16

Comparing the coarse- and high-resolution horizontal and vertical egg distribution patterns of three clupeiform species in the southern Benguela ecosystem

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This dissertation is submitted in fulfillment of the requirements for the degree of
MASTER OF SCIENCE
Department of Zoology
UNIVERSITY OF CAPE TOWN
September 2003

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DECLARATION

I hereby declare that unless otherwise stated this dissertation embodies my own work. The data used for this study were collected during a sardine survey in September/October 2000 by Marine and Coastal Management staff under the leadership of Dr Carl van der Lingen. I have carried out all egg sample extraction, counting, staging and analysis of the data by myself at Marine and Coastal Management during the duration of the study.

Marc Perreaux, a French student, used some of the high-resolution data from the CUFES (Phase II experiment 3) to perform a variogram analysis during his internship at Marine and Coastal Management. Excluding the aforementioned case this work has not been submitted for a degree at any other university. Any assistance I received is fully acknowledged and all references have been accurately reported. I lay claim to uncited conclusions contained in this dissertation.

…………………………..

Mbulelo Tomie Dopolo

I dedicate this thesis to the memory of my father Ntondo Dopolo, who did not live to witness my hard work, to my mother Nongenguwe Dopolo for her heroic efforts to make sure I reach my aspirations, my son Pumelele Dopolo, my brother Loyiso Dopolo and my fiancée Lungiswa Ndlovu for their gratifying confidence in me even in moments of stress, which made this MSc dissertation a success.

"… the problem of spatial distribution of plankton, and in particular the problem of patterns, remains the key predicament to modern ecological studies and theories …"

Robert P. McIntosh (1985)

On the patchiness of organisms

"… it surprised me to find, for instance, that patchiness problems are not much different in other ecosystems …"

M.R Reeve (1979)

ACKNOWLEDGEMENTS

The National Research Foundation (NRF) and the Department of Environmental Affairs and Tourism funded this study through the Benguela Ecology Programme (BEP) of the South African Network for Coastal and Oceanic Research (SANCOR). University Council B, Marine Biology Research Institute (MBRI), and Deutscher Akademischer Austausch Dienst (DAAD) Scholarships are all thanked for providing extended financial support to cover my living expenses during the course of the study. Justice Matshili is thanked for his commendable suggestions in drafting application letters for the extended financial assistance. I am indebted to all MCM staff who participated during the collection of the samples for this study. I am grateful to Dagmar Merkle who at times helped using the Surfer® program in creating egg distribution maps. Dr Laurent Drapeau is thanked for his assistance in getting S-Plus commands for general additive models running. Jan van der Westhuizen of MCM is thanked for providing catch data of the three pelagic fish species studied. Thanks to Cathy Boucher for producing Figure 1.2. I owe much thanks to an extraordinary teacher/lecturer, Prof. Rod Bally, who brought me an appreciation for marine ecology. I am grateful to both of my supervisors, Drs Carl van der Lingen and Coleen Moloney for improving the contents and the English through their critical scrutiny of my drafts and for their indulgence, encouragement and willingness to assist me when needed, the completion of this thesis would have been extremely difficult if not impossible without them. I am particularly indebted to Dr Carl van der Lingen for his tireless endeavours to get me to actively participate in some of the projects within the section, Surveys and Fish Behaviour and to get me in contact with experts in the field of my research, in particular, Prof. Dave Checkley of Scripps Institution for Oceanography (SIO) at the University of California, USA, who is the senior co-developer of the continuous underway fish egg sampler. Prof Dave Checkley is very much appreciated for his humble motivations and suggestions for my research. Dr Nicolas Bez of the Centre de Géostatistique, Ecole des Mines de Paris, France, is thanked for being instrumental in applying his geostatistical techniques that he co-developed with Rivoirard. I am sincerely grateful to Prof. John Field who at times gave freely of his time to listen to my ideas and give his perspective, specifically on which statistical techniques to use for the vertical egg data. Dr Larry Hutchings devoted a considerable share of his time to review my dissertation and was particularly ethical in criticising the technical aspects of this dissertation and provided invaluable suggestions on aspects that needed proper scientific presentation. Finally, I owe a special thanks to my friends Buyisile Dyantyi, Mandisi Mrwebi and Mzukisi Tyupu who lent me an odd shoulder when things got hectic at times.

TABLE OF CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

CHAPTER 2

GENERAL MATERIALS AND METHODS

page

CHAPTER 3

HORIZONTAL EGG PATTERNS

page

CHAPTER 4

VERTICAL EGG DISTRIBUTION PATTERNS

CHAPTER 5

RELATING SPAWNING TO TEMPERATURE AND GEOGRAPHIC LOCATION

CHAPTER 6

ABSTRACT

Three fish species, anchovy *Engraulis encrasicolus*, sardine *Sardinops sagax* and round herring *Etrumeus whiteheadi*, co-exist over the western Agulhas Bank (WAB) in the southern Benguela upwelling ecosystem. To date, data on high-resolution distributions in ichthyoplankton in the region is lacking. To address this need, coarse and high-resolution ichthyoplankton data were collected using a California vertical egg tow (CalVET) net and a continuous, underway fish egg sampler (CUFES) for horizontal data, respectively, and plankton pump and a closing WP II net for vertical data. Simultaneous measurements of temperature were recorded during the CalVET net hauls. Eggs from vertically stratified samples were grouped into three categories based on their developmental stage using established protocols.

Volumetric estimates of egg density (eggs.m^{-3}) from on-station CUFES samples were significantly correlated with normalized areal (eggs.m^{-2}) estimates of egg density from CalVET net samples at these stations for sardine ($r = 0.91$), round herring ($r = 0.80$) and anchovy ($r = 0.73$), demonstrating the validity of the CUFES sampling technique. Underway sample collection reduced the survey coefficient of variation for sardine and round herring eggs but not for anchovy eggs.

Horizontal egg distribution patterns for anchovy showed that high egg densities were centered at two specific regions: midshelf (east of Cape Point) and offshore in the southern region of the study area, whereas those of sardines and round herring were more continuously distributed above and beyond the 200 m isobath. Egg abundances for all species generally increased from inshore towards offshore. The mean egg densities for all three species were found to be spatially associated, whereas the station by station distribution patterns of anchovy eggs were spatially distinct from sardine and round herring.

Eggs of all three species were concentrated near the surface (0-20 m), although proportions varied among species. All developmental stages of anchovy egg were confined close to the surface indicating that this species spawns near the surface. Sardine and round herring vertical egg profiles varied ontogenetically, even though all developmental stages were concentrated near the surface. A significant (> 50%) proportion of early egg stages were widely distributed between 0-60 and 0-80 m for sardine and round herring, respectively. These results therefore show that the spawning depths of these three clupeiforms overlap to a large extent, but the intensity of spawning varied at depth for each species. In general the vertical thermal structure seemed to affect only how deeply the eggs are distributed rather than the mean egg abundance. The differences in vertical egg profiles among and within (with respect to ontogenetic variation) species were found to be statistically significant (Chi-squared test, $P < 0.05$).

Investigations of spawning patterns in relation to temperature, longitude and latitude were used to compare the spawning habitats of the three species. Quotient rule analysis was used to distinguish the preferred temperature ranges of all three species. Round herring spawn over a wider temperature range (16.5-18.1°C) than anchovy (17.4-17.8°C) and sardine (17-18.1°C). Two spawning peaks were apparent for round herring (main peak at 17.2°C and secondary peak at 17.8°C), whereas only one peak at 18.0°C was apparent for anchovy and sardine. Anchovy spawning showed a domed-shape relationship with geographic location and the relationship was viewed to be more informative ($r^2 = 0.13$ longitude, $r^2 = 0.11$ latitude) compared to sardine ($r^2 = 0.08$ longitude, $r^2 = 0.07$ latitude) and round herring ($r^2 = 0.04$ longitude, $r^2 = 0.06$ latitude). These results indicate that anchovy were more specific in their selection of spawning habitat than sardine and round herring.

Generally, these results suggest that the overall spawning habitats of anchovy, sardine and round herring on the WAB overlap to a large extent, but their exact locations of the point of spawning are spatially distinct.

Key words: Anchovy *Engraulis encrasicolus*, Sardine *Sardinops sagax*, Round herring *Etrumeus whiteheadi*, southern Benguela, Eggs, Horizontal and vertical, Distribution and abundance, Spawning, Geographic location, Depth

CHAPTER 1. GENERAL INTRODUCTION

1.1. CLUPEIFORMES: GENERAL OVERVIEW

Clupeiformes are small, fast-growing, planktivorous fishes known to show huge fluctuations through time in their population sizes because of their short life span and huge variability in their recruitment (Blaxter and Hunter, 1982; Shackleton, 1987; Lluch-Belda *et al.*, 1992). Although these fishes are often placed under severe pressure from fishing (Blaxter and Hunter, 1982; Beverton, 1990), studies on fossil fish scales have shown that major changes in clupeiform population sizes could not be entirely accounted for by fishing mortality (Lasker, 1985; Shackleton, 1987). Clupeiform species include clupeids (sardines, herrings, round herrings, sardinellas and menhadens) and engraulids (anchovies). These fish form an essential intermediate trophic level in many marine ecosystems; this trophic level being often dominated by one or at most a few species (Bakun, 1996), which are preyed on by larger marine fish, seabirds and marine mammals (Hunter and Alheit, 1995). In the southern Benguela ecosystem these small pelagic species include anchovy, sardine and round herring (Jarre-Teichmann *et al.*, 1998). The behaviour, life-history traits, and dynamics of the clupeiforms differ in many ways from large pelagic fish species, hence it could be expected that such differences would play an important role in the functioning of the ecosystem (Cury *et al.*, 2000). For example, the collapse of major population stocks that form a crucial intermediate food link within an ecosystem would have detrimental effects on the biological structure and functioning of such ecosystems (Beverton, 1990).

Clupeiforms form a great asset for commercial and socio-economic value in areas where they are harvested (Hunter and Alheit, 1995). Their global contribution to the total marine fish landings has ranged from about 50% in 1950 to over 64% in 1994 (Cury *et al.*, 2000). In 1993, five of the 10 dominant commercially exploited marine fish species landed were Clupeiforms: Peruvian anchoveta *Engraulis ringens*, Japanese and South American sardine *Sardinops sagax*, Atlantic herring *Clupea harengus*, and European sardine *Sardina pilchardus* (FAO, 1995).

Sardines (*Sardinops*) and anchovies (*Engraulis*) are both subtropical species, coexisting particularly in areas of subpolar and tropical mixing, where they constitute some of the most abundant fisheries species in the world (Lluch-Belda *et al.*, 1991). Anchovy and sardine species pairs co-exist in five nutrient rich regions of the world's oceans: Benguela, California, Canary, Humboldt and Kuryoshio (off Japan) (Fig. 1.1, Crawford *et al.*, 1987; Shannon *et al.*, 1996). Round herring (*Etrumeus*) species are known to be distributed along the Atlantic and Pacific coasts of America, the SW coast of Africa, the south coast of Australia, the coasts of Japan, the Galapagos Islands, the eastern Mediterranean and the Red Sea (Whiteheadi, 1963).

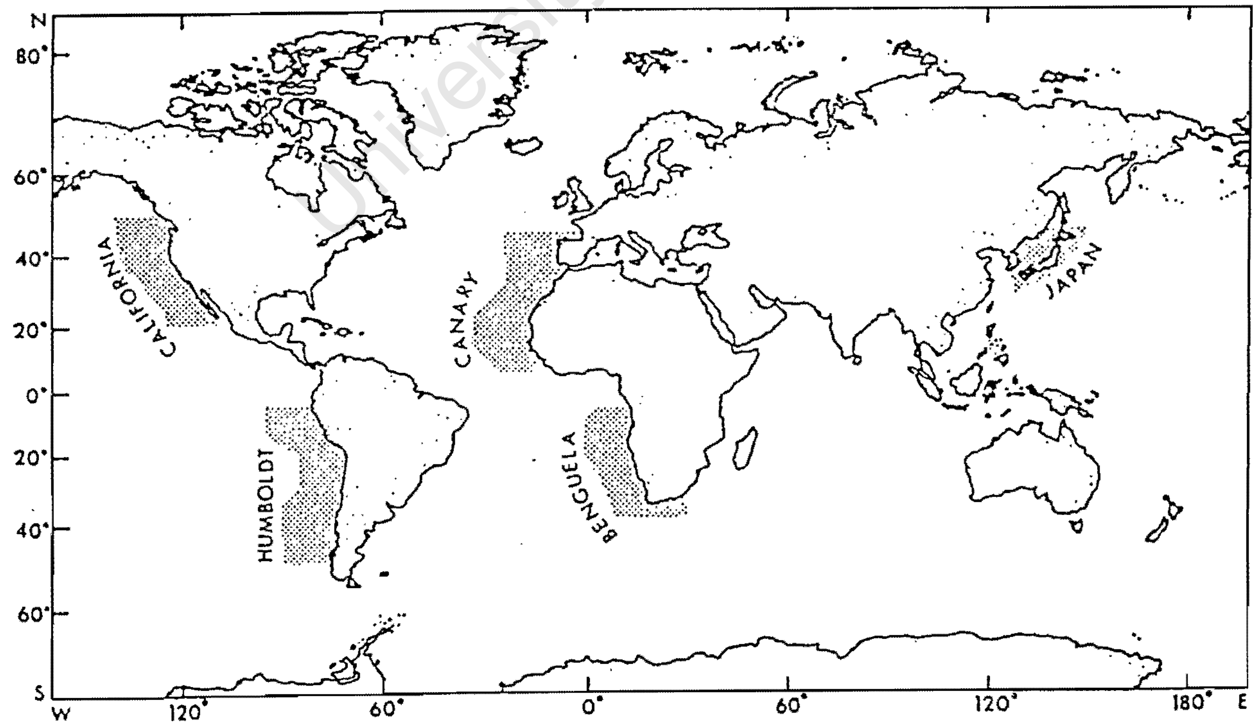


Figure 1.1. Map of the world's ocean basins illustrating the five ecosystems where anchovy/sardine species pairs exist (after Crawford *et al.*, 1987).

The genus *Etrumeus* is distributed along the entire South African coast, where there are two species: *E. teres* (tropical round herring) and *E. whiteheadi* (red-eye or Cape round herring often referred to as round herring) (O'Toole and King, 1974; Roel and Armstrong 1991). The former species is a tropical inhabitant found off the coast of KwaZulu Natal (KZN) on the east coast of South Africa (Indian Ocean). The latter species is a temperature inhabitant often found in water temperatures of 10-20°C. Although anchovy *Engraulis encrasicolus*, sardine *Sardinops sagax* and round herring *Etrumeus whiteheadi* are found in most parts of the South African coastal waters, they are mainly distributed in the rich shelf waters off the southern and western coasts (Fig. 1.2).

1.2. VARIABILITY IN POPULATION SIZES OF CLUPEIFORMS

The population sizes of clupeiforms are known to vary markedly through time (Blaxter and Hunter, 1982; Lluch-Belda *et al.*, 1992), and these fish are considered to be highly sensitive to changes in environmental conditions especially at their early life stages (Hjort, 1914; Houde, 1987; Armstrong and Shelton, 1990). The overall survival of a cohort of developing larvae could be influenced by growth rates, as the duration of the larval stage determines the period over which individuals are at high risk to environmental conditions and mortality (Pepin and Myers, 1991). It is therefore not surprising that slight variations in rates of development, growth and mortality during the egg and larval stages of these fish could result in immense fluctuations in recruitment (Houde, 1987). Variability in population sizes of clupeiforms is observed to occur over two time-scales: decadally (regime shift), and interannually.

1.2.1. Regime shifts

Alternating dominance between anchovy and sardine population sizes over decades has been and still is a subject of controversy among ecologists in the field, and to date no consensus has been reached on the underlying mechanisms prompting this process. However, there is one aspect that most ecologists seem to have common ground about; the expansion and contraction in geographic ranges in population sizes is the manifestation of regime shift (Lluch-Belda *et al.*, 1989; Lluch-Belda *et al.*, 1992; Schwartzlose *et al.*, 1999; Ward *et al.*, 2001). The spawning area of the Japanese sardine *Sardinops melanostictus* has been reported to have expanded from the continental shelf to further offshore with increasing abundance in the biomass of the spawners (Watanabe *et al.*, 1996 and 1997). In the southern Benguela upwelling ecosystem, anchovy distribution range has been observed to be significantly related with the biomass of the species, but not for sardine (Barange and Hampton, 1997).

Despite the controversy related to the subject of regime shifts, several postulations have been forwarded. Many of the fluctuations in pelagic fish stocks appear to have fluctuated in synchrony. For example, changes in *S. sagax* along the five heavily fished regions (Benguela, California, Canary, Humboldt and Kuroshio (off Japan) systems), from a regime dominated by either sardine or anchovy to a high level of abundance of the other have occurred synchronously (Kawasaki, 1983; Lluch-Belda *et al.* 1989; Crawford *et al.*, 1991 in Lluch-Belda *et al.*, 1992). This phenomenon of abundance-related expansion and contraction in geographic range has been observed for several clupeiform stocks (Murphy, 1967; Watanabe *et al.*, 1996 and 1997). In the southern Benguela ecosystem, van der Lingen *et al.* (2001) reported that sardine biomass has shown a steady increase since 1984 and that of anchovy a substantial decline, despite large fluctuations. This prompted Crawford (1998) to speculate that the southern Benguela region might be experiencing a regime shift, but this has still to be seen through time. More recently, Marine and Coastal Management unpublished data indicate that anchovy biomass has been consistently high for three successive years since 2000 until 2002, while that of sardine has continued its steadily increase.

Synchronous fluctuations in *S. sagax* population sizes have been attributed to global warming (Kawasaki, 1983; Lluch-Belda *et al.*, 1992). Those authors postulated that the disruption of the thermohaline circulation of the oceans would cause an increase or decrease in spawning and distribution ranges of either species. Verheye *et al.* (1998) and Schwartzlose *et al.* (1999) have suggested that continual modification of the environment (e.g. a change in food composition) would favour one of the species over the other. Most recently, Schwartzlose *et al.* (1999) suggested that episodic events that trigger changes in population numbers or the ambient environment that could lead to an altered dominance regime. For example, an episodic event could result in a formation of strong year class would favour rapid and enhanced population growth and production rate as observed for sardine off Japan (Kondo, 1980). In the southern Benguela upwelling ecosystem, Berruti and Colclough (1987) postulated that the strong year-class (two-year old) of sardines in 1983could have led to enhanced population growth. Similarly, mass mortalities could occur; after the mass mortality of Australian sardine caused by a virus or bacterium (Griffin *et al.*, 1997). After the mass mortalities, the adult population size and egg and larval distributions were severely reduced, whereas the adult population size, eggs and larval densities, as well as spawning areas of anchovy were enormously increased (Ward *et al.*, 2001).

1.2.2. Interannual variability

Inter-annual fluctuations in clupeiform population sizes are largely attributed to recruitment variability (Hunter and Alheit, 1995), resulting from variable survival of eggs and larvae. Early studies in Europe suggested that recruitment strength in fish is determined during the "critical period" of post larval first feeding (Hjort, 1914; Parrish *et al.*, 1981). Since then, extensive interest has been focused on egg and larval ecology of fishes in an attempt to understand the mechanism of recruitment. Despite the fact that the "critical period" hypothesis is still highly regarded, field investigations on links between feeding conditions at the time of first feeding and larval survival are not decisive (Anderson, 1988).

Since then, more hypotheses have been suggested in an effort to derive generalisations that could be useful for prediction models. Among these is the "match-mismatch" hypothesis Cushing (1972; 1973), which considers the coincidence of timing of spawning by fish and production of plankton, and how this affects the survival of the larvae. It is proposed that if the annual phytoplankton bloom (i.e. "spring" bloom) and subsequent copepod nauplii production occurs too early or too late, fish larvae will be mismatched with food supply and high mortality and poor recruitment would result (particularly for systems in high latitudes).

Lasker (1975) established the "stability" hypothesis, which predicts that the survival of larvae is determined by the presence of a sufficient concentration of food that is found during calm oceanographic conditions when plankton patches are intact so that the encounter rate between larvae and high concentration plankton layer is maximal.

Too much turbulence disrupts the concentration layer and encounter rate is reduced, but moderate turbulence increases the encounter rate of predator and prey (Frank, 2001; Megrey and Hinckley, 2001; Seuront *et al.*, 2001). Parrish *et al.* (1981) "larval transport" hypothesis proposes that variations in prevailing currents would result in variable egg and larval survival as these reproductive products are either retained in or advected away from nursery areas. Peterman *et al.*'s (1988) established that larval abundance does not seem to be correlated with subsequent recruitment, thus the extent to which starvation alone controls larval survival and subsequent year-class strength is not substantiated.

Cury and Roy (1989) proposed the "optimal environmental window" (OEW) hypothesis, which proposes a dome-shaped relationship between recruitment success and upwelling intensity, where moderate upwelling provides the best suite of feeding and advective processes for recruitment success. The OEW hypothesis stresses that both too little and too much upwelling could be detrimental to recruitment success. Too little upwelling would lead to little production that could lead to poor egg and larval condition, whereas, too much upwelling would lead to too much turbulence that would disrupt food concentration layer. This concept has been evident in clupeiform stocks off Peru, West Africa and California where a consistent environmental "window" exists wherein optimal wind speeds of about 5-6 $m.s^{-1}$ result in enhanced annual recruitment (Cury and Roy, 1989). In the southern Benguela the OEW hypothesis has been supported by Waldron *et al.* (1989), who showed a domed relationship between annual potential new production and anchovy spawner biomass. However, recent events of both high and weak upwelling have been observed in the southern Benguela (Roy *et al.*, 2001) and, contrast to the OEW hypothesis, high recruitment for both anchovy and sardine has been observed. This shows that the OEW concept alone not can be used to generalise recruitment variability, and again stresses the importance of the "match-mismatch" hypothesis.

Bakun (1993) later proposed a triad hypothesis to explain recruitment variability, which entails the interaction of three factors to promote growth and survival of eggs and larvae, and therefore increase their recruitment success. The factors are: (1) enrichment by physical processes such as upwelling, (2) concentration of food particles to enhance the encounter rate between prey and predator through stable ocean conditions or strong convergence in frontal zones, and (3) larval retention in, or transport to, suitable areas (Bakun, 1993). The "triad rule" still holds a prominent feature in the explanation of recruitment variability (Bakun and Parrish, 1991).

1.3. PELAGIC FISH SPECIES IN THE SOUTHERN BENGUELA ECOSYSTEM

1.3.1. Fishery

1.3.1.1. *Past and present*

Anchovy *Engraulis encrasicolus* (Linnaeus, 1758) formerly known as *E. capensis* (Gilchrist, 1913), sardine *Sardinops sagax* (Jenys, 1842) and round herring *Etrumeus whiteheadi* (Whiteheadi, 1963) are commercially important clupeiforms in the southern Benguela (Crawford *et al.*, 1987; Armstrong & Thomas, 1989; Armstrong *et al.*, 1991). Overall landings in the pelagic fishery for all species have averaged some 360 000 tonnes per annum for the past decade, and the aforementioned three species contributed more than 90% of the landings (Geromont *et al.*, 1999).

The exploitation of pelagic fish species dates back to 1935, but their commercial exploitation only began in 1943 to meet wartime demand for canned fish (Butterworth, 1983; Cochrane *et al.*, 1997). Major developments in the industry only began in the 1950s (Geromont *et al.*, 1999).

Generally, clupeiforms are caught using large nets that are set around visible shoals of fish. In particular, in the southern Benguela upwelling ecosystem are caught using purse-seine nets, and fishing generally takes place at night. Each night a fleet of purse-seiners goes out from harbours, which are often located near the areas where fish tend to aggregate to locate dense aggregations of target fish. Once target fish shoals are located, a curtain of purse-seine net is launched into the water column and the target shoal rapidly surrounded. When the net has surrounded the shoal, the bottom of the net is enclosed by means of a foot-rope and then the head-rope is slowly hauled alongside the vessel. The catch is then sucked out of the net with a suction pump, direct into the icy hold of the vessel. The purse-seiners return to harbour where various factories process the catch (Armstrong and Thomas, 1989; Beckley and van der Lingen, 1999).

Beach-seine netting is another fishing gear used to a lesser extent to capture those species that come very close to the shore, such as anchovy and sardine; mainly in the southern KwaZulu-Natal (KZN) and some parts of the west coast (Lambert *et al.*, 1994, 1997; Beckley and Fennessy, 1996). Generally, one end of the net is held ashore and a small boat is used to take the net encircle the target fishes, which are close to the beach. When the boat gets to the shore, the net is pulled in by hand, by the netters. In some years during the 'sardine run' when the coastal waters of the

KZN coast are cool, sardines come very close to the shore and get easily accessible to people in the surf zone and people use baskets, dipnets or whatever means to catch the sardines (Beckley and van der Lingen, 1999).

Harvested fish are processed for various purposes in different ways. Fresh, frozen and canned sardines in good condition are used for direct human consumption and to a large extent frozen sardines are also used as bait. To a lesser extent sardines in bad condition are processed for fish meal and oil extraction. Anchovies and round herrings are mainly processed for fish meal and oil extraction (Beckley and van der Lingen, 1999). However, round herring has a potential to canned if it could be caught by vessels with cooling facilities to cater for direct human consumption.

The industry was initially confined to the St Helena Bay region (Fig. 1.2) when sardine was the main pelagic target species. With the decrease in sardine landings in the mid 1960s, the fishery expanded the area of its operation to the north and south of St Helena Bay (Crawford *et al.*, 1987). Currently, the industry operates from south of Doring Bay on the west coast to west of Cape Agulhas on the south coast and around Port Elizabeth on the south-east coast (Fig. 1.2). A small fishery off the KwaZulu-Natal (KZN) coast operates during the annual "sardine run" in the form of beach seine-net fishing, but this fishery is considered to make an insignificant contribution in terms of catches (Armstrong *et al.*, 1991). Extensive fishing for anchovy occurs mainly in the area between Doring Bay and Cape Columbine, whereas most fishing for sardine occurs south of Cape Columbine and off Gans Bay on the south-west cape coast (Fig. 1.2). Round herring directed fishing is confined to the west coast area, mainly between Cape Point and Cape Columbine. However, a significant proportion of juvenile round herring fish are caught north of Cape Columbine together with anchovy and juvenile sardine (Marine and Coastal Management unpublished data).

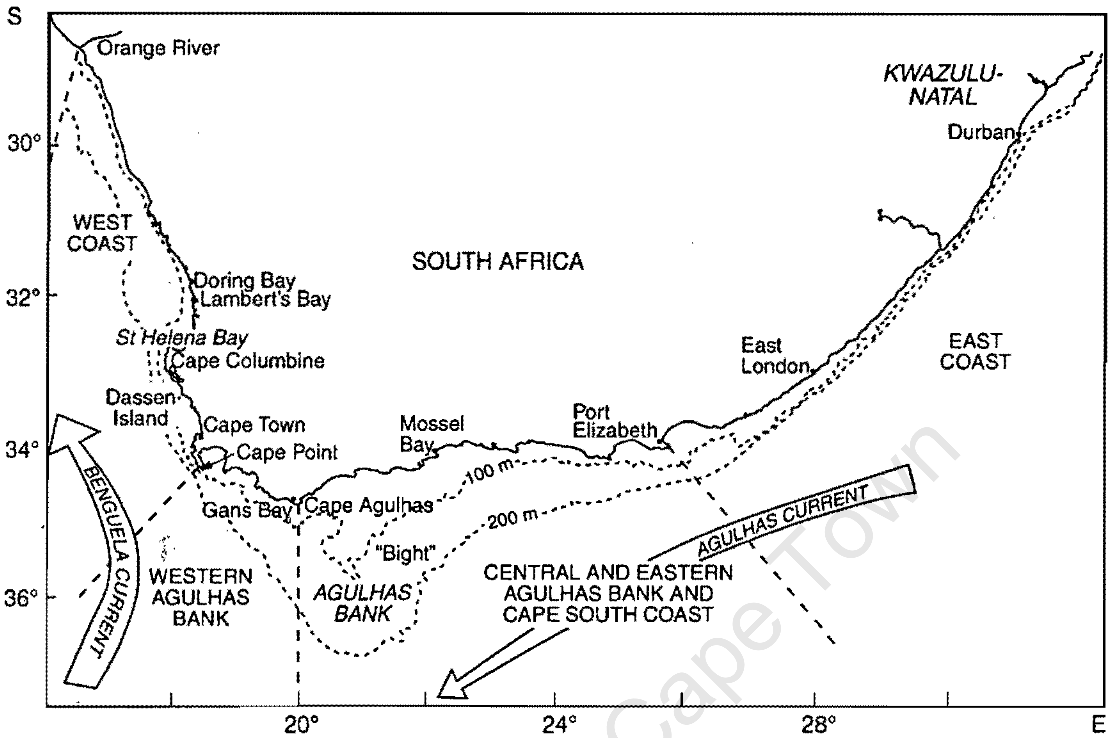


Figure 1.2. South African coastline map illustrating the spatial distribution of fishing grounds for pelagic fish species and some names mentioned in this dissertation.

1.3.1.2. *Catches*

Pelagic fish landings in South Africa, as in any other parts of the world where these resources are being exploited, have fluctuated from hundreds to tens of thousands of tonnes. Over the period 1956-1963 landings were mainly dominated by sardine (Fig. 1.3), reaching a peak catch of just over 410 000 tonnes in 1963 before collapsing to 16 000 tonnes in 1974. The collapse of the South African sardine fishery is believed to have resulted from intensive fishing and recruitment failure (Stander, 1964). After the collapse of sardine fishery, anchovy directed fishery began in 1964 with the introduction of small mesh (12.7mm) net (Geromont *et al.*, 1999). Since 1964 until 2002, anchovy catches ranged from 41 000-596 000 tonnes and sardine from 16 000-410 000 tonnes. There are no recorded landings for round herring prior to 1966.

Over the period 1966-2002 round herring catches have ranged from 1 300-92 000 tonnes (Fig.1.3).

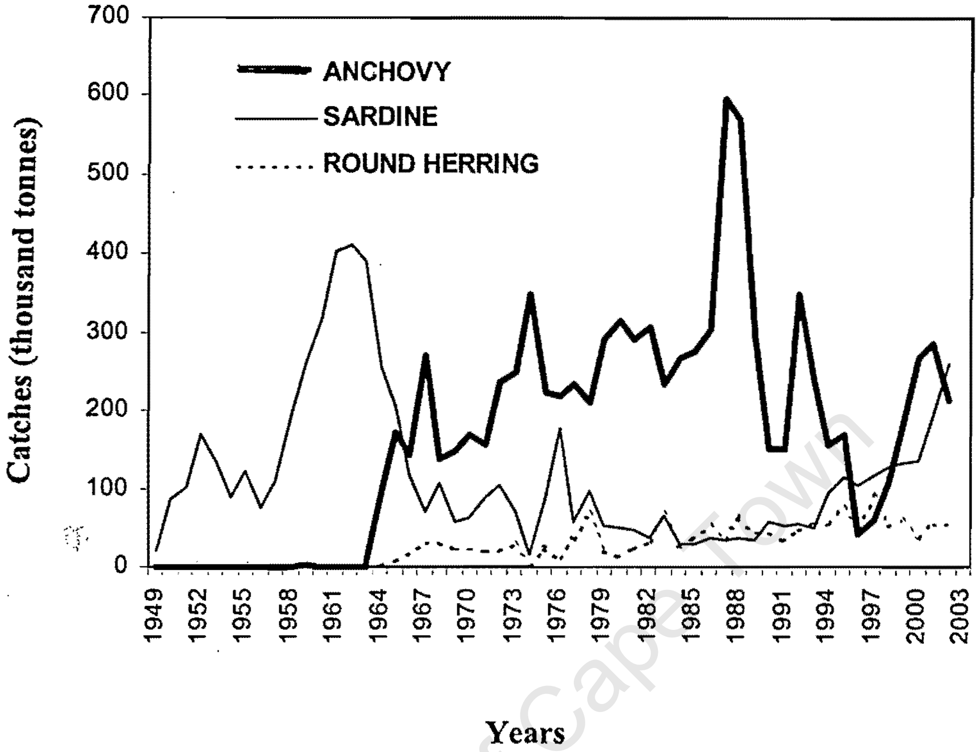


Figure 1.3. South African catches of anchovy *Engraulis encrasicolus*, sardine *Sardinops sagax* and round herring *Etrumeus whiteheadi* over the period 1950-2002 (Marine and Coastal Management data).

The introduction of a smaller mesh net brought about several changes in the pelagic fishery landings since 1964, and included:

i. change in age composition of the sardine catch (from old fish-dominated landings to recruit-dominated landings),

ii. anchovy-dominated landings and

iii. a juvenile sardine bycatch during the anchovy-directed fishing season (de Oliveira *et al.*, 1998).

Maximum catches for both anchovy and sardine within species pairs have never been recorded simultaneously in all the five main ecosystems where these two genera co-exist (Crawford *et al.*, 1987; Schwartzlose *et al.*, 1999; Shannon *et al.*, 1996). Such a phenomenon has been attributed to "regime shifts", where it is hypothesized that only one genus can be abundant in an ecosystem at any given time (Lluch-Belda *et al.*, 1992). In the southern Benguela upwelling ecosystem maximum (410 000 tonnes) sardine catches were made in 1962, whereas maximum (596 000 tonnes) catches for anchovy were made in 1987. Whereas landings made by South Africa's pelagic fishery have historically been dominated by either sardine or anchovy, the catches of both anchovy and sardine species have been simultaneously high in recent years (Marine and Coastal Management unpublished data). This phenomenon has prompted Crawford (1998) to speculate that the southern Benguela ecosystem is experiencing a regime shift. This speculation is based on the hypothesis that such phenomenon is observed during the process of regime shift whereby the biomass of the dominant species remains high while the biomass of the subordinate species concurrently increases (Schwartzlose *et al.*, 1999).

