TEMPORAL VARIABILITY IN THE FATTY ACID COMPOSITION OF SUSPENSION-FEEDERS AND GRAZERS ON A SOUTH AFRICAN ROCKY SHORE

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ABSTRACT

Numerous ecological studies have used lipids to determine trophic pathways in aquatic systems, as fatty acid profiles provide time-integrated information on an organism's assimilated diet. Many of these studies have, however, been based on sample collections with a limited temporal scale. The trophic ecology of pelagic systems has been studied intensively using fatty acid analyses, but very little work has been directed toward benthic communities, with the intertidal being especially neglected. The investigation of trophic pathways within rocky shore communities will help us to better understand system responses to environmental changes. The determination of long term temporal variation of the food web within a community could reveal the type, magnitude, duration and frequency of highly seasonal productivity. Changes in fatty acid profiles through time in primary consumers of intertidal rocky shores are poorly understood, but represent an important step towards a more comprehensive understanding of rocky shore food webs, compared with those derived from snapshot or short-term studies. The aim of this thesis was to clarify the temporal variability in the diets of rocky shore intertidal suspension-feeders (the brown mussels *Perna perna* and the Cape reef worm, *Gunnarea gaimardi*) and grazers (the Cape sea urchin *Parencinus angulosus* and the Goat-eye limpet, *Cymbulus oculus*) on the south east coast of South Africa using fatty acid profiles, and to investigate the effects of life style (e.g. feeding mode) and life cycle on temporal variations in tissue fatty acid profiles. I had three hypotheses: firstly, that suspension-feeders experience high levels of variability in their diets through time because water quality has the potential to change quickly and drastically, whereas grazers experience less variability in their diets over time since their food sources are more constant. Secondly, the reproductive cycles of the suspension-feeder *P. perna* and the grazer *P. angulosus* affect the fatty acid composition of their gonads, with temporal variations in lipid composition reflecting changes in reproduction investment. Thirdly, the total amount of energetic reserves available for reproduction are different for each gender (females allocate more energy to egg production than males allocate to gamete production). To address these aims, fatty acid profiles of suspension-feeders and grazers were investigated over a period of twelve months

(from July 2010 to June 2011) at a single site on the south east coast of South Africa. The results showed high variability in the fatty acid composition of both the suspension-feeders strongly related with changes in their food source (suspended particulate material). Furthermore, similar temporal changes in fatty acid profiles of the two suspension-feeders were observed over time, reflecting their common diet and life style. There were some inter-specific differences in the suspension-feeders, likely originating from differences in their particle capturing mechanisms. Grazers showed less variability through time compared with the suspension-feeders, with the limpets being more consistent than the sea urchins. The temporal variability in the sea urchin diets may have resulted from the highly diverse and heterogeneous food sources available to them, whereas limpets may be more selective and have a limited range of diet items. Differences between the two grazer species may have arose from differences in their feeding strategies and intertidal zonation.

The fatty acid compositions of gonad tissues in both *P. perna* and *P. angulosus* showed temporal variability strongly related to reproductive cycle. Differences in the fatty acid values between females and males were apparent, with females richer in total and polyunsaturated fatty acids than males. Spawning and gametogenesis influenced the variability of fatty acids through time in both species, suggesting the importance of considering the reproductive cycle when studying lipids in rocky shore species. Little evidence of lipid transfer between muscles and gonads was seen, suggesting the importance of direct lipid storage into the reproductive tissues. The influence of diet and life history of intertidal consumers on the temporal variability of their fatty acid compositions is important to understand, as it provides us with a better understanding of the functioning of rocky shore systems. There is an enormous potential for future research in this field of study.

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I am most grateful to the National Research Foundation (NRF) Masters bursary for providing the financial support for this research, you allowed a mature student to pay her own way - a privilege indeed. Lastly but not least, thank you Jehovah for seeing me through my Masters degree.

This thesis is dedicated to my brother the late Precious Nkosinathi Ndhlovu.

If you cannot be a pine on the top of the hill

Be a scrub in the valley-but be

The best little scrub by the side of the rill,

Be a bush if you can't be a tree

We can't all be captains, we've got to be crew,
There's something for all of us here,
There's big work to do, and there's lesser to do,
And the task you must do is the near.

If you can't be a highway just be a trail

If you cannot be the sun be a star;

It is not by size that you win or fail-

Be the best of whatever you are.

Douglas Mallock

To my lovely cousin the late Buti Solly Ndhlovu may your soul rest in peace.

Masungulo ya vuthlarhi I ku chava Jehovah (Khani Mamba)

DECLARATION

I, Rachel Tintswalo Ndhlovu, declare that this dissertation was submitted to Rhodes University for the degree of Master of Science, and work contained herein is my original work carried out under the supervision of Dr NB Richoux.

1 GENERAL INTRODUCTION AND OVERVIEW

1.1. The coastal environment

Coastal environments account for nearly 4 % of Earth's total land area (UNEP, 2006), and accommodate some of the most diverse ecosystems in the world (Di Gregorio, 2005; Martinez *et al*., 2007). Terrestrial and oceanic habitats interact within the coastal regions. These areas constitute complex and dynamic environments (Di Gregorio, 2005; Bode *et al*., 2006; Hill *et al*., 2008) characterized by high ecological productivity and they encompass a broad range of habitat types that harbour a wealth of species and genetic diversity. Coastal ecosystems serve a variety of ecological roles, as they store and cycle nutrients, filter pollutants from inland freshwater systems, and help to protect shorelines from erosion and storms (Burke *et al*., 2001; McLean *et al*., 2001). Coastal ecosystems also provide a wide array of goods and services; for example, they are important producers of fish, shellfish and seaweed for both human and animal consumption (McLean *et al*., 2001). Coastal regions accommodate one third of the world's population (UNEP, 2006) and yet the ecological significance of coastal ecosystems is far greater than commonly realised.

1.2. The South African coastline

The South African coastline stretches for some 3000 km from Kosi Bay near the Mozambique border in the east to the Gariep (Orange) River at the Namibian border in the west. South Africa has a diverse coastline that is considered relatively pristine by international standards (Branch *et al*., 2002). The southern African coast can be broadly divided into three biogeographic regions: namely the cool-temperate Namaqua region, the warm-temperate Agulhas region and the subtropical East Coast region (Stephenson and Stephenson, 1972; Emanuel *et al*., 1992; Whitfield, 1998; Turpie *et al*., 2000). These three regions are distinct physically and biologically mainly due to differences in the oceanographic current regimes (Lutjeharms, 2006). The east coast, which borders the Indian Ocean, is influenced by the south-flowing Agulhas Current, which brings warm water between 22-26 ºC from the Mozambique Channel (Lutjeharms *et al*, 2000). The west

(Atlantic) coast is influenced by the cold, north-flowing Benguela Current (Lutjeharms, 2007), with wind-driven upwelling giving rise to productive coastal ecosystems and fisheries (Lutjeharms *et al*., 2000). These coastal oceanographic conditions lead to the co-occurrence along the South African coastline of a wide array of marine fauna and flora assemblages (von der Heyde, 2009).

1.3. Rocky intertidal zone

Among coastal environments, rocky shore ecosystems are subject to various hydrodynamic conditions which determine the structure and the composition of communities (Riera *et al*., 2009). Rocky intertidal areas range from those that are seldom exposed to tidal seawater to those that are covered most of the time (Zottoli, 1973). Accordingly, organisms that live within this zone are exposed to marine conditions during high tide and dry conditions during low tide. The intertidal portion of rocky shores supports a great diversity of invertebrate grazers and suspension-feeders. These invertebrates serve as essential links between primary producers/microbial communities and higher level consumers such as fish, birds and mammals (Fiske *et al*., 2003). Intertidal community structure is profoundly influenced by the environment through several physical processes (Menge and Olson, 1990; Leichter and Witman, 1997) such as water flow, salinity, temperature and wave exposure (McQuaid and Branch, 1984; Menge *et al*., 1997) that drive food and nutrient delivery (Jenkins and Hartnoll, 2001). In this regard, water velocity and the degree to which organisms are exposed to wave action influence the amount of food that reaches suspension-feeding sessile organisms (Bustamante and Branch, 1996; Knox, 2000). The majority of intertidal animals are able to feed only when submerged and so the opportunities for feeding are limited by their position on the shore (Brehaut, 1982).

1.4. Rocky shore food webs

The southern African shoreline consists of approximately 27 % rocky shore, 42 % sandy beach, and 31 % mixed shore, the latter mostly comprising sand on the upper shore above a wave-cut rocky platform (Griffiths *et al*., 2010). The rocky shores exposed to strong wave action tend to be dominated by sessile suspension-feeders such as bivalves, barnacles and polychaetes (Bustamante *et al*., 1995). The dynamic nature of the rocky shore leads to large variations in the food available to these invertebrates through space and time. Studying the diets of primary consumers will help us to better understand the trophic structure and functioning of these diverse habitats, particularly as consumer diet plays an important role in

shaping the distribution and structure of marine communities (Howell *et al*., 2003; Laurand and Riera, 2006). Invertebrate feeding ecology represents an important aspect of carbon cycling and other important elements in aquatic food webs (Fiske *et al*., 2003).

Ecologists have long been interested in feeding relationships, which represent the nutrient and energy pathways, within animal communities. Modern understanding of aquatic ecosystems has been shaped by concepts of energy flow, food webs, and trophic levels (Kemp and Boynton, 2004). Rocky shore ecosystems are ideal for food web investigations because they provide large populations of small and easily manipulated organisms that are accessible on a daily basis. Food webs, or descriptions of "who eats what" within a community, provide a framework for integrating population dynamics, community structure, species integrations, community stability, biodiversity and ecosystem productivity (Belgrano *et al*., 2005). The assimilation and retention of key nutrients in consumers is fundamental to the optimal physiological performance of animals (Kainz *et al*., 2004). Detailed characterizations of energy flow through food webs highlight the trophic level of each species and the relative strength of the interactions based on the amount of energy flowing between producers and consumers (Trites, 2003). What remains lacking is our understanding of the spatial and temporal variability of these feeding relationships.

1.4.1. Primary producers and feeding modes of rocky intertidal organisms

On intertidal rocky shores, the food sources potentially available to invertebrate consumers include macroalgae (Phaeophyta, Rhodophyta, and Chlorophyta), benthic biofilms and suspended particulates (Riera *et al*., 2009). The relative amounts of primary production largely contribute to determine the productivity of the entire community (Persson *et al*., 1992). For instance, nutrient enhancement from coastal upwelling allows intertidal algae and higher-level consumers to increase productivity (Polis *et al*., 1997). Detritus originating from primary producers is mainly composed of refractory material that is degraded by microorganisms (i.e. bacteria, fungi; Anderson and Sedell, 1979). The detrital complex (i.e. detritus and associated microorganisms) is a readily available food source for benthic consumers (Tenore *et al*., 1984). On the South African coasts, detritus can dominate the suspended particulate material, rather than phytoplankton or bacteria (Simon and McQuaid, 1999).

Much shallow water benthos consists of sessile particle feeders that rely on organic material suspended in the water column (Polis *et al*., 1997). These consumers are active

feeders that can assimilate the different components of suspended particulate organic matter (e.g. bacteria, phytoplankton, zooplankton and detritus) (Teal and Teal, 1969; Knox, 2000; Kharlamenko *et al*., 2008). Suspension-feeders in intertidal environments are subject to a fluctuating and unpredictable food supply (Page and Lastra, 2003). Temporal variability in water motion and submergence time, resulting from tidal currents and waves, influences the availability of food to these organisms over the short term (hours, days), while the type, magnitude, duration, and frequency of highly seasonal productivity can be major relative influence to the changes in a high-quality food for consumers over the long term (weeks, months, years).

The diet of suspension-feeders is commonly based on macroalgae (e.g. kelps) and detritus (Bustamante and Branch, 1996; Miller and Page, 2012). However, because phytoplankton production is variable temporally and spatially according to local coastal oceanography features (e.g. upwelling cells, embayments, estuaries, etc.), it is expected that suspension-feeders could also base a large part of their diet on pelagic production. Phytoplankton, especially diatoms, are considered a high quality food for suspension-feeding species, and variability in this resource is reflected in the ecological performance (e.g. growth, reproduction) of suspension-feeders in the field (Widdows *et al*., 1979; Kiorboe *et al*., 1981; Blanton *et al*., 1987; Cranford and Hill, 1999). In addition, strong benthic-pelagic coupling can occur between suspension-feeding species and phytoplankton, with suspension-feeders altering phytoplankton abundance and species composition (Dame, 1996). Suspended particulate organic matter (POM) originating from terrestrial and/or riverine production also enters the near shore (Canuel *et al*., 1995; Deegan and Garritt, 1997; Cloern *et al*., 2002) and can be considered as a potential food source to suspension-feeders (Hackney and Haines, 1980; Stephenson and Lyon, 1982; Riera and Richard, 1997). River-derived POM may include a mix of freshwater phytoplankton, sloughed periphyton, vascular plant detritus, and terrestrially derived particulate material (Raikow and Hamilton, 2001).

Food webs on the intertidal rocky shores contain variable numbers of trophic levels. Alga-grazer interactions, herbivory and/or grazing effects include two trophic levels, the basal, represented by primary producers (algae), and primary consumers represented by herbivores (or grazers). Variability in grazing effects has been explained by gradients of wave exposure and desiccation (Hawkins and Hartnoll, 1983; Lubchenco, 1986; Menge and Olson, 1990). However, gradients of nutrient concentrations and oceanographic conditions can also

influence the variability in grazing effects at scales of kilometres (Bustamante *et al*., 1995, Menge *et al*., 1997).

The diets of grazers are variable; some feed on macroalgae (Underwood and Jernakoff, 1981; Steneck, 1982, 1983; Fletcher, 1987), others on microscopic living or dead plants or animals by means of appendages (muscular jaws with or without teeth, e.g. sea urchin) or a radula (Zottoli, 1973, e.g. limpets). Among grazers, the limpets are abundant herbivorous consumers that remove large quantities of unicellular microbes, algal germlings and detritus (Ruiz Sebastián *et al*., 2002; Brazão *et al.*, 2003, Maneveldt *et al*., 2009) using their radula (Brehaut 1982; Knox 2000). Limpets appear to feed unselectively during feeding excursions around their home scars (Brazáo, 2009). Limpets are also capable of consuming algae and diatoms assemblages (Branch, 1971; Chess, 1993). Camus *et al*. (2009) conducted feeding trails on three common Chilean herbivores (the key-hole limpets *Fissurella limbata* and *F. picta* and the polyplacophoran *Chiton granosus*) and found that their diets were comprised of both animal and algal food sources. Bustamante *et al*. (1995) demonstrated feeding behavior of *Patella granatina* and *P. argenvillei*, with both limpets feeding on sporelings and diatoms. Encrusting coralline algae have also been cited as important food sources for many intertidal herbivores (Steneck *et al*., 1991; Raffaelli and Hawkins, 1996). A number of grazers seem to perform best when foraging on mixed diets of intertidal algae, rather than one source that is dominant and readily available (Kitting, 1980; Hagele and Rowell-Rahier, 1999; Cruz-River and Hay, 2003).

Sea urchins are benthic omnivorous grazers, with a diet composed mostly of epilithic microalgae and green ephemeral algae (Santelices *et al*., 1986), in addition to some invertebrates (Aguilera, 2005; Camus *et al*., 2008; González *et al*., 2008, Navarrete *et al*., 2008). Analysis of the gut contents of sea urchin *Tetrapygus niger* showed crustose calcareous algae were the most frequent and abundant food item in its diet, although drift of the kelp *Lessonia nigrescens* and *Macrocystis pyrifera* were also consumed in low intertidal habitats (e.g. Rodríguez, 2003; Navarrete *et al*., 2008). Scheibling and Hatcher (2001) indicated that the distribution range of the sea urchin *Strongylocentrotus droebachiensis* was commonly associated with *Laminarian* kelps, and at high population densities, the sea urchins destructively grazed kelp beds and formed extensive barrens dominated by encrusting coralline red algae. Kelly and Cook (2001) demonstrated that small echinoid *Psammechinus miliaris* is an opportunistic omnivore, feeding on a wide range of material including macroalgae and encrusting invertebrates as well as scavenging on larger dead animals. This

variety of food sources and feeding habits reported indicates how complex grazer diets can be in natural habitats.

1.4.2. Study species

For this study, I selected four intertidal primary consumers: two suspension-feeders (the brown mussel *Perna perna* and the Cape reef worm *Gunnarea gaimardi*) and two grazer species (the goat's eye limpet *Cymbula oculus* and the Cape urchin *Parechinus angulosus*). The species were selected as model organisms on the basis of their feeding modes, generally high abundances and wide distributions along the South African coastline (Branch and Branch, 1981; Bustamante and Branch, 1996; Bownes, 2005).

The brown mussel *Perna perna* (class Bivalvia, family Mytilidae) is widely distributed in the tropical and subtropical regions of the Indian and Atlantic oceans (Viladomiu, 2004). This species is the dominant indigenous mussel along the warmer south and east coasts of South Africa (Berry and Schleyer, 1983). It is a species that inhabits wave exposed shores, and it reaches its maximum abundance in the mid-lower intertidal zone (Bownes, 2005). Berry and Schleyer (1983) found that *P. perna* is capable of filtering particles as small as 0.46 µm, such as free-living coccoid bacteria (Schleyer, 1981). As many mussels, *P. perna* seems to be able to regulate the organic content of ingested food, controlling its feeding mechanisms. Consequently, *P. perna* appears to selectively enrich the organic content of ingested matter by rejecting particles of higher inorganic content before ingestion (Suplicy *et al.*, 2003). This feeding selectivity is emphasized by several authors (Prato *et al.*, 2010; Cheung and Shin, 2011; Pernet *et al.*, 2012) studying the role of food availability in mussel diet preferences.

The Cape reef worm *Gunnarea gaimardi* (class Polychaeta, family Sabellidae), is a common sedentary polychaete found all around the southern African coast. The family Sabellidae includes tubicolous, reef-building polychaetes that are commonly found in shallow water where there is a good supply of sand (Fauchald and Jumars, 1979). These suspension-feeding polychaetes are among the most abundant marine metazoans in benthic environments (Fauchald and Jumars, 1979). Polychaetes are major controllers of sediment ecosystems and their mixing of sediment particles is an important driving force behind chemical reactions and transport of organic matter in marine sediments (Levinton, 1995; Merz and Woodin, 2000). The suspension-feeding tube-builder polychaetes use their branchial crowns to suspension-feed on particulate matter (Zottoli, 1973; Merz, 1984). Particles suspended in the water

column are filtered from the water and sorted according to size and composition (Charlmes, 2002). Large sand particles are used for tube construction, while smaller organic particles (3-5 µm for small worms, 6-8 µm for larger worms) are channelled by the cilia lining the radioles, to the mouth where they are ingested (Fitzsimons, 1965; Fauchald and Jumars, 1979; Charlmes, 2002). The polychaetes mostly filter small plankton composed of bacterio-, myco- and phytoplankton, filamentous green algae and detritus; these tube-forming polychaetes can filter bacterial cells about 0.5 µm in diameter from a suspension of single cells (Sorokin, 1973; Sieburth *et al*., 1978; Merz, 1984).

The Goat's eye limpet *Cymbula oculus* (phylum Mollusca, class Gastropoda) is a non territorial species that occurs in high abundances on the rocky shores of South Africa (Branch, 1976), and they are normally exposed to wave action and total exposure to air (Reinecke *et al*., 2012). Like other limpets, *C. oculus* uses "radula" teeth to scrape off food from the substrate (Steneck and Watling, 1982). Their diet includes a wide variety of food including coralline algae, lichens, organic debris and benthic microalgae (Brehaut, 1982; Knox, 2000; Ruiz Sebastián *et al*., 2002; Lasiak, 2006; Maneveldt *et al*., 2009; Reinecke *et al*., 2012).

The Cape urchin *Parechinus angulosus* (phylum Echinodermata, class Echinoidea) is common along the southern African coast (Day and Branch, 2000, 2002). Cape sea urchins live in vast numbers on shallow reefs, and primarily occupy hard substrata (Farquhar, 1994; Day and Branch, 2000). *P. angulosus* occurs in pools of the rocky shore and remains submerged most of the time during low tide. Most rocky shore urchins including *P. angulosus* have an Aristotle's lantern which they use to scrape material from the sea floor, and tubular feet to trap drift macroalgae (Tarr *et al*., 1996; Day and Branch, 2002). Many species of sea urchin occupy crevices by day, but emerge to feed by night (Shepherd, 1973; Day and Branch, 2002). Urchins graze on kelp, marine algae (McClintock, 1994; Raffaelli and Hawkins, 1996) and can selectively feed on detritus from terrestrial plants which are transported into their habitats and encrusting coralline algae on rocks (Rowley, 1990; Guillou and Lumingas, 1998, 1999; Day and Branch, 2000).

1.5. Trophic biomarkers

Traditional approaches to food web analysis include direct observation of feeding, laboratory feeding assays and gut content analysis of field-collected animals (Michener and Lajtha, 2007). These techniques have helped resolve food web structures and processes, yet

each method has its disadvantages. Gut content analysis has the major disadvantage in that it provides only a snapshot view (Verkuil, 1996; Sakano *et* al., 2005) on dietary composition of animals based on recent ingestion rather than assimilation (Hesslein *et al*., 1993). This method does not provide reliable quantitative results as it can lead to bias due to differences in the presence of unidentifiable, highly decomposed material (Haywood, 1995; Créach *et al*., 1997; Reñones *et al*., 2002). Regurgitation and post-mortem digestion of stomach contents is also frequent, and can make identifications challenging (Haywood, 1995). Biochemical techniques such as fatty acid and stable isotope analyses provide time integrated information on food sources that have been incorporated into organism tissues (Hesslein *et al*., 1993; Maruyama *et al*., 2001; Sakano *et al*., 2005), and they generally require fewer samples compared to stomach content analysis (Abrantes *et al*., 2010).

Fatty acids have been successfully used in many ecological studies to help define diets of marine organisms (Hughes *et al*., 2005; Alfaro, *et al*., 2007; Kelly, *et al*., 2008; Allan *et al*., 2010). Many potential food sources contain specific fatty acids which are consumed and retained by organisms occupying different trophic levels (Kharlamenko *et al*., 2008). As such, the fatty acid composition of a consumer is a reflection of its food sources, as fatty acids with 14 or more carbons are deposited in consumer tissues in a relatively unmodified manner (Budge *et al.*, 2006). Moreover, because most animals cannot efficiently synthesise polyunsaturated fatty acids (PUFA) *de novo*, some can be used as tracers or biomarkers to trace the origin of the food to a specific source (Dalsgaard and John, 2004). The use of individual fatty acids as tracers is, however, most useful if those fatty acids can be traced to only one of the possible food sources (Iverson, 1993).

1.6. Thesis overview

Although ecological studies utilizing biochemical tracer methods have been successful in determining trophic pathways in aquatic systems, many studies have been based on sample collections of limited temporal scale (Kharlamenko *et al*., 2001; Alfaro *et al*., 2006; Budge *et al*., 2008; Jaschinski *et al*., 2008; Hyndes and Hanson, 2009; Allan *et al*., 2010), and relatively few studies have focused attention on rocky intertidal invertebrates. It is critical that the scientific community gains an understanding of how fatty acid profiles change in primary consumers through time, is only then can we begin to interpret and validate ecological data derived from snapshot or short-term studies. To address these

important gaps in our knowledge of the temporal variability in the diets of rocky shore organisms, I performed a year long field study of several invertebrate species.

The main aim and objectives of this study were to determine:

1. the applicability of using fatty acids for assessing the diets of suspension-feeders and grazers on the rocky shore,
2. the temporal variations in the diets of rocky intertidal suspension-feeders (brown mussel *Perna perna* and Cape reef worm *Gunnarea gaimardi*) and grazers (goat's eye limpet *Cymbula oculus* and Cape sea urchin *Parechinus angulosus*) using fatty acid signatures,
3. how different life styles affect the temporal variability in the parameters measured,
4. how the reproduction cycle of each invertebrate could potentially bias signatures intended to measure diet.

For point #3, I hypothesized that the suspension-feeders experience high levels of variability in their diets through time because water quality has the potential to change quickly and drastically (Bastamante and Branch, 1996; Fiske *et al.*, 2003), whereas grazers experience less variability in their diets over time since their food sources are more constant (Galbraith and Vaughn, 2009).

For point #4, I first hypothesized that the reproductive cycles of the suspension-feeder *Perna perna* and the grazer *Parechinus angulosus* affect the fatty acid composition of their gonads, with temporal variations in lipid composition reflecting changes in reproduction investment (Castell, 1982; Ackman, 1983; Brazão *et al*., 2003). Secondly, I hypothesized that the total amount of energetic reserves available for reproduction is different for each gender (females allocate more energy to egg production than males allocate to sperm production; Robards *et al*., 1999; Guijarro *et al*., 2003).

The structure of the thesis is as follows:

Chapter 2 includes descriptions of the study area and the methods used. Chapter 3 explores temporal variations in the diets of suspension-feeders and grazers in the rocky intertidal zone. The changes in the fatty acid profiles of reproductive organs are explored in Chapter 4, with the aim towards understanding the effects of the reproductive cycle on the temporal variations in fatty acids. Chapter 5 includes the general discussions and conclusions.

2 GENERAL METHODS

2.1. Sample collection

Collections were carried out at one site near the mouth of the Kariega Estuary on the eastern coastline of South Africa (Fig. 2.1).

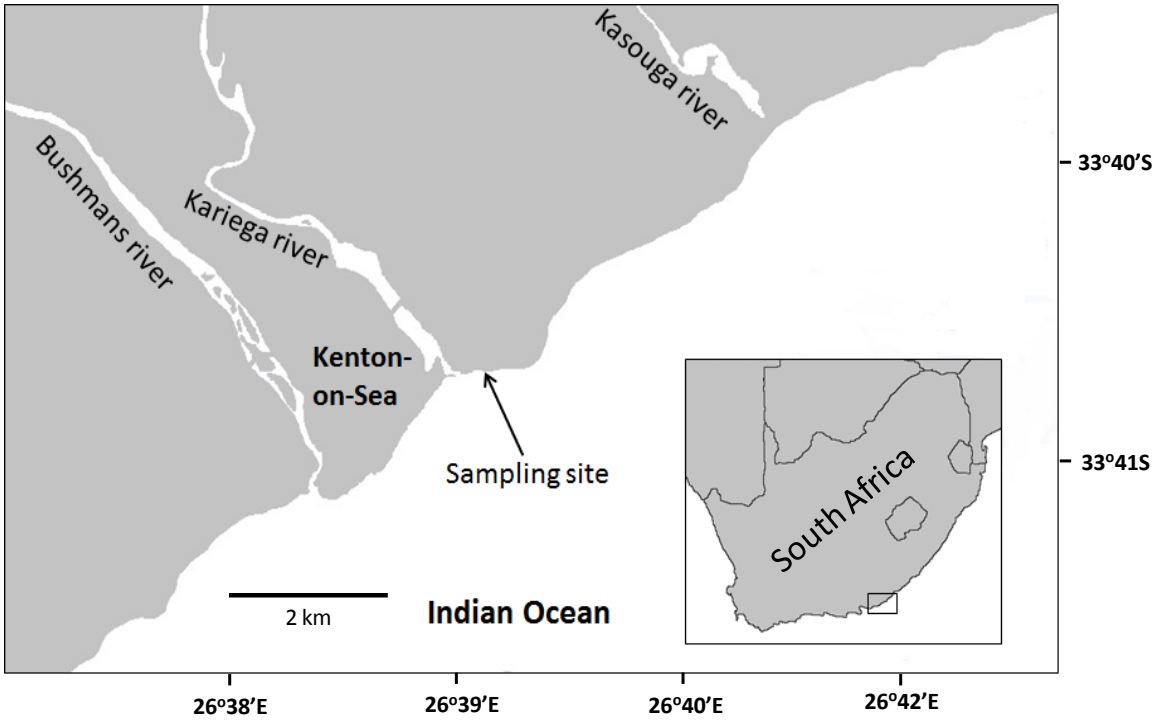

Figure 2.1. Map of the southeast coast of South Africa showing the position of the study area

The study site (33°40'50.1"S, 26°41'51.0"E) was approximately 2 km north-east from the permanently open Kariega river mouth. The environment around the study location is dominated by an extensive sandy beach and some rocky outcrops. The exposed rocks constituting the shore form a horizontal platform with some rock pools extending about 500m along the shore (Fig. 2.2).

Figure 2.2. Study site near Kariega River mouth. Note the dominance of red algae and the reefs of the polychaete *Gunnarea gaimardi* on the right side of the picture (photo: Marco Fusi).

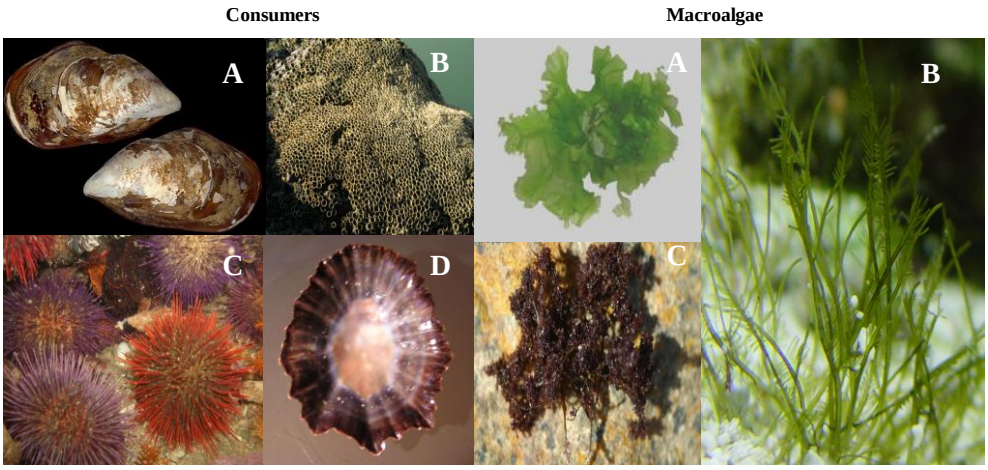

Figure 2.3. Study consumers: Suspension-feeders (A) *Perna perna*, (B) *Gunnarea gaimardi* and grazers: (C) *Parechinus angulosus*, (D) *Cymbula oculus*; and three species of macroalgae (A) *Ulva* sp., (B) *Bryopsis* sp., (C) *Gelidium pristoides*, (photos are taken from the internet).

Sampling was conducted for almost a year during low tide on each date. Note: samples were not colleted during the month of September due to technical problems. Four invertebrate species were selected for collection: the mussel *Perna perna* and the reef-building polychaete *Gunnarea gaimardi* (suspension-feeders; with the polychaetes located generally lower in the intertidal zone than the mussels), and the limpet *Cymbula oculus* and the echinoid *Parechinus angulosus* (grazers; the limpets occupying exposed open rock and the urchins in sheltered rock pools during low tide)(Fig. 2.3). Sea urchins were collected by hand from intertidal pools, whereas limpets, mussels and polychaetes were dislodged from rocks using a chisel. A preliminary study was done to determine the minimum number of individuals needed per species to make up at least 20 mg dry weight (DW) per sample, depending on their lipid content. Ten mussels (ranged from 35-50 mm in total length), ten sea urchins (ranged from 24-41 mm in total width and height of 21-33 mm) five limpets (ranged from 41-73 mm in total length) and five polychaetes were used for one fatty acid sample. Macroalgae were collected including three locally abundant species (the chlorophytes *Ulva* sp. and *Bryopsis* sp., and the rhodophyte *Gelidium pristoides*). The three species of macroalgae were collected by hand from the same areas as the animals (Fig. 2.3). However, *Bryopsis* sp. was scarce in some months and was collected only when available. Additionally, three samples of 5-litre surface sea water were collected as a measure of suspended particulate matter (SPM). Specimens were transported to the laboratory in buckets filled with seawater, and the animals were kept in aerated seawater over night to allow for gut clearance.

Water temperature was measured once a month using a YSI probe 550A and data are reported in degrees Celsius (°C) (Figure 2.4). Photoperiod was obtained from the internet, area Port Elizabeth, South Africa and data are reported in hours (h). Data from the internet were reported in hours/minute/seconds (hh/mm/ss) of day length and I therefore transformed it into decimal units (http://www.timeanddate.com/worldclock/astronomy.html?n=1485).

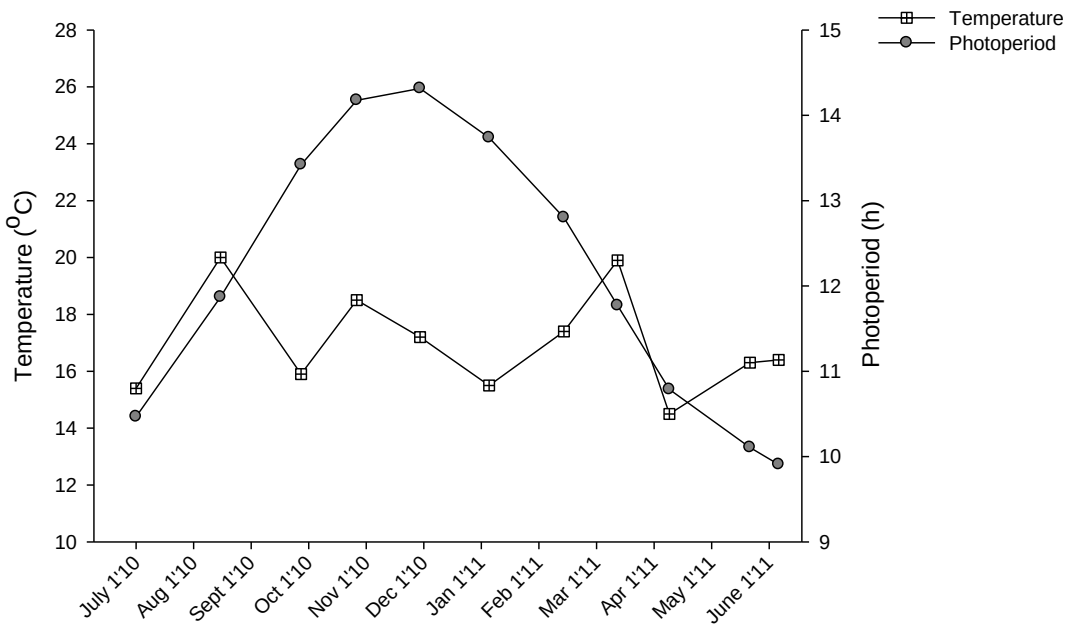

Figure 2.4. The annual variations in water temperature (°C) and photoperiod (h) measured from the study area.

2.2. Sample treatment

Water samples were vacuum-filtered onto pre-combusted Whatman GF/F filters (47mm) after each day of collection. Fresh macroalgae were placed in boiling filtered seawater for 2 minutes to ensure that all lipolytic enzymes were denatured (Budge and Parrish, 1999). Each animal was measured to the nearest millimetre using vernier callipers and dissected on ice with the aid of a dissecting microscope. The muscles and gonads of the sea urchins and mussels were analysed separately, as were the muscles of the limpets, whereas the polychaetes were analysed whole. The gender of each sea urchin and mussel was determined based on gonad colour, texture and thickness (Morias *et al*., 2003; Kawashima *et al*., 2008). All samples (filters of SPM and animal tissues) were stored in aluminium foil, sealed in Ziplock® bags and frozen at -80 °C until further analysis.

2.3. Fatty acid analysis

All samples were lyophilized with a VirTis BenchTop K freeze dryer at -60 °C for a minimum of 24 hours, then returned to -80 °C. All materials used in the preparation of tissue samples for fatty acid determinations were lipid-cleaned [three rinses each of methanol followed by chloroform ($CHCl_3$)] prior to and during processing. Clean glass tubes were

ashed at 450 °C for a minimum of four hours in a muffle furnace to remove any remaining organic contaminants. Algae and animal tissues were homogenized individually using a mortar and pestle. Subsamples were weighed (ranged from 20 to 100 mg of dry weight, depending on their lipid content), then stored at -20 °C in 2 ml $CHCl_3$ with 0.01 % butylated hydroxytoluene under nitrogen (N_2) atmosphere and sealed with Teflon tape.

Total lipids were extracted and fatty acid methyl esters (FAMEs) produced using a modified one-step procedure from Indarti *et al*. (2005). A known quantity of internal standard (nonadecanoic acid; 19:0) was added to each sample to allow for FAME quantification. Two ml of a freshly mixed solution of sulphuric acid and methanol (0.3:1.7 *v*: *v*) dried with anhydrous sodium sulphate (Na_2SO_4) were added to each homogenized sample in $ChCl_3$. Test tubes were topped with N_2, sealed with Teflon-lined caps and placed in an oven at 100 °C for 30 minutes. The tubes were cooled to room temperature, vortexed, and 1 ml of MilliQ water was added. Samples were vortexed for 10 seconds and centrifuged at 3000 rpm for 3 minutes. After centrifugation, the upper aqueous layer was discarded. Anhydrous Na_2SO_4 was added to each tube now containing only FAMEs in $ChCl_3$. FAME extracts were transferred into 2 ml vials through Pasteur pipettes containing columns of pre-cleaned cotton wool and Na_2SO_4. The resultant extract was concentrated to dryness under a gentle stream of N_2 and covered in lml hexane. All vials were topped with nitrogen, capped and sealed with Teflon tape until injection into the gas chromatogram (GC).

Fatty acid composition of each sample was determined by GC analysis using an Agilent 7890A GC equipped with a flame ionization detector and a capillary column (30 m length x 0.32 mm internal diameter I.D., 0.25 µm film thickness, ZB-Waxplus). The oven temperature was set at 70 ºC and held for 1 min, then ramped at 40 °C/min to 170 °C and held for 3 min and increased to 250 °C at 2.5 ºC min^{-1} and held for 4.5 min (for a total run time of 40 min). Helium was the carrier gas at a flow rate of 1.6641 ml min^{-1}, and the injector and detector were maintained at 250 °C and 300 °C, respectively.

Peaks were integrated using Chemstation, and their identities confirmed on a subset of samples using an Agilent 7000A GC/MS-QQQ coupled with the NIST 08 MS library and also by comparing retention times with those of known external standards (37 component FAME mix, marine PUFA no. 1, Bacterial acid methyl esters mix, Supelco). Each fatty acid was measured qualitatively as a proportion of the total identified fatty acids (% TFA) and

quantitatively as fatty acid per dry weight (µg FA mg^{-1} DW) using the internal standard peak areas.

Fatty acids are reported in the shorthand form x: aωb, where x is the number of carbon atoms in an acyl chain, a is the number of double bonds, and b is the position of the first double bond from the methyl end of the molecule. Saturated fatty acids (SFAs) have no double bonds between carbons (e.g. 14:0); monounsaturated fatty acids (MUFAs) contain one double bond (e.g. 16:1ω7), while polyunsaturated fatty acids (PUFAs) have >1 double bond (e.g. 16:4ω3). Essential fatty acids, or EFAs, include the 18 carbon FAs 18:2ω6, 18:3ω3, 18:4ω3; and long chain fatty acids 20:4ω6, 20:5ω3 and 22:6ω3 (Arts *et al*., 2001). Bacterial fatty acids (BAFAs) are those having odd-numbered carbon chains and/or *iso*- (*i*-) and *anteiso*- (*ai*-) branches (Budge and Parrish, 1998), and two of the EFAs, 18:2ω6 and 18:3ω3, are also of interest as indicators of green algae and HPFAs (or higher plant fatty acids), these markers are suitable only for environments where these food sources are dominant (Dalsgaard *et al*., 2003).

