Observations on the biology and seasonal variation in feeding of the East Coast redeye round herring (Etrumeus wongratanai) (Clupeiformes), off Scottburgh, KwaZulu-Natal, South Africa

By

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Abstract

The basic biology and ecology of *Etrumeus wongratanai* was investigated from samples of fish collected by jigging off Scottburgh, Kwazulu-Natal throughout 2014 and 2015. A total of 516 otoliths, 401 gonads, 140 stomachs and 163 samples of white muscle tissue was examined, and length and weight data of 3 637 fish caught between 2013 and 2016 was also analysed. Counts of annuli deposited on sagittal otoliths indicate that sampled *E. wongratanai* ranged from 0 to 3 y. (year/s) of age. Length-at-age for males (females) was estimated at 15.5 cm (16.2 cm) for 1 y. olds, 17.7 cm (17.4 cm) for 2 y. olds and 19.6 cm (18.8 cm) for 3 y. olds. The length at 50% maturity was estimated to be 15.6 cm for males, and 16.1 cm for females. *Etrumeus wongratanai* showed high gonadosomatic index values from June to December indicating that the breeding season lasts for 6 and 8 months for males and females, respectively, and that spawning takes place from the onset of winter to early summer. Condition factor was lowest in May through to August and increased from September. The low condition factor values from May through to August could be due to the physiological strain before and during the spawning season. Fish larvae were the most important food item in samples collected in summer, whereas eucalanids were the most important prey item in autumn and winter. Overall, large copepods were the dominant prey items in terms of frequency and importance. Stable isotope data suggests that there are gradual changes in the trophic level and diet of *E. wongratanai* as it increases with size, and whilst $\delta^{15}$N values differed between seasons $\delta^{13}$C did not. The results obtained here are compared with other species of *Etrumeus*, regionally and globally.

**Keywords**: age and growth, *Etrumeus wongratanai*, reproduction, trophic ecology
Declaration

I declare that: *Observations on the biology and seasonal variation in feeding of the East Coast redeye round herring (Etrumeus wongratanai) (Clupeiformes), off Scottburgh, KwaZulu-Natal, South Africa* is my own work, that it has not been submitted for any degree or examination at any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Lyle Dennis Vorsatz

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Table of contents

Abstract ........................................................................................................................... ii
Declaration ....................................................................................................................... iii
Table of contents ............................................................................................................ iv
Acknowledgements ....................................................................................................... vi

Chapter 1: Introduction ................................................................................................ 1
1.1. South African fisheries for small pelagic species ................................................. 1
1.1.1. History of South Africa’s commercial pelagic fishery .................................. 3
1.2. The genus Etrumeus .............................................................................................. 6
1.3. Fisheries for Etrumeus ......................................................................................... 7
1.4. Stock assessment and management of fisheries ................................................... 11
1.5. Ecosystem Approach to Fisheries (EAF) .............................................................. 14
1.6. Age and growth .................................................................................................... 15
1.7. Reproduction and condition factor ..................................................................... 18
1.8. Trophic ecology .................................................................................................. 19
1.9. Study objectives .................................................................................................. 23

Chapter 2: Materials and methods .............................................................................. 24
2.1. Sampling ................................................................................................................. 24
2.2. Age and growth ..................................................................................................... 24
2.2.1. Precision and bias in age determination ......................................................... 26
2.2.2. Growth modelling ......................................................................................... 26
2.3. Maturity estimates ............................................................................................... 27
2.4. Condition factor (CF) and Gonadosomatic index (GSI) .................................... 28
2.5. Stomach content analyses ................................................................................... 30
2.5.1. Frequency of occurrence .............................................................................. 33
2.5.2. Numerical abundance .................................................................................... 33
2.5.3. Volume ........................................................................................................... 34
2.5.4. Index of relative importance ......................................................................... 34
2.6. Stable isotope analysis ....................................................................................... 35

Chapter 3: Results ....................................................................................................... 37
3.1 Morphometrics ....................................................................................................... 37
3.1.1 Age and growth .............................................................................................. 37
3.1.2. Bias and precision ....................................................................................... 40
3.1.3. von Bertalanffy growth model ...................................................................... 43
3.1.4 Length at maturity ......................................................................................... 45
3.2. Gonadosomatic index (GSI) .............................................................................. 46
3.3. Average fish size and condition factor (CF) ......................................................... 49
3.4. Seasonal variation in feeding ............................................................................. 52
3.4.1. Diet composition .......................................................................................... 53
3.5. Stable isotope analysis ....................................................................................... 61

Chapter 4: Discussion ................................................................................................ 65
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Chapter 1: Introduction

1.1 South African fisheries for small pelagic species

The marine fishery sector plays a globally important role in food security (FAO 2013), and there has been a steady increase in global fish \(^1\) supply, outpacing world population growth over the last 50 years (FAO 2014). A record catch of 82.6 million tons (MT) was recorded in 2011 for global fishery production, of which the ten most productive species accounted for 24% of marine capture fisheries. However, most fish stocks are fully fished and have no potential for expansion, and ~ 29% of fish stocks exploited in 2011 were estimated to be fished at biologically unsustainable levels (FAO 2012). The development of increasing fishery production has been brought about by rising population growth and rising incomes, and has been facilitated through more effective channels for distribution (FAO 2014). In spite of the increase in availability of fish to consumers, the patterns of consumption are globally uneven; remaining static or decreasing in developing countries such as Congo, Gabon, Liberia, Malawi, and South Africa (FAO 2014).

Most South African marine capture fisheries are conducted off the west and south coasts in the Benguela upwelling ecosystem (Smith \textit{et al.} 2011). The Benguela upwelling ecosystem is highly dynamic and productive in nature and extends from the southern boundary of the Agulhas Bank northward to the Angola-Benguela front off the southern coast of Angola (Shannon \textit{et al.} 1987; ________________

\(^1\) Including molluscs, crustaceans and, other aquatic invertebrates but excluding mammals and plants
Hutchings et al. 2009). Large populations of living marine resources that support industrial fisheries are maintained in the system due to its high productivity. Many productive marine ecosystems around the world, such as seasonal upwelling regions, have a crucial intermediate trophic level that is occupied by small pelagic fishes of one or a few schooling species (Cury et al. 2000). Their role in the ecosystem is critical, as these pelagic fish exert top-down control on zooplankton and phytoplankton and bottom up control on predators in a “wasp-waist” manner (Cury et al. 2000; Duarte and Garcia 2004; c.f. Freon et al. 2009)

Four common small pelagic fish species occur off the coast of South Africa: sardine (Sardinops sagax), anchovy (Engraulis encrasicolus), west coast redeye round herring (Etrumeus whiteheadi) and east coast redeye round herring (Etrumeus wongratanai) (hereafter west and east coast redeye). In South African waters the east coast redeye only occurs off the coast of KwaZulu-Natal (KZN) whereas the other three species are found off the west, south and east coasts of South Africa (Heemstra and Heemstra 2004).

The small pelagic fishing industry accounts for around one quarter of the fish catch globally, and of the 17 fishery sectors in South Africa is the largest in terms of volume of landed catch and second largest in terms of value (Smith et al. 2011). Purse-seining is typically used for catching pelagic fish, where a purse-seine is made up of netting framed with a floatline and leadline, and fitted with purse rings hanging from the lower edge of the gear through which a steel purse line runs allowing the pursing of the net (FAO 2014). The basic equipment for purse-seine operations include; a hydraulic power block, a powerful purse-seine winch, a number of derricks which includes a brailler or a fish pump, small winches and an auxiliary boat (FAO 2014). Pelagic fish
are a cheap and high-quality source of protein (DAFF 2014), and anchovy and round herring are mostly reduced to fish meal and oil in industrial scale factories and used as a protein supplement in agri- and aquaculture feeds (DAFF 2014). Sardine are canned for human and pet consumption with a small fraction of the catch used as whole bait (DAFF 2014).

Fishing efforts in the South African pelagos have rather been more focused on anchovysardine and anchovy (DAFF 2014), with fluctuations in catches of west coast redeye being attributed to changes in the Total Allowable Catch (TAC) for anchovysardine and anchovy rather than to changes in west coast redeye biomass. The ease of capture and difference in retail value of sardine and anchovy compared to west coast redeye also impacts the targeting of west coast redeye; when anchovy and sardine are abundant from May to September, they are closer to the shore and therefore easier to catch than west coast redeye and thus become targeted in these periods, whereas west coast redeye are mainly targeted from March to May (van der Lingen et al. 2006, DAFF 2012).

1.1.1 History of South Africa’s commercial pelagic fishery

South Africa’s commercial pelagic fishing operations commenced in 1943 in St. Helena Bay in response to the high demand for canned products during the Second World War, with purse-seiners operating between Lambert’s Bay and Cape Hangklip (DAFF 2014). In the early years pelagic catches were dominated by sardine and horse mackerel (Trachurus capensis). Sardine catches increased rapidly during the 1950s when less than 200 000 t were landed to more than 400 000 t by the early 1960s (DAFF 2014). Horse mackerel catches which had peaked at ~ 120 000 t in the 1950s decreased to less than 30 000 t annually by the end of the 1960s (DAFF 2014).
As the sardine and the horse mackerel stocks started collapsing in 1960, the fishery changed to target anchovy which was the dominant catch from 1966 to 1995 (Hutchings et al. 1998). Sardine catches started to increase from 1995 to 2005, under a conservative management strategy throughout the 1990s and early 2000s, and following rapid population growth on the Cape South Coast. Low recruitment of sardine has resulted in annual catches of sardine of ~90 000 t over the last 5 years, and anchovy are dominating the fishery once again with average catches of ~200 000 t over the past 5 years (DAFF 2014). West coast redeye have been caught and reported since the 1960s in the commercial pelagic fishery, but have never dominated pelagic landings or exceeded 100 000 t per annum (which is the upper catch limit imposed by DAFF) despite considerable effort to increase the catches of this species (DAFF 2014).

In KZN, off the east coast of South Africa, line fishing is a popular pursuit for recreational, subsistence and commercial purposes (Pradevand and Fennessey 2009). Of the 2200 South African marine fish species, between 95 and 200 species are targeted in the linefishery (Penney et al. 1999), including the carpenter (Argyrozona argyrozoza), slinger (Chrysoblephus punicus), seventyfour (Polysteganus undulosus), red steenbras (Petrus rupestris), rockcod (Epinephelus spp.), santer (Cheimerius nufar), blueskin (Polysteganus coeruleopunctatus) and king mackerel (Scomberomorus commerson), all of which have centres of distribution off the coast of KZN (Penney et al. 1999). A total of 18 bait types are offered by retailers in the region to linefishers (Pradevand and Fennessey 2009) and whilst sardine is the most widely reported bait sold (accounting for 71% of annual bait sales), it is followed by the east coast redeye and chub mackerel (Scomber japonicus), which account for 10% and 7% of sales respectively. These latter bait species are highly desired by fishermen and are sourced illegally because the commercial
linefishery and industrial purse-seine fishery do not satisfy the demand because they are not allowed to target these species (Pradevand and Fennessey 2009). Given the strong demand for east coast redeye, the commercialisation of a legal fishery for these species should be assessed from a stock viability, as well as from an economic perspective (Pradevand and Fennessey 2009). A fishery for east coast redeye will partially address one of the initial priorities of ‘Operation Phakisa’ which strives to unlock the economic potential of South Africa’s oceans, estimated to have the potential to contribute R177 billion to gross domestic product (GDP) by 2033 in comparison to R54 billion in 2010 (Zuma 2014).

An exploratory permit to fish east coast redeye, using purse-seine nets, was applied for in the early 2000’s but initial attempts were only made in 2006 (van der Lingen et al. 2014). Purse-seining for east coast redeye proved to be unsuccessful for a variety of reasons. These included difficulties deploying purse-seine nets off small vessels in regions with strong currents, launch site limitations, the high probability of by-catch and the fact that purse-seining is not allowed during the sardine run or when the KZN shark nets have been lifted (van der Lingen et al. 2014). An exploratory permit for jigging was granted in 2012, with rights’ holders restricted to an upper catch limit of 50 tons per annum (van der Lingen et al. 2014). This has proven to be successful, and catch and effort data have been reported by the rights’ holders for 2013, 2014, 2015 and 2016. The data indicate that the fishery is predominately a winter one (van der Lingen et al. 2014), and the absence of fishing activity during summer months could be due to reduced fish availability (van der Lingen et al. 2014). A total catch of ~ 37 000 fish (~ 2 tons landed) was recorded for 2013 and 2014, which is very small compared to the single biomass estimate for this
species of ~13 000 tons, collected during a hydro-acoustic survey off the east coast of South Africa between East London and Richard’s Bay in June/July 2005 (Coetzee et al. 2010).

1.2 The genus *Etrumeus*

There are 8 species of *Etrumeus*, which are distributed globally across the world’s oceans from 40°N to 40°S (Figure 1) (Whitehead 1985, DiBattista et al. 2014). They mainly occur in subtropical and temperate regions and occur from inshore out to the edge of the continental shelf. Three species of *Etrumeus* occur off the coast of Africa; *E. whiteheadi* which has a distribution extending from Walvis Bay in Namibia to Durban off the east coast of South Africa; *E. wongratanai* whose range extends from Durban to north-eastern Somalia, and *E. golanii*, whose range extends from the northern Red Sea to the eastern Mediterranean Sea (DiBattista et al. 2014). Of the other species, *E. micropus* occurs off Japan and Taiwan; *E. jacksionensis* is from southern Australia; *E. sadina* is from the west coast of North America, *E. makiawa* is from the Hawaiian Islands and *E. acuminatus* is from the west coast of South America.
1.3 Fisheries for *Etrumeus*

The west coast redeye is currently considered to be under-utilized in South Africa (Roel and Armstrong 1991), with average annual catches of 50 000 t (DAFF 2014). Over 90% of west coast redeye are adults taken using purse-seine nets in directed-fisheries operations (DAFF 2014), with some juveniles caught as by-catch in the anchovy-directed fishery and a very small number of adults taken as by-catch in the sardine-directed fishery (DAFF 2014). Catch data collected since 1987 indicate that most west coast redeye are caught off the west coast, with very little to none being caught east of Cape Agulhas (DAFF 2014). Fish are typically caught across the shelf with most adult redeye being caught between January and May, with very few being caught for the remainder of the year (DAFF 2014). Catches of west coast redeye peaked in 1997
with 92 000 t caught, with the second highest catch of 88 000 t recorded in 2010 (Figure 2). Sixty-eight thousand tons of west coast redeye were caught in 2012, the second highest recorded catch since 2000, and similar to the 65 000 t caught in 2011. Similar to the 28 000 t caught in 2005, only 32 000 t were caught in 2013, although, the biomass was double that recorded in 2012. A total catch of 13 500 t was caught in 2015 which is the lowest recorded catch since the 11 000 t caught in 1976, which possibly reflect increased availability of anchovy (DAFF 2014).

![Figure 2. Catch time series for west coast redeye in the South African pelagic fishery, 1958-2015 (DAFF, unpublished data).](image)

*Etrumeus golanii* (formerly known as *E. teres*) forms an integral component of the Egyptian sector of the Red Sea fisheries. Mainly caught by purse-seiners in the region, it contributes approximately 25% of the total purse-seine catch (Mehanna and El-Gammal 2005). Catches of *E.*
E. golanii peaked in 1993 with 14 000 t being caught, with the second highest catch of 11 800 t recorded in 1990 (Mehanna and El-Gammal 2005). According to Sanders et al. (1984) the fishery in the Gulf of Suez is fully exploited, and any additional fishing effort would be coupled with reduced annual catches leading to a situation of economic overexploitation. This species accounted for 38% of total landings by the pelagic fishery in the Gulf of Suez and is canned for human and pet consumption and reduced to fish meal (El-Sayed 1996). In the USA, E. sadina is mainly fished from the Gulf of Mexico and landings are estimated at 50 000 to 250 000 t annually; these fish are marketed fresh, reduced to fishmeal or used as bait in sport fishing (Munroe and Nizinski 2002). However, there are no records of landings pre 1993 (Posadas et al.1996).