1.4. LIFE HISTORY OF CLUPEIFORMES IN THE SOUTHERN BENGUELA ECOSYSTEM

1.4.1. General features of the southern Benguela ecosystem

The Benguela upwelling ecosystem is one of the world's four (Benguela, California, Canary, and Humboldt) major eastern boundary coastal upwelling systems (Fig. 1.1). These systems are characterized by low diversity of nekton life but with high biomass, but low diversity compared to western boundary systems or tropical areas where species diversity is high. The probable cause for such low diversity may be attributed to the high variability in wind-driven upwelling that causes rapid fluctuations in temperature (Branch *et al.*, 1981), which will prove to be detrimental for species that can not adjust accordingly. Coastal upwelling is a process whereby warm surface waters move offshore under the influence of equatorward winds and the Coriolis force, and are replenished by upwelled deep, cold, nutrient-rich waters (Shannon, 1985). Introduction of these nutrients into the euphotic zone results in phytoplankton blooms, which cause the multiplication of the zooplankton populations as they have plenty of phytoplankton to feed on. Subsequently both phytoplankton and zooplankton are fed on by planktivorous fish such as anchovies (James, 1987), sardines (van der Lingen, 1994) and round herring feeds to a lesser extent in phytoplankton (Wallace-Fincham, 1987). Because of high productivity characteristic of upwelling systems, the southern Benguela upwelling ecosystem is able to support large assemblages of fishes like clupeiforms and other valuable fish species. This renders the southern Benguela ecosystem to be rich grounds for commercial exploitation of pelagic and other fisheries resources.

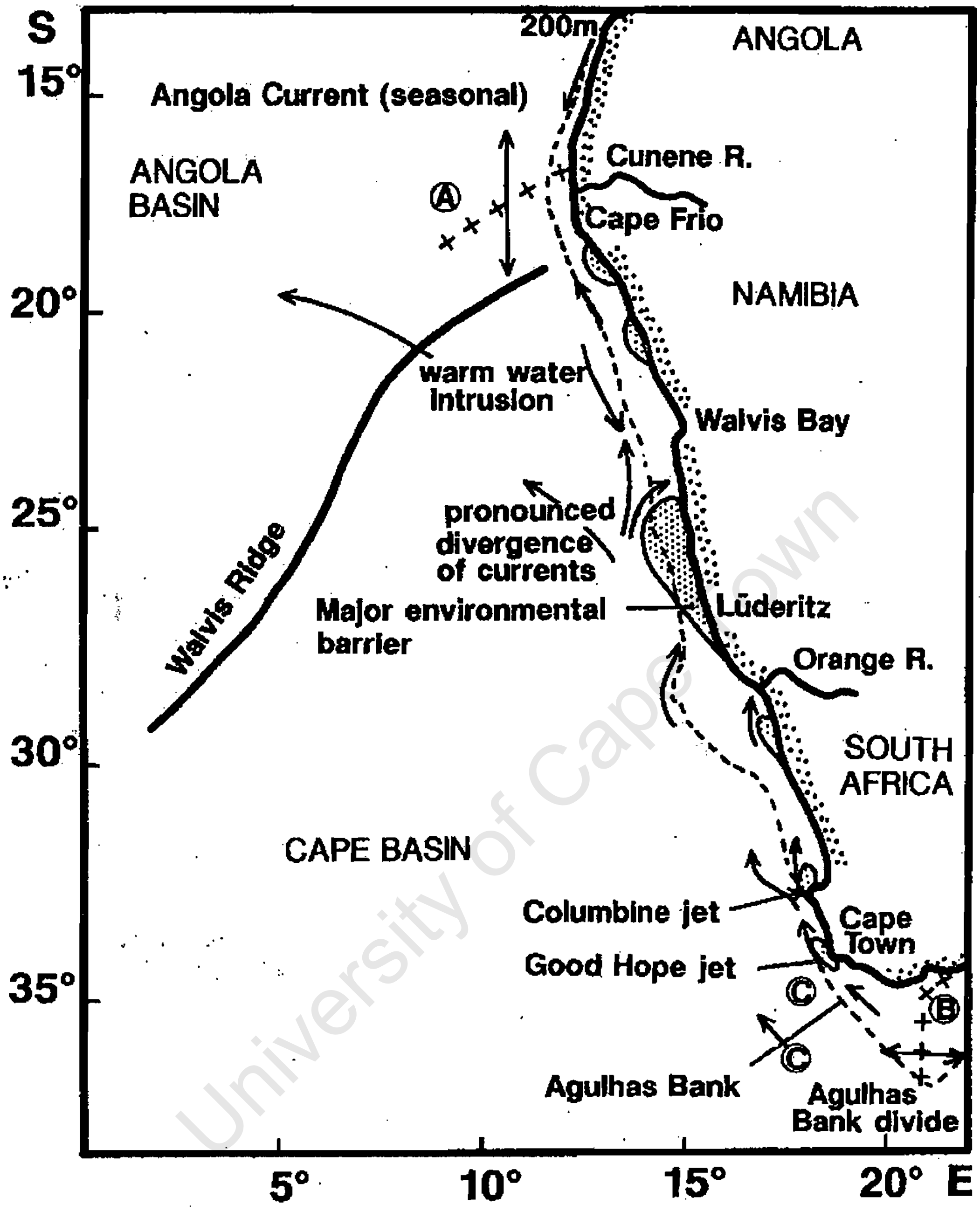


Figure 1.4. Important features of the Benguela upwelling ecosystem (after Shannon, 1985). Shaded areas illustrate upwelling cells. (A) northern boundary (Angola/Benguela front), which shifts occasionally: (B) southeastern boundary of the Benguela that shifts seasonally; (C) rings, eddies, and filaments from the Agulhas Current advected during summer into the southern Benguela ecosystem.

The Benguela upwelling system extends along the west coast of southern Africa, extending from 15°S (Angola) to 35°S (South Africa) and is subdivided into northern and southern Benguela regions. The two regions are said to be isolated by an area of intensive upwelling in the region of Luderitz (26°38′S, 15°09′E), Namibia (Fig. 1.4;

Olivar and Shelton, 1993). The northern Benguela is occasionally (i.e. every ten years on average) "flooded" by tropical waters from Angola and offshore (Bakun, 1996). Upwelling in the southern Benguela is frequent and variable, and the dynamics of the system are driven by wind stress and the penetration of warm Agulhas Current (AC) waters (Shannon, 1985; Bakun, 1996). In addition, the southern Benguela region has an exposed narrow continental margin, with very few embayments (Shannon, 1985; Hutchings *et al.*, 2002), and this might prove to be detrimental to early life stages of many fish species. For example, eggs and larvae are prone to offshore advection by Ekman transport, and this could impact negatively on recruitment success of the species.

1.4.2. Life history of pelagic fishes in the southern Benguela region

The life history of anchovy in the southern Benguela is relatively well studied and understood, and the current hypothesis on the life history of anchovy is shown in Fig. 1.5. The biology and ecology of anchovy has been well documented (Anders, 1965; King *et al.*, 1978; Shelton and Hutchings, 1982; Armstrong *et al.*, 1988; Shelton and Hutchings, 1990; Armstrong *et al.*, 1991; Hutchings, 1992; Beckley and Hewston, 1994; Melo, 1994; Roel *et al.*, 1994; Shannon *et al.*, 1996; Fowler and Boyd, 1998; Huggett *et al.*, 1998; Hutchings *et al.*, 1998; Painting *et al.*, 1998; Richardson *et al.*, 1998; Huggett *et al.*, 2003). That of sardine has been relatively well studied but remains less understood, although it is relatively well documented (de Jager, 1955; Davies, 1957; King *et al.*, 1978; Crawford, 1981; Armstrong *et al.*, 1989; Beckley and van der Lingen, 1999). Very few efforts have been made to study the life history of round herring in the region and it remains less understood and relatively poorly documented (Crawford, 1981b; Shelton *et al.*, 1985; Roel and Melo, 1990; Roel *et al.*, 1994). Roel and Armstrong (1991) provide a good synthesis of the biology and ecology of round herring in the southern Benguela upwelling ecosystem.

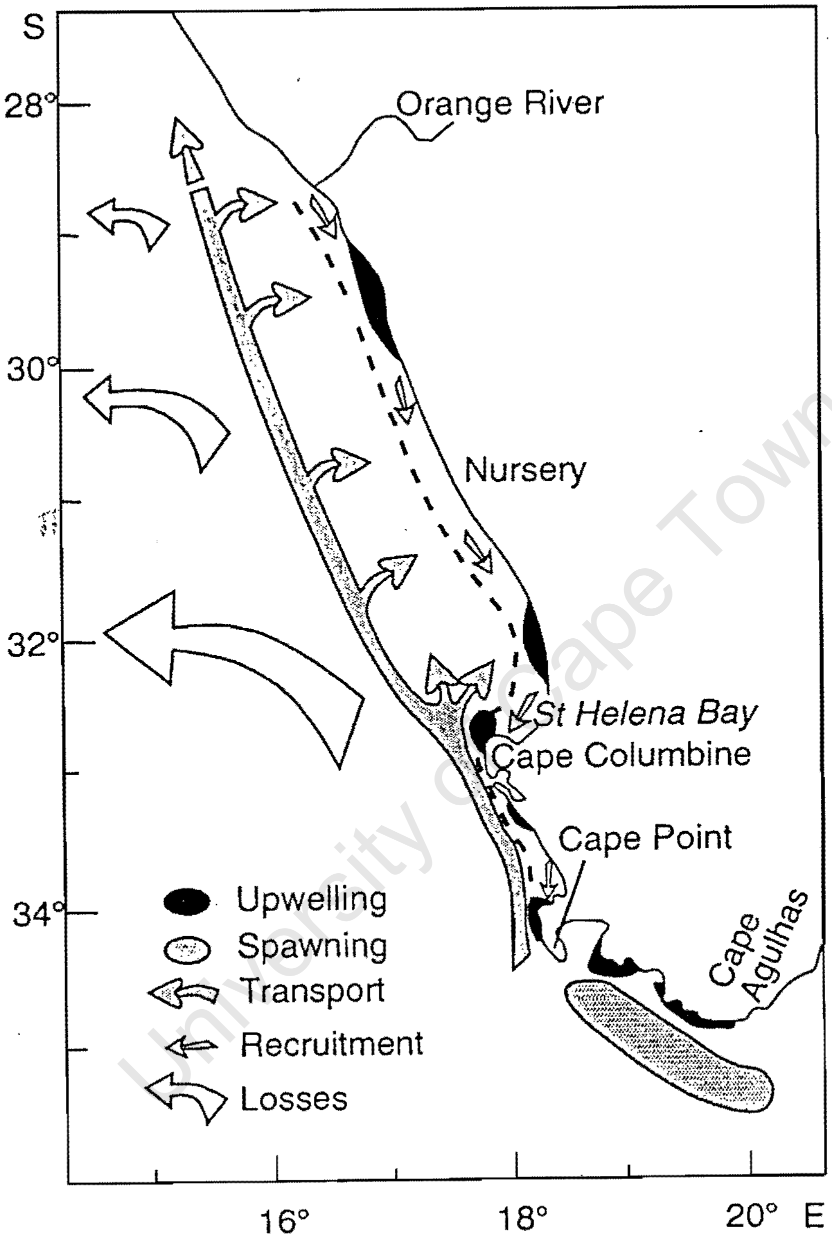


Figure 1.5. Life-history of anchovy in the southern Benguela (after Hutchings, 1992).

The narrow width of the southern Benguela continental margin and the orientation of the smooth coastline, combined with strong southerly winds, subject the eggs and larvae of the pelagic fishes to offshore advection, driven by turbulent mixing and offshore Ekman transport (Shannon, 1985). Generally the reproductive strategy of many pelagic species in the upwelling regions is characterised by distinct spawning and nursery grounds, with the spawning grounds characterised by weak coastal upwelling (Parrish *et al.*, 1981). For example, clupeiforms in the southern Benguela region mainly spawn in the Agulhas Bank region, which is characterised by the absence of intense upwelling activity (Roel *et al.*, 1994). Thus the reproductive strategies of many marine species are adapted to fit the prevailing currents so as to help transport eggs and larvae from spawning to nursery grounds (Parrish *et al.*, 1981). Typically, spawning grounds of pelagic fishes in coastal upwelling regions are "upstream" of the nursery grounds (Parrish *et al.*, 1981; Hutchings *et al.*, 2002)

The African continent does not extend nearly as far poleward as do the Eurasian and two American continental land masses (Bakun, 1996), because of that pelagic fishes in the southern Benguela region are able to migrate around the southern end of the continent to the Agulhas Bank region to spawn. Anchovies spawn from October to March (Shelton and Hutchings, 1982; Melo, 1994) mainly to the east of Cape Point in the WAB region (Shelton and Hutchings, 1989; Hutchings, 1994). Sardines spawn all year round, mainly from August to March (Akkers et al., 1996; Fowler, 1998; Huggett et al., 1998). Round herring is also observed to spawn throughout the year with a winter peak, mainly along the edge of the continental shelf (Shelton et al., 1985; Shelton, 1986; Roel and Melo, 1990).

The Agulhas Bank has ideal temperatures and food supply to ensure sustained spawning (Shelton and Hutchings, 1982; Swart and Largier, 1987; Largier *et al.*, 1992; Hutchings, 1994; Fowler, 1998; Richardson *et al.*, 1998), which promotes rapid development and shortens the exposure of eggs and larvae to predators, and thus enhances their survival (Checkley *et al.*, 1988). Furthermore, the Agulhas Bank has an east-west orientation, as opposed to the north-south orientation of the west coast, so offshore advection is minimised (Hutchings and Nelson, 1985; Shannon, 1966; 1985). Eggs and larvae from the AB are carried upstream to the WC nursery grounds by means of a shelf-edge jet currents (Bang and Andrews, 1974; Shelton, 1979 and 1986; Shelton and Hutchings, 1982; Hutchings, 1992; Hutchings *et al.*, 1998; Hutchings *et al.*, 2002), this movement away from the spawning area reduces cannibalism (Fowler and Boyd, 1998), and transports early life stages to the productive west coast nursery grounds. The jet current is considered to diverge at Cape Columbine into offshore, alongshore and inshore components (Boyd *et al.*, 1992).

A large proportion of the sardine and round herring population, spawns over the Agulhas Bank region during October to November, but their spawning also extends up to the west coast (Roel *et al.*, 1994; van der Lingen *et al.*, 2001; Hutchings *et al.*, 2002). However, spawning on the west coast is likely to be detrimental to recruitment when temperatures are not conducive for optimal development of eggs and larvae. Furthermore, as mentioned previously that the continental margin on the west coast is relatively thin and smooth; so propagules produced in this area are highly susceptible to Ekman transport. Similarly to the small proportion of spawners on the west coast, the proportion of spawners on the east coast is considered to make insignificant contribution to the population the three species (Hutchings *et al.*, 2002).

In general, anchovy, sardine and round herring shoal together during the first year of their life, then diverge in behaviour and distribution as they grow (Crawford, 1980; Roel and Armstrong, 1991). Their juveniles aggregate inshore at the nursery areas off the west coast during autumn and winter before migrating back to the south and south-west coasts to spawn (Crawford, 1980; Hampton, 1987). Adult anchovy and young adult sardine (of comparable size) aggregate towards the eastern boundary of the southern Benguela region, where warm Agulhas Bank water mixes with cooler upwelled waters (Armstrong *et al.*, 1987). Large, mature sardine and round herring extend widely within the habitat (Armstrong *et al.* 1991; Roel and Armstrong, 1991), occurring mostly towards the shelf-edge with round herring occurring in deeper waters than sardine (Roel and Armstrong, 1991; Roel *et al.*, 1994). The seasonal occurrences of sardine at the extremes of the species range off the KZN coast (known as the "sardine run") is believed to be triggered by the expansion and prevalence during winter of cool conditions in that region that are suitable for sardine. Phenomena such as current reversals, upwelling of cool water onto the narrow shelf or even pursuit by predators results in inshore movement of shoals along the shelf between Port St Johns and extending along the KZN coast (Armstrong *et al.*, 1991).

1.5. OBJECTIVES OF THIS STUDY

Horizontal and vertical patterns in ichthyoplankton of marine fishes holds a prominent position in world fisheries research and is among the main methods used to assess the recruitment dynamics, biomass of the spawning adults and capacity of their productivity (Haug *et al.*, 1986). In the southern Benguela upwelling ecosystem transport of eggs and larvae to, and retention in, in suitable environments seems to play a major role in the recruitment success of anchovy (Shelton and Hutchings, 1982;

Hutchings, 1992; Shannon *et al.*, 1996; Panting *et al.*, 1998. Successful transport of anchovy eggs and larvae will partly dependent on the horizontal dispersion from spawning grounds, which in turn is dependent on the vertical position of eggs and larvae (Haug *et al.*, 1986; Sundby, 1991). Both horizontal and vertical distributions of anchovy eggs and larvae are therefore likely to be significant factors determining the transport success, retention of reproductive products and ultimately recruitment of anchovy in the southern Benguela upwelling ecosystem. Although sardine and round herring appear to spawn more widely than anchovy, their intense spawning over the Agulhas Bank region means that transport is also likely to be an important factor determining their recruitment success. The overall objective of this study was to examine and compare the horizontal and vertical distribution patterns of eggs of the three species, and assess the possible implications of different distributions for transport success and offshore advection. The results obtained in this study will make a significant scientific contribution towards further understanding the ecology of the pelagic species.

The objectives of this study are:

- To compare and contrast the egg density estimates and egg distribution patterns derived from the continuous, underway fish egg sampler (CUFES) and California vertical egg tow (CalVET) net samples,
- To map, describe and compare the horizontal and vertical egg distribution patterns for anchovy, sardine and round herring using both low and high-resolution data over a part of the spawning habitat,
- To compare and contrast the horizontal egg distribution patterns of the species as observed during different sampling strategies,

- To compare and contrast the mean and station by station egg distribution patterns, and
- To evaluate the preferred spawning temperature and geographic location.

1.6. OUTLINE OF THE DISSERTATION

In order to avoid repetition, materials and methods common to Chapters 3, 4 and 5 are combined into Chapter 2. The horizontal egg distribution patterns of the three species, as assessed using CUFES and CalVET are examined in Chapter 3. Firstly, the null hypothesis that the egg patterns of the three species is random will be tested. Because pelagic fish species are known to spawn in aggregations it is expected that the egg distribution patterns would be aggregated. Secondly, geostatistical analysis techniques will be used to evaluate whether egg patterns of the three species are associated. It is expected that they will be, given the small size of the study area.

The vertical egg distribution patterns of the three species will be examined in Chapter 4. The null hypotheses that: (i) the egg profile is independent of the species and (ii) the egg profile is independent of ontogenetic variation (i.e. changes in developmental stage) will be tested. Elsewhere in the world anchovy eggs have been reported to concentrate closer to the surface than those of sardine (Olivar *et al.*, 2001), so similar patterns are expected.

In Chapter 5, I will use quotient analysis to evaluate the preferred spawning temperature range for the species within the study area and establish whether it overlaps among the species, and if so, to what extent. General additive models (GAMs) will be used to establish the form of any relationship between egg abundance and geographic location, and assess whether variation in egg abundance can be explained in terms of spawning location.

Finally, in Chapter 6 I will synthesize the results and relate them to our current understanding of the three species' early life history in the southern Benguela.

CHAPTER 2. GENERAL MATERIALS AND METHODS

2.1. ABSTRACT

This chapter describes general materials and methods that are appropriate for all subsequent chapters. Both coarse and high-resolution data for the horizontal and vertical egg samples were collected aboard FRS Algoa over the western Agulhas Bank in September 2000. Horizontal samples were collected using the continuous, underway fish egg sampler that collected samples over 1 and 3 nautical miles (n.miles) intervals apart as well as with CalVET nets at 10 n.miles apart. For vertical samples, a plankton pump and a closing WP II net were used to collect samples, from fixed depths and over 20 m depth ranges, respectively at systematically selected stations spaced at approximately 3 n.miles during phases II and III. Horizontal samples were counted on-board to ensure total spatial coverage of the eggs. Ashore, eggs were classified as anchovy, sardine and round herring, then counted, and staged. The data was recorded and punched into MS Excel spreadsheets for subsequent examination.

Key words: Study area, Resolution, Samples, Eggs, Horizontal and vertical, Patterns, CUFES, CalVET and closing WP II net, Plankton pump

2.2. INTRODUCTION

Sample collection for this study was conducted aboard the FRS Algoa from 22nd September to 2nd October 2000 during a daily egg production method (DEPM) survey conducted by Marine and Coastal Management (MCM). September is generally considered a peak spawning period for sardine (Beckley and van der Lingen, 1999) in the southern Benguela ecosystem. The primary objective of the survey was to estimate the spawner biomass of sardines, and secondarily to examine and compare the horizontal and vertical egg distribution patterns of anchovy, sardine and round herring using coarse, medium and high-resolution data. The intent in this Chapter is to describe in detail the general materials and methods applicable in Chapters 3, 4 and 5.

2.3. SURVEY DESIGN

The objective of this study was to examine both coarse and high-resolution egg distribution patterns, thus the survey was sub-divided into two categories: coarse and high-resolution sampling. Phase I (PI) consisted of both coarse and medium-resolution sampling conducted concurrently in a similar fashion to that followed by standard pelagic spawner biomass surveys conducted in the southern Benguela, whereby transect lines are perpendicular to the coastline (Jolly and Hampton, 1990). Phase I sampling lasted for approximately eight days from the 22nd September to 30th, however, because Phase II (PII, a high-resolution study) was embedded within PI, PI was interrupted and put on hold while busy with PII. PII was only conducted in areas of high egg densities, and was further divided into three experiments: Phase II experiment 1 ($P_{II}E_I$) that lasted for about nine hours on the 24th, Phase II experiment 2 ($P_{II}E_2$) that lasted for about five hours on the 25-26th and Phase II experiment 3 ($P_{II}E_3$)

which lasted for approximately 14 hours from the 28-29th. However, only $P_{II}E_I$ and $P_{II}E_3$ results are presented for this study, because $P_{II}E_2$ data was not sufficient enough as only very few eggs were encountered for the three species. Phase III (PIII) was also a coarse-resolution study, but was conducted in a different manner to PI, at midnight of the first of October through to the second lasting about 24 hours. Samples were collected along a zig-zag transects above the 200 m isobath centered in the region of high egg densities observed during PI.

Coarse-and medium-resolution ichthyoplankton samples and oceanographic data during PI were collected from stations positioned 10 n.miles and intervals spaced at 3 n.miles apart, along 13 randomly spaced inshore/offshore transects along the western Agulhas Bank (Fig. 2.1). Ichthyoplankton samples were collected using both a CalVET net (Smith *et al.*, 1985) and the continuous underway, fish egg sampler (CUFES, Checkley *et al.*, 1997). The CalVET net is a vertically towed net at discrete stations, whereas the CUFES is a fixed pump into the ships hull and samples on station and while underway. Underway CUFES samples were collected at 3 n.miles intervals while the research vessel was underway.

When high densities of eggs were encountered, the standard PI grid was interrupted and a fine-scale, PII survey of the egg patch was undertaken. During Phase II (PII), high-resolution horizontal and vertical ichthyoplankton samples and oceanographic data were collected along four (Phase II experiment 1 ($P_{II}E_I$), Fig. 2.1b) and six (Phase II experiment 3 ($P_{II}E_3$, Fig. 2.1c) with transects spaced at 1 M apart. Horizontal samples were collected underway using CUFES, whereas vertical samples were collected using a plankton pump (only used during $P_{II}E_I$; Pillar, 1984) and a closing WP II net (Fraser, 1966).

Sampling during Phase II began offshore and moved inshore in a direction parallel to the transect along which high egg density was located, and in the direction of the prevailing currents, while PI was put on hold. When two successive underway CUFES intervals were completed with no eggs for either of the three species the vessel steamed another parallel transect in the opposite direction, but 1 n.mile away. The process was repeated until the entire patch centre was completely covered. At the end of the patch survey, the vessel then steamed back to the cut-off point and the standard grid survey was continued.

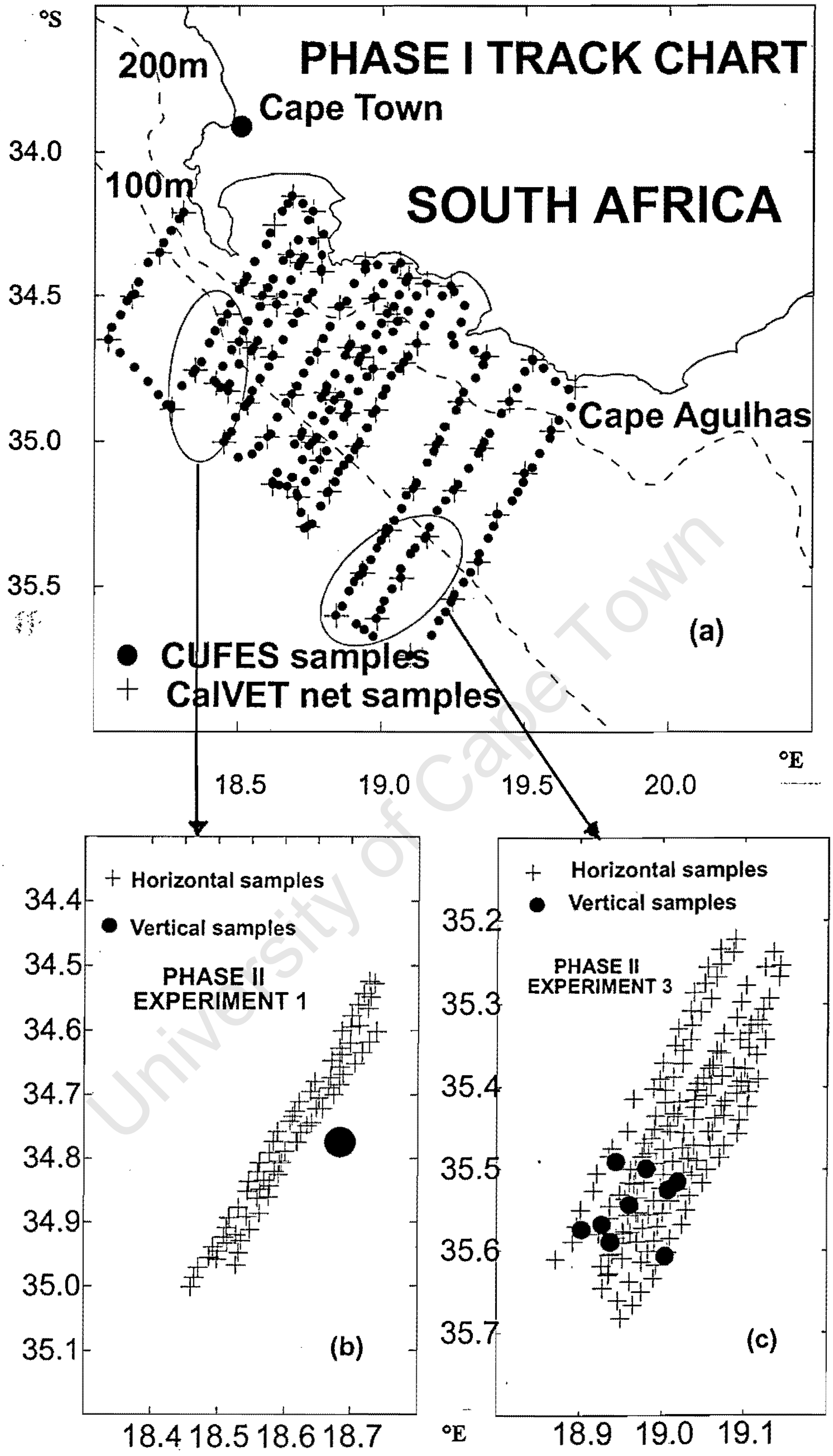


Figure 2.1. Site map showing the bathymetry of the WAB and the location of (a) Phase I stations for which CalVET net, on-station and underway CUFES samples were collected. The ellipses roughly indicate the areas where (b) Phase II experiment 1 stations were located, (c) Phase II experiment 3 stations were located.

During PIII survey, horizontal and vertical ichthyoplankton samples and oceanographic information were collected along zig-zag transects centered above the 200 m depth contour (Fig. 2.2). Horizontal and vertical samples were collected using the CUFES and a closing WP II net, respectively.

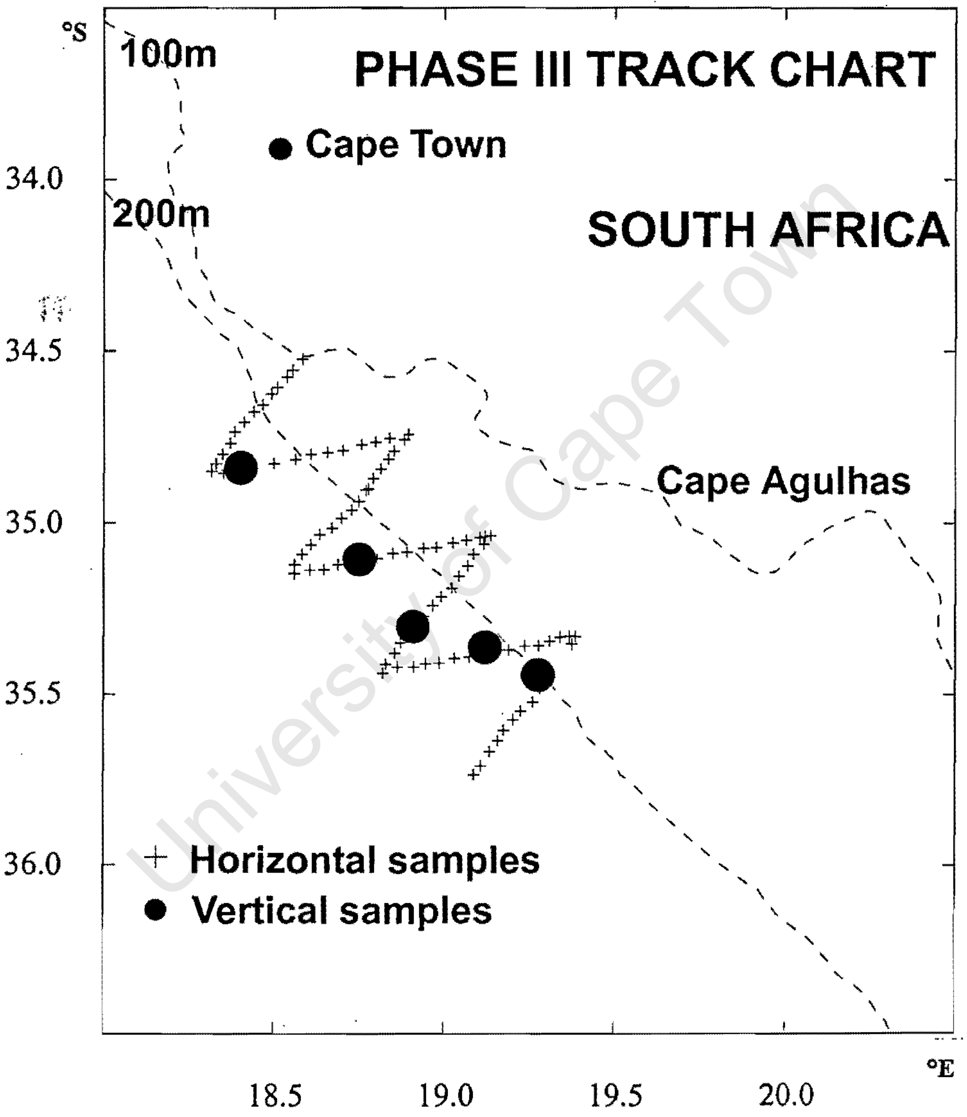


Figure 2.2. Site map showing the bathymetry of the WAB, the positions of the location of the zig-zag transects, and Phase III stations at which horizontal and vertical samples were collected.

2.4. SAMPLE COLLECTION

2.4.1. Horizontal egg distribution patterns

A full description of the CUFES can be obtained elsewhere (Checkley *et al.* 1997). Basically, the system consists of a submersible pump fixed rigidly to the ship's hull, a sample concentrator, and a mechanical sample collector (Fig. 2.4). Water is sucked from 3m depth to the concentrator, where egg-size particles are retained by a 500µm conical mesh and get concentrated in a reduced flow. The flow is then directed to the mechanical sample collector, which allows sequential sample collection. On-station CUFES samples were collected over 10 minutes duration at the same time that CalVET net samples were taken, so as to ensure simultaneous sampling and ensuring that CUFES samples an equivalent to that sampled by CalVET net. Also, CUFES underway samples were collected while the research vessel steamed at full ship speed of about 10 knots over 20, 6 and 12-15 minutes intervals respectively during PI, PII and PIII. The sample concentrator net was frequently cleaned every 2-6 h, often in areas where there were no eggs (e.g. nearshore) and in order to avoid loss of spatial coverage. The vessel's position was recorded from the ship's GPS (geographic positioning system) and stations were assigned station numbers with reference to their position in each transect (e.g. 01-01 for Line 1 station 1, 10-03 for Line 10 station 3, etc.). Temperature and the water volume filtered by the CUFES were recorded and stored on the computer every second during the CUFES interval from the ship's sensors. The CUFES operated continuously throughout the survey, sampling from 3 m depth at a pump flow rate of about 454 l.m^{-1} (±28%) and with a flow to the sample collector of 18 l. m^{-1}.

