

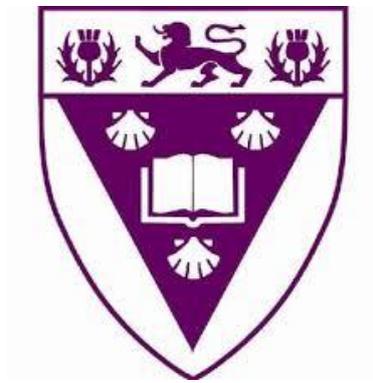
**AN ASSESSMENT OF TEMPORAL CHANGES IN SELECTIVE BIOLOGICAL
CHARACTERISTICS OF CHOKKA SQUID (*LOLIGO REYNAUDII* D'ORBIGNY, 1845)**

BY

MPHO AUDREY MMETHI

ORCID ID: 0000-0003-1769-0418

A thesis presented in the fulfilment of requirements for Master of Science degree in
Ichthyology and Fisheries Sciences in the Faculty of Science at Rhodes University



NOVEMBER 2019

TABLE OF CONTENTS

ABSTRACT	i
ACKNOWLEDGEMENTS	ii
CHAPTER 1	1
1. INTRODUCTION	1
CHAPTER 2	8
2. GENERAL BACKGROUND: LITERATURE REVIEW	8
2.1. THE CLASS CEPHALOPODA: GENERAL CHARACTERISTICS	8
2.2. CEPHALOPOD FISHERIES	10
2.3. CEPHALOPODS IN DIFFERENT REGIONS OF THE WORLD	13
2.3.1. Loliginid squid	13
2.3.2. Ommastrephid squid	13
2.3.3. Octopus	14
2.3.4. Cuttlefish	14
2.4. FISHERIES FOR SQUIDS	14
2.5. <i>LOLIGO REYNAUDII</i> (CHOKKA SQUID)	15
2.5.1. Life history	15
2.5.2. Distribution and habitat	17
2.5.3. Effects of environmental factors	18
2.5.4. Fishery and economic importance of chokka squid	20
2.5.5. Stock assessment and management	21
CHAPTER 3	23
3. MATERIALS AND METHODS	23
3.1. SQUID COLLECTION	23
3.2. SEA TEMPERATURE DATA COLLECTION	23
3.3. LENGTH FREQUENCY ANALYSIS	29
3.4. LENGTH-WEIGHT RELATIONSHIP ANALYSIS	30
CHAPTER 4	32
4. RESULTS	32
4.1. EXPLORATION OF LENGTH FREQUENCY DATA OF <i>LOLIGO REYNAUDII</i> COLLECTED BETWEEN PLETTENBERG BAY AND PORT ALFRED.	32
4.2. EXPLORATION OF LENGTH-WEIGHT DATA OF <i>LOLIGO REYNAUDII</i> OFF THE SOUTH COAST OF SOUTH AFRICA, BETWEEN PLETTENBERG BAY AND PORT ALFRED.	37

CHAPTER 5	49
DISCUSSION	49
REFERENCES.....	54
APPENDIX 1.....	62
EXPLORATION OF LENGTH FREQUENCY DATA.....	62
APPENDIX 2.....	80
EXPLORATION OF SEA TEMPERATURE RECORDED AT 9, 14, 18 AND 21 M DEPTH IN THE AREAS BETWEEN PLETTENBERG BAY AND PORT ALFRED.	80
APPENDIX 3.....	84
EXPLORATION OF LENGTH-WEIGHT DATA OF <i>LOLIGO REYNAUDII</i> OFF THE EAST COAST OF SOUTH AFRICA, SPECIFICALLY BETWEEN PLETTENBERG BAY AND PORT ALFRED.	84
ADDITIONAL FIGURES TO EXPLORE LENGTH FREQUENCY AND LENGTH-WEIGHT DATA OF <i>LOLIGO REYNAUDII</i> OFF THE EAST COAST OF SOUTH AFRICA, SPECIFICALLY BETWEEN PLETTENBERG BAY AND PORT ALFRED.	85

TABLE OF FIGURES

Figure 1: Distribution of the stations where samples of <i>L. reynaudii</i> were collected between Plettenberg Bay and Port Alfred for different years (1994–2017), during spring season (between October and December).	25
Figure 2: Graph showing the pattern of length frequency distribution of <i>L. reynaudii</i> from 1996 to 2017.	33
Figure 3: Summary of the temporal trend in mean length (95% confidence interval) for both male and female <i>L. reynaudii</i> from 1996 to 2017.	34
Figure 4: Plot of the mean length piecewise linear regression to show the trend in mean length of male and female <i>L. reynaudii</i> before and after 2013.	36
Figure 5: Association between overall catch from jig fishery and median length of males and females <i>L. reynaudii</i>	36
Figure 6: The length-weight relationship for both male and female <i>L. reynaudii</i> , over 10 years between 1994 and 2016 (caecum colour is also indicated).	38
Figure 7: The distribution of different caecum colours of <i>L. reynaudii</i> over time by sex and year.....	39
Figure 8: Overall pattern in distribution of caecum colour for male and female <i>L. reynaudii</i> over the years.....	40
Figure 9: The predicted length-weight relationship with the corresponding confidence interval based on parameter estimate from bootstrapping.	41
Figure 10: Distribution of the estimated parameters: ‘a’ and ‘b’ of $W = aL^b$ from bootstrap for both sexes and all the years for which data exist	42

Figure 11: Estimated parameters of length-weight relationship of <i>L. reynaudii</i> with the corresponding confidence interval calculated, based on estimated standard error.	43
Figure 12: Bootstrap-based estimated parameters for length-weight relationship of <i>L. reynaudii</i> and their corresponding confidence interval.	44
Figure 13: Standard model diagnostic for plots of fitted values vs residuals; a) – males, b) – females.	45
Figure 14: Model diagnostic showing the plot of residuals vs observed value; a) – males, b) – females.....	46
Figure 15: Exploratory plot of relationship between shape parameter of Length-Weight relationship and catch of <i>L. reynaudii</i> between Plettenberg Bay and Port Alfred.	47
Figure 16: Exploratory relationship between intercept of the Length-Weight model and catch of <i>L. reynaudii</i>	48
Figure 17: Histograms showing the pattern of length frequency distribution and mean length at 95% confidence interval of <i>L. reynaudii</i> , from 1996 to 2017.	85
Figure 18: Length-weight relationship of male and female <i>L. reynaudii</i> and model prediction of the data.	86

Table 1: Details of length frequency data collection of <i>Loligo reynaudii</i> in the areas between Plettenberg Bay and Port Alfred from 1996 to 2017.	26
Table 2: Details of length and weight relationship data collection of <i>Loligo reynaudii</i> in the area between Plettenberg Bay and Port Alfred.....	28
Table 3: Dorsal mantle length summary statistics (minimum, maximum, mean and median values) for <i>Loligo reynaudii</i> by sex, at the south-eastern coast of South Africa, in areas between Plettenberg Bay and Port Alfred at the depth of up to 50 m.	62
Table 4: Linear regression assessing the temporal trends in mean length of male and female <i>L. reynaudii</i> between the years for which data were available (1996 and 2017).	65
Table 5: Result of the test of piecewise regression for the data before and after 2013 of males and females <i>L. reynaudii</i> in the areas between Plattenberg bay and Port Alfred.....	66
Table 6: Result of linear regression fit for jig catch versus mean length of males and females <i>L. reynaudii</i> in the areas between Plattenberg bay and Port Alfred	66
Table 7: Result of ANOVA to analyse the variation of females <i>L. reynaudii</i> length frequency between the years 2014, 2015 and 2016 within Plettenberg Bay and Port Alfred.	67
Table 8: Result of ANOVA to analyse the variation of females <i>L. reynaudii</i> length frequency between 2014 and 2015 within Plettenberg Bay and Port Alfred.	68
Table 9: Result of ANOVA to analyse the variation of females <i>L. reynaudii</i> length frequency between the years 2015 and 2016 within Plettenberg Bay and Port Alfred.	68
Table 10: Result of ANOVA to analyse the variation of females <i>L. reynaudii</i> length frequency between the years 2014 and 2016 within Plettenberg Bay and Port Alfred.	69
Table 11: Summary statistics to show analysis of the variation of males <i>L. reynaudii</i> length frequency between the years 2014, 2015, 2016 in areas between Plettenberg Bay and Port Alfred.	69

Table 12: Result of ANOVA to analyse the variation of males <i>L. reynaudii</i> length frequency between the years 2014 and 2015 within Plettenberg Bay and Port Alfred.	70
Table 13: Result of ANOVA to analyse the variation of males <i>L. reynaudii</i> length frequency between the years 2014 and 2016 within Plettenberg Bay and Port Alfred.	70
Table 14: Result of ANOVA to analyse the variation of males <i>L. reynaudii</i> length frequency between the years 2015 and 2016 within Plettenberg Bay and Port Alfred.	71
Table 15: Summary of analysis of medians test between the years 2014, 2015, 2016 for females <i>L. reynaudii</i> length frequency.	72
Table 16: Result of median test to analyse the variation of females <i>L. reynaudii</i> length frequency between the years 2014 and 2015 within Plettenberg Bay and Port Alfred.	73
Table 17: Result of median test to analyse the variation of females <i>L. reynaudii</i> length frequency between the years 2015 and 2016 within Plettenberg Bay and Port Alfred.	74
Table 18: Result of median test to analyse the variation of females <i>L. reynaudii</i> length frequency between the years 2014 and 2016 within Plettenberg Bay and Port Alfred.	75
Table 19: Summary of analysis of medians test between the years 2014, 2015, 2016 for males <i>L. reynaudii</i> length frequency.	76
Table 20: Result of median test to analyse the variation of males <i>L. reynaudii</i> length frequency between the years 2014 and 2015 within Plettenberg Bay and Port Alfred.	77
Table 21: Result of median test to analyse the variation of male <i>L. reynaudii</i> length frequency between the years 2014 and 2016 within Plettenberg Bay and Port Alfred.	78
Table 22: Result of median test to analyse the variation of male <i>L. reynaudii</i> length frequency between the years 2015 and 2016 within Plettenberg Bay and Port Alfred.	79
Table 23: Result of Paired T-Test to analyse the sea temperature difference in the depth stratum of 9 m in the areas between Plettenberg Bay and Port Alfred for the years 2002 and 2015 (6–12 November for both years).	80
Table 24: Result of Paired T-Test to analyse the sea temperature difference in the depth stratum of 14 m in the areas between Plettenberg Bay and Port Alfred for the years 2002 and 2015 (6–12 November for both years).	81
Table 25: Result of Paired T-Test to analyse the sea temperature difference in the depth stratum of 18 m in the areas between Plettenberg Bay and Port Alfred for the years 2002 and 2015 (6–12 November for both years).	82
Table 26: Result of Paired T-Test to analyse the sea temperature difference in the depth stratum of 21 m in the areas between Plettenberg Bay and Port Alfred for the years 2002 and 2015 (6–12 November for both years).	83
Table 27: Result of GLM to analyse the interaction of sex and caecum colour over the selected years.	84

ABSTRACT

The biological characteristics of chokka squid, *Loligo reynaudii*, sampled off the south coast of South Africa, were compared between 1994 and 2017 to ascertain any temporal changes using analysis of length frequency (LF) data over 15 years (1996–2017), and mantle length (ML) and total weight (TW) relationships over 9 years (1994–2016). To investigate feeding, two caecum colours were chosen for analysis (white: no food ingested, and yellow: six to seven hours after food ingestion). Other factors were kept constant throughout: similar sampling period of 60 days in spring-summer, similar depths and areas sampled, and identical maturity stage. Anecdotal evidence from fishers suggested that the length frequency and ML_TW relationship of chokka have changed over years, with possibilities to be linked to fishing activities and environmental conditions. Also, there is evidence in the published literature that both fisheries and environment may influence length distributions in populations of fish and cephalopods over time. The results from this study showed no significant differences between length frequencies over the time series. However, a significant decrease in length frequencies between 2014 and 2016 was noted when paired data was analysed by Anova (2014:2015, 2014:2016 and 2015:2016), which was initially noted in kernel density, Figure 2, hence an additional analysis was done. These changes were not linked to sea temperature (at 9 m, 14 m, 18 m, and 21 m depth strata), with not significant ($P > 0.05$) results when temperature was analysed by t-test between 2002 and 2015. There was also a weak correlation between length frequency and the total squid catch in a given year (F Statistic (df = 1; 13) is 3.686 and 5.394 for males and females respectively, R^2 is 0.221 for males and 0.293 for females), but too weak to interpret, given the lack of other supporting data and the short time series. The ML_TW relationship showed no significant trends between the years for either sex. There was also no correlation between the ML_TW and total squid catch or temperature. A white caecum occurred significantly more often in males than in females (dof = 1; $p < 0.05$) from General Linear Model (GLM), indicating that the presence of non-feeding males in the spawning grounds may be linked to the behaviour of spawning squid.

Key words: Marine Biology; Fisheries Management; *Loligo reynaudii*; Biological Characteristics; Length Frequency, Mantle Length–Total Weight; Caecum colour

ACKNOWLEDGEMENTS

- I give my special thanks and appreciation to the South African Squid Management Industrial Association for their generous funding of this work.
- All thanks to the Department of Agriculture, Forestry and Fisheries (DAFF: Fisheries Research) for providing the data collected over years which made this work possible.
- My special thanks to the Department of Ichthyology and Fisheries Science (Rhodes University) and their staff for their help with logistics; especially to Bulelwa Mangali who was there for me whenever I needed some help, as was Yvain Erasmus.
- I would also like to extend my warmest gratitude to my supervisor, Prof Marek Lipiński, for his input, support, advice, and guidance; and for his efforts and patience with me throughout the duration of this study.
- To my co-supervisor, Prof Warwick Sauer, special thanks for his contribution and effort in improving the quality of this work.
- My special thanks to our 'Stats Guru', Dr Dawit Yemane, for his guidance and assistance with statistical analysis and his patience.
- My Special thanks to Prof Michael J Roberts and Marcel van den Berg for providing information concerning temperature data collection.
- My warmest gratitude to Tsamaelo Malebu 'Bosoo' for his advice and assistance with creating the maps.
- Thanks to Moletjie Library for hosting me throughout my study period, to the library staff (Francinah Hopane, Nare Poopedi, Matsiri Mokgokong, Windy Lesudi, Anna Rasakane, 'Aus' Khomotso) for their deep care and support, and for allowing me to use their facilities, even during weekends and holidays: from kitchen, tea materials, and the heater during cold weather.
- A special thanks to my Mom, Phuti Lucy Mmethi, for her support throughout the period of this study; to my little sister, Nkgodisheng Mmethi, for her wise advice, and for always being ready to search for papers from the University of Cape Town library whenever I needed them; and for her full support. To my little brothers, Kamogelo and Tshwarelo Mmethi, thank you for your support and for cheering me up on any day throughout this study.

- A special thanks to my lovely friends: Khutso Malebana, Kgaogelo Shibambo, Marie Mmowa, Dr Moloto, Mmaphuti Mabitsela, Collette Simwanza, Dr Mbokane, Thapelo Mokonyane, Tlou Maponya, Khangwelo Rathogwa, Motsatsi Mogale, Thabisile Pheko, Dr Masehela, Dr Cheddie, for being there for me during the period of this study.
- Last but not least I thank GOD for giving me this opportunity, and seeing me through it all ... 'YAWEH.'

CHAPTER 1

1. INTRODUCTION

The population dynamics of cephalopods in general is driven principally by phenotypic plasticity in response to environmental influence: the interactive effects of food availability, stage of life cycle, and temperature (Forsythe et al. 2001; Jackson & Domeier 2003; Rodhouse et al. 2014). Understanding these dynamics is important in order to manage sustainable exploitation of these resources (Mirzaei et al. 2015; Schultz et al. 2016). Cephalopods typically live only one or two years – living fast and dying young – with high growth rates and variable fecundity (Mather & Kuba 2013; Rodhouse et al. 2014; Arkhipkin et al. 2015). Growth rates have been shown to vary and are strongly influenced by environmental factors such as temperature and food availability, especially during the juvenile growth phase (Jackson & Domeier 2003).

The physiology of squid enables them to respond rapidly to environmental changes. The pattern of growth of the cephalopods, together with the short life span and rapid turnover of their populations, show cephalopods to be unique marine models for studying the relationship between growth and population dynamics (Jackson & Moltschaniwskyj 2002). Over the years fishers perceived that there are changes in Length Frequency and Mantle Length_Total Weight relationship of chokka squid, possibly linked to the environmental conditions and fishing activities. Environmental conditions together with changing seasons affects the growth pattern of squid especially in the early life stages (Forsythe 1993, 2004).

The growth strategies of *Loligo reynaudii* have the following characteristics: 1) efficient digestion, coupled with a protein-based metabolism; 2) continued growth using a combination of an increase in muscle fibre size (hypertrophy) with continual recruitment of new muscle fibres (hyperplasia); 3) efficient use of oxygen, and 4) low levels of anti-oxidative defence (Jackson & Domeier 2003). The short life span and non-asymptotic growth (Jackson & Moltschaniwskyj 2002) are unlike that of marine mammals, which are well-known squid predators.

Cephalopod growth shows an increase in total length and body weight over time, as in other molluscs (Mirzaei et al. 2015). Other components of typical biological characteristics include length frequency, maturity, length-weight relationship, and longevity. An analysis of age and growth has been used to describe the effects of environmental factors on growth and comparisons of growth rates in different geographic areas (Mirzaei et al. 2015). Multiple methods have been used to estimate growth rates of molluscs, and how the biological characteristics change and differ in different seasons, or between sexes, or in different geographic areas. For example, Mirzaei et al. (2015) used length frequency data to investigate the size frequency, growth rate, age, and mortality of *Anadara granosa* in Penang Island between 2011 and 2012. The growth rate and size frequency were given in the form of length-weight relationship plots and length frequency distributions.

Length frequency information can also provide insight into the dynamics of fish and cephalopod populations (Schultz et al. 2016) and has been used in a large number of studies to describe geographic and temporal patterns linked to squid abundance (Pierce et al. 1998; Hastie et al. 2009). Frequency distributions of fish or cephalopod length normally comprise a number of modes, suggesting groups of individuals of similar size and age (Fossen & Gordon 2015). This distribution can change over time; for example, the monthly size distribution of *L. forbesii* was found to vary considerably between the years 2006 and 2008 in the Moray Firth (Hastie et al. 2009). A cyclical annual pattern was observed in both sexes, with the modal size class influenced by the influx of large numbers of male and female squid in summer. Pierce et al. (1998) found *L. forbesii* patchily distributed in the North Sea of Scottish waters. The patterns appeared to be strongly related to bottom temperature (squid avoided waters at $<7^{\circ}\text{C}$) and to a lesser extent, salinity. In the same study, *L. forbesii* along the west coast at Rockall in Scotland was found to be more abundant in the areas of higher latitudes and shallow water. Sabrah et al. (2015) also used length frequency distributions of the common commercial species Indian squid, *Loligo duvauceli*, in the bottom-trawl survey catch in studies of biological and dynamic parameters.

However, different methods of sampling marine nektonic organisms may yield different results when looking at biological characteristics, such as length frequency, length-mass relationship (Lipiński 1994). For example, Lipiński (1994) showed that jigging selects a certain portion of the squid concentration, failing to catch certain size groups entirely, while under-representing others, including both male and female components of the population.

Sea surface temperature also influences recruitment strength and overall distribution. The general rise in carbon dioxide concentrations that has also been observed in recent years – a process associated with climatic change (Hastie et al. 2009) – may affect cephalopods. These conditions affect geographical distribution of species abundance and biological processes such as egg survival, growth, recruitment, and migration. Abundance fluctuations of cephalopods also influence the population dynamics of both their predators and their prey; hence it was suggested that cephalopods could act as indicators of environmental change and ecosystem conditions (Pierce et al. 2008).

The conditions of El Niño/La Niña events have also been found to have an impact on the growth and size of cephalopods. Jackson and Domeier (2003) studied the effects of these events on the biological characteristics of the squid, *Doryteuthis opalescens*, off southern California. Squid that hatched and grew through El Niño events were smaller and had slower growth rates than the squid that grew through a La Niña event. A positive correlation was found between squid mantle length and upwelling index, and a negative correlation was found between the sizes of the mantle length and sea temperature.

Squid growth can, therefore, be greatly modified or affected by temperature, season of hatching, and food supply (Jackson & Moltschaniwskyj 2001). Relatively small changes in temperature (especially during the juvenile phase) can have dramatic consequences on adult body size (Forsythe 1993; 2004): a small increase in temperature can cause an increase in the growth rate when food is not limited (Jackson & Moltschaniwskyj 2001; Jackson & Domeier 2003). A number of laboratory studies have demonstrated the clear effect of increased growth rate at increased temperatures in cephalopods (Hatfield 2000).

The studies conducted by Jackson et al. (1997) and Hatfield (2000) on the influence of seasonal temperature variation on the growth of different cohorts of *Doryteuthis gahi* and *Lolliguncula brevis* provided evidence that increased temperatures during the early growth period of squid may markedly accelerate growth, giving rise to significant differences in size-at-age for adult squid hatched at different temperatures. *Lolliguncula brevis* was found to have a clear seasonal pattern/trend of growth as a result of changes in seasonal temperatures (Jackson et al. 1997). The population that hatched during summer showed faster growth and a shorter lifespan than individuals that hatched during cooler seasons/periods of the year.

It is now generally agreed that higher temperatures accelerate squid growth (Forsythe et al. 2001; Jackson & Moltschaniwskyj 2002). However, this is not always the case; for example, for *Loligo vulgaris* off Galicia in Spain, and *Loliolus noctiluca* off Townsville (Rocha & Guerra 1999). It is clear that there are additional factors controlling the growth of squid.

Maturity is also dependent on season (Jackson & Moltschaniwskyj 2001; Augustyn et al. 1992); for example, in the case of *Loliolus noctiluca* males, those caught in autumn have larger gonads (Jackson & Moltschaniwskyj 2001); both sexes caught in winter show delayed maturity, and individuals of both sexes are smaller at maturity in summer. Forsythe et al. (2001) demonstrated that an increase in temperature during the juvenile growth phase may result in individuals with substantially faster growth rates and earlier maturation for *Sepioteuthis lessoniana*. Seasonal change also resulted in a variation of mantle length and body weight relationship for *Todarodes pacificus* in different waters off Hakodate-Esan fishing grounds (Murata 1978).

Generally, studies of length-weight relationships ($W=aL^b$) are made to determine the relationship between the two variables in order to calculate length if weight is known and vice versa. Generally, statistical analysis of basic biological characters provides a better understanding of relationships within the species and allows comparison of the same species between different geographical areas (Karnik & Chakraborty 2001).

Length and weight relationships also provide a useful indicator of the condition of individuals in the population and highlight various aspects of squid life history (Jackson & Moltschanivskyj 2001). Length-weight relationships, together with length frequency data can also be used to convert catch in weight to catch in numbers. Population size in numbers may, in fact, be more appropriate than biomass in analysing the status of the stock, especially in fast-growing species like squid (Lange & Johnson 1978).

Karnik and Chakraborty (2001) investigated the length-weight relationship and morphometry of *Uroteuthis duvaucelii* in Mumbai waters, and found no significant difference in length-weight relationship between the sexes, with a high degree of correlation among the compared factors of a single sex. The very same species of *U. duvaucelii* was then studied by Sukramongkol et al. (2007) to determine age in relation to mantle length, weight, and sexual maturity, by examining statoliths. The relationship between mantle length and total weight was found to be different between sexes, with females heavier than males of the same mantle length. The researchers also found that the estimated age increased with mantle length. Size at maturity was variable; the size range of mature females was smaller than that of mature males; males also showed a seasonal change in size at maturity.

