THE GEOGRAPHIC STOCK STRUCTURE OF CHOKKA SQUID, *LOLIGO REYNAUDI*, AND ITS IMPLICATIONS FOR MANAGEMENT OF THE FISHERY

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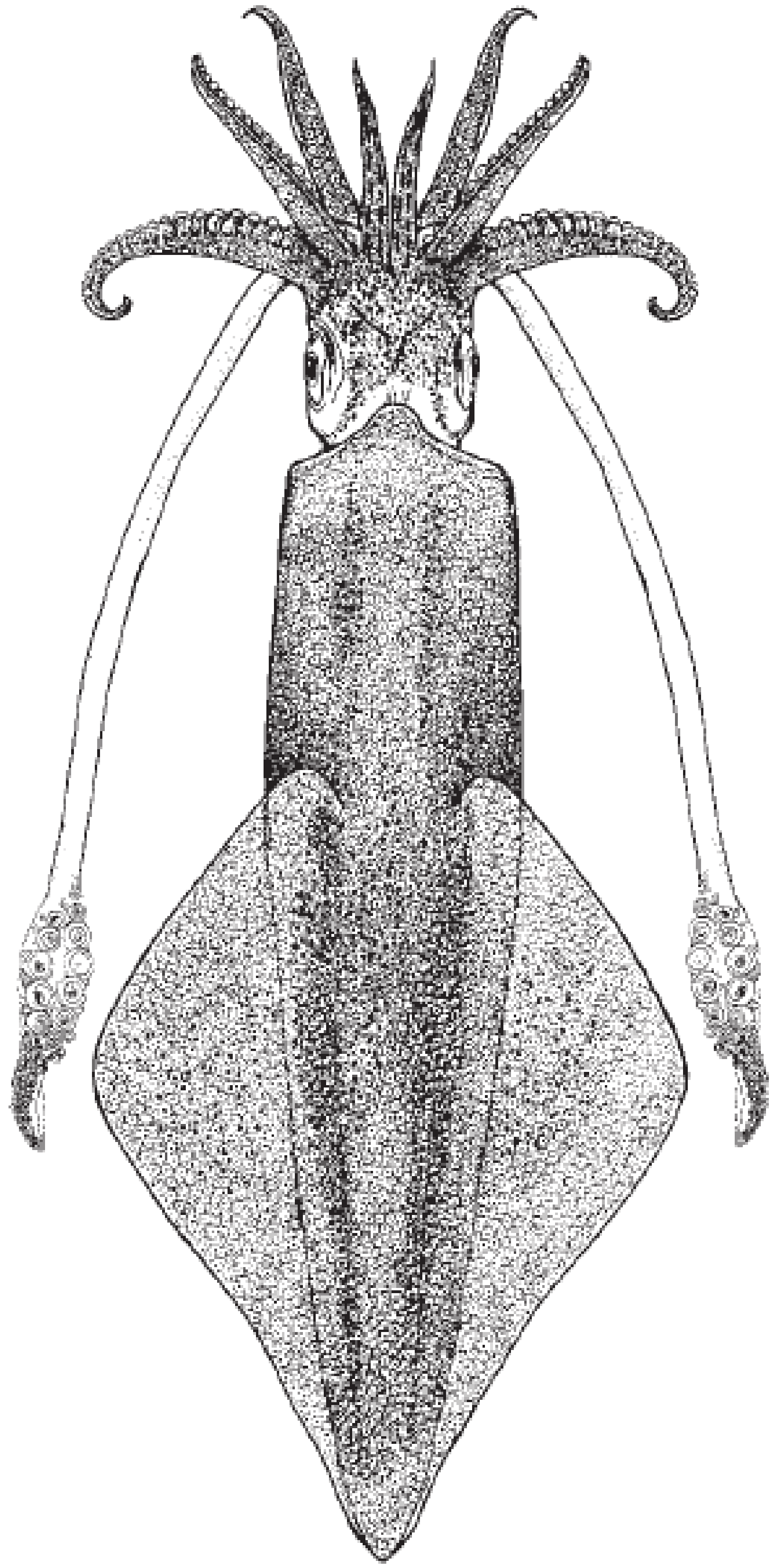

Loligo reynaudi

ABSTRACT

It is currently hypothesised that the chokka squid (*Loligo reynaudi*) consist of a single stock. This was tested through a spatial comparison of the morphology of this species. Forty three morphometric characters were measured from 1079 chokka squid collected from three regions: the south coast of South Africa, the west coast of South Africa, and southern Angola. While no significant differences were found for the hard body parts, results from discriminant function analyses showed the soft body morphometric characters from each of the three regions differed, with an overall correct classification rate of 100% for males and 99% for females in all three regions.

Due to the existing model being used to assess the resource currently being updated it was not feasible to apply this model to the area-disaggregated data from this study. Rather, the CPUE trends and catches from the area-disaggregated data were compared against those of the area-aggregated data, as a first attempt to discern any appreciable differences which would suggest the use of disaggregated data in future assessments. Both the trawl and jig CPUE trends from the area-disaggregated analysis differed only slightly from those of the area-aggregated data. Similarly, the spring and autumn biomass trends for the main spawning area (east of 22°E) followed the same trends as for the full area. It is therefore concluded that there is currently no need to assess the resource on an area-disaggregated basis.

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DECLARATION

The following thesis has not been submitted to a university other than Rhodes University, Grahamstown, South Africa. The work presented here is that of the author unless stated otherwise.

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Conserving biodiversity requires the definition of fine-scale population structure and reproductively isolated groups (Cadrin *et al.* 2007). The history of stock identification emphasizes a search for distinct allopatric patterns, in which homogenous stocks with closed life cycles, distinct genotypic frequencies, and migration patterns can be delineated (Cadrin and Secor 2009). Despite the recognition that many populations have complex, sympatric structures, the desire for allopatric management units results from the need to manage fishing grounds as a single resource (Cadrin *et al.* 2007). However, the realities of fish movement, mixed stock harvests, and advancements and accomplishments of stock composition analyses make this search for a one-to-one match between conservation unit and fishing ground outdated (Cadrin *et al.* 2007).

Recent application of advanced technologies and interdisciplinary approaches reveal complex patterns of spatial structure of many marine populations (Cadrin and Secor 2009). These heterogeneous, sympatric patterns present challenges to stock assessment modelling (Cadrin and Secor 2009). The continuing problems of exploiting sympatric populations, local depletions and recent issues of conserving essential habitats and designation of marine protected areas require new information from stock assessments (Cadrin and Secor 2009). Population modelling now needs to consider the incorporation of spatial patterns in sampling and stock assessment modelling (Cadrin and Secor 2009).

Stock composition analysis has improved over the last few decades through valuable developments in genetic markers and statistical mixing models (Cadrin and Secor 2009). Appropriately designed sampling of mixed-stock fisheries can support accurate estimates of stock composition and provide information for reliable stock assessment and real-time management decisions (Cadrin and Secor 2009).

Stock assessment involves the use of statistics and mathematics to make quantitative predictions about the reactions of exploited populations to alternative management options (Hilborn and Walters 1992). To make such predictions, an understanding of population dynamics is required (Goss *et al.* 2001). Data needed include measures of fecundity, population size, demography, distribution, age structure, recruitment and life-history features (Carvalho and Nigmatullin 1998). Most species are either temporarily or spatially fragmented into a number of distinct population units or ‘stocks’ (Carvalho and Nigmatullin 1998). These stocks may have different population dynamics depending on the degree of isolation/mixing between them, as well as the effects of the different environments in which they may occur. It is therefore important to estimate the extent to which any of the units differ in these measures (Carvalho and Nigmatullin 1998). This is critical to the formulation of meaningful estimates of mortality and recruitment, especially when the response to harvesting has to be predicted (Carvalho and Nigmatullin 1998).

The definition of stock boundaries can be problematic because of fish movement patterns (Cadrin *et al.* 2007). Stock boundaries of marine fishery resources are seldom distinct or stable over time, but their delineation remains necessary for the many objectives of fishery management (Cadrin *et al.* 2007). Such objectives may include: the monitoring of spatially and temporally explicit removals from the targeted fish stock; the design of research surveys that cover the entire area of a resource and allow for spatial disaggregation of separate stocks; ecological studies of biological processes conducted within geographic boundaries of individual unit stocks to represent the system of interest; stock assessment models and short-term fishery forecasts; long-term projections and management evaluations; and fishery regulations designed to conform to the spatio-temporal bounds of both fisheries and fishery resources to attain the desired catch, harvest rate or population response (Cadrin *et al.* 2007).

Stock identification plays an important role in the stock assessment and management of any fishery. It has important implications for optimal harvest, monitoring strategies, and to ensure that harvesting proceeds at sustainable levels (Kutuhn 1981, Grimes *et al.* 1987, Rathjen and Voss 1987, Coyle 1998, Carvalho and Nigmatullin 1998, Begg *et al.* 1999). However, such data are only useful in the context of fisheries management if fully incorporated into stock assessments (Carvalho and Nigmatullin 1998). Despite the long history of stock identification and much methodological advancement, the definition of management units remains a practical decision, because it depends on the management objective (Cadrin *et al.* 2007).

Disregard of stock structure and ineffective management may lead to dramatic changes in the biological attributes and productivity rates of an exploited species, as well as the genetic diversity of a species (Altukhov 1981, Ricker 1981, Smith *et al.* 1991, Ryman *et al.* 1981, Laikre and Ryman 1996, Allendorf *et al.* 2008). Failure to recognise stock complexity in management units has already led to overfishing, the depletion of less productive fish stocks and the erosion of spawning stocks (example Sadovy and Domeier 2005), resulting in a loss of genetic diversity and other unknown ecological consequences (Begg *et al.* 1999).

1.2 Population and "stock" definitions

Due to the large geographical scale of the physical systems in which many marine populations occur and the migrations of which they are capable, the definition of a population should be carefully considered (O'Dor 1998). Mayr (1942) defined biological species as "groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups". This means that a species is defined by effective barriers to gene flow, whereas populations are defined by restricted gene flow between them (O'Dor 1998).

In the fisheries context, a population of the same species is often referred to as a stock. It can be defined as a heterogeneous group of individuals that exists within a defined spatial range, shares the same gene pool, has little connection with adjacent groups of the same species and sustains itself over time (Booke 1981, King 2007). More importantly, a stock can also be characterised by having a specific range of variation, whether morphological, meristic, molecular, or reproductive (Carvalho and Nigmatullin 1998). Although different stocks may not be considered genetically distinct, they may still adapt morphologically to their respective environments (Coyle 1998). These are termed "environmental" or "phenotypic" stocks (Coyle 1998) and have been shown to occur in some species of squid (example Carvalho and Pitcher 1989, Hernandez-Garcia and Castro 1998, Vega *et al.* 2002). On the other hand, sympatric stocks which are phenotypically similar or identical, may comprise of cryptic species, for example Australian loliginid squid (Yeatman and Benzie 1994, Triantafillos and Adams 2001, 2005).

1.3 Stock assessment and management of cephalopod fisheries

In the past two decades, the increasing efficiency of commercial fishery techniques has placed severe pressure on cephalopod resources (Carvalho and Nigmatullin 1998). In particular, the general short life cycle (< 2 years) of most Loliginid squid, in which individuals grow rapidly, become reproductively mature, spawn and then die, has profound implications on both the rate of evolutionary change and susceptibility to harvesting (Augustyn *et al.* 1994, Pierce and Guerra 1994, Carvalho and Nigmatullin 1998, Goss *et al.* 2001). Therefore, their fisheries are very difficult to assess and manage (Boyle and Rodhouse 2005).

Although a large number of studies have in recent decades contributed to the biology of many squid species, knowledge of their life-cycles is needed to manage populations effectively (Goss *et al.* 2001). The population dynamics and distributions of many fished squid species are poorly understood (Jereb and Roper 2010).

A sound knowledge of stock structure is especially vital in annual species such as loliginid squid, where recruits are entirely dependent upon the survival of the previous generation (Carvalho and Pitcher 1989). It is therefore surprising that most stock assessment methods for Loliginid squid model the dynamics of closed populations assuming homogeneous life history characteristics, as this may produce misleading results (Begg *et al.* 1999).

1.4 Cephalopod stock identification techniques

Stock identification is a multidisciplinary field of fisheries science involving habitat, life history, mark-recapture, genetics, and biometric studies (Ihssen *et al.* 1981, Templeman 1982, Pawson and Jennings 1996, Begg and Waldman 1999). Given the diversity of stock identification approaches available for cephalopods and that different techniques may yield non-concordant results (example Kim 1993, Brierly 1993a, 1993b, Kang *et al.* 1996, Reichow and Smith 2001), it is recommended that data from more than a single source be used and the results compared in order to maximise the likelihood of correctly defining separate stocks (Hohn 1997, Carvalho and Nigmatullin 1998, Begg and Waldman 1999).

Although molecular techniques have become particularly popular (Cadrin and Secor 2009), they cannot be used as a “super-method” that leads to results preferable to those derived from phenotypic analysis; rather, both complement one another (Nesis 1998). Begg and Waldman (1999) recommended using at least one phenotypic-based and one genetic approach in stock identification. Ideally, genetic, phenotypic, and environmental approaches should be combined as the definition of a stock includes all three components (Dizon *et al.* 1992, Coyle 1998). This may allow for the examination of possible stock differences in life histories and patterns of recruitment (Carvalho and Nigmatullin 1998).

1.5 Chokka squid (*Loligo reynaudi*)

The chokka squid (*Loligo reynaudi*) is a commercially important Loliginid squid occuring from southern Angola to the southeast coast of South Africa (Augustyn 1989). In South Africa it occurs from the Orange River mouth (28°S) on the west coast to at least Port Alfred

(34°S) on the southeast coast (Figure 1) (Augustyn 1991). Its eastern limits remain unknown (Jereb and Roper 2010). The majority of squid biomass in South Africa is distributed over the Agulhas Bank shelf (Augustyn 1989, Roberts 2005), and particularly over the east of the Agulhas Bank. Chokka squid occurrence on the west coast is variable on both a spatial and temporal scale, with considerably lower biomass than on the south coast (Augustyn 1989). Depth distribution is mainly limited along the continental shelf down to 200m, however offshore distribution can reach depths of over 500m (Roberts *et al.* 2012).

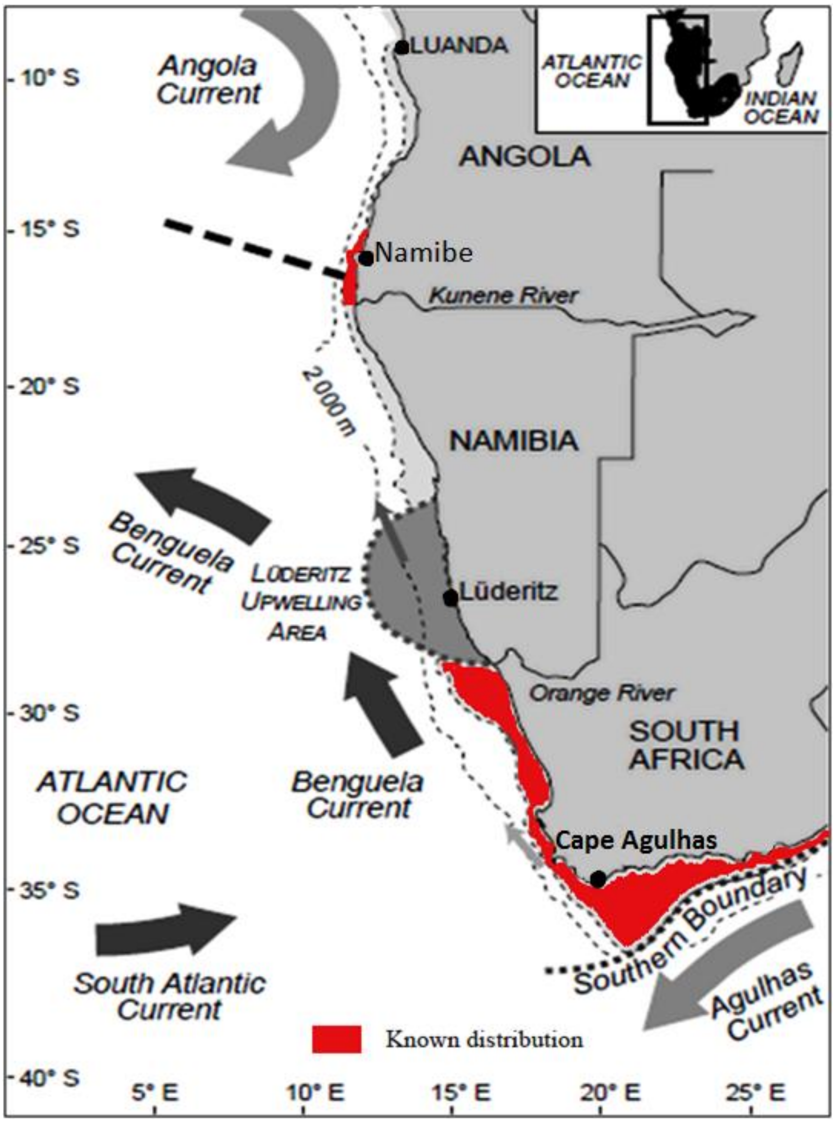


Figure 1. The current known distribution of chokka squid (*Loligo reynaudi*), with major oceanographic features of the region indicated (modified from Henriques *et al.* 2012).

The marine environment around southern Africa is one of the most diverse, complex and highly variable in the world (Lutjeharms *et al.* 2001). The distribution of chokka squid along the southern African coastline is largely influenced by the warm Angola current and the cold Benguela current upwelling system on the west, and the warm Agulhas current system on the south (Figure 1). Chokka squid inhabits these three different environments with what appears to be a break in its distribution off the coast of Namibia (Shaw *et al.* 2010, Roberts *et al.* 2012).

1.5.1 Life cycle synopsis

The currently accepted life cycle of chokka squid was reviewed by Olyott (2002). A short synopsis is provided below.

Egg hatching mainly occurs on the southeast coast between Plettenberg Bay and Port Alfred, where the major spawning grounds are located (Augustyn 1990, Sauer *et al.* 1992, Augustyn *et al.* 1994). The majority of chokka paralarvae are found in the vicinity of the main spawning grounds (Figure 2), both on the inshore and deeper regions (Augustyn *et al.* 1994). Initially after hatching, they passively drift with currents while feeding on abundant copepods (*Calanoides carinatus* and *Calanus agulhensis*) that exist in all regions of the Agulhas Bank (Augustyn *et al.* 1994, Venter *et al.* 1999, Roberts and van den Berg 2002). Augustyn *et al.* (1992, 1994) and Roberts (2005) hypothesised ("Westward Transport Hypothesis") that chokka paralarvae on the eastern Agulhas Bank are transported predominantly westwards to the "cold ridge" (Figure 2), where they feed and grow in this nutrient rich nursery area. Paralarvae hatched on the central Agulhas bank have the potential to be transported to the western Agulhas Bank where they may feed and grow. It is also possible for these paralarvae to reach the west coast via transportation of the strong and narrow equatorward shelf edge jet near Cape Town (34°S 18°E) (Augustyn *et al.* 1994). Paralarvae have been found on the west coast (Figure 2), however, it is highly unlikely that any would survive here due to unfavourably low average water temperatures (Martins *et al.* 2010).

According to Augustyn (1990) and Augustyn *et al.* (1994), juvenile chokka squid on the main spawning grounds move offshore in search of food, dispersing over the entire Agulhas Bank shelf. As sub-adults they reach either the area just offshore of their spawning grounds, or the southern and western Agulhas Bank, or even reach the southern west coast, depending on their individual circumstances of growth and maturity, and on the environmental conditions. From there they migrate back to the inshore waters of the south coast as mature adults. Adult chokka squid arrive in the general spawning areas throughout the year, although the major spawning migration is in spring (Augustyn 1989, 1990, Augustyn *et al.* 1994, Sauer *et al.* 1992, Roberts and Sauer 1994).

It has also been suggested that a large proportion of the eastern biomass does not undertake a westward feeding migration but instead spend most of their lives in the inshore and offshore waters of the Eastern Cape adjacent to the main spawning grounds between Plettenberg Bay and Port Alfred (Augustyn *et al.* 1994, Roberts and van den Berg 2002, Olyott *et al.* 2007). Some juvenile and adult chokka squid found on the western Agulhas Bank, as well as those hatched there, may also move up the west coast to take advantage of the Benguela Current system to feed and grow on fish, crustaceans, polychaetes and other cephalopods in the belt of enrichment formed by high primary productivity (Augustyn *et al.* 1994, Hutchings *et al.* 2009).

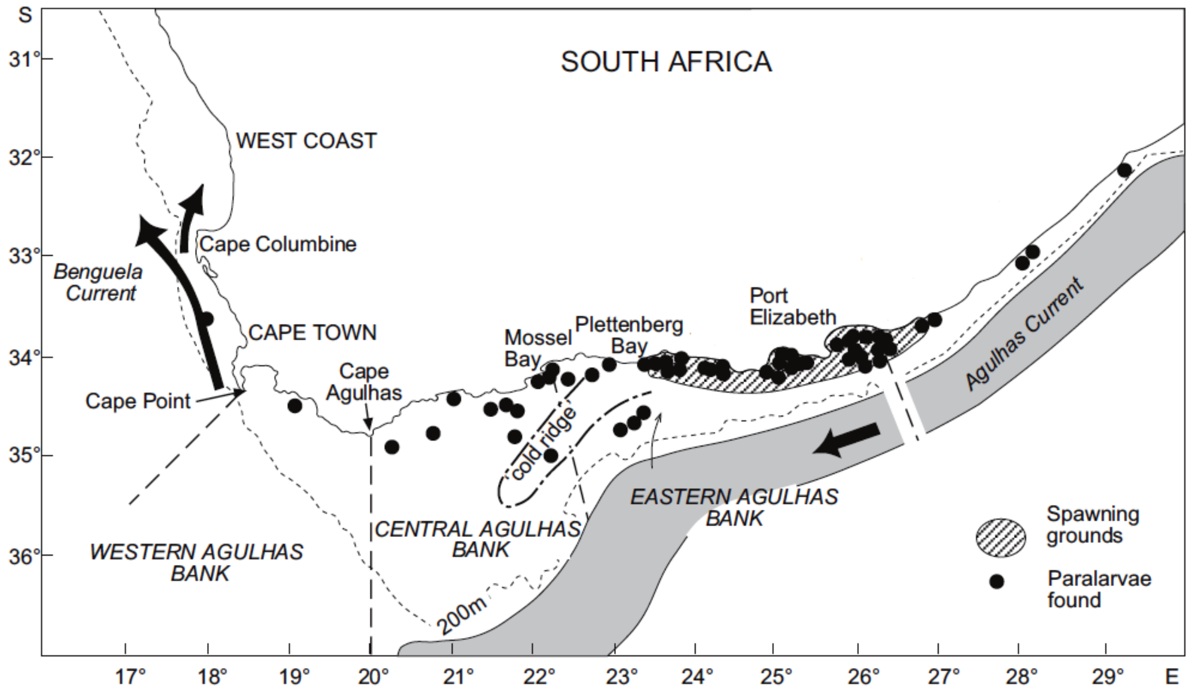


Figure 2. The main spawning grounds of chokka squid (*Loligo reynaudi*) on the eastern Agulhas Bank and east of the “cold ridge” (map taken from Roberts and van den Berg 2005). Locations where paralarvae have been found are indicated (data from Augustyn *et al.* 1994).

It is now commonly accepted that most of the mature adults occurring on the central and eastern Agulhas Bank undertake inshore return spawning migrations to the east where they form large aggregations (Figure 3) (Sauer *et al.* 1997, Augustyn and Roel 1998, Roberts and van den Berg 2002). It is possible that some of the matured and maturing adult males and females occurring on the west coast also undertake inshore return spawning migrations to the east, but possibly only reaching the inshore areas of the western and central Agulhas Bank, however no evidence of this phenomenon exists (Figure 3) (Olyott 2002). The maturity distribution patterns presented by Olyott (2002) adds little to verify this hypothesis because adults were predominantly found inshore and offshore on the eastern Agulhas Bank, with juveniles inshore on the eastern Agulhas Bank and offshore on the western and central

Agulhas Bank. Immature chokka squid showed an intermediate distribution pattern (Olyott 2002).

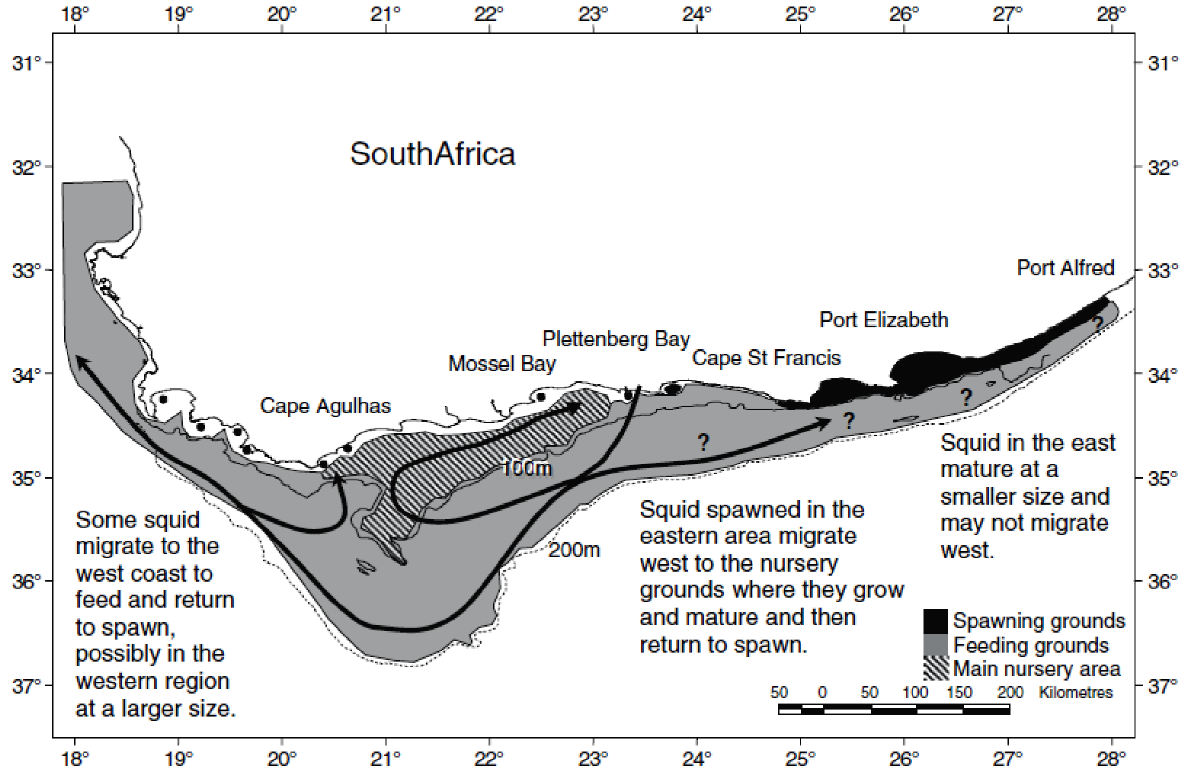


Figure 3. Schematic overview of the chokka squid (*Loligo reynaudi*) lifecycle along the coast of South Africa, taken from Olyott *et al.* (2007).

1.5.2 Exploiting fisheries

Chokka squid is currently targeted by recreational, artisanal, and commercial jig fisheries throughout its distribution in southern Africa. A demersal inshore and offshore trawl fishery landing chokka squid as by-catch has been operating in South Africa since the start of the 1900's (Augustyn and Roel 1998). In the last two decades the chokka squid bycatch in the demersal trawl fisheries has annually fluctuated between 200 and 1000 t.