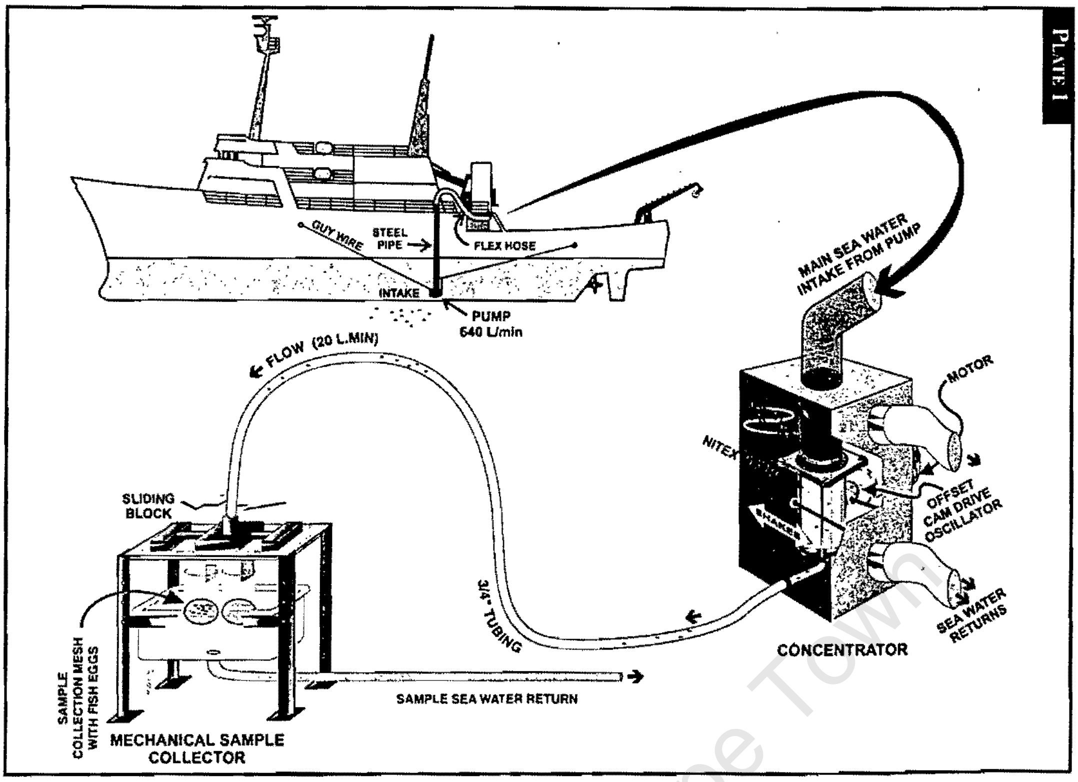


Figure 2.4. Schematic representation of a CUFES system shown as a submersible pump outside of ship's haul 3 m below the sea surface (after Checkley, 2000).

At each on-station, a paired 300μm mesh CalVET net of 25 cm mouth diameter was hauled vertically from a maximum depth of 100 m or from within 3 m of the bottom in shallow water, and the volume filtered by the net was calculated using standard protocols (Smith *et al.*, 1985). Each CalVET net haul was deployed and retrieved at 1 $m.s^{-1}$ simultaneously with the CUFES on-station sample so as to ensure simultaneous sampling. When the nets were brought back on-board they were thoroughly hosed down, thus flushing all clogged plankton down to the codend of the net. Samples were transferred from the codend of the net into plastic jars labelled with a data, station number, gear type and sample number, then preserved with 5% buffered formalin and labelled for ashore analysis. Vertical temperature profiles were recorded by means of an electronic temperature/depth sensor suspended below the CalVET net, which had an accuracy of 0.5°C (van der Lingen *et al.* 1998).

2.4.2. Vertical egg distribution patterns

The plankton pump is composed of a diesel-powered centrifugal pump that delivers water at a rate of about 200 $L.min^{-1}$ through a 7.6 cm diameter flexible hosing onto a 200μm mesh net. Sampling depths were located at 50 m, 30 m and 10 m, and the plankton pump was only used during PIIE1; with three replicate samples collected at each depth. To avoid contamination between sampling depths, 400 L of water (twice the dead volume of the pumping system) was flushed through before filtration commenced for successive depths.

A closing WP II net (Fraser, 1966) was used to collect samples was deployed on-station at five discrete depth ranges (100-80, 80-60, 40-20 and 20-0 m) during PIIE3 and PIII. The net was retrieved at $1m.s^{-1}$. A closing WP II net has a mouth area of 0.25 m^2 that can be closed by means of a messenger and has a mesh size of 300μm. Hauls were made vertically from as near as possible to the desired depth ranges; ship's drift gave rise to considerable tilting of the angle and an extra amount of wire was paid out to compensate this for. The net was deployed down to the lower limit of the depth range then lifted to the upper limit. A messenger was then released down the cable to close the mouth of the net, and resulted in the mouth being inverted. This was done before the net was retrieved to make sure that the net could not sample while being retrieved. After collection nets were brought back on-board they were thoroughly hosed down, thus flushing all clogged plankton down to the codend of the net. Samples were then transferred from the codend of the net into plastic jars labelled with a data, station number, gear type and sample number, then preserved with 5% buffered formalin and labelled for ashore analysis.

2.5. SAMPLE PROCESSING

2.5.1. On-board procedure

After collection, the CUFES samples from the sample collector were hosed into a 500μm mesh sized sieve (dish like) for immediate examination onboard. The samples were examined under the light microscope; and eggs classified using standard protocols described by Moser and Ahlstrom (1985), King *et al.* (1978) and O'Toole and King (1974), as anchovy, sardine and round herring, respectively, or other fish eggs. Eggs were then counted to facilitate adaptive sampling and ensure total coverage of the egg distribution; certain transects were extended offshore on an *ad hoc* basis to ensure total coverage. After the on-board laboratory examination was completed, samples were then emptied into plastic jars labelled with a data, station number, gear type and sample number; and fixed with a 5% seawater buffered formalin for subsequent shore-based laboratory analysis.

2.5.2. Shore-based procedure

Samples preserved in 5% buffered formalin were transferred into filtered seawater plastic jars in a fume hood in the laboratory for more detailed sample analyses. The samples were sorted and eggs removed by pipette and transferred to a petri-dish before being identified under a light microscope, counted and then stored in pill vials filled in 5% buffered formalin. The egg abundance at each station for CalVET net samples were expressed as numbers per m^2, and per m^3 for CUFES, plankton pump and closing WP II net samples. The egg abundance data for the three species were recorded in a logbook, and entered into a Microsoft Excel spreadsheet for subsequent data analysis.

CHAPTER 3. HORIZONTAL EGG DISTRIBUTION PATTERNS

3.1. ABSTRACT

On-station CUFES and CalVET net egg density estimates were compared using Model II regression to establish the compatibility between the two samplers. Variation in coarse, medium and high-resolution horizontal egg distributions of anchovy *Engraulis encrasicolus*, sardine *Sardinops sagax* and round herring *Etrumeus whiteheadi* in the southern Benguela upwelling ecosystem were investigated. Egg density estimates from on-station CUFES and CalVET net samples were significantly correlated ($P < 0.01$): anchovy ($r = 0.73$), sardine ($r = 0.91$) and round herring ($r = 0.80$), demonstrating the validity of the CUFES sampling technique. Underway CUFES (PI) samples reduced the survey coefficient of variation profoundly for sardine and very little for round herring but increased it for anchovy eggs. Inshore-offshore egg distribution patterns showed that egg abundances for the species increased from onshore towards offshore stations. Lloyd's patchiness index for horizontal egg distribution patterns showed that all species eggs were clumped. High-resolution egg distribution patterns showed that recently spawned eggs for anchovies were more structured and clumped into a single cluster, whereas late egg stages were less structured, and the opposite was true for sardine and round herring egg distribution of developmental egg stages. The mean egg densities of the species were found to be spatially associated, while their station-by-station patterns were spatially distinct. These results therefore suggest that the spawning areas among the species overlap broadly but their exact locations are spatially distinct.

Key words: Anchovy, Sardine, Round herring, southern Benguela, Eggs, Horizontal, Distribution, Spawning.

3.2. INTRODUCTION

Spatial and temporal distribution patterns in fish eggs are a function of the surrounding environment (Sundby, 1991; Brodeur *et al.*, 1996) and often reflect biological forcing such as the location, timing and the form of spawning of adults, the specific gravity of the eggs, physical forcing such as the distribution of sea surface temperature/salinity, and the influence of circulation features such as eddies, fronts and thermoclines (Doherty *et al.*, 1985; Chesney and Alonso-Noval, 1989;Brodeur *et al.*, 1996; Stabeno *et al.*, 1996). Understanding the mechanisms controlling the horizontal and vertical distribution patterns of fish eggs and larvae is one of the challenges in fisheries science (Aoki and Murayama, 1993). All the factors affecting the distributions of pelagic fish eggs can interact on various scales and in different ways to produce different egg and larval distribution patterns (Fletcher *et al.*, 1994; Brodeur *et al.*, 1996). In order for us to understand the processes affecting the overall egg abundance and distribution patterns of anchovy, sardine and round herring, we first need to describe these spatial patterns. Various statistical approaches can then be used to examine possible causes of observed distribution patterns. The description, analysis and interpretation of such ichthyoplankton patterns require data from a well-designed survey. It is difficult, but necessary to collect substantial data to perform such investigations, especially considering the fact that pelagic fish eggs are often highly aggregated.

In the south-east Atlantic Ocean, ichthyoplankton studies began at the beginning of the 20th century (Gilchrist, 1903; 1904; 1916; 1921; Gilchrist and Hunter, 1919). These studies tried to determine the distribution patterns of the eggs and larvae patterns of fish species in the South-East Atlantic waters. Later, Davies (1954; 1956), de Jager (1954) and King *et al.* (1978) studied the distribution patterns of eggs and larvae of sardine to establish the extent of spawning and the survival of the larvae, mainly as a tool to enhance management strategies of the species by forecasting recruits for commercial catches. They found that high egg abundances for sardines were consistently located close to the coast in a region <20 miles offshore between Lamberts Bay and Cape Columbine. The sampling methods used included a combination of horizontal (N100H net with a 950µm mesh), and vertical and oblique (N100H net with 200mm mesh) tows. These nets hardly captured anchovy eggs because of their large mesh sizes, but the discovery of elliptical anchovy eggs from a N100H net in False Bay and St. Sebastian Bay by Anders (1965) prompted the introduction of a N70H net, with a smaller mesh size than that of the N100H net (van der Lingen and Huggett, in press.).

The introduction of smaller meshed nets led to frequent capture of anchovy eggs, and eggs were observed to be concentrated on the south coast between Cape Point and Cape Infanta, with very few eggs collected off the west coast (Anders, 1965). Crawford (1981b) recorded highest egg densities for anchovy between Cape Point and Cape Agulhas and were associated with warm waters. Later, Shelton and Hutchings (1982) reported that high egg densities for the species extended up to the south-west coast whereas larvae were widespread over the Agulhas Bank and extended further up the west coast. Van der Lingen *et al.* (2001) showed that in the period 1984-2000 anchovy eggs were found in a continuous band between Cape Point and Algoa Bay, with highest densities mainly located on the WAB, the offshore portion of the central Agulhas Bank and eastern Agulhas Bank. The lack of anchovy spawning on the west coast is considered to be the consequence of its narrow spawning temperature tolerance range, as simulated in King *et al.*'s (1978) laboratory studies.

Continued investigations of sardine eggs and larval distributions during the period 1961-1963 showed that highest egg densities were located in two main regions: near Cape Columbine and between Cape Point and Danger Point off the south-west coast (Crawford, 1981a). In the period 1963-1967, egg densities declined off the south-west coast, coincidentally with the disappearance of adult sardines in the region. This shift in spawning was attributed to the disappearance of the west coast spawning population or a southward contraction of the species distribution (Armstrong *et al.*, 1991). Later, Roel *et al.*, (1994) reported that highest sardine egg abundances were mainly located towards the edge of the shelf waters in the WAB and EAB regions.

There is very little information about round herring early life history in the southern Benguela upwelling ecosystem (see O'Toole and King, 1974; Brownell, 1979; Shelton, 1986). The presence of round herring *Etrumeus whiteheadi* eggs and distribution patterns on the west and south-west coasts were reported off the Cape Peninsula in early studies (O'Toole and King, 1974; Brownell, 1979). Later, Shelton (1986) reported that the egg distribution of the species extends along the west coast down to the Agulhas Bank. Van der Lingen *et al.* (1998) reported that round herring eggs off the WAB were mainly distributed above and beyond the 200 m isobath. The east coast round herring *Etrumeus teres* eggs are also observed all year off Park Rynie, KZN region (Connell, 2001).

Off the east coast the continental margin is quite narrow, and Anders (1975) reported that both anchovy and sardine eggs were confined to continental shelf at inshore stations and neither of the species' distributions extended towards the offshore stations. Shelton and Kriel (1980), Beckley (1986), Armstrong *et al.* (1991) and Beckley and Hewston (1994) reported that round herring spawning on the east coast extend from the south-east coast up to KZN coast. More recently, Connell (2001), in his investigation of seasonal variation in spawning patterns of clupeoids off the KZN coast over the period 1987-1998, reported that after the "sardine run" along the KZN coast in June, the species remains in the region until November/December of each year before returning to the Agulhas Bank. He further reported that sardine was observed to spawn throughout their stay in the region, peaking during October-December, although adults were not being sighted.

Frequent monitoring of plankton in the southern Benguela region has provided substantial insights about the spawning strategy of anchovy, and anchovy spawning strategy is well understood. Anchovies are observed to spawn from October to March (Shelton and Hutchings, 1990; Melo, 1994) mainly to the east of Cape Point in the WAB region (Shelton and Hutchings, 1989; Hutchings, 1994; Roel *et al.*, 1994). In spite of all these reports, sardine spawning strategy still remains uncertain, but they seem to spawn all year round, mainly from August to March (Akkers *et al.*, 1996; Fowler, 1998; Huggett *et al.*, 1998). More recently, van der Lingen *et al.* (2001) reported that main spawning habitats for anchovy and sardine have diverged since 1994, with anchovy spawning shifting eastward whereas sardine spawning is mainly off the west coast. The spawning strategy of round herring, like that of sardine, is also poorly understood, but this species is observed to spawn throughout the year with a winter peak, mainly along the edge of the continental shelf (Shelton *et al.*, 1985; Shelton, 1986; Roel and Melo, 1990).

The sampling gear types used in most of the previous studies, such as the N100H, N70H and most recently, the California vertical egg tow (CalVET) nets, are only deployed at discrete stations and are therefore limited in accuracy, precision and sensitivity. Furthermore, such conventional methods are often limited by adverse sea conditions (Santos *et al.*, 2000). Checkley *et al.* (1997) suggested that patchily distributed pelagic fish eggs are best sampled at a fine-scale and the development of the continuous, underway fish egg sampler (CUFES) has improved sampling efficiency enormously.

In addition, the CUFES facilitates real-time assessments of egg abundance, which facilitates adaptive sampling (Checkley *et al.*, 1997; van der Lingen *et al.*, 1998; Santos *et al.*, 2000). Adaptive sampling permits the extension or truncation of transects according to observations of egg distributions, and so ensures better coverage of egg distribution, and allows enough time to concentrate on areas of interest and, in some cases, helps to cut down unnecessary running costs of the survey.

In the southern Benguela ecosystem, the coarse-resolution horizontal egg distribution patterns of anchovy, sardine and round herring have been described mainly to identify the spawning grounds of the species and make inferences regarding their reproductive strategies. However, inferences on reproductive strategies of the species from such coarse-resolution data may be misleading considering the fact of patchiness of plankton (including fish eggs) at the sea. Thus, high-resolution data would help us to better investigate the fine-scale features in egg distribution patterns and thus make relevant inferences about the distribution patterns of spawning adults. High-resolution data could also assist in designing sampling grid, especially if the data is analysed using variogram analysis, however, such analysis was not done for this study.

The objectives of this chapter are:

- To compare and contrast the egg density estimates derived from the CUFES and CalVET net samplers,
- To compare and contrast the horizontal egg distribution patterns of the species as observed during different sampling strategies,
- To examine fine-scale variation in horizontal egg distribution patterns.

This chapter is divided into two sections. In section one I aim to describe the type of data used for this study, collected during different survey designs or/and experiments, to compare the CUFES and CalVET net performance, and to describe and compare the horizontal egg distribution patterns for each species. In section two, I will then compare and contrast inter- and intra-species associations in horizontal egg distribution patterns using geostatistical analysis techniques.

SECTION ONE: EGG ABUNDANCE AND DISTRIBUTION PATTERNS

3.3. DATA ANALYSIS

3.3.1. CUFES-CalVET net comparison

Estimates of egg abundance from CalVET and on-station CUFES samples were compared using linear regressions. Linear regression equations are commonly used to establish the relationship between two variables collected either in the field or laboratory. In almost all cases, standard Model I regression techniques are used to establish the functional relationships between the variables, but the appropriateness of these techniques depends on the extent to which the data satisfy the assumptions that are explicitly defined for Model I theory (Laws and Archie, 1981). In such cases, it is assumed that the errors in the variable on the Y-axis (dependent variable) are normally distributed and independent of the variable on the X-axis (independent variable). An important and often violated assumption of the Model I theory is that the independent variable is under the control of the investigator. In this study both the dependent and independent variables are not under any control. For such cases, Ricker (1973) recommended that Model II regression theory should be applied, because the Model I regression slope would be expected to be lower than the real slope of the functional relationship (Sokal and Rohlf, 1969).

To examine the compatibility between CalVET and on-station CUFES samples, the estimated areal (eggs.m^{-2}) and volumetric (eggs.m^{-3}) egg density estimates from the CUFES and CalVET net samples were ln-transformed and estimated areal egg density further multiplied by 0.02 in order to force the regression through or close to the origin (van der Lingen *et al.*, 1998). The scientific implications for the normalisation of CalVET areal egg density are not clear, and in this study this was done for comparative purposes. During ln-transformation, estimated egg density estimates from the on-station CUFES and CalVET samples were expressed as plus one, because there were stations with zero density, and ln zero does not exist.

$$TransformedCUFESdata = \ln\left(eggs.m^{-3} + 1\right) \ldots\ldots\ldots 3.1$$

$$TransformedCalVETdata = \ln\left(eggs.m^{-2} * 0.02 + 1\right) \ldots\ldots\ldots 3.2$$

A simple Model I regression was calculated:

$$\ln\left(eggs.m^{-3} + 1\right) = a\ln\left(eggs.m^{-2} * 0.02 + 1\right) + b \ldots\ldots\ldots 3.3$$

where a is the slope of the regression and b is the intercept.

To evaluate whether the volumetric egg density estimates by the two samplers were even, the estimated volumetric (eggs.m^{-3}) egg density estimates from the CUFES and CalVET net samples were ln-transformed, and were expressed as plus one, because some stations had zero egg density, and ln zero does not exist.

$$TransformedCUFESdata = \ln\left(eggs.m^{-3} + 1\right) \ldots\ldots\ldots 3.4$$

$$TransformedCalVETdata = \ln\left(eggs.m^{-3} + 1\right) \ldots\ldots\ldots 3.5$$

A simple Model I regression was calculated for both CUFES and CalVET data:

$$\ln\left(eggs.m^{-3} + 1\right) = a\ln\left(eggs.m^{-3} + 1\right) + b \ldots\ldots\ldots 3.6$$

where a is the slope of the regression and b is the intercept.

To correct for the effect of errors in the CalVET net estimates on the values of the regression parameters from the Model I simple regression, the following equation suggested by Ricker (1973) was used:

$$V = \pm a / r \,.........3.7$$

where V is the slope of the Model II regression, a is the slope of the Model I regression and r is the correlation coefficient from the Model I regression.

To correct for the intercept of Model II regression, the formula below was used to compute the corrected intercept value of the Model II regression:

$$\overline{Y} = v\overline{X} + c \,.........3.8$$

where $\overline{Y}$ and $\overline{X}$ are the mean of ln-transformed volumetric egg densities from CalVET and on-station CUFES samples, and v and c are the slope and intercept of the Model II regression. To compute the intercept from equation 3.8, c was made the subject of the formula and the value of intercept was obtained.

3.3.2. Lloyd's patchiness index

Lloyd's patchiness index (LPI, Lloyd, 1967) is a density-independent statistic used to measure whether spatial patterns are random, uniform, and/or clustered (Hewitt, 1981):

$$LPI = 1 + \frac{(s^2 - \overline{X})}{\overline{X}} \,.........3.9$$

where S^2 is the sample variance and $\overline{X}$ is the sample mean density. In a randomly dispersed spatial pattern the LPI is equal to 1 (i.e. $S^2 = \overline{X}$), for a uniform pattern the LPI is < 1 ($\overline{X} >> S^2$) and for a clustered pattern the LPI is >1 (i.e. $S^2 >> \overline{X}$). Large LPI values indicate distributions that are relatively clustered, whereas small LPI values indicate several smaller clusters (patchy distributions). The LPI was applied to all CalVET, on-station CUFES and underway CUFES samples for anchovy, sardine and round herring. Sample means calculation included all samples, because the exclusion of samples with zero egg density is not justified when assessing patchiness.

3.3.3. Horizontal distribution maps

To illustrate the distributions of eggs, contour maps representing the interpolation of temperature (3-m depth), volumetric (100-m or 3 m depth off the bottom in shallow waters) egg densities of anchovy, sardine and round herring were generated using the kriging routine in SURFER® (1997). Kriging is a linear unbiased interpolation technique, where observations are weighted using the distance-variance relationship from the variogram in a way that minimises the estimation variance.

3.4. RESULTS

3.4.1. Description of the data

Before I can proceed to present ecological implications of the data obtained during this study it is important to first explore the data in order to have an idea of its properties. Summaries and descriptive statistics of the data collected during this study are presented below.

For on-station CUFES and CalVET net samples (coarse-resolution data) during PI, between 59 and 68% of the samples did not contain eggs of the three species. During underway CUFES (medium-resolution) spatial coverage, eggs were not found in 32-37% of samples collected during PI, and 23-23% of the samples during PIII (Table 3.2). These results reflect the effect of survey design of PIII, which was focused above the shelf edge where the eggs were distributed, and did not extend inshore where the eggs were seldom found. During high-resolution coverage (PIIE1 and PIIE3), fewer than 30% of the samples had no eggs (Table 3.2), but these results reflect the fact that sampling was only conducted in areas of high egg abundances as already highlighted during PIII. The CalVET net collected twice the number of anchovy eggs than on-station CUFES (Table 3.2). These results therefore indicate the high efficiency associated with the CalVET net in collecting anchovy eggs compared to CUFES. For sardine and round herring the reverse was true, indicating a high efficiency associated with the CUFES compared to CalVET net in these species' egg collection. The coefficient of variation (CV) of mean egg density for all three species was slightly smaller during PI (only phase when sample sizes were large) coarse-resolution study for the CalVET net than for on-station CUFES samples. The coefficient of variation (CV) depends on the deviation of the measurements around the mean (i.e. standard deviation), and the survey CV should decrease as the sample size is increased (i.e. increased the amount of water filtered). Thus, the underway CUFES samples reduced CVs substantially for all three as would be expected. Because high-resolution sampling was only conducted within the area of high egg abundance, the CVs for all species were generally low, with an exception of round herring eggs during PIIE1. Therefore, these results indicate that finer resolution improves the representation of the spatial heterogeneity between samples compared to coarse-resolution investigations.

Table 3.2. Basic statistics of the egg samples collected during Phases I, II and III, using the CUFES and CalVET net tows. The two gear types were either deployed on station (o/s) or while the ship was underway (u/w).

Phase (Expt)	Species	# Samples	# +ve Samples	Egg density per m^2 or m^3					
				Total	Min	Max	Mean	SD	%CV
CUFES o/s									
I	Anchovy	68	22	353	0	96	5	15.4	308
I	Sardine	68	25	723	0	391	11	48.3	439
I	Round herring	68	24	679	0	160	10	26.8	268
II(3)	Anchovy	9	6	185	0	81	21	31.9	152
II(3)	Sardine	9	8	376	0	100	42	31.1	74
II(3)	Round herring	9	8	108	0	19	12	5.3	44
III	Anchovy	5	5	147	1	109	29	46.1	159
III	Sardine	5	5	745	48	337	149	109.7	74
III	Round herring	5	5	116	8	48	23	16.3	71
CalVET o/s									
I	Anchovy	68	28	1154	0	289	17	49.3	290
I	Sardine	68	27	243	0	67	4	11.0	275
I	Round herring	68	25	247	0	36	4	7.9	198
II(3)	Anchovy	9	5	448	0	137	50	62.2	124
II(3)	Sardine	9	6	449	0	137	50	47.7	95
II(3)	Round herring	9	6	285	0	137	32	50.2	157
III	Anchovy	5	5	734	73	315	147	97.3	66
III	Sardine	5	5	760	30	445	152	170.3	112
III	Round herring	5	5	93	9	29	19	17.7	93
CUFES u/w									
I	Anchovy	137	86	1862	0	138	14	23.9	171
I	Sardine	137	93	3354	0	297	24	48.1	200
I	Round herring	137	87	2752	0	297	20	38.8	194
II(1)	Anchovy	75	45	398	0	25	5	6.9	138
II(1)	Sardine	75	60	591	0	53	8	10.2	128
II(1)	Round herring	75	32	497	0	74	7	15.9	227
II(3)	Anchovy	160	97	1715	0	87	11	17.8	162
II(3)	Sardine	160	107	2271	0	129	14	23.7	169
II(3)	Round herring	160	103	641	0	27	4	5.9	148
III	Anchovy	103	81	1628	0	143	17	26.8	158
III	Sardine	103	81	6173	0	1044	66	157.3	238
III	Round herring	103	77	1068	0	93	11	17.7	161

The dominance of zeroes in ichthyoplankton data makes the use of traditional statistical techniques to compare means problematic (Venrick, 1986). In addition, the ichthyoplankton samples are also characterized by the dominance of small values. The combination of these factors results in a lack of power of traditional statistical techniques, which indicates a need for more samples, a requirement that is frequently impractical. This factor becomes of serious concern when we consider the spatial heterogeneity exhibited by ichthyoplankton; thus the use of geostatistical techniques becomes useful due to their ability to account for spatial heterogeneity.

3.4.2. CUFES-CalVET net comparison

85 paired on-station CUFES and CalVET net samples were analysed using Model II regressions. The data were first plus one, then normalised by means of ln-transformation, and linear regression analysis was carried out using regression tools in MS Excel. High correlation coefficients were observed: $r = 0.73$, 0.91 and 0.80 for anchovy, sardine and round herring, respectively (Fig. 3.1a-c). The observed correlation coefficients for the three species were tested for significance using the product moment correlation coefficient test at 1% level of significance (Zar, 1999). The correlation coefficients for all three species were statistically significant ($P < 0.01$, $df = 84$). Therefore, despite any possible effects that may cause differences in the catchability of the eggs of the three species by the two samplers, these results indicate that the egg densities measured by the two samplers are statistically comparable. The null hypothesis volumetric egg density estimates from the two samplers were even (i.e. slope was equal to one) was tested. Regression slope for anchovy eggs (0.78) was found to be statistically significantly less than unity, whereas those of sardine (1.14) and round herring (1.07) were found to be statistically significantly greater than unity ($P < 0.05$, Fig. 3.1d-f). These results indicate that CUFES overestimates sardine and round herring eggs, whilst underestimates anchovy eggs, and this was probably caused by the low efficiency of the CUFES in retaining 100% of the anchovy eggs due to its large mesh-size (500μm) compared to the CalVET net (300μm). Furthermore, correlation coefficients for all species for the

volumetric egg density estimates were low: $r = 0.26$, 0.41 and 0.29 for anchovy, sardine and round herring, respectively. The correlation coefficients for all three species were statistically insignificant ($P > 0.01$, $df = 84$).

The mean volume of water filtered by the CalVET net during PI was $4.0 \pm 1.3\ m^3$ whereas that filtered during on-station CUFES was $4.2 \pm 0.8\ m^3$. These results therefore imply that the egg densities collected by the two samplers should be comparable. Indeed, the correlation of egg density estimates derived from on-station CUFES and CalVET net samples for all three species were found to be statistically significant ($P < 0.05$, Fig. 3.1). The results show that the amount of water filtered during underway CUFES deployment doubles the amount of water filtered during on-station CUFES or CalVET net deployment (Table 3.3).

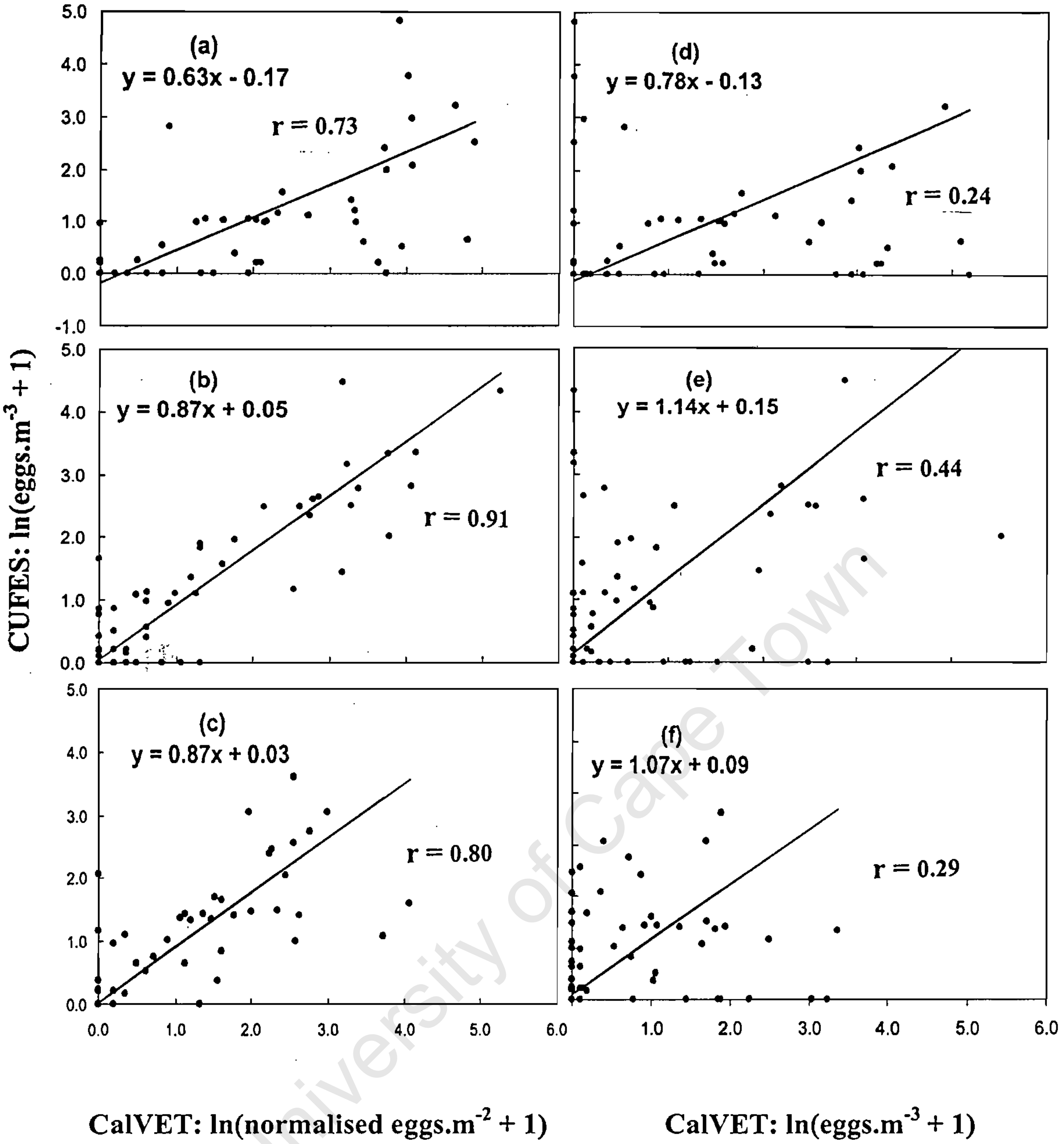


Figure 3.1. Comparison between ln-transformed volumetric egg densities (eggs.m^{-3}) from the CUFES (at 3m depth) and ln-transformed normalised areal egg densities (eggs.m^{-2}) from CalVET net samples for (a) anchovy, (b) sardine and (c) round herring, and between ln-transformed volumetric egg densities (eggs.m^{-3}) from the CUFES and CalVET net samples for (d) anchovy, (e) sardine and (f) round herring, eggs (eggs.m^{-3}).

Table 3.3. The total and mean volume of water filtered during Phases I, II and III, using the CUFES and CalVET net tows. The two gear types were either deployed on station (o/s) or while the ship was underway (u/w). Volume of water is expressed as m^3 for both CalVET and CUFES.

Phase (Expt)	Total	Mean	SD	n	CV(%)
CUFES o/s					
I	315	4.2	0.76	68	18
II(3)	37	4.2	0.39	9	9
III	22	4.5	0.09	5	2
CalVET o/s					
I	301	4.0	1.27	68	32
II(3)	44	4.9	0.02	9	0.4
III	24	4.9	0.03	5	0.6
CUFES u/w					
I	687	7.6	2.35	137	31
II(1)	204	2.8	0.33	75	12
II(3)	440	2.8	0.38	160	24
III	517	5.3	0.93	94	18

These results therefore suggest that CUFES may negatively bias anchovy egg density estimates, and positively bias sardine and round herring egg density estimates under those oceanographic conditions. Furthermore, the intercept for anchovy regression was negative whereas those of sardine and round herring were positive. This therefore implies that even though the CalVET net continues to collect anchovy eggs, CUFES might not be doing so as well, whereas for sardine and round herring eggs the opposite is true.

3.4.3. Oceanographic conditions

Oceanographic conditions (Fig. 3.2) observed during the survey were typical of the September/October period, whereby storms would homogenise properties (e.g. vertical temperature or plankton profile) in the water column. The sea was in a transition state from relatively well-mixed (during a storm) prior to the cruise, to a relatively stratified (post-storm) water column. Across the WAB, temperature (at 3 m depth) increased gradually from 15.5-16.5°C inshore and 16.5°C to 18.5°C offshore. A sharp temperature front inshore off Cape Point was apparent, as was an offshore front was also evident from 17.0-18.5°C. The water column was thermally stratified towards the east and west areas of the survey, but weakly stratified in the middle of the survey (Fig. 3.3). The inshore stations were nearly isothermal throughout the water column.