The study conducted by Lange and Johnson (1978) on the dorsal mantle length-weight relationship of two squid, *Doryteuthis pealeii* and *Illex illecebrosus* from the Northwest Atlantic, off the coast of the United States, revealed differences in length-weight relationship for *Doryteuthis* by sex, year (decreasing mean length sizes from 1975 to 1977), season and area (generally decreasing from south to north). Weight also varied between individuals of a given length, for both sexes but in different seasons. The differences in *Illex* length-weight relationships were also significant among areas and there was a significant decrease in length between 1975 and 1976. These changes and differences were attributed to physical and biological factors such as temperature and nutrients. These results are supported by Dawe (1988), who observed a difference in length-weight relationship of *Illex* as a result of seasonal changes, diet composition, and the level of competition for available prey associated with

squid density.

Many authors have documented a decrease in weight and length at maturity of teleosts linked to exploitation, and other fisheries-related disturbances (e.g. Bell et al. 1985; Sharpe & Hendry 2009). Age and size at given maturity are especially sensitive to altered mortality. To illustrate this, exploited species in marine protected areas (MPAs) may be more abundant and of a larger size and weight than those in associated reference sites (out of MPAs) (Barrett et al. 2009; Starr et al. 2015).

In the case of cephalopods, it has been pointed out that environmental changes and variability such as temperature, food availability, and geographic differences are the main factors responsible for changes in population characteristics, even in exploited populations (Roberts & Sauer 1994; Roberts 2005). Because of the rapid growth of chokka squid, limited suitable prey may result in relatively poor physical conditions, especially in years of high abundance (Dawe 1988).

Both the early life history and adult ecology of chokka squid is well researched (DAFF 2014); however, little is known about the possible changes of basic biological characteristics between years. Therefore, it is important to monitor, compare and explain such changes, preferably over a long time series. Unfortunately, not many such comparisons are available in the world (published or only reported) because of difficulties in accumulating large, comparable sets of data for such parameters over many years, and obtaining a long time series. For example, many comparisons have been made based on sex and area, but not years, and mostly do not withstand consistent application of the heuristic: “only one variable compared, all the rest have to be kept constant”. It is therefore important to look for changes which may possibly be linked to fishing activities (e.g. drop in an average mantle length for the same area and month between years). More research into these aspects can contribute to the present and future management of chokka resources (Augustyn et al. 1992).

According to fishers anecdotal evidence, it was suggested that the length frequency and

ML_TW relationship of chokka have changed over years. The length of squid was suggested to have dropped to smaller size with also the length weight relationship shifted over the years, with a possible link to fishing-related activities and environmental variations. This study aims to assess possible temporal changes in biological characteristics (discovering changes in length frequency and ML_TW relationship over time series available) of chokka squid, *L. reynaudii*, caught on the south coast of South Africa between Plettenberg Bay and Port Alfred, between years for which data were available. This assessment includes examining the length frequency, length-weight at maturity stage 5 (Lipiński 1979), and evidence of feeding through examination of the caecum fluid colour. More specifically the study aims to:

- compare length frequency data from 1996 to 2017;
- assess any correlation between length frequency and total squid catch over the same period;
- assess the length-weight relationship of squid between 1994 and 2016;
- assess selected feeding patterns via examination of the colour of the caecum fluid.

CHAPTER 2

2. GENERAL BACKGROUND: LITERATURE REVIEW

2.1. THE CLASS CEPHALOPODA: GENERAL CHARACTERISTICS

Cephalopods are a small class of molluscs containing about 800 species belonging to five main groups represented by different orders (Jereb & Roper 2005; Rodhouse et al. 2014). Their morphology, physiology, ecology, and behaviour were shaped by a co-evolutionary race with modern teleost fish, which resulted in developed abilities and properties convergent to vertebrates (Mather & Kuba 2013). About 800 species of coleoid cephalopods inhabit almost every marine environment, from the deep sea to the intertidal level, and from the tropics to the polar regions (Mather & Kuba 2013).

Around the world, species of cephalopods are predators of, and prey for, other species in the marine environment (Fries 2010). Squid dominate the diet of many species in the Antarctic zone, including toothed whales, elephant seals, albatrosses, and sperm whales. Thus there are important relationships between squid fisheries and marine ecosystems, especially in the context of ecosystem-based fishery management (EBFM) (Arkhipkin et al. 2015). Worldwide, cephalopod species reside in similar marine habitats to fish and are regarded as equally important in the ecological system.

Cephalopods can be considered as sub-dominant predators that tend to increase in biomass when other species (including their predators and competitors) are depleted by a combination of factors such as heavy fishing, human impacts, oceanographic fluctuations and competition for food (Jereb & Roper 2005). Therefore, removing cephalopods through fisheries would have the following impact on the environment and food web: the size of smaller mid-water fish populations would increase, while top predators such as seabirds, seals, and large fish populations would decrease (Jereb & Roper 2005).

The movement of cephalopods occurs during all phases of their life history. These phases

involve passive drifting of egg masses and paralarvae, daily vertical migrations of many oceanic species, and large-scale migrations of adult individuals from feeding grounds to spawning grounds (Semmens et al. 2007). The abundance and location of the population is strongly influenced by the prevalence and scale of their movement (Semmens et al. 2007).

Cephalopods are highly sensitive to environmental conditions and change in a range of spatial and temporal scales (Pierce et al. 2008; Rodhouse et al. 2014). Environmental parameters have a significant influence on population size, abundance and distribution (Semmens et al. 2007; Pierce et al. 2008). These parameters include sea temperatures and oceanic processes. Of all the environmental parameters, sea surface temperature is the most easily obtained and commonly used to assess the link between cephalopod distribution and environment. Other factors or parameters include rainfall, river discharge, water turbidity, solar flux, sea level pressure, wind speed, and salinity. The biological characteristics of cephalopods are closely linked to the immediate temporal environmental circumstances (Jereb & Roper 2005). Hence, cephalopods are good indicator species reflecting the changes in the environmental conditions, on both a large scale and a local scale.

Food availability is one of the major factors influencing the active movement of cephalopods (Rodhouse et al. 2014). Many species evolved migratory strategies to maximise high feeding rates. Populations undertaking large migrations are prone to large fluctuations in abundance. For example, Ommastrephid squid annual catch fluctuation was five orders of magnitude for a single species (Semmens et al. 2007). Unlike Ommastrephid squid, coastal cephalopod species such as octopods, loligonids and sepioids generally migrate in a small scale (tens to hundreds kilometres) between spawning and feeding grounds (Semmens et al. 2007). Pelagic species such as Ommastrephid squid are highly migratory and likely to be strongly affected by changes in current systems and large-scale oceanographic phenomena (e.g. El Niño) in addition to food availability (Pierce et al. 2010).

Exploited species of cephalopods typically live only one or two years – living fast and dying young – with high growth rates and variable fecundity (Mather & Kuba 2013; Rodhouse et al.

2014; Arkhipkin et al. 2015). These cephalopods include species of octopods, cuttlefish, and squid in which their population evolved to withstand substantial variations in abundance. The result of cephalopods non-overlapping generations is a lack of buffering of the population from the influence of varying environmental conditions on spawning and recruitment success (Pierce et al. 2008).

Despite their short lives, octopuses are clever animals with complex and diverse behaviour (Mather & Kuba 2013). The brain controls the behavioural plasticity, high cognition capacity, and flexibility of the body and skin. The short life cycle, fast growth and high metabolic rates of cephalopods are associated with high plasticity in life history characteristics and marked sensitivity towards environmental variations. However, the dynamics of cephalopod populations are poorly understood (Rodhouse et al. 2014).

Some species of cephalopods are characterised by low growth rate, relatively slow maturation and low fecundity, and are especially vulnerable to overfishing. These species include the chambered *Natutilus* spp. (Rodhouse et al. 2014), which are in demand for their ornamental shells. In the Philippines, the growing market for the species resulted in an almost 80% decline in the reported catch per unit effort (CPUE) from 1980 to the present time, when the fishery ended in instability (Rodhouse et al. 2014).

2.2. CEPHALOPOD FISHERIES

The interaction between human society and marine resources dates back many millennia, and underpins both social and economic benefits (Jereb & Roper 2005; Arkhipkin et al. 2015). However, marine communities have changed dramatically as a result of human impact through unsustainable fishing activities that reduce the abundance of predator fishes and mammals (Sauer et al. 2013). It is recognised that humankind fails in many instances to conserve and obtain optimal usage of marine species (Arkhipkin et al. 2015). Fisheries provide food, income and employment in many parts of the world (Jenning et al. 2009), hence sustainable usage is most important. At the same time, while some records indicate the South African earliest marine fisheries date as far back as 160 000 years (Rodhouse et al. 2014;

Arkhipkin et al. 2015), not much is known about ancient fisheries in terms of benefits and economic activities.

When it comes to exploited species, cephalopods are one of the most valuable and diverse (Jereb & Roper 2005). These species are also important biologically and scientifically (Fries 2010). Cephalopods are common in all marine habitats worldwide and provide a large portion of the total global biomass of all marine species (Fries 2010); they support large-scale industrial fisheries, together with smaller-scale and local fisheries, and artisanal fisheries (Lipiński et al. 1998; Rodhouse et al. 2014; Arkhipkin et al. 2015).

The large natural populations, shoaling behaviour and high-quality protein of many species make cephalopods eminently suitable for commercial exploitation (Hastie et al. 2009). The low-fat content also makes most species of cephalopods a healthy element in the human diet (Jereb & Roper 2005). Utilisation of cephalopod resources for human consumption is extensive and diverse: the products range from fresh food, raw food such as 'sashimi' in Japan, fresh cooked, and processed products (dried, frozen, fermented, etc.) (Jereb & Roper 2005). Cephalopod species are also used for bait in line fishing (Fries 2010). Cephalopods are an important component of the natural food chains in many areas (Fries 2010; Arkhipkin et al. 2015).

For multiple centuries cephalopods were known and valued by Greeks, Romans, and Chinese, and fished on an artisanal basis (Jereb & Roper 2005). Since the 1950s, the importance of cephalopods as a fishery resource has risen dramatically (Rodhouse et al. 2014). Moreover, as production and demand increased steadily, cephalopod fisheries began dominating in some fishing grounds (Lipiński et al. 1998). Around the 1960s, the Japanese demand for cephalopods increased, which resulted in the global expansion of commercial fisheries (Jereb & Roper 2005). The worldwide catches continued to increase, signifying a departure from traditional finfish resources and fishing grounds (Jereb & Roper 2005; Arkhipkin et al. 2015). The catch increased from approximately 1 million tonnes in 1970 to over a peak of 4.3 million tonnes in 2007 (Arkhipkin et al. 2015; Rodhouse et al. 2014). In 2009 and 2010, the world

cephalopod landings fell to 3.5 million tonnes (Arkhipkin et al. 2015; Rodhouse et al. 2014), and recovered to over 4 million tonnes again in 2012.

The importance of cephalopods as a fishery resource varies in different global regions (Lipiński et al. 1998; Rodhouse et al. 2014). The most commonly exploited species of cephalopods for commercial fisheries are squid from two families, the Ommastrephidae and Loliginidae, accounting for about 70% of world catch, followed by cuttlefish (species of *Sepia* and *Sepiella*), then octopuses (*Octopus* spp.) (Jereb & Roper 2005). Most global fisheries which exploit ommastrephid and loliginid squid (Arkhipkin et al. 2015) are relatively stable (Rodhouse et al. 2014).

The family Ommastrephidae dominates Loliginidae in terms of biomass with five main commercial species, namely *Todarodes pacificus*, *Nototodarus sloanii*, *Illex argentinus*, *Ommastrephes bartramii* (species complex) and *Dosidicus gigas*. Main species targeted of the family Loliginidae reported by FAO include *Loligo vulgaris*, *Loligo forbesii*, *Doryteuthis gahi*, *Doryteuthis pealeii*, *Heterololigo bleekeri*, and *Loligo reynaudii* (Arkhipkin et al. 2015). However, there are a few species that are underexploited, among them *Sthenoteuthis pteropus*, some species of the *Ommastrephes bartramii* complex, *Martialia hyadesi*, *Todarodes sagittatus*, *Sthenoteuthis oualaniensis* complex, *Nototodarus philippinensis*, and *Todarodes filippovae*. The species of *Dosidicus gigas* was also originally included in this list but since 2004, the global landings have risen to almost one million tonnes annually (Arkhipkin et al. 2015). There are also members of the families *Enoploteuthidae*, *Gonatidae*, *Onychoteuthidae*, and *Thysanoteuthidae* (Arkhipkin et al. 2015) targeted by fisheries, apart from ommastrephids and loliginids.

Historically, in European waters, cephalopods have been considered as a minor fishery resource, despite the local economic importance, especially in southern Europe's artisanal fisheries. In the northern part of Europe, the interest in cephalopods as a fishery source is evident from number of publications since the 1970s and grew substantially, especially from the 1980s (Pierce et al. 2010). In the 1990s, the potential of cephalopods as a source of both

protein and revenue to supplement, or even to replace over-exploited finfish stocks (Pierce et al. 2010) stimulated further interest.

The total cephalopod landings from the north-east Atlantic and Mediterranean routinely exceeded 116 000 tonnes in 2006, comprising 49 000 tonnes from the north-east Atlantic and 67 000 tonnes landed from the Mediterranean (Pierce et al. 2010). European landing statistics generally refer to broad commercial categories which include long-finned squid, short-finned squid, octopus, and cuttlefish, with some categories including two or more species (Pierce et al. 2010).

Statistics for 2001 show Argentina as the leading exporter of cephalopods in terms of quantity, followed by Spain, Morocco and the United States (Jereb & Roper 2005). The main markets for imported cephalopod products are European countries such as Spain, Italy, Greece, Portugal and France (Jereb & Roper 2005). Cephalopod fisheries are especially intense in Japan, Southeast Asia, China, and the Mediterranean Sea. Catches in these areas showed a decreasing trend in the last decade for all the three groups (squid, cuttlefish and octopus). In 2005, Japan reported to be a leading cephalopod fishing country, followed closely by China (Jereb & Roper 2005).

2.3. CEPHALOPODS IN DIFFERENT REGIONS OF THE WORLD

2.3.1. Loliginid squid

The species from this family that are exploited in Europe occur in relatively shallow waters and are caught mostly by demersal gear. They include *Loligo forbesii* and *Loligo vulgaris*. All the exploited species in this group are among the valued squid species and generally command higher prices in the market than short-finned squids (Pierce et al. 2010).

2.3.2. Ommastrephid squid

These cephalopods are less valuable in the market than loliginids. These species are also more prone to extreme variations in abundance (Pierce et al. 2010). Three species are most commonly landed in European waters: *Illex coindetii* and *Todaropsis eblanae*, which mainly

occur in the south, and *Todarodes sagittatus* which is more abundant in the north. These species are normally caught by bottom trawls (Pierce et al. 2010).

2.3.3. Octopus

This category comprises species belonging to the family Octopodidae. The main harvester of this group of species is Morocco, with a share of more than 35% of total production back in 2001, followed by Japan with about 14% of total world production. Mexico ranks first for catches and landings of *Octopus vulgaris*' landings (Jereb & Roper 2005).

2.3.4. Cuttlefish

This category of cephalopod production has increased significantly over the last two decades, and doubled between 1980 and 2001. The main producer is China (over 50%), followed by Thailand (western Pacific and central Indo-Pacific areas) and Morocco (eastern central Atlantic) (Jereb & Roper 2005). In the European countries, the highest landings are in western Europe, mostly from catches concentrated over the continental shelf in the region of the southern Celtic Sea. Most of the landings are composed of catches of a single species of *Sepia officinalis*, but landings of two more species are common in some European countries. The species are caught by demersal trawling and artisanal trapping (Pierce et al. 2010).

2.4. FISHERIES FOR SQUIDS

Squids possess ecological and behavioural features that are similar to those of fishes (Arkhipkin et al. 2015). Many of the nektonic species of squid migrate in large schools like those of pelagic fish. Most of the fishing methods applying to squid are also similar for fish, with the exception of jigging (Arkhipkin et al. 2015).

Squid have special characteristics that set them apart from many other commercially exploited marine species. These include a short life cycle, fast growth, high feeding rates and conversion efficiencies, and high reproductive rates. The squid fisheries make a relatively small contribution to the world landings from capture fisheries, but over recent decades the proportion has increased steadily, although recently, landings have levelled off. The

estimated global squid consumption by predators was suggested to be a greater mass than the total world catch of all marine species combined (Arkhipkin et al. 2015).

Squid have been caught with various types of fishing gear since the early days of exploitation; methods include trap nets, set nets and purse seines (Arkhipkin et al. 2015). Set nets are used for *I. illecebrosus*, *Doryteuthis pealeii*, and *Watasenia scintillans*, the latter in the Far East. Seine nets are combined with lights in the Californian *Doryteuthis opalescens* fishery, and pumps are used to remove the squid. Pelagic and bottom trawls are used to catch *Todarodes sagittatus* and *Todarodes angolensis* in the north and south-east Atlantic (Arkhipkin et al. 2015). Bottom trawls are used mainly to catch near-bottom aggregations of loliginid squids such as *Doryteuthis gahi* (Arkhipkin et al. 2015).

Jigging is less damaging to the environment and produces high-quality, valuable products. This method, used with lights, is the preferred technology to exploit squid, which move up in the water column towards the surface at night (Arkhipkin et al. 2015). The squid caught by this method are of high value because the process causes little damage to the skin. The large-scale fisheries for ommastrephid and loliginid squid use this method.

In many instances, mankind has failed to conserve marine species (Arkhipkin et al. 2015). Both direct (targeted) and indirect (by-catch) fishing may overexploit resources (Rodhouse et al. 2014). It seems that, until the turn of the century, the few species of coleoid cephalopods were locally over-exploited, with many other stocks also subjected to heavy fishing pressure (Rodhouse et al. 2014).

2.5. *LOLIGO REYNAUDII* (CHOKKA SQUID)

2.5.1. Life history

The population characteristics of chokka squid from the western parts of South African waters differ from those from the eastern parts (Arkhipkin et al. 2015). Generally, squid occurring in the west grow and mature more slowly and their distribution is more limited than the population in the east (Arkhipkin et al. 2015). Generally, chokka reach maturity at an age well

below one year, with a potential fecundity of about 18 000 eggs (Sauer et al. 2013). Individuals of this species breed once and then die (Hastie et al. 2009; Rodhouse et al. 2014), like most other species of cephalopods (Rodhouse et al. 2014).

The size at maturity of chokka squid varies, depending on the geographic location and times of the year (Augustyn et al. 1992). The size at maturity in females ranges from 100–180 mm (ML) while males can reach maturity at 90 mm (ML) or be immature at 250 mm (Augustyn et al. 1992).

Spawning has been observed to take place mainly inshore. The cold temperatures offshore were found to be caused by the downwelling that extends up to 120 m deep and 20 km offshore (Oosthuizen & Roberts 2009), leading to intrusions of water warmer than 10° C (Sauer et al. 2013). Chokka spawning also occurs in these deeper waters because inshore the water is limited by high turbidity near the seabed, forcing squid concentrations to the deeper waters (Roberts & Sauer 1994). Roberts (1998) estimated that turbidity events may interfere with pairing and guarding behaviour, and terminate spawning.

Spawning behaviour in chokka squid was found to be complex. Females mate with multiple males, which results in multiple paternities (Sauer et al. 2013). During spawning, chokka squid aggregate to form concentrations ranging from a few hundred to several thousand individuals (Roberts 1998). The basic pattern of the spawning process involves jostling, mating, sneaker male interception, egg deposition, cannibalism and predator avoidance (Roberts 1998). Chokka squid spawn in the protected bays of the south-east coast (Arkhipkin et al. 2015). The clusters of closely packed egg pods are deposited on the sea floor, then attach to the substratum (open sand, or rocky reef with small patches of sand) forming egg beds up to 4 m in diameter (Roberts 1998; Arkhipkin et al. 2015). The pods laid in the shallow coastal regions take approximately four weeks to hatch, depending on the surrounding water temperature (Roberts 1998).

According to the distribution scheme, most chokka squid spawn off the south east coast of

South Africa, and the paralarvae drift westwards (Shaw et al. 2010; Lipiński et al. 2016). Some of the stock spawn locally and others migrate to the west coast waters of South Africa, and some individuals return eastwards to spawn (Lipiński et al. 2016). The most important prey for chokka paralarvae are copepods, *Calanus agulhensis* (Arkhipkin et al. 2015). Adults feed mainly at night on the teleosts dominating the prey items on the spawning grounds. During the day cannibalism was also observed (Arkhipkin et al. 2015).

2.5.2. Distribution and habitat

The distribution range of chokka squid varies seasonally due to the complexity, variability and diversity of the marine environment on the southern African coast (Roberts 2005; Sauer et al. 2013; Van der Vyver et al. 2015). Chokka occupies the area between the Great Fish River east coast of South Africa to the Orange River on the west coast at depths of up to 400 m, and large numbers are also found in southern Angola (Shaw et al. 2010; Sauer et al. 2013) where spawning is also known to occur; however, there is little information from this region (Sauer et al. 2013).

The distribution of chokka squid along the southern African coastline is largely influenced by the warm Angola current in the north, the cold Benguela upwelling system along the west African coast, and the warm Agulhas system along the south-east coast (Roberts 2005; Van der Vyver et al. 2015).

In South African waters, most of the stock is concentrated on the south-east coast, where spawning and hatching is known to take place inshore in the shallow bays, mainly between Plettenberg Bay and Port Alfred (Shaw et al. 2010; Sauer et al. 2013; Lipiński et al. 2016), preferably at depths between 10 and 60 m, with a temperature range between 11 and 20° C (15.3° C average) (Oosthuizen & Roberts 2009). Less important spawning sites are found as far west as False Bay and as far east as beyond Port Alfred (Augustyn et al. 1992).

Juvenile squid ranging between 20 and 80 mm in mantle length were found throughout the year along the south coast from depths of 30 m to 150 m (Augustyn 1989; Augustyn et al.

1992; Lipiński et al. 2016). Their distribution at this development stage was detected mainly between Plettenberg Bay and Cape Agulhas (Augustyn et al. 1992; Lipiński et al. 2016), with high densities between Algoa Bay and Cape St Francis (Augustyn et al. 1992). Maturing and immature individuals were also found over an entire depth range on the south coast and to depths of more than 300 m on the west coast (Augustyn et al. 1992). Adult chokka were usually detected offshore, foraging in small schools across the Agulhas Bank (Lipiński et al. 2016). Paralarvae were mainly detected around spawning grounds, but probably dispersed along the whole coastline (Lipiński et al. 2016).

2.5.3. Effects of environmental factors

The marine environment off the South African coast is diverse, complex and highly variable (Van der Vyver et al. 2015). Of all South African fisheries, the chokka squid fishery fluctuates most, both in biomass and catch, and this variability has been found to be caused by the combination of environmental, biological, and fisheries-related factors. Biological factors include spawning distribution and survival rates of hatchling and juveniles (Sauer et al. 1991; Sauer et al. 2013; DAFF 2014). Temperature was acknowledged to vary with seasons, latitude and depth (Forsythe 2004). Chokka squid live within the diverse environment between Orange River and the Great Fish River throughout the stages of the life cycle as mentioned previously. Therefore, the variability and changes in the environment such as sea surface temperature from this shelf could either weaken or strengthen the stock well-being (Roberts 2005; Sauer et al. 2013; Rodhouse et al. 2014).

