TAXONOMY AND LIFE HISTORY OF THE ZEBRA SEABREAM, *DIPLODUS CERVINUS* (PERCIFORMES: SPARIDAE), IN SOUTHERN ANGOLA

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ABSTRACT

The zebra sea bream, *Diplodus cervinus* (Sparidae) is an inshore fish comprised of two boreal subspecies from the Gulf of Oman and the Mediterranean / north eastern Atlantic and one austral subspecies from South Africa and southern Angola. The assumption of a single austral subspecies has, however, been questioned due to mounting molecular and morphological evidence suggesting that the cool Benguela current is a vicariant barrier that has separated many synonymous inshore fish species between South Africa and southern Angola. The aims of this thesis are to conduct a comparative morphological analysis of *Diplodus cervinus* in southern Angola and South Africa in order to classify the southern Angolan population and then to conduct a life history assessment to assess the life history impact of allopatry on this species between the two regions.

Results of the morphological findings of the present study (ANOSIM, $p < 0.05$, $R_{meristic} = 0.42$) and ($R_{morphometric} = 0.30$) along with a concurrent molecular study ($F_{ST} = 0.4 – 0.6$), identified significant divergence between specimens from South Africa (n = 25) and southern Angola (n = 37) and supported stock separation and possibly sub-speciation, depending on the classification criteria utilised. While samples from the two boreal subspecies were not available for the comparative morphological or molecular analysis, comparisons of the colouration patterns between the three subspecies, suggested similarity between the southern Angola and the northern Atlantic / Mediterranean populations. In contrast, the colouration patterns between the southern Angolan and South African specimens differed substantially, further supporting the morphological and molecular results.

The distinct morphological divergence between the southern Angolan and South African populations was not reflected within the life history traits of both populations. A combination of methods, including length/age frequency analyses, adult sex ratios and histological analysis was used to determine that this species is a rudimentary hermaphrodite in southern Angola. Peak spawning season was observed between June and July. The overall sex ratio (M: F) was 1:1.52 with females dominating smaller younger size classes and 50% maturity was attained at 210 mm FL and 4.6 years. Females [$L(t) = 287.5(1-e^{-0.18(t-2.84)})$] grew significantly faster (LRT, $p < 0.05$) than males [$L(t) = 380.19(1-e^{-0.06(t-7.12)})$]. The higher maximum age of the southern Angolan population of *D. cervinus* (43 years) was older than that of South African individuals sampled in the tsitsikamma national park. The similarities in the life history of the two austral

populations are probably a consequence of similar selective pressures in the similar warm-temperate habitats.

Evidence to support the above comments was found in the feeding study which showed that the South African and Angolan populations were almost identical, with both populations feeding primarily on amphipods and polychaete worms throughout ontogeny. In contrast, the diet of their boreal conspecifics from the Mediterranean was different, where larger individuals tended to select larger, and more robust, prey items.

The life history differences observed between the boreal and austral populations can be attributed to either sampling bias or environmental factors. Sampling biases included the use of different age and growth estimation techniques, while the environmental factors would include differential selective pressures most likely driven by different resource availability and exploitation.

The present study provides crucial baseline life history information of a potentially exploitable species off southern Angola as well as information on the life history plasticity of the species. Unfortunately, the current lack of uniformity in the methods used to estimate life history parameters between studies conducted on the boreal and austral populations have complicated our understanding of the evolution of various life history trends in sparid fish. From a management perspective however, the results from the present study can be used to propose management strategies for an emerging trap fishery in southern Angola. Using a balanced exploitation fishery approach (harvesting up to the size-at-100% maturity), the size of the fish traps entrance was calculated based the morphological information from this and other small sparid species that are targeted and was estimated to be 62 mm.

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CHAPTER 1

Introduction

Marine biogeographic barriers, such as upwelling cells and frontal zones, can restrict gene flow between populations of temperature-sensitive species in marine environments (Floeter et al. 2008, Hemmer-hansen 2007). Restricted gene flow combined with contrasting selective pressures can result in local adaptation and populations in such regions may evolve alternate life history strategies (Reznick et al. 1990, Reznick and Ghalambor 2005, Ruzzante et al. 2006).

Gene flow between populations of coastal warm-temperate marine fishes from the north eastern Atlantic/ Mediterranean and southern Africa appears to be restricted (Floeter et al. 2008). The primary barriers to dispersal are thought to be the cold Lüderitz upwelling region and the warm eastern Atlantic tropical currents in the Gulf of Guinea (Floeter et al. 2008). These barriers have split this region into three distinct warm-temperate zones — the north eastern Atlantic/ southern Mediterranean, southern Angola and the south coast of South Africa — for at least two million years (Floeter et al. 2008, Krammer et al. 2006).

The zebra sea bream (*Diplodus cervinus*) is a coastal species that has a disjunct distribution in isolated temperate zones**.** No taxonomic comparisons have, however, been conducted on these Atlantic populations. Although several life history studies have been conducted in the Canary islands (Pajuelo et al. 2003a, 2003b), Algeria (Derbal and Kara 2006, 2010) and South Africa (Mann and Buxton 1992, 1997, 1998), no life history information is available from the Angolan region.

Previous comparative morphology, life history and molecular studies, conducted on a broad range of fish and cephalopod species common to the southern Angola and South African regions, have revealed a range of results. For example, in the family Scaenidae, southern Angolan and South African populations of *Atractoscion aequidens* have been found to exhibit significant divergence in terms of molecular characteristics and life history styles, while having

minimal morphological divergence (Henriques 2011). In comparison, *Diplodus capensis* (Sparidae) from the same regions shows both morphological (Richardson 2011) as well as molecular (Henriques 2011) divergence, but expresses very similar life history traits (Richardson et al. 2011a) when compared with the same species in South Africa. These contrasting findings suggest that the way in which different species have evolved or adapted, in response to a relatively recent isolation event, may vary. The following question therefore arises: how has the biology and morphology of *D. cervinus* changed (if at all) in the southern Angolan region, compared to that found in the other two isolated populations in South Africa and the north eastern Atlantic/ Mediterranean.

While it is important to understand the ways in which different fish species respond to reproductive isolation, from a genetic, morphological and life history perspective, biological information on many of the Angolan fish populations is non-existent or data-deficient (Duarte et al. 2005). However, since a large number of these species are being increasingly exploited by a large and growing subsistence and artisanal fishery (Duarte et al. 2005), biological information is critical to inform sustainable management practices. Unfortunately, Angola like many other developing African countries, lacks the capacity to develop the fundamental knowledge and implement suitable fisheries management frameworks to implement sustainable exploitation of their marine resources (Potts et al. 2009). To combat this problem, it has been suggested that a multidisciplinary strategy should be implemented through the collection of biological, ecological, economic and social information on the fishery species and their fisheries (Potts et al. 2009). It is therefore imperative to conduct accurate biological studies on exploited, or potentially-exploitable, species in order to form a critical component that will provide fundamental information necessary for the development of multidisciplinary management frameworks.

While *D. cervinus* is presently relatively under-exploited in the southern Angolan linefishery, it does contribute significantly to a large artisanal trap fishery in the sheltered waters of the Canary Islands (Pajuelo et al. 2003a, 2003b). The trap fishery in Angola, which is implemented by several artisanal fishing cooperatives, who are experimenting with these gears, owing to the relatively calm inshore ocean conditions. Currently there are no management regulations for this fishery. The present high exploitation rates of other small sparid species (seabream), such as *D. capensis* (Richardson et al. 2011a), *Dentex bernardi* (Richardson et al. 2012) and *Dentex macropthalmus* (Potts et al. 2010) in the linefishery, may lead to declines in these stocks and,

therefore, the development of alternative fishing methods, such as traps in required, to satisfy the high market demand for small sparid fishes.

In terms of food supply the southern Angolan region is heavily dependent on coastal fisheries and associated industries as it is situated in the non-arable Namibe Desert. However, the coastal fishing techniques are still relatively basic, with illegal beach-seine and legal hook-and-line gears dominating the artisanal sector. While this sector has been operating with rudimentary gear and infrastructure for some time, primarily due to the infancy of the sector as a whole, resulting from the 27-year civil war (which ended in 2002), significant investment has been directed into this sector since 2007. The greatest development, however, has been the proliferation of '*chatas*': 5–8 m vessels with 40hp outboard motors. Besides the traditional hook-and-line techniques, these vessels are also used to deploy crab traps and illegal gills nets. Individual fishers also make use of small (1.5–2 m) canoe-type craft (made of gillnet floats), targeting near-shore reef species. The additional mobility provided by the *chatas* has extended the fishery to regions further from the coastal towns, where previously-unexploited reef fish populations are now targeted. While most of this evidence is based on personal observation and reports from various members of the Angolan coastal fisheries research team, the development of an artisanal sea bream trap fishery appears most likely. For this reason biological information on possible target species, such as *D. cervinus,* is needed to implement appropriate future management frameworks.

The genus *Diplodus* is the largest of the family Sparidae, comprising of 13 species and 11 subspecies, of which five species inhabit the inshore coastal waters of Angola, including *Diplodus cervinus hottentotus* (Smith 1849). This subspecies attains 60 cm FL, 7 kg, and is distributed from approximately Lucira to Baia dos Tigres in the South of Angola and then from Cape Point to the southern border of Mozambique on the east coast, with a break in its distribution along a stretch of the South African–Namibian west coast (Heemstra and Heemstra 2004).

Like many small sparid fishes, *D. c. hottentotus* prefers rocky reef habitats, from the littoral zone rock pools to depths of 120 m (Heemstra and Heemstra 2004). Juveniles have also been found in the lower reaches of estuaries (Heemstra and Heemstra 2004). *D. c. hottentotus* is a predator, feeding predominantly on benthic invertebrates such as polychaete worms and amphipods (Mann and Buxton 1992). It exhibits rudimentary hermaphroditism (Mann and Buxton 1998) in South Africa and possibly protogyny off the Canary Islands (Pajuelo et al.

2003b). Sparids are generally slow growing, long lived and late maturing, as was found to be the case in previous studies (Mann and Buxton 1997, Pajuelo et al. 2003a).

Thesis outline

The overall aim of this thesis is to assess the biology of *D. cervinus* in the southern Angolan coastal region and compare the findings with those of previous studies in other geographic locations, so as to assess the life history variability within the species and shed light on the process of local adaptation. Such a study will provide baseline information for the design of appropriate management regulation for the species in southern Angola.

To achieve this aim the thesis is divided into four data-orientated chapters. Although the Angolan population of *Diplodus cervinus* is presently grouped within the South African *hottentotus* subspecies, a taxonomic study — in which fish from both these populations are compared — has never been conducted, so that much of the knowledge of this species is primarily based on assumption. Management of fishes hinges on identifying the unit stock to be managed, which is not possible in the absence of information on stock separation. In Chapter 2 of this thesis I have therefore investigated the taxonomic relationship between these two populations and whether there is sufficient morphological evidence for stock separation, or even species separation, between the two populations.

Chapter 3 provides ecological information associated with the feeding habits of *D. cervinus* in southern Angola compares these with those of the populations in South African and the Canary Islands and discusses how the nutrient-rich Benguela Current may influence the types and amount of food available to this species in the region.

Sparids are considered as one of the most sexually diverse families of fish, exhibiting contrasting styles of reproduction both within and between species. However, due to the complexity of diagnosing such reproductive styles, there is often disagreement when attempting such a diagnosis. In Chapter 4, a contemporary multi-method approach is used to determine this population's reproductive style as well as the two other reproductive parameters. This chapter also provides a detailed description of gametogenesis, reproductive seasonality, and reproductive style in the Angolan population of *D. cervinus*.

Chapter 5 quantifies important life history traits — such as growth rate, population structure and age and length at maturity — within this population and uses this information to assess

how the Angolan marine environment has affected important life history traits. Possible reasons for differences or similarities between other populations of the species are discussed.

Chapter 6 concludes the thesis and discusses the finding of the previous chapters, specifically their implications for our current understanding of this species complex, patterns of local adaptation, and possible management options for the species in the Angolan region. Future research priorities for the region are also discussed.

Study area

The study was conducted on the inshore coastal zone, between 0–15 m water depth, in the immediate area surrounding Flamingo Lodge, which is situated between the towns of Namibe (15° 11’ S, 12° 09’ E) and Tombua (15° 48’ S, 11° 50’ E) in southern Angola. Additional taxonomic specimens where obtained from the fishing settlements of Benguela, Lucira, Namibe and Tombua in Angola and from Port Elizabeth, St Francis and Port Alfred in South Africa (Figure 1.1).

Two oceanographic features predominantly influence this inshore coastal region: the northerly-flowing, cold Benguela Current and the southerly-flowing, warm Angolan Current. The confluence of the two currents gives rise to the Angola-Benguela Frontal Zone (ABFZ), which demonstrates seasonal variation in its location (Meeuwis and Lutjeharms 1990, Veitch et al. 2006). During autumn and winter the velocity of the Benguela Current is at its strongest, pushing the ABFZ further north, while during spring and summer the opposite occurs, with the Angolan Current gaining in velocity and pushing the ABFZ further south (Meeuwis and Lutjeharms 1990, Veitch et al. 2006). Seasonal variation in coastal water temperature is reflected in the inshore water temperature at Flamingo Lodge (Figure 1.2). The seasonal flux in current velocity and the location of the ABFZ have been shown to have pronounced effects on regional productivity, since the cool Benguela Current has been shown to be nutrient rich with a high chlorophyll *a* concentration, in comparison with the warm, oligotrophic Angolan Current (Hutchings et al. 2009). Thus, productivity is highest during the winter period, while the summer period is characterised by reduced productivity.

The inshore littoral zone at the study site was characterised by intermittent hard sand stone reefs and sandy beds. However, during the characteristically rough winter sea conditions, the sandbanks are often scoured, exposing the rocky sandstone reefs. In contrast, during the calmer

summer sea conditions, the reciprocal occurred with the deposition of sand reducing the primary reef habitat of *D. cervinus*.

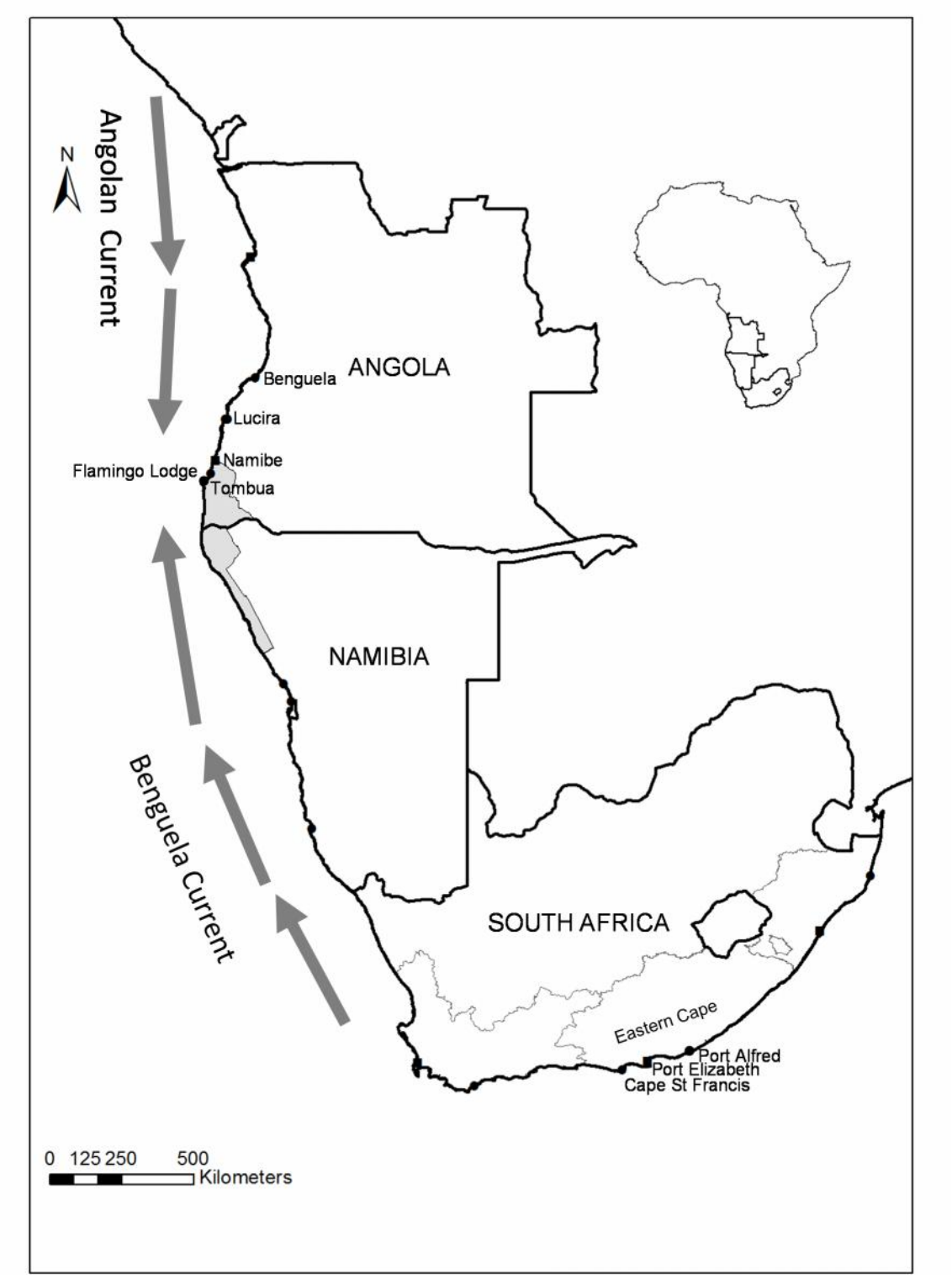

Figure 1.1: Map of sampling sites utilised in the present study. Biological samples were only collected from the inshore area surrounding Flamingo Lodge.

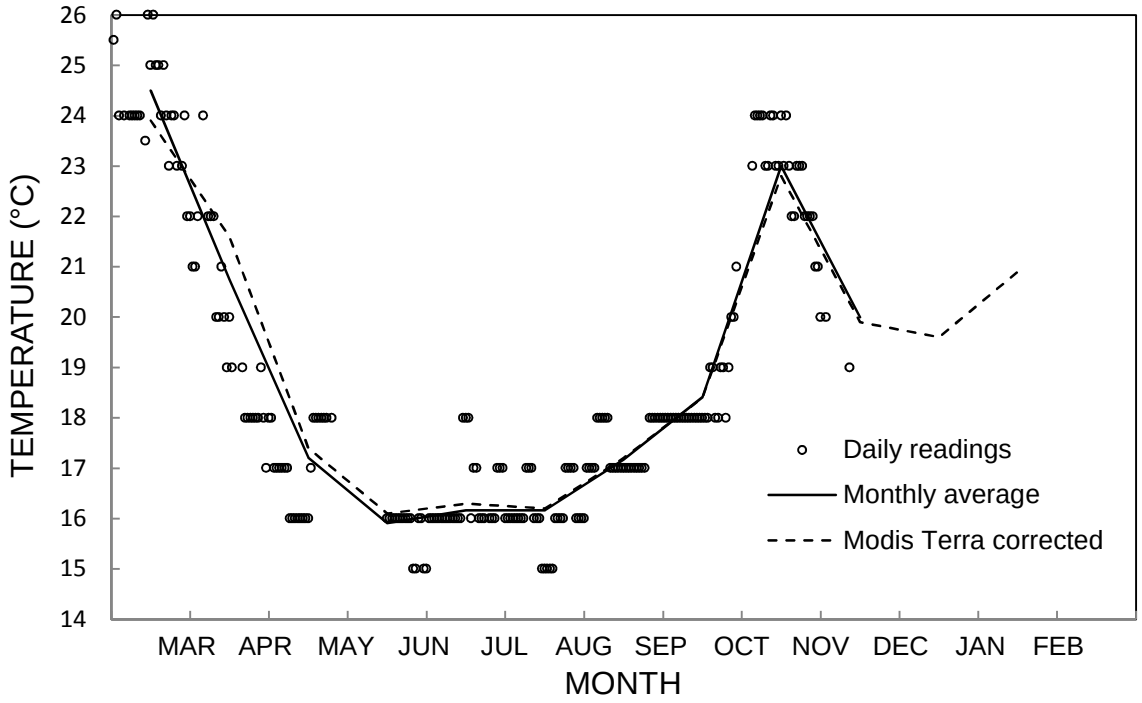

Figure 1.2: Monthly variation in the inshore coastal water temperature off Flamingo Lodge in southern Angola between March 2011 and February 2012. Due to unforeseen circumstances, the measurement of surf zone water temperature was not recorded during January and February 2012, but measurements from a remote sensing satellite (Modis Terra) were used (Figure 1.2) and adjusted, using a correction factor to acquire accurate surf zone temperature estimates (Munnik 2012).

CHAPTER 2

Morphological taxonomic comparison of *Diplodus cervinus* between South Africa and southern Angola

Introduction

The family Sparidae comprises 35 genera and 118 species (Hanel and Tsigenopoulos 2011) with its, current taxonomic classification based on morphological characters such as dentition and external characteristics (Day 2002). The genus *Diplodus* comprises 12 species (Hanel and Tsigenopoulos 2011) of which there are a number of sub-species. The *Diplodus argenteus, D. cervinus* and *D. sargus* species-complexes have been divided into two, three and six subspecies, respectively, based on geographic discontinuities in their distributions and, occasionally, subtle morphological differences (Hanel and Tsigenopoulos 2011). While this is the general scientific consensus relating to the taxonomy of the genus, Heemstra and Heemstra (2004) suggest raising the sub-species status to full species status of the southern African Diplodus contingent. For example they suggest the renaming of Diplodus cervinus hottentotus to Diplodus hottentotus. In my opinion this complicates the taxonomy of the genus even more instead of simplifying it, as it suggests that all previously described sub-species and full species are as closely related.

The centre of *Diplodus* diversity is thought to be the coastal waters of the Atlantic Ocean and Mediterranean Sea, but remnants of the genus are distributed throughout the temperate Atlantic and Indian oceans (Summerer et al. 2001). The biogeography of, and speciation within, the genus is thought to be a consequence of numerous colonisation and extinction events (Summerer et al. 2001, Floeter et al. 2008). These are thought to have been brought about by glacial (distribution constriction) and interglacial (distribution expansion) periods, which formed and degraded biogeographic barriers within the ancestral range of the genus (Summerer et al. 2001, Floeter et al. 2008).

The *Diplodus cervinus* species complex comprises three subspecies: *D. c. cervinus* (Lowe 1849), *D. c. hottentotus* (Smith 1844) and *D. c. omanensis* (Bauchot and Bianchi 1984) from the Mediterranean and North Eastern Atlantic, the South African south-east coast, and southern Angola and the Gulf of Oman (Figure 2.1). *D. c. hottentotus* is the only subspecies with a distinct break in its distribution (Figure 2.1). With no records of this species along the Namibian or the South African west coast, it is hypothesised that the southern Angolan and South African populations of *D. c. hottentotus* may be reproductively isolated. This isolation is thought to be a consequence of the cold-water marine biogeographic barrier formed by the Benguela Current (Floeter et al. 2008), which has been shown to reproductively isolate the populations of several warm-temperate species between the two regions, including *Diplodus sargus capensis*, *Atractoscion aequidens*, and *Lichia amia*, (Henriques 2011).

Until now, the taxonomic status of the Angolan population remains unknown. Modern taxonomy is primarily based upon the concept of binomial nomenclature proposed by Linnaeus (1735), which requires one to assign a genus and species name to a biological entity. However, the designation of a fish to "species" is dependent on the definition of a species, and there are a number of conflicting theories relating to this question (Turner 1999). In a review of fish species concepts, Turner (1999) highlighted the importance of alpha taxonomy in identifying species through the use of taxonomic keys and museum type specimens, to guarantee that the species can be unambiguously identified. Other than the strict rules associated with alpha taxonomy, which allows us to name an organism, two species concepts are particularly relevant in the context of the present study.

Firstly, the Darwinian species concept suggests that species are simply well-marked varieties with no discontinuity between individual variation (Darwin 1859). Although this definition is old, its contemporary application introduces the concept of "fuzzy-logic" to species definition, where a blurred discontinuity exists between species, due to the continual modification of individuals within a species by natural selection (Turner 1999). Secondly, the Biological Species Concept (BSC) uses the concept of reproductive isolation for species subdivision, where a species is defined as a population or group of populations which interbreed (Mallet 1995). The BSC has been around for about the fifty years but has recently gained support as it is easily testable: the introduction of modern genetics has enabled researchers to determine whether there is an absence of shared alleles on a nuclear DNA locus, which would provide evidence for reproductive isolation (Turner 1999).

Both alpha taxonomy and BSC have strict testable criteria, while the Darwinian concept is more blurred, since it lacks a strict testable criterion, rather relying on the discretion of the author. Turner (1999) suggested that researchers should use a standardised combination of all three concepts in an attempt to solve taxonomic conflicts between authors. Intraspecific divisions — such as local variants, races, subspecies, stocks and evolutionary-significant units — have been used by many researchers to define populations that do not conform to, or meet, the strict criteria of species status but do show variation from a designated type specimen (Dizon et al. 1992, Moritz 1994, Begg and Waldman 1999, Turner 1999). These divisions have not been accepted by all (Mallet 1995, Heemstra pers. comm) as they do not have strict criteria or a formal publication process, such as those associated with alpha taxonomy species descriptions. Nevertheless, they do provide us with clues on the evolutionary trajectory taken by a population of a species.

Since a molecular analysis was beyond the scope of the present study, a concurrent molecular analysis on *D. c. hottentotus* was conducted by Gwilliam (pers comm). The standard morphological techniques associated with alpha taxonomy were therefore used to evaluate the taxonomic relationship between the South African and Angola populations of *D. c. hottentotus*. These results will be used, along with those by Gwilliam (pers comm), to interrogate the species/population status based on the BSC and a decision on the taxonomic status of the southern Angolan population of *D. c. hottentotus* will be made after considering both methods. In terms of Alpha taxonomy, the morphological criteria used to diagnose the taxonomic status of the sympatric sparid *Diplodus sargus capensis* were followed (Richardson 2011). To diagnose "species", at least one meristic count or one morphometric ratio had to differ between the populations, with no overlap in their range. In order to diagnose "subspecies" or "stock separation", any multivariate statistically-significant differences in meristic or morphometric data between samples from both regions were considered sufficient. Results from the concurrent molecular study were also incorporated, where any evidence of limited gene flow between the two regions was utilized as evidence for the BSC.

The aim of this chapter is therefore to assess the possibility of stock separation, or possible speciation, in *Diplodus cervinus hottentotus* between southern Angolan and South African populations. This was done through the use of modern alpha-taxonomic methods and any other information that could be used to correctly classify these two populations correctly.

The objectives of this chapter are therefore to:

- Utilise modern multivariate statistics on morphological characters data sets from both South African and Southern Angolan specimens to provide a non-biased alpha taxonomic assessment of the morphology of both populations.
- Incorporate the results of a previous genetic study (on the reproductive connectivity of *D. c. hottentotus* between both regions in support of the biological species concept) with data generated during the present study, the aim being to correctly classify both populations**.**

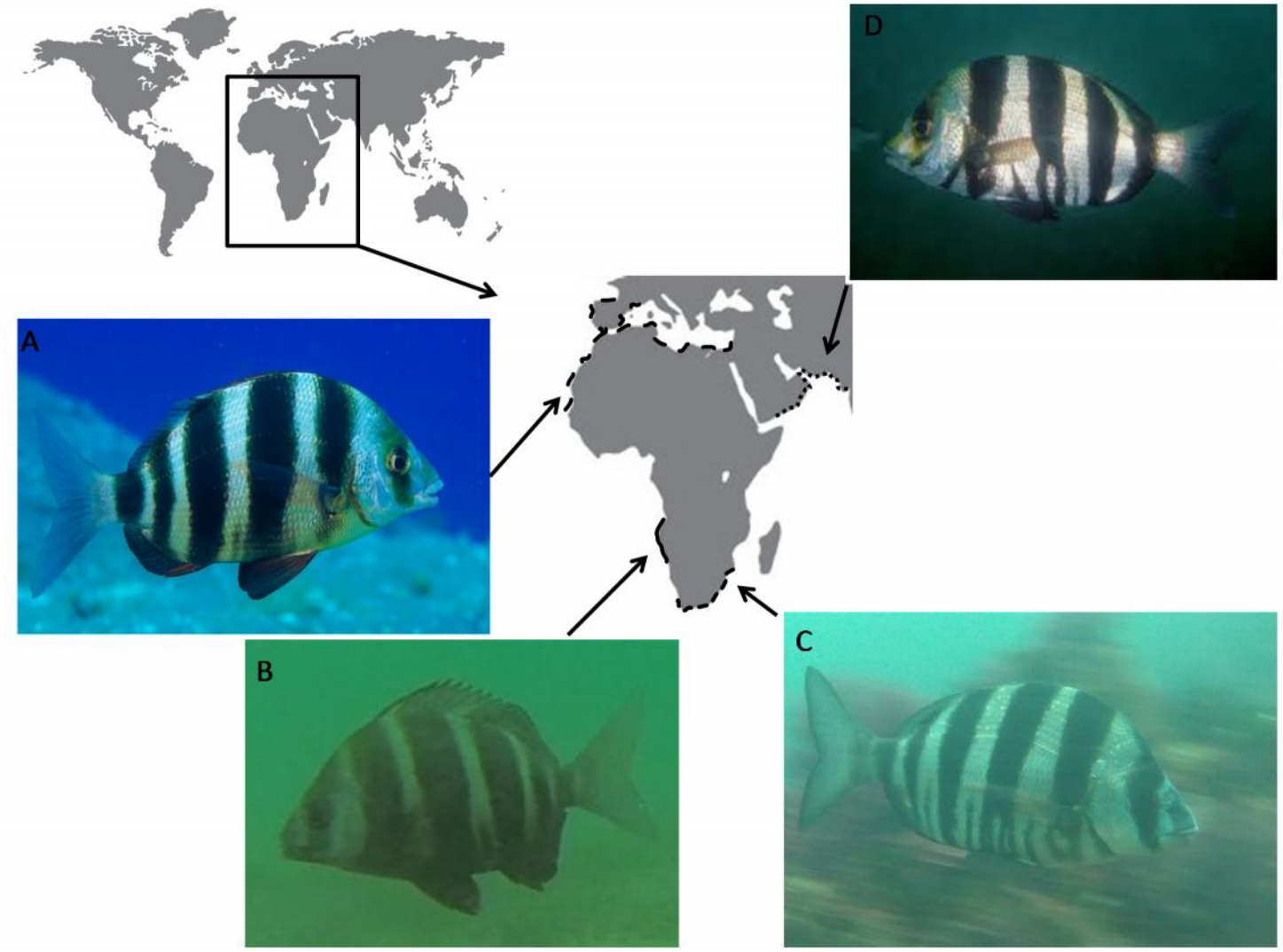

Figure 2.1: Geographic distribution of and comparative live pictures of individuals from the four isolated populations of the *Diplodus cervinus* species complex. Pictures of live specimens for corresponding populations: A) *D. c. cervinus* of the northern Atlantic and Mediterranean (Fishbase 2013); B) *D. c. hottentotus* of Angola; C) *D. c. hottentotus* of the east coast of South Africa; D) *D. c. omanensis* of the Omanian gulf (Fishbase 2013).

Materials and methods

Sample collection and preservation

Fish were collected using spear fishing, hook-and-line, or purchased from local fish markets from Benguela, Lucira, Namibe, Flamingo Lodge and Tombua in southern Angola (n=25) and from Port Alfred, Port Elizabeth and Cape St Francis in South Africa (n=47) (Figure 1.1). While this is a fairly large sample of 72 samples, most of the Angolan samples were collected from the Flamingo lodge area and the South African samples from the Port Elisabeth area with only a small number collected from the other areas. Due to these low sample sizes from the other locations, the resolution of the analysis was restricted. After capture, fish were sacrificed and immediately placed in 10% formalin. After at least one month, fish were transferred from the formalin to a 10% ethanol solution for three days, a 50% ethanol solution for three days, and final storage in a 70% ethanol solution.

Morphometric measurements

A total of 15 meristic counts and 47 morphometric measurements were made on each fish, following preservation. Measurements generally followed Hubbs and Larger (1947) and Richardson (2011) and are listed in Table 2.1. All morphometric measurements were made using digital callipers to the nearest 0.01 mm. If a fish was damaged and a particular measurement was not possible, the measurement was estimated from a linear regression of the form:

$$\boldsymbol{FL_i} = mx_i + c$$

where FL_i is the fork length of the damaged individual, *m* is is the slope of the model, x_i is the missing character and c is the y intercept, for that variable from the respective population.

Data analysis

Since morphometric data is continuous and the meristic data is discrete (Turan et al. 2006), statistical analyses were performed separately. Extreme outliers in the morphometric data from each region were defined as those greater than three times the inter-quartile range, below or above the first and third quartiles, and detected using a box plot analysis (Simon et al. 2010). Significant correlations between size (FL) and morphometric characters may accentuate such

size differences (Simon et al. 2010) and complicate the morphometric comparisons. To eliminate this common problem associated with allometric growth variation, all morphometric measurements were size-adjusted to an overall mean fork length of 206.09 mm (the mean size of all samples) using the following equation (Reimchen et al. 1985, Senar et al. 1994, Simon et al. 2010):

$$\boldsymbol{Y'_{ij}} = \log Y_{ij} - b_j(\log \mathrm{FL}_i - \log \overline{\mathrm{FL}})$$

where Y' is the adjusted value of character j for the individual i, Y_{ij} is the original value, b_j is the pooled regression coefficient of $\log Y$ on logFL, FL_i is the fork length of individual i, and $\overline{\mathrm{FL}}$ is the overall mean fork length. The efficacy of the size transformation was determined from the coefficient of determination (R^2) values of the $\log Y'$ vs logFL regression, where R^2 values below 0.05 were considered to have limited size bias.