3 TEMPORAL VARIABILITY IN DIET AND LIPID COMPOSITION OF SUSPENSION-FEEDERS AND GRAZERS ON A SOUTH AFRICAN ROCKY SHORE

3.1. INTRODUCTION

Intertidal rocky shores are heterogeneous environments that support a wide variety of living forms (Thompson *et al.*, 2002). There are two main classes of consumers which are commonly found in rocky intertidal zones: suspension-feeders and grazers (Fréchette and Bourget, 1985; Griffiths and Hockey, 1987; Kawashima *et al*., 2002). Suspension-feeders such as mussels and grazers such as limpets encounter a wide diversity of potential food sources including macroalgae, epilithic or epiphytic biofilms, detritus of various origins, zooplankton, phytoplankton (Teal and Teal, 1969; Fréchette and Bourget, 1985; Navarrete and Wieters, 2000; Bode *et al*., 2006), bacteria and dissolved organic matter (Riley, 1963; Williams and Yentsch, 1976; Zweifel *et al*., 1993).

Suspension-feeders have access to numerous food resources that are produced in the three-dimensional pelagic environment and are continually replenished by tidal currents and wave action flowing over the sessile or sedentary invertebrates (Fréchette and Bourget, 1985). Among suspension-feeders, mussels and polychaetes have been studied to determine the effects of their filtration activities on plankton communities. For example, Prins *et al*. (1996) measured the *in situ* exchange of suspended particulate matter (SPM) and phytoplankton between the water column and mussel beds (SW Netherlands) and found that the mussels acted as selective filters for phytoplankton. In bivalves in general, phytoplankton represents one of the primary sources of food, as growth and reproductive activities of bivalves follow the annual cycle of phytoplankton biomass (Kang *et al*., 2006). However, studies revealed that mussels can ingest and possibly assimilate mesozooplankton (200 μm - 2 cm) (Davenport *et al*., 2000; Lehane and Davenport, 2002; Wong *et al*., 2003; Zeldis *et al*., 2004; Alfaro, 2006; Wong and Levinton, 2006). Cannibalism of adult mussels on early stages of juvenile mussels has also been reported (Porri *et al*., 2008). For many suspension-feeders, phytoplankton is not always the greatest contributor to diets despite its high nutritional quality, because its production is so variable and erratic (Schleyer, 1981; Talbot and Bate, 1988), hence suspension-feeders can rely heavily on detritus and to lesser extent bacteria

(Langdon and Newell, 1990; Simon and McQuaid, 1999). On the South African coast, detritus forms the bulk of the SPM biomass, followed by phytoplankton and bacteria (Cliff, 1982; Talbot and Bate, 1988). On the east coast of South Africa, Berry and Schleyer (1983) found that 25 % of dry organic content of suspended food was available for *Perna perna* and of this, detritus comprised about 94.1 %, phytoplankton 5.4 % and free bacteria 0.5 %. How these contributions change through space and time, and across taxa, remains largely unknown. The quantity and quality of food for rocky shore suspension-feeders has a great potential to change through space and time due to a variety of physical factors (such as nutrients dynamic, coastal hydrodynamics, currents, tidal range and wave exposure) which reshape plankton assemblages in the water column.

In contrast to suspension-feeders, grazers utilise food that is produced in a relatively limited, two dimensional space on the rocky surface that is less frequently replenished (Bustamante *et al*., 1995). In coastal ecosystems, grazing by gastropods, arthropods, and echinoids can directly impact algal assemblages (Lubchenco and Gaines, 1981; Hawkins and Hartnoll, 1983), indirectly impact other herbivores through resource competition (Underwood *et al*., 1983; Branch, 1984), or create a combination of effects through habitat modification (Hori *et al*., 2006). As a feeding guild, grazers exhibit important controls on the species composition, distribution and dynamics of algal communities (Lubchenco and Gaines, 1981; Paine, 2002; Jenkins *et al*., 2005). Limpets, for example, are intertidal grazers that remove, almost continuously and apparently unselectively, both the microbial films that coat surfaces (Hill and Hawkins, 1991; Jenkins and Hartnoll, 2001; Thompson *et al.*, 2005) and macroalgae (Benedetti-Cecchi *et al*., 2001; Jenkins *et al*., 2005). The biofilm is a heterogeneous mixture of unicellular microbes, algal germlings, diatoms and detritus: a complex community affected by many physical and biological factors (Branch, 1981; Della *et al.*, 1995; Thompson *et al*., 2004). Similarly, local macroalgal communities are shaped by a variety of physical conditions (light, temperature, wave exposure, substrate slope, etc.) and biotic factors such as the presence of herbivores (Bulleri *et al*., 2012). Consequently, food availability and quality of rocky shore grazers can change through space and time (Fretter and Graham, 1976; Branch, 1981; Underwood *et al*., 1983; Farrell, 1991; Benedetti-Cecchi, 2000). However, because grazers inhabit demersal habitats and feed on benthic food sources, the extent of the aforementioned variations is potentially smaller than that faced by suspension-feeders relying on planktonic food sources (Galbraith and Vaughn, 2009; Lai *et al*., 2010).

Lipids, and especially fatty acids, have long been used as biological indicators of diets of marine organisms (Sargent *et al*., 1988). Fatty acids are the primary constituents of most lipids, and are carbon-rich compounds that are ubiquitous in all organisms and relatively easy to metabolize from the diet (Nooshin and Peyman, 2011). Therefore, to the organism, fatty acids are important because they contribute energy, essential nutrients for survival and growth, and components of cell membranes (Sargent *et al*., 1987; Hazel *et al*., 1991; Parrish, 1998; Parrish *et al*., 2000). In marine organisms, fatty acids are commonly composed of chains of 14 to 24 carbon atoms, with varying numbers of double bonds. Some ingested fatty acids can remain intact or be predictably modified through deposition in animal tissues (Fraser *et al*., 1989; Iverson *et al*., 1995; Kirsch *et al*., 2000), and animals can biosynthesize a relatively limited number of fatty acids (Cook, 1985). These biochemical restrictions, coupled with the fact that fatty acids in the marine food web are exceptionally complex and diverse, provide researchers the opportunity to use fatty acids to understand trophic interactions in food webs. The biological specificity of fatty acids, and their eventual transference from primary producers to higher trophic levels, makes them suitable for use as dietary indicators (Parrish *et al*., 2000). For example, previous studies have used fatty acids as biomarkers for bacteria (Rajendran *et al*., 1993), diatoms (Parrish *et al*., 2000), dinoflagellates (Parrish *et al*., 2000), zooplankton (Falk-Petersen *et al*., 2002), and macroalgae (Johns *et al*., 1979; Khotimchenko and Vaskovsky, 1990). As such, individual fatty acids can then be used to trace the origin and routes of organic matter in ecosystems (Alfaro *et al*., 2006). Furthermore, total fatty acid profiles can be analysed using ordination techniques to assess consumer diets and determine whether they are changing through space or time (Hudson *et al*., 2004; Kelly and Scheibling, 2012). Several researchers have used fatty acid signatures in this manner to investigate spatial or temporal variability in consumer diets (Galois *et al*., 1996; Gladyshev *et al*., 2007; Ventrella *et al*., 2008; Martinez-Pita *et al*., 2010; Prato *et al*., 2010), reproductive dynamics (Morais *et al*., 2003; Khotimchenko, 2006; Martinez-Pita *et al*., 2010; Lazzara *et al*., 2012), or environmental factors that affect food webs (Khotimchenko and Yakovleva, 2005; Leu *et al*., 2006; Prato *et al*., 2010). However, many of these studies have been based on data collected over a limited temporal scale (e.g. seasonal).

The primary aim of this study was to assess temporal changes in diets of two rocky shore suspension-feeders and two grazers. The quality and temporal variability in the invertebrate diets was assessed using both individual fatty acids and overall fatty acid profiles. I hypothesized that the diet quality and temporal variability of the organisms

depends on their lifestyle: suspension-feeders should experience great variability in their diets through time because of changes in water quality (Kattner and Krause, 1989; Bustamante and Branch, 1996; Fiske *et al*., 2003; Li *et al*., 2007) and plankton availability (Haury *et al*., 1978; Pitcher *et al*., 1992; Harding and Perry, 1997; Lai *et al*., 2010). Grazers, on the other hand, should experience little variability in their diets over time since their macroalgal food sources are relatively more consistent compared to those in the water column (Galbraith and Vaughn, 2009).

3.2. METHODS

3.2.1. Study area, sample collection and treatment

Detailed descriptions of the study area and sample collection (section 2.1), sample treatment (section 2.2) and fatty acid analysis (section 2.3) have been provided in Chapter 2.

3.2.2. Trophic and dietary fatty acid markers

An ideal trophic marker is a compound whose origin can be uniquely and easily identified, that is inert and not harmful to organisms and that is not selectively processed during food uptake and incorporation. Furthermore, an ideal marker is metabolically stable and hence transferred from one trophic level to the next in both qualitative and quantitative manner (Dalsgaard *et al*., 2003). Most of the different food types available to consumers have many fatty acids in common; however, some specific fatty acids, groups of fatty acids and fatty acid ratios can serve as biomarkers of different food sources (Sargent *et al*., 1987). In this chapter, a variety of trophic markers and ratios were determined to obtain information on the dietary contributions of different food sources (i.e. macroalgae, diatoms, dinoflagellates) to the suspension-feeders and grazers. Epiphytic diatoms can be grazed upon in benthic environments (Nichols *et al*., 1986, Latyshev *et al*., 2004; Richoux and Froneman, 2008), and both diatoms and dinoflagellates can be filtered from the water column by suspension-feeders (Graeve *et al*., 2001; Guest *et al*., 2008). Diatoms are characterized by relatively high concentrations of 16:1ω7, 16:4ω1, and 20:5ω3, while dinoflagellates are typically rich in 22:6ω3 and 18:4ω3 (Kharlamenko *et al*., 1995; Volkman *et al*., 1998; Parrish *et al*., 2000; Dalsgaard *et al*., 2003). The fatty acid marker 18:1ω9 can be a good indicator of carnivorous feeding, as reported in copepods (Daalsgard *et al*., 2003; Hudson *et al*., 2004). Two independent ratio markers were used to assess the prevalence of diatoms and dinoflagellates, respectively, in the suspension-feeder diets: (16:1ω7+16:1ω5)/16:0 and 22:6ω3/20:5ω3 (Budge and Parrish, 1998). The fatty acids 18:2ω6, 18:3ω3, 18:4ω3, 20:4ω6, 20:5ω3 and

22:6ω3 were summed together to represent the total essential fatty acids (ΣEFAs). Several additional sums were calculated: total polyunsaturated fatty acids (ΣPUFAs), total monounsaturated fatty acids (ΣMUFAs), total essential fatty acids (ΣEFAs), total bacterial fatty acids (BAFAs) are those with odd-numbered carbon chains and *iso- (i-)* and *anteiso- (ai-)* branches (Budge and Parrish, 1998), and higher plant fatty acids (HPFAs) include 18:2ω6 and 18:3ω3 (Dalsgaard *et al*., 2003). Refer to Chapter 2 for fatty acid nomenclature.

3.2.3. Data analysis

Principle components analysis (PCA, based on a covariance matrix) of proportional fatty acids data were completed to visualize temporal patterns in the food sources (separate PCAs for macroalgae and SPM) and the consumers (separate PCAs for each species). Proportional data were first arcsin square root-transformed to reduce the effect of the most abundant fatty acids, and improve graphical representations. PCA reduced the large number of fatty acid variables to fewer dimensionless axes, and provided coefficient components (loadings) to identify those fatty acids that contributed most to any separations between months (Richoux, 2011). All fatty acids occurring at levels > 1 % of total fatty acids (% TFA) were included in each multivariate analysis. All analyses were performed on percent composition data and results were confirmed by analysis of the quantitative data (not shown). The PCA factor loadings (> 2.5 % for at least one component) of the potential food sources and consumers were used to identify the fatty acids contributing the most to the variability in the data set. PCA results were summarised as principal components (PCs) 1, 2 and 3 scores. The first two PCs were plotted on the *x*- and *y*-axes, and the third component was presented in the appendix (Figures A1 to A3). To test for statistical differences among months within a PCA, PC-1 and PC-2 scores were used as dependent variables in two separate one-way ANOVAs, and the sources of any temporal variations were determined using Tukey post-hoc tests. Monthly groupings were considered distinct from one another if either or both of the PC1 or PC2 scores differed significantly (Richoux, 2011).

Additionally, the monthly variations characterizing the fatty acid composition of animals and their potential food sources were analyzed using a multidimensional scaling (MDS) approach, associated with analyses of similarity (ANOSIM) testing for monthly variations, and analyses of the contributions of the different fatty acids to these variations (SIMPER). An example of results obtained using the MDS/ANOSIM/SIMPER approach is presented in the Appendix (Fig. A5 and Table A20, case of *Perna perna*). Overall, the MDS

and PCA approaches produced equivalent statistical scores, comparable graphical visualization of monthly patterns in fatty acid composition of animals, and they identified similar fatty acids contributing to these differences. Consequently, only outputs from the PCA approach are detailed herein, since they allowed for a more synthetic representation of overall results (i.e. one 2-dimensional labeled graphic, including loadings, for each PCA).

All quantitative (Tables A2, A3) and proportional (Tables A4, A5) data are presented as untransformed values. The quantitative (µg FA mg^{-1} DW) and proportional (%TFA) data of primary producers (*Ulva* sp., *G. pristodes*, *Bryopsis* sp.) and consumers (*P. perna*, *G. gaimardi*, *P. angulosus*, *C. oculus*) revealed similar patterns, therefore most graphics are presented as percentage data. General Linear Models (GLM) followed by Tukey post-hoc tests were used to explore monthly differences of specific biomarker fatty acids (%TFA) in each potential food source and consumer. The GLM procedure uses the method of least squares, and is particularly suited to unbalanced data (Quinn and Keough, 2002). Data were checked for normality and homogeneity of variance by examination of residuals, and after transformations [arcsin, square root or log (x+1), depending on variables], both assumptions were satisfied.

PCA and n-MDS were performed using PAST 1.42 (Hammer *et al*., 2001) and PRIMER v6 (Clarke and Gorley, 2006), respectively, and SigmaPlot 10.0 was used for creating or improving graphics. All GLM or ANOVA were done using Statistica (v10), and results were considered statistically significant at $p < 0.05$. Letters ('a', 'b', etc.) were used to show significant differences between months in the appendix (Tables A3, A5, A9, A10, A11, A12 and A15). Coefficients of variation (CVs) were calculated by dividing each standard deviation by the mean and expressed as percentages (% CVs). Variations are reported as ± one standard deviation (SD) from the mean.

3.3. RESULTS

3.3.1. Temperature

Minimum water temperatures were recorded in July, October, January and April (14.5 to 15.9 °C), and maxima in August and March (19.9 to 20.0 °C) graphics are presented in chapter 2 Figure 2.4.

3.3.2. Primary producers

Thirty fatty acids (Table A1) were detected at concentrations > 1 % TFA in the macroalgae and suspended particulate matter (SPM); the mean ± SD of the specific biomarker fatty acids (% TFA) are reported as quantitative (Tables A2 and A3) and proportional data (Tables A4 and A5). PCs 1, 2 and 3 explained 35, 14 and 9 %, respectively, of the variance in the SPM proportional fatty acids data (Fig. 3.1A). One-way ANOVA of PC-1 ($F = 35.29_{10,\ 22}$; $p < 0.001$) and PC-2 ($F = 4.65_{10,\ 22}$; $p < 0.001$) scores detected significant differences in the fatty acid composition of SPM among months. Most notable was the complete separation of July, October and November 2010 from the rest of the year. All macroalgae (the chlorophytes *Ulva* sp. and *Bryopsis* sp. and the rhodophyte *Gelidium pristoides*) data were combined into one PCA. GLM of PC-1 ($F_{(months)} = 2.34_{9,\ 59}$; $p < 0.01$; $F_{(species)} = 537.14_{2,\ 59}$; $p < 0.001$) showed significant differences among months and species, and PC-2 ($F_{(months)} = 0.33_{9,\ 59}$; $p > 0.05$; $F_{(species)} = 0.48_{2,\ 59}$; $p > 0.05$) scores showed no significant variation among months or species using fatty acid composition (Fig. 3.1B). The corresponding figures of the third principal component for SPM and all macroalgae are provided as an appendix (Fig. A1).

The quantities of EFAs and TFAs showed no significant differences through time in each species of macroalgae (Table 3.1). Significant monthly variations were detected in TFA of SPM (Table 3.1). SPM generally showed low concentrations of TFAs and EFAs compared to all macroalgae species (Fig. 3.2). The relative proportions of PUFAs, MUFAs and SFAs in each macroalgae species did not change through time (Table 3.2, Fig. 3.3A, B, and C). SPM showed no significant changes through time in PUFAs or MUFAs (Table 3.2, Fig. 3.3D), but SFAs did (Table 3.2). The fatty acid categories (% TFA) in *Ulva* showed very low variability among-individuals and appeared fairly consistent through time (Table 3.2, Fig. 3.3A). *Gelidium* was also fairly consistent through time, although the among-individual variability was much larger as evidenced by large standard deviations (Table 3.2, Fig. 3.3B). *Bryopsis* was also very consistent with large among-individual variability, but very consistent through time (Table 3.2, Fig. 3.3C). SPM showed lower quantities of EFAs (Table A5; range 7.44±2.56 to 20.76±0.81 % TFA). The proportion of EFAs (% TFA) in SPM showed a clear trend (Fig. 3.4), with low values from July to November 2010 and increased values from December to June 2011. BAFAs, but not HPFAs, in SPM changed through time (Table 3.2, Fig. 3.4). The fatty acid marker 16:1ω7 did not change significantly through time in the SPM (GLM, with month as predictor: $F = 1.72_{10,\ 22}$; $p = 0.139$; Fig. 3.5A), but there was a distinct

increase in 20:5ω3 after November 2010 (GLM, with month as predictor: $F = 42.23_{10, 22}$; $p < 0.001$; Fig. 3.5A). Fatty acid markers 22:6ω3 and 18:1ω9 showed significant changes through time in the SPM (GLM, with month as predictor: $F_{22:6\omega3} = 3.69_{10, 22}$; $p < 0.005$, and $F_{18:1\omega9} = 5.71_{10, 22}$; $p < 0.001$; Fig. 3.5B), with a distinct increase in 18:1ω9 after November 2010.

The coefficients of variation of PUFAs, MUFAs and SFAs were the highest in *Bryopsis* (76, 89 and 39 %, respectively, 5-month average), followed by *Gelidium* (31, 73 and 29 %, respectively, 10-month average) and *Ulva* (9, 51 and 13 %, respectively, 10-month average; Table A6). The proportions of fatty acid categories varied considerably among-individuals for all macroalgal species (Table A6), but the greatest among-replicate variations concerned the concentrations of ω3 and ω6 PUFAs: 18:3ω3, 18:2ω6, 18:4ω3, 20:4ω6, 20:5ω3 and 22:6ω3 (EFAs, see Table A4). EFAs were most dominant in *Ulva* (range 33.34±20.29 to 44.01±4.95 % TFA), followed by *Gelidium* (8.83±0.02 to 40.82±15.18 % TFA), and *Bryopsis* (18.92±16.25 to 24.44±25.74 % TFA). SPM PUFAs, MUFAs and SFAs were moderately consistent among replicates (38, 28 and 13 %, respectively, 10-month average; Table A7).

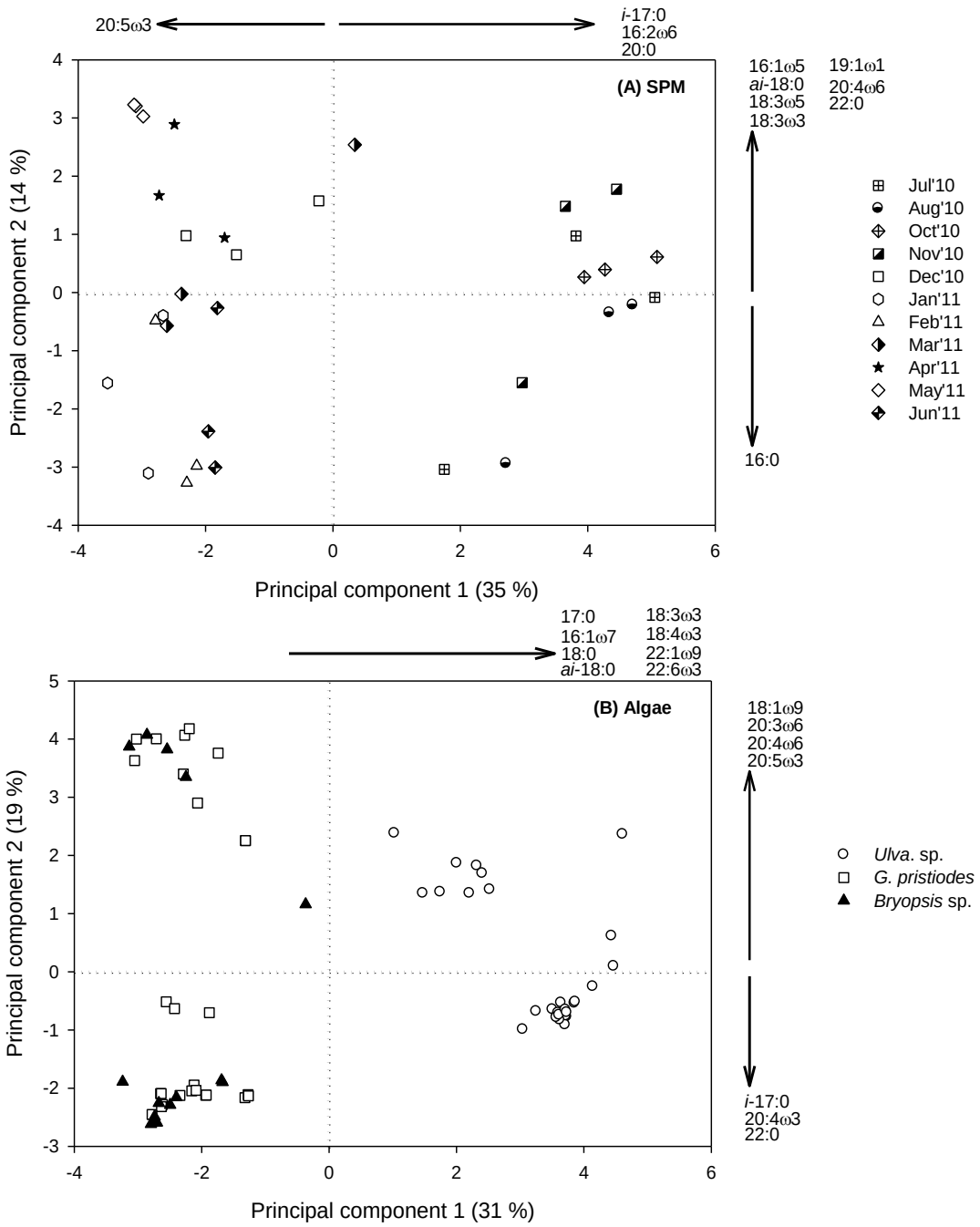

Figure 3.1. Principal component analysis (PCA) of arcsin square root-transformed proportional fatty acid data (% TFA) of (A) suspended particulate matter (SPM) and (B) macroalgae. Fatty acid >1% TFA were included in the analyses (30 fatty acids in total). Percentage values in PC (principal component) labels indicate the proportion of variation accounted for by each PC. Arrows running parallel to each axis denote the influence of the specific fatty acids that have loading values >2.5%. The cross-section of the dotted lines represents the origin.

Table 3.1. Results of the General Linear Models for the quantities (µg FA mg^{-1}DW) of essential fatty acids (EFAs) and total fatty acids (TFAs) from three macroalgae (*Ulva* sp., *Gelidium pristoides* and *Bryopsis* sp.) and suspended particulate matter (SPM). The symbol Σ indicates total abundance for each category.

	Ulva sp.			*Gelidium pristoides*			*Bryopsis* sp.			SPM		
Source	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
ΣEFA												
Months	9	0.95	0.510	9	0.53	0.831	4	0.08	0.988	10	16.27	<0.001
Error	18			17			10			22		
ΣTFA												
Months	9	0.92	0.528	9	0.99	0.482	4	0.20	0.932	10	4.51	<0.01
Error	19			17			10			22		

Factors: Fatty acid categories were the continuous dependent variables; and month the categorical independent variable or predictor.

Table 3.2. Results of the General Linear Models for the proportions of fatty acid categories from three macroalgae (*Ulva* sp., *Gelidium pristoides* and *Bryopsis* sp.) and suspended particulate matter (SPM). The symbol Σ indicates total abundance for each category.

	Ulva sp.			*Gelidium pristoides*			*Bryopsis* sp.			SPM		
Source	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
ΣBAFA												
Months	9	0.74	0.667	9	1.19	0.362	4	1.70	0.225	10	5.26	<0.001
Error	15			17			10			22		
ΣPUFA												
Months	9	2.17	0.084	9	0.56	0.811	4	0.05	0.995	10	1.45	0.223
Error	16			17			10			22		
ΣMUFA												
Months	9	0.78	0.640	9	0.41	0.913	4	0.25	0.906	10	0.87	0.573
Error	19			17			10			22		
ΣSFA												
Months	9	0.77	0.646	9	0.61	0.776	4	0.03	0.998	10	2.95	<0.05
Error	19			17			10			22		
HPFA												
Months	9	0.90	0.546	9	0.57	0.803	4	1.79	0.269	10	2.20	0.059
Error	19			17			5			22		
ΣEFA												
Months	9	0.78	0.641	9	0.53	0.834	4	0.00	1.000	10	28.77	<0.001
Error	19			17			10			22		

Factors: Fatty acid categories were the continuous dependent variables; and month the categorical independent variable or predictor; PUFA: polyunsaturated fatty acids; MUFA: monounsaturated fatty acids; SFA: saturated fatty acids; EFA: essential fatty acids.

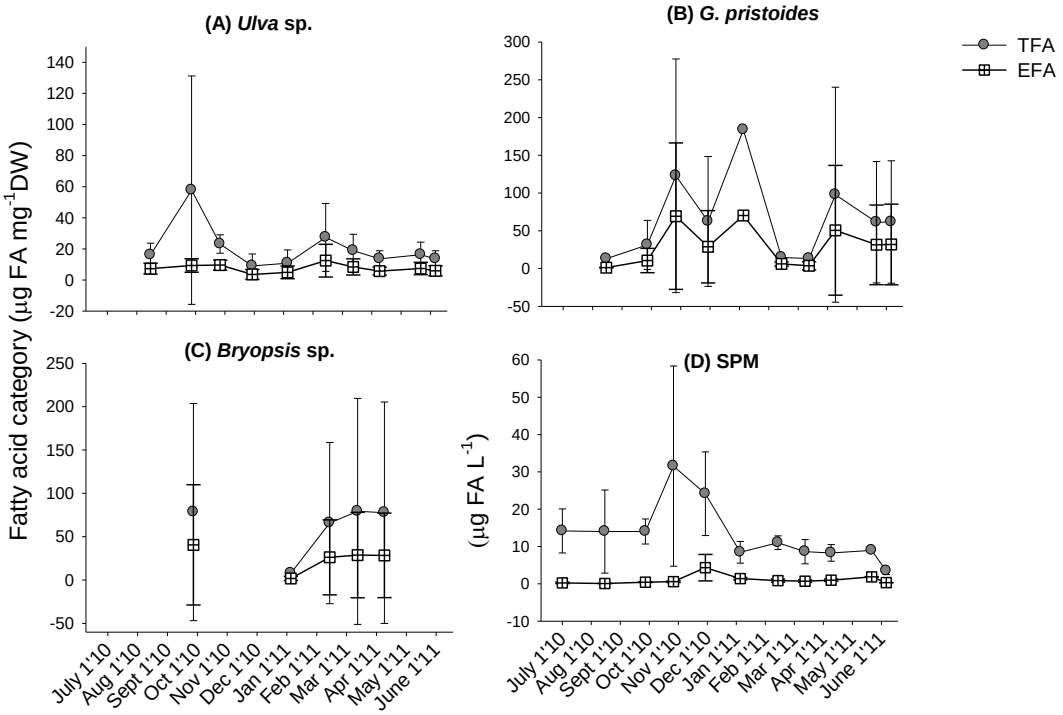

Figure 3.2. Monthly changes in total (Σ) concentrations of fatty acid (FA), over one year in both macroalgae species (A-C) and suspended particulate matter [SPM (D)]. The total fatty acids (TFA) and essential fatty acids (EFA) are presented in µg FA mg^{-1} DW or µg FA L^{-1} (for SPM). Values are expressed as mean ± SD; labels represent average values, and error bars standard deviations; lines connecting the monthly values represent hypothetical connections between months.

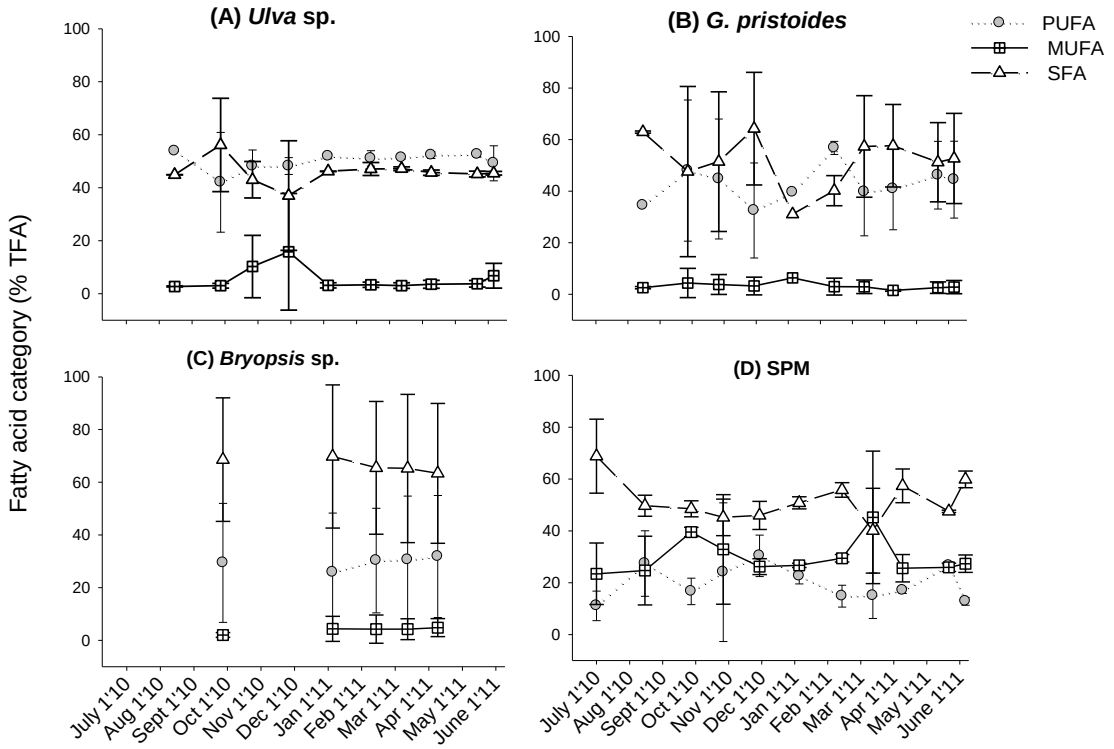

Figure 3.3. Monthly changes in the relative proportions of fatty acid categories of polyunsaturated (PUFA), monounsaturated (MUFA), and saturated (SFA) fatty acids over one year in three macroalgae species (A-C) and suspended particulate matter [SPM (D)]. Values are expressed as mean ± SD; labels represent average values, and error bars standard deviations; lines connecting the monthly values represent hypothetical connections between months.

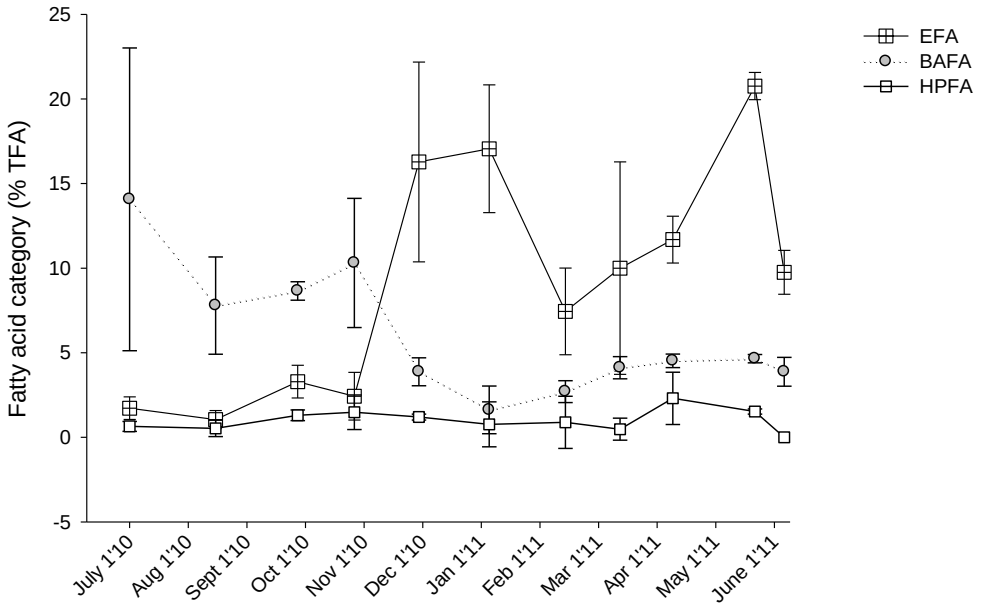

Figure 3.4. Monthly changes in the relative proportions of fatty acid categories of essential fatty acids (EFA), bacterial fatty acids (BAFA), and higher plant fatty acids (HPFA) over one year in the suspended particulate matter (SPM). Values are expressed as mean ± SD; labels represent average values, and error bars standard deviations; lines connecting the monthly values represent hypothetical connections between months.

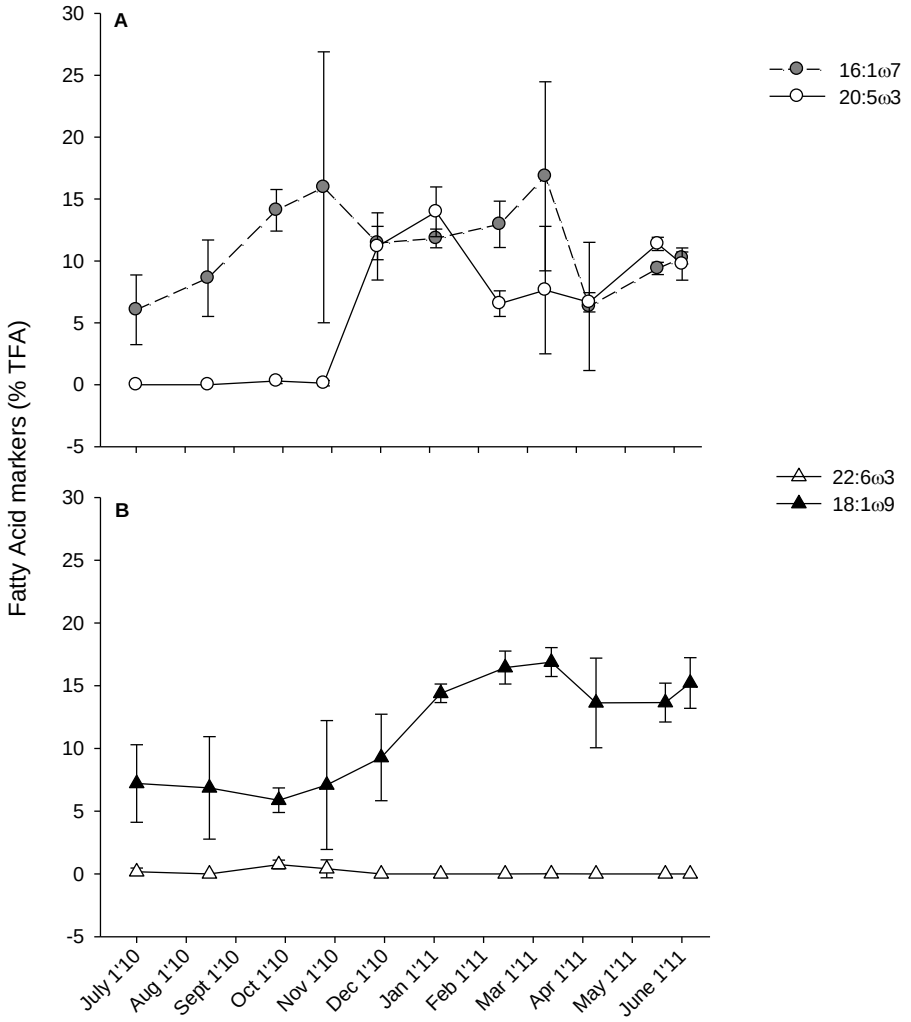

Figure 3.5. Monthly changes in the relative proportions of markers 16:1ω7, 20:5ω3 (A), and 18:1ω9, 22:6ω3 (B), over one year in suspended particulate matter [SPM]. Values are expressed as mean ± SD; labels represent average values, and error bars standard deviations; lines connecting the monthly values represent hypothetical connections between months.

3.3.3. Suspension-feeders and grazers

Thirty eight fatty acids (Table A8) were detected at concentrations > 1 % TFA in at least one of the consumers; the means ± SD of the specific biomarker fatty acids (% TFA) are reported as quantitative (Tables A9 and A10) and proportional data (Tables A11 and A12). As with the SPM (Fig 3.1A), the PCA results of suspension-feeders revealed a distinct group of the months July, August, October and November separated from the rest of the year (Fig. 3.6). PCs 1, 2 and 3 from the *P. perna* analysis explained 35, 23 and 10 %, respectively, of the variance in the data set (Fig. 3.6A). One-way ANOVA of PC-1 ($F = 53.84_{10, 38}$; $p < 0.001$) and PC-2 ($F = 128.55_{10, 38}$; $p < 0.001$) detected significant monthly differences in *P. perna*. PCs 1, 2 and 3 from the PCA of the *G. gaimardi* explained 37, 18 and 10 %, respectively, of the variance in the data set (Fig. 3.6B). One-way ANOVA of PC-1 ($F= 90.70_{10, 35}$; $p < 0.001$) and PC-2 ($F= 150.24_{10, 35}$; $p < 0.001$) detected significant monthly differences in *G. gaimardi*. As with PCA of the potential food sources, the corresponding figures of the third principal component for both suspension-feeders are provided as an appendix (Fig. A2).

