Lipid quantity, composition and provisioning in the New Zealand snapper/tamure *Chrysophrys auratus*

Hamish Allen

Auckland University of Technology

School of Science

Thesis submitted in partial fulfilment for the degree of Master of Science (MSc)

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

Signature: 

Date: 6/03/2017

Acknowledgments

I will now attempt to thank all the people who in some way contributed to the work in this thesis. First and foremost, I would like to thank my supervisor Dr Armagan Sabetian for his help, guidance and support from the very outset of this project. You have made this process enjoyable, have regularly offered invaluable direction and comments, and have been engaged and supportive in the many aspects of this thesis. Dr Chris Pook for his knowledge and assistance with all things lipid and chromatography. To Dr Elizabeth Tripp for her help and support. Tom Rowlands for his cheerful help through hours of lab work, fishing, dissecting and for his fatty acid chromatography and analyses. Justine and Matt for their help on long days fishing and dissecting and their work with histology and ageing.

I would like to thank the vast number of people that helped in the field work associated with this study, but a special mention to Jonny Pearce for catching the biggest fish and sharing his vast fishing knowledge, and to Evan 'No-Fish' Brown for his friendship and expert skippering throughout this project.

I would like to thank Dr John Perrott for his advice and help during the consultation process of this thesis, and to Fiona McKenzie and Lorena Cardenas at the Manuhiri Kaitiaki Charitable Trust for their interest, comments and feedback.

I would like to thank all my family and friends for their support, countless proof readings and constant encouragement. Thanks to my colleagues at Ambury Regional Park and the wider Southern Parks network for their ongoing support of my studies and their flexibility with working hours over the many years I have been at University. And lastly I would like to thank Dr Melanie Vaughan and Dr Jarrod Walker at Auckland Councils' Research and Evaluation Unit (RIMU), for their support, advice, and for the opportunity to sound ideas off them throughout my post graduate studies.

Contents

Abstract 1
Chapter 1 - Introduction 2
1.2 Aim and objectives 5
1.3 Life history of *Chrysophrys auratus* (snapper) 6
1.4 Snapper; a New Zealand icon 10
1.5 Justification for this study 11
Chapter 2 - Methodology 12
2.1 Location, specimen collection and sampling 12
2.2 Reproductive analyses 14
2.3 Ageing and growth estimation 14
2.4 Chromatography and lipidomics overview 18
2.4.1 TAG analysis methods 20
2.4.2 Fatty Acid Methyl Ester (FAME) Methods 21
2.4.3 Dry Weight 22
2.4.4 Statistical Analysis 22
Chapter 3 - Results 23
3.1 Population demographics and reproduction 23
3.1.2 Size, age and growth 24
3.1.3 Reproduction 28
3.2 Female lipid and fatty acid composition, quantity and provisioning 31
3.2.1 TAG gonad and liver quantity 32
3.2.2 TAG species-specific generalised additive models 34
3.2.3 TAG provisioning 35
3.2.4 TAG relative precentage and composition 36
3.3 Fatty Acid Methyl Esters 37
Chapter 4 - Discussion 42
4.1 Population demographics and reproductive parameters 42
4.2 Lipid composition, quantity and provisioning 46
4.2.1 Triglyceride (TAG) quantity 47
4.2.2 Triglyceride (TAG) composition 51
4.3 Lipid provisioning and breeding strategy 52
4.4 Fatty Acids Methyl Esters 54
4.4.1 Composition and concentration 54
4.4.2 Oocyte and tissue comparisons 55
5. Limitations 56
6. Conclusion 57
References 59

Abstract

Snapper (Sparidae: *Chrysophrys auratus*) are a demersal teleost, and are one of the most abundant and ecologically important species inhabiting coastal New Zealand waters. They are highly valued both commercially and recreationally, and are under continuing pressure from intensive fishing efforts. This has led to declines in genetic diversity amongst populations, changes in the ecosystem structure of reef communities, and a significant reduction in stock biomass.

Lipids and fatty acids are fundamentally important for growth, as sources of metabolic energy, in the structure and integrity of cell membranes and as endogenous energy reserves for the purpose of reproduction. Within a population, the quantity and concentration of an individual's lipids and fatty acids can vary significantly. During reproduction, older and larger individuals can produce larvae that are larger, grow faster and survive periods of starvation for longer, compared to larvae of smaller, younger individuals, due in part to a greater provisioning of energy rich and biologically important lipids. The composition of lipids may also vary; an important aspect as lipids such as triglycerides and certain fatty acids, play more integral roles than others in developmental processes.

It has been alleged that maternal age can have profound impacts on embryonic, larval and juvenile survival, growth rates, and functionality. We investigated triglyceride and fatty acid profiles of female New Zealand snapper/tamure (*Chrysophrys auratus*) across age, size, and condition, throughout its spawning season between November 2015 and February 2016.

A total of 113 *Chrysophrys auratus* were sourced from the Hauraki Gulf Marine Park, North Island east coast, New Zealand. Individuals were measured, aged and histologically staged, before High Pressure Liquid Chromatography was used to determine triglyceride concentrations in female liver and gonad tissue, and Gas Chromatography to determine fatty acid concentrations in female gonad tissue.

Results determined that maternal influences such as size, age, and condition, are not influencing lipid composition or concentrations. Therefore, the quality of oocytes is likely to be comparable across the population, placing importance on larger individuals whose fecundity is exponentially greater than that of their smaller counterparts. In addition to this, snapper appear to demonstrate a tendency towards a capital breeding strategy, using (to some degree) stored lipid reserves to fund reproductive needs across multiple spawning events. This removes any reliance on specific food sources being available at specific times, and affords adaptability in the timing of spawning, enabling reproduction to take place when it is environmentally optimum, and has likely been a contributing factor in the success and abundance of this species.

Chapter 1 - Introduction

Reproduction is a demanding physiological process requiring substantial amounts of energy and resource investment. A common reproductive method for teleost species involves broadcast spawning, with both males and females releasing gametes into the water column where they are fertilized externally. Typically, this process results in a large number of offspring and a subsequent high rate of mortality, with reproductive capacity and recruitment success being limited by a number of factors. These can include abiotic factors such as water temperature, salinity, and nutrient availability, whilst internally, molecular and biochemical characteristics such as oocyte morphology, membrane integrity and lipid content and composition can influence larval durability and survival (Bobe & Labbé, 2010; Brooks, Tyler, & Sumpter, 2007).

Lipids provide an important source of metabolic energy for processes such as somatic growth and are integral in the formation of cell and organelle membranes (Sargent, 1995). Furthermore, lipid quantity and composition is critical to both the growth and development of embryos, and is essential in sustaining larvae prior to organ development and their subsequent ability to feed independently (Denslow & Sepulveda, 2007; Zeldis et al., 2005). Therefore, analysis of the composition and quantity of lipids in oocytes and gonadal tissue can provide a good indication of maternal health, and the likelihood of larval survival (Grote et al., 2011; Salze et al., 2005).

Lipids are a naturally occurring diverse group of hydrophobic molecules ubiquitous in cells of living organisms (Harwood et al., 2016). The term lipids is broad, encompassing a number of sub-categories which include fatty acids, waxes, sterols, triglycerides and phospholipids. They provide a range of fundamentally important biological roles, such as acting as sources of metabolic energy, aiding in chemical signalling, providing insulation and as structural components of cell membranes (Wiegand, 1996). In teleosts, lipids also support buoyancy control, provide additional integumental waterproofing and constitute the major nutrient source for developing embryos during the reproductive process (Sargent, 1995). Weigand (1996) has identified three sources of lipids for developing oocytes; endogenous lipids mobilized from storage tissue, lipids supplied exogenously through diet, and lipids synthesized within the oocyte. Dietary input is particularly important for serial broadcast spawners who continue to feed in between spawning events (Johnson, 2009), particularly in light of the fact that the vast majority of triglycerides are sourced from exogenous means (Ruiz-Gutiérrez & Barron, 1995).

The reproductive cycle and lipid levels in fish species are often interrelated, with lipid development and provisioning changing pre, during, and post reproduction (Sargent, 1995). Certain lipids are considerably more valuable than others during early larval development. For example, triglycerides, also known as triacylglycerols (hereafter TAG's), along with wax esters are the main components of endogenous oil globules, and are the major contributors of energy for both growth and metabolic processes (Anderson, Anderson, & Arthington, 1990; Berkeley, Chapman, & Sogard, 2004; Norton, MacFarlane, & Mohr, 2001). TAG's are a class of neutral lipids consisting of a glycerol and three molecules of fatty acids (Berg, Tymoczko, & Stryer, 2012). They are the primary constituent of natural fats and oils and are the preferred long term form of stored energy in the animal kingdom, dominating the composition of fish oocytes (Harwood et al., 2016).

Both the composition and quantity of lipids in teleosts leading up to and during reproduction can vary significantly among species, ranging from between 10% - 30% of the total dry weight of their eggs (Sargent et al., 1993; Weigand, 1996). Some species such as sea bass (*Dicentrarchus labrax*), turbot (*Scophthalmus maximus*) and snapper (*Chrysophorus auratus*) have developed discrete oil globules contained within the egg yolk (Sargent, Tocher, & Bell, 2002). This process is referred to as oocyte lipidation, and fish who demonstrate this trait tend to have higher levels of neutral lipids (primarily TAG's) compared to those without, where polar lipids such as phospholipids dominate (Hiramatsu et al, 2015; Weigand, 1996). Levels of certain lipids have been shown to have an influence well beyond the reproductive stages, with larvae containing high levels of TAG's improving in survivability and overall condition (Berkeley et al., 2004; Giraldo et al., 2013). Along with TAG's, various polyunsaturated fatty acids (PUFA) also provide specific functions for larval development. PUFA are straight chained even numbered structures comprising of more than one double bond. Three PUFA in particular have been identified as being of specific importance in fish reproduction and in larval development. Referred to as essential fatty acids (EFA); docosahexaenoic acid (DHA), arachidonic (AA) and eicosapentaenoic acid (EPA) are critical components in fish development, acting as signalling molecules controlling growth, are significant components in cell membrane phospholipids, and have the ability to be catabolized for energy (Sargent et al., 1995; Grote et al., 2011). EFA have been shown to directly affect egg quality and hatching success, as well as larval formation and growth in several species such as cod (*Gadus morhua* L.), zebrafish (*Danio rerio*), yellowtail flounder (*Limanda ferruginea*) and capelin (*Mallotus villosus*) (Copeman et al., 2002; Jaya-ram et al., 2008; Sawaboonchun, 2009; Weigland, 1996). These EFA have the additional role of acting

as precursors for a number of intra and extracellular messengers known as eicosanoids (Sargent, 1995; Weigand, 1996). Eicosanoids are important localised hormones, helping to control growth, acting as messengers in the central nervous system, and playing an essential role in immune regulation, helping to maintain health and ensure correct larval development (Rowley et al., 1995; Sargent et al., 1999b). In particular, DHA is known to be specifically important in eye and brain development (Sargent, 1995; Sargent et al, 2002), and as such is of particular interest to most fish species that require proficient sight to selectively and visually feed once digestive systems have developed (e.g. Pankhurst, 1994).

The quantity and composition of lipids and their fatty acid constituents in fish oocytes can vary among species and within populations. These differences can be maternally derived, and have been identified as a contributing factor for larval survival in a number of teleost's such as yellowtail kingfish (*Seriola lalandi*), black rock fish (*Sebastes melanops*) and white sea bream (*Diplodus sargus*) (Berkley et al., 2004; Cejas et al., 2003; Hilton, Poortenaar, & Sewell, 2008). Typically, where differences occur, older and/or larger females have shown a better provisioning capacity, producing larvae that are bigger, grow faster and survive periods of starvation for longer, compared to larvae of smaller, younger individuals (Birkeland & Dayton, 2005). This may be as a result of an individual's increasing ability to provision effectively and efficiently over multiple years, or as a trade-off in allocation as somatic growth slows, allowing resources to be concentrated into developing gametes. Black rockfish (*Sebastes melanops*) larvae from older females were found to not only grow up to three times faster, but also possessed the ability to survive periods of starvation for longer compared with younger individuals (Berkeley et al., 2004). The underlying driver for this observation was posited to be a greater provisioning of maternally derived TAG, increasing in volume with the age of the individuals (Berkeley et al., 2004).

Even a slight increase in growth rates at the larval stage has been shown to have a profound impact on survival rates (Meekan & Fortier, 1996), enabling an individual to pass through its most vulnerable life stage quickly and successfully, improving as it does so in its ability to feed, escape predation and increasing in overall fitness and locomotion capabilities (Osse et al., 1997). This increased growth rate can extend beyond the larval stage, with fast growing larvae having been observed to continue to be fast growing juveniles (e.g. Sim-Smith, Jeffs, & Radford, 2012). Whilst the foundation for this is not clear, it is thought to potentially be a result of genetic or nutritional variations among individuals (Sim-Smith et al., 2012). Larger and older female fish have long been shown to

contribute significantly more to population replenishment (Beldade et al., 2012; Palumbi, 2004), a particularly important aspect when regarding exploited, ecologically significant or commercially valuable stocks. Whilst higher levels of fecundity observed in older and larger fish undoubtedly contribute to this, the quality of offspring produced may also be a significant factor in larval success, survival, and the establishment of strong year classes (Beldade et al., 2012; Birkeland & Dayton, 2005).

Reproduction in fish is dependent on the way energy reserves and lipid resources are either stored or allocated. As such, fish are referred to as either 'income' or 'capital' breeders (McBride et al., 2015). Income breeders rely on the ongoing intake of food to supply the necessary nutrients for reproduction, while alternatively, capital breeders use stored supplies of nutrients. A species can sit anywhere along this continuum, ranging from those that at the extreme income end that are entirely reliant on immediate food supplies, to those at the extreme capital end, that are storing lipids over several years in preparation for singular spawning events (McBride et al, 2015). Determining the location of a species on this continuum allows for better understanding of their biology and their reliance on local food sources. Serial spawners may require food between each spawning event to replenish their lipid reserves, or they may allocate discrete amounts of lipids to the gonads, leaving stores for the next spawning event. The liver is a key organ in this provisioning process, where synthesis, accumulation and transport of lipids are initiated. In teleosts, lipids sourced through diet and from adipose tissue are first held in the liver, where they are synthesized into vitelloginin (an egg yolk precursor), and lipoproteins, before being transported to the gonad and developing oocyte (Johnson, 2009; Weigand, 1996). For example, both heptosomatic and gonadosomatic indices have been shown to increase in female *C. auratus* during the reproductive cycle, indicating the important role the liver plays in storage and synthesis for this species (Scott & Pankhurst, 1992).

1.2 Aim and objectives

Fisheries management relies on spawning biomass as an indication of recruitment potential; however, biomass on its own may not provide an adequate measurement, such is the potential for variation in egg and larval quality. As such, more detailed knowledge on reproductive biology is required to accurately and effectively manage stocks (Fitzhugh et al., 2012). Determining the factors involved in stock-recruitment are fundamental to our understanding of marine ecosystems, as well as our ability to implement effective conservation and to manage fisheries sustainably.

The aim of this study was to compare lipid composition and quantity of the New Zealand snapper/tamure (*Chrysophrys auratus*) against maternal age, size and condition, and in relation to provisioning strategy. In order to achieve this aim, two specific objectives were formulated; firstly, to ascertain and analyse the composition and quantity of TAG's and fatty acids in gonadal tissue, and secondly, to analyse TAG provisioning in both liver and gonadal tissue.

These objectives have been addressed in the following chapters. In chapter two the specific methodologies pertaining to establishing age and reproductive stages are described, along with lipid and fatty acid analyses using liquid and gas chromatography, respectively. Chapter three presents and discusses the results of the demography and life history of *C. auratus*, along with the results of lipid and fatty acid analyses in gonadal and liver tissues. In chapter four the findings are summarised and discussed in relation to the aim and objectives of this study. Furthermore, the contribution of this thesis to the understanding of gamete quality and reproductive success in *C. auratus* is discussed in consideration of management and conservation efforts, and future research priorities suggested.

1.3 Life history of *Chrysophrys auratus*

Chrysophrys auratus is one of the most abundant and ecologically important species inhabiting coastal New Zealand waters (Parsons et al., 2014). They are demersal teleosts and a member of the Sparidae family, which consists of 115 species within 33 genera, distributed throughout both tropical and temperate waters worldwide (Chiba et al., 2009). *C. auratus* are distributed widely throughout Australasia, restricted by colder water temperatures in the south, which is why this species is most commonly found in northern New Zealand, with large stocks existing along the west coast, and the east coast north of East Cape (Crossland, 1981; Paulin; 1990). Found at a maximum depth of 200m, snapper is more commonly found in waters <70m, (Crossland, 1981), and are present over a wide range of substrates, inhabiting varying ecosystems throughout their life cycle (Parsons et al., 2014).

C. auratus has historically undergone numerous changes in nomenclature. Most recently, the genus *Pagrus* was instated in place of the previous genus *Chrysophrys* in the early 1990s, after study into previously separate stocks in Australian (*Chrysophrys unicolor; Chrysophrys guttulatus*) New Zealand (*Chrysophrys auratus)* and Japanese (*Pagrus major*) waters revealed indeterminate morphological and biochemical differences, prompting a call for all species be allocated a single name: *Pagrus auratus* (Paulin 1990).

This remained until mitochondrial DNA analysis revealed that the Australasian and Japanese stocks are genetically distinct, and are not in the same monophyletic grouping as other *Pagrus* species (Chiba et al., 2009). As such, it was suggested that the name *Chrysophrys auratus* be reinstated for this species in Australia and New Zealand. However, this suggestion has not been universally adopted, with a number of departments and researchers continuing to use the genus *Pagrus*. This thesis will use the most recently recommended *Chrysophrys auratus*, however the common name "snapper" will be employed to refer to citations where other names were used.

Snapper is an oviparous serial broadcast spawner, releasing gametes into the water during spawning aggregations between the months of October and March (Crossland, 1981). Fertilized eggs remain in the upper water column, hatching 0-2 days after insemination (Parsons et al., 2014). Snapper larvae do not feed until 4 – 6 days after hatching, relying on endogenous nutrients during this time, after which they visually and selectively feed, primarily on copepods and nauplii (Pankhurst, 1994). Larvae begin life with poor eyesight (Pankhurst, 1994), which is why the density of nutrients in the water can influence survival and recruitment rates (Zeldis et al., 2005). Larvae remain in open water for approximately 28 days, by which time they have grown to between 9 – 14mm, their swimming ability is good, and they are ready for settlement as juveniles, typically in sheltered, shallow, structured habitats such as sea grass or mussel beds, drawn in by hydrodynamic and olfactory cues (Parsons et al., 2014). These habitats are significantly higher in larval abundance compared to bare substrate (Parsons et al., 2016). This is thought to be as a result of structurally complex habitats offering enhanced rates of flow velocity, delivering high amounts of planktonic copepods, and thus providing juveniles with a good source of nutrients while minimising their energy expenditure (Parsons et al., 2015). Approximately 5-6 months after settlement juveniles reach 60 – 70mm in length and begin moving out beyond their sheltered settlement habitat to occupy more varied coastal environments (Parsons et al., 2013). Their ecological range continues to increase further throughout their first year, with individuals occupying sand, mud, and/or rocky reef habitats (Francis, 1995). Along with habitat, diet also diversifies during this period with pharyngeal apparatus and mouth size development leading snapper to change from planktonic copepods to a wide range of taxa, primarily crustaceans, but also including polychaetes, molluscs and echinoderms (Usmar, 2012).

Throughout the juvenile stage, an individual's eyesight improves, altering in both acuity and wavelength sensitivity, preparing snapper for changes in light intensity and

wavelength, as it moves further from sheltered to more open ocean environments (Robinson et al., 2011). Water temperature also plays an important role during larval development of snapper. Lipid concentration and growth analysis has revealed that snapper first utilise lipids in somatic growth, before a decrease in growth rate and an increase in stored lipids occurs during autumn, readying the larvae for the oncoming winter (Sim-Smith, Jeffs, & Radford, 2013b). Years with warmer water and longer autumn seasons allow larvae to maximise both growth and the subsequent storage of lipids, while years with an earlier winter are likely to see a lower overall survival rate (Sim-Smith et al., 2013b). Along with water temperature, other abiotic factors such as the presence and strength of onshore winds and large tidal coefficients also strongly influence larval success, assisting the migration of larvae into their settlement habitats (Sim-Smith et al., 2013b). Because of the planktonic nature of snapper eggs and larvae they are extremely vulnerable to both predation and starvation, with an estimated 83% mortality occurring between spawning and hatching, with this rising to 98% for larvae <8 days old (Zeldis et al., 2005).

After initial rapid growth over the first six months, rates slow comparatively over the next five years, with individuals reaching sexual maturity at 3 – 4 years and approximately 23cm in length (Walsh, McKenzie, & Armiger, 2006). Snapper exhibit remarkable spatial plasticity in growth rates around New Zealand, with populations from the east coast typically growing at a slower and more varied rate than that of other stocks. For example, a 16-year-old individual from the north east can be anywhere between 28 and 58cm in length (Walsh, Buckthought, & McKenzie, 2011a). The underlying drivers for these observed differences are unclear, however the historical high level of fishing exploitation on the east coast is thought to be a contributing factor (Parsons et al., 2014).

Snapper live for a maximum of 60 years, reaching a maximum length of 100cm (Leach, 2006). As protogynous hermaphrodites, all snapper begin life as females, with around half developing into males prior to reaching sexual maturity (Francis & Pankhurst, 1988). It is unclear whether endogenous or exogenous cues, or a combination of both, drive this process. Snapper becomes sexually mature as early as 23 cm, with 100% maturity by 30 cm (Crossland, 1981). As adults, snapper inhabit a variety of environments, ranging from sandy substrates to coraline algae reefs (Parsons et al., 2014). The home range of individuals varies significantly, with snapper roaming at scales varying from up to 20 km (Gilbert & McKenzie, 1999), down to a few hundred meters, with a high level of site fidelity demonstrated in some individuals (Parsons et al., 2003; Willis, Millar, & Babcock, 2003). This variance has important implications for the conservation of the species, with

both large and small individuals (> 23 cm) observed remaining entirely within marine protected areas (Willis et al., 2003), whilst highly migratory individuals challenge fisheries biomass interpretations and in turn their sustainable management (Gilbert & McKenzie, 1999). Further to variances in range, snapper also exhibit differences in spatial behaviour. There is anecdotal evidence to suggest that some snapper lead a more pelagic and highly mobile existence. Referred to as 'school snapper', these individuals can migrate large distances into the inshore areas of the Hauraki Gulf to take part in spawning aggregations, along with potentially slight variances in colouring and morphology, they also consume a diet dominated by pelagic fish species (Parsons et al., 2014), as opposed to benthic fish and crustaceans such as crabs, molluscs and echinoids, favoured by less transient snapper (Usmar, 2102).

Some snapper will travel considerable distances to aggregating sites while others will remain in their localised environment, and not all mature fish spawn every year (Parsons et al., 2014). Large harbours and embayments such as the Hauraki Gulf undoubtedly play an integral role in the spawning process, with areas within the Gulf known historically as favoured aggregating sites (Cassie, 1956). Plankton tows reveal high levels of snapper eggs to be present in inner and sheltered areas, however, some degree of spawning takes place right throughout the gulf (Zeldis & Francis, 1998). This includes the possibility that some resident snapper demonstrating short, vertical migrations during the spawning season, maybe spawning in situ, remaining within their home range (Parsons et al., 2003). Observations of snapper spawning taking place in the wild are limited, however, numerous controlled sightings have been reported involving captive snapper where a female (or group of females) swims vertically towards the surface, followed by several males, with gametes released close to the surface (Smith, 1986). The timing of spawning is initiated by water temperatures reaching between 14.8 - 16°C, and persist from late October to early March, typically peaking during November and December (Francis, 1994a). As asynchronous spawners, snapper contain gametes at varying stages of maturity simultaneously. These stages can be classified microscopically, for both males and females from immature, to resting, through to developing, spawning and spent. Snapper are thought to spawn daily throughout the spawning season, with spawning typically occurring in the late afternoon/early evening (Scott, Zeldis, & Pankhurst, 1993).

1.4 Snapper; a New Zealand icon

Snapper are regarded as having strong ecological, cultural, recreational and commercial value in New Zealand. As an apex predator, snapper provide an essential role in the trophodynamics of coastal ecosystems, modifying and influencing the environment they inhabit (Shears & Babcock, 2002). They are an important predator of the sea urchin (*Evechinus chloroticus*), which when in an environment free of predators can overgraze macroalgae, an important food source and habitat for a range of species, resulting in unproductive areas dominated solely by *E. chloroticus* known as urchin barrens (Shears & Babcock, 2002).

Snapper's relative abundance and coastal distribution has meant that they are accessible to both recreational and commercial fishers, with stocks under historic intensive fishing pressure (Willis et al., 2003). Snapper was a dominant and important source of protein for early Māori throughout New Zealand, with remains found in middens consisting 15% of the total fish catch (Leach, 2006). Snapper has been fished commercially in New Zealand for over 150 years, beginning in earnest with the deployment of steam trawlers in the Hauraki Gulf in the early 1900's (Paul, 2014), and continuing on to become one of the largest and most valued commercial and recreational fisheries in New Zealand (Ministry for Primary Industries, 2013). Fishing intensity steadily rose through the 1960's, peaking in 1978 at over 18, 000 tonnes, after which some stocks began showing significant signs of over fishing (Ministry for Primary Industries, 2013). The introduction of a quota management system (QMS) in 1986 aimed to provide sustainable management of fisheries within New Zealand's exclusive economic zone, setting and controlling harvest levels and total allowable catch limits to enable exploited stocks to replenish (Ministry for Primary Industries, 2013). In terms of recreational pressure, aerial and boat ramp surveys predict that over a single summer (2011 – 2012) along the east coast of the North Island, 3,754 tonnes of snapper were harvested, with two thirds of this coming from the Hauraki Gulf alone (Hartill et al., 2013). Continuing reductions in snapper biomass throughout this area prompted the Ministry for Primary Industries to take action, and on April 1, 2014, the number of fish allowed per recreational fisher per day was reduced (from 9 to 7) and an increase in size limits (from 27cm to 30cm) was established in an attempt to mitigate declines in the stock.

