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A Comparison of Biochemical Composition and Bioactivity of Abalone (*Haliotis* spp) Subjected to Different Diets and Treatments

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Abstract

The fast growth of the aquaculture sector has led to an unsustainable harvest of wild fish species used in the production of aquaculture feed. Integrated Multi-trophic Aquaculture (IMTA) promises ecological and socio-economic advantages that include the provision of sustainable aquaculture feed. However, the promotion of IMTA requires that the stakeholders have sufficient quantitative information associated with its implementation. This present study compared the biochemical composition and bioactivity of the abalone, *Halitosis tuberculata coccinea* that was fed IMTA-produced macroalgae with the same species samples fed with an artificial diet, and a hybrid diet of the macroalgae and the artificial diet. The biochemical analyses of *H. tuberculata coccinea* extended to the impact of two aquaculture systems, the flow-through system (FTS) and the recirculation system (RS), used within an IMTA framework. In an additional part of this project, the effects of heat processing on abalone using cultivated animals of raw and heat-processed *Haliotis midae* were evaluated. The biochemical analyses included quantification of fat and protein content, determination of lipid class, fatty acid, and amino acid composition. To compare the bioactivity of samples, antioxidative capacity and HMG-CoA reductase inhibitory activity were tested. Significant differences in biochemical composition were found among the compared groups. The abalone that was fed macroalgae from IMTA had higher protein and fat content than the abalone fed with the artificial diet. However, a higher content of the beneficial omega-3 fatty acids was found in the abalone fed with the artificial diet, whereas its amount of essential amino acids was less than that of the abalone fed IMTA-macroalgae. Biochemical differences between samples from FTS and RS were minor. For the *H. midae* samples, it was shown that heat processing reduced the content of fatty acids and amino acids; some antioxidative capacities were enhanced whereas the fat content was not affected. Findings from this study indicate that macroalgae produced in IMTA meet abalone's nutritional needs and that macroalgae can be used as a supplement or a replacement for the fishmeal-based diets used in abalone aquaculture.

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Abbreviations

AA Amino acid

AAPH 2,2'azobis (2- Amidinopropane) dihydrochloride

APH Abalone protein hydrolysate

Ala Alanine

Arg Arginine

Asp Aspartic acid

AUC Area under the curve

Cys Cysteine

DCM Dichloromethane

DGDG Digalactosyldiacylglycerol

DHA Docosahexaenoic acid

DMC Dry matter content

DPA Docosapentaenoic acid

DW Dry weight

EAA Essential amino acid

EPA Eicosapentaenoic acid

FA Fatty acid

FFA Free fatty acid

FRAP Ferric ion reducing antioxidant power

FTS Flow-through system

Glu Glutamate

Gly Glycine

His Histidine

Ile Ileucine

IMTA Integrated multi-trophic aquaculture

IS Internal standard

Leu Leucine

Lys Lysine

MAG Monoacylglycerol

MeOH Methanol

Met Methionine

MMA Mycosporine-like amino acid

MUFA Monounsaturated fatty acid

NaCl Sodium chloride

n-3 Omega-3

n-6 Omega-6

ORAC Oxygen radical absorbance capacity

PC Phosphatidylcholine

PG phosphatidylglycerol

Phe Phenylalanine

Pro Proline

PS Phosphatidylserine

PUFA Polyunsaturated fatty acid

RS Recirculation system

Ser Serine

SFA Saturated fatty acid

TAA Total amino acid

TAG Triacylglycerol

TE Trolox equivalents

Thr Threonine

TPTZ 2,4,6-Tri(2-pyridyl)-2-triazine

Tyr Tyrosine

Val Valine

1. INTRODUCTION

The value of the annual global fish trade exceeds the value of all other animal food sources combined (World Bank, 2011). It is estimated that 3.3 billion people rely on fish for 20% of their average per capita consumption of animal protein. In addition, 5.6 billion people depend on fish for 10% of their per capita intake (FAO, 2020). The statistics undoubtedly highlight the vital role fisheries and aquaculture may play in a framework of present and future policies that seek to ensure food security around the world. It is true that plant proteins, in comparison with animal proteins may hold some appeal in the developed world where they are perceived as relatively healthier and in the developing world where they cost significantly less (Grigg, 1995). However, many plant proteins do not contain adequate quantities of all essential amino acids, which are relatively abundant in many fishes. In addition to containing high-quality protein, fish contains essential fatty acids (FAs) and important micronutrients such as zinc, iodine, iron, calcium and vitamin A (Kawarazuka and Béné, 2010). Indeed, an informed reason for the inclusion of fish in many diets is that many fishes, especially fatty fishes, are rich sources of the beneficial marine omega-3 (n-3) polyunsaturated fatty acids (PUFAs) that the human body cannot produce with a considerable degree of efficiency (Calder, 2013).

There may be genuine reasons for concern over the current state of fisheries, which account for a sizable chunk of global fish production. The most recent data available indicates that capture production in 2018 reached an all-time high of 96.4 million tonnes (FAO, 2020). This surge in capture fisheries was mainly driven by marine capture fisheries, especially, catches of anchoveta by Peru and Chile (FAO, 2020). It is important to note that some studies suggest that high fishing pressures without a risk-based management scheme may lead to stock collapse (Worm et al., 2006, Essington et al., 2015). Even temporary collapses of low trophic-level fishes can affect a whole ecosystem by reducing food supply to higher trophic-level fishes, marine mammals and seabirds (Pinsky et al., 2011). Meanwhile, aquaculture has been discussed as a solution to overfishing (Longo et al., 2019), and the contribution of the sector to the total production of aquatic animals has risen steadily from 25.7% in 2000 to 46% in 2018 (FAO, 2020).

However, there are some indications that aquaculture, as it is practiced today, may not be sustainable. For instance, even though the volume of fish produced from aquaculture for direct human consumption surpassed the corresponding volume from wild catch for the first time in

2014 (FAO/OECD, 2016), the total production from fisheries has remained higher as a result of harvesting of forage fishes including anchoveta increasingly used in the production of fishmeal for aquaculture feed (Natale et al., 2013). Also, aquaculture generally cultivates fewer species than those caught in the wild (Golden et al., 2016). Hence a global fish market dominated by aquaculture as it is currently practiced would lead to a drop in the diversity and consequently to a drop in the supply of fishes used in many diets (Thilsted et al., 2016).

Integrated multi-trophic aquaculture (IMTA) is an aquaculture system that, amongst other benefits, has the potential to reduce the demand for fishmeal and to improve crop diversity in aquaculture (Shpigel et al., 2017, Troell et al., 2009). IMTA is a system that involves the integrated cultivation of a fed species (e.g., finfish) alongside an extractive species (marine invertebrates and/or algae) that feed on detritus and inorganic nutrients from the fed species (Alexander et al., 2016). This study compares the biochemical composition and bioactivity of abalone, *Haliotis tuberculata coccinea* produced in two IMTA systems; Flow-through system IMTA (FTS. IMTA) and Recirculation system IMTA (RS. IMTA). It also investigates the effect of IMTA diet on the biochemical composition and bioactivity of abalone. Three levels of diets are compared; algae produced in IMTA, a compound feed (ABFEED®), and a diet that combined IMTA algae and ABFEED®. The effect of heat processing on the biochemical composition and bioactivity of the abalone, *H. midae* is also investigated.

1.1 Aquaculture: Food for a Growing Population

It is inevitable that with a human population boom, rising incomes and changing food preferences, there will be a surge in global demand for nutritious food in the coming years (FAO, 2018). Estimations of population and income by 2050 project a future need of more than 500 megatonnes of meat per year for human consumption. Unfortunately, there is no clear indication that this future demand can be met by scaling up the production of land originated food without incurring serious ecosystem damages (Costello et al., 2020). Ironically, the ocean, which covers more than 70% of the earth's surface, currently accounts for only 2% of all human calorie intake and 15% of protein intake (European Commission, 2017). It is therefore likely that the nutritional needs of the 9.2 billion people expected to live on earth by 2050 can be secured

by boosting the contribution that the ocean, through sustainable aquaculture makes to global food production (Duarte et al., 2009).

1.2 IMTA: A Sustainable Aquaculture System

As mentioned above, IMTA, characterized by an integrated culture of species that belong to different trophic levels, is inherently designed to increase long-term sustainability and profitability per cultivation unit because the wastes of one crop are converted to food, energy and fertilizer for the other crops (Barrington et al., 2009). This section presents briefly key potential benefits that can be realized from implementing IMTA. The presentation does not go beyond sustainability and economic benefits. A brief overview of FTS and RS is also presented.

1.2.1 Benefits of IMTA

Perhaps the most widely cited benefit of IMTA is that it mitigates the impact of excess nutrients released from aquaculture water. A study conducted in 2009 on Norwegian salmon farms noted that of the total feed input, 70% carbon, 62% nitrogen and 70% phosphorous were released into the environment (Wang et al., 2012) Both the organic and inorganic nutrients traceable to aquaculture feeds are deposited via faeces, urine and uneaten feed (Barrington et al., 2009). IMTA studies have observed that the effects of the two nutrient streams are very different. Inorganic nutrients dissolve in water and are available as food for algae. In contrast, small particulate organic nutrients sink to the seabed where they are available as food for filter feeders such as sea cucumber, worms and sea urchins (Cranford et al., 2013, Brager et al., 2016, Wang et al., 2014). There is indeed some evidence that suggests that macroalgae can extract inorganic nutrients from fish wastes. For instance, brown algae, *Saccharina latissima,* was showed a 50% growth increase when it was grown near a salmon farm (Wang et al., 2014). In similar research conducted on a salmon farm in western Norway, the proportion of salmon derived nitrogen available for *S. latissima* decreased with distance from the farm and the kelp's growth rate decreased with increasing distance from the farm (Fossberg et al., 2018). It is important to conduct more research to gain a deeper understanding of the form and the extent to which IMTA systems extract excess nutrients from aquaculture wastes. For instance, it has been assumed that bivalves such as mussels can be used in IMTA systems to remove particulate organic fish

wastes. However, Cranford et al. (2013) found that mussels' waste extraction efficiency is very low as the fish waste matter may be too big for them to absorb, at least in a salmon driven IMTA. An alternative approach would be to recycle the inorganic waste nutrients using phytoplankton, which are then fed to the bivalves (Strand et al., 2019). In short, enhancing interactions between fish wastes and extractive species through optimizations in IMTA system design may yield intended economic and ecological benefits of the IMTA approach. However, this enhancement of farm design demands knowledge on particulate waste dynamics and transport pathways under applicable hydrographic conditions (Brager et al., 2016).

Another benefit, and indeed one that needs more attention is the potential to diversify aquaculture products with IMTA. The total aquaculture production of seaweed has more than doubled in the last two decades, and most of the growth happened in Asia (Ferdouse et al., 2018). Even though the demand for seaweed continues surging (García Poza et al., 2020), the dominant practice outside Asia remains to harvest wild stocks (Buschmann et al., 2017). In the western world, seaweed previously used mainly as phycocolloids is now increasingly used as food. By 2012, more than 75% of seaweed total tonnage was directly consumed by humans (Chopin, 2012). Hence, seaweed produced in IMTA may have a growing market to meet. Technically, an important advantage IMTA has over monoculture is the potential to generate uncorrelated sources of income, consequently reducing the effect of fed aquaculture loses when they occur and providing farm resilience from an economic standpoint (Ridler et al., 2007). More so, it is reasonable to expect aquaculture loses in the face of climate change that is expected to have species specific effects (Cochrane et al., 2009). Furthermore, it is possible that fed aquaculture produced in IMTA can be sold at premium market prices by simply differentiating and eco-certifying them as IMTA products (Chopin et al., 2012).

There is a consensus on the need to find new sustainable marine resources for fish feed (Ellis and Tiller, 2019). IMTA products could be used as alternative ingredients as kelp produced in the system have already been deployed in salmon-feeding trials (Chopin, 2012). Similarly, mussel meal has been described as a unique, sustainable ingredient in fish feed. The amino acid composition may be almost the same as fish meal, the difference being that methionine, lysine and isoleucine are slightly lower in mussel meal. On the other hand, taurine and glycine, used by mussel in osmoregulation are much higher in mussel meal than in fish meal (Árnason et al., 2015). More importantly, mussel meal could be a good source of omega-3 PUFAs as

eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) accounted for 17% and 16% of the fatty acids, respectively (Árnason et al., 2015). However, aquaculture producers may prefer to use the cheapest acceptable resources to produce feed, and IMTA derived products may be more expensive than conventional resources (Ellis and Tiller, 2019). Perhaps IMTA-derived feed ingredients could be used as a supplement to conventional aquaculture feed sources.

1.2.2 Two IMTA Systems: FTS and RS

IMTA has been practiced in China for centuries. However, the system is relatively new to the rest of the world, where a couple of IMTA systems are now running in temperate marine waters, although mostly at a pilot or research scale (Alexander and Hughes, 2017). Several IMTA models have been proposed. These ideas have expounded on simpler concepts such as trials of novel benthic extractive components (Nederlof et al., 2020) and optimizations in the density and harvesting frequency of the extractive species grown (Lamprianidou et al., 2015, Buck and Buchholz, 2004), as well as proposed a complex model such as an offshore biorefinery coupled to IMTA seaweed biomass production (Neori et al., 2019).

The underlying production systems in the emerging land-based IMTA models often involve FTS, RS, or a compromise of the two systems. FTS is the conventional aquaculture production system and as the name implies, the system has water flowing straight through without any recirculation (Villar-Navarro et al., 2021). This means that the system would usually require a large reservoir of water to meet production needs. Also, the setup of FTS usually results in a more significant land usage in contrast to the RS. However, the advantages of FTS are low setup costs and relative ease of operation (Martins et al., 2010).

On the other hand, RS is a system in which water is partially reused after some purification treatment (Rosenthal, 1986). It involves treatment steps where each step reduces the system water exchange to the needs of the next, limiting waste component (Martins et al., 2010). RS systems are often called ‘urban’ or ‘indoor’ aquaculture because of their independence of surface water to produce crops (Martins et al., 2010). This production system offers several benefits such as the ability to farm seafood products near markets (Schneider et al., 2010), an enhanced opportunity for nutrient recycling and waste management (Piedrahita, 2003), a

reduced water usage (Verdegem et al., 2006), and some opportunity for disease control (Tal et al., 2009). Despite its glaring benefits, the high initial capital investment associated with this production system has contributed to its slow adoption (Schneider et al., 2006). Additionally, due to the mechanical and biological sophistication associated with this system, poor water quality, component failures, diseases, stress and off-flavour are common in poorly managed RS (Masser et al., 1999).

The main difference between the two systems in IMTA models is how water is supplied to the crops. In FTS, water is passed from the fed component (member) down to the extractive component from where it is drained out. In the RS, water is passed from the fed component down to the extractive component and then made to flow back to the fed component (Neori et al., 2019).

1.3 Abalone (*Haliotis* spp.)

1.3.1 Abalone Biology

Haliotis, the only genus in the family Haliotidae, comprises at least 56 recognized abalone species distributed in tropical and temperate waters around the world (Geiger, 1999, Baldwin et al., 2007). These single-shelled marine herbivorous gastropods inhabiting the rocky intertidal and subtidal zones have their highest species diversity today in the Indo-Pacific waters (Boolootian et al., 1962, Geiger and Groves, 1999). Abalones are gonochoric with sexual differentiation and maturity of organisms characterized by distinct gonad coloration (Shepherd and Laws, 1974). These gastropod mollusks are broadcast spawners; both sexes release their gametes into the water column, where external fertilization occurs (Hobday et al., 2000).

The growth cycle of *Haliotis* is characterized by the five stages; embryo, larvae, post-larvae (spat), juvenile and adult (De Vicose et al., 2007). Fertilized eggs first go through the embryonic stage that lasts for a few hours before hatching to become ciliated trochophore larvae. The newly emerged larvae are lecithotrophic and pelagic. Depending on species and water temperature, the larvae swim in the water column for days or weeks before they acquire

‘competence’ to respond to metamorphosis inducing cue (Najmudeen and Victor, 2004, Sawatpeera et al., 2001). The nonfeeding larvae depend on maternally provided energy reserves and available dissolved organic material in seawater until they mature for settlement (Shilling et al., 1996). Competent larvae settle for metamorphosis once they come across appropriate settlement cues. Often, many chemical and biological materials are valuable cues for the settlement and metamorphosis of abalone larvae, and in general marine gastropods. However, neither their effects nor their effective concentrations have been resolved (Roberts, 2001, Pawlik, 1990). Likewise, the responses of individual species are significantly variable even to the same cues (Searcy-Bernal and Anguiano-Beltrán, 1998). Settlement is quite critical because it signals attachment and metamorphosis and, more importantly, marks a transition from pelagic to benthic life (Li et al., 2006). In some habitats, the settlement of larvae may be the major factor determining the local population of invertebrates (Menge and Sutherland, 1987, Roughgarden et al., 1985, Connell, 1985).

Once attached to a substrate, the larvae cease to swim while retaining their velum. Then metamorphosis ensues to produce some irreversible anatomical and physiological changes that commit the larvae to benthic life (Bonar, 1976). Thus, the abalone enters the post-larvae stage, where it starts to live on benthic microflora, usually biofilms consisting of diatoms and bacteria growing on surfaces of settlement rocks (Li et al., 2006, Kawamura and Kikuchi, 1992). The juvenile stage is marked by the appearance of the first respiratory pore and may involve rapid growth in some species. There is also a shift of diet from microalgae to macroalgae during this stage. (Slattery, 1992). See figure 1 below for an illustration of the main stages of the abalone life cycle.

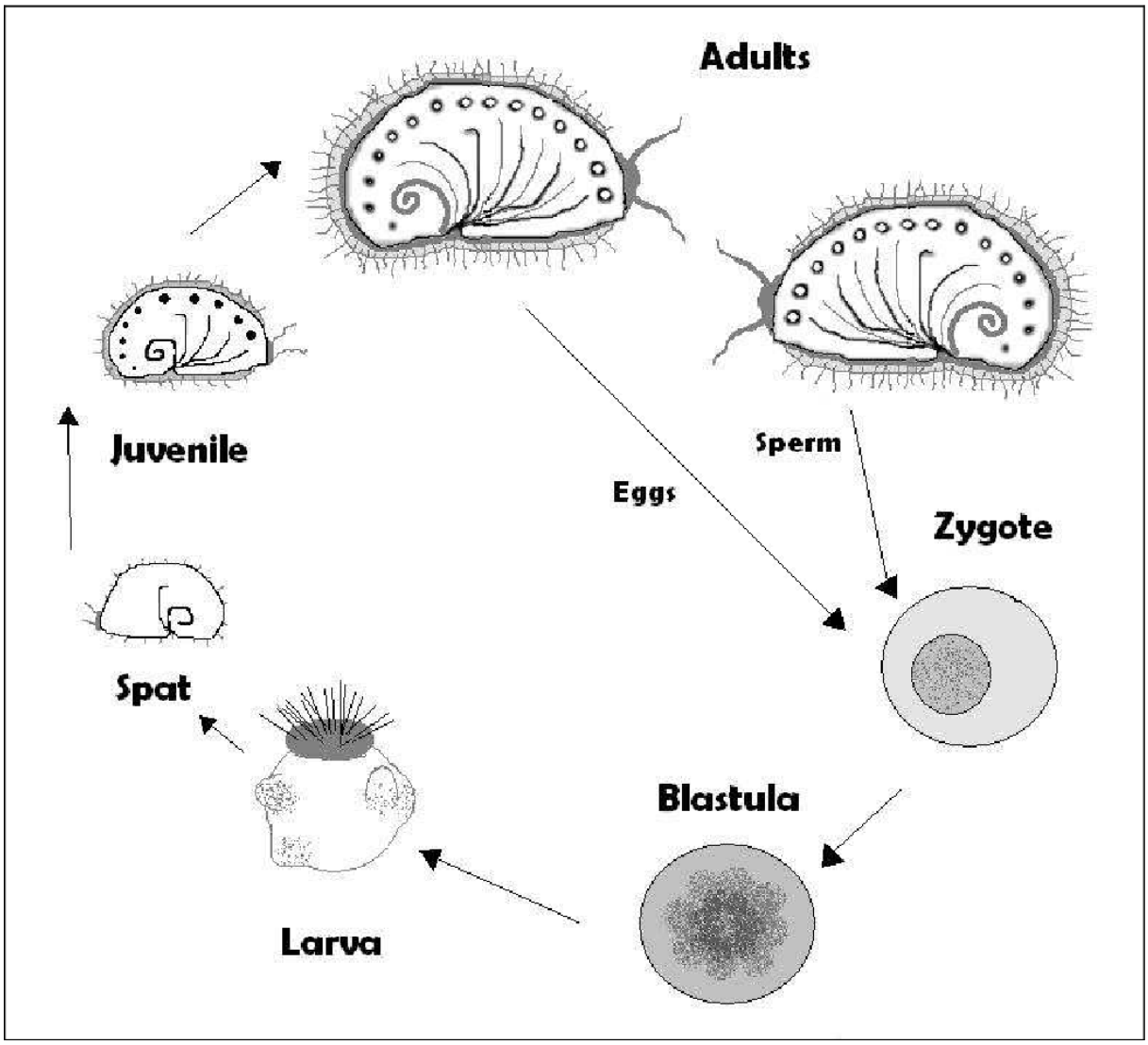


Figure 1. Abalone life cycle. The adult male and female animals release gametes that fuse to form an embryo. The larva develops until settlement as a juvenile abalone before growing to a sexually mature animal. Source (Hepple, 2010).

Growth from juvenile to adult tends to be less rapid in temperate species. Tropical abalone grows fast and can reach marketable size with shell lengths of 55 to 65 mm in a year in contrast to the temperate species, which take 3 to 4 years to reach this size range (Okuzawa et al., 2008). However, all the larger-bodied species are found in temperate waters. The largest recorded shell length of a tropical abalone is 122 mm for *H. mariae* of the Arabian Peninsula. About 50% of the temperate species have maximum recorded shell lengths that surpass this value (Estes et al., 2005).

1.3.2. *Haliotis midae*

Haliotis midae is one of the six species of abalone indigenous to South Africa. It is also the only species of commercial importance in the country (Reddy-Lopata et al., 2006). A study of the diet of wild *H. midae* reported that it found 18 species of algae in its stomach contents: *Ecklonia maxima* (56% by volume) and *Plocamium* spp. (21% by volume) (Barkai and Griffiths, 1986). *H. midae* growing in the wild can reach a maximum size of 20 cm shell length after 30 years. However, farm production can accelerate its growth to 10cm shell length in 5 years (Sales and Britz, 2001).

1.3.3 *Haliotis tuberculata coccinea*

Haliotis tuberculata coccinea is a small-sized abalone in comparison with other temperate water abalones. It attains a maximum size of 8 cm and can reach its commercial size of 4.5 to 6 cm in about 18-22 months. However, due to the growing demand for abalone in Europe, *H. tuberculata coccinea* Reeve, native to the Canary Islands, has been identified as a target species for aquaculture development. (Viera et al., 2016).

1.4 Abalone as Food

1.4.1 Production

Abalone is luxury seafood and one of the most expensive seafood worldwide (Hoshino et al., 2015). In 2016, Australian wild abalone was sold for more than double the price of the world's second-highest priced fresh whole fish or shellfish (FAO, 2017). A unique taste and a fanciful shell lined with 'mother-of-pearl' make them highly desirable on the plate (García-Bueno et al., 2016). It may be possible that the illegal, unreported, and unregulated harvest of this shellfish has persisted due to the high market value it commands (Raemaekers and Britz, 2009). It is, however, certain that because of excess harvesting, which has happened in many places, most abalone stocks have collapsed and failed to recover for decades (Munguía-Vega et al., 2015).

Abalone has been described as a vulnerable species and exhibits a higher risk of stock collapse than other marine organisms (McGarvey et al., 2008). *H. sorenseni*, the white abalone was the first marine invertebrate to be listed as endangered under the Endangered Species Act (Stierhoff et al., 2012). Consequently, legal landings from wild stock have steadily decreased from 19,720 mt in 1970 to 6,500 mt in 2015 (Cook, 2016). Australian abalone wild stock accounts for roughly 50% of wild-caught abalone in the global market (Young et al., 2020), which is mostly in Asia, where growth in both human population and household income is expected in the coming years.

Production volume from aquaculture has, in contrast to abalone fishery, steadily increased from 50 mt in 1970 to 129,287 mt in 2015 (Cook, 2016). Abalone aquaculture has been a reliable supplier of abalone products for decades and is expected to match any eventual increase in demand for this shellfish (Oakes and Ponte, 1996, Cook, 2016).

1.4.2 Nutrition

Abalone meat is low in lipid, like the lipid content of their natural diet. The mollusk, however, is very efficient at assimilating lipids from its diet (Dunstan et al., 1996). There is evidence of high utilization efficiency of exogenous lipids by abalone species. In one study, efficient lipid digestibility was observed in two abalone species fed with an artificial diet consisting of only 3.4% lipid (Wee et al., 1994). Hence many compound feed formulators may be adding excess lipid to their abalone feed products. A few studies have reported that the composition of FAs in algivores such as abalone differs significantly from those of plankton-feeders and carnivores. These FA composition differences warrant further studies to determine whether abalone lipid needs are met by the fish and vegetable oils added to their formulated diets. More so, several lipids and FAs found in macroalgae are not found in these oils (Fleming et al., 1996, Dunstan et al., 2000). It is worth mentioning that some studies that examined two abalone species, *H. midae* and *H. japonica* found some unusual lipids (De Koning, 1966a, De Koning, 1966b, Toshiko and Akira, 1982). Furthermore, in contrast to most other marine animals, wild abalone contains high levels of docosapentaenoic acid (DPA) and negligible levels of DHA. They also have high proportions of arachidonic acid (ARA); a unique feature of abalone and fish that feed on macroalgae (Dunstan et al., 1996).