As in the suspension-feeders, the PCA results of the grazers showed similar groupings: animals collected in the months July, August, October and November formed one group (Fig. 3.7). PCs 1, 2 and 3 from the analysis of *P. angulosus* explained 35, 15 and 9 %, respectively, of the variance in the data set (Fig. 3.7A). One-way ANOVA of PC-1 ($F= 28.90_{10, 34}$; $p < 0.001$) and PC-2 ($F= 21.38_{10, 34}$, $p < 0.001$) detected significant monthly differences in *P. angulosus*. PCs 1, 2 and 3 from the PCA of the *C. oculus* explained 33, 22 and 10 %, respectively, of the variance in the data set (Fig. 3.7B). One-way ANOVA of PC-1 ($F= 47.41_{10, 40}$, $p < 0.001$) and PC-2 ($F= 3.84_{10, 40}$, $p < 0.001$) detected significant monthly differences in *C. oculus*. As with PCAs of the potential food sources and suspension-feeders, the corresponding figures of the third principal component for both grazers are provided as an appendix (Fig. A3).

The quantities of TFAs showed variation through time in the different suspension-feeders (Table 3.3). The TFAs in *P. perna* and *G. gaimardi* showed similar trends (Fig. 3.8A and B), with decreasing values from July to November 2010 and increases thereafter. Similar fluctuations were apparent in the proportions of EFAs in the different suspension-feeders (Fig. 3.9A). The relative proportions of PUFAs, MUFAs and SFAs showed significant changes in the different suspension-feeders through time (Table 3.4, Fig. 3.10). *P. perna* and *G. gaimardi* showed similar trends in MUFAs and PUFAs, with generally decreased values

from July to November and then increased values, while SFAs showed the reverse trend (Fig. 3.10C). Based on the coefficients of variation, the fatty acid categories for both suspension-feeders revealed very low variability among-individuals (Table A13). Significant changes through time were detected in qualitative data of PUFAs, MUFAs and SFAs in the different grazers (Table 3.4, Fig. 3.11).

Significant monthly changes were detected in the quantities of TFAs in the different grazers (Table 3.3), with increases apparent in the sea urchins during December 2010 and January 2011, and a general increase in the limpets after October 2010 (Fig. 3.8C and D). The proportions of EFAs (Fig. 3.9B) in *P. angulosus* increased after November 2010, but did not show much change in C. *oculus* (Fig. 3.9B). *P. angulosus* showed similar trends in PUFAs and MUFAs, with generally decreased values from July to November, and then increased values, while SFAs showed the opposite trend (Fig. 11C). *C. oculus* fatty acid categories were relatively consistent through time (Fig. 3.12D, E and F). BAFAs and HPFAs changed through time in the two suspension-feeders (Table 3.4, Fig. 3.12A and B), with peaks in BAFAs occurring after July 2010 and decreases occurring in November or December. HPFAs in suspension-feeders were generally low throughout the year, with minimal fluctuations (Fig. 3.12). HPFAs and BAFAs in grazers *P. angulosus* and *C. oculus* showed significant changes through time (Table 3.4, Fig. 3.12C and D).

3.3.4. Diets of suspension-feeders and grazers using target fatty acids

To assess the changing importance of potential food sources in the suspension-feeders and grazers, specific fatty acid markers (Fig. 3.13) and ratios (Fig. 3.14) were examined in the muscle tissues of consumers. As different species tend to have different amounts of total lipids (Richoux and Froneman, 2008), quantitative differences between lipid-rich and lipid-poor organisms can overwhelm any potential differences due to dietary fatty acids. To avoid this problem, the proportional data (% TFA) were used for among-species comparisons (Table A14).

The fatty acids 16:1ω7, 20:5ω3, 18:1ω9 and 22:6ω3 changed through time in the two suspension-feeders (Table 3.5; Fig.3.13). All four fatty acids generally decreased from July to November 2010 in *P. perna* and then increased (Fig. 3.13A, B). The patterns in these same markers in *G. gaimardi* were somewhat different, although 16:1ω7 and 20:5ω3 also decreased from July to November and then increased (Fig. 3.13C, D). The two ratio markers (16:1ω7+16:1ω5)/16:0 and 22:6ω3/20:5ω3 changed through time in *P. perna* (Table 3.4; Fig.

3.14). *G. gaimardi* showed significant monthly differences in the ratio marker (16:1ω7+16:1ω5)/16:0, but not 22:6ω3/20:5ω3 (Table 3.5 and Fig. 3.14).

The fatty acids 20:5ω3 and 18:1ω9 changed through time in the two grazers (Table 3.15; particularly *P. angulosus*). The two fatty acids generally decreased from July to November 2010 in *P. angulosus* and then increased (Fig. 3.15A, B). *C. oculus* showed higher and more consistent concentrations of both 20:5ω3 and 18:1ω9 relative to the sea urchins, although the among-individual variability in the limpets was much larger as evidenced by large standard deviations (Table 3.6; Fig. 3.15C, D).

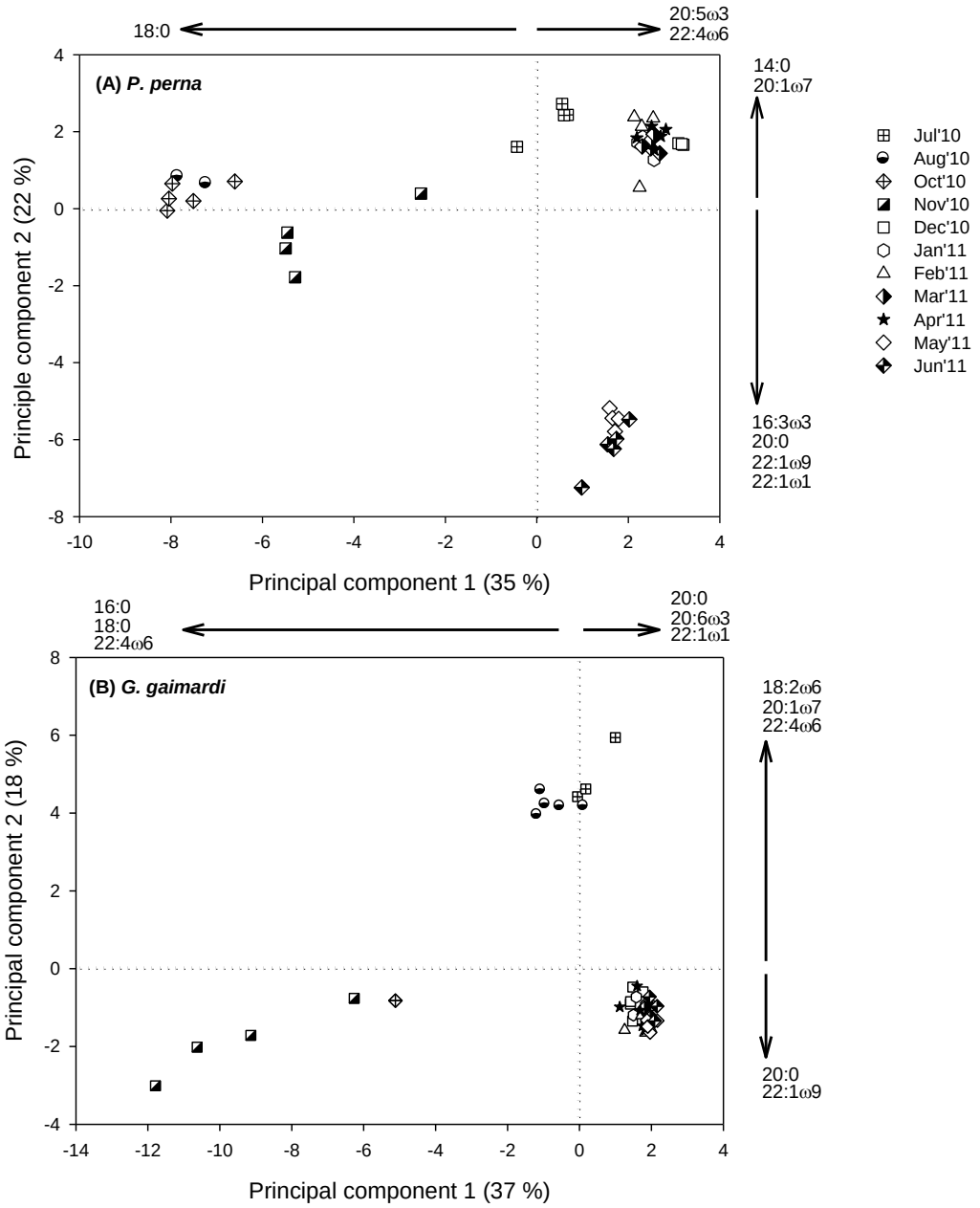

Figure 3.6. Principal component analysis (PCA) of arcsin square root-transformed proportional fatty acid data (% TFA) from the muscle tissues of (A) *Perna perna* and (B) *Gunnarea gaimardi*. Fatty acid >1% TFA were included in the analyses (38 fatty acid in total). Percentage values in PC (principal component) labels indicate the proportion of variation accounted for by each PC. Arrows running parallel to each axis denote the influence of the specific fatty acids that have loading values >2.5%. The cross-section of the dotted lines represents the origin.

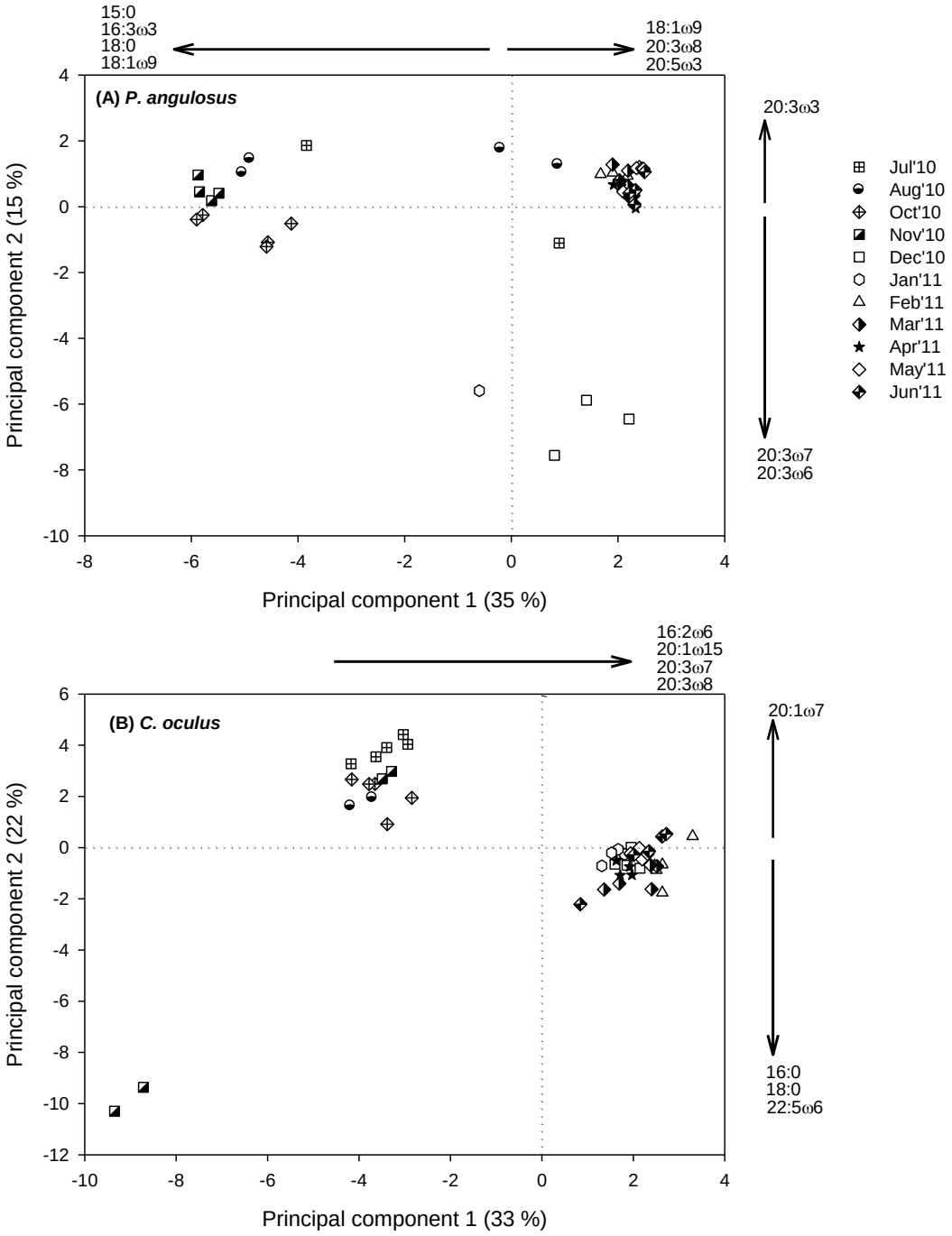

Figure 3.7. Principal component analysis (PCA) of arcsin square root-transformed proportional fatty acid data (% TFA) from the muscle tissues of (A) *Parechinus angulosus* and (B) *Cymbula oculus*. Fatty acid >1% TFA were included in the analyses (38 fatty acids in total). Percentage values in PC (principal component) labels indicate the proportion of variation accounted for by each PC. Arrows running parallel to each axis denote the influence of the specific fatty acids that have loading values >2.5%. The cross-section of the dotted lines represents the origin.

Table 3.3. Results of the General Linear Models for the quantities (µg FA mg^{-1}DW) of essential fatty acids (EFAs) and total fatty acids (TFAs) from the muscle tissues of suspension-feeders (*Perna perna* and *Gunnarea gaimardi*) and grazers (*Parechinus angulosus* and *Cymbula oculus*). The symbol Σ indicates total abundance for each category.

	Perna perna			*Gunnarea gaimardi*			*Parechinus angulosus*			*Cymbula oculus*		
Source	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
ΣEFA												
Months	10	9.64	<0.001	10	39.29	<0.001	10	13.84	<0.001	10	12.30	<0.001
Error	40			35			34			40		
ΣTFA												
Months	10	7.21	<0.001	10	27.63	<0.001	10	6.35	<0.001	10	13.37	<0.001
Error	40			35			34			40		

Factors: Fatty acid categories were the continuous dependent variables; and month the categorical independent variable or predictor.

Table 3.4. Results of the General Linear Models using the proportional fatty acid categories from the muscle tissues of suspension-feeders (*Perna perna* and *Gunnarea gaimardi*) and grazers (*Parechinus angulosus* and *Cymbula oculus*). The symbol Σ indicates total abundance for each category.

	Perna perna			*Gunnarea gaimardi*			*Parechinus angulosus*			*Cymbula oculus*		
Source	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
ΣBAFA												
Months	10	48.91	<0.001	10	163.19	<0.001	10	6.56	<0.001	10	12.04	<0.001
Error	39			35			34			40		
ΣPUFA												
Months	10	227.49	<0.001	10	37.87	<0.001	10	20.30	<0.001	10	3.72	<0.001
Error	39			35			34			40		
ΣMUFA												
Months	10	31.51	<0.001	10	23.66	<0.001	10	8.55	<0.001	10	4.35	<0.001
Error	39			35			34			40		
ΣSFA												
Months	10	347.17	<0.001	10	12.72	<0.001	10	23.91	<0.001	10	3.84	<0.001
Error	39			35			34			40		
HPFA												
Months	10	15.18	<0.001	10	48.06	<0.001	10	2.74	<0.01	10	6.31	<0.001
Error	39			35			34			40		
ΣEFA												
Months	10	132.92	<0.001	10	18.23	<0.001	10	20.57	<0.001	10	5.76	<0.001
Error	39			35			34			40		

Factors: Fatty acid categories were the continuous dependent variables; and month the categorical independent variable or predictor; PUFA: polyunsaturated fatty acids; MUFA: monounsaturated fatty acids SFA: saturated fatty acids; EFA: essential fatty acids.

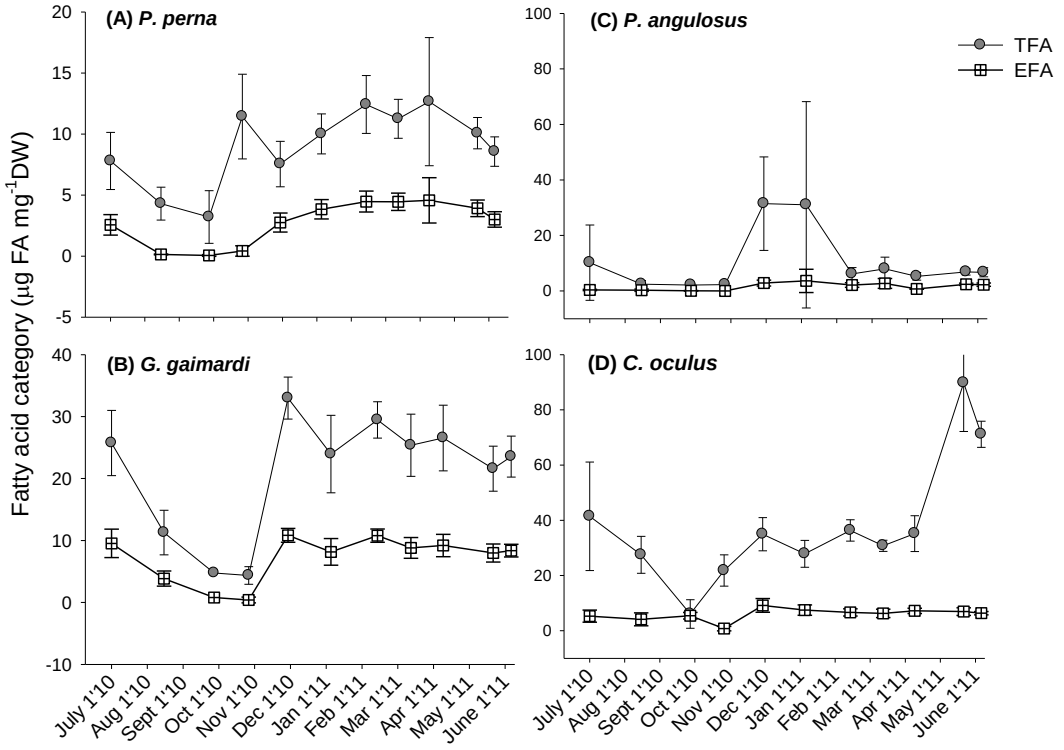

Figure 3.8. Monthly changes in total (Σ) concentrations of fatty acids over one year in suspension-feeders (A,B) and grazers (C,D). The total fatty acids (TFA) and essential fatty acids (EFA) are presented in quantities (µg FA mg^{-1} DW). Values are expressed as mean ± SD; labels represent average values, and error bars standard deviations; lines connecting the monthly values represent hypothetical connections between months.

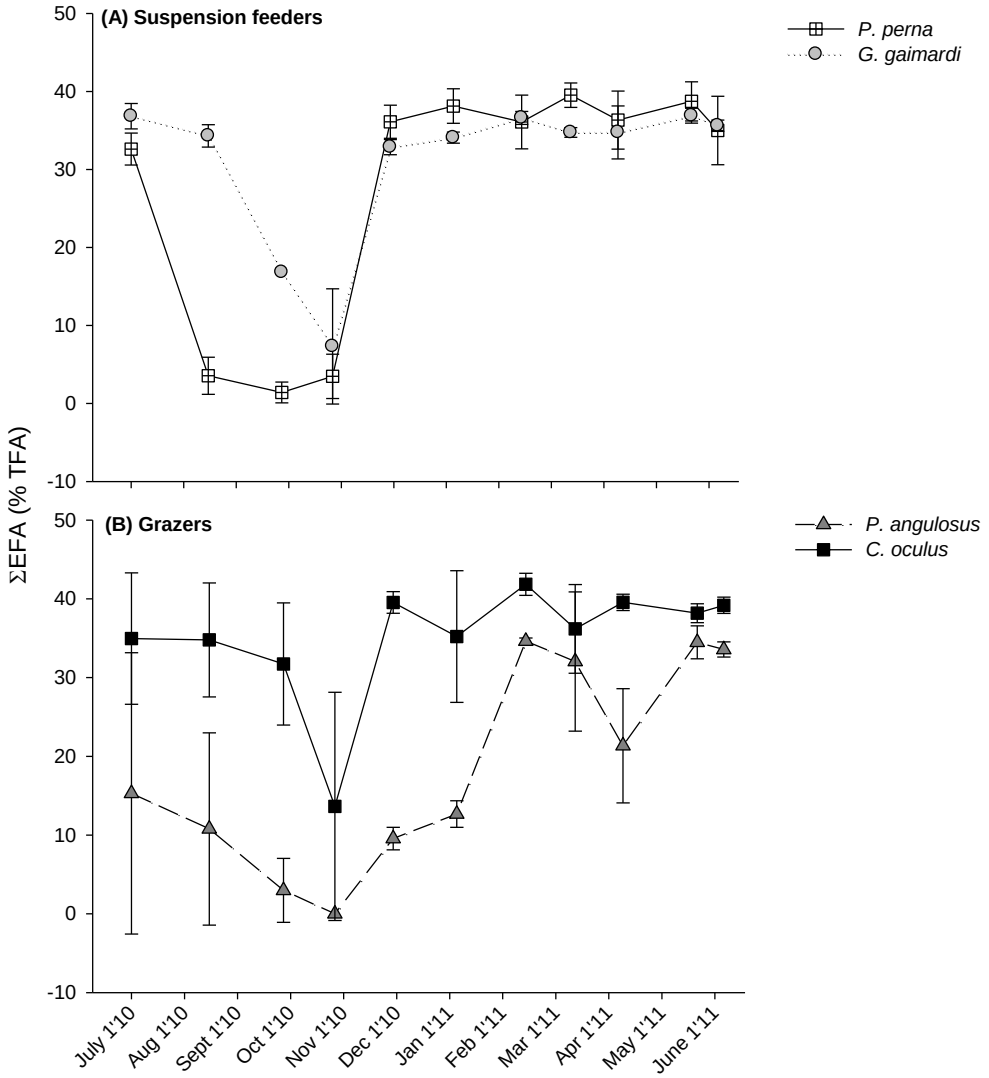

Figure 3.9. Monthly changes in the relative proportions of the total essential fatty acids (ΣEFA), over one year in the muscle tissues of (A) suspension-feeders and (B) grazer species. Values are expressed as mean ± SD; labels represent average values, and error bars standard deviations; lines connecting the monthly values represent hypothetical connections between months.

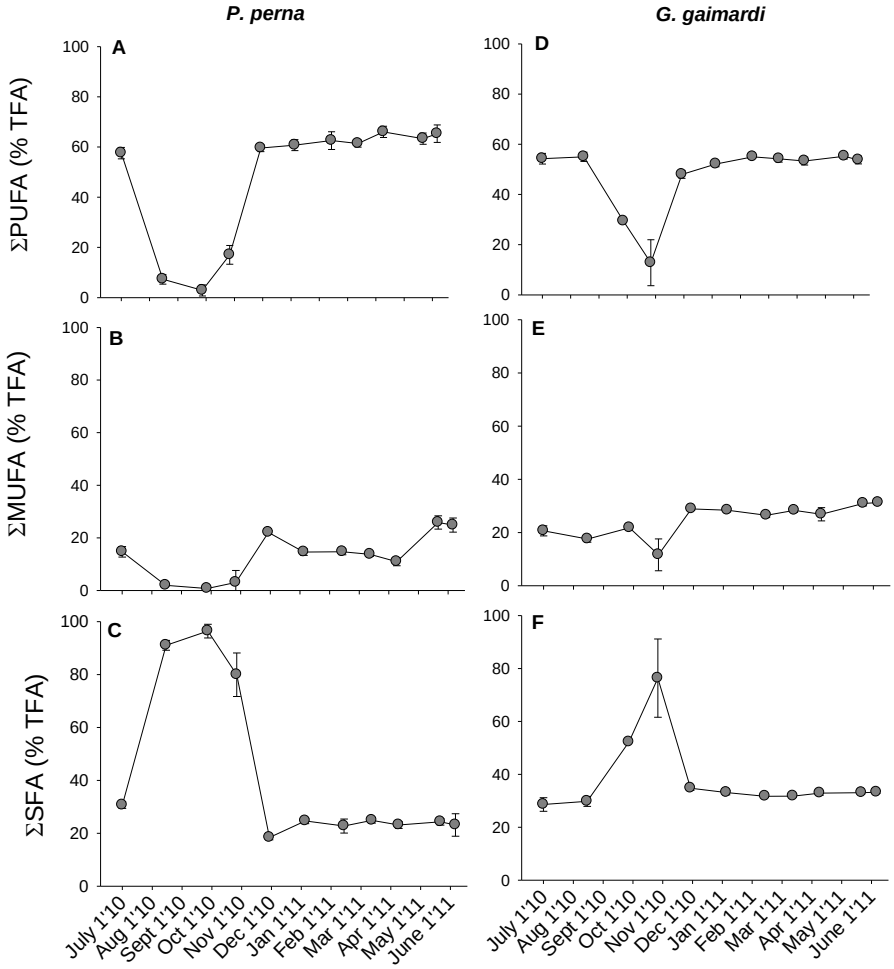

Figure 3.10. Monthly changes in the relative proportions of fatty acid (FA) categories. The FA categories are presented by percent polyunsaturated FAs (PUFA= A,D); monounsaturated FAs (MUFA= B,E); saturated FAs (SFA= C,F) over one year in the muscle tissues of suspension-feeding species. Values are expressed as mean ± SD; labels represent average values, and error bars standard deviations; lines connecting the monthly values represent hypothetical connections between months.

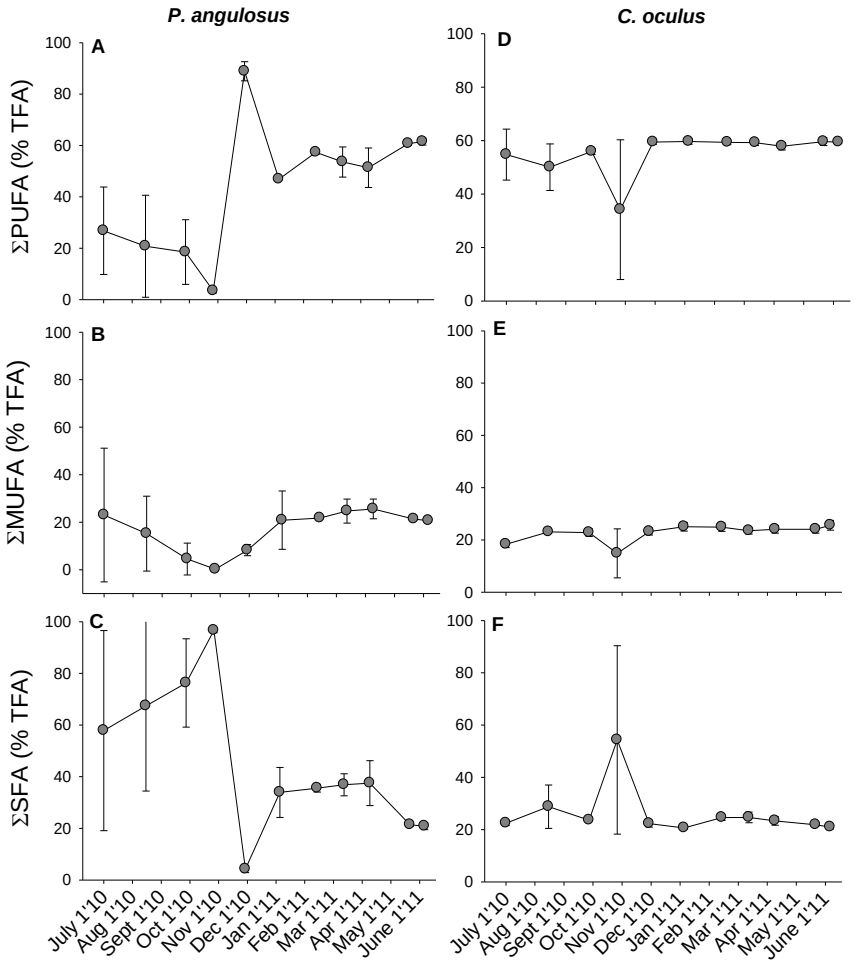

Figure 3.11. Monthly changes in the relative proportions of fatty acid (FA) categories. The FA categories are presented by percent polyunsaturated FAs (PUFA= A,D); monounsaturated FAs (MUFA= B,E; saturated FAs (SFA= C,F) over one year in grazers. Values are expressed as mean ± SD; labels represent average values, and error bars standard deviations; lines connecting the monthly values represent hypothetical connections between months.

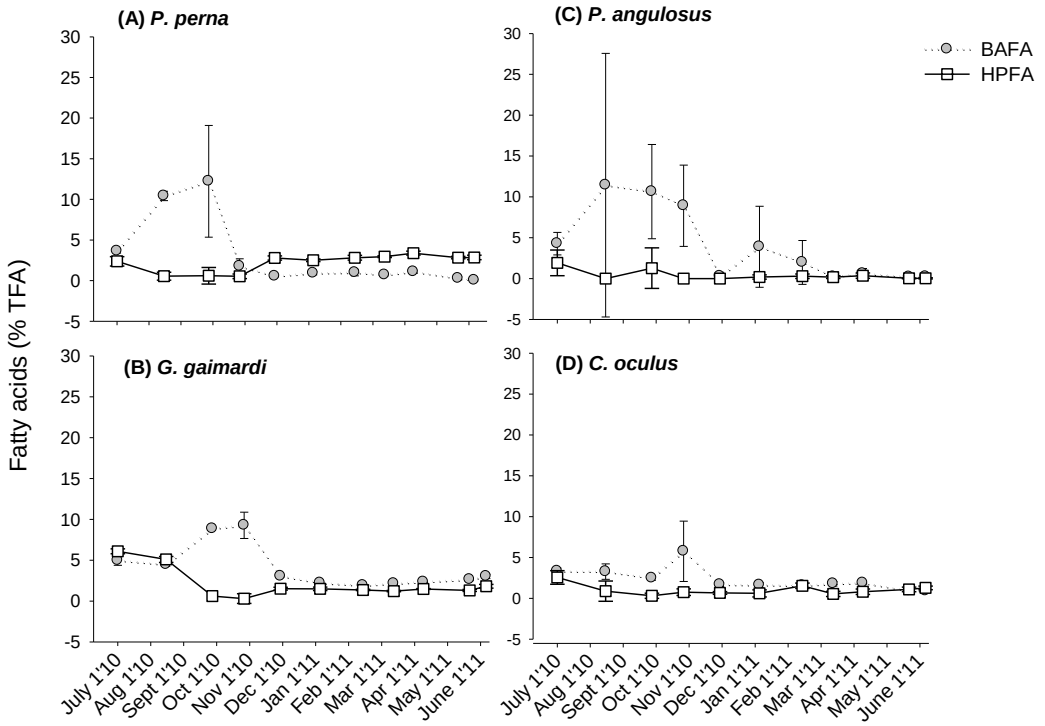

Figure 3.12. Monthly changes in the relative proportions of fatty acid (FA) categories. The FA categories are presented in percent of bacterial FAs (BAFA); higher plant FAs (HPFA) over one year in the muscle tissues of suspension-feeders (A-B) and grazers (C-D). Values are expressed as mean ± SD; labels represent average values, and error bars standard deviations; lines connecting the monthly values represent hypothetical connections between months.

Table 3.5. Results of the General Linear Models for the proportion of fatty acid markers from muscle tissues of suspension-feeders (*Perna perna* and *Gunnarea gaimardi*).

	Perna perna			*Gunnarea gaimardi*		
Source	*df*	*F*	*p*	*df*	*F*	*p*
16:1ω7						
Months	10	25.61	<0.001	10	27.88	<0.001
Error	40			35		
20:5ω3						
Months	10	62.10	<0.001	10	23.58	<0.001
Error	40			35		
22:6ω3						
Months	10	59.99	<0.001	10	5.43	<0.001
Error	40			35		
18:1ω9						
Months	10	31.48	<0.001	10	9.85	<0.001
Error	40			35		

Factors: Fatty acid categories were the continuous dependent variables; and month the categorical independent variable or predictor.

Table 3.6. Results of the General Linear Models for the proportion of fatty acid markers from muscle tissues of grazers (*Parechinus angulosus* and *Cymbula oculus*).

	Parechinus angulosus			*Cymbula oculus*		
Source	*df*	*F*	*p*	*df*	*F*	*p*
18:1ω9						
Months	10	6.42	<0.001	10	2.97	<0.01
Error	34			40		
20:5ω3						
Months	10	14.54	<0.001	10	2.92	<0.01
Error	34			40		

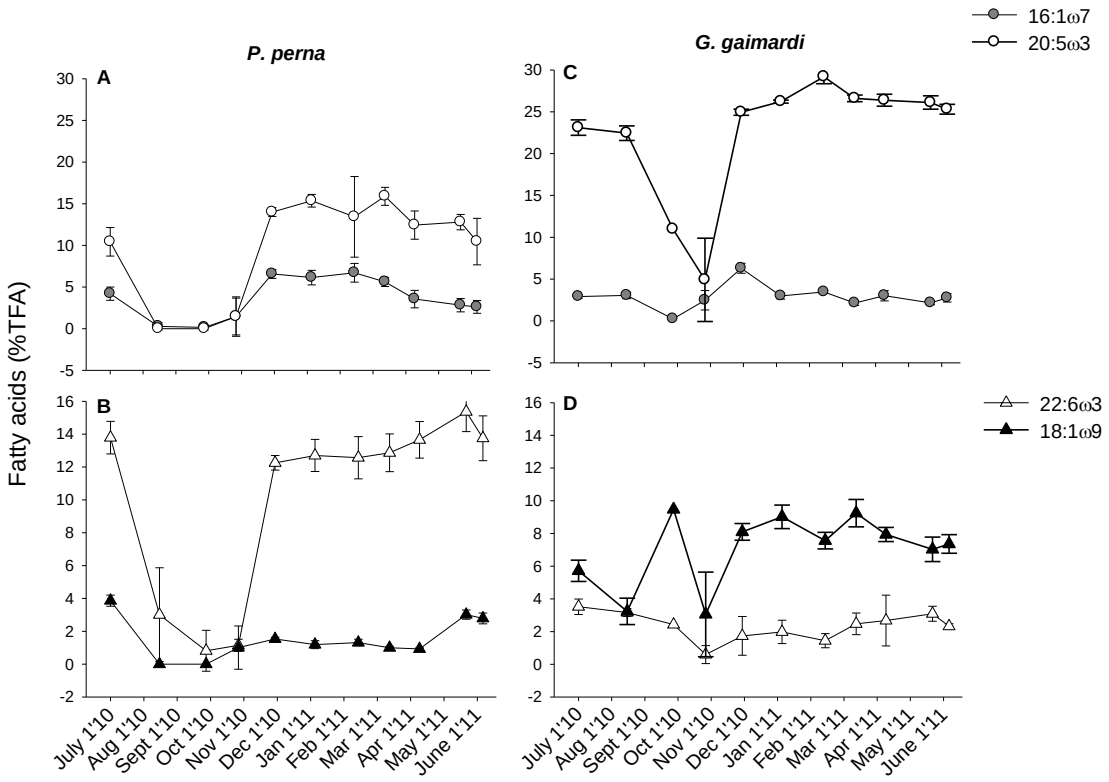

Figure 3.13. Monthly changes in the relative proportions of fatty acid (FA) markers 16:1ω7, 20:5ω3 (A,C), and FA markers 18:1ω9, 22:6ω3 (B,D) over one year in the muscle tissues of suspension-feeders. Values are expressed as mean ± SD; labels represent average values, and error bars standard deviations; lines connecting the monthly values represent hypothetical connections between months.

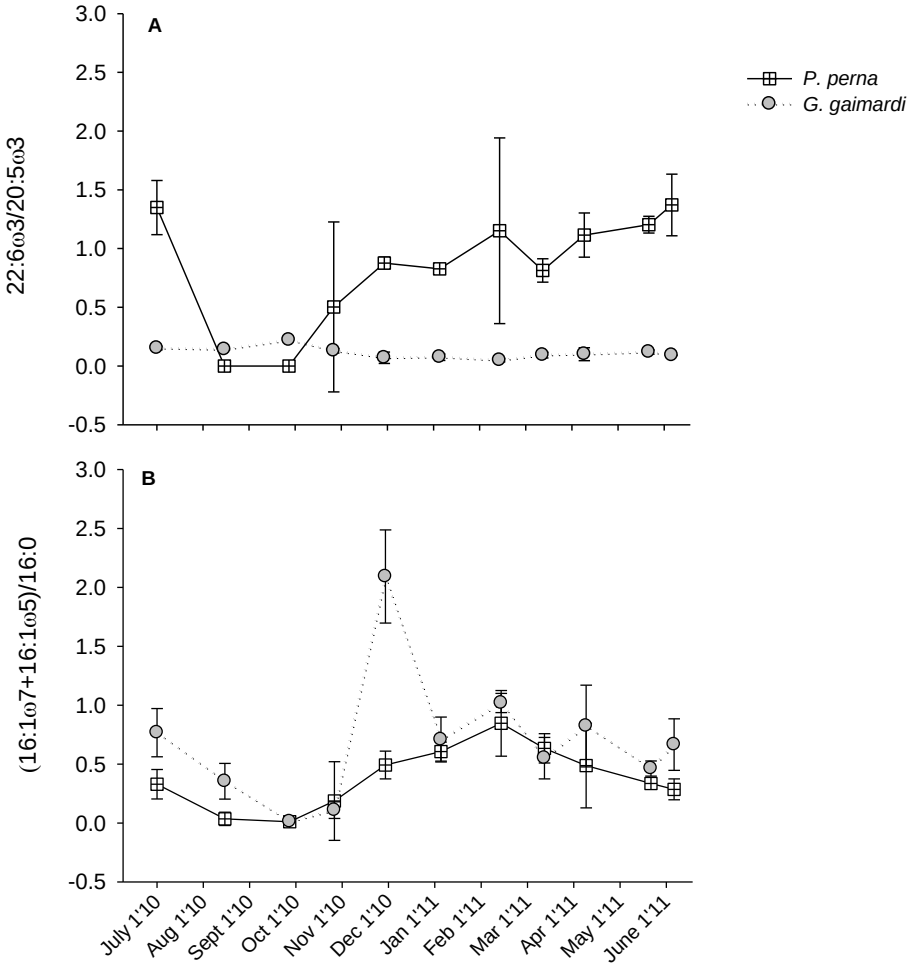

Figure 3.14. Monthly changes in the relative proportions of ratio marker 22:6ω3/20:5ω3 (A) and ratio marker (16:1ω7+16:1ω5)/16:0 (B) over one year in the muscle tissues of suspension-feeders. Values are expressed as mean ± SD; labels represent average values, and error bars standard deviations; lines connecting the monthly values represent hypothetical connections between months.