Increased international demand for squid in the 1980’s led to the establishment of a dedicated chokka squid jig fishery in the eastern Cape around 1985 (Augustyn 1989). Since then the average annual jig catch has increased from 4641 t in the 80’s, to 5330 t in the 1990’s, and ~10 000 t in the 2000’s. The jigging fleet operates from the ports of St Francis and Port Elizabeth, mainly targeting spawning aggregations in the shallow coastal waters (20 - 60 m) of the south-eastern Cape between Plettenberg Bay and Port Alfred. Currently very little jigging, if any, takes place west of Cape Agulhas (20°E) (J. van der Westhuizen, DAFF, pers. comm.).

Little is known about the operations or catch statistics of the artisanal jig fishery for chokka squid in Angola. Apparently fishers operate from small skiffs using similar hand-line gear as the South African jig fishery.

1.5.3 Management and stock status

Extensive studies of the biology and life cycle, and the application of some stock assessment techniques to the South African chokka squid have contributed toward formulating management approaches for this species (Augustyn and Roel 1998). The current management objective for the chokka squid jig fishery is to cap effort at a level that secures the greatest catches in the long term, without exposing the resource to the threat of reduction to levels at which recruitment success might be impaired or catch rates drop below economically viable levels (DAFF 2012). The jig fishery is currently regulated through the setting of an annual total allowable effort (TAE) to restrict the number of jigging vessels and crew operating in the fishery (Sauer *et al.* 2002). Stock assessment models for the South African stock are based on a single-unit stock assumption and therefore make use of area-aggregated models.

This assumption may be violated by greater stock complexity than what was originally believed.

1.5.4 Previous stock identification studies

Although numerous studies on the biology, ecology, fisheries and systematics of chokka squid have been carried out in the last three decades, its stock structure still remains unclear. Because chokka squid have lengthy planktonic paralarval stages with the potential for high dispersal rates (Roberts and Mullon 2010), highly migratory adult stages (Sauer 1995, Sauer *et al.* 2000), and the lack of obvious physical geographic barriers to movement along the south coast of South Africa, genetic homogeneity of this stock was previously assumed (Shaw *et al.* 2010). However, this assumption is questionable based on the large area of chokka squid's known distribution that covers different geographical regions, each with its own set of environmental variables. Due to the global concern over the importance of intra-species biodiversity and its necessity for adaptations to climate change (Brierly & Kingsford 2009), some investigations into the stock structure of chokka squid have been attempted in the last decade using various biological and genetic techniques (Olyott 2002, Olyott *et al.* 2006, 2007, Shaw *et al.* 2010, Stonier 2012).

Limited data are available on regional variation in the morphology of chokka squid. By investigating the distribution of maturity patterns from samples of the entire Agulhas Bank, Olyott *et al*. (2006) found no conclusive evidence of geographically distinct stocks. However, they found longitudinal differences in size-at-maturity with adult squid in the east maturing at a smaller size than squid further west. As the influence of temperature on the growth of squid have been shown (Forsythe *et al.* 1994, Carvalho and Nigmatullin 1998, Forsythe 2004), warmer temperatures on the eastern Agulhas Bank may be responsible for chokka squid living there to mature at a smaller size. Chokka squid have been shown to spawn over a significantly larger area of the Agulhas Bank than previously estimated (Roberts *et al.* 2002), therefore it was suggested that juveniles growing under different environmental conditions on the eastern and western Agulhas Bank respectively could result in discrete subpopulations with different biological characteristics (Olyott *et al.* 2007).

Chokka squid have been shown to exhibit fine-scale genetic structuring. Results of Shaw *et al.* (2010) and Stonier (2012) revealed a potentially complicated stock structure. Although no significant differences were found between genetic samples of different spawning aggregations across the main spawning range on the southeast coast, subtle differences were found between geographically more distant samples from the western Agulhas Bank (Cape Town) and the eastern Agulhas Bank (Tsitsikamma, Cape St. Francis, Port Elizabeth and Port Alfred) (Shaw *et al.* 2010). More recently (Stonier 2012), an isolation by distance effect was found between samples from Angola, the west coast and the south coast of South Africa respectively. These results suggested limited movement between the three regions with previous work indicating two possible boundaries, one off the Namibian coast and another somewhere between Cape Town and Plettenberg Bay (Stonier 2012). Subtle genetic differentiation of geographically distant populations has been noted in other Loliginid squid such as the veined squid (*Loligo forbesi*) (Shaw *et al.* 1999), and may be related to the retention of paralarvae within large scale current systems (Shaw *et al.* 2010).

1.6 Motivation and rationale

Physical barriers such as the Luderitz upwelling cell is believed to limit the dispersal of many reef fishes (Floeter *et al.* 2008). It may therefore also act as an effective barrier to interbreeding between chokka squid populations occurring across the Benguela region. Currently there is evidence from research cruises (bottom trawl data) that chokka squid occurs only sporadically off the coast of central Namibia (Cruises of Dr Fridtjof Nansen and Blue Sea; Lipinski, Sauer pers. comm.). There are also no tagging studies to suggest that individuals migrate between South Africa and southern Angola. Nevertheless, the high dispersal capability of chokka squid adults, combined with disruption of the upwelling during the Benguela *Niños* (Shannon *et al.* 1986, Hutchings *et al.* 2009), has the potential to allow some mixing of populations in certain years. This may explain the recent isolation by distance genetic effects found in samples collected between Angola and South Africa (Stonier 2012).

The presence of reproductively active adults in South Africa and Angola, their association with warm waters in both areas, the lack of mass occurrence off the coast of Namibia as well as recent genetic results (Shaw *et al.* 2010, Stonier 2012) combined provide a strong case for the existence of at least two distinct stocks, one in South Africa and another in southern Angola.

The further possibility of separate stocks in South Africa complicates the current resource assessment, given that it is based on an area-aggregated model. Establishing whether the commercially exploited chokka squid population is made up of separate stocks is therefore an important consideration for fisheries management. Clear evidence of isolated stocks may require a change in the current management strategy and perhaps a separation of the fisheries into distinct geographical zones (Olyott *et al*. 2006). Based on available data (Stonier 2012, van der Vyver unpublished data), future mathematical models should incorporate the possibility of separate stocks. This will allow for area-disaggregated assessment of the resource.

Throughout the history of the jig fishery, chokka squid have been targeted further west off Cape Agulhas. This has caused some concern over the question of a single stock, especially in the light of new evidence suggesting possible stock separation somewhere along the South Coast (Shaw *et al.* 2010, Stonier 2012). Gaylard and Bergh (2006) suggested that the biomass in these less fished western areas act as a buffer against the impact of fishing in the more productive eastern areas. Thus, from a population modelling point of view, there is a broader population to consider.

In order to further examine the topic of a geographically fragmented stock, it was concluded that population genetics and morphological studies covering the entire known distribution area of chokka squid be conducted on a finer scale.

1.7 Aims and objectives

Fishery management decisions should be based on accurate knowledge of population structure (Cadrin and Secor 2009). Therefore, this study aimed to provide a clearer understanding of the chokka squid stock structure by investigating samples from across its entire known distribution range to test the current hypothesis of a single stock. The study also aimed to evaluate the feasibility of an area-disaggregated assessment by investigating the extent to which stock separation might be determined. This will be achieved by examining catch and CPUE data for both the commercial jig and trawl fisheries, biomass estimates from research surveys, new morphological data and recent genetic results on stock separation in order to search for logical cut-off points. Important limitations that were considered included the availability of catch and effort data within the resultant disaggregated areas, and the validity of these data.

Key Questions:

- Based on the morphology of *Loligo reynaudi*, and previous genetic analysis, can likely stock boundaries be delineated? (Chapter 2)
- Are there different catch, CPUE and biomass estimate trends in the resultant disaggregated areas when compared to the aggregated data? (Chapter 3)

CHAPTER 2

CLASSIFICATION OF CHOKKA SQUID (*LOLIGO REYNAUDI*) FROM THREE REGIONS BASED ON DISCRIMINANT FUNCTION ANALYSIS.

2.1 Introduction

The field of morphometrics is concerned with methods for the description and statistical analysis of phenotypic shape variation within or among samples of organisms and of the analysis of shape change as a result of growth, experimental treatment or evolution (Rohlf and Marcus 1993). The analysis of morphometric shapes has been and still is an important part of much biological research (Adams *et al.* 2004). In fisheries it is primarily used to study relationships among stocks, such as stock membership, the spatial distribution of stocks and as the supporting information for the phylogeny of stocks (Coyle 1998). Developments in the field of statistics, along with the computation abilities of large computers, have resulted in increasing sophistication of the analysis of these types of data (Adams *et al.* 2004).

Morphometrics is characterized by the application of multivariate statistical analysis to sets of morphometric variables (Rohlf and Marcus 1993). The variables usually correspond to various measured distances on an organism, usually lengths and widths of structures and distances between certain characteristic landmarks (Rohlf and Marcus 1993). With this approach, covariation in the morphological measurements can be quantified, and patterns of variation within and among sample groups can be assessed (Adams *et al.* 2004). To enable the study of shape differences among samples of different sizes, applications are frequently concerned with allometry (Rohlf and Marcus 1993). However, much effort has been spent over the years developing methods for size correction, so that size-free shape variables can be extracted and patterns of shape variation elucidated (Adams *et al.* 2004).

Classical methods of morphometric analysis include both uni- and multivariate statistics (for example simple and multiple regression, Principle Component Analysis (PCA), Canonical Variate Analysis (CVA), Discriminant Function Analysis (DFA), and covariance) based on linear measurements of body dimensions (Cadrin 2000). DFA is one of the multivariate techniques most widely used to investigate patterns of variation (Borges 1995). Discriminant analysis finds combinations of the components which maximise differences between already known groups (Andersen 1966, Thorpe 1983).

Unlike molecular markers, phenotypic variation in body parts is markedly influenced by environmental factors (Carvalho and Nigmatullin 1998) and does not always result from genetic divergence (Cadrin 2000). Therefore, phenotypic variation can only provide indirect indication of stock structure (Begg *et al.* 1999). Although they do not provide direct evidence of genetic isolation between stocks, they can indicate separation of post-larval stocks living in different environmental regimes (Begg *et al.* 1999). Phenotypic markers may be more useful for studying short-term, environmentally-induced variation, as opposed to long-term genetic variation. A combination of these two approaches are advisable to assess the importance of each in solving stock identity problems (Begg *et al.* 1999).

The geographic variability of morphometric characters is a common feature among cephalopods (Lipinski 1981, Carvalho and Nigmatullin 1998). Morphometric characters may be influenced by interactive effects of environmental, selection, and genetic variables, causing variation in the growth and shape of soft parts (example Liao *et al.* 2010) and hard structures (examples Borges 1995, Durholtz and Lipinski 2000, Villanueva 2000a) within a species, especially in those with a wide distribution range (Darwin 1859, Neige and Boletzky 1997, O'Dor and Lipinski 1998, Roper and Mangold 1998, Cadrin 2000).

The use of morphometric data for studies of geographic variation has been an accepted method in fisheries stock discrimination for over a century (Ihssen *et al.* 1981), and provides a powerful complement to genetic and environmental stock identification approaches (Cadrin 2000). In the early phases of morphometric study, many fishery stocks were identified using the application of univariate statistical analyses (Cadrin 2000). More recently the multivariate statistical analysis has been applied to identify fish stocks (examples in Lee, 1971, Winans 1987, Saila and Martin 1987). This method has also been extended to morphometric data of cephalopods such as octopods (Voight 2002, Lefkaditou and Bekas 2004) and sepiids (Guerra *et al.* 2001, Kassahn *et al.* 2003, Neige 2006). It has been widely used to distinguish between species of squid (Haefner 1964, Lipinski 1981, Augustyn and Grant 1988, Pierce *et al.* 1994b, Sanchez *et al.* 1996, Bonnaud *et al.* 1998, Pineda *et al.* 2002) and to study the geographic variation of population units and fishery stocks within species (Kashiwada and Recksiek 1978, Kristensen 1982, Brunetti and Ivanovic 1991, Boyle and Ngoile 1993, Pierce *et al.* 1994a, Borges 1995, Zecchini *et al.* 1996, Carvalho and Nigmatullin 1998, Hernandez-Garcia and Castro 1998, Vega *et al.* 2002, Liao *et al.* 2010).

Character dimensions from soft body parts such as the body, head, arms and tentacles continue to be widely used to distinguish among squid populations, because they are relatively easy and cost-effective to measure (Carvalho and Nigmatullin 1998). However, working with soft-bodied animals such as squid may pose a number of difficulties. The description of morphometric data is difficult due to the plasticity of their body form. Once preserved, many parts become stiff making it difficult to obtain accurate measurements (Roeleveld 1998). Also, loss of arm tips and tentacles during sampling (trawling and jigging) can drastically reduce the value of the specimen for morphometric analyses, and loss of original body shape during fixation and repeated freezing/thawing can result in unnecessary warping of morphological characters (Lipinski 1981). However, chitinous hard structures such as the beaks, gladius, sucker rings and statoliths are also related to body size and in some studies have shown to be useful and more reliable than soft parts (Borges 1995, Martinez *et al.* 2002).

Multivariate discriminant analysis of morphometric characters has revealed patterns of geographic variation in veined squid (*Loligo forbesii*) (Boyle and Ngoile 1993, Pierce *et al.* 1994a), patagonian squid (*L. gahi*) (Vega *et al.* 2002, Carvalho and Pitcher 1989) and swordtip squid (*Photololigo edulis*) (Sin *et al.* 2009, Liao *et al.* 2010). However, in geographically distant populations of another Loliginid squid with a similar life history, the long-finned squid (*Doryteuthis pealei*), this method has shown insignificant levels of geographic variation (Cohen 1976). Nevertheless, being a neritic coastal species distributed along the south and west coast of South Africa, and southern Angola, chokka squid (*L. reynaudi*) provides a good example of a species living under different environmental conditions, and therefore the possibility of geographically separated stocks may be assumed.

This chapter describes the results of morphometric variation in chokka squid from samples caught across its entire known geographic range. It deals with the comparison of morphometric characters obtained from samples caught in three different regions:

1) Southern Angola (Angola-Benguela front)
2) West Coast and western Agulhas Bank of South Africa (southern Benguela Current system)
3) Central and eastern Agulhas Bank of South Africa (Agulhas Current system)

Multivariate statistics were applied to the morphometrics of chokka squid in an attempt to discover possible differences between populations from the three different regions. DFA was used with the aim of showing whether these populations are significantly different using soft parts and hard structure characters.

2.2 Materials and methods

2.2.1 Sampling

A total of 1079 individual chokka squid were collected from 69 localities on the west and south coast of southern Africa (Figure 4) between April 2011 and July 2012. This material comprised three subsets:

a) Angola, 76 (38 males, 38 females) caught off the coast of southern Angola in July 2012
b) West coast, 87 (63 males, 24 females) caught off the west coast and on the western Agulhas Bank from January to February 2012
c) South coast, 916 (443 males, 473 females) caught on the central and eastern Agulhas Bank from April to November 2011

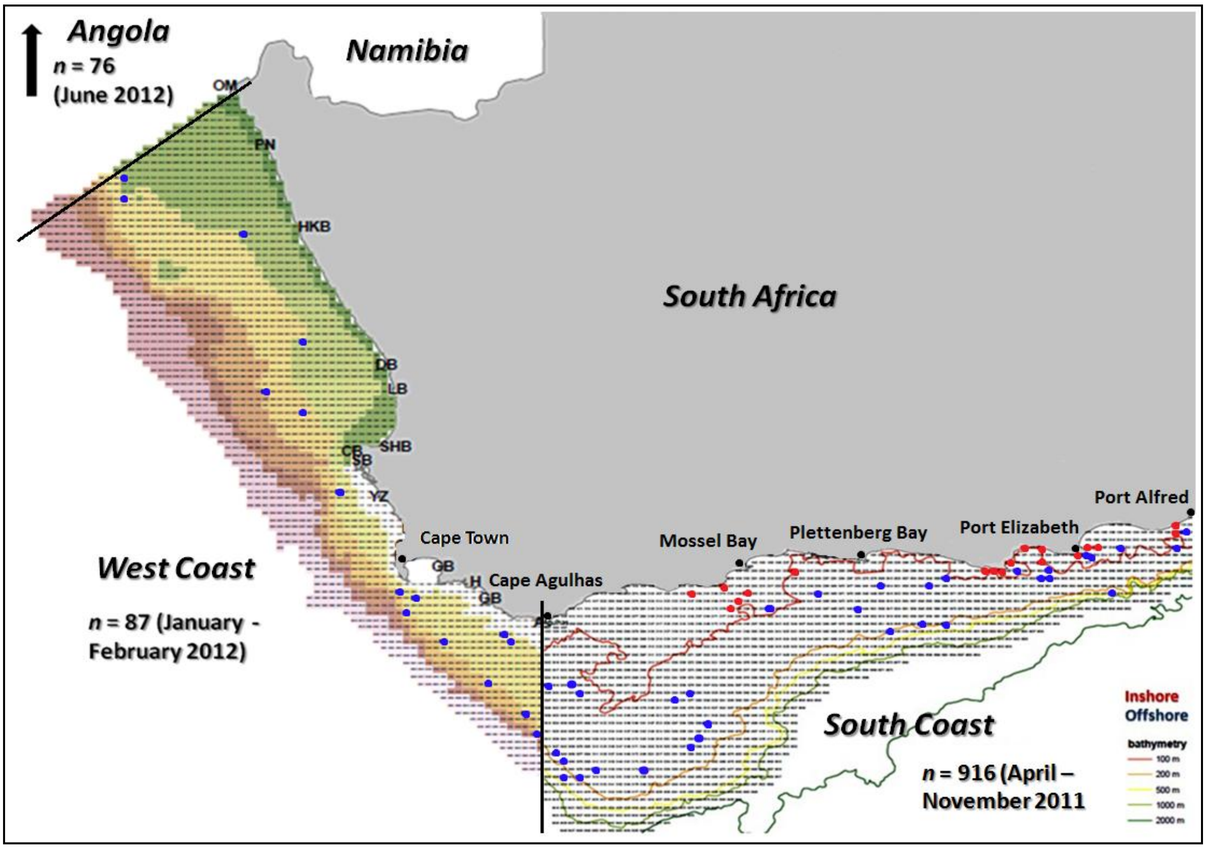


Figure 4. Fishing stations of all retained chokka squid (*Loligo reynaudi*) samples. The South African west and south coast catch locations were recorded using the demersal trawl survey grid numbers. *n* = number of individual chokka squid caught in each region.

Only adult specimens were collected with sizes of individuals ranging from 180 – 420 mm dorsal mantle length (DML) for males and 150 – 260 mm DML for females. Samples from the central and eastern Agulhas Bank were collected onboard various commercial jig vessels and a demersal trawl research vessel. West coast and western Agulhas Bank samples were collected on a demersal trawl research vessel only, and samples from southern Angola were collected at a fish market in Namibe.

The primary aim of the south and west coast demersal trawl surveys (onboard the F.R.S. Africana research vessel) was to estimate the biomass of shallow water hake (*Merluccius* *capensis*) and deep water hake (*M. Paradoxus*) over the depth range 20 – 500 m. The south coast survey covered the shelf between 20°E (Cape Agulhas) and 27°E (Port Alfred) and the west coast survey between 20°E (Cape Agulhas) and 29°S (Orange River). The surveys provided for the collection of random squid samples over a range of shallow and deep areas on the south and west coast. The south and west coast demersal survey areas were divided into 5′ x 5′ grids within depth bands of 0 – 50, 51 – 100, 101 – 200, and 201 – 500 m. Trawl blocks for each of the two surveys were selected on a semi-random basis along the coast, the number of stations per depth and longitude stratum being roughly proportional to the number of trawlable blocks in each stratum. Known areas of hard ground were avoided. A 180-ft German trawl net with V doors was used. The 75 mm codend mesh was lined with a 27.5 mm mesh in the form of a sleeve. Trawl duration was limited to actual bottom time of 30 minutes, which equated to a standard trawl area of ~0.0842 km^2 $trawl^{-1}$.

Four trips onboard various commercial jig vessels between June and November 2011 provided jigged samples from the main inshore spawning areas on the southeast coast that were not covered during the south coast demersal trawl survey (Figure 5). Samples caught by the artisanal jig fishery in southern Angola (in the coastal waters between 15° and 17°S) were collected from a single hawker at a fish market in Namibe, the species' northern-most known geographical limit (Shaw *et al.* 2010, Roberts *et al.* 2012).

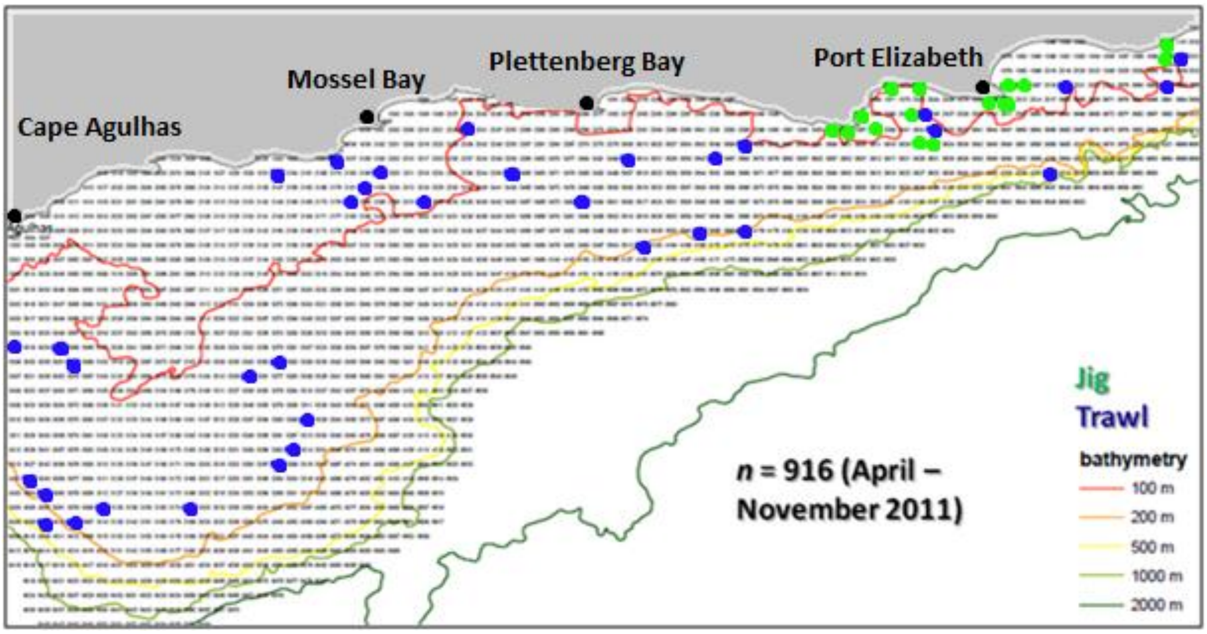


Figure 5. Jig (green) vs. trawl (blue) fishing stations for all retained chokka squid (*Loligo reynaudi*) on the south coast of South Africa. *n* = number of individual chokka squid caught on the south coast.

The aim during all sampling efforts was to collect 40 adult females and 40 adult males per catch location. However, this was not always possible due to low chokka squid availability, especially during the west coast demersal survey. Hence the grouping of samples for better statistical values. After capture, the sex of each specimen was determined before immediately being frozen onboard the vessel. At the end of research cruises and commercial trips all samples were transported frozen to Rhodes University, South Africa. Samples from Angola were also transported frozen to South Africa. To ensure standardization of effects of storage at Rhodes University, all squid were kept frozen at -20°C until analysis.

2.2.2 Morphometric data sources

A total of 43 morphometric characters (Table 1) of the soft parts (body, head, arms, tentacles) and hard structures (gladius, sucker rings, lower beak, statolith) were measured from each sample. Beak morphometric characteristics were modified from Clarke (1986), statolith morphometric characters from Clarke and Maddock (1988) and, gladius, sucker rings and soft parts were selected and modified from Lipinski (1981). Detailed specifics on the measurements taken for each soft part and hard structure can be seen in Table 1 and Figure 6 - 8. In order to prevent any unnecessary warping of morphological characteristics, which can happen with repeated freezing and thawing (Lipinski 1981), each specimen was defrosted only once at room temperature before morphometric measurements were taken. No measurements were made on soft parts or hard structures which appeared to be damaged or to have suffered previous damage (e.g. missing arm and tentacle tips; re-grown arms and tentacles; damaged gladius, lower beaks, sucker rings and statoliths). All morphometric measurements were made by the author and under standardised conditions to avoid unnecessary variation in measurements.

Table 1. Soft part and hard structure morphometric characters of chokka squid (*Loligo reynaudi*) measured in this study.