The feed given to abalone can affect the nutritional value of the animal as food for humans. Formulated feeds contain higher lipid concentrations than macroalgae as a result of fortification with high levels of vegetable and fish oils, targeting the enhancement of energy and nutritional value of feed (Uki et al., 1986a, Durazo-Beltrán et al., 2003, Xu et al., 2004, Mateos et al., 2012). However, the balance of the FA profile of the commonly used vegetable oils tilts towards saturated fatty acids (SFAs) and omega-6 (n-6) long-chain PUFAs (Mulvaney et al., 2015). Considering that biochemical analyses of abalone fed on various diets demonstrates how their lipid composition is a reflection of their dietary lipid intake and the bioavailability of the ingested lipids (Nelson et al., 2002, Mercer et al., 1993), it is quite easy to agree with Dunstan et al. (1996) and Su et al. (2004) who reported that abalone farmed on formulated diets contained lower levels of omega-3 (n-3) PUFAs than those fed with macroalgae. Farming abalone with more n-3 PUFAs and less n-6 PUFAs is a viable way to restore the human diet to have more of the beneficial n-3 PUFAs (Mulvaney et al., 2015).

When considered for the need of aquaculture animals, the protein, and nutritionally essential amino acids (EAAs) content of macroalgae shows great variability and is often very low. Harvest time, geographic origin, environmental conditions, and species differences are factors that explain the variability (Øverland et al., 2019). The perceived inadequacy of macroalgae has propelled fishmeal to become the main protein source in the formulation of abalone feed. It should, however, be taken into account that the significant variability in the nutritional quality of fishmeal between different sources entails that it cannot be considered as a single product, hence it must be analyzed to ascertain its value as a protein source (Fleming et al., 1996). Different abalone species may have different protein needs. One study found *H. midae* to achieve maximum growth rate on a diet with 47% protein (Britz, 1996), while another study found *H. tuberculata* to achieve maximum growth rate on a diet with 35% protein (Mai et al., 1995b).

The level of free amino acids (AAs) found in abalone tissues is heavily influenced by the diet they were fed. In contrast to abalone fed an artificial diet, those fed *Gracilaria* spp. contained lower arginine and taurine levels, but higher levels of glutamic acid, serine, glycine, proline, and alanine (Brown et al., 2008). Also, the levels of an amino acid (AA) found in the muscles, and to greater extents, the viscera of abalone correlates with the dietary requirement of that amino acid for somatic growth (Mai et al., 1994).

Abalone fed with a natural diet may contain some bioactive compounds that could promote abalone health. Many types of marine macroalgae are important sources of structurally diverse bioactive compounds with biomedical and pharmaceutical potentials (Øverland et al., 2019).

1.4.3. Heat Processing

Raw abalone has a shelf life of fewer than three days during refrigerated storage. Hence increasing the shelf life without compromising quality is a challenge for its commercial distribution (Jo et al., 2014). Previous studies suggest that heat processing affects both the texture and biochemical composition of abalone (Luo et al., 2019). Likewise, the concentrations of certain free amino acids (especially glycine and glutamate) that characterize the taste of abalone, have been indicated to be affected by heat processing (Brown et al., 2008). Abalone can be dried to increase the shelf-life; however, the dried abalone is often rated as lower quality by consumers (Jo et al., 2014).

2. AIM AND OBJECTIVES

The goal of this study is to analyze the biochemical composition and bioactivity of abalone subjected to different treatments. The study compares the biochemical composition and bioactivity of *H. tuberculata coccinea* samples that were subjected to different dietary treatments. It also compares *H. tuberculata coccinea* samples produced under RS. IMTA and FTS. IMTA. Furthermore, the study evaluates the effect of heat processing on the biochemical composition and bioactivity of *H. midae*.

The abalone samples are analyzed for fat and protein content, FA composition, AA composition, lipid class composition, antioxidant capacity, and HMG-CoA reductase inhibitory activity.

The study evaluates this goal further by looking into the following research questions:

1. Are there differences in the biochemical composition and bioactivity of *H. tuberculata coccinea* fed with IMTA-macroalgae diet, artificial diet, or a hybrid diet of IMTA-macroalgae and artificial feed? Does any of these diets produce abalone with a more beneficial composition of FAs and AAs?
2. Can the RS production system in IMTA result in significant changes in the biochemical composition and bioactivity of farmed *H. tuberculata coccinea*?
3. Are there any changes in the biochemical composition and/or bioactivity *of H. midae* subjected to the traditional South African form of heat processing?

To answer these questions, the goals are divided into the following objectives:

- Determine fat content gravimetrically after fat extraction using Folch's method
- Determine FA composition after methylation of the lipids into fatty acid methyl esters (FAME) and gas chromatography (GC)
- Analyze protein content based on AA composition after hydrolysis of proteins and quantification of AAs using Biochrom 30+ AA analyzer
- Determine the lipid class composition through High-Performance Liquid Chromatography
- Compare antioxidant capacities using the Oxygen radical absorbance capacity (ORAC) and the Ferric ion reducing antioxidant power (FRAP) methods
- Evaluate the HMG-CoA reductase inhibitory activity based on spectrophotometric measurement of reaction mixtures

3. MATERIALS AND METHODS

3.1 Biological Materials

Six sets of abalone samples were analyzed in this study. The first category consisted of five individual animals of farmed *H. midae*, received from West Coast Abalone, South Africa on September 16, 2019. This set of abalone was cooked, salted, and dried. This category is referred to as 'processed' abalone in this study. The second category consisted of five individual animals of farmed *H. midae*, also received from West Coast Abalone, South Africa. This second category was raw abalone freeze-dried at Stellenbosch University, South Africa before shipment to UiT, Tromsø. It was received on September 16, 2019. This category is referred to as 'raw' abalone in this study.

The other four categories were pooled samples of *H. tuberculata coccinea* received from aquaculture production in the GIA/IUECOAQUA facilities in the Canary Islands (27.9892° N, 15.3753° W; Gran Canaria, Spain). These samples were either raised in FTS- or RS-IMTA. Also, they were given three dietary treatments. One group was fed live macroalgae (*Ulva rigida and Gracilaria cornea*), another group was fed an artificial diet (Abfeed®), and the final group was fed a combined diet of the macroalgae and Abfeed®.

The following descriptive notation is used to refer to the *H. tuberculata coccinea* treatments in this study

- The control group - consisting of pooled animals produced in FTS. IMTA and fed macroalgae
- Hybrid diet group - consisting of pooled animals produced in FTS IMTA and fed both macroalgae and Abfeed®
- Recirculation system (RS) group - consisting of animals produced in RS IMTA and fed the macroalgae diet
- Artificial diet group - consisting of animals produced in FTS IMTA and fed with Abfeed®

Two batches of the control group were received. The first sample was received in May 2020 while the second was received in August 2020. Samples of the other groups were all received at once, in August 2020.

3.2 Sample Preparation and Storage

Table 1. Materials and equipment used for sample preparation and storage.

Equipment	**Distributor, Country**
IKA MF 10 analytical mill	IKA-Werke, Germany
Samsung Freezer	Samsung, Japan
Reagents/Chemicals	**Distributor, Country**
Liquid Nitrogen	Linde, Norway
Ethanol	VWR, Germany

Procedure

The raw and processed *H. midae* were first immersed in liquid nitrogen to soften. A hammer was then used to break the specimens into smaller chunks before they were grinded using an analytical mill. After grinding each sample to a consistent powder, a ball of cotton wool immersed in 70% ethanol was used to clean out sample remnants that remained on the grinding parts of the mill before the next sample was fed to the machine. The *H. tuberculata coccinea* samples were freeze-dried, pulverized and several specimens were pooled before shipment to the laboratory. Upon grinding or receipt of the samples, they were immediately stored in the freezer at -18 °C until use.

3.3 Dry Matter Content Estimation

Table 2. Material and equipment used for dry matter content estimation.

Equipment/Material	**Distributor, Country**
Sartorius Cubis weighing balance	Sartorius, Germany
Heating oven	Thermo Fisher, USA
Disposable aluminium weighing dishes	VWR, Germany

Procedure

About 1 g of sample was weighed into already weighed aluminium dishes and thereafter dried in the oven at 105 °C until constant weight. The water content of the sample was then determined using the following formula

$$Water\ content\ (\%) = \frac{Weight_{before\ drying} - wieght_{after\ drying}}{Weight_{before\ drying}} \times 100 \qquad \text{..... (Eq. 3.1)}$$

Dry matter content was then derived by subtracting the water content from the sample weight before drying. Analyses were performed in triplicates.

3.4 Quantitative determination of fat content

The quantity of lipid in all categories of samples was determined through the procedure for fat extraction described by Folch et al., (1957) with some modifications as described below. Three parallels of each individual sample were used in the analysis, and the procedure was conducted three times.

Table 3. Materials and equipment used in fat extraction and quantification.

Equipment	**Distributor, Country**
Multi Reax Shaker	Heidolph Instruments, Germany
Whatman 125mm Paper filters	GE Healthcare Life Sciences, Germany
Disposable glass Pasteur pipettes 150mm	VWR, Germany
Teflon tubes (PTFE)	VWR, Germany
Heraeus Multifuge 1 S-R Centrifuge	Thermo Scientific, USA
4ml Sample Vial Glass tubes	National, USA
Sample Concentrator SBHCONC/1	Stuart Equipment, Staffordshire, UK
Satorius AG Goettingen weighing scale	Satorius, Germany
Reagents/Chemicals	**Distributor, Country**
Dichloromethane	Honeywell, Germany
Methanol	VWR international, France

Sodium Chloride	VWR, Belgium
Nitrogen gas	AGA, AS, Norway

Procedure

Approximately 0.5g of each abalone specimen/pooled sample was weighed out in a labelled Teflon tube. The exact weight of the sample used was then recorded. Exactly 9.5 ml of dichloromethane/methanol (DCM:MeOH) (2:1, v/v) solution was then pipetted to the weighted sample. This mixture was capped and left on the shaker to extract for about 30 minutes. The extract and solvent were then collected in new Teflon tubes after removing solid matter with Whatman paper filters. Exactly 2 ml of 0.9% sodium chloride (NaCl) was added to the extract solution and inverted a few times to mix properly. The solution was then centrifuged at 2000g for 10 minutes to obtain a lower phase consisting of DCM and lipids, and an upper phase consisting of water, salts, methanol, and other polar substances. This upper phase was discarded using a glass pipette. The lower DCM/lipid phase was then transferred to pre-weighed glass tubes and evaporated to dryness using nitrogen gas. After drying, the glass tubes were weighed again, and the amount of lipid was then derived from calculating the observed weight difference. The percentage of lipid was finally determined using equation 3.2 below.

$$Lipid\ (\%) = \frac{Glass\ tube_{with\ content} - Glass\ tube_{empty}}{\text{Weight of sample}} \times 100 \qquad \text{..... (Eq. 3.2)}$$

3.5 Fatty Acid Composition Analysis

The quantitative composition of the FAs present in the samples was determined by a modification of the procedure described by Stoffel et. al, (1959). This involved esterification of the lipids and analysis through GC.

Table 4. Materials and equipment used in fatty acid composition analysis.

Equipment	Distributor, Country
Multi Reax Shaker	Heidolph Instruments, Germany
Duran tubes	DWK Life Sciences, Germany
Teflon tubes (PTFE)	VWR, Germany
Digital Dry Baths/Block heater	Thermo Scientific, USA
6890N Gas chromatograph	Agilent Technologies, USA
Gas chromatography (GC) tubes	VWR, Germany
Reagents/Chemicals	**Distributor, Country**
Dichloromethane	Honeywell, Germany
Methanol	VWR international, France
Heptadecanoic acid (Internal standard)	Sigma-Aldrich, USA
Sulfuric acid	VWR, Germany
Heptane	Honeywell, Germany
Sodium chloride	VWR, Germany
Nitrogen gas	AGA, AS, Norway

Procedure

Two parallels of approximately 0.5 g of each sample material were weighed out in Teflon tubes and the exact weights were recorded. Exactly 9.5 ml of DCM:MeOH (2: 1, v / v) was added to the weighted samples. The heptadecanoic acid internal standard (IS) was dissolved to a concentration of 10mg/ml in DCM:MeOH (2: 1), and exactly 0.5 ml (5 mg of IS) was added to each sample and mixed on a shaker for about 30 minutes. The lipid content of the samples was then extracted following the modified Folch's procedure described in section 3.4 above. The obtained lipid samples were then dissolved in DCM:MeOH (2: 1) to a concentration of 10 mg/ml. Exactly 100 µl of the sample was added to Duran tubes. Thereafter, 0.9 ml of DCM and 2 ml of 2% sulfuric acid in methanol was added to each Duran tube. Care was taken to ensure the lids were tightly screwed. The samples were then placed on the heating block at 100 °C for one hour. Following this heating, 3.5 ml of heptane and 3.5 ml of 5% NaCl were pipetted into the tubes and mixed well. Two phases were observed – the upper phase consisted of heptane and FAs.

This phase was pipetted into glass tubes and evaporated to dryness using nitrogen gas. Thereafter, the samples were dissolved in 100 µl of heptane and transferred to GC tubes.

The FA composition was determined using the Agilent 6890N gas chromatograph equipped with a 7683B autoinjector and flame ionization detector (FID). The carrier gas used was helium and the various FAs were separated due to different migration rates through a Varian CP7419 capillary column (50 m x 250 µm x 0.25 µm nominal). The temperature of the injector was 240 °C and the temperature of the detector was 250 °C. A temperature program was used in the column oven, which was designed to get the best possible separation of the FAs in the sample.

The individual FAs were identified based on their retention time, in comparison with known standards. The percentage of the individual FAs was determined as the area percentage and the amount of FA per 100 g of sample. The area percentage was calculated as the area of the individual FA peak in the chromatogram divided by the total area of all the FA peaks. The area percentage of the various FAs gave a good measure of the FA composition in the samples. The amount and proportion of the various FAs was calculated using equations 3.3 and 3.4 respectively.

$$Amount\ of\ FA\ \text{g}/100\text{g} = \frac{\text{Peak area FA}}{\text{Peak area IS}} \times \frac{\text{Amount of IS (g)}}{\text{Weight of sample (g)}} \times 100g \quad \text{..... (Eq. 3.3)}$$

$$FA\ ((\%\ of\ total\ FAs) = \frac{\text{Amount of FA in sample}}{\text{Total FA in the sample}} \times 100 \quad \text{..... (Eq. 3.4)}$$

3.6 Total Amino Acids Quantification

Following some modifications to the procedure described by Moore and Stein (1963), analysis of the total AA was performed after the breakdown of peptide bonds enabled by acid hydrolysis. A known concentration of the AA standard, norleucine was added to the samples to comparatively determine the quantity of each AA in the samples. However, the quantity of

tryptophan in the samples was not determined through this procedure as this AA breaks down during acid hydrolysis

Table 5. Materials and equipment used for quantification of amino acids.

Equipment/Software	Distributor, Country
Heat cabinet	Termaks-Labolytic AS, Norway
Eppendorf tubes	Eppendorf, Germany
Sartorius Cubis weighing balance	Sartorius, Germany
Eppendorf centrifuge 5424 R	Eppendorf, Germany
1.5ml Short Thread Vials	VRW, Germany
Biochrom 30 Amino Acid Analyzer	Biochrom Co. Cambridge, UK
Chromeleon software	Dionex, Sunnyvale, CA, USA
Reagents/Chemicals	**Distributor, Country**
Norleucine	Sigma Aldrich, USA
Hydrochloric acid	Honeywell, Germany
Nitrogen gas	AGA, Norway
Lithium citrate buffer	Biochrom Co. Cambridge, UK
Ninhydrin	Sigma-Aldrich, USA
A9906 physiological amino acid standard	Sigma-Aldrich, USA

Procedure

Two parallels of about 40 mg of each abalone sample were weighed out into glass tubes. Care was taken to record the exact weight of the samples. Thereafter, 0.7 ml of distilled water was then added to the tubes. Exactly 0.5 ml of 20mM norleucine was then added to the tubes. This was then mixed with 1.2 ml of 37% hydrochloric acid. The glass tubes were flushed with nitrogen gas for 10 seconds and capped immediately to prevent oxidation. The capped tubes were then placed in the heating cabinet set at 110 ° C and left for 24 hours. Afterward, the samples were removed from the heating cabinet and allowed to cool. About 500 µl of sample solution was pipetted into Eppendorf tubes and then centrifuged at 4000 g, for 10 minutes to obtain a clear supernatant. 100 µl of this supernatant was then transferred to short thread assay vials. Then 1ml of the loading buffer, lithium citrate kept at a pH of 2.2 was added to the assay vials.

The samples were analyzed using the Biochrom 30 Amino Acid Analyzer with lithium citrate equilibrated column and post-column derivatization with ninhydrin. The signals were analyzed with the Chromeleon Software and the identification of the amino acids was done by comparison with the A9906 physiological amino acid standard. The retrieved data was then copied to an Excel spreadsheet for further analysis. Following the guidelines drawn by FAO (2003), sums of individual amino acid residues (the molecular weight of each amino acid minus the molecular weight of water) were taken as the protein content of the sample.

3.7 Lipid Class Composition Analysis

The composition of the lipid classes was determined using a normal phase HPLC method developed by Abreu et al., (2017). A gradient program was used (Appendix 1). Three mobile phases namely - Mobile phase A: isooctane/ethyl acetate (99.8:0.2), Mobile phase B: acetone/ethyl acetate (2:1) 0.15 % acetic acid, Mobile phase C: isopropanol/H_2O (85:15) were used for the HPLC.

Table 6. Materials and equipment used for HPLC based determination of lipid classes.

Equipment	**Distributor, Country**
Waters e2795 Separations Module	Waters, USA
Supelcosil LC-SI 5 µm (25cm x 4.6 mm) column	Supelco HPLC products, USA
Waters 2424 ELS detector	Waters, USA
Mass Lynx software	Waters, USA
Reagents/Chemicals	**Distributor, Country**
Chloroform	Honeywell, Germany
Isooctane	Honeywell, Germany
Isopropanol	Sigma Aldrich, USA
Ethyl acetate	Honeywell, Germany
Acetone	Honeywell, Germany
Acetic acid	Honeywell, Germany
Lipid standards	Sigma Aldrich, USA; Larodan AB, Sweden

Procedure

Lipid was extracted from 0.5 g of each test sample using the modified Folch's method previously described in section 3. 4. The obtained lipid was then dissolved to concentrations of 1 mg/ml and 0.1 mg/ml in a solution of mobile phase A/Chloroform (4:1 v/v) before analysis. The higher concentration was used to detect analytes that may be present in small amounts while the lower concentration was used to avoid saturation of the peaks from the larger constituents. All samples were put in 1.5 ml sample vials and capped with HPLC pre-slit vial lids. Thereafter, the samples were passed through the Waters e2795 separations module, coupled to the Supelcosil™ LC-SI column that was set to a working temperature of 40 °C. Lipids were then quantified using a Waters 2424 ELS detector set to gain 100, nebulizer heating level set to 30%, drift tube temperature set to 45 °C, and pressure set to 40 PSI. The total run time was 41 minutes using the gradient profile and mobile phases. Chromatograms were retrieved using the Mass Lynx software. Lipids were then quantified based on peak area in the chromatograms and converted to absolute amounts based on standard curves obtained from the lipid standards used.

3.8 Bioactivity Tests

3.8.1 Preparation of Extracts

Table 7. Materials and equipment used in preparing extracts for bioactivity tests.

Equipment	**Distributor, Country**
Heraeus Multifuge 1 S-R Centrifuge	Thermo Scientific, USA
Rotary evaporator	IKA, Germany
Freeze dryer	Labogene, Denmark
Reagents/Chemicals	**Distributor, Country**
Hexane	Honeywell, Germany
DCM	Honeywell, Germany
Ethyl acetate	Honeywell, Germany
Methanol	VWR, Germany

All abalone samples were extracted for 24 hours using five solvents: Hexane, Dichloromethane (DCM), ethyl acetate, methanol, and distilled water. Afterward, the mixture was centrifuged at 4000g for 20 minutes. The supernatants were then collected in new bottles. The crude extracts were concentrated with the rotary evaporator set to automatic pressure adjustment, and temperature set at 40 °C. The aqueous extract was concentrated using the freeze drier. Thereafter the extracts were reconstituted to a concentration of 200 mg/ ml, from which dilutions were then prepared and used in the bioactivity assays. Extracts were stored at -18 °C and used within two weeks.

3.8.2 Ferric ion Reducing Antioxidant Power (FRAP) Assay

Table 8. Materials, equipment, and software used for FRAP assay.

Equipment/Software	**Distributor, Country**
White 96-well microplates	VWR, Germany
Spectramax i3 microplate reader	Molecular devices, USA
SoftmaxPro version 6 software	Molecular devices, USA
Reagents/Chemicals	**Distributor, Country**
Iron trichloride hexahydrate	Merck, Germany
2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ)	Sigma-Aldrich, USA
Hydrochloric acid	Honeywell, Germany
Sodium acetate	Merck, Germany
Acetic acid	Honeywell, Germany
6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox)	Sigma-Aldrich, USA
Methanol	VWR, Germany

Procedure

The FRAP assay was performed by following the procedure described in Benzie and Strain (1996), with some modifications. The FRAP-solution was prepared by mixing 50 mL acetate buffer (1.505 g sodium acetate and 8 mL acetic acid and then topped to 500 mL with distilled water) with 5

mL of 2,4,6-Tri(2-pyridyl)-2-triazine (TPTZ) solution (0.0312 g of TPTZ in 10 mL of 40 mM hydrochloric acid) and 5 mL of iron trichloride hexahydrate (19 mM) solution. The final solution was then incubated at 37 °C before use.

Dilutions of the five solvent extracts of abalone were made from a starting concentration of 100mg/ml down to a low concentration of 0.005mg/ml to ascertain the concentrations that fell within the standard curve. The assay standard, trolox was first dissolved in methanol before diluting with water. Water dilutions of trolox were then made from 1000 µM to 15.625 µM.

In the microplate 10 µL of sample or standard solution, 30 µL of distilled water, and 300 µL of FRAP-solution were added. Parallels of sample and standard concentrations were made in the microplate to reduce experimental error. This was incubated for 30 minutes at 37◦C before using the microplate reader to measure light absorbance at 593 nm in comparison with a blank. The absorbance value of the blank well was automatically subtracted from the absorbance value of the standard and sample wells. The absorbance data were then retrieved and analyzed using the SoftMax Pro software. The experiment was then repeated twice to reduce error. The linear equations derived from the standard curves were then used to estimate the FRAP values of the samples and finally expressed as millimole trolox equivalents (TE) per 100 g of dry weight (DW). The step to derive sample concentration in TE from the standard curve is illustrated in equation 3. 5 below.

$Y = aX + b$ ……. (Eq. 3.5; Y = Absorbance, X = Concentration in µMTE/ml)

3.8.3 Oxygen Radical Absorbance Capacity (ORAC) Assay

Table 9. Materials, equipment, and software used for ORAC assay.

Equipment/Softwares	**Distributor, Country**
Black 96-well microplates	VWR, Germany
Spectramax i3 microplate reader	Molecular devices, USA
SoftmaxPro version 6 software	Molecular devices, USA
Reagents/Chemicals	**Distributor, Country**
6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox)	Sigma-Aldrich, USA
Sodium phosphate dibasic	Sigma-Aldrich, USA

Hydrochloric acid	Honeywell, Germany
2,2'-Azobis(2-methylpropionamidine) dihydrochloride (AAPH)	Sigma-Aldrich, USA
Fluorescein sodium salt	Sigma-Aldrich, USA

Procedure

The ORAC assay was performed as described by Davalos et. al, (2004) with some modifications. Antioxidant samples and all reagents used in the assay were diluted with 75 mM phosphate buffer (pH 7.4). Abalone samples were diluted from the stock concentration of 200mg/ml to a low concentration of 0.005mg/ml. Four dilutions of the assay standard, Trolox ranging from 50 µM to 6.25 µM were used. In the black microplate wells, 25 µL of sample or standard and 125 µL of 50 mM fluorescein were added and the mixture was incubated using the plate reader for 15 minutes at 37 °C. Thereafter, 50 µl of 38mM AAPH was rapidly added to each well using a multichannel pipet. The microplate was immediately placed in the plate reader and the fluorescence was measured kinetically at 485 and 535 nm with excitation set at 9nm and emission at 15 nm. The microplate was automatically shaken for 5 seconds before the first reading and 3 seconds before subsequent readings every 60 seconds for 35 minutes. Plate settings and fluorescence readings were retrieved using the SoftMax Pro 6 software. A blank well, containing fluorescein, AAPH, and phosphate buffer instead of the antioxidant solution was used in each assay. All the reaction mixtures were prepared in duplicate, and three independent assays were performed for each sample.

ORAC values were calculated as described by Cao and Prior (1999). The area under the curve (AUC) and the Net AUC of the standards and samples were automatically calculated by SoftMax pro 6 using equations 3.6 and 3.7 respectively.

$$\mathrm{AUC} = (\mathrm{R1}/\mathrm{R1}) + (\mathrm{R2}/\mathrm{R1}) + (\mathrm{R3}/\mathrm{R1}) + \ldots + (\mathrm{Rn}/\mathrm{R1}) \quad \text{..... (Eq. 3.6)}$$

Where R1 is the fluorescence reading at the initiation of the reaction and Rn is the last reading.

$$\text{Net AUC} = \text{AUC Test} - \text{AUC blank} \quad \text{..... (Eq. 3.7)}$$

The SoftMax Pro 6 software was used to obtain the standard curve and a linear or quadratic standard curve equation (Depending on which model was a better fit) by plotting the Net AUC of different Trolox concentrations against their concentration. ORAC values of samples were then calculated and expressed as mmol TE, and then normalized to dry weight. Further data analyses for comparison of sample sets and extraction solvent were performed using Microsoft Excel and R software.

3.8.4 HMG Co-A Reductase Inhibitory Activity Assay

The HMG Co-A reductase inhibitory activity was performed using a commercial kit provided by Sigma-Aldrich. The analysis was an adaptation of the procedure described by Zhou et al., (2015) and the instructions on the assay kit's product bulletin. The kit's reagents were thawed, diluted, and stored according to the product's manual. All dilutions were made with ultrapure water.

Table 10. Materials, equipment, and software used for HMG Co-A reductase inhibitory assay.

