A factorial approach to defining the dietary protein and energy requirements of mulloway, *Argyrosomus japonicus*: optimising feed formulations and feeding strategies.

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The research presented and reported in this thesis was conducted within the guidelines for research ethics outlined in the *National Statement on Ethics Conduct in Research Involving Human* (1999), the *Joint NHMRC/AVCC Statement and Guidelines on Research Practice* (1997), the *James Cook University Policy on Experimentation Ethics*. *Standard Practices and Guidelines* (2001), and the *James Cook University Statement and Guidelines on Research Practice* (2001). The proposed research methodology received clearance from the James Cook University Experimentation Ethics Review Committee (approval number A1102).

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Conferences

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Abstract

The costs associated with feeds and feeding typically constitute the largest proportion of operating expenses in the production of fish in intensive culture. Sub-optimal feeds and inefficient feeding regimes result in direct economic losses through food wastage and sub-optimal growth, deterioration of water quality and increased environmental pressures from excessive waste production. The formulation of cost-effective, nutritionally optimal diets are therefore imperative to maximising profitability and reducing waste output on marine fish farms. Recent interest by industry in New South Wales and South Australia has focused on mulloway culture; however, little information exists on the protein and energy requirements for this species. Prior to the commencement of this research there were no published data on the requirements for digestible protein (DP) and digestible energy (DE) for mulloway and, as a consequence, no specific diet formulations or feeding standards were available.

Mathematical modelling in animal nutrition provides an extremely useful tool in the development of practical feed evaluation systems (i.e. feeding standards and practices) to describe and predict nutrient requirements, body composition and growth of the animal. Factorial bioenergetics is the quantitative study of energy gains, losses and transfers within the whole organism based on thermodynamic principles and has been widely applied to animal nutrition and the development of feed evaluation systems.

The general aim of this thesis was to establish a practical feed evaluation system for mulloway based on the factorial approach. This was achieved by

conducting a series of interrelated studies which determined the requirements for DP and DE for maintenance and growth and described aspects of metabolism relating to the fasting and feeding physiology of this species. The following is a brief overview of these studies.

A comparative study was undertaken to establish the routine metabolic rates (RMR) for similar sized mulloway, a sedentary species, and yellowtail kingfish, a highly active species, acclimated at one of several temperatures ranging from 10 – 35 ^{o}C. RMR increased linearly with increasing temperature (*T*) for both species. RMR for mulloway was 5.78*T* - 29.0 mg O_2 $kg^{-0.8}$ h^{-1} and for yellowtail kingfish was 12.11*T* -39.40 mg O_2 $kg^{-0.8}$ h^{-1}. The energetic cost of routine activity can be described as a function of temperature for mulloway as 1.93*T* – 9.68 kJ $kg^{-0.8}$ day^{-1} and for yellowtail kingfish as 4.04*T* – 13.14 kJ $kg^{-0.8}$ day^{-1}. RMR for mulloway was least thermally dependent at 28.5 ^{o}C and for yellowtail kingfish at 22.8^{o}C. The results of this study have direct implications with regard to the appropriate temperatures at which to culture these species.

Specific dynamic action (SDA) is the energy expended on the physiological processes associated with meal digestion and is strongly influenced by the characteristics of the meal and the body weight (BW) and temperature of the organism. The effects of temperature and body weight on the RMR and SDA response in mulloway were assessed at 3 temperatures (14, 20 or 26 ^{o}C). RMR and SDA were shown to represent significant energetic costs in the overall energy budget of mulloway. Many of the SDA indices measured in this study were within the ranges of those reported for other temperate marine fish; however, these values are not fixed and are highly dependent on temperature, body size and feed intake. The effect of body size on the mass-specific RMR (mg O_2 kg^{-1} h^{-1}) varied significantly depending

on the temperature with a greater relative increase in the mass-specific RMR demonstrated for smaller mulloway with increasing temperature. The gross RMR (mg O_2 $fish^{-1}$ h^{-1}) of mulloway can be described as function of temperature as: $(0.0195T - 0.0454)BW(g)^{0.8}$ and the mass-specific RMR (mg O_2 kg^{-1} h^{-1}) can be described as: $(21.042T - 74.867)BW(g)^{-0.2}$. SDA duration occurred within 41-89 h and was influenced by both temperature and body weight. The average proportion of energy expended over the SDA period (SDA coefficient) ranged from approximately 7 – 13 % of the total DE intake while the proportion of total energy expended on SDA above RMR ranged from approximately 16 to 27 %.

The utilization of DP and DE is dependant on the composition of the diet and the efficiency with which tissue deposition (growth) occurs. A detailed understanding of the relationships between nutrient intake, tissue deposition and body composition is necessary to accurately determine feed requirements. The effects of body weight, temperature and feed intake level on the utilization of DP and DE and the requirements for maintenance in mulloway were investigated. Utilization efficiencies for growth based on linear regression for DP (0.58) and DE (0.60) were found to be independent of fish size, temperature and feed intake level. The partial utilization efficiencies of DE for protein (k_p) and lipid (k_l) deposition, estimated using a factorial multiple regression approach, were 0.49 and 0.75 respectively. Maintenance requirements estimated using linear regression were independent of temperature for DP (0.47g DP $kg^{-0.7}$ day^{-1}) while maintenance requirements for DE increased with increasing temperature (44.2 or 49.6 kJ DE $kg^{-0.8}$ day^{-1} at 20 or 26 ^oC respectively).

The interactive effects of DP and DE on the feed intake, growth and body composition of mulloway were investigated using the dose-response method to identify the optimal DP content and DP:DE ratio for the growth of mulloway. This

was achieved by feeding mulloway diets containing one of four different DP levels (250 - 550 g kg^{-1}) at two DE levels (16 or 21 MJ kg^{-1}). The results indicated that feed intake was not governed solely by energy demands but was also dependant on the DP content of the diet. Protein utilization did not improve with diets containing decreasing protein and increasing lipid content indicating that mulloway have a limited capacity to spare dietary protein. Optimal DP content was found to be 444-491 g kg^{-1} depending on the DE content of the diet and the size of mulloway and is within the range reported for other sciaenid species. The use of formulated diets with 28.6 g DP MJ DE^{-1} will achieve optimal growth and protein deposition for 70 – 275g mulloway.

The final study consolidated the results of the previous experiments to establish a feed evaluation system for mulloway using a factorial approach based on the requirements for DP and DE. Assessments of the growth potential of mulloway and the allometric relationships between body size and protein and energy metabolism and protein and energy whole body composition were combined with data previously established on the utilization efficiencies and maintenance requirements for DP and DE. Factorial modeling of the data allowed estimations of the decreasing requirement of the ratio of DP:DE for mulloway with increasing body size through grow-out production up to 2 kg. Estimations using the factorial method were found to be close to those estimated independently using the dose-response method. From this information theoretical diet formulations and feeding regimes were iteratively derived to match the predicted shifting requirements for DP and DE dependant on body size and the grow-out stage of mulloway.

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List of Abbreviations

ABT Arrhenius breakpoint temperature

BW Body weight

BOD Biochemical oxygen demand

DE Digestible energy

DE_m Maintenance digestible energy

DO Dissolved oxygen

DP Digestible protein

DP:DE Ratio of g DP MJ DE^{-1}

ERE Energy retention efficiency

FCR Food conversion ratio

FE Feeding efficiency

GE Gross energy

GMBW Geometric mean body weight

IBW Initial body weight

k_l Partial energy efficiency for lipid

k_p Partial energy efficiency for protein

LD Lipid deposition

LDO™ Luminescent dissolved oxygen

LE Lipid energy

MBW Metabolic body weight

MO_2 Oxygen consumption

MO_{2rmr-g} Gross RMR

MO_{2rmr-s} Mass-specific RMR

MO_{2scope} Post-prandial metabolic scope

MO_{2sda} Cumulative MO_2 above RMR over the SDA period

MO_{2sda-d} SDA duration

MO_{2sda-p} Peak post-prandial MO_2

$MO_{2sda-pd}$ Time to peak post-prandial MO_2

NFE Nitrogen free extract

NSW New South Wales

OT Oxygen transfer

OTR Oxygen transfer rate

PD Protein deposition

PE Protein energy

PRE Protein retention efficiency

PSFI Port Stephens Fisheries Institute

QLD Queensland

RE Retained energy

RFI Relative feed intake

RMR Routine metabolic rate

SA South Australia

SDA Specific dynamic action

SDA_E SDA energy expenditure

SMR Standard metabolic rate

T Temperature

TE Total energy

Chapter 1

General Introduction

1.1 A brief overview of global aquaculture production

A total of 143.6 million tonnes of fish (marine fish, freshwater fish, molluscs, crustaceans etc.) were produced or harvested by the aquaculture and capture fishery industries in 2006 (FAO 2008). An estimated 110.4 million tonnes of food-fish were consumed, an average of 16.7 kg per capita, with the remaining 33.2 million tonnes used for non-food purposes such as fishmeal and fish oil. Almost half of all food-fish consumed are farm raised; 51.7 million tonnes worth US$78.8 billion (FAO 2008). By far the largest single producer is China with a reported 34.4 million tonnes, or 66.5% of global aquaculture production. Asia (excluding China) and the Pacific (22.8 %), Europe (4.2 %) Latin America and the Caribbean (3.0 %), Africa (1.5 %), North America (1.2 %) and the Near East (0.6 %) account for the remaining major global aquaculture producing regions by quantity (FAO 2008). Of the total global aquaculture production, freshwater fish (54 %) make up the majority by species group followed by molluscs (29 %) crustaceans (9 %), diadromous fish (6 %), marine fish (3 %) and other aquatic animals (1 %) (FAO 2008).

The huge global demand for fish has made aquaculture the fastest growing

animal food production sector in the world with an average annual growth rate of 8.8 % worldwide since 1970 (Tacon et al. 2006; Figure 1.1). Population growth, rising per capita incomes and urbanization are factors fueling the growing global demand for fish (Brugere and Ridler 2004). Since the mid 1980's capture fisheries production has, depending on the source of information, either largely remained static (FAO 2008) or has been steadily declining (Watson and Pauly 2001; Pauly 2008).

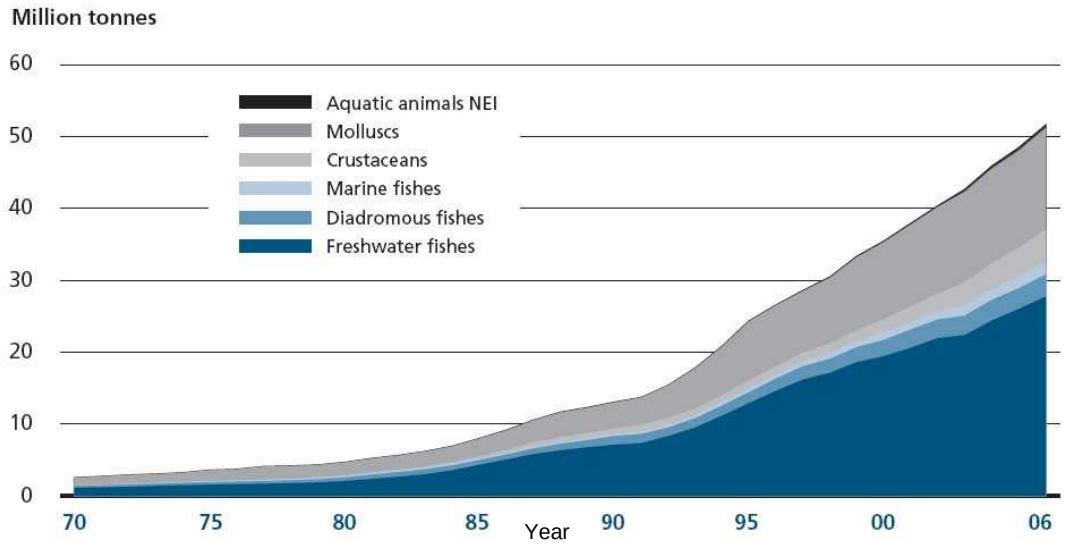

Figure 1.1. Trends in world aquaculture production by major species group. NEI = Not elsewhere included. Source: FAO (2008)

In 2007, 80% of the worlds natural fish stocks were estimated to be fully exploited (52 %), overexploited (19 %), depleted (8 %), or recovering from a period of depletion (1 %) (FAO 2008). This indicates that the maximum potential of capture fisheries from the world's oceans is limited to around 80 – 100 million tonnes which was likely reached in the 1970's or 1980's (Garcia and Grainger 2005; FAO 2008). While capture fisheries have reached their potential, increasing global

demand for fishery products suggest a greater reliance on aquaculture to supply the worlds demand for fish protein into the future (Brugere and Ridler 2004). Assuming capture fisheries production remains constant, and an average per capita consumption of 16.7 kg, it is estimated that an additional 80.5 million tonnes of fish will have to be produced by aquaculture to meet the demand of a world population of 8.3 billion by 2030 (Brugere and Ridler 2004; FAO 2008; UN 2008). If capture fisheries are in decline (Watson and Pauly 2001; Garcia and Grainger 2005) and average annual per capita consumption increases (Brugere and Ridler 2004), both likely scenarios, then the demand on aquaculture production will be even greater.