As previously stated, environmental conditions may affect all phases of the squid's life cycle as well as the growth pattern (Roberts & Sauer 1994). For example, temperature plays a critical role when it comes to egg incubation. Egg development requires an optimal temperature between 12 and 17° C (Oosthuizen et al. 2002), and paralarvae will not survive or develop well in temperatures greater than 20° C (Sauer et al. 2013). Clearly, temperature plays an important role in the spawning and development of the embryos (Augustyn et al. 1992; Roberts 1998). In 2005, Roberts linked good annual chokka squid yields off the South African east coast to low sea temperatures. These findings are also supported by other studies

of loliginids and other aquatic species, where temperature affects metabolism, ovary development, development of egg pods, dispersal and recruitment (Roberts 1998; Roberts 2005; Martins et al. 2010).

Dissolved oxygen, currents and temperature all have a direct impact on the demersal distribution of adult chokka in the feeding grounds (Augustyn 1990; Roberts & Sauer 1994). Most squid hatchlings are not strong swimmers and go through passive and active planktonic phases, which depend heavily on the availability of the right type of food for survival (Augustyn et al. 1992). Conditions have been shown to be suitable for mating, egg laying and embryonic development on the spawning grounds (Roberts & Sauer 1994; Martins et al. 2010), however, the greatest food concentration is found in the central part further west from the spawning grounds in the eastern Agulhas bank where there is a cold ridge. The cold ridge is associated with the enhanced levels of primary and secondary production. The current flow pattern is the key factor that controls the dispersion of hatchlings (Augustyn et al. 1992; Roberts 2005). The survival of paralarvae depends mostly on yolk reserves when moving between the spawning grounds and the paralarval nursery grounds (Martins et al. 2010). Environmental temperature also affects the utilization rates of the yolk by paralarvae. Martins et al. (2010) found that paralarvae maintained at warmer temperature utilize their yolk faster and survive less than their counterparts in cold temperature.

The south coast experiences limited wind-driven upwelling during summer seasons. Also, there are higher sea surface temperature and dissolved oxygen than on the west coast (Roberts & Sauer 1994; Kirkman et al. 2016). Hence, it is suggested that chokka squid distribution in the west coast regions could be limited by cold temperature and the lower levels of dissolved oxygen found on the outer shelf (Roberts & Sauer 1994). However, West coast forms an important part of feeding ground for chokka, with large scale upwelling system bringing cold nutrients rich water to the surface resulting in high productivity (Roberts & Sauer 1994). Paralarvae are transported by the current from the spawning grounds to the food-rich feature called cold ridge, which is 100-200 km away to the west coast (Martins et al. 2010). Recruitment is thought to be influenced by the successful transport of paralarvae

from the spawning grounds to a food-rich environment on the west. Paralarvae hatched on the deeper mid-shelf spawning grounds have greater risk of loss to the adjacent oligotrophic oceanic waters than those hatched inshore particularly within sheltered embayments (Martins et al. 2010). These loss result in high mortality because squid paralarvae are particularly sensitive to food shortage (Martins et al. 2010).

The morphometrics of chokka squid suggest that there are three geographical groups, originating from short migrations and different paralarval events. This morphological divergence is related to the influence of environmental heterogeneity and genetic isolation which led to phenotypic difference (Van der Vyver et al. 2015). Adult squid show little genetic diversity, even between sub-populations that are widely separated (Angola and Port Alfred) (Lipiński et al. 2016).

2.5.4. Fishery and economic importance of chokka squid

The chokka squid (*Loligo reynaudii*) is an important commercial species in the South African fishing industry, and third most important in economic terms (Augustyn 1990; Augustyn et al. 1992). The chokka squid fishery provides employment for up to 3000 people, with catches generating R400 million per year (Arkhipkin et al. 2015). Most of the chokka squid fishery operates out of the Eastern Cape, which makes the fishery an important economic engine for the province (Arkhipkin et al. 2015). This stock is targeted by a major commercial hand-jig fishery (Arkhipkin et al. 2015; Van der Vyver et al. 2015) that operates throughout the year, except for closed season(s), and during the peak spawning season (October–November) (DAFF 2014; Arkhipkin et al. 2015) and other *ad hoc* closed seasons (e.g. in winter).

In the jig fishery, catches of chokka increase with the intensity of coastal upwelling (Roberts & Sauer 1994). Chokka is also caught as by-catch in the commercial inshore demersal trawl fishery that targets Cape hake (*Merluccius capensis*) (Roel et al. 2000). Total annual catch varies from 2000 to 13 000 tonnes (Sauer et al. 2013). The National Regulator for Compulsory Species (NRCS) catch data indicate an increase in squid catches over the period between 2001 and 2004 (DAFF 2014). Artisanal fishers in southern Angola catch *L. reynaudii* close to shore from small individual rafts using homemade jigs and hand-lines (Sauer et al. 2013; Van der

Vyver et al. 2015).

2.5.5. Stock assessment and management

The first formal stock assessment for squid was conducted in 1998, although the fishery has been in operation since 1985 (Sauer et al. 2013; Arkhipkin et al. 2015). The 1998 stock assessment, which was based on an observation error estimator (Sauer et al. 2013), indicated that the resource was at risk of collapsing (Arkhipkin et al. 2015). The risk was defined as the probability of the spawning biomass dropping below 20% of carrying capacity at least once within a ten-year projection period under fixed level of effort (Sauer et al. 2013). Therefore, reduction in effort level was implemented (Sauer et al. 2013; Arkhipkin et al. 2015).

A more recent stock assessment incorporating data up to 2008 indicates that the *L. reynaudii* resource is in a healthy condition, due to successive years of above-average recruitment (Sauer et al. 2013). Currently, efforts are capped at 136 vessels and a maximum of 2422 crew (Sauer et al. 2013; Arkhipkin et al. 2015). The current primary management objective for the squid fishery is to cap efforts at a level which secures the greatest catch, on average, in the long term without exposing the resource to the threat of reduction to levels at which future recruitment success might be compromised, or at which catch rates drop below economically viable levels (Sauer et al. 2013). A closed season of variable duration is implemented each year to protect the spawning squid, improve recruitment for the year that follows, and curb effort (DAFF 2014).

The exponential growth phase of paralarval and juvenile cephalopods is highly dependent on temperature. The research conducted by Forsythe 1993 demonstrated that a small temperature change of 1 °C experienced by cephalopods in paralarval phase can produce significant differences in growth rates and population structure in later life stages (Forsythe 1993, 2004). *Loligo forbesi* reared at an average temperature of 1 °C warmer were over three times larger at 90 days after hatching than cooler siblings under laboratory experiment. Rapid exponential growth in weight and length of most cephalopods species is a fundamental trait in the first two to four months of life after hatching. Furthermore, cephalopods are

poikilotherms, the metabolic rate rises or drops directly with temperature, which further affects feeding and growth rates (Forsythe 1993). Moreover, Growth rate increases as temperature rises (Forsythe 1993), also with availability of food (Forsthe 2004).

CHAPTER 3

3. MATERIALS AND METHODS

3.1. SQUID COLLECTION

This study is based on historical data collected by observers using handline jigging from commercial vessels hired for scientific use, and from R/V Algoa by bottom-trawl surveys and jigging stations for different years (1994–2017), all during the closed season (between October and November). The samples of chokka squid were collected from the south coast of South Africa, where spawning is known to be taking place. The main sampling areas were between Plettenberg Bay and Port Alfred during spring, between October and December, but 90% of data were collected 1–15 November. The samples were taken up to the depth stratum of 50 m, except those that were collected in 2016 for length and weight relationships; these were taken up to depths of 100 m. All squid were sexed and analyses were conducted separately for males and females. All individuals were at maturity stage 5 (Lipiński & Underhill, 1995), and had empty stomach. Squid with a red or brown caecum were eliminated, to ensure consistency in terms of weight gain due to recent feeding (Lipiński 1987). Separate data were used for length frequency and length-weight relationship analysis, but both with the same characteristics (collected in the same areas, depth, maturity stage 5, empty stomachs). These data were used separately due to availability of data for both purposes (length frequency and length-weight relationship) and difficult logistics to collect data with limiting criterion (only maturities 5, stomach 0 and caecum "all yellows and white"). Collection of data with limited criterion as in this study consumes time. The number of individuals in any sample does not necessarily reflect the size of the survey catch or the relative abundance of species in any year. The origin of the materials, details of capture, and distribution of stations are given in Figure 1, and Tables 1 and 2.

3.2. SEA TEMPERATURE DATA COLLECTION

The Sea temperature used in this study was sourced from Michael Roberts project (Department of Environmental Affairs UTR database), collected in the areas between

Plattenberg Bay and Port Alfred specifically in Krom by the instrument called Starmon mini. This instrument records water temperature at a user defined interval and stores the data in its internal memory. The recorder in the instrument is supported by the Communication Cable and the Sea Star software, calibrated in a stable, high accuracy. The data was collected hourly, then used to create daily averages through Microsoft excel. The available daily average temperature used date between 6-12 November, for two years, 2002 and 2015, from depth stratum of 9 m, 14 m, 18 m and 21 m. The mantle length distribution of chokka in 2002 was typical and in 2015 dropped. The sea temperature was compared for two years (2002 and 2015) to detect if there is any link to the shift in mantle length between years 2013 and 2016.

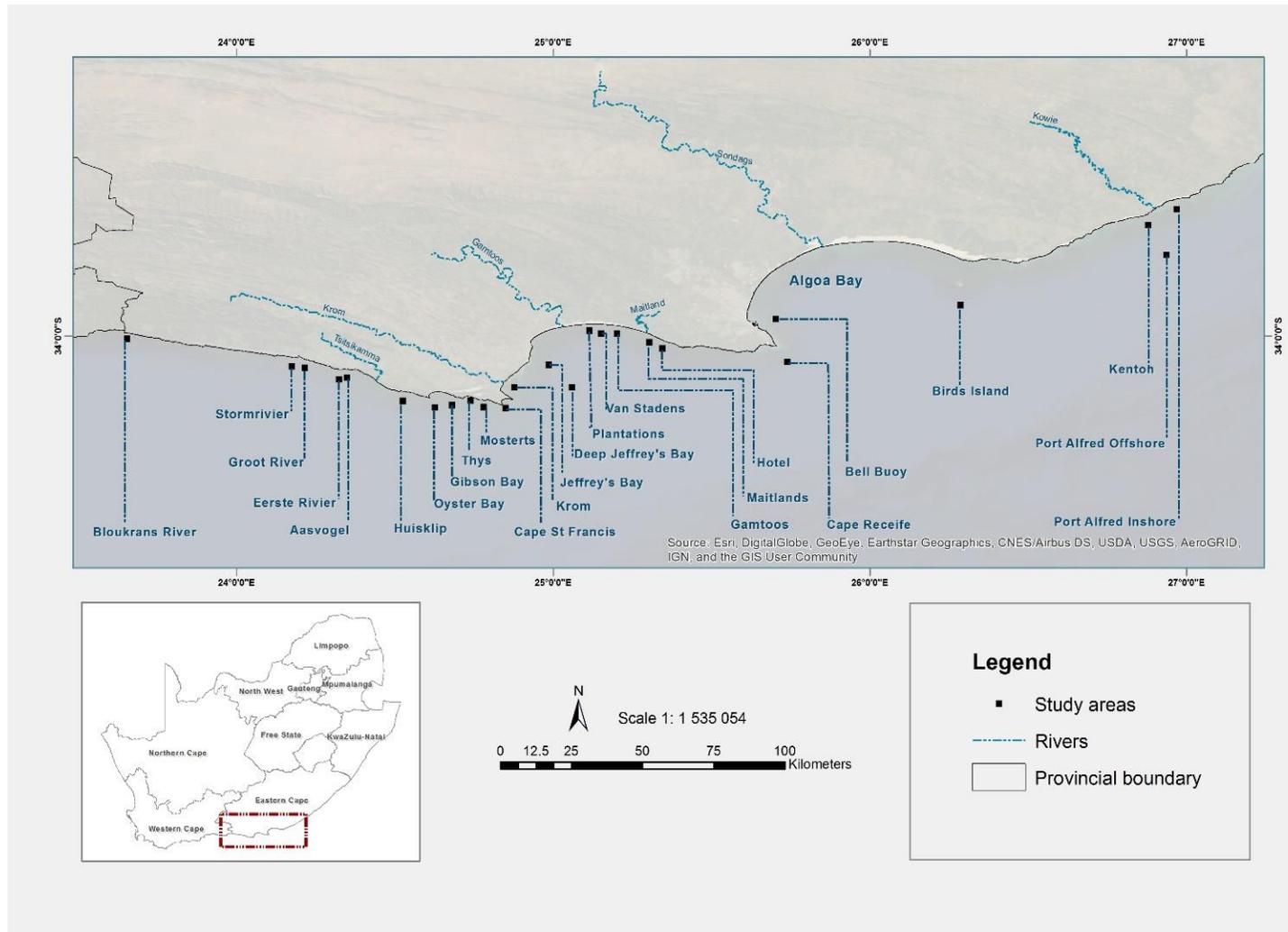


Figure 1: Distribution of the stations where samples of *L. reynaudii* were collected between Plettenberg Bay and Port Alfred for different years (1994–2017), during spring season (between October and December).

Table 1: Details of length frequency data collection of *Loligo reynaudii* in the areas between Plettenberg Bay and Port Alfred from 1996 to 2017.

Year	Day and Month	Vessel Name	Fishing Grounds	Number of males squid (N)	Number of females squid (N)	Depth (m)
1996	8 - 11 Nov	Koningsberg	Aasvogel	954	78	0-50
	27 Nov - 17 Dec	Mkuze	Krom, Jeffrey's Bay, Bird Island	716	844	0-50
2002	10-12 Nov	Pegasus	Deep Jeffrey's Bay, Gamtoos	499	453	0-50
2003	3-9 Nov	South Star	Groot Rivier, Aasvogel, Oyster Bay, Port Elizabeth	1040	402	0-50
2004	6-11 Nov	Silver Dorado	Aasvogel, Seal Island, Krom, Jeffrey's Bay	740	144	0-50
2006	5-10 Nov	Silver Explorer	Aasvogel, Thys, Krom, Huisklip	941	46	0-50
2007	2-13 Nov	Sparodon, Silver Star	Jeffrey's Bay, Oyster Bay, Maitlands, Huisklip, Krom	1299	241	0-50
2008	15-18 Nov	Erongo	Algoa Bay	575	259	0-50
	8-22 Nov	Maluti	Algoa Bay	1587	1206	0-50
2009	31 Oct - 3 Nov	Silver Explorer	Algoa Bay, St Francis, Gamtoos	346	248	0-50
	9-16 Nov	Silver Eagle	Thys	362	114	0-50
2010	2 -11 Nov	South Star, Sea Gull	Gamtoos, Algoa Bay	1271	342	0-50

2011	8 Nov	South Star, Sea Gull	Gamtoos, Algoa Bay	80	108	0-50
2013	31 Oct – 5 Nov	Sparodon	St Francis Bay, Oyster Bay, Eerste Rivier	605	235	0-50
	06 – 11 Nov	Megalodon	Cape St Francis, Eerste Rivier, Oyster Bay, Gibson Bay	1227	844	0-50
2014	03 – 08 Nov	Michelle	St Francis Bay, Oyster Bay, Eerste Rivier	177	29	0-50
	01 – 07 Nov	Megalodon	Maitland, Eerste Rivier, Oysters Bay, Gibson Bay	441	141	0-50
2015	09 – 18 Nov	Silver Eagle	Port Elizabeth (Clarendon Marine), Cape Recife, Gamtoos Mouth, St Francis Bay, Oyster Bay	842	182	0-50
	02 – 09 Nov	Silver Champion	Oyster Bay, Gibson Bay, St Francis Bay, Cape St Francis, Port Elizabeth, Jeffrey's Bay	2455	662	0-50
2016	31 Oct – 07 Nov	Silver Champion	Port Elizabeth, Eerste Rivier, Maitlands, Jeffrey's Bay, St Francis Bay, Oyster Bay.	2364	618	0-50
	08-17 Nov	Silver Eagle	Maitlands, Jeffrey's Bay, Eerste Rivier	975	440	0-50
2017	09 – 16 Nov	Silver Eagle	Eerste Rivier, Jeffrey's Bay. Gamtoos Mouth, Oyster Bay	1827	76	0-50
	01 – 05 Nov	Jenny	St Francis Bay, Oyster Bay, Maitlands, Eerste Rivier	127	103	0-50

Table 2: Details of length and weight relationship data collection of *Loligo reynaudii* in the area between Plettenberg Bay and Port Alfred.

Year	Date	Location/Site	Vessel Name	Number of males squid (N)	Number of females squid (N)	Depth (m)
1994	11-13 Nov	Oyster Bay	R/V Algoa	123	50	0-50
2000	17 – 23 Oct	Sundays River to Aasvogel	R/V Algoa	48	44	0-50
2001	9 – 21 Nov	Aasvogel, Krom, Maitlands	R/V Algoa	70	64	0-50
2002	6 - 21 Nov	Bloukrans, Eerste Rivier, Oyster Bay, Krom, Gamtoos Jeffrey's Bay, Maitlands	R/V Algoa	119	116	0-50
2003	18 Nov – 06 Dec	Krom, Jeffrey's Bay, Oyster Bay, Grootrivier, Aasvogel, Bird Island, Bell Buoy, van Stadens.	R/V Algoa	106	92	0-50
2004	14 Nov - 01 Dec	Maitlands, van Stadens, Gamtoos, Plantations, Bell Buoy	R/V Algoa	113	112	0-50
2005	15 Nov – 01 Dec	Oyster Bay, Thys, Maitlands, Jeffrey's Bay, Algoa Bay	R/V Algoa	95	99	0-50
2008	15 Nov – 04 Dec	Off Cape St. Francis, whole St. Francis Bay (Maitlands, Krom), Aasvogel, Algoa Bay, Port Elizabeth area	R/V Algoa	119	124	0-50
2016	9-17 Oct	Storms River, Port Elizabeth, Port Alfred, Kenton-on-Sea, Jeffrey's Bay, Oyster Bay.	R/V Africana	34	24	0-100

3.3. LENGTH FREQUENCY ANALYSIS

The length frequency data used were from 1996 to 2017, with no available data for the years 1997 to 2001, 2005 and 2012. The number of males and females that were analysed are given in Table 1. Length used was standard mantle length in cm (mantle length measured dorsally, as defined in Roper and Voss, 1983) taken to 1 cm below. Multiple R packages were used for processing raw data, data analysis, and visualisation of results. These include: Muggeo 2017; Aphalo 2018; R Core Team 2018; Wickham et al. 2018; Henry & Wickham 2019; Wickham & Bryan 2019; Wickham et al. 2019. Statistica programme, SPSS and Excel were also used to calculate means and medians and to explore significance of changes of mantle length over time.

The length frequency distributions for both males and females over the sampling years considered (1995 to 2017) are shown in exploratory graphs. The measure used was histogram and kernel density, which showed the density along observed values of the response variables (in this case observed length). A range of kernel algorithms can be used for the smoothing: Gaussian (usually the default). In this case, kernel density was used as it is usually preferred to histograms to display the distribution of continuous variables (Crawley 2012). Histogram was further used additionally to analyse length frequency data.

Temporal trends in mean length were shown at 95% confidence interval for both males and females for the years considered. Linear regression (Crawley 2012) was fitted to assess if the temporal trend in mean length was significant for the given years. This was paired with segmented regression (Muggeo 2008), where two regression models were fitted to the same data but split into two: in this case, data were analysed for years before and after 2013.

In addition, Analysis of Covariance was conducted together with a median test for years between 2014 and 2016 in pairs (2014:2015, 2014:2016, and 2015:2016) to test for changes in length frequency between the recent years after 2013. Further, the length frequency data were linked to the jig catch per year to investigate the relationship between the two by linear regression.

3.4. LENGTH-WEIGHT RELATIONSHIP ANALYSIS

The years examined were 1994, 2000, 2001, 2002, 2003, 2004, 2005, 2008 and 2016 within the period from 1994 to 2016. The parameters were dorsal mantle length in mm, total weight (g) per sex, maturity (Lipiński & Underhill 1995). The stomach fullness and caecum colour (Lipiński 1987) were also included at some stages of the analysis, as it was suspected to have an impact on data structure over the years. Feeding ecology is one of the most powerful domain providing sharp indicators in data structuring. To check the association between the numbers of differently coloured caecum and sexes, generalized linear model (GLM) was fitted, testing both for the main effect and the interaction of sex and caecum colour.

A standard length-weight model $W=aL^b$ (where W = weight of squid (g), L = mantle length of squid (mm), a = constant or intercept and b = regression coefficient) was fitted to length and weight from each sampling year considered (Ricker, 1975). Once the model was specified optimal (best fit), values of parameters a and b were done using *minpack.lm* package (Elzhov et al. 2016), which is one of the multiple packages in *R* that allow for fitting non-linear models. Estimation of non-linear models typically requires specification of initial values for the estimated parameters, which can be obtained in a number of ways (e.g. educated guess and exploratory plots) (Verzani 2014). For the sake of simplicity, a separate length-weight relationship was fitted for each sex and for all the years for which data were available. After fitting the model, standard diagnostics were checked. These included distribution of residuals across-fitted values. Estimated parameters a and b , with their corresponding standard error, were then extracted from the fitted model. It is generally recommended to estimate confidence intervals for nonlinear regressions, such as the length-weight relationship, either from a likelihood profile or by bootstrapping (Ritz & Streibig 2008). In this study the confidence interval was mainly computed using the bootstrap approach, but for the sake of comparison, the confidence interval was also estimated, based on the standard error estimated from the model. Bootstrapping was conducted by bootstrapping residuals; mean-centred residuals were repeatedly sampled (500 times) and added to the original response value, and the model was fitted. The confidence interval was then estimated

as 0.025 and 0.975 quantile of the distribution of the estimated parameters. Parameters from the bootstraps were then used to generate predicted weight for length classes for each year. The median, lower and upper confidence intervals were then extracted as the 0.5, 0.025 and 0.975 quantile of the predictions for each length class.

Further analysis was done to show parameter estimates with the correspondence interval obtained as estimate \pm Se $\times 1.96$, followed by linear regression to detect trends. Model diagnostics were looked at by plotting residuals versus actual observations (measured mantle length). This was done to see how well the model was doing, and whether there were any systematic patterns (bias) in the prediction from the model. The predicted model for length-weight relationship was also done with the corresponding confidence interval based on parameter estimate from bootstrap. The fit of the model was also added. Histograms were used to show distribution of the estimated parameters a and b of $W=aL^b$. The relationship of length and weight were also linked to the jig catch per year to investigate if there was any relationship between them.

A Paired t-test was used to analyse the difference between sea temperature for two years (2002 and 2015) in different depth stratum (9 m, 14 m, 18 m, 21 m) on the south-east coast, specifically between Plettenberg Bay and Port Alfred. This method compares the means of two samples, (in this case the sea temperature between 6-12 November, for two years, 2002 and 2015, for the same depth). Consequently, a depth of 9 m in 2002 was compared with 9 m from 2015, 14 m 2002:2015, 18 m 2002:2015, and 21 m 2002:2015. Since the aim here was to see if temperature difference may have been responsible for the drop of mean ML for three years from 2015 onwards, a comparison of temperatures with mantle length distribution recorded in a "typical" year (2002) was compared with temperatures recorded in 2015, where a drop in mean mantle length was significant.