Equipment	**Distributor, Country**
Flat bottom, clear bottom 96-well microplates	VWR, Germany
Spectramax i3 microplate reader	Molecular devices, USA
SoftmaxPro version 6 software	Molecular devices, USA
Reagents/Chemicals	**Distributor, Country**
Hexane	Sigma-Aldrich, USA
Ethyl Acetate	Sigma-Aldrich, USA
Methanol	VWR, Germany
Dichloromethane	Honeywell, Germany
HMG Co-A reductase assay kit	Sigma-Aldrich, USA

Procedure

The plate reader was first set to 37 °C and 340 nm, with a kinetic program to read the 96 well-plate every 45 seconds for 10 minutes. The program was also set to shake the plate vigorously for 10 seconds before the first absorbance measurement.

The assay buffer was first added to the microplates; 181 µl to the inhibition wells, 182 µl to the activity wells, and 184 µl to the blank. Then 1 µl of sample and pravastatin (positive control) were then added to the inhibition wells. Thereafter, 4 µl of reconstituted NADPH and 12 µl of HMG-CoA were added to all wells. Finally, 2 µl of HMG-CoA reductase was added to the inhibition and activity wells before mixing the plate thoroughly. The kinetics program was started, and the plate was read immediately after the addition of HMG-CoA reductase. Absorbance readings were retrieved using the SoftmaxPro software. Inhibition activity was calculated using equation 3.8 below.

$$\text{Inhibition } \% = \left(\frac{\Delta \text{ Absorbance activity} - \Delta \text{ Absorbance test}}{\Delta \text{ Absorbance activity}}\right) \text{X } 100 \qquad \text{..... (Eq. 3. 8)}$$

3.9 Statistical Analysis

Means and standard deviations (SD) were calculated for each parameter measured. At the end of the experiments, proximate composition, biochemical composition, and bioactivity were calculated and compared statistically using Student's t-test. Rstudio was used for data manipulation and visualization. The significance level was set to 5% ($P \leq 0.05$)

4. RESULTS

All abalone used for the analyses in this study, except for the processed *H. midae*, were received as freeze-dried samples. For ease of comparison, all results are presented on a dry weight (DW) basis unless when otherwise indicated. The abbreviation, ND is used in tables to denote amounts that fell below the detection limit.

4.1 Proximate Composition

All abalone samples contained less than 20 g water/100 g freeze-dried/processed sample. The processed *H. midae*, dried according to the traditional South African salt drying procedure, had the highest remaining water content with a dry matter content (DMC) of 84.52 ± 1.15 g/100 g. Compared with the processed sample, the raw *H.midae* had a significantly ($P < 0.05$) higher DMC (91.90 ± 0.66 g/100 g). There were no significant DMC differences between the *H. tuberculata coccinea* samples and DMC was between 95.80 ± 0.23 g/100 g and 96.91 ± 0.72 g/100 g. There were, however, significant ($P <0.05$) DMC differences between the *H. tuberculata coccinea* samples and the *H. midae* samples as can be observed in table 11 below.

Protein content was highest in the *H. midae* raw sample (49.3 g/100 g). This was significantly ($P < 0.05$) higher than the content of both the processed sample and the *H. tuberculata* coccinea control sample. Apart from the artificial diet group that had a comparatively low protein content (34.40 ± 0.47 g/100 g), protein contents in the *H. tuberculata* samples were marginally different. All protein content values are presented in table 11.

Conversely, the fat contents of both *H. midae* groups were lower than the contents of the *H. tuberculata coccinea* samples. There was no noticeable difference in fat content between the raw and the processed *H. midae*, with mean observed values at 2.36 ± 0.64 g/100 g and 2.55 ± 0.34 g/100 g, respectively. There was, however, a significant ($P < 0.05$) difference between the *H. midae* sample and the control sample, whose mean fat content was 4.82 ± 0.44 g/100 g: the highest observed value in the fat content analysis. The artificial diet group (3.58 ± 1.16 g/100 g) had the lowest fat content amongst the *H. tuberculata coccinea* samples. All fat content values are listed in table 11 below.

Table 11. Proximate composition of *H. midae** and *H. tuberculata coccinea*** samples.

	g per 100 g DW		
	Fat	Protein	Dry weight***
H. midae			
Processed	2.55 ± 0.34[a]	36.9 ± 1.46[a]	84.52 ± 1.15[a]
Raw	2.36 ± 0.64[a]	49.3 ± 4.3[c]	91.90 ± 0.66[b]
H. Tuberculata coccinea			
Artificial diet	3.58 ± 1.16	34.40 ± 0.47	96.91 ± 0.72[c]
Hybrid diet	4.24 ± 0.69	39.73 ± 0.13	96.21 ± 1.37[c]
Control	4.82 ± 0.44[b]	41.9 ± 2.84[b]	95.80 ± 0.23[c]
RS	4.59 ± 0.36	41.89 ± 1.44	95.88 ± 0.23[c]

* Five (individual animals) replicates from each experiment

** Pooled batches of animals from each treatment

*** g dry matter/100 g freeze dried/processed sample

[a, b, c] Means in a column bearing different superscripts differ significantly ($P < 0.05$)

4.2 Fatty Acid Composition

4.2.1 Fatty Acid Composition of *H. tuberculata coccinea* Treatments

The FA composition of the *H. tuberculata coccinea* samples was substantially different between the treatments and none of the different groups had a similar FA profile. The SFA C16:0 was the

most abundant FA in all the samples. The highest C16:0 levels were found in the control group (1.73 ± 0.69 g/100 g), and the lowest levels in the hybrid diet group (1.29 ± 0.10 g/100 g). Total SFA in the artificial diet group (2.26 ± 0.38 g/100 g) and the control group (2.28 ± 0.94 g/100 g) was higher than in the hybrid diet group (1.71 ± 0.06 g/100 g) and in the RS group (1.57 ± 0.01 g/100 g). However, the SFA percentage of total FA was highest in the RS group (42.63 ± 0.28%) and least in the hybrid diet group (29.93 ± 1.01%).

Five monounsaturated fatty acids (MUFAs) were identified and C18:1 n-7 and C18:1 n-9 were the most abundant. The artificial diet group had the highest amount and composition percentage of total MUFA (1.29 ± 0.35 g/100 g and 24.57 ± 3.05%, respectively). The RS group (0.673 ± 0.11 g/100 g) had the least amount of MUFA while the control group (14.80 ± 9.71%) had the least composition percentage. A detailed presentation is given in table 12 below.

The hybrid diet group (2.14 ± 1.23 g/100 g) contained the highest absolute amounts of PUFA while the artificial diet group (1.42 ± 0.07 g/100 g), control group (1.78 ± 0.89 g/100g), and the RS group (1.07 ± 0.12 g/100 g) contained significantly lower amounts of PUFA. As can be seen from Table 11, the higher PUFA amount in the hybrid diet group was contributed by a distinctly higher level of DHA (0.81 ± 0.88 g/100 g), and a slightly higher level of DPA (0.35 ± 0.16 g/100 g) than the artificial diet group (0.35 ± 0.11 g/100 g, 0.21 ± 0.03 g/100 g, respectively) and the control group (0.08 ± 0.002 g/100 g, 0.34 ± 0.19 g/100 g, respectively). The RS group had the least amount of DHA (0.17 ± 0.18 g/100 g), DPA (0.20 ± 0.02 g/100 g), and EPA (0.15 ± 0.06 g/100 g) while the artificial diet group and the control group had equal amounts of EPA (0.317 ± 0.12 g/100 g and 0.317 ± 0.16 g/100 g, respectively). The PUFA percentage of total FA was 29.24 ± 14.5% in the control group and 29.15 ± 3.14% in the RS group. It was relatively higher in the hybrid diet group (37.48 ± 21.5%) and relatively lower in the artificial diet group (24.22 ± 0.56%).

Table 12. Fatty acid composition of pooled samples from the four different treatments of *H. tuberculata coccinea* presented in absolute amounts (g per 100 g) and percentage of total fatty acids.

H. tuberculata coccinea

	ARTIFICIAL DIET		HYBRID DIET		CONTROL		RS	
FATTY ACID	Amount	Percentage	Amount	Percentage	Amount	Percentage	Amount	Percentage
C14:0	0.30 ± 0.02	5.15 ± 0.42	0.15 ± 0.004	2.65 ± 0.06	0.17 ±0.06	2.74 ± 1.03	0.13 ± 0.000	3.57 ± 0.01
C16:0	1.63 ± 0.29	30.03 ± 2.48	1.29 ± 0.10	22.56 ± 1.81	1.73 ± 0.69	28.42 ± 11.3	1.16 ± 0.08	31.39 ± 0.52
C18:0	0.30 ± 0.08	5.71 ± 0.68	0.18 ± 0.06	3.07 ± 1.07	0.15 ± 0.07	2.49 ± 1.17	0.15 ± 0.08	4.18 ± 2.14
C20:0	0.03 ± 0.01	0.41 ± 0.04	0.10 ± 0.02	1.66 ± 0.33	0.23 ± 0.11	3.79 ± 1.84	0.13 ± 0.05	3.50 ± 1.33
TOTAL SFA	2.26 ± 0.38	41.44 ± 3.33	1.71 ± 0.06	29.93 ± 1.01	2.28 ± 0.94	37.43 ± 15.4	1.57 ± 0.01	42.63 ± 0.28
C16:1 n-7	0.15 ± 0.01	2.73 ± 0.09	0.07 ± 0.01	1.27 ± 0.14	0.07 ± 0.03	1.15 ± 0.54	0.04 ± 0.02	1.06 ± 0.50
C18:1 n-7	0.50 ± 0.05	8.28 ± 0.47	0.45 ± 0.09	7.82 ± 1.58	0.48 ± 0.40	7.86 ± 6.51	0.33 ± 0.10	8.97 ± 2.82
C18:1 n-9	0.63 ± 0.37	13.10 ± 3.21	0.33 ± 0.08	5.69 ± 1.34	0.28 ± 0.13	4.65 ± 2.07	0.27 ± 0.03	7.29 ± 0.89
C20:1 n-9	0.02 ± 0.03	0.47 ± 0.22	0.01 ± 0.01	0.18 ± 0.25	ND		ND	
C22:1 n-9	ND		ND		0.07 ± 0.04	1.14 ± 0.59	0.04 ± 0.02	0.94 ± 0.51
TOTAL MUFA	1.30 ± 0.35	24.57 ± 3.05	0.85 ± 0.16	14.96 ± 2.81	0.90 ± 0.59	14.80 ± 9.71	0.67 ± 0.11	18.27 ± 2.93
C18:2 n-6	0.22 ± 0.03	4.08 ± 0.29	0.12 ± 0.02	2.15 ± 0.38	0.08 ± 0.03	1.22 ± 0.57	0.04 ± 0.01	1.09 ± 0.38
C18:3 n-4	ND		ND		ND		0.13 ± 0.18	3.40 ± 4.80
C18:4 n-3	0.16 ± 0.02	2.72 ± 0.14	0.21 ± 0.04	3.74 ± 0.70	0.39 ± 0.22	6.44 ± 3.61	0.06 ± 0.09	1.75 ± 2.47
C20:2 n-6	0.05 ± 0.01	0.88 ± 0.09	ND		ND		ND	
C20:4 n-6 ARA	0.11 ± 0.03	1.72 ± 0.27	0.38 ± 0.07	6.66 ± 1.24	0.58 ± 0.28	9.43 ± 4.58	0.33 ± 0.11	8.91 ±3.03
C20:5 n-3 EPA	0.32 ± 0.12	4.78 ± 1.02	0.27 ± 0.06	4.66 ± 0.99	0.32 ± 0.16	5.19 ± 2.66	0.15 ± 0.06	4.12 ± 1.57
C22:5 n-3 DPA	0.21 ± 0.03	3.38 ± 0.26	0.35 ± 0.16	6.04 ± 2.81	0.34 ± 0.19	5.61 ± 3.16	0.20 ± 0.02	5.39 ± 0.65
C22:6 n-3 DHA	0.35 ± 0.11	6.68 ± 0.93	0.81 ± 0.88	14.22 ± 15.4	0.08 ± 0.002	1.35 ± 0.04	0.17 ± 0.18	4.50 ± 4.82
TOTAL PUFA	1.42 ± 0.07	24.22 ± 0.56	2.14 ± 1.23	37.48 ± 21.5	1.78 ± 0.89	29.24 ± 14.5	1.07 ± 0.12	29.15 ± 3.14
n-3	1.03 ± 0.06	17.55 ± 0.49	1.64 ± 1.14	28.67 ± 19.9	1.13 ± 0.57	18.59 ± 9.40	0.58 ± 0.19	15.76 ± 5.07
n-6	0.39 ± 0.01	6.67 ± 0.07	0.50 ± 0.09	8.81 ± 1.62	0.65 ± 0.31	10.65 ± 5.15	0.37 ± 0.13	10.00 ± 3.40
n-6/n-3	0.38 ± 0.01	0.38 ± 0.01	0.38 ± 0.21	0.38 ± 0.21	0.58 ± 0.01	0.58 ± 0.01	0.71 ± 0.44	0.71 ± 0.44
UNIDENTIFIED	0.80 ± 0.07	13.45 ± 0.60	1.01 ± 0.87	17.63 ± 15.2	1.13 ± 0.75	18.53 ± 12.3	0.37 ± 0.28	9.95 ± 7.63

4.2.2 Fatty Acid Composition of the Raw and Processed *H midae*

Seventeen FAs were identified in *H. midae*, and all identified FAs were present in both the raw and processed samples. The FA profiles of the raw and processed samples were similar, and the most abundant FAs in both treatments were C16:0, C22:5 n-3, C18:0, C18:1 n-7, and C18:1 n-9, as presented in table 13. However, in absolute amounts, the samples differed significantly ($P < 0.05$) for six identified FAs, whereas the percentage of total FAs was significantly different for three FAs.

Three SFAs, C14:0, C16:0, and C18:0 was identified. C16:0 was the most abundant FA in both the raw (0.33 ± 0.03 g/100 g, 23.65 ± 4.86%) and the processed (0.25 ± 0.04 g/100 g, 22.65 ± 2.06%) samples. There was a significant difference ($P < 0.05$) in the amounts of C16:0 and C18:0 even though the percentage of total FAs were similar.

Four MUFAs were identified, and there were significant differences ($P < 0.05$) in the amounts of three FAs namely, C16:1 n-7, C18:1 n-7, and C20:1 n-9. The raw *H. midae* had higher amounts in each of these fatty acids. However, there was no significant difference in the percentage of total FAs across the MUFAs.

The only significant differences in the amount of PUFA were in two n-3 FAs. On one hand, the raw *H. midae* had EPA and DPA values of 0.09 ± 0.03 g/100 g and 0.18 ± 0.06 g/100 g, respectively. On the other hand, the processed *H. midae* had an EPA value of 0.07 ± 0.02 g/100 g and a DPA value of 0.13 ± 0.03 g/100 g. The raw *H. midae* contained significantly higher amounts of total n-3 and n-6 FAs than the processed sample. The PUFA percentages of total FAs were significantly ($P < 0.05$) different for C18:2n-6, C18:3n-4, and C20:4n-6. The processed sample had higher percentages of each of these PUFAs.

Table 13. Fatty acid composition of raw and processed *H. midae* in absolute amounts (g per 100 g) and percentage of total fatty acids. n = 5

	H. midae			
	PROCESSED		RAW	
FATTY ACID	Amount	Percentage	Amount	Percentage
C14:0	0.03 ± 0.01[a]	2.17 ± 0.50[a]	0.03 ± 0.004[a]	2.15 ± 0.36[a]
C16:0	0.25 ± 0.04[a]	22.65 ± 2.06[a]	0.33 ± 0.03[b]	23.65 ± 4.86[a]
C18:0	0.09 ± 0.02[a]	7.61 ± 0.45[a]	0.12 ± 0.02[b]	8.76 ± 1.86[a]
TOTAL SFA	0.36 ± 0.06[a]	32.43 ± 0.71[a]	0.49 ± 0.05[b]	34.56 ± 6.94[a]
C16:1 n-7	0.003 ± 0.005[a]	0.86 ± 0.13[a]	0.008 ± 0.007[b]	0.85 ± 0.13[a]
C18:1 n-7	0.08 ± 0.01[a]	7.34 ± 0.51[a]	0.10 ± 0.03[b]	7.04 ± 0.68[a]
C18:1 n-9	0.08 ± 0.02[a]	7.11 ± 0.42[a]	0.10 ± 0.02[a]	6.66 ± 0.54[a]
C20:1 n-9	0.02 ± 0.02[a]	2.69 ± 1.15[a]	0.05 ± 0.02[b]	3.28 ± 0.97[a]
TOTAL MUFA	0.19 ± 0.05[a]	16.59 ± 2.04[a]	0.25 ± 0.07[b]	17.17 ± 2.60[a]
C18:2 n-6	0.05 ± 0.01[a]	4.29 ± 0.29[a]	0.05 ± 0.01[a]	3.17 ± 0.58[b]
C18:3 n-3	0.05 ± 0.05[a]	3.65 ± 3.12[a]	0.06 ± 0.03[a]	4.33 ± 2.16[a]
C18:3 n-4	0.04 ± 0.02[a]	4.37 ± 0.86[a]	0.01 ± 0.02[a]	2.75 ± 0.21[b]
C18:4 n-3	0.02 ± 0.01[a]	1.69 ± 1.06[a]	0.02 ± 0.01[a]	1.21 ± 0.65[a]
C20:2 n-6	0.007 ± 0.01[a]	1.11 ± 0.24[a]	0.01 ± 0.01[a]	1.06 ± 0.39[a]
C20:4 n-6 ARA	0.003 ± 0.005[a]	5.00 ± 0.72[a]	0.06 ± 0.02[a]	4.14 ± 0.52[b]
C20:5 n-3 EPA	0.07 ± 0.02[a]	6.17 ± 0.18[a]	0.09 ± 0.03[b]	6.26 ± 1.08[a]
C22:4 n-6	0.01 ± 0.002[a]	0.83 ± 0.80[a]	0.002 ± 0.005[a]	0.67 ± 0.68[a]
C22:5 n-3 DPA	0.13 ± 0.03[a]	11.71 ± 0.54[a]	0.18 ± 0.06[b]	12.20 ± 1.77[a]
C22:6 n-3 DHA	0.02 ± 0.01[a]	2.08 ± 0.18[a]	0.03 ± 0.01[a]	1.80 ± 0.66[a]
TOTAL PUFA	0.44 ± 0.10[a]	38.71 ± 2.30[a]	0.51 ± 0.14[a]	34.71 ± 3.41[b]
n-3	0.29 ± 0.09[a]	25.12 ± 3.50[a]	0.38 ± 0.10[b]	25.80 ± 2.83
n-6	0.11 ± 0.02[a]	10.09 ± 0.71[a]	0.12 ± 0.04[b]	8.08 ± 1.39[b]
n-6/n-3	0.41 ± 0.07[a]	0.41 ± 0.05[a]	0.31 ± 0.05[a]	0.31 ± 0.05[a]
Unidentified	0.14 ± 0.05[a]	12.27 ± 2.04[a]	0.21 ± 0.10[a]	13.87 ± 4.51[a]

4.2.3 Fatty Acid Composition Differences Between *H. tuberculata coccinea* and *H. midae*

As previously described, seventeen FAs were identified in the *H. midae* raw sample. Its total unidentified FAs weighed 0.21 ± 0.10 g/100 g. The percentage of unidentified FAs was 13.87 ± 4.51% of its total FAs. On the other hand, only fourteen FAs were identified in the *H. tuberculata coccinea* control group. The percentage of its unidentified FAs was 15.75 ± 4.08%; in absolute amount, 0.78 ± 0.44 g/100 g sample. The FAs C20:0 and C22:1 n-9 were quantified only in the *H. tuberculata coccinea* control sample. On the other hand, C20:1 n-9, C18:3 n-3, C18:3 n-4, C20:2 n-6, and C22:4 n-6 were not detected in the control sample but were found in the raw *H.midae*. The absolute amount of the FAs detected in both samples were significantly ($P < 0.05$) different across nine FAs, while the percentage of total FAs was significantly different across three FAs.

SFA profile in the two samples was considerably different. The FA, C16:0 was the most abundant in both samples. Its weight was 0.33 ± 0.03 g/100 g in the *H. midae* raw sample. It was, however, significantly ($P < 0.05$) higher in the control sample (1.46 ± 0.78 g/100 g). The SFA percentage of total FA was not significantly different; 34.56 ± 6.94% in raw *H. midae*, and 39.59 ± 3.71% in the control sample. However, the total SFA amount in the control sample (2.02 ± 0.83 g/100 g) was four times the amount in the raw *H. midae* (0.49 ± 0.05 g/100 g).

Even though the number of unique MUFAs was equal in both species, there were qualitative differences. Total MUFA amount and percentage of total FAs in raw *H. midae* was 0.25 ± 0.07 g/100 g and 17.17 ± 2.60%, respectively. The corresponding values in the control sample were 1.16 ± 0.38 g/100 g and 18.03 ± 6.85%, respectively.

Four PUFAs, including C22:4 n-6, that were found in the raw *H. midae* were not detected in the control sample. However, the total PUFA amount was significantly ($P < 0.05$) higher in the control sample. EPA and DHA percentages of total FAs were similar in both samples. In raw *H. midae*, EPA and DHA were 6.26 ± 1.08% and 1.80 ± 0.66%, respectively. In the control group, it was 6.00 ± 1.39% and 1.57 ± 0.85%, respectively. Finally, the raw *H. midae* contained a significantly higher percentage of DPA than the control sample even though both species contained similar amounts of this FA. Table 14 below presents a detailed FA profile comparison of the two abalone samples.

Table 14. Fatty acid composition of raw *H. midae* (n =5) and the control *H. tuberculata coccinea* (n = 3) in absolute amounts (g/100 g) and percentage of total fatty acids.

	H. midae		***H. tuberculata coccinea***	
	RAW		CONTROL	
FATTY ACID	Amount	Percentage	Amount	Percentage
C14:0	0.03 ± 0.004[a]	2.15 ± 0.36[a]	0.23 ± 0.06[b]	3.46 ± 1.10[a]
C16:0	0.33 ± 0.03[a]	23.65 ± 4.86[a]	1.46 ± 0.78[b]	30.1 ± 3.01[a]
C18:0	0.12 ± 0.02[a]	8.76 ± 1.86[a]	0.26 ± 0.09[b]	3.51 ± 1.72[b]
C20:0	ND		0.23 ± 0.11	3.83 ± 0.15
TOTAL SFA	0.49 ± 0.05[a]	34.56 ± 6.94[a]	2.03 ± 0.83[b]	39.59 ± 3.71[a]
C16:1 n-7	0.01 ± 0.007[a]	0.85 ± 0.13[a]	0.09 ± 0.03[b]	1.44 ± 0.47[a]
C18:1 n-7	0.10 ± 0.03[a]	7.04 ± 0.68[a]	0.53 ± 0.20[b]	8.29 ± 2.83[a]
C18:1 n-9	0.10 ± 0.02	6.66 ± 0.54	0.45 ± 0.16	7.04 ± 0.68
C20:1 n-9	0.05 ± 0.02	3.28 ± 0.97	ND	
C22:1 n-9	ND		0.07 ± 0.02	1.26 ± 0.19
TOTAL MUFA	0.25 ± 0.07[a]	17.17 ± 2.60[a]	1.16 ± 0.38[b]	18.03 ± 6.85[a]
C18:2 n-6	0.05 ± 0.01[a]	3.17 ± 0.58[a]	0.06 ± 0.02[a]	1.15 ± 0.17[b]
C18:3 n-3	0.06 ± 0.03	4.33 ± 2.16	ND	ND
C18:3 n-4	0.05 ± 0.02	2.75 ± 0.21	ND	ND
C18:4 n-3	0.02 ± 0.01[a]	1.21 ± 0.65[a]	0.17 ± 0.20[b]	4.64 ± 3.00[a]
C20:2 n-6	0.02 ± 0.01	1.06 ± 0.39	ND	ND
C20:4 n-6 ARA	0.06 ± 0.02[a]	4.14 ± 0.52[a]	0.34 ± 0.22[b]	7.80 ± 3.00[a]
C20:5 n-3 EPA	0.09 ± 0.03[a]	6.26 ± 1.08[a]	0.37 ± 0.11[b]	6.00 ± 1.39[a]
C22:4 n-6	0.01 ± 0.008	0.67 ± 0.68	ND	ND
C22:5 n-3 DPA	0.18 ± 0.06[a]	12.20 ± 1.77[a]	0.25 ± 0.12[a]	5.03 ± 0.90[b]
C22:6 n-3 DHA	0.03 ± 0.01[a]	1.80 ± 0.66[a]	0.08 ± 0.002[b]	1.57 ± 0.85[a]
TOTAL PUFA	0.51 ± 0.14[a]	34.71 ± 3.41[a]	1.37± 0.55[b]	26.64 ± 4.87[a]
n-3	0.38 ± 0.10[a]	25.80 ± 2.83[a]	0.82 ± 0.37[b]	16.71 ± 3.39[b]
n-6	0.12 ± 0.04[a]	8.08 ± 1.39[a]	0.41 ± 0.24[b]	8.94 ± 3.16[a]
n-6/n-3	0.31 ± 0.05[a]	0.31 ± 0.05[a]	0.48 ± 0.08[b]	0.49 ± 0.06[b]
Unidentified	0.21 ± 0.10[a]	13.87 ± 4.51[a]	0.78 ± 0.44[b]	15.75 ± 4.08[a]

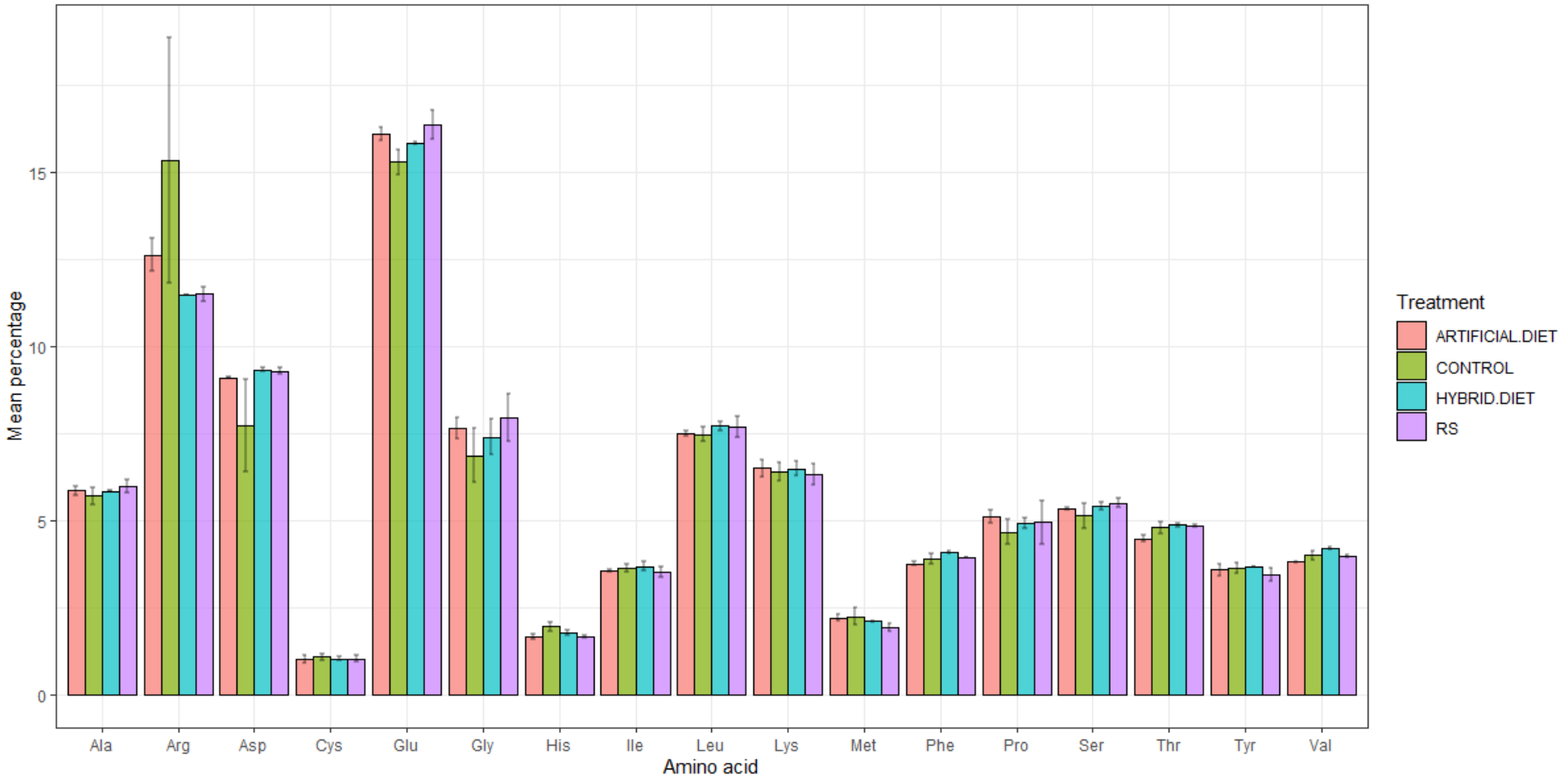


Figure 2. Percent composition of the amino acids quantified in samples from the four treatments of *H. tuberculata coccinea*.