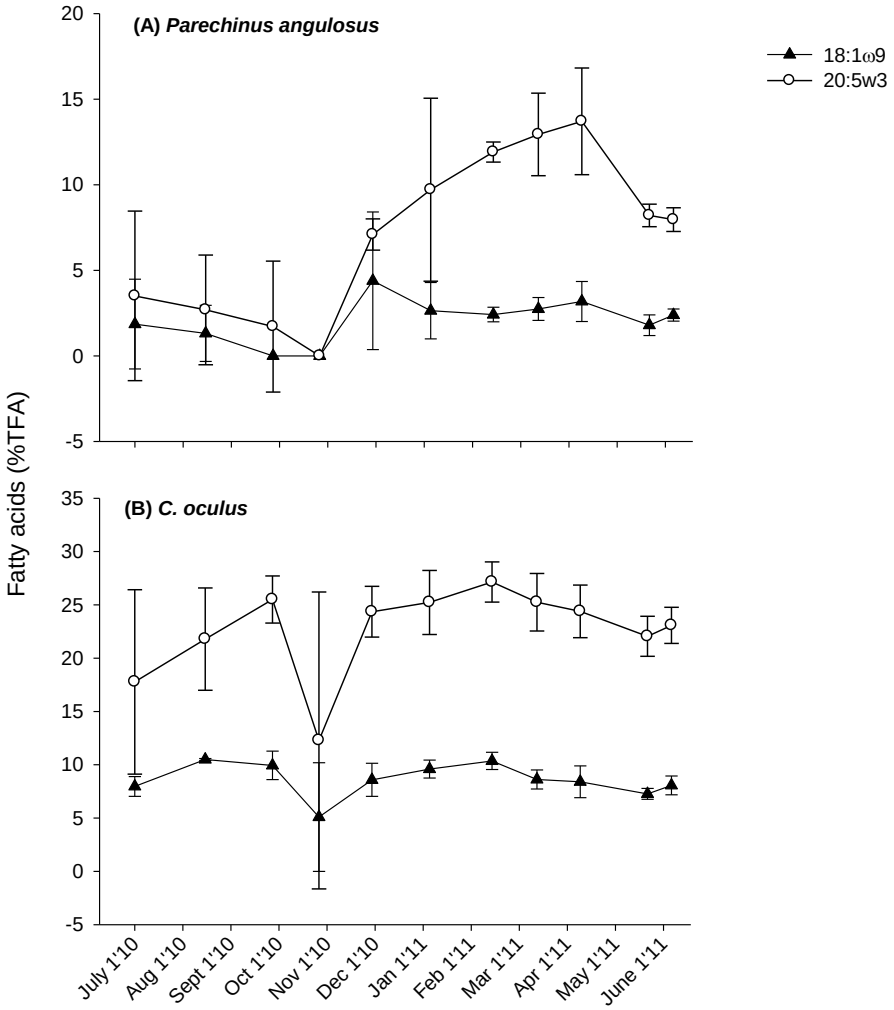

Figure 3.15. Monthly changes in the relative proportions of fatty acid (FA) marker 18:1ω9 (A), and FA marker 20:5ω3 (B) over one year in the muscle tissues of grazers. Values are expressed as mean ± SD; labels represent average values, and error bars standard deviations; lines connecting the monthly values represent hypothetical connections between months.

3.4. DISCUSSION

The consumers analysed in this study are represented by two different categories of invertebrates commonly found on rocky shores: suspension-feeders and the grazers. The main differences are the general feeding modes and life-styles: the suspension-feeders are sessile/sedentary and utilise a filtration apparatus to capture particles from the water, whereas the grazers are errant and use a scraping apparatus to forage on algae or other non-particulate food. The four taxa analysed also colonised different regions of the rocky shore, including the lower intertidal zone (polychaetes), tide pools (sea urchins) and middle intertidal zone (mussels and limpets). These differences in food collection, life-styles and habitat could all contribute to differences in diet among species and also the variability of these diets through time.

The grazers and suspension-feeders analysed in this study were characterized by different fatty acid compositions, which varied with time. Diets of organisms in the different categories were distinct in terms of temporal patterns and extent of temporal variations. These results were probably partially attributable to the animals feeding strategy, supporting the hypothesis of an effect of life style on the diets of rocky shore invertebrates. A similar high monthly variability was found in mussels (*Perna perna*) and polychaetes (*Gunnarea gaimardi*), while sea urchins (*Parechinus angulosus*) and limpets (*Cymbula oculus*) showed low and no variability through time, respectively. As expected, this difference is likely due to more variation through time in the suspension-feeder diets (seston vs macroalgae), as shown by the results for SPM and three representative macroalgae (that are not necessarily important food sources for these consumers).

Regarding the potential food sources analysed in my study, a different temporal variation was reported. Among the four different producers (chlorophytes *Ulva* sp. and *Bryopsis* sp., the rhodophyte *Gelidium pristoides* and SPM), only the SPM showed clear temporal variation in the TFAs (Fig. 3.2D) and EFAs (Fig 3.4) which showed increased values after November. The changing EFAs in the SPM support my hypothesis of higher variation of the food sources for the suspension-feeders (SPM) than for the grazers (macroalgae). Indeed, variation over time for macroalgae was evident only in *Gelidium pristoides* (Fig. 3.2 B), indicating high within-month variability. The relative low temporal variation in the composition of fatty acids for the grazers potential food sources is in accord with my aforementioned hypothesis. However, preliminary data on the stable nitrogen

isotope signatures of the grazers and macroalgae (Ndhlovu and Richoux, unpublished data) indicate very weak trophic linkages. Most likely, the two grazers species relied on different macroalgal species than those studied here. A study conducted by Allan *et al*. (2010) on the south coast of South Africa showed high levels of EFA 20:5ω3 in *Gelidium pristoides* (19.4%) and coralline algae (i.e. 17.6 %, *Corallina* sp. 34.9 %). The higher proportions of fatty acid marker 20:5ω3 of consumers (Fig. 3.15) correlates with higher levels of marker 20:5ω3 for coralline algae (Allan *et al*., 2010). In the West coast of South Africa, studies done by Maneveldt *et al*. (2009) and Reinecke *et al*. (2012) reported that *C. oculus* include coralline algae as part of their diet. Therefore, *C. oculus* collected for this study could be relying on coralline algae as a main food source rather than the macroalgae species I collected.

The suspension-feeders analysed in my study showed similar changes in their fatty acid profiles, supporting my hypothesis of similar variability within a feeding mode category. The polychaetes *G. gaimardi* and the mussels *P. perna* showed similar temporal trends in TFAs and EFAs, suggesting similarities in their diets through time and showing that the water samples collected could potentially capture evidence of variations in the food quality for the suspension-feeders throughout the year (with some of the discrepancies, such as high EFAs in consumers during July 2010 not reflected in the SPM, owed to the effects of time lags in the incorporation of dietary fatty acids into consumer tissues). The high monthly variability in diets of the suspension-feeders from my study agreed with the studies done by Bachok *et al*. (2009) on *Quidnipagus palatum* and Braeckman *et al*. (2012) on *Lanice conchilega.* The two studies indicated that the fatty acid markers of *Q. palatum* and *L. conchilega* differed between seasons and depended on the sources of organic material present in the sediment and water column (Bustamante and Branch, 1996; Hill *et al*., 2006; Bachok *et al*., 2009; Braeckman *et al*., 2012).

The linkage between rocky shore suspension-feeders and the water column has been studied by several authors (Berry and Schleyer, 1983; Jørgensen, 1983; Prins *et al*., 1996; Wong *et al*., 2008), and supension-feeders are able to filter large volumes of water and selectively feed and alter the species composition of the plankton (Cloern, 1982; Officer *et al*., 1982; Dame, 1996; Ward *et al*., 1997; Wong *et al*., 2003). However, the suspension-feeder diets are ultimately related to what is available in the SPM. Therefore, in terms of diet, the variations in suspension-feeder diets through time should follow the temporal fluctuation of food availability and quality of the water column. I found evidence of such dependence in

both of the study species included in the suspension-feeders category. In fact, the PCA ordination of fatty acid composition for both suspension-feeders showed similar monthly groupings (Fig. 3.6) which were comparable with the monthly groupings obtained for SPM (Fig. 3.1A). This similarity suggests a strong link between the diets of suspension-feeders and the composition of the SPM, as reported by several authors (Hily, 1991; Bustamante and Branch, 1996; Fiske *et al.*, 2003; Newell, 2004; Hill *et al*., 2006; Li *et al*., 2007). In both *P.perna* and *G. gaimardi* the fatty acids 20:5ω3 and 22:6ω3 (Fig. 3.13), markers of diatoms and dinoflagellates, respectively, were similar to changes in the SPM (Fig. 3.5; especially 20:5ω3). Similar results were recorded by Bachok *et al*. (2009), who analysed fatty acid markers in rocky intertidal consumers in Japan and suggested that the seasonal changes in the food sources (SPM and detritus) affected the diet of the bivalve *Quidnipagus palatum*. Bachok *et al*. (2009) revealed that the bilvalve *Q. palatum* seemed to feed on vascular plant detritus and bacteria from summer to autumn, macroalgae and phytoplankton during winter and diatoms during spring-early summer. Wong *et al*. (2008) investigated the mussel *Perna viridis* in Japan, and their study showed a strong relationship between the SPM composition and *P. viridis* tissue fatty acids. Furthermore, Wong *et al*. (2008) reported significant positive correlations in the monoenoic 16:1 and 17:1 and PUFAs 18:3 and 22:6ω3 between the mussel tissues and the SPM samples at several locations. In my study, the PCA of the SPM (Fig. 3.1A) and the suspension-feeders (Fig. 3.6) showed high accumulation of PUFAs (e.g. 20:5ω3, 20:6ω3 and 22:4ω6) from December to June. Braeckman *et al*. (2012) indicated that marine polychaetes *Lanice conchilega* showed similar increased accumulation of PUFAs from the suspended matter upon bloom deposition from a sandy coastal station of Oostende (Belgium). The increase of PUFAs may also suggest energy storage for gametogenesis (Braeckman *et al.*, 2012). The effect of reproduction cycle on the temporal variation of fatty acid composition in intertidal animals is an important factor and is discussed in detail in Chapter 4.

The increasing dominance of EFAs in SPM (Fig. 3.4) suggests that suspension-feeders accumulate highly productive food sources in the water column during summer period. In this regard, nearshore oceanographic conditions (i.e. phytoplankton concentration and productivity) could be considered important and may have strong influence on the intertidal rocky shore communities, in particular on suspension-feeder growth, recruitment and food sources (Menge *et al*., 1997; Menge, 2000). Hill *et al*. (2006) reported temporal changes in the nearshore coastal SPM which strongly influenced the diet of suspension-

feeding mussel *P. perna*. Using stable isotope methods, the authors found that the SPM samples were represented by a mixture of phytoplankton plus detritus from different macroalgal species (Hill *et al*., 2006).

Other non-phytoplankton sources could be considered important food sources for the suspension-feeders. Bivalves were once considered to be exclusively herbivorous, with phytoplankton being their sole food source, and it was assumed that bivalves influenced zooplankton communities only indirectly through competition for phytoplankton (Peharda *et al*., 2012). However, studies conducted since the 1980s have revealed that bivalves are in fact omnivores: their additional food sources include bacteria, detritus and zooplankton (Stuart *et al*., 1982; Davenport *et al*., 2000; Wong and Levinton, 2006; Davenport *et al*., 2011). Furthermore, several authors have pointed out that these additional food sources could be important during periods when phytoplankton biomass is too low to satisfy bivalve energy demands (Cranford and Grant, 1990; Langdon and Newell, 1990). In my study, the BAFA levels were high in muscle tissues of both suspension-feeders *P. perna* and *G. gaimardi,* with generally increasing values during spring-summer (Fig. 3.12), whereas the EFAs showed low values during this period (Fig 3.9A). High BAFA proportions in the two suspension-feeders over July to November 2010 (Fig 3.12 A, B) are reported concurrently with decreased values of diatom and dinoflagellate biomarkers (Fig. 3.13 A, B, C), suggesting higher microbial contributions compared with phytoplankton. This result reflects great detrital components in their diets over that period.

Another interesting aspect of suspension-feeder diets is the idea of a general feeding plasticity, as highlighted by several authors. Recent research on seasonal changes in mussel diets using fatty acid biomarkers highlighted the ability of suspension-feeders to switch between different sources of food (Prato *et al*., 2010; Cheung and Shin, 2011; Pernet *et al*., 2012), indicating an opportunistic feeding strategy (Freites *et al*., 2002; Page and Lastra, 2003). Feeding by mussels has been considered opportunistic in nature, dependent on food availability. Among the fatty acid biomarkers, 18:1ω9 is used to indicate carnivorous diet input, as reported by Falk-Petersen *et al*. (2002). The ratio of 18:1ω9/18:1ω7 can be used as an indicator of carnivory in marine animals (Drazen *et al*., 2008). Many animals can synthesize 18:1ω9 from its saturated precursor 18:0, and 18:1ω7 is of bacterial origin (Mayzaud *et al*., 1989; Nichols *et al*., 1991) or synthesized by chain elongation of 16:1ω7, which is algal or bacterial in origin (Graeve *et al*., 1997). Therefore, proportionally more 18:1ω9 indicates carnivory in some cases. For my study species, 18:1ω9 content in

G.gaimardi was greater than in *P. perna*, with more fluctuating values occurring in *G.gaimardi* throughout the year (Fig 3.13 B and D). These results may suggest differences in particle selection between the two suspension-feeders, with *G. gaimardi* showing more carnivory. Polychaetes incorporate more zooplankton into their diets than the mussels, partially due to the mussels being more selective for smaller particles in general compared with the polychaetes (Fauchald and Jumars, 1979; Schleyer, 1981; Berry and Schleyer, 1983). Polychaetes can also feed on a variety of food sources (Fauchald and Jumars, 1979). For example, *Lanice conchilega* showed selective feeding behaviour and could switch between a range of food sources, such as diatoms, bacteria, fragmented macroalgae and microphytobenthos (Braeckman *et al.,* 2012). Such feeding plasticity has also been reported for other taxa (i.e. crustaceans and pycnogonida) using fatty acid biomarkers, highlighting the ability of many animals to switch between different sources of food (Cartes, 2011; Soler-Membrives *et al*., 2011).

In general, the use of different biomarkers can potentially provide additional information over a wide range of food targets. The differences in 22:6ω3 in *P. perna* and *G.gaimardi* provide further evidence that the two suspension-feeders have different diets throughout the year (Fig. 3.13). The variation in fatty acid 22:6ω3 in addition to differences in fatty acids 20:5ω3 and 18:1ω9 in the two species over time could be interpreted as interspecific differences in diet, despite the fact that they are exposed to the same food source (SPM). Mussels and polychaetes have different particle capturing mechanisms (mussels capture particles using ciliated gills, whereas polychaetes capture particules using ciliated tentacles), which can help explain the diverse food intake (Fauchald and Jumars, 1979; Hawkins and Bayne, 1992; Manaham, 1990, 1993).

The two grazers sea urchin *P. angulosus* and limpet *C. oculus* showed different results in terms of temporal variability, partially contrasting the initial hypothesis of consistent diets throughout the year. Between the two species, the sea urchin diet showed more temporal variation than the limpets. Hughes *et al.* (2006) reported that all temporal variations in the fatty acid signatures of wild populations of the sea urchin *Psammechinus miliaris* may have resulted from changes in both diet and reproductive maturity. The PCA ordination of both grazers showed similar monthly groupings (Fig. 3.7) to the suspension-feeders (Fig. 3.6), with PUFAs dominating from December to June. The TFAs in the grazers showed differences between the two species (Fig. 3.8). The sea urchins had lower concentrations of TFAs than the limpets, with a peak in December/January. The limpets instead showed more

variation in the concentration of TFAs, with generally higher values and a peak in May 2011. Increasing values during late spring/early summer for both species could be related with higher food availability and quality during this period.

The sea urchin *P. angulosus* showed large variations in EFAs (Fig. 3.9) within the first three months of collection (i.e. July, August and October), which could be linked with large variations in the food sources available. Higher variations in EFAs were not reflected in the limpets, suggesting that the two grazers did not feed heavily on the macroalgae collected in the study. The macroalgae (*Ulva* sp., *Gelidium pristoides* and *Bryopsis* sp.) analysed did not show increasing values of TFAs during November/December, so the two species of grazers probably relied on different food sources. Grazers can indeed feed either on microalgae or macroalgae (Branch, 1981) depending on feeding anatomy, food preferences and food availability (Aguilera, 2011). Encrusting coralline algae has been suggested as an important food source for *C. oculus* on the south-west coast of South Africa (Maneveldt *et al*., 2006). *P. angulosus* is more of a generalist feeder (omnivore), with a diet composed mostly of epilithical microalgae and green ephemeral algae (Santelices *et al*., 1986), and perhaps small invertebrate species inhabiting the rock surface (Aguilera, 2005; Camus *et al*., 2008). Stable isotope signatures (mean $\delta^{15}N$ values ranged from 8.85 to 9.10 ‰, unpublished data by Ndhlovu and Richoux) indicated that *P. angulosus* feeds at slightly higher level of the food web relative to *C. oculus* (mean $\delta^{15}N$ values ranged from 8.28 to 9.00 ‰). The differences in the fatty acid profiles between the two grazers and the potential food sources analysed support my conclusion that the macroalgae (mean $\delta^{13}C$ values ranged from -12.33 to -16.63 ‰, -16.27 to -20.51 ‰, and -13.50 to -16.78 ‰ for *Ulva* sp., *Bryopsis* sp., and *G.pristoides*, respectively) collected in this study are not important parts of the diets for the grazers (mean ranged from -11.74 to -16.45 ‰ and -9.23 to -10.5 ‰ for *P. angulosus* and *C.oculus*) .

An additional energy source for the grazers could come from bacterial communities and biofilms associated with algae, as bacterial abundance is strongly correlated to algal biomass, an effect of healthy bacterial growth on algal extracellular excretions (Hepinstall and Fuller, 1994; Romani and Sabater, 1999). Between my two grazer species, only the sea urchins showed an increase of BAFAs from July 2010 to November 2010, even though the relative proportions are very low (Fig. 3.12 C). However, Morales and Ward (2000) reported preferential consumption of algae over bacteria by a grazing snail, and concluded that the

close spatial proximity of both components and the small prey-to-grazer ratios did not allow selective ingestion.

Differences in the diets of sea urchins and limpets may be attributable to several factors including different food capturing mechanisms, and different locations on the rocky shore. Sea urchins mainly occupy tidal pools and are likely to be under water during low tide, while limpets are more exposed throughout the tidal cycle (Stace, 1998; Anthoni, 2007). Some instances of high temporal or among-individual variability in the grazers seem to be in contrast with my hypothesis on more consistent diets in the grazers through time (relative to the suspension-feeders). However, variability in the grazer diets may reflect high levels of heterogeneity within the macroalgal/biofilm community. The analysis on the few select species of macroalgae collected have shown that large variability in fatty acid quality is even common within a macroalgal species, let alone within the entire complex community.

3.5. CONCLUSION

In this chapter I described variations over time in the fatty acid compositions of primary producers and consumers in the rocky intertidal zone. The large temporal scale covered in this study has provided valuable information about the limitations of short-term diet studies in the rocky intertidal. Changes in the composition of fatty acids between spring-summer and autumn-winter were evident, especially in the suspension-feeders. In this category, the trend was consistent between the food source (SPM) and the consumers analysed. The different categories of consumers (suspension-feeders and grazers) showed differences in their fatty acid compositions through time, which confirmed the prediction of an influence of the life style and feeding mode on temporal variability in diets.

The seasonality was more accentuated in suspension-feeders than grazers, since the suspension-feeders are probably subject to larger changes in their food quality. On the other hand, grazers responded differently through time, with *P. angulosus* having more variability in its diet as compared to *C. oculus*. This could be attributed to differences in food selectivity between the species, in addition to heterogeneity in the macroalgal/biofilm community. However, overall the limpet results provide general support for my initial hypothesis of more consistent diets (throughout the year) in the grazers compared with the suspension-feeders, although the sea urchin *P. angulosus* diets showed more variability than expected.

This study represents the first of its kind on the rocky shore from the south coast of South Africa. By covering a total of four species, eleven sampling months and one site, this study represents an original contribution towards a better understanding of the dynamics of rocky shore communities. In particular, my results emphasized the utility of fatty acid analyses to retrieve information on the diets of intertidal rocky shore animals. The large temporal scale covered by this study contributed to a better understanding of temporal changes in animal diets, giving a more comprehensive scenario than based only on snapshot collections. Furthermore, I demonstrated the importance of considering life style (i.e. feeding mode in this chapter and reproduction in Chapter 4) when examining the diets of rocky shore intertidal animals.

4 LIFE HISTORY RELATED VARIATIONS IN THE FATTY COMPOSITION OF ROCKY SHORE CONSUMERS

4.1. INTRODUCTION

Many studies have described the energetic shift from growth to reproduction that allows older/larger individuals to produce more gametes than younger/smaller individuals (Stearns, 1992; Heino and Kaitala, 1996; Kozlowski and Gawelczyk, 2002). The dynamic energy budget model presented by Kooijman (2000) showed that living organisms split their energy reserves between growth and maturity (juveniles) or reproduction (adults). In all organisms, there are two main life history strategies regarding when and how much energy to allocate to growth and reproduction: determinate and indeterminate growth (Kozlowski *et al*., 2004; Lord and Shanks, 2012). Organisms with determinate growth reach a maturation stage, at which point they stop growing and then put all excess energy into reproduction (Sebens, 1987). This group includes most terrestrial vertebrates (Winship *et al*., 2001), some crustaceans and fish (Sebens, 1987; Forseth *et al*., 1994). Organisms with indeterminate growth may slow down their growth rate with age, but even the largest individuals continue to grow (Sebens, 1987; Kozlowski *et al*., 2004). Organisms with indeterminate growth show a decrease in growth rate with age, reportedly due to an energy allocation shift from growth to reproduction (Sebens, 1987; Heino and Kaitala, 1996; Kozlowski *et al*., 2004). A broad range of organisms display indeterminate growth; this includes plants, many fish, amphibians, and most soft-bodied invertebrates (Sebens, 1987; Kozlowski, 1996; Rinke *et al*., 2008).

Intertidal rocky shore invertebrates show various modes of reproduction, development and dispersal, ranging from species that lay and brood benthic egg masses which develop into adult forms through larval stages, to those that undergo direct development releasing juveniles which are adult-like (Strathmann, 1985). Whatever the strategy involved, reproduction requires considerable investment of material and energy for gonad development, particularly in broadcast spawners (Martinez *et al*., 2000). Gonad development relies on the mobilization of nutrients either directly from ingested food (Simpson, 1982; Foster *et al*., 1999) or from mobilization of reserves stored in body tissues (Belisle and Stickle, 1978; Delgado and Perez-Camacho, 2005). In periods of high food availability, the cost of gonadal

maturation can be covered entirely by absorbed materials and energy, but when food availability is low, gonadal maturation and gametogenesis essentially depend on storage reserves (Martinez *et al*., 2000). These reserve materials generally accumulate during a pre-spawning refractory period (Belisle and Stickle, 1978), often corresponding with periods of high productivity when food is readily available (Mizrahi and Achituv, 1990). During periods of poor food supply, stored lipids and their constituent fatty acids are used by marine organisms as an energy source to support metabolism demands, growth and sexual maturation (Sargent *et al*., 1989; Ruiz *et al*., 1992; Mourente and Tocher, 1994). Lipids are the nutritive reserve product of the gonads (Brazão *et al.*, 2003), and they are important during gametogenesis (Gatenby *et al.*, 2003; Morais *et al.*, 2003; Sewell, 2005). Females generally have higher energy requirements than males during reproduction due to the high energetic cost of egg production (Robards *et al*., 1999; Guijarro *et al*., 2003).

Primary productivity in coastal ecosystems is important in the regulation of nutritional shortage by marine organisms, which ultimately affects their body condition, reproductive performance, and survival (Calder *et al*., 2002; Oftedal *et al*., 2007). Macronutrients thus have important effects on the structure and dynamics of near-shore and intertidal marine communities (Bosman *et al*., 1987; Wootton *et al*., 1996; Worm *et al*., 2002; Nielsen, 2003). Seasonal variations in nutrient dynamics, through their effects on primary productivity, influence the ultimate nutritional condition of marine invertebrates, which also varies depending on reproductive cycles (Gatenby *et al.*, 2003; Shin *et al.*, 2008). These variations in nutritional condition and reproductive cycle are reflected in the relative proportions of particular fatty acids within consumers. However, annual changes in the reproduction cycle can be a dominant influential feature and can obscure dietary influences on fatty acid profiles in consumers (Castell, 1982; Ackman, 1983; Brazão *et al*., 2003). For this reason, any study of temporal changes in marine invertebrate diets using fatty acid profiles must account for the influence of development and reproduction stages of the organisms.

Like other marine invertebrates, bivalves undergo seasonal cycles in their physiological condition in association with their reproductive cycle (Gabbott, 1976, 1983; Fearman *et al*., 2009). Gametogenesis and energy storage occur in the mantle tissue, where a large shift in cell types is evident throughout the annual cycle (Bayne *et al*., 1982). During the resting phase, before gametogenesis begins, adipogranular cells and vesicular connective tissue are dominant features of the mantle tissue. As gametes develop within follicles in the mantle tissue, the adipogranular and vesicular tissue diminishes as the number and size of gametes increase (Fearman *et al*., 2009). Mussels store energy as glycogen, which

accumulates in the mantle tissue during resting periods and is subsequently used during gametogenesis (Bayne *et al*., 1982). These concurrent cycles of glycogen storage and gametogenesis are well documented in many bivalve species, e.g., oysters (Honkoop, 2003; Ren *et al*., 2003), scallops (Farias *et al*., 1997), clams (Darriba *et al*., 2005), and mussels (Zwaan and Zandee, 1972). As stored glycogen is used as an energy source during gametogenesis, the quantity of glycogen stored during the resting period may substantially influence the reproductive capacity of an individual with respect to fecundity, oocyte quality and larval development (Fearman *et al*., 2009). Studies by Gabbott and Bayne (1973) showed that protein and lipid reserves are also built up, but mainly in the non-mantle tissues. The seasonal changes in lipid level are inversely related to the changes in glycogen level (Lubet and Le Feron de Longcamp, 1969; Williams, 1969; Pieters *et al*., 1979; Waldock, 1979).

Food and temperature are important factors in regulating the timing and rate of energy storage and reproduction in mussels and other bivalve species (Bayne *et al*., 1975; Bayne *et al*., 1982; Dix and Ferguson, 1984; Cranford and Hill, 1999; Alfaro *et al*., 2001; Chavez-Villalba *et al*., 2002; Darriba *et al*., 2004). The direct effect of food quantity is straightforward: when food is plentiful, the energetic costs of metabolism are met and excess energy is available for somatic growth, energy storage, and reproductive maturation (Widdows and Johnson, 1988). The brown mussel *Perna perna* is a dioecious rocky shore suspension-feeder which matures early (at sizes as small as 35 mm and aged less than one year old; Arrieche *et al*., 2002). Sexual maturation and the subsequent development of the gonads are sensitive to environmental conditions, in particular the availability and quality of food, physico-chemical conditions of the water (particularly temperature) and the energetic reserves previously accumulated by the mussel (Coll, 1991; Pérez, 1991).

Echinoid species also have strong links between changes in diet quality and reproductive development (Floreto *et al*., 1996). Sea urchins have a reproductive cycle mainly characterized by a growing stage, during which nutrients accumulate in the gonad nutritive phagocytes; a maturation stage, during which these nutrients are transferred to germ cells for gametogenesis, and finally a spawning stage when mature spermatozoa and ova are released from the gonad (Walker *et al*., 2007). This reproductive cycle is linked to seasonal changes of water and photoperiod (Walker *et al*., 2007), and the quantity and quality of food can also influence gonad growth (Marsh and Watts, 2007). The biological compositions of sea urchin gonads have been studied in different species in relation to seasonality (Marsh and Watts, 2007). Protein content in sea urchin gonads usually increases during growth and maturation stages and decreases with spawning, whereas glycogen accumulates in nutritive

phagocytes but declines when gametogenesis is initiated (Marsh and Watts, 2007). Changes in lipid content are less clear but tend to be similar to that of glycogen, with higher levels during the growing phase and a decrease before spawning (Fenaux *et al*., 1977; Fernandez, 1998; Montero-Torreiro and Garcia-Martinez, 2003). Although specific fatty acid markers characteristic of different groups of primary producers and consumers have been successfully used to identify their food sources (Perry *et al*., 1979; Volkman *et al*., 1989; Kharlamenko *et al*., 1995, 2001), more information is needed on how lipids obtained from the diet change through development and reproduction. The cape urchin *Parechinus angulosus* is a dioecious rocky shore grazer which reaches sexual maturity at 1-2 years (i.e. generation time), it spawns twice per year, size at sexual maturity ranges from 20-30 mm (test diameter), and mortality is highest early in its life cycle (shortly after metamorphosis; Muller, 2011). Few individuals can reach 60 mm, which approximates to an age of 4-7 years (Greenwood, 1980); individuals of this diameter can possibly be older as growth slows significantly with age in favour of gonad development (Greenwood, 1980).

In this chapter, my first objective was to determine how the fatty acid composition in the gonads of the suspension-feeder *Perna perna* (bivalve mussel) and the grazer *Parechinus angulosus* (echinoid sea urchin) change through an annual period. Furthermore, a second objective was to investigate gender differences in the fatty acid composition in gonads for each species. To achieve these objectives, I first hypothesized that the reproductive cycles of the suspension-feeder *P. perna* and the grazer *P. angulosus* affect the fatty acid composition of their gonads, with a temporal variations in lipid composition reflecting changes in reproduction investment. Secondly, I hypothesized that the total amount of energetic reserves available for reproduction is different for each gender (females allocate more energy to egg production than males allocate to gamete production).

4.2. METHODS

4.2.1. Study area, sample collection and treatment

Detailed descriptions of the study area, sampling methods (section 2.1), sample processing in the laboratory (section 2.2) and fatty acid analysis (section 2.3) are provided in Chapter 2. A brief summary is presented here, with particular focus on the methods relevant to the analysis of fatty acids in reproductive tissues.

4.2.2. Sample collection and treatment

Organisms were collected according to the protocols described in Chapter 2. For each specimen, gonads were separated from the rest of the body. Males and females were distinguished based on gonad colour, texture and thickness (Morais *et al*., 2003; Kawashima *et al*., 2008) and analysed separately. Although an effort was done to process individual sea urchins and mussels, samples of similar size and sex were occasionally pooled together to obtain enough material for analysis (i.e. minimum dry weight of 20 mg).

In this chapter, fatty acid composition is analysed according to several classes: total saturated fatty acids (ΣSFAs), total monounsaturated fatty acids (ΣMUFAs), total polyunsaturated fatty acids (ΣPUFAs), total fatty acids (ΣTFAs), and total essential fatty acids (ΣEFAs), which included the sum of fatty acids 18:2ω6, 18:3ω3, 18:4ω3, 20:4ω6, 20:5ω3 and 22:6ω3 (see Chapter 3 for more details about how and why these groups were obtained and targeted). Fatty acid profiles of male and female gonads (mean ± SD) were assessed monthly for the two species.

4.2.3. Data analysis

Principle components analyses (PCA, based on a covariance matrix) of proportional fatty acid data (% total fatty acid concentrations) were used to examine temporal patterns in the gonads of each consumer (separate PCA for each species). For details on the PCA analysis refer to Chapter 3 (section 3.2.3). The first two principal components (PCs) were plotted on the *x*- and *y*-axes, and the third component is presented in the Appendix (Figures A4). Two separate One-way ANOVA followed by Tukey post-hoc tests of PCA loadings were used to test for statistical differences in fatty acid profiles among months (see Chapter 3, section 3.2.3 for further details). Additionally, the monthly variations characterizing the fatty acid composition of animals were analysed using a multidimensional scaling (MDS) approach, associated with analyses of similarity (ANOSIM) testing for monthly variations, and analyses of the contributions of the different fatty acids to these variations (SIMPER). General linear models (GLM) followed by Tukey post-hoc tests were used to explore sex-related and monthly variations in the quantities of specific fatty acids in gonads for each species. Letters (‘a’, ‘b’, etc.) were use to show significant differences between months in the appendix (Tables A16, A17 and A18). Details on the softwares and methods used for the statistic analysis are given in Chapter 3 (Section 3.2.3).

A GLM was used to determine the effects of temperature and photoperiod on the concentrations of total fatty acids in the gonad tissues (TFAs, dependent variable). Data were

analyzed using (SYSTAT version 10.0) and photoperiod data was obtained from the internet, area Port Elizabeth (http://www.timeanddate.com/worldclock/astronomy.html?n=1485).

4.3. RESULTS

4.3.1. Environmental variables

Photoperiod was minimum in June (9.9 h) and maxima in December (14.3 h), refer to Chapter 2, Figure 2.4 for annual monthly variations.

4.3.2. Temporal variations in fatty acid proportional composition of gonads

PCA on fatty acids data from *P. perna* gonads indicated that November was completely separated from the rest of the year. PCs 1, 2 and 3 explained 42, 15 and 9 %, respectively, of the variance in the *P. perna* data set. One-way ANOVA of PC-1 ($F = 35.93_{10, 37}$; $p < 0.001$) and PC-2 ($F = 14.67_{10, 37}$; $p < 0.001$) detected significant monthly differences in *P. perna*. The results of the Tukey post-hoc test from PC-1 of *P. perna* clearly showed that the months July and November were significant different from the rest of the year. PCA on fatty acids data from *P. angulosus* gonads identified a separation of October from the other months. PCs 1, 2 and 3 explained 29, 12 and 10 %, respectively, of the variance in the *P.angulosus* data set. One-way ANOVA of PC-1 ($F= 15.51_{10, 46}$; $p < 0.001$) and PC-2 ($F=3.74_{10, 46}$, $p < 0.001$) detected significant monthly differences in *P. angulosus*. The Tukey post-hoc test from PC-1 of *P. angulosus* showed that October was significant different from the rest of the year. The corresponding figure of the third principal component for both suspension-feeder and grazer are provided as an appendix (Fig. A4).

4.3.3. Temporal variations in fatty acid quantities in gonads

Quantities of the different fatty acid categories in male and female gonads of *P. perna* and *P. angulosus* are shown in Tables A16 and A17. PUFAs, MUFAs and SFAs in *P. perna* varied significantly between sex and among months (Table 4.1, Fig. 4.2). MUFA and PUFA quantities in gonads of *P. perna* presented similar temporal patterns, with minimum values in November and maximum values through December to June (Fig. 4.2A and B). SFA quantities generally decreased from July to April and increased from May to June (Fig. 4.2C). In *P. angulosus* gonads, the quantities of PUFAs and MUFAs did not vary between males and females; however, SFAs varied significantly (Table 4.1, Fig. 4.2). All the quantities (PUFAs, MUFAs and SFAs) varied significantly among months, with minimum values in October and maximum values through December to June (Fig. 4.2D, E and F). For the two

species, the quantities of PUFAs, MUFAs and SFAs in gonads were generally slightly higher in females than in males (Fig. 4.2).

The quantities of TFAs in gonads from male and female *P. perna* (Fig. 4.3A) showed similar temporal trends, with higher values in May and June. For the grazer *P. angulosus* (Fig. 4.3B), quantities of TFAs also varied similarly in males and females, decreasing from July to October, and gradually increasing from November to June. As for TFAs, the quantities of EFAs in gonads from males and females of *P. perna* and *P. angulosus* showed similar temporal trends (Fig. 4.4), with significant differences between months and gender (*P.angulosus*, Table 4.1). The concentrations of EFAs in *P. perna* (Fig. 4.4A) gonads were generally higher than those in *P. angulosus* gonads (Fig. 4.4B), with the female gonads displaying consistently higher contents than male gonads for both species. In *P.angulosus*, patterns in EFA concentrations (Fig. 4.4B) matched the patterns in TFAs (Fig. 4.3B).

4.3.4. EFAs and TFAs in muscles and gonads

The quantities of TFAs and EFAs varied significantly between tissues (gonads vs. muscles) and among months in both *P. perna* and *P. angulosus* (Table 4.2, Fig. 4.5 and 4.6). TFAs in *P. perna* showed higher among-individual variability through time in the gonads than in the muscles (Fig. 4.5A; Table A19). For *P. angulosus*, the TFAs in gonad tissues were generally higher than in muscles (Table A19), and gradually increased from October 2010 to May/June 2011. The among-individual variability in muscle tissues of *P. angulosus* was almost always very small, except in July and January (CVs 133 and 120 %, respectively; Table A19). EFA quantities in both *P. perna* and *P. angulosus* were more variable among-individuals and through time in gonads than in muscles (Fig. 4.6; Table A19).

4.3.5. Linear regression model of TFA in gonad tissues

Simple linear regressions showed a significant effect of photoperiod on total fatty acid concentrations in both *P. perna* ($r^2_{(Male)}$ =0.53, F= $10.26_{1, 9}$, $p < 0.01$) ($r^2_{(Female)}$ =0.58, F=$11.45_{1, 8}$, $p < 0.01$) and *P. angulosus* ($r^2_{(Female)}$ =0.51, F= $9.37_{1, 9}$, $p < 0.01$). Analyses using data from the male sea urchins indicated no effects of photoperiod on total fatty acid concentrations in gonad tissues ($r^2_{(Male)}$ =0.57, F= $6.54_{1, 5}$, $p > 0.05$). Temperature had no effect on TFAs in both *P. perna* ($r^2_{(Male)}$ =0.03, F= $0. 37_{1, 9}$, $p > 0.05$) ($r^2_{(Female)}$ =0.01, F=$0.09_{1, 8}$, $p > 0.05$) and *P. angulosus* ($r^2_{(Male)}$ =0.04, F= $0. 21_{1, 5}$, $p > 0.05$) ($r^2_{(Female)}$ =0.06, F= $0.53_{1, 9}$, $p > 0.05$) (Fig. 4.3 A and B).