Snapper stocks are divided into four separately managed areas; SNA1- East coast North Island from Cape Reinga to East Cape, SNA2 – East Coast North Island south of

East Cape, SNA7 – Marlborough and Tasman, and SNA8 – West Coast North Island. Total allowable catch (TAC) rates are guided by stock biomass assessments, commercial and recreational catch rates and commercial catch per unit effort (Ministry for Primary Industries, 2013). This has allowed the different pressures, growth rates and localised dynamics to be managed independently within each area. Currently, commercial fishing of snapper in New Zealand continues at or above the allocated threshold, with a variety of long lining and netting methods used to harvest an annual catch limit of 6,357 tonnes (4 500 in SNA1 alone), valued at over $250 million NZD annually (Ministry for Primary Industries, 2013; Statistics New Zealand, n.d). Both commercial and recreational fishing pressure on snapper has had wide ranging impacts, leading to declines in genetic diversity amongst populations (Hauser et al., 2002), changes in the ecosystem structure of reef communities (Shears & Babcock, 2002), and ultimately brought about a significant reduction in overall stock biomass (Ministry for Primary Industries, 2013).

1.5 Justification for this study

Despite snapper being one of the most widely researched species in New Zealand (Parsons et al., 2014), little is known of the cytoplasmic quality of its gametes or the lipid and fatty acid composition or content of its gonads. Whilst female snapper have been shown to produce significantly more oocytes as they increase in size (Crossland, 1981), no data exists pertaining to the lipid quantity and composition of these oocytes in wild stock, nor on their levels in female gonadal tissue throughout the spawning season. Given the variability in biotic and abiotic factors influencing the success of snapper eggs and larvae post hatching, including the patchiness of available nutrients driven by oceanographic processes and the presence or absence of planktivorous predators (Zeldis et al., 2005), analysis of the quality of gametes prior to dispersal may provide a clearer indication of recruitment potential and future biomass of the stock. Parsons et al., (2014) identify that a better understanding of the early life stages of snapper, including determinants of larval survival and ecology and physiology of eggs, is an essential component to the effective and sustainable management of snapper.

This thesis will contribute to the knowledge base of *C. auratus* reproductive biology by investigating the relative influence of maternal size and age on oocyte TAG and fatty acid content. It will also explore lipid provisioning strategy to shed light on physiological developmental mechanisms and the place of *C. auratus* on the capital – income spectrum.

Chapter 2 - Methodology

2.1 Location, specimen collection and sampling

Chrysophrys auratus were sourced from recreational fishing trips in the Hauraki Gulf Marine Park, North Island east coast, New Zealand (see Figure 2.1). A total of 11 trips were used at 10-day intervals (where possible) beginning on 26/10/2015 and ending on 11/02/2016. The majority of specimens came from the near shore area (<45m depth) between Tiritiri Matangi and Kawau islands. This area is adjacent to the Mahurangi estuary, a well-established nursery for post settlement snapper (Sim-Smith et al., 2013a). In addition, six fish came from the Mokohinau Island group, and four from the Firth of Thames. Specimens were kept on salt ice until back at AUT Science laboratories where dissection and morphological measurements took place. Fork length (FL), standard length (SL) and total length (TL) was measured for each specimen, in addition to total weight (gm). After removal of the brain with a scalpel, the otic capsules were separated and the sagittal otoliths removed with a pair of fine tweezers. Later, both otoliths were cleaned with 70% ethanol and stored dry.

Gonads (Ovaries and Testes) were removed and weighed to the nearest gram for the purpose of calculating the Gonadosomatic index (GSI) using the formula GSI = 100 (Wg/Wt) (where Wg is gonad mass in grams and Wt is total body mass in grams) before being split in two, one preserved in 10% formalin for histological analysis, with the other placed in an air tight bag and stored in a -80°c freezer for lipid and fatty acid analyses. Livers were also removed, stored in air tight bags and placed in storage at -80°c for lipid analysis. Care was taken to ensure the specimens were kept on ice and stored as soon as possible in -80°c to avoid possible degradation.

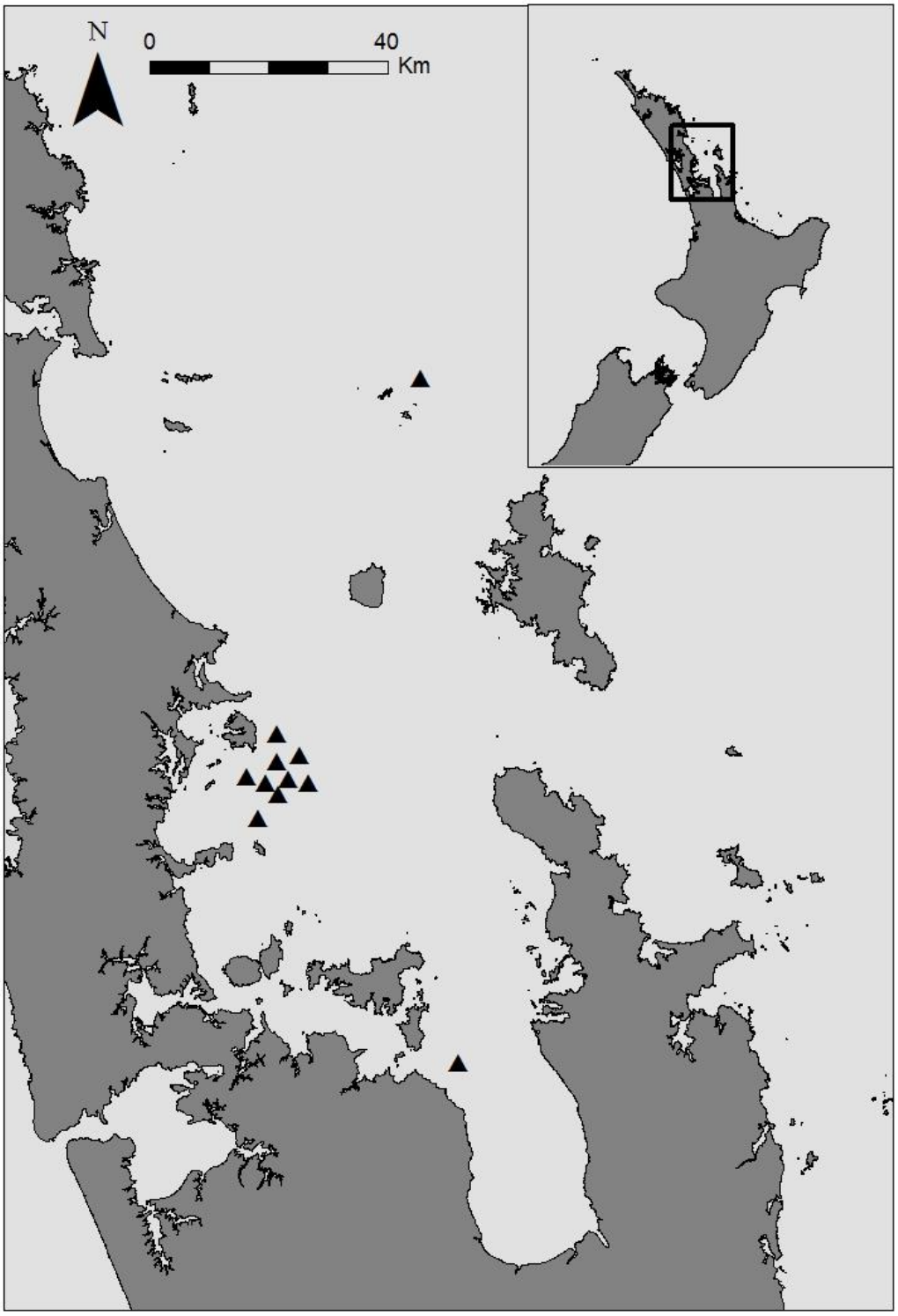


Figure 2.1: Location of Hauraki Gulf within New Zealand (inset) and specific fishing locations marked - ▲ Projection: NZGD 2000 New Zealand Transverse Mercator. Base layer source: www.koordinates.com.

2.2 Reproductive analyses

Transverse sections of each preserved gonad were dehydrated, embedded in paraffin wax, cut using a microtome at 5 micron and set to an adhesive histological slide. The slides were then stained using Mayer's Haematoxylin and Yong's Eosin-Erythrosin. For each specimen the gender, reproductive activity, and developmental stage of males and females were described and classified based on the most developed oocyte or sperm growth present (Grier, 1981; West, 1990). Reproductive activity was assessed with the knowledge that the population would be 100% sexually mature, based on the minimum size limit set by the ministry for primary industries. As such, no immature specimens were expected, or found to be present within the sample. Thus, three categories were established for female (Resting, Vitellogenic, Spawning) and male (Resting, Mature, Spawning) specimens. Microscopic descriptions of histological features were informed by Mackie et al., (2009) and are described in Table 2.1.

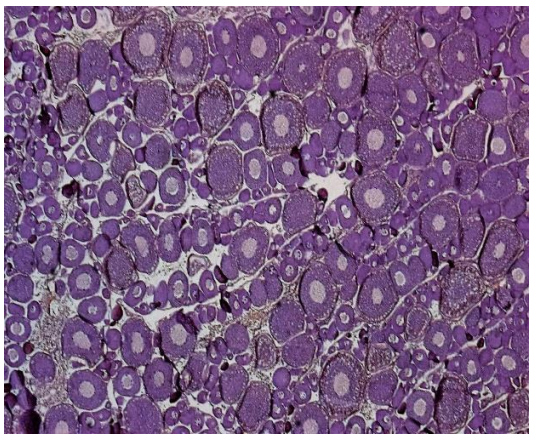
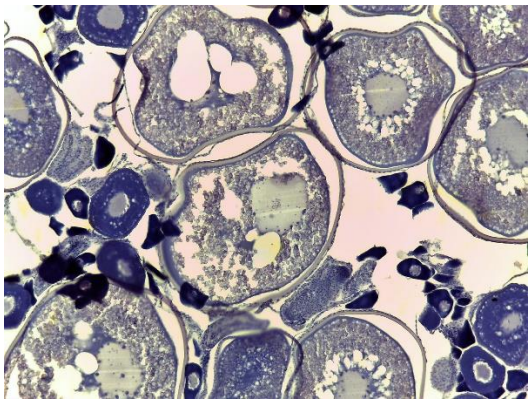
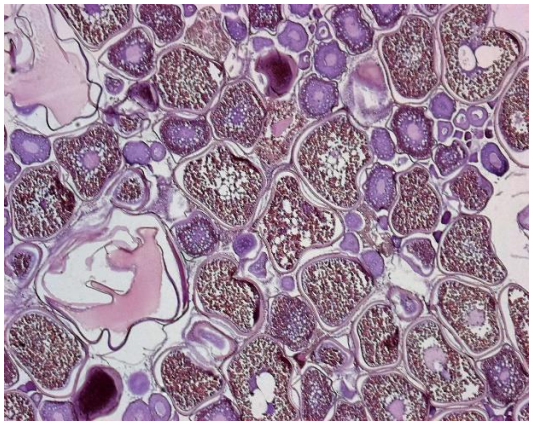
2.3 Ageing and growth estimation

Sagittal otoliths were ground down on both sides to retain a thin section of the centrum for the purpose of age analysis. This was carried out by mounting the otolith on the edge of a glass slide using the glue Crystalbond^{TM}. Using a 3000 grit diamond encrusted disc, each otolith was ground down to the edge of the slide. The otolith was then placed facing down on another slide, ground down again, reheated and smeared with mounting glue for better readability. Opaque growth rings, which appear as dark bands under transmitted light, were counted as representation of annual age using a camera-mounted compound microscope (Figure 2.2). The annual periodicity of opaque and translucent zones in snapper otoliths, caused as a result of slower, denser growth occurring during the winter, has been validated by Francis, Paul and Mulligan (1992). Because this study was conducted in the spawning season of snapper (i.e. birth months), ages deduced from the number of opaque zones on the otoliths were counted to whole numbers. The ages were then read by 4 individuals, with any between-reader differences discussed and a consensus reached.

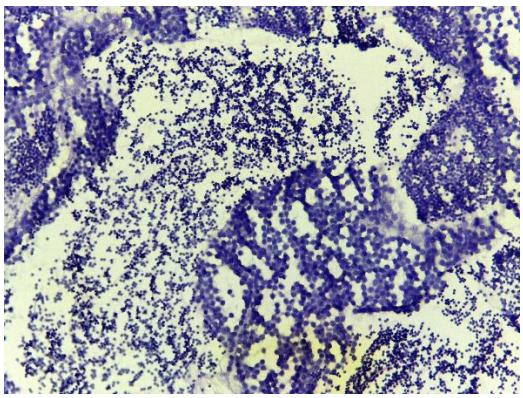
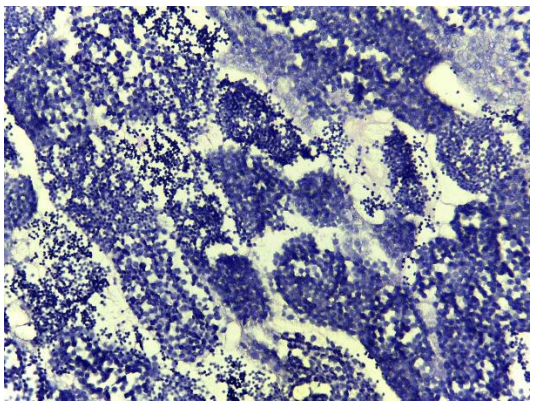
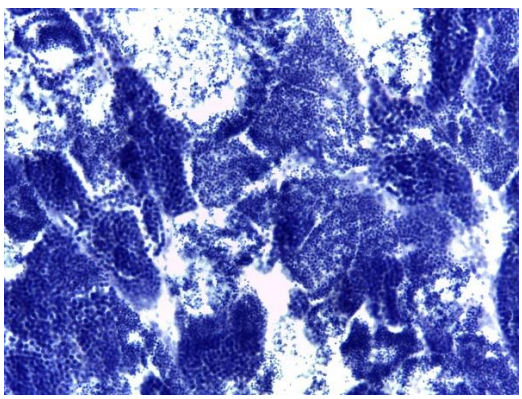
The relationship between size and age were assessed by fitting Francis' reparametrized von Bertalanffy Growth Function (rVBGF) to the length at age of each individual fish. The von Bertalanffy growth equation is represented by $L_t = L_\infty (1 - e^{-K(t-t_0)})$, where L_t is the length at age t (years), L_∞ is the asymptotic length, K is the growth coefficient and t_0 is the hypothetical age at which members of the population would have the length zero (Kimura, 1980).

Table 2.1: Photographic and written description of histological features used in classifying male and female gonads of *Chrysophrys auratus*.

Female

Developmental stage	**Histological description**
1. Resting (40x magnification) 	Ovary dominated by perinucleolus or cortical alveoli oocytes. Thick ovary wall suggests resting rather than immature individuals.
2. Vitellogenic (100x magnification) 	Ovary is in active vitellogenesis. Ovary dominated by vitellogenic oocytes with oil globules.
3. Spawning (40x magnification) 	Ovary contains ovulated and hydrated oocytes along with atritic and/or postovulatory follicles.

Male

Developmental stage	**Histological description**
1. Resting (400x magnification) 	Testis contain spermatocytes, small spermatids and spermatozoa. Sperm found within the radial sinuses but major ducts not filled with spermatozoa.
2. Mature (400x magnification) 	Testis is large in size. Spermatocytes, spermatids and spermatozoa present.
3. Spawning (400x magnification) 	Evidence of all the stages of spermatogenesis with spermatozoa packed in sinuses, lumen and/or sperm ducts, thus indicating a functional testis (i.e. spawning/running ripe or spawning capable).

The reparametrized version of the VBGF (rVBGF) provides mean body size at three ages of tau, omega, and nu. Tau and nu are randomly selected ages from the sample, with omega being the mean of tau and nu (Francis, 1988). Tau and Nu used in this study were set at 1 and 13 years, with Omega at 7. The rVBGF growth function used in this study was fitted to the dataset by constraining size-at-age zero to 11mm, which is in the mid-range of the established

9-14mm length of snapper at settlement (Hamer & Jenkins, 2004). The observed growth trends between males and females were compared using a likelihood ratio test (LRT) (Cerrato, 1990). The null hypothesis assumes that there is no difference in growth between populations and was rejected at alpha = 0.05, with degrees of freedom defined as the number of parameters being constrained. The relationship between length and weight was described as $W = a \times L^b$ (Rickter 1973), where W is the total weight in grams, L is the standard length (mm), a is the y-intercept and exponent b is the slope coefficient. Isometric growth (i.e. b=3.0) indicates equal increase in weight and length, where when b is < 3.0 negative allometric growth is experienced, and likewise >3.0 positive allometric growth is experienced where somatic growth precedes weight.

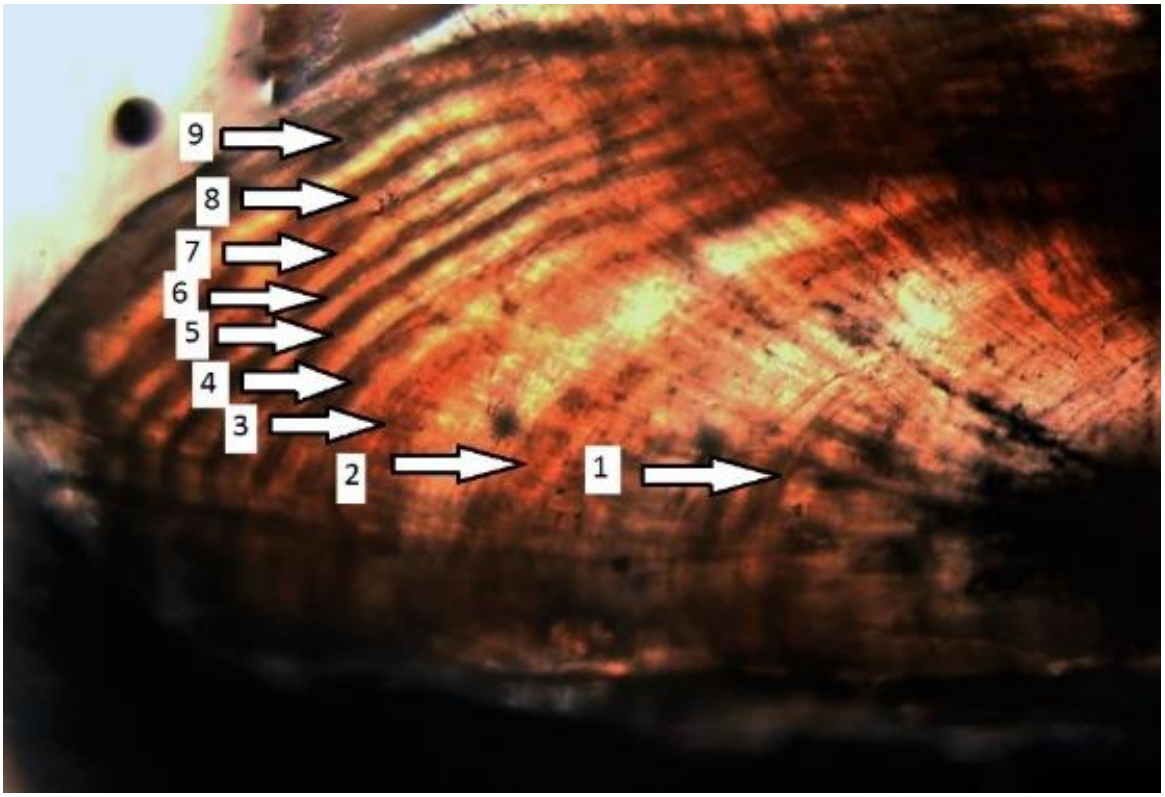


Figure 2.2: *Chrysophrys auratus* otolith cross section showing the annual growth rings. This male was 9 years old and 390 mm (FL).

Condition of individuals was determined by using the Fulton's Condition Factor (K), calculated using the following: $K = 100*W/L^3$ (Froese, 2006). Where W is wet weight in grams, L is fork length in centimetres and the equation is multiplied by 100 to bring the value close to one. Fulton's Condition Factor predicts that the weight of fish is proportional to its length cubed, allowing an individual's nutritional condition to be quantified (Jin et al., 2015).

This value can then be compared within a population and amongst species to access geographical, seasonal, climatic or biological changes in condition factor.

2.4 Chromatography and lipidomics overview

Chromatography is a scientific discipline involving the physical process of separating a substance into its individual components. This process utilizes the influence of differing intermolecular interactions as the analytes move within a mobile phase through a structure containing another material (stationary phase) (Wixom & Gehrke, 2010). Chromatography has been a recognized analytical process for over 100 years, and today is utilised heavily in chemical, biological, medical, health and environmental sciences (Wixom & Gehrke, 2010). Advancements in technology have allowed continuing improvements in this discipline to take place, particularly in the field of lipidomics, enabling more accurate and detailed profiling of lipid molecular species, along with their functions, interactions and dynamics (Harwood et al., 2016).

The complex nature and differing structure of analytes in natural lipid extracts means that there is rarely a situation where all lipid classes can be separated with one procedure. In order to analyse lipid content completely, it is necessary to fractionate lipids into simpler categories (Christie & Han, 2010). Of the various chromatography methods available, one major difference is the transfer of the sample from a liquid to a gas phase. Gas Chromatography (GC) is useful for complex, volatile substances, while for those that are less complex and less volatile, High Pressure Liquid Chromatography (HPLC) produces superior separations (Harwood et al., 2016). Whilst traditionally, Thin Layer Chromatography (TLC) has been the common method for lipid class analyses, HPLC has in more recent times surpassed TLC, offering both easier quantification and superior resolution (Christie & Han, 2010). This study has used HPLC to analyse TAG levels in gonads and livers, utilising adsorption chromatography with a silica gel column. This method can be achieved with a homogenised, diluted sample extract. In order to analyse fatty acids however, it is necessary for them to be first esterified to non-polar derivatives such as fatty acid methyl esters (FAME's). Derivatisation to FAME's reduces the complexity and polarity of the molecules, making the FAME's volatile at the temperature used for GC. GC is the most common method used for fatty acid analysis, allowing individual fatty acids to be identified against literature and internal standard retention times, while HPLC is the preferred method for the analysis of individual lipid classes such as TAG's (Christie & Han, 2010), and is preferable over other chromatographic techniques for the analysis of fish lipids (Silversand & Haux, 1997).

The next step in lipid chromatography is analysis of the output. The use of mass spectrometry (MS) as an accurate, sensitive and reliable method has increased consistently in recent times, particularly in the fields of proteomics and lipidomics (Pottiez, 2015). MS involves the detection and measurement of the mass of compounds, represented as the mass to charge ratio (m/z). This process first requires the compound to be ionized in order for the molecule to acquire a charge, before separation occurs dependent on the molecules mass, and finally detection (Pottiez, 2015). HPLC in conjunction with MS allows increased accuracy during analysis, enabling the isolation and recording of monoisotopic peaks and the removal of potentially misleading isotopes and isomers from the output.

Lipid composition and quantity has been examined in a wide variety of marine species at varying stages of their life cycle, ranging from the analysis of fish eggs (Anderson et al., 1990), to larvae (Hilton et al., 2008), to juveniles (Sim-Smith et al., 2013b), through to mature, reproducing individuals (Zudaire et al., 2014). Analysis of fatty acids is almost routinely carried out with the use of GC, while lipid classes have been studied using both High Performance Thin Layer Chromatography (Anderson et al., 1990; Zudaire et al., 2014), and High Performance Liquid Chromatography (Silversand & Haux, 1997).

With regards to *C. auratus*, lipid analysis has been undertaken on larvae and early juveniles (Sim-Smith et al., 2013b), revealing a metabolic strategy that switches from growth to storage prior to the onset of winter, however, to the best of our knowledge, no research has been carried out on mature wild snapper in New Zealand, or anywhere else the species inhabits. Lipid related research has been carried out on captive snapper (Martin, 2009), however, it involved the analysis of eggs from 18 females in an aquaculture setting, and did not involve the examination of specific lipid classes using the methodologies described here.

