Studies on the integrated feeding of *Sufflogobius bibarbatus* (von Bonde, 1923) in the Northern Benguela ecosystem using fatty acid biomarkers and stable isotopes ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$)

By

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DECLARATION

I declare that: **Studies on the integrated feeding of *Sufflogobius bibarbatus* (von Bonde, 1923) in the Northern Benguela ecosystem using fatty acid biomarkers and stable isotopes ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$)**, is my own work, that it has not been submitted for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Megan Geralda van der Bank May 2010

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ABSTRACT

The present study employs a multi-tracer approach to shed light on the integrated feeding of the bearded goby *Sufflogobius bibarbatus*. The study was conducted between 31 March and 10 April 2008 at two 48 hour stations off the Namibian coast, namely inshore (station A, 120 m) and offshore (station B, 180 m). Isotopes of $\delta^{13}C$ and $\delta^{15}N$ suggest that the goby diet at station A and B at the time of the study was primarily composed of the jellyfish *Chrysaora fulgida* and *Aequorea forskalea*, which comprised up to 73.85 % and 70.36 % of the goby diets respectively. However the importance of these cnidarian prey items, together with adult euphausiids, benthic polychaetes and shrimp varied with goby size, point of collection and habitat. Spatially pooled $\delta^{34}S$ results suggest that the sulphidic benthic sediment (containing diatoms and sulphur bacteria) overlying the central shelf off Namibia may contribute 34.2 % to the diet of the goby and that the goby may be able to drive sulphides from the benthos. Fatty acid analyses were limited to data collected only at station B and they suggest that gobies retrieve most of their essential fatty acids from pelagic zooplankton. Furthermore, small gobies at station B were high in the copepod markers 20:1ω9 and 22:1ω11, but their larger counterparts were high in the diatom FA 20:4ω6, 20:5ω3, diatom marker ratios 16:1/16:0 and 20:5ω3/22:6ω3 suggesting that small gobies fed more on pelagic copepods while large gobies fed directly on sedimented diatoms on the benthos. Fatty acid analyses also provided support for sulphur bacterial and jellyfish-feeding amongst gobies. The study highlights the advantages of using multiple trophic tracers to compliment each other, and identifies the bearded goby as an opportunistic feeder that plays an important role in terms of energy transfer within the northern Benguela ecosystem.

CHAPTER 1: INTRODUCTION

1.1 The Benguela ecosystem

The Benguela ecosystem is an eastern boundary current ecosystem and is one of the four major upwelling systems in the world. It stretches from Cape Agulhas in South Africa (35 ºS) along the Namibian coast to the Angola-Benguela frontal zone (14-16 ºS) (Shannon 1985). The Benguela ecosystem is characterized by cool nutrient-rich waters, and is unusual in that it is bordered at both ends by warm water currents, namely the Agulhas Current in the South and the Angola Current in the North (Shannon 1985). The Benguela ecosystem is rich in pelagic and demersal fish populations and sustains high biological productivity (Boyer *et al*. 2000; Boyer and Hampton 2001). Due to the instability and unpredictable nature of the Benguela system, it attains low species diversity (Gibbons and Hutchings 1996).

The Benguela ecosystem is effectively divided into the northern Benguela and the southern Benguela upwelling regions by the Lüderidz perennial upwelling cell (26 º S -28 º S) (Shannon 1985). This upwelling cell is the most intense and extensive upwelling centre off Namibia due to the steep, narrow continental shelf and strong year round prevailing southerly winds (Boyer *et al*. 2000; Boyer and Hampton 2001). The North-westerly deflection of turbulent upwelled water off Lüderidz serves as a semi-permanent barrier to the north-south transport of the fish eggs and larvae of certain fish species such as anchovy (*Engraulis encrasicolis*) and sardine (*Sardinops sagax*) (Agenbag and Shannon 1988; Cury and Shannon 2004). Lett *et al*. (2007) have attributed surface hydrodynamics and subsurface temperatures as the main mechanisms which limit the

movement of ichthyoplankton between the southern and northern Benguela. Along the rest of the Namibian coast, upwelling is more or less perennial and intensity varies with local bathymetry, wind intensity, coastally trapped waves disseminating from the equatorial Atlantic and warm tropical water intrusions from the Angola Current (Boyer *et al*. 2000). Inherent to an upwelling event is the replacement of deflected surface water by deep, cold water rich in dissolved nutrients facilitating algal blooms (Nelson and Hutchings 1983; Shannon 1985).

The central Namibian shelf is covered by an anoxic/hypoxic diatomaceous mud belt formed from sunken phytoplankton and faecal pellets (Boyer *et al*. 2000; Bakun and Weeks 2004). Monteiro *et al*. (2006) have suggested that this anoxia can also be the result of physical forcing such as the balance in the influx of Cape Basin South Atlantic Central Water (CB-SACW) and Eastern Tropical South Atlantic Central Water (ETSA-SACW) to the central Benguela shelf. The system becomes more anoxic as the influx of tropical water increases and returns to relative hypoxia when the influx of colder, less saline CB-SACW increases (Monteiro *et al*. 2006). In addition the sediment attains high sulphate reduction rates with associated high sulphide concentrations (Schulz *et al*. 1999; Brüchert *et al*. 2003). The surface sediment off the central Namibian shelf contain dense populations of the large sulphur bacteria *Thiomargarita* and *Beggiatoa* which results in a close coupling of the sulphur and nitrogen cycles (Schulz *et al*. 1999). These bacteria oxidize hydrogen sulphide (H_2S) to elemental sulphur or sulphate and ammonium (NH^+_4) by using dissolved nitrate (NO^-_3) (Schulz *et al*. 1999). Consequently sulphur bacteria consume H_2S at the sediment-water interface and have been suggested to limit H_2S from

escaping the sediment into the overlaying water column (Brüchert *et al*. 2003; Dale *et al*. 2009). However H_2S and other poisonous, corrosive gasses such as methane can effervesce (Monteiro *et al*. 2006) and become oxidized, leading to the presence of elemental sulphur near the ocean surface (Bakun and Weeks 2004; Brüchert *et al*. 2009). The emission of these gasses together with anoxia frequently causes large scale mortality amongst certain marine animals while others display avoidance behaviour (Bakun and Weeks 2004; Monteiro *et al*. 2006). For example, in 1994 a severely hypoxic event caused the displacement of hake juveniles (*Merluccius capensis*) offshore subjecting them to predation by their cannibalistic adult counterparts (Hamukuaya *et al*. 1998).

The northern and southern Benguela are very dynamic systems and have undergone numerous changes over the last few decades (Heymans *et al*. 2004). These changes were induced and sustained by either bottom-up (environmental forcing) or top-down (fisheries, predation) control factors (Cury and Shannon 2004). In both systems the sardine was the dominant fish species between the 1950s and 1960s. During the 1970s and 1980s sardine catches off Namibia started to decline due to continuous overfishing (reviewed by Boyer and Hampton 2001; Cury and Shannon 2004). During this time, the fishing industry started targeting anchovy in an attempt to decrease competition and allow the recovery of the sardine populations (Butterworth 1980, 1983; Cury and Shannon 2004). In the northern Benguela the anchovy was exploited to a greater extent than in the southern Benguela. Due to these different fisheries management systems the sardine stocks in the southern Benguela had recovered by 1982/1983 while both the

sardine and anchovy fisheries crashed in the northern Benguela during the late 1970s and early 1980s (Cury and Shannon 2004).

In the northern Benguela, sardine was largely replaced by a range of other small fishes such as horse mackerel, (*Trachurus trachurus capensis*), bearded goby (*Sufflogobius bibarbatus*), and to a much less significant extent by anchovy (Cury and Shannon 2004). This change in species dominance brought about associated changes in the structure and function of the ecosystem. Sardine started feeding at higher trophic levels (Heymans *et al*. 2004), while top predators such as seals (*Arctocephalus pusillus pusillus*), Cape gannets (*Morus capensis*), Cape cormorants (*Phalacrocorax capensis*) and African penguins (*Spheniscus demersus*) started feeding at lower trophic levels (Heymans *et al*. 2004). Many predators which had once fed on sardines started feeding on gobies subsequent to the collapse in the sardine fishery (Crawford *et al*. 1985). During the 1980s and 1990s the mean trophic level of catches decreased off Namibia as a result of "fishing down the marine food web" (Pauly *et al*. 1998; Heymans *et al*. 2004).

In the period 1986-1991, sardine and hake biomass increased again in the northern Benguela. This trace of sardine recovery was very short lived, because 1995 brought with it a Benguela Niño event (Gammelsrød *et al*. 1998). Such Benguela Niño events had occurred previously in 1963 and 1984 mainly due to warm water intrusions from the Angola Current (Boyer *et al*. 2000). This warm water intrusion causes the Angola-Benguela front to shift southward, and a warming of the water column causes the thermocline to deepen so that warm, nutrient deficient water wells up during favourable

wind conditions (Boyd *et al*. 1987). These Benguela Niño events resulted in the large scale mortality of fish stocks, as well as of their predators, with associated declines in productivity (Gammelsrød *et al*. 1998). Some fish species such as hakes were also displaced offshore (Hamukuaya *et al*. 1998).

As opposed to the southern Benguela, the regime shift that has taken place in the northern Benguela brought about changes in the functioning of the whole system (Cury and Shannon 2004; Heymans *et al*. 2004). It could be said that a new state has been reached in the northern Benguela and that it is unlikely that this ecosystem will return to the way it was a few decades ago (Cury and Shannon 2004). Recently the effects of "fishing down the marine food web" or selective fishing and environmental perturbations have been accompanied by dramatic increases in jellyfish such as *Chrysaora fulgida* (Reynaud, 1830) and *Aequorea forskalea* (Forskål, 1775) (Pauly *et al*. 1998; Boyer and Hampton 2001; Lynam *et al*. 2006). Lynam *et al*. (2006) estimated the biomass of jellyfish in the Northern Benguela in 2003 to be in the order of 12.2 million tons; nearly four times that of the previously dominant fish stocks. These jellyfish have changed the transfer and flow of energy through the system (with most energy thought to be channelled from jellyfish directly to the detrital food web) and are thought to prey on fish larvae thereby limiting the recovery of pelagic fish stocks (Heymans *et al*. 2004; Lynam *et al*. 2006). Furthermore, large quantities of phytoplankton are being left unutilized due to low pelagic fish biomass, and as a result this decaying organic material promotes anoxia which may further limit sardine recruitment (Boyer *et al*. 2001; Bakun and Weeks 2004, 2006).

1.2 *Sufflogobius bibarbatus*

Sufflogobius bibarbatus (common name: “bearded goby” or “pelagic goby”) is a largely demersal fish species which was described for the first time from South Africa by von Bonde in 1923 as *Gobius bibarbatus*. This species was re-described by Smith 1956 as belonging to the genus *Sufflogobius*. The bearded goby is discontinuously distributed between Tiger Bay (16º 50'S Southern Angola) and St. Sebastian Bay (34º40'S, 21ºE SE coast of South Africa) (Cruickshank *et al*. 1980; Staby and Krakstad 2006). The goby is most abundant, and has its widest distribution between Hollams Bird Island and Möwe Point (Staby and Krakstad 2006) in the northern Benguela, and it can be found up to 85 km from the coast and up to depths of 350 m (Staby and Krakstad 2006). This distribution roughly corresponds to that of the diatomaceous mud belt off central Namibia (Staby and Krakstad 2006). Juvenile gobies generally occur inshore of the 200 m isobath and up to 30 km offshore (Cruickshank *et al*. 1980; Melo and Le Clus 2005), while their larger demersal counterparts are more abundant offshore (O’Toole 1978).

Off Namibia, the biomass of *Sufflogobius bibarbatus* has been estimated to be between 0.6 million tons based on surveys conducted during 1978-1979 (Hewitson and Cruickshank 1993) and up to 1.45 million tons based on ECOPATH modelling in the 1980s (Shannon and Jarre-Teichmann 1999). More recent surveys (1990-2005) have suggested that goby biomass is probably between 10000-100000 tons which is substantially less than previously suggested (Staby and Krakstad 2006).

The bearded goby is long-lived and can live up to six years and reach a size of 130 mm (Melo and Le Clus 2005). *Sufflogobius bibarbatus* reaches maturity at two to three years of age (Melo and Le Clus 2005), with males maturing later and attaining a larger body size at maturation compared to females (Melo and Le Clus 2005). Bearded gobies are typically serial inshore batch spawners (Melo and Le Clus 2005; O'Toole 1978). Spawning takes place during July-April (Melo and Le Clus 2005) where the male probably territorially guards the nest (Staby and Krakstad 2006).

The bearded goby is well adapted to its largely demersal habit as it is torpedo-cylindrically shaped, has a reduced lateral line system, enhanced vision and fused pelvic fins (Staby and Krakstad 2006). The bearded goby is unusual in that it is able to tolerate extremely low levels of oxygen and high levels of hydrogen sulphide characteristic of the inner shelf of central Namibia (Staby and Krakstad 2006; Utne-Palm 2008). The bearded goby is an important prey species for commercial fishes such as hake, kingklip (*Genypteus capensis*), monk (*Lophius vomerinus*) and horse mackerel, in addition to seals and seabirds (Crawford *et al*. 1985; Cury and Shannon 2004).

Sufflogobius bibarbatus is indigenous to the Benguela ecosystem and occurs in both the benthic and pelagic environment, commonly displaying diel vertical migration. Small gobies are more frequent diel vertical migrators compared to their larger counterparts (D'Arcangues 1977; Staby and Krakstad 2006; Hundt 2009). Recent studies have shown that gobies join the sound scattering layers which are primarily composed of the jellyfish *Aequorea forskalea* and *Chrysaora fulgida* (Utne-Palm *et al*. 2008). Catches of

Sufflogobius bibarbatus as obtained during the "Hake and Goby Cruise" conducted on board the RV *G.O. Sars* in 2008 were frequently associated with *A. forskalea* and *C. fulgida* (Utne-Palm *et al*. 2008). Juveniles of other fish species have been associated with scyphomedusae where they seek shelter between the oral arms under the umbrella of the jellyfish (Lynam and Brierley 2007; Masuda *et al*. 2008). This association between jellies and *S. bibarbatus* is favourable because the goby acquires shelter, protection and possibly food (either by direct feeding on the jellyfish or by feeding on zooplankton entangled in the tentacles of medusae, although neither jellyfish tissue nor nematocysts have been found amongst the gut contents of *S. bibarbatus*). Laboratory experimentation and observations have shown that *S. bibarbatus* is not affected by the nematocysts of *C. fulgida* and does not display any typical avoidance behaviour in the presence of this jellyfish species (Utne-Palm *et al*. 2008).

Studies of stomach content analysis of the bearded goby have produced conflicting results. Barber and Haedrich (1969) suggested that juvenile gobies feed primarily on diatoms (*Fragilaria*, *Coscinodiscus* and *Cheatoceros*) based on the gut contents of three juvenile gobies. Similarly Crawford *et al*. (1985) reported that goby stomachs contained numerically 93 % phytoplankton and approximately 7 % zooplankton based on a sample size of 10 gobies. O'Toole (1978) showed that larval, juvenile and adult gobies (a total of 210 fish were analyzed) feed largely on diatoms but fragments of copepods and euphausiids were also present. In contrast, D'Arcangues (1977) showed that adult and juvenile gobies feed primarily on invertebrates such as euphausiids (*Nyctiphanes capensis*), amphipods and copepods (*Calanoides carinatus* and *Rhincalanus*) (no

indication of sample size was provided). Macpherson and Roel (1987) highlighted that benthic polychaetes can numerically form an important part of the diet of the goby after analyzing approximately 120 gobies. Similarly Cedras (2009) showed that polychaetes and euphausiids are more important in the diet of large gobies but that copepods are numerically more important in their smaller counterparts. Hundt (2009) reported diatoms and benthic polychaetes as the dominant prey items inshore (120 m). However, offshore, the stomachs of gobies caught in the pelagic were dominated by pelagic zooplankton such as euphausiids and copepods but those caught on the benthos were dominated by benthic crustaceans and benthic polychaetes (Hundt 2009). The variable diet of the goby suggests that it is an opportunistic feeder (Cedras 2009). The above observations on the gut contents of *Sufflogobius bibarbatus* were largely limited by their small sample sizes but data collected by Hundt (2009) and Cedras (2009) were by far the most comprehensive with sample sizes of 549 and 3739 gobies respectively. Given that the species shows pronounced diel vertical migration (D'Arcangues 1977; Staby and Krakstad 2006) it is likely its diet changes throughout the day. In addition, small gobies are seen to migrate vertically more frequently than large gobies (D'Arcangues 1977) and therefore the diet may also vary ontogenetically (Cedras 2009).

1.3 Using gut content and stable isotope analysis to infer trophic structure

Traditionally, gut content analysis is used to make inferences on the diet and feeding of fish species. This method, however, has a number of limitations. Analysing gut contents gives only a snap-shot, proximate reflection of the diet and requires that gut contents be in a relatively identifiable and in an undigested state (James 1988). Using stable isotopes to elucidate diet and feeding is a favoured method especially in marine environments

because animals frequently change their diets, and gut contents are often completely digested or regurgitated on capture (Staniland *et al*. 2001). Although gut content analysis may identify a food source it is unable to provide any information on the fate and relative importance of that food source in the food web (Thomas and Cahoon 1993; Fry 2006). Stable isotope analyses have the potential to allow one to elucidate assimilated food, the origin of the food source (whether benthic or pelagic, marine or freshwater) and determine the relative importance of the specific food source within the food web (DeNiro and Epstein 1978; Thomas and Cahoon 1993; Machás and Santos 1999; Pitt *et al*. 2007a). Stable isotope ratios provide a temporally and spatially integrated view of trophic structure and diet (Peterson and Fry 1987; Minagawa and Wada 1984; Hobson *et al*.1995; Pitt *et al*. 2007a) and can be used to identify food sources that can be difficult to identify using gut content analyses (Pitt *et al*. 2009).

Stable isotope analyses of food web structure are based on the fact that the stable isotope ratios in the tissue of prey items are reflected in that of the consumers (DeNiro and Epstein 1978; Peterson and Fry 1987). Consumers differentially assimilate heavier isotopes and expel lighter isotopes (Peterson and Fry 1987) resulting in a predictable stepwise enrichment (fractionation) in heavy isotopes from prey to consumer. This fractionation is minimal for δ^{13} C and δ^{34}S but ranges between 3 ‰ and 4 ‰ per trophic level for δ^{15}N (DeNiro and Epstein 1978; Minagawa and Wada 1984; Peterson and Fry 1987; Machás and Santos 1999; Post 2002). δ^{13} C (ratio of heavy to light carbon isotopes relative to a standard) provides information on the primary source of organic matter sustaining a particular food web (Peterson and Fry 1987; Godley *et al*. 1998; Post 2002). Where a foodweb has more than one basal organic component, δ^{13}C can provide

information on the relative importance of these organic components in sustaining the foodweb. Seeing that $\delta^{15}N$ increases predictably with an increase in trophic level it can be used to determine the relative trophic level (Minagawa and Wada 1984; Peterson and Fry 1987; Machás and Santos 1999; Post 2002) of a particular individual. The use of $\delta^{34}S$ is based on the knowledge that seawater sulphates are much more enriched in heavy sulphur compared to sulphides in reduced benthic sediments (Fry *et al*. 1982). $\delta^{34}S$ can hence assist in making inferences to possible links to the detrital or benthic food web (Thomas and Cahoon 1993; Machás and Santos 1999).

1.4 Using fatty acids to infer trophic structure

In addition to stable isotopes, fatty acids can also be used as powerful trophic tracers because the structure changes in a predictable way when being transferred from prey to consumer (Pitt *et al.* 2009). Lipids lend buoyancy to marine animals and play a role in energy conservation (Hagen 2000). Lipids are important compounds for vertical migrants due to the fact that they have limited compressibility and thermal expansion (Hagen 2000). Hence the total lipid content of an animal may provide information pertaining to its biology and ecology (Sargent and Whittle 1981; Hopkins *et al*. 1993). Fatty acids (FA) are a group of lipids which are diverse in structure and similar to stable isotope ratios in that the fatty acid composition of prey items are reflected in the tissues of its predators (Dalsgaard *et al*. 2003). Fatty acids can be used as markers for specific organisms because some fatty acids are specific to certain taxa (Sargent *et al*. 1987; Nichols *et al*. 2003; Meziane *et al*. 2007). Lipid trophic markers provide a temporally integrated view of food web structure (Graeve *et al*. 2001), as opposed to traditional feeding studies using gut content analysis.

Using stable isotope ratios and fatty acid composition in combination provides a more rigorous assessment of prey species at the base of a food web (Dahl *et al*. 2003, El-Sabaawi *et al*. 2010). These two methods can be used to make up for each other's shortcomings. Kharlamenko *et al*. (2001) used $\delta^{13}C$, $\delta^{34}S$ and fatty acids to study food web structure in a *Zostera marina* community of the Sea of Japan. In the latter study FA analyses were conducted to help clarify the contribution of heterotrophic microorganisms to the food web which was difficult to do using isotopes alone. The joint application is widely applied in marine (e.g. Kharlamenko *et al*. 2001; Pond *et al*. 2008) and terrestrial environments (e.g. Ruess *et al*. 2005).

Using FA as a tool in trophic studies has proven to be helpful in marine, freshwater and estuarine environments. In marine environments, Rossi *et al*. (2006) used fatty acid markers to study trophic relationships between larval anchovy (*Engraulis encrasicolus*), zooplankton and phytoplankton in the NW Mediterranean. Phleger *et al*. (1998) studied Antarctic zooplankton and used fatty acid markers to distinguish between herbivorous and carnivorous diets in addition to shedding light on survival and reproductive strategies. Sargent and Falk-Petersen (1981) were able to show that calanoid copepods were more important in the diet of the krill, *Meganyctiphanes norvegica* while phytoplankton was more important in the diet of *Thysanoessa raschi* and *T. inermis* by applying lipid and fatty acid techniques in the fjords of Northern Norway. Similarly, FA have been used in trophic studies in both freshwater (Bychek and Guschina 2001; Hessen and Leu 2006) and estuarine environments (Napolitano *et al*. 1997; Richoux and Froneman 2008).

1.5 Stable isotope and fatty acid research in Southern Africa

Stable isotope trophic research in aquatic environments around southern Africa is largely untapped as yet, with research in estuarine systems far exceeding that in marine pelagic environments. Southern African researchers have, however, taken advantage of the usefulness of stable isotopes in other areas of research. Isotopes in bone collagen and tooth enamel have been used to infer modern and pre-historic mammalian diets and habitats in east and southern Africa (Ambrose and DeNiro 1986; van der Merwe *et al*. 1988; Lee-Thorp *et al*. 1989). Stable isotope analyses have also been used in forensics and conservation to determine the geographic origin of elephant ivory and rhinoceros horns (Vogel *et al*. 1990). Terrestrial plant researchers have used stable isotopes to study metabolic plant types (Schulze *et al*. 1996), nitrogen cycling (Aranibar *et al*. 2003), nitrogen fixation (Schulze *et al*. 1991) and to study the relationship between isotopes, land use and water availability (Aranibar *et al*. 2008).

In aquatic environments, many isotopic trophic studies have been conducted in estuaries in southern Africa. For example, Schlacher and Wooldridge (1996) studied the trophic importance of detritus in the diet of three species of macroinvertebrates in the Gamtoos estuary, Richoux and Froneman (2007) studied the spatial variation in carbon flow from producers to consumers in the Kariega estuary, while estuarine feeding experiments were done by Henninger *et al*. (2009) to establish the dietary importance of macrophytes in the diet of the isopod *Exosphaeroma hylocoetes*. In the marine environment, $\delta^{15}N$ has been used to study the effect of eutrophication and upwelling on the growth of *Gracilaria* (Anderson *et al*. 1999) and *Ulva* (Monteiro *et al*. 1997) in Saldanha Bay, while Hill and

McQuaid (2009) conducted baseline experimental work on food quality and its effects on tissue turnover rates in a marine mussel. Marine trophic studies include those by Kohler *et al*. (2009) on the African Black Oystercatcher (*Haematopus mosquini*), Jaquemet and McQuaid (2008) on the Cape gannet, Hill *et al*. (2006) and Hill *et al*. (2008) on the temporal and spatial importance of nearshore primary production for intertidal mussels while Sholto-Douglas *et al*. (1991) and Monteiro *et al*. (1991) used $\delta^{13}C$ with the use of trophic position isotope spectrum modelling to study the trophic ecology of pelagic fish in the southern Benguela.

Isotope work in southern Africa far exceeds that on the use of fatty acids. Fatty acids have been used in estuarine environments (Richoux and Froneman 2008) and marine environments (Arnould *et al*. 2005) to study trophic ecology.

1.6 Aims

The present study is aimed at using stable isotope ratios of carbon, nitrogen and sulphur in conjunction with fatty acid analyses to study the diet and trophic position of *Sufflogobius bibarbatus* in the northern Benguela upwelling ecosystem. Information obtained from the study will assist in answering questions concerning ontogenetic changes in the diet of the goby, benthic-pelagic coupling and the relationship between *S. bibarbatus* and the jellyfish *Aequorea forskalea* and *Chrysaora fulgida*.

CHAPTER 2: MATERIALS AND METHODS

2.1 Sampling area

All samples were collected off Walvis Bay (Namibia) between 31 March and 10 April 2008 at 3 stations along a transect (S 23º20 and E 14º12 to S 23º40 and E 13º15). An inshore station was sampled for 24 hours at 60 m depth (station C), whilst a midshelf (station A) and an offshore (station B) station were each sampled for 48 hours at 120 m and 180 m respectively (Fig. 1). Due to limited sampling at station C, emphasis here was on the data collected from stations A and B of which station A was located inshore to station B. Physical conditions on the seabed at station A were largely hypoxic, with a characteristic sulphide rich mud bottom (containing large sulphur bacteria, *Thiomargarita namibiensis* and *Beggiatoa spp*.) and oxygen levels dropping to below 1 ml DO/L at 62 m depth, and below 0.17 ml DO/L on the mud bottom (Fig. 2a). In contrast, station B was more oxygenated throughout the water column with oxygen levels dropping below 1 ml DO/L at 100 m depth but never dropping below 0.4 ml DO/L at the bottom (Fig. 2b). At both stations A and B, temperature showed a decrease from 18 ºC at the surface to 13 ºC at the bottom but the salinity remained more or less constant throughout the water column (Figs. 2a, b).

2.2 Field sampling

A pelagic trawl 16 m wide and 12 m high fitted with a multi-sampler was used to acquire stratified pelagic fish and jellyfish samples. Mesh sizes at the trawl-mouth were 68 mm, 55 mm and 31 mm but 20 mm, 10 mm, 4 mm and 3 mm at the multi-sampler cod-end. Bottom trawls (14.5 m wide and 4.5 m high) were used to collect demersal fish and jellyfish samples. One of the aims of the “Hake and goby cruise” was to determine the

length frequency distribution of the pelagic goby, hake and horse mackerel as well as the abundance of fish and jellyfish. As a result, as soon as a trawl came onboard, the catch was poured into buckets which were clearly marked with a net number, and each bucket was weighed. A sample of the catch was sorted to species from a random number of buckets, and all individuals were weighed (to the nearest 100 g) and measured (to the nearest mm: total length TL). In total, 54 trawls were performed of which 14 were demersal and 40 were pelagic. Up to 50 individuals of each fish and jellyfish species were measured from each trawl and their subsample weight was then used in order to obtain an estimate of the contribution by each to the total catch. For isotope and fatty acid analyses, fresh goby (*Sufflogobius bibarbatus*) and jellyfish (*Chrysaora fulgida* and *Aequorea forskalea*) covering a wide size range were hand picked from pelagic and bottom trawl nets. A piece of the oral arm of *C. fulgida* and a piece of the bell perimeter of *A. forskalea* were removed on collection. The gobies and jellyfish tissue were washed with filtered seawater and subsequently frozen at -40 ºC.

Zooplankton samples were collected using an oblique MOCNESS (mouth area= 1 m^2; mesh size= 180 µm) tow. All zooplankton that were analyzed here were collected from station B and no zooplankton were collected from station A. The zooplankton were washed with filtered seawater and bulk frozen. When possible large zooplankton were handpicked and frozen at -40 ºC. No phytoplankton or water samples were collected.

A mini Van Veen grab (ca. 2 L sample volume) was used to acquire benthic sediment and infauna. Grab samples were sorted onboard separating sediments and various animal taxa

Sediment samples were taken immediately and frozen at -40 ºC. Isolated infauna were washed with filtered seawater and frozen at -40 ºC. Infauna was only recorded from station B.

2.3 General laboratory analyses

The lateral muscle tissue of gobies was removed whilst frozen in the laboratory but gobies were not sexed. Bulk-frozen zooplankton were thawed and then sieved through mesh nets of sizes of 1000, 500, 250, 150 µm. These size classes consisted primarily of copepods and juvenile euphausiids (Table 2) and were analyzed separately. All samples including those of the sediment were freeze-dried prior to isotope and fatty acid analyses.

2.4 Stable isotope laboratory analysis

2.4.1 *Carbon and nitrogen isotopes*

Individuals vary in their lipid and isotopic content, with individuals with high lipid contents having lower ^{13}C than those with low lipid content (DeNiro and Epstein 1977). Therefore, the lipids were removed from fish, cnidarian and zooplankton tissue and mud to limit bias in $\delta^{13}C$ (Sholto-Douglas *et al*. 1991; McCuthan *et al*. 2003). In order to effect this, freeze-dried tissue was first homogenised using a pestle and mortar and transferred to Pyrex tubes. Twenty times the volume of dichloromethane: methanol 2:1 (v/v) was added to each pyrex tube so that a tissue to solvent ratio of 1:20 was established (Folch *et al*. 1957). This was subsequently homogenized with a vortex for 3 minutes and filtered through a Whatman #1 filter to remove particulate matter. The tissue residue was washed again with approximately 5 ml of dichloromethane and filtered for a second time. Inorganic carbon was removed from zooplankton and sediment samples by washing dried

samples with dilute HCl (2 N), subsequently washing with distilled water and re-drying at 35-40 ºC. Tissue samples were weighed into tin cups to an accuracy of 1 mg on a Sartorius microbalance. The tin cups were subsequently squashed to enclose the sample.

The samples were combusted in a Flash EA 1112 series elemental analyzer (Thermo Finnigan, Italy) at the light stable isotope laboratory based at the University of Cape Town, South Africa. The gasses were passed to a Delta Plus XP IRMS (isotope ratio mass spectrometer) (Thermo electron, Germany), via a Conflo III gas control unit (Thermo Finnigan, Germany). Merck gel (a proteinaceous gel produced by Merck) and crushed sealbone were used as in-house standards. Both in-house standards were calibrated against IAEA (International Atomic Energy Agency) standards. Nitrogen and carbon is expressed relative to atmospheric nitrogen (N_2) and Vienna Pee-Dee Belemnite (PDB) respectively.

2.4.2 *Sulphur isotopes*

Sulphur isotopic data were not analyzed by station due to the fact that a limited number of samples were available and even these had to be pooled extensively because of the large amount of dried material that was required to obtain a reliable sulphur isotopic signal. As a result the sulphur isotope signatures of a limited number of spatially pooled samples and their associated carbon and nitrogen signatures were determined at the University of Stockholm. Freeze-dried, ground, chemically untreated sediment, jellyfish, zooplankton and fish tissue was weighed in tin capsules and analyzed by conventional GC-IRMS on a Delta Plus isotope ratio mass spectrometer. For sulphur isotope analysis, samples were combusted with fivefold weight excess of vanadium pentoxide. Nitrogen

and carbon is expressed relative to N_2 and PDB respectively. Sulphur is expressed relative to Vienna Canyon Diablo Troilite, and iron sulphide (VCDT). All isotope ratios are reported as per mil values (‰) according to the formula:

$$\delta Z = (R \text{ sample} / R \text{ standard} - 1) * 1000 \qquad 1$$

where Delta (δ) Z denotes the standardized isotope signature ($\delta^{13}C$, $\delta^{15}N$ or $\delta^{34}S$), and R represents the ratio of the heavier to lighter isotope (^{13}C: ^{12}C; ^{15}N: ^{14}N or ^{34}S: ^{32}S) in the sample and standard respectively.

2.5 Fatty acid laboratory analysis

A minimum of 20 mg of ground, freeze-dried fish white muscle, cnidarian tissue, zooplankton and sediment was weighed and placed in glass centrifuge tubes containing 2 ml chloroform ($CHCl_3$). The centrifuge tubes were capped with nitrogen, sealed with Teflon tape and subsequently stored at -20 ºC until further analysis.

All samples and extraction solvents were kept on ice at all times throughout fatty acid analyses. All containers and utensils were washed three times with methanol (CH_3OH) followed by chloroform ($CHCl_3$) and left to dry in a fume hood.

The methods used for lipid extractions and transesterification follow Budge *et al.* (2006). Ice-cold CH_3OH (1 ml) and 1 ml $CHCl_3$:CH_3OH (v/v 2:1) was added to each centrifuge tube. Centrifuge tubes were capped, vortexed for 20 seconds, sonicated (1510 BRANSON) for 4 minutes in an ice bath and vortexed again for 10 seconds. Each tube was flushed with nitrogen, recapped, sealed with Teflon tape and stored at -20 ºC for 24 hours or at least overnight.

After 24 hours, the homogenate was filtered through a glass pipette fitted with cotton wool prewashed with $CHCl_3$:CH_3OH (v/v 2:1). The centrifuge tube was washed twice with 1 ml $CHCl_3$:CH_3OH (v/v 2:1), the contents filtered as described above and the filtrates pooled to make up a total filtrate volume of 6 ml. A quarter of the total volume (1.5 ml) 0.9 % KCl was added to the filtrate and a $CHCl_3$:CH_3OH:H_2O ratio of 8:4:3 maintained. KCl was used to assist in the removal of dissolved non-lipid contaminants. The solution was subsequently vortexed for 30 seconds and centrifuged for 4 minutes at 3000 rpm. A lipid bilayer was formed from which the upper aqueous phase was carefully removed and discarded using a glass pipette. KCl (0.5 ml 0.9 %) and 0.5 ml CH_3OH was added to the lower phase, which was again vortexed for 30 seconds and centrifuged for 4 minutes at 3000 rpm. After separation, the upper phase was removed and discarded. The lower phase was filtered through a glass pipette fitted with washed ($CHCl_3$:CH_3OH v/v 2:1) cotton wool bedded with anhydrous Na_2SO_4 to effectively remove remaining water. The final filtrate was evaporated to dryness under a gentle stream of N_2. Dichloromethane (1.5 ml) was added to the lipid pellet. The centrifuge tube was flushed with N_2, sealed with Teflon tape and stored at -20 ºC until further analysis.

Fatty acids were converted to methyl esters using acid transesterification after addition of 23:0 [Tricosanoic acid ≥ 98.5 % (GC) FLUKA] as an internal standard. Internal standard (0.1 ml) was added for samples with a DW less than 100 mg, 0.2 ml to samples with DW between 100-200 mg, 0.25 ml to samples with a DW between 200 – 300 mg, and 0.3 ml to samples with a DW more than 300 mg. The standard concentrations used ranged between 1.04-1.17 mg STD/ 1 ml dichloromethane. Hilditch reagent (3 ml) was added to

each tube using a glass pipette. The aforementioned reagent was made up by adding 1.5 ml of concentrated H_2SO_4 to 100 ml of anhydrous CH_3OH (methanol was dried using anhydrous Na_2SO_4 crystals). Each tube was flushed with N_2, recapped and sealed with Teflon tape. The samples were vortexed and placed in an oven at 100 ˚C for 1 hour. Here, acid transesterification was used to remove individual fatty acids from their glycerol chains using heat as a primary catalyst. After this cleaving process the tubes were left to cool to room temperature.

Hexane (3ml) and 1 ml of MilliQ water were added to each of the tubes. The samples were subsequently capped, vortexed and centrifuged for 5 minutes at 3000 rpm. A bilayer formed, of which the top layer was carefully removed and transferred to a second tube. The original tube was washed with 1 ml of hexane and centrifuged twice, each time adding the top layer to the second tube. Thereafter 2 ml of MilliQ water was added to the pooled top layers (second tube), which was then capped, vortexed and centrifuged for a further 2 min at 3000 rpm. Again, a bilayer formed, from which the top layer was removed and transferred to a third tube. To remove any water from this sample, anhydrous Na_2SO_4 was added (± 0.5 g), the sample was shaken gently and the salt allowed settling. The sample was then concentrated down to about 1 ml under N_2 and the solvent transferred to a clean 2 ml glass vial. The original tube was washed once with 1 ml hexane, which was also added to the 2 ml vial. The extract was then evaporated to dryness under N_2. When dry, 0.5 ml hexane was added to the fatty acid methyl esters (FAME), the vial was flushed with N_2, capped, vortexed and stored at -20 until further analysis.

