Diet, nutrition, and growth in the temperate rocky reef fish *Girella tricuspidata* (Girellidae)

Tabea Salewski

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Abstract

This thesis investigated the relationship between nutritional ecology and growth in the marine reef fish *Girella tricuspidata* (Girellidae) found in northern New Zealand and temperate eastern Australia. The aim was to establish how diet, nutrition, and temperature affect the demography of the species/population, and in particular to test two hypotheses. The Temperature-Constraint Hypothesis predicts that digestion of algal foods in marine herbivorous fish is constrained by temperature at higher latitudes, and therefore restricts growth, whereas the Temperature-Size Rule predicts that temperature determines growth rate. This thesis investigated the effects of changes in diet and nutrition on growth and longevity between two populations of *Girella tricuspidata*, and how diet and nutrient intake were scheduled across the year in relation to growth and reproduction. The aim was also to identify the strategies omnivorous fishes on a mainly herbivorous diet employ to survive on a diet considered nutritionally poor and difficult to digest. Spatial differences in growth between populations were analysed to examine the effects of nutrition and temperature on growth.

Two populations (coastal and offshore, about 50 km apart) were sampled in the Outer Hauraki Gulf in north-eastern New Zealand. Diet analysis of stomach content samples revealed an omnivorous diet, with fish ingesting a wide array of food items. The diet consisted mainly of small epiphytic Rhodophyta, *Abroteia suborbicularis* in particular, complemented by a considerable amount of animal material. Epiphytes on *Carpophyllum maschalocarpum* varied in abundance seasonally, but the pattern differed between two coastal sampling locations, indicating that epiphytes are available throughout the year. *Ulva* species (Chlorophyta), which are ususally considered to be the preferred food, were also ingested but were not a dominant dietary item. Seasonal changes in diet compositions were mainly due to the seasonal appearance of salps, which formed a major part of the diet between spring and autumn. Salps appeared slightly earlier in stomach contents of fish offshore, where abundances peaked earlier in the season due to the hydrology of the Hauraki Gulf. When salps were not available fish increased their intake of other animal matter such as crustaceans.

Nutrient analysis (carbon, nitrogen, lipid, ash) of stomach contents revealed that diet items differed in nutrient compositions and that diet composition varied between locations and seasons. However *G. tricuspidata* mixed diet items so that the nutrient composition remained nearly constant throughout the year and was similar for both populations. The condition factor based on gutted weight remained nearly constant throughout the year, but increased slightly in spring based on the

total weight, a pattern associated with gonad development. Spawning peaked in December. Nutrient demand was expected to increase during spawning time in spring, but relative gut content mass and nutrient composition remained constant in both populations, indicating consistent nutrient intake throughout the year. Intake of salps, which contain high lipid levels needed for reproduction, increased during spawning time. Relative gut length varied between seasons in both populations, and coastal fish had longer guts in relation to body length than offshore fish.

G. tricuspidata is a long-lived temperate reef fish that displays an asymptotic growth pattern. Annual increments in sagittal otoliths revealed 54 years of age for the oldest fish, an offshore specimen, representing the maximum-recorded age reported for *G. tricuspidata*. The oldest fish caught from the coastal population was 44 years. Otolith chronologies showed that increment widths reflected increased growth during warmer years, while colder years resulted in increment widths narrower than average. Growth increment width indices correlated strongly with summer sea surface temperatures. There was a significant difference in growth rate between the coastal and offshore populations, but not between sexes. Calculations of the reparameterized von Bertalanffy growth function parameters showed that coastal fish grew faster as juveniles. Population growth curves crossed at about seven years. Coastal fish reached their adult size at about 13 years and offshore fish at 18 years. Coastal fish had smaller mean adult sizes (291.5 mm SL) than offshore fish (326.3 mm SL). The determining factor was most likely microhabitat. Juvenile coastal fish spend their first two years exclusively inside Whangateau Harbour before moving to the open coast, and adult fish also spend time inside the Harbour. Summer sea surface temperatures are about 2.9°C warmer inside Whangateau Harbour than along the coast and at the offshore location.

This study suggests that *G. tricuspidata* selectively feed on protein-rich algae and also employ a complementary feeding strategy gaining the majority of their energy from epiphytic algae. Epiphytic algae are complemented with animal matter rich in protein and lipid. Data on resource availability, nutrition, and digestion was inconsistent with the Temperature-Constraint Hypothesis. Rather, temperature variation influenced growth variation over the spatial scale of the study, and temperature differences between habitats were most likely the factor driving observed growth variation between the two populations, thereby supporting the Temperature-Size Rule.

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Glossary

Age	referred to in this thesis as the estimated age from otolith reading
ANCOVA	analysis of covariance
ANOSIM	analysis of similarity
ANOVA	analysis of variance
ARSTAN	dendrochronology software for chronology development, plotting, and analysis
C	carbon
C:N	carbon:nitrogen ratio
CI	confidence interval
COFECHA	dendrochronology software for dating and measurement control
df	degrees of freedom
diet category	diet items summarized into diet categories as described in 2.2.2 Diet analysis
diet item	diet item of stomach contents identified to species level
DW	dry weight
ENSO	El Niño/Southern Oscillation
FL	fork length
g	gram
GW	gutted weight
GSI	Gonadosomatic Index
HPLC	high performance liquid chromatography
IL	intestine length
kg	kilogram
K_{GW}	condition factor based on gutted weight of the fish
km	kilometre
K_{TW}	condition factor based on total weight of the fish
$L_{(0)}$	size at settlement
L_{∞}	asymptotic length
M	mean
MEI	Multivariate El Niño/Southern Oscillation Index
mg	milligram
min	minute/s
mm	millimetre

MS	mean sum of squares
N	nitrogen
n	variable quantity
NOAA	National Oceanic and Atmospheric Administration
µl	microliter
p	significance level of statistic test
PCO	principal coordinate analysis
PERMANOVA	permutational multivariate analysis of variance
PERMDISP	tests the homogeneity of multivariate dispersions within groups
PRIMER	software to analyse ecological data
Pseudo-F	F value by permutation
R	strength of the factors in the sample
RGL	relative gut length
rVBGF	reparameterized von Bertalanffy growth function
SCFA	short chain fatty acid
SD	standard deviation
SE	standard error
SL	standard length
SS	sum of squares
SST	sea surface temperature
TCH	Temperature-Constraint Hypothesis
THAA	total hydrolysable amino acid
TL	total length
T_{max}	longevity of oldest fish (years)
$T_{max10\%}$	mean of longevity of oldest 10% of fish in the sample (years)
$T_{max\ 25\%}$	mean of longevity of oldest 25% of fish in the sample (years)
TSR	Temperature-Size Rule
TW	total weight
VBGF	von Bertalanffy growth function
WW	wet weight
yrs	years
ZI	Zihler Index

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Name	Nature of Contribution
Prof. Kendall D. Clements	Discussion about experimental design and analysis, reviewed chapter draft
Dr. Richard B. Taylor	Discussion about experimental design and analysis, reviewed chapter draft

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Name	Signature	Date
Kendall Clements		22/12/16
Richard Taylor		21/12/16

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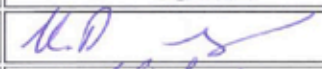
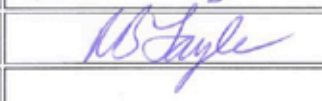
Kendall Clements		22/12/16
Richard Taylor		21/12/2016

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Richard Taylor		21/12/2016

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Chapter 1

General Introduction

Ecology is the study of how organisms interact with their physical and biological environments. Environmental factors are subject to change and influence the life history (patterns of survival and reproduction) of the organism. The life history of fishes is heavily influenced by their environment. Most fishes are ectothermic poikilotherms. As such, they cannot regulate their own body temperature and rely on external heat sources, and their body temperature conforms to that of the ambient temperature (Levinton, 2001). Fluctuating temperatures affect metabolic rate and life history traits such as growth rate, size- and age-at-maturity, timing and success of reproduction, final body size, ageing, and longevity (Bureau, Kaushik, & Cho, 2002; A. Clarke & Johnston, 1999; Pörtner, 2002). Life history traits thus reflect the process of adaptation of organisms to their natural environment to achieve reproductive success (Sala-Bozano & Mariani, 2011; Stearns, 2000). Organisms have to trade-off limited resources (e.g. energy, time, and essential nutrients) between life history traits such as growth, maintenance (survival), and reproduction (Stearns, 2000). Distinct populations living in heterogeneous habitats offer the opportunity to identify the cause of differences between these traits (Sala-Bozano & Mariani, 2011) and their influence on demography. However an understanding of basic life history is lacking for many species and even families. Ecological generalists provide good models to test hypotheses on the relationships between life history traits and environmental variation, and the omnivorous parore *Girella tricuspidata* (family Girellidae) will be used to examine this question in the present study.

1.1 Diet, food choice, and nutritional ecology in herbivorous fish

The diet of animals varies widely in terms of the type and range of foods they consume. These foods are used to classify them into trophic categories, namely, carnivore, herbivore, detritivore, and omnivore. Definitions of these terms may vary widely and thus make classification difficult (Clements & Raubenheimer, 2006; Clements, Raubenheimer, & Choat, 2009). Marine herbivorous fishes ingest a wide range of primary producers including algae, diatoms, cyanobacteria, seagrass, and phytoplankton (Horn, 1989). Species diversity of herbivorous fish is generally highest in the tropics and decreases towards the poles (Choat, 1991; Ebeling & Hixon, 1991; Floeter, Ferreira, Dominici-Arosemena, Zalmon, & Ferreira, 2004), although this varies from region to region (Clements et al., 2009). The ecology of herbivorous fishes in tropical regions has been studied extensively, but temperate regions have received less attention (Choat & Clements, 1998). Herbivorous fishes are important components of the reef ecosystem and coral reefs can yield 50-100% of their daily production to herbivores (Burkepile & Hay, 2008; Carpenter, 1986; Hatcher & Larkum, 1983; Hay, 1991). By feeding on autotrophs the fixed energy is released to higher trophic levels and the cycling

of nutrients is improved (Burkepile & Hay, 2006; Choat, 1991; Horn, 1989). Herbivorous fishes can affect the structure of plant communities by altering their distribution, abundance, and diversity (Belliveau & Paul, 2002; Bruno & O'Connor, 2005; Gobler et al., 2006; D. I. Taylor & Schiel, 2010; Vergés, Alcoverro, & Ballesteros, 2008). The evolution of the structural features of marine plants and their chemical composition might be affected by herbivory on a longer time scale, especially when individual plants are targeted as food items (Hay, 1991; Horn, 1989).

To understand diet choices in marine herbivores, studies have mainly focused on the avoidance of secondary metabolites, and most have failed to account for nutrient intake (Clements et al., 2009). The field of nutritional ecology aims to understand the nutritional relationship between animals and their environment (Foley & Cork, 1992). Three factors influence food choice, given the abundance of food: (1) an optimal balance of multiple nutrients is likely to best explain diet quality (Lobel & Ogden, 1981; R. P. Wilson, 2002a), (2) palatability and digestibility of algae is influenced by algal properties such as toughness and chemical composition (Horn, 1989; Montgomery & Gerking, 1980), and (3) the digestive physiology of the fish also needs to be taken into account as the ability of fish to access and assimilate nutrients varies between fish species (Choat & Clements, 1998; Horn, 1989). These aspects are well studied in terrestrial vertebrates, but knowledge is lacking in marine fishes (Choat & Clements, 1998) despite a greater focus on fish nutrition in recent years (Clements et al., 2009).

All fish require about the same 40 nutrients regardless of feeding mode and physiology (Rust, 2002), even though some fish species are able to synthesise certain essential nutrients (Monroig et al., 2012). The three macronutrients important in nutrition are carbohydrates, lipids, and proteins. These are processed by endogenous and/or exogenous digestive enzymes, which are highly variable in their levels of activity (Skea, Mountfort, & Clements, 2007; Stone, 2003). Macronutrients supply the organism with energy to varying degrees, with the energy yield from lipids twice as high as that from protein and carbohydrates (Gnaiger & Bitterlich, 1984). Protein is required for growth and usually the limiting nutrient (Bowen, Lutz, & Ahlgren, 1995).

Carbohydrates provide most of the bulk of available energy in macroalgae (Gatlin, 2002; Montgomery & Gerking, 1980), but the ability of fish to use carbohydrates varies widely between species (Stone, 2003). The storage polysaccharides in marine algae differ from and are more diverse than those found in terrestrial plants (Choat & Clements, 1998; Skea et al., 2007). Chlorophyta and Rhodophyta store starch and floridean starch, respectively, which both have α–linkages that can be degraded by endogenous carbohydrases. Phaeophytes are considered superior to Rhodophyta on

the basis of nutrient and energy content. However most carbon is stored in the form of the sugar alcohol mannitol (Graiff, Ruth, Kragl, & Karsten, 2016), which cannot be metabolised by animals (Solomon, Waters, & Oliver, 2007). Obtaining energy from mannitol requires the fermentative activity of gut symbionts, producing short chain fatty acids (SCFA) that can be assimilated and used for energy or lipid synthesis (Montgomery & Gerking, 1980; Neighbors & Horn, 1991; Skea, Mountfort, & Clements, 2005).

Lipids play a major role in fish as they form barriers and biological membranes, store energy, and are sources of metabolic energy for growth, reproduction, and movement (Gurr & Harwood, 1991; Tocher, 2003). In carnivorous fish lipids are largely, if not exclusively, gained from the diet rather than through biosynthesis (Clements & Raubenheimer, 2006; Tocher, 2003). But some fish, such as the herbivorous rabbitfish *Siganus canaliculatus*, are able to synthesise physiological essential long-chain polyunsaturated fatty acids (Monroig et al., 2012).

Vertebrates, including fish, derive the majority of nitrogen from total hydrolysable amino acids (THAA). These include dietary proteins, peptides, and free amino acids. Proteins and peptides are hydrolysed into free amino acids, which can then be absorbed (Crossman, Clements, & Cooper, 2000; Stevens & Hume, 1998; R. P. Wilson, 2002a). These amino acids are used to synthesize new proteins for growth and reproduction, and to replace existing ones. A surplus in amino acids will be converted to energy (R. P. Wilson, 2002a). The major organic constituents of fish tissues are proteins, which can make up 65% to 75% of dry weight of teleost tissue (R. P. Wilson, 2002a). To achieve maximum growth rates it has been proposed that fish require diets with high protein contents of 35-55%, whereas mammals and birds attain maximum growth on diets of only 12-25% protein (Horn, 1989; 1998). Protein is thus the major dietary requirement of fishes (Bowen et al., 1995) but the efficiency of protein utilization falls within the range for other vertebrates (R. P. Wilson, 2002a).

Plant matter is generally considered to be of low nutritional value, mainly due to their perceived low protein content compared to animal matter, and its difficulty to be digested (Bowen et al., 1995; Horn, 1989). Taking into consideration the digestibility and nutritional content, fleshy algae are usually preferred over calcareous algae, and epiphytic algae over macroalgae (Montgomery & Gerking, 1980). Algae taxa have been ranked with Chlorophyta being superior to Rhodophyta, and Rhodophyta superior to Phaeophyta (Montgomery & Gerking, 1980). Algae become less attractive as food if they are calcified or otherwise tough (leathery or rubbery), or contain secondary metabolites that deter herbivores (Dorenbosch & Bakker, 2011; Targett & Arnold, 2001). Algae vary in their

production of secondary compounds and responses to the same compound vary between different herbivores (Horn, 1989; Targett & Arnold, 2001).

In herbivorous fishes the alimentary tract is usually longer than that of non-herbivorous species of equivalent size, providing a larger gut capacity and an increased opportunity for nutrient absorption (Horn, 1989; Kapoor, Smit, & Verighina, 1976; Kramer & Bryant, 1995b). However, there are many exceptions and relative gut length can also vary with the nutritional status of the fish, temperature, and an ontogenetic shift in diet (Benavides, Cancino, & Ojeda, 1994; Clements & Raubenheimer, 2006; German & Horn, 2006; Horn, 1989). Consumption rates are generally higher and gut transit times shorter in herbivorous fish compared to omnivores and carnivores, with the latter having lowest consumption rates and longest gut transit times (Clements & Raubenheimer, 2006; Horn, 1989). Gut retention times vary depending on the diet and decrease in some herbivorous fish feeding on a lower protein diet (Fris & Horn, 1993; Horn, Mailhiot, Fris, & McClanahan, 1995).

The digestive tract in herbivorous fishes shows various adaptations to overcoming mechanical and chemical defence mechanisms in algae, thereby enabling the fish to access the nutrients within the cells (Horn, 1989). Different types of the alimentary tracts have been described in herbivorous fishes (Horn, 1989; Lobel, 1981). Some species have specialized jaw teeth, strong pharyngeal mills, and/or muscular stomachs that function as gizzards and mechanically destroy algae. Thin-walled, but highly acidic stomachs in other species enable the chemical lysis of algal cell walls (Horn, 1989; Lobel, 1981). Other species contain microbial symbionts in their enlarged hindgut that ferment ingested plant material, enabling the fish to access the energy in compounds including mannitol and structurally complex carbohydrates (Choat & Clements, 1998; Clements & Raubenheimer, 2006; Horn, 1989; Horn & Messer, 1992; Mountfort, Campbell, & Clements, 2002; Skea et al., 2005). Most of the assimilation of nutrients takes place in the postgastric regions, including the pyloric cecae, and fish can absorb a range of carbohydrate monomers, proteins from amino acids and intact peptides, lipids from medium- to long-chain fatty acids, and SCFAs (the end products of fermentation) (Clements & Raubenheimer, 2006).

Two theories attempt to explain how organisms feed to maximise the assimilation of specific nutrients and how they ingest a balanced amount of nutrients: optimal digestion theory and optimal diet theory (Baker, Clauss, & Clements, 2016; Simpson & Raubenheimer, 2001). Fish adapted to a herbivorous diet are to some extent capable of compensating for low or unbalanced energy and nutrient intakes (Bowen et al., 1995; Hemre, Mommsen, & Krogdahl, 2002; Horn, 1989). The optimal

diet theory predicts that an organism will increase intake of the most nutritious food when food is abundant to acquire body reserves and ingest less when food quality is low (anticipatory response) (Meyer, Hummel, & Clauss, 2010). Seasonal changes in algal abundances and species composition can thus force diet shifts and alter ingestion rates (Clements & Choat, 1993; Horn, Neighbors, & Murray, 1986; Schiel, 1985). On the other hand optimal digestion theory states that organisms feeding on a less nutritious diet item will increase food intake and/or gut capacity to compensate for low nutrient levels (compensatory response) (Simpson & Raubenheimer, 2001). However feeding on one dietary item may not provide the optimal proportions of required nutrients. Also secondary plant compounds or toxins can constrain the amount that can be ingested (for more details on these theories see introduction in Baker et al., 2016).

The value of any single dietary item can only be understood in the light of the nutrient composition of the overall diet (Simpson, Sibly, Lee, Behmer, & Raubenheimer, 2004). Omnivorous fish, with a mainly herbivorous diet, employ a mixed feeding strategy. By feeding on a range of dietary items fish are able to increase food intake and also avoid the accumulation of toxins (Lobel & Ogden, 1981). Omnivores increase ingestion rates for energy by feeding on algae, and complement their diet by selectively feeding on animal material for protein (Bowen et al., 1995; Choat & Clements, 1998; Clements et al., 2009; Clements & Raubenheimer, 2006; Horn, 1989). There are indications that this complementary feeding strategy is also employed by *Girella tricuspidata* (Raubenheimer, Zemke-White, Phillips, & Clements, 2005).

1.2 Growth and longevity in fishes

Knowledge about age and growth is essential in understanding the life history and ecological role of fishes (Katsanevakis, 2006). Variation in growth rate is driven by the dynamic interaction of various intrinsic and extrinsic factors such as age, fitness, reproduction, competition, nutritional quality and availability of food, temperature and climate, and fisheries exploitation (Black, Boehlert, & Yoklavich, 2005; Caldow & Wellington, 2003; Morrongiello & Thresher, 2015; Sala-Bozano & Mariani, 2011; Sarre & Potter, 2000; B. M. Taylor, Lindfield, & Choat, 2015; Trip, Clements, Raubenheimer, & Choat, 2013). As ectotherms the growth of fishes is heavily influenced by their environment, however fish, and organisms in general, have the capability to adapt to changing environmental conditions, which can result in distinct morphologies (phenotypic plasticity) (Levinton, 2001). Temporal and spatial variation in growth rate, longevity, and adult body size have been described within many fish species (Baudron, Needle, Rijnsdorp, & Marshall, 2014; e.g. Morrongiello & Thresher, 2015; Trip et al., 2013).

Age is a critical variable in population biology as it forms the basis for calculations of growth rate, age structure, mortality rate, and production. Thus it is critical to stock assessment and fisheries management (Campana, 2001). To determine the age of marine animals, different calcified structures have been used that reveal daily or annual growth zones, e.g. bivalve shells, corals, and mammalian teeth (Black, 2009; Campana & Thorrold, 2001; Pinedo & Hohn, 2000; Zohdy et al., 2014). In fish otoliths are the most commonly used ones (Campana, 2001; Campana & Thorrold, 2001) and are thus a fundamental tool for estimating age structure, growth, and longevity in teleosts (Ewing, Welsford, Jordan, & Buxton, 2003). Otoliths, also called 'ear bones' or 'ear stones', are calcareous structures in the inner ear of fish located directly behind the brain. There are three pairs of otoliths, one large pair (sagittae) and two small pairs (asteriscii and lapilli) (Kalish et al., 1995). Otoliths are used for balance, orientation, and sound detection (Popper, Ramcharitar, & Campana, 2005) and are formed at the embryonic stage growing throughout the fishes' lives (Campana & Thorrold, 2001; Maillet & Checkely, 1989). Protein-rich and protein-poor layers of calcium carbonate are deposited to the exposed surface of the otolith, producing different growth zones that are retained within the otolith. These growth zones, also called increments, reveal daily, seasonal, and annual changes and reflect the growth and metabolic history of the fish (Geffen & Morales-Nin, 2013). The age estimates coupled with the body size of the fish have been used to describe growth trajectories. These allow for the comparison of life history traits within and between taxa, populations, and species (Chen, Jackson, & Harvey, 1992).

The use of otoliths to determine annual age and growth is one of the most widely used applications (Campana, 2005) and in recent years chronological use is also gaining in popularity. Methods are derived from dendrochronology (tree-ring dating) based on the assumption that growth increments are formed at regular intervals and that increment width is influenced by the physical environment. Extreme climate events that last less than a year would result in conspicuously different increment widths. Favourable conditions produce wider increments while less favourable conditions result in narrower increments (Black, 2009). Developed growth chronologies can be used to estimate the environmental variability over the lifetime of a fish (Black et al., 2016).

1.3 The effects of temperature and nutrition on growth

The effects of temperature and resource availability on growth rate and adult body size have puzzled researchers for over a century (Angilletta, Steury, & Sears, 2004). The Temperature-Size Rule (TSR or Bergmann's rule) predicts that ectotherms growing up in a warmer environment (lower latitudes) have

faster initial growth rates, mature earlier at a smaller body size, reach a smaller adult body size, and have a shorter life span than individuals living in colder environments (higher latitudes) (Angilletta et al., 2004; Arendt, 2010; Baudron et al., 2014; Trip et al., 2013; Walters & Hassall, 2006). Optimization models also predict lower initial growth rate and delayed maturity at colder temperatures but at a smaller adult body size due to reduced resource availability or increased nutrient stress (Arendt, 2010; Berrigan & Charnov, 1994; Stearns, 2000). These two responses, which can be observed over latitudinal gradients, result in characteristic growth curves as depicted by Trip et al. (2013) (Figure 1.1). Growth responses that conform with the TSR show crossing growth trajectories (Figure 1.1a). By prolonging growth fish can reach relatively large body sizes in colder environments despite their slow growth rates but resources have to be traded-off. Maturity is delayed, which decreases the likelihood of surviving until reproduction (Angilletta et al., 2004). The advantages of early maturation include a shorter period of exposure to juvenile mortality before the first reproductive event and a shorter generation time. But a smaller body size at maturity leads to lower fecundity and possibly lower-quality offspring (Angilletta et al., 2004; Stearns, 2000). Higher temperatures decrease aerobic capacity, thus making smaller body sizes advantageous as they reduce the risk of oxygen deprivation (Baudron et al., 2014). Attempts to explain the pattern as a constraint on either growth or development have been unsatisfactory because each constraint appears to be system

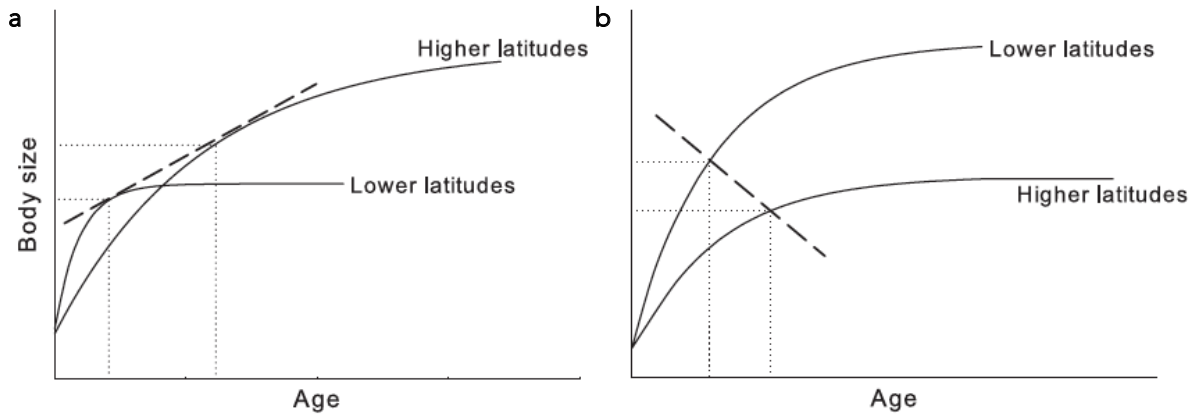


Figure 1.1: From Trip et al. (2013): Theoretical growth trajectories and maturation reaction norms in response to latitude (a) as predicted by the Temperature-Size Rule (TSR) as seen in the majority of ectotherms (Atkinson, 1994; Berrigan & Charnov, 1994) and (b) as predicted by the Temperature-Constraint Hypothesis (TCH) for ectothermic herbivores (Floeter, Behrens, Ferreira, Paddack, & Horn, 2005; Gaines & Lubchenco, 1982)). Note that (b) refers specifically to the hypothesis that the digestion of algal foods in marine piscine herbivores is constrained by temperature at higher latitudes (TCH). Continuous lines are growth trajectories, and dashed lines show the shape of the reaction norm in size- and age-at-maturity.

specific (Arendt, 2010). One optimization model, the Temperature-Constraint Hypothesis (TCH), established for aquatic ectothermic herbivores, argues that digestion is compromised at higher latitudes when fish feed on low quality algae and consequently fail to meet their nutritional demand (Behrens & Lafferty, 2007; Floeter et al., 2005; Gaines & Lubchenco, 1982). It is argued that the inability of herbivores to process and assimilate algae food in colder environments affects growth and results in nested growth trajectories (Figure 1.1b). This has thereby led to lesser species richness and abundances of herbivorous fish in temperate and polar regions compared to the tropics.

1.4 Ecology and hydrology of the study area

The Hauraki Gulf is located in north-eastern New Zealand, and covers an area of about 4,000 km^2 (Figure 2.1). It is situated between the North Auckland region in the west, the Hauraki Plains in the south, and the Coromandel Peninsula and Great Barrier Island in the east. Many estuaries and bays characterise the east coast of northern New Zealand (M. P. Francis, Morrison, Leathwick, Walsh, & Middleton, 2005). Rocky reefs are present along much of the coastline with varying rock type, topography and wave action (B. Ballantine, 1991; Brook, 2002; Choat & Schiel, 1982; Shears, Babcock, Duffy, & Walker, 2004). The main habitats in shallow rocky reefs are (1) areas of algal assemblages with fucoids (mainly *Carpophyllum* spp., *Sargassum* spp., *Cystophora* spp.), laminarians (*Ecklonia radiata* and *Lessonia* spp.), and rhodophytes providing shelter for fish and larger crustaceans, and (2) coralline paint-covered areas that are dominated by grazing sea urchins and a variety of molluscan grazers (B. Ballantine, 1991; Choat & Ayling, 1987; Schiel, 1988). The deep reef community, found below 15 to 20 meters depth, consists of mainly filter-feeding animals (B. Ballantine, 1991). Ecology and hydrology are dominated by the East Auckland Current, which flows south-east along the north-eastern coast of New Zealand (Figure 1.2). It originates in tropical regions and transports warmer water into the southern regions of New Zealand as far as the East Cape (Brook, 2002; Roberts, Ward, & Francis, 2012). The East Auckland Current has a stronger subtropical influence on the fish fauna at Great Barrier Island than the mainland, although it is not a major influence (Roberts et al., 2012). Sea surface temperatures in the Hauraki Gulf reach 20-22°C in summer and 14-16°C in winter (Brook, 2002) and are about 2°C warmer at Great Barrier Island than along the coast from early spring until late summer (Zeldis, Walters, Greig, & Image, 2004).

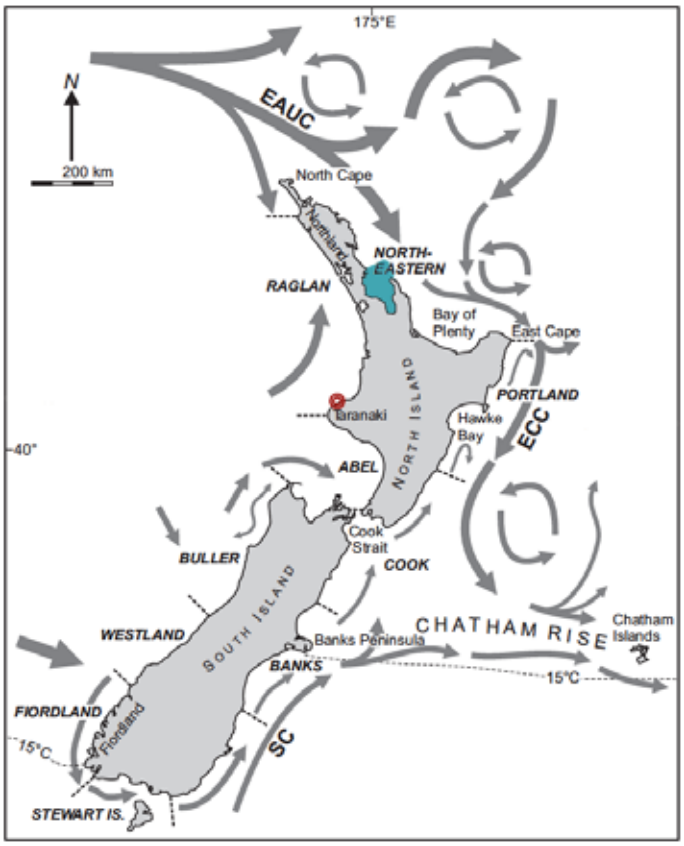


Figure 1.2: From Buchanan & Zuccarello (2012): Map of New Zealand showing the major currents and geographic regions with the Hauraki Gulf highlighted in blue. EAUC: East Auckland Current; ECC: East Cape Current; SC: Southland Current. Dashed lines show present day 15°C sea surface temperature isotherm. Red double circles mark the approximate location of the Waiwhakaiho River mouth (see Chapter 4.4.3 Growth variations and the effects of temperature).

1.5 *Girella tricuspidata*

Girella tricuspidata (Quoy & Gaimard, 1824) (Figure 1.3) is a marine fish species belonging to the family Girellidae (Knudsen & Clements, 2016), which contains two genera, *Girella* Gray, 1835 and *Graus* Philippi, 1887. Fifteen species of *Girella* occur in five regions in the subtropical and temperate waters of the Pacific and eastern Atlantic Ocean. Two species occur in northern New Zealand: *Girella cyanea* and *Girella tricuspidata* (Yagishita & Nakabo, 2003). *G. tricuspidata* is native to the coastal shores and shallow estuaries of the North Island of New Zealand and south-eastern Australia (southern Queensland to South Australia), including northern Tasmania. Its common names are parore in New Zealand and luderick, black bream, and blackfish in Australia.

The reported maximum length and mass are 710 mm and 4,000 g, respectively (Froese & Pauly, 2015; Kailola et al., 1993), though most adult fish are about 300 - 350 mm and 1,000 – 1,500 g (Gray

et al., 2012). The maximum reported age is 24 years in Australia (Gray et al., 2012; Gray, Ives, Macbeth, & Kendall, 2010) and 10 years in New Zealand (Morrison, 1990). Sexual maturity is reached at about 286 mm and 295 mm fork length (FL) and 4.1 and 4.5 years for males and females, respectively (Gray et al., 2012). In Australia spawning occurs between May and September in northern areas (Clearance River) and between October and March further south (Tuross River) (Gray et al., 2012).

Figure 1.3: Image of *G. tricuspidata*. Fish of this species have a silver-grey body with dark vertical stripes and a golden tinge to the face and lips. (Photography by K.D. Clements)

G. tricuspidata is one of the most abundant coastal reef fish in New Zealand (Cole, Creese, & Ayling, 1990; Meekan & Choat, 1997), where it contributes a large proportion to the total fish biomass (Choat & Ayling, 1987; Jones, 1988; Meekan & Choat, 1997; Russell, 1977). Abundances are higher on the east coast of northern New Zealand than on the west coast (M. P. Francis et al., 2005). Estimated population size in the Cape Rodney to Okakari Point Marine Reserve show a decline from 9,820 fish in 1978 to 5,902 in 2014 (Brown, 2015). At Great Barrier Island they dominate the central part of the western side of the island (Meekan & Choat, 1997). *G. tricuspidata* does not get specifically targeted by fisheries in northern New Zealand but is caught as by-catch in the grey mullet, flatfish, and trevally set-net fisheries. The total landings varied from 56 to 92 tons per year between 2004 and 2012. It is a low value recreational species and catches are likely to be low (Ministry of Primary Industries, 2013).

High abundances of *G. tricuspidata* occur in shallow waters down to 6 meters and they are rarely encountered at depths of more than 18 meters (Brook, 2002; Kingsford, 2002; Meekan & Choat, 1997). They are mainly found on rocky reefs and are common around coastal infrastructure such as marinas (Clynick, Chapman, & Underwood, 2007; Ferguson, Harvey, Taylor, & Knott, 2013; Meekan & Choat, 1997). In north-eastern New Zealand highest abundances occur in shallow *Carpophyllum* habitat (Brown, 2015). Juveniles recruit into estuaries and harbours, where they spend at least the first two years before moving to the open coast (M. P. Francis et al., 2005; Jones, 1988; Morrison, 1990; Rotherham & West, 2002). Young *G. tricuspidata* form large schools, though adult fish tend to be more solitary (Kilner & Akroyd, 1978; Morrison, 1990). Individuals exhibit strong site fidelity. Nevertheless, they are highly mobile and some fish migrate over hundreds of kilometres along the coast (Ferguson et al., 2013; Gray et al., 2012).

G. tricuspidata is a diurnal feeder (Pankhurst, 1989). Juvenile fish feed on plankton for the first year until they reach a size of 90 - 100 mm. They then move to the open coast and shift their diet to a broad range of Rhodophyta and Chlorophyta, complemented by animal and detrital material (Choat & Clements, 1992; Clements & Choat, 1997; Morrison, 1990; Raubenheimer et al., 2005). Even though a significant amount of the diet of *G. tricuspidata* consists of animal material, they have historically been classified as herbivores (Choat & Clements, 1992; Jones, 1988; Kilner & Akroyd, 1978; Kingsford, 2002; Meekan & Choat, 1997; Russell, 1983; Thomson, 1959), although more recent papers class them as omnivores (Clements & Choat, 1997; Raubenheimer et al., 2005; Willmott, Clements, & Wells, 2005).

The gastrointestinal tract of *G. tricuspidata* is differentiated into oesophagus, stomach, intestine with pyloric caeca that protrude from the anterior end, and rectum. A layer of circular muscles is present in the oesophagus and the stomach (T. A. Anderson, 1986). The gastric pH ranges from 1.8 to 3.5 and as a result the content is very acidic, while slightly alkaline conditions occur in the pyloric caeca, intestine and rectum (pH 6.0-9.0) (T. A. Anderson, 1991; Zemke-White, Clements, & Harris, 1999). Incubation of plant material in the acidic conditions of the stomach facilitate the lysis of plant cell walls and subsequently the leakage of cell contents (Lobel, 1981; Zemke-White et al., 1999; Zemke-White, Clements, & Harris, 2000). Endogenous enzymes break down starch and some degradation of laminarin can be recorded to a low degree but fish seem to mostly rely on endogenous digestion (Clements & Choat, 1997; Moran & Clements, 2002; Skea et al., 2007).

1.6 Thesis structure and research objectives

Diet and temperature are two of the most important factors affecting growth (Munday, Kingsford, O'Callaghan, & Donelson, 2008) but studies usually focus on only one of these and do not consider potential interactions between them. This study aims to investigate the effects of both factors. *G. tricuspidata* is an omnivore, with the major part of the diet consisting of epiphytic algae complemented with animal matter. This makes *G. tricuspidata* an excellent study species by investigating how a mainly herbivorous diet is supplemented with animal material and which strategies are used to meet nutrient requirements. This will be examined with regard to times of high nutrient demand such as reproduction, seasonal changes in the availability of their main food items (i.e. epiphytic rhodophytes and salps), and consequential changes in nutrient availability and intake. Habitats differ in environmental condition and influence life history traits such as growth rate, longevity, and adult size to varying degrees. Comparison of two distinct populations allows the finding of underlying causes to variations in life history characteristics.

For this study, sampling of wild specimens of *G. tricuspidata* took place at two locations in north-eastern New Zealand about 50 km apart. Investigating a population at different times or populations in distinct habitats allows for comparison. One population was sampled in the western Hauraki Gulf along the coast of Leigh (coastal) and the other in the east of the Outer Hauraki Gulf at Great Barrier Island (offshore) (Figure 2.1). Seasonal samples of fish were collected over two years from February 2010 until August 2012. The coastal population was sampled along the east coast of northern New Zealand around the small town of Leigh. Great Barrier Island, situated in the east of the Hauraki Gulf was sampled for offshore fish. It is unlikely that fish move between these two locations as *G. tricuspidata* is associated with shallow reefs where they forage for food (Meekan & Choat, 1997). Fish smaller than 300 mm do not cross large expanses of sand (Morrison, Jones, Consalvey, & Berkenbusch, 2014). They are not found at smaller offshore islands that are lacking suitable nursery habitats (e.g. Mokohinau Islands, Poor Knight Islands, Little Barrier Island) (Choat & Ayling, 1987; Hidas, 2001; Meekan & Choat, 1997; Morrison et al., 2014). These islands are within the same distance or even closer to the mainland than Great Barrier Island and would be expected to house populations of *G. tricuspidata* if fish were crossing the Hauraki Gulf.

The thesis aims to answer the following questions:

1. What is the diet of *G. tricuspidata*? Does diet vary with location or gender?

2. Do diet and nutrient composition vary seasonally between coastal and offshore sites? How does *G. tricuspidata* regulate diet and nutrient intake across the year? Is reproduction associated with increased nutrient intake?

3. Do growth differences exist between the two populations living in distinct habitats? Does sea surface temperature affect growth on spatial and temporal scales?

4. Is there a relationship between diet/nutrition and growth or temperature and growth? Can potential differences in growth be explained by the Temperature-Constraint Hypothesis or the Temperature-Size Rule?

Chapter 2 investigates the diet of *G. tricuspidata* and compares the differences between gender and location. This species is known to mainly feed on rhodophyte and chlorophyte algae and complement its diet with animal matter. Detailed knowledge about which algae they are feeding on is missing. Results of different studies are highly variable as to what extent they ingest animal matter (Clements & Choat, 1997; Raubenheimer et al., 2005; e.g. Thomson, 1959). This chapter will provide a detailed diet analysis for both populations and establish in which habitats they spend their time feeding. This knowledge will help to evaluate the ecological role these fish hold.

Chapter 3 examines the nutritional value of the diet, seasonal changes in diet and nutrient content. It will be examined how diet quality and availability of food influences diet choice in *G. tricuspidata*. Previous work suggests that they target animal material for protein and seaweed for energy (Raubenheimer et al., 2005) but it is unclear how they regulate nutrient intake on temporal and spatial scales. Fish are known to have an increased nutrient demand during reproduction (Bureau et al., 2002). Changes in nutrient intake during different times of the year will be investigated. This will be considered in relation to the changing nutrient demand during reproduction and with reference to the availability of epiphytic algae. Examining gut content mass data and gut length will show if fish have adapted to potential differences in the diet and nutrient intake between seasons or locations.

Chapter 4 explores differences of the life history traits of *G. tricuspidata* between the two populations as a response to differing and changing environmental factors, in particular temperature. Growth and longevity are investigated by ageing sagittal otoliths and establishing von Bertalanffy growth curves. *G. tricuspidata* showed growth variations between populations from three latitudinal regions in

Australia (Gray et al., 2010). No study so far has investigated spatial and intersexual variation in growth between populations in New Zealand. Chronologies from tree ring data have been widely used but chronologies from marine organisms remain greatly underutilized even though they can be used to reconstruct various environmental factors (Black, 2009). Growth chronologies have been established for *G. tricuspidata* before (Gillanders, Black, Meekan, & Morrison, 2012) but the authors did not investigate the effects of environmental factors between populations, which will be examined here.

In Chapter 5 (General Discussion) the effects of temperature and nutrition on growth will be considered simultaneously with regards to the TSR and TCH, thereby linking the results of the three data chapters.

Chapter 2

Herbivory or omnivory?

A comparison of the highly variable diet between the sexes and populations

2.1 Introduction

Knowledge of diet is central to understanding the biology and ecology of an animal and also contributes to the understanding of ecosystem function and population dynamics (Ahlbeck, Hansson, & Hjerne, 2012). *Girella tricuspidata* is one of the most abundant large reef fishes in north-eastern New Zealand where they account for up to 51% of the total fish biomass (Russell, 1977). Comprehensive diet studies for adult fish are lacking, which restricts the knowledge about their ecological role in the rocky reef community.

Feeding observations and stomach content analysis have been used for decades to gain insight into the diet of animals, including fish (Choat & Clements, 1992; Choat, Clements, & Robbins, 2002; e.g. Hynes, 1950; Swynnerton & Worthington, 1940). Feeding observations can remain inconclusive especially when fish are feeding on small organisms, e.g. detritus, microbial mats or epiphytes (Choat et al., 2002). The analysis of stomach and gut contents enables direct quantitative and qualitative analysis of the diet (Hyslop, 1980). Results of diet compositions can then be put in a wider ecological context providing information about feeding habitats, potential competitors, and predator-prey relationships (Ahlbeck et al., 2012). Trophic studies help to identify pathways of energy and assess the nutritional standing of the organism in context of the whole community.

Species belonging to the genus *Girella* have been described as either herbivores or omnivores (Barry & Ehret, 1993; Behrens & Lafferty, 2012; Kanda & Yamaoka, 1994; Lewis, 2012; Muñoz & Ojeda, 1997; Yagishita & Nakabo, 2003), with algae accounting for the major part of the diet. Algae as food, in comparison to animal matter, are generally considered to contain lower proportions of essential nutrients, in particular protein (Clements et al., 2009). Various mechanical and chemical defence mechanisms have developed in algae to withstand predators. Herbivorous fishes in turn have adapted to evade these strategies enabling them to access the nutrients within algal cells and hence meet their nutritional demands (for a detailed review see Horn, 1989). Girellids are no exception. They developed specialised tricuspid teeth and an intramandibular joint within the lower jaw that increases the gape, maximizing the contact with the substratum, which allows more effective removal of food (Ferry-Graham & Konow, 2010; Kanda & Yamaoka, 1994; Vial & Ojeda, 1992; Yagishita & Nakabo, 2000; 2003). This type of jaw modification is also seen in some acanthurids, blenniids, scarines, and poeciliids and is consistently associated with the capacity to scrape filamentous, particulate or microscopic autotrophic or detrital material from surfaces (Ferry-Graham & Konow, 2010; Gibb, Ferry-Graham, Hernandez, Romansco, & Blanton, 2008). Ingested food in *G. tricuspidata*

is exposed to a low gastric pH of 1.8 to 3.5 (T. A. Anderson, 1986; Zemke-White et al., 1999). Incubation of plant material in the acidic conditions of the stomach facilitates the lysis of algal cell walls and subsequently the leakage of cell contents providing access to the nutrients (Lobel, 1981; Zemke-White et al., 1999; 2000).

Historically *G. tricuspidata* has been classified as an herbivore, but more recent papers class them as omnivores that supplement their algal diet with animal matter. *G. tricuspidata* has been observed to selectively browse epiphytes of fucoids (Choat & Clements, 1992). Diet studies show that they feed on a wide range of epiphytic rhodophytes and some animal material, which is subject to seasonal changes (Choat & Clements, 1992; Clements & Choat, 1997; Raubenheimer et al., 2005; Russell, 1983). Besides epiphytic rhodophytes *G. tricuspidata* is also known to feed on Chlorophyta, especially algae belonging to the genus *Ulva* (Choat & Clements, 1992; Raubenheimer et al., 2005). *Ulva* is sometimes referred to as the preferred dietary alga (Curley, Jordan, Figueira, & Valenzuela, 2013; Ferguson, Harvey, Rees, & Knott, 2015; Gray et al., 2010). But of the seven diet analyses available (Choat & Clements, 1992; Clements & Choat, 1997; Kilner & Akroyd, 1978; Morrison, 1990; Raubenheimer et al., 2005; Russell, 1983; Thomson, 1959), only three have reported the presence of *Ulva* species in the stomach contents, and only in small proportions (Choat & Clements, 1992; Kilner & Akroyd, 1978; Raubenheimer et al., 2005). Published diet analyses record a maximum of 11.1% *Ulva* species (including algae of the junior taxon *Enteromorpha*) (Kilner & Akroyd, 1978). A detailed diet analysis for adult *G. tricuspidata* is lacking. Descriptions of the diet in previous studies were conducted using small sample sizes (Choat & Clements, 1992; Clements & Choat, 1997; Russell, 1983) and included juvenile and subadult fish (< 250 mm standard length) in the sample (Choat & Clements, 1992; Clements & Choat, 1997; Raubenheimer et al., 2005; Russell, 1983).

2.1.1 Study sites

The two sites selected for the present study lie at the northern end of the Hauraki Gulf (Figure 2.1). The coastal site, in the west of the Hauraki Gulf, is located along the coast of New Zealand's mainland near Leigh village. Sample sites were situated north and south of the Cape Rodney - Okakari Point Marine Reserve (Goat Island), which was established in 1975 as New Zealand's first marine reserve. The offshore site, Great Barrier Island, is located in the east of the Hauraki Gulf. *G. tricuspidata* tends to avoid highly exposed areas and sample sites were usually located in bays and sheltered areas, where fish spend time foraging for food. The two sample locations were chosen

as they harbour two distinct populations of *G. tricuspidata* separated by approximately 50 km of deep water.

2.1.2 Aims and objectives

The present study was undertaken to describe the diet of adult *G. tricuspidata* in north-eastern New Zealand, and to compare diet in these two distinct populations. Some fish species also exhibit variation in diet between gender. This has been observed in the temperate herbivore *Odax pullus* (Labridae) and might have been due to size- and sex-related differences in habitat utilisation or reflect a behavioural response to physiological changes in nutritional demand (Johnson, 2011). The main objective of this chapter was to establish a detailed list of the dietary items found in the stomach contents of this abundant reef fish, and to examine how diet varies with gender and location.

The chapter also aims to answer the following questions:

1. Is *G. tricuspidata* an omnivore or a herbivore? What animal material is targeted, and how important is it to the overall diet?

2. How much do *Ulva* spp. contribute to the diet?

3. Which are the main foraging habitats for *G. tricuspidata*?

Results will help to explain the ecological role of *G. tricuspidata* in New Zealand's shallow rocky reef community. Results of diet composition are especially valuable when placed into a nutritional context. This chapter forms the basis for the nutritional study, which will be discussed in the following chapter.

2.2 Methods

2.2.1 Fish sampling

Fish were collected from two populations of *Girella tricuspidata* in the Hauraki Gulf during daylight hours (between 08:45 to 18:15). Coastal fish were collected around Leigh from Okakari Point in the north (36°15.32S, 174°45.56E) to Kawau Island in the south (36°25.55S, 174°52.53E). Offshore fish were obtained from several locations around Great Barrier Island (36°05S – 36°20S, 175°18E – 175°30E) and ten specimens from Mercury Island (36°38S, 175°50E) (Figure 2.1). Offshore juvenile fish were collected in August 2014 and all other fish between February 2010 and August 2012

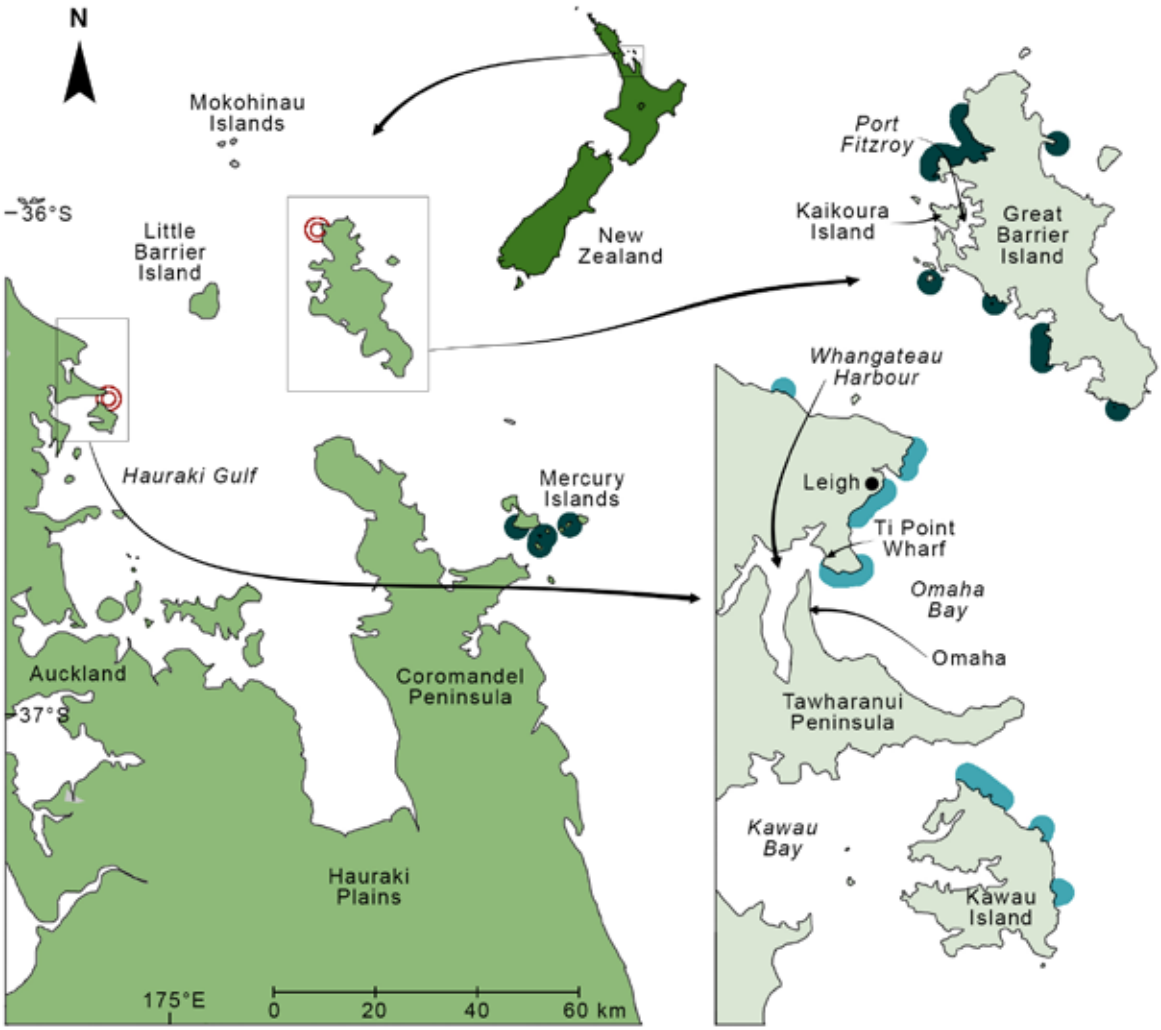


Figure 2.1: Map of study region showing the Hauraki Gulf (left) and the coastal (right bottom) and offshore (right top) areas in more detail. Sample sites are highlighted for coastal and offshore areas. Red double circles ◎ mark the approximate position of the NOAA temperature sampling points used in Chapter 4 (Figure 4.8).

(Table 2.1). Fish were collected on snorkel by spear and either immediately processed on board the research vessel 'Hawere' (all offshore and some coastal fish) or put on ice and returned to the Leigh Marine Laboratory (most coastal fish) and then processed. Sex was determined by examining the reproductive organs, which was feasible for adult and subadult fish > 205 mm SL. The stomach and gut were removed from the fish and their contents were placed in vials and frozen in liquid nitrogen (see Chapter 3.2.2 Fish sampling for gut processing). Upon return to the laboratory the samples were weighed (wet weight (WW)) and stored at -80°C. The Auckland Animal Ethics committee approved the collection of fish under approvals R717 and 001009.

Table 2.1: Number of sampled *G. tricuspidata* at coastal and offshore sites between summer 2010 and winter 2014

		Summer			**Autumn**			**Winter**				**Spring**		
		2010	2011	2012	2010	2011	2012	2010	2011	2012	2014	2010	2011	**Total**
Coastal	Adult	8	15	20	1	20	20	16	22	-	-	21	18	161
	Juvenile	1	1	-	10	-	1	-	-	-	-	-	1	14
Offshore	Adult	25	25	-	20	22	-	-	21	23	-	22	22	180
	Juvenile	-	-	-	-	-	-	-	-	-	21	-	-	21
											Total number of fish			376

2.2.2 Diet analysis

Fifty per cent (by weight) of each stomach content sample was used for diet analysis. The sample was thawed in filtered seawater and evenly distributed in a tray (50 x 50 mm or 100 x 100 mm) with a grid spaced at 1mm. The area each diet item covered was counted for 25% of the squares (5 or 25 squares of 10 x 10 mm, respectively). If samples were too big to fit into one tray, the process was repeated until the sample was used up. Algae were identified to species level following (Adams, 1994; Guiry & Guiry, 2007; Nelson, 2013; Womersley, 1984; 1987; 1994; 1996; 1998; 2003) and assistance from phycological experts (algae: Professor Wendy Nelson, NIWA Wellington; Mike Wilcox, Herbarium at the Auckland War Memorial Museum; cyanobacteria: Dr. Susie Wood, Cawthron Institute).

Algae and other dietary items were grouped into diet categories for further analysis as follows: Rhodophyta, Chlorophyta, Ochrophyta, animal matter (excluding salps), salps, cyanobacteria/diatoms, detritus, and others. Cyanobacteria and diatoms were grouped together as some fish had ingested microbial mats mainly consisting of unicellular diatoms and filamentous cyanobacteria of the order Oscillatoriales. Detritus was defined as particulate organic matter, which

could have been ingested or might have originated from algae that were already partly digested in the stomach. The category "others" included bark, sand, and seeds.

Algae were further categorized into functional groups depending on their morphological features (modified after Steneck & Watling, 1982): tiny filamentous, small filamentous, large filamentous, small foliose, large foliose, fleshy with no cortices, fleshy with light cortices, articulated calcareous, corticated macrophytes, leathery macrophytes, and microbial mats consisting of microscopic cyanobacteria, algae, and diatoms. Data were analysed separately for each phylum.

Dietary items were also grouped regarding the habitat they can be found in. Animals were classed as epifauna, pelagic, epifauna/pelagic, or benthic. Algae were placed into the habitats regarding their growth substrate: epiphytic, epilithic, epiphytic/epilithic, or unattached. Other dietary items were allocated to the habitat terrestrial or as unknown. As with the categories, unidentifiable organic matter was classed as detritus as the origin is unknown.

Appendix A7 lists each dietary item found within the stomach contents and which functional group and habitat each of them was grouped in.

2.2.3 Data analysis

The frequency of occurrence was calculated for each dietary item. Percentage compositions were calculated for each stomach content and averaged separately for sexes and locations.