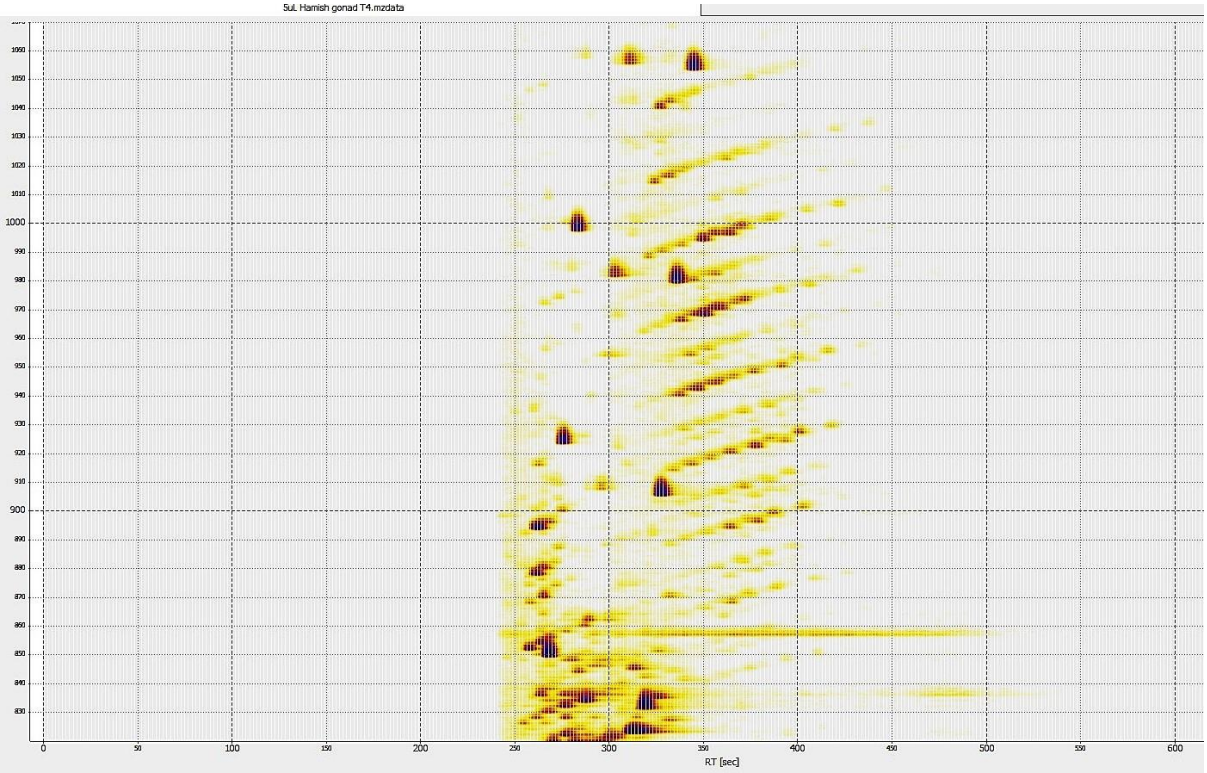


Figure 2.3: Example of triglyceride chains as viewed in Mass Hunter software.

2.4.1 TAG analysis methods

Female liver and gonad samples were first reduced in size to between two and five grams. Three cross sections from each gonad were cut, and then reduced further if the volume exceeded five grams. Cross sections were taken from near both ends and the middle of each gonad. Whilst Zeldis and Francis (1998) have shown that fecundity estimates do not depend on which part of *C. auratus* ovary is sampled, indicating an even distribution of oocytes, this method was employed regardless in order to achieve as much homogeneity as possible. The tissue was then weighed before being placed in a beaker with crushed ice.

Prior to analysis, lipids must first be extracted from tissue samples, requiring the tissue to be homogenised in the presence of solvents that will both dissolve the lipids and break the bonds that link lipids to various other cellular components. Therefore, ±50mL of isopropanol was added before a blender was used to homogenise the tissue until the texture was smooth and as much of the tissue as possible was combined. Whilst blending, a glass syringe was used to extract the homogenate and fill glass vials, along with a pre weighed Eppendorf tube. Vials were then centrifuged for 5 minutes at 1500 rpm. Care was taken to store samples in a -20 freezer wherever practical between extraction processes to avoid lipid degradation. Approximately 1mL of supernatant was then transferred into a 1.8mL autosampler brown

glass vial to be run in the LC/MS. Samples were run with a 10uL injection volume in an Agilent Technologies 1200 series with a 6420 Triple Quad LC/MS. Three mobile phase solutions were used consisting of: 1) 1/2L Milli Q, 500uL Formic Acid (HCOOH), 325uL concentrate ammonium (NH+4-), 2) 1/2L Isopropanol, 5mL Formic Acid (HCOOH), 325uL concentrate ammonium (NH+4-) and 3) 1/2L of Acetonitrile (CH_3CN), 25mL Milli Q, 500uL Formic Acid (HCOOH), 325uL Conc. ammonium (NH+4-).

The output was first analysed in the Mass Hunter software programme (see Figure 2.3), where the retention time and M/Z of all TAG chains were identified and recorded. This allowed for specific retention times and M/Z to be extracted from the data set, the surface area under the peak to be calculated, and an overall volume in mg/g as both a total, and for each individual TAG, to be determined. Starting concentrations of TAG's in mg/g were determined by dividing total TAG's extracted with the dry mass of tissue. A standard curve was run using a serial dilution of pure Triolein (glyceryl trioleate) to regulate tissue concentration values.

2.4.2 Fatty Acid Methyl Ester (FAME) Methods

The FAME extraction and analysis was conducted as part of a collaboration with Tom Rowlands. Fatty acid methyl esters were analysed using a variation on the extraction technique formulated by Bligh and Dyer (1959) where lipids, non-lipids and water are separated using methanol and chloroform homogenized with tissue. Toluene (a relatively safe and easily accessible solvent) was used in place of chloroform for this research. Firstly, each sample of wet gonad tissue was initially reduced in size, homogenised with isopropanol and extracted with a glass syringe as per the TAG extraction methods detailed above. From this point, 50 µL of each sample was then distributed into separate vials and spiked with 10 µL of 2g/L tridecanoic acid in toluene as an internal standard. In addition to this, Supelco's 37 component FAME internal standard mix was used to identify fatty acids ranging from C4:0 to C24:1. Each sample then had 750 µL of methanolic HCl and 490 µL of toluene added, before being vortexed thoroughly and then incubated in a heating block at 70°c for two hours. After being allowed to cool, each sample had 6% K_2CO_3 (6g anhydrous K_2CO_3 per 100 mL of ultrapure water) added, and the mixture vortexed again. Lastly, the top organic layer was removed via a glass pipette into four 1.8 mL autosampler vials, each containing a low volume glass insert and cap. Samples were then ready to be analysed in a GC 2010 Plus Gas Chromatograph by Shimazdu. The Oven temperature was set at 200°c, the autosampler injected samples at 1 µg, and a flame ionisation detector was used for identification of fatty

acid profiles and their concentrations. Final concentrations of FAMEs are presented in mg/g for consistency and to allow comparisons with TAG concentrations.

2.4.3 Dry Weight

The dry weight of samples was determined by the evaporation of solvent from subsamples in Eppendorf tubes, carried out in a Labonco CentriVap Benchtop Console for approximately 6 hours. The remaining tissue was then weighed and the dry weight calculated based on original wet weight records. Final concentrations are presented as mg/g of dry weight.

2.4.4 Statistical Analysis

All statistical analyses were conducted in R version 3. 2. 1 (R Development Core Team, 2016). Following exploratory data analyses using correlation and principal component analysis (data not shown), we applied more flexible generalised additive models (GAM) to test our hypothesis of maternal influence on lipid quantity and concentration. GAMs were fitted with a Gamma error distribution (GAMs, R-package mgcv) which allow us to analyse both linear and non-linear relationships between lipid concentration and each of the maternal parameters (age, size, condition factor). In an overarching model, we used total lipid concentration as response variable, followed by separate GAMs for individual TAGs and fatty acid species. We used model diagnostic plots to assess the underlying assumptions of variance homogeneity (residuals vs. fitted values plot) and normality (quantile-quantile plots). Scatter plots of total TAG levels for each maternal parameter (size, age and condition factor) are displayed, along with box plots representing the average TAG levels across each reproductive stage, and bar graphs to depict relative composition, oocyte/tissue comparisons and average mg/g of individual TAG species.

Chapter 3 - Results

3.1 Population demographics and reproduction

A total of 113 *C. auratus* were collected, 60 females and 53 males. Age ranged from 3 to 24 (years) with an average age of 7.6 for males and 8.6 for females. Length (fork length) ranged from 300 mm (the minimum allowable size) up to 620 mm, with an average length for males of 340 mm and 370 mm for females. The weight to length ratio for both sexes was closely correlated with an R value of .92 (Fig. 6), while length to age ratio was very weakly correlated with an R value of .34. (Fig. 5). The length-weight analysis indicate that this population is negatively allometric, producing a *b* coefficient of 2.7 for both sexes combined, 2.4 for males and 2.8 for females. Mortality demonstrates a rapid decline from age 6, with an 85% annual survivorship calculated from 6 – 12-years-old. The re-parametrized von Bertalanffey Growth function demonstrated differences in growths rates between the sexes, with females growing significantly larger than males. This was confirmed with significant results (p values < 0.0004) shown in the post hoc Likelihood Ratio Test. Histological analysis revealed the vast majority of both males and females (82%) to be in an active stage of spawning. Reproductive stages dependent on age (Fig. 10 and Fig. 11), fork length (Fig. 12 and Fig. 13) and Gonadosomatic Index (Fig. 14) are presented.

3.1.2 Size, age and growth

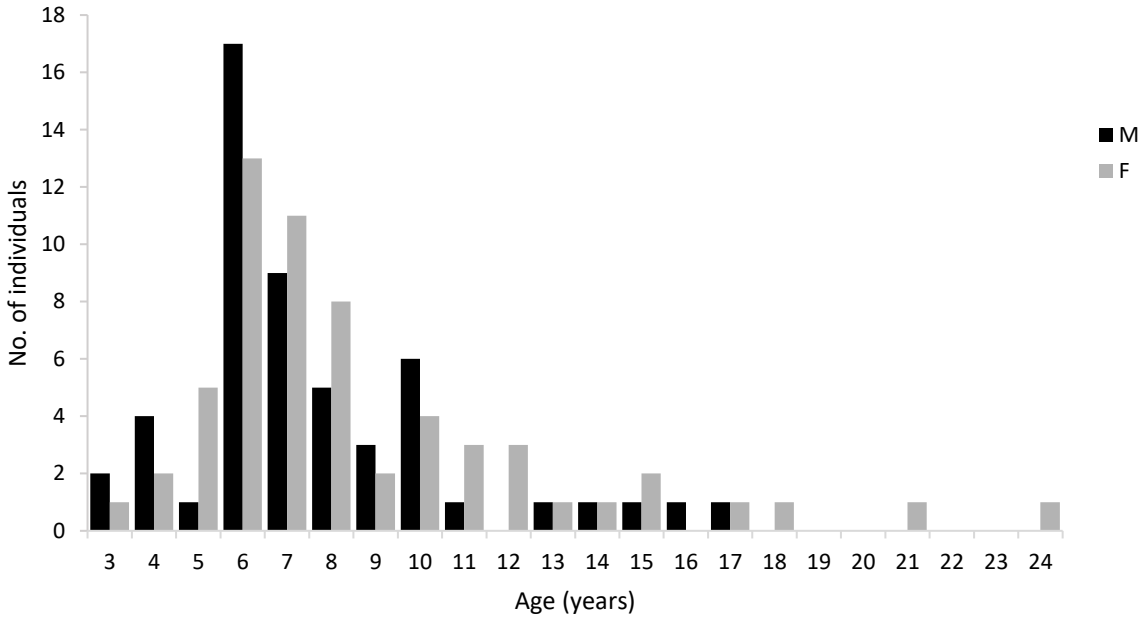


Figure 3.1.1: Age (years) frequency of *Chrysophrys auratus*.

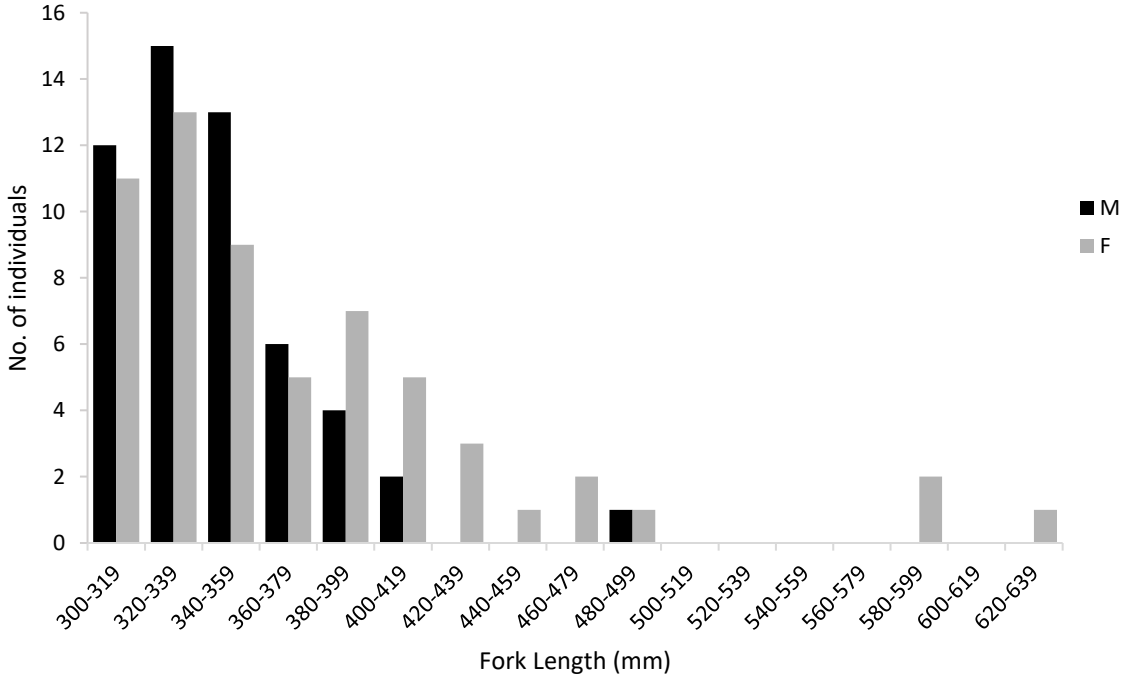


Figure 3.1.2: Fork length (mm) size frequency of *Chrysophrys auratus*.

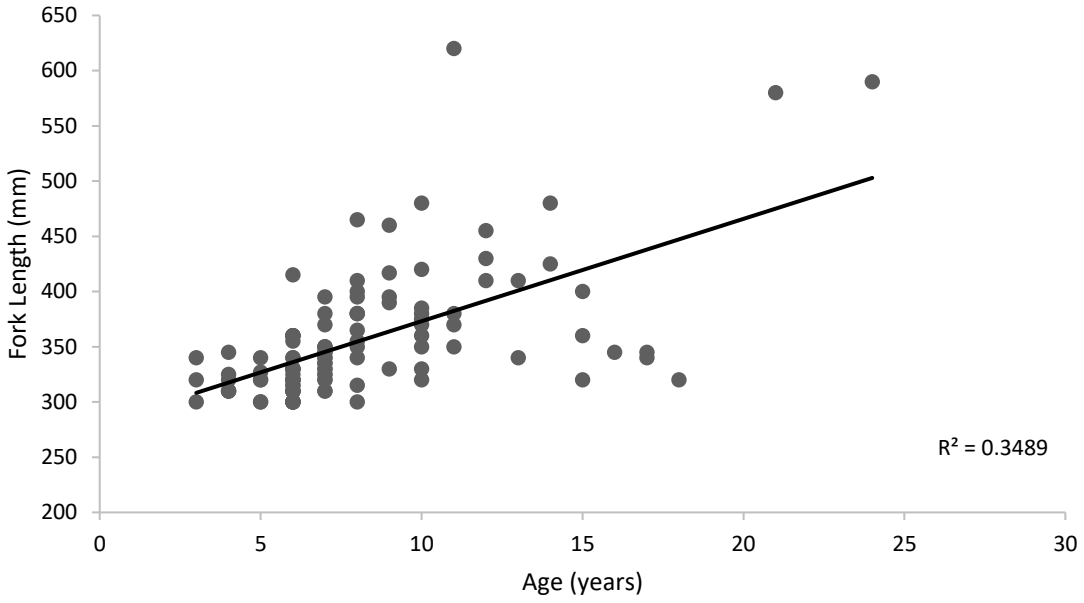


Figure 3.1.3: Age (years) and fork length (mm) of *Chrysophrys auratus*. Sexes combined.

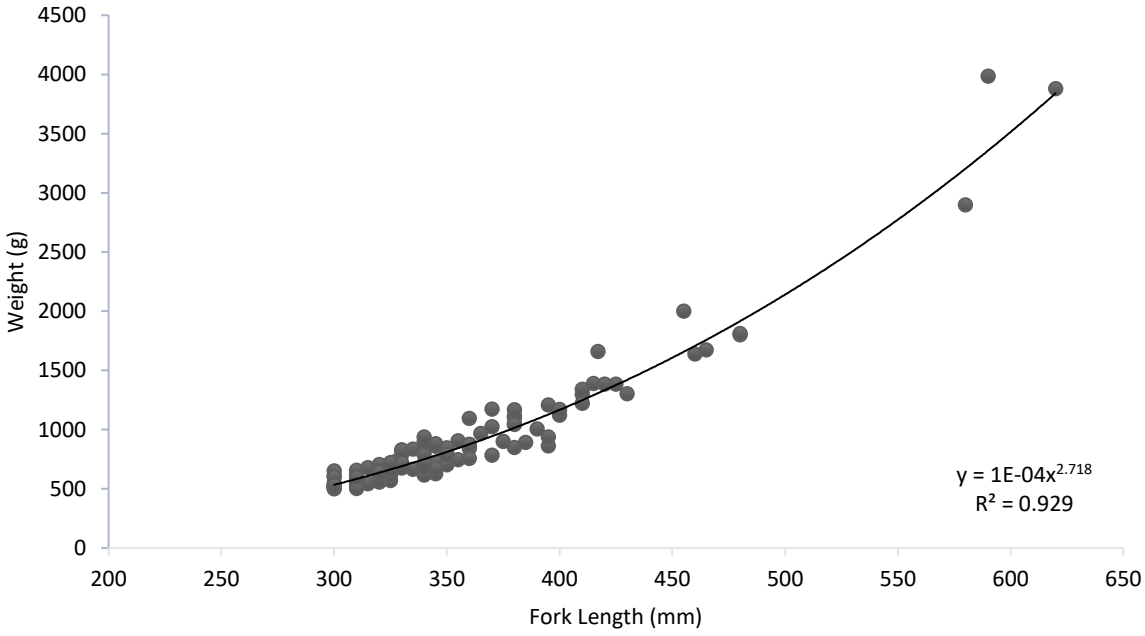


Figure 3.1.4: Fork length (mm) and weight (grams) ratio of *Chrysophrys auratus*. Sexes combined

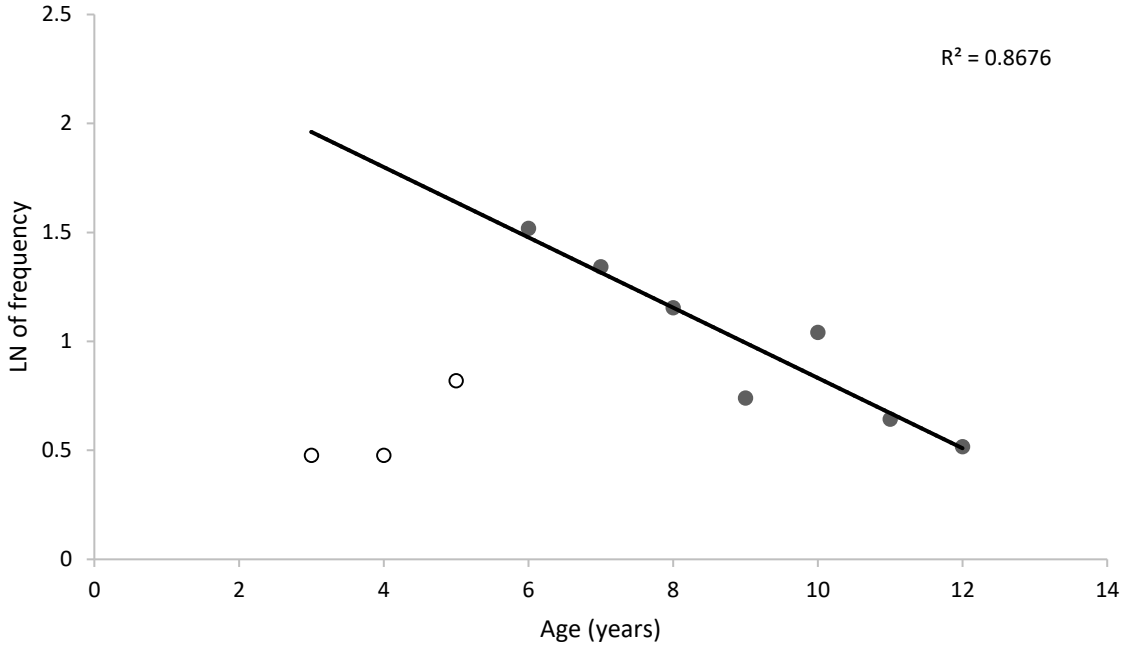


Figure 3.1.5: Mortality estimates for male and female *Chrysophrys auratus* for individuals 6 – 12 years.

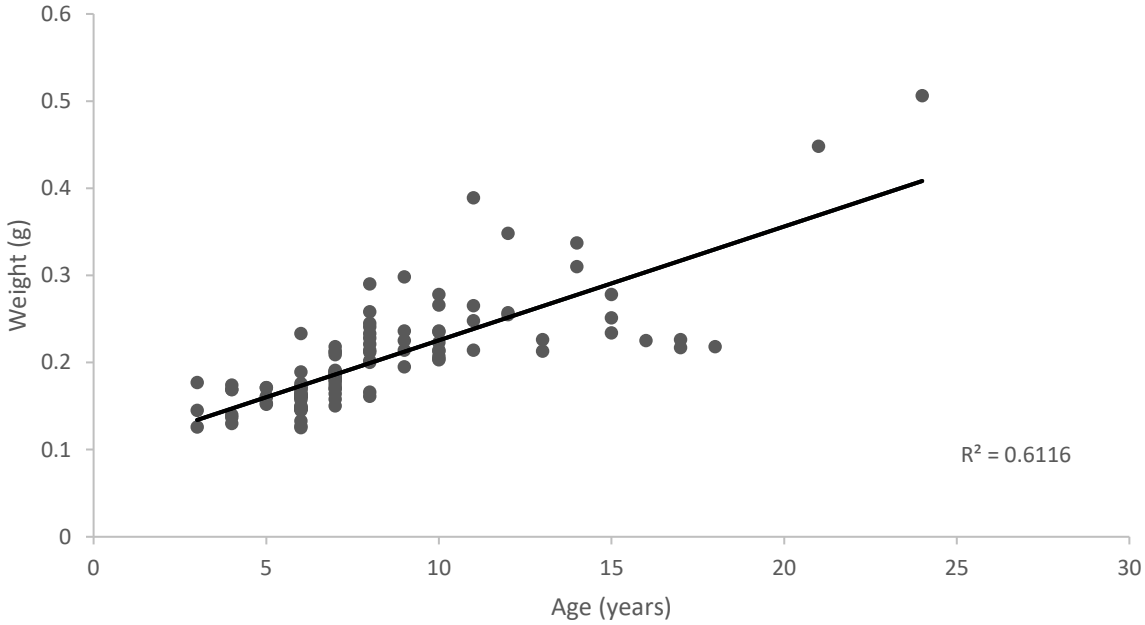


Figure 3.1.6: Otolith weight (grams) and age (years) for sampled *Chrysophrys auratus.*

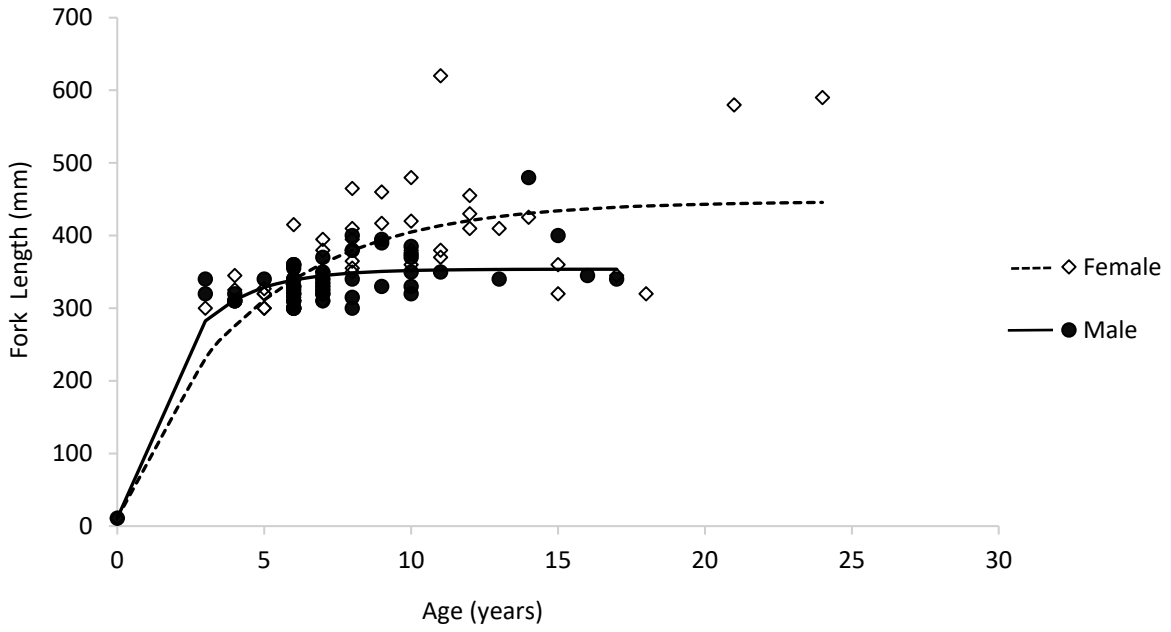


Figure 3.1.7: The re-parametrized von Bertalanffey Growth function for male and female *Chrysophrys auratus*.

Table 3.1: Parameter values for reparametrized von Bertallanfey Growth Function, Length-Weight relationship, Age-Weight relationship and Age-Length relationship for sampled *Chrysophrys auratus*.