1.2 Aquaculture in Australia

Aquaculture, as in the rest of the world, is Australia's fastest growing primary industry. Aquaculture production in Australia has grown by an average 6 % p.a. since 1997/98 (Figure 1.2) with production totaling 62,000 tonnes worth approximately AUS$800 (US$645) million in 2006/07 (O'Sullivan and Savage 2009). In a global context this contributes to approximately 0.12 % of the global aquaculture production by quantity or 0.82 % by value. While aquaculture is supplying almost half of the fish consumed in the world (FAO 2008), Australian production in 2006/07, by comparison, accounted for only 1/4 of the total gross Australian fisheries production of 240,000 tonnes (ABARE 2008; O'Sullivan and Savage 2009) indicating the relative infancy of the industry and the current reliance on capture fisheries in Australia.

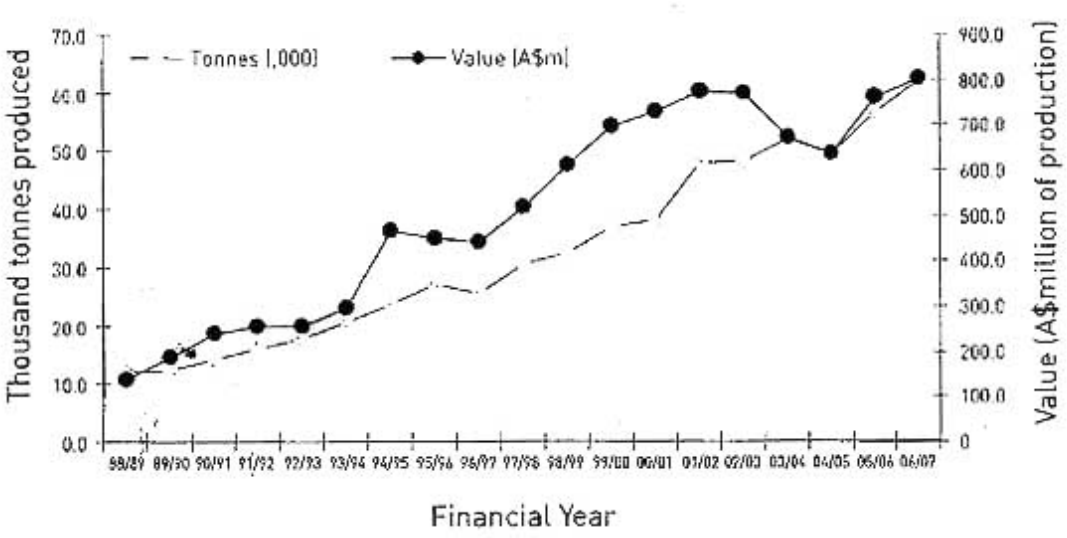

Figure 1.2. Trends in total aquaculture production and value in Australia. Source: O'Sullivan and Savage (2009).

Australia's aquaculture sector expansion took place relatively recently in the 1990's, until then the industry was dominated buy the cultivation of edible and pearl oysters (FAO 1997). The expansion in the 1990's was underpinned by innovation in southern bluefin tuna farming, growth in existing industries (including pearling, edible oyster, prawn and salmonid aquaculture) and significant development in other new industries (such as barramundi, abalone, silver perch, mulloway and yellowtail kingfish) (Gibson et al. 2005; Dundas-Smith and Huggan 2006). Finfish are now the key aquaculture production group in Australia in terms of both volume (62 %) and value (AUD$478.6 million) (Figure 1.3, Table 1.1). Southern bluefin tuna (South Australia), Atlantic salmon (Tasmania) and barramundi (all mainland states and Northern Territory) are the three main commercial finfish species cultured on a large scale (Table 1.1). Between 2002 and 2007, the volume and value of farmed salmonid sea-cage production in the state of Tasmania increased by 10,000 tonnes and AUD$154 million where now farmed salmonids have emerged

as the key production species, surpassing tuna as Australia's most valuable species group (ABARE 2008). Recent expansion of sea-cage production of yellowtail kingfish and mulloway in South Australia has also seen increased growth in that industry sector (ABARE 2008; O'Sullivan and Savage 2009).

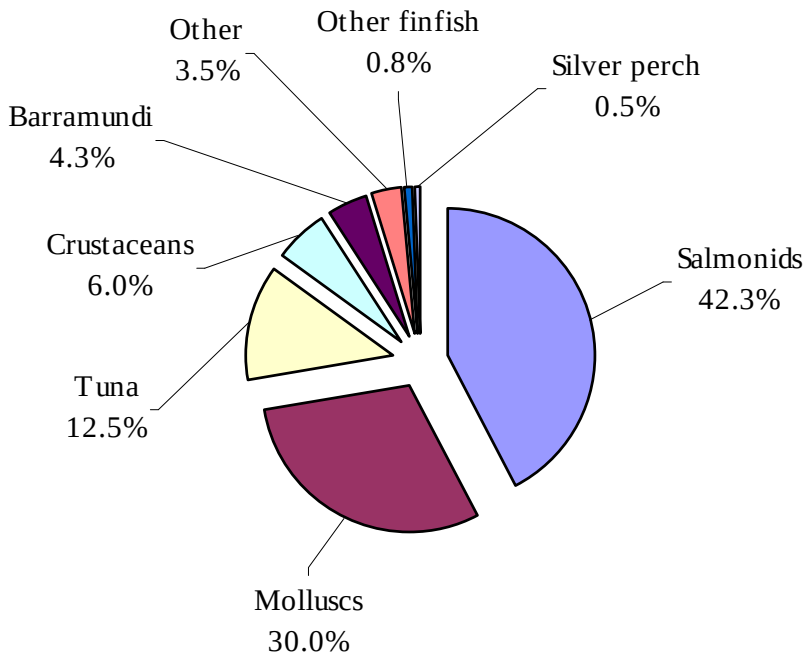

Figure 1.3. Australian aquaculture production by quantity 2006/07. "Other" includes aquaculture production not elsewhere specified due to confidentiality restrictions. In Victoria, this includes abalone, warm water finfish, ornamental fish, other shellfish, shrimps and aquatic worms. Data adapted from ABARE (2008).

Table 1.1. Australian aquaculture production of finfish in 2006/07

Common name	Scientific name	Production (tonnes)	Value (AUD$;000)
Atlantic salmon	*Salmo salar*	22,703.8	$266,629.9
Southern bluefin tuna	*Thunnus maccoyii*	7,588.0	$137,650.0
Barramundi	*Lates calcarifer*	3,037.7	$26,881.0
Rainbow trout (f/w)	*Oncorhynchus mykiss*	1,990.1	$12,951.3
Yellowtail kingfish	*Seriola lalandi*	1,355.0	$11,178.8
Mulloway	***Argyrosomus japonicus***	**607.7**	**$4,877.0**
Rainbow trout (s/w)	*Oncorhynchus mykiss*	500.5	$3,903.3
Silver perch	*Bidyanus bidyanus*	400.0	$4,253.1
Short finned eels[b]	*Anguilla australis*	183.6	$2,743.3
Murray cod[a]	*Maccullochella peelii*	99.2	$1,768.8
Brown trout	*Salmo trutta*	78.0	$438.5
Barcoo grunter	*Scortum barcoo*	51.2	$153.6
Golden perch	*Macquaria ambigua*	7.3	$466.9
Long finned eels[b]	*Anguilla reinhardtii*	5.2	$54.5
Australian bass	*Macquaria novemaculeata*	2.0	$384.1
Sleepy cod	*Oxyeleotris lineolata*	1.0	$24.0
Chinook salmon	*Oncorhynchus tshawytscha*	0.0	$18.0
Eel-tailed catfish	*Plotosus lineatus*	0.0	$16.4
Trout cod	*Maccullochella macquariensis*	0.0	$10.0
Seahorses		0.0	$48.0
Misc. marine[c]		0.0	$5.4
Native aquarium fish		0.0	$936.5
Exotic aquarium fish		0.0	$2,934.5
TOTAL		**38,610.3**	**$478,326.9**

[a]Includes some data for Mary River cod and sleepy cod, no production for Eastern freshwater cod. [b]In QLD and Vic. longfin eels are grouped with shortfin eels. [c]May include sand whiting, tarwhine, snapper, black bream, Australian salmon, Australian herring, silver bream and scad. Data adapted from O'Sullivan and Savage (2009).

Five of Australia's most valuable aquaculture species groups accounted for almost 90 % of the total gross value of aquaculture production in 2006/07. These were salmonids (AUD$283.9 million), pearl oysters (AUD$142.3 million), southern bluefin tuna (AUD$137.6 million), edible oysters (AUD$86.5 million) and prawns (AUD$47.3 million) (ABARE 2008). By comparison with global aquaculture production predominated by the cultivation of freshwater finfish, approximately 95 % of Australia's aquaculture is of marine species (ABARE 2008; FAO 2008).

1.3 Mulloway (*Argyrosomus japonicus*, Sciaenidae) aquaculture in Australia

As indicated above, temperate marine finfish species are at the forefront of the expansion of new commercial aquaculture enterprise in Australia. Production and research was initially focussed on Australian snapper (*Pagrus auratus*), however issues with product quality and growth rates have seen no production since 2002/03 (O'Sullivan and Savage 2009). Mulloway (Figure 1.4) and yellowtail kingfish are now farmed in preference and are regarded as important species which offer economic diversity amongst the tuna and salmonid industries and/or an opportunity to provide species for similar industries in new areas (Gibson et al. 2005).

Figure 1.4. Mulloway, *Argyrosomus japonicus* (Temminck & Schlegel, 1843).

Mulloway have many attributes which make them a suitable candidate for aquaculture in Australia. They are euryhaline (Fielder and Bardsley 1999; Harrison and Whitfield 2006), eurythermal (Harrison and Whitfield 2006) and hypoxia tolerant (Fitzgibbon et al. 2007b). They are a gregarious, fast growing and highly fecund species that are easily reproduced in captivity (Battaglene and Talbot 1994; Fielder et al. 1999). Mulloway are naturally widely distributed around the east, west

and southern seaboards of Australia (Kailola et al. 1993; Silberschneider and Gray 2008) and can be grown successfully in different culture systems including sea cages, ponds and recirculating aquaculture systems (Quartararo 1996; Fielder et al. 1999; O'Sullivan and Ryan 2001; Doroudi et al. 2006).

Aquaculture of mulloway is relatively new in Australia, beginning in the mid 1990's (Gooley et al. 2000) after being successfully produced under hatchery conditions in 1992 (Battaglene and Talbot 1994). Although the mulloway aquaculture industry is in its relative infancy, there has been a steady increase in production in recent years. A number of farms in NSW and SA are now producing small commercial quantities with production of mulloway in 2006/07 of over 600 tonnes for a mainly domestic market valued at approximately AUD$4.9 million (O'Sullivan and Savage 2009; Table 1.1). In a global context, production of sciaenids in 2007 was over 115,000 tonnes valued at over USD$156 million. Over 95 % by volume and 80 % by value was produced by China; although China had the lowest farm-gate price of approximately USD$1.18 / kg. In comparison, meagre from Portugal fetched the highest prices of USD$15.60 / kg while Australian mulloway fetched a median price of USD$6.36 / kg (Table 1.2). In terms of both global production volume and value, Australia's sciaenid production is third behind China and the USA (FAO 2008; O'Sullivan and Savage 2009; Table 1.2).

Table 1.2. Global sciaenid aquaculture production, 2007.

