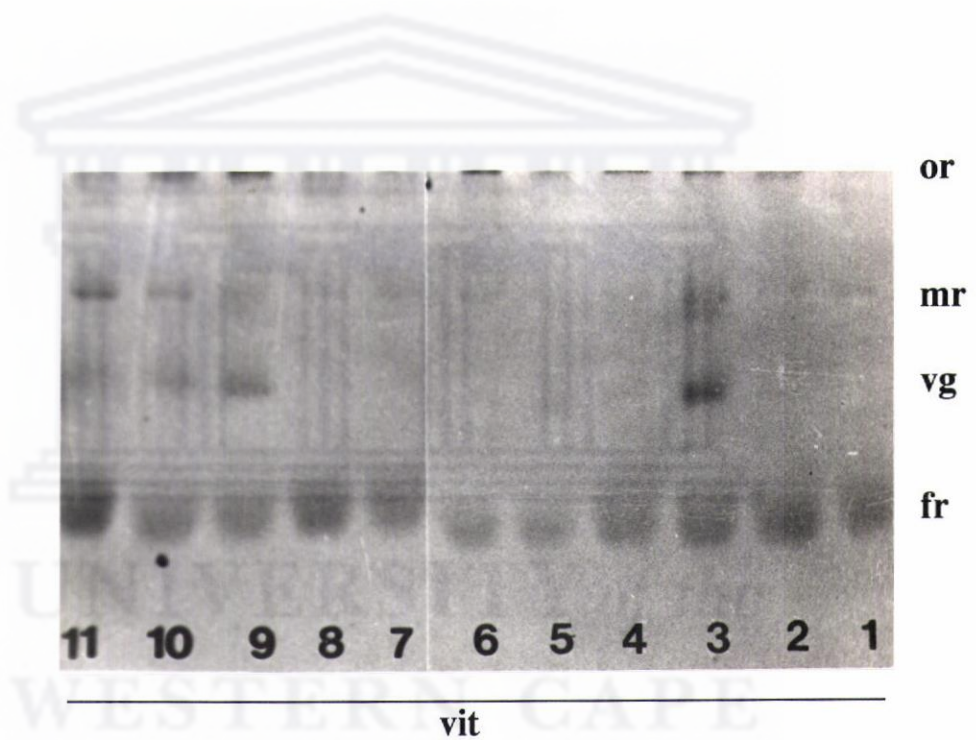
Figure 3.6.b: **Phosphoprotein bands (vg) are present between the mid (mr) and frontal (fr) regions of the gel in the vitellogenic female** *S. sagax*, **following methyl green staining.**

3.4   DISCUSSION

3.4.1   Hepatocellular activities

The liver is an important target organ for the binding of the steroid hormone, oestradiol-17-ß (E_2), in that during the vitellogenic process, E_2 activates numerous hepatocellular activities, including vitellogenin synthesis. In the present study, the *HSI* of *S. sagax* remained fairly constant in the different ovarian maturation stages and throughout most of the monthly sampling period. During the 48 h sampling period, however, the *HSI* fluctuated substantially, except for the occasional peaks in the morning, at midday and once in the early evening. The fluctuations and the peaks in the *HSI* could be attributed to the fact that pilchard are serial spawners, thereby producing and releasing vitellogenin at regular intervals, so as to maintain vitellogenesis. The peak *HSI* values could therefore be associated with the production of vitellogenin as estradiol-17-ß is taken up by the hepatocytes, while the lowered *HSI* value could be associated with the release of vitellogenin into the bloodstream of the fish. The correlation between *HSI* and *GSI* was generally weak and remained out of phase in the different ovarian maturation stages as well as in the monthly and 48 h sampling period. The fact that the *HSI* was highest in females in the partially spent condition and at 14h02 when Day 2 post-ovulatory follicles were observed in the ovaries of partially spent females, suggests that the liver plays a role in the gonadal maturation and ovulatory processes of *S. sagax..* Since pilchard are serial spawners (Le Clus, 1979; 1987), it is possible that the high *HSI* is maintained in order to satisfy the demands placed on the gonad during vitellogenesis.

The high *HSI* values recorded in February and March in this study (Chapter 2), suggest that the liver is activated and that vitellogenin is sequestered by the primary oocytes so as to ensure gonadal development and maturation. The correlation between the *HSI* and *GSI* prior

to these months was weak; indeed, the *HSI* was lowest in January, when the highest *GSI* was observed. In the latter month, it seems evident that a large proportion of the energy reserves of the liver had been utilized and that the vitellogenin that was released into the bloodstream, had been sequestered by the vitellogenic oocytes in preparation for final maturation and ovulation, at the time of sampling. Similar trends were observed in other species in the goldfish, *Carassius auratus* (Delahunty and de Vlaming, 1980). Hepatocellular activity was reported for the rainbow trout *Salmo gairdneri* (Campbell and Idler, 1980; van Bohemen *et al*, 1981), European flounder *Platichthys flesus* L. (Emmersen and Petersen, 1976) and red grouper *Epinepholus akaara* (Ng *et al*, 1984) during the vitellogenic and spawning period.

Nevertheless, the weak relationship between the *HSI* and *GSI* in *S. sagax* differs from the observation made in pike *Esox lucius* L. (Lenhardt, 1992) where, in the latter species, the two parameters were strongly correlated and remained in phase with one another for the entire duration of the vitellogenic and ovulatory cycle. The difference could be attributed to the serial mode of spawning observed in pilchard since the liver is activated throughout the reproductive period.

3.4.2   Plasma vitellogenin

The present study characterized the phospholipoprotein, vitellogenin, in the plasma of male as well as female pilchard in different ovarian maturation stages. Initially, vitellogenin could not be distinguished from the lipoprotein bands observed when gels were stained with Sudan black. Successful resolution of vitellogenin was achieved when gels were stained for phosphoprotein, using methyl green stain as described by Cutting and Roth (1973).

Vitellogenin was detected in the plasma of females in the vitellogenic and partially spent condition, but not in females in the immature, previtellogenic and totally spent ovarian maturation condition and, as expected, vitellogenin was not detected in the plasma of male pilchard. Previous studies showed that vitellogenin is a female-specific lipoprotein (European flounder *P. flesus,* Emmersen and Petersen, 1976; killifish *Fundulus heteroclitus*, Selman and Wallace, 1983). The fact that no difference in the lipoprotein bands before or after methyl green staining in male pilchard was observed, confirms the female specificity of vitellogenin in *S. sagax*.

Craik (1978) found that the vitellogenin levels dropped during the annual ovarian cycle of adult female elasmobranchs *Scyliorhinus canicula*. Higher vitellogenin levels were also found in mature, pre-spawning spotted trout *Cynoscion nebulosus*, as opposed to lower levels recorded in spawning fish (Copeland and Thomas, 1988). Similar observations were made in the rainbow trout, *S. gairdneri* (van Bohemen and Lambert, 1981) and English sole *P. vetulus* (Johnson *et al*, 1991). The fact that traces of vitellogenin were observed in partially spent female *S. sagax*, may reflect increased uptake of vitellogenin by the developing primary oocytes (i.e. vitellogenic oocytes) undergoing vitellogenesis. The high *HSI* values in females in the partially spent condition therefore reflects the increased energy demands placed on the liver by the developing gonads.

3.5 CONCLUSION

Vitellogenin presence in the plasma of *S. sagax* serves as an indicator of the vitellogenic process. The presence of vitellogenin in female pilchard in the vitellogenic and patially spent condition could be correlated with an increased *HSI*, indicating the liver's activity during the vitellogenic process. The high *HSI* values in females in the partially spent condition supports other lines of evidences that increased spawning occurred in February, while declining in March (Chapter 2) in this study.

3.6   REFERENCES

Benfey, T.J.; Donaldson, E.M. and Owen, T.G. (1989) - An homologous radioimmunoassay for Coho salmon (*Oncorhynchus kisutch*) vitellogenin, with general applicability to other Pacific salmonids. *Gen. Comp. Endoc.* **75:** 78 - 82.

Bradley, J.T. and Grizzle, J.M. (1989) - Vitellogenin induction by estradiol in channnel catfish, *Ictalurus punctatus*. *Gen. Comp. Endoc.* **73:** 28 - 39.

Campbell, C.M. and Idler, D.R. (1980) - Characterization of an estradiol-induced protein from rainbow trout serum as vitellogenin by the composition and radioimmunological cross reactivity to ovarian yolk fractions. *Biol. Reproduction.* **22:** 605 - 617.

Copeland, P.A., Sumpter, J.P., Walker, T.K. and Croft, M. (1988) - Vitellogenin levels in male and female rainbow trout (*Salmo gairdneri* Richardson) at various stages of the reproductive cycle. *Comp. Biochem. Physiol.* **83B:** #2, 487 - 493.

Copeland, P.A. and Thomas, P. (1988) - The measurement of plasma vitellogenin levels in a marine teleost, the spotted seatrout (*Cynoscion nebulosus*) by homologous radioimmunoassay. *Comp. Biochem. Physiol.* **91B:** #1, 17 - 23.

Craik, J.C.A. (1978) - Plasma levels of vitellogenin in the elasmobranch *Scyliorhinus canicula* L. (lesser spotted dogfish). *Comp. Biochem. Physiol.* **60B:** 9 - 18.

