THE EFFECTS OF FEED SUPPLEMENTED WITH OMEGA-3 POLYUNSATURATED FATTY ACIDS ON CULTURED ABALONE

A thesis submitted for the degree of Doctor of Philosophy

By

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I dedicate this thesis to my parents, father, Tadesse Mateos and mother, Elfinesh Eskiyas and to the entire Mateos family. Finally, to my wife Jerusalem, all my dearest thanks for your support, love, and encouragement in the past, now and future.

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Mateos, H.T., Lewandowski, P.A., and Su, X.Q. (2011). Dietary Fish Oil Supplements Increase Tissue n-3 Fatty Acid Composition and Expression of Delta-6 Desaturase and Elongase-2 in Jade Tiger Hybrid Abalone. *Lipids*, 46, 741–751.

Mateos, H.T., Lewandowski, P.A., and Su, X.Q. (2011). Effects of dietary fish oil replacement with flaxseed oil on tissue fatty acid composition and expression of desaturase and elongase genes. *Journal of the Science of Food and Agriculture,* Article first published online: 10 AUG 2011 DOI: 10.1002/jsfa.4594.

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Oral presentations at conferences

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Table of Contents

List of Tables and Figures

List of Abbreviations and Acronyms

AIHW	Australian Institute of Health and Welfare
ALA	alpha-linolenic acid,18:3n-3
ARA	arachidonic acid, 20:4n-6
Na_2SO_4	anhydrous sodium sulphate
ANOVA	analysis of variance
AOCS	American oil chemists' society
ß	beta
BF_3	boron trifluoride
Ca	calcium
cDNA	complementary DNA
cm	centimeter
CO	canola oil
CVD	cardiovascular diseases
°C	degree Celsius
Δ	delta
DAFF	Department of Agriculture, Fisheries and forestry
DG	digestive gland
DGRw	daily growth rate in weight
DG_{SL}	daily growth rate in shell length
DHA	docosahexaenoic acid, 22:6n-3
DPA	docosapentaenoic acid, 22:5n-3
EPA	eicosapentaenoic acid, 20:5n-3
FAME	fatty acid methyl ester

FA	fatty acid
Fe	iron
FID	flame ionization detector
FlaxO	flaxseed oil
FO	fish oil
g	gram
GC	gas chromatography
GLC	gas liquid chromatography
GSI	gonadosomatic index
H	Haliotis
HSI	hepatosomatic index
LC-PUFA	long chain polyunsaturated fatty acid (PUFA with twenty or more atoms of carbon)
ln	natural log
I	iodine
K	potassium
kg	kilogram
KOH	potassium hydroxide
L	liter
LDL	low-density lipoprotein
HDL	high-density lipoprotein
LA	linoleic acid, 18:2n-6
m	meter
mg	milligram
Mg	magnisum

min	minute
mL	milliliter
mg/L	milligram per liter
mm	millimeter
MUFA	monounsaturated fatty acid
mol/L	mole per liter
mRNA	messenger ribonucleic acid
n-3	omega 3
n-6	omega 6
NHFA	National Heart Foundation of Australia
N_2	nitrogen
OA	oleic acid, 18:1n-9
P	phosphorus
pH	the negative logarithm(base 10) of the molar concentration of dissolved hydrogen ions
ppt	parts per thousand
PUFA	polyunsaturated fatty acid
RNA	ribonucleic acid
Rpm	revolution per minute
RT-PCR	real-time polymerase chain reaction
s	second
SFA	saturated fatty acid
SGRw	specific growth rate weight
SGR_S	specific growth rate of shell
SLI	shell length increase

SPSS	statistical package for the social sciences
Sr	strontium
µL	microliter
v/v	volume per volume
WG	weight gain
Zn	zinc

Summary Abstract

The long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA) are associated with a broad range of health benefits including reducing the risk of cardiovascular diseases, arthritis and certain cancers. In addition, they have also been found to play a role in reducing the risk of Alzheimer's disease, depression and schizophrenia. Seafoods are the main sources of LC n-3 PUFA. The LC n-3 PUFA and total lipid contents of marine animals can be influenced by a range of biological and environmental factors such as the diet of animals, water temperature, and the reproductive cycle. This research looked at the effects of dietary fish oil (FO) supplements on tissue fatty acid composition, expression of desaturase and elongase genes and growth performance of adult cultured Jade Tiger hybrid abalone. It also examined the effects of replacement of FO with vegetable oils on fatty acid composition, expression of desaturase and elongase genes, and growth performance of abalone. In addition the contents of total lipid and fatty acid of tissues were also examined over four seasons.

The first study investigated the lipid and fatty acid contents of muscle, gonad and digestive glands (DG) of Jade Tiger hybrid abalone over four seasons. Higher contents of total lipid and saturated fatty acids (SFA) were found in summer from muscle. For gonad the higher total lipid content was found in summer and spring whereas the SFA content peaked in summer only. For DG the higher contents of total lipid and SFA were recorded in all seasons except autumn. Winter samples showed significantly higher content of PUFA in all three types of tissue. High contents of eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) were recorded in winter from muscle

although no marked variations were observed from gonad. For DG the high content of DHA was also observed in winter while EPA and DPA maintained high levels in all seasons except summer.

The second study investigated the effects of FO supplements on fatty acid composition and the expression of Δ-6 desaturase and elongase-2 genes in Jade Tiger abalone. Five test diets were formulated to contain 0.5, 1.0, 1.5, 2.0 and 2.5% of FO respectively, and the control diet was the normal commercial abalone diet with no additional FO supplement. The muscle, gonad and DG of abalone fed with all of the five test diets showed significantly higher levels of total n-3 PUFA, EPA, DPA and DHA than the control group. In all three types of tissue, abalone fed diet supplemented with 1.5% FO showed the highest level of these fatty acids ($P<0.05$). For DPA the higher level was also found in muscle and gonad of abalone fed diet supplemented with 2% FO ($P<0.05$). Elongase-2 expression was markedly higher in the muscle of abalone fed diet supplemented with 1.5% FO ($P<0.05$), followed by the diet containing 2% FO supplement. For Δ-6 desaturase, significantly higher expression was observed in muscle of abalone fed with diet containing 0.5% FO supplement ($P<0.05$).

The third study was conducted to evaluate the effects of total or partial substitution of dietary FO by flaxseed oil (FlaxO) in Jade Tiger hybrid abalone on fatty acid composition of muscle, gonad and digestive gland, and the expression of desaturase and elongase genes. Abalone were fed five different experimental diets in which FO (control diet) was serially replaced by 25, 50, 75 and 100% FlaxO respectively. Muscle, gonad and DG of abalone fed the control diet and the diets

containing 25, 50 and 75% FlaxO showed significantly higher ($P<0.05$) levels of EPA, DPA and DHA compared to those fed the 100% FlaxO. The results also showed that Δ-6 desaturase and elongase gene expression in muscle was increased in a graded manner by increasing dietary FlaxO. The expression of both genes was higher in abalone fed the FlaxO substituted diets compared to the abalone fed FO.

The fourth study investigated the effects of graded substitution of dietary FO with canola oil (CO) on fatty acid composition and expression of Δ-6 desaturase and elongase-2 genes in muscle of Jade Tiger hybrid abalone. The control diet contained 1.5% FO supplement (0% CO). Four other diets contained FO/CO in ratios of 3:1 (CO 25%), 1:1 (CO 50%), 1:3 (CO 75%) and 100% CO. The result demonstrated that abalone fed the diets supplemented with 25% and 50% CO showed similarly high levels of total n-3 PUFA, EPA and DHA ($P<0.05$) as the 0% CO group. The highest levels of total PUFA and total monounsaturated fatty acids (MUFA) were found in the 100% CO group ($P<0.05$). The results also showed that Δ-6 desaturase and elongase-2 gene expressions were increased in a graded manner by increasing dietary CO.

The final study was conducted to investigate the effects of FO supplementation on the growth performance of Jade Tiger abalone and to determine the impacts of dietary replacement of FO by FlaxO and CO on the growth performance of abalone. In experiment 1 (E1), five test diets were formulated to contain 0.5, 1.0, 1.5, 2.0 and 2.5% of FO respectively. The control diet was the normal commercial abalone diet with no additional FO supplementation. In experiment 2 (E2), abalone were fed five different experimental diets in which FO (control diet) was serially

replaced by 25, 50, 75 and 100% FlaxO respectively. The control diet was the normal commercial abalone diet with 1.5% FO supplement. In experiment 3 (E3), abalone were fed five different experimental diets in which FO (control diet) was serially replaced by 25, 50, 75 and 100% CO respectively. The control diet was the normal commercial abalone diet with 1.5% FO supplement. Results from E1 showed that abalone fed diet supplemented with 1.5% FO displayed a significantly higher daily growth rate (DGRw) compared to the other experimental diets. Abalone fed the control diet showed a significantly lower DGRw. The values of weight gain (WG) and specific growth rate (SGRw) were also the highest in abalone fed 1.5% FO diet relative to those on the control diet. In E2, abalone fed the control FO diet and the diets containing 25, 50 and 75% of FlaxO showed no significant differences in DGRw, WG and SGRw. The diet containing 100% FlaxO showed significantly lower values of these growth parameters. In E3, abalone fed the control diet, FO diet and the diets containing 25% CO and 50% of CO showed no significant differences in DGRw, WG and SGRw. The diets containing 75% CO and 100% CO showed significantly lower values of DGRw, WG and SGRw compared to the abalone fed the other diets.

In conclusion, the contents of total lipid, omega-3 series of fatty acids, as well as the main individual fatty acid in muscle, gonad and DG of Jade Tiger hybrid abalone varied seasonally. Variations of total lipid content are likely to be associated with energy transfers during the reproductive cycle. The seasonal changes in fatty acid content were possibly due to the temperature fluctuations throughout the year. Supplementation with FO in the normal commercial diet can significantly improve LC n-3 PUFA level in cultured abalone, with 1.5% being the most effective supplementation level. The replacement of FO with FlaxO in the commercial abalone

diets at the levels of 25 to 75% can maintain the composition of health-benefiting LC n-3 PUFA without significant reduction in the tissues of cultured hybrid abalone, and achieve similar outcomes as FO supplementation. Supplementation with FO in the normal commercial diet of cultured abalone can improve growth performance of cultured abalone. Consistent with the effects on LC n-3 PUFA, FO supplementation at a concentration of 1.5% also achieved the best outcome on growth performance. In addition, the study demonstrated that it is feasible to replace 75% of dietary FO with FlaxO and 50% of dietary FO with CO respectively, without any negative effect on the growth performance of cultured abalone.

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General Declaration

I certify that the thesis entitled"**THE EFFECTS OF FEED SUPPLEMENTED WITH OMEGA-3 POLYUNSATURATED FATTY ACIDS ON CULTURED ABALONE"** submitted for the degree of **Doctor of Philosophy** is the result of my own work and contains no material which has been accepted for the award of any other degree or diploma at any university, and that to the best of my knowledge, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes four original papers published in peer reviewed journals and one original paper submitted to peer review journal. I confirm that this thesis does not exceed 100,000 words.

Full Name ..Hintsa Tadesse Mateos..
(Please Print)

Signed .....[redacted].....

Date.....15/10/2011.....

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PART A: DETAILS OF INCLUDED PAPERS: THESIS BY PUBLICATION

Please list details of each Paper included in the thesis submission. Copies of published Papers and submitted and/or final draft Paper manuscripts should also be included in the thesis submission.

Item/ Chapter No.	Paper Title	Publication Status (e.g. published, accepted for publication, to be revised and resubmitted, currently under review, unsubmitted but proposed to be submitted)	Publication Title and Details (e.g. date published, impact factor etc.)
Four	Seasonal variations of total lipid and fatty acid contents in muscle, gonad and digestive glands of farmed jade tiger hybrid abalone in Australia	Published	Published April 2010 in **Food Chemistry**. Impact Factor: 3.458
Five	Dietary Fish Oil Supplements Increase Tissue n-3 Fatty Acid Composition and Expression of Delta-6 Desaturase and Elongase-2 in Jade Tiger Hybrid Abalone	Published	Published April 2011 in **Lipids.** Impact Factor: 2.151
Six	Effects of dietary fish oil replacement with flaxseed oil on tissue fatty acid composition and expression of desaturase and elongase genes	Published	Published July 2011 in **Journal of the Science of Food and Agriculture.** Impact Factor: 1.36
Seven	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	Published	Published October 2011 in **Food Chemistry.** Impact Factor: 3.458
Eight	The growth performance of cultured abalone fed diets supplemented with fish oil and vegetable oils	Submitted	Submitted March 2012 to **Journal of the Science of Food and Agriculture**

Declaration by [candidate name]: Hinta **Signature:** [redacted] **Date:** 15/10/2011

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Chapter 1: Introduction

The long chain ($\geq C_{20}$) omega-3 polyunsaturated fatty acids (LC n-3 PUFA) are associated with a broad range of health benefits including reducing the risk of cardiovascular diseases (Rasmussen et al., 2006; Schwellenbach et al., 2006), and reducing the risk of certain cancers (Kimura et al., 2007; Kuriki et al., 2007). In addition, they have also been found to play a role in reducing the risk of Alzheimer's disease (Morris et al., 2005), depression and schizophrenia (Su et al., 2008). Seafoods are the main sources of dietary LC n-3 PUFA (Nichols et al., 1998; Sinclair et al., 1998; Su et al., 2004). However, cultured abalone showed lower levels of LC n-3 PUFA than their wild counterparts. Previous study showed that muscle of cultured Australian adult blacklip abalone, *H. rubra* contained a lower concentration (mg/100 g wet weight) and composition (% of total FA) of EPA, DPA and total n-3 PUFA than wild blacklip abalone (Su et al., 2004). In addition, Dunstan et al. (1996, 1999) found that wild adult greenlip abalone contained higher levels of EPA, DPA and total n-3 PUFA proportion (% of total FA) compared to juvenile cultured hybrid abalone. In fish, despite the high lipid content in cultured pintado and pacu, the n-3 PUFA concentrations in muscle tissue were higher in wild pintado and pacu (mg/g flesh) than in their respective cultured varieties (Tanamati et al., 2009). A study on other fish also showed that wild fish contained high proportion (% of FA) of LC n-3 PUFA than cultured fish (Jankowska et al., 2008). This is in agreement with the recent review by Turchini et al. (2011) which indicated that cultured fish contain less LC-PUFA as a percentage than wild fish. Other studies on fish also showed that wild fish are a better source of LC n-3 PUFA than their cultured counterparts (Kriton et al., 2002; Alasalvar et al., 2002).

Abalone are highly sought-after and nutritionally important shellfish with approximately 100 species found worldwide, and over 15 species are farmed in several countries including Australia, China, South Africa and USA (Sales and Janssen, 2004). There are 11 species of abalone in Australia waters. Blacklip abalone (*H. rubra)*, greenlip abalone *(H. laevigata*) and hybrid abalone (*H. laevigata x H. rubra)* are the three main types of abalone farmed in Victoria, Australia (Fisheries Victoria, 2003; Department of Primary Industries, 2007). Whilst abalone fisheries have gradually decreased worldwide from almost 20,000 mt in the year 1970s to less than 9000 mt in 2008, farmed abalone production has rapidly increased. In the six years immediately preceding 2008 the farm production increased by more than 350%, culminating in an estimated output of 30760 mt in 2008 (Gordon and Cook, 2010). Australian abalone fishery supplies around 50% of wild catch product and 30% of global product, and both wild and farmed abalone generates hundreds millions of dollars for the country's economy (Australian seafood CRC, 2011).

Abalone are excellent source of protein (Bautista-Teruel et al., 1999; González et al., 2001; Lee et al., 2010) and vitamins A, B_6 and B_{12} (Soriguer et al., 1997; González et al., 2001)**.** Abalone is also rich in Fe, Ca, Mg, Zn, Sr, I, P.K and other trace elements important to human health (Wang et al., 2009). Abalone meat contains a component of metabolites that can destroy cancer cells (Lee et al., 2010). Cultured abalone also represent a source of LC n-3 PUFA and good source of DPA for consumers (Dustan et al., 1999; Nelson et al., 2002; Wee et al., 2004; Su et al., 2004, 2006; Bautista-Teruel et al., 2011).

The LC n-3 PUFA and total lipid contents of marine animals vary depending on various biological and environmental factors, such as diet of the animals, water temperature the reproductive cycle, and the latitude at which they were harvested (Dunstan et al., 1999; Linehan et al., 1999; Su et al., 2006). Studies on mollusks have also found that lipid content in these animals vary depending on the nutritional value of the food supply and the environmental influences on the metabolic activities (Pazos et al., 1996, 1997). Fish oil (FO) contained high levels of LC n-3 PUFA, and inclusion of FO in commercial feed has been found to improve the health benefiting n-3 PUFA content in marine animals. However to date, there have been no studies conducted investigating the effects of feed supplemented with FO on fatty acid composition and growth performance cultured adult Jade Tiger hybrid abalone (*H. laevigata x H. rubra*).

Lipid sources, rich in LC n-3 PUFA, mainly come from marine origins, such as FO. However, FO is increasingly in short supply globally, and intensive studies are underway on FO substitution in the aquaculture industry (Barlow, 2000; Myers and Worm, 2003). Researches on the substitution of dietary FO have indicated that it may be possible to replace FO by plant seed oils (Barlow and Pike, 2001). No significant differences were found in the growth of *H. fulgens* fed vegetable oils (olive, corn and linseed oils) and cod liver oil (Durazo-Beltran et al., 2003). A number of studies on fish have suggested that dietary vegetable oil inclusion does not result in reduced growth performance and feed conversion in comparison with FO supplementation (Torstensen et al., 2000; Bell et al., 2003). Flaxseed oil (FlaxO) contains high concentrations of n-3 PUFA, mainly ALA, whereas canola oil (CO) is an alternative to FO because of the low cost and availability**.** The replacement of FO

with FlaxO or canola oil does not appear to lead to significant adverse effects on fish growth (Raso and Anderson, 2003; Wonnacott et al., 2004), but there is an unavoidable decrease in the concentration of LC-PUFA in the fish flesh (Mourente et al., 2005). Consistently, Francis et al. (2006) reported that CO and FlaxO could be partially substituted for FO in diets for Murray cod (*Maccullochella peelii*) with no apparent negative effect on growth. No studies have investigated the effect of dietary FO replacement with FlaxO and CO on fatty acid composition and growth performance in Jade Tiger hybrid abalone.

Fatty acid biosynthesis in fish has been well studied and it has been found that the overall conversion of 18-carbon PUFA to LC-PUFA occurs poorly in the marine species (Sargent et al., 2002). For abalone, however, there is still no direct evidence on the pathways of fatty acid biosynthesis. Available information suggests that *H. discus hannai* (Wei et al., 2004) have a capacity to synthesize EPA, and DHA from alpha-linolenic acid (18:3n-3, ALA) and ARA from linoleic acid (18:2n-6, LA) through elongation and desaturation. However, this study was based on the feeding trial only. Similar studies also reported that *H. laveigata, H. fulgens* and *H. asinina Linne* have limited capacity to synthesize LC n-3 PUFA from ALA (Dunstan et al., 1996; Durazo-Beltran et al., 2003; Bautista-Teruel et al., 2011). The same results were recorded in the study of *H.discus hannai* with its capacity to synthesize ALA to LC n-3 PUFA (Uki et al., 1986). It appears likely that abalone is able to biosynthesize LC-PUFA to a certain extent, by elongation and desaturation of short-chain fatty acids. These studies were all based on fatty acid profiles results of abalone. Therefore further investigations are required to elucidate the detailed pathways of fatty acid biosynthesis, metabolic gene expression and their regulation in abalone.

Desaturases and fatty acid elongases are enzymes which found to be critical in the biosynthetic pathways of LC-PUFA from shorter chain PUFA (Zheng et al., 2004). No studies have yet been conducted on the effects of FO supplementation and replacement of FO by vegetable oil on fatty acid desaturase and elongase gene expression in abalone. Studying gene expression in abalone as it will provide an indication of how supplementation and replacement of FO with vegetable oil effects fatty acid metabolise. There are no previous studies on fatty acid metabolism in abalone, thus the experiments described in this thesis will provide novel insights for researchers who work in this area and industry bodies who may be able to adapt the outcomes of the research into improved abalone aquaculture practices.

This thesis comprises five papers that are presented in the specific chapters. In the respective chapters, author declarations accompany these papers. Four of these papers have been published and one manuscript is yet to be submitted. The following is a brief summary of each chapter in this thesis: Chapter Two contains a literature review of the current scientific knowledge on the proposed subject. Chapter Three describes the general methods and materials used throughout the thesis. Chapter Four examines the contents of total lipid and fatty acid of muscle, gonad and digestive glands in farmed Jade Tiger hybrid abalone over the four seasons. Chapter Five reports the results of studies investigating the effects of feed supplemented with fish oil on fatty acid composition in muscle, gonad and of cultured adult female Jade Tiger hybrid abalone. In addition the effects of FO supplementation on fatty acid Δ-6 desaturase and elongase-2 gene expressions in muscle tissue of this animal were also studied. Chapter Six determines the effects of total or partial substitution of FO with FlaxO on fatty acid composition of muscle, gonad and digestive gland of

cultured Jade Tiger hybrid abalone. In addition the effects of replacement of FO with FlaxO on the expression of Δ-6 desaturase and elongase genes in muscle tissue were also studied. Chapter Seven focuses on the effects of total or partial replacement of FO with CO on fatty acid composition in the muscle of farmed Jade Tiger hybrid abalone. Moreover, the effects of replacement of FO with CO on expression of Δ-6 desaturase and elongase genes in muscle tissue were also studied. Chapter Eight examines the effects of feed incorporated with FO on growth performance of Jade tiger hybrid abalone and to evaluate the influence of replacement of FO by FlaxO and CO on growth performance of Jade tiger hybrid abalone. Chapter Nine outlines the overall conclusion and future research direction and Chapter Ten has the list of references used in the thesis excluding those references in the papers.

Chapter 2: Literature review

2.1 Lipids

Lipids are a chemically diverse group of substances, which are poorly soluble or insoluble in water, but soluble in nonpolar organic solvents such as chloroform, hydrocarbons, alcohols or ethers. Lipids are mostly composed of carbon, hydrogen, and oxygen (sometimes nitrogen and phosphorous). The main types of lipids include triacylglycerols, phospholipids sterols and waxes. The triglycerides, neutral lipids which are composed of a glycerol and three fatty acids, are the predominant lipids that exist in both foods and in the body and usually serve as energy sources. Triglycerides can be utilized as energy or stored in the adipose tissue for later use within the body (Turchini et al., 2011). Phospholipids are polar lipids and are generally considered to be structural or functional lipids, which are incorporated to a large extent in the membrane structure of cells. A phospholipid is composed of two fatty acids, a glycerol unit, a phosphate group and a polar molecule. Waxes are esters of fatty acids combined with long chain alcohols. Waxes are mainly used as a waterproofing material by plants and animals. Steroids are constituents of the plasma membrane, influencing the membrane's structure and its role in transport and permeability. The most common plasma membrane steroid is cholesterol (Ikonen, 2008).