Figure 3. Catch time series for E. golanii in the Gulf of Suez adapted from Mehanna and El-Gammal (2005), 1990-2002.
*Etrumeus micropus* is mostly landed in prefectures in western Japan (Posadas et al. 1996). Average annual landings from 1980 to 1985 were 35 666 MT and from 1985 to 1990 was 49 240 MT, while Oozeki et al. (2007) reported average landings to be less than 100000 t from 1900 to 2000 (Figure 4). The Japanese round herring is primarily fished from September to October and sold fresh or more commonly as “dried in the round” for human consumption (Posadas *et al.* 1996). In some cases fish are frozen and sold as bait in the tuna longline fishery, but are not caught in large enough quantities for fishmeal production (Posadas *et al.* 1996).

![Figure 4. Landing records for sardine, anchovy and *E. micropus* off Japan given by Oozeki *et al.* (2007), 1905-2004.](image)
1.4 Stock assessment and management of fisheries

The success of a fishery can be defined in two ways. Biologically successful fisheries are maintained at a healthy stock size at near or above the levels that produce maximum harvestable surplus (Hilborn et al. 2003). Economically successful fisheries can be measured by the profitability of the fishing industry (Hilborn et al. 2003). In order to assess the potential viability of a fishery, information on the catch, abundance and biology of the species in question needs to be accurately and timely investigated and reported (Cadima 2003). The basic purpose of fish stock assessment is to provide advice on how to optimally exploit aquatic resources (Cadima 2003). A stock assessment uses information on a range of life history characteristics for a species which includes age and growth; natural mortality; sexual maturity; reproduction; geographical boundaries of the population and stock; environmental factors which affect the stock; diet; habitat preferences; and the historical and current estimates of the size (biomass) of the stock (Cooper 2006).

Data used in stock assessments can be categorised as fishery-dependant or fishery-independent (Cooper 2006). Fishery-dependent data are collected directly from commercial and recreational harvesters through avenues such as self-reporting, on-board observers, portside surveys or vessel monitoring programs (Cooper 2006). Self-reporting is the most common source of fishery-dependent data in the form of landing records globally, although in South Africa, fisheries monitors and inspectors record the catch. Landing records result directly from the sale of caught fish but only provide information on the landed catch and is usually in the form of total weight and seldom as total numbers of fish (Cooper 2006). On-board observers are trained to sample catch on-board commercial vessels for size frequency distributions and to estimate bycatch and
discards (Cooper 2006). The on-board observer’s main purpose is to observe and improve understanding of how these commercial fishermen interact with a variety of species that are in targeted fisheries (Cooper 2006). Port- or dockside surveys are carried out on the docks on some portion of both commercial and recreational catches. Commercial catch receipts are monitored for an accurate account of landings and provide information on species, mass, date and place of capture (Burst and Skrobe 2000). These surveys are carried out by government scientists known as portside observers or port agents (Cooper 2006). Vessel monitoring programs automatically record the location of a vessel that have been proved to be valuable for determining the spatial distribution and amount of effort in the fishery and these are used in conjunction with fishermen log books (Cooper 2006).

Fishery-independent data is data collected from systematic sampling carried out by government or contract scientists (Burst and Skrobe 2000). Scientists take samples throughout the potential range of the target fish using standardised sampling gear including trawls, seines, hydroacoustics and video (Cooper 2006). These surveys can target a group of several species; in South Africa, the biomass and distribution of anchovy, sardine, and a number of schooling fish such as west coast round herring and juvenile horse mackerel are assessed biannually using hydroacoustic surveys carried out by the Department of Agriculture, Forestry and Fisheries (DAFF 2014). These comprise a summer adult biomass survey and a winter recruit survey, and the survey programme has been ongoing without interruption for the past 30 years (DAFF 2014). Other key biological reference points such as sex, gonad maturity stage and fish condition and information pertaining to the environment are also collected during these surveys (DAFF 2014).
Population dynamics models are the underpinning of all stock assessments (Cooper 2006). The most basic way to predict the size of a fish stock for the following year is to calculate the number of fish alive this year, minus those that die due to natural causes or fishing, plus the number of new recruits entering the stock (Cooper 2006). More complex models of population dynamics include; the instantaneous mortality rate; age structure; stock recruitment functions; and biomass (Cooper 2006).

Two alternative approaches exist to assess the stock of a single-species, which depend on the data available (FAO 2005). Synthetic methods of stock assessment use the theoretical relationship between the level of fishing intensity and total catch. Alternatively, analytical methods of stock assessment use relationships between spawner stock size, recruitment, growth, and mortality, among others. Length-frequency data are usually a first step in stock assessment and growth estimates (FAO 2005; King 2007). These measurements are supplemented by hard part analysis which entails assessing otoliths and scales of fish to determine the age structure of the population, growth rates of the fish and how quickly the population is reduced either as an effect of natural mortality or fishing (FAO 2005; King 2007). Age and growth data are supplemented by information on the fecundity of a species, which is the number of ripe eggs produced during a spawning season or event (Cole 2010). Determining fecundity is important for making total population estimates as well as characterising specific populations and stocks of fish (Cole 2010). Morphometric data can be used to examine seasonal and/or inter-annual changes in the biology of fish species, an example being fish condition factor which can be derived through length-weight relationships (King 2007). The reproductive status can be
assessed through looking at the development of gonads to trace maturity and spawning times of species (King 2007).

1.5 Ecosystem Approach to Fisheries (EAF)

Historically, fisheries in South Africa have mainly used a single-species approach also known as TROM (Target Resource Oriented Management) to fisheries management. However, the global attitude to managing fisheries resources is shifting towards more holistic approaches such as ecosystem-based fisheries management (Pikitch et al. 2004). The objective of ecosystem-based fisheries management is to maintain healthy marine ecosystems and the fisheries they support to ensure that they benefit future generations (Cadima 2003). The importance of managing whole systems rather than single resources has become evident for fisheries ecologists, as a thorough understanding of the trophic relationships of the species involved is needed as predation not only influences community structures but influences population dynamics as well (Hobson and Welch 1992; Officer and Parry 1997). Information which can be measured and used for decision making in an ecosystem-based fisheries management strategy includes but is not limited to the feeding patterns of the targeted and associated species (food web metrics), the productivity of the ecosystem (systems analysis metrics) and diversity indices (community metrics) (Link 2002; Pikitch et al. 2004).

A food web describes relationships between predator and prey. All species within an ecosystem are connected through what they eat and in turn what eats them. Phytoplankton forms the base of food webs in the marine environment and provides energy to sustain top level carnivorous fish, marine mammals and seabirds (Burst and Skrobe 2000). Therefore the amount of primary
productivity in an ecosystem produced by phytoplankton or marine algae determines the numbers of fish that can be supported and because of these connections population dynamics of one species can have an effect on many others. Interpretation of stock assessment results can be contextualised through the understanding of these interactions. Outputs from food web and predator-prey models can provide input to a single species assessment model such as the time series of natural mortality to provide improved stock assessment outputs (Burst and Skrobe 2000).

In addition to understanding food webs and productivity of an ecosystem, the effects of the physical environment on population dynamics should also be taken into consideration. Climatic and ocean pattern variability should also be taken into account when fish stocks are examined as disruptions in the physical environment due to climate, or other perturbations, can impact natural stock behaviours such as spawning and migration (Burst and Skrobe 2000). A complete understanding of the impact of climate and other physical parameters on populations is therefore required to minimise un-intended impacts to fishery stocks due to mismatches between seasonal fisheries regulations, migration patterns and distributional patterns (Burst and Skrobe 2000).

1.6 Age and growth

Two approaches to ageing fish using otoliths exist; annual ageing is used for harvest calculations and population studies (Casselman 1987; Campana 2001), whereas daily ageing is mainly used to assess recruitment and in studies of young fish (Pannella 1971; Campana and Neilson 1985; Campana 2001). Annual ageing uses the annuli which can be defined as a hyaline ring laid down once a year resulting in a translucent band formed during the slow or no growth period in autumn
and winter and an opaque zone which forms during spring and summer (Victor and Brothers 1982). The annuli are viewed under a microscope; when viewed against a black background translucent rings appear to be conspicuous and these rings are assumed to represent the age of fish in years. Daily ageing uses electron-micrographs obtained via a Scanning Electron Microscope (SEM) to count the daily increments deposited on the otoliths by fish and in order to validate annual aging estimates.

The process of estimating the age of fish comes with possible error. These errors mainly arise from subjectivity originating with the preparation and interpretation of the periodic features on the otoliths which vary among readers and laboratories (Campana 2001). In order to alleviate potential bias between readers, visual assessments and precision estimates are calculated to detect these errors in ageing (see Campana et al. 1995). This is particularly important as there have been many cases where errors in ageing (overageing) of fish resulted in the overexploitation of stocks, and underageing of fish resulted in the estimation of growth and mortality rates that were overly optimistic (van der Broek 1983; Smith et al. 1995; Beamish and McFarlane 1995; Campana 2001).

To date there have been no studies on age and growth of *E. wongratanai* off the South African coast. Studies have been limited to its congener *E. whiteheadi*. Geldenhuys (1978) deduced the growth parameters of west coast redeye from counting the number of hyaline rings on otoliths, which determined their annual periodicity. It was noted that fish aged one and two years old had mean lengths of 92 and 163 mm respectively (Geldenhuys 1978).
Waldron et al. (1991) examined the growth of juvenile west coast redeye by counting the number of hyaline rings, the daily growth increments and by analysis using length frequency distributions. In contrast to Geldenhuys (1978) they found that the average length of one-year olds was 150 mm, based on the assumption that spawning mainly takes place between winter and spring. The analysis of daily rings indicated a faster growth rate of fish that were 6 months old than would be obtained by counting the number of hyaline rings (Waldron et al. 1991).

Geja et al. (in press) obtained age estimates of west coast redeye from counting the hyaline rings of otoliths; male fish ranged from 0-4 years (y) and female fish from 0-8 years old. Males were considered to be mature at a caudal length of 160 mm, which corresponded to a mean age greater than 2 years, while females were considered to be mature at a length of 180 mm which corresponded to a mean age of greater than 3 years. Mean length-at-age indicated that west coast redeye is a fast growing species, but not as fast as first described by Geldenhuys (1978) or Waldron et al. (1991).

Elsewhere in the world, age and growth studies of Etrumeus have mainly been carried out on E. golanii in the Mediterranean Sea. Yilmaz and Hossucu (2003) reported that standard lengths for E. golanii from the Gulf of Antalya, south of Turkey, were between 95-225 mm for females and 95-215 mm for males. Mean length-at-age values for females were reported to be 145, 179 and 208 mm for fish aged 1, 2, and 3 years, respectively. Male mean length-at-age values were recorded as 139, 179, and 208 mm for fish aged 1, 2 and 3 years. Average standard lengths for all specimens in the study were 142 (±0.148 SE), 179 (±0.145 SE), and 208 (±0.180 SE) mm for fish aged 1, 2 and 3 years, respectively. Farrag et al. (2014) carried out a study on E. golanii
along the Egyptian Mediterranean coast and noted that this species has a maximum lifespan of 5 years. The mean length-at-age for males was 101, 141, 177, 205 and 225 mm at ages 1 to 5 years respectively. Female mean length-at-age was reported as 102, 142, 176, 202 and 220 mm at ages 1 to 5 years respectively. The data suggested that male and females showed increased growth rates for the 1st year of their lives which gradually decreased with increasing in age.

1.7 Reproduction and condition factor

The gonadosomatic index (GSI) measures the seasonal changes in relative gonad size of fishes by looking at general gonad-body weight ratios (Cole 2010). By comparison females typically invest more energy in gonads than males, but for pelagic spawners the measures could be similar for males and females, as males have to produce more sperm in order to ensure fertilisation given the very large numbers of eggs released by a single female during spawning. For example, batch fecundity for west coast redeye ranges from 2000-17000 eggs per batch (Roel and Melo 1990). Connell (2001) described a seasonal pattern in egg abundance of *E. wongratanai* off KZN, which indicated spawning began in June, peaked in October to November and tailed off in January; no other data on the reproduction of this species are available.

Information on the reproduction of west coast redeye off South Africa is limited to a single study conducted by Roel and Melo (1990), who through histological analysis based on the presence of previtellogenic oocytes, noted that females who were smaller than 130 mm CL were classified as immature, half of them attained maturity at 14.5 cm and all were deemed to be mature at a length of 19 cm. Further, it was concluded that the west coast redeye shares many of its reproductive characteristics with the other well studied pelagic fish in South African waters. There was clear
evidence that the species is a multiple spawner, with reproduction taking place during a prolonged spawning season (primarily in winter) producing multiple “sub-cohorts” per year. Also, spawning usually takes place in the evening in the upper layer of the water column.

Plaza (2007) conducted a reproductive study on *Etrumeus micropus* off Japan and noted that it is a multiple spawning fish with a protracted spawning season. The species is reproductively active all-year round except for summer with peaks in winter and spring and has group synchronous ovarian development with indeterminate fecundity. Osman *et al.* (2011) concluded that *E. golanii* in Egyptian Mediterranean waters had a prolonged spawning season which started in December (boreal winter) for both sexes and ended in early July with a peak in April for males, while females extended their spawning season to May. The reproductive cycle restarted in July for females which first attained sexual maturity at 13.5 cm TL. Males restarted their reproductive cycle in August and first attained sexual maturity at a length of 13.8 cm.

1.8 Trophic ecology

The diet and trophic ecology of sardine off southern Africa is well studied, and has included both the examination of stomach contents (Davies 1957; King and Macleod 1976; van der Lingen 1998; 1999; 2002; Mketsu 2008) and laboratory experimentation (van der Lingen 1994; 1995). Sardine are omnivorous clupeids capable of ingesting phytoplankton and zooplankton. Sardine primarily employ filter feeding as it is energetically inexpensive when food is abundant, in contrast to particulate feeding, but switch to particulate feeding depending on the variability of prey size and abundance (van der Lingen 1995, van der Lingen *et al.* 2006). They can entrap particles as small as 0.02 mm in size with retention efficiency increasing with prey size up to 1.2
mm. When preys are larger than 1.2 mm the feeding mode switches to particulate feeding in which they display size selectivity of prey items (van der Lingen 1994). The bulk of their dietary carbon is obtained through ingesting zooplankton less than 1.2 mm in size. These typically consist of copepods, crustacean eggs and nauplii entrapped through non-selective filter feeding (van der Lingen 2002).

The diet and trophic ecology of Cape anchovy has also been relatively well studied, also via field (James 1987) and laboratory (James and Findlay 1989) studies. The bulk of the carbon content in the diet of anchovy is obtained from large zooplankton, usually greater than 1.5 mm in size. These typically consist of large calanoid copepods and euphausiids (James 1987). Size selective particulate feeding is the principal feeding mode of anchovy; however, filter feeding can contribute to dietary carbon through the entrapment of phytoplankton and smaller zooplankton (James and Findlay 1989). Anchovy switch from particulate to filter feeding when prey items are less than 0.7 mm in size and are in high abundance (James and Findlay 1989). The minimum particle size that an anchovy can entrap is 0.20-0.25 mm which results in a large fraction of phytoplankton being unavailable to them (van der Lingen et al. 2006).

Studies on the feeding and diet of the west coast red eye off South Africa are limited to Wallace-Fincham (1987) and Mketsu (2008). Wallace-Fincham (1987) concluded that west coast redeye were mainly zooplanktivorous, fed by particulate feeding and their diet consisted of, and was dominated by large copepods, euphausiids and decapods. James (1988) indicated that the west coast redeye is a particulate feeder after a review of the diets of commercially important clupeids. Literature on the diet of east coast redeye is limited to Mketsu (2008) and Vorsatz et al.
Mketsu (2008) noted that the diets of both west coast and east coast redeye off the east coast were dominated by fish eggs and large crustacean zooplankton and no phytoplankton was found in the stomach contents. Vorsatz et al. (2015) indicated that east coast redeye fed on large particles (1.5-2.5 mm), mainly large calanoid copepods, with prey ~ 2.5 mm making the greatest contribution to dietary carbon. It was noted that east coast redeye feeds on larger prey items than do sardine and anchovy but on particles of similar size to those consumed by west coast redeye.

