SPATIAL AND TEMPORAL VARIABILITY IN LIPID CONTENT AND GONADOSOMATIC INDEX OF SARDINE (*Sardinops sagax*) IN THE SOUTHERN BENGUELA ECOSYSTEM

Johannes de Goede

Spatial and temporal variability in lipid content and gonadosomatic index of sardine (*Sardinops sagax*) in the southern Benguela ecosystem

Johannes de Goede

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External Supervisor : Dr. Carl van der Lingen

Internal Supervisor : Mr. Conrad Sparks

Declaration

I declare that this thesis is my own work. It is being submitted for the degree of Master of Technologiae (Oceanography) to the Cape Technikon, Cape Town. It has not been submitted for any other degree or examination at any other University, Technikon or tertiary institution. The opinions and the conclusions are not necessarily those of the Cape Technikon.

J. de Goede

08/04/02

Date

". . . the most important indicators for the environmental variability you're looking at the animals themselves. Therefore, the scientist who studies fat content [of fish] is looking at one of the best biological health and status indicators available."
Sharp, 1987

There is something fascinating about science. One gets such a wholesale return of conjecture out of a trifling investment of fact.
Mark Twain, 1874

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List of Appendices

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Abstract

Determining lipid content for sardine (*Sardinops sagax*) in the Southern Benguela Ecosystem (SBE), through applying a method of fat staging, is a relatively easy method to use as a biological time-series to assess the condition of sardine. The condition of fish is an important indicator for fluctuations in the population size of post-recruit fish.

Depending on the amount of fat associated with the stomach, one of seven fat stages was assigned to each sardine. Visually assigned fat stages were then converted to a lipid content value, given as a percentage of wet body mass, by applying a conversion equation to these fat stages.

Time-series of lipid content and gonadosomatic index (GSI) were generated using general linear models (GLM). Results from these GLM's explained 34% and 39% of the observed variance in lipid content and GSI respectively. Monthly and annual least square means (LS mean) were derived from these GLM's to show seasonal variability in lipid content and GSI, for the period 1996 to 2003. Clear seasonal patterns in both lipid content and GSI were observed. Lipid content showed a decrease, but an interannual trend in GSI could not be observed, for the time series.

The study area, which ranged from the Orange River mouth (28°40′S and 16°30′E) on the west coast to Port Elizabeth (25°37′E and 33°57′S) on the east coast was divided into four smaller zones to allow for spatial tests. Zone 1 being on the west coast and Zone 4 on the east coast. Both lipid content and GSI showed strong spatial differences, with lipid content being at highest levels in Zone 4 throughout the time series, and the highest GSI levels being observed in Zone 1.

Lipid content and GSI were tested for relationships against adult spawner biomass and relative recruitment biomass. Both lipid content and GSI versus adult biomass were found to be significantly inversely related, for the time series. 49% of the variance in lipid content was explained by adult biomass in the linear regression trend line, at a significance level of 0.05 and 55% of the variance in GSI was explained by adult biomass in the linear regression trend line, at a significance level of 0.04. No relationships could be derived between both lipid content and GSI versus relative recruitment.

Fish parameters lipid content and GSI were tested for relationships between environmental parameters sea surface temperature (SST) and chlorophyll a (Chl a), and were found to be lag related: Lipid content lag SST – 2 months , lipid content lag Chl a – 3 months, GSI lag SST – 3 months and GSI lag Chl a – 2 months. Lipid content and GSI were inversely related.

A significant negative relationship was observed between lipid content versus SST.

Remarkable spatial differences were observed on all the scatter plots between the four zones for lipid content and GSI versus SST and Chl a. Significant negative relationships were observed in Zones 3 and 4, between lipid content and SST. A significant positive relationship was observed in Zone 3, between lipid content and Chl a.

This newly applied method of lipid content determination, through fat staging was tested against another method. The chosen method is described in Kreiner *et al.* (2001), where condition factor (CF) is determined through length-weight relationships. A GLM for CF explained 16% of the observed variance in CF. The lipid content method was more sensitive to changes in the seasonal patterns. Monthly LS mean values for both lipid

content and CF were normalized to the mean of monthly LS means. Differences in both peak and low values in the seasonal patterns were observed. The CF cycle showed low values for CF during July and lipid content showed low values during August. High CF values were reached in December and high lipid content values were reached during May. Zone specific scatter plots showed that CF versus lipid content was highly significant ($p < 0.001$), but CF explained a relatively low percentage of the variance in the predicted lipid content, i.e. 14%, 11%, 14% and 33% for Zones 1, 2, 3, and 4, respectively. Slope angles of these regression lines increased from Zones 2 to 4, which indicated that some effects incorporated by lipid c ntent were not incorporated by CF.

Some of the main advantages of this study are that it is accurate, cheap, quick and easy. The main implication of this study is that the condition and reproductive state of sardine temporally and spatially can be monitored on a continuous and real time basis. Striking spatial differences are shown for both lipid content and GSI, which will give the industry, which prefers good quality sardines, the advantage of knowing where and when to fish for better quality sardines. The reproductive state of sardines in the four zones is also known, which will allow fishermen to avoid areas where fish are spawning.

Abbreviations and terms

Chl a.....	Chlorophyll a (mg/m^3)
CF.....	*Condition factor* – condition of sardine determined by method of Kreiner *et al.* (2001). p2-13.
Fat St.....	Fat Stage according fig. 2.3a, p2-5.
FWT.....	Total wet fish weight to the nearest 0.1g
GSI.....	Gonadosomatic index (% WBM)
GWT.....	Gonad weight to the nearest 0.1g
LC.....	Caudal length - length from tip of snout to end of vertebra – fig. 2.2, p2-4.
Lipid content...	Given as a percentage of the wet body mass (% WBM). p2-6.
MCM.....	Department of Environmental Affairs and Tourism - Marine and Coastal Management
Recruit.....	Naught year old fish, available to the industry for the first time.
SBE.....	Southern Benguela Ecosystem: For this study - the area between the Orange river on the west coast of south Africa to Port Elizabeth on the East Coast (Fig. 2.1, p2-3).
SST.....	Sea Surface Temperature (°C)
Upwelling.....	Physical process where nutrients are welled up from the ocean bed.
% WBM.....	Percentage wet body mass

Zone 1..... The area between the Orange river on the west coast and St. Helena Bay on the west coast, i.e. north of the 32°45′ latitude (Fig. 2.1, p2-3).

Zone 2..... The area between St. Helena Bay and Cape Point on the west coast, i.e. between the 32°45′ latitude and 18°30′ longitude (Fig. 2.1, p2-3).

Zone 3..... The area between Cape Point and Cape Infanta on the south coast, i.e. between the 18°30′ longitude and 21°00′ longitude (Fig. 2.1, p2-3).

Zone 4..... The area between Cape Infanta and Port Elizabeth on the southwest and east coasts, i.e. east of the 21°00′ longitude (Fig. 2.1, p2-3).

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Chapter 1

Introduction

1.1 Clupeoids' global importance

Clupeoids are among the most important groups of fish exploited by the world's fisheries, in terms of biomass and value (Blaxter and Hunter 1982). In the 1980's, an average of 18.2 million tons of clupeoids were caught annually, which represented about 20% of the world's marine fish catch (Armstrong and Thomas 1989) and according to Anon. (1998), clupeoids made up 23% of the world's catch in 1998. The five main regions where both anchovies and sardines are harvested are the western (Japan system) and eastern (California Current) boundary areas of the North Pacific, the eastern boundary (Humboldt Current) of the South Pacific, and both the northern (Canary Current) and southern (Benguela system) boundaries of the eastern Atlantic (Lluch-Belda *et al.* 1989). Clupeoids dominate catches of these ecosystems, which are highly productive upwelling systems (Beckley and van der Lingen 1999).

1.2 Variability in clupeoid stocks

Populations of clupeoids are known to have undergone large-scale fluctuations (Schwartzlose *et al.* 1999), which are thought to be the result of inter-decadal variability in climate (Kawasaki and Omori 1995, Crawford 1991, Lluch-Belda *et al.* 1989). Prior to the World War II intensive fishing on sardine off Japan was thought to be one of the reasons for the collapse of the stock (Lluch-Belda *et al.* 1989). When the fishing activities increased after the war, catches remained low until the mid 1970's. There is general agreement that unfavourable environment conditions, rather than excessive exploitation

was the main cause of the decline (Lluch-Belda *et al.* 1989). Biological interactions like predation on ichthyoplankton by the same species are also known to play an important role in population variability of small pelagic fish (Crawford 1991). Cannibalism of eggs by adult anchovy has been estimated to account for more than 70 percent of the total egg mortality and is related to the density of the parents (Valdés-Szeinfeld 1995). Environmental influences during El Niño events tend to affect recruitment and therefore the size of the population. Changes in sea surface temperature are suggested to lead to habitat change in sardines, which is associated with changes in abundance (Lluch-Belda *et al.* 1989 and Lluch-Belda *et al.* 1992). The best example of such a stock fluctuation is probably that of the Peruvian anchoveta *Engraulis ringens* which increased from an almost negligible fishery in the late 1950's to one of 8-12 million tons in 1966-72 (Schwartzlose *et al.* 1999). Catches dropped to less than 2 million tons by 1973 and reached its smallest catch of 25 084 tons in 1984. The catches rose again to 12 million tons in 1994 and in 1995 and 1996 anchovy catch was 8 million tons (Lluch-Belda *et al.* 1989).

1.2.1 Processes responsible for fluctuations in clupeoids

Global change in climate alternates environmental conditions which favour one species over the other, e.g. anchovy over sardine and *vice versa.* (Schwartzlose *et al.* 1999, van der Lingen *et al.* submitted).

Off Tasmania periods of low zonal westerly winds cause a form of Tasmanian *El niño*, which causes sea surface temperatures to rise. Nutrients then become scarce in the surface waters, which leads to a decrease in clupeoid productivity. In Namibian waters in

the South-Eastern Atlantic, warm, more saline water moves from the north onto the Namibian shelf, because of Benguela *Niños*. This water may intrude about 600km farther south than normal (Schwartzlose *et al.* 1999).

Food and temperature are two mechanisms that may sustain large shifts in the abundance of anchovy and sardine populations. van der Lingen (1999) reported that sardine utilizes more phytoplankton than anchovy, although both species are primarily carnivorous. Any change in the size structure of zooplankton, may therefore initiate regime changes.

Unfavourable temperatures may result in mortality of eggs, which could lead to larvae and recruitment failure (Schwartzlose *et al.* 1999).

1.3 The South African fishery (Southern Benguela)

The commercial exploitation of pelagic fish off the Western Cape, South Africa started in 1943 during World War II (Davies 1954) in the St. Helena Bay area (Du Plessis 1959; Stander 1967). The current South African fleet operates from north of Lamberts Bay (18°19′E and 32°06′S) on the west coast to Port Elizabeth (25°37′E and 33°57′S) on the east coast. The annual South African pelagic fishing season starts mid January of each year and continues until the TAC is landed or until late December. Although catches and landings are made throughout the year and in different areas, fewer landings are usually made from mid December to mid January.

The most important pelagic species harvested in the South African pelagic fisheries include anchovy (*Engraulis encrasicolus*), sardine (*Sardinops sagax*) and red eye (*Etrumeus whiteheadi*). Juvenile horse mackerel (*Trachurus trachurus capensis*),

mackerel (*Scomber japonicus*), lantern (*Lampanyctodes hectoris*) and light fish (*Maurolicus muelleri*) also form a small part of the bycatch when anchovy, sardine and red eye are targeted.

These species are generally caught at night with large purse-seine nets which are set around a school of fish. Once the school is surrounded, the bottom of the net is closed by a footrope. The net is then pulled alongside the vessel and the fish are pumped directly into the hold of the vessel (Beckley and van der Lingen 1999). There are approximately 100 small (10-50m) wood- or steel-hulled purse-seines responsible for catching the South African pelagic fish, 80 of which are geared to catch and cool adult sardine for human consumption (van der Lingen 2004). The hold capacity of the vessels range from 30-500 tons (van der Lingen 2004). Adult sardine caught by the commercial fleet are usually cooled rapidly when pumped from the ocean into the hold of the vessel, once the fish has been pursed. The vessel hold contains a small amount of either refrigerated sea water (sea water cooled at sea) or chilled sea water (sludge ice taken in at the factory, prior to the trip). This cooling process keeps sardine fresher for longer periods, which also makes these sardine excellent sampling material. Anchovy, which have been the focus species of condition determination since the fat staging method was introduced, are not cooled down for commercial purposes. Anchovy decomposes too rapidly from the time of the catch until it is offloaded at the factory. Commercially caught anchovy were therefore not used in this study.

The various uses of the harvested fish include *inter alia* canned sardine and fish paste for human consumption and fishmeal, for animal feed or fertilizer. Sardine are a sought after bait species for rock and surf anglers, handline and longline fisheries. The value of the pelagic fishery in South Africa was worth more than one billion rand in 2003 (van der Lingen 2004).

The catches of clupeoids in the Southern Benguela have, like clupeoid catches in other upwelling systems in the world, fluctuated dramatically over the last 50 years (Fig. 1.1). Sardine was the primary target species of the pelagic industry in the early 1960's. Sardine catch levels were less than 100 000t in 1956 and peaked, after exceptional recruitment, to more than 410 000t in 1963. From the high catch level in 1963 sardine stock collapsed to 16 000t in 1974, due to poorly controlled increase in effort and catches, expansion to fishing grounds in the south and variable recruitment (Newman *et al.* 1979; Crawford 1981*a*; Armstrong and Thomas 1989). Anchovy catches increased from 41 000 - 596 000t between 1964 and 2003 (Marine and Coastal Management unpublished data).

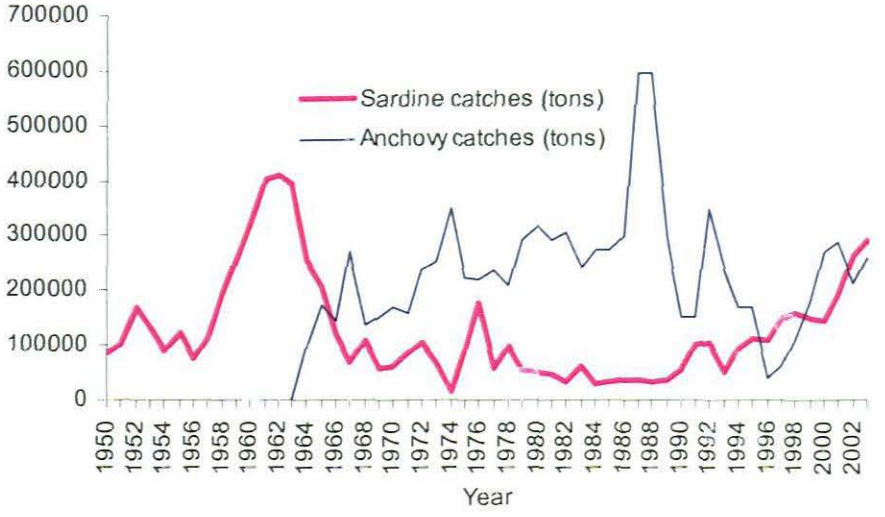


Fig. 1.1 – South African catches of anchovy *Engraulis encrasicolus* and sardine *Sardinops sagax* from 1950 to 2003 (Marine and Coastal Management data).

1.4 Assessment and management of clupeoids in the Southern Benguela

Armstrong *et al.* (1989) described that, as a result of high catch rates of sardine in the 1950's, the need for resource management was recognized. By the late 1940's the pelagic industry expanded to such an extent that scientists became concerned about the fish stocks and restrictions in the form of minimum mesh sizes, restrictions on industrial expansion and closed seasons were introduced.

Management of the pelagic industry is based on an Operational Management Procedure (OMP), which uses population assessment models which are build, using survey (from research vessels) and catch data (from commercial landings) (de Oliveira 2003). The OMP requires data on stock size for both recruits (juveniles) and spawners (adults), for anchovy and sardine. The research division of Marine and Coastal Management (section Offshore Resources) estimates adult biomass during November/December and recruit (juvenile sardine) biomass during May/June using the *R.S. Africana*, through hydro-acoustic echo integration. Echo-sounders transmit sound waves from the vessel, which are then reflected from the fish back to the ship. The strength of the returned acoustic energy is proportional to fish biomass (Johanneson and Mitson 1983).

Further requirements for the OMP include data on age, mass-at-age; natural mortality and fishing mortality which are collected or derived from the surveys, as well as from commercial catch. The survey is conducted from the Orange River mouth (28°40′S and 16°30′E) on the west coast to Port St. Johns (31°62′S and 29°54′E) on the east coast (see fig. 2.1), although commercial catches are not made much further north of Port Elizabeth (25°37′E and 33°57′S).

Total allowable catches (TAC's) are set separately for anchovy and sardine.

1.5 Biology of sardine in the Southern Benguela

1.5.1 Distribution

In the Northern and Southern Benguela Ecosystem, sardine are distributed from southern Angola to KwaZulu-Natal on the north-east coast of South Africa. Despite wide ranging migrations there appear to be two separate sardine stocks. The Northern and Southern Benguela stocks are separated by the Lüderitz upwelling cell (27°S) which forms a natural barrier to extensive alongshore migrations of fish (Schülein *et al.* 1995).

The distribution of sardine in South African waters showed age-specific patterns from 1964 to 1976, according to Crawford (1979, 1980, 1981*b*) and Crawford *et al.* (1980). Most of the spawning during 1964 to 1976 took place on the Agulhas Bank between Cape Point and St. Sebastian Bay (near Cape Infanta – between the 18°30′E longitude and 21°00′E longitude) and some spawning was also recorded off St. Helena Bay (18°01′E and 32°44′S). Most of the spawning took place during spring and summer.

The main nursery area off South Africa for sardine are between the Orange River and St. Helena Bay, on the west coast (Armstrong and Thomas 1989). These young fish (0-1 year old) occur on the nursery grounds from May onwards, reaching peak abundance in September and when autumn starts, sardine will migrate southwards to the Agulhas Bank (Beckley and van der Lingen 1999). The schools pass around Cape Point, into False Bay and Walker Bay. At this stage sardines are approximately 10cm in total length. Armstrong *et al.* (1987) investigated sardine distribution between 1983 and 1985 and also concluded that size related distribution was evident, similar to the findings of Crawford (1979, 1980, 1981*b*) and Crawford *et al.* (1980) from 1964 to 1976. Armstrong *et al.*

(1987) found intermediate-sized sardine of 14-20cm total length (2-4 year old) to dominate the south-east-coast.

1.5.2 Sardine life cycle

Sardine spawning may take place for the first time, from the age of two years (Armstrong and Thomas 1989). Prior to sexual maturity sardine are known as recruits. The main spawning period of sardine in South African waters are between September and February. Sardine are serial spawners and a spawning occurs at weekly intervals (Le Clus 1989a, 1989b; Beckley and van der Lingen 1999).

Le Clus (1989a, 1989b) found that the reproductive potential for the entire spawning season of the larger sardines was considerably more than of the smaller ones. Not only do the bigger fish spawn more often, but the bigger sardine also spawn over a longer period during the spawning season than the smaller sardine.

A large proportion of the sardine population spawn on the Agulhas Bank, but spawning also extends to the west coast and east coast (Roel *et al.* 1994; van der Lingen *et al.* 2001; Hutchings *et al.* 2002). Spawning on the west coast is likely to be detrimental to recruitment when temperatures are not conducive for optimal development of eggs and larvae.

King (1977) performed extensive laboratory experiments on artificially fertilized sardine eggs and found that incubation time is strongly influenced by water temperature, with mean incubation time ~89h in water at 11°C, 40-44h at 16°C and 23h at 22°C.