Differences between size adjusted morphometric and meristic character means between the two regions were tested using a student's *t*-test. Both data sets were then analysed using a multi-dimensional scaling (MDS) incorporating the Bray-Curtis similarity measure. The extent of similarity between sites was assessed using a one-way analysis of similarity (ANOSIM) using the statistical package PAST Version 2.16 (Hammer et al 2001) and were considered significant at $p < 0.05$. The significance of the ANOSIM results is based on the R statistic, which ranges between -1 and 1 but usually 0 and 1. An R value of 1 indicates that all replicates within sites are more similar to each other than to any replicates from other sites, while a value of zero indicates that similarities between sites are, on average, the same as those within each site (Clarke and Warwick 1994).

Table 2.1: Meristic and morphometric measurements and counts (with definitions) used for the taxonomic study on *Diplodus cervinus* populations from South Africa and southern Angola.

Character	Description	Acronym
Morphometric measurements		
Fork length	Tip of snout to fork of tail	FL
Total length	Tip of snout to tail maximum	TL
Caudal peduncle depth	Thickest part of caudal peduncle	CPD
Caudal peduncle length	Base of anal to end of vertebra (oblique measure)	CPL
Pre-dorsal length	Tip of snout to anterior of 1st dorsal	PDL
Pre-anal length	Tip of snout to first anal fin	PAL
Pre-pectoral length	Tip of snout to base of pectoral ray	PPL
Pre-pelvic length	Tip of snout to pelvic spine	PPEL
Front of dorsal - pelvic distance	Base of 1st dorsal spine to 1st anal spine	FD - P
Front of dorsal - front of anal distance	Base of 1st dorsal spine to 1st anal spine	FD - FA
Front of dorsal - back of anal distance	Base of 1st dorsal spine to last anal ray	FD - BA
Back of dorsal - back of anal distance	Base of last dorsal ray to last anal ray	BD - BA
Back of dorsal - front of anal distance	Base of last dorsal ray to 1st anal spine	BD - FA
Pelvic - anal fin distance	Base of 1st pectoral spine to base of 1st anal spine	P - AF
Dorsal base	Anterior to posterior of dorsal fin	DB
Anal base	Anterior to posterior of anal fin	AB
Length of pectoral fin	Pectoral fin length	LPEC
Length of pelvic fin	Pelvic fin length	LPEL
Head length	Base of front incisors to centre of orbit	HL
Depth of head	Last row of scales to bottom of head	DH
Interorbital distance	Between orbits from top	IOD
Snout length	Snout to centre of orbit	SL
Postorbital length of head	Centre of orbit to furthest part of operculum	POLH
Orbit to angle of pre-opercle	Centre of orbit to sub operculum/ inter-operculum angle under pre-opercula bone	O - APO

Length of eye	Horizontal distance between orbital rims	LE
Length of upper jaw	Base of incisors to most distant maxillary bone	LUJ
Length of mandible	Base of lower jaw incisors to point where angular bone meets the maxillary bone	LM
Width of gape	Maximum distance measured between where the angular bone meets the maxillary bone	WG
Least distance between orbit and maxilla	Middle orbit and closest part of maxilla	O - M
Right top incisor width	Width of front right middle incisor	TRI
Left top incisor width	Width of front middle left incisor	TLI
Average front incisor width	Average of the both incisor widths	AFIW
Top caudal ray length	Length of top caudal ray	TCRL
Middle caudal ray length	Length of middle caudal ray	MCRL
Bottom caudal ray length	Length of bottom caudal ray	BCRL
1st dorsal spine length	Base to the point of the first dorsal spine	1ST DS
2nd dorsal spine length	Base to the point of the second t dorsal spine	2nd DS
3rd dorsal spine length	Base to the point of the third dorsal spine	3rd DS
4th dorsal spine length	Base to the point of the forth dorsal spine	4th DS
5th dorsal spine length	Base to the point of the fifth dorsal spine	5th DS
6th dorsal spine length	Base to the point of the sixth dorsal spine	6th DS
7th dorsal spine length	Base to the point of the seventh dorsal spine	7th DS
1st anal spine length	Base to the point of the first anal spine	1ST AS
2nd anal spine length	Base to the point of the second anal spine	2nd AS
3rd anal spine length	Base to the point of the third anal spine	3rd AS
Pelvic spine length	Base to the point of the pelvic spine	PSL
Pelvic flap length	Length of flap of skin at the base of the pelvic fin	PFL
Meristic counts		
Dorsal spines	Number of dorsal spines	DS
Dorsal rays	Number of dorsal rays	DR
Anal spines	Number on anal spines	AS
Anal rays	Number of anal rays	AR
Pectoral rays	Number of pectoral rays	PecR
Pelvic spines	Number of pelvic spines	PelS

Pelvic rays	Number of pelvic rays	PelR
Pored lateral line scales (LL)	Number of pored lateral line scales	LL
Scales between LL and 4th dorsal spine	Number of scales between lateral line and forth dorsal spine	4th & LL
Scales between LL and anal region	Number of scales between lateral line and anal region	A &LL
Cheek scales rows	Number of scale rows on pre-operculum	CSR
Top incisor count	Number of top incisors	TI
Bottom incisor count	Number of bottom incisors	BI
Upper gill rakers	Numbers of upper gill rakers	UGC
Lower gill rakers	Number of lower gill rakers	LGC

Results

General results

For the mean length, min and max, and n values for both populations, refer to Table 2.2.

Data analysis

Only one individual from the morphometric dataset in the Angolan samples was identified as an extreme outlier and excluded from the subsequent analysis (Figure 2.2). The R^2 values for the linear regressions were all above 0.6 before transformation. These were however all below 0.05 after transformation, indicating that the transformed characters were free from a size bias. The differences in 25 of the 46 morphometric measurements were highly significant ($p < 0.01$), 7 where significant ($0.1 \leq p \leq 0.05$) and 14 were not significant ($p > 0.05$) between the South African and Angolan populations (Table 2.2). The relationship between the most significant morphometric characters and fork length further provides evidence for separation between the two regions (Figure 2.3). The differences between six of the 15 meristic counts were highly significant, one was significant, and eight were not significant (Table 2.3). Despite the large number of significantly different morphometric (69.5%) and meristic characters (46.0%) there was still a large amount of overlap in the ranges of all of these characters between the regions.

The MDS ordination plot for both morphometric and meristic characters separated individuals into two groups, either from South Africa or Angola, with marginal overlap (Figure 2.4, Figure 2.5). The ANOSIM results suggested a similar result to the MDS but also verified that the groupings were significantly different from one another ($p < 0.05$). The relatively low R statistic (< 0.5) observed for both datasets suggests that, based on morphometric and meristic characters, there is a relatively narrow degree of separation between the regions. The meristic characters ($R = 0.42$) were, however, more dissimilar between fish from the two regions than were the morphometric characters ($R = 0.30$).

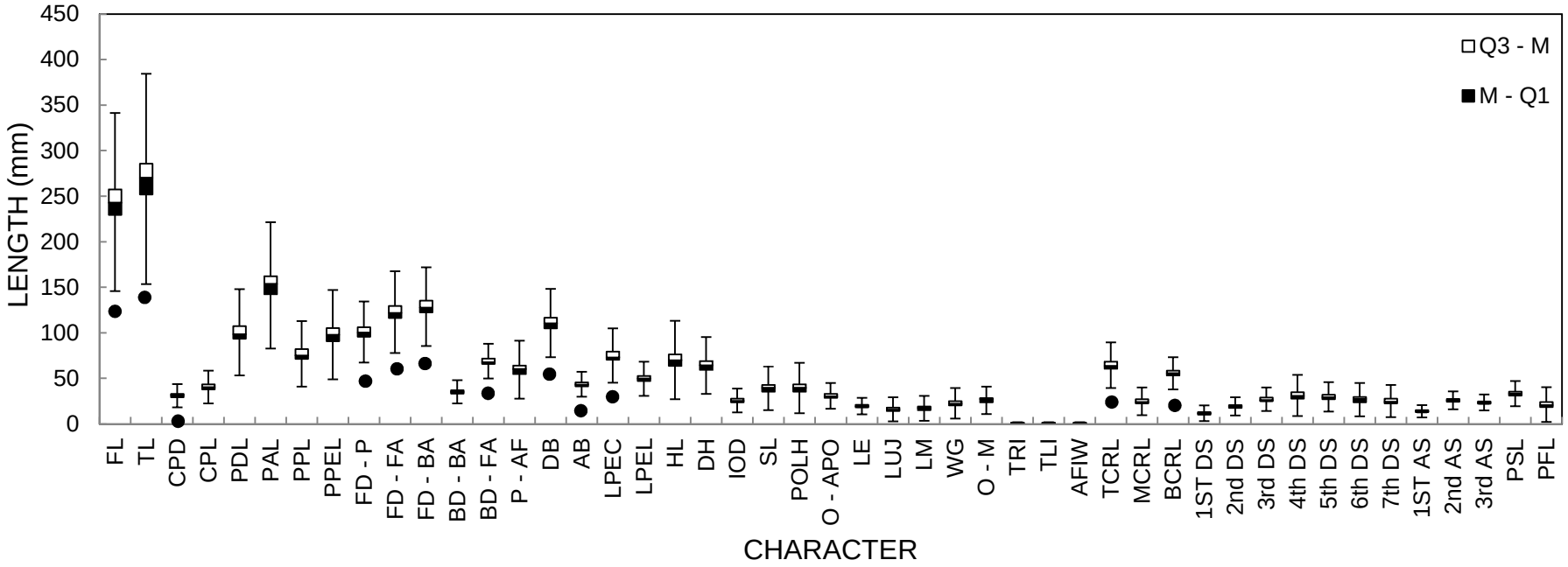

Figure 2.2: Box plot of 37 morphometric characters of *Diplodus cervinus* from southern Angola. Error bars depict three times the inter-quartile range and closed circles denote extreme outliers exhibited by a single fish from the sample.

Table 2.2: Student *t*-test results comparing mean size-adjusted morphometric character measurements between *Diplodus cervinus* populations from southern Angola (n = 25) and South Africa (n = 47).

	Southern Angola					South Africa					
Character	Mean	SD	Median	Min.	Max.	Mean	SD	Median	Min.	Max.	*p* -value
TL	5.43	0.02	5.44	5.37	5.47	5.45	0.02	5.44	5.41	5.49	< 0.05
CPD	3.27	0.08	3.28	3.09	3.46	3.38	0.13	3.38	3.11	3.76	< 0.01
CPL	3.54	0.08	3.54	3.35	3.65	3.60	0.09	3.61	3.34	3.78	< 0.01
PDL	4.45	0.04	4.45	4.32	4.51	4.44	0.04	4.45	4.35	4.51	> 0.05
PAL	4.85	0.02	4.85	4.63	4.91	4.83	0.02	4.83	4.79	4.88	> 0.05
PPL	4.16	0.03	4.16	4.11	4.24	4.11	0.04	4.11	4.01	4.21	< 0.01
PPEL	4.40	0.05	4.40	4.31	4.47	4.36	0.05	4.35	4.24	4.44	< 0.01
FD - P	4.45	0.03	4.45	4.39	4.52	4.47	0.04	4.47	4.40	4.57	< 0.05
FD - FA	4.65	0.03	4.66	4.60	4.70	4.67	0.03	4.67	4.59	4.75	< 0.01
FD - BA	4.69	0.02	4.70	4.66	4.75	4.71	0.03	4.72	4.62	4.78	< 0.01
BD - BA	3.41	0.04	3.40	3.34	3.48	3.47	0.05	3.47	3.33	3.58	< 0.01
BD - FA	4.09	0.03	4.09	4.04	4.15	4.14	0.04	4.15	4.06	4.22	< 0.01
P - AF	3.90	0.05	3.90	3.79	3.99	3.93	0.05	3.93	3.83	4.04	< 0.05
DB	4.54	0.02	4.54	4.48	4.58	4.56	0.04	4.56	4.47	4.65	< 0.01
AB	3.64	0.04	3.62	3.57	3.72	3.68	0.06	3.69	3.56	3.77	< 0.01
LPEC	4.15	0.06	4.17	4.00	4.22	4.17	0.08	4.18	3.85	4.31	> 0.05
LPEL	3.76	0.05	3.77	3.64	3.88	3.78	0.10	3.76	3.62	4.21	> 0.05
HL	4.07	0.05	4.08	3.98	4.16	4.03	0.04	4.03	3.96	4.13	< 0.01
DH	3.99	0.05	3.99	3.87	4.11	3.96	0.04	3.96	3.88	4.06	< 0.01
IOD	3.07	0.04	3.06	2.98	3.16	3.08	0.05	3.08	2.99	3.17	> 0.05
SL	3.49	0.05	3.49	3.40	3.61	3.45	0.05	3.45	3.35	3.56	< 0.01
POLH	3.49	0.06	3.49	3.38	3.61	3.42	0.12	3.44	2.92	3.58	< 0.01
O - APO	3.24	0.11	3.24	2.82	3.43	3.20	0.13	3.23	2.79	3.40	> 0.05
LE	2.81	0.03	2.80	2.75	2.93	2.72	0.06	2.72	2.58	2.83	< 0.01
LUJ	2.63	0.11	2.61	2.46	2.84	2.59	0.08	2.59	2.39	2.77	> 0.05
LM	2.60	0.10	2.62	2.34	2.77	2.48	0.13	2.47	2.19	2.78	< 0.01
WG	2.90	0.06	2.90	2.78	3.02	2.88	0.07	2.88	2.68	3.07	> 0.05
O - M	3.06	0.05	3.06	2.99	3.13	2.99	0.18	3.06	2.55	3.18	< 0.05
TRI	-0.22	0.13	-0.25	-0.42	0.10	-0.25	0.12	-0.25	-0.53	-0.01	> 0.05
TLI	-0.19	0.11	-0.18	-0.48	0.00	-0.29	0.13	-0.29	-0.60	-0.09	< 0.01
AFIW	-0.20	0.10	-0.18	-0.38	0.05	-0.27	0.10	-0.27	-0.51	-0.08	< 0.01
TCRL	3.98	0.05	3.99	3.78	4.07	3.99	0.07	4.00	3.80	4.12	> 0.05
MCRL	3.06	0.05	3.06	2.97	3.17	3.02	0.10	3.01	2.87	3.26	< 0.05
BCRL	3.87	0.05	3.88	3.73	3.94	3.90	0.06	3.91	3.74	4.00	< 0.05
1ST DS	2.33	0.18	2.30	1.85	2.69	2.31	0.12	2.31	2.03	2.46	> 0.05
2nd DS	2.85	0.11	2.87	2.58	2.97	2.81	0.12	2.80	2.51	3.01	> 0.05
3rd DS	3.15	0.09	3.16	2.99	3.33	3.09	0.10	3.11	2.85	3.23	< 0.05
4th DS	3.27	0.10	3.28	2.97	3.41	3.19	0.11	3.19	2.85	3.32	< 0.01
5th DS	3.22	0.08	3.23	3.09	3.36	3.15	0.11	3.18	2.87	3.30	< 0.01
6th DS	3.13	0.10	3.12	2.96	3.29	3.06	0.10	3.09	2.77	3.20	< 0.01
7th DS	3.05	0.09	3.06	2.85	3.18	2.95	0.12	2.98	2.66	3.12	< 0.01
1ST AS	2.50	0.11	2.50	2.18	2.69	2.38	0.11	2.39	2.12	2.56	< 0.01
2nd AS	3.13	0.07	3.11	2.98	3.26	3.03	0.08	3.05	2.77	3.18	< 0.01
3rd AS	3.00	0.07	3.02	2.83	3.11	2.88	0.09	2.89	2.68	3.05	< 0.01
PSL	3.36	0.09	3.36	3.07	3.49	3.38	0.11	3.36	3.25	3.81	> 0.05
PFL	2.84	0.13	2.84	2.58	3.10	2.85	0.12	2.86	2.54	3.04	> 0.05

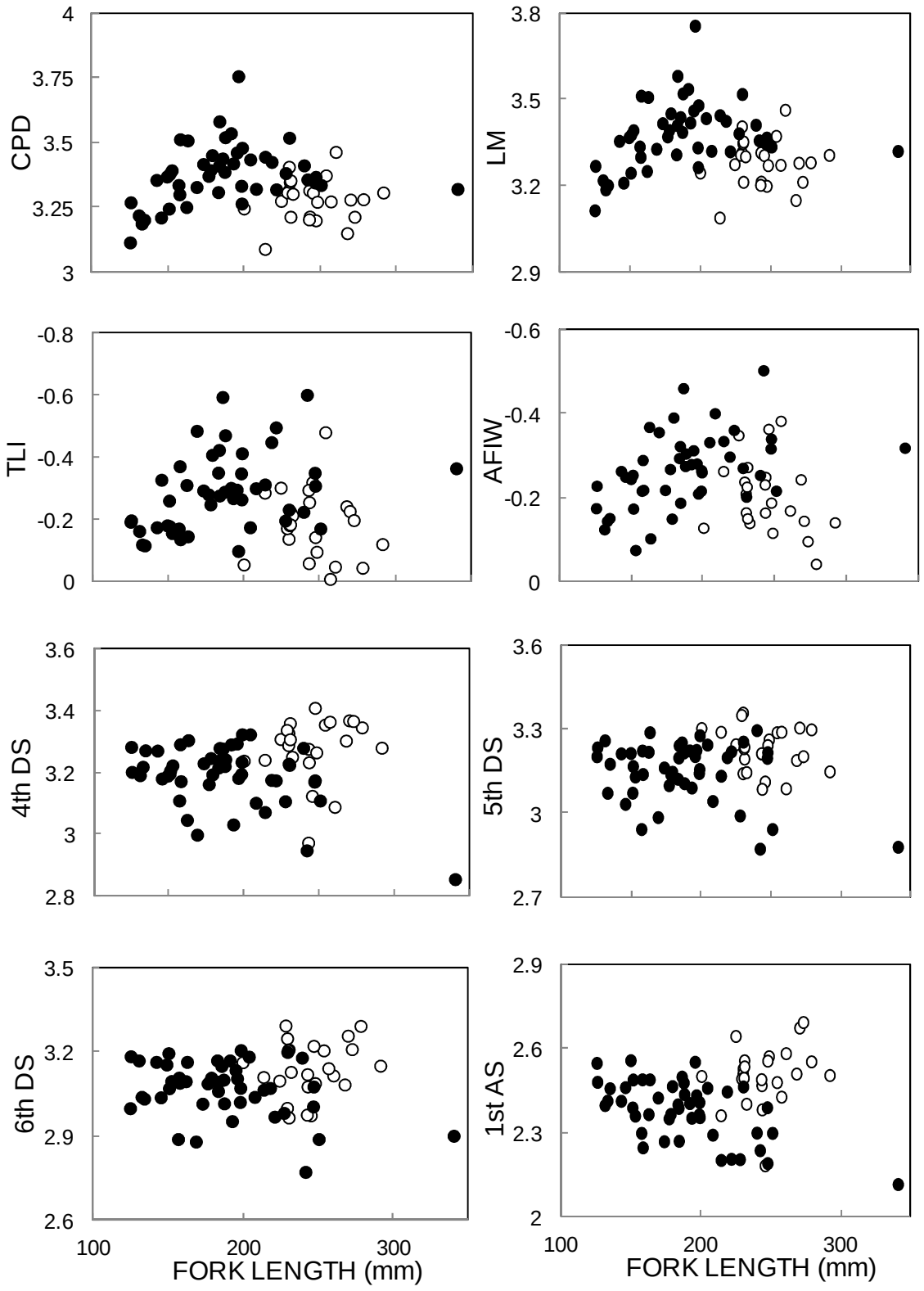

Figure 2.3: Relationship between fork length and selected size-adjusted morphometric character measurements for *Diplodus cervinus* from southern Angola (open circles) and South Africa (closed circles). Differences between these measurements from the two regions were highly significant (Student's *t*-test, $p < 0.01$).

Table 2.3: Student's t-test results comparing mean meristic character counts for *Diplodus cervinus* in southern Angola (n = 25) and South Africa (n = 47).

	Southern Angola					South Africa					
Character	Mean	SD	Median	Min.	Max.	Mean	SD	Median	Min.	Max.	*p*-value
DS	10.96	0.45	11	10	12	10.91	0.28	11	10	11	> 0.05
DR	12.76	0.97	13	11	15	13.43	0.68	13	12	15	< 0.01
AS	3.00	-	3	3	3	3.00	-	3	3	3	-
AR	11.24	0.66	11	10	13	11.74	0.53	12	11	13	< 0.01
PecR	15.68	0.48	16	15	16	14.77	0.81	15	11	16	< 0.01
PelS	1.00	-	1	1	1	1.00	-	1	1	1	-
PelR	5.00	-	5	5	5	5.00	-	5	5	5	-
LL	56.92	1.85	57	54	62	57.81	1.56	58	54	60	< 0.05
4th and LL	8.24	0.60	8	7	10	8.96	0.55	9	8	10	< 0.01
A and LL	13.92	0.86	14	12	16	14.32	0.86	14	12	17	> 0.05
CSR	3.4	0.87	3	2	6	4.30	0.59	4	3	6	< 0.01
TI	10.68	0.75	11	9	12	11.00	0.78	11	9	13	> 0.05
BI	8.04	0.54	8	7	10	7.91	0.46	8	6	9	> 0.05
UGC	6.84	1.03	7	5	8	5.83	1.17	6	3	8	< 0.01
LGC	10.12	0.83	10	8	11	9.79	0.62	10	8	11	> 0.05

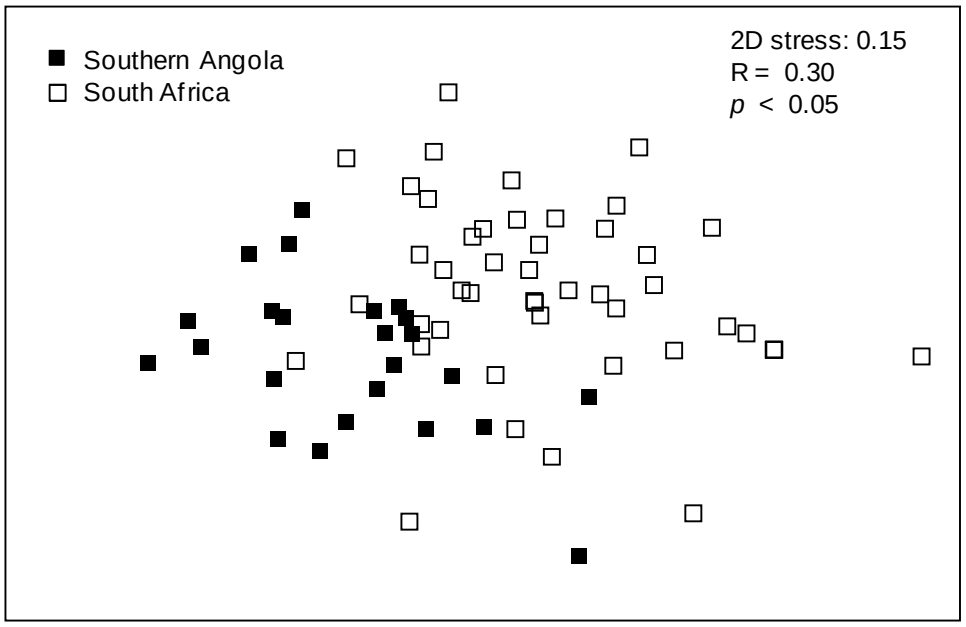

Figure 2.4: Multi-dimensional scaling (MDS) ordination plot of 46 size-adjusted morphometric measurements of *Diplodus cervinus* from southern Angola (n = 25) and South Africa (n = 47).

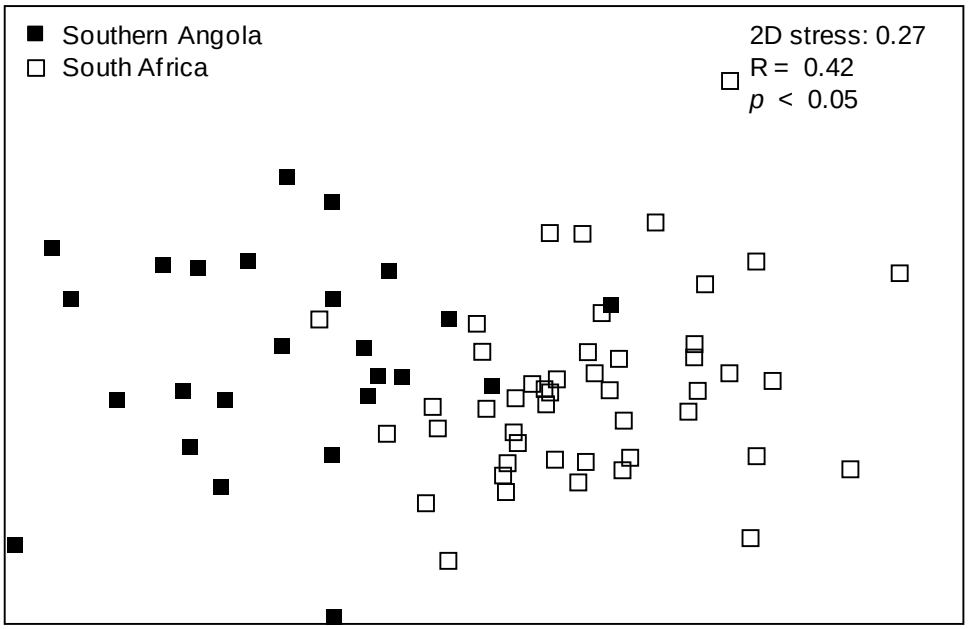

Figure 2.5: Multi-dimensional scaling (MDS) ordination plot of 15 meristic measurements of *Diplodus cervinus* from southern Angola (n = 25) and South Africa (n = 47).

Discussion

The morphological evidence for divergence between these two populations included divergence in the morphometric (R = 0.30, ANOSIM) and meristic (R = 0.42, ANOSIM) characters, differentiation in the MDS ordination plots (Figure 2.4, 2.5) and significantly different mean values for 69.5 % of the morphometric characters and 46.1 % of the meristic characters. However, the large degree of overlap in all character counts and the range of the measurement ratios provided alpha taxonomic evidence in support of stock separation or possible sub-speciation but did not support the hypothesis of speciation between the two *Diplodus cervinus* populations.

Similar ANOSIM R values (< 0.5) where observed for the meristic (R = 0.35) and morphometric (R = 0.34) characters on a similar morphological study done by Richardson (2011) on the closely related *Diplodus sargus capensis* between the same two regions. Fewer of the meristic and morphometric character means were significantly different between the two regions in *D. s. capensis*. Despite *D. cervinus* having a more divergent morphology, there appeared to be reduced molecular divergence between the populations of *D. cervinus* compared to *D. s. capensis*, with mitochondrial DNA sequences [F_{ST} = 0.4 – 0.6 for *D. cervinus* (Gwillam pers comm.), and F_{ST} = 0.8 – 0.9 for *D. s. capensis* (Henriques 2012). The F_{ST} is a common statistic used to infer genetic divergence between populations and is a measure of the observed

genetic diversity within populations relative to observed genetic diversity between populations, which can vary between 0 (no genetic divergence) and 1 (total genetic divergence). Although, slightly lower than those for *D. s. capensis*, the F_{ST} values for Angolan and South African pairwise comparisons in *D. cervinus* were very high, and suggest a breakdown of gene flow across the Benguela Current.

Interestingly, the genetic differences between these two species could be a consequence of differential generation turn over times. *D. s. capensis* matures earlier at 1.8 years (Richardson et al. 2011a) compared to 4.9 years in *D. cervinus* (Chapter 5, Mann and Buxton 1997) and does not live for as long with a maximum age of 31 years (Richardson et al. 2011a) compared to 43 years in *D. cervinus* (Chapter 5). Therefore, the faster generation time of the former may have resulted in a faster accumulation of molecular differences in *D. s. capensis* when compared with *D. cervinus*, if the time of divergence was similar for both species. The higher degree of morphological and reduced molecular divergence between the *D. cervinus* populations could indicate that this species is phenotypically plastic and has responded to divergent ecological pressures in the two regions.

This study focused on clarifying the taxonomic status of *D. cervinus* in southern Angola and a morphometric analysis comparing fish from all the subpopulations of *D. cervinus* was beyond its scope. There have also been no previous molecular studies conducted that compare samples from all of the subpopulations of the species. However, a comparison between the meristic characters from previous species descriptions and identification literature is possible (Table 2.4). Unsurprisingly the meristic counts overlapped between all the subpopulations except for the description provided by Smith (1949) and suggests that in all cases fish belonged to the same species. In Smith and Smith's (1949) description, the LL scale count was obviously greater than all the other descriptions (Table 2.4), including those done later on the same South African population (van der Elst 1993). The only possible cause for this is that Smith and Smith's LL count was conducted using a different counting protocol, possibly counting the scales until the start of the middle caudal ray and not using the standard procedure counting until the end of the peduncle. This unconventional method may be the cause of over estimation but was not specified in the text. Another possible reason could however be a simple printing error which has not been corrected and has been reproduced even until the latest edition (Smith and Heemstra 2003).

Table 2.4: A comparison of the meristic counts of the *D. cervinus* species complex.

		Meristic count							
Sub-species	Region	LL	DS	DR	PelS	PelR	PecR	AR	AS
D. c. hottentotus[1]	South Africa (False bay)	-	11	13	1	5	14	11	3
D. c. hottentotus[2]	South Africa	-	11	12-13	-	-	15	11	-
D. c. hottentotus[3]	South Africa	55-60	11	12-13	-	-	-	11	3
D. c. hottentotus[4]	South Africa	60-67	11	12-13	-	-	-	11	3
D. c. hottentotus[5]	South Africa (Eastern Cape)	54-60	10-11	12-15	1	5	11-16	11-13	3
D. c. hottentotus[5]	Southern Angola (Flamingo)	54-62	10-12	11-15	1	5	15-16	10-13	3
D. c. cervinus[6]	Eastern Atlantic	51-62	11-12	10-12	-	-	-	-	-
D. c. cervinus[7]	Portugal (Madeira)	-	11	12	1	5	15	11	3
D. c. omanensis[8]	Oman (Gulf of Oman)	61-63	11	12	-	-	-	10-11	3

[1]Smith 1844, [2]Heemstra and Heemstra 2004, [3]van der Elst 1993, [4]Smith and Smith 1949, [5]This study, [6]Fischer et al. 1981, [7]Lowe 1838, [8]Bauchot and Bianchi 1984

The question then arises: what should the taxonomic status of the Angolan *D. cervinus* population be? In the description of the subspecies *Diplodus cervinus omanensis* from the Arabian Gulf (north Indian Ocean), Bauchot and Bianchi (1984) — as was the case in the present study — found inconclusive morphometric and meristic variation between this subspecies and the Mediterranean *Diplodus cervinus cervinus* (Table 2.4). Their designation of subspecies was however based on the colouration and stripe pattern. This newly-described subspecies had similar ventral abdominal stripes, to that of *Diplodus cervinus hottentotus* of South Africa, but only had four dark bands, compared with the five in the South African population. In the present study, the external colouration and marking patterns between specimens from both South Africa and Southern Angola differed (Figure 2.1). Angolan fish were more bronze in colour and lacked ventral abdominal stripes while those from South Africa where more silver, with intermittent black belly stripes, similar to those observed in the Omanian population (Figure 2.1). The colour pattern of the Angolan fish is more synonymous with that of the Mediterranean fish, with its bronze colour and lack of belly stripes (Figure 2.1). The use of overall body colouration is generally not considered to be a robust diagnostic character as this feature can be highly variable in some fish species and can be particularly sensitive to environmental influences (Seehausen et al. 1999). However, based on the conclusions of Bauchot and Bianchi (1984), the presence or absence of abdominal stripes can be used to differentiate *D. c. hottentotus* from South Africa and southern Angola. Therefore, due to the significant differences in the mersitic and morphometric characters, the molecular

results that indicate clear genetic isolation, and based on the conclusions by Bauchot and Bianchi (1984) — which were based on colour patterns — the present study provides sufficient evidence to suggest that the Angolan population of *D. cervinus* is a different subspecies from the South African population.

Although one would assume that the Angolan population of *D. cervinus* may be a new subspecies, it is not possible to designate this, based on the results of the present study, as the relationship between the Angolan and north eastern Atlantic/ Mediterranean population was not considered in the present study. However, based only on colouration patterns, it is possible that the southern Angolan population may be a remnant of the northern Atlantic/ Mediterranean population of *D. cervinus* (Figure 2.1). With both populations of fish exhibiting a lack of abdominal stripes and a bronze colouration, which is not the case in both the South African and Omanian populations of the species, this may not be inconceivable, as many of the sparid species in the southern Angolan coastal fish assemblage are common to the northern Atlantic/ Mediterranean and not to South Africa. These include *Dentex barnardi*, *Dentex macrophthalmus*, *Dentex gibbosus* and *Pagrus auriga*, which suggests historical connections between these two regions. The assumption by previous authors (Smith and Smith and Smith 1949, Fisher et al. 1981, van der Elst 1993, Heemstra and Heemstra 2004) that *D. cervinus* in southern Angola is synonymous with the subspecies in South Africa is probably a consequence of the close proximity of the two regions and limited sampling undertaken in Angola.