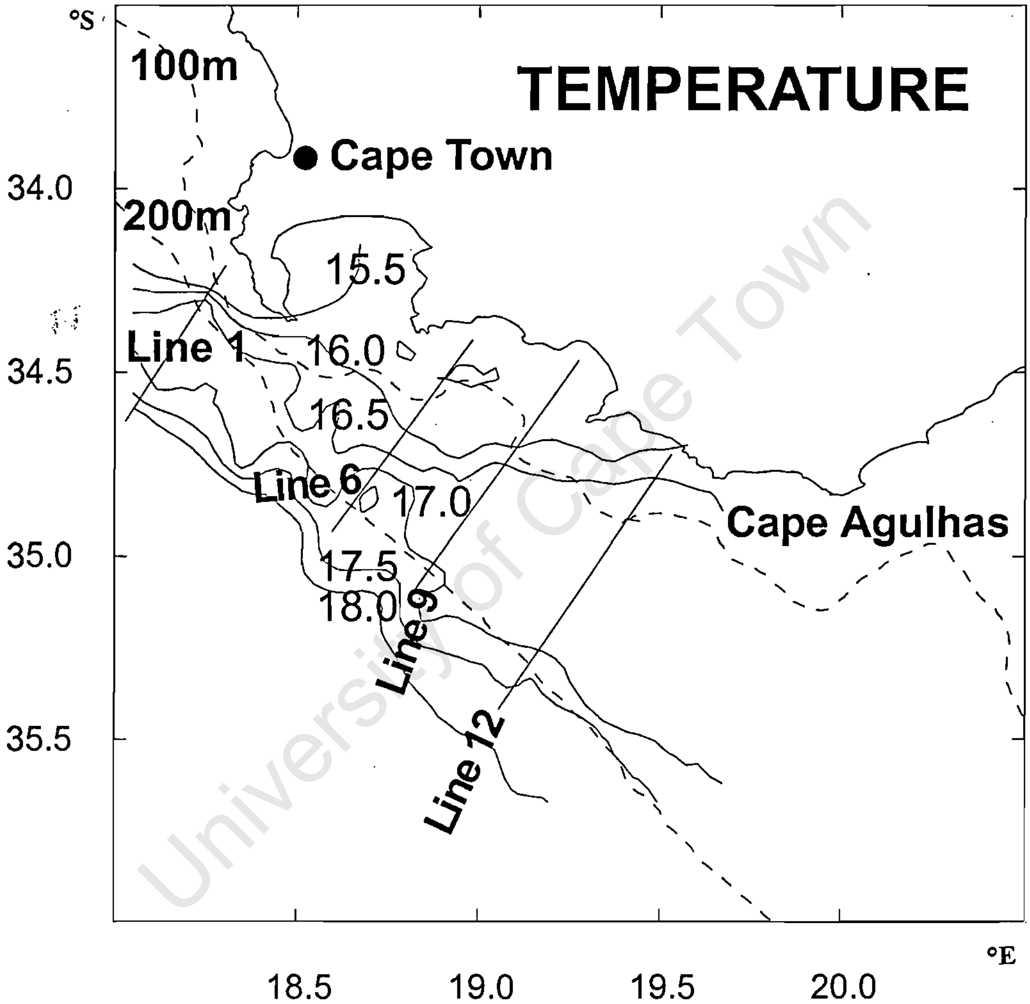


Figure 3.2. Bathymetry and temperature (°C) at 3m depth from Phase 1 sampling collected during the September/October Survey 2000 aboard FRS Algoa over the WAB of South Africa.

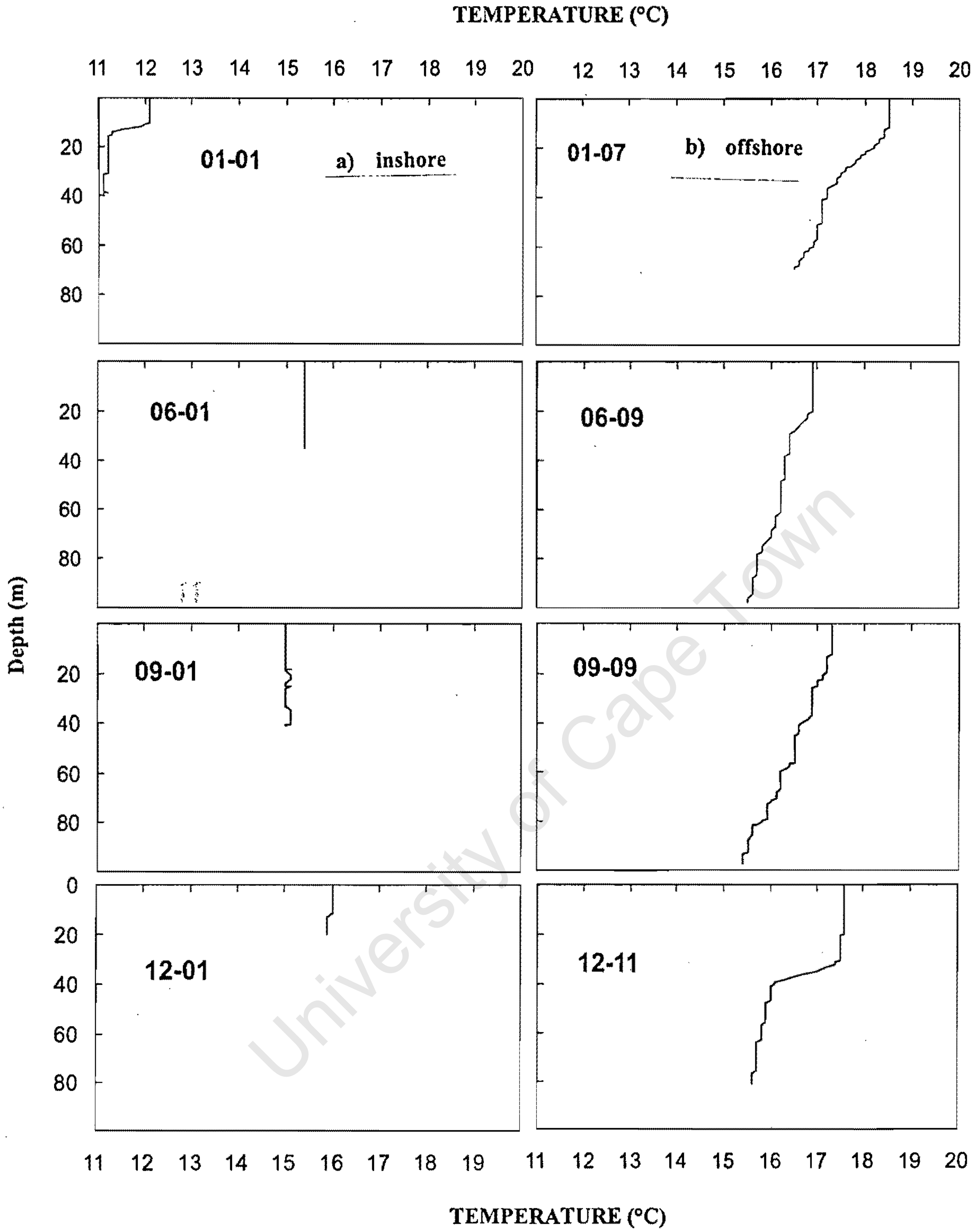


Figure 3.3. Vertical temperature profiles from the a) inshore and b) offshore stations along lines 1, 6, 9 and 12 (Fig. 3.2), collected during the September/October Survey 2000 aboard FRS Algoa over the WAB of South Africa.

3.4.4. Horizontal egg distribution patterns

The horizontal egg distribution patterns for all three species were found to be clustered, with LPI values larger than 1 (Table 3.6). The patterns of clusters varied among and within the species during different phases and experiments, probably because of the sample size or specific properties of the egg patches (i.e. developmental stage).

Table 3.6. Values of Lloyd's patchiness index (LPI) for anchovy, sardine and round herring, indicating the degree of aggregation of the eggs from the samples taken during Phases I, II and III, using the CUFES and CalVET net tows. The two gear types were either deployed on station (o/s) and the CUFES was also deployed while the ship was under way (u/w). High-resolution values are highlighted.

Phase		#	(n.miles)	LPI values		
(Expt)	Gear	Samples	Resolution	Anchovy	Sardine	Round herring
I	CalVET o/s	68	10	27	8	10
I	CUFES o/s	68	10	11	47	37
I	CUFES u/w	137	3	38	24	12
II(1)	**CUFES u/w**	**75**	**1**	**35**	**5**	**15**
II(3)	**CUFES u/w**	**160**	**1**	**11**	**14**	**3**
III	CUFES u/w	94	3	15	71	5

3.4.4.1. *Coarse- and medium-resolution egg distribution patterns*

Coarse- and medium-scale (PI) egg distribution maps for anchovy, sardine and round herring during CalVET net and CUFES deployment, are provided in Figures 3.4, 3.5 and 3.6. The highest egg abundances for anchovy from the CalVET net occurred mainly offshore along Lines 12 and 13 (Fig. 3.4a). During CUFES deployment eggs were centred in two distinct areas: a small patch in the midshelf region east of Cape Point and in the offshore region along Line 12 (Fig. 3.4b). Highest sardine egg densities from both CalVET net and CUFES were centred in two areas, largely overlapping with those of anchovy from the CUFES samples, but the patch north was further offshore of the east of Cape Point along Line 1 (Fig. 3.5a-b). Eggs of round herring collected using the CalVET net were relatively continuous above and beyond the 200 m isobath (Fig. 3.6a), CUFES samples also showed similar patterns to the CalVET net samples but densities were generally low for CUFES samples (Fig. 3.6b). The results indicate that CalVET and CUFES samplers provide similar egg distributions for round herring.

During PIII, egg distribution patterns similar to those observed for CalVET net PI were observed for all three species (Fig. 3.7a-c), for the same area of spatial coverage. These results indicate that agreeable distribution patterns were illustrated correctly by the CalVET net, and that sardine and round herring eggs might have drifted north, whereas those of anchovy were retained at midshelf.

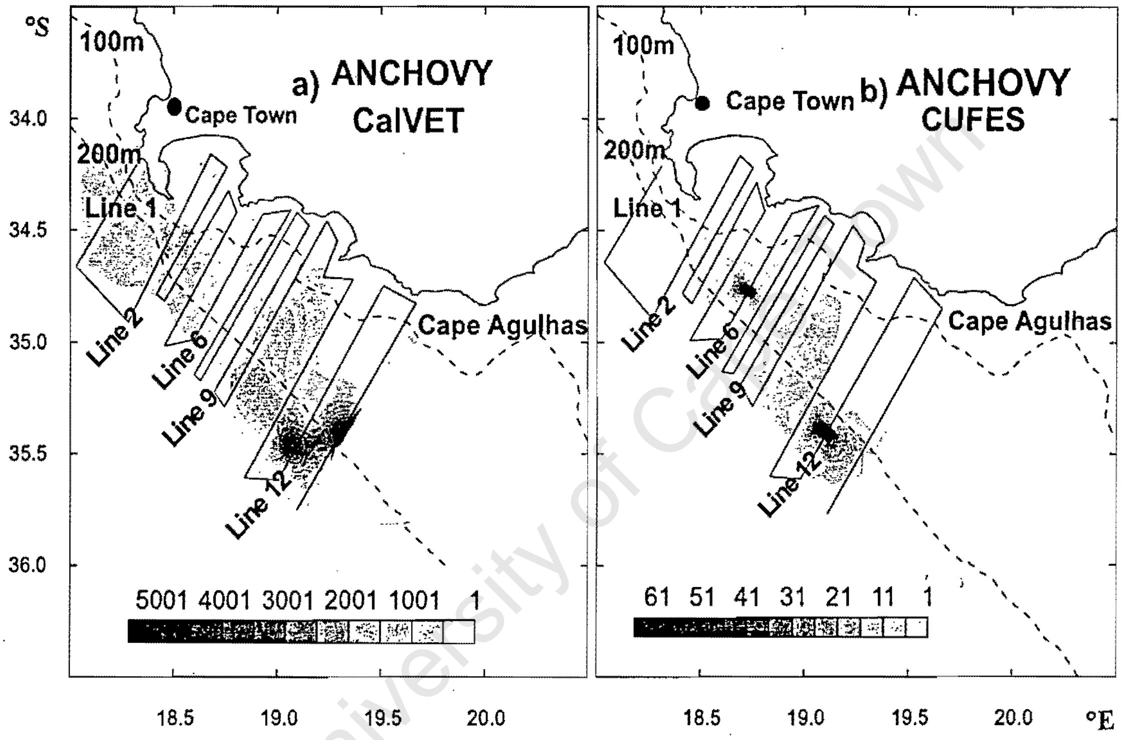


Figure 3.4. Results of coarse-resolution sampling, Phase I. Horizontal egg distribution patterns for anchovy *Engraulis encrasicolus* during September 2000. a) Results from CalVET net samples collected from 100-m (or 3 m depth off the bottom in shallow areas) to the surface (eggs.m^{-2}). b) Results from underway CUFES samples collected from 3 m depth (eggs.m^{-3}).

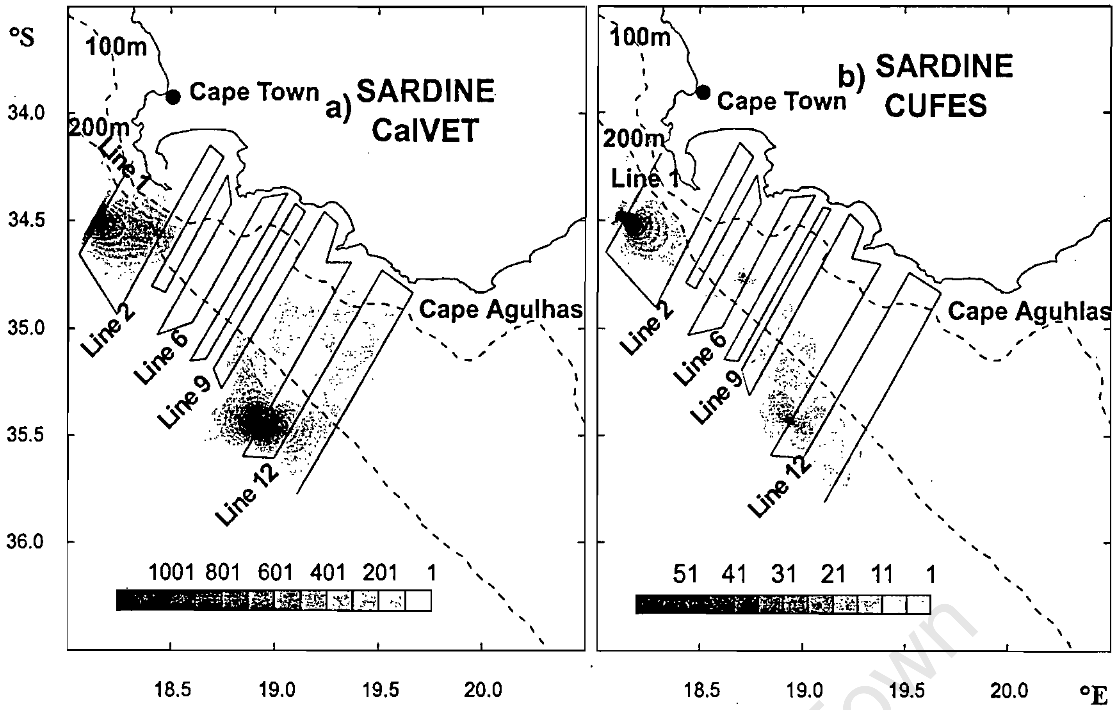


Figure 3.5. Results of coarse-resolution sampling, Phase I. Horizontal egg distribution patterns for sardine *Sardinops sagax* during September 2000. a) Results from CalVET net samples collected from 100-m (or 3 m depth off the bottom in shallow areas) to the surface (eggs.m^{-2}). b) Results from underway CUFES samples collected from 3 m depth (eggs.m^{-3}).

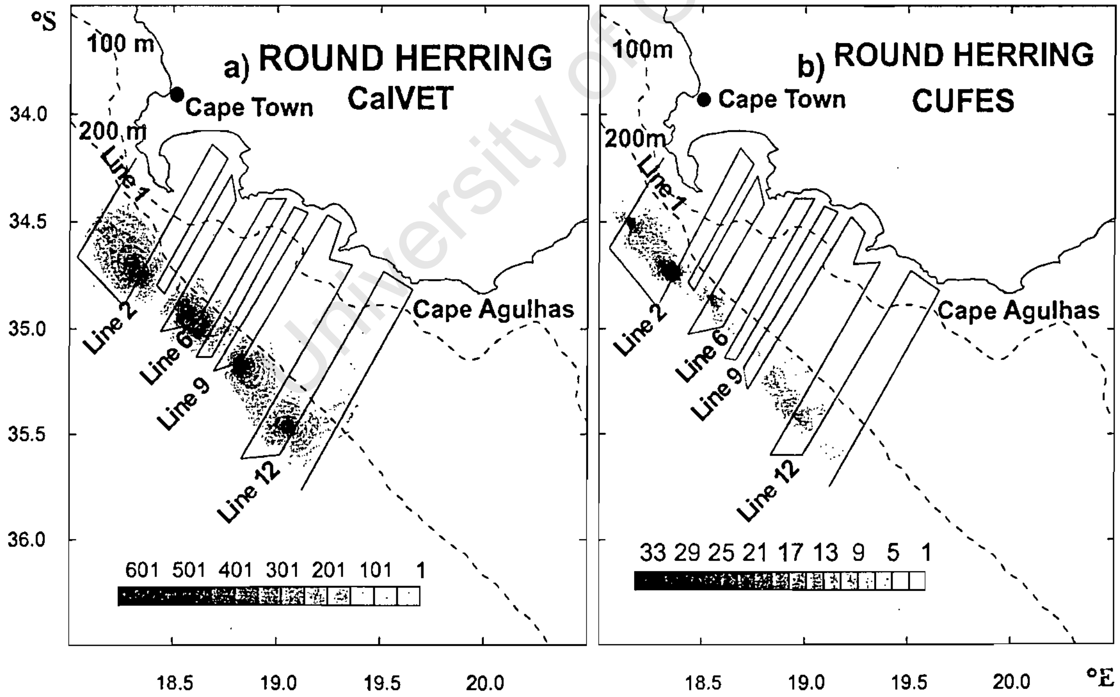


Figure 3.6. Results of coarse-resolution sampling, Phase I. Horizontal egg distribution patterns for round herring *Etrumeus whiteheadi* during September 2000. a) Results from CalVET net samples collected from 100-m (or 3 m depth off the bottom in shallow areas) to the surface (eggs.m^{-2}). b) Results from underway CUFES samples collected from 3 m depth (eggs.m^{-3}).

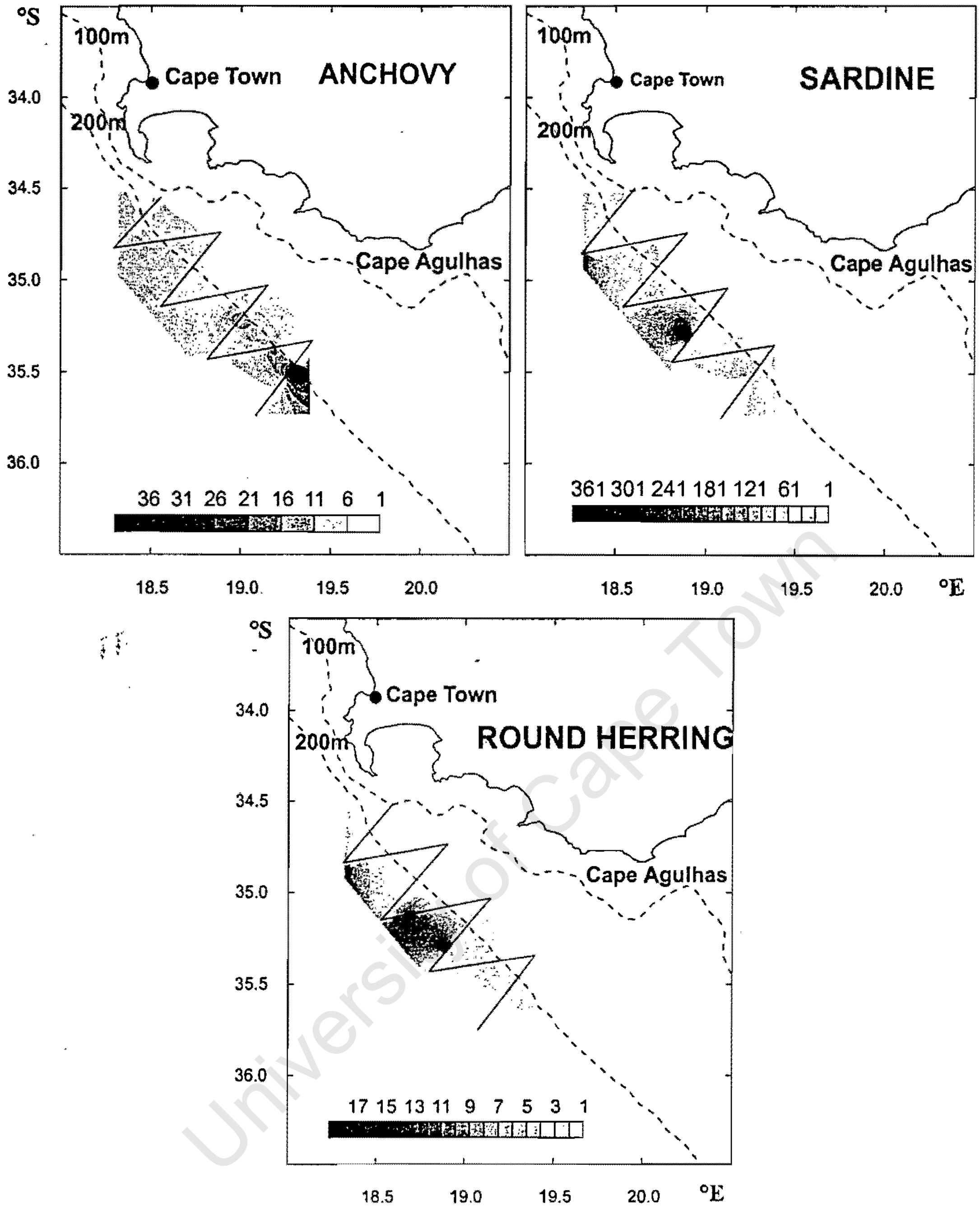


Figure 3.7. Results from underway CUFES samples collected from 3-m depth during Phase III. Horizontal egg patterns for a) anchovy, b) sardine and c) round herring (eggs.m^{-3}) at 3-m depth.

Inshore/offshore volumetric egg density profiles along transect 9 and 12 during PI, showed that all species' eggs increased progressively from inshore and/or midshelf to offshore (Fig. 3.8). However, the figure also shows that anchovy eggs tend to be slightly more inshore than those of sardine and round herring.

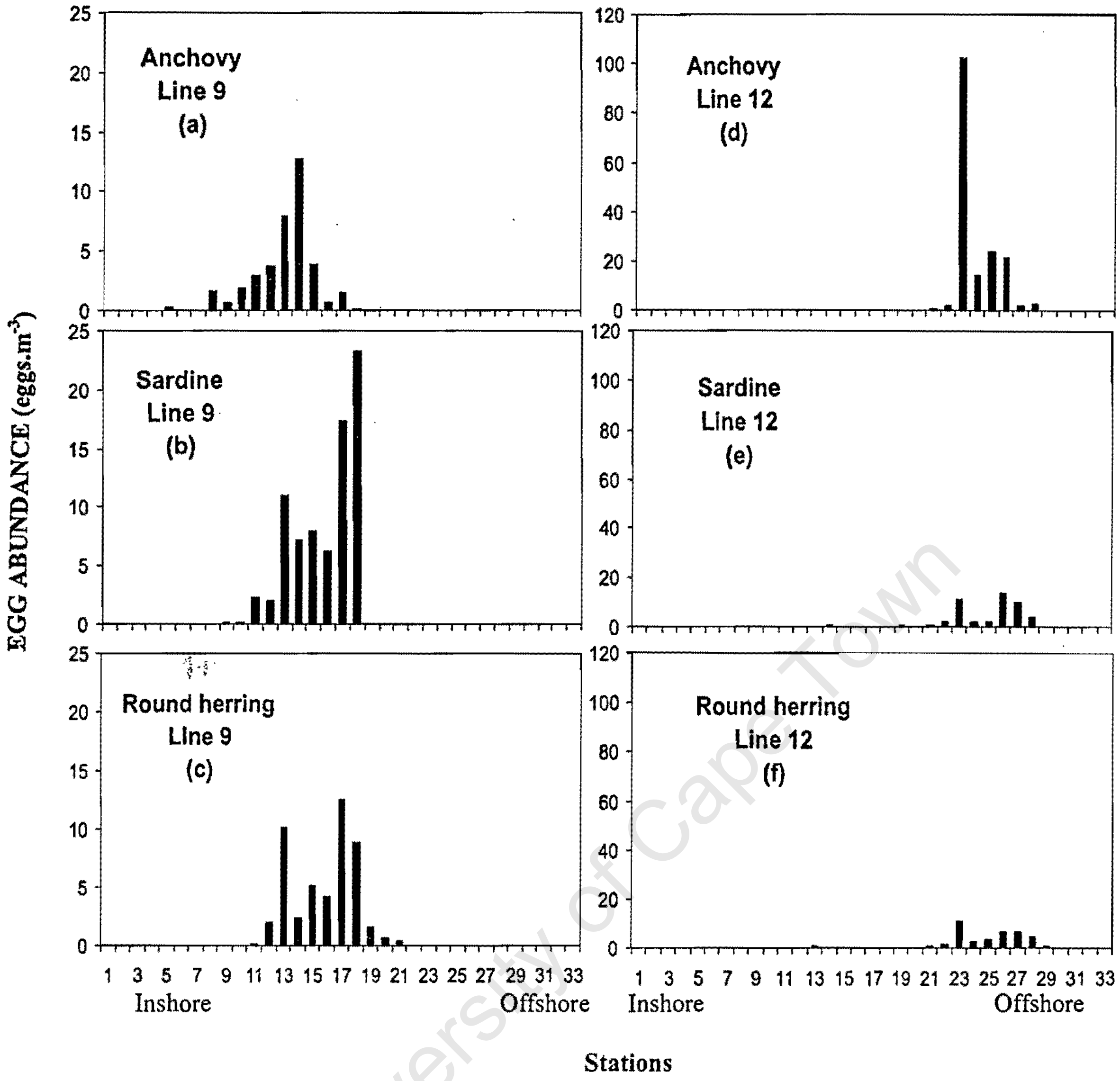


Figure 3.8. Results of coarse-resolution sampling in Phase I using on-station and underway CUFES samples. Cross-shelf distributions of eggs (eggs.m^{-3}) of anchovy *Engraulis encrasicolus*, sardine *Sardinops sagax* and round herring *Etrumeus whiteheadi*, along (a-c) transects 9, (d-f) transect 12.

3.4.4.2. *Fine-resolution egg distribution patterns*

High-resolution egg distribution maps obtained from $P_{II}E_1$ showed that the egg distributions for anchovy and round herring were structured and (i.e. egg density decreases as moving away from the core of the egg patch) and centred in a single cluster; whereas those of sardine were distributed into several clusters (e.g. less structured or patchily distributed, Fig. 3.9a-c). During $P_{II}E_3$, anchovy egg distribution was distinct from sardine and round herring, whereas the latter two species egg distributions were distributed relatively similarly. Sardine and round herring eggs were less patchily distributed, whereas anchovy eggs were patchily distributed (Fig. 3.10a-c). However, LPI (Table 3.6) indicated that anchovy and sardine eggs were more structured, whereas round herring eggs were less structured. These results highlight that even when looking on a finer scale, species' egg patterns were highly variable within the studied areas and this variability could be attributed to both biological (e.g. fish shoaling during spawning) and physical (e.g. currents) mechanisms.

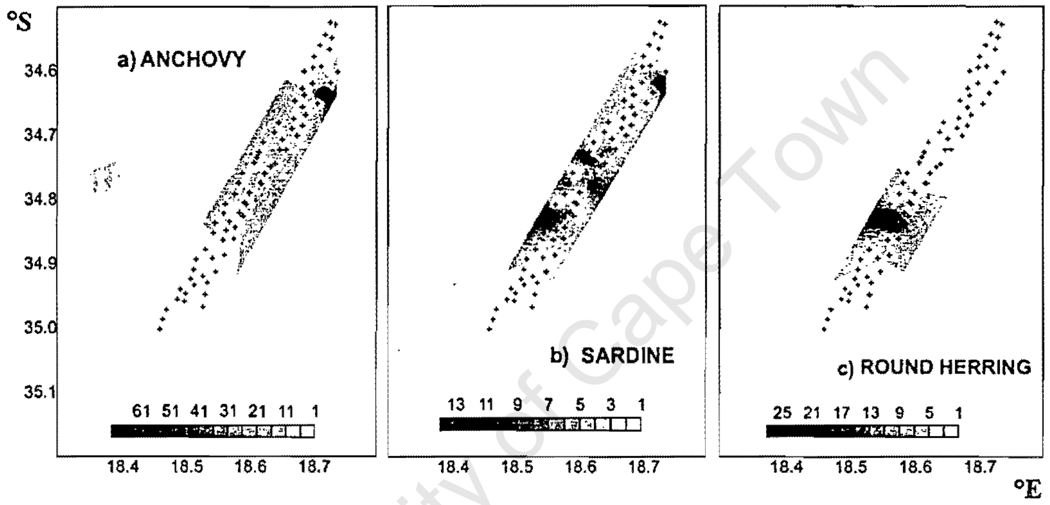


Figure 3.9. Results of high-resolution sampling during $P_{II}E_{I}$ using CUFES underway samples. Horizontal patterns of eggs of a) anchovy, b) sardine and c) round herring, (eggs.m^{-3}). The crosses represent the stations at which CUFES samples were taken.

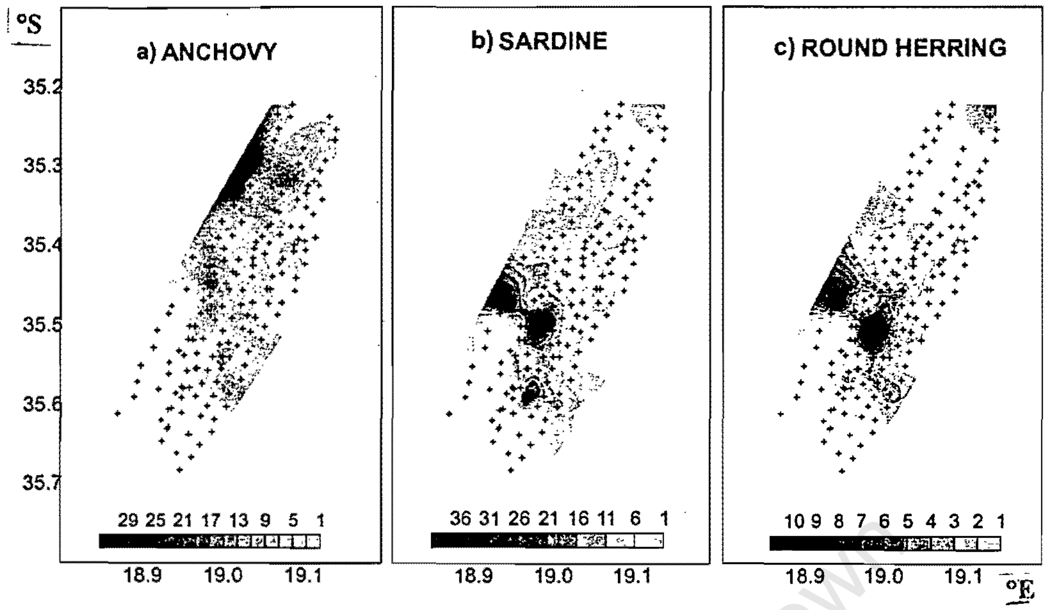


Figure 3.10. Results of high-resolution sampling during $P_{II}E_3$ using CUFES underway samples. Horizontal patterns of eggs of a) anchovy, b) sardine and c) round herring, (eggs.m^{-3}). The crosses represent the stations at which CUFES samples were taken.

SECTION TWO: GEOSTATISTICAL COMPARISONS OF HORIZONTAL EGG DISTRIBUTION PATTERNS

3.5. DATA ANALYSIS

3.5.1. Geostatistical analysis techniques

Geostatistical analysis techniques were used in this study to establish whether the mean and station-by-station egg patterns were spatially associated. Geostatistics (Matheron, 1962; 1963a; 1963b cited in Cressie, 1991) developed as a discipline combining mining, engineering, ecology, mathematics and statistics. Its advantage over traditional statistical approaches lies in its ability to recognise spatial variability at both coarse and fine-scales. However, geostatistical analysis techniques were generally designed for terrestrial studies, and such environments are ideally static, even though this is unlikely to be the case. In this study, the areas in which eggs were collected are regarded as the place where the eggs had been spawned; hence the use of geostatistical analysis techniques in this study is justified as it is assumed that the egg patterns of the three species were static. Specific techniques used in this study are quite novel and include global and local indices of collocation, which were developed by Bez and Rivoirard (2000). In this study, collocation/association refers to a manner with which eggs of different species are positioned relative to each other, and these are probably better terms to use than overlapping, which only refers to areas of intersections (Petitgas, 2000).