Patches of warm water from the Agulhas Bank which are shed through short-circuiting the Agulhas Return Current near the Cape Point form eddies which then flows north with

the Benguela jet current (Huggett and Boyd 1998). Eggs and larvae are transported from the Agulhas Bank to the west coast nursery area via these eddies (Fowler and Boyd 1998).

The larva, which is 3mm long at birth, first uses the yolk reserves from the yolk sac which is still connected to the gut. Once the yolk reserves are exhausted, it starts to eat small planktonic organisms (van der Lingen 1999).

Relaxation of upwelling allows for offshore water to flow shoreward bringing the larvae, which have limited swimming capabilities into shallow inshore water. These areas along the west coast are known as larval retention areas (Armstrong and Thomas 1989). Larvae of about 3-3.5cm (about two months old), undergo metamorphosis (flexion), which signals the juvenile stage. The juvenile stage ends when gonads start to develop and the adult phase starts prior to the first spawning on the spawning grounds (Beckley and van der Lingen 1999).

1.5.3 Feeding

Adult sardine are both filter and particulate feeders. Adult sardine on the west coast of South Africa consume more phytoplankton, whereas adult sardine on the south coast of South Africa consume more zooplankton (van der Lingen, 1996; 1999). King and Macleod (1976) suggested that juvenile sardine were feeding predominantly on calanoid copepods and at approximately 10cm standard length they switch to phytoplanktivory.

The accumulated energy (from food intake) in juvenile sardine are used for growth. In adult sardine lipids, which reach a peak prior to the spawning season are mainly used for gonad development (Davies 1956; Patterson 1992; Adams *et al.*, 1995; van der Lingen

1999). The abundance and quality of food in the adult sardine life cycle is therefore critically important to the condition or fatness of the fish, especially prior to the spawning season. Sardines with high lipid content values are considered to be in better condition than sardines with low lipid content values.

1.6 The condition of fish

The condition of fish is believed to be a good indicator of the "well-being or fitness" of the population under consideration (Bolger and Connolly 1988; Patterson 1992). The condition of fish is also an indicator of the food intake and of biological and physiological conditions of the fish (Matthews 1964). Condition is a rough measure of the state of fish, whether healthy or unhealthy, starved or well-fed, spawning or spent (Patterson 1992).

A number of methods to estimate condition in fish have been recorded, where the equation:

$$K = 100w/l^3$$

is the most frequently used (Davies 1956; Adams *et al.* 1995; Matthews 1964; Patterson 1992; Kreiner *et al.* 2001). In the above equation, K is known as the condition factor, w represents the fish weight and l is the represents the fish length.

Some other indicators of fish condition include: Oil-to-meal ratios (Schülein *et al.* 1995), lipid energy stored in the liver of fish (Melo 1992; Marshall *et al.* 1999), body water

content (Elliot 1976), visceral-somatic index (Delahunty and de Vlaming 1980; Adams and McLean 1985), gut index (Jensen 1980), protein-energy ratio (Bowen 1979), RNA/DNA ratios of liver and muscle (Bulow *et al.* 1981), calorific values of fish tissues, including protein and lipid fractions (Booth and Keast 1986; Hails 1983); and partial condition factors (Iles 1984).

1.6.1 Variability in condition

The condition of clupeoids is known to vary seasonally and annually (Matthews 1964). A direct relationship exists between the condition of fish and the gonad weight and gonad maturity. Gonad quality or maturity will be higher in better conditioned fish (Matthews 1964; Millán 1999). Energy levels (stored as lipids) build up to a peak prior to the annual spawning season and decline to a minimum after spawning (Hickling 1945; Lasker 1970). Gonad development is strongly affected by food intake prior to the spawning season (Morimoto 1996).

Hunter and Leong (1981) suggest that energy reserves are important for keeping the fish spawning during the prolonged spawning season. The quality of eggs appears to be dependent to some degree on parental condition. High-quality females will produce eggs which will hatch into bigger larvae. These bigger larvae have a higher survival probability (Morimoto 1996; Laine and Rajasilta 1999).

According to Morimoto (1996) a positive linear relationship exists between hydrated eggs and ovary weight, for the Japanese sardine *Sardinops melanostictus*. He noted a positive correlation between the lipid content in the ovary and in the muscle. Lipid content in the ovary also correlated to the ovarian weight. His findings suggested that

female condition would definitely influence the egg quality and quantity. He reported further that accumulation of lipid prior to the spawning season would be of great importance to produce higher quality eggs. Le Clus (1989a, and b) documented that the number of batches of eggs spawned over the spawning season, as well as the duration of the spawning season will depend in part on energy reserves stored prior to the spawning season.

1.6.2 A new technique to examine condition in sardine.

A new technique to examine fish condition by visual assessment of mesenteric fat has been developed, initially to collect information on anchovy condition during the spawning season, as a possible predictor variable for recruitment strength (van der Lingen, 1995; van der Lingen *et al.* 1998). Depending on the amount of mesenteric fat present in anchovy, one of five fat stages is assigned to individual fish. A similar technique using seven stages has been developed for sardine in the southern Benguela, which is the focus of this study. van der Lingen and Hutchings (in press) documented accurate predictions of body lipid content from fat stage of sardine, through general linear modeling. Their model used fat stage, fish weight and the interaction between fish weight and fat stage. The model accounted for 89% of the variability in body lipid content. This technique has the advantages of being quick, cheap, no specialized equipment is needed and it can be done at sea.

The precision of this novel technique has been documented by van der Lingen and Hutchings (in press). The average percent error (APE) was calculated, for a series of fat stage determinations, according to the method of Beamish and Fournier (1981). A

number of readers determined the fat stage of the same fish. The average error of each reader was then calculated as a percentage from the average fat stage of that fish. The precision estimates of sardine ranged between 9.4 – 22.8%, with a mean value of 16.6%. Low APE indicates better precision. The slight decrease over successive fat stage assessments suggests that by increased experience, improved reproducibility was evident. The APE values indicated that the fat staging method has a moderate to high level of precision.

1.7 Objectives of this study

A close relationship exists between gonad development and the condition of fish. Numerous factors like sea temperature, current, climate, food availability, food quality etc. play a very important role in the overall success of a stock (Schwartzlose *et al.* 1999). Determining the condition of fish usually requires time-consuming laboratory analysis (Bolger and Connolly 1989). The overall objective of this study is to apply the new technique of visually assessing mesenteric fat, to determine the condition of commercially caught sardine in the southern Benguela, as described by van der Lingen and Hutchings (in press). This new technique will be used to monitor spatio-temporal variability in sardine lipid content (condition), and to compare this variability with spatio-temporal variability in gonadosomatic index (GSI).

The objectives of this study are:

- To assess spatial and temporal variability in sardine lipid content and GSI for the period January 1996 to December 2003.

- To assess the relationship between lipid content and GSI.
- To examine the relationship between lipid content and GSI to biomass and recruitment.
- To assess possible relationships between sardine biological parameters (lipid content and GSI) and the environment.
- To assess how the fat stage method compares with another method of assessing fish condition.

1.8 Study motivation

1.8.1 Scientific

The results of this study will make a significant contribution towards further understanding of the ecology in the SBE. Real time monitoring of the condition and GSI of the short lived sardine throughout the fishing season means that information on stock health and spawning behaviour is readily available to scientists and the industry. This condition and GSI information, together with other environmental parameters like SST and Chl a, are useful indicators in understanding recruitment and longer term biomass fluctuations and general behaviour of the species in the dynamic pelagic environment. Initial studies done on anchovy (for recruitment related studies), based on the fat stage method (van der Lingen 1995) were done on survey data only, which concentrated on spatial results.

Fish in good condition during the spawning season may have a longer spawning season than those in poor condition (Le Clus 1989a). Monitoring of condition and GSI status gives an improved understanding of the biological behaviour in the species.

1.8.2 Industry

The pelagic fishing industry produces a preferred higher quality product from sardines which are in good condition. Spatial monitoring of condition throughout the year indicates where fish of higher quality are more likely to be found.

Chapter 2

2.1 Materials and methods

2.1.1. Sampling sites

Samples were collected from the commercial purse seines during the offloading process, at the various harbours and landing points. Collected samples were analyzed at laboratories, traditionally referred to as field stations, which are situated in St. Helena Bay (18°01′E and 32°44′S) and Saldanha Bay (17°54′E and 33°03′S) on the west coast, Hout Bay (18°22′E and 34°03′S) on the southwest coast, Gans Bay (19°21′E and 34°35′S) and Mossel Bay (22°08′E and 34°11′S) on the south and southeast coast and Port Elizabeth (25°37′E and 33°57′S) on the east coast (fig. 2.1).

Canneries are located in Laaiplek (1 factory), St. Helena Bay (3 factories) and Saldanha Bay (1 factory) on the west coast and Gans Bay (1 factory) on the south coast. More factories, which processes good quality sardine for bait and human consumption, but do not can sardine, are situated in the same areas as mentioned above, as well as in Hout Bay, Hermanus, Mossel Bay, Cape St. Francis and Port Elizabeth. Samples were collected at any of the above mentioned canneries, factories or at the vessels while offloading at the landing points.

2.1.2. Data collected

A bucket of sardine (±20kg) was taken from a vessel, cannery or factory and a sub-sample of twenty five sardines were used for biological analysis. The following data were recorded:

- Date of catch
- The catch area (study area grouped into four zones) (Fig. 2.1):

- Zone 1 – Orange River to St. Helena Bay i.e. north of the 32°45′S latitude.
- Zone 2 –St. Helena Bay to Cape Point i.e. between the 32°45′S latitude and 18°30′E longitude.
- Zone 3 - Cape Point to Cape Infanta i.e. between the 18°30′E longitude and 21°00′E longitude.
- Zone 4 - Cape Infanta to Port Elizabeth i.e. east of the 21°00′E longitude.

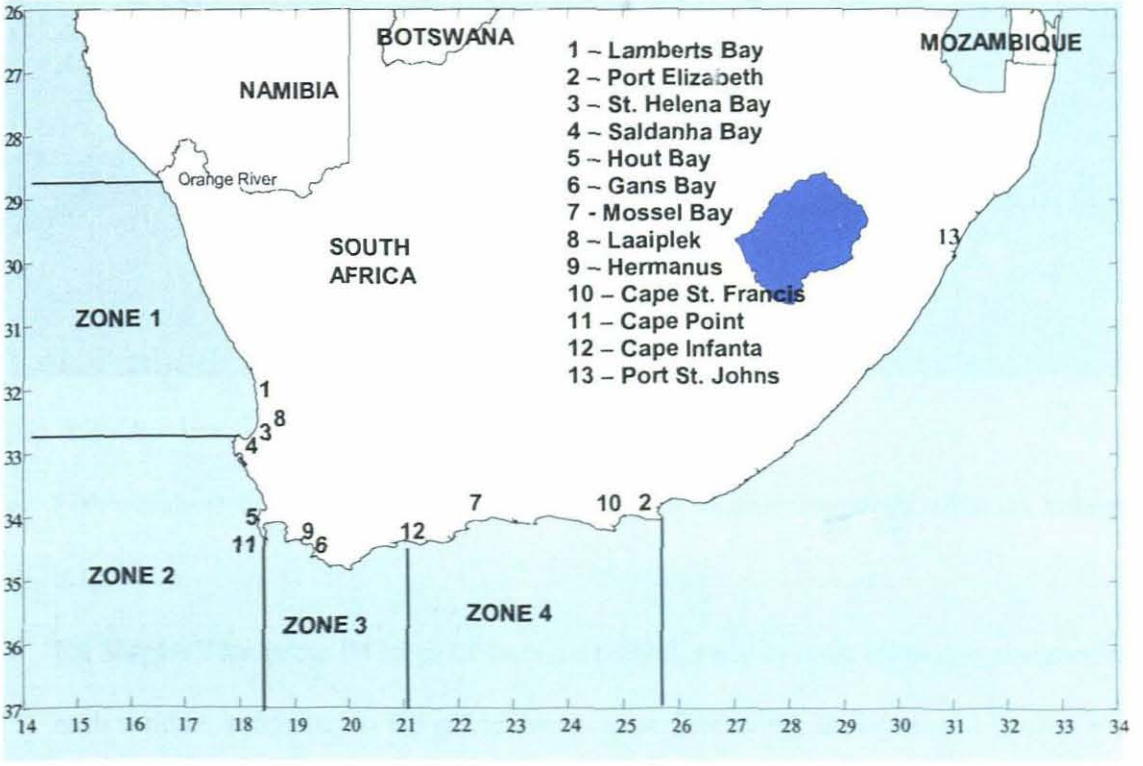


Fig. 2.1 - Map showing the four zones, as well as the localities of the field stations, factories and places mentioned in the text.

- When a skipper made more than one haul per trip, which were in different zones, the data were excluded.

The following data were recorded from each fish:

- The caudal length (CL). This measurement, as shown in fig. 2.2, was taken from the tip of the snout to end of the caudal vertebra for each fish. In order to exclude juvenile fish, sardine of less than 13.0cm CL were excluded from this study (Kreiner *et al.* 2001).

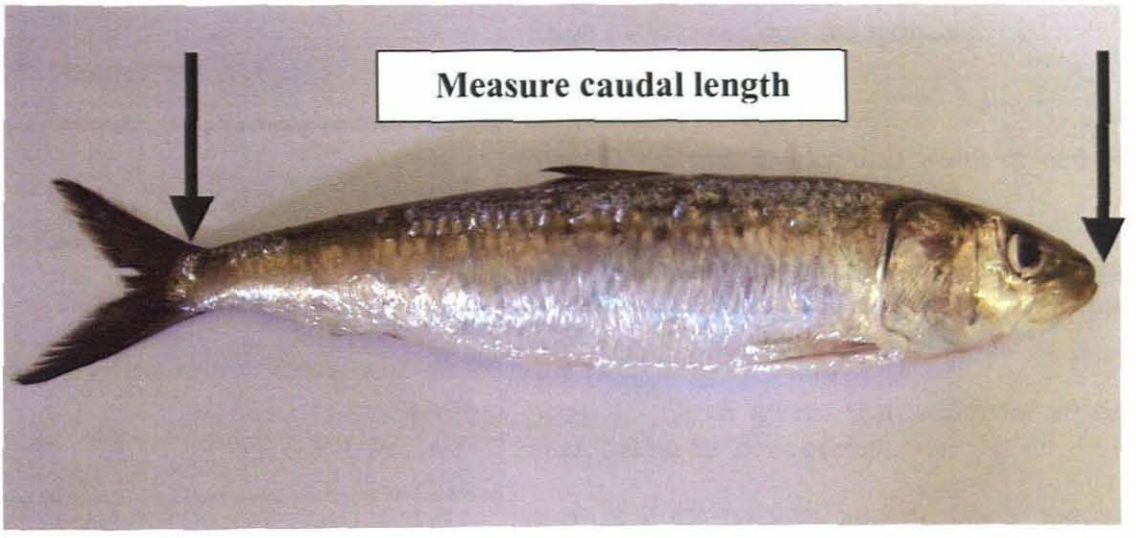
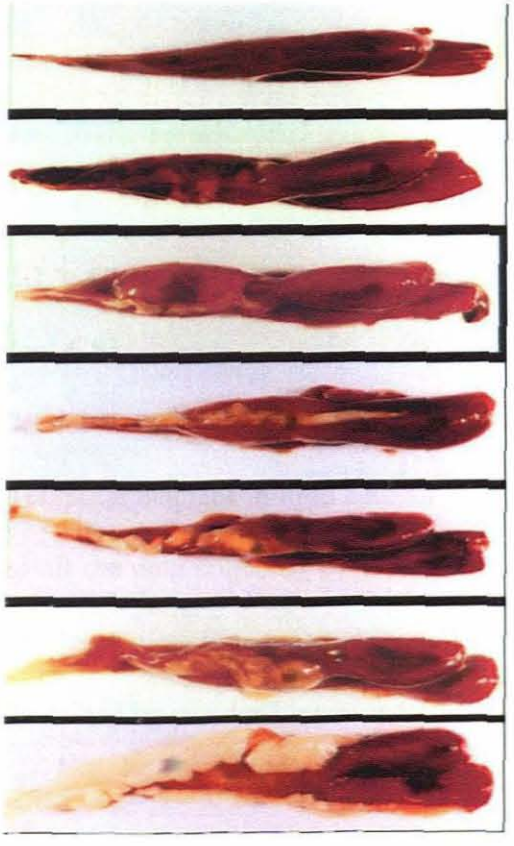


Fig. 2.2 – Sardine *Sardinops sagax*

- Fish weight (FWT) – The wet body mass of each sardine was recorded to the nearest 0.1g.
- Fat Stage – This is the fat stage of each individual sardine. A fat stage was assigned to each sardine, according to the guidelines as described in fig. 2.3a (van der Lingen and Hutchings in press). Each sardine was cut open from the anus to the gills and the stomach and other internal organs were moved to be able to examine the mesenteric fat and assign a fat stage (fig. 2.3b).
- Gonad weight (GWT) was recorded to the nearest 0.1g. From the gonad weight, the gonadosomatic index (GSI) was calculated, using:

$$GSI = \frac{GWT}{FWT - GWT} \times 100$$

- Sex – male, female or immature.



Stage 1 - Fat lines thin and indistinct.

Stage 2 - Depth greater than width of one or more fat lines.

Stage 3 - Pyloric fat line noticeably thicker than the other fat lines and about one-third the thickness of the pyloric junction.

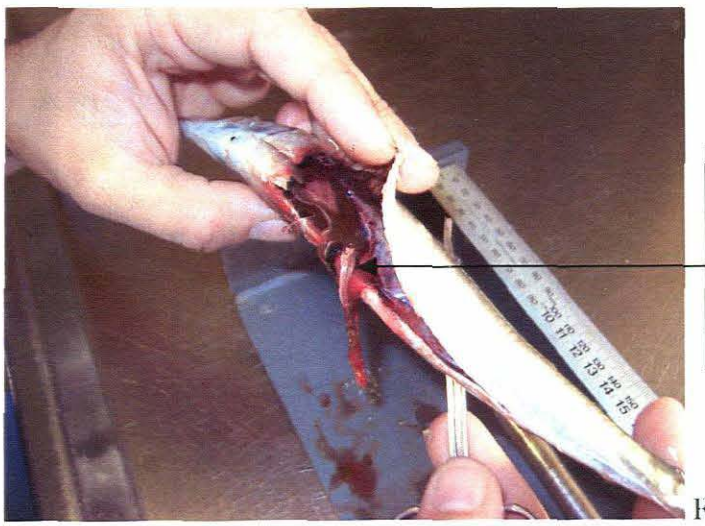
Stage 4 - Depth greater than width for all fat lines, but no fat lobes present.

Stage 5 - All fat lines slightly lobed, but no overlap between lobes.

Stage 6 - Fat line lobes obvious and show some overlap.

Stage 7 - Fat line lobes large, lots of overlap, and fundulus well covered with fat.

Fig. 2.3a - Description and pictures of fat stages for *Sardinops sagax*, from van der Lingen and Hutchings (in press).



The mesenteric fat is clearly visible on the ventral side of the stomach. The fish shown in this example would be assigned a fat stage of 3.

Fig. 2.3b – Assigning the fat stage.