In order to fully understand the taxonomy of this species complex, a molecular study incorporating samples from all four isolated populations is required. This will allow for a closer analysis, making use of the biological species concept, the purpose being to assign these populations to the correct taxonomic status (Turner 1999). Due to the complex nature of this species complex and problems associated with the taxonomic key for the South African population, as well as the possible recent connectivity with the Mediterranean population, subspecies names will not be used in the present study.

CHAPTER 3

The feeding biology of *Diplodus cervinus* in southern Angola

Introduction

"The survival, growth and reproduction of a fish depends on the income of energy and nutrients generated by feeding activity" (Wootton, 1998). Understanding the feeding biology of a fish is therefore critical to untangle the connections between the external environment and other aspects of its biology. Wootton (1998) suggested that three basic questions need to be addressed in an ecological analysis of feeding: 'What is being eaten?' 'When is it eaten?' and "How much is eaten?'. Asking the first question gives a perspective on what food items are present in the fish's environment or what the fish is able to successfully consume and convert into energy. 'When it is eaten?' leads to information about the times in a fish's life when particular food items are utilized. 'How much is eaten?' provides insight into the energy requirements of the fish and shows how it overcomes various challenges imposed on it by its environment.

Sparid fishes occupy a diverse range of trophic niches, from herbivores to generalist omnivores and specialist predators (Hanel and Tsigenopoulos 2011). Feeding specialization in this family is facilitated by the flexibility in the teeth type and anterior jaw arrangement (Vandewalle et al. 1995). Many species, such as those belonging to the family Cyrinidae and Labridae, have developed crushing pharyngeal teeth to process their food. Sparids, however, have retained a simple pharyngeal tooth arrangement, developing molariform teeth to process hard shelled molluscs and invertebrate prey. Hanel and Tsigenopoulos (2011) have suggested that this alternate strategy is a major factor facilitating the trophic diversity in sparid fishes.

Besides broad interspecific differences in sparid diets, intraspecific variation has also been observed in certain species (Tancioni et al. 2003). Some sparids have specialised their feeding mode to utilise vacant, or under-utilised, trophic niches (for example, in species that feed on hard-shelled molluscs) thus avoiding feeding competition with other species (Buxton and Clarke 1991, Wassef and Eisawy 1985). In resource-limited environments these adaptations can give a population a competitive advantage over possible competitors, but adaptations such as dentition or head shape can also provide us with clues as to what prey items can be consumed. Trophic morphology is however often a deeply-fixed phylogenetic trait that may not actually represent a corresponding diet in the immediate environment (Wainwright and Richard 1995). Diet is more often related to the immediately-available prey items that provide the most energy at the least cost. For example, *Pagellus erythrinus* has a jaw with large crushing molars that are well adapted for crushing hard prey items. Unsurprisingly, this species feeds on hard shelled brachyurans (Caragitsou and Papaconstantinou 1985) but in certain habitats its dominant prey items are soft bodied polychaetes (Jukic 1972). Thus the functional foraging morphology, such as teeth or mouth shape, is not always a good predictor of the trophic niche occupied by a species. Consequently, if dietary differences are found within a species, this may be driven by extrinsic ecological factors (e.g. competition or resource availability) (Hernandez and Motta 1997).

Significant divergence within certain morphological measurements belonging to the head region of *D. cervinus* between southern Angola and South Africa were found in Chapter 2. For example, fish from southern Angola had significantly deeper and longer heads and this may indicate that differential foraging habits may have placed selective pressures on these traits.

Feeding studies not only provide us with valuable information on the ecology and trophic niche occupied by a fish species but, if unbiased sampling methods are used, can also provide an understanding of the diversity and abundance of prey communities (Hyslop 1980, Tancioni et al. 2003). For example, the abundance of easily digested soft bodied prey items may be significantly underestimated when post-capture digestion is high, such as when passive sampling gears (gillnets and long lines) are used and are not checked regularly (Hyslop 1980). Passive gear has also been criticised for over-estimating food consumption in stomach contents as it may select for moving foraging fish. Hayward et al. (1989) found that the stomach content of *Perca flavescens* was overestimated when caught using gillnets (passive gear), compared to fish that were caught in otter trawls (active gear).

The diet of *Diplodus cervinus* in South African waters has been described in a number of studies. Christensen (1978) studied the diets of the early life history stages, while Mann and Buxton (1992) assessed the feeding biology of juveniles and adults in a no-take marine protected area. One other feeding study has been conducted on this species in the southern Mediterranean Sea off the coast of Algeria (Derbal and Kara 2006). Both of the South African studies concluded that juveniles (< 100 mm) predominantly feed on small invertebrates such as chironomid larvae and polychaete worms (Christensen 1978, Mann and Buxton 1992). Mann and Buxton (1992) concluded that adults were generalist invertebrate predators focussing on polychaetes and amphipods. Derbal and Kara (2006), however, found that *D. cervinus* was a generalist omnivore predominantly feeding on Caridae shrimp and other invertebrates but also browsing on benthic macrophytes. Both Derbal and Kara (2006) and Mann and Buxton (1992) suggested that larger fish (> 301 mm FL) were more opportunistic and preyed on larger food items.

Trophic seasonality in benthic reef fishes is well known. For example, in a study carried out on *Pagrus auratus* in New Zealand waters, Kingett and Choat (1981) found a shift from amphipods in summer to bivalves and polychaetes in winter and a significant decrease in feeding intensity in winter. Denny and Schiel (2001) observed changes in feeding intensity in *Notolabrus fucicola* and attributed this to reduced water temperatures and the onset of reproduction in winter. Previous studies have shown similar trophic seasonality in *D. cervinus*, in terms of feeding intensity in Algerian waters, with the highest intensity being observed during spring. Very little change was however observed in terms of dietary composition during different seasons (Derbal and Kara 2006). In South African waters the opposite was found, where there was no clear seasonal change in feeding intensity but a clear shift in prey selectivity (Mann and Buxton 1992).

The marked seasonality associated with the southern Angolan region (see Chapter 1) may have an influence on food availability and this may in turn be reflected in the diet of *D. cervinus.* Richardson et al. (2011b), who studied the closely-related *Diplodus sargus capensis* population in southern Angola, found that feeding intensity did not vary seasonally. However, algae was more important in the diets of these fish during winter, a possible influence of the nutrient-rich Benguela Current, which strengthened during this time.

The aim of the present study is to describe the diet of *D. cervinus* in southern Angola, to examine ontogenetic changes in the diet and to assess the impact of strong seasonal nutrient

and temperature gradients on the feeding of the species. It is hypothesised that, due to the differences found in the head morphology of *D. cervinus* between southern Angola and South Africa in Chapter 2, there may possibly be dietary differences between these two populations.

The objectives of this chapter are to:

- To identify stomach contents to the lowest possible taxon;
- To investigate the effects of seasonality on stomach content diversity and feeding intensity,
- To assess a possible ontogenetic shift in stomach content diversity and feeding intensity through ontogeny,
- To relate selected differences in the morphological features of the head to differences in the diets of the southern Angolan and South African *D. cervinus*.

Materials and methods

Collection and description of diet

A total of 190 *Diplodus cervinus* were collected, using a spear gun in the inshore zone (0 m - 15 m depths), in the vicinity surrounding Flamingo Lodge in southern Angola between March 2011 and February 2012. Fish were weighed (to the nearest 0.1g) and measured (FL mm). Stomachs were removed by severing the oesophagus (at the buccal cavity) and the intestine (immediately anterior to the pyloric caecae) and stored in 10% formalin for later laboratory analysis. The evicerated mass of fish (without internal organs) was then determined (to the nearest 0.1 g).

In the laboratory, stomachs were dissected and the contents weighed. Individual food items were identified to the lowest possible taxon, using a dissecting microscope, and weighed to the nearest 0.0001g after excess fluid had been drawn from the sample, using absorbent blotting paper. In cases where food items were inseparable, a visual proportion of each food type was estimated as a proportion and multiplied by the total mass of the conglomerate.

The importance of each food item was quantified using the frequency of occurrence (% of stomachs containing a specific food item) and percentage mass (weight of each food category expressed as a percentage of the total stomach content mass) (Berg 1979). The relative importance of each food item was assessed using the ranking index (RI) method which was

calculated as the product of the frequency of occurrence (%) and percentage mass (%) (Hobson 1974). Fish were split into small (< 225 mm FL), medium (225–275 mm FL) and large (> 275 mm FL) size classes that corresponded with the 50% and full reproductive maturity of the species (Chapter 5). In order to compare the "relative importance" of food items between size classes, the RI was expressed as a percentage of the sum of the RIs for all food items within a specific size class of fish (% RI). The stomachs of fish in all size classes contained large amounts of sand and unidentified matter. Most of the unidentifiable matter was recognisable as partially digested prey, such as shell fragments or flesh, and therefore contributed nutritionally to the fish and was subsequently included in analyses. Some of it, however, was comprised of white gelatinous material. As this was most probably stomach lining, it was excluded from the analysis. To test for differences in the average percentage mass between size classes, an analysis of variance (ANOVA) was performed for each food group. When the ANOVA indicted significant differences ($p < 0.05$), a Tukey's HSD post-hoc test was performed.

To test for similarity in dietary composition of each size class, the mass contribution of each prey category was square root transformed and subjected to a Bray-Curtis similarity analysis (Bray and Curtis 1957). The similarities in diet between the size classes was illustrated by means of dendograms and tested for differences using an analysis of similarity (ANOSIM) presumption test (Clarke and Warwick 1994), using the statistical package PAST Version 2.16 (Hammer et al 2001). The difference between size classes was considered significant at $p < 0.05$. The significance of the ANOSIM results is based on the R statistic, where values of R usually range between zero to one. An R value of zero indicates that the similarities between and within size classes will be the same, while an R value of one suggests that all replicates within size classes are more similar to each other than any replicates from the other size classes.

Feeding intensity

Feeding intensity between seasons and size classes was assessed using a stomach fullness index (SFI) (Man and Hodgekiss 1977) calculated as:

$$\boldsymbol{SFI} = \frac{Stomach\ content\ mass}{Eviserated\ fish\ mass} \times 100$$

Difference between seasons and size classes were tested using ANOVA.

Feeding seasonality

Descriptive seasonal variation in the dietary composition of *D. cervinus* was assessed by comparing the stomach contents and the % RI between autumn (February–April), winter (May–July), spring (August–September) and summer (November–January). Similarities in the dietary composition between seasons was analysed in the same way as in size classes and illustrated using dendrograms. To test for differences in the average percentage mass between seasons, an ANOVA was performed for each food group.

Results

Feeding intensity

Only 13.8% of the stomachs were empty. The mean stomach fullness index (SFI) of large fish was very similar (SFI = 0.14) and not significantly different from either the small (SFI = 0.13) and medium (SFI = 0.13) size classes (ANOVA, $p > 0.05$) (Figure 3.1). The SFI was highest in winter (0.16) and lowest in summer (0.09), although there were no significant differences in SFI between the seasons (Figure 3.2) (ANOVA, $p > 0.05$).

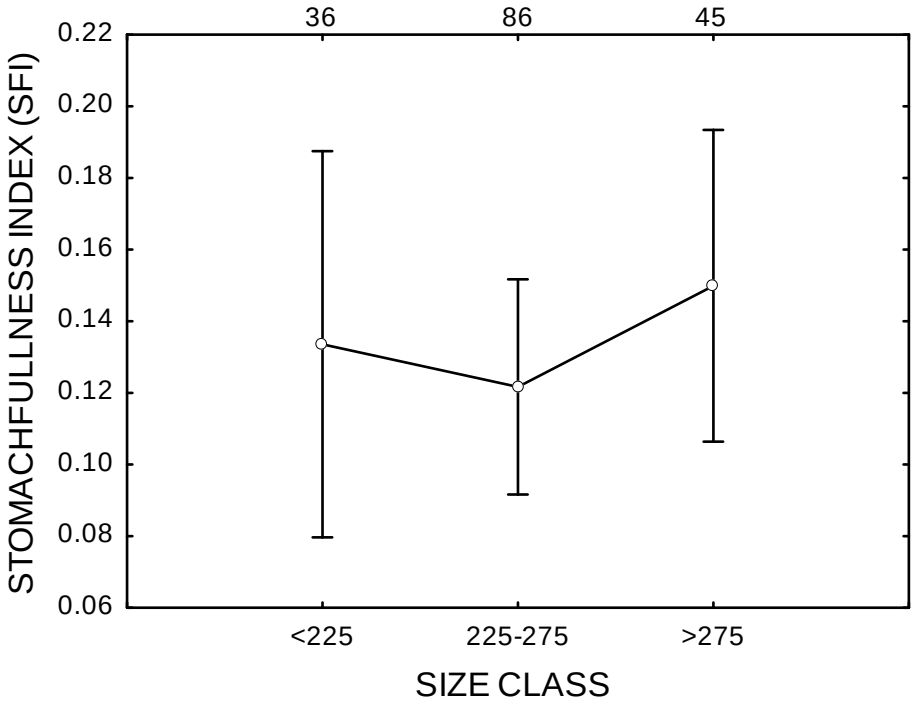

Figure 3.1: Stomach fullness index of three size classes of *Diplodus cervinus* from southern Angola between March 2011 and February 2012. Vertical bars denote 95% confidence intervals and samples sizes are indicted above the graph.

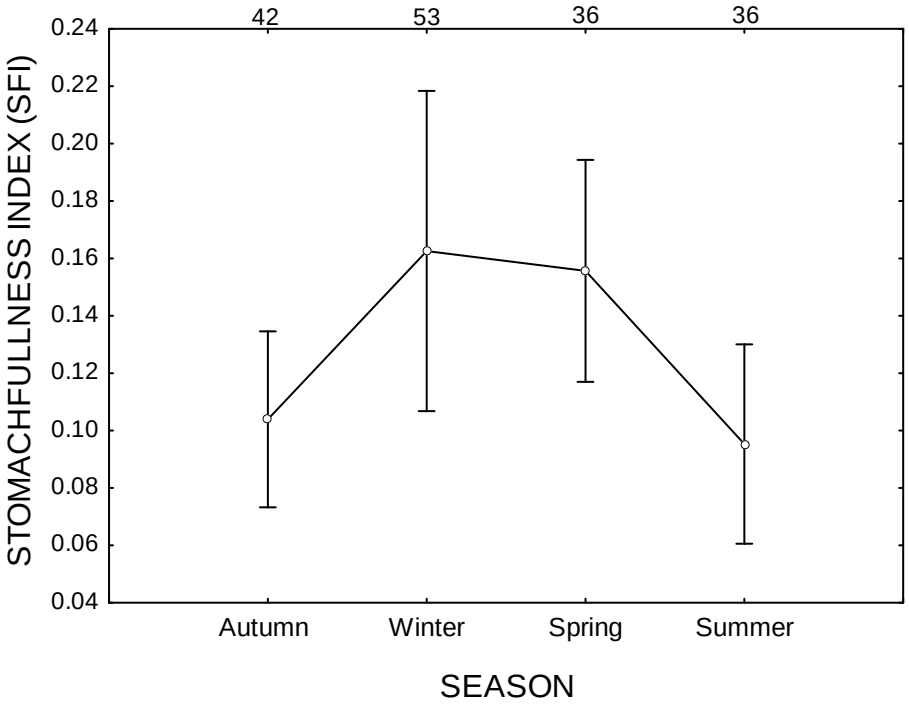

Figure 3.2: Seasonal variation in the mean stomach fullness index of *Diplodus cervinus* from southern Angola between March 2011 and February 2012. Vertical bars denote 95% confidence intervals and samples sizes are indicted above the graph.

Relative importance of food items

Invertebrates dominated the diet of *D. cervinus* (46.5% RI) and comprised Polycheata (30.7% RI), Amphipoda (8.7% RI), Cirripedia (1.9% RI) and Bivalvia (1.5% RI) (Table 3.1).

Larger prey items, such as Brachyura (crabs), fish, Anomura (burrowing shrimps), Gastropoda (snails) and Cephalpoda, were absent (or present in very low numbers) in the stomachs of the small *D. cervinus*, but fairly well represented in the diets of larger individuals (Table 3.1). The stomachs of fish in all size classes contained large amounts of sand and unidentified matter (Table 3.1).

There were no significant differences in the importance of the dominant prey items between the three size classes. There was however a significant difference in the percentage mass of unidentifiable matter between the size classes (ANOVA, $p < 0.05$) (Figure 3.3). The composition of the diet of small fish (< 225 mm) was 72.1%, similar to that of the large (> 275 mm) and medium (225-275 mm) fish, while the diets of large and medium fish were more similar (83.2% similarity index) (Figure 3.4). The ANOSIM results indicated that the Bray-

Curtis clusters were significantly different from one another (R = 0.095, $p < 0.01$). However, the low R value indicated that there was a large degree of similarity between the clusters.

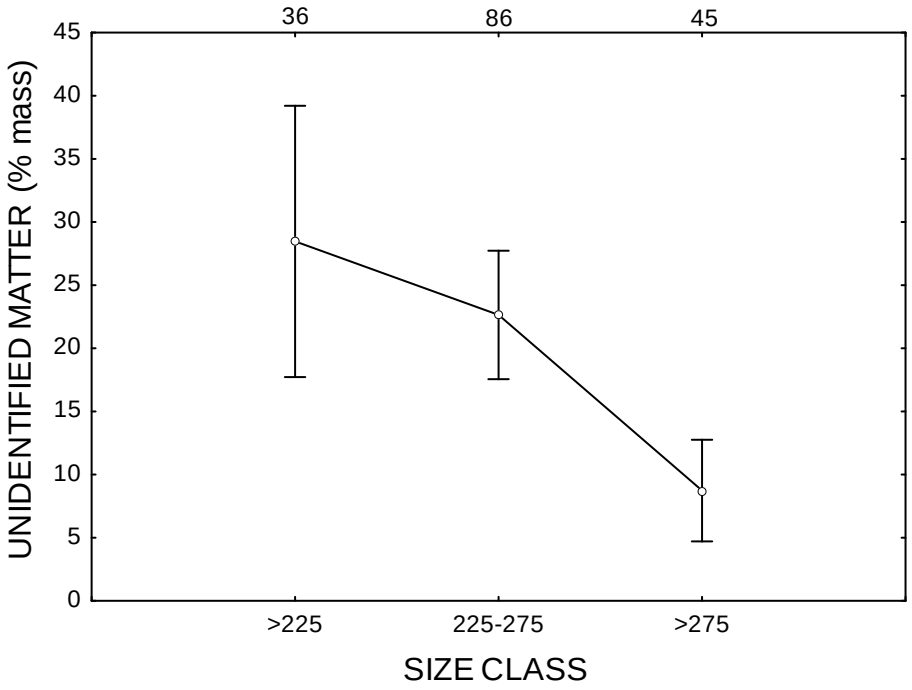

Figure 3.3: The change in the percent mass of unidentified matter between small (< 225 mm), medium (225-275 mm) and large (> 275 mm) *Diplodus cervinus* in southern Angola. Vertical bars denote 95% confidence intervals. Sample sizes indicted above the graph.

Table 3.1: Comparison of the stomach contents of small (< 225 mm), medium (225–275 mm), and large (> 275 mm) *Diplodus cervinus* from southern Angola (F = Frequency of occurrence, M = Mass, RI = Ranking index).

	All (n =167)			Small (n = 36)			Medium (n = 86)			Large (n= 45)		
Prey item	% F	% M	% RI	% F	% M	% RI	% F	% M	% RI	% F	% M	% RI
Annelida												
Polychaete	63.47	23.05	**30.73**	55.56	26.20	**29.21**	68.60	22.41	**28.22**	60.00	21.75	**34.24**
Aryhropoda												
Amphipoda	68.86	6.05	**8.75**	55.56	2.65	**2.96**	80.23	7.05	**10.38**	57.78	6.84	**10.37**
Cirripedia	22.16	4.06	**1.89**	2.78	0.25	0.01	25.58	4.92	**2.31**	31.11	5.47	**4.47**
Caridea	16.17	3.69	**1.25**	8.33	2.67	0.45	15.12	3.65	1.01	24.44	4.57	2.93
Brachyura	15.57	3.33	1.09	-	-	-	19.77	3.52	**1.28**	20.00	5.66	2.97
Tanaidacea	17.96	0.82	0.31	27.78	3.28	**1.83**	17.44	0.17	0.05	11.11	0.08	0.02
Isopoda	12.57	0.28	0.07	5.56	0.60	0.07	16.28	0.17	0.05	11.11	0.22	0.06
Anomura	2.40	1.20	0.06	-	-	-	4.65	2.32	0.20	-	-	-
Mysidacea	2.99	0.69	0.04	-	-	-	3.49	0.15	<0.01	4.44	2.26	0.26
Insecta	0.60	<0.01	<0.01	-	-	-	-	-	-	2.22	<0.01	<0.01
Mollusca												
Bivalva	22.16	3.30	1.53	13.89	5.15	**1.44**	17.44	1.88	0.60	37.78	4.51	**4.47**
Gastropoda	14.97	1.09	0.34	-	-	-	16.28	1.43	0.43	24.44	1.31	0.84
Polyplacophora	11.38	1.07	0.26	5.56	2.78	0.31	12.79	0.54	0.13	13.33	0.72	0.25
Cephalopoda	1.80	1.35	0.05	-	-	-	2.33	1.53	0.07	2.22	2.07	0.12
Plants												
Chlorophyta	17.96	2.97	1.12	13.89	3.46	0.96	16.28	3.23	0.97	24.44	2.10	1.34
Rhodophyta	7.19	1.64	0.25	2.78	2.78	0.15	5.81	0.53	0.06	13.33	2.85	1.00
Other												
Fish	8.38	3.03	0.53	5.56	0.09	< 0.01	6.98	0.70	0.09	13.33	9.84	**3.44**
Bryozoa	5.39	0.60	0.07	8.33	0.26	0.04	4.65	0.98	0.08	4.44	0.13	0.02
Holothuroidea	7.19	0.64	0.10	2.78	0.11	< 0.01	10.47	0.60	0.12	4.44	1.11	0.13
Ophiuroidea	1.20	0.05	<0.01	-	-	-	2.33	0.10	<0.01	-	-	-
Sand	59.88	20.85	26.22	61.11	21.25	26.06	66.28	22.64	27.54	44.44	19.62	22.87
Unidentified	59.88	20.15	25.34	63.89	28.46	36.49	67.44	21.32	26.39	44.44	8.73	10.18

*Bolded and highlighted cells indicate four most important prey items

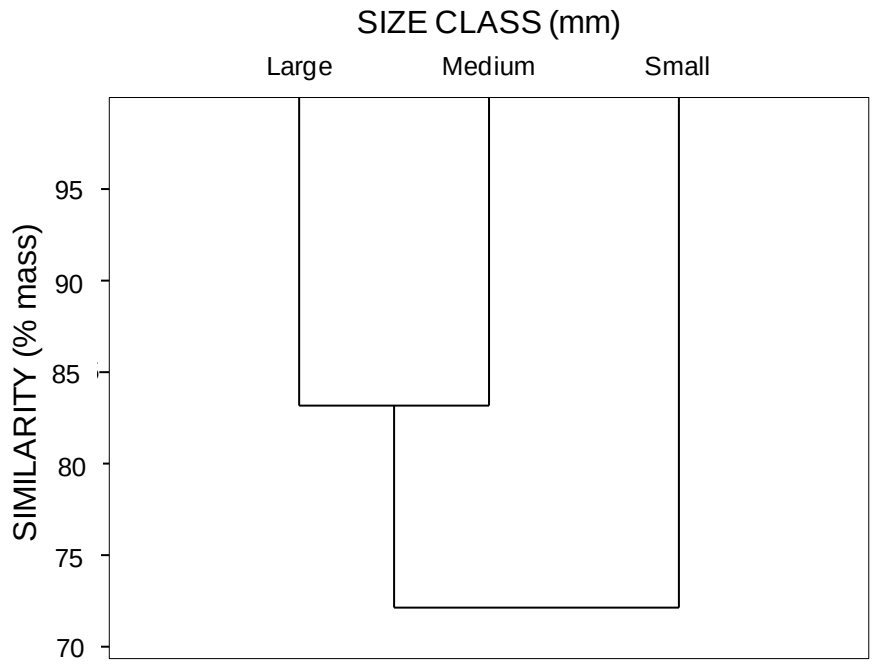

Figure 3.4: Similarity dendrogram of the dietary composition by per cent mass of small (< 225 mm), medium (225–275 mm) and large (> 275 mm) *Diplodus cervinus* in southern Angola.

Feeding seasonality

Polychaetes were the dominant prey item in all seasons especially during autumn (42.6% RI), winter (27.6% RI) and spring (31.2% RI) (Figure 3.5). Amphipods were also well represented during all seasons, except autumn 3.2% (RI) (Figure 3.5). The diets were the most diverse in winter and autumn when prey items other than polychaetes and amphipods comprised over 30% of the total RI (Figure 3.5). Unidentifiable animal matter increased from 4.6% (autumn), 15.9% (winter), 35.2% (spring) to 47.8% in summer (Figure 3.6), while the RI of sand varied from 17.7% during autumn to 27.6% during summer (Figure 3.5).

In terms of mass, the dietary composition of *D. cervinus* in the autumn/winter period was 66.9% similar to that of the spring/summer period (Figure 3.7). The diets of these fish were slightly less similar between winter and autumn than between spring and summer, at 73.5% and 74.6%, respectively (Figure 3.7). The ANOSIM results indicated that the Bray-Curtis clusters were significantly different from one another ($R = 0.062$, $p < 0.01$). However, the low R value indicated that there was a large degree of similarity between the clusters.

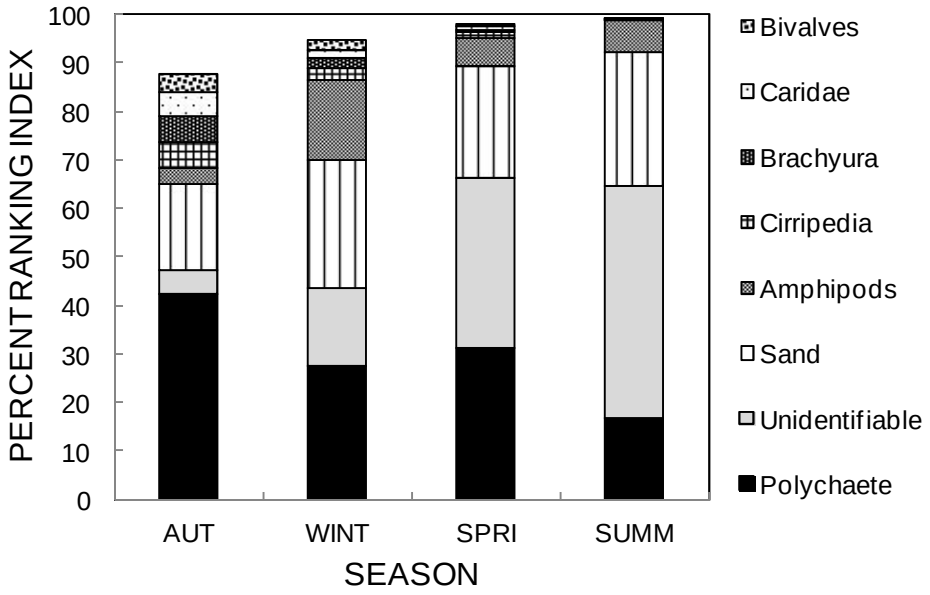

Figure 3.5: Seasonal per cent ranking index for the dietary items found in the stomachs of *Diplodus cervinus* in southern Angola between March 2011 and February 2012.

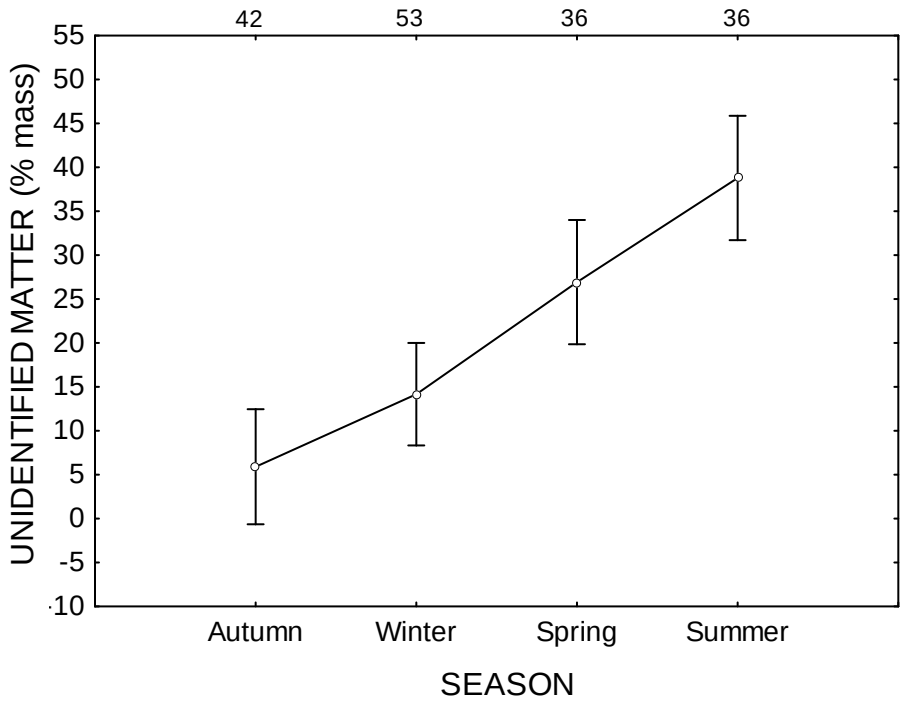

Figure 3.6: The change in the per cent mass of unidentified matter between seasons in the stomachs of *Diplodus cervinus* in southern Angola. Vertical bars denote 95% confidence intervals. Sample sizes indicted above the graph.

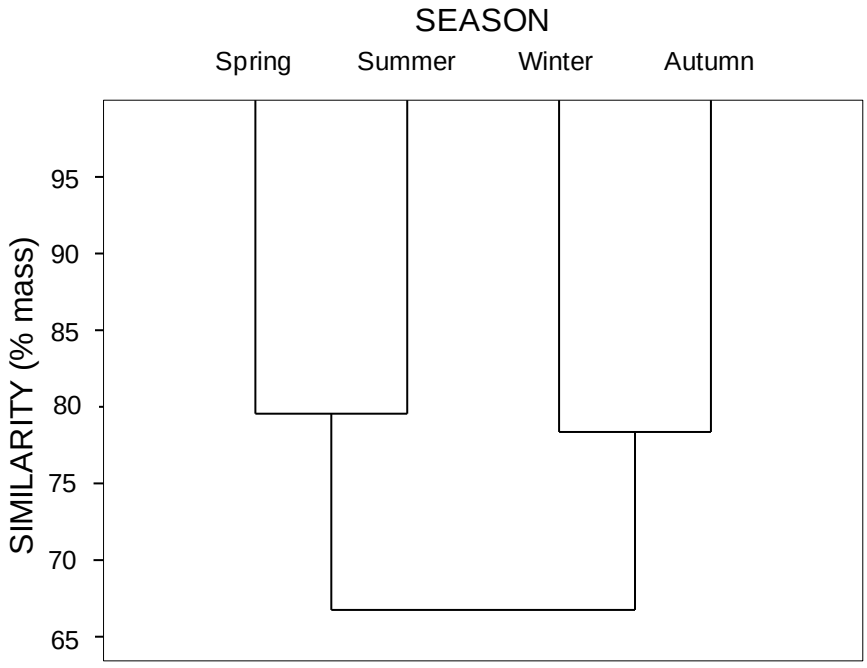

Figure 3.7: Similarity dendrogram of the seasonal dietary composition by per cent mass of *Diplodus cervinus* in southern Angola.

Discussion

D. cervinus in southern Angola occupies a bentivorous niche consuming mainly invertebrates. Although there were subtle changes in the contribution of certain prey items between size classes, the two main food groups, polychaetes and amphipods were the most important prey in all three size classes. This diet was very similar to the *D. cervinus* population found along the warm-temperate South African coast (Mann and Buxton 1992) and thus the hypothesis that the different dentition and jaw shape influences the diets of the two populations is rejected. However, the habitat in the southern Angolan region does differ from that in the Tsitsikamma National Park, which is dominated by high profile sandstone reef interspersed with sandy flats (Buxton and Smale 1984). The reefs are dominated by calcareous algae with echinoderms, octocorals, poriferans and ascidians present (Buxton and Smale 1984) where Mann and Buxton (1992) conducted a feed feeding study on *D. cervinus*. It is possible that while diet has remained fairly constant between the regions, the invertebrate prey maybe utilising different habitat types and the fish in both populations may have adapted to optimise their feeding on these prey items.

Although there was no comparative morphological information on *D. cervinus* off the coast of Algeria in the southern Mediterranean, the feeding habits of this population are different from those found in the present study. *D. cervinus* in the Mediterranean occupies a bentivorous niche

where they feed mainly on hard-bodied carid shrimps as well as mussels (bivalves). Differences in the stomach contents of the Algerian population, when compared to those found in South African studies, may be attributed to a number of factors. These include prey availability, competition, and sampling bias. Intraspecific variation in diet, which relates to differences in prey availability, is common in sparid fishes. For example, Tancioni et al. (2003) found that the diet of *Sparus aurata* was more diverse in a more species-rich estuary (high prey availability) compared to that of fish sampled from a neighbouring less-specious estuary (low prey availability). The diet of *D. s. capensis* has been shown to vary among southern African marine habitats. Algae dominated the stomach contents of *D. s. capensis* in southern Angola (Richardson et al. 2011b) and the Eastern Cape, South Africa (Coetzee 1986) but contributed little to the diet of these individuals along the Tsitsikamma coast, South Africa (Mann and Buxton 1992). Intraspecific dietary variation can often be attributed to prey density and the accessibility of the prey items to foraging individuals (Wootton 1998, Pita et al. 2002). Accessibility of prey is, in turn, dependent on whether the consumer is morphologically adapted to ingest the prey item in a particular environment (Hanel and Tsigenopoulos 2011, Hernandez and Motto 1997). For example, fish that lack crushing molars or robust pharyngeal teeth will not successfully feed on hard shelled molluscs as they lack the morphological features for manipulating the prey items into a manageable size, to allow for swallowing. Assuming that the *D. cervinus* population in Algeria has similar feeding morphology, it is likely that differential prey availability is a possible cause of the difference in diet found in the Algerian population of *D. cervinus,* compared to that of the austral two populations.