Abbreviation	Character	Description
Soft parts		
Body		
AN	Fin angle	Angle of fin to body on ventral side
DML	Dorsal mantle length	Taken from most anterior to most posterior point of mantle, along midline on dorsal side
VML	Ventral mantle length	Taken from most anterior to most posterior point of mantle, along midline on ventral side
FL	Fin length	Total length of a fin including the anterior fin lobe
FWL	Fin width length	Between widest points of fin lobes
MW1	Mantle width 1	Width of mantle at the anterior end of mantle (mantle opening width)
MW2	Mantle width 2	Width of mantle at the base of fin lobes
MW3	Mantle width 3	Width of mantle at the widest points between fin lobes
AF	Funnel cartilage length	Taken along central line of funnel to opening of funnel tube, on ventral side
GRNI	Nidamental gland length (female only)	Nidamental gland length taken along central line of gland
Head		
HL	Head length	Taken from the anterior neck groove (dorsal side) to V-junction between 1st pair of arms
HW	Head width	Taken between eyes
Arms		
A1	First arm length	1st arm of 1st pair, taken from 1st sucker at the base of arm to tip of arm
A2	Second arm length	1st arm of 2nd pair, taken from 1st sucker at the base of arm to tip of arm
A3	Third arm length	1st arm of 3rd pair, taken from 1st sucker at the base of arm to tip of arm
A4	Fourth arm length	1st arm of 4th pair, taken from 1st sucker at the base of arm to tip of arm
Tentacles		
TL	Left tentacle lenght	Taken from the base of the tentacle to the tip of the club
TR	Right tentacle length	Taken from the base of the tentacle to the tip of the club
HEC	Hectocotylus arm length (male only)	Taken on 3rd arm (left side), from 1st sucker (nearest to tip of arm) to tip of arm
CL	Club length	Taken from 1st carpal sucker to tip of club
Hard structures		
Gladius		
GLA	Gladius length	Taken from anterior to posterior tip
GW1	Gladius width 1	Free rachis width
GW2	Gladius width 2	Rachis width
GW3	Gladius width 3	Width taken at widest point of gladius
GRL	Free gladius length	Taken from anterior tip of gladius to rachis
Sucker rings		
S1	Sucker diameter 1	Diameter of largest sucker on the first arm, measurement taken inside sucker
S2	Sucker diameter 2	Diameter of largest sucker on the 2nd arm, measurement taken inside sucker
S3	Sucker diameter 3	Diameter of largest sucker on the 3rd arm, measurement taken inside sucker
S4	Sucker diameter 4	Diameter of largest sucker on the 4th arm, measurement taken inside sucker
T	Tentacle sucker diameter	Diameter of largest sucker on the left tentacle club, measurement taken inside sucker
Lower beak		
g	Hood length	Measured along midline of the beak, in profile
f	Crest length	Measured along midline of the beak, in profile
a	Rostral length	Distance between rostral tip and front edge of wing
b	Wing length	Taken from front edge of wing to base of wing
d	Baseline length	Taken from base of wing to base of crest, in profile
c	Rostral height to base	Taken from rostral tip to base of beak platform, in profile
Statolith		
TSL	Total statolith length	Taken from apex of dorsal dome to tip of rostrum
LDL	Lateral plus dorsal dome length	Taken from rostral angle to apex of dorsal dome
DLL	Dorso-lateral length	Taken from apex of dorsal dome to lateral tip of lateral dome
RSL	Rostral length	Taken from angle to tip of rostrum
RBLD	Rostral base to lateral tip of lateral dome	Taken from the base of rostrum to lateral tip of lateral dome
LDW	Lateral dome width	Taken from lateral tip of lateral dome to medial fissure
VLL	Ventro-lateral length	Taken from tip of rostrum to lateral tip of lateral dome

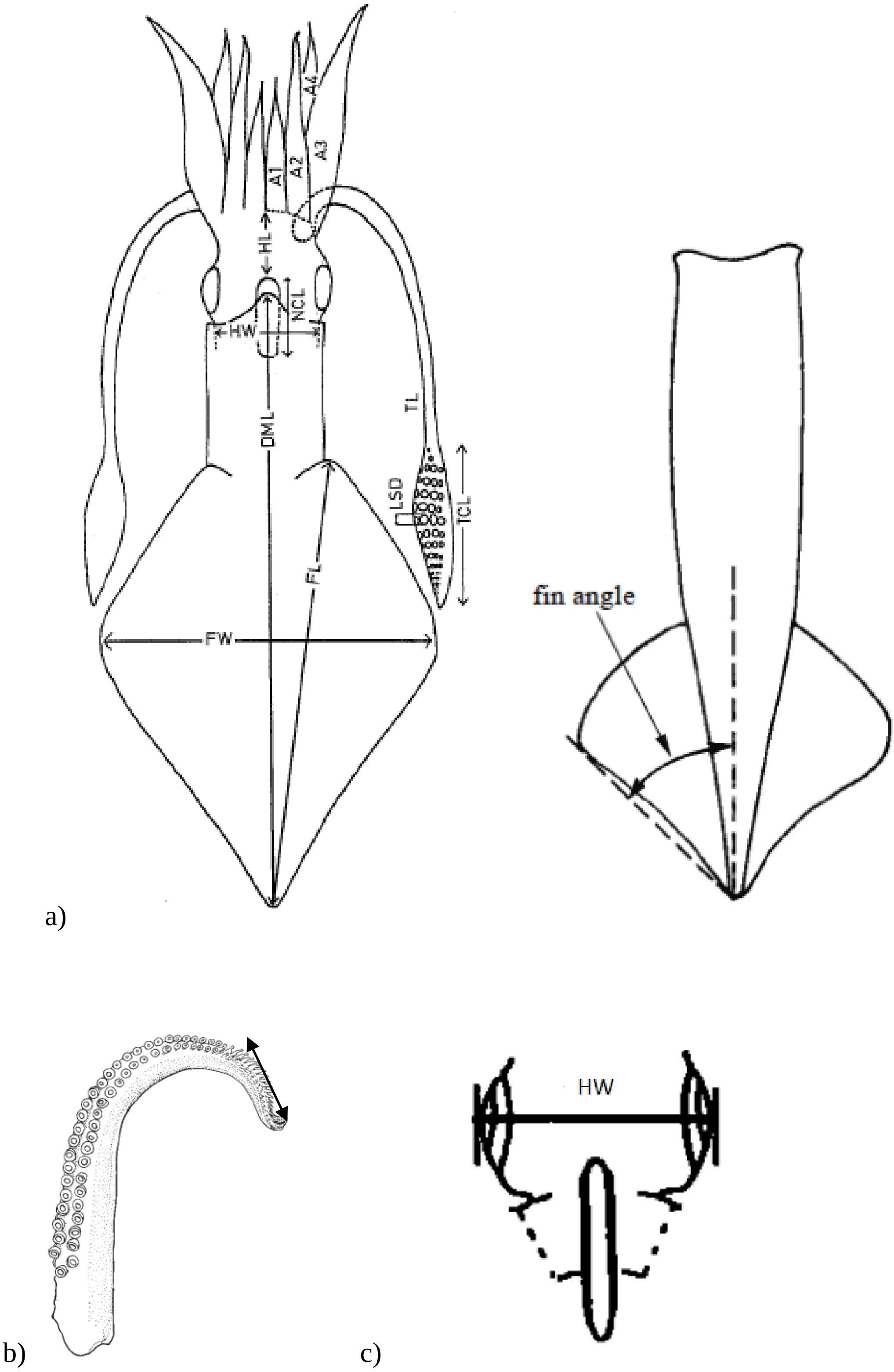


Figure 6. Soft part morphometric measurements recorded for chokka squid (*Loligo reynaudi*), based on the work by Lipinski (1981). a) soft part dimensions (Pierce *et al.* 1994a), b) modified male hectocotylized arm measurements (Jereb and Roper 2010), c) head dimensions (Cohen 1976).

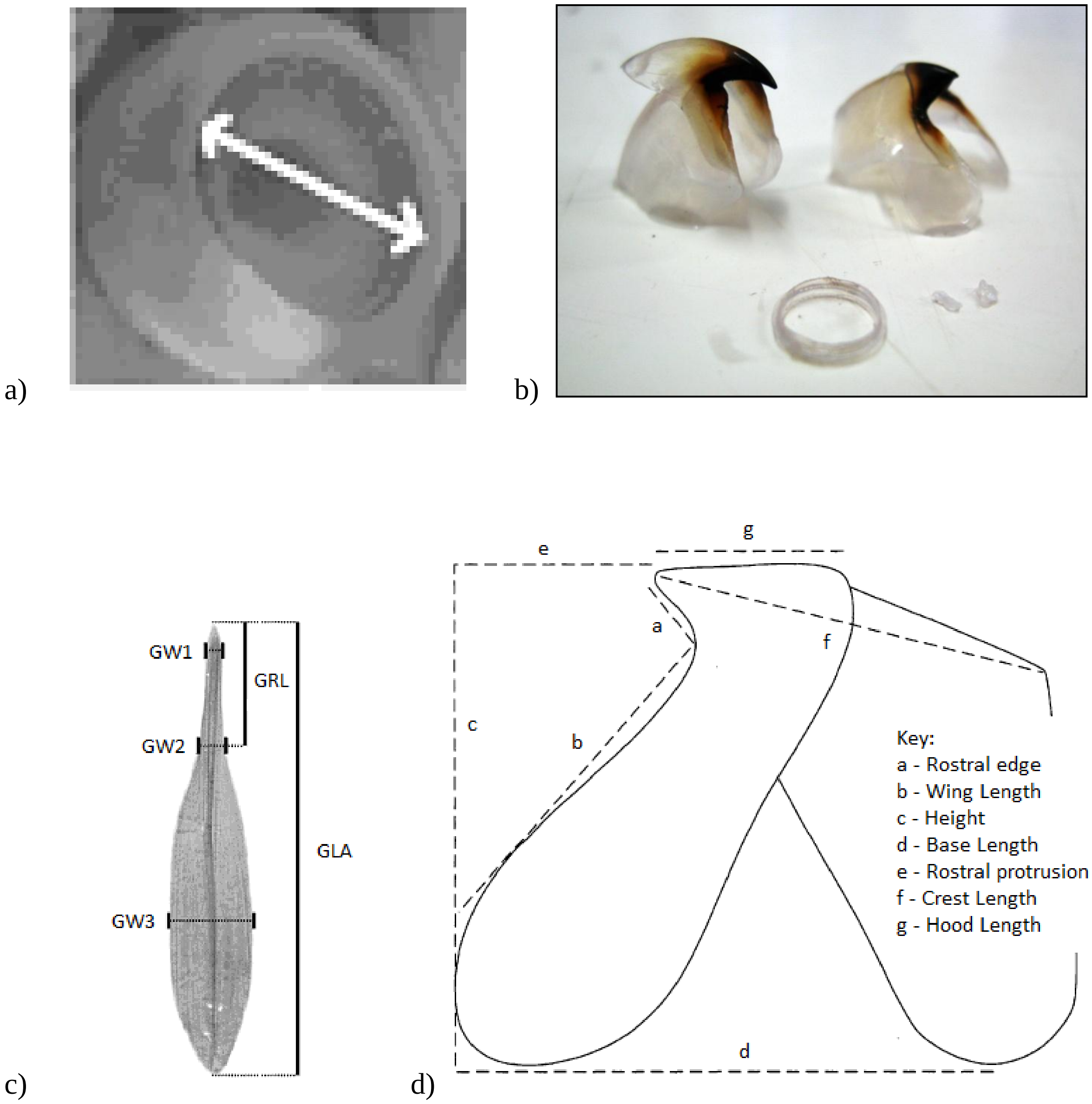


Figure 7. Hard structure morphometric measurements recorded for chokka squid (*Loligo reynaudi*), based on the work by Lipinski (1981). a) sucker ring diameter measurements (Baron and Re 2002), b) upper and lower beak, tentacle sucker ring and statoliths after removal from squid, c) Gladius dimensions (Baron and Re 2002), d) lower beak dimensions (Ogden *et al.* 1998).

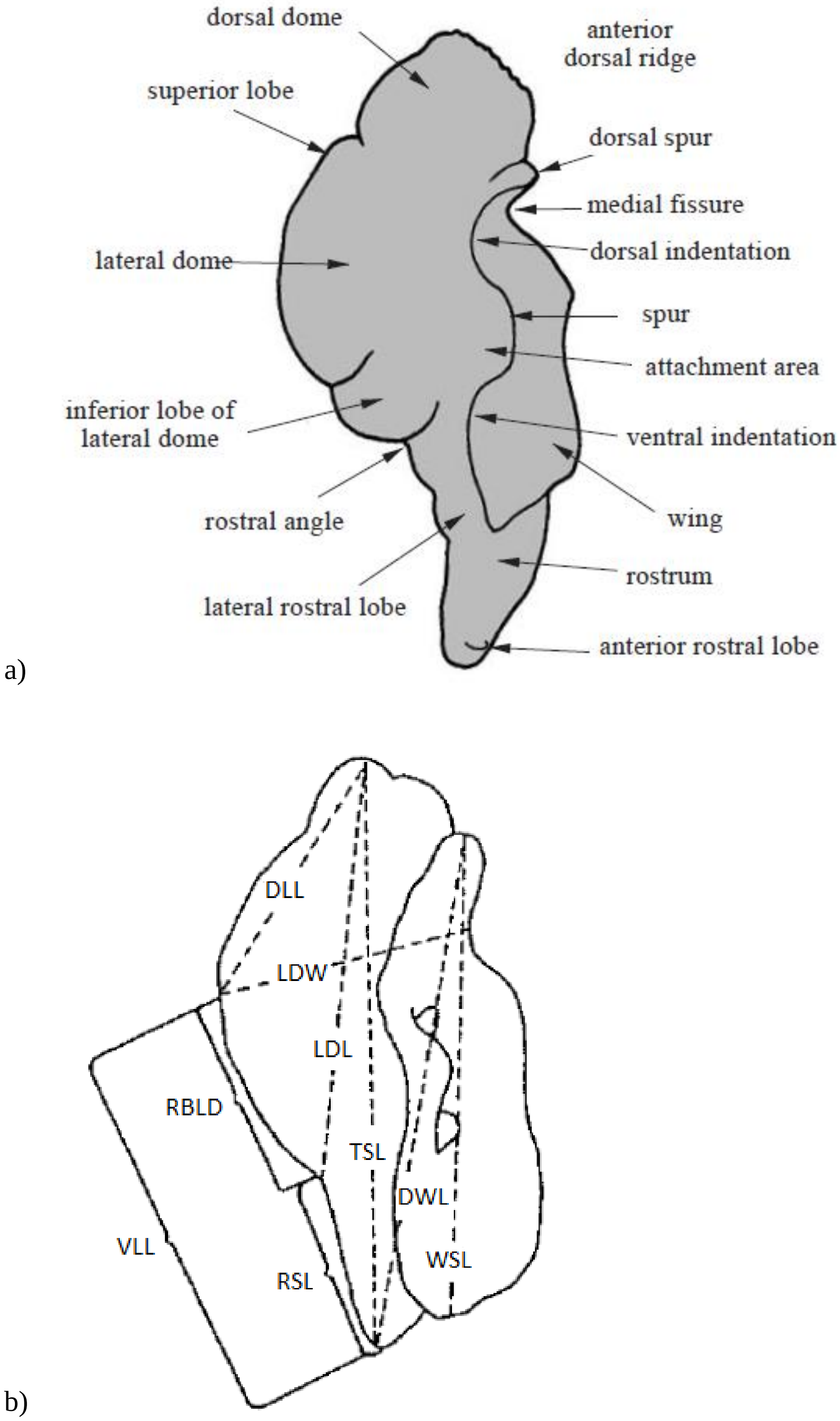


Figure 8. Statolith morphometric measurements recorded for chokka squid (*Loligo reynaudi*), based on the work by Lipinski (1981). a) basic terms of a generalized teuthoid statolith (Jereb and Roper 2010), b) Statolith dimensions (Lipinski *et al.* 1993).

All soft part morphometric data were measured to the nearest millimetre (mm) according to recommendations by Roper and Voss (1983), using a single set of vernier callipers. Measurements on the gladius and sucker rings were made after removing the structures from the squid. Gladius measurements were made to the nearest mm using vernier callipers and sucker ring diameter was measured using a low-powered microscope with an eyepiece micrometer. Beaks were carefully extracted from the buccal mass following the method described by Clarke (1986) and immediately frozen until further analysis. After defrosting at room temperature at a later stage, lower beaks were measured in profile to the nearest 0.01 mm using a single set of digital callipers. Statoliths were removed from the head with a small pair of tweezers and stored in empty vials until further analysis of only one statolith per pair (either left or right) under a low-powered microscope with an eyepiece micrometer.

2.2.3 Statistical method

To assess the existence of distinction among samples from the different regions the morphological measurements were analysed using DFA. DFA tests the significance of group differences by deriving a weighted combination of variables which maximizes differences among groups (Cadrin 2000). Cases are classified into one of several mutually exclusive groups on the basis of various characteristics, in this case, the combinations of morphological measurements (Carvalho and Pitcher 1989). Measurements are initially required to be made on a sample of known identity (i.e., sample number) and then an unknown classification assigns the members to the designated groups on the bases of the weighted scores obtained in the first part of the analyses (Carvalho and Pitcher 1989). The proportion of correctly classified cases gives a measure of the "morphological distinctness" of each sample compared (Carvalho and Pitcher 1989).

2.2.4 Statistical analysis

Discrimination between patagonian squid samples from different regions has been shown to be less distinct when combining soft parts and hard structures (Vega *et al.* 2002). Therefore, DFA in this study was done on the combination of all the soft parts, and then on each of the different hard structures (lower beak, gladius, sucker rings, and statolith). The data for males and females were also analysed separately due to differences in the allometric growth of sexes.

Prior to analysis, morphometric data were screened for errors using bivariate plots and regression analyses to identify outliers. Unfortunately soft part measurements could not be retaken as specimens were discarded after measurements. Errors in soft part data were therefore corrected by reference to the original data sheets, or data from those samples were deleted. Some hard structures such as the beaks and statoliths were re-measured where necessary.

All measurements were positively linearly related with size (DML and GLA-TL). Typically, the size of different body parts of an animal is allometrically related to the body size and takes the following form (Leonart *et al.* 2000):

$$Y = \alpha X^{\beta},$$

Where Y is the body size, α is the shape parameter, and β is the power parameter that scales the morphometric measurement to the body size.

It is therefore important to first remove the effect of size before conducting further statistical analysis (Leonart *et al.* 2000, Liao *et al.* 2010). In order to remove the effect of size on the different morphometric measurements, each morphometric character was log-transformed and standardized using the following allometric formula (Liao *et al.* 2000):

$$M_{std} = \log M - \beta(\log Ml - \log \overline{Ml})$$

Where M_{std} is the standardized morphometric measurement, $log\ M$ is the log of the morphometric measurement, β is the slope of regression of the morphometric measurement to the dorsal mantle length, *Ml*, *log Ml* is the log of the dorsal mantle length, and $\log \overline{Ml}$ is the mean of the log of the dorsal mantle length.

After removing the effect of size, DFA analysis was applied. Both the removal of the effect of size and application of the DFA was done separately for each group of the morphometric measurements (soft parts, lower beaks, gladius, sucker rings, statoliths).

Both Linear Discriminant Function Analyses (LDA) and Quadratic Discriminant Function Analysis (QDFA) were applied. For the results from LDA to be useful, a number of important assumptions had to be met. Some of the most important assumptions included: homogeneity of variance, sufficient sample size, and normality of the data. In this study the homogeneity of variance assumption was not met, in which case there were two recommended alternatives: Multinomial Logistic Regression or QDFA (Zuur *et al.* 2007).

After an initial graphical exploration of the data, using dot chart and box plots, the spread of variables among the three major areas (Angola, south coast, and west coast) was assessed and found to be different, therefore a QDFA was also conducted. The results from the DFA were summarized graphically, whereby the centroid of the three regions combined with their discriminant function analysis was assessed using the overall accuracy/misclassification error rate and also its performance in terms of correctly classifying the individuals from each region.

Assessment of the importance of variables was restricted to the soft body measurements as it was this aspect of *L. reynaudi*'s morphology that led to a significant separation of squid from the three regions. Variable importance was assessed by the stepwise-selection method using 'Correctness rate' as performance criterion where forward and backward selection was applied and variables that lead to 1% change in the performance criterion were retained in the model.

Discriminant function analysis was applied using the package 'MASS' (Venables and Ripley 2002) and the variable selection was conducted using the 'klaR' package (Weihs *et al.* 2005). All analyses were conducted using the R statistical and programming language (R Developement Core Team 2012).

2.3 Results

Note that in this section the term "west coast" refers to the West Coast and the western Agulhas Bank combined, "south coast" refers to the central and eastern Agulhas Bank combined, and "Angola" refers to southern Angola only.

2.3.1 Selection of individuals

A total of 544 male and 535 female samples from the three regions were used in the discriminant function analyses. The average DML length of males (279.3 mm Angola, 299.8 mm south coast, 250.6 mm west coast, see Table 2) and females (185.8 mm Angola, 207.4

mm south coast, 190.8 mm west coast, see Table 3) from each region differed only slightly (Figure 9). For both males and females the south coast subsample size was by far the largest.

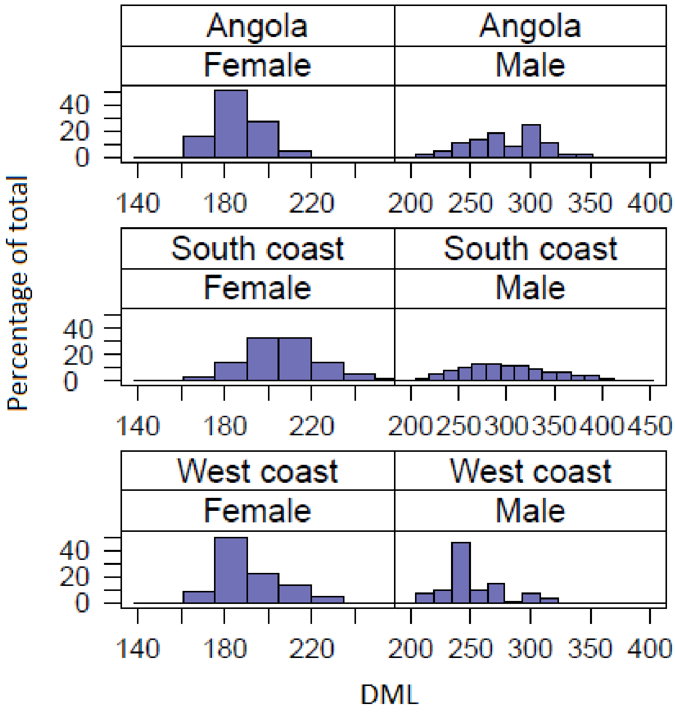


Figure 9. The percentage of male and female chokka squid (*Loligo reynaudi*) in each size class per region (Angola, south coast and west coast).

Table 2. Descriptive statistics of male chokka squid (*Loligo rynaudi*) character measurements from each of the three regions (Angola, south coast and west coast).

Male *Loligo reynaudi*															
Body	minimum			maximum			median			mean			standard deviation		
Characters	Angola	south	west	Angola	south	west	Angola	south	west	Angola	south	west	Angola	south	west
AN (°)	29	13	26	40	45	44	34.0	33.0	36.0	34.1	33.4	36.2	2.90	3.13	3.72
DML (mm)	218	196	212	344	430	320	278.0	295.0	244.5	279.3	299.8	250.6	30.93	45.08	24.00
VML (mm)	199	183	188	311	382	289	254.0	272.0	223.5	253.4	275.8	230.4	27.52	41.47	22.93
HL (mm)	33	30	31	49	59	51	43.0	45.0	41.0	42.5	45.4	41.1	3.91	5.10	3.89
HW (mm)	25	29	29	38	52	44	34.0	40.0	37.0	33.1	40.1	36.6	3.12	3.88	3.09
FL (mm)	135	134	133	220	274	219	180.0	196.0	166.0	181.4	199.6	169.1	20.90	29.49	18.75
FWL (mm)	111	94	104	172	217	165	144.0	150.5	128.0	141.0	152.1	130.8	14.89	23.20	12.63
MW1 (mm)	48	46	50	69	78	72	59.0	61.0	56.0	59.1	61.2	57.1	4.93	6.16	4.63
MW2 (mm)	55	34	51	78	89	77	68.0	66.0	61.0	66.7	66.6	62.0	6.33	7.52	5.67
MW3 (mm)	41	10	39	61	70	62	51.0	50.0	48.0	50.8	49.8	48.6	5.48	6.88	5.51
HEC (mm)	19	17	14	29	32	23	23.5	23.0	19.0	23.7	23.6	18.9	2.57	2.56	2.04
CL (mm)	69	28	61	103	130	130	85.0	96.0	89.5	84.4	95.8	90.0	9.75	12.58	11.53
AF (mm)	25	23	27	42	50	41	36.0	37.0	33.5	35.7	37.2	33.6	3.24	4.35	2.87
A1 (mm)	55	43	47	80	108	89	67.0	73.0	63.0	67.0	73.8	64.0	5.69	10.02	7.20
A2 (mm)	65	55	59	95	122	97	82.0	87.0	77.0	80.7	87.8	76.8	7.27	11.50	7.60
A3 (mm)	74	57	65	107	135	108	88.0	92.5	80.0	87.9	93.0	80.3	7.39	13.36	8.67
A4 (mm)	62	58	58	94	113	93	77.0	82.5	70.5	77.8	83.5	70.7	7.42	10.41	6.43
TL (mm)	243	189	218	349	413	355	280.0	317.0	290.0	283.7	314.7	289.2	26.58	34.64	25.05
TR (mm)	238	136	230	356	404	366	284.0	314.0	283.5	287.6	314.0	287.3	27.79	37.32	26.67
GLA (mm)	227	211	217	354	430	450	294.5	298.0	254.5	290.3	301.8	263.4	31.44	41.21	35.23
GW1 (mm)	5	4	6	10	12	11	8.0	8.0	8.0	7.9	8.1	8.0	1.08	1.37	0.84
GW2 (mm)	12	9	13	19	20	19	15.0	15.0	15.0	15.0	15.6	14.8	1.54	1.79	1.35
GW3 (mm)	30	27	30	43	53	43	37.0	37.0	34.0	36.6	37.0	34.9	3.09	3.97	3.15
GRL (mm)	172	160	163	271	397	255	225.5	229.0	192.0	220.3	230.9	198.2	25.06	33.22	21.27
S1 (mm)	1.10	0.88	1.30	1.65	2.75	2.20	1.45	1.70	1.70	1.41	1.68	1.69	0.13	0.29	0.16
S2 (mm)	1.23	1.00	1.30	1.80	3.44	2.20	1.50	1.79	1.80	1.53	1.80	1.83	0.15	0.36	0.17
S3 (mm)	1.25	1.10	1.60	2.38	3.38	2.70	1.78	2.08	2.00	1.76	2.08	2.08	0.21	0.35	0.18
S4 (mm)	1.00	0.88	1.20	1.70	3.50	2.00	1.30	1.50	1.50	1.31	1.53	1.56	0.15	0.28	0.15
T (mm)	4.40	4.00	5.20	6.90	9.00	8.00	5.70	6.90	6.80	5.83	6.88	6.80	0.63	0.92	0.57
g (mm)	4.68	4.07	5.36	7.26	8.74	7.74	5.81	6.17	6.34	5.89	6.17	6.33	0.55	0.71	0.48
f (mm)	10.49	6.83	11.61	14.88	18.08	15.72	12.52	13.35	13.06	12.47	13.35	13.21	0.98	1.38	0.85
a (mm)	2.73	2.53	3.17	3.90	4.72	4.46	3.42	3.75	3.70	3.39	3.77	3.74	0.31	0.39	0.26
b (mm)	8.75	7.42	8.87	12.15	16.15	13.84	10.48	10.78	10.33	10.57	10.83	10.45	0.81	1.23	0.91
c (mm)	8.88	7.55	9.35	12.22	14.65	12.99	10.63	11.15	10.55	10.62	11.18	10.73	0.80	1.13	0.79
d (mm)	9.19	10.58	12.27	14.91	19.49	16.44	13.43	14.42	14.10	13.17	14.51	14.16	1.24	1.41	0.95
TSL (calibrated units)	79	68	74	90	90	85	85.0	81.0	80.0	84.76	81.32	80.34	2.60	3.75	2.65
LDL (calibrated units)	53	45	49	60	62	60	57.0	55.0	55.0	56.54	54.77	54.95	1.99	2.73	2.25
DLL (calibrated units)	34	30	30	47	57	54	42.0	41.0	41.0	42.19	40.92	41.55	2.49	4.08	3.72
RSL (calibrated units)	27	22	25	42	44	36	35.0	32.0	30.0	33.89	31.59	33.89	3.08	3.43	2.96
RBLD (calibrated units)	34	31	31	49	56	43	40.0	38.0	38.0	40.86	38.17	37.69	2.85	2.77	2.04
LDW (calibrated units)	31	28	30	43	49	44	38.0	38.0	38.0	37.11	37.75	38.19	2.88	3.88	3.47
O (calibrated units)	31	29	32	40	42	40	34.0	37.0	36.5	35.03	36.66	36.74	2.05	2.33	1.90
VLL (calibrated units)	70	58	60	82	93	74	74.0	70.0	68.0	74.76	69.77	67.63	3.13	4.21	3.06

Table 3. Descriptive statistics of female chokka squid (*Loligo rynaudi*) character measurements from each of the three regions (Angola, south coast and west coast).