Multivariate analysis of all dietary items was performed to test for differences between sexes and regions using the software PRIMER v6.1.12 (K. R. Clarke & Gorley, 2006) with the PERMANOVA+ v1.0.2 add-on (M. J. Anderson, Gorley, & Clarke, 2008). Data were square root transformed and converted to resemblance matrices using Bray-Curtis dissimilarity coefficients. Principal coordinate analysis (PCO) was used to display patterns of similarities and differences between the diets. The permutational multivariate analysis of variance (PERMANOVA) was designed on the two factors sex and region to detect significant differences between regions or sexes. One factor PERMANOVA was used to test for differences between sexes for each location separately. PERMDISP was conducted to analyse homogeneity of multivariate dispersions on the basis of the resemblance measure.

Independent-samples t-tests were performed to determine statistically significant differences between sexes and locations for the mean values of each dietary category, functional group, and

habitat. Statistical tests were performed using the software IBM SPSS Statistics v22 with the SPSS statistics guide (Laerd Statistics, 2015).

2.3 Results

Diet analysis of the stomach contents of adult *Girella tricuspidata* revealed a diverse range of food items. In 335 stomach contents a total 110 different dietary items were identified. These included 88 different algal species: 68 Rhodophytes, 10 Chlorophyta, and 9 Ochrophyta (Appendix A7). *G. tricuspidata* also ingested considerable amounts of animal material, namely salps, amphipods, isopods, copepods, krill, polychaetes, nematodes, gastropods, bivalves, and hydroids. Other dietary items included cyanobacteria, diatoms, and sand. Any particulate organic matter that could not be identified, was classified as detritus and could have been ingested or could have originated from algae that were already partially broken down by acidic conditions in the stomach.

The alga *Gredgaria maugeana* (Womersley) was found in two stomach contents from fish caught at Great Barrier Island, and represents the first record for this species in New Zealand. It has previously been recorded in East Victoria and South-east Tasmania, Australia. After identifying the alga from this study, previously collected specimens from Tawharanui (peninsula south of Leigh) could also be identified as *Gredgaria maugeana*. Both specimens are held in the herbarium of the Auckland War Memorial Museum: Tawharanui, *M.D. Wilcox* 4186, 12 Aug 2010, AK 315900; Great Barrier Island, from stomach of a parore fish, *T. Salewski*, Aug 2014, AK 359886.

The most important dietary item was the rhodophyte *Abroteia suborbicularis*, which was present in 50.5% of the fish examined. *A. suborbicularis* comprised 21.6% of all stomach contents overall and thus made up the largest portion of the diet of *G. tricuspidata*, followed by the chain-forming salp *Thalia democratica*, which made up 18.4% of the diet. *T. democratica* was found in 43.0% of the stomach contents. The dietary item that was found in most fish (86.3%) was detritus, which formed 17.6% of the total diet. Arthropods and all three *Ceramium* species combined were found in 83.0% and 75.5% of the stomach contents, respectively, and made up a small portion of the overall diet (3.6% and 1.4%, respectively). 57.0% of *G. tricuspidata* had algae of the genus *Ulva* (six species) in their stomachs and they contributed 7.0% to the overall diet. Other algae species that contributed between 2.5 to 3.6% to the diet and were ingested frequently (in brackets: % of fish with alga) were Ceramiales sp5 (26.9%), *Metamorphe colensoi* (23.3%), and *Caulacanthus ustulatus* (22.4%). Some algae were frequently found in the stomach contents but did not contribute substantially to the overall diet (less than 0.9%): *Ceramium* sp1 (27.5%), *Ceramium* sp2 (32.2%), *Dasyclonium bipartitum* (28.7%), *Dipterosiphonia heteroclada* (28.1%), and *Carpophyllum maschalocarpum* (29.9%) (see Appendix A7 & A8 for frequency of occurrence and percentage composition of each species).

Results of the PERMANOVA revealed significant differences between diet of sexes and regions but no differences for the interactive effect of both factors (Table 2.2). Assumptions of homogeneity of dispersions were met for sexes (PERMDISP: F = 1.93, df1 = 1, df2 = 320, *p*(perm) = 0.188) but not for regions (PERMDISP: F = 10.94, df1 = 1, df2 = 320, *p*(perm) = 0.002). Testing for differences between the sexes for both locations separately revealed significant differences for the offshore location but not for coastal fish (Appendix A2).

Table 2.2: Results of the two factor PERMANOVA investigating the effect of genders and regions on diet composition (categories). Significant results are highlighted in bold. df = degrees of freedom, SS = sum of squares, MS = mean sum of squares, Pseudo-F = F value by permutation.

Source of variation	**df**	**SS**	**MS**	**Pseudo-F**	***p*(perm)**	**Unique perms**
Sex	1	12703	12703	3.936	**0.001**	997
Region	1	22833	22833	7.075	**0.001**	998
Sex*Region	1	4081.6	4081.6	1.265	0.249	997
Residual	317	1.02E5	3227.4			

PCO graphs indicated that 44.2% of the variation was explained by the first two PCO axes (Figure 2.2). Comparison between the sexes did not reveal any clear grouping. Evaluations of the locations showed only slight separation indicating that offshore fish ingested more salps than coastal fish. Vectors show the strongest correlation with the distribution of data points for salps, then Rhodophyta, followed by detritus.

PERMANOVA conducted on a lower taxonomic resolution (diet categories) resulted in the same significances and also showed homogeneity of dispersions for both factors. The first two axes explained 73.9% of variation at category level for the PCO (Appendix A1).

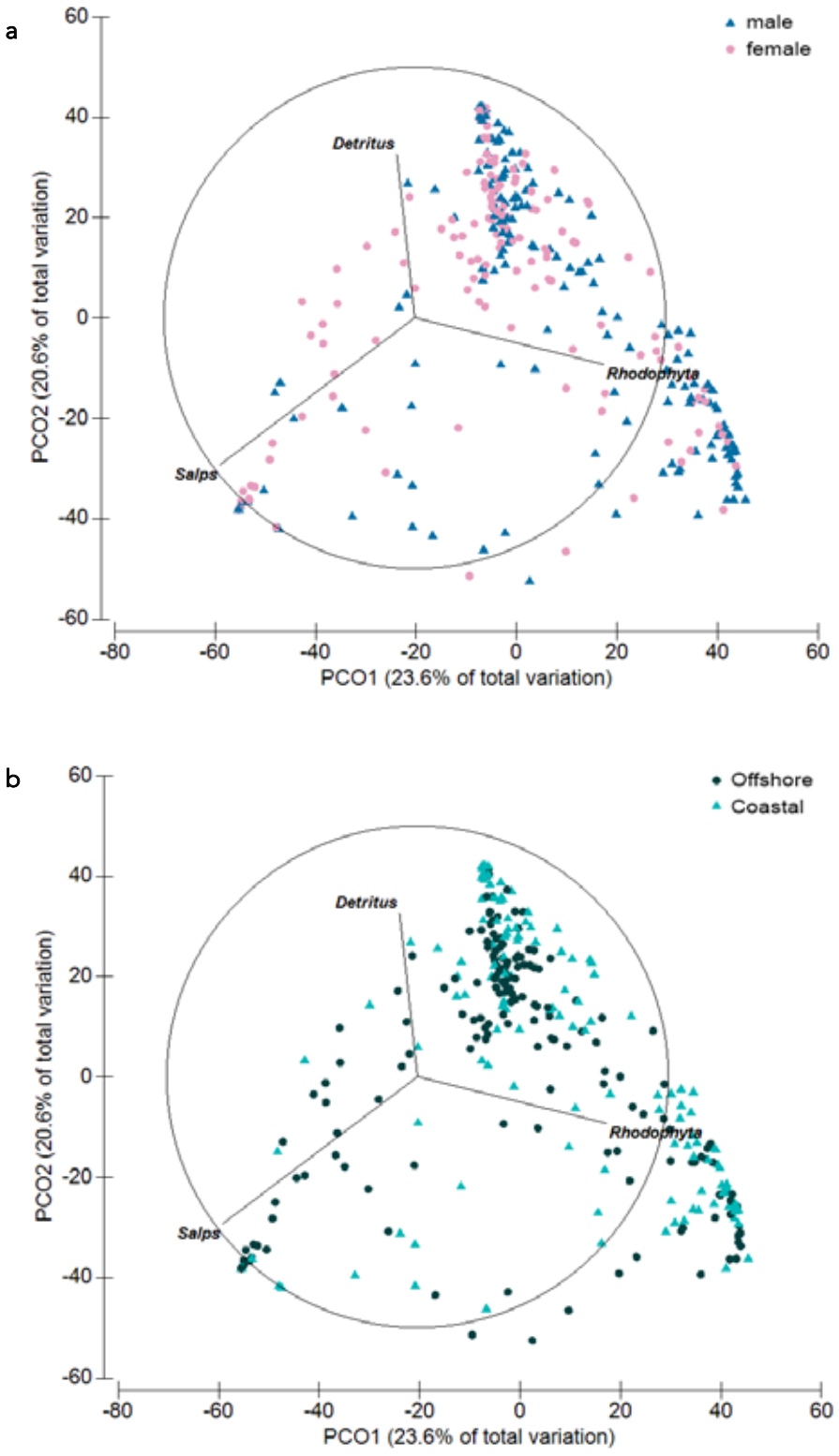


Figure 2.2: PCO ordination showing differences and similarities between the diet items for (a) male and female fish and (b) coastal and offshore populations. Vector plots present Pearson's correlations of untransformed diet categories for correlations > 0.25. The distance of the vectors extending to the circle indicates the strength of the correlation of that vector with the distribution of data points.

2.3.1 Diet categories

The main diet category for male (n = 177) and female fish (n = 144) was Rhodophyta followed by detritus and salps (Figure 2.3a). Rhodophyta were also the main dietary item for both coastal (n = 146) and offshore fish (n = 175). The second most important dietary item for coastal fish was detritus, followed by salps. The diet of offshore fish was characterised by higher levels of salps, followed by Chlorophyta and detritus (Figure 2.3b).

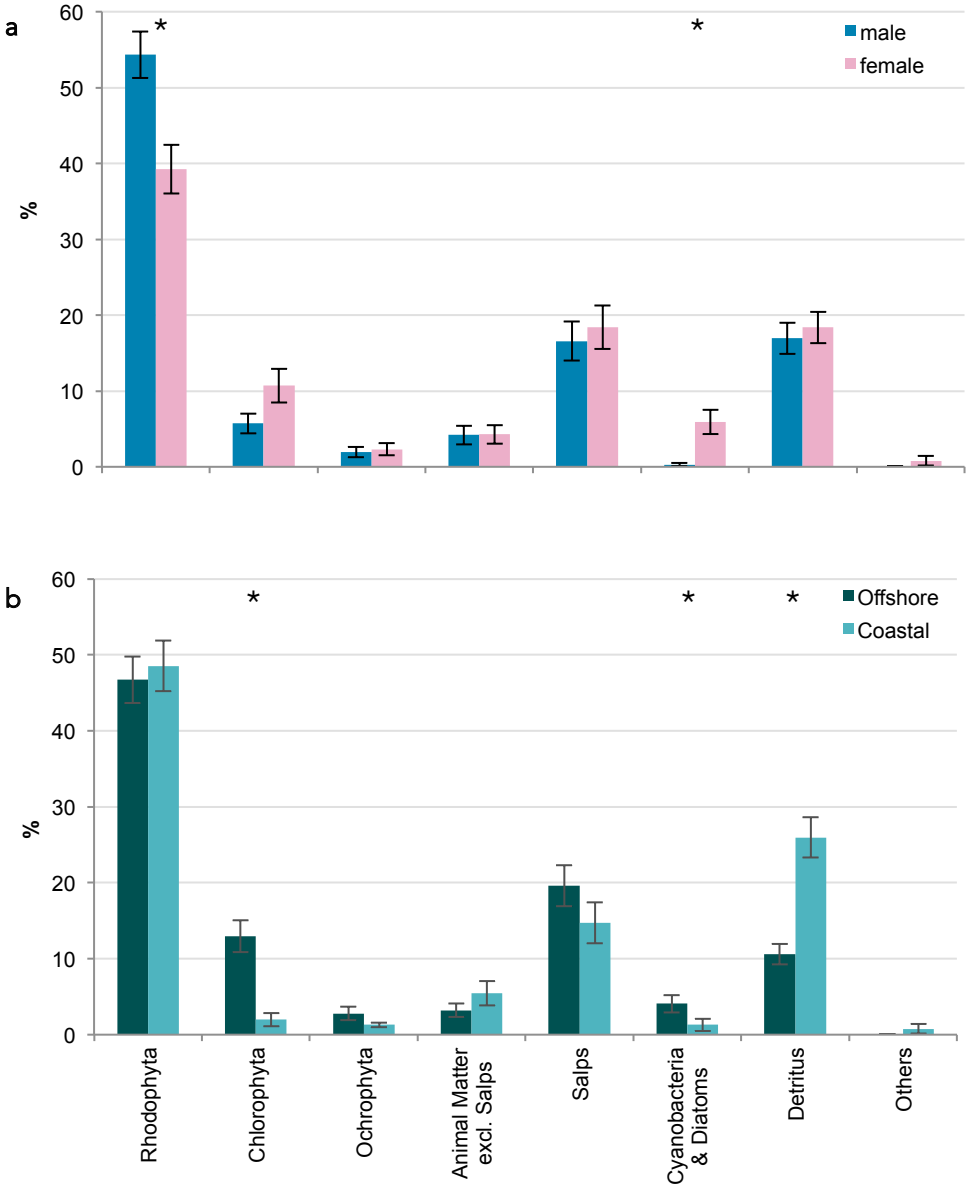


Figure 2.3: Comparison of the mean diet composition of (a) male and female *G. tricuspidata* and (b) coastal and offshore fish. Error bars indicate standard error. Asterisks mark diet categories with statistically significant differences between genders or locations (independent-samples t-test). For values of each category see Appendix A3.

Independent-samples t-tests were performed to compare means for each category between genders and locations. There were outliers in the data for all categories except for Rhodophyta and coastal detritus, as assessed by inspection of boxplots for values greater than 1.5 box-lengths from the edge of the box. They were considered as genuinely unusual data points and included in the analysis. The Shapiro-Wilk's test revealed that categories for each gender and location were not normally distributed ($p < 0.05$). All categories were positively skewed except for male Rhodophyta, which was slightly negatively skewed. Transformation of data did not achieve a normal distribution and the independent samples t-test produced the same results. Therefore the t-test was run using the untransformed data as it is also considered fairly robust to deviations from normality. As assessed by Levene's test for equality of variances ($p > 0.05$), there was homogeneity of variance for the following categories when comparing male and female fish: Rhodophyta, Ochrophyta, animal matter, salps, and detritus. The assumption of homogeneity of variances was violated for the categories Chlorophyta, cyanobacteria/diatoms, and others ($p < 0.05$). For the comparison between coastal and offshore fish Rhodophyta was the only category that met the assumption of equality of variances. In case of violation of the homogeneity of variances the Welch t-test was used.

Consumption of Rhodophyta was 15.1% (95% CI, 6.3 to 23.9) higher for male than female fish and lower in males for all other categories: Chlorophyta -5.0% (95% CI, -10.1 to 0.1), Ochrophyta -0.4% (95% CI, -2.4 to 1.6), animal matter -0.1% (95% CI, -3.6 to 3.4), salps -1.8% (95% CI, -9.4 to 5.7), cyanobacteria/diatoms -5.6% (95% CI, -8.8 to -2.5), detritus -1.4% (95% CI, -7.2 to 4.4), and others -0.7% (95% CI, -2.0 to 0.5). There was a statistically significant difference between male and female fish for the mean diet composition of Rhodophyta and cyanobacteria/diatoms (Figure 2.3a, Appendix A3). The comparison of male and female fish for each location separately revealed no significant differences for coastal fish (n: male / female = 85 / 61). For offshore fish (n: male / female = 92 / 83) results showed a significant difference in the consumption of two categories. Male fish ingested significantly more Rhodophyta but less cyanobacteria/diatoms (Appendix A4). These results are reflected in the same way for male and female fish combined for both locations, as well as the PERMANOVA results.

Coastal fish ingested higher proportions than offshore fish of the categories Rhodophyta 1.8% (95% CI, -7.1 to 10.7), animal matter 2.2% (95% CI, -1.4 to 5.9), detritus 15.4% (95% CI, 9.5 to 21.2), and others 0.7% (95% CI, -0.5 to 2.0), but lower proportions of the following: Chlorophyta -11.0% (95% CI, -15.4 to -6.6), Ochrophyta -1.5% (95% CI, -3.4 to 0.4), salps -4.9% (95% CI, -12.4 to 2.6), cyanobacteria/diatoms -2.8% (95% CI, -5.6 to 0.0). Results of the independent-samples t-test

indicated statistically significant differences between coastal and offshore fish for the mean diet composition of Chlorophyta, detritus, and cyanobacteria/diatoms (Figure 2.3b, Appendix A3).

2.3.2 Functional groups

The functional groups of algae were analysed separately for the three phyla Rhodophyta (n: male / female = 152 / 120, coastal / offshore= 122 / 150), Chlorophyta (n: m / f = 61 / 65, c / i = 32 / 94), and Ochrophyta (n: m / f = 86 / 74, c / i = 70 / 90). Within these phyla fish mainly consumed filamentous and foliose Rhodophyta, fleshy and foliose Chlorophyta, and leathery macrophytes of the phylum Ochrophyta (Figure 2.4). To determine if differences exist between the means of the two genders or locations for each phylum, independent-samples t-tests were performed (Appendix A5). As with the comparison of the mean diet composition, outliers were included in the analysis and the t-test was performed even though data for each functional group was not normally distributed (Shapiro-Wilk's test, $p < 0.05$). Homogeneity of variances was met for the comparison of male and female fish ($p > 0.05$) for filamentous Rhodophyta; filamentous, fleshy, and foliose Chlorophyta; fleshy Ochrophyta, corticated and leathery macrophytes; and for the comparison of coastal and offshore fish for filamentous and foliose Rhodophyta; and all functional groups of Ochrophyta. If the assumption of homogeneity of variances was not met, the Welch t-test was used.

Male fish consumed more calcareous Rhodophyta 0.8% (95% CI, -0.3 to 1.8), foliose Rhodophyta 19.0% (95% CI, 9.7 to 28.4), foliose Chlorophyta 4.6% (95% CI, -11.3 to 20.5), corticated macrophytes of the phylum Ochrophyta 4.8% (95% CI, -6.0 to 15.5), and fleshy Ochrophyta 0.13% (95% CI, -3.2 to 3.4) than female fish and less of all the other functional groups: corticated macrophytes of the phylum Rhodophyta -0.8% (95% CI, -2.5 to 0.9), filamentous Rhodophyta -11.0% (95% CI, -20.0 to -1.9), fleshy Rhodophyta -8.0% (95% CI, -14.5 to -1.6), filamentous Chlorophyta -3.6% (95% CI, -12.2 to 5.1), fleshy Chlorophyta -1.0% (95% CI, -17.1 to 15.1), filamentous Ochrophyta -1.4% (95% CI, -4.1 to 1.3), foliose Ochrophyta -3.4% (95% CI, -8.5 to 1.2), and leathery macrophytes of the phylum Ochrophyta -0.1% (95% CI, -12.1 to 11.8). Differences in means between male and female *G. tricuspidata* were statistically significant for filamentous, fleshy, and foliose Rhodophyta (Figure 2.4a,c,e, Appendix A5).

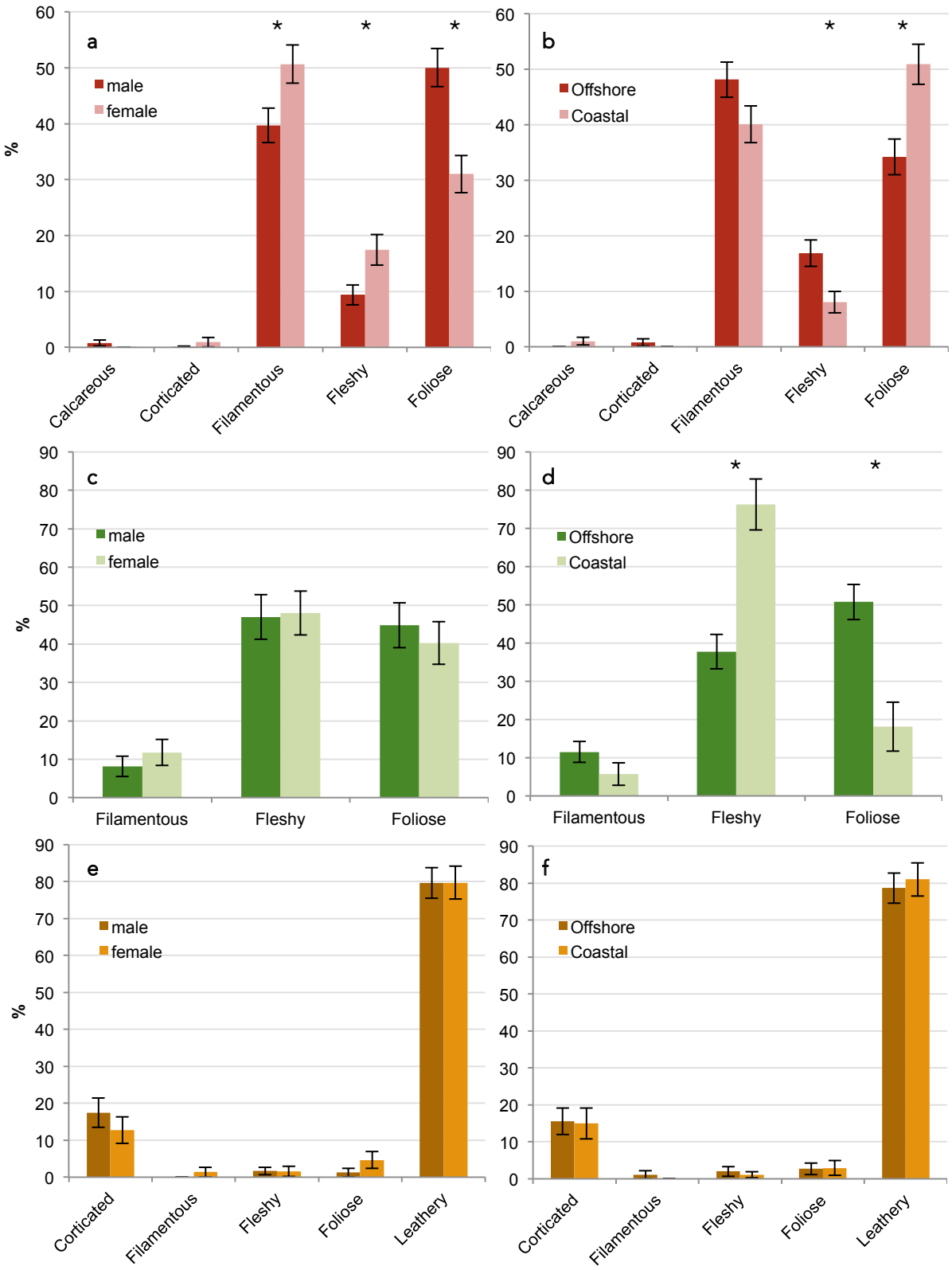


Figure 2.4: Mean composition of the functional groups of (a + b) Rhodophyta, (c + d) Chlorophyta, and (e + f) Ochrophyta. Graphs show the comparison between male and female fish (a, c, e) and coastal and offshore fish (b, d, f). Error bars indicate standard error. Asterisks mark diet categories with statistically significant differences between genders or locations (independent-samples t-test). For parameter values see Appendix A5.

Coastal fish ate more calcareous Rhodophyta 0.98% (95% CI, -0.4 to 2.3), foliose Rhodophyta 16.2% (95% CI, 6.6 to 25.7), fleshy Chlorophyta 38.5% (95% CI, -22.4 to 54.5), foliose Ochrophyta 0.2% (95% CI, -4.8 to 5.3), and leathery macrophytes of the phylum Ochrophyta 2.3% (95% CI, -9.6 to 14.3) than offshore fish, but ingested less corticated Rhodophyta -0.8% (95% CI, -2.1 to 0.5), filamentous Rhodophyta -8.3% (95% CI, -17.3 to 0.7), fleshy Rhodophyta -8.0% (95% CI, -14.1 to -2.0), filamentous Chlorophyta -5.8% (95% CI, -13.8 to 2.2), foliose Chlorophyta -32.7% (95% CI, -48.5 to -16.9), corticated macrophytes of the phylum Ochrophyta -0.5% (95% CI, -11.4 to 10.3), filamentous Ochrophyta -1.1% (95% CI, -3.6 to 1.4), and fleshy Ochrophyta -0.9% (95% CI, -4.2 to 2.4). There were statistically significant differences between means of coastal and offshore fish for the following functional groups: fleshy Rhodophyta, foliose Rhodophyta, fleshy Chlorophyta, and foliose Chlorophyta (Figure 2.4b,d,f, Appendix A5).

2.3.3 Habitat

G. tricuspidata mainly fed on epiphytic algae. These algae contributed 26.6% to the overall diet of female fish, 42.7% for male, 32.9% for offshore, and 38.2% for coastal fish. Pelagic animals and detritus also formed an important part of the diet for both genders (male: 17.0%, female: 18.4% for either category). Stomach contents of coastal fish contained 26.0% detritus and 14.7% pelagic animals, while pelagic animals (20.0%) were more important than detritus (10.6%) in the diet of offshore fish. Epilithic algae also contributed a considerable part to the diet of male (11.3%) and female fish (14.0%) as well as offshore ones (18.2%). Algae that can be found growing either epiphytic or epilithic were also important for female (13.8%) and offshore fish (11.1%) (Figure 2.5, Appendix A6).

Results of the independent-samples t-test displayed significant differences between male (n = 177) and female fish (n = 144) for the habitats epiphytic and epiphytic/epilithic (Appendix A6). Significant differences for the comparison of coastal (n = 146) and offshore (n = 175) populations were detected for the habitats epilithic, epiphytic/epilithic, and detritus. Outliers were present for all habitats except for epiphytic algae (gender comparison) and all habitats except epiphytic algae and coastal detritus (location comparison), which were evaluated by inspection of the boxplot for values greater than 1.5 box-lengths from the edge of the box. Outliers were considered true data points and were included in the analysis. The Shapiro-Wilk's test was used to determine whether data were normally distributed ($p < 0.05$). As the independent-samples t-test is considered fairly robust to deviations from normality, the tests were preformed even though data were not normally distributed for all

habitats (both genders and locations). Homogeneity of variances was met for the habitats epifauna, pelagic, unattached, detritus, and unknown, as assessed by the Levene's test for equality of variances ($p < 0.05$) and violated for all other habitats for the comparison of genders. For the comparison of locations the assumption was met for the habitats epifauna/pelagic, terrestrial, and unknown and was violated for the other habitats. In case of violation of the homogeneity of variances the Welch t-test was used.

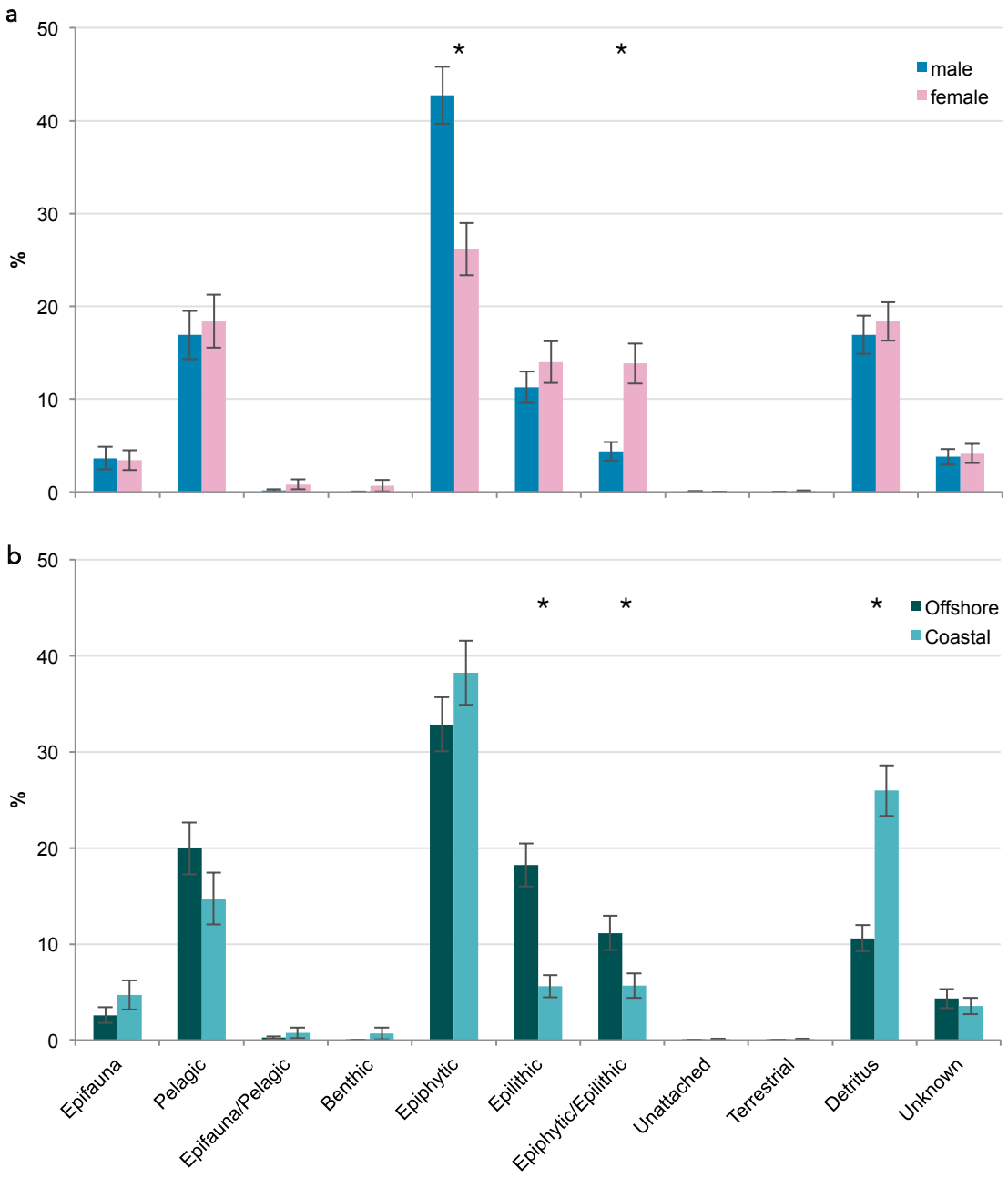


Figure 2.5: Mean percentage of different habitats that *G. tricuspidata* is obtaining its food items from. Graphs show the comparison between (a) genders and (b) locations. Error bars indicate standard error. Asterisks mark diet categories with statistically significant differences between genders or locations (independent-samples t-test). For parameter values see Appendix A6.

Male fish consumed more epifauna 0.2% (95% CI, -3.1 to 3.5), epiphytic algae 16.6% (95% CI, 8.4 to 24.7), and unattached algae 0.0% (95% CI, -0.1 to 0.1), but less food items from all other habitats: pelagic -1.5% (95% CI, -9.1 to 6.1), epifauna/pelagic -0.6% (95% CI, -1.7 to 0.5), benthic -0.6% (95% CI, -1.9 to 0.6), epilithic -2.7% (95% CI, -8.3 to 2.8), epiphytic/epilithic -9.4% (95% CI, -14.1 to -4.8), terrestrial -0.1% (95% CI, -0.2 to 0.0), detritus -1.4% (95% CI, -7.2 to 4.4), and unknown -0.3% (95% CI, -3.0 to 2.3) (Figure 2.5a, Appendix A6).

Coastal fish consumed more food items from the following categories: epifauna 2.1% (95% CI, -1.3 to 5.5), epifauna/pelagic 0.5% (95% CI, -0.5 to 1.5), benthic 0.7% (95% CI, -0.6 to 1.9), epiphytic 5.4% (95% CI, -3.2 to 13.9), unattached 0.1% (95% CI, -0.1 to 0.2), terrestrial 0.1% (95% CI, -0.1 to 0.2), and detritus 15.4% (95% CI, 9.5 to 21.2). Coastal fish consumed less food in the following categories: pelagic -5.2% (95% CI, -12.7 to 2.3), epilithic -12.6% (95% CI, -17.6 to -7.7), epiphytic/epilithic -5.5% (95% CI, -9.8 to -1.2), and unknown -0.8% (95% CI, 3.4 to 1.9) (Figure 2.5b Appendix A6).

2.4 Discussion

A great diversity of 110 different dietary items, which included 88 algal species, were found in the stomach contents. Rhodophyta made up nearly 50% of the diet followed by salps and detritus and comparison of the diet compositions revealed significant differences between gender and locations. Small foliose and filamentous, epiphytic Rhodophyta were the preferred algae. Less Chlorophyta and only small amounts of Ochrophyta were ingested. A considerable proportion of animal matter was ingested.

2.4.1 Diet composition

The detailed diet analysis shows that *Girella tricuspidata* should be classified as omnivorous with a mainly herbivorous diet. They feed on a wide range of epiphytic rhodophytes, supplemented with a substantial amount of animal matter, in particular salps (Figure 2.3). Animal material was not just ingested accidentally but accounted for 22.4% of the overall diet and was found in nearly 85% of fish. The high proportions of small filamentous and foliose epiphytic algae (Figure 2.4, 2.5) in the diet of *G. tricuspidata* shows the capability of the morphological specialised mouth to selectively remove epiphytic algae off the host macroalgae (Ferry-Graham & Konow, 2010; Kanda & Yamaoka, 1994; Vial & Ojeda, 1992; Yagishita & Nakabo, 2003). When foraging for food *G. tricuspidata* spends most of its time in the algae assemblages on rocky reefs browsing epiphytes of fucoid algae or rocks but, surprisingly, also spend a considerable amount of time in the water column feeding on zooplankton, namely salps (Figure 2.5). This feeding behaviour has not been described for *G. tricuspidata* in any previous literature. Arthropoda, mainly Gammaridea, Caprellidea, Isopoda, and Copepoda, were encountered in a large number of stomach contents. Many of these animals belong to the epifauna that lives on algae, and were most likely ingested when fish were browsing the epiphytes off the host plant. Only slight differences in diet compositions between both sexes and locations were detected (Figure 2.2). Significant differences between male and female fish might simply reflect sample differences. The higher consumption of Chlorophyta in offshore fish compared to coastal fish is most likely due to the higher abundances of *Ulva* spp. at Great Barrier Island than coastally (pers. observation, Hidas, 2001).

The most important alga *G. tricuspidata* fed on was *Abroteia suborbicularis* (Appendices A7, A8). This alga belongs to the family Delesseriaceae and is monotypic. It is also endemic to New Zealand. *A. suborbicularis* forms small, rounded to wedge-shaped blades that are rosy pink to crimson in

colour and usually less than two centimetres high. Several blades of various sizes are attached to one holdfast. They are soft and only one cell thick. *A. suborbicularis* grows subtidally as an epiphyte on *Carpophyllum* and *Landsburgia* species (Adams, 1994). *G. tricuspidata* has been observed browsing epiphytes growing on *Carpophyllum* species while avoiding ingesting the host plant (Choat & Clements, 1992). This is consistent with the findings of this study. Filamentous and foliose, epiphytic rhodophytes made up the largest portion of the diet. *Carpophyllum* fragments were recorded in the diet in nearly a third of the fish, but contributed little to the diet. Many *Carpophyllum* pieces found still had epiphytes attached to them, indicating that the fish was actually targeting the epiphyte and incidentally ingested parts of the host alga. In the literature filamentous algae (Thomson, 1959), rhodophytes (Choat & Clements, 1992; Russell, 1983), filamentous rhodophytes (Clements & Choat, 1997), and turfing rhodophytes (Raubenheimer et al., 2005) have previously been recorded as a major diet component of *G. tricuspidata*.

Detritus formed a large part of the diet and could be identified as of algal origin, but the fragments were too small to be identified. *G. tricuspidata* has a very acidic stomach enabling it to break down their food (T. A. Anderson, 1991). Therefore it is unknown whether detritus in this study was ingested as is or whether it derived from algae that were already partly digested in the stomach.

2.4.2 Ulva – the preferred alga? And other miscitations

Ulva spp. are sometimes referred to as the preferred algae (Curley et al., 2013; Gray et al., 2010) but diet analyses fail to reveal a major contribution to the diet of *G. tricuspidata* with a maximum of 11.1% (Choat & Clements, 1992; Clements & Choat, 1997; Kilner & Akroyd, 1978; Morrison, 1990; Raubenheimer et al., 2005; Russell, 1983; Thomson, 1959). In the present study all *Ulva* species combined only contributed 7.02% to the total diet and were found in nearly 38% of the fish. Other filamentous chlorophytes (5 species) were also recorded in nearly 12% of the fish, contributing 0.43% to the overall diet.

There have been numerous miscitations on diet in *G. tricuspidata*, leading to several misconceptions, including *Ulva* being the preferred dietary alga. The first diet analysis for *G. tricuspidata* was conducted at Lake Macquarie, NSW, Australia, by Thomson (1959), who recorded filamentous algae as the major diet component. Anderson (1987; 1988; 1991) used *Ulva* sp. in all his studies as food for *G. tricuspidata*, stating that it 'comprises a major portion of the diet', incorrectly referring to Thomson (1959). Gray et al. (2010) state that *G. tricuspidata*, besides seagrass and *Gracilaria* spp.,

predominantly feed on 'filamentous green algae *Enteromorpha* spp. and *Ulva* spp.'. They refer to Anderson (1987; 1991) and Raubenheimer et al. (2005). As discussed above, Anderson incorrectly cited Thomson (1959). Raubenheimer et al. (2005) performed seasonal gut content analysis on 54 subadult fish (150-250 mm SL) from Ti Point, New Zealand, with a maximum amount of 0.38 ± 0 % of *Ulva lactuca* and 4.83 ± 3.08% of *Enteromorpha intestinalis* in summer (n = 15), clearly not comprising a major part of the diet. Curley et al. (2013) cite Raubenheimer et al. (2005) and Clements and Choat (1997). They describe *G. tricuspidata* as omnivores that 'primarily consume chlorophytes and rhodophytes, and prefer chlorophytes such as *Enteromorpha intestinalis* and *Ulva lactuca*'. As discussed above, Raubenheimer et al. (2005) only recorded small amounts of *Ulva* species. Clements and Choat (1997) analysed six stomach contents from fish collected at Cronulla, NSW, Australia, and found a larger amount of filamentous chlorophytes (17.27 ± 14.49 %), but none were identified as *Ulva*. Ferguson et al. (2015) investigated the correlation between girellids and the cover of *Ulva* spp. at Jervis Bay, NSW, Australia, and, not unexpectedly, the abundance of *G. tricuspidata* was consistent across time and was not a response to the variation in *Ulva* spp. cover. They state that 'there is considerable evidence to suggest *G. tricuspidata* preferentially feed on *Ulva* spp.' but also admit that 'gut content analyses have failed to reveal significant amounts of *Ulva* spp. in the diet of *G. tricuspidata*'. They argue that '*in situ* feeding choice experiments (Gollan & Wright, 2006; R. B. Taylor & Steinberg, 2005), field observations (Kingsford, 2002), and fishers targeting *G. tricuspidata* using *Ulva* spp. primarily as bait (Gray et al., 2010) indicate *Ulva* spp. are the preferred algae of *G. tricuspidata*'. *G. tricuspidata* can be caught on various baits (Ring & Eccleston, 1986), including *Ulva* spp., but this does not demonstrate that this alga is an important dietary component for wild fish. The field observations by Kingsford (2002) conducted in NSW, Australia, did not reveal that *G. tricuspidata* were actually feeding on *Ulva*, but rather that they were 'observed feeding on turfing algae, particularly where solitary ascidians ('cunjei' *Pyura stolonifera*) and/or *Ulva* covered a large percentage of the substratum'. Results of feeding studies should be viewed with caution, as they only can show the preference of feeding on one alga over the other within the range of algae offered. During the in situ feeding experiments by Taylor and Steinberg (2005) and Gollan and Wright (2006) fish were offered nine and eight different algae, respectively. *Ulva* spp. was the only dietary alga in these studies and the other algae offered, if present in the diet at all, contributed less than 0.32% of the overall diet (present study). On several occasions large patches of sheet-forming *Ulva* sp. were observed during sampling at Great Barrier Island in the present study, but only fourteen fish from the entire sample set had more than 75% of this alga in their stomachs. The records in the literature, data in this study and personal observations lead to the conclusion that *Ulva* spp. are consumed by *G. tricuspidata* and form part of their diet, but are not the predominant dietary targets.

Gray et al. (2010) also drew incorrect conclusions from a feeding experiment (Raubenheimer et al., 2005), stating that *Gracilaria* spp. form a major part of the diet. Raubenheimer et al. (2005) used *Gracilaria chilensis* in their experiment, clearly stating that this is a non-dietary item. Fish only fed on this rhodophyte because they were given no other choice. Gray et al. (2010) and Conacher et al. (1979) also state that the seagrass *Zostera capricorni* is part of the diet of *G. tricuspidata*, but none of the studies they cited actually record seagrass as a dietary item. Juvenile fish have been observed to recruit into seagrass beds (Rotherham & West, 2002), but this is for shelter not to feed on the seagrass, as juvenile fish are planktivorous.

2.4.3 Conclusions

Results of the diet composition in the present study match the diet description of other *Girella* species, which have also been described as herbivores or omnivores (Froese & Pauly, 2015; Yagishita & Nakabo, 2003). Omnivorous species include *G. cyanea* (Lewis, 2012), *G. nigricans* (Barry & Ehret, 1993; Behrens & Lafferty, 2012), *G. laevifrons* (Muñoz & Ojeda, 1997), and *G. melanichthys* (Kanda & Yamaoka, 1994). *G. punctata* has been described as intermediate between omnivory and herbivory, while *G. mezina* is considered a herbivore (Kanda & Yamaoka, 1994). These girellids mainly feed on algae (Rhodophyta and Chlorophyta) but also include some animal material in their diet (crustaceans, bivalves).

The diet of *G. tricuspidata* is highly variable. Even though 110 different dietary items were identified, most were only encountered in a few fish or contributed little to the diet. Epiphytic rhodophytes formed the largest part of the diet, with *A. suborbicularis* being the most important. Differences in the diet are to be expected for other populations of *G. tricuspidata* within New Zealand. These fish are opportunistic browsers, and the diet will depend on the spatial and temporal abundance and distribution of their food (Chapter 3). Differences in the diet will be even greater for populations in Australia as the main dietary alga *A. suborbicularis* is endemic to New Zealand. *Ulva* spp. have often been described as the preferred algae. The results of the present diet analysis and records in the literature show *Ulva* spp. can be found in the diet of *G. tricuspidata* but it is not a major dietary item. Animal material was recorded in the stomach contents. The salp *T. democratica* forms a large part of the diet and it shows that the diet of this fish is best described as omnivorous. Due to the high abundances of *G. tricuspidata* in New Zealand they are likely to play an important role in transferring a considerable amount of energy from the lowest level of primary producers to higher trophic levels.

Chapter 3

Seasonal variation in diet and nutrition, and the availability of epiphytes

3.1 Introduction

Survival and ultimately growth and successful reproduction of every organism depends on the availability of suitable food. Seasonal changes in temperate regions and to a greater extent in subpolar regions, can lead to dramatic changes in the productivity of primary producers in terrestrial and marine ecosystems. This in turn affects heterotrophs, predominantly primary consumers such as herbivores that depend on primary producers for food (Townsend, Harper, & Begon, 2003). Algae exhibit seasonal changes in abundances and nutrient composition (Kaehler & Kennish, 1996; Lamare & Wing, 2001; McQuaid, 1985; Schiel, 1985). Epiphytic algae, the main food source of *Girella tricuspidata* (Chapter 2), are no exception and display seasonal variation in abundances and diversity in temperate locations such as Spain, Ireland, and south-west Australia (Arrontes, 1990; Kendrick & Burt, 1997; Otero-Schmitt & Pérez-Cirera, 1996; Rindi & Guiry, 2004). This variation will inevitably lead to seasonal diet variation in herbivores and may preclude herbivorous fish from obtaining their optimal diets (Horn, 1983; Horn et al., 1986; Horn, Murray, & Edwards, 1982).

Information on the diet of animals, as established for *G. tricuspidata* in the previous chapter, does not explain food choice. Nutritional ecology aims to understand the interaction between an organism and its environment by investigating the factors that drive food choice: nutrition, physiology, and behaviour (Clements & Raubenheimer, 2006; Foley & Cork, 1992; Raubenheimer & Boggs, 2009).

Understanding food choice requires knowledge of the nutritional properties of plants (positive choices) and the deterrent effects of secondary compounds (negative choices) (Baker et al., 2016; Choat & Clements, 1998). The value of food differs between algal taxa due to differences in macronutrient content and chemical composition (Kaehler & Kennish, 1996; Lamare & Wing, 2001; Montgomery & Gerking, 1980). Protein is usually argued to be the limiting nutrient in herbivorous fishes due to the low perceived content in algae, and protein levels are often used as an indicator of diet quality (Bowen et al., 1995; Horn, 1989). Nevertheless, total protein or energy levels do not always explain food choices by herbivores (Neighbors & Horn, 1991) as effectively as an optimal balance of nutrients (Choat & Clements, 1998; Simpson & Raubenheimer, 2001). Fish exhibit different feeding strategies. Some herbivorous fishes selectively feed on the most nutritious algae or algae parts (Clements & Choat, 1993; Horn et al., 1986; Lobel & Ogden, 1981), while other species defend territories against feeding activities of other herbivores to ensure access to the most nutritious food resources (Bruggemann, van Oppen, & Breeman, 1994; Vitelli, Hyndes, Kendrick, & Turco, 2015). The nutritional process in fishes is also subject to seasonal changes (Clements & Raubenheimer, 2006).

Nutrient and energy requirements of fishes vary during the course of a year in response to the metabolic demands of activity, growth, and reproduction (Bureau et al., 2002). Temperature affects these metabolic demands, and also digestive enzyme activities and assimilation (Bakke, Glover, & Krogdahl, 2011; A. Clarke & Johnston, 1999; Clements & Raubenheimer, 2006; Stevens & Hume, 1998). Fish respond to variation in temperature by modulating food intake, gut transit times, and assimilation efficiencies (Horn & Gibson, 1990).

Ontogenetic diet shifts are associated with changes in relative gut lengths (Benavides et al., 1994; German & Horn, 2006). Species of the family Stichaeidae increase their gut length and gut mass in association with an ontogenetic shift from carnivory to herbivory or omnivory as body size increases. Carnivorous stichaeid species did not show this pattern (German & Horn, 2006; German, Gawlicka, & Horn, 2014). Diet quality and quantity can also affect the gut structure over shorter time scales, and it has been shown that fish can down-regulate the surface area of the gastrointestinal tract or significantly reduce relative gut length when faced with starvation or a diet that fails to meet energy demands (German, Neuberger, Callahan, Lizardo, & Evans, 2010; Rios, Kalinin, Fernandes, & Rantin, 2004). Relative gut length can change in as little as eight weeks in herbivorous stichaeid fishes (Behrens & Lafferty, 2012).

Understanding food selection requires information on seasonal variation in availability of the dietary items and the nutritional ecology of the study species. How *G. tricuspidata* meets nutrient requirements through diet choice and whether seasonal differences in the nutrient demand and intake of adult fish affect this is unknown. The condition factor provides information on the well-being of fish (Froese, 2006), and can be used to evaluate the condition of fish over seasonal cycles and whether they are affected by additional energy expenditure during the reproductive season. Although some information about the digestive mechanisms in *G. tricuspidata* exist (T. A. Anderson, 1987; 1988; 1991; Clements & Choat, 1997; Moran & Clements, 2002; Raubenheimer et al., 2005; Skea et al., 2007; White, Coveny, Robertson, & Clements, 2010; Zemke-White et al., 1999), nutrient composition and nutrient intake of the diet has not received much attention. Determining the nutrient composition of stomach contents provides a means of measuring nutrient intake in wild, free-feeding fishes. In combination with gut content mass it can also provide a proxy for nutrient intake.

3.1.1 Aims and objectives

The three main objectives of this chapter are to establish (1) whether diet and nutrient composition vary seasonally between coastal and offshore sites, (2) how *G. tricuspidata* regulates diet and nutrient intake across the year, and (3) if increased nutrient intake is associated with reproduction.

These result in further questions, which this chapter also examines:

1. Are epiphytes, i.e. the main dietary item, available throughout the year, or do they show seasonal changes in abundance?

2. Is seasonal variation in food abundance reflected by seasonal changes in diet/nutrient composition?

3. What is the characteristic nutrient composition of the various dietary categories? Does *G. tricuspidata* adjust its nutrient intake by choosing food items accordingly?

4. When is the reproductive season? Is this associated with additional energy expenditure, and does this have an effect on the condition of fish?

5. If spatial and temporal differences in diet and nutrient composition/intake are present, are these associated with differences in relative gut length and relative gut content mass?

3.2 Methods

3.2.1 Seasonality of epiphytes on *Carpophyllum maschalocarpum*

The host fucoid macroalga *Carpophyllum maschalocarpum* was chosen to investigate seasonal variation in epiphyte abundances. *G. tricuspidata* has been observed browsing epiphytes off *Carpophyllum* species (Clements & Choat, 1997) and greatest abundances of *G. tricuspidata* were recorded in shallow *Carpophyllum* habitat (Brown, 2015). Samples of *C. maschalocarpum* were collected either snorkelling or diving from 1-2 metres below the low tide mark. Ten plants were gathered from each of two different locations in north-eastern New Zealand close to the village of Leigh. The first location, Mathesons Bay, lies on the southern coast of Leigh (36°302 S, 174°801 E) and is characterized by a rocky reef that steeply drops off to about 5 m depth. The second location, Kempts Bay, is situated at the northern end of Cape Rodney – Okakari Point Marine Reserve (Goat Island) (36°260 S, 174°765 E). Boulders dominate this shallow reef. All *C. maschalocarpum* plants were collected within 15 m of each other. Collection took place seasonally between November 2013 and December 2014 (Table 3.1).

Table 3.1: Collection dates of *C. maschalocarpum* for analysis of seasonality of epiphytes.

	Spring 2013	Summer 2014	Autumn 2014	Winter 2014	Spring 2014
Kempts Bay	11/11/2013	05/02/2014	13/05/2014	03/09/2014	05/12/2014
Mathesons Bay	21/11/2013	28/02/2014	13/05/2014	03/09/2014	05/12/2014

Sampled algae were weighed and measured within 24 hours. Each plant was divided into three equally long pieces (bottom, middle, and top) that were each weighed separately again. A subsample of 10 cm length was taken from each section and frozen until further analysis. Frozen subsamples were then thawed and epiphytic growth investigated. Five 1 cm^2 pieces were inspected to quantify epiphytic abundance by estimating the cover by epiphytes. A simple subjective scoring system, ranking from 0 to 6, was used to record epiphyte cover (modified after Arrontes, 1990; D. L. Ballantine, 1979):

0 - Host without epiphytes

1 - Very few epiphytes, less than 5%

2 - Some epiphytes, between 5% and 25%

3 - Epiphytes fairly numerous, between 25% and 50%

4 - Epiphytes numerous, between 50% and 75%

5 - Epiphytes very numerous, between 75% and 95%

6 - Host covered in epiphytes, 95% to 100%

All epiphytes were then identified to species level where possible and carefully removed from the host plant. The subsample of the host plant and each epiphyte species were weighed separately (WW). Samples were dried to constant weight in the oven at 60°C and dry weight (DW) recorded.

3.2.2 Fish sampling

Collection of fish was conducted at coastal and offshore sites as described in Chapter 2.2.1 Fish sampling. Each fish was measured to the nearest mm (fork length (FL) and standard length (SL)) and weighed to the nearest g (total weight (TW) and gutted weight (GW)). Immediately following capture the gut was removed from the fish, measured without stretching and divided into four sections with the stomach being section I and the rest of the intestine divided into five sections of equal lengths termed II-V following Mountfort et al. (2002). The contents of each section were placed in separate vials and immediately frozen in liquid nitrogen. Upon return to the laboratory the samples were weighed (WW) and stored at -80°C. 50% (by weight) of each stomach content sample and the complete samples of sections II to V were freeze dried to constant weight (DW) (Heto PowerDry LL 3000). Samples were stored desiccated at -20°C until further analysis. Gonads of mature fish were removed and weighed (to the nearest 0.1 g).

3.2.3 Diet analysis

Diet analysis was conducted as described in Chapter 2.2.2 Diet analysis.

3.2.4 Nutrient analysis

Stomach content samples were ground with a mixer mill (Retsch mixer mill MM301) for 10 seconds at 25 Hertz. Grinding jars were submerged in liquid nitrogen beforehand to avoid lipid burn-off. Samples were stored desiccated at -20°C until processed for nutrient analysis.

3.2.4.1 C:H:N analysis

The carbon and nitrogen content of macroalgae are frequently used as proxies for energy and protein content, respectively (Clements & Raubenheimer, 2006; Kaehler & Kennish, 1996; Rico &

Fernández, 1996). Protein content can be variable between different food items (Mariotti, Tomé, & Mirand, 2008). Amino acid analysis by high performance liquid chromatography (HPLC) yields a more accurate measure of nutritionally significant nitrogen than commonly used spectrophotometric methods (e.g. Bradford or Lowry assays) but HPLC is a time consuming and expensive procedure (Crossman et al., 2000). However, nitrogen content can be cautiously used as a proxy for protein content and to evaluate the nutritional value of the diet (Baker et al., 2016). The carbon:nitrogen (C:N) ratio is also used as a general estimate of food quality (Niell, 1976).

Total nitrogen and carbon contents of each stomach content were determined with a CE-440 Elemental Analyser (Exeter Analytical, Inc.) operated by Auckland University of Technology. Between 2.0 to 3.5 mg of each sample was weighed into tin capsules to the nearest 0.01 mg (Mettler Toledo Excellence, XS205 Dual Range). Duplicates were run for each sample. Samples were combusted at about 975°C in pure oxygen under static conditions. Helium was used to carry the combustion products through the analytical system at atmospheric pressure, as well as for purging the instrument. Carbon, hydrogen, and nitrogen output signals were recorded while the sample gas flowed through the detectors.

3.2.4.2 Lipid analysis

Lipids are a chemically heterogeneous group of substances having in common the property of insolubility in water, but solubility in non-polar solvents such as chloroform, hydrocarbons, or alcohols (Gurr & Harwood, 1991). Extraction procedures have therefore varied little since those outlined by Folch (1957) and Bligh and Dyer (1959). Following the basic principle of those methods lipids were extracted following the procedure as described by Mann and Gallager (1985) and Johnson (2011).

50 mg of the homogenized sample was weighed into glass vials to the nearest 0.01 mg (Mettler Toledo Excellence, XS205 Dual Range). Triplicates were run for each sample. If the remaining sample was too small 10 – 20 mg were used. Samples were mixed with 100 µl purified water and 1.5 ml chloroform : methanol (1:2, v:v) and vortexed until sample was well mixed. Samples were left to stand on ice for 10 minutes and then centrifuged for 5 minutes at 1000g (Eppendorf 5810R). The supernatant was removed into a glass vial and kept on ice. The pellet was re-extracted with 1.5 ml chloroform : methanol (2:1, v:v), vortexed, left to stand on ice for 10 minutes and centrifuged again (1000 g, 5 min). The supernatant was removed and added to the supernatant from the first extraction step. The pooled supernatants were then mixed with 950 µl NaCl (0.7%, w:v), vortexed and left to stand on ice for 30 minutes and centrifuged (1000 g, 5 min), which separated the upper and lower

phase. The chloroform layer, which contains the lipids, was extracted with a pipette and 1ml was placed into pre-weighed aluminium caps and left to evaporate in the fume cupboard overnight. Lipids remained and their quantity was measured gravimetrically to the nearest 0.01 mg.

3.2.4.3 Ash

Ash, water and fibre dilute the nutrient content of the diet (Baker et al., 2016). Ash content is a measure of the total amount of inorganic minerals and is usually seen as the indigestible part of the diet, in contrast to the ash-free organic matter containing the nutrients. Even though some minerals are assimilated (Bjorndal, 1985), the caloric content of algae is negatively correlated with the ash content (Lamare & Wing, 2001), and therefore provides a cautious estimate of the nutrient content of the diet.

