MSc Dissertation

Feeding habits of sardine (*Sardinops sagax*) in relation to their spawning activities

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Abstract

Feeding activity of sardine (*Sardinops sagax)* from St Helena Bay and Gans Bay in the Southern Benguela was studied between February and April 2011 and related to reproduction. Feeding intensity of 373 sardine was calculated and correlated with caudal length across different gonad maturity and fat stages. Feeding intensity tended to be higher in small than large sardine ($r = -0.14$, $n = 373$, $p = 0.005$) and similar for both sexes ($t = 1.567$, $df = 371$, $p = 0.12$). Feeding intensity was high in St Helena Bay, possibly because of food availability. Gonad maturity and GSI were highest in February and were greater in St Helena Bay than in Gans Bay. Fat staging and the relative weight index were used to represent fish condition. Fat reserves varied, being low in February during the spawning season and peaking thereafter in March and April. A GLM indicated that feeding intensity in sardine was affected by time of year and gonad maturity state. Sardine fed throughout the study period but feeding intensity was highest in periods before- and after- spawning. With the observed continuous feeding and the accumulation of fat reserves before spawning, it appears *S. sagax* in the Southern Benguela employs both capital and income breeding strategies.

Chapter 1

Review of literature on feeding biology, reproduction and condition in sardine

Reproduction in sardine is affected by a number of factors, such as environmental variability, condition of the fish and food availability (Cushing, 1990; Richardson *et al.*, 2003). Environmental variability and fish condition are the major limiting factors of larval survival in fish. Sardine and other small pelagic fish in the Southern Benguela match their reproductive season to favourable environmental conditions to maximise larval survival and enhance recruitment (Mullon *et al.*, 2002; Anderson and Lucas, 2008; Kreiner *et al.*, 2001). However, matching spawning and favourable environmental conditions is not the only strategy that fish species use to ensure survival of progeny. There are other strategies, including storage of energy prior to the spawning season and maximising feeding during spawning (Garrido *et al.*, 2008a).

Two contrasting strategies that are used for reproduction in fish are called capital and income breeding (Houston *et al.*, 2007). In capital breeding, fish accumulate fat reserves before the spawning season and utilise them as energy for spawning. In income breeding, fish utilize the food ingested and instantaneously convert it to energy for formation of eggs and spawning (Stearns, 1992). Most capital-breeding fish tend to be demersal species in cold marine waters and income breeders tend to be small, pelagic species in temperate waters (Murua and Saborido-Rey, 2003).

Sardinops sagax in the Southern Benguela is a species of small pelagic fish inhabiting the temperate waters of the Atlantic and Indian Oceans. Because of their lifestyle and habitat, sardine in the Southern Benguela might be expected to be income breeders. Studies conducted on Southern Benguela sardine indicate that accumulation of fat reserves occurs before the spawning season and decline as the gonads develop (van der Lingen and

Hutchings, 2005), supporting the hypothesis that sardine are capital breeders. Accumulation of fat reserves before the spawning season has also been observed in other clupeoid species (Blaxter and Hunter, 1982; García-Franco *et al*., 1999; Millan, 1999).

The breeding strategy and feeding activity of sardine in the Southern Benguela need to be thoroughly studied before any conclusions can be made about energetic investment in reproduction. Factors such as environmental variability, food production and seasonality can influence feeding intensity and reproduction of sardine. These and other factors will be considered in this study to help link reproduction of sardine to feeding activity.

1.1. Feeding biology of sardine

Sardinops sagax in the Southern Benguela are planktivourous, filter feeding on both zooplankton and phytoplankton (van der Lingen, 1995). Besides filter feeding, the Southern Benguela sardine has the ability to utilise another mode of feeding called particulate feeding (van der Lingen, 1994). In filter feeding, sardine are able to capture food particles of less than 1230 µm (van der Lingen, 1994). Larger food particles are captured in particulate feeding (van der Lingen, 1994).

Sardine in other upwelling ecosystems have also been found to utilise both filter and particulate feeding. The Portuguese sardine *Sardina pilchardus* particulate feed on prey >780 µm (Garrido *et al.,* 2007a). However, numerical dominance of prey <780 µm found in the stomachs of Portugese sardine indicate that filter feeding is the main feeding mode employed in this species (Garrido *et al.*, 2008a). Stomach fullness and colour indices used to assess

Sardina pilchardus feeding showed that most of their stomachs had different proportions of phytoplankton and zooplankton (Cunha *et al.*, 2005).

Studies on feeding biology, feeding periodicity and prey size in Southern Benguela sardine indicate that these depend on and are affected by the size of the fish (van der Lingen, 1994; 1998b). Differences in feeding behaviour between size classes of sardine from different regions have also been found. Juvenile sardine utilise particulate feeding more than adults and feed more on zooplankton than phytoplankton (Hand and Berner, 1959; King and Macleod, 1976; van der Lingen, 1998b). Small fish also consume more phytoplankton and zooplankton daily than bigger sardine (van der Lingen, 1998b).

Feeding and diet composition play an important role in growth, reproduction and in meeting other energy requirements in fish (Tocher, 2003; Bell and Sargent, 1996; Marshall *et al.*, 1999). Growth of sardine increased when feeding on both phytoplankton and zooplankton (van der Lingen and Durholtz, 2005). *Sardinops sagax* in the Southern Benguela showed increased growth for a wide variety of plankton, but growth was maximised when filter-feeding on high concentrations of microzooplankton (van der Lingen, 1999). Sardine obtain most of their dietary carbon from zooplankton, typically small (<1200 µm) zooplankton, consisting of nauplii, crustacean eggs, and calanoid and cyclopoid copepods (James, 1987; van der Lingen, 2002). Phytoplankton also play an important role as a contributor of dietary carbon at particular times of the year when there are blooms in different localized regions (van der Lingen and Durholtz, 2005). Sardine obtain fatty acids for reproduction and growth from zooplankton and phytoplankton as part of their diet (Garrido *et al.*, 2008b). Comparisons between the Northern and Southern Benguela revealed high primary productivity levels in the North, indicating that phytoplankton is the primary source of diet for sardine in the region (Brown *et al.*, 1991; Giraudeau and Rogers, 1994).

Zooplankton, phytoplankton, crustacean eggs and fish eggs have also been found to be important dietary items in Iberian sardine (Garrido *et al.*, 2008a). All these prey items have been found to contribute 90% of the estimated dietary carbon in Iberian sardine. Variation in contributions of phytoplankton to dietary carbon has also been found in Iberian sardine, with contributions being high during the spring-summer upwelling season and also in areas of strong upwelling (Fiúza *et al.*, 1983).

Feeding intensity in sardine is influenced by a number of factors such as reproduction, food availability, fish length and seasonality (Bode *et al.*, 2003; Garrido *et al.*, 2008a; Ganias, 2009; van der Lingen, 2002). Studies carried out to investigate feeding intensity in Southern Benguela sardine indicate differences in feeding intensity between sardine size classes (van der Lingen, 1998b), probably caused by different feeding behaviour of small compared to large individuals. *Sardinops sagax* of wet mass >25 g (~ >135 mm) appear to feed all day long and do not appear to be affected by periodicity (van der Lingen, 1998b). Feeding activity of small fish (<25 g) (~ <135 mm) was found to increase around sunset (van der Lingen, 1998b).

The relationship between reproduction and feeding activity of *Sardinops sagax* in the Southern Benguela is not well understood. However feeding biology studies have been carried out on the Southern Benguela sardine and can help in understanding the influence of feeding on reproduction and vice versa. Studies have been carried out on sardine in other parts of the world to try and link sardine reproduction and feeding. Ganias (2009) observed a strong link between sardine spawning dynamics and primary productivity in the Eastern Mediterranean Sea, which may reflect direct energetic flow from phytoplankton feeding to reproduction. This can also indicate an adaptive mechanism for enhancing offspring survival through matching egg production with favourable environmental conditions for early growth stages. Riveiro *et al.* (2000) have also shown that *Sardina pilchardus* in the Atlantic tend to intensify egg production under conditions of increased environmental productivity. The matching of breeding and increased environmental productivity in sardine implies that, besides using stored energy for reproduction, there is also a direct energetic transfer from concurrent food intake to reproduction. This suggests that some sardine may be deploying both capital and income breeding strategies.

1.2. Reproductive strategies and biology in sardine

Sardinops sagax in California are multiple spawners, with indeterminate annual fecundity and continuous oocyte size frequency distribution (Macewicz *et al.*, 1996). In indeterminate fecundity, oocytes continually develop in the ovary of the fish and are released when they are mature throughout the spawning season (Hunter and Macewicz, 1985; Hunter *et al.*, 1985). Sardine in the Southern Benguela were found to be indeterminate spawners, releasing batches of mature oocytes throughout the year during the spawning season (van der Lingen and Hutchings, 2005).

Southern Benguela sardine spawning occurs mainly on the Agulhas Bank and recruitment takes place off the west coast (Hutchings *et al.*, 2002; Miller *et al.*, 2006). Sardine spawn in this area as it offers advantages for egg and larval retention by transporting larvae into the nursery area off the west coast (Hutchings *et al.*, 2002). Frequent loss of eggs and larvae occurs when they are swept off the shelf area by the Agulhas Current (Anderson and Lucas, 2008). Since the west coast is the main nursery area for sardine, it is expected that feeding intensity, condition and gonadosomatic (GSI) index of the fish will be higher there than elsewhere around the South African coast as they have more food resources available.