3.5.1.1. *Global index of collocation*

The global index of collocation (I_g, Bez and Rivoirard, 2000) is a spatial statistic used to establish whether the spatial patterns between two populations are geographically distinct or associated, and uses the formula:

$$I_g = 1 - \frac{|CG_A - CG_B|}{(CG_A - CG_B)^2 + I_A + I_B} \cdots\cdots\cdots 3.10$$

where CG$_A$ and CG$_B$ are two centres of gravity of the populations being compared, and I$_A$ and I$_B$ are the inertias of the two centres of gravity. The centre of gravity refers to the mean location of each population, and inertia refers to the spatial dispersion around the centre of gravity (Bez and Rivoirard, 2000; de Oliveira *et al.*, 2002). The inertias I$_A$ and I$_B$ are calculated using the formulae:

$$I_A = \left[\frac{z(x_i)((x_i - xCG_A)^2 + (y_i - yCG_A)^2)dx}{z(x_i)dx}\right]dx \cdots\cdots\cdots 3.11$$

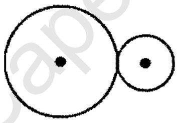
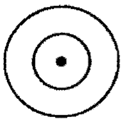
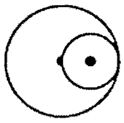
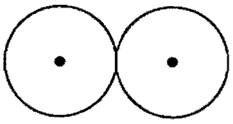
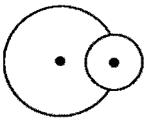
$$xCG_A = x_i \frac{z(x_i)}{\int z(x_i)dx} \cdots\cdots\cdots 3.12$$

$$yCG_A = y_i \frac{z(x_i)}{\int z(x_i)dx} \cdots\cdots\cdots 3.13$$

where z(x$_i$) is the density of species A at each point, and x$_i$ and y$_i$ are the co-ordinates of points in an x-y plane, where i = 1, 2, 3, …, n. The inertia represents the square of the radius of a circle around the centre of gravity and is proportional to the area of the circle.

The global index of collocation (I$_g$) is a spatial statistic that ranges from zero, in extreme cases where each population is concentrated at a single but different location (i.e. inertia = 0, where the inertia quantifies the global dispersion), through to one, where the two centres of gravity are coincident. Some typical cases of spatial association between two populations with various density distribution patterns are illustrated in Table 3.10. Because the I_g depends on the distance between the two centres of gravity and the respective inertias of the two populations, it is unable to differentiate between a situation where the two populations are effectively identical and a situation where the two populations are mixed. Such situations are resolved through computation of a local index of collocation (see next section).

Table 3.10. Summary of the typical situations observed for I_g between two populations (Bez and Rivoirard, 2000). Sketches are ordered by decreasing level of global collocation. The circles represent the two populations with each centred on its centre of gravity (dot).

Sketches	I_g	Sketches	I_g
	$1-\frac{0}{0+0+0}$ conventionally equal to 1		$1-\frac{1}{1+1+0}=0.50$
	$1-\frac{0}{0+1+1}=1$		$1-\frac{9}{9+1+4}=0.36$
	$1-\frac{0}{0+1+4}=1$		$1-\frac{4}{4+1+1}=0.33$
	$1-\frac{1}{1+1+4}=0.83$		$1-\frac{16}{16+4+4}=0.33$
	$1-\frac{0.25}{0.25+1+0}=0.80$		$1-\frac{4}{4+1+0}=0.20$
	$1-\frac{1}{1+1+1}=0.66$		$1-\frac{9}{9+1+1}=0.18$
	$1-\frac{4}{4+1+4}=0.56$		$1-\frac{9}{9+0+0}=0$

3.5.1.2. *Local index of collocation*

The local index of collocation (I_l, Bez and Rivoirard, 2000) ranges from zero, in extreme cases where no individuals of the two species share a common sample location, through one, where the distributions of the two species are identical or proportional. Unlike the I_g, which compares the overall geographic patterns between two populations, I_l only compares the point-by-point distributions of the two populations, without considering the overall spatial distribution. This property makes it sensitive to local heterogeneity in the spatial patterns. The I_l is calculated using the formula:

$$I_L = \frac{\int z_1(x) z_2(x) dx}{\sqrt{z_1^{\,2}(x)dx}\sqrt{z_2^{\,2}(x)dx}} \cdots\cdots\cdots 3.14$$

where $z_1(x)$ and $z_2(x)$ are the densities at each point of the two species.

Unlike the traditional coefficient of correlation, the problem of zeros is not relevant for this index. The I_l statistic is very sensitive to the level of spatial integration of the CUFES; e.g. change from 3 to 1 n.miles would yield different I_L values.

3.6. RESULTS

3.6.1. Global index of collocation

Mean egg densities for all three species were spatially associated during coarse-scale investigations (PI, 10 n.miles) for on-station CUFES and CalVET net samples (Table 3.11). The values of I_G were generally larger for CalVET net samples compared to those of CUFES. With increasing sampling resolution during medium-resolution (3 n.miles, PI and PIII) and high-resolution (1 n.mile $P_{II}E_1$ and $P_{II}E_3$) sampling using the underway CUFES samples, sardine and round herring eggs frequently showed a high level of spatial association (Table 3.11). During $P_{II}E_1$ underway CUFES samples anchovy and sardine eggs had a rather strange large association ($I_g = 0.73$) compared to that of sardine and round herring ($I_g = 0.58$). These unexpected results probably reflect the effect of various forms of aggregation of eggs due to different egg developmental stages.

Table 3.11. Results for the global index of collocation (I_g) showing the interspecies comparisons of I_g from the samples taken during Phases I, II and III, using the CUFES and CalVET net tows. The two gear types were deployed on station (o/s) and the CUFES while the ship was underway (u/w). Unexpected results are in bold font.

Phase (Expt)	Gear	# of Samples	(n.miles) resolution	Anchovy vs Sardine	Anchovy vs Round herring	Sardine vs Round herring
I	CalVET o/s	68	10	0.97	0.85	0.97
I	CUFES o/s	68	10	**0.36**	0.61	1.00
I	CUFES u/w	137	3	0.93	0.89	1.00
II(1)	CUFES u/w	75	1	**0.73**	**0.23**	**0.58**
II(3)	CUFES u/w	160	1	0.65	0.68	1.00
III	CUFES u/w	94	3	0.83	0.84	1.00

Intra-species comparisons of the mean egg distributions at on-station CUFES and CalVET net samples showed the largest I_g value for anchovy, and relatively smaller values for sardine and round herring (Table 3.12). These results highlight the fact that the mean egg distribution pattern for anchovy is unlikely to be misrepresented by the use of either of the two samplers. This is so, because anchovy eggs tend to be highly aggregated as highlighted by LPI values. Furthermore, inter-species comparisons showed a strong level of spatial association between anchovy eggs from the CalVET net samples and eggs of the other species from the CUFES samples. Both sardine and round herring CalVET net egg samples showed a reduced level of association with anchovy CUFES samples (Table 3.12). These results probably indicate that CalVET net retains all eggs of the species' equally, whereas the CUFES retains anchovy eggs to a lesser extent than other two species.

Table 3.12. Results of the global index of collocation showing the intra- and inter-specific comparisons of the I_g from the samples taken during Phase I using the CUFES and CalVET net tows. The two gear types were both deployed on station (o/s), at stations spaced 10 n.miles. Intraspecies comparison values are in bold.

Phase (Expt) Index	# Samples	Species	**CalVET o/s** Anchovy	 Sardine	 Round herring
CUFES o/s					
I	68	Anchovy	**0.74**	0.77	0.53
I	68	Sardine	0.98	**0.60**	0.98
I	68	Round herring	0.98	0.98	**0.66**

3.6.2. Local index of collocation

The comparisons of spatial point patterns represented by I_l values enables to identify small-scale differences in spatial patterns that are difficult to establish from I_G or when using ordinary statistical analysis techniques. Generally, interspecies comparisons (Table 3.13) indicate that anchovy eggs were spatially distinct from those of sardine and round herring. Anchovy eggs were moderately associated at times with round herring eggs, whereas sardine and round herring were consistently spatially associated (Table 3.13). The CUFES-CalVET net interspecies comparisons (Table 3.14) clearly demonstrated that all the spatial point patterns of all species were distinct. On the other hand, intra-species comparisons of the spatial point patterns for CUFES-CalVET net comparison showed sardine egg distributions were spatially associated, whereas those of anchovy and round herring were spatially distinct. These results indicate the effectiveness of the two samplers in collecting and retaining sardine eggs, and conversely highlight their weaknesses in collecting anchovy and round herring eggs at each point. The differences highlighted for round herring between the two samplers is rather strange as the diameter of these eggs is considerable larger so that eggs could not have been missed due to reduced retention efficiency in either of the samplers. However, these differences could arise from a probable variability in vertical egg distribution patterns.

Table 3.13. Results of the local index of collocation (I_l) showing the interspecific comparisons of I_l's from the samples taken during Phases I, II and III, using the CUFES and CalVET net tows. The two gear types were either deployed on station (o/s) or while the ship was under way (u/w).

Phase (Expt)	Gear	# Samples	(n.miles) Resolution	Anchovy vs Sardine	Anchovy vs Round herring	Sardine vs Round herring
I	CalVET o/s	68	10	0.29	0.40	0.46
I	CUFES o/s	68	10	0.08	0.52	0.48
I	CUFES u/w	137	3	0.26	0.13	0.66
II(1)	CUFES u/w	75	1	0.47	0.61	0.34
II(3)	CUFES u/w	160	1	0.31	0.31	0.84
III	CUFES u/w	94	3	0.16	0.33	0.77

Table 3.14. Results of the global and local indices of collocation showing the intra- and inter-specific comparisons of the local index of collocation (I_l) from the samples taken during Phase I using the CUFES and CalVET net tows. The two gear types were both deployed on station (o/s), at stations spaced 10 M. Intraspecies comparison values are in bold.

Phase (Expt) Index	# Samples	Species	CalVET o/s Anchovy	Sardine	Round herring
CUFES o/s					
I	68	Anchovy	**0.12**	0.04	0.04
I	68	Sardine	0.02	**0.68**	0.26
I	68	Round herring	0.03	0.29	**0.24**

3.7. DISCUSSION

3.7.1. CUFES-CalVET net comparison

Abundance, distribution and the spatial association of pelagic fish eggs on the WAB was assessed using the CUFES during September 2000, and its performance was verified by comparison with estimates of egg abundance derived from CalVET net samples. The significant correlation coefficients between the estimates of egg abundance from the two samplers, for all three species were statistically significant ($P < 0.05$), and confirms CUFES to be a reliable system with which to collect pelagic fish eggs (Checkley *et al.*, 1997; van der Lingen *et al.*, 1998). The success of CUFES as a sampler of pelagic fish eggs results in part from its substantially increased sampling effort, which would help improve the assessment of the spatial distribution of adult fish (Porteiro, 2000; van der Lingen and van der Westhuizen, 2000). Geostatistical analysis, using indices of collocation indicated that the mean egg distribution for each species between on-station CUFES and CalVET net samples had a good agreement between the two samplers. The I_g values were generally higher for anchovy eggs and decreased for round herring and sardine. Station-by-station comparisons of the egg density distribution showed poor agreement between on-station CUFES and CalVET net samples for anchovy and round herring eggs, but good agreement for sardine eggs. The poor agreement for round herring eggs between the two samplers is unexpected, as both samplers are efficient enough to retain eggs of the species. These differences may be attributed to a possible variability in vertical egg distribution patterns (see Chapter 4) or may be due to random effects.

Variability in vertical egg distribution patterns is considered responsible for the interspecies differences in CUFES-CalVET net relationships observed in this study and is most likely to have been the cause for a substantial amount of variation between these samplers. The regression slope for ln-transformed volumetric estimates of egg density for anchovy less than unity, whereas it was greater than a unity for sardine and round herring. Vertical egg profiles presented in the next chapter (Chapter 4) indicate that anchovy eggs were concentrated near the surface, suggesting that sampling by the CUFES should have been positively biased. However, this was not apparent from the CUFES-CalVET net regression slope, which was less than a unity. Anchovy eggs are oblate spheroids, with their short axis ranging from 400-600μm. The CUFES mesh size is 500 μm, and loss of some eggs was inevitable, this could have contributed to the value of the slope of the CUFES-CalVET net regression being less than unity, because the CUFES could not always retain eggs while CalVET net did. Ayón and Sánchez (2000) and Braun and Osses (2000) have reported similar results (although they used Model I regressions) for

Engraulis ringens, reporting slopes of 0.67 and 0.73, respectively. Checkley *et al.* (1997) reported a Model I slope of 0.75 for the northern anchovy *Engraulis mordax*.

Sardine eggs have a large diameter and are positively buoyant, therefore they will tend to concentrate at the surface (Coombs *et al.*, 2000; van der Lingen and van der Westhuizen, 2000); where they would be more accessible to CUFES than anchovy eggs, and slightly more accessible than round herring eggs. This was evident from the regression slope of the ln-transformed volumetric egg density estimates between the two samplers, which was greater than 1. These results therefore indicate that sardine eggs were indeed more accessible to the CUFES, even though more than 50% of the eggs were distributed below 20 m (see Chapter 4). Good agreement between estimates of sardine egg density from the two samplers has been reported locally and elsewhere. Locally, van der Lingen *et al.* (1998) reported a volumetric egg density estimate regression slope of greater than unity (although using Model I regression) in the same area as in this study. Elsewhere, Checkley *et al.* (1997) and Braun and Osses (2000) comparing volumetric egg density estimates from both CalVET and CUFES samplers reported regression slopes of 1.30 and 1.35, respectively for sardine *Sardinops sagax*. Results from these studies justify the hypothesis that sardine eggs concentrate near the surface. Collectively all the results from previous studies, those locally, elsewhere, and in this study supports the hypothesis that sardine eggs will always tend to concentrate near the surface because of their highly positive buoyancy, and their abundance is positively biased by the CUFES.

Round herring eggs have a deeper distribution than eggs of anchovy and sardine (Konishi, 1980), and the vertical egg profile data obtained during this study shows that round herring eggs are distributed deeper than those of sardine. The CUFES-CalVET net regression slope was greater than 1, suggesting that the CUFES overestimates the egg abundance for this species. Similar results (although they used Model I regressions) were also made locally by van der Lingen *et al.* (1998) where they reported that the regression slope was significantly greater than 1. Therefore, these results indicate that round herring eggs are positively buoyant, similar to those of sardine.

Generally, less than 100% retention efficiency of eggs by either of the samplers could be the result of a number of factors: (1) chance, (2) extrusion of eggs through the concentrator, and (3) egg collapse due to mechanical damaging (Checkley *et al.*, 1997).

3.7.2. Horizontal egg distribution patterns

Underway sampling by the CUFES helped to reduce the survey CVs for all three species, as expected. CalVET net data in the southern Benguela upwelling ecosystem are often used to make inferences about the reproductive behaviours such as the distribution patterns of spawning fish. As the CVs highlighted large magnitude of variability in samples collected using CalVET net compared to those collected using the CUFES, inferences about the reproductive behaviour of species is likely to be associated with large errors. The ability of the CUFES to sample small spatial scales was explored by investigating sampling at high-resolution to examine fine-scale features of egg patches.

3.7.2.1. *Coarse- and medium-resolution egg distribution patterns*

Coarse-resolution (10 n.miles) distribution maps were generated from CalVET net hauls that provided vertically integrated estimates of egg density at discrete stations. Medium-resolution (3 n.miles intervals) distribution maps for anchovy, sardine and round herring eggs were generated from underway CUFES samples, which provided a horizontally integrated estimate of egg density from a fixed depth (3 m) along the survey grid. CUFES distribution patterns would be expected to appear continuous with its continuous coverage of the horizontal egg distribution patterns, whereas CalVET net distribution patterns would appear well separated, because CalVET net may only pick up eggs from few isolated stations. However, Surfer® contours the isolated CalVET data, interpolating between those isolated stations. If the scale of egg patches is less than 10 n.miles (which it certainly must be immediately after spawning) sampling at 10 n.miles resolution may well miss egg patches and provide misleadingly continuous egg distribution patterns. Furthermore, CalVET data could be misleading especially when inferring about the reproductive strategy of the adults, whereas the CUFES data would give relevant information. However, CUFES is likely to miss low concentrations of anchovy eggs due to losses through the meshes of the concentrator and collector, as illustrated in CUFES-CalVET net comparisons.

Highest anchovy egg densities from CalVET net samples were distributed offshore of Cape Agulhas, whereas in CUFES samples they were distributed in two main areas: midshelf, along Line 6 and offshore along Line 12. The difference between the two samplers highlights the inefficiency of the CalVET data in describing horizontal egg distribution patterns for this species. CalVET data did not show eggs along line 6, whereas the CUFES did, but CalVET data showed eggs along Line 13 and CUFES did not. The differences between the two samplers is likely to arise from the fact that the CalVET net could only be deployed on discrete stations that are wide spread thus missing some spatial coverage, and CUFES inefficiency to obtain 100% retention of anchovy eggs, especially in areas of low concentrations. This is likely to be the for anchovy eggs, which appear high concentrations only in specific areas. Studies by Anders (1965), Crawford (1981a), Fowler (1998) and van der Lingen *et al.* (2001) showed similar patterns, that highest egg densities were recorded between the Cape Point and Cape Agulhas.

High sardine egg abundances from both the CalVET net and CUFES samples were limited to two isolated areas and overlapped with those of anchovy from the CUFES samples along Line 11. The egg patches located in the northern region were separated, with sardine eggs distributed along Line 1, mainly offshore, whereas anchovy eggs were distributed at midshelf. Round herring eggs were also found offshore of the continental shelf, but were distributed in a continuous band throughout the sampled area for the CalVET net samples. Van der Lingen *et al.* (1998) has reported similar distribution patterns for sardine and round herring eggs using CUFES data in the same area.

The horizontal egg distribution patterns results highlight a large degree of overlap in spawning of the species offshore in the southern region of the study area, and a slight distinction in the northern region, where anchovy seem to spawn closer to the shore (midshelf) whereas sardine and round herring spawn offshore. However, sardine and round herring spawning appear not to be separated, as both species seems to spawn further south. CUFES-CalVET net egg distribution comparisons therefore suggest that the CalVET net should be used cautiously for providing information about the spawning habitats of pelagic fish species as it misses much of the spatial variability and tends to contour the egg abundance over large distances. In addition, mean egg distribution comparisons consistently showed that CalVET data often overestimates the associations among the species. This is so because egg distributions are structured at a spatial scale smaller than 10 n.miles, and may lead erroneous conclusions if such information is regarded absolute when using that information in modelling studies, or making inferences on spawning patterns. Therefore, underway CUFES data best represents the variability in horizontal egg distribution patterns and should be used to identify or map spawning habitats of the pelagic fish species and in inferring the spawning patterns of the spawning adults, even though it undersamples anchovy eggs.

Looking at the comparisons of different sampling strategies (PI and PIII) during the medium-resolution investigation (3 n.miles) indicates that horizontal egg distribution patterns provided during both sampling designs largely overlapped with slight visible differences. Since the two strategies were conducted over different periods, and spatial distribution patterns of ichthyoplankton are a function of the medium (i.e. water masses). Therefore, they are most likely to have been passively transported with the movement of water masses carried by currents, because it is unlikely that the egg cohorts that were sampled during PI remained, about eight days before PIII sampling. In addition, all three species eggs hatch within the period of 3-4 days depending on the ambient temperature, thus slight differences were inevitable, but the main location of eggs should not be different, presumably spawned by the same shoal of fish.

Investigations into cross-shelf egg distribution patterns have shown that anchovy, sardine and round herring eggs increased from midshelf extending towards offshore, but those of sardine and round herring were broadly distributed offshore, whereas those of anchovy sharply declined. Fowler and Boyd (1998) showed that anchovy and sardine egg concentrations peaked over midshelf on the WAB, as in this study. Over the same area, van der Lingen *et al.* (1998) reported that sardine and round herring eggs were broadly distributed, and those of round herring more broadly distributed across shelf.

3.7.2.2. *High-resolution egg distribution patterns*

The determinant of a sample egg concentration is the volume of water filtered (Bez, 2000), in any given area where the eggs are present, if the absent the concentration is zero. In this study, the total volume filtered during underway CUFES deployment was double that filtered during on-station CUFES or CalVET net sampling (Table 3.3). When measuring the concentrations at different resolution, it would be expected that the mean abundance remains stable but the CV would vary (Bez, 2000). Considering a case where we sampled underway CUFES over 3 and 1 n.miles; for each 3 n.miles interval, the underway CUFES represents the concentration of eggs at 1.5 n.miles, whereas for every 1 n.miles interval represent the concentration of eggs at 0.5 n.miles. For a 3 n.miles intervals, there are fewer chances of getting zero egg density because we sample for longer, and patchiness would be masked as large values will be mixed with medium and zero areas. This would theoretically result in the same sample mean densities for each of the two sampling resolutions and small deviation for the coarse resolution, especially for small sample sizes. However, there are even chances of missing or getting eggs in the samples when sampling at 10 n.miles stations. In addition, these are theoretical expectations and are also dependent upon the spawning and egg distribution patterns for each specific species. Therefore, high-resolution sampling should be helpful in identifying reproductive and behavioural patterns of adult fishes, and an appropriate sampling distance or interval.

High-resolution interspecies comparisons showed the horizontal mean egg distribution patterns of the species were strongly associated, with sardine and round herring even showing perfect association. Results for comparisons in station-by-station egg distribution patterns indicated that sardine and round herring eggs were spatially associated whereas those of anchovy were spatially distinct from the other two species. These results highlight the fact that sardine and round herring adults probably share some of their behavioural and spawning patterns. For example, spawners (adults) grow to similar sizes and are mainly distributed beyond shelf waters (Armstrong *et al.*, 1991; Roel *et al.*, 1994), and spawn in several aggregations. Also, the eggs of sardine and round herring are more similar in size and shape to each other than they are to anchovy, which may have influence the differences between the two clupeids and the engraulid.

Fletcher and Sumner (1999) examined high-resolution (at stations 1-4 n.miles apart) egg distribution patterns for the Australian sardine *Sardinops sagax*, and used a geostatistical technique (variogram) to analyse their data. They found that recently spawned eggs were highly patchy with relatively small patch dimensions, while late egg stages showed a reduced level of patchiness and increased dimensionality. The main sources of variations in horizontal egg patterns include concentration of eggs in frontal regions and Langmuir circulation (Checkley *et al.*, 1997), the location, size and number of aggregations (groups) of the spawning population, and the developmental stage of the egg. The results of high-resolution survey are therefore presumed to illustrate three things: (i) the size of the spawning group(s) within and among the species, (ii) the affects of the sample size on the values of the sample mean and variance, and (iii) the affects of developmental egg stage on the level of aggregation.

Egg distribution patterns for the three species were found to vary during two high-resolution investigations during different experiments, the definite cause for this could not be established, but is believed to be influenced by both biological and physical mechanisms. If we were to assume that Fletcher and Sumner's (1999) observations on high-resolution egg distribution patterns of different developmental egg stages applies to local species. Fletcher and Sumner (1999) reported that young eggs are patchily distributed and occur in small patches, whereas older eggs are more dispersed. This should therefore apply to sardine and round herring eggs, and the opposite should apply to anchovy, because the behaviour of this species appears to be different from those of the other two species.

Most eggs collected during $P_{II}E_1$ and $P_{II}E_3$ from the vertical sampler (see Appendix 1) showed the dominance of embryo egg stages and their distribution patterns were structured (Table 3.6). Fletcher and Sumner (1999) reported opposite distribution patterns for sardine recently spawned egg stages, it is therefore supposed that anchovy spawn as a unit or/and in large groups. Late egg developmental stages would therefore be expected to have less patchy distribution patterns as currents disperse eggs.

For sardine eggs, LPI values were low during $P_{II}E_1$ and egg distributions highly patchy (Table 3.6), and most eggs collected near the surface from the vertical samplers were early stage eggs, and most eggs collected near the surface during $P_{II}E_3$ were middle stage eggs, they had larger LPI value and distribution patterns were less patchy. These patterns are similar to those reported for Australian sardine, thus our local sardine species is also considered to display a patchy spawning strategy; for example, they spawn in small groups or aggregations, possibly due to variable age classes. Variable age classes in sardines are highly probable considering the greater longevity of this species compared to anchovy.

If we were to assume a similar behaviour for round herring to that of sardine, as these two species share similar properties, such as a longer longevity than anchovy, and comparable adult sizes, age at maturity, and possibly fecundity then the egg distribution patterns of the latter species should resemble that of the former species. We would therefore, expect less patchy distribution patterns for round herring during $P_{II}E_1$ as the results from vertical samplers show that most of the eggs collected were late developmental egg stages. Indeed, the round herring egg patterns were less patchy during $P_{II}E_1$ (LPI = 15) experiment, but patchier during $P_{II}E_3$ (LPI = 3). Like sardine, these suggest that this species spawns in small aggregations or groups.

CHAPTER 4. VERTICAL EGG DISTRIBUTION PATTERNS

4.1. ABSTRACT

Vertical egg profiles of anchovy *Engraulis encrasicolus*, sardine *Sardinops sagax* and round herring *Etrumeus whiteheadi* in the southern Benguela upwelling ecosystem were investigated in relation to temperature and ontogenetic variation. The difference in vertical distributions of eggs at each depth among the species were found to be statistically significant ($\chi^2 = 329.330$, df = 8, $P < 0.05$). In spite of interspecies differences among the species, most eggs for all species were concentrated near the surface. Anchovy and sardine eggs were mainly distributed in the upper 60 m, whereas those of round herring extended further down to 80 m. The egg profile among the species varied ontogenetically, even though the majority of all egg stages for each species were concentrated near the surface. The differences in ontogenetic variations with depth for each species were found to be statistically significant, anchovy: $\chi^2 = 63.364$, $P < 0.05$, df = 8; sardine: $\chi^2 = 29.743$, $P < 0.05$, df = 8; and round herring: $\chi^2 = 40.515$, $P < 0.05$, df = 8. Recently spawned anchovy and sardine eggs were restricted to the upper 40 and 60 m, respectively, whereas those of round herring were found further down to 80 m. Thus anchovies are considered to spawn between 0-40 m, where the water is relatively warm all the time, whereas sardines spawn at approximately 0-60 m and round herrings between 0-80 m. Late stage eggs for all three species were restricted to the upper 40 m. In general, results in this chapter therefore indicate that even though the three species may show some overlap in their spawning depth range, the intensity of spawning varies among the species.

Key words: Anchovy, Sardine, Round herring, southern Benguela, Eggs, Vertical, Distribution, Spawning, Depth.

4.2. INTRODUCTION

Horizontal distribution patterns in ichthyoplankton suggest that the reproductive strategies of fish are adapted to environmental conditions, ensuring successful transport of early life history stages to nursery areas (Cushing, 1975; Iles and Sinclair, 1982; Brodeur *et al.*, 1996). However, there is a large variations of the horizontal flow field along the vertical water column (Sundby, 1991), which influences the extent and the direction of horizontal dispersion or transport of the eggs and larvae in the water column (Neilson and Perry, 1990; Sundby, 1991). In addition to the heterogeneity in horizontal flow field along the vertical water column, there is a great deal of vertical heterogeneity in physical, chemical and biological properties in the water column of the world's oceans basin (Sverdrup *et al.*, 1942 cited in Solow *et al.*, 2000; Hutchinson, 1967). Vertical distributions may also affect rates of development and mortality, depending on the level of coincidence with the predators, food and physio-chemical conditions (Neilson and Perry, 1990; Solow *et al.*, 2000).

The vertical egg distribution patterns are affected by many factors including spawning depth, specific gravity (or often referred as buoyancy) and the ascent rates of eggs (Coombs *et al.*, 1985; Sundby, 1991) and mixing due wind events (Fish, 1928 cited in Page *et al.*, 1989; Sundby, 1991). The specific gravity is a function of egg diameter, lipid and water content, and hydration levels of the oocytes. Generally, water content, egg diameter and the presence of an oil globule are considered to be main determinants of specific gravity of fish eggs. For those species with no oil globule, the water content is a principal determinant of their specific gravity (Craik and Harvey, 1987), for example, anchovy and round herring eggs.

Immediately before spawning the oocytes of marine teleosts with pelagic eggs swell abruptly during final maturation, resulting in eggs with a volume three to five times larger than the originating vitellogenic oocytes and a water content of about 90-94% (Thorsen *et al.*, 1996). The water uptake during final maturation appears to be mediated osmotically by hydrolysis of yolk proteins into free amino acids (FAA) and a simultaneous uptake of ions, mainly potassium and chloride (Craik and Harvey, 1987; Thorsen *et al.*, 1993). As a consequence of this process, newly spawned marine pelagic fish eggs store 15-40% of their total amino acids content as FAA (Thorsen and Fyhn, 1991). A high water content together with the eggs' lipid content balances the effect of heavy components (e.g. amino acids and salts) (Craik and Harvey, 1987), making them pelagic. Pelagic eggs are believed to remain hypotonic to surrounding seawater, which enhances their positive buoyancy during their development (Yin and Blaxter, 1987).

Kjesbu *et al.* (1992) and Thorsen *et al.* (1996) reported negative correlations between egg diameter and specific gravity for marine cod *Gadus morhua L.* eggs. Those authors identified the effect of the large diameter and presence of an oil globule as acting as lifting forces. However, variation in egg diameter among the eggs of the species has been reported, and was considered to be related to the condition of the fish, such as weight and size of the female fish (i.e. cod species, Kjesbu *et al.*, 1991). The production of eggs with a smaller diameter is most likely to occur in smaller fish (Kjesbu, 1989) or in fish that are in bad condition (i.e. starving, Kjesbu *et al.*, 1991). Egg size may also vary over the spawning period, for instance in cod, eggs with large diameter are produced initially and eggs with small diameter towards the end of the spawning period (Kjesbu *et al.*, 1992). Therefore, because of larger size and an oil globule, sardine eggs would be expected to rise faster than anchovy and round herring eggs, and concentrate closer to the surface.

Spawning depth has a strong effect on early ontogenetic stages because they are closest in time to the spawning event. If egg specific gravity decreases during early development, the eggs will be more positively buoyant at an early stage than at a late stage (Jacobsen and Johansen, 1908 cited in Page *et al.*, 1989; Moksness and Torstensen, 1985). A study by Parrish *et al.* (1981) has showed that a reasonable depth for the Ekman layer is the upper 20 m; Stenevik *et al.* (2001) reported that in the northern Benguela upwelling ecosystem below this depth, water moves inshore.

The significance of egg vertical distributions has been rarely considered in the southern Benguela ecosystem, but see Parada *et al.* (2003). Understanding the forces on the vertical distribution patterns of fish eggs and larvae is one of the compelling challenges in the understanding of fisheries science (Aoki and Marayama, 1993). In order for us to understand which factors and processes that affect the overall egg abundance and distribution patterns for anchovy, sardine and round herring eggs, we first need to describe these patterns. Various statistical approaches can then be used to examine possible causes.

In the southern Benguela upwelling ecosystem, anchovy, sardine and round herring all spawn over the western Agulhas Bank (WAB) (Armstrong *et al.*, 1991; Roel *et al.*, 1994; Hutchings *et al.*, 2002). However, there are very few or no data at all on the vertical egg patterns of any fish egg species. For anchovy, Shelton and Hutchings (1982) reported that eggs were restricted to the upper 50 m above the thermocline and concentrated in the top 30 m depth. No data exist for sardine and round herring egg vertical distribution patterns. In the northern Benguela upwelling ecosystem, all three species' eggs have been reported to be confined in the upper 50 m above the thermocline (Olivar, 1990; Olivar and Shelton, 1993). The effect of ontogenetic variability and its ecological implications remain poorly studied in the southern Benguela region.

Increasing use of the Daily Egg Production Method (DEPM) of estimation of egg abundance, which is used to back calculate the biomass of the spawners, requires that their vertical egg distribution be well understood to ensure unbiased estimates of egg abundances. This is also of particular interest considering the fact that the use of the CUFES samples are being considered to be used for the DEPM (Checkley *et al.*, 1997; Fletcher *et al.*, 1997).

The objectives of this Chapter are:

- To describe and compare the vertical egg profiles of anchovy, sardine and round herring in relation to the temperature profile, and
- To describe and compare the vertical egg profile in relation to ontogenetic variation.

Particular emphasis in this chapter is paid to egg abundance at depth in relation to ontogenetic development because it has significant implications for Ekman transport and early larval survival. Early egg stages are expected to be mainly located below the offshore moving horizontal flow field (0-20 m) to reduce losses of early stages due to Ekman transport.

4.3. DATA ANALYSIS

4.3.1. Vertical distribution plots

Eggs from different depths strata were identified, counted and expressed in numbers per m^3. To illustrate the relative proportion of eggs in the water column in relation to temperature, bar plots for the vertical egg abundance and distribution patterns at each station and temperature profiles were generated separately then overlaid.

To investigate the vertical egg distribution in relation to ontogenetic variation, eggs of each species were grouped into three categories, based on stage descriptions given by Moser and Ahlstrom (1985) and Lo *et al.* (1996):

1. eggs without embryo (early stage eggs, e.g. stages 1-3);
2. eggs with early stage embryos (middle stage eggs, e.g. stages 4-7, where the tail is still attached to the yolk); and
3. eggs with late embryos (late stage eggs, e.g. stages 8-11), where the tip or the entire tail was not attached to the yolk.

This classification allowed the assessment of whether different developmental egg categories show vertical different vertical distributions. O'Toole and King (1974) did provide formal description of round herring egg, but not in the 11 stages as has been done for anchovy and sardine. They only give five stages (A-F). Eleven developmental stages were identified using new protocols derived from established protocols prepared for anchovy and sardine (see Fig. 1 and 2, Appendix 1). The new protocols developed for round herring egg developmental stages are not discussed in this study.

4.3.2. Chi-square analysis

Chi-square (χ^2) analysis (Zar, 1999) was used to test whether the vertical egg abundance of anchovy, sardine and round herring eggs at all depth ranges were similar, and whether different developmental egg stages were distributed differently at all depth ranges by comparing observed against expected counts of eggs. For these calculations, samples collected using the closing WP II net samples during $P_{II}E_3$ and PIII were analysed separately then pooled by depth category.