The diet and feeding of other species within the genus *Etrumeus* has been studied in the East China Sea (Tanaka et al. 2006), the Gulf of Mexico (Chen et al. 1992) as well as in the Egyptian Mediterranean waters (Osman et al. 2013). Tanaka et al. (2006) indicated that the Pacific round herring’s (*E. micropus*) diet was characterised by a high number of calanoid copepods, and stomachs contents consisted mainly of copepods, amphipods, decapods and fish larvae. Chen et al. (1992) concluded that larval *E. sadina* fed primarily on copepods, nauplii and invertebrate eggs with some pteropods also found in the stomach contents. Osman et al. (2013) suggested that *E. golanii* fed on a variety of prey items but stomach contents mainly consisted of small crustaceans (shrimp larvae, isopods and amphipods) followed by fish larvae, molluscs and other digested material. Shrimp larvae were the most preferred food item followed by fish larvae. Feeding intensity (percentages of empty stomachs and stomachs that contained food in relation to the number of stomachs examined) was highest during winter and spring and lowest during summer and autumn (Osman et al. 2013). It was also suggested that males and fish of smaller lengths (90-150 mm) seemed to be more active in capturing different food items than females and larger fish. Also, autumn was the season with the most diverse prey items found in stomachs that contained food.
Dietary studies focusing on stomach content analysis allow only a snapshot into the consumer’s diet, whereas stable isotope analysis provides a temporally and spatially integrated view of an assimilated diet and trophic structure (Minagawa and Wada 1984; Peterson and Fry 1987; Hobson et al. 1995; Pitt et al. 2007; van der Bank et al. 2011; van der Lingen and Miller 2014). Stable isotope ratios of nitrogen (δ¹⁵N) are used to estimate the trophic position of a consumer, as δ¹⁵N of consumers are generally enriched by 2-5‰ relative to their diets with an average of ca 3.4‰ per trophic level (Post 2002). This is in contrast to the ratio of carbon stable isotopes (δ¹³C), which increases at a much slower rate than δ¹⁵N as carbon moves through food webs (Post 2002), making these differences in δ¹³C more appropriate for evaluating the origin of carbon source production for an organism when isotopic signatures of sources differ (Peterson and Fry 1987; Post 2002; Fry 2006, van der Lingen and Miller 2014). Qualitative dietary data through stomach content analysis combined with stable isotope analysis serves to improve the understanding of trophic interactions (Miller et al. 2010).

Stable isotope studies on west coast redeye in South Africa are limited to Sholto-Douglas et al. (1991) and van der Lingen and Miller (2011). Sholto-Douglas et al. (1991) indicated that west coast redeye had a lower δ¹³C value (i.e. less enriched in ¹³C) than that of co-occurring anchovy in the southern Benguela and the difference in δ¹⁵N was small, suggesting they occupy a similar trophic level. It was also observed that as west coast redeye and anchovy increased in size there appeared to be a depletion of δ¹³C as well as δ¹⁵N. Van der Lingen and Miller (2011) reported that west coast redeye had higher δ¹⁵N values than anchovy or sardine, and that anchovy appear to be more isotopically similar to sardine than to west coast redeye.
1.9 Study objectives

At present, there have been no studies on age and growth, two preliminary studies characterising the diet of *E. wongratanai*, and one study on reproduction limited to *E. whiteheadi* off the South African coast. Therefore, the objectives of the present study consist of four parts to address these gaps in knowledge and provide the first estimates of the various biological characteristics of this species in South African waters. Firstly, the age and growth of individual *E. wongratanai* will be estimated by reading the number of annual growth zones on their sagittal otoliths. The age data will then be used to construct von Bertalanffy growth curves and estimate growth rates. Secondly, the reproductive characteristics of *E. wongratanai* will be assessed using GSI and macroscopic assessment of maturity stages coupled with morphologically-derived estimates of condition factor. Thirdly, seasonality of the diet of east coast redeye will be investigated via stomach content analysis. Lastly, the trophic position of east coast redeye and seasonal variability therein will be evaluated through stable isotope analysis. The biology and ecology of east coast redeye is not well known, therefore the present study will provide new knowledge as a utility for management of the resource.
Chapter 2: Materials and methods

2.1 Sampling

Samples of east coast redeye were collected by rights holders in the exploratory fishery throughout 2014 and 2015 by jigging from a kayak off the coast of Scottburgh, KZN. Sampling was opportunistic and fish were kept cool on ice, before being frozen at the landing site ashore. The fish were thawed on returning to the laboratory, and the caudal length (CL, to the nearest mm) and wet body mass (WBM, to the nearest 0.1 g) measured. The specimens were then cut open, sexed, and assigned a gonad maturity stage. The gonads of individuals were then removed and weighed, to determine GSI, where possible. A small piece of white muscle tissue approximately 1 cm in length and 1 cm wide was cut from the anterior-dorsal region of each fish and thereafter placed in aluminium foil and labelled for stable isotope analysis. Fish stomachs were removed and preserved in 10% formalin for at least 24 hours to fix their contents for later analysis.

2.2 Age and growth

Otoliths from 535 fish were extracted using surgical blades and forceps; they were cleaned, labeled and stored dry in 5 ml pill vials. In the laboratory, the otoliths were cleaned, weighed (to the nearest 0.001 g) and measured (to the nearest 0.1 mm) under a dissecting microscope. Left and right otolith weights were then compared using a T-test. Otoliths were embedded in super clear casting resin (with a catalyst) on black Perspex trays and viewed under a dissecting microscope at 20 x magnification with the use of reflected light to identify annual growth zones. The annual growth zones were visible as concentric hyaline and opaque zones (Waldron et al. 1991).
The hyaline or translucent growth zones, which are relatively dark in appearance, were generally narrow and were assumed to reflect periods of reduced otolith growth following Geja et al. (in press). The opaque zones, which are white in appearance, are generally wider and were assumed to reflect periods of rapid growth. The hyaline growth zone was considered to represent the end of a year of growth and termed an annulus, while the opaque zone plus the hyaline zone was considered to represent a year of growth in the life of the fish and termed an annual growth zone. It was assumed that no annual growth zone could be wider than the preceding growth zone in accordance with the general pattern of fish growth (Geja et al. in press). Once the annual growth zones in the otoliths were identified, the number of growth zones that were counted was used as an estimate of the age of each fish. A fish with no visible annuli in the otolith was therefore considered to be still in its first year of growth, and was consequently assigned to the 0+ age group. This age group would therefore encompass fish ranging in age from 0 to 0.99 years of age. A fish that displayed one annulus in its otolith would have completed its first year of growth and be in the second year of growth at the time of sampling, and would be assigned to the 1+ age group. The 1+ age group would therefore encompass all fish of between 1 and 1.99 years of age. Similarly, the 2+ age group would encompass all fish of between 2 and 2.99 years of age displaying 2 annuli in their otoliths. An additional criterion was determined in order to reduce age overestimation by disregarding the juvenile ring which is a translucent margin of a growth zone which occurs in the 1st year of life (Kerstan 1995).
2.2.1 Precision and bias in age determination

In order to prevent potential bias in interpretation and to evaluate the precision with which fish were aged, three readers independently read a subsample of otoliths. Potential bias was assessed in two ways. Firstly a visual assessment was conducted, following Geja et al. (in press), where residuals were calculated for each reader, by computing the difference between the age reading from each reader and the mean of all readers for each otolith. An average of the residuals for all otoliths was then calculated for each reading session and plotted. If any bias was present it would appear as a directional trend in under- or overestimation in age of successive readings. Secondly, a more detailed approach, as outlined in Campana et al. (1995), was conducted in which ageing error was visually assessed by applying age bias plots. Each otolith was assigned to an age group based on the mode of the three readings. The average age of the estimates was then obtained for all otoliths in the subsample falling within a modal group age and plotted against the age group. This approach allowed for a visual assessment of potential age-specific bias and would appear as a directional trend in under- or overestimation in age within a given age group. This allows evaluation of the source of bias, if present. Precision was measured using average percentage error (APE) (Beamish and Fourier 1981), coefficient of variation (CV) (Campana et al. 1995) and the index of precision $D$ (Chang 1982).

2.2.2 Growth modelling

Using the solver function of Microsoft Office Excel, a von Bertalanffy growth curve was fitted to the caudal length-at-age data for 516 fish. The models were fitted for male ($n=123$) and female ($n=178$) data, and for all fish including unsexed (juveniles where gender could not be determined due to underdevelopment of the gonads) individuals combined ($n=516$):

\[ L(t) = L_{\infty} - L_{\infty} e^{-K(t-t_0)} \]

where $L(t)$ is the length at age $t$, $L_{\infty}$ is the theoretical maximum length, $K$ is the growth coefficient, and $t_0$ is the theoretical age at which length is zero.
\[ L_t = L_\infty \left( 1 - e^{-kt} \right) \]  

(Equation 1)

Where \( L_t \) (cm) is the caudal length at age \( t \) (y), \( L_\infty \) (cm) is the asymptotic caudal length, \( k \) (y\(^{-1}\)) is the growth coefficient and \( t_0 \) (y) is the theoretical age at zero length. To test for possible differences between growth curves of males and females, Kimura’s likelihood ratio test method (Kimura 1980, Haddon 2001) was used.

2.3 Maturity estimates

Gonads of individuals were macroscopically assessed using an adaptation of Davies (1956), and assigned to one of seven maturation stages (Table 1). Fish with stage I or II gonads were deemed to be incapable of spawning and labelled as juveniles immature.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Maturation</th>
<th>Ovaries</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Immature</td>
<td>less than half the length of the body cavity, pale pink or transparent</td>
</tr>
<tr>
<td>II</td>
<td>Maturing</td>
<td>Slight elongation, darker colouration</td>
</tr>
<tr>
<td>III</td>
<td>Mature</td>
<td>Half the body cavity, opaque yellow</td>
</tr>
<tr>
<td>IV</td>
<td>Nearly ripe</td>
<td>Two-thirds of the body cavity, bright yellow, transparent at posterior end</td>
</tr>
<tr>
<td>V</td>
<td>Ripe</td>
<td>Filling body cavity, dark yellow, semitransparent</td>
</tr>
<tr>
<td>VI</td>
<td>Spawning</td>
<td>Elongated, flat, bloodshot</td>
</tr>
<tr>
<td>VII</td>
<td>Spent</td>
<td>Elongated, flat, opaque yellow</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Testis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flat, pink or transparent</td>
</tr>
<tr>
<td>Elongated, white colouration</td>
</tr>
<tr>
<td>Elongated, thickened, half the body cavity</td>
</tr>
<tr>
<td>Two-thirds of the body cavity, opaque white; milky at posterior end</td>
</tr>
<tr>
<td>Almost filling Body cavity, opaque white, posterior half milky</td>
</tr>
<tr>
<td>Elongated, flat, bloodshot</td>
</tr>
<tr>
<td>As for ovaries</td>
</tr>
</tbody>
</table>
The length at which 50% of all individuals were sexually mature was estimated using the logistic function:

\[ P_i = \frac{1}{1+e^{-(L_i-L_{50})/a}} \]  
(Equation 2)

Where \( P_i \) is the proportion mature, \( L_i \) is the caudal length (cm), \( L_{50} \) is the caudal length (cm) at which 50% of individuals are mature and \( a \) (cm) is the width of the ogive. The analysis of residual sum of squares (Chen et al. 1992) was used to test for significant differences between maturity ogives of males and females.

2.4 Fish size, Condition factor (CF) and Gonadosomatic index (GSI)

Length and weight data were reported for individual fish caught between August 2013 and May 2016 by permit holders. Measurements taken by permit holders for each fish included total length (TL, to the nearest mm) and WBM. Where caudal instead of total length was reported, total length was calculated using the equation:

\[ TL \text{ (mm)} = 1.1024 \times CL \text{ (mm)} + 6.2482 \]  
(Equation 3)

Derived by fitting a linear equation (\( r^2 = 0.9523; n = 12 \)) to a subsample where total and caudal length data were available.

The number of fish caught each year was visually assessed by using a stacked bar graph to assess the time of the year most reported landings were made, along with the size frequency distribution of fish caught for 2013 to 2016. Expected mass of fish was derived from length weight
relationships by fitting power regressions to untransformed TL and WBM data for each year, using Marquardt’s (1963) iterative algorithm:

\[
\text{Expected WBM} = a \cdot \text{TL}^b \quad \text{(Equation 4)}
\]

where \(a\) and \(b\) are estimated parameters.

The average TL of fish was calculated each year, and raw data were log\(_{10}\) transformed to test for differences between years using a one-way ANOVA. Post hoc analyses (Tukey test) were undertaken in order to determine which years were different. The one-way ANOVA was computed in SPSS v.22 (Green and Salkind 2011), at 95% significance level.

Condition factor (CF) of each individual fish was then calculated using the equation:

\[
\text{Condition factor} = \frac{\text{Observed body mass}}{\text{Expected body mass}} \quad \text{(Equation 5)}
\]

The gonadosomatic index (GSI) of each individual fish (where data were available; \(n = 401\)) was calculated using the equation:

\[
\text{GSI} = \frac{\text{Gonad mass}}{\text{Wet body mass}} \times 100 \quad \text{(Equation 6)}
\]

A univariate General Linear Model was then computed in SPSS v.22 (Green and Salkind 2011) to assess the effects of week number, year and sex on CF, and of sex, week number and year for
GSI, in order to investigate temporal variability in these indices. Distribution of the residuals was then visually assessed for normality. The mean (± SD) for GSI (males, females and combined) and CF (all fish combined) were then plotted at a weekly temporal resolution.

2.5 Stomach content analyses

The stomach contents of 161 fish sampled in 2014 and 2015 were examined, of which 66 were caught in winter, 61 were caught in spring and 33 in summer; no fish caught in spring were available for analysis. Before the examination of stomach contents, the stomachs were left to soak in freshwater for a minimum of 24 hours to remove formalin, whereafter they were blotted dry and weighed to the nearest 0.001 g before being dissected and the contents flushed into a petri dish. The emptied stomachs were then blotted dry and reweighed, to determine the mass of the stomach contents. The stomach contents were then stained with rose Bengal and examined under a dissecting microscope at various magnifications up to 50x. Prey items were identified to the lowest taxonomic level and, where possible and appropriate their total length (TL, µm), prosome length (PL, µm) and prosome widths (PW, µm) were measured using a micrometer eyepiece.

Where prosome length was not recorded for copepods, regressions were fitted in order to predict prosome length from prosome width. Regression analysis and associated morphometric relationships were calculated for different copepod families found to be ingested in the stomachs of *Etrumeus wongratanai* in 2014 and 2015. There were strong, significant relationships between prosome length and prosome width for Calanidae, Centropagidae, Clausocalanidae, Metridinidae
and Pseudodiaptomidae. The rest of the families displayed weak but statistically significant relationships for prosome width and prosome length.

Identified prey items were grouped according to broad taxonomic categories, and prey size converted to dry mass and carbon content using published equations for length to mass, and mass to carbon relationships (Table 2).

Table 2. Equations used to estimate dry weight (DW) and carbon content (CC) of identifiable prey items found in the stomachs of *E. wongratanai* caught off the coast of Scottburgh, KZN. All dry weight and carbon content are reported in µg.