Function	Parameter	Male	Female	Combined
rVBGF	L1	150.7 mm	101.7 mm	111.5 mm
	L7	345.0 mm	362.0 mm	356.4 mm
	L13	353.4 mm	426.3 mm	398.8 mm
Length-Weight relationship	n	53	60	113
	b	2.47	2.80	2.71
Length - Age relationship	*b*	0.28	0.25	0.21

Table 3.2: Likelihood Ratio Test comparing re-parametrized von Bertalanffey Growth functions fitted to male and female *Chrysophrys auratus.*

Results	Base Case	Coincident	L-1	L-7	L-13
SSQ	290932.6904	280210.5927	246882.4093	246832.3545	279749.8172
MLE(norm)	1902.75693	606.86726	597.09259	596.06379	606.77168

N	713.6	334	327	325	334
Chi square		-12.54	-53.61	-53.42	-13.09
Df		3	1	1	1
P value		*0.00573*	*0.00000*	*0.00000*	*0.00030*

3.1.3 Reproduction

Table 3.3: Percentage of male, female and combined sexes of *Chrysophrys auratus* individuals at reproductive stages. Reproductive stages for females are: 1 = Resting, 2 = Vitellogenic and 3 = Spawning, and for males: 1 = Resting, 2 = Mature and 3 = Spawning.

Reproductive stage	Male	Female	Combined
1	9%	25%	18%
2	43%	52%	48%
3	48%	23%	34%

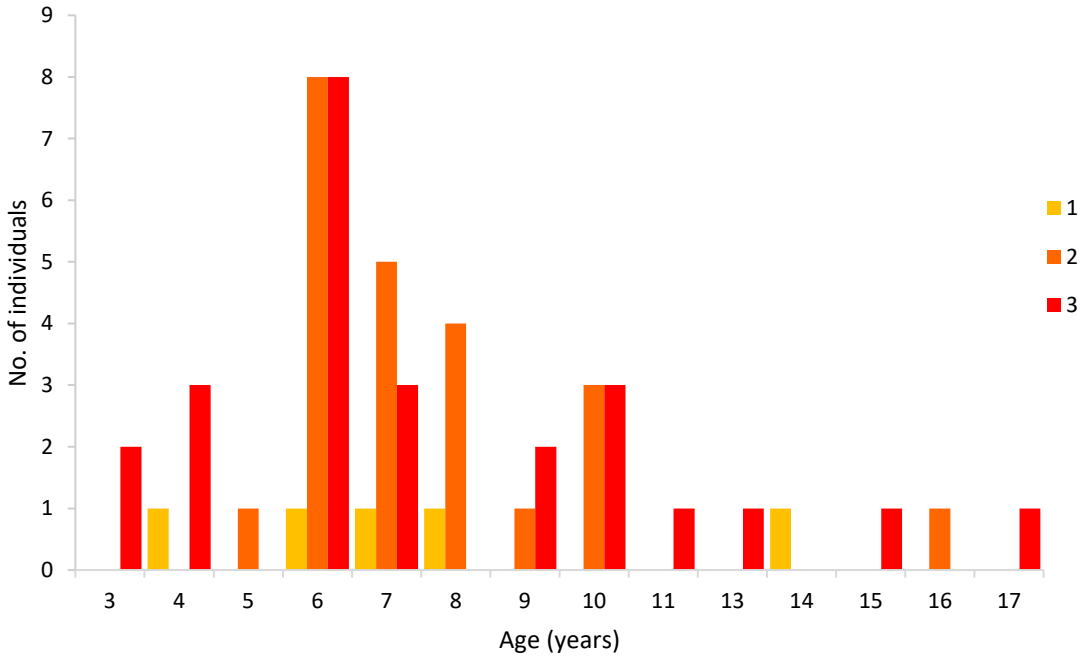


Figure 3.1.8: Male histology and age data for sampled *Chrysophrys auratus*. Reproductive stages are: 1 = Resting, 2 = Mature and 3 = Spawning.

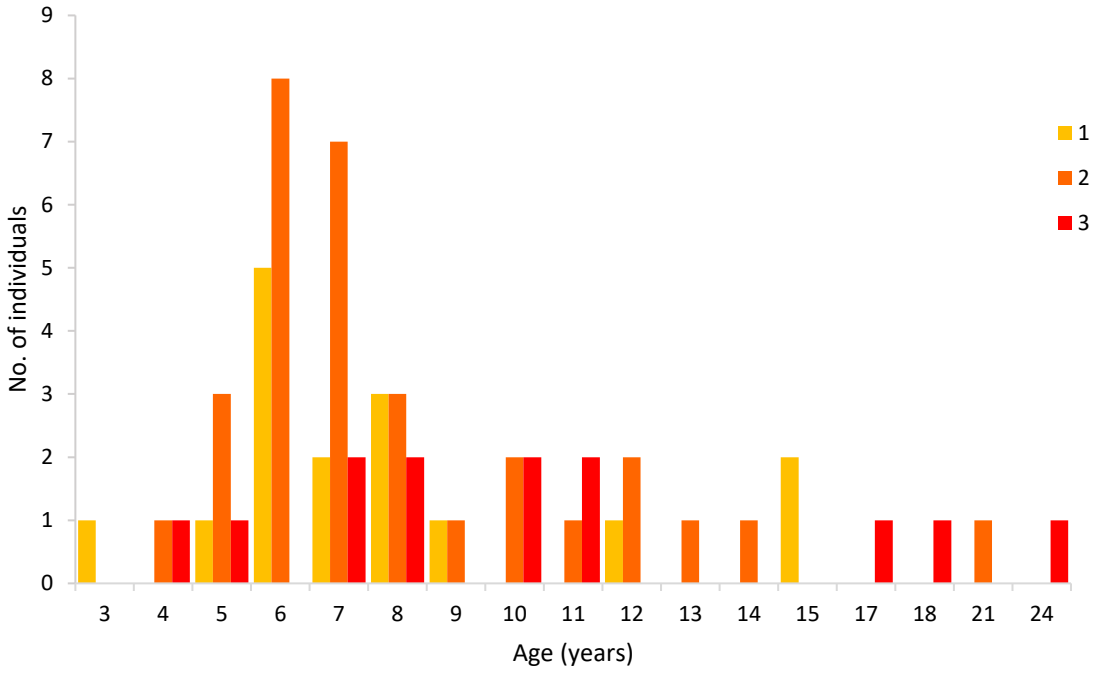


Figure 3.1.9: Female histology and age data for sampled *Chrysophrys auratus*. Reproductive stages are: 1 = Resting, 2 = Vitellogenic and 3 = Spawning.

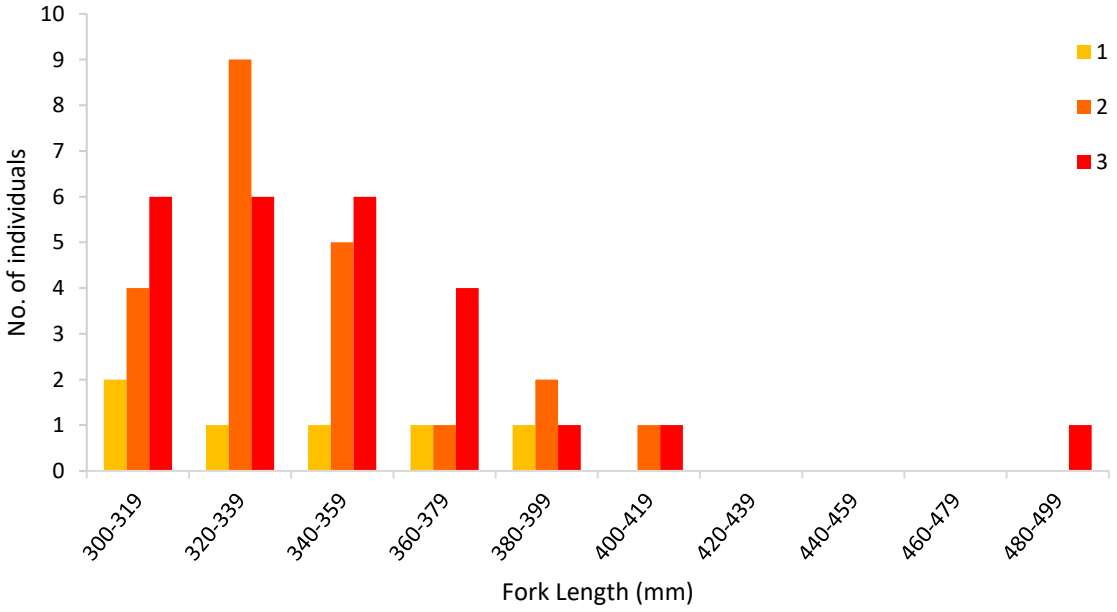


Figure 3.1.10: Male histology and fork length (mm) data for sampled *Chrysophrys auratus*. Reproductive stages are: 1 = Resting, 2=Mature and 3 = Spawning.

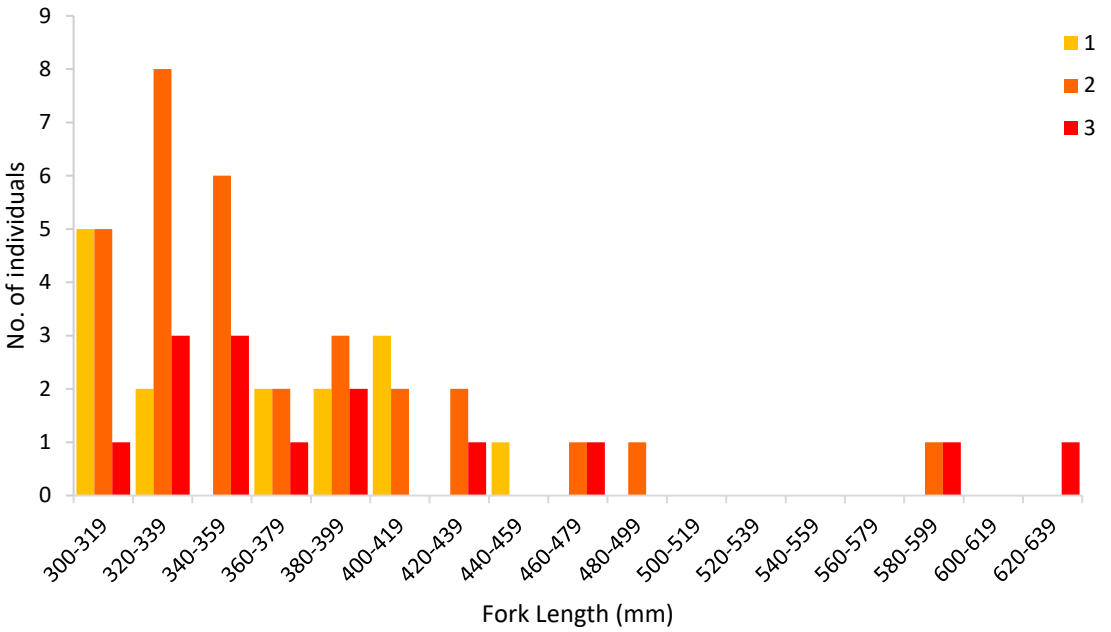


Figure 3.1.11: Female histology and fork length (mm) data for sampled *Chrysophrys auratus*. Reproductive stages are: 1 = Resting, 2 = Vitellogenic and 3 = Spawning.

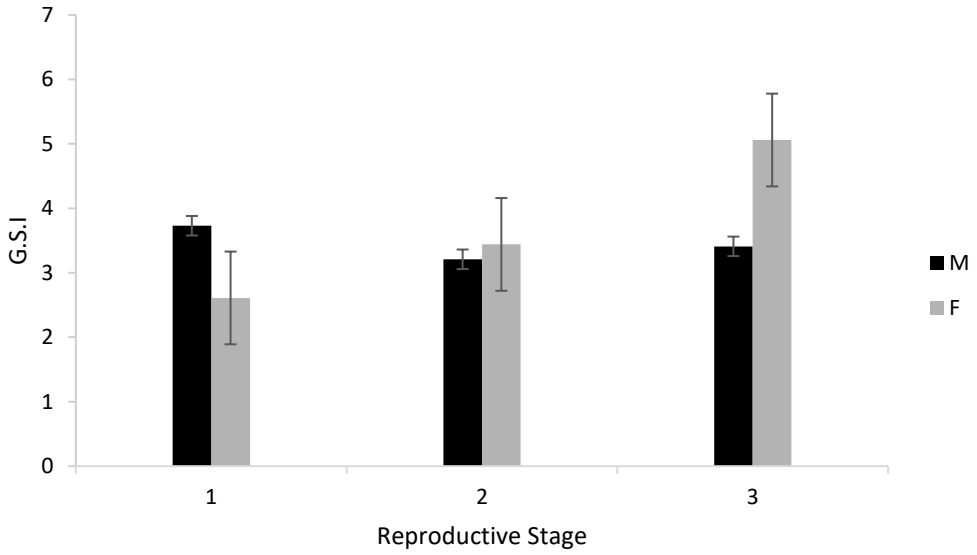


Figure 3.1.12: Average Gonadosomatic Index (G.S.I) for male and female *Chrysophrys auratus*. Reproductive stages for females are: 1 = Resting, 2 = Vitellogenic and 3 = Spawning, and for males: 1 = Resting, 2 = Mature and 3 = Spawning.

3.2 Female lipid and fatty acid composition, quantity and provisioning

A total of 60 species of triglycerides were found to be present within snapper gonads and 58 within liver tissue. Total TAG levels ranged from 2.3 mg/g up to 14.3 mg/g in gonad tissue with an average of 5.8, and from .3 mg/g to 3.7 mg/g with an average of 1.9 in liver tissue. The quantity of total TAG (mg/g) in gonad tissue showed no linear correlations with age, size or condition, with p values of .65, .74 and .79 respectively, likewise, liver quantity revealed no linear correlations, though did demonstrate declining trends in both age (p value .09) and condition factor (p value .26). Five species of triglyceride dominated the composition of both gonad and liver tissue, comprising approximately 35% of the overall quantity. Of these, 56:7 was the most dominate, consisting of approximately 10% of the overall TAG composition in both liver and gonads, followed by 60:12, 54:7 and 52:2 in gonad tissue, and 54:6, 54:7, 50:2 and 52:2 in liver. Both liver and gonad samples were dominated by medium to long chain TAG species (>6 double bonds), containing 64% and 72% respectively. When broken down to percentage of total TAG, medium to long chain species made up 75% of the total gonad composition, while this dropped to 63% in liver tissue. Two samples of oocytes were collected, each containing considerably higher lipid levels than was present in the gonadal tissue itself, 4.3mg/g (tissue) vs 11mg/g (oocytes) and 6.8mg/g (tissue) vs 11.5mg/g (oocytes).

The presence of nonlinear trends was determined using species-specific generalised additive models. Among all TAG species, only three (54:7, 56:8 and 50:4) showed a nonlinear trend in liver tissue, and two (60:8 and 58:7) in gonad tissue. In line with the spread of our sample and the thinning of data in the large/old range, the level of uncertainty increased with increasing predictor values, represented by widening 95% confidence intervals for TAGs 60:8 (age) and 50:4 (fork length). Analysis for condition factor revealed no significant trends for TAG species, irrespective of tissue type (data not shown).
GAM analysis of total TAG (mg/g) concentration in gonad tissue showed no significant relationship with age, size or condition factor, with p values of 0.75, 0.74 and 0.38, respectively. This was mirrored by results from the liver where no significant relationship was present for age ($p = 0.07$) size ($p = 0.27$) and condition factor ($p = 0.27$).

3.2.1 TAG gonad and liver quantity

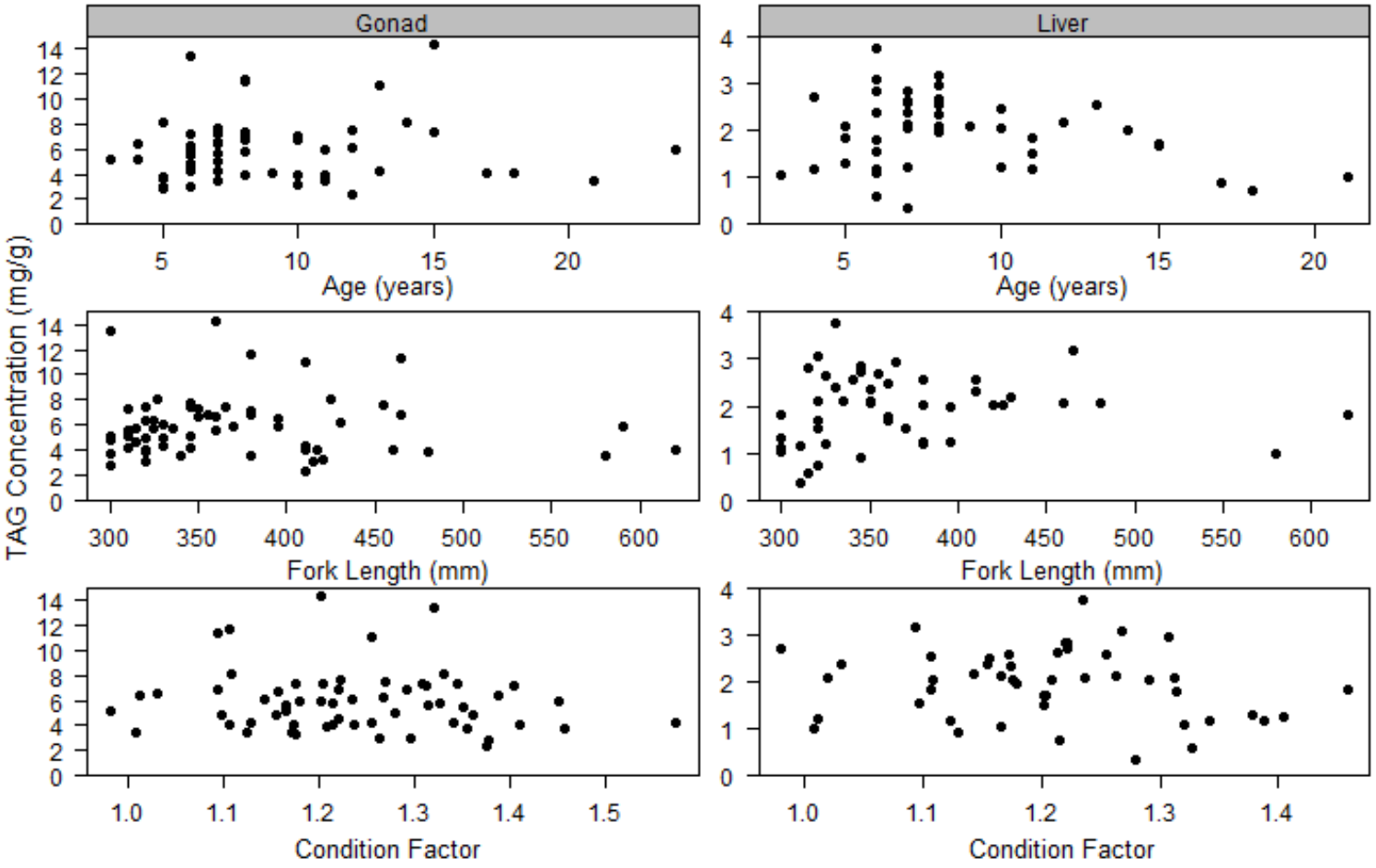


Figure 3.2.1. The relationship between total TAG concentration and age (years), fork length (mm) and condition (Fulton's condition factor) in gonad and liver tissue of female *Chrysophrys auratus*. Note the varying *x* and *y* axis values. Concentrations are in mg/g of dry weight.

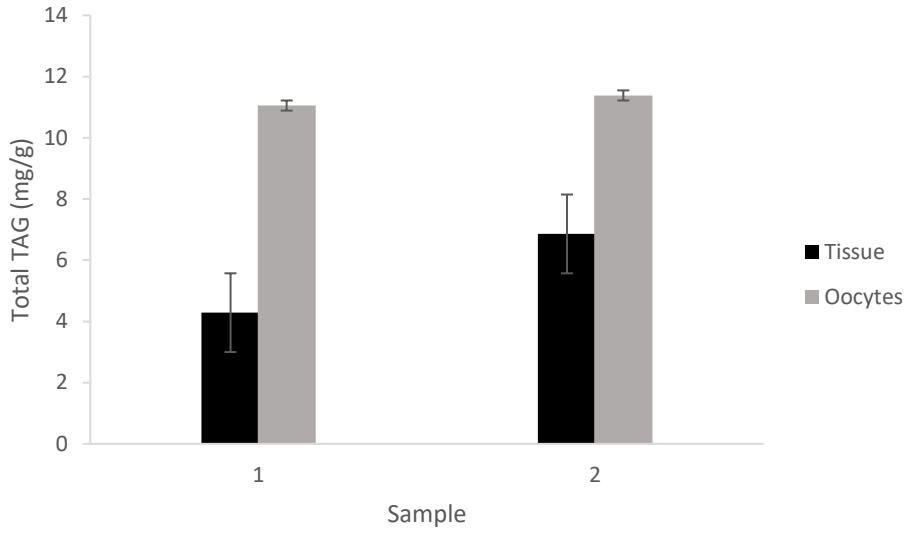


Figure 3.2.2: Tissue and oocyte total TAG (mg/g) for female *Chrysophrys auratus*. Sample 1 was 13 years old and 410mm fork length. Sample 2 was 8 years old and 465mm fork length.

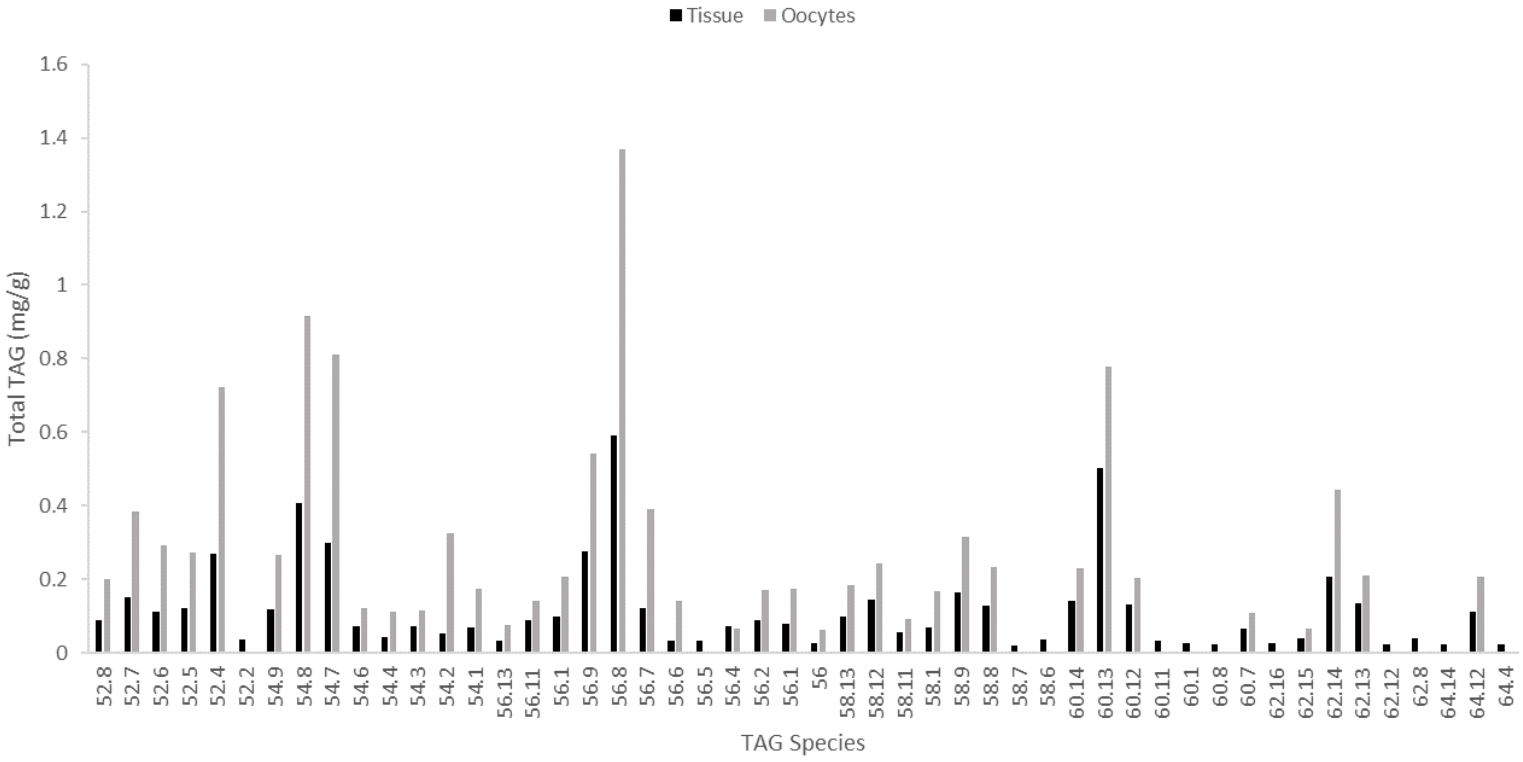


Figure 3.2.3: Average total TAG (mg/g) of individual TAG species in tissue and oocytes of 2 female *Chrysophrys auratus*.