CHAPTER 4

4. RESULTS

4.1. EXPLORATION OF LENGTH FREQUENCY DATA OF *LOLIGO REYNAUDII* COLLECTED BETWEEN PLETTENBERG BAY AND PORT ALFRED.

The lengths of *L. reynaudii* for females ranged from 10–26 cm (two outliers removed), with males ranging from 11–47 cm between the years 1996 to 2017. The lengths of *L. reynaudii* varied each year. The size range started at the lowest from 10 cm mantle length (2017) for females and 11 cm (2008) for males, with upper range sizes for females from 19 cm mantle length (2017) to 29 cm mantle length (2009; 28 and 29 cm being outliers) and from 35 cm mantle length (2016) to 47 cm mantle length (2013) for males (Appendix 1 Table 3). The lower part of the mantle length range is not informative as it depends upon the jigging method used to collect the data. The highest mean length of males was 35 cm in 2002, with females having a highest mean length of 22 cm in 2013 (Appendix 3 Figure 17, Appendix 1 Table 3). Some datasets for the same year were collected on different vessels and in different localities, which led to variation in ranges, means, and medians, but these differences are small. The summary statistics for length frequencies (minimum, maximum, mean, median) of *L. reynaudii* samples are given in Table 3 (Appendix 1).

The length frequency distribution for the selected years is shown in Figures 2 (kernel density) and Appendix 3 Figure 17 (histogram as an additional results). Generally, males had a wide range of lengths throughout the years, while females had sharp, well-defined peaks and a smaller range of lengths. The mean mantle length of males was larger than that of females. Female length distributions were skewed to the left, whereas male distributions were more uniform (Figures 2, Appendix 3 Figure 17). A general trend for the whole data series was that of relative stability, with the length frequencies, specifically, showing more consistency in females from 1996 to 2010, with some small shift between 2011 and 2013. Furthermore, a smaller mean length was apparent from 2013 to 2016, with two sharp peaks in 2017 (coming from two different vessels), situated in both the smaller and larger length range. Two more small and wide peaks were also seen in 2006 (one vessel). These results were further explored

using linear regression to test the difference. Moreover, there was a slight change of distribution in mean length throughout the years for both sexes, as illustrated in Figure 17 Appendix 3. In 2017 female length frequency distribution returned to the average.

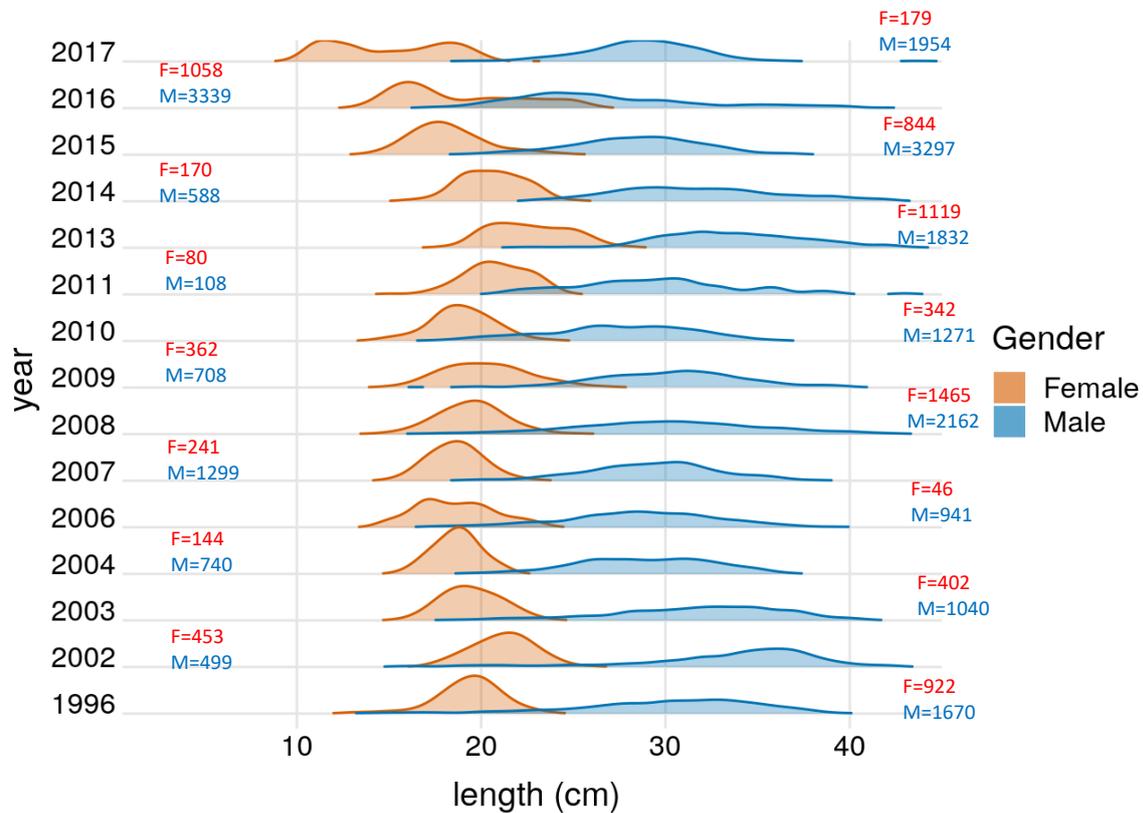


Figure 2: Graph showing the pattern of length frequency distribution of *L. reynaudii* from 1996 to 2017.

The results from testing for a temporal trend in mean length (95% confidence interval) are shown in Figure 3 for both males and females for all the years considered in the study. There is no apparent significant trend in the data when all years are considered together (F statistic for males 1.876 and for females 1.825; Figure 3, Appendix 1 Table 4). There are no major differences in means of both males and females over the years. The females show means closer to one another for the years 2003, 2004, 2007, 2010, while male length means are more widely spaced in all the years except in 2004, 2006, and 2007 (Figure 17).

Most importantly, there is a pattern of differences for females from 2013 to 2016 showing a decrease in length from year to year (Figures 2 and Appendix 3 Figure 17) as mentioned above, which was generally found to be significant for females but not significant for males (Appendix 1 Table 6). An Analysis of Covariance was conducted together with the median test to analyse this pattern of change in length frequency for years between 2014 and 2016, which showed that the decrease was statistically significant for both sexes (Tables 7-22, Appendix 1).

A piecewise linear regression was conducted to explore the mean mantle length before and after 2013 for both sexes to further investigate the change in observed mantle length in females. Results (Figure 4) show a visible change in mean length for the years after 2013 for both sexes. A Piecewise regression test was used to explore this pattern of change, which showed a significant decline in mantle length of females after 2013 (Figure 4 and Appendix1 Table 5).

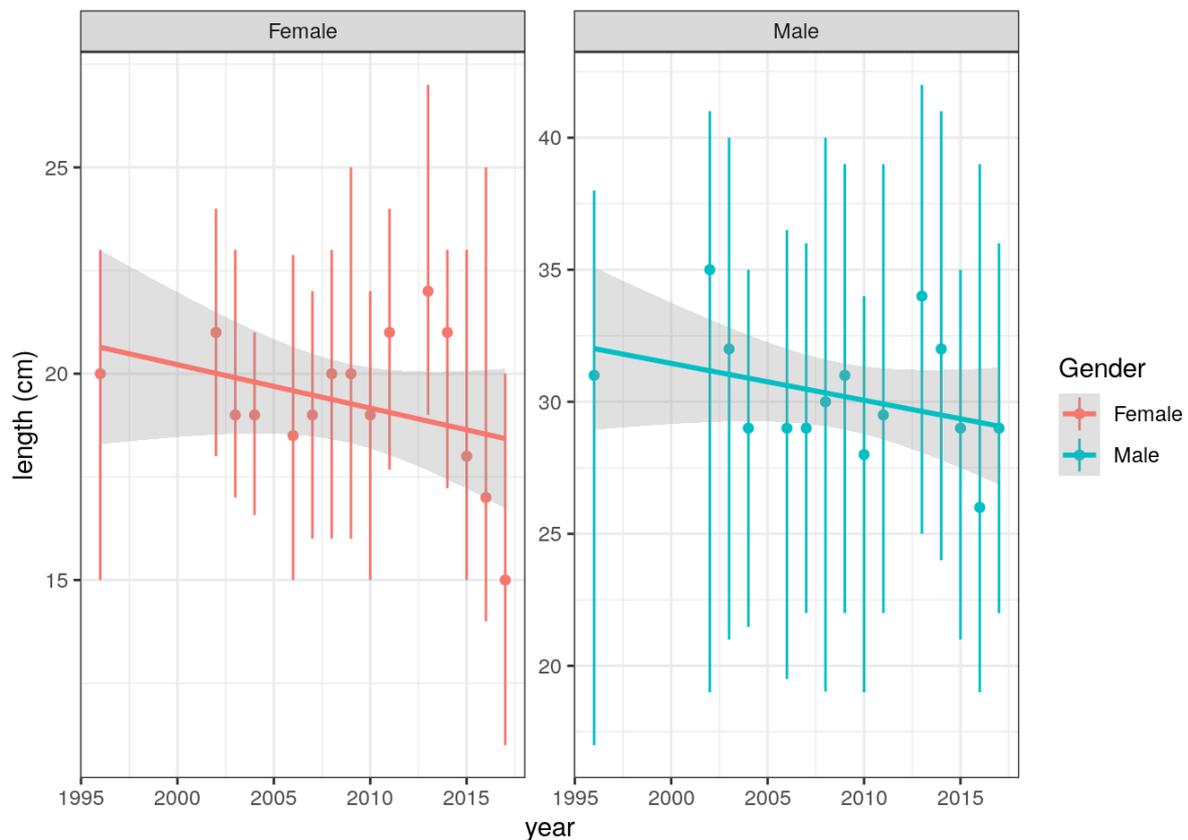


Figure 3: Summary of the temporal trend in mean length (95% confidence interval) for both male and female *L. reynaudii* from 1996 to 2017.

This result suggests that *Loligo reynaudii* length frequency distribution experienced a real short-term drop (three years in a row) in the investigated time series. The best-known environmental factor which may have been responsible for this drop was temperature. However, this link proved to be not significant (Tables 23-26, Appendix 2). The results from paired t-test indicated that there is no significant difference in sea temperature between 2002 and 2015 for depth strata (9 m, 14 m, 18 m, 21 m) (Tables 23-26, Appendix 2). Fluctuations in Length Frequency are common in squid. The most obvious biological factor which may have played a role was abundance, which was assumed to be reflected in the catch.

Results of linear regression illustrating the relationship between the mean length and jig catch are presented in Figure 5. This link between the catch and length frequency was not significant for males, but weakly significant for females in Appendix 1 Table 6 (F Statistic (df = 1; 13) is 3.686 and 5.394 for males and females respectively, R^2 is 0.221 for males and 0.293 for females).

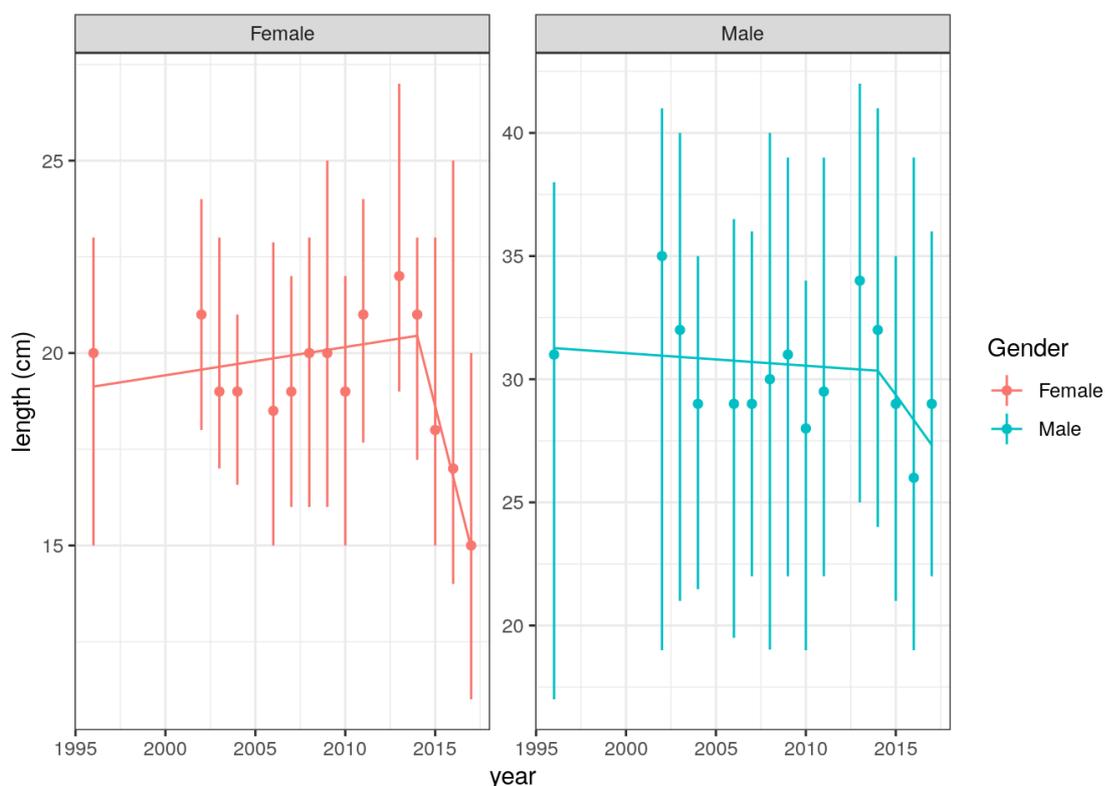


Figure 4: Plot of the mean length piecewise linear regression to show the trend in mean length of male and female *L. reynaudii* before and after 2013.

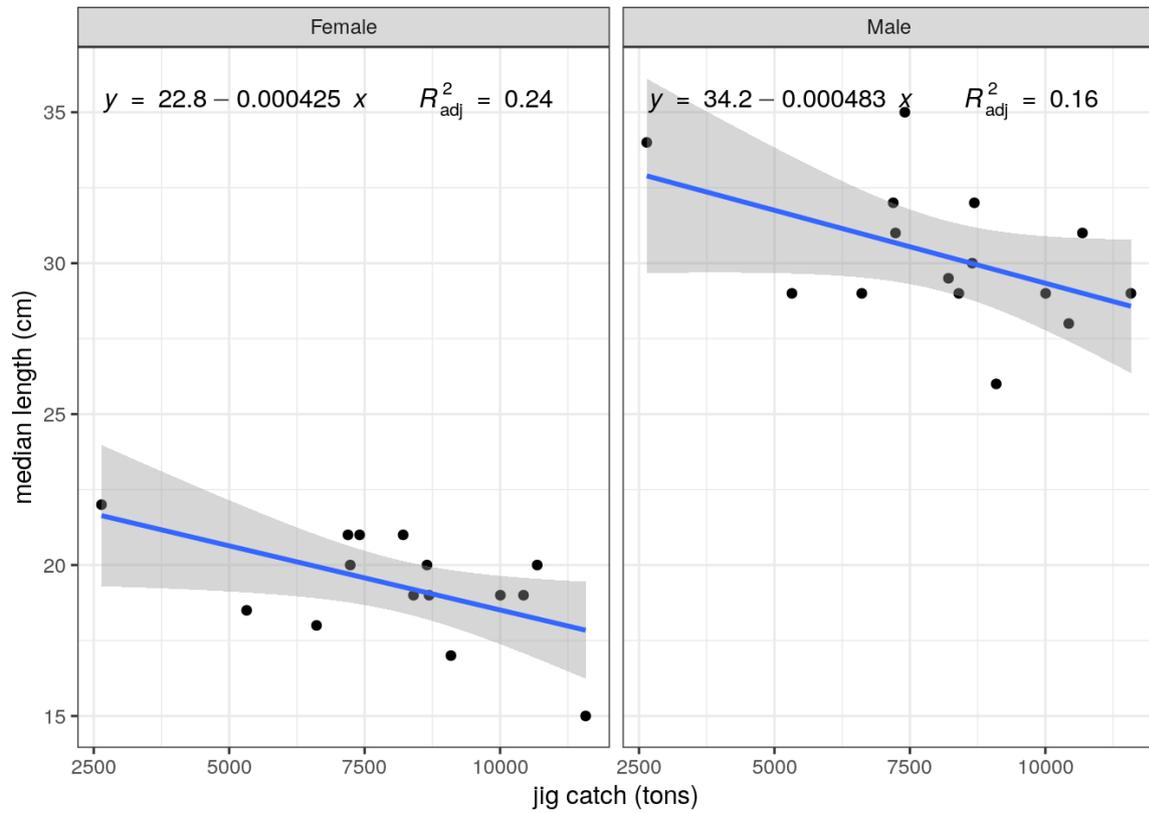


Figure 5: Association between overall catch from jig fishery and median length of males and females *L. reynaudii*.

4.2. EXPLORATION OF LENGTH-WEIGHT DATA OF *LOLIGO REYNAUDII* OFF THE SOUTH COAST OF SOUTH AFRICA, BETWEEN PLETTENBERG BAY AND PORT ALFRED.

The sample size of females ranged from 24 to 124 individuals, and males from 34 to 123 individuals from 1994 to 2016. Mantle length ranged from 110–278 mm for females weighing 47–378 g. The males were from 22–946 g, with a mantle length of 88–450 mm, from 1994 to 2016 (9 years considered in the study). The overall average length and weight varied between years. The size range of females both in length (mm) and weight (g) was smaller than that of males. Males grew to larger sizes than females (Figure 6).

The length-weight relationship patterns are shown in Figure 6 with the two caecum colours (composite yellow, and white) also given on Figures 7, 8. The results showed that in all the years, both sexes had more individuals with yellow fluid in the caecum (indicating six to seven hours after food ingestion) (Figure 6). However, white caecum fluid occurred significantly more frequently in males than in females (Appendix 3 Table 27 and Figure 8). A GLM model was performed to investigate link between caecum colour and sexes. There was a significant interaction of sex and caecum colour. A white caecum occurred significantly more often in males than in females (Table 27; dof = 1; $p < 0.05$).

Frequency distributions for the different caecum colours are shown in Figures 7, 8. The patterns indicate a higher density of yellow caeca than white throughout the selected years. Also, the incidence of white fluid between years varied considerably; however, the available time series was too short to identify any patterns. In 2002 and 2016 white caeca were not visible in either sex. The year 2005 had a higher density of males with a white caecum than other years, with 2002 having less. Females had a high density of white caeca in 2002 and lower incidences of this colour in 2000 and 2003.

The overall frequency distribution of yellow and white caeca are shown below (Figure 8). Males had white caeca more frequently than females.

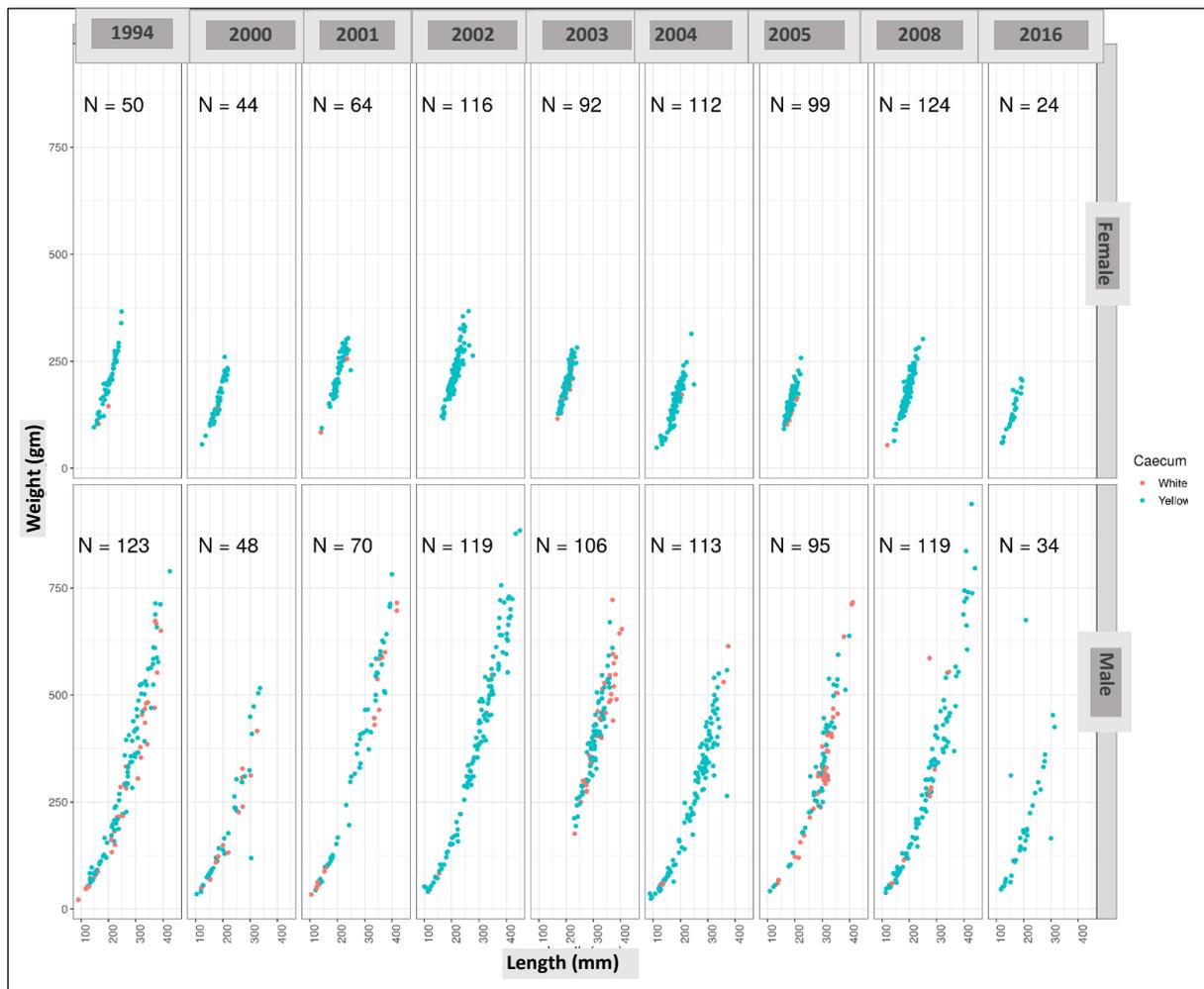


Figure 6: The length-weight relationship for both male and female *L. reynaudii*, over 10 years between 1994 and 2016 (caecum colour is also indicated).

The predicted ML_TW relationships with the corresponding confidence intervals (for possible future use beyond investigated ranges) are given in Appendix 3 Figure 18. The figure shows a correlation between the given variables (length and weight).

The pattern of predicted length-weight with the corresponding confidence interval based on parameter estimate from bootstrapping is shown in Figure 9. There is no large dispersal of individuals in most of the years for either males or females. This supports the fit of the model to the length-weight data (Figure 9). The distribution of the estimated parameter 'a' and 'b' from bootstrap parameters differed between the years for both sexes (Figure 10).