Craik, J.C.A. and Harvey, S.M. (1984) - A biochemical method for distinguishing between the sexes of fishes by the presence of yolk protein in the blood. *J. Fish Biol.* **25:** 293 - 303.

Davies, D.H. (1956) - The South African pilchard, *Sardinops ocellata*. Sexual maturity and reproduction, 1950 - 1954. *Investl. Rep. Div. Fish. S. Afr.* **22:** 1 - 155.

Delahunty, G. and de Vlaming, V.L. (1980) - Seasonal relationships of ovary weight, liver weight and fat stores with body weight in the goldfish, *Carassius auratus* (L.). *J. Fish Biol.* **16:** 5 - 13.

Cutting, J.A. and Roth, T.F. (1973) - Staining of phospho - proteins on acrylamide gel electropherograms. *Anal. Biochem.* **54:** 386 - 394.

Emmersen, B.K. and Petersen, I.M. (1976) - Natural occurrence, and experimental induction by estradiol - 17-ß, of a lipophosphoprotein (vitellogenin) in flounder (*Platichtys flesus*, L.). *Comp. Biochem. Physiol.* **54B:** 443 - 446.

Johnson, L.L.; Casillas, E.; Myers, M.S.; Rhodes, L.D. and Olson, O.P. (1991) - Patterns of oocyte development and related changes in plasma 17-ß estradiol, vitellogenin, and plasma chemistry in English sole *Parophrys vetulus* Girard. *J. Exp. Mar. Biol. Ecol.*, **152:** 161 - 185.

Korsgaard, B. and Petersen, I. (1979) - Vitellogenin, lipid and carbohydrate metabolism during vitellogenesis and pregnancy, and after hormonal induction in the blenny *Zoarces viviparus* (L.). *Comp. Biochem. Physiol. B* **63B:** 245 - 251.

Laemmli, U.K. (1970) - Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* **227:** 680 - 685.

Le Clus, F. (1979) - Oocyte development and spawning frequency in the South West African pilchard *Sardinops ocellata. Fish. Bull. S. Afr.* **12:** 53 - 68.

Le Clus, F. (1987) - Reproductive dynamics of female pilchard *Sardinops ocellatus* in the northern Benguela system, with particular reference to seasonality, the environment and fish condition. Ph.D. thesis, University of Port Elizabeth.

Le Guellec, K.L.; Lawless, K.; Valotaire, Y.; Kress, M. and Tenniswood, M. (1988) - Vitellogenin gene expression in male rainbow trout (*Salmo gairdneri*). *Gen. Comp. Endoc.* **71:** 359 - 371.

Lenhardt, M. (1992) - Seasonal changes in some blood chemistry parameters and in relative liver and gonad weights of pike (*Esox lucius* L.) from the River Danube. *J. Fish. Biol.* **40:** 709 - 718.

Martin, J.B. and Doty, D.M. (1949) - Determination of inorganic phosphate. *Anal. Chem.*, **21:** 965 - 967.

Melo, Y.C. (1994) - Multiple spawning of the anchovy *Engraulis capensis*. *S. Afr. J. mar. Sci.* **14:** 313 - 320.

Mommsen, T.P. and Walsh, P.J. (1988) - Vitellogenesis and oocyte assembly. In *Fish Physiology*, Vol. XIA. The Physiology of Developing Fish. Hoar, W.S. and Randall, D.J. (Eds). Academic Press, Inc. San Diego, New York, Berkeley: 347 - 406.

Ng, T.B. and Idler, D.R. (1983) - Yolk formation and differentiation in teleost fishes. In *Fish Physiology*, Vol. IXA. Hoar, W.S.,Randall, D.J. and Donaldson, E.M. (Eds). Academic Press, Inc. America: 373 - 404.

Ng, T.B.; Woo, N.Y.A.; Tam, P.P.L.; and Au, C.Y.W. (1984) - Changes in metabolism and hepatic ultrastructure induced by estradiol and testosterone in immature female *Epinepholus akaara* (Teleostei, Serranidae). *Cell Tissue Res*. **236:** 651 - 659.

Nichols, A.V.; Krauss, R.M. and Musliner, T.A. (1986) - Nondenaturing polyacrylamide gradient gel electrophoresis. In *Methods in enzymology*, Vol. 128. Academic Press, Inc. America: 417 - 431.

Pacoli, C.Q.; Grizzle, J.M. and Bradley, J.T. (1990) - Seasonal levels of serum vitellogenin and oocyte growth in the channel catfish *Ictalurus punctatus*. *Aquaculture*. **90:** 353 - 367.

Pan, M.L.; Bell, W.J. and Telfer, W.H. (1969) - Vitellogenic blood protein synthesis by insect fat body. *Science*. **165:** 393 - 394.

Retamales, R.G. and Gonzàlez, M. (1985) - Incidence of El Niño 1982 - 1983 phenomenon on the spawning of the Chilean pilchard, (*Sardinops sagax*). *Invest. Pesq.* (Chile) **32:** 161 - 165.

Selman, K. and Wallace, R.A. (1983) - Oogenesis in *Fundulus heteroclitus*. II.
Vitellogenesis. *J. Exp. Zool.* **226:** 441 - 457.

So, Y.P.; Idler, D.R. and Hwang, S.J. (1985) - Plasma vitellogenesis in Landlocked Atlantic salmon (*Salmo salar* Ouananiche): Isolation, homologous radioimmunoassay and immunological cross-reactivity with vitellogenin from other teleosts. *Comp. Biochem. Physiol.* **81B:** 63 - 71.

van Bohemen, CH.D.; Lambert, J.G.D. and Peute, J. (1981) - Annual changes in plasma and liver in relation to vitellogenesis in the female rainbow trout, *Salmo gairdneri*. *Gen. Comp. Endoc.* **44:** 94 - 107.

van Bohemen, C.G. and Lambert, J.G.D. (1981) - Estrogen synthesis in relation to estrone, estradiol and vitellogenin plasma levels during the reproductive cycle of the female rainbow trout, *Salmo gairdneri*. *Gen. Comp. Endoc.* **45:** 105 - 114.

CHAPTER FOUR

ENDOCRINE FLUCTUATIONS AND THE EFFECTS

ON THE REPRODUCTIVE PROCESSES IN

PILCHARD, *Sardinops sagax*

CHAPTER 4: ENDOCRINE FLUCTUATIONS AND THE EFFECTS ON THE REPRODUCTIVE PROCESSES IN PILCHARD, *Sardinops sagax*

ABSTRACT

Levels of oestradiol-17-ß (E_2) in the plasma of female pilchard, *Sardinops sagax* were determined by radioimmunoassay for different ovarian maturation stages during a monthly and 48 h sampling period. The E_2 levels were lowest in January and in immature and totally spent females. At the onset of vitellogenesis E_2 levels increased to approximately 95 % higher than those of immature, previtellogenic and totally spent pilchard. Similarly, the E_2 levels of partially spent females were 95 % higher than those of totally spent females. Spawning occurred in February - March when peak E_2 levels were observed. The E_2 levels measured during the 48 h sampling period showed a diurnal cycle, with peaks occuring after sunrise and once during midday, at 14h02. A weak correlation between E_2, *HSI* and *GSI* was observed throughout the sampling period.

4.1 INTRODUCTION

The seasonal reproductive period of animals is characterized by the process of vitellogenesis (Pan *et al*, 1969). During the vitellogenic period, numerous integrated activities at the level of the pituitary gland, the liver and the developing gonads occur. A series of events, orchestrated by the brain, causes the release of numerous hormones from the afore-mentioned

organs. Environmental cues such as photoperiod, temperature and feeding regulate the production of gonadotropic hormone (GtH) in the pituitary gland (Liley and Stacey, 1983). Under favourable environmental conditions the immediate release of gonadotropic hormone is triggered, which in turn stimulates the release of the steroid hormone, oestradiol-17-ß (E_2), from the follicle membrane of the developing ovary (Mommsen and Walsh, 1988). A complex interaction exists between the released hormones and the organs, since E_2 eventually stimulates the production of the lipophosphoprotein, vitellogenin (from the liver), destined for yolk deposition by the developing ovaries (van Bohemen et al, 1981).

The role of E_2 in the reproductive biology of fish has been investigated by means of radioimmunoassay in catfish, *Heteropneustes fossilis* (Sundaraj et al, 1982; Lamba et al, 1982), goldfish, *Carassius auratus* (Stacey et al, 1983), rainbow trout, *Salmo gairdneri* (van Bohemen et al, 1982) and plaice, *Pleurocnectes platessa* (Wingfield and Grimm, 1977). It was found that E_2 levels in rainbow trout, *S. gairdneri*, vary considerably during the change over from one vitellogenic phase to another, reaching maximal levels during exogenous vitellogenesis (van Bohemen and Lambert 1981). Fluctuations in E_2 levels have also been recorded for Amago salmon, *Oncorynchus rhodurus* (Kanamori et al, 1988) and white sucker, *Catostomus commersoni* (Scott et al, 1984) during exogenous vitellogenesis.

The role of E_2 in ovarian development has been studied previously in serial (multiple) spawners. Zohar et al (1988) found that E_2 levels fluctuated during the day in gilthead seabream, *Sparus aurata*, while Kadmon et al (1985), reported variable levels in different ovarian maturation stages of the same species.