Before making conclusions based on comparisons between this and previous studies, sampling techniques should be scrutinized to account for the effects of sampling bias (Hyslop 1980). In the present study, and that of Mann and Buxton (1997), shallow-water (0-15 m) spear fishing was the main sampling technique used in the collection of *D. cervinus*. In the study by Derbal and Kara (2006) passive gear (gillnets and long lines) were mainly used to capture the study specimens. Passive gears bring with them a range of potential biases, including post-capture digestion, regurgitation, and selectivity to feeding studies (Hyslop 1980). Post-capture digestion can cause over- and under-estimation of hard- and soft- bodied prey items, respectively. Regurgitation, while unavoidable in both active and passive methods, is seen to be higher in passive gears due to the often-longer period of time taken for a fish to die after capture. Passive gears also capture moving, hungry fish which increases the probability of capturing foraging fish that are likely to have digested the majority of the food in their

stomachs. This is particularly the case for easily-digestible prey items. Vinson and Angradi (2011), in a critique of fish feeding studies, found that the average percentage of fish with empty stomachs that were caught using passive gears was 35.9% compared with the 21.7% captured when using active gears.

The use of shallow-water spear fishing (< 15 m) as a sampling method in feeding studies is therefore not directly comparable to passive capture methods. In the former case death is instantaneous (resulting in reduced post-capture digestion and regurgitation) and actively foraging fish are not selected. Spear fishing has however been criticized by Vignon and Dierking (2011) who suggest that post-capture regurgitation may be increased when samples are brought up from depths and the stomach is inverted, due to the effects of barotrauma. This concern was, however, based on fish that had been speared at depths of over 20 m, using SCUBA gear. In the present study sampling took place in waters that were shallower than 15m, as was also the case for the study by Mann and Buxton (1997). Thus, the negative effects of barotraumas were not likely to be a problem in either of these two studies. It is therefore safe to assume that the sampling methods utilized in the present study and that of Mann and Buxton (1997) provided a relatively unbiased description of the diet of *D. cervinus*. Unsurprisingly both of these studies suggested that *D. cervinus* consumes high volumes of soft bodied prey items, such as polychaetes, which were insignificant in the diet described by Derbal and Kara (2006).

The Angolan population of *D. cervinus* did not seem to be exhibit a clear ontogenetic dietary shift with increasing size, as both polychaetes and amphipods were found to be the most important prey items throughout all size classes. Larger fish did however consume a wider range of prey items than was the case for smaller individuals. This included larger and harder prey items, such as crabs (Brachura), fish, mussels (Bivalvia) and barnacles (Cirripedia). This shift in dietary diversity was evident from the cluster analysis (Figure 3.2) which indicated that the diets of small fish (72.1% similarity index) were less similar than those of medium and large fish (83.2% similarity index). A similar trend was also observed in the South African population (Mann and Buxton 1992) but was less obvious in *D. cervinus* from the southern Mediterranean, probably due to the low representation of soft prey in all size classes. In this case, however, aquatic macrophytes were also included in the diet of larger fish (Derbal and Kara 2006). A shift to larger prey items is not uncommon in fish, as noted by Karpouzi and Stergiou (2003) who stated that, “As fishes grow, they tend to broaden the size range of prey items ingested, hence increasing the size of prey consumed”. Similarly, a shift towards more

robust prey items has also been found to be common, in cases where the species concerned is morphologically suited to a durophagous (feeding on hard prey items) diet (Hernandez and Motta 1997, Richardson et al. 2012).

A shift in diet, from smaller and softer prey items to larger hard items, is common in the family Sparidae (Buxton and Kok 1983, Buxton 1984, Buxton and Clarke 1986, 1989, 1991, Hernandez and Motto 1997, Fehri-Bedoui et al. 2009, Richardson et al. 2011b). Hernandez and Motto (1997) assessed the possible morphological cause of this dietary shift in the sparid, *Achosargus probatocephalus*. The adjustment towards durophagy in this species was found to be due the development of robust crushing molars and the force generated by the abductor mandibular muscle complex (cheek) with increasing size (Hernandez and Motto 1997). Similar findings were found by Richardson et al. (2011b) on *Diplodus sargus capensis*, a congeneric species to *D. cervinus*, in the Angolan region.

This selectivity of large prey items by larger fish could account for the significantly lower proportion of unidentifiable matter in the diets of such fish, when compared with small- and medium-sized fish (Figure 3.3). Larger prey items are more resistant to digestion; therefore, the ease of recognition of the larger prey items may have contributed to the reduced proportion of unidentifiable material found within this size class.

Mann and Buxton (1992) suggested that the sharp snout, thick lips and sharp outward-protruding incisors characteristic of adult *D. cervinus* have evolved to pick out sessile benthic invertebrates. Similarly, the dental configuration of *D. cervinus* in southern Angola, which includes sharp incisors and reduced molars (Figure 3.8A), appears to be suited to soft-shelled invertebrates, in comparison with the adults of certain other bentivorous sparids that have blunter snouts and larger molars (Buxton and Clarke 1991, Wassef and Eisawy 1985) and selectively feed on harder-shelled molluscs.

In the present study most of the shelled molluscs that were present in stomach contents were small and whole, suggesting that *D. cervinus* is unable to remove and crush harder, larger molluscs. However, the animal component of the diet of adult *D. s. capensis*, which coexists with *D. cervinus* but occupies a different trophic niche, is dominated by hard prey items such as gastropods and mussels (Richardson et al. 2011b). The dentition of this species is quite different (Figure 3.8B) to that of *D. cervinus* and the species appears to have evolved strong incisors and robust molars with which to remove and crush hard, sessile prey items. The results of the present study suggest that *D. s. capensis* and *D. cervinus* have evolved a food partitioning

strategy to avoid competition between each other. As the relative abundance of *D. s. capensis* is much greater than that of *D. cervinus* in southern Angola (pers. obs), differences in either species' feeding apparatus have probably allowed for cohabitation, where their distributions overlap.

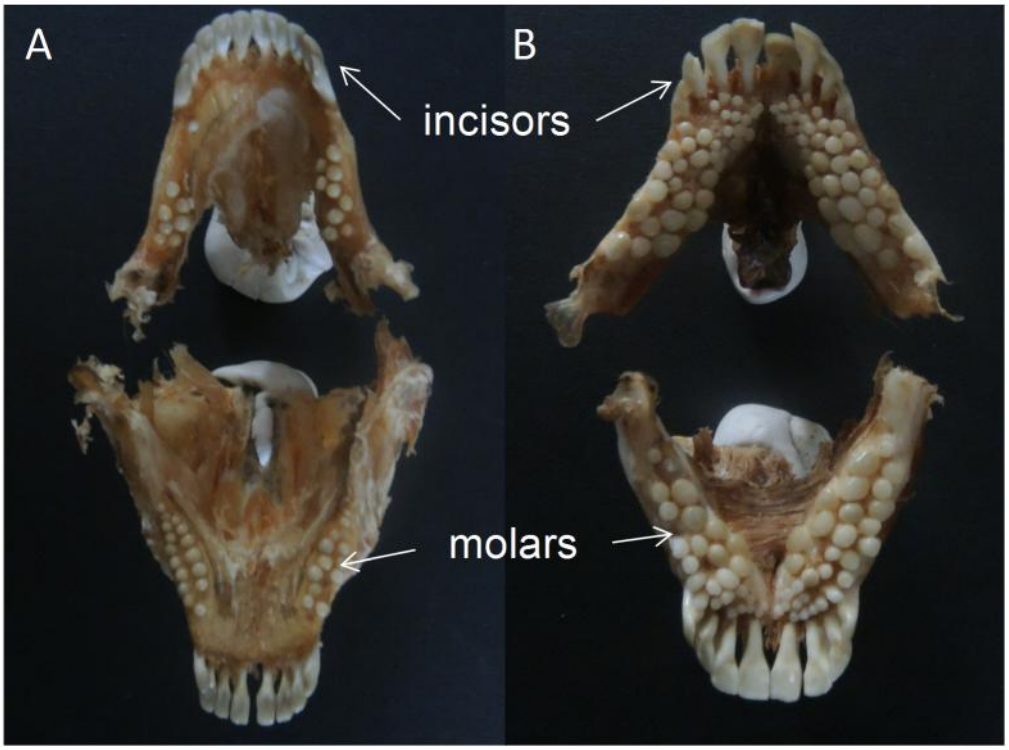

Figure 3.8: Photographs of the upper (top) and lower (bottom) jaw and dental morphology of A) *Diplodus cervinus* (413 mm FL) and B) *Diplodus capensis* (352 mm FL) from southern Angola.

Seasonal variation in dietary composition is largely dependent on the composition of available prey organisms, which change between seasons (Wootton 1998). Seasonal dietary changes reflect the adaptability of the consumer to shifting seasonal changes in the composition of available food items. Polychaetes and amphipods remain the most important prey items of *D. cervinus* in southern Angola, regardless of the season. It was however noted that their importance varied between seasons. Prey diversity was low in spring and summer when compared to that during winter and autumn (Figure 3.5). This trend was significant and is reflected in the cluster analysis (Figure 3.7). The nutrient-rich Benguela Current dominates the southern Angolan region during autumn and winter, which may have an influence on benthic productivity, increasing the abundance of various invertebrate communities that reside in low numbers during the nutrient-deprived seasons of spring and summer (Meeuwis 1990). High

nutrient levels in the food web could also reduce food competition between invertebrate communities, presenting a more diverse community of prey items to foraging fish.

The amount of unidentifiable matter differed significantly between seasons, increasing from autumn until summer (Figure 3.6). The most probable cause for this increasing trend could be the different sampling times in the respective seasons. Sampling during spring and summer usually occurred after 8:00 AM, while in autumn and winter this was delayed until after 11:00 AM, after the thick morning fog (a characteristic of the Benguela coastal region) had dissipated and the underwater visibility had improved. Watt-Pringle (2009), using hook-and-line catch data in the Tsitsikamma National Park, South Africa, showed that feeding activity of *D. cervinus* followed a crepuscular pattern. Thus, if the feeding activity is similar in the Angolan population, prey items would be more digested and more likely to be unidentifiable during winter and autumn (when fish were sampled well after crepuscular morning feeding) compared with that measured during earlier sampling times, in spring and summer when sampling more closely coincided with the maximum morning feeding intensity. This would leave less time for prey items to be digested, and thus represented as unidentifiable.

In the present study no significant changes were observed in feeding intensity as a function of size or season. Feeding intensity was however higher during winter and spring and this may have been due to the onset of the reproductive season, which extends from May to October (Chapter 4). During this period, primary production is seen to be the highest with a strong influence of the Benguela Current. This suggests that this species may have evolved to reproduce during the periods of highest food availability.

In the present study it was found that the largest size class of fish had the highest feeding intensity. Since gamete production is generally exponentially greater in larger fish (Roberts and Poulinin 1991), feeding requirements may also be greater. Reproduction is a costly exercise that diverts resources away from normal body function and growth (Jönsson 1997, Wootton 1998) and since gonad mass is generally expressed as an exponential function of body size (de Vlaming et al. 1982), the feeding requirements of larger fish should be greater. Jönsson (1997) describes two resource-acquisition strategies that may counteract this resource sink: an individual can either build up resources before (capital breeder) or during (income breeder) the energetically-expensive reproductive season. As an asynchronous spawning species (Chapter 4) *D. cervinus* in Angola is most likely an income breeder and increasing its resource intake

during the spawning season would be a strategy to ensure successful reproduction and improve survival (Jönsson 1997).

In conclusion, *D. cervinus* in southern Angola is a generalist carnivore that feeds on benthic invertebrates and has a very similar diet to that of its congeneric *D. s. capensis* population in South Africa, despite significant differences between the populations. Seasonal shifts in prey diversity could be attributed to the influence of the nutrient-rich Benguela Current on benthic invertebrate communities, while the differences in feeding intensity could be attributed to the energetic requirements during the extended seasonal spawning season.

CHAPTER 4

The reproductive strategy of *Diplodus cervinus* in southern Angola

Introduction

Fishes strive to maximise their lifetime contribution of genetic material to the population (Wootton 1998). To achieve this they must reproduce during times that are optimal for the survival of their eggs and larvae (Munro 1990) and adopt a reproductive style that maximises their lifetime reproductive output (Buxton and Garrat 1990, Sadovy 1996). While understanding the reproductive functioning of individuals and how endogenous and exogenous factors affect the reproduction of a species is of general interest, comparative studies between populations can provide more detailed information on the extent of local reproductive adaptation.

Reproduction is energetically expensive, it channels large amounts of energy away from somatic growth, general metabolic functions, movement, and foraging activity. This may be detrimental to the chances of survival in adult fishes, particularly if resources are limited (Toumi et al. 1983, Miguad et al. 2010). Toumi et al. (1983) suggested that reproduction can either reduce survival directly, due to its high resource demand on the body, or indirectly when reproduction increases the vulnerability of individuals to predators, disease or other environmental stressors. Due to the high risk and investment associated with reproduction, evolutionary forces have ensured that mature individuals synchronize their gonad maturation with optimal environmental conditions (Miguad et al. 2010). The survival of the eggs and larvae are equally, if not more important, to maximise the lifetime genetic contribution to the population. Thus, many species have evolved to reproduce during conditions that are optimal for egg and larval growth and survival (Sumpter 1990, Sadovy 1996, Miguad et al. 2010).

In the seasonal environments encountered by species living at high latitudes, spawning usually follows a timed seasonal cycle that is signalled by intra-annual variation in photoperiod and temperature (Miguad et al. 2010). In low-latitude environments, where seasonal fluctuations are less pronounced, spawning is often synchronised with periods of increased food availability, such as peak primary production during the monsoon (Sadovy 1996) or upwelling (Sumpter 1990).

Like many other temperate and tropical fishes, sparids have distinct spawning periods that are governed by temperature and photoperiod (Bye 1984, Sumpter 1990, Sheaves 2006). Sparids found at high latitudes generally spawn during the warmer summer temperatures, while those at low latitudes spawn during cooler winter temperatures (Sadovy 1996, Sheaves 2006). While Sadovy (1996) assumed that winter spawning in tropical regions reflected the mainly temperate distribution of the family, Sheaves (2006) suggested that the timing of spawning was influenced by the narrow optimal temperature range required by eggs and larvae.

The strongest selective pressure placed on individuals in a population is that encountered in its immediate environment (Wootton 1984). Factors such as temperature, photoperiod, food availability, predator abundance and habitat type influence the way in which individuals reproduce. Sparids have however developed a diverse array of reproductive life history styles (Atz 1964, Buxton and Garrat 1990, Sadovy et al. 2008) and this has enabled them to adapt to a wide diversity of habitats. Reproductive style diversity in this family has been assessed by Sadovy et al. (2008), using the criteria proposed by Sadovy and Shapiro (1987) in which thirteen species were confirmed to be gonochoristic (which includes rudimentary hermaphrodites), twenty are protandrous, and ten are protogynous. This diversity is probably made possible by the bipotential characteristic of the juvenile gonad of all sparids (Atz 1964, Buxton and Garrat 1990). The reproductive style of a species in a particular environment is thought to have evolved through the natural selection of successful individuals that are able to maximise their size-specific reproductive output in a specific environment (Buxton and Garrat 1990, Allsop and West 2003, Munday et al. 2006, Sadovy et al. 2008).

The plasticity in reproductive style is not restricted to family or genus level in sparids: it can even vary between populations of the same species. Several authors have found that different populations of the same species can have different reproductive life history styles. For example, *Rhabdosargus sarba* are gonochoristic in Australian waters (Hesp and Potter 2003) but protandrous in Asian waters (Yeung and Chan 1987). Varying diagnoses within species have

however resulted in much debate, with many (for example, Buxton and Garrat 1990) citing author's inconsistencies, concerning the criteria used for diagnosis, as a major factor contributing to these findings. In a review of the criteria used to diagnose hermaphroditism, Sadovy and Shapiro (1987) criticized the use of indirect methods such as adult sex ratios, length frequencies, age frequency and adult GSI, and stressed the importance of using a multi-method approach, that also incorporates gonad histology, to diagnose the reproductive style of fishes.

Although the above-mentioned indirect methods should not be used exclusively to detect hermaphroditism, they can provide useful evidence on the mating pattern exhibited by a particular population, especially when observations of natural spawning are lacking or extremely difficult to obtain. For example, a low mean GSI in male fish suggests that there is a low level of sperm competition during mating and it is therefore likely that pair spawning is preferred by the species concerned. In contrast, when male GSI is high or equitable to that of females, the species is most likely a group spawner that is subject to high sperm competition (Buxton and Garrat 1990, Molloy et al. 2007).

The aim of this chapter is to assess the reproductive strategies of the *D. cervinus* population in Angolan waters and to compare the findings with those of previous studies on other populations of the species.

The objectives of this chapter are:

- To use a combination of macroscopic stageing and mean monthly GSI to determine reproductive seasonality.
- To gain an understanding of the environmental factors driving reproductive activity.
- To use a combination of length frequency, age frequency, sex ratio and histological evidence to determine the reproductive style exhibited by *D. cervinus* in southern Angola.
- To compare these results with similar studies on this species complex from around the world.

Materials and methods

Monthly samples of *D. cervinus* were collected from the inshore zone at the southern Angolan study site by spear fishing between March 2011 and February 2012. Samples were measured (mm FL), weighed (0.1g), sexed and staged macroscopically according to the criteria described in Table 4.1. If a gonad contained both male and female portions, the fish was classified as an intersex individual. Fish were weighed whole and gutted, to the nearest 0.1g. At least ten representative samples of each macroscopic stage, as well as all intersex gonads, were kept in 10 % buffered formalin solution for histological examination. Histological samples were embedded in paraffin wax, sectioned at 5–6 microns, and stained using haematoxylin and eosin (HE) (Austin and Austin 1989), prior to examination.

Reproductive seasonality was assessed by examining the proportion of fish with macroscopically-staged ripe gonads as well as determining the peak gonadosomatic index (GSI) levels, expressed as follows:

$$GSI = \frac{gonad\ mass}{eviserated\ mass}$$

Differences between the GSI during, and out of, the spawning season, as well as the differences between males and females were tested using a student's t-test (significance level, $p < 0.05$).

In order to assess the effect of environmental parameters (photoperiod and water temperature) on the timing of spawning GSI was plotted against both parameters. Photoperiod data was obtained from the United States Naval Observatory website (http://aa.usno.navy.mil/data/docs/RS_OneYear.php). Inshore coastal water temperature was collected off Flamingo lodge for most of the sampling period. However, due to gaps in the data set measurements from a remote sensing satellite (Modis Terra) were used and adjusted, using a correction factor to acquire accurate surf zone temperature estimates without gaps in the data set (Munnik 2010).

Results

General

A total of 408 fish were collected, of which 392 gonads were macroscopically-staged and 371 were weighed (0.1 g).

Histological observations

Oogenesis

Oogenesis occurs within the two paired cylindrically shaped ovaries, located ventrally within the abdominal cavity. Oocyte development and propagation in *D. cervinus* was typical for a teleost (Yamamoto 1956, Wallace and Selman 1981, Booth and Weyl 2000, Rutaisire and Booth 2004) and similar to that described in previous studies on sparids, following asynchronous oocyte development (Coetzee 1983, Buxton 1990, Booth and Hecht 1997, Mann and Buxton 1998, Pajuelo et al. 2006, Pajuelo et al. 2008, Richardson 2011). The primary differences between these studies was the number of oogenesis stages and this tends to vary, based on the number of gonads examined and the interpretation by the authors. In this particular study, three pre-vitellogenic stages and three vitellogenic stages were identified.

Pre-vitellogensis

Oogonia stage

Oogonia (OO) were mainly found at the periphery the ovarian lumen or outer connective tissue in ovarian nests (Figure 4.1A). They are characterised as being round to oval in shape (being the smallest germ cells) and have a large nucleus: cytoplasm ratio, with a lightly basophilic cytoplasm (Figure 4.1A).

Chromatin nuclear stage

Chromatin nuclear oocytes (CN) are round in shape and have a highly basophilic large central nucleus in conjunction with a lightly basophilic cytoplasm (Figure 4.1A). They are associated with oogonia and are found in a similar region of the ovarian lumen (Figure 4.1A).

Perinucleolar stage

Perinucleolar oocytes are polygonal in shape and arise from the first meiotic division of chromatin nuclear oocytes (Figure 4.1B). There are three successional stages of perinucleolar

development, progressing away from the ovarian nests with increasing maturity (Figure 4.1B & C). All are characterised by a strongly basophilic cytoplasm and nucleoli with a weakly basophilic nuclear plasm (Figure 4.1B & C). Pre-perinucleolar oocytes (PPO) which are the closest and often in contact with ovarian nests, are the smallest in size, and have multiple nucleoli scattered randomly in the nucleus (Figure 4.1B). Early perinucleolar oocytes (EPO) are larger than PPOs, with less basophilic cytoplasm (Figure 4.1B). The nuclear region is characterised by three to four larger nucleoli and multiple smaller ones around the inner periphery of the nuclear membrane (Figure 4.1 B). Late perinucleolar oocytes (LPO) are larger than the previous two stages, more spherical in shape, and contain less basophilic cytoplasm (Figure 4.1C). The nuclear region of these cells contains multiple nucleoli arranged in an orderly fashion around the inner periphery of the nuclear membrane (Figure 4.1 C).

Vitellogenesis

Primary yolk vesicle oocyte stage

The beginning of this stage is characterised by the formation of cortical alveoli (ca) towards the periphery of the cytoplasm (Figure 4.1D). The outer cellular membrane begins to thicken and this continues as the zona radiata begins to develop, together with an increase in the number of nucleoli in the nucleus.

Secondary yolk vesicle oocyte stage

The appearance of acidophilic yolk granules becomes evident in the cytoplasm, congregating to the periphery of the cytoplasm (Figure 4.1E). The development of two rows of cortical alveoli around the exterior of the nucleus and to the periphery of the cytoplasm, make this stage easily identifiable (Figure 4.1E).

Tertiary yolk vesicle oocyte stage

This is the final stage of development before an oocyte is mature and ready to be spawned. The early stages are characterised by the presence of well-developed zona radiata (ZR) and zona granulosa (ZG) (Figure 4.1F). The cytoplasm becomes filled with acidophilic tertiary yolk vesicles and the cortical alveoli aggregate in the nuclear region (Figure 4.1G). Towards the end of this stage the nucleus begins to migrate to the periphery of the cytoplasm and lipid droplet formation occurs in the central nuclear region of the cytoplasm (Figure 4.1H). The completion of vitellogenesis is marked by the completion of the nucleus's migration to the periphery of the

cytoplasm (Figure 4.1H). Immediately prior to ovulation oocytes usually hydrate and dramatically increase in size before moving into the lumen of the ovary. This stage of oogenesis was not encountered in this study which was probably due to the tendency of hydrated oocyte to collapse during sectioning. Figure 4.1I depicts a late vitellogenic oocyte that is being reabsorbed by the ovary and is going through the process of atresia, characterised by a granular cytoplasm and the breakdown of the zona radiata.

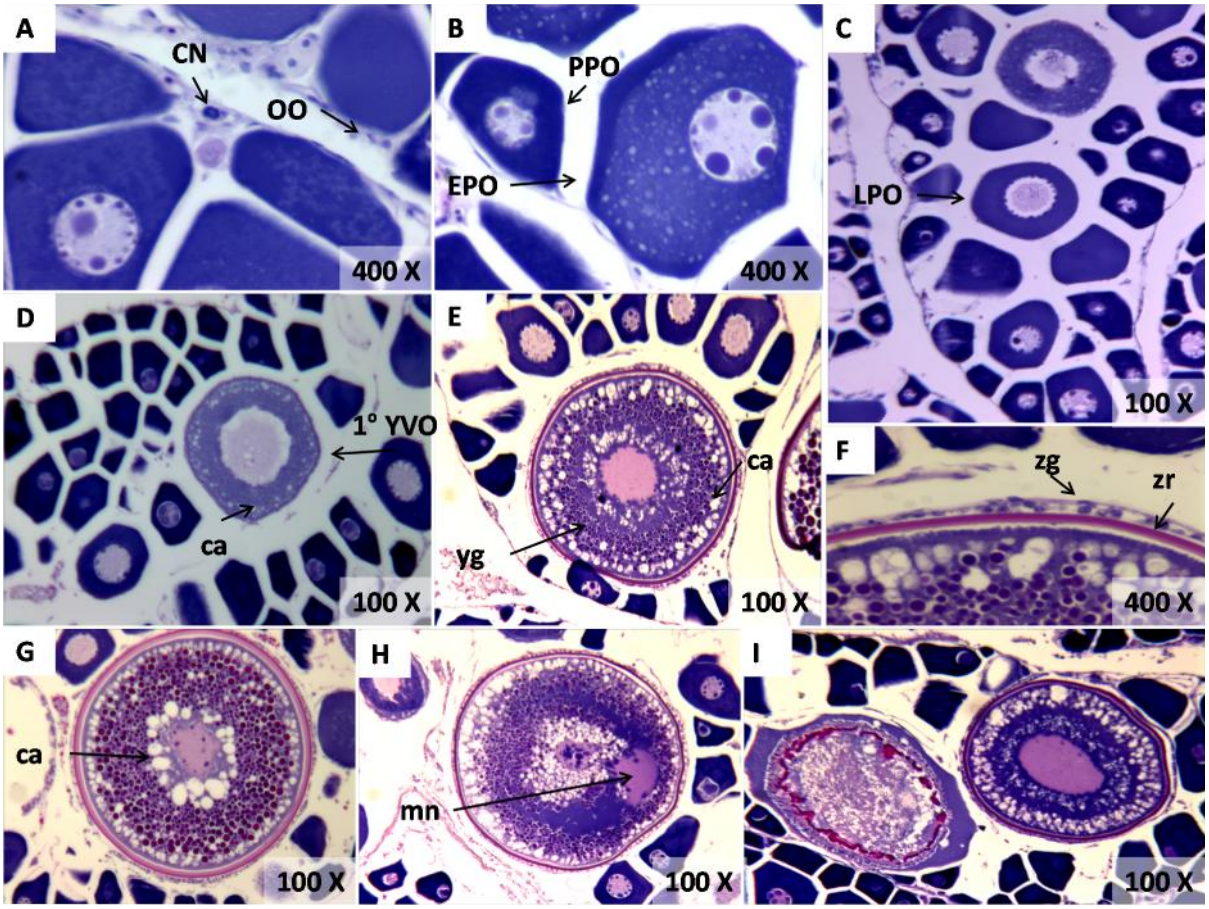

Figure 4.1: Transverse HE sections of the ovaries of *Diplodus cervinus* depicting successive stages of oogenesis stages from A–I. (A) Ovary containing nests of oogonia (OO) and chromatin nuclear oocytes (CN) bordering the ovarian lumen. (B) The first two perinucleolar stages – pre-perinuclear oocytes (PPO) and early-perinuclear oocytes (EPO) – following the meiotic division of chromatin nuclear oocytes. (C) A late perinuclear oocyte (LPO). (D) A primary yolk vesicle oocyte (1° YVO) characterizing the beginning of vitellogenesis, with the first presence of cortical alveoli (ca) in the cytoplasm. (E) A secondary yolk vesicle oocyte (2° YVO) characterized by the first appearance of red acidophilic yolk granules (yg) towards the periphery of the cytoplasm. (F) Well developed zona granulosa (zg) and zona radiata (zr), characterizing the formation of a tertiary yolk vesicle oocyte. (G) A tertiary yolk vesicle oocyte (3° YVO) showing aggregation of cortical alveoli in the nuclear region and red acidophilic yolk granules in the cytoplasm. (H) A tertiary yolk vesicle oocyte during the migrating nucleus (mn) phase, characterizing the end of vitellogenesis. (I) an atretic oocyte providing evidence for reabsorption.

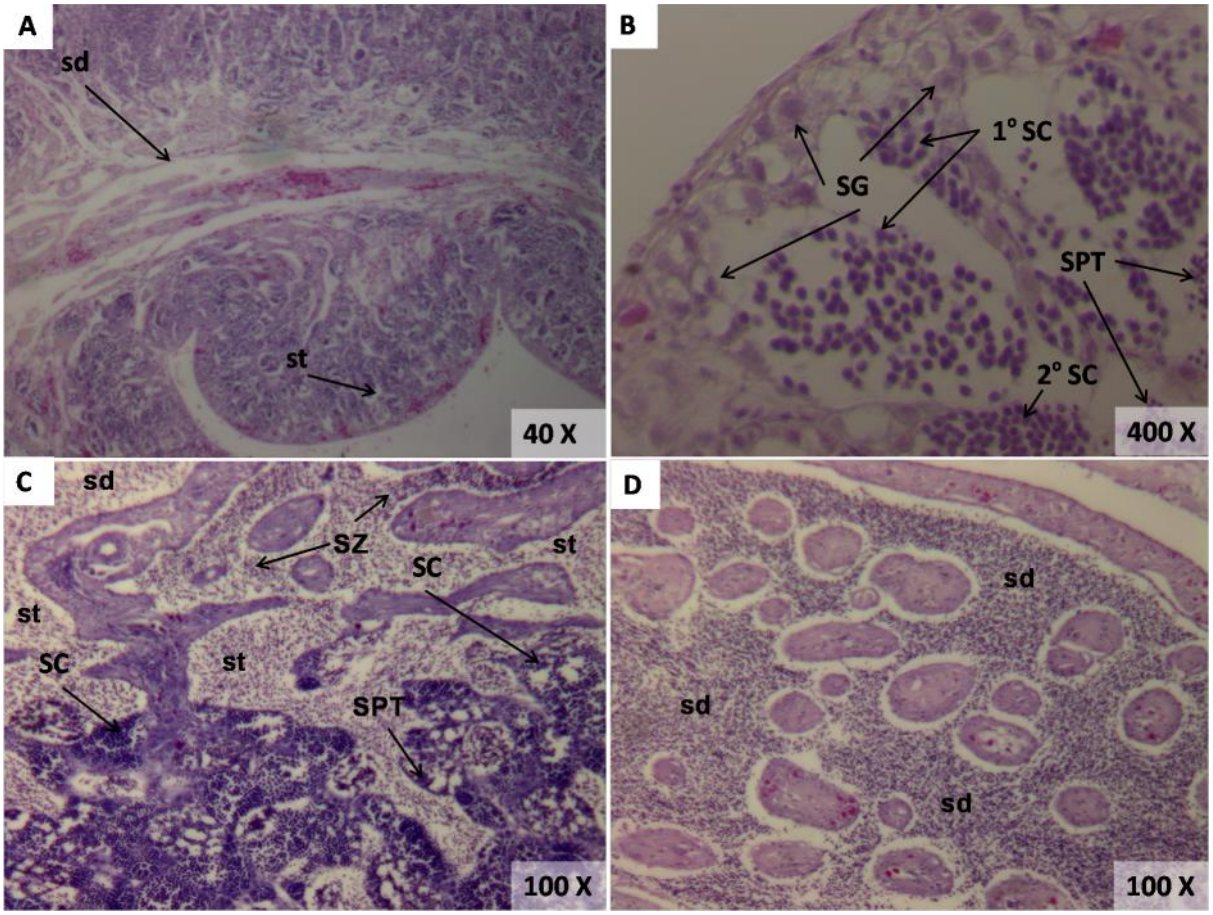

Figure 4.2: Transverse HE sections of the testes of *Diplodus cervinus* depicting the successive stages of spermatogenesis from A–D. (A) Typical structure of an immature testis with empty outer seminal tubules (st) and inner sperm ducts (sd). (B) Aggregations of spermatogonia (SG) towards the periphery of the seminiferous cysts, as well as the first signs of primary (1° SC) and secondary spermatocytes (2° SC). (C) Ruptured cysts containing spermatids (SPT) that will mature as spermatozoa (SZ) in the seminal tubules (st). (D) A ripe testis showing the proliferation of spermatids in seminal tubules (st).

Spermatogenesis

The testes are paired organs, triangular in cross section, and consisting of numerous seminiferous tubules running in between the seminiferous cysts of germinal cells in which spermatogenesis takes place. These tubules lead into secondary sperm ducts that finally lead posteriorly into the main sperm duct (Figure 4.2A). Four discreet stages of spermatogenesis can be recognised in the testes of *D. cervinus* (Figure 4.2 A – D) and follow a similar general developmental pathway as that of other sparid species, following asynchronous sperm development (Coetzee 1983, Buxton 1990, Booth and Hecht 1997, Mann and Buxton 1998, Richardson 2011). Spermatogonia (SG) are characterized as the largest cells within the seminiferous cysts, with a prominent cytoplasm and lightly basophilic nuclear chromatin (Figure 4.2 B).