About 100 mg of sample was weighed into pre-weighed and pre-muffled borosilicate culture tubes and placed in aluminium heater blocks. If enough sample material was available duplicates were run. If less than 100 mg was available as much material as possible was used to run one sample. Samples were placed in a muffle furnace and combusted for 6 hours at 500°C. Pre-experiments showed that running the experiment for 12 hours did not decrease the ash yield any further. Samples were left to cool in the muffle furnace and then re-weighed to determine the ash content of the samples.

3.2.5 Data analysis

Seasons were assigned based on the warmest (summer) and coldest (winter) sea surface temperatures resulting in January to March as summer, April to June as autumn, July to September as winter, and October to December as spring.

If not mentioned otherwise statistical tests were conducted using the software IBM SPSS Statistics v22 with the SPSS statistics guide (Laerd Statistics, 2015).

3.2.5.1 Seasonality of epiphytes on C. maschalocarpum

The average number of species and abundances were calculated as the average for the whole host plant and for each section of the host plant. The biomass was calculated as g DW epiphyte per kg DW host for each section of *C. maschalocarpum*. The biomass for each section was extrapolated from the subsample and the sum of all three sections resulted in the total algae epiphyte biomass for each

host. Hydroids, i.e. animals, were excluded from biomass calculations. *Lithophyllum* sp. was also excluded, as these are coralline algae and DW consists mainly of inorganic $CaCO_3$, which would result in erroneously high biomass values.

A Kruskal-Wallis H test was run to determine significant differences of the total epiphyte biomass between seasons. Pairwise comparisons were performed using Dunn's procedure with a Bonferroni correction for multiple comparisons. To test for differences between the two locations Mann-Whitney U tests were run for each season.

3.2.5.2 Seasonal changes in diet composition

Percentage compositions of the diet were calculated for each stomach content. The results for the eight main diet categories (as described in Chapter 2.2.2 Diet analysis) were averaged for each season. Multivariate analysis of the diet categories was performed to test for differences between seasons using the software PRIMER v6.1.12 (K. R. Clarke & Gorley, 2006) with the PERMANOVA+ v1.0.2 add-on (M. J. Anderson et al., 2008). Data were square root transformed and converted to resemblance matrices using Bray-Curtis dissimilarity coefficients. Principal coordinate analysis (PCO) was used to display patterns of similarities and differences between the diets for coastal and offshore fish. The permutational multivariate analysis of variance (PERMANOVA) was performed to detect significant differences between seasons for each location. PERMDISP was conducted to analyse homogeneity of multivariate dispersions on the basis of the resemblance measure. Pairwise tests (ANOSIM) revealed seasonal differences in the diet.

3.2.5.3 Nutrient analysis and reproducibility

The nutrient content of material in the foregut of captured fish was quantified on the basis of ash, nitrogen, lipid, and carbon. All results are reported as the percentage of DW. Hydrogen was not included in further analysis as it is highly influenced by the water content of the sample. Conversion factors have been established in numerous studies to estimate protein contents from nitrogen measurements. The universal factor of 5.0 has been suggested for algae (Angell, Mata, de Nys, & Paul, 2016), and will be used in this study for the algal categories, and conversion factor of 5.6 is used for the other categories (Mariotti et al., 2008). The crude amount of carbohydrate was estimated by difference, i.e. by subtracting % ash, % protein, and % lipid from 100% (Johnson, 2011; Montgomery & Gerking, 1980).

The accuracy and reproducibility of assays for carbon, protein, lipid, and ash were tested using replicate samples of stomach content material. For lipids and ash the difference of the mean was expressed as percentage for each replicate, and reproducibility was then calculated as the overall mean of these values. For carbon and protein all samples were used to calculate reproducibility. Differences between duplicates for each sample were expressed as a percentage of the mean of the two measurements. The mean of differences was then calculated across all samples.

3.2.5.4 Relationship between diet and nutrient composition

Two methods were used to estimate nutrient contents (carbon, nitrogen, lipids, ash) of individual diet categories from the measured values for total combined gut contents. (1) Gut contents of many individual fish were dominated (> 90%) by a single diet category. For these diet categories the nutritional content of the total stomach contents were averaged across the relevant fish assuming that they accurately reflect the nutritional content. For this, sufficient individual fish were available for sufficient quantities of Rhodophyta (n = 91), Chlorophyta (n = 9), animal matter (n = 5), salps (n = 35), and detritus (n = 10) for these categories to be analysed separately. (2) Nutritional contents of all dietary components were estimated by using the Excel add-in tool Solver to iteratively adjust single nutrient values of individual dietary components, so that when multiplied by the proportion of that dietary component in each individual fish, the sum of absolute differences between the estimated and measured total nutrient contents across all fish was minimised. The category others was excluded from the analysis due to its highly variable content and its negligible contribution to the overall diet.

Principal coordinates analysis (PCO) was used to visualise whether there was a relationship between diet and nutrient composition. Only fish that had ingested more than 90% of one dietary category were used, as the influence on the nutrient composition of the remaining 10% was considered negligible. Additionally, only diet categories with $n \geq 5$ were used. This applied to the following five dietary categories: Rhodophyta (n = 91), Chlorophyta (n = 9), animal matter (n = 5), salps (n = 35), and detritus (n = 10). Nutrient data were normalised and converted to resemblance matrices using Euclidean distance. PERMDISP was conducted to test for homogeneity of dispersions on the basis of the resemblance measure. One factor PERMANOVA was run to test for differences between dietary items. To test for significant differences among groups an ANOSIM was conducted. The same analysis was also performed on species level for the diet items *Abroteia suborbicularis* (n = 23), detritus (n = 10), other crustacean (n = 3), salps (n = 35), and *Ulva* spp. sheet (n = 5), resulting in a smaller sample size than on category level. The multivariate analysis was performed using the software PRIMER v6.1.12 (K. R. Clarke & Gorley, 2006) with the PERMANOVA+ v1.0.2 add-on (M. J.

Anderson et al., 2008). No statistical tests were performed to test for differences between coastal and offshore fish, as there were only two diet categories (salps and Rhodophyta) in fish collected from both locations.

3.2.5.5 Seasonal changes in nutrient composition

Multivariate analysis of nutrient compositions was performed to test for spatial and temporal differences. The similarity matrix was constructed on normalised data using the Euclidian distance similarity measure. Principal coordinates analysis (PCO) was used to display patterns of similarities and differences between the seasons for each region. One factor PERMANOVA was conducted for each region to detect significant differences between seasons. To locate statistically significant differences pairwise tests (ANOSIM) were performed for each season. PERMDISP was used to analyse homogeneity of multivariate dispersions on the basis of the resemblance measure. All tests were performed using the software PRIMER v6.1.12 (K. R. Clarke & Gorley, 2006) with the PERMANOVA+ v1.0.2 add-on (M. J. Anderson et al., 2008).

3.2.5.6 Gonadosomatic Index

The Gonadosomatic Index (GSI) is used as a measure of changes in reproductive state (Ebert, Hernandez, & Russell, 2011) and was calculated using the following equation:

$$GSI = 100 * GoW / TW$$

with GoW as gonad weight (g) and TW as the total somatic weight of the fish (Hostetter & Munroe, 1993).

3.2.5.7 Condition factor

The condition factor (K_{TW}) is used to compare the condition (fatness, well-being) of fish based on the assumption that heavier fish of the same length are in better condition (Froese, 2006). It was calculated for adult fish using the equation:

$$K_{TW} = TW * 100 / FL^3$$

with TW as the total weight of the fish and FL the fork length (Froese, 2006). The condition factor was also calculated using the gutted weight (GW) of fish instead of the TW:

$$K_{GW} = GW * 100 / FL^3$$

The second equation eliminates any impact that gut weight, gut content mass, or gonad weight might have on the fish.

3.2.5.8 Relative gut content mass

The wet weight of the gut contents for each gut section was recorded for 355 specimens. The results of 204 out of 1420 gut sections had to be excluded as gut sections were damaged and gut contents had leaked out. The following number of fish were included in the analysis: section I = 355 fish, II = 295, III = 308, IV = 298, and V = 310.

For each fish the relative gut content mass for the whole gut and each section of the gut was calculated as follows (Choat, Robbins, & Clements, 2004):

Relative gut content mass = wet weight gut content mass (g)/ total body weight (g)

Mann-Whitney U tests were run to test for differences in total relative gut content mass (sections I-V combined) between locations. Seasonal changes were analysed using Kruskal-Wallis H tests.

To test whether feeding on a particular diet category was linked to the the amount eaten (relative gut content mass) a two factor PERMANOVA was conducted with season and relative gut content mass as factors. Diet category data were square root transformed and converted to resemblance matrices using Bray-Curtis dissimilarity coefficients. Relative gut content mass data were ranked into eleven categories. The highest recorded value for relative gut content mass was 0.1415. Ten equal categories were assigned in 0.015 steps and an additional category for stomach contents with a relative gut content mass of 0.0.

3.2.5.9 Diel variation in relative gut content mass and nutrient composition

G. tricuspidata is a diurnal feeder with feeding rates higher in the afternoon than in the morning (Pankhurst, 1989; Raubenheimer et al., 2005). Combined with diel variation in nutrient levels in algae (Zemke-White, Choat, & Clements, 2002), this creates the possibility that variation in time of sampling could influence seasonal and location comparisons. Independent-samples t-tests were therefore performed to test for diel variation in gut content mass and nutrient composition (carbon, nitrogen, lipid, and ash) of stomach contents between fish sampled in the morning, early afternoon, and late

afternoon. Morning fish were classified as fish caught between 9.30 am and 10.00 am and early afternoon fish between 1.00 pm and 1.40 pm. Late afternoon fish were only collected from the offshore location, and these were caught between 5.35 pm and 6.15 pm. A Mann-Whitney U Test was also performed to test for differences in nutrient composition between the two regions.

3.2.5.10 Age related differences in relative gut content mass and nutrient composition

Independent-samples t-tests were run to determine whether relative gut content mass, composition of carbon, nitrogen, lipid, and ash, and C:N ratio differed between coastal and offshore populations in young and adult fish. Fish between the ages of 3 to 5 years were classed as young fish and fish between 18 to 20 years as adult fish. These ages were chosen as they are closest to the rVBGF parameters L_2 and L_{18} (see Chapter 4.2.1.2 Size-at-age modelling). Nutrient analysis was not performed on juvenile fish, therefore the youngest fish in the sample range were used for analysis. Fish were aged as described in Chapter 4.2 Methods.

3.2.5.11 Relative gut length and Zihler Index

Gut length can be an indication of the food fish consume and which trophic category they belong to (Horn, 1989). Relative gut length (RGL) is most commonly used to compare fishes with different diets (German & Horn, 2006; Kapoor et al., 1976):

$$\text{RGL} = \text{gut length (mm)} / \text{standard length (mm)}$$

However, RGL has been criticized as gut length can vary with different body shapes. For example, deeper-bodied fish can accommodate far longer, more coiled guts than elongate fish and differences in body mass can also produce misleading results (German & Horn, 2006; Kramer & Bryant, 1995a; 1995b). It has therefore been proposed that body mass be taken into account when comparing small and large fishes of the same species (German & Horn, 2006; Kramer & Bryant, 1995b). The Zihler Index includes body mass and is a potentially powerful approach:

$$\text{ZI} = \text{gut length (mm)} / 10\ [\text{body mass (g)}^{1/3}]$$

Results for both equations are presented. Independent-samples t-tests were run to determine differences in the RGL and the ZI between the two locations. A one-way ANOVA was performed to test for seasonal changes in the ZI for both locations, followed by a Tukey HSD post hoc test for multiple comparisons.

3.3 Results

3.3.1 Seasonality of epiphytes on *C. maschalocarpum*

A total of nine species of epiphytic algae were recorded on *C. maschalocarpum* (Table 3.2). Eight of these species belonged to the order Ceramiales and were present in the diet of *G. tricuspidata*.

Table 3.2: Species list with short description of epiphytes found on *C. maschalocarpum*. Presented are the average (bold) and maximum values (italics) (in g epiphyte per kg host) for the bottom, middle, and top part of the host. Dashes indicate that the epiphyte was not recorded on that part of the host plant.

Species	Bottom	Middle	Top	Appearance
Order Ceramiales				
Abroteia suborbicularis	**7.0** *147.3*	**10.6** *183.5*	**6.7** *107.7*	Multiple oval blades off one holdfast, less than 2 cm, often on edge of blades, bright red/purple
Nancythalia humilis	**0.2** *4.0*	**1.2** *20.0*	**4.0** *58.3*	Multiple elongate blades off one holdfast, up to 2.5 cm, often on edge of blades, red/greenish(/brownish)
Dasyclonium bipartitum	**1.3** *27.5*	**2.3** *64.0*	**0.1** *9.5*	Filamentous, creeping under 2 cm with many holdfasts, often on edges of blades and stipe, red/brownish
Dipterosiphonia heteroclada	-	**0.1** *3.8*	-	Filamentous, bushy, fern-like fronds, up to 2 cm, red(/brownish)
Ceramium sp1	**0.0** *2.1*	**0.3** *3.8*	**0.1** *5.6*	Filamentous, bushy, 2 cm, grows mostly in blade axis and next to receptacles, red(/brownish)
Ceramium sp2	**0.1** *2.8*	**0.2** *3.4*	**0.2** *5.4*	Similar to Ceramium sp1 but less than 1 cm, bushy, epiphytic on *Abroteia suborbicularis* and *Nancythalia humilis*, red(/brownish)
Ceramiales sp1	-	**0.2** *10.0*	**0.1** *6.2*	Filamentous, unicellular strings with whorls of 4-5 branches after each cell, forming dense clumps
Metamorphe colensoi	-	**0.1** *8.4*	-	Filamentous, creeping along blades with fernlike, pinnately-divided fronds, 1.5-2 cm high, red(/brownish)
Order Corallinales				
Lithophyllum sp1	**0.1** *10.1*	**0.1** *13.1*	**0.2** *15.6*	Red coralline algae, pink
Phylum Cnidaria, Class Hydrozoa				
Hydroids	**0.1** *10.1*	-	-	Clear, brownish, feathery

Lithophyllum sp. was the only exception as it did not form part of the diet and belongs to the order Corallinales. Hydroids were also found on *C. maschalocarpum* and in the stomach contents. The average number of epiphytic species found on the host plant ranged from 1.3 to 3.1 at Kempts Bay and 0.4 to 2.1 at Mathesons Bay and highest diversity was found in the middle part of the host (Figure 3.1).

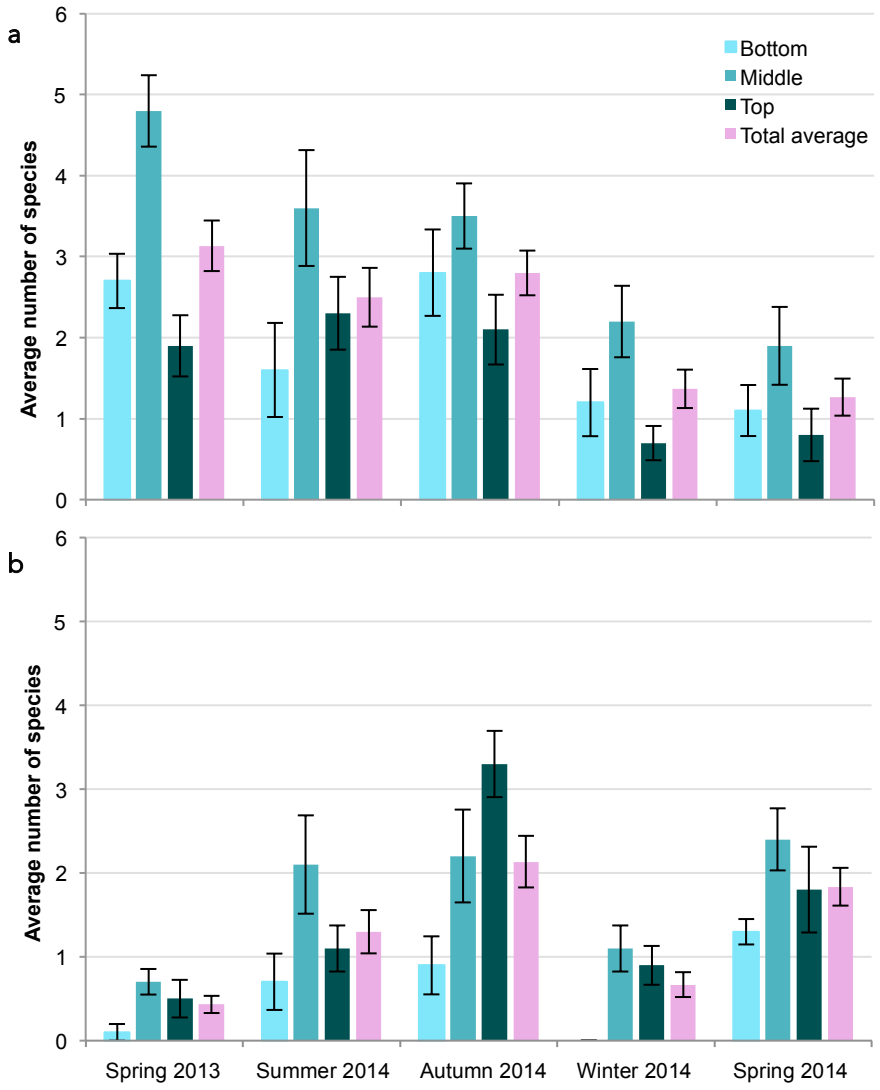


Figure 3.1: Species diversity expressed as the average number of epiphytic species on *C. maschalocarpum* on the top, middle, and bottom part of the plant and as total average for (a) Kempts Bay and (b) Mathesons Bay. Error bars indicate standard errors.

The middle part of the host plant was most heavily epiphytized (Table 3.2). *N. humilis* and *Lithophyllum* sp1 were the only epiphytes most abundant on the top part of the host. *Abroteia suborbicularis* accounted for the majority of the total epiphytic biomass (70.1%), while *Nancythalia*

humilis (14.5%) and *Dasyclonium bipartitum* (12.0%) contributed minor proportions. Biomass of all other epiphytes was negligible (Appendix B1).

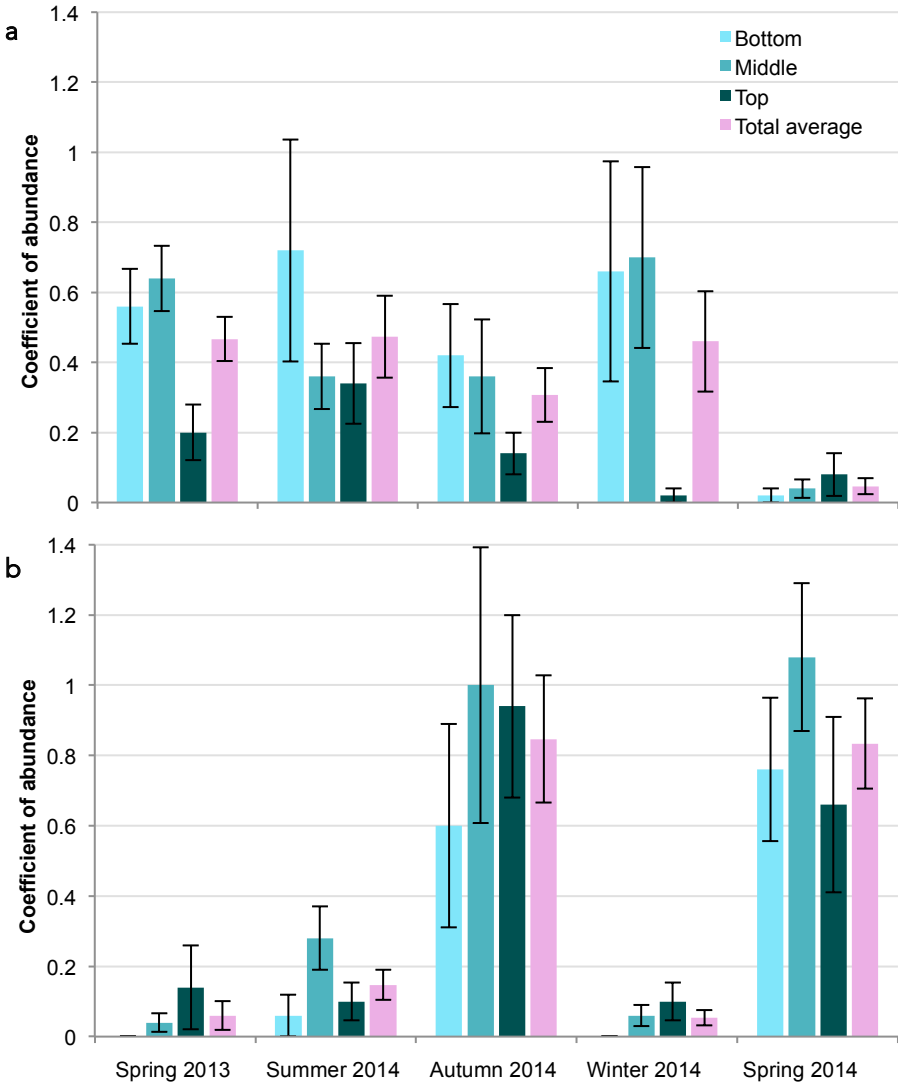


Figure 3.2: Average coefficient of abundance showing the cover of epiphytes on *C. maschalocarpum* on the top, middle, and bottom part of the plant and as total average at (a) Kempts Bay and (b) Mathesons Bay. Error bars indicate standard errors.

Epiphyte abundances varied between seasons and locations (Figure 3.2 & 3.3). At Kempts Bay the abundances were highest during winter 2014 (26.1 g epiphytes / kg host) and summer 2014 (17.3 g epiphytes / kg host). Lowest values were recorded in spring 2014 (1.7 g epiphytes / kg host). At Mathesons Bay the highest biomasses were recorded in autumn 2014 (29.5 g epiphytes / kg host) and spring 2014 (27.5 g epiphytes / kg host), while lowest were recorded in winter 2014, spring 2013, and summer 2014 (0.3 g, 1.3 g, and 1.9 g epiphytes / kg host, respectively).

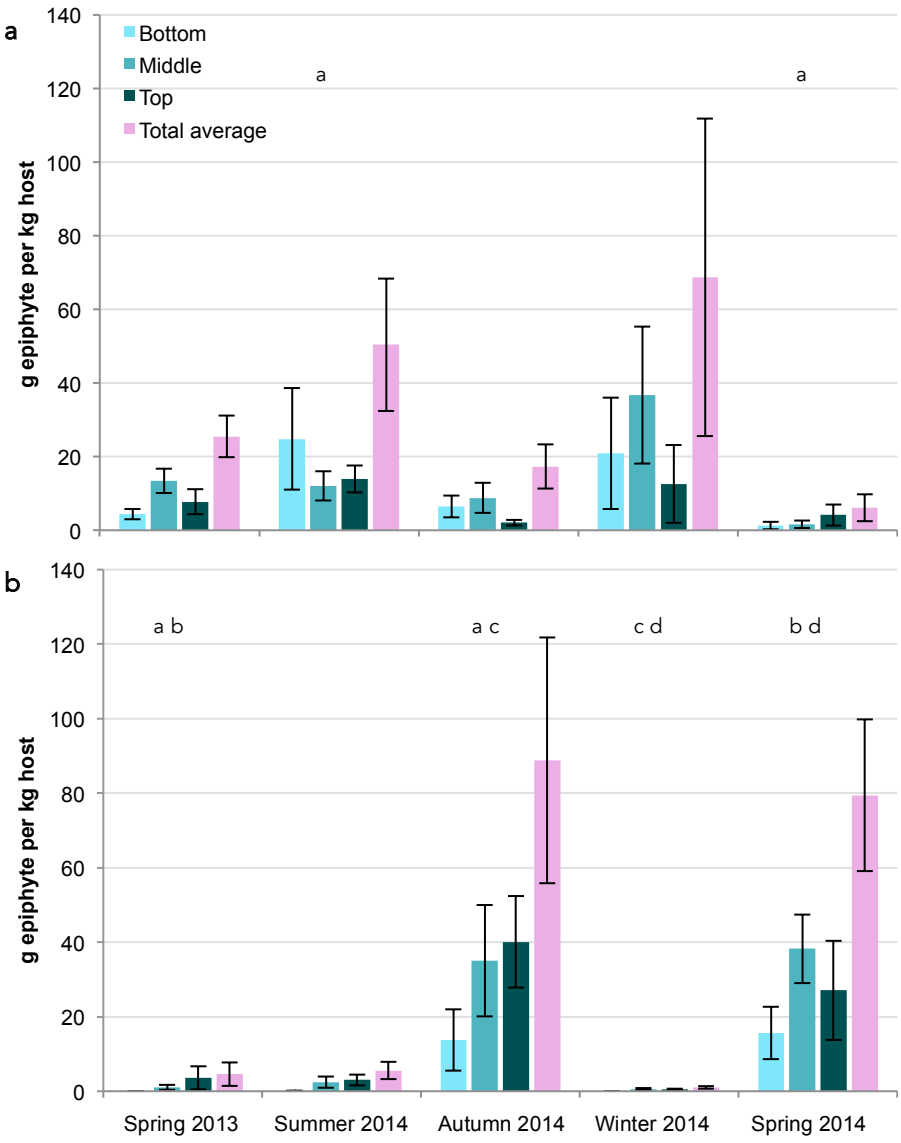


Figure 3.3: Seasonal variation in the abundance of epiphytes estimated as the biomass of epiphytes per kg of *C. maschalocarpum* (DW) on the top, middle, and bottom part of the plant and as total average for (a) Kempts Bay and (b) Mathesons Bay. Error bars indicate standard errors. Same letters indicate significant differences between total average values as assessed by Kruskal-Wallis H test.

A Kruskal-Wallis H test was conducted to detect significant differences in total epiphyte biomass between the five seasons sampled (n = 10 each). Distributions of epiphyte biomass were not similar for all groups, as assessed by visual inspection of boxplots. The distributions of epiphytic biomass were statistically significantly different between seasons at both Kempts Bay ($\chi^2(4) = 11.358$, $p = 0.023$) and Mathesons Bay ($\chi^2(4) = 23.236$, $p < 0.001$). Adjusted *p*-values for the post hoc analysis revealed statistically significant differences in epiphyte biomass ($p = 0.017$) only between summer 2014 (mean rank = 33.25) and spring 2014 (mean rank = 12.85) at Kempts Bay. No significant differences were detected between the other comparisons (spring 2013, mean rank = 28.90; autumn 2014, mean rank = 24.30; winter 2014, mean rank = 28.20). At Mathesons Bay significant differences

were detected between spring 2013 (mean rank = 17.75) and autumn 2014 (mean rank = 36.90) (p = 0.030), spring 13 and spring 2014 (mean rank = 37.55) (p = 0.021), autumn 2014 and winter 2014 (mean rank = 14.15) (p = 0.004), and winter 2014 and spring 2014 (p = 0.003). No differences were detected for summer 2014 (mean rank = 21.15) or any other combinations (Figure 3.3).

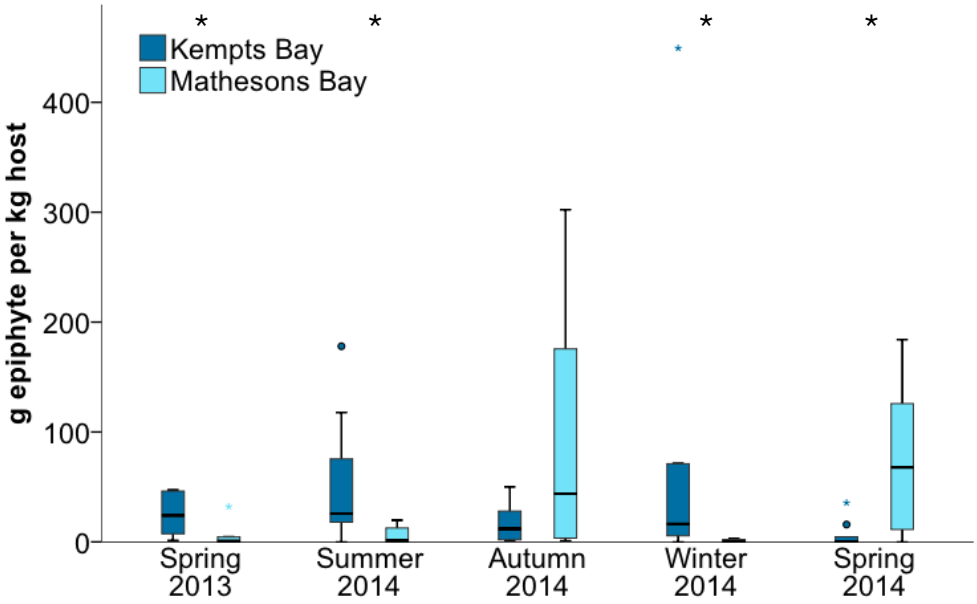


Figure 3.4: Seasonal variation in the abundance of epiphytes estimated as the biomass of epiphytes per kg of *C. maschalocarpum* (DW) as a comparison between Kempts Bay and Mathesons Bay. Black asterisks mark seasons with statistically significant differences between locations (Mann-Whitney U test).

The comparison of locations using Mann-Whitney U tests revealed statistically significant differences in the distribution of epiphytic biomass between Kempts Bay and Mathesons Bay for each season except for autumn 2014. Distributions of the biomass for the locations were not similar, as assessed by visual inspection. The biomass of epiphytes was significantly higher at Kempts Bay in spring 2013, summer 2014, and winter 2014, but significantly lower in spring 2014 (Figure 3.4, Table 3.3).

Table 3.3: Results of the Mann-Whitney U test analysing differences in the distribution of epiphytic biomass between locations. Statistically significant differences are highlighted in bold.

	Spring 2013	**Summer 2014**	**Autumn 2014**	**Winter 2014**	**Spring 2014**
Mean rank Kempts Bay	14.20	14.25	8.60	14.00	6.65
Mean rank Mathesons Bay	6.80	6.75	12.40	7.00	14.35
Mann-Whitney U	13.00	12.50	69.00	15.00	88.5
z – score	-2.818	-2.845	1.436	-2.703	2.949
p value	**0.004**	**0.003**	0.165	**0.007**	**0.002**

3.3.2 Seasonal changes in diet composition

Rhodophyta made up the largest portion of the diet in all seasons for both coastal and offshore populations (40.1% – 59.1%), with the only exception being in autumn, where coastal fish contained higher proportions of detritus (Figure 3.5). Unidentifiable material classed as detritus was more important in coastal (20.5% - 36.5%) than offshore fish (4.2% – 18.1%) throughout the year. Both

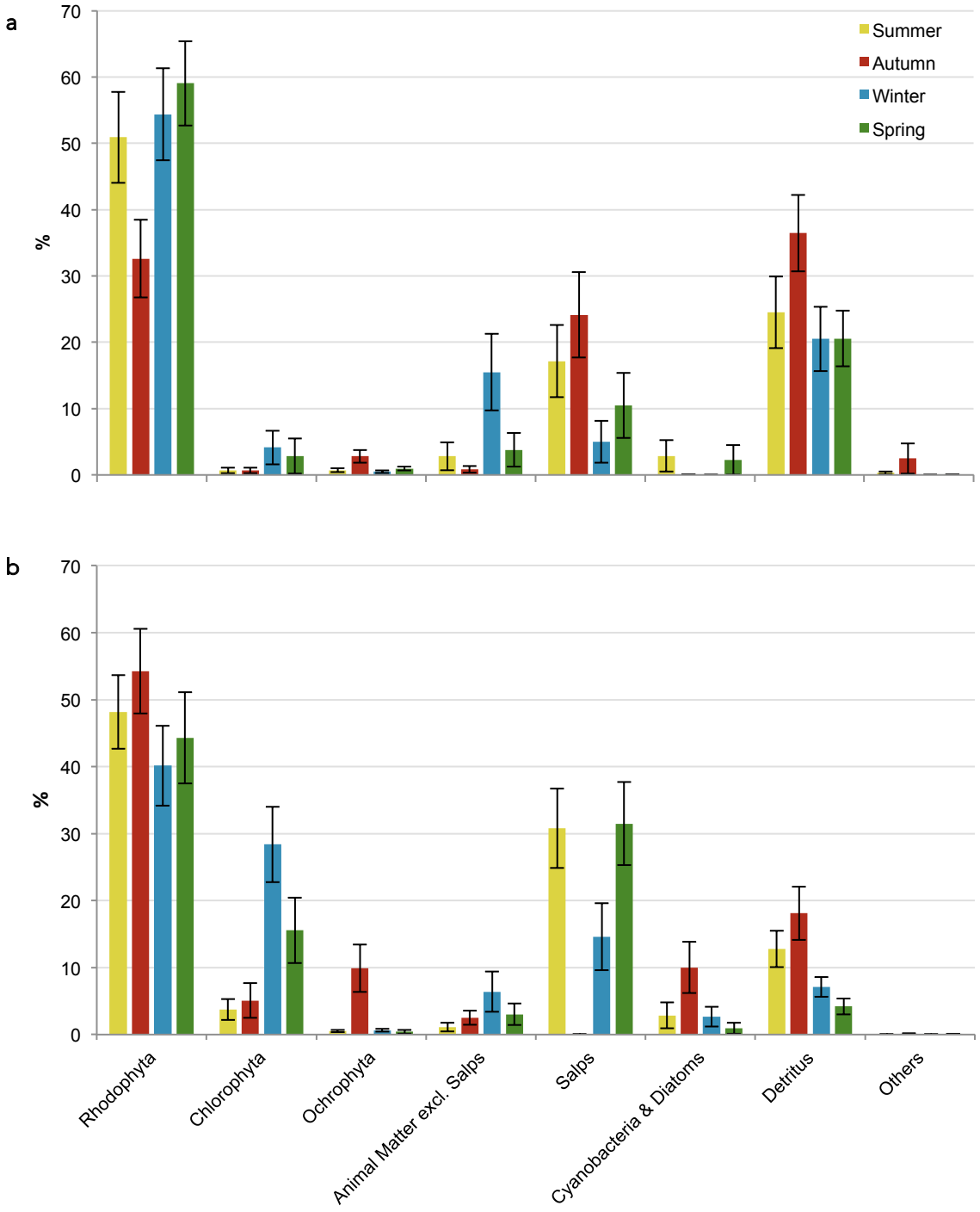


Figure 3.5: Seasonal changes in the percentage composition of the dietary categories in adult *G. tricuspidata* for (a) coastal and (b) offshore populations.

populations revealed the highest proportions of detritus in autumn. The amount of salps ingested showed seasonal variations and differences between regions. Coastal fish had the highest proportions of salps in summer (17.1%) and autumn (24.1%) and the lowest in winter (5.0%), while offshore fish ingested more salps during spring (31.5%) and summer (30.8%) and none in autumn (0.0%). In autumn when salps were absent in the diet of offshore fish the highest amounts of Rhodophyta and detritus were recorded. Coastal fish also had a considerable amount of animal matter in their stomachs during autumn (15.5%). Chlorophyta were an important dietary item for offshore fish in winter (28.4%) and spring (15.6%) and to a lesser degree for coastal fish (maximum 4.1% in winter). In autumn the contribution of cyanobacteria/diatoms and Ochrophyta in offshore fish increased to 10.0% and 9.9%, respectively, which is 15 times and 3.5 times more than during any other season. Ingestion of Ochrophyta in coastal fish also peaked in autumn when salps were absent in the diet. All other diet categories contributed less than 5% to the overall diet in any season for both populations.

Significant differences in the diet compositions between seasons were found for both locations (Table 3.4). The assumptions of homogeneity of dispersions were met for seasons at both locations (PERMDISP: coastal: F = 2.73, df1 = 3, df2 = 143, *p*(perm) = 0.165; offshore: F = 2.39, df1 = 3, df2 = 171, *p*(perm) = 0.181). Pairwise comparison of the seasons showed significant differences in diet compositions for coastal and offshore fish (Table 3.5). Coastal fish had statistically significant differences in the overall diet composition between autumn – winter, and autumn – spring. In offshore fish significant differences were detected between all seasons except for summer – spring comparison. PCO graphs for both locations did not reveal clear groupings of the seasons. The first two axes explain 80.6% of the variation for coastal fish and 76.8% for offshore fish (Figure 3.6).

Table 3.4: Results of the one factor PERMANOVAs comparing nutrient compositions among seasons for each region. Significant results are highlighted in bold. df = degrees of freedom, SS = sum of square, MS = mean sum of squares, Pseudo-F = F value by permutation.

Source of variation	df	SS	MS	Pseudo-F	*p*(perm)	Unique perms
Coastal						
Season	3	12310	4103.5	2.27	**0.025**	999
Residual	143	2.59E5	1810.9			
Offshore						
Season	3	38970	12990	7.19	**0.001**	999
Residual	171	3.09E5	1806			

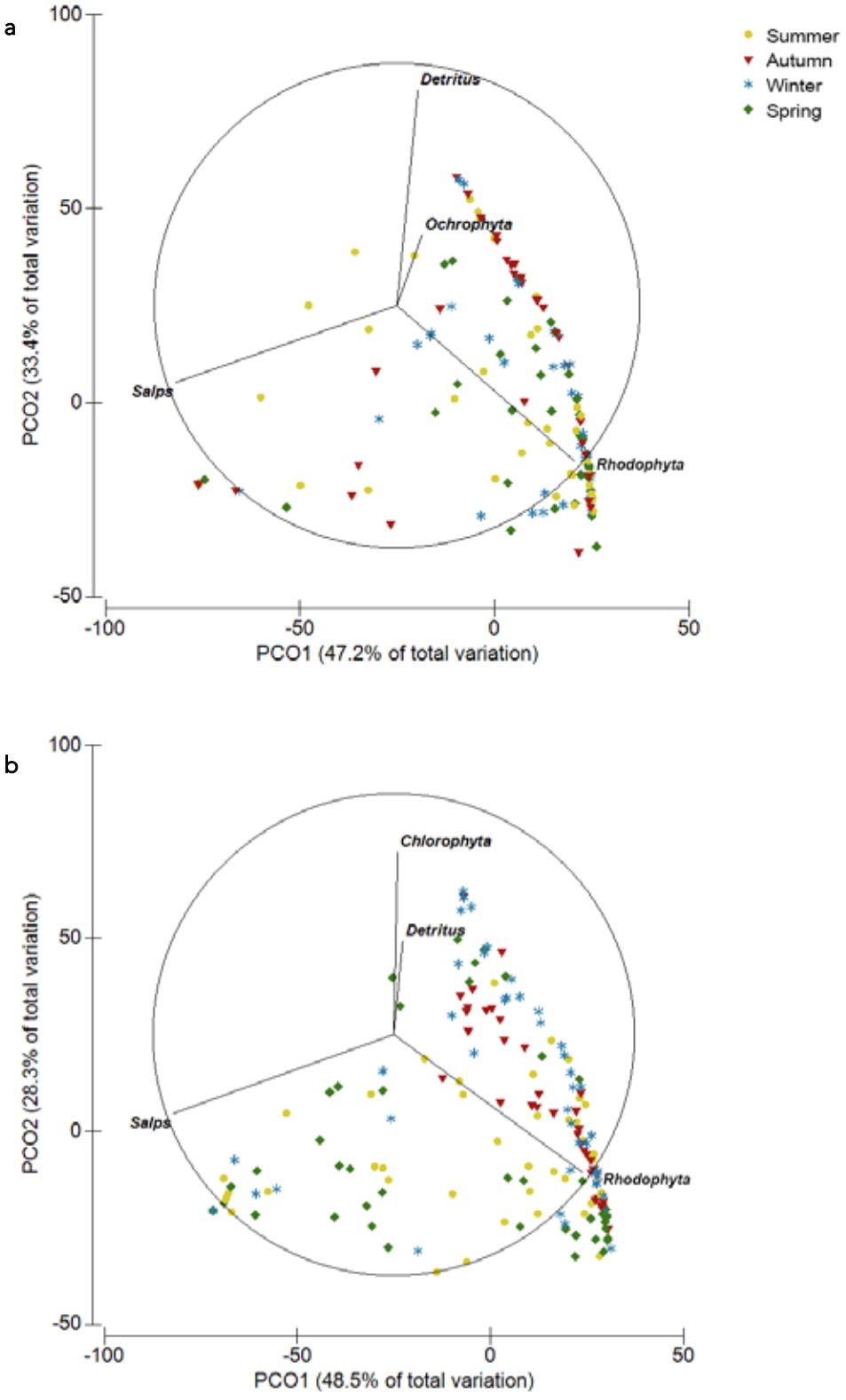


Figure 3.6: PCO of the diet categories showing seasonal similarities and differences for (a) coastal and (b) offshore *G. tricuspidata*. Vector plots present Pearson's correlations of untransformed diet category data for correlations > 0.25. The distance of the vectors extending to the circle indicates the strength of the correlation of that vector with the distribution of data points.

Table 3.5: ANOSIM results of the pairwise tests comparing diet compositions among seasons for coastal and offshore fish. Global test results: coastal R: 0.022, p = 0.044, nr of permutations: 999, nr of the permuted statistics greater than or equal to global R: 43; offshore: R: 0.099, p = 0.001, nr of permutations: 999, nr of the permuted statistics greater than or equal to global R: 0. Significant results are highlighted in bold.

	Coastal			Offshore		
Pairwise test	**R**	***p***	**Actual Permutation**	**R**	***p***	**Actual Permutation**
Summer – Autumn	0.019	0.119	999	0.137	**0.001**	999
Summer – Winter	0.012	0.202	999	0.092	**0.001**	999
Summer – Spring	-0.018	0.839	999	0.020	0.117	999
Autumn – Winter	0.070	**0.011**	999	0.114	**0.001**	999
Autumn – Spring	0.042	**0.047**	999	0.191	**0.001**	999
Winter – Spring	0.000	0.413	999	0.051	**0.017**	999

3.3.3 Reproducibility of nutrient analyses

For the reproducibility of lipids, one single sample with a low lipid content was chosen (3.8%) and results indicate reproducibility of 2.1% for 50 mg (n = 6) and 9.8% for 10 mg (n = 5), the latter being the least amount of sample used for analysis. Reproducibility of the ash analysis was also determined based on replicates of a single sample and was 0.8% for 100 mg (n = 5) and 7.0% for 10 mg (n = 5). Reproducibility of carbon and nitrogen analysis based on all samples (n = 315) was 0.6% and 1.1%, respectively.

3.3.4 Relationship between diet and nutrient composition

Estimating nutrient contents (C, N, lipids, ash) of individual diet categories from the measured values for total combined gut contents revealed differences in nutrient concentrations between diet categories. There were strong correlations for each nutrient and ash between the two methods of calculations as revealed by the Pearson's correlation coefficient ($r(5) > 0.820$, $p < 0.05$, Appendix B2). Therefore the Solver results were used for all categories including those that did not have sufficient samples for calculating the percentage composition. The lowest carbon values were in salps (23.8%) and detritus (19.5%) and the highest in Ochrophyta (35.0%) (Figure 3.7). Nitrogen values were highest for animal matter (8.1%) and salps (5.4%). Rhodophyta (4.2%) had higher nitrogen concentrations than Chlorophyta (2.6%) and Ochrophyta (2.3%). Protein calculations consequently followed the same trends. C:N ratios were lowest for animal matter (4.00:1) and salps (4.42:1) reflecting the high nitrogen concentrations observed. Comparing the algal phyla revealed lowest C:N values in

Rhodophyta (7.74:1), followed by Chlorophyta (11.13:1) and Ochrophyta (15.53:1), with the latter two showing the highest values of all categories. Lipid concentrations ranged from 3.9% (detritus) to 9.0% (cyanobacteria/diatoms), with values in the lower range for Ochrophyta (4.04%), Rhodophyta (5.31%), and Chlorophyta (5.40%). Ash was lowest in Ochrophyta (21.0%) and Rhodophyta (21.6%) and highest in detritus (50.2%) and salps (42.6%). Carbohydrate concentration was lowest for animal matter (19.24%) and salps (19.80%), intermediate for detritus (29.40%) and cyanobacteria/diatoms (35.40%) and highest for Chlorophyta (51.04%), Rhodophyta (52.10%), and Ochrophyta (63.75%) (Figure 3.7).

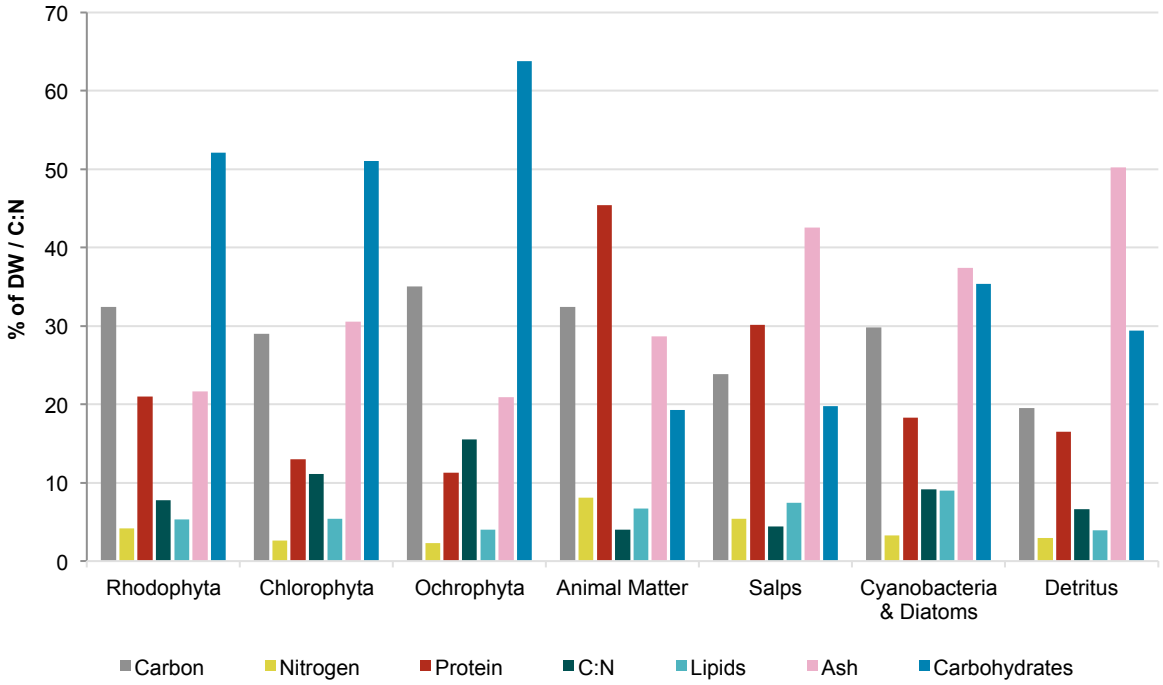


Figure 3.7: Percentage of the element/nutrient composition for each diet category. Values are based on measured values for carbon, nitrogen, lipids, and ash. All other values resulted from calculations (see 3.2.5.3 Nutrient analysis and reproducibility).

PCO results support the previous calculations and revealed clear groupings of diet categories on the basis of their nutrient contents with the first two axes explaining 90.6% of the variation (Figure 3.8). Rhodophyta and Chlorophyta contained higher amounts of carbon and lower amounts of ash in comparison to salps and detritus. Nitrogen and lipid content was higher in animal matter and salps compared to Rhodophyta, Chlorophyta, and detritus. Salps and Rhodophyta were the only diet categories that were included in both coastal and offshore samples. No clear separations were visible between locations for these two categories.

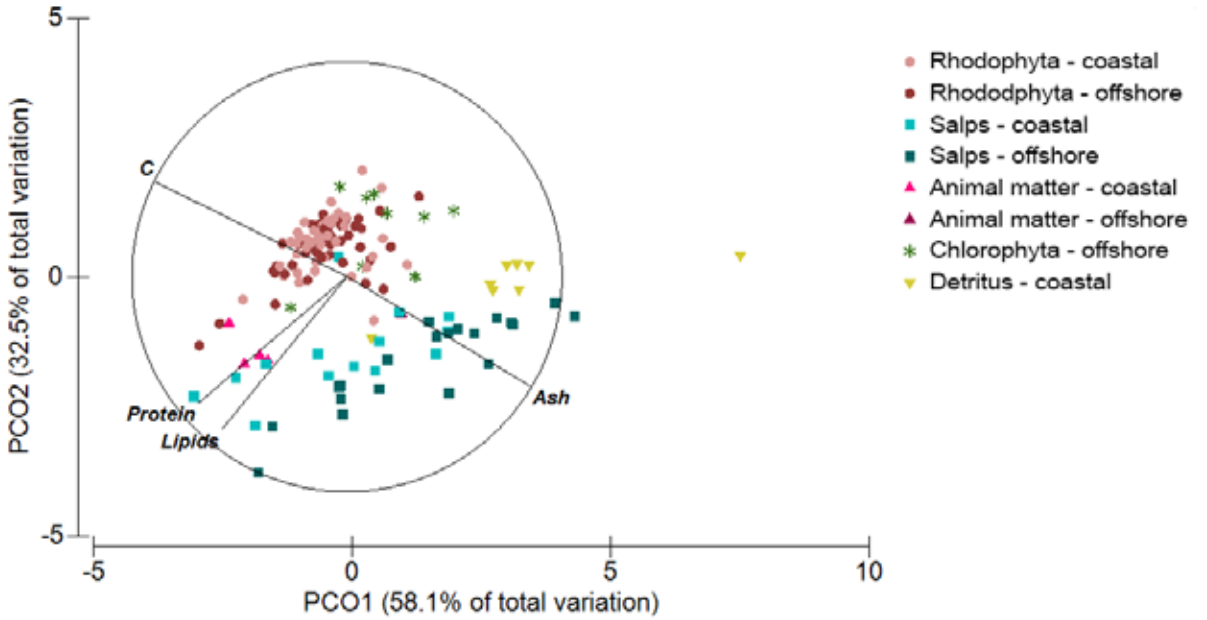


Figure 3.8: PCO of the element/nutrient compositions of stomach contents that contained more than 90% of one single diet category. Data points are displayed for coastal and offshore fish. Vector plots present Pearson's correlations of untransformed nutrient data for correlations > 0.25. The distance of the vectors extending to the circle indicates the strength of the correlation of that vector with the distribution of data points.

PERMANOVA results indicated significant differences in nutrient composition between diet categories (Table 3.6), but the assumption of homogeneity of dispersions was not met (PERMDISP: F = 12.34, df1 = 4, df2 = 141, *p*(perm) = 0.001). This indicated that there was only a category effect. Pairwise comparison revealed that there were significant differences in nutrient compositions between all diet categories (ANOSIM results see Appendix B3). As with diet categories the PCO graphs revealed the same trends of nutrient compositions for diet items (identified to species level) and axes explained 91.7% of the variation (Appendix B4). Species results showed that Rhodophyta are mostly influenced by *Abroteia suborbicularis*, which was the only rhodophyte that accounted for more than 90% in any stomach contents. Sheet forming *Ulva* spp. was the only alga in the category Chlorophyta, while other crustaceans comprised the category other animal matter. Salps were identified as the species *Thalia democratica*. Differences were also significant between diet items but were stronger between diet categories (larger Pseudo-F). Pairwise comparison also revealed significant differences between all diet items (Appendix B4).

Table 3.6: Results of the PERMANOVA comparing nutrient compositions among dietary categories. Significant results are highlighted in bold. df = degrees of freedom, SS = sum of square, MS = mean sum of squares, Pseudo-F = F value by permutation.

Source of variation	df	SS	MS	Pseudo-F	*p*(perm)	Unique perms
Diet category	4	291.39	72.848	35.59	**0.001**	999
Residual	141	288.61	2.047			

3.3.5 Seasonal changes in nutrient composition

The average proportional intake of elements/nutrients for all adult fish was 28.2% carbon, 4.3% nitrogen, 5.8% lipids, and 32.9% ash. These proportions remained nearly constant throughout the year (Figure 3.9). PCOs for coastal and offshore fish did not reveal any clear seasonal differences in nutrient composition and the first two axes explained 94.6% and 88.7% of the variation for coastal and offshore fish respectively (Figure 3.10). One-factor PERMANOVAs were conducted and results indicated no significant differences of nutrient compositions between seasons for each of the regions (Table 3.7). The assumptions of homogeneity of dispersions were met (PERMDISP: coastal: F = 2.47, df1 = 3, df2 = 133, *p*(perm) = 0.093; offshore: F = 1.35, df1 = 3, df2 = 165, *p*(perm) = 0.305).

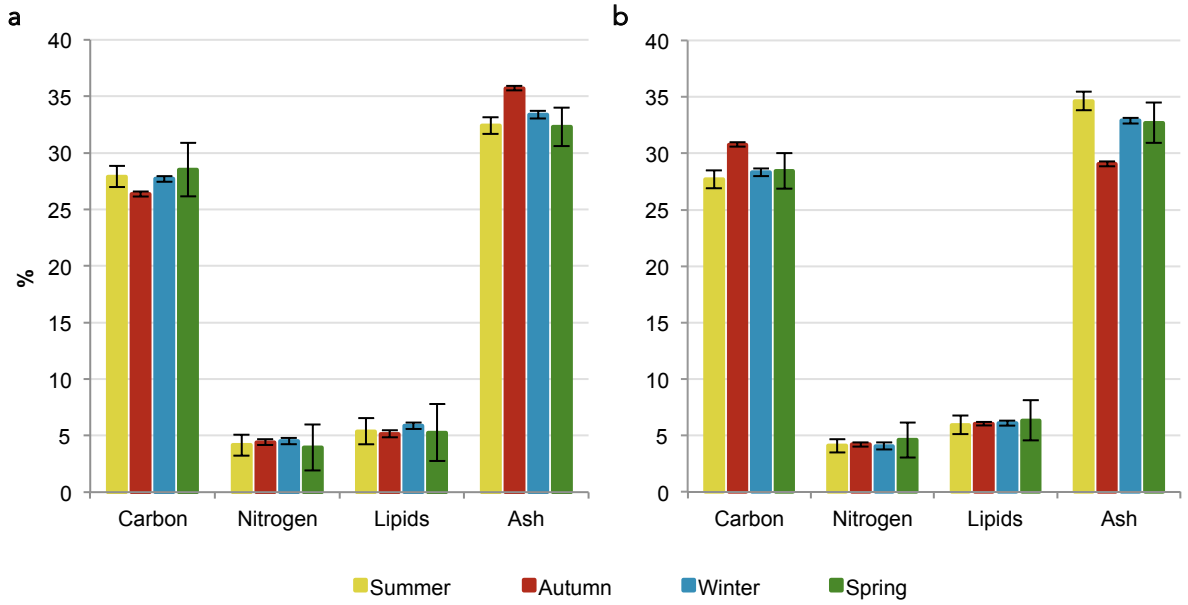


Figure 3.9: Seasonal changes in the element/nutrient proportions as per cent composition of *G. tricuspidata's* food intake for (a) coastal and (b) offshore fish. Error bars indicate standard errors.