<table>
<thead>
<tr>
<th>Prey item</th>
<th>L to DW (µg)/ DW to CC (µg)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calanoid Copepods</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acartiidae</td>
<td>Ln(DW) = 2.74ln(PL) – 16.41</td>
<td>DW= Chrisholm and Roff (1990)</td>
</tr>
<tr>
<td>Calanidae</td>
<td>CC = 0.424 DW</td>
<td>CC= van der Lingen (2002)</td>
</tr>
<tr>
<td>Candaciidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Centropagidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clausocalanidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eucalanidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Euchaetidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterorhabdidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mecynoceridae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metridinidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paracalanidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pontellidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudodiaptomidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temoridae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclopoid copepods</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corycaeidae</td>
<td>Ln(DW) = 1.96ln(PL) – 11.64</td>
<td>DW= Chrisholm and Roff (1990)</td>
</tr>
<tr>
<td>Taxon</td>
<td>Biomass Relationship</td>
<td>Authors</td>
</tr>
<tr>
<td>------------------------------</td>
<td>----------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>Oncaeidae</td>
<td>( CC = 0.424 \text{ DW} )</td>
<td>van der Lingen (2002)</td>
</tr>
<tr>
<td>Poecilostomatoid copepods</td>
<td>( \text{Ln(DW)} = 1.96 \text{ln(PL)} - 11.64 )</td>
<td>Chrisholm and Roff (1990)</td>
</tr>
<tr>
<td>Sapphrinidae</td>
<td>( CC = 0.424 \text{ DW} )</td>
<td>van der Lingen (2002)</td>
</tr>
<tr>
<td>Decapod</td>
<td>( \text{DW} = 0.0012 \text{TL}^{3.16} )</td>
<td>Espinoza and Bertrand (2008)</td>
</tr>
<tr>
<td>Decapod larvae</td>
<td>( CC = 0.424 \text{ DW} )</td>
<td>Espinoza and Bertrand (2008)</td>
</tr>
<tr>
<td>Euphausiacea</td>
<td>( \text{DW} = 0.0012 \text{TL}^{3.16} )</td>
<td>van der Lingen (2002)</td>
</tr>
<tr>
<td>Fish eggs</td>
<td>( \text{DW} = 0.093 \text{Vol} + 0.0012 )</td>
<td>van der Lingen (2002)</td>
</tr>
<tr>
<td>Fish larvae</td>
<td>( CC = 0.424 \text{ DW} )</td>
<td>van der Lingen (2002)</td>
</tr>
<tr>
<td>Hyperiid amphipods</td>
<td>( \text{DW} = 0.005 \text{TL}^{2.31} )</td>
<td>van der Lingen (2002)</td>
</tr>
<tr>
<td>Mysid</td>
<td>( \text{DW} = 0.0012 \text{TL}^{3.16} )</td>
<td>van der Lingen (2002)</td>
</tr>
<tr>
<td>Ostracoda</td>
<td>( \text{DW} = 3.946 \text{TL}^{2.46} )</td>
<td>van der Lingen (2002)</td>
</tr>
</tbody>
</table>

Feeding intensity was determined based on the Vacuity index (VI; which is the percentage of empty stomachs) and Fullness index (FI; which is the percentage of stomachs containing food) in relation to the total number of examined stomachs and grouped according to season (as AbdEl-Aziz and Gharib 2007).

The importance of a food category can be assessed using the number and volume or weight of that item in the diet of fish (Hyslop 1980). Considering both number and volume of prey items in diet studies gives a more accurate account of dietary importance than would be obtained using
either index in isolation (Hyslop 1980). The feeding activity of *E. wongratanai* was characterized and examined to assess whether diet changed seasonally using the following indices calculated as outlined in Hyslop (1980):

2.5.1 Frequency of occurrence

Frequency of occurrence (%F) is defined as the percentage of stomachs containing a specific food item in relation to all stomachs examined which contain food

\[
\%F = 100 \frac{n_i}{n} \quad \text{(Equation 7)}
\]

Where %F is the frequency of occurrence of food item *i* in the sample; *n* is the number of stomachs containing food across all seasons. A \( \chi^2 \) analysis was used to test, at 95% significance, for differences between main prey items and season, under the null hypothesis that there are no significant differences between main prey items and season.

2.5.2 Numerical abundance

The numerical abundance (%N) is defined as the number of individuals identified in a given prey category. It was calculated as the number of individuals of a given prey category expressed as the percentage in relation to the number of individuals in all prey categories

\[
\%N = 100 \frac{N_i}{N} \quad \text{(Equation 8)}
\]
where $%N$ is the numerical abundance of prey item $i$; $N_i$ is the number of individuals of prey item $i$; $N$ is the number of individuals in all prey categories.

2.5.3 Volume

The volume (%) is defined as the volume of individuals of a given prey category. Volume is expressed as a percentage of the total volume of all individuals measured in all prey categories. For the purpose of this study and being unable to physically measure the volume of prey items in three dimensions, the dry mass of each prey item was used as a proxy for volume.

$$%V = 100 \frac{v_i}{V}$$

(Equation 9)

where $%V$ is the volume of individuals in prey category $i$; $v_i$ is the cumulative volume of individuals of prey category $i$; $V$ is the sum of the volume of all individuals in all prey categories.

2.5.4 Index of relative importance

The index of relative importance (IRI) is defined as the importance of a given prey item relative to all other prey items found in the diet of fish by taking into account volume ($%V$), numerical abundance ($%N$) and frequency of occurrence ($%F$) (Pinkas et al. 1971).

$$\text{IRI} = (%N + %V) \times %F$$

(Equation 10)

The index of relative importance was then expressed as a percentage.

$$%\text{IRI} = 100 \frac{IRI}{\sum IRI}$$

(Equation 11)
A $\chi^2$ analysis was used to test, at 95% significance, for differences between dominant prey size class and season, under the null hypothesis that there is no difference in dominant prey size with season. Stomach contents were expressed as length frequency histograms illustrating prey size, and in terms of percentage numerical abundance and percentage contribution to carbon content and index of relative importance.

2.6 Stable isotope analysis

Samples of white muscle tissue from fish collected in 2014 and 2015 were freeze dried at -50 °C for 48-72h. A mortar and pestle was subsequently used to pulverise the freeze dried tissue into a fine powder, which was then placed into labelled Eppendorf tubes for stable isotope analysis. Samples were not lipid extracted as recommended by some studies (McConnaughy & McRoy, 1979; Post et al. 2007) mainly due to the negative effects of fractionation on $\delta^{15}$N values (Sweeting et al. 2006; Post et al. 2007).

Aliquots of approximately 0.6 to 0.7 mg fish muscle were weighed into tin capsules that were pre-cleaned in toluene. Isotopic analysis was done on a Flash EA 1112 Series coupled to a Delta V Plus stable light isotope ratio mass spectrometer via a ConFlo IV system (all equipment supplied by Thermo Fischer, Bremen, Germany), housed at the Stable Isotope Laboratory, Mammal Research Institute, University of Pretoria.

A laboratory running standard (Merck Gel: $\delta^{13}$C = -20.57‰, $\delta^{15}$N=6.8‰, C%=43.83, N%=14.64) and blank sample were run after every 12 unknown samples. All results are referenced to Vienna Pee-Dee Belemnite for carbon isotope values, and to air for nitrogen
isotope values. Results are expressed in delta notation using a per mille scale using the standard equation:

$$\delta X \, (\%o) = \left[ \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 1000$$

(Equation 12)

where X = $^{15}$N or $^{13}$C and R represents $^{15}$N/$^{14}$N or $^{13}$C/$^{12}$C respectively. Analytical precision was <0.09‰ for $\delta^{13}$C and <0.10‰ for $\delta^{15}$N. This is based on the mean and standard deviations for the Merck Gel carbon and nitrogen values.

The mean and standard deviation of $\delta^{15}$N, $\delta^{13}$C and C:N were calculated according to E. wongratanai size class (small: <131 mm, medium: 131-165 mm and large: >165 mm CL; with size classes selected so as to give roughly equal numbers of fish in each) and season caught (summer; autumn and winter). Data for each season were tested for normality using Levene’s test and where data violated the assumption of normality, a log$_{10}$ transformation was conducted on the data. A one-way ANOVA was conducted to test for differences in $\delta^{15}$N and $\delta^{13}$C amongst fish size classes and season. Post hoc comparisons were done with a Tukey test; all tests were done at 95% significance and computed in SPSS v.22 (Green and Salkind 2011). The relationships between fish size and $\delta^{15}$N and $\delta^{13}$C were also examined using regression analysis on individual fish data (Draper and Smith 1981).
Chapter 3: Results

3.1 Morphometrics

3.1.1 Age and growth

Length and weight data were reported for 3 637 individual fish for the period of August 2013 to May 2016. The highest number of fish for which data were reported in a single month was June 2014 (602 individuals: Figure 5), whilst the lowest number reported was in November 2015 (five individuals). *Etrumeus wongratanai* caught by jigging ranged in size from 112 to 250 mm, with the majority being between 180 to 200 mm TL (Figure 6). Fish ranged in WBW from 13 to 123 g, and significant length-weight relationships were derived per year, and overall (Table 3). Morphometric relationships showed a strong linear relationship between otolith length and caudal length (Figure 7a), whilst there were weaker but still significant non-linear relationships between caudal length and age with otolith weight (Figure 7b, c and d): and between otolith length and age (Table 4). There was no significant difference between the weight of left and right otoliths ($t (58) = 1.71, p = 0.09$), thus left otoliths were used for ageing.
Figure 5. Number of fish length-weight data reported by permit holders per month from August 2013 to May 2016.

Figure 6. Length-frequency distribution (TL) of *E. wongratanai* caught by jigging off the coast of Scottburgh, KZN from 2013 to 2016 (*n* = 3 637).
Table 3. Equations and statistical parameters for annual length-weight relationships of *E. wongratanai* from 2013 to 2016, and for all fish combined, reported by the permit holders for fish caught off the coast of Scottburgh, KZN.

<table>
<thead>
<tr>
<th>Year</th>
<th>Regression equation</th>
<th>n</th>
<th>r²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
<td>$WBM (g) = 0.00010(TL (mm))^{2.4972}$</td>
<td>1165</td>
<td>0.82</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2014</td>
<td>$WBM (g) = 0.00001(TL (mm))^{3.0245}$</td>
<td>1802</td>
<td>0.91</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2015</td>
<td>$WBM (g) = 0.00001 (TL (mm))^{2.8811}$</td>
<td>490</td>
<td>0.97</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2016</td>
<td>$WBM (g) = 0.00003(TL (mm))^{2.8089}$</td>
<td>180</td>
<td>0.90</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Combined</td>
<td>$WBM (g) = 0.00001(TL (mm))^{2.939}$</td>
<td>3637</td>
<td>0.89</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Figure 7. Relationships between (a) otolith length and caudal length, (b) otolith length and estimated age, (c) otolith weight and caudal length, and (d) otolith weight and estimated age for *E. wongratanai*. Regression equations and statistical parameters are given in Table 4.
Table 4. Equations and statistical parameters for regressions describing the relationships among caudal length (CL, cm), estimated age (y), otolith length (OL; mm) and otolith weight (OW; g) for *E. wongratanai* caught in 2014 and 2015 off the coast of Scottburgh, KZN.

<table>
<thead>
<tr>
<th>Relationship</th>
<th>Regression equation</th>
<th>(n)</th>
<th>(r^2)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Otolith weight vs. Lc</td>
<td>(OW = 0.0002(Lc)^{1.6447})</td>
<td>524</td>
<td>0.67</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Otolith weight vs. age</td>
<td>(OW = 0.0011e^{2.878(\text{age})})</td>
<td>512</td>
<td>0.39</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Otolith length vs. Lc</td>
<td>(OL = 0.256(Lc) + 2.515)</td>
<td>321</td>
<td>0.81</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Otolith length vs. age</td>
<td>(OL = 5.6277e^{0.1241(\text{age})})</td>
<td>322</td>
<td>0.67</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

### 3.1.2 Bias and precision

A total of 173 otoliths was independently read by three readers. The simple visual assessment by plotting the mean residuals of modal age between readers indicated that there was no apparent bias, but there was a slight underestimation by Readers 1 and 3, and a slight overestimation in aging of otoliths by Reader 2, who was the primary reader (Figure 8). The age-bias plots indicated that there was no deviation from the 1:1 equivalent line, which suggested no age-specific bias was present between all readers (Figure 9). The precision measurements between readers (Table 5) showed low values of the coefficient of variation (CV; <5%), average percent error (<5%) and the index of precision \(D\) (<2.5%) for males, females and all individuals including unsexed fish.
Figure 8. Comparison of mean residuals of modal age between readers for *E. wongratanai* caught off the coast of Scottburgh, KZN in 2014 and 2015.

Table 5. Precision measurements for age estimates provided by three independent readers, obtained from counts of annual growth zones on otoliths of *E. wongratanai*. Estimates are provided separately for males (*n* = 48), females (*n* = 38) and all fish combined including unsexed individuals (*N* = 173).

<table>
<thead>
<tr>
<th>Precision Measurements</th>
<th>CV (%)</th>
<th>APE (%)</th>
<th>D (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>3.13</td>
<td>2.35</td>
<td>1.81</td>
</tr>
<tr>
<td>Females</td>
<td>3.51</td>
<td>3.24</td>
<td>2.03</td>
</tr>
<tr>
<td>Males + females + unsexed</td>
<td>4.31</td>
<td>3.48</td>
<td>2.49</td>
</tr>
</tbody>
</table>
Figure 9. Age-bias plots indicating the differences in age estimates (years) between three paired age readers for *E. wongratanai* caught off the coast of Scottburgh, KZN in 2014 and 2015. The 1:1 equivalent line is shown.
3.1.3 von Bertalanffy growth model

Of the 535 otoliths extracted for ageing, a total of 516 otoliths could be used for length-at-age plots and fitting of the von Bertalanffy growth model (Figure 10). The remaining otoliths were either broken or difficult to interpret due to improper casting in resin. The size of fish used for age and growth analysis ranged from 9.6 to 20.2 cm CL for all fish including unsexed fish, 11.1 to 20.2 cm CL for females \((n=178)\) and 10.7 to 16.8 cm CL for males \((n=123)\). The estimated age of fish ranged from 0 to 3 years, where 0 is interpreted as a fish in its first year of life, 1 is interpreted as a fish being between its first and second year of life etc. (Waldron et al. 1991). Samples were characterized by a high percentage of 0 (28%) and 1 (54%) year olds. The males caught were predominantly 1 (65%) and 2 (22%) years old \((y)\), as too were females \((1\ y\ 61\%,\ 2\ y\ 26\%)\). The von Bertalanffy growth model indicates rapid growth in \(E.\ wongratanai\’s\) first year of life, with fish attaining sizes of 10 to 16 cm CL. The growth parameters are shown in Table 6. The mean asymptotic maximum length or length at infinity \((L_\infty)\) was estimated at 18.9, 20.2 and 16.2 cm for all individuals including unsexed fish, females and males, respectively. The growth rates \((k)\) were 0.64, 0.46 and 1.42 year\(^{-1}\) for all individuals including unsexed fish, females and males, respectively. The theoretical age where the fish would have length of 0 \((t_0)\) was estimated as \(-1.62\), \(-2.16\) and \(-1.08\ y\) for all individuals including unsexed fish, females and males. Kimura’s likelihood test indicated a significant difference between the male and female growth curves \(\chi^2 (3,\ N = 301) = 11.76,\ p=0.008\).
Figure 10. Length-at-age data for males (triangles; n=123) and female (squares; n=178) *E. wongratanai* collected during the study, including the three von Bertalanffy curves fitted to data for males (dotted line), females (dashed line) and all individuals (solid line; n=516). von Bertalanffy growth model parameter estimates are given in Table 6.

Table 6. von Bertalanffy growth model parameter estimates for length-at-age data of females, males and all fish combined of *E. wongratanai*. Sample sizes (n) are given, along with the estimated residual sums of squares (RSS) from the regressions.

<table>
<thead>
<tr>
<th></th>
<th>L∞ (cm)</th>
<th>k (y⁻¹)</th>
<th>t₀ (y)</th>
<th>n</th>
<th>RSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>20.20</td>
<td>0.46</td>
<td>-2.16</td>
<td>178</td>
<td>612.24</td>
</tr>
<tr>
<td>Males</td>
<td>16.25</td>
<td>1.42</td>
<td>-1.08</td>
<td>123</td>
<td>305.27</td>
</tr>
<tr>
<td>Males + females + unsexed</td>
<td>18.95</td>
<td>0.64</td>
<td>-1.62</td>
<td>516</td>
<td>1211.73</td>
</tr>
</tbody>
</table>
3.1.4 Length at maturity

Maturity ogives were fitted using logistic curves for length and age at maturity (Figure 11). A total of 189 females of which 99 were mature and 138 males, of which 66 were mature, was used for maturity ogives. The size range of females analyzed for length and age at maturity ranged between 11.7 and 20.2 cm CL, whereas males ranged from 12.3 and 20.1 cm CL.