4.3 Amino Acid Composition

Overall, there were notable differences in AA composition across the different treatments. These differences are highlighted in this section. The AA composition is reported as both percentage of total AAs, and as absolute amounts in mg/ g DW.

4.3.1 Amino Acid Composition of the *H. tuberculata coccinea* samples

Seventeen AAs were identified in all four treatments of the *H. tuberculata coccinea* samples. These AAs included all essential amino acids, except tryptophan, and nine non-essential amino acids. Figure 2 above is a bar chart of the mean percentage of total AAs for all identified AAs. The four treatments resulted in abalone with identical percent composition of AAs. The most abundant AAs were the non-essentials. Leucine was the only EAA that was over 7% across all treatment groups. Howbeit, four non-EAAs; arginine, glycine, glutamic acid, and aspartic acid (glutamic acid representing both glutamic acid and glutamine and aspartic acid representing both aspartic acid and asparagine) were all over 7% by composition.

The most abundantly AA was glutamic acid (representing both glutamic acid and glutamine). It was most abundant in the RS group (16.34 ± 0.42%) and least abundant in the control group (15.74 ± 0.23%). Arginine showed the most variability in composition across the four treatments. It was most abundant in the control diet group (15.35 ± 3.54%) and least abundant in the hybrid diet group (11.48 ± 0.01%). Cysteine was the least abundant AA, making up roughly 1% of total AAs across all treatment groups.

In absolute amounts, glutamic acid, the most abundant in all treatments, was highest in the RS group (68.52 ± 0.61 mg/g DW) and least in the artificial diet group (55.40 ± 1.38 mg/g DW), see table 15. Cysteine was the least abundant AA and lowest in the artificial diet group (3.48 ± 0.44 mg/g DW).

The highest amount of total EAA was found in the control group (144.32 ± 9.08 mg/g DW) while the least was found in the artificial diet group (115.44 ± 0.45 mg/g DW). The total EAA in the RS group was 141.97 ± 1.45 mg/g DW, and in the hybrid diet group was 138.96 ± 3.34 mg/g DW. Similarly, the total amino acid (TAA) was also highest in the control group (419.16 ± 9.35 mg/g DW) and least in the artificial diet group (344.04 ± 4.67 mg/g DW). TAA values in

the RS group and the hybrid diet group were 418.86 ± 14.40 mg/g DW and 397.27 ± 1.29 mg/g DW, respectively. A full comparison of the AA profiles for the four treatment groups is shown in table 15 below.

Table 15. Amino acids quantified (mg/g DW) from samples of *H. tuberculata coccinea* representing the four different treatments.

H. Tuberculata coccinea

AMINO ACID	ARTIFICIAL DIET	CONTROL	HYBRID DIET	RS
Histidine	5.73 ± 0.16	8.22 ± 1.11	7.08 ± 0.32	6.96 ± 0.04
Isoleucine	12.28 ± 0.07	15.21 ± 0.99	14.68 ± 0.54	14.72 ± 0.12
Leucine	25.85 ± 0.11	31.34 ± 1.72	30.66 ± 0.67	32.23 ± 0.12
Lysine	22.41 ± 0.53	26.82 ± 1.55	25.82 ± 0.96	26.51 ± 0.33
Methionine	7.62 ± 0.41	9.50 ± 1.59	8.37 ± 0.13	8.07 ± 0.17
Phenylalanine	12.95 ± 0.06	16.34 ± 0.72	16.29 ± 0.19	16.45 ± 0.57
Threonine	15.44 ± 0.51	20.13 ± 0.86	19.37 ± 0.29	20.35 ± 0.88
Tryptophan*	ND		ND	ND
Valine	13.16 ± 0.10	16.75 ± 0.82	16.69 ± 0.24	16.69 ± 0.70
EAA (Total)	115.44 ± 0.45	144.32 ± 9.08	138.96 ± 3.34	141.97 ± 1.45
Arginine	43.47 ± 1.01	65.15 ± 18.41	45.62 ± 0.20	48.23 ± 0.77
Alanine	20.16 ± 0.69	23.92 ± 1.06	23.27 ± 0.09	25.10 ± 1.65
Aspartic acid**	31.36 ± 0.47	32.16 ± 3.25	37.12 ± 0.28	38.93 ± 0.91
Cysteine	3.48 ± 0.44	4.56 ± 0.35	4.14 ± 0.27	4.37 ± 0.60
Glutamic acid**	55.40 ± 1.38	64.08 ± 3.27	62.95 ± 0.33	68.52 ± 0.61
Glycine	26.36 ± 1.40	28.69 ± 2.38	29.43 ± 1.89	33.38 ± 3.99
Proline	17.63 ± 0.89	19.56 ± 0.86	19.61 ± 0.49	20.79 ± 3.3
Serine	18.39 ± 0.34	21.47 ± 0.45	21.50 ± 0.35	23.09 ± 1.35
Tyrosine	12.34 ± 0.38	15.23 ± 0.65	14.68 ± 0.06	14.47 ± 0.25
TAA	344.04 ± 4.67	419.16 ± 9.35	397.27 ± 1.29	418.86 ± 14.40
EAA/TAA	0.34± 0.003	0.34± 0.008	0.35± 0.007	0.34± 0.008

* tryptophan is degraded during the acid hydrolysis

** Glutamic acid and aspartic acid represent the sums of aspartic acid + asparagine and glutamic acid + glutamine, respectively, as asparagine and glutamine are converted into their acidic forms during the acid hydrolysis

4.3.2 Amino Acid Composition of the Raw and Processed *H. midae*

Seventeen AAs were detected in the raw *H. midae* whereas sixteen AAs were detected in the processed sample. Cysteine was the least abundant AA in the raw *H. midae* (0.56 ± 0.16%). It was below the detection limit in the processed sample. The two most abundant AAs were non-EAAs as can be seen from figure 3 below. Glutamic acid was the most abundant AA in both samples, and it accounted for 18.57 ± 0.41% in the raw *H. midae* and 19.01 ± 0.47% in the processed *H. midae*. The proportion of arginine, the second most abundant amino acid, was 12.52 ± 0.50% in the raw *H. midae* and 12.49 ± 1.15% in the processed *H. midae*.

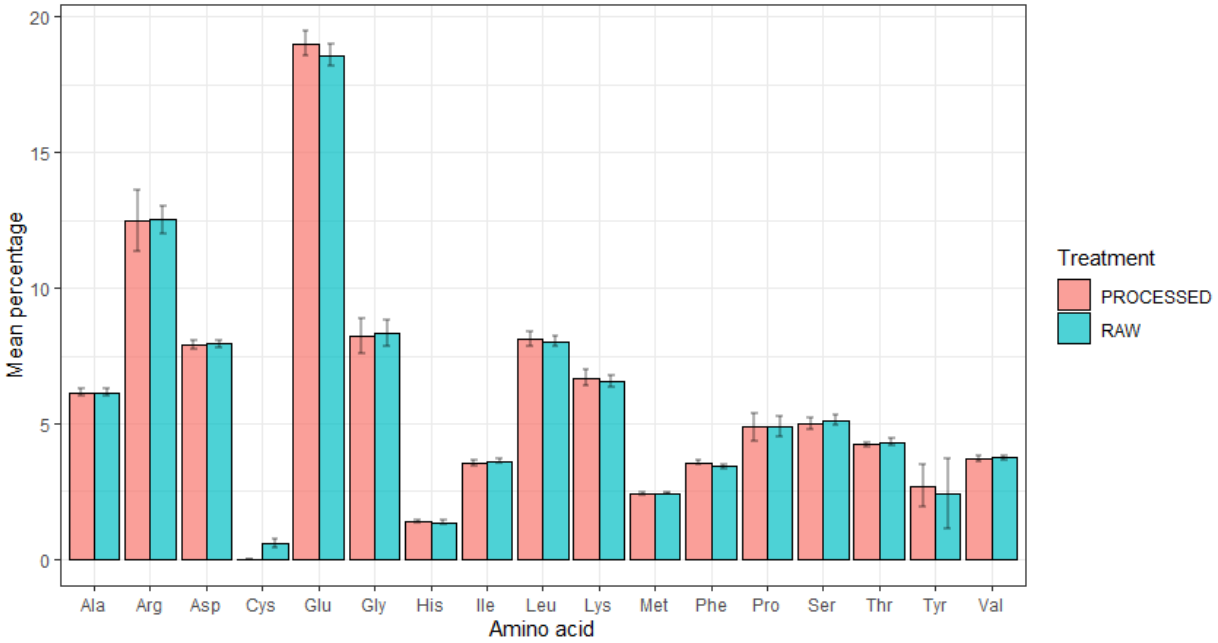


Figure 3. Percent composition of amino acids quantified in the raw and processed *H. midae* (n=5).

For all detected AAs, raw *H. midae* had higher absolute amounts than processed *H. midae*. Except for tyrosine, the difference between the absolute amounts across all AAs was significant ($P < 0.05$). EAA and TAA amounts for raw *H. midae* were 165.21 ± 15.1 mg/g DW and 493.66 ± 43.89 mg/g DW, respectively. The corresponding values for the processed *H.*

midae were 124.43 ± 5.67 mg/g DW and 369.70 ± 14.56 mg/g DW, respectively. The Full AA profile of the two samples is presented in table 16.

Table 16. Amino acids quantified (mg/g DW) in the raw and processed *H. midae* (n = 5).

	H. midae	
AMINO ACID	PROCESSED	RAW
Histidine	5.14 ± 0.17[a]	6.70 ± 0.49[b]
Isoleucine	13.10 ± 0.71[a]	17.88 ± 1.74[b]
Leucine	30.08 ± 1.65[a]	39.59 ± 3.52[b]
Lysine	24.67 ± 1.52[a]	32.29 ± 2.76[b]
Methionine	8.98 ± 0.47[a]	11.98 ± 1.02[b]
Phenylalanine	13.20 ± 0.48[a]	16.93 ± 1.63[b]
Threonine	15.61 ± 0.79[a]	21.37 ± 2.36[b]
Tryptophan*		
Valine	13.65 ± 0.51[a]	18.47 ± 1.96[b]
EAA (Total)	124.43 ± 5.67[a]	165.21 ± 15.1[b]
Arginine	46.15 ± 4.55[a]	61.76 ± 5.60[b]
Alanine	22.74 ± 0.97[a]	30.38 ± 2.87[b]
Aspartic acid**	29.18 ±1.20[a]	39.22 ± 3.62[b]
Cysteine	ND	2.82 ± 0.95[b]
Glutamic acid**	70.27 ± 3.09[a]	91.62 ± 7.51[b]
Glycine	30.43 ± 2.49[a]	41.26 ± 4.80[b]
Proline	17.99 ± 1.96[a]	24.22 ± 3.42[b]
Serine	18.51 ± 1.13[a]	25.35 ± 2.94[b]
Tyrosine	10.00 ± 3.02[a]	11.83 ± 6.39[a]
TAA	369.70± 14.56[a]	493.66 ± 43.89[b]
EAA/TAA	0.34± 0.008[a]	0.33± 0.005[a]

* tryptophan is degraded during the acid hydrolysis

** Glutamic acid and aspartic acid represent the sums of aspartic acid + asparagine and glutamic acid + glutamine, respectively, as asparagine and glutamine are converted into their acidic forms during the acid hydrolysis.

4.3.3 Amino Acid Composition Differences Between the control *H. tuberculata coccinea* and the raw *H. midae*

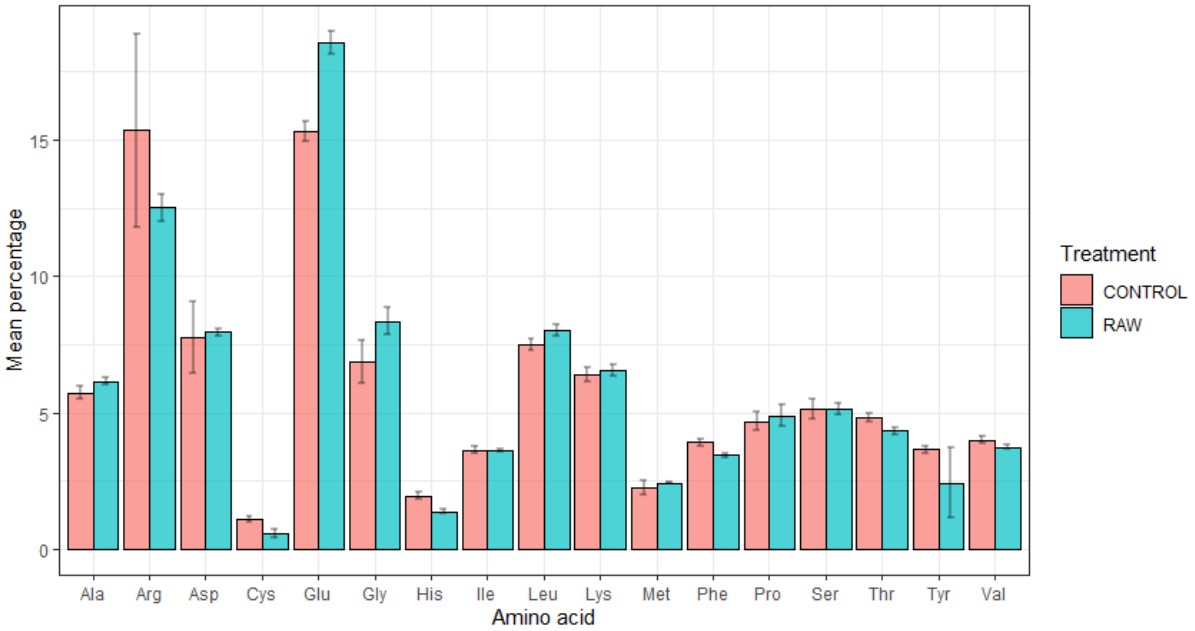


Figure 4. Percent composition of amino acids identified in the raw *H. midae* (n = 5) and the control *H. tuberculata coccinea* sample (n = 3).

Figure 4 above shows a comparison of the AA percentage of TAA recorded in the *H. tuberculata coccinea* control group and the raw *H. midae*. There were significantly ($P < 0.05$) higher percentages of glutamic acid (glutamic acid + glutamine), glycine, and leucine in the raw *H. midae* than in the *H. tuberculata coccinea* sample. The composition percentage of EAAs was generally higher in the *H. tuberculata coccinea* sample.

The absolute amounts of most of the EAAs in the two species were significantly different ($P < 0.05$), and only phenylalanine and threonine did not differ. A similar pattern was recorded across the non-EAAs, as only the amount of arginine did not differ significantly. Table 17 below presents a full comparison of the AA profile of the two samples.

Table 17. Amino acid quantified (mg/g DW) in raw *H. midae* (n = 5) and *H. tuberculata coccinea* control sample (n = 3).

	H. midae	***H. tuberculata coccinea***
AMINO ACID	RAW	CONTROL
Histidine	6.70 ± 0.49[a]	8.22 ± 1.11[b]
Isoleucine	17.88 ± 1.74[a]	15.21 ± 0.99[b]
Leucine	39.59 ± 3.52[a]	31.34 ± 1.72[b]
Lysine	32.29 ± 2.76[a]	26.82 ± 1.55[b]
Methionine	11.98 ± 1.02[a]	9.50 ± 1.59[b]
Phenylalanine	16.93 ± 1.63[a]	16.34 ± 0.72[a]
Threonine	21.37 ± 2.36[a]	20.13 ± 0.86[a]
Tryptophan*		
Valine	18.47 ± 1.96[a]	16.75 ± 0.82[b]
EAA (Total)	165.21 ± 15.1[a]	144.32 ± 9.08[b]
Arginine	61.76 ± 5.60[a]	65.15 ± 18.41[a]
Alanine	30.38 ± 2.87[a]	23.92 ± 1.06[b]
Aspartic acid**	39.22 ± 3.62[a]	32.16 ± 3.25[b]
Cysteine	2.82 ± 0.95[a]	4.56 ± 0.35[b]
Glutamic acid**	91.62 ± 7.51[a]	64.08 ± 3.27[b]
Glycine	41.26 ± 4.80[a]	28.69 ± 2.38[b]
Proline	24.22 ± 3.42[a]	19.56 ± 0.86[b]
Serine	25.35 ± 2.94[a]	21.47 ± 0.45[b]
Tyrosine	11.83 ± 6.39[a]	15.23 ± 0.65[b]
TAA	493.66 ± 43.89[a]	419.16 ± 9.35[b]
EAA/TAA	0.33± 0.005[a]	0.34± 0.008[b]

* tryptophan is degraded during the acid hydrolysis

** Glutamic acid and aspartic acid represent the sums of aspartic acid + asparagine and glutamic acid + glutamine, respectively, as asparagine and glutamine are converted into their acidic forms during the acid hydrolysis.

4.4 Lipid Class Composition

Both neutral lipids and polar lipids were detected in the samples. There were, however, several unidentified lipid classes. These unidentified lipid classes were not included in the calculations to derive the lipid class composition. The chromatograms obtained from the HPLC analyses showed that the majority of the unidentified lipid peaks corresponded to the retention time profile of polar lipids. As per the identified lipids, there was a striking difference between the class composition of the two species. *H. tuberculata coccinea* had relatively more neutral lipids, whereas *H. midae* had relatively more polar lipids. Figure 5 below is a collection of chromatograms from the analyses that depict this difference. Peaks identified as neutral lipids corresponded to the lower retention times while peaks identified as polar lipids corresponded to the higher retention times.

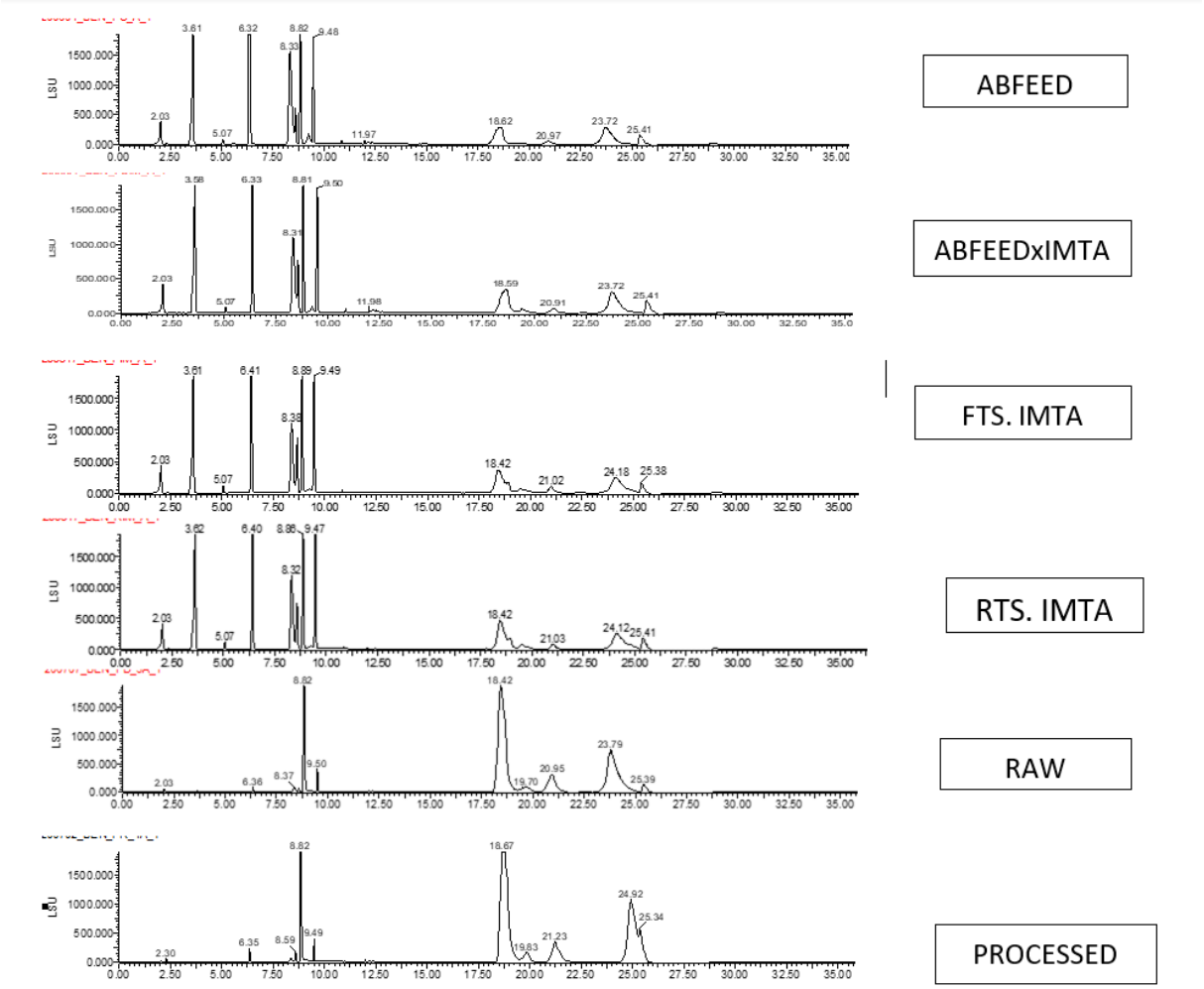


Figure 5. A collection of chromatograms from HPLC lipid class composition analyses. Peaks represent lipid classes while numbers on the horizontal plane of the chromatograms are retention times. (ABFEED = artificial diet, ABFEEDxIMTA = hybrid diet, FTS.IMTA = control, RTS.IMTA = Recirculation system)

The most abundant lipid class detected in the *H. tuberculata coccinea* samples was free fatty acid (FFA). The FFA composition was 25.6 ± 2.83% in the artificial diet group and 30.65 ± 2.76% in the hybrid diet group. It was a bit higher in the control (32.1 ± 0.85%) and highest in the RS (35.15 ± 0.07%). Triacylglycerol (TAG) was also prominent in the samples. The artificial diet group had 28.15 ± 8.84%, noticeably higher than the rest of the samples. The hybrid diet sample, control sample, and RS sample had 13.6 ± 0.85%, 11.4 ± 0.99%, and 6.5 ± 0.99%, respectively. Digalactosyldiacylglycerol (DGDG) comprised 2.3 ± 0.71% of the lipid classes in the artificial diet. This lipid class was not detected in any other *H. tuberculata coccinea* or *H.*

midae samples. The most abundant polar lipid in *H. tuberculata coccinea* was phosphatidylglycerol (PG). It was 10.1 ± 0.57% in the artificial diet sample and 3-4% higher in the control and RS samples. Details of all detected lipid classes are presented in table 18.