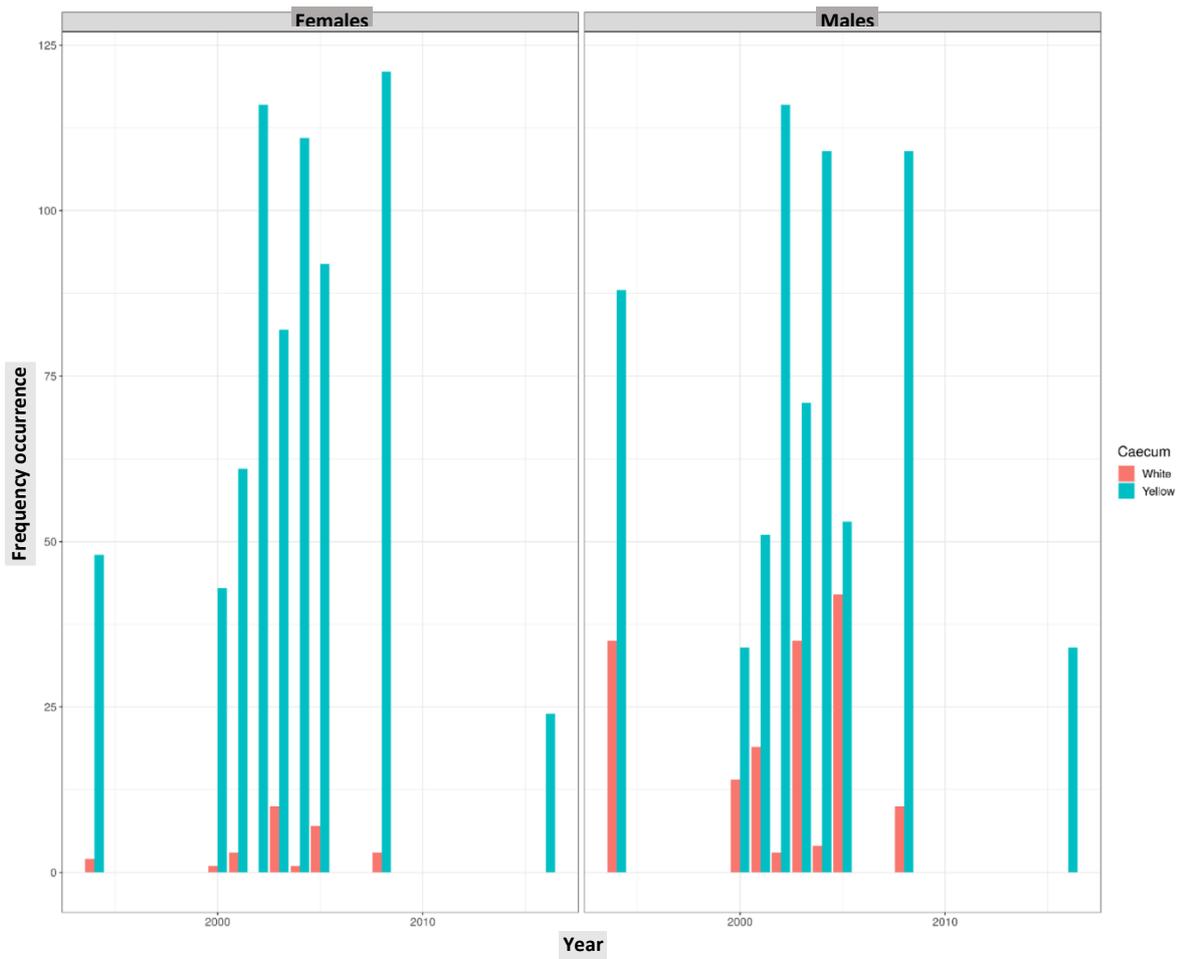


Figure 7: The distribution of different caecum colours of *L. reynaudii* over time by sex and year.

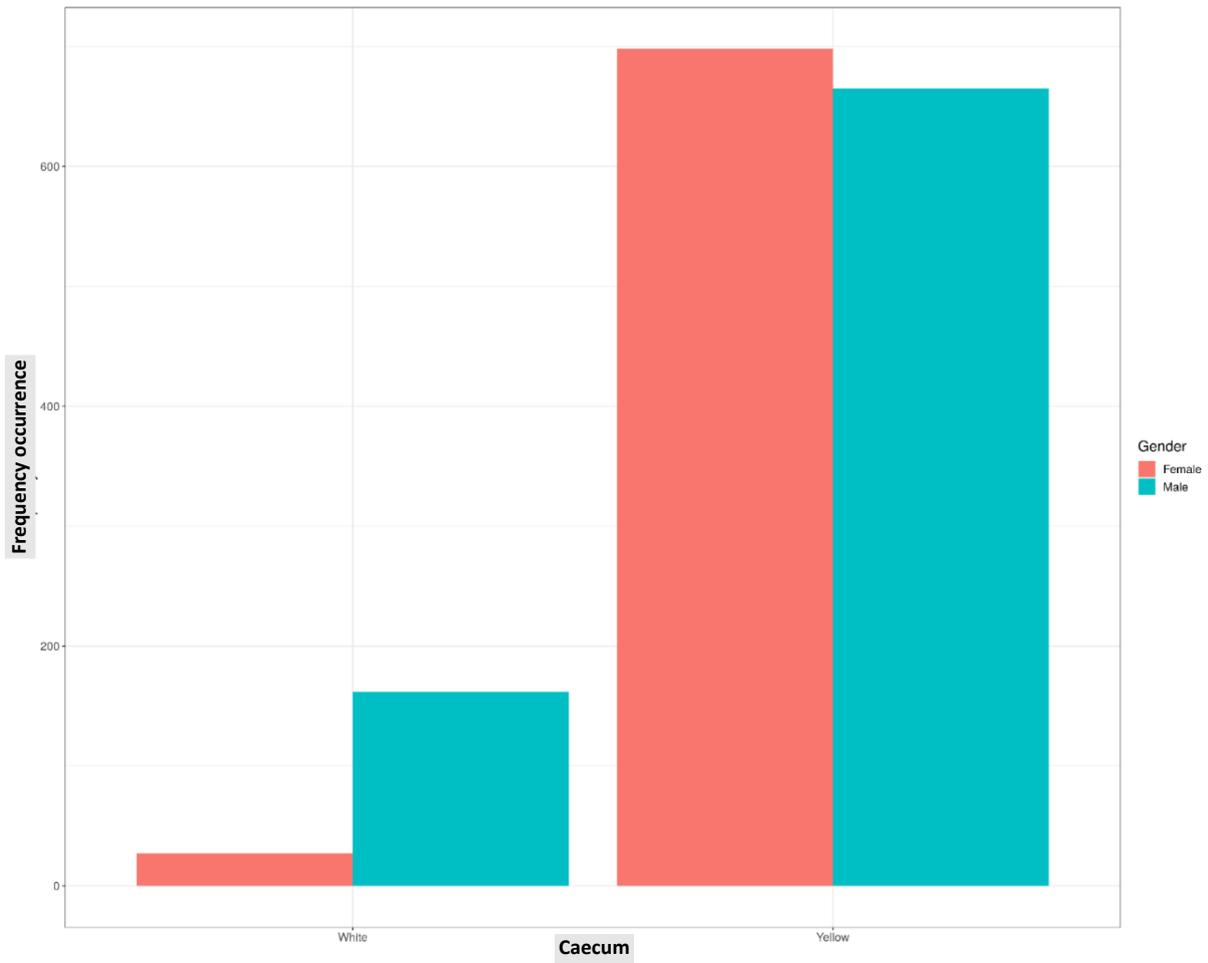


Figure 8: Overall pattern in distribution of caecum colour for male and female *L. reynaudii* over the years.

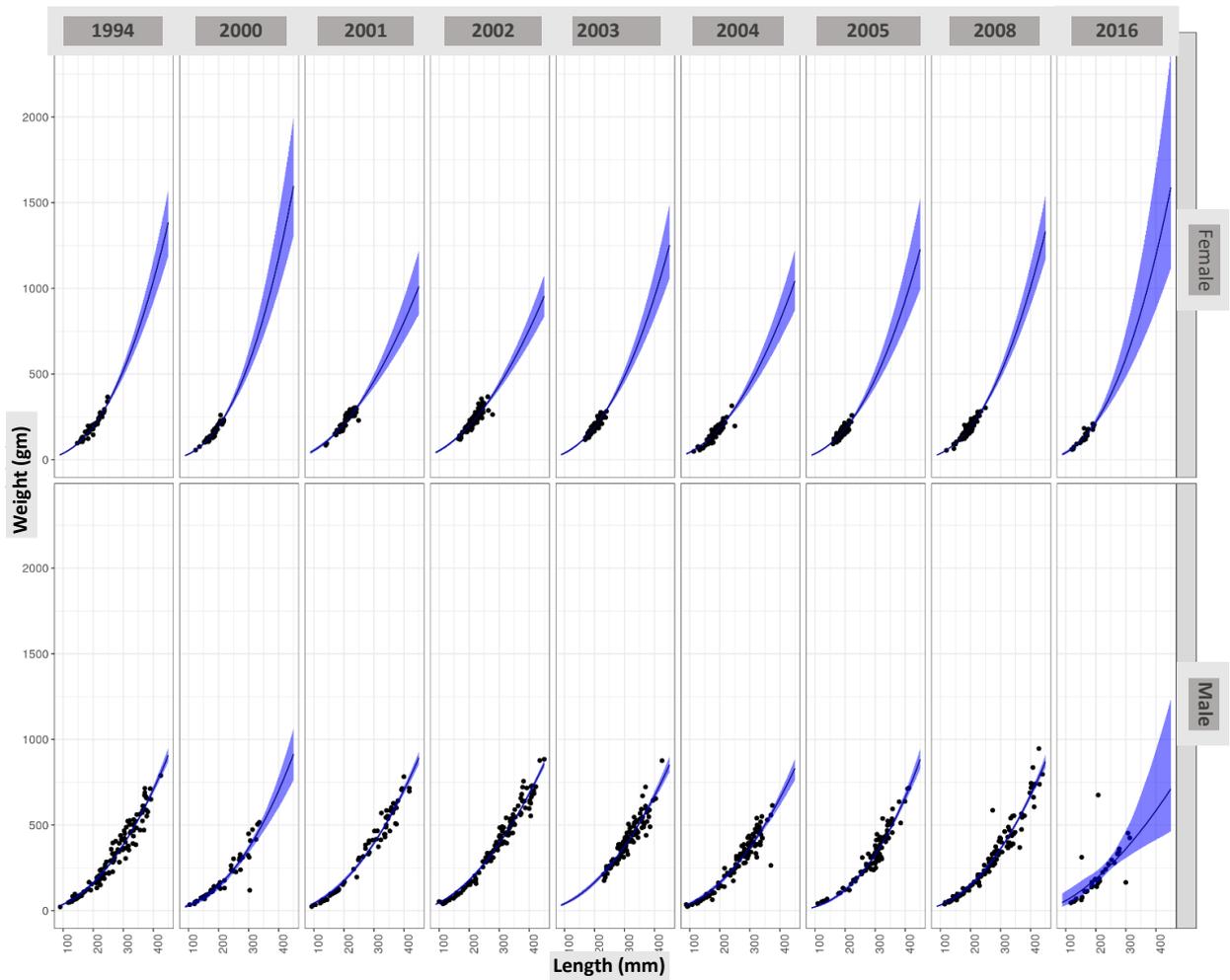


Figure 9: The predicted length-weight relationship with the corresponding confidence interval based on parameter estimate from bootstrapping.

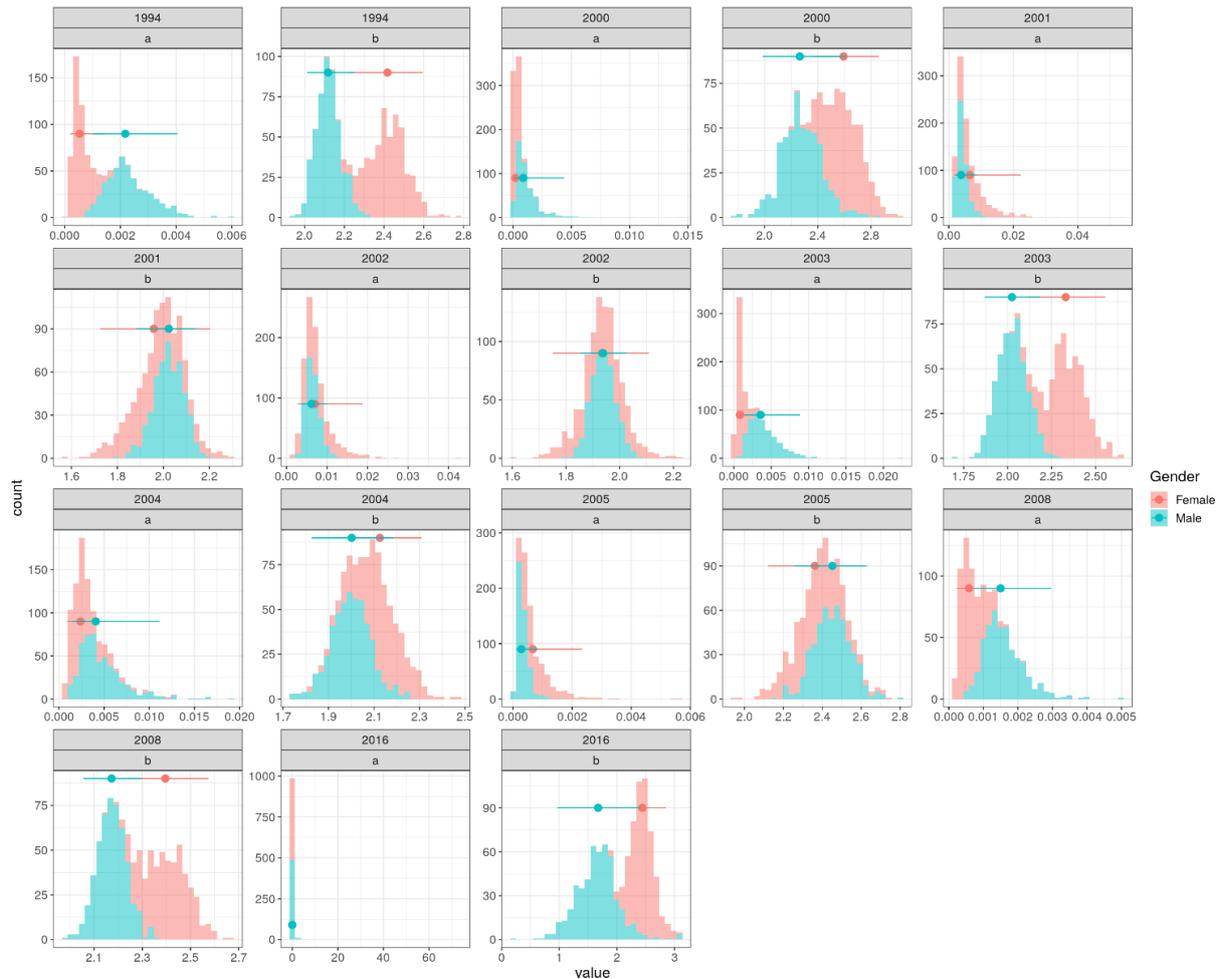


Figure 10: Distribution of the estimated parameters: 'a' and 'b' of $W = aL^b$ from bootstrap for both sexes and all the years for which data exist

The fit of linear regression from estimated parameters with the corresponding confidence interval showed that there is no clear trend of ML_TW between years and, on visual inspection, these relationships seem to be uniform (Figure 11). The linear regression from bootstrap distribution, which was added to the trends in the estimated parameters, also shows no trend in length-weight relationship over the years (Figure 12).

The two model diagnostic tests were performed and, to a large extent, no systematic patterns were found, which shows that the model performed fairly well (Figures 13(a,b) and 14(a,b)). These graphs generally show no systematic pattern (bias) in the predictions from the model, except that in 2002 the model tends to underestimate weight for larger size individuals and

overestimate weight for the smaller size class for females (Figure 14b).

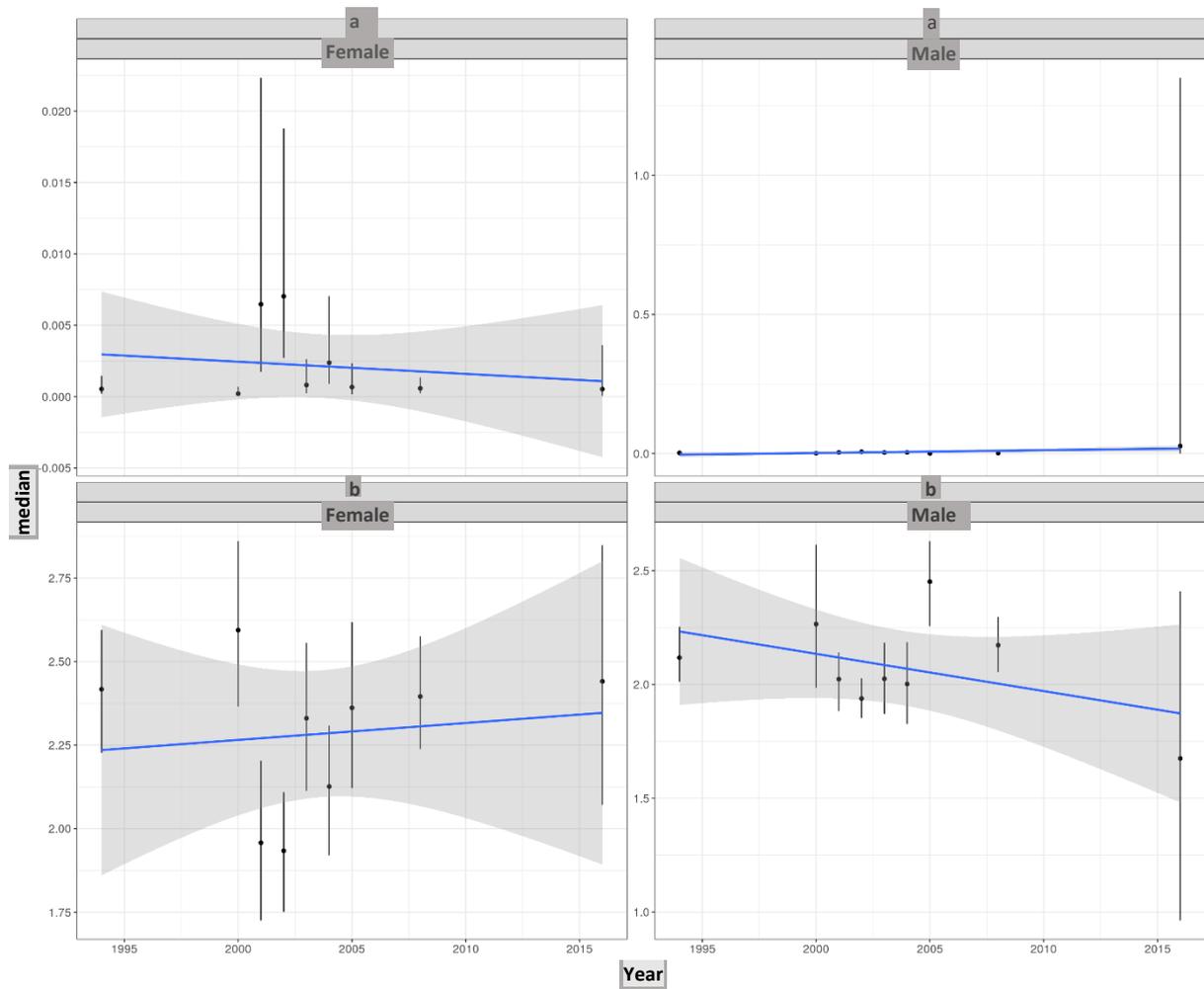


Figure 11: Estimated parameters of length-weight relationship of *L. reynaudii* with the corresponding confidence interval calculated, based on estimated standard error.

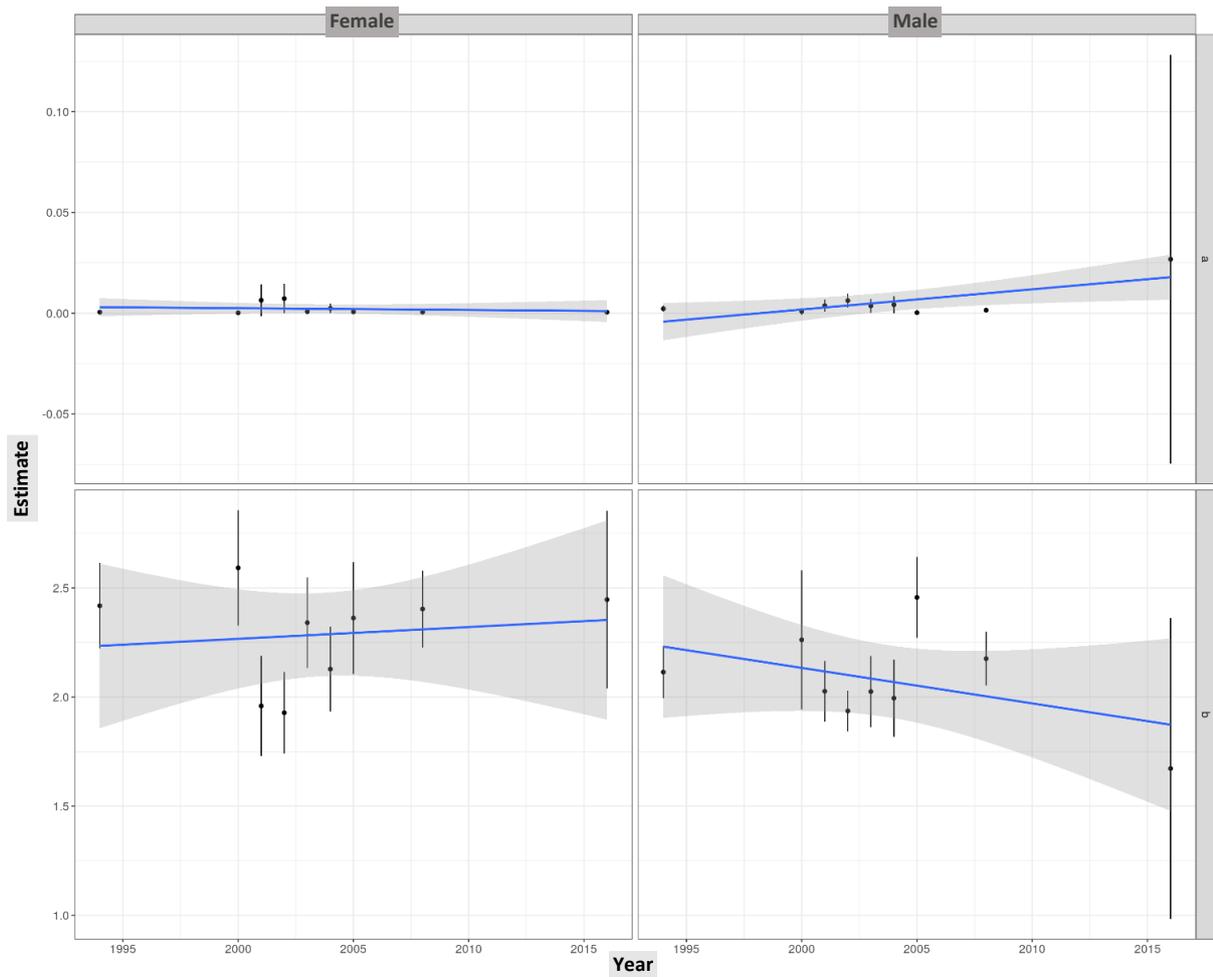


Figure 12: Bootstrap-based estimated parameters for length-weight relationship of *L. reynaudii* and their corresponding confidence interval.