Female *Loligo reynaudi*															
Body Characters	**minimum**			**maximum**			**median**			**mean**			**standard deviation**		
	Angola	**south**	**west**	**Angola**	**south**	**west**	**Angola**	**south**	**west**	**Angola**	**south**	**west**	**Angola**	**south**	**west**
AN (°)	34	29	34	48	49	35	40.0	39.0	38.0	40.4	38.6	38.8	3.33	2.81	3.33
DML (mm)	166	157	170	214	265	221	186.0	207.0	189.0	185.8	207.4	190.8	17.13	13.76	10.44
VML (mm)	152	141	156	194	241	205	168.0	190.0	173.0	167.0	189.8	175.9	15.74	13.03	9.32
HL (mm)	30	24	26	46	48	40	38.0	39.0	35.0	38.0	38.6	34.4	3.92	3.45	3.61
HW (mm)	23	24	26	34	45	36	29.0	34.0	32.0	28.9	34.1	31.8	3.24	2.83	2.50
FL (mm)	104	95	105	142	192	149	122.0	136.0	127.0	121.3	136.7	125.4	12.58	11.58	8.46
FWL (mm)	89	83	72	128	166	122	108.0	115.0	102.0	108.6	115.6	102.5	11.97	12.56	7.94
MW1 (mm)	43	38	41	57	62	53	49.0	50.0	49.0	49.6	49.9	48.3	3.84	3.32	2.68
MW2 (mm)	46	41	41	64	71	61	53.0	54.0	51.0	53.8	54.1	51.9	4.83	4.80	4.43
MW3 (mm)	35	30	32	50	61	50	43.0	43.0	40.0	43.1	42.7	40.7	4.92	4.10	3.88
CL (mm)	63	50	48	86	104	84	70.5	79.0	70.0	72.1	78.6	67.9	8.97	9.57	5.52
AF (mm)	25	20	20	35	39	31	29.0	30.0	27.0	29.5	30.1	26.5	2.75	2.83	2.46
A1 (mm)	46	36	41	70	80	63	59.0	59.0	52.0	58.6	58.4	50.9	6.87	5.81	4.67
A2 (mm)	61	35	50	81	94	75	72.0	70.0	58.0	71.3	70.0	59.6	7.86	6.50	5.02
A3 (mm)	65	45	52	87	104	72	77.0	74.0	64.0	76.9	73.9	63.3	9.27	4.71	5.18
A4 (mm)	56	47	40	76	90	67	67.0	69.0	57.0	66.8	68.3	56.0	7.79	7.09	5.25
TL (mm)	212	183	178	286	349	274	261.5	266.0	234.0	255.6	266.0	230.5	27.90	25.18	20.55
TR (mm)	210	166	174	308	345	265	261.0	267.0	230.0	257.1	265.9	225.8	30.28	23.55	21.43
GLA (mm)	179	164	174	222	267	230	196.0	210.0	200.0	195.2	211.7	198.4	16.56	14.22	9.90
GW1 (mm)	4	4	5	6	8	7	5.0	5.0	6.0	4.9	5.4	5.8	0.73	0.83	0.43
GW2 (mm)	11	6	9	13	17	13	12.0	12.0	12.0	12.1	12.2	11.7	1.30	1.11	0.80
GW3 (mm)	29	24	26	38	45	35	34.0	34.0	30.0	33.6	33.8	29.7	3.21	1.81	2.15
GRL (mm)	131	122	135	165	220	230	146.0	160.5	151.0	145.9	161.2	156.2	14.22	23.77	8.17
GRNi (mm)	50	28	20	75	87	65	63.0	63.0	34.5	63.2	62.0	38.0	10.31	12.99	5.34
S1 (mm)	0.95	0.70	1.00	1.38	1.79	1.60	1.13	1.26	1.30	1.12	1.26	1.30	0.17	0.14	0.11
S2 (mm)	0.93	0.88	1.10	1.55	1.89	1.50	1.25	1.37	1.40	1.23	1.35	1.37	0.17	0.13	0.15
S3 (mm)	1.23	0.99	1.30	1.70	2.27	1.90	1.50	1.65	1.60	1.47	1.64	1.64	0.19	0.16	0.10
S4 (mm)	0.85	0.60	1.00	1.25	1.70	1.60	1.01	1.10	1.20	1.05	1.15	1.23	0.15	0.14	0.12
T (mm)	4.30	3.70	4.00	5.70	7.50	6.90	4.90	5.90	5.60	4.94	5.85	5.55	0.71	0.67	0.44
g (mm)	4.10	3.92	4.11	5.30	6.80	5.57	4.79	5.04	5.02	4.74	5.03	4.96	0.38	0.41	0.29
f (mm)	9.55	8.61	9.52	11.83	13.82	11.93	10.41	11.21	10.81	10.55	11.16	10.68	0.85	0.74	0.56
a (mm)	2.33	2.50	2.33	3.31	4.07	3.56	3.02	3.28	3.20	2.97	3.29	3.13	0.28	0.32	0.23
b (mm)	7.14	6.81	7.04	10.16	13.05	9.16	8.76	8.83	8.45	8.69	8.86	8.39	0.79	0.57	0.71
c (mm)	7.78	7.27	7.11	10.22	11.61	9.64	8.88	9.31	8.79	8.92	9.30	8.76	0.75	0.64	0.63
d (mm)	10.03	8.21	9.83	12.53	14.58	12.69	11.10	12.10	11.67	11.21	12.03	11.54	0.95	0.80	0.67
TSL (calibrated units)	74	62	69	87	89	82	80	77	77	80.24	77.05	76.48	4.48	3.44	2.91
LDL (calibrated units)	50	37	47	60	61	56	55	53	53	54.57	52.48	52.35	3.11	2.60	2.26
DLL (calibrated units)	33	25	33	45	50	44	40	38	40	39.70	37.70	39.43	3.67	2.69	2.28
RSL (calibrated units)	24	18	23	35	58	38	31	30	28	31.00	29.64	28.83	3.88	3.02	2.86
RBLD (calibrated units)	35	30	30	47	46	42	41	37	37	40.65	37.48	37.09	2.93	2.52	2.42
LDW (calibrated units)	31	26	27	39	47	43	35	36	36	35.65	35.85	36.26	3.97	3.76	2.25
O (calibrated units)	31	29	29	38	49	39	34	35	35	33.95	35.34	35.35	2.35	2.52	1.72
VLL (calibrated units)	65	55	57	77	102	74	72	67	66	71.65	67.15	65.91	4.83	3.55	2.74

All chokka squid samples used in the DFA were classified as adults with maturity stages of 3 (preparatory), 4 (maturing) and 5 (mature), according to Lipinski's universal maturity scale for commercially-important squid (Lipinski 1979). The distribution and proportions of maturity stages in each region can be seen in Figures 10 and 11, and Table 4.

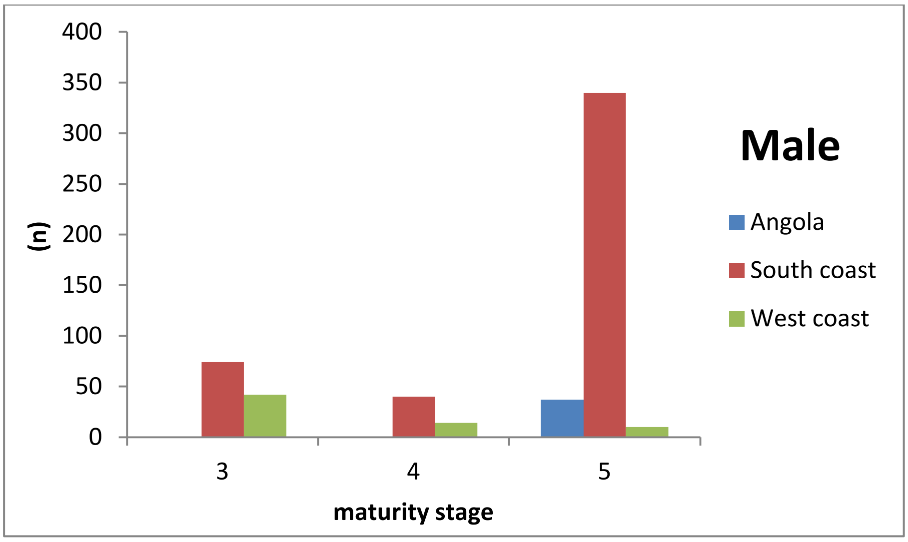


Figure 10. Male maturity scale distribution of chokka squid (*Loligo reynaudi*) by region (Angola, south coast, west coast). n = number of individual chokka squid.

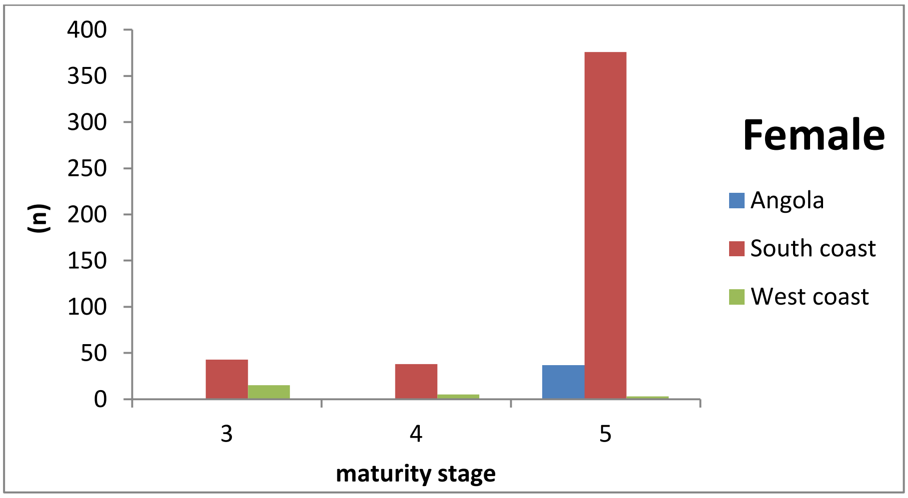


Figure 11. Female maturity scale distribution of chokka squid (*Loligo reynaudi*) by region (Angola, south coast, west coast). n = number of individual chokka squid.

Table 4. Proportion of chokka squid (*Loligo reynaudi*) individuals at different maturity stages across the three regions (Angola, west coast, south coast).

Angola	south coast	west coast	sex	maturity stage
0.00	0.64	0.36	male	3
0.00	0.74	0.26	male	4
0.10	0.88	0.03	male	5
0.00	0.74	0.26	female	3
0.00	0.88	0.12	female	4
0.09	0.90	0.01	female	5

2.3.2 Removal of the effect of size

Figures 12 - 15 compare the results of the regression that was used to remove the effect of size on the different morphometric measurements. Figure 12 indicates the plot results of the slope of the regression model for the different measurements on the soft parts as a function of DML. Similar results are presented for the lower beaks (Figure 13), sucker rings (Figure 14), and statoliths (Figure 15).

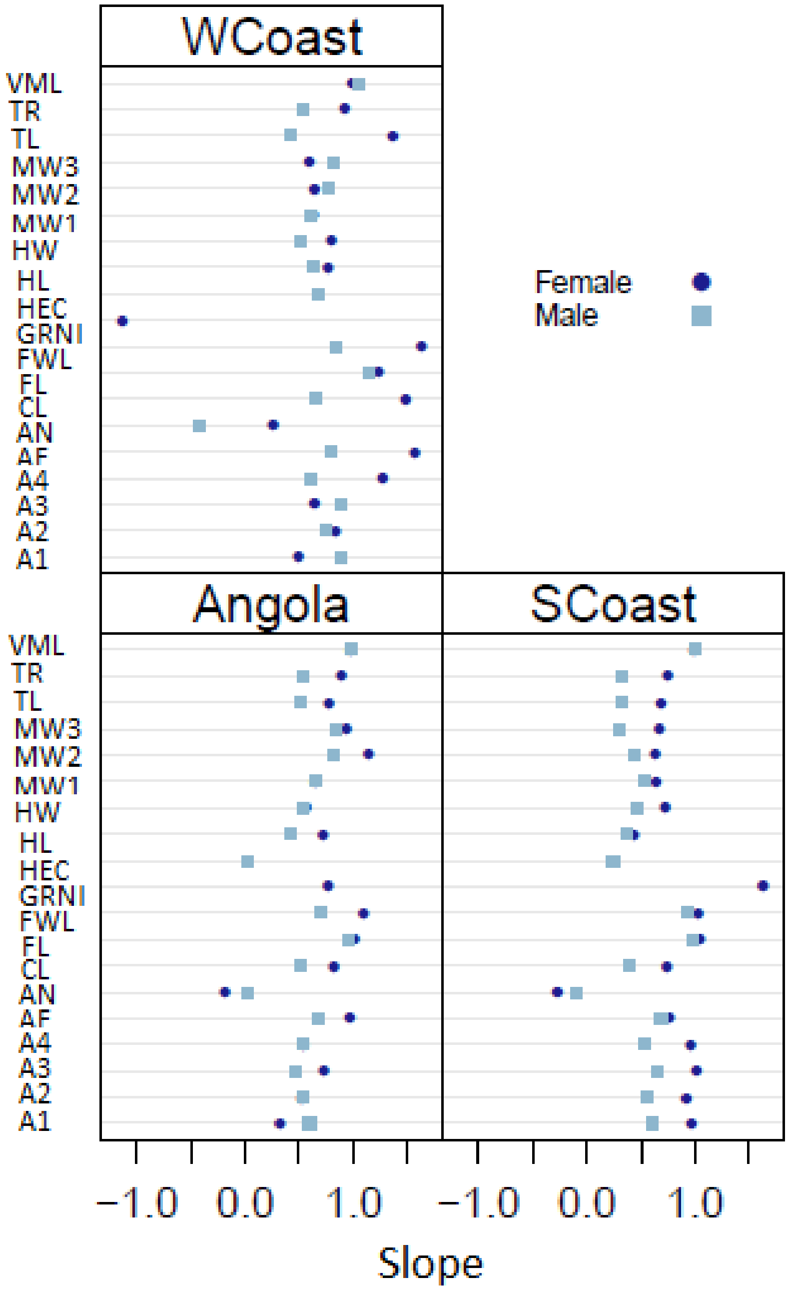


Figure 12. Dot plot distribution of the slope of the regression of chokka squid (*Loligo reynaudi*) soft part measurements vs. DML.

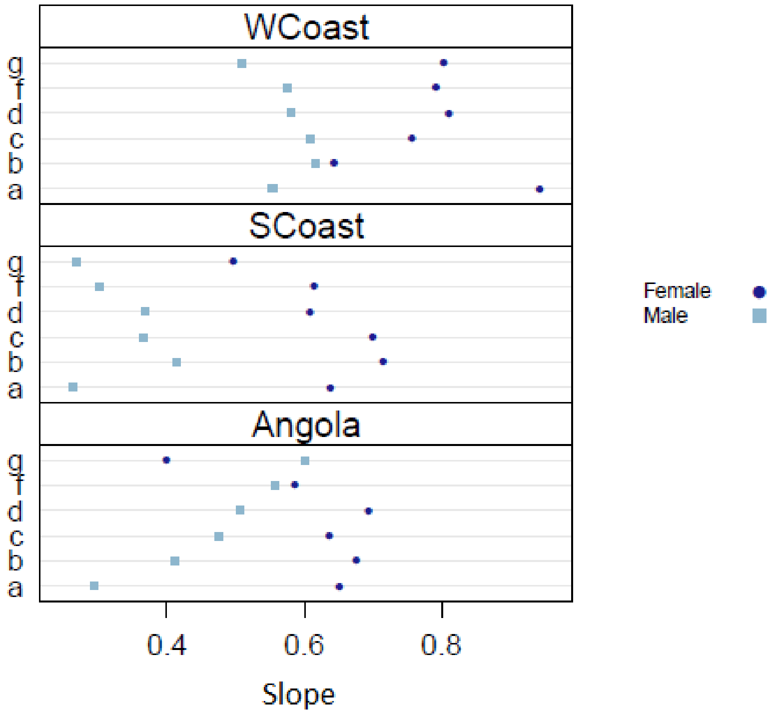


Figure 13. Dot plot distribution of the slope of the regression of chokka squid (*Loligo reynaudi*) lower beak measurements vs. DML.

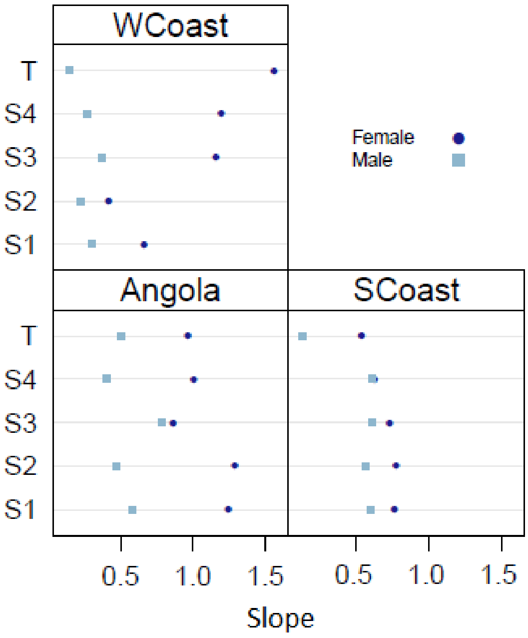


Figure 14. Dot plot distribution of the slope of the regression of chokka squid (*Loligo reynaudi*) sucker ring measurements vs. DML.

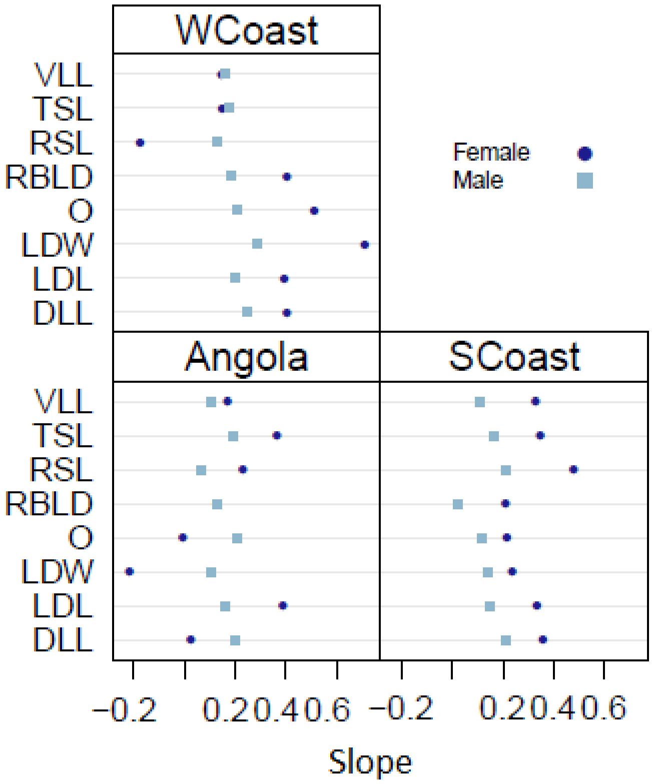


Figure 15. Dot plot distribution of the slope of the regression of chokka squid (*Loligo reynaudi*) statolith measurements vs. DML.

2.3.3. Discriminant Function Analysis

Plot results of the DFA conducted separately for male and female chokka squid from the three regions are indicated in Figure 16 - 19: using morphometric characters of the soft parts (Figure 16); beaks (Figure 17); sucker rings (Figure 18); and statoliths (Figure 19). Discriminant function analysis using soft parts only (20 characters) shows clear separation between male samples from the three different regions. In female samples there is only a slight overlap of soft parts between Angola and the west coast of South Africa. In contrast, although there are some slight differences between regions, no clear separation can be seen in the discriminant function analysis plot results of any of the male or female hard structures (lower beaks, sucker rings, and statoliths) as most labels occur in the centroid of the data clouds. The discrimination between the three regions, therefore, is only significant using soft parts (with an overall accuracy of more than 99%, Table 5).

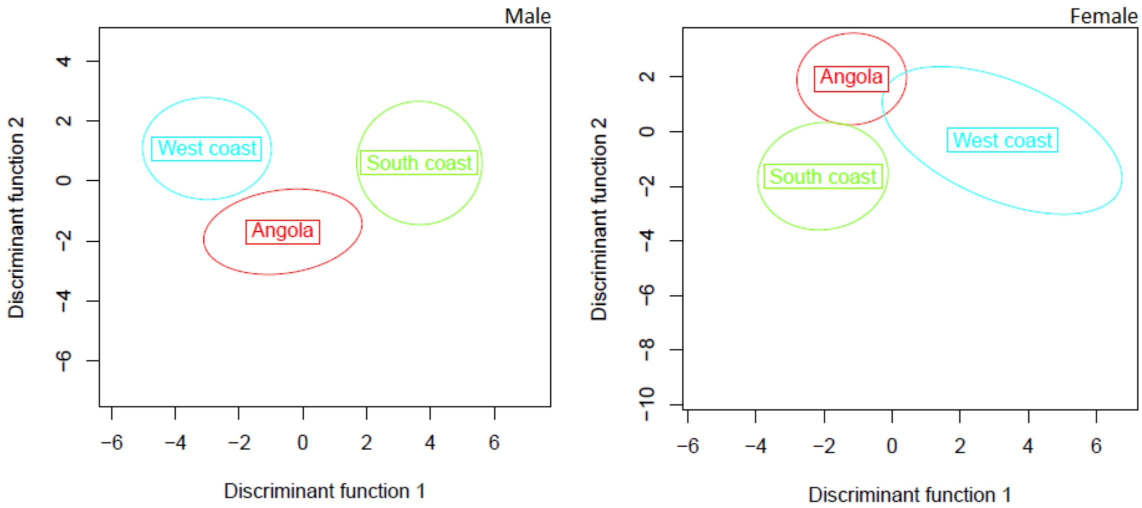


Figure 16. Plot results of the discriminant function analysis based on chokka squid (*Loligo reynaudi*) soft body part measurements for males (left) and females (right).

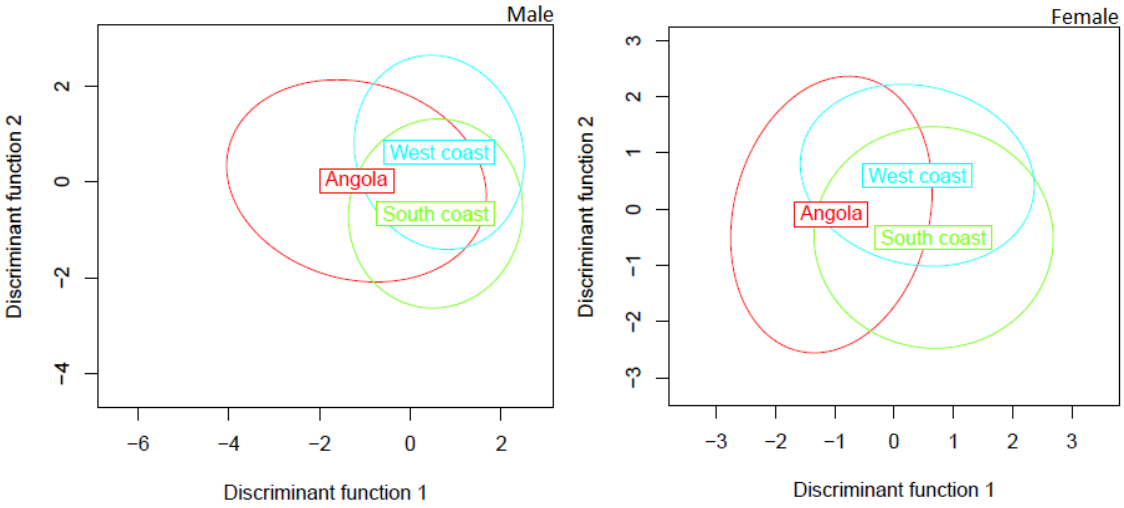


Figure 17. Plot results of the discriminant function analysis based on chokka squid (*Loligo reynaudi*) beak measurements for males (left) and females (right).

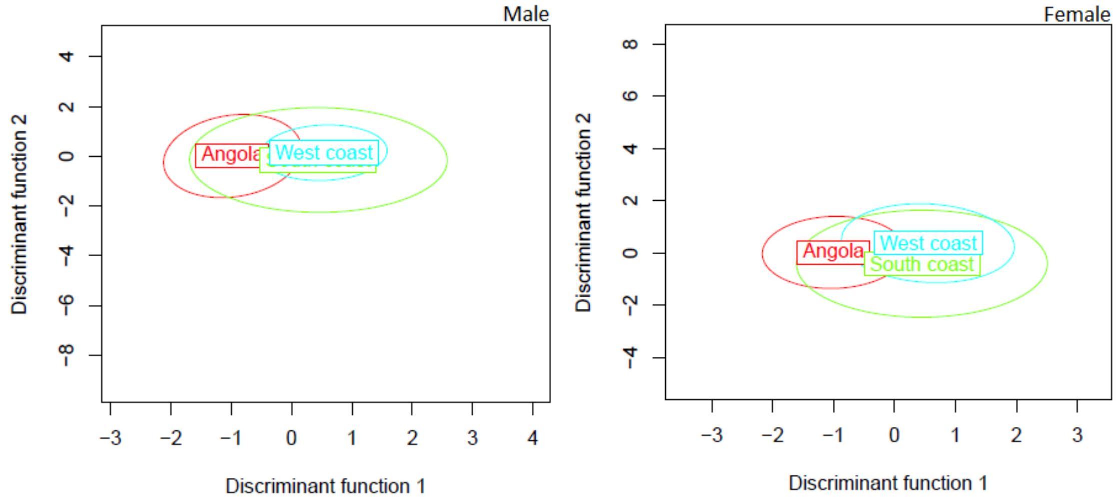


Figure 18. Plot results of the discriminant function analysis based on chokka squid (*Loligo reynaudi*) sucker ring measurements for males (left) and females (right).

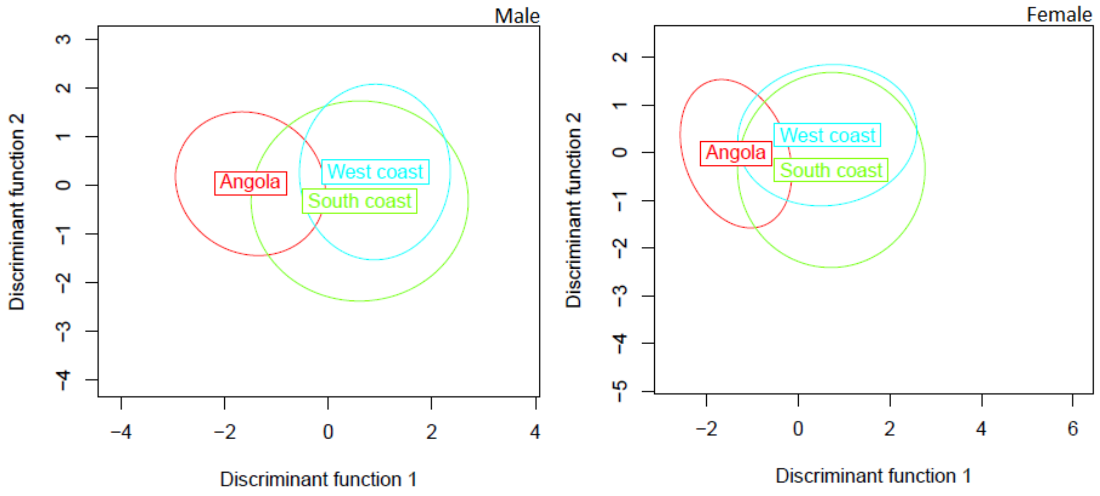


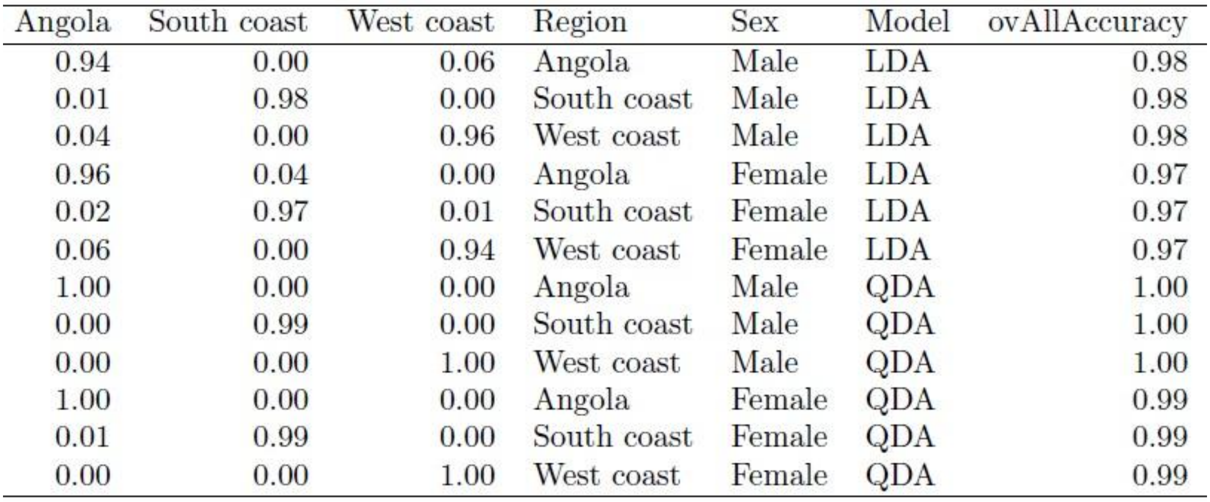
Figure 19. Plot results of the discriminant function analysis based on chokka squid (*Loligo reynaudi*) statolith measurements for males (left) and females (right).