No information is available on the E_2 levels during the vitellogenic period for the serial spawning pilchard, *S. sagax*. A key question in the study was to determine plasma E_2 levels during the reproductive cycle of *S. sagax*. Oestradiol-17-ß levels were measured at monthly and during a 48 h sampling period. Previous studies on rainbow trout, *Salmo gairdneri*, showed the relationship between E_2 levels at different ovarian maturation stages and ovary size (*GSI*) (Lambert *et al*, 1978) and these two parameters were thus compared in reproductively active pilchard. Furthermore, the relationship between E_2 and hepatosomatic index - *HSI* (Chapter 3), body mass, condition factor - *CF* and somatic condition factor - *SCF* (Chapter 2) was investigated, since they reflect the utilization of liver resources during ovarian recrudescence (Delahuntey and de Vlaming, 1980).

4.2   MATERIALS AND METHODS

4.2.1   Animals and sampling procedures

Sexually mature and immature pilchard, *S. sagax* were collected at monthly intervals from September 1993 to March 1994 during the SARP cruises. Samples of mature and immature female pilchard were also collected during a 48 h period in September 1994 during the RaFoS cruises. Complications in sampling strategies precluded the capture of pilchard during November and December 1994.

Pilchard were collected by means of an Engels midwater trawl net and transferred to aerated 100 *l* holding tanks with a continuous sea water flow system aboard the research vessel. Live pilchard were sexed and macroscopically staged according to the classification of Davies (1956). Approximately 1.5 ml blood was collected from the caudal vein of individually tagged fish for further physiological and biochemical analyses. Blood samples were stored

on ice for no longer than 1 h and centrifuged on site in a SIGMA 112 centrifuge at 3000 xg for 10 min. The supernatent was aliqouted and stored at -20 °C until further laboratory analysis. Thereafter, all fish were sacrificed by severing the spinal cord. The frozen plasma of all pilchard *S. sagax*, was analyzed for the presence of the steroid hormone, oestradiol-17-ß (E_2).

4.2.2 Procedures for the determination of oestradiol-17-ß (E_2)

4.2.2.1 Determination of plasma E_2

Plasma aliquots, viz: 100 μl and 50 μl, were diluted with 100 μl and 450 μl of 0 nmol/L oestradiol calibrator A, respectively. All dilutions (viz. 1:2 and 1:10) were made in duplicate and assayed on the same day. ^{125}I-labelled oestradiol and anti-oestradiol antiserum were obtained from the Double Antibody Estradiol radioimmunoassay (RIA) kit (Diagnostic Products Corporation - DPC). The RIA procedure was carried out according to kit specifications (DPC). After incubation, separation of bound from free ^{125}I-labelled oestradiol was achieved by the PEG-accelerated double-antibody method.

4.2.2.2 Internal standards

Three duplicate E_2 controls were assayed with each analysis, viz. control 1 (low), control 2 (medium) and control 3 (high), kindly donated by the Department of Chemical Pathology, Groote Schuur Hospital, Cape Town. In addition, duplicates of human plasma controls, of known E_2 concentration were assayed with each analysis in order to determine assay variation. The intraassay (within one assay) and interassay (between assays) variation, was

determined by replicate measurements of the human plasma control in a single and different assay/s, respectively. The variation was determined for samples collected monthly and during the 48 h sampling period.

Replicates of pilchard plasma samples assayed previously, were repeated on a different day in order to determine any variation which may have occurred as a result of differing environmental conditions within the laboratory. The coefficient of variation ($CV\%$) was determined as follows:

$$CV\% = \frac{\text{Standard Deviation}}{\text{Mean}} \times 100$$

4.2.2.3 *Quantitation of plasma E$_2$*

After precipitation of the antibody-bound complex, the samples were counted for 2 min on the Packard Crystal II Multidetector counting system. Standard curves were obtained by plotting the percentage binding against concentration of E_2 (pg/mL). The percentage binding for each unknown was derived from the standard curve.

4.2.3 Statistical analysis

Tests for significance were performed by Kruskal - Wallis Nonparametric Analysis of Variance (ANOVA), followed by Dunns' Multiple Comparisons test, using the INSTAT computer programme. Correlations were performed by Regression Analysis using the STATISTICA computer programme (Statsoft™, Inc.).

4.3   RESULTS

4.3.1   Precision of the radioimmunoassay

The inter-assay variation ranged between 3.2% (n = 11; 48 h sampling period) and 3.7% (n = 23; monthly period); while the intra-assay variation ranged between 5.62% (n = 4; monthly period) and 5.7% (n = 10; 48 h sampling period). The difference between the two variations were infintesimal and were therefore the assays were regarded as being accurate.

Pilchard plasma samples assayed, were repeated on a different day and yielded a coefficient of variation ($CV\%$) ranging between 0.67% and 4.5% (Table 4.1). The relatively small inter- and intra-assay variations observed in the E_2 levels in the study, indicate the reliability of the results obtained. Furthermore, the minor variation in the E_2 levels observed when identical plasma samples were measured on a different day is indicative of the repeatability of the assay binding affinity to E_2.

4.3.2   Changes in plasma oestradiol-17-ß (E_2) levels

Plasma E_2 levels were relatively low in immature and previtellogenic females (Fig. 4.1). With the onset of the vitellogenic process, plasma E_2 levels increased sharply to 395.97 $\pm$ 27.58 pg/mL in vitellogenic females, declining thereafter to 348.58 $\pm$ 195.75 pg/mL in partially spent females, until baseline levels were reached in totally spent females. The E_2 level in partially spent females was 94% higher than that of totally spent females ($p \leq 0.05$). Reproductively active pilchard (i.e. vitellogenic stage) had E_2 levels 89% and 95% higher than fish with ovaries in the immature and totally spent condition ($p \leq 0.001$),   and previtellogenic condition ($p \leq 0.01$), respectively.

Table 4.1: Variation between plasma samples of *S. sagax* assayed (assay 1) and repeated on a different day (assay 2).

ASSAY 1 Mean ± SD (pg/mL)	ASSAY 2 Mean ± SD (pg/mL)	VARIATION CV%
83.79 ± 5.37	82.83 ± 6.93	0.81
68.24 ± 1.99	64.45 ± 7.49	4.04
132.61 ± 5.37	124.43 ± 5.40	4.50
211.44 ± 0.77	200.60 ± 8.99	3.72
467.89 ± 6.56	444.03 ± 20.59	3.70
397.88 ± 1.51	401.69 ± 29.14	0.67

CV% = coefficient of variation
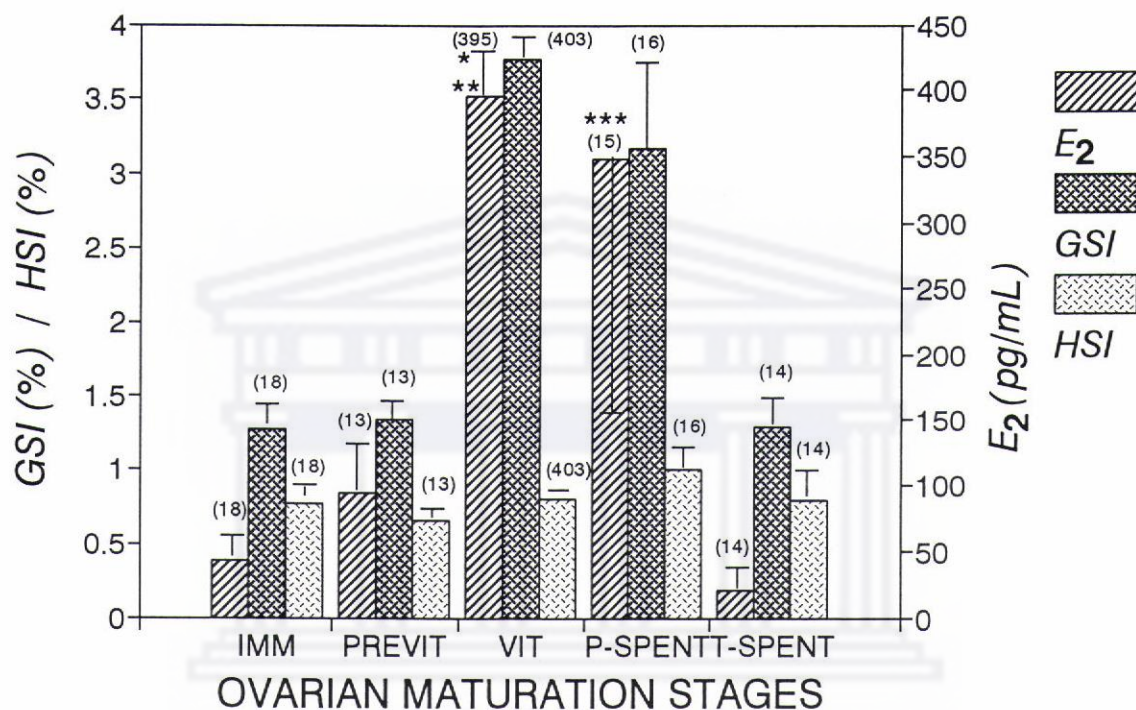
Figure 4.1: Changes in the plasma oestradiol-17-ß (E$_2$) levels, gonadosomatic index (*GSI*) and hepatosomatic index (*HSI*) in different ovarian maturation stages of *S. sagax*. Vertical bars represent Mean $\pm$ SEM; values in brackets = n; p values for E$_2$: * = p $\leq$ 0.001 (Vit vs Imm, T-Spent); ** = p $\leq$ 0.05 (Vit vs Previt); *** = p $\leq$ 0.05 (P-Spent vs T-Spent); p values for *GSI* and *HSI* are presented in Figures 2.2 and 3.2, respectively.