FAME were injected into a gas chromatograph (5890 series II, Hewlett Packard) using a 1 µl syringe. The oven, injector and detector temperature were maintained at 150 ºC, 250 ºC and 260 ºC respectively. The column was cleaned intermittently by hexane injection. The temperature profile used involved an initial temperature of 150 ºC for 5 minutes. The temperature then increased to a final temperature of 225 ºC at a rate of 2.5 ºC per minute. All chromatogram detection and integration was done using ClarityLite 2.6.2 software.

2.6 Statistical data analyses

Homogenate prey sources with less than two replicate samples were excluded from statistical analyses. For both stable isotope and fatty acid analyses, gobies were categorised as small (< 57 mm), medium sized (58-90 mm) and large (> 90 mm) based on a visual inspection of a $\delta^{13}C$ and $\delta^{15}N$ vs total length plot of goby signatures which suggested that gobies most likely change their diet at these lengths (Figs. 5a, b). These size classes are not fully in agreement with those used by D'Arcangues (1977) who classified gobies into larvae (4-21 mm SL ~ 12-31 mm TL), juveniles (21-49 mm SL ~ 31-59 mm TL) and adults (50-83 mm SL ~ 60-93 mm TL) based on the depth ranges they exploit during the dial vertical migratory (DVM) cycle. The sample sizes of each group used for both stable isotope and fatty acid analyses are summarized in Table 1.

2.6.1 *Stable isotopes* ($\delta^{13}C$, $\delta^{15}N$)

Many authors have stressed that the isotope signals of autotrophs sustaining a system may vary depending on the source of nutrients entering the system, also known as a "shifting baseline" (Melville and Connolly 2003; Pitt *et al*. 2009). Such changes are then disseminated to higher trophic levels because the isotope signatures of prey items will be

reflected in that of their consumers. This can then result in both spatial and temporal variation in isotope signatures of consumers (Post 2002; Melville and Connolly 2003). The two stations sampled in this study showed differences in terms of oxygenation, bottom sediment conditions and animal life (see Utne-Palm *et al*. 2008; Utne-Palm *et al*. 2009), and therefore it was first necessary to establish whether or not fish collected at the two main stations (Station A and Station B) showed differences in their $\delta^{13}C$ and $\delta^{15}N$-signatures. Univariate analysis of variance (one-way ANOVA) was used to test for difference in $\delta^{13}C$ and $\delta^{15}N$-signatures between fish collected at station A and B. If significant differences were found, fish isotope data would need to be analyzed separately by station. If no such differences were found, isotope station data would be pooled and analyzed together.

To determine whether there were any ontogenetic changes in the diet of gobies and jellyfish their $\delta^{13}C$ and $\delta^{15}N$- signatures were related to animal size. One- way ANOVA together with linear regressions, was used to analyze differences in the $\delta^{13}C$ and $\delta^{15}N$-signatures of the different goby size fractions. The jellyfish data failed Levene's test for homogeneity of variances ($p>0.05$), and therefore non-parametric Spearmans rank correlations were used to test for differences in the $\delta^{13}C$ and $\delta^{15}N$-signatures with jellyfish size.

In order to establish which of the sampled organisms the gobies likely assimilated, "trophic shift boxes" in 2- dimensional $\delta^{13}C$ and $\delta^{15}N$ space were used. For this analysis, the ranges in isotope signatures expected for gobies deriving nutrition exclusively from

jellyfish or other zooplankton were calculated. For these calculations, it was assumed that there was no trophic-level fractionation for $\delta^{13}C$, but 3 ‰ (DeNiro and Epstein 1978; Minagawa and Wada 1984; Peterson and Fry 1987) was added to $\delta^{15}N$ for jellyfish and other zooplankton. If the goby isotope signatures fell within the range of a given potential prey item, it would mean that the goby had assimilated carbon obtained from that given prey item (Demopoulos *et al*. 2007).

The minimum and maximum percent contribution of potential prey items to the goby diet were determined by using a multisource mixing model (Isosource 1.3.1) (Philips and Gregg 2003). To apply this model, goby $\delta^{15}N$ were first corrected for fractionation by subtracting 3 ‰. Again, no significant fractionation was assumed for $\delta^{13}C$. Delta ^{13}C and corrected $\delta^{15}N$ values of the gobies were plotted and a five-sided polygon that best constrained the fish signals was drawn narrowly around the fish data. The apices of the polygon represent the average $\delta^{13}C$ and $\delta^{15}N$ values of prey sources that are available for goby consumption. *It should be noted that the isosource model is a reflection of only the limited number of prey sources collected during this study*. Prey source $\delta^{13}C$ and $\delta^{15}N$ which were most similar to that of the gobies were used to constrain the fish data, as collected from the two sampling stations. This was done due to ambiguity when trying to extrapolate prey sources from the fish signals alone, as many other authors have done (e.g. Philips and Gregg 2003). These source isotope values together with the measured $\delta^{13}C$ and $\delta^{15}N$ values of gobies were used in the Isosource 1.3.1 program to calculate proportional contributions of five potential food sources. Source increments were set at 1 % with a tolerance level of 0.05 %. As stated previously, instead of extrapolating prey

sources from apex values (unknown sources) as is usually done, we used measured (known) $\delta^{13}C$ and $\delta^{15}N$-signatures of prey sources as apices and for the most part tried to include all fish data within the model polygon (Figs. 3, 4).

For station A data (Fig. 3), apices were allocated for adult euphausiids (collected from station B), polychaetes (collected from station B), the jellyfish *A. forskalea* (collected from station A), and to two groups of jellyfish *C. fulgida* 1 (collected from station A) and *C. fulgida* 2 (collected from station A), of which one had lower $\delta^{13}C$ and $\delta^{15}N$- signatures relative to the other. Despite the fact that the polychaetes and adult euphausiids and gobies were collected at different stations, here none of the other prey items collected at station A or B were closer to the signatures of the fish collected from station A. Since these prey items were not collected at station A, their isotope signatures had to be inferred from polychaetes and adult euphausiids collected at station B. Therefore, polychaetes and adult euphausiids were considered valid for the station A model. For station B data (Fig. 4), apices were allocated for station-sampled adult euphausiids, shrimp and the jellyfish *A. forskalea, C. fulgida* 1 and *C. fulgida* 2.

Percentage source contributions at station A were found to be normally distributed while those at station B were not normally distributed when tested using Kolmogorov-Smirnov test at an alpha level of 0.05. Consequently, Pearson's Product Moment and Spearmans correlations were used to correlate fish size with prey source contributions at station A and B respectively (Zar 1999). In order to reduce the probability of making at least one type one error (that is, detecting a significant relationship when no such relationship

exists) amongst these repeated significance tests, alpha levels were adjusted using the sequential Bonferroni correction of Holm (1979).

2.6.2 *Sulphur isotopes*

Sulphur isotopic data were not analyzed by station due to sample size limitations. In order to determine the contribution of benthic and pelagic food sources to the diet of *Sufflogobius bibarbatus*, a two-end member linear mixing model was calculated (Levin and Michener 2002) using δ^{34}S-signatures. Due to the fact that mixed zooplankton and medusae had very similar δ^{34}S-signatures, they were grouped together and referred to as an "enriched δ^{34}S food source". Benthic sediment was referred to as a "reduced δ^{34}S food source" and could potentially include contributions from diatoms, bacteria and/or benthic meiofauna. Ultimately, the "enriched δ^{34}S food source" was used as one end member and the "reduced δ^{34}S food source" and porewater hydrogen sulphide (H_2S) as the second end member. A value of -15 ‰ was used for porewater dissolved H_2S, based on previous measurements at a shelf station at 120 m water depth and 2-3 cm sediment depth (Brüchert, University of Stockholm, unpublished data).

2.6.3 *Fatty acids*

The quantitative and qualitative fatty acid content of the samples was determined. Quantitative FA content reflects the FA content of a particular sample in micrograms per milligram of sample dry weight (µg/mg DW) relative to a standard (23:0). Qualitative FA content essentially reflects the FA content as a % (percentage) relative to the total fatty acids in a particular sample and is useful in cases where samples vary greatly in their total fatty acid (TFA) contents.

All fatty acid data were divided into two sections which were analyzed separately: individual fatty acids and summary fatty acids. While individual fatty acids represent a single fatty acid variable, summary fatty acids are combinations of the individual fatty acids (Viso and Marty 1993), represented as sums or ratios. It is often easier and more useful to use summary fatty acids as markers for specific organisms (Volkman *et al*. 1989). For example, 22:6ω3 is sometimes used as a dinoflagellate marker (Richoux and Froneman 2008), although it may also occur in diatoms (Volkman *et al*. 1989, Viso and Marty 1993; Budge and Parrish 1998). Diatoms produce more 20:5ω3 than 22:6ω3, while the inverse is true for dinoflagellates (Volkman *et al*. 1989; Viso and Marty 1993). Therefore it is more useful to use 22:6ω3/20:5ω3 as an index of the relative contribution of either dinoflagellates or diatoms (Volkman *et al*. 1989; Viso and Marty 1993; Litz *et al*. 2010).

Fatty acids 18:1ω9 and 18:4ω3 are often characteristic of dinoflagellates and prymnesiophytes (Ackman *et al*. 1968, Volkman *et al*. 1981, Napolitano *et al*. 1997; Dalsgaard *et al*. 2003). The ratio of 18:1ω9/18:1ω7 has been used to reflect different levels of carnivory (Sargent and Falk-Petersen 1981; Graeve *et al*. 1997). If this ratio is high it indicates a high degree of carnivory, whereas low ratios indicate increased relative herbivory (Sargent and Falk-Petersen 1981; Graeve *et al*. 1997). Fukunda and Naganuma (2001) used the ratio of polyunsaturated ω3/ω6 as an indication of contributions from the herbivorous food web relative to that from the detrital food web. FAs 22:1ω11, 22:1ω9, 20:1ω11 and 20:1ω9 are known to be produced *de novo* by calanoid copepods (Sargent and Falk-Petersen 1988, Graeve *et al*. 1997, Falk-Petersen *et al*. 2002, Budge *et al*. 2006,

Kattner *et al*. 2007). Using the sum of these long chain monounsaturates can be useful as an indication of calanoid copepod consumption. Odd numbered and branched chain fatty acids are characteristic in bacteria (Sargent *et al*. 1987; Fukunda and Naganuma 2001), and the sum of these BAFA (bacterial fatty acids) can be used as a gross indication of dietary bacterial contributions in consumers. In contrast, authors such as Jacq *et al* (1989), McCaffrey *et al* (1989) and Zhang *et al* (2005) have shown that iso-anteiso-fatty acids which are characteristic of sulphate-reducing bacteria were minor components in sulphur-oxidizing bacteria such as *Thioploca* and *Beggiatoa* and that these bacteria were dominated by FA such as 16:1ω7, 18:1ω9, 16:0 and 18:0. Furthermore they suggested that 16:1ω7 and 18:1ω9 can be used as markers for sulphur-oxidizing bacteria in marine sediments rich in H_2S.

The first aim was to establish whether gobies show any changes in their fatty acid composition with changing body size. Quantitative fish data were normalized using mixed transformations and subsequently analyzed using a Principal Component Analysis (PCA), using PAST (Hammer *et al*. 2001). These mixed transformations involved log transforming all the data. Fatty acid variables that failed Levene's test for homogeneity of variances after log x+1 transformation were subsequently square root transformed or normalised using reciprocal transformation. The only variable that required reciprocal transformation was 19:0. Variables that occurred infrequently (15:0, ai-16:0, 18:2ω4, 17:1, 18:3ω6 and 24:1) were removed to limit noise. The aim of PCA is to reduce large multivariate data sets to a smaller number of unitless components which are linear

combinations of the original variables that account for as much of the variance in the original multidimensional data set as possible (McCune and Grace 2002).

Qualitative fish data (% data) failed Levene's test for homogeneity of variances ($p>0.05$) after being log x+1 transformed and they were therefore explored using non-metric multidimensional scaling (NMDS) in PRIMER v 6 (Clarke and Warwick 2001). NMDS was used as it makes the least number of assumptions about the data and does not assume multivariate normality (McCune and Grace 2002). NMDS analyzes the similarity or distance between samples and then ranks these in a similarity matrix based on Euclidean distance. It then constructs a configuration of these rank (dis)similarities in low dimensional space (Clarke and Green 1988). Hence the closer groups are to each other on the ordination plot, the higher the similarity or the smaller the dissimilarity is between them. The disparity or distortion between the similarity between samples in high dimensional space and their rank similarities in low dimensional space is termed "stress" (McCune and Grace 2002). Low stress values (< 0.2) are favourable if one is to avoid making misleading interpretations relating to ordination results (Clarke and Green 1988; Clarke and Warwick 2001).

Analysis of Similarity (ANOSIM) in PRIMER v 6 was used to test the null hypothesis that small, medium, sized and large gobies did not differ significantly from each other in terms of their fatty acid proportions. This method is based on the same similarity matrix (Euclidean distance matrix) underlying NMDS and operates by computing a Global R test statistic to reflect the observed differences between sample groups. The closer the

Global R is to unity, the greater the difference between groups. Groups were regarded as being significantly different from each other if $p < 0.05$ and R was relatively large (Clarke and Green 1988; Clarke and Warwick 2001). These two test statistics were always investigated together because although R is not inappropriately affected by the number of replicates within a sample group, its statistical significance, p, is (Clarke and Warwick 2001).

One-way Analysis of Variance (ANOVA) in SigmaStat was used to test if small, medium sized and large gobies were significantly different in their absolute and proportional concentrations of a selected number of key FA. In order to reduce the probability of making at least one type one error (that is, detecting a significant relationship when no such relationship exists) amongst these repeated significance tests, alpha levels were adjusted using Bonferroni corrections (Quinn and Keough 2002). Statistical significance was conducted at the 95 % level after Bonferroni corrections.

All the prey data (qualitative and quantitative) failed Levene's test for homogeneity of variances ($p>0.05$) after being log x+1 transformed, primarily due to small and unequal sample sizes. Consequently NMDS in PRIMER v 6 was used to explore the relationships among prey items (taxa or size classes of taxa) and to relate gobies to their potential prey (Clarke and Warwick 2001). ANOSIM in PRIMER v 6 was used to test the null hypothesis that the prey items of the goby did not differ significantly from each other, or the goby, in terms of their proportional and absolute fatty acid contents.

Due to incomplete sampling, only fatty acid trends at station B are explored here.

CHAPTER 3: RESULTS

3.1 Stable isotopes

3.1.1 Station and ontogenetic changes in isotope ratios

3.1.1.1 *Gobies*

The first aim was to determine whether there were any differences in the $\delta^{13}C$ and $\delta^{15}N$ signatures between the two stations. Results obtained from ANOVA suggested that there was a significant difference between small gobies (< 57 mm TL) collected at stations A and B in terms of $\delta^{13}C$ ($F= 7.05$, $p< 0.05$, station A: -17.09 ± SE 0.2 ‰, n= 8, station B: -16.58 ± SE 0.08 ‰, n= 41) and $\delta^{15}N$ ($F= 72.80$, $p< 0.05$, station A: mean= 12.75 ± SE 0.32 ‰, n= 8, station B: mean= 11.24 ± SE 0.05 ‰, n= 41). Medium sized gobies (58 mm- 90 mm TL) were significantly different in their $\delta^{13}C$ ($F= 6.26$, $p< 0.05$, station A: -16.24 ± SE 0.05 ‰, n= 33, station B: -16.06 ± SE 0.06 ‰, n= 21) but not in their $\delta^{15}N$ when comparing the two stations. Station related differences between large gobies could not be analyzed as no large gobies were collected from station A. These station-related differences prompted the separate analysis of station A and station B *fish* data.

Linear regressions showed a weak positive relationship between goby size and $\delta^{13}C$ ($R^2= 0.35$, $p< 0.05$) (Fig. 5a) and a weak negative relationship with $\delta^{15}N$ at station A ($R^2= 0.19$, $p< 0.05$) (Fig. 5b). By contrast, a strong positive relationship was seen between goby size and $\delta^{13}C$ ($R^2= 0.57$, $p< 0.05$) and $\delta^{15}N$ ($R^2= 0.79$, $p< 0.05$) at station B (Figs. 5a, b). Results of the ANOVA suggest that small gobies at station A were significantly different from medium sized gobies at station A in terms of their $\delta^{13}C$ ($F= 19.15$, $p< 0.05$,

n= 8 and n= 33 respectively) and $\delta^{15}N$- signatures (F= 13.66, p< 0.05, n= 8 and n= 33 respectively). Similarly small, medium and large gobies at station B were significantly different from each other in terms of their $\delta^{13}C$ (F= 37.55, p< 0.05, n= 41, n= 21 and n= 15 respectively) and $\delta^{15}N$- signatures (F= 255, p< 0.05, n= 41, n= 21 and n= 15 respectively).

Small gobies collected at station B fed at the same average trophic level ($\delta^{15}N$= 11.24 ± SE 0.05; n= 41) as medium sized gobies ($\delta^{15}N$= 11.82 ± SE 0.11; n= 21), but small and medium sized gobies fed at a lower trophic level compared to large gobies ($\delta^{15}N$= 14.12 ± SE 0.14; n= 15) (Fig. 5b). At the inshore station, small gobies were more enriched in $\delta^{15}N$ ($\delta^{15}N$= 12.75 ± SE 0.32; n= 8) compared to medium size gobies ($\delta^{15}N$= 11.58 ± SE 0.08; n= 34), but these two goby size fractions still fed at approximately the same average trophic level (Fig. 5b). Delta ^{15}N-signatures clearly support an ontogenetic change in goby diet at station B.

Furthermore $\delta^{13}C$-values also support an ontogenetic change in the diet of the goby as there was a significant difference in average $\delta^{13}C$ of small ($\delta^{13}C$= -17.09 ± SE 0.20; n= 8) and medium sized gobies ($\delta^{13}C$= -16.25 ± SE 0.04; n= 33) (F= 19.15, p< 0.05) at station A, as well as the average of small ($\delta^{13}C$= -16.58 ± SE 0.08; n= 41), medium sized ($\delta^{13}C$= -16.06 ± SE 0.06; n= 21) and large gobies ($\delta^{13}C$= -15.47 ± SE 0.12; n= 15) (F= 37.55, p< 0.05) at station B (Fig. 5a). Gobies became more enriched in the heavier carbon isotope as they increased in size at both station A and station B, although very large gobies were absent from station A (Fig. 5a). Medium sized gobies fed at the same trophic level at both

station A and B based on $\delta^{13}C$ (mean -16.06 to -16.25 ‰) and $\delta^{15}N$ (mean 11.58 to 11.82 ‰) (Figs. 5a, b). When plotting $\delta^{13}C$ against $\delta^{15}N$ (Fig. 5c), a shift in diet was again visible, although not as clearly as in Figs. 5a and b. According to Fig. 5c, small gobies at station A were enriched in $\delta^{15}N$ but still fed at the same average trophic level as medium sized gobies (Fig. 5c). Large gobies collected offshore fed at a higher trophic level compared to their smaller counterparts (Fig. 5c).

3.1.1.2 *Jellyfish*

To see if there was an ontogenetic shift in the diet of the two jellyfish species, $\delta^{13}C$ and $\delta^{15}N$-signatures were related to the bell diameter of *Aequorea forskalea* and *Chrysaora fulgida* (Figs. 6, 7). Based on Spearmans rank correlations, no significant correlation could be seen between the body size of *A. forskalea* and its $\delta^{13}C$- signatures at station A ($R= -0.393$, $p= 0.232$, $t= 1.28$, $n= 11$) and body size only explained 39.3 % of the variance. By contrast, *A. forskalea* at station B showed a significant increase in heavy carbon with increasing body size ($R= 0.829$, $p< 0.05$, $t= 4.452$, $n= 11$). Large *Aequorea forskalea* (>190 mm) were found only offshore (station B) and were more enriched ($\delta^{13}C= -13.36 \pm$ SE 0.15; $n= 3$) in heavy carbon compared to their smaller counterparts ($\delta^{13}C= -15.25 \pm$ SE 0.24; $n= 8$) (Fig. 6a). Spearman's rank correlations suggested that *A. forskalea* becomes more enriched in $\delta^{15}N$ at station A ($R= 0.663$, $p< 0.05$, $t= 2.65$, $n=11$) but more depleted in $\delta^{15}N$ at station B ($R= -0.617$, $p< 0.05$, $t= 2.35$, $n=11$) when these signatures were correlated with body size (Fig. 6b).

Chrysaora fulgida showed highly variable $\delta^{13}C$ values with no actual differences between sizes or between stations (Fig. 7a) indicating individual feeding habits or an opportunistic

diet irrespective of habitat. No correlation (Spearmans rank) could be seen between body size and $\delta^{13}C$ at station A (R= -0.163, p= 0.44, t= 0.791, n= 25) or at station B (R= -0.517, p= 0.103, t= 1.18, n= 25). The $\delta^{15}N$ signatures of *C. fulgida* also indicated that all sizes of this species fed at the same trophic level irrespective of whether they were found inshore or offshore (Fig. 7b). No correlation was seen between body size and $\delta^{15}N$ at station A (R= 0.289, p= 0.16, t= 1.45, n= 25) or at station B (R= 0.12, p= 0.725, t= 0.36, n= 25). Whereas *Aequorea forskalea* exhibited $\delta^{15}N$ values between 8.8-13.8 ‰, the co-occurring *C. fulgida* had $\delta^{15}N$ values between 7.3-10.9 ‰, suggesting that *A. forskalea* is capable of feeding on a broader range of prey and trophic levels (Figs. 6b, 7b).

3.1.1.3 *Benthic sediments*

Sediment at station A was primarily composed of diatomaceous mud of small particle size (Utne-Palm *et al*. 2008). Numerous fish scales, bones and calcareous shells of pteropods were present. Sediments contained no macrofauna and even foraminifera and radiolarians were scarce (Utne-Palm *et al*. 2008). The sediment at station B was primarily composed of organic mud containing bivalves, gastropod shells, polychaetes and foraminifera (see Utne-Palm *et al*. 2008 for more details).

No clear pattern can be seen when looking at the $\delta^{13}C$ and $\delta^{15}N$ signatures of the benthic sediments collected at stations A compared to that collected at station B, perhaps in part because of the very small sample sizes. The five homogenized sediment samples analyzed from station A showed variable isotope signatures: mean sediment $\delta^{13}C$ was -19.20 ‰ while $\delta^{15}N$ was 6.32 ‰. Only two homogenised samples were analyzed from

station B and they were remarkably similar for both isotopes: mean sediment $\delta^{13}C$ was -19.04 ‰ while $\delta^{15}N$ was 5.90 ‰ at station B (Fig. A.1).

3.1.2 Isotope ratios of the potential prey and gobies

Potential zooplankton prey items were collected only from station B, while jellyfish and mud were collected from both sampling stations. Zooplankton were not identified to species level, but mixed zooplankton samples consisted primarily of copepods and juvenile euphausiids with some chaetognaths, bivalve and gastropod larvae and cumaceans (Table 2).

3.1.2.1 *Jellyfish*

At station A, *Chrysaora fulgida* and *Aequorea forskalea* fed at the same average trophic level but lower in the food chain than small gobies. Medium sized gobies fed at a higher trophic level compared to *C. fulgida* which was generally more depleted in $\delta^{13}C$ compared to *A. forskalea* (Fig. 8a). Both of these jellyfish species were more enriched in carbon compared to small and medium sized gobies (Fig. 8a).

At station B, small and medium gobies were similar to *C. fulgida* in their $\delta^{13}C$-signatures while the enriched $\delta^{13}C$-signature of *A. forskalea* indicates a more carnivorous diet (Fig. 8b). Based on $\delta^{15}N$-signatures, *A. forskalea* fed at the same average trophic level as small and medium sized gobies but lower than large gobies and higher than *C. fulgida* (Fig. 8b).

Spatially pooled stable sulphur isotope signatures indicate that *Aequorea forskalea* ($\delta^{34}S$ = 21.44 ± SE 0.21; n= 3) and *Chrysaora fulgida* ($\delta^{34}S$ = 21.50 ± SE 0.12; n= 7) fed at the

same level in the foodweb but were more enriched in heavy sulphur when compared to gobies (Fig. 9).

3.1.2.2 *Zooplankton and sediment*

Shrimp ($\delta^{13}C$= -15.79 ± SE 0.15; n= 2), benthic polychaetes ($\delta^{13}C$= -18.40 ± SE 0.67; n= 3), mud ($\delta^{13}C$= -19.04 ± SE 0.02; n= 2) and adult euphausiids ($\delta^{13}C$= -19.25 ± SE 0.31; n= 6) gave carbon isotope signals closest to that of the goby at station B, while the other zooplankton had much lighter carbon isotope signals (Fig. 8b). Pteropods (exclusively *Cymbulia*) ($\delta^{13}C$= -19.87 ± SE 0.34; n= 4) and their shells ($\delta^{13}C$= -19.69 ± SE 0.14; n= 5) showed no clear difference in their $\delta^{13}C$-signatures (Fig. 8b). The $\delta^{13}C$ signature of adult euphausiids was similar to that of benthic polychaetes and pteropods, ranging between -20 and -18 ‰ (Fig. 8b). In addition no clear differences were seen between the $\delta^{13}C$-signatures of benthic polychaetes and mud collected at station B. Mixed crustacean zooplankton showed the lowest $\delta^{13}C$-signatures, with those sized 200 µm ($\delta^{13}C$= -22.60 ± SE 0.45; n= 2) being more enriched with heavier carbon compared to those sized 1000 and 500 µm respectively (Fig. 8b).

Pteropod shells were the least enriched in $\delta^{15}N$ followed by mud, pteropods and mixed zooplankton, adult euphausiids, polychaetes and shrimp (Fig. 8b). Based on $\delta^{15}N$, pteropods generally fed at the same trophic level as mixed zooplankton (Fig. 8b) but at a lower trophic level compared to small gobies, medium sized gobies, benthic polychaetes and shrimp which all fed at the same average trophic level (Fig. 8b). Large gobies fed the highest in the foodweb at station B (Fig. 8b). Zooplankton collected at station B which were collected in small sample sizes are presented in Fig A.2.

Spatially pooled benthic sediment gave the lowest $\delta^{34}S$- signature ($\delta^{34}S$= 5.75 ± SE 0.99; n= 3) when compared to that of gobies ($\delta^{34}S$= 16.45 ± SE 0.41; n= 7) and mixed zooplankton ($\delta^{34}S$= 19.78 ± SE 0.64; n= 8) (Fig. 9).

3.1.3 Diet of gobies inferred from isotope ratios

3.1.3.1 *"Trophic shift boxes using $\delta^{13}C$ and $\delta^{15}N$"*

Gobies collected at station A appeared to feed almost entirely on *Chrysaora fulgida* with only one individual falling outside of the *C. fulgida* isotope signature range (Fig. 10a) although it should be recognized that no other prey items were collected at station A. Gobies collected at stations A and B did not appear to feed on mud, as their $\delta^{13}C$ and $\delta^{15}N$ signatures were well outside the range of the diatomaceous mud (also known to contain large sulphur bacteria and diatoms) (Figs. 10a, b). Gobies collected at station B fed both on *Aequorea forskalea* and *C. fulgida* although it would appear as though the very large gobies fed almost exclusively on *A. forskalea* (Fig. 10b). These large gobies also appear to supplement their diet with shrimp (Fig. 10b).

The trophic shift box plot looking at potential prey items collected from station B related to gobies from station A and station B showed that most gobies collected at station A fed almost entirely on *Chrysaora fulgida* except for some small individuals (35 mm-52 mm TL) that could be supplementing their diet with polychaetes (Fig. 10c). Gobies collected at station B, fed on both *C. fulgida* and *Aequorea forskalea*. Large gobies (> 90 mm TL) collected at station B fed almost exclusively on *A. forskalea* (Figs. 10b, c). Some small gobies (37 mm- 55 mm TL) collected at station B were outside the isotopic range of *C. fulgida* and could possibly be supplementing their jellyfish diet with adult euphausiids or

polychaetes (Figs. 10c). Based on the "trophic boxes", no other potential prey items collected at station B appear to have been assimilated by the gobies collected at either station (Fig. 10). *It should be stressed that the above interpretations are limited by the number of collected prey sources during this study and should be treated as preliminary.*

3.1.3.2 *Isosource model using $\delta^{13}C$ and $\delta^{15}N$*

A five source mixing model (Philips and Gregg 2003; Demopoulos *et al*. 2007) based on stable C and N signatures was used to establish the minimum and maximum contribution of various prey sources to the diet of differently sized *Sufflogobius bibarbatus* at station A and B (Figs. 11, 12). *It should be noted that the isosource model used here is a reflection of only the limited number of prey sources collected during this study*. The maximum source contribution of polychaetes decreased with increasing fish size, while *Chrysaora fulgida* 1 and *C. fulgida* 2 were positively correlated with fish size at station A (Fig. 11). Maximum contributions of shrimp and *Aequorea forskalea* increased with increasing fish size, while the maximum contribution of *C. fulgida* 2 was negatively correlated with fish size at station B (Fig. 12).

Polychaete consumption was significantly correlated with fish size at station A ($R= 0.39$, $p< 0.05$ after Bonferroni, $n= 42$) (Fig. 11b). Polychaetes could potentially contribute up to an average of 47.63 % to the diet of small gobies at station A (Table 3). *Chrysaora fulgida* with elevated $\delta^{13}C$ and $\delta^{15}N$-signatures ($R= 0.59$, $p< 0.05$, $n= 42$) and *C. fulgida* with lighter $\delta^{13}C$ and $\delta^{15}N$-signatures ($R= 0.47$, $p< 0.05$, $n= 42$) were significantly correlated with fish size at station A, with maximum contributions increasing with an increase in fish size (Figs. 11d, e). The average source contributions by *C. fulgida* 1 and

C. fulgida 2 at station A reached up to 50.09 % and 69.91 % in larger fish respectively (Table 3).

At station B, the maximum contributions of shrimp (R= 0.86, p< 0.05, n= 76) and *Aequorea forskalea* (R= 0.91, p< 0.05, n= 76) were significantly correlated with fish size with source contributions increasing with an increase in fish size (Fig. 12b, c). Average source contributions from these two prey items reached maxima of 58.64 % and 70.36 % respectively in large gobies (Table 4). *Chrysaora fulgida* with lighter $\delta^{13}C$ and $\delta^{15}N$-signatures was significantly correlated with fish size (R= -0.82, p< 0.05, n= 76) with source contributions decreasing with an increase in fish size (Fig. 12e) and potentially reaching an average maximum contribution of 73.85 % in small fish (Table 4).

3.1.3.3 *Sulphur isotopes*

The depleted $\delta^{34}S$-signature of goby tissue ($\delta^{34}S$= 16.5 ± SE 0.4; n= 7) compared to that of mixed zooplankton ($\delta^{34}S$= 19.8 ± SE 0.6; n= 8) and jellyfish ($\delta^{34}S$= 21.45 ± SE 0.1; n= 8) indicates that gobies derived at least part of their diet from the depleted sulphidic benthos ($\delta^{34}S$= 5.7 ± SE 1; n= 3) (Fig. 9).

Based on the $\delta^{34}S$-signatures of seven gobies, the contribution of the benthic sediment (which may include diatoms, bacteria and/or benthic fauna) to the diet of the goby may reach up to 34.2 ± SD 6.9 % (Table 5, Fig. 13). Hydrogen sulphide dissolved in sediment porewater can make dietary contributions of up to 15 ± SD 2.95 % (Fig. 13; Table 5). Furthermore, "enriched $\delta^{34}S$ food sources" (which include medusae and/or mixed zooplankton) are able to make up 65.8 ± SD 6.72 % and 85 ± SD 6.9 % of the diet of the

goby when using benthic sediment (including porewater sulphates, elemental sulphur, organic sulphides and pyrite) and porewater dissolved sulphide as end members respectively (Table 5).

3.2 Fatty acids

A total of 42 fatty acids (FA) were identified in the tissue of fish and their potential prey items. *Fatty acid analyses were limited to station B (180 m) due to limited sample collection at station A.* All raw qualitative (Table A.1, A.2, A.3, A.4) and quantitative (Table A.5, A.6, A.7, A.8) individual and summary FA are presented as appendices.

3.2.1 Ontogenetic changes in the fatty acid composition of *Sufflogobius bibarbatus*

The FA composition of small gobies was dominated by 16:0 (21.5 % TFA) followed by 22:6ω3 (20.3% TFA), 20:5ω3 (11.5 % TFA), 18:0 (7.3 % TFA), 18:1ω9 (6.9 % TFA), 18:1ω7 (4.4 % TFA), 14:0 (4.3 % TFA) and 16:1ω7 (4.0 % TFA) (Table A.1). All of these were present at concentrations ranging between 7.29 and 1.35 µg/mg DW (Table A.5). The FA composition of medium sized gobies was dominated by 22:6ω3 (21.2 % TFA), followed by 16:0 (20.1 % TFA), 20:5ω3 (12.6 % TFA), 18:0 (8.0 % TFA), 18:1ω9 (5.8 % TFA), 18:1ω7 (3.6 % TFA), 16:1ω7 (3.5 % TFA) and 14:0 (3.2 % TFA) (Table A.1). All of these were present at concentrations ranging between 5.03 and 0.8 µg/mg DW (Table A.5). The dominant fatty acid in the tissues of large gobies was 22:6ω3 (19.9 % TFA), followed by 16:0 (18.4 % TFA), 20:5ω3 (14.2 % TFA), 18:0 (8.3 % TFA), 18:1ω9 (4.9 % TFA), 20:4ω6 (4.4 % TFA), 16:1ω7 (4.3 % TFA), 22:5ω3 (4.1 % TFA), 18:1ω7 (3.6 % TFA) and 14:0 (3.1 % TFA) (Table A.1). And these were present at concentrations ranging between 3.87 and 0.68 µg/mg DW (Table A.5).

NMDS based on the qualitative individual fatty acid content of fish suggests that the FA profiles of small gobies were different from that of large gobies, but those of medium sized gobies showed overlap with both small and large gobies (Fig. 14). The ANOSIM suggests that small gobies were significantly different from medium sized ($p < 0.05$, pairwise $R = 0.07$) and large gobies ($p < 0.05$, pairwise $R = 0.51$) based on their proportional FA composition. Medium sized gobies were significantly different from large gobies ($p < 0.05$, pairwise $R = 0.122$). The proportional FA composition of small, medium sized and large gobies were still fairly similar to each other as the global R statistic was only 0.119.

The results of the PCA based on the quantitative individual fatty acid content of gobies suggest that the saturated fatty acids (SFA) 14:0, 16:0 and 18:0, the monounsaturated FA (MUFA) 16:1ω7, 18:1ω7, 18:1ω9 and 20:1ω9, and the polyunsaturated fatty acids (PUFA) 18:2ω6, 20:5ω3 and 22:6ω3 were most responsible for the similarity amongst small gobies (Fig. 15a). Medium sized gobies showed a lot of overlap with small and large gobies in terms of their FA content (Fig. 15a). The PUFA 16:2ω4, 16:3ω3 16:3ω4 and 20:4ω6 were most responsible for similarities amongst large gobies (Fig. 15a). PC 1 (47.09 %) and PC 2 (10.24 %) jointly accounted for 57.3 % of the variation in the dataset (Fig. 15a). The PCA of FA marker ratios/summaries indicates that PC 1 (55.84 %) and PC 2 (20 %) jointly accounted for 75.8 % of the variation in the dataset (Fig. 15b). TFA, SFA, MUFA, PUFA, EFA, BAFA, sum ω3, ω3/ω6, the dinoflagellate marker 22:6ω3/20:5ω3 and the copepod markers sum 22:1 and sum 22:1+20:1 were highest amongst small gobies (Table A.7), and were most responsible for the difference in

similarity between small and large gobies (Fig. 15b). Medium sized gobies showed a lot of overlap with small and large gobies (Fig. 15b). The diatom markers 16:1/16:0 and 20:5ω3/22:6ω3 were elevated in the tissues of large gobies (Table A.7), and were most responsible for the similarity amoungst large gobies (Fig. 15b).