In all soft and hard part DFA (except for statoliths, Table) for males and females the overall accuracy of the QDFA model was higher (Tables 5 - 7), thereby making this model more suited to the data. Soft part QDFA was the most accurate with an overall accuracy of 100% for males and 99% for females (Table 5). This can be considered as a very good discrimination. For both males and females the QDFA correctly discriminated between south coast (99%), west coast (100%) and Angola (100%) samples.

Although there was significant overlap among all hard structure measurements from the three different regions (Figure 17 - 19), with low overall QDFA accuracy for males and females (Table 6 - 8), the correct classification rates for hard structure measurements from some regions was fairly high. The hard structure with the highest overall QDFA accuracy was lower beaks, 70% (males) and 68% (females), with a correct classification rate of 69% (south coast), 86% (west coast), and 65% (Angola) for males, and 65% (south coast), 82% (west coast), and 89% (Angola) for females respectively (Table 6). However, the hard structure with the highest classification rate for the west coast and Angola regions alone were statoliths (Table 7), with 81% (west coast) and 95% (Angola) for males, and 70% (west coast) and 97% (Angola) for females respectively.

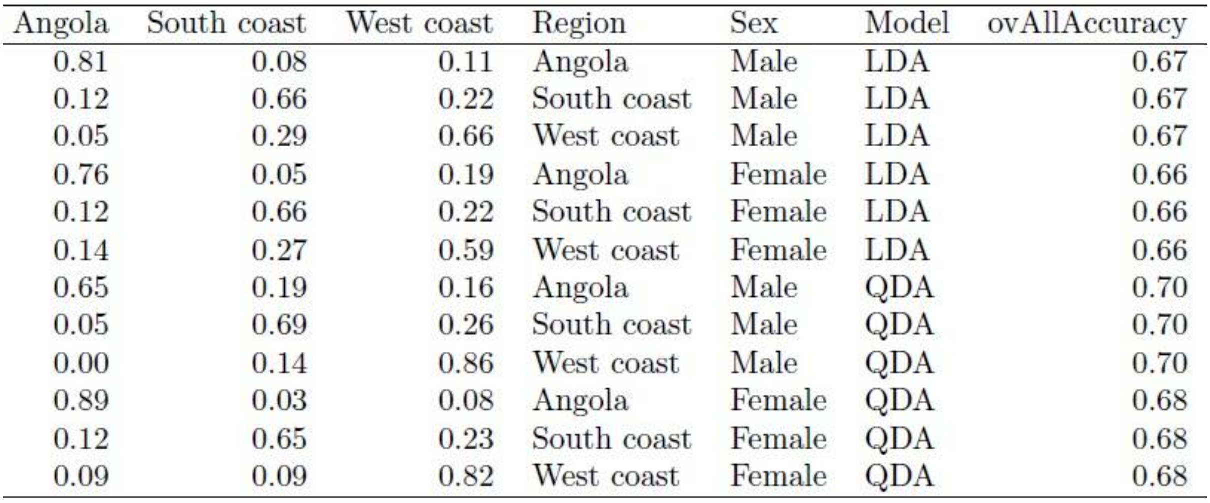
For all hard structure analyses (except for male beaks) the south coast repeatedly had the lowest correct classification rate, but was repeatedly more similar to the west coast than Angola. All hard structures (except for male beaks) from the Angolan samples were repeatedly slightly more similar to those from the west coast than the south coast. For the west coast all hard structures (except for female statoliths) were slightly more similar to the south coast than to Angola. Overall soft and hard structure analysis, the samples from Angola had the highest correct classification rate, making it the most distinct sample group by region. The most important soft part variables separating males and females from the three regions were FL and HW in males and DML, FL and AF in females (Table 9).

Table 5. Misclassification table of the results of the discriminant function analysis for chokka squid (*Loligo reynaudi*) soft parts.



Angola	South coast	West coast	Region	Sex	Model	ovAllAccuracy
0.94	0.00	0.06	Angola	Male	LDA	0.98
0.01	0.98	0.00	South coast	Male	LDA	0.98
0.04	0.00	0.96	West coast	Male	LDA	0.98
0.96	0.04	0.00	Angola	Female	LDA	0.97
0.02	0.97	0.01	South coast	Female	LDA	0.97
0.06	0.00	0.94	West coast	Female	LDA	0.97
1.00	0.00	0.00	Angola	Male	QDA	1.00
0.00	0.99	0.00	South coast	Male	QDA	1.00
0.00	0.00	1.00	West coast	Male	QDA	1.00
1.00	0.00	0.00	Angola	Female	QDA	0.99
0.01	0.99	0.00	South coast	Female	QDA	0.99
0.00	0.00	1.00	West coast	Female	QDA	0.99

Table 6. Misclassification table of the results of the discriminant function analysis for chokka squid (*Loligo reynaudi*) lower beaks.



Angola	South coast	West coast	Region	Sex	Model	ovAllAccuracy
0.81	0.08	0.11	Angola	Male	LDA	0.67
0.12	0.66	0.22	South coast	Male	LDA	0.67
0.05	0.29	0.66	West coast	Male	LDA	0.67
0.76	0.05	0.19	Angola	Female	LDA	0.66
0.12	0.66	0.22	South coast	Female	LDA	0.66
0.14	0.27	0.59	West coast	Female	LDA	0.66
0.65	0.19	0.16	Angola	Male	QDA	0.70
0.05	0.69	0.26	South coast	Male	QDA	0.70
0.00	0.14	0.86	West coast	Male	QDA	0.70
0.89	0.03	0.08	Angola	Female	QDA	0.68
0.12	0.65	0.23	South coast	Female	QDA	0.68
0.09	0.09	0.82	West coast	Female	QDA	0.68

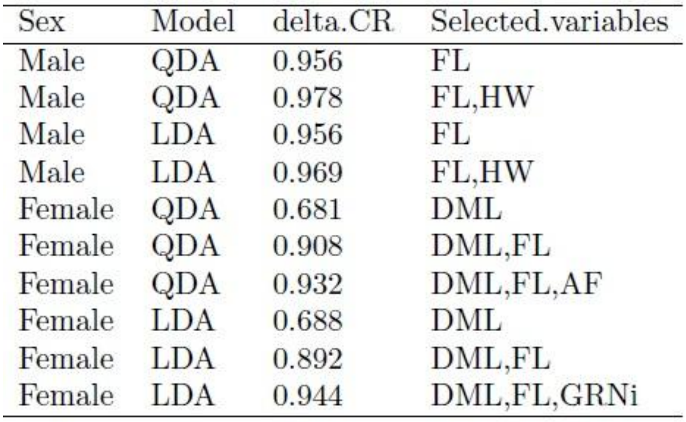
Table 7. Misclassification table of the results of the discriminant function analysis for chokka squid (*Loligo reynaudi*) sucker rings.

Angola	South coast	West coast	Region	Sex	Model	ovAllAccuracy
0.93	0.00	0.07	Angola	Male	LDA	0.45
0.24	0.41	0.35	South coast	Male	LDA	0.45
0.05	0.39	0.56	West coast	Male	LDA	0.45
0.90	0.07	0.03	Angola	Female	LDA	0.56
0.20	0.53	0.27	South coast	Female	LDA	0.56
0.14	0.27	0.59	West coast	Female	LDA	0.56
0.89	0.04	0.07	Angola	Male	QDA	0.51
0.17	0.44	0.38	South coast	Male	QDA	0.51
0.05	0.15	0.81	West coast	Male	QDA	0.51
0.90	0.00	0.10	Angola	Female	QDA	0.56
0.15	0.53	0.32	South coast	Female	QDA	0.56
0.09	0.09	0.82	West coast	Female	QDA	0.56

Table 8. Misclassification table of the results of the discriminant function analysis for chokka squid (*Loligo reynaudi*) statoliths.

Angola	South coast	West coast	Region	Sex	Model	ovAllAccuracy
0.89	0.05	0.05	Angola	Male	LDA	0.55
0.13	0.51	0.36	South coast	Male	LDA	0.55
0.06	0.31	0.63	West coast	Male	LDA	0.55
0.97	0.03	0.00	Angola	Female	LDA	0.58
0.13	0.56	0.31	South coast	Female	LDA	0.58
0.22	0.35	0.43	West coast	Female	LDA	0.58
0.95	0.03	0.03	Angola	Male	QDA	0.55
0.10	0.49	0.42	South coast	Male	QDA	0.55
0.00	0.19	0.81	West coast	Male	QDA	0.55
0.97	0.00	0.03	Angola	Female	QDA	0.58
0.08	0.55	0.37	South coast	Female	QDA	0.58
0.17	0.13	0.70	West coast	Female	QDA	0.58

Table 9. Selection of chokka squid (*Loligo reynaudi*) soft part variables based on stepwise-selection procedure.



Sex	Model	delta.CR	Selected.variables
Male	QDA	0.956	FL
Male	QDA	0.978	FL,HW
Male	LDA	0.956	FL
Male	LDA	0.969	FL,HW
Female	QDA	0.681	DML
Female	QDA	0.908	DML,FL
Female	QDA	0.932	DML,FL,AF
Female	LDA	0.688	DML
Female	LDA	0.892	DML,FL
Female	LDA	0.944	DML,FL,GRNi

2.4 Discussion

2.4.1 Difficulties of interpretation

The loss of tentacles and arm tips during capture, low chokka squid occurrence on the west coast and limited opportunity for sample collection in Angola allowed for a considerably reduced but still acceptable subsample size on the west coast (n = 87) and Angola (n = 76). This may have impacted negatively on the true representation of stocks sampled, affecting the range of measurement results. In both regions care was however taken to sample only maturing and mature squid to minimise these impacts. For both the south coast and Angolan samples, with an exception only to the west coast samples, majority of the samples were classified as mature individuals (Figure 10 and 11).

For comparative morphometric studies, Pierce *et al.* (1994a,b) recommended simultaneous sampling to minimize mixed-stock samples. In this case, samples for all three regions should ideally have been collected at least in the same season. However, due to the cost of sampling and large sampling area covered, it was not possible to collect all samples in the entire geographic range simultaneously or even during the same season. As squid are believed to be highly mobile (Boyle 1990), this may have had a temporal effect on the results of the morphometric analyses and should be kept in mind when interpreting results. Pierce *et al.* (1994a, b) also suggested that all measurements of morphometric characters be made by a single worker to avoid errors and this was accomplished during this study, with the author taking all the measurements.

Shea and Vecchione (2002) showed that the growth rates of morphometric characters varied with different stages of the life-history of Oegopsid squid. It is therefore important to sample squid at similar life-history stages. In this study all samples used were classified as adults with maturity stages ranging from 3 to 5 (Figure 10 and 11, as well as Table 4) according to Lipinski's universal maturity scale for commercially-important squid (Lipinski 1979). Results are therefore assumed to represent morphometric variation of adult squid at similar life stages, but not necessarily at similar age. Proving that samples were also more or less the same age would have made for a more convincing argument. Direct ageing using statoliths could unfortunately not be performed due to the time constraints of this study. Ageing samples should be of prime importance in future morphological studies focussing on the stock structure of this species.

2.4.2 Main findings

In contrast to the findings of Borges (1995), Vega *et al.* (2002), and Martinez *et al.* (2002), soft parts (FL, HW, DML and AF in particular) in this study proved to be more effective than hard structures (gladius, lower beaks, sucker rings, statoliths) in discriminating between chokka squid populations from different geographical regions. This is surprising as soft body parts are generally accepted as being less reliable than hard structures due to their plasticity and warping response to freezing and thawing (Carvalho and Nigmatullin 1998). Nevertheless, this is an important finding for which there may be various possible explanations.

Firstly, the geographic variation found may be related to the phenotypic response of squid derived from region-bound environmental conditions (Shea and Vecchione 2002). This is an evolutionary phenomenon that has been identified in other species of squid occurring in different habitats across large geographical areas (Carvalho and Pitcher 1989, Hernandez-Garcia and Castro 1998, Vega *et al.* 2002). As FL (fin length) in both males and females and AF (funnel length) in females were the most important variables associated with swimming ability among regions (Table 9), it is possible that phenotypic responses of these body parts may be related to the different environmental conditions in each region, possibly influencing swimming adaptations (Lipinski pers. comm).

Temperature regimes can have a significant influence on the growth and development of cuttlefish and squid, and growth at different temperatures can result in squid of markedly different size and growth-related parameters (Forsythe *et al.* 1994, Carvalho and Nigmatullin 1998, Forsythe *et al.* 2001). According to Portner and Zielinski (1998), oxygen availability can also limit performance levels in squid. Some squid may be able to operate at their functional and environmental limits, revealing a trade-off between oxygen availability, temperature, performance level, growth, and possibly body size (Portner and Zielinski 1998). This may explain why HW (head width) in males and DML (dorsal mantle length) in females act as some of the most important variables among squid from the three regions.

The marine environment around southern Africa is one of the most diverse, complex and highly variable in the world (Lutjeharms *et al.* 2001). In order to explain the possible effects of its region-bound environmental conditions on the geographic variation of chokka squid, it is necessary to highlight the main differences in each region.

Conditions on the south coast (central and eastern Agulhas Bank, 20° - 26°E) of South Africa are influenced by the warm south-westward flowing Agulhas current and associated with moderate water temperatures fluctuating between 9° and 24°C, and well oxygenated bottom waters (Augustyn *et al.* 1994). Conversely, conditions on the west coast (west coast and western Agulhas Bank, 29° - 35°S) are influenced by the cold equatorward flowing Benguela current and associated with much colder bottom water temperatures fluctuating between 5° and 11°C with an average of 10°C, and low bottom dissolved oxygen (BDO) of 1.5 – 4.5 ml/1 (Augustyn 1991, Roberts 2005). In southern Angola (15 - 17°S) conditions are influenced by two diverging currents, namely the warm subtropical Angola current from the north and the cold Benguela current from the south (FAO 2007). Coastal water temperatures in this region are generally much warmer than on the south coast of South Africa, fluctuating between 15.4 - 26.3°C with an average of 19.5°C (Winkler and Potts unpublished data). Therefore, given that water temperature and bottom dissolved oxygen considerably differ in each region, they may act as the main drivers of phenotypic variation found in squids from the three different regions. However, better defined and substantiated relations need to be further researched.

Another explanation involves genetic differences (Shaw *et al.* 2010, Stonier 2012) that may be caused by geographic or oceanographic barriers, or simply geographic distance. Globally, oceanographic features have been shown to shape the population structure of many marine species (e.g. Shaw *et al.* 2004, Bargelloni *et al.* 2005). In particular, the presence of the

Lüderitz strong upwelling cell is thought to be responsible for gene-flow disruption between adjacent reef fish populations, due to the interruption of the mechanisms of longshore movement from the southern to the northern Benguela ecosystems (Floeter *et al.* 2008). The Lüderitz upwelling cell could therefore also hinder genetic interchange between squid populations from southern Angola and the west coast of South Africa by preventing longshore movement of adult squid (Augustyn and Grant 1988). This is indeed possible, as water temperatures colder than the 8°C and BDO concentrations less than 3.5 ml 1^{-1} limiting adult squid distribution (Augustyn 1991) are almost permanent in the perennial Luderitz upwelling cell. However, the high dispersal ability of chokka squid adults, combined with disruption of the Luderitz upwelling zone during the Benguela *Niños* (Shannon *et al.* 1986, Hutchings *et al.* 2009), has the potential to allow occasional mixing of populations from southern Angola and the west coast of South Africa.

Subtle genetic differences recently found between samples from the western (Cape Town) and eastern (Mossel Bay, Tsitsikamma, Cape St. Francis, Port Elizabeth) Agulhas Bank (Shaw *et al.* 2010) were less pronounced than those between the western (Cape Town) Agulhas Bank and southern Angola (Stonier 2012), and therefore seem less responsible for the morphological differences found between the west coast (west coast and western Agulhas Bank) and south coast (central and eastern Agulhas Bank). Nevertheless, it is worth considering genetic differences as possible drivers of variation in the South African population.

The distribution of chokka squid paralarvae and adults in South Africa is highly influenced by the Agulhas and Benguela current systems (Roberts and van den Berg 2002, Roberts and van den Berg 2005, Roberts and Mullon 2010). Although it has been shown that paralarvae spawned on the eastern and central Agulhas Bank shelf can be transported either to the east or west, they are generally transported to the west and have the potential of being transported as far as the western Agulhas Bank (Roberts and Mullon 2010). Juvenile and adult squid occurring on the central Agulhas Bank certainly has the potential to move to the western Agulhas Bank (Olyott 2002), as there are no known permanent environmental barriers preventing movement between the two areas. They are also believed to undertake a westward feeding migration as far as the western Agulhas Bank and even up the west coast (via alongshore jet currents), as showed by demersal trawl survey data (Augustyn *et al.* 1994). It is believed that once matured, some of these squid undergo eastern inshore return spawning migration from at least the western Agulhas Bank back to the spawning grounds on the central Agulhas Bank and Eastern Cape (Olyott 2002).

With such a large degree of paralarval drift across the eastern, central and western Agulhas Bank, as well as adult migration from at least the western Agulhas Bank back to the central and perhaps eastern Agulhas Bank, it is presumed that mixing and interbreeding occurs to a large extent. However, recent genetic work by Shaw *et al.* (2010) and Stonier (2012) found slight differences between squid from the western and eastern Agulhas Bank. Their results suggest a partial environmental barrier hindering genetic exchange somewhere between the eastern (Plettenberg Bay) and western (Cape Town) Agulhas Bank.

The morphometric results of this study show that squid morphology on the western Agulhas Bank and west coast are different to that of squid found on the central and eastern Agulhas Bank, therefore complementing the genetic results of Shaw *et al.* (2010) and Stonier (2012). Together these results suggest a possible environmental barrier occurring at Cape Agulhas, which:

1) show major phenotypic differences between adjacent regions associated with different environmental conditions, and

2) the barrier may be responsible for slight interruption of genetic exchange causing slight genetic differences between adjacent regions.

Results from this morphometric study further complements and supports the genetic results of Stonier (2012) in that the phenotypic differences are also more accentuated between samples from the Angola-Benguela Frontal zone and the southern Benguela Current system, than between samples from the latter and the Agulhas Current system. It is therefore assumed that there is a much higher degree of mixing between individuals from the Agulhas Current and the southern Benguela Current than between the latter and southern Angola. Together, these results lead to the assumption that the Angolan population may be sufficiently isolated from the populations of South Africa as to hinder genetic flow, possibly due to the Lüderitz upwelling cell off the coast of southern Namibia acting as a partial environmental barrier to movement. Populations occurring on the west coast and western Agulhas Bank vs. those occurring on the central and eastern Agulhas Bank also seem to be phenotypically isolated from each other due to the different environmental conditions found on either side of Cape Agulhas, influencing the phenotypic response of chokka squid. Some slight genetic differences found between the same regions (Shaw *et al.* 2010) complements the suggestion that the confluence of the cold Benguela and warm Agulhas current at Cape Agulhas act as the approximate point of a phenotypic and possible genetic breakpoint. A graphical view of the two major environmental barriers suggested here can be seen in Figure 20.

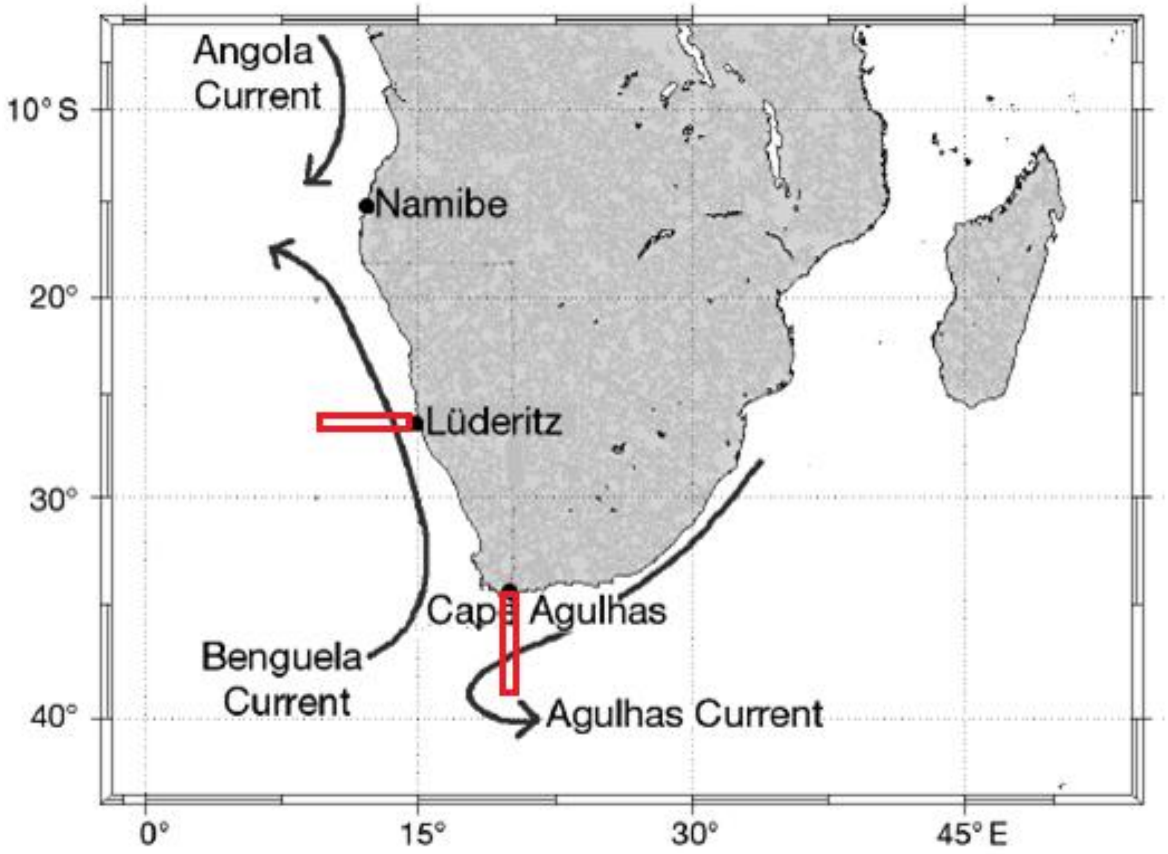


Figure 20. The two major potential barriers to chokka squid (*Loligo reynaudi*) movement, as suggested by the combined results of this study, Shaw *et al.* (2010) and Stonier (2012). Map taken and modified from Lett *et al.* (2007).

2.5 Conclusion

This chapter demonstrated morphometric variations between chokka squid populations from the south coast (central and eastern Agulhas Bank), the west coast (west coast and western Agulhas Bank), and southern Angola. The study presented results of phenotypic population sub-structuring of chokka squid, an important transboundary coastal fishery resource across the Angola, Benguela and Agulhas Currents of south-western Africa. DFA demonstrated that morphologically, based on soft part measurements at least, chokka squid from the south coast (central and eastern Agulhas Bank), west coast (west coast and western Agulhas Bank), and southern Angola are different, and may be separated into those three regions. Possible biogeographic reasons for variation such as different environmental conditions and varying degrees of genetic exchange were discussed. As water temperature and oxygen concentrations are the major factors that differ between each region, they may act as drivers of variation in the phenotypic response of chokka squid. It is possible that a phenotypic response to different environmental conditions best explains the most significant differences found in FL, HW, DML and AF. Even though this may imply plastic growth more than genetic differentiation, it still indicates that squid from the three regions belong to different stocks, as they may have different life histories and growth patterns.

The degree and reliability of these factors should be further investigated in order to achieve a better understanding of the stock structure of chokka squid. These results should be accompanied by an in-depth genetic analysis of samples from similar locations in the three regions sampled here, and indeed such a study is underway.

It is important to note that data on the stock structure of chokka squid are only useful in the context of management of the resource if fully incorporated into stock assessment models. This may ensure that harvesting proceeds at sustainable levels while aiming to maintain the species' genetic diversity and evolutionary potential (Carvalho and Hauser 1994). Given that a phenotypic boundary is indicated in the vicinity of 20°E (according to results presented here) it may be sensible to set a boundary to stock separation here and separate all data east and west along this line. However, the availability of sufficient data to the west of this line may be problematic for stock assessment purposes.

CHAPTER 3

INVESTIGATING THE FEASABILITY OF AN AREA-DISAGGREGATED ASSESSMENT FOR STOCK ASSESSMENT MODELLING AND MANAGEMENT OF THE CHOKKA SQUID (*LOLIGO REYNAUDI*) RESOURCE

3.1 Introduction

The challenges associated with managing fisheries that exploit mixed stocks or those with complex structures require the incorporation of spatial and temporal patterns in sampling and stock assessment modelling (Cadrin *et al.* 2007). Investigations into population structure have been at the forefront of fisheries research for decades and although recent applications of interdisciplinary stock identification techniques reveal complex spatial stock structure in many marine species, population structure is generally ignored in stock assessment models (Cadrin and Secor 2009, Goethel *et al.* 2011). As the complexity of marine population structure has been uncovered, models have attempted to accurately portray the complexity through the development of spatially explicit assessments that allow for movement between subpopulations (Goethel *et al.* 2011). As spatial management measures become more widely used, it is increasingly important that assessment models include the spatial complexities of population structure and patterns of fishery removals, in order for more reliable monitoring of population rebuilding to take place (Goethel *et al.* 2011).

Many seemingly isolated populations may receive new recruits from other distant reproducing populations through migration or paralarval drift (King 2007). This complicates the CPUE statistics of the commonly applied Leslie-DeLury forecast model (Beddington *et al.* 1990, Rosenberg *et al.* 1990, Basson *et al.* 1996) as it assumes no significant immigration or emigration and an insignificant level of natural mortality (Carvalho and Nigmatullin 1998), making it difficult to distinguish between a reduction in CPUE due to harvesting and that associated with the emigration of several schools (Carvalho and Nigmatullin 1998).

In cephalopod stock assessment models the incorporation of separate stocks is often neglected and many squid fisheries are modelled on an area-aggregated basis (Begg *et al.* 1999). A possible reason may be that stock boundaries are often difficult to determine and are therefore commonly based on jurisdictional and management convenience instead of biological inference (Goethel *et al.* 2011).

3.2 Chokka squid (*Loligo reynaudi*) stock assessment

3.2.1 History

Considerable attention has been given to the stock assessment approach of chokka squid (*Loligo reynaudi*) over the past two decades (see Augustyn *et al.* 1992, 1993, Augustyn and Roel 1998, Roel *et al.* 1998, Roel and Butterworth 2000, Roel *et al.* 2000, Glazer and Butterworth 2005, 2006, 2009, 2010, 2012a, 2012b, Gaylard and Bergh 2006, Lipinski and Soule 2007). Understanding the biology and life cycle of chokka squid combined with various stock-assessment techniques has shaped the management of the species as we know it today (Augustyn & Roel 1998), and the 2012 review panel for the International Fisheries

Stock Assessment Workshop (Smith *et al.* 2012) has recognised the very high quality of research on the resource.

3.2.2 Current management approach and stock status

The current management objective for the chokka squid directed jigging fishery is to cap effort at a level that secures the greatest catches in the longer term, without exposing the resource to the threat of reduction to levels at which recruitment success might be impaired or catch rates drop below economically viable levels (Status of the South African Marine Fishery Resource 2012, DAFF). Effort in the jig fishery has therefore been conservative with the Total Allowable Effort (TAE) (136 vessels and 2422 crew) being maintained at a constant level over the past four years (Glazer and Butterworth 2012a). Although there are concerns related to latent effort in the fishery, the current level of effort appears sustainable (Glazer pers comm.). The chokka squid resource has been categorized as optimally exploited at 300 000 man-days and the latest stock assessment by Glazer and Butterworth (2012a) suggests that there is little room for effort increases.