Region	Country	Common name	Scientific name	Production (tonnes)	Value (USD$;000)
Africa	Mauritius	Red drum	*Sciaenops ocellatus*	550	$3,167.0
	Mayotte	Red drum	*Sciaenops ocellatus*	122	$959.8
Americas	Mexico	Freshwater drum	*Aplodinotus grunniens*	70	$147.0
	USA	Red drum	*Sciaenops ocellatus*	1,814	$9,596.1
Asia	China	Large yellow croaker	*Larimichthys croceus*	61,844	$72,975.9
	China	Red drum	*Sciaenops ocellatus*	49,291	$58,163.4
	Israel	Red drum	*Sciaenops ocellatus*	400	$1,380.0
	Saudi Arabia	Croakers, drums nei*	Sciaenidae	5	$25.0
	Taiwan	Croakers, drums nei*	Sciaenidae	23	$173.6
Europe	France	Meagre	*Argyrosomus regius*	282	$2,705.6
	Italy	Meagre	*Argyrosomus regius*	192	$1,789.5
	Portugal	Meagre	*Argyrosomus regius*	25	$389.9
	Spain	Meagre	*Argyrosomus regius*	251	$1,004.0
Oceania	Australia	Mulloway	*Argyrosomus japonicus*	607.7	$3,867.5
			TOTAL	**115,477**	**$156,344.3**

Data adapted from FAO (http://www.fao.org/fishery/statistics/global-aquaculture-production/query/en). Original FAO data excluding mulloway production figures. Mulloway data adapted from O'Sullivan and Savage (2009) and AUD$0.793 (http://www.xe.com/; June 2009). *not elsewhere indicated

1.4 Need for research

The commercial viability of any aquaculture venture hinges on the successful development and integration of brood-stock management, hatchery, nursery and grow-out technologies and a good understanding of the basic nutritional requirements of the species (Gibson et al. 2005). Marine fish farmers and feed manufacturers alike need to implement management decisions based on biological data obtained from rigorous experimental design to ensure economic performance and environmental sustainability. One key area which currently restricts the development of the mulloway industry in Australia is a lack of knowledge of the nutritional requirements of the species. To date there is no published information on the requirements for digestible protein (DP) and digestible energy (DE) for

mulloway and, as a consequence, no specific diet formulations or feeding standards are available.

As a carnivorous species it is expected that mulloway will have a high requirement for DP and this is reflected in the current practice by industry of feeding commercial diets formulated for other carnivorous species such as barramundi, Atlantic salmon or more generic ‘marine fish’ formulations. Growth rates have been reasonable; however, these diets may not be optimal as it is not uncommon for food conversion ratio’s (intake/gain) in excess of 1.5 to be reported. Significant reduction of production costs can be achieved if feed formulations and feeding strategies are optimised. Feed is the primary source of waste output from aquaculture operations with phosphorus and nitrogen the major elements of concern. Excessive waste outputs can significantly impact on the environment which, in turn, has direct socio-economic ramifications and influences negative public perception of the industry (Burbridge et al. 2001; Ridler et al. 2007). Reduction of waste outputs from marine fish culture operations can be achieved by optimising nutrient utilisation through better diet formulation and implementing more efficient feeding strategies (Kaushik 1998; Cho and Bureau 2001).

1.5 Digestibility and utilisation of feeds

The nutritional value of a feed is not solely based on its chemical composition but also on the amount of nutrients and energy that can be absorbed and used; the digestibility being the difference between the amount of nutrient taken in and that excreted as faeces (NRC 1993). Determining the digestibility of feeds and ingredients is important as it indicates the availability of nutrients to the animal.

The daily requirements for maintenance and growth can then be described in terms of DP and DE and diets can be formulated on a digestible rather than gross nutrient basis (NRC 1993; Houlihan et al. 2001; Bureau et al. 2002).

It is important to note that digestibility is not a measure of nutrient utilization but an indication of the potential availability of energy and nutrients through the digestive process (NRC 1993; Bureau et al. 2002). The utilization of DP and DE is dependant on the composition of the diet and the efficiency with which tissue deposition (growth) occurs (van Milgen and Noblet 2003; Schroeder and Titgemeyer 2008). Modern commercial aquaculture farming practices demand the efficient conversion of feeds into the production of body tissue (usually protein deposition) to maximise economic returns and environmental sustainability. However, to achieve this, a detailed understanding of the relationships between nutrient intake, tissue deposition and body composition is needed. Patterns of protein deposition with increasing levels of DP intake can vary considerably between species, diet and environmental conditions. Responses in fish can be linear (Lupatsch et al. 2001a; Fournier et al. 2002; Lupatsch and Kissil 2005; Peres and Oliva-Teles 2005) or curvilinear (Huisman et al. 1979; McGoogan and Gatlin 1998; Watanabe et al. 2000b; Bureau et al. 2006) indicating that utilization efficiencies are either constant or tend to plateau with increasing protein intake. Understanding how growth is affected by the nutrient intake level is important in optimizing feeding strategies for aquaculture species. A curvilinear response indicates that restrictive feeding will optimise feeding efficiencies while supporting rapid growth, conversely a linear response indicates that satiated feeding is required to achieve maximum growth and feeding efficiencies. As the costs of aquafeeds represent the greatest proportion of costs in fish production, getting the feeding strategy wrong can result

in significant loss of revenue through either excessive feed wastage and/or undesirable body conditioning, or delayed production cycle turnover.

1.6 Bioenergetic approach to practical feed evaluation systems

Nutrient requirements in fish have traditionally been determined empirically using a dose-response approach, typically with weight gain or nutrient retention expressed as the response criteria and the relationship analysed using linear or non-linear regression. Evaluating diets by testing all combinations of nutrient inclusion levels against various response criteria and under various culture conditions will undoubtedly yield the most accurate definitions; however, this approach is neither cost effective nor practical to implement. Mathematical modelling in animal nutrition provides an extremely useful tool in the development of practical feed evaluation systems (i.e. feeding standards and practices) to describe and predict nutrient requirements, body composition and growth of the animal (Cho 1992; Dijkstra et al. 2007). Bioenergetics is the quantitative study of energy gains, losses and transfers within the whole organism based on thermodynamic principles (Jobling 1994; Haynie 2001; Bureau et al. 2002), and has been widely applied to animal nutrition and the development of feed evaluation systems over the past several decades (Brody 1945; Kleiber 1961; Cho et al. 1982; Bureau et al. 2002; Dumas et al. 2008).

Traditional bioenergetic systems are factorial; i.e. total energy requirements are calculated as the sum of energy required for maintenance, activity, growth, reproduction etc. (Baldwin and Sainz 1995). The partitioning and quantification of dietary energy is important in the study of nutritional energetics because it provides

a convenient platform to predict the energy balance of individuals based on body weight, sex, activity, physiological state, environment, and amount and nutritive value of the feed eaten (Baldwin and Bywater 1984). This information can then form the basis for practical diet formulation and evaluation (Baldwin and Bywater 1984; Bureau et al. 2002). It is important to recognise that the factorial method is empirical in form; models based on the digestion, metabolism and utilisation of nutrients need to be considered in the context of relevant culture conditions to accurately predict growth and feed requirements. Validation against independent feeding trials will determine the predictive accuracy of the models and assess the need for adjustment of the input data defining the model parameters.

It is recognised that the bioenergetic approach has its limitations; most notably the presumption of additivity of functions (factors) without interaction (Baldwin and Sainz 1995) and the fact that animals continue to deposit protein while losing lipids when fed maintenance levels of DE (Bureau et al. 2002; van Milgen and Noblet 2003; Sandberg et al. 2005a). There are indications that some bioenergetic models have not been well evaluated over the ranges of conditions to which they have been applied (Bajer et al. 2004), although this seems to indicate issues with the application of the models rather than the principles and fundamental concepts of bioenergetic theory. Bioenergetic models can therefore be regarded as relatively inflexible in their adaptability (Bureau et al. 2002) which is, in part, an artefact of the empirically derived nature of the sub-models. The adequacy of some feed evaluation systems has also been questioned as they are devised to meet animal requirements rather than predict animal response, which has seen a shift (back) towards nutrient-based mechanistic models to meet modern animal production demands (Dijkstra et al. 2007; Dumas et al. 2008). However, some mechanistic

models, while being theoretically correct, may be considered too complex for implementation in practical feed evaluation systems (Bureau et al. 2002).

In spite of the limitations noted above, the factorial approach remains a very useful and practical method in constructing feed evaluation systems. Several models have been successfully developed to predict growth, feed requirements and feed efficiencies in a number of fish species using these principles (Cho and Bureau 1998; Lupatsch et al. 1998; Lupatsch et al. 2001a; Lupatsch and Kissil 2005; Zhou et al. 2005; Glencross 2008). Factorial models based on bioenergetic principles which also integrate a nutrient-based approach have the greatest flexibility and can be adapted to formulate feeds based on specific nutrient requirements (e.g. Lupatsch et al. 1998) or predict waste outputs of inorganic compounds (e.g. Hua et al. 2008). Furthermore, these types of "hybrid" models (sensu Dumas et al. 2008) can provide greater and more relevant application in the context of commercial production when calibrated using on-farm data (e.g. Bureau et al. 2003; Lupatsch et al. 2003a; Glencross 2008).

1.7 This thesis

The work presented in this thesis forms part of the New South Wales Department of Primary Industries (NSW DPI), Port Stephens Fisheries Institute current research program "*Feed Technology for Temperate Marine Fish Species*" FRDC Project No. 2004/220, funded by the Fisheries Research and Development Corporation (FRDC) and the Aquafin Cooperative Research Centre (Aquafin CRC). Chapters 2, 3, 4, 5 and Appendix 1 are unabridged versions of the manuscripts

published in international peer-reviewed journals which have been re-formatted for this thesis.

The general aim of this thesis was to establish a practical feed evaluation system for mulloway based on the factorial approach. This was achieved by conducting a series of interrelated studies which determined the requirements for DP and DE for maintenance and growth and described aspects of metabolism relating to the fasting and feeding physiology of this species.

Mulloway have a strong shoaling instinct, particularly as juveniles, and are easily startled in culture systems. Therefore, a pilot study (**Appendix 1**) was initially carried out to identify appropriate stocking densities which would assist in mitigating the potential for adverse density dependent behavioural effects in the subsequent feeding and metabolic studies. **Chapter 2** investigated the fasting routine metabolic rates (RMR) of mulloway and yellowtail kingfish and provided the opportunity to compare the metabolic responses of a sedentary (mulloway) and a highly active species (yellowtail kingfish) over a broad range of temperatures. The results of this experiment also provided insight into the appropriate temperature at which to culture these species. **Chapter 3** builds on the results established in Chapter 2 by also testing the effect of body weight on the RMR and postprandial metabolic response in mulloway over a range of temperatures. **Chapter 4** further investigates the feeding physiology of mulloway by describing responses of body gain and composition to varying feed intake levels. DP and DE utilization efficiencies and the requirements for maintenance were established based on the patterns of protein and energy deposition as a function of DP and DE intake respectively. The interactive effects of DP and DE on the feed intake, growth and body composition of mulloway were investigated in **Chapter 5** using a classic dose-

response approach. This study identified the optimal DP content and DP:DE ratio for mulloway. The results in this study also served to validate estimations made using the factorial method in the following chapter. **Chapter 6** consolidates the results of the previous experiment chapters to establish a feed evaluation system for mulloway based on the requirements for DP and DE. A factorial approach was used to estimate the requirements for DP and DE for mulloway throughout the production cycle and diet formulations and feeding regimes were then iteratively derived. The overall results from this thesis are discussed and the main conclusions are presented in **Chapter 7.** Chapter 7 also presents a sensitivity analysis of the individual parameters used to populate the mathematical sub-models which form the framework of the factorial model.

The specific objectives of this thesis were to:

1. identify the effects of stocking density on the growth of mulloway
2. describe and compare the RMR of mulloway and yellowtail kingfish as a function of temperature
3. describe the influence of body mass and temperature on the RMR and specific dynamic action (SDA) of mulloway
4. determine i) the protein and energy utilization responses in mulloway to increasing DE and DP intake, ii) the efficiencies of DP and DE utilization, and iii) the DP and DE maintenance requirements of mulloway
5. i) describe the interactive effects of varying DP and DE content on feed intake, growth, protein utilization and whole body composition of mulloway, ii) determine the optimal DP content for mulloway and iii) to determine the optimal DP:DE ratio for growth.