2.2 Saturated and unsaturated fatty acids

Generally fatty acids are hydrocarbon chains with a carboxyl group at one end and a methyl group at the other (Mozaffarian et al., 2010). According to their degree of saturation (number of double bonds between carbon atoms) fatty acids can be

divided into three types or families: saturated, monounsaturated and polyunsaturated. Fatty acids also differ in the length of the carbon chain. Short-chain fatty acids have less than eight carbons. Medium-chain fatty acids have 8 to 14 carbons and long-chain fatty acids have 16 or more carbons. However, from a nutritional perspective, essential fatty acids (described below) are considered short-chain if they have 18 carbons and long-chain with 20 or more carbons**.**

2.2.1 Saturated fatty acids

Saturated fatty acids contain only carbon-carbon single bonds in their chain (Figure 1). The carbons in these fatty acids are fully loaded with hydrogen atoms, thus forming straight chains. SFA stack tightly, providing rigidity and making food fats, such as butter, solid at room temperature. They also have important structural properties and are a useful source of energy. The most prevalent SFA are palmitic acid, with 16 carbons, and stearic acid, with 18 carbons. They are found most commonly in animal products. Vegetable derivatives of SFA include palm oil, palm kernel oil, and coconut oil which are produced from vegetables that are common in tropical climates (Johnson and Sanders, 1994). They have important structural properties and are a useful source of energy. However, fats that are high in SFA have been found to be a key contributor to cardiovascular diseases (CVD), a major health threat worldwide (NHFA, 2008).

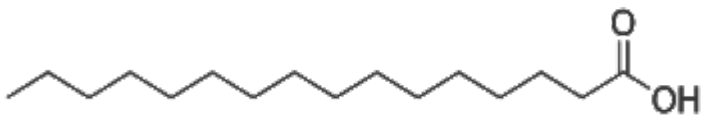


Figure 1. Chemical structure of palmitic acid

2.2.2 Monounsaturated and polyunsaturated fatty acids

Unsaturated fatty acids are so called because they have lost one or more pairs of hydrogen atoms from their carbon chain. Unsaturated fatty acids include monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) (Table 1). When pairs of hydrogen atoms are removed, double bond is formed, and the fatty acid molecule develops a kink or bends. The more hydrogen atoms missing, the more bent out of shape the fatty acid becomes. Unsaturated fatty acids, especially those with several double bonds, occupy more space, thereby making a fat containing them liquid (an oil) and cell membranes more fluid.

Table 1. Examples of unsaturated fatty acids

Common name	**Scientific name**	**Molecular name**	**Abbreviation**
Monounsaturated fatty acids			
Vaccenic acid	11-octadecenoic acid	18:1 n-7	VA
Oleic acid	9-octadenoic acid	18:1 n-9	OA
Omega 3 polyunsaturated fatty acids			
alpha-linolenic acid	9,12,15-octadecatrienoic acid	18:1 n-3	ALA
Eicosapentaenoic acid	5,8,11,14,17-eicosapentaenoic acid	20:5 n-3	EPA
Docosapentaenoic acid (Clupanodonic acid)	7,10,13,16,19-docosapentaenoic acid	22:5 n-3	DPA
Docosahexaenoic acid	4,7,10,13,16,19-docosahexaenoic	22:6 n-3	DHA
Omega 6 polyunsaturated fatty acids			
Linoleic acid	9,12-octadecadienoic acid	18:2 n-6	LA
gamma-linolenic acid	6,9,12-octadecatrienoic acid	18:3 n-6	GLA
Arachidonic acid	5,8,11,14-eicosatetraenoic acid	20:4 n-6	AA
Docosapentaenoic acid (Osbond acid)	4,7,10,13,16-docosapentaenoic acid	22:5 n-6	DPAn-6

(Footnote: all unsaturated FAs shown in this table are of the *cis* configuration.)

2.2.2.1 Monounsaturated fatty acids

The fatty acids containing one double bond are MUFA (Figure 2). Common MUFA are palmitoleic acid (16:1 n−7), cis-vaccenic acid (18:1 n−7) and oleic acid

(18:1 n−9, OA). Palmitoleic acid has 16 carbon atoms with the first double bond occurring 7 carbon atoms away from the methyl group (and 9 carbons from the carboxyl end). It can be lengthened to the 18-carbon cis-vaccenic acid. OA has 18 carbon atoms with the first double bond occurring 9 carbon atoms away from the carboxylic acid group. Vegetable oils such as olive oil and CO are a good source of MUFA (Schmalz and. Kerrigan. 2003). Beef fat and lard fats also contain MUFA. Other sources include macadamia nut oil, grape seed oil, peanut oil, sesame oil, corn oil, popcorn, whole grain wheat, cereal, oatmeal, safflower oil, sunflower oil, tea-oil, camellia, and avocado oil. MUFA have been found to reduce low-density lipoprotein (LDL) cholesterol, while possibly increasing high-density lipoprotein (HDL) cholesterol. A high-MUFA diet (45% energy from fat) based on olive oil, and showed a decrease of the dense LDL fraction in the MUFA group (Zambon et al., 1999). However, their true ability to raise HDL is still under debate.

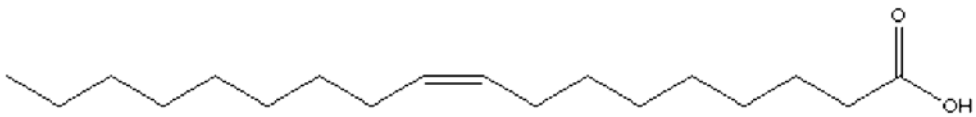


Figure 2. Chemical structure of oleic acid (OA, 18: n-9)

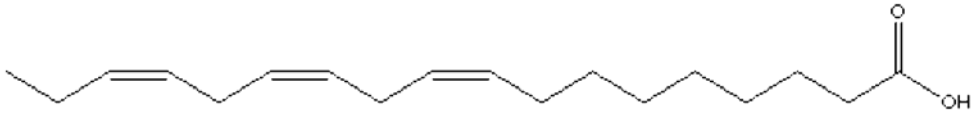
2.2.2.2 Polyunsaturated fatty acids

The fatty acids that contain more than one double bond are called PUFA (Figure 3). The position of the first double bond is given by the (n-x) notation, counting the number of carbon atoms from the methyl end, according to the international nomenclature. For example, omega-3 and omega-6 (also referred as n-3 and n-6 fatty acids) denote fatty acids, in which the first double bond starts at 3 and 6 carbons from the methyl end respectively. The symbol 18:3n-3 identifies a fatty acid, with 18 carbon atoms and 3 double bonds, the first double bond occurring after the third

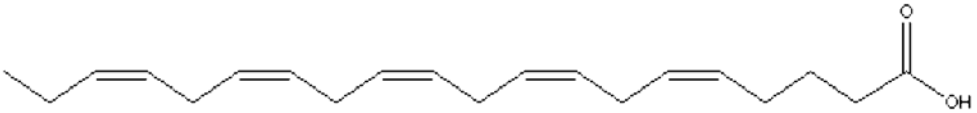
carbon atom (Gurr et al., 2002).

2.2.2.2.1 n-3 polyunsaturated fatty acids

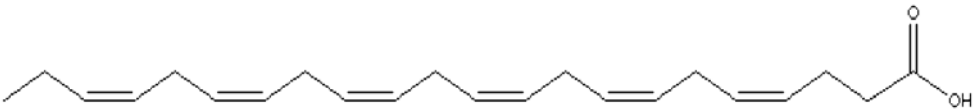
n-3 polyunsaturated fatty acids come in short and long-chain varieties. The short chain form is ALA. It has 18 carbons and 3 double bonds (Figure 3). ALA is considered essential because all vertebrates are not able to biosynthesie it denovo. EPA, DPA and DHA are the LC n-3 PUFA. Compared with ALA, these fatty acids are elongated and highly unsaturated; EPA has 20 carbons with 5 double bonds, DPA and DHA has 22 carbons with 5 and 6 double bonds respectively (Figure 3). Terrestrial sources rich in n-3 PUFA include flax, camelina seed, perilla and chia seed oils which contain the 18-carbon ALA as the major n-3 PUFA (Turchini et al., 2011). The carbon-20 and carbon-22 n-3 PUFA such as EPA and DHA have been found in abundance in seafood such as fish and shellfish. These fatty acids are essential for humans as well as for fish and shellfish. Fish that tend to have high concentrations of these fatty acids include tuna, sardines, salmon, mackerel and herring (NHFA, 2008). Shellfish such as abalone, oyster, mussel and scallop are also good sources of these health-benefiting LC n-3 PUFA (Su et al., 2004, 2006; NHFA, 2008). To reduce the risk of coronary heart disease, the Heart Foundation recommends that Australians should consume about 500 mg per day of combined DHA and EPA through a combination of two or three serves (150 g per serve) of oily fish per week, FO capsules or liquid, and food and drinks enriched with marine n-3 PUFA (NHFA, 2008). DPA is an n-3 elongation product of EPA. It may also be formed by the retroconversion of DHA. At present, there is insufficient evidence on the role of DPA in preventing CVD. The Heart Foundation has not made any recommendations about DPA.



Alpha-linolenic acid (ALA, 18:3n-3)



Eicosapentaenoic acid (EPA, 20:5n-3)

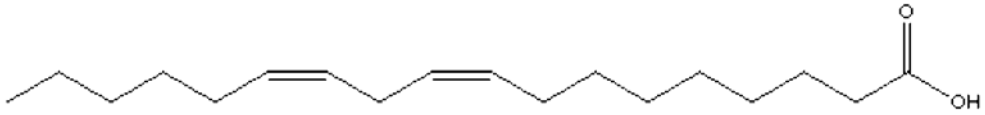


Docosahexaenoic acid (DHA, 22:6n-3)

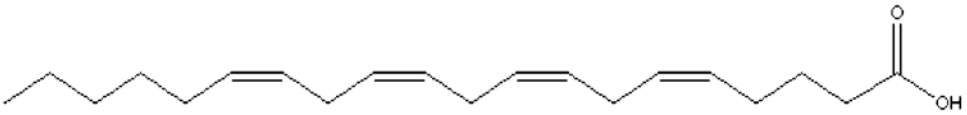
Figure 3. Chemical structure of alpha-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid.

2.2.2.2.2 n-6 polyunsaturated fatty acids

Like n-3s, this family of PUFA has its short-chain representative, LA an essential fatty acid and the most prevalent PUFA in western diets (Figure 4). LA is most prominent in nature, with a high proportion found in vegetable oils, such as, sunflower, safflower and corn, soybean and canola oils (Schmalz and Kerrigan. 2003). Evening primrose and borage oil are also high in LA. Another major source of n-6 PUFA is through animal products in the form of ARA (Figure 4), which are predominantly found in both muscle and organ meats (Sinclair, 1991). ARA is a vital constituent of cell membranes and an important source of substances involved in combating infection, generating protective inflammatory responses and promoting blood coagulation. It also has important functions in communication between and within cells.



Linoleic acid (LA, 18:2n-6)



Arachidonic acid (ARA, 20:4n-6)

Figure 4. Chemical structure of linoleic acid and arachidonic acid

2.3 Health benefits of n-3 polyunsaturated fatty acids

2.3.1 Cardiovascular disease

Cardiovascular disease, or diseases of the circulatory system, includes all diseases of the heart and blood vessels. Cardiovascular disease (CVD) is known to be one of the major causes of death in Australia and other western nations accounting for 34% of all deaths in Australia in 2006. In 2004-05, 18% (approximately 3.5 million) of Australians reported having a long-term cardiovascular condition (AIHW, 2010). The dietary habits of individuals have been shown to impact on heart health. Long-term consumption of a diet containing high SFA and low PUFA has been found to be a primary cause of CVD (AIHW, 2001).

A study by Mensink and Katan (1992) indicated that increased consumption of fish and shellfish, as a source of n-3 PUFAs is often associated with decreased mortality as well as morbidity from CVD. Similar studies have also found a favourable modifying effect of dietary FO on various CVD risk factors such as high blood

pressure and high blood cholesterol (Appeal et al., 1993). A high level of triacylglycerol is a risk factor associated with CVD. EPA and DHA found in FO have been shown to reduce triacylglycerol levels in the liver (Flaten et al., 1990), which has been found to decrease circulating triacylglycerol levels in humans (Harris, 1999). A diet high in LC n-3 PUFA has been shown to be associated with decreased plasma LDL cholesterol, therefore decreasing the risk of CVD (Li et al., 1999). On the other hand, ALA found in vegetable oil has been shown to have little effect on decreasing plasma lipid levels and reducing blood pressure compared to n-3 PUFA particularly EPA and DHA found in marine sources (Sinclair, 1991). Other study also indicated that long term consumption of fish appears to be associated with lower primary and secondary heart attack rates and death from CVD (Erkikla et al., 2006).

Studies also found that FO supplementation can reduce serum cholesterol levels and produce a gradual reduction resulted in LDL cholesterol, and a highly significant increase in HDL cholesterol, which competes with LDL cholesterol, (Zsigmond et al., 1990; Saynor and Gillott, 1992). The treatment with FO was also reported to have a dose response relationship (Harris et al., 1990), and with a level of around 6% n-3 PUFA of total energy, resulting in a significant plasma cholesterol lowering (Insull et al., 1994). n-3 PUFA intake was also associated with increases in HDL cholesterol concentrations in healthy volunteers and patients with familial hyperlipidemia (Engler et al., 2004; Breslow, 2006).

Inflammation is now recognized to be a major contributor to the underlying mechanism of atherosclerosis (Willerson and Ridker, 2004). Treatment with n-3

PUFA was associated with reductions in plasma levels of tumor necrosis factor-a and interleukin-1J3 in healthy subjects (Caughey et al., 1996). Philips et al. (2003) have found that dietary DHA supplementation decreased exercise-induced inflammation by reducing C-reactive protein and interleukin-6 in healthy subjects. Most of the above-mentioned immune-modulatory effects of n-3 PUFA may suggest prevention of atherosclerosis. Some of the anti-atherosclerotic associations of n-3 PUFA (Hino et al., 2004; Erkikla et al., 2006) may be the result of their beneficial effects on platelet activities.

n-3 PUFAs may also play a role in regulation of blood pressure (Mozaffarian, 2007); this effect may be mediated through an alteration in the balance between vasoconstrictive prostaglandins and increasing production of vasodilatory prostacyclin. A study by Mori and colleagues (2000) reported a significant reduction in systolic and diastolic blood pressure in overweight subjects by supplementing 4 g/day purified DHA.

Anti-arrhythmic properties of n-3 PUFA are another area of interest associated with CVD. These effects may be direct consequences of the incorporation of n-3 PUFA, especially DHA and EPA, into cell membranes (Gallai et al., 1995). Both EPA and DHA are readily incorporated into cell membranes following supplementation (Wilkinson et al., 2005). The membrane enrichment with EPA/DHA may result in increasing membrane fluidity in cardiac cells, thereby preventing atrial fibrillation (Schwalfenberg, 2006) and reducing the binding of inflammatory cytokines to their receptors (Ergas et al., 2002). This may explain the benefits of EPA/DHA in preventing cardiac events. Consuming 3 g/day

encapsulated FO for 6 weeks has reduced inducible ventricular tachycardia and risk of sudden cardiac death among patients with coronary artery disease (Metcalf et al., 2008). This could be the result of an attenuation in intracellular calcium and in the response to noradrenalin (Den Ruijter et al., 2008). Moreover, consumption of marine n-3 PUFAs was linked with particular heart rate variability constituents, including indexes of vagal activity, baroreceptor responses, and sinoatrial node function among American adults (Mozaffarian et al., 2008). Such enhancement of vagal control by fish consumption could explain, in part, improved endothelial function and reduced resting heart rate after FO supplements (Shah et al., 2007).

2.3.2 Cancer

Cancer is known to be one of the leading causes of death in Australia and around the world (Australian Bureau of Statistics, 2011). It has been previously found that SFA such as those found in meat and meat products are related to increasing cancer risks, whereas seafood, high in n-3 PUFA are found to reduce cancer risk (Zang et al., 2000). A positive association between high intake of fat and the incidence of breast, colon, pancreatic, and prostate cancers has been shown (Cohen, 1992). However, such an association may be independent of the energy contents of the fats (Cohen, 1992). Further studies revealed high n-3 PUFA content in erythrocyte membranes was inversely correlated with the development of colorectal cancer (Kuriki et al., 2006; Kimura et al., 2007;Theodoratou et al., 2007) and breast cancer (Kuriki et al., 2007). Similar studies have found that EPA and DHA, which are primarily found in fish and shellfish, have been shown consistently to inhibit the proliferation of cancer cell lines in vitro and to reduce the

risk and progression of these tumours in animal experiments (Bougnoux, 1999). On the other hand, studies have shown that, diets high in animal fat or n-6 PUFA intake contributed to an increased risk of colorectal cancer (Ames et al., 1995) and breast cancer (Larsson et al., 2004). Nonetheless, the n-6: n-3 PUFAs ratio of eicosanoid production seems to play a major role (Rose et al., 1995). ARA had been shown to stimulate pancreatic cancer cell growth in vitro, whereas EPA had suppressed such growth (Funahashi et al., 2008). Other mechanisms may include modifications in the hormonal status, cell membrane structure and function, cell signaling transduction pathways and gene expression, and immune function (Escrich et al., 2006).

2.3.3 Mental health

n-3 PUFA have been found to play a role in the preventation of mental disorders, in particular EPA and DHA are related to brain activity (Markrides et al., 1994) by helping the cells transmit electrical signal to the body (Bourre et al., 1991). It appears to improve the conduction of electrolyte through cell membranes (Crawford and Sinclair, 1996). Studies showed that a diet low in n-3 PUFA could impair the growth and functioning of neurons (Neuringer, 1988). Therefore, n-3 PUFA is a major component in mental disorders such as depression and dementia where low levels of DHA are found (Hibblen and Salem, 1995). Similar studies have found that dementia with a vascular component were most strongly associated with a diet high in SFA, whereas the consumption of seafood inversely associated with the incidence of dementia, in particular Alzheimer's disease (Neuringer, 1988).

Incorporation of DHA into brain cell membranes also improves membrane

fluidity, which may contribute to brain function via their ability to bind ligands and initiate a series of signal transduction processes (Michell et al., 2003). DHA and EPA may also influence brain function by affecting production and function of neurotransmitters such as serotonin and dopamine (du Bois et al., 2006), inhibition of phospholipase A2 (Bennett and Horrobin, 2000), and inhibition of protein kinase C (Seung et al., 2001).

Dementia and Alzheimer's disease, affects more than 30% of elderly people older than age 85 years (Ott et al., 1995). Studies have shown that dementia and CVD may share several common risk factors, including high intakes of dietary total fat, high SFA, high n-6: n-3 PUFA ratio, and low fish intake (Kalmijn, 2000). Because n-3 PUFA possess anti-inflammatory properties, and inflammatory markers have been located in the brain of patients with Alzheimer's disease, it seems reasonable to suggest that n-3 PUFA may delay the onset of Alzheimer's disease by reducing brain inflammatory state (Honig, 2005). This may be one of the reasons behind prevention of Alzheimer's disease/dementia by adequate DHA/EPA intake as suggested by the Framingham heart study (Schaefer et al., 2006).

2.3.4 Infant development

Several studies indicated that, n-3 PUFA are required for normal conception, growth, and development of an embryo. During the third trimester, approximately 50 to 60 mg/day of maternal DHA stores are transferred to a fetus via the placenta (Clandinin et al., 1980; Innis et al., 2001). DHA is particularly highly concentrated in the brain and retinal membranes, especially in photoreceptors, and is therefore

assumed to play a critical role in both vision and cognitive function (Jensen, 2006). A positive association has been observed between red blood cell DHA content and improved visual acuity as well as other indexes of brain development in human infants (Innis, 2003; Lauritzen et al., 2004). Several studies have also reported improved visual acuity (Hoffman et al., 2003; Decsi et al., 2005) and hand-eye coordination (Dunstan et al., 2008) in infants receiving greater DHA in the womb and during lactation. Cognitive function has also shown to be improved in formula-fed term infants supplemented with DHA for 17 weeks (Birch et al., 2000).

2.3.5 Rheumatoid arthritis

Rheumatoid arthritis is one of the most common inflammatory illnesses that have shown improvement by n-3 PUFA supplementation. In a recent study (Aksoy et al., 2006), it was found that n-3 PUFA supplementation may improve pain intensity, morning stiffness, number of affected joints, and amount of medication needed to alleviate symptoms of this disorder. The benefit most often observed with FO supplementation is an improvement in the number of tender joints on physical examination (Kremer et al., 1995), although some authors reported improvement in the Ritchie Articular Index (Kjeldsen-Kragh et al., 1992) and in morning stiffness (Kremer et al., 1990). n-3 PUFA supplements have been studied in comparison with a variety of dietary interventions, including corn oil (Kremer et al., 1995), and olive oil (Kremer et al., 1990). Improvements in the number of tender joints and in morning stiffness were confirmed in a meta-analysis of published studies that used a wide range of daily dietary supplementation with n-3 PUFA (Fortin et al., 1992).

2.4 Abalone classification, anatomy and reproduction

2.4.1 Classification

In the animal kingdom, abalone belongs to the phylum Mollusca, a group of invertebrates or animals lacking a backbone or other forms of internal skeleton. Molluscs also include scallops, oysters, mussels, pippies and cockles as well as octopus, squid and cuttlefish (Heasman and Savva, 2007). Abalone are the member of the class Gastropoda which includes snails. All members of this class are univalves, having one shell (Galindo et al., 2003), unlike the bivalve such as oysters which have two shells. All abalone belong to the family *Haliotida*e and are member of the genus *Haliotis* (Huchette et al., 2003). There are currently about 100 *Haliotis* species worldwide (Sales and Janssens, 2004), with 13 of them found in Australian waters (Department of Agriculture, Fisheries and Forestry (DAFF), 2005).

