The early life strategy of Cape hakes in the Benguela upwelling system off South Africa

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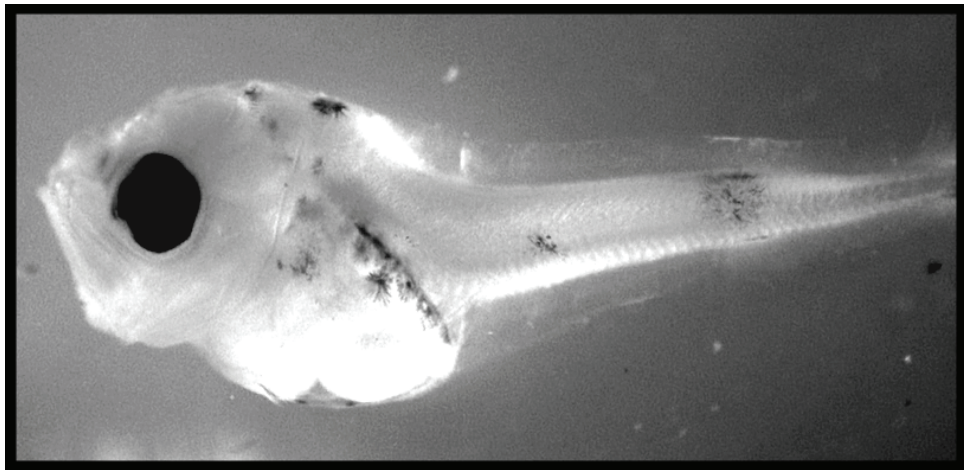
"The difficult is what takes a little time; the impossible is what takes a little longer."
Fritdjof Nansen

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Abbreviations

ARA	arachidonic acid, 20:4(n-6)
DHA	docosahexaenoic acid, 22:6(n-3)
DM	dry mass
EPA	eicosapentaenoic acid, 20:5(n-3)
FAME	fatty acid methyl ester
n	number of individuals
TFA	total fatty acid
PC	principal component
PCA	principal component analyses
PLD	pelagic larval duration
SGR	somatic growth rate
WE	wax ester

Abstract

Recruitment is a key process in the sustainability of marine fish populations. The dynamic and complex process of recruitment is integrated over several life stages, with many different factors affecting each stage leading to high variability in populations. This study aimed at gaining important knowledge on the early life history and the factors affecting recruitment of the two demersal Cape hake species *Merluccius paradoxus* and *M. capensis* in the southern Benguela upwelling system off South Africa. Eggs and larvae were collected by stratified hauls during four cruises in two consecutive years. Early juveniles were collected on one cruise in the northern part of the study area off the Orange River.

The spatial distribution patterns, growth and condition of early stages were investigated using a combination of classic and novel methods. Spatial distribution patterns were described by species-specific vertical and horizontal abundances. Growth was investigated by otolith analyses of larvae and juveniles. Larval conditions were analysed by measuring RNA:DNA ratios. Another method for estimating condition was applied to eggs and larvae by measuring the total lipid content. Fatty acid compositions were investigated in order to reveal the levels of essential fatty acids, needed for growth and development. Stomach content analyses complemented the information on early life stages as they elucidated the feeding of larvae in the study area.

Distribution patterns revealed that *M. paradoxus* was the dominant species during the study periods, indicating a temporal and possibly spatial separation of spawning of the two Cape hake species. This difference in the spawning strategy reduces competition during the early life stages between the two similar species, allowing their co-existence within the same ecosystem.

Furthermore, the distribution patterns of hake eggs and larvae indicated that a substantial part of spawning occurs on the western Agulhas Bank. Early stages are transported by the jet current, whereby the drift routes of the two species are separated, with *M. paradoxus* found further offshore than *M. capensis*. Complex retention mechanisms enable larvae on the inshore drift route to reach the coastal nursery area off Cape Columbine, whereas larvae from the offshore route are transported further north. This transport and dispersal mechanisms are important for larval connectivity. Therefore, the spawning strategies of the two hake species are adapted to the variable

environment of the southern Benguela system, as spawning takes place during a time of optimal transport conditions for eggs and larvae towards their specific nursery areas.

In addition, factors affecting the recruitment of hake were investigated by analysing growth and condition of early stages. Analyses indicated that the South African hakes are fast growing species. Survivors outgrow their conspecifics and cannibalism seems to play an important role in the survival strategy of hake. Larvae were generally in good condition, as indicated by high RNA:DNA ratios, and they can be regarded as the survivors, as starving larvae with poor condition are more vulnerable to predation. RNA:DNA ratios as condition proxy and growth did not correlate in hake larvae, as the RNA:DNA ratios were well above the threshold level for growth. Furthermore, low lipid contents were found in yolk-sac larvae of one investigated year, indicating maternal effects by poor conditions of spawners, possibly affecting recruitment. The life history strategy of hakes can be described as periodic with adaptations from an opportunistic strategy.

In conclusion, the interdisciplinary approach of this study provided new and important knowledge on the early life history and the recruitment of the Southern African hake species, which can be transferred to other demersal, long-lived and fast-growing fish species. Furthermore, this information can be applied to fisheries management and it can help to predict the consequences of climate change for *M. paradoxus* and *M. capensis* in the southern Benguela upwelling system.

Zusammenfassung

Rekrutierung ist ein Schlüsselprozess in der Beständigkeit mariner Fischpopulationen. Dieser dynamische und komplexe Prozess zieht sich über mehrere Lebensstadien und viele verschiedene Faktoren beeinflussen die Überlebensraten in jedem dieser Stadien, was zu großen Fluktuationen in Populationen führen kann. In dieser Arbeit wurden die frühe Lebensgeschichte der beiden demersalen Kap-Seehechtarten *Merluccius paradoxus* und *M. capensis* und die Faktoren, welche ihre Rekrutierung beeinflussen, untersucht. Eier, Larven und Juvenile wurden in Tiefenstufen während vier Expeditionen in zwei aufeinanderfolgenden Jahren im südlichen Benguelasystem vor der Küste Südafrikas beprobt.

Die räumlichen Verteilungsmuster, das Wachstum und die Kondition der frühen Lebensstadien wurden mit einer Kombination von klassischen und neuen Methoden untersucht. Räumliche Verteilungsmuster wurden durch artspezifische vertikale und horizontale Abundanzen beschrieben. Das Wachstum von Larven und Juvenilen wurde durch Analyse der Otolithen untersucht. Die Kondition von Larven wurde durch die Bestimmung des RNA:DNA -Verhältnisses analysiert. Die Bestimmung des Lipidgehalts ist eine weitere Methode zur Abschätzung der Kondition, welche an Eiern und Larven durchgeführt wurde. Außerdem wurden die Fettsäurezusammensetzungen untersucht um den Anteil essentieller Fettsäuren, welche für das Wachstum und die Entwicklung wichtig sind, in Seehechtlarven zu ermitteln. Mageninhaltsanalysen komplettieren die erhaltenen Informationen, denn sie geben Auskunft über die Nahrung der Larven im Untersuchungsgebiet.

Die Verbreitungsmuster zeigten, dass *M. paradoxus* die dominante Art im Untersuchungsgebiet auf allen vier Expeditionen war, was auf eine zeitliche und möglicherweise auch räumliche Trennung des Laichens der beiden Kap-Seehechtarten hinweist. Dieser Unterschied in der Laichstrategie reduziert die Konkurrenz zwischen den beiden sehr ähnlichen Arten während der frühen Lebensphase, was ihre Koexistenz im gleichen Ökosystem ermöglicht.

Des Weiteren deuteten die Verbreitungsmuster der Seehechteier und -larven daraufhin, dass ein beträchtlicher Anteil der Laichaktivitäten auf der westlichen Agulhas Bank statt findet. Frühe Lebensstadien werden durch den 'Jet-Strom' transportiert, wobei die Driftwege der beiden Arten unterschiedlich sind. Die frühen Lebensstadien von *M. paradoxus* wurden küstenferner gefunden als die Eier und Larven von *M. capensis*.

Komplexe Retentionsmechanismen ermöglichen es den Larven in der küstennahen Driftroute das Aufwuchsgebiet vor Cape Columbine zu erreichen, wogegen die Larven in der küstenfernen Driftroute weiter nach Norden transportiert werden. Diese Transport- und Verbreitungsmechanismen der Larven sind ausschlaggebend für die Konnektivität zwischen Laichgebiet und Aufwuchsgebiet. Die Laichstrategien der beiden Seehechtarten sind adaptiert an die variablen Umweltbedingungen im südlichen Benguelasystem, denn der Laichzeitpunkt stimmt mit einer Zeit für den schnellen und optimalen Transport der Eier und Larven in die artspezifischen Aufwuchsgebiete überein.

Faktoren, welche die Rekrutierung beeinflussen, wurden untersucht indem das Wachstum und die Kondition der frühen Stadien der Seehechte analysiert wurden. Dabei zeigte sich, dass die südafrikanischen Seehechte schnell wachsende Arten sind. Die überlebenden Seehechtlarven und -juvenile entwachsen ihren langsamer wachsenden Artgenossen und Kannibalismus scheint eine wichtige Rolle in der Überlebensstrategie der Seehechte zu spielen. Die gefangenen Larven waren generell in guter Kondition, bestimmt durch das RNA:DNA -Verhältnis, und können als die Überlebenden angesehen werden, weil hungernde Larven in schlechter Kondition eher gefressen werden. RNA:DNA -Verhältnisse von Larven als deren Konditionsindikator und das Wachstum der Larven korrelierten nicht miteinander, denn alle RNA:DNA - Verhältnisse waren über dem Grenzwert für Wachstum. Des Weiteren wurden niedrige Lipidgehalte in Dottersacklarven in einem der Untersuchungsjahre gefunden, was auf maternale Effekte durch niedrige Kondition der laichenden Fische hinweist, was einen Einfluss auf die Rekrutierung haben kann. Die Lebensstrategie der Seehechte kann als periodisch mit Anpassungen aus einer opportunistischen Strategie beschrieben werden.

Insgesamt konnten mit Hilfe dieses interdisziplinären Ansatzes neue Erkenntnisse über die Lebensgeschichte und die Rekrutierung der südafrikanischen Seehechtarten gewonnen werden, welche auf andere demersale, langlebige und schnell wachsende Fischarten übertragen werden können. Die gewonnenen Informationen können für das Management der Seehechte genutzt werden. Außerdem können die Erkenntnisse hilfreich sein um die Auswirkungen des Klimawandels auf *M. paradoxus* und *M. capensis* im südlichen Benguela-Auftriebssystem vorherzusagen.

Outline of Publications

The following overview outlines the three first author publications included in this PhD thesis and the aims and separation of themes. The general concept of this study was developed by myself with advice and scientific guidance by my supervisors Prof. W. Hagen and Dr. W. Ekau. Realisation of the applied analysis and methods was done by myself with provision of laboratories and equipment by the department of Marine Zoology (Prof. Dr. W. Hagen) at the University of Bremen, the Leibniz-Center for Tropical Marine Ecology in Bremen, the department of Evolutionary Ecology of Marine Fishes (Dr. C. Clemmesen) at the IFM-Geomar in Kiel, and the department of Biotechnology and Molecular Genetics (Prof. Dr. D. Blohm) at the University of Bremen.

CHAPTER I

Grote B, Stenevik EK, Ekau W, Lipinski MR, Verheye HM, Hagen W

Spatiotemporal distribution of early life stages and spawning strategy of two Cape hake species, *Merluccius paradoxus* and *M. capensis,* in the southern Benguela upwelling system.

I shared the field work with EK Stenevik, MR Lipinski and HM Verheye. I performed all genetic analyses. The manuscript was written by myself with scientific and editorial advice by all co-authors. The manuscript is submitted to Journal of Plankton Research.

CHAPTER II

Grote B, Ekau W, Stenevik EK, Clemmesen C, Verheye HM, Lipinski MR, Hagen, W

Characteristics of survivors - growth and condition of early life stages of the two hake species *Merluccius paradoxus* and *M. capensis* in the southern Benguela system*.*

I shared the field work with EK Stenevik, MR Lipinski and HM Verheye. The analytical work, namely otolith and RNA:DNA ratio analyses as well as genetic analyses were done by myself. All co-authors provided ideas and scientific advice for the manuscript. The manuscript is submitted to the Journal of Fish Biology and in revision.

CHAPTER III

Grote B, Hagen W, Lipinski MR, Verheye HM, Stenevik EK, Ekau W

Lipids and fatty acids as indicators of egg condition, larval feeding and maternal effects in Cape hakes (*Merluccius paradoxus* and *M. capensis*).

I shared the field work with EK Stenevik, MR Lipinski and HM Verheye. I carried out the lipid analyses and evaluated the results. I wrote the manuscript with scientific and editorial advice by all co-authors. The manuscript is submitted to Marine Biology.

1. SCIENTIFIC BACKGROUND AND OBJECTIVES

1.1 General introduction

Fisheries yields in upwelling systems, such as the Benguela current system in the southeast Atlantic, are outstanding compared to other regions of the world's oceans. The Benguela upwelling system off the coasts of South Africa and Namibia belongs to the most productive ecosystems in the Atlantic. Upwelling areas are characterised by nutrient-rich deep-water driven to the surface, inducing prolific primary production, followed by high secondary production, in turn sustaining large fisheries.

One of the dominant piscivores in the eastern boundary upwelling systems of the Atlantic and Pacific Oceans are the hakes. They not only play a key role as top predator within their food webs (Cohen et al. 1990), but furthermore, are among the most important commercially caught fishes in the world (Pitcher & Alheit 1995). The genus *Merluccius* (Rafinesque 1810) consists of 13 morphologically distinct species. They are distributed along the coasts of Europe (*Merluccius merluccius*) and West Africa (from north to south *M. senegalensis*, *M. polli, M. paradoxus, M. capensis*), northeast America (*M. bilinearis*, *M. albidus*), southeast America (*M. hubbsi*, *M. australis*), northwest America (*M. productus*, *M. angustimanus*), southwest America (*M. gayi*, *M. australis*), and east of New Zealand (*M. australis*) (Cohen et al. 1990).

Two hake species inhabit the waters of the west coast of South Africa: *M. paradoxus* and *M. capensis*. The deep-water hake, *M. paradoxus* Franca 1960*,* inhabits waters between 250 m and 850 m depth, whereas the shallow-water hake, *Merluccius capensis* Castelnau 1861*,* is distributed over the shelf from 50 m down to 550 m (Payne 1989). Both species are caught by commercial trawling and since the 1990s also by longline fishing, which targets larger fish in areas where trawling is not possible

(Field et al. 2008). The demersal trawl fishery, dominated by deep-sea trawling for Cape hakes, is the second largest sector of the South African fishing industry by tonnage landed and contributes the highest value of all fisheries landings (FAO 2009). In 2007, 141,360 t *Merluccius* spp. were caught in South African waters, whereby in 2008 catches dropped to 131,717 t. Both hakes are morphologically very similar and visually distinguishable only after maturity, and therefore initially managed as one stock (Botha 1985), although they are different species (Van Eck 1969, Bentz 1976, Grant et al. 1988). The need for a species-based management was pointed out by Butterworth & Rademeyer (2005) and has recently been accomplished (Field et al. 2008).

1.2 The competitive exclusion principle and the ecological niche theory

The co-occurrence of the two hake species (*M. paradoxus* and *M. capensis)* is used as an example to explain the mechanisms enabling the co-existence of two related and ecologically similar demersal fish species. The antitropical distribution of hakes is apparent, although the sister taxa relationships are not well resolved (Grant & Leslie 2001). At least two species in six different areas have a sympatric distribution (Lloris et al. 2003). These co-existing species might be sister taxa, if sympatric or parapatric speciation occurred.

Fish of the genus *Merluccius* most likely originate from the northwest Atlantic and the opening of the North Atlantic Basin in the mid Eocene, as well as a southward movement of fish due to decreasing temperatures might have let to a separation of the European-African lineage and the American lineage of hake (Grant & Leslie 2001). The European-African lineage can be separated by phylogenetic analyses into one group including *M. paradoxus*, *M. polli* and its subspecies *M. cadenati*, whereas the other group contains *M. merluccius*, *M. capensis* and *M. senegalensis* (Roldán et al. 1999, Quinteiro et al. 2000, Campo et al. 2007). The two lineages are likely to originate both

from an ancestral species in the northwest Atlantic, although their separation occurred most likely at different times (Quinteiro et al. 2000). The first lineage, including *M. paradoxus,* evolved probably several million years earlier than the second one, leading to sufficient genetic divergence to prevent introgression (Grant & Leslie 2001, Campo et al. 2007). Therefore, a sympatric speciation mechanism leading to several co-occurring African hake species can be rejected. More likely, vicariance and secondary contact through dispersal after divergence in allopatric isolation lead to the occurrence of sympatric distributions of hakes (Campo et al. 2007). This type of allopatric speciation seems to be common for temperate marine fish species (Grant & Leslie 2001).

The competitive exclusion principle (Gause 1934) states that no two species can occupy the same niche within the same ecosystem. If one species has a benefit over the other, it would better reproduce and outcompete the inferior species (Begon et al. 1996). Intra-specific competition occurs between individuals of the same species and was found to be generally density-dependent, whereas inter-specific competition exists between individuals of different species competing for the same resource. This competition can be direct, whereby the dominant species refuses access to the resource for the inferior species (interference competition) or indirect by reduction of the resource by the superior species without direct interaction with the inferior one (exploitation competition) (Begon et al. 1996). Direct and indirect competition can occur simultaneously and often have an effect on survival, growth and/or reproduction of the competing individuals. This can lead to a reduction in population size or even to the extinction of the inferior species.

Effects of competition can, however, be reduced through specialisation and niche separation (Werner & Gilliam 1984), which structures communities by interacting together with other mechanisms, e.g. predation (Schoener 1974). The ecological niche is the range of n-dimensional factors in which a species can exist (Gause 1934). Species usually do not occupy exactly the same niche but experience some

differentiation along at least one resource factor (Hardin 1960). As species primarily compete for space and food, niche separation in the oceanic habitat occurs mainly by three known mechanisms: 1) spatial separation, e.g. vertical partitioning, 2) temporal separation, e.g. in feeding or reproduction time, and 3) variations in physiology and behaviour, e.g. in feeding or reproduction (Madin & Madin 1995). Species with different developmental stages can occupy different niches during their lifecycle and the terms of competition, as well as mechanisms to reduce competition, can change during the different developmental phases (Werner & Gilliam 1984). Therefore, it is important to consider all ontogenetic stages when interpreting the mechanisms of co-existence in fish species.

1.3 Reproductive population connectivity

Many marine organisms have evolved a benthic adult stage and a pelagic larval phase, which allows a wide dispersal and can place offspring in habitats more favourable for their survival than the habitat of the adults (Cowen & Sponaugle 2009). Population connectivity is the exchange of individuals between spatially separated populations, and is a key process in population maintenance, local and metapopulation dynamics, genetic diversity, spread of invasive species and even in the resilience of populations to exploitation (Cowen et al. 2007). In this study, population connectivity measured at settlement time is differentiated from reproductive population connectivity, defined as the dispersal of populations combined with factors leading to survival of the dispersed organisms (Cowen & Sponaugle 2009). Part of the reproductive population connectivity is in turn demographic larval connectivity, meaning the transport and dispersal of larvae from spawning sites to nursery areas. Reproductive population connectivity also includes the migration of juveniles into an adult population and reproduction, which was not investigated in this study.

The knowledge of larval transport and dispersal is essential to understand the demographic connectivity between populations of different developmental stages of marine species (Pineda et al. 2007). First studies on connectivity investigated the self-recruitment level of coral reef fish (Jones et al. 1999, Jones et al. 2005, Almany et al. 2007) and until recently, larval connectivity was mainly investigated in coral reef fish and invertebrates (Bradbury et al. 2008a). As temperate fish have a longer pelagic larval phase and therefore greater dispersal probabilities, their populations seem to be more open than those of other marine species (Bradbury et al. 2008a). However, numerous recent studies showed that many species experience restricted dispersal with high levels of self-recruitment, partly due to high larval mortality or constrained transport possibilities (Pineda et al. 2007). Therefore, spatial connectivity of different life history stages of many species remains poorly understood. If larval dispersal and transport mechanisms fail, it can impact recruitment strength and population size. Thus, a better understanding of larval connectivity and its effect on cohort strength is important for fisheries management.

1.4 Recruitment variability

To understand recruitment variability, knowledge on larval connectivity can be regarded as the first step. However, larval connectivity also includes the survival of offspring, which in turn determines recruitment strength. Fluctuations in the population strength caused by recruitment variability are known from many fish species living in upwelling regions of the world's oceans (Hjort 1914, Cury & Roy 1989, Roy et al. 1992). Recruitment is defined as the number of fish from a year class reaching a certain age and adding to the exploitable stock. Recruitment variability is mainly caused by high mortality of fish eggs and larvae (Hollowed & Bailey 1989). Fish egg mortality may be caused by predation (Bailey & Houde 1989), but also, the maternal condition was found to affect egg viability and larval development in many fish species (Wiegand 1996). The

survival of larvae is influenced by predation and starvation and it is hypothesised that mortality is reduced with growth and development of larvae (Hjort 1914, Bailey & Houde 1989). During the larval stage, food availability and quality play an important role in development and survival (Hjort 1914, Jennings et al. 2001, Houde 2008), as low prey abundance and nutritional deficits can cause massive mortality during the early life of fish. Larvae are vulnerable to starvation, as they rely only on limited reserves of endogenous energy and are dependent on feeding success (Hjort 1914, Cushing 1975), which is below 25 % in most fish species (Hunter 1981).

Larval survival can also be influenced by different environmental factors such as variations in temperature, turbulence or currents (Hjort 1914, Cury & Roy 1989, Jennings et al. 2001, Houde 2008), which in turn can affect prey availability, transport or growth of larvae. Spawning and early life stage survival strategies can be adapted to biological and environmental factors to enhance larval survival. In ecology, different hypotheses were developed during the last century to explain mortality of early life stages, early life history strategies and the pronounced variability in recruitment of many marine fish species. In the 'aberrant drift' hypothesis, Hjort (1914) stated that the drift of eggs and larvae into an unfavourable environment for feeding could be responsible for high larval mortality and therefore variations in recruitment. The 'match-mismatch' hypothesis describes that spawning of fish has to be matched by high prey abundance to increase levels of larval survival rates in order to sustain recruitment (Cushing 1975). In addition, the retention of eggs and larvae was proposed to be critical for successful recruitment, as stated in the 'larval retention' hypothesis by Iles & Sinclair (1982) and Sinclair (1988). Two hypotheses were developed for recruitment in upwelling areas, explaining the high variability of fish recruitment in these systems. The first, the 'stable ocean' hypothesis by Lasker (1978) states that temporarily stable conditions in the water column cause the aggregation of fish larvae and their prey at stratification boundaries, supporting feeding and larval survival. Based on this, Cury and Roy (1989) and Roy et

al. (1992) then developed the second one, the 'optimal environmental window' hypothesis, which signifies a dome-shaped relationship between upwelling strength and recruitment. Intermediate upwelling intensity, which balances offshore losses and food availability and therefore prey encounter rates, can lead to optimal conditions for larval survival.

In general, no single one of all these hypotheses explains the recruitment variability of a fish species, as it is various factors or mechanisms acting together causing variances in the survival of early life stages. Predation is thought to be a major agent of mortality of eggs and larvae, but the extent of it is seldom quantified in the field (Houde 2008). Predation mortality is thought to be size-dependent, leading to the assumption that faster growth and a high condition improves survival ability (Houde 1987, Miller et al. 1988, Bailey & Houde 1989, Kamler 1992). Growth is influenced by temperature and condition (Buckley 1984), which is determined by maternal energy reserves incorporated into eggs and by the feeding of larvae (Clemmesen et al. 2003).

Since 2000, the *Merluccius* spp. stock sizes off South Africa have been decreasing, apparently due to low recruitment since the late 1990s (Butterworth & Rademeyer 2004). For a fish population to persist, a sufficient number of offspring must survive to replace the parental stock (Fogarty & O'Brian 2009). Mortality of the eggs and larvae can be extremely high and therefore even small changes in survival rate can cause extensive variability in recruitment (Houde 1989). Developing a refined understanding of the spawning and early life strategy, as well as of those larval traits leading to survival can be essential to understand the factors affecting recruitment. Investigation of the poorly understood early life history of *M. paradoxus* and *M. capensis* can provide information to explain the recruitment variability of demersal, long-lived fish species.

1.5 The biology of Merluccius paradoxus and M. capensis

Hakes are demersal gadoid species, widely distributed throughout the cool water systems of the Atlantic and the Pacific (Payne & Punt 1995). In most areas, the distribution ranges of two hake species partially overlap, e.g. that of *M. bilinearis* and *M. albidus* in the Northwest Atlantic, of *M. australis* and *M. hubbsi* in the southwest Atlantic and of the Cape hakes *M. paradoxus* and *M. capensis* in the Southeast Atlantic, investigated in this study (Cohen et al. 1990). The Cape hake adult composition off the west coast of South Africa and on the western Agulhas Bank was estimated to be 90% of *M. paradoxus*, whereas on the eastern Agulhas Bank and off Namibia 70% to 85% were thought to be *M. capensis* (Assorov & Berenbeim 1983, Payne 1989).
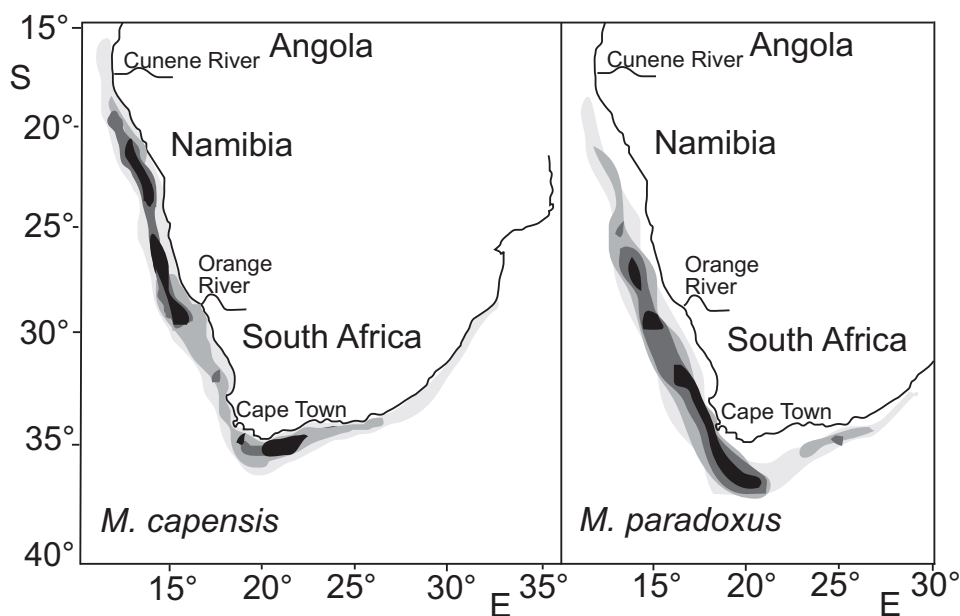

Figure 1 Distribution of adult fish of *M. capensis* and *M. paradoxus* (modified from Payne 1989). High relative abundances are indicated by darker colour.