Monthly changes in plasma E_2 levels are represented in Fig. 4.2. Females sampled at the onset of the sampling period in September, had high levels of E_2, decreasing steadily thereafter, until baseline levels were reached in January (54.91 $\pm$ 14.07 pg/mL). No data was available for November and December. Increased E_2 levels were observed in February and March. Basal levels of E_2 recorded in January were approximately 80% lower than the E_2 levels recorded in September and March (p $\leq$ 0.05) and February (p $\leq$ 0.001).

The plasma E_2 levels measured at various time points over a 48h period fluctuated considerably, with peaks occurring after sunrise at 07h56, 08h26, 07h46, with the exception of the high E_2 level at 14h02, (Fig. 4.3).

4.3.3 Correlations between plasma E_2 levels and gonadosomatic index (*GSI*)

The *GSI* of pilchard followed a similar pattern to that of plasma E_2 levels in different ovarian maturation stages (Fig. 4.1) and was directly correlated in females in the totally spent condition (Table 4.2). Significant correlations (p $\leq$ 0.001) were observed in vitellogenic and totally spent females.

Monthly *GSI* values were generally out of phase with plasma E_2 levels, the most notable difference being in January (Fig. 4.2). Significant correlations (p $\leq$ 0.05) were observed in September, February and March in relation to the other months (Table 4.3).
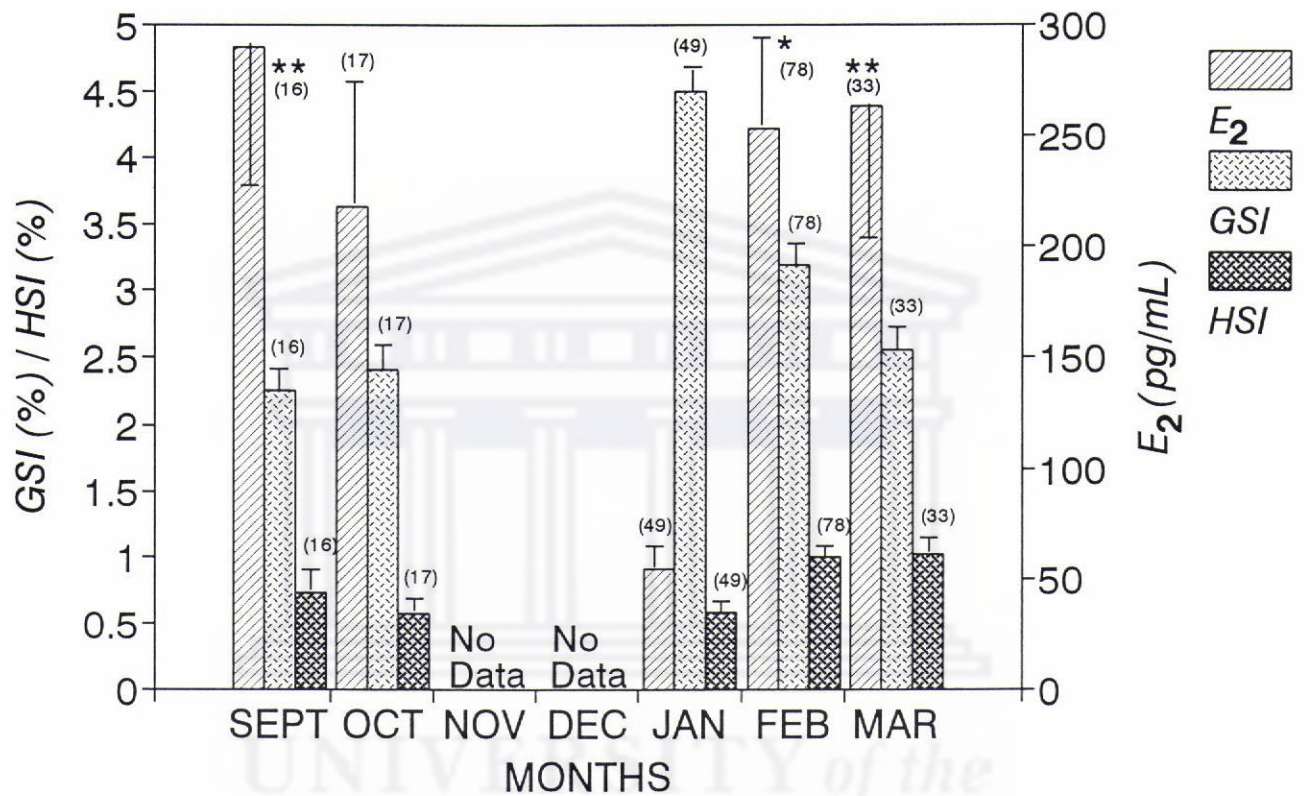
Figure 4.2: Monthly changes in plasma oestradiol-17-ß (E₂) levels, gonadosomatic index (*GSI*) and hepatosomatic index (*HSI*) of *S. sagax*. Vertical bars represent Mean ± SEM; values in brackets = n; p values for E₂: * = p ≤ 0.001 (Feb vs Jan); ** = p ≤ 0.05 (Sep, Mar vs Jan); p values for *GSI* and *HSI* are presented in Figures 2.2 and 3.3, respectively.
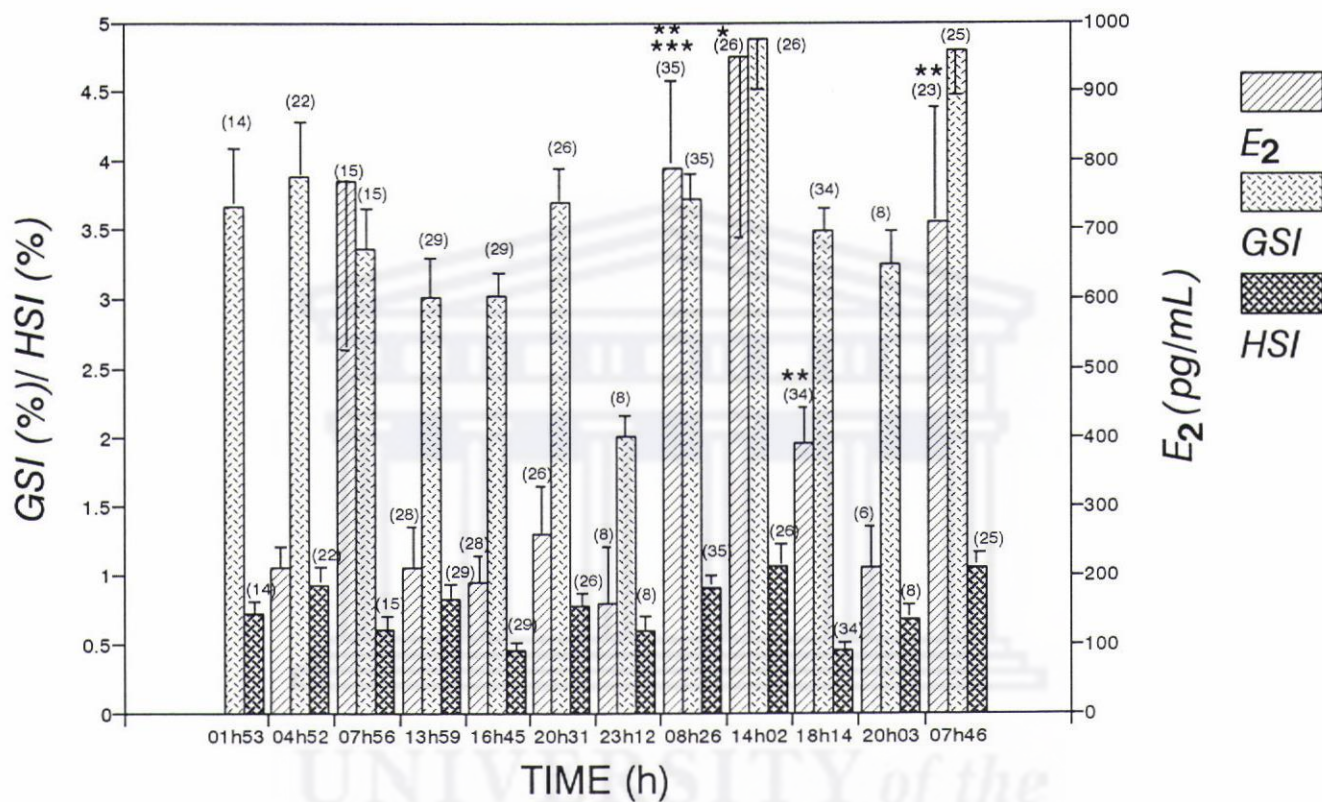
Figure 4.3: Changes in plasma oestradiol-17-ß (E₂), gonadosomatic index (*GSI*) and hepatosomatic index (*HSI*) of *S. sagax* over a 48 h period during September 1994. Vertical bars represent Mean ± SEM; values in brackets = n; p values for E₂: * = p ≤ 0.01 (14h02 vs 04h42, 13h59, 16h45, 20h31, 23h12); ** = p ≤ 0.05 (08h26, 07h46, 18h14 vs 04h42, 13h59); *** = p ≤ 0.05 (08h26 vs 16h45, 23h12); p values for *GSI* and *HSI* are presented in Figures 2.4 and 3.4, respectively.