Expected egg frequencies for each species at each depth range ($E_{species,\ depth}$) were calculated using the formula:

$$E_{species,depth} = \frac{C_{species} * R_{depth}}{N} \text{.........4.2}$$

where N is the total number of eggs of all species at all depths, $C_{species}$ is the total number of eggs of each species and R_{depth} is the total number of eggs for all species at each depth range. The Contingency table used for the χ^2 analysis is provided in the Appendix 1, Tables 4-7.

In cases where expected frequencies of less than five counts were more than 20% of the total, adjacent groups were combined. Furthermore, in cases where the joining of adjacent groups led to the degrees of freedom being one, a correction for continuity (Zar, 1984) was applied using the formula:

$$\chi^2 = \sum \frac{\left(|O - E| - 0.5\right)^2}{E} \text{.........4.3}$$

where O is the observed value, E the expected value and 0.5 the correction term.

In cases where a statistically significant difference among the species was established, additional χ^2 tests were performed in which species pairs in turn was compared, and differences were tested because the initial χ^2 analysis among the species does not indicate which species is/are responsible for the difference. However, because the additional χ^2 tests were developed after the initial χ^2 analysis, it is inappropriate to use the new tests to infer statistical differences (Zar, 1999).

4.4. RESULTS

4.4.1. Hydrographic conditions

$P_{II}E_1$ vertical station was located within shelf waters (< 200 m isobath) and far north of the study area compared to PIIE3 and PIII vertical stations, which were all located above and beyond shelf waters (> 200 m). At $P_{II}E_1$, temperatures steadily declined from 16.1°C at the surface to 15.6°C at 60 m, then a declined sharply to a minimum of 14.0°C at 80.2 m (Fig. 4.1). Vertical temperature profiles during $P_{II}E_3$ stations showed relatively stronger thermal stratification (Fig. 4.2) than was apparent at the $P_{II}E_1$ station. During PIII, the thermocline depth increased from south (26 m depth) towards the central region (45 m depth) and then declined further north (27 m depth) of the study area (Fig. 4.3).

4.4.2. Vertical distribution of eggs

The number of eggs collected at various depths (categories) from each sampler is given in Appendix 1, Tables 1-3.

4.4.2.1. *Egg profile in relation to temperature profile*

Samples from $P_{II}E_{1}$ vertical station were sampled using two different methods, namely a plankton pump and a closing WP II net. Plankton pump samples were collected at three depths (10, 30 and 50 m), but all three species' eggs only occurred at 10 and 30 m depths (Fig. 4.1a). Anchovy eggs were more abundant than those of sardine and round herring, and were concentrated near the surface at 10 m depth. Most sardine eggs were near the surface, whereas those of round herring were evenly distributed at both 10 and 30 m. Samples from the closing WPII net were collected from three depth ranges (0-20, 20-40 and 40-60 m). Anchovy eggs were found at all depth ranges, but sardine and round herring eggs were not. Sardine eggs were not found near the surface whilst round herring eggs were not found below 40 m depth (Fig. 4.1b).

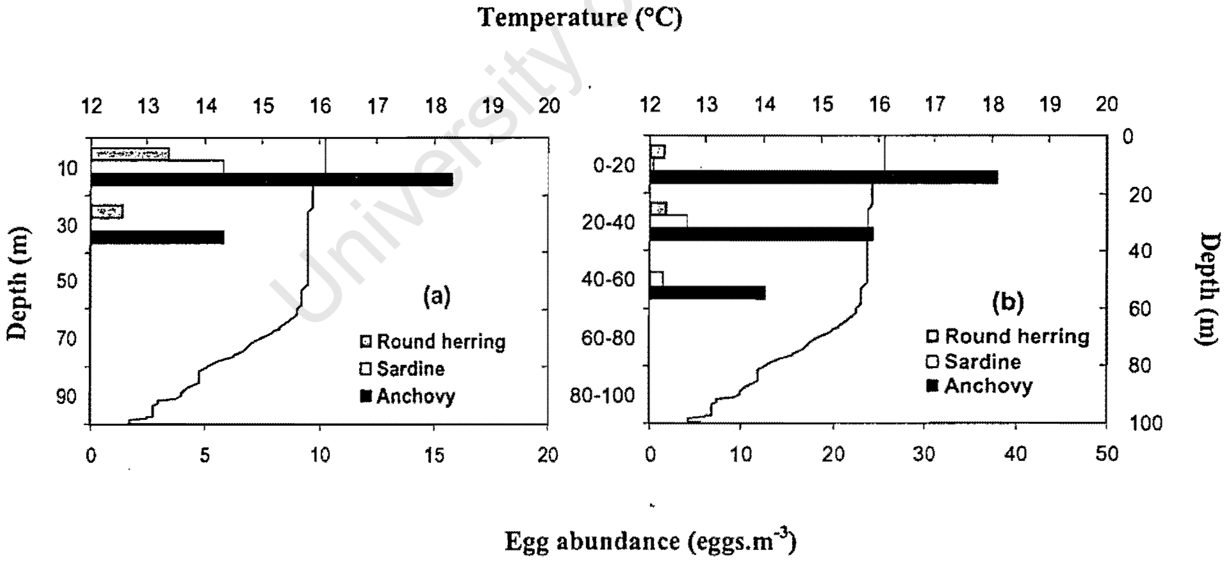


Figure 4.1. Vertical egg abundance for anchovy *Engraulis encrasicolus*, sardine *Sardinops sagax* and round herring *Etrumeus whiteheadi* (shown as bars), and temperature profiles (shown as line) from the station sampled during $P_{II}E_{1}$ using (a) plankton pump and (b) closing WP II net.

During $P_{II}E_{3}$, eggs were found over the entire depth range sampled from the surface to 100 m (Fig. 4.2). Low egg abundances for anchovy were observed at $P_{II}E_{3}$-001, and eggs were absent from stations $P_{II}E_3$-002, $P_{II}E_3$-004 and $P_{II}E_3$-005, whereas sardine and round herring eggs were present at all stations. Stations where eggs were absent for at least two species were not included for analysis, and such stations were $P_{II}E_3$-002, $P_{II}E_3$-003 and $P_{II}E_3$-004. Round herring egg abundances were generally low compared to those of anchovy and sardine. Highest anchovy egg abundances were generally concentrated near the surface and abundance decreased progressively with increasing depth, whereas those of sardine and round herring were distributed throughout the water column. Exceptional cases were sardine and round herring eggs were not concentrated close to the surface but were evenly distributed over first three depth ranges, and where anchovy egg distribution had a subsurface secondary peak at 40-60 m, occurred at two stations: P_{IIE}3-006 and $P_{II}E_3$-007. The water column at these stations was thermally stratified similarly to other stations, so the egg vertical distribution seems not to be entirely influenced by the thermal structure. Those differences in egg vertical distributions could be attributed to other factors, such as differences in the distribution of different ontogenetic stage, for details about the vertical distribution of different ontogenetic stages see next section.

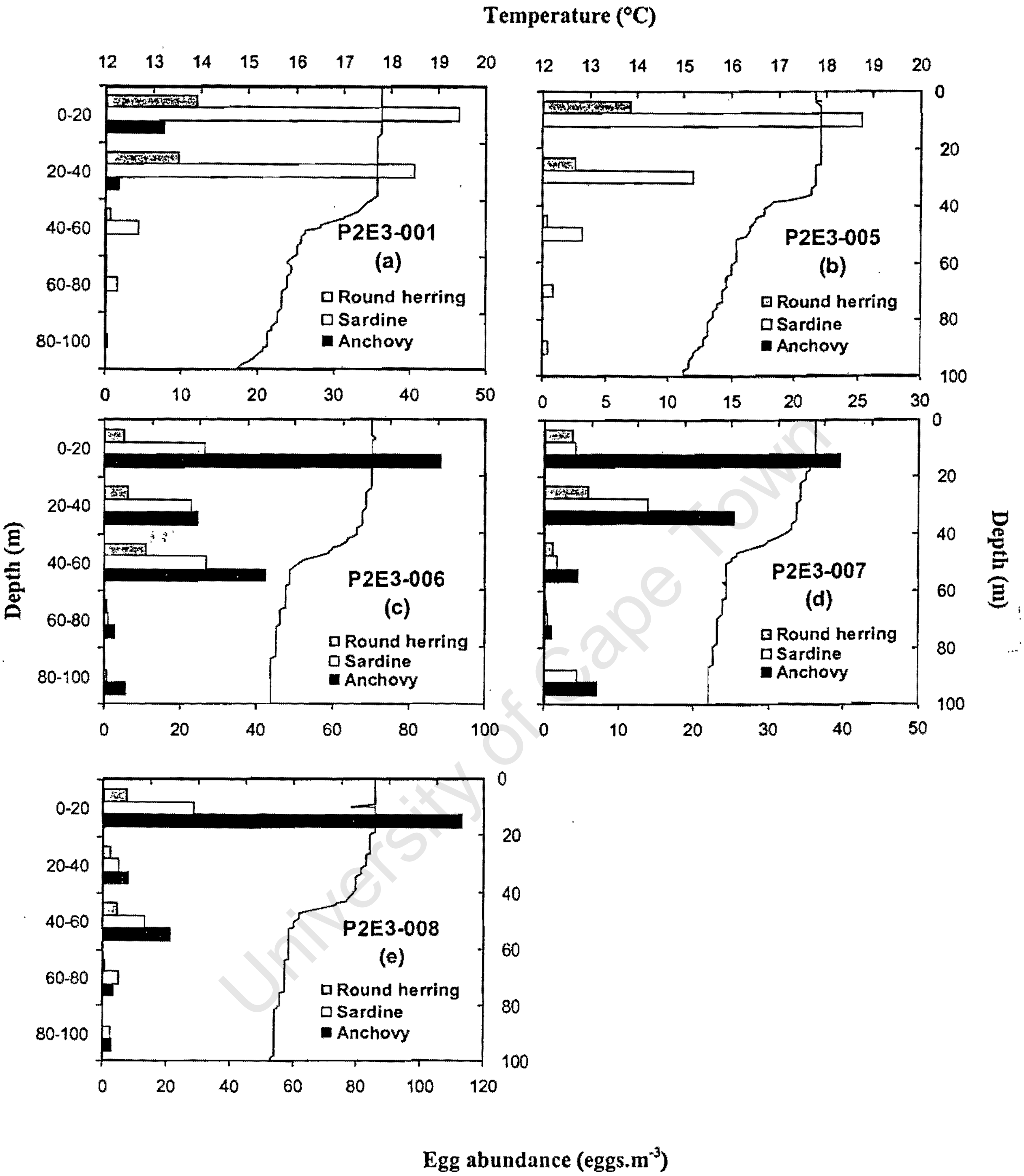


Figure 4.2. Vertical egg abundance for anchovy, sardine and round herring (shown as bars), and temperature profiles (shown as line) from stations sampled during $P_{II}E_3$ using a closing WP II net.

During PIII, eggs of all three species were found from all stations over the entire depth range sampled from the surface to 100 m (Fig. 4.3). Vertical egg distribution patterns for all species was the same at all stations: eggs were concentrated near the surface and concentration/abundance decreased with increasing depth.

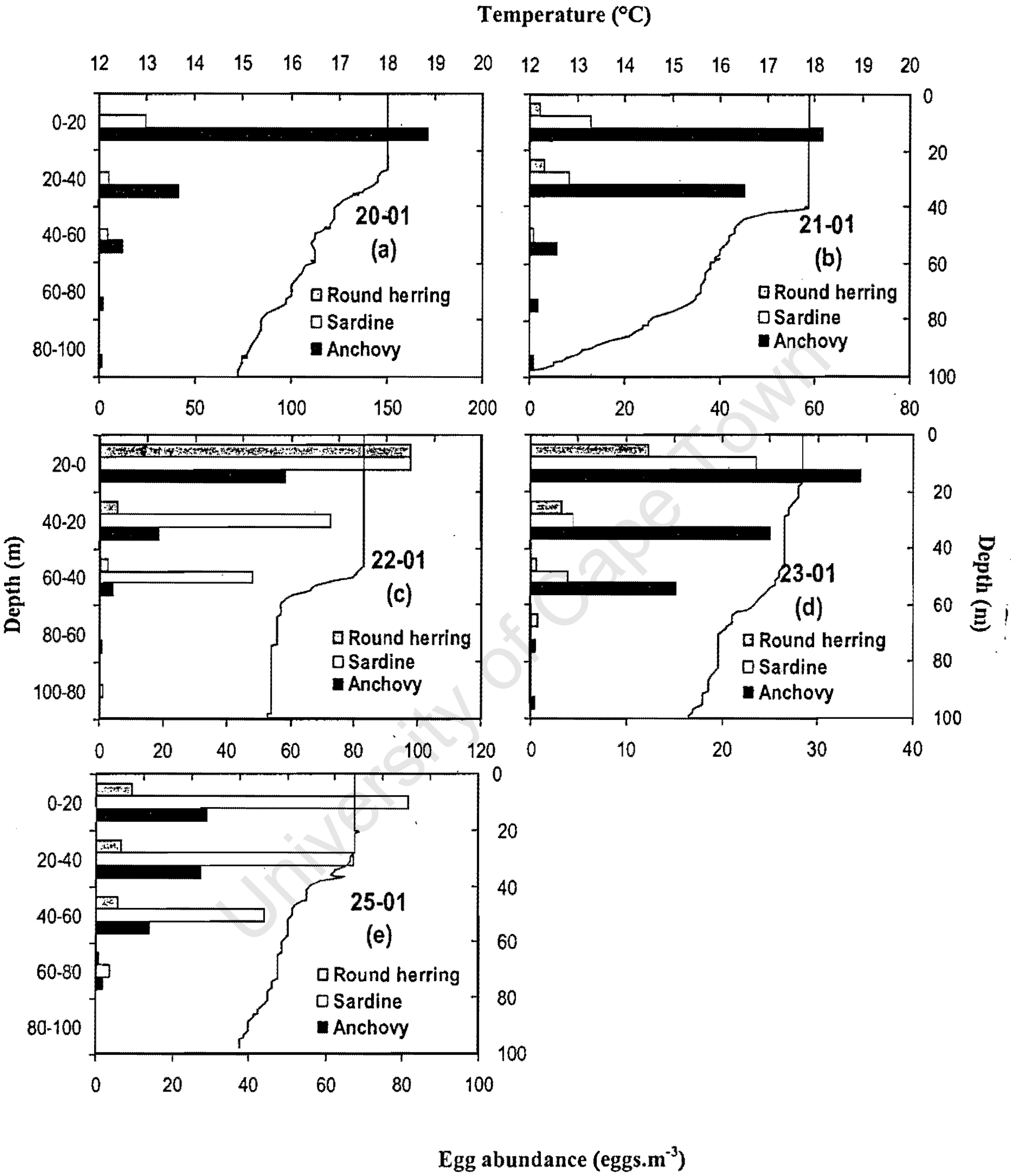


Figure 4.3. Vertical egg abundance for anchovy, sardine and round herring (shown as bars), and temperature profiles (shown as line) from station sampled during PIII using a closing WP II net.

In general, eggs of all three species were concentrated near the surface above the thermocline, particularly when the thermocline was strong; however some eggs were also found below the thermocline. Sardine and round herring vertical egg distribution was more variable at times than that of anchovy eggs, and causes for such cases are difficult to explain, considering that similar physical forces might have acted equally on all species. The variability in vertical egg distribution of the two species may be attributed to the probable wide spawning depth range and intraspecies variation in egg size.

The differences in vertical egg abundances during $P_{II}E_3$ and PIII (see Appendix 1 for data) at various depths among the species were found to be statistically significant ($\chi^2 = 243.341$, $P < 0.05$, df = 8) and ($\chi^2 = 349.897$, $P < 0.05$, df = 8), for $P_{II}E_3$ and PIII respectively. The percentage of anchovy and sardine eggs collected near the surface (0-20 m) was consistent for $P_{II}E_3$ and PIII, being 60 and 62% for anchovy and 45 and 47% for sardine, respectively. Consistent patterns were not observed for round herring: 45% and 80% of the total eggs were located in the top 20 m during $P_{II}E_3$ and PIII, respectively. The causes of such huge variations in the vertical distribution patterns for round herring eggs are hard to imagine, as similar physical forces might have acted equally on all species. As has already been suggested in previous pages such variations may be attributed to changes in biological features, such as ontogenetic variation.

The difference in egg abundances at various depths among the species was also found to be statistically significant ($\chi^2 = 329.330$, $P < 0.05$, df = 8) for the pooled data. More anchovy eggs than expected were consistently found at 0-20 m depth range and less than expected below the that depth range, if all the eggs of the three species were distributed in a similar pattern (see Appendix 1 for data). In contrast, fewer sardine eggs were encountered near the surface and more at depth than would be expected. For round herring eggs, similar patterns to those observed for anchovy were found, but the vertical egg distribution patterns for this species were found to vary between datasets.

Additional χ^2 tests showed that the differences between each species pair were statistically significant (Table 4.1), and implied that the differences to be caused mainly by sardine eggs that were less abundant on the surface during PIII and in the pooled data, mainly caused mainly by anchovy eggs, which were more abundant than expected on the surface during $P_{II}E_3$ if all species' eggs were distributed in the same pattern.

Table 4.1. Results of the chi-square (χ^2) analysis showing comparisons between the vertical egg distribution of species pairs from the samples taken during PIIE3, PIII and for the pooled data from the closing WP II net tows. All comparisons were made at 0.05 level of significance (P).

Phase (Expt)	df	Anchovy vs Sardine	Sardine vs Round herring	Round herring vs Anchovy
II(3)	4	211.164	10.321	82.367
III	4	173.125	253.729	91.070
Collective	4	271.913	149.447	12.969

4.4.2.2. *Egg profile in relation to ontogenetic variation*

A reasonable number of anchovy eggs were collected from the plankton pump and closing WP II net, but very few eggs of sardine and round herring were collected during $P_{II}E_1$ (see Appendix 1 for data). Most of the anchovy eggs collected from both samplers were middle egg stages (Fig. 4.4a,d), and were concentrated near the surface (0-20 or 10 m), but early egg stages at 40-60 m. Most of the sardine eggs collected from both plankton pump and WPII nets samples were late egg stages and were concentrated near the surface. Few round herring eggs were collected during $P_{II}E_1$, most of those collected from the plankton pump were late egg stages and concentrated near the surface, whereas those collected using a closing WP II net were early stage eggs, and were concentrated near the surface. The cause for discrepancy of egg stages collected by the two samplers is unclear, but could be considered to highlight the differences in the two samplers efficiency to capture different developmental egg.

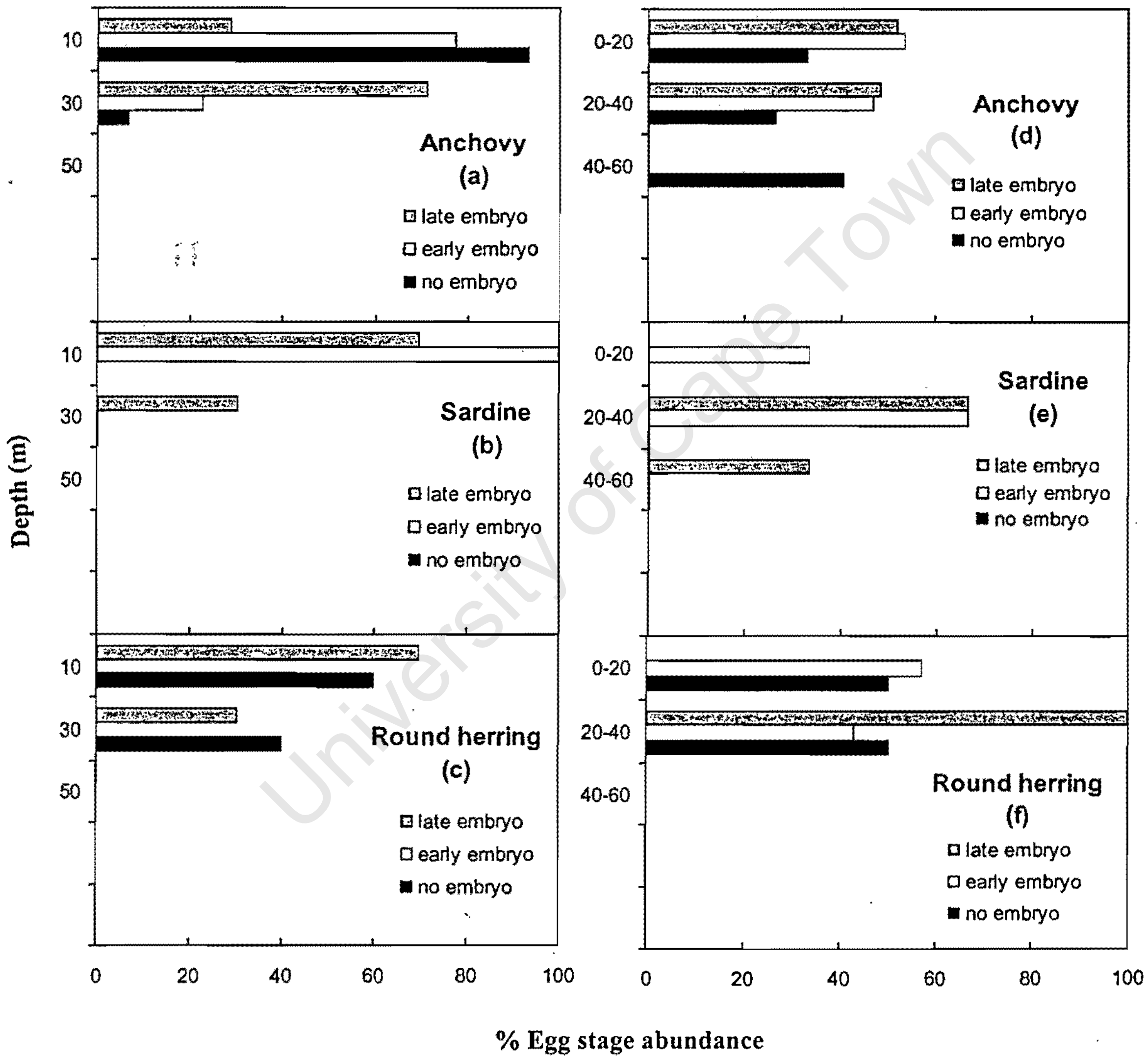


Figure 4.4. Percentage depth profile by stage category of anchovy, sardine and round herring, eggs sampled during $P_{II}E_1$ from (a-c) the plankton pump and (d-f) WP II net.

During $P_{II}E_3$, the majority (> 50%) of recently spawned (i.e. with no embryo) anchovy eggs were distributed within the top 20 m of the water column and their abundance decreased exponentially, and very small proportion of eggs were collected below 60 m depth (Fig. 4.5a). For sardine, the majority (> 50%) of early egg stages were located subsurface at 20-40 m (Fig. 4.5b), whereas those of round herring were evenly distributed at 0-20 and 20-40 m depths (Fig. 4.5c), and a considerable proportion of recently spawned eggs for both species were also found further down to 80 m depth. Early and late egg stages for all three species' eggs were mainly distributed within the upper 60 m depth of the water column.

The differences in egg abundances at each depth in relation to ontogenetic variation during $P_{II}E_3$ were found to be statistically insignificant for anchovy eggs ($\chi^2 = 5.040$, df = 8, $P > 0.05$), whereas this was significant for sardine ($\chi^2 = 57.048$, df = 8, $P < 0.05$) and round herring ($\chi^2 = 52.959$, df = 8, $P < 0.05$). Additional χ^2 tests for sardine and round herring eggs showed that the main differences were caused by the recently spawned egg stages, which were more abundant below the surface than would have been expected if all ontogenetic stages were distributed equally.

During PIII, over 50% of all developmental egg stages for anchovy and sardine were concentrated near the surface (0-20 m), then the abundance declined progressively to almost nothing below 60 m depth. In contrast, only 42% of early egg stages for round herring were distributed near the surface, and the rest were widely distributed down to 80 m depth and declined progressively. Middle egg stages of all species followed similar distribution patterns to those observed during $P_{II}E_3$.

The differences in egg abundance at each depth in relation to ontogenetic variation was found to be statistically significant, (anchovy: $\chi^2 = 20.148$, df = 8, $P < 0.05$; sardine: $\chi^2 = 18.031$, df = 8, $P < 0.05$; and round herring $\chi^2 = 24.152$, df = 8, $P < 0.05$). Additional χ^2 tests for anchovy eggs showed that middle stage eggs were responsible for the differences, they were less abundant near the surface if all egg stages were to be distributed equally. For sardine and round herring eggs the main source of the differences was in recently spawned eggs that were more abundant subsurface than would have been expected, if all ontogenetic stages were distributed similarly.

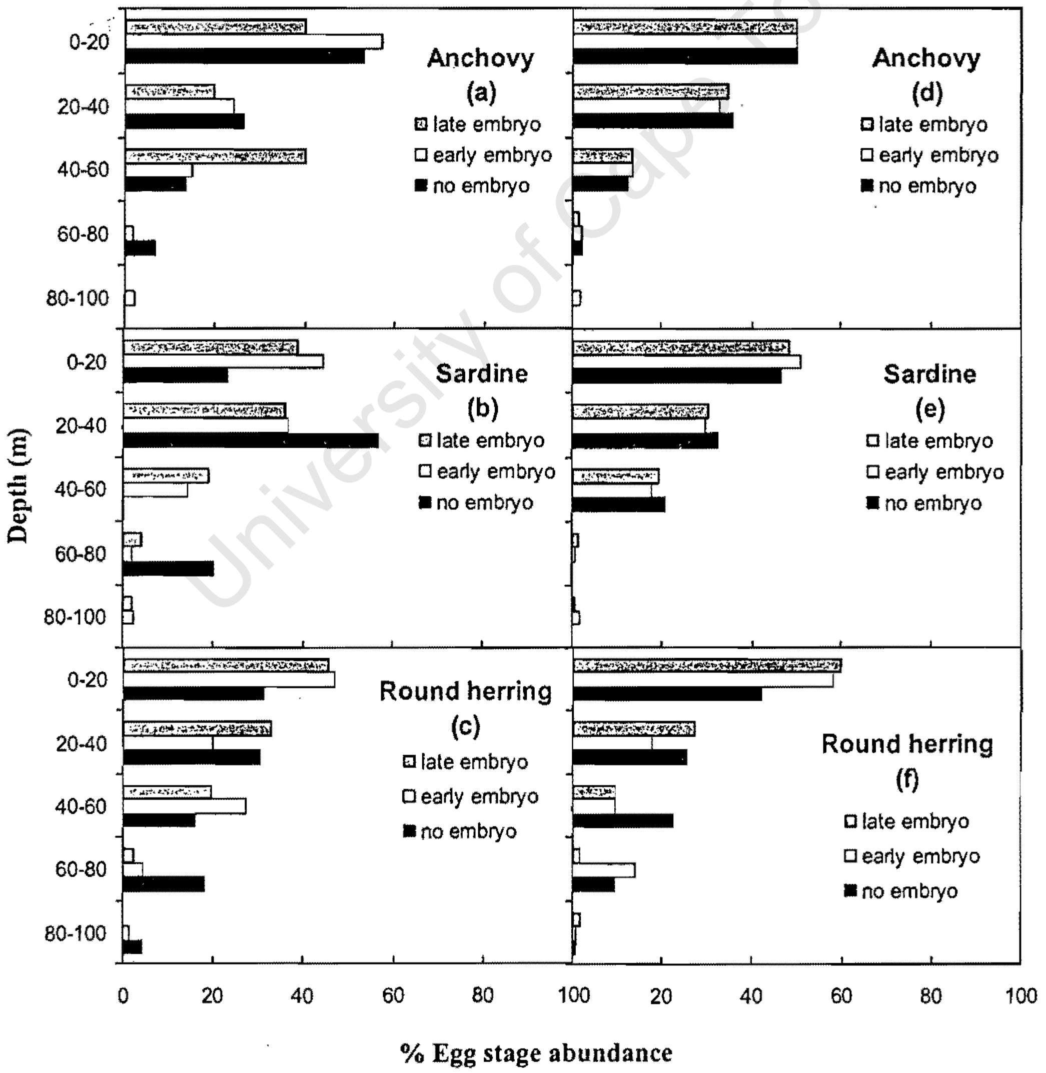


Figure 4.5. Percentage depth profile by stage category of anchovy *Engraulis encrasicolus*, sardine *Sardinops sagax* and round herring *Etrumeus whiteheadi*, eggs sampled during (a-c) $P_{II}E_3$ and (d-f) PIII from WP II net.

Pooled data showed that over 50% of early stage eggs for anchovy were located near the surface and restricted to the upper 60 m depth, whereas this value was slightly less than 50% for sardine and round herring (Fig. 4.6). At a depth range of 20-40 m, the abundance of the three stage categories for all species was even. Late egg stages for all species were restricted to the upper 60 m depth.

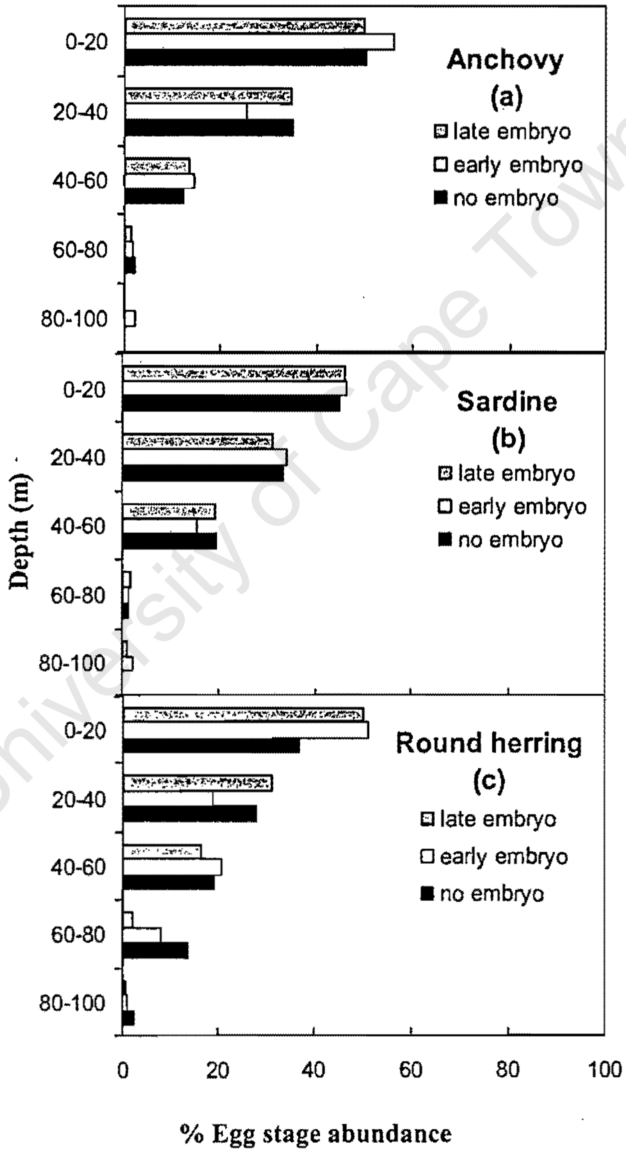


Figure 4.6. Percentage depth profile by stage category of anchovy, sardine and round herring, eggs from pooled data collected using a closing WP II net.

For the pooled data, the differences in egg abundances at each depth in relation to ontogenetic variation was found to be statistically significant for all species, (anchovy: $\chi^2 = 63.364$, df = 8, $P < 0.05$; sardine: $\chi^2 = 29.743$, df = 8, $P < 0.05$; and round herring: $\chi^2 = 40.515$, df = 8, $P < 0.05$). Additional χ^2 tests showed that early eggs were responsible for the differences among anchovy developmental egg stages as more of these stages were collected near the surface than would be expected if all ontogenetic stages were distributed similarly. For sardine and round herring eggs the main cause of the differences was recently spawned eggs, which were more abundant below near surface waters than would have been expected if all ontogenetic stages were distributed equally.

Collectively, the results of the distribution of eggs in relation to ontogenetic variation suggest that the spawning depth range of all three species overlap, but that of anchovy was mainly within the top 40 m of the water column, whereas that of sardine and round herring extended deeper. These results therefore indicate that the vertical egg profile for sardine and round herring is greatly influenced by ontogenetic variation.

4.5. DISCUSSION

4.5.1. Comparing vertical egg distribution patterns

This study has provided quantitative information on vertical egg distribution patterns of the most important pelagic fish species in the southern Benguela upwelling ecosystem. Knowledge of vertical egg distributions is crucial for understanding the ecological consequences that different vertical egg distributions may have on the early life history stages of pelagic fish species. Closing WP II net samples collected off the WAB show that anchovy, sardine and round herring eggs were distributed from the surface down to 100 m depth of the water column sampled. However, very few eggs of any of the three species studied was found below 80 m depth, and 61%, 45% and 64%, for anchovy, sardine and round herring, respectively, were found within the top 20 m, above the thermocline. Even though there was a weak thermocline at approximately 70 m during $P_{II}E_{I}$, eggs were consistently distributed in the top 50 m.