The life history of Cape hakes has been described by Botha (1986) and Payne (1989), who noted that juveniles are found more inshore and that the size increases with depth. Both species are important predators in the Benguela upwelling system and different studies have found that they are opportunistic feeders, adapting their feeding mode to

the availability and abundance of prey (Payne & Punt 1995, Pillar & Barange 1995). Juveniles prey mainly on euphausiids and other shoaling crustaceans, while larger hake become more piscivorous (Payne et al. 1987, Payne & Punt 1995). Cannibalism of large hake on their juvenile conspecifics was found for both species, and also pseudo-cannibalism, feeding of large *M. paradoxus* on smaller *M. capensis,* is common due to the species' distributional overlap (Prenski 1986, Macpherson & Gordoa 1994, Gordoa et al. 1995, Payne & Punt 1995). Spawning of hake takes place in mid-water and there is evidence that *M. paradoxus* and *M. capensis* spawn at different depths (Botha 1973, O'Toole 1978, Olivar et al. 1988).

Until now, gaps in the knowledge of the biology of the two *Merluccius* species remain. In particular, their early-life history has received very little research attention until recently (Payne and Punt 1995, Gordoa et al. 1995, Hutchings et al. 2002). The pelagic eggs, yolk-sac larvae and their development were described by Olivar and Fortuno (1991) for both species. *Merluccius* eggs are spawned at depth, ascend in the water column as they are slightly positively buoyant and yolk-sac larvae hatch after 98 h at 12 °C (Sundby et al. 2001). In most of the existing studies, species identification of *M. paradoxus* and *M. capensis* eggs and larvae was based on the distribution of adult specimens in the study area (Sundby et al. 2001) or on the only actually visible difference between the two related species during the early life stage, namely the number of vertebrae (Olivar et al. 1988). *M. paradoxus* has 26 to 28 vertebrae, while *M. capensis* has 23 to 26 (Olivar & Fortuño 1991), making a differentiation still a challenging task. Some studies did not distinguish between eggs and larvae of the two species due to the lack of distinct morphological characters (Porebski 1975). So far, only two studies from one survey in 2005 used species-specific data on the early life stages of *M. paradoxus* and *M. capensis* applying genetics for identification purposes (Von der Heyden et al. 2007, Stenevik et al. 2008).

1.6 Objectives

The present study focused on the mechanisms enabling the co-existence and the early life strategy of two closely-related and ecologically similar demersal fish species, living in a highly variable ecosystem. The widespread co-occurrence, overlapping ranges and sympatric distribution patterns of the early stages of the two hake species raise the question, which mechanisms effectively minimise interspecific competition, also during the early life stages. Egg and larval dispersal, as well as larval traits leading to survival, were investigated in order to clarify the spawning and early life strategy of these demersal, long-lived, piscivorous fish species. In a variable environment like the Benguela upwelling system it is most likely that various factors act together, influencing hake larval survival. It can also be assumed that the species have certain adaptations to compensate negative effects, such as low food availability or unfavourable oceanographic conditions for larval survival. Thus, to understand the recruitment variability of hake larvae, it is essential to detect which environmental and biological factors affect survival during early life phases.

These mechanisms and processes were investigated by applying a variety of methods including lipid extraction and fatty acid analyses, RNA:DNA ratio measurements and otolith analyses as well as genetic methods for the identification of species. In detail, this work addresses three objectives which are summarised below.

Objective 1

To enable the co-existence of species with similar requirements, speciation and niche partitioning must occur to prevent the inferior species from being reduced in population size or becoming extinct. Mechanisms reducing competition must be incorporated in the life strategy of the two hake species allowing their sympatric distribution throughout their various life stages. Based on this assumption, differences were investigated with regard

to spawning and early survival strategy that allow the closely-related hake species to find distinct ecological niches.

Hypothesis: Mechanisms exist that reduce competition and enable the co-occurrence of the two closely-related demersal fish species.

The distribution of eggs and larvae of both hake species was investigated in order to gain knowledge on their spawning strategies. To confirm this first hypothesis, the spawning strategy and the occurrence of different developmental stages of the two species will be discussed in the light of distribution and dispersal processes as well as specific adaptations.

Objective 2

To secure larval connectivity, spawning strategies of fish have to be adapted to the environmental conditions they are experiencing in their ecosystem. Transport and dispersal of eggs and larvae determine larval connectivity. In a highly variable upwelling ecosystem, larval dispersal can be variable and transport mechanisms like current flow and retention are important for larval arrival at the nursery sites.

Hypothesis: Larval demographic connectivity is not only important in tropical reef organisms, but also in temperate, long-lived, demersal fish species, which have adaptations for optimal larval connectivity included in their spawning strategy.

The distribution and abundances of eggs and larvae of the two hake species were analysed together with hydrographical data. To test this second hypothesis, the spawning strategies as well as the dispersal and transport of eggs and larvae of both hake species will be discussed.

Objective 3

The early life strategy of species includes adaptations to their environment improving the survival of offspring. These adaptations include spawning strategies and larval traits,

such as growth and condition, which lead to a better viability. Many marine fish species experience pronounced fluctuations in recruitment. However, early life strategies should enable these species to sustain their populations.

Hypothesis: Both hake species have developed an early life strategy to enhance survival of offspring exhibiting specific adaptations to their highly variable ecosystem.

The RNA:DNA ratios, lipid contents and fatty acid compositions as well as growth rates were investigated to gain knowledge on the growth and condition of larvae and juveniles. To prove this third hypothesis, the spawning strategy and the characteristic traits of larvae enhancing survival are discussed and the early life strategy of hakes is classified.

2. MATERIALS AND METHODS

2.1 Study area

The major eastern boundary upwelling system in the South Atlantic is the Benguela current system, situated off the west coast of the African continent and extending from Angola at 17°S to 28°E around the South African coast to Port Elizabeth (Fig. 2). It is the only eastern subtropical upwelling region with a low-latitude poleward boundary, consisting of warm Agulhas water from the Indian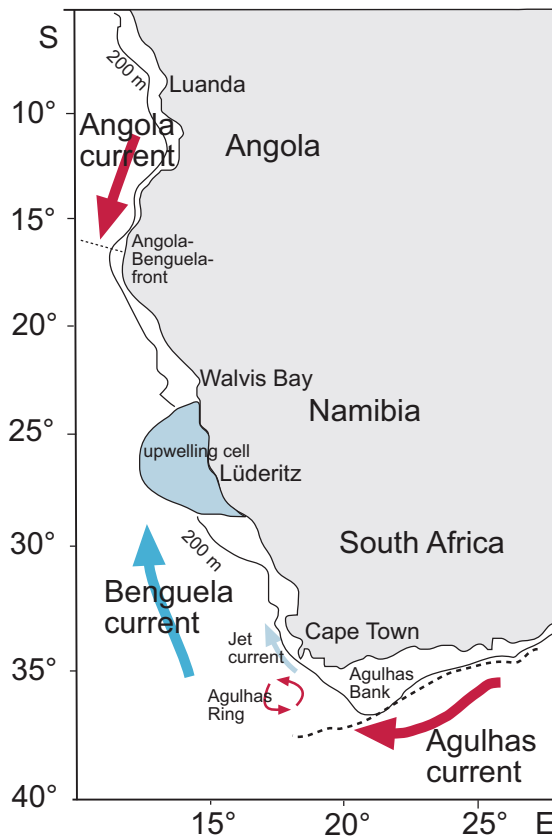 Ocean at the southern tip of Africa, and another border of warm tropical Angolan water to the north (Shelton et al. 1985). These fronts are very variable on temporal and spatial scales. Warm Agulhas surface water intrudes the system from the south mainly in form of Agulhas rings produced by perturbation in the Agulhas retroflection (Gordon 2003, Shannon & O'Toole 2003). The Benguela upwelling system can be divided into a northern and a southern system with differences in hydrographical dynamics and ecology, separated by the permanent Lüderitz upwelling cell (Shannon 1985).

Figure 2 The Benguela upwelling system.

Materials and methods

The upwelling is driven by coast-parallel, equatorward wind inducing offshore advection of surface water masses, which in turn leads to the ascent of nutrient-rich, cool South Atlantic central water to the surface near the coast (Shannon 1966). The nutrient rich water leads to high primary production, which supports high zooplankton production as well as small pelagic fisheries (Skogen 2005). Surface current flow is generally in equatorward direction with intense coastal upwelling cells, strongly seasonal between 30°-34°S, and a poleward undercurrent along the shelf slope and bottom as well as a narrow equatorward jet off the Cape Peninsula (Shannon 1966, Shannon 1985 , Veitch et al. 2009). The Agulhas Bank on the southern coast of South Africa extends 116 000 km² with a mean depth of 100 m. It is an important spawning ground for clupeid fish species of the Benguela system (Hutchings et al. 2002). Mainly in summer, warm Agulhas current water leads to stratification of the water column over the western Agulhas Bank (Shelton et al. 1985 , Largier et al. 1992).

2.2 Field work

Sampling of eggs and larvae of *Merluccius paradoxus* and *M. capensis* was carried out on four cruises, the first in January 2007, the second in April 2007, and the third in September 2007 on the RV *Dr. Fridtjof Nansen* and the fourth in September/October 2008 on the RV *Ellen Khuzwayo* along the west coast off South Africa (Table I).

Table I: Sampling details of four surveys indicating sampling date, number of stations, latitudinal range, research vessels and stages of fishes sampled.

Cruise	Stations	Latitudinal range	Research vessel	Total number of *M. paradoxus*			Total number of *M. capensis*		
				eggs	larvae	juve-niles	eggs	larvae	juve-niles
11.01.-2. 02.2007	42	34.8° - 31.1°S	Dr. Fridtjof Nansen	1	12	57	5	-	2
2.-15. 04.2007	90	34.8° - 27.9°S	Dr. Fridtjof Nansen	23	7	-	2	6	-
17.-21. 09.2007	52	34.7° - 33.8°S	Dr. Fridtjof Nansen	49	142	-	38	7	-
20.09.-20. 10.2008	121	36.0° - 31.1°S	Ellen Khuzwayo	95	224	-	7	17	-

Juveniles were collected on the first cruise in January 2007. Sampling areas covered the shelf and slope off South Africa and partly Namibia (Fig. 3). Sampling of larvae in September 2007 covered a smaller area due to time constraints. Details of sampling stations are mapped in chapter I (Grote et al. subm.-c). Temperatures, salinities and oxygen concentrations were measured with a Seabird CTDO probe between the surface and 10 m above the bottom at all stations.
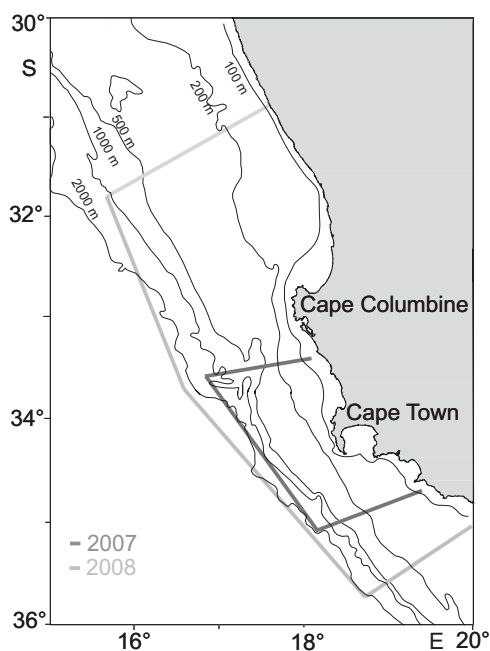

Figure 3 Study area in September 2007 (dark grey) and in January and April 2007 and September/October 2008 (light grey). Black dots indicate sampling stations for hake juveniles in January 2007.

Merluccius spp. eggs and larvae were sampled with a Hydrobios Multinet® plankton sampler (0.25 m² mouth area), equipped with five nets of 405 µm mesh size, collecting plankton samples in five depth intervals of 50 m from maximum 250 m to the surface. A Scanmar depth recorder with acoustic transmission to the vessel was mounted on top of the Multinet to monitor net depth. The plankton sampler was heaved in an oblique mode at a speed of 0.5 m s^{-1} with the ship maintaining a ground speed of 2 kn. Juvenile hake up to a size of 100 mm were collected in pelagic and bottom trawls. Eggs, larvae and juveniles were sorted based on the descriptions of both species by Porebski (1975) and Olivar and Fortuño (1991). In 2008, eggs were caught in sufficient amounts for biochemical analyses and they were staged into two groups, early (I) and late, indicating a visible embryo (II). All hake larvae and juveniles were measured to the nearest 0.1 mm or 1.0 mm (total length), respectively. Eggs and larvae of hake were briefly rinsed in distilled water and all samples were frozen in liquid nitrogen immediately

after length measurement or staging, but always within 30 min after the haul was on board.

2.3   *Analytical work*

Hake early life stage survival – biological and biochemical approaches

The presented biological and biochemical approaches were used in this study to investigate growth, condition and feeding of *M. paradoxus* and *M. capensis* early life stages in order to shed light on the factors influencing larval survival.

Survival of larvae can be influenced by factors acting at large spatial scales, such as environmental factors and primary productivity and therefore food supply (Lloret et al. 2001, Olivar et al. 2003). Food quality as well as intra- and interspecific competition may influence larval growth and condition (Lloret et al. 2002, Maynou et al. 2003). This in turn, can affect predation vulnerability and consequently the strength of a cohort (Bailey & Houde 1989). Thus, larval growth rate and condition measurements are effective methods for evaluating survival chances of individual larvae (Houde 1987, Clemmesen & Doan 1996, Meekan & Frontier 1996).

Growth

Daily increments are deposited in otoliths and can be used to determine growth rates, for ageing and back-calculation of hatch dates as well as to measure daily variations in growth of larvae and juveniles (Pannella 1971, Campana & Neilson 1985, Campana & Jones 1992, Campana & Thorrold 2001). Daily otolith increment deposition was validated for *M. productus* (Bailey 1981) and for *M. merluccius* (Arneri & Morales-Nin 2000). Previous studies on growth of *M. capensis* or *M. paradoxus* focused on length-weight relationships of juveniles and adults (Kono 1980, Chlapowski 1982, Prenski 1984) and otoliths were analysed in only one study of *M. capensis* juveniles (Roux 2006). Growth of juveniles was compared with that of larvae to estimate the growth rates of surviving larvae.

Condition

Biochemical indices can define the conditions of early life stages and can indicate growth potential (Buckley 1984, Clemmesen & Doan 1996, Caldarone et al. 2003). Such indices can be nucleic acid ratios or lipid contents (Buckley 1984, Rainuzzo et al. 1997, Tocher 2003, Caldarone 2005). The RNA:DNA ratio is an index for protein synthesis in a cell and an indicator of nutritional condition and starvation of fish larvae (Buckley 1979, Clemmesen et al. 1997). So far, only few studies analysed RNA:DNA ratios and growth rates together on the same larva, e.g. haddock, *Melanogrammus aeglefinus* (Caldarone 2005) and cod, *Gadus morhua* (Clemmesen & Doan 1996).

Lipid content analyses reveal information on the energy reserves of larvae and therefore indicate growth potential and survival probabilities, whereas the lipid content of eggs can indicate the condition of broodstock (Rainuzzo et al. 1997, Tocher 2003). The fatty acid compositions of fish larvae reveal the amount of certain essential fatty acids, which are important for development and growth of larvae (Sargent et al. 1999). Dietary lipids are an important source of essential fatty acids and they include some fatty acids, which can be used as lipid biomarkers (Sargent et al. 1987, Rainuzzo et al. 1997, Dalsgaard et al. 2003). In addition to stomach content analyses, these lipid biomarkers reveal longer-term information of the feeding regime (Sargent et al. 1987, St.John & Lund 1996).

Analytics

All analyses were conducted on the same individual larva to obtain a high-resolution data set. For the first time, these analyses were used to investigate hake growth and condition. Prior to further analyses, all deep frozen eggs, larvae and juveniles were lyophilised for 24 or 48 hours for bigger samples (Leybold-Heraeus, LYOVAC GT2 freeze-drier) and weighed to the nearest 0.1 µg (Sartorius microbalance MC21 S). After mass determination, larvae were moisturised with distilled water and sagitta otoliths and, if present, stomach contents were removed as quickly as possible from the samples with fine insect needles under a dissecting microscope and samples were returned to the

deep-freezer immediately afterwards. The sagitta otoliths of juveniles were dissected in the same way. Subsequently, eggs and larvae were stored in 1.5 ml dichloromethane:methanol (2:1 per volume) for seven days in the -80°C deep-freezer to extract all lipids. After the extraction of lipids, eggs were used for genetic analyses, whereas larvae were used for RNA:DNA ratio analyses, followed by genetic analyses. Details of these analytical methods are described in the 'Materials & Methods' parts of chapter II (Growth rates, RNA:DNA ratios and genetics, Grote et al. subm.-a) and chapter III (Lipid content, fatty acid analyses and genetics, Grote et al. subm.-b).

3.   RESULTS AND SYNOPTIC DISCUSSION

A major focus of this thesis was to elucidate the mechanisms leading to the co-existence of two related and ecologically similar fish species. In addition, the present work aimed at revealing larval demographic connectivity between spawning sites and nursery areas as well as the early survival strategy of long-lived, demersal fish species. South African Cape hakes *Merluccius paradoxus* and *M. capensis* were used as model organisms to investigate these scientific issues. The findings were applied to draw conclusions for fish recruitment, fisheries management and climate change scenarios. New scientific questions, which emerged during this study, are presented in an outlook on future research.

3.1 The co-occurrence of two related fish species

Sympatric distribution

The differentiation of the two Cape hake species was introduced by Franca (1960) based on small morphological differences such as the number of vertebrae and Van Eck (1969) by the pigmentation of the gill rakers. However, the two hake were still thought to be sympatric subspecies or sister species, possibly hybridising. Subspecies represent an independent lineage, but reproductive isolation from the other subspecies group is incomplete (Mayr & Ashlock 1991). In this study, all early life stages of the two hake species were allocated to one or the other species and no indication of hybridisation was apparent (Fig. 4).

Geographical distribution and morphological differences were used historically to assess subspecies until the new approach of molecular phylogenetic analyses was developed. Even the analyses of a few individuals to identify the phylogenetic lineages still defines
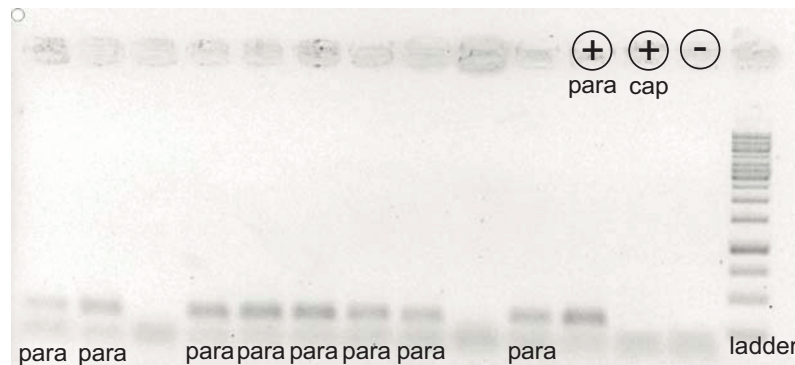
Figure 4 Genetic analyses of larval DNA samples with a primer for identification of *M. paradoxus*. Positive signs mark position of adult samples of *M. paradoxus* (para) and *M. capensis* (cap); negative sign marks negative control.

species, as long as the intraspecific differentiation among populations is lower than the divergence among species (Grant & Leslie 2001). Low levels of genetic differentiation were detected in hake populations, even if they were spatially well separated (Von der Heyden et al. 2009), which implies high migratory and dispersal potential between populations. Genetic analyses of mitochondrial DNA of the two related species *M. paradoxus* and *M. capensis* have determined that they represent well separated species from different lineages, despite many morphological similarities (Grant et al. 1988, Von der Heyden et al. 2007).

Co-existence and the ecological niche

The understanding of mechanisms enabling the co-existence of species with similar requirements is of basic ecological interest. The ecological niche concept states that no two species can occupy the same niche within the same ecosystem (Gause 1934). One species would be better adapted to certain conditions and would outcompete the other. Species with sympatric distribution and similar feeding requirements are likely to compete for the same resources (Evans 1983, Ross 1986, Gerking 1994), which will most likely result in adaptations to minimise competition and maximise resource

utilisation. Some of the mechanisms of co-occurrence of closely related fish species are thought to be feeding mode, resource-sharing or an unlimited resource itself (Smith & Tylor 1973, Jones 1978, Gerking 1994).

For species that change their life style with different developmental stages, the niche concept has to be reconciled, as the niche of the species changes with its various life-history phases (Werner & Gilliam 1984). Reducing competition between two sympatric species in the adult life stages by vertical partitioning is a mechanism to enable the co-existence of *M. capensis* and *M. paradoxus*. This study has shown that competition during the early life stages might play a crucial role as larvae of both species are feeding on the same prey. Larvae of the two species in both years preyed mainly on different life stages of small cyclopoid copepods (Fig. 5).
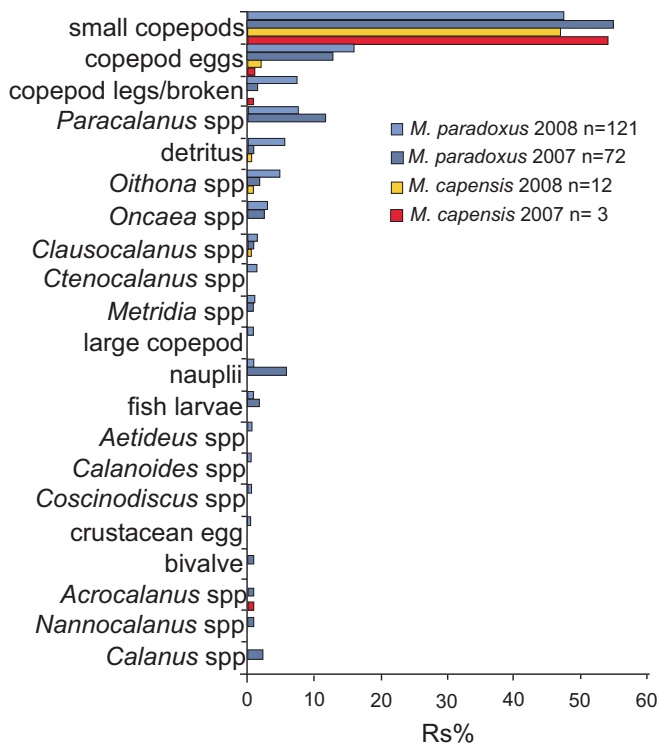

Figure 5 Simple resultant index (Rs, %) of stomach contents of *M. paradoxus* and *M. capensis* larvae in 2007 and 2008.

The temporal separation of larval stages of fish species is known as a possible mechanism to minimise competition for food (May 1974, Floyd et al. 1984). Peak spawning of *M. paradoxus* and *M. capensis* and therefore the occurrence of their larval

stages appears to be temporally and spatially separated (Chapter I, Grote et al. subm.-c). Since *M. capensis* larvae were not caught in the Multinet on the west coast, it can be assumed that they were spawned further inshore on the eastern Agulhas Bank. These larvae would have been in a more advanced developmental stage when reaching the west coast, which may have enabled them to avoid the sampling gear. Furthermore, the separation of spawning grounds is indicated by the difference in transport routes of eggs and larvae of the two hake species, with *M. capensis* found further inshore than *M. paradoxus* (Chapter I, Grote et al. subm.-c). The spatial difference in the spawning of *M. paradoxus* and *M. capensis* could minimise competition for food, as the larvae of the two species would be separated by size when co-occurring. At the same time, this would enhance the possibility of pseudo-cannibalism of larger *M. capensis* larvae or early juveniles on smaller *M. paradoxus* specimens. Variation in the timing of peak spawning of both species could be a way to avoid this negative effect (Chapter I, Grote et al. subm.-c). Peak spawning of *M. paradoxus* occurred in September to October, whereas peak spawning of *M. capensis* was not taking place during these months. Temporal and spatial separation between peak spawning of the two hake species can thus be regarded as a mechanism to avoid competition during the critical life phase of first feeding and the subsequent life stages. Furthermore, competition between juveniles of the two species is minimised by different transport routes to separate nursery grounds (Chapter I, Grote et al. subm.-c, Tore Strømme pers. comm.).

The co-occurrence of the two hake species *M. paradoxus* and *M. capensis* in the southern Benguela upwelling system during the adult life stage seems to be attributed to the difference in depth distribution of adult fish (Botha 1985, Payne & Punt 1995). Vertical separation of habitats enables the co-existence of two species, if their competitive efficiency differs in the two habitats, such that species *a* outcompetes species *b* in one habitat, while in the alternative habitat species *b* is dominant (Schreiber & Kenton 2005). Such competitive displacement has been described for the code gobies

Gobiosoma robustum and *Microgobius gulosus,* with a sympatric distribution in Florida Bay, where the former is most abundant in seagrass habitats, whereas the latter is found in areas of low vegetation (Schofield 2003). In aquarium experiments, both species preferred the seagrass habitat if separated, but in sympatry, *G. robustum* displaces *M. gulosus* towards bare habitat, demonstrating that interspecific competition appears to directly modify habitat choice of species. If a species is able to avoid competition by moving towards a habitat on the edge of its optimum requirements, it might be able to succeed there (Williams 1988). Apparently, *M. paradoxus* may be better adapted to a deeper, colder and possibly lower oxygen environment than *M. capensis*, which allows the former to inhabit a deeper habitat. Therefore, the difference in depth distribution might be a result of competition between the two species, by which the older species *M. paradoxus* is displaced by the later evolved species *M. capensis* (Von der Heyden et al. 2007).

Large *M. capensis* prey pseudo-cannibalistically on smaller *M. paradoxus* when their distributions overlap (Payne & Punt 1995). This confirms the dominance of the former species over the latter, regulating the distribution of *M. paradoxus* towards shallower waters. The varying distribution of different-sized hakes with depth is therefore a mechanism to reduce cannibalism and competition for food. However, it is likely that competition between the two hake species still occurs and that this is the reason for the relatively low population size of *M. paradoxus* (Von der Heyden et al. 2007). Another mechanism to avoid competition and to allow co-occurrence of species besides spatial separation is the utilisation of different resources (Werner & Gilliam 1984, Sabatés & Saiz 2000). The absence of niche partitioning through different feeding modes or prey types seems to be unusual in gadoid species, with hake being the exception. The two closely related and morphologically similar gadoid species saithe, *Pollachius virens*, and pollock, *P. pollachius*, with sympatric distributions in the Scottish Sea, can co-exist through differentiation in feeding strategies (Sarno et al. 1994). For the gadoid species

saithe (*Pollachius virens*), pollock (*Pollachius pollachius*), haddock (*Melanogrammus aeglefinus*) and cod (*Gadus morhua*) in the North Atlantic, it was found that their sympatric distributions are enabled by adaptations minimising the overlap in feeding resources and by taking advantage of very abundant prey (Høines & Bergstad 1999). Separation of feeding mode or prey type has not been reported for any co-existing hake species, when they were feeding within the same habitat (Payne et al. 1987, Bezzi et al. 1995, Martos & Peralta 1995), indicating that they compete for food. However, there are several examples of vertical habitat partitioning for co-occurring hake species (Grant & Leslie 2001). This strategy appears to be an important mechanism of sustaining co-existence of hakes within the same habitat.

In conclusion, two of the three processes most important in enabling co-occurrence of species, namely spatial and temporal divergence of the two hake species, act as stabilising factors allowing their co-existence during the larval stage. The third process, prey partitioning during the early life stages, was not detected during this study. In addition, the question emerged as to whether the co-existence of the two hake species could persist because of *M. paradoxus* being better adapted to a deeper environment.