Table 4.2: Correlations between plasma oestradiol-17-ß (E₂) and *GSI*ᵃ, *HSI*ᵇ, *SCF*ᶜ, *CF*ᵈ of *S. sagax* in different ovarian maturation stages.

	Immature n=18	Previtellogenic n=13	Vitellogenic n=380	Partially-Spent n=15	Totally-Spent n=13
GSI r	0.36	0.24	0.20	0.19	0.87
p	ns	ns	sc	ns	sc
HSI r	-0.17	-0.39	0.23	0.41	0.05
p	ns	ns	sc	ns	ns
SCF r	0.24	0.16	-0.08	-0.32	-0.24
p	ns	ns	ns	ns	ns
CF r	-0.29	0.11	-0.09	-0.22	-0.35
p	ns	ns	ns	ns	ns

a = gonadosomatic index

b = hepatosomatic index

c = somatic condition factor

d = condition factor

r = regression coefficient

ns = not significant

sc = significant correlation (p ≤ 0.05)

Daily *GSI* values generally followed those of E_2 levels (Fig. 4.3). Correlations were generally weak and not significant ($p > 0.05$), except for fish obtained at 07h56 and 16h45 ($p \leq 0.05$) (Table 4.4).

A weak, but significant correlation ($p \leq 0.001$) was observed between E_2 and *GSI* for pilchard collected throughout the sampling period (Fig. 4.4.a).

4.3.4 Correlations between plasma oestradiol-17-ß levels and hepatosomatic index (*HSI*)

The *HSI* value of pilchard in the different ovarian maturation stages was generally constant with the exception of the partially spent stage which was between 25% to 40% higher than that of the other stages (Fig. 4.1). The correlation between E_2 and *HSI* values per ovarian maturation stage was generally weak, except for the significant correlation ($p \leq 0.001$) in vitellogenic females (Table 4.2). Similarly, correlations between plasma E_2 levels and *HSI* values of pilchard collected monthly, were weak and not significant ($p > 0.05$), except for those obtained in February ($p \leq 0.05$) (Table 4.3).

HSI values during the 48 h sampling period generally followed those of E_2 levels (Fig. 4.3). Correlations between E_2 and *HSI* were weak and not significant ($p > 0.05$) (Table 4.4).

A weak, but significant correlation ($p \leq 0.001$) was observed between plasma E_2 and *HSI* of pilchard collected throughout the sampling period (Fig. 4.4.b).

Table 4.3: Monthly correlations between plasma oestradiol-17-ß and *GSI*[a], *HSI*[b], *SCF*[c], *CF*[d] of *S. sagax* during the spawning season.

	September n=16	October n=17	January n=49	February n=78	March n=33
GSI r	0.57	0.30	0.19	0.50	0.34
p	sc	ns	ns	sc	sc
HSI r	0.22	0.18	-0.03	0.39	0.21
p	ns	ns	ns	sc	ns
SCF r	0.47	0.00	-0.05	0.14	0.04
p	ns	ns	ns	ns	ns
CF r	0.35	0.08	0.01	0.17	0.05
p	ns	ns	ns	sc	ns

a = gonadosomatic index

b = hepatosomatic index

c = somatic condition factor

d = condition factor

r = regression coefficient

p = significance level

ns = not significant

sc = significant correlation (p ≤ 0.05)

Table 4.4: **Correlations between plasma oestradiol-17-ß and *GSI*[a], *HSI*[b], *SCF*[c] and *CF*[d] of *S. sagax* during a 48 h period in September 1994.**

	GSI %		HSI%		SCF		CF	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
01h53 n=14	No E$_2$ data		No E$_2$ data		No E$_2$ data		No E$_2$ data	
04h52 n=22	0.18	ns	-0.07	ns	0.48	sc	0.52	sc
07h56 n=15	0.65	sc	0.16	ns	0.30	ns	0.12	ns
13h59 n=29	0.23	ns	0.31	ns	0.30	ns	0.29	ns
16h45 n=26	0.39	sc	-0.29	ns	0.14	ns	-0.28	ns
20h31 n=26	0.23	ns	0.08	ns	-0.37	ns	-0.34	ns
23h12 n=8	-0.17	ns	-0.52	ns	-0.48	ns	-0.48	ns
08h26 n=35	0.15	ns	-0.06	ns	-0.06	ns	-0.03	ns
14h02 n=26	0.22	ns	0.29	ns	-0.12	ns	-0.15	ns
18h14 n=33	0.19	ns	0.01	ns	0.21	ns	0.26	ns
20h03 n=6	0.01	ns	0.71	ns	-0.15	ns	-0.13	ns
07h46 n= 23	-0.08	ns	0.39	ns	0.05	ns	0.04	ns

a = gonadosomatic index
b = hepatosomatic index
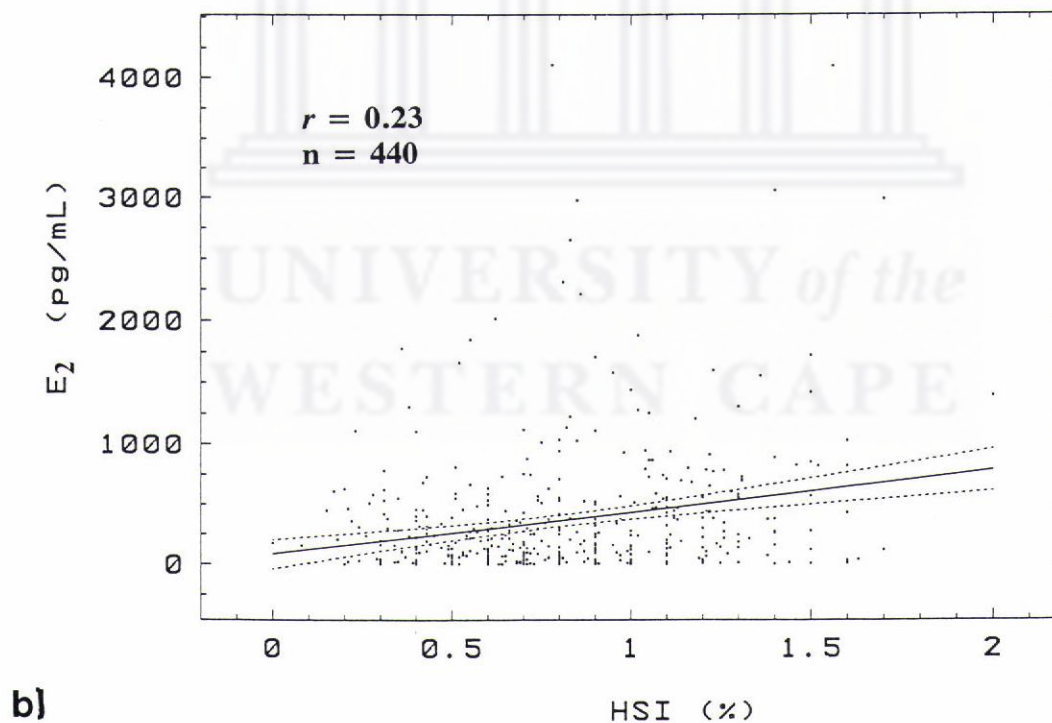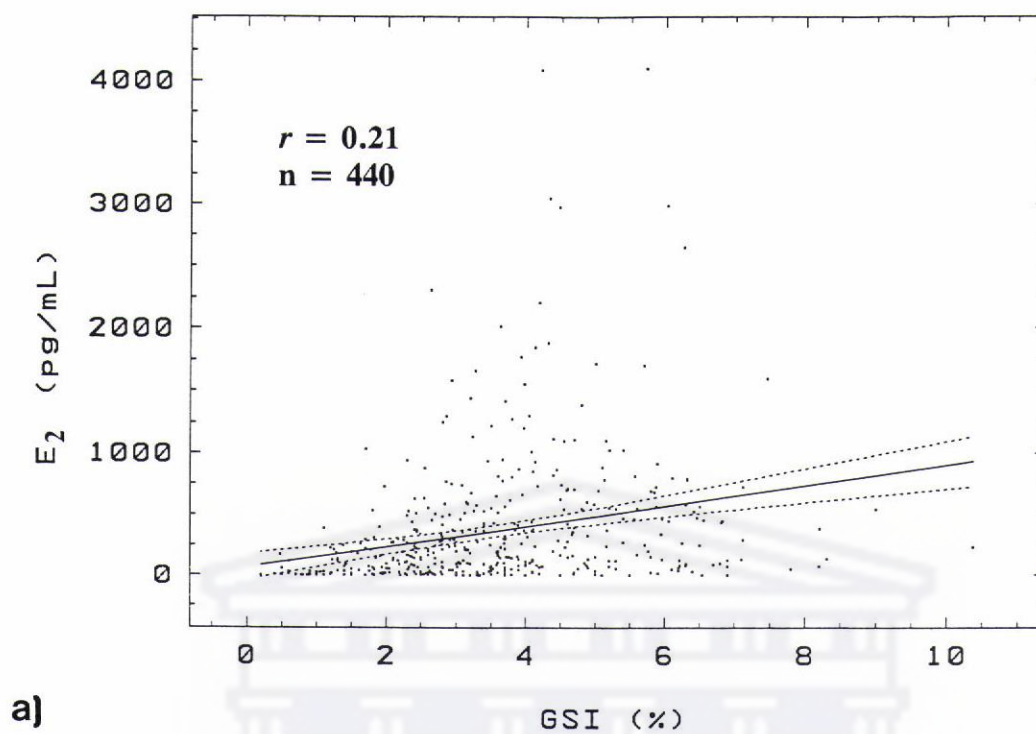*c* = somatic condition factor
d = condition factor
r = regression coefficient
ns = not significant
sc = significant correlation (p $\leq$ 0.05)

Figures 4.4: The general correlation between plasma oestradiol-17-ß levels (E_2) and (a) the gonadosomatic index (*GSI*) and (b) the hepatosomatic index (*HSI*) of *S. sagax* was weak, but significant ($p \leq 0.001$) throughout the sampling period.