The minimum length at maturity for female fish was 13.3 cm CL and 14 cm CL for males. The CL at which 50% of females attain maturity was estimated to be 16.1 cm CL, which corresponds to an age of 1.56 y. The caudal length at which 50% of males attain sexual maturity was estimated to be 14.5 cm CL which corresponds to a mean age of 1.58 y. All females were deemed to be mature at a CL above 19 cm (3 y of age) and all males were deemed to be mature at a CL above 18 cm (3 y of age). Likelihood ratio tests indicated there was a significant difference between the length at sexual maturity ($\chi^2 (1, N = 18) = 4.38, p = 0.036$), but no significant difference for age at sexual maturity ($\chi^2 (1, N = 26) = 0.29, p = 0.58$), between males and females.
Figure 11. Maturity ogives for (a) caudal length and (b) age of male (triangle; dashed line; \(n=138\)) and female (square; solid line; \(n=189\)) *E. wongratanai* caught during 2014 and 2015 off the coast of Scottburgh, KZN.

3.2 Gonadosmotic index (GSI)

A total of 401 fish was examined for GSI of which 123 were male, 159 were female and 119 were unsexed. GSI for all samples combined ranged from 0.03 to 4.28 with a mean (±SE) of 0.61
(± 0.02). GSI for females were typically lower than males and ranged from 0.11 to 3.38 with a mean (±SE) of 0.73 (± 0.18), while males ranged from 0.12 to 4.8 with a mean (±SE) of 0.78 (± 0.17) (Figure 12). Mean GSI values for females, males and unsexed individuals were highest between the months of July to December (Figures 12(a) and 12(b)). The generalized linear model indicated that there was a significant effect on mean GSI values between weeks ($F (1, 20) = 14.603, p < 0.001$), there was a significant difference in mean GSI at a weekly temporal resolution between males and females ($F (1, 2) = 19.26, p < 0.001$), and also that there was a significant effect on GSI between years ($F (1, 3) = 17.32, p < 0.001$) (Table 7).

Table 7. Model outputs for the GLM of gonadosomatic index (GSI) and condition factor (CF) of *E. wongratanai* caught off Scottburgh, KZN, indicating variables used in the analysis with its associated regression ($r^2$) values, Type III sum of squares, degrees of freedom (df), mean sum of squares, $F$-Value and significance ($p$-value).

<table>
<thead>
<tr>
<th>Source</th>
<th>Parameter estimate</th>
<th>Type III SS</th>
<th>df</th>
<th>Mean SS</th>
<th>$F$-Value</th>
<th>$p(&gt;F)$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GSI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week Number</td>
<td>0.43</td>
<td>47.189</td>
<td>20</td>
<td>2.359</td>
<td>14.6</td>
<td>0.001</td>
</tr>
<tr>
<td>Year</td>
<td>0.04</td>
<td>4.507</td>
<td>3</td>
<td>4.507</td>
<td>17.32</td>
<td>0.001</td>
</tr>
<tr>
<td>Sex</td>
<td>0.08</td>
<td>9.559</td>
<td>1</td>
<td>4.78</td>
<td>19.26</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>CF</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week number</td>
<td>0.32</td>
<td>1.362</td>
<td>21</td>
<td>0.065</td>
<td>12.14</td>
<td>0.001</td>
</tr>
<tr>
<td>Year</td>
<td>0.04</td>
<td>4.017</td>
<td>3</td>
<td>1.339</td>
<td>50.061</td>
<td>0.001</td>
</tr>
<tr>
<td>Sex</td>
<td>0.007</td>
<td>0.029</td>
<td>1</td>
<td>0.014</td>
<td>1.91</td>
<td>0.149</td>
</tr>
</tbody>
</table>
Figure 12. Mean ± standard error (SE) gonadosomatic index for (a) all individuals of *E. wongratnai* (*n* = 401) and (b) for male (triangle; dashed line; *n* = 123) and female (square; solid line; *n* = 159) *E. wongratnai* caught off the coast of Scottburgh, KZN in 2014 and 2015 at a weekly and monthly temporal resolution.
3.3 Average fish size and condition factor (CF)

Length data for fish caught from 2013 to 2016 indicated that the maximum size of fish per year ranged from 220 mm caught in 2013 and 2016, and 250 mm TL caught in 2014. The smallest fish was caught in 2015 with a size of 112 mm. Fish caught between June and late March had the largest average size, across all years. In 2013 fish ranged from 135 mm to 220 mm TL, while on average fish caught were 180 mm TL, and increased steadily from August to September, after which fish caught were on average more or less the same size (Figure 13(a)). In 2014 fish ranged from 112 mm to 280 mm with an average size of 180 mm, and average fish size increased from March to September. Fish caught in 2015 ranged from 109 mm to 238 mm in length, with the average being 175 mm, and on average larger fish occurred during the summer months with average fish size increasing from January to March, whereafter average size decreased and remained steady from April to July. The largest fish caught in 2016 was 220 mm TL and the smallest fish was 143 mm, the average fish caught in 2016 was 190 mm in total length, larger fish were found from March to April, whereafter average fish size decreased, possibly representative of new recruits to the stock. There was a significant difference in mean size of fish between years ($F (3, 3447) = 97.529, p<0.05$). No significant difference in TL was found between years 2013 and 2015 ($p= 0.981$), while the rest were significantly different ($p<0.001$).

Condition factor was found to be independent of total length ($p> 0.05$). There was, however, a significant effect of week number and year on condition factor, but sex had no significant effect (Table 7). Condition factor for all years at a weekly temporal resolution ranged from 0.61 to 1.84. The mean condition factor ($\pm$SE) in 2013 was 0.99 ($\pm$0.01), in 2014 it was 1.03 ($\pm$0.01), in 2015 it was 0.98 ($\pm$ 0.01) and in 2016 it was 0.87 ($\pm$0.01). In 2013, condition factor increased
from August to September, after which it remained relatively constant with the exception of low condition factor occurring in October and late December (Figure 13(b)). Condition factor was steady in 2014 but there was a slight increase from March to July. Condition factor was greatest in the summer months of 2015 (January to end of February) and decreased from March to July. In 2016, condition factor decreased from April to June. The lowest mean condition factor was seen to be between May to August (autumn to winter) whereafter, condition factor appeared to increase with the onset of September to January (spring and summer). There was a significant effect on condition factor between years and weeks, but not between sexes (Table 7).
Figure 13. Weekly temporal resolution of mean (± standard error) for (a) fish size (TL) (2013: blue circles, \( n = 1165 \); 2014: red circles, \( n = 1802 \); 2015: green circles, \( n = 490 \); 2016: purple circles, \( n = 30 \)) and (b) condition factor (CF) (2013: blue circles, \( n = 1165 \); 2014: red circles, \( n = 1802 \); 2015: green circles, \( n = 490 \); 2016: purple circles, \( n = 30 \)) for *E. wongratanai* caught by jigging over the period July 2013 to May 2016.
3.4 Seasonal variation in feeding

Seasonal changes in the feeding intensity of *E. wongratanai* are shown in Figure 14. Out of the 161 stomachs examined, 21 (13% of all stomachs examined) were found to be empty. The vacuity index (% empty stomachs) was highest in summer (21%), followed by autumn (15%) and was lowest in winter (8%). The fullness index, which is a proxy for feeding intensity, was highest during winter (92%), then autumn (85%), and summer (78%).

![Feeding intensity per season of *E. wongratanai* caught off Scottburgh, KZN in 2014 and 2015.](image-url)

Figure 14. Feeding intensity per season of *E. wongratanai* caught off Scottburgh, KZN in 2014 and 2015.
3.4.1 Diet composition

A total of 5 854 prey items was identified from stomachs that contained food: 3 314 were identified from winter-collected stomachs, 1 998 were identified from autumn stomachs and 521 from summer stomachs. The morphometric relationships between prosome width and length of copepods were all significant (Table 8). Twenty five different prey taxa could be identified (Table 9). Prey items ranged in size from 320 (ostracod) to 44 800 (fish larva) µm. Prey items in the size class 1 001-2000 µm dominated numerically in all seasons combined, while prey items in the size class 2 001-3 000 µm dominated in terms of contribution to dietary carbon (Figure 17). In summer and winter prey items in the size class 1001-2000 and 2001-3000 µm dominated numerically and in terms of contribution to dietary carbon, respectively (Figures 18 and 20). In autumn prey items in the size class 1001-2000 µm were numerically dominant but prey items in the size class >5000 µm dominated the contribution to dietary carbon (Figure 19). There was a statistically significant difference \( \chi^2 (10, n = 5854) = 963.34, p <0.001 \) between prey size classes with season.

Table 8. Morphometric relationship of prosome length (PL) and prosome width (PW) for different copepod groups found in the stomach of *E. wongratanai* caught off the coast of Scottburgh, KZN. In all cases prosome width was measured and where possible prosome length and/or total length (TL) were measured.

<table>
<thead>
<tr>
<th>Family</th>
<th>Morphometric relationship</th>
<th>n</th>
<th>( r^2 )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calanidae</td>
<td>PL = 0.3021(PW) + 74.003</td>
<td>986</td>
<td>0.57</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Candaciidae</td>
<td>PL = 0.3521(PW) + 1763.3</td>
<td>319</td>
<td>0.31</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
The most frequently occurring prey items recovered from all stomachs were calanoid copepods from the families Eucalanidae and Calanidae, which occurred in 69 and 66% percent of stomachs respectively: oncaeids occurred in 60% of stomachs and hyperiid amphipods occurred in 34% of all stomachs, respectively (Table 9). In summer, the most frequently occurring prey items were fish larvae (Clupeidae), and eucalinid and calanid copepods (Table 10). Oncaeids (57%) and eucalanids (54%) dominated diets during autumn (Table 11), while calanids (77%) and eucalanids (76%) were the most frequently occurring prey item in winter (Table 12). There was a statistically significant difference ($\chi^2 (26, n = 5691) = 1397.76, p <0.001$) between main prey items with season.
Figure 15. Contribution to dietary carbon (grey bars; %CC) and size frequency distributions (white bars; %F) by size class of identifiable prey items examined from the stomachs of (a) combined ($n = 140$), (b) summer ($n = 27$), (c) autumn ($n = 52$) and (d) winter ($n = 61$) of *E. wongratanai* caught off the coast of Scottburgh, KZN in 2014 and 2015.
Table 9. Diet items found in stomachs of *E. wongratanai* (*n* = 140) caught off the coast of Scottburgh, KZN in 2014 and 2015. %N = percentage of total prey items; %V = percentage volume of prey items; %F = percentage frequency of occurrence of prey items; %IRI = index of relative importance of prey items.

<table>
<thead>
<tr>
<th>Prey items</th>
<th>All seasons</th>
<th>%N</th>
<th>%V</th>
<th>%F</th>
<th>%IRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acartiidae</td>
<td></td>
<td>0.02</td>
<td>0.02</td>
<td>0.71</td>
<td>0.00</td>
</tr>
<tr>
<td>Calanidae</td>
<td></td>
<td>16.85</td>
<td>14.27</td>
<td>65.71</td>
<td>17.77</td>
</tr>
<tr>
<td>Candaciidae</td>
<td></td>
<td>5.45</td>
<td>5.92</td>
<td>47.14</td>
<td>4.66</td>
</tr>
<tr>
<td>Centropagidae</td>
<td></td>
<td>1.25</td>
<td>1.08</td>
<td>28.57</td>
<td>0.58</td>
</tr>
<tr>
<td>Clausocalanidae</td>
<td></td>
<td>0.21</td>
<td>0.18</td>
<td>4.29</td>
<td>0.01</td>
</tr>
<tr>
<td>Corycaeidae</td>
<td></td>
<td>0.41</td>
<td>0.23</td>
<td>6.43</td>
<td>0.04</td>
</tr>
<tr>
<td>Decapod</td>
<td></td>
<td>2.12</td>
<td>0.38</td>
<td>26.43</td>
<td>0.58</td>
</tr>
<tr>
<td>Decapod larvae</td>
<td></td>
<td>1.28</td>
<td>0.04</td>
<td>22.14</td>
<td>0.25</td>
</tr>
<tr>
<td>Eucalanidae</td>
<td></td>
<td>42.48</td>
<td>55.82</td>
<td>68.57</td>
<td>58.58</td>
</tr>
<tr>
<td>Euchaetidae</td>
<td></td>
<td>2.07</td>
<td>2.41</td>
<td>32.86</td>
<td>1.28</td>
</tr>
<tr>
<td>Euphausiacea</td>
<td></td>
<td>0.05</td>
<td>0.00</td>
<td>1.43</td>
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</tr>
<tr>
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<td>0.02</td>
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</tr>
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</tr>
<tr>
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<td>0.06</td>
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<td>0.00</td>
</tr>
<tr>
<td>Hyperiid amphipods</td>
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<td>0.03</td>
<td>33.57</td>
<td>0.75</td>
</tr>
<tr>
<td>Mecynoceridae</td>
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</tr>
<tr>
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</tr>
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</tr>
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<td>Sapphrinidae</td>
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<td>26.43</td>
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</tr>
</tbody>
</table>
Table 10. Diet items found in stomachs of *E. wongratanai* (n = 27) caught off the coast of Scottburgh, KZN in summer 2014 and 2015. %N = percentage of total prey items; V = percentage volume of prey items; %F = percentage frequency of occurrence of prey items; %IRI = index of relative importance of prey items.

<table>
<thead>
<tr>
<th>Prey items</th>
<th>%N</th>
<th>%V</th>
<th>%F</th>
<th>%IRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acartiidae</td>
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<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Calanidae</td>
<td>14.39</td>
<td>8.68</td>
<td>38.24</td>
<td>13.33</td>
</tr>
<tr>
<td>Candaciidae</td>
<td>3.60</td>
<td>2.66</td>
<td>11.76</td>
<td>3.81</td>
</tr>
<tr>
<td>Centropagidae</td>
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<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Clausocalanidae</td>
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<td>0.23</td>
<td>2.94</td>
<td>0.03</td>
</tr>
<tr>
<td>Corycaeidae</td>
<td>1.89</td>
<td>0.84</td>
<td>14.71</td>
<td>0.61</td>
</tr>
<tr>
<td>Decapod</td>
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<td>0.08</td>
<td>8.82</td>
<td>0.31</td>
</tr>
<tr>
<td>Decapod larvae</td>
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<td>0.00</td>
<td>2.94</td>
<td>0.01</td>
</tr>
<tr>
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<td>17.68</td>
<td>38.24</td>
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</tr>
<tr>
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<td>3.01</td>
<td>8.82</td>
<td>0.81</td>
</tr>
<tr>
<td>Euphausiacea</td>
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<td>0.02</td>
<td>2.94</td>
<td>0.02</td>
</tr>
<tr>
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<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
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<td>47.15</td>
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<td>0.00</td>
<td>0.00</td>
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<td>0.22</td>
<td>2.94</td>
<td>0.02</td>
</tr>
<tr>
<td>Metridinidae</td>
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<td>0.53</td>
<td>5.88</td>
<td>0.10</td>
</tr>
<tr>
<td>Mysid</td>
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<td>0.00</td>
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</tr>
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<tr>
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<td>0.29</td>
<td>5.88</td>
<td>0.06</td>
</tr>
<tr>
<td>Paracalanidae</td>
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<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Pontellidae</td>
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<td>2.94</td>
<td>0.02</td>
</tr>
<tr>
<td>Pseudodiaptomidae</td>
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<td>1.71</td>
<td>11.76</td>
<td>0.71</td>
</tr>
<tr>
<td>Sapphrinidae</td>
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<td>0.54</td>
<td>14.71</td>
<td>0.33</td>
</tr>
<tr>
<td>Temoriidae</td>
<td>1.89</td>
<td>1.16</td>
<td>20.59</td>
<td>0.95</td>
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</table>
Table 11. Diet items found in stomachs of *E. wongratanai* (*n* = 52) that contained food caught off the coast of Scottburgh, KZN in autumn 2014 and 2015. %N = percentage of total prey items; %V = percentage volume of prey items; %F = percentage frequency of occurrence of prey items; %IRI = index of relative importance of prey items.