These results for anchovy concur with the observations made by Shelton and Hutchings (1982) for the same species in the southern Benguela upwelling ecosystem. They reported that almost all anchovy eggs were restricted to surface waters above the thermocline, typically above 50 m depth, with the highest densities in the top 30 m. Elsewhere, Moser and Pommeranz (1999) and Motos and Coombs (1998) cited in Santos *et al.* (2000) with a vertical resolution of tens to hundreds of centimetres reported that 95% of anchovy *E. encrasicolus* and *E. mordax* eggs were restricted in the upper 30 m. Off the NW Mediterranean Sea, Olivar *et al.* (2001) conducted comparative studies of anchovy *E. encrasicolus* and sardine *Sardina pilchardus* egg vertical distributions, and found that anchovy eggs concentrated near the surface, whereas those of sardine were distributed deeper. In the northern Benguela upwelling ecosystem O'Toole (1977), Olivar (1990), and Olivar and Shelton (1993) reported that anchovy, sardine and round herring eggs were restricted to the upper 50 m, above the thermocline. Konishi (1980) reported that round herring eggs were most abundant near surface waters above the thermocline, but were distributed deeper than those of sardine. The vertical egg distribution of round herring in the southern Benguela ecosystem contrasts with the reports made for the northern Benguela round herring eggs by Olivar and Shelton (1993) in which they reported that over 50% of the eggs were mainly distributed in the upper 50 m. In this study, round herring vertical egg distribution was found to be broadly distributed and variable, where in one experiment eggs were mainly distributed subsurface, and in the other experiment at the surface. The cause for the discrepancy is unclear, but it is alleged that it may have resulted from a wide and variable vertical egg profile of this species, as manifested during this study.

Generally, these results therefore reaffirm the idea proposed by Lasker (1985) and Fletcher *et al.* (1997) that it is necessary to sample only to 70 m depth when conducting surveys to estimate the spawner biomass stocks for anchovy and sardine when using the DEPM. The kind of the results presented for this study suggest that similar protocols be applied for our local anchovy and sardine species, but not for round herring eggs. It is therefore suggested that it would be necessary to sample down to 90 m depth if samples were to be used for the DEPM, as this species has shown the possibly of spawning deeper than 70 m. In addition, the surface concentration of all three species' eggs necessitates a cautionary sampling measure that the net should not be dragged horizontally at the surface, because that could positively bias the egg abundance estimates. Despite that, (Lo pers. Comm. cited in Fletcher, 1997) surface sampling could be an efficient way of mapping spawning areas of pelagic spawning fishes, and the CUFES is particularly well suited for this purpose due to its advantage of continuous sampling.

4.5.2. Comparing ontogenetic egg vertical distribution patterns

Results presented above show that all anchovy developmental egg stages collected during this study were restricted to the warm, well-mixed surface layer (0-20 m) of the water column. Early staged eggs were confined in the top 40 m, therefore it appears that anchovy spawn near the between 0 ~ 40 m in the southern Benguela ecosystem. Matsuoka *et al.* (2002) has reported a similar finding for the Japanese anchovy *Engraulis japonicus* early egg stage distributions, and suggested that it spawned mainly in the top 20 m.

For sardine, a considerable proportion of early stage eggs were distributed down to 60 m during this study. During $P_{II}E_3$, most of the early stage eggs were located at 20-40 m, whereas during PIII were located near the surface, but a considerable proportion was located at 20-40 m depth. Although the spawning depth of sardine appears to overlap with that of anchovy, it seems likely that the former spawns deeper (approximately at 0 ~ 60 m) than the latter species. Furthermore, the fact that sardine eggs are more positively buoyant than those of anchovy, because they have a large diameter and contain an oil globule, which enhances the buoyancy of this species compared to small egg with no oil globule in anchovy. Sardine eggs therefore ascended to the surface at a faster rate even if they are spawned deeper. Fletcher (1999), Steinevik *et al.* (2001) and Matsuoka *et al.* (2002) reported similar spawning depths for the Australian, Namibian and Japanese sardines, except that these authors excluded the top 20 m of the water column.

Early stage eggs of round herring were distributed evenly down to 60 m and even extended down to 80 m. Thus it is believed that round herring in the southern Benguela environment spawns at greater depth (approximately at 0 ~ 80 m) than anchovy and sardine. This hypothesis is supported by Konishi (1980), who reported that round herring eggs had a deeper distribution than did sardine.

Worth mentioning from this study is the fact that the abundance and distribution of eggs collected appeared to be related to the time at which samples were collected. Modelling studies of vertical egg distribution show that after at least 10 hours, eggs would have reached an equilibrium depth distribution (Sundby, 1983). This suggests that eggs spawned at depth will reach an equilibrium depth (reach to the surface) after 10 hours. Samples during $P_{II}E_3$ were collected mainly during day light (09h30-18h30 GMT) and contained very few early egg stages. Samples collected after 18h30 contained more early egg stages for all species. During PIII, samples were collected during night time (02h50-03h59 GMT), and of all eggs collected most were early egg stages for all species and were located subsurface. At this period (02h50-03h59 GMT), eggs would not have reached the equilibrium depth distribution, thus the three species appeared to spawn mainly in the evening (e.g. 18h00-02h00) and most probably below the top 20 m.

Temperature affects the duration of the egg stage (Laurence and Rogers, 1976; Pepin, 1991) and since temperature in the southern Benguela ecosystem are highly variable due to irregular and frequent upwelling, the duration of the egg stages would be expected also to vary accordingly. Le Clus and Malan (1995) reported on temperature-dependent development rate studies of sardine eggs in the northern Benguela upwelling ecosystem and showed that early and late egg stages had a shorter duration than the middle egg stages. This therefore explains why most of the eggs collected for all species in this study were intermediate egg stages.

CHAPTER 5. RELATING SPAWNING TO TEMPERATURE AND GEOGRAPHIC LOCATION

5.1. ABSTRACT

Investigations were conducted on egg distribution patterns of anchovy *Engraulis encrasicolus*, sardine *Sardinops sagax* and round herring *Etrumeus whiteheadi* in relation to temperature and geographic location, indexed by longitude and latitude. Quotient rule analysis was used to characterize the preferred temperature range for spawning, and generalized additive models (GAMs) were used to characterize and distinguish the preferred spawning locations. Preferred spawning temperature range for anchovy and sardine was 17.6-18.2°C, whereas for round herring the preferred spawning temperature range appeared to be slightly wider (16.8-18.1°C), than the other two species. Quotient plots for round herring were bimodal and the primary peak was well separated from that of anchovy and sardine unimodal peaks, but the secondary peak overlapped. Generally, preferred temperature ranges for anchovy and sardine spawning overlapped completely during this study, but that of round herring appeared distinct. Anchovy spawning was found to be specific in relation to geographic location, whereas those of sardine and round herring appeared to be less specific.

Key words: Anchovy, Sardine, Round herring, Eggs, Abundance and distribution, Spawning, Temperature, Geographic location

5.2. INTRODUCTION

Temperature exerts a major influence on aquatic organisms, both in terms of development, distribution, composition and dynamics, in water temperature (Wood and McDonald, 1998) and the distribution, recruitment, productivity, and even the survival of key commercial fish species (Hubbs, 1984; Fields *et al.*, 1993; Bakun, 1996; Alheit and Hagen, 1997). The consequences of change in temperature disturb every rate process within an organism, including sensory organ function, respiration and metabolism, muscle contraction and swimming, feeding and assimilation, growth and development; regulation and timing of gametogenesis and spawning; and egg and larval development (Lluch-Belda *et al.*, 1991; Wood and McDonald, 1998). Adverse thermal effects on any of the aforementioned processes could negatively impact the population's recruitment potential. Kawasaki (1983) and Lluch-Belda *et al.* (1992) stated that fluctuations in fish population stocks around the world were synchronous with climatic changes. However, recent evidence (Renssen *et al.*, 2001) suggests that climatic changes and stocks fluctuations were not synchronous throughout the oceans. Instead, changes in heat transport by the global thermohaline circulation might have led to opposite regional warming or cooling trends (for details see Broecker, 1998). For example, Blunier *et al.* (1998) reported that in some instances opposite climatic trends seem to have operated at the same time in the southern and northern hemispheres.

The southern Benguela ecosystem, as with other coastal upwelling areas, is highly variable in terms of both physical and biological processes. In such dynamic environments, pelagic fishes are though to select specific sites and oceanographic conditions for spawning in order to maximize the survival of their reproductive products (Bakun, 1996). Among other factors though to prompt the selection of specific spawning sites is temperature, which is considered to be a good indicator of preferred spawning range, but otherwise it is a poor indicator if used by itself, as suitable temperature ranges may be wide (Wood and McDonald, 1998). In the southern Benguela ecosystem a great deal of effort has been focused on the physical and biological processes that influence the population and recruitment variability of anchovy *E. encrasicolus* (Roel *et al.*, 1994; Cochrane and Hutchings, 1995; Hutchings *et al.*, 1998; Richardson *et al.*, 1998).

Extensive efforts have been made to investigate the effects of temperature on the abundance and distribution patterns of anchovy and sardine eggs, larvae and adult fish in the southern Benguela ecosystem (Anders, 1965; King *et al.*, 1978; Shelton, 1986; Armstrong *et al.*, 1987; Shelton and Hutchings, 1990; Hutchings *et al.*, 1998; Richardson *et al.*, 1998; van der Lingen *et al.*, 2001). It has been reported that anchovy spawn primarily in waters of 16-19°C (Anders, 1965; King *et al.*, 1978; Armstrong *et al.*, 1991; Fowler, 1998; Richardson *et al.*, 1998), but more recently van der Lingen *et al.* (2001) and Twatwa (2002) reported preferred spawning temperatures ranges of 15.2-20.5°C and 12-21.5°C, respectively.

Very few efforts had been made to investigate the relationship between sardine spawning and temperature in the southern Benguela ecosystem. King's (1977) laboratory studies showed that maximal development rate of Namibian sardine *Sardinops sagax* eggs would occur at 16-21°C. In the southern Benguela ecosystem Fowler (1998) reported that sardine eggs were predominantly found in waters of between 14.5-21.5°C, van der Lingen *et al.* (2001) found a range of 15.2-20.5°C and Twatwa (2002) reported a 12-21.5°C temperature range.

Data on preferred spawning temperatures of round herring are still lacking in the southern Benguela ecosystem. O'Toole and King's (1974) laboratory studies for the Namibian round herring *Etrumeus whiteheadi* demonstrated development rate increased exponentially, with temperatures for round herring eggs within 11.5-20.5°C temperature range.

Variability in spawning across the shelf (i.e. longitude or inshore/offshore) and alongshore (i.e. latitude) has been examined from ichthyoplankton samples in the southern Benguela ecosystem (see Fowler, 1998; van der Lingen *et al.*, 1998; van der Lingen *et al.*, 2001; Twatwa, 2002). In those studies, anchovy has been reported to spawn mainly in shelf waters and restricted to low latitude areas (i.e. Agulhas Bank). Castro *et al.* (2002) reported that variation in spawning alongshore has a substantial influence on the ontogenetic traits, such as egg and larval size. In this report Castro *et al.* reported that the egg and larval size increased with latitude. Due to time constraints these aspects could not be investigated in this study. In this study I only looked at variation in egg abundance in relation to temperature and to both longitude and latitude.

A broad-scale investigation of spawning to relation with temperature for anchovy and sardine has been documented for southern Benguela ecosystem (van der Lingen et al., 2001; Twatwa, 2002). However, explicit investigations of the effect of longitude and latitude are still lacking. In addition, data indicating the preferred spawning areas of round herring in relation to temperature is lacking. Finally, there have been no data that allowed comparisons of the spawning sites of the three species in the region.

The objectives of this chapter are:

- To investigate and compare egg abundance and temperature in order to establish the preferred spawning temperature range for anchovy, sardine and round herring, and
- To investigate and compare the effects of longitude and latitude on egg abundance.

5.3. DATA ANALYSIS

5.3.1 Quotient rule analysis

Egg abundance and temperature data from all the samples taken during all three phases were combined in order to assess the preferred temperature range for spawning. First, the egg abundance (E_c) within each 0.1°C temperature class (c) was expressed as a percentage of total egg abundance (E):

$$\%E_C = \frac{E_C}{E} X100\% \ldots\ldots\ldots 5.1$$

The percentage egg abundance within each temperature class ($\%E_C$) was then divided by the percentage frequency of occurrence of that particular temperature class ($\%F_{TC}$). The resultant quotient value (Q_C) was used to reflect the preferred spawning temperature range, with an assumption that fish do select spawning sites on the basis of the temperature.

$$Q_C = \frac{\%E_C}{\%F_{TC}} \ldots\ldots\ldots 5.2$$

Values of $Q_c > 1$ are considered to indicate positive selection, whereas Q_c values <1 were considered to indicate the avoidance of spawning at that particular temperature. Quotient values were smoothed using a five-point running mean (for details see van der Lingen *et al.*, 2001, although they used an 11-point running mean), then plotted against temperature.

5.3.2 Generalized additive models (GAMs)

Egg abundance, longitude and latitude data from all the samples taken during all three phases were combined in order to assess the preferred geographic location for spawning. Relationships between two or more variables may be analyzed using statistical methods, often involving a prior transformation such as a logarithmic transformation. However, linear models are explanatory techniques that do not provide an insight into ecological processes (Austin *et al.*, 1990). These relationships are determined from statistical models that establish the strength of the relationship between the response variable (e.g. egg abundance) and a suite of one or more explanatory variables (e.g. temperature, food production, etc.) and presuppose a Gaussian function.

Generalized additive models (GAMs) are semi-parametric extensions of general linear models (GLMs). GAMs are less rigorous than GLMs but posses greater ability to identify non-linear and non-monotonic relationships, and use degrees of freedom (df) to define the equivalent number of factors used in the model. For a single factor additive model, hence single coefficient, such a model with an intercept only requires one df. The number of df is a function of the smoother of the predictor variable in the data set (Hastie and Tibshirani, 1990).

Generally, a generalized additive model provides an adjusted statistical relationship between the response and predictor variable(s) by a smoothing function for each predictor using smoothers and assumes that the observed response variable is independent of the predictor variable. Basically, smoothers are tools that summarize the general pattern of observations of the response (Y) variable as a function of a predictor (X_i), which is less variable than the actual pattern. Smoothers work by averaging the observed values of the response variable in each neighbourhood (span) in the data set. The bigger the span, the smaller the variance in response variable (Y), however, this may be misleading, because it forces a pattern that may not and conversely a smaller span results in high variances. The degree of smoothness may be selected automatically using cross-validation or may be specified in terms of degrees of freedom (Hastie and Tibshirani, 1990).

A number of different smoothers exist, but two smoothing functions are commonly used in transforming predictors an are: loess (lo) and spline smoothers. The loess smoother fits a locally weighted least-squares regression to estimate the smoother function. Smoothing splines are locally cubic splines that minimize a residual sum of squares.

The GAM uses a function that replaces linear terms with smoothers and thus accommodates non-linearities, and the GAM uses the formula:

$$Y = a_0 + s_1(X_1) + s_2(X_2) + \ldots + s_n(X_n) \ldots\ldots\ldots 5.3$$

Y is the adjusted value of the response variable, a_o is an intercept, s_1 is the first smoother, X_1 is the first observation of the predictor variable, etc.

Separate GAM scatter plots were constructed, and explained variance (i.e. r^2 value) computed for anchovy, sardine and round herring eggs using the S-Plus 4.5 computer package. Egg density estimates were log transformed to improve the degree of normality and homoscedasticity. The form of the relationship between egg abundance and the environmental variables was determined visually from GAM plots.

5.4 RESULTS

5.4.1 Quotient rule analysis

Temperatures at on-station and underway CUFES samples during this study ranged between 15.3 and 18.9°C, with the most samples collected from waters where the temperature ranged between 17.2 and 18.4°C (Fig. 4.1a). Anchovy, sardine and round herring eggs were collected over a similar temperature range mentioned above, but the quotient-temperature plot (Fig. 4.1b) illustrates some degree of distinction in preferred spawning temperature range between round herring and the other two species, whilst anchovy and sardine were similar.

The results indicate that the preferred spawning temperature range for anchovy ranged from 17.6-18.2°C, and 17.7-18.1°C for sardine, whereas that of round herring ranged from 16.8-18.1°C. The frequency distributions of quotient values for all species were bimodal, but secondary peaks for anchovy and sardine were less than the threshold quotient value (which is one), whereas both round herring peaks were greater than the threshold quotient value. The quotient values showed a preferred spawning peak at

17.7°C for both anchovy and sardine, and bimodal peaks for round herring with a main peak at 16.8-17.2°C and a secondary peak at 17.6-18.1°C. Round herring appeared to have a greater affinity for spawning in cooler waters than did the other two species. A rather unexpected result was the complete overlap between anchovy and sardine, and this could be attributed to the limited hydrographic information and time.

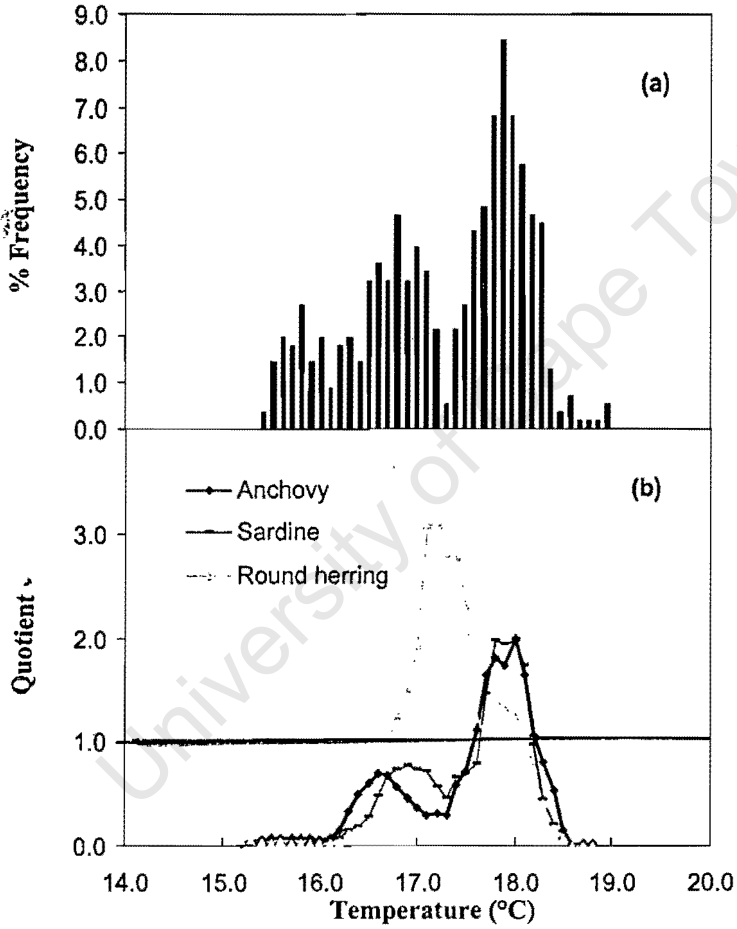


Figure 5.1. (a) Percentage frequency distribution of temperature from all on-station and underway CUFES samples collected during the September 2000 survey; and (b) Egg abundance-temperature quotients (the 5 – point running means are shown) for anchovy *Engraulis encrasicolus*, sardine *Sardinops sagax* and round herring *Etrumeus whiteheadi* eggs (eggs.m^{-3}) sampled by CUFES at 3 m depth during the survey.

5.4.2 Generalized additive models (GAMs)

5.4.2.1. *Spawning in relation to longitude*

GAM scatter plots of log egg abundance vs longitude indicate that anchovy spawning showed a dome-shaped relationship with a peak spawning at the midshelf region, whereas both sardine and round herring spawning were negatively related with longitude (i.e. spawning decreased towards the east or inshore) (Fig. 5.2). These results illustrate that sardine and round herring spawned further offshore (west) than did anchovy. Ticks on the x-axis indicate the number of samples collected over that range, and shows that most of the samples were collected over the midshelf (central) region of the study area.

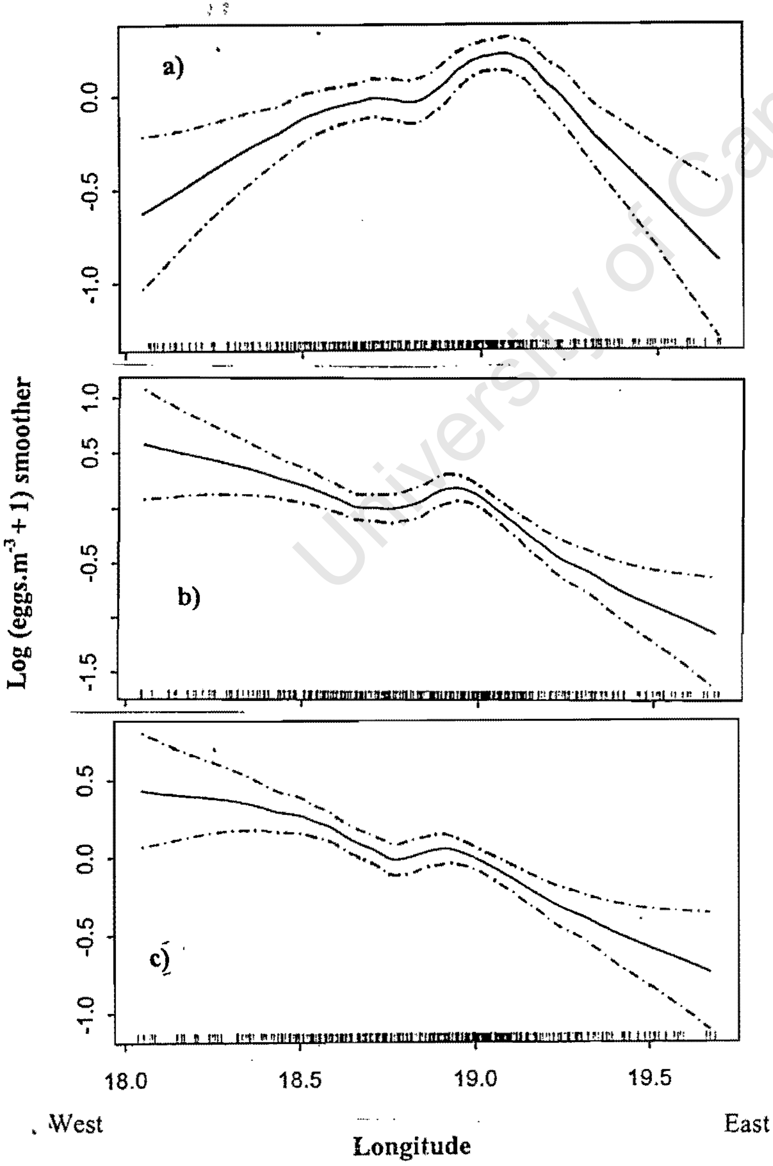


Figure 5.2. GAM scatter plot showing the relationship between egg abundance and longitude for (a) anchovy, (b) sardine and (c) round herring eggs (eggs.m^{-3}) sampled by CUFES at 3 m depth; dashed lines indicate approximate 95% confidence limits.

5.4.2.2. *Spawning in relation to latitude*

GAM scatter plots of log egg abundance vs latitude showed dome-shaped relationship for anchovy spawning, whereas sardine and round herring spawning increased with latitude (Fig. 5.3). These results indicate that sardine and round herring spawning intensity increases further south than did anchovy. Rug plots on the x-axis indicate that majority of the samples were evenly collected throughout the study area.

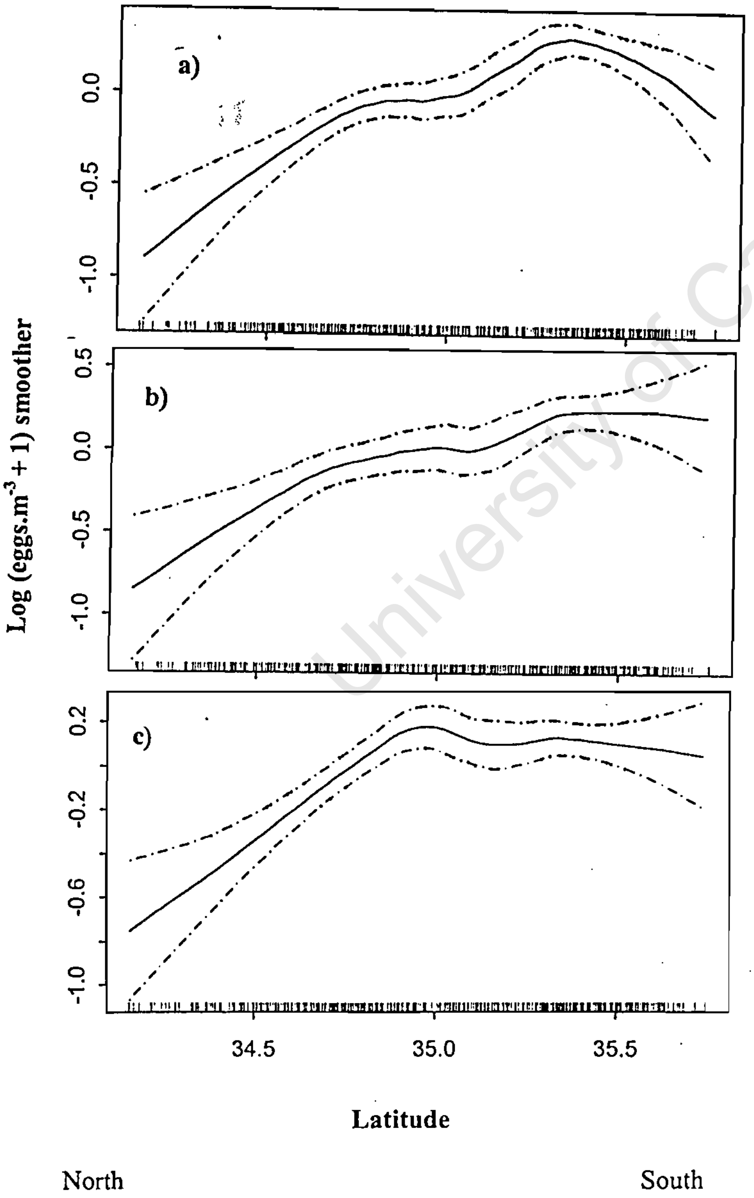


Figure 5.3. GAM scatter plot showing the relationship between egg abundance for (a) anchovy, (b) sardine and (c) round herring eggs (eggs.m^{-3}) sampled by CUFES at 3 m depth with the latitudinal evolution during this study; dashed lines indicate approximate 95% pointwise confidence limits.

5.5 DISCUSSION

The preferred spawning temperature ranges for anchovy, sardine and round herring were defined by constructing quotient curves derived from egg abundance and temperature data. Anchovy, sardine and round herring spawning in the southern Benguela ecosystem extends over the entire Agulhas Bank (Roel *et al.*, 1994) but mainly over the WAB. The WAB is chosen because eggs spawned in this area have enhanced chances for successful transport into the nursery grounds off the west coast (Hutchings, 1992; Huggett *et al.* 2003; Parada *et al.* 2003). It is often difficult to define absolute temperature limits for organisms, whether breeding or otherwise (Lluch-Belda *et al.*, 1991), especially when the entire distribution range has not been covered, as this was the case in this study. Despite that the "quotient rule" analysis has been used successfully to identify and characterize spawning habitats of anchovy and sardine (Lluch-Belda *et al.*, 1991; van der Lingen *et al.*, 2001), and this method has been successfully used in this study as well.

The main concentrations of anchovy eggs were centered in two areas (Fig 5.4a): midshelf, associated with temperatures of approximately 17.0°C, and offshore in temperatures between 17.5 and 18.0°C. These patterns further maintain the fact that spawning of this species is restricted mainly to warmer waters (Shelton and Hutchings, 1982; Shelton, 1986). High concentrations of sardine eggs were found in a band running parallel to the coast beyond the 200 m isobath and were associated with temperatures of between 17.0°C in the northern region and 18.0°C in the southern region of the study area (Fig. 5.4b). Those of round herring were distributed mainly above and beyond the 200 m isobath, and high densities were associated with cooler temperatures of 17.0-17.5°C (Fig. 5.4c). Generally, all three species seem to spawn over an area of relatively small change in temperature (17.0-18.0°C), and avoided temperatures greater than 18.0°C.

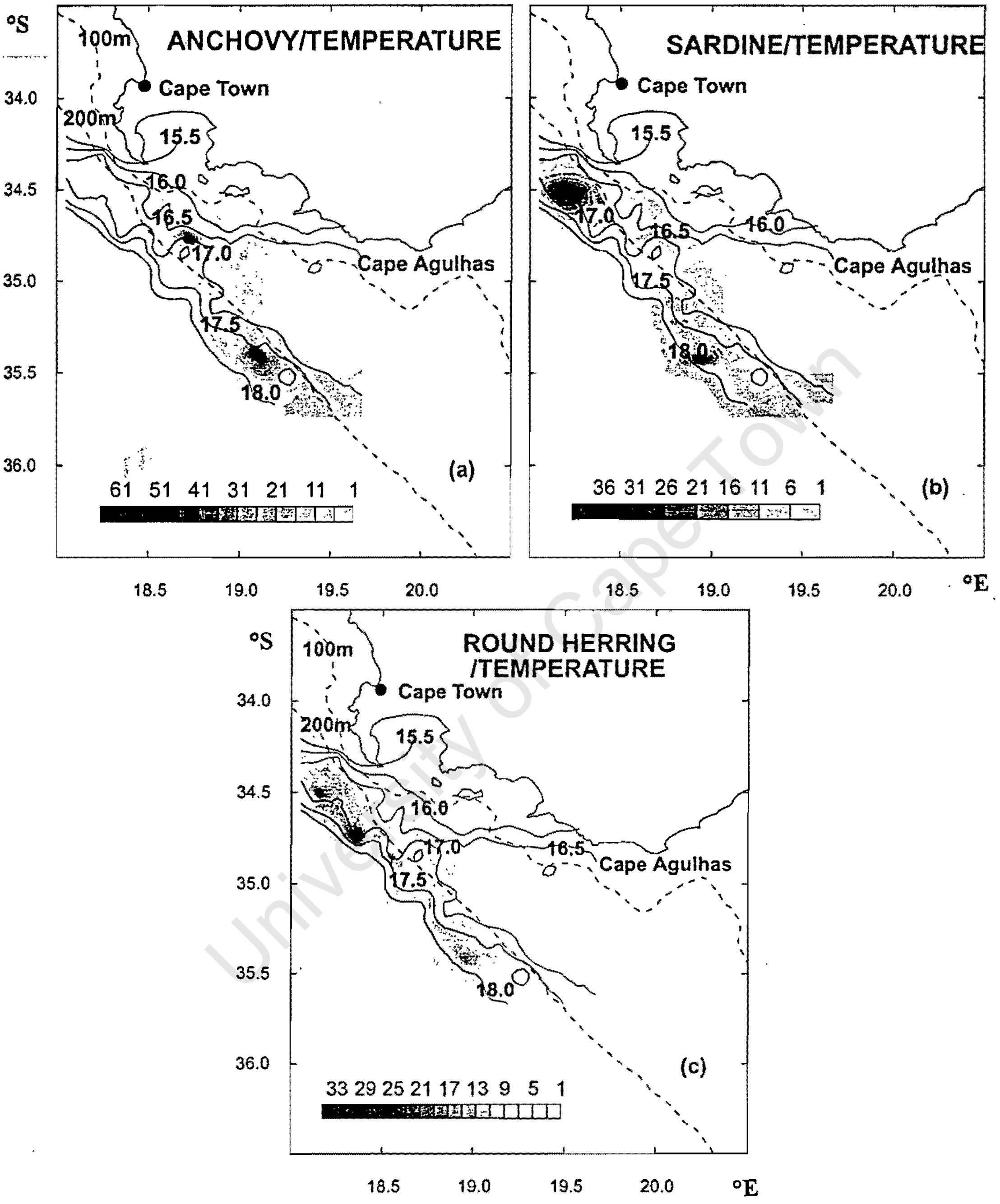


Figure 5.4. Spatial distributions (a) anchovy, (b) sardine and (c) round herring eggs, (eggs.m^{-3}) sampled by CUFES at 3 m depth in relation to the bathymetry and temperature at 3 m depth during PI of this study.

Results presented in this chapter demonstrate that the preferred spawning temperature ranges for anchovy and sardine overlapped completely. Interestingly, the preferred temperature range for spawning round herring was partially distinct from those of the other two species. All species showed a similar bimodal distribution of the quotient values, but only one peak for anchovy and sardine exceeded the quotient threshold, whereas both peaks did so for round herring. The main peak of round herring was distinct from those of the other two species, whereas the secondary peak overlapped with the peaks of other two species. These results therefore highlight the tolerance to spawn over a wide temperature range for round herring even though the data used in this study was from a small area and relatively small data set compared to those used to establish existing information on the preferred spawning temperature range for anchovy and sardine in the southern Benguela region. A summary of the preferred spawning temperatures in the southern Benguela ecosystem and other parts of the ocean basin is provided in Table 4.1. Sardines appeared to spawn in deeper and cooler waters than anchovy (see Chapter 4), even though their eggs ultimately concentrate near the warm surface waters. This signifies their tolerance of a wider temperature range than anchovies even though this was not evident from the quotient rule result, as shallower spawning and distribution of anchovy eggs possibly reflect a narrow, warm temperature affinity.

Table 4.1. Summary of the findings for long-term data studies of the preferred spawning temperature range of anchovy and sardine in the Southern and Northern Hemisphere.

Species	Region	Temperature range (°C)	Source
Anchovy	South Africa (Southern Hemisphere)	17.4-21.1	d
		16.0-23.0	c
	California	11.5-16.5	a
	(Northern Hemisphere)	11.5-17.0	b
Sardine	South Africa	15.2-20.5	d
		19.0-22.0	c
	California	15.0-23.0	b

[a]Green-Ruiz and Hinojosa-Corona (1997)

[b]Lluch-Belda *et al.* (1991)

[c]Twatwa (2002)

[d]van der Lingen *et al.* (2001)

The Californian anchovy *E. mordax* has been shown to spawn in cooler waters than sardine (Lluch-Belda *et al.*, 1991; Green-Ruiz and Hinojosa-Corona, 1997). Cubillos and Arancibia (1993 in Castro and Hernandez 2000) reported that the Chilean anchovy *E. ringens* had wide spread geographic spawning, which signifies a wide temperature tolerance. Contradictory, in the southern Benguela anchovy seem to be well adapted to spawn mainly in warm waters with a narrow spawning temperature range and are specific in selecting a location for spawning. Sardine appears to be better adapted to spawn over a wider temperature and geographic range in the southern Benguela region (Twatwa, 2002). Van der Lingen *et al.* (2001) compared the spawning of anchovy and sardine in the southern Benguela ecosystem and found that anchovies were stenothermic and sardines eurythermic. Compared to their counterparts in the California upwelling ecosystem, the southern Benguela species show opposite behaviours, and van der Lingen *et al.* (2001) suggested that these differences may be responsible for the observed out of phase differences in cycles of abundance between the genera.