4.3.5 Correlations between plasma E_2 levels and somatic condition factor (*SCF*) and condition factor (*CF*)

Correlations between plasma E_2 levels and *SCF* and *CF* were generally weak and not significant ($p > 0.05$) in the different ovarian maturation stages and the monthly sampling period, Table 4.2 and 4.3, respectively. Significant correlations in the *SCF* and *CF* were observed at 04h52 during the daily sampling period (Table 4.4). Similarly, a weak but non significant ($p > 0.05$) correlation was observed between plasma E_2 and *SCF* and *CF* of all pilchard collected throughout the sampling period, Fig. 4.5.a and b, respectively.

4.4 DISCUSSION

4.4.1 Plasma oestradiol-17-ß levels (E_2) in different ovarian maturation stages

The pattern followed by plasma E_2 levels of pilchard in different ovarian maturation stages corresponded well with that found in other non-synchronous spawners, except for the generally weak correlation with the gonadosomatic index. In *S. sagax*, plasma E_2 levels were low prior to and after the vitellogenic stage, reaching maximal levels during the vitellogenic stage. Kadmon *et al* (1985) found that the plasma E_2 levels in the non-synchronous spawning Giltheaded seabream *Sparus aurata* were low and constant during the previtellogenic period until maximum levels were reached at the onset and during the vitellogenic period, whilst declining in fish containing mature ova. Similarly, the E_2 levels observed in synchronous spawners such as rainbow trout *S. gairdneri* (Fostier *et al*, 1978; Lambert *et al*, 1978; Zohar *et al*, 1986), brown trout *Salmo trutta* (Crim and Idler, 1978), sweep *Scorpis lineolatus*

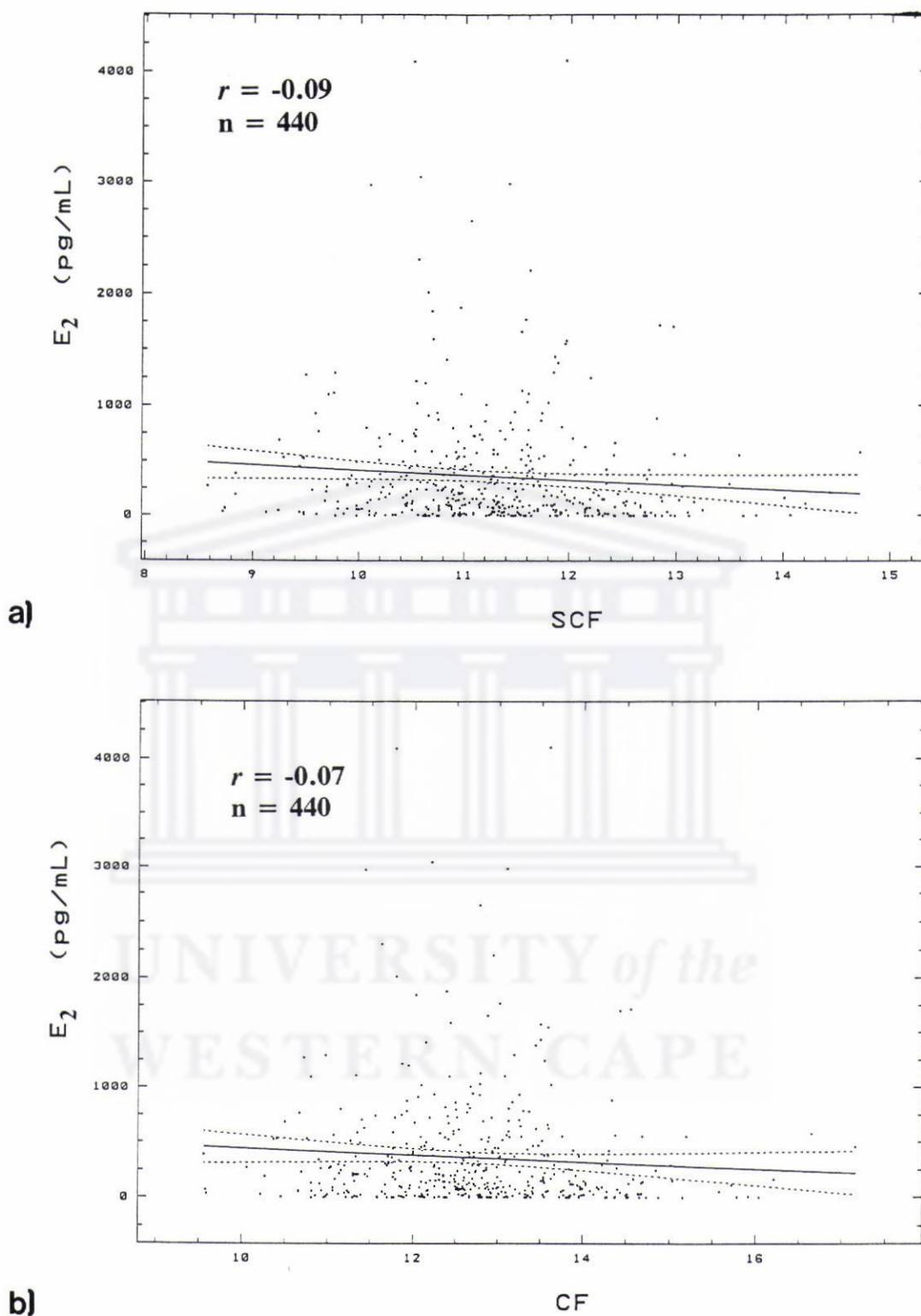**Figure 4.5:** The general correlation between plasma oestradiol-17-ß levels (E_2) and (a) the somatic condition factor (*SCF*) and (b) the condition factor (*CF*) of *S. sagax* was weak and not significant ($p > 0.05$) throughout the sampling period.

(Dedual and Pankhurst, 1992), catfish *H. fossilis* B. (Lamba *et al*, 1983) and white sucker *C. commersoni* (Stacey *et al*, 1984) were highest in vitellogenic females and declined immediately in mature and spent fish. In vitellogenic pilchard, therefore, plasma E_2 levels are highest when maximum ovarian maturation takes place prior to spawning.

The relatively high plasma E_2 levels observed in partially spent females in the present study may be related to the serial mode of spawning in pilchard since the active ovary contains oocytes in different degrees of maturation (i.e. the ovary contains secondary yolk oocytes, undergoing maturation and ovulation, as well as primary oocytes, undergoing vitellogenesis). Kagawa *et al* (1983) found that after ovulation, the vitellogenic follicles (i.e. primary oocytes) in the non-synchronous spawning goldfish *Carassius auratus*, produced high plasma E_2 levels that are required for continued ovarian maturation during the spawning season. One could therefore speculate that the primary oocytes release high levels of E_2 into the bloodstream of vitellogenic and partially spent pilchard, so as to ensure continued vitellogenesis during the spawning season. The differing degrees of maturing oocytes present in individual ovaries probably contributed to the high variation observed in the plasma E_2 levels of partially spent females.

4.4.2 Monthly fluctuations in plasma oestradiol-17-ß (E_2) levels

Seasonal variations in the plasma E_2 levels generally remained minimal throughout the sampling period of reproductively active *S. sagax*, except in January, when very low levels, relative to other months were observed. In the present study, the E_2 levels in September, February and March were approximately 80% higher than those recorded in January, indicating that pilchard were preparing for final maturation and spawning, since E_2 levels

were high in pilchard obtained in the vitellogenic and spawning condition. In the staghorn sculpin *Leptocottus armatus*, high E_2 levels were observed in the month when yolky oocytes appeared in the ovary in preparation for spawning (de Vlaming *et al*, 1984). That maximum spawning took place during in February is evident from the decline in the *GSI* and the increase in the proportion of totally spent ovaries. The high E_2 levels in February could further indicate that the primary and secondary oocytes, found in the ovary were actively secreting E_2 so as to maintain continued ovarian growth during vitellogenesis. Similarly, in the goldfish *C. auratus*, it was observed that the remaining vitellogenic follicles (i.e. primary and secondary oocytes) released high plasma E_2 levels in fish that had ovulated (Kagawa *et al*, 1983). In the present study, however, evidence of spawning was not observed in pilchard caught in September and thus it cannot be concluded that spawning occurred during this month.