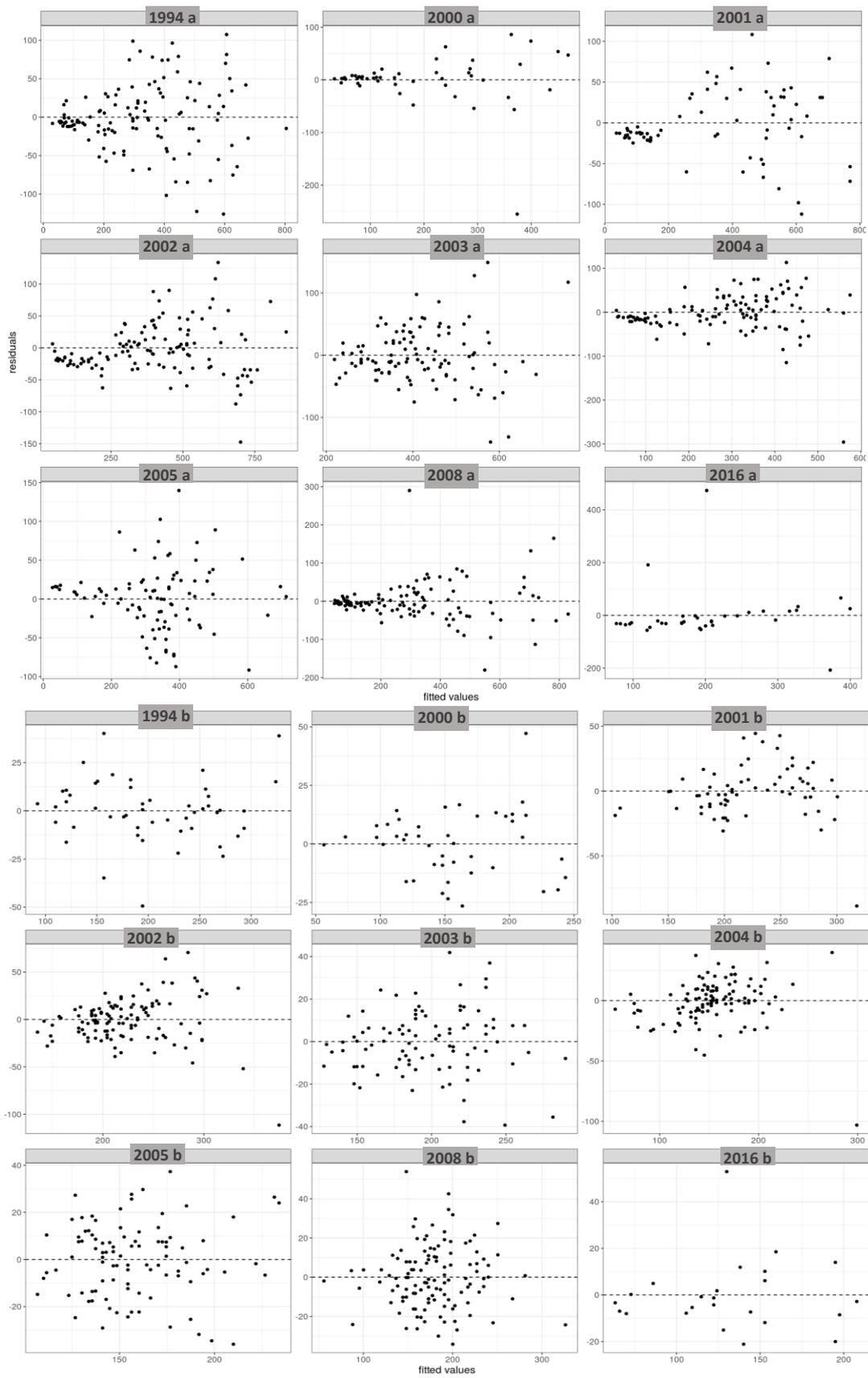


Figure 13: Standard model diagnostic for plots of fitted values vs residuals; a) – males, b) – females

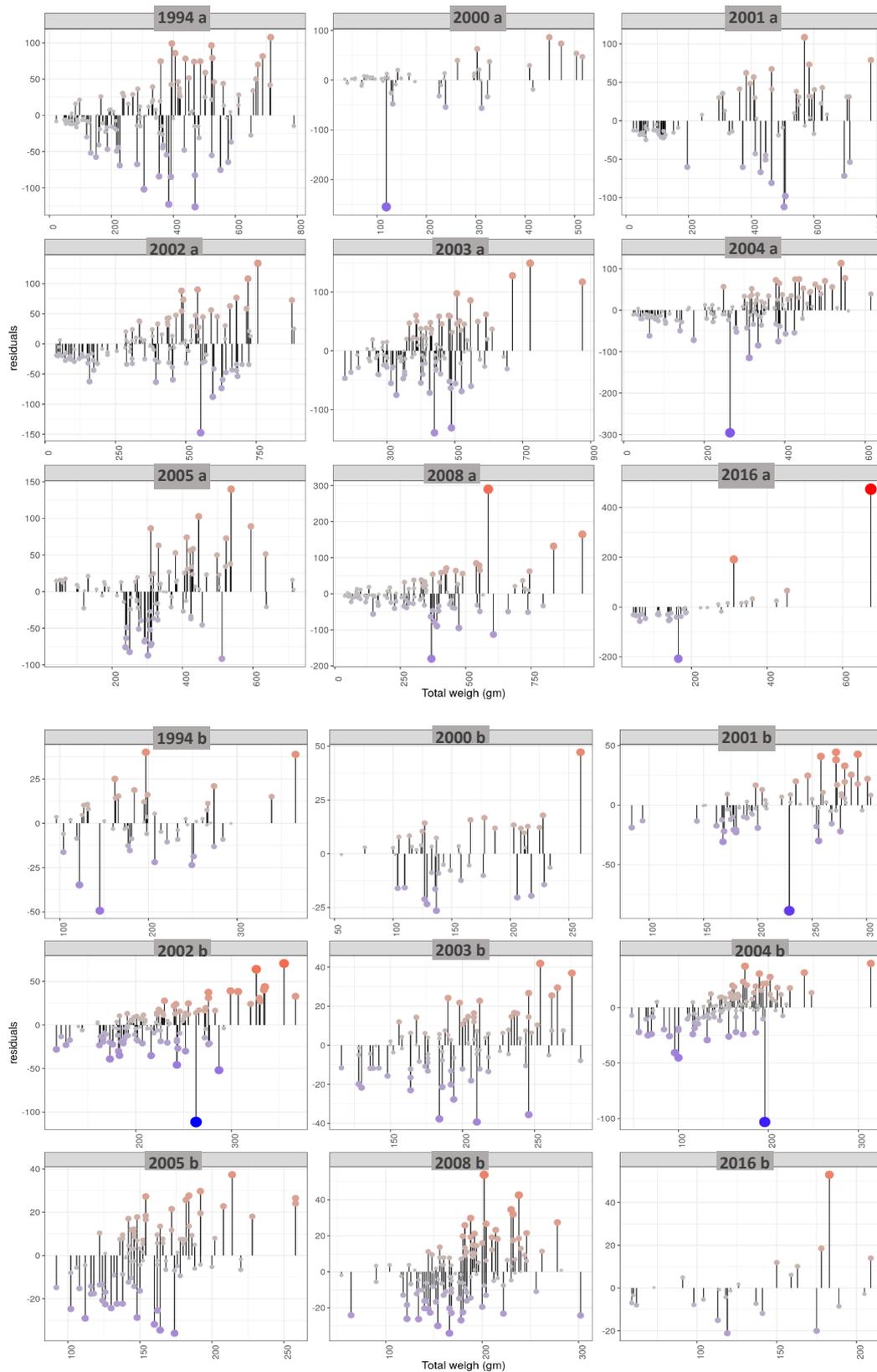


Figure 14: Model diagnostic showing the plot of residuals vs observed value; a) – males, b) – females.

Link between Length-Weight (LW) relationship and catch

The relationship between shape parameter b of LW model and jig catch (Figure 15), found no significant relationship for either sex (the value of R^2 is 0.029, 0.17 for females and males, respectively). There is no relationship between the jig catch and the intercept parameter of length-weight (the value of R^2 is 0.069, 0.1 for females and males, respectively) (Figure 16).

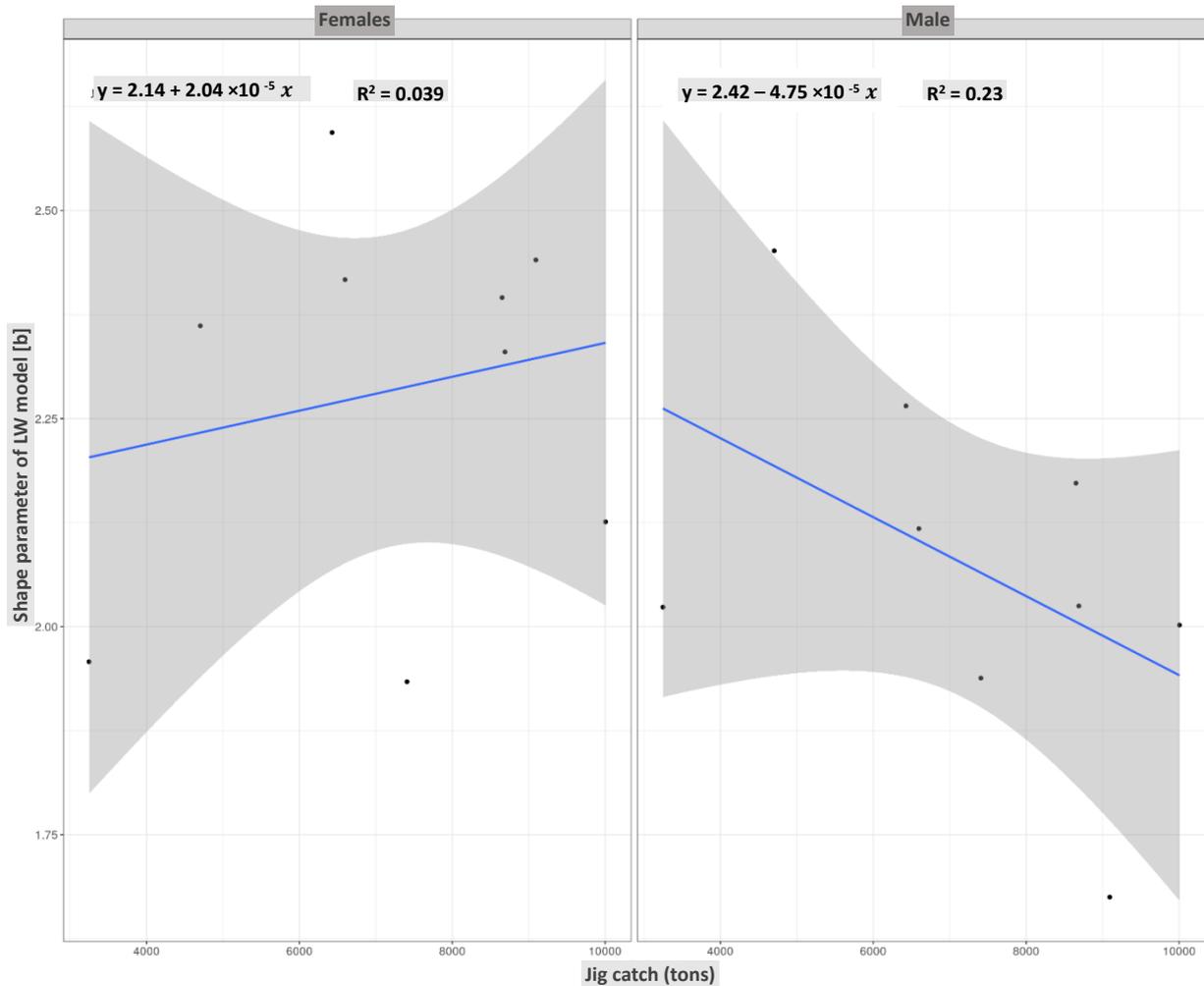


Figure 15: Exploratory plot of relationship between shape parameter of Length-Weight relationship and catch of *L. reynaudii* between Plettenberg Bay and Port Alfred.

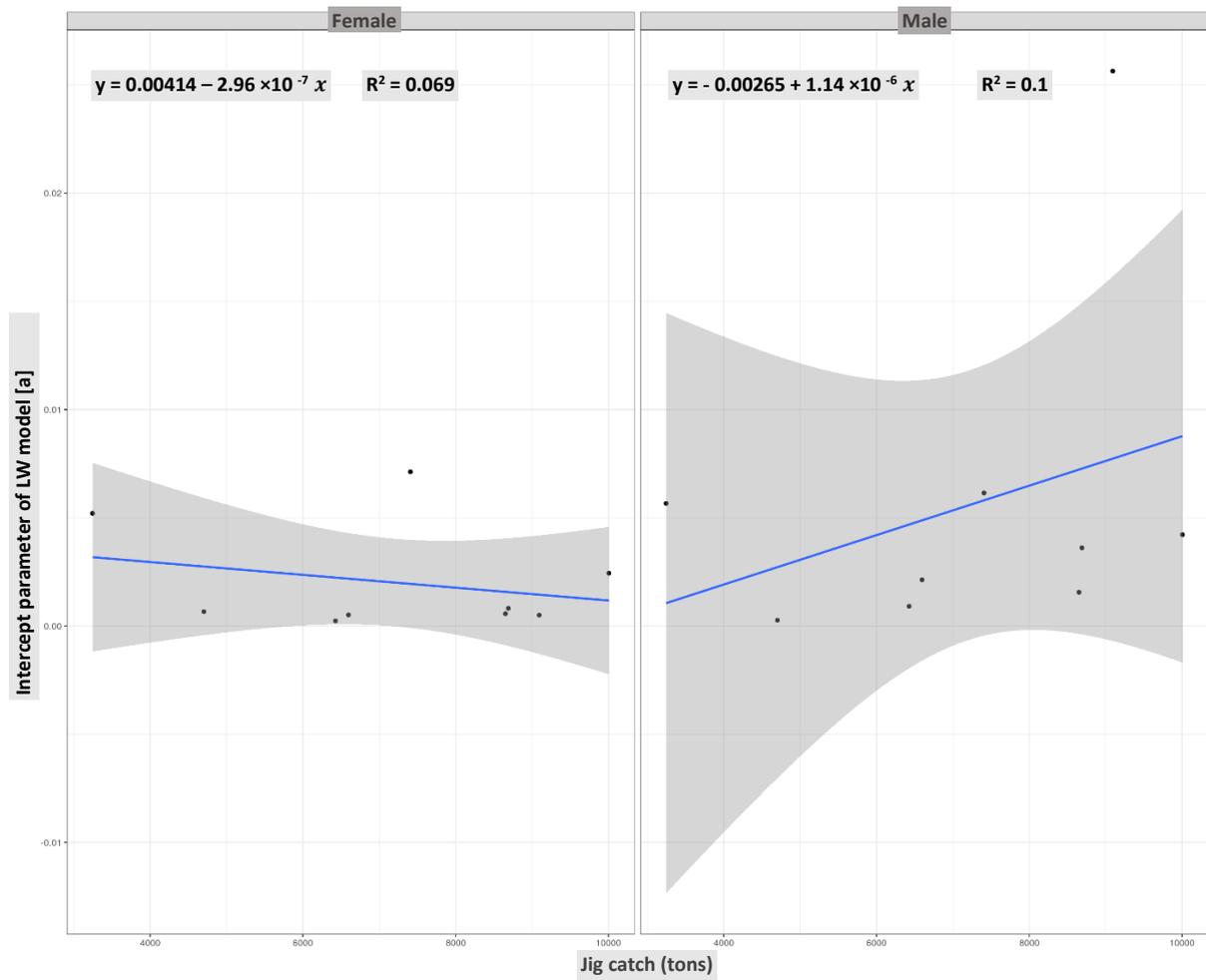


Figure 16: Exploratory relationship between intercept of the Length-Weight model and catch of *L. reynaudii*.

CHAPTER 5

DISCUSSION

This study provides an assessment of the temporal changes in length frequency, length-weight relationship and the effects of caecum colour of *Loligo reynaudii* over two periods of fifteen years and nine years. These parameters are important for population dynamics diagnostics and as indicators of well-being of the population of squid and fish (Jackson & Moltschaniwskyj 2001; Miller et al. 2008; Barrett et al. 2009; Starr et al. 2015; Sabrah et al. 2015). Generally, weight as a function of length in cephalopods is an important part of biological studies (Khaliluddin & Haq 1998).

The imbalance in numbers of investigated individuals in the samples (more males than females) of this study does not necessarily reflect the size of the survey catch or the relative abundance of species in any year. The reverse has been seen in the study of *Loligo chinensis* by Mulyono et al. (2017) in the waters of Lamongan Regency, East Java province, Indonesia, with higher numbers of females than males. In the latter instance this difference was assumed to be due to the behaviour of *L. chinensis*, and the fishing factors. Female growth is completed entirely before maturation of the gonads, while male growth continues even when the gonads are already mature (Mulyono et al. 2017). Our data showed that male *L. reynaudii* were larger than females, due to the physiology of squid.

Anecdotal evidence from fishers suggests that the length frequency and ML_TW relationship had changed over years. Results presented here suggest that there are no long-term major trends and variations of length frequency distributions and ML_TW relationships between years in the investigated time series in chokka squid. This result is useful for future investigations of variation of these parameters between regions and between sub-populations (van der Vyver et al. 2015) because it provides some fundamental understanding concerning possible temporal variability. The variation of these parameters is an important part of routine biological, fisheries-related analysis of fish and squid. Length structure and its dynamics in the population is often a substitute for age structure if the latter is not available

and it also provides information about size composition of a population (Miller et al. 2008; Fossen & Gordon 2015). Furthermore, lengths describe geographic and temporal patterns which are linked to squid abundance (Pierce et al. 1998; Hastie et al. 2009).

Food shortage in the natural environment may lead to change in the optimal ML_TW relationship (Dawe 1988; Jackson & Moltschaniwskyj 2001). Environmental factors (e.g. rise in the temperature) may lead to smaller lengths at maturity and subsequent smaller populations (Forsythe 1993, 2004; Jackson & Moltschaniwskyj 2001). Forsythe et al. (2001) demonstrated that an increase in temperature during the juvenile growth phase may result in individuals with substantially faster growth rates and earlier maturation. The influence of seasonality on length has also been seen on *L. chinensis*, where the summer population increased more quickly in length than the winter population (Mulyono et al. 2017). Large-scale population investigations based on length and length-weight relationships were conducted to highlight various and differing aspects of squid life history. Murata (1978) and Lange and Johnson (1978) carried out this research to investigate differences between areas, seasons and years (by sex) and to enable easy conversion of catch into numbers (Jackson & Moltschaniwskyj 2001). Dawe (1988) did research to investigate the effect of diet on condition and growth (e.g. feeding intensity increase individual weight of squid), and ML_TW relationships aided Clarke (1986) to develop formulae used to calculate the weight of the prey consumed by cephalopod predators. The same was true for work by Cooper (1979). Research by Olyott et al. (2006, 2007) investigated the dynamics of sexual maturation in chokka. Sometimes length frequencies and length-weight relationships are collected and presented without any particular aim except vague 'population characteristics' (e.g. Khaliluddin & Haq 1998; Karnik & Chakraborty 2001; Mulyono et al. 2017) or are used for market-related issues (e.g. Miyahara, 2004). However, none of these studies has considered comparing squid of the same maturity stage and same feeding status. Dawe (1988) did incorporate 'somatic condition' into the analysis but rather as a measure of squid condition (determination if the squid was feeding well before analysis or not). Collection of such comparable data as in this work (time, area, depth, maturity and stomach stage must be the same) is rather difficult and such long time series are rare.

The results presented here indicate that changes in length frequency from year to year do not show any significant trend when analysed as one dataset; only in a dataset limited to three years upon visual inspection (Figure 2) were highly significant changes detected. Statistical analysis of long-time series found small changes of length frequency and ML_TW relationship to be not significant, however, in a small scale these changes may be important. Moreover, what fisherman interpreted as long-time trend may be short flashes of change, which are unlikely to impact general life cycle. However, efforts to explain these changes were not successful. Fluctuations in Length Frequency of squid are common, which may happen from year to year as an indication of important changes in biology of squid. Sea temperature regime differences are most often seen as the possible cause of mantle length changes (Forsythe 1993, 2004; Jackson & Moltschaniwskyj 2001; Rodhouse et al. 2014). Furthermore, temperature is known to vary with season, latitude and depth (Forsythe 2004). A small change in temperature has a direct impact on growth rates of parlarvae squid and possibly population structure in the later stages of life (Forsythe 1993, 2004). These were supported by studies of loliginids and other aquatic species, where temperature affects metabolism, ovary development and development of egg pods (Roberts 1998). Squid is known to be a continuous, asymptotic growing animal, with the ability to generate new muscle fibres throughout their lifespan (Jackson & Moltschaniwskyj 2001, 2002). Changes in their life history features have been seen in combination with seasonal changes in their environment (Jackson & Domeier 2003). Furthermore, cephalopods are poikilotherms, the metabolic rate rises or drops directly with temperature, which further affects feeding and growth rates (Forsythe 1993). The small changes of length frequency were then investigated if they are not related to temperature. Simple correlations of carefully chosen temperature data with length frequency changes were not significant in our case. The temperature regimes between the selected years for the same depth strata were not significantly different. No possible connection was found between the sea temperature and length frequency, or the ML_TW relationship over the years. The negative correlation between temperature and length frequency and ML_TW do not mean that there is no relationship. This is the first attempt to look at correlation between length frequency and ML_TW relationship of chokka squid.

Broader, more representative, long time series analysis is required for the future research. Although there is some signal in correlations of length frequency changes for females with total squid catch for a given year, this signal is too weak to attempt to explain it. Further analyses with a longer available time series and more sophisticated catch datasets (such as males and females separated, various geographic limitations, etc.) may be worth exploring in the future. These data were not available for the present project. The study conducted by Barrett et al. (2009) shows that fishing substantially influences population characteristics of many species, including altering mean sizes and abundance of rock lobster. These influences have been noted on rock lobsters and their prey species, such as urchins and abalone (Barrett et al. 2009). The implementation of MPAs to protect some species from fishing has generated detectable positive changes in the recovery of good growth, lower mortality rates, conservation of the biodiversity of fish and other invertebrates, and in tracking the ecosystem effects of fishing. Furthermore, closed seasons protect squid spawner biomass against stock collapse.

The ML_TW data were uniform and there were no significant trends between the years for either sex. This feature has also been observed in other species of cephalopods, generally loliginids (Karnik & Chakraborty, 2001). However, the ML_TW for a single year showed a close relationship. The distribution of estimated parameters 'a' and 'b' differed between sexes throughout the years, indicating differences in the relationship of ML_TW of males and females. These differences have also been observed in other species of squid, where the 'b' value was closely related to the growing stages of squid (Murata 1978). Correlations between ML_TW relationships and total catches were not significant, indicating that there is no relationship between ML_TW and jig catch in this study. However, an interesting pattern emerged when the incidence of the caecum colour between males and females was investigated. Males had a significantly higher occurrence of white caeca than females did. A white caecum indicates non-feeding squid and various shades of yellow caecum indicate a period of six to seven hours after ingestion of food (Lipiński 1987). This is an important result, since published papers about chokka food and feeding (Lipiński 1987, 1990, 1992; Sauer & Lipiński 1991) do not provide enough information about feeding patterns of males and

females and differences thereof, especially on the incidence of empty stomachs per sex on their spawning grounds. The pattern reported here (consistent over years) may indicate that spawning behaviour of males on the spawning grounds (e.g. guarding egg-laying females) leaves them relatively less time than females to forage. Generally, condition of squid feeding is also influenced by seasonal changes and habitat (Mulyono et al. 2017).

Fishers often spend their lives out on the ocean and anecdotal reports frequently reflect real changes. In this case the results do not indicate a lasting change in the parameters tested but do indicate changes over a few years, after 2013. The total catch of squid was, in fact, very low in 2013 and 2014, prompting concern from both industry and researchers, which is also the period during which this study found a reduction in the average mantle length for both sexes. It is this observation that would be easily noticed by fishers. For *Loligo reynaudii*, spawning occurs throughout most of the year but peaks in early summer, and demonstrates a smaller peak over winter (Sauer & Smale 1991; Roberts 2005; DAFF 2014; Augustyn & Roel 1998). The reduction in average length may well be linked to poor recruitment for those years, however many factors may have been responsible. Results of this study are important as they contradict common perceptions by fishermen that the length frequency and weight of chokka have changed from the inception of the fishery in 1985, but may possibly reflect short-term changes which could be linked to years of poor recruitment. This needs further investigation.