3.2.2 TAG species-specific generalised additive models

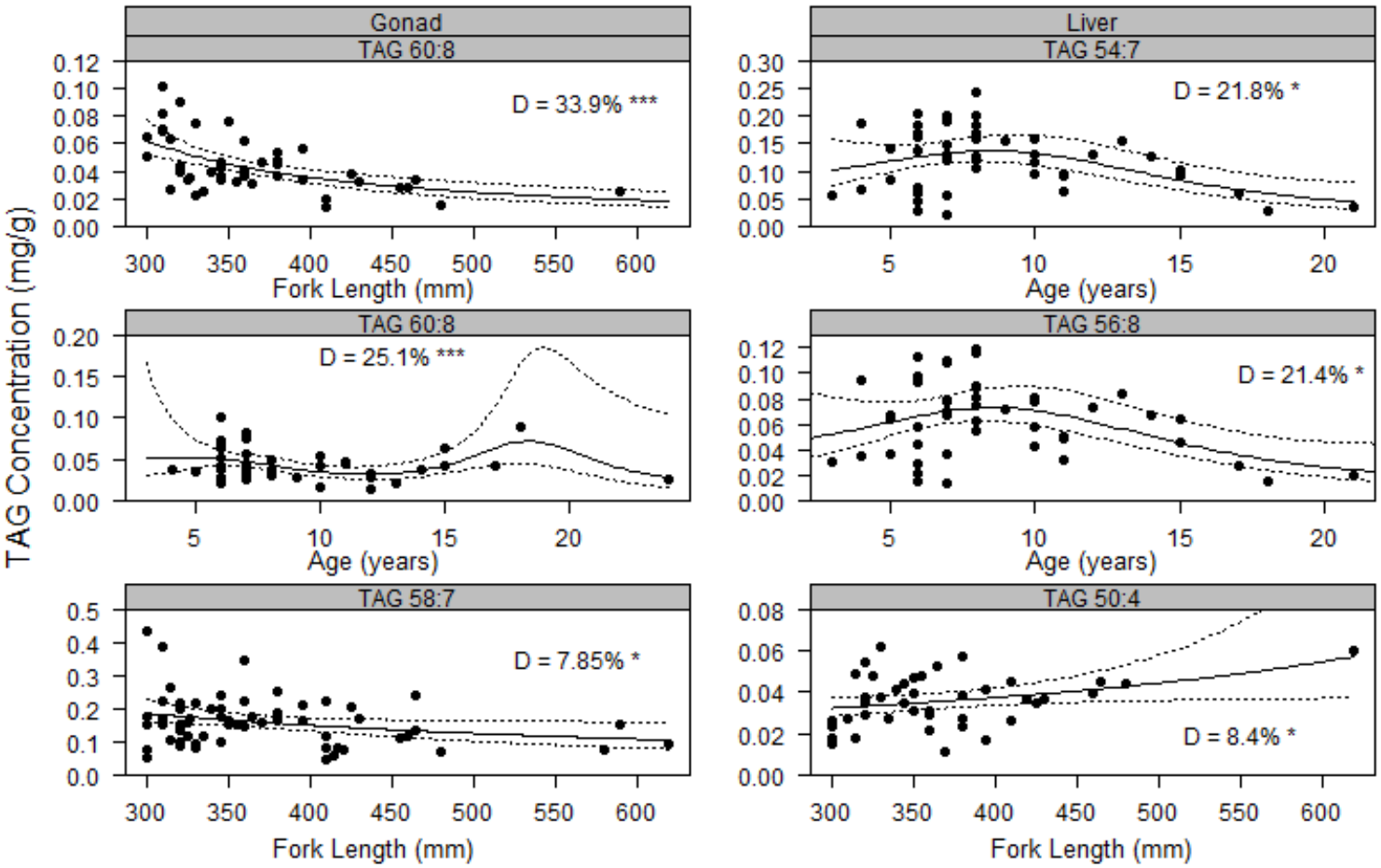


Figure 3.2.4. Concentration of TAG species that showed a significant non-linear trend as a function of fork length or age in gonad and liver tissue of female *Chrysophrys auratus*. Solid lines represent fits from generalised additive models with gamma error distribution, dotted lines indicate 95% confidence intervals. Concentrations are in mg/g of dry weight. Note the varying x and y axis values. D = deviance explained, * *P* < 0.001, * *P* < 0.05.**

3.2.3 TAG provisioning

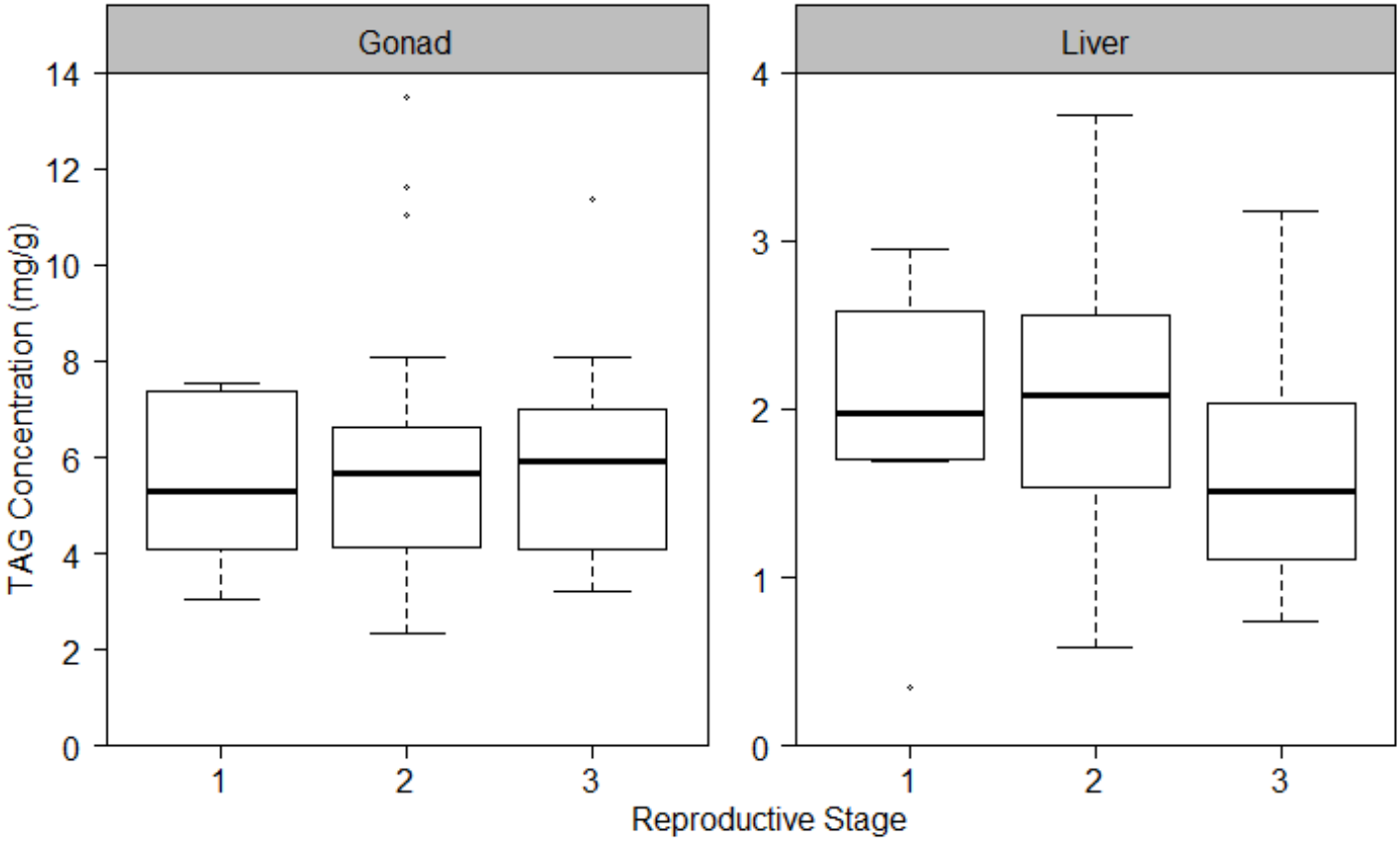


Figure 3.2.5. TAG concentration by reproductive stage for female *Chrysophrys auratus* in gonad and liver tissue; 1=Resting, 2=Vitellogenic, 3=Spawning. Concentrations are in mg/g of dry weight. Note the varying x axis for gonad and liver concentrations. N = 60.

3.2.4 TAG relative precentage and composition

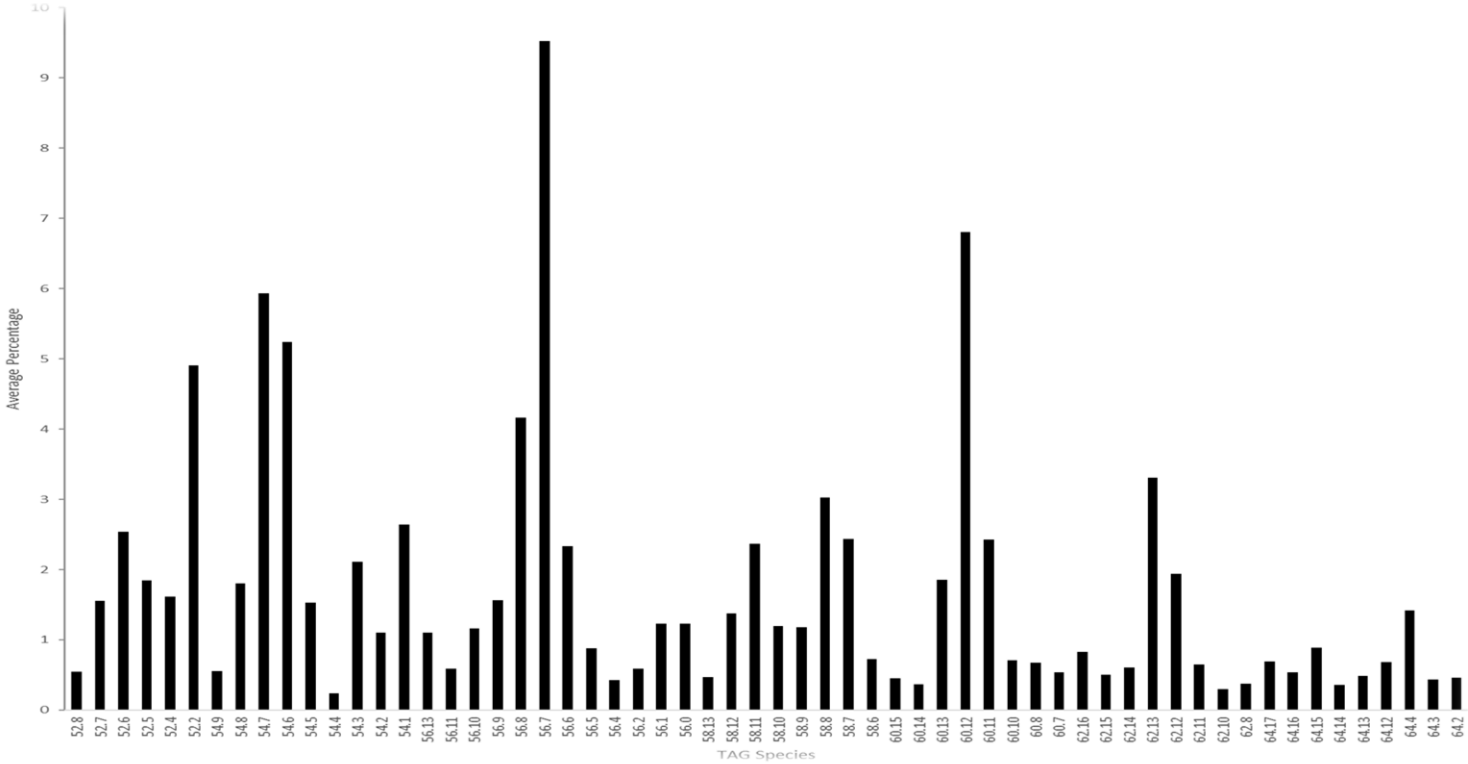


Figure 3.2.6: Average percentage of TAG species in gonad tissue of female *Chrysophrys auratus*.

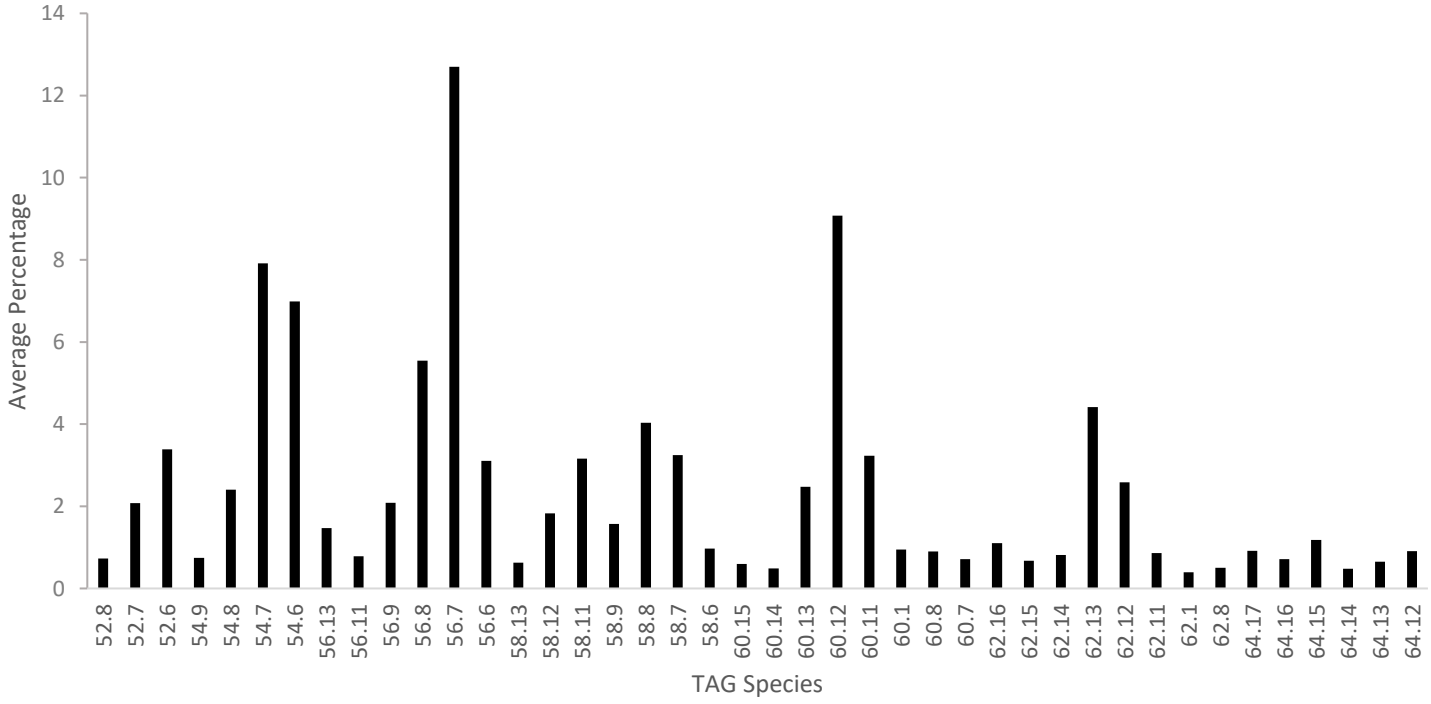


Figure 3.2.7: Average percentage of TAG species with >6 saturations in gonad tissue of female *Chrysophrys auratus*.

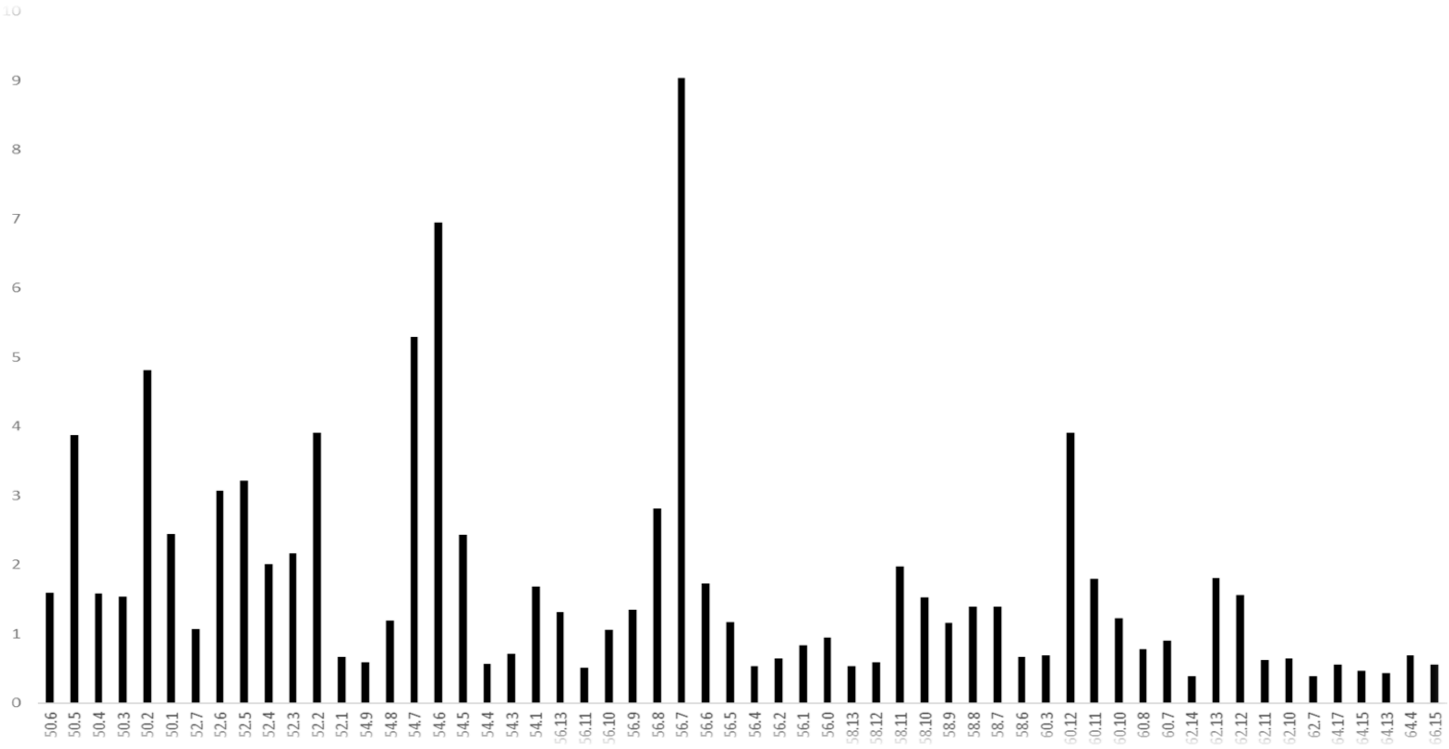


Figure 3.2.8: Average percentage of TAG species in liver tissue of female *Chrysophrys auratus*

3.3 Fatty Acid Methyl Esters

A total of twenty-eight fatty acid species were found to be present within *C. auratus* gonads (liver not tested), the twenty most abundant of which are shown in Figure 3.3.1. Palmitic (PAL), oleic (OLE), palmitoleic (PTO) and stearic (STE) acids occurred in average concentrations ranging from 4.1 (STE) to 17.4 (PAL) mg/g and together accounted for 68% of the total fatty acid pool. Following these are the biologically important omega's docosahexaenoic (DHA) and eicosapentaenoic (EPA), with averages of 3.7 and 3.07 mg/g respectively. The other omega fatty acid unique to fish and of importance to reproduction and larval development is α-lenolenic (ALA), which produced an average concentration of 0.5 mg/g. Results indicated that for fork length and age there was no significant relationships observed. Results have focused on the 3 above mentioned fatty acids (Fig.3.3.2 and Fig.3.3.3). In general concentrations followed a normal distribution, increasing between the ages of 5 and 12.

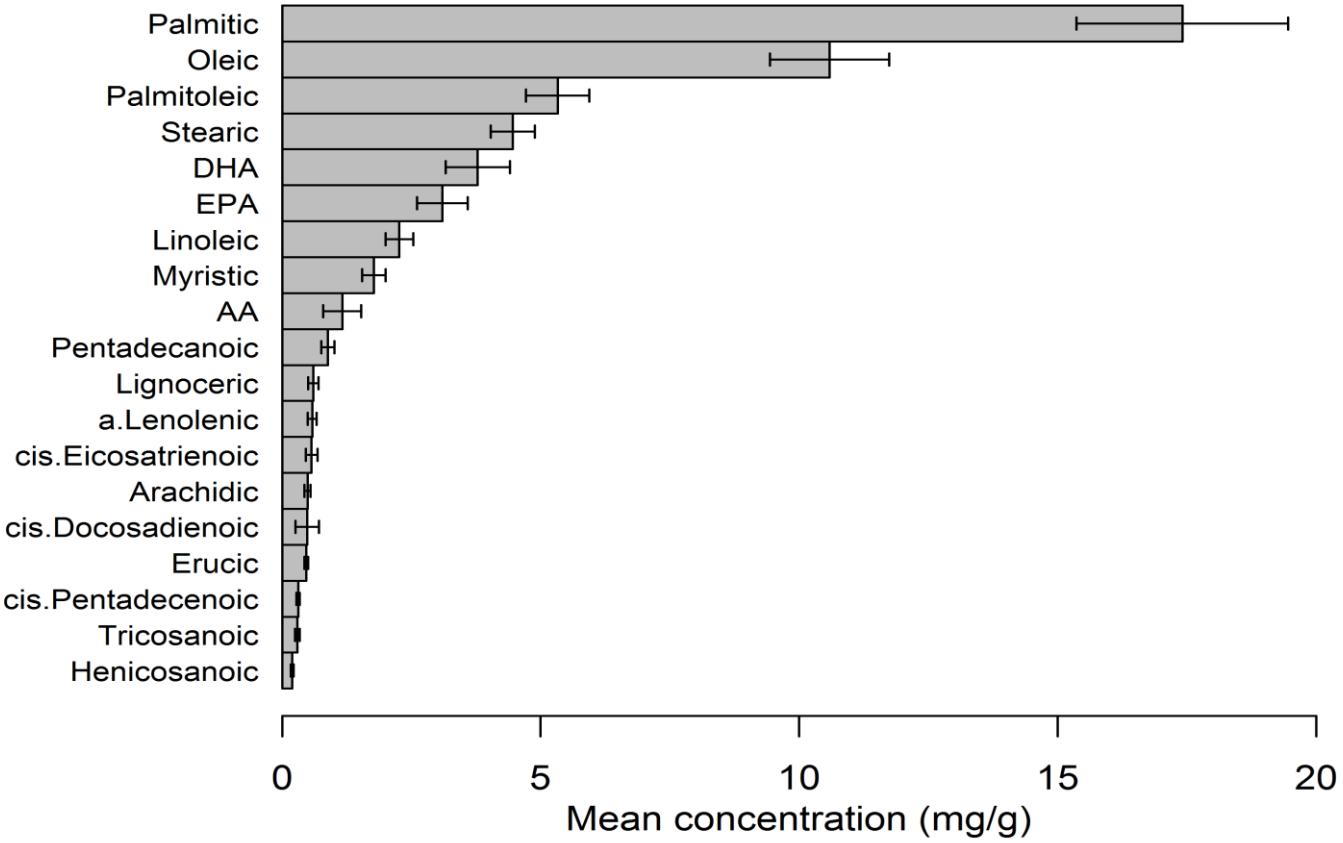


Figure 3.3.1: Fatty acids present in gonad tissue of female *Chrysophrys auratus*. Concentrations are in mg/g of dry weight. Bars represent means ± standard error.

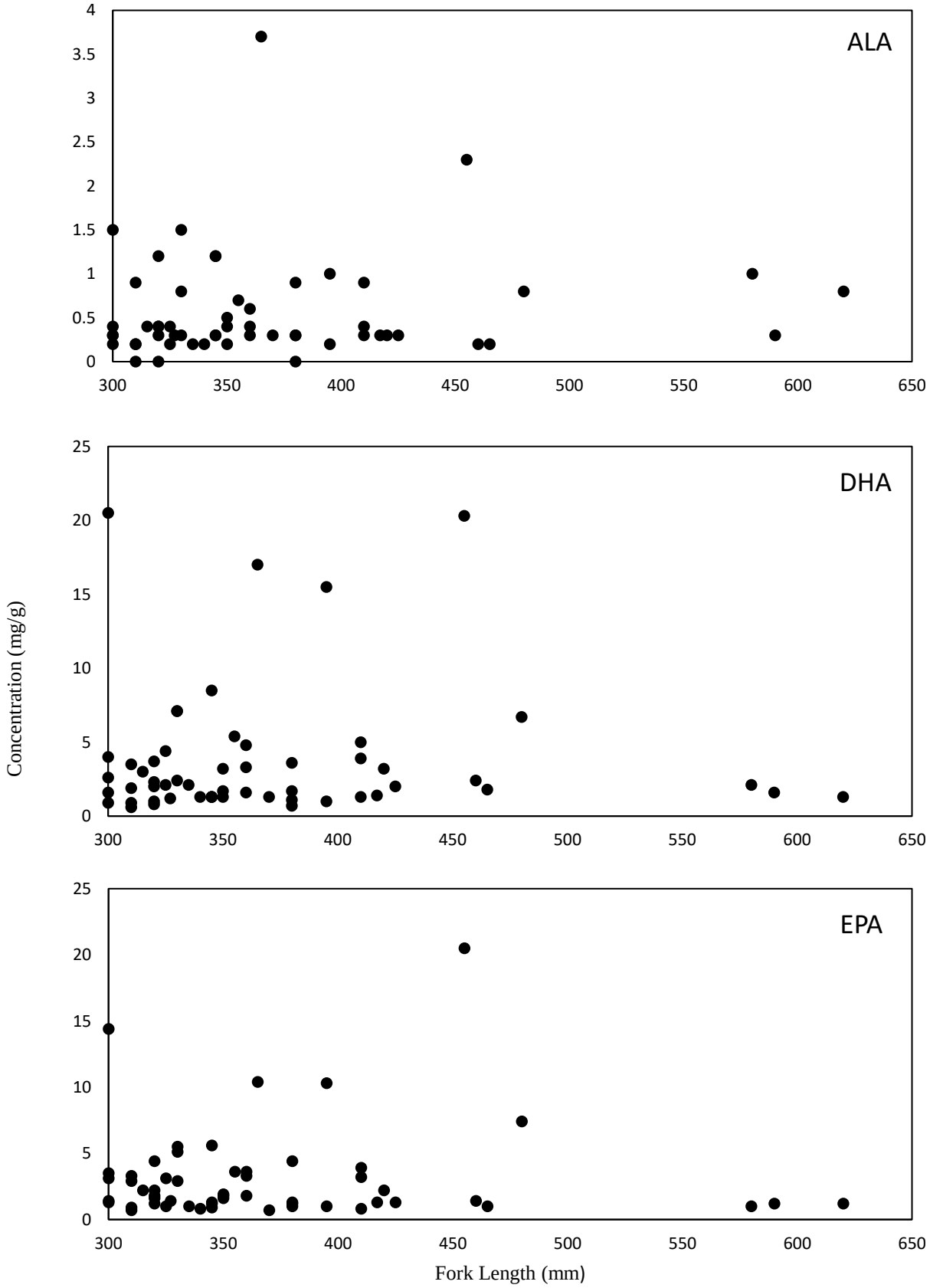


Figure 3.3.2: Concentrations of omega fatty acids α-linolenic (ALA), docosahexanoic (DHA) and eicosapentaenoic (EPA) against fork length in gonadal tissue of female *Chrysophrys auratus*.