6. describe the requirements for DP and DE for mulloway throughout the production cycle using the factorial method and to derive diet formulations and feeding regimes based on the requirements for protein and energy.

Chapter 2

The Routine Metabolic Rate of Mulloway and Yellowtail Kingfish Acclimated to Six Different Temperatures[1]

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[1]The following chapter is published as:

Pirozzi, I., Booth, M.A., 2009. The routine metabolic rate of mulloway (*Argyrosomus japonicus*: Sciaenidae) and yellowtail kingfish (*Seriola lalandi*: Carangidae) acclimated to six different temperatures. Comp. Biochem. Physiol. A-Mol. Integr. Physiol. 152, 586-592.

2.1 Abstract

This study compared the mass-specific routine metabolic rate (RMR) of similar sized mulloway (*Argyrosomus japonicus*), a sedentary species, and yellowtail kingfish (*Seriola lalandi*), a highly active species, acclimated at one of several temperatures ranging from 10 – 35 ^{o}C. Respirometry was carried out in an open-top static system and RMR corrected for seawater-atmosphere O_2 exchange using mass-balance equations. For both species RMR increased linearly with increasing temperature (T). RMR for mulloway was 5.78T - 29.0 mg O_2 $kg^{-0.8}$ h^{-1} and for yellowtail kingfish was 12.11T -39.40 mg O_2 $kg^{-0.8}$ h^{-1}. The factorial difference in RMR between mulloway and yellowtail kingfish ranged from 2.8 to 2.2 depending on temperature. The energetic cost of routine activity can be described as a function of temperature for mulloway as 1.93T – 9.68 kJ $kg^{-0.8}$ day^{-1} and for yellowtail kingfish as 4.04T – 13.14 kJ $kg^{-0.8}$ day^{-1}. Over the full range of temperatures tested Q_{10} values were approximately 2 for both species while Q_{10} responses at each temperature increment varied considerably with mulloway and yellowtail kingfish displaying thermosensitivities indicative of each species respective niche habitat.

RMR for mulloway was least thermally dependent at 28.5 ^{o}C and for yellowtail kingfish at 22.8^{o}C. Activation energies (E_a) calculated from Arrhenius plots were not significantly different between mulloway (47.6 kJ mol^{-1}) and yellowtail kingfish (44.1 kJ mol^{-1}).

2.2 Introduction

Mulloway (*Argyrosomus japonicus*) and yellowtail kingfish (*Seriola lalandi*) are marine carnivores that are important food species and highly sort after sport fish. They are both important aquaculture species in Australia and are cultured in sea cage or on-shore recirculation systems. While both species sometimes naturally co-occur each occupy distinct niche habitats. Mulloway are found in estuarine, near-shore waters and surf zones (Griffiths 1996; Griffiths 1997; Silberschneider and Gray 2008). Yellowtail kingfish are a schooling pelagic species with a circumglobal distribution and are found in both near-shore and off-shore waters (Kailola et al. 1993). In Australia both species are similarly distributed around the eastern and southern seaboards although yellowtail kingfish are also found in cooler temperate waters of the Bass Strait and Tasmania while mulloway are also found in the warmer temperate waters to the North West Cape of Western Australian (Kailola et al. 1993). Both species possess distinct physiological and morphological characteristics adapted to exploit their respective niche environments. Mulloway are a relatively sedentary species (Silberschneider and Gray 2008) that are euryhaline (Fielder and Bardsley 1999; Harrison and Whitfield 2006), eurythermal (Harrison and Whitfield 2006), hypoxia tolerant (Fitzgibbon et al. 2007b) and have a low aerobic capacity similar to rainbow trout and Atlantic cod

(Bushnell et al. 1984; Schurmann and Steffensen 1997; Fitzgibbon et al. 2007b). In contrast, yellowtail kingfish are a high-performance high-energy-demand species which share many of the specialized morphological adaptations of the tunas (Dewar and Graham 1994; Clark and Seymour 2006) and have an aerobic metabolic scope similar to that of other highly active teleost species such as salmon (Clark and Seymour 2006).

Metabolism reflects the uptake, transformation and allocation of energy and materials for maintenance, growth and reproduction; the rate at which this occurs determines the pace of an organism's life (Brown et al. 2004). Metabolic theory links metabolic rate on a broad scale to the ecology of populations and ecosystem processes (Brown et al. 2004; van der Meer 2006) while at the organismal level life history traits such as development time (Finn et al. 2002; Gillooly et al. 2002), mortality rate (Brown et al. 2004) and reproductive rate (Savage et al. 2004a) can also be predicted (Brown et al. 2004). An individual's metabolic rate is predominately a function of its mass (Schmidt-Nielsen 1975; West et al. 2002) but will also vary considerably depending on its activity level (Boisclair and Tang 1993), health (Barton 1997) and nutritional status (Jobling 1982). Temperature is one of the most important extrinsic factors influencing metabolic rate in ectotherms, directly governing the speed at which biochemical and physiological processes proceed (Clarke and Johnston 1999; Hochachka and Somero 2002) as well as having a direct influence on activity (Jobling 1982). The relationship between temperature and metabolic rate is strongly linked to the temperature dependence of enzymatic reactions (Hochachka and Somero 2002).

Respiration rate, measured in terms of oxygen consumption (MO_2), is an accurate proxy for metabolic rate (Withers 1992). Deriving an organism's metabolic

rate in this way is useful as it firstly provides a direct measure of the animals requirement for oxygen, information which is critical for the culture of any aquatic species and secondly, it allows indirect estimations of the requirements for energy (Elliott and Davison 1975). Metabolic rates of mulloway and yellowtail kingfish have been measured previously; however only at a single temperature for mulloway (22 °C; Fitzgibbon et al. 2007b) and over a small temperature range for yellowtail kingfish (20-25 °C; Clark and Seymour 2006). Those studies showed that the standard metabolic rate (SMR) of mulloway and yellowtail kingfish, scaled for body weight and temperature, were similar (Clark and Seymour 2006; Fitzgibbon et al. 2007b).

In this study, we examine the influence of a wide range of temperatures on the routine metabolic rate (RMR) of mulloway and yellowtail kingfish where RMR reflects the MO_2 during routine activity and spontaneous movement of unfed, but not starving fish (Fry 1957; Beamish 1964). The overall objectives were twofold; firstly to validate an open-top respirometry system and secondly, to describe the RMR of mulloway and yellowtail kingfish as a function of temperature. As biogeographic patterns of distribution and abundance are closely linked to the temperature-adaptive physiology of ectotherms (Hochachka and Somero 2002; Somero 2005), we hypothesize that the RMR of mulloway and yellowtail kingfish will closely reflect those characteristics noted above; i.e. relative to yellowtail kingfish, mulloway will 1) have a lower RMR 2) show a reduced thermosensitivity response to different acclimation temperatures and 3) exhibit a higher temperature at which RMR is least thermally dependant.

2.3 Materials and methods

2.3.1 Respirometry validation

MO_2 readings used to calculate the metabolic rates of mulloway and yellowtail kingfish in this study were measured in an open-top system; i.e. the surface water was exposed to the atmosphere. Therefore, O_2 transfer coefficients at each temperature treatment were established in a separate trial to account for atmospheric transfer of O_2 into seawater at sea level (1013 hPa). This was achieved by depleting DO levels down to 60% saturation using nitrogen gas and measuring the rate of increase to resaturation. Duplicate 200 l experiment tanks (dimensions: top diameter = 78 cm; bottom diameter = 68 cm; height = 55 cm) were placed in the sumps (fiberglass tanks 2.7 x 1.2 x 0.6 m) of each recirculation system (described below) which acted as water baths maintaining constant temperatures. Inside tank surfaces were scrubbed down and disinfected with sodium hypochlorite (NaOCl) before the start of each temperature reaeration trial, rinsed and refilled with seawater which had also been treated with NaOCl and neutralized with sodium thiosulphate ($Na_2S_2O_3$). A small submersible aquarium pump (flow rate approximately 5 l min^{-1}) was placed at the bottom of each 200 l tank and positioned to create a small turbulent flow to mimic fish movement in the tanks; initial DO readings taken at the near-surface, middle and bottom of tanks were virtually identical indicating complete mixing. DO Readings for each temperature trial were taken at intervals of approximately 0, 1, 6, 18 and then every 24 h up to10 d or until resaturation was achieved.

2.3.2 RMR experiment design and fish handling

The mass-specific routine metabolic rate (RMR; mg O_2 $kg^{-0.8}$ h^{-1}) was established at 6 temperatures for mulloway (10, 15, 20, 25, 30 or 35 ^oC) and yellowtail kingfish (10, 15, 20, 25, 30 or 32.5 ^oC). Fish were F1 juveniles of wild caught broodstock held at the New South Wales Department of Primary Industries, Port Stephens Fisheries Institute (PSFI).

Eight mulloway (181.8±4.4 g; mean±SD) or 5 yellowtail kingfish (206.0±7.0 g) were stocked in triplicate groups into 200 l tanks for each of the 6 temperature treatments with each tank constituting an experimental unit. The recirculation system supplying the tanks was split into two independent banks with one bank designated as cool water (10 – 20 ^oC) while the other designated as warm water (25 – 35 ^oC). Fish were initially stocked at ambient water temperature (23 ^oC) then, depending on which system they were assigned, adjusted up or down to specific temperature treatments in increments of 1^oC day^{-1}, which is in excess of the 1^oC h^{-1} required for complete temperature acclimation in ectotherms (Mora and Maya 2006) . Fish were held for one week at that temperature before MO_2 readings were taken using a Luminescent Dissolved Oxygen (LDO™) meter (model HQ30d-LDO101-03; Hach Company, Loveland, CO, USA) which was calibrated at each temperature treatment according to manufacturers instructions. The temperature for each unit was controlled with a chiller and immersion heater operating in an antagonistic mode which allowed precise temperature control of ±0.5 ^oC of the set temperature. Constant water flow (360 l h^{-1}) and air was supplied to all tanks when MO_2 was not being recorded. 100% medical grade O_2 was injected into the main water supply manifold for the high temperature treatments (30 and 35 ^oC). After fish were initially stocked PVC tubes 300 mm long and 32 mm diameter perforated with

10 mm holes were positioned vertically down from the centre surface of each tank to act as sleeves through which the LDO probe could be introduced discretely into tanks without disturbing the fish. Black plastic sheets were also placed across the front top half of tanks to prevent disturbance to fish from the presence of workers taking MO_2 readings.

A power failure occurred over night during the acclimatization period from 33 to 34 ^{o}C resulting in the loss of 37.5 % of the mulloway and 100% of the yellowtail kingfish from the warm water system. Mortalities were likely due to low DO levels. There was 100 % survival of both mulloway and yellowtail kingfish in the corresponding cool water system (12 to 11 ^{o}C). Both species were restocked for the warm water treatment group at ambient temperatures (26 ^{o}C) and re-acclimated following the above protocols. No further mortalities occurred. Some yellowtail kingfish were previously observed to regurgitate food at 33 ^{o}C; therefore it was decided upon restocking to end MO_2 readings at 32.5 ^{o}C for that species.

Water quality parameters were consistent between systems and pH (7.93 – 8.16), NH_4^+ (<0.1 mg l^{-1}) and salinity (33.4 – 35.0 ppt) were monitored regularly. All fish were fed a maintenance ration once daily of a commercial diet (Ridley AquaFeed Pty. Ltd., Narangba, Qld. Australia; 45.5 % crude protein, 18.7 % crude fat, 22.2 MJ kg^{-1} gross energy). All fish were fasted for 48 h prior to MO_2 readings to ensure MO_2 readings were not confounded due to post-prandial effects (refer to Chapter 3).

2.3.3 MO_2

MO_2 was established by measuring the decrease of DO in standing water

over approximately 1 hourly intervals. Measurements were repeated three times over several hours for each replicate tank and the mean used to calculate RMR. For all MO_2 trials DO levels were at or near saturation at start readings and always remained above 60 % after 1 h. After each end reading water flow was re-established for approximately 1 h to flush tanks of metabolites and return DO to saturated levels. Each temperature treatment group was independent and fish were removed from the system and re-weighed after completion of MO_2 readings for that particular temperature and species group. Background biochemical oxygen demand (BOD) was then determined for each replicate tank after fish were removed and water re-saturated with O_2.