2.4.2 Anatomy

The most conspicuous part of any abalone is the shell, with its row of respiratory pores. Shells are prized because of their inner, iridescent layer (http://www.fishtech.com). The shell protects the soft body tissues including a very large and undulating muscular foot (Figure 5) that allows the abalone to remain firmly attached to rocky substrates even while moving and feeding (Heasman and Savva, 2007). A column of shell muscle attaches the body to its shell. The mantle circles the foot as does the epipodium, a sensory structure and extension of the foot which bears tentacles. The epipodium projects beyond the shell edge in the living animal. The epipodium surface may be smooth or pebbly in appearance and its edge may be frilly or scalloped. It is the most reliable structure for identifying abalone species. The internal organs are arranged around the foot and under the shell. The gonad

extends around the side opposite the pores and to the rear of the abalone. Mature males and females can easily be recognized by differences in gonad color. Male abalone can be identified by their white to cream gonads (Heasman and Savva, 2007). Female abalone gonads vary widely from green to olive and from maroon to brown (Freeman, 2001). Abalone have a pair of eyes, circular mouth with a tongue-like organ called a 'radula' with rows of tiny teeth that allow abalone to rasp particles off large kelps and smaller algae (Shepherd et al., 1997). The gill chamber is next to the mouth and under the respiratory pores. Water is drawn in under the edge of the shell, and then flows over the gills and out the pores. Waste and reproductive products are carried out in the flow of water. Since it has no obvious brain structure, the abalone is considered to be a primitive animal. However, it does have a heart on its left side and blood flows through the arteries, sinuses and veins, assisted by the surrounding tissues and muscles (http://www.fishtech.com).

2.4.3 Reproduction

The sexes in abalone are separate, and males and females are easily recognised by the colour of the gonad (Figure 6) (Brown, 1991a) and fertilization in abalone is external (Joll, 1996). The eggs or sperm are released through the pores with the respiratory current. This is known as broadcast spawning (Freeman, 2001). Most abalone species generally have one annual maturation period, however a study found that not all eggs are necessarily released in a single spawning and that an individual may be able to release eggs over an extended period (Shepherd et al., 1992). In Australia, blacklip abalone have been observed to have multiple spawnings within one spawning season (Brown, 1991a). Abalone are relatively fecund and there is a

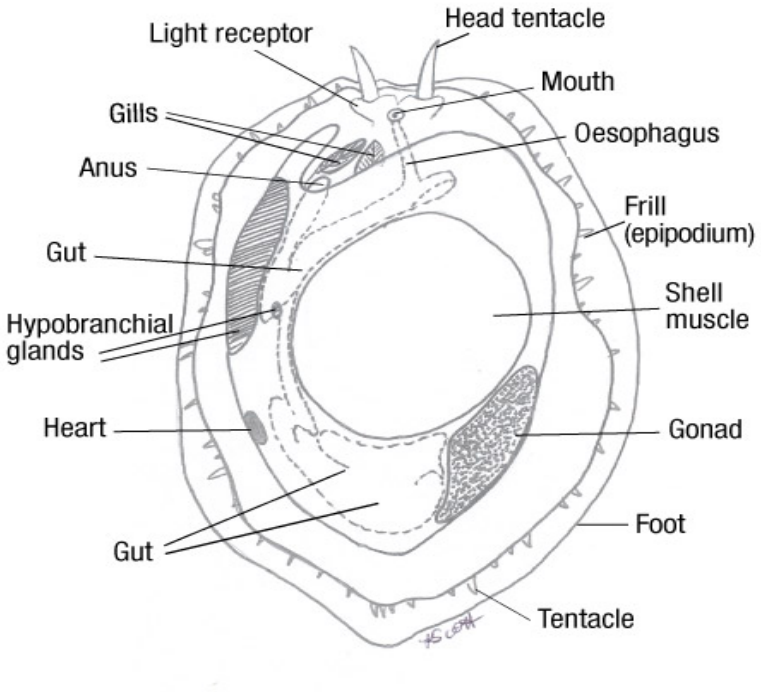


Figure 5. Anatomy of Abalone (from DAFF, 2008).

relationship between size and fecundity for different species of abalone (Wells and Mulvay, 1992). For example, abalone with a 37.5 mm shell diameter may spawn 183,000 eggs or more at a time, while a 122 mm abalone may spawn 8.6 million or more (Wells and Keesing, 1989). Environmental factors such as temperature, photoperiod and food abundance are known to influence the spawning cycle of abalone (Freeman, 2001). The egg hatches as microscopic, free living larvae. Larvae drift with the currents for about a week, then the abalone larvae settles to the bottom, sheds its swimming hairs and begins to develop the adult shell form. If suitable habitat is located it may grow to adulthood. The chance that an individual larva will survive to adult hood is very low. Fortunately abalone are prolific spawners. Mortality, however, is also thought to be high (McShane, 1992). Hybrid abalone are not uncommon in areas where several species occur together. All species can hybridize (http://www.fishtech.com).

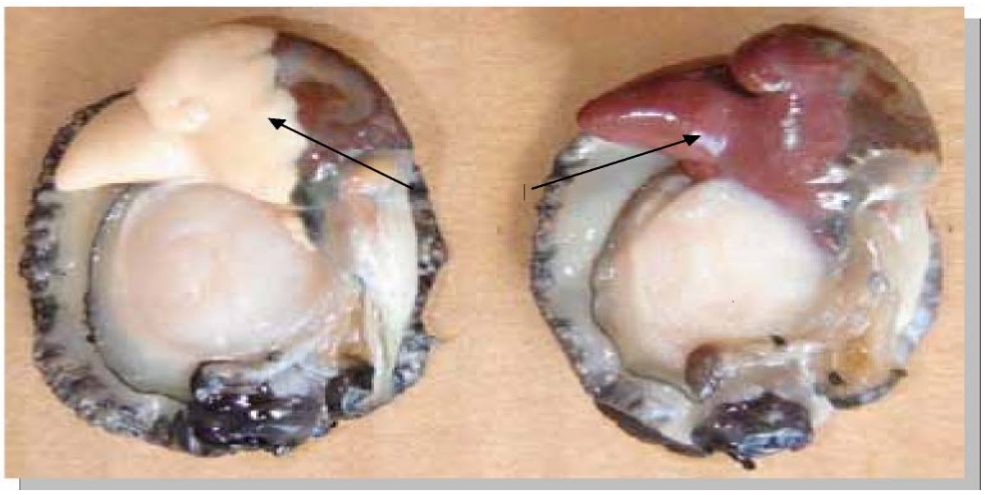


Figure 6. The ripe gonads of adult blacklip abalone, characterized in the case of males (left specimen) by a large cream-colored testis and in females (specimen on the right), by a maroon-colored ovary (from Liu, 2005).

2.5 Abalone fisheries and aquaculture

2.5.1 Abalone fisheries

Abalone have been fished since early history, with the first reference to abalone divers in Japan dating from 30 A.D (Hahn, 1989b). However, it is not known whether the practice started in Japan or China (Grubert, 2005). The popularity of abalone in Southeast Asia spread with the gradual movement of the Chinese to other regions including Taiwan and Korea (Cuthbertson, 1978), but continuous unsustainable levels of harvesting in most of these countries has depleted and destroyed their fisheries (Grubert, 2005). The commercial catch of abalone worldwide has declined from almost 20.000 mt in the 1970s to less than 9.000 mt in 2008 (Gordon and Cook, 2010). During this time, two of the world's major abalone fisheries (United States and South Africa) have been decommercialized (California Department of Fish and Game, 2009), and the only country that has shown a slight

increase in abalone fishery production is Mexico (Gordon and Cook, 2010). The major reasons for the decline include over exploitation, illegal harvesting, disease, and habitat degradation (Cook and Gordon, 2010). Predation is a substantial cause of high mortality of abalone (Shepherd et al., 2000). Ecological interactions with other organisms, not necessarily predators within the natural habitat, may also have a substantial effect on the survival of abalone, in positive or negative ways. Sea urchins are typically abundant organisms in subtidal, barren rocky-reefs (Jones and Andrew, 1990). Most countries with viable abalone fisheries are attempting to reduce human impacts on abalone stocks by minimizing industrial discharge and placing restrictions (e.g. quotas, gear controls and seasonal closures) on recreational and/or commercial fisheries (Grubert, 2005). The major abalone fishing countries are Australia, Japan, New Zealand, South Africa, Mexico, and the United States. Recent (2008) annual catch statistics (legal landing from abalone fisheries) for these countries are 5000 t, 1200 t, 1500 t, 0 t, 750 t and 0 t, respectively (Gordon and Cook, 2010). Illegal catches in 2008 for those countries was 1500 t, unknown t, 1000 t, 2000 t, 400 t and 200 t, respectively (Gordon and Cook, 2010). The world market for abalone relies on about 15 species, with those sold in greatest quantities being *H. diversicolor* and *H. discus hannai*. The largest consumer nations are Japan, Hong Kong and China.

Australia is one of the major abalone fishing countries and abalone is one of Australia's most valuable fisheries resources. Australia produces about 50 per cent of the global wild abalone harvest. In 2003-04, Australia produced an estimated 5,585 tonnes of wild-caught abalone (Australian Bureau of Agricultural and Resource Economics, 2004), making it Australia's fourth most valuable fishery export after rock

lobster, tuna and pearls. However recent annual catch statistics (2008) showed that legal landing from abalone fisheries for Australia is 5000 t, which showed a gradual decrease in wild caught abalone like the rest of the world (Gordon and Cook, 2010). The abalone industry provides a wide range of economic, social and recreational benefits to the Australian community and, in particular, to the coastal areas of the abalone-producing states of New South Wales, South Australia, Tasmania, Victoria and Western Australia (Table 2 and Figure 7). In Victoria, for example, more than 1,100 jobs are estimated to be directly linked to abalone harvesting or processing. The combined value of commercial abalone licenses in the states is estimated at more than $500 million (DAFF, 2005). Abalone is not harvested in commercial quantities in Queensland or the Northern Territory, though both states export some quantities, as has the Australian Capital Territory, but in smaller quantities.

Table 2: Species of abalone taken and number of license, by region in Australia (DAFF, 2005).

State	**Species**	**No. of license**	**Total allowable (ton**
NSW	Blacklip	42 licensed divers	206
Victoria	Blacklip, Greenlip	71 licence holders	1359
Tasmania	Blacklip	125 diving licenses	2509.5
South Australia	Blacklip, Greenlip	35 holders	880
Western Australia	Greenlip, Brownlip and Roe's	42 licenses	196.3
	Total		4,608

Figure 7. Distribution of abalone species in Australia (from Freeman, 2001).

2.5.2 Abalone aquaculture

As a result of wild stock depletion, abalone farming began in the late 1950s and early 1960s in Japan and China. During the 1960s, several government and private researchers began studies to advance knowledge of hatchery systems, leading to successful production of juvenile abalone for release in the wild (Gorden and Cook, 2010). A very rapid development of abalone cultivation has taken place in the 1990s and it is now widespread in many countries including Australia (Dustan et al.,1999), USA (McBride, 1998), Mexico (Searcy-Bernal et al., 2010), South Africa (Troell et al., 2006), China (Nie and Wang, 2004), Chile (Flores-Aguilar et al., 2007), Japan, Taiwan, Korea, Ireland, Iceland and others (Gorden and Cook, 2010; http://www.fishtech.com). The largest cultured abalone producer in the world is China with over 300 farms (Nie and Wang, 2004). As a result of the huge increase in farmed abalone from China estimated farm production for 2008 to 2009 is 23,000

mt, mostly *H. supertexta* (Gorden and Cook, 2010). Japan has many major seed farming operations, most of which are involved in ocean enhancement, totals of which are included under Fisheries. 30 to 40 million seeds are planted annually. Taiwan currently has over 400 farms (many are small family-run operations) (http://www.fishtech.com). Abalone aquaculture in California is expanding. There are currently 13 abalone farms on the coast of California. The largest farm produces over 100 tons and the smaller less than 10 tons (McBride, 1998). Farm production in Korea, which is estimated to have increased by more than 60 times during the past 9 years, totaled more than 4,500 mt in 2007(Gorden and Cook, 2010). Abalone aquaculture is rapidly developing in Chile although this mollusc does not occur naturally in this country. The red abalone *H. rufescens* was introduced in 1977 and the ezo abalone *H. discus hannai* in 1982. After several years of research and development, the industry began in 1992 and Chile is currently one of the top ten producers of cultured abalone in the world with 304 tons in 2006 culturing mainly red abalone (Flores-Aguilar et al., 2007). In combination, all of Europe, Iceland and Pacific Rim countries have a farm production of over 300 metric tons in 2007. Australia/New Zealand farmed production is estimated at 600mt in 2007 and Thailand is the newest and fastest growing farming nation, mostly *H.diversicolor supertexta* (http://www.fishtech.com). Worldwide, there are over 15 species of abalone which are farmed and commercially important. Species can live in water temperatures ranging from 2°C to 30°C (http://www.fishtech.com). The South African abalone cultivation industry with *H. midae* started in the 1990s and has developed rapidly. It is now the largest producer outside Asia. With a rapid decline in wild abalone fisheries, farming now dominates the abalone export market in South Africa. There are 15 farms in South Africa and some farms have reached 100 tones

or more per annum (Troell et al., 2006). In Mexico currently, three private farms culture abalone mostly producing *H. rufescens,* and 29.6 t of this abalone product were sold in 2008, mostly to markets in Asia and the United States. In addition, six hatcheries of fishermen cooperatives produce larvae and seed for restocking purposes and at least two of these have started grow-out trials. During the past two years, more than 130 million larvae and 350 thousand seeds of *H. fulgens* and *H. corrugata* have been released in the wild by these cooperative hatcheries (Searcy-Bernal et al., 2010). At the same time as world landings from legal fisheries were declining, world farm production was rapidly increasing in several countries. In the six years immediately preceding 2008, for example, farm production increased by more than 350%, culminating in an estimated output of 30.760 mt in 2008 (Cook and Gordon, 2010).

Abalone aquaculture began in Australia in the early 1980s in South Australia and Tasmania and spread to other states including Victoria (Weston et al, 2001). From about the mid-1990s specialised diets and improved tank technology allowed the production cycle to be shortened with minimum market size of 70 mm attained in 3 to 4 years (Weston et al, 2001). The abalone aquaculture industry is still developing in Victoria, but is the fastest growing aquaculture sector (Anonymous, 2005). Production has increased exponentially over the past five years with the value of grow out production trebling between 2002/03 and 2003/04. In Australia as a whole, production has reached 300 tonnes and is expected to increase by 20% per year as more farms reach commercial levels (Fleming, 2005). Blacklip *(H. rubra)*, greenlip *(H. laevigata)* and a hybrid abalone *(H. rubra x H. laevigata)* (Figure 8) are currently farmed in Victoria on farms spread across the state's coastline. A hybrid

abalone, Jade tiger abalone, has been produced that apparently combines the better features of greenlip and blacklip abalone. There are 18 license holders in the state; however not all were producing commercially in 2004/05. Figure 7 shows the location of both the land-based and offshore sites currently licensed to culture abalone in Victoria. Most production currently comes from land-based, flow-through culture systems. A small number of farmers are trialling offshore culture in cages suspended off long-lines, but production from these systems is not yet commercially significant (Department of Primary Industries, 2007). It is anticipated that production will continue to increase in the near future as there has been considerable investment in recent years (Aquaculture Advisory Group, 2005).

Greenlip abalone

Jade Tiger abalone

Blacklip abalone

Figure 8. The three species of abalone farmed in Victoria, Australia

The greenlip abalone culture has been developing in South Australia since the early 1990's and is now recognised as a significant contributor to the state's aquaculture industry. The stimulus for continued growth of the industry is the highly lucrative Asian shellfish market. Commercial abalone farms currently operate near Port Lincoln and Streaky Bay on the Eyre Peninsula, and on Kangaroo Island. Several pilot programs are also currently being undertaken to utilise other waters of the state to culture abalone, with significant interest in marine grow out sites. In addition, one mobile abalone farming system is currently licensed to operate in South Australian waters. South Australia is considered an ideal location for continued development in

farming abalone, as it offers suitable protected areas with accessible pristine water and well-established abalone industry association. South Australia is one of the principal states within Australia that have investment in abalone culture. There are 17 land-based farms in South Australia, with production in 1999 estimated at 72 tonnes (Gordon, 2000). The abalone cultured in South Australia are greenlip (*H. laevigata*), blacklip (*H. rubra*), and a hybrid of these two species. Also, some farmers have trialled Roe's abalone (Freeman, 2001). In South Australia, abalone aquaculture efforts are concentrated on greenlip abalone, to cater for the Asian shellfish market, with the Chinese market favouring greenlip abalone whilst the Japanese market prefers blacklip (PIRSA, 2010). Tasmania also has investment in abalone aquaculture. There are three land based farms in Tasmania, with production in 1999, estimated at 10 tonnes (Gordon, 2000). The abalone cultured in Tasmania are greenlip, blacklip, and a hybrid of these two species. Eleven abalone species occur along the Western Australian coast with three species; greenlip abalone, brownlip abalone (which is closely related to blacklip abalone), and staircase abalone, considered to have the highest potential as candidates for culture. A hybrid abalone, Tiger abalone, has also been produced. In addition there been attempts to farm the Roe's abalone in Western Australia. Staircase and Roe's abalone are probably better suited to warmer culture sites between Augusta and Geraldton than greenlip abalone, although good growth and survival rates have been achieved in indoor grow out systems with this species in Perth (Freeman, 2001).

2.6 Abalone nutrition

Abalone are herbivorous invertebrates and naturally feed on macroalgae (Sales and Britz, 2001). Their preference varies worldwide, depending on their habitat and

availability of macroalgal species (Nelson et al., 2002). In the wild, abalone juveniles generally inhabit the intertidal zone, preferring water less than 2 m in deep, where turbulent water and high levels of dissolved oxygen can be found (McShane et al., 1994). When abalone reach sexual maturity, they begin to migrate into deeper waters. Small abalone (less than 5 mm) graze microscopic algae, especially diatoms and ingest a range of bacteria (Garland et al., 1985). Larger juveniles and adults are more sedentary, feeding predominantly on drifting macroalgae by raising the front of their foot to catch drifting algae pieces (Tong, 1992). The natural feeding pattern of abalone is one of consuming different foods at various stages of their life cycle (De Waal et al., 2003). That is attributed not only to the increased mouth size, but also to morphological changes of the radula as the abalone grows (Onitsuka et al., 2004). Changes in radular structure and organization in abalone have been shown to prepare the animals for the feeding transition from micro- to macroalgae (Kawamura et al., 2001). As abalone grow, changes in diet could also take place due to transformations in the gut's micro-flora, such as bacteria and transformations of enzymes within the digestive system, which enable them to digest macroalgae (Tanaka et al., 2003).

From the perspective of an aquaculture operator, macroalgae are more difficult to obtain, to process for feeding, and to clean and store. In comparison with artificial pellets, macroalgae are more difficult to use and sustain the current commercial production of abalone (Johnston et al., 2005). Artificial diets are also easier to produce than growing macroalgae (Viana et al., 1993; Emmanuel and Corre, 1996). The basic nutrients in a formulated diet are proteins, lipids, carbohydrates, vitamins and minerals.

2.6.1 Protein

Fish meal, defatted soybean meal and casein are all commonly used sources of protein for abalone diets. Fish meal is the only protein source that can solely support good growth performance. Others, such as soybean meal and casein need to be fed together with other protein sources in order to support good growth (Fleming et al., 1996). Soybean is potentially a good protein source because its amino acid profile is close to fish, and its protein is highly digestible (Fleming et al., 1996). A combination of abalone viscera silage and soybean meal as protein sources in abalone *H. fulgens* diet was found effective in supporting good growth (Guzman and Viana, 1998). Uki et al. (1985), in their search for various protein sources for abalone, identified casein as a suitable protein. Later, Bautista-Teruel et al., (2003) and Viana et al. (1993) contended fish meal and casein used as protein sources for abalone had achieved similar growth rates. The reason for the better growth was mainly attributed to protein quality due to differences in digestibility of each protein source by juvenile abalone. However, because of its relatively high cost, casein is not suitable for practical use in abalone feeds. *Spirulina*, likewise, was shown to have a very good potential as a protein source for abalone (*H. midae*) diets. *H. midae* fed fishmeal and *Spirulina*-based diets exhibited good growth and high efficiency of feed conversion to body weight (Britz et al., 1996b). Furthermore, plant protein sources such as field peas, faba beans, yellow lupins, defatted soy flour and vetch were tested and used by Vandepeer et al. (1999) for *H. laevigata*. Their results showed that all these legumes were well digested by various abalone species (Kemp et al., 1999).

Dietary protein levels also vary between different abalone species. Mai et al. (1995a)

estimated that the optimum crude protein level for growth of *H. discus hannai* was between 25% to 37%; Taylor (1997) and Coote et al. (2000) reported that dietary crude protein requirements of *H. kamtschatkana* and *H. laevigata* were 30% and 27%, respectively; *H. midae* exhibited optimum growth rates when crude protein levels were 36% to 47% in the diets (Britz, 1996a). The optimum protein level in a casein-based diet for *H. discus* was determined to be at 20–30% level (Uki et al., 1986). Britz1996) found that the growth rate of *H. midae* increased with an increasing protein content from 27% to 47%. Bautista-Teruel and Millamena (1999) found the optimum protein level for juvenile tropical abalone, *H. asinina Linne* to be 27%. Further, Bautista-Teruel et al. (2003) have shown that an abalone dietary formulation would need a combination of plant and animal protein sources in order to be more effective when it comes to attainment of higher growth rate. Thongrod et al. (2003) reported that a combination of soybean meal and *Spirulina* sp at 4.4:1 can provide best growth for juvenile abalone compared with a fish meal-based diet. Gomez-Montes et al. (2003) showed that abalone diet with protein/energy ratios of 99.7 and 108.5 mg protein/kcal resulted in significantly higher growth rate for *H. fulgens*. Montano-Vargas et al. (2005) have recommended the inclusion levels of protein at 35% to meet requirements for energy and maximum growth of abalone, *H. corrugate*. Garcia-Esquivel et al. (2007) showed in their experiment that formulated diets with protein content of 38% and 25% gave significantly higher growth rate compared with kelp which contained only 9.5% of the same nutrient.

These optimum dietary protein levels for different abalone species are between 20-44%, which are lower than those for most carnivorous and omnivorous fish, but similar to those of several herbivorous fresh-water fish, such as common carp and

Nile tilapia (NRC, 1993). These differences may be due to abalone size, feeding trial periods, the ratios of digestible protein to energy, essential amino acid patterns in the diets, culture surroundings, or statistical analysis methods used in the studies. Differences in leaching rates of nutrients from the diets into water could also have resulted in the differences in the protein requirements, as these studies had revealed (Guzman and Viana, 1998).