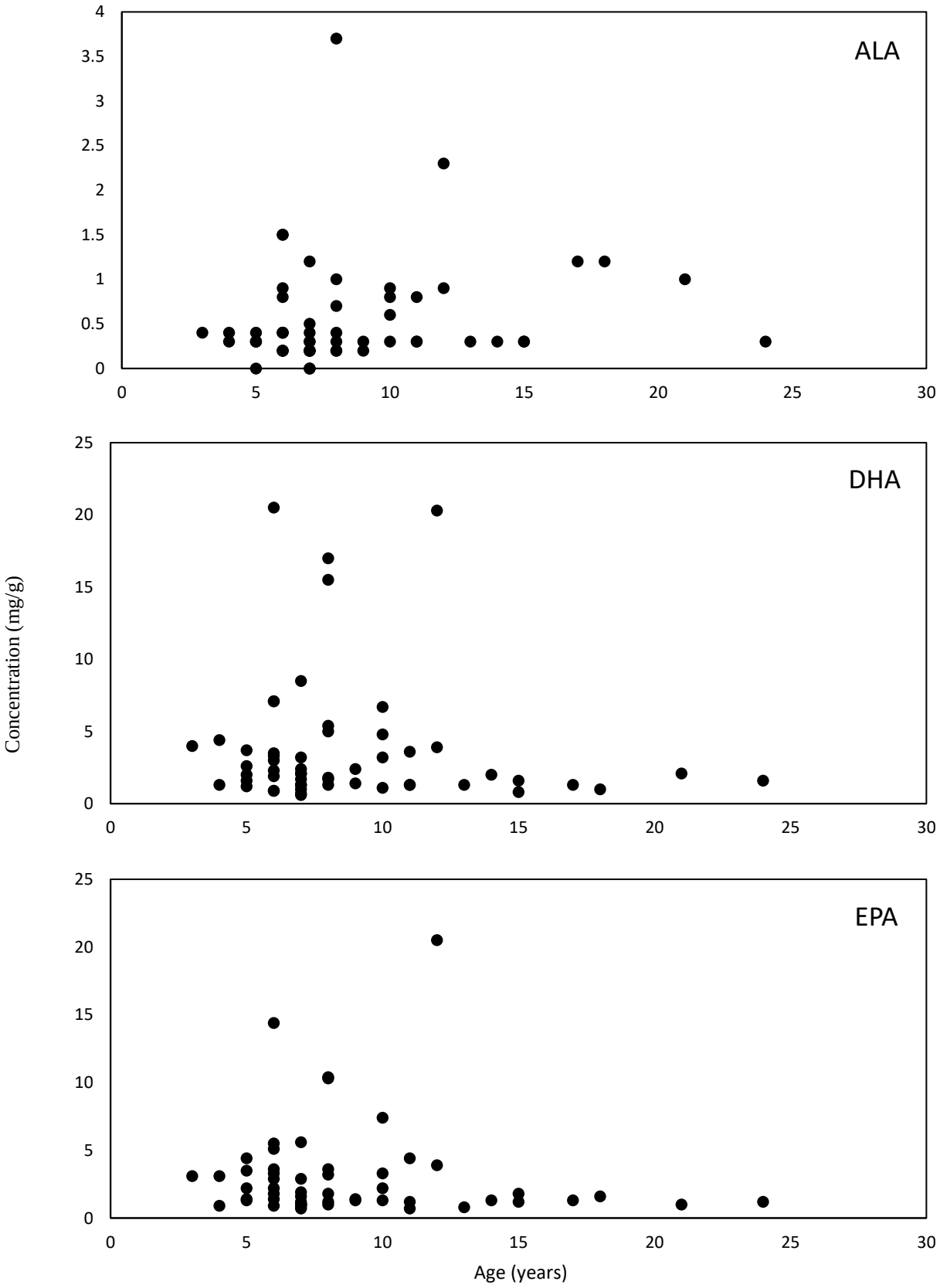


Figure 3.3.3: Concentrations of omega fatty acids α-linolenic (ALA), docosahexanoic (DHA) and eicosapentaenoic (EPA) against age in gonadal tissue of female *Chrysophrys auratus*.

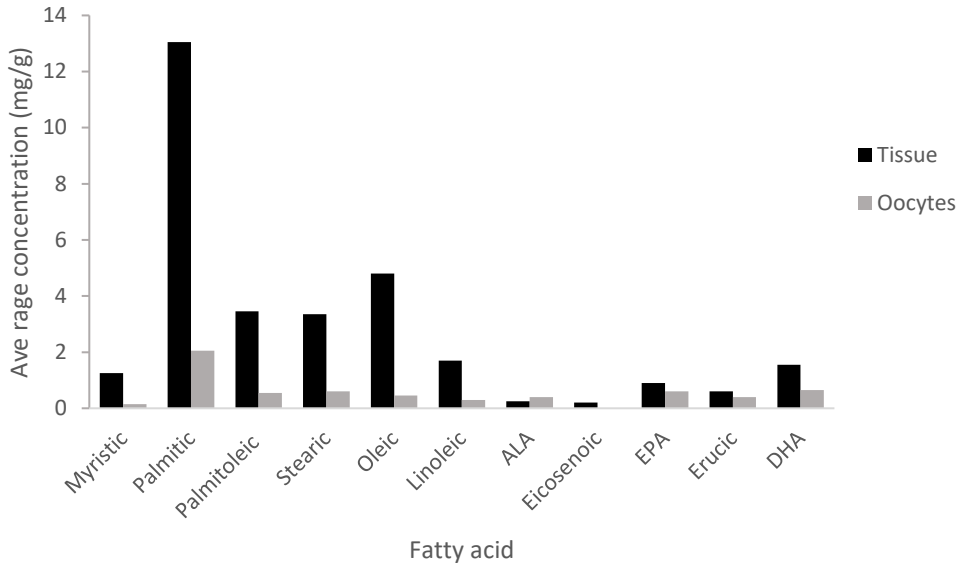


Figure 3.3.4: Average concentrations (mg/g) of individual fatty acid species in tissue and oocytes of 2 female *Chrysophrys auratus*.

Chapter 4 - Discussion

4.1 Population demographics and reproductive parameters

The age and size distribution of *C. auratus* from the Hauraki Gulf provided some interesting characteristics. Firstly, the modal age class was 6 (Figure 3.1.1), followed by 320-339mm FL as the modal size class (Figure 3.1.2), for both sexes, respectively. These two parameters represented the highest frequencies of age and size from our dataset and indicate a relatively young and small population in the Gulf. Furthermore, females outnumbered males beyond 380-399mm FL, making up 9 out of the 10 largest specimens. Male and female frequencies varied between age classes, however older age classes were dominated by females, making up the three oldest specimens (i.e. 18, 21,24yrs).

As indicated in previous work on snapper population demographics (Walsh et al., 2011b), *C. arautus* in the Hauraki Gulf exhibit highly variable size at age, leading to the conclusion that size is not an accurate indicator of age. This was confirmed in this study (Figure 3.1.3) by a very weak correlation ($R^2 = 0.35$) between age and size (FL). At the most extreme, 10-year-old individuals in this study could range between 320mm and 480mm in length and between 642g and 1 800g in weight. However, this plasticity appears to be localised to the Hauraki Gulf and does not extend to other areas such as the West coast, Northland, and the Bay of Plenty (Walsh et al., 2014; Walsh et al., 2011b). A number of hypotheses have been proposed for explaining the wide size range for age groups in the Hauraki Gulf, including variations in water temperature, food availability and genetic diversity (Francis, 1994b), as well as historic fishing pressure (Parsons et al., 2014).

The relationship between length and weight ($W = a \times L^b$) was highly correlated ($R^2 = 0.93$), exhibiting a *b* coefficient of 2.71 (Figure 3.1.4) which indicates negative allometric growth, where a population grows faster in length than weight (Karachle & Stergiou, 2012). Sparid species have shown varying degrees of both negative and positive allometrey, ranging from 2.4 to 3.5 (Karakulak, Erk, & Bilgin, 2006). Despite a limited seasonal and geographical range within our sample, our value agrees with historical *b* coefficient values for snapper from the Hauraki Gulf (2.79) (Froese & Pauly, 2005). Snapper from the north island's west coast however, grow in a considerably more isometric manner, with a *b* coefficient of 3.01 (Froese & Pauly, 2005). Previous research on length-weight relationships within teleosts have revealed a number of potential causes for observed differences. These include annual or seasonal changes in population structure, competition, disease, as well as environmental conditions such as food availability, temperature, salinity or habitat fitness (Froese, 2006).

Additionally, recent studies on fresh water species have proposed allometric growth to occur as a result of increased pollution levels in water ways (Hussain et al., 2015; Naz, Javed, & Tahir, 2013). A number of the above mentioned causes are possibilities for the observed difference in allometry between Hauraki Gulf and west coast snapper populations. However, the differences in population structure due to current and historic high levels of fisheries exploitation in the Hauraki Gulf (Ministry for Primary Industries, 2014), along with differences in environmental conditions due to the geographic location and prevailing weather systems are perhaps the most probable causes. It should be noted that whilst snapper in the Hauraki Gulf are negatively allometric, the value remains within the calculated range for most fish species (2.5 – 3.5), and is not considered to be at the extreme end of the spectrum (Froese, 2006). When divided by sex, males showed greater allometry (2.4) than females (2.8), indicating that males exhibit a slightly slenderer body shape than females. Protogynous species may exhibit a growth spurt at the time of sex change (Matthias et al., 2016), due to the lower energetic costs of producing sperm compared to eggs, allowing additional energy for somatic growth. This has been observed in the Sparid species *Chrysoblephus puniceus*, where recently changed males demonstrated a short burst of accelerated growth (Garratt, Govender & Punt, 1993). Considering our male population contained mostly young individuals (average 7.6 years), and given that snapper change sex between the ages of 2 and 5 years and 20 and 30 cm (FL) (Francis & Pankhurst, 1988), younger, smaller males may potentially have undergone a relatively recent period of quicker growth. Whilst this may account for changes in allometry at a young age, it does not extend to the entire population, with the largest and oldest individuals in our sample being females.

Mortality analysis (Figure 3.1.5) shows a strong correlation ($R^2 = 0.87$) with a consistent decline (approximately 25% per year) from the modal age class of 6 years. Snapper is considered to be moderately long lived, with otoliths unearthed from middens throughout New Zealand indicating that this species can live to a maximum age of 60 years, and a maximum size of 100cm (Leach, 2006), making 6 years a remarkably early onset for such a rapid decline. The impact of significant historical fishing pressure from the commercial sector, as well as ongoing and increasing pressure from recreational anglers in the Hauraki Gulf may be responsible for a compensatory response. The prevailing consensus on the impact of chronic fishing pressure is that population dynamic changes occur as a result of a skewed population distribution (in this case a dominance of small, young individuals), and can lead to changes such as earlier age of maturation, slower growth, skewing of sex

ratio, reduced egg size, and changes in morphological traits (Polunin & Graham, 2003; Vincent & Sadovy, 1998).

The relationship between otolith weight and age (Figure 3.1.6) was linear ($R^2 = 0.61$), indicating otolith accretion with age. However, the relatively low R^2 value can be attributed to two factors. According to Choat et al., (2003), accretion slows with increasing age leading to outliers. This was evident in our population sample. Furthermore, our sample was relatively small (n=113) and age-biased towards younger individuals, which also affected the R value.

The relationship between age and size was modelled using a reparameterised Von Bertalanffy growth model (Figure 3.1.7). Tau, Omega, and Nu were set at L1, L7, and L13, respectively, representing a spread across the most abundant age classes. The expected mean body size for these parameters were as follows; L1 111.5mm FL, L7 356.4mm FL, and L13 398.8mm FL. However, the more significant aspect of this analysis is the sexually dimorphic growth pattern, where females reach a higher size at equivalent age than males. Table 3.1 provides these values partitioned by gender; where L7 and L13 for males are 345.0mm and 353.4mm FL, respectively, and the same parameters for females were 362.0mm and 426.3mm FL, respectively. These growth parameters were subjected to the Likelihood Ratio Test (LRT) which found L7 and L13 to be significantly different ($P < .0004$) between males and females (Table 3.2). The significance of this finding may be profound as to date the snapper fishery in New Zealand has been managed under the assumption that snapper show no differential growth between sexes (Paul, 1976). The sexually dimorphic growth pattern exhibited by *C. auratus* in this study is typical of gonochor teleosts (e.g. Alós et al., 2010; Dutney, Elizur, & Lee, 2016; Wilson et al., 1991), and furthermore, has been observed in a number of species within the Sparidae family (e.g Buxton & Clark, 1985; Griffiths et al., 2002; Pajuelo & Lorenzo, 2004; Pajuelo et al., 2006). Notably, Jackson et al., (2010) found male and female growth differences in *C. auratus* in a resident population in Shark Bay, Western Australia. Females in a subpopulation in Denham Sound, were found to grow at a significantly faster rate than males, despite closely neighbouring populations showing no differences (Jackson et al., 2010). Whilst the underlying cause for this particular case of sexual growth dimorphism remain unclear, the potential for fine scale (as small as tens of kilometres in some instances) variation in biological and life history traits between *C. auratus* populations is possible (Jackson et al., 2010), and warrants further investigation throughout the ecological range the species inhabits. Hypothetically, there is benefit for snapper to exhibit larger growth as females than males. Larger female fish are typically more fecund

than their smaller counterparts (Palumbi, 2004), and this is true for female snapper, with the number of oocytes being produced increasing in proportion with the size of the individual (Crossland, 1981). Furthermore, male gametes are typically smaller than females, and lack the larval provisioning requirements associated with oocytes. Indeed, females have been shown to grow at faster rates than males for a number of Sparid species including *Pachymetopon aeneum* (Buxton & Clark, 1985), *Rhabdosargus globiceps* (Griffiths et al., 2002), and *Pagrus auriga* (Pajuelo et al., 2006). Snapper's developmental pattern has been described as functional gonochorism, where protogynous sex change occurs from non-functional juvenile females (Francis & Pankhurt, 1988). The triggers for such sex change are as yet unknown, however it is possible that size may be an influence, as with other teleost species, where either endogenous or sociodemographic controls can enable larger or faster growing individuals to remain as females, whilst smaller individuals change to male, or vice versa (Parker, 1992; Ross, 1990). Griffith et al., (2002) suggest that slower growth rates in males compared to females observed in the Sparid *Rhabdosargus globiceps*, may be as a result of a need for greater energy demands associated with spawning behaviour, where males are required to defend territory and compete for females. Little is known of the spawning behaviour of *C. auratus*. Early reports described a lethargic process where fish (both male and female) roll on their side near the surface releasing clouds of milt or oocytes into the water column (Cassie, 1956). More recently, multiple males have been observed following females near the surface (Smith, 1986), potentially invoking competition between males for fertilization of eggs, requiring both energy and agility from males in order to successfully fertilize oocytes. However, this theory remains somewhat contradictory in its concept, considering if competition between males during spawning is apparent, then large size would be a profound advantage, as is the case for many coral reef species (Warner, 1984).

Histological analysis of male and female gonads, partitioned by reproductive stage revealed several interesting observations. The reproductive stages used for males were 1) Resting, 2) Mature, and 3) Spawning, while for female were 1) Resting, 2) Vitellogenic, and 3) Spawning. Given that this study was conducted during the spawning season of snapper, the majority of individuals were likely to be some way through the reproductive cycle. This was evident with only 9% of males and 25% of females in a resting state (Table 3.3). This was further confirmed by age and size class analysis (Figures 3.1.8 – 3.1.11) partitioned by reproductive stage for males and females, where the majority of age and size classes were in the latter stages of spawning capable (i.e. stage 2) or spawning (i.e. stage 3). The Gonadosomatic Index (Figure 3.1.12) also shows very close similarity in values between

males and females, confirming that the vast majority of individuals sampled were in an active stage of reproduction. Snapper are known to spawn in the Hauraki Gulf from November to February (Crossland, 1981), and the location of the bulk of fish caught was in close proximity to the Mahurangi Harbour, a known settlement site for juveniles (Sim-Smith et al., 2013a).

Strong inter annual variations have been observed in the temporal scale of snapper spawning, and are thought to be primarily influenced by sea surface temperature (SST) (Scott & Pankhurst, 1992). Snapper typically begin spawning when SST reaches between 14.8 - 16°C (Francis, 1994), and continue until SST reaches between 19 - 21°C (Scott & Pankhurst, 1992). SST during sampling rose steadily from 16.2°C in late October, to 23.4°C in early February, and snapper continued to show evidence of active spawning throughout this time, with individuals caught on February 11 (23.4°C) at reproductive stages 2 or 3. Thermoclines can develop in the Hauraki Gulf during summer, capable of having temperature differences of up to 3°C between surface and benthic waters (Harris, 1985). With snapper being a predominantly demersal teleost, sea surface temperature may then (at times) vary considerably from that which the species predominantly inhabits, and may explain the extension in spawning activity beyond the literary cited temperature induced conclusion.

4.2 Lipid composition, quantity and provisioning

This study aimed to compare the lipid and fatty acid composition and quantity of the New Zealand snapper/tamure (*Chrysophrys auratus*) against maternal age and size, as well as examine the provisioning strategy used by this species. Results from TAG quantity analyses indicate that there are no significant differences in total TAG concentrations across maternal age, fork length, or condition factor (Figure 3.2.1). It is in some respects unsurprising that correlation tests across our entire sample yielded insignificant results. Cellular degeneration occurs as individuals age, resulting in a number of outcomes for teleosts such as reproductive, anatomical and physiological senescence, along with potential changes in lipid, carbohydrate and protein metabolism (Patnaik, Mahapatro & Jena, 1994). As this occurs an individual will cease to function at their peak, surpassing the time when physiological processes are running at their optimum potential. The timing of this optimum zone may well be individualized, dependent on a number of both internal and external factors such as diet, heredity and environmental conditions, and cannot be assumed to be a species wide trait. This fact, along with the sparse number of samples at the high end of both age and size spectrums, lends itself to the potential for high variances at the top end of the sample, and the potential for distorted correlation results. A humped response was perhaps more likely, as individuals

reach their prime at some stage through the middle of their lives, post full development and prior to any degradation occurring. This was tested using a generalized additive model (GAM), calculating the smoothness of any possible trends in the data. However, GAM results indicated no significance when tested against age, size and condition (p values .26, .83 and .66 respectively). The potentially long life span (60 years) and large maximum size (100cm) of snapper mean that the majority of this sample was at the lower end of the range for this species, with the oldest female (24 years), less than half the maximum age. While snapper population demographics in the Hauraki Gulf have undoubtedly been influenced by historic and ongoing commercial and recreational fishing pressure, it remains that the species today are rarely reaching their maximum age or size. This may be inconsequential to overall stocks if degradation is already beginning to occur in individuals at or near the present day maximums, or alternatively it may be having a significant impact, if individuals are being removed before they reach their reproductive prime.

Whilst no trends or differences were observed owing to any particular maternal parameters, TAG levels did vary significantly between individuals (e.g 1.5 to 11 mg/g). The variances observed for individual snappers are potentially caused by a number of factors, including diet, genetic or heredity differences, varying life history characteristics, and capital breeding strategies. These possibilities, along with others, will be further discussed below.

4.2.1 Triglyceride (TAG) quantity

Exploratory analysis revealed no apparent difference in total TAG or fatty acid concentrations across any of the three maternal parameters. Similarly, GAM results indicated no significant relationship between total TAG concentration in gonad and liver tissue when tested against age, size, and condition factor. Whilst there was no overall significance in total TAG concentrations, GAM analysis revealed five out of the sixty individual TAG species showed significant nonlinear trends (Figure 3.2.4). These species showed varying responses; either a gradual decline in TAG concentration with size and age or, as seen in the liver with 54:7 and 56:8, a humped response suggesting peak TAG concentration between 7 and 8 years of age. Whilst the biological significance of this finding is unclear, and it could be argued that five statistically significant TAG species from a total of sixty is more a product of chance or sample bias than true statistical significance. However, we do believe that presenting these specific TAG species does serve as a baseline result, which could be examined or compared in future studies.

Diet undoubtedly plays a major role in the quantity of TAG's found in gonad tissue. Considering the vast majority of TAG's are derived exogenously through an individual's diet (Ruiz-Gutiérrez & Barron, 1995), and TAG's constitute a major lipid class in the diet of marine fishes (Tocher, 2003), what an individual feed's on prior to reproduction will have a strong influence on their lipid quantity and composition. Adult snapper have a wide and varied diet ranging from crabs to bivalves to teleosts (Usmar, 2012). This variety is enhanced by the differing habitats and the associated diets of 'resident' or 'reef' snapper and 'schooling' snapper. Variations in environment such as substrate and depth, as well as in the geographic location, result in schooling snapper from the outer Hauraki Gulf acquiring a preference for teleosts (Godfriaux, 1969). These diet changes due to life characteristics are highly likely to in turn play a role in lipid sourcing and impact an individual's total lipid content, and may go some way to explaining the observed individual variances.

Some anecdotal seasonal changes in diet have been observed in snapper, however this has not been studied in detail. Closer examination of this may indicate whether certain foods are preferred as reproduction approaches and/or throughout the spawning season, or whether feeding is purely opportunistic and availability based. Gut content analysis coupled with lipidomics may indicate what impact an individual's primary food source is having on lipid levels, and indicate how much of a role food plays in this species lipid composition and provisioning. The importance of lipids in the diet of marine teleosts cannot be understated. The failure of Newfoundland cod (*Gadus morhua*) to recover after heavy fishing has been linked to the absence of the previously abundant oil rich capelin (*Mallotus villosus*) in their diet (Rose & O'Driscoll, 2002).

Interestingly, Martin (2009) also observed high variability in biochemical content (including total lipid) of oocytes between snapper within different size classes. Considering that the specimens in Martin's study had been captive for a number of years and fed exactly the same food source, it can be concluded that diet alone cannot account for all variances in snapper lipid levels, it therefore may be possible that through heredity, certain individuals are better capable of converting food into effective lipid resources for reproductive processes than others.

A number of concepts attempt to explain the influence maternal age and size can have on reproductive capacity. McBride et al., (2015) suggest that age related differences in egg quality may arise due to younger fish having poorer condition, inhabiting poorer habitats or having lower levels of cognitive ability. It is the later which may play a role in snapper diet, with older fish being more prolific feeders or better selective feeders with age as cognitive

ability improves. Similarly, age may produce an increase in proficiency when it comes to reproduction, as older fish have had more practise/experience and may be more proficient at reproducing. Snapper growth slows after the first 5 years (Walsh et al., 2006). Beyond this age, as energy required for somatic growth reduces, resources may then be more readily available to be distributed into gonadal tissue in preparation for reproduction, as is the case for many other fish species (Roff, 1983). Despite these possibilities, it appears in this study age is not influencing female snapper TAG levels during reproduction, with an even distribution observed across 4 to 24 year old females. Size and age are not closely related in snapper from the Hauraki Gulf (Walsh et al., 2011b), and as such either of these parameters had the potential to influence lipid levels independently.

Maternal length has influenced the biochemical composition of eggs from the common nase (*Chondrostoma nasus*), as well as influencing larval, oil globule and egg size for a range of species (Berkley et al., 2004; Green & McCormick, 2005; Raventos & Planes, 2008). However, findings from this study indicates that TAG levels for *C. auratus*, although variable between individual specimens, were not significantly different across size class, with no obvious relationship observed. This concurs with Martin (2009), who examined size as a potential variable for total lipid levels.

Martin (2009) studied 18 captive snapper held at the Leigh Marine Laboratory of Auckland University. This research included the analysis of three broodstocks in separate size classes, with total lipid, total protein and free amino acids of expelled eggs examined. Whilst Martin found differences in parameters such as the length of spawning season and the size and viability of eggs produced in relation to fork length, biochemical analysis revealed no significant differences. There are difficulties extrapolating these findings to wild stock, due to the environmental, nutritional, and subsequent biological and morphological differences that can occur between wild and aquacultured individuals (Arechavala-Lopez et al., 2012; Fallah, Saei-Dehkordi, & Nematollahi, 2011). Fatty acid and total lipid content in broodstock has been compared to wild stock in both the white sea bream (*Diplodus sargus*) and the gilthead seabream (*Sparus aurata)*, with significant differences found in all the major classes examined (Cejas et al., 2003; Lenas et al., 2011). Furthermore, the analysis of broad lipid classes doesn't allow for the identification and analysis of particularly important lipid subclasses and overall lipid composition, which may have a disproportionate influence on larval success. It is for these reasons that it was deemed worthwhile undertaking further lipid composition and quantity research on this species, from a larger sample size of wild stock.