Key findings

➢ **Peak *M. capensis* spawning occurs earlier than the peak spawning of *M. paradoxus,* which takes place in September to October.**

➢ ***M. capensis* spawning takes place on the eastern Agulhas Bank, whereas the main spawning ground of *M. paradoxus* lies partly on the western Agulhas Bank and partly off the South African west coast.**

> **The two hake species can co-exist as their niches during their early life stages are separated**

- o **by temporal and spatial divergence of spawning.**
- o **by spatial separation of nursery grounds.**

This explains to a large extent the co-existence mechanisms enabling the two related species to live in the same ecosystem. However, to sustain a sufficient population size in a variable environment, both species need efficient spawning and survival strategies for their early life stages…

3.2 The dispersal of larvae and implications for connectivity

Larval connectivity

Understanding larval transport and dispersal provides the basis of knowledge on population demographic connectivity and growth. The transport and dispersal of offspring can be regarded as larval connectivity and is a part of population connectivity (Pineda et al. 2007). Many demersal fish populations have pelagic early life stages and rely on the transport of eggs and larvae towards nursery grounds, where conditions for survival of the early life stages are better than in the adult habitats (Leis 2006, Bradbury et al. 2008a). Large-scale circulations, for example eastern boundary currents, or processes like coastal upwelling and coastally trapped waves, are energetic and coherent in the alongshore direction. They influence small-scale processes, which in turn can enhance or suppress larval transport (Pineda et al. 2007). These small-scale processes are extremely important for species with inshore nursery areas, as once larvae have been transported to the inshore nursery area, they need to be retained there.

In the southern Benguela, the spawning strategy of hakes is well adapted to a time of optimal transport to nursery areas and retention for larvae that have reached these inshore areas (Chapter I, Grote et al. subm.-c). The spawning grounds of the two hake species are located upstream and nursery grounds downstream of the main current flow, which is influenced by bottom topography (Chapter I, Grote et al. subm.-c). Furthermore, the assumed earlier inshore spawning of *M. capensis* leads to the transport of larvae to the inshore area of St. Helena Bay, South Africa (Chapter I, Grote et al. subm.-c), whereas later offshore spawning of *M. paradoxus* supports main transport of eggs and larvae to a nursery area off Orange River, Namibia.

A patchy distribution or schooling behaviour of larvae and juveniles, as found for Cape hakes (Chapter I, Grote et al. subm.-c), are common in marine fish species (Houde 1987). Early larvae with no swimming ability are passively transported by currents, whereas more developed larvae and juveniles have swimming capabilities, which can improve accumulation and schooling (Leis 2006). Thus, the transport of older larvae and juveniles can also be influenced by small-scale vertical and horizontal larval behavioural responses (Leis 2006). The extent, to which these behavioural responses can influence larval transport, depends on the development time of larvae. Pelagic larval duration (PLD) and development time are considerably affected by temperature (Houde 1989, O'Connor et al. 2007). Larval dispersal is thus a complex function of PLD, development stage and environmental factors, such as temperature and current flow from spawning kernel to nursery habitats. For *M. paradoxus*, the spawning kernel is located on the western Agulhas Bank and PLD seems to be adapted to the prevailing environmental conditions during the main spawning period (Chapter I, Grote et al. subm.-c). This adaptation includes fast growth during the early life phases and transport by the relatively warm water masses of Agulhas Bank filaments merging into the fast shelf-edge jet current (Grote et al. 2007, Chapter II, Grote et al. subm.-a, Chapter I, Grote et al. subm.-c). If transport or retention of eggs and larvae failed, a substantial number of

hake offspring could be advected offshore into the unfavourable open ocean environment leading to high mortality.

The whole population connectivity of hake is not fully understood, although temperate demersal species populations are generally regarded to be open (Cowen 2000). For Newfoundland cod (*Gadus morhua*), localised dispersal and self-recruitment was found, despite the high dispersal potential of larvae and juveniles (Bradbury et al. 2008b). In general, studies on dispersal of higher latitude demersal species, e.g. hakes, are underrepresented to date (Bradbury et al. 2008a). The deep-water hake, *M. paradoxus,* was thought to consist of only one population off Namibia and South Africa, as its population can distribute at depth along the whole west coast of southern Africa. For *M. capensis*, it was assumed that populations off South Africa and Namibia are separated by the permanent Lüderitz upwelling cell, presenting a barrier for the distribution of this shallow-water hake. Analyses of the genetic structure of these populations suggest exactly the opposite for both hake species (Von der Heyden et al. 2006, 2009). *M. paradoxus* seems to maintain a self-recruiting population off South Africa with a northward nursery area off Orange River, whereas *M. capensis* has a more open population with connectivity between habitats off Namibia and South Africa. Larval distribution and hydrography patterns do not indicate pronounced dispersal of *M. capensis* larvae along the coast as far north as Namibia (Chapter I, Grote et al. subm.-c). Hence, only migration of juveniles or adults could lead to the observed genetic population structure. It is assumed that both species of hake migrate along the South African and Namibian coasts, although the extent of this migration remains unknown (Botha 1973). Genetic analyses did not reveal the full extent of the population structure of both African hake species (Von der Heyden et al. 2006, 2009), and further research of population connectivity is needed in this regard.

In conclusion, larval connectivity is a major factor in the survival of the Cape hake species. The spawning strategies of hakes seem to be well adapted to ensure larval connectivity, which generally involves larval dispersal and transport towards nursery areas. This implies that larval connectivity might be a factor influencing survival and recruitment in other demersal species. Population connectivity remains a complex issue, which requires more research as genetic and demographic connectivity are not well resolved and, so far, have yielded somewhat contradictory results.

Key findings

> **The two hake species have developed a spawning strategy in which larval connectivity, namely dispersal and transport to nursery areas, is very important.**

> **Small-scale retention mechanisms play a major role in larval connectivity of the two hake species.**

Reaching a nursery site does not secure larval survival as larvae need specific traits to survive and recruit, other than transport and dispersal…

3.3 Early survival strategy

Spawning strategy

No single factor is responsible for the survival of fish larvae and different factors often act in combination. Early life strategies have to be adapted to these factors to secure maximum survivorship to maintain the population. As shown in chapter 3.2, spawning needs to be tuned to optimal environmental conditions for larval transport to nursery

sites. A similar synchronisation of spawning strategies to optimal environmental conditions were found in other fish species, e.g. for anchovy (*Engraulis encrasicolus*), cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) (Sherman et al. 1984, Huggett et al. 2003). In addition, other seasonally varying factors, e.g. food availability and temperature, can influence larval survival (Hunter 1981). Voss et al. (2006) showed that the 'windows of survival' do not necessarily coincide with peak spawning, as Baltic sprat, *Sprattus sprattus*, larvae from late spawning had a higher survival rate than larvae from the earlier peak spawning time, due to differences in food availability. Peak spawning of the Cape hakes occurs during a time of intermediate upwelling (Grote et al. 2007), when transport and feeding conditions are favourable for survival of offspring (Chapter I, Grote et al. subm.-c). Hakes have an indeterminate fecundity and a protracted spawning season, which is unusual for gadoid species (Murua & Motos 2006), but is likely to be an adaptation to a highly variable environment, which only provides very pulsed optimal feeding conditions. All year spawning with a peak during the most optimal season for larval survival secures recruitment for population maintenance.

Effect of traits on larval survival

Survival potential is highly affected by stage duration, which is dependent on the growth rate (Houde 1987). The survivors should be those larvae which grow faster, as they gain an advantage over their smaller conspecifics, which are more vulnerable to predation (Sogard 1997). Higher growth rates are generally found in temperate and tropical species experiencing unstable environments (Fonseca & Cabral 2007). The Cape hakes *M. paradoxus* and *M. capensis* are able to enhance survival through very high growth rates during early life stages (Chapter II, Grote et al. subm.-a).

Species with fast growth rates often experience high RNA:DNA ratios, indicating higher metabolic investment in protein synthesis (Fonseca & Cabral 2007). The development of RNA:DNA -temperature-growth models proved to be possible for some temperate species, such as haddock (*Melanogrammus aeglefinus*) and cod (*Gadus morhua*) (Buckley 1984, Clemmesen & Doan 1996, Peck et al. 2003, Caldarone 2005). This relationship between RNA:DNA ratio and growth was not confirmed for hake in this study (Chapter II, Grote et al. subm.-a). In addition, no correlation was found between the two proxies for condition, lipid content and RNA:DNA ratio, as well as between lipid content and growth rate. One explanation for this could be that the growth rates of early larvae (≤ 22 days) and the different proxies used to describe the condition of hake larvae, act on different time scales. The condition proxies showed no correlation, as the lipid reserves are diminished faster than the protein metabolism is slowed down, when feeding conditions are worsening. Lipid content shows an immediate response to low prey availability, whereas RNA:DNA ratios react more slowly and growth rates respond with an even greater time lag. In a highly variable environment like an upwelling system, fast growth secures survival and therefore all energy of hake early life stages will be allocated to fast growth, thus rapidly depleting lipid reserves, but maintaining high growth rates as long as possible. However, this time lag was not detectable in the samples, as the age range of larvae was relatively small and since all parameters changed with development stages of larvae (Fig. 6). Another reason for the lack of correlation between growth rate and condition proxies could be that the condition of hake larvae, in terms of lipid content and RNA:DNA ratios, was generally sufficient to sustain growth (Fig. 6). Generally, the surviving larvae are caught in the field, because the starving larvae are the most vulnerable to predation. This latter explanation is supported by the finding that all investigated hake larvae had RNA:DNA ratios above the calculated critical threshold levels for growth at the temperature they were experiencing (Chapter II, Grote et al. subm.-a).

Measurement of condition, in order to relate environmental variability to larval growth potential and fitness, is a recent subject in fish larval ecology. Feeding during the early life stages is probably not as critical for larval survival as previously thought, since micro-turbulences provide mechanisms to increase prey encounter rates (MacKenzie & Miller 1994). However, prey availability and food quality are still regarded as major factors influencing the condition of feeding larvae (Houde 2008). Although the main prey of hake larvae are very abundant small copepods (Fig. 5), energy reserves of larvae are not very high (Chapter III, Grote et al. subm.-b). Energy gained by feeding is presumably directly allocated to fast growth, allowing only few days of starvation resistance (Chapter III, Grote et al. subm.-b). The abundance of small copepods has increased during the last decades in the southern Benguela system (Verheye et al. 1998), securing the feeding of early hake larvae. In addition, the quality of food may be important for the development of *M. paradoxus* and *M. capensis* larvae (Chapter III, Grote et al. subm.-b),
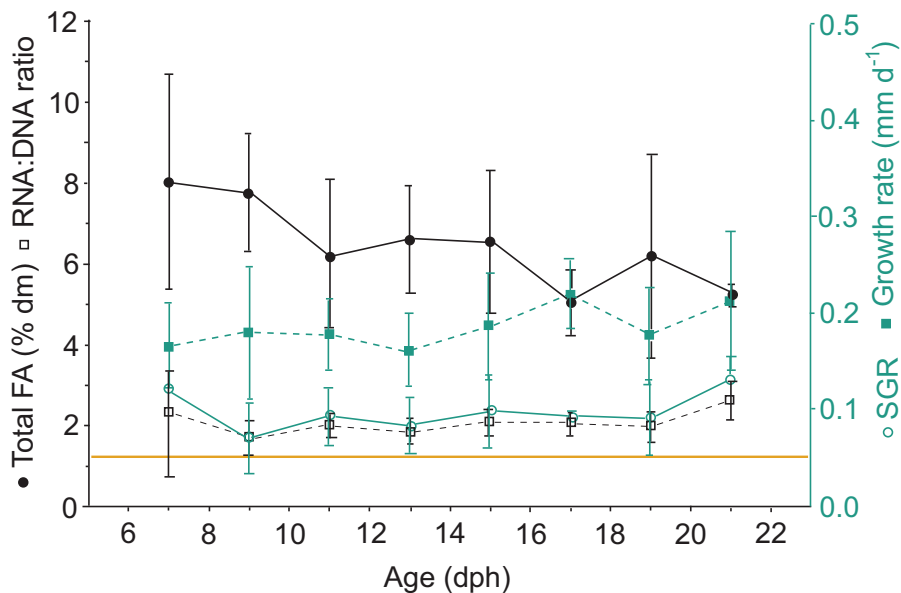

Figure 6 Total fatty acid content (% dry mass), RNA:DNA ratios, somatic growth rate (SGR; calculated from RNA:DNA ratios and temperature (Chapter II, Grote et al. subm.-a)) and individual growth rate (mm/d; calculated from the last three otolith increments) versus age (days post hatching) of *M. paradoxus* larvae in 2008.

as essential fatty acids mainly originate from incorporation into eggs by spawners or from food sources (Rainuzzo et al. 1997). For instance, the sufficient amount of the essential fatty acid docosahexaenoic acid (DHA) is crucial for the development of the neural system and a DHA deficiency can be detrimental to fast growing fish species (Mourente 2003), such as hake.

For the survival of juvenile stages, hake most likely adapted the strategy of cannibalism, securing survival during times of low food abundance in a variable environment (Chapter II, Grote et al. subm.-a), where food for larger size classes seems to be pulsed due to variable upwelling intensities (Van der Lingen et al. 2006). Hake show a dietary preference for small (Macpherson & Gordoa 1994), indicating that it could not only be a strategy to overcome times of low food abundance, but also to recycle energy within the population (Cushing 1991). Based on the findings in this study and the knowledge of cannibalism in some other gadoid species, such as cod (*Gadus morhua*) (Folkvord 1997) or walleye pollock (*Theragra chalcogramma*) (Wespestad et al. 2000), it can be inferred that cannibalism is not an unusual component in the life strategies of large, piscivorous fish species.

Maternal effects

Fish species with a high fecundity and longevity are able to produce strong recruitment, giving rise to a cohort capable of sustaining the population over a number of potential reproductive periods, as stated in the 'storage model' (Warnerand & Chesson 1985). This model was often revived by coral reef fish studies to explain species co-existence (e.g. Abrams 1984, Munday 2004), but it also accounts for long-lived fecund species such as hake. These species rely on older age classes to maintain the population (Warnerand & Chesson 1985), as the large, old females produce more and/or bigger eggs compared to younger females and are responsible for a large part of the

recruitment (Longhurst 2002). Maternal condition has only recently been studied as a factor to explain larval survival in fish species (Houde 2008), although it was recognised relatively early to play an important role in offspring survival (Hjort 1914). The nutritional condition of spawners affects the energy reserves of yolk-sac larvae and therefore their resistance to starvation and predation (Domínguez-Petit et al. 2009). Therefore, low lipid contents in yolk-sac larvae of hake indicate poor condition of spawners (Chapter III, Grote et al. subm.-b). Environmental factors influencing prey availability on the spawning sites can cause a decline in female condition, especially in fish such as hake, which feed continuously during their spawning season (Domínguez-Petit et al. 2009). As poor condition of female spawners can reduce reproductive potential or even larval viability of long-lived fish species (Marteinsdottir & Steinarsson 1998, Óskarsson et al. 2002, Blanchard et al. 2003), recruitment variability could result partially from variations in spawner conditions. Thus, maternal condition is a major factor in hake recruitment and highlights that hake larval survival can be affected as early as the egg stage.

Life history strategy

According to Winemiller and Rose (1992), three different types of life-history strategies exist in fish species. The 3-endpoint life-history model is based on trade-offs among survival, reproduction and age at maturation (Table II):

Table II 3-endpoint model for life-history strategies of fish species (Winemiller & Rose 1992).

	opportunistic strategy	periodic strategy	equilibrium strategy
fecundity	low	high	low
maturity	early	late	delayed
individual size	small	large	small to medium
spawning	repeated	few	parental care
egg size	small	small	large
spawning season	prolonged	short	prolonged

Hake have a high fecundity, late maturation, large individual size, fast growth and small eggs (Pitcher & Alheit 1995), indicating that they follow a periodic strategy. However, hakes also have a protracted spawning season and several spawning events (Chapter I, Grote et al. subm.-c), representing the opportunistic strategy. Such an intermediate life strategy, like for hakes being between the periodic and opportunistic endpoints in the life-history model, is known for several other marine species, such as rockfishes (*Sebastes* spp.) and coho salmon (*Oncorhynchus kisutch*) (Winemiller & Rose 1992). The protracted spawning period of hakes is an adaptation to a variable environment to enhance survival chances of larvae (Chapter 3.3). Therefore, hakes appear to follow a periodic life-history strategy, with some adaptations towards an opportunistic strategy. This example shows that life histories of fish species are not always distinctive, but that intermediate strategies can be favourable in unstable environments.

In conclusion, hake eggs have to be spawned at the right time for optimal transport and retention of larvae to nursery areas. They need sufficient energy reserves from their mothers and they have to feed to gain energy and to sustain fast growth in order to belong to the survivors. Hake early life strategy is thus well adapted to a variable environment due to characteristics, such as a protracted spawning season, fast growth and cannibalism.

Key findings

> **Hakes are fast-growing species, feeding mainly on small cyclopoid copepods during their first 22 days of life.**
> **Cannibalism seems to be a common strategy to overcome periodic food shortages during the early life stages of large, piscivorous fish species.**
> **Lipid content is a valuable proxy for the condition of hake yolk-sac larvae.**
> **Maternal condition is an important factor for egg quality and thus for larval survival and recruitment in long-lived species, such as hake.**

> ➢ **The life history strategy of hakes can be described as intermediate between a periodic strategy and an opportunistic one.**
>
> ➢ **Investigation of larval growth rates and characteristics of survivors is a useful approach for further studies of recruitment processes.**

3.4 Implementation of results and perspectives

…for recruitment

One of the goals of fisheries science is to gain a better understanding of recruitment by following the life cycle of a cohort from egg to recruitment (Cushing 1985), which can be achieved by investigating the spatial and temporal differences in early life stage survival (Irigoien et al. 2008). The information on the early life history of hakes, gained through this study, provides the basis for understanding mechanisms of recruitment for demersal fish species living in variable ecosystems. Although in this study it was not possible to follow the same cohort, the combination of conventional and ecophysiological methods to investigate traits of the different life stages of hake has revealed new insights into their early life stage survival and biology. No single concept can explain recruitment variations for all marine species. However, there seem to be basic patterns of life strategy and adaptations for species experiencing the same environment.

Hydrographical features such as current flow, retention mechanisms and meso- and small-scale processes play a significant role in egg and larval transport of hake (Chapter I, Grote et al. subm.-c). These features can reduce or enhance larval survival and therefore recruitment. Furthermore, the larval traits condition and growth rate were found to be important factors influencing larval survival (Chapter II, Grote et al. subm.-a, Chapter III, Grote et al. subm.-b). Cannibalism may decrease overall recruitment, but secures survival of the fastest growing juveniles in the cohort and therefore also secures a certain level of recruitment (Chapter II, Grote et al. subm.-a). In addition, evidence

suggests that maternal condition has been underestimated in the recruitment of hake (Chapter III, Grote et al. subm.-b) and probably of many other long-lived, demersal fish species. Finally, recruitment of hake is impacted by many different factors influencing egg and larval survival chances, as well as possibly the viability of juvenile stages. Growth rates and larval condition are likely to provide good proxies for recruitment and cohort strength estimates.

<div align="center">

…for fisheries

</div>

For hake, as for most other long-lived species, 'big old fat fecund female fish' (BOFFFF) produce a substantial number of viable offspring (Field et al. 2008). These fish are also the main target of the longline fishery, which was established in the 1990s, as the demand for fresh hake in Europe increased. The longline fishery explored rocky areas, which had previously given shelter to large old fish of both hake species, as these places could not be trawled. As the large old females are thought to contribute substantially to recruitment (Berkeley et al. 2004), hence, the reduction of large, fast-growing females through longline fisheries could explain the reduced recruitment since the late 1990s. As suggested by Field et al. (2008), fisheries management has to take age-related stock-recruit relationships into account, since in long-lived fish species assumptions of proportionality between biomass and reproduction or a constant sex ratio and mean fecundity do not have to be generally valid. An age-structure based management, which accounts for reproductive potential of large, old females, would be a better option to manage long-lived fish species such as hake. Survival patterns of larval stages can be used to improve management strategies, e.g. area or season closures, to protect those fish likely to produce viable offspring. These results emphasise the need of a profound understanding of the biology, including the early life history of commercially exploited species in order to assure their sustainable management.

…for climate change

Climate change has many expected impacts on the world's oceans including increasing temperature, sea level rise, ocean acidification and alterations in currents (IPCC 2007). These changes are likely to impact fish and fisheries, because they will affect fish migrations and recruitment, which will be reflected in the distribution and abundance of fish species (Roessig et al. 2004). Spawning behaviour of Pacific hake (*Merluccius productus*) was found to be influenced by changes in climate regime (McFarlane et al. 2000). Furthermore, changes in current flow induced by El Niño Southern Oscillation (ENSO) seemed to influence abundance and distribution of adult Pacific hake (*M. productus*) in the California current (Agostini et al. 2008). Changes in the North Atlantic Oscillation (NAO) were linked to variations in recruitment and habitat shifts of cod (*Gadus morhua*) (Rose et al. 2000). For the Benguela upwelling system, a change in climate is thought to alter wind stress resulting in increased upwelling and primary production (Bakun & Weeks 2004). The identification of the early life strategy of fish and of factors influencing year class strength will help to identify the species response to climate change.

The decadal change in small copepod abundance can be attributed partly to increased upwelling (Verheye et al. 1998, Verheye 2000). An increase in upwelling can therefore be regarded as positive for the food supply of early larvae, since they prey mainly on small copepods (Chapter 3.1, Fig. 5). The 'gadoid outburst' during the 1970's is an example of a change in zooplankton abundance leading to high cod recruitment in the North Sea, which co-occurred with a change in the copepod community (Beaugrand 2003). On the other hand, increased upwelling can drive primary production higher, which can lead to lower oxygen levels in the southern Benguela coastal region (Van der Lingen et al. 2006). Event-driven low-oxygen waters occur between Port Nolloth and St. Helena Bay (Van der Lingen et al. 2006), which was identified as a nursery area for

hake juveniles (Chapter I, Grote et al. subm.-c, Hutchings et al. 2002). This could lead to mortality of early life stages of hake or to an offshore displacement of juveniles, increasing their vulnerability to cannibalism. Off central Namibia, low-oxygen events led to a distribution shift of juvenile *M. capensis* in 1994 and to reduced recruitment (Hamukuaya et al. 1998).

The alteration of current patterns or boundaries through climate induced change could also have negative impacts on hake recruitment. Larval transport in the jet current and retention mechanisms are important hydrological features for the larval survival of both *Merluccius* species (Chapter 3.2) and changes in wind forcing have been related to the strength of the jet current and fish recruitment (Boyd et al. 1998). In addition, hydrographic conditions on the Agulhas Bank could be affected by climate change, as a reduction in the stabilisation of the water column will lead to lower food abundance for spawners (Hutchings et al. 2002). This, in turn, can induce poor maternal condition, causing low energy reserves for reproduction and offspring, possibly resulting in reduced recruitment (Chapter 3.3). A change of spawning sites, forced by differences in currents or temperatures, as shown for Pacific hake (*M. productus*) (McFarlane et al. 2000, Agostini et al. 2008), could thus result in altered transport routes of eggs and larvae, possibly lowering recruitment.

In conclusion, the various effects of climate change on the southern Benguela ecosystem may be both, beneficial or detrimental to the recruitment of hake, depending on the timing and intensity of alterations of the upwelling system. Predicting the consequences of climate change for hake populations will depend on additional information of their biology and their ability to cope with changes in the environment. This study provides important information on the early life history of long-lived, demersal species which may improve predictions of possible effects of climate change on fish recruitment.

4. PERSPECTIVES

This study focused on the co-existence mechanisms of two related predatory fish species, the importance of larval demographic connectivity in temperate demersal fish, the early life strategy of hake and some important traits of survivors, such as condition and growth. During this study, some questions arose, which could not be completely addressed within the framework of this PhD thesis, but the emerging research perspectives will be outlined shortly in this chapter.

The importance of small copepods as the main food source for early larvae of hake has emerged from this study. Zooplankton abundance and productivity were not monitored during the sampling periods of this study, but they are clearly needed to assess and predict food and energy supply and to relate growth rate and condition to prey availability. In addition, food quality should be investigated as it may be of importance for larval survival and recruitment success (Cutts et al. 2006). Further analyses of prey availability and quality are required to fully understand the importance of feeding in the early life history of hake. Another parameter of larval survival, namely predation mortality, was not investigated during this study. Predation is thought to be the major agent of larval mortality, which severely influences recruitment availability (Bailey & Houde 1989). Predation is difficult to study in the field, as fish larvae are quickly digested by their predators. Laboratory studies on the other hand can often alter the behaviour of prey and predators and they are frequently restricted to a small number of species (Houde 2008). Therefore, studying predation on hake larvae is a challenging task, but the predation impact should be included in future hypotheses on recruitment.

The temporal and spatial distribution and the transport of eggs and larvae were resolved in the present work. However, larvae were mainly regarded as planktonic, i.e. passive

particles drifting with ocean currents and moving only for feeding and predator avoidance (Pineda et al. 2007). This does not take into account vertical and horizontal larval swimming behaviour, which improves with development (Leis 2006). Thus, active behaviour influencing the transport of larvae needs further attention. Hake larvae are difficult to hatch and further research is required to conduct laboratory experiments on the swimming behaviour of different larval stages. Laboratory results on the larval behaviour should be tested in field studies, using drift and tagging experiments. Modelling approaches of transport and distribution together with hydrographical data could also be useful, as field studies on *Merluccius* larvae are complicated by the protracted spawning season and the patchy distribution of eggs and larvae.

In the present study, the growth of early larvae and juveniles was investigated and revealed new insights into hake growth, early life history patterns and traits of survivors. Nonetheless, larger larval stages and early juveniles are difficult to sample, due to their greater mobility and net avoidance. For all hake species the juvenile size class of 1-3 cm is not well studied and further research is needed focusing on growth, feeding and condition as well as survival rates of this stage. A better knowledge on the role of cannibalism in this size class and also in larger juveniles is highly desirable. Furthermore, detailed information on juvenile nursery areas is required, as the multitude of processes in these areas influencing recruitment is not well studied so far.

Given the high variability in the Benguela upwelling system, an ecosystem approach might help to improve our knowledge of hake recruitment variability. This approach should target all developmental stages, including female spawners, as maternal condition most likely plays a decisive role in larval condition and survival. Furthermore, it should include prey abundance and quality, as well as a high spatio-temporal resolution to account for spawning time and for different nursery areas of both hake species.

It is evident that more research is needed to fully understand the recruitment variations of large, long-lived, demersal species. The South African hake species represent a good example of a model organism to study the early survival strategies of long-lived, demersal fishes and the co-occurrence mechanisms of two related fish species. In order to understand and to support the concepts established in this study and to investigate whether these are general strategies accounting for other species, the hypotheses of this thesis should be applied to a broader set of species, for instance the less studied South American Atlantic hakes and other gadoid or large, long-lived species.

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CHAPTER I

Spatiotemporal distribution of early life stages

and spawning strategy of two Cape hake species,

Merluccius paradoxus and *M. capensis*,

in the southern Benguela upwelling system.