There were some striking differences in the lipid class composition of the raw and processed *H. midae* samples. The processed and raw *H. midae* differed considerably in TAG composition (2.9 ± 1.70% and 0.2 ± 0.001%, respectively) and FFA composition (3.75 ± 1.06% and 8.15 ± 0.49%, respectively). In contrast to the raw sample, some monoacylglycerol (MAG) was quantified in the lipids from the processed sample (1.3 ± 0.99%). In both samples, polar phospholipids were the dominant lipid classes. Precisely, the most abundant lipid class detected was PG, which was 31.35 ± 2.47% of the lipid classes in the processed sample and 34.2 ± 1.13% in the raw sample. The second most abundant class was phosphatidylserine (PS) that was 23.65 ± 1.48% in the processed and 24.8 ± 0.14% in the raw *H. midae*. The third most abundant lipid class was Phosphatidylcholine (PC). It was 17.8 ± 1.56% and 10.56 ± 0.78% in the processed and raw samples respectively. A succinct comparison of the lipid class composition of the *H. midae* samples is also presented in table 18 below.

Table 18. The relative composition of identified lipid classes in lipid extracts of *H. tuberculata coccinea* and *H. midae* extracted with DCM/MeOH (2:1 v/v).

	H. tuberculata coccinea				*H. midae*	
CLASS	ARTIFICIAL DIET	HYBRID DIET	CONTROL DIET	RS	PROCESSED	RAW
EE	6.65 ± 0.64	7 ± 0.28	6.4 ± 0.28	7.9 ± 0.14	0.25 ± 0.07	1.0 ± 0.00
TAG	28.15 ± 8.84	13.6 ± 0.85	11.4 ± 0.99	6.5 ± 0.99	2.9 ± 1.70	0.2 ± 0.00
FAIC	2.15 ± 0.07	3.05 ± 0.35	3.55 ± 0.07	3.35 ± 0.49	0.85 ± 0.35	0.7 ± 0.14
DAG	6.65 ± 0.64	6.35 ± 0.21	6.35 ± 0.49	6.75 ± 0.35	11.25 ± 0.35	12.05 ±0.78
FFA	25.6 ± 2.83	30.65 ± 2.76	32.1 ± 0.85	35.15 ± 0.07	3.75 ± 1.06	8.15 ± 0.49
MAG	1.5 ± 0.14	2.35 ± 0.64	1.35 ± 0.07	1.4 ± 0.14	1.3 ± 0.99	ND
MGDG	0.35 ± 0.07	0.2 ± 0.28	0.35 ± 0.21	0.2 ± 0.00	0.1 ± 0.00	0.3 ± 0.14
DGDG	2.3 ± 0.71	ND	ND	ND	ND	ND

PG	10.1 ± 0.57	12.6 ± 0.00	12.95 ± 0.07	14.7 ± 0.28	31.35 ± 2.47	34.2 ± 1.13
PE	ND	5.85 ± 1.06	ND	ND	ND	ND
DGTS	ND	ND	6.4 ± 0.28	5.15 ± 0.07	6.75 ± 0.92	8 ± 0.42
PS	7.05 ± 1.48	9.45 ± 0.01	11.25 ± 1.20	8.25 ± 0.35	23.65 ± 1.48	24.8 ± 0.14
PC	9.55 ± 1.77	8.85 ± 1.63	7.85 ± 0.35	10.65 ± 0.49	17.8 ± 1.56	10.56 ± 0.78

*EE – Ethyl esters, TAG -Triacylglycerol, FAIC – Fatty acids in conjugation, DAG – Diacylglycerol, FFA – Free fatty acids, MAG – Monoacylglycerol, MGDG – Monogalactosyldiacylglycerol, DGDG – Digalactosyldiacylglycerol, PG – Phosphatidylglycerol, PE – Phosphatidylethanolamine, DGTS – Diacylglyceryl trimethyl homoserine, PS – Phosphatidylserines, PC – Phosphatidylcholine ND = Not detected

4.5 Antioxidant Capacity

4.5.1 FRAP

There was a clear difference in the pattern of FRAP values obtained from the analyses. Ethyl acetate extracts of *H. tuberculata coccinea* were at least double the value of any other extract. Ethyl acetate extract of the control sample had the highest FRAP value (1.81 ± 0.02 mmol TE/g DW). This value was significantly ($P < 0.05$) different from the value of the corresponding extract of the RS sample (1.60 ± 0.07 mmol TE/g DW), and those from both raw *H. midae* (0.29 ± 0.004 mmol TE/g DW) and processed *H. midae* (0.37 ± 0.01 mmol TE/g DW). It was, however, not significantly ($P < 0.05$) different from ethyl acetate extracts of the hybrid diet sample (1.67 ± 0.1 mmol TE/g DW) and the artificial diet sample (1.81 ± 0.02 mmol TE/g DW). This is illustrated in figure 6 below.

Water extracts had the highest FRAP values for both *H. midae* samples. The water extract of raw *H. midae* had a FRAP value of 0.5 ± 0.02 mmol TE/g DW. It was significantly different from the equivalent extract of processed *H. midae* (0.43 ± 0.01 mmol TE/g DW) even though the numerical difference is not very pronounced

Generally, in terms of FRAP values, samples with relatively stronger water extracts were also samples with relatively weaker ethyl acetate extracts. Pearson's correlation coefficient for this relationship was – 0.91.

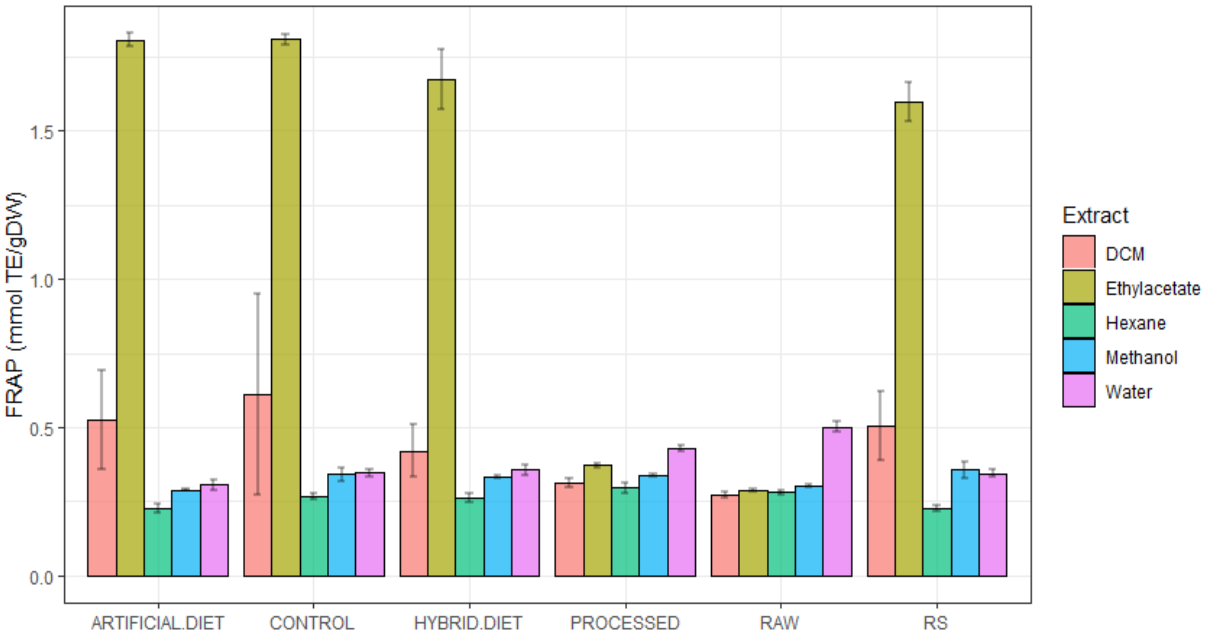


Figure 6. Mean FRAP (mmol TE/g DW) of *H. tuberculata coccinea* and *H. midae* samples extracted with five different solvents. n = 3

4.5.2 ORAC

Across all samples, the methanol extracts had the highest antioxidant capacity measured by ORAC. This was most pronounced in the methanol extract of the processed *H. midae* that had a mean ORAC value of 94.23 ± 18.1 mmol TE/g DW. This value was not significantly ($P < 0.05$) different from the values of the methanol extracts from the other samples.

The water extracts had the second highest mean ORAC values in all treatments of the two species. The mean ORAC value of the water extract of the control sample (48.82 ± 1.2 mmol TE/g DW) was significantly ($P < 0.05$) lower than those of the other *H. tuberculata coccinea* samples.

In the analysis of ORAC value by extraction solvent, the processed *H. midae* had higher mean ORAC values than the raw *H. midae* on every count. This can be seen in figure 7 below.

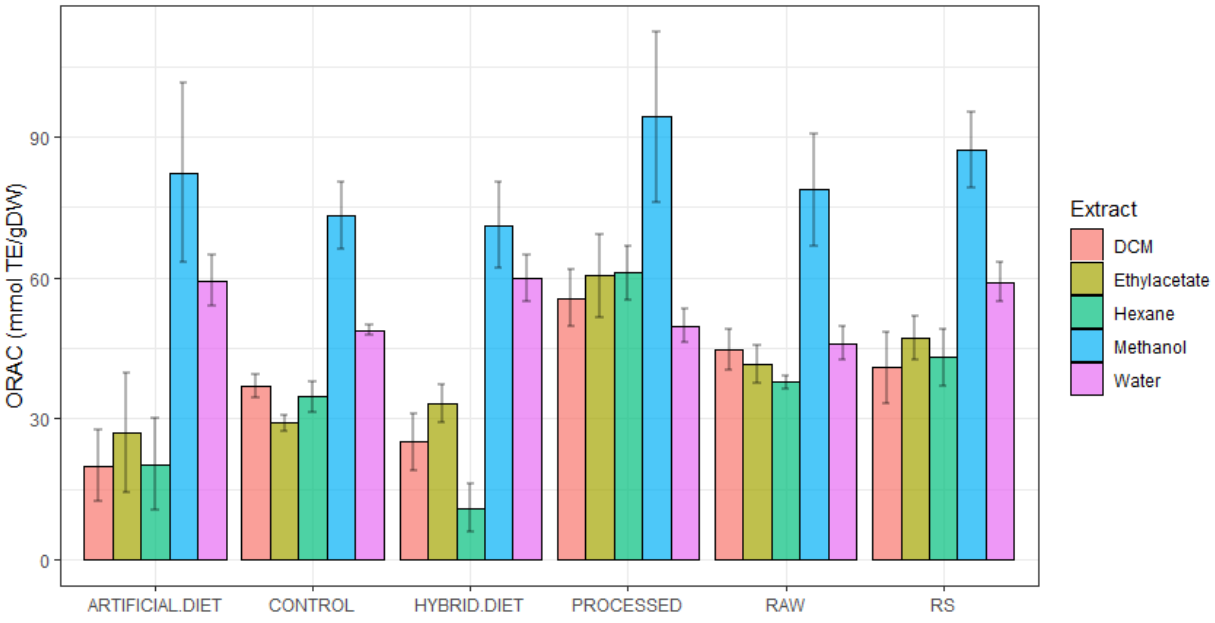


Figure 7. Mean ORAC (mmol TE/g DW) of *H. tuberculata coccinea* and *H. midae* samples extracted with five different solvents. n = 3

4.6 HMG-CoA Reductase Inhibitory Activity

The preliminary results obtained from the HMG-coA reductase inhibitory assay using a sample concentration of 1 mg/ml and pravastatin as the positive control are presented in table 19 below. The highest inhibitory activities were seen in the extracts from the *H. tuberculata coccinea* hybrid diet sample. All extracts of the hybrid diet at 1mg/ml showed a strong ability to inhibit the HMG-CoA reductase activity and this effect varied from 66% to 75%.

Table 19. HMG-CoA reductase inhibitory activity of 1 mg/ml extracts of *H. midae and H. tuberculata coccinea*. (n = 1, Pravastatin inbibitory activity =79.36%)

	% Inhibition					
	H. tuberculata coccinea				***H. midae***	
Extract	ARTIFICIAL DIET	HYBRID DIET	CONTROL	RS	RAW	PROCESSED
DCM	25.35	67.91	45.35	16.40	NT	2.44
Ethyl acetate	20.93	67.30	39.88	1.05	NT	NT
Methanol	30.47	66.74	62.33	23.95	NT	12.21
Water	32.33	75.00	48.02	33.72	NT	13.14

*NT = Not tested

5. DISCUSSION

Abalone that was fed IMTA-macroalgae diet had a different biochemical composition compared to abalone fed an artificial diet. Notably, the abalone that was fed the macroalgae diet had a relatively higher fat and protein content. Even though a lesser amount of DHA was quantified in the sample fed macroalgae diet, its EPA content was comparable to that of the artificial diet group. Also, the macroalgae diet resulted in increased DPA and ARA levels. Furthermore, higher amounts of EAAs were quantified in the abalone fed with macroalgae diet. Lipid class analyses showed a higher composition of TAG in the sample that was fed an artificial diet. Interestingly, the biochemical composition of the abalone fed the hybrid diet was more similar to that of the abalone fed with the macroalgae diet and less similar to that of the abalone fed the artificial diet. More importantly, the potential HMG-CoA reductase inhibitory activity of the hybrid diet group was distinctively outstanding.

There were some minor differences between the biochemical composition of abalone produced in FTS and RS. For instance, there was a higher concentration of taste active amino acids (glutamic acid and glycine) in the abalone produced in RS. On the other hand, the total of EPA, DPA, and DHA was higher in the abalone produced in FTS. In summary, the biochemical and bioactivity differences noted in the two groups were not sufficient to suggest that the RS production either enhanced or lowered the nutritional quality of the abalone.

The South African traditional heat and salt processing impacted the biochemical composition and bioactivity of *H. midae*. The processing neither resulted in a change of fat content nor caused striking changes in the percentage composition of FAs and AAs. However, it resulted in significantly lower levels of FAs and AAs per gram of sample. Lower levels of EAAs and the n-3 PUFAs could be the main concern about the nutritional quality of the processed *H*. *midae.* However, there was some indication that the processing slightly increased the antioxidant capacity measured by ORAC and FRAP. The following sections discuss the findings from this study in more detail.

5.1 Proximate Composition

It was not possible to determine the water content of the abalone samples in this study because samples were received as either salt-dried or freeze-dried. No information about the quantity of water removed during the drying process was documented.

5.1.1 Proximate Composition of *H. tuberculata* Treatments

Amongst the *H. tuberculata coccinea* samples, the artificial diet group (3.58 ± 1.16 g/100 g DW) had the lowest total fat content while the control group (4.82 ± 0.44 g/100 g DW) had the highest total fat content. The total fat contents of the RS group (4.59 ± 0.36 g/100 g DW) and the hybrid diet group (4.24 ± 0.69 g/100 g DW) were not significantly lower than that of the control group. The total fat contents of *H. tuberculata coccinea* analyzed in this study were in a similar range to the results previously reported on both farmed and wild abalone fed on macroalgae and artificial diets (Grubert et al., 2004, Floreto et al., 1996, Chiou et al., 2001, Nelson et al., 2002, Mai et al., 1995a, Bilbao et al., 2012). However, the result from this study revealed a gradient increase of total fat content from samples fed the artificial diet to the ones fed macroalgae; the median total fat content was found in the hybrid diet group.

Unfortunately, it was not possible to obtain any samples of the diets used in the cultivation of the abalone samples included in this study, and considering that analysis of the abalone diets was beyond the scope of this study, it is difficult to explain why the control group of *H. tuberculata coccinea* contained slightly more crude fat than those fed the formulated diet and the hybrid diet. It is however unlikely that the formulated diet applied had an insufficient amount of lipid source. This is because the lipid content of formulated abalone diets usually ranges from 1.5% to 5.3% (Fleming et al., 1996). A previous study that analyzed the proximate composition of the commercial abalone diet, Abfeed®, reported that it contained 5.3% crude fat (Simon et al., 2002). Moreover, abalone is known to be very efficient in the utilization of lipids, growing well on diets with as low as 2.2% fat (Bautista-Teruel et al., 2011)

Likely, the dietary lipid requirement of the *H. tuberculata coccinea* control group was met by the combination of the two macroalgae in the feed. These macroalgae, *U. rigida*, and *G. cornea* are estimated to have crude lipid contents of 1.4% DW and 0.7% DW, respectively (Valente et al., 2006). Assuming that the formulated diet contained more crude fat than the macroalgae, the fat contents could have been different as previous studies found that soft-

body fat content positively correlated with dietary fat levels (Dunstan et al., 1996, Mai et al., 1995a)

Amongst the *H. tuberculata coccinea* samples, the protein contents of the control group (41.9 ± 2.84 g/100 g DW), and the RS group (41.89 ± 1.44 g/100 g DW) were similar; slightly higher than that of the group fed the hybrid diet (39.73 ± 0.13 g/100 g DW) and much higher than that of the group fed the artificial diet (34.40 ± 0.47 g/100 g DW). These results demonstrate that protein content increased with an increased quantity of macroalgae fed to the abalone. Previous studies found that Abfeed® contains about 35% protein mainly from fishmeal and soya bean meal while *U. rigida* and *G. cornea* produced out of fish farm wastewater effluents contain about 34% and 29% protein respectively (Troell et al., 2006, Viera et al., 2011). Since the optimal dietary protein level of *H. tuberculata coccinea* is 35% (Bansemer et al., 2016), it could be assumed that the protein in the macroalgae and to a greater extent, in the formulated feed met the abalone's protein requirements. However, the higher protein levels in the macroalgae fed samples could mean that the protein in the macroalgae was more digestible than the protein in the formulated feed, and perhaps the combination of the two macroalgae provided a more suitable amino acid balance (Mulvaney et al., 2013). The protein content of *H. tuberculata coccinea* in this study was much lower than the 74-76% reported by Viera et al., (2011) but much closer to the range reported by Mai et al. (1995a).

5.1.2 Proximate Composition of Raw and Processed *H. midae*

The crude fat content of *H. midae* presented in this study was similar to that reported in a previous study (Knauer et al., 1994). There was no significant difference between the crude fat content of the raw *H. midae* (2.36 ± 0.64 g/100 g DW) and the processed *H. midae* (2.55 ± 0.34 g/100 g DW). No fat was lost during the boiling of the processed *H. midae*. This is explained by the fact that abalone is a low-fat shellfish. Hence it did not readily lose fat to the surrounding boiling water. A decrease in fat content after boiling is more common in fatty species; there is often a drip of fat from the meat to the boiling water (Domiszewski et al., 2011). On the other hand, likely, the increased lipid levels observed in some individual processed *H. midae* was as a result of interactions in the meat during boiling. Heat treatment

may increase or decrease lipid extractability (Pikul and Wojciechowska, 1994, Kolakowska and Bienkiewicz, 1999). Even though lipid extraction in this study was done using a combination of a polar and a non-polar solvent (methanol and DCM), due to interactions between tissue components, some quantity of lipid in the raw *H. midae*, probably held by covalent bonding, might not have been extracted (Domiszewski et al., 2011).

The protein content of raw *H. midae* reported in this study was close to the 44.67% reported by Knauer et al., (1994). Howbeit, it was reasonably higher than the reported protein content of the *H. tuberculata* coccinea samples. This made sense because the dietary protein requirement of *H. midae* (47%) is significantly higher than the dietary protein requirement of *H. tuberculata coccinea* (Bansemer et al., 2016). Also, the protein content of processed *H. midae* (36.9 ± 1.46 g/100 g DW) was significantly ($P < 0.05$) lower than that of the raw *H. midae* (49.3 ± 4.3 g/100 g DW). A partial explanation for this may be that the processed samples have accumulated a high content of salt during the preservation process (Desmond and Vasilopoulos, 2019). Unfortunately, the challenges associated with the COVID-19 lockdown prevented ash analyses which could have confirmed this assumption.

5.2 Fatty Acid Composition

Recent report suggests that DPA contributes to the numerous health benefits previously attributed solely to EPA and DHA (Richter et al., 2016). Hence in describing the nutritional quality of the abalone samples in terms of the beneficial n-3 PUFAs, the sum of the amounts of EPA, DPA, and DHA quantified was taken.

The artificial diet produced a higher sum of these FAs than the macroalgae diet (see appendix 2) mainly due to a relatively higher quantity of DHA. The RS, on the other hand, had a lower sum of EPA, DPA, and DHA than the control. The RS system used in the abalone farm may have been suboptimal at some point. A typical RS is not easy to maintain as they are technology-biology interaction systems that demand constant high-level monitoring (Lekang, 2007). A drop in the water quality, which is common in poorly managed RS systems results in a drop in the quality of the farmed animals (Badiola et al., 2012).

Finally, the processed *H. midae* contained a lower sum of EPA, DPA, and DHA as some loss of these FAs may have occurred due to thermal degradation caused by heat processing (Wang et al., 2014a). The following subsections discuss the FA composition results in detail.

5.2.1 Fatty Acid Composition of the *H. tuberculata* Treatments

There were differences in the amount and composition of several FAs found in the *H. tuberculata coccinea* samples. The major FAs across the four treatments were C16:0, C18:0, C18:1 n-7, C18:1 n-9, C20:4 n-6, C20:5 n-3 (EPA)**,** C22:5 n-3(DPA)**,** and C22:6 n-3 (DHA)**.** This FA dominance pattern was consistent with some previous studies (Olley and Thrower, 1977, Uki et al., 1986b, Nelson et al., 2002, Shimma and Taguchi, 1964, Hernández et al., 2013). The proportion of total PUFA in the control and RS group was identical. However, there was a slightly higher proportion of both total MUFA and SFA in the RS. group. It would be hard to make any conclusions from this given that the total amount of unidentified FAs in the control group was twice the total unidentified FAs in the RS group.

The SFA C14:0 was relatively more abundant in the artificial diet group (5.15 ± 0.42%) than in the other groups. The C14:0 proportion in the control (2.74 ± 1.03%), RS (3.57 ± 0.01%), and hybrid diet(2.65 ± 0.06%) groups was similar to those found in *H. fulgens* fed a mix of macroalgae (Nelson et al., 2002). The higher proportion of C14:0 in the artificial diet group might have been caused by the deposition of this FA from SFA-rich oils used in the formulated feed (Mulvaney et al., 2015). On the other hand, Nelson et al., (2002) stated that a moderate abundance of C14:0 similar to what was observed in the control, RS, and hybrid diet groups are common in abalone that was fed with red and brown macroalgae diets.

Also, there was a sharp difference between the amounts of ARA in the artificial diet group (0.11 ± 0.03 g/100 g DW) and the other groups. This difference was higher amounts in the hybrid diet group (0.38 ± 0.07 g /100 g DW), RS group (0.33 ± 0.11 g/100 g DW), and the control group (0.58 ± 0.28 g/ 100 g DW). This finding was in agreement with Dunstan et al., (1996) who also noted that higher levels of ARA are peculiar to marine animals that feed on algae. In comparison to the rest of the samples, the artificial diet group contained high proportions of C18:2 n-6. Dunstan et al., (1996) found similar elevated proportions of this FA in the muscle of juvenile abalone fed artificial diet. Also, C20:2 n-6 was only quantified in the artificial diet group. This suggests that the abalone fed artificial diet could have synthesized this C20 PUFA from C18 PUFA (Dunstan et al., 1996)

The same amounts and proportions of EPA were found in both the control group and the artificial diet group. There was no much variation in EPA across the four treatments (4.12% to 5.19%). EPA proportions in abalone are typical of macroalgae diets. Its proportions in wild abalone species range from 2.8% in *H. japonicus* to 10.4% in *H. iris* (Joseph, 1982). Similar proportions of EPA in both the control group and the artificial diet group suggest that the artificial diet indeed contained adequate EPA. Also, If the artificial diet contained higher levels of EPA than the macroalgae diets, the excess EPA did not result in any additional deposition in the abalone tissues.

DHA was relatively more abundant in the artificial diet group (6.68 ± 0.93%) than in the control group (1.35 ± 0.04%). Low proportions (1.4% to 0.4%) and even the absence of DHA in many wild abalone species were documented by Joesph (1982). Thus the DHA proportion in the control group is within range of what is known. The elevated levels of DHA in the artificial diet group might be a result of deposition from some marine oil in the artificial feed (Dunstan et al., 1996). The DHA proportions and amounts within the hybrid diet group (14.22 ± 15.4%) and the RS group (4.50 ± 4.82%) showed great variations so much that it was difficult to determine the effect size. The uneven surface area of the pulverized *H. tuberculata coccinea* samples used in the analyses might have contributed to this inconsistency of FA values in the replicates.

On the other hand, DPA was less abundant in the artificial diet group(3.38 ± 0.26%) than in the rest of the groups where it was more or less twice the value in the artificial diet group (5.39% to 6.04%). A higher proportion of DPA in macroalgae fed abalone may result from an

adaptation to a low lipid diet, and a low rate of conversion of DPA to DHA (Shimma and Taguchi, 1964, Uki et al., 1986b).

The n-6:n-3 ratio of the artificial diet group (0.38 ± 0.01%) and the hybrid diet group (0.38 ± 0.21%) was lower than the ratio of the control group (0.58 ± 0.01%) and the RS group (0.71 ± 0.44%). The lower n-6:n-3 in the artificial group was mainly due to a relatively lower percentage of ARA and a higher percentage of DHA. However, All the samples of *H. tuberculata coccinea* in this study have a desirable n-6:n-3 ratio considering that the western diet is estimated to have a ratio of 15-20:1 (Husted and Bouzinova, 2016).

5.2.2 Fatty Acid Composition of Raw and Processed *H. midae*

The major FAs in the *H. midae* samples were C16:0, C18:0, C18:1 n-7, C18:1 n-9, C20:4 n-6, C20:5 n-3, and C22:5. This pattern was not strikingly different from the profile of the *H. tuberculata coccinea* samples. Generally, the FA profile in *H. midae* was similar to the findings from previous studies that analyzed several abalone species (Joseph, 1982, Shimma and Taguchi, 1964, Olley and Thrower, 1977, Uki et al., 1986b, Hernández et al., 2013).

The composition of SFAs, MUFAs, and PUFAs reported in those previous studies were around 40%, 20%, and 40%, respectively. This corresponded with the proportions in the raw *H. midae* (35%, 17%, and 35%, respectively). The numerical difference was accounted for by 13% of unidentified FAs in this study. The n-6/n-3 ratio was not significantly different; 0.41 ± 0.07 in processed *H. midae* and 0.31 ± 0.05 in raw *H. midae*. This ratio suggests that both raw and processed *H. midae* can be classified as food with a low n-6/n-3 ratio (Mulvaney et al., 2015)

Generally, there were no striking changes in percentages of FAs between heating conditions. Only significant ($P < 0.05$) differences were noticed in three PUFAs; C18:2 n-6, C18:3 n-4, and ARA where the proportion in each case increased slightly in the processed *H. midae*. A previous study also noted no significant changes in percentages of total FAs of *H. discus hannai* subjected to heat treatment (Wang et al., 2014a). Different cooking processes have been shown to have a negligible effect on percentages of FAs in meat (Gerber et al., 2009).