Lipid composition and quantity is considered to be specific beyond orders or families down to the species level (Kaitaranta & Ackman, 1981), making direct comparisons between species somewhat irrelevant (Wiegand, 1996). Additionally, the role of lipids in some species may be complimented or even superseded by other nutritional sources (Kaitaranta & Ackman, 1981). Whilst this research has demonstrated that the maternal influences on snapper larval growth rates and survivability appear not to be related to TAG or fatty acid levels, it is possible (however unlikely) that other components may provide the majority of sustenance for larvae. Whilst TAG's, along with other essential fatty acids have been identified as vital to embryo development (Wiegand, 1996), proteins and carbohydrates have also been identified as important sources of endogenous larval nutrition. The Baltic herring (*Clupea harengus*) is perhaps the most striking example of this, where oocytes have been found to contain upwards of 80% protein (V'uorela, Kaitaranta & Linko, 1979), undoubtedly providing the main energy source for larvae of this species. In addition to some species reliance on protein as an energy source, some, such as turbot (*Scophthalmus maximus*) also rely heavily on amino acids as a major source of energy (Ronnestad, Fyhn, & Gravningen, 1992). Indeed, free amino acids have been proposed to play an important role in the nutrient supply for developing embryos and early larval growth for fish in general (Fyhn, 1989).

Condition too has been correlated with positive larval and egg characteristics. Marteinsdottir and Steinarsson (1998) observed condition, as well as size and age, to influence parameters such as the size of the egg, the development of a swim bladder, and early larval growth rates in the Atlantic cod (*Gadus morhua*). Condition is likely to play a role in larval success of snapper too, given that healthier fish typically produce better offspring (Donelson, McCormick & Munday, 2008), however this is not correlated with TAG provisioning, with no relationship observed in this study.

A seminal study relating to maternal influence on larval success was carried out by Berkley, Chapman and Sogard (2004), who established that offspring from older black rockfish (*Sebastes melanops*) grew faster and survived starvation for longer due to a greater provisioning of energy rich TAG. This work continued with a further five species of rockfish, all demonstrating some form of maternal influence from older/larger females (Sogard, Berkeley & Fisher, 2008). TAG levels in these studies were calculated without the use of any chromatographic techniques, instead calculating the volume of larval oil globules. The assumption that the oil within the globule was mostly comprised of TAG's, relied on previous work by Norton et al., (2001) who analysed lipid class composition in the shortbelly rockfish (*Sebastes jordani*) finding TAG to be the dominant lipid. Extrapolating this for other

species can be somewhat ambiguous; such is the species specific nature of lipid composition and quantity (Kaitaranta & Ackman, 1981). Furthermore, Norton et al., (2001) found TAG levels to increase significantly during a larvae's development, with a rapid increase observed with fish at the juvenile stage (71 – 150 days old). Berkley examined oil globule volume from rockfish during the preflexion and early flexion stages (up to a maximum of 30 days of age), during which TAG levels are relatively constant, with increases rarely occurring before the post flexion stage, when larvae are at least 46 days old (Norton et al., 2001). This indicates that factors such as larval diet or feeding ability, or a specific lipid composition or genetic disposition that improves or enhances a fishes' ability to process and store TAG may be contributing to larval condition and survivability, more or as much as, maternally provisioned TAG. Whilst certain species of older and/or larger black rockfish may indeed produce offspring that grow faster and have greater survivability, the assumption that these traits are purely from maternally provisioned TAG may be somewhat erroneous. Whilst the survivability of larvae was not tested in this study, it appears that TAG levels are not driving this process. Older or larger females may potentially be producing larvae that grow at faster rates and survive periods of starvation for longer than those from smaller, younger individuals, however the mechanisms driving this are unclear, and for snapper at least, are not related to either TAG quantity or composition.

4.2.2 Triglyceride (TAG) composition

A small number of TAG species dominated the overall composition, and broadly speaking medium/long chain species also dominated (Figure 3.2.6). The species 56:7 (three acyl groups containing 56 carbons and 7 double bonds), which is a long chained and highly unsaturated triglyceride, comprised approximately 10% of both liver and gonad TAG's, along with other dominating species.

The fatty acid tails of higher numbered TAG species (60+ carbons) are likely to consist of more biologically important fatty acids such as DHA (22:6), EPA (20:5) and AA (20:4) due to their own high carbon and saturation number; for example, a TAG with a carbon number of 60 may include 3 x 20 carbon chains, 1x 18, 1x 20, and 1x 22, 2x 22 and 1x 16, and so on. While less biologically important fatty acids such as lauric acid (C12:0), palmitic acid (C16:0) and stearic acid (C18:0), have no double bonds and generally possess lower carbon chains. As such, separate analyses were conducted on TAG species with a

saturation number greater than 6, in order to ascertain if quantities of these species were impacted by size or age (Figure 3.2.7).

Variations in lipid composition between liver and gonad tissue were observed (Figure 3.2.8 and 3.2.6), with medium to long chain species taking up 64% of the overall lipid composition in liver tissue, and 72% in gonad tissue. The liver converts polar lipids and very low density lipoproteins to vitellogen, where it is then transferred to the gonad and synthesised further into TAG's (Wiegand, 1996). This synthesis occurring within the gonads may explain why TAG species with a lower unsaturation were more prevalent in livers than gonads, while a lower numbered carbon chain (50) was only present in the liver.

It was theorised that as the female ovary progresses through reproductive stages from resting to vitellogenic, to spawning, changes in the TAG species composition would become evident in gonad tissue. This was based on the assumption that a shift from stocking or energy based TAG's (lower carbon numbers) occurs to more biologically important species as spawning approaches. However, this trend was not observed in this study, with only minor and insignificant differences being observed between reproductive stage and TAG species composition. A longer temporal study may be required in order to better understand snapper lipid composition, as gonadal and liver lipid class and composition have been observed to change seasonally in other teleost species (Bandarra, et al., 1997; Murzina et al., 2012).

4.3 Lipid provisioning and breeding strategy

Along with comparisons of TAG and fatty acids across population demographics, this study also sought to shed light on to the lipid provisioning and breeding strategy used by snapper. Some fish species feed and spawn in separate areas and at separate times, stocking lipids in preparation for spawning events and drawing on them when needed. This 'capital' breeding strategy differs from 'income' breeding, where a species typically uses locally acquired energy, often throughout a prolonged spawning season (McBride et al, 2015).

Provisioning of lipids by snapper in the liver and gonad revealed consistent trends over the spawning stages, with gonad concentration remaining around 7 mg/g and liver around 2/mg (Figure 3.2.5). Scott and Pankhurst (1992) have shown an increase to occur in the hepatosomatic index for females leading up to spawning, followed by a slightly decreasing trend as the spawning season progresses. Our TAG results demonstrated slightly decreasing concentrations in the liver over the reproductive stages, most likely as lipids begin being transferred into the ovaries during vitellogenisis. The gonadosomatic index measured in this study revealed female gonads to be increasing in size as they neared reproduction. This

however did not correspond with an increase in TAG concentration, with average concentrations only slightly increasing with reproductive stage (Figure 3.2.5). It is evident from this research that snapper supplement their cost of reproduction with stored lipid reserves. The lack of extremely low levels of TAG's seen in recently spawned individuals, and the consistent average of TAG concentrations across the reproductive stages, place snapper towards the capital end of the breeding spectrum. Undoubtedly snapper remain feeding throughout the spawning season, however it appears this intake of food is not critical to stock the lipids they require for reproduction.

Although not observed in this study, it is possible that snapper may demonstrate a mixed capital and income breeding pattern. This may be explained by differences in the life characteristics of schooling and resident snapper. Schooling snapper undergo energy costly migrations prior to spawning, a trait often combated with a 'capital breeding' provisioning strategy, thus eliminating a conflict of resources during migrations (McBride et al, 2015). Whereas resident snapper may be more reliant on consistent and dependable food sources available throughout the year, eliminating a need to bulk up on lipids or replenish stocks between spawning events, enabling a drip feeding of reserves to be distributed into the ovaries throughout multiple spawns. Species such as snapper that are serial spawners and demonstrate asynchronous oocyte development, are generally thought to be at the income end of the spectrum (McBride et al., 2015), however there is considerable benefit for a species adopting a capital provisioning approach, given that this allows them more flexibility in the timing of their spawning, rather than being constricted temporally, and in the rate of reproduction by the availability of food sources, such is the case for income breeding species (Varpe et al., 2009).

The concept of income and capital breeding has not been without its critics. Stephens et al., (2009) acknowledged that while these terms are useful explanations of physiology and behaviour, interpretations can be varied and lack definitive definition, leading to subjectivity in their understanding. Furthermore, the boundary between capital and income breeding is not always clear and species may adopt a mixed strategy (Houston et al., 2007), or their strategy may shift over time due to environmental characteristics or ontogeny (McBride et al., 2015). It is therefore beneficial to focus on the tendency for a species to be either capital or income dependent, rather than classifying them into one of two separate, distinct strategies (Houston et al., 2007).

Whilst this research indicates snapper have a tendency to provision lipids with a capital strategy, the extent of this is unclear, and an extended data set, allowing the months

prior to and post spawning to be examined is required to provide more information on snappers provisioning strategy.

4.4 Fatty Acids Methyl Esters

One of the aims of this thesis was to determine whether maternal traits such as age, size and condition were influencing fatty acid concentrations or compositions in female snapper during reproduction. Results indicate that this is not occurring, with post hoc analysis revealing no significant differences between fatty acids dependent on any maternal parameter. However, GAM analysis revealed two out of the twenty-eight fatty acids (tricosanoic and behenic) to show a significant relationship with size and condition factor, respectively, but this was attributed to outliers and subsequently ignored. Furthermore, tricosanoic and behenic were ranked outside of the top twenty most abundant fatty acids. As with TAG's however, individual levels varied, and this, along with the selective catabolism and fatty acid composition of *C. auratus* will be discussed below.

4.4.1 Composition and concentration

The most abundant fatty acids observed in gonads of *C. aurautus* were palmitic (16:0), oleic (18:1n-9), palmitoleic (16:1n-7) and stearic (18:0) (Figure 3.3.1). These particular fatty acids are often among the most commonly observed in fish (Wiegand, 1996). Stearic and palmitic are the dominant saturated fatty acids found not only in fish lipids but in all animal fats, while palmitoleic and oleic are the predominant monounsaturated fatty acids found in most lipids (Tocher, 2003). Following these, comes docosahexaenoic (DHA, 22:6n-3), eicosapentaenoic (EPA, 20:5n-3), linoleic (18:2n-6), and myristic (14:0). Myristic acid is a common saturated fatty acid, and is able to be synthesized internally, while the other three fatty acids (along with *alpha*-linolenic (ALA, 18:3n-3)), are considered essential fatty acids, unable to be produced within the body and therefore must be derived exogenously. It is the fatty acids that are derived from the diet that are thought to be particularly important in the intracellular metabolism of other lipids, allowing the conversion to more biologically important lipids (Sargent et al., 1995). Fatty acids are a key source of metabolic energy for fish, allowing growth, reproduction and locomotion to take place (Tocher, 2003). Certain fatty acids play more integral roles than others during embryonic and larval development. Some, such as DHA and EPA, are vital in the development of the brain, eyes, and for cell membrane structure and function (Sargent et al., 1999b), and as such, these particular fatty acids have

garnered special attention in this field (see Osman, Suriah & Law, 2001; Watanabe, 1993; Wiegand, 1996), and consequently have been a focus in this study (Figure 3.3.2 and 3.3.3). DHA and EPA originate in aquatic ecosystems, where certain microalgae have the capacity to synthesise these fatty acids de novo, before they are transferred up the trophic levels via predation (Gladyshev, Sushchik & Makhutova, 2013). Along with these two fatty acids, *alpha*-linolenic (ALA) has also been given close attention in this study. Another essential fatty acid, ALA is also a likely homologue for DHA and EPA in marine teleosts (Sargent et al., 1995).

As with TAG, the composition of fatty acids observed among fish species are highly variable, due to differences in a range of factors such as habitat, diet, season, temperature and salinity (Özogul et al., 2009). Whilst the same fatty acid species were observed in snapper as in other species, quantity can vary significantly. For example, a selection of 34 Mediterranean fish ranged in DHA from 3 – 31% and in EPA from 1 to 10% (Özogul et al., 2009). In comparison, snapper contained an average concentration of 6.8% DHA and 5.6% EPA respectively.

The concentration (mg/g) of fatty acids in gonadal tissue of female snapper was not affected by maternal age or size. This analysis was carried out for all fatty acids, and a selection is represented here by ALA, DHA and EPA (Figure 3.3.2 and 3.3.3). Individual variations were significant and at their extreme a 30cm individual could range in DHA from .09 mg/g to 20.5 mg/g. Similarly to TAG, this may be explained by factors such as an individual's life history characteristics, diet or genetic or heredity influences.

4.4.2 Oocyte and tissue comparisons

Certain species (such as some tuna and capelin) are capable of selective catabolism of specific fatty acids, with an apparent inherent ability to provision oocytes with a particular assortment of fatty acids (Tocher, 2003). This typically involves a higher ratio of DHA and EPA being observed in oocytes, largely at the expense of eicosenoic (20:1n-9) and erucic acids (22:1n-11), found in higher levels in tissue and very low levels in eggs (Tocher, 2003). This ability has been observed in snapper in this study, with variances in gonad and oocyte TAG species observed (Figure 3.2.4), as well as variations of fatty acid concentration (Figure 3.3.4). These results did not follow literature values however, with both DHA and EPA showing slightly lower concentrations in oocytes than eggs, and erucic and eicosenoic acids found to be in relatively low quantities (Figure 3.3.4). When broken down to a percentage

however, the amount provisioned into the oocytes of DHA, EPA and ALA were much higher (42, 66 and 160%), than those of palmitic, stearic and palmitoleic (15, 17 and 15%), indicating a preference for these fatty acids to be kept, rather than provisioned. ALA is the parent chain for EPA and DHA, so may well go on to produce more of these essential fatty acids as further larval development takes place.

This selectivity gives further rise to the notion that fatty acid regulation and provisioning may well have an intrinsic or individual capacity, rather than simply an environmental or dietary influence.

5. Limitations

A number of limitations emerged as this research progressed. Firstly, acquiring a data set with a large range of sizes was limited due to the minimum legal size limit set by the Ministry for Primary Industries. This was further compounded by a relative absence of large snapper in the gulf, due to historical commercial and recreational fishing pressure (Willis, Millar & Babcock, 2003).

Small inaccuracies exist in the quantification of TAG concentrations, due to certain ‘split peaks’ being unable to be isolated during analysis. This led to a very slight under or over estimation of some TAG species peak values.

Assumptions made regarding biologically important TAG species are a best estimate, as while the carbon number is set, the fatty acid chain configuration can differ and can’t be confirmed. Therefore, the analysis of TAG species with carbon chains >60 is done so with a degree of uncertainty, however, the probability of such TAG’s being biologically important is considerable.

Ideally, it had been a goal to test primarily oocytes. However, we were unable to acquire many samples of running oocytes (only 2 from 62 females), despite nearly all individuals being histologically staged close to spawning. It is evident that lipid levels in oocytes are considerably higher than in gonadal tissue (4.3mg/g vs 11mg/g and 6.8mg/g vs 11.5mg/g). Examining oocytes from wild snapper stocks is extremely challenging, due in part to the sporadic and variable nature of spawning events. Coupled with this, strip spawning of wild females may result in either unripe and therefore un provisioned oocytes being included, while the option of holding caught individuals in tanks until they naturally spawn, results in the female undergoing significant levels of stress, potentially resulting in a lower reproductive capacity. Using lipid levels in gonadal tissue as a precursor to oocyte is plausible, however it is apparent from this study that the final content of TAG in oocytes may

be 2 to 3 times that of the tissue. Furthermore, testing gonadal tissue can limit the accuracy of oocyte lipid prediction by both picking up trace amounts of lipids held in ovarian follicle cells and ovarian stromal tissue, and through the selective catabolism of certain fatty acids (Wiegand, 1996; Tocher, 2003). However, it is thought that once vitellogenisis takes place (as was the case for the vast majority of *C. auratus* sampled), then the significance of these interferences will be minimal (Wiegand, 1996).

Snapper are thought to spawn from midday through to early evening (Scott et al., 1993; Crossland, 1980). Due to logistical and weather conditions field trips generally finished between 2 and 4 pm, potentially missing the peak period for spawning snapper. Alternatively, the timing of fishing trips (11 in total) may have fallen outside spawning times.

As well as liver and gonad, fish hold TAG's in their adipose and muscle tissue (Tocher, 2003), and as such measuring this would have been interesting, particularly in provisioning. Lipid levels in muscle tissue and perigonadal fat have been shown to decrease as spawning approaches in the northern bluefin tuna (*Thunnus thynnus*), indicating the utilization of these as important storage tissues for this species (Mourente, Megina & Díaz-Salvago, 2002). Snapper may also utilize tissue and fat as lipid storage, although it is thought that the liver is the primary storage organ for the majority of fish species, as well as providing for the synthesis of exogenous lipids into biologically important lipoproteins (Sheridan, 1988).

6. Conclusion

The results of this study indicate a lack of maternal influence in TAG or fatty acid composition or quantity, and a likely capital breeding strategy for *C. auratus*. Whilst these lipids appear to not be increasing with age or size in this species, the influence of other proteins and amino acids is as yet unknown, and may be having some influence in the survivability and development of larvae. Although no increases where observed, it is important to note that alternatively no decreases were apparent, despite individual differences being observed, overall a relatively even spread of quantities and compositions was seen across our sample. This shows that the quality of oocytes is likely to be comparable across maternal size and age. In regards to the future conservation and management of snapper in New Zealand, it is therefore pertinent that an increase of large and/or old individuals remain in the population, given the fact that their fecundity is exponentially greater, and their oocytes are of a similar quality. Management options to encourage this could include adopting a maximum size limit for the species, along with an increase in marine reserves, across a wide

range of habitats, enabling an increase in larger individuals and protection of this species throughout its many life stages.

The tendency towards a capital breeding strategy indicates the importance of year round feeding for this species. The benefits of adopting such a strategy for snapper are numerous, including removing the concern of unpredictable food sources during and leading up to reproduction, an ability to alter the specific timing of spawning events so as to maximise environmental conditions, and a reduction in energy expenditure from foraging during spawning, thus allowing reserves to be provisioned into reproductive processes. This adaptability during their reproductive cycle may well be a contributing factor to this species success and abundance.

While the reproductive season for snapper is relatively short, information obtained prior to and post spawning may hold as many answers. It is recommended that where applicable future lipid related studies adopt an annual temporal scale extending well beyond the start and conclusion of the reproductive cycle, an unfeasible option given the capacity of this study. The scope for further study in the field of lipidomics for both snapper and other species in New Zealand is considerable. This field has the potential to help identify a species nutritional requirements, as well as shedding light on the link between nutrition and physiological and biological processes. This will enable a better understanding of the parameters influencing larval success, as well as providing insight into wider marine ecosystem functions, and will subsequently lead to better informed management decisions.

References

Alós, J., Palmer, M., Alonso-Fernández, A., & Morales-Nin, B. (2010). Individual variability and sex-related differences in the growth of *Diplodus annularis* (Linnaeus, 1758). *Fisheries Research, 101,* 60-69.

Anderson, A. J., Anderson, S., & Arthington, A. H. (1990). Lipid classes and fatty acid composition of the eggs of some Australian fish. *Comparative Biochemistry and Physiology -- Part B: Biochemistry and Molecular Biology, 96*(2), 267-270.

Arechavala-Lopez, P., Sanchez-Jerez, P., Bayle-Sempere, J. T., Sfakianakis, D. G., & Somarakis, S. (2012). Morphological differences between wild and farmed Mediterranean fish. *Hydrobiologia, 679*(1), 217-231. doi:10.1007/s10750-011-0886-y

Bandarra, N. M., I. Batista, M. L. Nunes, J. M. Empis, and W. W. Christie. (1997). Seasonal changes in lipid composition of sardine (*Sardina pilchardus*). *Journal of Food Science 62*(1), 40-42.

Beldade, R., Holbrook, S. J., Schmitt, R. J., Planes, S., Malone, D., & Bernardi, G. (2012). Larger female fish contribute disproportionately more to self-replenishment. *Proceedings Biological Sciences / The Royal Society*, *279*(1736), 2116-2121.

Berg, J. M., Tymoczko, J. L., & Stryer, L. (2012). *Biochemistry.* New York: W.H. Freeman.

Berkeley, S. A., Chapman, C., & Sogard, S. M. (2004). Maternal age as a determinant of larval growth and survival in a marine fish, *Sebastes melanops*. *Ecology, 85*(5), 1258-1264. doi:10.1890/03-0706

Birkeland, C., & Dayton, P. K. (2005). The importance in fishery management of leaving the big ones. *Trends in Ecology and Evolution*, *20*(7).

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*(8), 911-917.

Bobe, J., & Labbé, C. (2010). Egg and sperm quality in fish. *General and Comparative Endocrinology, 165*(3), 535-548. doi: 10.1016/j.ygcen.2009.02.011

Brooks, S., Tyler, C. R., & Sumpter, J. P. (1997). Egg quality in fish: What makes a good egg? *Reviews in Fish Biology and Fisheries*, *7*(4), 387-416. doi:10.1023/A:1018400130692

Buxton, C. D., & Clarke, J. R. (1986) Age, growth and feeding of the blue hottentot *Pachymetopon aeneum* (Pisces: Sparidae) with notes on reproductive biology. *South African Journal of Zoology, 21*(1), 33-38. doi: 10.1080/02541858.1986.11447953

Cassie, R., M. (1956). Spawning of the snapper, *Chrysophrys auratus* in the Hauraki Gulf. *Transactions of the Royal Society of New Zealand, 84*(2), 309 – 328.

Cejas, J. R., Almansa, E., Villamandos, J. E., Badía, P., Bolaños, A., & Lorenzo, A. (2003). Lipid and fatty acid composition of ovaries from wild fish and ovaries and eggs from captive fish of white sea bream (*Diplodus sargus*). *Aquaculture, 216,* (299-313). doi:10.1016/S0044-8486(02)00525-2

Cerrato, R, M. (1990). Interpretable statistical tests for growth comparisons using parameters in the von Bertalanffy equation. *Canadian Journal of Fisheries and Aquatic Science 47*, 1416–1426.

Chiba, S. N., Iwatsuki, Y., Yoshino, T., & Hanzawa, N. (2009). Comprehensive phylogeny of the family Sparidae (Perciformes: Teleostei) inferred from mitochondrial gene analyses. *Genes & Genetic Systems, 84*(2), 153.

Choat, J. H., Robertson, D. R., Ackerman, J. L., & Posada, J. M. (2003). An age-based demographic analysis of the Caribbean stoplight parrotfish *Sparisoma viride*. *Marine Ecology Progress Series, 246,* 265-277.

Christie, W. W., and Han, X. (2010). *Lipid Analysis - Isolation, Separation, Identification and Lipidomic Analysis (4th edition*). Somerset, England: The Oily Press.

Copeman, L., Parrish, C., Brown, J., & Harel, M. (2002). Effects of docosahexaenoic, eicosapentaenoic, and arachidonic acids on the early growth, survival, lipid composition and pigmentation of yellowtail flounder (*Limanda ferruginea*): a live food enrichment experiment. *Aquaculture, 210,* 285-304.

Crossland, J. (1980). The number of snapper, *Chrysophrys auratus* (Forster), in the Hauraki Gulf, New Zealand, based on egg surveys in 1974-75 and 1975-76. *Fisheries Research Bulletin-Ministry of Agriculture and Fisheries (New Zealand). no. 22.*

Crossland, J. (1981). The biology of the New Zealand snapper. *Fisheries Research Division: Occasional Publication No. 23*. Wellington, New Zealand: Ministry of Agriculture and Fisheries.

Denslow, N., & Sepulveda, M. (2007). In: Babin, P. J., Cerdà, J., & Lubzens, E. (Eds.). (2007). *The fish oocyte: from basic studies to biotechnological applications*. Dordrecht, Netherlands: Springer.

Donelson, J. M., McCormick, M. I., & Munday, P. L. (2008). Parental condition affects early life-history of a coral reef fish. *Journal of Experimental Marine Biology and Ecology*, *360*(2), 109-116.

Dutney, L., Elizur, A., & Lee, P. (2016). Analysis of sexually dimorphic growth in captive reared cobia (*Rachycentron canadum*) and the occurrence of intersex individuals. *Aquaculture, In press*.

Fallah, A. A., Siavash Saei-Dehkordi, S., & Nematollahi, A. (2011). Comparative assessment of proximate composition, physicochemical parameters, fatty acid profile and mineral content in farmed and wild rainbow trout (*Oncorhynchus mykiss*). *International Journal of Food Science & Technology*, *46*(4), 767-773.

Fitzhugh, G. R., Shertzer, K. W., Kellison, G. T., & Wyanski, D. M. (2012). Review of size- and age-dependence in batch spawning: Implications for stock assessment of fish species exhibiting indeterminate fecundity. *Fishery Bulletin, 110*(4), 413. *Freshwater Research, 39*(5), 625. doi:10.1071/MF9880625

Francis, R. I. C. (1988). Are growth parameters estimated from tagging and age-length data comparable? *Canadian Journal of Fisheries and Aquatic Sciences 45*, 936–942.

Francis, M. (1994a). Duration of larval and spawning periods in *Pagrus auratus* (Sparidae) determined from otolith daily increment. *Environmental Biology of Fishes, 39*(2), 137 – 152.

Francis, M. (1994b). Growth of juvenile snapper, *Pagrus auratus*. *New Zealand Journal of Marine & Freshwater Research, 28*(2), 201. doi:10.1080/00288330.1994.9516608

Francis, M. (1995). Spatial and seasonal variation in the abundance of juvenile snapper (*Pagrus auratus*) in the north-western Hauraki gulf. *New Zealand Journal of Marine and Freshwater Research, 29*(4), 565-579.