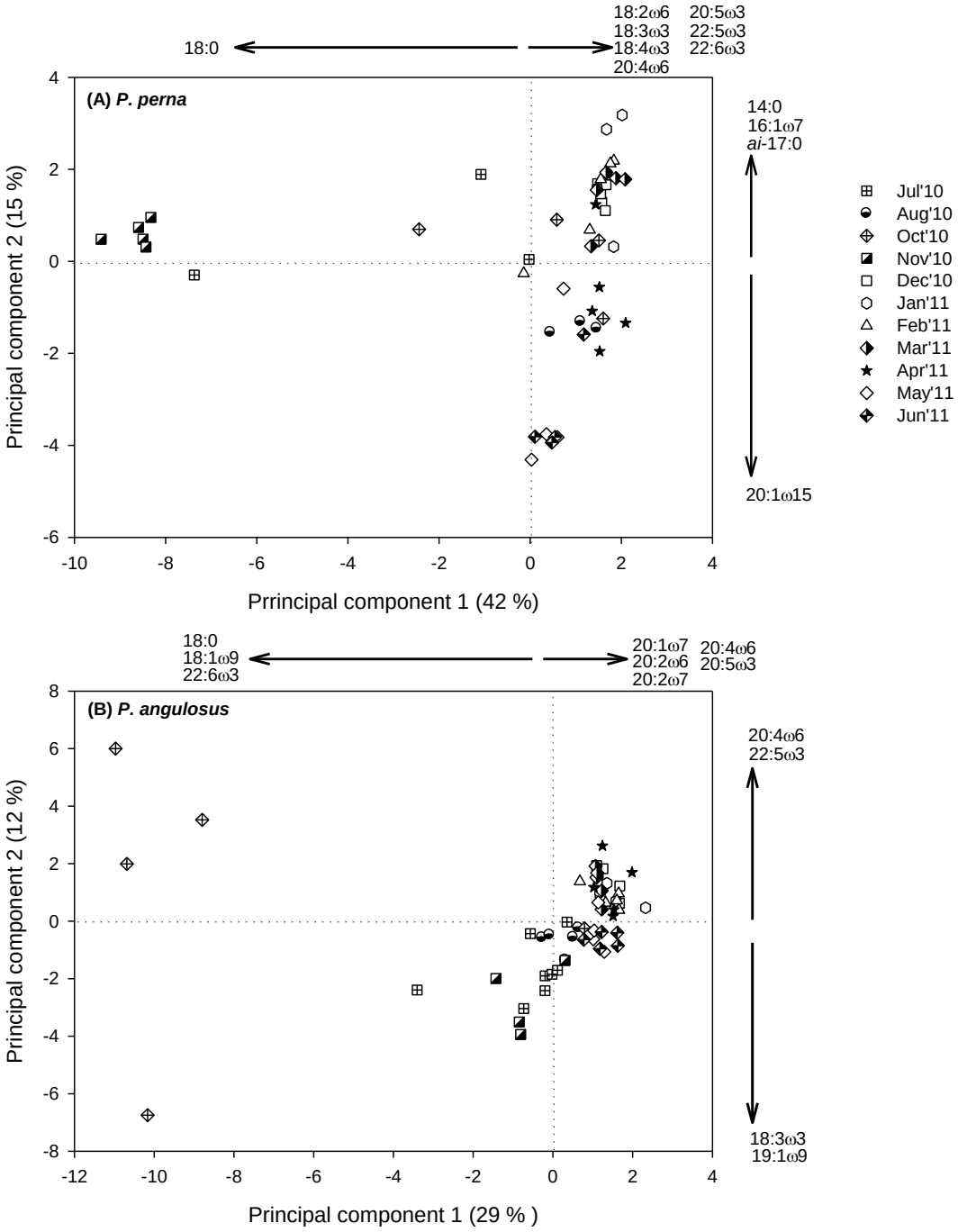

Figure. 4.1. Principal component analysis (PCA) of arcsin square root-transformed proportional fatty acid data (% TFA) from the gonad tissues of (A) *Perna perna* and (B) *Parechinus angulosus*. Fatty acid >1% TFA were included in the analyses (38 fatty acids in total). Percentage values in PC (principal component) labels indicate the proportion of variation accounted for by each PC. Arrows running parallel to each axis denote the influence of the specific fatty acids that have loading values >2.5%. The cross-section of the dotted lines represents the origin.

Table 4.1. Results of the General Linear Models (GLM) for the quantities (µg FA mg^{-1}DW) of fatty acid saturation category from gonad tissues of suspension-feeder *Perna perna* and grazer *Parechinus angulosus*. The symbol Σ indicates total abundance for each category.

	Perna perna			*Parechinus angulosus*		
Source	*df*	*F*	*p*	*df*	*F*	*p*
ΣPUFA						
Month	9	10.79	<0.001	6	11.15	<0.001
Gender	1	12.72	<0.001	1	0.32	0.577
Months*Gender	9	1.34	0.267	6	0.26	0.951
Error	25			23		
ΣMUFA						
Month	9	4.04	<0.01	6	8.80	<0.001
Gender	1	14.11	<0.001	1	0.08	0.784
Months*Gender	9	1.08	0.408	6	0.29	0.934
Error	25			23		
ΣSFA						
Month	9	4.85	<0.001	6	18.59	<0.001
Gender	1	12.31	<0.01	1	7.91	0.010
Months*Gender	9	1.21	0.333	6	1.43	0.247
Error	25			23		
ΣEFA						
Month	9	4.40	<0.01	6	5.06	<0.01
Gender	1	0.44	0.512	1	4.46	0.046
Months*Gender	9	0.79	0.625	6	0.74	0.621
Error	25			23		
TFA						
Month	9	7.02	<0.001	6	13.96	<0.001
Gender	1	16.40	<0.001	1	1.57	0.223
Months*Gender	9	1.38	0.248	6	0.36	0.896
Error	25			23		

Factors: Fatty acid categories were the continuous dependent variables; month and sex as categorical independent variable or predictors; PUFA: polyunsaturated fatty acids; MUFA: monounsaturated fatty acids; SFA: saturated fatty acids; EFA: essential fatty acids; TFA: total fatty acids.

Table 4.2. Results of the General Linear Models (GLM) for the quantities (µg FA mg^{-1}DW) of essential fatty acids (EFAs) and total fatty acids (TFAs) from muscles and gonads of *Perna perna* and *Parechinus angulosus*. The symbol Σ indicates total abundance for each category.

	Perna perna			*Parechinus angulosus*		
Source	*df*	*F*	*p*	*df*	*F*	*p*
ΣEFA						
Month	10	14.37	<0.001	10	30.95	<0.001
Tissue	1	155.92	<0.001	1	529.29	<0.001
Months*Tissue	10	9.23	<0.001	10	5.84	<0.001
Error	77			80		
ΣTFA						
Month	10	3.60	<0.001	10	18.38	<0.001
Tissue	1	116.70	<0.001	1	298.97	<0.001
Months*Tissue	10	4.68	<0.001	10	11.92	<0.001
Error	77			80		

Factors: Fatty acid categories were the continuous dependent variables; month and tissue as categorical independent variable or predictors; EFA: essential fatty acids; TFA: total fatty acids.

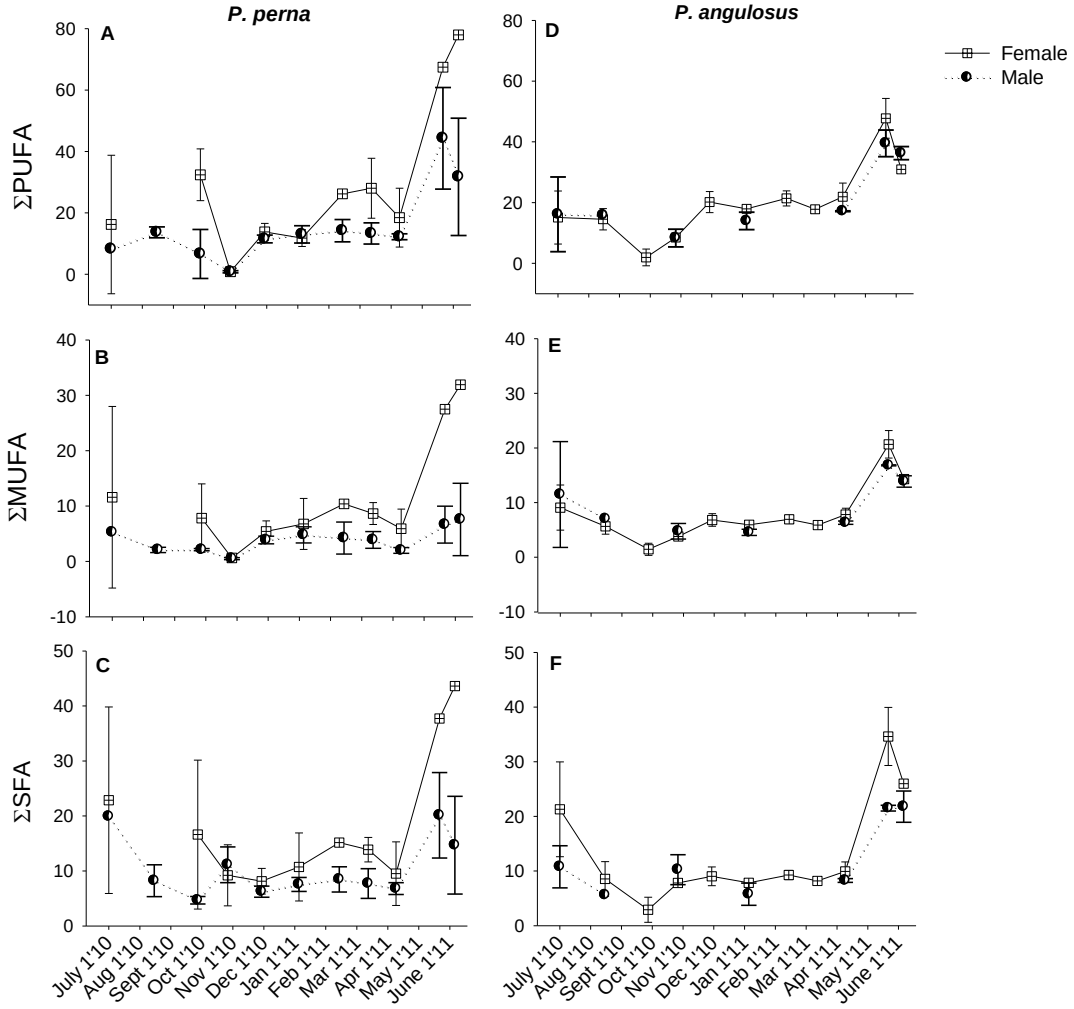

Figure. 4.2. Monthly changes in the quantities of fatty acids (FA) categories over one year in the gonads of male and female *Perna perna* (A-C) and *Parechinus angulosus* (D-F). FA categories are presented in µg FA mg^{1} DW of polyunsaturated FAs (ΣPUFA, A and D); monounsaturated FAs (ΣMUFA, B and E); saturated FAs (ΣSFA, C and F). Values are expressed as mean ± SD; labels represent average values, and error bars standard deviations; lines connecting the monthly values represent hypothetical connections between months.

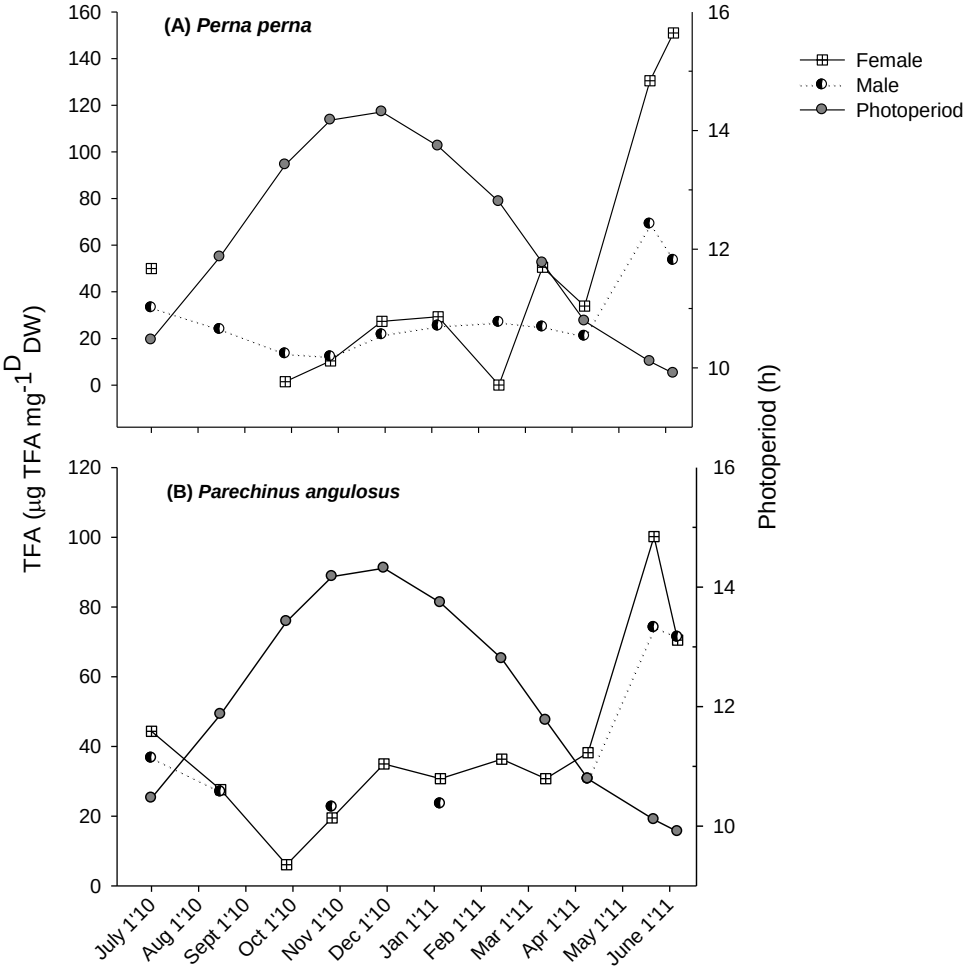

Figure. 4.3. Mean monthly trends of total fatty acid concentrations (TFA) plotted against mean daily photoperiod (hours of day length). Results of a linear regression model are reported for (A) suspension-feeder *Perna perna* females: $r^2 = 0.58$, $p < 0.01$, $n = 9$; males: $r^2 = 0.53$, $p < 0.01$, $n = 10$ and (B) grazer *Parechinus angulosus* females: $r^2 = 0.51$, $p < 0.01$, $n = 10$; male: $r^2 = 0.57$, $p > 0.05$, $n = 6$.

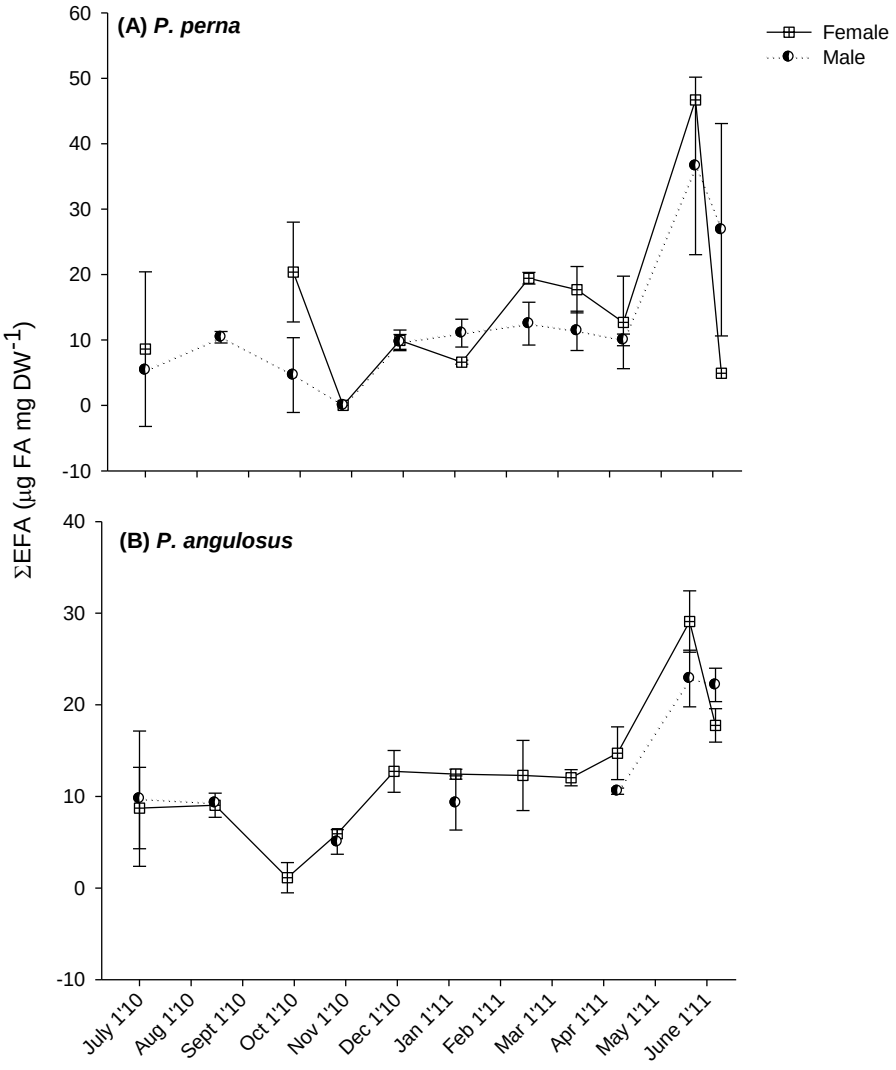

Figure. 4.4. Monthly changes in sum of essential fatty acids (ΣEFAs µg FA mg^{1} DW) over one year in the gonads of male and female of *Perna perna* (A) and *Parechinus angulosus* (B). Values are expressed as mean ± SD; labels represent average values, and error bars standard deviations; lines connecting the monthly values represent hypothetical connections between months.

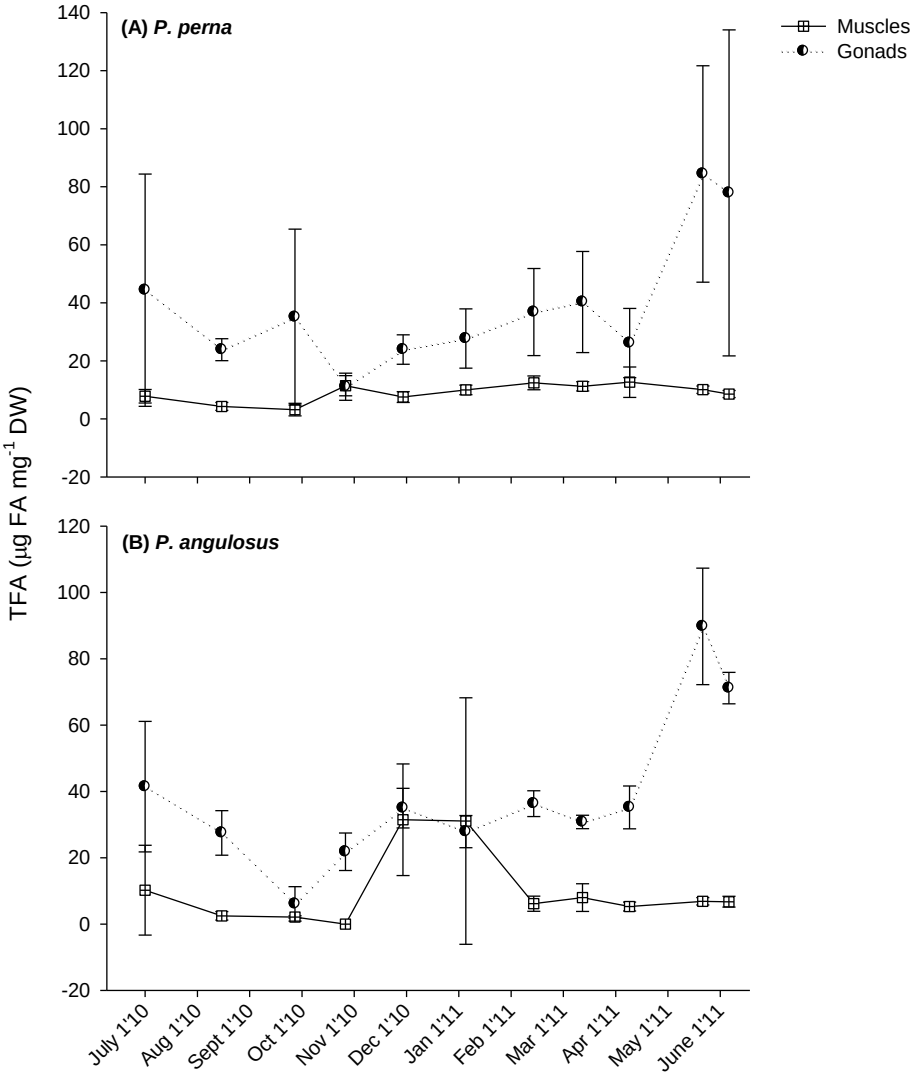

Figure. 4.5. Monthly changes in concentrations of total fatty acids (TFA µg mg^1 DW) over one year in a combined male and female muscles and gonads of *Perna perna* (A) and *Parechinus angulosus* (B). Values are expressed as mean ± SD; labels represent average values, and error bars standard deviations; lines connecting the monthly values represent hypothetical connections between months.

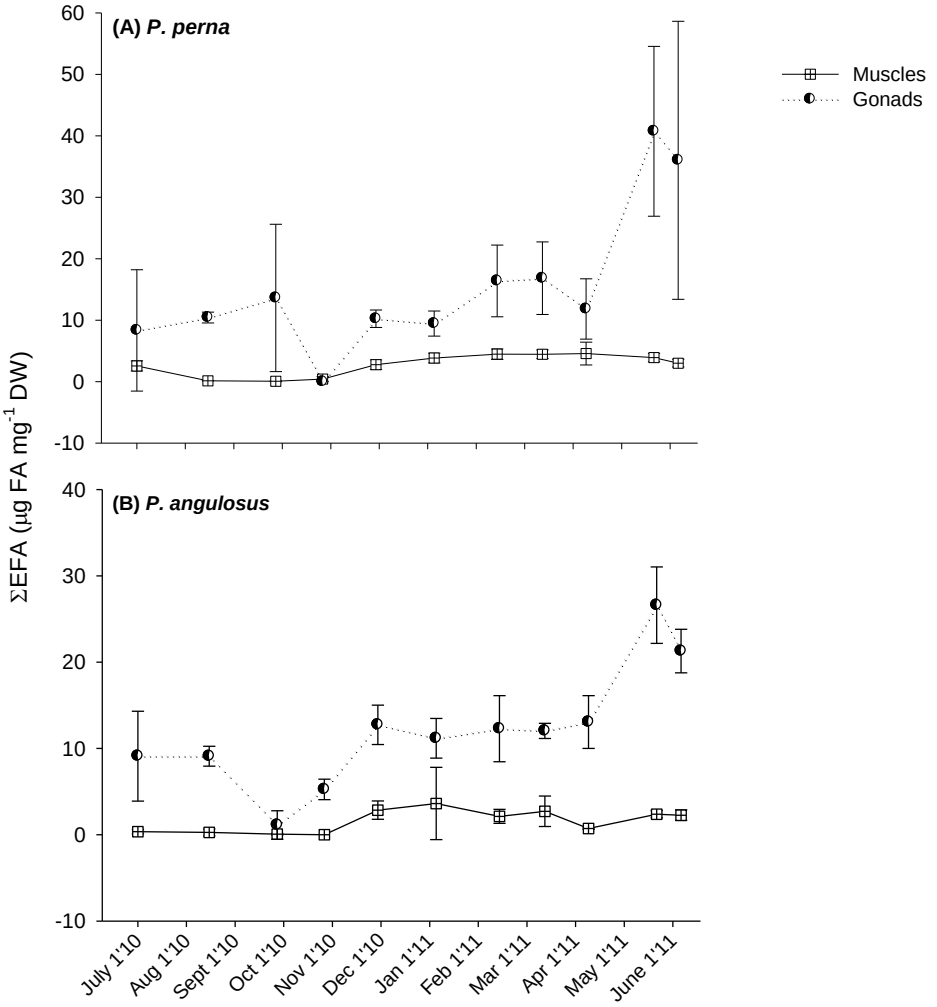

Figure. 4.6. Monthly changes in concentrations of total essential fatty acids (ΣEFA µg mg^1 DW) over one year in a combined male and female muscles and gonads of *Perna perna* (A) and *Parechinus angulosus* (B). Values are expressed as mean ± SD; labels represent average values, and error bars standard deviations; lines connecting the monthly values represent hypothetical connections between months.

4.4. DISCUSSION

In Chapter 3, I showed that fatty acid composition varied through time in rocky shore suspension-feeders and grazers. Reproductive cycles of marine invertebrates have been well studied, as they influence community composition and diversity, species dispersal patterns, and biogeography (Ramirez-Llodra, 2002). For many invertebrate species, spawning is usually preceded by an increase in the size and activity of gonads, which requires a significant amount of energy (Ramirez-Llodra, 2002). Lipids are efficient sources of energy for fueling gamete production; therefore, it is of ecological interest to examine fatty acid composition of consumers in relation to their reproductive cycles in a natural environment (Cook *et al*., 2000; Morais *et al*., 2003; Martinez-Pita *et al*., 2010).

Researchers studying sea urchin reproductive cycles have reported some variability in gametogenesis and spawning times. Drummond (1995), studying the reproductive cycle of two species of sea urchin *Echinometra mathaei* and *Diadema savignyi* from the Natal coasts in South Africa, found substantial evidence of spawning activity during summer to autumn (December to March/April). He also found significant differences in reproductive timing between the species (Drummond, 1995). Greenwood (1980) investigated *Parechinus angulosus* in South Africa and indicated that spawning occurs during late winter (August). Internationally, a study on the reproductive cycle of the sea urchin *Loxechinus albus* from the Beagle channel, Argentina, reported higher gonad indices during August and subsequent spawning activity between September and December (Pérez *et al*., 2009). A similar spawning period was described for *L. albus* populations in the Magellan region in Argentina (Bay-Schmith *et al*., 1981; Arana *et al*., 1996; Oyarzun *et al*., 1999). In my study, the gonads of the cape sea urchin *Parechinus angulosus* were most lipid rich during May, June and July (late autumn to winter), followed by clear decreased levels for the rest of the year. A refractory period has been documented after spawning for marine invertebrates (Belisle and Stickle, 1978), and this seems to coincide with spring to the beginning of summer (Figs. 4.3 and 4.4). These findings coincide with the literature on the gonad development (winter) and spawning (post-winter) times of *P. angulosus*. Overall, gonad development and spawning times were very similar in both *P. perna* and *P. angulosus*, with the winter season as the main period for gonad development. These findings indicate that the life cycles of both species are probably strongly influenced by similar environmental factors.

Links have been investigated between environmental variability, especially temperature, photoperiod and food availability, and gamete maturation and spawning of

marine invertebrates (Giese, 1959; Pearse and Cameron, 1991; Brockington *et al.*, 2001; Brockington *et al*., 2007). Regarding sea urchins, several studies have documented an effect of photoperiod on gametogenesis and spawning. Pérez *et al*. (2009) showed an inverse proportion between photoperiod and gonad index of the sea urchin *Loxechinus albus*, and gametogenesis was initiated during autumn, when light period was shortening. A similar influence of photoperiod was described in sea urchin *Centrostephanus rodgersii* (Byrne *et al*., 1998) and several populations of *Strongylocentrotus purpuratus* (Pearse *et al*., 1986; Bay-Schmith and Pearse, 1987). The effect of photoperiod on gametogenesis in bivalves is still not clear; while several experiments have shown that an increasing photoperiod stimulates reproduction in *Pecten maximus* (Couturier and Aiken, 1989; Devauchelle and Mingant, 1991), others indicated no influence in scallop *Argopecten* v*entricosus* and zebra mussel *Dresseina polymorpha* (Janzel and Villalaz, 1994; Borcherding, 1995). Saout *et al*. (1999) showed that a simultaneous increase of temperature and photoperiod enhances gonad growth when food is not limited, although the authors experimental design did not permit determination of which factor was the more influential. Photoperiod may also have an indirect effect on the spawning of coastal invertebrates, as light levels influence primary production: Himmelman (1975) speculated that a chemical released by phytoplankton (increasing in abundance during growth seasons) initiates nervous and physiological events that ultimately cause spawning in marine invertebrates such as the sea urchin *Strongylocentrotus droebachiensis*. For my study species, the photoperiod showed significant effects on TFAs in both *P. perna* and *P. angulosus*, underlining its influence on the reproductive cycle (Fig 4.3). The increase in gonad TFAs is likely due to gametogenesis which coincided with decreasing photoperiods after April. The spawning period is coincident with decreases in fatty acids and increasing of the day lengths after mid-winter (Fig. 4.3).

Temperature had an effect on the spawning activity of sea urchin *Tripneustes gratilla* from the Southwestern Indian Ocean, inducing the release of gametes during mid and later winter, when temperatures and day lengths were lowest (Vaïtilingon *et al*., 2005). Rise in temperatures have been described to favour gamete release in temperate echinoids e.g. *Paracentrotus lividus* (Fenaux, 1968; Byrne, 1990; Spirlet *et al*., 1998), although temperature effects have not been consistently found (Pearse, 1974; Himmelman, 1978; Pérez *et al*., 2009). Stott (1931) reported, for the sea urchin *Echinus esculentus*, spawning activity when the sea temperature was rising in late April, May, and June. The author observed the same for marine bivalve *Mytilus* (mussels) and *Ostrea* (oysters) in the same region (Port Erin, Isle of

Man). In my study, temperature did not have any effect on the reproductive cycle. This could be attributed to my methodology, which produced only instantaneous measures of temperature (see Chapter 2 for details) rather than integrated information like photoperiod. Further investigations of the effects of temperature should include an integrated estimate of temperature changes using data loggers, which provide a very suitable method for abiotic data capturing on rocky shores (Xavier *et al*., 2007; Lathlean *et al*., 2011).

Females and males typically differ in their concentrations of lipids in reproductive tissues, since females allocate more energy to accumulate within eggs (i.e. to allow larvae growth and survival in early stages) than males allocate to sperm production (Lawrence and Giese, 1969; Robards *et al*., 1999; Guijarro *et al*., 2003). In the present study, fatty acid contents of *P. perna* and *P. angulosus* gonads were different between males and females, in accordance with my second hypothesis, with female gonads tending to be more lipid rich than the males.

These findings are consistent with those from echinodermata *Psammechinus miliaris* from the the south shore of Loch Creran, west Scotland, and *Paracentrotus lividus* and *Arbacia lixula*, from the south coast of Spain, indicating that mature males and females had significant differences in the fatty acid composition of their gonads (Hughes *et al*., 2006; Martinez-Pita *et al*., 2010). Martinez-Pita *et al*. (2010) suggested that these differences between sexes resulted from different metabolic requirements of males and females during gametogenesis. Lower fatty acid contents in male testes is a common phenomenon in marine invertebrates, as sperm contain no reserves (only the gonad tissue stores lipids), whereas eggs must contain reserves for the developing embryos (Lawrence and Giese, 1969). In my study, although the temporal patterns in gonad fatty acid composition were coarsely similar between sexes in *P. angulosus*, some noticeable gender differences were apparent. All of the major fatty acid categories increased substantially in *P. perna* females after May 2011, with increases in the males substantially decreased (Fig. 4.2). The increase of fatty acids during early winter in both species was likely coincident with the development of gonads prior to spawning, when lipid stores need to build up for gametogenesis (Fig. 4.3 A and B). Dry mass measurements of the gonads of *P. perna* from the Cape and Transkei South African coasts indicated that gonad development occurred in early winter (van Erkom Schurink and Griffiths, 1991). Similarly, on the southern coast of South Africa, assessments of female condition and larval density in the near shore water column showed that *P. perna* spawns around May/June (McQuaid and Lawrie, 2005). In other regions of the world, Shafee (1989)

reported spawning activity of mussel *Perna picta* from southern Mediterranean coasts during the austral late winter, preceded by the development of gonads in early winter. As such, the published literature on *Perna* spawning cycles corroborates the timing of fatty acid accumulation in the mussel gonads from early winter in my study. The spawning period coincides with the lipid-depleted gonads from August to October/November (Fig. 4.3).

Researchers often investigate the lipid compositions in total body material, pooling data from different tissues (Lubet *et al*., 1985; Napolitano and Ackman, 1992; Alkanani *et al*., 2007; Ekin *et al*., 2008; Martínez-Pita, 2012). However, separating tissues with different functions (such as reproductive vs. non-reproductive) can help to distinguish between seasonal changes in diets and seasonal changes in life cycle dynamics (Ahn *et al*., 2003; Richoux *et al*., 2004; Ekin *et al*., 2012; Martínez-Pita, 2012). In my study, both intertidal species had striking differences in quantities of TFAs and EFAs between muscle and gonad tissues. Lipids were generally much greater in gonads compared with muscle tissues, although the patterns were highly variable through time (Figs. 4.5 and 4.6). The concentration of lipids was similar in gonads and muscles during October, December and January (*P.angulosus*) and November (*P. perna*), after both species had already spawned. During winter, the mature gonads reached their maximum fatty acid concentrations, whereas the muscles of *P. perna* remained consistently lipid-rich since the summer before (see Chapter 3, Fig. 3.8). *P. angulosus* gonad fatty acids also showed a decoupling from the muscle tissues (Fig. 4.5). Ahn *et al*. (2003) investigated the mass and biochemical composition in several tissues of the bivalve *Laternula elliptica* from King George Island, Antarctica. The authors suggested that muscle tissues served as a major energy depot for both maintenance of metabolic components and gonad development in winter, and also reported a considerable increase of gonad mass during the development season. Conversely, Giese (1966) indicated that non-reproductive tissues in mussels were not storage sites for lipid reserves since the fatty acid content was seasonally constant. Both species in my study showed discrepancies between muscles and gonads in terms of fatty acid composition (Fig. 4.5 and Fig 4.6). Some evidence of the use of lipid reserves of the muscle tissues for gonadal growth could be found in either *P. perna* or *P. angulosus*, as there was a slight drop of muscle lipids when the gonad lipids increased up to the maximum (Fig. 4.5). Muscle lipid levels also dropped during the period of main spawning activity (Fig. 4.5). Such findings agree with the hypothesis that lipid reserves of the muscle tissues are utilised at least partially for gonadal growth, as suggested by Ahn *et al*. (2003) and also reported by Lawrence and Giese (1969) for the chiton

Katharina tunicate. However, the patterns of muscles and gonads lipid composition of *P.perna* and *P. angulosus* do not match perfectly, thus indicating a large dietary influence through time (in terms of quality of food sources; Chapter 3).

4.5. CONCLUSION

In conclusion, the results confirm my hypotheses that temporal variations in lipid composition of rocky shore invertebrates are affected by reproduction cycles. In particular, the gonadal composition of fatty acids seems to reflect the reproductive cycle with increasing levels before the spawning activity for both species, when the gonads need high amounts of energy. The muscle lipid composition seems to be driven more by food quality and quantity (as discussed in Chapter 3) than by reproduction. Only small evidence of the invertebrates using non-reproductive tissues as storage of energy emerged in this study. Differences between sexes, with higher values in females than males for both species, confirmed that more energy was allocated to egg than sperm development. Possibly the increases in quantity and/or quality of food from December to June (Chapter 3: Figs. 3.8 and 3.9) influenced the reproductive strategies for both *P. perna* and *P. angulosus*. The effect of food quality on muscles and gonads was particularly pronounced in the suspension-feeder *P. perna*, which has strong dependency on highly variable changes in suspended particulates, as highlighted in Chapter 3.

5 GENERAL DISCUSSION

The intertidal rocky zone is a highly dynamic ecosystem, where a whole range of biotic and abiotic factors determine its structure, dynamics and trophic linkages (Fiske *et al.*, 2003; Riera and Adin, 2003; Riera *et al.*, 2009). Such factors include predation, intra- and inter-specific competition, temperature, hydrodynamics, wave exposure, photoperiod and food availability (McQuaid and Branch, 1984; Field and Griffiths, 1991; Bustamante and Branch, 1996; Raffaelli and Hawkins, 1996). Fundamental ecological concepts such as energy flow, food webs, and trophic levels have been targeted by many researchers studying rocky shores, and their results have provided many insights into how organisms respond to environmental changes (Menge and Sutherland, 1976; Raffaelli and Hawkins, 1996; Laurand and Riera, 2006). There is, however, still much to clarify on how rocky intertidal systems vary at different spatial and temporal scales. Long-term studies on the diet variations in consumers inhabiting these habitats in relation to their changing environment are particularly needed.

Investigating the diets of marine animals is essential for the understanding of their basic ecology, trophic interactions, and community-level responses to biotic and abiotic changes (Fiske *et al.*, 2003; Howell *et al*., 2003; Kelly *et al*., 2012). Fatty acids in organism tissues provide a useful tool to derive long-term dietary information (Iverson *et al*., 2004; Hughes *et al*., 2005; Alfaro *et al*., 2007; Kelly *et al*., 2012). Fatty acid analyses have been intensively applied to pelagic trophic systems, but less effort has been dedicated to benthic communities, and even less to the intertidal zone (Gatenby *et al.*, 2003; Kharlamenko *et al*., 2008; Kelly *et al*., 2008, 2012). In this thesis, I firstly aimed to provide evidence for the applicability of fatty acid techniques to intertidal rocky shore communities, and the utility of this ecological tool was confirmed in this habitat. The high diversity of potential food sources available on the rocky shores is indeed a limiting factor for the utility of fatty acids as dietary indicators (Brazão, 2009; Kelly *et al*., 2012). For future studies, combined analyses of fatty acid profiles analysed on organisms reared in the laboratory and on organisms collected in the field could help us to better understand relationships between consumers and their potential

food sources. In particular, the research field requires more information on the turn-over rates of fatty acids in different tissues and species. In my study, the mussels *P. perna* and sea urchins *P. angulosus* were feasible model species to demonstrate temporal changes in muscle tissue fatty acid profiles as a result of changes in their food sources, and changes in their gonad fatty acids through development. My study is the first of its kind to provide important insights on the temporal variability in the diets of rocky intertidal suspension-feeders and grazers in South Africa using fatty acid techniques. I confirmed that this approach is indeed suitable to detect the temporal changes in organism diets and reproductive cycles in such a dynamic habitat.

In my thesis, I demonstrated that rocky shore consumers (suspension-feeders and grazers) showed differences in their fatty acid compositions through time, which confirmed my prediction of an influence of the life style and feeding mode on diets. The efficiency of fatty acids for classifying consumers into broad dietary groups (such as suspension-feeders vs grazers) has recently been emphasized (Hughes *et al*., 2005; Budge *et al.*, 2006; Hyndes and Hanson, 2009; Allan *et al*., 2010; Kelly *et al*., 2012). In the present study, temporal variability in diets was more accentuated in suspension-feeders than grazers, since the suspension-feeders are potentially subject to larger changes in their food quality. The two suspension-feeding species (the mussel *P. perna* and the polychaete *G. gaimardi*) generally showed similar trends, while the two grazers responded differently through time, with the sea urchin *P. angulosus* having more variability in its diet as compared to the limpet *C. oculus*.

Suspension-feeders are key components of rocky shore communities, and represent a core link between the suspended organic matter and the benthos (Hill *et al*., 2006; Wing and Jack, 2012). Researchers have shown that nearshore productivity influences the diet of the brown mussel *P. perna* from the south coast of South Africa (Hill *et al*., 2006). My findings from the SPM provided further support for this linkage and indicated that a significant proportion of the total fatty acids in the suspension-feeders may be affected by changes in nearshore biological production. These variations in food quality and quantity are likely influenced by environmental variables such as temperature, light intensity, coastal hydrodynamics and wind (Khotimchenko and Yakovleva, 2005; Khotimchenko, 2006; Becker *et al*., 2010). Both of the suspension-feeders analyzed in my study showed similar changes in their fatty acid profiles, with similar trends in TFAs and EFAs. This result demonstrated the similar functional roles of the mussel *P. perna* and the polychaete *G.gaimardi* within the rocky shore community.

Suspension-feeders are often considered opportunistic as a group (Coma *et al*., 2001), but inter-specific dissimilarities in diet selection can lead to differences in ecological function, even within one trophic level (Wing and Jack, 2012). Differences in biomarker tracers (such as 22:6ω3, 20:5ω3 and 18:1ω9) were found between the two species of suspension-feeders, underlying the importance of interspecific differences in diets allowing for the partitioning of the food niche. In this case, the interspecific differences in diets were most likely due to differences in feeding mechanisms and selection. Despite such discrepancies, the similar temporal trend found between the two species of suspension-feeders and the water samples (SPM) supported my hypothesis of a strong link between this category and its variable food source.