2.3.4 Data analyses

2.3.4.1 Atmosphere-seawater oxygen transfer

Predicted rates of reaeration were derived by first establishing the relationship between O_2 saturation (%) and O_2 concentration (mg l^{-1}) at temperature (T) according to the linear relationship of the coefficients when x and y-intercepts = 0 (r^2=0.998) (Figure A2.1). Equivalent oxygen concentrations (mg l^{-1}) could then be established for Top (100 %) and Bottom (60 %) saturation levels at any temperature (T) (Figure A2.2). O_2 transfer (OT; mg l^{-1}) (Figure A2.3) and O_2 transfer rates (OTR; mg l^{-1} h^{-1}) (Figure A2.4), applicable to the system and conditions used in the current study, can be described by the exponential associations:

$$\mathrm{OT} = \mathrm{Bottom} + (\mathrm{Top} - \mathrm{Bottom}) \times (1 - \exp(-k \times t)) \qquad (2.1)$$

Where Bottom = O_2 concentration (mg l^{-1}) at 60% saturation at temperature *T*; Top = O_2 concentration (mg l^{-1}) at 100 % saturation at temperature *T*; *k* = rate constant; *t* = time (h)

$$\text{OTR} = (\text{Top} - \text{Bottom}) \times \exp(-k \times t)) + \text{Bottom} \quad (2.2)$$

Where Top = max. OTR (mg l^{-1} h^{-1}) at temperature *T* when *t* = 0; Bottom = 0 (saturated; fixed); *k* = rate constant; *t* = time (h)

Rate constants were described as a function of temperature according to the linear relationship of *k* (*y*-axis) and *T* (*x*-axis) for OT (when *x* and *y*-intercepts = 0; $r^2 = 0.98$) and also for OTR (*y*-intercept $\neq 0$; $r^2 = 0.78$). By solving for *t* in equation 1 at a known OT value (geometric mean of beginning and end reading) the OTR could then be derived at any point of the O_2 gradient between 60-100% saturation and for any temperature between 10-35^{o}C.

2.3.4.2 Metabolic indices

References to temperature (*T*) are in ^{o}C unless otherwise stated where temperature is absolute temperature in ^{o}K. Mass-specific data are scaled using the metabolic body weight exponent of 0.8 (Brett and Groves 1979). Once OTR and background BOD were established the mass-specific RMR for each species at each temperature could then be calculated as:

$$\text{RMR (mg kg}^{-0.8}\text{ h}^{-1}) = (\text{V} / \text{BW} / \text{n}) \times (\Delta \text{O}_2 - \text{O}_{2\text{otr}} + \text{O}_{2\text{bod}}) \quad (2.3)$$

Where V = tank water volume (l); BW = mean body weight ($kg^{-0.8}$); n = number of fish $tank^{-1}$; ΔO_2 = net change in O_2 concentration (mg l^{-1} h^{-1}) inclusive of fish respiration, atmospheric re-aeration and background BOD; O_{2otr} = atmospheric OTR (mg l^{-1} h^{-1}); O_{2bod} = background BOD rate (mg l^{-1} h^{-1})

Interspecific differences in the relationship between RMR and temperature were analyzed using linear regression. ANCOVA was used to compare slopes and elevations (Motulsky and Christopoulos 2003). Factorial difference was calculated as $RMR_{yellowtail\ kingfish}$ / $RMR_{mulloway}$. All results were regarded as significant at $p<0.05$. Data are presented as mean ± standard error.

Kinetic function was indexed by the effects of temperature on the apparent activation energy (E_a) of each species with E_a determined directly from the slope of Arrhenius plots using the equation (Kotz and Treichel 1996):

$$E_a = -slope \times R \qquad (2.4)$$

Where slope = $\Delta \ln RMR / \Delta(1/K)$, K is absolute temperature (^oK) and R is the universal gas constant (8.3145 x 10^{-3} J mol^{-1} K^{-1}). Slope discontinuities in Arrhenius plots can indicate perturbations in the underlying rate process and are identified at the Arrhenius breakpoint temperature (ABT) (Hochachka and Somero 2002). ABT's were not detected for either species i.e. respiration rates did not fall at high temperatures, however the temperature sensitivity of RMR was described by applying nonlinear regression to temperature quotient (Q_{10}) values plotted as a function of the geometric mean temperature (^oC) with the asymptote describing the

temperature which has the minimum influence on RMR with respect to a 10^{o}C shift in temperature. Q_{10} values were established at each temperature interval using the equation:

$$Q_{10} = (K_2 / K_1)^{10/(T_2 - T_1)} \quad (2.5)$$

Where K_1 and K_2 are the RMR values at temperatures T_1 and T_2 respectively.

Table 2.1. Parameter values used to populate Eqns. (2.1) and (2.2) describing the re-aeration rates of seawater as a function of temperature (10-35 ^{o}C) applicable to the system and conditions used in this study.

Parameter	**OT (mg l^{-1})**	**OTR (mg l^{-1} h^{-1})**
k	$0.0014T$	$0.0009T + 0.0098$
Top	$8.2377 \times \exp(-0.0371T) + 3.6963$	$\mathbf{0.0036}\boldsymbol{T}$
Bottom	$4.9389 \times \exp(-0.0369T) + 2.2106$	**0**

2.4 Results

2.4.1 Respirometer validation

Table 2.1 describes the theoretical parameters applicable to the system and conditions used in the current study to estimate reaeration rates in Eq. (2.1) and (2.2).

Background BOD rates at low temperatures (10 and 15 ^{o}C) were typically beyond the resolution limits of the LDO probe to detect a change (i.e. <0.01 mg l^{-1}) and were therefore assumed to equal that of the theoretical OTR applicable to that temperature. Background BOD generally increased with increasing temperature with BOD in yellowtail kingfish tanks tending to be higher in warmer water than

mulloway tanks (Figure 2.1). BOD slopes between species were significantly different (p<0.05). Average (OTR adjusted) background BOD ranges were approximately 0.005-0.030 (mgO_2 l^{-1} h^{-1}) depending on temperature and species tank.

There was no significant differences found between the RMR of uncorrected data when compared to values that were corrected for OTR and background BOD (p>0.5; Figure 2.2). The proportion of ΔO_2 attributed to fish respiration far exceed that due to OTR and background BOD; ranging from the lowest at 97.1±0.1 % for mulloway tanks at 35 ^{o}C to 99.9±0.1 % for yellowtail kingfish tanks at 10 ^{o}C.

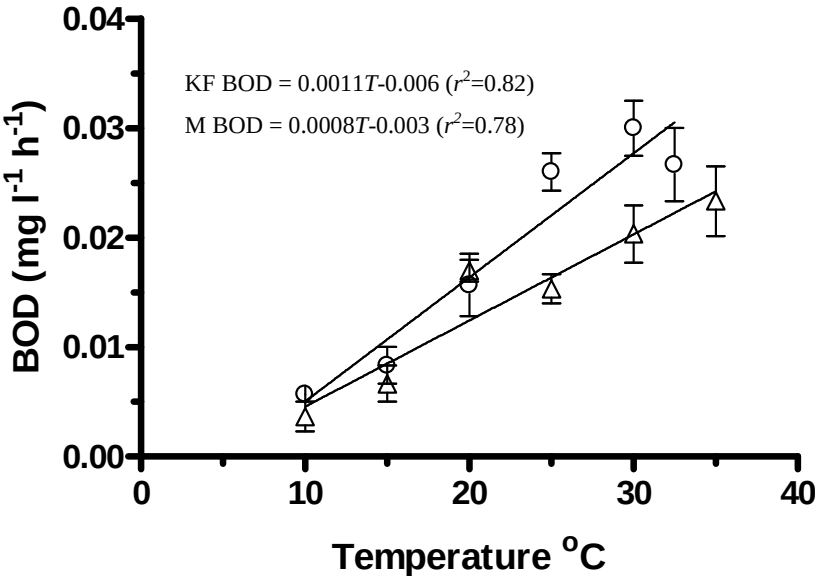

Figure 2.1. Background BOD (mg l^{-1} h^{-1}) in mulloway (M; triangle) and yellowtail kingfish (KF; circle) tanks adjusted for OTR (mean±se; n=3).

2.4.2 Metabolism

The following results are reported as corrected data. RMR of both mulloway and yellowtail kingfish was shown to increase significantly with temperature (Figure 2.2). RMR was significantly different (p<0001) between species at each

temperature. RMR for mulloway ranged from 33.0±0.6 mg O_2 $kg^{-0.8}$ h^{-1} at 10^{o}C to 180.2±11.7 mg O_2 $kg^{-0.8}$ h^{-1} at 35 ^{o}C while the RMR of yellowtail kingfish ranged from 85.3±4.5 mg O_2 $kg^{-0.8}$ h^{-1} at 10 ^{o}C to 382.3±8.9 mg O_2 $kg^{-0.8}$ h^{-1} at 32.5 ^{o}C. The relationship between temperature and RMR was linear for both species (Figure 2.2) and can be described as:

$RMR_{mulloway} = 5.783T - 29.004$ ($r^2 = 0.97$) (2.6)

$RMR_{yellowtail\ kingfish} = 12.113T - 39.402$ ($r^2 = 0.95$) (2.7)

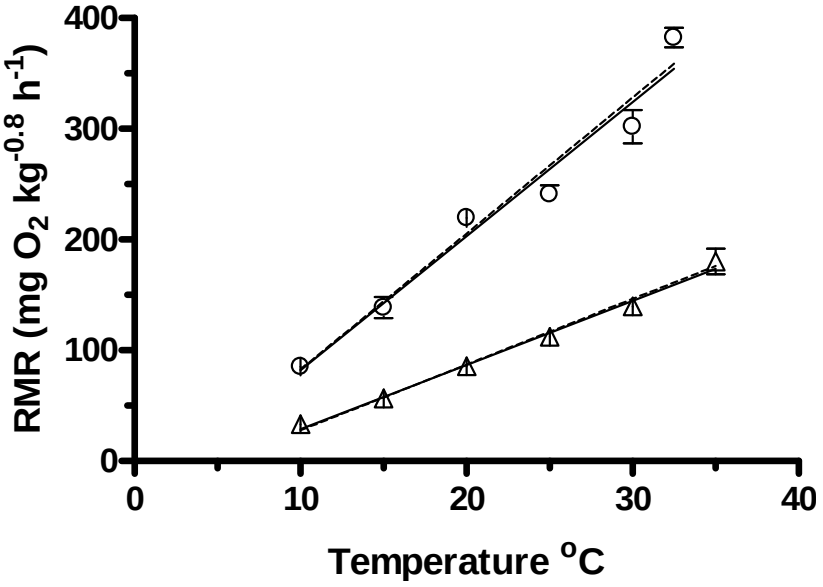

Figure 2.2. Linear relationship between temperature and the mass-specific RMR of mulloway (triangle) and yellowtail kingfish (circle). Data points and solid regression lines represent corrected data (mean±se; n = 3). Dashed regression lines representing uncorrected data also shown for comparison.

The factorial difference between RMR of mulloway and yellowtail kingfish derived from Eq. (2.6) and (2.7) was not constant but decreased exponentially with increasing temperature:

RMR factorial difference = $3.469 \times \exp(-0.174T) + 2.222$ ($r^2 = 0.99$) (2.8)

There was no significant difference between the slopes of Arrhenius plots for mulloway and yellowtail kingfish (p>0.25; Figure 2.3). The Arrhenius relationship can be described as:

$$\ln RMR_{mulloway} = -5.729(1/Kx10^3) + 23.876 \quad (r^2 = 0.97) \qquad (2.9)$$

$$\ln RMR_{yellowtail\ kingfish} = -5.320(1/Kx10^3) + 23.360 \quad (r^2 = 0.95) \qquad (2.10)$$

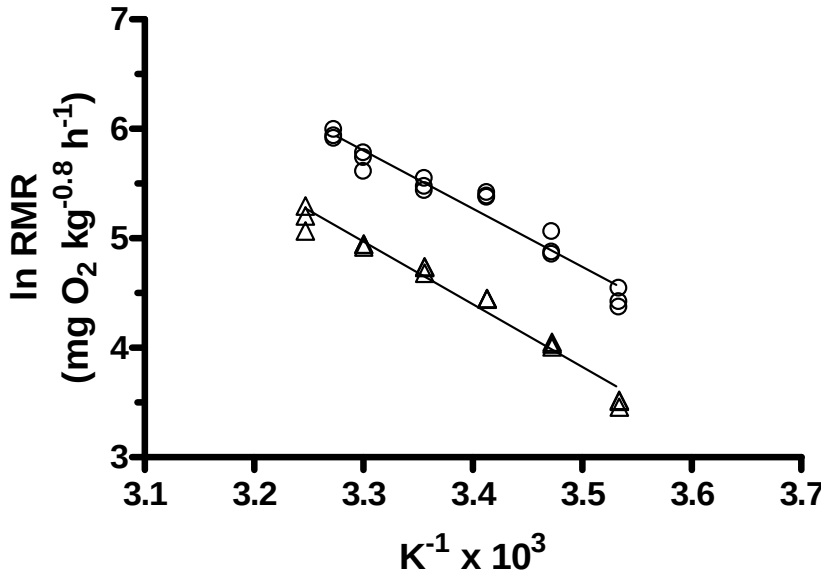

Figure 2.3. Arrhenius plot of for mulloway (triangle) and yellowtail kingfish (circle) where K = absolute temperature.