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Spatiotemporal distribution of early life stages

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ABSTRACT

Distribution of early life stages of *Merluccius paradoxus* and *M. capensis* was investigated in order to derive species-specific information on spawning location, spawning season, and transport of eggs and larvae. Samples were collected during four surveys between January 2007 and October 2008 in the southern Benguela upwelling system off South Africa. *M. paradoxus* was the dominating species during all surveys. *M. paradoxus* eggs and larvae were mainly found from September to October, indicating one peak spawning time during late austral winter to spring. The western Agulhas Bank was identified as spawning ground for most eggs and larvae, smaller spawning events occurred on the west coast. Larvae mainly occurred in subsurface waters between 25 and 100 m. Early stages of the two species followed separate drift routes with *M. capensis* being found further inshore than *M. paradoxus* early stages. This distribution pattern most likely evolved through differences in spawning locations and periods. The difference in spawning may reduce competition during the early life phases and enable the coexistence of the two related species. In addition, the spawning strategy of

M. paradoxus is well adapted to a time frame of optimal environmental conditions favourable for larval survival in the highly variable environment of the southern Benguela upwelling system.

KEYWORDS

Cape hake; early life history; Spawning strategy; drift pattern; Benguela current;

INTRODUCTION

Two species of Cape hake, *Merluccius capensis* Castelnau 1861 and *M. paradoxus* Franca 1960, live as top predators within the same ecosystem in the southern Benguela system off South Africa. Both species are caught in commercial trawling and long-line fisheries, the second largest sector of the South African fishing industry by tonnage landed (FAO 2009). Although they are described as different species (Van Eck 1969, Bentz 1976, Grant et al. 1988), both are morphologically similar and therefore were initially managed as one stock (Botha 1985). Differences in the biology and adult distribution of both species have revealed the need for a different management of the two Cape hakes and various attempts have been made to resolve this issue (Butterworth & Rademeyer 2005), which led to a species-based management (Field et al. 2008).

Adults of *M. capensis*, the shallow water Cape hake, live inshore between 150 and 400 m depth, whereas *M. paradoxus*, the deep-water Cape hake, inhabits waters from 250 down to 800 m depth (Payne 1989). Older fish of both species are generally occurring at greater depths than younger specimens and the main overlap between *M. paradoxus* and *M. capensis* distribution is thought to be between 335 and 425 m depth (Macpherson & Roel 1987). Off the west coast of South Africa and on the western Agulhas Bank 90% of hake are estimated to be *M. paradoxus*, whereas on the eastern Agulhas Bank and off

Namibia 70% to 85% are thought to be *M. capensis* (Assorov & Berenbeim 1983, Payne 1989).

The main spawning time of *M. capensis* and *M. paradoxus* in the southern Benguela system lasts from August to October, without identifying species-specific spawning periods (Grote et al. 2007). It is assumed that the two species spawn at different depths, however, knowledge on their spawning period and area as well as their early life history is limited (Botha 1985, Hutchings et al. 2002, Kainge et al. 2007) due to the lack of species-specific data on early life stages. Only two studies from one survey in 2005 identified hake eggs and larvae to species level based on genetic identification (Von der Heyden et al. 2007, Stenevik et al. 2008). Stenevik et al. (2008) proposed the hypothesis of different transport routes of early stages of the two Cape hake species along the west coast of South Africa. Different drift routes of eggs and larvae may be a result of separate spawning locations (Olivar & Shelton 1993). The transport of fish eggs and larvae from the Agulhas Bank along the west coast off South Africa in a jet current is known for several fish species of the southern Benguela upwelling system (Hutchings et al. 2002). The jet current follows the topography transporting different water masses from the western Agulhas Bank along the coast to the north (Veitch et al. 2009), The meandering structures of the jet current, the development of eddies and filaments as well as the intrusion of warm Agulhas current filaments close to the Cape Peninsula upwelling area are common features of the southern Benguela system (Lutjeharms & Stockton 1987, Nelson et al. 1998).

The purpose of the present study was to gain knowledge on the spawning strategies of *M. capensis* and *M. paradoxus* along the west coast of South Africa. Egg and larval distribution patterns were investigated to derive more information on spawning locations, transport routes and depth distribution of early life stages of the two southern African

hake species and relate these patterns to the environmental setting.

MATERIALS AND METHODS

Sampling strategy

Sampling of eggs and larvae of *Merluccius capensis* and *M. paradoxus* was carried out during three cruises in January, April and September 2007 on the R/V Dr. Fritdjof Nansen and in September 2008 on the R/V Ellen Khuzwayo along the west coast off South Africa (Table I). Sampling grids consisted of cross-shelf transects between 31° and 36°S covering the shelf and slope (Fig. 1). Transects were spaced about 15 nm apart, with stations 10 to 15 nm apart, and an adaptive sampling strategy was applied, adding stations for sampling of larvae in areas of highest larval abundance. Sampling in September 2007 covered a smaller area due to time constraints.

Table I Details of sampling on the four surveys indicating duration, number of station, latitudinal range, ships and total number of *Merluccius capensis* and *M. paradoxus* eggs and larvae collected.

Sampling dates	No. of stations	Latitudinal range	Research vessel	Total number of *M. capensis*		Total number of *M. paradoxus*	
				eggs	larvae	eggs	larvae
11. Jan- 2. Feb 2007	42	34.8° - 31.1°S	Dr. Fridtjof Nansen	5	-	1	12
2.-15. Apr 2007	90	34.8° - 27.9°S	Dr. Fridtjof Nansen	2	6	23	7
17.-21. Sep 2007	52	34.7° - 33.8°S	Dr. Fridtjof Nansen	38	7	49	149
20. Sep - 20. Oct 2008	121	36.0° - 31.1°S	Ellen Khuzwayo	7	17	95	224

Temperature, salinity and dissolved oxygen data were collected at all stations with a Seabird CTD probe between the surface and 10 m above the bottom. Water bottle samples for the calibration of salinity profiles were taken at most stations.
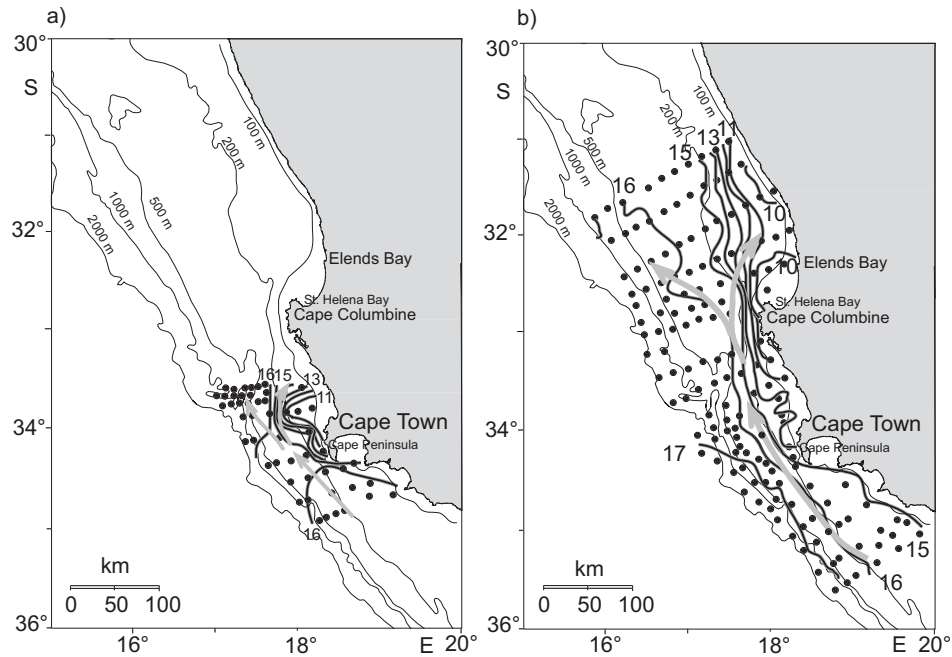
Figure 1 Temperature (°C) at 50 m depth 17.- 21. Sep. 2007 (a) and 20. Sep. - 20. Oct. 2008 (b). Bathymetric lines are drawn as black contours (100 m, 200 m, 500 m, 1000 m, 2000 m); grey arrows indicate main current flow; black dots indicate sampling stations in 2007 (a) and in 2008 (b).

On all cruises, hake eggs and larvae were sampled with a Hydrobios Multinet® plankton sampler (0.25 m² mouth area), equipped with 5 nets of 405 µm mesh size. A Scanmar depth recorder with acoustic transmission to the vessel was mounted on top of the Multinet to monitor net depth. The plankton sampler was heaved in an oblique mode at a speed of 0.5 m s^{-1} with the ship maintaining a ground speed of 2 knots. During the ascent, plankton samples were collected in five depth intervals of 50 m depth from 250 m depth to the surface. Calibrated mechanical flowmeters were mounted inside each net recording flow-through for the respective depth strata to calculate the volume of water filtered.

Immediately after the net was on board, hake eggs and larvae were sorted from the other plankton based on characterisations of hake by Olivar and Fortuño (1991) and Porebski (1975). Eggs were staged into two groups, early (I) (without embryo) and late (II) (containing embryo). All hake larvae were measured to the nearest 0.1 mm (total length). The numbers of eggs and larvae were corrected based on the volume filtered and sampling depth. Numbers were then standardised to the number under 10 m² of sea surface. Vertical distributions were presented as individuals per 100 m³. Proportions of number of early life stages 100 m⁻³ were calculated as in Hare and Govoni (2005). Distribution figures were produced with the software SURFER 8.01 using a constrained spline algorithm for interpolation (smoothing) and linear interpolation (kriging). Depth distributions of proportions of early life stages were compared by using the Fisher's exact test (two tailed) of independence calculated in R programming system.

Genetic identification

As eggs and larvae of *Merluccius capensis* and *M. paradoxus* are not visually distinguishable, mitochondrial DNA (mtDNA) of each single egg and larva was used to identify the species. DNA of specimens was extracted using the QIAGEN DNeasy tissue kit following the manufacture's protocol. The methods for genetic analyses are described in von der Heyden *et al.* (2007). Species-specific primers for the two hake species were used in polymerase chain reactions (PCR) to screen the extracted DNA from the sampled eggs and larvae in order to identify them to species level. Positive controls of identified adult samples of both species as well as negative controls were run in each PCR to check the accuracy of the genetic analyses.

RESULTS

Hydrography

Temperatures at 50 m depth were used to describe the hydrographic situation in the investigation area during the cruises in 2007 and 2008, because they represent the depth strata with the highest larval abundance. Temperatures ranged from 9 to 16.5 °C in both years (Fig. 1), generally increasing from inshore to offshore. Lowest temperatures <10 °C, indicating strong upwelling, were detected off the Cape Peninsula in 2007 (Fig. 1 a) and in St. Helena Bay near Cape Columbine in 2008 (Fig. 1 b).

The investigation area in 2008 extended further north and south than in 2007 and is used to describe the overall temperature distribution. Isotherms were running almost parallel to the coast, obviously following the topography of the shelf and slope (Fig. 1 b). The 15°C and 16°C isotherms represent the general flow of water masses from the Agulhas Bank in the south to the shelf area off Cape Columbine and Elends Bay. The compression of the two isotherms west of Cape Town coincides with the increase in current velocity and the formation of the jet stream along the coast between Cape Peninsula and Cape Columbine. North of Cape Columbine the isotherms spread out and meander along the 200 m depth contour. The meanders in the 14°C inshore isotherm between Cape Town and Cape Columbine, the 15°C and 16°C isotherms north of Cape Columbine and the offshore movement of the 17°C isotherm indicate the existence of small-scale eddies that move along with the jet stream and dominate the small-scale hydrographic background for distribution patterns of planktonic organisms, e.g. fish larvae (Fig. 1 b).

ADCP data collected during the cruises were too noisy to produce a reasonable current pattern for the investigation area. Preliminary analyses suggest a large-scale pattern as indicated in Fig 1. Mean current flow, averaged for the 20 to 80 m depth stratum, was in northward direction for both years.

Cape hake egg and larval horizontal distribution

A total of 220 hake eggs and 422 larvae caught during the 2007 and 2008 cruises were analysed in the laboratory. In January and April 2007, a total number of 6 and 25 hake eggs and a total number of 12 and 13 hake larvae were found, respectively (Table I). In January, five eggs belonged to *Merluccius capensis* and only one *M. paradoxus* egg was found, whereas in April two eggs were *M. capensis* and 23 eggs belonged to *M. paradoxus*. In January 2007, the larvae belonged exclusively to *M. paradoxus*, while in April 2007, 6 larvae were *M. capensis* and 7 larvae *M. paradoxus* (Table I). In contrast to the amount of eggs and larvae found on the cruises in January and April 2007, the September surveys of both investigated years showed much higher numbers of eggs and larvae. In September 2007, 38 eggs (43.7%) and 7 larvae were *M. capensis* (4.7%), whereas 49 eggs (56.3%) and 149 larvae were *M. paradoxus* (95.3%). In September 2008, 7 eggs (6.9%) and 17 larvae (7.1%) were identified as *M. capensis*, while 95 eggs (93.1%) and 224 larvae (92.9%) were *M. paradoxus*. All eggs of *M. capensis* and 95% of *M. paradoxus* eggs from 2007 and 2008 were early stage eggs.

In September 2007, maximum concentrations of *M. capensis* eggs were found closer inshore than those of *M. paradoxus* (Fig. 2 a, c). Larvae of both species were found in a large patch in the warm offshore filament between Cape Point and Cape Columbine (Fig. 2 b, d). Concentrations of *M. paradoxus* larvae, ranging between 5 and 171 larvae 10 m^{-2}, were much higher in the patch than those of *M. capensis,* reaching only 4-13 larvae per 10 m^{-2}. Smaller patches of *M. paradoxus* larvae were also found south of the Cape Peninsula (up to 70 larvae 10 m^{-2}) (Fig. 2 d).
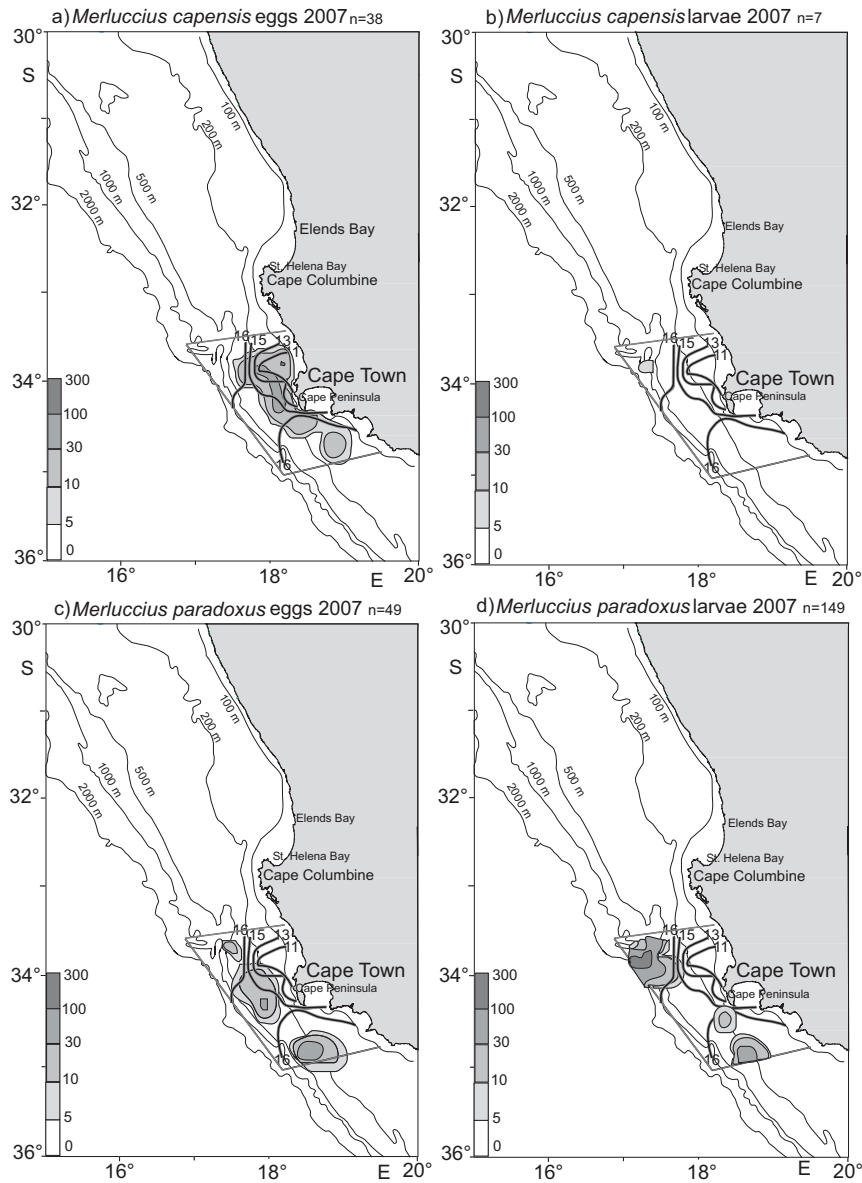
Figure 2 Distribution of *Merluccius capensis* eggs (a) and larvae (b) and *M. paradoxus* eggs (c) and larvae (d) in September 2007 (number 10 m⁻²). Grey lines indicate sampling area, black lines are bathymetric contours.

In September 2008, early life stages of hake were collected throughout most of the survey area, from about 31°S to the western Agulhas Bank in the south (Fig. 3). Peak concentrations of eggs of both species were found in a large patch off the Cape Peninsula (Fig. 3 a, c), while another patch of *M. paradoxus* eggs was sampled off Cape Columbine (Fig. 3 b). Peak abundance of larvae of both species occurred further north

than those of eggs (Fig. 3). *M. capensis* eggs and larvae were found closer inshore compared to those of *M. paradoxus* (Fig. 3). Concentrations of early life stages of *M. capensis* ranged between 7 and 35 eggs 10 m^{-2} and between 4 and 25 larvae 10 m^{-2} (Fig. 3 a, b), whereas ranges of *M. paradoxus* were between 6 and 185 eggs 10 m^{-2} and between 4 and 158 larvae 10 m^{-2} (Fig. 3 c, d).
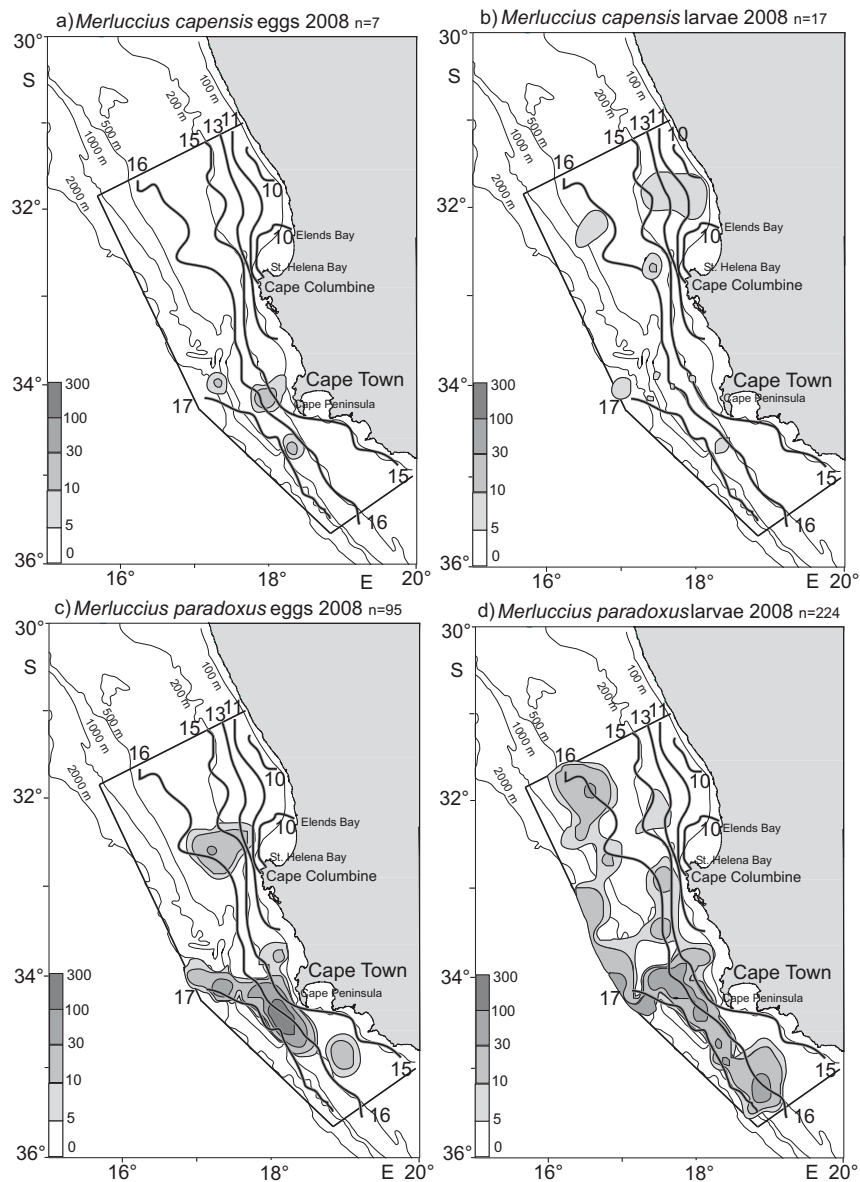
Figure 3 Distribution of *Merluccius capensis* eggs (a) and larvae (b) and *M. paradoxus* eggs (c) and larvae (d) in September 2008 (number 10m^{-2}). Grey lines indicate sampling area, black lines are bathymetric contours.

Cape hake egg and larval vertical distribution

Hake eggs and larvae were found throughout the sampled water column from 250 m depth up to the surface (Fig. 4). In September 2007 and 2008, proportions of eggs of *M. capensis* compared to those of *M. paradoxus* were almost evenly distributed throughout the water column (Fisher's exact test p= 0.488 and p= 0.425, respectively). In September 2007, egg densities of both species were in a similar range at each depth stratum. In 2008, highest mean numbers per 100 m³ of *M. capensis* eggs were caught in the 200 to 250 depth stratum, whereas *M. paradoxus* eggs were most abundant in the 100 to 150 m depth stratum (Fig. 4 b). In September 2008, maximum mean egg density of *M. paradoxus* per depth stratum was higher (5.9 eggs 100 m^{-3}) compared to that of *M. capensis* (2.9 eggs 100 m^{-3}). Maximum mean densities of eggs of both species were generally found deeper than those of larvae (Fig. 4), indicating spawning at depth below 100 m.

Figure 4 Depth distribution (x ± s per depth stratum of 50m) of eggs in September 2007 (a) and September 2008 (b) and larvae in September 2007 (c) and September 2008 (d) of both hake species (number 100 m^{-3}).
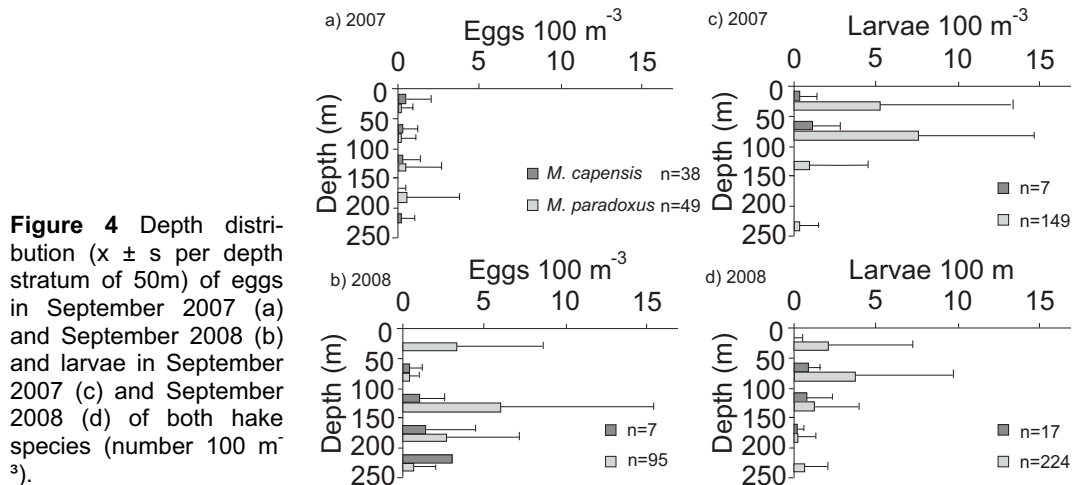
In both investigated years, larvae of both species were found mainly in the upper part of the water column (Fig. 4 c, d). In September 2007, mean peak density for *M. capensis* was 1.1 larvae 100 m^{-3} and for *M. paradoxus* 7.6 larvae 100 m^{-3} at the same depth stratum of 50 to 100 m. In September 2008, mean peak density of *M. capensis* larvae occurred again at 50 to 100 m depth with 0.9 larvae 100 m^{-3} and for *M. paradoxus*, mean

larval density was highest with 3.8 larvae 100 m⁻³ in the same depth interval. Larval depth distributions of proportions of both species were not significantly different (Fisher's exact test p= 1). Finer scaling of depth intervals at the stations 50 to 52 in September 2007 revealed that larvae mainly occurred between 25 and 100 m depth, above the thermocline (Fig. 5). These stations are located north-west of the study area in 2007 and represent stations with high larval abundances.
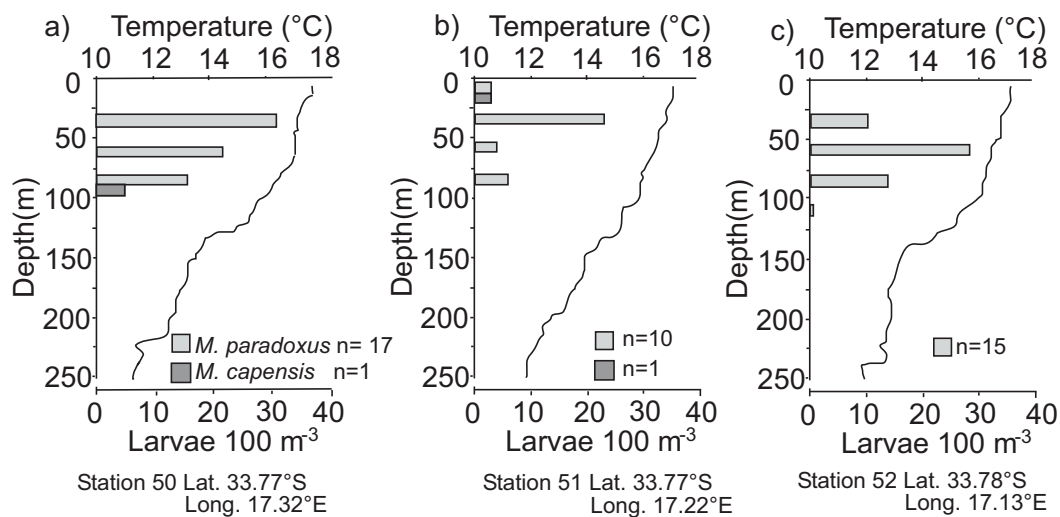
Figure 5 Depth distribution of larvae of both *Merluccius* species at station 50 to 52 in September 2007 (larvae 100 m⁻³). Temperature profile data (°C) for each station is indicated by the black line.