Although the west coast is the main recruitment and nursery area, sardine are distributed along the entire South African coastline from the Orange River to KwaZulu Natal (Beckley and van der Lingen, 1999). The sardine spawning season along the coast occurs all year round with two peaks, in September/October and February/March during the main spawning season (van der Lingen and Huggett, 2003).

Sardine employ a bet-hedging strategy and release pelagic eggs, as is the case with most clupeids (Blaxter and Hunter, 1982). During their spawning season, sardine produce a number of successive egg batches over extended periods. This strategy improves the chances of progeny survival whenever environmental conditions are favourable, since sea conditions and food resources vary with time and locations (Cury and Roy, 1989; Fuiman and Cowan, 2003). Surviving sardine eggs and larvae in the Southern Benguela grow into juveniles and mature in their second or even third year. During spawning, mature sardine produce around 260 eggs.g^{-1} of egg mass released (van der Lingen and Durholtz, 2005).

Egg production in sardine relies on energy from stored fat reserves and therefore this may indicate that sardine deploy the “capital breeding” strategy (Stearns, 1992). Capital breeding together with bet-hedging offer benefits under unfavourable environmental conditions (Jonsson, 1997). It is also a means of sustaining the energy required for egg production through winter by capitalising on reserves accumulated during the most productive periods of the year, i.e. in summer and spring, as observed in *Sardina pilchardus* in the Eastern Mediterranean Sea (Ganias *et al.*, 2007).

As is the case with feeding, spawning in *Sardinops sagax* also depends on the size of the fish. Le Clus (1989) found that large sardine spawned more frequently than small ones, with the increase in spawning being 50-70% when length was increased by 1 cm. Sardine spawning also differed seasonally across the various length classes sampled (Le Clus, 1989). Spawning in the Chilean and Iberian sardine was also found to vary seasonally with length classes (Herrera *et al.*, 1994; Silva *et al.*, 2006). Large sardine have extended periods of reproduction compared to small size classes (Parrish *et al.*, 1986; Millan, 1999). Sexual maturity in *Sardinops sagax* starts at a caudal length of 16cm and most of the fish reach maturity at 17-17.5 cm (Akkers *et al.*, 1996). The dependence of reproduction on size is clear from these findings.

There are other factors that have been found to influence reproduction in sardine. Reproduction in sardine is affected by density dependence, seasonality and environmental variability (Amstrong *et al.*, 1989; Le Clus, 1989; 1990). Fecundity in *Sardina pilchardus* depends on food availability and duration of the spawning season (Sinovčić, 1983).

Reproduction in sardine and other clupeids has been shown to be dependent on fat reserves and fish condition (Blaxter and Hunter, 1982; van der Lingen and Hutchings, 2005).

1.3. Fat reserves and seasonal cycles in condition

Fat reserves are important in fish species as they play an important role in growth, reproduction and survival (Shulman & Love, 1999). All life history stages of fish depend on fatty acids as one of the major sources of energy (Tocher, 2003). Most of the energy used for oocyte development in teleosts is derived from lipid reserves accumulated by the females before or during the spawning season (Wiegand, 1996). Exhaustion of fat reserves in fish can lead to starvation, susceptibility to diseases, reduced larval survival, and reduction in fecundity and quality of eggs (Shulman & Love, 1999; Lambert *et al.*, 2003; Koops *et al.,* 2004).

Studies of reproduction of clupeid species have found that gonad maturation is dependent on stored lipid reserves (Blaxter and Hunter, 1982; García-Franco *et al.*, 1999; Millan 1999). In these studies, clupeids have been found to accumulate fat reserves before the spawning season and convert them into energy for egg production during the spawning season. A study of reproduction in the northern anchovy *(Engraulis mordax)* found that almost two thirds of the required energy for spawning is derived from lipid reserves (Hunter and Leong, 1981).

Similar to most clupeids, sardine in the Southern Benguela appear to follow the trend of fat reserves peaking before the spawning season and declining after (van der Lingen and Hutchings, 2005). The pattern in fat reserves over time alternated with the GSI in *Sardinops* *sagax*, indicating a transfer of energy to gonad maturation during the spawning season. Hunter and Leong (1981) suggested that stored fat reserves sustain energy requirements of fish during spawning. The GSI is the ratio of fish gonad weight to body weight and is used to identify spawning days and seasons, as fish gonads increase in size prior to spawning.

Condition factor of a fish is defined as an index of the physiological well being of the fish. Condition factor is affected by availability of food resources, physical factors, density and the physiology and reproductive stage of the fish (Kreiner *et al.*, 2001). The condition factor of *Sardinops sagax* in the Southern Benguela has also been found to be affected by Chl-a, sea surface temperature and the GSI (De Goede, 2004). Sardine condition lagged sea surface temperature and Chl-a by two and three months respectively (De Goede, 2004). A negative linear relationship was also found between sardine GSI and lipid, also indicating dependence of sardine reproduction on stored fat reserves. It appears it takes sardine up to three months to convert food into fat reserves (De Goede, 2004).

Different trends for biomass and condition factor of Southern Benguela sardine during the period 1989-2004 have been observed (Kreiner *et al.*, 2001), with a suggestion that the observed trends in condition factor may be at least partly density-dependent. The condition factor of sardine has been found to be high at small population sizes, and low at large population sizes (Le Clus, 1987). This inverse relationship between condition and population size has been attributed to intraspecific competition and provided verification of density-dependence in sardine (Kreiner *et al.,* 2001; van der Lingen *et al.,* 2006).

Fish condition is very important for egg production, spawning, and survival of larvae. In *Sardina pilchardus* the fatty acid content of eggs produced varied throughout their spawning season, decreasing as the female lost condition, and therefore having a significant effect on egg quality and on the amount of reserves available to larvae (Garrido *et al.,* 2008b*)*. This has been found to affect resistance of larvae to starvation and decrease the appropriate fatty acid content required for normal growth. Parental condition is therefore important in determining size and survival probability of larvae (Laine and Rajasilta, 1999).

From the literature reviewed, it appears reproduction and feeding activity in sardine are affected, among others, by food availability, condition of the fish, length of the fish and location. All these mentioned factors are considered to be important for studying and finding out the interaction between feeding and reproduction in the Southern Benguela sardine.

1.4. Objectives of this study

This study aims to investigate the feeding activity of *Sardinops sagax* and to link it to reproduction. Season of sampling and location will be taken into consideration as they have effects on feeding and reproduction through differences in primary and secondary productivity.

The differences between areas and sexes for gonad maturity, fish condition and feeding intensity across different caudal lengths will be investigated. To determine seasonal trends, differences between weeks for gonad maturity, GSI, fat stage, feeding intensity and stomach content weight for *Sardinops sagax* in the Southern Benguela will be investigated. To determine if *Sardinops sagax* in the Southern Benguela is a capital or income breeder, feeding intensity at different fat stages and gonad maturity will be studied. Lastly a General Linear Model will be computed to determine variables which had an effect on feeding intensity.

Chapter 2

Feeding habits of sardine (Sardinops sagax) *in the Southern Benguela in relation to their spawning activities*

2.1. Introduction

Small pelagic fish dominate in the Southern Benguela ecosystem, with the main species including sardine *Sardinops sagax* and anchovy *Engraulis encrasicolus*, as is the case in most of the world's upwelling ecosystems (van der Lingen *et al.*, 2002). These species have supported a substantial pelagic fishery over the past 50 years in this region and similar species have long supported large fisheries in other systems around the world (Schwartzlose *et al.*, 1999). Sardine and anchovy are the primary targets of purse-seine vessels in the established pelagic fisheries in the Southern Benguela (van der Lingen and Durholtz, 2005). Sardine are canned or frozen for human consumption or as pet food, used for bait, or reduced to fish meal and oil. Sardine are not only commercially important, but are an important component of the pelagic food web, being the major prey item for several species of fish, seabirds and marine mammals (van der Lingen, 2002). They play a significant role in energy flow to higher trophic levels because of their low position on the food chain (Cushing, 1978; James, 1988).

Sardine are distributed along the entire South African coastline (Fig. 1) from ~29°S on the west coast to ~27°S on the east coast (Beckley and van der Lingen, 1999). A consistent separation is evident between sardine found west and east of Cape Agulhas, with overlap in the area between the two parts of the population (Cape Agulhas to Mossel Bay) only at very high levels of biomass (Coetzee *et al.*, 2008).