3.2.3 The Assessment Model

The model specifications and results for the latest assessment of chokka squid are available in Glazer and Butterworth (2012a). It appears that the model is quite sensitive to changes in data inputs and assumptions made. The target effort limit of 300 000 man-days is exceeded in certain years, and there is a need to translate the target effort level into more practical management quantities (currently number of vessels and crew).

A Bayesian analysis to take full account of model parameter uncertainty was first undertaken in 2005 to assess the status of the chokka squid resource (Glazer and Butterworth 2010). At that time, the input data were not informative enough to allow for the precise estimation of all parameters and as a result, the stock recruitment steepness parameter *h* was fixed (Glazer and Butterworth 2010). Twelve models were considered, each for a different value of *h* (ranging from 0.4 to 0.95 in units of 0.05). Results were then integrated over the models using Deviance Information Criterion (DIC) weighting (Glazer and Butterworth 2010).

For the purpose of in-season advice regarding the additional closed season for 2010, an updated analysis was conducted for one of the twelve models, namely that which assumed *h*=0.7 (Glazer and Butterworth 2010). This model incorporated updated data where certain series e.g. jig catches, had been revised based on more reliable data sources (Glazer and Butterworth 2010). A further advancement was to allow for the estimation of *h* given that 6 more years of data were then available and included in the assessment (Glazer and Butterworth 2010). The adoption of this model removed the need to integrate results over twelve separate models (Glazer and Butterworth 2010). The latest Bayesian assessment of the squid resource was performed in 2012 as data were available for more years (the previous analysis included data to 2008; the updated analysis included data to 2011) (Glazer and Butterworth 2012a).

Due to the previously assumed homogenous stock structure of chokka squid, the existing assessment model is area-aggregated. The population model splits a year into two time periods (January-March and April-December) to better reflect the dynamics of the stock and the two fisheries (jig and trawl) that exploit it (Glazer and Butterworth 2010). Hardly any recruitment takes place in the January – March period and jig and trawl catches are disproportionately divided between this and the April – December period (Roel and Butterworth 2000). The biomass time series is estimated by projecting the assumed pristine biomass at the start of the period (=$B^*_{1971} = K$) forward given the historic annual catches (Glazer and Butterworth 2010). Both process and observation error are taken into account. The likelihood of the data is calculated by assuming the abundance indices to be log-normally distributed about their expected values. A Beverton-Holt stock recruitment relationship is assumed and prior distributions are selected for the estimable parameters. An assumption that disturbance of spawning by jig fishing has a negative effect on recruitment is required to fit the available indices of abundance, particularly that of the decline in the trawl CPUE index at the time that the jig fishery started (Roel and Butterworth, 2000). Currently there is a study underway to prove that the disturbance is indeed important (Lipinski pers. comm.)

Recently, the chokka squid stock assessment model was critically evaluated by a panel of international experts who made numerous recommendations with respect to improving both the model itself and the associated input data. Details of these recommendations can be found in Smith *et al.* (2012). As per the recommendations made, the assessment model is currently being updated with the intention to move away from utilizing discrete Pope catch equations to one that incorporates the differential Baranov catch equations.

3.3 Motivation and rationale

Although the chokka squid jig fishery mainly operates on the main spawning grounds off the southeast coast of South Africa, trawl and jig catches are also being made further west off Cape Agulhas (20°E). This has caused some concern over the possible consequences of future increased effort in this area, especially in the light of new morphometric (Chapter 2, this study) and recent genetic (Stonier 2012) evidence suggesting possible stock separation. In addition, there are some suggestions that the biomass in these less-fished areas act as a buffer against fishing in the more productive areas (Gaylard and Bergh 2006). Thus, from a population modelling point of view there is a broader population to consider.

Gaylard and Bergh (2006) suggested that evidence of different catch and CPUE trends observed in the two fisheries (jig and trawl) exploiting the chokka squid resource, and the fact that each dataset has a specific spatial bias, may make it sensible to introduce spatial disaggregation into the assessment and management of the resource. They indicated that this would facilitate the use of all available data and would also better reflect the dynamics of the chokka squid resource.

Due to the existing chokka squid assessment model currently being updated, it is not feasible to apply this model to the area-disaggregated data produced by this study. Rather, the CPUE trends and catches from the area-disaggregated data will be compared against those of the most recent area-aggregated assessment performed by Glazer and Butterworth (2012a). It must be noted that the intention of this chapter was not to use advanced statistics to compare aggregated vs. disaggregated data but rather a first broad look at the overall trends observed, to suggest future options for data collection and management.

3.4 Materials and methods

3.4.1 Exploration of the data

The following data, included in the assessment of Glazer and Butterworth (2012a), were made available for developing the area-disaggregated catches and CPUE indices.

- Commercial trawl catch data from inshore and offshore demersal fisheries: 1971 – 2011 (Figure 21).
- Commercial jig catch data from the chokka squid directed jigging fishery: 1985 – 2011 (Figure 21).
- Commercial trawl CPUE data: 1978 – 1999 (Figure 22).
- Commercial jig CPUE data: 1995 – 2011 (Figure 23).
- Survey biomass indices collected by the RV Africana in its biannual demersal surveys: 1986 - 2010 (Figure 24 and 25).

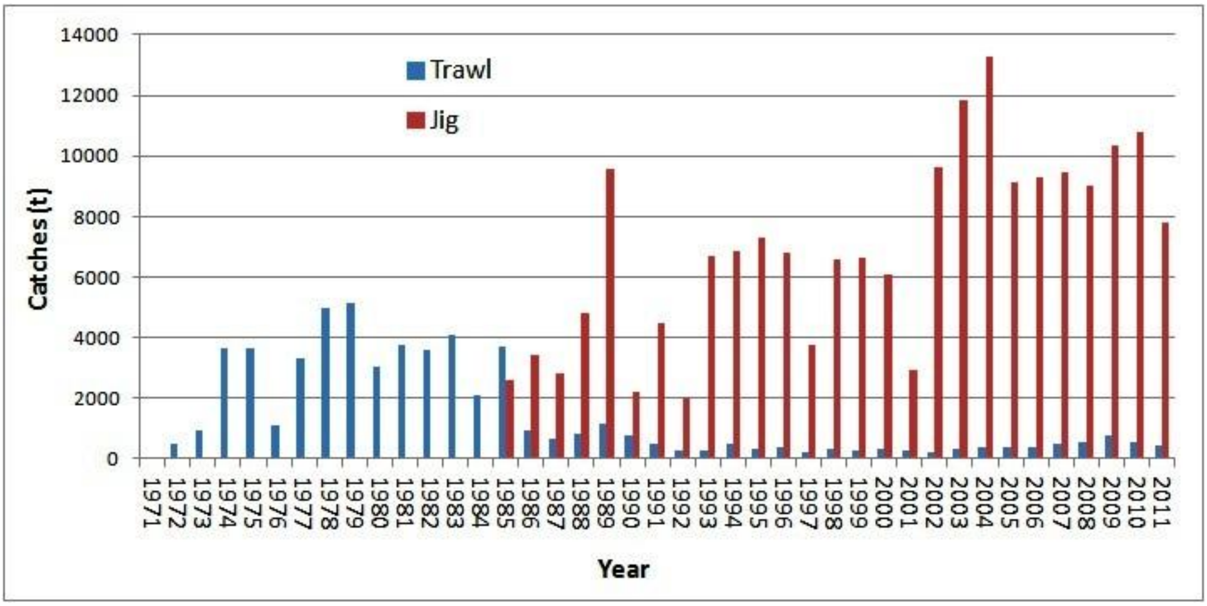


Figure 21. Annual commercial trawl (blue) and jig (red) catches of chokka squid (*Loligo reynaudi*) from 1971 – 2011 (Glazer and Butterworth 2012a).

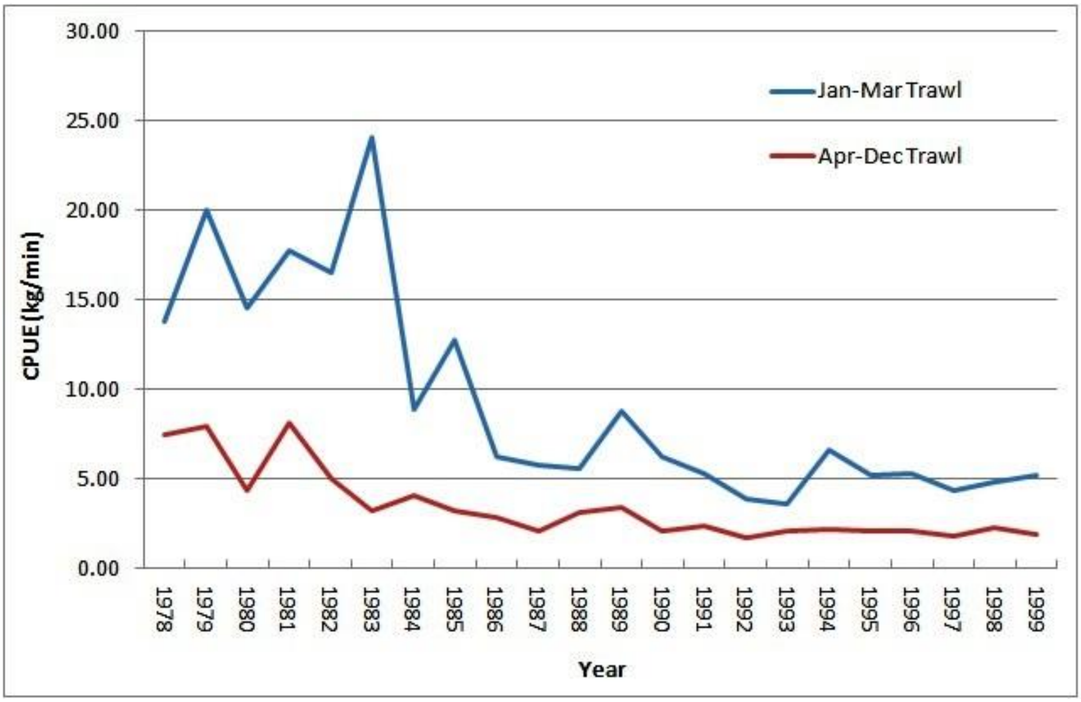


Figure 22. Trawl CPUE (kg/min) of chokka squid (*Loligo reynaudi*) for the periods January to March and April to December from 1978 – 1999 (Glazer and Butterworth 2012a).

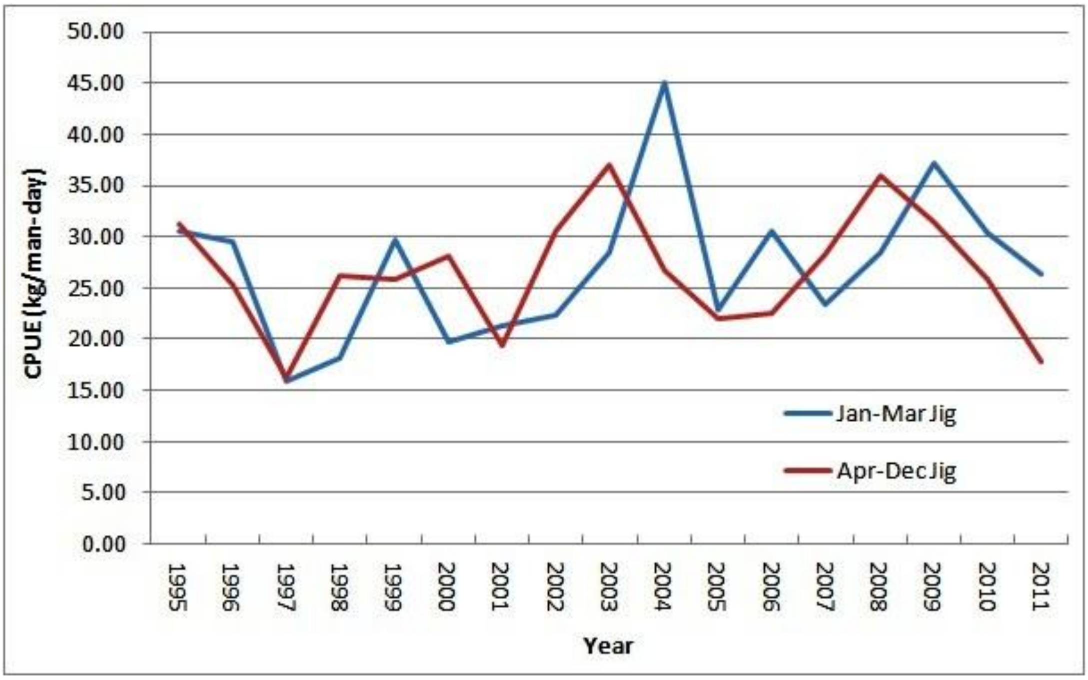


Figure 23. Nominal jig CPUE (kg/man-day, restricted to data from the core 19 vessels and to 3 ≤crew≤20) of chokka squid (*Loligo reynaudi*) for the periods January to March and April to December from 1995 – 2011 (Glazer and Butterworth 2012a).

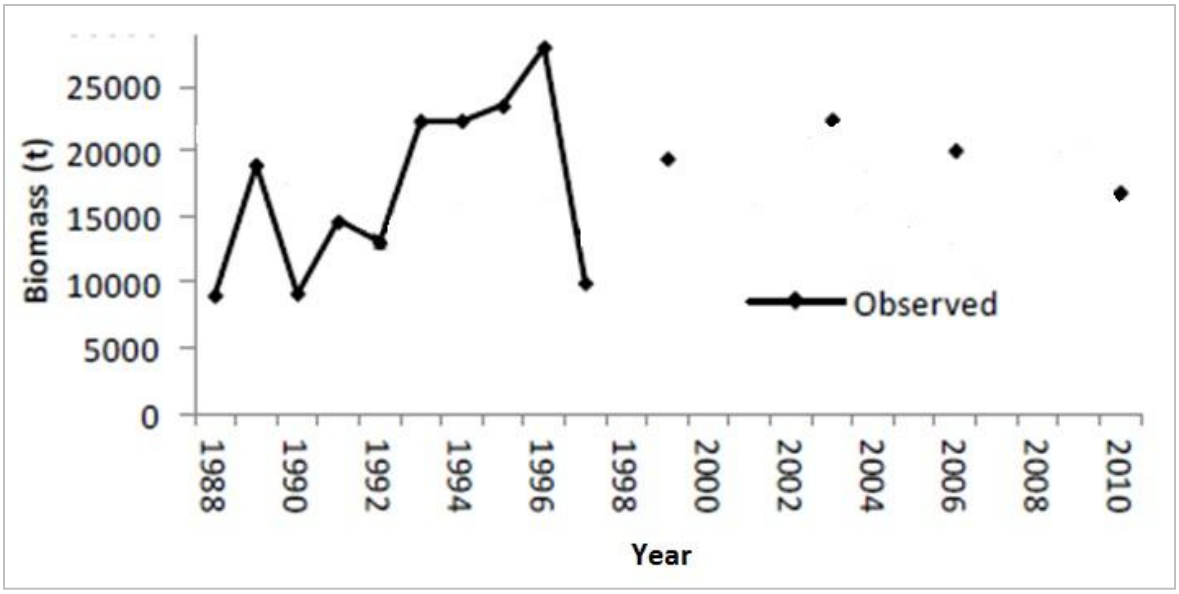


Figure 24. Autumn survey biomass indices (tons) of chokka squid (*Loligo reynaudi*) for the latest assessment (taken from Glazer and Butterworth 2012a).

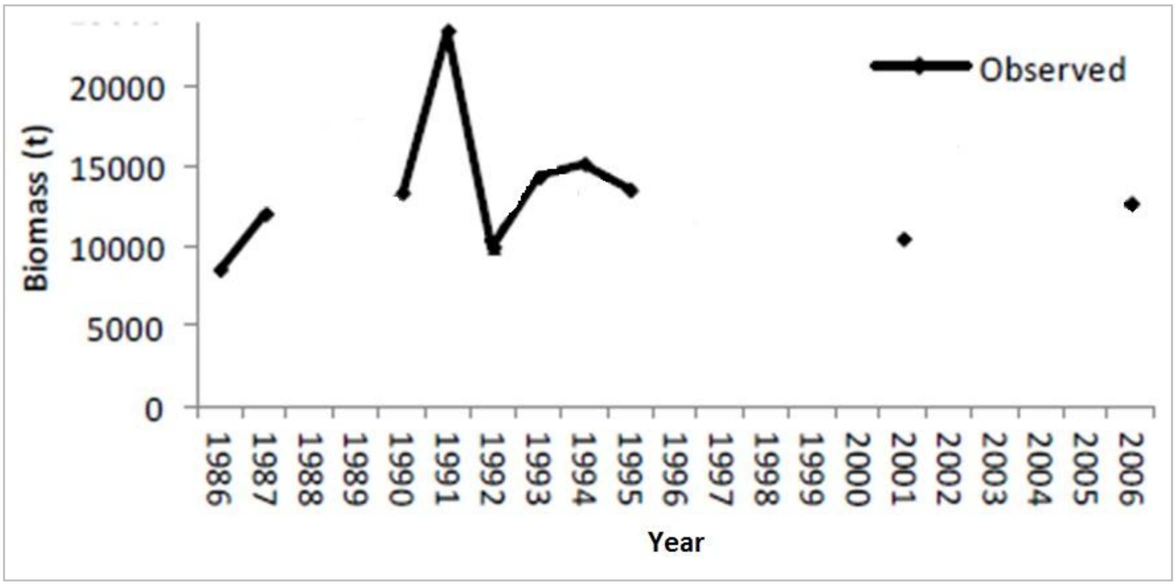


Figure 25. Spring survey biomass indices (tons) of chokka squid (*Loligo reynaudi*) for the latest assessment (taken from Glazer and Butterworth 2012a).

3.4.2 Description of data sources

Data on jig, trawl and survey catches were extracted from the Department of Agriculture, Forestry and Fisheries (DAFF) database. Data fields used in the comparisons are presented in Table 10.

Table 10. Data fields extracted from DAFF chokka squid (*Loligo reynaudi*) trawl and jig databases:

Jig	Trawl	Survey
trip ID	drag date	drag date
catch date	effort	grid number
effort	grid number	chokka catch weight
block number	depth	depth
depth	mesh size	gear type
vessel number	target species	wing spread
crew	chokka catch weight	area swept
catch weight	CPUE (kg/hour)	density

The following datasets were extracted:

Trawl catch (1978 – 2011) and CPUE (1978 – 1999) data (source: DAFF demersal database)

Trawl catch and effort information is recorded in skipper log books in the inshore and offshore trawl fisheries. Spatial information for the trawl records is given at the level of grid cells with dimension 20 minutes of latitude by 20 minutes of longitude, as well as depth. Although these fisheries are generally directed at species other than squid (hake, horse mackerel, sole etc.), chokka squid is caught as a by-product and their catches are recorded along with the targeted and other by-catch species. The total squid catch represented by this dataset is roughly 7% of the total annual chokka squid catch, therefore this dataset is a valuable additional source of information about trends in the population (Gaylard and Bergh 2006).

Roel (1998) applied Generalised Linear Modelling (GLM) analyses to the trawl CPUE data to obtain standardized indices of abundance for the two periods (January – March and April - December) into which the data were separated for assessment purposes. The aim of the CPUE analyses was to determine reliable historic trends in the resource abundance of chokka squid in the period covered by the data. The basic assumption in fisheries science is that CPUE is directly proportional to fish density and that fish density is proportional to resource abundance (Gaylard and Bergh 2006). Because CPUE is influenced by factors other than abundance, these other factors need to be addressed, where possible, in producing an unbiased and fair comparison of year to year changes in CPUE (Gaylard and Bergh 2006). Hence the need for the standardisation of CPUE trends by means of GLM analyses or similar techniques.

Although trawl CPUE data are available post-2000, it was not possible to include them in the analyses since they cannot be treated in the same manner as the pre-2000 data, and a complete re-analysis of the trawl CPUE data is required. The data supplied by DAFF exclude all records north of Cape Columbine on the West Coast and all records at depths greater than 300m, the rationale being that chokka squid are unlikely to be found in records of catches further north or at greater depths.

Jig catch (1985 – 2011, NRSC) and CPUE (1995 – 2011, DAFF jig catch and effort database)

Annual commercial jig catches are made available by the SABS/NRCS and cover the period 1985 to 2011.

In the past, the chokka squid jig data were recorded along with catches of linefish and stored in the National Marine Linefish System (NMLS). In 2006, a new log book was introduced specifically for the jig fishery, which allowed for the recording of more detailed catch and effort information using grid block numbers unique to the fishery. These data are now stored in a dedicated database. This new reporting system has indicated that the previous data may not be as reliable as what had originally been assumed. Efforts continue to improve the quality of data used for assessment of the resource and to develop reliable indices for input to future assessment models. Methods to achieve this include skipper education, comparison of catch and effort data with factory landing data, improved skipper catch return book collection and data-checking systems.

The nominal jig CPUE data (1995 - 2011) included as indices of abundance in the assessment model are restricted to a core set of 19 vessels which historically showed the most reliable catch returns. These data are further constrained to records where $3 \leq crew \leq 20$. Roel (1998) justified this constraint by indicating that vessels carrying more than 20 crew members have some members involved in packing and processing so that the proportional relationship between CPUE and the number of crew members on board breaks down.

Autumn and spring survey biomass index (source: DAFF demersal database)

The survey data comprise trawl-by-trawl records from surveys conducted with the RV Africana on the South Coast from September 1986 to April 2010. Ten of these surveys were conducted in spring (September/October) and fourteen in autumn (April/May).

The autumn and spring biomass indices are obtained from those surveys that utilized the old trawl gear (a new trawl gear was introduced in June 2003, but a calibration factor between gears has yet to be determined for squid). The autumn index indicates an increasing trend in biomass, while the spring index is relatively stable; both being in contrast to the trawl CPUE indices, which show a decline in abundance (Figure 22, 24 and 25).

3.4.3. Determining a stock separation boundary

Morphometric results from Chapter 2 indicated that two separate phenotypic stocks of chokka squid were present in South Africa, with a boundary to separation at Cape Agulhas (20°E) (Figure 20). However, very little data are available to the west of 20°E as biomass on the west coast is considerably less than on the south coast (Augustyn 1991). Commercial trawl and survey data shows few catches being made in the area west of 20°E and at irregular levels (Figures 29 and 30) (Augustyn 1991). Jig catches take place almost entirely to the east of 20°E with almost no catches taken to the west (Figure 28).

Van der Westhuizen (2013) separated the catch area of the chokka squid jig fishery by half degree blocks (B, C, D, E, F, and G) (Figure 26) and plotted the spatial and temporal distribution of the catch by area and year (Figure 27). The Areas A and H extend to the western and eastern boundaries respectively of the main jig catch area. Squid from area A are mainly caught off the Cape Agulhas and those from area H in the vicinity of Port Alfred (van der Westhuizen 2013). Figure 27 shows majority of the jig catches being taken between Plettenberg Bay and Port Elizabeth with no catches being made wets of 20°E.

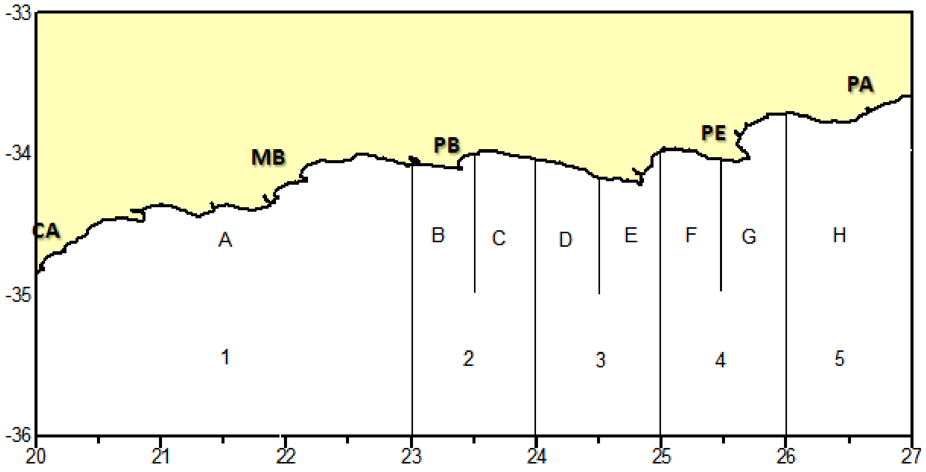


Figure 26. Map illustrating catch areas for spatial and temporal representation of the chokka squid (*Loligo reynaudi*) commercial jig catches (taken from van der Westhuizen 2013). CA (Cape Town); MB (Mossel Bay); PB (Plettenberg Bay); PE (Port Elizabeth); PA (Port Alfred).

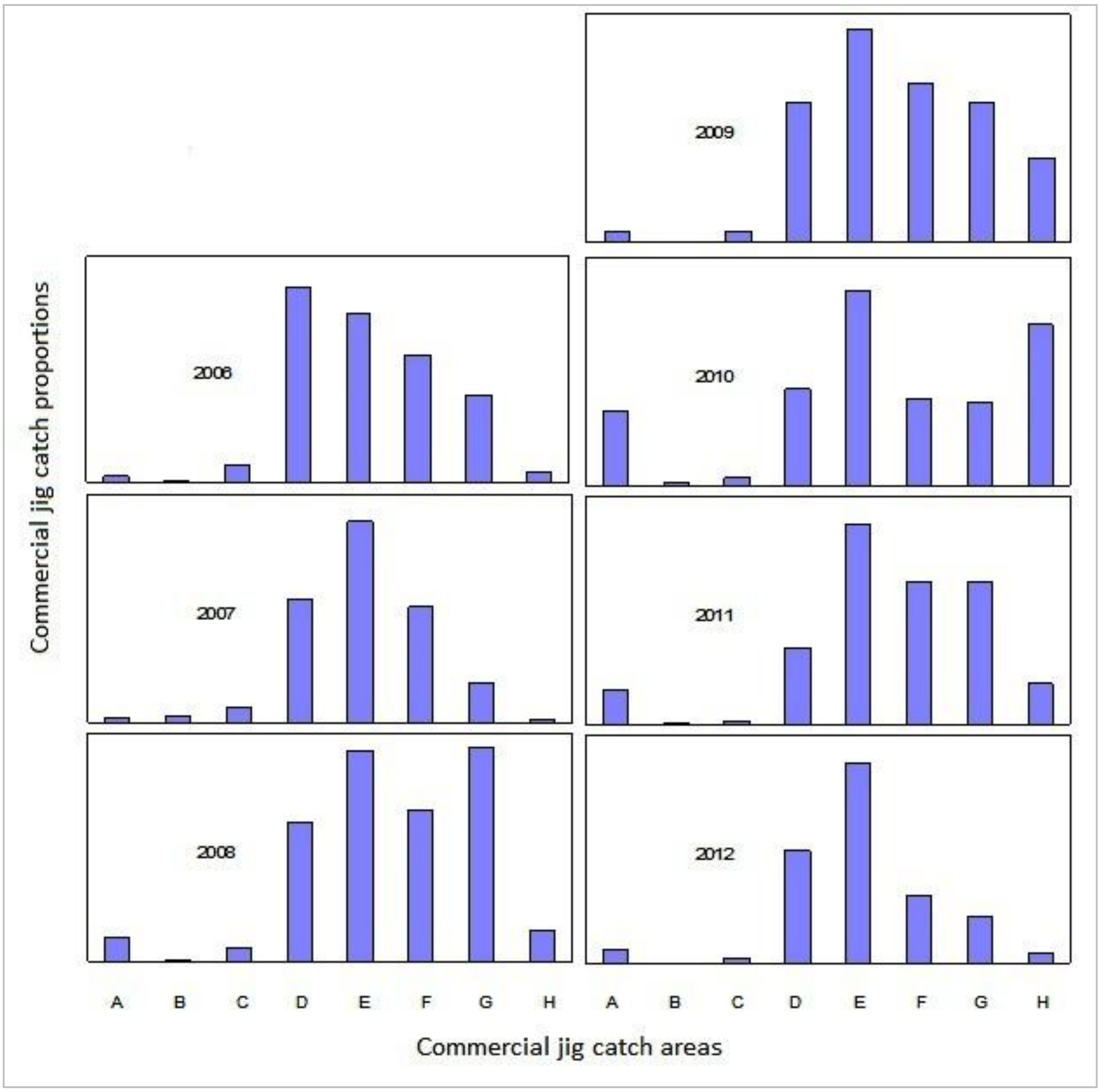


Figure 27. Comparison of the chokka squid (*L. reynaudi*) commercial jig catch proportions by area (see Figure 6) over the period 2006 - 2012 (taken from van der Westhuizen 2013).