Grazing is also a critical and important biological process in the rocky intertidal, where grazers like limpets, snails and sea urchins may directly or indirectly influence patterns of abundance and distribution of other consumers and macroalgal communities (Menge, 1995; Benedetti-Cecchi, 2000). Many studies have investigated the direct effects of grazing on macroalgal communities, and the indirect effects of grazing on the overall community (Benedetti-Cecchi *et al*., 2001), highlighting the relative importance of spatial and temporal variability in grazing (both at large and small scales) (Underwood, 1985; Chapman and Underwood, 1992). The timing and intensity of recruitment of algae or other organisms (such as barnacles) affects the impact of grazers on intertidal assemblages, and such events are themselves spatially and temporally variable (Underwood *et al*., 1983; Farrell, 1991; Benedetti-Cecchi, 2000). Seasonal or other temporal changes in foraging patterns influence the relative importance of grazing at different scales of time (Benedetti-Cecchi *et al*., 2001). In my thesis I focussed on how the diets of two grazers (the sea urchin *P. angulosus* and limpet *C. oculus*) can change through time using fatty acid techniques. The two grazers showed different patterns in temporal variability, partially contrasting the initial hypothesis of consistency in diets throughout the year. Within the grazer category, the sea urchin diet showed more temporal variations than the limpets, which showed more consistency throughout the year. These results indicate that grazer diets are probably very complex in some species, and that temporal variability may be closely related to the diversity of food sources available. Further studies comparing grazer diets on rocky shores hosting different macroalgal diversities may shed further light on this question.

Fatty acid analyses from gonadal tissue through time also revealed important results in the mussel *P. perna* and the sea urchin *P. angulosus.* Seasonal variations in nutrient

dynamics, through their effects on primary productivity, influences the nutritional condition of marine invertebrates and are highly influenced by the reproductive cycles (Gatenby *et al.*, 2003; Shin *et al.*, 2008). Variations in relation to nutritional condition and reproductive cycle are reflected in the relative proportions of particular fatty acids within consumers. This thesis provides an important contribution on the strong effect of reproductive cycle on the temporal variability of fatty acid composition in the gonads of two intertidal invertebrates on the South African rocky shore. The results confirmed my hypotheses that the reproductive cycle affects the lipid composition of rocky shore invertebrates over time. The results also emphasized the importance of considering different tissues when investigating consumer diets. Indeed, lipid composition in muscle tissues was more reflective of food quality and quantity for both of the species, whereas the gonadal tissues provided information specific to the reproductive cycles. In periods of high food availability, the cost of gonadal maturation can be covered entirely by absorbed materials and energy, but when food availability is low, gonadal maturation and gametogenesis essentially depend on storage reserves (Martinez *et al*., 2000). In both of the study species, some evidence of lipid utilisation and transport between non-reproductive (muscles) and reproductive tissues (gonads) was evident. Possibly the two species acquired most of the energy, in form of lipids, from the food sources available and directly transferred these to reproductive tissues for gametogenesis and spawning activities. I recommend that further investigations on fatty acid compositions in consumers must account for (a) the influence of development and reproduction, and (b) the effects of potentially changing diets through time.

Lipids represent an important basis of energy that animals can use during gametogenesis or gonad development (Brazão *et al.*, 2003; Gatenby *et al.*, 2003; Morais *et al.*, 2003; Sewell, 2005). Females generally have higher energy requirements than males due to the high egg production costs, as reserves need to be stored in the egg for embryogenesis (Robards *et al*., 1999; Guijarro *et al*., 2003). The fatty acid contents of *P. perna* and *P.angulosus* (i.e. SFA and EFA, Table 4.1) gonads were different between males and females, in accordance with my second hypothesis, with female gonads tending to be more lipid rich than the males. The temporal difference in females within the same species (with regards to lipid composition) is a fascinating aspect of reproduction and could be linked with the ability of females to invest more (in term of energy allocation during the embryogenesis) based on what they experienced in their environment. Further studies could consider the

effect of food availability as an important environmental factor influencing the female investment into their offspring.

In summary, the temporal scale (monthly sampling rate) covered in this study has provided valuable information about the limitations of diet studies considering short-term sampling rate (seasonal rate) in the rocky intertidal. These short-term studies represent snapshots in time and give us little information on highly complex organisms and habitats like the intertidal rocky shore. A seasonal sampling rate would be appropriate, in case the fatty acids turnover rate of the animals is known to occur in season length. For this reason investigating on the fatty acids turnover rate of animals would give a strong contribute to understand the diet dynamics of marine communities. Indeed, the transformation that may occur during the assimilation of fatty acids by consumers and the rate of which it occurs is crucial and highly variable among consumer taxa (McLeod *et al*., 2013). Although, this study provided clear temporal trends in the fatty acids composition of rocky shore consumers, I did not take into account any analysis of the fatty acids turnover rate of the study animals. Regarding rocky intertidal organisms, very few studies addressed the importance of fatty acids turnover and a handful analysed it in the field. Freites *et al*. (2002) transplanted the mussel *Mytilus galloprovincialis* to a new location with similar environment to the original site. They found that the fatty acid profiles of these mussels closely resembled the fatty acid profiles of the local mussels after an average of three weeks. These results suggest that a monthly rate of fatty acids analyses in mussels could be more appropriate. Further works on the temporal variations of fatty acids should consider the importance of the turnover rate, in order to optimize the sampling rate. This is certainly an important matter as fatty acids are considered a time integrated signal for diet studies. However, analysing temporal changes over one year allowed me to detect some drastic changes in the diets and nutritional condition of several species. A field collection at only one of these time points would not capture this variability, and we could develop serious misunderstandings of organism diets based on only one time point. A snapshot analysis is sometimes suitable for researchers examining spatial variability in organism diets, thus temporal variations are not part of the study questions.

I also highlighted the importance of considering life style when examining the trophic ecology of rocky shore intertidal animals. Ecologists have used a range of biomarkers such as fatty acids to study diets of marine organisms (Hughes *et al*., 2005; Alfaro *et al*., 2006; Kelly, *et al*., 2008; Allen *et al*., 2010). According to Kharlamenko *et al*. (2008), many potential food sources contain specific biomarker fatty acids which are utilized by consumers and may be

traced over a number of organisms occupying different trophic levels. Given the scarce amount of information on the use of fatty acids to understand the diet of intertidal organisms, my contribution is particularly important, as it provides new insights into a highly dynamic and biomass-rich ecosystem.

Future ecological investigations over long-term temporal scales should incorporate additional tracer methods such as stable isotope ratios. By using additional methods, we may gain further insights into the complex feeding habits of organisms living in this dynamic habitat and in general of marine coastal ecosystem. My thesis provides a foundation for future investigations into rocky intertidal trophic ecology. Ideally the temporal and spatial scales should be increased, and additional consumers included for comparative purposes. The influence of riverine inputs to coastal habitats could also provide relevant information to link adjacent systems trophically. Links between the intertidal and subtidal benthos could also be an interesting study theme, as the subtidal region may provide important sources of nutrients for intertidal consumers. The more we learn about consumer diets and how they change through space and time, the better understanding we will have of how the rocky shore ecosystem functions as a whole and what the consequences are for any disruptions.

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APPENDIX

Table A1. Fatty acid composition (mean % TFA ± SD) of dominant species and suspended particulate matter (SPM). Data represents all fatty acids at concentrations > 1 % TFA in at least one sample; means ± standard deviation (n = 3).

Fatty acid	*Ulva* sp.	*G. pristoides*	*Bryopsis* sp.	SPM
14:0	1.42 ± 0.70	3.31 ± 2.12	3.17 ± 2.20	6.51 ± 2.65
i-15:0	0.00 ± 0.00	0.02 ± 0.05	0.00 ± 0.00	1.01 ± 0.60
ai-15:0	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.04	1.37 ± 1.57
15:0	0.24 ± 0.54	0.05 ± 0.10	0.05 ± 0.09	1.06 ± 0.56
i-16:0	0.00 ± 0.02	0.07 ± 0.13	3.00 ± 2.46	0.00 ± 0.00
16:0	26.38 ± 5.80	33.14 ± 7.63	34.63 ± 7.08	28.02 ± 6.63
i-17:0	0.70 ± 0.45	1.26 ± 0.82	2.64 ± 1.84	0.48 ± 0.71
ai-17:0	1.97 ± 2.72	0.53 ± 0.30	0.82 ± 1.53	1.12 ± 2.38
17:0	0.61 ± 1.25	0.05 ± 0.24	0.06 ± 0.24	0.97 ± 1.33
ai-18:0	0.35 ± 0.27	0.04 ± 0.11	0.00 ± 0.00	0.48 ± 1.12
18:0	12.93 ± 4.32	0.43 ± 0.38	0.41 ± 0.25	9.75 ± 6.32
20:0	0.17 ± 0.18	0.00 ± 0.00	0.00 ± 0.00	0.59 ± 0.99
22:0	1.04 ± 0.79	13.10 ± 12.14	21.70 ± 17.04	0.42 ± 0.71
ΣSFA	45.81 ± 17.05	51.99 ± 24.00	66.49 ± 32.77	51.78 ± 25.58
16:1ω7	2.36 ± 6.89	0.70 ± 0.71	1.60 ± 1.10	11.25 ± 5.14
16:1ω5	0.09 ± 0.22	0.01 ± 0.04	0.05 ± 0.14	2.36 ± 5.63
18:1ω9	0.85 ± 1.90	2.44 ± 2.21	2.28 ± 2.61	11.50 ± 4.74
18:1ω7	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
20:1ω7	0.10 ± 0.46	0.00 ± 0.00	0.00 ± 0.00	2.19 ± 3.90
22:1ω9	1.22 ± 0.89	0.00 ± 0.00	0.00 ± 0.00	0.50 ± 0.75
ΣMUFA	4.61 ± 10.36	3.15 ± 2.96	3.93 ± 3.85	27.80 ± 20.15
16:2ω6	0.60 ± 0.56	0.00 ± 0.00	0.00 ± 0.00	0.54 ± 0.86
18:2ω6	9.26 ± 2.92	5.35 ± 2.05	5.32 ± 4.47	0.35 ± 0.61
18:3ω5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.69 ± 1.02
18:3ω6	3.56 ± 1.69	1.04 ± 0.73	3.70 ± 7.29	0.02 ± 0.12
18:3ω3	16.76 ± 5.76	0.58 ± 0.69	0.96 ± 1.41	0.66 ± 0.77
18:4ω3	11.98 ± 8.23	0.32 ± 0.49	0.25 ± 0.37	0.24 ± 0.34
18:4ω6	0.04 ± 0.20	1.70 ± 6.12	0.00 ± 0.00	ND ±
20:2ω6	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 3.31

Table A1. (cont.)

Fatty acid	*Ulva* sp.	*G. pristoides*	*Bryopsis* sp.	SPM
20:3ω6	0.00 ± 0.00	0.34 ± 0.59	0.08 ± 0.18	0.25 ± 0.51
20:4ω6	0.07 ± 0.24	7.06 ± 11.17	5.11 ± 12.06	1.70 ± 2.53
20:3ω3	0.09 ± 0.18	1.76 ± 5.65	0.08 ± 0.21	3.23 ± 8.64
20:4ω3	0.49 ± 0.46	12.61 ± 11.26	4.77 ± 3.64	0.36 ± 0.82
20:5ω3	0.97 ± 0.99	13.10 ± 15.23	9.20 ± 15.88	6.14 ± 5.33
22:4ω6	0.03 ± 0.17	0.00 ± 0.00	0.04 ± 0.16	0.03 ± 0.09
22:5ω3	1.51 ± 2.02	0.04 ± 0.15	0.03 ± 0.08	2.40 ± 2.32
22:6ω3	2.19 ± 1.41	0.17 ± 0.38	0.03 ± 0.12	0.12 ± 0.32
∑PUFA	47.54 ± 24.84	44.07 ± 54.50	29.58 ± 45.86	17.74 ± 27.58
∑EFA	3.24 ± 1.60	20.33 ± 23.41	14.34 ± 21.21	7.97 ± 7.08
BAFA	3.51 ± 4.59	1.97 ± 0.85	6.58 ± 3.46	6.01 ± 4.46
HPFA	26.02 ± 6.79	5.93 ± 2.18	6.27 ± 4.35	1.01 ± 0.95
16:1ω5+16:1ω7/16:0	3.16 ± 16.27	0.06 ± 0.18	0.10 ± 0.15	2.87 ± 6.27
22:6ω3/20:5ω3	2.88 ± 2.18	0.01 ± 0.02	0.15 ± 0.34	0.32 ± 0.91
TFA	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00

Table A2. The monthly variation of fatty acid saturation categories from three species of macroalgae. Units for quantitative data are in µg FA mg^{-1}DW (mean ± standard deviation). The symbol Σ indicates total abundance for each category.

Month	ΣBAFA	ΣPUFA	ΣMUFA	ΣSFA	ΣEFA	ΣHPFA	TFA
Ulva sp							
25-Aug-10	0.75 ± 0.35	8.65 ± 4.07	0.44 ± 0.24	7.22 ± 3.41	7.33 ± 3.45	4.51 ± 2.13	16.07 ± 7.61
06-Oct-10	12.89 ± 21.17	15.07 ± 12.11	2.17 ± 3.09	41.08 ± 58.91	9.33 ± 4.36	2.95 ± 1.66	57.78 ± 73.50
04-Nov-10	0.51 ± 0.36	11.09 ± 3.21	2.45 ± 2.95	9.89 ± 2.96	9.58 ± 3.21	6.02 ± 1.93	23.11 ± 5.94
07-Dec-10	0.25 ± 0.35	4.39 ± 3.65	0.56 ± 0.34	4.28 ± 4.35	3.54 ± 3.17	2.49 ± 1.89	9.06 ± 7.71
12-Jan-11	0.29 ± 0.32	5.65 ± 4.46	0.36 ± 0.34	5.01 ± 3.89	4.91 ± 4.12	2.85 ± 1.82	10.85 ± 8.44
20-Feb-11	0.98 ± 0.96	14.29 ± 11.57	1.06 ± 1.01	12.56 ± 9.82	12.43 ± 10.48	6.81 ± 5.19	27.35 ± 21.86
20-Mar-11	0.64 ± 0.55	9.65 ± 5.35	0.65 ± 0.45	8.94 ± 5.05	8.40 ± 5.17	4.75 ± 2.05	18.86 ± 10.52
16-Apr-11	0.32 ± 0.23	7.06 ± 2.62	0.53 ± 0.34	6.24 ± 2.49	5.75 ± 2.54	3.46 ± 0.61	13.58 ± 5.23
28-May-11	0.45 ± 0.34	8.50 ± 4.12	0.67 ± 0.44	7.41 ± 3.78	7.34 ± 3.98	4.16 ± 1.38	16.28 ± 8.08
12-Jun-11	0.38 ± 0.27	6.90 ± 3.24	0.76 ± 0.15	6.14 ± 2.28	5.88 ± 3.27	3.25 ± 1.10	13.56 ± 5.17
Gelidium pristoides							
25-Aug-10	0.45 ± 0.16	4.49 ± 1.52	0.35 ± 0.18	8.20 ± 2.73	1.15 ± 0.39	0.93 ± 0.33	13.04 ± 4.43
06-Oct-10	0.50 ± 0.37	20.76 ± 28.24	2.54 ± 4.13	7.98 ± 6.87	10.65 ± 16.18	1.11 ± 0.75	31.27 ± 32.52
04-Nov-10	2.09 ± 2.30	72.99 ± 97.79	7.66 ± 10.63	42.31 ± 46.27	69.36 ± 97.01	9.66 ± 12.58	122.96 ± 154.68
07-Dec-10	1.08 ± 1.30	30.87 ± 48.64	4.00 ± 6.65	27.56 ± 30.80	28.83 ± 47.88	1.65 ± 0.82	62.43 ± 86.09
12-Jan-11	3.64 ± 0.00	72.73 ± 0.00	11.77 ± 0.00	57.13 ± 0.00	70.29 ± 0.00	11.17 ± 0.00	183.75 ± 0.00
20-Feb-11	0.26 ± 0.07	8.43 ± 0.99	0.47 ± 0.54	5.92 ± 0.49	6.15 ± 2.76	0.64 ± 0.39	14.82 ± 1.05
20-Mar-11	0.19 ± 0.10	5.61 ± 3.89	0.44 ± 0.49	7.13 ± 1.45	3.68 ± 5.36	0.45 ± 0.26	13.18 ± 3.56
16-Apr-11	1.12 ± 1.42	54.95 ± 86.64	1.63 ± 2.45	41.16 ± 53.28	50.64 ± 85.91	7.36 ± 11.12	97.74 ± 142.37
28-May-11	0.45 ± 0.33	35.38 ± 51.22	2.78 ± 4.48	23.23 ± 24.60	31.47 ± 52.78	3.53 ± 4.49	61.39 ± 80.31
12-Jun-11	0.47 ± 0.27	35.51 ± 52.23	3.13 ± 5.08	23.09 ± 23.76	31.90 ± 53.37	4.06 ± 5.35	61.73 ± 81.07
Bryopsis sp							
06-Oct-10	5.99 ± 9.98	41.91 ± 70.83	2.33 ± 3.87	34.26 ± 50.53	40.67 ± 69.42	4.64 ± 7.12	78.49 ± 125.23
12-Jan-11	0.70 ± 0.29	2.24 ± 2.22	0.38 ± 0.46	5.21 ± 1.68	1.84 ± 2.47	0.32 ± 0.44	7.83 ± 1.85
20-Feb-11	4.20 ± 5.10	32.22 ± 51.86	6.12 ± 10.35	27.45 ± 30.80	26.24 ± 43.32	1.16 ± 0.26	65.79 ± 92.97
20-Mar-11	1.60 ± 2.22	45.23 ± 77.20	6.75 ± 11.55	27.25 ± 41.56	28.84 ± 49.24	0.27 ± 0.24	79.23 ± 130.31
16-Apr-11	1.49 ± 2.25	44.45 ± 75.72	6.63 ± 11.30	26.65 ± 40.74	28.54 ± 48.78	0.48 ± 0.21	77.73 ± 127.75

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; EFA: essential fatty acids; BAFA: bacterial fatty acids; HPFA: higher plant fatty acids; TFA: total fatty acids.

Table A3. The monthly variation of fatty acid saturation categories from suspended particulate matter (SPM). Units for quantitative data are in µg FA L^{-1} (mean ± standard deviation). The symbol Σ indicates total abundance for each category.

Month	ΣBAFA	ΣPUFA	ΣMUFA	ΣSFA	ΣEFA	ΣHPFA	TFA
SPM							
12-Jul-10	2.04 ± 1.83	1.52 ± 0.85	3.38 ± 2.43	9.65 ± 3.74	0.26 ± 0.20^{a,b}	0.10 ± 0.09	14.16 ± 5.91^{a}
25-Aug-10	0.80 ± 0.27	4.77 ± 5.61	2.49 ± 0.20	6.87 ± 5.24	0.11 ± 0.03^{a}	0.04 ± 0.04	14.00 ± 11.14^{a,b}
06-Oct-10	1.09 ± 0.19	2.24 ± 0.36	5.58 ± 1.60	6.85 ± 2.00	0.45 ± 0.09^{a,b,c}	0.18 ± 0.03	14.02 ± 3.37
04-Nov-10	2.13 ± 0.92	12.37 ± 18.96	6.60 ± 1.02	13.05 ± 8.90	0.58 ± 0.22^{a,b,c}	0.29 ± 0.08	31.54 ± 26.81^{a}
07-Dec-10	0.74 ± 0.46	7.60 ± 4.24	6.23 ± 2.78	11.01 ± 5.20	4.33 ± 3.54^{e}	0.30 ± 0.18	24.15 ± 11.20^{a}
12-Jan-11	0.08 ± 0.06	1.88 ± 0.60	2.25 ± 0.76	4.32 ± 1.59	1.39 ± 0.34^{d,e}	0.05 ± 0.08	8.45 ± 2.91^{a,b}
20-Feb-11	0.22 ± 0.08	1.67 ± 0.74	3.23 ± 0.44	6.12 ± 0.79	0.85 ± 0.43^{c,d,e}	0.11 ± 0.20	11.03 ± 1.85^{a,b}
20-Mar-11	0.24 ± 0.05	1.10 ± 0.43	4.45 ± 4.14	3.10 ± 0.46	0.73 ± 0.34^{c,d}	0.04 ± 0.04	8.63 ± 3.25^{a,b}
16-Apr-11	0.28 ± 0.03	1.43 ± 0.45	2.19 ± 0.94	4.67 ± 0.86	0.97 ± 0.31^{c,d,e}	0.17 ± 0.07	8.29 ± 2.21^{a,b}
28-May-11	0.33 ± 0.03	2.38 ± 0.09	2.32 ± 0.18	4.26 ± 0.06	1.86 ± 0.08^{d,e}	0.14 ± 0.01	8.96 ± 0.13^{a,b}
12-Jun-11	0.07 ± 0.02	0.45 ± 0.16	0.95 ± 0.34	2.05 ± 0.48	0.33 ± 0.05^{b,c,d}	0.00 ± 0.00	3.45 ± 0.95^{b}

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; EFA: essential fatty acids; BAFA: bacterial fatty acids; HPFA: higher plant fatty acids; TFA: total fatty acids; Mean values marked by the same letters indicates that no statistically significantly differences were present (GLM, Tukey post-hock test, $p < 0.05$); only fatty acid categories presented in figures were checked for significant differences between months.

Table A4. The monthly variation in percentage of total fatty acid saturation categories of the three species of macroalgae. Units are in mean % TFA ± standard deviation. The symbol Σ indicates total abundance for each category.

Month	ΣBAFA	ΣPUFA	ΣMUFA	ΣSFA	ΣEFA	ΣHPFA
Ulva sp						
25-Aug-10	4.66 ± 0.05	53.82 ± 0.15	2.72 ± 0.20	44.90 ± 0.05	45.60 ± 0.14	28.05 ± 0.02
06-Oct-10	11.62 ± 12.61	42.05 ± 18.83	3.01 ± 0.88	56.17 ± 17.61	33.34 ± 20.29	16.88 ± 13.88
04-Nov-10	2.29 ± 1.59	48.13 ± 6.10	10.24 ± 11.77	43.01 ± 6.86	41.67 ± 8.59	25.86 ± 4.17
07-Dec-10	2.22 ± 1.64	48.17 ± 3.19	15.80 ± 22.02	37.03 ± 20.71	38.93 ± 3.17	27.99 ± 5.65
12-Jan-11	2.38 ± 1.64	51.84 ± 0.92	3.13 ± 0.96	46.17 ± 0.08	44.01 ± 4.95	28.24 ± 8.13
20-Feb-11	2.75 ± 1.75	51.05 ± 2.91	3.37 ± 0.98	47.06 ± 2.46	41.75 ± 8.52	26.26 ± 2.91
20-Mar-11	2.62 ± 2.20	51.31 ± 0.49	3.05 ± 1.06	47.17 ± 0.71	42.55 ± 5.38	27.71 ± 6.83
16-Apr-11	2.05 ± 1.17	52.29 ± 1.25	3.55 ± 1.51	45.68 ± 1.00	41.46 ± 3.57	27.53 ± 7.85
28-May-11	2.38 ± 1.27	52.58 ± 1.07	3.71 ± 1.24	45.14 ± 1.09	43.85 ± 3.74	27.97 ± 7.58
12-Jun-11	2.50 ± 1.29	49.23 ± 6.61	6.75 ± 4.67	45.46 ± 0.69	40.59 ± 11.02	24.37 ± 1.54
Gelidium pristoides						
25-Aug-10	3.44 ± 0.02	34.47 ± 0.04	2.59 ± 0.47	62.94 ± 0.43	8.83 ± 0.02	7.14 ± 0.08
06-Oct-10	2.35 ± 1.09	47.99 ± 27.37	4.40 ± 5.65	47.61 ± 33.01	26.13 ± 18.19	5.85 ± 3.33
04-Nov-10	2.52 ± 1.30	44.74 ± 23.25	3.82 ± 3.84	51.45 ± 27.09	32.49 ± 38.03	6.79 ± 1.69
07-Dec-10	2.25 ± 0.57	32.52 ± 18.41	3.25 ± 3.43	64.23 ± 21.85	23.54 ± 24.63	6.74 ± 4.46
12-Jan-11	1.98 ± 0.00	39.58 ± 0.00	6.40 ± 0.00	31.09 ± 0.00	38.25 ± 0.00	6.08 ± 0.00
20-Feb-11	1.77 ± 0.55	56.77 ± 2.55	3.01 ± 3.28	40.22 ± 5.83	40.82 ± 15.18	4.47 ± 2.82
20-Mar-11	1.56 ± 0.95	39.75 ± 17.04	2.92 ± 2.62	57.34 ± 19.66	22.85 ± 30.61	3.77 ± 2.43
16-Apr-11	1.66 ± 0.52	40.87 ± 15.82	1.51 ± 0.34	57.61 ± 15.99	23.53 ± 29.13	6.60 ± 0.96
28-May-11	1.31 ± 0.67	46.20 ± 13.10	2.58 ± 2.24	51.22 ± 15.34	24.43 ± 30.78	6.06 ± 0.36
12-Jun-11	1.56 ± 0.91	44.49 ± 14.90	2.83 ± 2.57	52.68 ± 17.46	24.90 ± 30.58	6.53 ± 0.06
Bryopsis sp						
06-Oct-10	5.02 ± 2.46	29.39 ± 22.57	2.01 ± 0.90	68.60 ± 23.46	24.44 ± 25.74	7.60 ± 1.60
12-Jan-11	9.48 ± 4.42	25.85 ± 22.46	4.36 ± 4.74	69.79 ± 27.16	20.28 ± 26.52	3.79 ± 5.18
20-Feb-11	8.82 ± 2.60	30.29 ± 19.84	4.25 ± 5.36	65.46 ± 25.20	21.46 ± 19.58	6.70 ± 5.24
20-Mar-11	5.81 ± 3.48	30.54 ± 24.21	4.22 ± 3.95	65.24 ± 28.14	19.24 ± 15.64	6.81 ± 5.89
16-Apr-11	3.80 ± 1.74	31.82 ± 23.15	4.80 ± 3.42	63.37 ± 26.56	18.92 ± 16.25	6.46 ± 5.33

SFA: saturated fatty acids MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; EFA: essential fatty acids: BAFA: bacterial fatty acids; HPFA: higher plant fatty acids; TFA: total fatty.

Table A5. The monthly variation in percentage of total fatty acid saturation categories of suspended particulate matter (SPM). Units are in mean % TFA ± standard deviation. The symbol Σ indicates total abundance for each category.

Month	ΣBAFA	ΣPUFA	ΣMUFA	ΣSFA	ΣEFA	ΣHPFA
SPM						
12-Jul-10	14.06 ± 8.95[a]	11.07 ± 5.69	23.44 ± 11.85	68.81 ± 14.28	1.72 ± 0.67[a]	0.65 ± 0.29
25-Aug-10	7.79 ± 2.87[a,b,c]	27.39 ± 12.62	24.69 ± 13.25	49.69 ± 4.04	1.05 ± 0.54[a]	0.53 ± 0.49
06-Oct-10	8.65 ± 0.54[a,b]	16.63 ± 5.11	39.54 ± 1.80	48.49 ± 3.10	3.29 ± 0.96[a]	1.30 ± 0.32
04-Nov-10	10.30 ± 3.82[a,b]	24.07 ± 26.75	32.84 ± 21.14	45.21 ± 7.05	2.43 ± 1.41[a]	1.48 ± 1.02
07-Dec-10	3.87 ± 0.83[b,c]	30.37 ± 8.00	26.19 ± 3.06	45.96 ± 5.42	16.27 ± 5.90[b,c,d]	1.20 ± 0.18
12-Jan-11	1.62 ± 1.41[c]	22.51 ± 3.00	26.69 ± 0.85	50.80 ± 2.32	17.06 ± 3.78[c,d]	0.77 ± 1.33
20-Feb-11	2.70 ± 0.64[b,c]	14.81 ± 4.23	29.41 ± 1.43	55.78 ± 2.82	7.44 ± 2.56[b]	0.89 ± 1.54
20-Mar-11	4.11 ± 0.66[a,b,c]	14.97 ± 8.79	45.21 ± 25.55	40.06 ± 16.36	10.00 ± 6.28[b,c]	0.48 ± 0.65
16-Apr-11	4.52 ± 0.40[a,b,c]	17.05 ± 1.21	25.58 ± 5.30	57.37 ± 6.50	11.69 ± 1.39[b,c,d]	2.31 ± 1.55
28-May-11	4.65 ± 0.25[a,b,c]	26.59 ± 1.38	25.90 ± 1.62	47.51 ± 0.45	20.76 ± 0.81[d]	1.53 ± 0.16
12-Jun-11	3.87 ± 0.85[b,c]	12.80 ± 1.49	27.32 ± 3.37	59.88 ± 3.22	9.75 ± 1.30[b,c,d]	0.00 ± 0.00

SFA: saturated fatty acids MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; EFA: essential fatty acids: BAFA: bacterial fatty acids; HPFA: higher plant fatty acids; TFA: total fatty acids; Mean values marked by the same letters indicates that no statistically significantly differences were present (GLM, Tukey post-hock test, $p < 0.05$); only fatty acid categories presented in figures were checked for significant differences between months.

Table A6. The coefficients of variation (expressed as percentages) of fatty acid saturation categories from three species of macroalgae. Coefficients of variation were calculated using qualitative data. The symbol Σ indicates total abundance for each category.

Month	ΣBAFA	ΣPUFA	ΣMUFA	ΣSFA	ΣEFA	ΣHPFA
Ulva sp.						
25-Aug-10	1	0	7	0	0	0
06-Oct-10	109	45	29	31	61	82
04-Nov-10	69	13	115	16	21	16
07-Dec-10	74	7	139	56	8	20
12-Jan-11	69	2	31	0	11	29
20-Feb-11	63	6	29	5	20	11
20-Mar-11	84	1	35	2	13	25
16-Apr-11	57	2	43	2	9	29
28-May-11	53	2	33	2	9	27
28-Jun-11	52	13	69	2	27	6
Average	**64**	**9**	**51**	**13**	**17**	**27**
Gelidium pristoides						
25-Aug-10	1	0	18	1	0	1
06-Oct-10	46	57	128	69	70	57
04-Nov-10	52	52	101	53	117	25
07-Dec-10	25	57	106	34	105	66
12-Jan-11	0	0	0	0	0	0
20-Feb-11	31	4	109	14	37	63
20-Mar-11	61	43	90	34	134	64
16-Apr-11	32	39	22	28	124	15
28-May-11	51	28	87	30	126	6
28-Jun-11	59	33	91	33	123	1
Average	**33**	**31**	**73**	**29**	**79**	**33**
Bryopsis sp.						
06-Oct-10	49	77	45	34	105	21
12-Jan-11	47	87	109	39	131	137
20-Feb-11	30	66	126	39	91	78
20-Mar-11	60	79	94	43	81	87
16-Apr-11	46	73	71	42	86	83
Average	**46**	**76**	**89**	**39**	**99**	**81**

SFA: saturated fatty acids MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; EFA: essential fatty acids: BAFA: bacterial fatty acids; HPFA: higher plant fatty acids; TFA: total fatty acids.

Table A7. The coefficients of variation (expressed as percentages) of fatty acid saturation categories from suspended particulate matter (SPM). Coefficients of variation were calculated using qualitative data. The symbol Σ indicates total abundance for each category.

Month	ΣBAFA	ΣPUFA	ΣMUFA	ΣSFA	ΣEFA	ΣHPFA
SPM						
12-Jul-10	64	51	51	21	39	45
25-Aug-10	37	46	54	8	51	92
06-Oct-10	6	31	5	6	29	24
04-Nov-10	37	111	64	16	58	69
07-Dec-10	21	26	12	12	36	15
12-Jan-11	87	13	3	5	22	173
20-Feb-11	24	29	5	5	34	173
20-Mar-11	16	59	57	41	63	136
16-Apr-11	9	7	21	11	12	67
28-May-11	5	5	6	1	4	10
28-Jun-11	22	12	12	5	13	0
Average	**31**	**38**	**28**	**13**	**35**	**80**

SFA: saturated fatty acids MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; EFA: essential fatty acids: BAFA: bacterial fatty acids; HPFA: higher plant fatty acids.

Table A8. Fatty acid composition (mean % TFA ± SD) of consumer species. Data represents all fatty acids at concentrations > 1 % TFA in at least one sample; means ± standard deviation (n = 3 to 5).

Fatty acid	*Perna perna*		*Parechinus angulosus*		*Cymbula oculus*	*Gunnarea gaimardi*
	Muscles	Gonads	Muscles	Gonads	Muscles	Whole body
14:0	2.02 ± 1.35	2.98 ± 1.64	7.42 ± 5.32	9.03 ± 4.23	0.86 ± 0.61	4.83 ± 2.05
15:0	0.52 ± 1.38	0.20 ± 0.37	1.85 ± 3.64	1.67 ± 5.03	0.75 ± 0.31	0.94 ± 0.48
16:0	19.49 ± 9.45	18.85 ± 8.49	16.62 ± 10.32	16.04 ± 6.03	14.34 ± 5.84	15.26 ± 7.05
ai-17:0	0.23 ± 0.33	0.78 ± 0.91	0.04 ± 0.22	0.37 ± 0.25	0.07 ± 0.22	0.38 ± 0.73
17:0	0.89 ± 1.69	2.02 ± 1.47	0.64 ± 1.18	0.27 ± 0.19	0.87 ± 0.71	0.94 ± 0.57
18:0	13.86 ± 16.71	12.62 ± 14.29	11.64 ± 13.39	5.37 ± 5.99	6.94 ± 5.22	8.53 ± 4.70
20:0	0.26 ± 0.54	0.11 ± 0.24	4.50 ± 6.13	0.50 ± 0.52	0.94 ± 1.47	4.62 ± 3.10
22:0	0.13 ± 0.26	0.16 ± 0.21	0.01 ± 0.07	0.00 ± 0.01	0.09 ± 0.20	0.07 ± 0.34
∑SFA	37.39 ± 31.72	37.71 ± 27.63	42.72 ± 40.29	33.25 ± 22.25	24.87 ± 14.59	35.57 ± 19.02
16:1ω7	3.86 ± 2.49	5.37 ± 4.13	1.27 ± 2.89	1.67 ± 2.99	0.52 ± 0.36	3.01 ± 1.38
18:1ω9	1.65 ± 1.33	5.24 ± 4.26	2.00 ± 1.71	3.71 ± 3.94	8.37 ± 2.38	6.98 ± 2.27
18:1ω7	1.46 ± 1.63	0.00 ± 0.03	0.01 ± 0.03	0.00 ± 0.00	ND	0.00 ± 0.00
19:1ω9	0.09 ± 0.29	0.00 ± 0.00	0.74 ± 0.75	1.15 ± 0.75	0.19 ± 0.25	0.00 ± 0.00
20:1ω9	0.12 ± 0.40	0.57 ± 0.83	0.06 ± 0.28	0.14 ± 0.55	3.52 ± 1.74	0.34 ± 1.66
20:1ω15	1.56 ± 1.73	0.28 ± 0.71	4.50 ± 6.18	6.60 ± 1.53	3.01 ± 2.18	0.41 ± 1.56
20:1ω7	1.67 ± 1.18	2.00 ± 1.61	1.32 ± 2.73	3.12 ± 1.14	1.29 ± 1.53	3.00 ± 2.67
22:1ω9	2.01 ± 3.88	0.55 ± 1.14	1.68 ± 1.24	2.89 ± 1.15	1.92 ± 1.38	2.24 ± 1.34
22:1ω1	0.45 ± 0.96	0.00 ± 0.00	0.35 ± 0.62	0.00 ± 0.00	1.91 ± 0.96	3.43 ± 2.49
∑MUFA	12.87 ± 13.88	14.03 ± 12.71	11.94 ± 16.42	19.28 ± 12.05	20.73 ± 10.78	19.41 ± 13.36
16:3ω3	0.38 ± 0.58	0.27 ± 0.60	0.75 ± 1.70	0.42 ± 1.40	0.00 ± 0.00	0.00 ± 0.00
16:3ω4	2.63 ± 3.80	0.00 ± 0.00	0.05 ± 0.16	0.00 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
16:2ω6	0.43 ± 1.17	0.13 ± 0.31	0.06 ± 0.37	0.02 ± 0.08	0.43 ± 0.34	0.00 ± 0.00
18:2ω6	1.51 ± 0.63	1.05 ± 0.47	0.23 ± 0.94	0.64 ± 0.38	0.18 ± 0.38	1.61 ± 1.59
18:3ω3	0.78 ± 0.44	0.94 ± 0.54	0.09 ± 0.17	0.76 ± 0.56	0.83 ± 0.54	0.48 ± 0.21
18:4ω3	0.99 ± 0.71	1.70 ± 1.16	0.04 ± 0.17	0.29 ± 0.25	0.69 ± 0.52	0.36 ± 0.35
20:2ω9	2.46 ± 2.08	1.17 ± 1.19	9.33 ± 11.04	0.05 ± 0.15	0.23 ± 0.16	1.06 ± 1.03
20:2ω6	0.76 ± 0.71	0.08 ± 0.41	0.32 ± 1.44	6.66 ± 2.95	0.37 ± 1.05	0.28 ± 0.58
20:2ω7	0.20 ± 0.76	0.19 ± 0.73	0.03 ± 0.18	2.24 ± 0.98	0.05 ± 0.12	0.08 ± 0.50
20:3ω8	0.02 ± 0.08	0.00 ± 0.00	2.59 ± 3.79	0.00 ± 0.00	1.74 ± 1.31	0.43 ± 0.29
20:3ω7	0.00 ± 0.00	0.02 ± 0.08	0.73 ± 2.17	0.00 ± 0.00	0.32 ± 0.27	0.27 ± 0.74
20:3ω6	0.17 ± 0.97	0.05 ± 0.14	0.51 ± 1.32	0.39 ± 0.25	1.26 ± 3.83	0.04 ± 0.09
20:4ω6	4.64 ± 2.69	4.13 ± 2.08	11.59 ± 10.75	12.90 ± 4.50	10.62 ± 5.54	4.01 ± 1.47
20:3ω3	0.03 ± 0.16	0.06 ± 0.41	0.98 ± 0.73	1.07 ± 0.56	3.48 ± 2.07	0.00 ± 0.02
20:4ω3	0.08 ± 0.23	0.46 ± 1.06	0.01 ± 0.05	0.24 ± 0.19	1.02 ± 3.15	0.01 ± 0.03
20:5ω3	10.26 ± 5.86	17.84 ± 9.09	7.82 ± 5.20	14.74 ± 6.40	22.96 ± 6.08	22.25 ± 8.05
22:2ω9	5.09 ± 3.94	3.37 ± 3.06	5.32 ± 8.51	1.64 ± 0.77	0.96 ± 1.13	2.90 ± 1.99
22:4ω6	1.39 ± 0.84	0.92 ± 0.53	0.67 ± 1.18	0.72 ± 0.63	0.59 ± 0.95	1.22 ± 2.00
22:5ω6	0.82 ± 0.73	0.47 ± 0.39	0.04 ± 0.19	0.14 ± 0.30	0.45 ± 1.33	0.44 ± 0.36
22:5ω3	2.41 ± 1.46	2.25 ± 1.12	0.33 ± 0.45	0.81 ± 0.58	5.52 ± 1.46	4.27 ± 1.68
22:6ω3	10.50 ± 5.37	11.39 ± 6.18	0.68 ± 0.94	1.11 ± 1.78	0.30 ± 0.34	2.35 ± 1.07
∑PUFA	45.56 ± 33.22	46.48 ± 29.55	42.18 ± 51.46	44.85 ± 22.72	52.01 ± 30.57	42.04 ± 22.07
∑EFA	25.41 ± 13.04	33.36 ± 15.60	20.09 ± 14.10	28.75 ± 9.45	33.89 ± 8.88	28.61 ± 9.22
BAFA	2.71 ± 4.33	4.24 ± 2.66	3.68 ± 6.48	2.52 ± 4.98	2.17 ± 1.58	3.85 ± 2.40
HPFA	2.30 ± 1.04	1.98 ± 0.93	0.33 ± 0.93	1.40 ± 0.70	1.01 ± 0.73	2.09 ± 1.71
16:1ω5+16:1ω7/16:0	0.40 ± 0.33	0.30 ± 0.21	0.51 ± 1.02	4.30 ± 26.53	0.14 ± 0.20	0.26 ± 0.19
22:6ω3/20:5ω3	1.06 ± 0.41	1.24 ± 2.97	0.09 ± 0.13	0.04 ± 0.04	0.01 ± 0.01	0.72 ± 3.50
TFA	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	99.93 ± 0.54	100.00 ± 0.00

Table A9. The monthly variation of fatty acid saturation categories in the muscle tissues of suspension-feeders. Units are in µg FA mg^{-1}DW ± standard deviation. The symbol Σ indicates total abundance for each category.