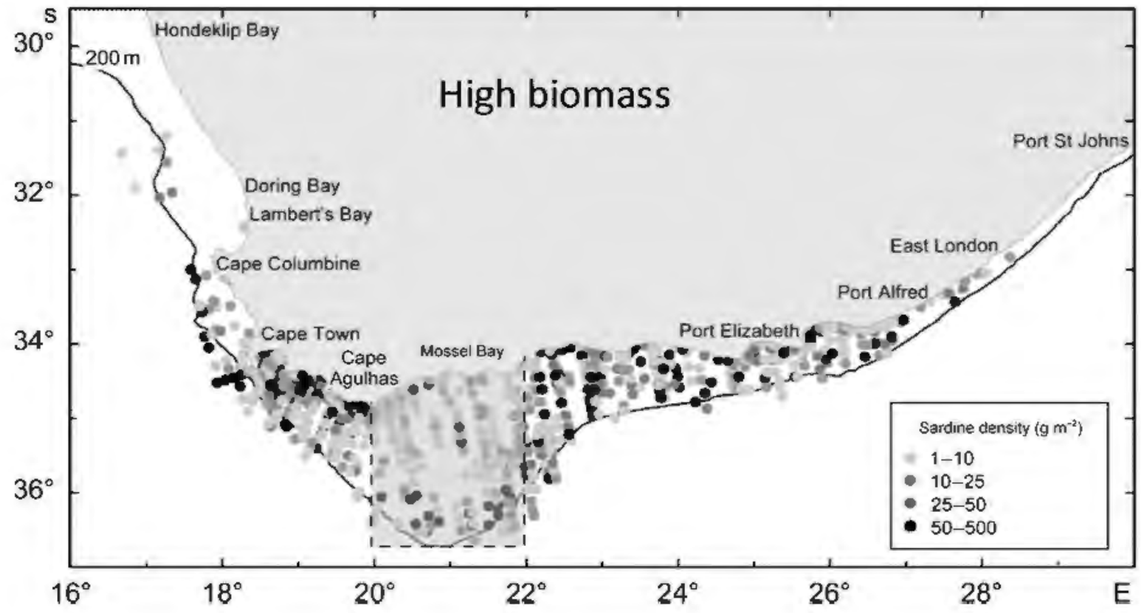


Figure 1. Composite density map showing the distribution of sardine along the South African coastline during periods of high biomass. The grey block indicates the transition zone between the west coast system and the Agulhas Bank system. (Coetzee *et al.*, 2008).

Although food resources are generally favourable in the Southern Benguela, the circulation makes the upwelling system a hazardous environment for the fish in terms of losses of eggs and juvenile stages. Offshore transport or starvation can occur when larvae are being transported by currents from the spawning area towards the nursery areas (Fig. 2, Anderson and Lucas, 2008). These factors result in a complex network of spawning grounds, transport pathways, migration patterns and nursery grounds for sardine in the Southern Benguela system (Fig. 2). The inshore area of the west coast is the principal sardine nursery area (Hutchings *et al.*, 2002), although it has been suggested that the south coast may be an important sardine nursery ground on occasion (Miller *et al.,* 2006).

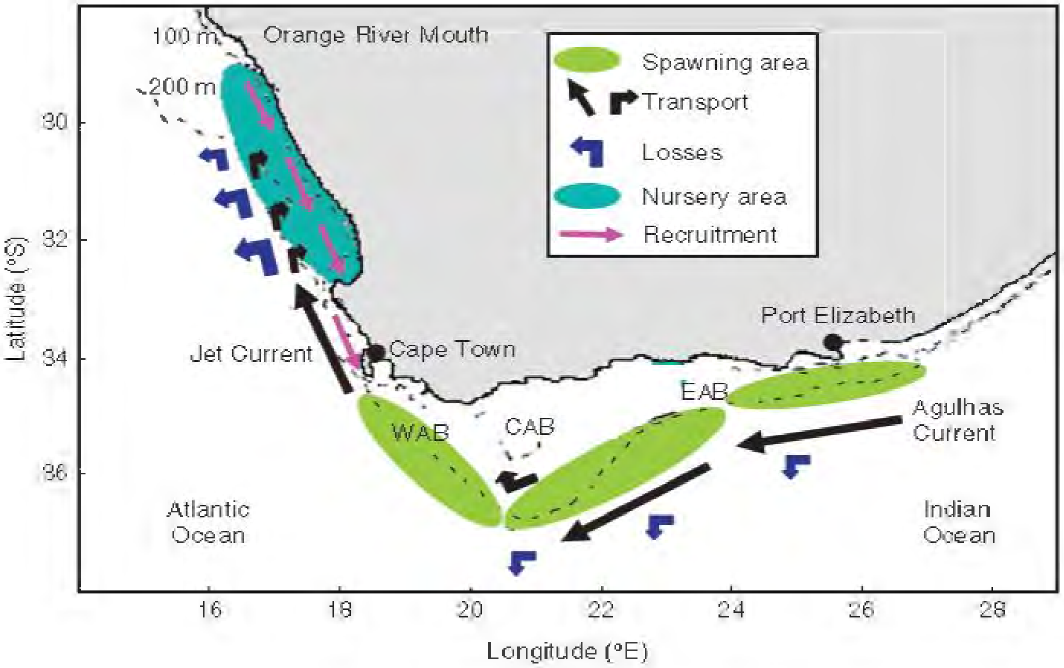


Figure 2. Map of the Southern Benguela off South Africa showing the locations of small pelagic fish spawning and nursery grounds and transport and loss processes that impact on eggs and larvae. WAB, CAB, and EAB indicate the Western, Central, and Eastern Agulhas Banks, respectively. Redrawn from Lehodey *et al* (2006) by Anderson and Lucas (2008).

Sardine spawning has been found to show substantial inter-annual variability, with both the west coast and the south coast being important spawning grounds during different periods (van der Lingen *et al*., 2001). Sardine in the Southern Benguela have a long spawning season, peaking in October and March, with spawning taking place mostly on the Western Agulhas Bank (Fig. 2) (Anderson and Lucas, 2008). From 2001 to 2005, virtually no sardine spawning was observed off the west coast, the majority of eggs collected during spawner biomass surveys being found over the shelf edge of the Central and Eastern Agulhas Banks between Cape Agulhas and Port Elizabeth (van der Lingen *et al*., 2005).

Sardine exhibit indeterminate fecundity, in which potential annual fecundity is not fixed before the onset of spawning and unyolked oocytes continue to mature and be spawned during the spawning season (Hunter *et al*., 1985). Since indeterminate spawners have an extended spawning period, they need high, continuous amounts of energy because yolk production is costly (Ganias, 2009). Gonad maturation in clupeids is believed to depend on stored energy, with fish condition and lipid reserves peaking before the spawning season and decreasing after (Blaxter and Hunter, 1982). Seasonal variations in lipid reserves have been observed for sardine in the Southern Benguela (De Goede, 2004). It is likely that seasonal variation in the feeding environment is related to the reproductive cycle. The dynamics of feeding and spawning activities probably involve biological and ecological trade-offs related to environmental conditions.

Energy storage is an important component of life-history variation in fish and other organisms. There is a difference between species that support reproduction using energy gained concurrently (income breeders) and those that support offspring using energy stores accumulated at an earlier time (capital breeders) (Houston *et al*., 2007). Sardine *Sardinops sagax* in the Southern Benguela could be using a capital breeding strategy because of observed seasonal cycles in lipid reserves and fish condition (van der Lingen and Hutchings, 2005). The lipid reserves alternate in time with the gonadosomatic index (GSI), peaking before the spawning season and being depleted during spawning (van der Lingen and Hutchings, 2005). The observed seasonal decrease in lipid reserves for Southern Benguela sardine can also be attributed to tradeoffs between spawning and feeding, environmental variability, or spawning migrations. The apparent dependence of gonad maturation on stored lipid reserves has been observed in other clupeids (Blaxter and Hunter, 1982; García-Franco *et al*., 1999; Millan, 1999) as well as the sardine in the Southern Benguela.

The aim of this chapter is to describe the feeding habits of sardine in the Southern Benguela in relation to reproduction. The first hypothesis to be tested is that sardine continue feeding during spawning, therefore utilising income breeding to sustain energy lost during spawning. The second hypothesis is that feeding intensity is greatest in fish that have recently spawned, because they need to gain energy lost during spawning. This information will allow evaluation of some basic aspects of the feeding biology in relation to reproduction of this ecologically and commercially important species. The results will be useful for investigating the impact of variable feeding environments on possible feeding and spawning migrations, as well as for providing an improved understanding of sardine's reproductive trade-off strategies.

2.2. Materials and Methods

Samples of *Sardinops sagax* were collected from commercial landing spots and research surveys conducted by Offshore and High Seas Fisheries Management (Department of Agriculture, Forestry and Fisheries). Fish samples were collected between February and April 2011, and were obtained from purse-seine catches from St Helena Bay (33°34'S & 17°48'E) and Gans Bay (34°36'S & 19°15'E). Biological data were recorded for each sardine: length (total length-TL and caudal length-CL, mm), gutted weight (fish weight minus viscera, g), sex, gonad weight (g), stomach weight (g), macroscopically-determined indices of gonad maturity stage (1-10, Davies, 1956; Sinovčić, 2000) (Table 1) and fat stage (1-7) (Table 2). The fish samples were divided into three length classes (CL): small (135-158 mm), medium (159-181 mm) and large (182-206 mm).

2.2.1. Gonad maturity stages and GSI

The fish were gutted and sexed based on visual inspection. The gonads were removed and weighed to the nearest 0.1 g. Fish gonad maturity stage was determined following descriptions provided by Davies (1956) (Table 1). The fish samples were divided into three groups according to gonad maturity stage: before spawning (stages 1-3), during spawning (4-6) and post spawning (7-10).

Table 1. Gonad maturity stages for *Sardinops sagax* (from Davies 1956).