E_a can be calculated as 47.6(±2.1) and 44.1(±2.8) kJ mol^{-1} for mulloway and yellowtail kingfish respectively.

The lowest Q_{10} for mulloway (Q_{10} = 1.5) occurred between 25-30 ^{o}C and for yellowtail kingfish (Q_{10} = 1.2) between 20-25 ^{o}C (Figure 2.4). Q_{10} values were similar for both species over the entire temperature range ($Q_{10\ (10\ –\ 35)}$ = 2.0 for mulloway and $Q_{10\ (10\ –\ 32.5)}$ = 1.9 for yellowtail kingfish). The relationship between Q_{10} and temperature can be described as:

$$Q_{10\text{mulloway}} = 5.797 - 0.297T + 0.005T^2 \qquad (r^2 = 0.99) \qquad (2.11)$$

$$Q_{10\text{yellowtail kingfish}} = 7.805 - 0.5489T + 0.012T^2 \qquad (r^2 = 0.65) \qquad (2.12)$$

The asymptotes of equations 10 and 11 occurred at 28.5 and 22.8 ^{o}C for mulloway and yellowtail kingfish respectively.

Using the oxyenergetic coefficient of 13.59 kJ mg^{-1} O_2 (Elliott and Davison 1975) the daily energetic cost of post-absorptive routine activity can be described as a function of temperature as:

$$\text{mulloway (kJ kg}^{-0.8}\text{ day}^{-1}) = 1.929T - 9.677 \quad (r^2=0.97) \qquad (2.13)$$

$$\text{yellowtail kingfish (kJ kg}^{-0.8}\text{ day}^{-1}) = 4.041T - 13.141 \qquad (r^2=0.95) \qquad (2.14)$$

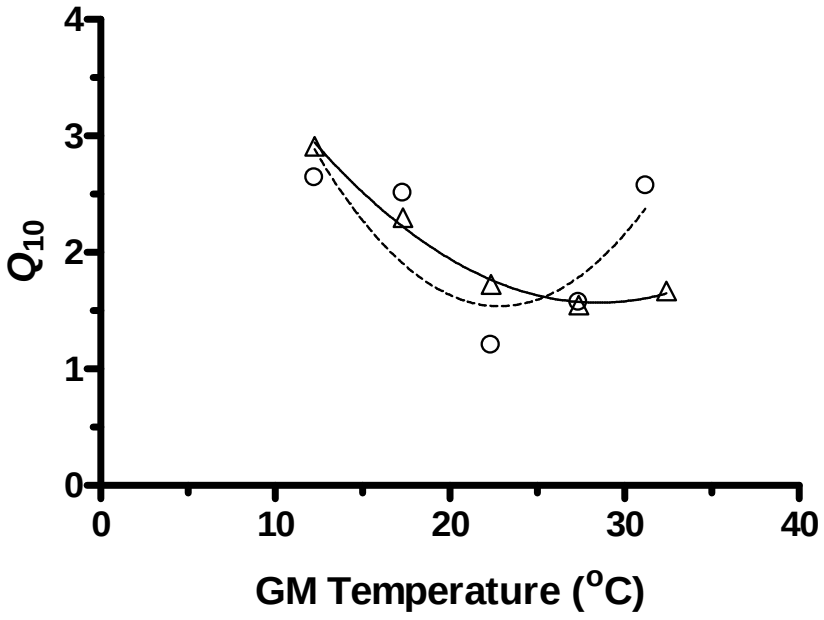

Figure 2.4. Relationship between mean Q_{10} (n = 3) and geometric mean temperature for mulloway (triangle) and yellowtail kingfish (circle).

2.5 Discussion

By nature of the gaseous phase, respirometers designed to estimate metabolism in air breathing terrestrial animals require air-tight chambers to account for O_2 and CO_2 budgets (e.g. Misson 1974; Moors 1977). This study demonstrated that the air-water interface in a static system provides a sufficient boundary layer to establish reliable metabolic estimates and, although this boundary layer is permeable, MO_2 through fish respiration can easily be accounted for through simple mass-balance equations. Re-aeration and background BOD rates were shown to have a numerically small but statistically insignificant influence on the measured RMR of mulloway and yellowtail kingfish. This was because the proportional contribution of fish respiration to the change in DO concentration at all temperatures far exceeded that due to re-aeration and background BOD. The advantages of establishing MO_2 in an open system such as the one used in this study are many. MO_2 of groups of fish is less likely to be confounded by elevated stress which may occur with individually housed fish, particularly with gregarious species such as mulloway. Acclimation periods can easily be of a sufficient duration to ensure relatively normal stress levels. Logistically, the open-top system is much more practical and cost effective than traditional respirometers and, as such, allows for greater replication and experimental power. Contiguous measurements over longer periods in a static system can be achieved by intermittent flow to avoid hypoxic conditions (Forstner 1983; Kaufman et al. 1989).

RMR is a useful index of metabolic requirement as most fish maintain routine swimming velocities. The RMR of similar size mulloway and yellowtail kingfish was shown to be linearly dependant on water temperature while comparisons between the species clearly demonstrated a greater demand for oxygen

by yellowtail kingfish; the RMR of both species being equivalent when yellowtail kingfish are at temperatures approximately half that of mulloway. This difference was made evident during the power failure at 33-34^{o}C when 100% of the yellowtail kingfish were lost while 62.5% of mulloway survived. The high oxygen demand of yellowtail kingfish has clear implications for the aquaculture of this species. While mulloway are known to be relatively hypoxia tolerant (Fitzgibbon et al. 2007b) it is critical that high DO levels are maintained for yellowtail kingfish, preferably at saturated levels.

During routine activity yellowtail kingfish will consume, depending on temperature, approximately two to three times as much oxygen as mulloway and will therefore require two to three times as much energy intake to fuel routine metabolism. In the feeding study in Chapter 4, carried out using the same recirculation system and experiment tanks as the current study, the daily maintenance digestible energy (DE) requirements for mulloway were found to be 44.2 and 49.6 kJ DE kg$^{-0.8}$ day^{-1} at 20 and 26^{o}C respectively. These values are higher than the energy requirements estimated from equation 13 (*cf.* 28.9 and 40.5 kJ kg$^{-0.8}$ day^{-1} at 20 and 26 ^{o}C respectively). Maintenance energy requirements derived from feeding studies in this way are inclusive of the increased energetic costs associated with prandial metabolism including specific dynamic action (SDA) as well as general feeding activity. Clearly the maintenance energy requirements of fasted fish at routine swimming velocities compared to that of actively feeding fish represent different levels within the metabolic scope of activity. This has important implications for the construction of bioenergetic models used to make predictions of energy requirements, which in turn provide the foundation for diet formulations and feeding strategies for cultured fish (Bureau et al. 2002). A clear delineation between

the different levels of activity must be made to ensure the integrity of models predicting “maintenance” energy requirements.

Fish have a lower metabolism and a correspondingly lower activity level at colder temperatures (Jobling 1982; Fonds et al. 1992). While swimming velocities were not recorded in this study, the stark contrast in activity exhibited by the two species is likely to have contributed towards the significant differences seen in overall MO_2; mulloway activity was generally restricted to maintaining their position at the bottom of the tank while yellowtail kingfish continued to swim actively throughout the water column, although this activity was quite noticeably reduced in cold temperatures. Normalized for temperature and size, the standard metabolic rates (SMR) of mulloway and yellowtail kingfish have been shown to be similar although the aerobic scope of yellowtail kingfish is almost 3 times that of mulloway (Clark and Seymour 2006; Fitzgibbon et al. 2007b). The decrease in the factorial difference in MO_2 with increasing temperatures between mulloway and yellowtail kingfish may give some insight into the thermoregulatory responses between the two species. The relative difference between standard and routine metabolism in some species has been shown to decrease with increasing temperature (Hölker 2003) and this may be because cold temperatures are known to enhance the oxidative capacity of skeletal muscle and other tissues (Guderley and Johnston 1996).

The RMR of mulloway at 22 ^{o}C (98 mg $kg^{-0.8}$ h^{-1}), derived from Eq. (2.6), is very close to the MO_2 at the slowest swimming velocity recorded by Fitzgibbon et al. (2007b) for mulloway at this temperature in normoxic conditions using a tunnel respirometer. Fitzgibbon et al. (2007b) point out that MO_2 of mulloway at the slowest test velocity (7.5 cm s^{-1}) was slightly higher than that observed at 15 cm s^{-1}

(86 mg $kg^{-0.8}$ h^{-1}) and attribute this to the energetic cost associated with maintaining stability at low velocities. Similar responses in MO_2 as a function of low swimming velocity have also been recorded for the European seabass (*Dicentrarchus labrax*) (Chatelier et al. 2005) and Pacific bonito (*Sarda chiliensi*) (Sepulveda et al. 2003). While estimations of RMR, by definition, include MO_2 associated with spontaneous activity (Fry 1957), estimates of SMR derived by extrapolating relative swimming speed to 0 velocities can be influenced by the energetic cost of stability at low swimming velocities (Magnuson 1973; Webb 2002) particularly in obligate ram-ventilating species such as the tunas and sharks (Sepulveda et al. 2003; Sepulveda et al. 2007).

While yellowtail kingfish are a high-energy-demand, high-performance species that share similar morphological characteristics of the tunas (Clark and Seymour 2006) the RMR of tuna species is much higher. Southern bluefin tuna (*Thunnus maccoyii*), a species that are also cultured in Australia, have a RMR of 834 mg $kg^{-0.8}$ h^{-1} at 19 ^{o}C (Fitzgibbon et al. 2008) which is more than four times that of yellowtail kingfish and more than ten times than that of mulloway at the same temperature (*cf.* 191 and 81 mg $kg^{-0.8}$ h^{-1} for yellowtail kingfish and mulloway respectively). The high metabolic rate of tunas is related in part to their elevated endothermy and obligate ram-ventilating requirement (Sepulveda et al. 2003); characteristics which are absent in other teleosts such as mulloway and yellowtail kingfish.

From Eq. (2.6) the predicted RMR of mulloway at 26 ^{o}C (121 mg O_2 $kg^{-0.8}$ h^{-1}) is slightly higher than that of the similarly sedentary barramundi (*Lates calcarifer*) held between 26-32 ^{o}C (92 mg O_2 $kg^{-0.8}$ h^{-1}) (data adapted from Glencross and Felsing (2006) based on a 180 g fish). Barramundi are a catadromous

species that are also cultured in Australia, and while the study by Glencross and Felsing (2006) was carried out on barramundi in freshwater, the energetic cost associated with osmoregulation by euryhaline species such as mulloway in seawater may not necessarily equate to a significant relative increase in MO_2. The influence of salinity on MO_2, and therefore energy metabolism, varies considerably depending on the species and rearing conditions (Claireaux and Lagardere 1999; Altinok and Grizzle 2003; Wuenschel et al. 2005) as well as life history (Morgan and Iwama 1991) making generalizations very difficult and necessitating the establishment of MO_2 and salinity relationships for each species as required.

The thermosensitivity of RMR in yellowtail kingfish demonstrated a clear parabolic response with the lowest Q_{10} occurring between 20-25 ^{o}C and the asymptote at 22.8 ^{o}C. While not strictly stenotherms, the increased thermosensitivity of RMR outside these ranges indicate that yellowtail kingfish have a narrower temperature range for optimal metabolic function (compared to mulloway) indicative of a temperate pelagic species. At all temperatures yellowtail kingfish appeared to feed well, albeit noticeably less vigorously at 10 ^{o}C. As mentioned previously, initially stocked yellowtail kingfish were observed to sometimes regurgitate feed at 33 ^{o}C and although no ABT was detected, this response may indicate that yellowtail kingfish were approaching their upper thermal limit.