2.6.2 Carbohydrate

Carbohydrates are the main components of the macroalgae that fed abalone naturally. They exist in many forms, such as starch, polysaccharides, alginate, agar, and carrageena in the marco-algae. Abalone have enzymes capable of hydrolyzing complex carbohydrates. After digestion by digestive enzymes, the carbohydrates become monosaccharides and are then catabolized for energy. Assays of the hepatopancreatic enzymes of *H. midae* showed that abalone produce their own cellulase, alginate lyase, laminarinase, agarase and carrageenase. A number of studies dealing with the digestive enzymes of abalone have concluded that these animals could synthesize their own cellulase (Ostgaard et al., 1994). These research supported the notion that carbohydrate is a main energy source for abalone in their natural diets. A result from Tayler (1997) also indicated that carbohydrate in the formulated diets can be used for energy, thus amino acids would be used for growth rather than waste on energy metabolism. For abalone, carbohydrates feature at the top on the list of energy sources rather than lipids or proteins. The carbohydrate requirements for most *Haliotis* species range from 43 to 48% (Sales and Janssens, 2004) in formulated diets. Cheap sources of carbohydrates include wheat, corn flour, soybean meal, maize and rice starch. It is believed that too

much carbohydrate in the diet may lead to poor utilization of protein (Fleming et al., 1996).

2.6.3 Lipids

Dietary lipid is one of the major nutrients that play a major role in abalone nutrition. It provides essential fatty acids, concentrated energy and fat soluble nutrients for normal growth of abalone (Wei et al., 2004). Further, it monitors the physiological and nutritional status of mollusc larvae for potential metamorphosis (Gallager et al. 1986). It provides other compounds such as sterols and polar lipid which are structural components of the cell membrane (Bautista-Teruel et al., 2011).

Abalone are highly efficient in utilizing lipid (Castanos, 1997). Moreover; Wee et al. (1994) reported a high lipid digestibility (84.7%) in abalone. Fleming and colleges (1996), in their review also pointed out that abalone have very high lipid absorption efficiency. Several researchers have studied the lipid requirements of different abalone species. Uki et al. (1985) have shown that the optimum lipid requirement of *H. discus hannai* is 5% in an artificial diet. Mai et al. (1995) have reported that significantly higher weight gains were observed when *H. discus hannai* were fed with lipid levels ranging from 3.11 to 7.09% while *H. tuberculata* grew best when fed diets containing 3.11% lipid. Growth in terms of protein gain was best when both *H. discus hannai* and *H. tuberculata* were fed 3.11 and 5.15% dietary lipid (Mai et al., 1995). Another study showed that abalone (*H. midae)* from South Africa grew well at up to 6% dietary lipid (Britz and Hecht, 1997). Thongrod et al. (2003) have shown in their experiments that abalone, *H. asinina* diet containing lipid to carbohydrate ratio of 1.3%:47.8% provided significantly the highest growth rates among the other ratios tested. Lee

(2004), on the other hand has noted that *H. discus hannai* require 2-5% lipid in a formulated diet. Bautista-Teruel et al. (2011) have reported that significantly higher growth rate was observed when *H. asinina Linne* were fed diets with lipid levels ranging from 2.2 to 6.1%. In Australia, the maximum growth rate of juvenile greenlip abalone was obtained when the animals were fed formulated feed that contained lipids at 2.5% in summer and 3.5% in winter (Dunstan et al., 1997). In another study, Dunstan et al. (1999) found that growth rate of greenlip abalone increased when abalone fed a diet containing 3.8% total lipid. Furthermore, other formulated diets with total lipid contents of 2.6 and 4.2% also improved growth rates (Dunstan et al., 1996). Van Barneveld et al. (1998) suggested that the addition of marine or vegetable oils in abalone diets should be limited to 3% due to the negative influence on the digestibility of amino acids in juvenile *H. laveigata*. Dunstan et al. (1996) found that the digestibilities of marine origin fatty acid rich lipid classes, i.e. triacyglycerols from fish oil were higher in greenlip abalone than that of lipid classes from vegetable oils. In general, studies showed that formulated abalone diets from around the world contained a wide range of total lipid contents (1-11% wet wt) (Uki et al., 1985; Mai et al., 1995; Dunstan et al., 1996, 1999; Thongrod et al., 2003). In most cases the total lipid comprised less than 5% of the diet (Dunstan et al., 1999). In general these studies indicated that lipid requirement of abalone varies between the species.

2.6.3.1 Essential fatty acids

The essential fatty acid (EFA) nutrition of mollusks is less studied compared to those of fish and crustaceans. Some studies have shown that LC n-3 PUFA plays a more important role in EFA nutrition of bivalves than C18 PUFA such as ALA and LA (Caers et al., 2000; Navarro and Villanueva, 2000; Nelson et al., 2002). EPA and

DHA are generally considered EFA for mariculture species (Nichols et al., 1994). However, for gastropods like abalone, studies on EFA requirements have not been given much attention (Hanns and Sinclair, 1996). Uki et al. (1986) reported that *H. discus hannai* require n-3 and n-6 LC PUFA as EFA for growth. Additionally it has been suggested that only LA and ALA are EFA in abalone (Hanns and Sinclair, 1996). Floreto et al (1996) indicated that ARA, and not EPA is an EFA. Mai et al. (1996) showed that LA, ALA, ARA and EPA are important fatty acids for the growth of abalone, *H. discus hannai*. However, *H. tuberculata* appeared to be dependent largely on LC n-3 PUFA (Mai et al., 1995). Nelson et al. (2002) suggested that LA and ALA may not be EFA in green abalone, *H. fulgens* compared to ARA and EPA. Similar study by Durazo-Beltran and Colleges (2003) has shown that LC-PUFA such as ARA, EPA and DPA are essentials in the diet of green abalone, *H. fulgens.* Recent study by (Bautista-Teruel et al., 2011) also showed that LA, ALA, and LC n-3 PUFA are essential fatty acids for growth of juvenile abalone, *H. asinina Linne*. Addition of these EFA also resulted in an increase in both n-3 and n-6 fatty acids. Abalone research in Australia showed that the growth rate of juvenile greenlip abalone was lowest when abalone were fed diets containing mostly short chain PUFA (Dunstan et al. (1996). Feeding trials on greenlip abalone also showed a poor growth rate with a diet containing only short chain PUFA, while diets with both EPA and DHA have been found to produce the highest growth rates (Dunstan et al., 1997).

Studies also suggest that many marine fish and prawns require LC n-3 PUFA, particularly EPA and DHA to maintain normal physiology and growth (Xu et al., 1993, 1994; Glencross, 1999, 2001). For some marine species it was believed that DHA appears to be superior to EPA (Mourente and Tocher, 1993: Xu et al., 1994;

Rodriguez et al., 1997). Several studies have also shown the benefits of dietary LC-PUFA in freshwater species despite their known ability to synthesize these fatty acids endogenously (Castle et al., 1994; Ling et al., 2006). The ALA fatty acid series (n-3) have generally been observed to have a greater essential fatty acid value in marine animals than the LA series (n-6) (Glencross, 2001). Conversely, fresh water species have been observed to have a greater requirement for the LA series (n-6) (Shikata and Shimeno, 1994; Glencross, 2000). Studies focusing on the requirements of Chinese prawn, determined that AA had greater essential fatty acid value than LA and ALA, but less than EPA and DHA (Xu et al., 1993, 1994). Furthermore, the requirements for dietary essential fatty acids differ between species (Sargent et al., 1999; Watanabe and Vassallo-Agius, 2003).

2.6.4 Minerals and vitamins

Information on mineral requirements for aquatic animals is limited. Although many studies have been conducted on osmoregulation, heavy metal toxicity and related physiological functions, few of these findings are relevant to nutrition. Unlike other nutrient elements, a significant amount of minerals can be absorbed from the surrounding water, thus making it harder to get clear picture of the dietary intake of the minerals being studied. Especially in experimental tracing of minerals such as iron, zinc, manganese, copper and cobalt, it is very difficult to measure their requirements. On the other hand, this provides a significant benefit for farms except those operating with a closed recirculation water system, because mineral insufficiency will not take place unless an extremely unbalanced mineral diet is provided. Coote et al. (1996) showed that abalone did not require high levels of Ca in their diet, but increasing phosphate supplementation could improve their growth rates.

Another interesting element is zinc, which was found to improve both growth rates and the body's alkaline phosphatase activity (Tan and Mai, 2001).

Few studies have been conducted on vitamin requirements for abalone. The effect of dietary vitamin C on survival, growth and tissue concentration of ascorbic acid was investigated by Mai (1998), who claimed that dietary ascorbic acid (AsA) had clear effect on the carcass AsA concentration, but had no significant effect on growth and survival. Furthermore, Tan and Mai (2001) suggested that vitamin K might contribute to mineralization of shells in abalone. Boarder and Maguire (1998) found that increasing the dietary vitamin mix levels from 0.3% to 0.6% and 1.2% improved the growth rate of the Greenlip abalone but inclusion of a mineral mix in the diet depressed growth unless there were elevated levels of vitamin mix.

2.7 Artificial feed

Abalone artificial diets are very similar in their proximate composition (Fleming et al. 1996). The Japanese company, Nihon Nosan Kogyo K.K. (NNKKK) is the producer of the most common reference diet consisting of 20 % defatted soybean meal, 15 % fishmeal, 1.5 % total lipid (20 % n-3 and 3 % n-6 PUFA), 46 % total carbohydrate, 3 % crude fiber, 1.5 % vitamin mix, 4 % mineral mix, 2.5 % calcium, 1 % phosphorus and 12 % moisture (Fleming et al. 1996). The amino acid balance of the diet when compared with the amino acid balance of the soft tissue of abalone reveals a similar profile. With the exception of threonine, arginine and histadine, all essential amino acids are in slight excess relative to lysine. The artificial feed was formulated by identifying protein sources, which when integrated, match the amino acid profile of the abalone flesh (Fleming et al. 1996).

According to Fleming et al. (1996), carbohydrates were the largest portion with an average inclusion of 47 % and ranging from 30 - 60 %. Next was crude protein ranging from 20 - 50 % with an average of 30% followed by lipid concentration of 1.5 - 5.3 % averaging around 4 %. There was less than 3 % crude fiber in all of the diets tested because of the limited capacity of abalone's intestinal micro flora to digest fiber (Fleming et al. 1996). After the diet composition has been identified, certain attractants such as Taremal A40 can be added to feed to increase intake and thus growth rate for abalone (Fleming et al. 1996). According to Freeman (2001) in general a typical abalone diet contains protein, carbohydrate, lipid, fiber, minerals, vitamins, binder, feed stimulants and attractants (Freeman, 2001).

2.8 Factors that influence lipid content, fatty acid composition and growth of abalone

Fish and shellfish are the rich sources of LC n-3 PUFA for human diet. It is evident that several biological and environmental factors such as diet, reproduction cycle and taxonomy of the animals, seasonal changes in water temperature and the latitude at which they were harvested, have significant influence on the lipid content, fatty acid composition and growth of marine animals (Linehan et al., 1999; Nelson et al., 2002; Litaay and De Silva, 2003; Su et al., 2006; Li et al., 2007). In abalone, diets and water temperature are among the main factors that have been found to influence the lipid content, fatty acid composition and growth.

2.8.1 Effect of diet

Many species of algae that are commonly consumed by abalone in the wild have very low lipid levels (Fleming et al. 1996), however EFA are very important in abalone nutrition (Mai et al., 1996). The main C20 PUFA in red and brown

macroalgae are ARA and EPA with brown algae also containing significant levels of ALA (Dunstan et al. 1996). However, green macroalgae contained very low levels of C20 PUFA, whereas C16 and C18 (n-3) PUFA were abundant. In all groups of macroalgae, C22 PUFA are present at low levels. Uki et al. (1986) concluded that abalone are able to convert the C18 PUFA they receive in their diet to the C20 and C22 PUFA they require.

A study by Su and colleagues (2004) analysed the fatty acid contents of wild abalone fed a natural microalgae diet and cultured Australian adult blacklip abalone fed an artificial diet. It was found that wild abalone contained significantly higher levels (mg/100g) of total n-3 PUFA, EPA, DPA and ALA than cultured abalone. Furthermore, cultured abalone showed a significantly higher percentage composition of DHA than wild abalone. Significantly higher levels of ARA and total n-6 PUFAwere found in wild abalone than in cultured animals. Su et al (2004) suggested that manipulation of nutrient sources of cultured abalone may influence their lipid composition.

Dunstan et al. (1996, 1999) found that the muscle of wild adult greenlip abalone fed a natural diet of macroalgae contained higher EPA, DPA and total n-3 PUFA proportion (% of total FA) compared to juvenile cultured hybrid abalone fed artificial diets. Similarly wild adult blacklip abalone fed a natural diet of macroalgae contained a higher proportion of DPA compared to hybrid abalone fed artificial diets. In addition Dunstan et al. (1996) reported that muscle of juvenile cultured greenlip and hybrid abalone fed an artificial diet showed lower levels of EPA, DPA and total n-3 PUFA than those fed green algae. The observed differences in fatty acid

composition were attributed to the accumulation of lipid in the foot muscle of abalone fed an artificial diet (Dunstan et al., 1996). In a growth assessment of greenlip, Dunstan et al. (1996) found that the artificial diet that contained only C18 PUFA and none of the LC-PUFA resulted in the poorest growth. The addition of ARA (high in seaweed) was less effective than the addition of DHA (high in fish) whereas diets with both EPA (high in diatoms) and some DHA produced the highest growth rates.

Grubert et al. (2004) studied lipid and fatty acid composition (% of total FA) of wild-caught blacklip and greenlip abalone fed a formulated feed containing 5% lipid, of which the major fatty acids were LA, OA and 16:0 and found that, the lipid content of foot, testis, ovary and digestive gland (DG) were similar for both species. Each tissue had a different fatty acid signature, with the foot, testis and ovary characterized by elevated levels of ARA, EPA and LA, respectively. The proportions of LA and EPA in the DG were intermediate between those of the testis and ovary.

Floreto et al. (1996) investigated the effects of seaweed diets on the lipid and fatty acid composition of juvenile *H.discus hannai* by feeding seaweeds with different FA profiles: green (*U. pertusa*, rich in 16:4n-3, ALA, and 18:4n-3 PUFA), red (*G. sparsa*, high in ARA and EPA PUFA, and brown (*U. pinnatifida*, rich in ALA, ARA, and EPA PUFA). The results identified best growth in juveniles fed *U. pinnatifida*. The data showed that none of the seaweed diets affected the major lipid classes of abalone tissues.

Mai et al. (1996) evaluated the nutritional role of PUFA in dietary macroalgae for two abalone species, *H. tuberculata* and *H. discus hannai*. These authors found that

PUFA, such as ALA, ARA and EPA, were dominant in the brown algae, *A. esculenta L. digitata and L. saccharina*. The red alga, *P. palmata* was characterised by the highest proportion of EPA among the selected algae. In the green alga, *U. lactuca*, however, the dominant PUFA were C16 and C18 fatty acids, while C20 fatty acids were minimal. All the selected algae consistently contained very low levels of DHA. Mai et al. (1996) also found that EPA played a prominent role in the nutrition of both abalone species. ALA as well as other n-3 PUFA, and LA together with n-3 and other LC n-6 PUFA also contributed to the faster growth of *H. tuberculata* and *H. discus hannai*, respectively. The study suggests that both the n-3 and n-6 PUFA seem to be essential for growth of *H. discus hannai.* But for *H. tuberculata*, however, growth enhancement appeared to depend largely on n-3 PUFA.

Durazo-Beltra and colleagues (2003) conducted a trial to compare the growth and fatty acid composition of juvenile green abalone *(H. fulgens)* fed formulated diets containing four different sources of triacylglycerols (olive, corn, linseed and cod liver oils) at three levels (1.5, 3.0 and 5.0%, total added dietary lipid was 5.0%) and a reference diet that contained no added lipids (0.25% total lipids). No significant differences in growth were found among abalone fed the different oil types. Furthermore, the authors reported that responses to different dietary levels of lipid were significantly different but not to sources of oils. Maximum growth was achieved at a 1.5% inclusion of oil. The relationship between fatty acid profiles of tissue and the diets fed to the abalone suggests that diet can influence essential fatty acids of abalone.

Nelson et al. (2002) examined lipid and fatty acids profiles of tissues in green abalone (*H. fulgens)* over a one-year interval by feeding five diet treatments: the

macrophytic algal phaeophyte *Egregia menziesii,* rhodophyte *Chondracanthus canaliculatus,* chlorophyte *Ulva lobata,* a composite of the three algae and a starvation control and found that, the major fatty acids were 16:0, 18:0, 18:1n-7, OA, ARA, EPA and DPA as well as 14:0 for abalone fed brown and red algae. Highest growth rates were observed in abalone fed the phaeophyte *E. menziesii,* an alga containing the highest levels of C20 PUFA. This study provides evidence of the influence of diet and temperature on seasonal changes in abalone lipid profiles, where diet is most strongly related to body mass and temperature to shell length. The allocation of lipids to specific tissues in green abalone clarifies their lipid metabolism. These results provide a basis for improving nutrition of abalone in mariculture through formulation of artificial feeds.

Investigations were carried out to evaluate the effects of six diets containing graded dietary lipid (0.63 to 11.58%) and a red alga, *P. palmata* control diet on the survival, growth and body composition of *H. tuberculata* and *H. discus hannai* and it was found that, *P. palmata* produced similar or better growth performance for both abalone species than the artificial diets. Among the artificial diets, significantly higher weight gains were observed for *H. discus hannai* at dietary lipid levels ranging from 3.11 to 7.09%, and for *H. tuberculata* at 3.11%. Soft-body lipid content positively correlated with dietary lipid levels. There were no significant differences in survival of abalone fed the experimental diet (Mai et al., 1995).

The effect of feeding different levels of LC n-3 PUFA on the fatty acid profile was evaluated in soft tissue from juvenile *H. tuberculata coccinea*. Five diets were formulated with different oil sources (palm, colza, fish, sunflower, and soybean),

containing from 2.52 to 12.33% LC n-3 PUFA, while fresh *Ulva rigida* was used as reference diet. No significant differences in growth were recorded among all formulated diets but a tendency to accumulate different fatty acids in muscle was observed. It was also found that the diet containing 6.8% LC-PUFA resulted in higher lipid accumulation in muscle tissue of this abalone than that observed in the other treatments (Toledo-AgÃero, and Viana, 2009).

Thongrod et al. (2003) studied the effect of five diets, a control diet containing 1.3% lipid with no FO added and four other diets 5.8, 10.2, 14.8 and 19.0% of lipids (FO as the only lipid source) on fatty acids profiles and growth of *H. asinina*, Linne. The fatty acid profile of abalone reflected the influence of fatty acid profiles of the diets. The data showed that abalone fed the diet containing 1.3% lipid (no FO added) resulted in significantly lower accumulation of LC n-3 PUFA compared to the other diets containing FO supplementation. However, Thongrod et al. (2003) found that the abalone fed a diet containing 1.3% lipid obtained the final average body weight that was significantly higher than those fed the other diets, indicating that high levels of dietary lipid may negatively affect abalone growth.

Capinpin and Corre (1996) evaluated the growth rate of *H. asinina* fed three diets: an artificial diet and red alga *G. heteroclada* and *K. alvarezii* for 120 days. These researchers found that abalone fed the red alga *G. heteroclada* and an artificial diet grew faster in terms of both total body weight and shell length than those fed the red alga *K. alvarezii*. However, *G. heteroclada* promoted high growth rates over a long-term period (360 days) and is considered to be best suited for abalone farming in the Philippines. Similarly Chen and Lee (1999) found that the growth rate of abalone fed

an artificial diet was higher than abalone fed *Gracilaria tenuistipitata*. Furthermore, hatchery-reared paua *(H. iris)* were investigated to determine the effect of two artificial and one natural diet on growth and it was found that paua fed either of the artificial feeds consistently grew better than did those fed dried Gracilaria (Clarke and Creese, 1998). A further study was conducted to determine the growth rate of juvenile *H. discus hannai* and *H. rufescens* fed with fresh algae diets, *Lessonia trabeculata, Macrocystis integrifolia,* and *Ulva rigida* and one artificial diet. The best growth rate of abalone was obtained with the artificial diet compared to the fresh algae (Corazani and Illanes, 1998).

The growth performance of juvenile abalone, *H. midae*, fed diatoms and a pelleted, practical diet was evaluated by Knauer et al. (1996). The growth of abalone did not differ significantly in terms of the increase in shell length and weight daily growth rate. Another study was conducted to evaluate the effects of dietary lipid sources on the growth and fatty acid composition of juvenile abalone *H. discus hannai* Ino fed one of four diets containing 3.5% of dietary lipid from either tripalmitin (TP), soybean oil (SO), linseed oil (LO), or EPA-enriched FO and it was found that the growth rate of abalone was significantly affected by dietary lipid source. FO produced the highest weight gain rate, closely followed by LO and SO. These three WGR values were not significantly different to each other. However, abalone fed the TP diet showed a significantly lower WGR. The fatty acid profile in abalone reflected that of dietary lipids, especially the unsaturated fatty acids (Wee et al., 2004). Moreover a study was conducted to examine the effects of the ratio of dietary LA to EPA on the growth and fatty acid composition of juvenile *H. discus hannai* fed five diets with 35 g kg^{-1} total lipid containing graded LA/EPA ratios (1: 0,

0.75: 0.25, 0.5: 0.5, 0.25: 0.75 and 0:1, respectively). The results showed that abalone survival rates were generally high and independent of the dietary treatments. However, abalone growth was significantly affected by LA/EPA ratio. The LA/EPA ratio of 0.5: 0.5 produced the highest weight gain rate, closely followed by the ratio of 0:1. The abalone fed the diet without EPA (1:0) had the lowest WGR. Fatty acid profiles in the abalone body reflected those of dietary lipids, especially for the PUFA (Xu et al., 2011).

A recent study by Bautista-Teruel et al. (2011) evaluated the growth, survival, and fatty acid composition of juvenile *H. asinine* tissues fed six diets containing lipid levels at 0, 2, 4, 6, 8, and 10%. The results identified significantly higher growth rates with abalone fed diets with lipid levels of 2.2, 3.6, and 6.1% compared with those containing lipid levels of 7.6% and 9.8%. Abalone fed the lipid-free diet showed a significantly lower growth rate among treatments. The optimum lipid requirement for growth is 3.59%. It was also found that addition of LA, ALA and LC n-3 PUFA showed significant improvement in weight gains up to 1.6% supplementation. Fatty acid composition of the lipid samples reflected those of the diets. Addition of essential fatty acids resulted in an increase in both n-3 and n-6 fatty acids. Lipid incorporation at 3.6% using a 1:1 ratio of cod-liver oil and soybean oil with essential fatty acids supplementation (1.6%) is the best in juvenile abalone diet formulation.