Stage	**Description**	**Approximate gonad Mass (g)**	
		Female	**Male**
1	Ovaries either immature or inactive, less than half the length of the body cavity, cylindrical but thin, pale pink in colour or transparent. Testis flat and leaf-like, pink or transparent	0.2–5.5	0.2–4.5
2	Ovaries inactive generally, but with the beginnings of enlargement taking place. Slight elongation, thickening and darker colouration; mainly translucent pinkish yellow. Testis beginning to thicken and become elongated with slight white colouration	2–7	2–6
3	Ovaries elongated and filling over half the body cavity. Colour opaque yellow with discrete pigmented ova present. Testis elongated, thickened and filling over half the body cavity	3–17	2–15
4	Ovaries elongated, distended, filling approximately two-thirds of the body cavity. Colour bright yellow, gonads vascular, ova discrete and becoming transparent at posterior end. Testis further enlarged, filling approximately two-thirds of the body cavity. Colour opaque white; milkiness apparent at posterior end	6–20	5–18
5	Ovaries at maximum size, almost filling body cavity. Colour darker yellow, no longer opaque but semi-transparent due to even dispersal of ripe ova throughout the gonad. Testis at maximum size, almost filling the body cavity. Colour opaque white; posterior half of gonad milky	10–30	9–28
6	Stage 5, except that perhaps greater enlargement has taken place and pressure on the belly causes extrusion of ova or milt through the vent. Fish in this condition almost in the act of spawning	12–35	10–30
7	Ovaries elongated, but flat due to recent evacuation of ova, very bloodshot, sometimes gelatinous. Testis elongated, flat, strap-like and very bloodshot	1–7	1–6
8	Ovaries much like those of previous stage, but obvious signs of recovery and reversion to Stage 1, i.e. further shrinkage, less bloodshot in appearance and beginning to become transparent. Testis — as for ovaries	2–7	2–6
9	Ovaries showing signs of having been recently spent, recovery to Stage 1 apparent, nevertheless, evident that reversion to Stage 1 is only temporary as signs of 'making' to Stage 3 already evident. Testis — as for ovaries	4–10	3–8
10	Ovaries still showing signs of having been recently spent, but evidence that gonads are about to become active almost immediately by 'making' to Stage 3. Testis — as for ovaries	2–5	2–4

The gonadosomatic index (GSI) is expressed as a percentage of wet body mass, and was calculated using the equation:

$$\text{Gonadosomatic index} = \frac{\text{Gonad Mass}}{\text{Wet Body Mass} - \text{Gonad Mass}} \text{ x } 100\% \qquad (1)$$

2.2.2. Fat stages

A technique developed by van der Lingen and Hutchings (2005) was used in this study for estimating lipid content of sardine by visual assessment of their mesenteric fat. Lipids in sardine have been associated with the stomach and appear as fat lines that extend from the posterior end of the fundulus towards the anterior end of the cardiac stomach and occasionally up to the oesophagus. The size and appearance of these fat lines were used to assign fish to low to high fat stages. Seven fat stages for sardine were used, based on the size and appearance of fat lines on the fundulus (Table 2). These fat stages were grouped for further analyses: 1-2, 3-4 and 5-7.

Table 2. Description of fat stages for sardine (van der Lingen and Hutchings, 2005).

Fat Stage	**Description**
1	Fat lines invisible or thin and distinct
2	Depth greater than width of one or more fat lines
3	Pyloric fat line noticeably thicker than the other lines, and about one third the thickness of the pyloric junction
4	Depth greater than width for all fat lines but no fat lobes present
5	All fat lines slightly lobed, but no overlap between lobes
6	Fat line lobes obvious and show some overlap
7	Fat line lobes large, lots of overlap, and fundulus well covered with fat

2.2.3. Feeding Intensity

The stomach of each fish was removed and weighed individually. The stomach was then cut open, the contents removed and the empty stomach weighed again. Feeding intensity (FI) of the fish was calculated using the following expression (Garrido *et al.*, 2008a):

$$\text{Feeding Intensity} = \frac{\text{Stomach Content Mass}}{(\text{Total Body Mass} - \text{Gonad Mass})} \text{ x } 100\% \qquad (2)$$

The data on feeding intensity did not meet the assumptions of parametric statistical tests. Inspection of the data indicated that the standard deviation varied proportionally to the mean, so an arcsine transformation was performed to stabilize the variance.

2.2.4. Relative weight index

Relative weight was used as an index for calculating fish condition (Ndjaula *et al.*, unpublished). In this case, the ratio of the established modelled-length-based standard weight (Ws) and observed weight (Wo) at length of that individual fish was used to give a ratio in a form of relative weight (Wr) as:

$$\text{Relative Weight (Wr)} = \frac{\text{Observed Weight (Wo)}}{\text{Standard Weight (Ws)}} \text{ x } 100\% \qquad (3)$$

This method indicates that a fish is at the expected (median) weight when its relative weight index is 100, and it is below the median expected weight if its relative weight index is below 100.

2.2.5. Statistical analyses

Non-parametric Kruskal-Wallis one-way analysis of variance was used to test differences in the frequency of gonad maturity states and fat stages among weeks and length classes, since the data came from independent ranked measures of more than two groups. Parametric one-way ANOVA was used to test differences in feeding intensity, stomach content weight and GSI among weeks, since the data came from independent normally distributed measures of more than two groups. One-way ANOVA was also used to test differences in feeding intensity among grouped fat stages and reproductive stages.

Non-parametric Mann-Whitney tests were used to test differences in the frequency of gonad maturity and fat stages between areas and sexes since the data came from two independent ranked groups. Parametric Students-t tests were used to test area and sex differences in feeding intensity and the relative weight index, since the data came from two independent equal variance groups.

The Pearson correlation coefficient was used to correlate feeding intensity with caudal length, grouped fat stages and reproductive stages. The Spearman rank correlation was used to correlate fat stage with caudal length.

The one way ANOVA test assumes that the values in each of the groups follow the normal distribution curve, with equal population variances. Post hoc comparisons of means between pairs of categories were tested using the Tukey's test for unequal sample sizes at a confidence level of 0.05. The ANOVA *F* statistic, Tukey's *q* statistic*,* degrees of freedom (*df*) and mean differences (*MD*) between groups were used to report statistics for one way ANOVA.

The Kruskal-Wallis rank test assumes that all samples come from populations having the same continuous distribution and all observations are mutually independent. Dunn's multiple post hoc comparison tests were used to test differences in rank sum between groups. Kruskal-Wallis results were reported using the *H* statistic and difference in rank sums (*D*).

The Students t-test assumes that observations from each group are normally distributed and the variances are equal in the two groups. The Mann- Whitney test makes no assumptions about the distribution. The Pearson correlation coefficient assumes that the data are normally distributed and the Spearman rank correlation uses ranked data. All statistical analyses were calculated using *GraphPad Prism 5* for Windows.

2.2.6. General Linear Model

The relationship between feeding intensity and gonad maturity and other measured variables for sardine was examined using a general linear model (GLM), a technique that combines ANOVA and regression analyses to yield a predictive equation. The GLM fitted to arcsine-transformed feeding intensity data used area, sex, week, fat stage and gonad maturity as categorical variables, and caudal length as a continuous predictor variable. The *STATISTICA* version 10 for Windows was used to generate the GLM.

The GLM was computed to assess which of the measured variables affected feeding intensity in *Sardinops sagax*. The GLM equation with feeding intensity as the dependent variable had the following form:

$$Arcsine\ (FI) = intercept + a_{area} + b_{week} + c_{sex} + d_{\mathrm{FS}} + e_{GM} + f * CL \qquad (4)$$

where intercept, *a*, *b, c, d, e* and *f* are parameters representing the effects of area, different weeks of sampling, sex, fat stage (FS), gonad maturity stage (GM) and caudal length (CL). The GLM was fitted by systematically adding and removing variables to account for correlations among explanatory variables.

2.3. Results

A total of 373 fish was analyzed for this study, 189 females and 184 males. Table 3 shows the number of fish that were sampled in St Helena Bay and Gans Bay during different weeks.

Table 3. Numbers of sardine sampled at different dates at St Helena Bay and Gans Bay, February to April 2011.

Week	St Helena Bay	Gans Bay	Total
1 (02/Feb)	42	42	124
2 (13/Feb)	39	40	39
4 (03/Mar)	40	21	84
7 (26/Mar)	23	42	42
9 (10/Apr)		84	84
Total	144	229	373

2.3.1 Differences between areas and sexes.

The results in this section present exploratory, bivariate analyses. It is recognised that there are correlations and interactions among the explanatory variables and these will be addressed by the GLM analysis.

Differences in length distributions were observed between the two locations (Fig. 3). The mean length (± SE) of sardine caught from St Helena Bay (172.0 ± 1.4) was bigger than sardine from Gans Bay (165.7 ± 0.7) ($t = 4.364$, $df = 371$, $p < 0.0001$).

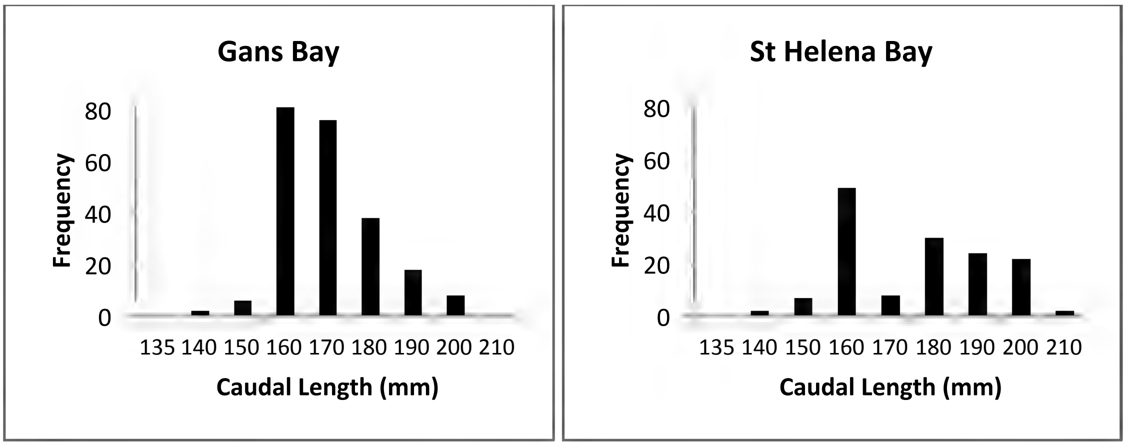


Figure 3. Length frequency distributions of sardine obtained from Gans Bay and St Helena Bay.