2.1.3. Objective 1: To assess temporal and spatial variability in sardine lipid content and GSI.

2.1.3.1 Temporal changes in lipid content and GSI

Temporal changes in lipid content were analyzed by fitting a general linear model (GLM) to all the data collected from 1996 to 2003 (n = 29051 observations). Visually assigned fat stages were first converted to a lipid value, given as a percentage of wet body mass, as described by van der Lingen and Hutchings (in press) and using the following equation:

Lipid content % = a (Fat stage) + b (Fish weight) + c (Fat stage * Fish weight) + d

Parameter values for the GLM are given below:

Fat stage 1, a = -2.576150815,
Fat stage 2, a = -2.463955870,
Fat stage 3, a = -2.006219678,
Fat stage 4, a = -1.313122619,
Fat stage 5, a = -0.424204434,
Fat stage 6, a = -0.043716993,

Fat stage 7, a = 0.000000000,

Fish weight, b = 0.097270216,

Fish weight*Fat stage 1, c = -0.079603402,
Fish weight*Fat stage 2, c = -0.056736018,
Fish weight*Fat stage 3, c = -0.040113991,
Fish weight*Fat stage 4, c = -0.035271619,
Fish weight*Fat stage 5, c = -0.036791044,
Fish weight*Fat stage 6, c = -0.012238821,
Fish weight*Fat stage 7, c = 0.000000000, and intercept d = 3.226033372.

(The above constants were not part of the published results in van der Lingen and Hutchings (in press), but were obtained from the authors for the purposes this study.)

Data from 1996 to 2003 were documented in this thesis to ensure eight full year's cycles. The SAS Proprietary Software Release 8.2 software package was used to run the GLM's (SAS institute inc. 1998).

The GLM for lipid content used Year, Month, Zone and Sex as independent class variables and CL as independent continuous variable, plus their two-level interactions. This was the optimal GLM model which used all variables and their interactions. Thereafter, a sub-optimal model was manually selected, after following stepwise rejection of non-significant variables or interactions terms from the optimal model. The sub-optimal model used Month, Zone and the interaction between Year and Month and Month and Zone. This sub-optimal model explained a high degree of variance (as assessed using the r^2 value), but with a reduced number of parameters. This was done because the optimal model, which would include all significant variables, would be over-parameterized and some parameter estimates would be biased and/or not unique estimators. As documented by Kreiner *et al.* (2001), it is stressed by Lebreton *et al.*

(1992) that, instead of intending to get the ideal model explaining the highest percentage of variance; it is preferred to allow some secondary and hypothetical effects in the residuals and to focus on the main effects of the model. A visual residual analysis was done, also using SAS software, to confirm normality in the distribution of residuals and to ensure that there was no trend in the mean and variance of residuals plotted against observed values. Monthly least square estimates of marginal means (LS means) were derived from this sub-optimal model. Sample size was unbalanced for the different class and continuous variables, which would result in biased results if simple means were used. LS means were therefore used instead of simple means. The monthly LS means for lipid content were plotted on line plots, using Microsoft Excel (Microsoft corp., 2000) spreadsheets to show the temporal changes in lipid content for the time series, from 1996 to 2003.

Annual LS means were also derived from the lipid content sub-optimal GLM. The annual LS means were plotted in Microsoft Excel (Microsoft corp., 2000) to track interannual changes in lipid content.

A GLM fitted to the gonad data (GSI) used the same approach as for the lipid content GLM. The optimal model for GSI which used all variables and interactions used Year, Month and Sex as independent class variables and LC (caudal length) as an independent continuous variable, plus their two-level interactions, and after stepwise rejection of non-significant variables and interactions, a manually selected sub-optimum model, was used. This sub-optimal model used Month, Zone, LC and the interaction between Month and Zone.

Monthly and yearly derived LS mean plots were plotted separately in Microsoft Excel (Microsoft corp., 2000), to show monthly and interannual changes in GSI. Zone specific GLM's were also derived from the sub-optimum models, for both lipid content and GSI.

2.1.3.2 Spatial variability in lipid content and GSI

Spatial patterns in lipid content and GSI were assessed by conducting separate GLM's for each zone.

Monthly, as well as annual lipid LS means were then derived for each of the four zones. These GLM derived monthly LS means were plotted on Microsoft Excel (Microsoft corp., 2000) line graphs for each of the four zones, to assess lipid content seasonal patterns, spatially.

Annual LS means for lipid content were plotted on Microsoft Excel (Microsoft corp., 2000) line graphs for each of the four zones separately, to assess lipid content interannual patterns, spatially.

Zone specific monthly and annual GSI LS means, which were derived from the GSI GLM, were plotted in the same manner as for the lipid content cycles. Seasonal changes (monthly LS means), as well as interannual changes (annual LS means) were then monitored for spatial effects in GSI.

2.1.4 Objective 2: To examine whether any relationship between lipid content and GSI existed.

Scatter plots of monthly LS mean values of lipid content and GSI were derived firstly for the entire dataset to determine a temporal relationship between lipid content and GSI and

then for each of the four zones separately to show spatial difference in the relationship between lipid content and GSI. Linear regression trend lines were fitted to each of these plots. The slope of the regression line provides an indication of the condition of sardine in relation to the GSI and how it varied per zone.

2.1.5 Objective 3: Examination of the relationship between lipid content and GSI versus biomass and recruitment.

The annual LS means for lipid content and GSI, were plotted on scatter plots, using Microsoft Excel (Microsoft corp., 2000) software, against (a) adult biomass and (b) recruitment strength estimates, i.e. scatter plots for:

a. Lipid versus adult biomass (for the same year).

b. Lipid [lipid in year (n)] versus recruitment strength the following year [recruitment strength in year (n+1)].

c. GSI versus adult biomass (for the same year).

d. GSI [GSI in year (n)] versus recruitment strength the following year [recruitment strength in year (n+1)].

An indication of relative recruitment strength ($R_{Strength}$) is given as (van der Lingen *et al.* 2002):

$$R_{Strength} = R_n / SB_{n-1},$$

Where R_n is the recruitment biomass of year (n) and SB_{n-1} is the spawner biomass in year (n-1).

Regression trend lines were then fitted to the above mentioned scatter plots, to show relationships.

2.1.6 Objective 4: The relationships between lipid content and GSI to the environmental parameters SST and Chl a.

Temperature and Chl a data were used from December 1997 to July 2003, because SST satellite images were not available prior to 1997 and at the time of writing this thesis, Chl a data were not available after July 2003. The data used included the monthly and annual LS mean data for lipid content and GSI, for the entire dataset, as well as being spatially categorized by Zone.

SST data were made available to Marine and Coastal Management via the Interactions and Spatial Dynamics of Renewable Resources in Upwelling Ecosystems (IDYLE) programme. The images were produced at the Institut de Recherché pour le Développement in France. Five-day composites were computed from half-hourly Meteosat SST images (Demarcq and Citeau 1995). The SST's were corrected by using ships data and the resolution was 0.5°C. Pixel size was approximately 5.07km (E-W dimension) x 5.98km (N-S) at 32°S. The N-S dimension is fixed, but E-W dimension varies with latitude. Monthly averages of SST were then determined, to show seasonal changes in SST. SST means were determined to an offshore depth extent of 1000m.

Chl a data were made available by the National Ocean and Atmospheric Administration (NOAA) and the National Atmospheric and Space Administration (NASA). The original data were acquired from the AVHRR and SeaWiFS sensors, on board the NOAA and

OrbView-2 satellites. The resolution was 4.5km. Monthly averages of Chl a were then determined, to show seasonal changes in Chl a (Demarcq *et al.* 2003).

Relationships were tested between:

i. Lipid content and SST
ii. Lipid content and Chl a
iii. GSI and SST and
iv. GSI and Chl a

For each of the above four options the following were done:

1. The temporal (seasonal) changes were shown, by plotting the monthly LS means of both parameters for all data.
2. Lag effects were tested through shifting a seasonal cycle of one parameter by one month at a time (1 lag) towards the right on the same axis (i.e. later in the year). Significance and r-squared values were calculated by using each lag test's results, derived from linear regression analysis in Microsoft Excel (Microsoft corp., 2000) data analysis tools.
3. The interannual changes were tracked, by plotting the annual LS means of both parameters.
4. The spatial effects were tested by showing Zone specific scatter plots, for annual data.

2.1.7 Objective 5: Assess the fat ranking method (as an indicator of body lipid content) against a different method to determining fish condition.

The morphometrically derived method to determine condition in sardine as documented by Kreiner *et al.* (2001), was used to compare the fat ranking method, where condition factor (CF) was determined by:

$$CF = \frac{Observed\ wet\ body\ mass}{Expected\ wet\ body\ mass},$$

and

$$\text{expected wet body mass} = a\ CL^{b}$$

Where a and b are constants determined from the length-weight regression (a = 0.0108, b = 3.0629) (Fig. 2.4).

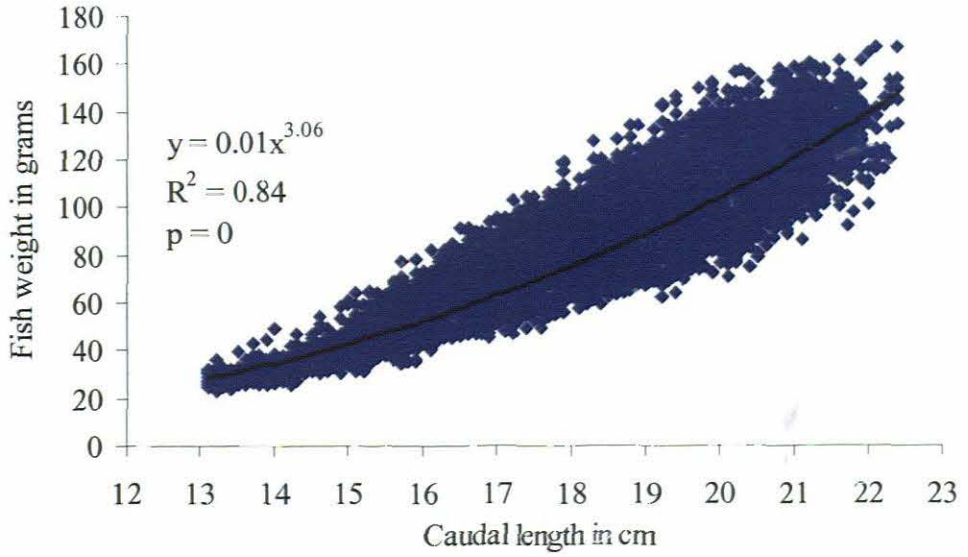


Fig. 2.4 – Length -Weight relation - all data 1996 to 2003.

A condition factor (CF) was derived for all the collected data for the time series, using the method of Kreiner *et al.* (2001). This CF was compared to the lipid content value,

which was derived separately from the same data, according to the visual fat stage method (fat stage converted to lipid content).

GLM derived monthly LS means for both CF and lipid content were then normalized to the mean of the monthly LS means. Annual tests were done in the same manner as for the monthly data.

Spatial comparison was tested by plotting lipid content versus CF on zone specific scatter plots using all individual data points.

Chapter 3

3.1 Results

A total of 29051 sardines were analyzed for this study. Table 3.1 shows the number of fish that were sampled by month and year, and Tables 3.2, 3.3, 3.4 and 3.5 shows the numbers of fish sampled for each of the four zones, by month and year.

Table 3.1 - Numbers of sardine sampled by month and year between the Orange River and Port Elizabeth, 1996 – 2003.

YEAR	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	TOTAL
1996	244	452	521	405	492	414	449	349	498	296	400	150	4670
1997	50	469	636	670	542	547	750	278	274	125	74	24	4439
1998	75	372	598	342	446	543	423	571	445	374	50		4239
1999	100	25	482	407	614	617	425	300	348	174	98	25	3615
2000		124	266	207	274	348	223	300	323	321	349	50	2785
2001	150	199	299	225	225	268	224	295	200	219	197	99	2600
2002		225	322	249	225	225	423	500	524	444	367	250	3754
2003	123	448	518	224	293	245	274	274	175	50	175	150	2949
TOTAL:	742	2314	3642	2729	3111	3207	3191	2867	2787	2003	1710	748	29051

Key:	
0 - 150	
151-300	
301-450	
451-600	
>600	

Table 3.2 - Numbers of sardine sampled by month and year in Zone 1 (Orange River to St. Helena Bay), 1996 – 2003.

YEAR	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	TOTAL
1996	25	175	248	169	50								667
1997		25	75	149	25	24		50				24	372
1998		24		22									46
1999						25							25
2000													0
2001		25							25				50
2002		25					25						50
2003													0
TOTAL:	25	274	323	340	75	49	25	50	25	0	0	24	1210

Key:
0 - 80
81 - 160
161 - 240
241 - 320
>320

Table 3.3 - Numbers of sardine sampled by month and year in Zone 2 (St. Helena Bay to Cape Point), 1996 – 2003.

YEAR	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	TOTAL
1996	169	174	124	25	192	291	350	274	373	198	275	75	2520
1997		120	138	75	93	149	300	179	225	125	74		1478
1998	50	200	200	125	274	174	75	100	100	124	25		1447
1999	50		150	63	125	100	150	150	175	74	73		1110
2000		50	68	33				50	175	171	174	50	771
2001	100	75	25	25		50	25			8	97	49	454
2002		100	99	49		25		25	125	125	199	125	872
2003	75	250	270						50	25	100	25	795
TOTAL:	444	969	1074	395	684	789	900	778	1223	850	1017	324	9447

Key:
0 - 80
81 - 160
161 - 240
241 - 320
>320

Table 3.4 - Numbers of sardine sampled by month and year in Zone 3 (Cape Point to Cape Infanta), 1996 – 2003.

YEAR	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	TOTAL
1996	25	75	99	113	150	23	24		25	23	50		607
1997	25	324	349	372	324	275	375	25	25				2094
1998		148	298	120	97	270	273	446	297	225			2174
1999		25	261	271	414	394	225	125	149	75			1939
2000		25	124	174	200	273	223	175	148	125	150		1617
2001		49	225	200	175	143	149	170	100	211	75		1497
2002		50	148	200	125	100	299	300	274	219	94	75	1884
2003	48	100	149	224	218	245	123	49			75	50	1281
TOTAL:	98	796	1653	1674	1703	1723	1691	1290	1018	878	444	125	13093

Key:
0 - 80
81 - 160
161 - 240
241 - 320
>320

Table 3.5 - Numbers of sardine sampled by month and year in Zone 4 (Cape Infanta to Port Elizabeth), 1996 – 2003.

YEAR	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	TOTAL
1996	25	28	50	98	100	100	75	75	100	75	75	75	876
1997	25		74	74	100	99	75	24	24				495
1998	25		100	75	75	99	75	25	48	25	25		572
1999	50		71	73	75	98	50	25	24	25	25	25	541
2000		49	74		74	75		75		25	25		397
2001	50	50	49		50	75	50	125	75		25	50	599
2002		50	75		100	100	99	175	125	100	74	50	948
2003		98	99		75		151	225	125	25		75	873
TOTAL:	175	275	592	320	649	646	575	749	521	275	249	275	5301

Key:
0 - 80
81 - 160
161 - 240
241 - 320
>320

3.1.1 Model results for lipid content:

The first sub-optimal GLM, with lipid content as dependent variable, took into account Month, Zone, and interactions between Year and Month and Month and Zone, and had the form:

$$\text{Lipid Content}_{Year,\,Month,\,Zone,\,i} = n + a_{Month} + b_{Zone} + c_{Year,\,Month} + d_{Month,\,Zone} + \varepsilon_{i\,Year\,Month\,Zone}$$

where n is a constant, a, b, c and d are parameters, and ε is the residual. This model explained 47% of the observed variance in Lipid Content, with most of the observed variance being explained by the interaction between Year and Month and Zone (Table 3.6). The sub-optimal GLM results show that Zone had the second strongest effect, indicating that there was a strong spatial effect. Zone was therefore removed from this GLM, in order to better assess annual / seasonal effects.

Table 3.6 – Model output for sardine lipid content: GLM no. 1 (r^2 = 0.47, n = 29 051). The degrees of freedom (df), Type III and Mean sum of squares (SS), F Value and significance (p-value) are shown for each of the variables.

Source	df	Type III SS	Mean SS	F Value	p (> F)
Month	11	14883.431	1353.039	295.030	<0.0001
Zone	3	16271.816	5423.939	1182.710	<0.0001
Year*Month	80	18703.195	233.790	50.980	<0.0001
Month*Zone	30	12377.647	412.588	89.970	<0.0001

The second sub-optimal GLM, with lipid content as a dependent variable, took the effects of Month, Year, caudal length and interactions between Year and Month into account and had the form:

$$\text{Lipid Content}_{Year,\,Month,\,i} = n + a_{Month} + b_{Year} + c_{Year,\,Month} + dCL_{Year,\,Month} + \varepsilon_{i\,Year\,Month}$$

where n is a constant, *a*, *b*, *c* and *d* are parameters, and ε is the residual. The model explained 34% of the observed variance in lipid content (Table 3.7), and indicates that most of the variance is explained by month and by the interactions between year and month.

Table 3.7 - Model output for sardine lipid content: GLM no. 2 (r^2 = 0.34, n = 29 051). The degrees of freedom (df), Type III and Mean sum of squares (SS), F Value and significance (p-value) are shown for each of the variables

Source	df	Type III SS	Mean SS	F Value	p (> F)
Year	7	4 067.549	581.078	102.44	<0.0001
Month	11	50 422.004	4 583.818	808.09	<0.0001
LC	1	1 156.258	1 156.258	203.84	<0.0001
Year*Month	74	10 515.481	142.101	25.05	<0.0001

3.1.2 Model results for GSI:

Two sub-optimum GLM's were derived for GSI as dependent variable. The first sub-optimum GLM took into account Month, Zone, LC and the interactions between Month and Zone, and had the form:

$$GSI_{Month,\ Zone,\ i} = n + a_{Month} + b_{Zone} + c_{Month,\ Zone} + dLC_{Month,\ Zone} + \varepsilon_{i\ Month\ Zone}$$

where n is a constant, *a*, *b*, *c* and *d* are parameters, and ε is the residual. This model explained 50% of the observed variance in GSI, with most of the observed variance being explained by Zone (Table 3.8), indicating that there was a strong spatial effect. Zone was therefore removed from this GLM, in order to better assess annual - seasonal effects.

Table 3.8 - Model output for sardine GSI: GLM no. 1 (r^2 = 0.50, n = 29 051). The degrees of freedom (df), Type III and Mean sum of squares (SS), F Value and significance (p-value) are shown for each of the variables.

Source	df	Type III SS	Mean SS	F Value	p (> F)
Month	11	8620.958	783.723	328.280	<0.0001
Zone	3	15655.482	5218.494	2185.900	<0.0001
LC	1	9679.522	9679.522	4054.520	<0.0001
Month*Zone	31	6148.433	198.337	83.080	<0.0001

The second sub-optimal GLM, with GSI as a dependent variable, took the effects of Month, Sex, caudal length and interactions between Year and Month into account and had the form:

$$GSI_{Month,\ Year,\ Sex,\ i} = n + a_{Month} + b_{Sex} + cLC_{Month,\ Year,\ Sex} + d_{Month,\ Year} + \varepsilon_{i\ Month,\ Year,\ Sex}$$

where n is a constant, a, b, c and d are parameters, and ε is the residual. The model explained 39% of the observed variance in GSI (Table 3.9), and indicates that most of the variance is explained by Month and by the length (LC).

Table 3.9 - Model output for sardine GSI: GLM no. 2 (r^2 = 0.39, n = 29 051). The degrees of freedom (df), Type III and Mean sum of squares (SS), F Value and significance (p -value) are shown for each of the variables.