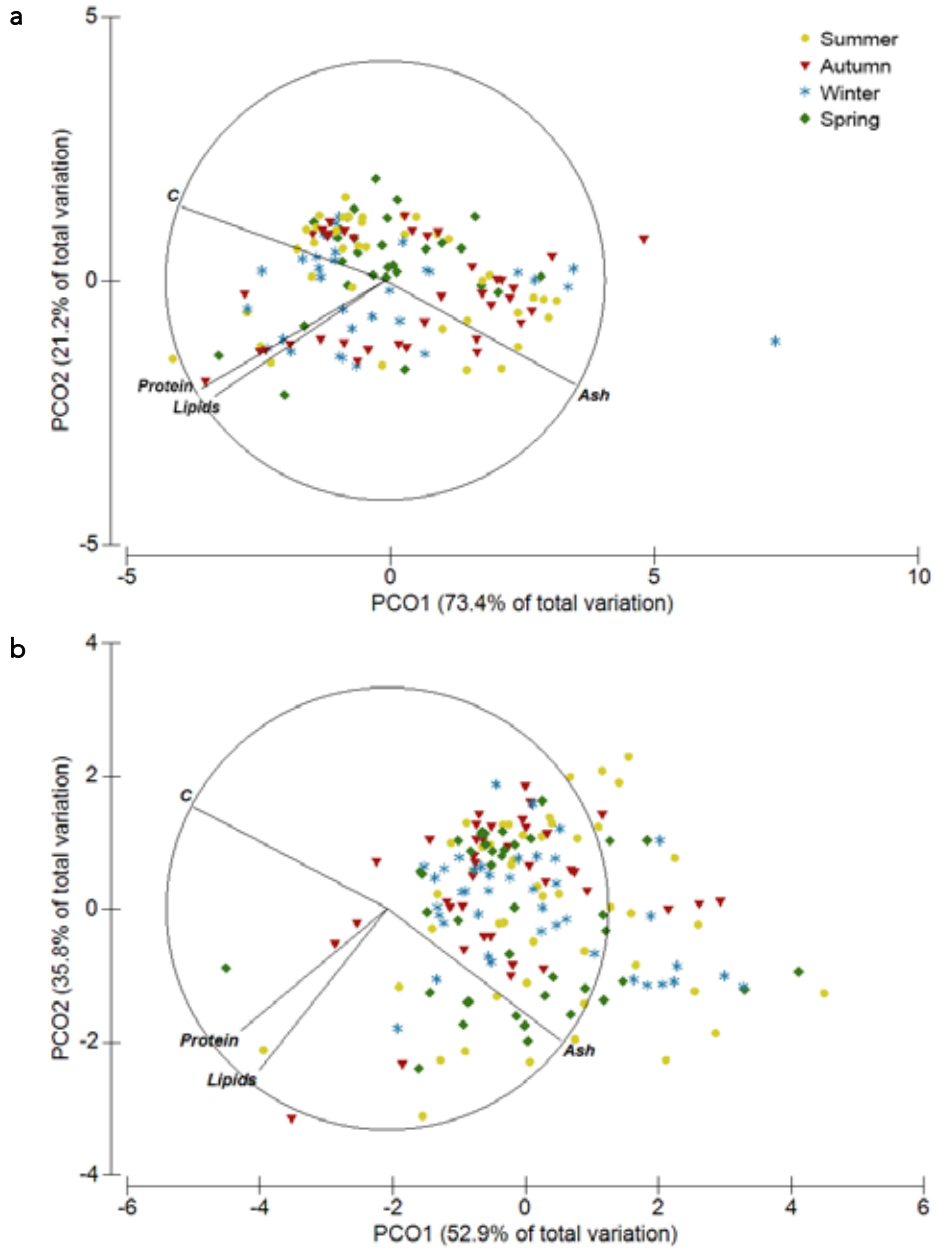


Figure 3.10: PCO comparing seasonal differences and similarities of the nutrient composition for (a) coastal and (b) offshore *G. tricuspidata*. Vector plots present Pearson's correlations of untransformed nutrient composition data for correlations > 0.25. The distance of the vectors extending to the circle indicates the strength of the correlation of that vector with the distribution of data points.

There was no clear separation of data points in the PCO comparison of nutrient compositions between coastal and offshore fish (Figure 3.11). However, one-factor PERMANOVAs were conducted and results indicated significant differences in nutrient composition between populations (Table 3.7). The assumption of homogeneity of dispersions was met (PERMDISP: F = 1.93, df1 = 1, df2 = 304, *p*(perm) = 0.208). Pairwise comparison for each season showed that nutrient compositions were

significantly different between coastal and offshore populations in autumn only (ANOSIM $p = 0.001$, Appendix B5).

Table 3.7: Results of the one factor PERMANOVAs comparing nutrient compositions among regions and seasons. Significant results are highlighted in bold. df = degrees of freedom, SS = sum of square, MS = mean sum of squares, Pseudo-F = F value by permutation.

Source of variation	df	SS	MS	Pseudo-F	*p*(perm)	Unique perms
Region	1	15.38	15.38	3.88	**0.022**	999
Residual	304	1204.6	3.96			
Coastal						
Season	3	13.66	4.55	1.05	0.377	998
Residual	133	577.01	4.34			
Offshore						
Season	3	19.23	6.41	1.78	0.077	996
Residual	165	594.71	3.60			

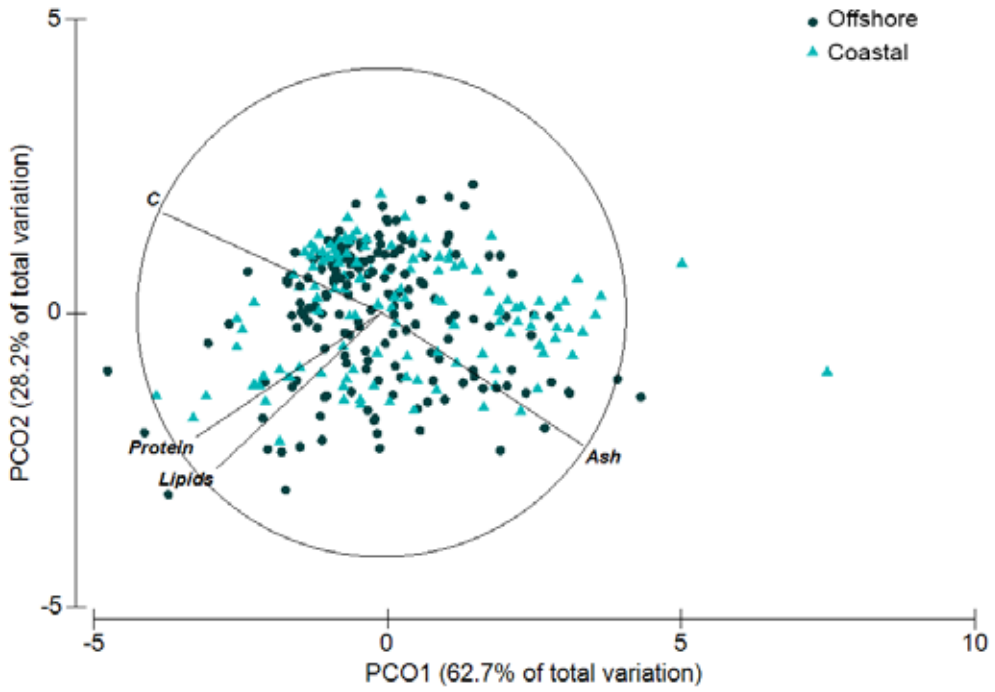


Figure 3.11: PCO with data points comparing the dietary nutrient composition between coastal and offshore fish. Vector plots present Pearson's correlations of untransformed nutrient data for correlations > 0.25. The distance of the vectors extending to the circle indicates the strength of the correlation of that vector with the distribution of data points.

3.3.6 Gonadosomatic Index

Plotting of the GSI showed that the gonad weight slowly started to increase with rising sea surface temperatures (September) for coastal and offshore *G. tricuspidata* (Figure 3.12). Highest values were recorded in spring. This suggests that spawning took place from October through to December, reaching peak values in December (coastal: mean of 13.73 ± 1.02 SE). Values rapidly decreased again and stayed low from March through to August (mean GSI less than 1.236).

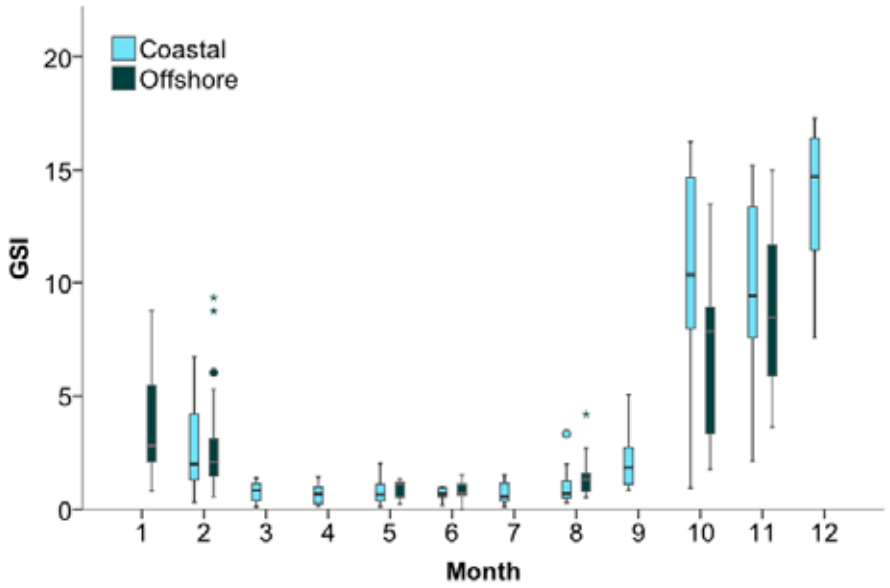


Figure 3.12: Monthly variations in the Gonadosomatic Index comparing coastal and offshore populations of *G. tricuspidata*.

3.3.7 Condition factor

The condition factor K_{TW} calculated for the total weight of the fish stayed nearly constant throughout the year for coastal and offshore fish with a slight increase in spring (22.2% and 18.6%, respectively) (Figure 3.13a). Highest values were recorded for coastal fish in December (mean ± SE: 2.31 ± 0.05) and lowest in August (1.89 ± 0.03) (22.2% difference). Offshore fish displayed highest values in October (2.17 ± 0.03) and lowest in May (1.83 ± 0.02) (18.6% difference). The condition factor K_{GW} calculated for the gutted weight of the fish was also fairly constant through the year (Figure 3.13b), with highest values for coastal fish in September (1.80 ± 0.05) and lowest in October (1.58 ± 0.02). For offshore fish highest values were recorded in August (1.71 ± 0.02) and lowest in January (1.55 ± 0.02)

and May (1.55 ± 0.03). K_{GW} did not increase in spring in contrast to the slight increase recorded for K_{TW}.

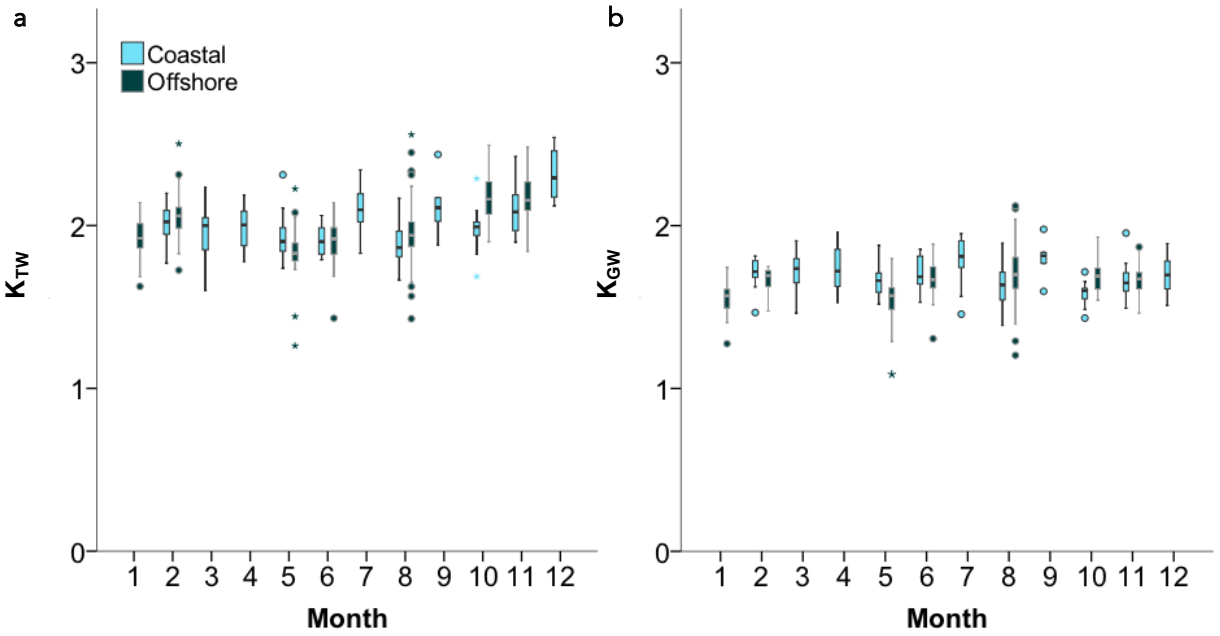


Figure 3.13: Monthly variation of the condition factor calculated on the basis of (a) total weight and (b) gutted weight comparing coastal and offshore populations of *G. tricuspidata*.

3.3.8 Relative gut content mass

The wet weight of stomach contents contributed an average of 1.0% to 1.8% of the TW of coastal and offshore fish respectively. For both locations the relative gut content mass decreased along the gut from section II to IV and then increased again in section V (Figure 3.14). Section II was on average the fullest section of the gut for all fish (relative gut content mass: 0.013 – 0.014). Section IV had the lowest relative gut content mass values of 0.006 - 0.008. Statistic results of the Mann-Whitney U test for the comparison of the total relative gut content mass indicated that there were significant differences between the locations ($p = 0.001$) (Appendix B6). On average offshore fish had 18.0% higher relative gut content mass values than coastal fish. Stomach fullness was significantly different between coastal and offshore fish (median values: 0.008 and 0.015 respectively) and coastal fish had 43% less content mass in their stomach than offshore fish. The median weights of the gut contents were significantly greater for offshore fish compared to coastal fish for sections III to V ($p < 0.05$).

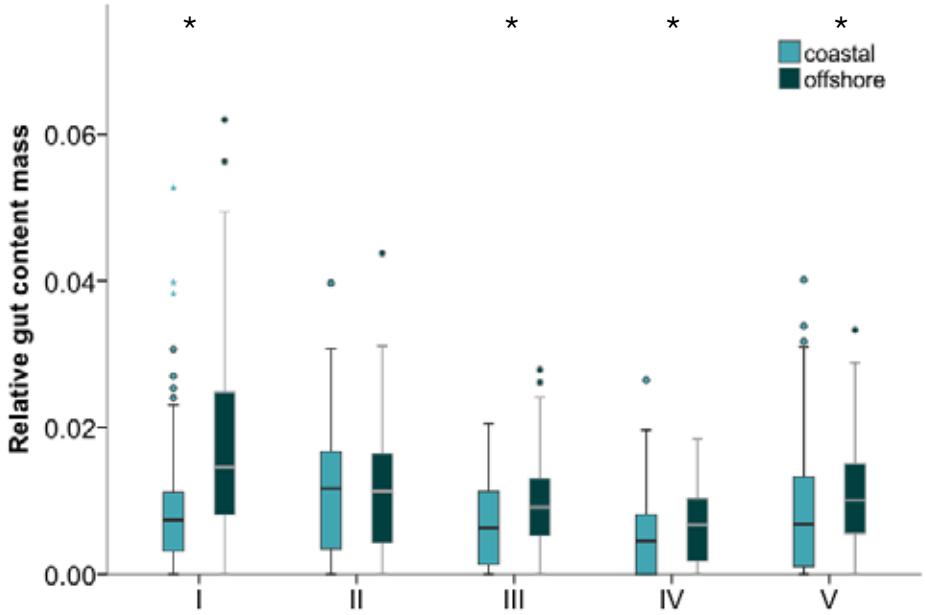


Figure 3.14: Relative gut content mass for each gut section of *G. tricuspidata* comparing coastal and offshore fish with I being the stomach and II to V sections of equal lengths from the anterior to the posterior, respectively. Black asterisks mark gut sections with statistically significant differences between locations (Mann-Whitney U tests, Appendix B6).

The Kruskal-Wallis H test was run to detect seasonal changes in total relative gut content mass. The distributions of relative gut content mass values were not similar for all seasons for both locations, as assessed by visual inspection of both boxplots. Distributions were also not significantly different between seasons for coastal fish, $\chi^2(3) = 1.177$, $p = 0.759$, and offshore fish, $\chi^2(3) = 5.839$, $p = 0.120$ (Figure 3.15) indicating that there were no differences in the seasonal pattern of relative gut content mass between the two locations.

Two factor PERMANOVA showed that diet changed with season but relative gut content mass did not, and there was no interactive effect between seasonal changes in diet composition and seasonal changes in relative gut content mass (Table 3.8). The assumption of homogeneity of dispersions was met (PERMDISP: F = 2.29, df1 = 1, df2 = 320, *p*(perm) = 0.232).

Table 3.8: Results of the two factor PERMANOVA analyzing the effects of season and relative gut content mass (RGCM) on diet composition. Significant results are highlighted in bold. df = degrees of freedom, SS = sum of square, MS = mean sum of squares, Pseudo-F = F value by permutation.

Source of variation	df	SS	MS	Pseudo-F	*p*(perm)	Unique perms
Season	3	13549	4516.3	2.338	**0.016**	999
RGCM	10	16602	1660.2	0.859	0.697	998
Season*RGCM	24	41271	1719.6	0.890	0.741	998
Residual	317	5.49E5	1932			

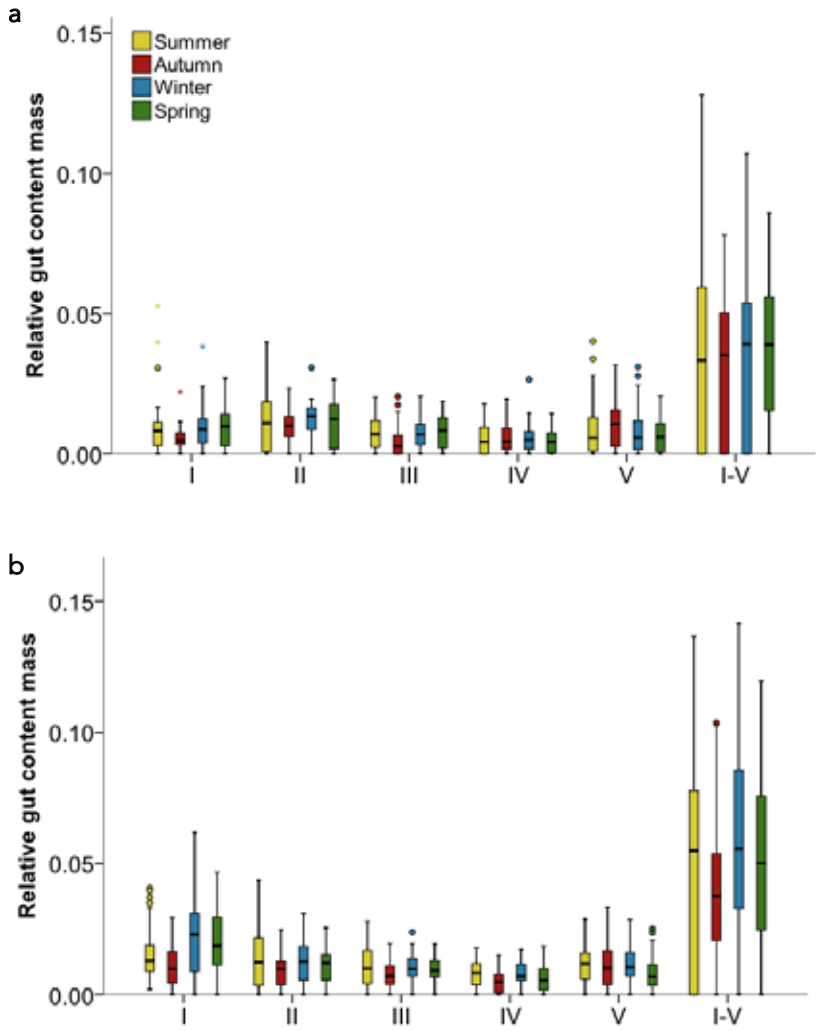


Figure 3.15: Seasonal changes of the relative gut content mass for (a) coastal and (b) offshore fish for each gut section with I being the stomach, II to V the gut sections from the anterior to the posterior end respectively, and I-V the total relative gut content mass. For median and mean values of total gut content masses see Appendix B6.

3.3.9 Diel variation in relative gut content mass

Shapiro-Wilk's tests revealed that data were normally distributed ($p > 0.05$) in all categories except for two (see table Appendix B7). Nevertheless, the t-test was run for all comparisons as it is considered fairly robust to deviations from normality. As assessed by Levene's test for equality of variances ($p > 0.05$), all but two comparisons displayed homogeneity of variance. The assumption of homogeneity of variance was violated for the comparison of carbon in coastal fish and ash in offshore fish ($p < 0.05$). In these cases of non-homogeneity of variance the Welch t-test was used.

The total average relative gut content mass (I-V) in coastal fish varied little through the day (Figure 3.16a), with sections I and III staying nearly constant, and II and V only slightly increasing during the day. Section IV gut content mass declined through the day (figures in Appendix B7). There was no significant difference in relative gut content mass between coastal fish sampled in the morning (9.30 a.m. to 10.00 a.m.) and the early afternoon (1.00 p.m. to 1.40 p.m., $p = 0.746$, Appendix B7).

In offshore fish all five gut sections increased in relative gut content mass (figures in Appendix B7) during the day (9.00 a.m. to 6.15 p.m.). Total average relative gut content mass also increased through the day (Figure 3.16b), and was significantly higher in the early (1.00 p.m. to 1.40 p.m.) and late afternoon (5.35 p.m. to 6.15 p.m.) than in the morning (9.30 a.m. to 10.00 a.m., $p = 0.045$ and $p = 0.001$ respectively, Appendix B7).

There were no statistical differences between the nutrient levels of stomach contents (section I) for carbon, nitrogen, lipids, and ash between morning and afternoon samples for either population ($p > 0.05$, Appendix B7). Results of the Mann-Whitney U test revealed significant differences in lipid concentrations between populations ($p = 0.02$), but not for carbon, nitrogen and ash ($p > 0.05$, Appendix B8).

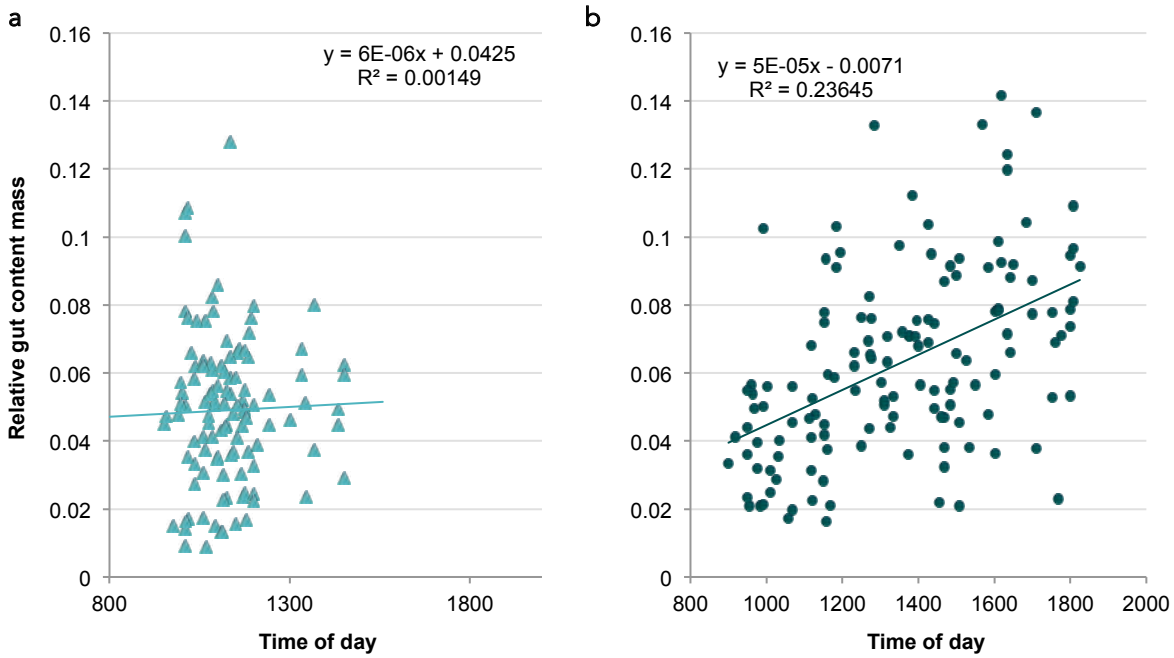


Figure 3.16: Changes of total relative gut content mass in relation to time of day for (a) coastal and (b) offshore fish

3.3.10 Age related differences in relative gut content mass and nutrient composition

In coastal fish the relative gut content mass slightly increased with age but did not vary with age in offshore fish. Independent-samples t-tests indicated no differences between coastal and offshore populations of young fish (p = 0.249), but significantly higher values in offshore adult fish (p = 0.014, see Appendix B9 for graphs and statistical results). Carbon, nitrogen, lipid, ash concentrations, and C:N ratio did not change with increasing age in coastal fish, and only exhibited slight changes in offshore fish. Statistical tests revealed no differences in compositions of carbon, nitrogen, lipid, ash, and C:N ratio between coastal and offshore populations in either young or adult fish (p > 0.05, Appendix B9).

3.3.11 Relative gut length and Zihler Index

The gut lengths of 323 *G. tricuspidata* were recorded. The mean gut lengths (± SE) were 672.2 mm (±1.1) for coastal (n = 160) and 753.9 mm (±1.0) for offshore fish (n = 162), and the mean RGL (± SE) was 2.539 ± 0.039 and 2.308 ± 0.038 respectively (Figure 3.17a). RGL values for each region were normally distributed, as assessed by Shapiro-Wilk's test (p > 0.05) and there was homogeneity of variances, as assessed by Levene's test for equality of variances (p > 0.05). The independent-samples t-test revealed that mean values of RGL between coastal and offshore fish differed significantly, with the coastal mean value higher than the offshore mean value differing by 0.231 (95%CI, 0.124 to 0.337). The difference in the mean values was statistically significantly different, t(321) = 4.245, p < 0.001, d = 0.472.

The ZI (± SE) revealed results as follows: 7.836 ± 0.115 for coastal and 7.267 ± 0.117 for offshore *G. tricuspidata* (Figure 3.17b). Data were normally distributed for both regions (Shapiro-Wilk's test, p > 0.05). Homogeneity of variances was given (Levene's test, p > 0.05). The independent-samples t-test indicated a statistically significant difference between coastal and offshore fish, t(320) = 3.460, p = 0.001. The coastal mean index was higher than the offshore mean index, 0.568 (95%CI, 0.245 to 0.891), t(320) = 3.460, p = 0.001, d = 0.386.

A one-way ANOVA was performed to determine seasonal changes in the ZI. There was a statistically significant difference between the seasons for coastal fish (F(3,143) = 5.541, p = 0.002) and offshore

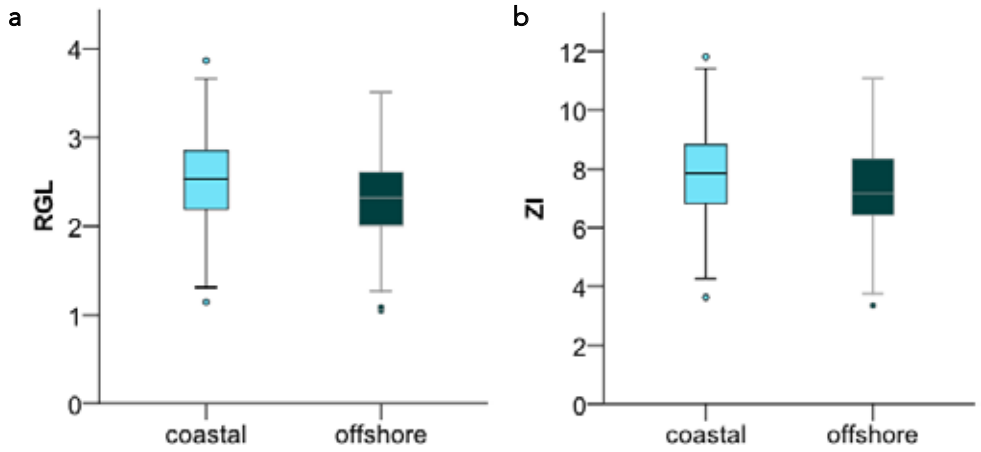


Figure 3.17: (a) Relative gut length and (b) Zihler index for *G. tricuspidata* comparing coastal and offshore fish.

fish ($F(3,158) = 6.032$, $p = 0.002$). The post hoc tests revealed significant differences in ZI between summer and winter ($p = 0.001$), summer and spring ($p = 0.048$), and autumn and winter ($p = 0.047$) for coastal fish with the highest ZI values in summer and autumn and the lowest in winter and spring. For offshore fish, significant differences were detected between summer and autumn ($p = 0.007$) and autumn and winter ($p = 0.001$), where autumn shows the lowest ZI values, and summer and winter the highest. Data displayed normal distribution at both locations for all seasons (Shapiro-Wilk test $p > 0.05$). The assumption of homogeneity of variances was met for coastal and offshore fish, as assessed by Levene's test for equality of variances ($p = 0.407$ and $p = 0.742$, respectively) (Figure 3.18).

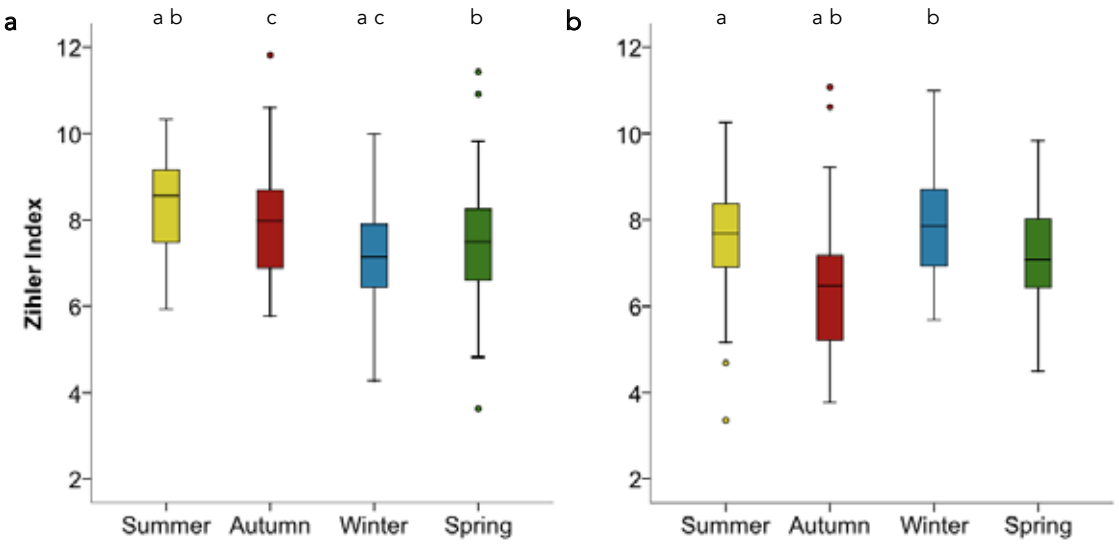


Figure 3.18: Seasonal changes of the Zihler Index of (a) coastal and (b) offshore fish. Same letters indicate statistically significant differences as assessed by one-way ANOVA.

3.4 Discussion

Epiphytes displayed a different pattern of seasonal variation between the two locations but overall were available year round. The diet of *Girella tricuspidata* varied throughout the year and even though each diet category had a distinct nutrient composition the overall composition of nutrients and intake of food stayed relatively constant throughout the year. Offshore fish had overall higher gut content mass but smaller relative gut content length than coastal fish. The Gonadosomatic Index increased considerably from October to December however the condition factor of fish did not indicate any seasonal variation in somatic weight. Each of these results is discussed in detail below.

3.4.1 Epiphyte availability and their importance as a food source

Abroteia suborbicularis was the most abundant epiphyte on *Carpophyllum maschalocarpum* and was also the most common alga in *G. tricuspidata* guts (Table 3.2, Chapter 2.3 Results). Biomass and diversity of epiphytes were highest in the middle part of the host plant (Figure 3.1, 3.2, 3.3). Lower abundance of epiphytes on the basal part of the plant were most likely due to a lack of light, whereas lower abundance at the top part of the plant could be attributed to a lack of time for epiphytes to establish, as the host tissue was only recently formed (Arrontes, 1990; D. L. Ballantine, 1979; Jennings & Steinberg, 1997). Even though most epiphyte species were distributed in the middle part of the plant, *Nancythalia humilis* displayed highest abundances at the top part of the host, indicating a preference for higher light levels.

The morphology of the epiphyte is likely to influence the ability of fish to feed on it (Steneck & Watling, 1982). Herbivorous fishes including *G. tricuspidata* have well-developed visual capabilities that enable them to detect plants that might otherwise be missed by nonvisual grazers such as urchins (Gaines & Lubchenco, 1982). Some epiphytes were inconspicuous on the host plant, as they were similar in colour, e.g. *Dipterosiphonia heteroclada*, *Ceramium* sp1, *Dasyclonium bipartitum*, and *Metamorphe colensoi*. The latter two species exhibited very low growth forms that creep along the blades of the host plant and numerous holdfasts make removal of the epiphytes difficult. *Ceramium* sp1 was mainly found growing along the blade axis and/or together with the receptacles, which makes feeding on the epiphyte impossible without also ingesting parts of the host plant. The foliose blades of *A. suborbicularis* and *N. humilis* that float and protrude and were often attached at the edge of the host's blades make these epiphytes an easier target.

The success of epiphytism can be related to various factors such as availability of space, age and structure of the host tissue, height in the water column, grazing pressure, nutrient availability, wave exposure, and light availability (Arrontes, 1990; D'Antonio, 1985; Jennings & Steinberg, 1997; Kersen, Kotta, Bučas, Kolesova, & Deķere, 2011; Otero-Schmitt & Pérez-Cirera, 1996). Small-scale spatial variation of epiphytes can be very high at the scale of meters to tens of meters (Lavery & Vanderklift, 2002; Rindi & Guiry, 2004). As a result of spatial differences, varying seasonal patterns of epiphyte abundance on *C. maschalocarpum* were observed between the two locations (Figure 3.4). Storms can reduce epiphyte abundance considerably (Berthelsen & Taylor, 2014) and arthropod mesograzers reduce the abundance of algal epiphytes on coralline algal turf in north-eastern New Zealand (Berthelsen & Taylor, 2014). Mesograzers might have similar effects on epiphyte abundance on *C. maschalocarpum*. Epiphytes decrease the growth rate of their host, increase the probability of axis breakage, decrease reproductive success, and can compete for nutrients and light (D'Antonio, 1985; Lee, Park, & Kim, 2007). The removal of epiphytes by herbivores might therefore be beneficial to the host alga. Despite variations in abundance epiphytes were available as food for *G. tricuspidata* throughout the year. However, seasonal and spatial variation in epiphyte abundance requires that *G. tricuspidata* move between locations supporting fucoid algae with high epiphytic abundances. *G. tricuspidata* is highly mobile and capable of moving hundreds of kilometres along the coast (Gray et al., 2012), allowing them to detect food even on a large spatial scale.

Epiphyte samples were collected at two coastal sites but not offshore sites, and sampling times differed between fish sampling (summer 2010 to winter 2012) and epiphyte sampling (spring 2013 to spring 2014). These aspects of the sampling design potentially limit the conclusions that can be reached on resource availability and confound direct comparison between the two sampling sites. Sampling of algal epiphytes was added as an element of the study once the importance of this food resource to the diet was established, since no data on epiphyte abundances and seasonality were available for New Zealand. This necessarily resulted in synchronous diet and algal epiphyte datasets. Logistical limitations prevented sampling epiphytes at offshore locations after the completion of fish sampling. Nevertheless, results of the epiphyte sampling showed that this resource was available throughout the year, even though the seasonal patterns of abundances differed between the two coastal sampling sites. This year-round availability is also reflected by the diet composition in coastal fish, where epiphytes contributed on average >26% to the diet in any season. Epiphytes also accounted for >27% of the diet in offshore fish in any thus indicating that epiphytes are also available at the offshore location throughout the year. The fact that epiphytes were present in the diet of both coastal and offshore fish throughout the year indicates that local variations in epiphyte abundance

throughout the year do not prevent *G. tricuspidata* from using this food resource, although they may incur additional foraging costs at times.

3.4.2 Nutritional value of the diet

Although Ochrophyta contain higher levels of carbohydrates than Chlorophyta and Rhodophyta, these algae are considered of lower nutritional value as most of the carbohydrate is in a form that cannot be assimilated or metabolised by the fish (Baker et al., 2016; Montgomery & Gerking, 1980; Pillans, Franklin, & Tibbetts, 2004). Mannitol, a sugar alcohol found in phaeophytes, was not assimilated by *G. tricuspidata* (White et al., 2010). Some degradation of laminarin, a storage polysaccharide of ochrophyta and diatoms, was recorded to a low degree (Moran & Clements, 2002; Skea et al., 2007) and very low levels of short-chain fatty acids (SCFA), which are a product of fermentation by endosymbiotic microorganisms (Choat & Clements, 1998; Clements & Choat, 1997). This indicates that *G. tricuspidata* might be able to degrade a small proportion of the carbohydrate in Ochrophyta and diatoms, but overall the microbial fermentation is not important in these fish which appear to mainly rely on endogenous digestive enzymes for algal digestion (Clements & Choat, 1997; Skea et al., 2007). Studies of endogenous carbohydrase activity revealed that *G. tricuspidata* are capable of breaking down starch, the main storage polysaccharides in chlorophytes and rhodophytes (Moran & Clements, 2002; Raubenheimer et al., 2005; Skea et al., 2007).

Carbohydrates and carbon both correlate with the energy content in plants including algae (Kaehler & Kennish, 1996; Rico & Fernández, 1996). A negative correlation between ash and energy content has also been reported (Kaehler & Kennish, 1996; Lamare & Wing, 2001; Montgomery & Gerking, 1980). Lamare and Wing (2001) investigated 118 macroalgae species, including 28 species from New Zealand, and ash content explained 85.9% of the observed variability in calorific content. An assessment of the energy value can therefore be made for diets in the present study by using carbon, ash, and carbohydrate concentrations, however estimates have to be viewed cautiously, especially in regard to carbon. Carbon is a major dietary component that is spread across several functionally distinct biochemicals (e.g. cellulose, starch, sugars, lipids, amino acids), and depending on the source will follow different post-ingestive pathways (Raubenheimer, Simpson, & Mayntz, 2009). Therefore it might be impossible to predict an animals' food choice based on carbon measurements as carbon concentrations of the diet do not reflect the animals' ingestive responses in distinguishing between nutritional and non-nutritional sources of carbon (Raubenheimer et al., 2009; Raubenheimer & Jones, 2006).

The majority of carbon intake of *G. tricuspidata* is derived from algae. These make up the major proportion of the diet (Figure 3.5) and contained higher carbon concentrations than salps and detritus, the other two diet categories that contribute a considerable proportion of carbon to the diet (Figure 3.7, 3.8). Ash content in the present study was about one third lower in Rhodophyta and Ochrophyta compared to Chlorophyta. Carbon concentrations were similar between algae with slightly higher values in Ochrophyta than Rhodophyta and lowest values in Chlorophyta. On the basis of ash and carbon content, it can be assumed that Ochrophyta had higher or similar energy levels compared to Rhodophyta, and that both contained higher levels than Chlorophyta. Carbohydrates are known to contribute the bulk of energy in algal diets (Montgomery & Gerking, 1980). Calculations of crude carbohydrate content of the algal phyla in the present study are consistent with previous results and revealed about 20% higher concentrations in Ochrophyta compared to Rhodophyta and Chlorophyta. But *G. tricuspidata* is, as discussed above, not capable of utilising most of the carbohydrate in Ochrophyta, making them nutritionally irrelevant. Crude carbohydrates in detritus were about 60% of that in Rhodophyta and Chlorophyta, contributing less to the overall diet than algae. Quantities of crude carbohydrates were also considerably lower in salps and animal matter than in algae. Calculated values in the present study were even higher than reported carbohydrate levels from direct measurements in animal matter (amphipods, copepods, euphausiids) and *T. democratica* (0.63-2.04% and 0.66-1.11% respectively) (Wang, O'Rorke, Nodder, & Jeffs, 2014). Due to these low values other carbohydrates derived from salps and animal matter are expected to be negligible.

Lipid concentrations were highest in cyanobacteria/diatoms (9.0%), followed by salps (7.5%), and animal matter (6.7%) (Figure 3.7, 3.8). Of these categories only salps contributed a major proportion to the overall diet (Figure 3.5) and thus provide an important source of lipids for *G. tricuspidata*. Lipid levels in algae are usually low (< 5% of dry weight) (Crossman, Choat, & Clements, 2005; Gressler et al., 2010; Johnson, 2011; McDermid & Stuercke, 2003; Montgomery & Gerking, 1980). This is consistent with lipid concentrations calculated in this study. Lipid was lowest in Ochrophyta with 4.0% and over 30% higher in Rhodophyta (5.3%) and Chlorophyta (5.4%). These results imply that *G. tricuspidata* derives most lipids from epiphytic algae and salps. Lipids contain the highest levels of gross energy with about 39.5 kJ/g compared to carbohydrates and proteins with 17.5 kJ/g and 23.9 kJ/g, respectively (Gnaiger & Bitterlich, 1984).

Adequate intake of protein is especially important for animals on a mainly herbivorous diet (Bowen et al., 1995; Cruz-Rivera & Hay, 2000). Results in the present study showed that ingested Rhodophyta

contained higher amounts of nitrogen than Chlorophyta and Ochrophyta (Figure 3.7). Protein estimates based on nitrogen contents were 21.0% for Rhodophyta, 13.0% for Chlorophyta, and 11.3% for Ochrophyta. Algae in the category Rhodophyta consisted mainly of *A. suborbicularis* and other small epiphytes. Protein analysis of epiphytic rhodophytes collected off *C. maschalocarpum* in a previous study in the Hauraki Gulf recorded 15.8% protein (measured as THAA (total hydrolysable amino acids)) per dry mass, twice as much compared to the relatively low content of 6.5% and 7.3% measured in *C. maschalocarpum* and *Ecklonia radiata*, respectively (Johnson, 2011). Results of the latter study are lower than the protein content estimations for Rhodophyta and Ochrophyta obtained in the present study. Algae can contain high concentration of non-protein nitrogenous substances and use of the nitrogen-to-protein conversion factor consequently can lead to an overestimation of the protein content (Angell et al., 2016; Barbarino & Lourenço, 2005; Lourenço, Barbarino, Lavín, Marquez, & Aidar, 2004). Nevertheless, results support the comparatively higher protein values found in Rhodophyta. C:N ratios for the three algae phyla in the present data support that Rhodophyta (7.74:1) are superior to Chlorophyta (11.13:1) and both are superior to Ochrophyta (15.53:1) from a nutritional perspective. Smit et al. (2006) also showed that epiphytic algae on seagrass provided the best source of protein for herbivores compared to seagrass or drift rhodophytes. The results of the present study suggest that *G. tricuspidata* selectively feeds on algae with higher nitrogen/protein concentrations, which differs from the findings of Raubenheimer et al. (2005) on captive fish. They conducted feeding experiments offering two dietary (*Ulva lactuca*, *Enteromorpha intestinalis*) and one non-dietary (*Gracilaria chilensis*) algae species to *G. tricuspidata* and concluded that fish chose algae with higher starch contents and lower protein content. But results were based on three algae species only and protein and carbohydrate levels in algae can be highly variable even within a genus (McDermid & Stuercke, 2003) and between seasons (Kaehler & Kennish, 1996).

Salps, besides epiphytic Rhodophyta, were another major and at certain times even more important source of protein for *G. tricuspidata*. Salps contained 28% more nitrogen than algae in the present study. The C:N ratio of salps was the second lowest of all diet categories indicating its high nutritional value (Figure 3.7, 3.8). Gelatinous zooplankton has often been seen as a dead end in the food web (Mianzan, Pájaro, Colombo, & Madirolas, 2001), but in recent years many fish species have been reported to feed on salps (Arkhipkin & Laptikhovsky, 2013; Janssen & Harbison, 1981) and gelatinous plankton in general (see review Arai, 2005; Henschke, Everett, Richardson, & Suthers, 2016). Due to their low mobility salps make an easy prey target, allowing *G. tricuspidata* to access an easily digestible food source with high nitrogen content. The category animal matter, which consisted mainly of crustaceans, revealed the highest protein levels and lowest C:N ratio of all diet categories

in the present study, but contributed little to the overall diet. Animal matter such as invertebrates are considered a good food source due to their high protein and gross energy levels compared to algae, macrophytes, and detritus, but require considerably more foraging effort (Bowen et al., 1995).

The source of detritus in stomach contents is unknown and might have resulted from partly digested algae or might have been ingested by fish from turf algae. Detritus forms an important part of the epilithic algae community on coral reefs and consists of a complex mixture of dead organic matter (e.g. algae, fish faeces, and coral mucus), inorganic material, microorganisms, microalgae (diatoms, dinoflagellates, and cyanobacteria), and associated meiofauna (Crossman, Choat, Clements, Hardy, & McConochie, 2001). Such detritus contains similar or even higher levels of amino acids than algae making it a valuable food source (Crossman et al., 2001; S. K. Wilson, 2002b).

3.4.3 Seasonal changes in diet and nutrient intake

In coastal fish Chlorophyta composed more of the diet in autumn and considerably more of the diet of offshore fish during winter and spring (Figure 3.5). *Ulva lactuca* is an annual, and abundances peak in late spring (Lamare & Wing, 2001). Nevertheless *G. tricuspidata* ingested more salps and Rhodophyta in spring even though Chlorophyta would be abundant at this time. Abundance of Chlorophyta and *Ulva* spp. in particular is generally higher at Great Barrier Island than along the coast (Hidas, 2001), which resulted in a larger contribution of Chlorophyta to the overall diet of offshore fish.

The diet of coastal fish contained the least amount of salps in winter, intake increased during spring and summer and peaked in autumn (Figure 3.5). Salps made up a larger portion of the overall diet for offshore fish with a third of the diet consisting of salps in spring and summer, while considerably less were ingested in winter and none in autumn (Figure 3.5). In the Hauraki Gulf the salp *Thalia democratica* displays interannual variation in abundance and a patchy occurrence in early spring. Salps are also more abundant in the outer than the inner Gulf (Zeldis, 1995; Zeldis & Willis, 2014). Licandro et al. (2006) studied *T. democratica* in the north-western Mediterranean Sea over two and a half decades and found interannual and seasonal variation in abundance. Water temperature was the only parameter significantly linked to salp abundance and annual peaks were related to SSTs >15.5°C. Average monthly SSTs at the two sampling locations in the present study were above 15.5°C from November to June (Figure 4.8). The increased feeding on salps during these times of the year at both locations reflects the seasonal peaks of salp abundances during spring and early

summer, which are triggered by temperature. Seasonal peaks in salp ingestion happened slightly earlier in offshore fish. This might be related to the physical oceanography in the Hauraki Gulf. From early spring to early summer upwelling can occur on the shelf north of the Hauraki Gulf. Upwelling ceases between early to late summer when conditions shift towards downwelling and strong stratification on the shelf and in the Hauraki Gulf (Zeldis et al., 2004). This results in warmer SSTs further away from the coast from early spring to early summer, after which SSTs align between the shelf and Hauraki Gulf (Zeldis et al., 2004). Warmer SSTs and upwelling, which directs water away from the coast, result in the higher abundances of salps offshore, and thus in the earlier appearance of salps in the diet of offshore fish.

During times of low salp abundance *G. tricuspidata* increased intake of other prey items with high nitrogen and lipid contents. For coastal fish the quantity of ingested animal matter revealed the opposite pattern to salp quantity: the lower the amount of salps in the diet, the higher the amount of animal matter. During winter when salp intake was lowest in coastal fish, the amount of other animal matter ingested was four times higher than during other seasons. For offshore fish salp intake was lowest in autumn and winter, during winter the amount of animal matter in the diet doubled. Juvenile *G. tricuspidata* are carnivores (Morrison, 1990). Even though animal matter (salps excluded) contributes little to the overall diet in adult fish, it appears to be important in complementing the diet with nitrogen and protein when salps are not available.

The diet analysis of *G. tricuspidata* revealed significant differences between seasons (Figure 3.5), and dietary items had differing characteristic element/nutrient compositions (Figure 3.7). Despite this variation the composition of nitrogen, carbon, lipids, and ash remained nearly constant throughout the year, with no significant seasonal changes at either study location (Figure 3.9, 3.10, Table 3.7). Differences in nutrient composition between populations were only detected in autumn, where diets differed as discussed above. These seasonal changes in the diet also resulted in slight variations of the C:N ratio. For coastal fish the lowest C:N ratio was recorded in autumn (5.97:1) and the highest in spring (7.21:1) and intermediate values in summer (6.72:1) and winter (6.13:1). The overall C:N ratio was 6.51:1, slightly lower than 6.80:1 in offshore fish. For offshore fish summer (6.76:1) and winter (6.00:1) was also in the intermediate range but highest values were recorded in autumn (7.30:1) and lowest in spring (6.19:1). Raubenheimer et al. (2005) demonstrated that during a short period of a few days the intake of algae in *G. tricuspidata* appeared to be regulated by protein rather than starch intake. When offered three algae species individually, fish ingested similar amounts of protein but variable amounts of starch. Results of the present study showed that when feeding in the wild where

G. tricuspidata can choose from a wide variety of food items (Appendix A7) they have the potential to not only regulate protein intake but the intake of all nutrients through the year. This has been observed in other animals, including cockroaches, locusts, and fish (Behmer, Cox, Raubenheimer, & Simpson, 2003; Raubenheimer & Jones, 2006; Vivas, Sánchez-Vázquez, García, & Madrid, 2003). Results of wild caught fish in the present study support the conclusion that Raubenheimer et al. (2005) drew from their feeding experiment, i.e. that *G. tricuspidata* is an omnivorous fish that complements its mainly algal based diet with animal matter (Raubenheimer et al., 2005). This complementary feeding strategy enables *G. tricuspidata* to regulate the balance of nutrients they ingest and improves the nutritional quality of the diet. The overall resource availability is increased, as fish do not depend on one food source alone. It might enable them to survive on nutritionally imbalanced food over prolonged periods and subsequently restore nutrient imbalances through selective feeding (Clements et al., 2009; Raubenheimer & Jones, 2006). Protein requirements of fish are estimated to be as high as 29-55% for maximum growth rates (Horn, 1989; R. P. Wilson, 2002a), but the present results show that *G. tricuspidata* maintains a diet through the year containing average nitrogen levels of less than 4.6%, which is equivalent to about 25% protein. In summer and spring when there is a plethora of salps *G. tricuspidata* could increase ingestion rates of salps. For the freshwater fish *Tilapia aurea* it has been shown that the protein assimilation rate depends on the ratio of assimilable protein to assimilable energy (Bowen et al., 1995). This could explain why *G. tricuspidata* still included algae into the diet even though fish could have easily increased their intake of protein. Mixing the diet items allowed for a balanced intake of nutrients through the year and probably an optimized nutrient assimilation.

3.4.4 Physiological responses to a variable food supply

Zihler Index and relative gut length have been used as potential indices to identify the dietary strategy of a fish based on its gut length. The relative gut length is widely applied but comparable data on the Zihler Index for large herbivores does not exist, which makes comparison with other species impossible. Relative gut lengths were 2.3 times the standard length in offshore fish and 2.5 in coastal *G. tricuspidata* (Figure 3.17a), which are within the range of values previously determined for this species (1.9 to 2.9) (T. A. Anderson, 1986). In general, the relative gut length is greatest in herbivorous fish (2.0 - 21.0), followed by omnivores (1.3 - 5.0), and carnivores with the shortest relative gut lengths (0.5 – 2.4) (Al-Hussaini, 1947; Kapoor et al., 1976). This classification is imprecise as categories overlap considerably and *G. tricuspidata* lies within the range of omnivorous species, or herbivores with relative short gut lengths, or carnivorous species with relative long guts. Therefore

any conclusions drawn from relative gut lengths need to be viewed cautiously as previously discussed by several authors (Clements et al., 2009; Clements & Raubenheimer, 2006; German, Horn, & Gawlicka, 2004; Horn, 1989; Kramer & Bryant, 1995b). Relative gut length should only be applied to identify broad categories because it is influenced by fish size, body shape, recent feeding history, ontogeny, and phylogeny. In consideration of nutritional significance, an increase in gut length does not consequently lead to a larger absorptive surface area in the gut (Montgomery, 1977). Relative gut length also does not take pyloric caeca into account, which increase the area for digestion and absorption (Buddington & Diamond, 1987). These are numerous in *G. tricuspidata* (100-150) (T. A. Anderson, 1986). The existence of pyloric caeca, which are generally better developed in carnivores than herbivores (Buddington & Diamond, 1987) might facilitate the comparably short gut length of *G. tricuspidata* even though algae contribute the largest part to the diet. Kramer and Bryant (1995b) showed that there was no relationship between the proportion of plant material in the diet and intestine length among omnivorous fishes in a tropical stream. The present findings highlight the caution required when drawing conclusions about dietary preferences based on gut length.

Due to these problems it has been proposed that body mass be taken into account when comparing gut lengths of small and large fishes of the same species (German & Horn, 2006; Kramer & Bryant, 1995b), thus the Zihler Index is used for discussing intraspecies and seasonal variation. The Zihler Index was 7.8% greater in coastal than offshore *G. tricuspidata* (Figure 3.17b). As mentioned above, gut length is influenced by a range of factors and a long gut does not necessarily imply that fish have an increased absorptive capacity. It is more likely that gut length relates to throughput rate and in longer guts the exposure of ingested food to the digestive enzymes would be increased (Clements & Raubenheimer, 2006; Horn, 1989). Seasonal differences in the Zihler Index within the population were greater than differences between populations. Through the year the Zihler Index varied by a maximum of 17.3% for coastal and 19.8% for offshore fish but differences were not consistent between populations (Figure 3.18). Diet can influence gut length in fishes such as stichaeds (Behrens & Lafferty, 2012; German & Horn, 2006), but the factors influencing spatial and temporal variation in relative gut length in *G. tricuspidata* were not resolved in the present study. Neither diet composition nor nutrient composition showed clear patterns of variation between seasons and locations, so the relationships between these variable and gut length remain unclear.

A longer gut should also increase total gut volume (Horn, 1989), but the relative gut content mass in coastal fish was about 18% less than in offshore fish. Rising water temperatures are generally associated with an increase in food intake and growth rate (R. P. Wilson, 2002a). During reproduction

energy demand increases (Bureau et al., 2002), which would lead to a higher demand for nutrients/food in *G. tricuspidata* during spring. However, relative gut content mass remained constant throughout the year (Figure 3.15), despite seasonal changes in relative gut length (Figure 3.18). Even though diet composition varied seasonally, feeding on a particular diet item was not linked to the amount eaten (Table 3.8). Relative gut content mass in relation to nutrient composition of the stomach contents provide a proxy for nutrient intake. The lack of variation in relative gut content mass, in combination with a constant nutrient composition throughout the year indicates that each population had a similar level of nutrient intake throughout the year and did not increase food intake during reproduction, which is considered a time of higher nutrient demand.

Higher relative gut content mass in offshore fish indicated that the overall nutrient intake is higher than in coastal fish. Comparison of relative gut content mass between locations must take into account differences in daily sampling time between the two locations. This difference was caused by the logistics of spearing fish at each location. It has been hypothesized that diurnal feeding takes advantage of the increased starch content and thus nutritional value of algae in the afternoon (Zemke-White et al., 2002). Bite rates of *G. tricuspidata* increase from sunrise to late afternoon, and decrease again towards sunset (Raubenheimer et al., 2005). Coastal fish were speared between 8.45 am and 4.00 pm, with the majority caught between 9.00 am and 12.00 pm. Offshore fish were caught between 9.00 am and 6.15 pm. Only 24 of 112 fish were caught in the afternoon at the coastal site, compared to 121 of 181 offshore fish. Furthermore, 44 offshore fish were caught later during the day (after 4.00 pm) than any coastal fish (Figure 3.16). There is thus the potential for a comparison of gut content mass and nutrient compositions between locations to be confounded by this sampling discrepancy, and hence the effect of sampling time on relative gut content mass and nutrient content was tested.

There were no statistical differences in levels of carbon, nitrogen, lipids, and ash in stomach contents (section I) between morning and afternoon samples from either population (Appendix B7). Furthermore, no differences were found in levels of carbon, nitrogen, and ash between coastal and offshore populations. Only lipid levels differed significantly between populations. This indicates that differences between populations in capture times did not confound food comparisons between populations. In offshore fish the total average relative gut content mass was significantly higher in the early and late afternoon than in the morning. No significant difference between morning and early afternoon samples was detected for coastal fish (Figure 3.16, Appendix B7). A significant difference between the coastal and offshore population was also detected in total relative gut content mass

(Appendix B6) and between adult fish (Appendix B9). Thus time of day when fish were sampled potentially confounds the comparison of relative gut content mass between locations.