Larval size distribution

On all cruises, only early-stage larvae (<8 mm total length) were caught, except for three *M. paradoxus* larvae > 10 mm in September 2007 (Fig. 6). The yolk-sac was mostly absorbed at a size of 3 mm total length (TL). The smallest larva was newly hatched with 1.7 mm TL . More than 50% of *M. paradoxus* larvae were of a size between 3 and 4 mm TL in both years. In 2007, *M. capensis* larvae were yolk-sac larvae of 2-3 mm TL, whereas in 2008, larvae of *M. capensis* were generally larger than 3 mm.
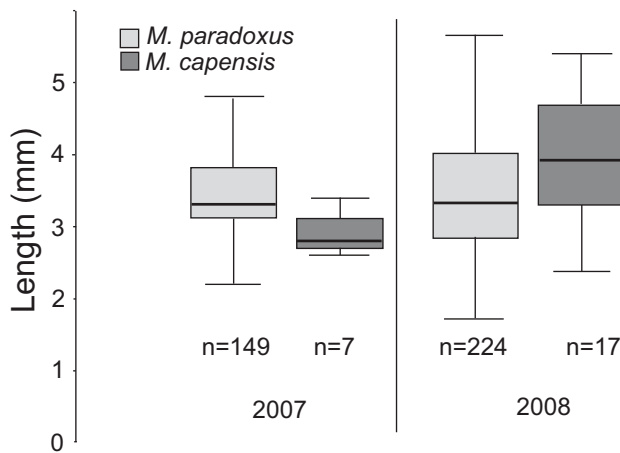
Figure 6 Larval size distribution of *Merluccius capensis* and *M. paradoxus* larvae in September 2007 and 2008. Lines indicate median, boxes indicate percentiles, whiskers indicate range without outliers.

The average size of *M. paradoxus* larvae increased significantly (Kruskal-Wallis-test p = 0.0002) with decreasing latitude (Fig. 7), indicating that larvae were spawned in the south and transported northward. It is highly unlikely that this shift represents differences in growth rates for different locations (Grote et al. subm.-a).
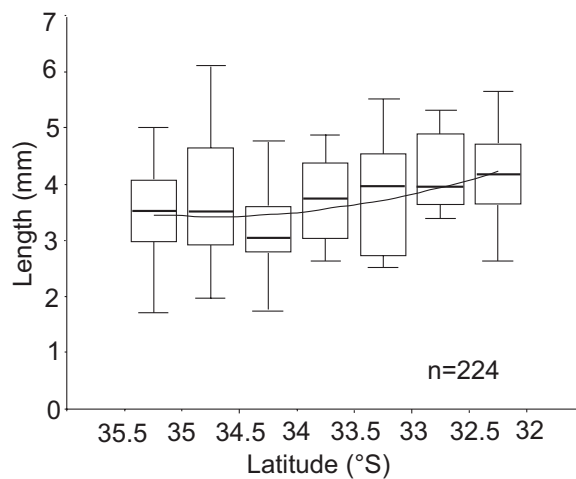
Figure 7 Size of *Merluccius paradoxus* larvae in September 2008 per latitude (°S). Lines indicate median, boxes indicate percentiles (25%-75%), whiskers indicate range without outliers.

No significant difference (Kruskal-Wallis-test p = 0.289) was found in larval size distribution per depth stratum (Fig. 8), possibly a result of the generally high abundance of larvae (number per 100 m³) in the depth stratum of 50 to 100 m.
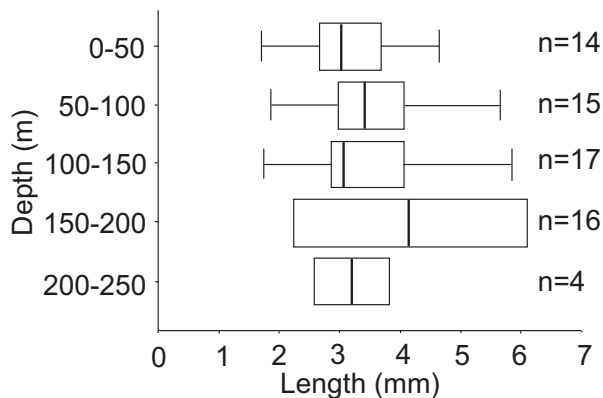
Figure 8 Size distribution for larvae of *Merluccius paradoxus* in September 2008 per depth stratum. Lines indicate median, boxes indicate percentiles (25%-75%), whiskers indicate range without outliers.

Size range of larvae in 2008 was 1.7 to 7.2 mm TL. Assuming an egg duration time of 90 h, a growth rate of 0.2 mm d^{-1} (Bjelland & Skiftesvik 2006, Grote et al. subm.-a) and a mean current speed of 20-35 cm s^{-1} in the jet current measured by ADCP in 2007 off the Cape Peninsula, the distance travelled by hake eggs until hatching is approx. 65 to 113 km. For a five days old larva, the distance range would increase to approx. 151 to 265 km.

DISCUSSION

The aim of this study was to gain knowledge of the early life history and spawning strategies of two southern African hake species. This study addressed the gap in information on *Merluccius paradoxus* and *M. capensis*, since species-specific data on spawning grounds and distribution were very limited as yet.

Published data suggested that Cape hake spawn throughout the year with distinct peak seasons (Kono 1980, Shelton 1986, Payne 1989). European hake show an indeterminate fecundity and a protracted spawning season, which is unusual for gadoid species (Murua & Motos 2006). In this study, eggs and larvae of *M. paradoxus* were found during all

cruises, with maximum abundance from mid-September to early October, which can be interpreted as a peak spawning time for this species. This is supported by a preceding study, where the peak spawning season of hake off South Africa was identified to last from August to October (Grote et al. 2007). *M. capensis* peak spawning time or location was not hit by any of our surveys and it was surprising that *M. paradoxus* was the prevalent hake species during all surveys, since both species were thought to spawn on the western Agulhas Bank (Botha 1973). Furthermore, Botha (1973) found active gonads of Cape hake from June to August and ripe gonads mainly in September, but without specifying the species. This indicates that peak spawning times of the two South African hakes do not coincide.

For European hake, *M. Merluccius,* it is postulated that temperature or temperature and salinity are the driving factors for timing of spawning (Arbault & Lacroix- Boutin 1969, Coombs & Mitchell 1982, Valencia et al. 1989). In austral winter, there is a high water column stability on the western Agulhas Bank (Shelton & Hutchings 1990, Largier et al. 1992). These conditions could trigger spawning of *M. paradoxus.* Furthermore, there is evidence from previous studies that time of spawning of *M. paradoxus* in the southern Benguela and *M. merluccius* in the central Mediterranean matches with low to medium upwelling (Grote et al. 2007, Bartolino et al. 2008). Reason for this can be the dome-shaped relationship between turbulence in the water column caused by upwelling and feeding success of fish larvae (Cury & Roy 1989, MacKenzie & Miller 1994). Hence, feeding rates of larvae are likely to be higher at intermediate than at high turbulence level. Thus, *M. paradoxus* spawning during a time of intermediate upwelling can therefore be interpreted as an adaptation to an optimal environmental window, probably favourable for feeding and thus for growth and survival of the larvae.

The dispersal of eggs and the size distribution of larvae of *M. paradoxus* in the study area showed that a substantial number of these early stages were released on the western Agulhas Bank and only a smaller fraction of *M. paradoxus* eggs on the west coast off St. Helena Bay (33°S). This supports findings of other studies, where the western Agulhas Bank was previously identified as spawning ground for hake, without specification of the species (Crawford et al. 1987, Grote et al. 2007). Furthermore, spawning of other related hake species occurs mainly over the shelf or at the shelf break zone (Olivar et al. 2003, Alvarez et al. 2004, Landaeta & Castro 2006). The low abundance of *M. capensis* early stages during all study periods could also be related to sampling location and method. If *M. capensis* spawns further east on the Agulhas Bank, as the adult distribution of this species might suggest (Payne 1989), *M. capensis* larvae would have been advanced-stage larvae, which were probably able to avoid the sampling gear used.

Hake spawn at depth and eggs rise to the surface (Sundby et al. 2001). In this study, eggs of both species were found deeper than the larvae indicating spawning at depths below 100 m. Larvae of both species were located mainly in the subsurface stratum between 25 and 100 m with decreasing numbers towards the surface and greater depths, supporting the results of Stenevik et al. (2008). A similar larval vertical distribution was found for European hake (Coombs & Mitchell 1982, Motos et al. 2000) and Pacific hake (Bailey 1981, Mullin & Cass-Calay 1997, Cass-Calay 2003). The subsurface distribution of larvae could be a strategy to avoid loss through offshore transport and also places larvae in favourable conditions for feeding (Verheye & Hutchings 1988, Sundby et al. 2001, Hutchings et al. 2002, Olivar et al. 2003).

In both investigated years, high abundances of *M. paradoxus* eggs and larvae were found offshore, whereas *M. capensis* early stages occurred further inshore. This distribution strongly supports the hypothesis discussed by Stenevik et al. (2008) that the drift routes

of early life stages of the two species are separated. At the same time, this indicates that the spawning locations of the two hake species are different. Further offshore-spawned *M. paradoxus* will be mainly transported on the fast outer branch of the jet current, whereas inshore-spawned *M. capensis* are likely to be transported by an inshore branch of the upwelling-driven current from the western Agulhas Bank towards northern inshore areas. A divergence of the jet current off Cape Columbine into a fast outer branch of the current and a slower inshore branch has been observed before (Penven 2000). The different transport routes of early stages of hake would result in separate destinations, which is supported by the assumed locations of juvenile nursery grounds for both species. One known nursery area of *M. paradoxus* is located over the Orange Bank shelf (Strømme *et al.*, unpublished data), whereas that of *M. capensis* seems to be located just north of St. Helena Bay (Hutchings et al. 2002). Slow currents near the coast north of Cape Columbine and the circulation mechanisms in St. Helena Bay are mostly favourable for the retention of larvae and this bay is also known as nursery ground for other species, such as sardine and anchovy. However, strong offshore currents near Cape Columbine may indicate a loss of eggs and larvae from the shelf. In this study we are not in the position to resolve the question, whether this is a permanent loss or if larvae drift within a warm eddy back towards the coast. The prevailing current patterns on the west coast of South Africa during austral spring are beneficial for the transport of eggs and larvae of the two hake species from their suggested separate spawning locations to their species-specific nursery grounds.

Conclusions

In complex ecosystems, species have to develop fine-tuned spawning- and early life strategies to be able to react to the most crucial factors affecting their survival and recruitment (Bakun 1985). In order to survive, the spawning strategy of *M. paradoxus* has

to be adapted to the complex environment of the southern Benguela system, where upwelling, and thus temperature, turbulence and food availability vary strongly throughout the year. Spawning on the western Agulhas Bank at depth below 100 m is most advantageous for larval survival, as these larvae are transported to the northern nursery areas mainly in subsurface layers, where they are less vulnerable to offshore loss and experience good feeding conditions. Therefore, the spawning strategy of *M. paradoxus* is adjusted to a time frame of optimal environmental conditions favourable for larval survival. Although data on *M. capensis* still remain scarce, it is surmised that the early life strategy of this species is similarly well adapted to the highly variable environment. The peak spawning periods of the two southern African hake species, *M. paradoxus* and *M. capensis*, are most likely spatially and temporally separated, which reduces competition between the two species and enables their coexistence within the same ecosystem.

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CHAPTER II

Characteristics of survivors –

growth and condition of early life stages of the two

hake species *Merluccius paradoxus* and *M. capensis*

in the southern Benguela system

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Characteristics of survivors -

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ABSTRACT

Hake is an important commercial gadoid fish in South Africa represented by two species, *Merluccius paradoxus* and *M. capensis.* The species show wide overlap in various life features such as distribution, spawning, growth etc. We tried to identify if and how early life stages differ in growth and condition, and what leads to different development in their population dynamics. Juveniles were caught in 2007 in the southern Benguela upwelling system off the west coast of South Africa. Larvae were caught in the two consecutive years, 2007 and 2008. By means of genetic identification, higher abundances of *M. paradoxus* eggs and larvae were found in the study area in September 2007 and 2008 compared to those of *M. capensis*. Larval and juvenile otolith microstructures were analysed to estimate age, hatch date distributions and growth rates. *M. paradoxus* larval age ranged from 2 to 29 days post hatching (dph) and *M. capensis* age ranged from 6 to 26 dph, whereas age of juveniles was 67 to 152 dph. Mean growth rate of age 0-group *M. paradoxus* was 19 mm month^{-1}, supporting the 'fast growth hypothesis' stated for hake. *M. paradoxus* juveniles hatched in 2006, were significantly bigger than those hatched in 2007 and 2008, indicating survival of fastest growing individuals.

RNA:DNA ratios of the same larvae were used to evaluate nutritional condition of the wild caught hake larvae. RNA:DNA ratios of larvae of both species were above the relevant threshold levels known from literature, indicating no food shortage for larvae caught. Key characteristics of hake survivors are a good condition and a high growth rate to avoid predation and cannibalism.

KEYWORDS

Survival strategy; growth; condition; Cape hake; Benguela current; South Africa

INTRODUCTION

The Cape hakes, *Merluccius paradoxus* Franca, 1960, and *M. capensis* Castelnau, 1861, are demersal gadiform species living in the southeast Atlantic Ocean in the Benguela upwelling system. The demersal trawl fishery, dominated by deep-sea trawling for Cape hakes, is the second largest sector of the South African fishing industry by tonnage landed and contributes the highest value of all fisheries landings in the country (FAO 2009). The two hake species are morphologically very similar and until very recently they were managed as one stock (Butterworth & Rademeyer 2005, Field et al. 2008). The species-based management has been realised with increasing knowledge on the differences of distribution and biology of the two species.

Life history of the two Cape hake species has been described by Botha (1985, 1986) and Payne (1989). The size of hake individuals increases with depth, although *M. capensis* lives more inshore in shallower waters than *M. paradoxus*, with some overlap at approximately 330 - 430 m (Macpherson & Roel 1987). Presumably, *M. paradoxus* and *M. capensis* spawn at different depths, but knowledge on their early life history and recruitment is still very limited (O'Toole 1978, Botha 1979, 1985, Hutchings et al. 2002, Kainge et al. 2007). The gap in species-specific data on the early life stages is due to the

lack of visible morphological differences between eggs and larvae of the two related species. So far only two studies from one survey in 2005 (Von der Heyden et al. 2007, Stenevik et al. 2008) and one co-study to the present (Grote et al. subm.-c), have succeeded in identifying hake eggs and larvae to species level. Greater knowledge on the early life history of the two species, especially on the characteristics of the surviving larvae, is needed in order to better understand recruitment processes of the two hake species.

Growth performance has a profound impact on larval and juvenile survival (Cushing 1975, Hollowed & Bailey 1989, Houde 1996). Variations in growth and condition of larval and juvenile fish influence predation vulnerability and consequently the strength of a cohort (Bailey & Houde 1989). They can be influenced by a combination of abiotic and biotic factors, e.g. temperature, food availability, turbulence (Hjort 1914, Cury & Roy 1989, Jennings et al. 2001, Houde 2008). Hence, larval growth rate and condition, the latter as a measure of growth potential, are effective measures for evaluating survival chances of individual larvae (Houde 1987, Clemmesen & Doan 1996, Meekan & Frontier 1996).

Growth of larvae and juveniles of *Merluccius* species has been studied in European hake, *M. merluccius* (Alvarez & Cotano 2005, Morales-Nin et al. 2005, de Pontual et al. 2006), in Argentinean hake, *M. hubbsi* (Brown et al. 2004, Santos & Renzi 2006) and also in Pacific hake, *M. productus* (Sumida & Moser 1980, Bailey 1982, Cass-Calay 1997). Previous studies on growth of *M. capensis* or *M. paradoxus* focused on length-weight relationships of juveniles and adults (Kono 1980, Chlapowski 1982, Prenski 1984) and otoliths were analysed in only one study of *M. capensis* juveniles (Roux 2006). Otolith daily increments represent a powerful tool to estimate growth rates during the early life stages, for ageing of early life stages of fish and to back-calculate hatch date distribution

(Campana & Neilson 1985, Campana & Jones 1992, Campana 2005). Validation of daily otolith increment deposition was conducted for *M. merluccius* and for *M. productus* (Bailey 1981, Arneri & Morales-Nin 2000), but not verified for *M. capensis* and *M. paradoxus*. In this study, it was not possible to perform validation experiments hence it was assumed that the deposition of increments in the South African species was also daily as in the related species *M. productus* and *M. merluccius*.

The analysis of condition of larvae complements otolith analyses, as it indicates changes in growth potential and nutritional constitution over short periods (Clemmesen 1996, Clemmesen & Doan 1996, Buckley et al. 2004). A method to estimate condition and growth potential of larvae is the nucleic acid ratio measurement (Buckley 1984, Caldarone 2005, Buckley et al. 2008). The relationship between RNA and DNA is an index for protein synthesis in a cell and also an indicator of nutritional condition and starvation of fish larvae(Buckley 1979, Clemmesen et al. 1997). Thus a change in protein synthesis or growth rate will be reflected in a changing RNA:DNA ratio (R:D) (Buckley 1984, Caldarone 2005).

Few studies addressed RNA:DNA ratios in conjunction with growth analyses of fish larvae. In haddock, *Melanogrammus aeglefinus* (Caldarone 2005) it was found that the RNA:DNA ratio and the protein:DNA ratio correlated with growth. In cod, *Gadus morhua* (Clemmesen & Doan 1996) it could be shown that RNA:DNA ratios correlated with increment width of otoliths. In herring, *Clupea harengus* (Clemmesen 1994, Folkvord et al. 1996), anchovy, *Engraulis anchoita* (Clemmesen et al. 1997) and sprat, *Sprattus sprattus,* larvae (Dänhardt et al. 2007) RNA:DNA ratios were investigated as a measure of condition in field-caught and laboratory-reared larvae.

The primary objective of this study was to identify the characteristic traits of those hake larvae most likely to survive until recruitment. Investigations of growth rates and condition of wild-caught hake larvae and juveniles were combined to analyse how they congruently influence larval survival. Analyses of genetic identity, otolith microstructure, and RNA:DNA ratios were carried out on the same individual larva to compare species-specific condition and growth of different larval stages. Furthermore, hatch dates of larvae and juveniles were back-calculated from otolith readings for identification of spawning time.

MATERIALS AND METHODS

Sampling

Larvae and juveniles of *Merluccius paradoxus* and *M. capensis* were sampled during three cruises, the first in January 2007 and the second in September 2007 on the RV *Dr. Fridtjof Nansen,* and the third in September/October 2008 on the RV *Ellen Khuzwayo* along the west coast off South Africa (Fig. 1; Table I). Sampling grids contained onshore/offshore transects, which were about 15 nm apart, covering shelf and slope.

Figure 1 Sampling area in September 2007 (dark grey) and in September 2008 (light grey). Black dots indicate sampling stations for hake juveniles in January 2007.
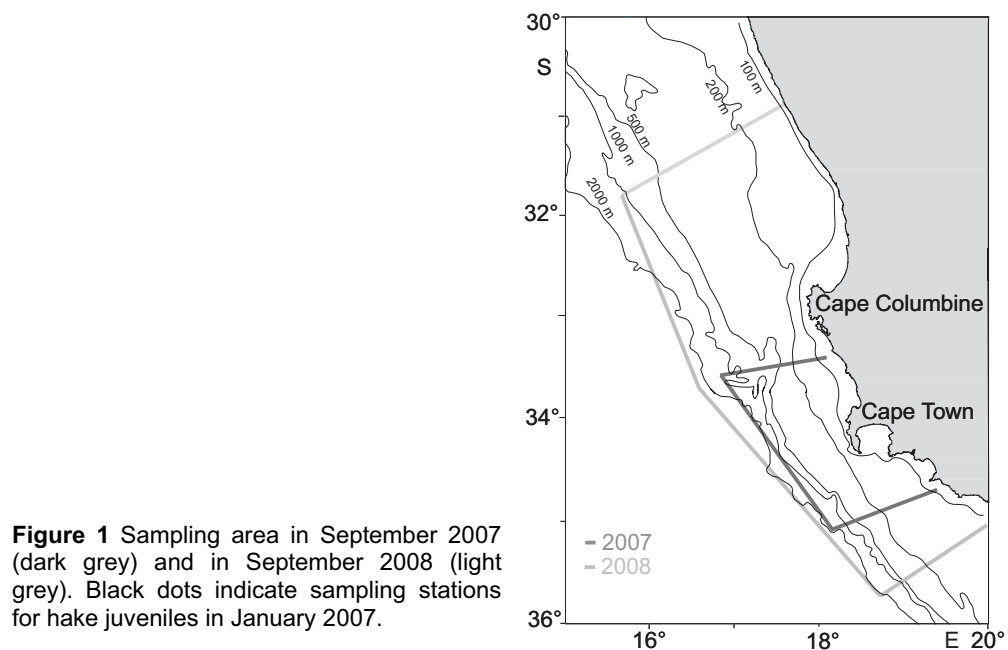
Stations were spaced 10 to 15 nm apart, although an adaptive sampling strategy was applied, adding stations for plankton sampling in areas of highest larval abundance. Sampling of larvae in September 2007 covered a smaller area due to time constraints. Temperatures were measured with a Seabird CTD probe between the surface and 10 m above the sea floor at all stations. Mean monthly sea surface temperatures (SST) were computed from Integrated Global Ocean Services System (IGOSS 2009) as a proxy to reconstruct temperature histories of hake juveniles.

Table I	**Sampling time**	**No. of stations**	**Stage**	**Latitudinal range (°S)**	**Research vessel**
Sampling details of three surveys indicating number of stations, stages of fishes sampled, latitudinal range and ships.	29. Jan - 2. Feb 2007	42	juveniles	31.2 - 29.4	Dr. Fridtjof Nansen
	17.-21. Sep 2007	52	larvae	34.7 - 33.8	Dr. Fridtjof Nansen
	20. Sep – 20. Oct 2008	121	larvae	36.0 - 31.1	Ellen Khuzwayo

Fish larvae were sampled with a Hydrobios Multinet® plankton sampler (0.25 m² mouth area), equipped with 5 nets of 405 µm mesh size, collecting plankton samples in five depth intervals of 50 m each from a maximum depth of 250 m to the surface. A *Scanmar* depth recorder with acoustic transmission to the vessel was mounted on top of the Multinet to monitor net depth. The plankton sampler was heaved in an oblique mode at a speed of 0.5 m s^{-1} with the ship maintaining a ground speed of 2 kn. Hake larvae were sorted from other plankton based on the descriptions of both species by Olivar and Fortuño (1991) and Porebski (1975), but not identified to species level. Juvenile hake up to a size of 100 mm were collected in pelagic and bottom trawls. All hake larvae and juveniles were measured to the nearest 0.1 mm or 1 mm (total length), respectively, and they were frozen in liquid nitrogen immediately after length measurement, but always

within 30 min after the haul was on board. Prior to further analyses, all larvae and juveniles were freeze-dried for 24 or 48 hours, respectively, using a Leybold-Heraeus, LYOVAC GT2 freeze-drier and weighed to the nearest 0.1 µg (Sartorius microbalance MC21 S).

Growth and age

For growth rate and age determination, a total of 184 sagitta otoliths of the hake larvae and 59 right sagitta otoliths of the early juveniles (30-10 mm TL) were removed as quickly as possible from the samples with fine insect needles under a dissecting microscope and samples were returned to the deep-freezer immediately afterwards. Otoliths were cleaned and mounted either with two components epoxy resin or embedded in *Euparal* on a glass slide. If not all increments were visible within the same plane (larvae > 5 mm), otoliths were grinded with silicon carbide paper of 2400 and 4000 granulation.

Right juvenile otoliths were polished with frequent microscopic control until the core was reached. The otoliths were magnified at 400 to 1000 x in Zeiss immersion oil under a stereomicroscope and digital images were taken with a Zeiss Axio Cam ICc1. The computer image-analysis system Image Pro Plus 5.1 was used to measure individual increment width and radius. Three consecutive measurements of otolith radius were made and the average radius was calculated for each otolith. Daily increments were enumerated from the otolith core to the edge, measuring in an area of the otolith, where the most complete increment sequence was found (Morales-Nin & Aldebert 1997). Increments were counted two times by two different observers and the mean of the readings was used to estimate the specimen's age. However, if the differences exceeded 5%, the otolith readings were rejected. Of the total number of otoliths read, 83% (n= 201) were used for further analyses.

Larval and juvenile age was analysed by counting the number of increments from a more prominent dark ring near the nucleus, which was defined as hatch check and increments were assumed to be formed daily according to (Bailey 1982). Time until hatching and time from hatching until complete yolk absorption are temperature-dependent (Bailey 1982). Hatching usually occurs 2 to 3 days after fertilisation (Bailey 1982, Sundby et al. 2001, Bjelland & Skiftesvik 2006). The age in days post hatching (dph) was defined as number of increments counted from hatch check. For back-calculation of spawning dates two days were added to the age (dph) of larvae, taking into account the egg duration time and relatively high ambient water temperatures (15°-17°C), in which larvae were caught.

The relationship between larval/juvenile length and age (increment number from otolith readings) was analysed by fitting the following Laird-Gompertz equation:

$$L_t = L_0 * e^{k*(1-e^{-G*t})}$$

where L_t is length at age t, L_0 is the size at hatching, k is the specific growth rate at hatching, G is rate of exponential decay of the specific growth rate, and t is age in days (dph).

A power function was fitted to describe the allometric relationship between larval/juvenile size and otolith radius:

$$TL = a*OR^b$$

where TL is total length (mm) measured, a and b are regression parameters and OR is the otolith radius (µm).

This allometric growth function was used to back-calculate a TL_{bci} of *M. paradoxus* larvae and juveniles for comparison of growth within the first 30 days of life:

$$TL_{bci} = a*OR_i^b$$

where TL_{bci} is the total length back-calculated for the ith increment, OR_i is the otolith radius at increment i and a and b are regression parameters.

Mean growth rates (MGR) of larvae and juveniles were calculated for certain age classes k = 10, 20, 30, 50, 75 by the equation:

$$MGR = \sum_{1}^{k} \frac{TL_{bci\,k} - TL_{bci\,k\text{-}1}}{n}$$

RNA:DNA ratio

To estimate condition and growth potential, RNA:DNA ratios of larvae were measured according to Clemmesen et al. (2003) and Belchier et al. (2004), Whole freeze-dried larvae were used in these analyses. All samples were rehydrated in Tris-SDS buffer (Tris 0.05 mol l^{-1}, NaCl 0.01 mol l^{-1}, ethylenediaminetetracetic acid (EDTA) 0.01 mol l^{-1}, sodium dodecyl sulfate (SDS) 0.01%) for at least 15 min cooled on ice. Larvae bigger than 6 mm were rehydrated for 30 min cooled on ice. Cells were disrupted by shaking in a cell mill (Mixermill MM2 by Retsch) with different sized glass beads (diameter 2.0 mm and 0.17– 0.34 mm) for 15 min for homogenisation. Next, the resulting homogenate was centrifuged at 3829 *g* (6,800 rpm) at 0°C for 8 min (Sigma Laboratories Centrifuge 3-18k). The sum of RNA and DNA in the homogenate was determined fluorometrically in a microtiter fluorescence reader (Labsystems, Fluorescan Ascent). Subsequently, the enzyme RNase was added to the samples for RNA digestion (30 min. at 37°C) and the remaining DNA was measured. The RNA fluorescence was calculated by subtracting the DNA fluorescence from the total one. By using the calibration curve fitted to the standard measurements (23s r RNA Boehringer) the amount of RNA was calculated. Following Le Pecq and Paoletti (1966) the DNA concentration was calculated using the relationship between RNA and DNA fluorescence with a slope ratio of standard DNA to standard RNA of 2.2, which adjusts for the relative fluorescence intensity difference of RNA and DNA. Minimum dry mass for which confident RNA:DNA ratios were obtained was 23 µg.

RNA:DNA ratios were standardised with a reference slope ratio (Caldarone et al. 2006). Standardised RNA:DNA ratio values were used to calculate instantaneous growth rates

(G_i) with the equation from Buckley et al. (2008), using the cod and haddock equation, as these species are closely related to hake:

$$G_i = (0.0254 \times srd) + (0.0037 \times srd \times t) - 0.0873$$

where G_i is the instantaneous growth rate, srd is the standardised RNA:DNA ratio and t is the median *in situ* temperature (°C) of the depth strata in which the larva was caught. To estimate the RNA:DNA ratio threshold level for growth of hake larvae, the turning point from positive G_i to negative G_i, was calculated, followed by back-calculating the related RNA:DNA ratio of this turning point.