Source	df	Type III SS	Mean SS	F Value	p (> F)
Month	11	19 119.19	1 738.11	595.86	<0.0001
Sex	1	3 252.49	3 252.49	1 115.02	<0.0001
LC	1	13 243.99	13 243.99	4 540.30	<0.0001
Year*Month	81	8 879.78	109.63	37.58	<0.0001

3.2 Objective 1: To assess temporal and spatial changes in sardine lipid content and GSI.

3.2.1 The temporal / seasonal variability in lipid content and GSI for the time series 1996 to 2003.

Monthly LS means for both lipid content and GSI showed significant ($p<0.001$) seasonal trends, with peaks in lipid content clearly associated with troughs in GSI: highest lipid content values were reached during May and lowest values reached during August and September, and the lowest GSI values were recorded during April and May and the highest GSI levels during November (Fig. 3.1).

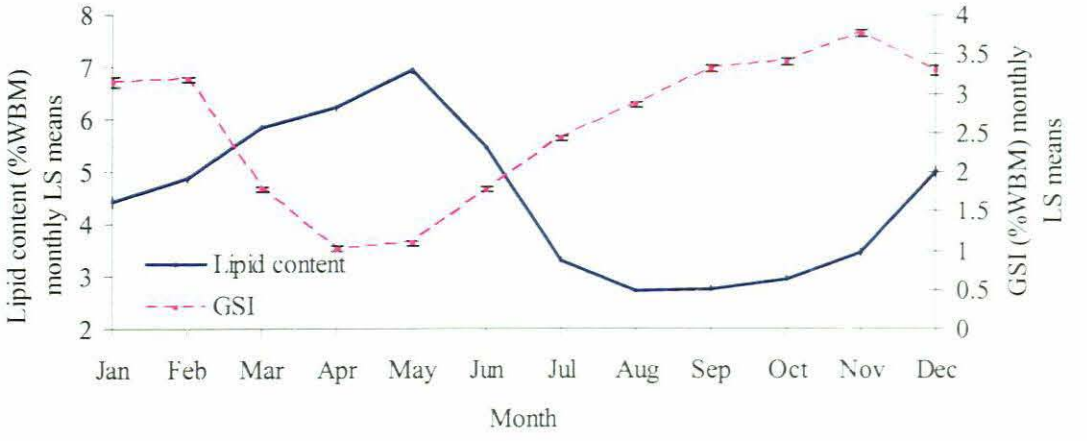


Fig. 3.1 – Temporal / seasonal variability in lipid content and GSI (given as % of wet body mass), derived from GLM no. 2 results from lipid content (Table 3.7) and results from GLM no. 2 from the GSI GLM (Table 3.9), using the entire data series, i.e. between the Orange River and Port Elizabeth, 1996 to 2003. Standard deviations are shown for both lipid content and GSI. Standard deviation bars are not clearly visible on the figure, due to the low degree of standard deviation of the data.

3.2.2 Interannual lipid content and GSI variability for the time series 1996 to 2003:

A significant year effect has been indicated by the GLM for lipid content (Table 3.7). Annual LS means showed a steady decline for lipid content from 1996 to 1998 and again

from 2000 to 2003. A significant year effect was not indicated by the GLM for GSI (Table 3.9) and no interannual trend is shown for GSI in Fig. 3.2.

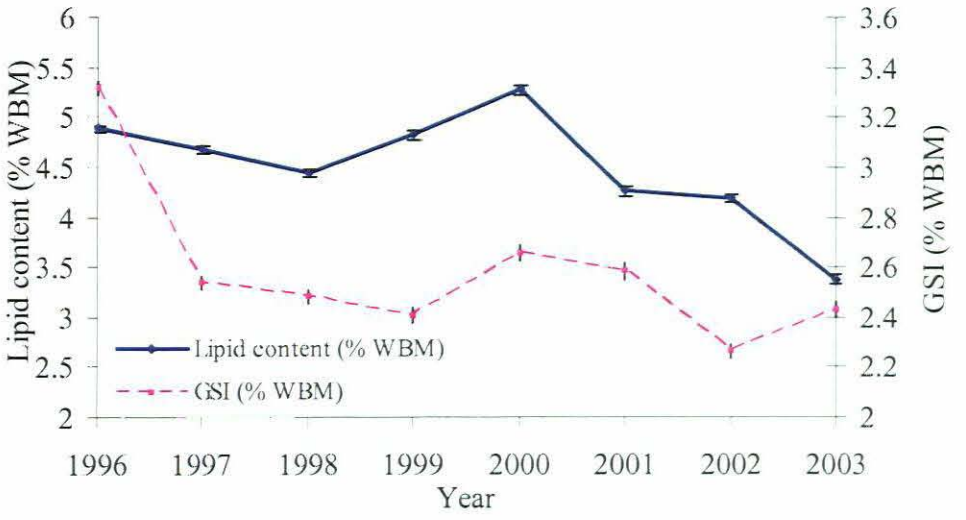


Fig. 3.2 – GLM derived time series of annual LS means of lipid content (% of wet body mass) and GSI (% of wet body mass) between the Orange River and Port Elizabeth, 1996 to 2003. Standard deviations are shown. Standard deviation bars are not clearly visible on the figure, due to the low standard deviation degree of the data.

3.2.3 Zone specific model results for lipid content:

The second sub-optimal GLM, with lipid percentage as a dependent variable, which took the effects of Month, Year, caudal length and interactions between Year and Month into account, were run for each of the four zones separately. For Zone 1, 17% of the observed variance in lipid content was explained by the GLM, indicating that Month explained most of the variance in the GLM (Table 3.10). For Zones 2, 3 and 4, 40%, 38% and 64% of the observed variance in lipid content was explained by the model respectively, also indicating that Month explained most of the variance in the GLM (Tables 3.11, 3.12 and 3.13 respectively).

Table 3.10 - Model output for sardine lipid content: Zone 1 (r^2 = 0.17, n = 1 210). The degrees of freedom (df), Type III and Mean sum of squares (SS), F Value and significance (p -value) are shown for each of the variables.

Source	df	Type III SS	Mean SS	F Value	p (> F)
Year	5	120.33	24.07	4.05	0.0012
Month	9	490.86	54.54	9.18	<0.0001
LC	1	6.08	6.08	1.02	0.312
Year*Month	4	116.24	29.06	4.89	0.0007

Table 3.11 - Model output for sardine lipid content: Zone 2 (r^2 = 0.40, n = 9 447). The degrees of freedom (df), Type III and Mean sum of squares (SS), F Value and significance (p -value) are shown for each of the variables.

Source	df	Type III SS	Mean SS	F Value	p (> F)
Year	7	258.31	36.90	11.55	<0.0001
Month	11	8 437.64	767.06	240.14	<0.0001
LC	1	297.81	297.81	93.23	<0.0001
Year*Month	57	4 794.66	84.12	26.33	<0.0001

Table 3.12 - Model output for sardine lipid content: Zone 3 (r^2 = 0.38, n = 13 093). The degrees of freedom (df), Type III and Mean sum of squares (SS), F Value and significance (p -value) are shown for each of the variables.

Source	df	Type III SS	Mean SS	F Value	p (> F)
Year	7	4 702.06	671.72	165.67	<0.0001
Month	11	9 859.13	896.28	221.06	<0.0001
LC	1	384.89	384.89	94.93	<0.0001
Year*Month	59	4 984.21	84.48	20.84	<0.0001

Table 3.13 - Model output for sardine lipid content: Zone 4 (r^2 = 0.64, n = 5 301). The degrees of freedom (df), Type III and Mean sum of squares (SS), F Value and significance (p -value) are shown for each of the variables.

Source	df	Type III SS	Mean SS	F Value	p (> F)
Year	7	2 977.82	425.40	72.53	<0.0001
Month	11	28 385.86	2 580.53	439.95	<0.0001
LC	1	19.15	19.15	3.26	0.0708
Year*Month	57	6 796.69	119.24	20.33	<0.0001

3.2.4 Zone specific model results for GSI:

The second sub-optimal GLM, with GSI as a dependent variable, which took the effects of Month, Sex, caudal length and interactions between Year and Month into account, were run for each of the four zones separately. For Zones 1, 2, 3 and 4, 57%, 44%, 42% and 36% of the observed variance in GSI were explained by the GLM respectively, indicating that Month explained most of the variance in the GLM (Tables 3.14, 3.15, 3.16 and 3.17 respectively).

Table 3.14 - Model output for sardine GSI: Zone 1 (r^2 = 0.57, n = 1 210). The degrees of freedom (df), Type III and Mean sum of squares (SS), F Value and significance (p -value) are shown for each of the variables.

Source	df	Type III SS	Mean SS	F Value	p (> F)
Month	9	1 745.55	193.95	68.68	<0.0001
Sex	1	184.17	184.17	65.21	<0.0001
LC	1	1 016.02	1 016.02	359.78	<0.0001
Year*Month	9	257.44	28.60	10.13	<0.0001

Table 3.15 - Model output for sardine GSI: Zone 2 (r^2 = 0.44, n = 9 447). The degrees of freedom (df), Type III and Mean sum of squares (SS), F Value and significance (p -value) are shown for each of the variables.

Source	df	Type III SS	Mean SS	F Value	p (> F)
Month	11	8 365.23	760.48	266.8	<0.0001
Sex	1	1 771.21	1 771.21	621.39	<0.0001
LC	1	6 788.90	6 788.90	2 381.75	<0.0001
Year*Month	64	2 287.51	35.74	12.54	<0.0001

Table 3.16 - Model output for sardine GSI: Zone 3 (r^2 = 0.42, n = 13 093). The degrees of freedom (df), Type III and Mean sum of squares (SS), F Value and significance (p -value) are shown for each of the variables.

Source	df	Type III SS	Mean SS	F Value	p (> F)
Month	11	3 836.86	348.81	261.9	<0.0001
Sex	1	342.73	342.73	257.34	<0.0001
LC	1	1 925.74	1 925.74	1 445.93	<0.0001
Year*Month	66	2 833.83	42.94	32.24	<0.0001

Table 3.17 - Model output for sardine GSI: Zone 4 (r^2 = 0.36, n = 5 301). The degrees of freedom (df), Type III and Mean sum of squares (SS), F Value and significance (p -value) are shown for each of the variables.

Source	df	Type III SS	Mean SS	F Value	p (> F)
Month	11	3122.61	283.87	133.28	<0.0001
Sex	1	355.62	355.62	166.97	<0.0001
LC	1	823.24	823.24	386.53	<0.0001
Year*Month	64	1157.28	18.08	8.49	<0.0001

3.2.5 Zone specific seasonal patterns of lipid content and GSI:

A seasonal trend in lipid content was observed (in each of the four zones), peaking in April / May, followed by low values in August / September (Fig. 3.3).

A complete monthly lipid content LS mean cycle for Zone 1 could not be derived, because of the lack of availability of samples during all the months: however, the full annual cycle was sampled in the other zones. In Zone 1 the mean lipid content peaked during March at a level of 6.7%.

Lipid content monthly LS means peaked in May for Zones 2, 3, and 4, at different levels of 5.8%, 6.1% and more than 10%, respectively.

The lowest lipid content LS mean levels were reached as follows:

- Zone 2 – September, at a value of 2.55%.
- Zone 3 – August, at a value of 2.79%.
- Zone 4 – September, at a value of 3.23%.

A complete monthly GSI LS mean cycle for Zone 1 could not be derived, because of the lack of availability of samples during all the month: however, the full annual cycle was sampled in the other zones. For data collected in Zone 1, the mean GSI peaked during February.

GSI monthly LS means peaked in December and November for Zones 2 and 3 respectively and in July for Zone 4. The GSI peak in July suggests that the spawning season commenced earlier in Zone 4, compared to the other zones. A decline in GSI for Zone 4 during November occurred when a peak in GSI was observed Zone 3, for the same month. The lowest GSI LS mean levels were reached in April, for all zones, except for Zone 1, which reached the lowest GSI level in June.

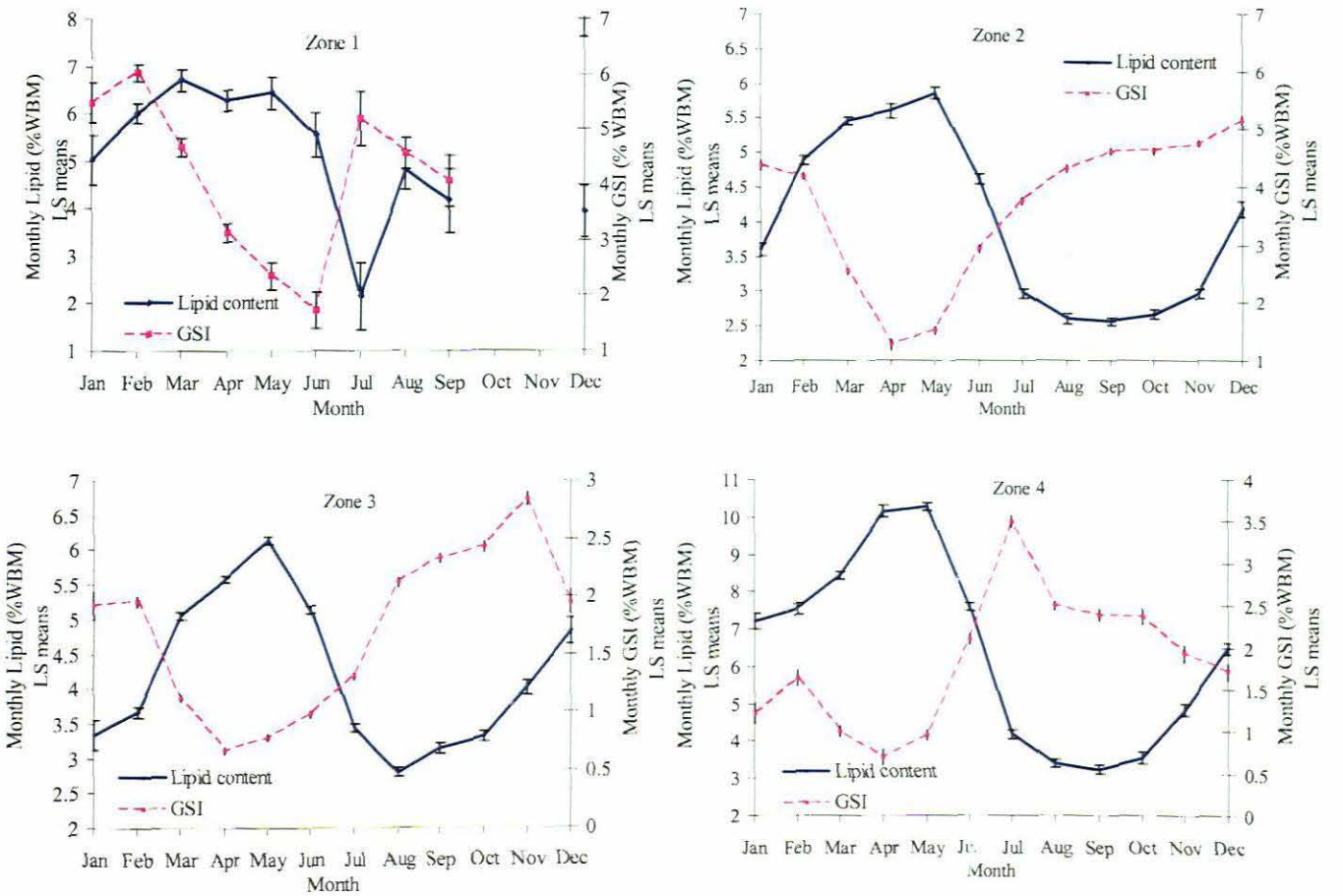


Fig. 3.3 - GLM derived time series of monthly LS means of lipid content (% of wet body mass) and GSI (% of wet body mass), 1996 to 2003, showing zone specific differences. Standard deviations are shown.

3.2.6 Interannual trends per zone in lipid content and GSI:

A complete annual cycle was not derived for Zone 1; due to sample availability for the years 2000 and 2003 (Fig. 3.4). According to the lipid content GLM (Table 3.7) Year had a significant effect on lipid content. The annual effect on lipid content has been the strongest in Zone 3, followed by Zone 4, Zone 2 and Zone 1. Zone 3 showed most declines in lipid content LS means over the period. Zone 4 showed a decline in annual lipid content LS means from 1996 to 2003, at the highest lipid content LS mean values for all the years, compared to the other three zones during the same year.

The interannual GSI LS mean cycle for Zone 1 could not be completed, due to sample availability of sardine in this area for the years of 2000 and 2003 (Fig. 3.4). The highest annual GSI LS mean values were calculated for Zone 1, followed by Zone 2, 3 and 4. GSI LS means were low and similar in Zone 3 and Zone 4. Interannual GSI LS means has been relatively constant for Zones 1, 2, 3 and 4, which is why Year was not a significant parameter in the GSI GLM (Table 3.9). GSI was markedly higher in Zones 1 and 2, compared to Zones 3 and 4.

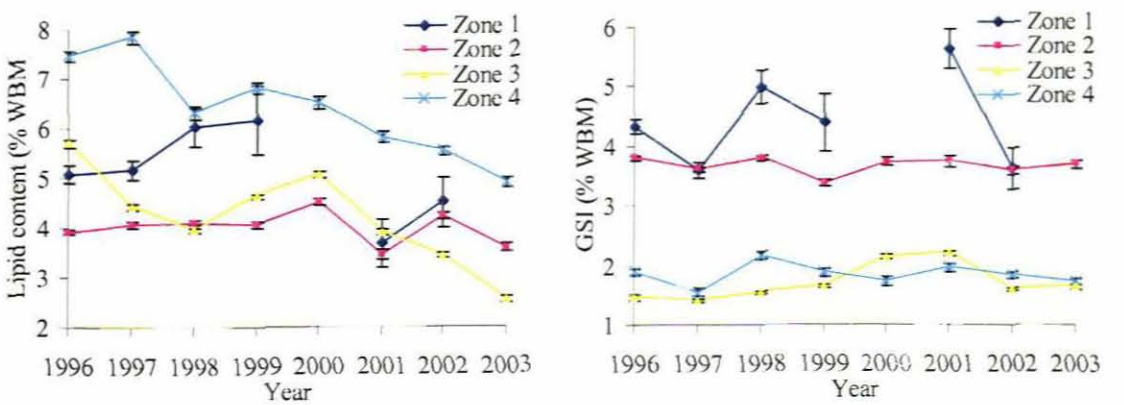


Fig. 3.4 - GLM derived time series of annual LS means of lipid content (% of wet body mass) and GSI (% of wet body mass), 1996 to 2003, to show interannual zone specific differences. Standard deviations are shown.

3.3 Objective 2: To examine whether any relationship between lipid content and GSI exists:

A significant ($p<0.001$) negative linear relationship was found between monthly GSI LS means and monthly lipid content LS means for the time series 1996 to 2003, where 60% of the variance in the GSI was explained by lipid content (Fig 3.5).