Gonad maturity stages (1-10) generally increased with caudal length (Spearman's $r = 0.72$, $n = 373$, $p < 0.0001$) (Fig. 4a-b). Small (135-158 mm CL) sardine had higher feeding intensity than medium (159-181 mm CL) and large (182-206 mm CL) size classes ($H = 179.2$, $df = 3$, $p < 0.05$). This resulted in the gonad maturity stages from the two locations varying significantly (Fig. 4a) ($U = 9791$, $n_1 = 229$, $n_2 = 144$, $p < 0.0001$), as sardine from Gans Bay showed lower gonad maturity stages compared to St Helena Bay sardine. Gonad maturity stages were similar for both sexes ($U = 16761$, $n_1 = 189$ $n_2 = 184$, $p = 0.47$) (Fig. 4b).

Fat stage of sardine was negatively correlated with caudal length (Spearman's $r = -0.58$, $n = 373$, $p < 0.0001$) (Fig. 4c-d). Fat stage was high in small (135-158 mm CL) sardine and low in large (182-206 mm CL) sardine ($H = 90.9$, $df = 2$, $p < 0.0001$). The Mann Whitney test showed that fat stages of sardine collected in Gans Bay were significantly higher than those in St Helena Bay ($U = 12775$, $n_1 = 229$, $n_2 = 144$, $p = 0.0003$) (Fig. 4c). Fat stages were similar for both sardine sexes ($U = 16210$, $n_1 = 189$ $n_2 = 184$, $p = 0.25$) (Fig. 4d).

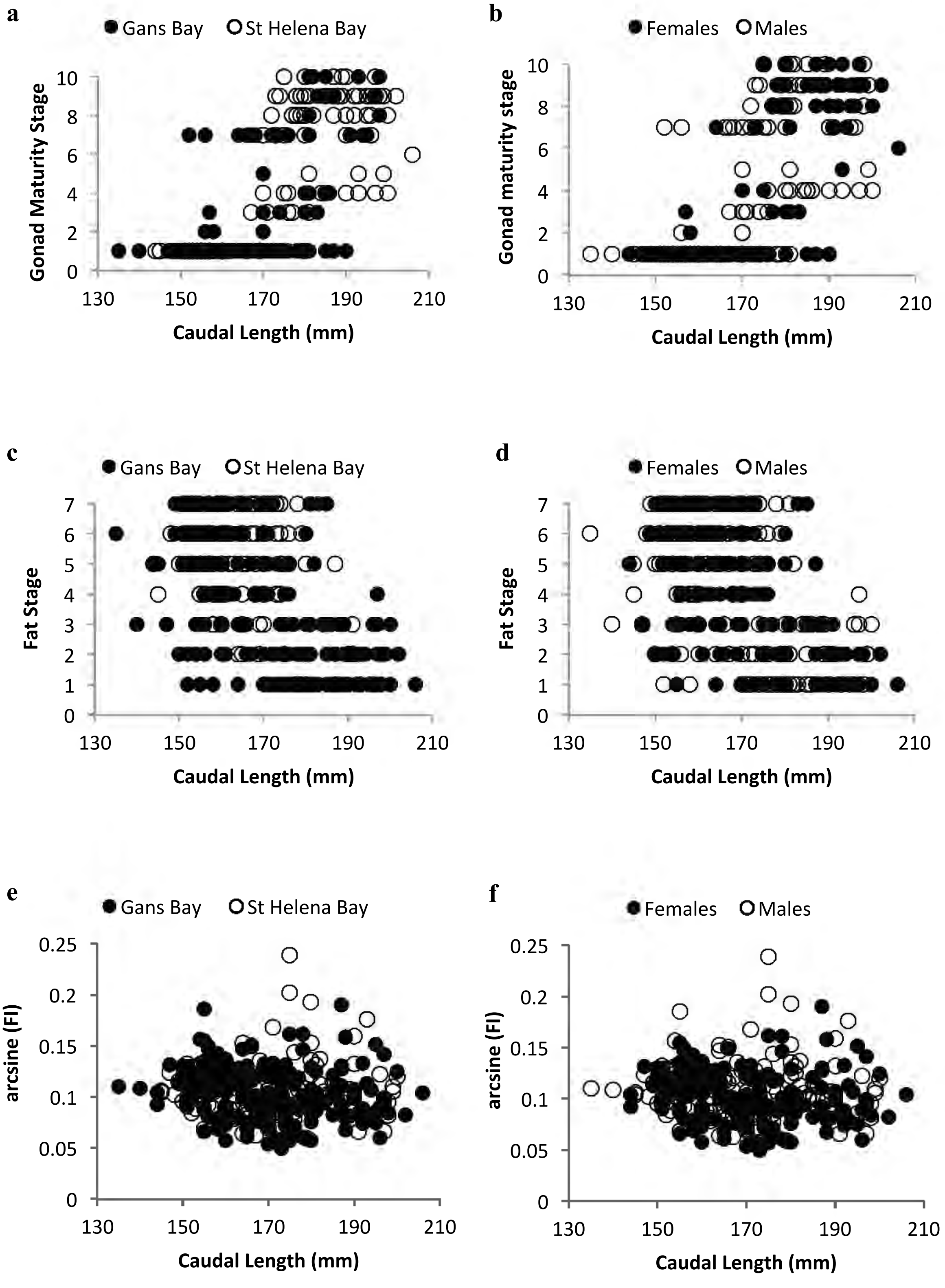


Figure 4. Gonad maturity stages, fat stages and feeding intensity of *Sardinops sagax* across different caudal lengths for location (a,c,e) of sampling and sexes (b,d,f).

Feeding intensity was negatively correlated with caudal length (Pearson's $r = -0.14$, $n = 373$, $p = 0.0053$). Small (135-158 mm CL) fish had higher feeding intensity than medium (159-181 mm CL) and large (182-206 mm CL) fish (Fig. 4e-f) ($F = 7.656$, $df = (2, 370)$, $p = 0.0006$). Mean (± SE) feeding intensity varied significantly between the two sampling locations, being higher in St Helena Bay (0.1120 ± 0.0024) than Gans Bay (0.1040 ± 0.0015) ($t = 3.023$, $df = 371$, $p = 0.0027$) (Fig. 4e). Statistical analysis using Student's-t test revealed that feeding intensity was similar for both sexes ($t = 1.567$, $df = 371$, $p = 0.12$) (Fig. 4f).

The mean (± SE) relative weight index of sardine from St Helena Bay (101.9 ± 0.2) was higher than that from Gans Bay sardine (100.4 ± 0.1) ($t = 6.745$, $df = 370$, $p < 0.0001$).

2.3.2. Differences among weeks

The results in this section present exploratory, bivariate analyses. It is recognised that there are correlations and interactions among the explanatory variables and these will be addressed by the GLM analysis.

Gonad maturity of sardine was higher during the first two weeks of sampling in February (Fig. 5a) and decreased in late March and April ($H = 157.7$, $df = 4$, $p < 0.0001$). Post hoc comparisons of gonad maturity stages indicated significant differences between weeks 1 and 2 compared to the rest of the weeks. No significant differences in gonad maturity were found for weeks 4 and 7 ($D = 31.54$, $p > 0.05$), weeks 4 and 9 ($D = 19.68$, $p > 0.05$) and weeks 7 and 9 ($D = -11.86$, $p > 0.05$).

GSI of sardine also peaked during the second week of sampling in February (Fig. 5b) and decreased in late March and April (F = 83.63, df = (4, 367), $p < 0.0001$). Post hoc comparisons of the GSI of sardine for the sampled weeks indicated significant differences between week 1 and 2 when compared to all other weeks. No significant differences in GSI were found for weeks 4 and 7 (mean difference (*MD)* = 0.5674, q = 2.304, $p > 0.05$), weeks 4 and 9 (*MD* = 0.4593, q = 2.284, $p > 0.05$) and weeks 7 and 9 (*MD* = -0.1081, q = 0.4390, $p > 0.05$).

Feeding intensity differed significantly (Fig. 5c) among the sampling weeks (F = 30.95, df = (4, 368), $p < 0.0001$). Tukey post hoc comparisons indicated no differences among feeding intensity of fish collected during weeks 1 to 7. Fish collected during week 9 had low feeding intensity when compared to week 1 (*MD* = 0.023, q = 13.67, $p > 0.05$), week 2 (*MD* = 0.024, q = 7.950, $p > 0.05$), week 4 (*MD* = 0.029, q = 12.21, $p > 0.05$) and week 7 (*MD*= 0.035, q = 11.87, $p > 0.05$).