However, the absolute amounts in g/100 g DW of FAs generally decreased with heating as was seen in table 13. This is likely due to thermal degradation and fat-melting during cooking (Wang et al., 2014a)

5.2.3 Fatty Acid Composition Differences Between *H. tuberculata coccinea* and *H. midae*

Exactly 17 FAs were identified in the *H. midae* samples while either 14 or 15 FAs were identified in the *H. tuberculata coccinea* samples. The variability in the number and nature of FAs identified in each *H. tuberculata coccinea* sample was likely due to the different dietary treatments. The number and nature of FAs identified in both *H. midae* samples did not vary and tallied with the FAs reported in a similar study that evaluated the effect of heat treatment on the FA composition of *H. discus hannai* (Wang et al., 2014a). It seems that the abalone diet had more impact on its biochemical composition than the heat treatment did.

5.3 Amino Acid Composition

5.3.1 Amino Acid Composition of *H. tuberculata coccinea* Treatments

A total of 17 AAs was detected in all four treatments of *H. tuberculata coccinea*. The different treatments did not cause any considerable variations in individual AA percentages. The most abundant AA was glutamic acid, representing the sum of glutamic acid and glutamine. Previous studies have also reported glutamic acid as the most abundant AA in abalone (Latuihamallo et al., 2015, Knauer et al., 1995).

Arginine is considered an EAA for abalone (Mai et al., 1994), and was the second most abundant AA quantified. It was most abundant in the artificial diet group (12.64 ± 0.46%) and least abundant in the control group (10.79 ± 0.17%). Mai et al., (1994) noted that due to high levels of arginine in abalone protein, this AA could be the first limiting EAA in abalone

foodstuff. Thus, it makes sense to increase the concentration of this amino acid as well as the concentration of other potential limiting EAA to optimum levels when selecting food for abalone. An effective method of increasing the concentration of potential limiting EAAs is to select a well-balanced mixed algal diet where the individual algae contribute unique EAAs in a synergistic relationship as demonstrated by Mercer et al., (1993).

Leucine and Lysine were the next most abundant EAAs. Very similar findings were reported by Mai et al. (1994). The relative abundance of arginine, alanine, and glycine found in this study is characteristic of marine mollusks, requiring these AAs to maintain glycolysis under hypoxic conditions (Latuihamallo et al., 2015). There were no considerable differences in the proportions of glutamic acid and glycine across the four treatments. Concentrations of glutamic acid, adenosine monophosphate (AMP), and glycine are thought to be responsible for the characteristic taste of abalone meat, and higher concentrations of these molecules give a better taste quality to the abalone meat (Brown et al., 2008).

The amount (mg/g DW) of EAAs was directly proportional to the quantity of IMTA-macroalgae in the abalone diet (see appendix 3). This indicates that the macroalgae was a better protein source for the abalone, and highlights that the two macroalgae species fed to the *H. tuberculata coccinea* control group provided a better amino acid balance than the artificial feed (Mulvaney et al., 2013). Hence the protein quality of the control group was higher than that of the artificial diet group (Shi et al., 2020). The EAA amounts in the RS group was only slightly lower than the control group. It is possible that this drop was caused by stress on the abalone induced by a lower quality of water in the RS set-up (Masser et al., 1999).

5.3.2 Amino Acid Composition of Raw and Processed H.midae

All the amino acids detected in the *H. tuberculata coccinea* samples were detected in the *H.* midae in similar percentages. As seen in the *H. tuberculata coccinea* samples, there were no considerable differences in the AA percentages of the two *H. midae* samples. However, cysteine, which was about 0.56% of the TAAs in the raw *H. midae*, was below the detection limit in the processed sample. Cysteine is heat-labile and is readily destroyed at temperatures just above 100 °C (Kirk, 1984). Hence the loss of cysteine could be one of the

most critical factors in the evaluation of the nutritional value of abalone processed under high-temperature treatment.

Glutamic acid and arginine were the most abundant AAs in both samples. The composition percentage of glutamic acid was slightly higher in the processed sample. Cooking abalone under certain temperature and time conditions has been shown to increase taste active AAs such as glutamic acid (Brown et al., 2008).

In absolute amounts, the processed sample contained significantly ($P < 0.05$) lower amounts in 16 out of the 17 identified AAs. Tyrosine was the only AA in the processed *H. midae* that was not significantly lower in absolute amount. These differences in absolute amount were most pronounced in arginine, glycine, glutamic acid, and leucine. Heat treatment and the use of salt in food processing can alter the concentration of AAs. Some previous studies suggest that while prolonged heat treatment can reduce the concentration of certain amino acids (Taira, 1973), the concentration of salt used in food processing can either increase or reduce the concentration of some AAs (Altahir et al., 2015).

5.3.3 Amino Acid Composition Differences Between *H. tuberculata coccinea* and *H. midae*

Apart from some considerable differences in the proportions of glutamic acid, aspartic acid, and arginine, both species showed a very similar amino acid pattern. This indicates that they may have similar amino acid requirements for body growth (Mai et al., 1994). The higher proportion of glutamic acid and glycine in the raw *H. midae* could mean that it has a more desirable taste (Brown et al., 2008). The raw *H. midae* contained significantly ($P < 0.05$) higher amounts of EAA and TAA than the *H. tuberculata coccinea.* The EAA level is an important factor that affects the nutritional value of protein (Shi et al., 2020) From a nutritional point of view, this means that the *H. midae* has a better protein quality than the control *H. tuberculata coccinea*.

5.4 Lipid class composition

Due to the amount of unidentified lipid classes, which by indications from the HPLC chromatogram was not a minor quantity, it is not possible to determine the actual percentage

composition of lipid classes in this study. The unidentified lipid classes were lipids that did not correspond to any of the available standards necessary for identification. These are suspected to be lipids not normally found in Arctic marine fish and shellfish. The table presented in the result section and the discussion below aim to describe differential patterns noticed amongst the samples, and more importantly, serve as a basis for a more comprehensive *H. tuberculata coccinea* and *H. midae* lipid class analysis.

The most abundant lipid class in the *H. tuberculata coccinea* was FFA. This was unexpected, given that the major lipids identified by previous studies on abalone were polar lipids and sterols (Nelson et al., 2002). It is not clear as to why there were 25 -35 % FFAs in the samples. One possible explanation is that free fatty acids might have accumulated if the samples have been treated suboptimally or kept at ambient temperatures before freeze-drying as the samples might be sensitive to endogenous lipase activity (Xu et al., 2018). Unfortunately, it was not possible to obtain a detailed description of the initial treatment of these samples.

TAG was the second most abundant lipid class in *H. tuberculata coccinea*. TAG was elevated in the artificial diet group (28.15 ± 8.84%) and relatively low in the control group (11.4 ± 0.99%) and RS group (6.5 ± 0.99%). The carbohydrate sources in the artificial diet and the macroalgae are quite different (Naidoo et al., 2006). A previous study that examined lipid compositions in *H. discus hannai* reported that different dietary carbohydrate sources and levels affected lipid metabolism in the abalone and thus the TAG levels (Wang et al., 2009). It is, however, worth noting that in juvenile flounder, blood TAG level reached a peak 11-24 hours after feeding and afterward declined gradually, irrespective of the dietary carbohydrate sources and levels (Lee et al., 2003)

Most of the identified lipids in the *H. midae* samples were polar lipids – mainly phospholipids. This agreed with previous research demonstrating that it is made up of mostly membrane lipids, and very low amounts of lipid energy reserves, and the main energy reserves in adult abalones have been reported to be packed in the foot tissues as glycogen (Nelson et al., 1999).

5.5 Antioxidant capacity

Several studies have investigated abalone for antioxidant molecules using various methods that probe their polysaccharides, proteins, and fatty acids. For example, some previous studies found that the antioxidant capacity of abalone viscera was enhanced through enzymatic hydrolysis (Zheng et al., 2019, Zhou et al., 2012). In another study, an antioxidative polysaccharide containing uronic acid was isolated from the abalone shell (Wang et al., 2014c). In this study, the method was to test crude extracts of abalone samples made from selected solvents that offered a good range of polarity.

Antioxidant capacity can be analyzed using different methods. However, it is a well-known fact that each method has its limitations (de Torre et al., 2019). The two assays used in this study, FRAP and ORAC, measure antioxidant capacity based on different mechanisms (Payne et al., 2013). Results from both assays thus offered a better approximation of the antioxidant capacity of *H. tuberculata coccinea* and *H. midae*. As noted in the result section, the measured antioxidative capacities of the different abalone samples varied between the tests. The recovery of antioxidant compounds was likely dependent on the polarity of the chemical constituents in the abalone as well as the polarity of the solvent used.

The highest FRAP values were found in the *H. tuberculata coccinea* samples extracted with ethyl acetate and DCM. The polarity index of the two solvents suggests that the antioxidant compounds in *H. tuberculata coccinea* with the highest FRAP are weakly polar. More precisely, the highest FRAP values were obtained with the ethyl acetate extracts, which ranged from 1.81 ± 0.02 mmol TE/g DW in the control group to 1.60 ± 0.07 mmol TE/g DW in the RS group.

The water extract of the raw *H. midae* had a significantly ($P < 0.05$) higher FRAP value than that of the processed *H. midae*. It is possible that the heat treatment destroyed some water-soluble antioxidants in the processed *H. midae*. However, loss of water-soluble antioxidative compounds due to heat treatment on fruits and vegetables is well documented (Jiménez-Monreal et al., 2009, Jeong et al., 2004). On the other hand, the processed *H. midae* had a higher FRAP value for the other four solvent extracts tested. This points out that the heat treatment enhanced the content or capacity of some putative antioxidants whereas others were reduced.

The methanol extracts had the highest ORAC values for all the samples. Except for the processed *H.* midae, the water extract had the second highest ORAC value in all samples. This

indicates that the antioxidants detected are polar compounds. Across the *H. tuberculata coccinea* samples, there were no significant ($P < 0.05$) differences in ORAC values of the methanol extracts indicating that changing the dietary treatments and the cultivation system had no considerable effect on the antioxidative capacity measured by ORAC. However, the non-polar extracts of processed *H. midae* had ORAC values significantly ($P < 0.05$) higher than the corresponding extracts of raw *H. midae*. Indeed, this was a further indication that the heat treatment enhanced the capacity of some non-polar antioxidative compounds.

Previous studies on the antioxidative capacity of *H. tuberculata coccinea* and *H. midae* using FRAP or ORAC methods are not easy to find. It is also difficult to make comparisons with previous studies on the antioxidative capacity of abalone in general. For instance, a previous study that investigated the ORAC of an abalone protein hydrolysate (APH) made from *H. discus hannai* reported a value of 0.46 mmol TE/mg sample (Park et al., 2015). Considering that this test was conducted on APH, it is not clear how this ORAC value compares with the present study's methanol extract of the processed *H. midae* (94.23 ± 18.1 mmol TE/g DW).

5.5.1 Antioxidant Capacity in Relation with n-3 PUFA Content, Polar Lipid Composition and Extraction Solvent

Previous studies have suggested that lipid class composition and n-3 PUFA content may play a role in the antioxidative capacity of biological extracts. Some of these studies documented synergistic effects of these bioactive lipids and FAs with other molecules such as pigments and phenolic compounds (Lopes et al., 2020, Shimoni et al., 1998, Vázquez et al., 2018, Bourlieu et al., 2018, Olcott, 1958, Richard et al., 2008, Gülcin, 2012). A chart of n-3 PUFA amount (g/100 g DW) and antioxidant capacity measured by either ORAC or FRAP is presented in figure 10. The plot shows the mean FRAP and ORAC expressed by the amount of n-3 PUFA in the *H. midae* and *H. tuberculata coccinea* samples. No clear-cut pattern was found between the antioxidative capacity (both ORAC and FRAP results) and the content of n-3 PUFAs. However, all samples with less than 0.4 g/100 g DW n-3 PUFA had a mean FRAP less than 0.5 mmol TE/g DW.

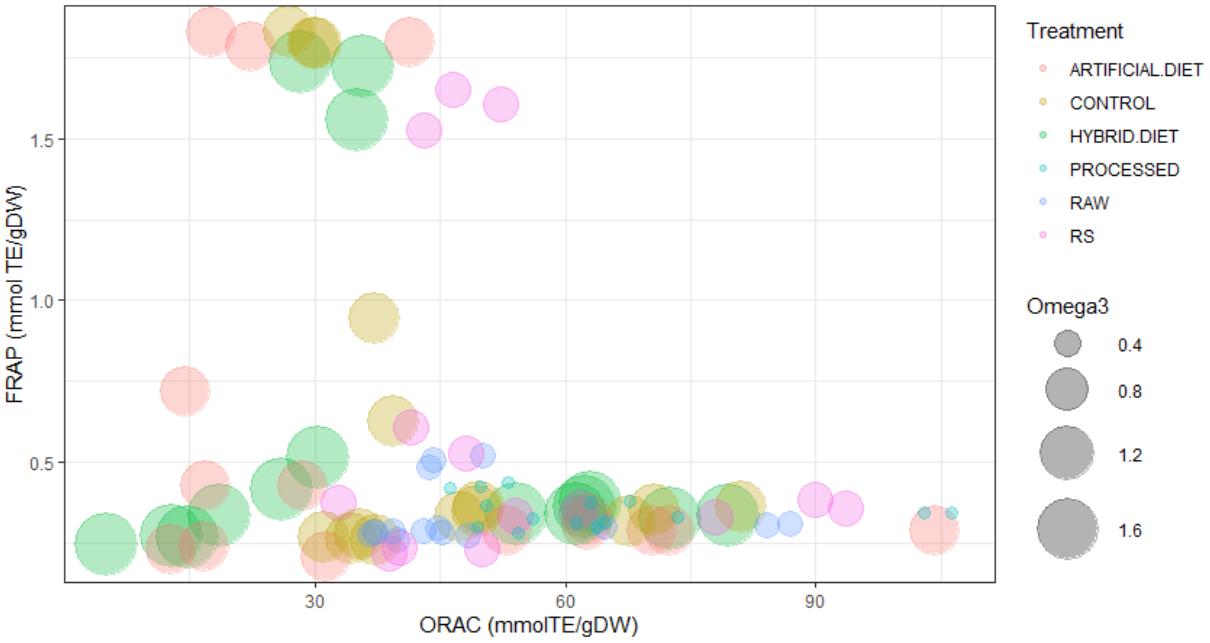


Figure 8. Relationship between antioxidant capacity and n-3 PUFA amount in g/100 g DW.

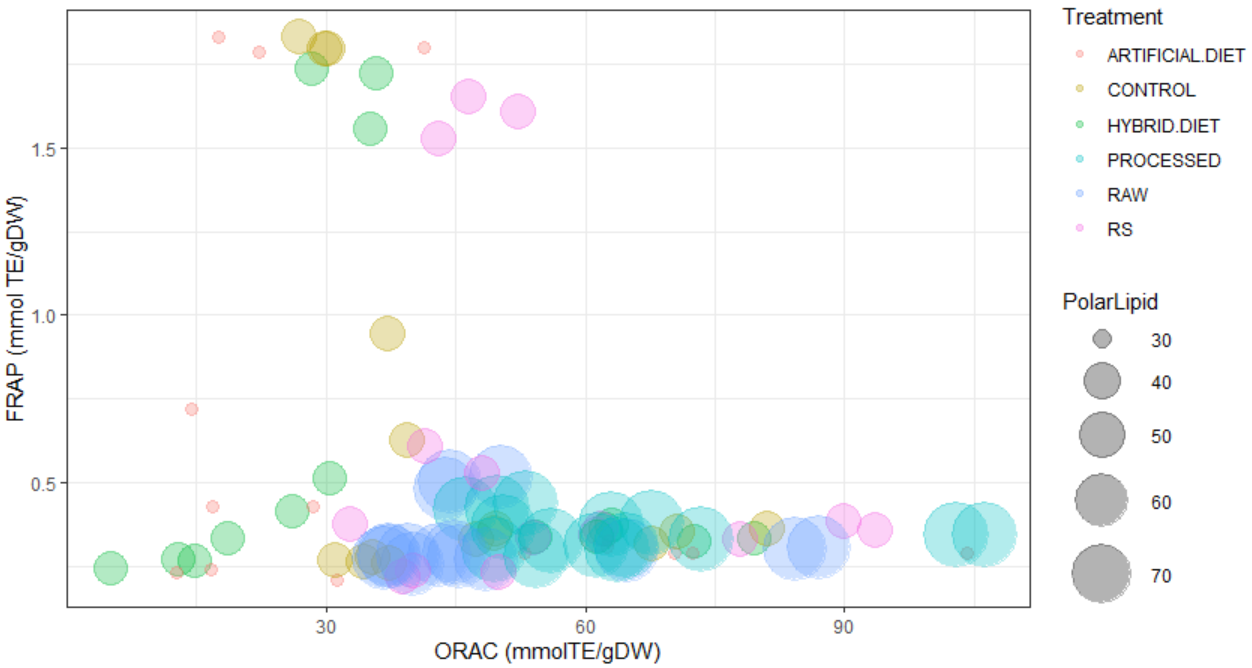


Figure 9. Relationship between antioxidant capacity and polar lipid composition in percentage.

A similar chart mapping ORAC and FRAP, expressed by polar lipid percentage proportions, is presented in figure 11. This chart did not reveal any convincing patterns. However, the *H. midae* samples with the higher composition of polar lipids generally had relatively low FRAP values but had relatively high ORAC values. Considering that there were several unidentified lipid classes in this study, it is not possible to get an accurate estimate of the extent to which polar lipids have contributed to the antioxidative capacity measured by the two antioxidant assays.

However, the plot of antioxidant capacity and extraction solvent indicate that extraction solvent had the most significant impact on the ORAC and FRAP values. This is depicted in figure 12 below.

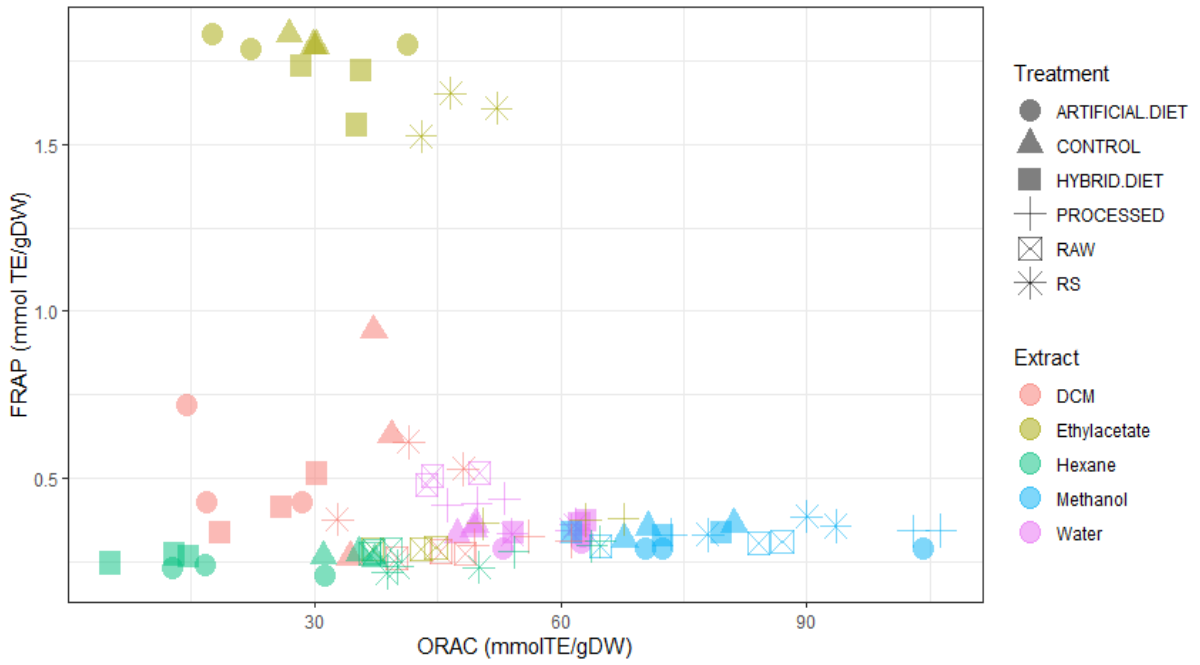


Figure 10. Relationship between antioxidant capacity and extraction solvent.

5.6 HMG-CoA reductase inhibition

Compounds that inhibit the rate-limiting enzyme in cholesterol biosynthesis are generally referred to as statins. These compounds competitively inhibit HMG-CoA reductase resulting in a decrease in the synthesis and secretion of low-density lipoproteins and ultimately resulting in lowering of blood cholesterol levels (Maron et al., 2000, Manzoni and Rollini, 2002). Hence, statins has become the primary medication in the management of hyperlipidemia and atherosclerotic disease. The first generation of natural statins were fungal secondary metabolites (Wierzbicki, 2001). However, statins in clinical use today range from natural compounds such as lovastatin and pravastatin to full synthetics such as cerivastatin and atorvastatin (Maron et al., 2000). Statins have pleiotropic effects and due to the side effects associated with the use of statins (Thompson et al., 2016), it is an important task to find new statin-like compounds. Although natural statins are typically obtained from species of filamentous fungi (Manzoni and Rollini, 2002), continued investigations have shown that statin-like compounds can be obtained from diverse sources (Baskaran et al., 2015, Hafidz et al., 2017, Ha et al., 1998, Koo et al., 2008). When assessing the extracts from *H. tuberculata*

coccinea for their potential to inhibit the HMG-CoA reductase activity, results were promising. The preliminary results from this study indicates that the extracts comprises some compound(s) of that might have pharmaceutical interest. The control sample showed relatively higher inhibitory activity than the artificial diet and RS groups. The 75% inhibitory activity recorded in the aqueous extract of the hybrid diet group was produced by 1µg of the sample. This inhibitory activity was greater than the 74.1% reported in a 50 µg methanol extract of *Basella alba* (Baskaran et al., 2015). Therefore, it would be interesting to continue investigating the HMG-CoA reductase inhibitory activity of these samples.

Due to lack of material, the raw *H. midae* sample was not tested for HMG-CoA reductase inhibitory activity. Hence, it was not possible to compare the cholesterol-lowering potential of the two *H. midae* samples. However, the analysis indicated that the *H. midae* did not show a similar potential to inhibit the HMG-CoA reductase activity as the samples from *H. tuberculata coccinea.*

5.7 Biotechnological value of IMTA macroalgae cultivation

Macroalgae produced in IMTA can replace some of the feed provided by fishmeal and other unsustainable sources. In addition, the macroalgae cultivation may be an important reservoir of compounds of industrial and biotechnological importance (Beaulieu, 2019). Glycolipids and phospholipids isolated from seaweeds have been used in a wide spectrum of industries such as food, cosmetic and pharmaceutical (Azevedo, 2016). In a recent study of the red algae *Gracilaria vermiculophylla* grown in an IMTA system, mycosporine-like amino acids (MMAs), useful as photoprotector and antioxidant compounds in the cosmetic industry were recovered. The study showed that in one year, 71.33 g MAA can be produced in a 100 m^2 culture (Barceló-Villalobos et al., 2017). Also, a recent study that examined the DCM and methanol extracts of red algae, *Osmundea pinnatifida* found that human breast adenocarcinoma cell line proliferation was reduced 61% and 75% by the DCM extracts (1mg/ml; 24h) obtained from wild and IMTA-cultivated algae, respectively (Silva et al., 2014). It is worth mentioning that when seaweed is cultivated as a crop for bioactive compounds, IMTA potentially offers the flexibility to increase biomass yield, and to customize production

to target the final applications (da Costa et al., 2015). Additionally, harvesting macroalgae biomass from IMTA removes the risk of destroying local biodiversity and ecosystem integrity often associated with macroalgae trawler-based wild stock harvesting (Stévant et al., 2017).

6. Conclusion

IMTA holds great potential to transform modern aquaculture into a more sustainable practice. One of the key issues it can address is the current overexploitation of fisheries due to overreliance on fishmeal for aquaculture feed production. This study has reported that the nutritional quality of abalone fed two macroalgae species produced in IMTA was, by some key indications, better than that of the abalone that was fed an artificial diet. Moreso, a hybrid diet of the IMTA-macroalgae and the artificial feed produced abalone of better nutritional quality than the artificial diet. Hence, future research effort is needed to understand practical solutions that will make macroalgae cultivation and harvesting within the IMTA framework a common practice.

The RS offers some sustainability-related advantages over the conventional FTS in aquaculture production. However, it is not widely practiced due to the associated cost of implementation. Findings from this study showed some biochemical differences between abalone produced in FTS and RS. Notably, there was a slight decrease in the essential AAs and FAs of abalone cultivated with the RS system. Due to the sensitivity of the system, high-level management is required to maintain water quality, and thus the nutritional quality of farmed animals.

Finally, the biochemical changes produced by the heat and salt applied during the South African processing procedure of *H. midae* are acceptable. The processing might indeed have resulted in lower amounts of some EAAs and FAs. However, abalone is a luxury food that is mainly consumed for its unique taste. The heat processing enhanced the percentage composition of glutamic acid, which contributes to the unique taste of abalone. It also improved the antioxidative capacity of *H. midae* by enhancing the activity of some non polar compounds.

7. REFERENCES

ABREU, S., SOLGADI, A. & CHAMINADE, P. 2017. Optimization of normal phase chromatographic conditions for lipid analysis and comparison of associated detection techniques. *Journal of Chromatography A,* 1514**,** 54-71.

ALEXANDER, K. A., ANGEL, D., FREEMAN, S., ISRAEL, D., JOHANSEN, J., KLETOU, D., MELAND, M., PECORINO, D., REBOURS, C., ROUSOU, M., SHORTEN, M. & POTTS, T. 2016. Improving sustainability of aquaculture in Europe: Stakeholder dialogues on Integrated Multi-trophic Aquaculture (IMTA). *Environmental Science & Policy,* 55**,** 96-106.