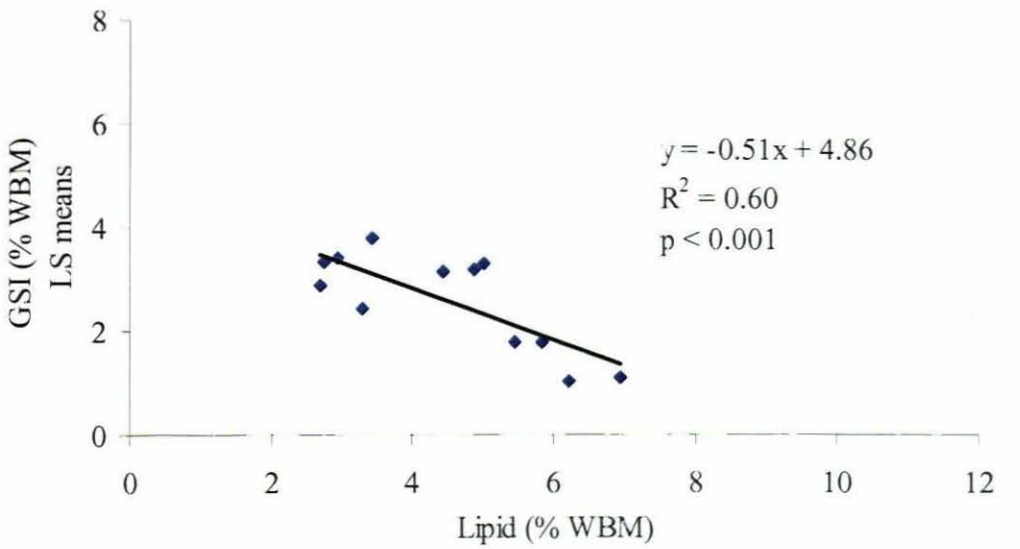


Fig. 3.5 – Scatter plot of monthly LS mean values for lipid content versus GSI, for the time series 1996 to 2003, all data combined.

3.3.1 Zone specific lipid content and GSI relationships:

Scatter plots of monthly GSI LS means against lipid content are shown in Fig. 3.6 for each of the four zones. Significant negative linear relationships were found between monthly GSI LS mean values and lipid content for each of the four zones for the time series 1996 to 2003 (see Fig. 3.6 for p-values). For Zone 1, only 18% of the variance in GSI was explained by lipid content, whereas for Zones 2, 3 and 4, 63%, 56% and 71% of the variance were explained by lipid content in GSI, respectively.

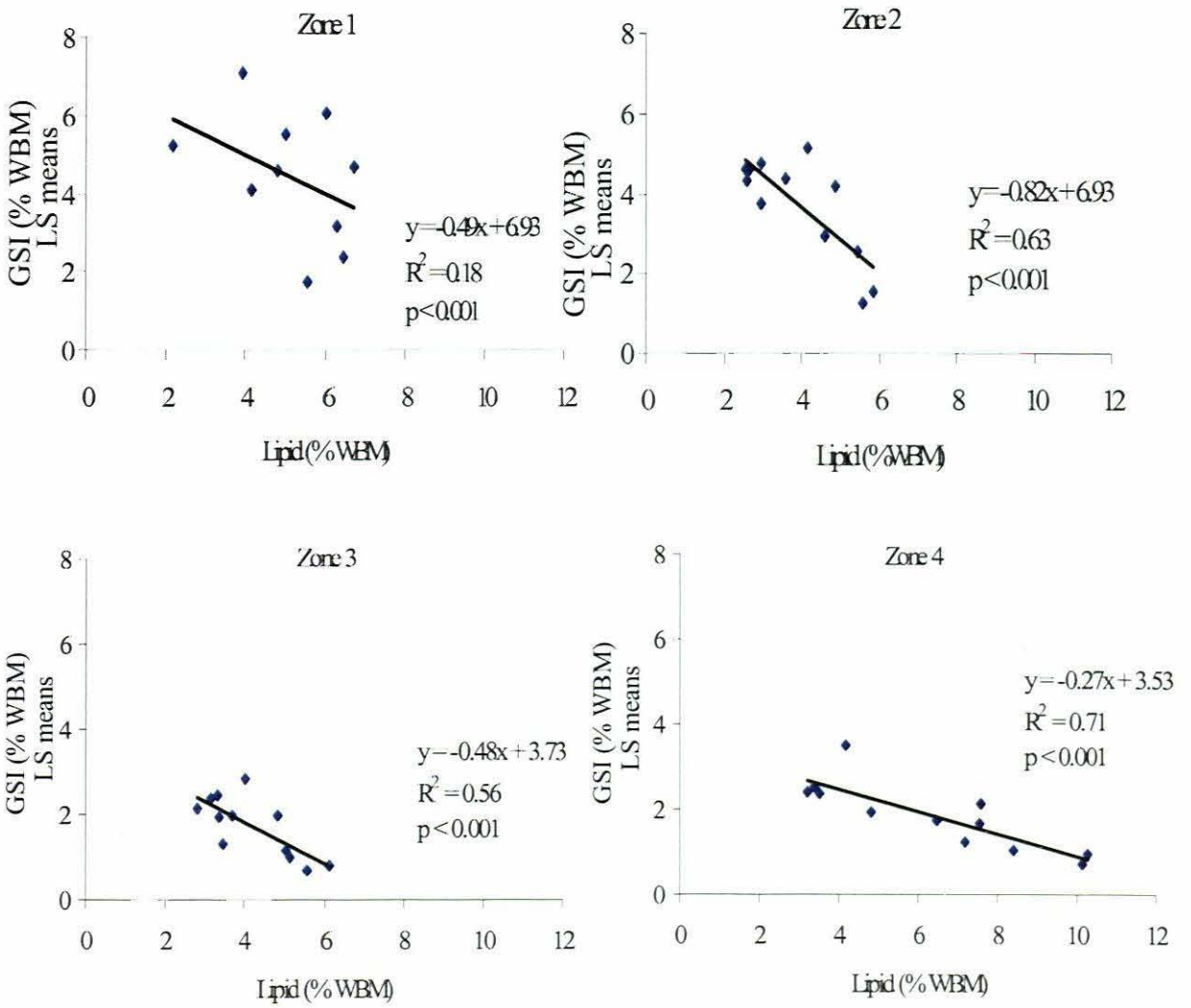


Fig. 3.6 – Scatter plots of monthly GSI LS means against lipid content (% of wet body mass) are shown for each of the four zones.

3.4 Objective 3: To examine the relationship between lipid content and GSI to biomass and recruitment:

Spawner- and recruitment biomasses are shown in Fig. 3.7. This figure was included to show variability in adult biomass and recruitment biomass from 1996 to 2003. Adult biomass is determined during predicted peak spawning season, i.e. during November and recruitment biomass is estimated during May/June from research biomass surveys. Recruitment biomass is determined on sardine less than 13.0cm total length (±0-2 years).

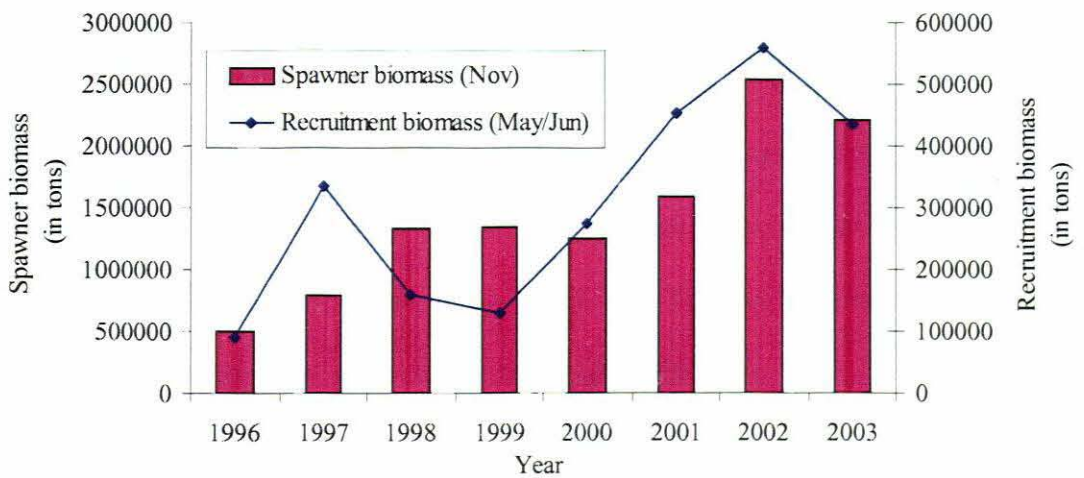


Fig. 3.7 - Recruit and adult biomass estimates obtained from Marine and Coastal Management (MCM, unpublished data).

3.4.1 The relationship between lipid content and adult biomass.

A scatter plot of annual lipid LS means (from sub-optimal GLM no. 2 – Table 3.7) versus adult biomass is shown in Fig. 3.8. Annual lipid content and adult biomass were found to be significantly (p=0.05) inversely related, for the time series. A Pearson correlation test was included for this relationship. The Pearson product moment correlation coefficient (-0.70017) also shows the data to have a weakly negative relationship.

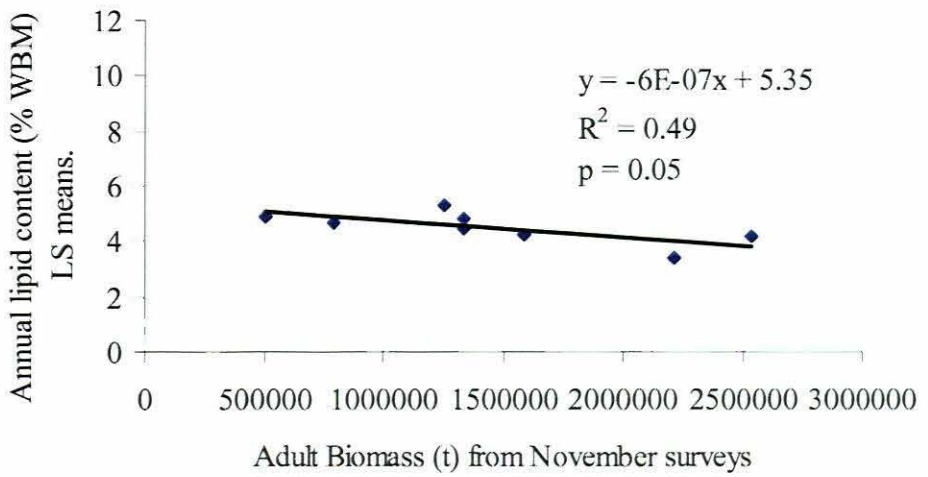


Fig. 3.8 - Adult biomass (t) and lipid relation (%WBM), 1996 - 2003.

3.4.2 The relationship between lipid content and relative recruitment strength.

A scatter plot of annual lipid content LS means (from sub-optimal GLM no. 2 – Table 3.7) versus relative recruitment is shown in Fig. 3.9. No relationship between lipid content and subsequent relative recruitment was observed.

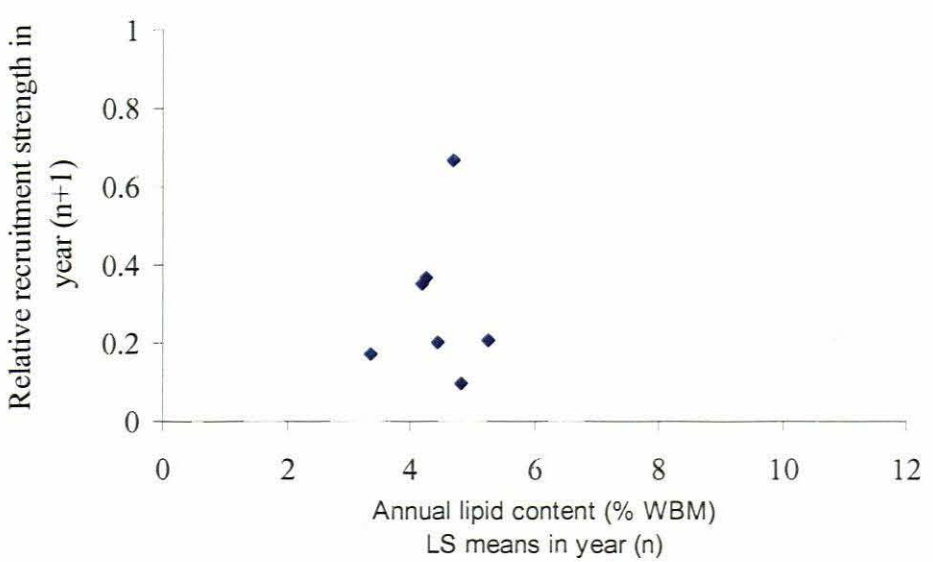


Fig. 3.9 – Relationship between annual lipid content (% WBM) LS means and the next year's relative recruitment strength.

3.4.3 The relationship between GSI and adult biomass.

A scatter plot of annual GSI LS means (from sub-optimal GLM no. 2 – Table 3.9) versus adult biomass is shown in Fig. 3.10. A negative linear relationship was derived between adult biomass and annual GSI LS means, where 55% of the variance in adult biomass was explained by annual GSI LS means for the time series, at a p-value of 0.036. A Pearson correlation test was included for this relationship. The Pearson product moment correlation coefficient (-0.73923) also shows the data to have a weakly negative relationship.

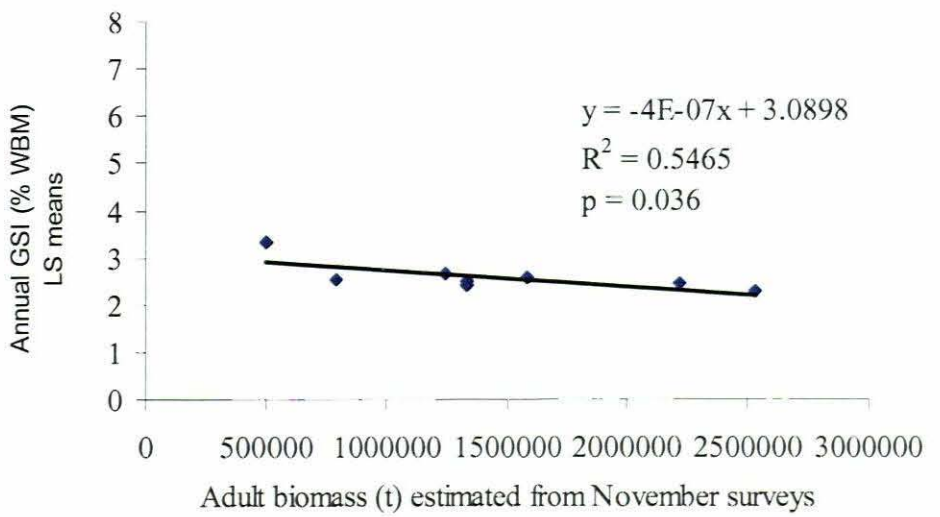


Fig. 3.10 - Adult biomass (t) and GSI (% WBM) relation - 1996 - 2003.

3.4.4 The relationship between GSI and relative recruitment strength.

A scatter plot of annual GSI LS means (from sub-optimal GLM no. 2 – Table 3.9) versus relative recruitment is shown in Fig. 3.11. A significant relationship between annual GSI (for year n) and relative recruitment strength (for year n+1) could not be derived for the time series.

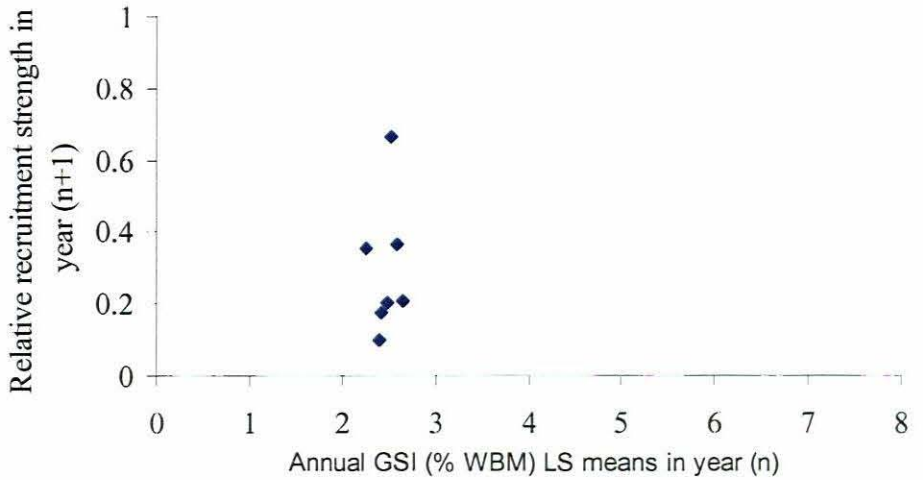


Fig. 3.11 – Relationship between annual GSI LS means and the previous year's relative recruitment strength.

3.5 Objective 4: Lipid content and GSI's relation to the environmental parameters SST and Chl a, for the time series December 1997 to July 2003:

3.5.1 The seasonal variation between lipid content and SST.

Seasonal variability was observed for SST (Fig. 3.12). Low lipid levels were associated with low SST levels during the month of August. Lipid content LS means values peaked three months later than the SST monthly means.

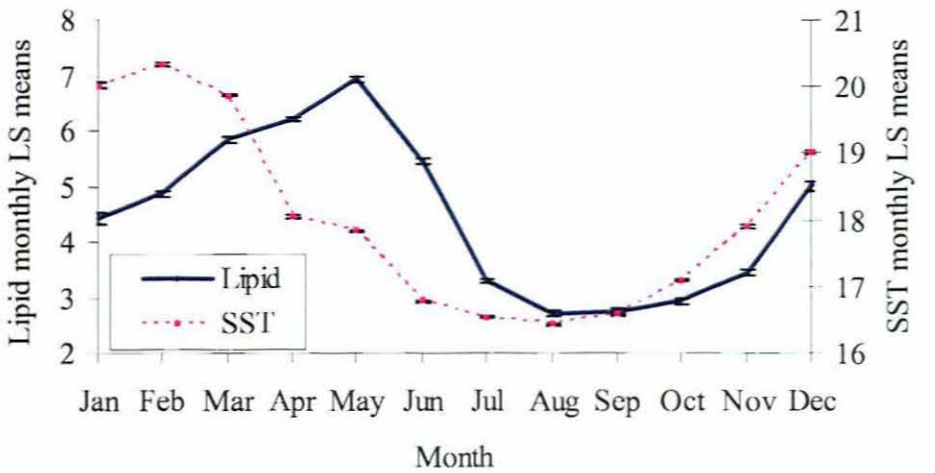


Fig. 3.12 - Monthly lipid content (% WBM) and SST (°C) LS means for the time series December 1997 to July 2003 – all Zones. Standard deviations are shown.

Lipid content lagged SST by 2 months (Table 3.18).

Table 3.18 – Lag results between lipid content and SST.

Parameter	No lag	1 month lag	2 month lag	3 month lag
R Square	0.22	0.54	0.76	0.60
Significance (p)	0.123	0.007	0.000	0.003

3.5.2 Interannual variation between lipid content and SST for the time series December 1997 to July 2003.

Lipid content decreased from 1997 to 1998 and from 2000 to 2003. Lipid content increased from 1998 to 2000. SST decreased from 1997 to 1999 and then showed a steady increase from 1999 to 2003 (Fig. 3.13).

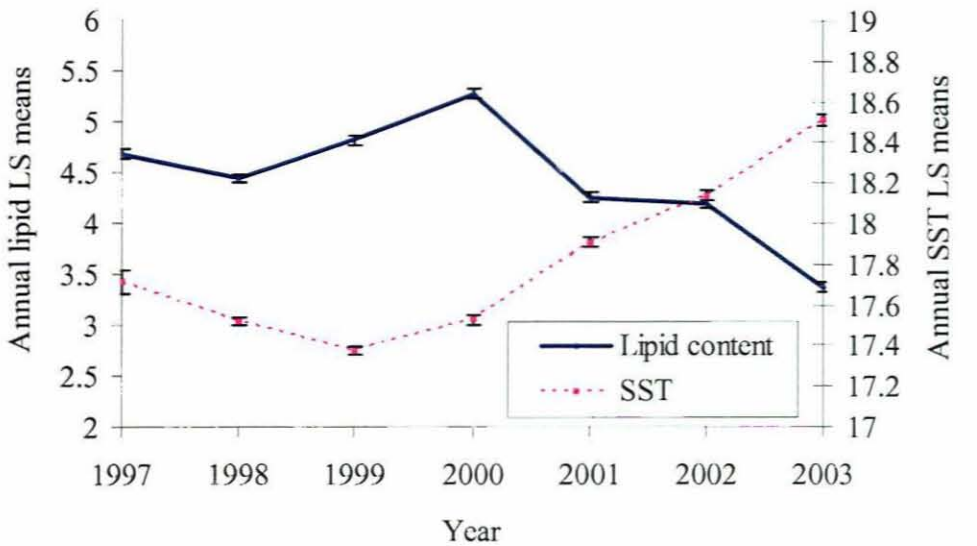


Fig. 3.13 - Annual lipid content (% WBM) and SST (°C) LS means for the time series December 1997 to July 2003. Standard deviations are shown.