The general pattern of the condition factor in fish is well known and usually shows a decrease during times of low temperatures and/or low availability of food, an increase towards the spawning season with a sharp decline after spawning, especially in females, followed by a second increase (Froese, 2006). The condition factor K_{TW} in *G. tricuspidata* increased in spring with a sharp decline after and stayed constant throughout the rest of the year (Figure 3.13a). There are two factors that suggest that the increase of total body mass in spring was due to an increase in gonad mass from October to December. First, K_{GW}, which does not include the weight of the gonads, liver, and intestine only showed slight annual variation and no increase in spring (Figure 3.13b). Second, the GSI resembles the pattern of K_{TW} (Figure 3.12) and shows that the increase in spring is due to the ripening gonads and their increasing weight. Condition factor and GSI data were analyzed monthly rather than seasonally in an attempt to detect changes in greater detail. This resulted in the lack of data for some months when fish were not sampled at either of the locations (lack of samples: coastal: January, offshore: March, April, Juli, September, and December). Nevertheless, data can be compared to seasonal nutrient composition results as the GSI and K_{TW} displayed highest values during the three month of spring (October to December), and remained low during the other three seasons. During the reproductive season energy demand increases (Bureau et al., 2002; Hendry & Berg, 1999). Lipids and fatty acids are the primary dietary requirements for egg development that determine successful reproduction and offspring survival (Hendry & Berg, 1999; Izquierdo, Fernández-Palacios, & Tacon, 2001). During spring *G. tricuspidata* increases the intake of salps, which contain high lipid concentrations compared to the other dietary categories. Some fish species store energy prior to reproduction, cease feeding, and use their stored energy resources to reproduce (capital breeding) (Stephens, Boyd, McNamara, & Houston, 2009). In some species, such as sockeye salmon, this strategy results in a drastic loss of weight and ultimately death (Hendry & Berg, 1999). But the lack of significant variation in body mass, food intake, and dietary nutrient composition through the year indicate that *G. tricuspidata* did not reallocate resources from somatic to reproductive tissues, but rather managed to derive adequate energy and nutrients for reproduction from their food (income breeding). If they used stored energy reserves for reproduction then this was not evident in changes in condition.

3.4.5 Conclusions

G. tricuspidata is an omnivorous fish that mainly feeds on epiphytic Rhodophyta throughout the year. These epiphytes supply fish with the majority of their energy in the form of carbohydrates, and also majority of protein and lipid. The epiphytic red alga *A. suborbicularis* was the preferred alga; a species that is clearly visible and easy to remove from the host plant. It is available as a food source year round but does appear to vary in abundance over local spatial scales throughout the year. *G. tricuspidata* is a highly mobile fish species that swims along the coast enabling it to locate areas of fucoid algae with high abundances of epiphytes. Epiphytic Rhodophyta contained more protein than Chlorophyta and Ochrophyta and displayed a lower C:N ratio, making these epiphytes more favourable from a nutritional perspective.

G. tricuspidata is a browser with a morphologically specialised mouth to selectively feed on algae (Yagishita & Nakabo, 2003). Interestingly, a large amount of the diet consisted of the pelagic salp *T. democratica*. Salps are easy prey due to their low mobility and do not require an excessive amount of energy to capture. They offer a nutritious addition to the mainly herbivorous diet of *G. tricuspidata* as they have a more favourable C:N ratio and contain higher amounts of protein and lipid than algae. Salps are highly efficient non-selective filter feeders and are capable of removing large amounts of the total phytoplankton production (Madin, 1974; Zeldis, 1995) and are likely to play an important role in the carbon cycle (Henschke et al., 2016). Mesozooplankton is estimated to remove 368% of the daily primary production in the outer Hauraki Gulf during early spring with salps contributing 87% to the clearance rates (Zeldis & Willis, 2014). At times of high abundances during the warmer months *T. democratica* contributed a significant proportion of the diet of *G. tricuspidata* (up to 31.5%). In combination with the large amounts of algae consumed, a considerable amount of energy is thus quickly transferred to higher trophic levels by *G. tricuspidata*.

When salp abundances decreased, fish of both populations increased their intake of animal matter and detritus. Stomach contents of offshore fish contained higher amounts of cyanobacteria/diatoms and Chlorophyta. These offer an alternative source of protein and lipid. The mainly herbivorous diet of *G. tricuspidata* is supplemented with animal material, and fish selectively fed on a large range of food items with different nutrient concentrations resulting in a balanced intake of nutrients throughout the year.

In conjunction with diet and nutrient intake, the ability of fish to access and absorb nutrients also has to be considered (Horn et al., 1986; Pillans et al., 2004). Pillans (2004) showed that a herbivorous rabbitfish chose to feed on algae based on the highest amounts of assimilable biomass, energy, carbon, and nitrogen rather than on total nutritional content. During collection of *G. tricuspidata* it became clear that the dietary composition of the stomach contents often differed from the contents at the posterior end of the gut (section V). This variation in food intake throughout the course of a day made calculation of absorption efficiencies impossible and hence these could not be included in this study. Results from feeding experiments offering *Ulva intestinalis* (former *Enteromorpha intestinalis*) showed that *G. tricuspidata* is capable of absorbing high proportions of starch (94.1 ± 0.52%), protein (81.7 ± 1.95%) (Raubenheimer et al., 2005), total nitrogen (83.50 ± 0.58%), protein nitrogen (79.17 ± 1.51%), total carbon (81.92 ± 0.71%) and to a lesser degree lipid (55.28% ± 5.34%) (T. A. Anderson, 1988). Overall, 78.41 ± 0.89% of the whole algae were utilized by fish (T. A. Anderson, 1988). There was only a small difference in the absorption of total nitrogen and protein nitrogen, indicating that *G. tricuspidata* does not use an alternative source of nitrogen and most of the nitrogen is derived from protein (T. A. Anderson, 1988; Raubenheimer et al., 2005).

Caceres et al. (1994) showed that the herbivore *Aplodactylus punctatus* was to some extent capable of compensating for variation in food quality and food intake. Populations living in habitats with lower food availability and quality, increased food intake and had larger digestive tracts. Contrary to this compensatory feeding strategy, the herbivore *Odax pullus* reduced food intake on a low quality diet and increased intake on a high quality diet in feeding experiments, following a strategy described in mammals as 'anticipatory' (Baker et al., 2016). An increase in gut content mass could not be observed in *G. tricuspidata* on particular dietary items. Hence, three factors could potentially contribute to the difference in gut length and gut content mass between populations of *G. tricuspidata*. First, coastal fish might have had less available food, resulting in lower relative gut content mass values but greater relative gut length that allows for slower transit times. Second, the nutritional value of food available to coastal fish was inferior to that of offshore fish and resulted in a reduced food intake (anticipatory strategy). Third, the opposite might be true and the diet of offshore fish might have been less nutritious such that fish increased food intake to meet their nutrient demands (compensatory strategy). However, nutritional results indicated that there were no differences in the nutrient composition of dietary items between the two populations (Figure 3.8) and relative gut content mass differences might have been confounded by the sampling discrepancy between populations. Thus food availability might have caused differences in relative gut lengths between populations but the opportunities to determine causation lie outside the scope of this study.

Chapter 4

The effects of temperature on growth and longevity

4.1 Introduction

Being ectotherms, growth in fish is heavily influenced by the environment. Variation in growth rate is driven by the dynamic interaction of various intrinsic and extrinsic factors such as age, fitness, reproduction, competition, nutritional quality and availability of food, temperature and climate, and fisheries exploitation (Black et al., 2005; Caldow & Wellington, 2003; Morrongiello & Thresher, 2015; Sala-Bozano & Mariani, 2011; Sarre & Potter, 2000; B. M. Taylor et al., 2015; Trip et al., 2013). Environmental changes can lead to alterations in growth rate, longevity, age at maturity, and mortality and thus change the productivity and composition of the population (Campana & Thorrold, 2001). Information about these life history traits is important to help understand the ecological role of fishes and assist in stock assessment and fisheries management (Campana, 2001; Campana & Thorrold, 2001; Pikitch et al., 2004).

Age is the most important determinant of growth rate variation in almost all fishes i.e. growth rates decline with increasing age (Morrongiello & Thresher, 2015). Otoliths display daily and/or annual increments in many fish species allowing for age and growth estimations (Campana & Thorrold, 2001). Otoliths continue to increase in mass as growth increments are deposited, even if somatic growth is negligible (Caldow & Wellington, 2003; Maillet & Checkely, 1989; Popper et al., 2005).

Remarkably little is known about how a particular body size is achieved and which factors regulate growth rate (Arendt, 2010). Temperature has been identified as an important factor influencing growth. In marine fish these growth responses to the Temperature-Size Rule (TSR) can be observed in species whose distributions span a wide range of latitudes. Populations living in habitats at diverging latitudes experience differences in sea surface temperatures (SST) that affect growth rates. Examples are the herbivorous fish *Odax pullus* and the carnivorous fish *Notolabrus fucicola*, which were sampled over 11° of latitude in New Zealand (Trip et al., 2013). Another example is *Girella elevata*. Growth curves were established for two populations in eastern Australia living about 300 km apart (about 2° of latitude) (Stocks, Gray, & Taylor, 2014). Stocks et al. (2014) did look at the effects of temperature/climate on growth and found faster growth rates for the population living in warmer waters. This population also reached a smaller adult body size, however authors did not clearly state that growth responded to the TSR in this species. Growth responses conforming to the TSR can be observed along a latitudinal temperature gradient as well as on smaller local scales. Demography and life history traits varied between regional populations of the omnivorous reef fish *Stegastes beebei* in

the Galápagos Islands that were separated by less than 150 km (Ruttenberg, Haupt, Chiriboga, & Warner, 2005). Several currents create hydrogeographic regions at this archipelago with strong environmental gradients causing SST differences of up to 5°C or more over only 150 km distance. Individuals in the coldest region grew larger, lived longer, and also occurred in higher densities than fish in the warmer regions. Ruttenberg et al. (2005) concluded that these differences were most likely caused by temperature mediated life history trade-offs between growth and reproduction as well as food availability and/or quality.

Girella tricuspidata is one of the most common reef fishes in north-eastern New Zealand, they have a broad distribution spanning the coast around the north island of New Zealand and also occur about 3,000 km along the south-east coast of Australia including northern Tasmania. They are ecological generalists in terms of both diet and habitat. These biological characteristics make it a useful species to study yet data on fundamental life-history characteristics are scarce and detailed ageing and growth studies are lacking for New Zealand. *G. tricuspidata* can reach a maximum fork length (FL) of 710 mm (common TL mm) and maximum weight of 4000 g in Australia (Kailola et al., 1993). Maximum FL measurements from New Zealand have not been reported, but fish with 450 mm FL were used in a study by Taylor and Willis (1998) (based on data set from Morrison, 1990). The first studies on growth and longevity of *G. tricuspidata* were based on scale counts and recorded a maximum age of 10 years in New Zealand (Morrison, 1990) and 11 years in Australia (Pollock, 1981). Scale counts can be inaccurate due to scale turnover and it has been demonstrated that this method underestimates the age of *G. tricuspidata* (Campana, 2001; Gray et al., 2012). Recent otolith ageing studies on *G. tricuspidata* in Australia recorded a maximum age of 24 years (Gray et al., 2010; 2012), indicating that this species lives longer than previously believed. To understand the ecological role of these fish it is crucial to obtain accurate age estimations for New Zealand populations based on otolith increment counts, which also includes the validation of annual increment formation. Gray et al. (2010) validated annual increment formation for *G. tricuspidata* in Australia. Gillanders et al. (2012) indirectly validated the formation of yearly rings in fish from north-eastern New Zealand by matching increment widths with annual mean sea surface temperatures. Studies conducted on *G. tricuspidata* inhabiting the east coast of Australia showed spatial differences in growth rates and asymptotic lengths between sexes and estuaries (Gray et al., 2010; 2012) that were not clearly correlated with temperature effects alone as female fish grew fastest in the central region and males grew fastest in the northern region. Other parameters such as variable spawning periods and differences in the initial growth rates of juveniles were considered to have an additional effect. Further, the movement of

individual fish between estuaries might have masked spatial differences in growth (Gray et al., 2010). These factors will have to be considered in the present study.

Spatial gradients of environmental conditions create ecological variations. These variations can affect demography and life history traits of populations and are usually observed on large spatial scales, associated with latitude, but have also been observed on smaller spatial scales (Ruttenberg et al., 2005; Trip et al., 2013). Investigating a population at different times or populations in distinct habitats allows for comparison of life history traits and the variability of the environment they live in. In this chapter spatial differences between two distinct populations of *G. tricuspidata* in the Hauraki Gulf in north-eastern New Zealand are investigated. The coastal population was sampled along the east coast of northern New Zealand around the small town of Leigh. Great Barrier Island, situated in the east of the Hauraki Gulf was sampled for offshore fish. Temperature differences can be observed between the outer shelf (offshore) and along the coast. The warm East Auckland Current has a stronger influence at Great Barrier Island. Upwelling on the shelf north of the Hauraki Gulf occurs from early spring to early/late summer resulting in colder SST along the coast during that time (Zeldis et al., 2004). (For more details on the locations see Chapters 1.4 Ecology and hydrology of the study area and 2.1.1. Study sites).

The intensity of environmental factors such as temperature also fluctuates over time causing temporal growth variations. Otolith growth reflects these fluctuations resulting in variable growth increment widths. Thereby the sensitivity of species to environmental factors can be determined. Long-term otolith growth chronologies can be used to reconstruct various aspects of climate and the effects of temperature on growth rates in natural environments on a temporal scale (Black, 2009; Doubleday et al., 2015; Rountrey, Coulson, Meeuwig, & Meekan, 2014; Stocks et al., 2014). Gillanders et al. (2012) constructed otolith growth chronologies over a period spanning 27 years for *G. tricuspidata* from fish sampled around Leigh, which is the same location as coastal fish of the present study. Chronologies revealed high frequency variation and growth was strongly correlated with summer sea surface temperatures (SST). Temperature might be identified as a factor influencing growth in the present study and support the findings of Gillanders et al. (2012) if similarities can be detected between their chronology and newly developed ones of the present study. Comparison of coastal and offshore chronologies might also provide further understanding of factors causing potential spatial differences in growth between populations. Black (2005) suggested that ageing accuracy can be improved by comparing increment widths of otoliths to existing master chronologies in long-lived fish. This is a novel approach and usually not feasible due to the limited number of existing master chronologies.

But this method will be tested in the present study as a master chronology exists for *G. tricuspidata* in north-eastern New Zealand.

4.1.1 Aims and objectives

The purpose of this chapter is to analyse growth patterns in the two populations (coastal and offshore), and to investigate the effects of sea surface temperature on these over time. Based on current knowledge it is hypothesised that opaque and translucent increments are expected to form annually in otoliths of *G. tricuspidata*, enabling the ageing of fish. The formation of annual increments will be validated. Longevity is expected to be greater than previously estimated in New Zealand for this species. Ageing data will be used to investigate differences in growth curves between sexes and the two populations. Environmental differences between the coastal and offshore sampling locations might cause differences in growth, with temperature being one of the most important factors affecting growth. Growth curves will be used to investigate whether differences in growth curves conform to the Temperature-Size Rule.

4.2 Methods

The collection of fish is described in detail in Chapter 2.2.1 Fish sampling. Sagittal otoliths were collected and cleaned with 70% ethanol and stored dry until further processing. Additionally to the fish collected in this study 33 specimens that were collected for field courses and speared at Great Barrier Island each year in August between 2010 and 2014 were integrated in this study.

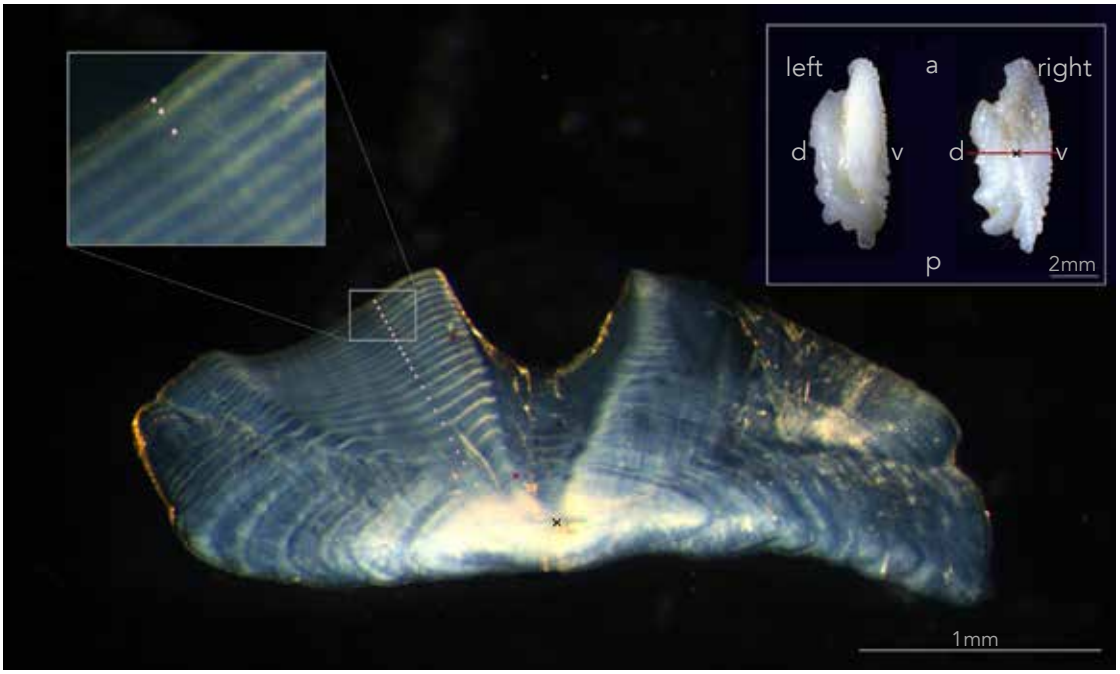


Figure 4.1: Transverse section of the left otolith of a twenty-year-old *G. tricuspidata*, viewed under reflected light. X = nucleus. Pink dots = edge of a finished opaque zone, the distance between two dots (one dark translucent and one white opaque zone) is equivalent to the growth of one year. Red dot = Measurement point for the increment width of the first year. Insert top left: Pink dots mark points for measuring the marginal increment width and the second last increment width. Insert top right: Left otolith shown from the medial side and right otolith shown from the lateral side. X = nucleus, red line shows position of transverse section, d = dorsal, v = ventral, a = anterior, p = posterior.

The left otolith was used for ageing analysis unless it had not been collected, was broken or was not clearly readable, in which case the right one was used. In total the otoliths of 409 fish were prepared but one was rejected as both otoliths were deformed and annuli could not be read. Otolith preparation followed the protocol of Trip et al. (2008; 2011). The anterior and posterior ends were sanded with wet grinding paper (Norton, grit P400) until a thin transverse section through the nucleus remained. Grinding was finished off with wet P800 paper and the otolith was glued onto a glass slide and covered with Crystal bond thermoplastic cement (Aremco) for reading (Figure 4.1).

Otoliths were viewed against a black background with reflected light. The annuli were counted along a radius from the primordium to the outer edge of the dorsal lobe close to the margin of the sulcus acusticus and were counted three times by the same person (TS) on different days. To avoid potential bias, all counts were made without knowledge of fish size, sex, date of collection, or collection location. One translucent zone (summer growth) and one opaque zone (winter growth) were counted as one year. The first reading was done using a stereomicroscope at 40x magnification (Leica MS 5). The second reading was also done with a stereomicroscope at 60x magnification (Leica MZ 9.5) and a photo was taken at 40x magnification with a digital camera attached to the microscope (Leica DFC 320, 3.3 megapixel). The third reading was made with the photo using the digital image analysis software ImageJ 1.84v (http://rsb.info.nih.gov/ij/) with the plugin ObjectJ (https://sils.fnwi.uva.nl/bcb/objectj/index.html, downloaded 10.04.13). A marker was placed on each opaque zone to allow for re-checking the otolith.

The age (in years) was calculated as the mean of the three separate readings. 43% of the results were consistent throughout the three readings. For 54% of the otoliths the readings differed by only one year from the mean and 2% by two years. Less than 1% of the readings varied by more than three years. Where triplicate readings did not coincide, the photo with the markers was analysed again and where possible the source of errors identified (these were usually due to difficulties in identifying the first rings, deciding on the completed formation of the last newly formed ring, or inclusion of faint false rings had been counted; see below). Based on the outcome of this process, the appropriate age was chosen.

Analysis of the otoliths revealed that occasionally the first increment was difficult to determine and false rings might have been put down within the first year. Campana (2001) stressed the importance of validating the first growth increment to ensure correct results. The procedure used to validate the first annual increment in the present study was as follows. The width of the first increment was measured using ImageJ and ObjectJ. Measurements were taken from the centre of the nucleus and along the edge between the ventral lobe and the sulcus acusticus. A scatterplot revealed questionably wide or narrow increments, which were inspected again. As a result of this process, an extra ring was added to thirteen fish. Their increment measurements revealed a very wide first ring and by inspecting the otolith again a faint first ring made it reasonable to add this ring as a yearly ring. The first ring was deleted for seven fish as the measurement indicated that it was a sub-annual ring.

The marginal increment of the otolith was examined to estimate a date on which the outermost opaque zone was completed. The edge type in relation to the assigned birth date was then taken into account, as the correct age might not be determined correctly by counting annuli only (Ewing et al., 2003; Morison, Coutin, & Robertson, 1998). The timing of peak spawning was inferred from the point at which annual peak GSI values commenced their decline. This time of peak spawning can be established as the birth date for age estimations (Ewing et al., 2003). Thus for *G. tricuspidata* the 1st of January was assigned as the birthday as peak spawning takes place in December (Chapter 3.3.6 Gonadosomatic Index). Formation of a new translucent zone took place from December to February, thus completing the annual increment (Figure 4.2). One fish caught in December showed a narrow translucent zone, which indicates an early increment formation and the age was reduced by one year. Twenty-six fish that were caught in January or February had otoliths with a wide translucent zone and an incomplete opaque zone. They were considered to have formed an increment late and the age count was increased by one.

Marginal increment analysis was also used to validate the periodicity of the opaque zone formation in adult *G. tricuspidata*. The percentage of completion of the marginal increment was calculated in relation to the second last ring formed (Ewing et al., 2003). Increment widths were measured on the dorsal lobe with the last increment ranging from the completed opaque zone of the second last increment to the edge of the otolith (Figure 4.1, insert top left). An opaque zone was considered complete if translucent material appeared between it and the edge of the otolith. All results were combined as there was no difference in time of formation between the sexes, locations, and the years sampled.

4.2.1 Data analysis

All Statistical tests were performed using the software IBM SPSS Statistics v22 with the SPSS statistic guides (Laerd Statistics, 2015).

4.2.1.1 Longevity

Estimation of longevity has been calculated in different ways (Beverton, 1992; Kritzer, Davies, & Mapstone, 2001; Trip et al., 2008). To enable comparison with other data longevity was calculated as follows: (1) as the oldest individual of the sampled population (T_{max}), (2) as the mean of the oldest 10% of fish in the sample ($T_{max\ 10\%}$), and (3) as the mean of the oldest 25% of fish in the sample

($T_{max\ 25\%}$). A Mann-Whitney U-test was performed to test for differences in longevity between males and females or coastal and offshore fish for $T_{max\ 10\%}$ and $T_{max\ 25\%}$.

4.2.1.2 Size-at-age modelling

The generalized von Bertalanffy growth function (VBGF) is the most commonly used equation in fisheries literature to describe growth in fish (Chen et al., 1992; Haddon, 2011; Katsanevakis & Maravelias, 2008). Its equation is as follows:

$$L_t = L_\infty * (1-e^{-k(t-t_0)})$$

where L_t is the fork length at age t, L_∞ is the asymptotic average maximum fork length, k is the growth coefficient (a measure of how rapidly the fish approaches L_∞), and t_0 is the theoretical age at which the species has zero length.

Even though the VBGF describes fish growth adequately it has been criticized for various reasons (Cerrato, 1990; 1991; R. I. C. C. Francis, 1988; Haddon, 2011; Schnute & Fournier, 1980; Welsford & Lyle, 2005). Limitations include the lack of biological relevance of its parameters and the uncertainty of the statistical properties of the parameters when comparing them between populations. These limitations are important considerations for this study and thus a re-parameterized version of the VBGF was also used.

One of these reparameterized von Bertalanffy growth functions (rVBGF) was developed by Francis (1988) and has often been utilized to describe fish growth (Claisse, Kienzle, Bushnell, Shafer, & Parrish, 2009; Trip et al., 2008; 2011; Welsford & Lyle, 2005). The rVBGF equation is as follows:

$$L(t) = L(\tau) + \frac{(L(\mu) - L(\tau)) \left[1 - r \left(2 \frac{t - \tau}{\mu - \tau}\right)\right]}{1 - r^2}$$

where

$$r = \frac{L(\mu) - L(\omega)}{L(\omega) - L(\tau)}$$

The rVBGF includes the three parameters $L(\tau)$, $L(\mu)$ and $L(\omega)$, which are the mean length for the three ages τ, μ and $\omega = (\tau + \mu)/2$. The ages τ and μ are randomly chosen within the dataset and should be

dispersed through the period of growth and well represented within the data set. The selected ages for the present study are τ = 2 as the juvenile age, μ = 18 as the adult age and thus resulting in ω = 10. There was only one fish aged 1 year in the sample set, therefore 2 years was chosen as the juvenile age. Data showed that fish reached their adult length at 18 years. 18 years was therefore selected as the adult age for analysis. Inclusion of the expected length-at-age parameters improve the statistical properties of the rVBGF dramatically for age classes drawn from the data set (Cerrato, 1991). It also results in parameters that have direct biological relevance.

The growth curves for the two locations and sexes within locations were modelled separately. Juvenile fish were not included in the sex-specific models, resulting in the youngest fish included being three and four year old for males and females, respectively.

To closely represent the growth of species and obtain accurate estimates of mean size-at-age, especially for the early years, it is also important to include recruit-size individuals in the data set, especially when juveniles are under-represented in the sample (Berumen, 2005; Kritzer et al., 2001). Thus for each VBGF and rVBGF model fitted, the y-intercept was constrained to the approximate size at settlement $L_{(0)}$. In New Zealand the size of *G. tricuspidata* at settlement is $L_{(0)}$ = 17 mm FL (based on Morrison (1990)).

The best-fit curves for the VBGF and rVBGF were obtained by minimizing the negative log of the likelihood, assuming that length at age t is a normal probability density function (Haddon, 2011). Comparative tests between re-parameterized curves were done using likelihood ratio tests (Cerrato, 1990; Haddon, 2011; Kimura, 1980). The null hypothesis of no difference in growth was rejected at α = 0.05. The hypothesis of equal $L_{(2)}$ between the sexes was omitted from the analysis as the youngest sexed fish was three years old and the y-intercepts for the curves were constrained to size at settlement.

4.2.1.3 Crossdating

Crossdating ensures that the correct calendar year is assigned to each growth increment. Each otolith is inspected for conspicuously narrow and wide rings, and such increments should correspond among samples (Black et al., 2005; Yamaguchi, 1991). Therefore all fish from the same location should reveal synchronous patterns of increment deposition (Gillanders et al., 2012). Missing or falsely added increments offset the synchronous growth pattern by a year relative to that in other samples, identifying the error (Black, 2009). Otoliths were crossdated both visually and statistically.

Measurement of otolith growth increments was conducted using ObjectJ. Increment widths were measured along a straight line perpendicular to the axis of the increment boundaries as close to the sulcus acusticus as possible. The location of the line thus varied to some degree among otoliths, and the first years were usually excluded. The year of capture was assigned to the otolith margin. Black et al. (2005) suggested that visual crossdating can be done by comparing individual otoliths with existing master chronologies. The increment widths of each otolith was plotted as a graph and compared to the master otolith chronology of *G. tricuspidata* in Gillanders et al. (2012). Visual crossdating ensured that conspicuously narrow or wide increments coincided with lower or higher values of the chronology respectively.

Statistical crossdating was conducted using the computer program COFECHA (Grissino-Mayer, 2001; Holmes, 1983). COFECHA was originally written by Holmes to be used in dendrochronology and it assesses the quality of crossdating and measurement accuracy of tree-ring series. In recent years it has also been applied to sclerochronology and otolith growth chronologies (Black, 2009; Black et al., 2005; 2016; Gillanders et al., 2012; Stocks et al., 2014) and can increase the quality of these studies.

Due to the rapidly changing geometry of otoliths that occurs during early growth, all fish with ten or fewer annual increment readings were excluded from statistical crossdating. Analysis was conducted separately for coastal and offshore fish. As fish age the width of growth increments decreases due to the asymptotic growth of the fish. Each series is transformed via spline fitting, autoregressive modelling and log transformations, which removes long-term age related trends. A cubic smoothing spline with a 50% frequency cut-off set at 22 years was fit to each series. A spline of 22 years was chosen as Black et al. (2005) showed that it produced the highest average correlations for the marine splitnose rockfish (*Sebastes diploproa*). Each detrended and standardized series was then correlated with the average of all standardized series, yielding a correlation value. Grissino-Mayer (2001) recommends examining segment lengths that are approximately half the average length of all series tested. But segment length should not be under 30 years, as it tends to yield spurious high and low correlation values. The mean length of the series was 19 and 20 years for coastal and offshore *G. tricuspidata*, respectively. Thus a segment length of 30 years lagged by 15 years was chosen. Low correlation values (< 0.42) between the master and the individual chronology indicated potential mistakes and the photo or the otoliths were inspected again to make a final decision. The master otolith growth increment width chronologies were created and reflect a residual time series that has been standardized to a mean of zero and a standard deviation of 1.0. An overall average correlation of all series with the master series is reported as the series intercorrelation. This value assesses the

strength of the crossdating for a site and preferably should be above 0.50. All these steps of statistical crossdating are automated by COFECHA. COFECHA also calculated the average mean sensitivity, which is an index of high-frequency, year-to-year growth variability, where high values (> 0.3) indicate stronger variability.

4.2.1.4 Otolith chronologies and climate

The master otolith growth increment width chronology (referred to as master chronology) was developed using the software ARSTAN (Cook, 1985). Age related low-frequency trends were removed by fitting negative exponential curves to individual increment width series. If these functions did not fit, a negative linear regression was fitted to the raw increment series. Values for each year were divided by the predicted increment width from the fitted function and the average of all residual increment time series was used to create a final master chronology for each location (Stocks et al., 2014). Only years with readings of eight fish or more were included to develop master chronologies.

Correlations between the coastal and the offshore growth increment width indices as well as for SST and indices for both regions were investigated by running a Pearson's correlation. SST data from the Leigh Marine Laboratory, University of Auckland, were used as they date back as far as otolith chronologies. The otolith chronologies were also correlated to the Mulivariate ENSO Index (MEI). El Niño/Southern Oscillation (ENSO) remains the most important coupled ocean–atmosphere phenomenon to cause global climate variability on seasonal to interannual time scales (Wolter & Timlin, 2011). The MEI is based on six variables observed over the tropical Pacific: sea-level pressure, zonal and meridional components of the surface wind, sea surface temperature, surface air temperature, and total cloudiness fraction of the sky. Even though New Zealand is usually not affected as strongly by El Niño conditions as are parts of Australia, there is nevertheless a significant influence (Wratt, Basher, Mullan, & Renwick, n.d.).

4.3 Results

The smallest *G. tricuspidata* caught measured 98 mm and weighed 35 g. The heaviest fish was a female offshore fish with a TW of 1930 g and SL of 380 mm (FL 437 mm). Another female offshore fish was also recorded as the longest fish with a SL of 390 mm (FL = 441 mm, TW = 1657 g). The majority of fish were between 310 mm and 410 mm FL (see Appendix C1 for length-frequency composition).

4.3.1 Marginal increment analysis

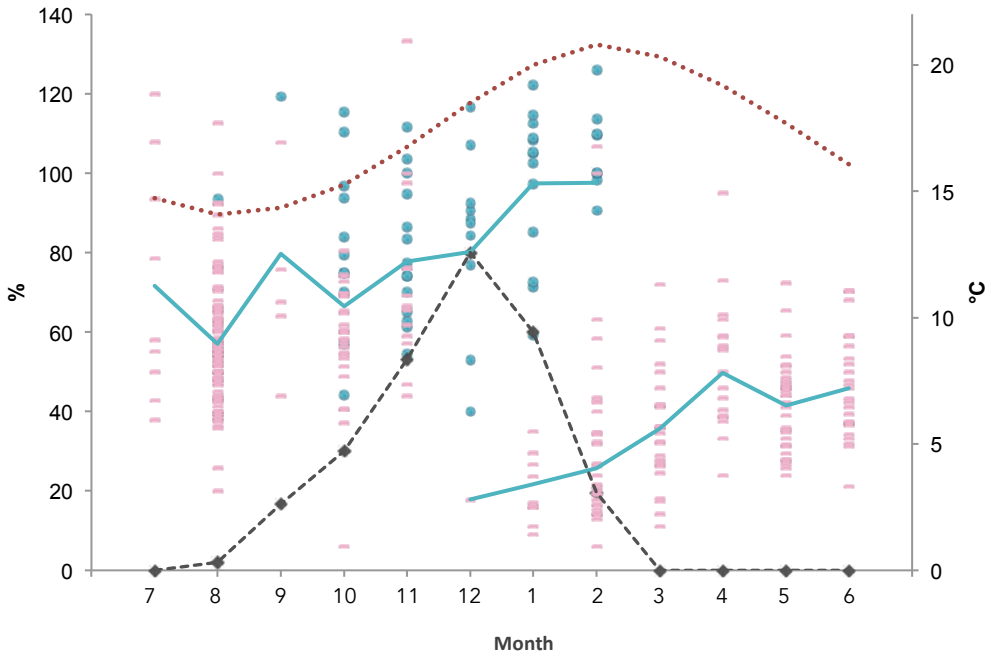


Figure 4.2: Formation of the opaque zone in relation to temperature for each month of the year. Primary axis: The percentage of completion of the last increment in relation to the second last one is displayed (1) by the turquoise dots for otoliths with an opaque zone at the edge and (2) by the pink dashes for otoliths with a translucent zone at the edge. The turquoise line connects the average values of all otoliths combined for each month. The short dashed black line and black diamonds show the percentage of fish that display an opaque zone at the outer margin of the otolith. Secondary axis: The dotted red line illustrates the average monthly sea surface temperature just south of Leigh (36.375 S, 174.875 E) (NOAA High Resolution SST data provided by the NOAA/OAR/ESRL PSD, Boulder, Colorado, USA, http://www.esrl.noaa.gov/psd/) (see map Figure 2.1 for sampling point).

The percentage of fish with an opaque zone at the edge of the otolith increased from August through to December and then dropped off rapidly in January and February (Figure 4.2). No opaque zones were visible at the edge of the otolith margin from March to July. The narrowest increments

were measured in December, January and February. Increment widths steadily increased throughout the year and the widest increments were also recorded in December, January and February. It can be concluded that opaque zone formation is thus completed in December, January and February and that the annual growth includes one translucent and one opaque zone. The same trend was observed regardless of gender, location, or age of fish.

4.3.2 Longevity

The maximum age recorded was 54 years for a male offshore fish. The oldest female fish was 50 years and the oldest coastal fish 44 years (Table 4.1, Figure 4.3). The majority of fish were between 2 and 26 years of age, and only a few exceeded 41 years (see Appendix C2 for age-frequency composition).

A Mann-Whitney U-test was run to determine differences in age between male and female fish as well as between coastal and offshore fish. Distributions of the ages were similar, as assessed by visual inspection. There was no statistically significant difference in age for $T_{max\ 10\%}$ between the median values for male (41 yrs) and female fish (40 yrs), $U = 157.5$, $z = -0.128$, $p = 0.898$, nor for $T_{max\ 25\%}$ ($U = 952.0$, $z = -0.815$, $p = 0.415$), with 34 years as the median value for both sexes. The difference of median ages of coastal and offshore fish differed statistically for $T_{max\ 10\%}$ ($U = 303.5$, $z = 2.653$, $p = 0.008$) and $T_{max\ 25\%}$ ($U = 1905.5$, $z = 4.322$, $p < 0.001$). Longevity was significantly higher in offshore (medians: $T_{max\ 10\%} = 41$ yrs, $T_{max\ 25\%} = 35$ yrs) than in coastal fish (medians: $T_{max\ 10\%} = 39$ yrs, $T_{max\ 25\%} = 27$ yrs).

Table 4.1: Longevity of *Girella tricuspidata* calculated for the oldest fish (T_{max}), the mean of the oldest 10% of the fish ($T_{max\ 10\%}$) and mean of the oldest 25% of the fish ($T_{max\ 25\%}$). All results are in years.

	All fish	Male	Female	Coastal	Offshore
T_{max}	54	54	50	44	54
$T_{max\ 10\%}$	40	42	41	37	42
$T_{max\ 25\%}$	34	35	34	30	36

4.3.3 Size-at-age modelling

The size of collected fish ranged from 98 to 390 mm SL (120 to 441 mm FL). The youngest fish was 1 year old. Gonads developed and could be sexed at a standard length of > 205 mm (250 mm FL)

and three years of age, at which stage they also showed ripening (increasing GSI values) during spring months.

Size-at-age for *G. tricuspidata* followed an asymptotic curve and was appropriately described by both models tested (Table 4.2). Growth in length was rapid for the first 7 to 8 years and then declined sharply, resulting in little change in size for the remaining life. Comparison of the rVBGF growth trajectories showed that there was no statistical difference in size-at-age between male and female fish as assessed by the likelihood ratio test ($p = 0.820$, Table 4.3, Appendix C3). Comparison between coastal and offshore fish revealed a significant difference between the growth rates of these two populations ($p = 0.001$) (Table 4.3, Figure 4.3). Coastal fish reached 52.4% of their adult length ($L_{(18)}$) by the age of two and 83.2% at five years. Offshore fish reached 40.2% and 70.7% of their adult length after two and five years respectively. This indicates that early somatic growth is faster in coastal than in offshore fish, with coastal fish reaching their adult size at about 13 years and offshore fish at 18 years. Growth curves cross at seven years of age with offshore fish reaching larger asymptotic lengths (381.0 mm FL) than coastal fish (344.6 mm FL). Further analysis of the rVBGF parameters strongly indicates that there was a significant difference between the sizes of adult fish ($L_{(18)}$: $p < 0.001$), with offshore fish being larger than coastal ones. There was no indication of a difference in the $L_{(10)}$ parameter ($p = 0.093$), but an indication that growth rates differed slightly in juvenile fish was provided by the likelihood ratio test ($L_{(2)}$: $p = 0.044$).

Table 4.2: Gender- and region-specific growth of *Girella tricuspidata* showing the results for the best fit VBGF and rVBGF model with $L_{(0)} = 17$ mm. The VBGF parameters are the asymptotic fork length L_∞ (mm), the growth coefficient k (yrs^{-1}), and the age t_0 at length zero (yrs). The rVBGF parameters are the mean size at age two (L_2), ten (L_{10}) and eighteen (L_{18}). n is the sample size, -λ the negative log-likelihood, and σ the standard deviation. For a description of the parameters refer to the method section Chapter 4.2.1.2 Size-at-age modelling.

	n	VBGF			rVBGF				
		L_∞ (mm)	k (yr^{-1})	t_0 (yr)	$L_{(2)}$ (mm)	$L_{(10)}$ (mm)	$L_{(18)}$ (mm)	-λ	σ (mm)
All fish	408	372.5	0.26	-0.18	162.6	347.0	369.4	1932.8	27.61
Male	194	366.3	0.31	-0.15	177.8	350.3	364.9	913.6	26.22
Female	174	376.1	0.26	-0.18	160.9	348.3	372.5	829.8	27.74
Coastal	172	345.3	0.34	-0.15	180.6	334.8	344.6	771.9	20.97
Offshore	236	387.0	0.23	-0.20	153.0	349.6	381.0	1097.3	24.80

Table 4.3: Results of the comparison of the rVBGFs for male vs. female and coastal vs. offshore *G. tricuspidata* using likelihood ratio tests. The header row refers to the hypotheses tested. The base case is where two individual curves are fitted separately to each data set (= best-fit curves) and subsequent hypotheses are compared to the resulting -λ value. For the coincident column one curve is fitted to the whole data set. The columns $L_{(2)}$, $L_{(10)}$, and $L_{(18)}$ show the results where curves were tested for that single parameter. -λ = sum of the negative log-likelihood for both curves combined, χ^2 = likelihood ratio, df = degrees of freedom. Statistically significant results are highlighted in bold.

		Base case	Coincident	$L_{(2)}$	$L_{(10)}$	$L_{(18)}$
male-female	-λ	1743.45	1747.81	-	1743.61	1746.04
	χ^2		0.923	-	0.033	0.549
	df		3	-	1	1
	P		0.820	-	0.856	0.459
coastal-offshore	-λ	1869.19	1941.08	1887.74	1882.14	1937.09
	χ^2		15.472	4.047	2.829	14.629
	df		3	1	1	1
	P		**0.001**	**0.044**	0.093	**<0.005**

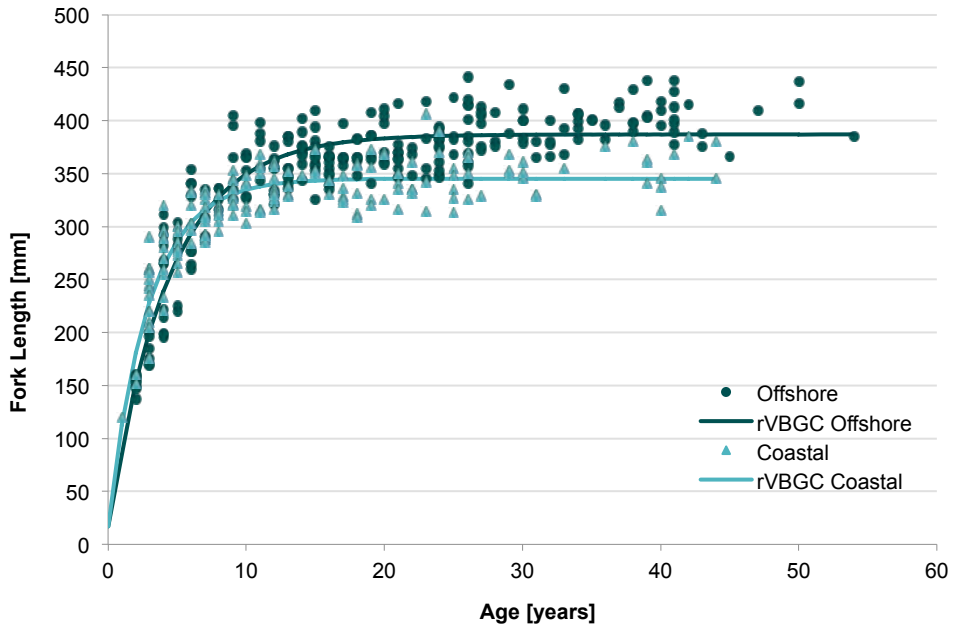


Figure 4.3: The reparameterized von Bertalanffy growth curves fitted for coastal and offshore populations of *G. tricuspidata* based on the best-fit model. For parameter values see Table 4.2.

4.3.4 Otolith chronologies and climate

Increment measurements revealed age related trends with increased increment widths for young fish. No long-term changes in growth rates were detected for either population but synchronous patterns were visible between fish in both coastal and offshore populations (Appendix C4 and C5). The

average correlation between each detrended time series (using a 22-year cubic spline) and the average of all other detrended measurement time series was 0.572 for coastal and 0.592 for offshore fish. Mean sensitivity, an index of high-frequency variability, was 0.174 for coastal and 0.161 for offshore fish. This is close to the values of 0.512 for the interseries correlation and 0.176 for the mean sensitivity as calculated for *G. tricuspidata* by Gillanders et al. (2012).

The coastal master chronology was generated based on measurements from 58 fish spanning 35 years from 1977 to 2011. For the offshore master chronology 131 fish were used spanning 39 years from 1973 to 2011. Detrended master chronologies revealed the synchronous growth patterns among individuals and large interannual variation (Figure 4.4, Appendix C6). Wider increments are reflected by positive values that indicate increased growth, while narrower increments have negative values indicating decreased growth. In coastal fish years of remarkable decreased growth were 1983, 1992, 1997, 2000, 2004, and 2006, while increased growth took place in 1978, 1981, 1995, 1999, 2009, 2010, and 2011. In offshore fish growth decreased in 1976, 1977, 1980, 1983, 1993, 2000, 2001, and 2003 to 2006, while growth increased in 1974, 1981, 1986, 1989, 1996, 1999, 2009, and 2010.

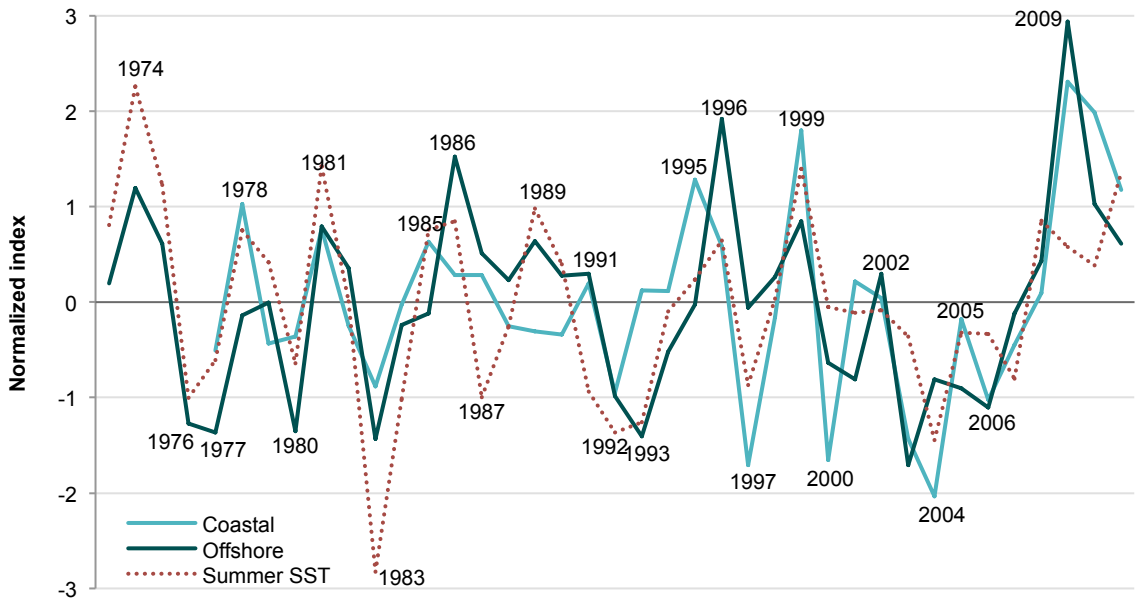


Figure 4.4: Relationship between master otolith increment width chronologies and sea surface temperature from 1973 to 2011. All time series are normalized to a mean of 0 and a standard deviation of 1. SST data were recorded at the Leigh Marine Laboratory near the coastal collection sites (36.272 S, 174.805 E) and are averaged for summer months (January to March).

Positive linear correlations between the average yearly SST and growth indices were moderately strong for both regions (coastal: $r(35) = 0.449$, $p = 0.007$; offshore: $r(39) = 0.496$, $p = 0.001$).

Correlations between the chronology indices and summer SST (January to March) were also investigated and showed stronger positive correlations for both populations (coastal: $r(35) = 0.585$, $p < 0.001$; offshore: $r(39) = 0.647$, $p < 0.001$). All data were normally distributed (Shapiro-Wilk's test: $p > 0.05$). Summer SST data are also plotted with indices visualizing the correlation (Figure 4.5).

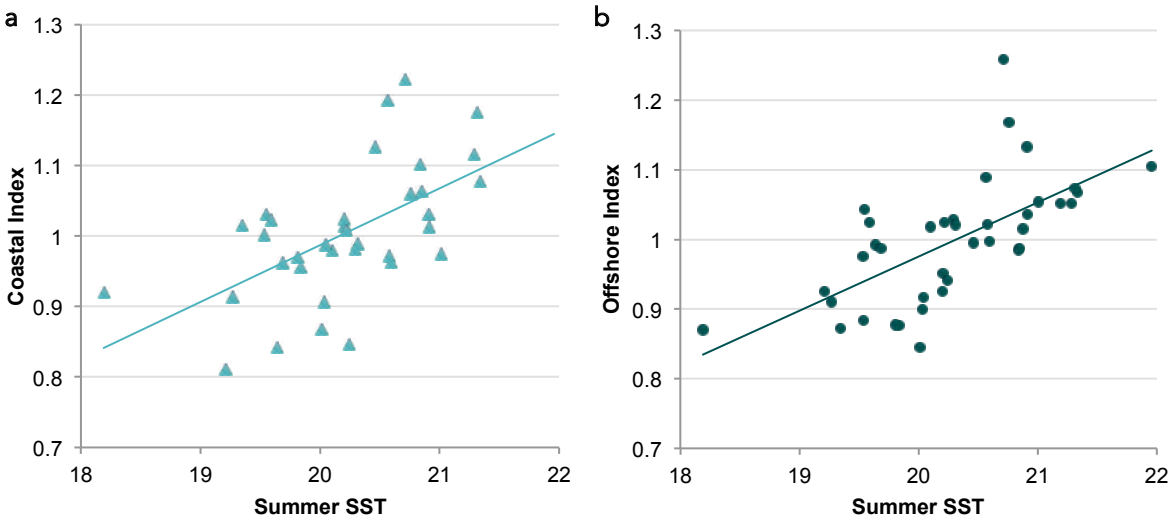


Figure 4.5: Correlations between summer SSTs and the (a) coastal and (b) offshore growth increment width indices. Each data point marks one year.

Coastal and offshore growth increment width indices were positively correlated. A Pearson's correlation was run to assess the strength of the relationship. Both variables were normally distributed, as assessed by Shapiro-Wilk's test ($p > 0.05$). The correlation was strong between the two indices, with $r(35) = 0.653$, $p < 0.001$ (Figure 4.6).

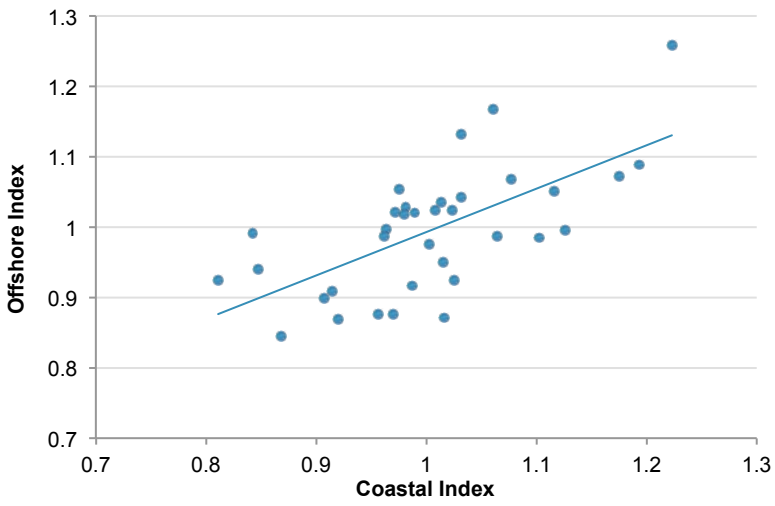


Figure 4.6: Correlation between coastal and offshore growth increment width indices with each data point marking one year.

Moderate negative correlations existed between the MEI and coastal ($r(35) = -0.375$, $p = 0.027$) and offshore fish ($r(39) = -0.340$, $p = 0.034$). All data showed a normal distribution (Shapiro-Wilk's test: $p > 0.05$) (Figure 4.7).

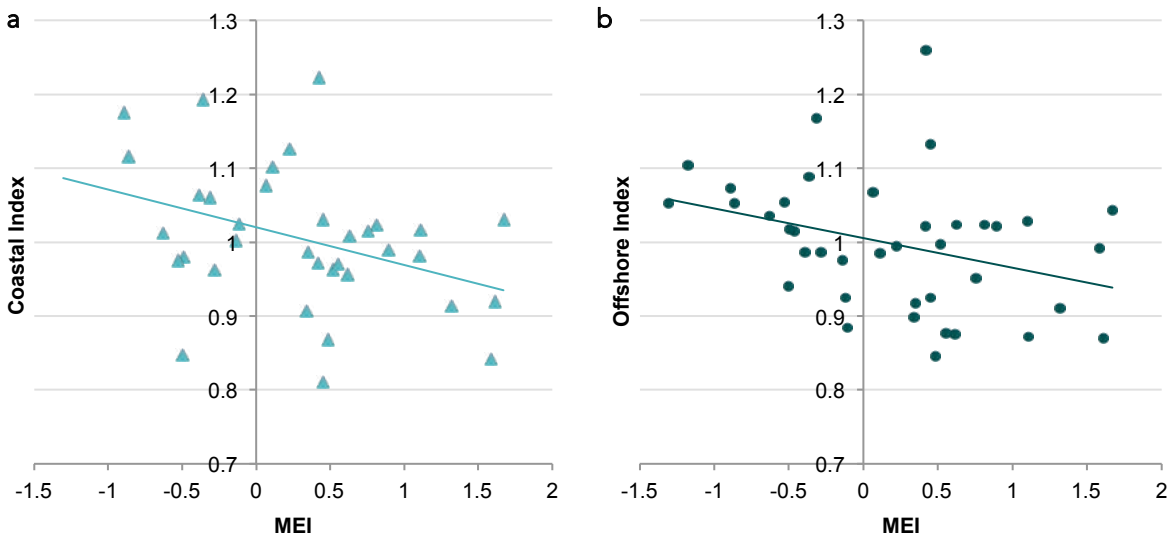


Figure 4.7: Correlations between the MEI and the (a) coastal and (b) offshore growth increment width indices. Each data point marks one year.

4.4 Discussion

Otolith increments were deposited annualy. Fish were as old as 54 years and there was a significant difference in length between adult coastal and offshore fish at any given age. Growth did not differ significantly between genders. Otolith increment widths were strongly correlated with summer SSTs and moderately negatively correlated with the MEI. Growth was faster in warm summers than in cold summers. Each of these results is discussed in detail below.

4.4.1 Annual growth increment formation in otoliths

Edge analysis of the otoliths confirmed the annual formation of increments (Figure 4.2). The opaque zone was formed during spring and early summer months, while the translucent zone was formed from summer through to winter. It is still unclear which biological processes govern the deposition of annuli in otoliths. Studies have linked the deposition of the opaque zone to factors such as spawning, faster and slower somatic growth, metabolic stress, and temperature (Caldow & Wellington, 2003; Ewing et al., 2003; Hostetter & Munroe, 1993; Millner, Pilling, McCully, & Høie, 2011; Pilling, Millner, Easey, Maxwell, & Tidd, 2007; Smith & Deguara, 2003). Reproduction in *G. tricuspidata* takes place in spring and early summer. Gonadosomatic Indices slowly increased from August/September and reached their peak values in December. Values rapidly decreased again and stayed low from March through to August (Figure 3.12, Chapter 3.3.6 Gonadosomatic Index). The spawning season thus coincides with formation of the opaque zone in *G. tricuspidata*. However, sexually immature fish collected for this study formed the opaque zone at the same time as adult fish. This indicates that spawning is not the sole factor influencing annual increment formation.

Temperature is a factor often linked to the formation of otolith increments. In north-eastern New Zealand the lowest sea surface temperatures are recorded in August and the highest in February (NOAA High Resolution SST data provided by the NOAA/OAR/ESRL PSD, Boulder, Colorado, USA, http://www.esrl.noaa.gov/psd/, Figure 4.8). Thus the formation of the opaque zone coincides with rising water temperatures, a pattern which has been reported in other marine fish in the southern hemisphere (Ewing et al., 2003; Fowler, 1990; Smith & Deguara, 2003; Trip et al., 2011) including *G. tricuspidata* at Clarence River, Australia (Gray et al., 2010). From these results alone it cannot be concluded whether the zonation is related to the onset of the spawning season or is due to accelerating fish metabolism associated with food availability or increasing water temperatures.

4.4.2 Longevity

The oldest fish recorded in this study were a 54-year-old male and 50-year-old female (Table 4.1), the maximum ages reported for *G. tricuspidata* in the literature. Previous studies documented a maximum age of 10 years in north-eastern New Zealand (Morrison, 1990), which is less than a fifth of the age found in the present study. Gillanders et al. (2012) published data from New Zealand on otolith increment widths in relation to climate. They did not report a maximum age but graphs show otolith increment widths were recorded for one individual from 1972 to 2005 or 2006. This would result in a maximum age of at least 34 years. Research in Australia reported maximum ages of 11 years (Pollock, 1981) and 24 years (Gray et al., 2010; 2012). The low counts of Pollock (1981) and Morrison (1990) are most likely due to methodological differences as the authors used scales instead of otoliths to count increments. Gray (2012) compared both methods and showed that scale counts underestimate the age of *G. tricuspidata* for fish older than 5 years due to scale loss and replacement, and should therefore not be used for ageing fish.