Normal distribution and homogeneity of variances of growth rate data of juveniles at the ages of 10, 20, 30, 50 and 75 days were checked using the Shapiro-Wilk- and the Levene-test, respectively. Growth rates of juveniles at the ages 10, 20, 30, 50 and 75 days per spawning group were analysed using a one-way ANOVA followed by a Tukey's HSD test for post-hoc comparison. Size and age of *M. paradoxus* larvae in 2008 were included in the canonical discriminant analyses to estimate the turning point in growth from yolk-sac to feeding larvae. This analysis derives canonical variates that have the highest possible multiple correlation with previously defined classes to maximally separate the groups. For identification of coherences between RNA:DNA ratios, temperature and growth rates, which were not visible in direct correlations, a PCA was performed on the correlation matrix, extracting non-rotated components with eigenvalues >1. STATISTICA 9.0 (StatSoft Inc.) was used to perform all statistical tests.

Genetic identification

As larvae and juveniles of *M. paradoxus* and *M. capensis* are not visually distinguishable, mitochondrial DNA (mtDNA) was used to identify the species. Tissue homogenates, obtained from RNA:DNA analyses, were used for genetic identification as only a small proportion of that extract was used for RNA:DNA ratio measurement. This allowed genetic identification and condition measurements on the same individual. Von der

Heyden et al. (2007) described the methods for genetic analyses. Species-specific primers for each of the two hake species were used in polymerase chain reactions (PCR) to screen the mtDNA of larvae and juveniles in order to identify them to species level. Previously identified adult samples of both species were used as positive controls and negative controls were run in each PCR to check the precision of the analyses.

RESULTS

Age and length distribution

To estimate age and growth rates of hake larvae, a total of 167 sagitta otoliths of *Merluccius paradoxus* larvae and 17 sagitta otoliths of *M. capensis* larvae could be analysed (Table II). In addition, 57 sagitta otoliths of *M. paradoxus* juveniles and two of *M. capensis* juveniles from January 2007 were investigated. Otoliths of the two hake species were not visually distinguishable in the larval or juvenile stage. Hake larvae have relatively small, round otoliths, which become more oval with increasing age of the larvae.

Table II	Sampling time	*M. paradoxus*		*M. capensis*	
		larvae	juveniles	larvae	juveniles
Number of otoliths and RNA:DNA ratio analyses (number in parenthesis) per survey for different hake species and stages.	29. Jan - 2. Feb 2007	-	57	-	2
	17.-21. Sep 2007	72 (111)	-	6 (8)	-
	20. Sep – 20. Oct 2008	95 (160)	-	11 (17)	-

The first ring after the primordium, assumed to be the hatch check (HC) (Alvarez & Cotano 2005), was observed at 7.5 ± 0.8 µm (Fig. 2). The regular pattern of L-zones and D-zones observed from the hatch check to the margin of the otoliths were interpreted as daily increments. Length of the *M. paradoxus* larvae used for age analysis was 1.7 to 11.2 mm (Fig. 3 a), corresponding to an age ranging from 2 to 29 days post hatching

(dph) in September 2007. In September/October 2008, *M. paradoxus* larval length ranged from 2.1 to 7.2 mm (Fig. 3 b), corresponding to an age of 4 to 24 dph. *M. capensis* larval size had a narrower range in September 2007 with 2.6 to 3.4 mm (Fig. 3 a) and an age range from 7 to 12 dph. In September/October 2008, size of *M. capensis* larvae was between 2.3 and 7.6 mm (Fig. 3 b), corresponding to an

Figure 2 Larval otolith of hake, *Merluccius paradoxus* (5.8 mm larval total length), with 17 daily increments at 1000x magnification. Hatch check (HC).

age of 6 to 26 dph. However, 14 and 24% of hake larvae represented yolk-sac stage (1.8 to 3 mm) in 2007 and 2008 respectively. The results presented here are related to larvae up to 12 mm TL. Larger larvae were not present in the samples, possibly indicating avoidance of the sampling gear. In January 2007, *M. paradoxus* juvenile size varied from 35 to 95 mm, with the majority between 60 to 105 mm lengths (Fig. 3 c), corresponding to an age range of 67 to 152 dph.

Dry mass (DM) of the early larvae follows an isometric growth in relation to total length (TL) while in the juveniles the relation of dry mass to total length becomes allometric with an exponent <3:

M. paradoxus $DM=0.00181* TL^{2.9545}$ $R^2=0.9828$
       $s_b=0.0231$ $n=227$; L range=2-7 and 35-100 mm

M. capensis $DM=0.00195* TL^{3.0161}$ $R^2=0.7672$
       $s_b=0.3812$ $n=17$; L range=2.4-5.4 mm

a)

b)

c)

Figure 3 Length frequency of *M. capensis* and *M. paradoxus* larvae caught with a Multinet (405µm) in September 2007 (a) and in September 2008 (b) grouped by 1 mm intervals and length frequency of *M. paradoxus* juveniles (c) caught with a trawl in January 2007 (c).

For back-calculation of the standardised total length (TL_s), total length (TL_s) was plotted against otolith radius (OR). The regression slopes of data for larvae of the same species from the two cruises showed no significant differences (t-test, p> 0.01) and these were fitted in single power functions (Fig. 4):

M. paradoxus

$TL_s = 0.3941 * OR^{0.7046}$ $R^2 = 0.9835$ n= 227 ; L range= 2-7 mm and 35-100 mm

M. capensis larvae

$TL_s = 0.5391 * OR^{0.5785}$ $R^2 = 0.8132$ n= 17 ; L range= 2.4-5.4 mm

a)
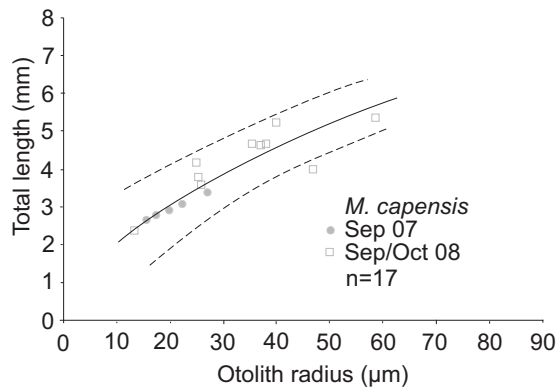
b)
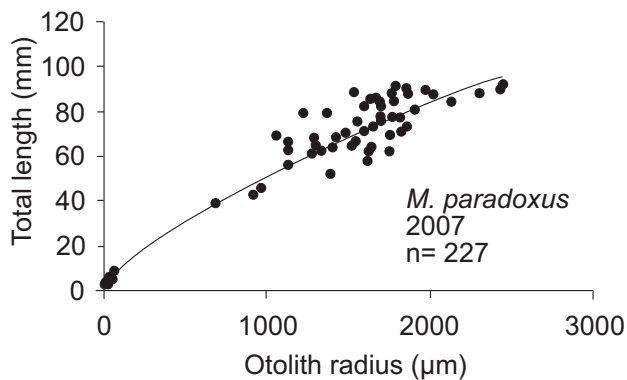
Figure 4 Relationship between larval length (mm) and otolith radius (μm) for *M. paradoxus* (a) larvae and juveniles and for *M. capensis* larvae (b) in 2007 and 2008. Lines indicate 95% confidence interval for estimates.

Birth dates retrieved from back-calculation in *M. paradoxus* larvae caught in September 2007 ranged between August 17th and September 16th 2007, while *M. paradoxus* larvae from September/October 2008 were spawned between August 29th and October 2nd 2008 (Fig. 5). For *M. capensis*, most larvae caught in 2007 were spawned between September 6th and 11th and in 2008 between September 16th and 23rd. *M. paradoxus* juveniles caught in January 2007 were spawned between August 31st and November 22nd 2006 and four

separate spawning batches (separated by ≥3 d) could be identified (Fig. 5). The majority of these juveniles had back-calculated spawning dates between the 24th of September and the 22nd of October (2006), a period that was not completely covered by the cruises in 2007 and 2008 and thus back-calculated spawning peaks in the three years did not overlap (Fig. 5).
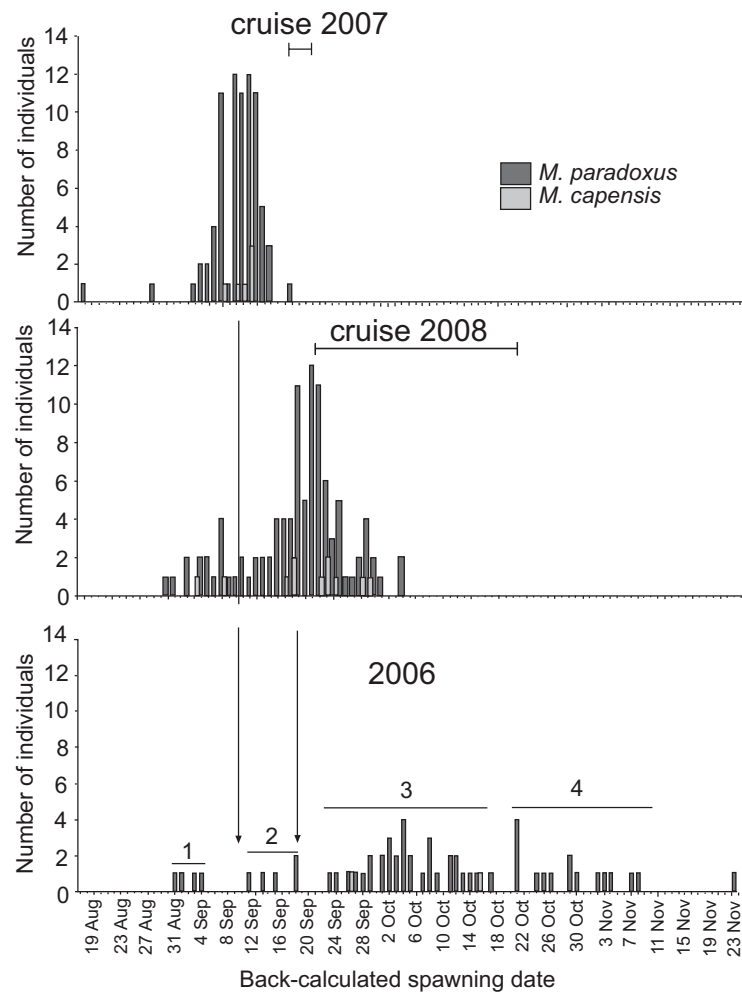
Figure 5 Back-calculated spawning dates for *M. paradoxus* and *M. capensis* larvae spawned in 2008, 2007, and 2006 (juveniles caught in 2007). Numbers indicate spawning groups in 2006.The two plankton sampling campaigns in 2007 and 2008 are indicated as horizontal bars.

Growth

The growth patterns of larvae were analysed to detect a inflexion point in growth at the transition from yolk-sac to feeding larvae using size-at-age data in a canonical discriminant analysis (CDA). The CDA showed a separation into two groups with a percentage of 96.9% (Wilks' λ 0.33) of cases correctly classified, marking a turning point in growth at the age of 12 days (Fig. 6).
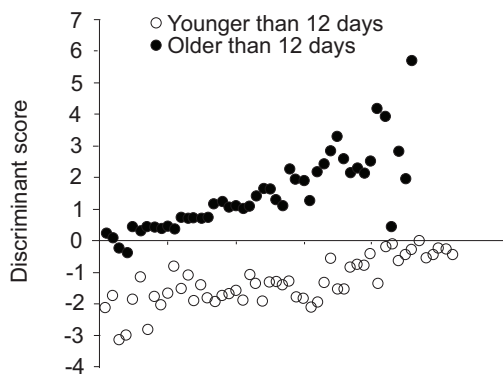
Figure 6 Discriminant scores from canonical discriminant analysis of size/age shown by individual *M. paradoxus* larvae in 2008, roughly separating larvae younger and older than 12 days.

The age-length relationship was analysed by fitting number of increments (age in days) and total length (mm) data using the Gompertz equation as best for description of growth of early life stages of fish. As growth rates of larvae of the same species were not significantly different for the cruises in 2007 and 2008 (t-test, p>0.05), these data were fitted into a single Gompertz function for each species:

$$L = 1.528 * e^{4.205*(1-e^{-0.023*t})}$$ for *M. paradoxus*   n=227 (Fig. 7)

$$L = 1.747 * e^{4.950*(1-e^{-0.012*t})}$$ for *M. capensis* n=17

The extrapolated size at hatching of 1.5 mm for *M. paradoxus* and 1.7 mm for *M. capensis* in the Gompertz function correspond to the hatching size found for European hake, *M. merluccius*, ranging from 1.6 to 2.9 mm (Maralle et al. 1996, Bjelland & Skiftesvik 2006).
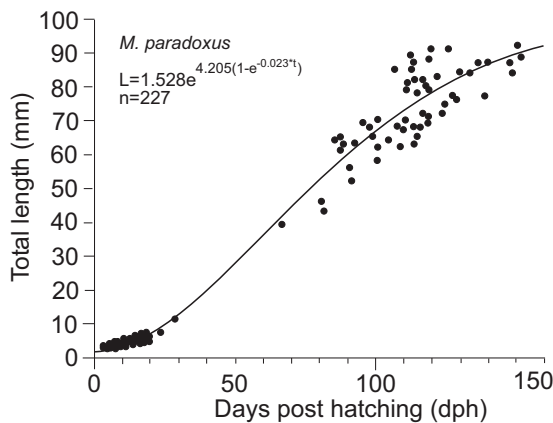
Figure 7 Length (mm)-age (days post hatching) relationship for *M. paradoxus* larvae in September 2007 and September 2008 combined and juveniles caught in January 2007 fitted by Gompertz equation.

The back-calculation of total length (TL_{bci}) was used to compare growth patterns of *M. paradoxus* juveniles from January 2007 and larvae from 2007 and 2008. TL_{bci} of juveniles continuously increased with age and did not show such a distinctive decrease in growth rates as predicted by the Gompertz model for the age of 110 days (Fig. 8). As the juveniles caught in 2007 represent the surviving larvae from spawning events in 2006, TL_{bci} patterns of these juveniles were compared with those of larvae to detect growth patterns of the survivors. However, it should be noted that no larval sampling was conducted in austral spring 2006. TL_{bci} of *M. paradoxus* juveniles and larvae was similar
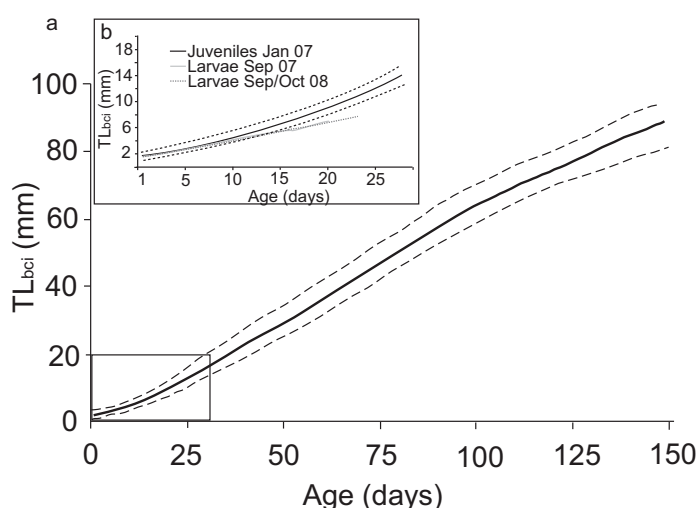
Figure 8 a) Mean back-calculated length-at-age relationship and standard deviation of *M. paradoxus* juveniles hatched in September to November 2006 over the first 150 days. Length as TL_{bci} in mm, age in days. b) Mean back-calculated length-at-age relationship of larvae for the first 30 days of life from the September 2007 and September 2008 plankton samples and from juveniles caught in January 2007 that hatched in September to November 2006.

until the age of 8 days (Fig. 8b). From the age of 9 days, mean TL_{bci} of *M. paradoxus* juveniles were higher than those of larvae at the same age in 2007 and 2008, indicating higher growth rates of the surviving *M. paradoxus* larvae from spawning events in 2006 than of larvae in 2007 and 2008.

To analyse if survival of spawning cohorts was influenced by spawning time and if growth rates of different cohorts as proxy for survival chance showed a seasonal pattern, growth rates of the cohorts for different length groups were compared. Mean growth rates of the first two spawning groups at the age of ten days were higher than those of spawning group 3 and 4, although the only significant difference found was between spawning groups 2 and 3 (ANOVA, Tukey's test $p < 0.05$) (Fig. 9). At the age of 20 and 30 days, this pattern was reversed, as spawning group 3 had the highest mean growth rates with 0.55 mm d^{-1} and 0.71 mm d^{-1}, respectively. At the age of 50 days, spawning group 4 had the highest mean growth rate with 0.75 mm d^{-1} and that of group number 2 was lowest with 0.58 mm d^{-1}. Lowest mean growth rate at the age of 75 days was found for spawning group 1, whereas the highest mean growth rate was observed in spawning group 4 (Fig. 9). However, all variances were not
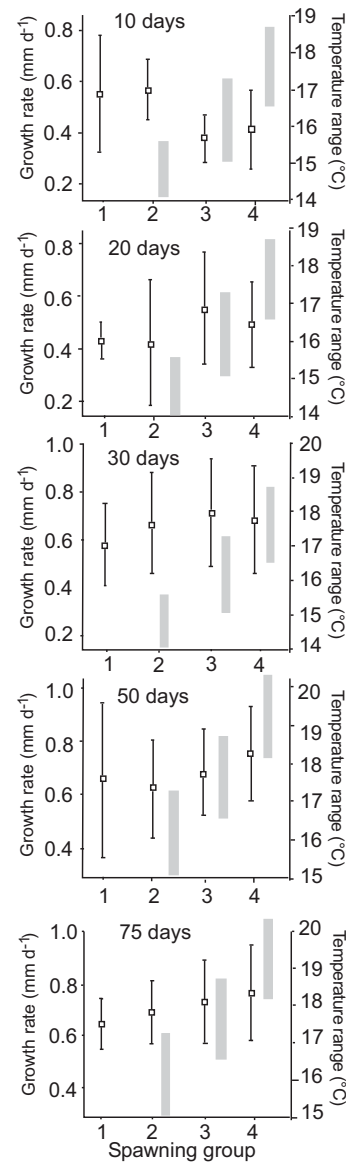
Figure 9 Mean growth rates (mm d^{-1}) with standard deviation separated by spawning groups 1-4 of *M. paradoxus* juveniles caught in 2007. Growth rates were calculated for ages of 10, 20, 30, re 5 Mean content (+ SD, Only drawn in positive direction) of the fatty

significant, but revealed clear trends in juvenile growth rates. Mean overall growth rate of *M. paradoxus* juveniles (L range= 35-100 mm) caught in January 2007 was 19 mm month^{-1}.

There was an increase observed in mean monthly SST from September to November 2006 of about 2.7 °C (Tab. 3; computed from Integrated Global Ocean Services System (IGOSS 2009). Mean monthly SST was higher in September 2007 than in September 2006 and 2008. Comparing SST and growth rates in the different months, a significant correlation could be found for juveniles at 50 and 75 days of age with r^2 =0.96 and r^2 = 0.83 ($p< 0.1$), respectively.

RNA:DNA ratio

In addition to the analysis of the growth increments in otoliths, larval condition and growth potential were estimated by means of RNA:DNA ratios. The RNA:DNA ratio threshold level for growth was calculated from instantaneous growth rates (Gi), using CTD-measured temperatures experienced by the larvae. This resulted in RNA:DNA ratio threshold levels for hake larvae of 1.3 at 10° to 11 °C, 1.2 at 12° to 14 °C and 1.0 at 15° to 17 °C. RNA:DNA ratios of larvae of both species were above the relevant threshold levels in both investigated years (Fig. 10 a, b) indicating instantaneous growth of all larvae. The median RNA:DNA ratios of *M. paradoxus* larvae changed with developmental stage, decreasing during the yolk-sac stage, being relatively stable for first feeding larvae and slightly increasing in feeding larvae (Fig. 10 a). For *M. capensis*, RNA:DNA ratios were low in the yolk-sac stage, increasing during first feeding and decreasing in more advanced larvae (Fig. 10 b). The small difference in the pattern of RNA:DNA ratio during development of *M. capensis* larvae compared to *M. paradoxus* larvae is probably related to the smaller sample size of the former.

a)

b)
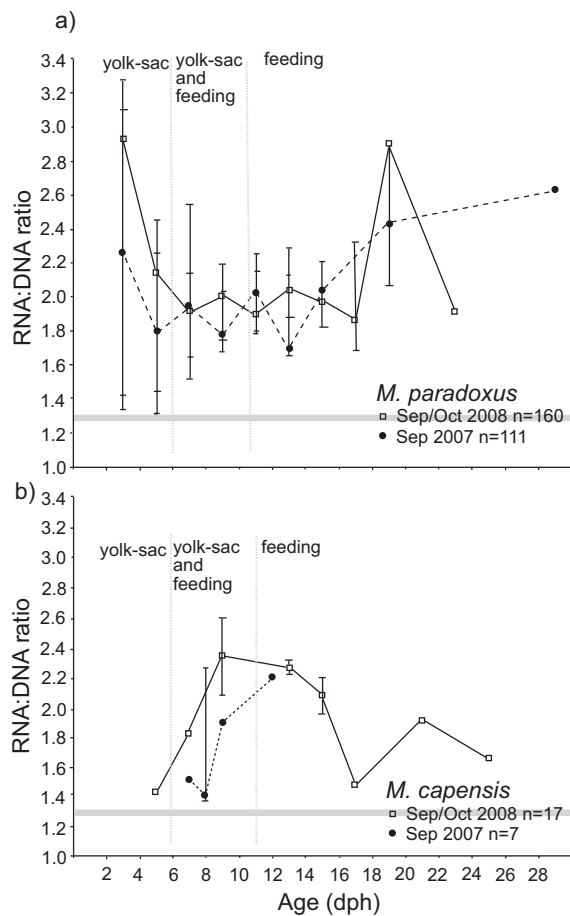
Figure 10 Median RNA:DNA ratio versus age (days post hatching) for *M. paradoxus* (a) and *M. capensis* (b) larvae. Whiskers indicate 25-75 percentiles; grey area indicates threshold RNA:DNA ratio level for growth at a temperature of 10°C.

Combined analysis of growth and condition

To measure a possible coupling between growth and condition in wild-caught larvae, RNA:DNA ratios, growth rates and medial water temperatures were analysed in conjunction. Principal component analysis for feeding larvae of *M. paradoxus* separated larvae based on RNA:DNA ratio, mean growth rates (MGR in mm d^{-1}) calculated from the last three otolith increments and medial temperature of the depth strata, where the larva was caught (Fig. 11). PC1 (38% of variance) was characterised by positive loadings of mean growth rates (0.8) and temperature (0.7). Contribution of RNA:DNA ratio to PC1 was not significant (0.3), whereas PC2 (33% of variance) was almost exclusively explained by the positive loading of RNA:DNA ratio (0.9) and contribution of mean growth rates (0.004) and temperature (-0.4) was minor or negative. This indicates that growth

rate and temperature are closely coupled factors, whereas RNA:DNA ratio seems not to be directly related to growth rate.

In general, one big cluster of data was found except for two *M. paradoxus* larvae from 2007 with RNA:DNA ratios >3 and MGR >0.2 mm d^{-1}. . A few other larvae were also placed outside of the cluster, aged 11 to 22 days, with low mean growth rates (<0.2 mm d^{-1}) and with lower RNA:DNA ratios (<3). They were caught at temperatures below 13°C (Fig. 10). Furthermore, some larvae aged 7 up to 14 days, found at the outer edge of the cluster had very low RNA:DNA ratios (<2), but high MGR (>0.2 mm d^{-1}). High MGR (>0.2 mm d^{-1}) were never found when larvae experienced temperatures below 15°C, confirming the coupling between temperature and growth rates in hake larvae.
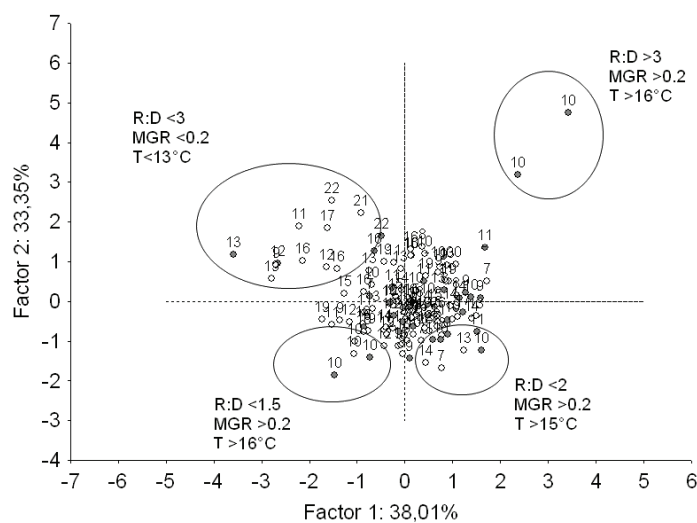
Figure 11 Principal component analysis of RNA:DNA ratio (R:D), mean growth rate calculated from the last three otolith increments (MGR in mm d^{-1}) and temperature (T in °C) of feeding larvae of *M. paradoxus* from September 2007 (dark circles) and 2008 (light circles). Numbers indicate larval age.

DISCUSSION

The aim of this study was to investigate the survival strategies of hake larvae by determining characteristic daily growth rates and condition patterns. One goal was to detect if the early stages of the two species differ in their growth behaviour. To achieve these goals and improve the significance of the results, for the first time the analyses of

growth rates and RNA:DNA ratios of *Merluccius paradoxus* and *M. capensis* larvae and juveniles in the same specimens were combined. However, due to restrictions in ships time, it was not possible to sample larvae over the entire spawning period and juveniles of the same cohort as larvae. Nevertheless, as juvenile *M. paradoxus* originate from a nursery area off the Orange River*,* which is downstream of the larval transport route from the western Agulhas Bank (Grote et al. subm.-c), this study contributes significantly to our knowledge on survival strategies of early life stages of South African hake.

In contrast to the balanced abundance in the adult stock of the species, a high dominance of *M. paradoxus* was found in the collected larval and juveniles, which was attributed to the sampling period and area (Grote et al. 2007, Grote et al. subm.-c). Hake species are known to be serial batch spawners with distinct peak spawning (Shelton 1986, Macchi et al. 2004, Murua & Motos 2006). In a previous study, Cape hake eggs and larvae were predominantly found from June to October (Grote et al. 2007). Hatch-date distribution of juvenile *M. paradoxus* in this study was reduced to a period from August to November 2006 with a peak of survivors from late September to mid-October 2006 for the analysed size spectrum. Sampling size was restricted to max. 100 mm, thus missing fast-growing larvae spawned between August and mid-September 2006.

The growth pattern of *M. paradoxus* could be separated into two phases: the first from hatching to 12 days post hatching (dph), when growth was slower and the larva was still in the yolk-sac or first feeding stage, and a second phase from 12 dph onwards, when the larva had a completely developed head and the yolk-sac was totally depleted. This indicates a similar, though not as clear growth pattern as found in *M. merluccius*, where growth within the first 12 dph is shown to be greater in thickness for the development of the anterior body part than in length (Palomera et al. 2005). Only after complete yolk-sac depletion, which seems to occur shortly after 11 dph (Bjelland & Skiftesvik 2006), the

larva starts growing in length (Palomera et al. 2005). This indicates that the full development of the head occurs during yolk-sac depletion until 12 dph and only when the larva starts feeding, it grows mainly in length. Furthermore, larval growth follows an isometric growth pattern which changes to allometric growth in juveniles, indicating further growth mainly in length.