A scatter plot of annual lipid content versus SST shows a significant negative relationship (Fig. 3.14).

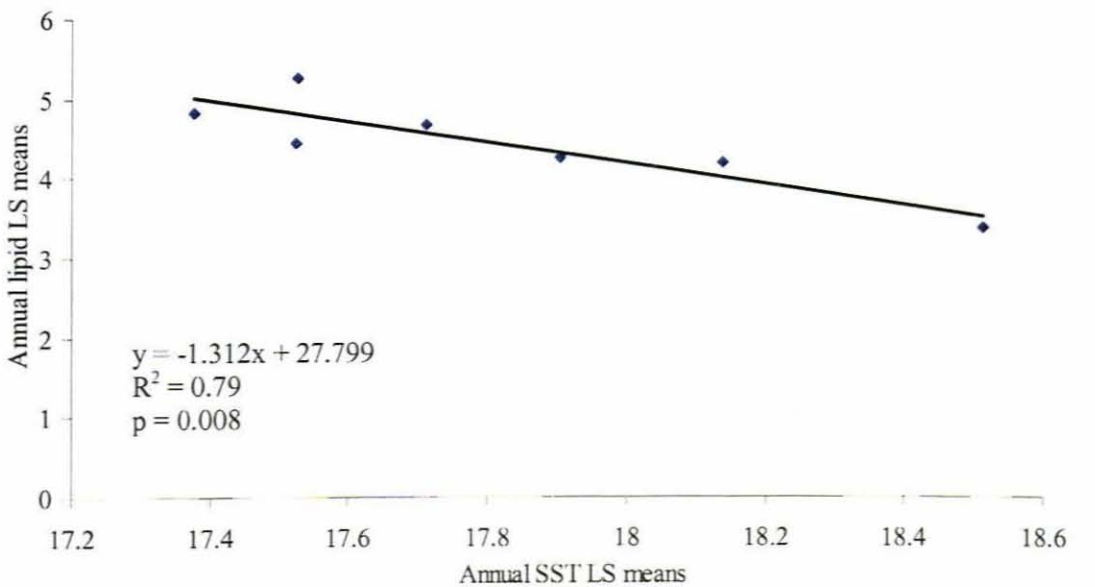


Fig. 3.14 – Scatter plot of annual lipid content (% WBM) LS means versus annual SST (°C) LS means, for all the data, 1996 to 2003.

3.5.3 Zone specific interannual lipid content and SST relationships for the time series December 1997 to July 2003.

Spatial difference between annual lipid content and annual SST were observed between all four zones (Fig. 3.15). It can be noted that both the lowest annual SST, as well as the highest annual SST value was observed in Zone 1. This was expected, because of known fluctuations of SST, which is common in upwelling areas (like Zone 1 – on the west coast). Although not as high as in Zone 4, annual values of lipid content in Zone 1 also reached high values. Although a significant relationship between annual lipid content and annual SST were not derived for Zone 2, it is still notable that a striking spatial difference exists between Zones 1, 2 and 4, for annual lipid content and annual SST. Annual lipid content values versus annual SST value were observed at lower levels in Zone 2, than observed in Zone 4. A significant negative linear relationship exists in both Zones 3 and 4, which indicates that with an increase in SST, lipid content decreases significantly in these Zones.

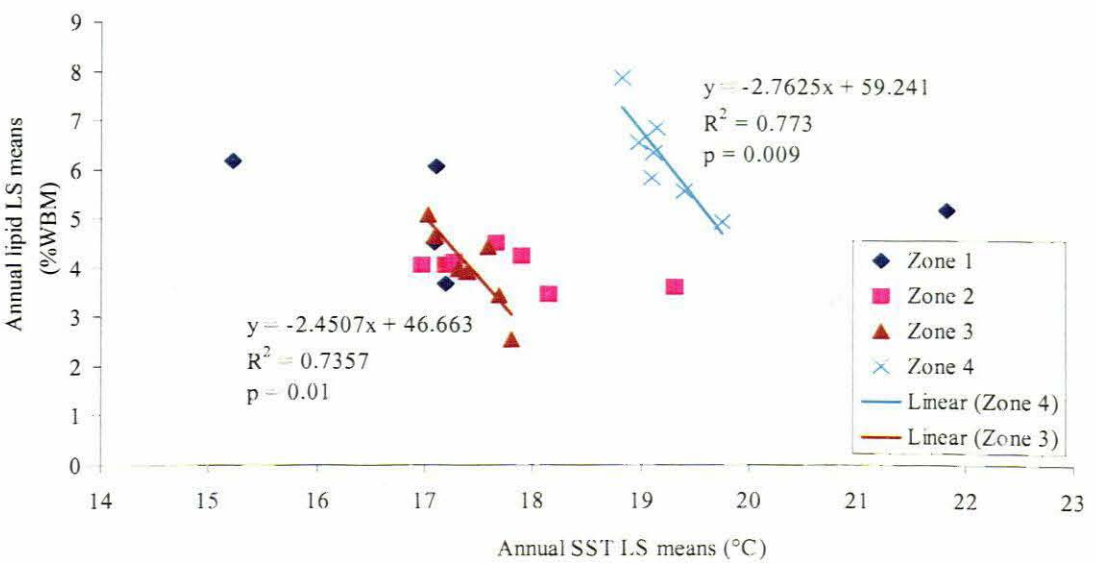


Fig. 3.15 Scatter plots of annual lipid content (% WBM) LS mean versus annual SST (°C) LS mean for each of the four zones, for the series 1997 to 2003.

3.5.4 The seasonal variation between lipid content and chlorophyll a (Chl a):

A seasonal trend was also observed for Chl a, from December 1997 to July 2003 (Fig. 3.16). High values of Chl a were reached during the summer months (November to February) and minimum values occurred during the winter period (May to August).

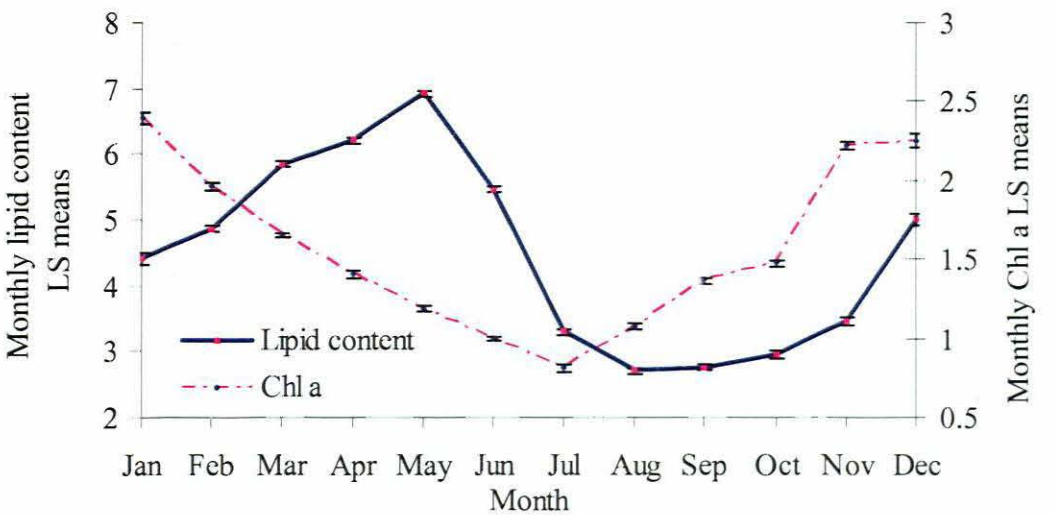


Fig. 3.16 – The seasonal relationship between lipid content (% WBM) and Chl a (mg/m^3) for the time series 1996 to 2003, all zones combined. Standard deviations are shown.

Lag effects between lipid and Chl a were tested and the following results were obtained:

Linear regression results in Table 3.19 show that the best correlation was observed at a three month lag, i.e. at an r-square value of 0.699 and a significant value of 0.001.

Table 3.19 – Lag results between lipid content and Chl a.

Parameter	No lag	1 month lag	2 month lag	3 month lag	4 month lag
R Square	0.002	0.201	0.462	0.699	0.626
Significance (p)	0.891	0.143	0.015	0.001	0.002

3.5.5 The interannual relationship between lipid content and chlorophyll a (Chl a):

A long-term trend was not observed in Chl a (Fig. 3.17) and a significant relationship between annual lipid LS means and annual Chl a LS mean could not be derived.

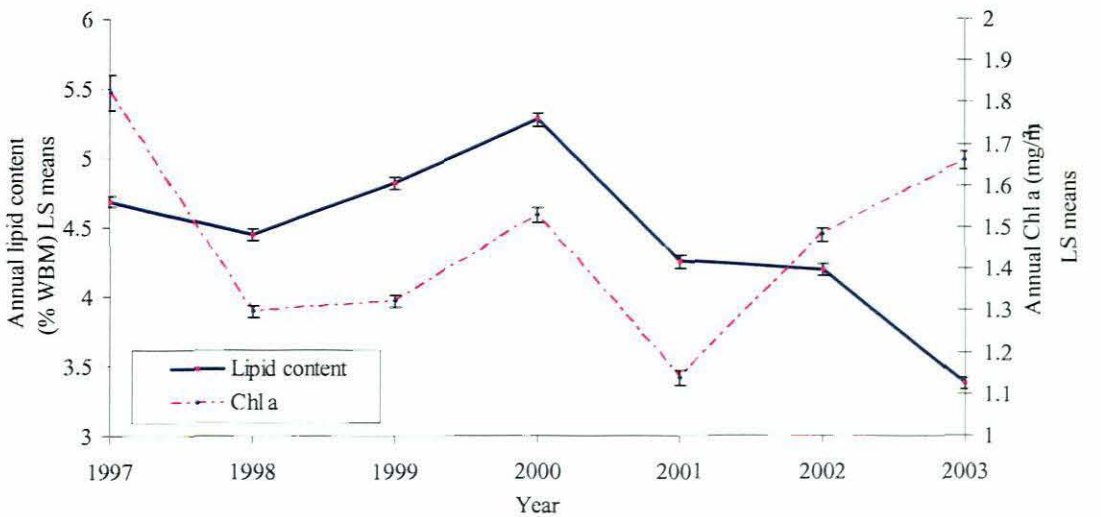


Fig. 3.17 - Interannual lipid content (% WBM) and Chl a (mg/m³) LS mean relationship for the data series December 1997 to July 2003. Standard deviations are shown.

3.5.6 Zone specific interannual relationships between lipid content and Chl a LS means.

Spatial differences between all four zones were observed between annual lipid content and Chl a (Fig. 3.18). Chl a values fluctuated over a larger range in Zones 1 and 2 than in Zones 3 and 4. Chl a values were higher in Zones 1 and 2 than in Zones 3 and 4. High Chl a concentrations (primary production) is commonly known to be associated with upwelling areas of the west coast (Zone 1 and 2). A significant positive relationship between annual lipid content and Chl a were derived for Zone 3. It is interesting to note that, although Chl a (which is considered to be an important food source on the west

coast) values were low in Zone 4; lipid content was noticed to be the highest of all four zones.

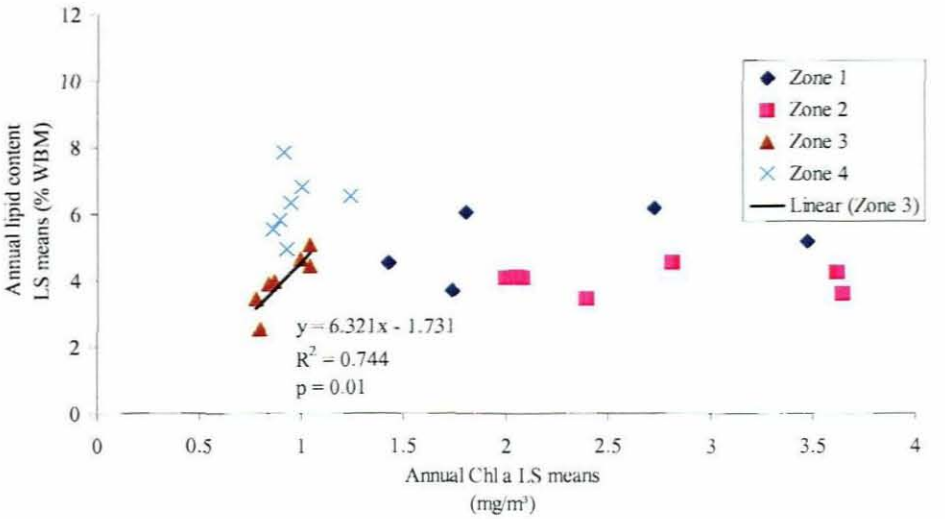


Fig. 3.18 Scatter plots of annual lipid content LS mean (% WBM) versus annual Chl a (mg/m³) LS mean for each of the four zones, for the series 1997 to 2003.

3.5.7 The seasonal variation between GSI and SST for the time series December 1997 to July 2003:

High GSI values corresponded with high SST values, but low GSI values were reached during April and May, whereas low SST levels were reached during August (Fig. 3.19).

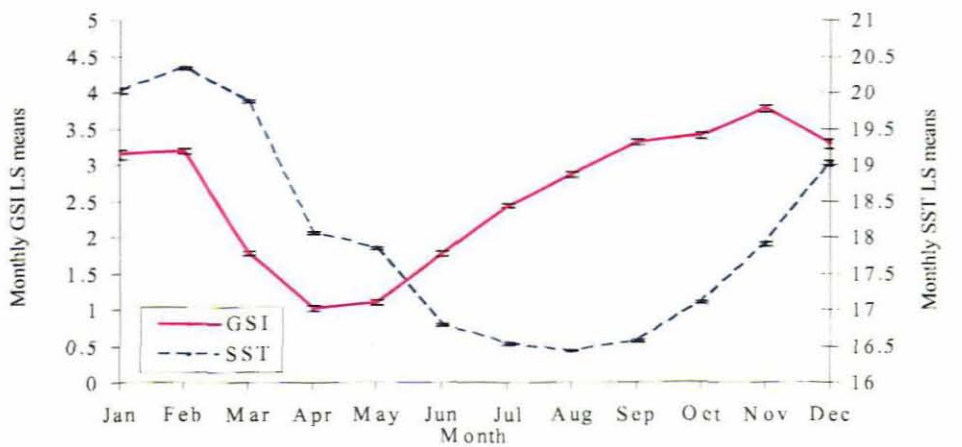


Fig. 3.19 – The seasonal variation between monthly GSI (% WBM) and SST (°C) LS means for the time series December 1997 to July 2003. Standard deviations are shown.

The following results were derived from lag tests done on the above GSI and SST cycles:

Linear regression results in Table 3.20 show that the best correlation between monthly GSI LS mean and monthly SST LS mean was observed at a 3 month lag, i.e. at an r-square value of 0.779 and a significance value of less than 0.001, where GSI lagged SST.

Table 3.20 – Lag results between GSI and SST.

Parameter	No lag	1 month lag	2 month lag	3 month lag	4 month lag
R Square	0.003	0.241	0.568	0.779	0.595
Significance (p)	0.872	0.105	0.005	0.000	0.003

3.5.8 Interannual relationship between GSI and SST:

No distinct long-term relationship could be observed between GSI and SST (Fig. 3.20).

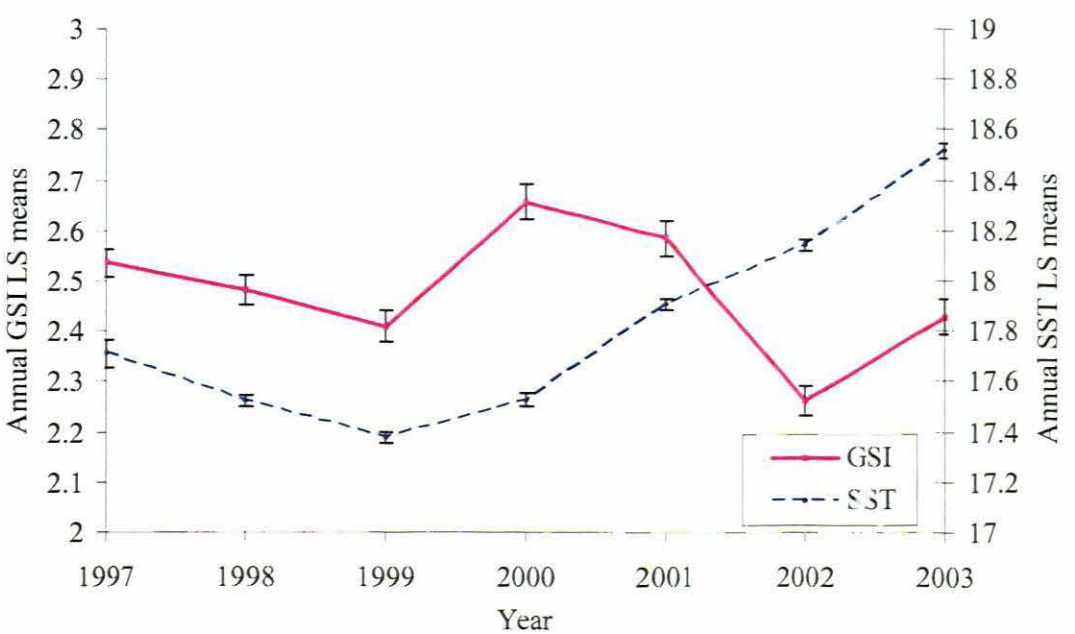


Fig. 3.20 – Annual means of GSI (% WBM) and SST (°C) for the time series December 1997 to July 2003. Standard deviations are shown.

3.5.9 Zone specific interannual relationships between GSI LS means and SST LS means for the time series December 1997 to July 2003:

Spatial differences between annual GSI and annual SST were observed between all four zones (Fig. 3.21). The highest annual GSI values were reached in Zone 1, and between the widest annual SST ranges of 15°C - 22°C. The second highest annual GSI values were reached in Zone 2, between annual SST ranges of 16.9°C – 19.4°C. Zones 3 and 4 showed similar lowest annual GSI levels, but at different annual SST ranges. Zone 3 annual GSI values were reached at annual SST ranges of 17°C – 18°C, whereas Zone 4 annual GSI values were reached at annual SST ranges of 18.6°C – 19.8°C.