Spermatogonia divide mitotically and mature into primary spermatocytes (1° SC) that have smaller and denser nuclei. Meiotic division of these cells gives rise to smaller secondary spermatocytes (2° SC) that congregate towards the outer edge of the seminiferous cysts (Figure 4.2B). As the testes ripen, the seminiferous cysts rupture and the 2° SC spermatids are released into the lumen of the seminiferous tubules, to mature into spermatozoa (Figure 4.2C). Spermatozoa are easily identifiable, due to their highly basophilic nucleus and small size. These aggregate in the lumen of the secondary sperm ducts and the main sperm duct in spawning males (Figure 4.2C).

Histological validation of macroscopic stageing

The histological validation of the macroscopically-staged gonads suggests that the macroscopic stageing and microscopic development are comparable. The macroscopic stageing criteria are therefore considered to be valid and the comparative microscopic description is presented in Tables 4.1 and 4.2. Although there was slight microscopic developmental variation in the gonads of individual fish classified as the same macroscopic stage, all fish still fell within the microscopic descriptions (Table 4.1 and 4.2). Juvenile gonads were predominantly comprised of germ cells or cells in the early stages of male and female gametogenesis (Figure 4.3A and 4.4A).

The gonads of virgin/resting female fish were characterised by a high density of oocytes in all stages of the perinuclear phase of development, with some oocytes going through early vitellogenesis (Figure 4.3B). The microscopic structure of active female gonads was similar to that of the gonads of the previous stage but had a higher proportion of early vitellogenic oocytes (Figure 4.3C). Ripe females were the easiest to differentiate from the other macroscopic stages, being the only stage with a very high proportion of large late vitellogenic oocytes present (Figure 4.3D). The microscopic appearance of spent ovaries was not consistent and therefore not validated.

Virgin/resting males showed all stages of spermatogenesis but no spermatozoa were present in the seminal tubules or ducts (Figure 4.4B). Active and ripe male gonads were microscopically very similar, with the main difference being higher densities of spermatozoa in tubules and ducts of ripe individuals and the lack of spermatozoa in the main sperm duct of active gonads (Figure 4.4 C & D). The relative size difference between the gonads of the two stages also influenced the determination of stage. Only two males were macroscopically staged as spent but the microscopic appearance was more consistent with that of a virgin/resting gonad.

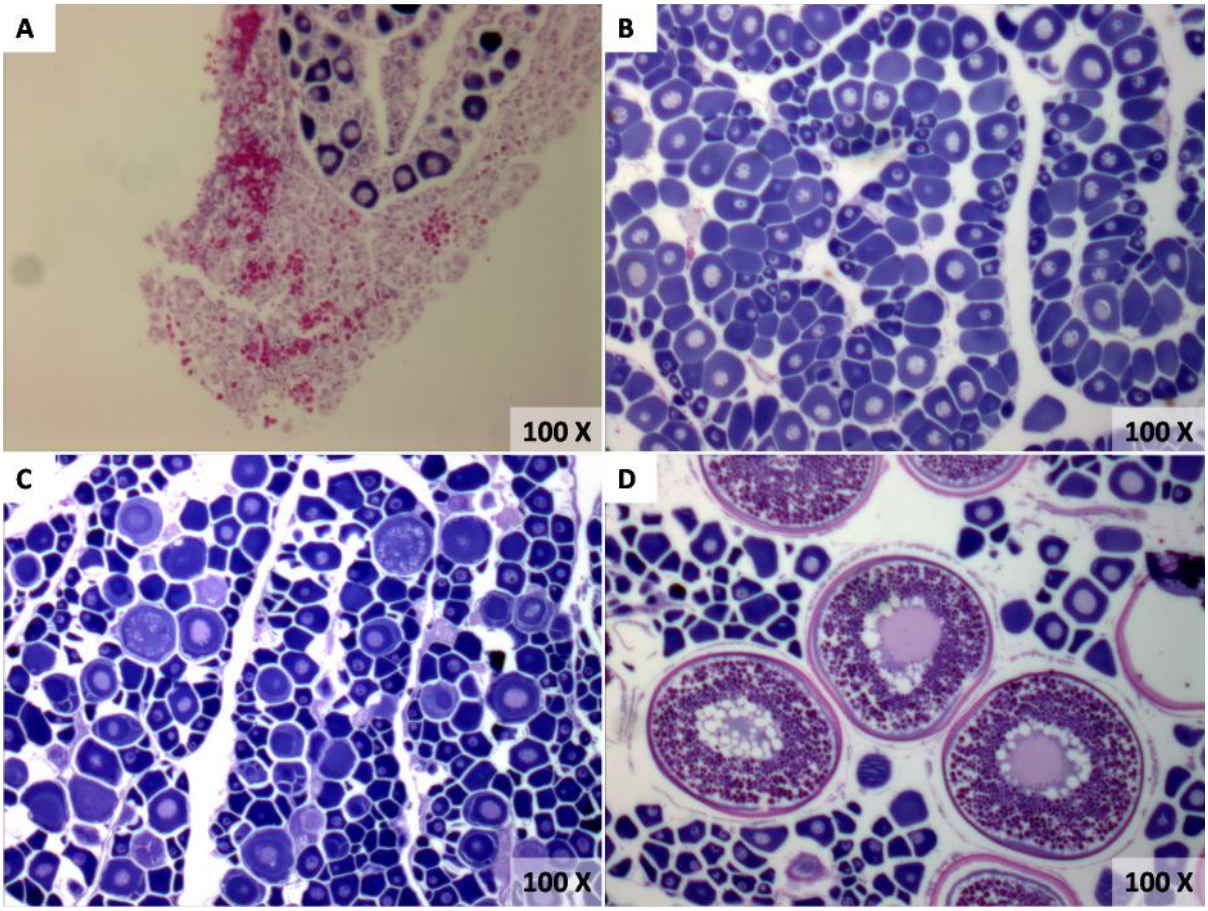

Figure 4.3: Transverse HE sections of *Diplodus cervinus* ovaries illustrating the microscopic appearance of macroscopic stages. (A) A juvenile ovotestis with oogonia and perinuclear oocytes present in the ovarian section. (B) A virgin/resting ovary comprised mostly of early to late perinuclear oocytes. (C) An active ovary showing the presence of early vitellogenic oocytes characterised by the presence of cortical alveoli. (D) A ripe ovary showing the appearance of large tertiary yolk vesicle oocytes.

Table 4.1: Macroscopic and equivalent histological stageing of female *Diplodus cervinus* gonads sampled from southern Angola between March 2011 and February 2012. Adapted from Buxton 1990, Booth and Buxton 1997 and Booth and Weyl 2000.

Stage	Macroscopic description	Microscopic description
1. Juvenile	Thin and filamentouse, white to transparent in colour.	Both ovarian and testicular tissue can be present in similar proportions. In ovarian tissue only oogonia and perinuclear oocytes present.
2. Resting/virgin	Long and elongated, orange in colour, eggs are not visible.	Oogonia and perinuclear oocytes dominate with few to no primary yolk vesicle oocytes.
3. Active	Gonads larger in size and deep orange in colour, eggs visible with naked eye.	Comprised mostly of late perinuclear oocytes and primary yolk vesicle oocytes with a few seconadry yolk vesicle oocytes in certain individuals.
4. Ripe	Eggs clearly visble and yellow in colour, gonad swollen and turgid.	All stages of vittelogenic oocytes present but tertiary yolk vesicle oocyes dominate. Migratory nuclei present.
5. Spent	Gonads decrease in size and lose turgidity, yellow in colour and eggs visible but blood shot.	Undetermined.

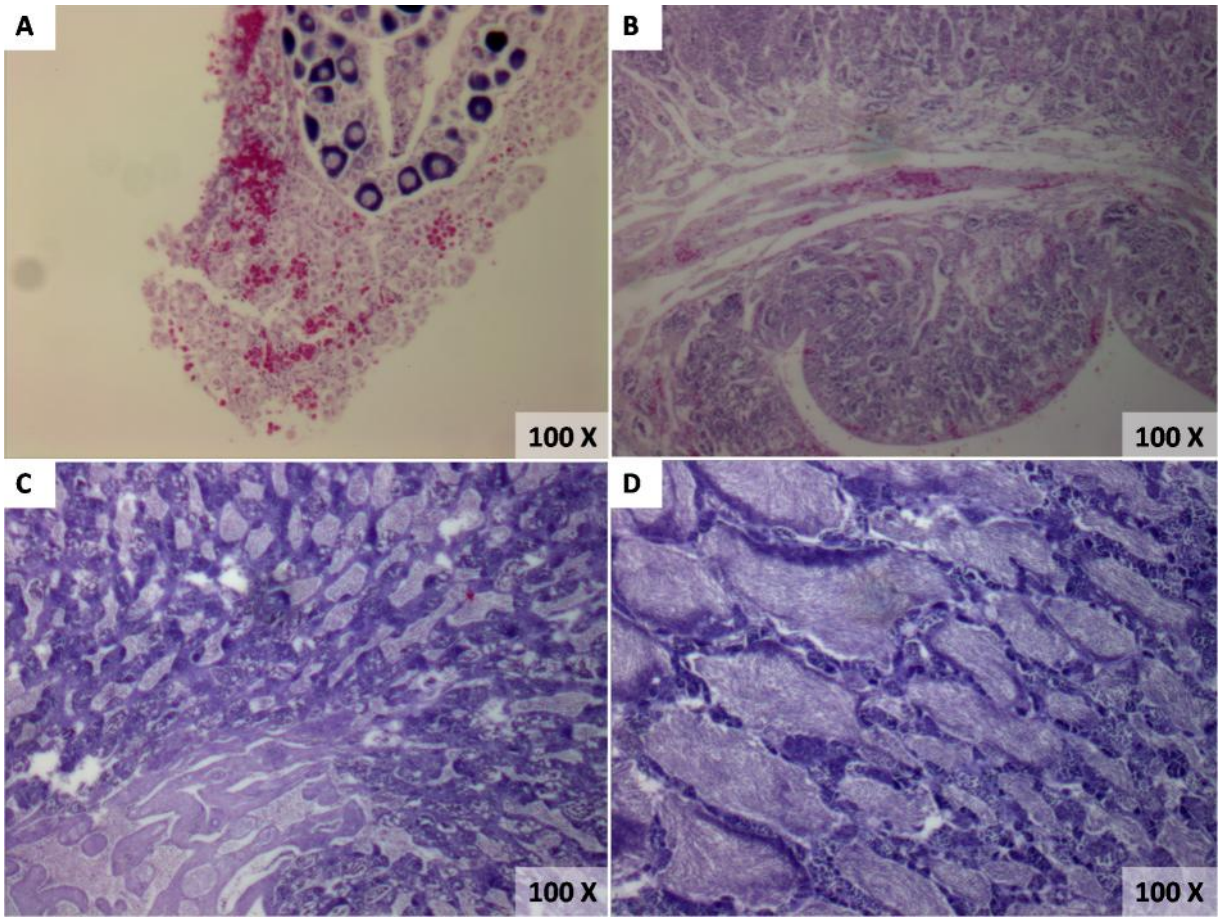

Figure 4.4: Transverse HE sections of *Diplodus cervinus* testis illustrating the microscopic appearance of macroscopic stages. (A) A juvenile ovotestis with spermatogonia present in the ovarian section. (B) A virgin/resting testis depicting the empty seminiferous tubules and ducts. (C) An active testis showing all stages of spermatogenesis and the presence spermatozoa in the seminiferous tubules but not in a portion of the secondary ducts. (D) A ripe testis showing high densities of spermatozoa in secondary sperm ducts.

Table 4.2: Macroscopic and equivalent histological stageing of male *Diplodus cervinus* gonads sampled from southern Angola between March 2011 and February 2012. Adapted from Buxton 1990, Booth and Buxton 1997 and Booth and Weyl 2000

Stage	Macroscopic description	Microscopic description
1. Juvenile	Thin and filamentouse, white to transparent in colour.	Both ovarian and testicular tissue can be present in similar proportions. In testicular tissue only spermatogonia present.
2. Resting/virgin	Increase in size, greyish in colour with small lobes extending out laterally.	Spermatogonia dominate with low densities of spermatocytes, seminiferous tubules and sperm duct empty.
3. Active	Larger in size, white with grey edges in colour with extended lobes.	All stages of spermatogenisis present. Spermatozoa begin to fill seminiferous tubules, leaving the main sperm duct empty.
4. Ripe	Large and swollen, white in colour often with a pinkish tinge, when cut semen is released.	All stages of spermatogenisis present. Spermatozoa fill seminiferous tubules and the main sperm duct.
5. Spent	Testes decrease in size and appear deflated and flacid, white to pink in colour with large numbers of blood vessles.	Undetermined

Reproductive seasonality

The patterns of gonad development in the *D. cervinus* were clearly seasonal (Figure 4.5). The highest proportions of ripe gonads were observed between May and October, indicating a protracted spawning period, but a small peak was also observed in February. Developing gonads were found during all sampled months, while resting/active gonads were at their highest proportion immediately before and after the main spawning period. Additional evidence, provided by GSI, validated these findings, with an elevated GSI being observed between May and October and a small peak in February (Figure 4.6). Females had a significantly higher mean GSI during the entire sampling period (*t*-test, $p < 0.05$) (Table 4.3).

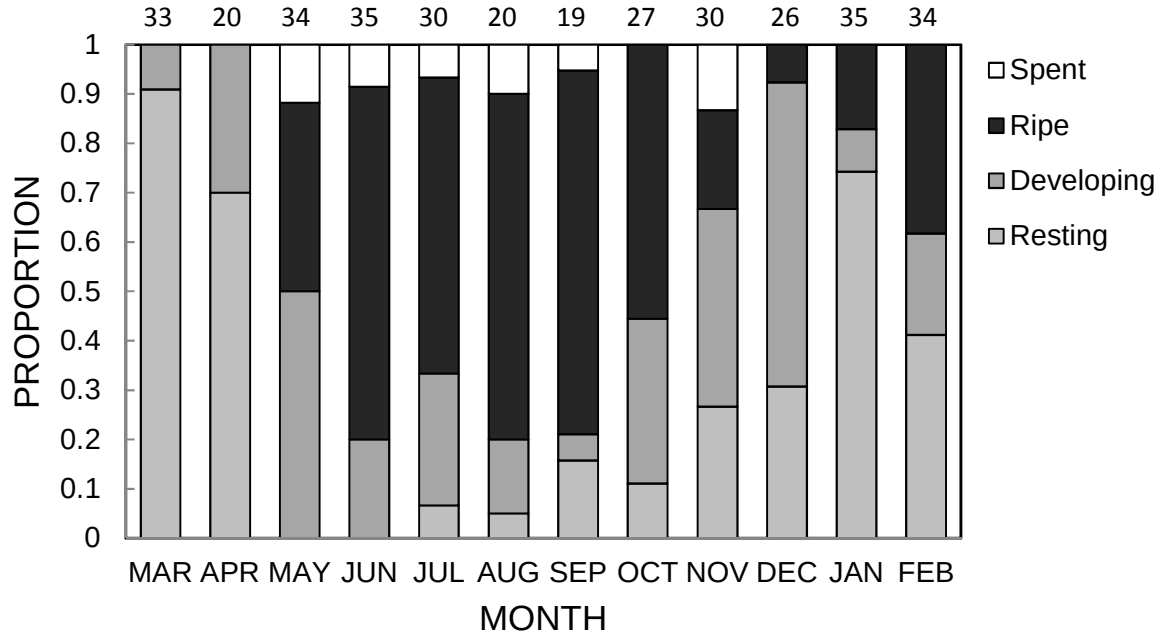

Figure 4.5: Monthly proportion of each macroscopic stage for gonads of mature *Diplodus cervinus* from southern Angola. Monthly sample size is depicted above columns.

Table 4.3: Average gonadosomatic index for female and male *Diplodus cervinus* sampled from southern Angola. *p* - values calculated using a student's *t* – test.

	Average GSI ± SD		
	Females	Males	*p* - value
Entire sample	1.53 ± 1.27	0.87 ± 1.05	<0.01
Out of the spawning season	0.98 ± 0.98	0.44 ± 0.64	<0.01
During the spawning season	2.15 ± 1.28	1.37 ± 1.21	<0.01

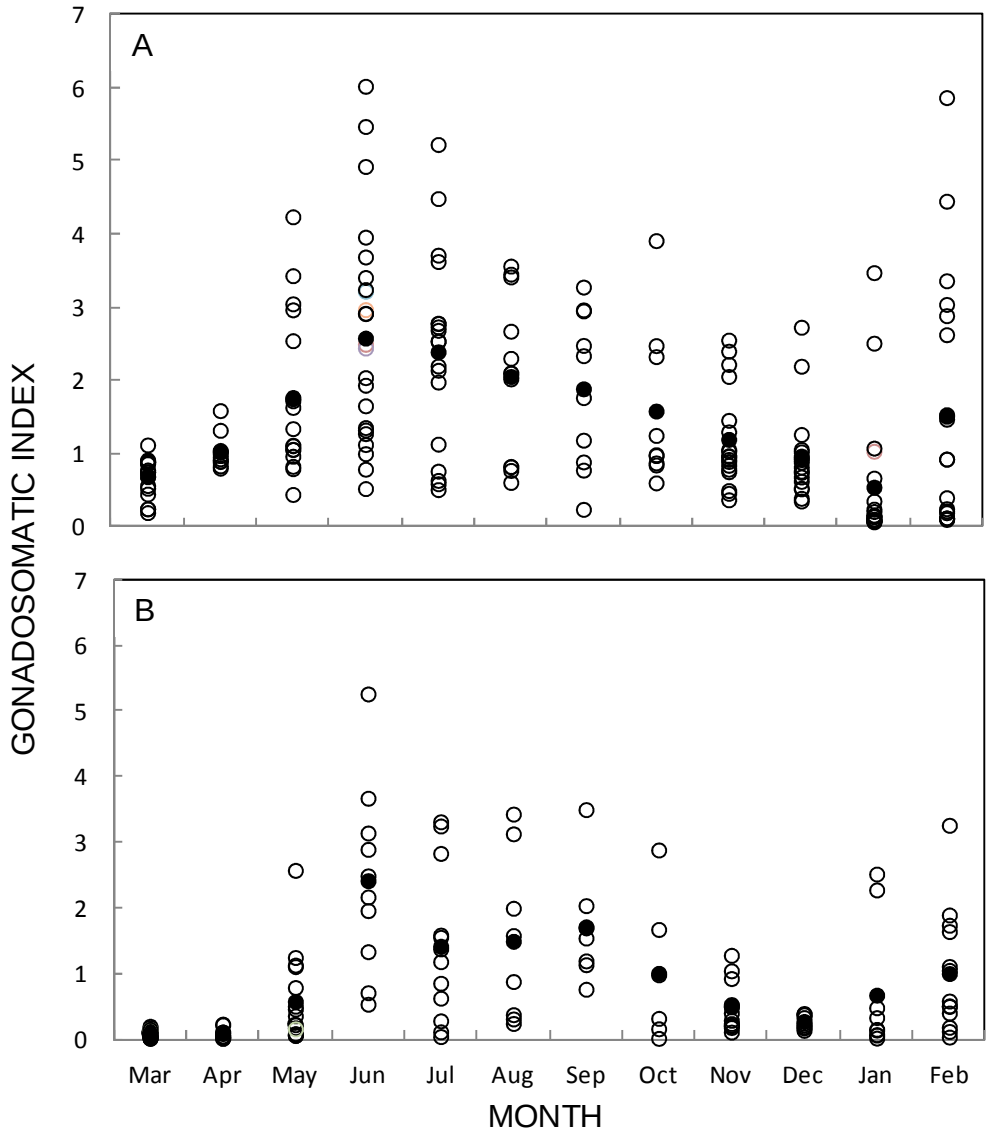

Figure 4.6: Individual (open circles) and mean monthly (solid black circles) gonadosomatic index (GSI) values for female (A) and male (B) *Diplodus cervinus* from southern Angola.

The influence of environmental factors on spawning

A gradual increase in GSI was observed as water temperature and photoperiod decreased (Figure 4.7) in autumn. A small peak in GSI was also observed in January and February. This occurred soon after an anomalous drop in water temperature in December and January.

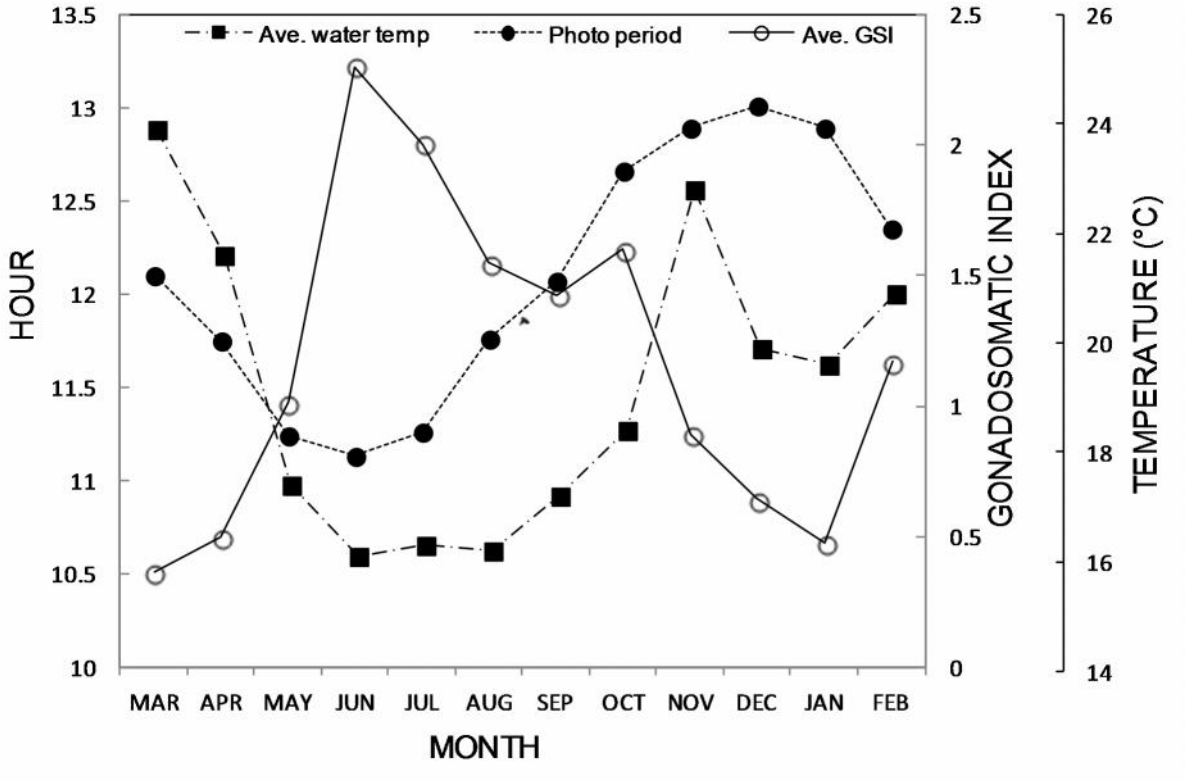

Figure 4.7: Average monthly gonadosomatic index (GSI) of male and female *Diplodus cervinus*, mean monthly inshore sea surface temperature and photoperiod from southern Angola between March 2011 and February 2012.

Reproductive style

Population structure

There was little difference in the distribution of the male and female length and age frequency histograms (Figure 4.8). The proportion of males was greater in the larger size classes compared to that in the smaller size classes (Figure 4.8 A). This trend was not however observed in the age frequency analysis, where males were found in similar proportions throughout the age classes (Figure 4.8 B). The overall sex ratio for mature fish was 1 male : 1.52 females.

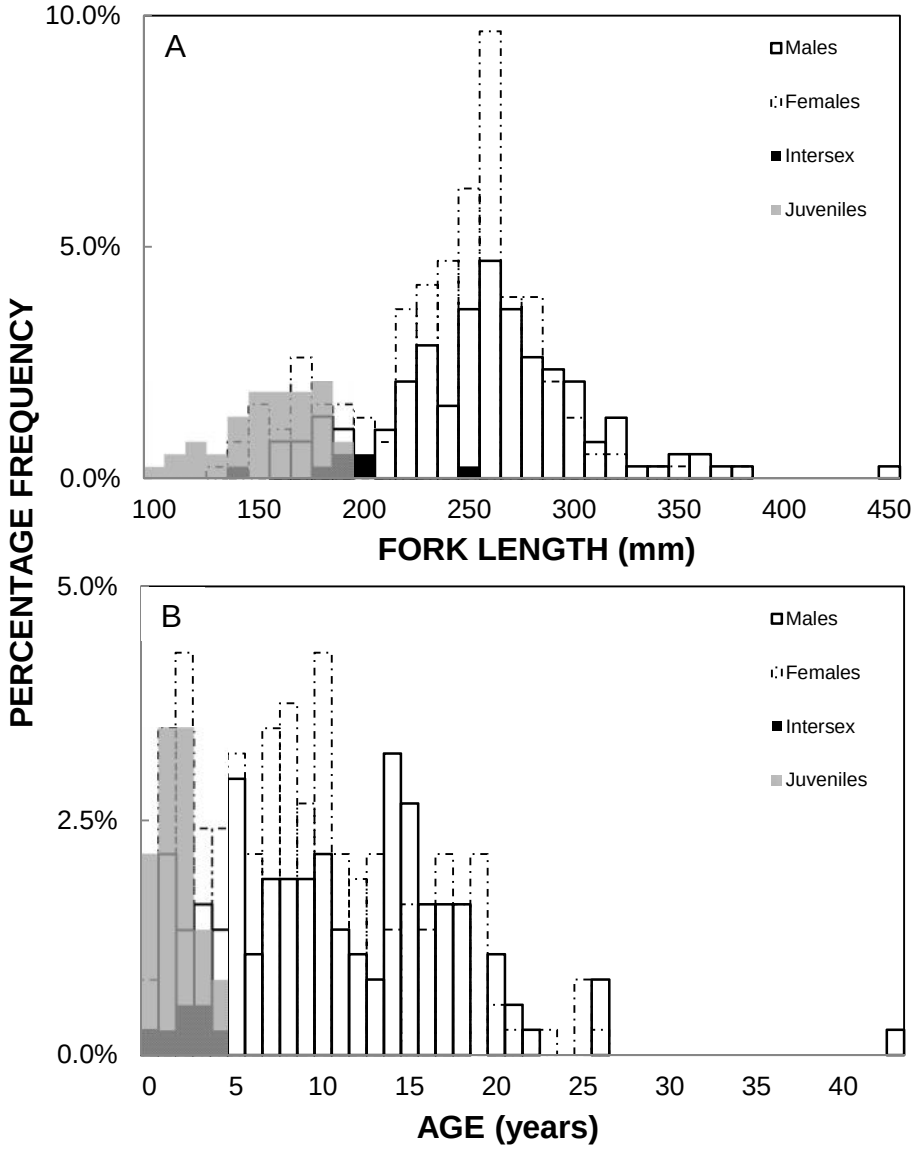

Figure 4.8: Length (A) and age (B) frequency distributions of male, female, juvenile and intersex *Diplodus cervinus* sampled from southern Angola from March 2011–February 2012.

Histological analysis

Juvenile reproductive tissue consisted of a non-functional bisexual gonad containing equal proportions of underdeveloped male and female parts separated my connective tissue, comprised mainly of spermatogonia in the male portion and oogonia in the female portion (Figure 4.9A & B). In functional females the ovarian tissue dominated and the testicular tissue occasionally resided as a reduced band on the periphery of the gonad (Figure 4.9C & D). However, this was not common and most functional female gonads did not contain any residing testicular tissue. In functional males the testicular tissue dominated and the ovarian tissue persisted as a few perinuclear oocytes in the region of the sperm duct (Figure 4.9E & F). As

with males, this was not common and most functional males showed no sign of residual ovarian tissue.

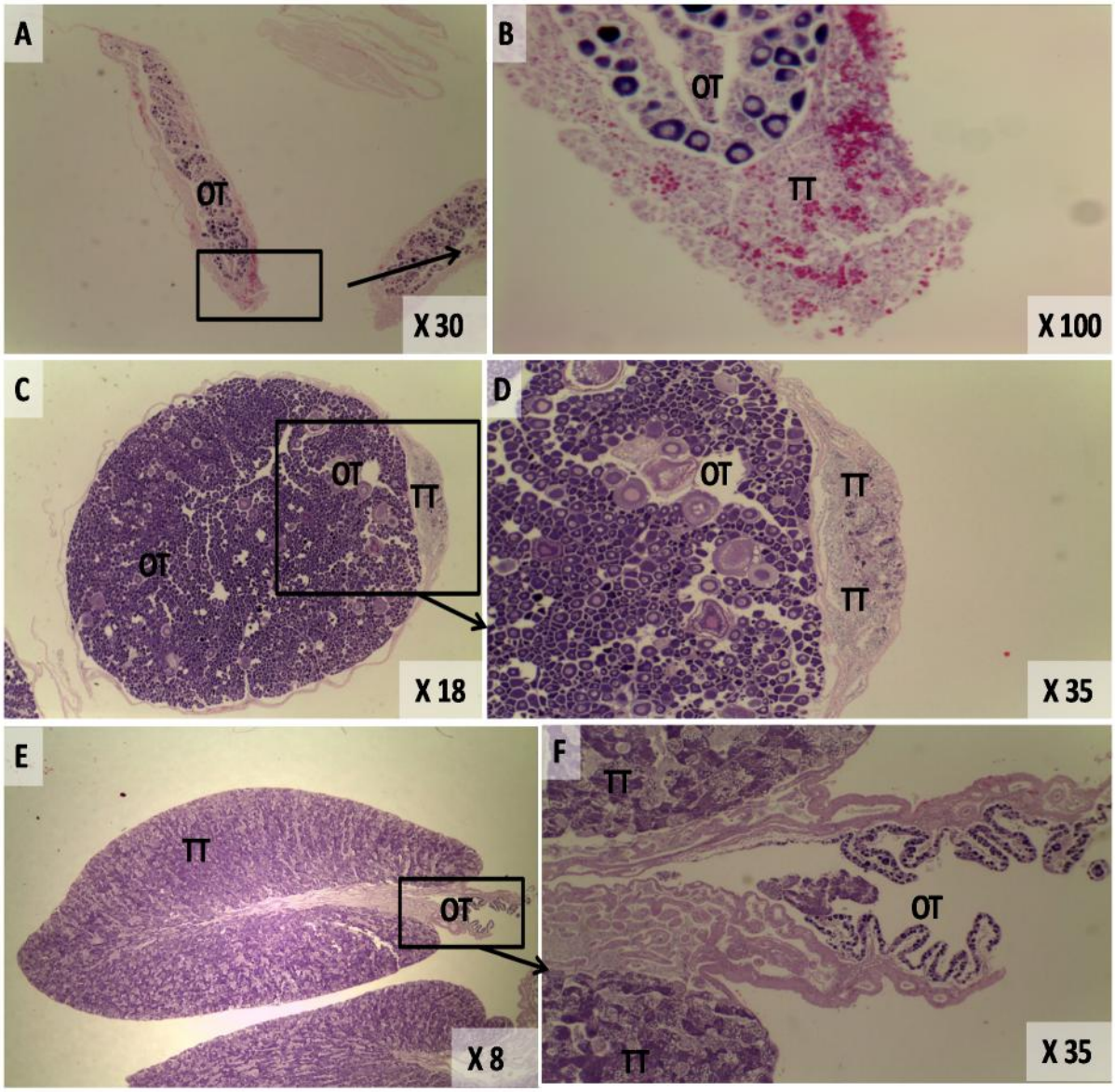

Figure 4.9: Transverse sections of the gonads of *Diplodus cervinus* illustrating their developmental pathway. A and B, Non-functional juvenile (140 mm FL) with ovarian (OT) and testicular tissue (TT). Oocytes at the early perinuclear stage were present in the ovarian portion and spermatogonia in the testicular portion. C and D, Functional female (182 mm FL) with a band of residual testicular tissue persisting from the intersexual juvenile gonad. The ovarian tissue was developing, with some vitellogenic oocytes present, while the testis were mostly comprised of spermatogonia and primary spermatocytes. E and F, Functional male testis (226 mm FL) with residual ovarian tissue persisting from the juvenile intersex stage. The testicular tissue was well developed, with the presence of spermatozoa in the seminal tubules and ducts while the persistent ovarian tissue was only comprised of pre-perinuclear oocytes.