Small gobies were significantly higher in their absolute concentration of the copepod marker 20:1ω9 compared to medium sized ($F= 17.65$, $p< 0.05$ after Bonferroni, $n= 24$ and $n= 20$ respectively) and large gobies ($F= 17.65$, $p< 0.05$ after Bonferroni, $n= 24$ and $n= 20$ respectively) (Fig. 16). Small gobies contained significantly higher concentrations of the dinoflagellate fatty acid 22:6ω3 compared to medium sized ($F= 30.65$, $p< 0.05$ after Bonferroni, $n= 24$ and $n=20$ respectively) and large gobies ($F= 30.65$, $p< 0.05$ after Bonferroni, $n= 24$ and $n=20$ respectively) (Fig. 16). Small gobies also had a higher ratio of the dinoflagellate marker 22:6ω3/20:5ω3 when compared to large gobies ($F= 16.63$, $p< 0.05$ after Bonferroni) (Fig. 17). This suggests that small gobies fed more on copepods in the pelagic environment, compared to medium sized and (especially) large gobies. Large gobies had significantly higher ratios of the diatom marker 16:1/16:0 (Fig. 17) compared to small gobies ($F= 22.37$, $p< 0.05$ after Bonferroni, $n= 20$ and $n= 24$ respectively) and medium sized gobies ($F= 22.37$, $p< 0.05$ after Bonferroni, $n= 20$ and $n= 20$ respectively). Furthermore, large gobies were significantly higher in their ratio of the diatom marker 20:5ω3/22:6ω3 (Table A.7) when compared to small ($F= 28.80$, $p< 0.05$ after Bonferroni) and medium sized gobies ($F= 28.80$, $p< 0.05$ after Bonferroni). Medium sized and small gobies contained similar concentrations of the diatom markers 16:1/16:0 ($F= 22.37$, $p= 0.97$) and 20:5ω3/22:6ω3 ($F= 28.80$, $p= 0.34$) (Fig. 17; Table A.7). Based

on their absolute fatty acid concentrations, small gobies were significantly higher in TFA (F= 9.84), EFA (F= 13.06), SFA (F= 7.99), MUFA (F= 9.57) and PUFA (F= 10.51) compared to large gobies ($p < 0.05$ after Bonferroni) (Fig. 17).

The proportional FA data have suggested similar trends as those noted above, since the large gobies were significantly higher in the diatom FA 20:4ω6 (F= 33.59), 20:5ω3 (F= 16.13) (Fig. 18) and diatom marker ratios 20:5ω3/22:6ω3 (F= 11.52) (Table A.3) and 16:1/16:0 (F= 9.53) (Fig. 19) relative to small gobies at an alpha level of 0.05 after Bonferroni adjustments. Small gobies were also proportionally higher in MUFA (F= 10.72, $p < 0.05$ after Bonferroni, n= 20 and n= 24 respectively) and proportionally lower in PUFA (F= 7.29, $p < 0.05$ after Bonferroni, n= 20 and n= 24 respectively), compared to large gobies (Fig. 19). These size related changes in absolute FA concentration and FA proportions may suggest that small gobies have a more pelagic feeding habit while large gobies may be more benthic, feeding on sedimented diatoms.

3.2.2 Prey environment related to gobies

Gobies were related to their prey environment using NMDS and ANOSIM. Results obtained from these analyses, based on quantitative fatty acid contents, suggest that prey items form three distinct groups. All the mixed zooplankton size fractions, which consisted primarily of copepods and juvenile euphausiids (Table 2) grouped together, based on their absolute FA content (Figs. 20a, b). Amphipods, pteropods and adult euphausiids were most similar to each other (Figs. 20a, b). Mud, jellyfish (*Aequorea forskalea* and *Chrysaora fulgida*) and pteropod shells were most similar to each other in

terms of their quantitative fatty acid content (Figs. 20a, b). These prey groupings were reinforced by the results obtained from cluster analysis based on ANOSIM (Figs. 21a, b).

When gobies were included in the quantitative analyses, they were found to be most similar to amphipods, pteropods and adult euphausiids (Figs. 22a, b). The results of the ANOSIM suggested that the fatty acid composition (based on single fatty acids) of gobies were significantly different from mud collected at station A ($p< 0.05$, pairwise R= 0.97) and B ($p< 0.05$, pairwise R= 0.98), pteropod shells ($p< 0.05$, pairwise R= 0.92) and the two jellyfish species *Aequorea forskalea* ($p< 0.05$, pairwise R= 0.96) and *Chrysaora fulgida* ($p< 0.05$, pairwise R= 0.94) (Table 6a). Gobies were also very different from the different zooplankton size fractions ($p< 0.05$, pairwise R= 0.73-0.99) (Table 6a). Gobies were similar to pteropods ($p> 0.05$; R= 0.17). ANOSIM suggested that gobies were different from amphipods ($p< 0.05$) and adult euphausiids ($p< 0.05$) but even so, gobies were similar to both these groups with pairwise R test statistics of 0.442 and 0.367 respectively (Table 6a). ANOSIM based on quantitative FA summaries/ratios (Table 6b) suggested very similar patterns as those based on individual FA. However, based on FA marker ratios, gobies were not very different from mixed zooplankton sized 150 µm ($p> 0.05$; R= 0.421) and 200 µm ($p> 0.05$; R= 0.386) (Table 6b). It is evident that the differences between sample groups as suggested by NMDS and cluster analysis based on ANOSIM (Figs. 20, 21) were largely based on sheer fatty acid content. TFA, EFA, SFA and MUFA concentrations were highest amongst the mixed zooplankton samples, followed by the gobies, euphausiids, amphipods and pteropods while mud, pteropod shells and the cnidarians had the lowest total fatty acid concentrations (Fig. 17). In order

to avoid this type of lipid content bias among very different types of samples, a more qualitative approach was thought to be appropriate for comparing the FA composition of gobies to different potential prey sources.

Based on the FA proportions (qualitative approach) of individual FA, small and large gobies were significantly different from all potential prey items ($p < 0.05$ ANOSIM) (Table 7a). Medium gobies were similar to amphipods ($p > 0.05$, pairwise R= 0.42) and mixed zooplankton sized 150 µm ($p > 0.05$, pairwise R= 0.28) (Fig. 23a; Table 7a). Although medium sized gobies were significantly different from pteropods ($p < 0.05$) and euphausiids ($p < 0.05$), they were still very similar with pairwise R statistics of 0.32 and 0.46 respectively (Fig 23a; Table 7a). Medium sized gobies were significantly different from mixed zooplankton sized 200 µm ($p < 0.05$, pairwise R= 0.58), 250 µm ($p < 0.05$, pairwise R= 0.52), 500 µm ($p < 0.05$, pairwise R= 0.78), 1000 µm ($p < 0.05$, pairwise R= 0.67), pteropod shells ($p < 0.05$, pairwise R= 0.78), mud ($p < 0.05$) and the two jellyfish species ($p < 0.05$) (Table 7a). NMDS and ANOSIM of qualitative FA summaries/ratios suggested that small gobies were significantly different from pteropods ($p < 0.05$), euphausiids ($p < 0.05$) and mixed zooplankton sized 150 µm ($p < 0.05$) but these were still very similar with pairwise R statistics of 0.46, 0.47 and 0.39 respectively (Fig. 23b; Table 7b). Small gobies were similar to mixed zooplankton sized 250 µm ($p > 0.05$; pairwise R= 0.40) (Fig. 23b; Table 7b). Furthermore, small gobies were significantly different from all other potential prey items ($p < 0.05$) (Table 7b). Medium sized gobies were significantly different from mixed zooplankton sized 200 µm ($p < 0.05$) and 500 µm ($p < 0.05$) but these were still very similar with pairwise R statistics of 0.49 and 0.46

respectively (Fig. 23b; Table 7b). Medium sized gobies were similar to pteropods ($p > 0.05$, pairwise R= 0.16), euphausiids ($p > 0.05$, pairwise R= 0.23) and mixed zooplankton sized 150 µm ($p > 0.05$, pairwise R= 0.25) and 250 µm ($p > 0.05$, pairwise R= 0.2) (Fig. 23b; Table 7b). Medium sized gobies were significantly different from mixed zooplankton sized 1000 µm ($p < 0.05$) but even so, these were very similar with an pairwise R statistic of 0.27 (Fig. 23b; Table 7b). Large gobies were similar to pteropods ($p > 0.05$, pairwise R= 0.08), euphausiids ($p > 0.05$, pairwise R= 0.10) and mixed zooplankton sized 150 µm ($p > 0.05$, pairwise R= 0.41) but significantly different from all other prey items in terms of their summary FA proportions (Table 7b). Gobies were very different from jellyfish in their absolute FA concentrations ($p < 0.05$, pairwise R statistics between 0.94 and 1) (Figs. 22a, b; Table 6a, b) and this was supported by the proportional FA data with $p < 0.05$ and R statistic of approximately 0.65 (Table 7a, b), although there was some similarity between the proportional FA content of gobies and jellyfish (Figs. 23a, b).

3.2.3 Fatty acid links between gobies, jellyfish and mud

NMDS analyses suggested that gobies are different from jellyfish based on individual absolute fatty acid concentrations (Fig. 22a) and summaries/ratios (Fig. 22b). These differences were reinforced by results obtained from ANOSIM which generated pairwise R values close to unity (Table 6a, b). However these differences were seen to be largely based on total fatty acid content (Fig. 17). The TFA concentration of *Aequorea forskalea* and *Chrysaora fulgida* was as low as 1.36 and 2.5 µg/mg DW respectively (Table A.8), while the TFA concentrations of small, medium sized and large gobies were 32.73, 23.77 and 20.16 µg/mg DW respectively (Table A.7). Futhermore, no marker specific to

jellyfish was identified during this study and hence it was difficult to link jellyfish to gobies using a fatty acid approach. The only potential link was the high ratio of the dinoflagellate marker 22:6ω3/20:5ω3 in jellyfish which were reflected in the tissues of the goby (Fig. 17).

When the fact that jellyfish are so devoid of lipids was discounted, *Aequorea forskalea* and *Chrysaora fulgida* was shown to be dominated by fatty acids such as 16:0 (11.38 % and 16.85 % TFA respectively), 18:0 (8.8 % and 14.29 % TFA), 22:6ω3 (13.04 % and 14.46 % TFA), 20:4ω6 (13.07 % and 7.17 % TFA) and 20:5ω3 (7.68 % and 9.26 % TFA) (Table A.2). *A. forskalea* and *C. fulgida* was proportionally high in i-17:0 (1.16 and 1.14% TFA respectively) and 19:0 (1.27 and 1.34 % TFA respectively) compared to other prey items (Fig. 18; Table A.2). Similarly, high proportions of these fatty acids were seen in the tissues of small, medium, sized and large gobies (Fig. 18; Table A.1).

NMDS suggested that gobies differ significantly from mud based on their absolute FA concentrations (Figs. 22a, b) but these differences were primarily due to the fact that mud was very depleted in FA compared to gobies. The TFA concentration in mud was as low as 0.85 µg/mg DW and 0.56 µg/mg DW at station A and B respectively. These differences were reinforced by ANOSIM which generated pairwise R statistics of close to unity (Table 6a, b). After adjusting for the low FA content of mud, it would appear that the FA composition of mud was still significantly different from that of gobies as ANOSIM generated pairwise R statistics close to unity (Figs. 23a, b; Tables 7a, b). However, the proportional data highlighted that mud at station A was dominated by 16:0

(20.99 % TFA), 14:0 (8.73 % TFA) and 18:0 (7.98 % TFA), while mud at station B was dominated by 16:0 (20.28 % TFA), 18:1ω7 (14.27 % TFA) and 16:1ω7 (12.92 % TFA) (Table A.2). Odd chain and branched FA were less abundant, for example 15:0, i-15:0, i-16:0, i-17:0, 17:0, 21:0 and 19:0 each accounted for less than 2 % of the TFA in mud (Table A.2). Mud was substantially higher in ai-15:0 (3.13-5.41 % TFA), 24:0 (1.29-5.59 % TFA), 16:1ω7 (6.24-12.92 % TFA), 18:1ω7 (6.66-14.27 % TFA) (Fig. 18) and the sum of its BAFA (12.06-14.3 % TFA) (Fig. 19) when compared to most other prey items.

CHAPTER 4: DISCUSSION

In the present study the diet of the goby was studied using trophic tracers. However the limited range of potential prey items, and small sample sizes of zooplankton and benthic components (such as mud and benthic polychaetes) somewhat constrains analysis and interpretation of the results obtained. Based on $\delta^{13}C$ and $\delta^{15}N$-signatures, small gobies appear to have fed mainly on polychaetes and shifted to a diet mainly composed of *Chrysaora fulgida* with increasing body size at station A (Fig. 11). Small gobies at station B fed primarily on *C. fulgida* and shifted to a diet mainly composed of *Aequorea forskalea* and shrimp with increasing body size (Fig. 12). Based on $\delta^{34}S$-signatures, pelagic food sources enriched in $\delta^{34}S$ made up the bulk of the goby diet (Fig. 13; Table 5). Benthic sediment which may include sulphur bacteria, diatoms and benthic infauna contributed up to 34.2 % of the diet, exceeding that from porewater dissolved H_2S (Table 5). Based on fatty acid signatures of gobies and their potential prey collected offshore, small gobies fed more on copepods found in the pelagic environment (Figs. 16) but large gobies fed more on diatoms (Figs. 17, 18, 19; Tables A.3, A.7) presumably from the benthos. Furthermore, FA results provide support for jellyfish feeding amongst gobies (Figs. 17, 18) and suggest that gobies may derive at least some nutrition from sulphur-oxidizing bacteria (Fig. 18). These results contrast with those obtained from stable isotopes because stable isotope analyses failed to identify copepods and bacteria-containing mud as a component of the goby diet.

4.1 Gobies feeding on mud (diatoms, bacteria)

Barber and Haedrich (1969), O'Toole (1978), Crawford *et al*. (1985) and Hundt (2009) have all highlighted diatoms (primarily *Fragilaria* sp., *Coscinodiscus* sp., *Chaetoceros*

sp., and *Thalassiosira* sp.) as being major components of the stomach contents of gobies collected inshore off the Namibian coast. The former three studies limited their sampling to 60 m depth or less, while Hundt (2009) sampled inshore to a maximum depth of 120 m. It is unlikely that gobies are able to feed on diatoms by filter feeding in the pelagic based on the fact that the goby has a relatively short gut and short, relatively broadly spaced gill rakers (Hundt 2009), unlike other filter feeding fish species (James 1987; van der Lingen 1994). Rather it is more likely that the diatoms recovered from the goby stomachs reflect material ingested from the seafloor. The $\delta^{13}C$ and $\delta^{15}N$ isotope results collected here could not shed any light on the feeding of diatoms by the goby as diatoms were not collected directly from the water column. Support for the benthic feeding hypothesis is also weak from the $\delta^{13}C$ and $\delta^{15}N$, as these signatures in gobies collected at station A and B were well outside the range of the diatomaceous mud (also known to contain large sulphur bacteria) that were collected and analyzed (Figs. 10a, b). The $\delta^{13}C$ and $\delta^{15}N$- signatures of benthic mud collected in the present study (Figs. 8a, b) is similar to that of Struck *et al*. (2002), who found that diatomaceous sediment off the Namibian coast had $\delta^{13}C$- signatures of between -20 and -21 ‰, and $\delta^{15}N$-signatures of between 5.5 and 7 ‰.

The use of $\delta^{34}S$ proved to be helpful because these results showed that (unfortunately, spatially pooled) benthic sediment (which is known to contain diatoms) may contribute up to 34.2 % to the diet of the goby (Fig. 13, Table 5). However it should be noted that this benthic sediment is also known to contain large populations of sulphur bacteria such

as *Thiomargarita* (Schulz *et al*. 1999) and so the benthic sediment contribution as suggested by $\delta^{34}S$ includes bacterial feeding.

Fatty acid analyses done in the present study at the offshore station (180 m depth) suggest that the tissues of large gobies contained higher absolute concentrations of the diatom markers 16:1/16:0 and 20:5ω3/22:6ω3 compared to small and medium sized gobies (Fig. 17; Table A.7). Large gobies were also proportionally higher in the diatom FA 20:4ω6, 20:5ω3 (Fig. 18) and the diatom marker ratios 16:1/16:0 (Fig. 19) and 20:5ω3/22:6ω3 (Table A.3) when compared to small gobies. This suggests that large gobies may spend more time on the diatomaceous mud bottom compared to smaller gobies which are known to be more regular diel vertical migrators (D'Arcangues 1977; Staby and Krakstad 2006; Hundt 2009). Whereas large gobies likely directly feed on diatoms in the mud, small and medium sized gobies may acquire diatom fatty acids such as 20:5ω3 from pelagic zooplankton and/or by direct feeding on diatoms in the mud (Fig. 18). Based on the fact that the absolute concentrations of the copepod markers 20:1ω9 and 22:1ω11 showed a decrease with increasing goby size (Fig. 16), it is likely that small and medium sized gobies are acquiring fatty acids characteristic of diatoms by feeding on zooplankton probably while in the pelagic at night and directly from sedimented diatoms while on the benthos during the day.

Despite its very low absolute FA concentration, mud collected at station A was dominated by 16:0 (20.99 % TFA), 14:0 (8.73 % TFA) and 18:0 (7.98 % TFA) while mud at station B was dominated by 16:0 (20.28 % TFA), 18:1ω7 (14.27 % TFA) and

16:1ω7 (12.92 % TFA) (Table A.2). Iso-anteiso fatty acids which are typically found in sulphate-reducing bacteria (Taylor *et al*. 1983; Zhang *et al*. 2002) were minor components of the TFA content of mud. The fatty acid content of mud as obtained in the present study is consistent with the fatty acid profiles of *Thioploca*, *Beggiatoa* and other sulphur-oxidizing bacteria in which 16:1ω7 and 18:1ω7 have been proposed as signature biomarkers for sulphur-oxidizing bacteria in hydrogen sulphide rich marine sediments (Jacq *et al*. 1989; McCaffrey *et al*. 1989; Zhang 2005). Certainly, 16:1ω7 and 18:1ω7 were proportionally higher in mud compared to other potential prey items collected in the present study (Fig. 18) and could indicate the presence of sulphur-oxidizing bacteria such as *Beggiatoa* and *Thiomargarita* which are well know to the central shelf of Namibia (Schulz *et al*. 1999). However, such interpretations are constrained by the small number of mud samples that were collected during the present study. In addition to 16:1ω7 and 18:1ω7, ai-15:0 and 24:0 was also higher in mud compared to other prey items (Fig. 18). This suggests that gobies could obtain many of these FA from bacteria-containing mud. However, gobies would need to ingest very large quantities of bacteria to make up for the overall dearth in the absolute FA concentration of mud (Figs. 16; Tables A.5, A.6).

4.2 Carnivory

4.2.1 *Pelagic and benthic food components*

The results obtained by analyzing isotopes of carbon and nitrogen suggest that mixed pelagic zooplankton of sizes 150 µm-1000 µm (refer to Table 2 for the composition of mixed zooplankton), pteropods and pteropod shells were not significantly assimilated by small, medium sized or large gobies at the offshore station (Fig. 10b). This is in contrast to all earlier studies that found copepods in the stomachs of gobies (D'Arcangues 1977;

O'Toole 1978; Macpherson and Roel 1987; Cedras 2009; Hundt 2009). D'Arcangues (1977) showed that copepods were the most abundant prey item in the stomachs of adult (50 mm-83 mm) and juvenile gobies (21 mm-49 mm). Cedras (2009) highlighted the increased consumption of copepods amongst small gobies, while Hundt (2009) showed that copepods were abundant in the stomachs of gobies collected in the pelagic environment offshore (180 m).

Large gobies may supplement their diet with shrimp at the offshore station (Fig. 10c) while small gobies may supplement their diet with benthic polychaetes or adult euphausiids at the inshore station (Fig. 10c), but these did not appear to form the bulk of the goby diet. Adult euphausiids were included as a possible source in the isosource model at the offshore station, but benthic polychaetes were not included. This was done because more fish data points could be included in the model and these data points could be better constrained when benthic polychaetes were excluded from the model (Fig. 4). Adult euphausiids could potentially make contributions up to 26.85 %, 33.14 % and 18 % to the diet of small, medium sized and large gobies respectively at the offshore station (Table 4). However, the percentage contribution of adult euphausiids was not significantly correlated with goby size (Fig. 12). Although gobies fed on adult euphausiids, these prey items were of secondary importance. In agreement with the aforementioned, D'Arcangues (1977) showed that euphausiids were the second most important prey items (copepods were the most abundant) in the guts of adult and juvenile gobies. In addition Hundt (2009) showed that euphausiids could contribute substantially to the diet of gobies caught in the pelagic environment offshore. In contrast, Cedras

(2009) showed that the occurrence of euphausiids in the guts of gobies increased with increasing goby size although he argued that this may have reflected their consumption whilst they were in deeper water during the day (close to the bottom). The potential contribution of shrimp was significantly positively correlated with goby size (Fig. 12) with shrimp potentially making contributions of up to 58.64 % to the diet of large gobies at the offshore station (Table 4).

No firm conclusions can be reached about the contribution of pelagic or benthic prey to the diet of gobies collected at the inshore station because unfortunately no prey items except jellyfish and mud were collected at the inshore station. The carbon and nitrogen signal of benthic polychaetes and adult euphausiids collected at the offshore station were similar to the signatures of gobies collected at the inshore station and therefore benthic polychaetes and adult euphausiids were included as possible sources in the isosource model of station A (Fig. 3). Another reason for the inclusion of benthic polychaetes and euphausiids collected at station B in the isosource model for station A, was because the inclusion of these sources allowed more fish data points to be included into the model and better constrained those data points (Fig. 3). The results obtained from this model indicate that the maximum percentage contribution of benthic polychaetes was negatively correlated with goby size at station A (Fig. 11). Furthermore benthic polychaetes could potentially contribute up to 47.63 % and 23.82 % to the diet of small and medium sized gobies respectively (Table 3). It should be stressed here that large gobies were not collected from station A and that the sample size of medium sized gobies were four times that of small gobies so caution should be exercised in their interpretation. Regardless,

these results are in contrast with those of Cedras (2009), who found that benthic polychaete consumption increased with increasing goby size. The results from the present study are however in agreement with Hundt (2009) who highlighted that benthic prey (polychaetes) were the second most abundant prey items in the stomachs of gobies between the sizes 79 mm- 83 mm TL (corresponding to medium sized gobies) collected inshore (120 m). The maximum percentage contribution of adult euphausiids was not correlated with goby size inshore but euphausiids could potentially contribute up to 44 % and 31.47 % to the diet of small and medium gobies respectively (Table 3). Again the latter is in agreement with D'Arcangues (1977).

Where isotopes of carbon and nitrogen could not identify links between gobies and zooplankton, fatty acid analyses suggest that gobies found offshore may derive most of their essential fatty acids from pelagic zooplankton such as copepods, pteropods, amphipods and euphausiids (Fig. 17). Small gobies offshore fed more on copepods compared to medium and large gobies as indicated by their significantly higher absolute concentrations of the copepod FA 20:1ω9 and 22:1ω11 when compared to medium and large gobies (Fig. 16). No comments concerning benthic polychaetes based on fatty acids can be made as these were unfortunately not sampled for FA.

4.2.2 *Jellyfish*

Although not directly relevant to the present study, the carbon and nitrogen content (as a percentage of dry weight) of the hydromedusa *Aequorea forskalea* and scyphomedusa *Chrysaora fulgida* were determined and are presented in Table 8. The nitrogen content of *A. forskalea* and *C. fulgida* as obtained in the present study is broadly similar to that of

the hydromedusa *Phacellophora camtschatica* and scyphomedusa *Cyanea capillata* (Larson 1986). *P. camtschatica* is however higher in %C composition compared to *A. forskalea* and *C. capillata* is substantially lower in carbon compared to *C. fulgida* (Table 8).

Pitt *et al*. (2009) have highlighted the fact that the isotopic composition and lipid content of different parts of the body of jellyfish vary. In the present study the "most tissue-rich" parts of the jellyfish body were used for both isotope and fatty acid analyses and may have caused some bias in the results obtained. Towanda and Thuesen (2006) did a study on the jellyfish *Phacellophora camtschatica* and found that the mesoglea was much heavier in carbon compared to the whole body, gonad and oral arm tissue. Similar variations in % C and % N were shown by Larson (1986) between the umbrella (% C= 8.3; % N= 2) (Table 8) and gonads (% C= 40; % N= 9.4) of the hydromedusae *Phialidium gregarium*. In the case of *Aequorea forskalea* and *Chrysaora fulgida*, this type of information was not obtained and certainly deserves more attention so that a more informed decision can be made concerning which tissue to use in tracer studies.

The goby (*Sufflogobius bibarbatus*) is frequently found in association with the two dominant jellyfish off the Namibian coast, *Chrysaora fulgida* and *Aequorea forskalea* (Utne-Palm *et al*. 2008; Utne-Palm *et al*. 2009). The results obtained in the present study contrast with other studies on the gut contents of *S. bibarbatus* as no trophic links have previously been made between gobies and these jellyfish, possibly due to the fast digestion rate of jellyfish tissue (Arai *et al*. 2003; Arai 2005) and prolonged and

inefficient sample storage which promotes rapid degradation of their soft tissues (Arai 2005).

Based on isotopes of carbon and nitrogen, gobies fed on jellyfish at the two sampling stations (120 m and 180 m respectively) off the Namibian coast. Inshore *Chrysaora fulgida* was a dominant prey source for medium sized gobies where it could potentially make dietary contributions of between 50 % and 69.9 % (Table 3). Offshore, *C. fulgida* with reduced $\delta^{13}C$ and $\delta^{15}N$-signatures dominated the diet of small gobies (maximum contribution of 73.85 %) while *Aequorea forskalea* dominated the diet of large gobies (maximum contribution of 70.36 %) (Table 4).

Jellyfish were almost completely devoid of lipids and no marker specific to jellyfish could be identified. However quantitative fatty acid analysis highlighted that gobies and jellyfish (both *Chrysaora fulgida* and *Aequorea forskalea*) had high absolute concentrations of the dinoflagellate marker 22:6ω3/20:5ω3 (Fig. 17). Information on jellyfish feeding is novel but gut content studies have shown that *Aequorea* species feeds on copepods (Purcell 1991; Purcell 2003) but prefers softer bodied prey such as larvaceans, hydromedusae and siphonophores (Purcell 1989; Purcell and Sturdevant 2001), in addition to fish larvae and cladocerans (Purcell 1989; Purcell 1991; Purcell 2003). *Chrysaora* spp. have been shown to have a diverse diet, including copepods and euphausiid eggs (Brodeur *et al*. 2008), and they take advantage of both pelagic and benthic prey items (Flynn and Gibbons 2007). With the exception of Flynn and Gibbons (2007), none of the aforementioned studies have identified dinoflagellates in the diet of

either *Aequorea* or *Chrysaora*. Flynn and Gibbons (2007) identified dinoflagellates as a minor prey item in the gut of *Chrysaora fulgida.* It can therefore be suggested that the dinoflagellate fatty acid signature (22:6ω3/20:5ω3) present in both *A. forskalea* and *C. fulgida* is due to both primary and secondary prey consumption and that these jellyfish were most likely eaten by *Sufflogobius bibarbatus* as the goby is unlikely to feed directly on dinoflagellates in the water column. In addition, *A. forskalea* and *C. fulgida* were proportionally higher in i-17:0 (1.14-1.16 % TFA) and 19:0 (1.27-1.34 % TFA) when compared to other prey items (Fig. 18). Similarly, high proportions of these FA were observed in goby tissues (Fig. 18) and suggest that gobies could be feeding on these two species of jellyfish. With that said, gobies would have to consume large quantities of jelly material in order to compensate for the overall dearth in absolute FA concentration of these jellyfish (Tables A.5, A.6).

4.3 Ontogenetic shift in goby diet

Previous authors have shown that the pelagic goby is a generalist feeder that feeds opportunistically depending on what is available, whether it is found offshore or inshore and whether it is found is the pelagic or benthic environment (Cedras 2009, Hundt 2009; Utne-Palm *et al*. 2009). Based on the results obtained in the present study, there appear to be gradual changes in the diet of the goby as it increases in size, and these changes in the diet may be primarily due to a change in the habit. Ontogenetic changes in diet have been observed for gobies elsewhere, for example small *Pomatoschistus microps* (common goby) in the northern Wadden Sea feed on meiofauna but large individuals tend to eat more macrofauna (del Norte-Campos and Temmings 1994). Similar size-related dietary shifts have also been observed for the giant goby *Gobius cobitis* (Gibson 1970),

landlocked goby *Rhinogobius sp*. (Maruyama *et al*. 2001) and amphidromous Hawaiian gobies (Sorensen and Hobson 2005) and may reflect changes in metabolic demands (Levy 1990), morphology, changes in habitat, prey availability (Grossman 1980) or behaviour (Grossman *et al*. 1980).

The isotope work done in the present study suggested that small gobies located inshore (120 m) fed primarily on polychaetes and adult euphausiids and supplemented their diet with *Chrysaora fulgida* and *Aequorea forskalea* (Fig. 11, Table 3). These gobies moved to a diet dominated by *C. fulgida* (both reduced and elevated $\delta^{13}C$ and $\delta^{15}N$-signatures) as they increased in size while supplementing their diet with polychaetes, adult euphausiids and *A. forskalea* (Fig. 11, Table 3). The patterns of diet shift with size could have been clearer inshore if fish larger than 100 mm had been caught and analyzed. In addition to the aforementioned limitation, the sample size of small fish was four times smaller than that of medium sized fish collected inshore.

Based on isotope analyses small gobies collected offshore fed primarily on *Chrysaora fulgida* with reduced $\delta^{13}C$ and $\delta^{15}N$-signatures and supplemented their diet with *C. fulgida* with enriched $\delta^{13}C$ and $\delta^{15}N$-signatures, *Aequorea forskalea* and adult euphausiids (Fig. 12 and Table 4). Fatty acid analyses, at least offshore, suggest that small gobies also fed on copepods based on their high absolute copepod marker (20:1ω9 and 22:1ω11) concentrations (Fig. 16). Based on isotope analyses these gobies moved to a diet dominated by *A. forskalea* and shrimp with increasing size while supplementing their diet with *C. fulgida* and adult euphausiids (Fig. 12, Table 4). Based on fatty acid analyses an

increase in goby size was also associated with higher diatom input (Figs. 17, 18, 19; Tables A.3, A.7).

Small gobies, at least offshore, contained high absolute concentrations of EFA, MUFA, PUFA and SFA obtained from pelagic zooplankton, and these concentrations show a gradual decrease with increasing goby size (Fig. 16). Such heavy fatty acid loadings are essential for growing individuals. Juvenile and larval fish usually are high in polyunsaturates (Bell and Sargent 1996) such as 20:5ω3 and 22:6ω3 as well as other ω3 and ω6 fatty acids because these are critical for membrane function, growth, survival and subsequent reproduction (Henderson *et al*. 1984; Bell and Sargent 1996; Kattner *et al*. 2007). Ishizaki *et al*. (2001) have shown that 20:5ω3 and 22:6ω3 are important for schooling behaviour and larval brain development of fish while Kanazawa (1997) suggested that 22:6ω3 may increase stress tolerance in fish. Fatty acids such as 16:0, 18:1ω9, 20:1ω9 and 22:1ω11 are important in providing metabolic energy (Henderson and Almater 1989). Some fatty acids may act as precursors for important hormones; for example, 20:4ω3 and 20:5ω3 are precursors for eicosanoids (Tocher 2003).

The ontogenetic changes in diet of the goby observed in the present study appear to be a function of changing habit and food availability. Small gobies are known to be more pelagic, frequently migrating vertically in the water column (D'Arcangues 1977; Staby and Krakstad 2006) and hence one would expect them to take advantage of pelagic prey items (mixed zooplankton, pteropods, amphipods and/or euphausiids) as seen when looking at their fatty acid profiles. Large gobies are more demersal, migrating

infrequently (D'Arcangues 1977; Cedras 2009; Hundt 2009) which explains why they had much higher benthic diatom (Figs. 17, 18, 19; Tables A.3, A.7) and less pelagic copepod input (Fig. 16) as suggested by fatty acid analysis.

The abundance of cnidarian prey items vary with distance from the shore. *Chrysaora fulgida* is more abundant inshore (Fearon *et al*. 1992; Brierley *et al*. 2001; Sparks *et al*. 2001; Utne-Palm *et al*. 2008) with small *C. fulgida* peaking closer to the coast than their larger counterparts (Fearon *et al*. 1992). In contrast *Aequorea forskalea* is more abundant offshore (Fearon *et al*. 1992; Brierley *et al*. 2001; Sparks *et al*. 2001; Utne-Palm *et al*. 2008). Research on the vertical distribution of these two jellyfish species suggests that *C. fulgida* is more abundant in shallow waters while *A. forskalea* is more abundant at greater depths inshore (Utne-Palm *et al*. 2008). Offshore, both *C. fulgida* and *A. forskalea* are more abundant at greater depths (Utne-Palm *et al*. 2008). It can be suggested that small and medium sized gobies feed more on *C. fulgida* as opposed to *A. forskalea* inshore, because *C. fulgida* is more abundant in shallow waters inshore. Furthermore, large gobies offshore probably feed more on *A. forskalea* as opposed to *C. fulgida* because *A. forskalea* is more abundant in deeper waters there.

The change in goby diet with body size may also be linked to sex. Since no conclusive evidence is available about the breeding of *Sufflogobius bibarbatus*, it is likely that its breeding biology is similar to that of gobies elsewhere (Lindström 1988, Forsgren *et al*. 1996). Typically, gobies build nests on the seafloor in which they spawn and the male remains to protect the developing eggs (Lindström 1988). Certainly, bearded gobies may

show changes in their diet during the reproductive process as has been shown for the sand goby *Pomatoschistus minutus* in southwest Europe where females broaden their food-niche to obtain richer caloric prey and meet the energetic cost of reproduction (Salgado *et al*. 2004). Unfortunately sex-related information was not obtained during the present study but certainly deserves further attention.

In order to be confident that a particular signature provides a temporally integrated view of feeding behaviour one must have some knowledge of how that signature is integrated (O'Reilly *et al*. 2002). The way in which fatty acids and isotopes are integrated into the tissue of a consumer depends on factors such as growth and metabolism (Miller 2000), which in turn are influenced by size/age and environmental factors such as temperature (Tocher and Sargent 1990; Kiessling *et al*. 2001).

A change in the FA profile in white muscle tissue due to a change in diet was not complete after 8 weeks in turbot *Psetta maxima* (Regost *et al*. 2003) and brown trout (*Salmo trutta*) (Robin *et al*. 2003). However Robin *et al*. (2003) highlighted that a change from a plant oil to a fish oil diet over the 8 week period was associated with a decrease in key plant oil FA such as 18:2ω6 and 18:3ω3 and an increase in key fish oil FA such as 22:6ω3, 20:5 ω3 and ω3 monounsaturates in both turbot and brown trout. Similar results were obtained by Benedito-Palos *et al*. (2009) after changing the diet of gilthead sea bream (*Sparus aurata*) from vegetable oil to fish oil over a 12 week period. Rather than total restoration of FA profiles after a change in diet, literature suggests that changes in fatty acid profiles follow a "dilution hypothesis" by which pre-existing fatty acids

become increasingly diluted with increased consumption and deposition of dietary fatty acids as a fish grows (Robin *et al*. 2003; Benedito-Palos *et al*. 2009). In juvenile or fast growing fish the time required to reach a stable FA composition relative to a changed diet is rather short (Skonberg *et al*. 1994) but in large or slow growing fish such changes continue over long periods of time (Tidwell and Robinette 1990). No conclusive comments can be made concerning the FA turnover time of the goby but if one is to assume that the goby incorporates FA in the same way as slow growing sea bream, the diet of the goby as found in the present study, may be representative of feeding behaviour over approximately 12 weeks (months not years).

The isotopic turnover of goby white muscle is likely similar to that of the slow-growing Pacific herring (*Clupea pallasi*) in which in rate of nitrogen isotopic change was shown to be 0.033 ‰ per day, reaching equilibrium with its diet in approximately 12 weeks (Miller 2000).

4.4 Global jellyfish increase and ecosystem effects

The apparent global increase in gelatinous zooplankton populations (Mills 2001; Purcell 2005; Lynam *et al*. 2006) has stimulated research relating to their potential effects on marine ecosystems. Many explanations have been proposed to explain the increase in gelatinous zooplankton, including factors such as climate change (Brodeur *et al*. 1999; Lynam *et al*. 2004; Purcell 2005), species introductions (Shiganova 1998; Mills 2001), eutrophication (Arai 2001), overfishing (Lynam *et al*. 2006) and possibly an interaction between two or more of the aforementioned factors (Purcell *et al*. 2007; Richardson *et al*. 2009).

Researchers have recognised the potential of gelatinous zooplankton to dominate marine pelagic ecosystems (Brodeur *et al*. 2008) and many have described them as trophic dead-ends (Sommer *et al*. 2002). Jellyfish feed on zooplankton and may therefore compete with zooplanktivorous fish species in areas where prey is limited as well as acting as predators of their early life history stages (Arai 1988; Purcell and Arai 2001). In addition pelagic jellyfish can serve as intermediate hosts for fish parasites (Arai 1988). Contrary to these negative effects on marine ecosystems, jellyfish can serve as prey for some species (Arai 1988; Purcell and Arai 2001) and engage in commensal associations with some pelagic fish species (Mansueti 1963; Purcell and Arai 2001) both to the benefit of fish populations.