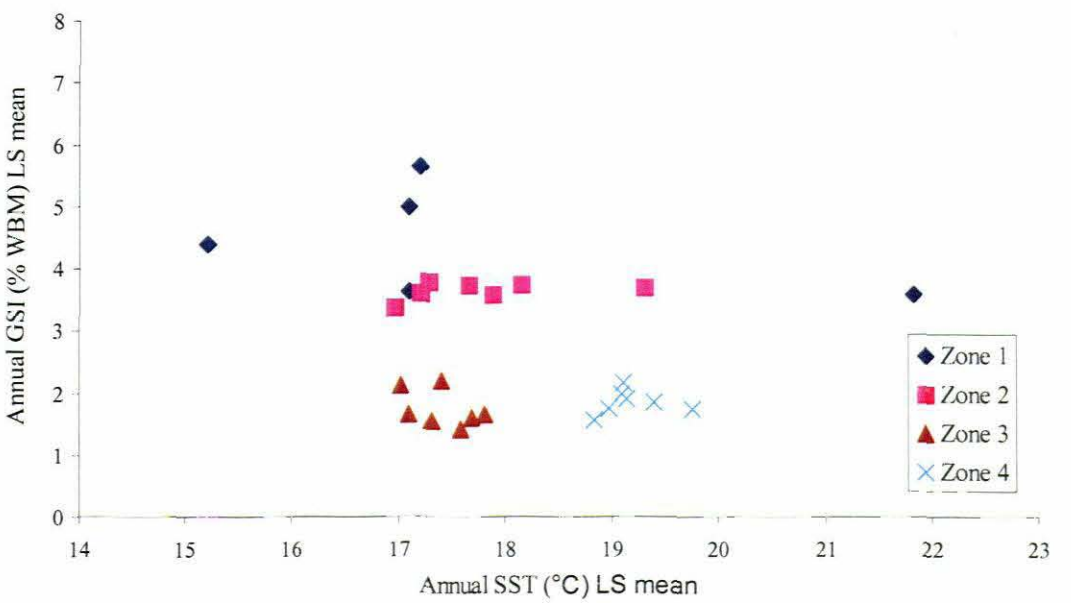


Fig. 3.21 Scatter plot of annual GSI LS mean (% WBM) versus annual SST (°C) LS mean for each of the four zones, for the series 1997 to 2003.

3.5.10 The seasonal variability between GSI and chlorophyll a LS means for the time series December 1997 to July 2003:

High GSI levels were associated with high Chl a levels, which occurred during the summer months. Low levels of GSI and Chl a were reached during April and July respectively (Fig. 3.22).

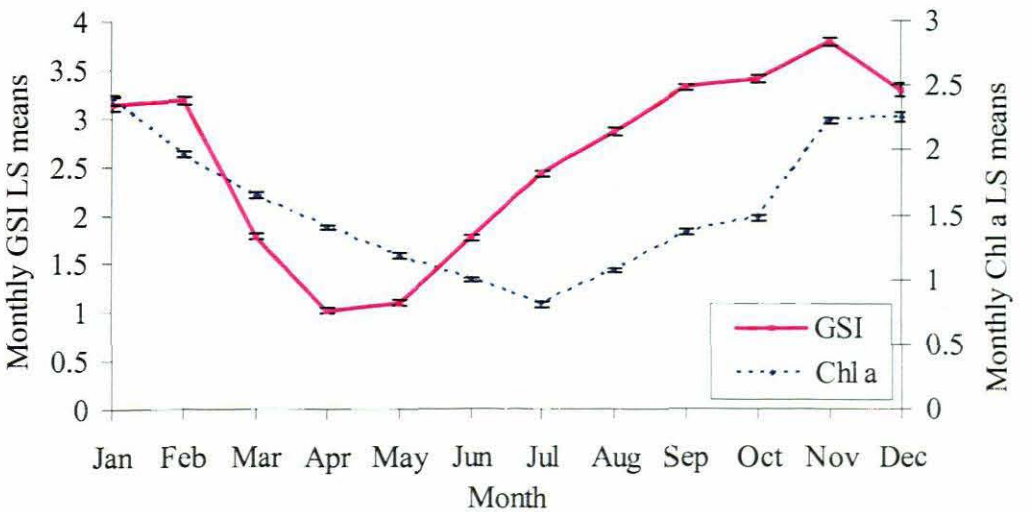


Fig. 3.22 - Seasonal variability in GSI (% WBM) and Chl a (mg/m³) LS means for the time series December 1997 to July 2003, all zones combined. Standard deviations are shown.

Lags were tested, with the following results:

Linear regression results in Table 3.21 show that GSI lags Chl a significantly by two months.

Table 3.21 – Lag results between GSI and Chl a.

Parameter	No lag	1 month lag	2 month lag	3 month lag	4 month lag
R Square	0.28	0.62	0.81	0.55	0.156
Significance (p)	0.07	0.002	< 0.001	0.005	0.202

3.5.11 Interannual relationship between GSI and Chl a LS means for the time series December 1997 to July 2003:

Interannual relationships between GSI and Chl a could not be observed (Fig. 3.23).

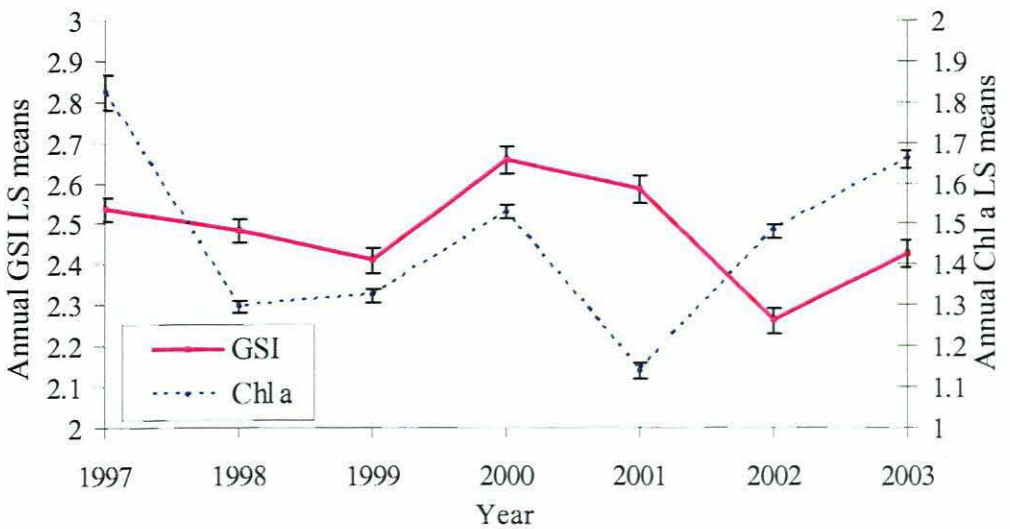


Fig. 3.23 - Annual variability in GSI (%WBM) and Chl a (mg/m³) LS means for the time series December 1997 to July 2003, data from all zones combined. Standard deviations are shown.

3.5.12 Zone specific interannual relationship between GSI and Chl a LS means for the time series December 1997 to July 2003:

Spatial differences between annual GSI and annual Chl a were observed between Zones 1, 2 and 3 and Zones 1, 2 and 4, but not between Zones 3 and 4 (Fig. 3.24). There were no significant linear relationships derived between annual GSI and annual Chl a in any one of the four zones. Zone 1 showed the highest annual GSI values and between a wide Chl a range of 1.4mg/m³ - 3.5mg/m³. Zone 2 showed the second highest annual GSI values and between a wide annual Chl a range of 1.9mg/m³ - 3.7mg/m³. Zones 3 and 4 showed similar low annual GSI values results between a narrow annual Chl a range of 0.7mg/m³ - 1.3mg/m³.

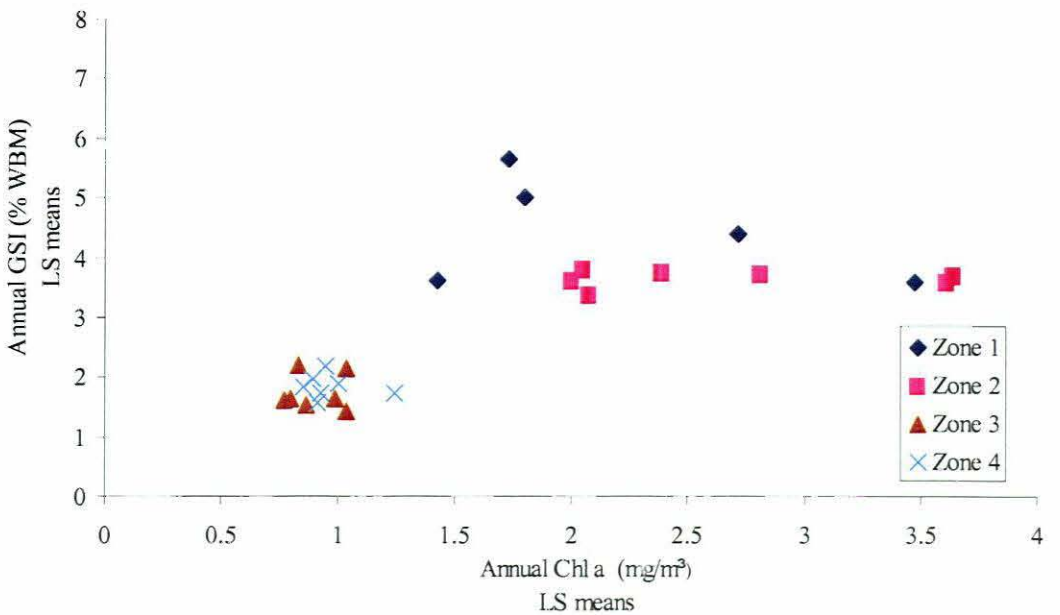


Fig. 3.24 Scatter plot of annual GSI LS mean (% WBM) versus annual Chl a (mg/m³) LS mean for each of the four zones, for the series 1997 to 2003.

3.6 Objective 5: To assess the fat ranking method (as an indicator of body lipid content) against a different method determining fish condition.

The GLM output for lipid content explained 34% of the observed variance, whereas that for morphometrically-derived CF explained 16% of the observed variance. The GLM for lipid content indicate that most of the variance was explained by Month and then by the interaction between Year and Month and the GLM for CF indicate that most of the variance was explained by the interaction between Year and Month, followed by Month.

3.6.1 GLM results: Lipid content

R-Square	Coeff Var	Root MSE	Lipid percentage Mean
0.341229	51.61579	2.381688	4.614263

Source	df	Type III SS	Mean Square	F Value	p (> F)
Year	7	4 067.55	581.07852	102.44	<0.0001
Month	11	50 422	4 583.81858	808.09	<0.0001
LC	1	1 156.258	1 156.25829	203.84	<0.0001
Year*Month	74	10 515.48	142.1011	25.05	<0.0001

3.6.2 GLM results: CF.

Table 3.22 – GLM results for CF

R-Square	Coeff Var	Root MSE	Kreiner_CF Mean
0.157687	10.85436	0.109243	1.006447

Source	df	Type III SS	Mean Square	F Value	p (> F)
Year	7	5.643514	0.80621631	67.56	<0.0001
Month	11	24.55066	2.23187843	187.02	<0.0001
LC	1	0.625404	0.62540434	52.4	<0.0001
Year*Month	74	28.48756	0.38496696	32.26	<0.0001

3.6.3 Assessing normalized monthly LS means of lipid content against normalized CF monthly LS means for the time series 1996 to 2003:

Normalized values for both lipid content and CF were used to best compare variation between the two parameters. Normalized values for both lipid content and CF were calculated according to both lipid content and CF mean value of all monthly LS means, as follows:

Normalized value = Monthly LS mean / mean of all Monthly LS mean values.

Normalized monthly LS mean values of lipid content and CF showed a different trend from February to April (Fig. 3.25) During that period lipid content showed an upward trend, whereas a downward trend was observed in CF. Minimum values reached in lipid

content and CF were also different: lipid content reached a minimum in the seasonal cycle, during the months August and September, whereas for CF, a minimum was observed in July and August.

Seasonal peaks were different between lipid content and CF. Lipid content seasonal peak was observed in May, whereas CF showed a seasonal peak in December.

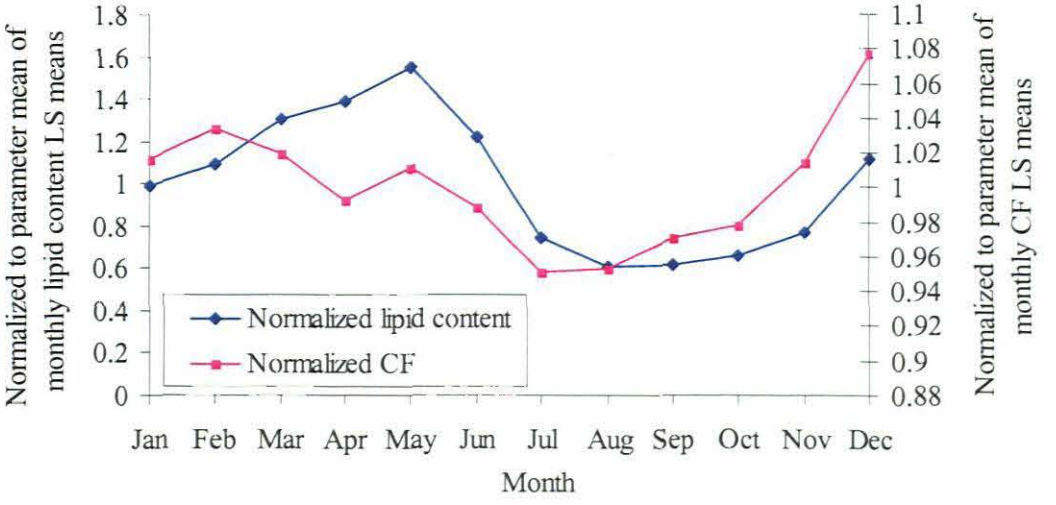


Fig. 3.25 - Normalized monthly LS means of lipid content against standardized CF, according to the mean of monthly LS means for lipid content and CF for the time series 1996 to 2003, all data combined.

3.6.4 Assessing normalized annual LS means of lipid content against normalized CF annual LS means for the time series 1996 to 2003:

Normalized values for both lipid content and CF were also calculated according to both lipid content and CF mean value of all annual LS means, as follows:

Normalized value = Annual LS mean / mean of all Annual LS mean values.

Fig. 3.26 showed similar interannual trends for lipid content and CF.

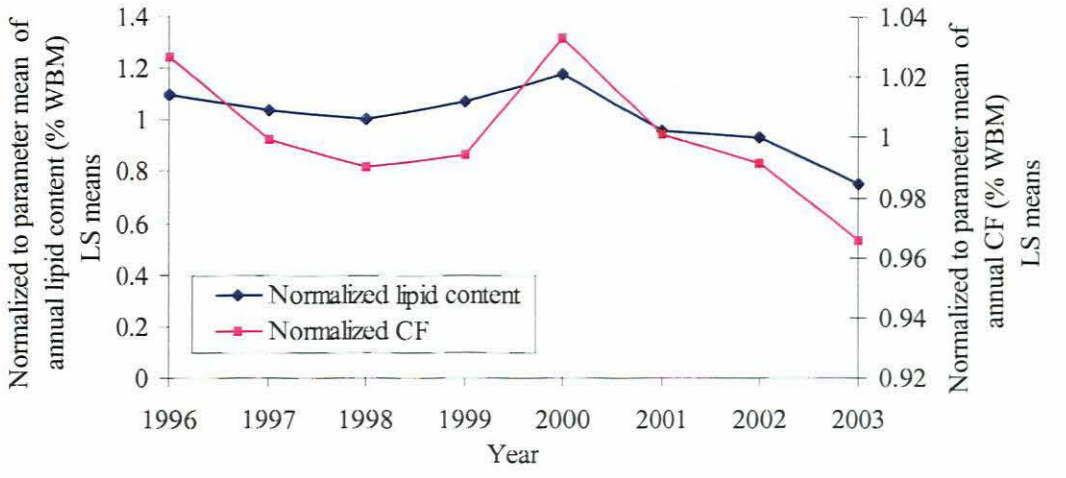


Fig. 3.26 - Normalized annual LS means of lipid content against standardized CF, according to the mean of annual LS means for lipid content and CF for the time series 1996 to 2003, all data combined.

3.6.5 Assessing zone specific relationships between lipid content and CF:

Significant relationships were derived between lipid content and CF, for each of the four zones, although the variances explained was low, especially for Zones 1, 2 and 3. The low variances could be due to the high number of data points (Fig. 3.27).

The slope angles differed from the one Zone to the next, which indicates that some differences occur between results from the two methods: lipid content and CF. Fig. 3.25 shows that the method used to determine lipid content is more sensitive.

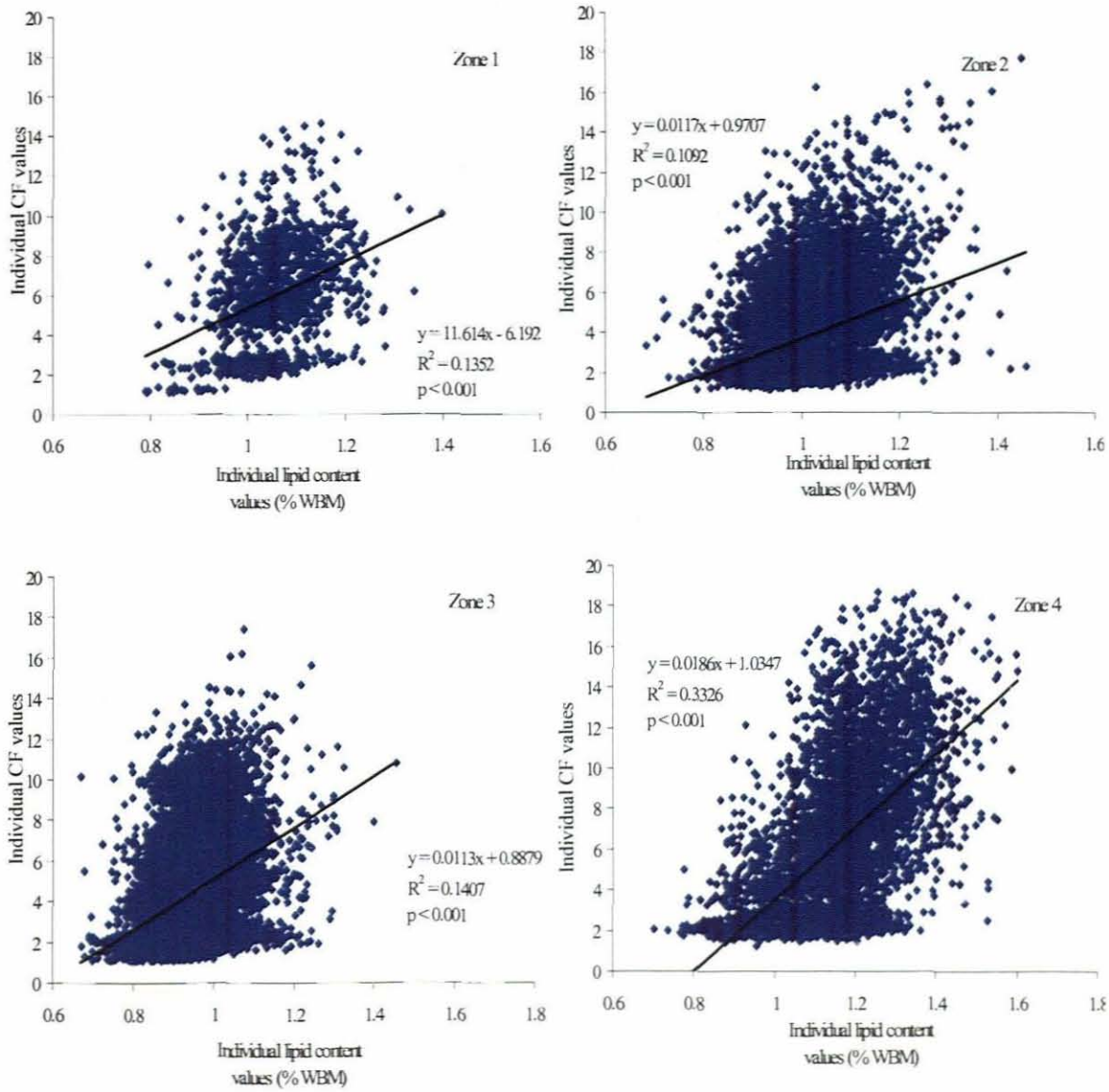


Fig. 3.27 - Zone specific relationship between lipid content and CF, using all the data for the time series 1996 to 2003.