If indeed a stock separation boundary were to be incorporated at 20°E (as results of Chapter 2 indicates), then there would be insufficient data in the resultant disaggregated area west of 20°E, making the comparison of trends from the disaggregated areas west and east of 20°E impossible.

Another approach may be to search for a possible alternative boundary by combining the genetic results of Shaw *et al.* 2010 and Stonier 2012. Due to the uncertainty and absence of genetic information between Tsitsikamma and Cape Town (Shaw *et al.* 2010, Stonier 2012), the assumption that a genetic boundary also occurred at Cape Agulhas could not be used as a basis for separating stocks at that position (20°E), especially as it had been shown that genetic and phenotypic response to environmental conditions could be non-concordant (example Kim 1993, Brierly 1993a, 1993b, Kang *et al.* 1996, Reichow and Smith 2001).

Therefore, combining the morphometric results of this study (Chapter 2) with the available genetic results of Stonier (2012), as well as available jig and trawl catch data for the areas under consideration, a compromise was made for illustrative purposes of an alternative stock assessment strategy. An alternative boundary to stock separation was chosen further east near Mossel Bay at 22°E (Figure 28). A dividing line at this position separated the current jig fishery into its two main areas of operation:

a) the main area on the spawning grounds of the south-east coast where over 90% of the annual jig catch was taken, and
b) a much smaller area off Cape Agulhas on the central Agulhas Bank (mainly between Cape Agulhas and Struisbaai) where less than 10% of the total annual jig catch was taken (Figure 28).

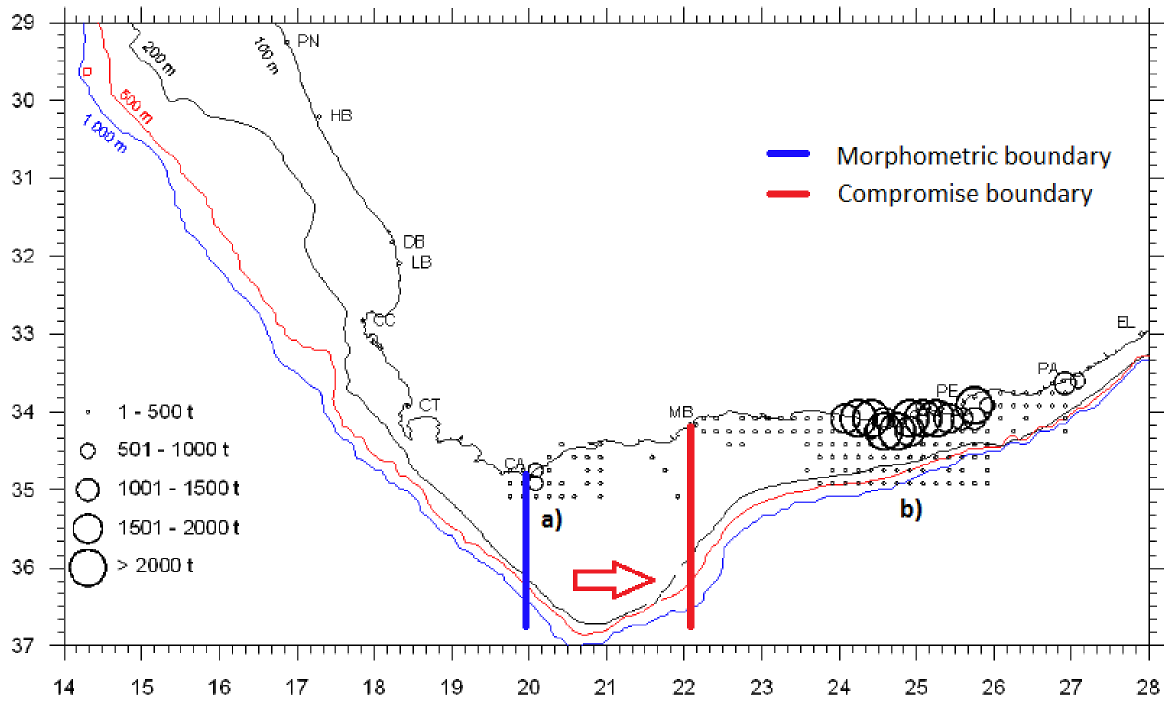


Figure 28. Map illustrating the distribution of recent (2006 – 2012) chokka squid (*Loligo reynaudi*) commercial jig catches split by the morphometric (blue line at Cape Agulhas, 20°E) and compromise boundary (red line at Mossel Bay, 22°E) to stock separation chosen for this study (adapted from Durholtz and van der Westhuizen 2012).

Placement of the compromise boundary line in relation to where the commercial trawl fishery and demersal research surveys operate can be seen in Figures 29 and 30 respectively. Placement of the boundary at 22°E ensures sufficient data availability for both areas west and east of the boundary.

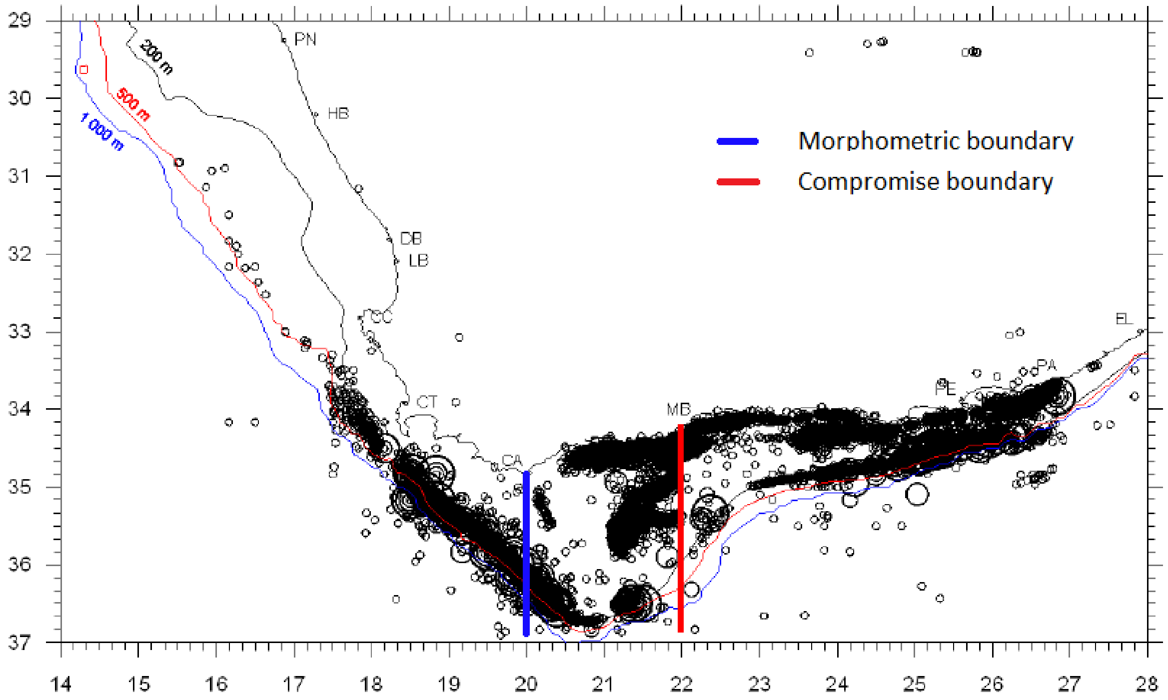


Figure 29. Map illustrating the distribution of commercial trawl catches containing chokka squid (*Loligo reynaudi*) over the period 2007 – 2011. The red line at 22°E (Mossel Bay) marks the position where the compromise boundary to stock separation was drawn (adapted from Durholtz and van der Westhuizen 2012).

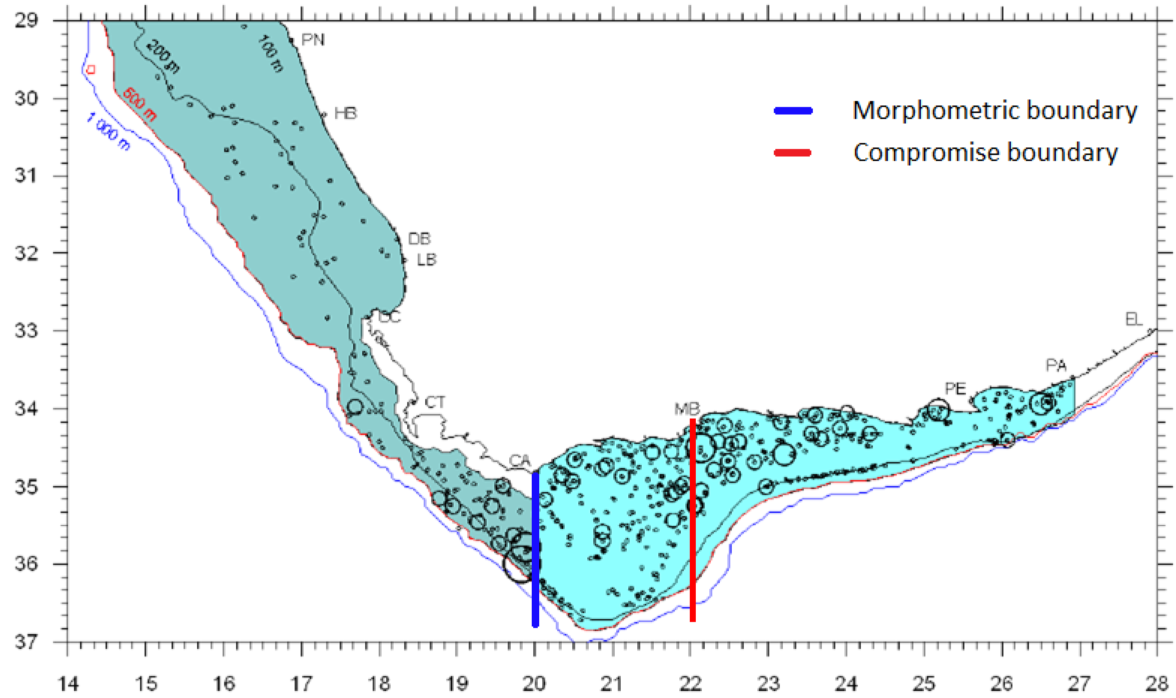


Figure 30. Map illustrating the distribution of chokka squid (*Loligo reynaudi*) catches recorded during demersal research surveys. The areas encompassed by the West Coast and South Coast surveys were illustrated in dark and light blue respectively. The South Coast demersal survey area was split by the compromise boundary to stock separation (red line at 22°E) chosen for this study (adapted from Durholtz and van der Westhuizen 2012).

3.4.4 Statistical analysis

For each of the six data sets, all data were filtered by grid block numbers to separate data occurring east and west of 22°E. Filters were also used to separate catches by year and catch period (January – March and April - December) for each area.

Trawl catches

In order to get the true estimated quantity of trawl catch per area, and to make the data comparable to those of Glazer and Butterworth (2012a), the trawl CPUE dataset was used to calculate the proportion of chokka squid catches taken in the area east of 22°E. These proportions were then applied to the total annual chokka squid catches included in the assessment (Glazer and Butterworth 2012a) in order to split the catches by area.

Jig catches

The jig CPUE dataset was used to calculate the proportion of chokka squid catches taken in the area east of 22°E. This ensured calculation of the true estimated quantity of jig catch per area, and to make the data comparable to those of Glazer and Butterworth (2012a), These proportions were then applied to the total annual chokka squid catches included in the assessment (Glazer and Butterworth 2012a) in order to split the catches by area.

Trawl CPUE

Commercial trawl chokka squid CPUE indices per period were calculated using the dataset which provided the area-aggregated indices of abundance for the trawl fishery for the two periods of interest. Glazer (DAFF) modified the existing code to separate the data east and west of 22°E and then applied the period-specific GLMs of Roel (1998) to those data in order to derive area- and period-specific standardized indices of abundance for the trawl fishery.

Jig CPUE

Nominal jig CPUE indices per period were calculated using the DAFF jig catch and effort database that provided the area-aggregated indices of abundance for the chokka squid jig fishery for the two periods of interest. Glazer (DAFF) modified the existing code to separate these data east and west of 22°E and derived area- and period-specific nominal CPUE indices of abundance for the jig fishery (constrained to the core 19 vessels and records where $3 \leq \text{crew} \leq 20$).

Survey biomass estimates

Spring and autumn survey data were filtered by grid block numbers to separate data occurring east of 22°E.

As per method used by Fairweather and Leslie (2009), the survey biomass estimates for chokka squid in this area were obtained as follows:

1. The swept area for each trawl was calculated as the product of trawl speed, net width, and trawl time.
2. Four strata were defined by depth ranges 0 to 50 m, 50 to 100 m, 100 to 200 m and 200 to 500 m.
3. The swept area in each stratum was then the sum of swept areas for all trawls in that stratum.
4. The chokka squid density was the total catch divided by total swept area.
5. The stratum biomass was the product of the stratum density and the stratum area.
6. Finally the total chokka squid biomass was the sum of the stratum biomasses.
7. Since some of the surveys did not trawl deeper than 200 m, only the first three depth strata were included in the final sum.

It should be noted that the choice of the strata for the survey were oriented to optimise the results for hake, not chokka squid. Furthermore, the demersal nature of the sampling gear might have resulted in the survey missing a portion of the stock higher up in the water column.

3.5 Results

3.5.1 Annual catches per area

Most chokka squid trawl catches were taken in the area west of 22°E, particularly in the early years (1971 – 1985) prior to the establishment of the directed jig fishery. Although there has been a reduction in chokka squid catches in both areas east and west of 22°E since around the time of the onset of the jig fishery (1983 – 1985), there has been a much sharper decline in chokka squid catches in the area west of 22°E (Figure 31). It should be noted that early catches included catches taken by the foreign fleet which were banned from fishing within the South African Economic Exclusive Zone (EEZ) after the mid-1980’s.

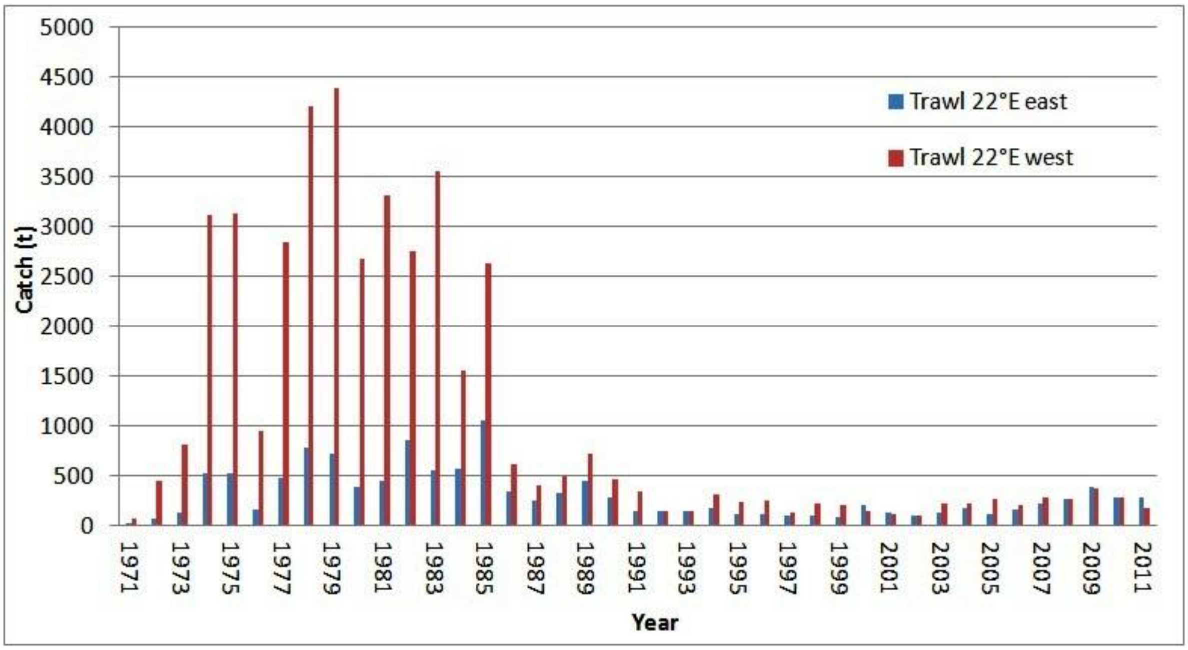


Figure 31. Annual commercial trawl catches of chokka squid (*Loligo reynaudi*) taken east and west of 22°E (source: DAFF demersal database, SABS/NCRS).

Most of the jig catches were taken east of 22°E since the onset of the jig fishery in 1985 (Figure 32). In the area west of 22°E, jig catches have remained relatively stable and are fairly small in comparison to the eastern area (Figure 32).

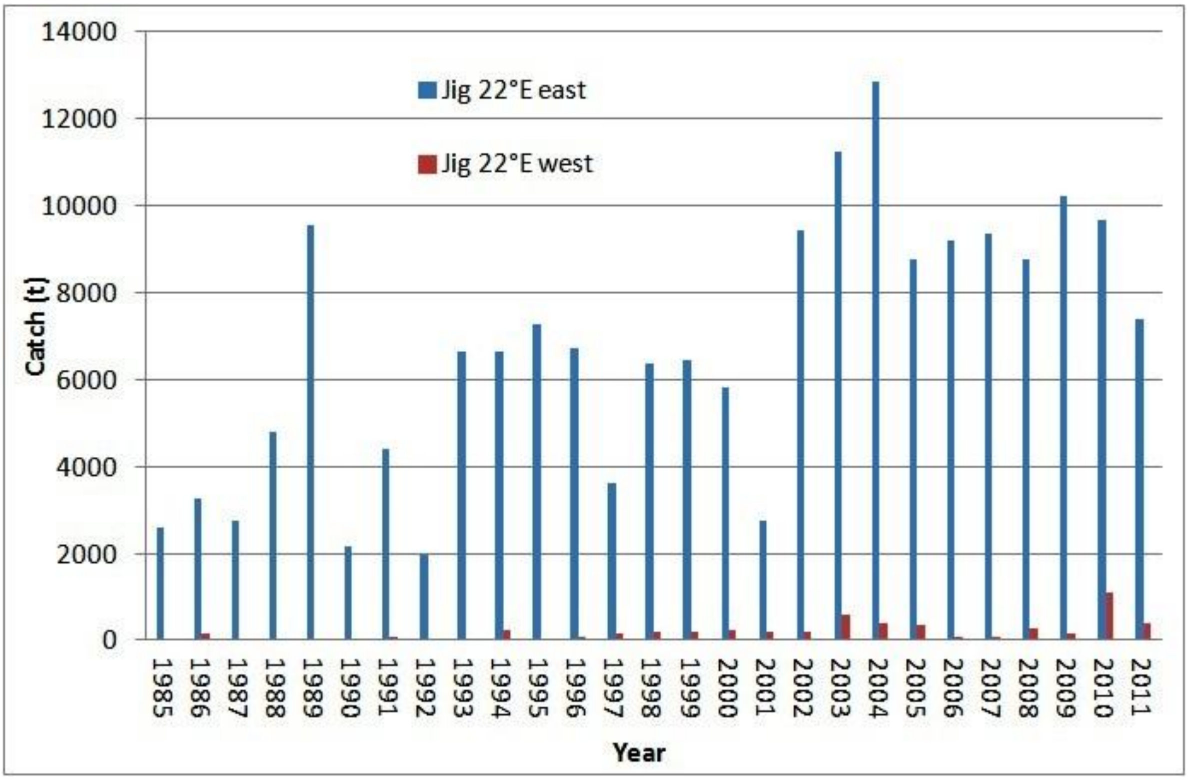


Figure 32. Annual commercial jig catches of chokka squid (*Loligo reynaudi*) taken east and west of 22°E (source: DAFF demersal database, SABS/NCRS).

3.5.2 Component of catches per area

The percentage of total chokka squid catch taken per year in each sector (jig and trawl) and area (east and west of 22°E) is shown in Figures 33 and 34 respectively.

Jig catches to the east of 22°E have remained relatively stable over the period (ranging between 90 – 100% per annum) with a slight decrease in the last two years. As a result, there has been an increase in the percentage of jig catches (thus effort) made to the west of 22°E.

Trawl catches east of 22°E are more variable, ranging between 12 and 61% over the period considered and display an increasing trend over the period. Conversely, there has been a decrease in the percentage of trawl catch taken to the west of 22°E.

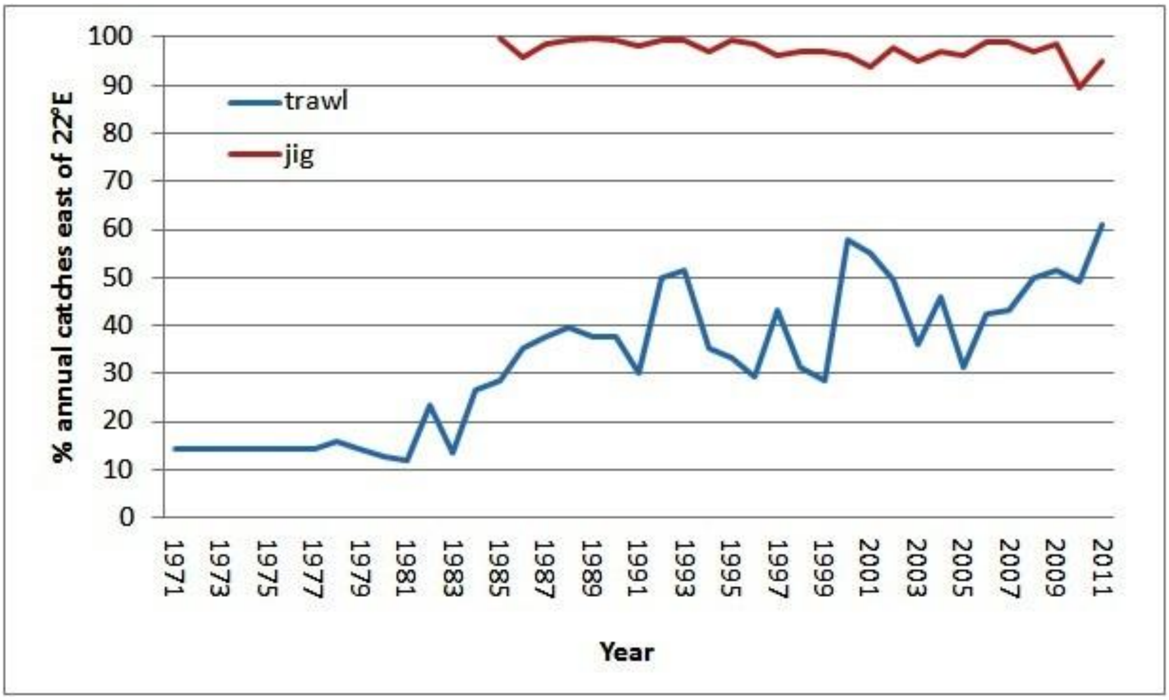


Figure 33. The percentage of total annual trawl (blue) and jig (red) catches of chokka squid (*Loligo reynaudi*) taken east of 22°E (source: SABS/NCRS; DAFF demersal database).

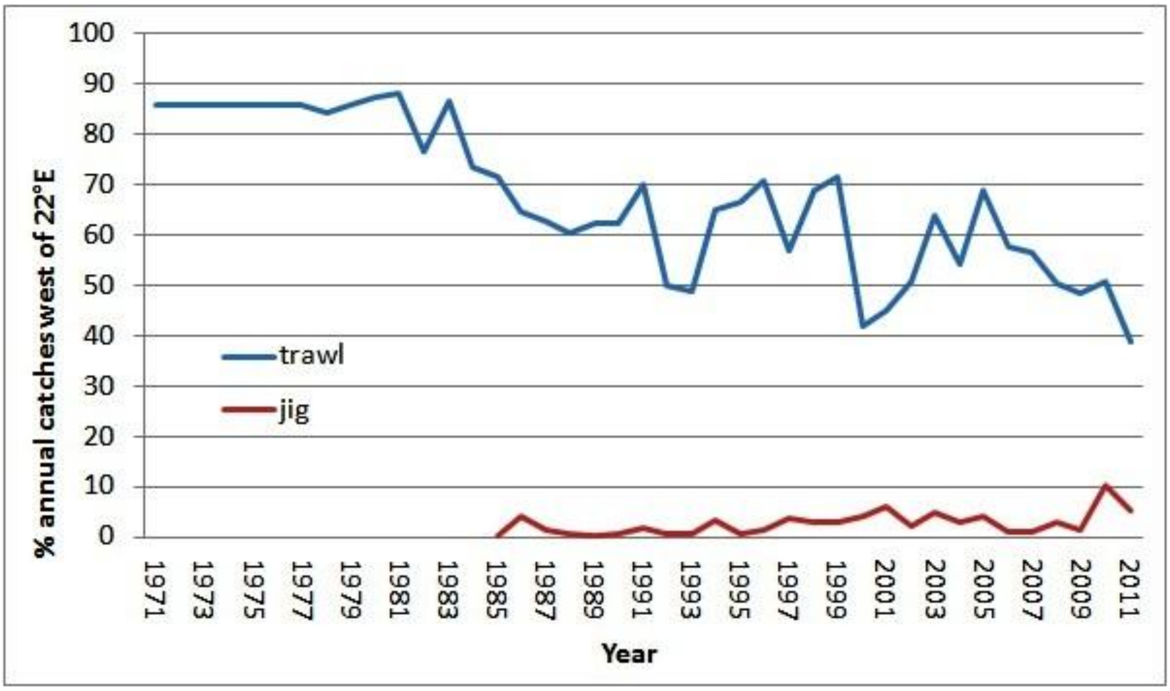


Figure 34. The percentage of total annual trawl (blue) and jig (red) catches of chokka squid (*Loligo reynaudi*) taken west of 22°E (source: SABS/NCRS; DAFF demersal database).

3.5.3 Trawl CPUE

The standardized CPUE indices derived from a GLM applied to the area- and period-specific trawl data are shown in Figures 35 – 36. The high CPUE evident in the January – March early period (1978 – 1982) for the aggregated data is not as pronounced in the area east of 22°E (Figure 35). Overall, the trends shown by both the aggregated index and that east of 22°E are similar.