Differences (Fig. 5d) in the stomach content weight were found for the different sampling weeks (F = 26.71, df = (4, 368) $p < 0.0001$). Post hoc Tukey's showed significant differences in stomach content weight for sardine between week 1 and 9. No significant differences in stomach content weight were found between weeks 1 and 7 (*MD* = 0.142, q = 2.472, $p > 0.05$) and weeks 4 and 7 (*MD* = -0.052, q = 0.864, $p > 0.05$).

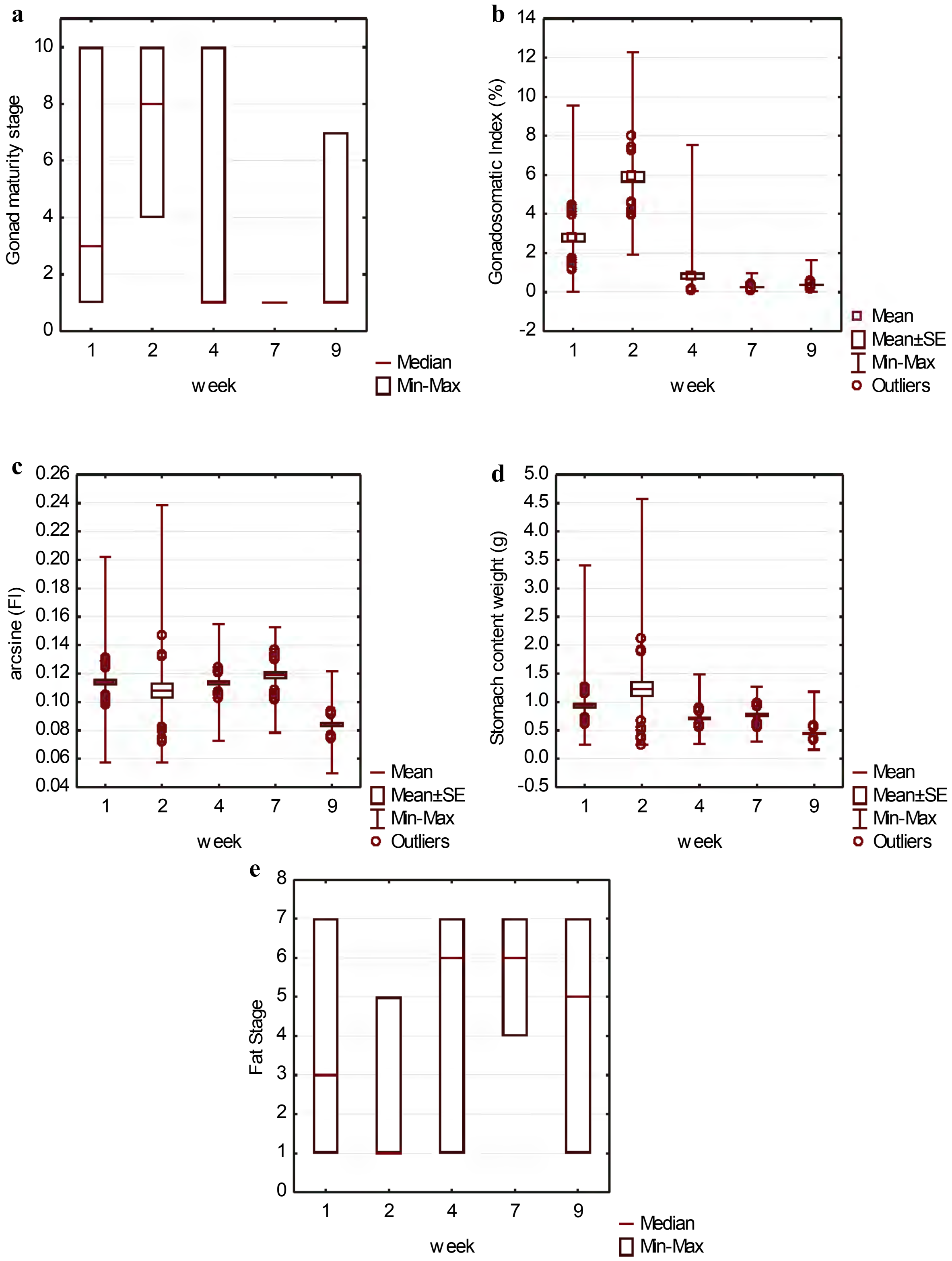


Figure 5. Differences among weeks of (a) gonad maturity stages, (b) gonadosomatic index, (c) feeding intensity, (d) stomach content weights and (e) fat stages of sardine. The dates corresponding to the weeks are given in Table 3.

Mean sardine fat stages were low in the first two weeks of sampling and high during the last three sampling weeks ($H = 131.6$, $df = 4$, $p < 0.0001$) (Fig. 5e). Multiple comparisons of sardine fat stages among weeks indicated that weeks 1 and 2 were significantly different from other weeks. No significant differences in fat stage were found between weeks 4 and 7 ($D = -12.01$, $p > 0.05$), weeks 4 and 9 ($D = 9.31$, $p > 0.05$), and weeks 7 and 9 ($D = 21.32$, $p > 0.05$).

2.3.3. Feeding intensity of *Sardinops sagax* at different fat stages and gonad maturity.

The results in this section present exploratory, bivariate analyses. It is recognised that there are correlations and interactions among the explanatory variables and these will be addressed by the GLM analysis.

No correlation was found between caudal length and feeding intensity for grouped fat stages 1 to 2 ($r = -0.060$, $n = 104$, $p = 0.55$) and grouped fat stages 3 to 4 ($r = -0.18$, $n = 68$, $p = 0.14$) (Fig. 6a-b). Feeding intensity was negatively correlated with caudal length for grouped fat stages 5 to 7 ($r = -0.28$, $n = 201$, $p < 0.0001$) (Fig. 6c). There were no differences in the mean feeding intensity of sardine between the three different grouped fat stages ($F = 0.2868$, $df = (2, 370)$, $p = 0.75$).

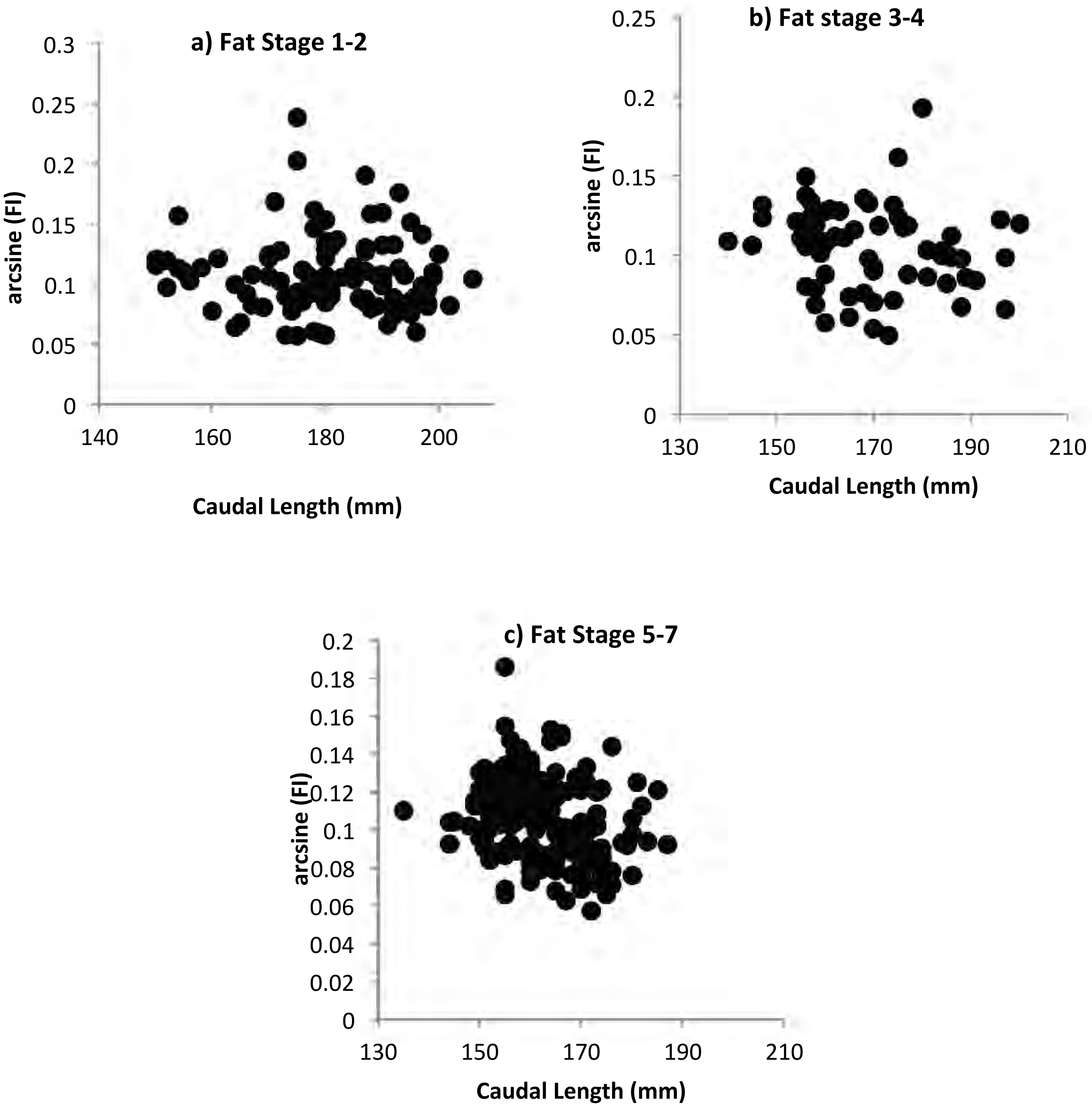
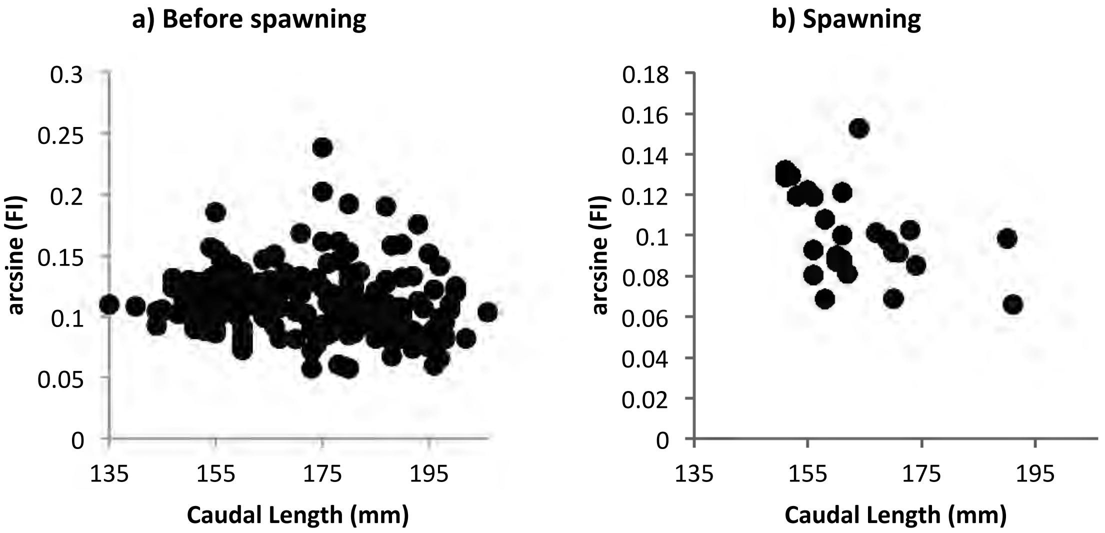