<table>
<thead>
<tr>
<th>Prey items</th>
<th>Autumn</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%N</td>
<td>%V</td>
<td>%F</td>
<td>%IRI</td>
</tr>
<tr>
<td>Acartiidae</td>
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<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Calanidae</td>
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<td>16.60</td>
<td>45.90</td>
<td>16.31</td>
</tr>
<tr>
<td>Candaciidae</td>
<td>4.73</td>
<td>6.42</td>
<td>42.62</td>
<td>5.08</td>
</tr>
<tr>
<td>Centropagidae</td>
<td>2.59</td>
<td>2.75</td>
<td>36.07</td>
<td>2.06</td>
</tr>
<tr>
<td>Clausocalanidae</td>
<td>0.10</td>
<td>0.15</td>
<td>1.64</td>
<td>0.00</td>
</tr>
<tr>
<td>Corycaeidae</td>
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<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Decapod</td>
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<td>0.88</td>
<td>24.59</td>
<td>0.74</td>
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<td>0.00</td>
<td>1.64</td>
<td>0.00</td>
</tr>
<tr>
<td>Fish eggs</td>
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<td>0.07</td>
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</tr>
<tr>
<td>Fish larvae</td>
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<td>6.62</td>
<td>24.59</td>
<td>2.26</td>
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<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Hyperid amphipods</td>
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<td>0.32</td>
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<td>Mecynoceridae</td>
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<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Metridinidae</td>
<td>0.40</td>
<td>0.64</td>
<td>8.20</td>
<td>0.09</td>
</tr>
<tr>
<td>Mysid</td>
<td>0.05</td>
<td>0.34</td>
<td>1.64</td>
<td>0.01</td>
</tr>
<tr>
<td>Oncaeidae</td>
<td>29.15</td>
<td>10.08</td>
<td>57.38</td>
<td>24.06</td>
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<tr>
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<td>0.24</td>
<td>6.56</td>
<td>0.03</td>
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<td>2.54</td>
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<tr>
<td>Pontellidae</td>
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<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Pseudodiaptomidae</td>
<td>0.90</td>
<td>1.10</td>
<td>16.39</td>
<td>0.35</td>
</tr>
<tr>
<td>Sapphrinidae</td>
<td>0.65</td>
<td>0.62</td>
<td>14.75</td>
<td>0.20</td>
</tr>
<tr>
<td>Temoriidae</td>
<td>1.59</td>
<td>1.42</td>
<td>27.87</td>
<td>0.90</td>
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</tbody>
</table>
Table 12. Diet items found in stomachs of *E. wongratanai* (*n* = 61) that contained food caught off the coast of Scottburgh, KZN in winter 2014 and 2015. %N = percentage of total prey items; %V = percentage volume of prey items; %F = percentage frequency of occurrence of prey items; %IRI = index of relative importance of prey items.

<table>
<thead>
<tr>
<th>Prey items</th>
<th>Winter</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>%N</td>
<td>%V</td>
<td>%F</td>
</tr>
<tr>
<td>Acartiiidae</td>
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<td>0.03</td>
<td>1.52</td>
<td>0.00</td>
</tr>
<tr>
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<td>14.34</td>
<td>77.27</td>
<td>18.12</td>
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<tr>
<td>Candaciidae</td>
<td>6.18</td>
<td>6.36</td>
<td>54.55</td>
<td>5.06</td>
</tr>
<tr>
<td>Centropagidae</td>
<td>0.63</td>
<td>0.55</td>
<td>27.27</td>
<td>0.24</td>
</tr>
<tr>
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<td>0.24</td>
<td>0.19</td>
<td>6.06</td>
<td>0.02</td>
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<tr>
<td>Corycaeidae</td>
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<td>0.22</td>
<td>3.03</td>
<td>0.01</td>
</tr>
<tr>
<td>Decapod</td>
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<td>0.22</td>
<td>28.79</td>
<td>0.52</td>
</tr>
<tr>
<td>Decapod larvae</td>
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<td>0.02</td>
<td>13.64</td>
<td>0.08</td>
</tr>
<tr>
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<td>53.78</td>
<td>67.38</td>
<td>75.76</td>
<td>67.90</td>
</tr>
<tr>
<td>Euchaetidae</td>
<td>2.17</td>
<td>2.28</td>
<td>40.91</td>
<td>1.35</td>
</tr>
<tr>
<td>Euphausiacea</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Fish eggs</td>
<td>0.03</td>
<td>0.00</td>
<td>1.52</td>
<td>0.00</td>
</tr>
<tr>
<td>Fish larvae</td>
<td>1.42</td>
<td>2.72</td>
<td>15.15</td>
<td>0.46</td>
</tr>
<tr>
<td>Heterorhabdidae</td>
<td>0.09</td>
<td>0.11</td>
<td>4.55</td>
<td>0.01</td>
</tr>
<tr>
<td>Hyperiid amphipods</td>
<td>2.89</td>
<td>0.03</td>
<td>36.36</td>
<td>0.79</td>
</tr>
<tr>
<td>Mecynoceridae</td>
<td>0.12</td>
<td>0.16</td>
<td>3.03</td>
<td>0.01</td>
</tr>
<tr>
<td>Metridinidae</td>
<td>0.75</td>
<td>0.98</td>
<td>18.18</td>
<td>0.23</td>
</tr>
<tr>
<td>Mysid</td>
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<td>0.00</td>
<td>1.52</td>
<td>0.00</td>
</tr>
<tr>
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<td>7.98</td>
<td>2.44</td>
<td>57.58</td>
<td>4.44</td>
</tr>
<tr>
<td>Ostracoda</td>
<td>0.15</td>
<td>0.11</td>
<td>7.58</td>
<td>0.01</td>
</tr>
<tr>
<td>Paracalanidae</td>
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<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Pontellidae</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Pseudodiaptomidae</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Sapphrinidae</td>
<td>1.51</td>
<td>0.98</td>
<td>28.79</td>
<td>0.53</td>
</tr>
<tr>
<td>Temoriidae</td>
<td>1.02</td>
<td>0.54</td>
<td>19.70</td>
<td>0.23</td>
</tr>
</tbody>
</table>
Large calanoid copepods (eucalanids and calinids) dominated numerically and in the index of relative importance, followed by oncaeids and fish larvae for all stomachs examined. Fish larvae and eucalanids were indicated to be the most important food item in summer according to the index of relative importance whereas eucalanids were the most important prey item in autumn and winter followed by oncaeids and calanids respectively (Figure 16).

Figure 16. Index of relative importance (IRI %) of main prey items found in stomachs that contained food caught off the coast of Scottburgh, KZN in summer, autumn and winter 2014 and 2015.
3.5 Stable isotope analysis

Stable isotope ratios were calculated for 163 fish from May 2014 to April 2015. The overall mean $\delta^{15}$N and $\delta^{13}$C obtained for all fish were 9.63 ± 0.42 ‰ and -17.84 ± 0.57 ‰, respectively. The mean $\delta^{15}$N for small, medium and large size classes were 9.51 ± 0.47 ‰; 9.54 ± 0.43 ‰ and 9.8 ± 0.30 ‰, respectively (Table 13) whereas mean $\delta^{13}$C values were -18.01 ± 0.55 ‰, -17.69 ± 0.43 ‰ and -17.81 ± 0.65 ‰ for small, medium and large *E. wongratanai*, respectively (Table 13). The mean ratio of carbon-to-nitrogen (C:N) was greatest for large *E. wongratanai*, followed by small and medium sized fish (Table 13). Mean $\delta^{15}$N was greatest for fish caught in summer, followed by autumn, and then winter, while fish caught in winter were the most depleted in terms of $\delta^{13}$C, followed by caught in summer, then autumn (Table 14).

**Table 13.** Stable isotope measurements ‰ (± standard deviation) of *E. wongratanai* of different size classes; the number of samples (*n*), mean caudal length (CL; mm), mean ($\delta^{15}$N) values, mean ($\delta^{13}$C) values and mean Carbon: Nitrogen ratios (C:N) are shown.

<table>
<thead>
<tr>
<th>Size class</th>
<th><em>n</em></th>
<th>Mean (± std. dev.) CL (mm)</th>
<th>Mean (± std. dev.) $\delta^{15}$N</th>
<th>Mean (± std. dev.) $\delta^{13}$C</th>
<th>Mean (± std. dev.) C:N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small (&lt;131 mm)</td>
<td>53</td>
<td>118.77 ± 8.71</td>
<td>9.51 ± 0.47</td>
<td>-18.01 ± 0.55</td>
<td>4.22 ± 0.52</td>
</tr>
<tr>
<td>Medium (131-165 mm)</td>
<td>50</td>
<td>150.70 ± 10.32</td>
<td>9.54 ± 0.43</td>
<td>-17.69 ± 0.43</td>
<td>4.01 ± 0.52</td>
</tr>
<tr>
<td>Large (&gt;165 mm)</td>
<td>60</td>
<td>178.35 ± 9.98</td>
<td>9.80 ± 0.30</td>
<td>-17.81 ± 0.65</td>
<td>4.49 ± 0.53</td>
</tr>
</tbody>
</table>
Table 14. Stable isotope measurements ‰ (± standard deviation) of *E. wongratanai* caught in different seasons; the number of samples (*n*), mean caudal length (CL; mm), mean (δ\(^{15}\)N) values, mean (δ\(^{13}\)C) values and mean Carbon: Nitrogen ratios (C:N) are shown.

<table>
<thead>
<tr>
<th>Season</th>
<th>n</th>
<th>Mean (± std. dev.) CL (mm)</th>
<th>Mean (± std. dev.) δ(^{15})N</th>
<th>Mean (± std. dev.) δ(^{13})C</th>
<th>Mean (± std. dev.) C:N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summer</td>
<td>40</td>
<td>170.37 ± 7.45</td>
<td>9.84 ± 0.33</td>
<td>-17.83 ± 0.64</td>
<td>4.61 ± 0.46</td>
</tr>
<tr>
<td>Autumn</td>
<td>10</td>
<td>142.66 ± 28.66</td>
<td>9.61 ± 0.43</td>
<td>-17.79 ± 0.41</td>
<td>4.05 ± 0.45</td>
</tr>
<tr>
<td>Winter</td>
<td>22</td>
<td>150.32 ± 20.28</td>
<td>9.3 ± 0.31</td>
<td>-18.08 ± 0.91</td>
<td>4.56 ± 0.70</td>
</tr>
</tbody>
</table>

All data were normally distributed except for δ\(^{13}\)C values between seasons which failed Levene’s test for normality and homogeneity of variances *P* < 0.05. There was a significant difference (*F* (2, 162) = 9.517 *p* < 0.001) in δ\(^{15}\)N between small, medium and large *E. wongratanai*. Large *E. wongratanai* were different in δ\(^{15}\)N to medium (*p* = 0.02) and small (*p* < 0.01) sized fish, while there was no significant difference between medium and small *E. wongratanai* (*p* = 0.932). There was a significant difference (*F* (2,162) = 4.326, *p* = 0.015) in δ\(^{13}\)C between size classes. Small and medium sized *E. wongratanai* were statistically different in terms of δ\(^{13}\)C (*p* = 0.012) while there was no difference between small and large (*p* = 0.152), and large and medium sized *E. wongratanai* (*p* = 0.481). There was a significant difference (*F* (2,162) = 13.745, *p* < 0.001) in δ\(^{15}\)N between fish caught in different seasons, while δ\(^{13}\)C was not statistically different between fish caught in different seasons (*F* (2,162) = 2.320, *p* = 0.102). In terms of δ\(^{15}\)N fish caught in summer was different to autumn (*p* = 0.004) and winter (*p* < 0.001), and those caught in autumn were also different to fish caught in winter (*p* = 0.003).
Regression analysis showed a weak but significant positive relationship of $\delta^{15}$N with fish size (Figure 217(a)) and of $\delta^{13}$C with fish size (Figure 17(b)). There was no significant relationship between $\delta^{15}$N and $\delta^{13}$C ($\delta^{15}$N = 0.078$\delta^{13}$C + 11.009, $R^2 = 0.011$, $p = 0.187$) (Figure 18).

Figure 17. Changes in (a) $\delta^{15}$N ($\delta^{15}$N = 0.006CL + 8.7621, $R^2 = 0.131$, $p < 0.001$, $n = 163$) and (b) $\delta^{13}$C ($\delta^{13}$C = 0.005CL - 18.525, $R^2 = 0.046$, $p = 0.006$, $n = 163$) signatures with caudal length of *E. wongratanai* sampled off Scottburgh, KZN in 2014 and 2015 with fitted regression.
Figure 18. Stable carbon and nitrogen values for *E. wongratanai* white muscle tissue collected from Scottburgh, KZN in 2014 and 2014.
Chapter 4: Discussion

4.1 Age and growth

There were significant, positive length-weight relationships which were best described with power curves for fish examined in this study (Table 3). These strong length-weight relationships observed in males, females and all individuals are in accordance with studies done on other *Etrumeus* species. As expected, the relationship between otolith- length with caudal length for *E. wongratanai* was best described by a linear relationship (Figure 7) (Araya *et al.* 2001). Geja *et al.* (in press) observed that the relationships between otolith- length and weight with caudal length for *E. whiteheadi* were best described by a power curve, which contradicts results observed in this study. However, the relationship between otolith-length and –weight with age was best described by a non-linear relationship, which is in accordance with the results obtained by both Geldenhuys (1978) and Francis and Campana (2004), who suggested that the relationship between otolith weight and age should be non-linear. Because as fish grow older, growth in total length, otolith length and otolith width slow down but otolith weight increases due to the continued deposition of material on otoliths (Blacker 1974; Boehlert 1985; Anderson *et al.* 1992; Francis and Campana 2004). There was no significant difference in the weight of left and right otoliths, suggesting that either side could be used for ageing, as Hunt (1979) observed that otolith pairs are mirror images of each other.

Information on the age of a species forms the basis of calculations of growth rate, productivity and mortality, which make it one of the most important biological variables (Campana 2001). These data on age of fish are especially useful when conducting stock assessments, since any estimated growth rate requires age data in their computation (Campana 2001). Although there are
several calcified structures that can be used for aging fish, otoliths have been applied over the broadest age range of species (Secor et al. 1995). As noted elsewhere, the aging of fish can be done at two scales; annual aging which is used in the harvesting calculations and population studies, and daily aging which is primarily used in the studies of young fish and recruitment (Casselman 1987). Age-bias plots of estimating age of *E. wongratanai* in this study (Figures 7, 8) indicated that there was no reader bias in the interpretation of annual growth zones to age estimates.

Precision estimates are given in Table 5. Precision can be defined as the reproducibility of repeated measures on any otolith, whether they are accurate or not (Campana *et al.* 1990). Consequently, precision cannot be used as a proxy for accuracy between readers, but rather the ease at which age can be determined from an otolith or an indication of the skill-level from one reader to another (Campana *et al.* 1990). When there is no obvious bias, CV and APE are similarly sensitive to differences in precision among readers, even though the CV is statistically more rigorous and flexible (Chang 1982). There are no designated target values for precision in ageing studies, due to the influence of life history traits. The CV in this study was calculated to be less than 5%, with a corresponding APE value of around 3% (Table 5). These values are a reflection of the ease at which annual growth zones of *E. wongratanai* were interpreted between readers. The general consensus in the literature is reporting CV values of less than 7.6%, which serves as a reference point for many fish of short to moderate longevity and reading complexity (Campana 2001).
No previous work has been conducted on the age and growth of *E. wongratanai*, and only a limited number of studies have been conducted on congeners elsewhere such as *E. whiteheadi* and *E. golanii* (Geldenhuys 1978; Waldron *et al.* 1991; Geja *et al.* in press; El-Sayed 1996; Yilmaz and Hossucu 2003; Mehanna and El-Gammal 2005; Farrag *et al.* 2014). *Etrumeus wongratanai* examined in this study ranged from 0 to 3 y. of age. Length-at-age for males in this study was 15.5 cm for 1 y. olds, 17.7 cm for 2 y. olds and 19.6 cm for 3 y. olds (Figure 10). In comparison, females were 16.2 cm at 1 y. old, 17.4 cm at 2 y. old and 18.8 at 3 y. old (Figure 10).