ALEXANDER, K. A. & HUGHES, A. D. 2017. A problem shared: Technology transfer and development in European integrated multi-trophic aquaculture (IMTA). *Aquaculture,* 473**,** 13-19.

ALTAHIR, M. O., ELGASIM, E. A. & AHMED, I. A. M. 2015. Effect of Heat Treatment and Salt Concentration on Free Amino Acids Composition of Sudanese Braided (Muddaffara) Cheese during Storage. *Turkish Journal of Agriculture-Food Science and Technology,* 3**,** 9-16.

ÁRNASON, J., BJÖRNSDÓTTIR, R., LARSEN, B. K., THRANDUR BJÖRNSSON, B., SUNDELL, K., HANSEN, A.-C., HOLEN, E., ESPE, M., LINDAHL, O. & KALSDÓTTIR, S. 2015. Local fish feed ingredients for competitive and sustainable production of high-quality aquaculture feed LIFF.

AZEVEDO, V. M. M. 2016. *Lipidomic study of the red marine macroalgae as source of bioactive compounds.* Universidade de Aveiro.

BADIOLA, M., MENDIOLA, D. & BOSTOCK, J. 2012. Recirculating Aquaculture Systems (RAS) analysis: Main issues on management and future challenges. *Aquacultural Engineering,* 51**,** 26-35.

BALDWIN, J., ELIAS, J. P., WELLS, R. M. G. & DONOVAN, D. A. 2007. Energy metabolism in the tropical abalone, Haliotis asinina Linné: Comparisons with temperate abalone species. *Journal of Experimental Marine Biology and Ecology,* 342**,** 213-225.

BANSEMER, M. S., QIN, J. G., HARRIS, J. O., HOWARTH, G. S. & STONE, D. A. J. 2016. Nutritional requirements and use of macroalgae as ingredients in abalone feed. *Reviews in Aquaculture,* 8**,** 121-135.

BARCELÓ-VILLALOBOS, M., FIGUEROA, F. L., KORBEE, N., ÁLVAREZ-GÓMEZ, F. & ABREU, M. H. 2017. Production of Mycosporine-Like Amino Acids from Gracilaria vermiculophylla (Rhodophyta) Cultured Through One Year in an Integrated Multi-trophic Aquaculture (IMTA) System. *Marine Biotechnology,* 19**,** 246-254.

BARKAI, R. & GRIFFITHS, C. L. 1986. Diet of the South African abalone Haliotis midae. *South African Journal of Marine Science,* 4**,** 37-44.

BARRINGTON, K., CHOPIN, T. & ROBINSON, S. 2009. Integrated multi-trophic aquaculture (IMTA) in marine temperate waters. *FAO Fisheries and Aquaculture Technical Paper***,** 7-46.

BASKARAN, G., SALVAMANI, S., AHMAD, S. A., SHAHARUDDIN, N. A., PATTIRAM, P. D. & SHUKOR, M. Y. 2015. HMG-CoA reductase inhibitory activity and phytocomponent

investigation of Basella alba leaf extract as a treatment for hypercholesterolemia. *Drug design, development and therapy,* 9**,** 509-517.

BAUTISTA-TERUEL, M. N., KOSHIO, S. S. & ISHIKAWA, M. 2011. Diet development and evaluation for juvenile abalone, Haliotis asinina Linne: Lipid and essential fatty acid levels. *Aquaculture,* 312**,** 172-179.

BEAULIEU, L. 2019. Insights into the regulation of algal proteins and bioactive peptides using proteomic and transcriptomic approaches. *Molecules,* 24**,** 1708.

BENZIE, I. F. & STRAIN, J. J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry,* 239**,** 70-76.

BILBAO, A., URIARTE, I., DEL PINO VIERA, M., SOSA, B., FERNÁNDEZ-PALACIOS, H. & HERNÁNDEZ-CRUZ, C. M. 2012. Effect of Macroalgae Protein Levels on Some Reproductive Aspects and Physiological Parameters for the Abalone, Haliotis tuberculata coccinea (Reeve 1846). *Journal of the World Aquaculture Society,* 43**,** 764-777.

BONAR, D. B. 1976. Molluscan metamorphosis: a study in tissue transformation. *American Zoologist,* 16**,** 573-591.

BOOLOOTIAN, R. A., FARMANFARMAIAN, A. & GIESE, A. 1962. On the reproductive cycle and breeding habits of two western species of Haliotis. *The Biological Bulletin,* 122**,** 183-193.

BOURLIEU, C., CHEILLAN, D., BLOT, M., DAIRA, P., TRAUCHESSEC, M., RUET, S., GASSI, J.-Y., BEAUCHER, E., ROBERT, B., LECONTE, N., BOUHALLAB, S., GAUCHERON, F., GÉSAN-GUIZIOU, G. & MICHALSKI, M.-C. 2018. Polar lipid composition of bioactive dairy co-products buttermilk and butterserum: Emphasis on sphingolipid and ceramide isoforms. *Food Chemistry,* 240**,** 67-74.

BRAGER, L. M., CRANFORD, P. J., JANSEN, H. & STRAND, Ø. 2016. Temporal variations in suspended particulate waste concentrations at open-water fish farms in Canada and Norway. *Aquaculture Environment Interactions,* 8**,** 437-452.

BRITZ, P. J. 1996. Effect of dietary protein level on growth performance of South African abalone, Haliotis midae, fed fishmeal-based semi-purified diets. *Aquaculture,* 140**,** 55-61.

BROWN, M. R., SIKES, A. L., ELLIOTT, N. G. & TUME, R. K. 2008. Physicochemical Factors of Abalone Quality: A Review. *Journal of Shellfish Research,* 27**,** 835-842, 8.

BUCK, B. H. & BUCHHOLZ, C. M. 2004. The offshore-ring: a new system design for the open ocean aquaculture of macroalgae. *Journal of Applied Phycology,* 16**,** 355-368.

BUSCHMANN, A. H., CAMUS, C., INFANTE, J., NEORI, A., ISRAEL, Á., HERNÁNDEZ-GONZÁLEZ, M. C., PEREDA, S. V., GOMEZ-PINCHETTI, J. L., GOLBERG, A., TADMOR-SHALEV, N. & CRITCHLEY, A. T. 2017. Seaweed production: overview of the global state of exploitation, farming and emerging research activity. *European Journal of Phycology,* 52**,** 391-406.

CALDER, P. C. 2013. Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology? *British Journal of Clinical Pharmacology,* 75**,** 645-662.

CHIOU, T.-K., LAI, M.-M. & SHIAU, C.-Y. 2001. Seasonal variations of chemical constituents in the muscle and viscera of small abalone fed different diets. *Fisheries science,* 67**,** 146-156.

CHOPIN, T. 2012. Seaweed aquaculture provides diversified products, key ecosystem functions.

CHOPIN, T., COOPER, J. A., REID, G., CROSS, S. & MOORE, C. 2012. Open-water integrated multi-trophic aquaculture: environmental biomitigation and economic diversification of fed aquaculture by extractive aquaculture. *Reviews in Aquaculture,* 4**,** 209-220.

COCHRANE, K., DE YOUNG, C., SOTO, D. & BAHRI, T. 2009. Climate change implications for fisheries and aquaculture. *FAO Fisheries and aquaculture technical paper,* 530**,** 212.

CONNELL, J. H. 1985. The consequences of variation in initial settlement vs. post-settlement mortality in rocky intertidal communities. *Journal of experimental marine biology and ecology,* 93**,** 11-45.

COOK, P. A. 2016. Recent Trends in Worldwide Abalone Production. *Journal of Shellfish Research,* 35**,** 581-583, 3.

COSTELLO, C., CAO, L., GELCICH, S., CISNEROS-MATA, M. Á., FREE, C. M., FROEHLICH, H. E., GOLDEN, C. D., ISHIMURA, G., MAIER, J., MACADAM-SOMER, I., MANGIN, T., MELNYCHUK, M. C., MIYAHARA, M., DE MOOR, C. L., NAYLOR, R., NØSTBAKKEN, L., OJEA, E., O'REILLY, E., PARMA, A. M., PLANTINGA, A. J., THILSTED, S. H. & LUBCHENCO, J. 2020. The future of food from the sea. *Nature,* 588**,** 95-100.

CRANFORD, P. J., REID, G. K. & ROBINSON, S. M. 2013. Open water integrated multi-trophic aquaculture: constraints on the effectiveness of mussels as an organic extractive component. *Aquaculture Environment Interactions,* 4**,** 163-173.

DA COSTA, E., MELO, T., MOREIRA, A. S. P., ALVES, E., DOMINGUES, P., CALADO, R., ABREU, M. H. & DOMINGUES, M. R. 2015. Decoding bioactive polar lipid profile of the macroalgae Codium tomentosum from a sustainable IMTA system using a lipidomic approach. *Algal Research,* 12**,** 388-397.

DÁVALOS, A., GÓMEZ-CORDOVÉS, C. & BARTOLOMÉ, B. 2004. Extending applicability of the oxygen radical absorbance capacity (ORAC− fluorescein) assay. *Journal of agricultural and food chemistry,* 52**,** 48-54.

DE KONING, A. 1966a. Isolation of 2-aminoethylphosphonic acid from phospholipids of the abalone (Haliotis midae). *Nature,* 210**,** 113-113.

DE KONING, A. 1966b. Phospholipids of marine origin IV. The abalone (Haliotis midae). *Journal of the Science of Food and Agriculture,* 17**,** 460-464.

DE TORRE, M. P., CAVERO, R. Y., CALVO, M. I. & VIZMANOS, J. L. 2019. A Simple and a Reliable Method to Quantify Antioxidant Activity In Vivo. *Antioxidants (Basel, Switzerland),* 8**,** 142.

DE VICOSE, G. C., VIERA, M., BILBAO, A. & IZQUIERDO, M. 2007. Embryonic and larval development of Haliotis tuberculata coccinea Reeve: an indexed micro-photographic sequence. *Journal of Shellfish Research,* 26**,** 847-854.

DESMOND, E. & VASILOPOULOS, C. 2019. 7 - Reducing salt in meat and poultry products. *In:* BEEREN, C., GROVES, K. & TITORIA, P. M. (eds.) *Reducing Salt in Foods (Second Edition).* Woodhead Publishing.

DOMISZEWSKI, Z., BIENKIEWICZ, G. & PLUST, D. 2011. Effects of different heat treatments on lipid quality of striped catfish (Pangasius hypophthalmus). *Acta Scientiarum Polonorum, Technologia Alimentaria,* 10**,** 359-373.

DUARTE, C. M., HOLMER, M., OLSEN, Y., SOTO, D., MARBÀ, N., GUIU, J., BLACK, K. & KARAKASSIS, I. 2009. Will the Oceans Help Feed Humanity? *BioScience,* 59**,** 967-976.

DUNSTAN, G. A., BAILLIE, H. J., BARRETT, S. M. & VOLKMAN, J. K. 1996. Effect of diet on the lipid composition of wild and cultured abalone. *Aquaculture,* 140**,** 115-127.

DUNSTAN, G. A., VOLKMAN, J. K. & MAGUIRE, G. B. 2000. *Optimisation of essential lipids in artificial feeds for Australian abalone*, CSIRO Marine Research Hobart, Australia.

DURAZO-BELTRÁN, E., D'ABRAMO, L. R., TORO-VAZQUEZ, J. F., VASQUEZ-PELÁEZ, C. & VIANA, M. A. T. 2003. Effect of triacylglycerols in formulated diets on growth and fatty acid composition in tissue of green abalone (Haliotis fulgens). *Aquaculture,* 224**,** 257-270.

ELLIS, J. & TILLER, R. 2019. Conceptualizing future scenarios of integrated multi-trophic aquaculture (IMTA) in the Norwegian salmon industry. *Marine Policy,* 104**,** 198-209.

ESSINGTON, T. E., MORIARTY, P. E., FROEHLICH, H. E., HODGSON, E. E., KOEHN, L. E., OKEN, K. L., SIPLE, M. C. & STAWITZ, C. C. 2015. Fishing amplifies forage fish population collapses. *Proceedings of the National Academy of Sciences,* 112**,** 6648-6652.

ESTES, J., LINDBERG, D. & WRAY, C. 2005. Evolution of large body size in abalones (Haliotis): Patterns and implications. *Paleobiology,* 31**,** 591-606.

FAO 2018. The State of World Fisheries and Aquaculture. Rome: Food and Agriculture Organization of the United nations.

FAO 2020. Fishery and Aquaculture Statistics. Rome: Food and Agriculture Organization of the United nations.

FAO/OECD 2016. OECD–FAO Agricultural Outlook 2015–2024.

FERDOUSE, F., HOLDT, S. L., SMITH, R., MURÚA, P. & YANG, Z. 2018. The global status of seaweed production, trade and utilization. *GLOBEFISH Research Programme,* 124.

FLEMING, A. E., VAN BARNEVELD, R. J. & HONE, P. W. 1996. The development of artificial diets for abalone: A review and future directions. *Aquaculture,* 140**,** 5-53.

FLORETO, E. A. T., TESHIMA, S.-I. & KOSHIO, S. 1996. The Effects of Seaweed Diets on the Lipid and Fatty Acids of the Japanese Disc Abalone Haliotis discus hannai. *Fisheries science,* 62**,** 582-588.

FOSSBERG, J., FORBORD, S., BROCH, O. J., MALZAHN, A. M., JANSEN, H., HANDÅ, A., FØRDE, H., BERGVIK, M., FLEDDUM, A. L., SKJERMO, J. & OLSEN, Y. 2018. The Potential for Upscaling Kelp (Saccharina latissima) Cultivation in Salmon-Driven Integrated Multi-Trophic Aquaculture (IMTA). *Frontiers in Marine Science,* 5.

GARCÍA-BUENO, N., TURPIN, V., COGNIE, B., DUMAY, J., MORANÇAIS, M., AMAT, M., PÉDRON, J.-M., ATUCHA, A. M., FLEURENCE, J. & DECOTTIGNIES, P. 2016. Can the European abalone Haliotis tuberculata survive on an invasive algae? A comparison of the nutritional value of the introduced Grateloupia turuturu and the native Palmaria palmata, for the commercial European abalone industry. *Journal of Applied Phycology,* 28**,** 2427-2433.

GARCÍA POZA, S., LEANDRO, A., COTAS, C., COTAS, J., MARQUES, J., PEREIRA, L. & GONÇALVES, A. 2020. The Evolution Road of Seaweed Aquaculture: Cultivation Technologies and the Industry 4.0. *International Journal of Environmental Research and Public Health,* 17**,** 6528.

GEIGER, D. L. 1999. Distribution and biogeography of the recent Haliotidae world-wide.:(Gastropoda: Vetigastropoda). *Bollettino Malacologico***,** 57-118.

GEIGER, D. L. & GROVES, L. T. 1999. Review of fossil abalone (Gastropoda: Vetigastropoda: Haliotidae) with comparison to Recent species. *Journal of Paleontology,* 73**,** 872-885.

GERBER, N., SCHEEDER, M. & WENK, C. 2009. The influence of cooking and fat trimming on the actual nutrient intake from meat. *Meat science,* 81**,** 148-154.

GIANGRANDE, A., PIERRI, C., ARDUINI, D., BORGHESE, J., LICCIANO, M., TRANI, R., CORRIERO, G., BASILE, G., CECERE, E. & PETROCELLI, A. 2020. An Innovative IMTA System: Polychaetes, Sponges and Macroalgae Co-Cultured in a Southern Italian In-Shore Mariculture Plant (Ionian Sea). *Journal of Marine Science and Engineering,* 8**,** 733.

GOLDEN, C. D., ALLISON, E. H., CHEUNG, W. W., DEY, M. M., HALPERN, B. S., MCCAULEY, D. J., SMITH, M., VAITLA, B., ZELLER, D. & MYERS, S. S. 2016. Nutrition: Fall in fish catch threatens human health. *Nature,* 534**,** 317-20.

GRUBERT, M. A., DUNSTAN, G. A. & RITAR, A. J. 2004. Lipid and fatty acid composition of pre- and post-spawning blacklip (Haliotis rubra) and greenlip (Haliotis laevigata) abalone conditioned at two temperatures on a formulated feed. *Aquaculture,* 242**,** 297-311.

GÜLCIN, I. 2012. Antioxidant activity of food constituents: an overview. *Archives of toxicology,* 86**,** 345-391.

HA, T.-Y., CHO, I.-J. & LEE, S.-H. 1998. Screening of HMG-CoA reductase inhibitory activity of ethanol and methanol extracts from cereals and regumes. *Korean Journal of Food Science and Technology,* 30**,** 224-229.

HAFIDZ, K. A., PUSPITASARI, N., AZMINAH, A. Y., ARTHA, Y. & MUN'IM, A. 2017. HMG-CoA Reductase inhibitory activity of Gnetum gnemon seed extract and identification of potential inhibitors for lowering cholesterol level. *Journal of Young Pharmacists,* 9**,** 559.

HEPPLE, J.-A. 2010. *An integrated linkage map of Perlemoen (Haliotis midae).* Stellenbosch: University of Stellenbosch.

HERNÁNDEZ, J., DE LA PARRA, A. M., LASTRA, M. & VIANA, M. T. 2013. Effect of lipid composition of diets and environmental temperature on the performance and fatty acid composition of juvenile European abalone (Haliotis tuberculata L. 1758). *Aquaculture,* 412**,** 34-40.

HOBDAY, A., TEGNER, M. & HAAKER, P. 2000. Over-Exploitation of a Broadcast Spawning Marine Invertebrate: Decline of the White Abalone. *Reviews in Fish Biology and Fisheries - REV FISH BIOL FISHERIES,* 10**,** 493-514.

HOSHINO, E., GARDNER, C., JENNINGS, S. & HARTMANN, K. 2015. Examining the Long-Run Relationship between the Prices of Imported Abalone in Japan. *Marine Resource Economics,* 30**,** 000-000.

HUSTED, K. S. & BOUZINOVA, E. V. 2016. The importance of n-6/n-3 fatty acids ratio in the major depressive disorder. *Medicina,* 52**,** 139-147.

JEONG, S.-M., KIM, S.-Y., KIM, D.-R., JO, S.-C., NAM, K. C., AHN, D. U. & LEE, S.-C. 2004. Effect of Heat Treatment on the Antioxidant Activity of Extracts from Citrus Peels. *Journal of Agricultural and Food Chemistry,* 52**,** 3389-3393.

JIMÉNEZ-MONREAL, A. M., GARCÍA-DIZ, L., MARTÍNEZ-TOMÉ, M., MARISCAL, M. & MURCIA, M. A. 2009. Influence of Cooking Methods on Antioxidant Activity of Vegetables. *Journal of Food Science,* 74**,** H97-H103.

JO, Y.-J., JUNG, K.-H., LEE, M.-Y., CHOI, M.-J., MIN, S.-G. & HONG, G.-P. 2014. Effect of high-pressure short-time processing on the physicochemical properties of abalone (Haliotis discus hannai) during refrigerated storage. *Innovative Food Science & Emerging Technologies,* 23**,** 33-38.

JOSEPH, J. D. 1982. Lipid composition of marine and estuarine invertebrates. Part II: Mollusca. *Progress in lipid research,* 21**,** 109-153.

KAWAMURA, T. & KIKUCHI, S. 1992. Effects of Benthic Diatoms on Settlement and Metamorphosis of Abalone Larvae. *Aquaculture Science,* 40**,** 403-409.

KAWARAZUKA, N. & BÉNÉ, C. 2010. Linking small-scale fisheries and aquaculture to household nutritional security: an overview. *Food Security,* 2**,** 343-357.

KIRK, J. R. 1984. Biological availability of nutrients in processed foods. ACS Publications.

KNAUER, J., BRADY, D., DUNCAN, J. & HECHT, T. 1995. Amino acid, fatty acid and mineral element profile of juvenile South African abalone, Haliotis midae L. *Aquaculture Research,* 26**,** 283-288.

KNAUER, J., HECHT, T. & DUNCAN, J. 1994. Proximate composition of the South African abalone, Haliotis midae (Haliotidae: Gastropoda). *Aquaculture Research,* 25**,** 351-354.

KOLAKOWSKA, A. & BIENKIEWICZ, G. 1999. Stability of fish lipids during microwave heating. *Acta Ichthyologica et Piscatoria,* 1**,** 101-111.

KOLETZKO, B., UAUY, R., PALOU, A., KOK, F., HORNSTRA, G., EILANDER, A., MORETTI, D., OSENDARP, S., ZOCK, P. & INNIS, S. 2010. Dietary intake of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in children – a workshop report. *British Journal of Nutrition,* 103**,** 923-928.

KOO, M., KIM, S. H., LEE, N., YOO, M. Y., RYU, S. Y., KWON, D. Y. & KIM, Y. S. 2008. 3-Hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitory effect of Vitis vinifera. *Fitoterapia,* 79**,** 204-206.

LAMPRIANIDOU, F., TELFER, T. & ROSS, L. G. 2015. A model for optimization of the productivity and bioremediation efficiency of marine integrated multitrophic aquaculture. *Estuarine, Coastal and Shelf Science,* 164**,** 253-264.

LATUIHAMALLO, M., IRIANA, D. A. & APITULEY, D. 2015. Amino Acid and Fatty Acid of Abalone Haliotis Squamata Cultured in Different Aquaculture Systems. *Procedia Food Science,* 3**,** 174-181.

LEKANG, O.-I. 2007. *Aquaculture engineering*, Wiley Online Library.

LI, H., WEI, L., ZHANG, G., CAI, Z., CAI, G., CHANG, Y. & XING, K. 2006. Enhancement of larval settlement and metamorphosis through biological and chemical cues in the abalone Haliotis diversicolor supertexta. *Aquaculture,* 258**,** 416-423.

LONGO, S. B., CLARK, B., YORK, R. & JORGENSON, A. K. 2019. Aquaculture and the displacement of fisheries captures. *Conservation Biology,* 33**,** 832-841.

LOPES, D., MELO, T., REY, F., MENESES, J., MONTEIRO, F. L., HELGUERO, L. A., ABREU, M. H., LILLEBØ, A. I., CALADO, R. & DOMINGUES, M. R. 2020. Valuing Bioactive Lipids from Green, Red and Brown Macroalgae from Aquaculture, to Foster Functionality and Biotechnological Applications. *Molecules,* 25**,** 3883.

LUO, Q., HAMID, N., OEY, I., LEONG, S. Y., KANTONO, K., ALFARO, A. & LU, J. 2019. Physicochemical changes in New Zealand abalone (Haliotis iris) with pulsed electric field (PEF) processing and heat treatments. *LWT,* 115**,** 108438.

MAI, K., MERCER, J. P. & DONLON, J. 1994. Comparative studies on the nutrition of two species of abalone, Haliotis tuberculata L. and Haliotis discus hannai Ino: II. Amino acid composition of abalone and six species of macroalgae with an assessment of their nutritional value. *Aquaculture,* 128**,** 115-130.

MAI, K., MERCER, J. P. & DONLON, J. 1995a. Comparative studies on the nutrition of two species of abalone, Haliotis tuberculata L. and Haliotis discus hannai Ino. III. response of abalone to various levels of dietary lipid. *Aquaculture,* 134**,** 65-80.

MAI, K., MERCER, J. P. & DONLON, J. 1995b. Comparative studies on the nutrition of two species of abalone, Haliotis tuberculata L. and Haliotis discus hannai Ino. IV. Optimum dietary protein level for growth. *Aquaculture,* 136**,** 165-180.

MANZONI, M. & ROLLINI, M. 2002. Biosynthesis and biotechnological production of statins by filamentous fungi and application of these cholesterol-lowering drugs. *Applied microbiology and biotechnology,* 58**,** 555-564.

MARON, D. J., FAZIO, S. & LINTON, M. F. 2000. Current Perspectives on Statins. *Circulation,* 101**,** 207-213.

MARTINS, C. I. M., EDING, E. H., VERDEGEM, M. C. J., HEINSBROEK, L. T. N., SCHNEIDER, O., BLANCHETON, J. P., D'ORBCASTEL, E. R. & VERRETH, J. A. J. 2010. New developments in recirculating aquaculture systems in Europe: A perspective on environmental sustainability. *Aquacultural Engineering,* 43**,** 83-93.

MASSER, M. P., RAKOCY, J. & LOSORDO, T. M. 1999. Recirculating aquaculture tank production systems. *Management of recirculating systems. SRAC Publication,* 452.

MATEOS, H. T., LEWANDOWSKI, P. A. & SU, X. Q. 2012. The effect of replacing dietary fish oil with canola oil on fatty acid composition and expression of desaturase and elongase genes in Jade Tiger hybrid abalone. *Food chemistry,* 131**,** 1217-1222.

MCGARVEY, R., MAYFIELD, S., BYTH, K., SAUNDERS, T., CHICK, R., FOUREUR, B., FEENSTRA, J. E. F. E., PREECE, P. & JONES, A. 2008. A diver survey design to estimate absolute density, biomass, and spatial distribution of abalone. *Canadian Journal of Fisheries and Aquatic Sciences,* 65**,** 1931-1944.

MEHBUB, M. F., LEI, J., FRANCO, C. & ZHANG, W. 2014. Marine sponge derived natural products between 2001 and 2010: trends and opportunities for discovery of bioactives. *Marine drugs,* 12**,** 4539-4577.

MENGE, B. A. & SUTHERLAND, J. P. 1987. Community regulation: variation in disturbance, competition, and predation in relation to environmental stress and recruitment. *The American Naturalist,* 130**,** 730-757.

MERCER, J., MAI, K.-S. & DONLON, J. 1993. Comparative studies on the nutrition of two species of abalone, Haliotis tuberculata Linnaeus and Haliotis discus hannai Ino I. Effects of algal diets on growth and biochemical composition. *Invertebrate reproduction & development,* 23**,** 75-88.

MOORE, S. & STEIN, W. H. 1963. [117] Chromatographic determination of amino acids by the use of automatic recording equipment.