Month	ΣBAFA	ΣPUFA	ΣMUFA	ΣSFA	ΣEFA	ΣHPFA	TFA
Perna perna							
12-Jul-10	0.28 ± 0.08	4.51 ± 1.48	1.17 ± 0.45	2.37 ± 0.62	2.56 ± 0.84^{a}	0.18 ± 0.03	7.80 ± 2.34^{a,b}
25-Aug-10	0.45 ± 0.12	0.30 ± 0.07	0.09 ± 0.03	3.92 ± 1.29	0.13 ± 0.04^{b,c}	0.03 ± 0.02	4.29 ± 1.35^{b,c}
06-Oct-10	0.52 ± 0.71	0.09 ± 0.07	0.04 ± 0.07	3.07 ± 2.04	0.06 ± 0.07^{b}	0.04 ± 0.07	3.20 ± 2.16^{c}
04-Nov-10	0.25 ± 0.18	1.99 ± 0.89	0.38 ± 0.65	9.06 ± 2.69	0.42 ± 0.43^{a,c}	0.07 ± 0.05	11.43 ± 3.47^{a}
07-Dec-10	0.08 ± 0.02	4.50 ± 1.15	1.65 ± 0.38	1.39 ± 0.35	2.75 ± 0.78^{a}	0.21 ± 0.07	7.54 ± 1.87^{a,b}
12-Jan-11	0.14 ± 0.02	6.09 ± 1.12	1.45 ± 0.20	2.46 ± 0.39	3.84 ± 0.79^{a}	0.25 ± 0.05	10.01 ± 1.64^{a,b}
20-Feb-11	0.16 ± 0.08	7.75 ± 1.32	1.83 ± 0.43	2.84 ± 0.78	4.47 ± 0.85^{a}	0.35 ± 0.09	12.42 ± 2.37^{a}
20-Mar-11	0.14 ± 0.03	6.90 ± 0.94	1.55 ± 0.26	2.80 ± 0.44	4.45 ± 0.71^{a}	0.33 ± 0.04	11.25 ± 1.60^{a}
16-Apr-11	0.18 ± 0.05	8.31 ± 3.25	1.41 ± 0.77	2.94 ± 1.27	4.57 ± 1.85^{a}	0.42 ± 0.15	12.65 ± 5.24^{a}
28-May-11	0.06 ± 0.01	6.39 ± 0.97	2.58 ± 0.19	2.44 ± 0.28	3.92 ± 0.68^{a}	0.29 ± 0.04	10.07 ± 1.28^{a,b}
12-Jun-11	0.03 ± 0.02	5.58 ± 0.73	2.11 ± 0.17	2.00 ± 0.55	3.00 ± 0.63^{a}	0.24 ± 0.04	8.56 ± 1.20^{a,b}
Gunnarea gaimardi							
12-Jul-10	1.29 ± 0.37	13.93 ± 2.66	5.24 ± 0.65	7.43 ± 2.01	9.53 ± 2.30^{a}	1.58 ± 0.37	25.74 ± 5.27^{a,b}
25-Aug-10	0.51 ± 0.17	6.18 ± 1.91	1.96 ± 0.55	3.40 ± 1.21	3.86 ± 1.21^{c}	0.58 ± 0.21	11.28 ± 3.60^{d}
06-Oct-10	0.42 ± 0.00	1.40 ± 0.00	1.03 ± 0.00	2.48 ± 0.00	0.80 ± 0.00^{b}	0.03 ± 0.00	4.75 ± 0.00^{c,d}
04-Nov-10	0.40 ± 0.12	0.65 ± 0.63	0.56 ± 0.44	3.19 ± 0.55	0.39 ± 0.47^{b}	0.02 ± 0.04	4.36 ± 1.42^{c}
07-Dec-10	1.00 ± 0.11	15.81 ± 1.46	9.52 ± 1.07	11.46 ± 1.22	10.84 ± 1.11^{a}	0.50 ± 0.10	32.99 ± 3.39^{b}
12-Jan-11	0.52 ± 0.14	12.51 ± 3.38	6.77 ± 1.69	7.91 ± 2.03	8.16 ± 2.15^{a}	0.35 ± 0.09	23.95 ± 6.25^{a,b}
20-Feb-11	0.56 ± 0.06	16.20 ± 1.65	7.80 ± 0.74	9.34 ± 0.93	10.79 ± 1.08^{a}	0.40 ± 0.04	29.47 ± 2.94^{a,b}
20-Mar-11	0.54 ± 0.10	13.70 ± 2.43	7.20 ± 1.52	8.05 ± 1.62	8.80 ± 1.67^{a}	0.31 ± 0.08	25.37 ± 5.02^{a,b}
16-Apr-11	0.62 ± 0.11	14.10 ± 2.45	7.18 ± 1.88	8.74 ± 1.86	9.19 ± 1.81^{a}	0.39 ± 0.09	26.54 ± 5.3^{a,b}
28-May-11	0.56 ± 0.08	11.96 ± 2.22	6.66 ± 1.05	7.14 ± 1.17	7.98 ± 1.46^{a}	0.28 ± 0.03	21.60 ± 3.63^{a}
12-Jun-11	0.72 ± 0.16	12.62 ± 1.42	7.36 ± 1.11	7.83 ± 1.21	8.37 ± 1.02^{a}	0.43 ± 0.11	23.54 ± 3.31^{a,b}

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; EFA: essential fatty acids; BAFA: bacterial fatty acids; HPFA: higher plant fatty acids; TFA: total fatty acids; Mean values marked by the same letters indicates that no statistically significantly differences were present (GLM, Tukey post-hock test, $p < 0.05$); only fatty acid categories presented in figures were checked for significant differences between months.

Table A10. The monthly variation of fatty acid saturation categories in the muscle tissues of grazers. Units are in µg FA mg^{-1}DW ± standard deviation. The symbol Σ indicates total abundance for each category.

Month	ΣBAFA	ΣPUFA	ΣMUFA	ΣSFA	ΣEFA	ΣHPFA	TFA
Parechinus angulosus							
12-Jul-10	0.34 ± 0.44	1.58 ± 1.89	0.44 ± 0.25	8.54 ± 11.80	0.35 ± 0.25^{a,b,c}	0.09 ± 0.10	10.21 ± 13.56^{a,b}
25-Aug-10	0.39 ± 0.64	0.51 ± 0.65	0.32 ± 0.37	1.71 ± 1.39	0.27 ± 0.40^{b,c}	0.00 ± 0.00	2.47 ± 1.29^{a}
06-Oct-10	0.25 ± 0.10	0.38 ± 0.25	0.10 ± 0.17	1.62 ± 0.50	0.07 ± 0.10^{b}	0.02 ± 0.04	2.12 ± 0.38^{a}
04-Nov-10	0.23 ± 0.14	0.08 ± 0.02	0.01 ± 0.01	2.22 ± 0.24	0.00 ± 0.00^{b}	0.00 ± 0.00	2.30 ± 0.24^{a}
07-Dec-10	0.38 ± 0.09	28.29 ± 16.07	2.66 ± 1.80	1.18 ± 0.20	2.85 ± 1.06^{d}	0.00 ± 0.00	31.45 ± 16.83^{c}
12-Jan-11	2.67 ± 3.69	14.60 ± 17.50	4.20 ± 3.95	12.33 ± 15.62	3.62 ± 4.19^{a,d}	0.01 ± 0.01	31.05 ± 37.17^{b,c}
20-Feb-11	0.19 ± 0.21	3.54 ± 1.36	1.33 ± 0.48	2.17 ± 0.76	2.13 ± 0.81^{a,d}	0.02 ± 0.02	6.14 ± 2.28^{a,b}
20-Mar-11	0.08 ± 0.04	4.40 ± 2.60	1.88 ± 0.77	2.84 ± 1.27	2.72 ± 1.76^{d}	0.01 ± 0.01	7.99 ± 4.18^{a,b}
16-Apr-11	0.06 ± 0.01	2.66 ± 1.03	1.35 ± 0.25	2.13 ± 0.43	0.72 ± 0.09^{a,d}	0.02 ± 0.01	5.28 ± 1.36^{a,b}
28-May-11	0.05 ± 0.01	4.18 ± 0.70	1.47 ± 0.25	1.45 ± 0.21	2.38 ± 0.41^{d}	0.00 ± 0.00	6.86 ± 1.11^{a,b}
12-Jun-11	0.06 ± 0.01	4.15 ± 1.05	1.39 ± 0.34	1.39 ± 0.29	2.27 ± 0.61^{a,d}	0.00 ± 0.01	6.73 ± 1.63a,b
Cymbula oculus							
12-Jul-10	0.49 ± 0.13	8.25 ± 3.12	2.75 ± 0.81	3.34 ± 0.84	5.27 ± 2.20^{a}	0.36 ± 0.07	41.43 ± 19.66^{a}
25-Aug-10	0.35 ± 0.04	5.91 ± 3.20	2.65 ± 1.07	3.11 ± 0.32	4.14 ± 2.36^{a}	0.07 ± 0.10	27.48 ± 6.70^{a,d}
06-Oct-10	0.43 ± 0.07	9.72 ± 1.17	3.95 ± 0.46	4.10 ± 0.46	5.45 ± 1.19^{a,b}	0.06 ± 0.06	6.08 ± 5.19^{a,b,c}
04-Nov-10	0.32 ± 0.21	1.93 ± 1.49	0.82 ± 0.54	3.01 ± 2.05	0.77 ± 0.82^{c}	0.04 ± 0.02	21.81 ± 5.67^{d}
07-Dec-10	0.38 ± 0.08	13.71 ± 3.19	5.36 ± 1.33	5.18 ± 1.52	9.18 ± 2.48^{b}	0.15 ± 0.02	34.96 ± 5.99^{c}
12-Jan-11	0.34 ± 0.06	12.67 ± 0.68	5.31 ± 0.17	4.39 ± 0.23	7.48 ± 1.87^{a,b}	0.13 ± 0.09	27.86 ± 4.84^{b,c}
20-Feb-11	0.24 ± 0.04	9.39 ± 1.79	3.90 ± 0.53	3.88 ± 0.66	6.63 ± 1.30^{a,b}	0.24 ± 0.08	36.32 ± 3.88^{a,b}
20-Mar-11	0.30 ± 0.02	10.21 ± 1.44	4.04 ± 0.49	4.24 ± 0.64	6.27 ± 1.51^{a,b}	0.09 ± 0.06	30.76 ± 2.02^{a,b,c}
16-Apr-11	0.34 ± 0.04	10.55 ± 1.48	4.37 ± 0.48	4.28 ± 0.80	7.20 ± 0.96^{a,b}	0.14 ± 0.05	35.18 ± 6.46^{a,b,c}
28-May-11	0.16 ± 0.03	10.84 ± 2.03	4.40 ± 1.11	3.99 ± 0.86	6.95 ± 1.38^{a,b}	0.20 ± 0.06	89.76 ± 17.56^{a,b,c}
12-Jun-11	0.15 ± 0.04	9.64 ± 0.75	4.14 ± 0.35	3.41 ± 0.35	6.36 ± 0.59^{a,b}	0.21 ± 0.03	71.17 ± 4.74^{a,b,c}

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; EFA: essential fatty acids; BAFA: bacterial fatty acids; HPFA: higher plant fatty acids; TFA: total fatty acids; Mean values marked by the same letters indicates that no statistically significantly differences were present (GLM, Tukey post-hock test, $p < 0.05$); only fatty acid categories presented in figures were checked for significant differences between months.

Table A11. The monthly variation in percentage of total fatty acids saturation categories in the muscle tissues of suspension-feeder. Units in mean % TFA ± standard deviation. The symbol Σ indicates total abundance for each category.

Month	ΣBAFA	ΣPUFA	ΣMUFA	ΣSFA	ΣEFA	ΣHPFA
Perna perna						
12-Jul-10	3.63 ± 0.23^{a}	57.57 ± 2.26^{a}	14.70 ± 2.01^{a,b,c}	30.63 ± 1.23^{a}	32.61 ± 2.04^{a}	2.38 ± 0.57^{a}
25-Aug-10	10.42 ± 0.58^{b}	7.30 ± 1.93^{c}	1.96 ± 0.31^{d}	91.03 ± 1.86^{e}	3.56 ± 2.38^{b}	0.55 ± 0.48^{b}
06-Oct-10	12.23 ± 6.87^{b}	2.88 ± 2.20^{b}	0.72 ± 1.07^{d}	96.41 ± 2.60^{e}	1.41 ± 1.33^{c}	0.60 ± 1.02^{b}
04-Nov-10	1.76 ± 0.91^{a,c}	17.03 ± 3.74^{d}	3.04 ± 4.57^{d}	79.94 ± 8.22^{b}	3.48 ± 2.84^{b}	0.54 ± 0.28^{b}
07-Dec-10	0.54 ± 0.06^{c,d}	59.57 ± 1.39^{a}	22.04 ± 0.70^{a,b}	18.39 ± 0.85^{d}	36.11 ± 2.12^{a}	2.78 ± 0.26a
12-Jan-11	0.91 ± 0.20^{c}	60.74 ± 2.17^{a}	14.60 ± 1.24^{a,b,c}	24.66 ± 1.01^{c}	38.13 ± 2.21^{a}	2.51 ± 0.16^{a}
20-Feb-11	0.98 ± 0.40^{c}	62.57 ± 3.53^{a}	14.70 ± 1.12^{a,b,c}	22.73 ± 2.67^{c}	36.08 ± 3.44^{a}	2.81 ± 0.29^{a}
20-Mar-11	0.69 ± 0.10^{c,d}	61.37 ± 1.45^{a}	13.75 ± 0.79^{a,c}	24.88 ± 1.04^{c}	39.53 ± 1.57^{a}	2.95 ± 0.11^{a}
16-Apr-11	1.08 ± 0.15^{c}	66.03 ± 2.24^{a}	10.91 ± 1.51^{c}	23.13 ± 1.30^{c}	36.33 ± 3.72^{a}	3.38 ± 0.24^{a}
28-May-11	0.27 ± 0.18^{c,d}	63.33 ± 2.27^{a}	25.85 ± 2.53^{a,b}	24.32 ± 1.25^{c}	38.73 ± 2.50^{a}	2.83 ± 0.15^{a}
12-Jun-11	0.04 ± 0.08^{d}	65.30 ± 3.48^{a}	24.86 ± 2.67^{b}	23.12 ± 4.26^{c,d}	35.00 ± 4.38^{a}	2.85 ± 0.28^{a}
Gunnarea gaimardi						
12-Jul-10	4.95 ± 0.57^{a}	54.27 ± 2.13^{a}	20.61 ± 1.90^{a,b}	28.58 ± 2.58^{a}	36.82 ± 1.63^{a,b}	6.09 ± 0.30^{a}
25-Aug-10	4.47 ± 0.22^{a}	54.99 ± 1.84^{a}	17.53 ± 1.18^{b}	29.76 ± 1.87^{a,c}	34.30 ± 1.43^{a,b}	5.09 ± 0.36^{a}
06-Oct-10	8.84 ± 0.00^{b}	29.40 ± 0.00^{c}	21.76 ± 0.00^{a,b,e}	52.18 ± 0.00^{b,d}	16.82 ± 0.00^{b}	0.61 ± 0.00^{c}
04-Nov-10	9.27 ± 1.61^{b}	12.80 ± 9.17^{b}	11.61 ± 5.98^{c}	76.34 ± 14.80^{b}	7.31 ± 7.39^{c}	0.30 ± 0.60^{b}
07-Dec-10	3.03 ± 0.09^{d}	48.00 ± 1.59^{a}	28.85 ± 0.61^{d,e}	34.73 ± 0.81^{c,d}	32.86 ± 0.97^{a,b}	1.51 ± 0.17^{c,d}
12-Jan-11	2.19 ± 0.09^{e,f}	52.12 ± 1.07^{a}	28.36 ± 0.65^{d,e}	33.09 ± 0.83^{c,d}	34.07 ± 0.71^{a}	1.49 ± 0.20^{c,d}
20-Feb-11	1.90 ± 0.05^{c}	54.98 ± 0.29^{a}	26.49 ± 0.90^{a,d,e}	31.69 ± 0.43^{c}	36.61 ± 0.83^{a}	1.36 ± 0.08^{c,d}
20-Mar-11	2.13 ± 0.08^{e}	54.19 ± 1.31^{a}	28.31 ± 0.76^{d,e}	31.73 ± 0.35^{c}	34.74 ± 0.63^{a}	1.22 ± 0.07^{c,d}
16-Apr-11	2.32 ± 0.05^{f}	53.31 ± 1.58^{a}	26.84 ± 2.50^{a,d,e}	32.89 ± 0.83^{c,d}	34.75 ± 3.39^{a}	1.48 ± 0.06^{c,d}
28-May-11	2.62 ± 0.11^{d}	55.25 ± 1.12^{a}	30.91 ± 0.92^{d}	33.06 ± 0.43^{c,d}	36.89 ± 0.95^{a}	1.30 ± 0.12^{c,d}
12-Jun-11	3.02 ± 0.26^{d}	53.73 ± 1.57^{a}	31.24 ± 0.34^{d}	33.22 ± 0.49^{c,d}	35.60 ± 0.73^{a}	1.81 ± 0.23^{d}

SFA: saturated fatty acids MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; EFA: essential fatty acids: BAFA: bacterial fatty acids; HPFA: higher plant fatty acids; TFA: total fatty acids; Mean values marked by the same letters indicates that no statistically significantly differences were present (GLM, Tukey post-hock test, $p < 0.05$); only fatty acid categories presented in figures were checked for significant differences between months.

Table A12. The monthly variation in percentage of total fatty acids saturation categories in the muscle tissues of grazers. Units in mean % TFA ± standard deviation. The symbol Σ indicates total abundance for each category.

Month	ΣBAFA	ΣPUFA	ΣMUFA	ΣSFA	ΣEFA	ΣHPFA
Parechinus angulosus						
12-Jul-10	4.27 ± 1.38^{a,b,c,d}	26.79 ± 17.02^{a,c}	23.03 ± 28.14^{a,b}	57.85 ± 38.74^{a,b,c}	15.29 ± 17.86^{a,b,c}	1.92 ± 1.58^{a}
25-Aug-10	11.44 ± 16.14^{b,c,d}	20.77 ± 19.87^{c,d}	15.19 ± 15.75^{a,b}	67.38 ± 32.94^{b,c}	10.78 ± 12.21^{b,c}	0.00 ± 0.00^{b}
06-Oct-10	10.64 ± 5.77^{d}	18.50 ± 12.59^{c,d}	4.49 ± 6.69^{b,c}	76.29 ± 17.11^{c}	2.98 ± 4.07^{c,d}	1.27 ± 2.49^{a,b}
04-Nov-10	8.91 ± 4.98^{c,d}	3.40 ± 0.85^{d}	0.22 ± 0.45^{c}	96.60 ± 0.85^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}
07-Dec-10	0.28 ± 0.49^{a}	88.89 ± 3.75^{b}	8.23 ± 2.34^{a,b,c}	4.22 ± 1.33^{d}	9.55 ± 1.43^{b}	0.00 ± 0.00^{a,b}
12-Jan-11	3.89 ± 4.96^{a,b,c,d}	46.88 ± 0.23^{a,b,c}	20.86 ± 12.26^{a,b}	33.92 ± 9.68^{a,b,e}	12.68 ± 1.69^{a,b}	0.19 ± 0.27^{a,b}
20-Feb-11	1.97 ± 2.70^{a,b,c}	57.35 ± 0.83^{a,b}	21.76 ± 0.42^{a}	35.54 ± 1.49^{a,e}	34.63 ± 0.40^{a}	0.29 ± 0.27^{a,b}
20-Mar-11	0.20 ± 0.11^{a}	53.58 ± 5.87^{a,b}	24.69 ± 5.08^{a}	36.87 ± 4.26^{a,b,e}	32.04 ± 8.83^{a}	0.18 ± 0.17^{a,b}
16-Apr-11	0.59 ± 0.65^{a,b}	51.35 ± 7.67^{a,b}	25.59 ± 4.17^{a}	37.49 ± 8.70^{a,b,e}	21.34 ± 7.25^{a,b}	0.36 ± 0.32^{a,b}
28-May-11	0.19 ± 0.39^{a}	60.74 ± 0.62^{a,b}	21.31 ± 0.33^{a}	21.37 ± 0.59^{e}	34.48 ± 2.09^{a}	0.02 ± 0.05^{b}
12-Jun-11	0.22 ± 0.03^{a,b,c,d}	61.51 ± 1.34^{a,b}	20.65 ± 0.57^{a}	20.83 ± 1.31^{e}	33.57 ± 0.96^{a}	0.03 ± 0.06^{b}
Cymbula oculus						
12-Jul-10	3.31 ± 0.40^{a,b}	54.76 ± 9.54^{a}	18.32 ± 1.25^{a,b}	22.48 ± 0.98^{a}	34.95 ± 8.35^{a}	2.55 ± 0.83^{a}
25-Aug-10	3.27 ± 0.95^{a,b,d}	50.06 ± 8.69^{a,b}	23.07 ± 0.48^{a,b}	28.78 ± 8.30^{a,b}	34.78 ± 7.24^{a}	0.88 ± 1.24^{b,c}
06-Oct-10	2.46 ± 0.25^{a,d}	55.94 ± 1.09^{a}	22.78 ± 1.4^{a}	23.62 ± 0.24^{a}	31.73 ± 7.75^{a}	0.31 ± 0.32^{c}
04-Nov-10	5.75 ± 3.68^{b}	34.20 ± 26.12^{b}	14.86 ± 9.36^{b}	54.33 ± 36.07^{b}	13.65 ± 14.48^{b}	0.75 ± 0.41^{b,c}
07-Dec-10	1.67 ± 0.24^{c,d}	59.45 ± 0.87^{a}	23.18 ± 1.33^{a}	22.28 ± 1.29^{a}	39.54 ± 1.38^{a}	0.66 ± 0.17^{b,c}
12-Jan-11	1.62 ± 0.31^{c,d}	59.67 ± 1.01^{a}	25.07 ± 1.65^{a}	20.66 ± 0.49^{a}	35.21 ± 8.37^{a}	0.60 ± 0.44^{b,c}
20-Feb-11	1.54 ± 0.12^{c,d}	59.34 ± 0.85^{a}	24.87 ± 1.61^{a}	24.59 ± 1.09^{a}	41.84 ± 1.40^{a}	1.54 ± 0.33^{b}
20-Mar-11	1.78 ± 0.29^{a,c,d}	59.22 ± 1.04^{a}	23.49 ± 1.22^{a}	24.66 ± 2.05^{a}	36.18 ± 5.63^{a}	0.54 ± 0.31^{b,c}
16-Apr-11	1.86 ± 0.12^{a,c,d}	57.87 ± 1.39^{a}	24.07 ± 1.48^{a}	23.37 ± 1.66^{a}	39.55 ± 1.04^{a}	0.81 ± 0.34^{b,c}
28-May-11	0.91 ± 0.07^{c}	59.62 ± 1.47^{a}	24.04 ± 1.45^{a}	21.86 ± 0.58^{a}	38.18 ± 1.20^{a}	1.09 ± 0.28^{a,b}
12-Jun-11	0.90 ± 0.19^{c}	59.48 ± 0.62^{a}	25.60 ± 1.85^{a}	21.00 ± 0.73^{a}	39.18 ± 1.04^{a}	1.27 ± 0.22^{a,b}

SFA: saturated fatty acids MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; EFA: essential fatty acids: BAFA: bacterial fatty acids; HPFA: higher plant fatty acids; TFA: total fatty acids; Mean values marked by the same letters indicates that no statistically significantly differences were present (GLM, Tukey post-hock test, $p < 0.05$); only fatty acid categories presented in figures were checked for significant differences between months.

Table A13. The coefficients of variation (expressed as percentages) of fatty acid saturation categories from different suspension-feeders. Coefficients of variation were calculated using qualitative data. The symbol Σ indicates total abundance for each category.

Month	ΣBAFA	ΣPUFA	ΣMUFA	ΣSFA	ΣEFA	ΣHPFA
Perna perna						
12-Jul-10	6	4	14	4	6	24
25-Aug-10	6	26	16	2	6	88
06-Oct-10	56	76	149	3	56	169
04-Nov-10	52	22	151	10	52	53
07-Dec-10	11	2	3	5	11	9
12-Jan-11	22	4	9	4	22	6
20-Feb-11	40	6	8	12	40	10
20-Mar-11	14	2	6	4	14	4
16-Apr-11	14	3	14	6	14	7
28-May-11	67	4	10	5	67	5
28-Jun-11	224	5	11	18	224	10
Average	**29**	**15**	**38**	**5**	**29**	**38**
Gunnarea gaimardi						
12-Jul-10	12	4	9	9	4	5
25-Aug-10	5	3	7	6	4	7
06-Oct-10	0	0	0	0	0	0
04-Nov-10	17	72	51	19	101	200
07-Dec-10	3	3	2	2	3	12
12-Jan-11	4	2	2	3	2	14
20-Feb-11	3	1	3	1	2	6
20-Mar-11	4	2	3	1	2	6
16-Apr-11	2	3	9	3	10	4
28-May-11	4	2	3	1	3	9
28-Jun-11	9	3	1	1	2	12
Average	**5**	**9**	**9**	**5**	**13**	**26**

SFA: saturated fatty acids MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; EFA: essential fatty acids: BAFA: bacterial fatty acids; HPFA: higher plant fatty acids.

Table A14. The coefficients of variation (expressed as percentages) of fatty acid saturation categories from different grazers. Coefficients of variation were calculated using qualitative data. The symbol Σ indicates total abundance for each category.

Month	ΣBAFA	ΣPUFA	ΣMUFA	ΣSFA	ΣEFA	ΣHPFA
Parechinus angulosus						
12-Jul-10	32	64	122	6	117	82
25-Aug-10	141	96	104	6	113	0
06-Oct-10	54	68	149	56	136	195
04-Nov-10	56	25	200	52	0	0
07-Dec-10	173	4	28	11	15	0
12-Jan-11	128	0	59	22	13	141
20-Feb-11	137	1	2	40	1	93
20-Mar-11	57	11	21	14	28	93
16-Apr-11	110	15	16	14	34	91
28-May-11	200	1	2	67	6	200
28-Jun-11	13	2	3	224	3	224
Average	**109**	**29**	**70**	**29**	**46**	**90**
Cymbula oculus						
12-Jul-10	12	17	7	4	24	33
25-Aug-10	29	17	2	29	21	141
06-Oct-10	10	2	6	1	24	103
04-Nov-10	64	76	63	66	106	54
07-Dec-10	14	1	6	6	3	25
12-Jan-11	19	2	7	2	24	73
20-Feb-11	7	1	6	4	3	21
20-Mar-11	16	2	5	8	16	58
16-Apr-11	6	2	6	7	3	42
28-May-11	8	2	6	3	3	26
28-Jun-11	21	1	7	3	3	18
Average	**19**	**12**	**11**	**13**	**23**	**58**

SFA: saturated fatty acids MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; EFA: essential fatty acids: BAFA: bacterial fatty acids; HPFA: higher plant fatty acids.

Table A15. Monthly changes in proportions (% TFA) of ratio marker (16:1ω7+16:1ω5)/16:0 and ratio marker (22:6ω3/20:5ω3) in muscle tissues of suspension-feeders (*Gunnarea gaimardi* and *Perna perna*) and grazers (*Parechinus angulosus* and *Cymbula oculus*).

Month	(16:1ω5+16:1ω7) /16:0	22:6ω3/20:5ω3	(16:1ω5+16:1ω7) /16:0	22:6ω3/20:5ω3
Perna perna			*Parechinus angulosus*	
12-Jul-10	0.33 ± 0.12^{a,b,c}	1.35 ± 0.23^{a}	0.06 ± 0.08^{a}	0.00 ± 0.00^{a,b,c}
25-Aug-10	0.04 ± 0.06^{d}	0.00 ± 0.00^{b}	0.01 ± 0.01^{a,b}	0.01 ± 0.01^{a,b,c}
06-Oct-10	0.01 ± 0.02^{d}	0.00 ± 0.00^{b}	0.00 ± 0.00^{a,b}	0.00 ± 0.00^{b,c}
04-Nov-10	0.19 ± 0.33^{d,e}	0.50 ± 0.72^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}
07-Dec-10	0.49 ± 0.12^{a,b}	0.88 ± 0.05^{a}	0.68 ± 0.69^{a,b}	0.00 ± 0.00^{a}
12-Jan-11	0.61 ± 0.08^{b}	0.83 ± 0.04^{a}	2.30 ± 3.08^{a,b}	0.23 ± 0.33^{a,b,c}
20-Feb-11	0.85 ± 0.28^{a,b}	1.15 ± 0.79^{a}	0.09 ± 0.03^{a,b}	0.00 ± 0.01^{a,b,c}
20-Mar-11	0.63 ± 0.12^{a,b,c}	0.81 ± 0.10^{a}	0.12 ± 0.04^{a,b}	0.03 ± 0.02^{a,b}
16-Apr-11	0.49 ± 0.36^{a,b,c}	1.11 ± 0.19^{a}	0.08 ± 0.05^{a,b}	0.03 ± 0.02^{a,b}
28-May-11	0.34 ± 0.05^{a,c}	1.20 ± 0.07^{a}	0.04 ± 0.02^{a,b}	0.29 ± 0.05^{a,b,c}
12-Jun-11	0.29 ± 0.09^{c,e}	1.37 ± 0.26^{a}	0.04 ± 0.02^{a,b}	0.22 ± 0.05^{a,b,c}
Gunnarea gaimardi			*Cymbula oculus*	
12-Jul-10	0.77 ± 0.20^{a,b}	0.15 ± 0.01	0.04 ± 0.02	0.00 ± 0.00
25-Aug-10	0.35 ± 0.15^{a,b}	0.14 ± 0.01	0.12 ± 0.04	0.00 ± 0.00
06-Oct-10	0.01 ± 0.00^{c}	0.22 ± 0.00	0.16 ± 0.15	0.01 ± 0.01
04-Nov-10	0.11 ± 0.07^{a,b}	0.13 ± 0.02	0.03 ± 0.02	0.00 ± 0.00
07-Dec-10	2.09 ± 0.40^{d}	0.07 ± 0.05	0.12 ± 0.05	0.02 ± 0.02
12-Jan-11	0.71 ± 0.19^{a,b}	0.08 ± 0.03	0.13 ± 0.03	0.02 ± 0.01
20-Feb-11	1.02 ± 0.08^{b}	0.05 ± 0.01	0.11 ± 0.03	0.03 ± 0.01
20-Mar-11	0.55 ± 0.18^{a}	0.09 ± 0.02	0.07 ± 0.04	0.01 ± 0.01
16-Apr-11	0.82 ± 0.35^{a,b}	0.10 ± 0.06	0.10 ± 0.03	0.02 ± 0.00
28-May-11	0.46 ± 0.06^{a}	0.12 ± 0.02	0.07 ± 0.02	0.00 ± 0.00
12-Jun-11	0.67 ± 0.22^{a,b}	0.09 ± 0.00	0.08 ± 0.04	0.01 ± 0.01

Mean values marked by the same letters indicates that no statistically significantly differences were present (GLM, Tukey post-hock test, $p < 0.05$); only fatty acid categories presented in figures were checked for significant differences between months.

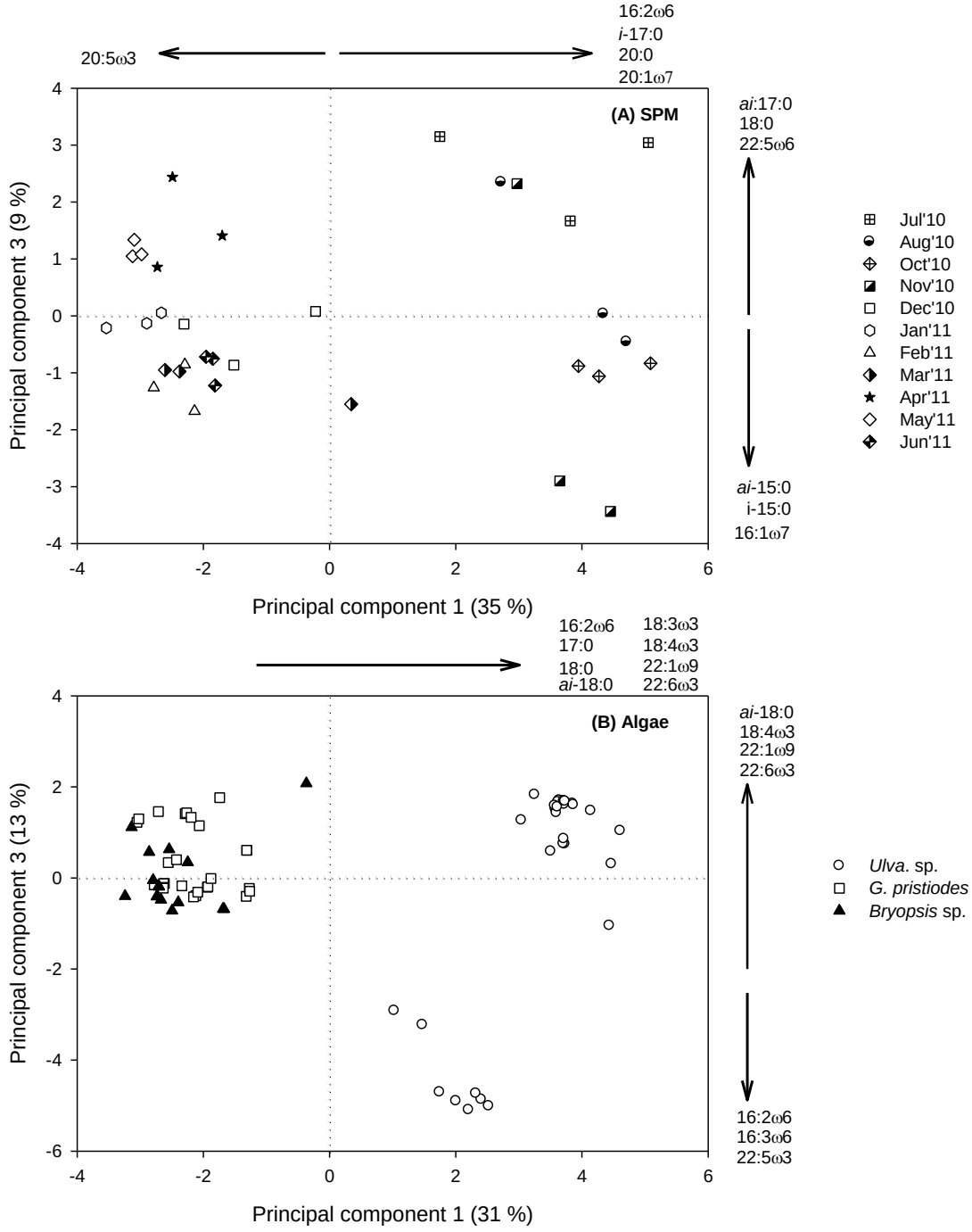

Figure A1. Principal component analysis (PCA) of arcsin square root-transformed proportional fatty acid data (% TFA) of (A) suspended particulate matter (SPM) and (B) macroalgae. Fatty acid >1% TFA were included in the analyses (30 fatty acid in total). Percentage values in PC (principal component) labels indicate the proportion of variation accounted for by each PC. Arrows running parallel to each axis denote the influence of the specific fatty acids that have loading values >2.5%. The cross-section of the dotted lines represents the origin.