*Etrumeus wongratanai* differs from its congener in South African waters, in this respect. In samples of *E. whiteheadi*, Geldenhuys (1978) and Waldron *et al.* (1991) reported individuals to range in age from 0 to 5 years old. Geja *et al.* (in press) observed *E. whiteheadi* to range between 0 to 4 years old for males and 0 to 8 year olds for females. These differences in age estimates could be attributed to differences in the environments occupied by the two species, with *E. whiteheadi* distributed primarily in the productive waters of the cold Benguela Current system off the west and southwest coasts whereas *E. wongratanai* is found in the warmer, oligotrophic waters of the east coast. Pauly (1998) noted that fishes from warmer waters tend to reach a lower maximum age and smaller maximum size than those fish of colder climes. Furthermore these differences in maximum ages could be a result of the sampling limitations in the fishery, as it is a spatially restricted area and may not have encompassed larger, older fish.
The values obtained for length-at-age of *Etrumeus wongratanai* in this study are also different to those reported for *E. whiteheadi* by Geldenhuys (1978), Waldron *et al.* (1991) and Geja *et al.* (in press). Geldenhuys (1978) reported length at age data to be 9.2 cm for 1 year olds and 16.3 cm for 2 year old fish. Waldron *et al.* (1991) reported mean length-at-age of fish aged 1.5 years old to be between 12.3 cm and 15.4 cm, 2.5 year olds to be 15.4 to 16.7 cm and 3.5 year olds to be 16.7 to 18.3 cm. Geja *et al.* (in press) observed mean length-at-age to be 11.6 cm at 1 year of age, 14.3 cm at 2 years of age, 16.3 at 3 years of age and 17.7 cm at 4 years of age. Parameter estimates in this study (Table 6) indicate a much faster growth rate when compared with that of Geja *et al.* (in press) for females, and this is even more pronounced in males. When all fish were combined and compared the two species appear to differ as well, with this study reporting a growth rate of 0.64 per year for *E. wongratanai* compared to 0.43 per year for *E. whiteheadi* reported by Geja *et al.* (in press). *Etrumeus wongratanai* appears to be a fast growing species in its first year of life, faster than has been reported for its congener *E. whiteheadi*. Although these differences in mean length-at-age could be attributed to errors in age estimation in the other studies (e.g. by mistaking the juvenile ring for an annual ring), that they are due to interspecific differences in the environment seems likely because fish tend to grow faster and mature quicker in warmer waters, than fishes that occur in cold or temperate waters (Pauly 1998). Identifying the first growth increment proves to be an important component of any age and growth study because with an incorrectly defined starting point, the age estimates will consistently be incorrect by a constant amount. Increased precision is imperative in age and growth studies because if a random error of one year is observed, it could introduce unacceptable error into all individual age estimates. Thus age validation or counts of daily rings are important for determining the precision of age estimations (Campana 2001).
Mean age-at-length of *Etrumeus wongratanai* in this study seems to be in accordance with previous studies conducted on *E. golanii* in the Gulf of Antalya in the Mediterranean Sea by Yilmaz and Hossucu (2003) and in the Egyptian sector of the Red Sea by Mehanna and El-Gammal (2005). Both studies noted that *E. golanii*, lived to a maximum age of 3 years. In contrast, Farrag *et al.* (2014) reported that *E. golanii* in the Egyptian Mediterranean Sea lived to a maximum age of 5 years. Growth rates of *E. wongratanai* were similar to those reported by Mehanna and El-Gammal (2005), but slightly higher than those reported by Yilmaz and Hossucu (2003) and Farrag *et al.* (2014) for *E. golanii*. The difference in maximum age groups between studies could be due to variation in habitats, length range and exploitation rate. Habitats could affect the maximum age to which fish survive due to shallow and deep habitats offering different amounts of food (Harvey and Nakamoto 1997). With a greater length range, fish are expected to be older at greater sizes and if fish are not overly exploited it gives an individual fish a chance to reach its maximum age. Similar to *E. wongratanai*, *E. golanii* has a high growth rate during its first year of life, which slows down as it increases in age, and this deceleration of the growth rate observed could associated with the onset of maturity.

4.2 Length at maturity

Length-at-maturity is another important parameter in fisheries research as it indicates the “ideal” length to remove individuals from the population to ensure that a healthy spawning stock is maintained (Farrag 2010). The length at 50% maturity in this study was estimated to be 16.1 cm for females, which corresponds to an age of 1.1 y. The CL at which 50% of males attain sexual maturity was estimated to be 15.6 cm, which corresponds to a mean age of 1.5 y. All females
were deemed to be mature at a CL above 19 cm (3 y.) and all males were deemed to be mature at a CL above 18 cm (3 y.) (Figure 11). There was scatter with age-at-maturity data which could be a reflection of error in identifying gonad maturity stages accurately, and the absence of a properly conducted validation study which contributed to inaccuracy in age estimation, and these data should thus be treated with caution.

When compared to the length at maturity results published on *E. whiteheadi* by Roel and Melo (1990), *E. wongratanai* attains maturity at a greater caudal length than its South African congener which was found to attain first sexual maturity at 14.5 cm for both sexes, which is counter-intuitive given that fish mature faster and at smaller sizes when in warmer waters in comparison to cooler waters (Pauly 1998). The difference in length at sexual maturity could be due to the difference in assessing maturation stages between the two studies. Roel and Melo (1990) applied macroscopic and histological techniques, the former being more susceptible to subjectivity as maturity can only be recognized with confidence in a more advanced stage of the maturation cycle. The latter technique detects the first signs of the onset of maturation and is therefore more accurate, even though females used in that study were past the peak of the spawning season. Histological techniques were not used in this study.

*Etrumeus golanii* in Egyptian Mediterranean waters attained sexual maturity at a TL of 12.6 cm for males and 13.1 cm for females (Osman 2011), while in the Gulf of Suez maturity was attained at a TL of 12 and 12.2 cm for males and females, respectively (El-Sayed 1996). The present results of length at sexual maturity for *E. wongratanai* are not consistent with these previously mentioned studies, but are in accordance with Sanders *et al.* (1984) who reported
length at maturity for female at 16.36 cm (corresponding to an age of 1.73 years) and 16.17 cm (1.70 y.) for male *E. golanii*. Differences in age at maturity between studies on the same species likely depends on the environmental conditions under which maturation takes place and the nature of the population (density-dependent growth) when length-at-maturity was determined. This is because growth is inhibited by intraspecific competition for food if stock sizes are large and this has been reported for many marine fish (Overholtz 1989; Helser and Almeida 1997; Sinclair *et al.* 2002; Moyle and Cech 2004).

4.3 Gonadosomatic index and condition factor

The gonadosomatic index (GSI) is a cost effective method to indicate changes in reproductive condition and time of spawning (Plaza 2007). *Etrumeus wongratanai* showed high GSI values from June to December for the pooled data (Figure 12), whereas females showed high GSI values from May to December and males from July to December (Figure 13). These values indicate that the breeding season lasts between 8 and 6 months for females and males respectively, and that spawning takes place from the onset of winter to early summer. These findings are consistent with the observations of Connell (2001), who observed that *E. wongratanai* eggs were seen in small numbers through summer, but that spawning peaked in June to December based on the density of eggs at Park Rynie along the Kwazulu-Natal coast. There was a significant difference in GSI values between sexes (Table 7), which can be attributed to the need of male pelagic fish to produce large amounts of sperm to ensure fertilization of eggs, which is counter-intuitive since females typically have higher GSI values than males (Cole 2010). Spawning behaviour is often associated with ecological factors that influence the timing of spawning activity. Food availability is an example of an important
ecological factor in determining the timing of spawning activity, as the number of food items with high nutritious value available in the environment should ensure that larval growth is optimal; other ecological factors that can potentially influence spawning include temperature, day length, latitude, photoperiod, salinity and exposure (Cole 2010). For most fish, temperature controls maturation and the onset of spawning, but the mechanism by which it is controlled is still uncertain (Cole 2010). The temperature range in which spawning occurs is quite narrow, which is a limiting factor for geographical distribution of species. In low latitudes there is little variation in day length and as such food selection could play a vital role in gonadal development (Cole 2010). The reproductive period of fishes tends to decrease with increasing latitude; therefore fish that occur at high latitudes tend to have a short reproductive period, whereas fish that occur in the tropics spawn almost continuously (Cole 2010).

The monthly fluctuations in GSI values suggest that *E. wongratanai* is a serial spawner. This hypothesis is consistent with the observations of Roel and Melo (1990), who showed that *E. whiteheadi* is a serial spawner with a prolonged spawning period and it shares the reproductive characteristics of other well studied pelagic species that occur in South African waters such as sardine and anchovy. However, older fish frequently spawn earlier than younger fish; therefore a prolonged spawning season might not be accurate for individual fish (Cole 2010). The prolonged spawning season suggested here could be due to differences in spawning time between age groups.

Osman (2011) reported that the highest GSI values for *E. golani* were observed during December to May for females and December to July for males in the Mediterranean Sea, with
the start of the spawning season coinciding with the onset of winter. El-Sayed (1996) reported results for *E. golanii* sampled off the Gulf of Suez and Plaza (2007) observed *E. micropus* in southern Japan. These authors indicated that spawning occurs with the onset of winter towards the end of spring and concluded that their respective species are serial spawners, which are in agreement with the present study. Fahay (1983) and Shaw and Drollinger (1990) reported that *E. sadina* in the Gulf of Mexico spawns from winter to summer during late January to early June, which agree with the results of the present study. In the case of *E. sadina* off the Eastern Gulf of Mexico, Houde (1977) reported that they spawn offshore from mid-October to the end of May.

Average fish size varied between years. In 2013, fish were (on average) smaller in July and August, whereas in 2014 fish were largest from May onwards. In 2015, fish were smallest in May through to June. There seems to be inter-annual variability in average fish size but average length data reported do not reflect a full year of catches due to the opportunistic nature of the fishery and thus should be treated with caution.

Condition factor is an indicator of the health of fish, and provides information on the variation in physiological status (Kreiner *et al.* 2001): it can be used for comparing populations as condition factor varies with weight, length and season for the same species (Parrish and Mallicoate 1995). The highest average values for condition factor in the present study were recorded in 2013, and were lowest in 2016. Condition factor was lowest in May through to August and increased from September in 2013, 2014 and 2015. The low condition factor values from May through to August could be due to the physiological strain during the spawning season (Farrag 2010). It is also likely that the condition of fish could be influenced by temperature, day length, food
availability, maturity stage and density-dependent effects (Parrish and Mallicoate 1995; Samat et al., 2008). Density-dependent effects have been reported for sardine from the southern Benguela where CF and standardized gonad mass were lower when fish were abundant and higher when abundance was low (van der Lingen et al. 2006).

In this study, week number explained 32% while year explained 4% of the variation in condition factor of *Etrumeus wongratanai* (Table 6); this suggested a seasonal pattern of condition factor with a more consistent seasonal signal from year to year. Due to a lack of data in the summer months when fish are scarce, the whole seasonal cycle in CF (or GSI) could not be well established.

The findings in this study are in accordance with Farrag et al. (2014) who indicated that *E. golani* collected in the Egyptian Mediterranean Sea showed the highest condition factor during July (summer), while the lowest was recorded from December to May (winter to spring) for females and early July (winter) for males. By contrast, El-Sayed (1996) and Yilmaz and Hossucu (2003), all of whom reported peak condition in January (winter) and spring for *E. golani* from the Gulf of Suez and the Gulf of Antalya in the Mediterranean. These differences could be attributed to change in temperature and photoperiod and its knock-on effects of food availability which contributes to somatic growth and wellbeing of fish (Samat et al. 2008).

4.4 Seasonal variation in diet

Highest feeding intensity in *E. wongratanai* was observed during winter, followed by autumn and summer (Figure 16). The vacuity index (VI) was highest in summer, followed by autumn and winter. The vacuity index is an inverse indication of feeding intensity, which is influenced
by fish abundance, spawning time, as well as seasonal changes in surface water temperature and available food items (Sakamoto et al. 1982). When fish are abundant density-dependent factors such as intraspecific competition for food affect the populations’ feeding ability and this has been reported for many marine fish (Overholtz 1989; Helser and Almeida 1997; Sinclair et al. 2002; Moyle and Cech 2004). The vacuity index is expected to be low during the spawning season as fish need a greater energy input in the spawning season to meet the requirements of reproduction (Tsikliras et al. 2005). Surface water temperature, which is a good proxy for nutrient enrichment, influences the size and structure of plankton communities. When ocean water is cold and well mixed, sporadic nutrient enrichment of the euphotic zone occurs and this promotes the development of phytoplankton and in turn affect zooplankton communities and size structure. In cold water zooplankton communities are dominated by crustaceans such as large copepods and can support large numbers of fish (van der Lingen et al. 2006; Richardson 2008). Whereas, if surface waters are warm, stratified and stable they are nutrient deficient, and the phytoplankton community is dominated by small dinoflagellate and nanoflagellate populations, which results in the zooplankton community being dominated by gelatinous zooplankton and small crustaceans (van der Lingen et al. 2006; Richardson 2008). This is an indication of how water surface temperature influences the composition of zooplankton which becomes available to fish and thus affects their feeding intensity. That feeding intensity was highest during the spawning season (winter), and is in accordance with Farrag et al. (2010), who suggested that spawning fish need a higher energy input in order to meet the physiological demands of reproduction (Froese and Pauly 2000).
When data were pooled and considered by season, the diet of *E. wongratanai* was numerically dominated by prey items in the 1001-2000 µm size class, though prey in the 2001-3000 µm size class made the greatest contribution to dietary carbon (except during autumn, when prey >5000 µm were most important). Due to a lack of samples, the difference in prey size classes per season was more pronounced at prey size class extremes, but could be a reflection of the available food environment at the time of feeding (Lazarro 1987). Feeding on larger zooplankton increases the food consumption and net energetic gain, as feeding would be influenced by the increased handling time needed to ingest an equivalent biovolume when only small zooplankton are available for particulate feeders (van Deurs *et al.* 2014). The fish examined were caught during the day, most likely at different depths and this could have had an influence on our understanding of their diet, particularly if they also feed at night. This is because zooplankton and especially copepods are patchily distributed, occupy different layers of the water column and display different diel vertical migration behaviours (Daro 1988; James 1988; van Deurs *et al.* 2014).

The *Etrumeus wongratanai* collected here generally fed on large prey items, and this finding concurs with those of Mketsu (2008) and Vorsatz *et al.* (2015). In comparison to other local clupeoid species on the east coast, *E. wongratanai* tends to feed on the same size prey items as *E. whiteheadi*, but on larger prey items than do either anchovy and sardine (Vorsatz *et al.* 2015).

No phytoplankton was found in any of the stomachs examined, which is consistent with previous observations (Mketsu 2008; Vorsatz *et al.* 2015) and can perhaps be attributed to the low productivity of the environment and the minimum particle size *E. wongratanai* is able to
efficiently retain due to its gill-raker gap size (Vorsatz et al. 2015). The stomach contents documented in this study were dominated by zooplankton, which is in agreement with the findings of Blaxter and Hunter (1982) who noted that clupeoid fish from areas of low productivity tend to feed mainly on zooplankton. Fish larvae and large copepods were the most frequently occurring prey items in summer (Table 10), small (oncaeids) and large (eucalanids) copepods were the most frequently occurring prey items in autumn (Table 11), and large copepods (eucalanids and calanids) were the most frequently occurring prey items in winter (Table 12). The presence of copepods, fish larvae and hyperiid amphipods suggests that E. wongratanai feeds throughout the water column as zooplankton tend to concentrate at different depths of the water column throughout the day through diel vertical migrations to escape predators during the day by descending further down the water column and ascending at night to feed (Osman 2013).