MULVANEY, W. J., JAHANGARD, S., INGRAM, B. A., TURCHINI, G. M. & WINBERG, P. C. 2015. Recovery of omega-3 profiles of cultivated abalone by dietary macroalgae supplementation. *Journal of Applied Phycology,* 27**,** 2163-2171.

MULVANEY, W. J., WINBERG, P. C. & ADAMS, L. 2013. Comparison of macroalgal (Ulva and Grateloupia spp.) and formulated terrestrial feed on the growth and condition of juvenile abalone. *Journal of Applied Phycology,* 25**,** 815-824.

MUNGUÍA-VEGA, A., SÁENZ-ARROYO, A., GREENLEY, A. P., ESPINOZA-MONTES, J. A., PALUMBI, S. R., ROSSETTO, M. & MICHELI, F. 2015. Marine reserves help preserve genetic diversity after impacts derived from climate variability: Lessons from the pink abalone in Baja California. *Global Ecology and Conservation,* 4**,** 264-276.

NAJMUDEEN, T. M. & VICTOR, A. C. C. 2004. Seed production and juvenile rearing of the tropical abalone Haliotis varia Linnaeus 1758. *Aquaculture,* 234**,** 277-292.

NATALE, F., HOFHERR, J., FIORE, G. & VIRTANEN, J. 2013. Interactions between aquaculture and fisheries. *Marine Policy,* 38**,** 205-213.

NEDERLOF, M. A., FANG, J., DAHLGREN, T. G., RASTRICK, S. P., SMAAL, A. C., STRAND, Ø., SVEIER, H., VERDEGEM, M. C. & JANSEN, H. M. 2020. Application of polychaetes in (de) coupled integrated aquaculture: an approach for fish waste bioremediation. *Aquaculture Environment Interactions,* 12**,** 385-399.

NELSON, M. M., LEIGHTON, D. L., PHLEGER, C. F. & NICHOLS, P. D. 2002. Comparison of growth and lipid composition in the green abalone, Haliotis fulgens, provided specific

macroalgal diets. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology,* 131**,** 695-712.

NELSON, M. M., NICHOLS, P. D. & LEIGHTON, D. L. 1999. The occurrence and possible significance of diacylglyceryl ether lipids in abalone. *Lipids,* 34**,** 423-427.

NEORI, A., GUTTMAN, L., ISRAEL, A. & SHPIGEL, M. 2019. Israeli-Developed Models of Marine Integrated Multi Trophic Aquaculture (IMTA). *Journal of Coastal Research,* 86**,** 11-20.

OAKES, F. R. & PONTE, R. D. 1996. The abalone market: opportunities for cultured abalone. *Aquaculture,* 140**,** 187-195.

OKUZAWA, K., MALIAO, R., QUINITIO, E., BUEN-URSUA, S., LEBATA, J., GALLARDO, W., GARCIA, L. & PRIMAVERA, J. 2008. Stock Enhancement of Threatened Species in Southeast Asia. *Reviews in Fisheries Science,* 16**,** 394-402.

OLCOTT, H. S. 1958. The role of free fatty acids on antioxidant effectiveness in unsaturated oils. *Journal of the American Oil Chemists Society,* 35**,** 597-599.

OLLEY, J. & THROWER, S. J. 1977. Abalone-An Esoteric Food. *In:* CHICHESTER, C. O., MRAK, E. M. & STEWART, G. F. (eds.) *Advances in Food Research.* Academic Press.

ØVERLAND, M., MYDLAND, L. T. & SKREDE, A. 2019. Marine macroalgae as sources of protein and bioactive compounds in feed for monogastric animals. *Journal of the Science of Food and Agriculture,* 99**,** 13-24.

PARK, S. Y., JE, J.-Y., HWANG, J.-Y. & AHN, C.-B. 2015. Abalone Protein Hydrolysates: Preparation, Angiotensin I Converting Enzyme Inhibition and Cellular Antioxidant Activity. *Preventive nutrition and food science,* 20**,** 176-182.

PAWLIK, J. R. 1990. Natural and artificial induction of metamorphosis of Phragmatopoma lapidosa californica (Polychaeta: Sabellariidae), with a critical look at the effects of bioactive compounds on marine invertebrate larvae. *Bulletin of Marine Science,* 46**,** 512-536.

PAYNE, A. C., MAZZER, A., CLARKSON, G. J. & TAYLOR, G. 2013. Antioxidant assays–consistent findings from FRAP and ORAC reveal a negative impact of organic cultivation on antioxidant potential in spinach but not watercress or rocket leaves. *Food science & nutrition,* 1**,** 439-444.

PIEDRAHITA, R. H. 2003. Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. *Aquaculture,* 226**,** 35-44.

PIKUL, J. & WOJCIECHOWSKA, K. 1994. Effect of coating and immersed frying of chicken carcasses on a lipids oxidation during cold storage. *Gospodarka Miesna (Poland)*.

PINSKY, M. L., JENSEN, O. P., RICARD, D. & PALUMBI, S. R. 2011. Unexpected patterns of fisheries collapse in the world's oceans. *Proceedings of the National Academy of Sciences,* 108**,** 8317-8322.

PRIOR, R. L. & CAO, G. 1999. In vivo total antioxidant capacity: comparison of different analytical methods1. *Free Radical Biology and Medicine,* 27**,** 1173-1181.

RAEMAEKERS, S. J. P. N. & BRITZ, P. J. 2009. Profile of the illegal abalone fishery (Haliotis midae) in the Eastern Cape Province, South Africa: Organised pillage and management failure. *Fisheries Research,* 97**,** 183-195.

REDDY-LOPATA, K., AUERSWALD, L. & COOK, P. 2006. Ammonia toxicity and its effect on the growth of the South African abalone Haliotis midae Linnaeus. *Aquaculture,* 261**,** 678-687.

RICHARD, D., KEFI, K., BARBE, U., BAUSERO, P. & VISIOLI, F. 2008. Polyunsaturated fatty acids as antioxidants. *Pharmacological Research,* 57**,** 451-455.

RICHTER, C. K., SKULAS-RAY, A. C. & KRIS-ETHERTON, P. M. 2016. Chapter 3 - Recommended Intake of Fish and Fish Oils Worldwide. *In:* RAATZ, S. K. & BIBUS, D. M. (eds.) *Fish and Fish Oil in Health and Disease Prevention.* San Diego: Academic Press.

RIDLER, N., WOWCHUK, M., ROBINSON, B., BARRINGTON, K., CHOPIN, T., ROBINSON, S., PAGE, F., REID, G., SZEMERDA, M., SEWUSTER, J. & BOYNE-TRAVIS, S. 2007. INTEGRATED MULTI − TROPHIC AQUACULTURE (IMTA): A POTENTIAL STRATEGIC CHOICE FOR FARMERS. *Aquaculture Economics & Management,* 11**,** 99-110.

ROBERTS, R. 2001. A review of settlement cues for larval abalone (Haliotis spp.). *J. Shellfish Res.,* 20**,** 571-587.

ROSENTHAL, H. 1986. *Flow-through and Recirculation Systems: Report of the Working Group on Terminology, Format, and Units of Measurement*, Food & Agriculture Org.

ROUGHGARDEN, J., IWASA, Y. & BAXTER, C. 1985. Demographic theory for an open marine population with space-limited recruitment. *Ecology,* 66**,** 54-67.

SALES, J. & BRITZ, P. J. 2001. Research on abalone (Haliotis midae L.) cultivation in South Africa. *Aquaculture Research,* 32**,** 863-874.

SAWATPEERA, S., UPATHAM, E. S., KRUATRACHUE, M., CHITRAMVONG, Y. P., SONCHAENG, P., PUMTHONG, T. & NUGRANAD, J. 2001. Larval development in Haliotis asinina Linnaeus. *Journal of Shellfish Research,* 20**,** 593-601.

SCHNEIDER, O., BLANCHETON, J., VARADI, L., EDING, E. & VERRETH, J. Cost price and production strategies in European recirculation systems. Conference: Aqua 2006: Linking Tradition & Technology Highest Quality for the Consumer, Firenze (Florence), Italy, 9-13 May 2006, 2006. 855.

SCHNEIDER, O., SCHRAM, E., POELMAN, M., ROTHUIS, A., VAN DUIJN, A. & VAN DER MHEEN, H. Practices in managing finfish aquaculture using RAS technologies, the Dutch example. OECD Workshop on Advancing the Aquaculture Agenda. OECD, Paris, France, 2010.

SEARCY-BERNAL, R. & ANGUIANO-BELTRÁN, C. 1998. Optimizing the concentration of gamma-aminobutyric acid (GABA) for inducing larval metamorphosis in the red abalone Haliotis rufescens (Mollusca: Gastropoda). *Journal of the World Aquaculture Society,* 29**,** 463-470.

SHEPHERD, S. & LAWS, H. 1974. Studies on southern Australian abalone (Genus <I>Haliotis</I>). II. Reproduction of five species. *Marine and Freshwater Research,* 25**,** 49-62.

SHI, L., HAO, G., CHEN, J., MA, S. & WENG, W. 2020. Nutritional evaluation of Japanese abalone (Haliotis discus hannai Ino) muscle: Mineral content, amino acid profile and protein digestibility. *Food Research International,* 129**,** 108876.

SHILLING, F. M., HOEGH-GULDBERG, O. & MANAHAN, D. T. 1996. Sources of Energy for Increased Metabolic Demand During Metamorphosis of the Abalone Haliotis rufescens (Mollusca). *The Biological Bulletin,* 191**,** 402-412.

SHIMMA, Y. & TAGUCHI, H. 1964. A comparative study on fatty acid composition of shellfish. *Bull. Japanese Soc. Sci. Fish,* 30**,** 153-160.

SHIMONI, E., ARMON, R. & NEEMAN, I. 1998. Note: Antioxidant properties of lipid fractions from Deinococcus radiophilus strain UWO1055. *Journal of Applied Microbiology,* 84**,** 461-465.

SHPIGEL, M., GUTTMAN, L., SHAULI, L., ODINTSOV, V., BEN-EZRA, D. & HARPAZ, S. 2017. Ulva lactuca from an Integrated Multi-Trophic Aquaculture (IMTA) biofilter system as a

protein supplement in gilthead seabream (Sparus aurata) diet. *Aquaculture,* 481**,** 112-118.

SILVA, P., ALVES, C., SILVA, J., ABREU, M. H., PEDROSA, R. & PEREIRA, L. IMTA-cultivated Osmundea pinnatifida inhibited cell proliferation on MCF-7 cell line. Front. Mar. Sci. Conference Abstract: IMMR| International Meeting on Marine Research, 2014.

SIMON, C., KAISER, H., BOOTH, A. & BRITZ, P. 2002. The effect of diet and live host presence on the growth and reproduction of Terebrasabella heterouncinata (Polychaeta: Sabellidae). *Invertebrate Reproduction & Development,* 41**,** 277-286.

SLATTERY, M. 1992. Larval settlement and juvenile survival in the red abalone (Haliotis rufescens), an examination of inductive cues and substrate selection. *Aquaculture,* 102**,** 143-153.

STÉVANT, P., REBOURS, C. & CHAPMAN, A. 2017. Seaweed aquaculture in Norway: recent industrial developments and future perspectives. *Aquaculture International,* 25**,** 1373-1390.

STIERHOFF, K. L., NEUMAN, M. & BUTLER, J. L. 2012. On the road to extinction? Population declines of the endangered white abalone, Haliotis sorenseni. *Biological Conservation,* 152**,** 46-52.

STOFFEL, W., CHU, F. & AHRENS, E. 1959. Analysis of long-chain fatty acids by gas-liquid chromatography. *Analytical Chemistry,* 31**,** 307-308.

STRAND, Ø., JANSEN, H. M., JIANG, Z. & ROBINSON, S. M. 2019. Perspectives on Bivalves Providing Regulating Services in Integrated Multi-Trophic Aquaculture. *Goods and Services of Marine Bivalves.* Springer, Cham.

TAIRA, H. 1973. Heat destruction of amino acids in soybean products. *Food Analysis and Nutrition Division, National Food Research Institute,* 7**,** 267-273.

TAL, Y., SCHREIER, H. J., SOWERS, K. R., STUBBLEFIELD, J. D., PLACE, A. R. & ZOHAR, Y. 2009. Environmentally sustainable land-based marine aquaculture. *Aquaculture,* 286**,** 28-35.

THILSTED, S. H., THORNE-LYMAN, A., WEBB, P., BOGARD, J. R., SUBASINGHE, R., PHILLIPS, M. J. & ALLISON, E. H. 2016. Sustaining healthy diets: The role of capture fisheries and aquaculture for improving nutrition in the post-2015 era. *Food Policy,* 61**,** 126-131.

THOMPSON, P. D., PANZA, G., ZALESKI, A. & TAYLOR, B. 2016. Statin-Associated Side Effects. *Journal of the American College of Cardiology,* 67**,** 2395-2410.

TOSHIKO, M. & AKIRA, H. 1982. Structural studies on glycolipid of shellfish: IV. A novel pentaglycosylceramide from abalone, Haliotis japonica. *Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism,* 711**,** 551-553.

TROELL, M., JOYCE, A., CHOPIN, T., NEORI, A., BUSCHMANN, A. H. & FANG, J.-G. 2009. Ecological engineering in aquaculture — Potential for integrated multi-trophic aquaculture (IMTA) in marine offshore systems. *Aquaculture,* 297**,** 1-9.

TROELL, M., ROBERTSON-ANDERSSON, D., ANDERSON, R. J., BOLTON, J. J., MANEVELDT, G., HALLING, C. & PROBYN, T. 2006. Abalone farming in South Africa: An overview with perspectives on kelp resources, abalone feed, potential for on-farm seaweed production and socio-economic importance. *Aquaculture,* 257**,** 266-281.

UKI, N., SUGIURA, M. & WATANABE, T. 1986a. Dietary value of seaweeds occurring on the Pacific coast of Tohoku [Japan] for growth of the abalone Haliotis discus hannai. *Bulletin of the Japanese Society of Scientific Fisheries (Japan)*.

UKI, N., SUGIURA, M. & WATANABE, T. 1986b. Requirement of essential fatty acids in the abalone Haliotis discus hannai. *Bulletin of the Japanese Society of Scientific Fisheries*.

VALENTE, L., GOUVEIA, A., REMA, P., MATOS, J., GOMES, E. & SOUSA PINTO, I. 2006. Evaluation of three seaweeds Gracilaria bursa-pastoris, Ulva rigida and Gracilaria cornea as dietary ingredients in European sea bass (Dicentrarchus labrax) juveniles. *Aquaculture,* 252**,** 85-91.

VÁZQUEZ, L., CORZO-MARTÍNEZ, M., ARRANZ-MARTÍNEZ, P., BARROSO, E., REGLERO, G. & TORRES, C. 2018. Bioactive Lipids. *Bioactive Molecules in Food***,** 1-61.

VERDEGEM, M., BOSMA, R. & VERRETH, J. 2006. Reducing water use for animal production through aquaculture. *Water resources development,* 22**,** 101-113.

VIERA, M. D. P., COURTOIS DE VIÇOSE, G., FERNÁNDEZ-PALACIOS, H. & IZQUIERDO, M. 2016. Grow-out culture of abalone Haliotis tuberculata coccinea Reeve, fed land-based IMTA produced macroalgae, in a combined fish/abalone offshore mariculture system: effect of stocking density. *Aquaculture Research,* 47**,** 71-81.

VIERA, M. P., DE VICOSE, G. C., GÓMEZ-PINCHETTI, J. L., BILBAO, A., FERNANDEZ-PALACIOS, H. & IZQUIERDO, M. S. 2011. Comparative performances of juvenile abalone (Haliotis tuberculata coccinea Reeve) fed enriched vs non-enriched macroalgae: Effect on growth and body composition. *Aquaculture,* 319**,** 423-429.

VILLAR-NAVARRO, E., GARRIDO-PÉREZ, C. & PERALES, J. A. 2021. Recycling "waste" nutrients back into RAS and FTS marine aquaculture facilities from the perspective of the circular economy. *Science of The Total Environment,* 762**,** 143057.

WANG, H.-X., QIN, L., WANG, Y., ZHOU, D.-Y., SONG, S., WANG, X.-S. & ZHU, B.-W. 2014a. Effects of heating conditions on fatty acids and volatile compounds in foot muscle of abalone Haliotis discus hannai Ino. *Fisheries Science,* 80**,** 1097-1107.

WANG, X., BROCH, O. J., FORBORD, S., HANDÅ, A., SKJERMO, J., REITAN, K. I., VADSTEIN, O. & OLSEN, Y. 2014b. Assimilation of inorganic nutrients from salmon (Salmo salar) farming by the macroalgae (Saccharina latissima) in an exposed coastal environment: implications for integrated multi-trophic aquaculture. *Journal of Applied Phycology,* 26**,** 1869-1878.

WANG, X., OLSEN, L. M., REITAN, K. I. & OLSEN, Y. 2012. Discharge of nutrient wastes from salmon farms: environmental effects, and potential for integrated multi-trophic aquaculture. *Aquaculture Environment Interactions,* 2**,** 267-283.

WANG, Z., LIANG, H., GUO, W., PENG, Z., CHEN, J. & ZHANG, Q. 2014c. Isolation, identification, and antioxidant activity of polysaccharides from the shell of abalone (Haliotis discus hannai Ino). *Genetics and Molecular Research,* 13**,** 4883-4892.

WEE, K., MAGUIRE, G. & HINDRUM, S. Methodology for digestibility studies with abalone. 2. Comparisons of markers in an artificial diet for blacklip abalone (Haliotis rubra) and greenlip abalone (H. laevigata). Third Asian Fisheries Forum, 1994. 152-155.

WIERZBICKI, A. S. 2001. Synthetic statins: more data on newer lipid-lowering agents. *Current medical research and opinion,* 17**,** 74-77.

WORM, B., BARBIER, E. B., BEAUMONT, N., DUFFY, J. E., FOLKE, C., HALPERN, B. S., JACKSON, J. B. C., LOTZE, H. K., MICHELI, F., PALUMBI, S. R., SALA, E., SELKOE, K. A., STACHOWICZ, J. J. & WATSON, R. 2006. Impacts of Biodiversity Loss on Ocean Ecosystem Services. *Science,* 314**,** 787-790.

XU, W., MAI, K., ZHANG, W., LIUFU, Z., TAN, B., MA, H. & AI, Q. 2004. Influence of dietary lipid sources on growth and fatty acid composition of juvenile abalone, Haliotis discus hannai Ino. *Journal of Shellfish Research,* 23**,** 1041-1045.

XU, Y., LI, L., REGENSTEIN, J. M., GAO, P., ZANG, J., XIA, W. & JIANG, Q. 2018. The contribution of autochthonous microflora on free fatty acids release and flavor development in low-salt fermented fish. *Food Chemistry,* 256**,** 259-267.

YOUNG, M. A., TREML, E. A., BEHER, J., FREDLE, M., GORFINE, H., MILLER, A. D., SWEARER, S. E. & IERODIACONOU, D. 2020. Using species distribution models to assess the long-term impacts of changing oceanographic conditions on abalone density in south east Australia. *Ecography,* 43**,** 1052-1064.

ZHENG, P., HAO, G., WENG, W. & REN, H. 2019. Antioxidant Activities of Hydrolysates from Abalone Viscera Using Subcritical Water-Assisted Enzymatic Hydrolysis. *Food and Bioprocess Technology,* 12**,** 910-918.

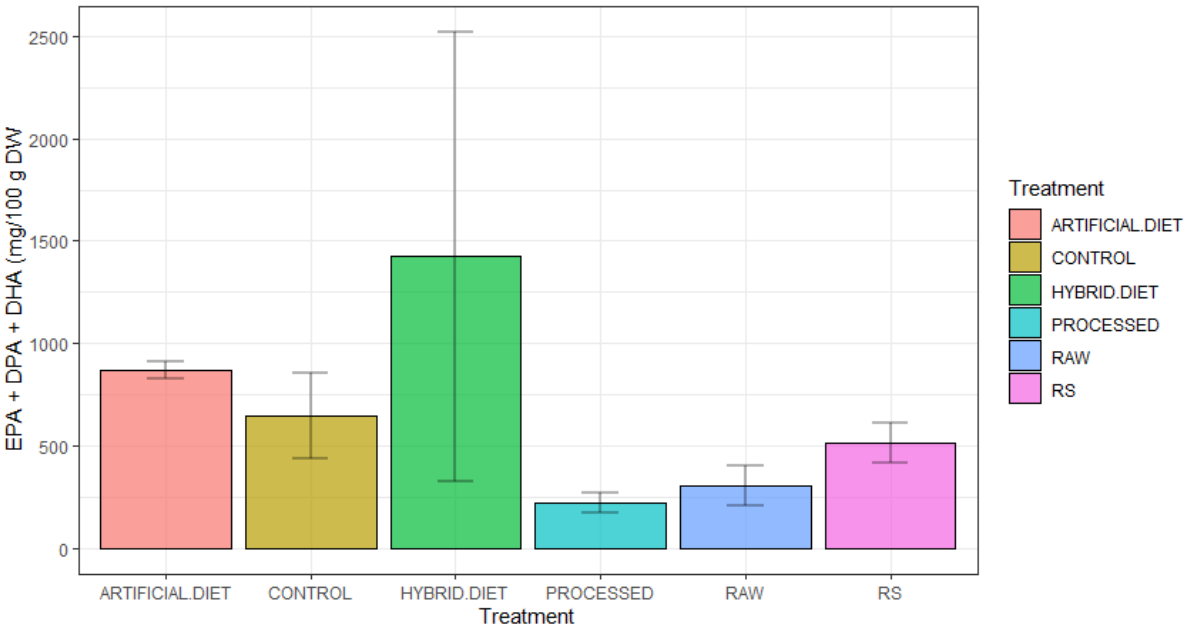
ZHOU, D.-Y., TANG, Y., ZHU, B.-W., QIN, L., LI, D.-M., YANG, J.-F., LEI, K. & MURATA, Y. 2012. Antioxidant activity of hydrolysates obtained from scallop (Patinopecten yessoensis) and abalone (Haliotis discus hannai Ino) muscle. *Food Chemistry,* 132**,** 815-822.

ZHOU, Y., WU, W.-H., ZHAO, Q.-B., WANG, X.-Y. & BAO, B. 2015. Inhibition of HMG-CoA reductase by MFS, a purified extract from the fermentation of marine fungus FusaFusarium solani FG319, and optimization of MFS production using response surface methodology. *Chinese Journal of Natural Medicines,* 13**,** 346-354.

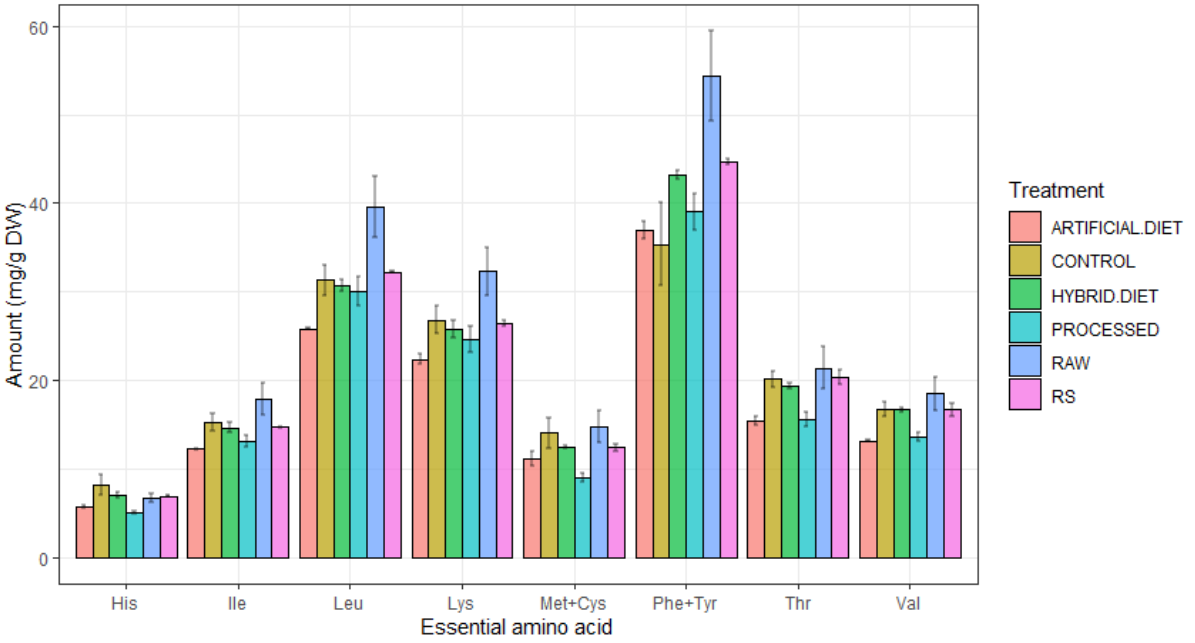
8. Appendix

Appendix 1 Solvent gradient program used for normal-phase liquid chromatography of lipid classes. Mobile phase A = isooctane/ethyl acetate (99.8:0.2), Mobile phase B = acetone/ethyl acetate (2:1) 0.15 % acetic acid. Mobile phase C = isopropanol/H2O (85:15).

Time (min)	Solvent A	Solvent B	Solvent C	Flow (ml $min^{-1)}$	Curve
0.0	100	0	0	1.5	1
1.5	100	0	0	1.5	6
1.6	97	3	0	1.5	6
6.0	94	6	0	1.5	6
8.0	50	50	0	1.5	6
8.1	46	39	15	1.5	6
14.0	43	30	27	1.5	6
14.1	43	30	27	1.5	6
18.0	40	0	60	1.5	6
23.0	40	0	60	1.5	6
24.0	0	100	0	1.5	6
25.0	0	100	0	2.0	6
27.0	0	100	0	2.0	6
27.1	100	0	0	2.0	6
36.0	100	0	0	2.0	6
36.1	100	0	0	1.5	6



Appendix 2. The sum of the amounts (in mg/100 g DW) of EPA, DPA and DHA in all *H. midae* and *H. tuberculata coccinea* samples.



Appendix 3. The amount (in mg/g DW) of essential amino acids in all samples of *H. tuberculata coccinea* and *H. midae*.