REFERENCES

- Aphalo, P.J., 2018. *Ggpmisc: Miscellaneous Extensions to 'Ggplot2'*. <https://CRAN.R-project.org/package=ggpmisc>.
- Arkhipkin, A.I., Rodhouse, P.G., Pierce, G.J., Sauer, W., Sakai, M., Allcock, L., Arguelles, J., Bower, J.R., Castillo, G., Ceriola, L., Chen, C.S., Chen, X., Diaz-Santana, M., Downey, N., González, A.F., Granados Amores, J., Green, C.P., Guerra, C.P., Hendrickson, L.C., Ibáñez, C., Ito, K., Jereb, P., Kato, Y., Katugin, O.N., Kawano, M., Kidokoro, H., Kulik, V.V., Laptikhovskiy, V.V., Lipinski, M.R., Liu, B., Mariátegui, L., Martin, W., Medina, A., Miki, K., Miyahara, K., Moltschanivskyj, N., Moustahfid, H., Nabhitabhata, J., Nanjo, N., Nigmatullin, C.M., Ohtani, T., Pecl, G., Perez, J.A.A., Piatkowski, U., Saikiang, P., Salinas-Zavala, C.A., Steer, M., Tian, Y., Ueta, Y., Vijai, D., Wakabayashi, T., Yamaguchi, T., Yamashiro, C., Yamashiro, N. and Zeidberg, L.D., 2015. World squid fisheries. *Reviews in Fisheries Science & Aquaculture*, 23(2), pp.92–252.
- Augustyn, C.J., 1989. Systematics, life cycle and fisheries potential of the chokka squid *Loligo vulgaris reynaudii*. PhD thesis, University of Port Elizabeth, South Africa.
- Augustyn, C.J., 1990. Biological studies on the chokka squid *Loligo vulgaris reynaudii* (Cephalopoda; Myopsida) on spawning grounds off the south-east coast of South Africa. *South African Journal of Marine Science*, 9(1), pp.11–26.
- Augustyn, C.J. and Roel, B.A., 1998. Fisheries Biology, stock assessment and management of the chokka squid, *Loligo vulgaris reynaudii* in South African waters: an overview. California Cooperative Oceanic Fisheries Investigations Report 39, pp.71–80.
- Augustyn, C.J., Lipiński, M.R. and Sauer, W.H.H., 1992. Can the *Loligo* squid fishery be managed effectively? A synthesis of research on *Loligo vulgaris reynaudii*. *South African Journal of Marine Science*, 12(1), pp.903–918.
- Barrett, N.S., Buxton, C.D. and Edgar, G.J., 2009. Changes in invertebrate and macroalgal populations in Tasmanian marine reserves in the decade following protection. *Journal of Experimental Marine Biology and Ecology*, 370(1–2), pp.104–119.
- Bell, J.D., Craik, G.J.S., Pollard, D.A. and Russell, B.C., 1985. Estimating length frequency distributions of large reef fish underwater. *Coral Reefs*, 4(1), pp.41–44.
- Clarke, M.R., 1986. *Handbook for the Identification of Cephalopod Beaks*. Oxford Science Publications: New York.
- Cooper, J., 1979. Length-mass relationships, water content and energy values for two species of squid, *Loligo reynaudi* and *Todaropsis eblanae*, off the southwestern Cape. *Fisheries Bulletin South Africa*, 11, pp.43–45.

- Crawley, M.J., 2012. *The R book*. John Wiley & Sons Publications: United Kingdom.
- DAFF *Annual Report, 2013/2014*. Cape Town. South Africa. [http://www.gov.za/sites/www.gov.za/files/DAFF Annual Report 2013–2014.pdf](http://www.gov.za/sites/www.gov.za/files/DAFF%20Annual%20Report%202013-2014.pdf)
- Dawe, E.G., 1988. Length-weight relationships for short-finned squid in Newfoundland and the effect of diet on condition and growth. *Transactions of the American Fisheries Society*, 117(6), pp.591–599.
- Elzhov, T.V., Mullen, K.M., Spiess, A. and Bolker, B., 2016. *Minpack.lm: R Interface to the Levenberg-Marquardt Nonlinear Least-Squares Algorithm Found in Minpack, Plus Support for Bounds*. <https://CRAN.R-project.org/package=minpack.lm>.
- Forsythe, J.W., 1993. A working hypothesis of how seasonal temperature change may impact the field growth of young cephalopods. In *Recent advances in Cephalopod fisheries and biology*. (Eds Okutani, T., O’Dor, R. K. and Kubodera, T). Tokyo: Tokai University Press, pp.133–143.
- Forsythe, J.W., 2004. Accounting for the effect of temperature on squid growth in nature: from hypothesis to practice. *Marine and Freshwater Research*, 55(4), pp.331–339.
- Forsythe, J.W., Walsh, L.S., Turk, P.E. and Lee, P.G., 2001. Impact of temperature on juvenile growth and age at first egg-laying of the Pacific reef squid *Sepioteuthis lessoniana* reared in captivity. *Marine Biology*, 138(1), pp.103–112.
- Fossen, I. and Gordon, J.D., 2015. Frequency distributions of fish length and otolith weight as means of estimating age of blue hake, *Antimora rostrata*. Scottish Association for Marine Science: United Kingdom, pp.87-105. <https://www.researchgate.net/publication/295073242>
- Fries, A., 2010. A gap analysis of the distributions of cephalopod species worldwide with a focus on commercially important species. Duke University Libraries: United States.
- Hastie, L., Pierce, G., Pita, C., Viana, M., Smith, J. and Wangvoralak, S., 2009. Squid Fishing in UK Waters: A Report to SEAFISH Industry Authority. http://www.seafish.org/media/Publications/AberdeenUni_SquidFishinginUKWaters.pdf
- Hatfield, E.M., 2000. Do some like it hot? Temperature as a possible determinant of variability in the growth of the Patagonian squid, *Loligo gahi* (Cephalopoda: Loliginidae). *Fisheries Research*, 47(1), pp.27–40.
- Henry, L. and Wickham, H., 2019. *Purrr: Functional Programming Tools*. <https://CRAN.R-project.org/package=purrr>.
- Jackson, G.D. and Moltschanivskyj, N.A., 2001. Temporal variation in growth rates and

- reproductive parameters in the small near-shore tropical squid *Loliolus noctiluca*; is cooler better? *Marine Ecology Progress Series*, 218, pp.167–177.
- Jackson, G. and Moltschaniwskyj, N., 2002. Spatial and temporal variation in growth rates and maturity in the Indo-Pacific squid *Sepioteuthis lessoniana* (Cephalopoda: Loliginidae). *Marine Biology*, 140(4), pp.747–754.
- Jackson, G.D. and Domeier, M.L., 2003. The effects of an extraordinary El Niño/La Niña event on the size and growth of the squid *Loligo opalescens* off Southern California. *Marine Biology*, 142(5), pp.925–935.
- Jackson, G.D., Forsythe, J.W., Hixon, R.F. and Hanlon, R.T., 1997. Age, growth, and maturation of *Lolliguncula brevis* (Cephalopoda: Loliginidae) in the north-western Gulf of Mexico with a comparison of length-frequency versus statolith age analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, 54(12), pp.2907–2919.
- Jennings, S., Kaiser, M. and Reynolds, J.D., 2009. *Marine fisheries ecology*. John Wiley & Sons Publications: United Kingdom.
- Jereb, P. and Roper, C.F. eds., 2005. *Cephalopods of the World: Myopsid and Oegopsid squids* (Vol. 2). Food and Agriculture Organization of the United Nations.
- Karnik, N.S. and Chakraborty, S.K., 2001. Length-weight relationship and morphometric study on the squid *Loligo duvauceli* (d'Orbigny)(Mollusca/Cephalopoda) off Mumbai (Bombay) Waters, West Coast of India. *International Journal of Medical Students*, 30(4), pp. 261-263.
- Kirkman, S.P., Blamey, L., Lamont, T., Field, J.G., Bianchi, G., Huggett, J.A., Hutchings, L., Jackson-Veitch, J., Jarre, A., Lett, C. and Lipiński, M.R., Mafwila, S.W., Pfaff, M.C., Samaai, T., Shannon, L.J., Shin, Y.J., Van der Lingen, C.D. and Yemane, D., 2016. Spatial characterisation of the Benguela ecosystem for ecosystem-based management. *African Journal of Marine Science*, 38(1), pp.7-22.
- Khaliluddin, M. and Haq, M.M., 1998. Determination of length-weight relationship of squid (*Loligo duvauceli*) found along Pakistan coast. *Pakistan Journal of Marine Sciences*, 7(1), pp.57–60.
- Lange, A.M. and Johnson, K.L., 1978. Dorsal Mantle Length-Total Weight Relationships of Squid (*Loligo pealei* and *Illex illecebrosus*) from the Northwest Atlantic, off the Coast of the United States. *International Commission for the North-west Atlantic Fisheries*. Res Doc.
- Lipiński, M., 1979. Universal maturity scale for the commercially-important squids (Cephalopoda: Teuthoidea). The results of maturity classification of the *Illex illecebrosus* (LeSueur, 1821) populations for the years 1973–1977. *International Commission for the*

Northwest Atlantic Fisheries, pp.1–39.

Lipiński, M.R., 1987. Food and feeding of *Loligo vulgaris reynaudii* from St Francis Bay, South Africa. *South African Journal of Marine Science*, 5(1), pp.557–564.

Lipiński, M.R., 1990. Changes in pH in the caecum of *Loligo vulgaris reynaudii* during digestion. *South African Journal of Marine Science*, 9(1), pp.43–51.

Lipiński, M.R., 1992. Cephalopods and the Benguela ecosystem: trophic relationships and impact. *South African Journal of Marine Science*, 12(1), pp.791–802.

Lipiński, M.R., 1994. Differences among basic biological parameters in a population of chokka squid *Loligo vulgaris reynaudii* (Cephalopoda: Loliginidae) sampled by three methods. *South African Journal of Marine Science*, 14(1), pp.281–286.

Lipiński, M.R. and Underhill, L.G. 1995. Sexual maturation in squid: quantum or continuum? *South African Journal of Marine Science*, 15(1), pp.207–223.

Lipiński, M.R., Butterworth, D.S. and Augustyn, C.J., 1998. Cephalopod fisheries: a future global upside to past overexploitation of living marine resources? Results of an international workshop, 31 August–2 September 1997, Cape Town, South Africa. *African Journal of Marine Science*, 20, pp.463–469.

Lipiński, M.R., van der Vyver, J.S.F., Shaw, P. and Sauer, W.H.H. 2016. Life cycle of chokka-squid *Loligo reynaudii* in South African waters. *African Journal of Marine Science*, 38(4), pp.589–593.

Martins, R.S., Roberts, M.J., Vidal, É.A. and Moloney, C.L., 2010. Effects of temperature on yolk utilization by chokka squid (*Loligo reynaudii* d'Orbigny, 1839) paralarvae. *Journal of Experimental Marine Biology and Ecology*, 386(1-2), pp.19–26.

Martins, R.S., Roberts, M.J., Chang, N., Verley, P., Moloney, C.L. and Vidal, E.A., 2010. Effect of yolk utilization on the specific gravity of chokka squid (*Loligo reynaudii*) paralarvae: implications for dispersal on the Agulhas Bank, South Africa. *ICES Journal of Marine Science*, 67(7), pp.1323–1335.

Mather, J.A. and Kuba, M.J., 2013. The cephalopod specialties: complex nervous system, learning, and cognition. *Canadian Journal of Zoology*, 91(6), pp.431–449.

Miller, E.F., Beck, D.S. and Dossett, W., 2008. Length-weight relationships of select common nearshore southern California marine fishes. *Bulletin, Southern California Academy of Sciences*, 107(3), pp.183–187.

Mirzaei, M.R., Yasin, Z. and Hwai, A.T.S., 2015. Length-weight relationship, growth and mortality of *Anadara granosa* in Penang Island, Malaysia: an approach using length-

- frequency data sets. *Journal of the Marine Biological Association of the United Kingdom*, 95(2), pp. 381–390.
- Miyahara, K., 2004. Mantle length-body weight relationship of the diamondback squid, *Thysanoteuthis rhombus*, caught in the western part of the Sea of Japan. *Bulletin of Hyogo Prefecture Technical Center for Agriculture and For Fisheries (Fisheries Section)*, 37, pp.1–8.
- Muggeo, V.M., 2008. Segmented: an R package to fit regression models with broken-line relationships. *R news*, 8(1), pp.20–25.
- Muggeo, V.M.R., 2017. Segmented: regression models with break points/change-points estimation. *R package version 0.5–2.1*. <https://CRAN.R-project.org/package=segmented>.
- Mulyono, M., Nuraini, A., Dewi, I.J., Kritiani, M.G. and Syamsudin, S., 2017. Biology aspects and length-weight relationship of squid *Loligo chinensis* in the waters of Lamongan Regency, East Java Province, Indonesia. *Aquaculture, Aquarium, Conservation & Legislation*, 10(5), pp.1221–1225.
- Murata, M., 1978. The relation between mantle length and body weight of the Squid, *Todarodes pacificus* Steenstrup. *Bulletin of the Hokkaido Regional Fisheries Research Laboratory (Japan)*.
- Olyott, L.J.H., Sauer, W.H.H. and Booth, A.J., 2006. Spatio-temporal patterns in maturation of the chokka squid (*Loligo vulgaris reynaudii*) off the coast of South Africa. *ICES Journal of Marine Science*, 63(9), pp.1649–1664.
- Olyott, L.J.H., Sauer, W.H.H. and Booth, A.J., 2007. Spatial patterns in the biology of the chokka squid, *Loligo reynaudii* on the Agulhas Bank, South Africa. *Reviews in Fish Biology and Fisheries*, 17(2–3), pp.159–172.
- Oosthuizen, A. and Roberts, M. J., 2009. Bottom temperature and *in situ* development of chokka squid eggs (*Loligo vulgaris reynaudii*) on the deep spawning grounds, South Africa. *ICES Journal of Marine Science*, 66, pp.1967–1971.
- Oosthuizen, A., Roberts, M.J. and Sauer, W.H., 2002. Temperature effects on the embryonic development and hatching success of the squid *Loligo vulgaris reynaudii*. *Bulletin of Marine Science*, 71(2), pp.619–632.
- Pierce, G.J., Bailey, N., Stratoudakis, Y. and Newton, A., 1998. Distribution and abundance of the fished population of *Loligo forbesi* in Scottish waters: analysis of research cruise data. *ICES Journal of Marine Science*, 55(1), pp.14–33.
- Pierce, G.J., Valavanis, V.D., Guerra, A., Jereb, P., Orsi-Relini, L., Bellido, J.M., Katara, I., Piatkowski, U., Pereira, J., Balguerias, E. and Sobrino, I., 2008. A review of cephalopod–

- environment interactions in European Seas. *Hydrobiologia*, 612(1), pp.49–70.
- Pierce, G.J., Allcock, L., Bruno, I., Bustamante, P., Gonzalez, A., Guerra, A., Jereb, P., Lefkaditou, E., Malham, S., Moreno, A. and Pereira, J., 2010. Cephalopod biology and fisheries in Europe. *ICES Cooperative Research Report*.
- Ritz, C. and Streibig, J.C., 2008. *Nonlinear Regression with R*. Springer Science & Business Media: New York.
- Roberts, M.J., 1998. The influence of the environment on chokka squid *Loligo vulgaris reynaudii* spawning aggregations: steps towards a quantified model. *African Journal of Marine Science*, 20, pp. 267-284.
- Roberts, M. J., 2005. Chokka squid (*Loligo vulgaris reynaudii*) abundance linked to changes in South Africa's Agulhas Bank ecosystem during spawning and the early life cycle. *ICES Journal of Marine Science*, 62(1): pp.33–55.
- Roberts, M.J. and Sauer, W.H.H., 1994. Environment: the key to understanding the South African chokka squid (*Loligo vulgaris reynaudii*) life cycle and fishery. *Antarctic Science*, 6(2), pp.249–258.
- Rocha, F. and Guerra, A., 1999. Age and growth of two sympatric squid *Loligo vulgaris* and *Loligo forbesi*, in Galician waters (north-west Spain). *Journal of the Marine Biological Association of the United Kingdom*, 79(4), pp.697–707.
- R Core Team., 2018. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. <https://www.R-project.org/>.
- Rodhouse, P.G., Pierce, G.J., Nichols, O.C., Sauer, W.H., Arkhipkin, A.I., Laptikhovskiy, V.V., Lipiński, M.R., Ramos, J.E., Gras, M., Kidokoro, H. and Sadayasu, K., Pereira, J., Lefkaditou, E., Pita, C., Gasalla, M., Haimovici, M., Sakai, M. and Downey, N., 2014. Environmental effects on cephalopod population dynamics: implications for management of fisheries. In *Advances in Marine Biology*, 67, pp. 99–233.
- Roel, B.A., Cochrane, K.L. and Field, J.G., 2000. Investigation into the declining trend in chokka squid *Loligo vulgaris reynaudii* catches made by South African trawlers. *African Journal of Marine Science*, 22, pp.121–135.
- Roper, C.F. and Voss, G.L., 1983. Guidelines for taxonomic descriptions of cephalopod species. *The Biology and Resource Potential of Cephalopods. Memoirs of the National Museum of Victoria* 44, pp.49–63.
- Sabrah, M.M., El-Sayed, A.Y. and El-Ganiny, A.A., 2015. Fishery and population characteristics of the Indian squids *Loligo duvauceli* Orbigny, 1848 from trawl survey along the north-west Red Sea. *The Egyptian Journal of Aquatic Research*, 41(3): pp.279–285.

- Sauer, W.H.H and Lipiński M.R., 1991. Food of squid *Loligo vulgaris reynaudii* (Cephalopoda: Loliginidae) on their spawning grounds off the Eastern Cape, South Africa. *South African Journal of Marine Science*, 10, pp. 193–201.
- Sauer, W.H.H. and Smale, M.J., 1991. Predation patterns on the inshore spawning grounds of the squid *Loligo vulgaris reynaudii* (Cephalopoda: Loliginidae) off the south-eastern Cape, South Africa. *South African Journal of Marine Science*, 11, pp.513-523.
- Sauer, W.H.H., Goschen, W.S. and Koorts, A.S., 1991. A preliminary investigation of the effect of sea temperature fluctuations and wind direction on catches of chokka squid *Loligo vulgaris reynaudii* off the Eastern Cape, South Africa. *South African Journal of Marine Science*, 11, pp.467-473.
- Sauer, W.H.H., Downey N.J., Lipiński M.R., Roberts M.J., Smale M.J., Glazer J. and Melo Y., 2013. *Loligo reynaudii*. In: Rosa R., Pierce G. and O’Dor R. (eds) *Advances in squid biology, ecology and fisheries*. New York, NY: Nova Science Publishers: pp.33–72.
- Schultz, L.D., Mayfield, M.P. and Whitlock, S.L., 2016. Sample sizes needed to describe length-frequency of small-bodied fishes: An example using larval pacific lamprey. *Journal of Fish and Wildlife Management*, 7(2), pp.315–322.
- Semmens, J.M., Pecl, G.T., Gillanders, B.M., Waluda, C.M., Shea, E.K., Jouffre, D., Ichii, T., Zumholz, K., Katugin, O.N., Leporati, S.C. and Shaw, P.W., 2007. Approaches to resolving cephalopod movement and migration patterns. *Reviews in Fish Biology and Fisheries*, 17(2–3), p.401.
- Sharpe, D.M. and Hendry, A.P., 2009. SYNTHESIS: life history changes in commercially exploited fish stocks: an analysis of trends across studies. *Evolutionary Applications*, 2(3), pp.260–275.
- Shaw P.W., Hendrickson L., McKeown N., Stonier T., Naud M-J. and Sauer W.H.H., 2010 Discrete spawning aggregations of Loliginid squid do not represent genetically distinct populations. *Marine Ecology Progress Series*, 408, pp. 117–127.
- Sukramongkol, N., Tsuchiya, K. and Segawa, S., 2007. Age and maturation of *Loligo duvauceli* and *L. chinensis* from the Andaman Sea of Thailand. *Reviews in Fish Biology and Fisheries*, 17(2–3): pp.237–246.
- Starr, R.M., Wendt, D.E., Barnes, C.L., Marks, C.I., Malone, D., Waltz, G., Schmidt, K.T., Chiu, J., Launer, A.L., Hall, N.C. and Yochum, N., 2015. Variation in responses of fishes across multiple reserves within a network of marine protected areas in temperate waters. *PLoS One*, 10(3), p.e0118502.

Van Der Vyver, J.S.F., Sauer, W.H.H., McKeown, N.J., Yemane, D., Shaw, P.W. and Lipiński, M.R., 2015. Phenotypic divergence despite high gene flow in chokka squid *Loligo reynaudii* (Cephalopoda: Loliginidae): implications for fishery management. *Journal of the Marine Biological Association of the United Kingdom*, 96(7), pp.1507–1525.

Verzani, J., 2014. *Using R for Introductory Statistics*. Chapman and Hall/CRC: New York.

Wickham, H., Chang, W., Henry, L., Pedersen, T.L., Takahashi, K., Wilke, C. and Woo, K., 2018. Ggplot: Create Elegant Data Visualisations Using the Grammar of Graphics. <https://CRAN.R-project.org/package=ggplot2>.

Wickham, H, and Bryan, J. 2019. *Readxl: Read Excel Files*. <https://CRAN.R-project.org/package=readxl>.

Wickham, H., François, R., Henry, L. and Kirill Müller. 2019. *Dplyr: A Grammar of Data Manipulation*. <https://CRAN.R-project.org/package=dplyr>.

APPENDIX 1

EXPLORATION OF LENGTH FREQUENCY DATA

Table 3: Dorsal mantle length summary statistics (minimum, maximum, mean and median values) for *Loligo reynaudii* by sex, at the south-eastern coast of South Africa, in areas between Plettenberg Bay and Port Alfred at the depth of up to 50 m.