The growth patterns of *M. capensis* and *M. paradoxus* were almost identical during the early life stages. Growth rates measured for *M. capensis* and *M. paradoxus* larvae were higher (0.21 mm d^{-1} at age 10 d) than those published for other species of the same genus. Mean growth rates of *M. hubbsi*, *M. bilinearis* and *M. productus* were found to be 0.16 mm d^{-1} (Brown et al. 2004). However, small differences in growth rates published are most likely due to differences in temperature or differences in sampling procedure, i.e. no correction for shrinkage was applied, or the calculation method was different. For *M. merluccius* larvae, growth rates ranged from 0.15 to 0.19 mm d^{-1} at a temperature of 15°C (Palomera et al. 2005).

Growth rates of larvae of both *Merluccius* species were slightly higher in 2007 compared to 2008, which might be related to higher water temperatures. *M. paradoxus* juveniles of the four spawning groups collected showed different growth rates, which can be attributed to differences in water temperatures experienced by juveniles during growth. As no direct measurements of in situ water temperatures for the fishing trawls were available, we used SST as a proxy. South African hake larvae usually occur at depths between 50 and <150 m (Stenevik et al. 2008), where slightly lower temperatures than SST prevail (Grote et al. subm.-c). At the age of 10 days, spawning groups 1 and 2 had higher growth rates than groups 3 and 4, which was in general reversed for the ages of 20, 30, 50, and 75 days. At the age of 10 days, differences in growth rates of hake larvae could be related to maternal effects (Grote et al. subm.-b). The trend of higher growth

rates of the spawning groups 3 and 4 from the age of 20 days onwards are assumed to result from higher water temperatures, as suggested by the increasing SST towards the end of the year. Similar relationships were found for European hake, *M. merluccius*, and Argentinean hake, *M. hubbsi*, which showed seasonal variations in growth rates (Norbis et al. 1999, Lleonart 2001), because batches spawned at different times of the year experienced different temperatures. Growth of larvae and juveniles is responsive to temperature in many fish species (Houde 2008). However, changes in feeding conditions can also influence growth, which were not investigated for juveniles in this study.

For 0-group hake, the fast growth hypothesis (12–25 mm month^{-1}) versus the slow growth hypothesis (7-12 mm month^{-1}), have been discussed in the literature for a long time already (Hickling 1933, Belloc 1935). Belloc (1935) established his fast-growth hypothesis suggesting a better survivorship for those specimens that pass through the early stages more quickly, and it was supported by various authors (García-Rodríguez & Esteban 2002, Kacher & Amara 2005, Piñeiro et al. 2008). On the other hand, some growth studies of different hake species supported the slow growth hypothesis (Morales-Nin & Aldebert 1997, Santos & Renzi 2006). Recent tagging experiment studies on hake validated the fast growth hypothesis (de Pontual et al. 2003, Piñeiro et al. 2007, Mellon-Duval et al. 2010). Our results showing a mean growth rate of *M. paradoxus* juveniles of 19 mm month^{-1} supported the fast growth hypothesis. The differences of the various growth rates of hake can be attributed to differences in methodology, sampling period or geographic area (population and species level).

The RNA:DNA ratio as a proxy for the condition of the hake larvae was found to change with ontogenetic development. The ratio declined during the yolk-sac stage of *M. paradoxus* larvae until first feeding stabilised it. The ratio slightly increased, when the yolk-sac was completely consumed and the larvae relied exclusively on external feeding.

A similar pattern in the development of the RNA:DNA ratio can be traced in *M. capensis*, although the small sample size resulted in a low significance. Clemmesen et al. (1994) described an analogical pattern of RNA:DNA ratios for herring larvae. RNA:DNA ratios of larvae of both *Merluccius* species were well above the relevant threshold levels of growth, indicating that larvae were in good condition, with no indication of starvation.

The strength of a year class is defined by the survival rates of early life stages of fish (Hollowed & Bailey 1989). Larval survival may be mainly influenced by a combination of different environmental factors such as variations in temperature, currents or food availability (Cury & Roy 1989, Jennings et al. 2001), which determine growth, development rate and condition of fish larvae, and therefore affect predation mortality (Bailey & Houde 1989). Our results indicate that in the southern Benguela upwelling system food availability in 2007 and 2008 was not a limiting factor for larval hake survival, as all larvae caught were in good condition, suggesting that there is no vital food limitation for the larval stages in the highly productive system. However, starving larvae are more vulnerable to predation, and thus may not have been caught. It can only be stated that there are enough survivors in good condition to sustain recruitment. Nonetheless, the growth patterns of juveniles lead to the assumption that not all larvae caught are potential survivors, which indicates that other factors than food availability influence the mortality of the early stages. Growth and mortality are closely coupled processes determining stage-specific survival of fish larvae (Houde 1996). Growth data of *M. paradoxus* juveniles suggest that only the fastest growing larvae survived in 2006. If this comprises larvae from 2007 and 2008, slower growing larvae would have suffered greater mortality, as growth of juvenile *M. paradoxus* spawned in 2006 was much faster than that of larvae caught in 2007 and 2008 at the same age. Based on otolith size-at-age data, Brown (2004) found evidence of low survival rates for slow-growing Pacific hake larvae in otolith size-at-age data. This indicates that slow-growing larvae suffer

higher mortality due to various factors, notably predation. Threshold levels of growth could be different in cohorts and vary between years, depending on environmental factors such as temperature. Therefore, growth of different developmental stages of hake of the same cohort should be investigated in the future.

An indirect reason for such high mortality can be the tight aggregating behaviour of early stage hake in specific nursery areas (Field et al. 2008, Grote et al. subm.-c). Hake are known to be cannibalistic on younger or slower growing individuals (Payne et al. 1987, Garrison & Link 2000). This was observed in older specimens of Cape hake (Macpherson & Gordoa 1994) and reported for the European hake, *M. merluccius*, from a semi-intensive culture system (Bjelland & Skiftesvik 2006) as well as in the field for the Pacific hake, *M. productus* (Sumida & Moser 1980). Folkvord (1997) showed that cod (*Gadus morhua*) were cannibalistic in the metamorphosis stage at 12 to 30 mm length. In European hake, *M. merluccius*, metamorphosis is completed at a length of 16 mm (Palomera et al. 2005), similar to *M. paradoxus* (Olivar et al. 1988). Thus, most *M. paradoxus* larvae caught in 2007 and 2008, could have been preyed upon by their faster growing or earlier-spawned conspecifics.

The spawning strategy of hake as described by Hunter (1981) is a 'large prey- fast growth'-strategy: fast-growing predators, who speed through the most vulnerable early life stages. Due to high energy consumption in the fast-growth phases, this survival strategy can fail during times of low food availability (Froese 1990). However, hake seem to follow a strategy favouring large numbers of small eggs rather than producing few large, oil-rich eggs, which are more likely to survive (Rainuzzo et al. 1997, Nishimoto & Hoshino 1999). As suggested by Polis (1981), an enormous number of eggs secures survival of the fast-growing conspecifics through the early life stages in times of low food availability, even in the pelagic environment. Due to the strong schooling behaviour fast-

growing individuals may rely on slow-growing larvae as resource. This strategy presents a unique mechanism of energy recycling within the same population.

The combined analysis of growth and condition of larvae showed that a very small number of *M. paradoxus* larvae had high mean growth rates and very high RNA:DNA ratios, and therefore the highest survival potential. No strong coupling between RNA:DNA ratios and growth rates or temperature was detected, as it has been described e.g. for laboratory reared cod larvae (Clemmesen & Doan 1996). For wild-caught larvae, the relationship between temperature, RNA:DNA ratio and growth rate seems to be more complex than results from laboratory experiments suggest. As RNA:DNA ratios and temperatures do not fully explain significant parts in the variability of growth rates and hence survival and mortality patterns, additional factors seem to influence larval growth as well. Compared to the long-latency growth rates, RNA:DNA ratios are a medium-latency parameter to measure condition (Dänhardt et al. 2007). During the early life stages of hake, growth could be influenced to a larger extent by factors detectable in short-latency parameters. These factors are most likely maternal effects (Field et al. 2008) or influence of food quality (St. John et al. 2001), which could be detectable in short-latency parameters such as lipid stores (triacylglycerols) or fatty acid compositions.

In conclusion, key characteristics of hake survivors are good condition and most importantly a high growth rate to speed through the vulnerable early life stages to avoid predation, also by cannibalism. The findings imply that various environmental and biological factors influencing larval growth can significantly affect recruitment strength of hake. The results show that in an ecosystem with highly pulsed food availability early life strategies have to include specific adaptations to overcome food shortages and hake is adapted to that in being a fast-growing, cannibalistic species.

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CHAPTER III

Lipids and fatty acids as indicators

of egg condition, larval feeding and maternal effects

in Cape hakes (*Merluccius paradoxus* and *M. capensis*).

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ABSTRACT

Cape hakes, *Merluccius paradoxus* and *M. capensis*, are important gadoid fish that are commercially fished in the Benguela system off Namibia and South Africa. The aim of this study was to elucidate the nutritional condition and feeding preferences of Cape hake larvae. Eggs and larvae were caught in the two consecutive years, 2007 and 2008 off the west coast of South Africa. To compare the condition of different larval stages of both hake species, genetic identification, total lipid contents and fatty acid compositions were always analysed on the same individual larva. Genetic identification of species, lipid content and fatty acid composition analyses were also done on eggs. Higher abundances of *M. paradoxus* eggs and larvae were found in the study area in both years compared to those of *M. capensis*. Eggs contained wax esters and they had significantly higher lipid contents per dry mass than larvae. Lipid content as well as fatty acid composition changed with the developmental stage of larvae. Essential fatty acid amounts of larvae increased with feeding due to dietary lipid incorporation. In 2007, yolk-sac larvae had significantly lower total lipid contents than those in 2008, apparently due to lower lipid levels transferred to the eggs by the spawners. These findings indicate that maternal effects can be an important factor for condition of hake larvae and that this may have an effect on hake recruitment.

KEYWORDS

Benguela system; *Merluccius capensis*; *Merluccius paradoxus*; fatty acids; eggs; larvae; lipids;

INTRODUCTION

Cape hakes, *Merluccius paradoxus* and *M. capensis*, are gadoid fish that sustain an important fishery in the Benguela upwelling system off Namibia and South Africa. The former species inhabits in deeper waters (from 250 to 660 m) and the latter shallower waters (50 to 350 m). The two hake species are morphologically very similar and until very recently they were managed as one stock (Butterworth and Rademeyer, 2005; Field *et al.*, 2008). The life history of these two species has been described in Botha (1985; 1986) and Payne (1989), who found that the size of hake individuals increased with depth. However, information on their early life history and recruitment, especially species-specific data of early life stages, were absent until very recently (Grote *et al.*, 2007; Von der Heyden *et al.*, 2007; Stenevik *et al.*, 2008). The Cape hake stocks undergo strong fluctuations in recruitment, as found for other hake species (Voges *et al.*, 2002; Murua and Motos, 2006).

Year class strength may be determined by the survival rates of early life stages (Hollowed and Bailey, 1989). In particular, egg viability and development of yolk-sac larvae are influenced by maternal condition in many fish species (Wiegand, 1996). Later in the larval phase, survival is influenced by a combination of different factors such as variations in temperature, turbulence or food availability and quality (Hjort, 1914; Cury and Roy, 1989; Jennings *et al.*, 2001; Houde, 2008). Mortality The agent of is mainly predation on eggs and larvae (Bailey and Houde, 1989). Especially during the early life stages fish are

vulnerable to starvation, since larvae rely on only very limited reserves of endogenous energy (lipids) and are dependent on feeding success (Cushing, 1975).

Lipids of eggs can reveal the condition of broodstock (Rainuzzo *et al.*, 1997; Tocher, 2003). In addition, lipid contents and fatty acid compositions can reflect the condition and diet of fish larvae (Sargent *et al.*, 1987; 1999b). Dietary lipids are an important source of essential fatty acids which are needed for normal development, growth and survival of fish larvae (Rainuzzo *et al.*, 1997). During ontogenesis, fatty acid compositions might change due to a dynamic lipid metabolism at all stages and due to the influence of dietary lipids starting with first feeding (Wiegand, 1996; Rainuzzo *et al.*, 1997).

The primary objective of this study was to estimate the condition and feeding of early-stage hake larvae. Therefore, the fatty acid compositions of eggs and larvae of both hake species, *M. paradoxus* and *M. capensis*, were measured in order to investigate egg quality and the importance of first feeding for the survival of hake larvae. In addition, dietary requirements were investigated during the initial feeding period. To compare the condition of different larval stages of both hake species, genetic identification, total lipid contents and fatty acid compositions were always analysed on the same individual larva.

MATERIALS AND METHODS

Sampling

Sampling of *Merluccius paradoxus* and *M. capensis* eggs and larvae was carried out on two surveys, one in September 2007 on the RV *Dr. Fridtjof Nansen* and the second in September/October 2008 on the RV *Ellen Khuzwayo* along the west coast off South Africa (Table I). Sampling grids comprised onshore/offshore transects spaced 15 nm apart, covering shelf and slope. Stations were 10 to 15 nm apart, while an adaptive

sampling strategy was applied, adding stations for plankton sampling in areas of high larval hake abundance. (Temperatures were measured at all stations with a Seabird CTD probe between the surface and 10 m above the bottom.)

Table I Sampling details of surveys indicating number of stations, number of eggs and larvae sampled, latitudinal range and ships.

Sampling time	No. of stations	Total number of *M. capensis*		Total number of *M. paradoxus*		Latitudinal range (°S)	Research vessel
		eggs	larvae	eggs	larvae		
17.-21. Sep 2007	52	38	7	49	149	34.7 - 33.8	Dr. Fridtjof Nansen
20. Sep – 20. Oct 2008	121	7	17	95	224	36.0 - 31.1	Ellen Khuzwayo

Eggs and larvae of both hake species were sampled with a Hydrobios Multinet® plankton sampler (0.25 m² mouth area), equipped with 5 nets of 405 µm mesh size. Oblique hauls were made between the surface and a maximum depth of 250 m, with the water column divided into 5 depth strata of fixed 50 m intervals. A Scanmar depth recorder with acoustic transmission to the vessel was mounted on top of the Multinet to monitor net depth. The plankton sampler was heaved at a speed of 0.5 m s^{-1} with the ship maintaining a ground speed of 2 kn.

Merluccius spp. eggs and larvae were identified based on the characterisations by Porebski (1975) and Olivar and Fortuño (1991). In 2008, eggs were caught in sufficient amounts for biochemical analyses and they were staged into two groups, early (I) (without embryo) and late (II) (containing embryo). All hake larvae were measured to the nearest 0.1 mm (total length). Eggs and larvae of hake were quickly rinsed in distilled water and shock-frozen in liquid nitrogen immediately after length measurement or staging, but always within 30 min after the haul was on board. Prior to further analyses,

all deep-frozen eggs and larvae were lyophilised for 24 h (Leybold-Heraeus, LYOVAC GT2 freeze-drier) and weighed to the nearest 0.1 µg (Sartorius microbalance MC21 S). Subsequently, otoliths and gut contents of larvae were dissected with fine insect needles under a stereo microscope. Larvae were grouped into 'yolk-sac' (larvae with yolk), 'non-feeding' (larvae without yolk and empty stomach) and 'feeding' (larvae without yolk and with stomach contents). Otoliths were used for ageing of larvae as described in Grote et al. (subm.).

Analytics

For the analyses of fatty acid and fatty alcohol compositions, single eggs and larvae were stored frozen in 1.5 ml dichloromethane/methanol (2:1 per volume) for at least seven days to extract all lipids. The high efficiency of this extraction method for fish larvae of the investigated size spectrum was successfully tested before (pers. comm. Dr. J. Peters, Institute for Hydrobiology and Fisheries Science, Hamburg).

After lipid extraction, mitochondrial DNA (mtDNA) was used to identify each single egg and larva of the two hake species, since it is not possible to distinguish eggs and larvae of *M. paradoxus* and *M. capensis* based on morphological characters. DNA extraction and conditions for polymerase chain reactions (PCR) were described in von der Heyden et al. (2007). In order to identify eggs and larvae to species level, species-specific primers for the two *Merluccius* species were used for amplification (Von der Heyden *et al.*, 2007). DNA of previously identified adult samples of both species were used as positive and negative controls and were run parallel in each PCR to check the precision of the analyses.

Absolute amounts of fatty acids and alcohols were quantified by adding tricosanic acid (23:0) as an internal standard to the 1.5 ml dichloromethane/methanol before storage(Peters *et al.*, 2006). Fatty acids were converted to their methyl ester

derivatives (FAME) and analysed together with the fatty alcohols by gas-liquid chromatography, according to Kattner and Fricke (1986). FAMEs were prepared by transesterification with methanol containing 3% concentrated sulphuric acid at 80°C for four hours. After cooling, 4 ml of aqua bidest. were added, and FAMEs were extracted with hexane (3 x1.7 ml). Fatty acid methyl esters and free fatty alcohols were analysed using a Hewlett-Packard gas chromatograph (HP 6890A) and an Agilent Technologies gas chromatograph (7890A), equipped with a DB-FFAP column (30 m length and 0.25 mm diameter, 0.25 µm film thickness) using temperature programming and helium as carrier gas. Peaks were identified according to retention times of a fish oil standard of known fatty acid composition. The accuracy of peak identification was confirmed for selected peaks using GC-MS.

Lipid classes were analysed by high performance thin layer chromatography (HPTLC) modified after Olsen and Henderson (1989). Since *M. paradoxus* larvae were most abundant in 2008, they were chosen for lipid class analyses. 6 to 19 µl of the total lipid extracts, in some cases pooled for five to seven larvae for size classes of 0.5 mm (Table II), were applied in duplicate by means of a CAMAG Linomat IV on pre-developed HPTLC plates (silica gel 60, Merck). For polar lipids, the plates were developed for 5 min with isopropanol : methyl acetate : chloroform : methanol : 0.25% KCl (25:25:25:10:9, v/v). For neutral lipids, plates were developed for 17 min with hexane : diethyl ether : acetic acid (80:20:2, v/v). After each development, the plates were dried for 30 min in an evacuated desiccator. The plates were then derivatised in manganese (II) chloride sulphuric acid for 1 min with a CAMAG Chromatogram Immersion Device III and charred at 120 °C for 20 min. Lipid bands were quantified with a CAMAG TLC-Scanning Densitometer 3 at 560 nm wavelength and calibrated using commercial standards (SIGMA) for each detected lipid class.

Calculations and statistical analyses

Fatty acid concentrations of < 1% TFA are not shown in the table and were excluded from statistical analyses. The proportions of wax esters in hake eggs relative to total lipid and dry mass, respectively, were calculated from the fatty alcohol content of the samples, assuming equal masses for the fatty alcohol and fatty acid chains of each wax ester molecule.

For all statistical analyses that require normal distribution, percentage data of FA were arcsine square root transformed. Normal distribution and homogeneity of variances were checked using the Shapiro-Wilk- and the Levene-test, respectively. For the identification of species- or stage-specific patterns in the fatty acid compositions, a hierarchical cluster analysis with group-linkage was performed on the basis of a similarity matrix (Bray-Curtis Similarity BCS). This analysis was performed using the software Primer 5. ANOVAs and post-hoc tests were performed with the software STATISTICA 9.0. Essential fatty acid (EFA) compositions of yolk-sac, non-feeding and feeding larvae of *Merluccius paradoxus* in 2007 and 2008 were analysed using a one-way ANOVA followed by a Tukey's HSD test for post hoc comparison. For the identification of coherences of fatty acid compositions in the different larval categories, principal component analyses (PCAs) were performed on the correlation matrix, extracting non-rotated components with eigenvalues >1.

RESULTS

Lipid content and lipid class composition

To investigate changes in lipid content during the first ontogenetic phases of *Merluccius paradoxus* and *M. capensis*, fatty acid and alcohol compositions of eggs and larvae caught in 2007 and 2008 were analysed. The lipid contents of eggs in terms of total fatty acids and alcohols ranged between 1.6 and 9.0 µg (3.5-17.0% of dry mass (%DM))

for early-stage eggs of *M. paradoxus* and from 4.1 to 6.8 µg (6.8-12.5%DM) for *M. paradoxus* late-stage eggs, and between 4.7 and 7.9 µg per egg (9.2-11.9%DM) for *M. capensis* early-stage eggs. Lipid contents were significantly higher in eggs than in yolk-sac larvae of both hake species (t-test p <0.05) (Fig.1a,b). During the yolk-sac stage, total lipid contents ranged between 0.8 and 5.6 µg (3.5-10.3%DM) for *M. paradoxus* and between 1.5 and 4.1 µg (6.3-7.9%DM) for *M. capensis* and increased with age of larvae of both species. Total fatty acid and alcohol contents as percentages of dry mass were higher in eggs than in larvae and did not increase with age of the latter (Fig.1b).

a)

b)
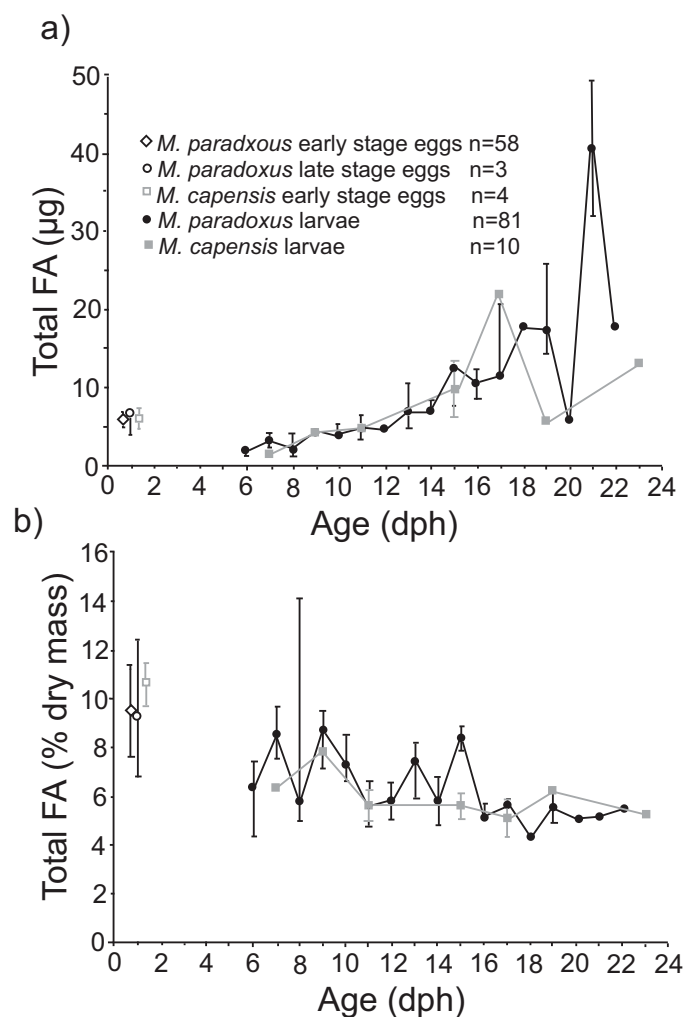
Figure 1 Lipid content in terms of fatty acids and alcohols in µg (a) and % of dry mass (b) of early-stage eggs, late-stage eggs and larvae per age in days post hatching (dph) of *Merluccius paradoxus* (black) and *M. capensis* (grey) in 2008.

Lipid class compositions, determined for *M. paradoxus* larvae from 2008, were very uniform for all larval size classes, containing high levels of phospholipids (PL) (Table II). Phosphatidylcholine (PC) was the major lipid class with 40 to 52% of total lipids, slightly decreasing with increasing larval length. The second dominant lipid class was phosphatidylethanolamine (PE) with around 20% of total lipid in all size classes. Triacylglycerol (TAG) levels were low and wax esters (WE) were not found in hake larvae, indicating that they rely on low energy reserves.

Table II Lipid class composition per length class (0.5 mm intervall) of larvae of *M. paradoxus* caught in September 2008. PC= Phosphatidylcholine; PE= Phosphatidylethanolamine; PI= Phosphatidylinositol; LPC= Lysophosphatidylcholine; PS= Phospahtidylserin; CL= Cardiolipin; ST= Sterole; FFA= Free Fatty Acids; DAG= Diacylglycerol; TAG= Triacylglycerol. n= number of pooled larvae.

Lipid class	2.5-3.0 mm (pooled n=7)	3.0-3.5 mm (pooled n=6)	3.5-4.0 mm (pooled n=6)	4.0-4.6 mm (pooled n=5)	4.7 mm (n=1)	5.5 mm (n=1)	5.6 mm (n=1)	Mean ± SD
PC	50.2	52.1	50.8	49.7	48.7	40.2	47.5	48.5 ± 3.9
PE	20.2	20.3	20.2	21.5	20.2	17.0	18.8	19.8 ± 1.4
PI	5.9	5.8	6.0	6.1	5.4	5.6	6.6	5.9 ± 0.4
LPC	2.7	3.0	3.9	3.5	3.3	5.2	4.1	3.7 ± 0.8
PS	3.0	2.7	2.4	2.6	2.7	1.9	2.8	2.6 ± 0.4
CL	2.0	2.7	2.1	3.0	2.6	2.5	1.9	2.4 ± 0.4
ST	6.9	6.8	7.5	6.3	6.3	9.6	9.1	7.5 ± 1.3
FFA	2.3	2.0	3.0	2.4	2.6	4.8	3.1	2.9 ± 0.9
DAG	1.8	1.8	3.2	2.2	3.1	5.7	3.3	3 ± 1.4
TAG	5.1	2.9	0.8	2.5	4.9	7.5	2.8	3.8 ± 2.2

Total lipid content in terms of fatty acids and fatty alcohols (µg) of *M. paradoxus* and *M. capensis* larvae increased with increasing dry mass (Fig. 2a,b). In both species, TFA contents were lower in larvae caught in 2007 than in larvae of the following year (Fig. 2a,b). This significant difference in TFA content of *M. paradoxus* larvae in 2007 and 2008 (t-test $p < 0.05$) was already noticeable in the yolk-sac larvae and persisted in larvae of older age (Fig. 2a).
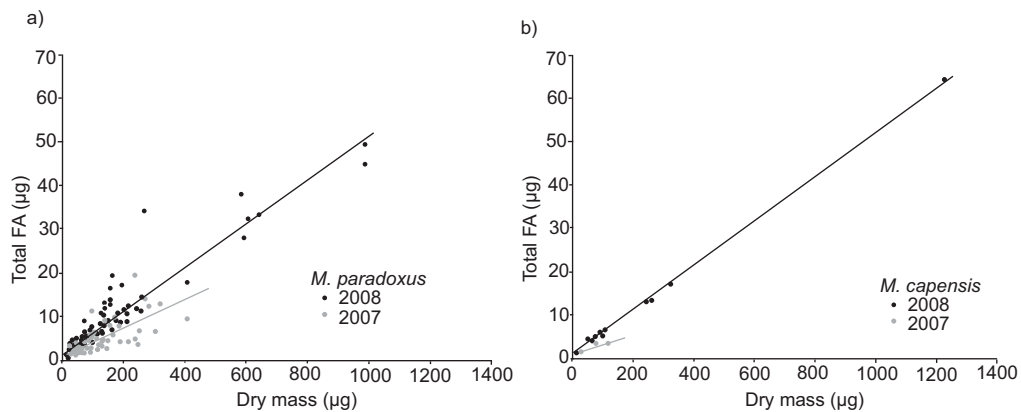
Figure 2 Total fatty acid content (µg) per dry mass (µg) of larvae of *Merluccius paradoxus* (a) and *M. capensis* (b) in 2007 and 2008.