The relatively low plasma E_2 levels observed in January could indicate that the vitellogenic process had reached its maximum and that depletion of the steriod hormone from the pilchard's bloodstream had occurred. The high *GSI* observed in pilchard in the same month suggests that ovarian maturation had proceeded beyond the point of exogenous vitellogenesis and that the developing ovaries were independent of high E_2 levels whilst preparing for ovulation and spawning. Similarly in plaice *P. platessa*, final ovarian maturation was independent of high E_2 levels prior to spawning (Wingfield and Grimm, 1977).

4.4.3 Fluctuations in plasma oestradiol-17-ß (E_2) levels during a 48 h period

A diurnal cycle in the plasma E_2 levels during the 48 h sampling period was observed in the present study, with high E_2 levels obtained after sunrise and once at midday. The high E_2 level in pilchard obtained after sunrise was followed by a consistently low E_2 level during the latter part of the day, except for the very high plasma E_2 level in pilchard caught at 14h02. It was discerned from this study that the oocytes in the vitellogenic maturation stage produced high E_2 levels so as to maintain gonadal maturation. It is therefore apparent that the pilchard in the vitellogenic ovarian maturation condition produced high E_2 levels after sunrise and once at midday, hence the high E_2 levels observed. The fact that high E_2 levels were observed mostly after sunrise suggests that the ovaries were actively producing E_2 at this time to maintain vitellogenesis and hence gonadal maturation in preparation for the next spawning event, irrespective of the low E_2 levels maintained during the latter part of the day. According to Sundaraj and Nath (1981), it is likely that the initial exposure to high plasma E_2 levels primes the liver so that high vitellogenic rates can be maintained, irrespective of the low E_2 levels which follow. Similarly, the daily peaks in the plasma E_2 levels observed in giltheaded seabream *S. aurata*, seemed sufficient to maintain hepatic production of the yolk precursor protein, vitellogenin (Kadmon *et al*, 1985; Zohar *et al*, 1988).

In the present study it was established that pilchard in the partially spent ovarian maturation condition had relatively high E_2 levels which may be related to the serial mode of spawning of the species. The active ovary in this condition in non-synchronous spawning fish contains primary and secondary oocytes (vitellogenic oocytes) which is known to produce high plasma E_2 levels so as to maintain continued ovarian maturation during the spawning season (Kagawa et al, 1983). The plasma E_2 level was high only once in the afternoon and could be

attributed to the production of high E_2 levels from the primary and secondary oocytes found in the partially spent ovaries obtained at that sampling time. The production of E_2 from the developing ovaries (i.e. primary and secondary oocytes) therefore ensures that gonadal maturation continues. The conspicuously high E_2 level observed only once during midday, could therefore be attributed to the presence of partially spent fish containing primary and secondary oocytes.

The plasma E_2 levels in giltheaded seabream, *S. aurata*, increased 6 hours prior to spawning (Kadmon *et al*, 1985; Zohar *et al*, 1988), while in rainbow trout, *S. gairdneri* maximum E_2 levels were reached two weeks prior to spawning (Lambert *et al*, 1978). The spawning frequency and exact time of spawning in *S. sagax* was not determined and thus it can only be inferred that the E_2 levels were high during gonadal maturation. Similarly, it can only be inferred that the high E_2 levels after sunrise were sufficient to maintain gonadal development during vitellogenesis.

4.4.4 Correlations between plasma oestradiol-17-ß (E_2) levels and gonadosomatic index (*GSI*).

Plasma E_2 levels and *GSI* values were generally correlated in the different ovarian maturation stages as well as the 48 h sampling period of *S. sagax*. The trend observed between E_2 and *GSI* in the monthly study was different, however, in that the two parameters were out of phase for most months, except September and February. Correlations between plasma E_2 and *GSI* were generally weak. The significant correlation ($p \leq 0.05$) observed in fish obtained in the vitellogenic and spent condition, during the monthly study in September and February and the daily study at 07h56 and 16h45 indicated that the primary oocytes were actively producing E_2.

The good correlation between E_2 and *GSI* in *S. sagax* in the totally spent stage suggests that the remaining follicles (i.e. primary oocytes) were actively producing E_2. The weak, but significant correlation ($p \leq 0.05$) in the vitellogenic stage could be an indication of continued E_2 synthesis by the primary oocytes undergoing vitellogenesis. The weak correlation between *GSI* and E_2 levels in *S. sagax* is not unique, since in plaice *P. platessa*, a lack of correspondence was observed between the two parameters during the spawning season (Wingfield and Grimm, 1977). The good correlation between E_2 and *GSI* observed in February, however, suggest that the primary and secondary oocytes in the ovary were actively producing E_2 in the month when peak spawning took place. de Vlaming *et al* (1984) observed good correlations between the two parameters in the month when spawning occurred during the reproductive season of staghorn sculpin, *L. armatus*.

The general relationship between individual *GSI* and plasma E_2 levels in *S. sagax* (i.e. all data collected) was extremely weak. Similarly, a weak correlation between the two parameters was observed during the 48 h sampling period, except that the general correlation was significant ($p \leq 0.001$). The weak correlations observed in the present study are similar to that observed in the staghorn sculpin, *L. armatus*, where the correlation coefficient between ovarian size and plasma E_2 levels was relatively low (de Vlaming *et al*, 1984). In the staghorn sculpin, however, the correlation between *GSI* and *HSI* was not significant. In the present study, the significant correlation between the *GSI* and E_2 could be attributed to the large sample size used and was not investigated further.

It is generally inferred that a larger ovary secretes more E_2 than a smaller one during the phase of ovarian growth (Yaron *et al*, 1977). This is true for most teleosts and has been widely reported in goldfish *C. auratus* (Kagawa *et al*, 1983), catfish *H. fossilis* (Lamba *et*

al, 1983), brown trout *S. trutta* (Crim and Idler, 1978), rainbow trout *S. gairdneri* (Lambert *et al*, 1978) and tilapia *Tilapia aurea* (Yaron *et al*, 1977) where good correlations between E_2 and *GSI* provided indirect evidence for the importance of the steroid hormone during the spawning season. The above-mentioned pattern was not observed in *S. sagax* during this study.

4.4.5 Correlations between plasma oestradiol-17-ß (E_2) levels and hepatosomatic index (*HSI*)

The correlation between the plasma E_2 and *HSI* was generally weak and non significant ($p > 0.05$) throughout the monthly and 48 h sampling period as well as in different ovarian maturation stages, except for the significance ($p \leq 0.05$) observed in females in the vitellogenic stage and those obtained in February. The weak, but significant ($p \leq 0.001$) correlation between the E_2 and *HSI* in *S. sagax* collected throughout the year does not conform to the general hypothesis that a good relationship exists between the two parameters and can probably be ascribed to the serial mode of spawning of this species. In rainbow trout, *S. gairdneri*, the *HSI* increased drastically after introperitoneal administration of E_2 (van Bohemen *et al*, 1981). Furthermore, a good correlation between E_2 and *HSI* was observed during the vitellogenic period of plaice, *P. platessa* (Wingfield and Grimm, 1977) and group-synchronous spawning English sole, *P. vetulus* (Johnson *et al*, 1991).

4.5 CONCLUSION

In the present study, the high E_2 levels recorded in vitellogenic and partially spent females, in February - March, after sunrise and at 14h02 in the 48 h study supported the evidence that the pilchard *S. sagax* underwent gonadal maturation during these times (Chapter 2 and 3). It is recommended, however, that E_2 not be used as an indicator of spawning in this species, since a high variance in the E_2 levels in the different ovarian maturation stages was observed. The high E_2 variance observed could be attributed to the serial mode of spawning of *S. sagax*, since oocytes in different degrees of maturation were prevelant in the ovaries.

The weak correlation between E_2, *HSI* and *GSI* in the present study provides further evidence that E_2 levels cannot be used to predict the maturation condition of the ovary. The seasonal pattern observed in the E_2 levels of *S. sagax* is different from that found in many teleosts. It is possible that rapid vitellogenin production, under the influence of E_2, takes place in the hepatocytes of pilchard, as is as the case in the rainbow trout *S. gairdneri* (van Bohemen *et al*, 1982). The turnover rate from plasma E_2 to vitellogenin production in *S. sagax* was not investigated.

4.6 REFERENCES

Crim, L.W. and Idler, D.R. (1978) - Plasma gonadotropin, estradiol, and vitellogenin and gonad phosvitin levels in relation the seasonal reproductive cycles of female brown trout. *Ann. Biol. anim. Bioch. Biophys.* **18:** 1001 - 1005.

Davies, D.H. (1956) - The South African pilchard, *Sardinops ocellata*. Sexual maturity and reproduction, 1950 - 1954. *Investl. Rep. Div. Fish. S. Afr.* **22:** 1 - 155.

Dedual, M. and Pankhurst, N.W. (1992) - Plasma steroid hormone concentrations in relation to reproductive cycle of the Sweep *Scorpis lineolatus* (Kyphosidae) caught from the wild. *Aust. J. Mar. Freshwater Res.* **43:** 753 - 763.