2.8.2 Effect of temperature

It has been reported that the PUFA content in fish and shellfish varies inversely with water temperature (Nelson et al., 2002; Su et al., 2006; Li et al., 2007), while the SFA content changes positively with water temperature (Dunstan et al. 1999; Pazos et al. 2003). The increased PUFA at colder temperatures are mainly due to higher n-3 PUFA concentrations (Dunstan et al. 1999). Bell et al. (1986) indicated that an increase in PUFA and a decrease in SFA levels when temperature falls could significantly contribute to the maintenance of cell membrane fluidity as PUFA have a lower melting point than SFA. To a lesser extent, increased unsaturation of nonmembrane lipids in the flesh (e.g., subcutaneous storage lipids) would also be expected to help maintain body flexibility for movement at reduced temperatures (Dunstan et al. 1999).

Although there are limited studies on molluscs, few studies revealed that water temperature can influence PUFA content of abalone. Su et al. (2006) studied the total lipid and n-3 PUFA contents of two cultured abalone species, blacklip and greenlip over four seasons. The water temperatures recorded during this study ranged from 12^{o}C in winter to 22^{o}C in summer, with 16^{o}C and 17^{o}C in autumn and spring, respectively. The total lipid content in both species varied significantly through the seasons with summer samples having the highest levels compared to the other seasons. The highest total n-3 PUFA content (mg/100g) was recorded in winter when the water temperature was low. In contrast the highest total SFA content is observed in summer when the water temperature was high. This study suggested the influence of water temperature on seasonal changes in abalone lipid and fatty acid

profiles. Consistently, Nelson et al (2002) also found that SFA content of muscle tissue of green abalone changed positively with water temperature and PUFA content varied inversely with water temperature. There is a positive correlation between the temperature and the degree of SFA of cell membrane (Phleger, 1991). Temperature may directly affect the fatty acid composition of abalone. Additionally, temperature may be an indirect influence, by affecting fatty acids in their macroalgal diet (Nelson et al., 2002).

Fluctuations in water temperature have been also found to influence seasonal changes in lipid and fatty acid profiles of other molluscs. Ozyuret et al. (2006) studied seasonal changes of fatty acids in cuttlefish, *Sepia officinalis*. Water temperature had a remarkable influence on the PUFA of *Sepia officinalis.* DHA and EPA levels of cuttlefish of mantle tissue were significantly lower in summer when water temperature was high. Pazos et al. (2003) conducted an experiment to investigate seasonal changes in lipid classes and fatty acid composition in the digestive gland of scallop, *Pecten maximus* and found positive correlations between the levels of SFA and temperature, whereas the levels of PUFA negatively correlated with temperature. A similar study on the giant lion's paw scallop (*Nodipecten subnodosus*) also showed a positive correlation between high temperature and levels of SFA and also a positive correlation between low temperature and levels of PUFA. Li et al. (2007) investigated the seasonal variations of lipid content and composition of mussel (*Perna viridis*). The proportion of PUFA was higher for phospholipids and phosphatidylcholine in winter, for triacylglycerol in summer and for phosphatidylethanolamine in autumn, whereas SFA were higher in summer than in the other three seasons in triacylglycerol, phospholipids and phosphatidylcholine.

These authors concluded that the fatty acid composition in different lipid fractions may be caused by many factors including temperature. Many other researchers have also reported that the amount and types of fatty acids in most marine organisms were influenced by seasonal changes (Aro et al., 2000; Orban et al., 2002a; Luzia et al., 2003).

A study by Britz et al. (1997) investigated the effect of temperature on growth rate and nutritional indices of abalone fed a dry formulated diet. These authors found that, between 12 and 20°C, growth rate and feed consumption increased, but FCR did not differ significantly. However, between 20°C and 24°C, growth and feed consumption declined sharply, and FCR deteriorated. The condition factor of abalone decreased with increasing temperature. Britz et al. (1997) concluded that temperatures between 12 and 20°C are physiologically optimal for abalone, *Haliotis midae.*

Temperature preference was determined in a horizontal thermal gradient and was found to be 25.4°C for green abalone and 25.0°C for pink abalone. The optimum temperature for growth calculated for both abalone species was 24.6°C and 24.5°C respectively (Díaz et al., 2006). Red abalone, *H. rufescens*, acclimatizated at 17 ± 1°C had a preferred temperature of 18.8 ± 2.1°C. The optimum growth temperature was calculated as 18.4°C for *H. rufescens* (Díaz et al., 2000).

Juvenile green abalone, *H. fulgens* were reared in laboratory for six months in order to determine their survival, growth rate, tissue composition, feed consumption (C) and feed conversion ratio (FCR) under two temperatures (20°C and 25°C) and three photoperiods (00:24, 12:12 and 24:00 light: dark hours). Survival was 100% at 20

°C, and between 68% and 75% at 25°C. The highest gross growth rate was observed in abalone from the combination 20°C-00:24 L: D. Slowest GGR was observed in the combination 25°C-24:00 L:D. Feed consumption markedly increased at night, decreased with age, and was higher at 25°C than 20°C, irrespective of photoperiod. Highest C was observed under continuous darkness and was lowest under continuous light. FCR was affected by temperature but not by photoperiod. Lower FCR was observed at 20°C, when compared to 25°C. It was concluded that *H. fulgens* can be best cultured at 20°C and 00:24 or 12:12 L: D regimes, while sustained temperatures at or above 25°C may result in cumulative stress (García-Esquivel et al., 2007). Nelson et al (2002) also found a strong relationship between mean growth rate and water temperature on green abalone.

The preferred temperature and critical thermal maximum of Australian blacklip abalone, and greenlip abalone, were found to differ only slightly; the blacklip abalone exhibited lower temperature tolerance and preference, as expected from its habitat distribution. Preferred temperatures were 16.9°C and 18.9°C, and 50% critical thermal maxima were 26.9°C and 27.5°C for blacklip and greenlip abalone, respectively. The optimum temperatures for growth calculated from each of these indices and averaged were 17.0°C and 18.3°C, respectively (Gilroy and Edwards, 1998).

Growth and survival of juvenile greenlip and blacklip abalone were investigated at high dissolved oxygen levels (95-120% saturation) between 17°C and 19°C. Abalone were fed the same artificial diet. Blacklip abalone held at 16.9°C and 97% oxygen saturation grew in shell length significantly faster than all other treatments of blacklip abalone held at 19°C, and significantly faster than blacklip abalone

maintained at 111% oxygen saturation and 17.5°C. Both temperature and oxygen saturation significantly affected the survival of this species. Blacklip abalone held at 19°C had significantly lower survival for both 96% oxygen saturation and 120% oxygen saturation, compared with blacklip abalone maintained at either 110% oxygen saturation and 19°C, or for any 17°C treatment. No significant differences were noted for greenlip abalone within the range tested in terms of growth rate, food consumption rate or survival, indicating that greenlip abalone tolerated these conditions better than did blacklip abalone (Harris et al., 2005).

Growth and feeding of juvenile triploid and diploid blacklip abalone were investigated at temperatures of 17°C and 21°C respectively. There were no differences in growth between triploid and diploid abalone as measured by shell length and body weight. Both triploid and diploid abalone increased in length but not in weight at 21°C. Condition indices were similar for triploid abalone maintained at both temperatures; however, those for diploid abalone were significantly higher at 17°C than at 21°C. Food intake was significantly greater yet feed conversion efficiency was significantly lower in triploid than in diploid abalone. Both the feeding variables were independent of temperature (Liu et al., 2006).

Growth rates of *H. tuberculata* were evaluated at three different temperatures (15°C, 18°C, and 22°C) when provided two artificial diets (fish meal and casein meal). Juveniles fed the fish meal diet and cultured at 22°C produced higher growth rates on shell length and body weight. In addition, the soft tissue weight/shell weight ratio was found to be the highest in these juveniles. No differences in proportions of crude protein, crude lipids, and moisture content were found between diets. Survival of juveniles during the feeding trial was not affected by the dietary treatments. The feed

conversion ratio did differ significantly between low (15°C) and highest (18 and 22°C) temperatures (Lopez et al., 1998).

2.9 The influence of dietary fish oil and vegetable oil

2.9.1 Fish oil supplementation

Although little is known on the comparison of lipid content and fatty acid composition of wild and farmed abalone, previous studies showed that farmed abalone contained significantly lower levels of total n-3 PUFA, EPA, and DPA (% of total FA as well as mg/100g) than wild abalone (Dunstan et al., 1996; Su et al., 2004). Studies on fish also showed that wild fish are a better source of n-3 PUFA than their cultured counterparts (Serot et al., 2002; Kriton et al., 2002; Alasalvar et al., 2002). Previous studies demonstrated that the commercial diet supplemented with FO could increase the content of LC n-3 PUFA in farmed abalone significantly (Durazo-Beltran et al., 2003; Thongrod et al., 2003; Wei et al., 2004; Bautista-Teruel et al., 2011). The improvement of the health benefiting LC n-3 PUFA in farmed abalone are attributed to the high levels of LC n-3 PUFA in particular, EPA and DHA in FO.

2.9.2 Replacement of fish oil by vegetable oils

Lipid sources, rich in LC-PUFA, mainly come from marine origins, such as fish oils. However, fish oils are increasingly in short supply globally, and intensive studies are underway on FO substitution in the aquaculture industry (Barlow, 2000; Myers and Worm, 2003). Research on the substitution of dietary FO has indicated that it may be possible to replace FO by plant seed oils (Barlow and Pike, 2001).

A number of studies on fish have suggested that dietary vegetable oil inclusion does not result in reduced growth performance and feed conversion in comparison with FO supplementation (Torstensen et al., 2000; Bell et al., 2001, 2003). However, at levels of vegetable oil inclusion above 50%, there was a significant accumulation of LA and reduction of EPA and DHA in the flesh (Bell et al., 2003). Flaxseed oil (FlaxO) contains a high concentration of n-3 PUFA, mainly ALA. Canola oil (CO) is an alternative to FO because of the cost and availability, and also the significant concentration of ALA. The replacement of FO with FlaxO and CO does not appear to lead to significant adverse effects on fish growth (Martino et al., 2002; Raso and Anderson, 2003; Wonnacott et al., 2004) but there was an unavoidable decrease in the concentration of LC-PUFA in the fish flesh (Mourente et al., 2005). Consistently, Francis et al. (2006) reported that FlaxO and CO could be partially substituted for FO in diets for Murray cod with no apparent negative effect on growth. Several studies involving freshwater and salmonid species have demonstrated the possibility of high inclusion levels of plant oil without compromising growth rate of fish (Fonseca-Madrigal et al., 2005; Francis et al., 2006; Ling et al., 2006). However, utilization of plant based oils such as linseed oil as sole lipid source significantly lowered deposition of DHA, EPA and ARA in livers, muscles and ovaries of fish (Ling et al., 2006).

Although there are limited studies on abalone, studies on fatty acid profiles of abalones suggested that both LA and ALA fatty acids contribute to faster growth in *H. discus* (Uki et al., 1986; Mai et al., 1996a, 1996b). Furthermore no significant differences were found in the growth of *H. fulgens* fed vegetable oils (olive, corn and linseed oils) and cod liver oil (Durazo-Beltran et al., 2003). Abalone research in

Australia showed that the growth rate of juvenile greenlip abalone was lowest when abalone were fed diets containing mostly short chain PUFA found in vegetable oils (Dunstan et al. (1996). Feeding trials on greenlip abalone also showed a poor growth rate with a diet containing only the short chain PUFA and enrichment with vegetable oils was less effective than diets with both EPA and DHA which have been found to produce the highest growth rates (Dunstan et al., 1997).

2.10 Conversion and biosynthesis of PUFAand mRNA expression of desaturase and elongase

Delta-6 desaturase (Δ-6) is the first and rate-limiting enzyme for LC-PUFA synthesis that consists of a series of elongation and desaturation reactions (Figure 9) (Roqueta-Rivera et al., 2010). Δ-6 desaturase adds a double bond to the 18 carbon fatty acids (ALA and LA) by converting a single bond to a double bond. LA and ALA are substrates for this enzyme and compete for active sites on the enzyme. The products of Δ-6 desaturation, 18:3n-6 and 18:4n-3, are elongated to 20:3n-6 and 20:4n-3, respectively by the elongase enzyme which adds a 2-carbon moiety to the 18 carbon fatty acids to make the 20 carbon fatty acids (Sprecher, 2000). The next step in the pathway is performed by the delta-5 desaturase (Δ-5) enzyme which adds another double bond to the fatty acids, 20:4n-3 and 20:3n-6 and 20:4n-3 to form EPA and ARA, respectively. EPA and ARA are further elongated to DPA and 22:4n-6 respectively, finally to their respective elongation products, 24:5n-3 and 24:4n-6, which are then desaturated by Δ-6 enzyme to 24:6n-3 and 24:5n-6 respectively, all in the microsomes, and that this intermediate is then chain shortened to DHA and 22:5n-6 (DPAn-6) respectively at an extra microsomal site, presumably peroxisomes

(Tocher et al., 2003) (Figure 9).

Long chain polyunsaturated fatty acid biosynthesis in fish has been well studied, in contrast very little are known on abalone. Available information suggested that *H. discus hannai* (Wei et al., 2004) might have the ability to bio-convert LA to ARA and ALA to EPA. Similar findings were also reported by Dunstan et al. (1996) from *H. laveigata* and Durazo-Beltran et al. (2003) from *H. fulgens*. Recent study by (Bautista-Teruel et al., 2011) also showed that abalone, *H. asinine Linne* can convert short chain PUFA to LC n-3 PUFA. It appears likely that abalone is able to biosynthesize LC-PUFA to a certain extent, by elongation and desaturation of short-chain fatty acids (Su et al., 2004). However none of these studies was based on the data from gene expression. Therefore, further investigations are required to elucidate detailed pathways of fatty acid biosynthesis, metabolic gene expression and their regulation in abalone.

The biosynthesis of EPA and DHA from ALA is inefficient in marine fish, an evolutionary consequence of a natural diet rich in LC n-3 PUFA (Sargent et al., 2002; Tocher, 2003). Conversion is better in freshwater fish, possibly due to higher concentrations of ALA and limited DHA in their natural diet (Sargent et al., 2002). Freshwater fish species possess the capacity to synthesize EPA and DHA from ALA and ARA from LA respectively, through two separate pathways involving desaturation and elongation of their respective precursors (Zheng et al., 2004). The extent to which animals, including fish, can convert ALA and LA, to LC-PUFA differs according to species and depend on the activities of the desaturase and elongase enzymes. In most freshwater fish, biosynthesis of LC-PUFA involves sequential

desaturation and elongation of precursor essential PUFA, ALA and LA. Synthesis of ARA is achieved by Δ-6 desaturation of LA to produce 18:3n-6 that is elongated to 20:3n-6 followed by Δ5 desaturation (Cook, 1996). Synthesis of EPA from ALA uses the same enzymes and pathway as for ARA, but DHA synthesis requires two further elongation steps, a second Δ6 desaturation and a chain shortening step (Sprecher, 2000). The extent to which any species can produce LC-PUFA varies and is dependent on their complement of fatty acid desaturase and elongase enzymes (Tocher, 2003).

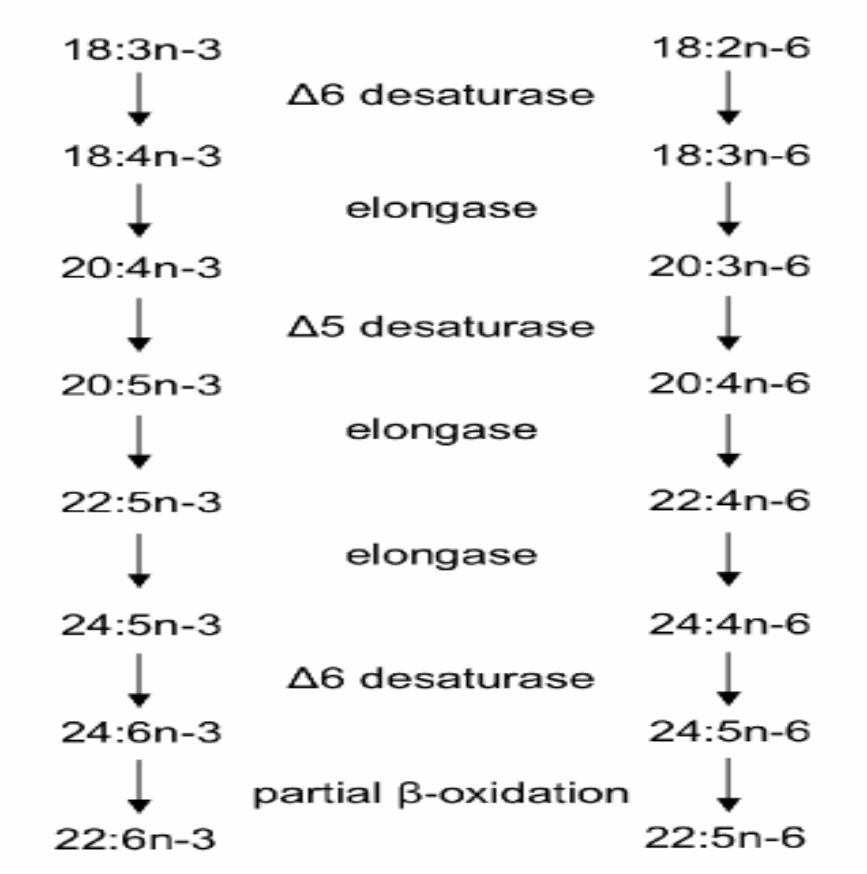


Figure 9. Biosynthesis pathways of long chain polyunsaturated fatty acids from C18 precursors, ALA and LA (Monroig et al., 2009).

Many studies investigated mRNA expression of desaturase and elongase in many species of fish, in contrast no study are available on abalone. Dietary studies

indicated that tissue desaturase and elongase mRNA expression can be influenced by dietary fatty acids found in vegetable or marine oils. Ling et al. (2006) studied the effect of different levels of dietary LC-PUFA on tissue fatty acid profiles and expression of desaturase and elongase genes in swordtail fish. Three diets utilizing different ratio of squid oil and LO (100% squid oil, 1:1 squid oil: LO and 100% LO) as lipid source were fed to swordtails. The study showed increased levels of hepatic expression for both swordtail desaturase and elongase mRNAs with increasing dietary levels of LA and ALA. Similarly, Zheng et al. (2004b) conducted a feeding trial in which Atlantic salmon were fed on diets consisted of a control diet containing FO and four diets in which the FO was replaced in a graded manner by LO. The results showed that after 20 weeks of feeding, desaturase and elongase gene expression in liver was increased in a graded manner by increasing dietary LO. Expression of both genes was positively and negatively correlated with dietary ALA and LC n-3 PUFA, respectively. Increased desaturase transcript level in fish in response to a vegetable oil diet has also been reported, with liver transcript levels of putative Δ-6 cloned from rainbout being higher in trout fed LO compared to trout fed FO (Seiliez et al., 2001). Similar study showed that the transcript of putative Δ-6 cloned from sea bream was highly expressed in liver from fish fed a LC-PUFA diet and only slightly expressed in livers from fish fed a LC-PUFA rich diet (Seiliez et al., 2003). Both the trout and sea bream putative desaturase were later confirmed as Δ-6 (Zheng et al., 2004). Study by Miller and Colleges (2008) also showed that gene expression of liver elongase and Δ-5 was up regulated in fish fed diet contained echium oil compared with FO fish. There was also significantly higher elongase and both Δ-5 and Δ-6 gene expression in liver of fish fed diet contained canola oil than FO. Increased hepatocyte desaturation and elongation activities in other species of fish such as

tilapia and zebra fish were also reported with vegetable oils based diet (Tocher et al., 2002). Ling et al. (2006) also observed that increasing levels of LO resulting in relatively higher expression of desaturase and elongase in swordtail muscle. Several studies also established that the nutritional regulation of desaturase in fish muscle with increased activities reported with diets containing low levels of LC-PUFA (Seiliez et al., 2001, 2003; Zheng et al., 2005). Many investigations at both, enzymatic and mRNA levels, have shown that higher desaturation and elongation activities in fish fed diets with limited LC-PUFA (Tocher et al., 2006a,b; Turchini et al., 2006; Ling et al., 2006). Another study by Zheng et al. (2005) reported that elongase gene expression was not increased by feeding vegetable oil blend and did not correlate with LC-PUFA biosynthetic pathway activity. Ling et al. (2006) also found that, while desaturase showed higher expression with increasing levels of LO, expression of elongase did not appear to show a similar trend, with diet contained combination of squid and LO (1:1 squid oil: LO) showing highest activity. Each step along the LC n-3 PUFA pathway depends on the amount of substrate and therefore activity of the preceding enzymatic steps if it is not supplied by the diet and also the removal of the subsequent products (Miller et al., 2008). Therefore increased elongase and desaturase genes could be influenced by either increased dietary concentration of substrate or removal or absence of the product (Zheng et al., 2004).

Δ-5 and Δ-6 fatty acid desaturases and fatty acid elongases are enzymes which found to be critical in the biosynthetic pathways of LC-PUFA from shorter chain PUFA (Zheng et al., 2004). A fatty acid desaturase has been cloned from zebra fish that has been shown to have both Δ-5 and Δ-6 activities (Hastings et al., 2001). Desaturases genes have been also cloned and characterised from rainbout (Seiliez et al., 2001).

Genes specifically involved in the elongation of fatty acids in the LC-PUFA biosynthetic pathway have been cloned and characterized from zebra fish (Agaba et al., 2004) and Atlantic salmon (Hastings et al., 2004). For abalone, however, there is still no direct evidence on the gene expression and activities of Δ-5 and Δ-6 fatty acid desaturases and elongases enzymes which are important in the biosynthetic pathways of LC-PUFA from shorter chain PUFA.

In summary, available information suggested that lipid requirement of abalone varies between the species. Furthermore, the requirements for dietary essential fatty acids differ between species. It was also evident that diet and water temperature have significant influence on the lipid content, fatty acid composition and growth performance of abalone. Previous studies also indicated that commercial diet supplemented with FO could increase LC n-3 PUFA content in cultured abalone significantly. The improvement of LC n-3 PUFA in cultured abalone are attributed to the high levels of EPA and DHA in FO. A number of studies suggested that dietary vegetable oil such as FlaxO and CO could be partially substituted for FO in abalone diets with no apparent negative effect on growth. Information also suggested that abalone may have the ability to bio-convert short chain PUFA to LC-PUFA. However, more extensive research is required to investigate the effects of FO and vegetable oil supplementation in the diet of cultured abalone on fatty acid composition, gene expression and growth performance, as there are still questions that remain unanswered. The main aims of this thesis were: (1) to investigate the effects of feed supplemented with FO on fatty acid composition and fatty acid Δ-6 desaturase and elongase-2 gene expressions in tissues of cultured Jade Tiger hybrid abalone; (2) to determine the effects of total or partial substitution of

FO with FlaxO and CO on fatty acid composition and the expression of Δ-6 desaturase and elongase gene in tissues of Jade Tiger abalone; and (3) to examine the effects of feed incorporated with FO on growth performance of Jade tiger hybrid abalone and to evaluate the influence of replacement of FO by FlaxO and CO on growth performance of Jade tiger hybrid abalone. It was hypothesized that dietary FO would increase the LC n-3 PUFA accumulation in the tissues as well as improve the growth performance of Jade Tiger abalone. In addition, it was also expected that dietary FlaxO and CO could partially replace FO in Jade Tiger abalone diet without detriment to growth performance.