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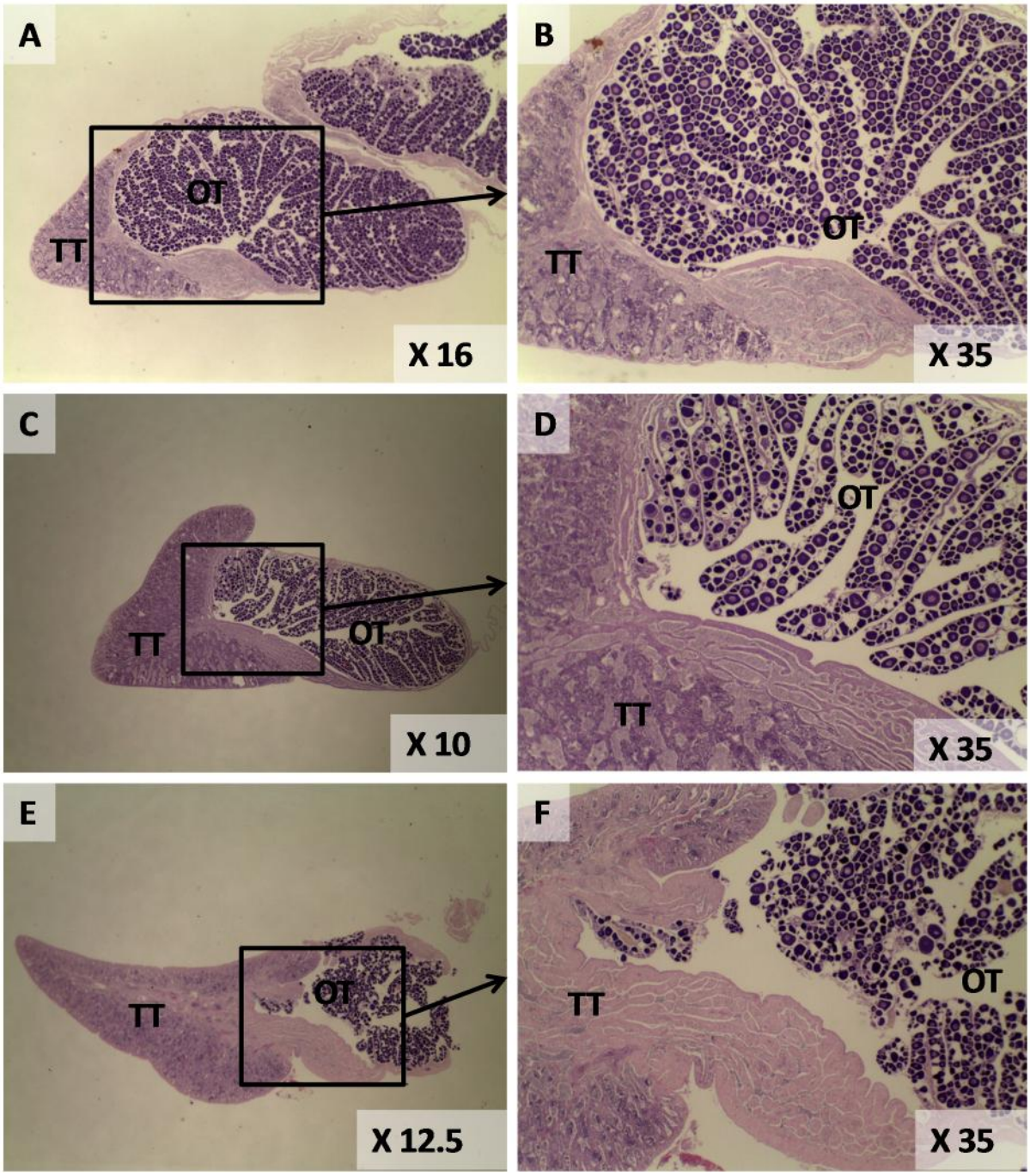

Figure 4.10: Transverse sections of the gonads of "intersex" *Diplodus cervinus* (A & B = specimen of 195 mm, C & D = specimen of 200 mm, E & F = specimen of 249 mm FL).

Five intersex fish (2.1 %) were identified and the gonads of all of these were histologically validated as ovotestes. Most of these individuals were smaller than the length-at-50% maturity, while only one was the size below the length-at-100% maturity and age-at-50% maturity (see Chapter 5) (Figure 4.10E & F). The oocytes in the ovarian portion of the ovotestis did not develop past the perinuclear phase in all intersex individuals, suggesting that it had persisted from the juvenile intersexual phase (Figure 4.10). The testicular portion of these gonads was well developed and contained all stages of spermatogenesis and no signs of testicular

degeneration (Figure 4.10 B, D, & F). Due to the functional nature of the testicular portion and the immature nature of the ovarian portion, these individuals where all considered to be functional males

Discussion

The spawning season of *D. cervinus* in southern Angola was prolonged, from May to October. The asynchronous gamete development of ripe gonads in both male and female fish indicates that this species is a batch spawner. An extended, asynchronous spawning period is a characteristic of many sparids (Coetzee 1983, Smale 1988, Buxton and Clarke 1991, Buxton and Clarke 1992, Booth and Buxton 1997, Hesp and Potter 2003) and specifically the genus *Diplodus* (Mann and Buxton 1998, Goncalves and Erzini 2000, Richardson et al. 2011c, Potts et al. in press). McEvoy and McEvoy (1992) described the main advantages of this reproductive strategy, including the ability to increase fecundity, as hydrated oocytes just prior to ovulation take up a large amount of space in the ovary. Other advantages include spreading of the risk of predation on larvae and eggs over a longer period, thus reducing intraspecific competition for food items amongst larvae and juveniles and spreading the risk of spawning during times of unfavourable climatic, hydrographic, or feeding, conditions. It is likely that the stochastic nature of the coastal marine environment and vulnerability of eggs and larvae has provided sufficient selective pressure for the evolution of an asynchronous spawning strategy for this and other closely related species.

Both water temperature and photoperiod appeared to have an influence on the timing of *D. cervinus* spawning during the study period (Figure 4.7). These factors have also been identified as important cues for the reproduction of this species complex in South Africa (Mann and Buxton 1997), the Canary Islands (Pajuelo et al. 2003b) and off the coast of Algeria in the Mediterranean Sea (Derbal and Kara 2010). It should however be noted that the gonadal development in the Angolan population appeared to be related to a drop in temperature and a decrease in photoperiod (Figure 4.7, Table 4.4), whereas gonadal development in the South African, Canary Island, and Mediterranean populations was associated with an increase in photoperiod and temperature (Table 4.4).

One of the main differences between the populations studied was their latitude. The present study was conducted at 15° S, well north of the tropic of Capricorn (23.5° S), a latitude with reduced variability in the photoperiod between seasons, when compared with previous studies

which were conducted at latitudes higher than 28° N/S. Sumpter (1990) classified organisms as either “long-day” or “short-day” species, occupying either high or low latitudes, respectively. Increases in day length stimulated gonad development in “long day” species, while decreases in day length stimulated gonad development of “short day” species. This hypothesis of Sumpter (1990) could explain the differences in the relationship between photoperiodicity and gonad maturation in these populations.

One of the problems associated with attempts to uncouple the environmental factors influencing gonadal development is that the variables are often correlated. For example, a decrease / increase in temperature is usually associated with season and is therefore correlated with photoperiod. In a review on the spawning seasonality of the family Sparidae, Sheaves (2006) noted that sparids tend to spawn during months of the coldest sea surface temperature (SST) and shortest photoperiod in tropical waters and during months with the warmest SST and longest photoperiod at high latitudes. This relationship was not only found at family level, as intraspecific variation in spawning seasons was also found to be correlated with SST and photoperiod (Sheaves 2006). For example, subpopulations of *Pagrus auratus* along the Australian coast spawn during winter at a lower latitude, of 25° S, and in summer at a higher a latitude, of 35° S (Sheaves 2006), and *Diplodus sargus capensis* populations in South African waters reproduce in summer in the high latitudinal (34° S) waters off the Southern Cape (Mann and Buxton 1998) and in winter in lower latitudinal (28° S) waters of KwaZulu–Natal (Joubert 1981). Sheaves (2006) speculated that the reduced tolerance of eggs and larvae to high water temperatures, when compared with that of adult fishes, could explain differences in the timing of spawning between fishes in low and high latitudes. If this holds true, utilization of warmer, low latitude waters would result in an adjustment of fish spawning season to coincide with cooler temperatures in which their eggs and larvae can survive. In South Africa and southern Angola *D. cervinus* spawn at temperatures between 16-20°C (Table 4.4) in summer and winter respectively, thus conforming to the hypothesis proposed by Sheaves (2006). Similarly, the Canarian and the Mediterranean populations partly conform to Sheaves’s (2006) hypothesis as they are summer spawners and occupy high latitudes, of 28° N and 35° N, respectively. However, these populations spawn at temperatures between approximately 16-23°C in July (Table 4.4), which is unusually high for sparids (Sheaves 2006) particularly for *Diplodus* spp. (Potts et al. in press).

Differences in preferred spawning temperatures, observed for the *D. cervinus* populations in the southern and northern hemisphere, suggest that spawning periodicity may be regulated by

other factors, such as food availability. In the case of *D. cervinus*, the frequency and timing of eutrophic upwelling, or the presence of nutrient-rich currents, may have influenced the evolution of reproductive timing in *D. cervinus*. In southern Angola, cold winter SSTs are a consequence of the northerly-retreating oligotrophic warm Angolan current and the advancing eutrophic cold Benguela Current from the south (see Chapter 1). Peak upwelling intensity in the South African study site in the Tsitsikamma National Park (Schumann et al. 1982), around the Canarian Archipelago (Van Camp et al. 1991, Marcello et al. 2011), and off the coast of Algeria (Bakun and Aciostini 2001) all occur during the peak summer spawning period of *D. cervinus* in the respective regions.

Table 4.4: Preferred spawning temperature and the state of photoperiodicity in four populations of *D. cervinus.*

Region	Latitude	Temperature Range (°C)	Spawning temperature (°C)	Photoperiod
Angola	15° S	16-24	16-20	↓
South Africa	34° S	12-24	17-20	↑
Canarian Archipelago	28° N	18-24	20-23	↑
Algeria	35° N	15-24	16-22	↑

[1]This study, [2]Mann and Buxton 1998, [3]Pajuelo et al. 2003, [4]Derbal and Kara 2010, *Based on sea surface temperature climatology in the region which was obtaind at: [http://emis.jrc.ec.europa.eu/index.php#

The synchronization of spawning and larval recruitment into an environment with the highest annual productivity has been observed by a number of authors (Bye 1984, Bromage et al. 2001, Pankhurst and Porter 2003, Tsikliras et al. 2010), especially in oligotrophic environments such as the Mediterranean (Ridame et al. 2011). Bromage et al. (2001) argued that there is a strong selective pressure towards reproduction during times of peak larval food availability. This is primarily because this life history phase is subject to the highest mortality rate and thus survival will only be significant during periods with optimal foraging .

Although temperature, photoperiod and food availability all appear to play a role in determining the timing and duration of reproductive activity in *D. cervinus*, as with other teleosts, the relatedness of these variables makes it difficult to uncouple the ultimate mechanism triggering reproduction in field studies (Pankhurst and Porter 2003). However, during a low temperature anomaly that occurred during December and January 2011 in southern Angola (Figure 4.7), *D. cervinus* responded with a small peak in the GSI, with several individuals categorised to have reproductively-active gonads. This suggests that temperature may override photoperiod in triggering reproductive activity. However, as measures of

productivity were not available during the present study, the relative importance of food availability on reproductive activity could not be quantified.

D. cervinus in southern Angola had a similar reproductive style to most sparid fishes with the intersexual juvenile stage preceding maturation the development of either male or female gonads. Sadovy and Shapiro (1987) stressed the importance of not relying on length and age frequency and adult sex ratios as evidence of sex change in diagnosing reproductive style, due to the possibility of a variety of unrelated causatives other than sex change such as differential mortality, growth rate, habitat preference and migration patterns between the sexes. The results of this study support this view. For example, the adult sex ratios were significantly female biased, a characteristic of protogyny. Similarly, the high proportion of males in the larger size classes may also have provided indirect evidence for protogyny. However, based on the histological analysis, this trend could most likely be ascribed to other factors, such as slower growth and higher mortality in females after sexual maturation. This is not unusual in teleosts and may be attributed to the significant energy that is dedicated to egg production (Sadovy 1996). Since the female GSI was significantly higher than that of males in this *D. cervinus* population, this is quite likely.

There were several lines of evidence that suggesting that *D. cervinus* in southern Angola are rudimentary hermaphrodites. This included a lack of intersexual individuals above the age and length at 100% maturity (see Chapter 5) and a lack of histological evidence for sex change amongst the macroscopically staged ‘hermaphrodites’ or any other individual. Similarly, the intersex individuals, which were all < 200 mm, were well below the length and age at 50% maturity (see Chapter 5) and had similar portions of early spermatic and previtellogenic tissue. The one large intersex individual (249mm) that was above the length-at-50% maturity may have provided evidence for sex change, however, there was no histological evidence of degeneration and development of either the male or female portions of the gonad and this individual was aged to be 4 years of age, which falls well below the age-at-50% maturity. This fish was therefore thought be an unusually fast growing juvenile. Booth and Buxton (1997) found similar intersex individuals of *Pterogymnus laniarius* but when histological analysis was used to assess the gonads, they amended the diagnosis of the species from protogynous to rudimentary hermaphrodite.

The reproductive style exhibited by *D. cervinus* in southern Angola is similar to that observed in the South African population (Mann and Buxton 1998). This was based on very similar

histological, GSI, and length-frequency evidence. These findings are however contradictory to those of Pajuelo et al. (2003b) who classified this species as a protogynous hermaphrodite, based on his studies of the population off the Canary Islands. This decision was based on data relating to male: female sex ratios, differential mean GSI between sexes, and the presence of intersex individuals well after length-at-50% maturity. Interestingly, if this information was the only evidence used in the present study, one could also conclude that this population in southern Angola is protogynous. This lends additional support to the opinions of Sadovy and Shapiro (1987) who stated that "conclusive evidence should only be formulated though histological examination throughout male and female gonad development".

There appears to be a correlation between the mean GSI and the reproductive style of sparids (Buxton and Garrat 1990) and teleost species in general (Molloy et al. 2007). This is thought to be associated with the different mating patterns that have evolved with each reproductive style. Protogynous species tend to have a significantly higher mean female GSI, usually greater than twice that of males during the spawning season, while gonochorists usually have a more equitable mean GSI during the spawning season (Buxton and Garrat 1990, Allsop and West 2004). Molloy et al. (2007) suggested that sperm competition in pair-spawning polygamous, protogynous fishes was lower than that observed in group-spawning gonochoristic species which encounter higher sperm competition, in which the ovary and testis sizes are usually more equitable. The male and female GSI values where significantly divergent from each other in *D. cervinus* in Angola, although the magnitude of divergence was not as great as that usually found in other sex changing sparids. This large size of the male testis in *D. cervinus* in comparison to other sex changing sparids may therefore be advantageous under conditions of high sperm competition. Unfortunately, wild spawning behaviour was not observed in this study and the GSI evidence alone is not sufficient to conclude whether this fish is a "pair" or "group" spawner. Nevertheless, the findings of the present study are similar to those of other studies done on rudimentary hemaphroditic sparids (Buxton and Garratt 1990, Buxton and Clarke 1992, Booth and Hecht 1997, Mann and Buxton 1998, Brouwer and Griffiths 2005) and suggest that *D. cervinus* in Angola may be group spawners.

Results from the present study lead to the conclusion that *Diplodus cervinus* has a protracted winter spawning season, between May and October, in Southern Angola. This appears to be related to a drop in SST and a reduction in photoperiod during the period of highest productivity. The reproductive seasonality and the cues for the timing of spawning differ from all previous studies on the species and this is probably due to the low latitude and unique

oceanography of the area. Male and female gametogenesis is consistent with previous studies on sparids. Histological analysis of the gonads, as a comparison of the male and female mean GSI and the length and age frequencies, all suggest that fish belonging to this population of *D. cervinus* are rudimentary hermaphrodites.

CHAPTER 5

A life history assessment of an unexploited population of *Diplodus cervinus* in southern Angola

Introduction

Life history theory suggests that life history traits evolve in response to extrinsic ecological conditions acting on survival during an organism's life, resulting in trade-offs among specific traits to maximise fitness (Stearns 1992). Wootton (1998) suggests that "the ideal fish would have a long life, grow quickly, reach maturity at an early age, then reproduce often and produce numerous young at each breeding attempt". However, the "ideal" life history is not possible, primarily due to the interconnectivity between specific traits. Therefore, where there is an increase in the time or resources invested in one trait, a trade-off, of one trait against another, will inevitably be made (Wootton 1998). For example, a fish may reproduce early, at a small size, but this strategy will divert resources away from somatic growth, resulting in an increase in the period when the individual is small and vulnerable to predation. Thus a specific life history strategy, expressed by an individual, is moulded around trade-offs between three main traits — growth, reproduction and mortality — in order to maximise fitness in its environment.

Several authors have attempted to characterize populations, based on their specific life history strategy in relation to the type of habitat occupied (Wooton 1998). An early attempt was made by MacArthur and Wilson (1967) who used the concept of *r*- and *K*- selection when trying to explain island biogeography in terms of life history strategies in relation to disturbed and stable habitats. According to this characterisation, *r*- selected species that were found in disturbed habitats with density-independent mortality, exhibited life history traits such as early reproduction, high fecundity and short life expectancy. However, in stable habitats where organisms encountered density-dependent mortality, *K*-selected species, with delayed reproduction, low fecundity, and long life expectancy, dominated. While these criteria can be applied to fish, the massive diversity of fish life history expression, which is probably due to

the heterogeneity of aquatic ecosystems, led to a revision of the topic by Winemiller and Rose (1992). In their revision of 219 fish species, three general categories of fish life history strategy were proposed: 'Opportunistic', 'Equilibrium' and 'Periodic' (Winemiller and Rose 1992). Opportunistic species are characterized by early reproduction, small clutches and high mortality and are mostly associated with frequently-changing stochastic environments such as floodplains. Equilibrium species are characterised by delayed reproduction, small clutches and low mortality, and are mostly associated with highly stable environments where there is high competition for resources, such as the Rift Valley great lakes. Periodic species are characterised by delayed reproduction, large clutches, and high juvenile mortality and are often associated with large-scale cyclical, or spatially variable, environments such as tropical and temperate reefs.

Life history variation in fishes is thought to be driven by immediate environmental conditions and the expression of life history traits can be modified within a genetically determined range (Baker 1994), within and between generations (Stearns 1992). Life history variation may have a genetic basis, where environmental conditions experienced by an individual in the past may have moulded some aspects of their life history that are not currently adaptive or responsive to the immediate environment (Baker 1994). Due to this interconnectivity between the remnants of an organism's evolutionary history and the immediate adaptive variability, uncoupling the driver of a particular life history trait is particularly difficult. Phylogenetic constraints and historical relationships must therefore be considered when attempting to isolate the potential factors influencing a particular trait (Bell and Foster 1994). In this chapter, the life history traits of Angolan *D. cervinus* will be compared to those of the South African population of *D. cervinus,* to assess the effects of environmental variability associated with a relatively recent vicariant event (2 million years) (see Chapter 1).

The temperate southern Angolan marine ecosystem has numerous conspecific and congeneric teleost species, shared with the south east coast of South Africa as well as the north eastern Atlantic and Mediterranean marine ecosystems. The most taxonomically-related region is that of the south east coast of South Africa, which is thought to be a consequence of the recent separation (2 million years) of the fauna of the two regions during the formation of the cold Benguela Current (Floeter et al. 2008). The vicariant populations of some species (for example, *Atractoscion* spp, Scianidae) show significant genetic and life history divergence, without morphological divergence (Henriques 2011) while others (*Diplodus sargus capensis*) show changes in life history and morphological divergence (Richardson 2011) but limited molecular

divergence (*Diplodus sargus capensis*) (Henriques 2011). *Diplodus cervinus* exhibits similar morphological and molecular divergence between the two regions (Chapter 2) as that found by Richardson (2011) in *D. capensis*. Wellington and Robertson (2001) conducted a study that compared the life history traits of the larvae of congeneric reef fish across the Panamanian Isthmus, in an attempt to negate phylogenetic effects when assessing environmental effects. The isthmus, like the Benguela Current, is a biogeographic barrier that has split populations of a range of coastal species for approximately 3.5 million years (Coates 1997 in Wellington and Robertson (2001). They hypothesised that the larval phase would be longer in the productive Pacific waters than in the less productive Atlantic waters, due to differences in plankton abundance as a larval food source. However, the study showed that there was no consistent change in this larval life history trait across the isthmus, in relation to environmental variables. On this basis, the differences observed in life history traits across the isthmus were concluded to be within the range of normal, within-location intra-population variability. This suggested that the life history traits of these larvae were either influenced by ecological factors that were not considered, or were phylogenetically fixed, and that the selective pressure was not sufficient to result in local adaptation and divergence during the 3.5 million years of isolation. This evidence suggests that certain life history traits may not be highly affected by contrasting environmental variables and thus large amounts of time and/or greater selective pressures are required for the divergence of life history traits. Based on these findings it is clear that the evolution of life history traits in natural environments is complicated, as hidden environmental drivers may be selecting for specific traits. The interactions between traits in relation to intrinsic and extrinsic trade-offs can also mask the outcomes in relation to any specific environmental variables.

The zebra sea bream *Diplodus cervinus* has been described from South Africa (Smith 1849), the Mediterranean, the north eastern Atlantic (Lowe 1838) and Omanian waters (Bauchot and Bianchi 1984). Nevertheless, life history information is only available for the South African (Mann and Buxton 1997) and the Canary Archipelago populations (Pajuelo et al. 2003a). Although *D. cervinus* in both populations, was found to be a slow growing, late maturing and long lived species, some noticeable differences were observed. These included greater longevity and a reduced growth rate in the South African population when compared with the Canary Islands.

The aim of this study was to describe the life history of the southern Angolan population of *D. cervinus.* This is then compared with that of the South African population. Anthropogenic

effects such as fishing pressure have been shown to affect fish life history strategies (Buxton 1993, Tripple 1993, Richardson et al. 2011a), and thus confound studies that associate environmental parameters with life history traits. However, the relatively unexploited nature of both the Angolan population, and the South African population, which was conducted in a marine protected area, negate the influence of this factor.

The objectives of this chapter were:

- To validate otolith increment deposition and successfully age *D. cervinus*.
- To model the growth of *D. cervinus* using estimated ages.
- To use a logistic model to predict the age and length at maturity from histologically validated macroscopic gonad stages.
- To use a combination of sex-specific length frequency and age frequency information to explain biphasic growth rates between sexes.
- To compare these results with those of the South African study and similar studies on this species complex from around the world.

Materials and methods

Sampling

Samples were collected from March 2011 to February 2012 from the unexploited research fishing area on the southern Angolan coast line. All fish were collected using a spear gun and sacrificed whilst free diving. In the laboratory, the FL and TL of each specimen was measured to the nearest mm. Each fish was weighed whole, and without viscera, to the nearest gram (0.1g), sexed (juvenile, male, female, intersex) and the gonads were macroscopically staged and weighed to the nearest 0.1g. The saggital otoliths were removed and stored for later preparation and analysis.

Data analysis

Length weight relationships

The relationship between fork length (FL) and total length (TL) was expressed using a linear relationship:

$$FL = mTL + c$$

where m is the slope and c is the intercept coefficient. The relationship between FL and weight (Wt) was described using the following exponential relationship:

$$Wt = \alpha FL^{\beta}$$

Where α and β are the model parameters to be estimated.

Otolith preparation and reading pilot study

To choose a suitable preparation method for accurately estimating age, 30 random otolith pairs were assessed. All 60 otoliths were set in clear polyester resin using 25 mm (D) x 20 mm (W) x 300 mm (L) latex setting trays. Otoliths were then sectioned at 0.3 mm using a twin-bladed diamond edged geological saw. In the first pilot study the sectioning plane was assessed, with one otolith from each pair sectioned transversely and the other longitudinally. Sectioned otoliths were mounted on glass slides using DPX mountant and read using a low-power dissecting microscope (magnification between 10 and 40 times) under transmitted light. The numbers of visible opaque zones were counted by the author and two other independent readers. A readability index (from 0 = unreadable to 5 = easily readable) was also assigned to each otolith section. The average of the readability indices (ARI) was calculated for each method and the reliability of growth zone counts was assessed by calculating an index of average percentage error (IAPE) (Beamish and Fournier 1981)

$$IAPE = \frac{1}{n}\sum_{j=1}^{n}\left[\frac{1}{R}\sum_{i=1}^{R}\left|\frac{X_{ij} - \bar{X}_j}{\bar{X}_j}\right|\right]$$

Where *n* fish are aged, *R* is the number of times each fish *j* is aged, *Xij* is the *i*th age determined for the *j*th fish, and *Xj* is the average age calculated for the *j*th fish.

Based on the results of the initial pilot study (Table 5.1) the remaining 15 otolith pairs were sectioned transversely. One of the otolith sections from each pair was baked at 400 °C for three minutes in a furnace, as per the methods of Robillard et al. (2009). Otoliths were then mounted, read and interpreted, based on the methods described for the first pilot study.

Based on the results from both pilot studies (Table 5.1), the otoliths from the remaining 408 fish were sectioned transversely and left unburned. Otoliths were then read once by three independent readers. If two of the three counts coincided, then the count was accepted as the age of the fish. If three counts produced a succession of numbers, for example 3, 4 and 5, then the middle count was accepted as the age of the fish. As there was a greater chance of inconclusive counts for older fish (Richardson et al. 2011a), the reading was accepted if all three counts differed by two or less (e.g. 13, 14 and 16) for fish with counts between 14 and 19. In otoliths with more than 20 counts, and if the counts differed by two, the two closest counts were averaged and the count was accepted (for example, 34, 36 and 37 was accepted as 36.5).

Age validation of increment deposition

The seasonality of growth zone deposition was investigated through the use of a marginal zone analysis, where the optical appearance of the otolith edge was either categorized as opaque or translucent (Wright et al. 2002). The proportion of opaque: translucent otolith edge zones was plotted by month. To validate the periodicity of the edge analysis, a periodic logistic regression (Flury and Levri 1999) was used to model the probability of either an annual or biannual distribution of opaque increment deposition using the methods described by Winker et al. (2010).

Growth model

A three-parameter specialized von Bertalanffy growth function (VBGF) (Ricker 1975) was used to model the growth of *D. cervinus* from the observed length-at-age data. This model was used because of the lower parameter requirement, compared to other similar growth models, making it more statistically robust (Booth 1997). An age correction value was applied to observed age data, using a theoretical birth date of the 1st of June, which corresponded to the peak reproductive activity during the sampling period (Chapter 4). The VBGF models the observed data according the following equation:

$$\boldsymbol{L(t)} = L_{\infty}\left(1 - e^{-K(t-t_0)}\right)$$

Where $L_{(t)}$ is the length of an individual at a given age, L_{∞} is the asymptotic maximum length of the population, K is the growth coefficient and t_0 is the theoretical age-at-zero length. The model's three parameters were estimated, using a downhill simplex search routine (Nelder and Mead 1965). Variability within the model was calculated through the use of a parametric

bootstrapping procedure (Efron 1982) by computing 1000 iterations. 95 % confidence intervals were constructed from the bootstrapped data using the percentile method described by Buckland (1984). To maintain biological realism, all juveniles were included in both the male and female models. A likelihood ratio test (LRT) was used to determine significant differences in the model parameters between sexes.

The macroscopic stageing information was used to assess the maturity of sampled fish (see Chapter 4). Fish with gonads categorised as stages 1 and 2 were considered to be immature, while fish at stages 3, 4, and 5 were considered mature and incorporated into the ogive. Maturity data was only used from the spawning season between the months of May and October (see Chapter 4) in order to achieve the most reliable results from the ogive. The length-at-50% maturity (L_{50}) as well as an age-at-50% maturity (A_{50}) was calculated by fitting a logistic ogive to the proportion of mature fish per 10 mm length class and one year age class, respectively, of mature fish.

The two-parameter ogive used to calculate L_{50} is described by the equation:

$$\mathbf{P}(\boldsymbol{L}) = \frac{1}{1 + e^{-(L-L_{50})/\delta}}$$

and the A_{50} by the equation:

$$\mathbf{P}(\boldsymbol{A}) = \frac{1}{1 + e^{-(L-A_{50})/\delta}}$$

where P *(L)* and P *(A)* is the proportion of mature fish in the length class or age class respectively and δ is the width of the ogive relating to either observed length or age at maturity. Juveniles that were indistinguishable as either male or female were included in the models to simulate biological realism. Size- and age-at-maturity was estimated by sex and differences between the size and age between sexes was assessed using a LRT

Results

Length and weight relationships

The relationship between FL and mass was best described by the following equation:

Wt = 0.0000543 $FL^{2.84}$ (where R^2 = 0.96) (Figure 5.1B). There was a linear relationship (TL = 1.12 FL + 6.28 (R^2 = 0.99) (Figure 5.1A) between TL and FL in D. *cervinus* (Figure 5.1A).

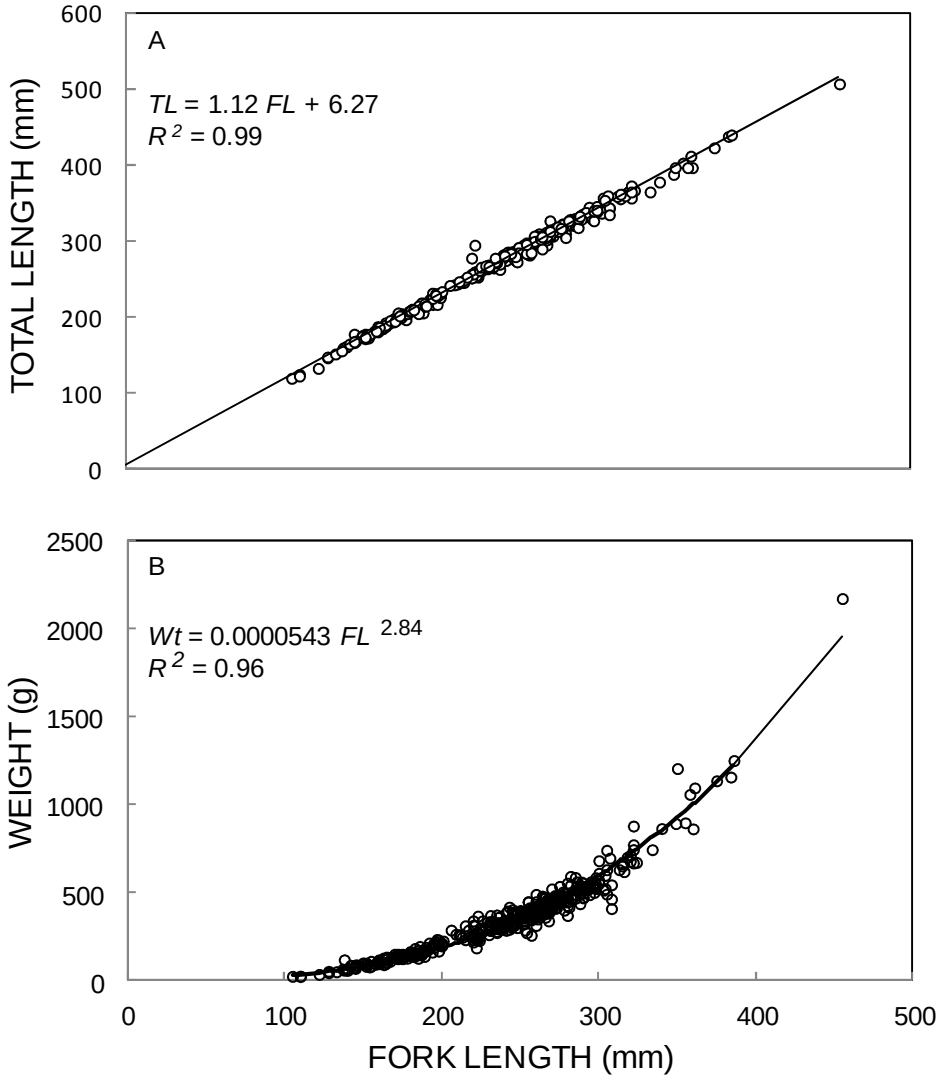

Figure 5.1: (A) Fork length–total length relationship, and (B) length-weight relationship for *Diplodus cervinus* in southern Angola.

Otolith preparation and reading pilot studies

The lowest index of average percentage error (IAPE) and the highest average readability index (ARI) were found for the unbaked transversely sectioned otoliths (Table 5.1).

Table 5.1: Assessment trials of possible otolith preparation methods in which Pilot Study 1 assessed sectioning plane and Pilot Study 2 assessed the effect of baking on readability of otoliths.

	Pilot study 1		**Pilot study 2**	
Method	Unbaked Transverse	Unbaked longitudinal	Baked Transverse	Unbaked Transverse
Mean ARI	2.98	2.84	2.87	3.13
IAPE	21.52	25.73	17.79	12.32

Age validation and increment deposition

No consensus was obtained on the optical property (opaque or hyaline) of 30 (7.93 %) of the otoliths read and these were not considered in the marginal zone analysis. There was no significant difference found between the full periodic model (where modal periodicity was estimated) and the annual model with a hypothesised single annual opaque increment deposition period ($\chi^2 = 1.32$, d.f. = 1, $P > 0.05$) (Table 5.2). However, when comparing the full model with a hypothetical biannual opaque increment deposition period, the results of the LRT showed significant differences between the model parameters ($\chi^2 = 104.10$, d.f. = 1, $P < 0.05$) (Table 5.2). Therefore, the probability of annual opaque increment deposition greatly outweighed the probability of a biannual increment deposition (Figure 5.3).

Table 5.2: Parameter estimates for the periodic regression fitted to the marginal zone analysis of *D. cervinus* between March 2011 and February 2012 in southern Angola.