Incorrect age estimations can have severe implications for species that are targeted by fisheries. Fisheries management have begun to slowly shift towards utilizing ecosystem-based management, which also requires life history information for non-targeted species (Pikitch et al., 2004). Total fisheries landings of *G. tricuspidata* are relatively low in New Zealand to date (56 to 92 tons per year between 2004 and 2012), and fish are usually caught as by-catch in the grey mullet, flatfish and trevally set-net fisheries. They are a low-value recreational species and catches are likely to be low (Ministry of Primary Industries, 2013). *G. tricuspidata* is under higher fishing pressure in Australia (New South Wales alone: commercial catch 300 – 600 t, recreational 270 – 550 t per year (Rowling, Hegarty, & Ives, 2011)). Nearly 96 % (2131 fish) of 2225 fish collected in Australia (Gray et al., 2012) were 10 years or less of age, about 4 % (92 fish) between 11 and 18 years and only one fish aged 19 years and one fish aged 24 years. One reason for the lack of older specimen might be that longevity is shorter in Australia. This would be expected as a response to the Temperature Size Rule due to higher annual mean SSTs in Australia compared to New Zealand (see Chapter 4.4.3 Growth variations and the effects of temperature). Alternatively, the low number of older specimens in Australia might indicate that this species is already overfished in Australia. A decline of the annual catch per unit effort and a large reduction in total commercial catch indicate a major decline in the population of *G. tricuspidata* in southern Queensland, but this was mainly related to increasing temperatures in this region (Pollock, 2016). However, age under-estimation results in overly optimistic estimates of growth, under-estimation of longevity, and over-estimation of natural mortality and can

thus lead to overexploitation of a population or species (Campana, 2001; Tracey & Horn, 1999). It is therefore crucial to obtain accurate information on age and growth, as it is fundamental to the management of any fish stock.

4.4.3 Growth variations and the effects of temperature

VBGF and rVBGF adequately described the length-age relationship of *G. tricuspidata* in the present study (Figure 4.3, Table 4.2, Appendix C3). Several previous studies describe growth in this species using VBGFs. Taylor and Willis (1998) published VBGF parameter values in their study, which was also conducted in north-eastern New Zealand. Their results suggested that fish took longer to reach their asymptotic length (k = 0.18 vs. 0.23 to 0.34 in the present study), which was greater (L_∞ = 454 mm FL) than the one modelled in the present study (L_∞ = 345.3 to 387.0 mm FL). The dataset in Taylor and Willis (1998) was taken from Morrison (1990), who used scale counts, which, as discussed above, resulted in erroneous age estimates. Gray et al. (2010) fitted growth curves to the length-age data for *G. tricuspidata* from three latitudinal regions in eastern Australia. Growth coefficient values (k = 0.15 to 0.28) and asymptotic length data (L_∞ = 332.0 to 364.8) were comparable with smaller values of the present study. K values are probably underestimated as growth curves were truncated and the y-intercept not restricted to size-at-settlement (Berumen, 2005). Gray et al. (2012) also established VBGFs for three different estuaries in eastern Australia, showing significant differences in growth between male and female fish for two locations. Growth also differed significantly among estuaries for fish of the same sex. VBGFs were restricted (t_0 = -0.3) and parameters indicated that fish reach their asymptotic length faster (k = 0.33 to 0.59) than observed in present study. Asymptotic lengths (L_∞ = 307.8 to 385.5 mm FL) lie within the range of the present study.

Von Bertalanffy growth models showed that there were no differences in growth between male and female fish (Appendix C3), but significant differences existed between the coastal and offshore populations (Figure 4.3, Table 4.3). Coastal fish displayed faster initial growth, and the curves of the two populations crossed at seven years, resulting in a significantly larger adult size for offshore fish. Differences in juvenile growth between coastal and offshore locations were only marginally significant (p = 0.044), and may have no biological importance. The crossing rVBGF trajectories and differences in longevity, initial growth rates, and adult body size indicate that growth of *G. tricuspidata* responded to temperature as predicted by the Temperature-Size Rule (TSR) (Arendt, 2010; Ohlberger, 2013; Trip et al., 2013). The TSR predicts that ectotherms growing up in a warmer environment (lower latitudes) have faster initial growth rates and reach a smaller adult body size. They

also mature at a smaller body size and have a shorter life span than individuals living in colder environments (higher latitudes). Hence the TSR would predict that the coastal population in the present study lives in a warmer environment. Sea surface temperature (SST) records show that yearly averaged temperatures are 0.13°C to 0.44°C higher at Great Barrier Island than along the Leigh coast. Averaged maximum temperatures are similar for both locations, while averaged minimum temperatures are up to 1.36°C colder in coastal areas (Figure 4.8a, NOAA High Resolution SST data provided by the NOAA/OAR/ESRL PSD, Boulder, Colorado, USA, from their web site at http://www.esrl.noaa.gov/psd/, Figure 2.1 displays temperature sampling points). Gillanders et al. (2012) found that growth of *G. tricuspidata* showed peak correlations with summer temperatures during February and March, which was supported by the strong correlation of summer SSTs with otolith increment widths in the present study (Figure 4.5). Monthly data for both locations reveal that temperatures coincide between locations from November over the summer months through to March. For the remaining months temperatures are slightly lower for the coastal area with a maximum

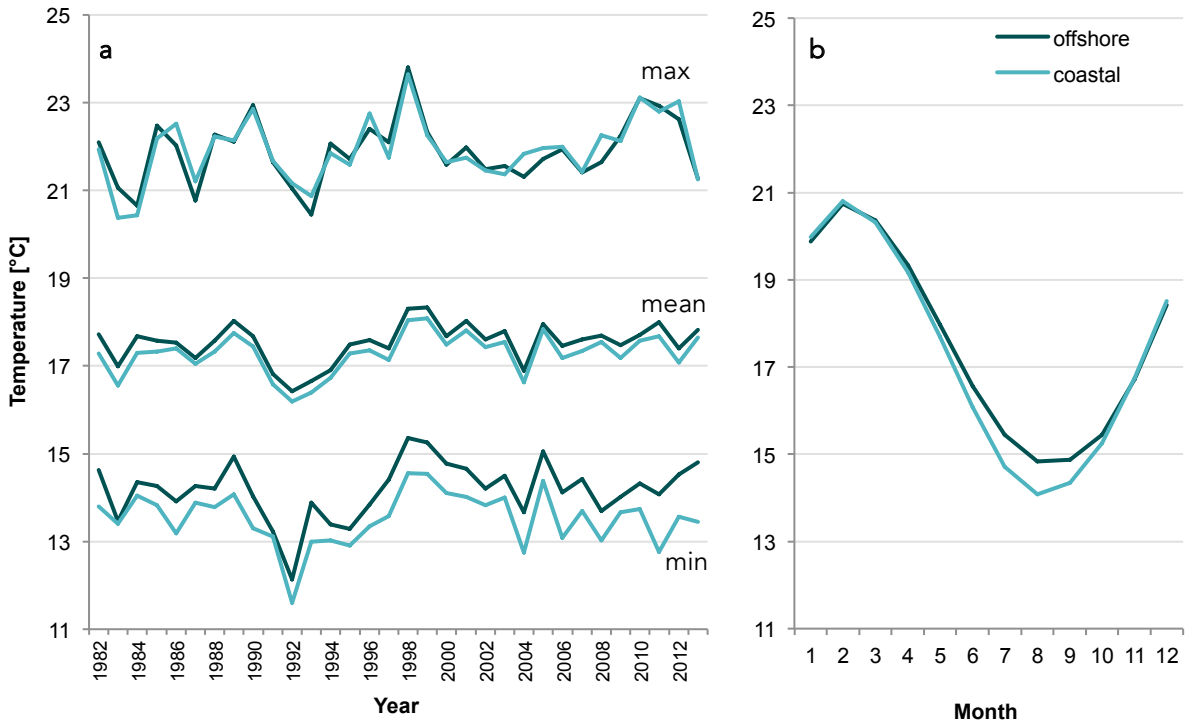


Figure 4.8: NOAA High Resolution SST data for Great Barrier Island (-36.125, 175.375) and Leigh Coast (-36.375, 174.875) (for data points see map Figure 2.1) from 1982 to 2013. (a) Centre lines display the mean annual temperatures based on daily mean temperature measurements (mean). Top and bottom lines show the maximum (max) and minimum (min) recorded daily temperatures during that year, respectively. (b) Daily temperature measurements averaged for each month.

difference of 0.76°C in August (Figure 4.8b). As summer temperatures are the same for both locations and somatic growth shows peak correlations with these summer temperatures, growth of the two populations would, based on temperature, be expected not to differ significantly.

One explanation for this anomaly could be that coastal fish spend more time in estuaries than offshore fish, and that coastal estuaries are warmer than offshore habitats. At Great Barrier Island *G. tricuspidata* are more abundant in the central western part of Great Barrier Island rather than northern sites (Meekan & Choat, 1997). Great Barrier Island does not have as many shallow estuaries or sheltered harbours compared to the coastal site. Juvenile fish were only found at Port Fitzroy where they seem to spend the first years of their lives, but might also retreat into other sheltered areas around the island. Juvenile fish were not encountered at exposed sites around the island where adult fish were collected. Port Fitzroy, located on the western side, is a harbour (about 1000 ha) sheltered by Kaikoura Island. Water depth is generally 10-30 meters apart from some shallow areas in Kaiarara Bay, Kiwiriki Bay, and Wairahi Bay (Hickman, 1979). Morrison (1990) showed that coastal *G. tricuspidata* of all size classes were found inside Whangateau Harbour and at Ti Point Wharf. Whangateau Harbour is a large shallow tidal lagoon (about 750ha, 1.56 m mean depth) fed by the Omaha River with a small opening to Little Omaha Bay that lies close to Ti Point Wharf. About 80% of the water is exchanged with the tides (Kelly, 2009). Morrison (1990) studied distribution and abundance of *G. tricuspidata* within Whangateau Harbour and the adjacent coast. Juveniles of up to 100 mm inhabited the estuary and estuary mouth, but not the open coast. Subadults (100 – 200 mm FL, 1 – 3 years) were also found inside Whangateau Harbour, the estuary mouth, and in smaller numbers along the coast. Adult fish (> 200 mm FL) were caught at Ti Point Wharf and the open coast and a few individual (up to 250 mm FL) inside Whangateau Harbour (Morrison, 1990). This indicates that juvenile fish spend their first years exclusively inside Whangateau Harbour and subsequently move towards the open coast. Adult fish are highly mobile (Gray et al., 2012) and move along open coasts, but in the outer Hauraki Gulf they also seem to spend a considerable amount of time close to the estuary mouth and inside Whangateau Harbour (Grace, 1971; 2015; Morrison, 1990). During summer months large schools of sub-adult and adult *G. tricuspidata* can be found inside the harbour, especially around the reef structures (pers. communication Mark A. Morrison). Temperature will have the biggest influence on growth in juvenile fish when most of growth in length takes place. Coastal fish reach 52.4% of their adult length (181 mm FL) by the age of two. During this time they will have spend most if not all of their time inside the Whangateau Harbour. Water temperatures within the estuary are expected to be higher than along the surrounding coast because on sunny days, especially during summer, the sun heats the shallow water. Additionally, the mudflats heat up when

exposed at low tide, increasing temperatures of the incoming water. Detailed temperature records from inside the Harbour that record peak temperatures are lacking. But Osunakoya & Creese (1997) report an average temperature of 15.7°C with a minimum and maximum of 4.1°C and 26.6°C respectively. Minimum and maximum temperatures recorded by NOAA along the coast between 1982 and 2013 are 11.6°C and 23.7°C respectively (Figure 4.8). Data show that temperatures inside the Harbour are more variable with the water body heating up more in summer than the waters along the coast but also cooling down more in winter. Maximum temperatures inside the Harbour are close to and might sometimes even exceed the thermal tolerance limit of 25.4°C determined for adult *G. tricuspidata* in Australia (Payne et al., 2016), which would lead to decreased growth performance. But a negative effect on growth in juveniles was not observed in the present study. The water in Whangateau Harbour is warmer in summer than that of the surrounding coast and the waters around Great Barrier Island. In consideration of the fact that summer SSTs have the strongest correlation with growth, the temperature differences between Whangateau Harbour and Great Barrier Island could be triggering the different growth responses following the TSR as observed for the two populations of *G. tricuspidata*. These findings highlight the importance of considering environmental factors at the level of the microhabitat.

One fish collected near New Plymouth, in the Taranaki region, provides further support to the observed response to the TSR. This female *G. tricuspidata* was collected at the beginning of March 2016 at the Waiwhakaiho River mouth, which is located just north-east of New Plymouth on the western coast of the North Island in New Zealand (39°02.13S, 174°06.16E). This location is about 3° further south than the other two sampling locations in the present study (Figure 1.1), and SSTs are on average about 2°C below those recorded in Leigh (Greig, Ridgway, & Shakespeare, 1988). The fish measured 430 mm SL (478 mm FL, 505 mm TL) with a total weight of 2488 mm, and was therefore 40 mm longer and 558 g heavier than the longest and heaviest fish recorded at the coastal and offshore sites in the Hauraki Gulf. Otolith reading provided an age estimate of 44 years, showing that this fish is significantly larger than coastal and offshore fish of the same age (Figure 4.9). This is consistent with the TSR, which predicts that fish living in colder environments reach a larger adult body size.

The growth patterns and the fish collected near New Plymouth indicate effects of temperature rather than nutritional differences and the latter will be discussed in Chapter 5.2 The effects of temperature and nutrition on growth.

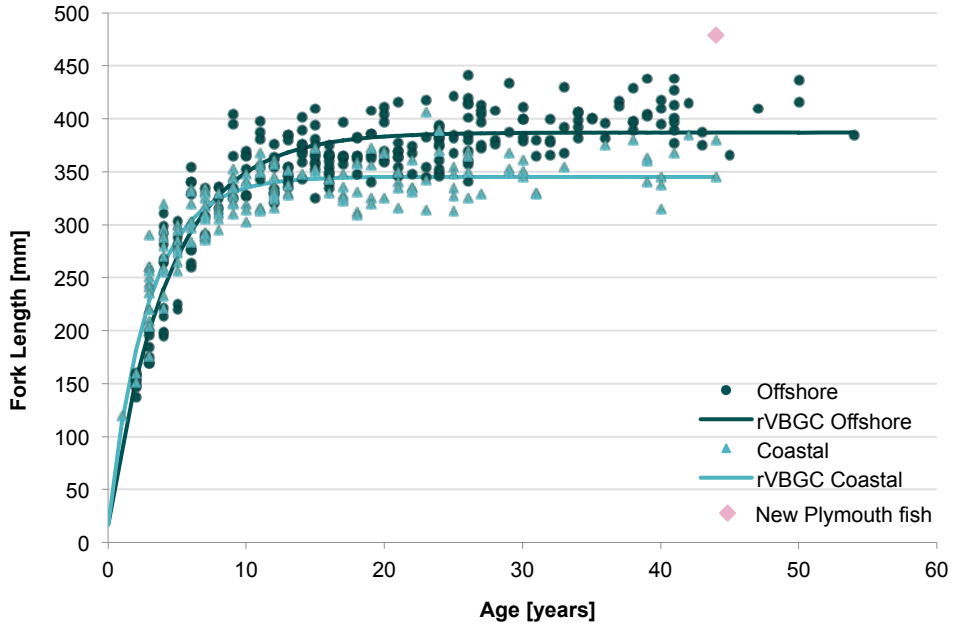


Figure 4.9: Reparameterized von Bertalanffy growth curves fitted for coastal and offshore populations of *G. tricuspidata* based on the best-fit model. The female specimen collected near New Plymouth is shown as a pink diamond.

4.4.4 Climate induced growth variation

Otolith growth increment width chronologies and the strong correlation with temperature (Figure 4.4, 4.5) support the hypothesis that temperature has a major influence on growth in *G. tricuspidata*. Detrended otolith chronologies might mask long-term changes in growth rates (Figure 4.4) but raw data indicated that growth rates did not change over the observed time period (Appendices C4 and C5). Growth indices also revealed a moderate negative correlation with the MEI (Figure 4.7), which is negatively related to temperature. Negative values of the MEI are associated with La Niña events that result in warmer SSTs around New Zealand, while positive values are associated with El Niño events that result in cooler SSTs (Renwick, Hurst, & Kidson, 1998). Both populations responded to lower and higher SSTs with decreased and increased growth respectively (Figure 4.6). Gillanders et al. (2012) previously developed an otolith chronology for *G. tricuspidata* for fish collected near Leigh that shows similarities with the chronologies presented in the present study for coastal and offshore fish (Figure 4.10). The results in Gillanders et al. (2012) are consistent with those of the present study as the former identified the strongest correlations between increment widths and sea surface temperatures during summer as well as a negative correlation with the MEI. Climate and SSTs are correlated with growth and abundance of a wide range of marine organisms, including bivalves and other fish species including *Girella elevata* (Black, 2009; Black, Copenheaver, Frank, Stuckey, &

Kormanyos, 2009; Doubleday et al., 2015; Hernández-Miranda & Ojeda, 2006; Rountrey et al., 2014; Stocks et al., 2014). Rising water temperatures are related to increasing metabolic rates (Floeter et al., 2005; Levinton, 2001), which in turn affect somatic growth. Growth rate increases as long as temperatures do not rise above species-specific thermal thresholds (Neuheimer, Thresher, Lyle, & Semmens, 2011; Pörtner, 2002). No negative responses to increased temperatures were found in the present study as temperatures along the coast and at Great Barrier Island were not close to the thermal tolerance limit of 25.4°C in *G. tricuspidata* (Payne et al., 2016). The present study investigated correlations with temperature and temperatures were never experimentally manipulated. It might therefore be possible that some unmeasured covariate of temperature is driving the relationship. Increased temperatures can also influence growth by extending growing seasons and influencing the productivity of other species. Increased abundances in salps, a major food item for *G. tricuspidata* (Figure 2.3), occurred when SST exceeded 15.5°C in the Mediterranean Sea and water temperature was the key factor regulating the annual peak (Licandro et al., 2006). Warmer than average years thus provide *G. tricuspidata* with a nutritious food resource high in protein and lipid (Figure 3.7, 3.8) for an extended period of the year.

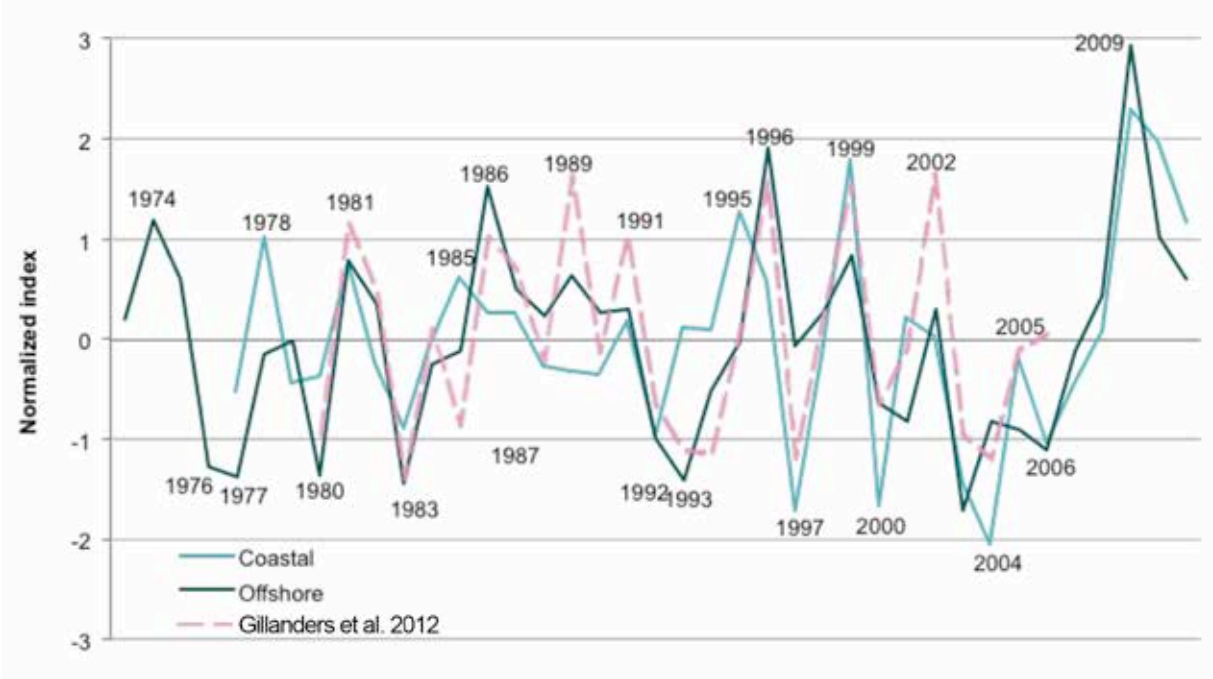


Figure 4.10: Relationship between master otolith growth increment width chronologies for coastal and offshore fish developed in the present study and the chronology by Gillanders et al. (2012). All time series are normalized to a mean of 0 and a standard deviation of 1.

4.4.5 Conclusions

Validation of periodic increment formation revealed that *G. tricuspidata* deposits the opaque zone annually between September and February. *G. elevata* collected in south-east Australia also forms the opaque zone in spring (Stocks et al., 2014). Counting of yearly otolith increments in *G. tricuspidata* enables accurate ageing of this species. Increment counts can be difficult to interpret in juvenile fish therefore the otolith weight-age relationship can be useful to verify the accuracy of increment counts (see Appendix D2) (Britton & Blackburn, 2014; Pawson, 1990). If this method is used for ageing, calibration curves would have to be established separately for different locations and sampling times due to spatial and temporal differences in growth (Worthington, Doherty, & Fowler, 1995). Using otolith weight to predict age is only suitable to verify otolith age counts in juvenile *G. tricuspidata* (Appendix D2).

Longevity and growth data presented here show that *G. tricuspidata* live five times longer than previous studies from New Zealand have shown. The few ageing studies that exist on other *Girella* species corroborate the findings of this study and showed that most species belonging to this genus are long-lived and live for 35 years or more. Maximum ages from otolith readings were recorded as follows: 10 years for *Girella nigricans* (Bredvik, Boerger, & Allen, 2011), 41 years for *Girella cyanea* (Ferrell, 2005; Lewis, 2012), and 45 years for *Girella elevata* (Stocks et al., 2014). All these species also showed asymptotic growth curves with fast initial growth. *G. elevata* also revealed significant spatial variation in growth but no significant differences between sexes (Stocks et al., 2014).

Growth increment widths were visually crossdated with an otolith chronology previously developed for *G. tricuspidata* (Gillanders et al., 2012), resulting in similar master chronologies. Years of comparably wide or narrow increments that correlate between all three chronologies are 1981, 1983, 1992, 1999 and 2002. But chronologies also showed some discrepancies in 1985/86, 1993, 1995/96, with one chronology having lower or higher values than the other two (Figure 4.10). This might be a result of the small sample size of six fish (each with two sets of independent measurements) used by Gillanders et al. (2012) or differences between the environmental factors on a microhabitat scale (Allen & Baltz, 1997). Not many master chronologies exist as this approach is not commonly used in sclerochronology and has only gained popularity over the last decade (Black et al., 2005; 2016). To my knowledge this is the first comparison of increment widths to an existing master chronology. It is an effective approach for dating annual increments and validating age counts. An internet database

containing master chronologies, as suggested by Black et al. (2005), would be beneficial to researchers for dating increments, especially when only small sample sizes of otoliths are aged.

Otolith chronologies and growth curves have been successfully used in this study to demonstrate the effects of temperature on growth. *G. tricuspidata* in the present study showed differences in growth between the two populations separated by only approximately 50 km of deep water which was in keeping with the TSR. The distribution of *G. tricuspidata* extends further north in Australia than in New Zealand, where Australian populations experience temperatures close to their thermal limit. The warm boundary temperature for *G. tricuspidata* has been identified as 25.4°C lying within the 95% confidence interval (23.2-26.2°C) for temperatures associated with the upper critical temperature for body activity in the wild (Payne et al., 2016). The Tasman Sea is one of the most rapidly warming regions in the Southern Hemisphere Ocean (Hobday & Lough, 2011). Growth in populations living close to their thermal threshold might decrease and/or distributions shift polewards if climate change and extreme La Niña events result in SSTs exceeding the thermal threshold (Neuheimer et al., 2011). The southern limit for *G. tricuspidata* in Tasmania has already extended more than two degrees of latitude (about 250 km) southwards associated with an 1°C increase in SSTs between 1994 and 2006 (Stuart-Smith, Barrett, Stevenson, & Edgar, 2009). Populations in southern Queensland, the northern range limit for *G. tricuspidata*, have experienced a major decline in the last two decades that has been associated with an increase in temperature (Pollock, 2016). There is little indication of warming in north-eastern New Zealand (Figure 4.8). Climate models can predict changes in temperatures and if the critical threshold temperature is known resulting changes in growth, longevity, productivity, and range can be forecasted. Data suggest a direct growth response to temperature, which would presumably also occur in many other fish species. The results of this study will help to assess biomass and yield and thus to manage and maintain healthy stock sizes by setting fisheries quota. Even though *G. tricuspidata* is not highly targeted in New Zealand to date, this might change in the future as a result of fishing down the food web.

Chapter 5

General Discussion

Abiotic factors and particularly temperature affect fishes in various ways as most are ectothermic poikilotherms and thus heavily influenced by their environment. Fluctuations in temperature directly affect metabolic rate and life history traits (Bureau et al., 2002; A. Clarke & Johnston, 1999). These can also have indirect effects by triggering changes at various ecosystem levels thereby prompting bottom-up or top-down effects. Limited resources (e.g. energy, time, and essential nutrients) have to be traded-off between life history traits (Stearns, 2000). These factors affect the demography of the whole species/population. As ecological generalists *Girella tricuspidata* provide good study models. The research was aimed at understanding how diet/nutrition and temperature influence life history characteristics, particularly growth rates, and thereby potentially affecting the demography of this temperate marine fish species. The aim was also to identify the strategies employed by omnivorous fishes on a mainly herbivorous diet employ to survive on a diet considered poor in quality, and how diet and nutrient intake were scheduled across the year in relation to reproduction.

Two populations of *G. tricuspidata* were sampled in the northern Hauraki Gulf over two years. Chapter 2 examined diet in both populations through the analysis of stomach contents. Chapter 3 quantified the nutrient composition of stomach contents by assessing carbon, nitrogen, lipid, and ash concentrations. Seasonal changes in diet and nutrient compositions were analysed with respect to both food availability and varying nutrient demand. Chapter 4 investigated the life history traits growth, ageing, and longevity based on the age of fish as derived from sagittal otolith increments. Sagittal otoliths were examined to confirm the annual formation of the opaque zone. Growth differences between populations and temporal variations in growth rates were related to sea surface temperatures at both spatial and temporal scales, respectively.

One of the main aims of this thesis besides investigating the effects of temperature on growth (Chapter 4) was to test for a relationship between diet/nutrition and growth. This chapter discusses the effects of differences in diet and nutrition on growth and longevity between the two populations of *G. tricuspidata*, and whether growth conforms with the Temperature-Constraint Hypothesis. The thesis thereby contributes to the understanding of herbivory and life history characteristics of fish in temperate regions and how these factors influence demography.

5.1 Diet and nutrition in marine herbivorous fish and the advantages of omnivory

Marine herbivorous fish play an important ecological role in reef ecosystems. By feeding on primary producers the fixed energy is released to higher trophic levels (Burkepile & Hay, 2006), and consequently these fish have key roles in the flow of carbon through ecosystems (Clements et al., 2009). To understand the nutritional relationship between animals and their environment it is crucial to understand the factors influencing food choice (Foley & Cork, 1992). Diet analysis provides information on food selectivity and is central to understanding the biology and ecology of a species (Ahlbeck et al., 2012). Stomach content analysis in the present study supported previous work showing that adult *G. tricuspidata* are omnivores with a predominantly herbivorous diet (Chapter 2). A wide range of algae was found in stomach contents, but only a small number of algal species contributed significantly to the diet. The bulk of the diet consisted of epiphytic Rhodophyta, which *G. tricuspidata* removed selectively off the host plant. This has also been documented in previous studies (e.g. Choat & Clements, 1992; Clements & Choat, 1997; Raubenheimer et al., 2005). No data were previously available on the seasonality and abundance of algal epiphytes in New Zealand, a gap addressed by the present study. Even though abundances varied on small spatial scales, the epiphytic algae on *C. maschalocarpum* that *G. tricuspidata* fed on were available throughout the year (Chapter 3).

Algae provided the majority of dietary nutrients, particularly carbohydrates (Chapter 3). Herbivorous diets are generally considered to be of low nutritional value, mainly due to their perceived low protein content compared to animal matter, and the difficulty of digesting algal material (Bowen et al., 1995; Horn, 1989). Protein occurs at higher concentrations in epiphytic algae in than in corticated macroalgae (Smit et al., 2006), providing *G. tricuspidata* with higher protein intake compared to herbivores feeding exclusively on macroalgae. Selective feeding has also been observed in other herbivores. The temperate fish *Odax pullus* selectively feeds on the reproductive structures of *Carpophyllum* spp., which have a higher protein content than thallus tissue (Clements & Choat, 1993; Johnson, 2011). Additionally, the mainly herbivorous diet of *G. tricuspidata* was complemented with salps when available. During the part of the year when salp abundances were low these were substituted with other animal matter, mostly crustaceans. Salps and animal matter had the lowest C:N ratios of the diet categories ingested by *G. tricuspidata*, providing an adequate and balanced intake of protein throughout the year. Salps also contained high levels of lipid, providing a seasonally

abundant prey item that can be captured without an excessive expenditure of energy. Demand for lipid increases during the reproductive season (Hendry & Berg, 1999; Izquierdo et al., 2001). Peak spawning of *G. tricuspidata* takes place in December, coinciding with increasing abundances of salps, which are then ingested in higher proportions (Chapter 3).

Feeding observations of wild fish can be misleading where fish feed selectively on small epiphytes, cyanobacteria, or microbial mats growing on other algae or seagrasses, as it might seem that fish are feeding on the host plant (Clements, German, Piché, Tribollet, & Choat, 2016). Only through the detailed diet analysis provided in this study was it possible to determine the importance of epiphytes to *G. tricuspidata* throughout the year, and also to identify microbial mats as occasional dietary items. The results of Raubenheimer et al. (2005) on sub-adult fish are supported here by investigating the relationship between food availability, diet composition (and thus food selection), and macronutrient intake, showing that adult *G. tricuspidata* also employ a complementary feeding strategy (Chapter 2 & 3). A balanced intake of nutrients was achieved by selectively feeding on a wide range of epiphytic algae and salps, protein-rich foods that are abundant and easily harvested. This highlights the importance of the omnivorous feeding strategy in *G. tricuspidata* where organisms gain energy by feeding on an abundant food source (e.g. algae), and complement their diet by selectively feeding on scarce animal matter (Bowen et al., 1995). Results of the present study showed that the value of each diet item can only be understood in the light of the nutrient composition of the overall diet (Simpson et al., 2004).

5.2 The effects of temperature and nutrition on growth – which factor drives the observed differences in *G. tricuspidata*?

Differences in growth rate in herbivorous fish have been related to variation in both food quality and digestion and to changes in sea surface temperatures. If resource availability, nutrition, and digestion were driving differences in growth between the two populations of *G. tricuspidata*, growth curves of the two populations were expected to be nested and not crossing (Figure 1.1b). Gut content mass was about 18% higher in offshore compared to coastal fish. Differences were even more pronounced in stomach contents, with about 43% less mass in stomachs of coastal fish (Chapter 3). Composition of stomach contents did not differ between either populations of young nor adult fish, and over time only differed between populations in autumn. Relative gut content mass was only higher in adult fish and no differences were found between young fish. Composition data in combination with relative gut content mass data indicate that the overall intake of food/nutrients was higher in adult offshore

fish (Chapter 3). However, comparison between populations of relative gut content mass may have been confounded by sampling discrepancies. This was due to differences in daily sampling times between the two populations.

The Temperature-Constraint Hypothesis (TCH) states that growth differences are caused by differences in digestion, where digestion is expected to be constrained in colder environments (Behrens & Lafferty, 2007; Floeter et al., 2005; Gaines & Lubchenco, 1982). Plant material is considered low-quality food and difficult to digest and absorb (Horn, 1989). If intake of animal matter increases, growth as a measure of performance is expected to be more rapid (Behrens & Lafferty, 2007). Salps are plentiful in summer and more abundant offshore (Zeldis, 1995; Zeldis & Willis, 2014) and thus contribute a slightly but not significantly larger portion to the overall diet in offshore fish. Even when salps are plentiful, *G. tricuspidata* still ingests algal material. This mixed diet supplies them with a balanced intake of nutrients, which does not differ between populations (Chapter 3). Differences in absorption efficiencies could not be determined as it was impossible to calculate these due to the highly variable diet confounding comparisons of anterior and posterior gut contents. Considering that annual mean and maximum sea surface temperatures (SSTs) were very similar between populations during the growing seasons (summer and autumn) (Figure 4.8b), it seems unlikely that these small temperature differences would affect digestion considerably and thereby cause such significant growth differences. Coastal fish spend a considerable amount of time (Grace, 2015; Morrison, 1990) in the warmer waters of the Whangateau Harbour (Osunkoya & Creese, 1997), and in keeping with Behrens & Lafferty (2007) these fish would be expected to have enhanced digestive abilities and hence were expected to grow bigger. Furthermore, coastal fish would be expected to have greater longevity but it was significantly less than longevity in offshore fish. Also, coastal fish in the present study had greater relative gut lengths (Chapter 3), which might allow for slower gut transit times and increased opportunity for nutrient absorption (Horn, 1998). All these findings are inconsistent with the TCH and it is therefore unlikely that a constraint in digestion would drive growth differences in *G. tricuspidata*.

In the present study growth trajectories of *G. tricuspidata* of coastal and offshore populations crossed (Figure 4.3), and thereby followed the characteristics as described by the Temperature-Size Rule (TSR, Figure 1.1a). Likelihood-ratio tests supported the observed trend, showing slightly significant differences between coastal and offshore juveniles ($L_{(2)}$). Coastal fish had faster initial growth, and there were significant differences between coastal and offshore populations for adult fish ($L_{(18)}$), where offshore fish reached larger body sizes. But no significant differences existed for fish aged 10 years,

which is close to where curves crossed (about 7 years) (Chapter 4). Three circumstances can cause growth trajectories to cross: a highly seasonal life-history, protandry, and temperature differences, with the latter one being the best known one (Arendt, 2010). *G. tricuspidata* neither exhibits a highly seasonal life-history, as found in Atlantic salmon (Økland, Jonsson, Jensen, Hansen, 1993), nor protandry, indicating that temperature is likely to be the driving factor (see discussion in Chapter 4.4.3 Growth variations and the effects of temperature). Furthermore, one fish caught near New Plymouth in an environment on average about 2°C colder than the Hauraki Gulf, was larger than any fish caught in the Hauraki Gulf. At an estimated 44 years the New Plymouth specimen was one of the oldest fish in the present study. Even so, this specimen was smaller than most other *G. tricuspidata* observed in the New Plymouth region (pers. communication Pat Swanson), and thus larger specimens are expected to be even older, thereby supporting the TSR. It is unkown whether the differences in growth were the direct effects of temperature (e.g. accelerating metabolism, extended growing season) or some unmeasured covariate that is influenced by temperature (e.g. availability and productivity of other food resources, in particular epiphytic algae and salps).

No evidence was found in the present study to support the TCH, i.e. that cooler temperatures restrict the ability of fish to digest algae (Behrens & Lafferty, 2007; Floeter et al., 2005; Gaines & Lubchenco, 1982). All data combined suggests that nutritional differences did not drive differences in growth, but rather that these were associated with differences in SSTs. Summer SSTs had even stronger correlations with growth than annual SSTs. Unexpectedly, the microhabitat appeared to be the determining factor, as coastal fish spend a considerable amount of time in the Whangateau Harbour (Grace, 2015; Morrison, 1990) where they experience higher temperatures than the offshore population during summer months. Differences in growth as seen in the present study can be caused by various factors with the most important ones being temperature and nutrition (Munday et al., 2008). Studies usually investigate either of these factors. The present study analyzed the effects of both and could thus eliminate nutrition as the driving factor. It also showed that temperature differences should be considered on a small spatial scale, which can result in growth differences between distinct populations.

5.3 Potential drivers of demographic changes

The comparison between coastal and offshore populations in the present study sheds some light on the effects that environmental factors have on different traits such as growth, longevity, and asymptotic body size. Besides linking spatial growth variations to temperature differences between

the two populations, the present study also successfully correlated variations in growth to temperature fluctuations on a temporal scale. Otolith growth chronologies provided an opportunity to assess the effects that annual variations of environmental factors have on growth, which was successfully conducted in the present study (Chapter 4). Results were consistent with an existing study of *G. tricuspidata* (Gillanders et al., 2012), as variation in increment widths showed similar patterns. This showed the potential of verifying age counts by crossdating increment width measurements with existing master chronologies, which is a new method successfully conducted in the present study. Climate change and associated temperature changes affect fish populations at all levels of biological organization, from cellular- and organism-level eco-physiology to population- and ecosystem-level responses (Rijnsdorp & Peck, 2009). In combination with chronologies and knowledge of the critical threshold temperature, models can be generated that can predict effects of climate change i.e. changes in temperature, and thereby predict changes in growth, longevity, and productivity. Estimates of growth and longevity are crucial in stock assessment and fisheries management and results of the present study can be used to mitigate and prevent mismanagement for this species and, if used in ecosystem-based management, also for other fish species by assessing the whole ecosystem rather than the target species only (Pikitch et al., 2004).

An active area of climate research is to investigate direct and indirect effects of temperature changes on the phenology of natural systems, i.e. how these changes influence the timing of life history events (Genner et al., 2010). Because the level of response to climate change may vary across functional groups and multiple trophic levels, these changes in phenology may be important to ecosystem function (Edwards & Richardson, 2004). For example, the ripening of gonads and timing of migration to spawning grounds changes in some fish species depending on sea surface temperatures (Genner et al., 2010; Jansen & Gislason, 2011; Sims, Wearmouth, & Genner, 2004). This in turn can have severe consequences for fish larvae and other zooplankton grazers as peak abundances might mismatch with phytoplankton blooms (Edwards & Richardson, 2004). The lack of the essential food resource can then lead to failed recruitment. Several factors in the present study were influenced by temperature, such as food availability and growth, and reproductive scheduling was also associated with rising water temperatures. Changes in the timing of these factors would influence demography. The ontogenetic habitat shift that *G. tricuspidata* exhibits poses additional challenges. Fish require suitable abiotic conditions, food, and shelter in each of the habitats they inhabit at some stage of their life (Rijnsdorp & Peck, 2009).

Predicted temperature changes due to climate change could thus also potentially affect *G. tricuspidata* in various ways. (1) If annual temperatures increase this could result in changes in food availability, in particular an earlier availability of salps with higher abundances and longer availability. Changes in the phenology of food availability and species composition can influence body condition and thus have consequences for the success of reproduction and ultimately population growth (Beaugrand, Brander, Alistair Lindley, Souissi, & Reid, 2003; Proffitt, Hebblewhite, Peters, Hupp, & Shamhart, 2016). (2) Most of the growth in *G. tricuspidata* takes place in summer. Increasing temperatures would also lead to longer summer seasons with an extended growing period. Higher annual SSTs might lead to faster initial growth but adult size will be reduced, following the TSR. This would lead to decreased productivity of the population. (3) Ripening of the gonads in *G. tricuspidata* was associated with warming SSTs. If reproduction is triggered by temperature then changes in SSTs might shift the timing of reproduction. This in turn can have consequences for the planktivorous fish larvae and juveniles as discussed in the example above. (4) Climate change and changes in phenology can result in changes of habitats to less favorable conditions and could ultimately result in a shift of the distribution of *G. tricuspidata*.

5.4 Future research

Foraging theories attempt to explain feeding behaviour and food choice in organisms based on the optimal amount of nutrients that needs to be provided at an optimal rate (Simpson & Raubenheimer, 2001). Based on the foraging theories either or both of nutritional value or food availability might influence relative gut content mass and relative gut length. Differences in relative gut content mass were detected between the populations and differences in relative gut length were detected between populations and seasons. Nevertheless it was not possible to determine causation, and feeding in *G. tricuspidata* followed neither the optimal diet theory nor optimal digestion theory. Epiphyte sampling at both locations to estimate available biomass and analysis of the nutritional value of these epiphytes might help to identify whether these factors caused the observed differences. Diet and nutrient analysis of other populations in different locations around New Zealand and especially in Australia will inevitably provide different results due to differences in food availability and might also provide further insight into the foraging strategy of *G. tricuspidata*.

Fishes are expected to have increased nutrient demand during reproduction (Hendry & Berg, 1999; Izquierdo et al., 2001), but a seasonal increase in food intake was not detectable. Experiments on captive fish offer great potential for controlled studies. Feeding experiments would enable the

determination of assimilation efficiencies for carbohydrates, protein, and lipids. Feeding studies so far have used *Ulva* spp. and other non-dietary species (T. A. Anderson, 1987; 1988; 1991; Gollan & Wright, 2006; R. B. Taylor & Steinberg, 2005) but future experiments should use Phaeophyta covered in epiphytes and/or salps to establish their true digestive potential. Regulating food intake and nutritional quality of the food offered could provide additional information regarding foraging strategies. Conducting experiments at different temperatures would reveal whether digestion is restricted at colder temperatures as predicted by the Temperature Constraint Hypothesis (Behrens & Lafferty, 2007; Floeter et al., 2005). Results on assimilation efficiencies, food intake, and nutritional value of food can help to identify the underlying causes of variation in relative gut length and relative gut content mass between populations.

Size- and age-at-maturity are important life history traits (Arendt, 2010) that were not established in the present study, as gonads were only visually inspected to sex fish. Size- and age-at-maturity could be determined by performing gonad histology during the reproductive season and would provide information about the reproductive development of ovaries and testes and whether fish have reached maturity. These traits are factors characterizing the Temperature-Size Rule, as organisms in colder environments are expected to mature later and have a larger body size upon maturity compared to fish in warmer environments leading to larger fecundity and possibly higher-quality offspring (Angilletta et al., 2004; Stearns, 2000). Establishing size- and age-at-maturity in *G. tricuspidata* could thereby support or contradict the TSR.

Sampling other populations from New Zealand or Australia to establish additional growth curves could further test the TSR. By encompassing a larger latitudinal range temperature differences are expected to be more pronounced. Fish around New Plymouth offer a starting point as size and age differences were already detected in one fish from this location. If possible, distinct populations e.g. around islands should be sampled to avoid trends being masked by migratory fish.

5.5 Conclusions

The present study investigated diet, nutrition, and life history traits in *G. tricuspidata*. *G. tricuspidata* is an omnivore with a mainly herbivorous diet. Even though herbivorous diets are often seen as low-quality (Bowen et al., 1995; Horn, 1989) *G. tricuspidata* employs a mixed feeding strategy that supplies them with the optimal balance of nutrients throughout the year by selectively feeding on small epiphytic algae that contain higher levels of protein compared to macrophytes. Additionally,

the diet is complemented with animal matter, particularly salps, which are rich in protein and lipid. Fermentative activity has not been found in the digestive tract of *G. tricuspidata* (Clements & Choat, 1997; Moran & Clements, 2002; Skea et al., 2007). Rather food is broken down by acid lysis in the stomach (T. A. Anderson, 1991; Zemke-White et al., 1999) and further by endogenous digestive processes in the gut (Clements & Choat, 1997; Skea et al., 2007), which enables fish to access the nutrients. Information on the availability of epiphytes was lacking and the present study filled this gap, showing that despite small scale variations in biomass epiphytes are available as food year round. Even though offshore fish had a higher overall food intake, the diet in both populations did not show any differences in nutrient composition.

Methods developed in dendrochronology to assess the impact of climate variations on growth have been used for many decades (Holmes, 1983). These methods have been applied to sclerochronology and otolith studies in marine organisms only in the last three decades (Campana & Thorrold, 2001). The present study therefore contributes to the understanding of the effects that environmental factors have on growth and other life history characteristics. Long-term otolith growth chronologies indicated that temporal variations in the growth rate of *G. tricuspidata* were positively correlated with temperature. This characteristic could also be used to crossdate otoliths to an existing master chronology (Gillanders et al., 2012). This methodical approach was suggested by Black et al. (2005) but has not been conducted previously due to the limited number of existing master chronologies. Spatial variations in growth were detected between the coastal and offshore populations and the observed growth patterns agreed with the TSR (Trip et al., 2013). Results of the present study suggest that temperature variations over small spatial scales (a distance of about 50 km) can lead to differences in growth, thereby supporting the TSR. Other factors influenced by temperature, such as the availability of food, might have an additional influence on growth. However, results on resource availability, nutrition, and digestion were inconsistent with the TCH (Behrens & Lafferty, 2007) and these factors are considered unlikely to drive spatial variation in growth.

Even though the knowledge about the nutritional ecology of temperate herbivores has increased in recent years, several aspects are still poorly understood (Clements et al., 2009). Particularly, which factors influence diet choice, and how diet and nutrition affect life history traits such as growth, survival, and reproduction. Environmental factors can have contradictory influences on growth rates (Angilletta et al., 2004). More studies are needed that simultaneously investigate the effects of diet, nutrition, and temperature on life history traits and demography. The present study provided answers to these questions for one abundant temperate reef fish and provides a starting point for further

studies on *G. tricuspidata* and other temperate herbivorous/omnivorous species, thereby helping to answer these fundamental questions.

Appendices

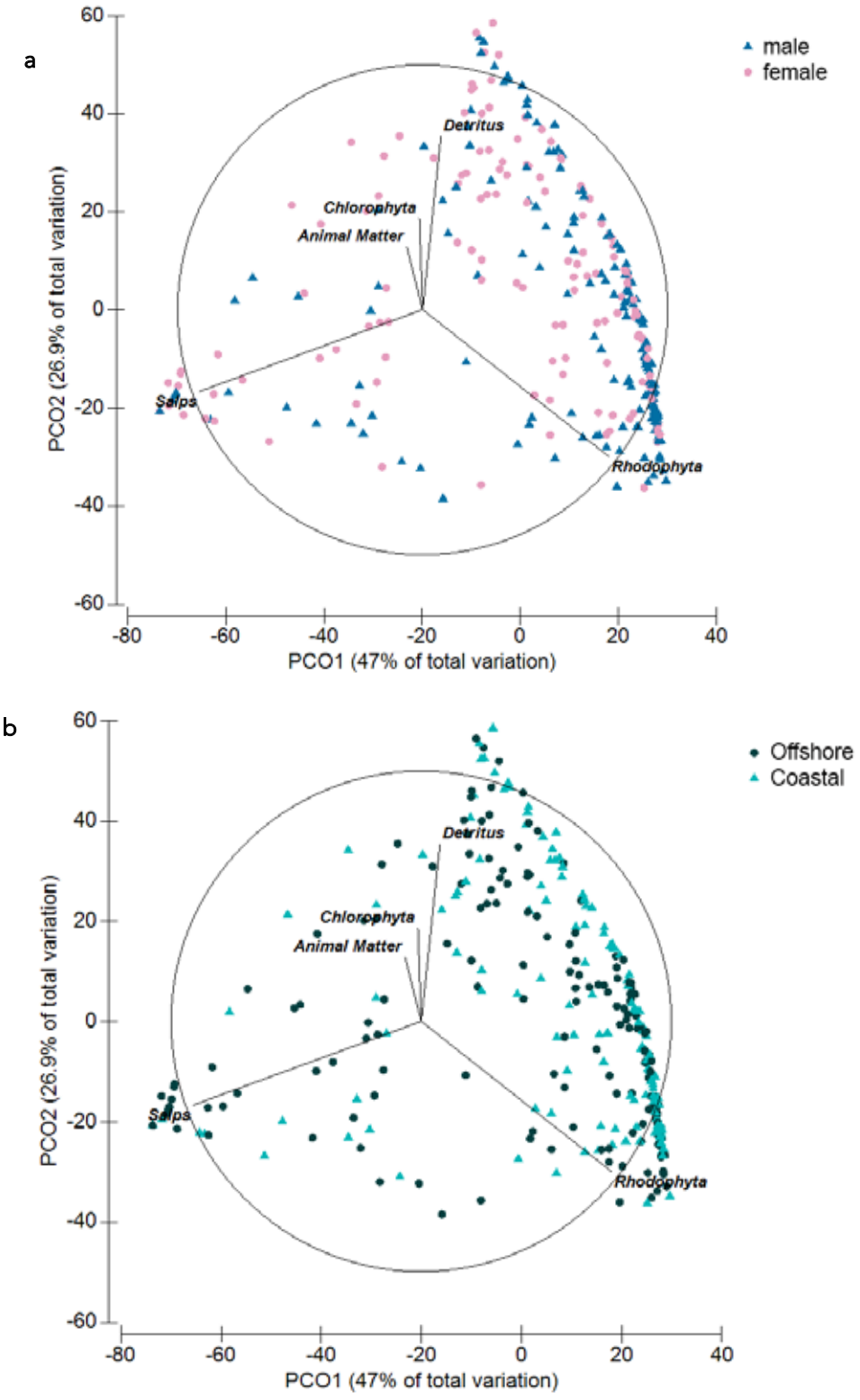
Appendix A: Chapter 2

Appendix A1: PERMANOVA results and PCO comparing the dietary categories between sexes and regions

Results of the PERMANOVA revealed significant differences of diet categories between sexes and regions. Data were square root transformed and converted to Bray-Curtis resemblance matrices. Assumptions of homogeneity of dispersions were met for sexes (PERMDISP: F = 3.88, df1 = 1, df2 = 319, *p*(perm) = 0.119) and regions (PERMDISP: F = 2.03, df1 = 1, df2 = 319, *p*(perm) = 0.247).

Statistically significant results are highlighted in bold.

Source of variation	df	SS	MS	Pseudo-F	*p*(perm)	Unique perms
Sex	1	9286.7	9286.7	4.854	**0.005**	999
Region	1	15082	15082	7.883	**0.001**	999
Sex*Region	1	2023.3	2023.3	1.058	0.372	998
Residual	317	6.06E5	1913.3			



PCO ordination showing differences and similarities in the diet categories between (a) male and female fish and (b) coastal and offshore fish. Vector plots present Pearson's correlations of untransformed diet categories for correlations > 0.25. The distance of the vectors extending to the circle indicates the strength of the correlation of that vector with the distribution of data points.

Appendix A2: PERMANOVA results for the comparison of dietary items comparing sexes for both locations

One factor PERMANOVA results of dietary items comparing sexes for both regions. Assumptions of homogeneity of dispersions were met for coastal (PERMDISP: F = 1.556, df1 = 1, df2 = 144, *p*(perm) = 0.292) and offshore fish (PERMDISP: F = 5.19E-2, df1 = 1, df2 = 173, *p*(perm) = 0.804).

Statistically significant results are highlighted in bold.

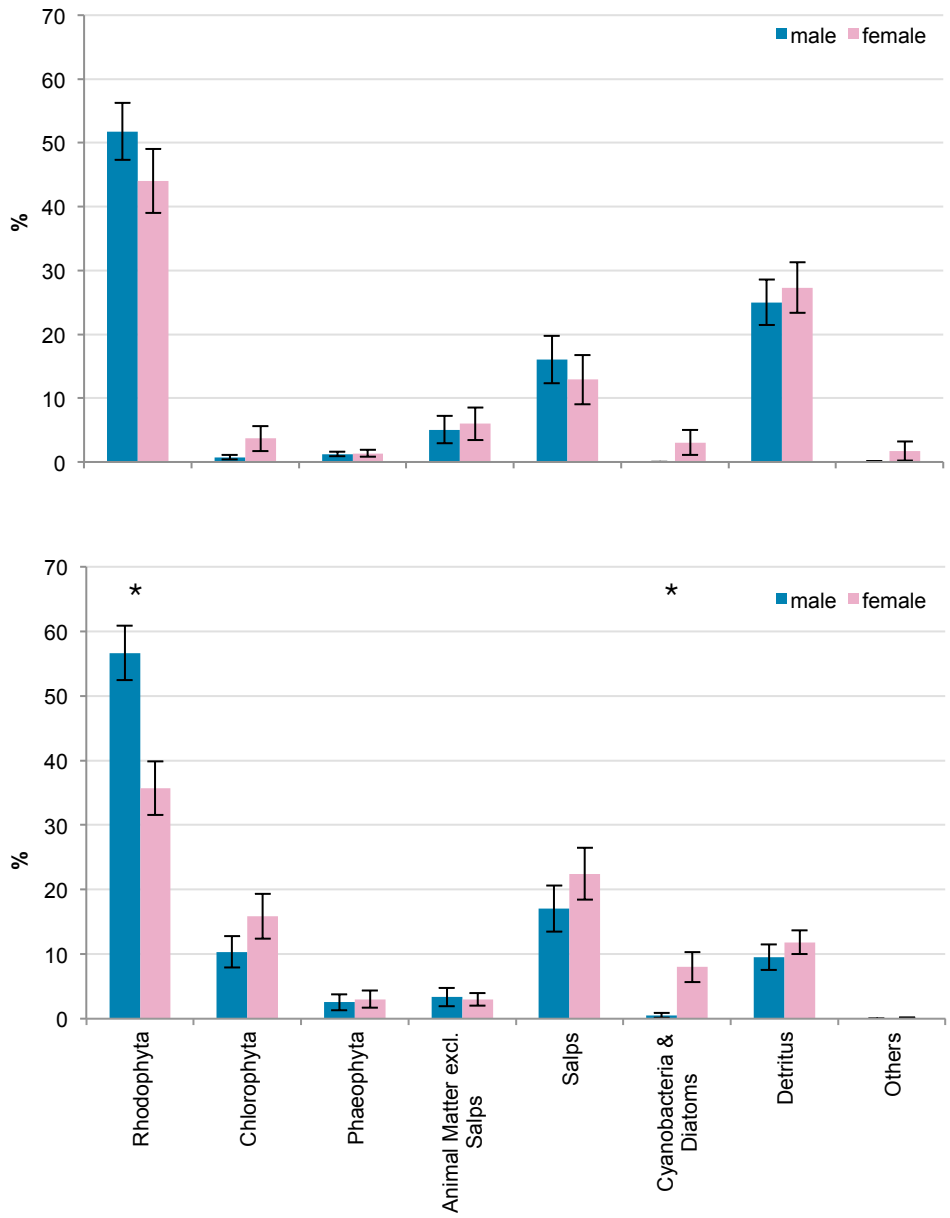
Source of variation	df	SS	MS	Pseudo-F	*p*(perm)	Unique perms
Coastal						
Sex	1	4748.1	4748.1	1.575	0.138	999
Residuals	144	4.34E5	3015.8			
Offshore						
Sex	1	12870	12870	3.781	**0.002**	999
Residuals	173	5.88E5	3403.5			

Appendix A3: Mean values of the diet categories, and results of the independent-samples t-tests for comparison of male and female fish, and coastal and offshore fish

Statistically significant results are highlighted in bold.