Chapter 3: General materials and methods

This section describes the general material and methods used in the thesis. More specific descriptions of the procedures could be found in Chapter 4 - Chapter 8 (papers 1- 5) of this thesis.

3.1 Experimental diets

A commercial diet (Adam and Amos Abalone Foods Pty Ltd, Mount Barker, Australia) for Australian abalone was used in the formulation of the experimental diets. Adam and Amos also assisted with formulation of the experimental diets. In Chapter 5, there were six diets, diet 1 (control diet) was the normal commercial abalone diet with no additional fish oil (FO) supplement. Diets 2 to 6 were supplemented with 0.5, 1, 1.5, 2, and 2.5% FO respectively. The control diet contained 57.6% total saturated fatty acid (SFA), 5.6% monounsaturated fatty acid (MUFA) and 36.8% polyunsaturated fatty acid (PUFA). The diets with FO supplements (diets 2 to 6) contained 21-24.7% total SFA, 18.6-22.9% total MUFA, and 52.3-60.3% total PUFA. The control diet contained 0.42g/100g total lipids while other diets contained lipids ranging from 1.10-2.72 g/100g. In Chapter 6, there were five experimental diets. The control diet contained 100% FO while the remaining diets contained increasing levels of flaxseed oil (FlaxO) at 25, 50 and 75 and 100% of total lipid added. The control diet contained 29.3% total SFA, 24.9% total MUFA and 46.4% total PUFA. The diets with FlaxO contained 14.9-26.6% SFA, 20.2-23.5% MUFA and 46.3-63.8% PUFA. The control diet contained 20 g kg^{-1}(2.0 g/100g) total lipids while other diets contained lipids ranging from 26.0 - 36.0 g kg^{-1}(2.6 - 3.6 g/100g). In Chapter 7, there were five experimental diets,

consisting of a control diet containing FO (0% canola oil (CO), and four diets in which the FO was replaced in a graded manner by 25, 50, 75 and 100% CO. The control diet contained 27.3% total SFA, 21.6% total MUFA and 50.8% total PUFA. The diets with CO contained 11.2-23.7% SFA, 31.0-45.2% MUFA and 42.1-45.0% PUFA. The control diet contained 2.0 g/100g total lipids while other diets contained lipids ranging from 2.3-3.4 g/100g. All ingredients used in the treatment diets were identical except for the added oil. All of these experimental diets were stored at 4 °C until use. The FO and FlaxO used in the feeding trials were obtained from Melrose Laboratories Pty Ltd, Victoria, Australia, whereas the CO was bought from Cole's supermarket, Victoria, Australia).

3.2 Experimental animals, husbandry and sampling

The research presented was carried out on Jade Tiger hybrid abalone (*H. rubra x H. laevigata*) (Figure 10). As mentioned in Chapter 2, this is one of the three abalone farmed in Victoria, Australia. Adult female Jade Tiger abalone were obtained from Great Southern Waters abalone farm, Victoria, Australia under a Fisheries research permit (99/RP/884). Abalone were transported to the aquaculture research laboratory of Victoria University, located at St Albans campus, Melbourne, Australia. All abalone were translocated and treated according to Victorian Abalone Aquaculture Translocation Protocol and the Fisheries act 1995. Before arrival these animals were maintained in the farm with a continuous flow of seawater and were fed a commercial artificial diet.

Figure 10. Adult Jade Tiger abalone.

In Chapter 4, live female Jade Tiger abalone (2.5 years old) were obtained in November (spring), January (summer), May (autumn) and July (winter) between 2007 and 2008. Ten individuals were sampled in each season. Upon arrivals to our laboratory, each individual abalone was cleaned and blotted dry with paper towel before shell length and total body weight were determined. After dissection, fresh muscle, gonad and digestive glands (DG) were obtained for lipid and fatty acid analyses.

In Chapter 5, 90 abalone and in Chapter 6, 75 abalone with an average body weight of 44.0 ± 0.3 grams, and in Chapter 7, 75 abalone with initial mean weight and shell length of 20.9 ± 0.4 g and 37.3 ± 0.5 mm were used in the feeding trials. Upon arrival abalone were acclimatized to laboratory conditions and fed a normal commercial diet for 1-2 weeks before initiation of the feeding trials. After acclimatization period completed, each individual abalone was gently blotted dry

with paper towel, tagged, measured and weighed and then five abalone were placed into a 30 L plastic aquarium supplied with filtered seawater via a flow through system and assigned one of the six experimental diets in Chapter 5 and one of the five diets in Chapter 6 and Chapter 7. There were three replicates for each of the dietary treatments in Chapter 5 - Chapter 7. In total, there were 18 aquariums in Chapter 5 and 15 aquariums each in Chapter 6 and Chapter 7. The filter sea water was obtained from Department of Primary Industries, Queenscliff, Victoria, Australia. The seawater was also re-filtered in our laboratory to avoid any contaminations. Flow rate to each aquarium were set at 1.5 L min^{-1}. Animals were maintained under a 24 hour dark photoperiod. Temperature was maintained at 16°C ± 1°C throughout the feeding trial. Salinity (31 - 35ppt), dissolved oxygen (no less than 6 mg/L), nitrite, ammonia and pH (7 to 8) were monitored daily. Aquaria were cleaned three times a week. The experimental diets were hand-fed every day in the evening at a rate of 2% of body weight. Each feeding trials in Chapter 5 - Chapter 7 lasted for 90 days.

At the end of each feeding trial, abalone were fasted for one day and collected from their aquaria. They were then measured and weighted. Fourteen abalone per treatment were sampled to obtain muscle, gonad and DG tissues for lipid and fatty acid analyses and investigation of gene expression. One abalone per treatment was sacrificed for histological analysis. In Chapter 8, 10 abalone from each treatment in Chapter 5 - Chapter 7 were used for growth performance and survival analysis.

3. 3 Lipid analysis

The lipid analysis of abalone muscle, gonad and digestive gland and feed samples

involved lipid extraction and fatty acid analysis. Prior to analysis, abalone were removed from their shell. Samples of tissues were finely cut and dried feeds were ground using a food grinder to provide fine particles and hence homogenous samples.

3.3.1 Lipid extraction and fatty acid analysis

Fresh dissected samples and feed from each treatment were extracted in 100 ml Scott glass bottles with 50ml of Chloroform: Methanol (2:1) (Merck, Germany), containing 10 mg/L antioxidant, butylated hydroxytoluene (BHT Sigma Chemical Co, St Louis, USA) overnight in dark. Each sample was processed using a separation funnel. The extraction bottle of samples was rinsed with 10ml of chloroform: methanol 2:1 twice and these solutions were filtered into the separation funnel. 0.6% of physiological saline was then added into the separation funnel and the solution was left to partition overnight. When two clear layers were observed, the bottom layer was collected into a round bottom flask and the solution was dried in a Heidolph WB 2000 rotary evaporator at 40°C. The lipid extract was immediately reconstituted with chloroform and transferred to a 10 ml volumetric flask, made up to the mark. The 10 ml solution was separated into three tubes with 1 ml transferred into a pre-weighed 4 ml glass vial, another 1 ml transferred into a glass methylation tube, and the remainder 8 mls was transferred into a glass screw cap tube for storage at -20^{o}C. The solution in the 4 ml vial was dried up under high purity nitrogen (N_2) and the lipid extract was then weighed.

For the fatty acid analysis, 1 ml internal standard, C23:0 methyl ester (Nuchek Prep Inc, USA) was added to the 1 ml of lipid extract in the glass methylation tubes. This solution was then dried under high purity N_2. The sample was reconstituted with 2

mls of 7.6% potassium hydroxide (KOH) (Univar, AJAX chemicals, Australia) in methanol solution (Merck, Germany) and flushed with N_2. After being placed in a 105^{o}C oven for 5 minutes the solution was shaken vigorously and placed back for a further 5 minutes, and left in a cold-water bath for 10 minutes. Two millilitres of 14% Boron Trifluoride-Methanol Complex (BF_3) (Merck, Germany) was added to each tube and again flushed with N_2 and heated in a 105^{o}C oven for 10 minutes and shaken after 5 minutes. After 10 minutes in cold water bath 3 mls of distilled water and the same volume of petroleum ether were added and each tube was centrifuged in a Beckman CS-15 at 1000 rpms for 10 minutes to break any emulsion formed, and to yield a clear petrol phase. This phase was carefully removed with a glass Pasteur pipette and washed in a tube containing 2 mls of distilled water. As the extract and water separates the petrol was removed again using a Pasteur pipette and placed into a tube containing a small amount of anhydrous sodium sulphate (Na_2 SO_4) to remove any water from the petrol phase. The solution was then transferred into a screw-capped test tube previously rinsed with petroleum ether.

Fatty acids and sterols were separated using the sep-pack silica cartridges (Waters Corporation, Massachusetts). After pre-washing the cartridge with 5 mls of petroleum ether the sample (FAME and non-saponifiable matter) was pushed through the cartridge by syringe, discarding the solvent. The sample tube was rinsed with 2.5 mls of petroleum ether (Sigma-Aldrich, USA) and pushed through the cartridge. A further 2 millilitres of petroleum ether was added to the tube and again pushed through the cartridge. Five mls of 5% diethyl ether in petroleum ether solution was added to the syringe, and the FAMES were then collected carefully under the cartridge. The solutions were dried up under N_2 and immediately

reconstituted to 2 mls of petroleum ether and placed in a 2 ml vial for aliquots to be injected into the gas liquid chromatography.

3.3.1.1 Gas liquid chromatography

FAMES were separated by capillary gas liquid chromatography (Varian 3400) equipped with an auto sampler and a flame ionization detector (FID) using a 50 m x 0.32 mm (I.D) fused silica bonded phase capillary column (BPX70, SGE, Melbourne, Australia**).** Air was set at 60psi and hydrogen at 40psi, along with helium, the carrier gas, at a pressure reading of about 70-80psi and the column head pressure was adjusted to 30psi. The column oven was programmed from 140°C to 220°C at 5°C/min and held for 3 min. The oven temperature was then increased to 260°C at a rate of 8°C/min and held for 8 min. The injector and detector were maintained at 250°C and 300°C respectively. For each sample 2 µL was injected using an SGE micro-syringe into the injector. The GC injection was split. Fatty acids were identified by comparison of retention times with those of standard mixtures of fatty acids methyl esters (GLC reference standard 403; Nu-Check Prep, Elysian, MN, USA). This was achieved by comparing the retention times of each peak to the standard, injected under identical conditions. The report displaying the retention times, line up with the area counts, which are used to calculate the FA concentration.

3.4 RNA extraction and quantitative polymerase reaction

3.4.1 RNA Extraction

After the completion of each feeding trials foot muscle samples of four abalone were randomly selected from each treatment group. They were frozen immediately in

liquid nitrogen and stored at -80°C prior to RNA extraction. The RNA extraction procedure began with the removal of the muscle tissues from the -80°C freezer and subsequent thawing of the tissue for 5 minutes. The samples were collected quickly and all unwanted surrounding connective tissue was removed before cutting and weighing 100 mg of muscle tissue sample on an analytical balance. 100 mg of tissue was harvested as mentioned above and placed into tubes containing 1000 µl of tri reagent (Molecular Research Centre, USA) and 500 mg of ceramic beads (MO BIO Laboratories, Inc, USA). All of the tubes were placed into fast prep was pulsed X2 for 40 seconds at speed 6. Following the homogenisation the samples were allowed to cool at room temperature for 5 minutes and the homogenate was transferred to a new eppendorf with the addition of 100 µL of 1-Bromo-3-chloropropane (BCP, Sigma-Aldrich, USA**)** and mixed via vortex for 20 seconds. The samples containing the homogenate and BCP mixture were stored for 15 minutes at room temperature pulsed to centrifuge at 12,000g for 15 minutes at 4°C. Following centrifugation the solution was separated into three clear phases. The upper aqueous phase containing RNA was transferred to a new eppendorf tube. The RNA was then precipitated by the addition of 500 µL of isopropanol which was mixed via vortex for 20 seconds. Samples were then stored for 5 minutes at room temperature and centrifuged at 12,000g for 10 minutes at 4°C. The subsequent RNA precipitate was washed with 1000 µl of 75% ethanol. Ethanol was subsequently removed via pipette and the palette was left to air dry for 5 minutes. The RNA was res-suspended by the addition of 20 µL of nucleus free water (Promega Corporation, USA).

3.4.2 RNA quantification

All RNA samples of muscle tissue were quantified using Cary UV-Visible

spectrophotometer (UV-S), Varian, Melbourne. The UV-S uses a wavelength of 260 nanometres (nm) to detect suspended RNA. A 1:200 RNA dilution was used for the spectrophotometer analysis by pippetting 1 µL into a quartez cuvette with 199 µL of millq water. Before samples were placed into the UV-S the spectrophotonanometer was blanked with 2000 µL of millq water in a quartez cuevette. The diluted RNA was then placed into the spectrophotonanometer to be analysed at 260 and 280 nm. The absorbance of RNA was read at 260 and DNA at 280 nm, whist the ratio was calculated by dividing the two. A ratio of above 1.4 was considered appropriate for further RNA analysis.

3.4.3 Quantitative polymerase reaction

One microgram of RNA was reverse transcribed into cDNA using AMV reverse transcriptase first strand cDNA synthesis kit (Marligen Biosciences, USA). The expression of Δ-6 desaturase and elongase genes in muscle tissue from abalone fed with the different experimental diets were studied by real-time polymerase chain reaction (RT-PCR). PCR primers known to be specific for, Δ-6 desaturase (F 5'-ACCTAGTGGCTCCTCTGGTC-3' and R 5-'CAGATCCCCTGACTTCTTCA-3', AF301910) and elongase (F 5'-GAACAGCTTCATCCATGTCC-3' and R 5'-TGACTGCACATATCGTCTGG-3', AY605100) were used for PCR amplification of cDNA. ß-actin (F 5-'CAAGCAGGAGTACGACGAGT-3' and R 5-'CTGAAGTGGTAGTCGGGTGT-3', AJ438158) was used as a housekeeping gene for normalising mRNA levels of the target genes. Due to lack of primers from abalone the primers used in this study were from trout. Therefore, before we started analysing these genes in abalone with trout primers, we took all the necessary measures to ensure the accurate analyses of those genes; including considerable

number of optimizing trials on gene expression analysis techniques. The amplification of cDNA samples was carried out using the SYBR green PCR kit (Bio-Rad, USA). Fluorescent emission data were captured and mRNA levels were analyzed using the critical threshold (CT) value (Schmittgen et al., 2000). Thermal cycling and fluorescence detection were conducted using the Biorad IQ50 sequence detection system (Biorad USA).

3.5 Growth performance

Growth performance and survival of abalone were evaluated according to the recommendations by Mai et al. (1995, 1996), Mercer et al. (1993), Nelson et al. (2002), Durazo-Beltran et al. (2003),Wee et al.(2004) and Bautista-Teruel et al. (2011). The following parameters were considered:

1) Daily growth rate of weight (DGRw) (mg / day) was computed as: 1000 (Final weight (g) -Initial weight (g) / no. of days.

2) Weight gain (WG) (%) was calculated as (Final weight - Initial weight) / Initial weight × 100.

3) Specific growth rate of weight (SGRw) (% / day) as 100 [ln (final weight) - ln (initial weight] / no. of days.

4) Daily growth rate of shell length (DGR_{SL}) (µm / day) = 1000 (Final shell length (mm) - Initial shell length (mm) / no. of days.

5) Shell length increase (SLI) (%) = (Final shell length - Initial shell length) / Initial shell length × 100.

6) Specific growth rate of shell length (SGRs) (% / day) = 100 [ln (final shell length) - ln (initial shell length] / no. of days.

7) Percent survival was calculated as: final no. of abalone / initial no. of abalone × 100.

3.6 Statistical data analysis

Results are presented as mean ± SD. The data were tested for homogeneity of variances using a Levene test. All data were analyzed using one-way analysis of variance (ANOVA) to determine significance of differences among total lipid content, fatty acid composition, gene expression and growth performance of abalone fed the different experimental diets. Tukey HSD tests were used for post-hoc comparison. P-values of less than 0.05 were considered statistically different. Statistical analysis was performed using the SPSS package (version 15 to 17.0)

Chapter 4: Seasonal variations of total lipid and fatty acid contents in muscle, gonad and digestive glands of farmed Jade Tiger hybrid abalone in Australia

Introduction

Chapter Four examines the contents of total lipid and fatty acid of muscle, gonad and digestive glands in farmed Jade Tiger hybrid abalone over the four seasons.

The paper titled Seasonal variations of total lipid and fatty acid contents in muscle, gonad and digestive glands of farmed Jade Tiger hybrid abalone in Australia by H. T. Mateos, P.A. Lewandowski, and X.Q. Su was published in the peer review journal , *Food Chemistry*, 123, 436-441, 2010.

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Declaration by [candidate name]: Hintsa Signature: [redacted] Date: 15/10/2011

Paper Title:

Seasonal variations of total lipid and fatty acid contents in muscle, gonad and digestive glands of farmed jade tiger hybrid abalone in Australia

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In the case of the above publication, the following authors contributed to the work as follows:

Name	Contribution %	Nature of Contribution
Hintsa Mateos	65	Sampled the tissue of interest Performed all lipid extraction and fatty acids analyses Evaluated analytical data Performed statistical analysis Prepared major part of the manuscript
Xiao Su	28	Contribution to writing of manuscript and journal submission
Paul Lewandowski	7	Contribution to writing of paper

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DECLARATION BY CO-AUTHORS

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Seasonal variations of total lipid and fatty acid contents in muscle, gonad and digestive glands of farmed Jade Tiger hybrid abalone in Australia by H. T. Mateos, P.A. Lewandowski, and X.Q. Su was published in the peer review journal , *Food Chemistry*, 123, 436-441, 2010.

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Chapter 5: The effects of diets supplemented with different concentration of fish oil on fatty acid composition and expression of desaturase and elongase genes in abalone

Introduction

This study investigated the effects of feed supplemented with fish oil on fatty acid composition in muscle, gonad and digestive gland of cultured adult female Jade Tiger hybrid abalone. In addition the effects of fish oil supplementation on fatty acid Δ6 desaturase and elongase 2 gene expressions in muscle tissue of this animal were also studied.

The paper titled Dietary fish oil supplements increases tissue n-3 fatty acid composition and expression of delta-6 desaturase and elongase-2 in Jade Tiger hybrid abalone by Hintsa T. Mateos, Paul A. Lewandowski, and Xiao Q. Su was published in the peer review journal, Lipids, 46, 91–751, 2011.

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Declaration by [candidate name]: Hintsa Signature [redacted] Date: 15/10/2011

Paper Title:

Dietary Fish Oil Supplements Increase Tissue n-3 Fatty Acid Composition and Expression of Delta-6 Desaturase and Elongase-2 in Jade Tiger Hybrid Abalone

In the case of the above publication, the following authors contributed to the work as follows:

Name	Contribution %	Nature of Contribution
Hintsa Mateos	70	Conduct feeding trial and Sampled abalone Carried out all lipid extraction and lipid and fatty acids analyses Performed all RNA extraction and analysis of gene expression Performed statistical analysis Prepared major part of the manuscript
Xiao Su	20	Contribution to writing of paper and journal submission
Paul Lewandowski	10	Contribution to writing of paper

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Chapter 6: Effects of dietary fish oil replacement with flaxseed oil on tissue fatty acid composition and expression of desaturase and elongase genes

Introduction

The aim of this work was to determine the effects of total or partial substitution of fish oil with flaxseed oil on fatty acid composition of muscle, gonad and digestive gland of cultured Jade Tiger hybrid abalone. In addition the effects of replacement of fish oil with flaxseed oil on the expression of Δ-6 desaturase and elongase genes in muscle tissue were also studied.

The paper titled Effects of dietary fish oil replacement with flaxseed oil on tissue fatty acid composition and expression of desaturase and elongase genes by Hintsa T. Mateos, Paul A. Lewandowski, and Xiao Q. Su was published in the peer review journal, *Journal of the Science of Food and Agriculture*, Article first published online: 10 AUG 2011 DOI: 10.1002/jsfa.4594.

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Declaration by [candidate name]: Hintsa Signature: [redacted] Date: 15/10/2011

Paper Title:

Effects of dietary fish oil replacement with flaxseed oil on tissue fatty acid composition and expression of desaturase and elongase genes

In the case of the above publication, the following authors contributed to the work as follows:

Name	Contribution %	Nature of Contribution
Hintsa Mateos	76	Conduct feeding trial Sampled abalone and carried out all lipid extraction and lipid and fatty acids analyses Performed all RNA extraction and analysis of gene expression Performed statistical analysis Prepared major part of the manuscript
Xiao Su	15	Contribution to writing of paper and journal submission
Paul Lewandowski	9	Contribution to writing of paper

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It is available at http://onlinelibrary.wiley.com/doi/10.1002/jsfa.4594/abstract

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Chapter 7: 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

Introduction

The aim of this study was to determine the effects of total or partial replacement of fish oil with canola oil on fatty acid composition in the muscle of farmed Jade Tiger hybrid abalone. In addition, the effects of replacement of fish oil with canola oil on expression of Δ-6 desaturase and elongase 2 genes in muscle tissue were also studied.

The paper titled 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 by H. T. Mateos, P.A. Lewandowski, and X.Q. Su was published in the peer review journal, *Food Chemistry*, Article first published online: 1 Oct 2011. doi:10.1016/j.foodchem.2011.09.107.

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Paper Title:

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

In the case of the above publication, the following authors contributed to the work as follows:

Name	Contribution %	Nature of Contribution
Hintsa Mateos	77	Conduct feeding trial Sampled abalone and carried out all lipid extraction and lipid and fatty acids analyses Performed all RNA extraction and analysis of gene expression Performed statistical analysis Prepared major part of the manuscript
Xiao Su	15	Contribution to writing of paper and journal submission
Paul Lewandowski	8	Contribution to writing of paper

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Chapter 8: The growth performance of cultured abalone fed diets supplemented with fish oil and vegetable oils

Introduction

The aims of the present study were to examine the effects of feed incorporated with fish oil on the growth performance of Jade Tiger hybrid abalone and to evaluate the influence of replacement of fish oil by flaxseed oil and canola oil on growth performance of Jade Tiger hybrid abalone.