In the NW Mediterranean Sea, García and Palomera (1996) found that the spawning of anchovy in this region was greatly affected by changes in longitude. They reported that anchovies tend to spawn in shelf waters. In this study, the r^2 values were higher for anchovy ($r^2 = 0.13$ longitude, $r^2 = 0.11$ latitude) than sardine ($r^2 = 0.08$ longitude, $r^2 = 0.04$ latitude) and round herrings ($r^2 = 0.07$ longitude, $r^2 = 0.06$ latitude), suggesting that anchovy were more specific in selecting the spawning location or range. In regard to changes in latitude, Castro *et al.* (2002) reported that egg size, yolk size and the length of the recently hatched larvae were larger at low (southern regions) latitudes, where temperatures are relatively warmer than at high (northern regions) latitude, where temperatures are relatively cooler. Twatwa (2002) made similar observations, in which she found that anchovy spawning was confined to specific conditions compared to sardine, which appeared to have wide range of tolerance to spawn at variable environmental conditions.

CHAPTER 6. CONCLUSIONS

This study was limited in its scope in that it was conducted over a relatively small area with limited hydrographic information. The depth resolution of the vertical sampling was coarser than I would have liked, nonetheless, it is the best information available, and samples were collected over a relatively short period of time. Despite these constraints, interesting horizontal and vertical distribution patterns for anchovy, sardine and round herring eggs were established in this study. The horizontal egg distribution patterns of sardine and round herring were strongly associated for both mean and station-by-station distributions, whereas those of anchovy were spatially distinct from the other two species. On the other hand, the vertical distributions of recently spawned eggs for the three species largely overlapped, but eggs of sardine and round herring were distributed deeper than those of anchovy. Furthermore, eggs of round herring were distributed deeper than those of sardine. These findings help fill gaps in our understanding of the early life history of the species in the region and provide valuable information for future studies.

Comparisons between CUFES and CalVET net derived egg distributions indicate that CalVET net derived distributions should be treated with caution in providing information about the spawning habitats and distribution patterns because it misses much of the spatial variability, as the net is only deployed at 10 n.miles stations. The derived egg densities from isolated CalVET net samples are interpolated and sometimes result in continuous distribution patterns, which we know necessarily do not exist, because pelagic fishes spawn in aggregations (Checkley *et al.*, 1997). The fact that CalVET net is being deployed at discrete 10 n.miles stations is inevitable, because it would be time consuming and expensive to deploy CalVET net at 1 n.mile stations. Therefore, the CUFES is considered adequate for mapping spawning habitats of the pelagic fishes and inferring about their spawning patterns.

The spatial egg distribution of all species was found to be aggregated at all levels of resolution. Therefore, the null hypothesis that the spatial egg distribution of the species is random was rejected, and the alternative hypothesis that the spatial egg distribution of the species is aggregated, has been adopted. This results highlight the fact that fish do not spawn anywhere, but chose specific areas for specific purposes. For example, they may chose a specific site to enhance their transport success, rapid development, etc.

Investigation into different sampling strategies used to map the egg distribution patterns during this study demonstrated that sampling design has a direct effect on the kind of distribution patterns. In particular, the PI sampling strategy covered the entire spatial range of the species distribution ranges, and therefore gave real distribution patterns of the species' eggs. On the other hand, the PIII sampling strategy only covered the area at which eggs were concentrated, therefore did not consider the complete spatial coverage. Therefore, the PI sampling strategy provides the most appropriate egg distribution patterns, if the interest of the survey is to infer about the distribution patterns. However, PIII sampling strategy is considered to be appropriate if the survey transects could be extended further inshore to ensure total spatial coverage of the eggs distribution. This would cover almost the same area as PI, but in less time, which would prove to be cost-effective. However, in order to estimate the variance properly, when estimating spawner biomass, a Jolly and Hampton (1990) randomised grid would be necessary.

The global indices of collocation showed that the mean egg density distribution patterns of the three species were found to be spatially associated from samples collected at large distances apart (coarse- and medium-resolution data). However, samples collected at smaller distances apart (high-resolution data) showed that coarse-resolution data often overestimated the spatial associations among the species. The station-by-station comparisons from both high and low-resolution data sets showed that all species were spatially distinct. It is, however, recommended that both global and local indices of collocation be used in combination, as these tools complement each other and allow the evaluation of different aspects (e.g. mean and station-by-station spatial patterns).

Investigations into high-resolution egg distribution patterns (Fig.'s 3.9-3.10) revealed that early egg stages for anchovy were clumped into a single cluster whereas those of sardine and round herring were clumped into several clusters (i.e. patchily distributed). Late egg stages for sardine and round herring were clumped into a large cluster characterised by a relatively uniform distribution, whereas those of anchovy were distributed into patchily clusters. These patterns therefore imply that anchovy in the southern Benguela spawns in large aggregations with probably huge gap between the cohorts, whereas sardine and round herring are assumed to spawn in small aggregations (patchily), which result in a series of different length size classes of cohorts. Therefore these results imply that in an event of egg or larval transport, if successful transportation is only observed at particular events, then some of sardine and round herring cohorts are likely to be transported successfully to the nursery areas in any case. However, anchovy cohorts are only likely to be successfully transported at specific events or conditions.

The southern Brazilian anchovy *E. anchoita* and sardine *Sardinella brasiliensis* share a common spawning area, and show clear segregation in terms of their spawning depths (Matsuura *et al.*, 1985 in Matsuura and Kitahara, 1995). In this study, large overlap in the vertical egg distributions among the species was evident, but almost all recently spawned egg stages for anchovy were restricted in the top 40 m near the surface. It is thus alleged that anchovies in the southern Benguela spawn within a relatively narrow depth range (0 ~ 40 m) in warm upper layer. On the other hand, over 50% of sardine and round herring recently spawned egg stages were widely distributed down to 60 and 80 m depths, respectively. Thus it is suggested these two species spawn in a variable and wider depth ranges (sardine 0 ~ 60 m and round herring 0 ~ 80-m), mainly below the surface at relatively cool and variable water temperatures. It is alleged that all three species spawn mainly in the evening as most early egg stages were collected in the evenings. All late egg stages for the species are confined to the top 60 m, and it is alleged that during late egg developmental stages, the vertical egg distribution patterns of the species are not entirely determined by passive wind-induced mixing and acquired buoyancy. Instead, the active developing embryo determines the vertical egg distribution. However, the mechanisms by which this is achieved could not be established. It is however, imagined that because the larvae are active and almost all their organs are developed during these stages, their sensory systems are also active. They therefore adjust their vertical position through the adjustment of their gas volume by using light or pressure sensors to sense their vertical position.

The potential for dispersal or Ekman transport of the recently spawned egg stages for the species is greatly reduced because these egg developmental stages are located below the offshore moving Ekman layer (0-20 m, Parrish *et al.*, 1981; Sundby, 1991). Strong wind mixing, which transports eggs deeper (Coombs *et al.*, 1985; Sundby, 1991; Stenevik *et al.*, 2001) helps to further reduce offshore advection of the eggs.

Even though the three species studied share their spawning area (Hutchings *et al.*, 2002; Roel *et al.*, 1994), the three species show some level of segregation in both horizontal and vertical niches. Anchovy spawning appeared to be more specific with respect to depth, longitude and latitude compared to sardine and round herring, which proved to be less specific. These segregations are believed to enhance optimal exploitation of the favourable spawning conditions inherited along the WAB.

Quantitative investigations of ichthyoplankton require adequate information on both horizontal and vertical dispersion of eggs and larvae; therefore one needs to have proper knowledge about the vertical distribution dynamics (Haug *et al.*, 1986). Individual based models (IBMs) used to investigate the effect of buoyancy and the effects of spatio-temporal variability in spawning on recruitment success and to integrate diverse information relevant to the recruitment processes in the southern Benguela ecosystem (Huggett *et al.*, 2003; Parada *et al.*, 2003), indicate that the vertical distribution of eggs has a significant impact on the ascent rate and subsequent transport or dispersion. The null hypotheses: that (i) the egg profile is independent of the species and (ii) the egg profile is independent of ontogeny are rejected, and the alternative hypotheses: that (i) the egg profile is dependent of the species and (ii) the egg profile is dependent of ontogeny are adopted.

This study has provided ecologically significant information about adult spawning patterns, and quantitative information on the vertical egg patterns of the ecologically and commercially important species in the southern Benguela ecosystem. These IBMs require adequate information from the field about the spatial variation in the distribution patterns of eggs and larvae within the water column, particularly at a fine-scale; to date, data on fine-scale distributions in ichthyoplankton in the southern Benguela ecosystem has been lacking. Therefore, these results of this study will help improve our understanding of the early life history of pelagic fishes in the southern Benguela upwelling ecosystem, and will provide useful inputs for further IBM studies.

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APPENDIX 1

Table 1. Number of eggs per each depth from the plankton pump and closing WPII net from station P2E1-001 during PIIE1.

Phase (Expt)	Type of Gear	(m) Depth	Actual no. of eggs per each depth Anchovy	Sardine	Round herring
II(1)	Plank pump	10	79	29	17
		30	29	0	7
		50	0	0	0
II(1)	WP II net	0-20	191	2	8
		20-40	121	20	9
		40-60	63	7	0

Table 2. Number of eggs per each depth from the closing WP II net during PIIE3.

Phase	**type**	**#**	(m)	Actual no. of eggs per each depth		
(Expt)	**Gear**	**Stations**	**Depth**	**Anchovy**	**Sardine**	**Round herring**
II(3)	WP II net	P2E3-001	0-20	39	232	60
			20-40	9	203	48
			40-60	0	22	4
			60-80	0	8	0
			80-100	0	2	1
II(3)	WP II net	P2E3-002	0-20	0	111	1
			20-40	0	2	18
			40-60	0	0	0
			60-80	0	0	0
			80-100	0	6	1
II(3)	WP II net	P2E3-003	0-20	0	0	0
			20-40	0	0	1
			40-60	0	0	0
			60-80	0	0	1
			80-100	0	3	3
II(3)	WP II net	P2E3-004	0-20	0	47	28
			20-40	0	8	3
			40-60	0	0	0
			60-80	0	1	0
			80-100	0	0	0
II(3)	WP II net	P2E3-005	0-20	0	127	35
			20-40	0	60	13
			40-60	0	16	2
			60-80	0	4	0
			80-100	0	2	0
II(3)	WP II net	P2E3-006	0-20	442	133	26
			20-40	123	114	33
			40-60	212	134	55
			60-80	14	5	4
			80-100	28	4	1
II(3)	WP II net	P2E3-007	0-20	198	21	19
			20-40	127	70	30
			40-60	23	9	2
			60-80	5	3	2
			80-100	36	22	1
II(3)	WP II net	P2E3-008	0-20	565	144	38
			20-40	41	25	12
			40-60	107	67	23
			60-80	18	26	4
			80-100	14	12	2
II(3)	WP II net	P2E3-009	0-20	64	251	69
			20-40	75	212	45
			40-60	26	107	27
			60-80	4	81	9
			80-100	3	13	4

Table 3. Number of eggs per each depth from the closing WP II net during PIII.

Phase (Expt)	**type Gear**	**# Stations**	**(m) Depth**	**Actual no. of eggs per each depth**		
				Anchovy	**Sardine**	**Round herring**
III	WP II net	20-01	0-20	858	121	1
			20-40	207	25	0
			40-60	62	23	1
			60-80	9	1	1
			80-100	7	1	0
III	WP II net	21-01	0-20	308	64	11
			20-40	226	41	16
			40-60	29	4	1
			60-80	9	1	0
			80-100	4	1	1
III	WP II net	22-01	0-20	173	488	488
			20-40	125	362	30
			40-60	76	241	15
			60-80	2	2	0
			80-100	2	7	1
III	WP II net	23-01	0-20	292	118	62
			20-40	95	22	16
			40-60	22	19	3
			60-80	5	1	4
			80-100	0	1	0
III	WP II net	25-01	0-20	144	406	48
			20-40	137	336	33
			40-60	70	220	29
			60-80	9	19	4
			80-100	1	2	1

Table 4. The 3X5 contingency table that was used for the χ^2 analysis. Each column contains the observed and expected number of eggs during PIIE3, PIII and collective data.

Phase	(m)	Anchovy		Sardine		Round herring		
	Depth	Observed	Expected	Observed	Expected	Observed	Expected	Total
PII(3)	0-20	1308	1099	908	1077	247	288	2463
	20-40	375	553	684	542	181	145	1240
	40-60	368	373	355	365	113	98	836
	60-80	41	83	127	82	19	22	187
	80-100	81	65	55	63	9	17	145
		2173		**2129**		**569**		**4871**
PIII	0-20	1775	1669	1197	1468	610	445	3582
	20-40	790	779	786	685	95	208	1671
	40-60	259	380	507	334	49	101	815
	60-80	34	31	24	27	9	8	67
	80-100	14	14	12	12	3	4	29
		2872		**2526**		**766**		**6164**
Collective	0-20	3083	2764	2105	2550	857	731	6045
	20-40	1165	1331	1470	1228	276	352	2911
	40-60	627	755	862	696	162	200	1651
	60-80	75	116	151	107	28	31	254
	80-100	95	80	67	73	12	21	174
		5045		**4655**		**1335**		**11035**

Table 5. The 3X5 contingency table that was used for the χ^2 analysis. Each column contains the observed and expected number of eggs during PIIE1.

Phase		(m)	**(No embryo)**		**(Early embryo)**		**(Late embryo)**		
Species	Sampler	Depth	Observed	Expected	Observed	Expected	Observed	Expected	Total
Anchovy									
	Plankton	10	14	12	31	32	10	11	**55**
	pump	30	1	3	9	9	4	3	**14**
		50	0	0	0	0	0	0	**0**
			15		**40**		**14**		**69**
	WP II net	0-20	31	45	128	113	30	31	**189**
		20-40	25	39	111	98	28	27	**144**
		40-60	38	11	0	27	7	7	**45**
			94		**239**		**65**		**398**
Sardine									
	Plankton	10	0	0	1	1	16	16	**17**
	pump	30	0	0	0	0	7	7	**7**
		50	0	0	0	0	0	0	**0**
			0		**1**		**23**		**24**
	WP II net	0-20	0	0	2	0	0	2	**2**
		20-40	0	0	4	4	16	16	**20**
		40-60	0	0	0	2	8	6	**8**
			0		**6**		**24**		**30**
Round herring									
	Plankton	10	3	3	0	0	16	16	**19**
	pump	30	2	2	0	0	7	7	**9**
		50	0	0	0	0	0	0	**0**
			5		**0**		**23**		**28**
	WP II net	0-20	4	4	4	4	0	1	**8**
		20-40	4	4	3	4	1	1	**8**
		40-60	0	0	0	0	0	0	**0**
			8		**7**		**1**		**16**

Table 6. The 3X5 contingency table that was used for the χ^2 analysis. Each column contains the observed and expected number of eggs during PIIE3 and PIII.

	Phase	(m)	**(No embryo)**		**(Early embryo)**		**(Late embryo)**		
Species	(Expt)	Depth	Observed	Expected	Observed	Expected	Observed	Expected	Total
Anchovy	PII(3)	0-20	8	9	1266	1265	2	3	**1276**
		20-40	4	4	534	534	1	1	**539**
		40-60	2	2	327	328	2	1	**331**
		60-80	1	0	39	40	0	0	**40**
		80-100	0	0	46	46	0	0	**46**
			15		**2212**		**5**		**2232**
	PIII	0-20	101	101	220	220	647	647	**968**
		20-40	72	69	143	151	451	445	**666**
		40-60	25	27	60	59	174	173	**259**
		60-80	4	3	9	8	20	22	**33**
		80-100	0	1	8	2	2	7	**10**
			202		**440**		**1294**		**1936**
Sardine	PII(3)	0-20	7	13	701	682	112	125	**820**
		20-40	17	11	580	583	104	107	**701**
		40-60	0	4	227	235	55	43	**282**
		60-80	6	1	31	41	12	7	**49**
		80-100	0	1	38	37	6	7	**44**
			30		**1577**		**289**		**1896**
	PIII	0-20	251	263	405	338	557	562	**1213**
		20-40	175	165	235	243	351	353	**761**
		40-60	113	103	140	153	224	221	**477**
		60-80	2	5	5	7	15	10	**22**
		80-100	0	5	13	7	10	11	**23**
			541		**798**		**1157**		**2496**
Round herring									
	PII(3)	0-20	43	58	121	109	58	54	**222**
		20-40	42	36	51	66	42	33	**135**
		40-60	22	31	70	58	25	29	**117**
		60-80	25	11	12	20	3	10	**40**
		80-100	6	3	4	4	0	2	**10**
			138		**258**		**128**		**524**
	PIII	0-20	58	72	91	82	37	32	**186**
		20-40	35	31	28	35	17	14	**80**
		40-60	31	20	15	23	6	9	**52**
		60-80	13	14	22	16	1	6	**36**
		80-100	1	1	1	1	1	1	**3**
			138		**157**		**62**		**357**

Table 7. The 3X5 contingency table that was used for the χ^2 analysis. Each column contains the observed and expected number of eggs for the collective data.

Phase	(m)	**(No embryo)**		**(Early embryo)**		**(Late embryo)**		
Species	Depth	Observed	Expected	Observed	Expected	Observed	Expected	Total
Anchovy								
	0-20	109	117	1486	1428	649	699	**2244**
	20-40	76	63	677	767	452	376	**1205**
	40-60	27	31	387	375	176	184	**590**
	60-80	5	4	48	46	20	23	**73**
	80-100	0	3	54	36	2	17	**56**
		217		**2652**		**1299**		**4168**
Sardine								
	0-20	258	264	1106	1099	669	669	**2033**
	20-40	192	190	815	791	455	481	**1462**
	40-60	113	99	367	410	279	250	**759**
	60-80	8	9	36	38	27	23	**71**
	80-100	0	9	51	36	16	22	**67**
		571		**2375**		**1446**		**4392**
Round herring								
	0-20	101	128	212	192	95	88	**408**
	20-40	77	67	79	101	59	46	**215**
	40-60	53	53	85	80	31	36	**169**
	60-80	38	24	34	36	4	16	**76**
	80-100	7	4	5	6	1	3	**13**
		276		**415**		**190**		**881**

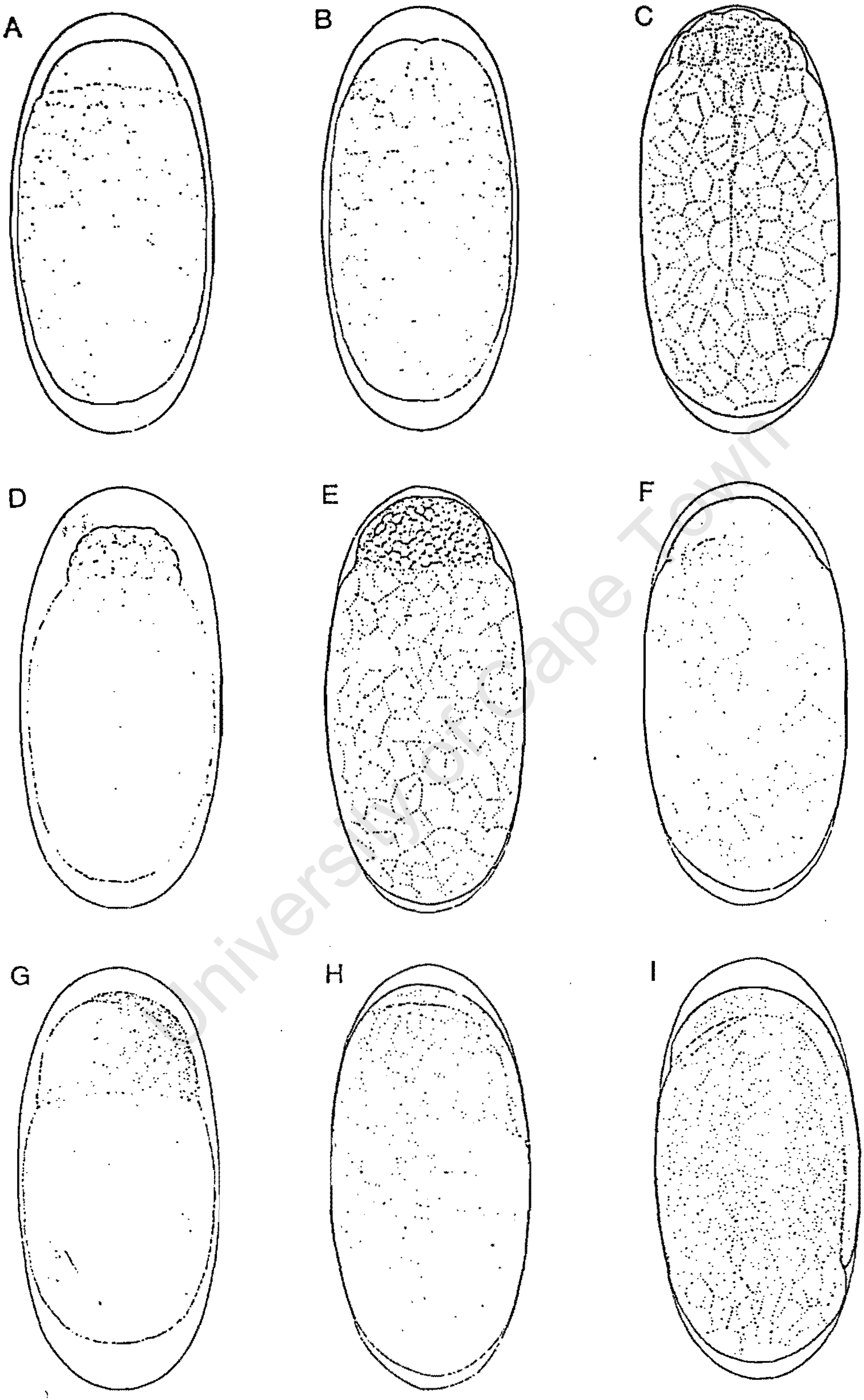


Figure A. 1a. Anchovy developmental egg stages used to stage anchovy eggs during this study, A. Stage 1; B. Stage 2 (2 cells); C, Stage 2 (16 cells); D. Stage 2 ("Mulberry"); E. Stage 2 (late); F, Stage 3 (mid); G. Stage 3 (late); H. Stage 4 (mid); I. Stage 4 (after Moser and Ahlstrom, 1985).

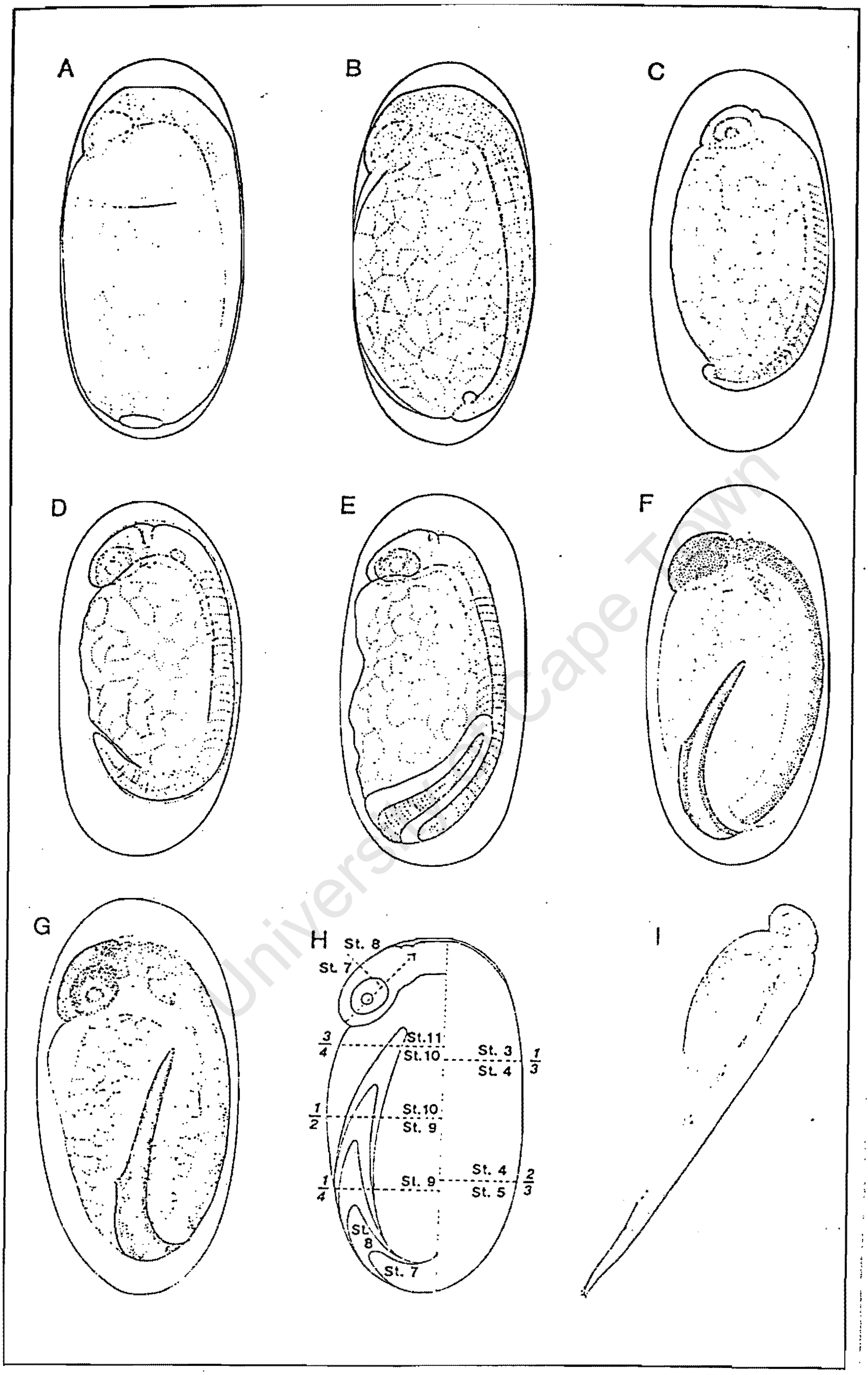
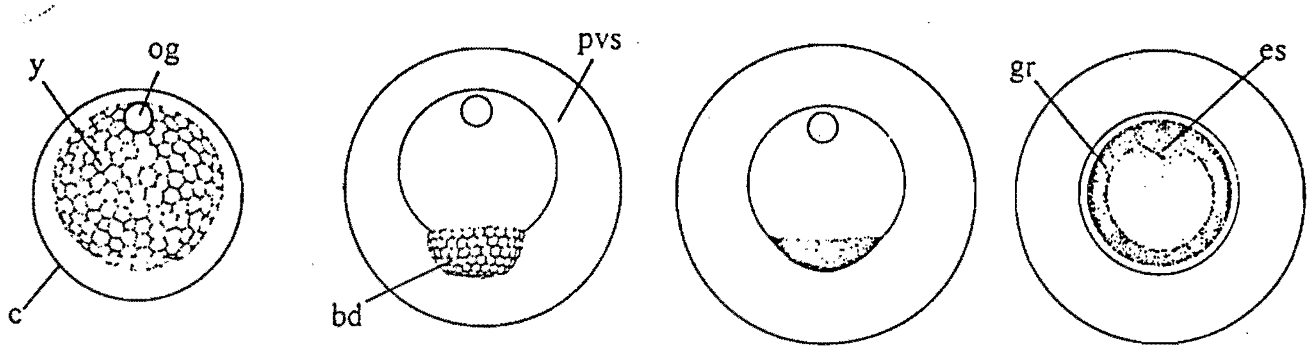
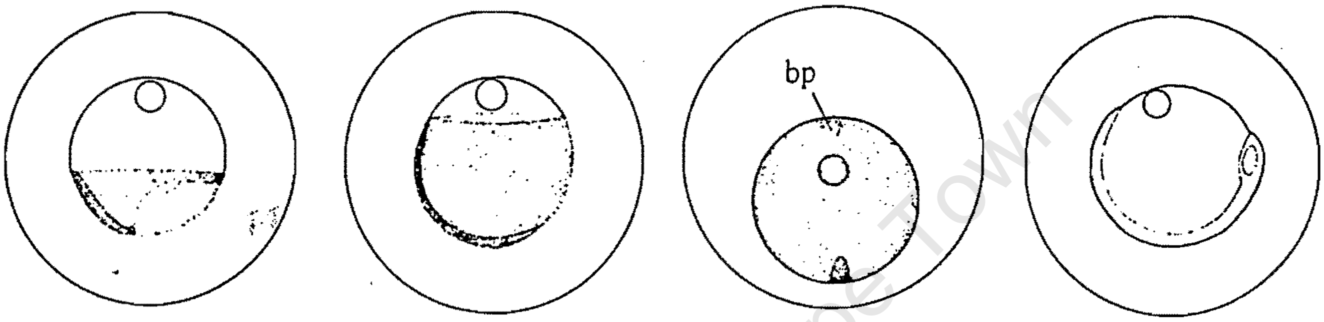


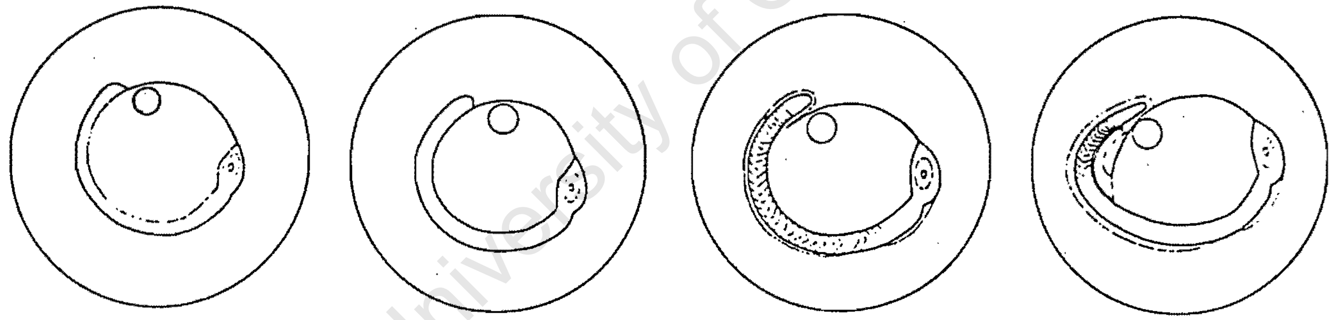
Figure A. 1b. Anchovy developmental egg stages used to stage anchovy eggs during this study, A. Stage 5 (late); B. Stage 6; C, Stage 7; D. Stage 8; E. Stage 9; F, Stage 10; G. Stage 11; H and I is the newly hatched larvae (after Moser and Ahlstrom, 1985).



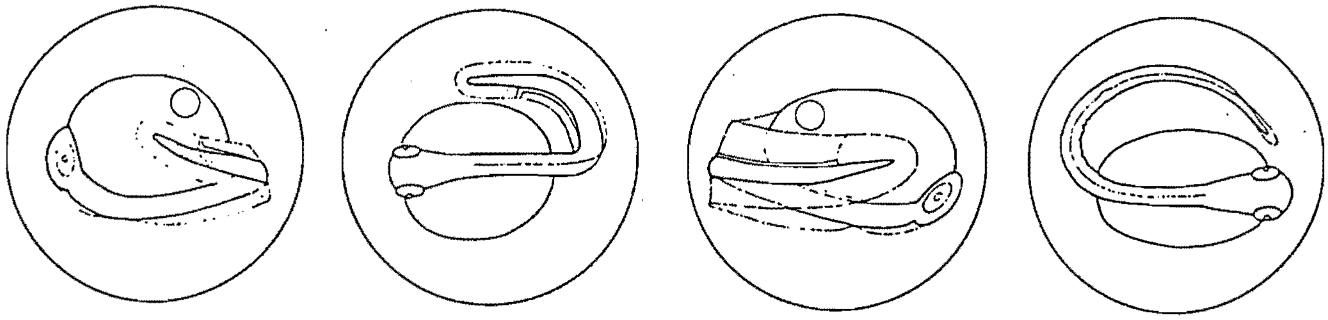
Stage 1 Stage 2 Stage 3 Late Stage 3 (polar view)



Stage 4 Stage 5 Late Stage 5 (polar view) Stage 6



Late Stage 6 Stage 7 Stage 8 Stage 9



Stage 10 Stage 10 (polar view) Stage 11 Stage 11 (polar view)

Figure 2. Sardine developmental egg stages (after Lo *et al.*, 1996) used to stage sardine eggs during this study, and from which round herring staging was mimicked. Symbols: bd, blastodisc; bp, blastopore; c, chorion or shell; es, embryonic shield; gr, germ ring; og, oil globule; pvs, perivitelline space; y, yolk.

APPENDIX 2

Examples of S-Plus 4.5 commands used to develop GAM scatter plots between egg abundance and longitude and/or latitudinal evolution.

```
win.graph( )
long <- gam{log(eggs.m^-3 + 1) ~ lo(long, span = 0.6, degree = 1), na.action = omit,
family = gaussian, data = Longitude)
graphsheet (pages = T)
Plot.gam(long, se = T, rug = T, residuals)
R2long <- (long$deviance/long$null.deviance)
```

Definition of some commands

lo~ allows the use of a Loess fit in a GAM formula that gives a robust, local smooth of scatterplot data

span~ specifies the wideness for the local smoother

na.action = omit, allows filtering out of missing data

plot.gam(long, se = T, rug = T, residuals), allows gam scatter plot together with rug (bars that represent count and location of data) plots

long$deviance/long$null.deviance), gives an explained variance