4.4.1 *Cnidaria as prey for fish*

Numerous studies have documented the presence of gelatinous organisms or nematocysts in stomachs of a variety of fish species (Arai 2005). Most fish that feed on jellyfish usually have very broad diets (Purcell and Arai 2001) however, there are some species that feed primarily but not exclusively on jellyfish (Arai 2005). Examples of fish that include jellyfish in their diet are the spiny dogfish *Squalus acanthias* (Arai 1988; Brodeur and Pearcy 1992), chum salmon *Oncorhynchus keta* (Arai 1988) and the subarctic Pacific myctophid *Stenobrachius leucopsarus* (Balanov *et al*. 1994; Beamish *et al*. 1999). The dietary inclusion of gelatinous prey items may vary temporally amongst fish. For example, juvenile chum salmon only start including gelatinous material in their diet during the summer following spawning (King and Beamish 2000). Similarly, Mianzan *et al*. (1996) examined 69 fish species on the Argentine coast and found that ctenophore consumption increases during spring, which they argued was likely linked to an increase

in ctenophore abundance (*Mnemiopsis maccradyi*, *Pleurobrachia pileus* and *Beroe ovata*) at that time (Mianzan *et al*. 1996).

If gobies were to show temporal variation in jellyfish feeding, it may likely be due to temporal variation in jellyfish abundance or due to variation in the abundance of small pelagic zooplankton. Although (Kramp 1961) highlighted that *Chrysaora* sp. are known to vary in abundance seasonally in other regions of the world, seasonal abundance data are lacking for both *C. fulgida* and *Aequorea forskalea* in the northern Benguela region. However according to Shannon (1985) waters in the northern Benguela are less seasonal than in the southern Benguela and therefore seasonal trends are not likely to be significant except in the North (18º) where coastal upwelling is seasonal and peaks from July-November (Shannon 1985). In addition Timonin *et al*. (1992) showed that although upwelling in the northern Benguela is not strictly seasonal, zooplankton biomass does vary depending on hydrological conditions. They showed that species such as the copepods *Nannocalanus minor* and *Neocalanus gracilis* that prefer warmer waters change their latitudinal and bathymetric boundaries, peaking closer to shore during quiescent upwelling periods at 25ºS off the coast of Walvisbay (Timonin *et al*. 1992). Furthermore they showed that species that prefer cooler waters, such as the offshore neritic species *Rhinocalanus nasutus* and *Calanoides carinatus*, increased in abundance inshore during upwelling periods (Timonin *et al*. 1992) and were able to constitute up to 92 % of the total zooplankton during that time. Therefore it can be suggested that the dietary inclusion of jellyfish may vary seasonally in the northern reaches of the northern

Benguela but most likely stay relatively constant in the rest of the region, but that jellyfish feeding may vary with hydrological conditions and requires further research.

Gelatinous feeding fish have various physical adaptations to their diet. Stromateoid species, of which many are specialist gelatinous feeders, have deep bodies and underslung jaws (Harbison 1993) e.g. butterfish *Poronotus triacanthus*, harvestfish *Peprilus alepidotus* which have been seen in association and feeding on the scyphomedusa *Chrysaora quinquecirrha* in Chesapeake Bay (Mansueti 1963). However generalist gelatinous feeders may be streamlined much like Atlantic mackerel *Scomber scombrus* which may feed on the hydromedusan *Aglantha digitale* (Runge *et al*. 1987). Other features include a large stomach and long digestive tract as seen in generalist jellyfish feeding fish (Arai 2005). The stomach of the opportunistic, chum salmon is enlarged compared to other species belonging to the genus *Oncorhynchus* and is able to hold about 3.5 times more than other species of comparable body size (Welch 1997; Arai 2001). The enlarged stomach in chum salmon allows it to exploit jellyfish and reduce competition with other salmon species (Welch 1997) such as pink salmon *Oncorynchus gorbuscha* in the Pacific and Bering Sea (Tadokoro *et al*. 1996). Furthermore, oesophageal or pharyngeal modifications are also common amongst jellyfish feeders (Purcell and Arai 2001). Chum salmon have been shown to have a thick and muscular oesophagus and stomach wall (Azuma 1995); stomateoid fish possess toothed pharyngeal sacs in the oesophagus (Isokawa *et al*. 1965) while masked angelfish *Genicanthus personatus* have oesophageal papillae which point posteriorly (Howe 1993). Such modifications assist with preventing regurgitation, promoting the mechanical break down

of tissue and protection against stinging nematocysts (Purcell and Arai 2001). *Sufflogobius bibarbatus* possess short gill rakers with generous spacing (0.7 mm) in addition to a simple digestive tract of equal or slightly longer than its standard length (Hundt 2009). Hundt (2009) found no pyloric cacae in the gut of *S. bibarbatus*. Despite its lack of apparent digestive adaptations, further anatomical research is needed to establish what equips *S. bibarbatus* for its gelatinous diet.

4.4.2 *Associations between fish and jellyfish*

Numerous fish (usually juveniles) are associated with jellyfish (Lynam and Brierley 2007; Masuda *et al*. 2008). Jellyfish potentially provide shelter (especially to juvenile fish) and food, entangled in the tentacles or situated in the oral cavity of the jellyfish (Masuda *et al*. 2008). These associations are usually facultative and are not species specific. Lynam and Brierley (2007) showed that association of 0-group whiting (*Merlangius merlangus*) with the jellyfish *Cyanea lamarckii* and *C. capillata* increased survival and first year recruitment of this gadoid fish in the North Sea. They suggested that the jellyfish provided protection against predators and a convenient foraging area where the fish could feed on crustacean zooplankton parasitizing the jellyfish. Masuda *et al*. (2008) showed similar associations between Jack mackerel (*Trachurus japonicus*) and the moon jellyfish (*Aurelia aurita*) collected from the coast of Japan, but in addition they suggest that assemblages of juvenile Jack mackerel use the giant jellyfish (*Nemopilema nomurai*) as an aggregating point for school formation or migrating objects to travel to frontal areas were planktonic food is abundant. Associations may exist between *Sufflogobius bibarbatus* and the two dominant jellyfish in the northern Benguela. If so, it would be favourable as the goby would be able to acquire shelter, protection and possibly

food (either by direct feeding on the jellyfish or by feeding on zooplankton entangled in the tentacles of medusae).

The likely answer to the question of why gobies would feed on jellyfish despite their low nutritional content may be a balance between the fact that jellyfish may provide protection and the fact that these jellyfish are so abundant (Lynam *et al*. 2006). Arai (2005) suggested that predators of jellyfish may be able to compensate for the low nutritional value of jelly tissue by consuming extremely large amounts of jelly tissue very frequently. She also suggested that predators of jellyfish are able to do the aforementioned based on the fact that jelly tissue is digested very quickly (Arai 2005). The chum salmon *Oncorhynchus keta* is able to digest ctenophore tissue (*Pleurobrachia bachei*) 20 times faster than an equal wet weight of shrimp (Arai *et al*. 2003). Since jellyfish are larger than other zooplankton prey items, their individual caloric value is higher and they can be caught at a lower energetic cost because of their slow movement (Arai 1988).

4.5 Importance of the obtained results for the Northern Benguela system

From Ecopath modelling done by Heymans *et al*. (2004) for the period 1970's-1990's, the northern Benguelan food chain was considered to be very structured and channel-like during the 1970's and the system sustained large populations of a few planktivorous fish such as sardine, anchovy and horse mackerel. This meant that energy flowed through a few trophic links (Heymans *et al*. 2004). During the 1980's catches of sardine and anchovy declined but catches of horse mackerel were still high (Boyer and Hampton 2001, Cury and Shannon 2004). In addition to sardine, anchovy and horse mackerel, the

system acquired a range of mesopelagic and small pelagic fish species (Cury and Shannon 2004; Heymans *et al*. 2004), which made the food chain of the system more web-like (Heymans *et al*. 2004). The system operated at a higher trophic level compared to the 1970's and had many pathways of energy transfer (Heymans *et al*. 2004). However, during the 1990's there were clear signs of "fishing down the marine foodweb" and the system started functioning at a lower average trophic level (Pauly *et al*. 1998; Heymans *et al*. 2004). The system operated at its lowest efficiency because of overfishing, the arrival and bloom of jellyfish (Pauly *et al*. 1998; Boyer and Hampton 2001; Lynam *et al*. 2006) and the 1995 Benguela Niño (Gammelsrød *et al*. 1998). Energy was transferred through few trophic links and was transferred inefficiently up the foodweb because energy from jellyfish was thought to be directly transferred to the detrital food chain (Heymans *et al*. 2004).

The fact that gobies are eating jellyfish that are generally regarded as ecological dead-ends to pelagic foodwebs (Sommer *et al*. 2002), means that energy previously thought to be lost to the system (Heymans *et al*. 2004) can now be exchanged within the pelagos and between the pelagic and the benthic environment. Another trophic link has been added to the system which means that commercially harvested fish, that feed on gobies, such as Cape hake effectively feed at a higher trophic level than previously thought. Bearded gobies have an advantage over other "pelagic" species because they are able to tolerate extended periods of anoxia and elevated levels of hydrogen sulphide (Utne-Palm *et al*. 2008; Utne-Palm *et al*. 2009). In the face of climate change where increased greenhouse gas build-up is hypothesised to result in more intense upwelling events (Bakun and

Weeks 2004), jellyfish blooms (reviewed by Purcell 2005; Bakun and Weeks 2006), consequent increases in the build-up of organic material on the sea floor (Pitt *et al.* 2007b) and increased sulphur eruptions and anoxia (Bakun and Weeks 2004), gobies are likely to thrive. Isotopic comparisons between gobies, anchovy and sardines in the northern Benguela suggests that the goby fed at the same average trophic level as the anchovy but both gobies and anchovy fed at a higher trophic level compared to sardines based on δ^{15}N-signatures (Fig. 24). This is in agreement with van der Lingen (1994) who suggested that adult anchovy and sardines feed mainly on zooplankton but that juvenile sardines are omnivorous and are able to filter feed on phytoplankton. James (1987) suggested that anchovy feeds on copepods and euphausiids which also form part of the diet of the goby as shown in the present study, hence the similarity in δ^{15}N between gobies and anchovies. The δ^{13}C of anchovy and sardines in the northern Benguela were similar and the reasons for this similarity are unclear (Fig. 24 unpublished data). One would expect that sardines would be lighter in δ^{13}C compared to anchovy considering its phytoplankton consumption. Nevertheless, information on the δ^{13}C and δ^{15}N-signatures of phytoplankton in the region is lacking and so it is possible that the suite of phytoplankton consumed by sardines in the northern Benguela were somewhat enriched in δ^{13}C.

Gobies are substantially lighter in δ^{13}C compared to sardines and anchovy collected in the northern Benguela (Fig. 24) possibly due to their consumption of jellyfish (Tables 3, 4), sedimented diatoms (Figs. 13, 17, 18, 19) and sulphur bacteria (Fig. 18). Such δ^{13}C depleted signatures as seen in goby tissue compared to sardines and anchovy can

potentially be indicative of a diet composed of large sulphur bacteria. Gilhooly *et al*. (2007) found that white mats of *Beggiatoa* off the northern Gulf of Mexico were depleted in $\delta^{13}C$ (-26.1 ‰). Carbon and nitrogen isotope signatures of Namibian sulphur bacteria are lacking, but are likely similar to the aforementioned. In this light it can be suggested that although many predators have replaced their once sardine diet with that of gobies (Crawford *et al*. 1985), gobies have not taken over the role of sardines and anchovy within the northern Benguela system because sardines, anchovy and gobies each have different diets and each play individual roles within the system. Cury *et al*. (2000) highlighted that small pelagic fish can play a structuring role in upwelling systems because they can affect other pelagic fish species as well as species at lower and higher trophic levels. Certainly, from the results obtained from the present study, the bearded goby plays an important role in energy transfer in the system. In addition $\delta^{34}S$ results obtained in the present study suggest that gobies are able to filter through dissolved sulphide-containing benthos by either assimilating diatoms, bacteria and/or benthic fauna (Fig. 13, Table 5). The aforementioned together with the fact that gobies have a high tolerance for anoxia (Utne-Palm *et al*. 2008; Utne-Palm *et al*. 2009) makes it feasible to suggest that gobies are able to incorporate sulphide into their metabolic processes hence driving sulphides from the benthos. However the actual sulphur species precursor for the goby is not clear and could include sulphur species such as elemental sulphur from bacteria, H_2S and/or iron sulphide.

Sardines, when abundant, removed large quantities of phytoplankton from the intensely divergent upwelling zone that zooplankton, gobies and anchovy could not remove (Bakun

and Weeks 2006). Sardines are strong swimmers and are able to overcome strong offshore surface flow whereas zooplankton would be swept out of the system (Bakun and Weeks 2004). Presently the low biomass of sardines in combination with the high jellyfish biomass has been suggested as one of the reasons for the increased phytoplankton build-up, and its associated effects on the system (Boyer *et al*. 2001). Although both gobies and anchovy are able to remove zooplankton from the system, the areas which they exploit are different. Gobies are tolerant of low oxygen and high levels of hydrogen sulphide (Utne-Palm *et al*. 2009) and are therefore able to feed on prey items in the demersal and pelagic environment whereas the anchovy is a pelagic zooplanktonic feeder.

CONCLUSIONS

The use of tracer techniques has proven to be helpful in the present study because it enabled the identification of the jellyfish *Chrysaora fulgida* and *Aequorea forskalea* as prey sources of the goby *Sufflogobius bibarbatus*, something that previous gut content studies have failed to do. Previous studies have suggested that *S. bibarbatus* is an opportunistic and generalist feeder (Cedras 2009) and this is supported from the results of the present study, which also indicate that the goby diet changes ontogenetically and likely reflects changes in habitat and prey environment. Previous stomach content studies have suggested that gobies feed on phytoplankton, while others have shown that the goby diet is dominated by zooplankton. The present study suggests that gobies assimilate sedimented diatoms, pelagic zooplankton, benthic fauna, sulphur-oxidizing bacteria and jellyfish but that the importance of these prey items vary depending on whether gobies are large and demersal or small and more pelagic in habitat.

The present study highlights the value of employing multiple stable isotope and fatty acid tracer techniques to compliment each other. Stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ failed to identify pelagic zooplankton and mud (containing diatoms and sulphur bacteria) as prey items but these were identified by $\delta^{34}S$ and fatty acid analyses. Similarly fatty acid techniques were less effective in tracing jellyfish, whereas stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ identified *Chrysaora fulgida* and *Aequorea forskalea* as the dominant prey items in the goby diet.

The present study should be viewed as preliminary and the way forward with this type of research would involve more intensive and extensive sampling of a wider range of potential prey items. It would be advantageous to obtain sedimented diatoms, infauna and sulphur bacteria from the benthic environment to establish the link between gobies and these potential prey sources with more certainty. It is very important to sample individual zooplankton taxa to species level to avoid interspecific variation in isotope ratios and fatty acid markers. Where jellyfish are concerned, further laboratory studies are needed in order to find any specific markers that could make tracing jellyfish as a prey source less ambiguous. The station-related changes in isotope ratios of $\delta^{13}C$ and $\delta^{15}N$ as seen in the present study suggest that future studies should include sampling at different geographic locations and at different times of year, in order to get an understanding of how changes in hydrological conditions and the ambient prey environment influence dietary assimilation. Further work is needed to determine the degree of variation in isotopic and fatty acid signatures between the different tissues of especially the pelagic goby, *A. forskalea* and *C. fulgida* so that a more informed decision can be made concerning which tissue to use in future tracer studies.

Previous authors have suggested that non-commercial mesopelagic and demersal fish such as gobies play an important role in ecosystem nutrient transfer (Cury *et al*. 2000). The present study has shown that jellyfish are perhaps not “dead-ends” to the pelagic foodweb as previously thought (Sommer *et al*. 2002; Heymans *et al*. 2004) and that gobies may allow energy previously thought to be lost to the system to be exchanged between the pelagic environment and the benthic environment. Another trophic link has

been added to the system which may suggest that we are “fishing up the marine foodweb”.

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FIGURES

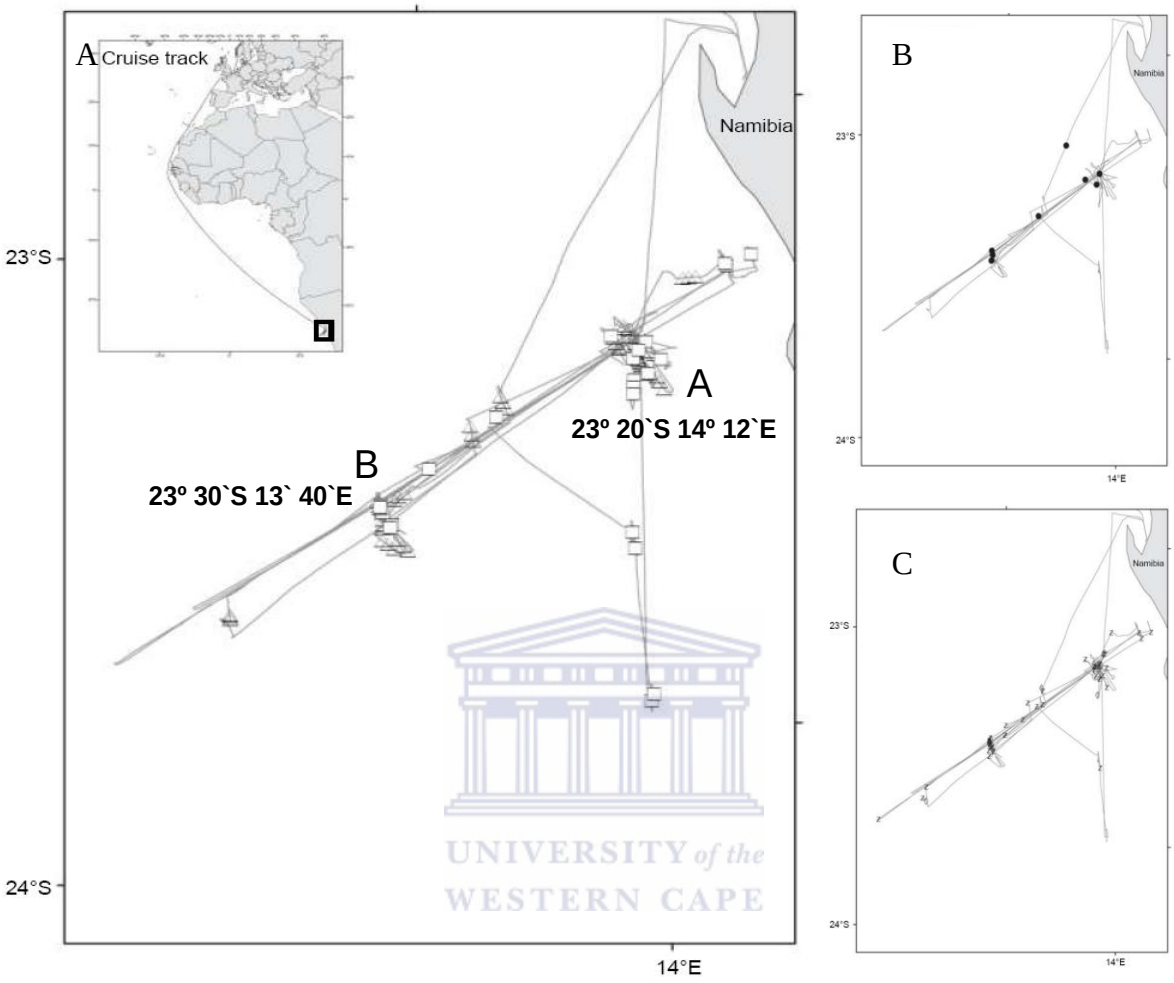


Figure 1: Map of the cruise track sampled off the Namibian coast during March 2008. Presented here is the positioning of pelagic and bottom trawls (A), grab samples (B) and Mocness zooplankton sampling tows (C) at the two major 48 hour stations. The positioning of the two 48 hour sampling stations is also indicated: Station A (23º20`S 14º12`E) sampled at a maximum depth of 120 m, and station B (23º30`S 13º40`E) sampled at a maximum depth of 180 m.

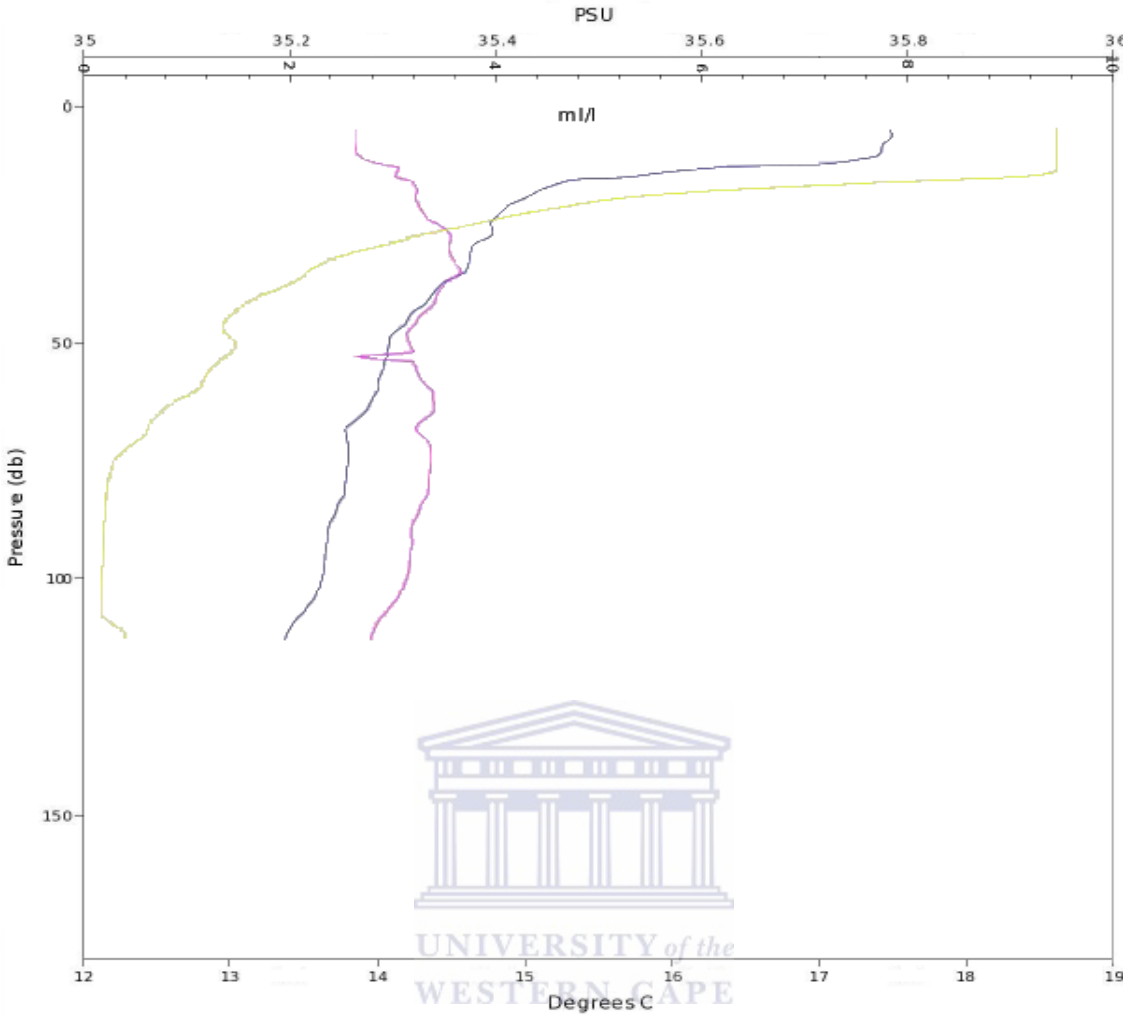


Figure 2a: Vertical oxygen (green, ml DO/L), salinity (pink, PSU) and temperature (blue, ˚C) profiles at station A. Taken from Utne-Palm *et al*. (2008).

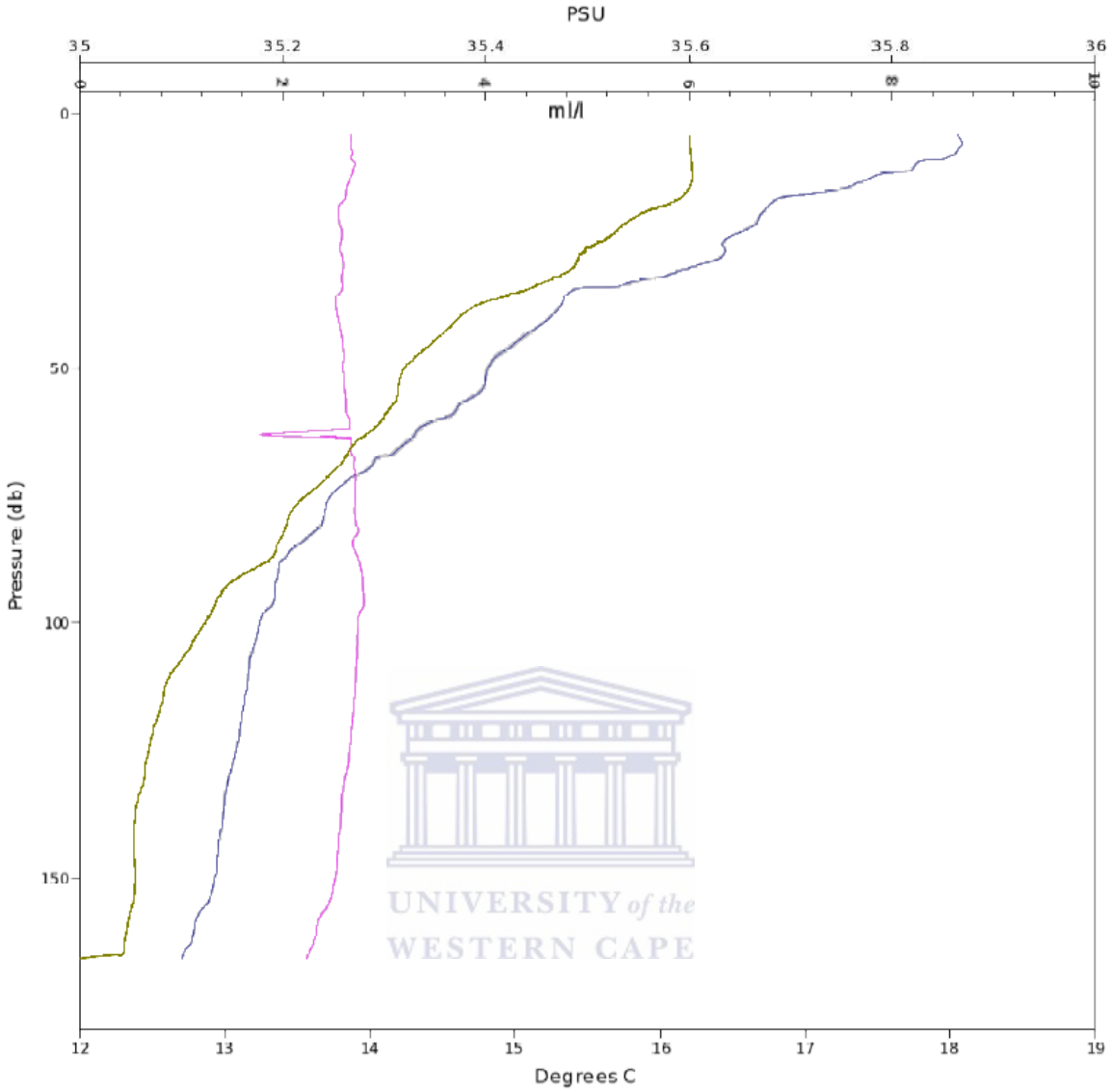


Figure 2b: Vertical oxygen (green, ml DO/L), salinity (pink, PSU) and temperature (blue, ˚C) profiles at station B. Taken from Utne-Palm *et al*. (2008).

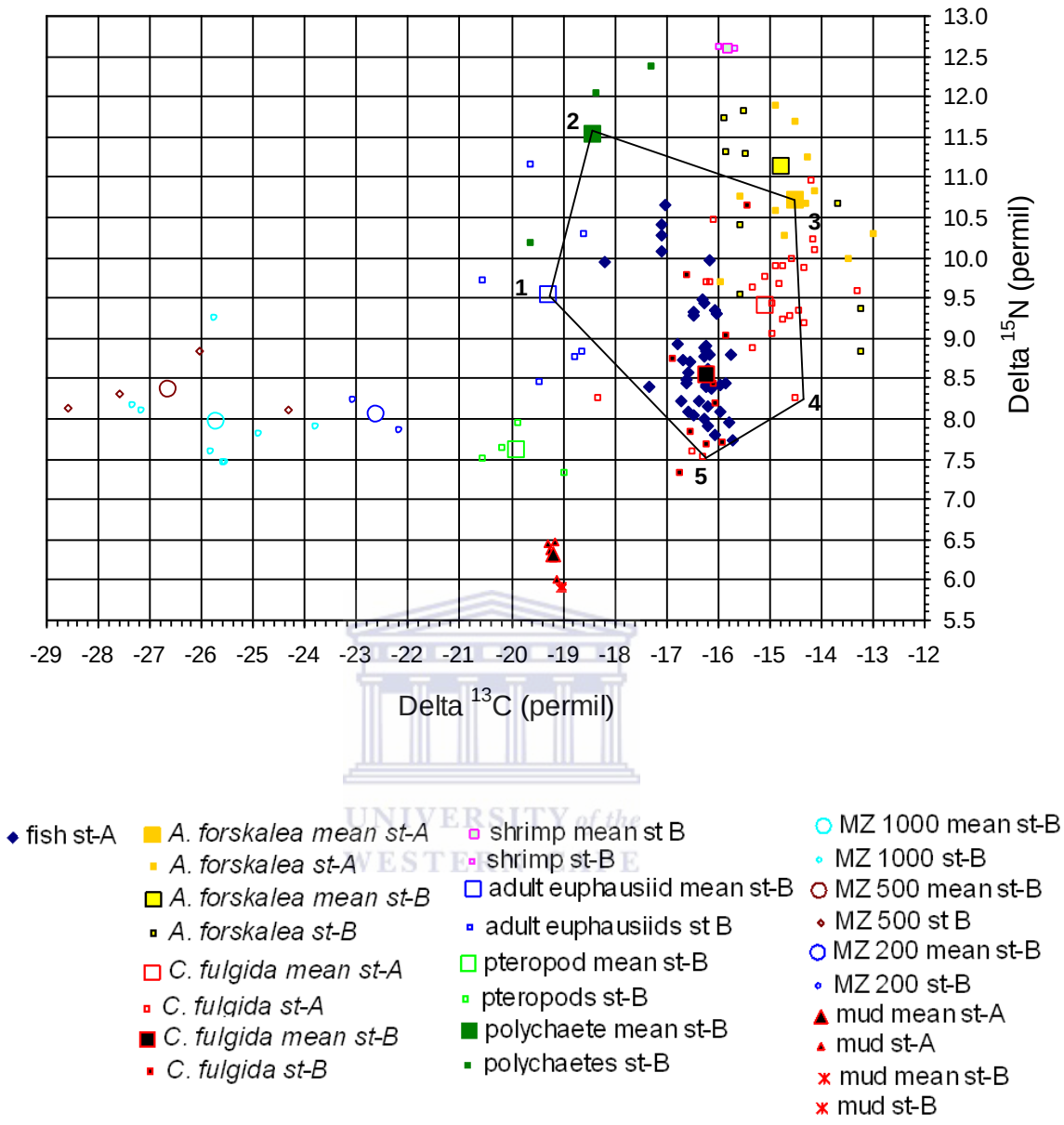


Figure 3: Isosource model used for fish data collected at station A. Apex prey source allocations are adult euphausiids (1), polychaetes (2), *Aequorea forskalea* (3), *Chrysaora fulgida* 1 (4) and *C. fulgida* 2 (5). All prey sources were collected at station A, except adult euphausiids and polychaetes which were collected at station B.

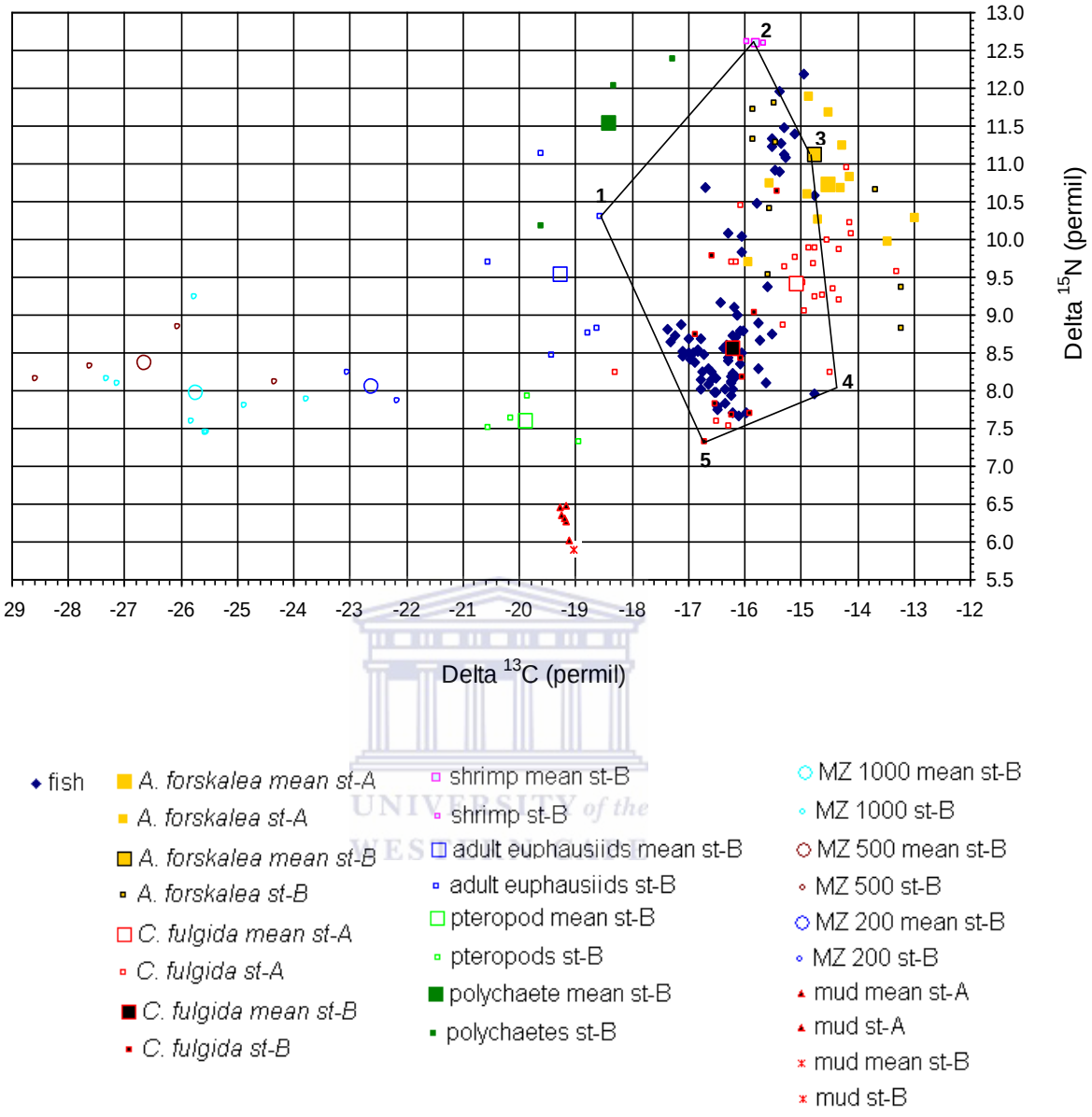


Figure 4: Isosource model used for fish data collected at station B with apex sources allocations as adult euphausiids (1), shrimp (2), *Aequorea forskalea* (3), *Chrysaora fulgida* 1 (4) and *C. fulgida* 2 (5). All prey sources were collected at station B.