Fish larvae and eucalanids were indicated to be the most important food item in summer according to the relative index of importance, whereas eucalanids were the most important prey item in autumn and winter followed by oncaeids and calanids respectively (Figure 21). Overall, large copepods were the dominant prey items in terms of frequency and relative importance. When primary productivity is similar in certain areas or seasons it is expected that different species of large and small copepods are abundant (van Deurs et al. 2014). Given this, it is not possible to exclude the fact that prey items found in the stomachs of E. wongratanai reflect what is available in its food environment at the time of feeding, rather than a mechanism of selective feeding on larger copepods and other zooplankton, because samples of the ambient environment at the time of fish sampling were unavailable. However, fish have the ability to adjust their diet
according to the seasonal abundance of prey items (Nieland 1982). The dominance of large copepods and other zooplankton suggests that there is some degree of selectivity in this species. If optimal foraging is employed by *E. wongratanai* it is highly unlikely that it would mainly focus on small prey items simply due their low contribution to ingested carbon, however, this needs to be corroborated with zooplankton data of the environment fish were caught in. The dominance of fish larvae in stomachs in summer months is indicative of spawning of clupeoid and other species along the east coast of South Africa, as *E. wongratanai*, *Sardinops sagax* and chub mackerel *Scomber japonicus* all display a peak spawning period between May to December off the coast of KwaZulu-Natal (Connell 2001). It is likely that these species spawn offshore; as this could partially explain the low frequency of fish eggs in the stomachs of *E. wongratanai* and only once they become larvae do they move inshore into an environment where they become vulnerable to predation by *E. wongratanai*. This is in accordance with Beckley and van Ballegoojen (1992), who observed that fish larval concentration decreased as you move further offshore in the Agulhas Current.

Species of *Etrumeus* across the globe tend to display similar zooplanktivorous feeding behaviours. Osman (2013) observed *E. golanii* in the Egyptian Mediterranean Sea to feed mainly on small crustaceans, fish larvae and mollusks. Small crustaceans were the major prey item in all seasons except for summer where fish larvae were preferred, which was proposed to be due to the increase in the abundance of fish larvae during the summer. Chen *et al.* (1992) reported that *E. sadina* from the Gulf of Mexico feeds primarily on euphausiids, fish larvae, copepods and gastropods, while Bianchi *et al.* (1993) reported that *E. whiteheadii* from Namibian waters feed mainly on copepods. In the East China Sea, Tanaka *et al.* (2006) reported that the stomach
contents of *E. micropus* were dominated by large calanoid copepods. These slight differences in prey items can be attributed to the food environment of each region in different habitats.

4.5 Stable isotope analysis

The stable isotope data suggest that there are gradual changes in the diet of *E. wongratanai* as it increases with size. Large *E. wongratanai* had greater $\delta^{15}$N values than medium and small sized fish, although there were no significant differences in $\delta^{15}$N of medium and small size classes of *E. wongratanai*. There were also differences in $\delta^{13}$C values between size classes, however these results are inconclusive as the Tukey test was not powerful enough to recognise which group the large fish belonged to, and both $\delta^{15}$N and $\delta^{13}$C values of individual fish were significantly positively correlated with CL. These variations in isotopic signatures with size likely indicate that there is an increase in trophic level arising from dietary change and could also have been an effect of size-dependent metabolic turnover, or an accumulation of specific biochemical components (Rossi *et al.* 2004). The variations in isotopic signatures with size can further be attributed to shifts in metabolic demands (Levy 1990), prey availability, habitat, morphology or behaviour (Grossman *et al.* 1980). A consumer’s growth rate can explain most of the variation observed in stable isotope ratios (Van der Zanden *et al.* 1998). Fish growth is a function of temperature, prey quality, and mass-specific consumption, as the respiration rates of fish decrease as fish size increases (Kitchell *et al.* 1977). Fish tend to grow faster in warmer waters and water temperature in turn affects the nutritional quality of prey items, as large prey items with high nutritious quality are found in cooler waters with high productivity and fish need to consume enough food in order to meet their metabolic demands (Pauly 1998).
Classical resource partitioning theory suggests that two competitors for the same resource can co-exist if that resource is partitioned differently amongst them (Schoener 1974). Fish generally grow in size during their ontogeny (Werner and Gilliam 1984) and this growth could lead to different resource use at different life stages. The most commonly mentioned explanation for ontogenetic dietary shifts in planktivorous fish is the development of the feeding apparatus (King and Macleod 1976, Gerking 1994). Therefore differences in the diet are associated with the size of the prey items that can be efficiently retained in the gill-rakers (Costalgo et al. 2012). Vorsatz et al. (2015) have shown that *E. wongratanai*’s gill raker gap increases with increasing fish size, thus affecting the prey size it could retain. The ontogenetic shift in the diet of *E. wongratanai* could then be considered to be consistent with the hypothesis that trophic relationships in pelagic food webs are size based (Cousins 1980) and could be an explanation for the increase in δ¹⁵N values of larger fish.

Stable isotope values for δ¹⁵N were significantly different between seasons, and were highest in summer, followed by autumn then winter. And while δ¹³C values were highest in autumn, followed by summer then winter, there was no significant difference between seasons. The differences in δ¹⁵N could be attributed to differences in the available prey environment in the different seasons, as observed in the gut content analysis (this study) where fish larvae were the most important food item in summer. Such prey would tend to have a higher δ¹⁵N than copepods, which were found to be more important in autumn and winter. Furthermore, seasonal variations in isotopic signatures could be due to seasonal differences at the base of the food chain and thus be caused by biogeochemical factors that affect stable isotope ratios of primary producers (Michener and Schell 1994). These factors, which affect δ¹³C, include the available forms of
dissolved oxygen (which is determined in part by temperature), the metabolic pathways used to fix carbon and phytoplankton growth rates, the latter of which are influenced by light and trace nutrient concentrations (Michener and Schell 1994). Nitrogen values ($\delta^{15}$N) are similarly dependent on the composition of dissolved inorganic nitrogen which differs on the rate of nitrogen recycling in surface waters (Michener and Schell 1994).

Carbon-to-Nitrogen (C:N) ratios were all greater than 3.5, which suggests samples of *E. wongratanai* had a high lipid content, and this could introduce measurement error when interpreting $\delta^{13}$C in stable isotope analyses. It has been found that measurement error increases in $\delta^{13}$C analyses with an increase in lipid content (Post *et al.* 2007). Lipids are generally more depleted in $\delta^{13}$C than proteins and carbohydrates, meaning that a variation in lipid content between organisms can introduce bias in $\delta^{13}$C in stable isotope analyses (Post *et al.* 2007). Analyses of $\delta^{13}$C and C:N ratios can also be influenced by variations in the fractionation of $\delta^{13}$C during lipid synthesis, which results in differences between lipids, protein and carbohydrates by 6-8‰ (De Niro and Epstein 1977). Due to the heterogeneity of tissue types found in aquatic organisms, considerable bias could also be introduced by large differences in $\delta^{13}$C in lipids and other tissue types and the difference in lipid content between organisms (McConnaughey and McRoy 1979). Therefore in order to accurately estimate $\delta^{13}$C in aquatic animals it is necessary to account for lipid content when C:N ratios are consistently high (>3.5) (Post *et al.* 2007), either by chemical extraction of the lipid or by mathematical correction. Carbon-to-Nitrogen (C:N) ratios may also have an effect on $\delta^{15}$N values (Logan and Lutcavage 2008) but this is considerably smaller than for $\delta^{15}$C. Due to time and logistical constraints, fractionation was
precluded from this study and could be a reason for the depleted $\delta^{13}C$ value observed in this study.

The mean $\delta^{15}N$ and $\delta^{13}C$ obtained here were 9.63 ± 0.42 ‰ and -17.84 ± 0.57 ‰, respectively. Nitrogen and carbon values were generally (substantially) lower than those reported for other *Etrumeus* species from around the world. In southern Africa, Sholto-Douglas *et al.* (1991) reported *E. whiteheadi* in the southern Benguela to have $\delta^{15}N$ and $\delta^{13}C$ values of 13.7 ± 0.8 ‰ and -16.2 ± 0.9 ‰, respectively, whilst van der Lingen and Miller (2011) reported a $\delta^{15}N$ value of 12.2 ± 0.9 ‰. Erasmus (2015) reported $\delta^{15}N$ and $\delta^{13}C$ values of 11.09 ± 1.77 ‰ and -16.86 ± 1.15 ‰, respectively for *E. whiteheadi* from the northern Benguela. Ahmad-Syazni *et al.* (2001) reported that *E. micropus* sampled in Hiroshima Bay, Japan had $\delta^{15}N$ and $\delta^{13}C$ values of 16.41 ± 1.0 ‰ and -15.6 ± 0.4 ‰, respectively. According to Gendron *et al.* (2001) *E. sadina* from Bahia de la Paz was shown to have $\delta^{15}N$ and $\delta^{13}C$ values of 13.9 ‰ and -17.9 ‰ (no standard deviations reported). In comparison to these authors, *E. wongratanai* appear to sit at a lower trophic level. These differences could be due to regional baseline differences as these fish come from different ecosystems and are predisposed to different sources and types of nitrogen integrated into production (Waser *et al.* 2000, Montoya *et al.* 2002, Waite *et al.* 2007, van der Lingen and Miller 2014). In the Benguela Current ecosystem, upwelling plays a major role in making nitrogen available to organisms (van der Lingen and Miller 2014), in comparison to the Agulhas Current ecosystem where recycled and Nitrogen-fixed nitrogen are important (Montoya *et al.* 2002, Waite *et al.* 2007, van der Lingen and Miller *et al.* 2014). The hydrographic conditions of Hiroshima Bay, with its variable salinity and sea-surface temperature, results in a well-mixed water column year-round, which in turn, results in continued nutrient regeneration.
and high levels of primary productivity (Ahmad-Syazni et al. 2001). These differences in oceanographic processes, thus make comparisons between ecosystems difficult.

*Etrumeus wongratanai* appear to have lower values of $\delta^{15}N$ and $\delta^{13}C$ than reported for sardine sampled off the east coast in 2009 and 2010, with sardine there having a mean $10.2 \pm 0.6 \%$ and $-13.3 \pm 1.0 \%$ for $\delta^{15}N$ and $\delta^{13}C$, respectively (van der Lingen pers. comm.). This is counter-intuitive, considering that sardine are primarily filter-feeders that feed on small zooplankton (and phytoplankton) whereas east coast redeye feed on larger zooplankton (this study), but could be a reflection of inter-annual variation in trophic conditions on the east coast (van der Lingen and Miller 2014). de Lecea (2012) reported that isotopic signatures for pelagic zooplankton in the KZN Bight ranged from $4.38 \%$ to $9.66 \%$ during the summer and $5.29 \%$ to $9.41 \%$ during the winter for $\delta^{15}N$, while $\delta^{13}C$ values ranged from $-21.64 \%$ to $-17.91 \%$ during summer and $-20.61 \%$ to $-18.70 \%$ during winter. The isotopic signatures reported in this study for *E. wongratanai* were higher in terms of $\delta^{15}N$ and $\delta^{13}C$ than for majority of the zooplankton which included copepods and decapods reported by de Lecea (2012). This suggests that *E. wongratanai* likely feeds on this zooplankton. However, pelagic zooplankton with similar isotopic signatures to that of *E. wongratanai* can be attributed to nitrogen being recycled into ammonia which leads to an enrichment of $\delta^{15}N$ values of plankton (de Lecea 2012).
Chapter 5: Conclusions

5.1 Age and growth

The present study provides the first set of data on the age and growth of *Etrumeus wongratanai* and indicates that annuli found on otoliths of *E. wongratanai* caught off Scottburgh, KwaZulu-Natal can be read with relative ease and with a small margin of imprecision between readers. It was observed that *E. wongratanai* appears to be a relatively short-lived species, not growing older than 4 years with a high growth rate during its 1st year of life. The results of other studies on *Etrumeus* species in South African waters and abroad tend to agree with the results attained in the present study as they indicated their respective species to be fast growing and short-lived with some attaining a maximum age of 8 years old. This study focused only on estimating age based on counts of annual growth zones and thus should be treated as preliminary and further work on the age and growth of *E. wongratanai* should incorporate counts of daily rings to validate the age of fish. This is important as determination of absolute age via daily ring analysis is required to determine the accuracy of the age estimate obtained from counts of annual growth zones and the counts of daily rings could provide strong corroboration with regard to the frequency of formation of these annual growth zones.

5.2 Length at maturity, gonadosomatic index and condition factor

It was observed that female *Etrumeus wongratanai* attained sexual maturity at a greater caudal length than males. *Etrumeus wongratanai* on the east coast of South Africa appears to attain sexual maturity at a greater length than that of its congeners found in South African waters and around the world. The scatter in the data with the fitted maturity ogives highlights the difficulty in using a macroscopic assessment of gonads to accurately determine whether a fish is sexually
mature. Microscopic examination of histological sections of gonads is suggested in future work to validate the macroscopic assessment of the size and age at which fish mature. The findings of the present study are consistent with that of reproductive work previously done on *Etrumeus* species from different regions. GSI data suggested that *E. wongratanai* is a multiple spawner and has a prolonged spawning season which lasts 6 months for males and 8 months for females. The spawning season extends from the onset of winter to summer, which has previously been observed in other *Etrumeus* species. There was a difference in GSI values between sexes which can be explained by the need for males to produce large amounts of sperm to ensure fertilization, although this is counter-intuitive since females typically have higher GSI values than males. A recommendation for further reproductive studies on *E. wongratanai* includes a more robust sampling procedure where a lot more fish are caught throughout the year and fish should be preserved in formalin instead of being frozen to give a more accurate estimates of gonad mass and hence GSI values.

Different seasonal patterns in condition factor from year to year were observed which could reflect inter-annual variability in environmental conditions. Condition factor was lowest from May to August in most years, before the onset of spawning, which suggests that the energy demands of reproduction seem to influence condition factor of fish within these months. Future research on condition factor should include data from all seasons to analyze the seasonal cycle for a full year.
5.3 Seasonal variation in diet

The findings of the present study are in accordance with previous studies in terms of the diet composition of *E. wongratanai*. The present study indicates that *E. wongratanai* appear to display a seasonal variation in feeding intensity with a higher percentage of full stomachs being recorded in winter than any other season. There was a difference in the size class of prey items found in the stomachs of *E. wongratanai* by season as well as some degree of possible selectivity in prey items from season to season, with fish larvae being the most important prey in summer compared to large copepods in winter and autumn. This suggests a seasonal variation in diet. However, the assumption of selectivity could not be corroborated due to the lack of samples of the prey environment at the time of feeding, which emphasizes a need for sampling of the zooplankton environment at time of feeding in future research. Additionally, more robust sampling techniques are needed in order to get information on where exactly fish were caught, at which depth, and at what time of the day. Better representation of samples from each season is also required, as no data were collected in spring and data for summer are sparse in this study.

5.4 Stable isotope analysis

The present study provides the first set of isotope data for *E. wongratanai* off the east coast of South Africa. Stable isotope data suggest that there is an ontogenetic shift in diet of *E. wongratanai* in terms of $\delta^{15}$N. This likely reflects the increased prey size spectrum available to larger fish and could possibly serve to reduce the effects of intraspecific competition between small, medium and large fish. The data also suggest that there was a seasonal shift in $\delta^{15}$N signatures of fish which supports the findings of stomach content analysis and can be attributed to a different availability of prey items in the different seasons. When compared to *Etrumeus*
species in other parts of the world, *E. wongratanai* showed substantially lower $\delta^{15}$N and $\delta^{13}$C values but different environmental properties of the different ecosystems where the other species of *Etrumeus* occur makes comparison difficult. When compared to $\delta^{15}$N and $\delta^{13}$C values of pelagic zooplankton from the east coast *E. wongratanai* tend to have higher $\delta^{15}$N and $\delta^{13}$C values than the majority of pelagic zooplankton sampled in the KZN Bight, which suggests they feed on these organisms. Future research should include the removal of lipids from the muscle tissue of *E. wongratanai* for $\delta^{13}$C analysis given that the C:N ratios suggested high lipid content in muscle tissue which could introduce bias in interpretation of $\delta^{13}$C. Furthermore, the ambient environment where fish were caught needs to be sampled and isotope signatures analyzed to establish a baseline for trophic level interactions off the east coast of South Africa.
Chapter 6: References


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