Chapter 4

4.1 Discussion

4.1.1 Summary of objectives and results

The 29051 sardines analyzed were used to assess interannual variation between 1996 and 2003. Only 4% of all fish sampled were caught in Zone 1, while 33%, 45% and 18% were caught in Zones 2, 3 and 4 respectively.

The first objective was to show whether the fat staging method could be used effectively as an indicator of lipid content and accurately to show seasonal and spatial variation in sardine lipid content and GSI. Results showed seasonal trends in both lipid content and GSI (Fig. 3.1). Zone specific results showed that spatial differences were evident for both lipid content and GSI (Fig. 3.3).

The second objective was to investigate the relationship between lipid content and GSI. The results indicate that there was a significant inverse relationship ($p < 0.001$) between lipid content and GSI (Fig. 3.5), as well as for each of the four Zones separately (Fig. 3.6). One of the limitations of this study was that stomach contents were not subtracted from fish mass in order to define somatic mass and therefore GSI more accurately.

The relationships of lipid content and GSI to adult biomass and relative recruitment biomass were addressed in objective 3. Annual lipid content and adult biomass were significantly inversely related ($p = 0.05$) (Fig. 3.8). Lipid content and relative recruitment showed no relationship (Fig. 3.9). GSI and adult biomass also showed a significant

inverse relationship (p = 0.036) (Fig. 3.10). GSI and relative recruitment showed no relationship (Fig. 3.11).

The relationships between sardine lipid content and GSI were tested against SST and Chl a in objective 4. Seasonal results showed that lipid content and GSI lagged both SST and Chl a (Tables 3.18 – 3.21). Annual relationship tests between lipid content and GSI and SST and Chl a showed spatial differences.

The method of fat staging sardines as an indicator of the lipid content of sardine were compared to another method of condition determination, where condition factor (CF) was determined through length-weight relationships, as documented in Kreiner *et al.* (2001). Comparing these two methods were addressed in objective 5. These two methods showed similar interannual trends, but seasonal trends showed different peaks and lows.

4.1.2 Temporal and annual variability

Seasonal variability in fish condition and GSI has been reported before (Davies 1956; van der Lingen *et al.* 1998; Kreiner *et al.* 2001). Kreiner *et al.* (2001) showed highest CF values between February and April and lowest CF values between July and September. GSI from Kreiner *et al.* (2001) were highest between January and March and lowest from April to June. Results from the fat staging method showed differences between peaks in lipid content and CF (Fig. 3.25). Lipid content (according to the fat staging technique used in this study) showed a peak in May, for the time period 1996 to 2003. According to Davies (1956), CF was high during summer months (December-February), reaching a maximum in March, for the time period 1950 to 1954. van der Lingen *et al.* (1998) reported that peaks in lipid content were reached between March and June, for the time

period 1996 to 1997. Although differences were found in peaks for the different time periods between both CF and lipid content, in the above mentioned papers, seasonal variability in both lipid content and CF were clearly evident in all of them.

Seasonal patterns for GSI from this study showed similar trends to the results documented by Kreiner *et al.* (2001). Both studies showed GSI peaks during the summer and low levels during April / May. Davies (1954) and Miller *et al.* (submitted) also documented spawning peaks to be from September to February. Armstrong *et al.* (1989) also showed similar seasonal cycles for GSI for three separate time periods (1953-1964, 1965-1975 and 1976-1987), although overall GSI levels differed between the three time periods. Armstrong *et al.* (1989) and Miller *et al.* (submitted) suggest that spawning may occur throughout the year and declines in GSI might be due to an increase in the interval between batch spawning events rather than a return to an inactive state.

Annual lipid content showed a decline trend from 1996 to 2003, which was similar to CF results from Kreiner *et al.* (2001), for the period 1985 to 1999. Both this study and Kreiner *et al.* (2001) showed biomass increases during the study periods, which suggest density dependant effects (see below). Interannual GSI trends were not observed during this study period and no interannual trend was found for GSI during the study period of Kreiner *et al.* (2001) either. Armstrong *et al.* (1989) and van der Lingen *et al.* (submitted) results showed variability in standardized gonad mass, which were suggested to be at least partly density-dependent.

4.1.3 Spatial variability

A change in the slope angle of scatter plots between lipid content versus GSI was observed from Zones 1 to Zone 4 (Fig. 3.6). The results from Zone 4 suggest that sardine in this zone have higher lipid content in relation to GSI than the other Zones. Kreiner *et al.* (2001) reported that "samples landed at Port Elizabeth had different characteristics from those landed elsewhere," but did not say what it was. This study shows that sardine in the Port Elizabeth region have better condition (higher lipid) in relation to the other three zones. van der Lingen and Hutchings (in press) documented that adult anchovy condition increased in an eastward direction over the Agulhas Bank, van der Lingen (2002) attributed this to diet. Sardine consume more zooplankton on the east coast (van der Lingen 2002), which is a superior food source to phytoplankton. Higher absorption efficiency is reported from zooplankton than from phytoplankton (van der Lingen 1998). An increased metabolic rate is associated with an increase in temperature (van der Lingen 1995), and therefore more energy (lipid) would be needed to keep up the required increased metabolic rates.

Lipid content, which is high on the east coast and GSI levels which were found to be low, were not expected. It seems that sardine on the west coast are more efficient in translating lipid into gonads than sardine on the east coast, probably due to the higher metabolic rates of sardines in higher sea temperatures on the east coast (van der Lingen 1995).

It has been reported that sardine do not appear to have a fixed spawning area and are known to spawn throughout their distribution range (Anders 1975; van der Lingen and Huggett 2003). Results from Miller *et al.* (submitted) suggested year round spawning in sardine with two spawning peaks occurring, i.e. September-October and February-March.

An incomplete sample from Zone 1 could not confirm their September-October peak, but their February-March peak was similar to this study. The main spawning period for sardine was suggested to be between August to late March (van der Lingen and Huggett 2003). This study showed similar results to both van der Lingen and Huggett 2003 and Miller *et al.* (submitted) for all four zones.

Miller *et al.* (submitted) proposed that sardine life history strategy could be divided into two systems: firstly a west coast system, where spawning occurs on the western Agulhas Bank and juvenile fish recruit to the west coast and secondly the Agulhas Bank system, where spawning occurs on the central and eastern Agulhas Bank and juveniles recruit to the south coast. It is difficult to speculate which of the systems are best for recruitment, because of the numerous factors involved in determining recruitment success e.g. temperature, offshore lost, food availability, egg mortality, predators, etc. (Crawford 1979 and 1991; Hutchings *et al.* 1998). Miller *et al.* (submitted) suggested that depth of spawning on the west coast and avoiding offshore loss are important factors for successful recruitment. Eggs can be retained on the south coast when released on the eastern Agulhas Bank, but this retention does not necessarily imply successful recruitment, due to the above mentioned factors. The advantage of more than one closed recruitment system was explained by Miller *et al.* (submitted) who showed that when recruitment from one system fails in a given year the other may have more favourable conditions for retention of eggs and larvae in that year. Their model showed that in only two years, of an eight year period was recruitment poor in both systems. For five of the years, poor recruitment in one system was contrasted by good recruitment in the other, thereby ensuring successful recruitment in at least one area.

4.1.4 Lipid content and GSI relationships to adult biomass and relative recruitment.

Lipid content showed a significant negative relationship ($p = 0.05$) to adult biomass (Fig. 3.8). This result indicated that, as in other studies (Kreiner *et al.* 2001) lipid content may be at least partly density-dependent. Both Kreiner *et al.* (2001) and Le Clus (1987) observed condition indices to be low when sardine biomass was high and high when biomass was low. This study supports their results and suggests that condition of fish is to some degree affected by adult biomass. Kawasaki and Omori (1995) reported a decrease in lipid content at high biomass levels for the Far Eastern sardine *Sardinops sagax*, which further supports results from this study.

van der Lingen *et al.* (submitted) documented that marked changes in standardized gonad mass and reduced length-at-maturity at low population size occurred in sardines over the last 50 years. An increased standard gonad mass was observed when population size was low. This study showed a significant negative relationship ($p = 0.036$) from 1996 to 2003 for GSI versus adult biomass (Fig. 3.10), which supports the finding of van der Lingen *et al.* (submitted). van der Lingen *et al.* (submitted), Li *et al.* (1993) and Kawasaki and Omori (1995) hypothesized density-dependant impacts on recruitment success, these data supports this hypothesis.

A relationship between lipid content and GSI to relative recruitment (Fig's. 3.9 and 3.11) could not be found during this study. Recruitment is a complex process and is affected by a variety of factors, particularly the environment, where food availability for larvae, temperature, wind, currents, etc. affects larval survival (Hutchings *et al.* 1998). Because of the complexity of recruitment, this result was not unexpected. High egg and larval

mortality which has lead to high recruitment variability in the past are not uncommon in sardine populations.

This study used the calendar year to calculate annual GSI means. As sardine spawn during austral summer, the spawning year was thus split in half. The annual GSI therefore did not represent spawning activity over one spawning season, but includes two "half" spawning seasons. Results from comparing annual GSI (based on the calendar year) with recruitment would therefore not be accurate. It is suggested for further studies that annual GSI means should be recalculated based on the spawning year (June to June or July to July), rather than use the annual means based on the calendar year.

4.1.5 The relationships between sardine lipid content and GSI to SST and Chl a.

SST is an important parameter in shallower waters of continental shelves where the rates of plankton production are highest and sardine are most abundant. In these regions, upwelling enriches the supply of nutrients to the upper layers of the sea, stimulating the growth of plankton (Armstrong and Thomas 1989).

Seasonal cycles were observed for both SST (Fig. 3.12) and Chl a (Fig. 3.16), which are similar to results published by Demarcq *et al.* (2003). For the entire data series (1996 to 2003), monthly SST means were the highest during the summer (January to March) and the lowest during the winter (July). Chl a monthly averages also showed high values during the summer months and lowest means during July. Lag tests for the seasonal cycle for the whole data series showed the best correlation between lipid content and SST to be at a two month lag ($r^2 = 0.76$; $p < 0.001$) (Table. 3.18). Lag tests between lipid content and Chl a and showed that it took three months to translate peak food (Chl a) into lipid.

Davies (1954) documented that spawning in sardine would take place in favourable conditions. Knowledge of environmental factors, like SST and Chl a (food source), which may prevent, limit or promote reproduction are therefore of considerable importance. Davies (1954) results showed that sardine gonad weights increased quickly when SST rose and spawning decreased when SST dropped. The seasonal cycles for gonads documented by Davies (1954), were similar to results from this study.

A significant negative linear relationship ($p = 0.008$) between SST versus lipid content was observed (Fig. 3.14), which suggested that lipid content is at least to some degree affected by SST. This result can be linked to the higher energy levels required at higher temperatures, due to increased respiration rates, more active feeding and swimming behaviour, etc. as documented by van der Lingen (1995). These data do not seem to agree with the monthly variation data e.g. in Fig. 3.12 there is a lag but not for the entire year as from September to February as SST increases so lipid content increases. But there is a much stronger signal shown with lipid and GSI, therefore reproductive patterns of sardine may be more closely linked to body lipid content and therefore overshadow any SST effect.

Significant spatial differences were observed between annual SST and annual lipid content (Fig. 3.15), for Zones 3 ($p = 0.01$) and 4 ($p = 0.009$). SST had a strong effect on lipid content in Zones 3 and 4. Lipid content values were the highest in Zone 4, also at highest SST levels. Annual SST means in Zone 1 ranged between about 15°C to over 21°C during this study. This wide SST range in Zone 1 is most probably due to upwelling on the west coast and wind induced turbulence (Demarcq *et al.* 2003). Unfavourable

SST's, which may occur during prolonged South Easterly winds, might have a detrimental effect on eggs and larvae in the area (Armstrong and Thomas 1989).

Zone specific interannual relationships between lipid content and Chl a LS means also showed marked spatial differences (Fig. 3.18). Probably most notable of these results was observed in the results of Zone 4; at lowest Chl a values, lipid content values were the highest. This result supports the suggestion by van der Lingen (2002) that sardine on the east coast has a different and more superior diet than sardine on the west coast.

Lag tests for the seasonal cycle for GSI for the whole data series showed the best correlation to SST at a three month lag ($r^2 = 0.78$; $p < 0.001$) (Table. 3.20). Lag tests for the seasonal cycle for GSI for the whole data series showed the best correlation to Chl a at a two month lag ($r^2 = 0.81$; $p < 0.001$) (Table 3.21). Chl a concentrations varied between the four zones, which was supported by Demarcq *et al.* (2003). Highest Chl a values were associated with the west coast upwelling region. It is difficult to explain the Chl a versus GSI lags, but the answer probably lies in SST effects on both GSI and Chl a peaks and food availably for larvae, in this area, which is considered to be one of the most productive nursery areas (Miller *et al.* submitted).

Spatial differences were observed on scatter plots between the four zones for GSI versus SST, but significant linear relationships were not observed between these two parameters (Fig. 3.21). It can be noted that annual GSI values were equally low (at different SST means), compared to annual GSI values from Zones 1 and 2, and from this result, it could be speculated that Zones 1 and 2 are more productive spawning areas, than Zones 3 and 4, supporting the suggestion by Miller *et al.* (submitted) that the lower west coast system

would be a better spawning area if offshore loss could be avoided. Miller *et al.* (submitted) documented that eggs and larvae contained in an intermediate depth of 25-50m proved to be important, to avoid both Ekman drift (offshore loss) and deep cold water.

Although strong spatial differences were also observed on scatter plots between the four zones for GSI versus Chl a, significant linear relationships were also not observed, within each of the four zones, between these two parameters (Fig. 3.24). Reasons for this are difficult to explain.

4.1.6 CF versus lipid content

The method used to determine CF assumes that if CF is high, then lipid content would be high. CF does not measure lipid content. The GLM results for lipid content (Table 3.7) explained 34% of the observed variance in lipid content, whereas the GLM for CF (Table 3.22) explained 16% of the observed variance in CF. Normalized data for both lipid content and CF showed that lipid content had a higher resolution than CF, which made the lipid content method more sensitive to changes in the seasonal patterns. This made trends easier to follow (Fig. 3.25). Scatter plots of CF versus lipid content showed that CF explained a relatively low percentage of the variance in predicted lipid content, i.e. 14%, 11%, 14% and 33% for Zones 1, 2, 3, and 4, respectively (Fig. 3.27). Fig. 3.27 within each zone there seems to be two groupings of data points, a top "wide group" and a bottom "narrow" group. This probably has something to do with post spawning fish and within year variation and needs further investigation. The CF method of determining fish condition uses length-weight data. Bolger and Connolly (1989) reported that care should

be taken when assuming that fish with heavier weight of a given length are in better condition. Changes in condition, based on length-weight data could be reflected easily, by normal seasonal fluctuations in metabolic balance, patterns of maturation and even the state and fullness of the alimentary canal.

Chapter 5

5.1 Conclusions

Lipid content in fish has been widely acknowledged to be a good indicator of the general condition of a population (Bolger and Connolly 1988; Patterson 1992). The fat staging method, as a predictor of lipid content is shown to be an accurate method to monitor the condition of sardine. The condition of sardine in the SBE is now monitored through the fat staging technique, on a real time basis, which is a good indicator of the biological behaviour of the species. The west coast (Zone 1) is known to be a highly productive system, for spawning as well as recruitment (Beckley and van der Lingen 1999; Miller *et al.* submitted). It is unfortunate that data from Zone 1 was very limited; therefore results from this zone are probably not as accurate, although the results from this study were supported by other studies (Davies 1956; van der Lingen *et al.* 1998; Kreiner *et al.* 2001).

A seasonal pattern for lipid content was derived, by using the fat staging method. A long term decline in lipid content was observed during this study. This trend was independently observed by Kreiner *et al.* 2001, which shows that the fat staging method can effectively be used as a quick, accurate and easy method to determine sardine condition. Seasonal spawning cycles were derived, which were similar to results by Le Clus (1989a, 1989b) and Beckley and van der Lingen (1999). Both lipid content and GSI cycles were found to be spatially different. These spatial differences were found to be *inter alia* food and temperature related, which was supported by van der Lingen (1995) and Miller (submitted).

Lipid energy is important in forming ova (Matthews 1964; Millán 1999). A significant inverse relationship was derived between lipid content versus GSI, which supports the findings by Matthews (1964) and Millán (1999). These relationships were also found to be spatially different, which suggests that all areas are not equally productive in terms of spawning and feeding (Miller *et al.* submitted). This suggests that Zones 1 and 2 would be better spawning areas than Zones 3 and 4, provided that offshore loss can be minimized.

Lipid content decreased when sardine adult biomass increased, between 1996 and 2003. This result was expected and was supported by van der Lingen *et al.* (submitted). Similar relationships between relative recruitment biomass versus lipid content and GSI were not found and this was attributed to the highly variable nature of recruitment. GSI values decreased significantly with an increase in biomass, which was supported by van der Lingen *et al.* (submitted).

SST had a significant effect on lipid content. Higher SST levels corresponded to lower lipid content values. These results can be linked to feeding, swimming and respiratory processes, which are increased with an increase in SST (van der Lingen 1995). Spatial differences also occurred between SST versus lipid content.

GSI levels were high when SST levels were high. Fig. 3.19 shows that GSI peaks ranged between the SST ranges of 19°C to 20.5°C which shows that sardine prefer warmer water to spawn. Spatial differences also occurred between SST versus GSI.

It was expected that Chl a values would be high on the west coast, due the productivity of upwelling systems on the west coast. Sardine would have been expected to have similar high lipid values as well in this area. It was expected to see that Zone 4 had lower Chl a values, but it was surprising to see that this Zone contained the highest lipid content values. It has to be surmised that sardine food source on the east coast is superior to that on the west coast. High lipid levels on the east coast did not correspond with high GSI values, which lead to the conclusion that more lipid energy are invested into metabolic processes like feeding and respiratory processes than in forming gonads.

From a fishery point of view; this study has shown that spatial differences occur in both lipid content and GSI. The fishing industry prefers fish which are in good condition. The lipid content method shows when and where sardine with high lipid content occur.

The lipid content method can not be used directly into management procedures (OMP's), only as an indicator of condition status of the stock.

5.2 Recommendations

It has been observed in Zone 4 that although fish were in good condition, GSI remained at low levels. It would be useful to examine lipid content of eggs in Zones 1 to 4, which will give more insight as to whether fish in high lipid content actually do produce more and/or better quality eggs. Laine and Rajasilta (1999) documented on the hatching success of Baltic herring eggs and its relation to female condition. They did laboratory experiments on females of different size-classes, determining individual fish's CF and muscle fat content, as well as the hatching success in relation to that female's determined CF and muscle fat content. Their results suggests that females with a higher CF or fat

content produced eggs which suffered lower levels of early mortality and also had better total survival and hatching success. A repeat of work done by Laine and Rajasilta (1999) would give more insight into whether fish with high lipid content actually do produce more and/or better quality eggs, especially on the east coast.

Chapter 6

6.1 References

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