	Models		
Parameter	Annual	Biannual	Full
β_0	-1.11	-0.78	-1.95
β_1	0.14	0.18	1.96
β_2	-1.82	0.14	-1.65
P	12	6	16.15
d.f.	3	3	4
lnL	183.87	235.26	183.21

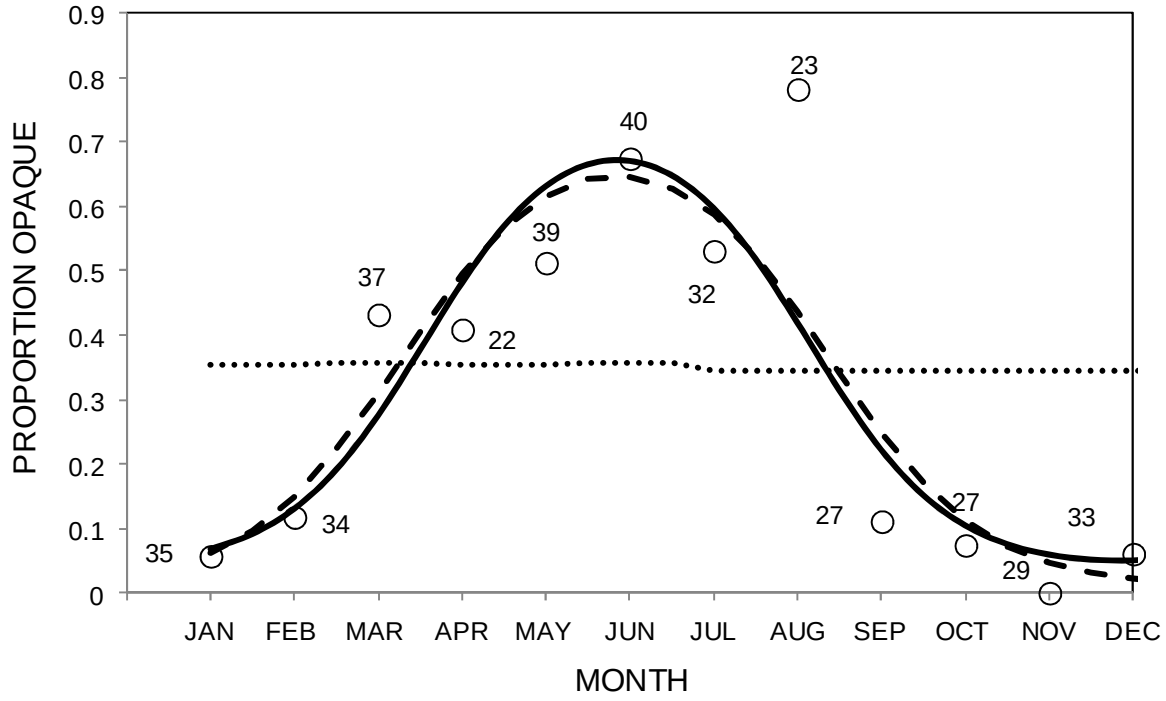

Figure 5.3: Periodic regression fitted to the marginal zone analysis showing observed (open circles), annual model (solid line), full model (dashed line), and bi-annual model (dotted line) for *Diplodus cervinus* collected in southern Angola between March 2011 and February 2012.

Growth

There was no significant difference between the mean length-at-age of males and females (student's t-test, $p > 0.05$) although, on average, males were larger. The oldest male was aged at 43 years (455 mm FL) while the oldest female was 26 years (318 mm FL). The von Bertalanffy growth equation was $L(t) = 287.5(1-e^{-0.18(t-2.84)})$ and $L(t) = 380.19(1-e^{-0.06(t-7.12)})$ for females and males, respectively (Figure 5.4). The point estimates of L_∞ were significantly higher for males than females (LRT, d.f. 3, $p < 0.05$) while K was significantly lower in males than in females (LRT, d.f. 3, $p < 0.05$) (Table 5.3).

Table 5.3: Point estimates and summary statistics for the von Bertalanffy growth function parameters for male and female *Diplodus cervinus* from southern Angola.

		Summary statistics						
	Parameter	Point estimate	Mean	Coefficient of variation	Standard error	Lower and upper 95% CI	Min	Max
Males (n = 176)	L_∞ (mm FL)	380.20	383.62	7.70%	29.26	344.01 - 441.92	330.61	512.05
	K (years^{-1})	0.06	0.07	20.88%	0.01	0.04 - 0.09	0.03	0.11
	t_0 (years)	-7.12	-7.21	18.34%	1.31	-9.82 - -5.13	-13.03	-4.35
Females (n = 238)	$L_{\infty\ (mm\ FL)}$	287.54	288.14	1.77%	5.09	280.27 - 300.73	273.96	309.33
	K (years^{-1})	0.18	0.18	11.21%	0.02	0.14 - 0.21	0.12	0.25
	t_0 (years)	-2.84	-2.88	19.51%	0.55	-3.92 - -2.07	-5.39	-1.63

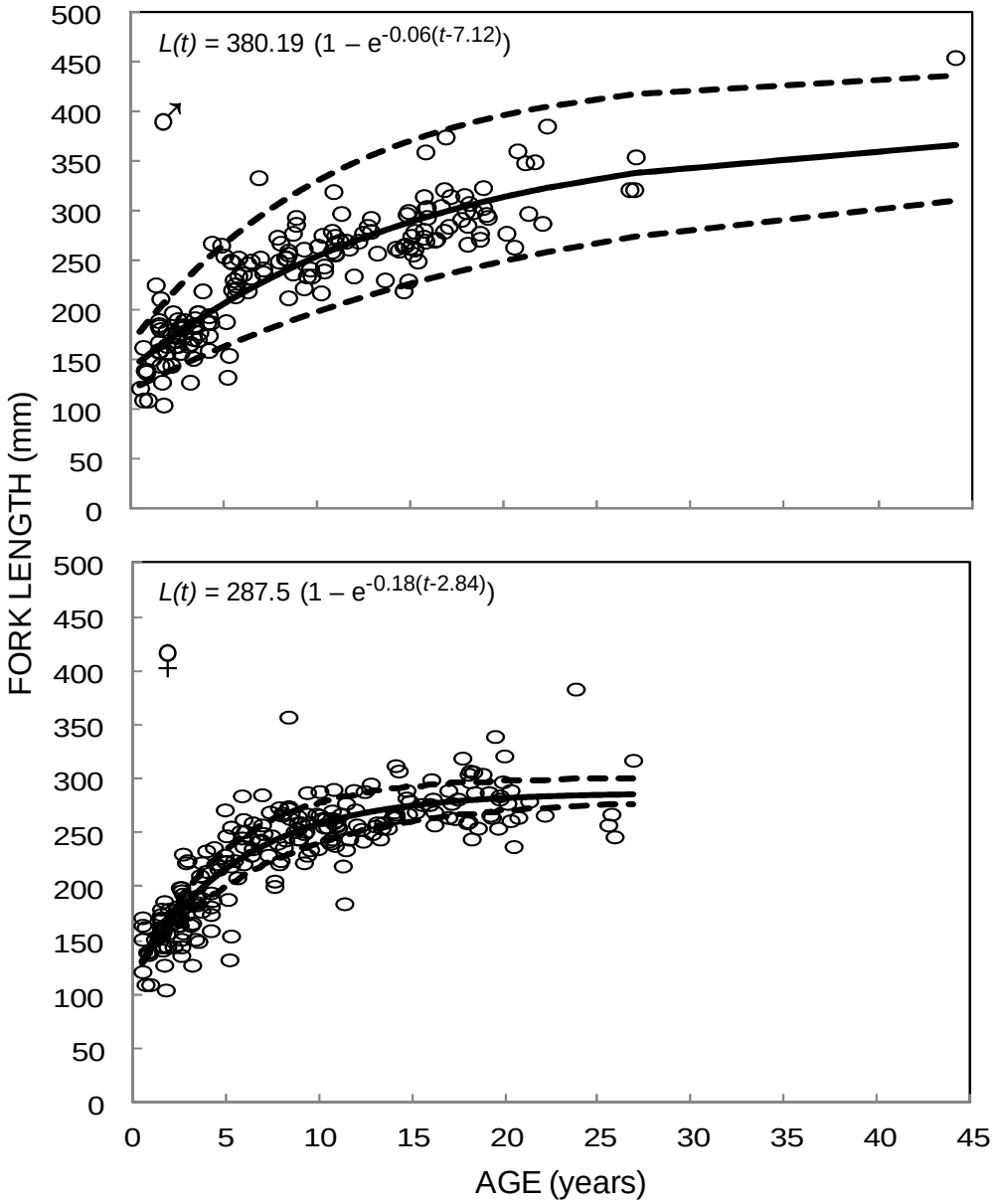

Figure 5.4: Growth of male and female *Diplodus cervinus* collected between March 2011 and February 2012 in southern Angola. Dotted lines represent 95% confidence intervals estimated from the bootstrapped predicted lengths-at-age.

Size and age at sexual maturity

Male and female age and length-at-50% maturity assessments were, respectively, 4.9 years and 220.6 mm FL (for males) and 4.4 years and 201.0 mm FL (for females) (Table 5.4). There was no significant difference between the age-at-50% maturity and the length-at-50% maturity between males and females (LRT, d.f. 2, $p > 0.05$) (Table 5.3). Males did, however, reach their full maturity at around 320 mm FL, which was larger than that of females (of 240 mm FL), thus indicating that female maturity is knife-edged compared to that of males (Table 5.5).

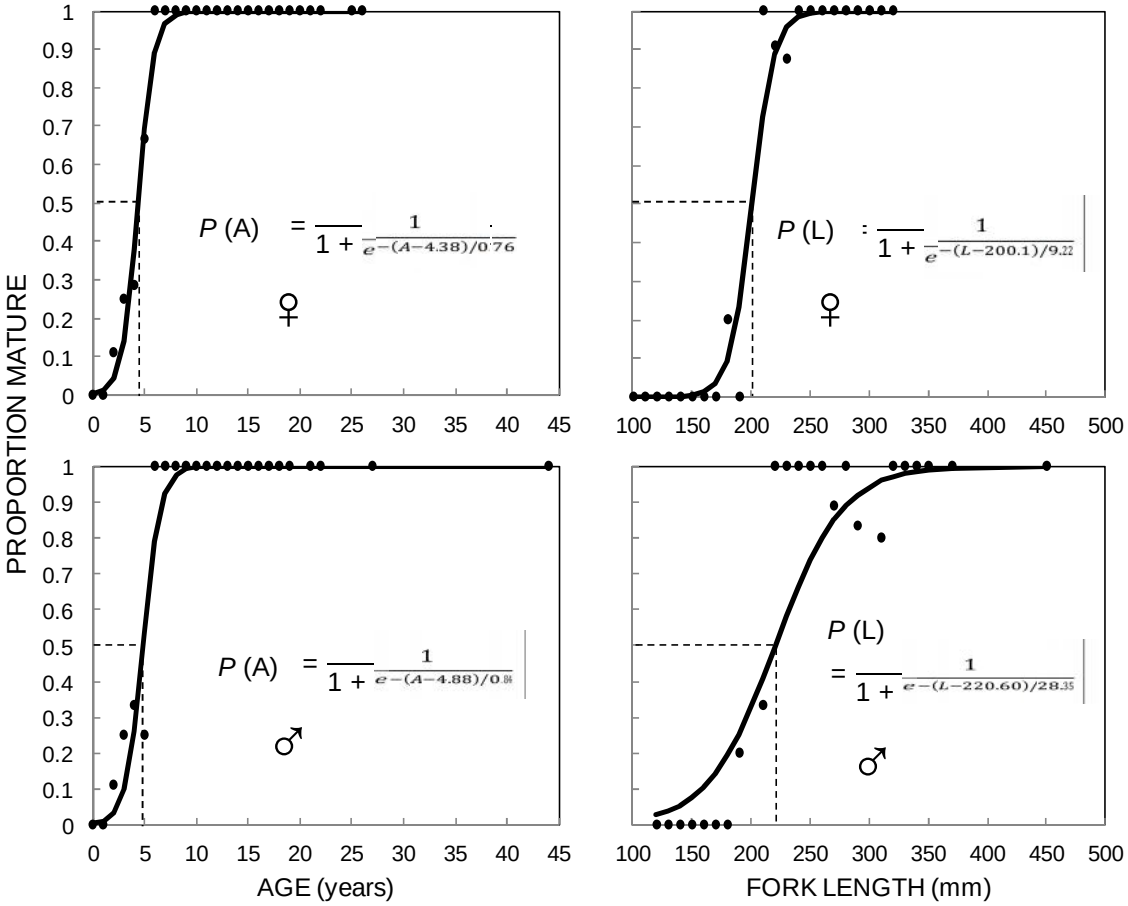

Figure 5.5: Logistic ogive of the maturation pattern of female (top row) and male (bottom row) *Diplodus cervinus* by age (left) and length in southern Angola.

Table 5.4: Logistic ogive maturity parameters for male and female *Diplodus cervinus* from southern Angola.

	Maturity	
Parameter	Males	Females
L 50%	220.6 mm FL	201.0 mm FL
L 100%	320 mm FL	240 mm FL
δ_L	28.3* mm FL^{-1}	9.2* mm FL^{-1}
A 50%	4.9 years	4.4 years
A 100%	8 years	8 years
δ_A	0.8 years^{-1}	0.8 years^{-1}

* significant differences ($p < 0.05$)

Population structure

Male fish were absent from the small size classes (<160 mm FL) but dominated the large size classes (Figure 5.6). Females dominated the overall sample and the sex ratio was 1 M:1.52 F. The length and age frequency distribution patterns were similar for males and females (Figure 5.6).

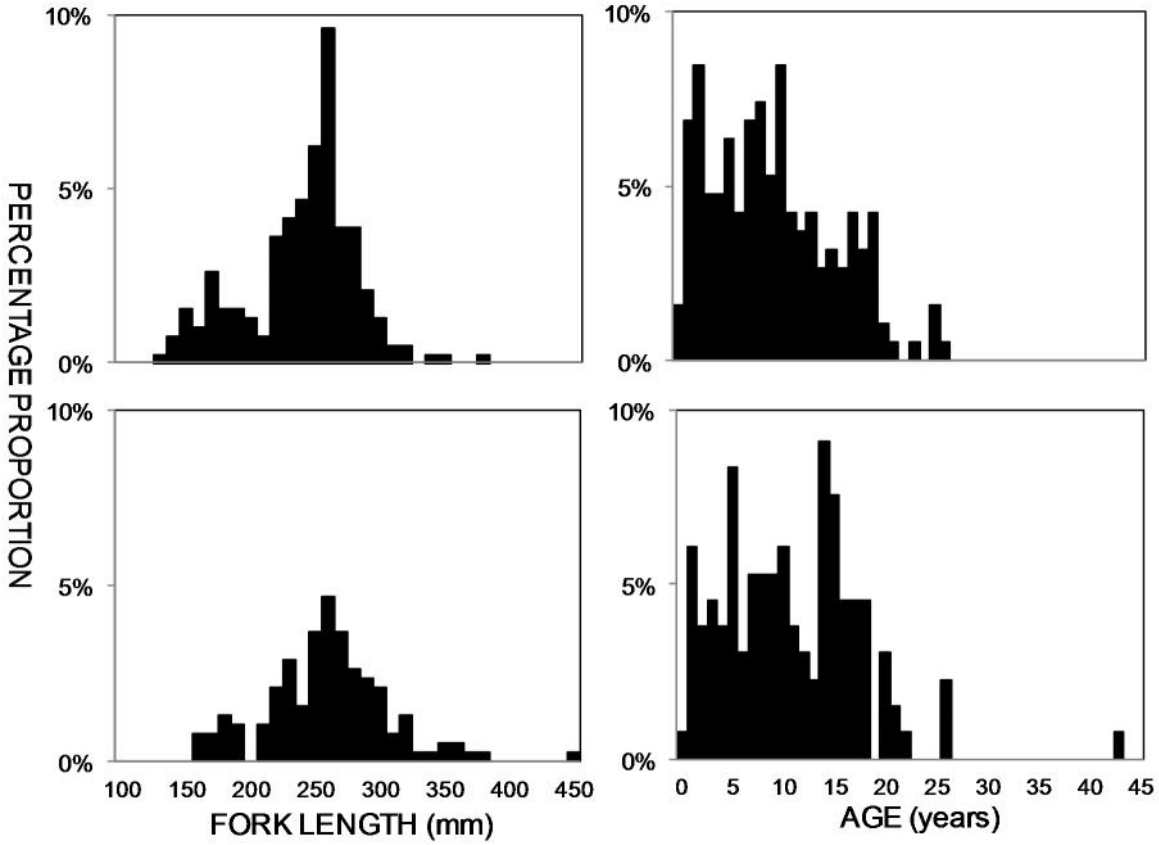

Figure 5.6: Length and age frequency distributions of female (top) and male (bottom) *Diplodus cervinus* from southern Angola.

Discussion

The *Diplodus cervinus* population in southern Angola is slow-growing, late-maturing and long-lived. The Food and Agriculture Organisation developed a categorisation system for the productivity of exploited fishes, based on life history parameters (FAO 2001). Based on these criteria, this population of *D. cervinus* falls into the low productivity category, which suggests that the stock will only support low levels of exploitation. This is similar to many other sparid fishes (Hanel and Tsigenopoulos 2011).

When comparing previous studies done on *D. cervinus* to the present study, it is clear that there were differences in life history parameters between the populations in the Canary Archipelago, South Africa and southern Angola (Table 5.4). The comparative growth parameter Phi prime Φ' ($\Phi' = \log10K + 2\log10L_\infty$), proposed by Munro and Pauly (1983), is widely used to compare growth performances between species as it incorporates the primary VBGF parameters. Using this measure, the growth of *D. cervinus* appeared to be the slowest in southern Angola, although

this was similar to that of the South African population (Mann and Buxton 1997). The *D. cervinus* population in the Canarian Archipelago had the highest growth rate (Pajuelo et al. 2003a) (Table 5.4).

Table 5.5: Reproduction, age and growth parameters for *D. cervinus* from the Canary Archipelago, South Africa and southern Angola.

		Life history parameters										
Region	Sex	L 50%	A 50%	M:F	$\overline{T}$ (°C)	A_{max}	L_{max}	L_∞	L_∞ (cm)	K	t_0	Φ'
Canary archipelago [1]	♂	327*	5.0	1:2.2	20.7 [4]	17	565	537.62	53.76	0.15	-0.73	2.64
Canary archipelago [1]	♀	284*	4.5			14	528	518.89	51.89	0.16	-0.80	2.63
South Africa [2]	♂	285[#]	6.0	1:1.3	19.1 [3]	33	480	409.64	40.96	0.14	-1.99	2.36
South Africa [2]	♀	285[#]	6.0			30	426	371.58	37.16	0.19	-1.51	2.41
Southern Angola [3]	♂	220	4.9	1:1.5	19.9 [5]	43	455	383.62	38.36	0.07	-7.12	2.01
Southern Angola [3]	♀	200	4.4			26	384	288.14	28.81	0.18	-2.84	2.17

[1]Pajuelo et al. 2003, [2] Mann and Buxton 1997, [3] this study, [4] based on the sea surface climatology in the region obtained at [http://emis.jrc.ec.europa.eu/index.php#], [5] Hannekom 1989.

* estimate transformed from total length (TL) to fork length (FL) for camparison using the equation TL = 1.1211 FL + 6.2755.

[#]not modeled for individual sexes.

The differences in the growth rate of these three populations may, as is the case with fishes in general, be attributed to a multitude of exogenous (environmental) and endogenous factors relating to the genotype and physiology of the fish (Wootton 1998). Exogenous factors can include variables such as food availability, temperature, oxygen and salinity (Wootton 1998) while endogenous factors, are determined by physiological constraints and evolutionary history, those that regulate the range in which a fish grows in response to exogenous factors (Wootton 1998).

Of the four main exogenous factors that are likely to affect growth rates, two (i.e. salinity and oxygen) were not measured in any of the biological studies, so no conclusions can be drawn on their relative effects on the growth of the populations. Nevertheless, temperature and food availability can be considered. Berrigan and Charnov (1994) reviewed the mechanisms by which temperature and food availability influence fish growth and concluded that they are remarkably different. A reduction in food resources was shown to affect the growth curve in such a way that L_∞ remains constant with a reduction in the growth coefficient *K* (Berrigan and Charnov 1994). Populations that are exposed to lower water temperatures, however, generally have lower L_∞ and *K* values (Berrigan and Charnov 1994). Based on these findings, and the

higher L_∞ and K values of the *D. cervinus* population in the Canarian Archipelago (Table 5.4), it appears that temperature may be the most important exogenous factor influencing the growth rate of this species. Nevertheless, the combined influence of food availability and temperature cannot be discounted.

Endogenous factors relating to the evolutionary history of a fish population can also have an effect on the growth rates (Wootton 1998). While the three fish populations assessed in the present study are grouped in the same species complex (*Diplodus cervinus*), possibly negating phylogenetic effects on growth, the isolation of these populations and the apparent divergence observed between the South African and southern Angolan populations (Chapter 2) suggest that all populations are most likely divergent, and that stocks are probably influenced by different evolutionary trajectories (Chapter 2). With this in mind, genetic divergence may also account for the variation in the growth rates between the populations. Evidence of the influence of genetic divergence on growth has been documented by Conover (1992) who compared the growth of Atlantic silversides along the North American east coast (*Menidia menidia*) (Conover 1992). Here fish from different latitudes were shown to exhibit different growth rates when grown under constant temperature and food regimes (Conover 1992). Interestingly, fish from populations living in cool, nutrient-poor habitats grew the fastest under laboratory conditions. This suggests that these fish had evolved to utilise better growing conditions more efficiently than fish that had evolved under less harsh conditions (Conover 1992). Because such an experiment was not feasible in the present study, it was very difficult to quantify the influence of genetic factors on the growth of the three *D. cervinus* populations.

Exploitation can also have an impact on the growth of long-lived species. Exploitation has been shown to increase growth rates due to reduced intraspecific competition for resources (Craig 1985, Tripple 1995, Jennings et al. 1999). The present study and the South African study were conducted on unexploited populations of *D. cervinus,* protected by isolation and inadequate fishing techniques (Chapter 6), and by legislation in the form of a marine protected area, respectively. It is therefore likely that the higher population growth rate estimated for the Canarian population may partially be ascribed to the impacts of exploitation. However, since both the southern Angola and South African sites are situated in areas characterised by episodes of nutrient-rich upwelling, it is unlikely that competition for food will be a significant driver of reduced growth rates.

While endogenous and exogenous factors may have played a significant role in the different growth rates observed in the three populations of *D. cervinus*, sampling bias and error must also be considered. There are two main areas of concern. Firstly, the estimation of growth based on an unrepresentative sample of the population may skew the growth estimate. For example, if fish below the size at sexual maturity are under-represented, then the growth rate will be under-estimated. This is primarily because adult fish channel much energy away from somatic growth and into reproduction (Wootton 1998). Naturally, an over-representation of immature fish would have the opposite effect.

Although very small fish (below 100 mm FL) were not captured in this study, 11 fish were estimated to be zero years of age. As these individuals were relatively large (110-172 mm FL) it is likely that they were close to one year of age and this may explain the low t_0 values observed in this study. While this was mitigated to an extent by ageing the fish from a hypothetical birth date corresponding with the peak of the reproductive season, this poor representation appeared to skew the predicted growth rate between the ages of 0 and 1. However, due to the good representation of fish age 1 and up, it is unlikely that this influenced the growth curves of the southern Angolan population.

Although the maximum size of this species is 550 mm TL (Fisher et al. 1981), no individuals near this size were observed during the sampling period. The largest fish in this study was 455 mm FL and was comparable with the largest fish in the South African study (480 mm FL). While larger fish where aged by Pajuelo et al. (2003a) (Table 5.4), it is possible that the fish in the North Eastern Atlantic may grow to a considerably larger size as a consequence of either local adaptation to the environment or endogenous factors (see above).

The second potential bias is an erroneous age estimation technique. Since growth is a function of time, inaccurate estimates of fish age can lead to an incorrect estimation of growth. The methods used in otolith preparation can drastically effect the estimation of fish age (Campana 2001) especially in sparids, where ages exceeding 20 years are not uncommon (Brouwer and Griffiths 2003, Potts et al. 2010, Richardson et al. 2011a). The use of whole otoliths for ageing sparids and other long-lived species has been shown to significantly underestimate the ages of older fish, and consequently leads to an overestimate of population growth rates (Beamish 1979, Hyndes et al. 1992, Brouwer and Griffiths 2003, Abecasis et al. 2006, Potts et al. 2010). Otoliths were sectioned in both this and the South African study (Mann and Buxton 1997) while whole otoliths were used in the Canarian study (Pajuelo et al. 2003a). When comparing

the age estimates for single fish, using sectioned and whole otoliths (Figure 5.7), it was clear that the age was underestimated. It therefore appears that the difference in the otolith preparation may have influenced the results in these studies. For example the oldest fish (17 years) in the Canary Islands study was 497 mm FL (Pajuelo et al. 2003a), compared to a 43 year old fish in this study which was only 455 mm FL. Several other studies have found similar discrepancies between the use of whole and sectioned otoliths in sparid fishes. For example, Brouwer and Griffths (2003) showed that after an age of approximately 10 years, whole otolith age estimation became invalid and greatly underestimated the age of *Argyrosona argyrosona.* This underestimation of age in the Canarian study may therefore be the main cause for the relatively rapid growth estimate of this population.

The validation of the periodicity of otolith increment deposition is a critical aspect of the ageing process (Beamish and McFarlane 1983, Campana 2001). Incorrectly validated increment periodicity can grossly underestimate age and consequently overestimate growth rates if validation undervalues increment periodicity, with the reciprocal occurring if validation overvalues increment periodicity (Beamish and McFarlane 1983, Campana 2001). All three studies used similar indirect validation techniques (Marginal Zone or Increment Analysis), which are based on the appearance of the edge of the otolith, and each found that one opaque and one hyaline zone were deposited each year. This result is characteristic for numerous other sparid species (Buxton and Clarke 1986, 1989, 1991, 1992, Smale and Punt 1991, Buxton and Clarke 1992, Bennett 1993, Chale-Matsau et al. 2001, Brouwer and Griffiths 2004, Potts and Cowley 2005) including other sparid fishes in Angola (Richardson et al. 2011a, Potts et al. 2010).

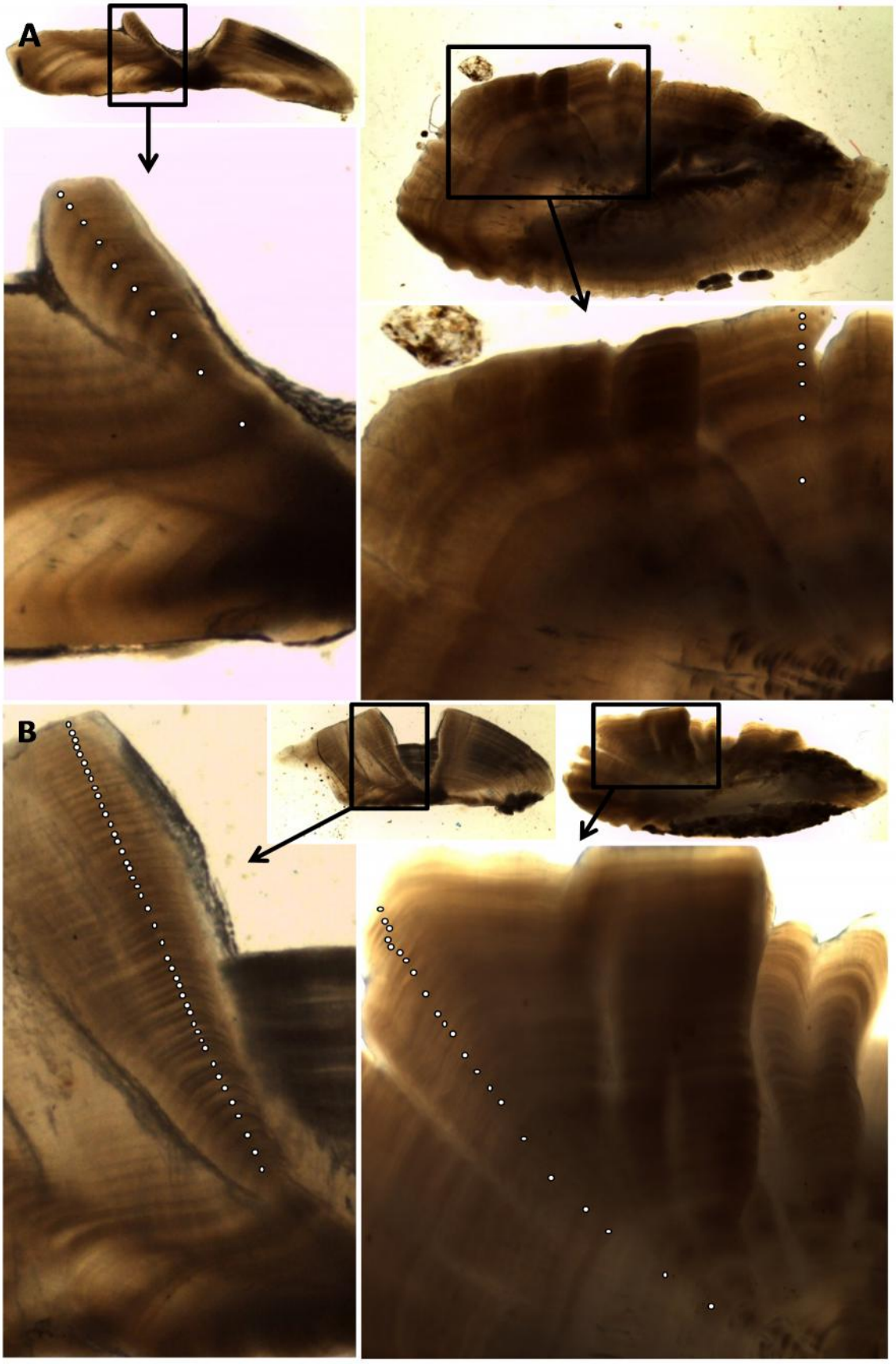

Figure 5.7: Comparisons of the number of opaque zones (white dotes) visible on a sectioned (left) and whole (right) otolith of A) 265 mm FL and B) 455 mm FL *Diplodus cervinus* specimen from southern Angola.

The reason for the annual deposition is not fully understood, but it is probably a function of environmental influences as well as metabolic processes (Wright et al. 2002). The general assumption is that decreases in somatic growth lead to opaque-zone deposition and the reciprocal to translucent-zone deposition (Wright et al. 2002). This assumption has been directly validated in two *Diplodus* species (*D. s. capensis* and *D. cervinus*) in South Africa by analysing the daily growth increment deposition of laboratory-reared juvenile fish under different environmental conditions (Lang and Buxton 1993, Mann-Lang and Buxton 1996). Similarly, a direct chemical validation study on wild *D. capensis* in southern Angola suggested that one opaque increment was deposited each year (Richardson *et al.* 2011a). In both previous studies done on *D. cervinus,* and in the present study, the formation of the opaque annuli coincided with peak spawning activity. Although peak spawning in the previous two studies was associated with warmer environmental conditions (Mann and Buxton 1997, Pajuelo et al. 2003a) in the present study it was associated with cooler temperatures (see Chapter 4). This suggests that slower growth periods associated with reproduction, and not temperature, influenced the formation of the opaque zone. As asynchronous species that are capable of spawning several times during a protracted spawning season (Chapter 4), this is hardly surprising. However, while it is clear that reproductive development may influence somatic growth in adult fishes, it is unclear if and when increment formation will occur in juvenile fishes. It is most likely that this will be influenced by lower temperature and associated slower somatic growth (Lang and Buxton 1993).

All three populations of *D. cervinus* reached sexual maturity (A50) at a similar age (4.5–6 years) but, due to differences in their growth rates, the corresponding size at maturity (L50) differed between populations (Table 5.4). The population with the slowest growth also matured at the smallest size (Angola) while the fastest growing population matured at the largest size (Canary archipelago). Individuals generally mature at a size that maximizes individual fitness in the face of one or more specific trade-offs (Belligan and Charnov 1994). For example, by delaying maturity or maturing at a larger size, the individual may increase its reproductive output (fecundity is positively and even exponentially related to size) (de Vlaming et al. 1982) but by delaying maturity the probability that the individual will reach maturity is drastically reduced, particularly in an environment where juvenile mortality is high. It is therefore not surprising that the age and size at maturity in a number of commercially exploited species often decreases with increased exploitation (Tripple 1995). This is particularly significant when

larger reproducing individuals are removed, as the pressure to reproduce increases for the smaller, earlier-maturing individuals (Tripple 1995).

While fish populations subjected to higher mortality often respond by decreasing their size at maturity, the opposite reaction has also been found (Tripple 1995). Where fishing pressure reduces the overall population size, there may be reduced competition for resources and this can result in an increase in population growth rates. In this case, an exploited population may attain maturity at a larger size as the increased growth rates could pass them quickly through size ranges that would previously require several years of slower growth (Tripple 1995). Thus, the higher growth rates, induced by the reduced competition for food in exploited populations, could hypothetically induce a larger size at maturity. This was shown in the Pacific halibut (*Hippolglossus stenolepis*), which attained a larger size at maturity after population reduction through exploitation (Schmitt and Skud 1978) and may be a factor contributing the larger size at maturity in the Canarian Archipelago population.

The population structure of all three populations of *D. cervinus* were female-biased, with the smaller age and size classes being dominated by females and the older, larger-size classes being dominated by males. While Pajuelo et al. (2003a) suggested that protogynous sex change as the probable cause of this in the Canarian population, evidence from both this and the South African population suggests that this is unlikely (see Chapter 4 discussion). A more probable cause is a higher female mortality and slower female growth rate because of the reallocation of resources away from somatic growth, for reproductive development (Chapter 4). If the Canarian population of *D. cervinus* is regarded as being late gonochorists, the higher M: F sex ratio of 1:2.16 compared to 1:1.5 and 1:1.3 in Angola and South Africa, respectively, may be a consequence of the exploitation of this stock (Table 5.2). As the proportion of males in all the populations is skewed towards the larger size classes, selective exploitation, as is the case in a trap fishery in the Canary Archipelago, would possibly selectively remove males from the population increasing the observed M: F sex ratio.

The later maturation of males, compared to that of females, in both the Angolan and Canarian population [not considered in Mann and Buxton (1997)] could be associated with the trade-offs between delaying growth and optimising individual fitness. A group spawning strategy implies that there may be high sperm competition during spawning aggregations. It might then be advantageous for males to delay maturation to a larger size in order to produce enough sperm

to compete with rival males when mature (Parker 1992). It is well known that small, or even dwarf, males have evolved in species with unusually high sperm competition (Parker 1992).