Diet category	Rhodophyta	Chlorophyta	Ochrophyta	Animal Matter	Salps	Cyanobacteria & Diatoms	Detritus	Others
Gender								
Mean male	54.32	5.73	1.92	4.18	16.56	0.28	16.95	0.05
SE male	3.06	1.31	0.67	1.26	2.58	0.19	2.06	0.02
Mean female	39.25	10.70	2.31	4.27	18.39	5.90	18.37	0.79
SE female	3.21	2.23	0.79	1.22	2.85	1.58	2.07	0.63
t-value	3.381	-1.925	-0.382	-0.050	-0.476	-3.524	-0.482	-1.168
df	319	236.61	319	319	319	147.12	319	143.20
p value	**0.001**	0.055	0.703	0.960	0.634	**0.001**	0.630	0.245
Region								
Mean coastal	48.55	1.97	1.29	5.44	14.72	1.29	25.96	0.78
SE coastal	3.34	0.86	0.30	1.62	2.69	0.81	2.64	0.63
Mean offshore	46.73	12.96	2.77	3.20	19.60	4.07	10.60	0.06
SE offshore	3.06	2.09	0.90	0.89	2.68	1.16	1.35	0.03
t-value	0.400	-4.873	-1.567	1.213	-1.284	-1.969	5.192	1.145
df	319	229.73	212.35	227.36	316.28	300.35	218.12	145.55
p value	0.689	**<0.001**	0.119	0.226	0.200	**0.050**	**<0.001**	0.254

Appendix A4: Comparison of the diet categories between male and female fish for coastal and offshore fish separately. Mean values and results of the independent-samples t-tests



Comparison of the mean diet composition of *G. tricuspidata* with standard errors for (a) male and female coastal fish and (b) male and female offshore fish. Asterisks mark diet categories with statistically significant differences between genders (independent-samples t-test).

Independent-samples t-tests were performed to compare means for each category between genders of the two locations. Boxplots were inspected for outliers greater than 1.5 box-lengths from the edge of the box. Outliers were present for coastal fish for male and female fish in the categories Chlorophyta, Ochrophyta, animal matter, salps, cyanobacteria/diatoms, and others. Rhodophyta was

the only category that did not have any outliers for male and female offshore fish. They were considered as genuinely unusual data points and included in the analysis. The Shapiro-Wilk's test revealed that categories for each gender and location were not normally distributed ($p < 0.05$). All categories were positively skewed except for male Rhodophyta for both locations, which was slightly negatively skewed. The t-test was run regardless as it is considered fairly robust to deviations from normality. As assessed by Levene's test for equality of variances ($p > 0.05$), there was homogeneity of variance for the following categories for both locations: Rhodophyta, Ochrophyta, animal matter, salps, and detritus. The assumption of homogeneity of variances was violated for the categories Chlorophyta, cyanobacteria/diatoms, and others ($p < 0.05$). In case of violation of the homogeneity of variances the Welch t-test was used.

Statistically significant results are highlighted in bold.

Diet category	Rhodophyta	Chlorophyta	Ochrophyta	Animal Matter	Salps	Cyanobacteria & Diatoms	Detritus	Others
Coastal								
Mean male	51.79	0.74	1.25	5.06	16.04	0.03	25.00	0.10
SE male	4.45	0.36	0.36	2.12	3.71	0.02	3.55	0.03
Mean female	44.04	3.68	3.68	1.35	12.89	3.04	27.30	1.71
SE female	5.02	1.98	1.97	0.52	3.85	1.93	3.94	1.49
Mean difference	7.75	-2.94	-0.10	-0.93	3.14	-3.01	-2.30	-1.61
95% CI lower	-5.61	-6.96	-1.32	-7.46	-7.65	-6.89	-12.89	-4.60
95% CI upper	21.10	1.07	1.11	5.60	13.94	0.86	8.29	1.38
t-value	1.147	-1.464	-0.168	-0.281	0.575	-1.556	-0.430	-1.08
df	144	63.98	144	144	144	60.01	144	60.06
p value	0.253	0.148	0.867	0.779	0.566	0.125	0.668	0.285
Offshore								
Mean male	56.66	10.34	2.55	3.37	17.05	0.51	9.51	0.01
SE male	4.21	2.41	1.24	1.44	3.60	0.36	1.96	0.00
Mean female	35.73	15.87	3.02	3.01	22.43	8.01	11.81	0.12
SE female	4.16	3.48	1.31	0.99	4.01	2.34	1.83	0.06
Mean difference	20.94	-5.53	-0.47	0.36	-5.39	-7.50	-2.30	-0.11
95% CI lower	9.21	-13.90	-4.03	-3.15	-15.99	-12.20	-7.62	-0.22
95% CI upper	32.67	2.84	3.08	3.87	5.22	-2.80	3.02	0.00
t-value	3.524	-1.305	-0.264	0.203	-1.002	-3.171	-0.853	-1.939
df	173	148.77	173	173	173	85.99	173	82.95
p value	**0.001**	0.194	0.792	0.839	0.318	**0.002**	0.395	0.056

Appendix A5: Mean values of the functional groups, and results of the independent-samples t-tests for comparison of male and female fish, and coastal and offshore fish

Data were analysed for each phylum separately. Statistically significant results are highlighted in bold.

Diet category	Calcareous Rhodophyta	Corticated Macrophytes - Rhodophyta	Filamentous Rhdopyhta	Fleshy Rhdophyta	Foliose Rhodophyta	Filamentous Chlorophyta	Fleshy Chlorophyta	Foliose Chlorophyta
Gender								
Mean male	0.79	0.09	39.70	9.39	50.04	8.16	47.02	44.82
SE male	0.54	0.09	3.07	1.80	3.40	2.66	5.79	5.83
Mean female	0.04	0.90	50.65	17.41	30.99	11.74	48.03	40.23
SE female	0.02	0.83	3.43	2.74	3.32	3.41	5.70	5.52
t-value	1.390	-0.971	-2.380	-2.449	4.008	-0.820	-0.124	0.572
df	151.50	121.70	270	212.34	267.50	124	124	124
p value	0.166	0.334	**0.018**	**0.015**	**<0.001**	0.414	0.902	0.568
Region								
Mean coastal	1.00	0.01	39.94	8.50	50.55	5.70	76.23	18.07
SE coastal	0.67	0.01	3.27	1.97	3.62	2.96	6.64	6.41
Mean offshore	0.02	0.81	48.26	16.53	34.38	11.48	37.77	50.75
SE offshore	0.01	0.67	3.20	2.36	3.25	2.73	4.52	4.61
t-value	1.459	-1.194	-1.804	-2.612	3.327	-1.435	4.790	-4.139
df	121.08	149.02	270	268.39	270	85.84	61.993	65.439
p value	0.147	0.235	0.072	**0.010**	**0.001**	0.155	**<0.001**	**<0.001**

Diet category	Corticated Macrophytes - Ochrophyta	Filamentous Ochrophyta	Fleshy Ochrophyta	Foliose Ochrophyta	Leathery Macrophytes - Ochrophyta
Gender					
Mean male	17.49	-	1.67	1.24	79.60
SE male	4.00	-	1.00	1.16	4.14
Mean female	12.73	1.37	1.54	4.65	79.72
SE female	3.60	1.35	1.36	2.31	4.42
t-value	0.874	-1.014	0.076	-1.317	-0.020
df	158	73	158	108.82	158
p value	0.384	0.314	0.939	0.191	0.984
Region					
Mean coastal	14.99	-	1.09	2.95	80.97
SE coastal	4.18	-	0.78	2.01	4.50
Mean offshore	15.52	1.13	2.01	2.71	78.63
SE offshore	3.60	1.11	1.34	1.57	4.07
t-value	-0.097	-0.894	-0.550	0.092	0.385
df	158	158	158	158	158
p value	0.923	0.373	0.583	0.926	0.701

Appendix A6: Mean values of the habitats *G. tricuspidata* is obtaining its food items from, and results of the independent-samples t-tests for comparison of male and female fish, and coastal and offshore fish

Statistically significant results are highlighted in bold.

Diet category	Epifauna	Pelagic	Epifauna/ Pelagic	Benthic	Epiphytic	Epilithic	Epiphytic/ Epilithic	Unattached	Terrestrial	Detritus	Unknown
Gender											
Mean male	3.65	16.90	0.19	0.05	42.72	11.27	4.40	0.05	-	16.95	3.81
SE male	1.20	2.60	0.12	0.02	3.05	1.70	1.00	0.05	-	2.06	0.85
Mean female	3.44	18.40	0.82	0.70	26.18	13.99	13.84	0.01	0.10	18.38	4.15
SE female	1.09	2.85	0.55	0.63	2.83	2.24	2.16	0.01	0.06	2.07	1.06
t-value	0.125	-.388	-1.117	-1.019	3.974	-0.967	-3.968	0.805	-1.520	-0.482	-0.257
df	319	319	156.78	143.20	318.80	279.93	203.81	319	143.00	319	319
p value	0.901	0.699	0.266	0.310	**<0.001**	0.334	**<0.001**	0.422	0.131	0.630	0.798
Region											
Mean coastal	4.70	14.73	0.74	0.71	38.23	5.60	5.65	0.06	0.07	25.96	3.54
SE coastal	1.53	2.69	0.54	0.62	3.33	1.16	1.28	0.06	0.06	2.64	0.87
Mean offshore	2.59	19.95	0.26	0.04	32.86	18.23	11.13	0.02	0.02	10.60	4.31
SE offshore	0.80	2.71	0.13	0.02	2.81	2.24	1.79	0.02	0.02	1.35	0.98
t-value	1.221	-1.370	0.941	1.074	1.231	-5.005	-2.491	1.042	0.812	5.192	-0.574
df	221.39	316.72	319	145.21	298.42	256.91	302.19	145.26	319	218.12	319
p value	0.223	0.172	0.348	0.284	0.219	**<0.001**	**0.013**	0.299	0.417	**<0.001**	0.566

Appendix A7: List of dietary items found in stomach contents of *G. tricuspidata*

Algae were identified to species level wherever possible. Plantae taxonomy after www.algaebase.org, animalia after www.itis.gov. The list also includes the functional group dietary items were classed in and the habitat they can be found in. Column '% F' is the frequency of occurrence and shows the percentage of fish that fed on the dietary item, column 'mean % all' the percentage of this dietary item averaged for all stomach contents, and 'mean %' the average percentage of this dietary item only for the stomach contents where this dietary item was present.

Filamentous tiny = fil-t, filamentous small = fil-s, filamentous large = fil-l, foliose small = fol-s, foliose large = fol-l, fleshy – no cortices = fl-noco, fleshy – light cortices = fl-lco, leathery macrophytes = leamac, corticated macrophytes = cormac, articulated calcareous = artcal, microbial mat tiny = mat-t, Epiph/Epil = Epiphytic/Epilithic.

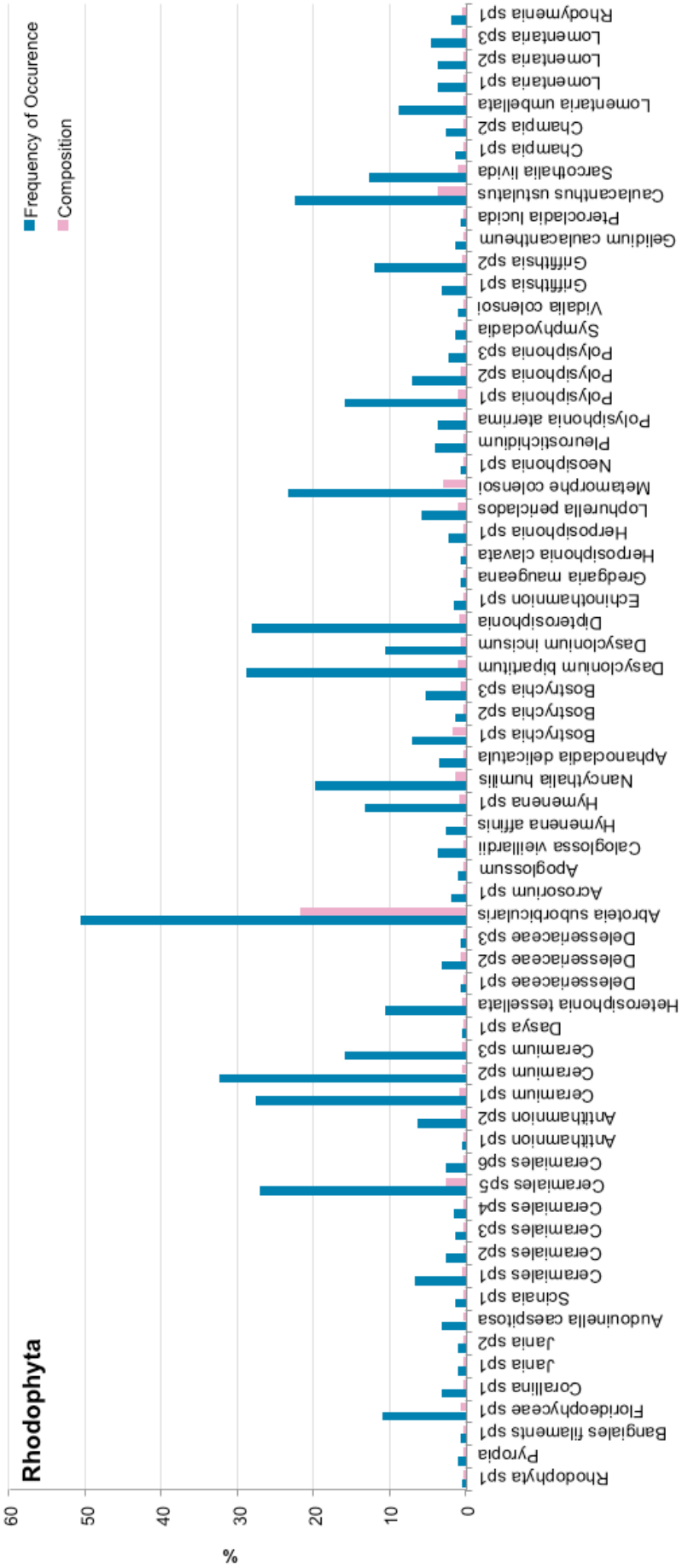
Phylum	Class	Order	Family	Genus	Species	Functional Group	Habitat	% F	Mean % all	Mean %
Chordata	Thaliacea	Salpida	Salpidae	*Thalia*	*democratica*	-	Pelagic	42.99	18.38	42.76
Arthropoda	Malacostraca	Euphausiacea	?	?	?	-	Pelagic	2.09	0.19	8.88
		Amphipoda	?	?	?	-	Epifauna	51.34	0.70	1.36
		Isopoda	?	?	?	-	Epifauna	2.39	0.01	0.21
	Maxillopoda	Copepoda (subclass)	?	?	?	-	Epifauna/ Pelagic	6.87	0.45	6.50
	?	?	?	?	?	-	Epifauna	17.91	1.94	10.82
	? Moults	?	?	?	?	-		2.39	0.35	14.81
Nematoda	?	?	?	?	?	-	Epifauna	0.30	<0.00	0.01
Annelida	Polychaeta	?	?	?	?	-	Epifauna	3.58	0.01	0.33
	?	?	?	?	?	-	Epifauna	1.19	<0.00	0.15
Mollusca	Bivalvia	?	?	?	?	-	Epifauna	2.39	0.01	0.36
	Gastropoda	?	?	?	?	-	Epifauna	14.33	0.05	0.34
Cnidaria	Hydrozoa	?	?	?	?	-	Epifauna	9.85	0.35	3.52
? Eggs	?	?	?	?	?	-	Epifauna/ Pelagic	0.60	0.01	1.57

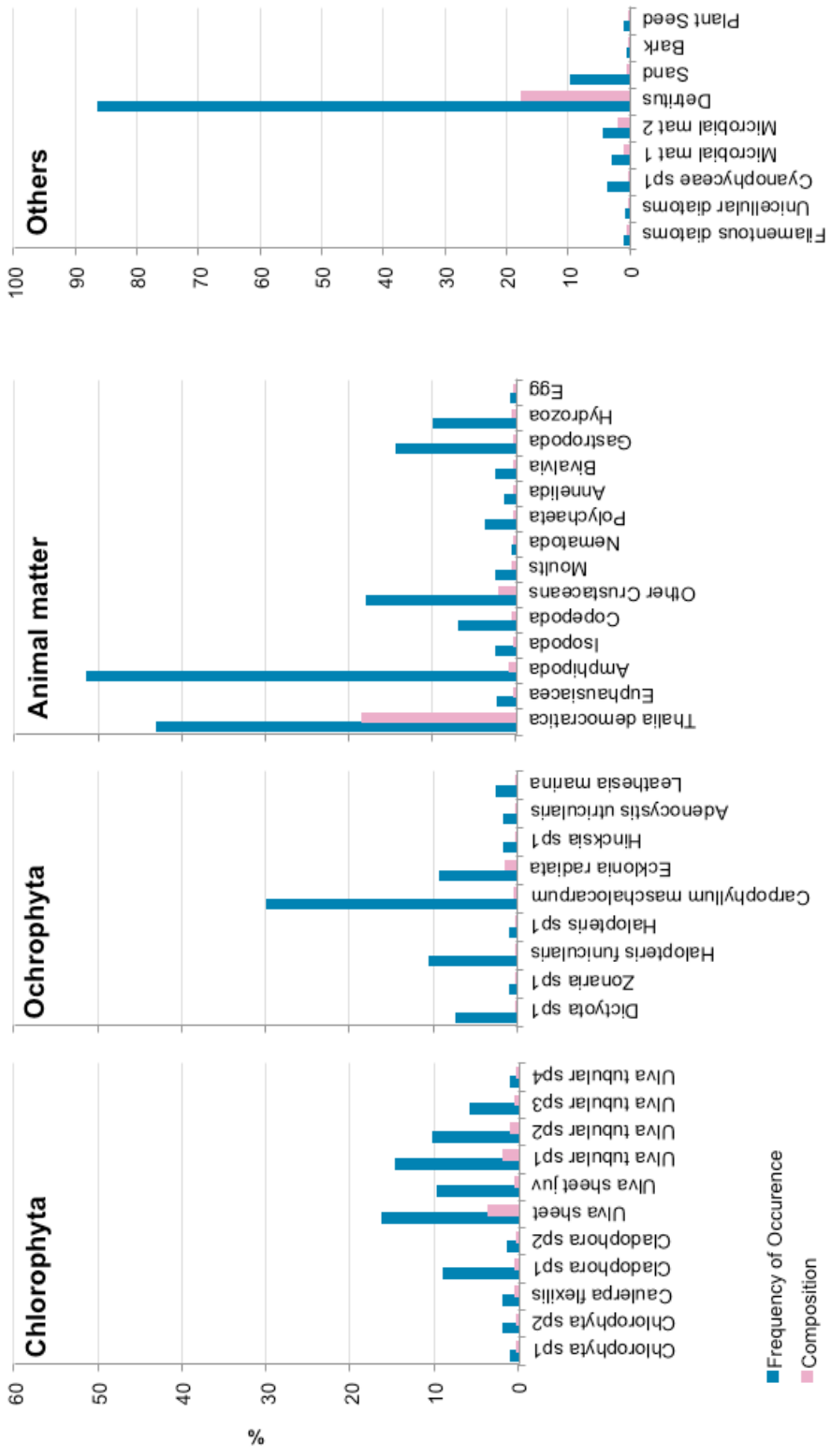
Phylum	Class	Order	Family	Genus	Species	Functional Group	Habitat	% F	Mean % all	Mean %
Rhodophyta	Bangiophyceae	Bangiales	Bangiaceae	Bangiales blades	*Pyropia*	fol-l	Epilithic	0.90	<0.00	0.10
	Florideophyceae			Bangiales filaments	*sp1*	fil-t	Epilithic	0.60	0.04	6.03
		Corallinales	Corallinaceae	*Corallina*	*sp1*	artcal	Epilithic	2.99	0.02	0.78
				Jania	*sp1*	artcal	Epilithic	0.90	<0.00	0.15
					sp2	artcal	Epilithic	0.90	<0.00	0.36
		Acrochaetiales	Acrochaetiaceae	*Audouinella*	*caespitosa*	fil-t	Epiph/Epil	2.99	0.01	0.39
		Nemaliales	Scinaiaceae	*Scinaia*	*sp1*	fl-noco	Epilithic	1.19	0.04	3.05
		Ceramiales	Ceramiaceae	*Antithamnion*	*sp1*	fil-t	Epiphytic	0.30	<0.00	1.35
					sp2	fil-t	Epiphytic	6.27	0.60	9.63
				Ceramium	*sp1*	fil-s	Epiphytic	27.46	0.61	2.24
					sp2	fil-s	Epiphytic	32.24	0.37	1.15
					sp3	fil-s	Epiphytic	15.82	0.41	2.60
			Dasyceae	*Dasya*	*sp1*	fil-l	Epiphytic	0.30	<0.00	0.80
				Heterosiphonia	*tessellata*	fil-l	Epilithic	10.45	0.41	3.88
			Delesseriaceae	*Abroteia*	*suborbicularis*	fol-s	Epiphytic	50.45	21.57	42.76
				Acrosorium	*sp1*	fol-s	Epiphytic	1.79	0.07	3.90
				Apoglossum	*montagneanum*	fol-s	Epiphytic	0.90	<0.00	0.15
				Caloglossa	*vieillardii*	fol-s	Epiphytic	3.58	0.05	1.46
				Hymenena	*affinis*	fol-s	Epiph/Epil	2.39	0.07	2.97
					sp1	fol-s	Epiph/Epil	13.13	0.68	5.51
				Nancythalia	*humilis*	fol-s	Epiphytic	19.70	1.25	6.35
			Rhodomelaceae	*Aphanocladia*	*delicatula*	fil-s	Epiph/Epil	3.28	0.05	1.59
				Bostrychia	*sp1*	fil-l	Epiph/Epil	6.87	1.61	23.52
					sp2	fil-l	Epiph/Epil	1.19	0.21	17.77
					sp3	fil-l	Epiph/Epil	5.07	0.45	8.93
				Dasyclonium	*bipartitum*	fil-s	Epiphytic	28.66	0.85	2.98
					incisum	fil-s	Epiphytic	10.45	0.52	5.01
				Dipterosiphonia	*heteroclada*	fil-s	Epiphytic	28.06	0.74	2.65
				Echinothamnion	*sp1*	fil-s	Epiph/Epil	1.49	0.08	5.03
				Gredgaria	*maugeana*	fil-s	Epiphytic	0.60	0.01	2.04
				Herposiphonia	*clavata*	fil-s	Epiphytic	0.60	0.02	2.71
				Lophurella	*periclados*	fil-s	Epilithic	5.67	0.96	16.94
				Metamorphe	*colensoi*	fil-s	Epiphytic	23.28	2.83	12.15
				Neosiphonia	*sp1*	fil-t	Epiph/Epil	0.60	0.20	33.30

Phylum	Class	Order	Family	Genus	Species	Functional Group	Habitat	% F	Mean % all	Mean %
				Pleurostichidium	falkenbergii	fl-noco	Epiphytic	3.88	0.09	2.30
				Polysiphonia	atterima	fil-s	Epiphytic	3.58	0.05	1.36
					sp1	fil-t	Epiph/Epil	15.82	0.95	5.98
					sp2	fil-t	Epiph/Epil	6.87	0.49	7.07
					sp3	fil-t	Epiph/Epil	2.09	0.04	1.83
				Symphyocladia	marchantioides	fol-s	Epilithic	1.19	0.01	0.87
				Vidalia	colensoi	cormac	Epilithic	0.90	0.01	0.72
			Wrangeliaceae	Griffithsia	sp1	fil-s	Epiphytic	2.99	0.03	1.09
					sp2	fil-s	Epiphytic	11.94	0.28	2.33
		Gelidiales	Gelidiaceae	Gelidium	caulacantheum	fl-lco	Epilithic	1.19	0.04	2.95
			Pterocladiaceae	Pterocladia	lucida	cormac	Epilithic	0.60	0.02	2.58
		Gigartinales	Caulacanthaceae	Caulacanthus	ustulatus	fl-lco	Epiphytic	22.39	3.58	15.99
				Sarcothalia	livida	fl-lco	Epilithic	12.54	0.84	6.72
		Rhodymeniales	Champiaceae	Champia	sp1	fl-noco	Epilithic	1.19	0.10	8.27
					sp2	fl-noco	Epilithic	2.39	0.03	1.05
			Lomentariaceae	Lomentaria	umbellata	fl-noco	Epiphytic	8.66	0.22	2.51
					sp1	fl-noco	Epiph/Epil	3.58	0.06	1.65
					sp2	fl-noco	Epiph/Epil	3.58	0.06	1.65
					sp3	fl-noco	Epiph/Epil	4.48	0.36	8.01
			Rhodymeniaceae	Rhodymenia	sp1	fol-s	Epilithic	1.79	0.27	15.27
Unidentified Rhodophyta	?	?	?	?	Rhodophyta sp1	fol-s	Unknown	0.30	0.01	2.39
	Florideophyceae	?	?	?	Florideophyceae sp1	fil-s	Unknown	10.75	0.57	5.31
	Florideophyceae	Ceramiales	?	?	Ceramiales sp1	fil-t	Epiphytic	6.57	0.32	4.92
			?	?	Ceramiales sp2	fil-t	Unknown	2.39	0.01	0.37
			?	?	Ceramiales sp3	fil-s	Unknown	1.19	0.01	1.00
			?	?	Ceramiales sp4	fil-s	Unknown	1.49	0.01	0.65
			?	?	Ceramiales sp5	fil-s	Unknown	26.87	2.53	9.42
			?	?	Ceramiales sp6	fil-s	Unknown	2.39	0.11	4.45
	Florideophyceae	Ceramiales	Rhodomelaceae	Herposiphonia?	sp1	fil-s	Epiphytic	2.09	0.04	1.86
	Florideophyceae	Ceramiales	Delesseriaceae	?	Delesseriaceae sp1	fol-s	Unknown	0.6	0.01	1.23
				?	Delesseriaceae sp2	fol-s	Unknown	2.99	0.49	16.25
				?	Delesseriaceae sp3	fol-s	Unknown	0.60	0.04	6.39

Phylum	Class	Order	Family	Genus	Species	Functional Group	Habitat	% F	Mean % all	Mean %
Chlorophyta	Ulvophyceae	Bryopsidales	Caulerpaceae	Caulerpa	flexilis	fl-lco	Epilithic	1.79	0.30	16.57
		Cladophorales	Cladophoraceae	Chaetomorpha	linum	fil-t	Unattached	2.69	0.03	1.08
				Cladophora	sp1	fil-t	Epiph/Epil	8.96	0.30	3.30
					sp2	fil-t	Epiph/Epil	1.19	0.02	1.69
		Ulvales	Ulvaceae	Ulva	sheet	fol-l	Epilithic	16.12	3.50	21.74
					sheet juv	fol-l	Epilithic	9.55	0.41	4.30
					tubular sp1	fl-noco	Epilithic	14.63	1.82	12.47
					tubular sp2	fl-noco	Epilithic	10.15	0.89	8.80
					tubular sp3	fl-noco	Epilithic	5.67	0.31	5.45
					tubular sp4	fl-noco	Epilithic	0.90	0.08	8.38
Unidentified Chloropyta	?	?	?	?	Chlorophyta sp1	fil-t	Unknown	0.90	0.03	3.19
	?	?	?	?	Chlorophyta sp2	fil-t	Epiphytic	1.79	0.06	3.42
Ochrophyta	Phaeophyceae	Dictyotales	Dictyotaceae	Dictyota	sp1	leamac	Epilithic	7.16	0.06	0.80
				Zonaria	sp1	leamac	Epilithic	0.90	0.02	2.77
		Sphacelariales	Stypocaulaceae	Halopteris	funicularis	cormac	Epilithic	10.45	0.14	1.34
					sp1	cormac	Epilithic	0.90	0.03	2.99
		Fucales	Sargassaceae	Carpophyllum	maschalocarpum	leamac	Epilithic	29.85	0.32	1.69
		Laminariales	Lessoniaceae	Ecklonia	radiata	leamac	Epilithic	9.25	1.40	15.14
		Ectocarpales	Acinetosporaceae	Hincksia	sp1	fil-t	Epilithic	1.49	0.17	11.69
			Adenocystaceae	Adenocystis	utricularis	fl-noco	Epilithic	1.49	0.01	0.55
			Chordariaceae	Leathesia	marina	fol-s	Epilithic	2.39	0.03	1.33
Bacillariophyta	?	?	?	?	Filamentous diatoms	fil-t	Epilitic	0.90	0.41	45.42
	?	?	?	?	Unicellular	-	Unknown	0.60	0.01	2.47
Cyanobacteria	Cyanophyceae	?	?	?	Cyanophyceae sp1	fil-t	Epiph/Epil	3.58	0.17	4.85
Cyanobacteria & Baccilariophyta	?	?	?	?	Microbial mat 1	mat-t	Epiph/Epil	2.69	0.84	31.16
	?	?	?	?	Microbial mat 2	mat-t	Epiph/Epil	4.18	1.84	44.07
Detritus	?	?	?	?	?	-	Detritus	86.27	17.57	20.37
Sand	-	-	-	-	-	-	Benthic	9.55	0.34	3.60
Bark	-	-	-	-	-	-	Terrestrial	0.30	0.01	3.82
Plant Seeds	-	-	-	-	-	-	Terrestrial	0.90	0.03	3.39

Appendix A8: Frequency of occurrence and percentage composition for each dietary item

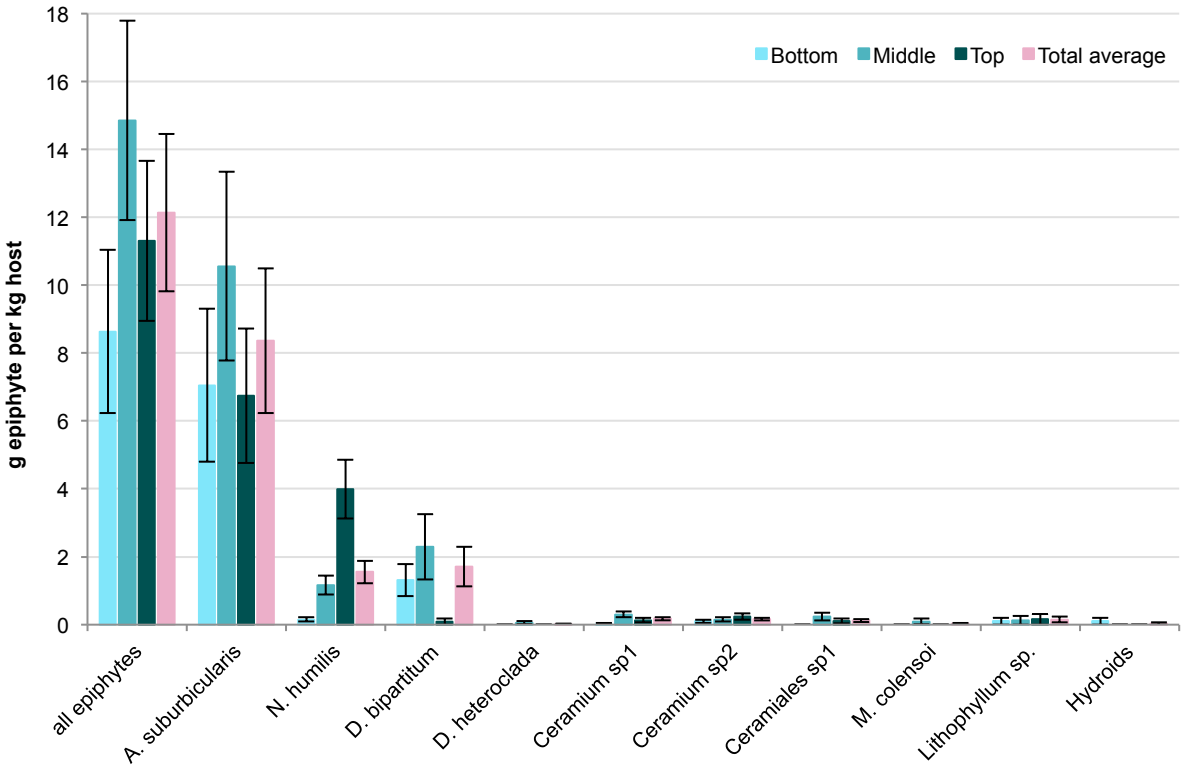



Chlorophyta
Ochrophyta
Animal matter
Others
%
0
10
20
30
40
50
60
70
80
90
100
Frequency of Occurence
Composition
Chlorophyta sp1
Chlorophyta sp2
Caulerpa flexilis
Cladophora sp1
Cladophora sp2
Ulva sheet
Ulva sheet juv
Ulva tubular sp1
Ulva tubular sp2
Ulva tubular sp3
Ulva tubular sp4
Dictyota sp1
Zonaria sp1
Halopteris funicularis
Halopteris sp1
Carpophyllum maschalocarpum
Eckonia radiata
Hincksia sp1
Adenocystis utricularis
Leathesia marina
Thalia democratica
Euphausiacea
Amphipoda
Isopoda
Copepoda
Other Crustaceans
Moults
Nematoda
Polychaeta
Annelida
Bivalvia
Gastropoda
Hydrozoa
Egg
Filamentous diatoms
Unicellular diatoms
Cyanophyceae sp1
Microbial mat 1
Microbial mat 2
Detritus
Sand
Bark
Plant Seed

Appendix B: Chapter 3

Appendix B1: Variation in abundance of epiphytes on the host plant estimated as biomass of epiphytes per kg of host (DW)

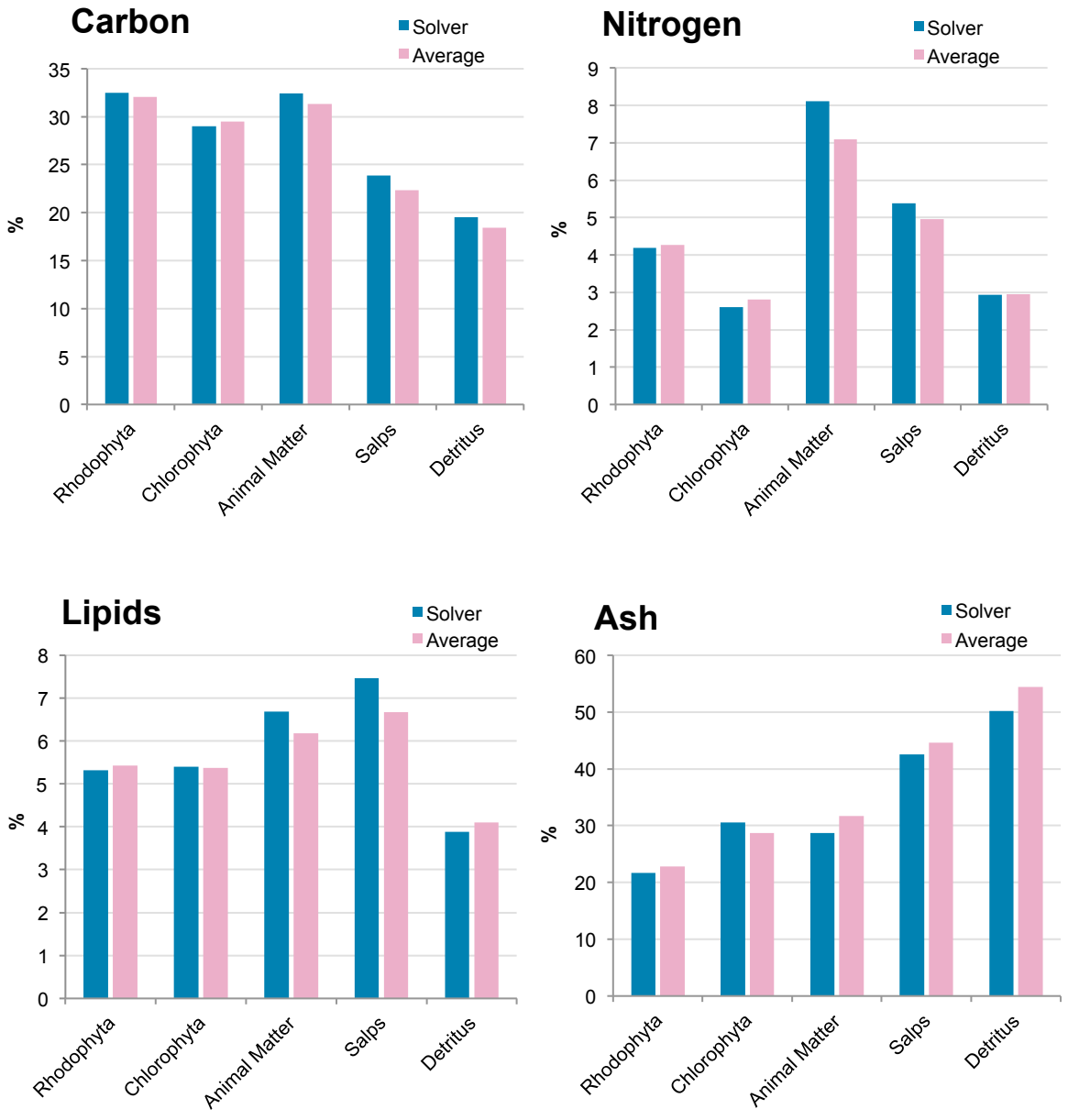
Error bars indicate standard errors.



Appendix B2: Pearson's correlations between the two methods used to calculate nutrient concentrations on the basis of gut content composition, results of the Shapiro-Wilk's tests and graphs displaying differences between results of the two methods

Significant results are highlighted in bold.

			Shapiro-Wilk's tests	
	Pearson's *r*	*p*	Solver *p*	Calculated *p*
C	0.900	**0.006**	0.126	0.446
N	0.990	**<0.001**	0.827	0.852
Lipids	0.939	**0.002**	0.812	0.044
Ash	0.820	**0.024**	0.821	0.653



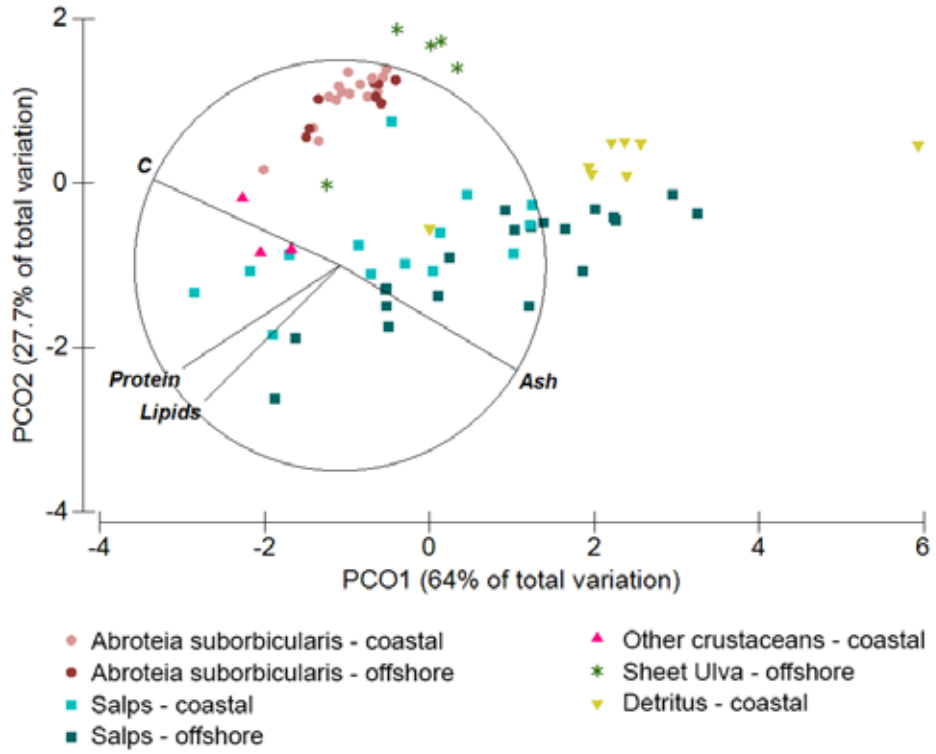
Appendix B3: ANOSIM results of the pairwise comparison of nutrient compositions between diet categories

Global test results: R: 0.802, p = 0.001, nr of permutations: 999, nr of the permuted statistics greater than or equal to global R: 0

Significant results are highlighted in bold.

Pairwise test	R	*p*	Actual permutations
Salps – Rhodophyta	0.850	**0.001**	999
Salps – Chlorophyta	0.456	**0.001**	999
Salps – Detritus	0.241	**0.023**	999
Salps – Animal matter	0.312	**0.009**	999
Rhodopyhta – Chlorophyta	0.620	**0.001**	999
Rhodopyhta – Detritus	0.958	**0.001**	999
Rhodopyhta – Animal matter	0.918	**0.001**	999
Chlorophyta – Detritus	0.574	**0.002**	999
Chlorophyta – Animal matter	0.873	**0.001**	999
Detritus – Animal matter	0.718	**0.004**	999

Appendix B4: PCO, PERMANOVA, PERMDISP and ANOSIM results for the comparison of nutrient compositions between diet items



PCO of the nutrient composition of stomach contents that contained more than 90% of one single diet item. Data points are displayed for coastal and offshore fish. Vector plots present Pearson's correlations of untransformed nutrient data. The distance of the vectors extending to the circle indicates the strength of the correlation of that vector with the distribution of data points.

PERMANOVA:

Source of variation	df	SS	MS	Pseudo-F	*p*(perm)	Unique perms
Diet item	4	155.15	38.79	19.854	**0.001**	998
Residual	68	132.85	1.95			

PERMDISP: F = 7.59, df1 = 4, df2 = 68, *p*(perm) = 0.002

ANOSIM: Global test results: R: 0.561, $p = 0.001$, nr of permutations: 999, nr of the permuted statistics greater than or equal to global R: 0.

Significant results are highlighted in bold.

Pairwise test	**R**	***p***	**Actual permutations**
Salps – *Ulva* sp. sheet	0.499	**0.001**	999
Salps – *A. suborbicularis*	0.591	**0.001**	999
Salps – Detritus	0.245	**0.010**	999
Salps – other crustaceans	0.501	**0.005**	999
Ulva sp. sheet – *A. suborbicularis*	0.765	**0.001**	999
Ulva sp. sheet – Detritus	0.567	**0.003**	999
Ulva sp. sheet – other crustaceans	1	**0.018**	56
A. suborbicularis – Detritus	0.956	**0.001**	999
A. suborbicularis – other crustaceans	1	**0.001**	999
Detritus – other crustaceans	0.874	**0.006**	165

Appendix B5: ANOSIM results of the comparison of the nutrient composition between coastal and offshore fish

Results of the pairwise comparison between coastal and offshore fish for each season showing differences in nutrient intake. Significant results are highlighted in bold.

Pairwise Tests	R	*p*	Actual permutations	Nr of the permuted statistics greater than or equal to global R:0
Summer	0.011	0.205	999	999
Autumn	0.131	**0.001**	999	999
Winter	0.034	0.094	999	999
Spring	0.019	0.228	999	999

Appendix B6: Statistical results of the Mann-Whitney U tests for differences in gut content mass between locations and mean/median values for each season

Median values of the relative gut content mass for the whole gut (I-V) and each section of the gut (I, II, III, IV and V), and results of the Mann-Whitney U test. Statistically significant results are highlighted in bold.

Section	I-V	I	II	III	IV	V
All fish						
Median	0.055	0.011	0.013	0.009	0.007	0.010
Region						
Median coastal	0.051	0.008	0.014	0.009	0.006	0.009
Median offshore	0.059	0.015	0.013	0.010	0.008	0.011
Mann-Whitney U	10386.5	22321.0	10266.5	13931.5	13011.5	14267.5
z – score	3.239	6.799	-0.821	2.671	2.576	2.884
p value	**0.001**	**<0.001**	0.411	**0.008**	**0.010**	**0.004**

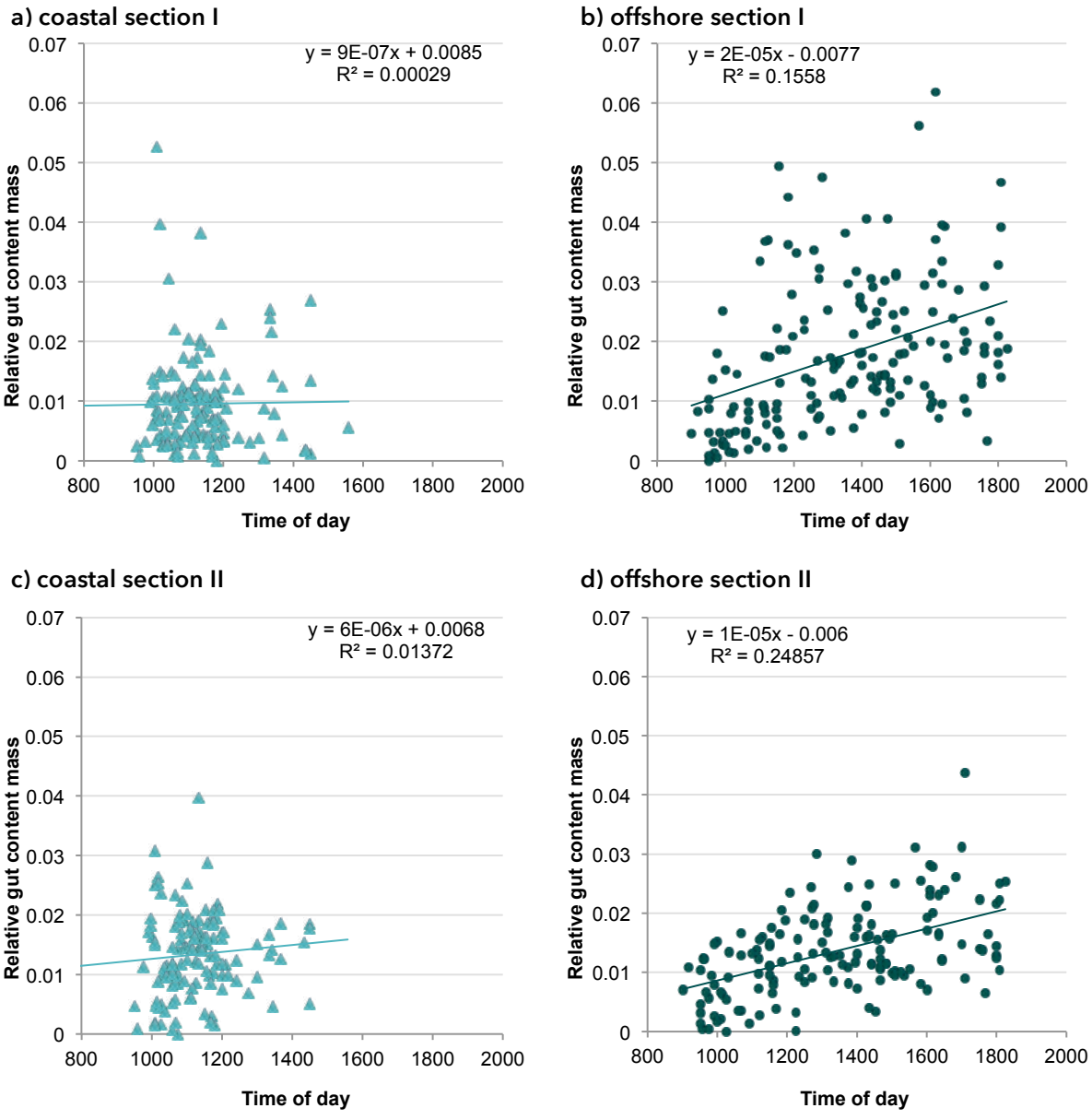
Number of fish (n), means, and medians of the relative gut content mass for all seasons for coastal and offshore *G. tricuspidata*.

	Coastal			Offshore		
	n	Mean	Median	n	Mean	Median
Summer	44	0.036	0.033	50	0.048	0.055
Autumn	41	0.031	0.035	42	0.038	0.038
Winter	38	0.032	0.039	44	0.057	0.056
Spring	39	0.037	0.039	44	0.049	0.050

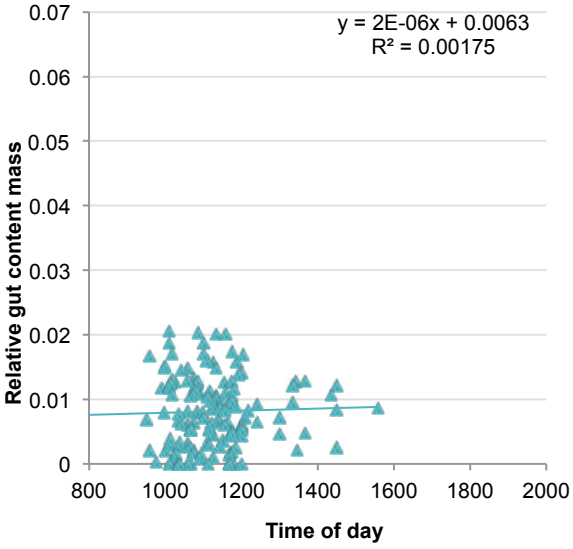
Appendix B7: Changes in relative gut content mass and results of the independent-samples t-tests analysing diel variation in relative gut content mass and nutrient composition

Morning fish were classified as fish caught between 9.30 am and 10.00 am and early afternoon fish between 1.00 pm and 1.40 pm. Late afternoon fish only existed for the offshore location and these were caught between 5.35 pm and 6.15 pm.

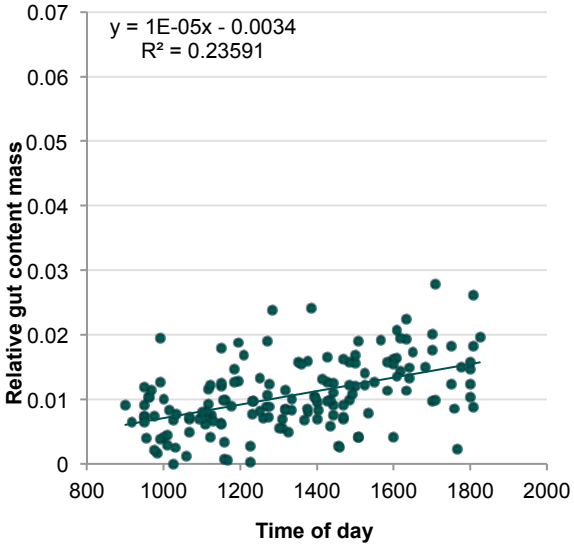
Figures showing the changes in relative gut content mass in relation to time of day for coastal fish (left-hand figures a, c, e, g, i) and offshore fish (right-hand figures, b, d, f, h, j) for the stomach (a,b) and each of the gut sections (c-j)



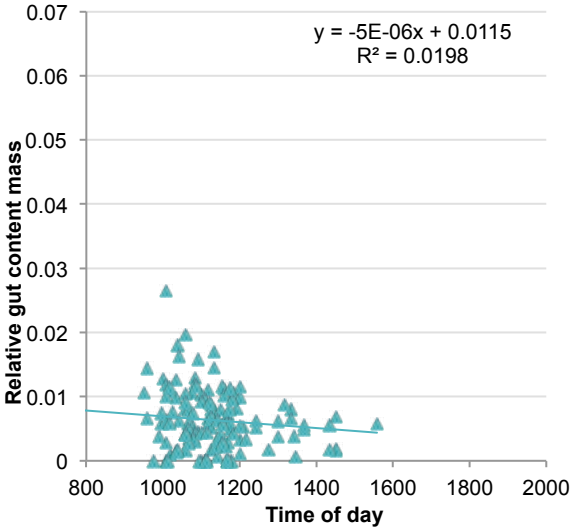
e) coastal section III



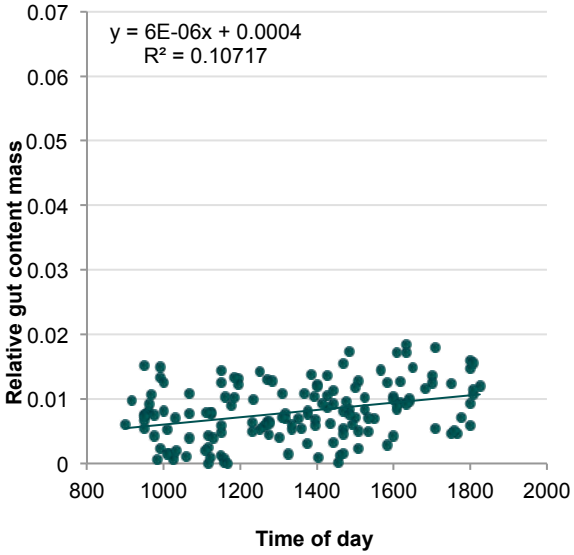
f) offshore section III



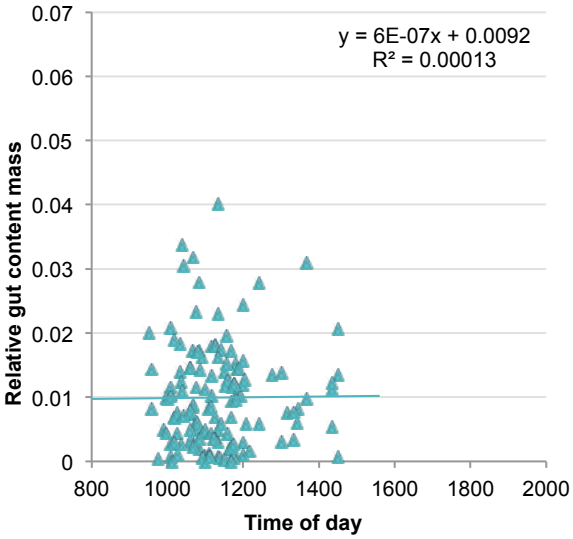
g) coastal section IV



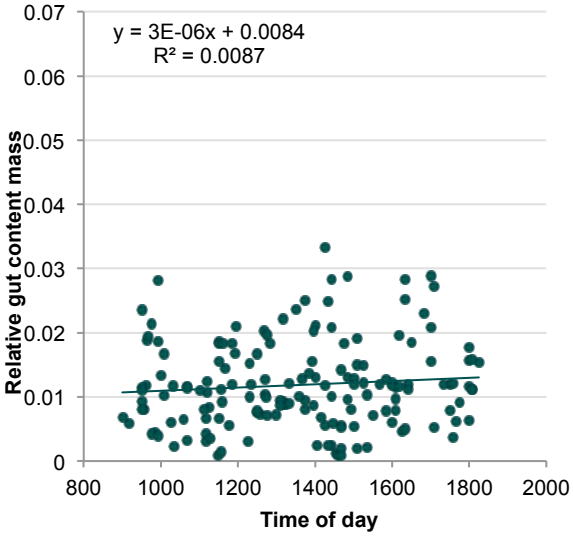
h) offshore section IV



i) coastal section V



j) offshore section V



Results of the Shapiro-Wilk test (*p*-values). Statistically significant results are highlighted in bold indicating that samples are not normally distributed ($p < 0.05$).

	Relative gut content mass	C [%]	N [%]	Lipids [%]	Ash [%]
Coastal					
Morning	0.856	0.582	0.230	0.239	0.552
Early afternoon	0.361	0.872	0.278	0.892	0.563
Offshore					
Morning	**0.019**	0.143	0.311	0.513	0.176
Early afternoon	0.102	**0.019**	0.354	0.738	0.388
Late afternoon	0.423	0.717	0.843	0.592	0.241

Analysis of diel variation for coastal and offshore populations. Presented are the mean values and results of the independent-samples t-test testing if time of day affects relative gut content mass and nutritional value of stomach contents. Statistically significant results are highlighted in bold ($p < 0.05$).