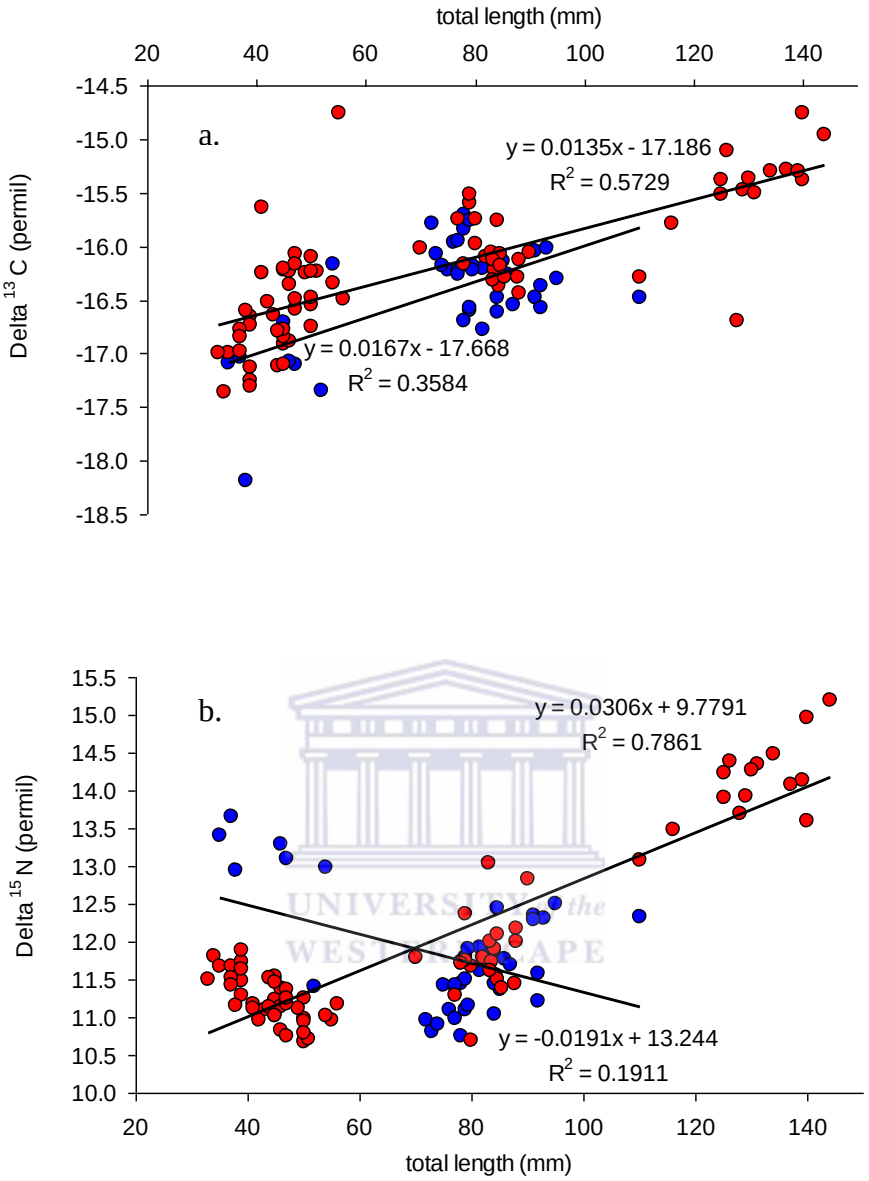


Figure 5: Delta ^{13}C (a) and $\delta^{15}N$ (b) signatures of individual gobies of various sizes as collected from the two stations: station A (blue) and station B (red).

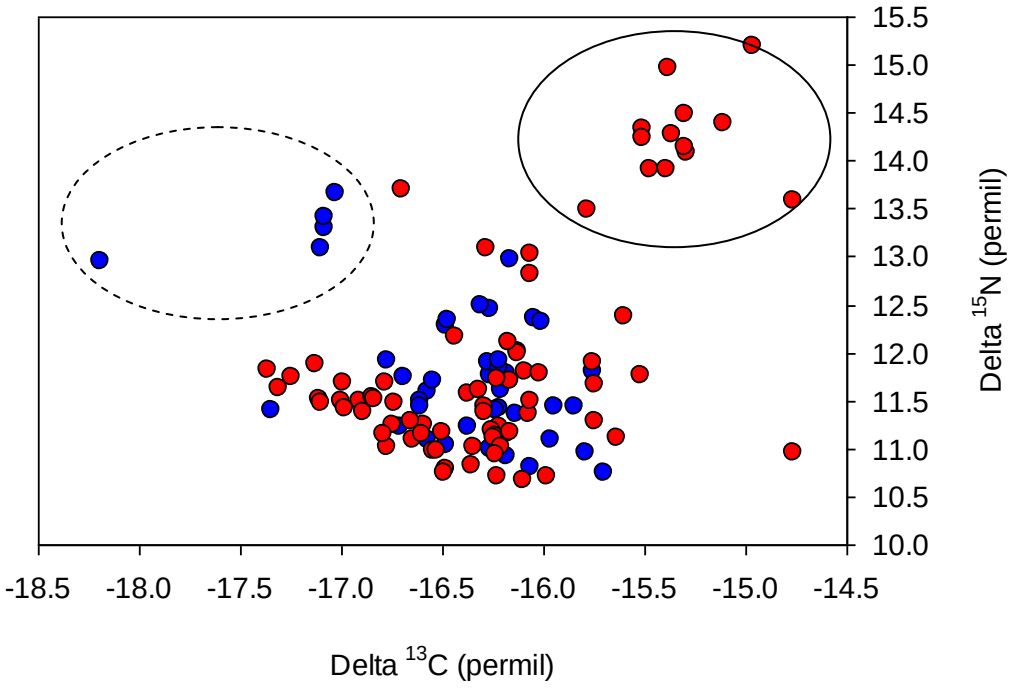


Figure 5c: Delta ^{13}C and δ^{15}N signatures of individual gobies of various sizes as collected from the two stations: station A (blue) and station B (red). The dotted line corresponds to small gobies collected inshore while the solid line represents large gobies collected offshore.

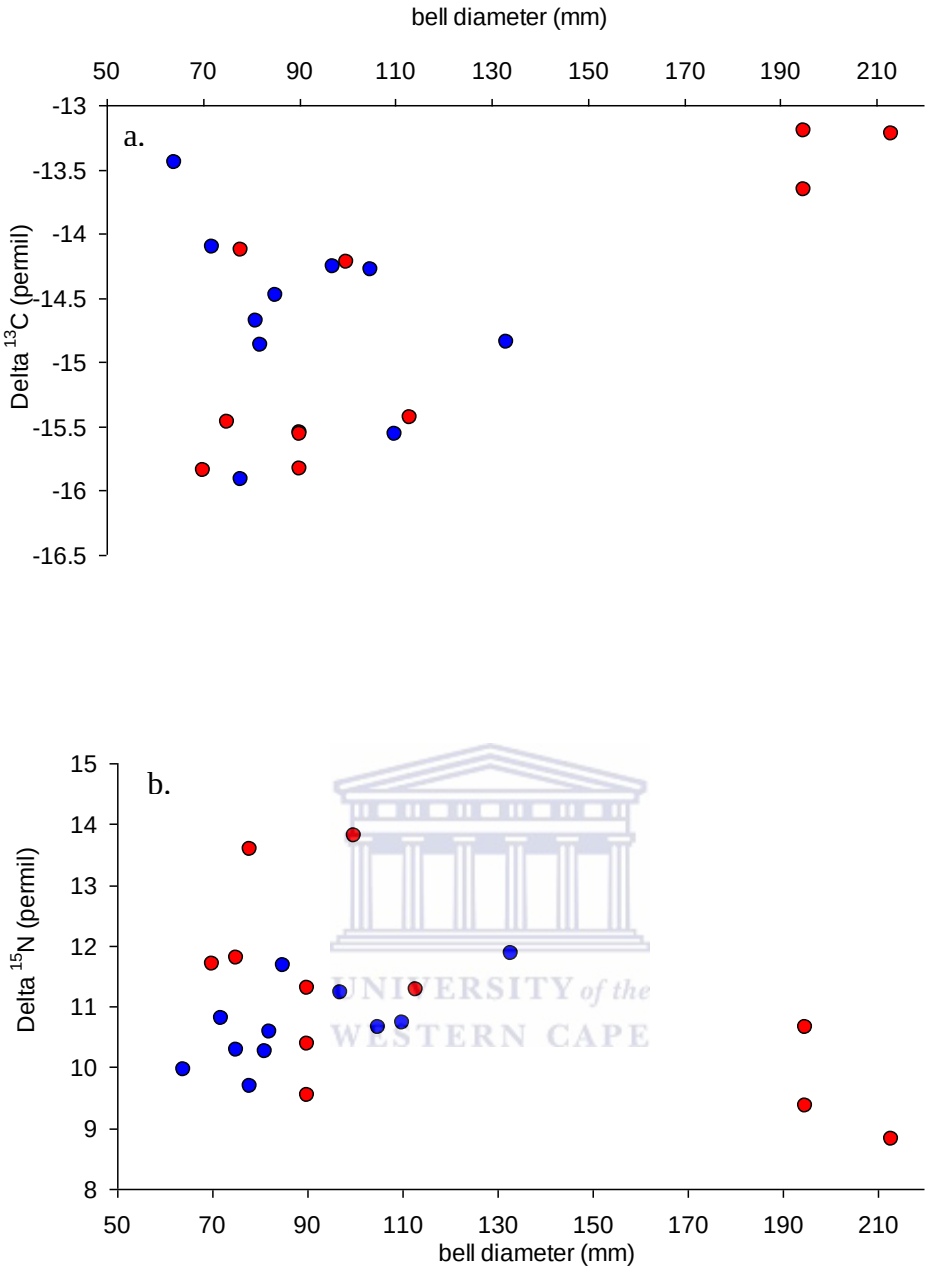


Figure 6: Delta ^{13}C (a) and $\delta^{15}N$-signatures (b) of individual *Aequorea forskalea* of various sizes from the two main sampling stations: station A (blue) and station B (red).

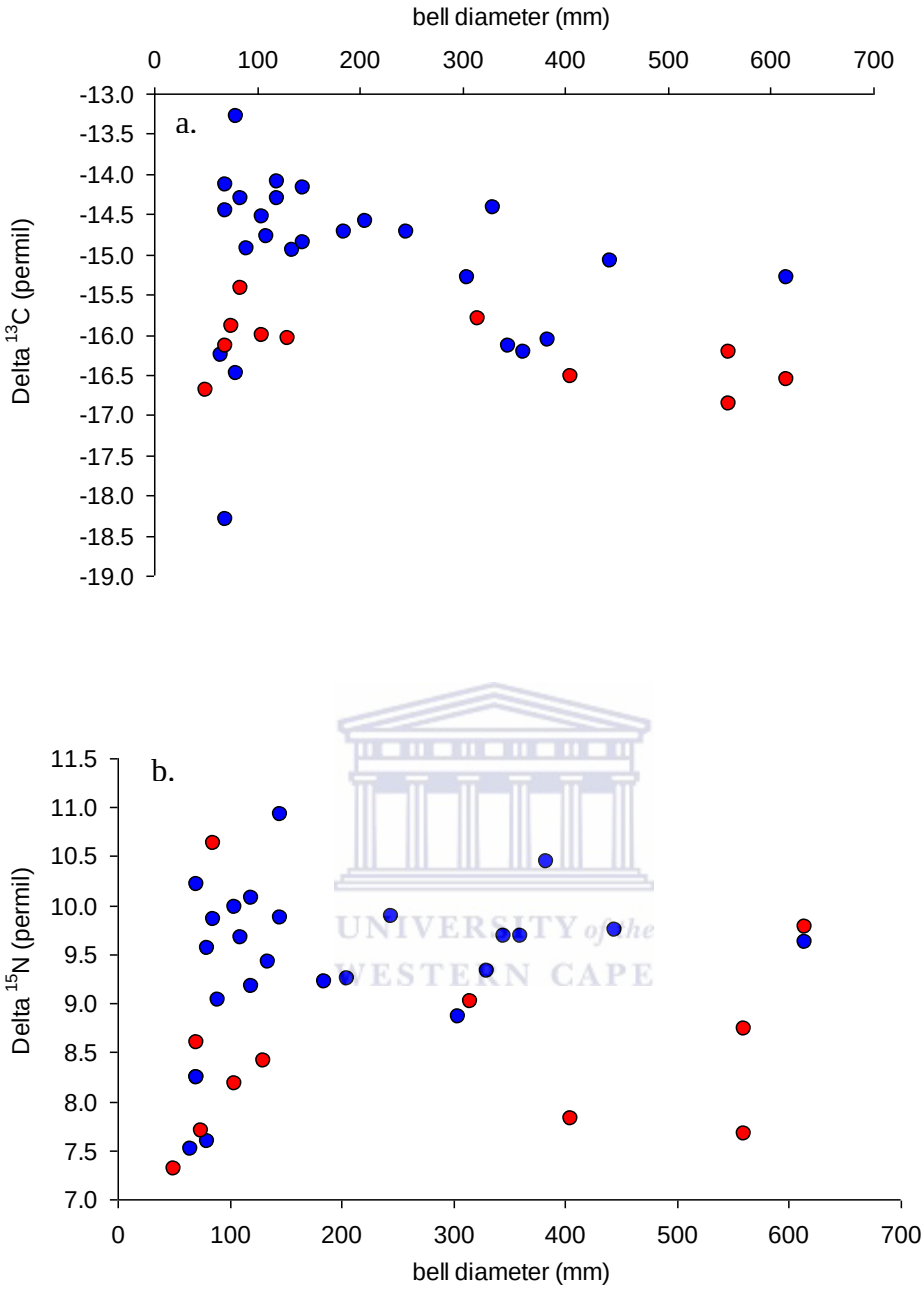


Figure 7: Delta ^{13}C (a) and δ^{15}N-signatures (b) of individual *Chrysaora fulgida* of various sizes from the two main sampling stations: station A (blue) and station B (red).

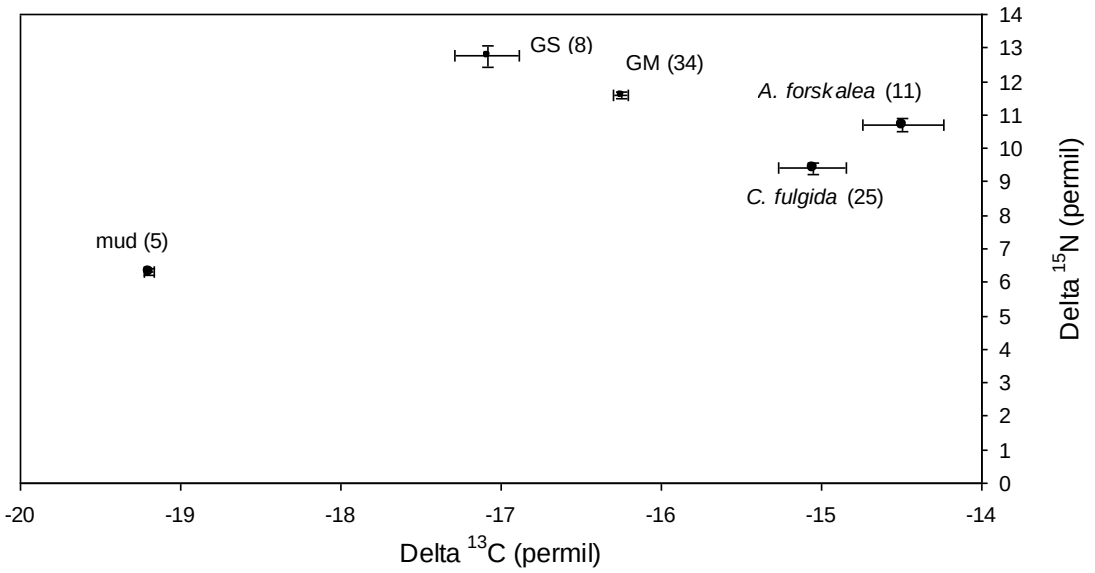


Figure 8a: Mean (± SE) $\delta^{13}C$ and $\delta^{15}N$ of goby (*Sufflogobius bibarbatus*) size fractions and its potential prey items collected from station A. Sample sizes are shown in brackets. GS= small gobies; GM= medium sized gobies. No large gobies or zooplankton samples were collected at station A.

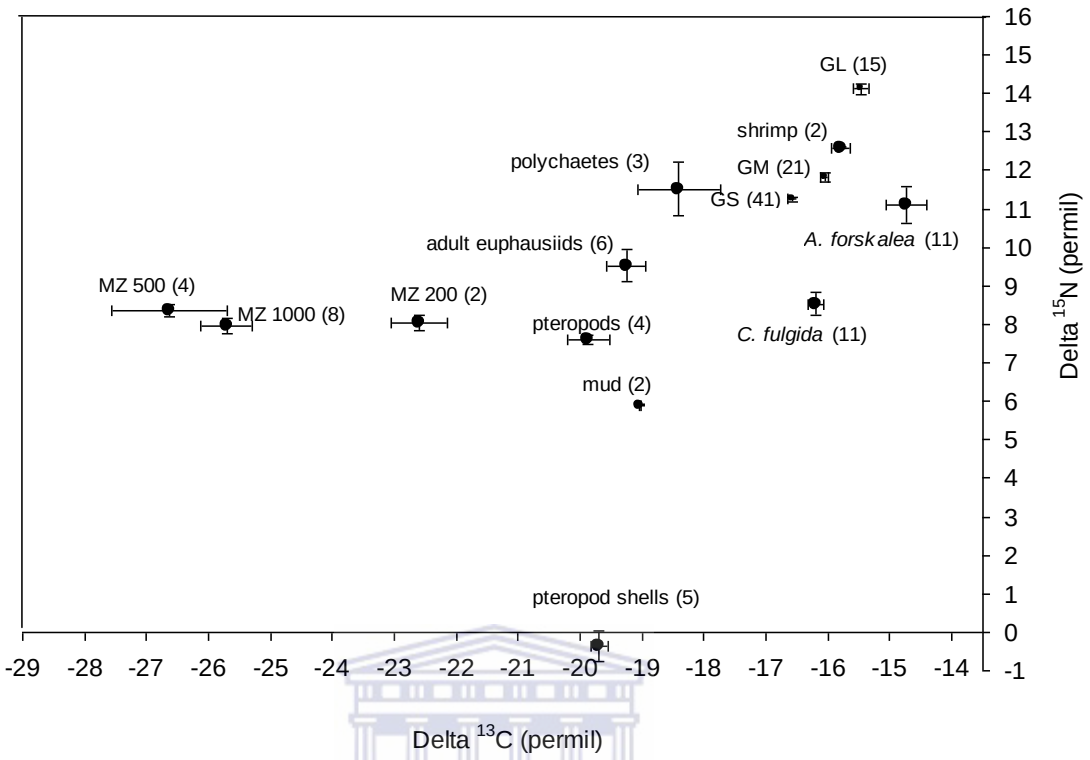


Figure 8b: Mean (± SE) $\delta^{13}C$ and $\delta^{15}N$ of goby (*Sufflogobius bibarbatus*) size fractions and its potential prey items collected from station B. Sample sizes are shown in brackets and only zooplankton samples that provided sufficient material for two or more homogenate samples are presented here. The remaining single homogenate zooplankton samples are included in Fig. A.2. GS= small gobies; GM= medium sized gobies; GL= large gobies.

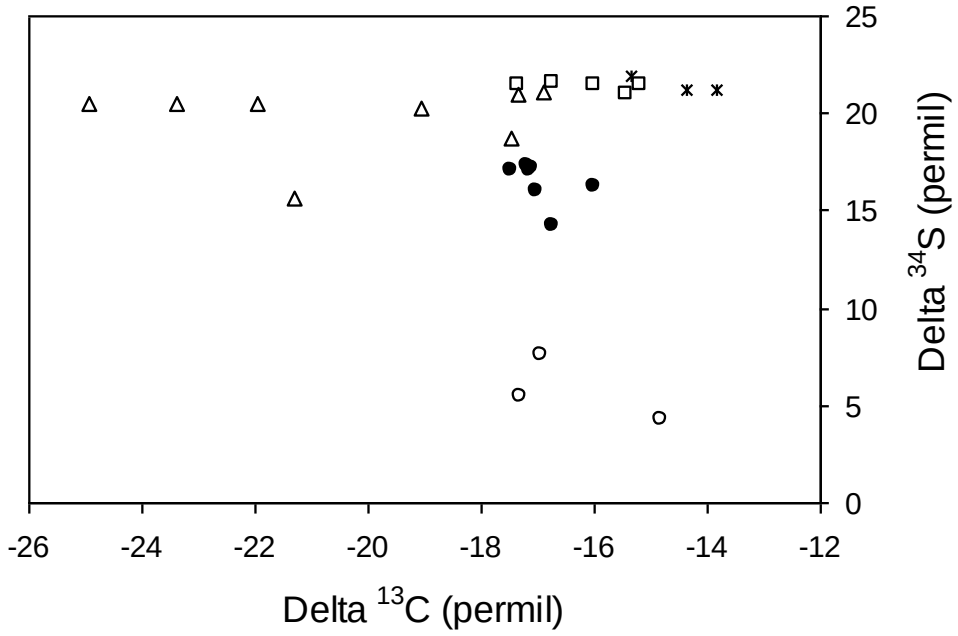


Figure 9: A Crossplot of $\delta^{13}C$ versus $\delta^{34}S$ of benthic sediment (white circles; n= 3), *Aequorea forskalea* (stars; n= 3), *Chrysaora fulgida* (white squares; n= 5), mixed zooplankton (triangles; n= 8) and *Sufflogobius bibarbatus* (black circles; n= 7).

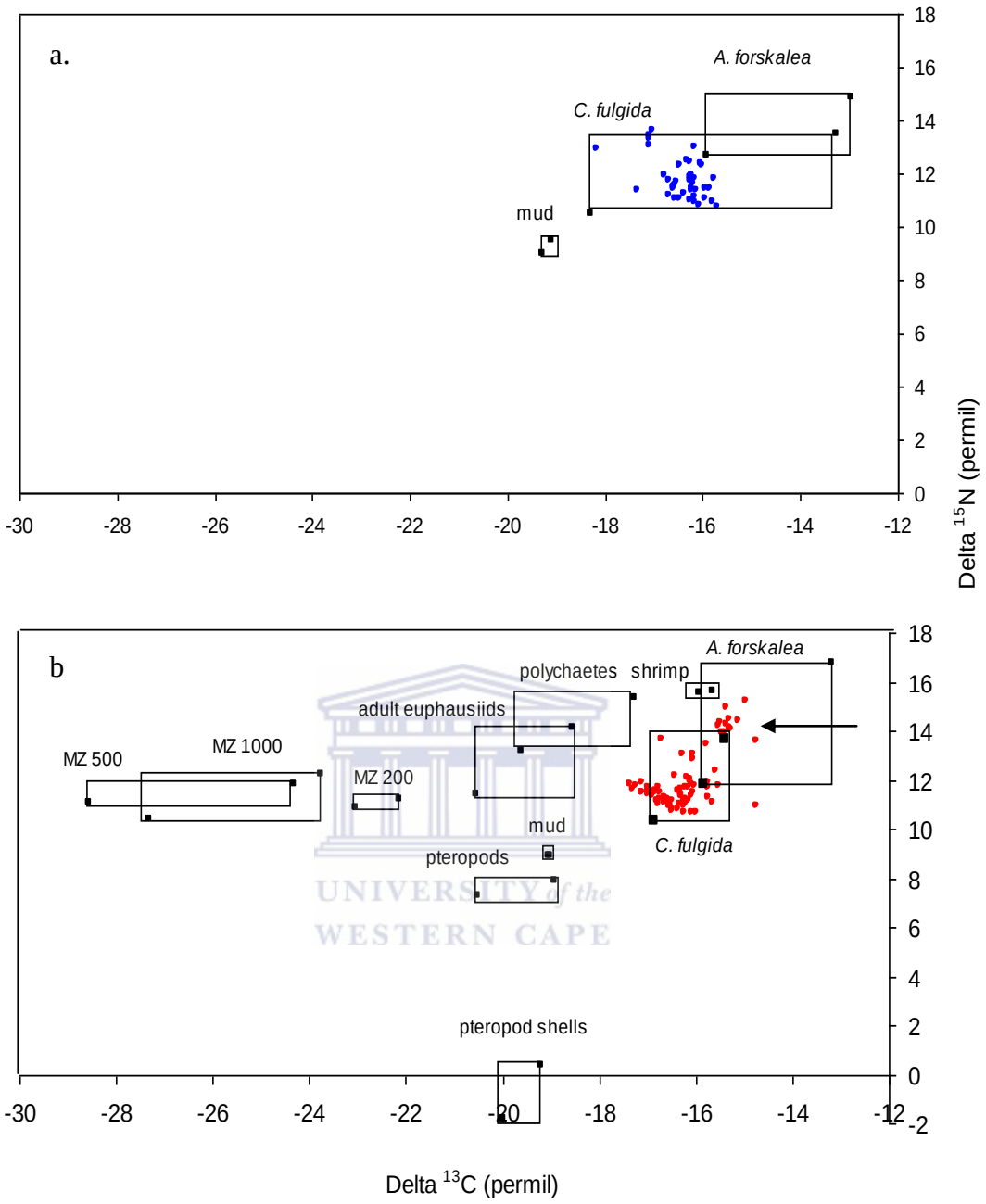


Figure 10: Trophic shift boxes of gobies collected at station A (a) and station B (b), related to the upper and lower limits of the $\delta^{13}C$ and $\delta^{15}N$- signatures found in the potential prey items collected from station A and station B respectively. The position of large gobies collected at station B is indicated with an arrow. These large gobies fed almost exclusively on *Aequorea forskalea.*

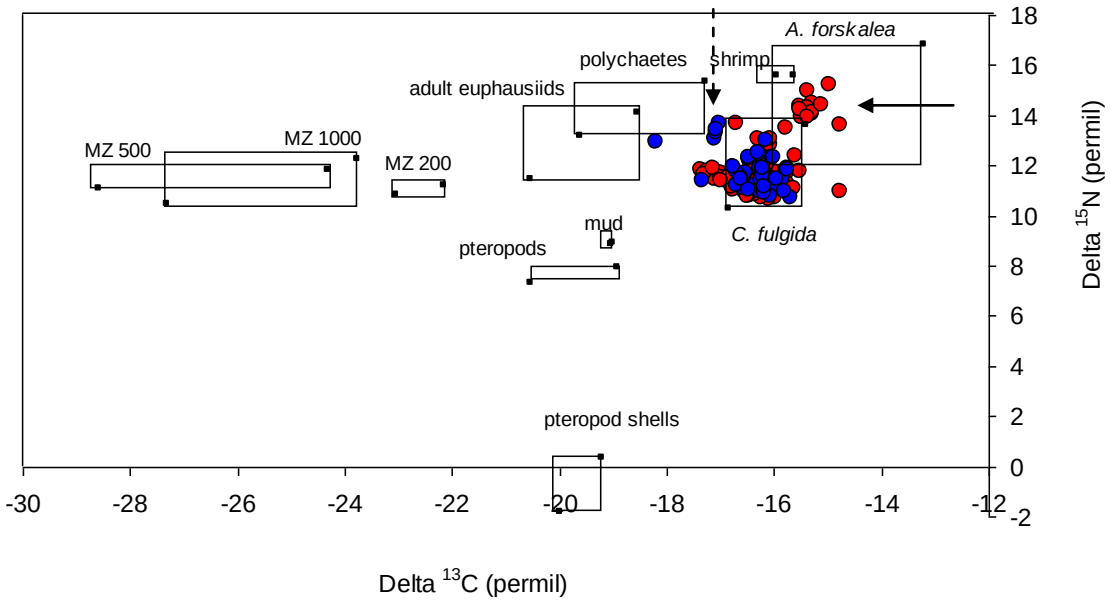


Figure 10c: Trophic shift box of gobies collected at station A (blue) and B (red), related to the upper and lower range of the $\delta^{13}C$ and $\delta^{15}N$-signatures of potential prey items collected from station B. Large gobies collected at station B are indicated with a solid arrow while small gobies collected at station A are indicated with by a dashed arrow.

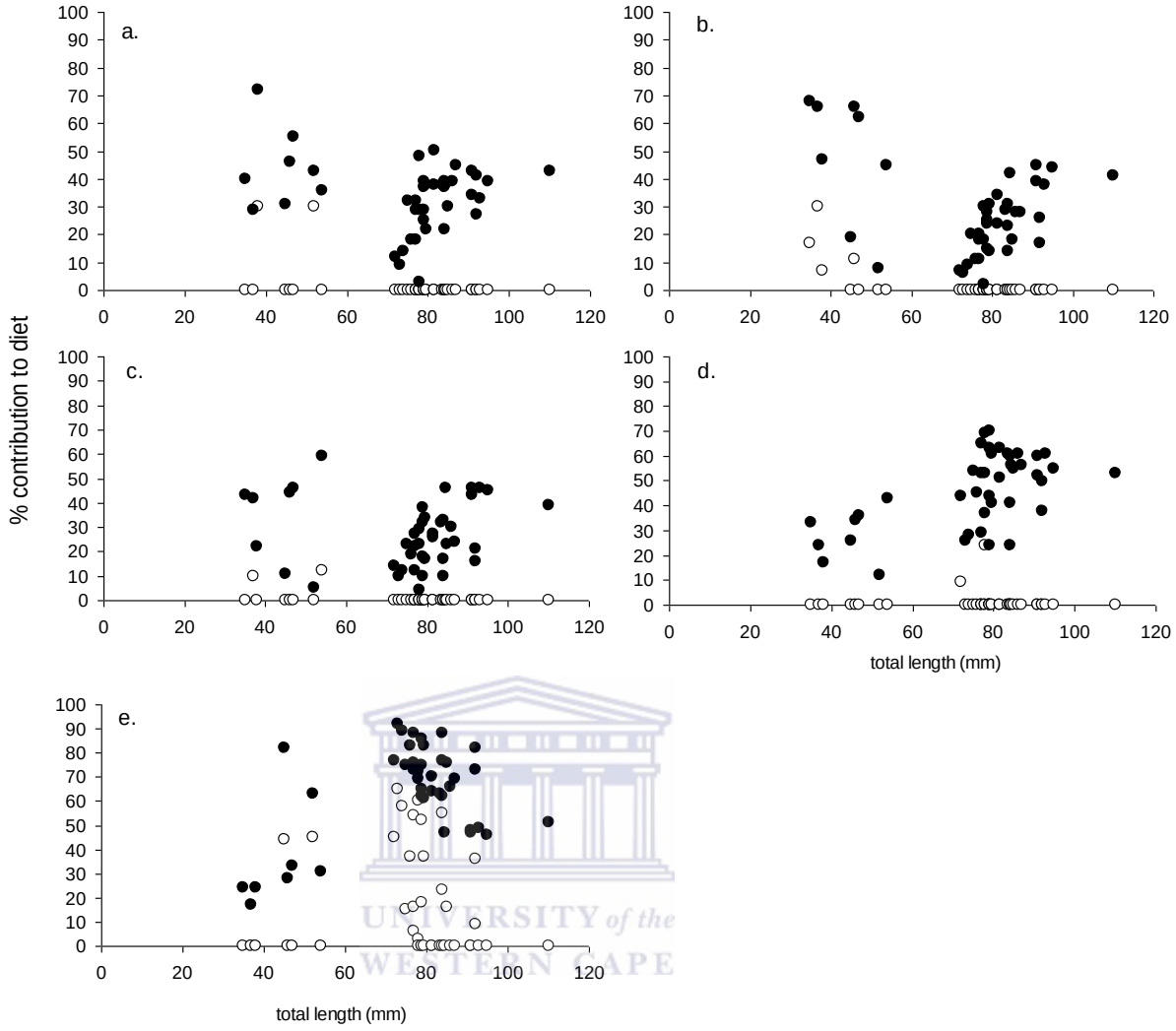


Figure 11: Minimum (white) and maximum (black) percentage contribution of adult euphausiids (a), polychaetes (b), *Aequorea forskalea* (c), *Chrysaora fulgida* 1 (d) and *C. fulgida* 2 (e) to the diet of *Sufflogobius bibarbatus* of varying sizes. *S. bibarbatus* and jellyfish used in this model were collected from station A but zooplankton prey are from station B.

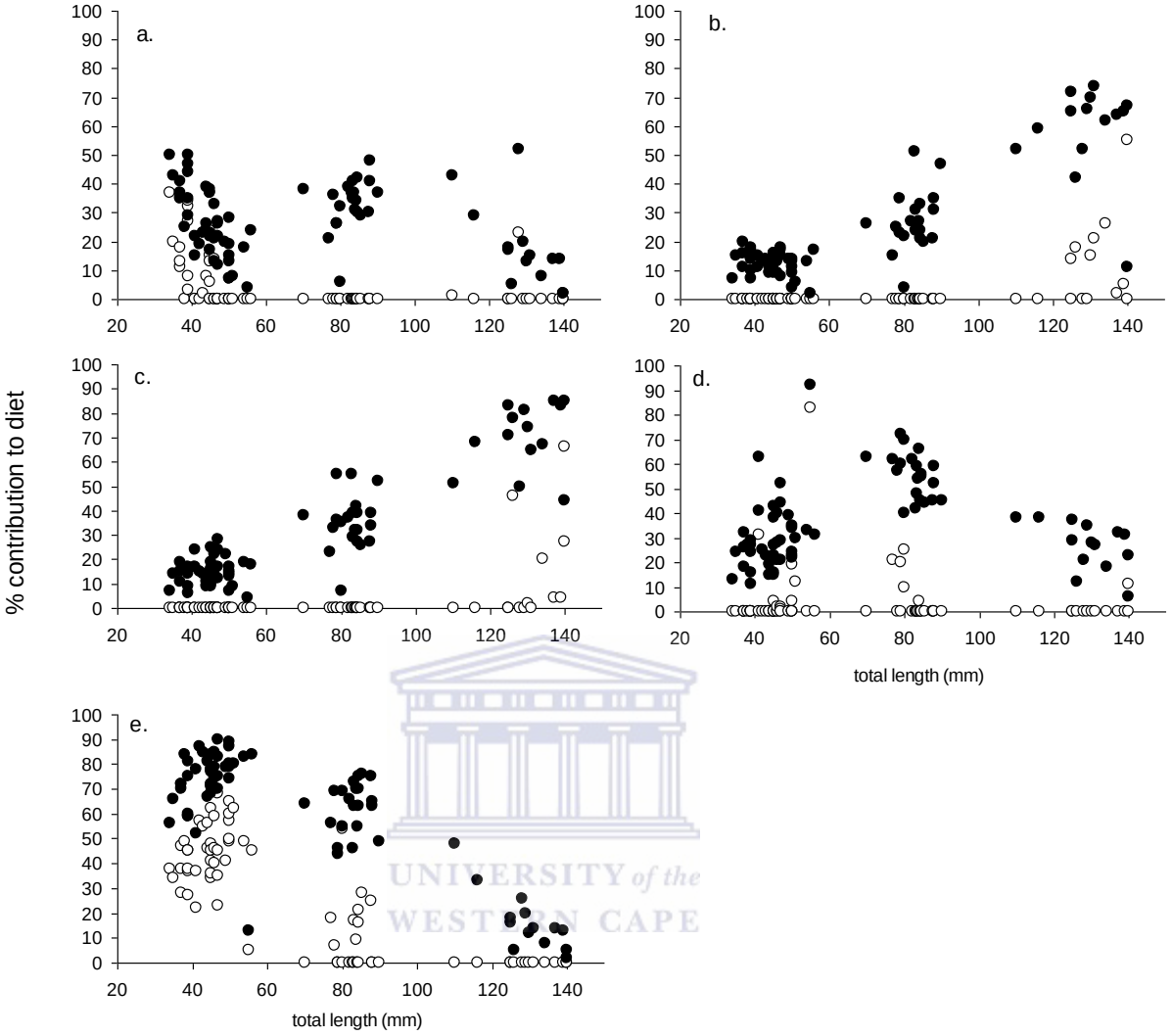


Figure 12: Minimum (white) and maximum (black) percentage contribution of adult euphausiids (a), shrimp (b), *Aequorea forskalea* (c), *Chrysaora fulgida* 1 (d) and *C. fulgida* 2 (e) to the diet of *Sufflogobius bibarbatus* of varying size. *S. bibarbatus*, jellyfish and zooplankton prey were collected from station B.

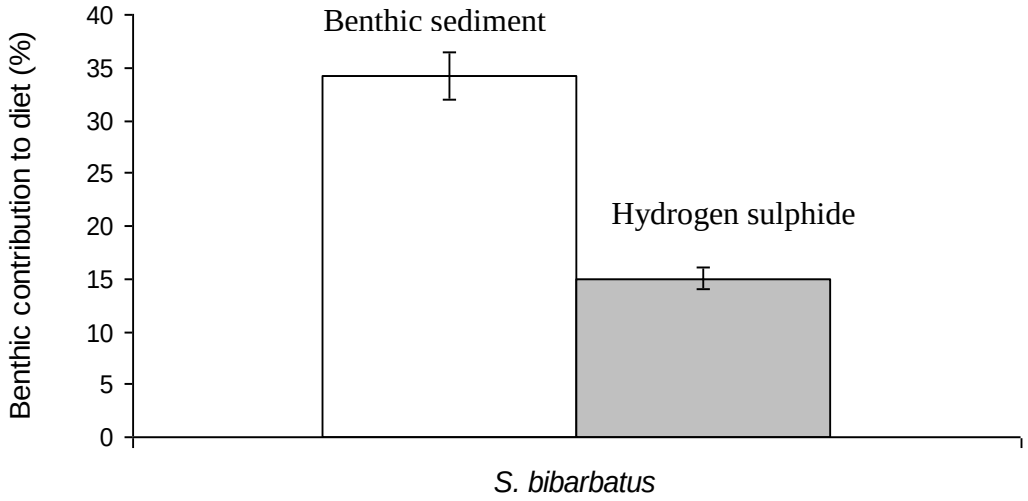


Figure 13: Relative benthic dietary contributions (mean percentage ± SD) from benthic sediment and porewater hydrogen sulphide respectively as calculated from a two-end member mixing model based on the δ^{34}S-signatures of seven gobies.