To detect yolk-sac and dietary influences on fatty acid contents and compositions of *M. paradoxus* larvae, the proportion of yolk-sac, feeding and non-feeding larvae for size classes of 1 mm was compared (Fig. 3). In 2007, 14% of larvae caught were yolk-sac larvae (Fig. 3a). Of the 86% of larvae without yolk sac in 2007, 40% as non-feeding (without stomach contents) and 60% were classified as feeding (with stomach contents). In 2008, 24% of *M. paradoxus* larvae had a yolk-sac (Fig. 3b). At a length of 2.6 mm in 2007 and 3.0 mm in 2008 more than 50% of hake larvae caught had a completely depleted yolk-sac. In 2008, 62% of larvae, belonging to the 76% of larvae without yolk-sac, were classified as feeding and 38% as non-feeding.
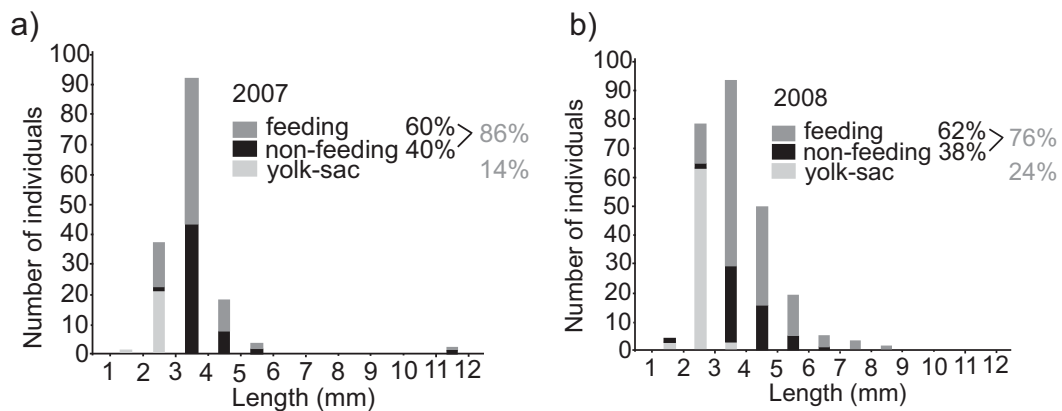
Figure 3 Number and size of yolk-sac, non-feeding and feeding larvae of *Merluccius paradoxus* caught in 2007 (a) and 2008 (b).

Fatty acid and alcohol compositions

The fatty acid of eggs and larvae of both species were analysed to elucidate possible differences in condition and feeding of hake larvae. The fatty acid compositions of early- and late-stage eggs as well as yolk-sac and older larvae of *M. paradoxus* were very similar within the same year, whereas fatty acid compositions of larvae slightly differed between years (Table III, Fig. 4). The total fatty acid compositions of *M. paradoxus* early- and late-stage eggs were dominated by the fatty acids 18:1(n-9) (28.9% and 33.7% of total fatty acid (TFA), respectively), 22:6(n-3) (25.2% and 22.7% TFA), 16:0 (16.3% and 15.4% TFA) and 20:5(n-3) (9.3% and 7.7% TFA). Overall high fatty alcohol contents and a high percentage of the 16:0 alcohol prevailed in the eggs (8-9% TFA), whereas *M. paradoxus* larvae had low amounts of fatty alcohols (1.7-2.0% TFA). The high alcohol levels in hake eggs indicate the occurrence of wax esters, which can be estimated from the overall alcohol content in eggs and account for 16.0-17.3% TFA. In both years, fatty acid compositions of *M. paradoxus* larvae were characterised by high amounts of 22:6(n-3) with 25-39% TFA content, 16:0 with 10- 19% TFA, 18:0 with 9-10% TFA, 18:1(n-9) with 8-15% TFA, and 20:5(n-3) with 7-12% TFA (Table III). In 2007, minor amounts of the fatty acids 16:4(n-1) and 20:4(n-3) were found (>1%) in hake larvae, but they did not occur in the eggs. In both investigated years, higher levels of 18:4(n-3) (0.3-5.1% TFA) and 20:2(n-6) (0.5–3.7% TFA) occurred in hake larvae compared to the eggs (< 0.4% TFA).

M. capensis yolk-sac and older larvae caught in 2008 had very similar fatty acid compositions (Bray-Curtis Similarity BCS 92.4) (Table IV, Fig. 4), while those of yolk-sac larvae differed slightly between years (BCS 87.5) (Table IV, Fig. 4). The total fatty acid compositions of *M. capensis* eggs were very similar to those of *M. paradoxus* eggs, dominated by the fatty acids 18:1(n-9) (28.4%TFA), 22:6(n-3) (23.9%TFA), 16:0 (16.7%TFA) and 20:5(n-3) (9.7%TFA) (Table IV). The eggs of *M. capensis* were
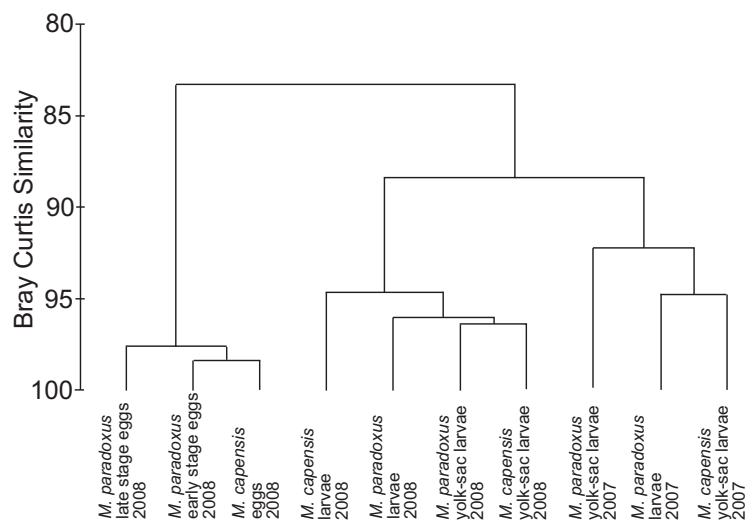
Figure 4 Bray-Curtis similarity index of fatty acid compositions of *M. capensis* and *M. paradoxus* early stage eggs, late stage eggs, yolk-sac larvae and larvae in 2007 and 2008.

characterised by high fatty alcohol contents, with 16:0 prevailing (Table III). The fatty acid compositions of both hake species were very similar for larvae hatched in the same year (BCS 94.8 in 2007 and 94.6 in 2008 (Tables III and IV, Fig. 4). The total fatty acid compositions of *M. capensis* larvae were characterised by high amounts of 22:6(n-3) with 25-39%TFA, 16:0 with 15-20%TFA, 18:0 with 9-11%TFA, 18:1(n-9) with 9-12%TFA, and 20:5(n-3) with 8-12%TFA (Table IV). In 2007, the fatty acids 16:4(n-1) and 20:4(n-3) were found in larvae of both *Merluccius* species. In addition, the fatty acid 20:2(n-6) occurred in *M. capensis* larvae (>1%) in 2007, but not in eggs or larvae from 2008. As found before in *M. paradoxus* in both years, *M. capensis* larvae had slightly higher levels of 18:4(n-3) (0.6-1.2%TFA) compared to the eggs (< 0.4%TFA).

The classification of yolk-sac, non-feeding and feeding *M. paradoxus* larvae was also used to investigate the occurrence of certain fatty acids, which were found in larval stages, but not in relevant amounts in the eggs (Fig. 5). In 2007, yolk-sac larvae showed the highest amount of the FA 18:4(n-3) and 20:2(n-6) and lowest amounts of these FA occurred in non-feeding larvae (Fig. 5). A significant difference in the amount of 18:4(n-3)

was found for yolk-sac and non-feeding, as well as feeding larvae (ANOVA, $p<0.05$) (Fig. 5a). The amount of 20:2(n-6) differed significantly between yolk-sac and non-feeding larvae (ANOVA, $p<0.05$) (Fig. 5b). In 2008, the amounts of FA 18:4(n-3) and 20:2(n-6) were lowest in yolk-sac and highest in feeding larvae (Fig. 5). Significant differences (ANOVA, $p<0.05$) in the amounts of 18:4(n-3) and 20:2(n-6) were found between feeding larvae and yolk-sac, as well as non-feeding larvae (Fig. 5).
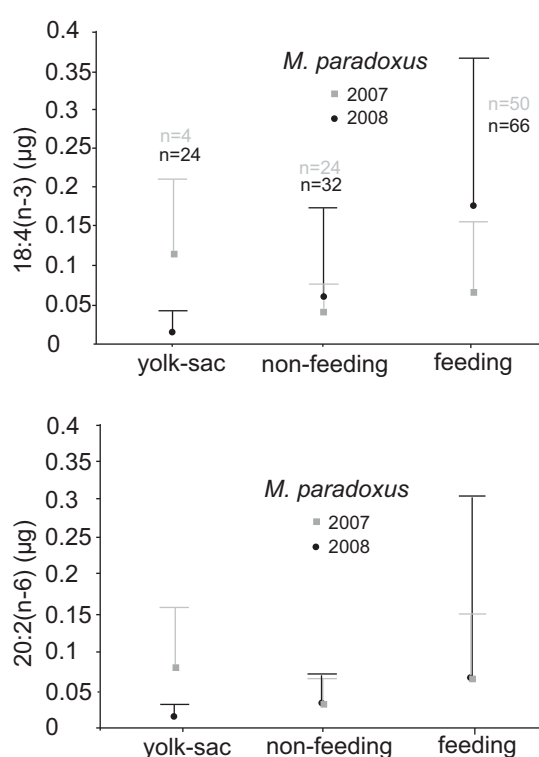
Figure 5 Mean content (+ SD, only drawn in positive direction) of the fatty acids 18:4(n-3) and 20:2(n-6) in *Merluccius paradoxus* larvae in 2007 and 2008 separated by yolk-sac, non-feeding and feeding larvae.

The essential fatty acids (EFA) arachidonic acid (20:4(n-6), AA), eicosapentaenoic acid (20:5(n-3), EPA) and docosahexaenoic acid (22:6(n-3), DHA) were compared in the classification of yolk-sac, non-feeding and feeding *M. paradoxus* larvae (Fig. 6). In 2007, all three EFA amounts (µg) were lowest in yolk-sac and highest in feeding larvae, although significant differences (ANOVA, $p<0.05$) only occurred between feeding and non-feeding larvae for 20:4(n-6) and 22:6(n-3) (Fig. 6a,c). For 20:5(n-3) significant differences were only found for feeding and yolk-sac larvae (ANOVA, $p<0.05$). In 2008, all

 three EFA showed minima in yolk-sac larvae and maxima in feeding larvae, with significant differences between feeding and yolk-sac, as well as non-feeding larvae (ANOVA, p<0.05) (Fig. 6). For 20:5(n-3), there were also significant differences (ANOVA, p<0.05) between yolk-sac and non-feeding larvae (Fig. 6b).

Principal component analyses (PCA) of fatty acid compositions of *M. paradoxus* larvae caught in 2007 and 2008 extracted four components and three components, respectively, with eigenvalues >1 (Fig. 7). In the PCAs, the first two components are presented, together explaining the highest variation in fatty acid composition (59.3% and 59.8% in 2007 and 2008, respectively). Principal component (PC) 1 explained 42.8 and 39.9% of variability in 2007 and 2008, respectively.

In the PCA of FA data from 2007, PC1 showed negative loadings for 16:0, 18:1(n-7) and 20:5(n-3) and positive

Figure 6 Mean amount (µg, ± SD) of arachidonic acid 20:4(n-6) (AA), eicosapentaenoic acid 20:5(n-3) (EPA) and docosahexaenoic acid 22:6(n-3) (DHA) for yolk-sac, non-feeding and feeding larvae of *Merluccius paradoxus* in 2007 and 2008.
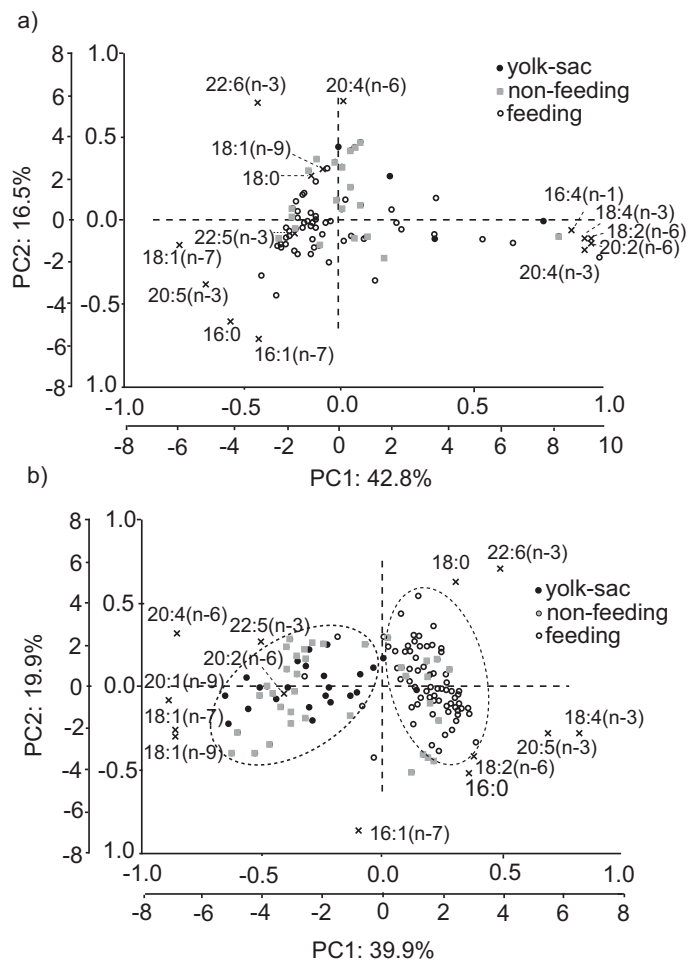
Figure 7 Principal component analyses of the relative fatty acid compositions of yolk-sac, non-feeding and feeding larvae of *Merluccius paradoxus* collected in 2007 (a, n= 78) and 2008 (b, n= 122).

loadings for 16:4(n-1), 18:2(n-6), 18:4(n-3), 20:2(n-6) and 20:4(n-3) (Fig. 7a). Along this component, feeding larvae were found in the negative section compared to the positive direction. The fatty acids 22:6(n-3), 20:4(n-6), 16:1(n-7) and 16:0 affected the separation of samples along PC2, which explained 16.5% of the variability. However, there was no other clear separation of the different larval stages due to different levels of fatty acids. In contrast, in 2008, the different larval stages were clearly separated due to different levels of certain fatty acids (Fig. 7b). This separation was mainly due to the different fatty acid clusters along PC1 with negative loadings for 18:1(n-7), 18:1(n-9),20:1(n-9) and 20:4(n-6), where feeding larvae where distributed and positive loadings for 18:4(n-3) and 20:5(n-3), where the majority of non-feeding and yolk-sac larvae clustered together. 22:6(n-3), 18:0 and 16:1(n-7) were the main fatty acids explaining the 19.9% variability of PC2. Samples of yolk-sac and feeding larvae of *M. paradoxus* were separated along PC1.

DISCUSSION

The lipids of eggs and larvae of *Merluccius paradoxus* and *M. capensis* were analysed to investigate the nutritional condition and feeding preferences of Cape hake larvae. The aim was to characterise egg and larval conditions in terms of total lipid content, lipid class and fatty acid and alcohol compositions of both hake species in two consecutive years. For the first time, the fatty acid compositions of *M. paradoxus* and *M. capensis* eggs and larvae were analysed in single specimens.

The lipid class compositions of hake larvae revealed phospholipids (PL) to be the most important lipid class, with phosphatidylcholine (PC) as major component. This composition is typical of early developmental stages dominated by biomembrane lipids, but low energy reserves (low TAG), as also known from other gadiform fish larvae (Olsen *et al.*, 1991; Plante *et al.*, 2007). The low percentage of free fatty acids (FFA) indicates the high quality of the samples with a very limited degradation.

Lipid class compositions of eggs were not measured due to insufficient sample size. However, eggs of *M. paradoxus* and *M. capensis* contained significant levels of fatty alcohols, which indicate that eggs of both species contain wax esters (16.0-17.3%), as previously found for *M. capensis* (18.3%) (Kayama *et al.*, 1974) and for the south-west Atlantic species *M. hubbsi* (Méndez *et al.*, 1992). Wax esters in fish eggs serve mainly as buoyancy aid and as energy store (Nevenzel, 1970) and wax esters were found in many species, whose eggs inclose oil globules (Kaitaranta, 1981). *M. paradoxus* and *M. capensis* eggs contain one oil globule (Olivar and Fortuño, 1991) of unknown lipid composition. Generally, oil globules of fish consist of one or more neutral lipid classes, usually TAG, wax esters or sterols, which are rich in essential polyunsaturated fatty acids (PUFA) needed for development (Wiegand, 1996). For *M. australis*, lipid droplet adherence (i.e. localisation of the lipid droplet in the posteriormost part of the yolk-sac) was found to be important for larval survival (Bustos *et al.*, 2007). For *M. paradoxus* and

M. capensis, it is not known whether they can synthesise their wax esters *de novo* or if they ingest them with their diet.

Lipid amounts (i.e., total fatty acids and alcohols) per dry mass were higher in the hake eggs than in the larvae of 2008, as the spawning females incorporate lipids in their ovaries. These lipid levels are utilized by the embryo and decrease during development (Wiegand, 1996). A lower overall lipid content was observed in larvae in 2007 compared to larvae in 2008. Furthermore, reduced DHA levels were detected in hake larvae from 2007 compared to those of 2008. The difference in lipid contents and DHA amounts was already detectable in the yolk-sac larvae, apparently due to lower lipid levels transferred to the eggs by the spawners. In European hake, *M. merluccius*, females with a lipid-rich liver had higher lipid levels in their ovaries (Lloret *et al.*, 2008), showing a relationship between maternal condition and lipid content of eggs and yolk-sac larvae. Maternal condition is related to feeding and age of female spawners (Izquierdo *et al.*, 2001).

Dietary lipids of the broodstock seem to directly affect the lipid content and fatty acid composition in fish eggs (Morimoto, 1996). The importance of parental diet for the fatty acid composition of eggs, and in turn for egg quality, has been shown in aquaculture. The influence of food availability and food quality on maternal condition was demonstrated for gilthead sea bream, *Sparus aurata* (Fernández-Palacios *et al.*, 1995) and cod, *Gadus morhua* (Pickova *et al.*, 1997). Eggs from farmed cod exhibited significantly lower levels of essential fatty acids, a lower fertilisation rate and an increased mortality than eggs in the wild (Izquierdo *et al.*, 2001; Salze *et al.*, 2005).

Fish larvae require polyunsaturated fatty acids (PUFA) for their normal development (Bell and Sargent, 1996; Sargent *et al.*, 1997; 1999a). The most important PUFAs for fish larvae are eicosapentaenoic acid, docosahexaenoic acid and arachidonic acid (EPA,

DHA and AA, resp.) (Sargent *et al.*, 1999b; Bell and Sargent, 2003). Especially DHA levels were found to correlate positively with growth and survival of larvae of various marine species (Cutts *et al.*, 2006; Yanes-Roca *et al.*, 2009) and they are essential for the development of the nervous system (Mourente, 2003). A DHA threshold level content is not known for *M. paradoxus* larvae. The amount of DHA incorporated into the eggs during vitellogenesis can be dependent on the DHA levels in the broodstock diet (Izquierdo *et al.*, 2001; Masuda, 2003). Low DHA levels in offspring can therefore indicate insufficient feeding conditions for the broodstock, possibly due to low food availability and/or quality. Energy reserves in European hake remained constant during their spawning period, indicating sufficient feeding during the reproductive season (Domínguez-Petit *et al.*, 2009). Thus, the low DHA levels in larvae of 2007 may indicate that *M. paradoxus* females on the western Agulhas Bank spawning ground experienced poorer feeding conditions compared to the next season in 2008.

The low lipid and DHA levels in hake eggs and larvae could also be explained by maternal age. The 'Big old fat fecund female fish' (BOFFFF) hypothesis states that in long-lived fish big, old, fat, fecund females produce more offspring with higher viability (Longhurst, 2002). The BOFFFF hypothesis was confirmed for a couple of long-lived species, such as the swordfish *Xiphias gladius* and the black rockfish *Sebastes melanops* (Berkeley *et al.*, 2004; Poisson and Fauvel, 2009). Berkeley et al. (2004) could relate maternal age to larval survival and larval survival to oil globule size, suggesting that older female fish produce larger, lipid-richer eggs. Furthermore, the effect of maternal age on larval performance has been shown for the gadiform species haddock, *Melanogrammus aeglefinus* (Hislop, 1988; Probst *et al.*, 2006), and Atlantic cod, *Gadus morhua* (Marteinsdottir and Steinarsson, 1998; Trippel *et al.*, 2005). Hakes are long-lived species with a protracted spawning season and spawning females can be found throughout the year (Murua and Motos, 2006). Field et al. (2008) stated that the large, older hake females produce more eggs. Moreover, it was found that condition of spawners of *M.*

hubbsi and *M. merluccius* varied with age and length, but no seasonal variation was detected (Macchi *et al.*, 2006; Domínguez-Petit *et al.*, 2009). Therefore, the age of female spawners could also influence the lipid levels of their eggs and yolk-sac larvae. This also shows that early developmental stages of species with extended longevity can experience strong maternal effects, possibly a common trait in long-lived species such as hake.

Maternal condition of hake is likely to affect potential reproductive success, as well as viability. Ovarian lipids provide the energy for embryos and yolk-sac larvae and are important for their survival (Rainuzzo *et al.*, 1997). Therefore, suboptimal maternal conditions can influence egg quality (Tocher, 2003). This was confirmed by a decrease in total egg dry mass and egg diameter in cod, which was related to poor conditions of female spawners (Lambert and Dutil, 2000; Ouellet *et al.*, 2001). Furthermore, low lipid levels in pre-spawning females led to reduced reproductive potential in cod, *Gadus morhua* (Marteinsdottir and Begg, 2002), herring *Clupea harengus* (Óskarsson *et al.*, 2002) and haddock *Melanogrammus aeglefinus* (Blanchard *et al.*, 2003). These results lead to the assumption that hake larvae caught in 2008 were in better condition and had a higher survival potential than larvae in 2007.

Accumulation of lipids, especially of the essential fatty acids, can be a key factor in larval survival and therefore in fish recruitment (Domínguez-Petit *et al.*, 2009). Recruitment failure can be induced by a cascade of factors starting with environmental conditions, which result in the reduction of food abundance or food quality for broodstock. This may lead to poor conditions in spawning females and in turn to low lipid reserves and insufficient transfer of essential fatty acids into eggs and yolk-sac larvae followed by high larval mortality with major negative effects on recruitment. For example, low lipid reserves in Atlantic cod in the Gulf of St. Lawrence may have caused the collapse of the stock

(Lambert and Dutil, 2000). In addition, big old productive females of long-lived species such as hake, are targeted by the long-line fishery, which reduces the reproductive potential and most likely recruitment of the stock. This makes maternal condition a possible key factor in recruitment fluctuations of hake.

Fatty acid compositions of *Merluccius paradoxus* and *M. capensis* larvae were not significantly different within the same year and very similar to that of other marine gadiform species, such as haddock or cod (Klungsøyr *et al.*, 1989; Plante *et al.*, 2007). The influence of dietary lipids on the lipid composition of larvae was evident in both investigated years. The significant increase of 18:4(n-3) and 22:2(n-6) in feeding larvae compared to yolk-sac or non-feeding larvae can be ascribed to the dietary influences. In addition, EPA, AA and DHA concentrations were higher in feeding than in non-feeding and yolk-sac larvae indicating accumulation of all three essential fatty acids through dietary lipids in larvae during 2007 and 2008. In both years, around 60% of larvae were feeding and food items consisted mainly of small (cyclopoid and calanoid) copepods (pers. obs.). These small copepods fed presumably on flagellates and ciliates *inter alia* (Wickham, 1995), which usually contain high amounts of the fatty acids 18:4(n-3) and 22:6(n-3) as well as 20:2(n-6) (Sargent *et al.*, 1987; Dalsgaard *et al.*, 2003). The amounts of 18:4(n-3) and 20:2(n-6) were significantly higher in yolk-sac larvae from 2007 compared to those of 2008, due to a more pronounced incorporation of these FA during vitellogenesis. Possible reasons for this could be a superior feeding regime for female spawners in 2007 as compared to 2008 or a different age structure of female spawners, as indicated before.

The variation in fatty acid compositions of larvae in 2007 and 2008 is reflected in the PCAs. In 2007, yolk-sac, non-feeding and feeding larvae did not cluster in the same way as larvae did in 2008, due to differences in the fatty acid composition. The importance of

DHA was emphasised in the PCA of fatty acid compositions of larvae from 2008. It is evident that some non-feeding larvae had a fatty acid composition resembling that of yolk-sac larvae, whereas other non-feeding specimens had a fatty acid pattern more similar to that of feeding larvae. This indicates that the latter larvae were feeding before, but had evacuated stomachs at the time of catch. The former larvae were in the transition phase towards first feeding or were not successful in first feeding. The PCA pattern of the fatty acid compositions of larvae from 2008 seems to be more usual, since it can be expected that the fatty acid composition changes between the ontogenetic stages of yolk-sac and feeding larvae due to dietary lipids. In summary, these findings suggest that in 2007 conditions for hake recruitment on the western Agulhas Bank and on the west coast off South Africa may have been poorer than in 2008.

Conclusions

The quantity of lipid deposits in yolk-sac larvae determine the time of larval survival before first-feeding, whereas the fatty acid composition influences larval development and growth. This study indicates that maternal effects can be an important factor for larval condition of hake, which in turn can be crucial for larval survival. Determination of broodstock condition during the spawning season could provide important information to allow predictions on the reproductive potential of hake. Furthermore, analyses of DHA levels in wild-caught eggs and larvae of hake can be a useful tool for the assessment of egg quality and larval viability. These findings highlight the importance of understanding early life history patterns and their implications for the recruitment of hake.

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Presentations

First author presentations on international conferences

Grote B., Ekau W., Hagen W., Clemmesen C., Verheye H.M. 2008: Nutritional status and growth of Cape hake *Merluccius capensis* and *M. paradoxus* larvae in the southern Benguela upwelling system off South Africa. 32nd Annual Larval Fish Conference, Kiel, Germany

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The man thinks.
The horse thinks.
The sheep thinks.
The cow thinks.
The dog thinks.
The fish doesn't think.
The fish is mute.
Expressionless.
The fish doesn't think
Because the fish knows.
Everything.

Iggy Pop: 'This is a film', from "Arizona Dream"(1993)

Eidesstattliche Erklärung

(Gem. § 6(5) Nr. 1-3 PromO)

Hiermit versichere ich, dass ich die vorliegende Arbeit:

1. ohne unerlaubte Hilfe angefertigt habe.

2. keine anderen, als die von mir im Text angegebenen Quellen und Hilfsmittel benutzt habe.

3. die den Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Bremen, 30.Juni 2010

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Britta Grote