The following paper entitled "The growth performance of cultured abalone fed diets supplemented with fish oil and vegetable oils" by H. T. Mateos, P.A. Lewandowski, and X.Q. Su has not been submitted for publication.

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Declaration by [candidate name]: Hintsa **Signature** [redacted] **Date:** 15/10/2011

Paper Title:

The growth performance of cultured abalone fed diets supplemented with fish oil and vegetable oils.

In the case of the above publication, the following authors contributed to the work as follows:

Name	Contribution %	Nature of Contribution
Hintsa Mateos	80	Conduct feeding trial Sampled abalone and carried out all statistical analysis Prepared major part of the manuscript
Xiao Su	13	Contribution to writing of paper
Paul Lewandowski	7	Contribution to writing of paper

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The growth performance of cultured abalone fed diets supplemented with fish oil and vegetable oils

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Abstract

BACKGROUND: The effects of fish oil (FO) supplementation and dietary replacement of FO with flaxseed oil (FlaxO) and canola oil (CO), on the growth of cultured abalone was investigated. The study involved three growth experiments; E1) diets containing 0.5, 1.0, 1.5, 2.0 and 2.5% of FO respectively, E2) diets in which FO was serially replaced by 25, 50, 75 and 100% FlaxO respectively, E3) diets in which FO was serially replaced by 25, 50, 75 and 100% CO respectively.

RESULTS: In E1 Abalone fed a diet supplemented with 1.5% FO showed a significantly higher daily growth rate of weight (DGRw) compared to control. In E2, abalone fed 1.5% FO diet and diets containing 25 - 75% FlaxO showed no significant differences in DGRw. The diet containing 100% FlaxO showed significantly lower growth parameters. In E3 abalone fed diets containing 25% and 50% CO showed the same DGRw as those fed a 1.5% FO diet. The diet containing 75% and 100% CO showed significantly lower DGRw.

CONCLUSION: Supplementation with 1.5% of dietary FO can improve growth performance in cultured abalone. It is feasible to replace 75% of dietary FO with FlaxO and 50% of dietary FO with CO, without negatively effecting growth performance.

Keywords: Abalone · Growth · Fish oil · Flaxseed oil · Canola oil · n-3 Fatty acids

Introduction

Dietary lipids play an important role in providing essential fatty acids and concentrated energy to organisms.[1, 2] In addition, they are important nutrients for maximising growth rate and health of many marine animals.[3] The dietary lipid requirement of abalone is very low as these animals are highly efficient in utilizing lipid.[4] Moreover; abalone have a high lipid digestibility (84.7%).[5] Dietary lipid contents of 3% to 5% have been recommended in the form of either FO, vegetable oil or a combination of both, to obtain maximum growth rate in juvenile abalone, *H. discus hannai, H. tuberculata* and *H. asinine*.[6-8] Mai et al., [9] reported that the optimum lipid concentration in the formulated diet of *H. discus hannai* should be controlled at between 3% to 7%. Another study showed that abalone *(H. midae)* grew well at up to 6% dietary lipid.[10] Durazo-Beltran et al., [11] have shown that maximum growth of green abalone, *H.flugens* was achieved at a 1.5% inclusion of lipid. The Japanese feed Nihon Kogyo contained only 1.5% lipid but produced significantly higher growth rates in abalone relative to those with 3% lipid.[12] Thongrod et al., [8] have shown in their experiments that abalone, *H.asinina* fed a diet containing lipid to carbohydrate ratio 1.3%:47.8% provide the highest growth rate among the other ratios tested. Lee [13], on the other hand has noted that *H. discus hannai* required 2.5% lipid in formulated diet. Bautista-Teruel et al., [14] found fastest growth when juvenile abalone, *H. asinine* Linne were fed with dietary lipid levels of 2.2-6.1%. In Australia, the maximum growth rate of juvenile greenlip abalone was obtained when the animals were fed formulated feed that contained lipids at 2.5% in summer and 3.5% in winter.[15] In another study, Dunstan et al.,[12] found that growth rate of greenlip abalone increased when abalone fed a diet containing 3.8% total lipid. Furthermore, other formulated diets with total lipid contents of 2.6 and

4.2% also improved growth rates.[12] Van Barneveld et al., [16] suggested that the addition of marine or vegetable oils in abalone diets should be limited to 3% due to the negative influence on the digestibility of amino acids in juvenile *H. laveigata*. Dunstan et al., [12] found that the digestibilities of marine origin fatty acid rich lipid classes, i.e. triacyglycerols from FO, were higher in greenlip abalone than that of lipid classes from vegetable oils. Studies demonstrated that formulated abalone diets from around the world contained a wide range of total lipid contents (1-11% wet wt) [6-8, 12] however, in most cases the total lipid comprised less than 5% of the diet.[12]

Long chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) are essential for survival and growth of molluscs.[6] Studies on fatty acid profiles of abalone fed natural diets suggested that EPA might play a dominant role in growth of juvenile *H. discus hannai and H. tuberculata.*[7] While LA, ALA and ARA fatty acids contributed to faster growth in *H. discus* [17, 18, 19] However, *H. tuberculata* appeared to be dependent largely on LC n-3 PUFA.[7] Durazo-Beltran et al., [11] have shown in their experiment that LC n-3 PUFA (e.g. ARA, EPA and DPA) are essentials for *H. fulgens*. No significant differences were found in the growth of *H. fulgens* fed vegetable oils (olive, corn and linseed oils) and cod liver oil.[11] Abalone research in Australia showed that the growth rate of juvenile greenlip abalone was lowest when abalone fed diets containing mostly short chain PUFA which are found in vegetable oils.[12] The adult greenlip abalone also showed a poor growth rate when fed a diet containing only the short chain PUFA. The enrichment with vegetable oils was less effective than diets containing both EPA and DHA which have been found to produce the highest growth rates.[15]

Abalone fishery is an important industry in Australia and its production accounts for

about 50% of the global market and generates hundreds millions of dollars for the country's economy.[20] However, due to rapidly increasing demands from global market, wild fisheries are under strict and limited production quotas and there is now a growing investment and research interest in aquaculture production of abalone.[21,22] Blacklip abalone *(H. rubra*), greenlip abalone *(H. laevigata)* and hybrid abalone *(H. laevigata x H. rubra)* are the three main types of abalone farmed in Australia.[23] Information about the fatty acid requirements for growth and development of cultured adult Jade tiger abalone was unavailable.

The aims of the present study were to examine the effects of feed incorporated with FO on the growth performance of Jade Tiger hybrid abalone and to evaluate the influence of replacement of FO by FlaxO and CO on growth performance of Jade Tiger hybrid abalone.

2. Materials and methods

2.1. Experimental animals

Abalone with initial mean weight and shell length of 40.1 ± 6.0 g and 56.3 ± 4.2 mm (experiment 1, E1), 40.2 ± 5.4g and 55.9 ± 4.0 mm (experiment 2, E2), and 19.7 ± 2.2g and 37.1 ± 1.8 mm (experiment 3, E3) respectively, were obtained from Great Southern Waters abalone farm, Victoria, Australia and used in the study. The abalone were acclimatized to the rearing conditions for two weeks prior to the feeding trial. During this period, abalone were fed the control diet. After acclimation each individual abalone was gently blotted dry on a paper towel, tagged, measured, and weighed for the experimental work.

2.2. Feed preparation

2.2.1. Experiment 1

A commercial diet for Australian abalone (Adam and Amos Pty Ltd) was used in the formulation of experimental diets. Diet 1 (control diet) was the normal commercial abalone diet with no additional FO supplement. Diets 2 to 6 were supplemented with 0.5, 1, 1.5, 2, and 2.5% FO respectively. The control diet contained 57.6% total SFA, 5.6% MUFA and 36.8% PUFA. The diets with FO supplementation (diets 2 to 6) contained 21-24.7% total SFA, 18.6-22.9% total MUFA, and 52.3-60.3% total PUFA. The composition of EPA, DPA, DHA and total n-3 PUFA of the diets with FO supplement were gradually increased in the experimental diets. The control diet contained 4.2 g kg^{-1} total lipids while other diets contained lipids ranging from 11.0 – 27.2 g kg^{-1}. All of these experimental diets were stored at 4°C until use. Fatty acid composition and total lipid content of the experimental diets are shown in Table 1

Insert Table 1 here

2.2.2. Experiment 2

The five diets consisted of a control diet containing 1.5% FO as the only added oil component (100% FO), and four testing diets in which the FO was replaced in a graded manner by FlaxO. The ratios of FO/FlaxO in these four testing diets are: 3:1 (FlaxO 25%), 1:1 (FlaxO 50%), 1:3 (FlaxO 75%) and 100% FlaxO. The control diet, formulated with 100% FO, contained 29.3% total SFA, mainly 16:0; almost 24.9% total MUFA and over half of which was OA MUFA; 16.5% n-6 PUFA predominantly LA; and 26.9% n-3 PUFA, predominantly the LC n-3 PUFA,

EPA and DHA, and less than 1.5% ALA. Inclusion of graded amounts of FlaxO resulted in graded increased percentages of ALA and LA with concomitant decreased proportions of LC n-3 PUFA such as EPA and DHA, 16:0, total SFA, 20:1 and total MUFA. Thus, in the diet formulated with 100% FlaxO, the levels of ALA and LA had risen to 39.5% and 23.1% of total fatty acids, respectively, whereas EPA and DHA totaled only 0.2%, and total SFA and MUFA were reduced to 14.9% and 20.2%, respectively. The fatty acid compositions of the diets are shown in Table 2.

Insert Table 2 here

2.2.3. Experiment 3

The five diets consisted of a control diet containing 1.5% FO supplements as the only added oil component to the commercial abalone diet (100% FO), and four experimental diets in which the FO was replaced in a graded manner by CO. The ratios of FO/CO in these four experimental diets were: 3:1 (CO 25%), 1:1 (CO 50%), 1:3 (CO 75%) and 100% CO. The control diet was formulated with 1.5% FO supplemented to the commercial abalone diet (100% FO). It contained 27.3% total SFA, mainly 16:0, almost 21.6% total MUFA and over half of which was the OA MUFA. The FO control diet also contained 23.3% n-6 PUFA, predominantly LA, and 27.5% n-3 PUFA, mainly the LC n-3 PUFA, EPA and DHA. Less than 2.3% ALA was also found in the control diet (Table 1). Inclusion of graded amounts of CO resulted in graded increased percentages of OA, LA and ALA as well as total MUFA with concomitant decreased proportions of LC n-3 PUFA such as EPA and DHA, 16:0 and total SFA, and total n-3 PUFA. Thus, in the diet

formulated with 100% CO, the levels of OA, LA and ALA had increased to 44.6%, 30.1% and 10.7% of total fatty acids, respectively, whereas EPA and DHA totaled only 0.6% and 0.9%, respectively. Total SFA was reduced to 11.2%, whereas total MUFA was increased to 45.2% of total fatty acids. The fatty acid compositions of these diets are shown in Table 3.

Insert Table 3 here

2.3. Experimental design and husbandry

For E1, eighteen 30L plastic aquaria, and for E2 and E3, fifteen each 30L plastic aquaria filled with filtered seawater were stocked with five abalone each. Dietary treatments were randomly allocated to each tank (3 replicates per treatment). During the experiments water temperature was maintained at 16°C ± 1°C, pH 7 - 8 and salinity 31 - 32ppt with constant oxygenation. Aquaria were cleaned three times a week. Any uneaten food was collected the following morning and dried and weighed. Abalone were fed 2% of their body weight using the dry diets. The daily ration was given once in the late afternoon (1700h). Each feeding trial lasted for 90 days.

2.4. Biological analysis

Daily growth rate of weight (DGRw), Weight gain (WG) and Specific growth rate of weight (SGRw) as well as Daily growth rate of shell length (DG_{SL}), Shell length increase (SLI) and Specific growth rate of shell length (SGRs) were determined to evaluate the dietary performance of each treatment. In addition survival was also determined for each experimental diet. DGRw was computed as: 1000 (Final weight

- Initial weight) / no. of days. WG was calculated as (Final weight - Initial weight) / Initial weight × 100. SGRw as 100 [ln (final weight) - ln (initial weight)] / no. of days. DGR_{SL} = 1000 (Final shell length - Initial shell length) / no. of days. SLI = (Final shell length - Initial shell length) / Initial shell length × 100. SGRs = 100 [ln (final shell length) - ln (initial shell length)] / no. of days. All animals measured and weighted at the begging and end of each experimental period. Percent survival was calculated as: final no. of abalone / initial no. of abalone × 100. All growth parameters and survival of abalone were evaluated according to previous studies on abalone.[2, 11, 14]

2.5. Lipid and fatty acid analysis

Ten grams of feed from each experimental diet were cut finely and left to extract lipids overnight in the dark in chloroform: methanol (2:1, v/v) which contained 0.01 $mgmL^{-1}$ butylated hydroxyltoluene as an antioxidant. The lipid extracts were washed according to the Folch procedure.[24] The solvent containing lipids were evaporated under vacuum in a rotary evaporator (Heidolph Standard evaporator, VV2000, UK). The lipid content was determined gravimetrically.[25]

Fatty acid methyl esters (FAME) were prepared by saponification of approximately 10 mg lipid, using KOH (0.68 mol L^{-1} in methanol), followed by esterification with 14% boron trifluoride in methanol, with 0.25 mg tricosanoic acid (23:0) added as an internal standard. FAME were separated by capillary gas–liquid chromatography (Varian 3400, Palo Alto, CA, USA) equipped with an auto sampler and a flame ionization detector using a 50 m x 0.32 mm (i.d.) fused-silica bonded phase capillary column (BPX70, SGE, Melbourne, Australia). The column

oven was programmed from 140 to 220 °C at 5 °C min^{-1} and held for 3 min. The oven temperature was then increased to 260 °C at a rate of 8 °C min^{-1} and held for 8 min. Helium was used as the carrier gas at a flow rate of 1.6 mL min^{-1} and a linear velocity 43 cm s^{-1}. The injector and detector were maintained at 250 and 300 °C respectively. Fatty acids were identified by comparison of retention times with those of standard mixtures of fatty acid methyl esters (GLC reference standard 403) and the results were calculated using response factors derived from chromatograph standards of known composition (Nu-Chek-Prep, Elysian, MN, USA).

2.6. Statistical analyses

Results are presented as mean ± SD. The data were tested for homogeneity of variances using a Levene test. All data were analyzed using a one-way analysis of variance (ANOVA). Multiple comparisons among treatments were performed using a Tukey HSD post hoc test. P-values of less than 0.05 ($P < 0.05$) were considered statistically significant. Statistical analysis was performed using the SPSS package (version 17).

3. Results

3.1. Experiment 1

The growth response of adult abalone fed the experimental diets for the 90 days feeding trial is presented in Table 4. The results revealed that FO supplementation affected the DGRw, WG, and SGRw of abalone. In addition, DG_{SL}, SLI as well as SGRs of abalone were also affected by the inclusion of FO to the commercial abalone diet. Abalone fed diet supplemented with 1.5% FO (diet 4) showed significantly higher value of DGRw while those fed the control diet (diet 1) showed significantly lower DGRw values. The

values of WG and SGRw were also the highest in abalone reared on the 1.5% FO diet relative to those on the control diet. The values of $DG_{SL,}$ SLI and SGR_S were significantly higher in abalone fed 0.5, 1, 1.5, and 2% FO (diets 2 -5) compared to those fed the control diet (diet 1) and the diet containing 2.5% FO (diet 6).

Insert Table 4 here

3.2. Experiment 2

The growth performances of abalone fed the experimental diets supplemented with FlaxO are shown in Table 5. At the end of the 90-day feeding trial, abalone fed the control diet (100% FO) and the diets containing 25, 50 and 75% of FlaxO showed no significant differences in DGRw, WG and SGRw (Table 5). The diet containing 100% FlaxO showed significantly lower values of DGRw, WG and SGRw compared to the abalone fed the other diets. No significant differences were recorded in the values of $DG_{SL,}$ SLI and SGR_S of abalone fed the control diet and the diets containing 25, 50 and 75% FlaxO. The values of DG_{SL}, SLI and SGR_S of abalone significantly declined when they were given diet with 100% FlaxO compared to the other diets.

Insert Table 5 here

3.3. Experiment 3

Results showed that, at the end of the 90-day feeding trial, abalone fed the control diet (100% FO diet) and the diets containing 25% and 50% of CO showed no significant differences in DGRw, WG and SGRw (Table 6). The diet containing 75% CO and 100% CO showed significantly lower values of DGRw, WG and SGRw compared to the abalone fed the other diets. In addition, no significant differences were recorded in

$DG_{SL,}$ SLI and SGR_S between abalone fed the control diet and those fed the diets containing 25% and 50% CO. The $DG_{SL,}$ SLI and SGR_S of abalone significantly declined when they were given diets with 75% CO and 100% CO compared to the other diets.

Insert Table 6 here

Discussion

Supplementation of abalone diets in E1 with FO increased the growth rate of Jade Tiger abalone. These results are consistent with previous reports on other abalone species, such as *H.laevigata, H.fulgens* and *H.discus hannai* Ino.[2, 11, 12] The improvement in DGRw, WG and SGRw as well as DG_{SL} and SGRs in abalone fed the diets containing FO may well define the important role that LC n-3 PUFA played in abalone nutrition. The significant increase in growth rate of abalone in this study with the supplementation of LC n-3 PUFA in their diet may well indicate that these nutrients are necessary for incorporation in every abalone diet formulation. Dunstan et al., [12] reported that maintaining adequate dietary concentration of the LC n-3 PUFA from FO is important to ensure high growth rate in abalone. Dunstan et al., [12] also found that abalone growth rate was higher when fed formulated diets containing LC n-3 PUFA (as found in FO) than when fed those containing LC n-6 PUFA (as found in red seaweeds). Mai et al., [7] reported that LC n-3 PUFA such as EPA play a dominant role in growth of *H.discus hannai* and *H.tuberculate*. While ALA, LA and ARA contribute to faster growth in *H.discus* [7, 17], *H. tuberculate* appears to depend on n-3 PUFA.[7] Durazo-Beltran et al., [11] have shown that LC n-3 PUFA such as EPA and DPA are

essential in the diet of *H.fulgens*. Other studies reported that LC n-3PUFA play a more important role in essential fatty acids nutrition in bivalves than C18 PUFA.[26] Generally these studies indicated that LC n-3 PUFA play a dominant role in the growth of abalone while ALA, LA and ARA contributes to the growth of abalone.

The present study also showed that abalone fed diet supplemented with 1.5% FO (diet 4) displayed a significantly higher value of DGRw compared to the abalone fed the other diets. The values of WG and SGRw were also significantly higher in abalone reared on this diet relative to those on the control (diet 1) diet. In addition abalone fed diets containing 0.5-2% FO (diets 2-5) showed significantly higher values of DGSL and SGRs compared to the abalone fed the control and the diet containing 2.5% FO (diet 6), with diet 4 (1.5% FO inclusion) being the most effective one. In greenlip abalone, growth rates were improved when abalone fed diets containing 1 and 2.5% added FO, at total lipid content of 2.6 and 4.2% respectively, during winter. Other diets with 1.5% added FO at total lipid content of 3.8% also increased growth rates.[12] Dunstan et al., [12] also reported that larger greenlip abalone fed a diet with FO inclusion at the level of 2.5% (total lipid content 3.2–4.1%) produced the highest average growth rates compared to the greenlip abalone fed a diet containing 4% FO (total lipid content 4.5-5%) inclusion. Generally it has been reported that greenlip abalone showed an improved optimal growth when fish oil levels are at 1.0-2.5% in the diets. Durazo-Beltran et al., [11] have shown that maximum growth of green abalone, *H. flugens* was achieved at a 1.5% inclusion of lipid. Similarly study by Wei et al., [2] showed highest growth rates in *H.discus hannai* Ino at a level of 2% FO (3.43% total lipid content) inclusion. The Japanese feed Nihon Kogyo contained only 1.5% lipid but produced

significantly increased growth rates in abalone relative to those with 3% lipid.[12] In contrast, some species showed an improved growth rate at the higher lipid levels. It appears that the lipid requirements of abalone are species specific although the overall range is lower in comparison with other animals. In our study the total dietary lipid contents of diets 1 to 6 ranged from 0.42 to 2.72% dry wt. The diet supplemented with 1.5% FO had 1.92% of total lipid and this probably is the optimal lipid level for Jade Tiger hybrid abalone, and thus achieved the best outcome. In general these results suggested that certain levels of FO inclusion in commercial abalone diet are important to improve growth rate of abalone. Our previous study also showed that supplementation with FO has significantly improved the LC n-3 PUFA levels, such as EPA and DHA in the tissues of Jade Tiger hybrid abalone.[23]

The fact that 1.5% FO supplementation significantly improved than other diets might also be attributed to the efficiency in utilization of dietary lipids by abalone. A previous study on abalone showed that their lipid requirement is very low as they are highly efficient in utilizing lipids.[4] Another study also showed that, addition of marine or vegetable oils to commercial abalone diets should be limited to 3% in order to maintain the optimal digestibility of nitrogen and amino acids, and gross energy.[16] In general, levels of dietary lipid in excess of 7% have been found to negatively affect growth and reduce the uptake of other nutrients in abalone diets.[16] The inability of abalone to utilize higher levels of dietary lipids has been linked to the low levels of lipase in their digestive tracts.[27-29] Formulated abalone diets from around the world contain a wide range of total lipid content varied from 1 to 11% wet wt.[12] It was also found that in the same FO diet, the SGRw was higher for smaller abalone compared to larger abalone.[12] This might also be the

case in the present study as the WG and SGRw in our trial were lower than in smaller abalone from other studies.[2,11,14]

The results of E1 showed that the control diet produced the lowest proportion of the growth parameters compared to other diets. This suggests that the total lipid content and LC n-3 PUFA of the control diet might not be adequate enough to produce comparable growth as FO diets. The highest total SFA in the control diet may also contribute to the lowest proportion of growth in abalone fed the control diet. Insufficient quantities of particular dietary lipids, especially the LC- PUFA have been shown to be responsible for low growth rates, reduced survival and poor disease resistance in many marine animals.[12] The low growth rate in abalone fed diet 6 might be a result of inefficient digestion of these fatty acids at higher levels. Abalone showed a poor response to elevated lipid levels due to the lower capacity for dealing with high dietary oil and this might be attributed to the low digestibility of lipids in this animal.[4, 11, 12, 27] This probably explains why at high FO levels in diet 6 at the present study, the growth or weight gain tended to decrease in comparison with the diet containing 1.5% FO. Supporting our study, a decrease in weight gain and utilization of dietary fat in juvenile fat cod with excessive dietary level of LC n-3 PUFA has been reported by Lee and Cho.[30] Similar results were also observed in other studies on fish. [31-33] The negative effects of excessive LC n-3 PUFA may be due to the disturbance of membrane polar lipids caused by the excessive accumulation of EPA and DHA in tissues. However, in the studies on rockfish and starry flounder, negative effects of excessive LC n-3 PUFA on growth and feed utilization were not observed.[34, 35] The underlying reason for different responses among fish fed the excessive LC n-3 PUFA is not clear, but it may be

attributed to differences in fish species, interaction with other nutrients, oil differences etc. Furthermore, the excess LC n-3 PUFA may also inhibit the metabolism of other fatty acids. Considering the poor growth in abalone fed higher levels of LC n-3 PUFA diets (2% and 2.5% FO) from the present study, partial replacement of FO by certain vegetable oils to meet the requirements of LC n-3 PUFA for abalone can also result in a good growth rate.