Figure 6. Feeding intensity of sardine across different caudal lengths for fat stage 1-7 (a-c).

Feeding intensity was negatively correlated with caudal length for sardine before spawning ($r = -0.29$, $n = 265$, $p < 0.0001$) (Fig. 7a). No significant correlation between feeding intensity and caudal length was found for sardine during spawning ($r = 0.10$, $n = 19$, $p = 0.67$) and post spawning ($r = -0.15$, $n = 89$, $p = 0.16$) (Fig. 7b-c). Statistical analysis indicated no differences in the mean feeding intensity of sardine for gonad maturation stages before, during and after spawning ($F = 1.275$, $df = (2, 370)$, $p = 0.28$).



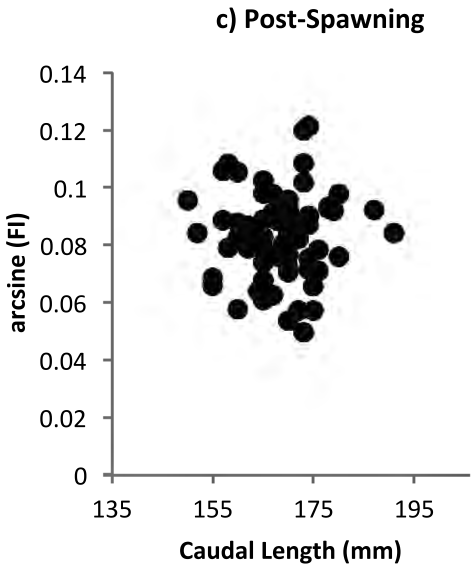


Figure 7. Feeding intensity of sardine across different caudal lengths before spawning, during spawning and post-spawning

2.3.4. General Linear Model

The period of sampling (week) (F = 28.13, df = (4, 350) $p < 0.0001$) and gonad maturity (F = 3.33, df = (9, 350), p = 0.0006) were the only two variables that had a significant effect on feeding intensity (Table 4).

Table 4. Results of the GLM showing the effects of each of the explanatory variables on feeding intensity

Effect	SS	Degr. of Freedom	MS	F	P
Intercept	0.005728	1	0.005728	13.01650	0.000354
Area	0.000521	1	0.00521	1.18288	0.277519
Sex	0.001020	1	0.001020	2.31782	0.128803
Week	0.049507	4	0.012377	28.12610	0.000000
Fat stage	0.002118	6	0.000353	0.80210	0.568789
Gonad Maturity	0.013198	9	0.001466	3.33257	0.000633
Caudal Length	0.000046	1	0.000046	0.10442	0.746779
Error	0.154015	350	0.000440		

The GLM output showed that in sardine with an average caudal length of 168 mm the mean feeding intensity was high before spawning (stage 1 to 3) and recently after spawning (stage 7, 8 and 10) (Fig. 8). Feeding intensity decreased slightly during spawning (stage 4 to 6) and late recovery stages (stage 9).

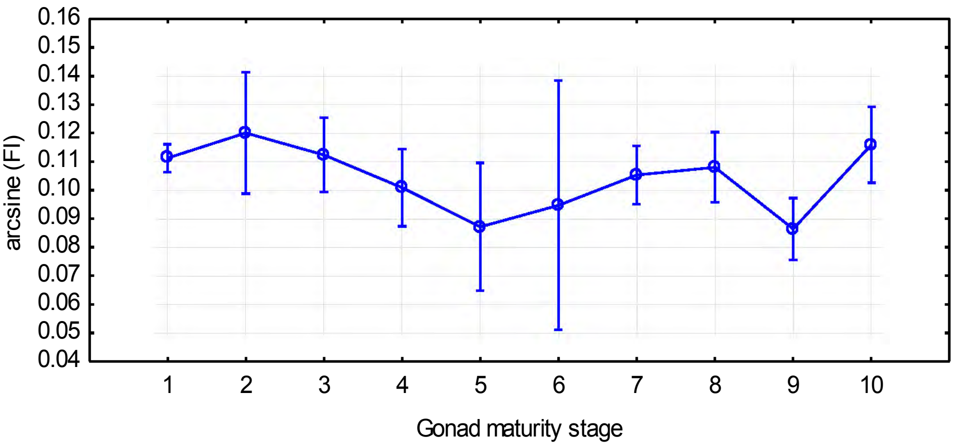


Figure 8. Least square means of sardine (caudal length 168 mm) feeding intensity at various gonad maturity stages. Bars denote 0.95 confidence intervals.

The GLM output showed that in sardine with an average caudal length of 168 mm the mean feeding intensity was high during weeks 1 to 7 and decreased during week 9 (Fig. 9).

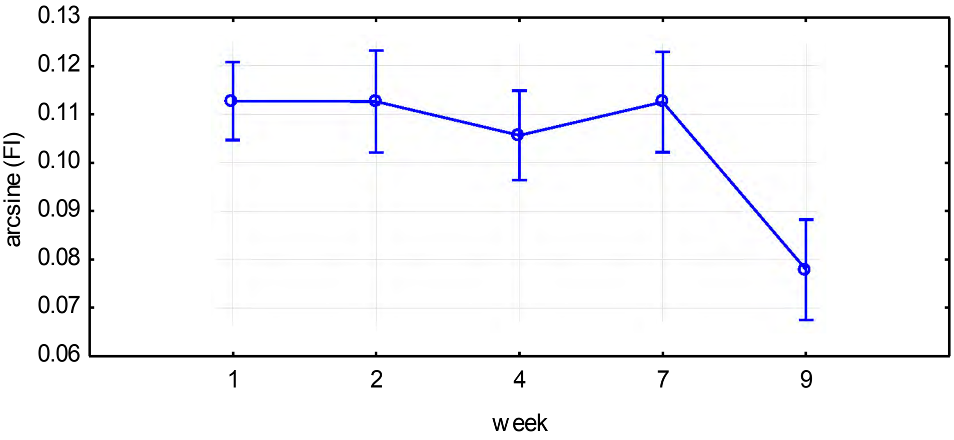


Figure 9. Least square means of sardine (caudal length 168 mm) for the period of sampling. Bars denote 0.95 confidence intervals.

2.4. Discussion

The present study investigated the feeding activity of *Sardinops sagax* in relation to reproductive state and fish condition. Selected variables which influence feeding and reproduction in sardine were measured to investigate possible trade-offs. Feeding intensity in sardine depends on the time of year and gonad maturity, being highest pre- and post-spawning. Both hypotheses formulated for this study were accepted; sardine continue feeding during spawning and increase their feeding intensity directly after spawning.

Since the data were collected from two areas, it was important to explore the possible spatial differences between Gans Bay and St Helena Bay. Fish from St Helena Bay were bigger than those from Gans Bay. The observed differences in gonad maturity stage, fat stage, relative weight index and feeding intensity were probably a result of the difference in size observed between the two locations, food availability and diet (Akkers *et al.*, 1996; Morimoto, 1996; van der Lingen, 1998b; van der Lingen, 2002; van der Lingen and Hutchings, 2005). Small fish of less than 158 mm (CL) had low gonad maturity stages because of sexual immaturity (Fig. 3a-b). Sexual maturity in *Sardinops sagax* has been found to start at 160 mm (CL), with fish reaching full maturity at 170 -175 mm (Akkers *et al.*, 1996).