	Relative gut content mass	C [%]	N [%]	Lipid [%]	Ash [%]
Coastal: morning – early afternoon					
Mean morning	4.009	26.937	4.415	6.296	36.540
SE morning	0.721	2.092	0.472	0.793	4.320
Mean early afternoon	4.367	30.553	5.001	5.403	29.564
SE early afternoon	0.809	1.079	0.731	0.644	2.393
Mean difference	-0.359	-3.616	-0.586	1.522	6.976
95% CI lower	-2.720	-8.829	-2.452	-0.683	-3.617
95% CI upper	2.003	1.598	1.281	3.727	17.569
t-value	-0.331	-1.536	-0.673	1.504	1.412
df	12	10.479	14	12	14
p value	0.746	0.154	0.512	0.158	0.180
Offshore: morning – early afternoon					
Mean morning	4.407	27.650	4.033	5.440	33.211
SE morning	0.547	1.434	0.245	0.444	3.523
Mean afternoon	6.076	28.560	4.251	5.748	34.402
SE afternoon	0.505	1.464	0.395	0.515	2.945
Mean difference	-1.669	-0.910	-0.218	-0.307	-1.191
95% CI lower	-3.299	-5.133	-1.160	-1.703	-10.670
95% CI upper	-0.039	3.313	0.724	1.088	8.286
t-value	-2.118	-0.444	-0.477	-0.453	-0.260
df	23	25	25	25	24
p value	**0.045**	0.661	0.638	0.654	0.797
Offshore: morning – late afternoon					
Mean morning	4.407	27.650	4.033	5.440	33.211
SE morning	0.547	1.434	0.245	0.444	3.523
Mean late afternoon	7.647	29.190	4.580	7.050	31.427
SE late afternoon	0.709	1.039	0.419	0.772	2.253
Mean difference	-3.240	-1.540	-0.547	-1.610	1.783
95% CI lower	-5.057	-5.180	-1.544	-3.441	-6.923
95% CI upper	-1.422	2.100	0.451	0.222	10.490
t-value	-3.680	-0.870	-1.127	-1.806	0.433
df	24	26	26	26	20.632
p value	**0.001**	0.392	0.270	0.082	0.674

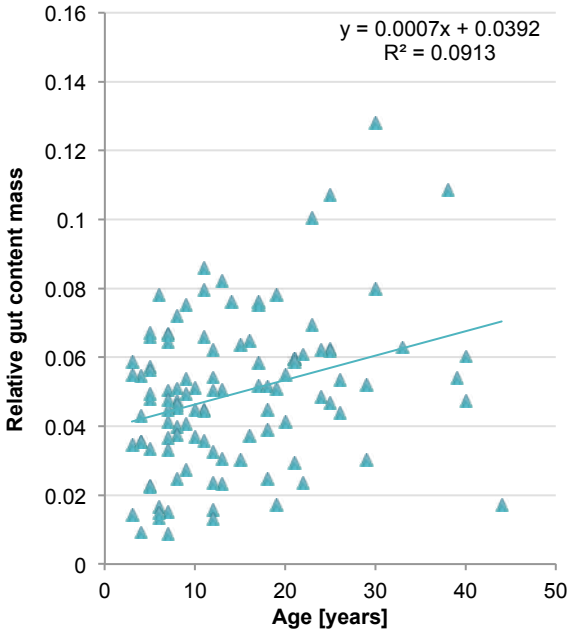
Appendix B8: Results of the Mann-Whitney U tests analysing differences between populations in nutrient and ash concentrations

Median values of the concentrations for carbon, nitrogen, lipid, and ash for coastal and offshore fish, and results of the Mann-Whitney U test. Statistically significant results are highlighted in bold.

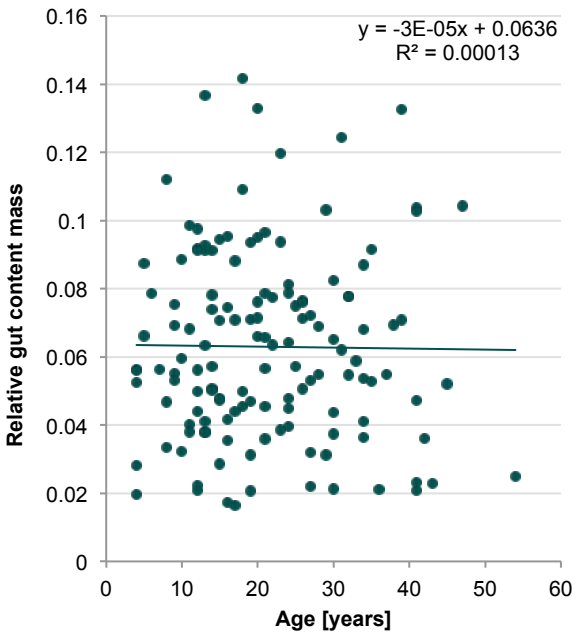
	Carbon	**Nitrogen**	**Lipid**	**Ash**
Median coastal	28.67 %	4.21 %	5.29 %	31.45 %
Median offshore	30.29 %	4.07 %	5.82 %	29.92 %
Mann-Whitney U	13597.0	12160.0	14317.0	12049.0
z – score	1.827	0.024	3.078	-0.222
p value	0.068	0.981	**0.002**	0.825

Appendix B9: Graphs showing changes in relative gut content mass, carbon, nitrogen, lipid, ash and C:N ratio with age and results of the independent-samples t-tests for the comparisons between locations

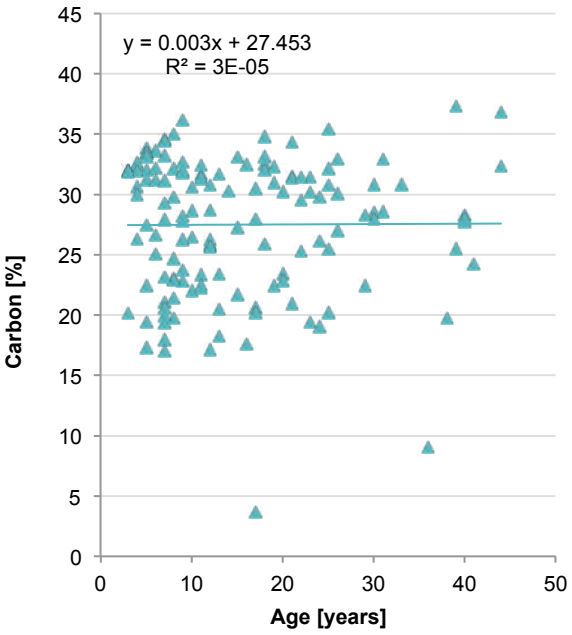
Coastal



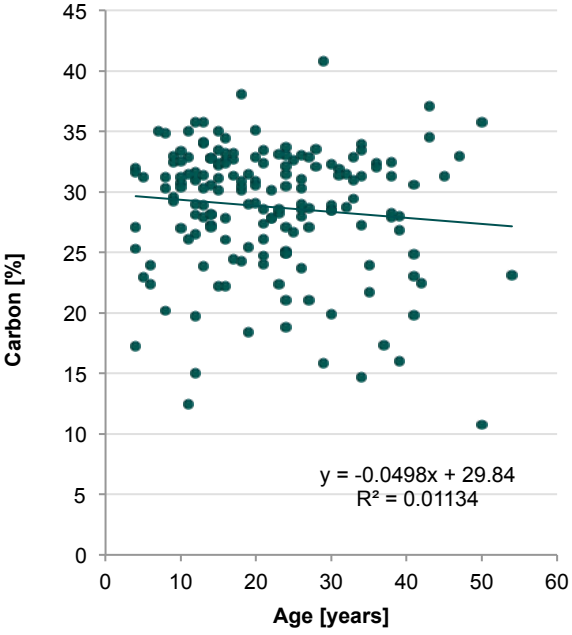
Offshore



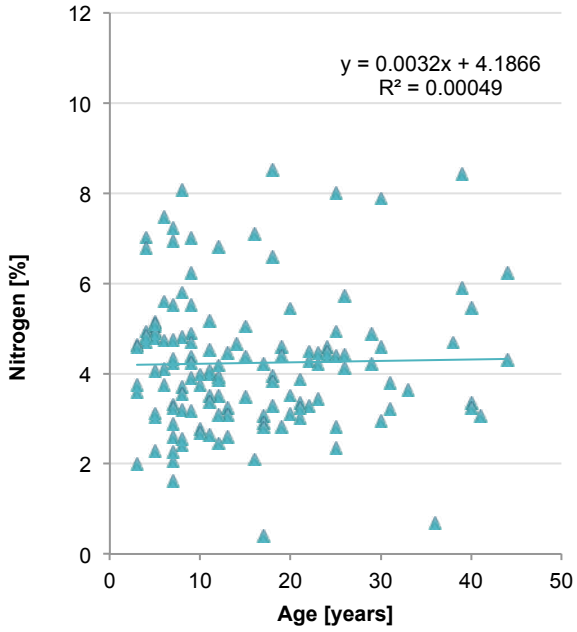
Coastal



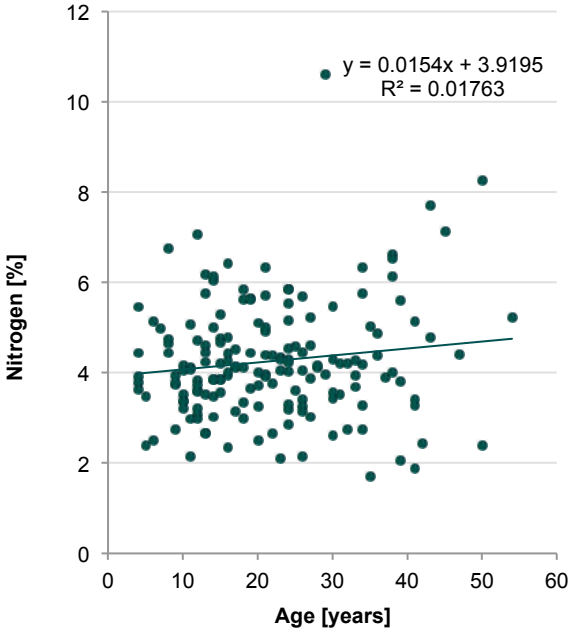
Offshore



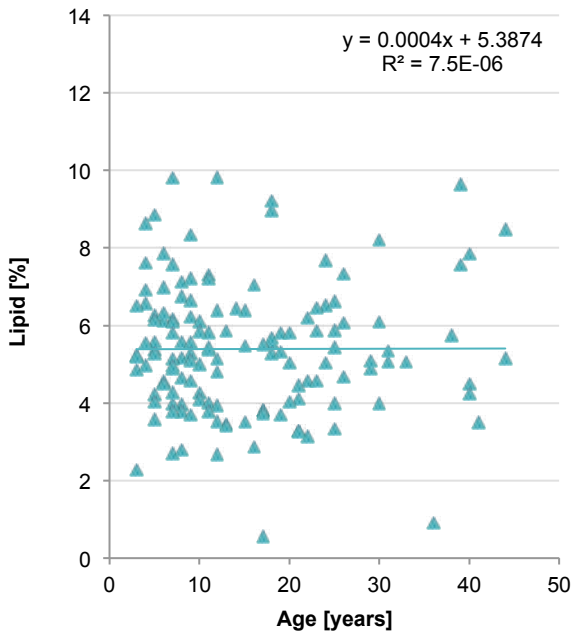
Coastal



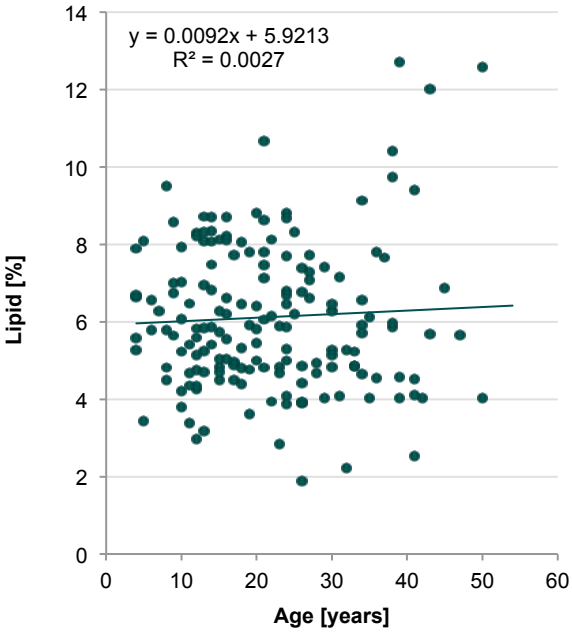
Offshore



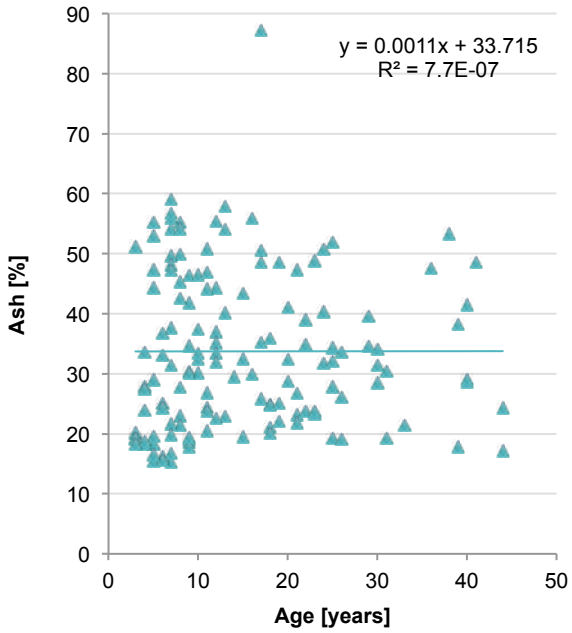
Coastal



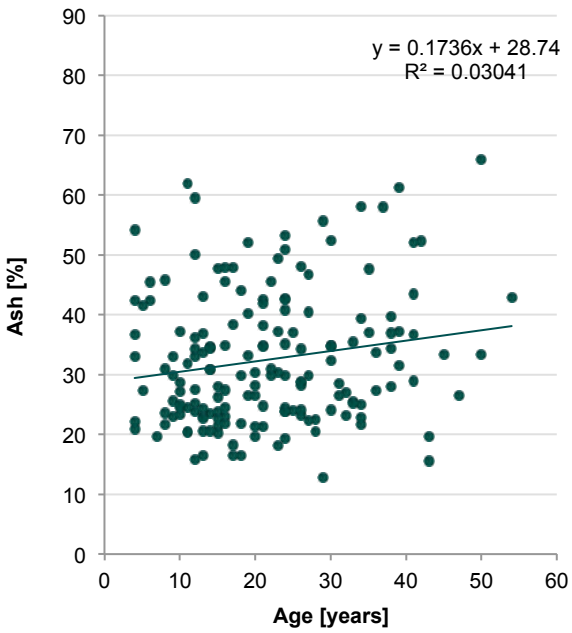
Offshore



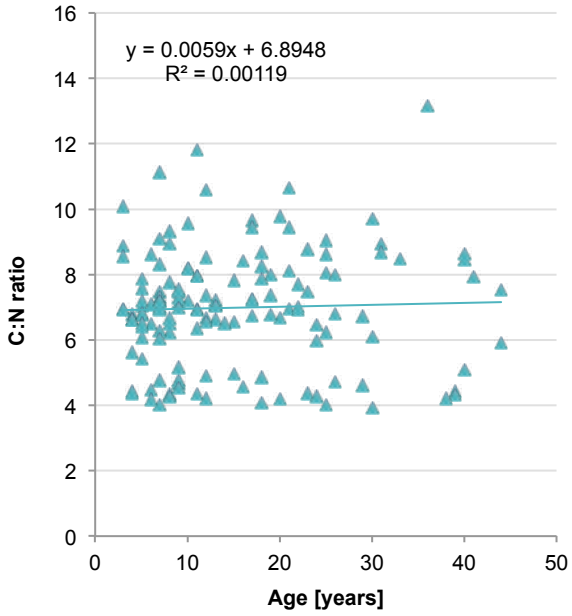
Coastal



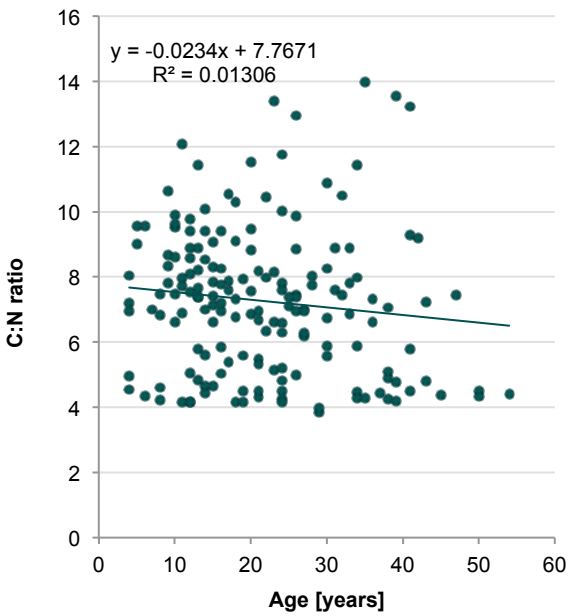
Offshore



Coastal



Offshore



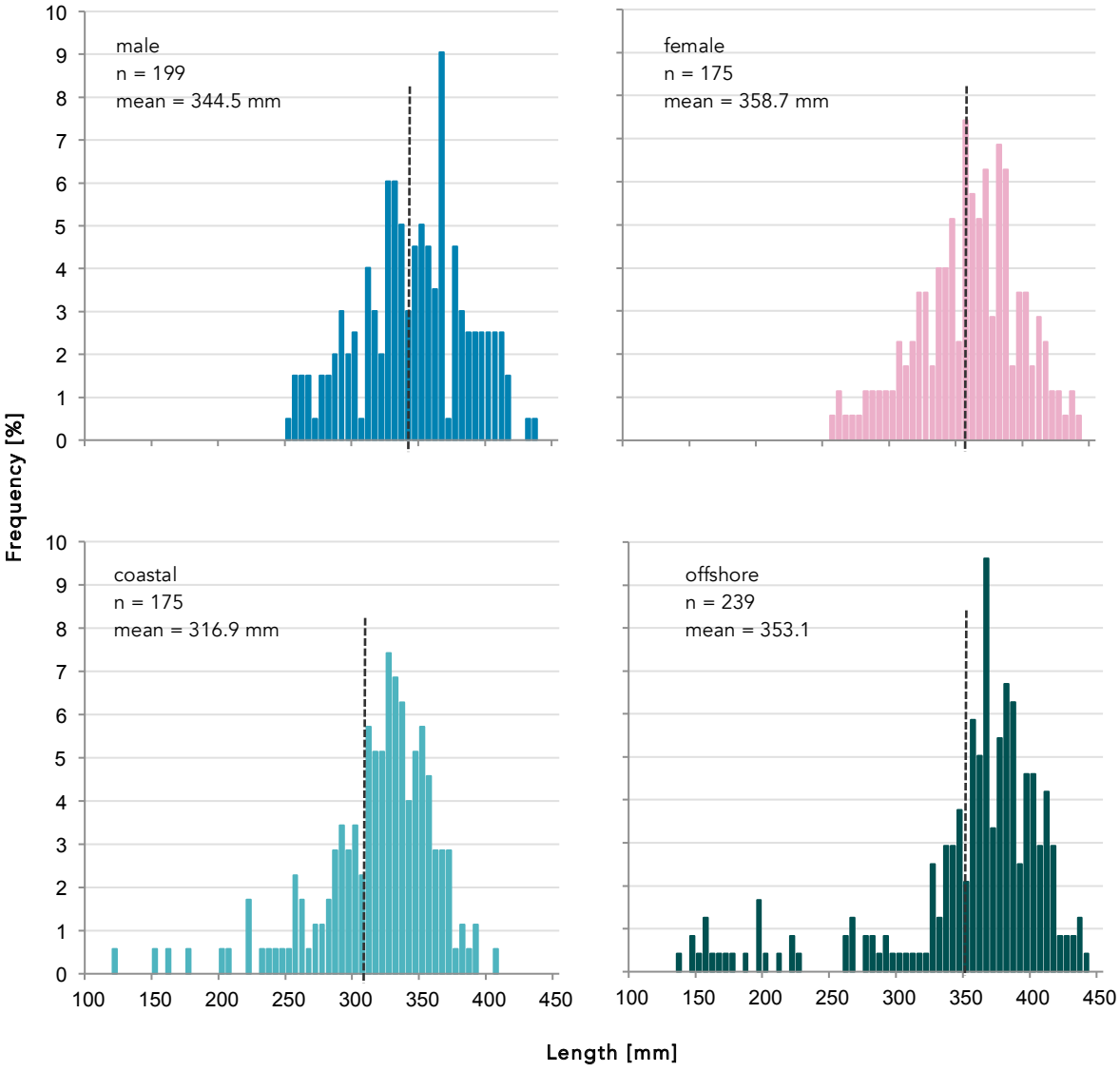
Results of the independent-samples t-tests for comparison of coastal and offshore populations. Statistically significant results are highlighted in bold.

	Relative gut content mass	Carbon	Nitrogen	Lipid	Ash	C:N ratio
Juveniles (3-5 years)						
Mean coastal	0.042	29.332	4.477	5.683	28.857	6.837
SE coastal	0.004	1.119	0.269	0.358	3.045	0.292
Mean offshore	0.052	26.792	3.877	6.235	34.767	7.185
SE offshore	0.009	2.066	0.356	0.611	3.995	0.719
t-value	1.184	1.118	1.174	0.784	1.084	0.536
df	23	26	26	25	26	26
p value	0.249	0.137	0.251	0.440	0.288	0.597
Adults (18-20 years)						
Mean coastal	0.045	29.164	4.546	5.857	29.549	6.957
SE coastal	0.006	1.385	0.518	0.526	2.743	0.564
Mean offshore	0.075	29.779	4.283	5.904	30.018	7.440
SE offshore	0.010	1.268	0.298	0.397	2.841	0.614
t-value	2.686	0.326	0.463	0.074	0.118	0.565
df	20	23	23	23	22	23
p value	**0.014**	0.747	0.648	0.942	0.908	0.577

Appendix C: Chapter 4

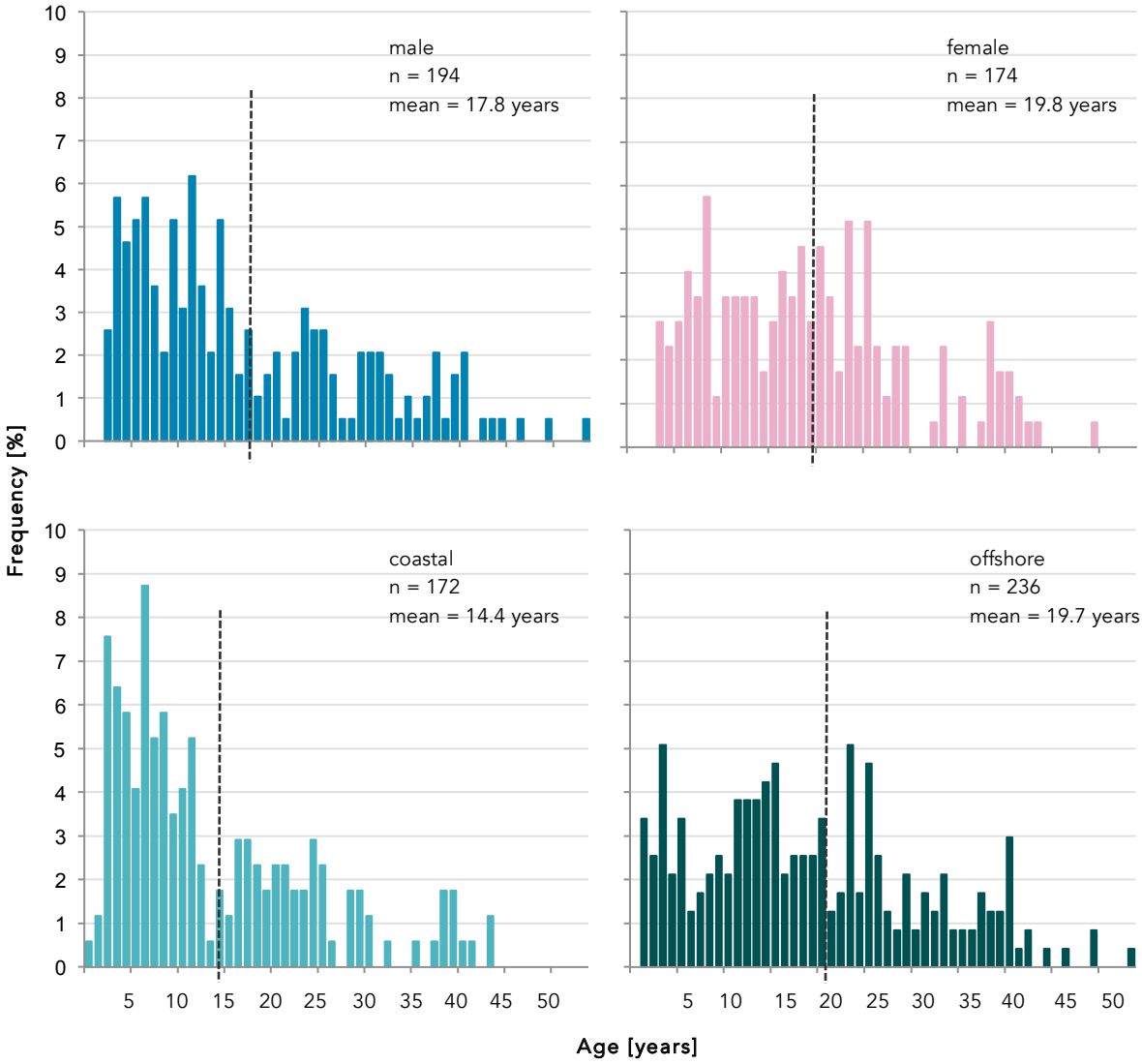
Appendix C1: Length-frequency composition of *G. tricuspidata*

Lengths were grouped to the nearest 5 mm below true length. The dashed line marks the mean.



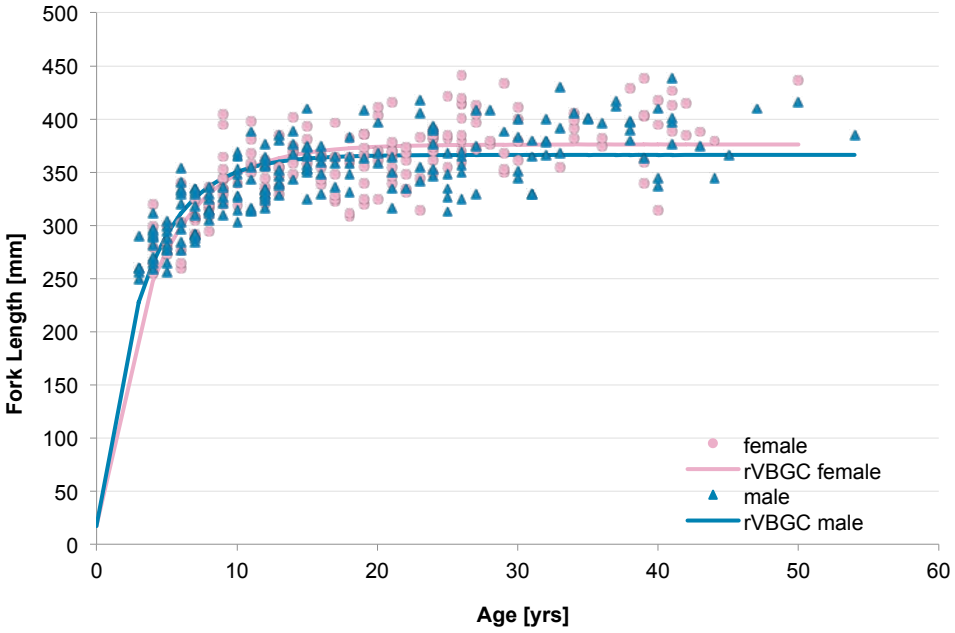
Appendix C2: Age-frequency composition of *G. tricuspidata* for both gender and locations

The dashed line marks the mean value.

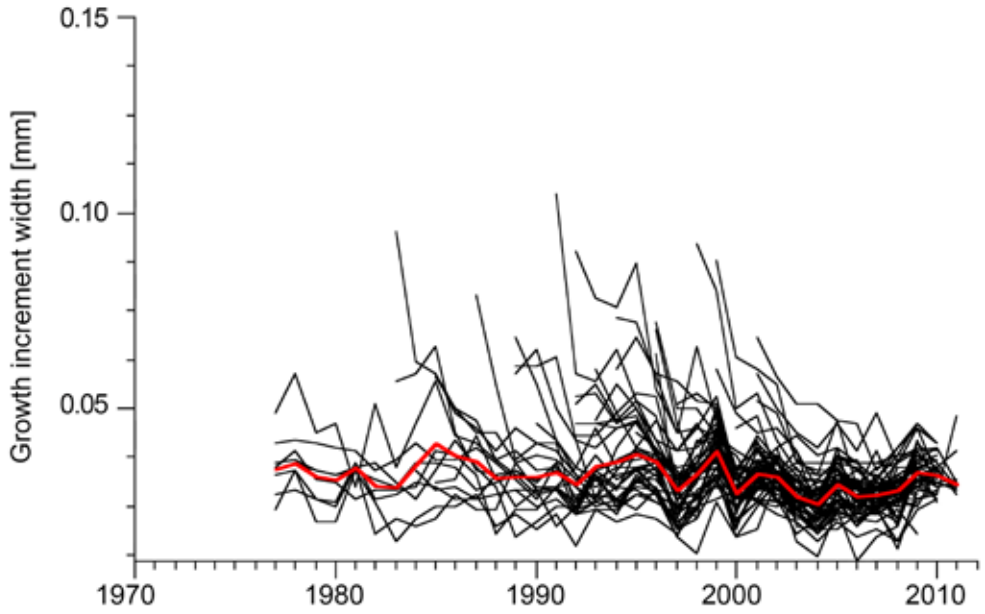


Appendix C3: The reparameterized von Bertalanffy growth curves fitted for male and female *G. tricuspidata* based on the best-fit model

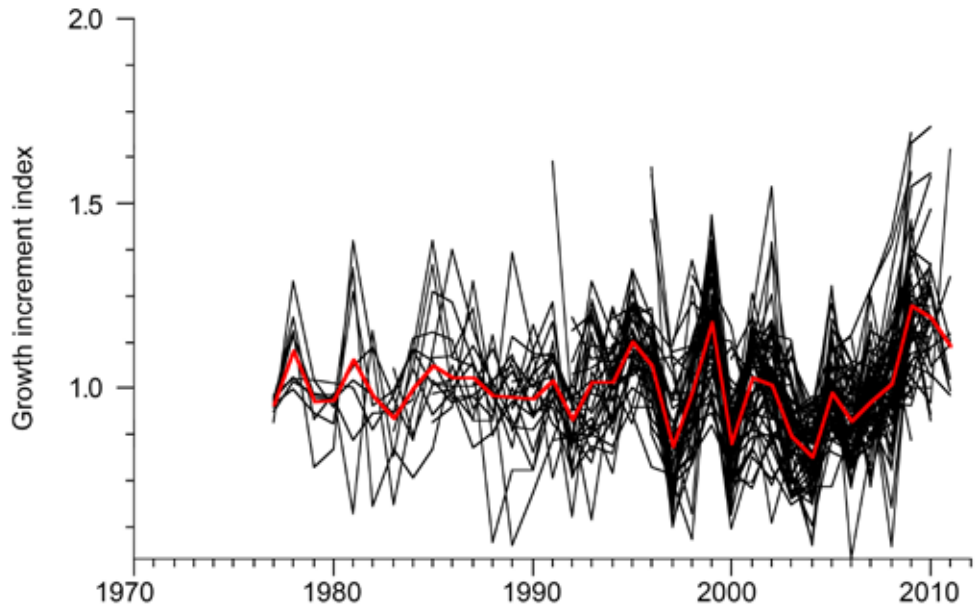
For parameter values see Table 4.2.



Appendix C4: Chronologies of growth increment widths and growth increment indices of coastal fish

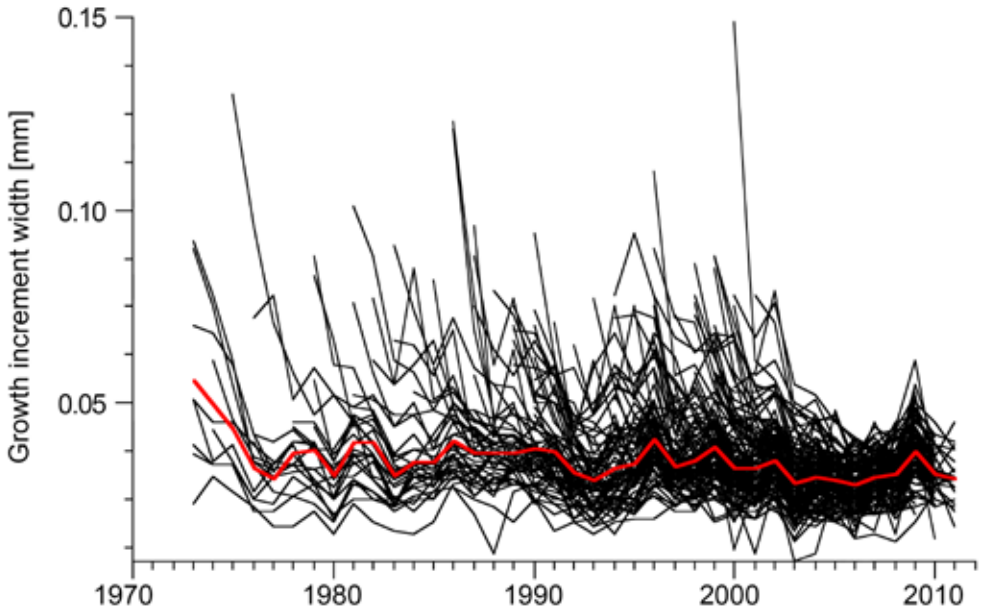


Growth increment widths of the raw data for individual coastal fish from 1977 to 2011 showing age related trends. The red line marks the average.

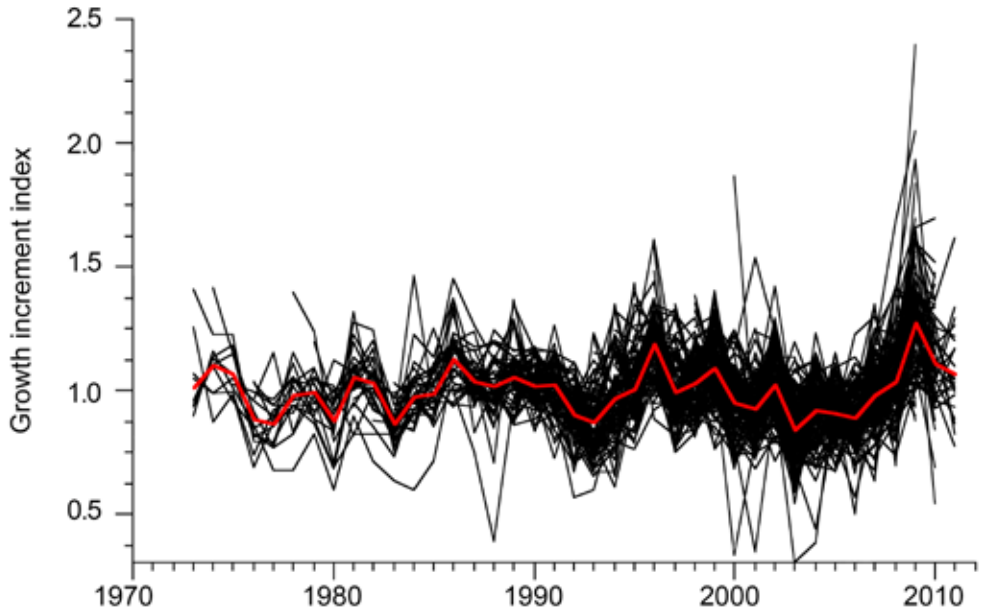


Detrended growth increment indices of coastal fish from 1977 to 2011. The red line marks the average.

Appendix C5: Chronologies of growth increment widths and growth increment indices of offshore fish



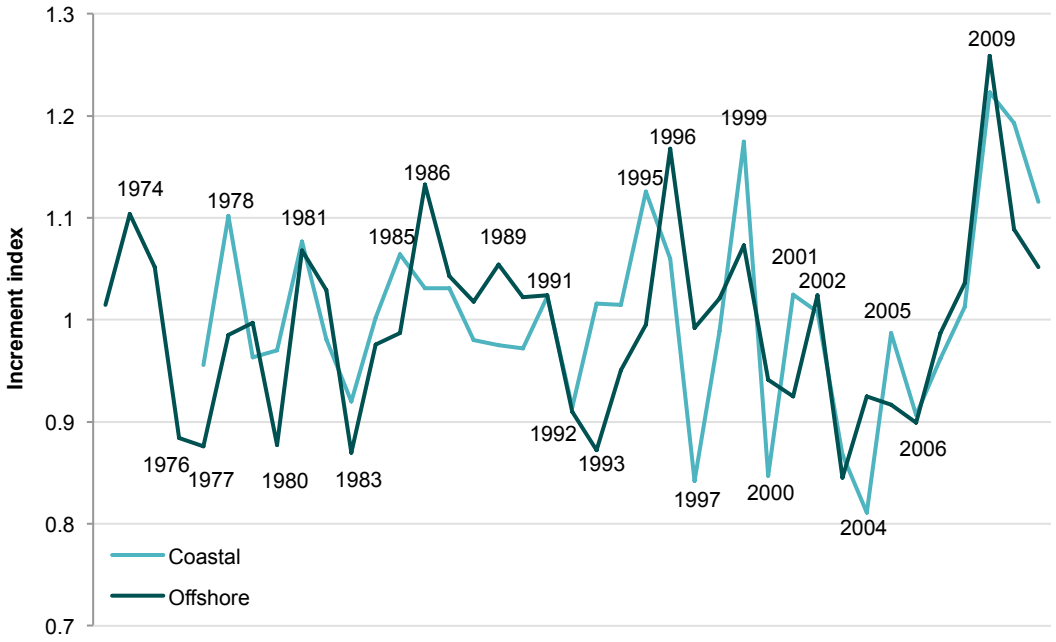
Growth increment widths of the raw data from 1973 to 2011 for individual offshore fish showing age related trends. The red line marks the average.



Detrended growth increment indices of each offshore fish from 1973 to 2011. The red line marks the average.

Appendix C6: Comparison of the master otolith growth increment width chronologies between coastal and offshore fish

Detrended and standardized increment widths created with ARSTAN. Values smaller than 1 indicate narrower than average increment widths and values greater than 1 indicate wider than average increments that can be correlated to years of decreased and increased growth, respectively.



Appendix D: Additional data

Appendix D1: Length-weight relationship

Body length and weight are the most obvious biological characteristics of a species and the weight of an organism, including fish, is related to its length. The relationship between those two parameters contains information about the growth of the fish and reveals whether a fish is growing isometrically, hypoallometrically (weight is proportional to length raised to a power of < 3) or hyperallometrically (weight is proportional to length raised to a power of > 3) (Froese, Tsikliras, & Stergiou, 2011). The length-weight relationship is also commonly used in fisheries research and management because (1) it enables the estimate of biomass from length observations, (2) it provides a means to estimate the condition of the fish, (3) it provides information on the body shape and thus vulnderability to different types of fishing gear, and (4) it can be used for comparisons between populations (Froese et al., 2011; Stergiou & Moutopoulos, 2001). Interspecific differences of the weight-length relationship occur due to interspecific differences in body shape. Intraspecific differences are influenced by seasonal and yearly changes of the condition of each fish and also sex and gonad development (Schneider, Laarman, & Gowing, 2000).

Length-weight regressions were calculated for all fish and separately for both genders and regions using the length-weight relationship equation ($W = aL^b$) in its logarithmic form:

$$\log_{10} W = \log_{10} a + b \log_{10} L$$

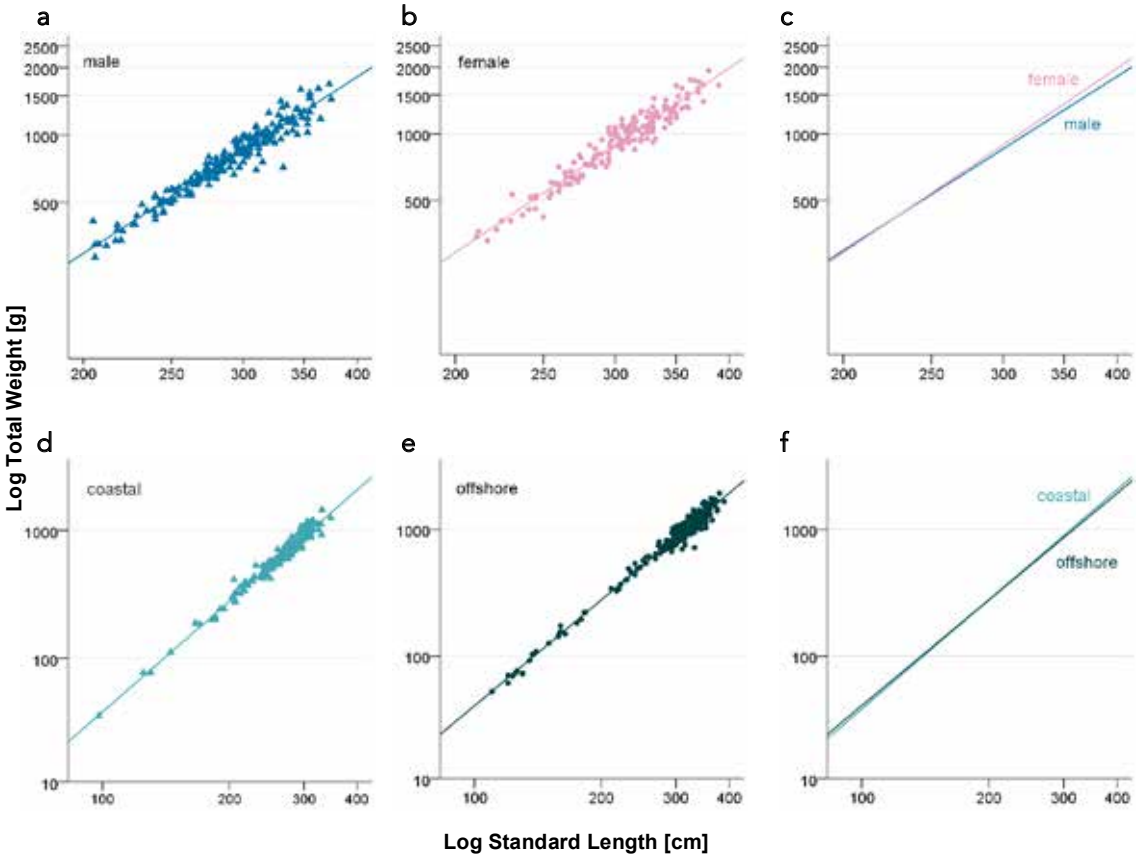
where W = total weight (g), L = standard length (cm), a = y-intercept, and b = slope (Froese, 2006; Weymouth, 1922). A Pearson's correlation was conducted to measure the strength and direction of the linear length-weight relationship. To compare the curves between adult fish of both genders and regions an analysis of covariance (ANCOVA) was performed. Post hoc analysis was performed with a Bonferroni adjustment.

Length-weight relationships are commonly calculated using total length (TL) of fish in centimetres (2006). In this study the parameters were calculated using the standard lengths (SL), which results in values for log a that are slightly higher (Froese, 2006). Values were transformed using the following equation given by Froese (2006):

$$a_{TL} = a_{SL} (TL/SL)^{-b}$$

with a_{TL} and a_{SL} as the a values of total length and standard length, respectively, and b as the slope of the length-weight relationship. The average ratio TL/SL was 1.170, based on two length measurements obtained from FishBase (Froese & Pauly, 2015).

The measurement of 413 specimens revealed a strong positive relationship between total weight (TW) and standard length (SL) for all fish, irrespective of sex and location ($r > 0.960$, $p < 0.001$, see figures and table below). Pearson's correlations were run despite data not being normally distributed, as the test is considered somewhat robust to deviations from normality.



Double-logarithmic plots of *G. tricuspidata* total weight vs. standard length. The top row shows the values for (a) male and (b) female fish separately including the best-fitted regression, and (c) compares the regression of both genders. The bottom row depicts the values for (d) coastal and (e) offshore fish with the best-fitted regressions and (f) the comparison of the regression for both locations. For parameter values see table below.

ANCOVA was run to compare the slopes between male and female fish and coastal and offshore fish using log10-transformed data (see table below). Regression slopes were homogeneous as the interaction term was not statistically significant (gender: $F(1,369) = 3.616$, $p = 0.058$; region:

$F(1,409) = 3.520$, $p = 0.061$). Standardized residuals for the two variables and for the overall model were not normally distributed, as assessed by Shapiro-Wilk's test ($p < 0.05$). The ANCOVA was run anyway as it is fairly robust to deviations from normality. Visual inspection of a scatterplot and Levene's test revealed homoscedasticity and homogeneity of variance (sex: $p = 0.117$; region $p = 0.288$). There were four outliers (1 for gender, 3 for regional comparison) in the data, as assessed by the absence of cases with standardized residuals greater than ±3 standard deviations. These outliers were kept in the dataset, as they seem to be true data points. After adjustment for SL, there was a statistically significant difference in TW between male and female *G. tricuspidata*, $F(1,370) = 14.444$, $p = < 0.001$, partial $\eta 2 = 0.038$. Post-hoc analysis revealed that female fish were significantly heavier than male fish ($p = < 0.001$). The difference between offshore and coastal fish was also statistically significant, $F(1,410) = 9.144$, $p = 0.003$, partial $\eta 2 = 0.022$ with coastal fish being significantly heavier than offshore ones ($p = 0.003$).

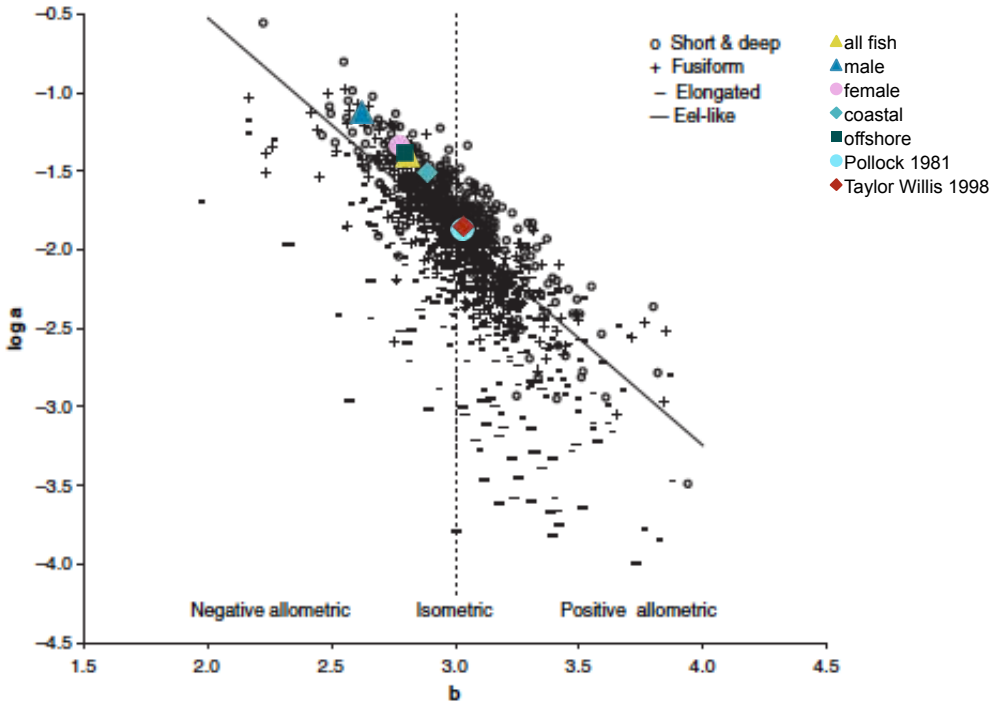
Table of the parameters of the length-weight relationship of *G. tricuspidata* for $\log_{10}(y) = a + b * \log_{10}(x)$ including results of the Pearson's correlation and ANCOVA. The parameters for the curve estimation are y-intercepts (a) for standard length (a_{SL}) and the calculated value for total length (a_{TL}), slope (b), coefficient of determination (R^2), p-value (*p*). The parameters for the Pearson's correlation are Pearson's correlation coefficient (*r*), degrees of freedom (df), and p-value (*p*), and for the ANCOVA M = mean, SD = standard deviation, and SE = standard error. Significant results are highlighted in bold.

	All fish	Male	Female	Coastal	Offshore
Length-weight relationship					
N	413	199	174	175	238
a_{SL}	0.0646	0.1174	0.0731	0.0502	0.0658
a_{TL}	0.0416	0.0779	0.0473	0.0320	0.0425
b	2.797	2.615	2.767	2.879	2.788
R^2	0.977	0.929	0.923	0.970	0.979
p	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Pearson's correlation					
r	0.988	0.964	0.961	0.985	0.989
df	411	197	172	173	236
p	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
ANCOVA					
Unadjusted M	-	2.892	2.962	2.787	2.909
Unadjusted SD	-	0.156	0.156	0.226	0.305
Adjusted M	-	2.917	2.934	2.865	2.852
Unadjusted SE	-	0.003	0.003	0.003	0.003

As expected, *G. tricuspidata* displayed a strong positive relationship between total weight and standard length as described for many fish species (İlkyaz, Metin, Soykan, & Kinacigil, 2008; Karakulak, Erk, & Bilgin, 2006; Morato et al., 2001). The development of the length-weight

relationship allows for estimation of the biomass from length measured or estimated in the field. The slope of the length-weight relationship equation revealed that the growth of *G. tricuspidata* followed a negative allometric pattern (b = 2.615 to 2.879). It can be concluded that *G. tricuspidata* (a) increased less in weight than predicted by their increase in length, and (b) acquired a more elongated body shape with age. Alternatively, young fish might have been in a better nutritional condition (Froese et al., 2011). Values increased as follows: coastal < all fish < offshore < female < male, indicating that coastal fish had the shortest body length or increased more in weight than predicted by their length, while male fish had the most elongated bodies or increased least in weight compared to their increase in length. Female fish were on average 0.58% heavier than male fish of equivalent length and coastal fish 0.46% heavier than offshore ones.

The parameters log a and b can be plotted against each other showing the interdependence of these two parameters (Froese, 2006; Kulbicki, Guillemot, & Amand, 2005). The figure below shows the plot of log a over b published by Froese (2006). It includes the values for *G. tricuspidata*, which are in the range of values for fish with a body shape that is either short and deep or fusiform.



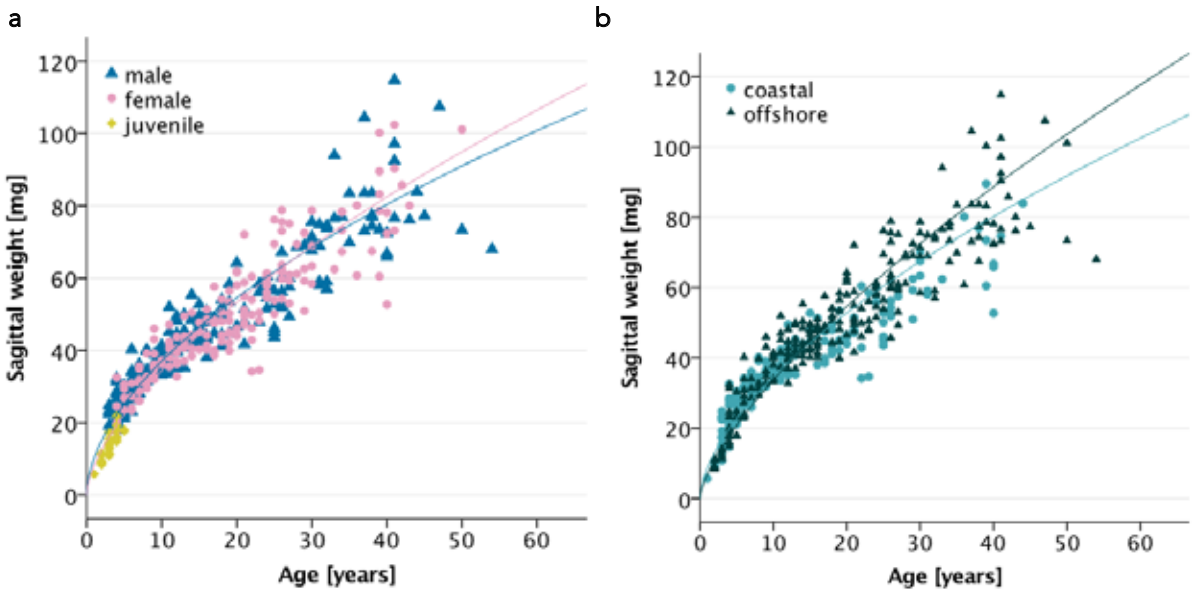
After Froese (2006): Scatter plot of mean log a (TL) over mean b for 1223 fish species with body shape information (see legend). Areas of negative allometric, isometric and positive allometric change in body weight relative to body length are indicated. Regression line based on robust regression analysis for fusiform species, with n = 451. Values of the relationship of log a (TL) over b for *G. tricuspidata* for this study and previous ones are also marked in the graph.

Two studies have previously investigated the length-weight relationship of *G. tricuspidata*. Pollock (1981) analysed 2848 fish from Moreton Bay (eastern Australia) that were collected from January 1977 to February 1978. His calculations resulted in a_{FL} = 0.0156 (a_{TL} = 0.0138) and b = 3.02 with R^2 = 0.92 for fish larger than 220 mm FL. The second study was conducted by Taylor and Willis (1998) and included 363 fish, resulting in a_{FL} = 0.0163 (a_{TL} = 0.0144), b = 3.022, and R^2 = 0.997. These fish were collected near Leigh by Morrison (1990), i.e. is the same location as for coastal fish in the present study. Small juvenile fish were included in the data set with FL ranging from 27 to 450 mm. Both studies reveal b values that are very close to isometric growth and a values that have a smaller y-intercept than found in the present study.

Appendix D2: Otolith weight-age relationship

Due to the continuous growth of otolith their weight can be used to predict the age of fish. This economic and objective method is sometimes favoured over counting otolith increments and is often more reliable than fish length as a predictor of age (Cardinale & Arrhenius, 2004) and is a fast method to age fish in comparison to counting increments (Pilling, Grandcourt, & Kirkwood, 2003; Worthington et al., 1995). A number of studies have shown a significant relationship between otolith weight and the age of fish (Britton & Blackburn, 2014; Choat, Robertson, Ackerman, & Posada, 2003; Pawson, 1990; Worthington et al., 1995). The relationship between otolith weight and age provides a check on the precision of the age count (Choat et al., 2003; Choat & Axe, 1996; Pilling et al., 2003). Growth rates must be consistent for the investigated species or a size class and a calibration curve has to be developed (Pawson, 1990; Worthington et al., 1995). Fish can then be aged rapidly and precisely using otolith weights, which is of particular interest for fisheries management, where large numbers of fish have to be aged for stock assessments.

The right otolith was weighed to the nearest 0.01g. If the right one was missing, broken or deformed the left one was used instead. A curve was fitted using least square regression analysis for the correlation between age and sagittal otolith weight with sagittal weight as the independent variable (Choat & Axe, 1996). A Spearman's rank-order correlation was run to assess the relationship.



Weight of the sagittal otoliths of *G. tricuspidata* in relation to age for (a) male, female, and juvenile fish and (b) coastal and offshore populations. Lines show the best-fit curves.

Sagittal otoliths weighed from 5.74 mg to 114.80 mg. Otoliths showed curvi-linear growth, with otolith weight increasing throughout the whole lifetime of the fish. Growth was proportionally faster in young fish (0 - 7 years) than in adults as reflected by the slope of the curve, in which slope decreases with age (see figures above). The correlation was strong between sagittal otolith weight and the age of all *G. tricuspidata* collected, $y = 0.048x^{1.510}$, $R^2 = 0.888$, $p < 0.001$; $r_s(391) = 0.948$, $p < 0.001$. Correlations were also strong for both gender (male: $y = 0.016x^{1.789}$, $R^2 = 0.883$, $p < 0.001$; $r_s(184) = 0.951$, $p < 0.001$; female: $y = 0.037x^{1.585}$, $R^2 = 0.836$, $p < 0.001$; $r_s(168) = 0.917$, $p < 0.001$, Figure a) and both locations (coastal: $y = 0.028x^{1.655}$, $R^2 = 0.853$, $p < 0.001$; $r_s(159) = 0.946$, $p < 0.001$; offshore: $y = 0.065x^{1.433}$, $R^2 = 0.910$, $p < 0.001$; $r_s(230) = 0.944$, $p < 0.001$, Figure b).

Body measurements, such as the length of fish, can be used to predict the age of fish (Morales-Nin & Aldebert, 1997). Length-frequency analysis is restricted in its use and is only applicable to young, fast growing fish where size classes are easily discernable and assumes one spawning period per year. But length is a poor predictor for older fish as somatic growth slows and fish approach their asymptotic length (Campana, 2001). *G. tricuspidata* also displayed considerable variation in size at any given age, making length a poor proxy for age. Another parameter that is often preferred over body length to predict age of fish is the weight of the sagittal otoliths. Even though otolith weight and age were strongly correlated ($r_s > 0.917$), sagittal weight was a poor predictor of age in older specimen of *G. tricuspidata* due to the variation in weight at any given age. For example, an otolith of about 60 mg could belong to a fish between 20 and 40 years of age. Otolith weight as a proxy for age is less suitable for species where growth slows with age as otolith weights will overlap among ages and thus result in erroneous predictions (Cardinale & Arrhenius, 2004; Pilling et al., 2003; Worthington et al., 1995). Eliminating this error can only be achieved by counting increments using microscopy.

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