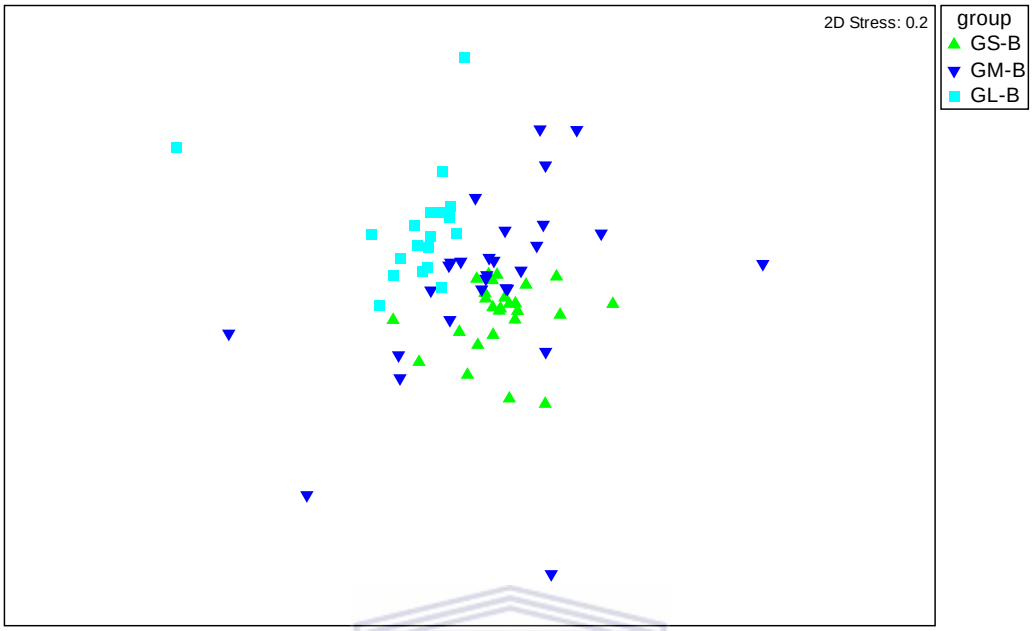
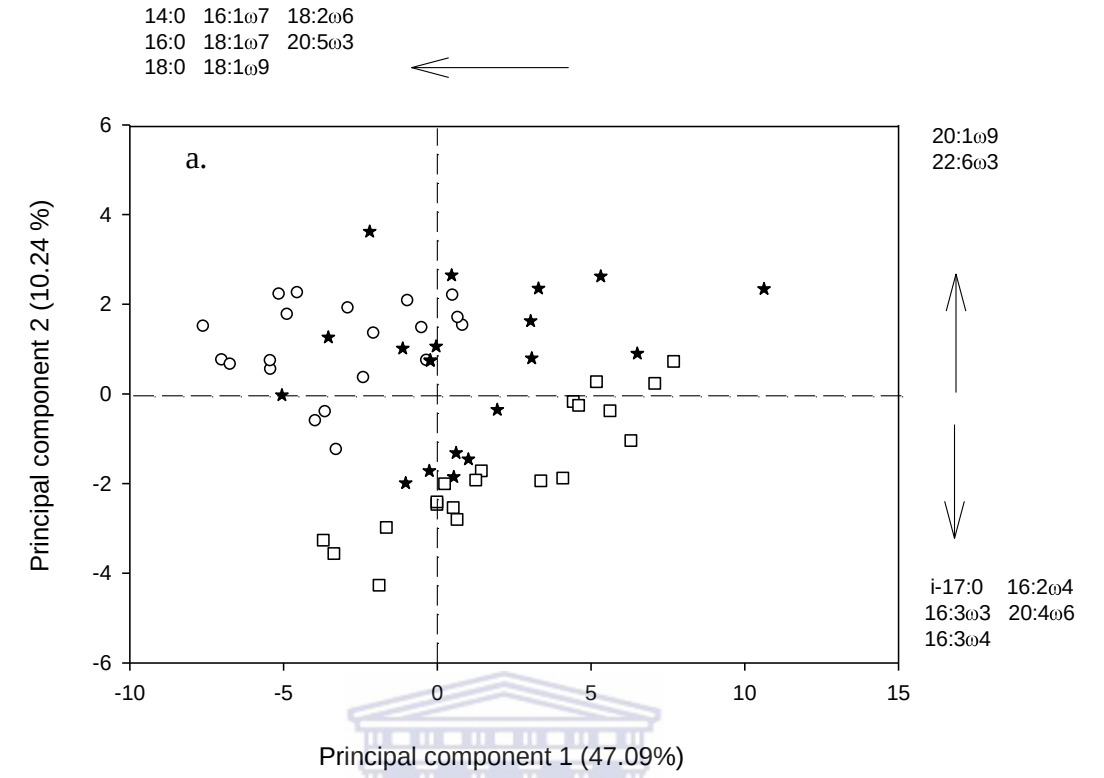


Figure 14: MDS ordination based on proportional individual fatty acids of *Sufflogobius bibarbatus* collected from station B. GS-B= small gobies, GM-B= medium sized gobies, GL-B= large gobies.



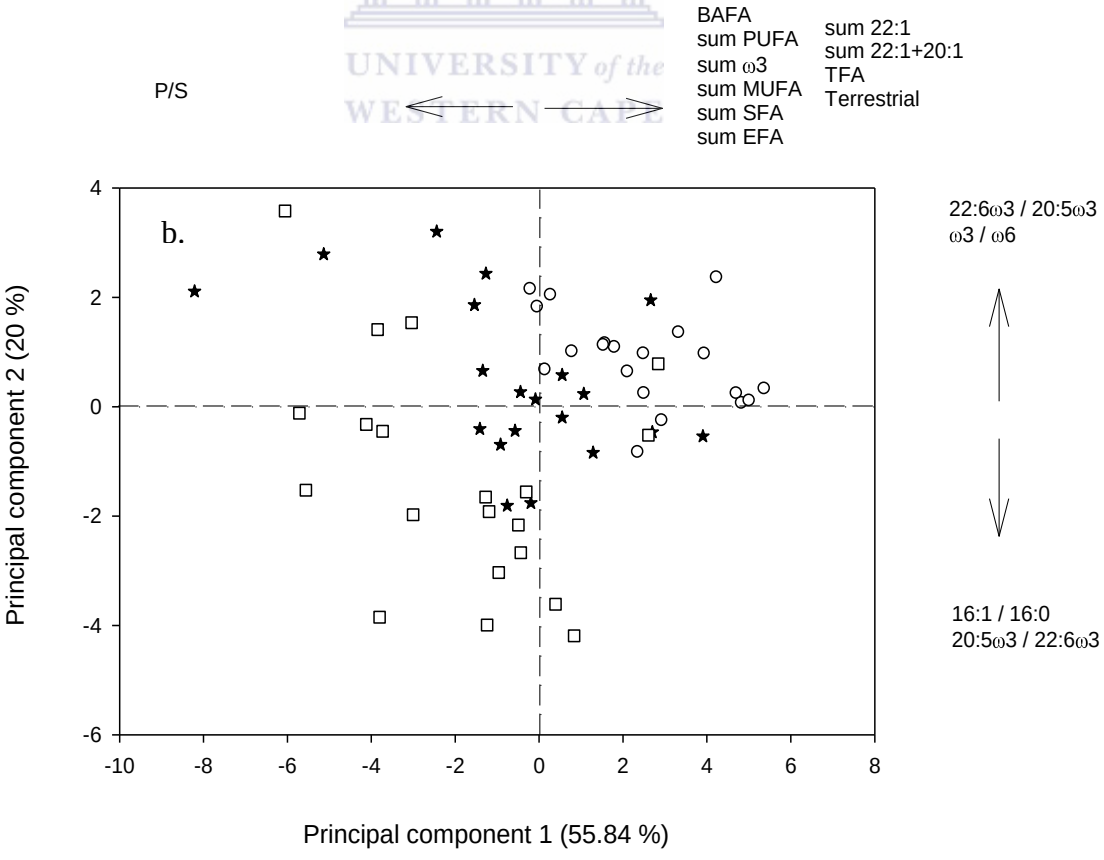


Figure 15: Principal component ordination based on quantitative fatty acid composition (µg/mg DW) of gobies collected from station B. Goby size classes are small indicated as circles, medium sized gobies indicated with stars and large gobies indicated with squares. Presented here are fatty acids (a) and summaries of these fatty acids (b). Arrows running parallel to axes represent the most influential fatty acids with loading values greater than 0.2. Dashed lines denote the origin. Percentage values represent the proportion of the variance explained by each principal component. Data presented here were transformed using mixed transformations but see text for more details.

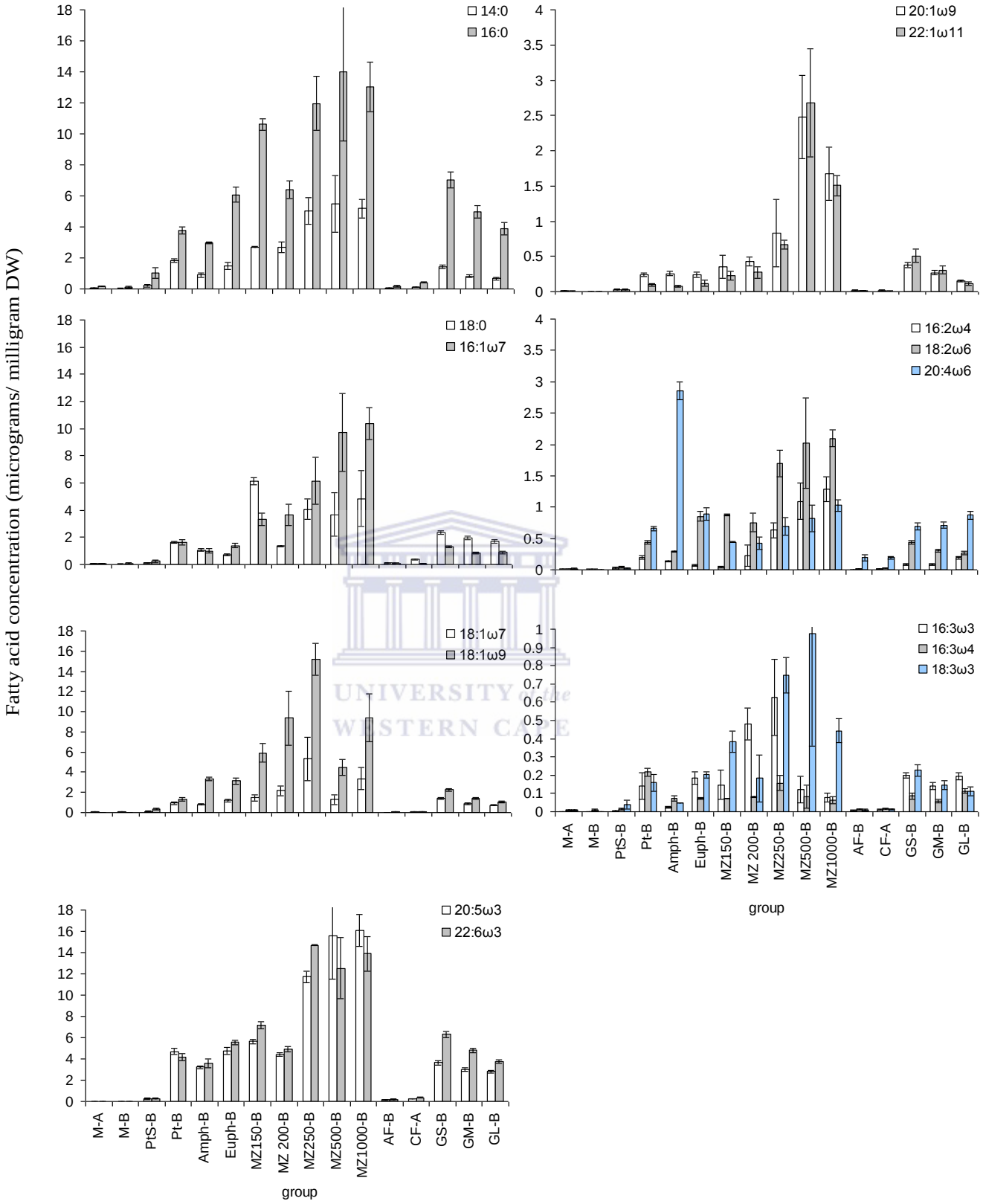

Fatty acid concentration (micrograms/ milligram DW)
14:0
16:0
20:1ω9
22:1ω11
18:0
16:1ω7
16:2ω4
18:2ω6
20:4ω6
18:1ω7
18:1ω9
16:3ω3
16:3ω4
18:3ω3
20:5ω3
22:6ω3
M-A
M-B
PtS-B
Pt-B
Amph-B
Euph-B
MZ150-B
MZ 200-B
MZ250-B
MZ500-B
MZ1000-B
AF-B
CF-A
GS-B
GM-B
GL-B
group

Figure 16: Quantitative comparison of the concentrations of fatty acid variables most responsible for differences between sample groups (as generated by PCA). Values are mean ± SE. M-A= mud collected at station A; M-B= mud collected at station B; PtS-B= pteropod shells; Pt-B= pteropods; Amph-B= amphipods; Euph-B= adult euphausiids; MZ150-B - MZ1000-B= mixed zooplankton of sizes 150 µm-1000 µm; AF-B= *Aequorea forskalea*; CF-A= *Chrysaora fulgida* collected at station A; GS-B= small gobies; GM-B= medium sized gobies; GL-B= large gobies. All samples presented here were collected offshore at station B except *C. fulgida* which at station A and mud which were collected at both sampling stations.

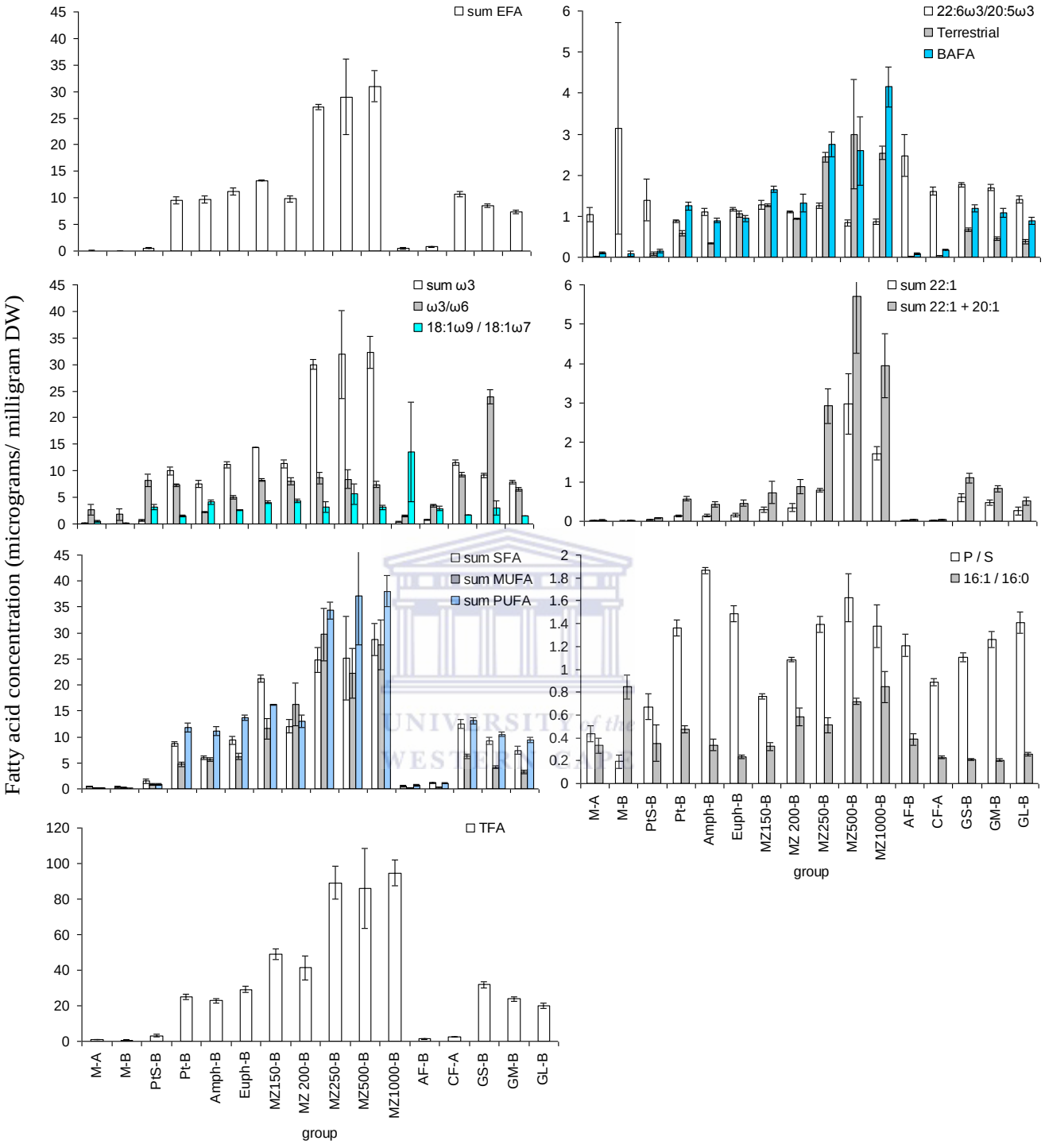

Fatty acid concentration (micrograms/ milligram DW)
sum EFA
22:6ω3/20:5ω3
Terrestrial
BAFA
sum ω3
ω3/ω6
18:1ω9 / 18:1ω7
sum 22:1
sum 22:1 + 20:1
sum SFA
sum MUFA
sum PUFA
P / S
16:1 / 16:0
TFA
M-A
M-B
PtS-B
Pt-B
Amph-B
Euph-B
MZ150-B
MZ 200-B
MZ250-B
MZ500-B
MZ1000-B
AF-B
CF-A
GS-B
GM-B
GL-B
group

Figure 17: Quantitative comparison of the concentrations of fatty acid summary/ratio variables most responsible for differences between sample groups (as generated by PCA). Values are mean ± SE. Sample group descriptions are as described in Fig. 16.

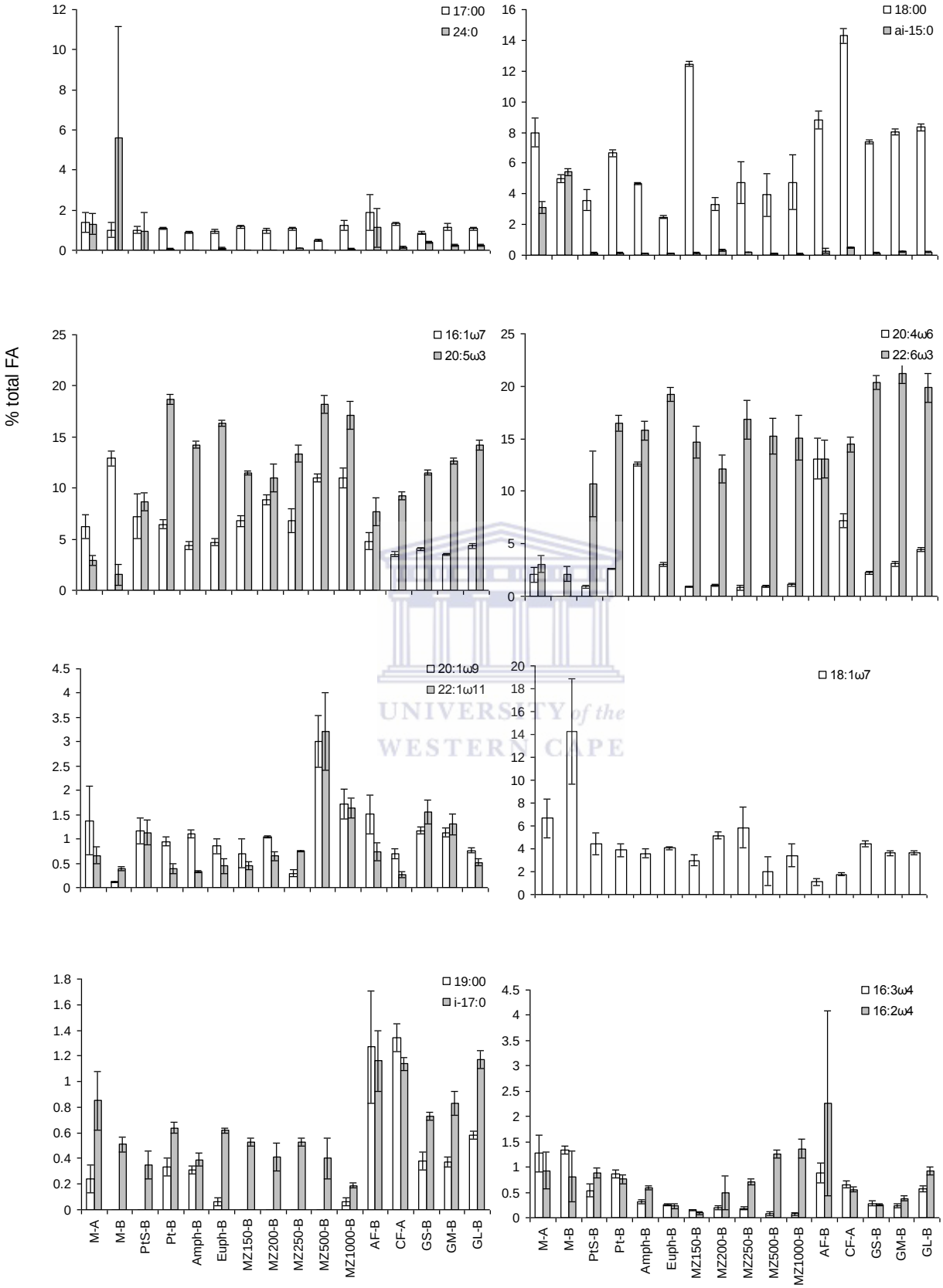


Figure 18: Qualitative comparison of the concentrations of a selected number of key fatty acid variables of gobies and their potential prey items. Values are mean ± SE. M-A= mud collected at station A; M-B= mud collected at station B; PtS-B= pteropod shells; Pt-B= pteropods; Amph-B= amphipods; Euph-B= adult euphausiids; MZ150-B - MZ1000-B= mixed zooplankton of sizes 150 µm-1000 µm; AF-B= *Aequorea forskalea*; CF-A= *Chrysaora fulgida* collected at station A; GS-B= small gobies; GM-B= medium sized gobies; GL-B= large gobies. All samples presented here were collected offshore at station B except *C. fulgida* which at station A and mud which were collected at both sampling stations.

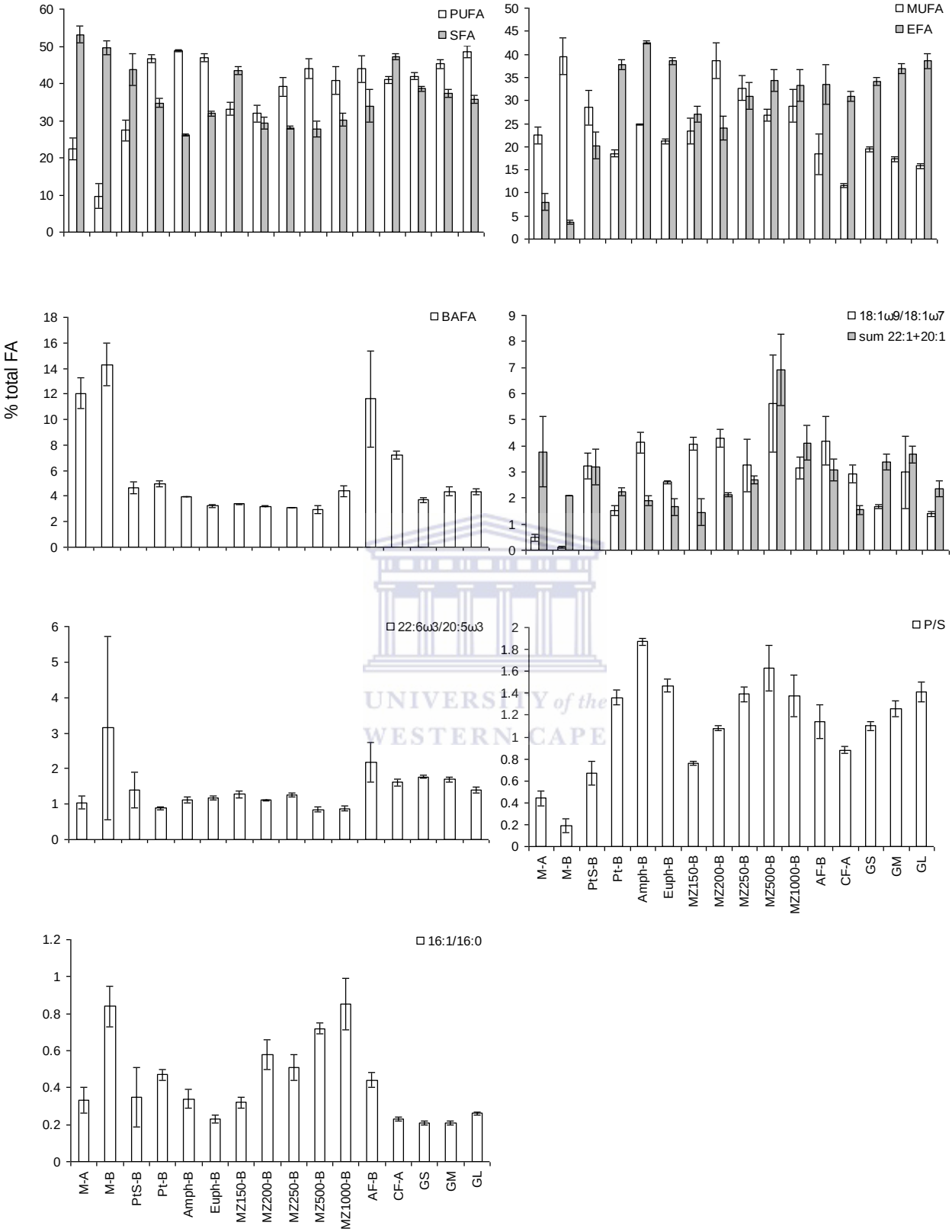
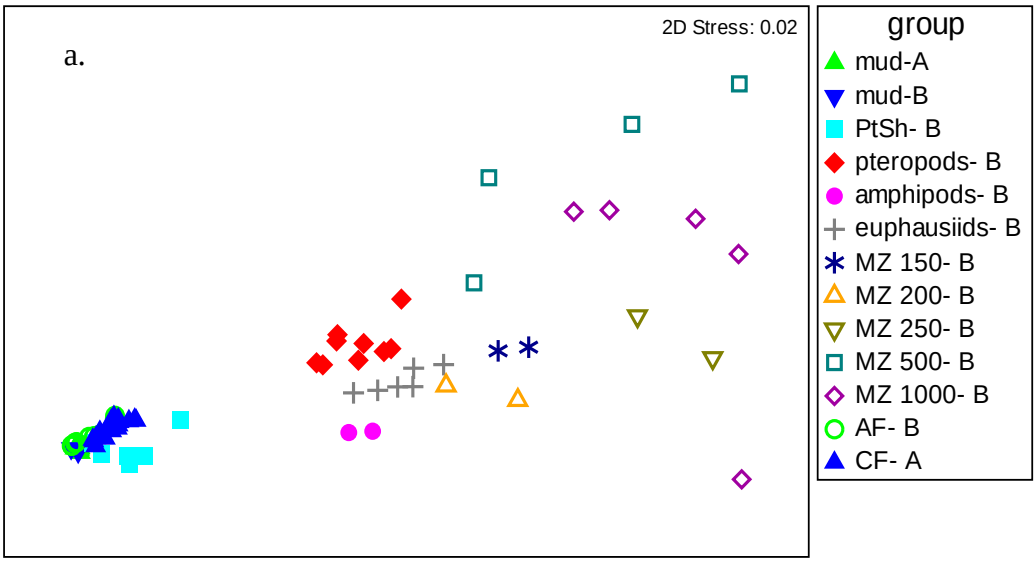


Figure 19: Qualitative comparison of a selected number of key fatty acid summary/ratio variables of gobies and their potential prey items. Values are mean ± SE. Sample group descriptions are as described in Fig. 18.



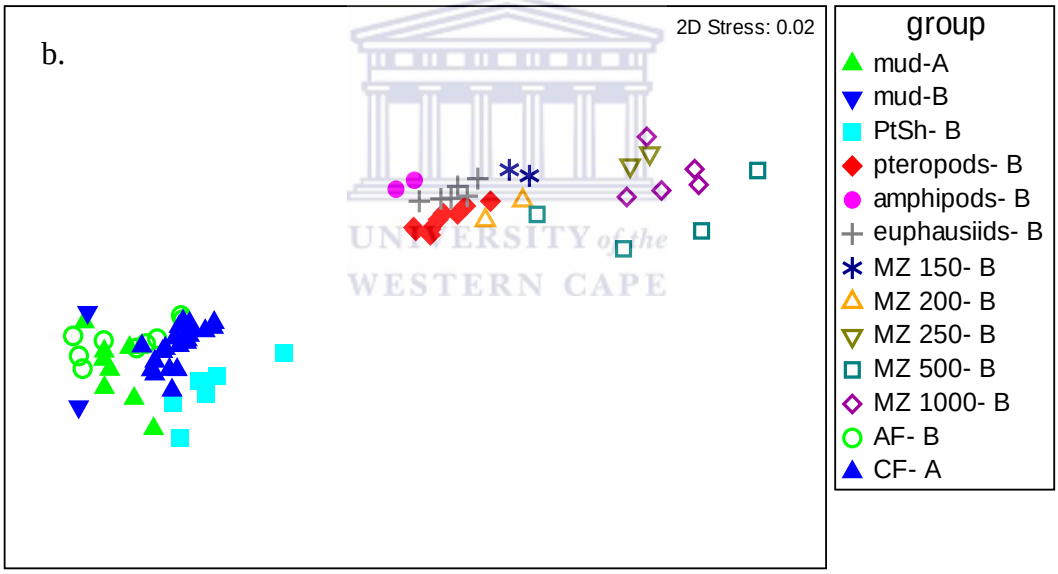
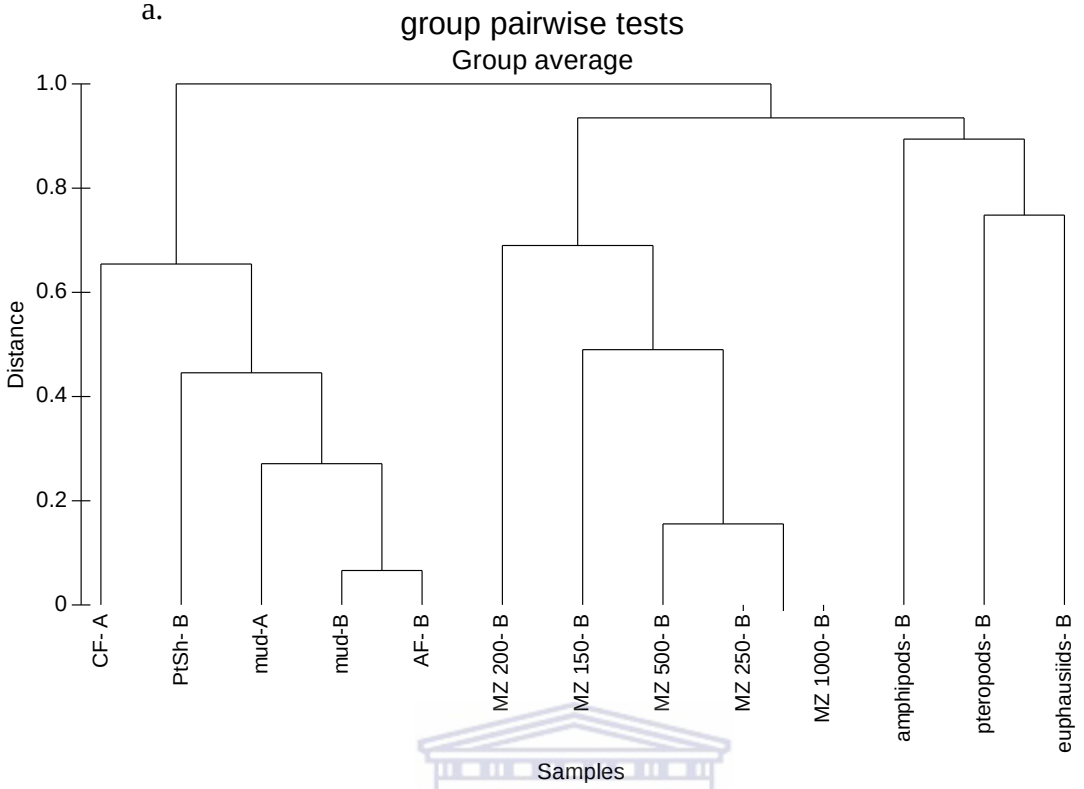
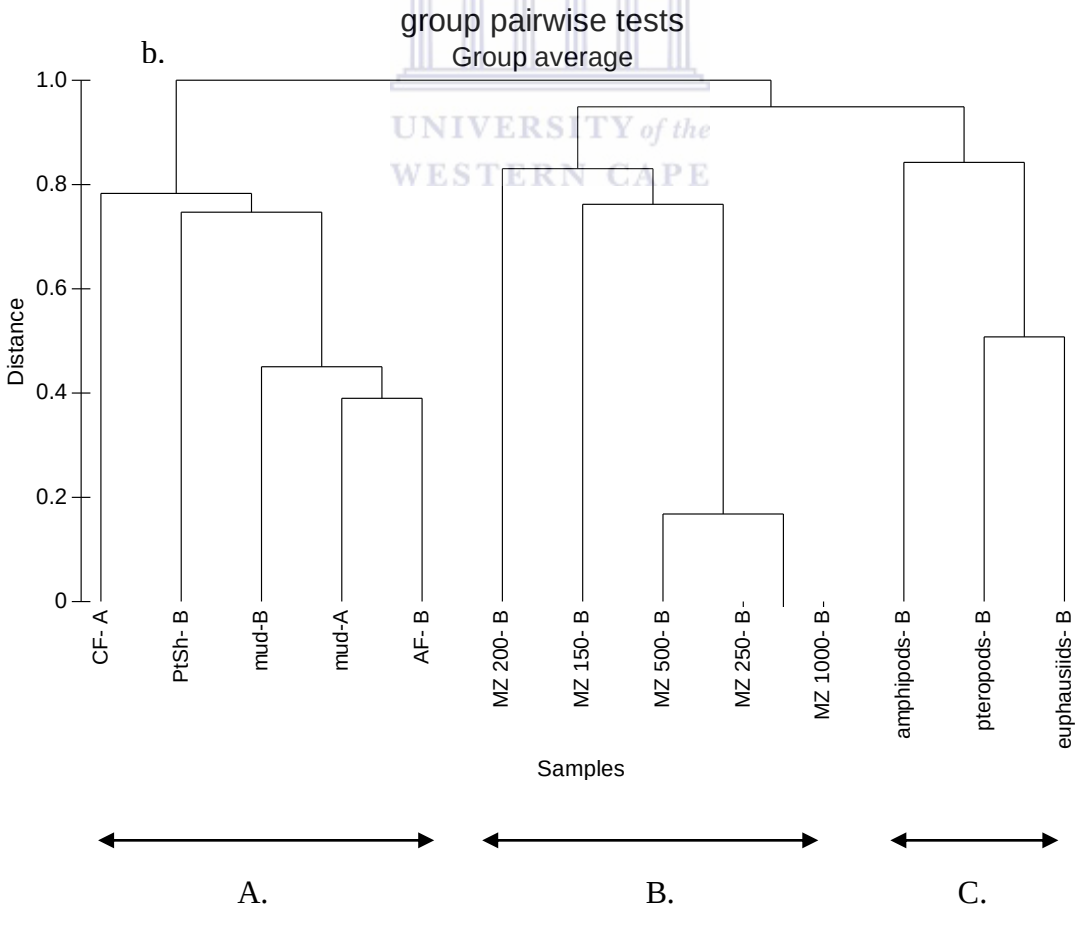


Figure 20: MDS ordination based on the absolute FA concentrations of the potential prey items of *Sufflogobius bibarbatus*. Individual fatty acids (a) and fatty acid summaries/ratios (b) are presented here. PtS-B= pteropod shells; MZ 150-B - MZ 1000-B= mixed zooplankton of sizes between 150 µm -1000 µm; AF-B= *Aequorea forskalea*; CF-A= *Chrysaora fulgida*. All samples presented here were collected offshore at station B, except *C. fulgida* which were collected at station A and benthic mud which were collected at both station A and station B.

a.



b.