The results of partial or total substitution of dietary FO (control) by FlaxO and CO in E2 and E3 suggest that up to 75% FlaxO and 50% of CO can be used to replace FO without significantly compromising the growth of Jade Tiger abalone. This is in agreement with previous reports showing that feeding abalone with commercial diets containing a certain amount (1.5%) of vegetable oil such as flaxseed, olive and corn oils can also reach similar performance as FO does without negative effects on growth.[11] Supplementation of abalone diets with ALA, LA and LC n-3 PUFA has also been found to vastly improve the condition of abalone, thereby increasing their growth rate.[14] ALA and LA have been found to contribute to faster growth in *H. discus*.[17,18] Although the FlaxO and CO diets contained lower levels of LC n-3 PUFA compared the FO control diet, the comparable increase in growth of abalone fed these diets suggests that this species of abalone has at least a limited capacity to synthesize LC n-3 PUFA from ALA. Similar results have been reported on other abalone species, such as *H. laevigata*, *H. fulgens*, *H. discus hannai Ino* and *H. asinina Linne.*[2, 11, 12, 14] The provision of LC n-3 PUFA may have likewise contributed to the increased growth rate. The results also indicate that the replacement of FO by 100% FlaxO and over 50% CO can have a negative effect on abalone growth. This suggests that diets containing lower levels of LC n-

3 PUFA may not be recommended for abalone as these diets produced lower values of all growth parameters compared to the other experimental diets. Previous feeding trials on abalone also identified that poor growth occurred with a diet containing only the C18 PUFA and none of the LC n-3 PUFA.[12] Inadequate contents of LC n-3 PUFA may also give rise to several behavioral and morphological alterations such as poor feeding and thereby may result in poor growth. Furthermore, inappropriate dietary contents of such fatty acids in diets for brood stock may reduce fecundity and fertilization rates, originate embryonic deformities and damage larva qualities.[36] The diets containing high lipid level and no fish products are not recommended.[12] In agreement with our study, several studies on fish suggested that vegetable oils such as FlaxO and CO can partially replace FO in fish compounded aquatic feeds without a negative effect on growth.[37-39] However, higher level of dietary FO substitution up to 80% has resulted in remarkably reduced growth performance and feed utilization efficiency in fish.[38,40] Although some concerns exist that feeding diets with vegetable oil may affect health of marine animals [40], no mortalities and health issue were observed in the current study.

In conclusion, the present study demonstrates that supplementation with FO in the normal commercial diet of abalone can improve growth performance of cultured abalone. FO supplementation at a concentration of 1.5% which contained 1.92% total dietary lipid appears to achieve the best outcome. In addition, the present study indicates that it is feasible to replace 75% of dietary FO with FlaxO and 50% of dietary FO with CO respectively, without significantly negative effect on growth performance of Jade Tiger hybrid abalone.

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Table 1. Fatty acid composition (% of total fatty acids) and total lipid content (g/100g) of experimental diet (Experiment 1).

	Diet					
Fatty acid	1	2	3	4	5	6
14:0	0.9	2.5	3.6	4.8	4.7	4.6
16:0	57.6	17.7	18.4	17.6	15.0	14.8
17:0	0.3	0.1	0.3	0.3	0.4	0.2
18:0	0.8	2.4	3.5	4.3	4.6	4.9
Total SFA	59.9	23.1	25.9	27.3	24.8	24.7
16:1	0.3	2.9	4.0	5.4	5.6	6.0
17:1	0.0	0.4	0.9	0.8	0.9	1.2
18:1n9	3.7	12.6	13.0	13.1	11.8	10.2
18:1n7	0.3	1.1	0.9	1.0	0.9	0.8
20:1	0.1	0.9	1.9	2.0	2.6	3.2
Total MUFA	4.4	17.5	20.6	21.5	21.0	21.3
18:2n6	16.6	36.4	30.5	22.1	20.8	17.7
18:3n3	1.3	3.1	2.8	2.4	3.0	2.7
20:2n6	0.5	0.1	0.1	0.2	0.1	0.1
20:3n6	0.3	0.1	0.1	0.1	0.2	0.1
20:4n6	0.4	1.5	0.5	0.6	0.8	0.8
20:5n3	9.1	9.5	10.1	14.6	16.0	18.1
22:4n6	0.4	0.2	0.4	0.1	0.7	0.8
22:5n6	0.2	0.3	0.4	0.4	0.4	0.3
22:5n3	1.0	1.3	1.3	1.4	1.8	1.9
22:6n3	5.8	6.9	7.3	9.4	10.5	11.4
Total n-3	17.2	20.8	21.5	27.8	31.2	34.2
Total n-6	18.4	38.6	32.0	23.4	23.0	19.8
Total PUFA	35.5	59.4	53.5	51.2	54.2	54.0
n-3/n-6 PUFA	0.9	0.5	0.7	1.2	1.4	1.7
Total lipid(g/100g)	0.42	1.10	1.59	1.92	2.23	2.72

SFA = Saturated fatty acids; MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids.

Table 2. Fatty acid composition (% of total fatty acids) and total lipid content (g/100g) of experimental of experimental diets (Experiment 2).

	Diet				
Fatty acid	100% FO	25% FlaxO	50% FlaxO	75% FlaxO	100% FlaxO
14:0	6.6	5.2	3.7	2.7	1.3
16:0	17.6	17.1	16.7	13.6	10.1
17:0	0.3	0.1	0.1	0.1	0.1
18:0	4.3	3.9	3.3	3.2	3.1
Total SFA	29.3	26.6	24.1	19.8	14.9
16:1	5.6	4.3	3.4	2.2	0.6
17:1	0.8	0.4	0.3	0.1	0.1
18:1n9	13.1	14.6	15.6	16.9	18.4
18:1n7	3.3	2.8	2.3	1.8	1.0
20:1	2.0	1.4	1.2	0.7	0.1
Total MUFA	24.9	23.5	22.8	21.7	20.2
18:2n6	15.1	17.7	20.5	21.3	23.1
18:3n3	1.5	13.6	21.8	28.7	39.5
20:2n6	0.2	0.1	0.1	0.1	0.1
20:3n6	0.1	0.1	0.1	0.3	0.3
20:4n6	0.6	0.4	0.3	0.3	0.3
20:5n3	14.6	7.7	4.5	2.0	0.1
22:4n6	0.1	0.3	0.2	0.1	0.1
22:5n6	0.4	0.3	0.3	0.2	0.1
22:5n3	1.4	0.9	0.6	0.3	0.1
22:6n3	9.4	5.2	2.9	1.3	0.2
Total n-3	26.9	27.4	29.8	32.3	39.8
Total n-6	16.5	18.9	21.5	22.3	24.0
Total PUFA	43.4	46.3	50.3	54.6	63.8
n-3/n-6	1.6	1.4	1.4	1.4	1.7
Total lipid(g/100g)	2.0	2.6	3.0	3.5	3.6

SFA = Saturated fatty acids; MUFA = Monounsaturated fatty acids; PUFA =Polyunsaturated fatty acids.

Table 3. Fatty acid composition (% of total fatty acids) and total lipid content (g/100g) of experimental diets (Experiment 3).

	Diet				
Fatty acid	100% FO	25% CO	50% CO	75% CO	100% CO
14:0	4.7	3.2	2.4	2.1	1.0
16:0	17.5	15.6	12.5	10.7	8.3
17:0	0.3	0.7	0.7	0.7	0.5
18:0	4.3	3.8	3.3	2.0	1.0
Total SFA	27.3	23.7	19.3	15.9	11.2
16:1	5.4	3.7	2.0	1.7	0.5
17:1	0.3	0.4	0.2	0.1	0.1
18:1n9	13.0	25.6	30.7	36.0	44.6
18:1n7	1.0	0.7	0.5	0.3	0.2
20:1	1.9	0.6	0.9	0.8	0.5
Total MUFA	21.6	31.0	34.1	38.9	45.2
18:2n6	22.0	28.7	29.8	30.0	30.1
18:3n3	2.3	6.4	7.5	9.1	10.7
20:2n6	0.2	0.2	0.2	0.1	0.1
20:3n6	0.1	0.1	0.1	0.1	0.1
20:4n6	0.5	0.4	0.2	0.1	0.1
20:5n3	14.4	3.4	2.6	1.6	0.6
22:4n6	0.1	0.1	0.1	0.1	0.1
22:5n6	0.4	0.4	0.2	0.2	0.1
22:5n3	1.4	0.8	0.5	0.3	0.3
22:6n3	9.4	4.1	3.8	3.0	0.9
Total n-3	27.5	14.7	14.4	14.0	12.5
Total n-6	23.3	29.9	30.6	30.8	30.6
Total PUFA	50.8	44.6	45.0	44.8	42.1
n-3/n-6	1.2	0.5	0.5	0.5	0.4
Total lipid(g/100g)	2.0	2.3	2.7	3.1	3.4

SFA = Saturated fatty acids; MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids; FO = fish oil; CO = canola oil.

Table 4. Biological indices of adult Jade Tiger hybrid abalone measured before and after feeding treatment with different levels of FO (n=10)

	Diets					
	1	2	3	4	5	6
Initial weight (g)	39.9±7.6	36.8±6.9	41.1±9.9	40.0±7.1	40.9±6.3	39.6±7.9
Final weight (g)	46.2±11.7	45.7±6.7	50.8±9.9	50.9±7.1	50.8±6.1	47.1±9.9
DGRw (mg/day)	70.1 ± 1.7^{a}	99.2 ± 3.8^{b}	108.1 ± 1.6^{b}	121.2 ± 1.1^{c}	110.0 ± 3.6^{b}	83.2 ± 15.2^{d}
Weight gain (%)	16.8 ± 4.3^{a}	25.3 ± 4.9^{ab}	24.9 ± 6.1^{ab}	27.9 ± 4.4^{b}	24.8 ± 4.6^{ab}	20.6 ± 7.9^{ab}
SGRw(% /day)	0.17 ± 0.02^{a}	0.25 ± 0.04^{ab}	0.25 ± 0.04^{ab}	0.27 ± 0.05^{b}	0.25 ± 0.04^{ab}	0.21 ± 0.03^{ab}
Initial length (mm)	56.4±6.3	53.4±2.6	59.2±6.0	56.1±2.9	56.3±4.7	56.6±2.6
Final length (mm)	63.1±6.2	63.6±2.8	69.7±5.8	67.7±2.8	66.6±3.6	63.4±6.3
DG_{SL} (µm/day)	74.7 ± 7.8^{a}	113.8 ± 6.1^{b}	116.9 ± 9.7^{b}	129.2 ± 10.4^{b}	114.3 ± 8.8^{b}	76.4 ± 6.0^{a}
Shell increase (%)	12.1 ± 1.3^{a}	19.2 ± 1.9^{b}	17.9 ± 1.8^{b}	20.8 ± 2.2^{b}	18.5 ± 1.9^{b}	12.2 ± 1.4^{a}
SGR_S (% /day)	0.13 ± 0.01^{a}	0.20 ± 0.02^{b}	0.18 ± 0.02^{b}	0.21 ± 0.03^{b}	0.19 ± 0.02^{b}	0.13 ± 0.01^{a}

Means in the same row with different superscripts are significantly different ($P < 0.05$). FO = fish oil

Table 5. Biological indices of adult Jade Tiger hybrid abalone measured before and after feeding treatment with different levels of FlaxO (=10)

	Diets				
	Control	25% FlaxO	50% FlaxO	75% FlaxO	FlaxO
Initial weight (g)	37.3 ± 5.4	37.8 ± 4.7	42. 5 ± 4.7	40.0 ± 4.8	43.1± 7.5
Final weight (g)	48.0 ± 6.2	47.8 ± 9.1	52.3 ± 4.3	49.7 ± 4.9	48.8 ±7.8
DGRw (mg/day)	119.0 ±12.3[a]	111.6 ±13.0[a]	108.8 ± 9.0[a]	107.7 ± 6.6[a]	63.3 ± 6.7[b]
Weight gain (%)	29.0 ± 3.4[a]	26.1 ± 2.6[a]	23.5 ±2.2[a]	24.5 ± 3.1[a]	13.5 ± 1.5[b]
SGRw (% /day)	0.28 ± 0.03[a]	0.25 ± 0.02[a]	0.23 ± 0.01[a]	0.24 ± 0.02[a]	0.14 ± 0.01[b]
Initial length(mm)	53.8 ± 3.1	58.4 ± 6.4	57.4 ± 3.1	57.2 ± 2.3	52.6 ± 5.0
Final length (mm)	64.8 ± 2.0	69.2 ± 4.0	67.9 ± 3.0	67.3 ± 1.9	58.8 ± 11.8
DG_{SL} (µm/day)	122.1 ± 20.6[a]	119.7 ± 16.0[a]	116.7 ± 15.4[a]	112.8 ± 8.7[a]	68. 3 ± 5.4[b]
Shell increase (%)	20.6 ± 3.5[a]	19.1 ± 3.0[a]	18.4 ± 2.9[a]	17.8 ± 1.9[a]	12.1 ± 2.0[b]
SGR_S (% /day)	0.21 ± 0.03[a]	0.19 ± 0.02[a]	0.19 ± 0.02[a]	0.18 ± 0.02[b]	0.13 ± 0.01[b]

Means in the same row with different superscripts are significantly different ($P < 0.05$).
FO=fish oil; FlaxO= flaxseed oil.

Table 6. Biological indices of adult Jade Tiger hybrid abalone measured before and after feeding treatment with different levels of CO (n=10)

	Diets				
	Control	25% CO	50% CO	75% CO	100% CO
Initial weight (g)	20.4 ± 1.3	20.2 ± 2.2	19.6 ± 2.4	20.0 ± 2.8	18.5 ± 2.1
Final weight (g)	31.0 ± 1.2	30.6 ± 2.4	29.6 ± 2.8	28.6 ± 2.5	24.2 ± 2.6
DGRw (mg/day)	118.3 ± 6.6[a]	115.1 ± 6.1[a]	111.0± 5.5[a]	95.4 ± 5.1[b]	63.7 ± 5.0[c]
Weight gain (%)	52.5 ± 4.2[a]	51.6 ± 5.3[a]	51.5 ± 5.3[a]	44.0 ± 9.6[a]	31. 2 ± 7.1[b]
SGRw (% /day)	0.47 ± 0.03[a]	0.46 ± 0.04[a]	0.46± 0.04[a]	0.40 ± 0.02[a]	0.30± 0.02[b]
Initial length (mm)	37. 6 ± 2.1	36.9 ± 2.0	36.1 ± 2.1	37.6 ± 1.2	37.2 ± 1.7
Final length (mm)	48.7 ± 2.3	47.6 ± 3.1	46.1 ± 2.0	44.0 ± 1.1	42.2 ± 1.8
DG_{SL} (μm/day)	124.3 ± 4.2[a]	119.1 ± 3.6[a]	111.1± 2.1[a]	71. 1 ± 1.5[b]	55.5 ± 1.3[b]
Shell increase (%)	29.8 ± 1.4[a]	29.1 ± 5.1[a]	27.8 ± 2.0[a]	17.0 ± 1.0[b]	13.5 ± 1.5[b]
SGRs(%/day)	0.29 ± 0.03[a]	0.28 ± 0.02[a]	0.27± 0.02[a]	0.17±0.01[b]	0.14±0.01[b]

Means in the same row with different superscripts are significantly different (*P <0.05*). FO = fish oil; CO = canola oil.

Chapter 9: General conclusions and future research direction

Abalone is a nutritionally important shellfish. Due to overfishing and illegal collection, the number of wild abalone has declined over the past decades and as a result, the abalone aquaculture industry is one of the fastest growing aquaculture industries in the world. In order to ensure abalone farming is a sustainable and beneficial practice, more studies are necessary to gain an overall understanding of feed optimization and fatty acid metabolism in abalone.

This thesis investigated the effects of fish oil and vegetable oil (flaxseed and canola oils) supplementation in the diet of farmed abalone on fatty acid composition, gene expression and growth performance of Jade Tiger hybrid abalone. In order to better understand the lipid and fatty acid profile variation during the year, the seasonal variation of total lipid and fatty acid of muscle, gonad and digestive glands (DG) has also been examined.

The present study showed that the contents of total lipid and fatty acids in muscle, gonad and DG of Jade Tiger hybrid abalone varied seasonally. Higher contents of total lipid were found in summer from muscle and in spring/summer from gonads. Winter samples showed significantly higher content of PUFA in muscle, gonad and DG. High contents of EPA, DPA and DHA were recorded in winter from muscle. Variations of total lipid content are likely to be associated with energy transfers during the reproductive cycle. The seasonal changes in fatty acid content were possibly due to the temperature fluctuations throughout the year.

Furthermore, the present study demonstrated that supplementation with fish oil in the normal commercial diet of abalone can increase the level of LC n-3 PUFA in cultured abalone. Fish oil supplementation at the concentration of 1.5% achieves the best outcome. It appears that the lipid requirements of abalone might be species specific although the overall range is lower in comparison with other animals. In our study the total dietary lipid contents ranged from 0.42 to 2.92% dry wt. The diet supplemented with 1.5% fish oil had 1.92% of total lipid and this probably is the optimal lipid level for Jade Tiger hybrid abalone and thus achieved the best outcome. The study also found that Jade Tiger abalone fed a diet containing 1.5% fish oil supplement obtained similar amounts of EPA, DPA and total n-3 PUFA, and even higher DHA than in wild-caught abalone. In addition, the expression of Δ-6 desaturase and elongase 2 genes in muscle of Jade Tiger abalone could be enhanced with fish oil supplementation. Elongase 2 expression in the muscle of abalone fed with the diet containing 1.5% fish oil supplement coincided with the level of LC n-3 PUFA in the tissue.

Moreover, this study demonstrated that, flaxseed oil is a potential substitute for fish oil in cultured abalone. The replacement of fish oil with flaxseed oil in the commercial abalone diets at the levels of 25 to 75% can maintain the composition (% of total FA) of health-benefiting LC n-3 PUFA without significant reduction in the tissues of cultured abalone, and achieve similar growth performance as fish oil supplementation. However, a 100% flaxseed oil replacement decreased markedly the concentration of EPA and DHA in abalone tissues as well as growth rate. The present study also showed that the expression of both Δ-6 desaturase and elongase 2 genes was higher in abalone fed the flaxseed oil substituted diets compared to the

abalone fed fish oil. As a result of the increased in gene expression, some of the ALA was desaturated and elongated to LC n-3 PUFA. These findings suggest that abalone may have the ability to synthesize highly unsaturated fatty acids in their tissues.

In addition, the results of the present study showed that canola oil can be also used successfully as a substitute for fish oil in the commercial diet for abalone aquaculture although at levels greater than 50% canola oil substitution, reductions occur in muscle EPA, DHA and the n-3/ n-6 PUFA ratio. It was also found that the expression of both elongase Δ-6 desaturase and elongase 2 genes was higher in abalone fed the canola oil substituted diets compared to the abalone fed fish oil.

Overall, this study demonstrated that supplementation with fish oil in the normal commercial diet of abalone can improve growth performance of cultured abalone. Fish oil supplementation at a concentration of 1.5% appears to achieve the best outcome. In addition, the present study indicates that it is feasible to replace 75% of dietary fish oil with flaxseed oil and 50% of dietary fish oil with canola oil respectively, without significantly negative effect on growth performance and survival of cultured abalone.

It is worth noting that all three types of tissue of Jade Tiger hybrid abalone in the present study represent a good source of LC n-3 PUFA. Traditionally only the foot muscle of abalone is used for human consumption. The possibility of manufacturing food products using the gonad and digestive glands of abalone can provide consumers with alternative sources of LC n-3 PUFA and encourage the intake of these health-

benefiting fatty acids.

In general, the results obtained from the present study suggest that consumption of cultured abalone species could possibly provide similar levels of LC n-3 PUFA as the wild species if the diets of cultured abalone were supplemented with certain levels of fish oil. For nutrition claims to be made, a food must contain 30 mg of total EPA + DHA per serving (100 g). Cultured abalone clearly meets this criterion. For a food to be deemed a "good source" of LC n-3 PUFA, the food must contain not less than 60 mg of EPA + DHA per serving. In this study, after the addition of FO, cultured abalone contains 31.2–62.3 mg of EPA and DHA per serving. With these results abalone could be also deemed as a good source of EPA and DHA. In addition due to its high proportion of n-3 PUFA and especially EPA, and being a good source of high level of DPA, abalone could readily be promoted as healthy low fat seafood and that is rich in essential n-3 PUFA. Abalone has got potential to supply a niche market for health conscious consumers. It is expected that the results of this study will provide valuable information for food and aquaculture industries on the improvement of nutritional value of cultured abalone, and boost Australian seafood market. It will also provide useful information to nutrition and consumer groups.

Below are points that should be considered for future work:

- Further investigations are needed on the effects of fish oil and vegetable oils on fatty acid composition over longer periods in both juvenile and adult abalone.

- Future studies on the gene expression in other tissues as well as the analysis of actual enzymatic activities of both Δ6 desaturase and elongase 2 are required to further support the suggestion of LC n-3 PUFA biosynthesis activities within the muscle.

- Future studies on Δ-5 desaturase gene expression are required to further support the suggestion of LC n-3 PUFA biosynthesis within the muscle.

- Further research using other vegetable oils in extended feeding trials is warranted, considering the encouraging results from the present study. Studies are needed to identify other lipid sources, as alternatives to FO, which can provide LC n-3 PUFA without any negative impact on abalone.

Chapter 10: List of references

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