Ship	Locations	Year	Dorsal Mantle Length for Females (cm)				Dorsal Mantle Length for Males (cm)			
			Sample N	Range	Mean	Median	Sample N	Range	Mean	Median
Koningsberg Bay	Aasvogel	1996	78	17-23	20,40	20	954	14-40	32.42	33
Mkuze	Krom, Jeffrey's Bay, Bird Island	1996	844	12-25	19.26	19	716	12-39	26.737	27
Pegasus	Deep Jeffrey's Bay, Gamtoos	2002	453	17-26	21.20	21	499	15-43	33.29	35
South Star	Groot Rivier, Aasvogel, Oyster Bay, Port Elizabeth	2003	402	15-24	19.50	19	1040	16-42	31.65	32
Silver Dorado	Aasvogel, Seal Island, Krom, Jeffrey's Bay	2004	144	16-21	18.67	19	740	16-37	28.87	29
Silver Explorer	Aasvogel, Thys, Krom, Huisklip	2006	46	15-23	18.61	18.5	941	17-41	28.64	29
Sparodon, South Star	Jeffrey's Bay, Oyster Bay, Maitlands, Huisklip, Krom	2007	241	15-23	18.63	19	1299	18-40	29.31	29
Erongo	Algoa Bay	2008	259	13-27	19.22	19	575	16-40	26.24	26
Maluti	Algoa Bay	2008	1206	13-28	19.54	20	1587	11-46	31.63	32

Silver Eagle	Thys	2009	114	14-27	20.25	20	362	16-42	29.91	30
Silver Explorer	Algoa Bay, St Francis, Gamtoos	2009	248	15-29	20.31	20	346	16-42	30.74	31
South Star, Sea Gull	Gamtoos, Algoa Bay	2010	342	14-24	18.97	19	1271	16-38	27.41	28
South Star, Sea Gull	Gamtoos, Algoa Bay	2011	80	15-24	20.83	21	108	21-43	29.66	29.5
Sparodon	St Francis Bay, Oyster Bay, Eerste Rivier	2013	235	17-26	20.83	21	605	20-41	31.95	32
Megalodon	Cape St Francis, Eerste Rivier, Oyster Bay, Gibson Bay	2013	884	20-28	23.13	23	1227	28-47	34.58	34
Megalodon	Maitland, Eerste Rivier, Oysters Bay, Gibson Bay	2014	141	19-25	21	21	411	23-46	32.81	33
Michelle	St Francis Bay, Oyster Bay, Eerste Rivier	2014	29	16-22	18,66	19	177	23-36	29,84	30
Silver Eagle	Port Elizabeth (Clarendon Marine), Cape Recife, Gamtoos Mouth, St Francis Bay, Oyster Bay	2015	182	14-22	17.30	17	842	19-37	28.45	29
Silver Champion	Oyster Bay, Gibson Bay, St Francis Bay, Cape St Francis, Port Elizabeth, Jeffrey's Bay	2015	662	11-27	18.48	18	2455	16-41	28.38	28
Silver Champion	Port Elizabeth, Eersterivier Strand, Maitlands, Jeffrey's Bay, St Francis Bay, Oyster Bay	2016	618	13-24	16.20	16	2364	13-35	24.43	24

Silver Eagle	Maitland, Jeffrey's Bay, Eerste Rivier	2016	440	16-26	22.29	22	975	22-46	33.10	33
Jenny	St Francis Bay, Oyster Bay, Maitlands, Eerste Rivier	2017	103	10-19	12,83	12	127	15-46	28.91	26
Silver Eagle	Eerste Rivier, Jeffrey's Bay. Gamtoos Mouth, Oyster Bay	2017	76	13-23	17,97	18	1827	17-39	28,86	29

Table 4: Linear regression assessing the temporal trends in mean length of male and female *L. reynaudii* between the years for which data were available (1996 and 2017).

Result of linear regression		
	MALE 1	FEMALE 2
year	-.140 (.102)	-.105 (.078)
Constant	311.027 (204.985)	230.927 (156.647)
Observations	15	15
R ²	.126	.123
Adjusted R ²	.059	.056
Residual Std. Error (df = 13)	2.252	1.721
F Statistic (df = 1; 13)	1.876	1.825
Notes:	*P < .05 **P < .01 ***P < .001	

Table 5: Result of the test of piecewise regression for the data before and after 2013 of males and females *L. reynaudii* in the areas between Plattenberg bay and Port Alfred.

Females					
	Est.	St.Err.	t value	CI(95%).l	CI(95%).u
slope1	0.073292	0.061095	1.1996	-0.061177	0.20776
slope2	-1.841300	0.731760	-2.5163	-3.451900	-0.23074
Males					
	Est.	St.Err.	t value	CI(95%).l	CI(95%).u
slope1	-0.050935	0.13703	-0.37170	-0.35254	0.25067
slope2	-1.018500	1.64130	-0.62054	-4.63090	2.59400

Table 6: Result of linear regression fit for jig catch versus mean length of males and females *L. reynaudii* in the areas between Plattenberg bay and Port Alfred

Result of linear regression		
	MALE	FEMALE
	1	2
Jig	-0.000	-0.000*
	(0.000)	(0.000)
Constant	34.170***	22.760***
	(2.122)	(1.542)
Observations	15	15
R ²	.221	.293
Adjusted R ²	.161	.239
Residual Std. Error (df = 13)	2.126	1.545
F Statistic (df = 1; 13)	3.686	5.394*
Notes:	*P <.05	
	**P <.01	
	***P <.001	

The results below show the analysis of male and female mean variations of length frequency between 2014, 2015, and 2016, all in pairs (2014:2015, 2014:2016, 2015:2016).

Table 7: Result of ANOVA to analyse the variation of females *L. reynaudii* length frequency between the years 2014, 2015 and 2016 within Plettenberg Bay and Port Alfred.

ANOVA: Single Factor						
SUMMARY						
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
Females 2014	170	3502	20,6	2,951479		
Females 2015	844	15379	18,22156	4,52855		
Females 2016	1058	19821	18,7344	12,02116		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	807,1934	2	403,5967	49,05449	0	3,000074
Within Groups	17022,74	2069	8,227518			
Total	17829,93	2071				

Table 8: Result of ANOVA to analyse the variation of females *L. reynaudii* length frequency between 2014 and 2015 within Plettenberg Bay and Port Alfred.

ANOVA: Single Factor						
SUMMARY						
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
Females 2014	170	3502	20,6	2,951479		
Females 2015	844	15379	18,22156	4,52855		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	800,454	1	800,454	187,6716	2,6E-39	3,850664
Within Groups	4316,368	1012	4,265185			
Total	5116,821	1013				

Table 9: Result of ANOVA to analyse the variation of females *L. reynaudii* length frequency between the years 2015 and 2016 within Plettenberg Bay and Port Alfred.

ANOVA: Single Factor						
SUMMARY						
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
Females 2015	844	15379	18,22156	4,52855		
Females 2016	1058	19821	18,7344	12,02116		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	123,4759	1	123,4759	14,19785	0,00017	3,846358
Within Groups	16523,94	1900	8,696808			
Total	16647,41	1901				

Table 10: Result of ANOVA to analyse the variation of females *L. reynaudii* length frequency between the years 2014 and 2016 within Plettenberg Bay and Port Alfred.

ANOVA: Single Factor						
SUMMARY						
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
Females 2016	1058	19821	18,7344	12,02116		
Females 2014	170	3502	20,6	2,951479		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	509,7664	1	509,7664	47,32795	9,55E-12	3,849055
Within Groups	13205,17	1226	10,77094			
Total	13714,93	1227				

Table 11: Summary statistics to show analysis of the variation of males *L. reynaudii* length frequency between the years 2014, 2015, 2016 in areas between Plettenberg Bay and Port Alfred.

ANOVA: Single Factor						
SUMMARY						
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
Males 2014	588	18765	31,91327	19,71478		
Males 2015	3297	93622	28,39612	12,66829		
Males 2016	3339	90023	26,96107	28,56889		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	13123,18	2	6561,589	318,6575	0	2,996975
Within Groups	148690,2	7221	20,59136			
Total	161813,4	7223				

Table 12: Result of ANOVA to analyse the variation of males *L. reynaudii* length frequency between the years 2014 and 2015 within Plettenberg Bay and Port Alfred.

ANOVA: Single Factor						
SUMMARY						
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
Males 2014	573	18345	32,01571	19,81968		
Males 2015	3282	93292	28,42535	12,53831		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	6288,455	1	6288,455	461,732	7,88E-97	3,843873
Within Groups	52475,07	3853	13,61928			
Total						
	58763,52	3854				

Table 13: Result of ANOVA to analyse the variation of males *L. reynaudii* length frequency between the years 2014 and 2016 within Plettenberg Bay and Port Alfred.

ANOVA: Single Factor						
SUMMARY						
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
Males 2016	3324	89635	26,966	28,69189		
Males 2014	573	18345	32,01571	19,81968		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	12462,83	1	12462,83	455,0311	1,4E-95	3,843847
Within Groups	106680	3895	27,38896			
Total						
	119142,8	3896				

Table 14: Result of ANOVA to analyse the variation of males *L. reynaudii* length frequency between the years 2015 and 2016 within Plettenberg Bay and Port Alfred.

ANOVA: Single Factor						
SUMMARY						
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
Males 2015	3282	93292	28,42535	12,53831		
Males 2016	3324	89635	26,966	28,69189		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	3517,04	1	3517,04	170,181	2,01E-38	3,842867
Within Groups	136481,4	6604	20,66647			
Total	139998,4	6605				

The results below show the median test to analyse males and females *L. reynaudii* median variations of length frequency between 2014, 2015, and 2016 all in pairs (2014:2015:2016, 2014:2015, 2014:2016, 2015:2016).

Table 15: Summary of analysis of medians test between the years 2014, 2015, 2016 for females *L. reynaudii* length frequency.

		Statistics			
		2014	2015	2016	
N	Valid	170	844	1058	
	Missing	1902	1228	1014	
Median		21.0000	18.0000	17.0000	
Frequencies					
		Year			
		2014	2015	2016	
Length Frequency	> Median	156	317	454	
	<= Median	14	527	604	
Test Statistics ^a					
	LFs2014:2015:2016				
N		2072			
Median		18.0000			
Chi-Square		171.087 ^b			
df		2			
Asymp. Sig.		.000			

Table 16: Result of median test to analyse the variation of females *L. reynaudii* length frequency between the years 2014 and 2015 within Plettenberg Bay and Port Alfred.

Frequencies				
		Years		
		2014	2015	
Length Frequency	> Median	0	29	
	<= Median	170	815	
Test Statistics ^a				
				LFs 2014:2015
N				1014
Median				2.0000
Chi-Square				6.013
df				1
Asymp. Sig.				.014
Yates' Continuity Correction	Chi-Square		4.840	
	df		1	
	Asymp. Sig.		.028	

Table 17: Result of median test to analyse the variation of females *L. reynaudii* length frequency between the years 2015 and 2016 within Plettenberg Bay and Port Alfred.

Frequencies			
		Year	
		2015	2016
Length Frequency	> Median	317	454
	<= Median	527	604
Test Statistics ^a			
			LFs 2015:2016
N			1902
Median			18.0000
Chi-Square			5.579
df			1
Asymp. Sig.			.018
Yates' Continuity Correction	Chi-Square		5.359
	df		1
	Asymp. Sig.		.021

Table 18: Result of median test to analyse the variation of females *L. reynaudii* length frequency between the years 2014 and 2016 within Plettenberg Bay and Port Alfred.

Frequencies			
		Length Frequency	
		2014	2016
Length Frequency	> Median	158	454
	<= Median	14	604
Test Statistics ^a			
			LFs 2014:2016
N			1228
Median			18.0000
Chi-Square			139.832 ^b
df			1
Asymp. Sig.			.000

Table 19: Summary of analysis of medians test between the years 2014, 2015, 2016 for males *L. reynaudii* length frequency.

Statistics				
		2014	2015	2016
N	Valid	573	3282	3324
	Missing	6606	3897	3855
Median		32.0000	29.0000	26.0000
Frequencies				
		Year		
		2014	2015	2016
Length Frequency	> Median	446	1671	1067
	<= Median	127	1611	2257
Test Statistics^a				
	LFs 2014:2015:2016			
N		7179		
Median		28.0000		
Chi-Square		519.728 ^b		
df		2		
Asymp. Sig.		.000		

Table 20: Result of median test to analyse the variation of males *L. reynaudii* length frequency between the years 2014 and 2015 within Plettenberg Bay and Port Alfred.

Frequencies			
		Length Frequency	
		2014	2015
Length Frequency	> Median	394	1294
	<= Median	179	1988
Test Statistics ^a			
			LFs 2014:2015
N			3855
Median			29.0000
Chi-Square			170.538
df			1
Asymp. Sig.			.000
Yates' Continuity Correction	Chi-Square		169.348
	df		1
	Asymp. Sig.		.000

Table 21: Result of median test to analyse the variation of male *L. reynaudii* length frequency between the years 2014 and 2016 within Plettenberg Bay and Port Alfred.

Frequencies				
		Years		
		2014	2016	
Length Frequency	> Median	479	1226	
	<= Median	94	2098	
Test Statistics ^a				
				LFs 2014:2016
N				3897
Median				27.0000
Chi-Square				402.963
df				1
Asymp. Sig.				.000
Yates' Continuity Correction	Chi-Square		433.346 ^b	
	df		1	
	Asymp. Sig.		.000	

Table 22: Result of median test to analyse the variation of male *L. reynaudii* length frequency between the years 2015 and 2016 within Plettenberg Bay and Port Alfred.

Frequencies				
		Years		
		2015	2016	
Length Frequency	> Median	2021	1226	
	<= Median	1261	2098	
Test Statistics ^a				
			LFs 2015:2016	
N			6606	
Median			27.0000	
Chi-Square			402.963	
df			1	
Asymp. Sig.			.000	
Yates' Continuity Correction	Chi-Square		401.975	
	df		1	
	Asymp. Sig.		.000	

APPENDIX 2

EXPLORATION OF SEA TEMPERATURE RECORDED AT 9, 14, 18 AND 21 M DEPTH IN THE AREAS BETWEEN PLETTENBERG BAY AND PORT ALFRED.

Table 23: Result of Paired T-Test to analyse the sea temperature difference in the depth stratum of 9 m in the areas between Plettenberg Bay and Port Alfred for the years 2002 and 2015 (6–12 November for both years).

Paired Samples Statistics					
		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	9 m (2002)	17.4229	7	.13388	.05060
	9 m (2015)	17.3500	7	.76245	.28818

Paired Samples Correlations				
		N	Correlation	Sig.
Pair 1	9 m (2002) & 9 m (2015)	7	.896	.006

Paired Samples Test									
		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	9 m (2002) – 9m (2015)	.07286	.64526	.24388	-.52391	.66962	.299	6	.775

Table 24: Result of Paired T-Test to analyse the sea temperature difference in the depth stratum of 14 m in the areas between Plettenberg Bay and Port Alfred for the years 2002 and 2015 (6–12 November for both years).

Paired Samples Statistics					
		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	14 m (2002)	17.3186	7	.10511	.03973
	14 m (2015)	17.3114	7	.82431	.31156

Paired Samples Correlations				
		N	Correlation	Sig.
Pair 1	14 m (2002) & 14 m (2015)	7	.615	.142

Paired Samples Test									
		Paired Differences				t	df	Sig. (2-tailed)	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower				Upper
Pair 1	14 m (2002) & 14 m (2015)	.00714	.76417	.28883	-.69960	.71388	.025	6	.981

Table 25: Result of Paired T-Test to analyse the sea temperature difference in the depth stratum of 18 m in the areas between Plettenberg Bay and Port Alfred for the years 2002 and 2015 (6–12 November for both years).

Paired Samples Statistics					
		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	18 m (2002)	17.1886	7	.20029	.07570
	18 m (2015)	16.3743	7	1.70570	.64470

Paired Samples Correlations				
		N	Correlation	Sig.
Pair 1	18 m (2002) & 18 m (2015)	7	.000	.999

Paired Samples Test									
		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	18 m (2002) & 18 m (2015)	.81429	1.71734	.64909	-.77399	2.40256	1.254	6	.256

Table 26: Result of Paired T-Test to analyse the sea temperature difference in the depth stratum of 21 m in the areas between Plettenberg Bay and Port Alfred for the years 2002 and 2015 (6–12 November for both years).

Paired Samples Statistics					
		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	21 m (2002)	17.1086	7	.25557	.09660
	21 m (2015)	15.9857	7	1.97226	.74544

Paired Samples Correlations				
		N	Correlation	Sig.
Pair 1	21 m (2002) & 21 m (2015)	7	-.063	.893

Paired Samples Test									
		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	21 m (2002) – 21 m (2015)	1.12286	2.00468	.75770	-.73116	2.97687	1.482	6	.189

APPENDIX 3

EXPLORATION OF LENGTH-WEIGHT DATA OF *LOLIGO REYNAUDII* OFF THE EAST COAST OF SOUTH AFRICA, SPECIFICALLY BETWEEN PLETTENBERG BAY AND PORT ALFRED.

Table 27: Result of GLM to analyse the interaction of sex and caecum colour over the selected years.

	<i>Dependent variable:</i>
	N
Gender Male	1.792 *** (0.208) t = 8.620 p = 0.000
Caecum Yellow	3.252 *** (0.196) t = 16.582 p = 0.000
Gender Male: Caecum Yellow	-1.840*** (0.215) t = -8.566 p = 0.000
Constant	1.099*** (0.192) t = 5.161 p = 0.00000
Observations	36
Log Likelihood	-289.924
Akaike Inf. Crit.	587.849
Residual Deviance	414.145 (df = 32)
Null Deiance	1,523.571 (df = 35)

ADDITIONAL FIGURES TO EXPLORE LENGTH FREQUENCY AND LENGTH-WEIGHT DATA OF *LOLIGO REYNAUDII* OFF THE EAST COAST OF SOUTH AFRICA, SPECIFICALLY BETWEEN PLETTENBERG BAY AND PORT ALFRED.

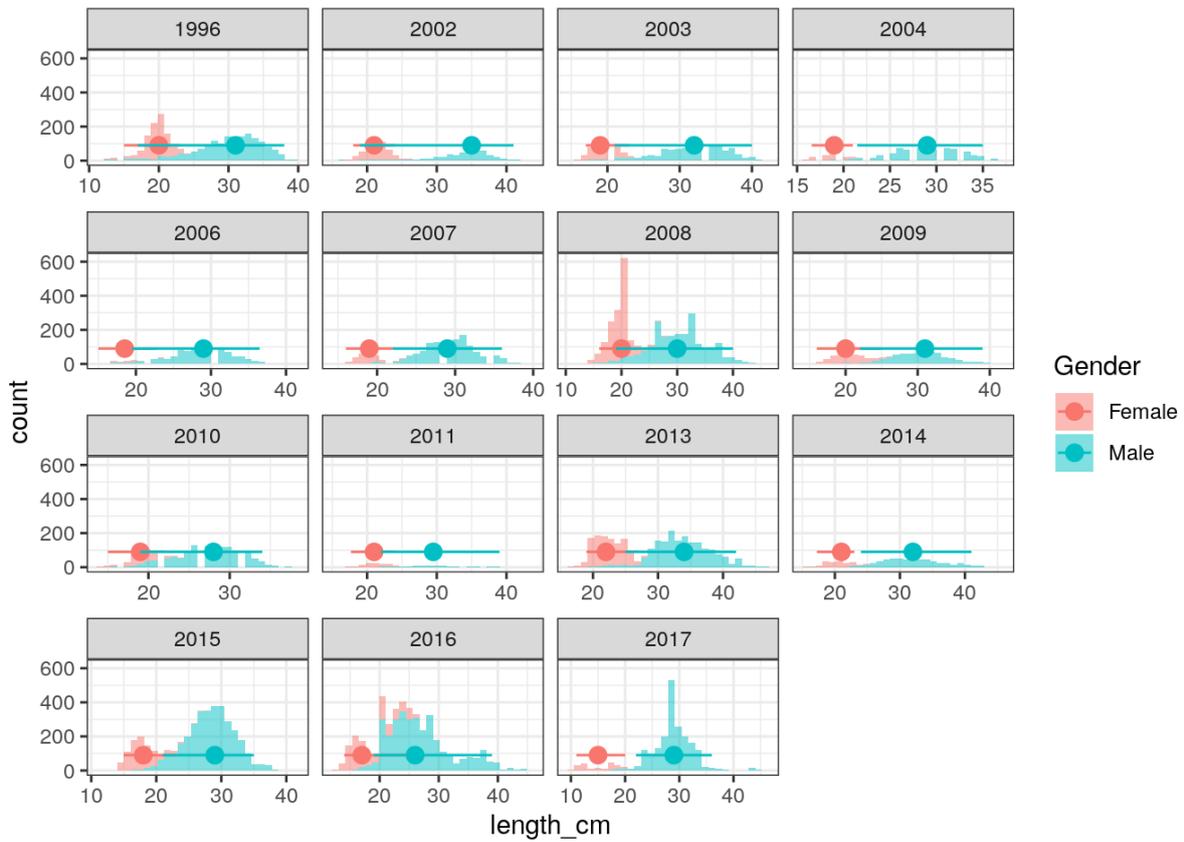


Figure 17: Histograms showing the pattern of length frequency distribution and mean length at 95% confidence interval of *L. reynaudii*, from 1996 to 2017.

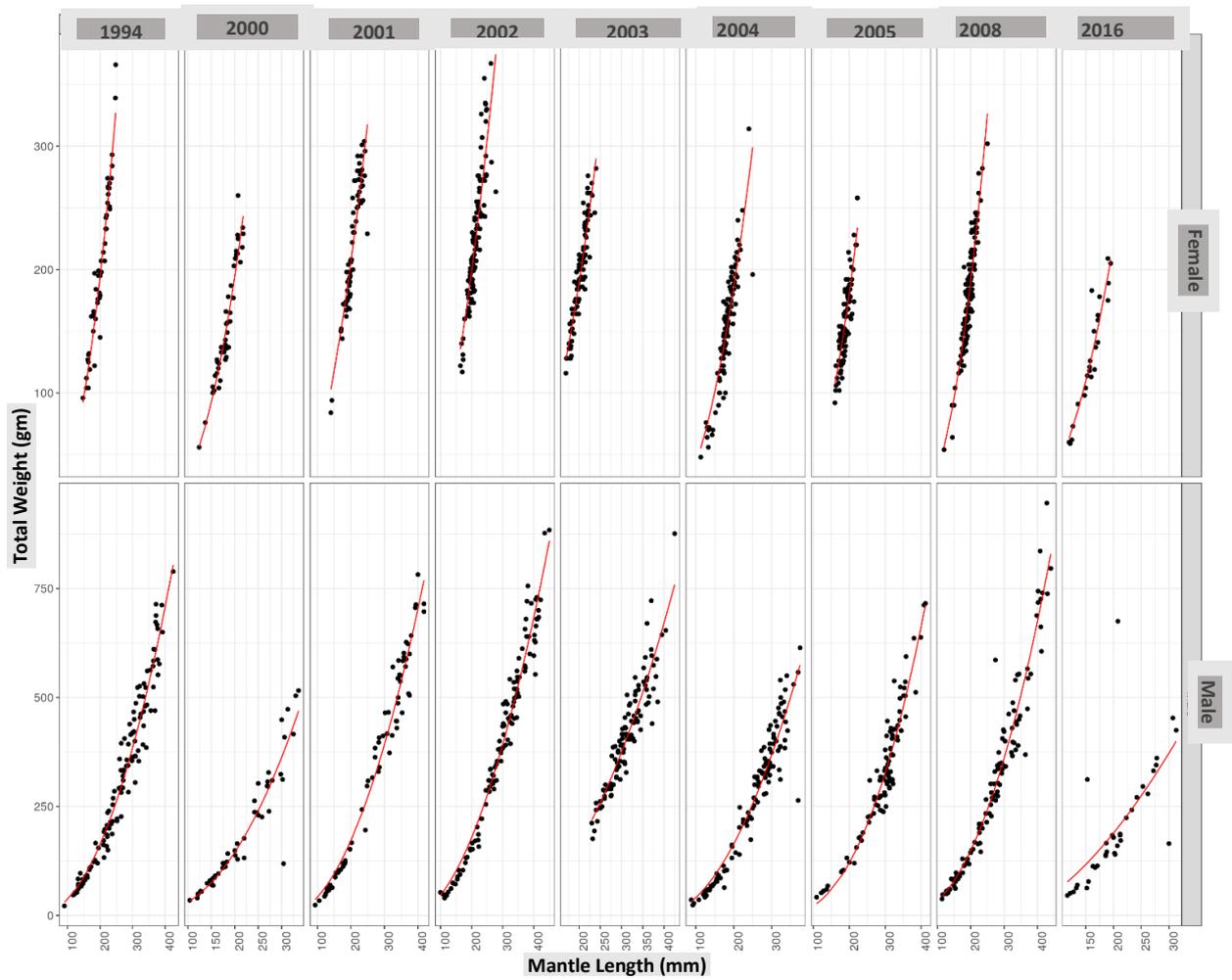


Figure 18: Length-weight relationship of male and female *L. reynaudii* and model prediction of the data.