A. B. C.

Figure 21: Dendrogram based on pairwise R comparisons generated by ANOSIM of the quantitative FA profiles of individual fatty acids (a) and fatty acid marker ratios (b) of the potential prey items of *Sufflogobius bibarbatus*. Global R= 0.789 and 0.879 respectively. PtS-B= pteropod shells; MZ 150-B - MZ 1000-B= mixed zooplankton of sizes between 150 µm -1000 µm; AF-B= *Aequorea forskalea*; CF-A= *Chrysaora fulgida*. All samples presented here were collected offshore at station B, except *C. fulgida* which were collected at station A and benthic mud which were collected at both sampling stations. A, B and C indicate the different groupings within the dataset based on individual fatty acids (a) and fatty acid ratios (b).

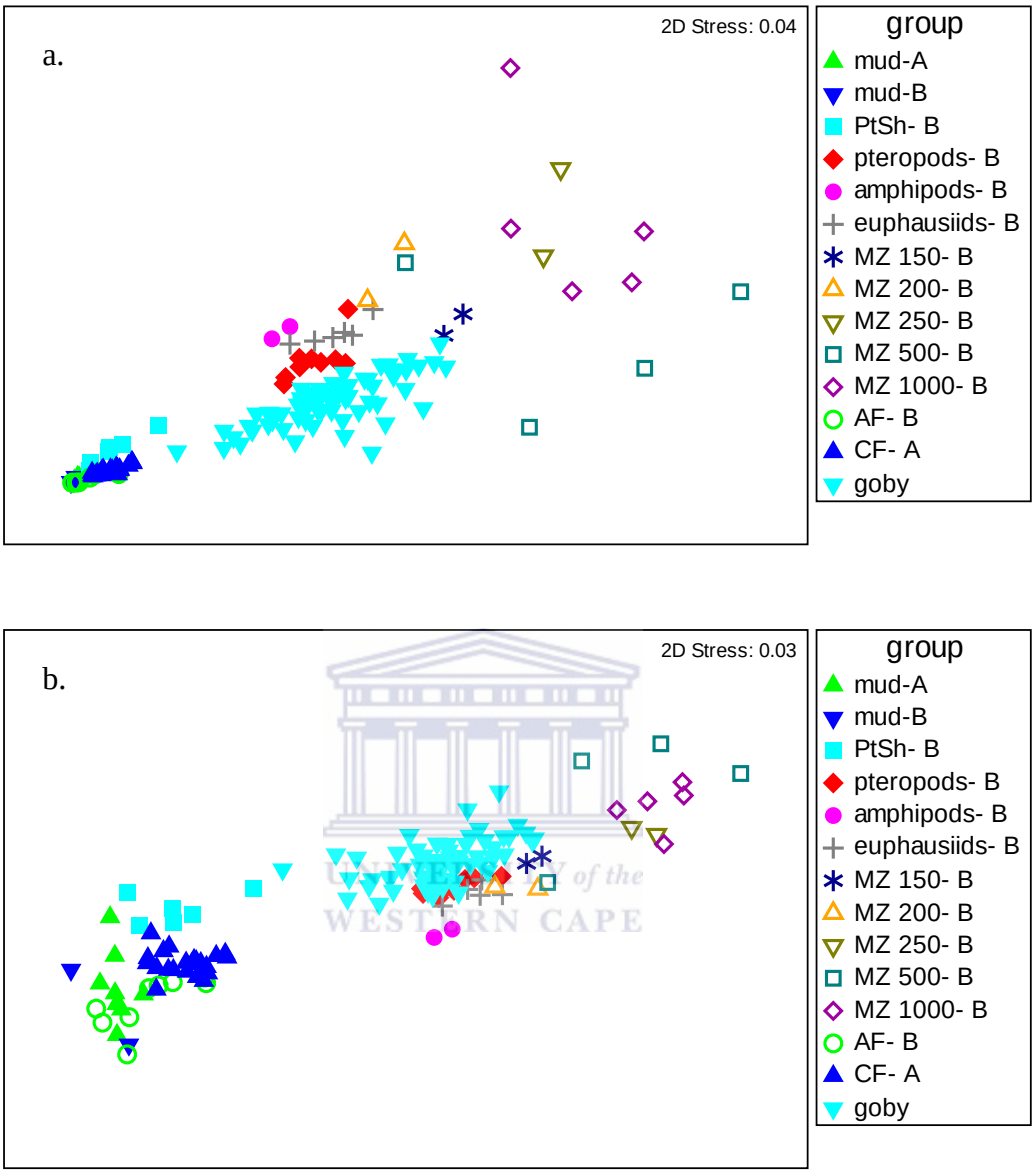
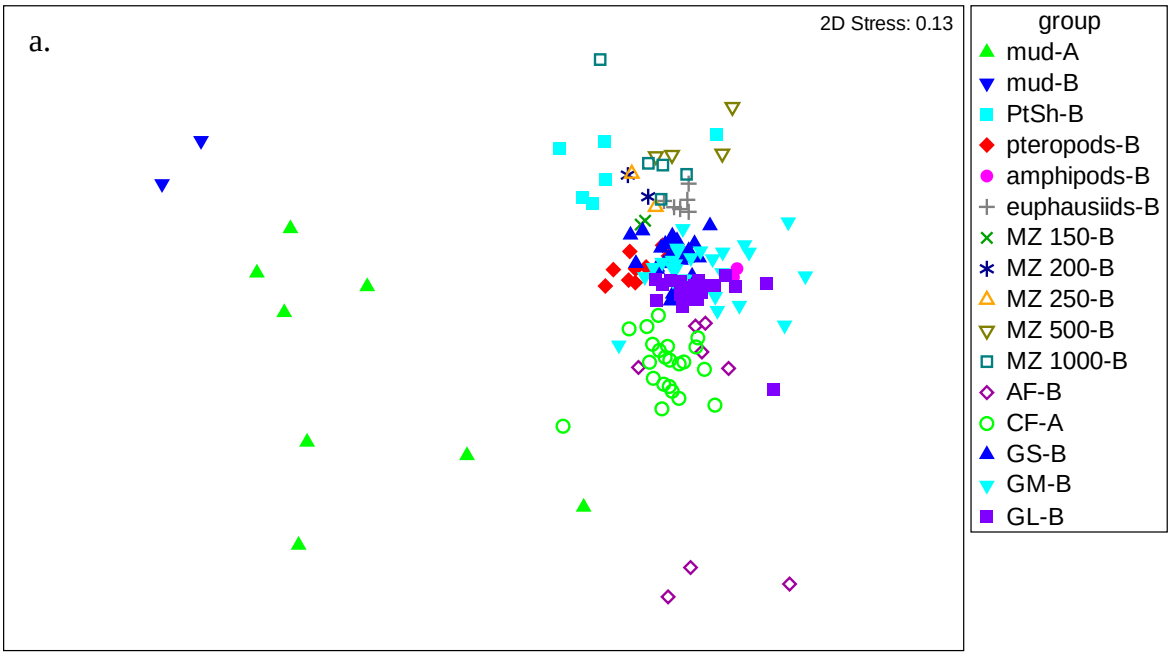


Figure 22: MDS representation of the quantitative profiles of individual fatty acids (a) and fatty acid summaries/ratios (b) of *Sufflogobius bibarbatus* and its potential prey items. Sample group descriptions are as described in Fig. 21.


a.
2D Stress: 0.13
group
mud-A
mud-B
PtSh-B
pteropods-B
amphipods-B
euphausiids-B
MZ 150-B
MZ 200-B
MZ 250-B
MZ 500-B
MZ 1000-B
AF-B
CF-A
GS-B
GM-B
GL-B

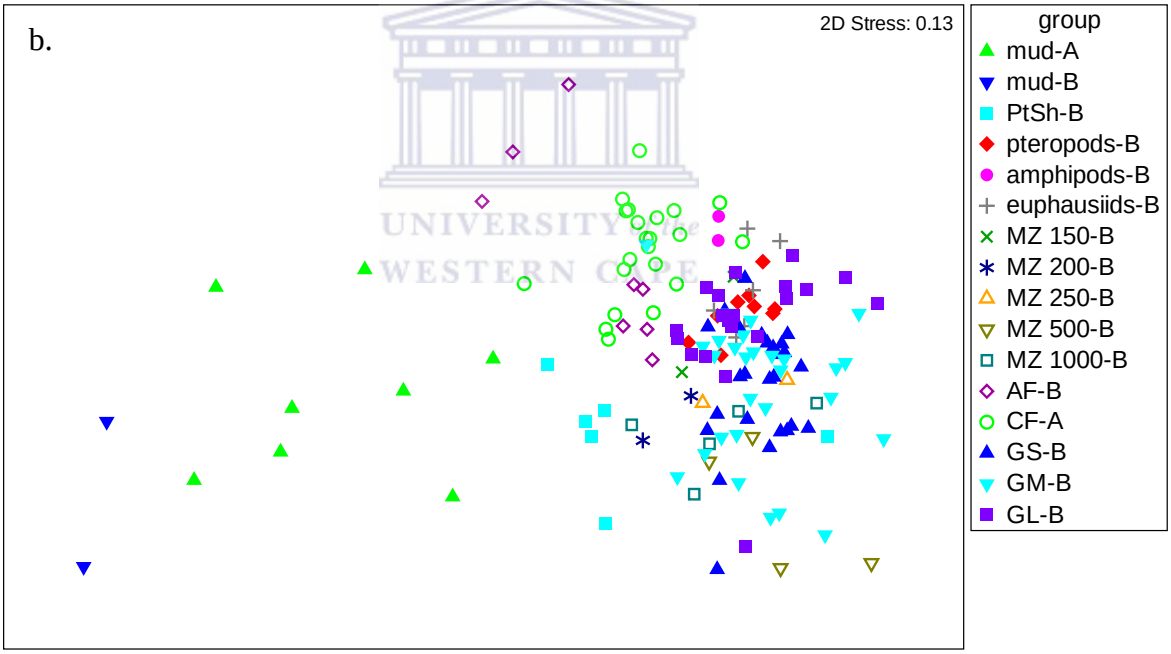

b.
2D Stress: 0.13
group
mud-A
mud-B
PtSh-B
pteropods-B
amphipods-B
euphausiids-B
MZ 150-B
MZ 200-B
MZ 250-B
MZ 500-B
MZ 1000-B
AF-B
CF-A
GS-B
GM-B
GL-B

Figure 23: MDS ordination of the qualitative FA profiles of *Sufflogobius bibarbatus* and its potential prey items. Individual fatty acids (a) and fatty acid summaries/ ratios (b) are presented. PtS-B= pteropod shells; MZ150-B - MZ1000-B= mixed zooplankton of sizes between 150 µm -1000 µm; AF-B= *Aequorea forskalea*; CF-A= *Chrysaora fulgida*. GS-B= small gobies, GM-B= medium sized gobies, GL-B= large gobies. All samples presented here were collected offshore at station B, except *C. fulgida* which were collected at station A and benthic mud which were collected at both station A and station B.

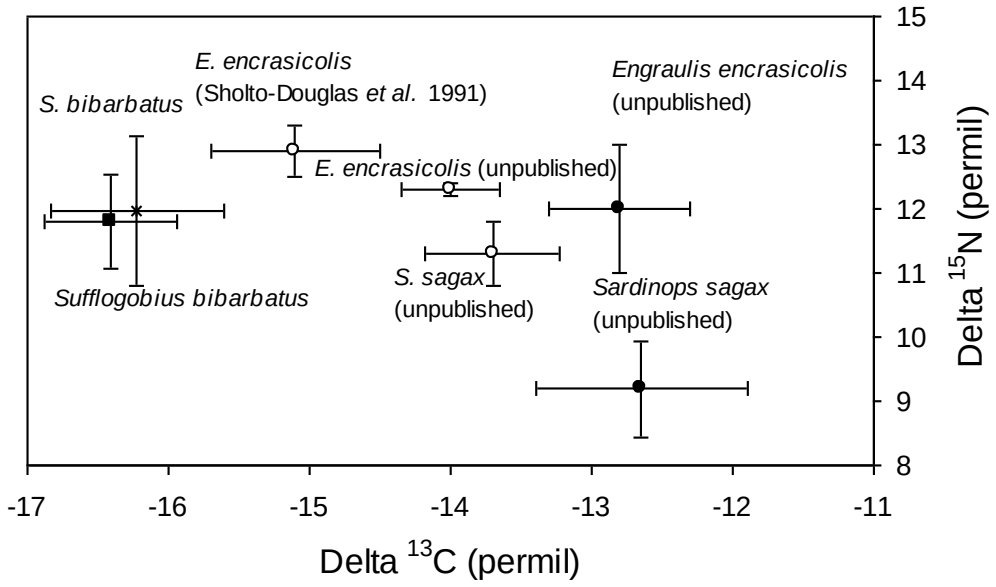


Figure 24: Mean (± SD) $\delta^{13}C$ and $\delta^{15}N$ of the goby (*Sufflogobius bibarbatus*) as collected from station A (black square) and B (star) in addition to sardine (*Sardinops sigax*) and anchovy (*Engraulis encrasicolis*) as collected from the southern (white circles) and northern Benguela (black circles) region respectively.

Credit to: T. Miller, Ehime University, Japan, and C.D. van der Lingen, MCM, South Africa (unpublished data).

TABLES

	[1]Stable isotopes (C, N)		[2]Stable isotopes (S,C,N)	Fatty acids	
	station A	station B	spatially pooled	station A	station B
Mud	5	2	3	8	2
Pteropod shells	NI	5	NI	NI	6
Amphipods	NI	NI	NI	NI	2
Pteropods	NI	4	NI	NI	9
Adult euphausiids	NI	6	NI	NI	6
MZ 150	NI	NI	NI	NI	2
MZ 200	NI	2	NI	NI	2
MZ 250	NI	NI	NI	NI	2
MZ 500	NI	4	NI	NI	4
MZ 1000	NI	8	NI	NI	5
MZ total	NI	14	8	NI	15
Aequorea forskalea	11	11	3	NI	9
Chrysaora fulgida	25	11	5	22	NI
goby large	NI	15	NI	NI	20
goby medium	34	21	NI	NI	20
goby small	8	41	NI	NI	20
goby total	42	77	7	NI	60

[1] Samples analyzed at the University of Cape Town, South Africa.

[2] Samples analyzed at the University of Stockholm.

Table 1: Summary of the number of samples included in stable isotope and fatty analyses. NI= groups that were not included in statistical analyses. MZ= mixed zooplankton. Samples that were analyzed at the University of Stockholm were spatially pooled and chemically untreated.

	size class	copepods	juvenile euphausiids	fish eggs	chaetognaths	bivalve larvae	gastropod larvae	echinoderm larvae	cumaceans	medusae
1	MZ 1000	66.3	26.0	0.0	7.7	0.0	0.0	0.0	0.0	0.0
2	MZ 1000	63.9	36.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	MZ 1000	40.5	59.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	MZ 500	84.5	1.4	0.0	0.0	0.0	0.0	0.0	8.5	5.6
5	MZ 500	96.0	0.9	0.6	2.3	0.2	0.0	0.0	0.0	0.0
6	MZ 500	98.7	0.4	0.4	0.4	0.0	0.0	0.0	0.0	0.0
7	MZ 500	80.4	15.0	1.8	0.0	0.0	0.0	0.0	2.9	0.0
8	MZ 250	70.0	0.0	0.0	0.0	22.1	0.2	0.0	2.9	0.0
9	MZ 250	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	MZ 150	85.0	0.0	0.0	0.0	14.2	0.6	0.2	0.0	0.0
11	MZ 150	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 2: Taxonomic composition and abundance of mixed zooplankton used for stable isotope analyses. Values are expressed as percentage (%) of the total number of individuals in a particular sample. MZ= mixed zooplankton of different size (µm).

prey source	fish size	N	minimum % contribution	maximum % contribution
adult euphausiids	<57 mm	8	7.5±4.91	44±4.97
	58mm - 90 mm	34	0	31.47±1.95
polychaetes	< 57 mm	8	8.13±3.85	47.63±8.12
	58mm - 90 mm	34	0	23.82±1.96
A. forskalea	< 57 mm	8	2.75±1.81	34±6.72
	58mm - 90 mm	34	0	25.53±2.03
C. fulgida 1	< 57 mm	8	0	28.13±3.65
	58mm - 90 mm	34	0.97±0.75	50.09±2.23
C. fulgida 2	< 57 mm	8	11.13±7.28	37.75±7.98
	58mm - 90 mm	34	17.79±3.87	69.91±2.26

Table 3: Average (± SE) minimum and maximum percentage contribution of prey items to the diet of *Sufflogobius bibarbatus* as collected from station A. Minimum and maximum contributions were generated using a 5-end member Isosource model. See text for more details.

prey source	fish size	N	minimum % contribution	maximum % contribution
adult euphausiids	< 57 mm	41	8.17±1.73	26.85±1.87
	58 mm- 90 mm	21	0	33.14±1.93
	> 90 mm	14	1.71±1.64	18±3.89
shrimp	< 57 mm	41	0	12.44±0.62
	58 mm- 90 mm	21	0	27.05±2.2
	> 90 mm	14	11.14±4.18	58.64±4.34
A. forskalea	< 57 mm	41	0	14.73±0.85
	58 mm- 90 mm	21	0	35.1±2.39
	> 90 mm	14	12.07±5.55	70.36±3.68
C. fulgida 1	< 57 mm	41	3.85±2.18	29.56±2.29
	58 mm- 90 mm	21	3.81±1.74	55.05±2.03
	> 90 mm	14	0.79±0.79	26.79±2.62
C. fulgida 2	< 57 mm	41	44.1±1.99	73.85±2.1
	58 mm- 90 mm	21	9.29±3.07	62.48±2.21
	> 90 mm	14	0	16.71±3.28

Table 4: Average (± SE) minimum and maximum percentage contribution of prey items to the diet of *Sufflogobius bibarbatus* as collected from station B. Minimum and maximum contributions were generated using a 5-end membered Isosource model. See text for more details.

		Benthic contribution to diet	
		Scenario 1	Scenario 2
	$\delta^{34}S$	$\delta^{34}S_{sediment}$ = 5.8‰ vs. VCDT	$\delta^{34}S_{H2S}$ = -15‰ vs. VCDT
S. bibarbatus - 1	16.0	37.0	16.3
S. bibarbatus - 2	17.1	30.0	13.2
S. bibarbatus - 3	17.2	29.8	13.1
S. bibarbatus - 4	17.4	28.6	12.6
S. bibarbatus - 5	16.2	35.6	15.6
S. bibarbatus - 6	17.0	30.5	13.4
S. bibarbatus - 7	14.3	47.6	20.9
mean ± SD benthic contribution		34.2 ± 6.92	15.0 ± 2.95
mean ± SD "enriched pelagic" contribution		65.8 ± 6.72	85 ± 2.95

Table 5: Relative percentage dietary contribution from benthic sediment, porewater hydrogen sulphide and pelagic "enriched $\delta^{34}S$ food sources" to the diet of seven gobies as calculated from a two-end member mixing model. See text for more details.

a.

	mud- A	mud- B	PtSh- B	pteropods- B	amphipods- B	euphausiids- B	MZ 150- B	MZ 200- B	MZ 250- B	MZ 500- B	MZ 1000- B	AF- B	CF- A	goby
mud- A														
mud- B	0.19													
PtSh- B	0.67	0.20												
pteropods- B	1.00	1.00	1.00											
amphipods- B	1.00	1.00	1.00	0.91										
euphausiids- B	1.00	1.00	1.00	0.75	0.88									
MZ 150- B	1.00	1.00	1.00	1.00	1.00	0.99								
MZ 200- B	1.00	1.00	1.00	0.98	1.00	0.84	1.00							
MZ 250- B	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00						
MZ 500- B	1.00	1.00	1.00	0.90	0.64	0.79	0.18	0.25	0.29					
MZ 1000- B	1.00	1.00	1.00	0.97	1.00	0.91	0.29	0.51	-0.11	0.03				
AF- B	0.35	0.07	0.47	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00			
CF- A	0.69	0.79	0.75	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.39		
goby	0.97	0.98	0.92	0.17	0.44	0.37	0.73	0.75	0.99	0.95	0.99	0.96	0.94	

b.

	mud- A	mud- B	PtSh- B	pteropods- B	amphipods- B	euphausiids- B	MZ 150- B	MZ 200- B	MZ 250- B	MZ 500- B	MZ 1000- B	AF- B	CF- A	goby
mud- A														
mud- B	0.41													
PtSh- B	0.72	0.81												
pteropods- B	1.00	1.00	1.00											
amphipods- B	1.00	1.00	1.00	0.86										
euphausiids- B	1.00	1.00	1.00	0.51	0.82									
MZ 150- B	1.00	1.00	1.00	0.96	1.00	0.95								
MZ 200- B	1.00	1.00	1.00	0.87	1.00	0.92	1.00							
MZ 250- B	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00						
MZ 500- B	1.00	1.00	1.00	0.93	0.75	0.85	0.29	0.32	0.04					
MZ 1000- B	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	-0.18	0.30				
AF- B	0.39	0.50	0.70	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00			
CF- A	0.85	0.99	0.78	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.51		
goby	1.00	1.00	0.98	-0.06	0.40	0.05	0.42	0.39	0.94	0.88	0.96	1.00	0.99	

Table 6: ANOSIM pairwise R comparison of the profiles of quantitatively measured individual fatty acids (a) and fatty acid summaries/ratios (b) of *Sufflogobius bibarbatus* and its potential prey items. Global R= 0.707 and R= 0.761 respectively. PtS-B= pteropod shells; MZ150-B - MZ1000-B= mixed zooplankton of sizes between 150 µm -1000 µm; AF-B= *Aequorea forskalea*; CF-B= *Chrysaora fulgida*. All samples presented here were collected offshore at station B, except *C. fulgida* which were collected at station A and benthic mud which were collected at both station A and station B. Highlighted values are where gobies are very similar to a potential prey item.

a.

	mud-A	mud-B	PtSh-B	pteropods-B	amphipods-B	euphausiids-B	MZ 150-B	MZ 200-B	MZ 250-B	MZ 500-B	MZ 1000-B	AF-B	CF-A	GS-B	GM-B	GL-B
mud-A																
mud-B	0.14															
PtSh-B	0.62	1.00														
pteropods-B	0.72	1.00	0.70													
amphipods-B	0.75	1.00	0.30	0.92												
euphausiids-B	0.80	1.00	0.39	0.82	0.82											
MZ 150-B	0.49	1.00	-0.21	0.90	1.00	0.84										
MZ 200-B	0.67	1.00	-0.27	0.94	1.00	0.69	1.00									
MZ 250-B	0.65	1.00	-0.24	0.94	1.00	0.84	1.00	1.00								
MZ 500-B	0.81	1.00	0.33	0.97	1.00	0.91	0.71	0.79	0.79							
MZ 1000-B	0.79	1.00	0.10	0.76	0.47	0.60	0.15	0.04	0.05	-0.04						
AF-B	0.78	1.00	0.75	0.66	0.16	0.54	0.34	0.52	0.54	0.60	0.58					
CF-A	0.93	1.00	0.98	0.81	0.82	0.96	0.89	0.98	0.97	0.99	0.98	0.55				
GS-B	0.96	1.00	0.93	0.73	0.94	0.83	0.54	0.81	0.81	0.99	0.89	0.93	0.89			
GM-B	0.97	1.00	0.78	0.32	0.42	0.46	0.28	0.58	0.52	0.78	0.67	0.68	0.64	0.07		
GL-B	0.95	1.00	0.93	0.63	0.66	0.80	0.78	0.89	0.84	0.98	0.91	0.75	0.66	0.51	0.13	

b.

	mud-A	mud-B	PtSh-B	pteropods-B	amphipods-B	euphausiids-B	MZ 150-B	MZ 200-B	MZ 250-B	MZ 500-B	MZ 1000-B	AF-B	CF-A	GS-B	GM-B	GL-B
mud-A																
mud-B	0.28															
PtSh-B	0.58	1.00														
pteropods-B	0.91	1.00	0.75													
amphipods-B	0.81	1.00	0.72	1.00												
euphausiids-B	0.91	1.00	0.65	0.68	0.63											
MZ 150-B	0.78	1.00	0.01	0.99	1.00	0.75										
MZ 200-B	0.79	1.00	-0.05	0.99	1.00	0.92	0.75									
MZ 250-B	0.87	1.00	0.08	0.96	1.00	0.79	1.00	0.50								
MZ 500-B	0.91	1.00	0.57	0.95	0.86	0.91	0.93	0.82	0.57							
MZ 1000-B	0.83	1.00	0.38	0.86	0.91	0.82	0.75	0.35	0.18	0.11						
AF-B	0.77	1.00	0.85	0.70	0.25	0.51	0.53	0.67	0.51	0.69	0.58					
CF-A	0.94	1.00	0.95	0.82	0.88	0.81	0.84	0.99	0.99	1.00	0.99	0.68				
GS-B	0.98	1.00	0.75	0.46	0.87	0.47	0.39	0.66	0.40	0.73	0.59	0.86	0.85			
GM-B	0.98	1.00	0.62	0.16	0.68	0.23	0.25	0.49	0.20	0.46	0.27	0.66	0.67	0.01		
GL-B	0.96	1.00	0.76	0.08	0.56	0.10	0.41	0.65	0.51	0.74	0.59	0.64	0.61	0.32	0.14	

Table 7: ANOSIM pairwise R comparison of the profiles of qualitatively measured individual fatty acids (a) and fatty acid summaries/ratios (b) of *Sufflogobius bibarbatus* and its potential prey items. Global R= 0.624 and R= 0.548 respectively. PtS-B= pteropod shells; MZ150-B - MZ1000-B= mixed zooplankton of sizes between 150 µm -1000 µm; AF-B= *Aequorea forskalea*; CF-B= *Chrysaora fulgida*. All samples presented here were collected offshore at station B, except *C. fulgida* which were collected at station A and benthic mud which were collected at both station A and station B. Highlighted values are where gobies are very similar to a potential prey item.

	% C	% N
Hydromedusae		
Aequorea forskalea	5.6±0.1	2±0
*Phialidium gregarium**	8.3±0	2±0
Scyphomedusae		
Chrysaora fulgida	9.2±0	3.1±0
*Cyanea capillata**	3.5±0	2.9±0

* Larson (1986)

Table 8: Carbon and nitrogen content (mean percentage ± SE) of the hydromedusae *Aequorea forskalea* (this study) and *Phialidium gregarium* (Larson 1986), and the scyphomedusae *Chrysaora fulgida* (this study) and *Cyanea capillata* (Larson 1986). Bell tissue was used for hydromedusae measurements but oral arm tissue was used for scyphomedusae.

APPENDIX A

Figures

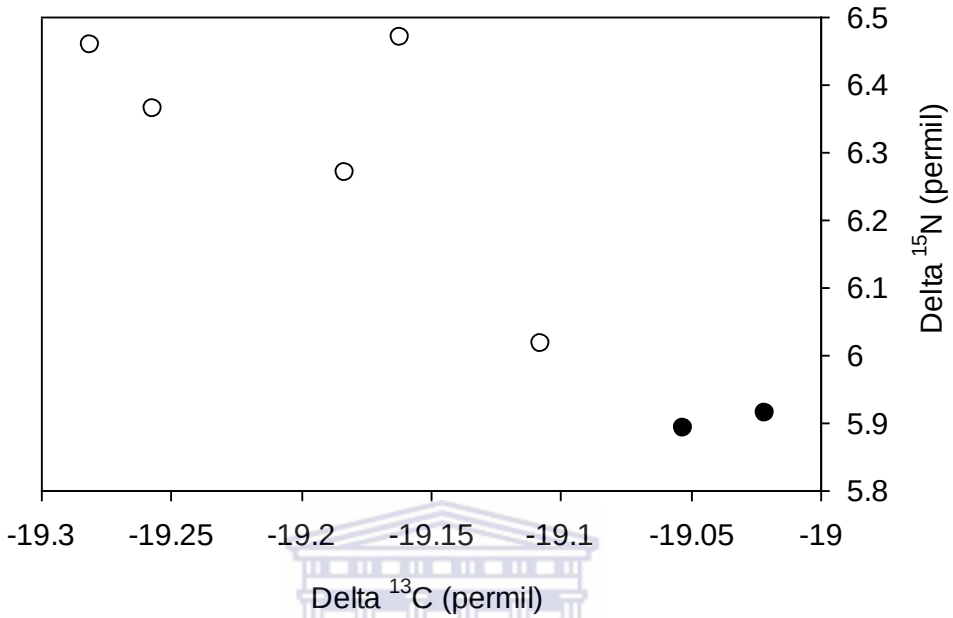


Figure A.1: $\delta^{13}C$ and $\delta^{15}N$ isotope signatures of benthic mud collected inshore at station A (white) (n= 5) and offshore at station B (black) (n= 2). NOTE FINE SCALE OF THE AXES.

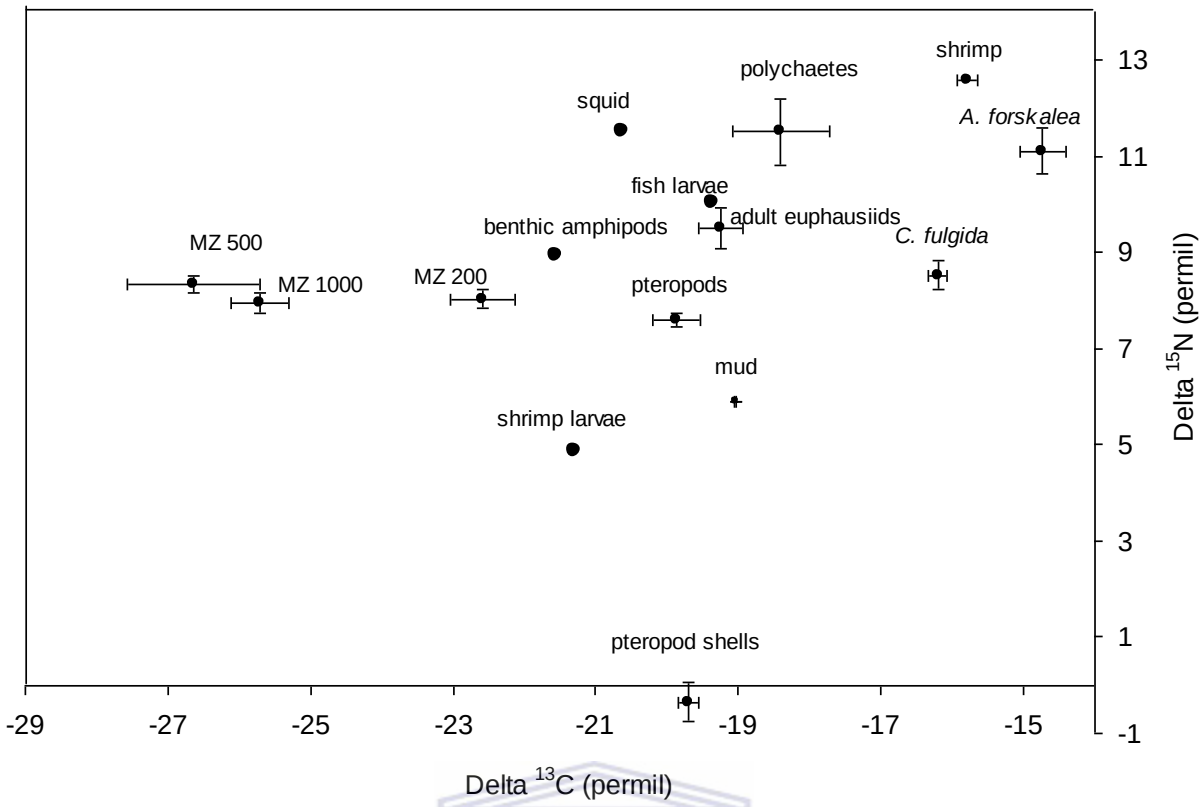


Figure A.2: Mean ± SE $\delta^{13}C$ and $\delta^{15}N$- signatures of the potential prey items of *Sufflogobius bibarbatus* collected from station B. These include pteropod shells (n= 5), pteropods (n= 4), adult euphausiids (n= 6), shrimp (n= 2), benthic amphipods (n= 1), fish larvae (n= 1), shrimp larvae (n= 1), benthic polychaetes (n= 3), mixed crustacean zooplankton sized 1000 µm (n= 8), 500 µm (n= 4), 200 µm (n= 2) , *Aequorea forskalea* (n= 11) and *Chrysaora fulgida* (n= 11).