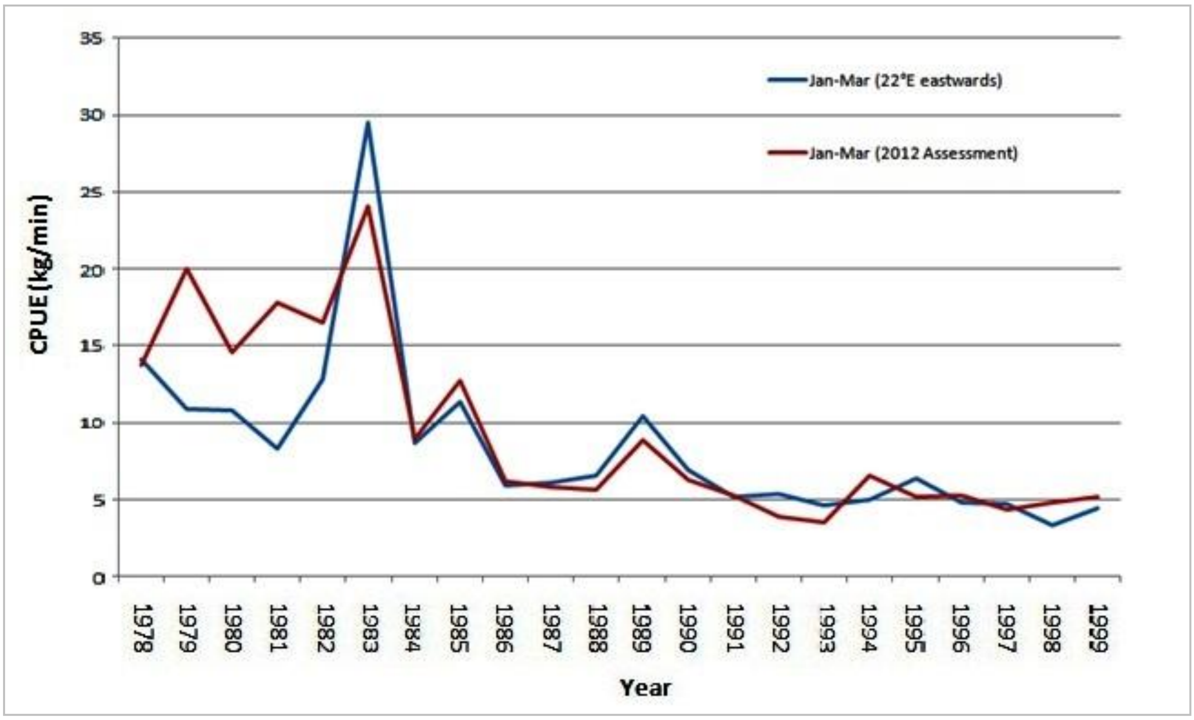


Figure 35. Standardized chokka squid (*Loligo reynaudi*) trawl CPUE indices (kg/min) per annum for the period January to March period for the area-aggregated data (red) *vs.* data from the area east of 22°E (blue).

Similar results are also evident for the April – December period (Figure 36) when comparing the area-aggregated standardized CPUE index with that for the area east of 22°E. It is interesting to note that CPUE is estimated to be higher for the area east of 22°E than that for the entire area.

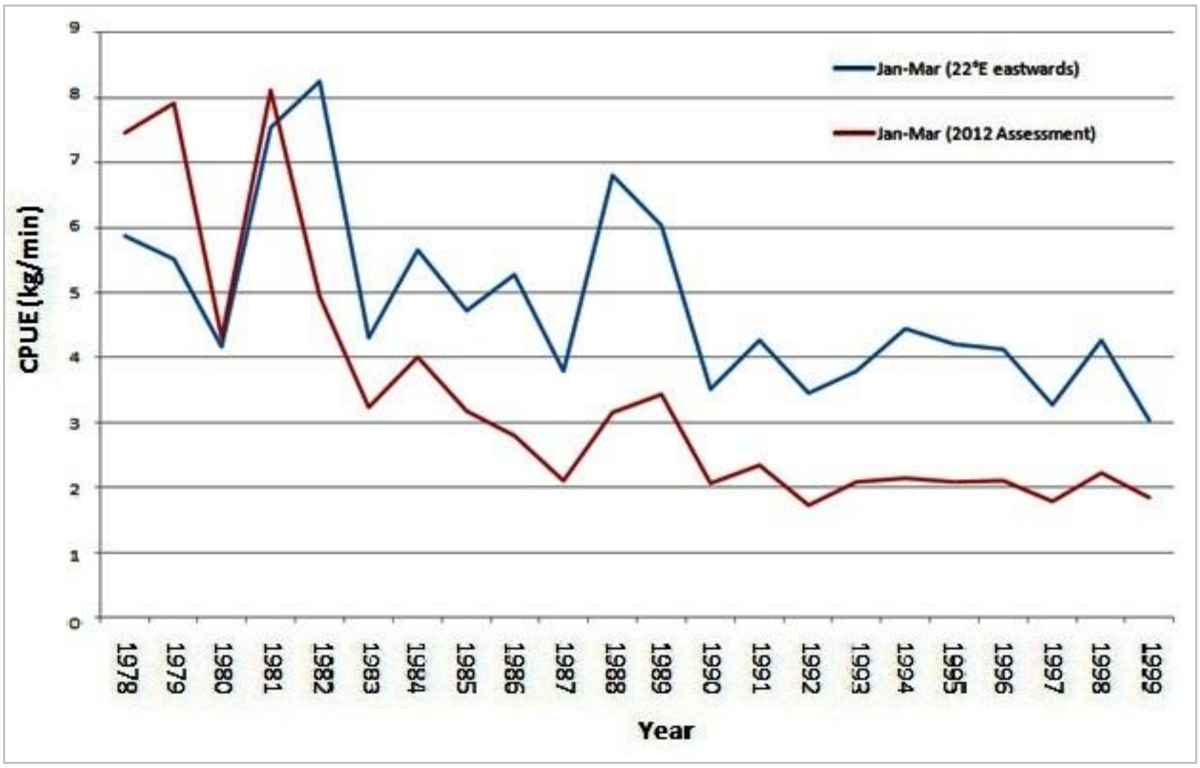


Figure 36. Standardized chokka squid (*Loligo reynaudi*) trawl CPUE indices (kg/min) per annum for the April to December period for the area-aggregated (red) data *vs.* data from the area east of 22°E (blue).

3.5.4 Nominal Jig CPUE

The nominal jig CPUE trend for the area east of 22°E for both periods does not differ much from that of the aggregated area evident in Figures 37 and 38.

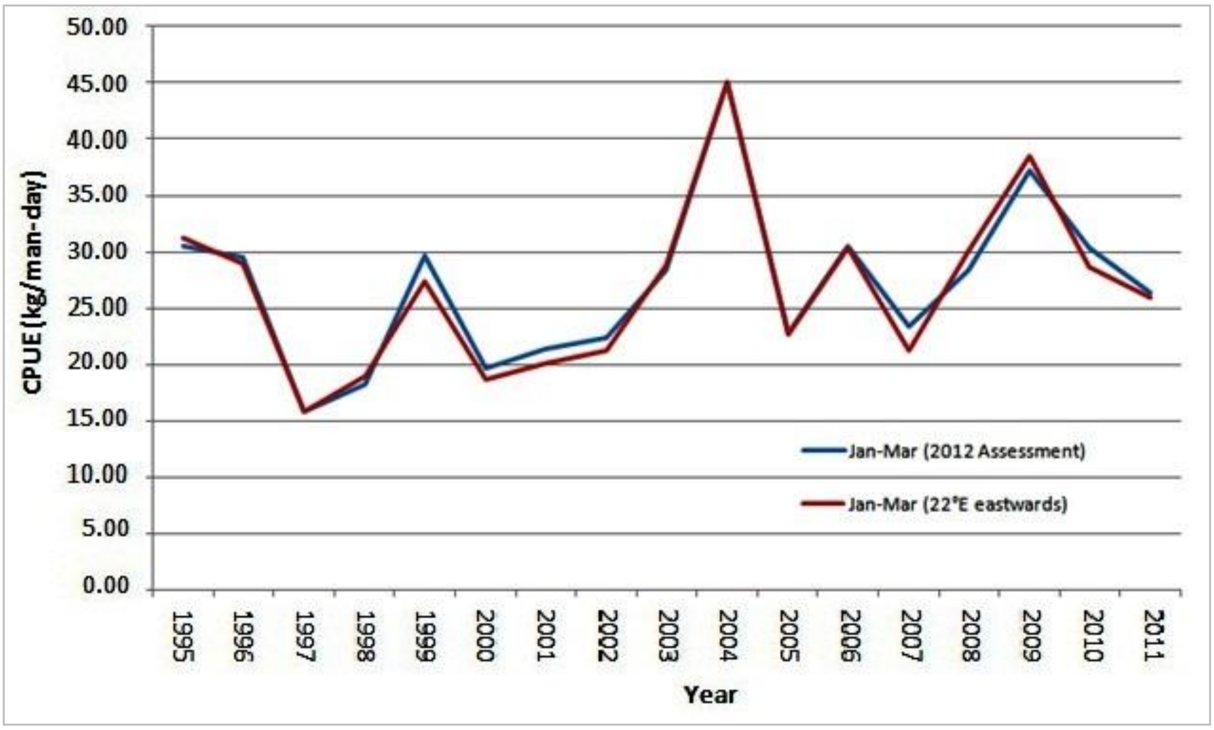


Figure 37. Nominal chokka squid (*Loligo reynaudi*) jig CPUE (kg/man-day) per annum for the period January to March for the area-aggregated (blue) data *vs.* data from the area east of 22°E (red), restricted to data from the core 19 vessels and 3≤crew≤20.

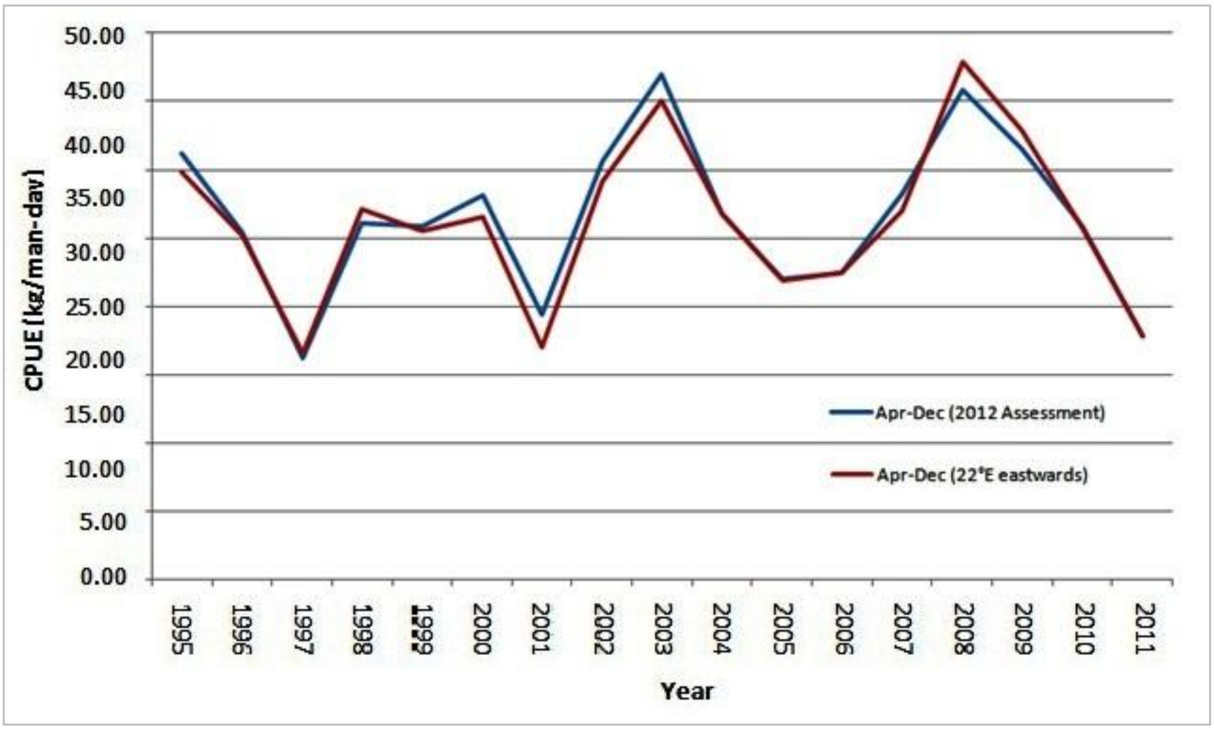


Figure 38. Nominal chokka squid (*Loligo reynaudi*) jig CPUE (kg/man-day) per annum for the period April to December for the area-aggregated (blue) data *vs.* data from the area east of 22°E (red), restricted to data from the core 19 vessels and 3≤crew≤20.

3.5.5 Survey abundance estimates

Figures 39 and 40 show the Autumn and Spring survey abundance estimates respectively for the full area compared to the area east of 22°E. Both indices show similar trends. The Autumn biomass estimate in the early years (1988 – 1991) are similar for the two areas, with greater divergence evident in subsequent years (Figure 39). The Spring biomass estimates are similar for the two areas (Figure 40).

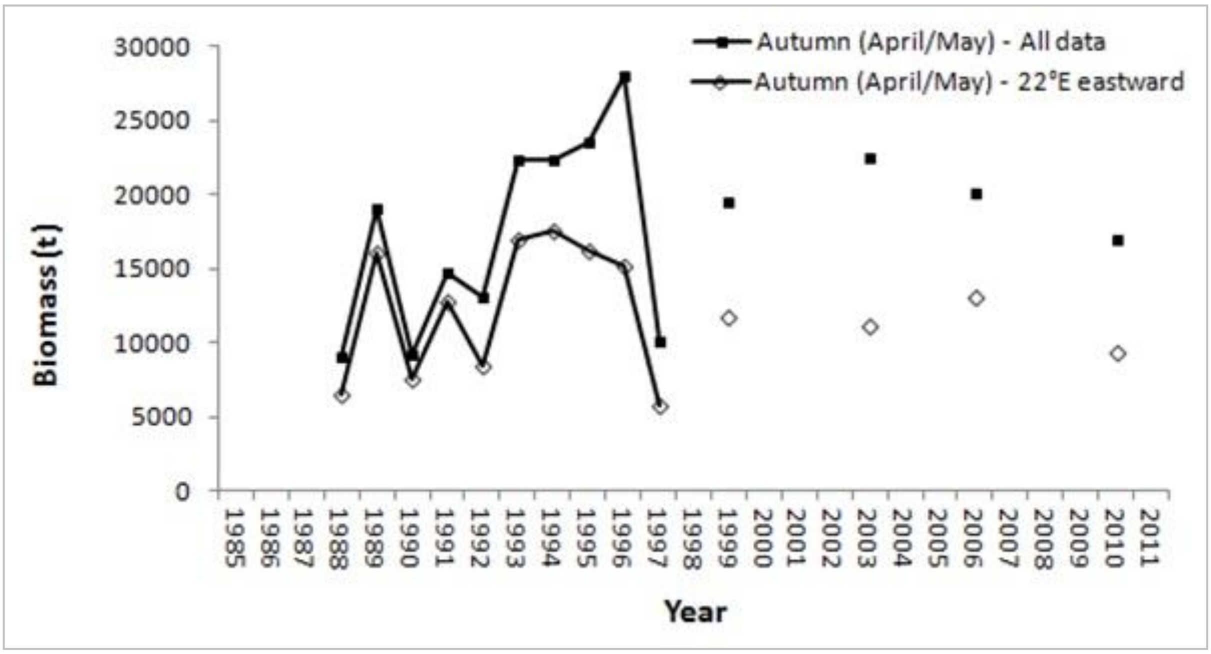


Figure 39. Chokka squid (*Loligo reynaudi*) autumn survey biomass estimates for the whole area compared to the area east of 22°E.

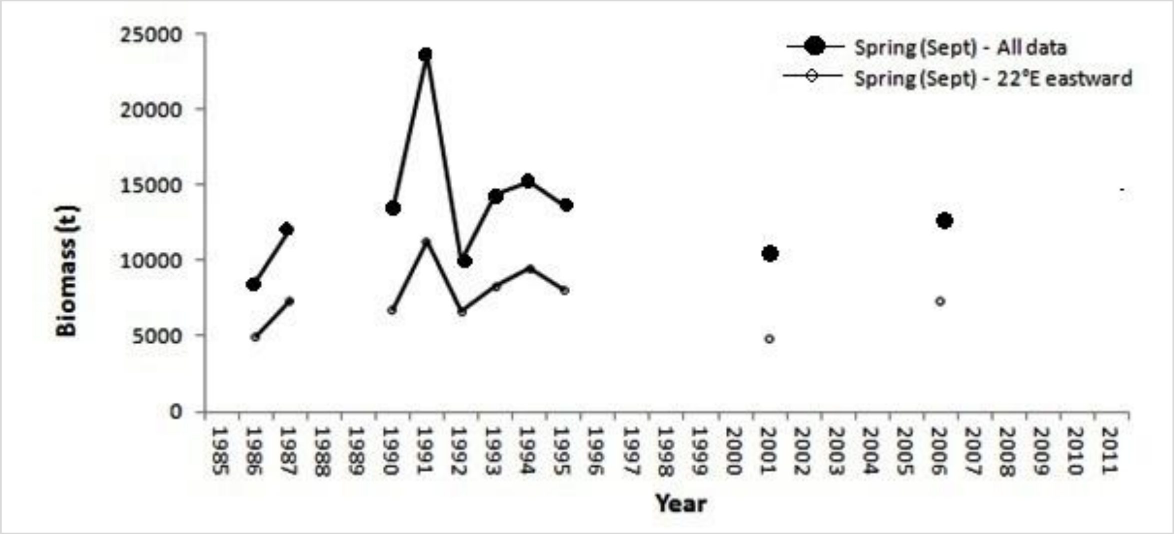


Figure 40. Chokka squid (*Loligo reynaudi*) spring survey biomass estimates for the whole area compared to the area east of 22°E.

3.6 Discussion

3.6.1 Trawl catch and CPUE

In the mid-1980's trawlers were forced to move away from targeting bycatch species (phasing out of trawl bag liners), and certain parts of the coast (example bays) were closed to trawling (Gaylard and Bergh 2006). Foreign activity was also phased out during this time. Roel (1998) suggested that these factors may have accounted for the steep decline in chokka squid bycatch recorded in the trawl fisheries around the period 1985 (Figure 31). The onset of the jig fishery in 1983 also appears to have had an influence as trawled chokka squid catches sharply declined during this period (Gaylard and Bergh 2006).

Figure 31 illustrates the reduction in chokka squid by-catch taken in the demersal trawl fishery in the areas both east and west of 22°E, with a sharper decline in catch evident to the west. This is due to a much larger portion of the catch being taken in this area during the early years (76 – 98% during 1971 to 1983) (Figure 31). As the newly established jigging fleet started targeting the main spawning stock on the main inshore spawning grounds (east of 22°E) in 1983, it may have affected recruitment onto the feeding grounds west of 22°E, possibly causing the sharp decline in catches in this area during that period. The sharp decline in trawled chokka squid bycatches around 1983 to 1985 in the area east of 22°E may have been due to competition with the newly established jigging fleet, the phasing out of foreign activity and especially the closure of shallow bays to trawling on the main spawning grounds east of 22°E.

The standardized CPUE indices for the area-disaggregated trawl CPUE data per period show the same sharp decline in CPUE in the early period as do the area-aggregated indices (Figures

35 and 36). This finding remains consistent with the declining trawl catch in this area, as well as the perception of trawlerman (Roel *et al.* 2000) that the quantity of chokka squid (therefore abundance) available to trawlers decreased substantially after the onset of the jig fishery in 1983. Both chokka squid trawl catch and CPUE results were thus not affected when analysing the data on an area-disaggregated basis given that similar trends were found.

3.6.2 Jig catch and CPUE

Jig catches to the east of 22°E make up 90 – 100% of the total annual catch over the period 1985 to 2011 (Figure 33), as a result of most of the effort being concentrated on the main spawning grounds. This percentage has decreased slightly in the past few years with an increase in percentage of jig catches made to the west of 22°E (mainly off Cape Agulhas) (Figure 34). The recent increase in chokka squid catches made in this area may be due to a lack of squid availability in the east and an increase in fishing effort to the west off Cape Agulhas, an area known by squid fisherman to produce good catches from February to April in some years (John Henrick pers comm.)

Jig catches in the area east of 22°E increased since the onset of the jig fishery (1985) as effort increased with the development of the fishery. After the capping of effort (number of vessels and crew) there were still increases in effort, due to new technological developments (bigger vessels with increasing range of operation, blast freezers increasing time spent at sea, strong lights allowing fishing at night, satellite image detection of chlorophyll concentration patterns affecting squid abundance). In the area west of 22°E, jig catches remained fairly stable but might slightly have increased in the last decade due to more fishing effort deployed in this area during recent years, as indicated by the DAFF jig effort database (Van der Westhuizen pers. comm. 2013).

Jig CPUE trends from the area east of 22°E are similar to those included in the area-aggregated assessment (Glazer and Butterworth 2012) for both the January – March and April – December periods (Figure 37 and 38). Jig CPUE trends east of 22°E are also consistent with the positive trend in jig catches made east of 22°E (Figure 32).

A key difference between the nominal jig and standardized trawl CPUE indices for the area east of 22°E is that the jig index shows an increasing trend while the trawl index exhibits a sharp decline in CPUE since 1983. The positive jig CPUE trend therefore contradicts the perception of trawlerman that there has been a general decline in chokka squid abundance. However, as mentioned previously, a number of factors may have contributed to this perception (e.g. the phasing out of foreign activity and liners, closure of bays, competition with the jigging fleet on the main spawning grounds, different areas of operation etc.). Another explanation of the difference may also lie in the fact that the jig effort is concentrated within 10km offshore in the area around Port Elizabeth, whereas the commercial trawl effort is predominantly west of Knysna (Gaylard and Bergh 2006). The commercial trawl CPUE results represents mainly the "smaller" or "immature" population (targeting deeper areas than the jig fishery), while the commercial jig CPUE data are subject to fishing selectivity and thus represent larger fish (targeting shallow inshore spawning areas) (Gaylard and Bergh 2006). Although, according to Gaylard and Bergh (2006) the larger size classes of squid tend to reside at shallower depths in the east than in the west (due to the inshore spawning aggregations in the east). Both jig catch and CPUE results were thus not affected when analysing the data on an area-disaggregated basis as similar trends to those of the area-aggregated catches and indices resulted.

3.6.3 Survey abundance estimates

Survey data play an important role in fisheries management and contain some inherent advantages over commercial data in that fishing methods are dictated by scientific goals and are not subject to economic pressures that may change commercial practise and thus exert non-biological influences over quantities such as CPUE (Gaylard and Bergh 2006).

Trends in the survey biomass estimates at an area-disaggregated level were similar to those for the area-aggregated data. Lower biomass estimate for the area east of 22°E in both autumn and spring surveys (Figure 39 and 40) are expected when using data from only a portion of the total area surveyed.

The opposing trends displayed by the survey indices and the trawl CPUE indices might be due to the spatial and temporal differences between survey and commercial effort (Gaylard and Bergh 2006). In particular, the surveys took place at a certain time of year (autumn and spring), whereas commercial trawling takes place year-round; and the survey samples cover the entire South Coast whereas the commercial trawl effort is predominantly west of Knysna. The surveys, aimed primarily at hake, tend not to survey the shallow inshore areas (main spawning grounds) intensively, whereas the commercial trawl fisheries use to target squid in these areas pre-1980’s. Another factor playing a possible role may be differences in trends between large and small squid, with the smaller mesh size in the survey gear likely to catch a greater proportion of small fish (Gaylard and Bergh 2006).

These differences are not addressed directly by the GLM modelling analyses but should be borne in mind and accounted for appropriately when using the GLM-standardised CPUE series within an assessment framework (Gaylard and Bergh 2006).

3.7 Conclusion

The objective of this chapter was to investigate trends in the chokka squid jig CPUE indices, trawl CPUE indices and demersal survey biomass indices in the context of an area-disaggregated framework. A key aim/challenge has been the selection of a boundary to separate stocks utilizing results from both morphometric (Chapter 2) and genetic (Stonier 2012) studies. As a consequence of the distribution pattern of chokka squid, the boundary used had to be a compromise from the apparent real boundary south of Cape Agulhas (at 20°E as determined by results from Chapter 2) to a more eastward position, 22°E. As a result the model outcomes may have been influenced but the boundary shifting could not be avoided due to the distribution patterns of squid catches along the coast.

Due to similarities in catch and CPUE trends in the analyses undertaken for the area-disaggregated data compared to the area-aggregated data, it is concluded that there is no need to assess the resource on a area-disaggregated basis if one accepts the compromised boundary for stock separation set at 22°E.

CHAPTER 4

GENERAL CONCLUSION AND MANAGEMENT IMPLICATIONS

Advancements in spatial modelling have wide application in fishery science. Such aspects include ecological theory (for example population stability, and climate effects); more effective fishery management plans (for example rebuilding and mixed-stock sustainable yield); design, implementation and evaluation of marine-protected areas, defining the appropriate spatial scale of ecosystem processes and management; designation and protection of essential fish habitat; improved assessment of environmental and economic impacts of management alternatives; and optimization of spatial harvest strategies (Cadrin and Secor 2009).

Population concepts have evolved to the point of accepting complex spatial patterns that can accommodate problems related to how mixed-stock fisheries and climate affect recruitment and resource sustainability (Cadrin and Secor 2009). The extension of conventional stock assessment methods to incorporate spatial patterns is relatively straightforward and available (Cadrin and Secor 2009). It appears that the missing link in applying spatially explicit population models is the lack of information on movement rates and patterns, reproductive isolation, and stock composition (Cadrin and Secor 2009). Therefore, advancement in modelling spatial population structure for stock assessment requires more extensive sampling of stock composition (genetic and morphological sampling of mixed stocks, analysis of environmental signals for contingents) and tagging studies designed to estimate movement rates and patterns of movement with respect to natal homing (Cadrin and Secor 2009).

Although extensive sampling of chokka squid stock composition (genetic, morphometric, environmental effects) and tagging studies have been undertaken, the advancement in modelling its spatial population structure is hampered by the distribution pattern of the species and the availability of CPUE and catch data across its known distribution range.

This thesis examined whether there was any evidence that the chokka squid stock should be managed as subunits should there be any evidence of stock separation along the South African coast. Chapter two used discriminant function analysis to assess differences between squid stocks from three regions (Angola, west coast and south coast of South Africa). Surprisingly soft body parts were more robust in these analysis than the hard part measurements and several possible explanations were proposed. Morphologically, based on soft part measurements, chokka squid from the south coast (central and eastern Agulhas Bank), west coast (west coast and western Agulhas Bank), and southern Angola were different, and may be separated into those three regions, however exact geographical stock boundaries are still speculative. In general these results supported genetic evidence (Stonier 2012) of slight but significant differences between squid collected from the three areas along the southern African coast.

Due to the existing assessment model used to assess the squid resource in South African waters currently being updated, it is currently not feasible to apply this model to the area-disaggregated data produced by this study. Therefore, Chapter three rather investigated the results of analysing the CPUE and catch data on an area-aggregated versus area disaggregated-basis. As a consequence of the distribution of chokka squid, the boundary used had to be a compromise from the apparent real stock boundary south of Cape Agulhas (20°E) to a more eastward position, 22°E. This may have influenced the model outcomes but the boundary shifting could not be avoided because of the distribution patterns of chokka squid catches along the South African coast.

No support for major differences in CPUE trends was found in the analysis undertaken on data with a stock boundary set at 22°E. Furthermore, a future area-disaggregated assessment of the resource (using an updated assessment model) with a stock boundary set at 20°E (as determined in Chapter 2) is not feasible due to limited available CPUE and catch data to the west of this boundary. It is therefore concluded that the stock should be managed on an area-aggregated basis as it currently is. However, it is recommended that catch monitoring continue in order to account for spatio-temporal patterns of both the commercial jig and trawl fishing fleets (e.g. stratified by fishing grounds and fishing seasons). The genetic structure should be sampled intensively in each fishery (including the Angolan artisanal jig fishery) to provide fine scale comparable estimates to the phenotypic stock composition results of this study.

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