The $Q_{10(20\text{-}25)}$ value of 1.2 for yellowtail kingfish recorded in this study is considerably lower than that reported by Clark and Seymour (2006) on the SMR of the same species ($Q_{10(20\text{-}25)}$ = 4.5; BW = 2.1 kg). The short acclimation period, although noted as ecologically relevant in their study, of 5 ^{o}C over 3 h (*cf*. 2-3 ^{o}C in 10 d this study) is likely the main reason for such discrepancy and highlights the

need for acclimation periods of adequate duration if acute temperature related responses in MO_2 are not desired. The thermosensitivity of RMR in mulloway demonstrated a reverse J-curve response with the lowest Q_{10} occurring between 25-30 ^{o}C and the asymptote at 28.5 ^{o}C. In contrast to yellowtail kingfish, the curve relating to Q_{10} values between 20-35 ^{o}C was very shallow showing little difference over this range indicating that mulloway have a much broader ranging thermal tolerance on metabolic function, typical of eurythermal species inhabiting estuarine and near-shore coastal habitats (Harrison and Whitfield 2006). The thermosensitivity of RMR has implications for the aquaculture of both species particularly in terms of seasonal temperature profiles at site locations for sea cage operations. Temperatures consistently above or below those least thermally dependent ranges may have negative impacts on productivity.

Ectotherms exhibit thermoregulatory behaviour by altering spatial and temporal patterns of activity to maintain their body temperature within a narrow "optimal" range (Beitinger and Fitzpatrick 1979; Hochachka and Somero 2002). This is linked with the idea that final thermal preferenda and thermal physiology are closely co-adapted and that thermal preferences coincide with temperatures that maximize Darwinian fitness (Beitinger and Fitzpatrick 1979; Angilletta et al. 2006; Martin and Huey 2008). Martin and Huey (2008) however proposed the concept of "suboptimal is optimal" in ectotherms whereby the preferred temperature may be lower than the physiologically optimal temperature. Their model predicts that animals will select temperatures that are somewhat lower than the temperature at which fitness is maximal (Martin and Huey 2008). Thermal studies on mulloway by Bernatzeder and Britz (2007) demonstrated a final preferred temperature range of 25 – 26.4 ^{o}C while the predicted temperature in the current study at which the RMR

of mulloway was the least thermally dependent was somewhat higher at 28.5 ^{o}C. The Martin and Huey model (2008) may apply in this case only if we consider 28.5 ^{o}C near the physiologically "optimal" temperature for mulloway. We may then speculate that yellowtail kingfish will select temperatures slightly below 22.8 ^{o}C if given a choice; however this remains to be tested. It should be remembered however that the RMR values established in this study are of the routine activity of post-absorptive juvenile fish and therefore exclude the influence of post-prandial effects and specific dynamic action (SDA); thermal sensitivities may shift slightly depending on physiological (and reproductive) states (Angilletta et al. 2002). Indeed, studies on the thermal effects of post-prandial metabolic responses in juvenile mulloway (Chapter 3) show a shift in Q_{10} values of approximately 0.2 when comparing the metabolic thermosensitivities between peak SDA MO_2 and RMR of 240 g fish. Furthermore a change in the direction of thermosensitivity ($Q_{10peakSDA}$- Q_{10RMR}) was seen depending on the shift in temperature from -0.2 ($Q_{10(14-20)}$) to +0.2 ($Q_{10(20-26)}$).

As the body temperature of ectotherms conforms to the temperature of their immediate environment it is therefore reasonable to consider that their metabolism also responds in the same way of simple chemical reactions. According to kinetic theory chemical reactions only proceed once they have attained a minimum required energy of activation (E_a). Generally, at around room temperature (25 ^{o}C) reaction rates with an E_a of ~50 kJ mol^{-1} double for every 10^{o}C rise in temperature (Kotz and Treichel 1996). Over the range of temperatures tested in this study the E_a values of mulloway (47.6 kJ mol^{-1}) and yellowtail kingfish (44.1 kJ mol^{-1}) were found to be very close to this value and generally conform to the overall Q_{10} for most fish species (Cameron 1989; Clarke and Johnston 1999). For similar reactions at a given

temperature the greater the energy barrier (i.e. the higher the E_a) the slower the reaction rate (Kotz and Treichel 1996); we can therefore expect reaction rates within mulloway to proceed at a similar rate as yellowtail kingfish. The difference in routine activity between mulloway and yellowtail kingfish appeared to influence the elevation but not the slope (Figure 2.4) indicating that E_a may be independent of activity level. Similar differences between the elevation but not the slope of Arrhenius plots can also be seen between the SMR and RMR of carp (*Cyprinus carpio*) (Becker et al. 1992).

To conclude, re-aeration rates and BOD levels of seawater in the open-top system used in this study were shown to have an insignificant influence on estimations of RMR; even so, metabolic rates can be accurately quantified using simple mass balance equations to account for minor influences not directly associated with fish respiration. Comparable results to published data on the same species using more traditional flow-through respirometers also lend confidence to the system and methods used. The thermosensitivity response of RMR appeared indicative of the temperature profiles where mulloway and yellowtail kingfish are naturally found. This has direct implications for the aquaculture of the species particularly with regard to appropriate site locations with exposure to optimal temperature ranges; i.e. 20-25 ^{o}C for yellowtail kingfish and 25-30 ^{o}C for mulloway, although mulloway should still perform well if temperatures remain above 20 ^{o}C. The high oxygen demand of yellowtail kingfish necessitates the supply of high levels of DO in any culture system for this species.

Chapter 3

The Effect of Temperature and Body Weight on the Routine Metabolic Rate and Postprandial Metabolic Response in Mulloway[2]

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[2]The following chapter is published as:

Pirozzi, I., Booth, M.A., 2009. The effect of temperature and body weight on the routine metabolic rate and postprandial metabolic response in mulloway, *Argyrosomus japonicus*. Comp. Biochem. Physiol. A-Mol. Integr. Physiol. 154, 110-118

3.1 Abstract

Specific dynamic action (SDA) is the energy expended on the physiological processes associated with meal digestion and is strongly influenced by the characteristics of the meal and the body weight (BW) and temperature of the organism. This study assessed the effects of temperature and body weight on the routine metabolic rate (RMR) and postprandial metabolic response in mulloway, *Argyrosomus japonicus*. RMR and SDA were established at 3 temperatures (14, 20 and 26 ^{o}C). 5 size classes of mulloway ranging from 60 g to 1.14 kg were used to establish RMR with 3 of the 5 size classes (60, 120 and 240 g) used to establish SDA. The effect of body size on the mass-specific RMR (mg O_2 kg^{-1} h^{-1}) varied significantly depending on the temperature; there was a greater relative increase in the mass-specific RMR for smaller mulloway with increasing temperature. No statistical differences were found between the mass exponent (*b*) values at each temperature when tested against H_0: $b = 0.8$. The gross RMR of mulloway (mg O_2 $fish^{-1}$ h^{-1}) can be described as function of temperature (T; 14-26 ^{o}C) as: ($0.0195T$ –

0.0454)BW(g)$^{0.8}$ and the mass-specific RMR (mg O_2 kg^{-1} h^{-1}) can be described as: (21.042*T* – 74.867)BW(g)$^{-0.2}$. Both SDA duration and time to peak SDA were influenced by temperature and body weight; SDA duration occurred within 41-89 h and peak time occurred within 17 – 38 h of feeding. The effect of body size on peak metabolic rate varied significantly depending on temperature, generally increasing with temperature and decreasing with increasing body size. Peak gross oxygen consumption (MO_2: mg O_2 $fish^{-1}$ h^{-1}) scaled allometrically with BW. Temperature, but not body size, significantly affected SDA scope, although the difference was numerically small. There was a trend for MO_2 above RMR over the SDA period to increase with temperature; however, this was not statistically significant. The average proportion of energy expended over the SDA period (SDA coefficient) ranged from approximately 7 – 13 % of the total DE intake while the proportion of total energy expended on SDA above RMR ranged from approximately 16 to 27 %.

3.2 Introduction

The obligatory increase in oxygen consumption (MO_2) that occurs in animals after feeding represents the energy expended on ingestion, digestion, absorption and assimilation of a meal and is often termed specific dynamic action (SDA) (Jobling 1981; Withers 1992). In ectotherms, an increase in temperature is generally accompanied by an increase in routine and peak metabolic rates and a decrease in the SDA duration (Robertson et al. 2002; Wang et al. 2003; Luo and Xie 2008). Body size also influences the SDA response and, in absolute terms, an increase in body size will generally correspond to an increase in metabolic rate, SDA duration and peak metabolism (Tandler and Beamish 1981; Boyce and Clarke

1997). The increased O_2 demand associated with feeding has practical implications for the management of intensively cultured aquatic animals. While dissolved O_2 may be at normoxic levels for fish during routine activity, the increased O_2 demand associated with feeding may, depending on stocking densities, induce periods of oxygen debt. If chronic hypoxic conditions occur, voluntary feed intake is reduced (Glencross 2009) and production potential is then likely to be compromised.

The mechanical costs of processing food are considered to be negligible and are in the order of 1 – 3 % of the energy expended on SDA (Cho and Slinger 1979; Peck 1998) while in most animals, as much as 60-80 % of SDA results from post-absorptive metabolism associated with the anabolic cost of protein and lipid synthesis, protein turnover and growth (Wieser 1994; Willmer et al. 2000). The magnitude and duration of the SDA response is greatly influenced by the characteristics of the meal such as composition (Ross et al. 1992; Peres and Oliva-Teles 2001; Fu et al. 2007), ration size (Secor and Diamond 1997; Fu et al. 2005) and feeding frequency (Guinea and Fernandez 1997; de la Gandara et al. 2002). The proportion of the energy expended during the SDA period above routine metabolism can also vary depending on species (Fu et al. 2005; Fu et al. 2006).

The partitioning and quantification of dietary energy is important in the study of nutritional energetics because it provides a convenient platform to predict the energy balance of individuals based on body weight, sex, activity, physiological state, environment, and amount and nutritive value of feed eaten (Baldwin and Bywater 1984). This information can then form the basis for diet formulation and evaluation (Bureau et al. 2002). Energy exchanges in biological systems can be studied in terms of their biochemical thermodynamics or bioenergetics, which involves the examination of energy gains, losses and transfers within the whole

organism (Jobling 1994; Haynie 2001). Energy budgets account for the energy ingested (IE) and the energy used for metabolism (M), nitrogenous waste (UE), fecal waste (FE) and production (P; somatic and non-somatic growth). This can be expressed in the general form:

$$IE = M + UE + FE + P$$

M can be further partitioned as the sum of routine metabolic rate (RMR; basal metabolism + metabolism associated with routine activity) + SDA.

The energetic requirements for RMR, maintenance and growth have been established for mulloway (Chapter 2; 4); however, information on the allometric relationships with temperature and metabolism are limited and there is currently no information on the SDA response for this species. The objectives of this study were to describe the influence of body mass and temperature on the RMR and SDA of mulloway.

3.3 Materials & Methods

3.3.1 Experiment design

The influence of temperature on oxygen consumption (MO_2) was tested using five size classes of juvenile mulloway (mean initial body weight (g) ± SD; 60.4± 0.9, 122.2± 2.6, 240.5±3.6, 496.7± 2.5 or 1140.6± 1.6). Sizes classes are referred to as XS, S, M, L or XL respectively. Fish were stocked in triplicate groups for each of the 3 temperature treatments (14, 20 or 26 ^{o}C) into 200 l open-top tanks at n = 22, 12, 8, 3 or 2 fish per tank for the XS, S, M, L and XL fish respectively.

Stocking densities were chosen to mitigate the potential for density dependent behavioral effects (Appendix 1).