The west coast of South Africa is known to be highly productive and regarded as a suitable location for spawning and recruitment of pelagic fish (Beckley and van der Lingen, 1999; Miller *et al.,* 2006). High productivity in St Helena Bay suggests that there are more food resources available for sardine to feed on and store as fat reserves for supporting gonad maturation (Beckley and van der Lingen, 1999). Morimoto (1996) found that gonad maturity in Japanese sardine is affected by food intake prior to the spawning season. De Goede (2004) hypothesised that sardine on the west coast appear to be more efficient than those on the east coast in translating lipid reserves into gonad maturity, hence the high GSI values in St Helena Bay. The low GSI values of sardine on the east coast were attributed to higher metabolic rates of sardine in warmer sea temperatures towards the east coast (van der Lingen, 1995).

The observed high fish condition in Gans Bay can also be attributed to size as fat stage was negatively correlated with caudal length. Van der Lingen and Hutchings (2005) recorded an increase of anchovy condition in an eastward direction over the Agulhas Bank. This was attributed to diet, since the Eastern Agulhas Bank has higher copepod biomass than the west coast (van der Lingen, 2002). Sardine on the east coast consume more zooplankton than phytoplankton because of the plankton community structure (van der Lingen, 1998a). The high gonad maturity stages in St Helena Bay indicate that fat reserves of the fish were converted during reproduction, explaining the low fat stages.

The observed high feeding intensity in immature sardine may indicate that small (135-158 mm CL) sardine feed more than large (182-206 mm CL) fish, perhaps to accumulate fat reserves for growth and other metabolic activities. Previous studies of sardine biology have shown that small sardine consume more plankton daily compared to large sardine (van der Lingen, 1998b), partially explaining the high feeding intensity in small sardine. Different results were found in *Sardina pilchardus* off the coast of Portugal, with large sardine having a higher feeding intensity than small fish (Garrido *et al.*, 2008a). The observed differences in feeding intensity between the two locations were possibly caused by food availability (De Goede, 2004). St Helena Bay is located in an area with high upwelling intensity in the Southern Benguela. This suggests that sardine from St Helena Bay had more food resources available and fed more, resulting in higher feeding intensity. Feeding intensity of *Sardina* *pilchardus* has also been found to be high in areas where upwelling events are stronger and recurrent during summer and spring (Garrido *et al.*, 2008a).

The data were also explored to investigate sex differences in gonad maturity, fat stage and feeding intensity. No sex effect was found in the variables, suggesting that gonad maturity, fish condition and feeding intensity occur at the same rate for both sardine sexes. The results for feeding intensity are similar for *Sardina pilchardus* off the coast of Portugal (Garrido *et al.*, 2008a). With the observed similarities, sex cannot be used to explain differences in feeding intensity for sardine.

Since the data were collected during different weeks and months, it was important to assess the temporal variability as well. Sardine gonad maturity stages in this study peaked in February and early March and declined to their lowest in late March and April. Previous studies have found that the sardine spawning season peaks during February-March in the Southern Benguela (van der Lingen and Huggett, 2003), explaining the advanced gonad maturity stages recorded during that period. Kreiner *et al,* (2001) reported seasonal variability in GSI of *Sardinops sagax* in the Northern and Southern Benguela*.* The GSI values reported in Kreiner *et al*, (2001) also peaked during January and March and declined from April to June. De Goede (2004) found that Chl-a monthly averages along the South African coast were high during summer months (January to March), indicating that sardine match their spawning to periods of high productivity. These results also suggest that income breeding may be utilised by *Sardinops sagax* in the Southern Benguela as a breeding strategy. *Sardina pilchardus* in the Eastern Mediterranean Sea were also found to intensify egg production under periods of high productivity (Ganias, 2009). The results were interpreted as an indication of direct energy flow from phytoplankton to gonadal development. Other studies have also found that sardine and other small pelagic fish in the Southern Benguela match their spawning season to favourable environmental conditions to maximise larval survival and enhance recruitment (Anderson and Lucas, 2008; Davies, 1954; Kreiner *et al.*, 2001; Mullon *et al.*, 2002). The favourable environmental conditions include current conditions for larval transport and retention, and availability of food resources to avoid starvation and ensure survival of offspring.

The declining fat stages during the spawning period and peaking after indicated a transfer of fat reserves to reproduction, as already found in previous studies (van der Lingen and Hutchings, 2005; Blaxter and Hunter, 1982; García-Franco *et al.*, 1999, Millan, 1999). Fish condition in the form of relative weight index of sardine showed that the fish sampled were in a good condition. Condition of sardine peaked during February and was the highest during the second week of sampling. Sardine condition declined in March and April. The condition factor of *Sardinops sagax* was related to Chl-a and GSI (De Goede, 2004).

The sardine feeding intensity peaked in February and March, which is the peak spawning season for this species in the Southern Benguela, and declined in April. The seasonal pattern of sardine feeding intensity might be more related to Chl-a concentration as a proxy of primary production than reproduction, as found for Portugese sardine *Sardina pilchardus* (Garrido *et al.,* 2008a). Seasonal changes in wind forcing indicate maximum upwelling in summer in the Southern Benguela, resulting in maximum phytoplankton biomass, estimated as Chl-a (Hutchings *et al.*, 2006). Feeding intensity of the Iberian sardine has also been found to be more related to plankton production than reproductive stage of sardine (Garrido *et al.,* 2008a). Feeding intensity was also found to be seasonal in horse mackerel, peaking with increasing abundance in euphausiids during spring and summer when seasonal upwelling enhances primary productivity (Garrido *et al.*, 2008c). This suggests that food availability has a major influence on feeding intensity in sardine. Large (182-206 mm CL) sardine have big stomachs, which contain more food. The low food content weight recorded in April correlates with the low feeding intensity recorded during that period. The low stomach food content results also correlate to low Chl-a concentrations in the southern Benguela in April (De Goede, 2004).

Feeding intensity showed a negative correlation with caudal length in fat stages 5-7, suggesting that large (182-206 mm CL) sardine with high fat reserves fed less. A number of sardine exhibited both high feeding intensity and low fat stages but this relationship was not statistically significant. The observed low feeding intensity in fat stages 5-7 was attributed to fish size. This was confirmed by the lack of significant differences in the mean feeding intensity of sardine across the three grouped fat stages.

Feeding intensity was negatively correlated with caudal length before spawning, suggesting that small (135-158 mm CL), immature sardine feed more than large (182-206 mm CL), sexually mature fish. Lack of correlation between feeding intensity and caudal length in spawning and post spawning sardine suggested that there are no trade-offs between feeding and reproduction. However the results were not satisfactory because of the size effect on fat stage and reproduction. There were only few data points in spawning fish and future work could investigate more on these observations.

A GLM was used to identify main variables influencing feeding intensity. The results of the GLM revealed trade-offs between feeding intensity and gonad maturity. Sardine feed during spawning but their feeding intensity is lower compared to pre- and post- spawning.

The GLM output also showed a seasonal trend in feeding intensity. The results correlate to Chl-a concentrations in the Southern Benguela which peak in summer and decline in autumn (De Goede, 2004). The results suggest that sardine utilise the income breeding strategy by matching reproduction to periods of high food availability

2.5. Conclusions

Feeding intensity is high in small sardine of caudal length less than 158 mm. This suggests that small (135-158 mm CL) sardine feed more to accumulate fat for growth and other metabolic activities. Feeding intensity is similar for both sexes in *Sardinops sagax*. Sardine feeding intensity varies, peaking during February and declining in April. Feeding intensity in sardine is dependent on time of year and gonad maturity. The matching of spawning to high productivity suggests that, in addition to capital breeding, sardine utilise the income breeding strategy.

Chapter 3

Critique, recommendations and future work

3.1. Critique and recommendations

The period of sampling in the present study was short since the AMS masters require a mini-dissertation to complete the course. Sampling for the whole year is needed to follow the trend of feeding intensity and reproduction in the Southern Benguela which will enable drawing of solid conclusions.

Macroscopic evaluation of gonad maturity stages as compared to GSI is a good representative of reproduction to determine which fish are spawning and which are not, but the method is not accurate as there are some difficulties in detecting differences between gonad stages before spawning and post spawning.

Many variables were considered in this study, some of which were correlated with one another whereas others interacted. This complicated the GLM analysis. The relative weight index proved to be an effective indicator of condition in sardine but it was negatively influenced by gonad maturity. For that reason fat stage was used as the indicator of fish condition in the GLM.

Gonad maturity and GSI peaked during February but fewer fish were sampled that were in the process of spawning. More sampling needs to be done on sardine while they are actively spawning to strengthen the conclusions drawn in this study. The effects of size on gonad maturity and feeding intensity need to be eliminated to correctly determine tradeoffs between feeding and reproduction in sardine. The GLM used in this study was found to be a good tool for doing that, but more data are required.

3.2. Future work

Study of the relationship between feeding intensity and reproduction of sardine in the Southern Benguela needs to be conducted throughout the year. This information can be matched with Chl-a concentrations and zooplankton biomass to understand sardine migrations. The Chl-a concentrations and zooplankton biomass can further be linked to egg and larval distributions to further understand how sardine strategise and match reproduction with productivity to avoid starvation.

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