Delahuntey, G. and de Vlaming, V.L. (1980). Seasonal relationships of ovary weight, liver weight and fat stores with body weight in the goldfish, *Carassius auratus* (L.). *J. Fish Biol.* **16:** 5 - 13.

de Vlaming, V., Fitzgerald, R., Delahuntey, G., Cech, J.J., Selman, K. and Barkley, M. (1984) - Dynamics of oocyte development and related changes in serum estradiol-17 -ß, yolk precursor, and lipid levels in the teleostean fish, *Leptocottus armatus*. *Comp. Biochem. Physiol.* **77A:** #4, 599 - 610.

Fostier, A.; Weil, C.; Terqui, M.; Breton, B. and Jalabert, B. (1978) - Plasma estradiol-17-ß and gonadotropin during ovulation in rainbow trout (*Salmo gairdneri* R.). *Ann. Biol. anim. Bioch. Biophys.* **18:** #4, 929 - 936.

Johnson, L.L.; Casillas, E.; Myers, M.S.; Rhodes, L.D. and Olson, O.P. (1991) - Patterns of oocyte development and related changes in plasma 17-ß estradiol, vitellogenin, and plasma chemistry in English sole *Parophrys vetulus* Girard. *J. Exp. Mar. Biol. Ecol.* **152:** 161 - 185.

Kadmon, G.; Yaron, Z. and Gordin, H. (1985) - Sequence of gonadal events and oestradiol levels in *Sparus aurata* (L.) under two photoperiod regimes. *J. Fish. Biol.* **26:** 609 - 620.

Kagawa, H.; Young, G. and Nagahama, Y. (1983) - Changes in plasma steroid hormone levels during gonadal maturation in female goldfish *Carassius auratus*. *Bull. Jap. Soc. Sci. Fish.* **49:** #12, 1783 - 1787.

Kanamori, A.; Adachi, S. and Nagahama, Y. (1988) - Developmental changes in steroidogenic responses of ovarian follicles of Amago salmon (*Oncorhynchus rhodurus*) to chum salmon gonadotropin during oogenesis. *Gen. Comp. Endoc.* **72:** 13 - 24.

Lamba, V.J.; Goswami, S.V. and Sundaraj, B.I. (1982) - Radioimmunoassay for plasma cortisol, testosterone, estradiol-17-ß, and estrone in the catfish *Heteropneustes fossilis* (Bloch): Development and Validation. *Gen. Comp. Endoc.* **47:** 170 - 181.

Lamba, V.J.; Goswami, S.V. and Sundaraj, B.I. (1983) - Circannual and circadian variations in plasma levels of steroids (cortisol, estradiol-17-ß, estrone, and testosterone) correlated with the annual gonadal cycle in the catfish, *Heteropneustes fossilis* (Bloch). *Gen. Comp. Endoc.* **50:** 205 - 225.

Lambert, J.G.D.; Bosman, G.I.C.G.M.; van den Hurk, R. and van Oordt, P.G.W.J. (1978) - Annual cycle of plasma oestradiol-17-ß in the female trout *Salmo gairdneri. Ann. Biol. anim. Bioch. Biophys.* **18:** #4, 923 - 927.

Liley, N.R. and Stacey, N.E. (1983) - Hormones, pheromones, and reproductive behaviour in fish. In *Fish Physiology*, Vol. XIB. Behaviour and Fertility Control. Hoar, W.S.; Randall, D.J. and Donaldson, E.M. (Eds). Academic Press, New York, London: 1 - 63.

Mommsen, T.P. and Walsh, P.J. (1988) - Vitellogenesis and oocyte assembly. In *Fish Physiology*, Vol. XIA. The Physiology of Developing Fish. Hoar, W.S. and Randall, D.J. (Eds). Academic Press, Inc. San Diego, New York, Berkeley: 347 - 406.

Pan, M.L.; Bell, W.J. and Telfer, W.H. (1969) - Vitellogenic blood protein synthesis by insect fat body. *Science.* **165:** 393 - 394.

Scott, A.P.; Mackenzie, D.S. and Stacey, N.E. (1984) - Endocrine changes during natural spawning in the White sucker, *Catostomus commersoni*. *Gen. Comp. Endoc.* **56:** 349 - 359.

Stacey, N.E.; Mackenzie, D.S.; Marchant, T.A.; Kyle, A.L. and Peter, R.E. (1984) - Endocrine changes during natural spawning in the white sucker, *Catostomus commersoni*. *Gen. Comp. Endoc.* **56:** 333 - 348.

Stacey, N.E.; Peter, R.E.; Cook, A.F.; Truscott, B.; Walsh, J.M. and Idler, D.R. (1983) - Changes in plasma concentrations of gonadotropin, 17-ß-estradiol, testosterone, and 17α-hydroxy-20ß-dihydroxyprogesterone during spontaneous and brain lesion induced ovulation in goldfish. *Can. J. Zool.* **61:** 2646 - 2652.

Sundaraj, B.I.; Goswami, S.V. and Lamba, V.J. (1982) - Role of testosterone, estradiol-17ß, and cortisol during vitellogenin synthesis in the catfish, *Heteropneustes fossilis* (Bloch). *Gen. Comp. Endoc.* **48:** 390 - 397.

Sundaraj, B.I. and Nath, P. (1981) - Steroid-induced synthesis of vitellogenin in the catfish, *Heteropneustes fossilis* (Bloch). *Gen. Comp. Endoc.* **43:** 201 - 210.

van Bohemen, C.G. and Lambert, J.G.D. (1981) - Estrogen synthesis in relation to estrone, estradiol and vitellogenin plasma levels during the reproductive cycle of the female rainbow trout, *Salmo gairdneri*. *Gen. Comp. Endoc.* **45:** 105 - 114.

van Bohemen, CH.D.; Lambert, J.G.D.; Goos, H.J.TH. and van Oordt, P.G.W.J. (1982) - Estrone and estradiol participation during exogenous vitellogenesis in the female rainbow trout, *Salmo gairdneri*. *Gen. Comp. Endoc.* **46:** 81 - 92.

van Bohemen, CH.D.; Lambert, J.G.D. and Peute, J. (1981) - Annual changes in plasma and liver in relation to vitellogenesis in the female rainbow trout, *Salmo gairdneri*. *Gen. Comp. Endoc.* **44:** 94 - 107.

Wingfield, J.C. and Grimm, A.S. (1977) - Seasonal changes in plasma cortisol, testosterone and oestradiol-17-ß in the plaice, *Pleuronectes platessa* L. *Gen. Comp. Endoc.* **31:** 1 - 11.

Yaron, Z.; Terakatin-Shimony, A; Shaham, Y. and Salzer, H. (1977) - Occurrence and biological activity of estradiol-17-ß in the intact and ovariectomized *Tilapia aurea* (Cichlidae, Teleostei). *Gen. Comp. Endoc.* **33:** 45 - 52.

Zohar, Y.; Breton, B. and Fostier, A. (1986) - Short-term profiles of plasma gonadotropin and estradiol-17-ß levels in the female rainbow trout, from early ovarian recrudescence and throughout vitellogenesis. *Gen. Comp. Endoc.* **64:** 172 - 188.

Zohar, Y.; Pagelson, G. and Tosky, M. (1988) - Daily changes in reproductive hormone levels in the female gilthead seabream *Sparus aurata* at the spawning period. *Reproduction in fish - Basic and applied aspects in endocrinology and genetics.* pp. 119 - 125.

GENERAL SUMMARY

The reproductive physiology of the pilchard, *Sardinops sagax* was investigated from monthly samples obtained during September - March and during a 48 h sampling period. The gonadosomatic index (*GSI*) and hepatosomatic index (*HSI*) values were determined from general body parameters, while the steroid hormone, oestradiol-17-ß (E_2) levels were determined by radioimmunoassay for different ovarian maturation stages. A histological analysis of the ovaries of pilchard was undertaken in order to verify the spawning pattern determined from the *GSI* values.

The *GSI* and *HSI* values were lowest in immature pilchard until maximal values were reached in vitellogenic females and declined in partially spent and totally spent females. Spawning took place mainly in February and declined in March. The high plasma E_2 and *HSI* values observed supported the fact that spawning activity was optimal during February - March, as evidenced by the dramatic decline in the *GSI*. During the 48 h sampling period, it was found that spawning probably took place prior to midnight, as evidenced by the presence of new post-ovulatory follicles (Day 0) and the *GSI*, *HSI* and plasma E_2 levels observed. The distinct diurnal cycle in the E_2 levels during the 48 h sampling period suggested that the E_2 levels were sufficient to maintain vitellogenesis throughout the latter part of the day.

In this study, the use of the *GSI* value proved to be a useful indicator of spawning activity, when used in association with histological assessment of the gonads and that the liver is activated during the vitellogenic period, as evidenced by the higher *HSI* value in vitellogenic females. Plasma E_2 levels within specific ovarian maturation stages, however, showed a high variance and it is therefore recommended that the latter parameter not be used to determine spawning activity in *S. sagax*.

A further recommendation eminating from this study is that physiological studies on serial spawners be confined to a simulated environment or that sampling be undertaken over a longer time period so as to incorporate the entire spawning season of the species without spatial and temporal differences influencing results.