Francis, R., Paul, L., & Mulligan, K. (1992). Ageing of Adult Snapper (*Pagrus auratus*) from Otolith annual ring counts: Validation by tagging and Oxytetracycline Injection. *Marine and Freshwater Research, 43*(5), 1069.

Froese, R. (2006). Cube law, condition factor and weight–length relationships: history, meta-analysis and recommendations. *Journal of Applied Ichthyology, 22*(4), 241-253.

Froese, R. & Pauly, D. (2005). *Fishbase.* Retrieved October 19, 2016, from: http://www.fishbase.org/PopDyn/LWRelationshipList.php?ID=6426&GenusName=Pagrus&SpeciesName=auratus&fc=330

Fyhn, H. J. (1989). First feeding of marine fish larvae: are free amino acids the source of energy? *Aquaculture, 80*(1), 111-120.

Garratt, P. A., Govender, A & Punt, A. E. (1993). Growth acceleration at sex change in the protogynous hermaphrodite *Chrysoblephus puniceus* (Pisces: Sparidae). *South African Journal of Marine Science*, *13*(1), 187-193.

Gilbert, D. J., & McKenzie, J. R. (1999). *Sources of bias in biomass estimates from tagging programmes in the SNA1 snapper (Pagrus auratus) stock.* New Zealand Fisheries Assessment Research Document No. 16. Wellington, New Zealand: Ministry of Fisheries.

Gladyshev, M. I., Sushchik, N. N., & Makhutova, O. N. (2013). Production of EPA and DHA in aquatic ecosystems and their transfer to the land. *Prostaglandins & other lipid mediators*, *107*, 117-126.

Godfriaux, B. L. (1969). Food of predatory demersal fish in Hauraki Gulf: 1: Food and feeding habits of snapper. *New Zealand Journal of Marine and Freshwater Research*, *3*(4), 518-544.

Giraldo, C., Mayzaud, P., Tavernier, E., Irisson, J. O., Penot, F., Becciu, J., ... & Koubbi, P. (2013). Lipid components as a measure of nutritional condition in fish larvae (*Pleuragramma antarcticum*) in East Antarctica. *Marine biology*, *160*(4), 877-887.

Green, B. S., & McCormick, M. I. (2005). Maternal and paternal effects determine size, growth and performance in larvae of a tropical reef fish. *Marine Ecology Progress Series*, *289*, 263-272.

Grier, H. (1981). Cellular Organization of the Testis and Spermatogenesis in Fishes. *Intergrative and Comparative Biology 21*(2), 345-357.

Griffiths, M. H., Wilke, C., Penney, A. J., & Melo, Y. (2002). Life history of white stumpnose *Rhabdosargus globiceps* (Pisces: Sparidae) off South Africa. *South African Journal of Marine Science*, *24(*1), 281. doi:10.2989/025776102784528394

Grote, B., Hagen, W., Lipinski, M. R., Verheye, H. M., Stenevik, E. K., & Ekau, W. (2011). Lipids and fatty acids as indicators of egg condition, larval feeding and maternal effects in Cape hakes (*Merluccius paradoxus* and *M. capensis*). *Marine Biology*, *158*(5), 1005-1017.

Hamer, P. A., & Jenkins, G. P. (2004). High levels of spatial and temporal recruitment variability in the temperate sparid *Pagrus auratus*. *Marine and Freshwater Research*, *55*(7), 663.

Harris, T. F. W. (1985). North Cape to East Cape: Aspects of the Physical Oceanography. *Leigh Laboratory Bulletin, 28.*

Harwood, J. L., Gurr, M. I., Michell, R.H., Murphy, D. J., & Frayn, K. N. (2016). *Lipids: Biochemistry, biotechnology and health (6th edition).* Sussex, England: Wiley.

Hartill, B., Bian, R., Rush, N., & Armiger, H. (2013). *Aerial-access recreational harvest estimates for snapper, kahawai, red gurnard, tarakihi and trevally in FMA 1 in 2011-2012*. Wellington: Ministry for Primary Industries.

Hauser, L., Adcock, G. J., Smith, P. J., & Carvalho, G. R. (2002). Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (*Pagrus auratus*). *Proceedings of the National Academy of Sciences of the United States of America, 99*(18), 11742-11747.

Hilton, Z., Poortenaar, C. W., & Sewell, M. A. (2008). Lipid and protein utilisation during early development of yellowtail kingfish (*Seriola lalandi*). *Marine Biology, 154*(5), 855-865. doi:10.1007/s00227-008-0978-z

Hiramatsu, N., Todo, T., Sullivan, C. V., Schilling, J., Reading, B. J., Matsubara, T., & ... Hara, A. (2015). Ovarian yolk formation in fishes: Molecular mechanisms underlying formation of lipid droplets and vitellogenin-derived yolk proteins. General and Comparative Endocrinology, 221, 9-15. doi: 10.1016/j.ygcen.2015.01.025

Houston, A. I., Stephens, P. A., Boyd, I. L., Harding, K. C., & McNamara, J. M. (2007). Capital or income breeding? A theoretical model of female reproductive strategies. *Behavioural Ecology, 18*(1), 241-250.

Hussain, A., Shakir, H. A., Ali, S., & Qazi, J. I. (2015). Growth coefficient and fecundity of *Chitala chitala* (Osteoglossiformes: Notopteridae) from the river Ravi, Pakistan. *Journal of Animal & Plant Sciences, 25*(2), 401.

Jackson, G., Norriss, J. V., Mackie, M. C., & Hall, N. G. (2010) Spatial variation in life history characteristics of snapper (*Pagrus auratus*) within Shark Bay, Western Australia. *New Zealand Journal of Marine and Freshwater Research, 44*(1), 1-15. doi: 10.1080/00288331003641646

Jaya-ram A, Kuah M, Lim P, Kolkovski S & Shu-Chien A. (2008). Influence of dietary HUFA levels on reproductive performance, tissue fatty acid profile and desaturase and elongase mRNAs expression in female zebrafish (*Danio rario). Aquaculture, 277* (3-4). 275-281.

Jin, S., Yan, X., Zhang, H., & Fan, W. (2015). Weight–length relationships and Fulton's condition factors of skipjack tuna (*Katsuwonus pelamis*) in the western and central Pacific Ocean. *PeerJ, 3*, e758.

Johnson, R. (2009). Lipid deposition in oocytes of teleost fish during secondary oocyte growth. *Reviews in Fisheries Science, 17*(1), 78-89. doi:10.1080/10641260802590004.

Kaitaranta, J. K., & Ackman, R. G. (1981). Total lipids and lipid classes of fish roe. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, 69*(4), 725-729.

Karachle, P. K., & Stergiou, K. I. (2012). Morphometrics and allometry in fishes. *INTECH: Open Access Publisher.*

Karakulak, F. S., Erk, H., & Bilgin, B. (2006). Length–weight relationships for 47 coastal fish species from the northern Aegean Sea, Turkey. *Journal of Applied Ichthyology, 22*(4), 274.

Kimura, D. K. (1980). Likelihood methods for the von Bertalanffy growth curve. *Fishery Bulletin 77*, 765–776.

Leach, F. (2006). *Fishing in pre-European New Zealand*. *Journal of Archaeology Special Publication, 15*. Wellington, New Zealand: Journal of Archaeology and Archaeofauna.

Lenas, D. S., Triantafillou, D. J., Chatziantoniou, S., & Nathanailides, C. (2011). Fatty acid profile of wild and farmed gilthead sea bream (Sparus aurata). *Journal für Verbraucherschutz und Lebensmittelsicherheit*, *6*(4), 435-440.

Mackie, M., Jackson, G., Tapp, N., Norriss, J. and Thomson, A. (2009). Macroscopic and microscopic description of snapper (*Pagrus auratus*) gonads from Shark Bay, Western Australia. *Fisheries Research Report No. 184*. Department of Fisheries, Western Australia.

Marteinsdóttir, G., & Steinarsson, A. (1998). Maternal influence on the size and viability of Iceland cod *Gadus morhua* eggs and larvae. *Journal of Fish Biology*, *52*(6), 1241-1258.

Martin, J. L. (2009). *Investigating maternal effects in a batch spawning teleost.* Unpublished MSc thesis. Auckland: University of Auckland.

Matthias, B., Ahrens, R., Allen, M., Lombardi-Carlson, L., & Fitzhugh, G. (2016). Comparison of growth models for sequential hermaphrodites by considering multi-phasic growth. *Fisheries Research, 179*, 67-75.

McBride, R. S., Somarakis, S., Fitzhugh, G. R., Albert, A., Yaragina, N. A., Wuenschel, M. J., & ... Basilone, G. (2015). Energy acquisition and allocation to egg production in

relation to fish reproductive strategies. *Fish & Fisheries*, 16(1), 23. doi:10.1111/faf.12043

Meekan, M., & Fortier, L. (1996). Selection for fast growth during the larval life of Atlantic cod G*adus morhua* on the Scotian shelf. *Marine Ecology Progress Series, 137*, 25-37. doi:10.3354/meps137025

Ministry for Primary Industries. (2013). *Fisheries Assessment Plenary, May 2013: stock assessments and yield estimates*. Compiled by the Fisheries Science Group, Ministry for Primary Industries, Wellington, New Zealand: Author.

Ministry for Primary Industries. (2014). *Fisheries Assessment Plenary, May 2014: stock assessments and stock status*. Wellington, New Zealand: Author.

Mourente, G., Megina, C., & Díaz-Salvago, E. (2002). Lipids in female northern bluefin tuna (*Thunnus thynnus L*.) during sexual maturation. Fish Physiology & Biochemistry, 24(4), 351. doi:10.1023/A:1015011609017

Murzina, S. A., Ottesen, C. A. M., Falk-Petersen, S., Hop, H., Nemova, N. N., & Poluektova, O. G. (2012). Oogenesis and lipids in gonad and liver of daubed shanny (*Leptoclinus maculatus*) females from Svalbard waters. *Fish physiology and biochemistry*, *38*(5), 1393-1407.

Naz, S., Javed, M., & Tahir., A. (2013). A Study on Length-Weight Relationships (LWR) and growth responses of major carps exposed to lead (Pb). *Journal of Biology, Agriculture and Healthcare*, *3*(19).

Norton, E. C., MacFarlane, R. B., & Mohr, M. S. (2001). Lipid class dynamics during development in early life stages of shortbelly rockfish and their application to condition assessment. *Journal of Fish Biology*, *58*(4), 1010-1024. doi:10.1111/j.1095-8649.2001.tb00551.

Osman, H., Suriah, A. R., & Law, E. C. (2001). Fatty acid composition and cholesterol content of selected marine fish in Malaysian waters. *Food chemistry*, *73*(1), 55-60.

Osse, J. W. M., van den Boogaart, J. G. M., van Snik, G. M. J., & van der Sluys, L. (1997). Priorities during early growth of fish larvae. *Aquaculture, 155*(1), 249-258.

Özogul, Y., Özogul, F. H., Çi˙ çek, E., Polat, A., & Kuley, E. (2009). Fat content and fatty acid compositions of 34 marine water fish species from the Mediterranean Sea. *International journal of food sciences and nutrition*, *60*(6), 464-475.

Pajuelo, J., & Lorenzo, J. (2004). Basic characteristics of the population dynamic and state of exploitation of Moroccan white seabream *Diplodus sargus cadenati* (Sparidae) in the Canarian archipelago. *Journal of Applied Ichthyology*, *20*(1), 15-21.

Pajuelo, J., Socorro, J., Gonzalez, J., Lorenzo, J., Perez-Penalvo, J., Martinez, I., & Hernandez-Cruz, C. (2006). Life history of the red-banded seabream *Pagrus auriga* (Sparidae) from the coasts of the Canarian archipelago. *Journal of Applied Ichthyology,22*(5), 430-436.

Palumbi, S. R. (2004). Fisheries science: why mothers matter. *Nature, 430* (7000), 621-622.

Pankhurst, P. M. (1994). Age-related changes in the visual acuity of larvae of New Zealand snapper, *Pagrus auratus*. *Journal of the Marine Biological Association of the United Kingdom, 74*(2), 337-349.

Parker, G. A. (1992). The evolution of sexual size dimorphism in fish. *Journal of Fish Biology*, *41*, 1-20.

Parsons, D., Babcock, R., Hankin, R., Willis, T., Aitken, J., O'Dor, R., & Jackson, G. (2003). Snapper *Pagrus auratus* (Sparidae) home range dynamics: Acoustic tagging studies in a marine reserve. *Marine Ecology Progress Series, 262*, 253-265.

Parsons, D., Morrison, M., Paul, L., Radford, C., Ross, P., Spong, K., . . . & McKenzie, J. (2014). Snapper (*Chrysophrys auratus*): A review of life history and key vulnerabilities in New Zealand. *New Zealand Journal of Marine and Freshwater Research, 48*(2), 256-283. doi:10.1080/00288330.2014.892013

Parsons, D. M., Middleton, C., Spong, K. T., Mackay, G., Smith, M. D., & Buckthought, D. (2015). Mechanisms explaining nursery habitat association: How do juvenile snapper (*Chrysophrys auratus*) benefit from their nursery habitat? *PloS One, 10*(3), e0122137. doi:10.1371/journal.pone.0122137

Parsons, D. M., Morrison, M. A., Thrush, S. F., Middleton, C., Smith, M., Spong, K. T., & Buckthought, D. (2013). The influence of habitat structure on juvenile fish in a New Zealand estuary. *Marine Ecology, 34*(4), 492-500. doi:10.1111/maec.12050

Parsons, D. M., Buckthought, D., Middleton, C., & MacKay, G. (2016). Relative abundance of snapper (*Chrysophrys auratus*) across habitats within an estuarine system. *New Zealand Journal of Marine and Freshwater Research, 50*(3), 358-370. doi: 10.1080/00288330.2016.1146310

Patnaik, B. K., Mahapatro, N., & Jena, B. S. (1994). Ageing in fishes. *Gerontology, 40*(2-4), 113-132.

Paul, L. J. (1976). A study on age, growth, and population structure of the snapper, *Chrysophrys auratus* (Forster), in the Hauraki Gulf, New Zealand. *Fisheries Research Bulletin No. 13*. 62 p.

Paul. L. J. (2014). History of, and trends in, the commercial landings of finfish from the Hauraki Gulf, 1850−2006. *New Zealand Aquatic Environment and Biodiversity Report No. 124.* Wellington, New Zealand: Ministry for Primary Industries.

Paulin, C. (1990). *Pagrus auratus*, a new combination for the species known as "snapper" in Australasian waters (Pisces: Sparidae). *New Zealand Journal of Marine and Freshwater Research, 24*(2), 259-265.

Polunin, N. V. C.; Graham N. A. J. (2003). Review of the impacts of fishing on coral reef fish populations. *Western Pacific Management Council*, 48pp.

Pottiez, G. (2015). *Mass spectrometry: developmental approaches to answer biological questions*. Oxford, England: Springer.

R Development Core Team. (2016) R: A language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna: Austria.

Raventos, N., & Planes, S. (2008). Maternal size effects on early life traits of the temperate fish *Symphodus roissali*. *Aquatic Biology, 4*(1), 1-6.

Rickter, W. E. (1973). Linear regressions in fisheries research. *Journal of the Fisheries Research Board of Canada 30,* 409–434.

Robinson, E., Jerrett, A. R., Black, S. E., & Davison, W. (2011). Visual acuity of snapper *Pagrus auratus*: Effect of size and spectral composition. *Journal of Fish Biology, 79*(7), 1883-1894. doi:10.1111/j.1095-8649.2011.03130.x

Roff, D. A. (1983). An allocation model of growth and reproduction in fish. *Canadian Journal of Fisheries and Aquatic Sciences, 40*(9), 1395-1404.

Ronnestad, I., Fyhn, H. J., & Gravningen, K. (1992). The importance of free amino acids to the energy metabolism of eggs and larvae of turbot (*Scophthalmus maximus*). *Marine biology, 114*(4), 517-525.

Rose, G. A., & O'driscoll, R. (2002). Capelin are good for cod: can the northern stock rebuild without them? *ICES Journal of Marine Science: Journal du Conseil, 59*(5), 1018-1026.

Ross, R. M. (1990). The evolution of sex-change mechanisms in fishes. *Environmental Biology of Fishes, 29*(2), 81-93.

Rowley, A., Knight, J., Lloyd-Evans, P., Holland, J., & Vickers, P. (1995). Eicosanoids and their role in immune modulation in fish – a brief overview. *Fish & Shellfish Immunology, 5*(8), 549-567.

Ruiz-Gutiérrez, V., & Barron, L. J. (1995). Methods for the analysis of triacylglycerols. *Journal of Chromatography. B, Biomedical Applications, 671*(1-2), 133.

Salze, G., Tocher, D. R., Roy, W. J., & Robertson, D. A. (2005). Egg quality determinants in cod (*Gadus morhua L.*): Egg performance and lipids in eggs from farmed and wild broodstock. *Aquaculture Research, 36*(15), 1488-1499.

Sargent, J. R., Bell, J. G., Bell, M. V., Henderson, R. J., & Tocher, D. R. (1993). The metabolism of phospholipids and polyunsaturated fatty acids in fish. *Aquaculture: Fundamental and Applied Research*, 103-124.

Sargent, J., Bell, G., McEvoy, L., Tocher, D., & Estevez, A. (1999a). Recent developments in the essential fatty acid nutrition of fish. *Aquaculture, 177*(1), 191-199.

Sargent, J., McEvoy, L., Estevez, A., Bell, G., Bell, M., Henderson, J., & Tocher, D. (1999b). Lipid nutrition of marine fish during early development: current status and future directions. *Aquaculture, 179*, 217-229. doi: 10.1016/S0044-8486(99)00191-X

Sargent, J. R. (1995). Origins and functions of lipids in fish eggs: nutritional implications pp. 353-372. In: Bromage, N. R., Roberts R. R. (eds), *Broodstock Management and Egg and Larval Quality*. Blackwell Science, Oxford: England.

Sargent, J. R., Bell, J. G., Bell, M. V., Henderson, R. J., & Tocher, D. R. (1995). Requirement criteria for essential fatty acids. *Journal of applied Ichthyology, 11*(3-4), 183-198.

Sargent, J. R., Tocher, R. D., & Bell, G. (2002). *The Lipids*. Pp 182 – 246. In: Halver, J. E., & Hardy, R., W. (eds), *Fish nutrition*. Academic Press, California, USA.

Sawaboonchun J. 2009. *Atlantic cod (Gadus morhua L.) broodstock nutrition: the role of arachidonic acid and astaxanthin as determinants of egg quality.* Unpublished MSc Thesis, Institute of Aquaculture, University of Stirling, Scotland.

Scott, S. G., & Pankhurst, N. W. (1992). Interannual variation in the reproductive cycle of the New Zealand snapper *Pagrus auratus* (Bloch & Schneider) (Sparidae). *Journal of Fish Biology, 41*(5), 685.

Scott S., G., Zeldis J., R., & Pankhurst., N., W. (1993). Evidence of daily spawning in natural populations of the New Zealand snapper *Pagrus auratus* (Sparidae). *Environmental Biology of Fishes, 36*(2), 149 – 156.

Shears, N., & Babcock, R. C. (2002). Marine reserves demonstrate top-down control of community structure on temperate reefs. *Oecologia, 132*(1), 131-142. doi:10.1007/s00442-002-0920-x

Sheridan, M. A. (1988). Lipid dynamics in fish: aspects of absorption, transportation, deposition and mobilization. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, 90*(4), 679-690.

Silversand, C., & Haux, C. (1997). Improved high-performance liquid chromatographic method for the separation and quantification of lipid classes: application to fish lipids. *Journal of Chromatography. B, Biomedical Sciences and Applications, 703*(1-2), 7-14.

Smith, P. J. (1986). Spawning behaviour of snapper, *Chrysophrys auratus*, in captivity (note). *New Zealand Journal of Marine & Freshwater Research, 20*(3), 513.

Sim-Smith, C. J., Jeffs, A. G., & Radford, C. A. (2012). Variation in the growth of larval and juvenile snapper, *Chrysophrys auratus* (Sparidae). *Marine and Freshwater Research* 63, 1231–1243.

Sim-Smith, C. J., Jeffs, A. G., & Radford, C. A. (2013a). Environmental influences on the larval recruitment dynamics of snapper, *Chrysophrys auratus* (Sparidae). *Marine and Freshwater Research, 64*(8), 726. doi:10.1071/MF12255

Sim-Smith, C. J., Jeffs, A. G., & Radford, C. A. (2013b). Balancing the odds: the relationship between growth and energy storage in juvenile snapper (*Chrysophrys auratus*: Sparidae). *Marine & Freshwater Research*, (11).

Sogard, S. M., Berkeley, S. A., & Fisher, R. (2008). Maternal effects in rockfishes Sebastes spp.: a comparison among species. *Marine Ecology Progress Series, 360*, 227-236.

Statistics New Zealand. (n.d). *Fish monetary stock account: 1996 – 2009*. Retrieved August 17, 2015, from http://www.stats.govt.nz/browse_for_stats/environment/environmental-economic-accounts/fish-monetary-stock-account-1996-2009/results.aspx

Stephens, P. A., Boyd, I. L., McNamara, J. M., & Houston, A. I. (2009). Capital breeding and income breeding: their meaning, measurement, and worth. *Ecology*, *90*(8), 2057-2067.

Tocher, D. R. (2003). Metabolism and functions of lipids and fatty acids in teleost fish. *Reviews in fisheries science, 11*(2), 107-184.

Usmar, N. (2012). Ontogenetic diet shifts in snapper (*Pagrus auratus*: Sparidae) within a New Zealand estuary. *New Zealand Journal of Marine and Freshwater Research, 46*(1), 31-16.

Varpe, O., Jorgensen, C., Tarling, G. A., & Fiksen, O. (2009). The adaptive value of energy storage and capital breeding in seasonal environments. *Oikos*, *118*(3), 363-370.

Vincent, A., Sadovy, Y. (1998). Reproductive ecology in the conservation and management of fishes. In: *Behavioural Ecology and Conservation Biology (Ed T. Caro)*. Oxford University Press, New York, pp 209-245.

V'uorela, R., Kaitaranta, J., & Linko, R. R. (1979). Proximate composition of fish roe in relation to maturity. *Canadian Institute of Food Science and Technology Journal*, *12*(4), 186-188.

Walsh C., McKenzie J. A., & Armiger H. (2006). *Spatial and temporal patterns in snapper length and age composition and movement, west coast North Island, New Zealand*. New Zealand Fisheries Assessment Report No. 6. Wellington, New Zealand: Ministry of Fisheries.

Walsh, C., Buckthought, D., McKenzie, J, A. (2011a). *Length and age composition of commercial snapper landings in SNA8, 2009–10*. New Zealand Fisheries Assessment Report No. 16. Wellington, Ministry of Fisheries. 27 p.

Walsh, C., McKenzie, J, A., Buckthought, D., Armiger, H., Ferguson, H., Smith, M., Spong, K., & Miller, A. (2011b). Age composition of commercial snapper landings in SNA1, 2009–10. *New Zealand Fisheries Assessment Report No. 54*. Wellington, Ministry of Fisheries.

Walsh, C., Horn, P., McKenzie, J., Ó Maolagáin, C., Buckthought, D., Sutton, C., & Armiger, H. (2014). Age determination protocol for snapper (*Pagrus auratus*). *New Zealand Fisheries Assessment Report 2014/51. 33 p*.

Warner, R. R. (1984). Mating Behaviour and Hermaphroditism in Coral Reef Fishes: The diverse forms of sexuality found among tropical marine fishes can be viewed as adaptations to their equally diverse mating systems. *American Scientist, 72*(2), 128 - 136.

Watanabe, T. (1993). Importance of docosahexaenoic acid in marine larval fish. *Journal of the World Aquaculture Society, 24*(2), 152-161.

West, G. (1990). Methods of Assessing Ovarian development in Fishes: a Review. *Australian Journal of Marine and Freshwater Research 41*(2), 199–222.

Wiegand, M. D. (1996). Composition, accumulation and utilization of yolk lipids in teleost fish. *Reviews in Fish Biology and Fisheries, 6*(3), 259-286.

Willis, T. J., Millar, R. B., & Babcock, R. C. (2003). Protection of exploited fish in temperate regions: High density and biomass of snapper *Pagrus auratus* (Sparidae) in Northern New Zealand marine reserves. *Journal of Applied Ecology, 40*(2), 214-227.

Wilson C.A., Dean J.M., Prince E.D., Lee D.W. (1991). An examination of sexual dimorphism in Atlantic and Pacific blue marlin using body weight, sagittae weight, and age estimates. *Journal of Experimental Marine Biology and Ecology, 151*, 209–225.

Wixom, R. L., & Gehrke, C. W. (2010). *Chromatography: a science of discovery.* Hoboken, N.J.: Wiley.

Zeldis, J. R., Oldman, J., Ballara, S. L., & Richards, L. A. (2005). Physical fluxes, pelagic ecosystem structure, and larval fish survival in Hauraki Gulf, New Zealand. *Canadian Journal of Fisheries and Aquatic Sciences, 62*(3), 593-593.

Zeldis J.R, & Francis R.I.C.C. (1998). A daily egg production method estimate of snapper biomass in Hauraki Gulf, New Zealand. *ICES Journal of Marine Science, 55*(3), 522-522. doi:10.1006/jmsc.1997.0277

Zudaire, I., Murua, H., Grande, M., Pernet, F., & Bodin, N. (2014). Accumulation and mobilization of lipids in relation to reproduction of yellowfin tuna (Thunnus albacares) in the Western Indian Ocean. *Fisheries Research, 160 (Advances in Fisheries Research in Ibero-America),* 50-59.