Tables

	pteropods-B (N= 9)	amphipods-B (N= 2)	euphausiids-B (N= 6)	MZ 150-B (N= 2)	MZ 200-B (N= 2)	MZ 250-B (N= 2)	MZ 500-B (N= 4)	MZ 1000-B (N= 5)	GS-B (N= 24)	GM-B (N= 20)	GL-B (N= 20)
14:0	7.44±0.57	3.9±0.3	4.99±0.42	5.54±0.35	6.51±0.24	5.65±0.31	6.03±0.48	5.73±1	4.34±0.2	3.21±0.19	3.07±0.25
15:0	1.17±0.05	0.92±0.12	0.59±0.03	0.74±0.13	0.66±0.02	0.82±0.02	0.53±0.12	0.88±0.38	0.97±0.11	1.15±0.18	1.18±0.08
16:0	14.92±0.38	13.11±0.65	20.82±0.7	21.65±0.48	15.68±1.17	13.47±0.51	15.57±1.01	13.79±1.19	21.51±0.49	20.09±0.75	18.39±0.66
17:0	1.07±0.05	0.9±0.04	0.95±0.09	1.18±0.07	0.97±0.12	1.07±0.07	0.49±0.05	1.25±0.25	0.86±0.09	1.16±0.16	1.07±0.08
18:0	6.64±0.25	4.68±0.06	2.48±0.1	12.46±0.18	3.33±0.44	4.75±1.37	3.93±1.38	4.75±1.78	7.38±0.12	8.04±0.19	8.33±0.21
19:0	0.33±0.07	0.31±0.03	0.06±0.03	0±0	0±0	0±0	0±0	0.06±0.03	0.38±0.07	0.37±0.04	0.58±0.03
20:0	0.27±0.08	0.32±0.01	0.17±0.06	0.28±0.02	0.29±0.1	0.42±0.14	0.21±0.04	0.35±0.09	0.53±0.04	0.56±0.06	0.45±0.04
21:0	0.09±0.04	0.13±0.01	0±0	0±0	0±0	0.2±0.19	0.13±0.05	0.12±0.11	0.03±0.02	0.07±0.05	0.01±0.01
22:0	0.1±0.03	0.15±0.01	0.11±0.03	0.15±0.01	0.38±0.14	0.56±0.31	0.05±0.02	1.63±1.46	0.34±0.04	0.43±0.19	0.25±0.05
24:0	0.06±0.02	0±0	0.09±0.04	0±0	0±0	0.09±0.01	0.01±0.01	0.05±0.05	0.41±0.04	0.27±0.05	0.23±0.05
i-15:0	0.39±0.05	0.41±0.02	0.25±0.02	0.53±0	0.51±0.05	0.41±0.05	0.34±0.07	0.59±0.18	0.53±0.08	0.4±0.07	0.39±0.06
ai-15:0	0.16±0.02	0.16±0	0.15±0.01	0.15±0.04	0.3±0.06	0.19±0	0.1±0.03	0.08±0.03	0.15±0.03	0.24±0.05	0.22±0.05
i-16:0	1.55±0.2	0.93±0.07	0.66±0.08	0.25±0.06	0.37±0.09	0.09±0.02	0.12±0.04	0.46±0.08	0.42±0.06	0.49±0.09	0.3±0.04
i-17:0	0.64±0.04	0.39±0.05	0.62±0.02	0.53±0.03	0.41±0.11	0.53±0.03	0.4±0.16	0.19±0.02	0.73±0.03	0.83±0.09	1.17±0.07
14:1ω5	0.12±0.04	0±0	0.08±0.02	0.01±0	0.07±0.06	0.08±0.01	0.13±0.05	0.1±0.04	0.2±0.02	0.22±0.03	0.13±0.03
16:1ω5	0.58±0.15	0±0	0.09±0.02	0.22±0.01	0.21±0.03	0.08±0	0.12±0.06	0.1±0.05	0.43±0.03	0.5±0.06	0.47±0.05
16:1ω7	6.45±0.41	4.4±0.41	4.7±0.31	6.79±0.55	8.83±0.49	6.82±1.18	11±0.4	10.99±0.94	4.04±0.12	3.52±0.09	4.33±0.26
18:1ω7	3.87±0.57	3.6±0.41	4.06±0.13	2.97±0.48	5.17±0.27	5.84±1.78	2.04±1.25	3.4±1	4.43±0.26	3.63±0.23	3.64±0.15
18:1ω9	5.26±0.55	14.73±0.23	10.61±0.51	11.97±1.23	22.25±2.91	17.17±0.07	5.58±0.58	9.56±2.07	6.94±0.15	5.76±0.2	4.86±0.28
20:1ω9	0.95±0.1	1.11±0.08	0.85±0.15	0.7±0.3	1.05±0.02	0.29±0.07	3±0.53	1.72±0.3	1.17±0.07	1.13±0.09	0.76±0.05
22:1ω9	0.15±0.02	0.27±0.09	0.14±0.06	0.14±0.02	0.16±0.03	0.15±0.03	0.39±0.08	0.25±0.09	0.28±0.05	0.84±0.25	0.6±0.33
20:1ω11	0.75±0.12	0.18±0.01	0.23±0.03	0.17±0.1	0.27±0.01	1.49±0.17	0.32±0.1	0.51±0.33	0.37±0.04	0.39±0.03	0.49±0.04
22:1ω11	0.39±0.1	0.33±0.02	0.44±0.16	0.45±0.09	0.65±0.09	0.75±0.01	3.21±0.8	1.64±0.21	1.55±0.24	1.3±0.22	0.52±0.08
16:2ω4	0.76±0.09	0.59±0.03	0.23±0.05	0.09±0.03	0.49±0.34	0.71±0.06	1.26±0.08	1.36±0.19	0.26±0.02	0.38±0.05	0.93±0.08
16:3ω3	0.48±0.22	0.1±0.01	0.62±0.11	0.29±0.15	1.16±0.03	0.69±0.16	0.17±0.12	0.08±0.03	0.67±0.07	0.59±0.09	1.04±0.14
16:3ω4	0.87±0.08	0.31±0.04	0.25±0.02	0.15±0.01	0.2±0.04	0.18±0.03	0.07±0.04	0.07±0.03	0.28±0.05	0.24±0.04	0.57±0.05
16:4ω3	0.05±0.01	0.08±0.08	0.03±0.01	0.08±0.02	0.06±0.06	0.02±0.02	0.05±0.03	0.06±0.03	0.14±0.02	0.16±0.04	0.1±0.03
18:2ω6	1.72±0.05	1.29±0.11	2.91±0.08	1.79±0.06	1.82±0.05	1.91±0.03	2.25±0.24	2.25±0.17	1.37±0.06	1.28±0.09	1.3±0.08
18:3ω3	0.6±0.14	0.21±0.02	0.71±0.07	0.79±0.16	0.5±0.39	0.87±0.2	0.86±0.38	0.47±0.06	0.73±0.1	0.61±0.08	0.5±0.1
18:4ω3	1.22±0.24	0.55±0.1	0.47±0.04	1.05±0	1.26±0.43	1.23±0.09	1.22±0.22	0.78±0.1	1.08±0.09	1.1±0.13	0.76±0.11
20:3ω3	0.36±0.04	0.19±0.03	0.28±0.1	0.09±0	0.1±0.02	0.1±0.1	0.16±0.07	0.06±0.02	0.11±0.05	0.15±0.06	0.1±0.03
20:3ω6	0.55±0.07	0.21±0	0.36±0.07	0.29±0.18	0.18±0.01	0.17±0.07	0.24±0.02	0.19±0.02	0.26±0.12	0.24±0.07	0.41±0.09
20:4ω6	2.61±0.07	12.56±0.2	3.03±0.19	0.9±0.04	1.02±0.08	0.81±0.25	0.97±0.1	1.11±0.13	2.23±0.15	3.08±0.21	4.44±0.18
20:5ω3	18.68±0.47	14.24±0.35	16.34±0.3	11.49±0.2	10.97±1.34	13.34±0.83	18.15±0.88	17.12±1.34	11.52±0.22	12.63±0.29	14.22±0.48
22:2ω6	0.5±0.15	0.12±0.04	1.41±0.74	0.37±0.31	0.2±0.11	0.54±0.2	1.34±0.98	1.01±0.36	0.31±0.07	0.46±0.12	0.22±0.06
22:5ω3	1.77±0.07	1.92±0.31	0.86±0.08	0.86±0.11	1.58±0.03	1.17±0.11	1.63±0.28	1.03±0.08	2.57±0.1	3.09±0.13	4.06±0.2
22:6ω3	16.44±0.76	15.79±0.88	19.2±0.66	14.67±1.49	12.12±1.28	16.82±1.82	15.25±1.74	15.06±2.13	20.34±0.65	21.22±0.99	19.86±1.35

Table A.1: Qualitative comparison of the concentrations of different fatty acids (% of TFA) of *Sufflogobius bibarbatus* and its potential prey items. Values are mean ± SE. x: aωb = x: number of C-atoms in the acyl chain; a: number of double bonds; b: position of double bond from the methyl end of the molecule. Pt-B= pteropods; Amph-B= amphipods; Euph-B= adult euphausiids; MZ150-B - MZ1000-B= mixed zooplankton of sizes 150 µm-1000 µm; GS-B= small gobies (< 57 mm); GM-B= medium sized gobies (58 mm-90 mm); GL-B= large gobies (> 90 mm). All samples presented here were collected offshore at station B. Sample sizes are indicated in brackets.

	mud-A (N= 8)	mud-B (N= 2)	PtSh-B (N= 6)	AF-B (N= 9)	CF-A (N= 22)
14:0	8.73±1.27	4.66±0.44	5.56±0.81	3.63±0.61	4.91±0.27
15:0	1.3±0.38	1.51±0.36	0.77±0.09	1.36±0.29	1.73±0.12
16:0	20.99±1.28	20.38±3.75	29.17±4.78	11.38±1.49	16.85±0.63
17:0	1.39±0.48	1.01±0.37	1.01±0.17	1.88±0.89	1.32±0.06
18:0	7.98±0.95	4.98±0.26	3.59±0.68	8.8±0.58	14.29±0.49
19:0	0.24±0.11	0±0	0±0	1.27±0.44	1.34±0.11
20:0	1.73±0.4	1±0.13	0.49±0.12	1.21±0.33	1.06±0.16
21:0	0.54±0.18	1.27±1.27	0.38±0.19	0.45±0.17	0.21±0.06
22:0	1.43±0.32	1.74±0.22	0.52±0.14	1.19±0.38	1.47±0.34
24:0	1.29±0.52	5.59±5.59	0.94±0.94	1.12±0.95	0.14±0.05
i-15:0	1.88±0.18	2.33±0.14	0.52±0.09	2.19±1	1.19±0.1
ai-15:0	3.13±0.4	5.41±0.23	0.14±0.06	0.27±0.16	0.5±0.05
i-16:0	2.26±1	0.44±0.04	0.53±0.23	1.52±0.33	1.23±0.12
i-17:0	0.85±0.23	0.51±0.06	0.35±0.11	1.16±0.24	1.14±0.05
14:1ω5	1.73±0.35	1.97±0.81	0.27±0.06	0.19±0.12	0.07±0.03
16:1ω5	0.73±0.33	3.89±0.24	0±0	0.26±0.26	0.27±0.03
16:1ω7	6.24±1.15	12.92±0.73	7.23±2.17	4.79±0.85	3.54±0.26
18:1ω7	6.66±1.7	14.27±4.63	4.42±0.93	1.1±0.29	1.78±0.16
18:1ω9	2.06±0.23	1.31±0.03	12.19±1.11	3.36±0.53	4.26±0.25
20:1ω9	1.38±0.71	0.12±0.01	1.17±0.27	1.51±0.4	0.7±0.1
22:1ω9	0.86±0.26	1.52±0.05	0.23±0.03	0.33±0.09	0.35±0.09
20:1ω11	0.88±0.69	0.06±0.02	0.66±0.21	0.74±0.34	0.22±0.05
22:1ω11	0.66±0.17	0.38±0.04	1.13±0.26	0.74±0.19	0.27±0.06
16:2ω4	0.93±0.36	0.81±0.5	0.89±0.1	2.26±1.82	0.56±0.05
16:3ω3	0.1±0.02	0.13±0.01	0.15±0.09	0.74±0.43	0.49±0.11
16:3ω4	1.27±0.36	1.34±0.08	0.54±0.13	0.88±0.2	0.65±0.07
16:4ω3	0.5±0.15	0.08±0	0.07±0.01	0.07±0.02	0.07±0.01
18:2ω6	1.28±0.34	0.72±0.32	1.37±0.14	1±0.2	1.21±0.15
18:3ω3	0.84±0.32	0±0	0.81±0.35	0.41±0.21	0.61±0.11
18:4ω3	2.88±1.71	0±0	1.28±0.2	1.31±0.42	1.32±0.15
20:3ω3	1.69±0.81	0±0	0.31±0.19	0.02±0.02	0.29±0.06
20:3ω6	1.27±0.53	0±0	0.19±0.07	2.62±1.95	0.4±0.08
20:4ω6	2.04±0.67	0±0	0.9±0.15	13.07±1.94	7.17±0.68
20:5ω3	2.92±0.44	1.53±1.04	8.65±0.88	7.68±1.4	9.26±0.37
22:2ω6	1.29±0.23	1.32±0.5	0.43±0.13	1.22±0.35	0.87±0.13
22:5ω3	1.68±1.1	0±0	1.02±0.16	2.95±0.66	3.52±0.16
22:6ω3	3.05±0.8	2.13±0.67	10.72±3.14	13.04±1.79	14.46±0.7

Table A.2: Qualitative comparison of the concentrations of different fatty acids (% of TFA) of *Sufflogobius bibarbatus* and its potential prey items. Values are mean ± SE. x: aωb = x: number of C-atoms in the acyl chain; a: number of double bonds; b: position of double bond from the methyl end of the molecule. M-A= mud collected at station A; M-B= mud collected at station B; PtS-B= pteropod shells collected at station B; AF-B= *Aequorea forskalea* collected at station B; CF-A= *Chrysaora fulgida* collected at station A. Sample sizes are indicated in brackets.

	pteropods-B (N= 9)	amphipods-B (N= 2)	euphausiids-B (N= 6)	MZ 150-B (N= 2)	MZ 200-B (N= 2)	MZ 250-B (N= 2)	MZ 500-B (N= 4)	MZ 1000-B (N= 5)	GS-B (N= 24)	GM-B (N= 20)	GL-B (N= 20)
BAFA	4.99±0.23	3.94±0.02	3.24±0.1	3.38±0.05	3.21±0.01	3.1±0	2.97±0.31	4.39±0.43	3.71±0.19	4.37±0.37	4.33±0.24
sum-pufa	46.65±1.12	48.82±0.3	46.84±1.06	33.12±1.71	31.93±2.25	39.09±2.53	44.03±2.64	40.85±3.78	41.93±0.88	45.24±1.17	48.52±1.52
sum-ω3	39.61±1.05	33.08±0.77	38.51±0.81	29.33±1.61	27.76±2.51	34.24±2.79	37.51±2.66	34.67±3.52	37.17±0.84	39.55±1.15	40.65±1.62
sum-mufa	18.53±0.75	24.87±0.09	21.2±0.55	23.43±2.78	38.67±3.85	32.67±2.73	26.77±1.25	28.77±3.53	19.45±0.49	17.35±0.54	15.8±0.59
sum-sfa	34.74±1.23	26.18±0.22	31.96±0.68	43.45±1.07	29.4±1.6	28.03±0.39	27.79±2.09	30.26±1.64	38.56±0.69	37.29±0.98	35.64±1.09
P/S	1.36±0.07	1.87±0.03	1.47±0.06	0.76±0.02	1.08±0.02	1.39±0.07	1.63±0.21	1.38±0.19	1.1±0.04	1.26±0.07	1.41±0.09
16:1/16:0	0.47±0.03	0.34±0.05	0.23±0.02	0.32±0.03	0.58±0.08	0.51±0.07	0.72±0.03	0.85±0.14	0.21±0.01	0.21±0.01	0.26±0.01
sum16/sum18	1.3±0.07	0.74±0.02	1.28±0.06	0.94±0.03	0.77±0.08	0.66±0.05	1.79±0.3	1.34±0.23	1.26±0.03	1.26±0.04	1.3±0.05
sum-EFA	37.73±1.13	42.59±0.32	38.57±0.75	27.07±1.72	24.1±2.55	30.96±2.9	34.37±2.42	33.29±3.47	34.1±0.8	36.92±1.14	38.52±1.59
22:6ω3/20:5ω3	0.88±0.04	1.11±0.09	1.18±0.05	1.27±0.11	1.11±0.02	1.26±0.06	0.84±0.07	0.87±0.07	1.77±0.05	1.69±0.08	1.4±0.09
20:5ω3/22:6ω3	1.15±0.05	0.91±0.07	0.86±0.04	0.79±0.07	0.9±0.01	0.8±0.04	1.22±0.1	1.18±0.1	0.58±0.02	0.62±0.02	0.76±0.04
sum22:1	0.54±0.11	0.6±0.11	0.58±0.15	0.6±0.11	0.81±0.12	0.9±0.05	3.6±0.75	1.89±0.27	1.83±0.27	2.15±0.31	1.12±0.31
sum22:1+20:1	2.24±0.16	1.9±0.19	1.66±0.33	1.46±0.5	2.13±0.09	2.68±0.15	6.91±1.37	4.12±0.65	3.38±0.29	3.67±0.32	2.36±0.31
ω3/ω6	7.36±0.25	2.23±0.12	5.02±0.31	8.28±0.3	7.98±0.63	8.67±1.1	8.39±1.77	7.4±0.55	9.26±0.44	8.53±0.48	6.53±0.37

Table A.3: Qualitative comparison of the concentrations of different fatty acid marker ratios (% of TFA) of *Sufflogobius bibarbatus* and its potential prey items. x: aωb = x: number of C-atoms in the acyl chain; a: number of double bonds; b: position of double bond from the methyl end of the molecule. BAFA: Bacterial fatty acids; PUFA: Polyunsaturated fatty acids; sum ω3 = sum of all fatty acids with a double bond on the 3rd carbon from the terminal methyl end; MUFA: Monounsaturated fatty acids; SFA: Saturated fatty acids; P/S= PUFA/SFA; sum 16:1/16:0 = Monounsaturates with 16 carbon atoms / Palmitic acid; sum 16/sum 18 = sum of FA's with 16 carbon atoms / sum of FA's with 18 carbon atoms; EFA: Essential fatty acids; sum 22:1 = sum of monounsaturates containing 22 carbon atoms; TFA= Total fatty acids.

Sample descriptions are as in Table A.1

	mud-A (N= 8)	mud-B (N= 2)	PtSh-B (N= 6)	AF-B (N= 9)	CF-A (N= 22)
BAFA	12.06±1.22	14.31±1.66	4.67±0.48	11.6±3.75	7.22±0.29
sum-pufa	22.43±2.92	9.64±3.32	27.34±2.77	43.88±3.62	41.01±0.97
sum-ω3	13.66±2.66	3.87±0.35	23.03±2.72	25.7±2.8	30.02±0.87
sum-mufa	22.44±1.9	39.54±3.93	28.48±3.79	18.41±4.4	11.57±0.38
sum-sfa	53.19±2.22	49.56±1.88	43.77±4.34	33.98±4.51	47.18±0.85
P/S	0.44±0.07	0.19±0.06	0.67±0.11	1.14±0.15	0.88±0.03
16:1/16:0	0.33±0.07	0.84±0.11	0.35±0.16	0.44±0.04	0.23±0.01
sum16/sum18	1.55±0.23	1.72±0.02	1.68±0.25	4.39±3.17	0.99±0.04
sum-EFA	8.02±1.77	3.66±0.36	20.27±2.86	33.51±4.28	30.89±1.12
22:6ω3/20:5ω3	1.04±0.18	3.14±2.57	1.39±0.51	2.18±0.55	1.61±0.1
20:5ω3/22:6ω3	1.23±0.24	0.97±0.79	1.06±0.22	0.47±0.09	0.67±0.04
sum22:1	1.51±0.27	1.9±0.02	1.36±0.29	1.46±0.35	0.63±0.11
sum22:1+20:1	3.77±1.35	2.08±0.01	3.19±0.67	3.09±0.42	1.54±0.16
ω3/ω6	2.71±0.95	1.78±1.08	8.24±1.13	1.45±0.25	3.39±0.3

Table A.4: Qualitative comparison of the concentrations of different fatty acid marker ratios (% of TFA) of *Sufflogobius bibarbatus* and its potential prey items. Sample descriptions are as in Table A.2. Fatty acid summaries/ratio descriptions are as in Table A.3.

	pteropods- B (N= 9)	amphipods- B (N= 2)	euphausiids- B (N= 6)	MZ 150-B (N= 2)	MZ 200- B (N= 2)	MZ 250- B (N= 2)	MZ 500- B (N= 4)	MZ 1000- B (N= 5)	GS-B (N= 24)	GM-B (N= 20)	GL-B (N= 20)
14:0	1.82±0.1	0.89±0.13	1.49±0.21	2.71±0.01	2.67±0.33	5.03±0.83	5.47±1.83	5.18±0.61	1.51±0.12	0.8±0.09	0.68±0.09
15:0	0.29±0.02	0.21±0.04	0.17±0.01	0.36±0.04	0.27±0.04	0.72±0.06	0.42±0.09	0.75±0.25	0.32±0.04	0.24±0.04	0.25±0.03
16:0	3.75±0.23	2.97±0.05	6.08±0.48	10.61±0.38	6.4±0.55	11.97±1.77	14±4.48	13.03±1.58	7.29±0.5	5.03±0.48	3.87±0.4
17:0	0.27±0.03	0.2±0	0.28±0.04	0.58±0.07	0.41±0.11	0.96±0.16	0.42±0.13	1.19±0.27	0.29±0.03	0.34±0.07	0.21±0.02
18:0	1.65±0.07	1.07±0.08	0.73±0.07	6.11±0.26	1.35±0.04	4.07±0.75	3.67±1.61	4.84±2.04	2.42±0.15	1.98±0.16	1.68±0.13
19:0	0.09±0.02	0.07±0.01	0.02±0.01	0	0	0	0	0.05±0.02	0.12±0.03	0.08±0.01	0.12±0.01
20:0	0.07±0.03	0.07±0.01	0.05±0.01	0.14±0	0.13±0.06	0.36±0.08	0.2±0.08	0.32±0.09	0.17±0.02	0.14±0.02	0.09±0.01
21:0	0.03±0.01	0.03±0	0	0	0	0.19±0.19	0.13±0.08	0.12±0.12	0.01±0	0	0
22:0	0.02±0.01	0.03±0	0.03±0.01	0.07±0.01	0.16±0.08	0.52±0.33	0.05±0.02	1.61±1.47	0.1±0.01	0.07±0.03	0.05±0.01
24:0	0.01±0	0	0.02±0.01	0	0	0.08±0	0.01±0.01	0.04±0.04	0.12±0.01	0.06±0.01	0.04±0.01
i-15:0	0.1±0.01	0.09±0	0.07±0	0.26±0.01	0.21±0.05	0.35±0	0.33±0.15	0.59±0.19	0.16±0.02	0.08±0.01	0.08±0.01
ai-15:0	0.04±0	0.04±0	0.04±0	0.07±0.02	0.13±0.04	0.16±0.02	0.08±0.03	0.07±0.03	0.05±0.01	0.05±0.01	0.05±0.01
i-16:0	0.39±0.06	0.21±0	0.19±0.02	0.12±0.02	0.15±0.01	0.08±0.01	0.08±0.04	0.43±0.08	0.14±0.03	0.11±0.03	0.06±0.01
i-17:0	0.16±0.01	0.09±0.02	0.18±0.02	0.26±0.03	0.16±0.02	0.47±0.07	0.41±0.26	0.18±0.02	0.24±0.02	0.17±0.02	0.24±0.02
17:1	0	0.05±0.01	0	0	0	0	0.85±0.26	0.46±0.11	0.01±0.01	0.01±0	0
14:1ω5	0.03±0.01	0	0.02±0	0.01±0	0.03±0.03	0.07±0	0.14±0.07	0.1±0.04	0.07±0.01	0.05±0.01	0.03±0.01
16:1ω5	0.16±0.05	0	0.03±0.01	0.11±0.01	0.09±0.03	0.07±0.01	0.08±0.02	0.09±0.04	0.14±0.01	0.12±0.02	0.1±0.01
16:1ω7	1.65±0.19	1.01±0.16	1.39±0.17	3.35±0.46	3.68±0.78	6.15±1.71	9.73±2.86	10.38±1.17	1.35±0.09	0.86±0.07	0.9±0.1
18:1ω7	0.96±0.14	0.81±0.04	1.19±0.11	1.47±0.32	2.16±0.45	5.34±2.14	1.29±0.5	3.38±1.07	1.37±0.09	0.95±0.11	0.72±0.06
18:1ω9	1.35±0.19	3.35±0.17	3.13±0.32	5.91±0.94	9.39±2.67	15.19±1.61	4.46±0.78	9.39±2.39	2.31±0.16	1.41±0.12	1.05±0.09
20:1ω9	0.24±0.03	0.25±0.03	0.24±0.04	0.35±0.16	0.43±0.06	0.83±0.47	2.48±0.59	1.67±0.38	0.4±0.03	0.27±0.03	0.15±0.02
22:1ω9	0.04±0.01	0.06±0.02	0.04±0.02	0.07±0.01	0.07±0.02	0.13±0.02	0.3±0.05	0.22±0.06	0.1±0.02	0.15±0.06	0.15±0.1
20:1ω11	0.19±0.04	0.04±0	0.06±0.01	0.09±0.05	0.11±0.01	1.3±0	0.24±0.09	0.55±0.36	0.11±0.01	0.1±0.01	0.1±0.01
22:1ω11	0.09±0.02	0.08±0.01	0.12±0.04	0.23±0.06	0.28±0.08	0.67±0.06	2.68±0.77	1.51±0.14	0.53±0.1	0.25±0.06	0.11±0.03
24:1	0	0	0	0	0	0	1.27±1.27	0	0.01±0.01	0.01±0.01	0.01±0
16:2ω4	0.19±0.03	0.13±0	0.07±0.02	0.04±0.01	0.23±0.17	0.63±0.12	1.1±0.29	1.28±0.2	0.08±0.01	0.08±0.02	0.19±0.02
16:3ω3	0.14±0.07	0.02±0	0.18±0.03	0.15±0.08	0.48±0.09	0.63±0.21	0.12±0.07	0.08±0.03	0.2±0.01	0.13±0.02	0.19±0.02
16:3ω4	0.22±0.02	0.07±0.01	0.07±0.01	0.07±0	0.08±0	0.16±0.04	0.08±0.06	0.06±0.02	0.08±0.02	0.07±0.01	0.11±0.01
16:4ω3	0.01±0	0.02±0.02	0.01±0	0.04±0.01	0.03±0.03	0.02±0.02	0.06±0.04	0.06±0.03	0.05±0.01	0.04±0.01	0.02±0
18:2ω6	0.43±0.03	0.29±0.01	0.85±0.07	0.88±0.02	0.76±0.14	1.7±0.22	2.02±0.72	2.1±0.13	0.45±0.03	0.29±0.03	0.27±0.03
18:3ω6	0.01±0	0.14±0.02	0.04±0.01	0.09±0.04	0.1±0.01	0.51±0.28	0.27±0.07	0.19±0.06	0.02±0.01	0	0
18:3ω3	0.16±0.05	0.05±0	0.2±0.02	0.38±0.06	0.18±0.13	0.75±0.1	0.98±0.61	0.44±0.06	0.22±0.03	0.13±0.02	0.11±0.03
18:4ω3	0.32±0.08	0.12±0.02	0.14±0.02	0.52±0.03	0.55±0.26	1.1±0.2	1.09±0.43	0.72±0.08	0.35±0.03	0.23±0.04	0.16±0.03
20:3ω3	0.09±0.01	0.04±0	0.08±0.03	0.05±0	0.04±0.02	0.08±0.08	0.16±0.1	0.06±0.02	0.04±0.02	0.05±0.02	0.02±0.01
20:3ω6	0.14±0.02	0.05±0	0.1±0.01	0.14±0.08	0.07±0.02	0.15±0.04	0.2±0.05	0.18±0.03	0.07±0.04	0.06±0.02	0.09±0.03
20:4ω6	0.66±0.04	2.85±0.14	0.89±0.1	0.44±0.01	0.43±0.1	0.69±0.14	0.82±0.21	1.02±0.1	0.68±0.06	0.68±0.05	0.87±0.06
20:5ω3	4.7±0.29	3.23±0.13	4.77±0.34	5.63±0.23	4.44±0.17	11.72±0.57	15.62±4.12	16.09±1.48	3.75±0.2	3.09±0.21	2.79±0.16
22:2ω6	0.12±0.03	0.03±0.01	0.36±0.17	0.19±0.16	0.08±0.03	0.5±0.23	0.79±0.42	0.87±0.29	0.09±0.02	0.05±0.01	0.05±0.01
22:5ω3	0.45±0.03	0.44±0.1	0.25±0.03	0.42±0.08	0.65±0.09	1.02±0.01	1.36±0.3	0.97±0.09	0.82±0.04	0.73±0.04	0.78±0.04
22:6ω3	4.15±0.34	3.61±0.43	5.55±0.24	7.16±0.32	4.92±0.27	14.7±0.03	12.53±2.88	13.9±1.62	6.46±0.26	4.81±0.26	3.76±0.2

Table A.5: Quantitative comparison of the concentrations of different fatty acids (µg/ mg DW). Values are mean ± SE. x: aωb = x: number of C-atoms in the acyl chain; a: number of double bonds; b: position of double bond from the methyl end of the molecule. Pt-B= pteropods; Amph-B= amphipods; Euph-B= adult euphausiids; MZ150-B - MZ1000-B= mixed zooplankton of sizes 150 µm- 1000 µm; GS-B= small gobies (< 57 mm); GM-B= medium sized gobies (58 mm-90 mm); GL-B= large gobies (> 90 mm). Sample sizes are indicated in brackets. All samples presented here were collected offshore at station B.

	mud-A (N= 8)	mud-B (N= 2)	PtSh- B (N= 6)	AF- B (N= 9)	CF- A (N= 22)
14:0	0.07±0.01	0.02±0.01	0.2±0.08	0.06±0.02	0.12±0.01
15:0	0.01±0	0.01±0.01	0.03±0.01	0.02±0	0.04±0
16:0	0.17±0.01	0.1±0.05	1.03±0.34	0.18±0.05	0.41±0.03
17:0	0.01±0	0.01±0.01	0.03±0	0.02±0	0.03±0
18:0	0.07±0.01	0.03±0.01	0.1±0.02	0.13±0.03	0.36±0.03
19:0	0	0	0	0.02±0	0.04±0.01
20:0	0.02±0	0.01±0	0.02±0.01	0.01±0	0.02±0
21:0	0	0	0.01±0	0	0
22:0	0.01±0	0.01±0	0.02±0.01	0.01±0	0.04±0.01
24:0	0.01±0	0.05±0.05	0.01±0.01	0	0
i-15:0	0.02±0	0.01±0.01	0.02±0.01	0.01±0	0.03±0
ai-15:0	0.03±0	0.03±0.02	0.01±0	0.01±0	0.01±0
i-16:0	0.02±0.01	0	0.02±0.01	0.02±0	0.03±0
i-17:0	0.01±0	0	0.01±0	0.02±0	0.03±0
17:1	0.01±0.01	0.01±0	0.03±0.01	0	0
14:1ω5	0.01±0	0.01±0.01	0.01±0	0	0
16:1ω5	0	0.02±0.01	0	0	0.01±0
16:1ω7	0.05±0.01	0.07±0.04	0.24±0.09	0.08±0.02	0.09±0.01
18:1ω7	0.06±0.01	0.06±0.02	0.12±0.02	0.02±0.01	0.05±0.01
18:1ω9	0.02±0	0.01±0	0.37±0.08	0.05±0.01	0.1±0.01
20:1ω9	0.01±0	0	0.03±0.01	0.02±0	0.02±0
22:1ω9	0.01±0	0.01±0.01	0.01±0	0.01±0	0.01±0
20:1ω11	0.01±0	0	0.02±0.01	0	0.01±0
22:1ω11	0.01±0	0	0.03±0.01	0.01±0	0.01±0
24:1	0.01±0.01	0	0±0	0	0
16:2ω4	0.01±0	0.01±0.01	0.03±0.01	0.01±0	0.01±0
16:3ω3	0	0	0	0.01±0	0.01±0
16:3ω4	0.01±0	0.01±0	0.01±0	0.01±0	0.02±0
16:4ω3	0	0	0	0	0
18:2ω6	0.01±0	0.01±0	0.05±0.01	0.02±0	0.03±0
18:3ω6	0.01±0	0.01±0.01	0	0	0
18:3ω3	0.01±0	0	0.04±0.02	0.01±0	0.01±0
18:4ω3	0.03±0.02	0	0.04±0.01	0.02±0	0.03±0
20:3ω3	0.02±0.01	0	0.02±0.01	0	0.01±0
20:3ω6	0.01±0.01	0	0.01±0	0.01±0	0.01±0
20:4ω6	0.02±0.01	0	0.03±0	0.2±0.05	0.19±0.02
20:5ω3	0.03±0.01	0.01±0.01	0.27±0.07	0.13±0.05	0.24±0.02
22:2ω6	0.01±0	0.01±0.01	0.01±0	0.01±0	0.02±0
22:5ω3	0.02±0.01	0	0.04±0.01	0.03±0.01	0.1±0.01
22:6ω3	0.03±0.01	0.01±0	0.28±0.06	0.21±0.06	0.35±0.02

Table A.6: Quantitative comparison of the concentrations of different fatty acids (µg/ mg DW). Values are mean ± SE. x: aωb = x: number of C-atoms in the acyl chain; a: number of double bonds; b: position of double bond from the methyl end of the molecule. M-A= mud collected at station A; M-B= mud collected at station B; PtS-B= pteropod shells collected at station B; AF-B= *Aequorea forskalea* collected at station B; CF-A= *Chrysaora fulgida* collected at station A.

Sample sizes are indicated in brackets.

	pteropods- B (N= 9)	amphipods- B (N= 2)	euphausiids- B (N= 6)	MZ 150-B (N= 2)	MZ 200- B (N= 2)	MZ 250- B (N= 2)	MZ 500- B (N= 4)	MZ 1000- B (N= 5)	GS-B (N= 24)	GM-B (N= 20)	GL-B (N= 20)
BAFA	1.25±0.1	0.9±0.05	0.94±0.07	1.65±0.07	1.33±0.22	2.74±0.3	2.59±0.84	4.15±0.49	1.23±0.09	1±0.12	0.89±0.09
sum-pufa	11.78±0.85	11.11±0.8	13.66±0.54	16.2±0.09	13.04±1.17	34.35±1.58	37.2±9.47	38.03±2.99	13.37±0.54	10.43±0.58	9.42±0.49
sum-ω3	10.02±0.76	7.54±0.67	11.18±0.61	14.34±0.04	11.3±0.79	30.02±0.87	31.92±8.28	32.32±3	11.89±0.48	9.2±0.52	7.83±0.37
sum-mufa	4.71±0.4	5.66±0.35	6.22±0.54	11.57±2.02	16.23±4.14	29.74±5.04	22.24±4.78	27.73±4.73	6.41±0.43	4.17±0.31	3.32±0.33
sum-sfa	8.67±0.45	5.95±0.34	9.35±0.77	21.28±0.7	12.04±1.28	24.77±2.39	25.14±8.02	28.76±3.05	12.94±0.83	9.15±0.85	7.42±0.71
P/S	1.36±0.07	1.87±0.03	1.49±0.07	0.76±0.02	1.08±0.02	1.39±0.07	1.63±0.21	1.38±0.19	1.09±0.04	1.27±0.09	1.41±0.09
16:1/16:0	0.47±0.03	0.34±0.05	0.23±0.02	0.32±0.03	0.58±0.08	0.51±0.07	0.72±0.03	0.85±0.14	0.21±0	0.2±0.01	0.26±0.01
sum16/sum18	1.3±0.07	0.74±0.02	1.25±0.06	0.94±0.03	0.77±0.08	0.66±0.05	1.79±0.3	1.34±0.23	1.29±0.02	1.26±0.04	1.29±0.04
sum-EFA	9.51±0.66	9.7±0.71	11.21±0.65	13.23±0.08	9.79±0.54	27.12±0.46	28.98±7.12	31.02±2.92	10.89±0.48	8.58±0.46	7.42±0.35
22:6ω3/20:5ω3	0.88±0.04	1.11±0.09	1.18±0.05	1.27±0.11	1.11±0.02	1.26±0.06	0.84±0.07	0.87±0.07	1.77±0.06	1.62±0.08	1.4±0.09
20:5ω3/22:6ω3	1.15±0.05	0.91±0.07	0.86±0.04	0.79±0.07	0.9±0.01	0.8±0.04	1.22±0.1	1.18±0.1	0.58±0.02	0.64±0.03	0.76±0.04
sum22:1	0.13±0.03	0.14±0.03	0.16±0.04	0.3±0.07	0.34±0.1	0.79±0.05	2.98±0.77	1.72±0.16	0.63±0.11	0.4±0.07	0.26±0.1
sum22:1+20:1	0.57±0.06	0.43±0.07	0.46±0.08	0.73±0.29	0.89±0.18	2.92±0.43	5.7±1.43	3.95±0.81	1.14±0.13	0.77±0.07	0.51±0.11
ω3/ω6	7.36±0.25	2.23±0.12	5.02±0.31	8.28±0.3	7.98±0.63	8.67±1.1	8.39±1.77	7.4±0.55	9.51±0.44	9.04±0.49	6.58±0.36
TFA	25.19±1.55	22.76±1.49	29.23±1.81	49.05±2.82	41.32±6.59	89.05±9.2	85.99±22.27	94.63±7.19	32.73±1.73	23.77±1.67	20.16±1.48
terrestrial	0.59±0.07	0.34±0.01	1.06±0.07	1.26±0.04	0.94±0.01	2.44±0.12	2.99±1.33	2.54±0.17	0.67±0.05	0.42±0.05	0.38±0.05

Table A.7: Quantitative comparison of the concentrations of different fatty acid marker ratios (µg/ mg DW). x: aωb = x: number of C-atoms in the acyl chain; a: number of double bonds; b: position of double bond from the methyl end of the molecule. BAFA: Bacterial fatty acids; PUFA: Polyunsaturated fatty acids; sum ω3 = sum of all fatty acids with a double bond on the 3rd carbon from the terminal methyl end; MUFA: Monounsaturated fatty acids; SFA: Saturated fatty acids; P/S= PUFA/SFA; sum 16:1/16:0 = Monounsaturates with 16 carbon atoms / Palmitic acid; sum 16/sum 18 = sum of FA's with 16 carbon atoms / sum of FA's with 18 carbon atoms; EFA: Essential fatty acids; sum 22:1 = sum of monounsaturates containing 22 carbon atoms; TFA= Total fatty acids. Sample descriptions are as in Table A.5.

	mud-A (N= 8)	mud-B (N= 2)	PtSh- B (N= 6)	AF- B (N= 9)	CF- A (N= 22)
BAFA	0.11±0.02	0.09±0.06	0.15±0.04	0.09±0.01	0.18±0.02
sum-pufa	0.2±0.04	0.06±0.05	0.82±0.21	0.66±0.17	1.04±0.08
sum-ω3	0.13±0.03	0.02±0.01	0.69±0.18	0.41±0.11	0.75±0.05
sum-mufa	0.19±0.02	0.21±0.11	0.85±0.19	0.19±0.05	0.28±0.02
sum-sfa	0.44±0.04	0.28±0.17	1.49±0.46	0.5±0.11	1.17±0.09
P/S	0.44±0.07	0.19±0.06	0.67±0.11	1.21±0.1	0.89±0.03
16:1/16:0	0.33±0.07	0.84±0.11	0.35±0.16	0.39±0.05	0.23±0.01
sum16/sum18	1.55±0.23	1.72±0.02	1.68±0.25	1.15±0.08	0.99±0.04
sum-EFA	0.07±0.02	0.02±0.01	0.58±0.13	0.54±0.15	0.78±0.07
22:6ω3/20:5ω3	1.04±0.18	3.14±2.57	1.39±0.51	2.48±0.51	1.61±0.1
20:5ω3/22:6ω3	1.23±0.24	0.97±0.79	1.06±0.22	0.51±0.07	0.67±0.04
sum22:1	0.01±0	0.01±0.01	0.04±0.01	0.02±0.01	0.02±0
sum22:1+20:1	0.03±0.01	0.01±0.01	0.08±0.02	0.04±0.01	0.04±0.01
ω3/ω6	2.71±0.95	1.78±1.08	8.24±1.13	1.55±0.2	3.44±0.3
TFA	0.85±0.09	0.56±0.33	3.17±0.84	1.36±0.33	2.5±0.18
terrestrial	0.02±0	0.01±0	0.08±0.04	0.02±0.01	0.04±0

Table A.8: Quantitative comparison of the concentrations of different fatty acid marker ratios (µg/ mg DW). Sample descriptions are as in Table A.6. Fatty acid summaries/ratio descriptions are as in Table A.7.