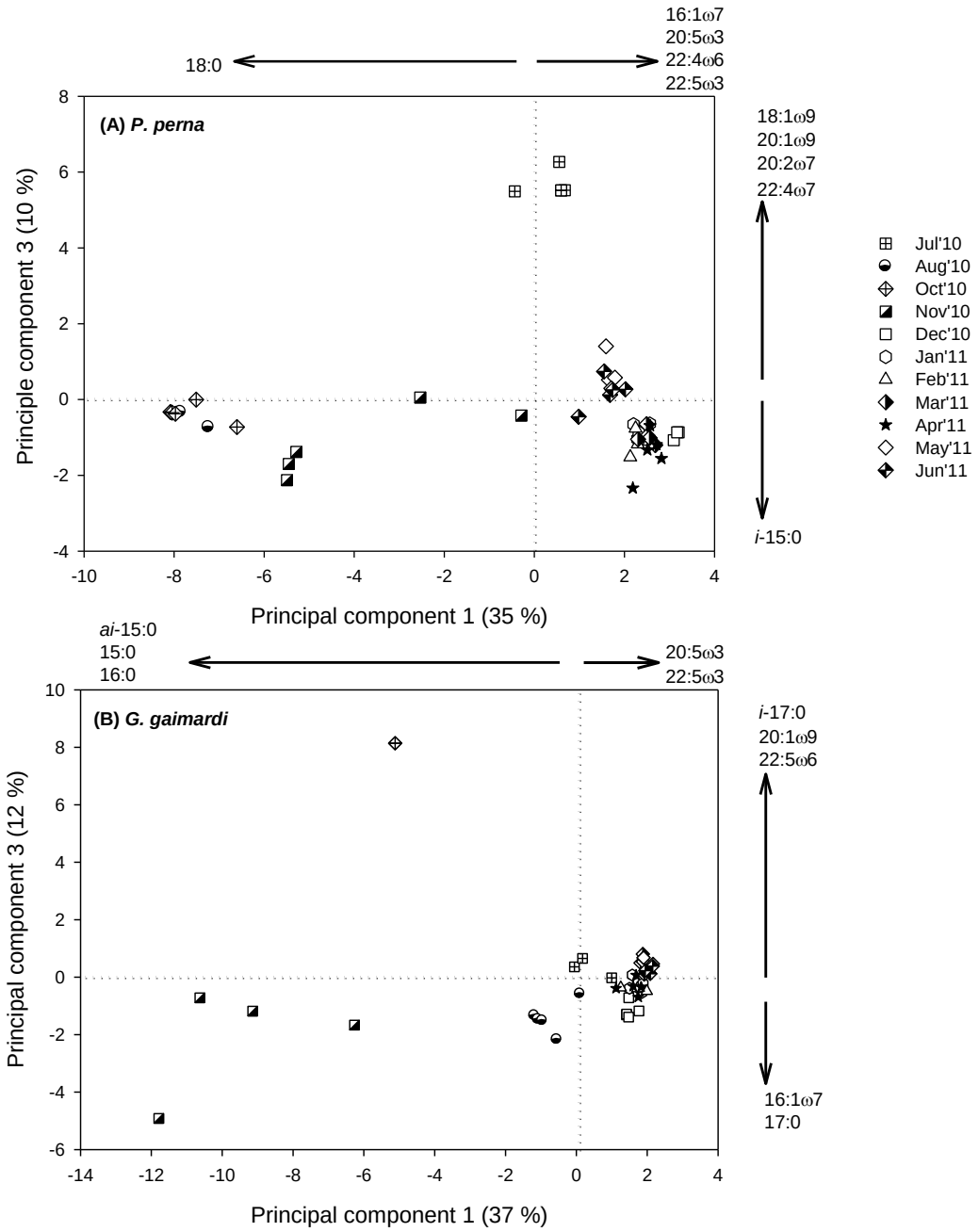

Figure A2. Principal component analysis (PCA) of arcsin square root-transformed proportional fatty acid data (% TFA) from the muscle tissues of (A) *Perna perna* and (B) *Gunnarea gaimardi*. Fatty acid >1% TFA were included in the analyses (38 fatty acid in total). Percentage values in PC (principal component) labels indicate the proportion of variation accounted for by each PC. Arrows running parallel to each axis denote the influence of the specific fatty acids that have loading values >2.5%. The cross-section of the dotted lines represents the origin.

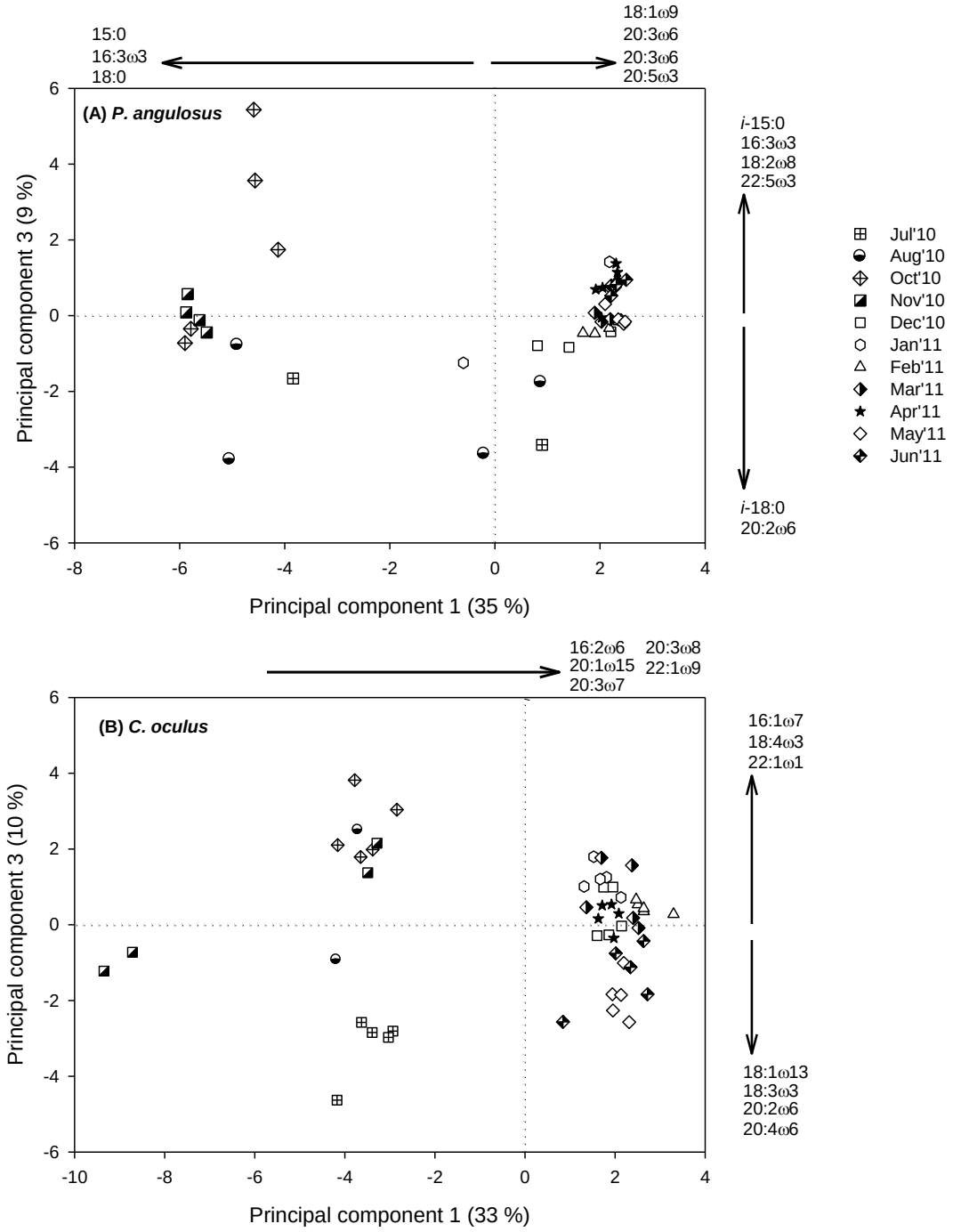

Figure A3. Principal component analysis (PCA) of arcsin square root-transformed proportional fatty acid data (% TFA) from the muscle tissues of (A) *Parechinus angulosus* and (B) *Cymbula oculus*. Fatty acid >1% TFA were included in the analyses (38 fatty acid in total). Percentage values in PC (principal component) labels indicate the proportion of variation accounted for by each PC. Arrows running parallel to each axis denote the influence of the specific fatty acids that have loading values >2.5%. The cross-section of the dotted lines represents the origin.

TABLES AND FIGURES FOR REPRODUCTION CHAPTER 4

Table A16. Monthly variation in fatty acid categories of *Perna perna*. Units are in µg FA mg^{-1}DW gonad tissues (mean ± standard deviation). The symbol Σ indicates total abundance for each category.

Month	ΣPUFA	ΣMUFA	ΣSFA	ΣEFA	TFA
Perna perna					
Female					
12-Jul-10	$16.21 \pm 22.57^{a,b,c,d}$	$11.60 \pm 16.40^{a,b,c}$	$22.86 \pm 16.96^{a,b,c}$	$9.80 \pm 13.49^{a,b}$	$49.96 \pm 54.92^{a,b,c,d}$
25-Aug-10	nd	nd	nd	nd	nd
06-Oct-10	$32.38 \pm 8.42^{c,d,e}$	$7.82 \pm 6.19^{a,b,c}$	$16.61 \pm 13.54^{a,b,c}$	$22.60 \pm 8.65^{a,b}$	$1.49 \pm 28.15^{a,b,c,d}$
04-Nov-10	0.83 ± 0.23^{a}	0.62 ± 0.31^{a}	$9.21 \pm 5.55^{a,b,c}$	$0.00 \pm 0.00^{a,b}$	10.37 ± 5.93^{a}
07-Dec-10	$13.76 \pm 2.79^{a,b,c,d,e}$	$5.42 \pm 1.89^{a,b,c}$	$8.13 \pm 2.34^{a,b,c}$	$11.03 \pm 1.86^{a,b}$	$27.32 \pm 7.02^{a,b,c}$
12-Jan-11	$11.77 \pm 2.75^{a,b,c,b,e}$	$6.78 \pm 4.61^{a,b,c}$	$10.73 \pm 6.19^{a,b,c}$	$8.38 \pm 1.34^{a,b}$	$29.29 \pm 13.53^{a,b,c}$
20-Feb-11	$26.16 \pm 1.39^{b,c,d,e}$	$10.40 \pm 0.34^{a,b,c}$	$15.16 \pm 0.69^{a,b,c}$	22.23 ± 1.13^{a}	$0.07 \pm 2.42^{a,b,c}$
20-Mar-11	$28.02 \pm 9.77^{c,d,e}$	$8.65 \pm 1.97^{a,b,c}$	$13.87 \pm 2.22^{a,b,c}$	$20.44 \pm 4.04^{a,b}$	$50.54 \pm 13.54^{a,b,c,d}$
16-Apr-11	$18.43 \pm 9.59^{a,b,c,d,e}$	$5.92 \pm 3.53^{a,b,f}$	$9.52 \pm 5.7^{a,b,c}$	$14.52 \pm 8.38^{a,b}$	$33.90 \pm 18.85^{a,b,c,d}$
28-May-11	$67.43 \pm 0.00^{d,e}$	$27.49 \pm 0.00^{b,c}$	$37.71 \pm 0.00^{b,c}$	$53.11 \pm 0.00^{a,b}$	$130.49 \pm 0.00^{c,d}$
12-Jun-11	77.95 ± 0.00^{e}	31.91 ± 0.00^{c}	43.62 ± 0.00^{c}	$63.49 \pm 0.00^{a,b}$	150.99 ± 0.00^{d}
Male					
12-Jul-10	$8.20 \pm 0.00^{a,b,c,d,e}$	$5.26 \pm 0.00^{a,b,c}$	$19.90 \pm 0.00^{a,b,c}$	$5.41 \pm 0.00^{a,b}$	$33.17 \pm 0.00^{a,b,c,d}$
25-Aug-10	13.64 ± 1.78	2.08 ± 0.47	8.23 ± 2.88	10.42 ± 0.89	23.86 ± 3.78
06-Oct-10	$6.60 \pm 7.98^{a,b,c}$	$2.12 \pm 0.19^{a,b,c}$	4.67 ± 0.68^{a}	4.63 ± 5.71^{a}	$13.39 \pm 8.85^{a,b}$
04-Nov-10	$0.80 \pm 0.33^{a,b}$	0.51 ± 0.18^{a}	$11.12 \pm 3.25^{a,b,c}$	0.00 ± 0.00a,b	$12.15 \pm 3.61^{a,b}$
07-Dec-10	$11.50 \pm 1.26^{a,b,c,d}$	$3.87 \pm 0.69^{a,b,c}$	$6.24 \pm 1.02^{a,b}$	$9.71 \pm 1.14^{a,b}$	$21.61 \pm 2.73^{a,b}$
12-Jan-11	$12.98 \pm 2.82^{a,b,c,d,e}$	$4.80 \pm 1.45^{a,b,c}$	$7.55 \pm 1.27^{a,b,c}$	$11.05 \pm 2.13^{a,b}$	$25.33 \pm 5.54^{a,b,c}$
20-Feb-11	$14.18 \pm 3.64^{a,b,c,d}$	$4.23 \pm 2.88^{a,b,c}$	$8.47 \pm 2.30^{a,b,c}$	$12.50 \pm 3.27^{a,b}$	$26.88 \pm 8.82^{a,b,c}$
20-Mar-11	$13.28 \pm 3.46^{a,b,c,d,e}$	$3.88 \pm 1.50^{a,b,c}$	$7.73 \pm 2.70^{a,b,c}$	$11.40 \pm 3.00^{a,b}$	$24.90 \pm 7.66^{a,b,c}$
16-Apr-11	$12.19 \pm 0.96^{a,b,c,d}$	$1.98 \pm 0.52^{a.b}$	$6.80 \pm 1.07^{a,b}$	$10.01 \pm 0.89^{a,b}$	$20.97 \pm 2.38^{a,b}$
28-May-11	$44.28 \pm 16.53^{d,e}$	$6.64 \pm 3.33^{a,b,c}$	$20.12 \pm 7.77^{a,b,c}$	$36.61 \pm 13.57^{a,b}$	$69.05 \pm 25.87^{b,c,d}$
12-Jun-11	$31.71 \pm 19.11^{c,d,e}$	$7.59 \pm 6.53^{a,b,c}$	$14.68 \pm 8.89^{a,b,c}$	$26.85 \pm 16.24^{a,b}$	$53.50 \pm 34.20^{a,b,c,d}$

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; EFA: essential fatty acids; BAFA: bacterial fatty acids; HPFA: higher plant fatty acids; TFA: total fatty acids; nd: no data; Mean values marked by the same letters indicates that no statistically significantly differences were present (GLM, Tukey post-hock test, $p < 0.05$); only fatty acid categories presented in figures were checked for significant differences between months and gender.

Table A17. Monthly variation in fatty acid categories of *Parechinus angulosus*. Units are in µg FA mg^{-1}DW gonad tissues (mean ± standard deviation). The symbol Σ indicates total abundance for each category.

Month	ΣPUFA	ΣMUFA	ΣSFA	ΣEFA	TFA
Parechinus angulosus					
Female					
12-Jul-10	15.08 ± 8.74^{a}	9.09 ± 4.13^{a,b}	21.30 ± 8.68^{b,d,f}	8.73 ± 4.44^{a}	44.33 ± 20.36^{a,b,c}
25-Aug-10	14.51 ± 3.51^{a,c}	5.65 ± 1.42^{a,b}	8.57 ± 3.17^{a}	9.04 ± 1.32^{a}	27.61 ± 7.73^{a}
06-Oct-10	1.93 ± 2.74	1.46 ± 1.11	2.92 ± 2.30	1.13 ± 1.65	6.08 ± 5.19
04-Nov-10	8.51 ± 0.00^{a,b,c}	3.81 ± 0.00^{a,b}	7.82 ± 0.00^{a,b,c,d,e}	5.93 ± 0.00^{a,b}	19.49 ± 0.00^{a,c}
07-Dec-10	20.17 ± 3.47	6.82 ± 1.15	9.02 ± 1.71	12.74 ± 2.28	34.96 ± 5.99
12-Jan-11	17.93 ± 0.82^{a,b,c}	5.94 ± 0.28^{a,b}	7.84 ± 0.23^{a}	12.43 ± 0.54^{a}	30.75 ± 0.98^{a,c}
20-Feb-11	21.39 ± 2.49	6.93 ± 0.78	9.26 ± 0.84	12.29 ± 3.83	36.32 ± 3.88
20-Mar-11	17.80 ± 1.28	5.90 ± 0.33	8.18 ± 0.51	12.04 ± 0.88	30.76 ± 2.02
16-Apr-11	21.94 ± 4.47^{a,b,c,d}	7.83 ± 1.16^{a,b}	9.93 ± 1.74^{a,b,c,e}	14.72 ± 2.88^{a}	38.21 ± 6.98^{a,b,c}
28-May-11	47.75 ± 6.54^{d}	20.67 ± 2.51^{c}	34.61 ± 5.31^{c}	29.09 ± 3.35^{a,b}	100.21 ± 14.19^{d}
12-Jun-11	30.96 ± 0.00^{a,b,c,d}	14.30 ± 0.00^{a,b,c}	25.99 ± 0.00^{b,d,e,f}	17.76 ± 1.83^{a,b}	70.51 ± 0.00^{a,b,c,d}
Male					
12-Jul-10	16.12 ± 12.30^{a,c}	11.48 ± 9.67^{a,b,c}	10.77 ± 3.86^{a,b,c,d}	9.75 ± 7.39^{a}	36.61 ± 21.60$^{a.b.c}$
25-Aug-10	15.84 ± 0.00^{a,b,c,d}	7.03 ± 0.00^{a,b,c}	5.60 ± 0.00^{a,c}	9.29 ± 0.00^{a,b}	26.94 ± 0.00^{a,b,c}
06-Oct-10	nd	nd	nd	nd	nd
04-Nov-10	8.33 ± 2.93^{a}	4.75 ± 1.42^{a}	10.24 ± 2.74^{a,b,c,e}	5.03 ± 1.35^{b}	22.58 ± 6.67^{a}
07-Dec-10	nd	nd	nd	nd	nd
12-Jan-11	13.96 ± 2.86^{a,b,c}	4.55 ± 0.57^{a,b}	5.77 ± 2.034^{a}	9.29 ± 2.96^{a,b}	23.52 ± 5.38^{a}
20-Feb-11	nd	nd	nd	nd	nd
20-Mar-11	nd	nd	nd	nd	nd
16-Apr-11	17.16 ± 0.12^{a,b,c}	6.32 ± 0.29a,b	8.27 ± 0.33^{a,c,e}	10.57 ± 0.33^{a,b}	30.63 ± 0.76^{a,b,c}
28-May-11	39.51 ± 4.38^{b,c,d}	16.82 ± 0.09^{b,c}	21.50 ± 0.53^{b,c,d,e,f}	22.87 ± 3.08^{a,b}	74.09 ± 3.52^{b,c,d}
12-Jun-11	36.28 ± 2.16^{b,d}	13.87 ± 1.046^{a,b,c}	21.78 ± 2.87^{c,f}	22.17 ± 1.83^{a,b}	71.33 ± 5.45^{b,d}

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; EFA: essential fatty acids; BAFA: bacterial fatty acids; HPFA: higher plant fatty acids; TFA: total fatty acids; nd: no data; Mean values marked by the same letters indicates that no statistically significantly differences were present (GLM, Tukey post-hock test, $p < 0.05$); only fatty acid categories presented in figures were checked for significant differences between months and gender.

Table A18. Comparisons of the total essential fatty acids (ΣEFAs) in muscle and gonad tissues of suspension-feeder *Perna perna* and grazer *Parechinus angulosus*. Values are combined male and female tissues and expressed in µg TFA mg^{-1} DW and percentage (% TFA). The symbol Σ indicates total abundance for essential fatty acids.

	Perna perna		*Parechinus angulosus*	
Month	ΣEFA	ΣTFA	ΣEFA	ΣTFA
Muscles				
12-Jul-10	2.56 ± 0.84^{a,b,c,d}	7.80 ± 2.34^{a,b,d}	0.35 ± 0.25^{a,b,c}	10.21 ± 13.56^{a,b,c,d}
25-Aug-10	0.13 ± 0.04^{a,b}	4.29 ± 1.35^{a,b}	0.27 ± 0.40^{a,b}	2.47 ± 1.30^{a}
06-Oct-10	0.06 ± 0.07^{a}	3.20 ± 2.16^{a}	0.07 ± 0.10^{a}	2.12 ± 0.38^{a}
04-Nov-10	0.42 ± 0.43^{a,b,c}	11.43 ± 3.47^{a,b,c,d,e}	0.00 ± 0.00^{a}	2.30 ± 0.24^{a}
07-Dec-10	2.75 ± 0.78^{a,b,c,d}	7.54 ± 1.87^{a,b,d}	2.85 ± 1.06^{c,d,e}	31.45 ± 16.83^{d,e}
12-Jan-11	3.84 ± 0.79^{b,c,d}	10.01 ± 1.64^{a,b,c,d}	3.62 ± 4.19^{b,c,d,e,f}	31.05 ± 37.17^{b,c,d,e}
20-Feb-11	4.47 ± 0.85^{b,c,d}	12.42 ± 2.37^{a,b,c,d,e}	2.13 ± 0.81^{b,c,d}	6.14 ± 2.28^{a,c}
20-Mar-11	4.45 ± 0.71^{b,c,d}	11.25 ± 1.60^{a,b,c,d,e}	2.72 ± 1.76^{c,e}	7.99 ± 4.18^{a,b,c}
16-Apr-11	4.57 ± 1.85^{b,c,d}	12.65 ± 5.24^{a,b,c,d,e}	0.72 ± 0.09^{a,c}	5.28 ± 1.36^{a,c}
28-May-11	3.92 ± 0.68^{b,c,d}	10.07 ± 1.28^{a,b,c,d}	2.38 ± 0.41^{c,d}	6.86 ± 1.11^{a,b,c}
12-Jun-11	3.00 ± 0.63^{a,b,c,d}	8.56 ± 1.20^{a,b,d}	2.27 ± 0.61^{b,c,d}	6.73 ± 1.63^{a,b,c}
Gonads				
12-Jul-10	8.34 ± 9.87^{b,c,d,e}	44.36 ± 40.03^{c,d,e,f,g}	9.11 ± 5.21^{e,f,g,h}	41.43 ± 19.66^{e}
25-Aug-10	10.42 ± 0.89^{c,d,e}	23.86 ± 3.78^{a,b,c,d,e,f}	9.09 ± 1.15^{f,g,h}	27.48 ± 6.70^{d,e}
06-Oct-10	13.62 ± 11.98^{d,e}	35.10 ± 30.31^{b,c,d,e,f}	1.13 ± 1.65^{a,b,c}	6.08 ± 5.19^{a,c}
04-Nov-10	0.00 ± 0.00^{a}	11.08 ± 4.67^{a,b,c,d,e}	5.26 ± 1.19^{d,e,f,g}	21.81 ± 5.67^{b,d,e}
07-Dec-10	10.23 ± 1.43^{d,e}	23.89 ± 5.08^{b,c,d,e}	12.74 ± 2.28^{h,i}	34.96 ± 5.99^{e}
12-Jan-11	9.45 ± 2.042^{c,d,e}	27.70 ± 10.19^{b,c,d,e,f}	11.18 ± 2.30^{g,h}	27.86 ± 4.84^{d,e}
20-Feb-11	16.39 ± 5.83^{e,f}	36.82 ± 15.02^{c,e,f,g}	12.29 ± 3.83^{h,i}	36.32 ± 3.88^{e}
20-Mar-11	16.82 ± 5.91^{e,f}	40.28 ± 17.42^{e,f,g}	12.04 ± 0.88^{h,i}	30.76 ± 2.02^{e}
16-Apr-11	11.82 ± 4.91^{d,e}	26.14 ± 11.91^{b,c,d,e}	13.06 ± 3.06^{h,i}	35.18 ± 6.46^{e}
28-May-11	40.73 ± 13.81^{g}	84.41 ± 37.28^{g}	26.60 ± 4.42^{j}	89.76 ± 17.56^{f}
12-Jun-11	36.01 ± 22.61^{f,g}	77.87 ± 56.17^{f,g}	21.29 ± 2.53^{i,j}	71.17 ± 4.74^{f}

Mean values marked by the same letters indicates that no statistically significantly differences were present (GLM, Tukey post-hock test, $p < 0.05$); only fatty acid categories presented in figures were checked for significant differences between months and tissues.

Table A19. The coefficients of variation (expressed as percentages) of essential fatty acids (EFA) and total fatty acids (TFA) from suspension-feeding mussel *Perna perna* and grazer *Parechinus angulosus*. Coefficients of variation were calculated using quantitative data. The symbol Σ indicates total abundance for each category.

	Perna perna		*Parechinus angulosus*	
Month	ΣEFA	TFA	ΣEFA	TFA
Muscle				
12-Jul-10	6	30	117	133
25-Aug-10	67	31	113	52
06-Oct-10	94	67	136	18
04-Nov-10	82	30	0	10
07-Dec-10	6	25	15	54
12-Jan-11	6	16	13	120
20-Feb-11	10	19	1	37
20-Mar-11	4	14	28	52
16-Apr-11	10	41	34	26
28-May-11	6	13	6	16
12-Jun-11	13	14	3	24
Average	**28**	**27**	**42**	**49**
Gonads				
12-Jul-10	75	90	34	47
25-Aug-10	16	16	11	24
06-Oct-10	51	86	61	85
04-Nov-10	0	42	17	26
07-Dec-10	7	21	8	17
12-Jan-11	31	37	6	17
20-Feb-11	7	41	28	11
20-Mar-11	11	43	3	7
16-Apr-11	7	46	7	18
28-May-11	12	44	7	20
12-Jun-11	10	72	9	7
Average	**21**	**49**	**17**	**25**

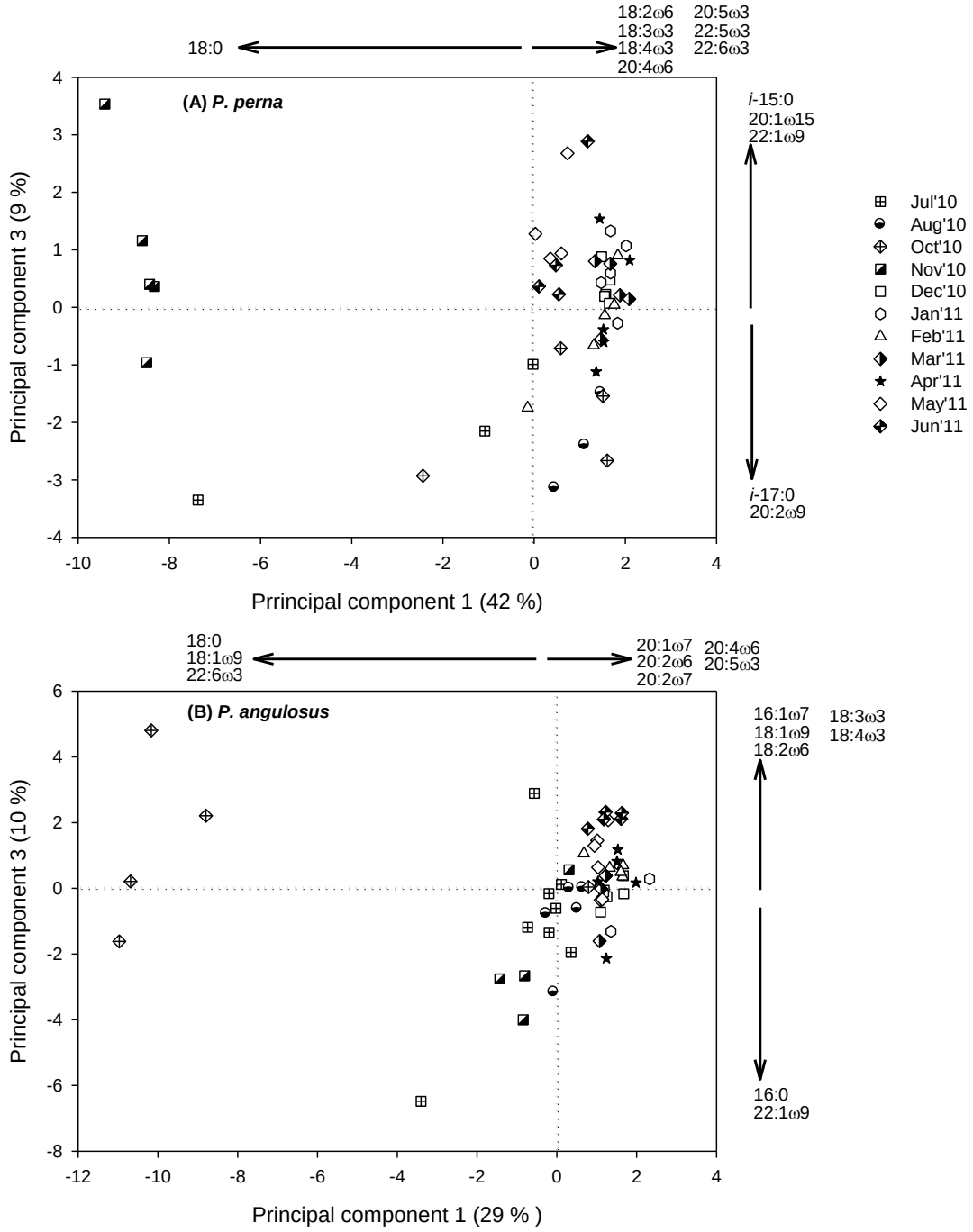

Figure A4. Principal component analysis (PCA) of arcsin square root-transformed proportional fatty acid data (% TFA) from the gonad tissues of (A) *Perna perna* and (B) *Parechinus angulosus*. Fatty acid >1% TFA were included in the analyses (38 fatty acid in total). Percentage values in PC (principal component) labels indicate the proportion of variation accounted for by each PC. Arrows running parallel to each axis denote the influence of the specific fatty acids that have loading values >2.5%. The cross-section of the dotted lines represents the origin.

TABLE AND FIGER FOR RESULTS OF NMDS, ANOSIM AND SIMPER

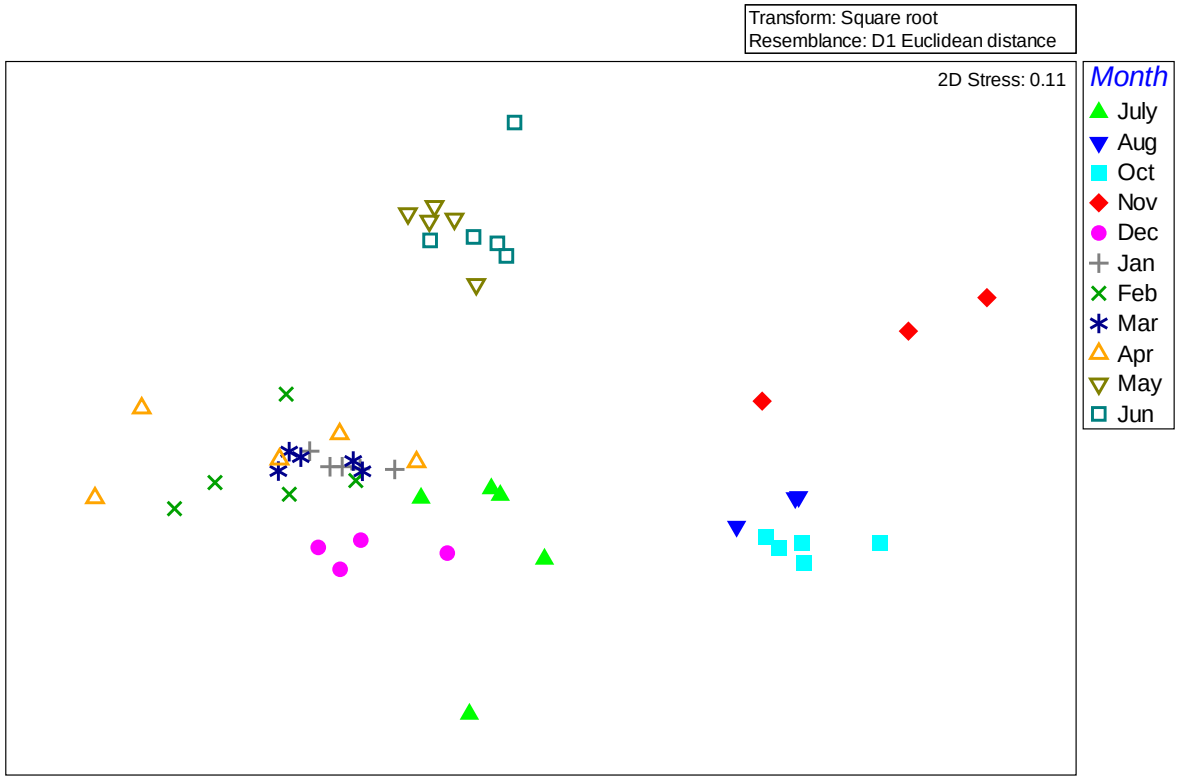

Figure A5. Multidimensional scaling (MDS) plot based on the results of analysis of similarity (ANOSIM) analysis of fatty acid (FA) compositions (% TFA) of suspension-feeder *Perna perna*. A stress level of 0.11 indicates an excellent representation of the data.

Table A20. Results of the analysis of similarity (ANOSIM) and similarity percentage (SIMPER) for the proportional fatty acid composition from the muscle tissues of suspension-feeders (*Perna perna*).

Pair	*R*	*P*	Fatty acids	Contributor (%)
July, Aug	0.938	0.018	20:5ω3	10.2
			22:6ω3	8.09
			20:4ω6	7.29
July, Oct	0.964	0.008	22:6ω3	10.02
			20:5ω3	9.53
			20:4ω6	6.82
July, Nov	1	0.018	18:0	12.15
			20:5ω3	6.49
			16:3ω4	5.89
July, Dec	0.688	0.008	18:0	8.96
			18:1ω13	8.94
			18:1ω7	6.96
July, Jan	0.668	0.008	18:1ω7	8.63
			22:2ω9	7.64
			20:1ω15	5.39
July, Feb	0.756	0.008	18:1ω7	7.84
			22:2ω9	6.7
			16:3ω4	6.49

Table A20. (cont.)

Pair	*R*	*P*	Fatty acids	Contributor (%)
July, Mar	0.716	0.008	18:1ω7	7.84
			22:2ω9	7.35
			20:5ω3	5.79
July, Apr	0.588	0.008	22:2ω9	6.88
			16:2ω6	5.79
			18:1ω7	5.77
July, May	0.956	0.008	22:1ω9	7.24
			16:3ω4	7.13
			20:1ω15	6.96
July, Jun	0.992	0.008	16:3ω4	10.11
			20:1ω15	6.84
			20:2ω9	6.23
Aug, Oct	0.241	0.107	18:0	14.07
			16:0	14.02
			22:6ω3	8.86
Aug, Nov	0.926	0.1	18:0	13.62
			16:3ω4	11.25
			16:3ω6	8.75

Table A20. (cont.)

Pair	*R*	*P*	Fatty acids	Contributor (%)
Aug, Dec	1	0.029	18:0	10.67
			20:5ω3	8.55
			18:1ω13	5.55
Aug, Jan	1	0.018	20:5ω3	10.54
			22:6ω3	6.88
			20:4ω6	6.56
Aug, Feb	1	0.018	20:5ω3	9.36
			22:6ω3	6.83
			16:1ω7	6.31
Aug, Mar	1	0.018	20:5ω3	10.68
			22:6ω3	7.07
			20:4ω6	6.6
Aug, Apr	1	0.018	20:5ω3	9.01
			22:6ω3	7.06
			22:2ω9	5.58
Aug, May	1	0.018	20:5ω3	8.87
			22:1ω9	7.71
			22:6ω3	7.24

Table A20. (cont.)

Pair	*R*	*P*	Fatty acids	Contributor (%)
Aug, Jun	1	0.018	16:3ω4	7.92
			20:5ω3	7.58
			22:1ω9	7.25
Oct, Nov	0.99	0.018	18:0	12.41
			16:0	11.5
			16:3ω4	9.31
Oct, Dec	1	0.008	18:0	9.88
			20:5ω3	8.39
			22:6ω3	7.12
Oct, Jan	1	0.008	20:5ω3	10.08
			22:6ω3	8.44
			22:2ω9	6.93
Oct, Feb	1	0.008	20:5ω3	8.88
			22:6ω3	8.09
			22:2ω9	6.23
Oct, Mar	1	0.008	20:5ω3	10.14
			22:6ω3	8.45
			22:2ω9	6.8

Table A20. (cont.)

Pair	*R*	*P*	Fatty acids	Contributor (%)
Oct, Apr	1	0.008	20:5ω3	8.64
			22:6ω3	8.42
			22:2ω9	6.74
Oct, May	1	0.008	22:6ω3	8.94
			20:5ω3	8.78
			22:1ω9	7.63
Oct, Jun	1	0.008	22:6ω3	8.25
			16:3ω4	8.13
			20:5ω3	7.78
Nov, Dec	1	0.029	18:0	15.39
			20:5ω3	6.6
			16:0	5.6
Nov, Jan	1	0.018	18:0	10.25
			20:5ω3	8.51
			20:4ω6	5.76
Nov, Feb	1	0.018	18:0	9.4
			20:5ω3	7.89
			22:6ω3	5.95

Table A20. (cont.)

Pair	*R*	*P*	Fatty acids	Contributor (%)
Nov, Mar	1	0.018	18:0	9.53
			20:5ω3	8.87
			22:6ω3	6.01
Nov, Apr	1	0.018	18:0	8.76
			20:5ω3	7.61
			22:6ω3	6.18
Nov, May	1	0.018	18:0	9.21
			20:5ω3	7.41
			22:1ω9	7.05
Nov, Jun	1	0.018	18:0	10.64
			22:1ω9	6.76
			20:5ω3	6.32
Dec, Jan	0.994	0.008	18:0	18.86
			18:1ω13	16.14
			20:5ω3	5.75
Dec, Feb	0.781	0.008	18:0	12.74
			18:1ω13	11.14
			16:4ω1	6.68

Table A20. (cont.)

Pair	*R*	*P*	Fatty acids	Contributor (%)
Dec, Mar	1	0.008	18:0	16.88
			18:1ω13	13.69
			20:5ω3	6.56
Dec, Apr	0.563	0.016	18:0	12.71
			18:1ω13	9.69
			16:2ω6	7.25
Dec, May	1	0.008	22:1ω9	9.91
			18:0	8.62
			18:1ω13	6.65
Dec, Jun	1	0.008	22:1ω9	9.07
			16:3ω4	7.76
			18:0	7.39
Jan, Feb	0.268	0.04	16:4ω1	12.31
			16:2ω6	11.13
			16:3ω4	6.82
Jan, Mar	–0.024	0.5	20:5ω3	7.37
			15:0	6.67
			22:6ω3	6.54

Table A20. (cont.)

Pair	*R*	*P*	Fatty acids	Contributor (%)
Jan, Apr	0.32	0.024	16:2ω6	11.06
			16:4ω1	9.93
			20:4ω6	6.05
Jan, May	1	0.008	22:1ω9	11.24
			22:2ω9	8.17
			20:2ω9	7.05
Jan, Jun	1	0.008	22:1ω9	9.51
			22:2ω9	7.66
			16:3ω4	6.81
Feb, Mar	0.332	0.024	16:4ω1	11.7
			16:2ω6	10.58
			20:5ω3	6.43
Feb, Apr	0	0.397	16:4ω1	7.17
			16:2ω6	6.95
			16:1ω7	6.37
Feb, May	1	0.008	22:1ω9	9.59
			22:2ω9	7.31
			20:2ω9	6.46

Table A20. (cont.)

Pair	*R*	*P*	Fatty acids	Contributor (%)
Feb, Jun	1	0.008	22:1ω9	8.19
			22:2ω9	6.92
			20:2ω9	6.06
Mar, Apr	0.324	0.008	16:2ω6	10.87
			16:4ω1	9.76
			20:4ω6	5.78
Mar, May	1	0.008	22:1ω9	10.79
			22:2ω9	8.33
			20:2ω9	6.88
Mar, Jun	1	0.008	22:1ω9	8.94
			22:2ω9	7.64
			20:2ω9	6.26
Apr, May	0.964	0.008	22:1ω9	9.17
			22:2ω9	7.52
			20:2ω9	6.63
Apr, Jun	0.968	0.008	22:1ω9	7.84
			22:2ω9	7.11
			20:2ω9	6.23
May, Jun	0.324	0.016	16:3ω4	10.61
			αι–18:0	9.34
			20:1ω7	9.18