The 2-million-year isolation of the South African and Angolan *D. cervinus* populations has resulted in only small difference in their life history styles. Differences include slower growth in the southern Angolan population, which may be a consequence of a lower mean annual water temperature (Table 5.4.). Although the South African population matured at an older age and larger length (Mann and Buxton 1997), the lack of sex-specific maturity parameters in the South African study prevents further comparison. It is however possible that endogenous genetic differences have evolved over two million years of isolation and are now reflected as life history differences.

In conclusion the life history parameters of *D. cervinus* from southern Angola are similar to the two previous studies done on the species, characterising the species as a slow growing long-lived fish (Mann and Buxton 1997, Pajuelo et al. 2003a). The growth parameters of the South African population were similar to the Angolan population, while the differences in the Canary islands population could most likely be attributed to their exploitation as well as the ageing methods used.

CHAPTER 6

General discussion

Taxonomy

The taxonomic analysis presented in Chapter 2, as well as the molecular analysis conducted by (Gwillian unpublished data), seems to have resolved any previous confusion over the identity of the southern Angolan population of *D. cervinus*. While previously described as the South African sub-species *D. c. hottentotus*, both genetic and morphological assessments suggest a degree of divergence between the two regions. External colouration of fishes between the two regions differs noticeably and, while this may be argued by some as a superficial character, it has been used as a distinguishing character in previous descriptions of sub-species in this species complex (Bauchot and Bianchi 1984). When considering colour patterns, the similarities in the Angolan and northern eastern Atlantic fish may suggest mixing between these populations even though there is said to be a break in their distribution in the tropical waters of the Gulf of Guinea. While this break is not unlikely since this warm-temperate species has not been found in the near shore areas in tropical environments, it is possible that the individuals of this species have not been recorded in this region due to the limited sampling effort. There is also evidence to suggest that this species does occur in the deeper cooler water in the more tropical areas. For example, Heemstra and Heemstra (2004) reported that *D. cervinus* is out of its distributional range at depths of 120 m, based on photographic evidence collected during an underwater submersible survey to assess coelacanth populations in sub-tropical South African waters. Since *D. cervinus* is known to occur to depths of 200 m (Fischer et al. 1981), it is possible that this species could bypass warm water barriers by moving at depth. The presumed sensitivity of sparid eggs and larvae to warm water (Sheaves 2006) does however suggest that these equatorial waters are barrier to larval dispersal between the north Atlantic and south Atlantic populations.

The only current information on the phylogeography of this species complex comprises the results of this study and the preliminary molecular results from Gwillain (unpublished data). These suggest that the cold Benguela Current acts as a biogeographic barrier for this species and supports the findings of Richardson (2011) and Henriques (2011) who studied a sympatric sparid, *D. s capensis*, from the same region. While this biogeographic barrier appears to limit the dispersal of resident species, there is also similar evidence relating to the distribution of several migratory coastal species, such as the leervis, *Lichia amia* (Henriques et al. 2012), geelbek, *Atractoscion aequidens* (Henriques 2011) and the dusky kob, *Argyrosomus spp* (Henriques pers comm). It therefore seems that the cold Benguela Current is a major barrier to the dispersal of a number of inshore species in the region.

While the formation of the Benguela Current clearly had a major impact on the biogeography of the coastal fish fauna, it is not the only feature responsible for the disjunctions in the distributions of this species complex around Africa. To understand the underlying oceanographic and geographic patterns responsible for their present day distribution, a comprehensive molecular study incorporating samples from all populations, that tests for the absence of shared alleles on a nuclear DNA locus, should be conducted.

Accuracy of life history comparison

One of the aims of this study was to assess the effects of environmental factors on the life history traits of a reproductively isolated population of *Diplodus cervinus*. This was done by assessing the effects of environmental variables on the expression of life history traits established in previous life history studies on this species complex in other geographic regions. One of the major limitations of comparing life history traits between these studies was the inconsistency in the methods used to determine the life history parameters. Direct comparisons were therefore only possible between this study and a South African study (Mann and Buxton 1992, 1997, 1998) as the methods were similar. If all of the previous studies conducted on this species had utilized standardized method-protocols, accurate comparisons across all regions would have been possible. This would have made it easier to determine the environmental factors that influence the life history traits of this species.

One of the primary concerns was the ageing protocol used by various researchers. Accurate fish ageing is critical for the estimation of individual and population growth rates, age-at-maturity, and other related indices (de Pontual et al. 2002). Otoliths are considered to be the

most reliable hard structures for ageing fish. This is due to their metabolically-inert formation, which makes them resistant to re-absorption, unlike other structures, such as scales (Simkiss 1974). Most reviews on fish ageing correctly scrutinize the effects of poor age validation techniques and specifically address the timing and periodicity of increment formation (Beamish and McFarlane 1983, Horn and Sutton 1996, Campana 2001). While this is an important consideration, I also propose that the preparation technique, when examining otoliths, should also be taken into consideration so as to ensure that fish are accurately aged. Otoliths can be prepared for observation using a number of techniques. They can be read whole, sectioned, broken, or ground and polished (McCurdy et al. 2002). After preparation otoliths can be left as is, heat treated (burned or baked) or stained, rendering growth zones more visible (McCurdy et al. 2002). Once an otolith has been prepared it is usually read under a dissecting microscope. Sections are usually mounted on a slide while whole and broken otoliths are read under an immersion medium (McCurdy et al. 2002).

Generally, the most accepted preparation technique is sectioning, which is considered to be reliable, time consuming (Hyndes et al. 1992). Whole otoliths are usually observed directly in fast growing fish, or in fish with small otoliths particularly those with small otoliths that are too small to section and have distinct central rings (Morales-Nin and Panfili 2002). The use of whole otoliths for the ageing of slow-growing fish, or fish with thick otoliths, has however been questioned, primarily due to the difficulty of observing growth bands in the opaque structure, with many authors suggesting that this method provides an underestimate of age (Buxton and Clarke 1989, 1991, Hyndes et al. 1992, Horn and Sutton 1996, Brouwer and Griffiths 2004, Abecassis et al. 2006, Potts et al. 2010).

Sparids are slow growing and long lived (Hanel and Tsigenopoulos 2011) and generally possess relatively large otoliths. Despite this, scientists working in the north eastern Atlantic and Mediterranean, have mostly used whole otoliths for ageing such species. In my review, covering of 37 Sparid ageing studies — which included ageing studies published after 1995 on 18 different species from the above-mentioned region, only seven studies, covering eight species, made use of sectioned otoliths (Table 6.1). This in spite of the wealth of literature denouncing the use of whole otoliths (Buxton and Clarke 1989, 1991, Hyndes et al. 1992, Horn and Sutton 1996) and scales (Simkiss 1974, Carlander 1987, Hutchings et al. 2006) for ageing long-lived species.

Underestimation of fish age often results in an over estimation of individual and population growth rates and also limits the use of these studies for comparisons and for use in studies that attempt to uncouple the environmental factors influencing growth, such as in the present study. Of greater concern is the implication of age underestimations on stock assessments of economically-important species, where incorrect analysis can lead to overexploitation (Tyler et al. 1989). This is most likely when mortality indices are calculated and, more importantly, when using yield per recruit models, which incorporate maximum age to calculate the sustainable yields of a fishery.

The reproductive styles of sparids relating to sex change in Sparidae is one of the least understood life history traits exhibited by this family. Although the understanding of this phenomenon is in its infancy, sex change is most likely influenced by social and environmental factors (Munday et al. 2006). To improve our understanding of sex change in sparid fishes, studies that compare the intraspecific reproductive style of populations with their associated social and environmental factors, should be encouraged. However, as with the ageing protocols, standard protocols for the diagnosis of sex change (Sadovy and Shapiro 1987) should be implemented. Up to now, the misdiagnosis of a population's reproductive style is common, and without sufficient histological evidence incorporated into the diagnostic, little inference can be made (Sadovy and Shapiro 1987). As with an erroneous ageing protocol, the misdiagnosis of a population's reproductive style can have implications for fisheries (Buxton 1992, Punt et al. 1993) and for studies that aim to compare intraspecies trends. For example, a population that is diagnosed as a late gonochorist might be managed with a minimum size limit, which is conventionally set at the length-at-50% maturity. However, if the population was in fact protogynous, the fishery would primarily exploit male fishes. This would significantly skew the sex ratio, reduce the reproductive capacity of the population and have implications for the resilience of the population to overfishing.

Since the incomparability of the results reported in the majority of sparid life history studies is a consequence of poor research design, this continues to limit our understanding of the family as a whole. I would therefore also recommend a degree of standardization in the methods used for the determination of the ageing and reproductive style of sparid fishes. In this regard, the use of sectioned otoliths as recommended by Buxton and Clarke (1989, 1991), Hyndes et al. (1992), Horn and Sutton (1996), and the implementation of the criteria for diagnosing reproductive style in Sadovy and Shapiro (1987) is strongly encouraged in future studies on this family. While some may argue that these techniques are expensive and time consuming,

the accuracy of the information produced would justify the extra costs. Once standardised, our understanding of environmental effects on life history traits will be greatly enhanced.

Table 6.1: Review of 37 Sparidae ageing studies conducted since 1995 assessing the use of different otolith preparation methods.

Species	Structure	Preparation	Study
Diplodus sargus	Otolith	Sectioned	Martinez-Pastor et al. (1996)
Dentex gibbosus	Otolith	Sectioned	Pajuelo and Lorenzo (1995)
Pagrus pagrus	Otolith	Sectioned	Pajuelo and Lorenzo (1996)
Pagellus erythrinus	Otolith	Sectioned	Pajuelo and Lorenzo (1998)
Spondyliosoma cantharus	Otolith	Sectioned	Pajuelo and Lorenzo (1999)
Sparus aurata	Otolith	Sectioned	Mercier et al. (2011)
Diplodus puntazzo	Otolith	Sectioned	Vigliola et al. (2000)
Diplodus vulgaris	Otolith	Sectioned	Vigliola et al. (2000)
Diplodus Sargus	Otolith	Sectioned	Vigliola et al. (2000)
Boobs boobs	Otolith/ scale	Whole	Abecasis et al. (2008)
Diplodus vulgaris	Otolith/ scale	Whole	Abecasis et al. (2008)
Diplodus sargus	Otolith/ scale	Whole	Abecasis et al. (2008)
lithognathus mormyrus	Otolith/ scale	Whole	Abecasis et al. (2008)
Pagellus erythrinus	Otolith/ scale	Whole	Abecasis et al. (2008)
Spondyliosoma cantharus	Otolith/ scale	Whole	Abecasis et al. (2008)
Diplodus annularis	Otolith	Whole	Alos et al. (2010)
Dentex gibbosus	Otolith	Whole	Alves and Vasconcelos (2012)
Pagellus erythrinus	Otolith	Whole	Al-Zahaby et al. (1996)
Diplodus sargus sargus	Otolith	Whole	Benchalel and Kara (2013)
Pagellus bogaraveo	Otolith	Whole	Anna et al. (2006)
Pagellus acarne	Otolith	Whole	Coelho et al. (2005)
Pagellus erythrinus	Otolith	Whole	Coelho et al. (2005)
Diplodus cervinus cervinus	Otolith	Whole	Pajuelo et al. (2003)
Diplodus puntazzo	Otolith	Whole	Dominguez-Seoane et al. (2006)
Diplodus valgaris	Otolith	Whole	Dulcic et al. (2011)
Boobs boobs	Otolith	Whole	El-Okda (2008)
Pagellus erthrinus	Otolith	Whole	Fassatoui et al. (2012)
Diplodus valgaris	Otolith	Whole	Goncalves et al. (2003)
Diplodus valgaris	Otolith	Whole	Gordoa and Moli et al. (1997)
Diplodus sargus	Otolith	Whole	Gordoa and Moli et al. (1997)
Diplodus annularis	Otolith	Whole	Gordoa and Moli et al. (1997)
Lithognathus mormyrus	Otolith	Whole	Kallianiotis et al. (2005)
Diplodus annularis	Otolith	Whole	Koc et al. (2002)
Diplodus puntazzo	Otolith	Whole	Kraljevic et al. (2007)
Diplodus annularis	Otolith	Whole	Matic-Skoko et al. (2007)
Boobs boobs	Otolith	Whole	Monteiro et al. (2006)
Diplodus annularis	Otolith	Whole	Pajuelo and Lorenzo (2001)
Pagellus acarne	Otolith	Whole	Pajuelo and Lorenzo (2000)
Diplodus sargus cadenati	Otolith	Whole	Pajuelo and Lorenzo (2002)
Diplodus valgaris	Otolith	Whole	Pajuelo and Lorenzo (2003)
Pagrus auriga	Otolith	Whole	Pajuelo and Lorenzo (2006)
Pagellus erythrinus	Otolith	Whole	Somarakis and Machias (2002)
Pagellus acarne	Otolith	Whole	Velasco et al. (2011)
Lithognathus mormyrus	Otolith	Whole	Vitale et al. (2011)

Evolutionary patterns

Evidence from the morphometric study (Chapter 2) and the preliminary molecular work (Gwillian pers comm) suggest that the South African and Angolan population have been reproductively isolated by the cold Benguela Current. With its present day features fully formed approximately 2 million years ago (Shannon 1985), the results of this study and those of Mann and Buxton (1997, 1998) provide an opportunity to comparatively examine the consequences of allopatry on morphology (Chapter 2), life history (Chapter 5) and reproduction (Chapter 4) in two unexploited populations.

Multivariate analysis on morphological characters suggests a degree of morphological variation between South African and Angolan populations (Chapter 2). However, the lack of distinguishable characters to discriminate these two populations does suggest that stabilizing selection may result in the maintenance of a relatively constant phenotype (morphology) in both populations (Williamson 1987). Stabilizing selection may occur when divergent populations occupy a similar trophic niche and are exposed to similar environmental selective pressures (Chapter 3). In the absence of divergent selection pressures, evolutionary changes in morphological characters can be slow (Colborn et al. 2001). For example, the bonefishes (*Albula spp*) comprise a number of species, but there is very little morphological variation between species in this genus, despite deep molecular divergence. In some cases the most recent common ancestor, estimated using molecular clock techniques, existed between 8–10 million years ago (Colborn et al. 2001). Colborn et al. (2001) suggest that stabilising selection was the most likely cause for this lack of morphological divergence between these reproductively-isolated populations, a consequence of the similar habitats occupied by the different species.

While morphology is generally a relatively fixed trait (unless acted upon by significant selective pressures), life history traits are generally considered more plastic in response to environmental variability within physiological and genotypic constraints (Stearns 1992, Nylin and Gotthard 1998). However, as with morphological comparison, only relatively small differences were found in the life history traits between the two populations. The most obvious differences were the marginally-slower growth rates, smaller size at maturity, and the lower maximum size in the Angolan population (Chapter 5, Table 5.4). The latter two traits are most probably a consequence of reduced growth rates. The reduced growth rate may be a consequence of the lower mean annual sea temperature in southern Angola. As with

morphology, the low variation in life history traits could also be a consequence of stabilising selection where homogenous habitat and environmental factors could be selecting against divergence in these traits.

While comparisons between the two austral populations suggested that there was limited plasticity in reproductive traits, differences where obvious in the Canary Archipelago population. Since all three populations seem to inhabit similar warm-temperate reef habitats, these findings suggest that there is a degree of plasticity in the life history traits of *D. cervinus.* However, this finding could be a consequence of reduced intraspecific competition, brought about by the exploitation of the northern population (Chapter 5). Water temperature may also play a role. The mean water temperature in the Canary Archipelago (20.7 °C) was one and two degrees higher than the South African and southern Angolan and coastal waters, respectively. However, the difference in the growth rate of the population in the Canary Islands was far greater between the South African population than the difference between the South African and southern Angolan populations (Chapter 5, Table 5.4) and suggests that temperature was not the single factor responsible for the documented differences in the growth rate of the populations. Thus, although temperature, exploitation and differential selective pressure may all have an effect on the life history of the Canarian population, without being able to compare life history traits from the two southern populations with that of an unexploited population from the northern hemisphere it is impossible to uncouple the effects of fisheries induced selective pressures on the life history of the northern population.

Adaptability to climate change

Sparid species make up a large proportion of subsistence and commercial catches in the regions discussed in this thesis (Hanel and Tsigenopoulos 2011). However, since the southern Angolan region is heating approximately 10 x faster (0.6 - 0.8 °C / decade) (Monteiro et al. 2008, Munnik 2012) than the global mean (0.07°C / decade) (Burrows et al 2011), ocean warming may threaten the *D. cervinus* population. Predicting the impacts of climatic change on fishery species is critical for promoting adaptive, sustainable fisheries management strategies. As a predominantly resident species, juvenile and adult sparids are generally resilient to a broad range of temperatures (Sheaves 2006) and warming conditions will probably not result in rapid distributional shifts, as one would expect for temperature-sensitive, migratory species. Temperature changes are however more likely to have an impact on the growth rates,

reproduction, and mortality of these populations. Compared with the juvenile and adult sparid life history stages, the early life stages appear to be more temperature sensitive and Sheaves (2006) suggested that the distribution of these fishes is limited by the temperature sensitivity of the eggs and larvae. Understanding the impact of ocean warming on sparid reproduction is therefore critical for our understanding of the likely changes in the distribution of sparid fishes. The rapid rate of warming in southern Angola is particularly interesting in terms of climate change: due to its above-average decadal rate of warming this ocean warming "hot spot" provides an ideal natural laboratory for investigating the impacts of ocean warming on fish life history traits (Potts et al. in press). Based on the present rate of warming and the environmental requirements for the reproduction of the congeneric *D. s. capensis*, it is expected that this species will no longer be able to spawn successfully in Angola within the next 60 years (Potts et al. in press). This conclusion is based on a review by Potts et al. (in press) of the temperature requirements for spawning, which identified a narrow temperature range (15 - 20˚C) in which these fish spawned. These findings highlighted the impact of warming temperatures on the distribution of these fishes and the methods should be considered for other resident species.

If this warming trend continues in southern Angola, it may have implications for *D. cervinus* as it has similar reproductive temperature requirements to *D. s. capensis* in the same region. One would therefore expect similar changes in the reproductive seasonality and distribution. However, unlike the *D. sargus* species complex, *D. cervinus* spawning occurs at temperatures up to 23°C in the Northern Atlantic and Mediterranean (Chapter 4, Table 4.4). This suggests a degree of adaptability associated with the optimal spawning temperature of *D. cervinus* and that this species may be more tolerant to ocean warming.

The greater depth preference of *D. cervinus* (maximum depth: 200 m) when compared to *D. s capensis* (maximum depth: 40 m) (Heemstra and Heemstra 2004) may also influence the success of reproduction in a warming environment. It is possible that the deeper waters in southern Angola could provide suitable temperatures for reproduction in a warming environment. Monteiro et al. (2008) demonstrated that the mean annual temperature at 15° S in the Benguela region was 15°C at depths between 100 and 200 m. However, temperature is not the only factor involved in successful spawning and some may argue that, at these depths, oxygen may be limiting for the survival of eggs and larvae (Portner and Knust 2007). However without experimental information on the lower limits of oxygen tolerance of *D. cervinus* eggs and larvae, the potential for the use of the deeper waters remains unknown.

While the above approach will provide some information, the effect of climate change on fishes is not straight forward as it does not only have direct effects on their physiology and life history traits; it also affects the entire ecosystem (Rijnsdorp et al. 2009). For example, physiological responses between fish and organisms lower down in the food chain can cause trophic mismatches between different consumer levels (Freitas et al. 2007). The complexity of the interconnectivity in aquatic ecosystems (not only that of fishes) make predicting the effects of climate change extremely complicated.

Future exploitation and management considerations

Angola falls within the Benguela Current Large Marine Ecosystem (BCLME), along with South Africa and Namibia (Cardosa et al. 2006). Between 130 000 and 140 000 people were engaged in artisanal fishing in Angola in 2005 (Duarte et al. 2005) making it the largest marine artisanal fishery in the region (Cardosa et al. 2006). Development in this fisheries sector has been encouraged by the Angolan government, in an attempt to solve food shortages following a period of civil war (Duarte et al. 2005). Although there is a multi-species total allowable catch (TAC) of 150 000 t/annum, data from the Angolan Fisheries Department indicates that this tonnage has never been reached, probably as a consequence of a lack of monitoring. Currently there are no restrictions on the harvest of any inshore sparid species, including *D. cervinus.* Without such controls, the long-term sustainability of the fishery is unlikely. Currently the Angolan population of *D. cervinus* is largely underexploited, as very low numbers are encountered in the artisanal fish markets in the southern coastal towns of Namibe, Pria Prinda and Tombwa (pers. obs.). The low exploitation of this species is most probably a consequence of the fishing methods, which include primarily hook and line (both shore- and boat-based), beach seining, and gill nets. While *D. cervinus* is an important recreational shoreline fish in South Africa, it very seldom caught in Angola even with similar techniques. This may be because of the high abundance of the sympatric *D. s. capensis* (Richardson 2011). However, in the Canary Islands, *D. cervinus* makes up a considerable proportion of the artisanal inshore trap fishery, along with other small sparids (Pajuelo et al. 2003a, Tuya et al. 2006), which suggests that this species may be susceptible to this method of fishing.

The artisanal trap fishery in the Canary Islands presently has a low economic value, but it supports the livelihoods of approximately 2000 fishers (Pascual 2004, Tuya et al. 2006). The use of fish traps in the coastal waters has gained momentum in Angola over the last three years.

The traps used, which are presently designed for the capture of deep water crabs, are placed in the inshore zone with relative success and, as is the case for the Canary Island fishery, the species composition is dominated by small sparid species (pers. obs). Despite rapid growth, the trap fishing sector has not yet been recognised by the Angolan government and is consequently not managed. Although the increasing numbers of traps may benefit the local artisanal communities, as they are able to target less-accessible species such as *D. cervinus,* the sustainability of this fishing method, if left unregulated, remains questionable. This study and others from the region have provided some information that may be relevant to the management of this developing fishery.

One would consider a trap fishery to be non-selective, however, since it targets reef fish that are small enough to enter through the trap entrance and large enough not to escape through the mesh of the trap can be considered a size selective gear. In the case of the Canary Islands, however, this trap fishery is relatively selective, with small sparids comprising approximately 50 % of the catch (Malnychuk et al. 2001). No data currently exists on the selectivity of fish traps in the Angolan region and it can only be hypothesised that, based on the above findings of Malnychuk et al. (2001), the catch composition of this fishery would mainly comprise small inshore sparids, such as *D. cervinus, D. s. capensis, Sarpa salpa*, *Dentex barnardi* and *Lithognathus mormyrus*. Management suggestions for this fishery should therefore be based on the available information on these species in the Angolan region.

Biological information in Angola is only currently available for three of these sparid species: *D. cervinus* (the present study), *D. s. capensis* (Richardson et al. 2011a, 2011b, 2011c) and *D. barnardi* (Richardson et al. 2012). Supplementary biological information on the other two species of *small sparid species* is only available from other regions, such as South Africa and the north eastern Atlantic. Recent evidence does however suggest that the two *Diplodus spp* populations are at least morphologically (Chapter 2, Richardson 2011) and genetically (Gwillian unpublished data, Henriques 2011) discreet stocks that are not connected to temperate north eastern Atlantic or South African populations. It is therefore advisable that caution should be exercised when considering the incorporation of biological information from different regions into management plans relating to this species complex.

Previous studies suggest that the small Sparidae species in Angola are slow growing and long lived (Table 6.1), particularly with respect to the two *Diplodus* species that attain ages in excess

of 20 years. Based on their relative susceptibility to exploitation, it appears that the risk of overexploitation of sparid fish is highest for fishes belonging to the genus *Diplodus.*

Table 6.2: Life history parameters of potentially exploitable inshore Sparidae species through a trap fishery in southern Angola.

		Life History Parameters								
Species	Region	Sex	A50 %	L 50 %	L 100 %	Spawning	L_∞	K	A_{max}	*Productivity
D. bernardi[1]	ANG	♀♂	4	220	270	Aug - Feb	332.14	0.14	13	low
D. s. capensis[2]	ANG	♂	1.8	149	160	May - Oct	236.3	0.31	24	low/medium
D. s. capensis[2]	ANG	♀	1.7	149	160	May - Oct	349.2	0.09	31	low/very low
D. cervinus[3]	ANG	♂	4.9	220	240	May - Oct	509	0.06	43	low/very low
D. cervinus[3]	ANG	♀	4.5	200	240	May - Oct	383	0.18	26	low/medium
S. salpa[4]	RSA	♀♂	1.5	145	200	Mar - Sep	224	0.55	6	high
L. mormyrus[5]	CAN	♂	2	200	250	Jun - Nov	407	0.22	8	medium
L. mormyrus[5]	CAN	♀	3	250	300	Jun - Nov	448	0.19	10	medium

[1]Richardson et al 2012, [2]Richardson et al 2011, [3]This study, [4]Vandervalt et al 1997, [5]Lorenzo et al 2002, *Based upon criteria proposed by Musick (1999)

Conventional fisheries management tools adopt the use of size limits, bag limits, gear restrictions, closed seasons and restricted area access (McClanahan and Castilla 2007). There has however been large amount of debate on the efficacy of such restrictions in terms of maintaining sustainability (Bohnsack and Ault 1996, Stergiou 2002, Garcia et al. 2012, Law et al. 2012). The primary cause of concern relates to the high mortality of released fish, poor enforcement, and compliance by users who do not understand, or who are not sufficiently informed of, the benefits that such regulation will bring in the long term (Bohnsack and Ault 1996). Size limits are conventionally set at a length at which the fish matures, in order to allow individuals within the population to reproduce at least once within its life time. This policy has promoted selection for larger individuals in the population. There is however some evidence to suggest that larger female fish produce exponentially more eggs than smaller fish (Roberts and Poulinin 1991) and that the quality of the eggs and larvae produced by larger fish is far greater than that of smaller fish (Birkeland and Dayton 2005).

"Balanced exploitation" is a new fisheries management paradigm that attempts to negate the selective harvest of large adult fishes. This is done through the distribution of fishing pressure across species and body size, in an attempt to balance fishing mortality with the natural productivity of different organisms (Law et al. 2012). The rationale for a reduction in the exploitation of older fish is the relatively higher resilience to exploitation of juvenile fish,

which is a consequence of higher growth and mortality rates. This strategy is however unlikely to be sustainable if the population is already overexploited and the majority of the adult population are close to the size at sexual maturity. Since southern Angola's small sparid fishes remain relatively unexploited, the implementation of a balanced exploitation model, using the trap fishery, may be more successful. My recommendation is the unselective harvest of small *Diplodus* species below the size at full maturity. This will ensure that the largest, slowest-growing and most reproductively-productive individuals are protected.

A fish trap easily allows for such a limit without the complication of post release mortality which would require fisherman to release all fish above the allowable size, as is the case of line fisheries. This could be better achieved by limiting the size of the entrance to the trap, to ensure that fish larger than Full maturity are excluded from the trap. The problem however is that the reproductive parameters and body depth for each of the species will be different. As the two *Diplodus* species are likely to be the dominant species in the trap fishery, traps should be designed with these species in mind. The body depth-at-full maturity for *D. s. capensis* and *D. cervinus* is 63.2 mm (Chapter 2) and 61.4 mm (Richardson 2011), respectively, suggesting that a trap opening of 62 mm would be ideal for the fishery (Figure 6.1). Using this trap design, the balanced exploitation approach could be implemented. While there may be opposition to these restrictions, due to the lower market price fetched by smaller fish, such fish could possibly fill a gap in the local market and such a policy would also function as an important "safety net" for the protein needs of the local population.

The developing trap fishery will inevitably have consequences for the inshore Sparidae community, but early implementation of these proposed gear restrictions could mean that limited additional enforcement measures would be required, other than the occasional dock-side spot check of trap entrance sizes. While a large proportion of adult fishes from all of the smaller sparids, such as *D. bernardi*, *S. salpa* and *L. mormyrus*, will be exploited, these fishes have faster growth rates and are not as susceptible to over-exploitation as is the case for the two *Diplodus* species (Table 6.2).

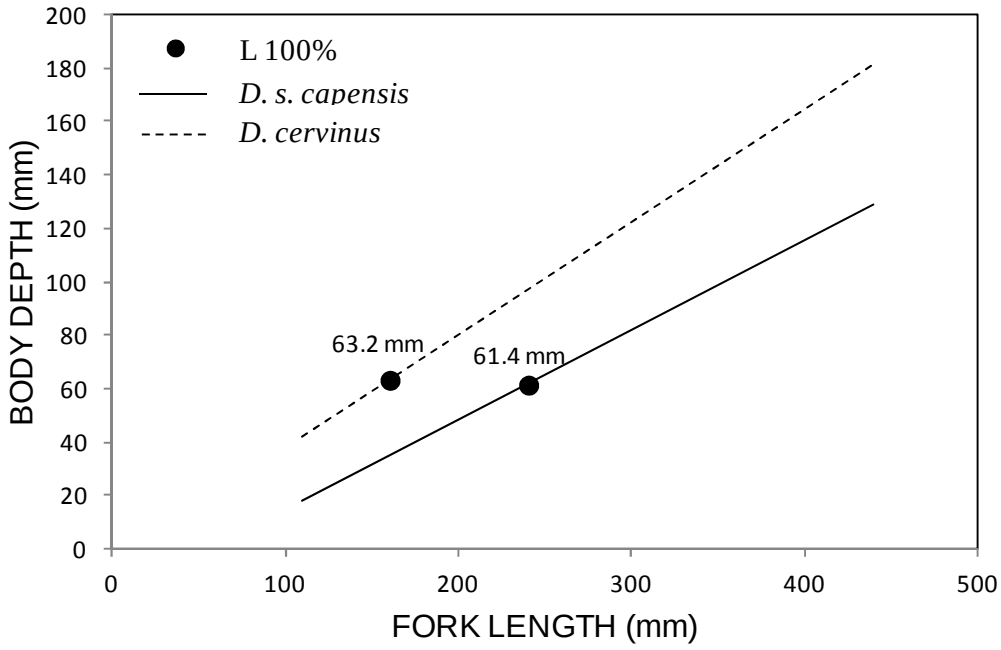

Figure 6.1: Linear relationships between body depth and fork length as well as point Full maturity estimates of *Diplodus sargus capensis* and *Diplodus cervinus* from southern Angola.

Other than the gear restrictions, additional measures, such as the implementation of a closed season, may be required to maintain the sustainability of the fishery. Inshore gears such as fish traps are susceptible to gear loss, which as ghost-gear can have severe impacts on the fishery. In southern Angola, rough sea conditions coincide with the peak spawning season and, therefore, a closed season between July and August could have significant benefits for the fishery.

A further management consideration which has recently gained in popularity, is the use of marine protected areas (MPAs). The advantages of MPAs in the management of important reef associated fisheries species such as *D. cervinus* include the maintenance of spawner biomass, improvement of yield, simplified enforcement, recruitment spill over, maintenance of natural population age structure and the maintenance ecosystem integrity (Bohnsack 1990, Attwood et al. 1997). Currently there are no established MPAs in Angola, and motivating the establishment of one for the conservation of one possibly exploitable species is impossible. However, MPAs are not single species management tools, they protect entire marine ecosystems and there biotic users. Richardson (2011) suggested a similar use of MPAs in preserving *D. cervinus's* congener *D. s. capensis* in the region and motivated the establishment of such a plan to its potential efficacy at conserving 13 other commercially important reef associated species and three migratory species which could use the MPA as a refuge. In terms of preserving Southern Angolas' in shore fisheries sustainably the proclamation of MPAs may be crucial in the future.

Summary and future research considerations

In summary, this study has found that the Angolan population of *D. cervinus* should not be classified as the same subspecies *D. c. hottentotus* due to obvious morphological (Chapter 2), molecular (Gwillian pers comm) and colouration pattern (Chapter 2) variation between the populations. The trophic niche of the South African and Angola populations was however almost identical with both populations feeding heavily on amphipods and polychaetes worms throughout ontogeny. In contrast, their boreal conspecifics from the Mediterranean mainly fed on Carid shrimps, and macrophytes. Life history parameters such as reproductive style, growth and spawning temperature were also similar within the two austral populations but differed greatly with those reported in studies conducted in the northern hemisphere. Similarities found within the two austral populations are probably a consequence of stabilising selection, which is a consequence of the similar habitats, competitive interactions and reduced levels of exploitation. The differences observed between the boreal and austral populations could be attributed to a number of factors. These include the use of different life history estimation techniques, the effects of exploitation on the population dynamics or differential selective pressures experience by fish in contrasting habitats.

This thesis has provided information on the taxonomy and the life history of *D. cervinus* in the southern Angolan region in an attempt to understand the effects of allopatry on the biology of this species. Future research on this topic should include the following:

1) A detailed molecular and morphometric study incorporating samples from the gulf of Oman, South Africa, Angola, the Canary Islands and the Mediterranean should be conducted in order to clarify taxonomic confusion within the species complex and understand some of the biogeographic patterns shaping the Africa coastal fish fauna.
2) Comparative life history assessments, using contemporary methods on sparid species, e.g. *Pagrus auriga*, *Dentex gibbosus* and *Pagellus bellottii* that are common to Angola and the Mediterranean to gain a better understanding of local adaptations in these regions.
3) Conduct ichthyoplankton and phytoplankton surveys in an attempt to understand the relationship between reproductive periodicity and ocean productivity in warm-temperate environments.

4) Initiate long-term projects to monitor the life history, movement patterns and species composition of the rapidly warming southern Angolan region to obtain predictive information for regions warming at slower rates.

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