All fish (i.e. all temperature x size groups) were initially stocked into the experiment system at ambient water temperature (16 ^{o}C) and then adjusted 1 ^{o}C day^{-1} until the start temperature of 14 ^{o}C was reached. Fish were then held for two weeks at that temperature to acclimate to the system before MO_2 readings were taken for the 14 ^{o}C treatment group. During acclimation all fish were fed a maintenance ration once daily of a 6mm sinking commercial diet (Ridley AquaFeed Pty. Ltd., Narangba, Qld. Australia; 45.5 % crude protein, 18.7 % crude fat, 22.2 MJ kg^{-1} gross energy). The apparent digestibility coefficient for energy of the diet was 0.84 (Booth, unpublished data, 2008). Each tank was supplied with constant water flow (6 l min^{-1}) and air when MO_2 was not being recorded. Tanks were exposed to indirect natural lighting (photoperiod 11L:13D) and water quality parameters (pH 7.5-7.84; NH_4^+ <0.1 mg/L; salinity 30.6-33.0 ppt) were monitored regularly.

3.3.2 MO_2

MO_2 readings were established as per Chapter 2 . Fish were fasted for 96, 72 or 48 h depending on the temperature treatment (i.e. 14, 20 or 26 ^{o}C respectively) prior to establishing routine metabolic rates (RMR). MO_2 measurements for each temperature were repeated three times over approximately 2 h intervals for each replicate tank and the mean regarded as the RMR. After RMR was established fish were fed the commercial diet slowly from 16:30 over approximately 1 h to slightly in excess of apparent satiation. Any uneaten pellets remaining after the feeding period were counted then siphoned from tanks. Total feed intake was then adjusted

accordingly using a predetermined individual pellet weight of 0.21± 0.02 g (mean ± SD; n = 202). MO_2 during the SDA period was monitored up to 72 h or until MO_2 rates fell within the standard error of RMR levels. MO_2 rates remained elevated after 72 h for the mulloway at 14 ^{o}C however readings taken at day 6 post-feeding showed that MO_2 had returned to RMR levels. The L and XL size mulloway did not feed well and were excluded from SDA analyses.

Fish that were assigned as the 14 ^{o}C treatment were re-weighed after MO_2 readings were completed for that temperature and removed from the system. Background biochemical oxygen demand (BOD) was determined for each replicate tank after fish were removed and water had been re-saturated with O_2. The temperature was then adjusted up 1 ^{o}C day^{-1} until the next experiment temperature (20 ^{o}C) was reached. The remaining fish in the system were acclimated for a further week before MO_2 was recorded. This protocol was again repeated for the final temperature (26 ^{o}C). After MO_2 measurements had been completed a sub-sample of 5 individual fish from each replicate tank were euthanized with an overdose of benzocaine (ethyl-*p*-aminobenzoate) and dissected to determine the presence or absence of feed remaining in the digestive tract. Fish from the 14 ^{o}C treatment were sub-sampled on day 6 post-feeding.

3.3.3 RMR and SDA Parameters

Mass-specific MO_2 was calculated as:

$$(\mathrm{V}/\mathrm{BW}/\mathrm{n})\times(\Delta\mathrm{O}_2-\mathrm{O}_{2\mathrm{otr}}+\mathrm{O}_{2\mathrm{bod}})$$

Where V = tank water volume (l); BW = mean body weight (kg); n = number of fish tank^{-1}; ΔO_2 = net change in O_2 concentration (mg l^{-1} h^{-1}) inclusive of fish respiration, atmospheric re-aeration and background BOD; O_{2otr} = atmospheric oxygen transfer rate (OTR; mg l^{-1} h^{-1}); O_{2bod} = background BOD rate (mg l^{-1} h^{-1}). OTR was calculated using seawater-atmosphere O_2 transfer coefficients established in Chapter 2.

The following MO_2 and SDA indices were calculated:

MO_{2rmr-g}: Routine metabolic rate (RMR) expressed as gross MO_2 (mg O_2 fish^{-1} h^{-1}) defined as the metabolic rate associated with standard metabolism and spontaneous swimming activity of post-absorptive fish fasted for 48 h – 96 h (depending on temperature treatment)

MO_{2rmr-s}: Mass-specific RMR (mg O_2 kg^{-1} h^{-1})

MO_{2sda-d}: SDA duration (h) defined as the time from initial feeding to the point when MO_2 rates returned to within the SE of MO_{2rmr-s}. 14^{o}C treatments were estimated by fitting a quadratic function and deriving the *x*-intercept when *y* = +SE of MO_{2rmr-s}

MO_{2sda-p}: Peak post-prandial MO_2 derived as *y*-intercept of asymptote of quadratic function fitted to MO_{2sda-d}

$MO_{2sda-pd}$: SDA duration from feeding to peak post-prandial MO_2 derived as *x*-intercept of asymptote of quadratic function fitted to MO_{2sda-d}

MO_{2scope}: Factorial scope calculated as MO_{2sda-p} MO_{2rmr-s}^{-1}

MO_{2sda}: The cumulative measurement of MO_2 (mg O_2 kg^{-1}) above RMR over the SDA period. Calculated as the area under the curve (AUC) (Motulsky and

Christopoulos 2003). 14^{o}C treatment was estimated based on predicted $MO_{2sda\text{-}d}$.

SDA coefficient: Estimate of the proportion (%) of total digestible energy (DE) intake from feed expended on SDA. Based on an oxyenergetic conversion factor of 13.59 kJ mg O_2^{-1} (Elliott and Davison 1975) applied to MO_{2sda}.

SDA_E: Proportion (%) of SDA energy expenditure above RMR

TE: Total metabolic expenditure (kJ $kg^{-0.8}$) over SDA period (sum of SDA + RMR)

3.3.4 Data analyses

The effect of temperature on the $MO_{2rmr\text{-}s}$ and $MO_{2rmr\text{-}g}$ of the 5 different size classes of mulloway (XS, S, M, L, XL) was tested with 2-way ANOVA (temperature x size). SDA variables were compared between 3 sizes (XS, S, M) using 2-way ANCOVA (temperature x size) with relative feed intake (RFI: g feed BW kg^{-1}) as the co-variate. Two-way ANOVA was used on SDA variables where the covariate was not significant ($MO_{2sda\text{-}d}$, MO_{2scope}). RFI was also compared between XS, S and M size mulloway using 2-way ANOVA. All data were normally distributed according to skewness, kurtosis and omnibus normality tests (NCSS 2004, Kaysville, Utah). All variances were homogeneous according to modified Levenes' equal variance test. Tukey-Kramer test was used for *a posteriori* multiple comparison of means on significant terms. Comparisons of individual model parameters were made using the extra sum-of-squares *F*-test. Results of all statistical tests were regarded as significant at $p<0.05$.

The effect of body weight on RMR at each temperature was described by the allometric equation:

$$RMR = aBW^{b}$$

Where a is the normalizing constant, BW is the body mass in g and b is the scaling exponent describing the influence of mass on metabolism. Power functions were iteratively derived using the non-linear least squares method in Graphpad Prism® v 4.0.

The SDA responses for XS, S and M size mulloway at each temperature were fitted with a quadratic function in the form:

$$MO_{2SDA} = a + bt + ct^{2}$$

Where MO_{2SDA} is the mass-specific MO_2 (mg O_2 kg^{-1} h^{-1}) over the SDA period expressed as function of time (t) in hours.

3.4 Results

3.4.1 Temperature and fish size interactions

3.4.1.1 RMR

The effect of body size on the MO_{2rmr-s} of mulloway varied significantly depending on the temperature (Table 3.1). There was a significantly greater relative increase in MO_{2rmr-s} for smaller mulloway with increasing temperature (Table 3.2). Partitioning of the data demonstrated no significant interaction (ANOVA; temperature x size; $p>0.5$) between MO_{2rmr-s} at 20 and 26 ^{o}C while comparisons at

14 and 20 ^{o}C were significant (temperature x size; $p<0.01$). The interaction with size and temperature is reflected in the different scaling exponent (b) values (Table 3.3, Figure 3.1). Exponent values for $MO_{2rmr\text{-}g}$ at 20 and 26 ^{o}C were very similar and were higher at 14 ^{o}C (Table 3.3). However; no significant difference was found between each exponent value when tested against H_0: $b = 0.8$ ($p>0.2$, 0.05 and 0.1 for 14, 20 and 26 ^{o}C respectively). The gross RMR of mulloway (mg O_2 $fish^{-1}$ h^{-1}) can therefore be described as function of temperature (T; 14-26 ^{o}C) as:

$$MO_{2rmr\text{-}g} = (0.0195T - 0.0454)BW(g)^{0.8} \qquad (r^2 = 0.999) \qquad (3.1)$$

and the mass-specific RMR (mg O_2 kg^{-1} h^{-1}) can be described as:

$$MO_{2rmr\text{-}s} = (21.042T - 74.867)BW(g)^{-0.2} \qquad (r^2 = 0.99) \qquad (3.2)$$

Table 3.1. 2-way ANOVA on $MO_{2rmr\text{-}s}$ and $MO_{2rmr\text{-}g}$ for all sizes classes. ns = not significant at $p<0.05$, * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$

Source of Variation		$\boldsymbol{MO_{2rmr\text{-}s}}$			$\boldsymbol{MO_{2rmr\text{-}g}}$		
	DF	**MS**	***F***	***P***	**MS**	***F***	***P***
A: Temperature	2	28290.2	377.4	***	2159.5	118.1	***
B: Size	4	5755.3	76.78	***	8648.6	473.1	***
AB	8	359.1	4.8	***	209.6	11.5	***
Residual	30	75.0			18.3		

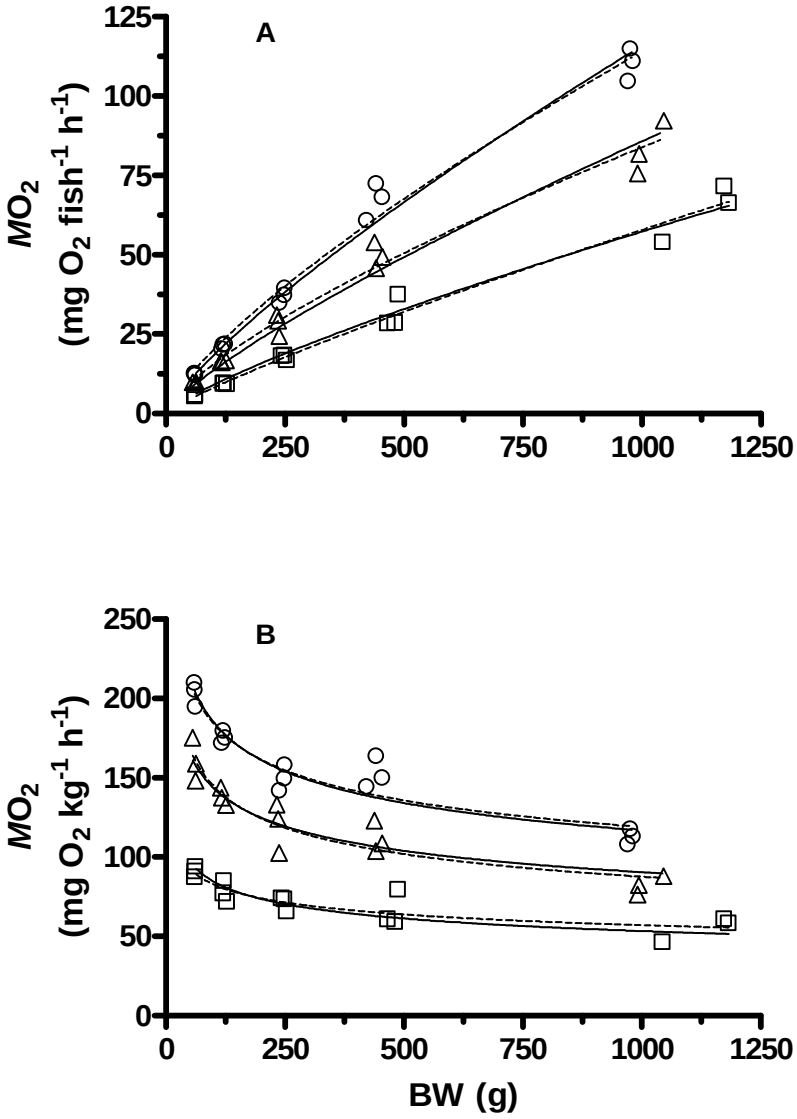

Figure 3.1. Relationship between BW (g) and A. $MO_{2rmr\text{-}g}$ and B. $MO_{2rmr\text{-}s}$ at 14 (squares), 20 (triangles) and 26 ^{o}C (circles). Solid lines represent power functions with exponent (*b*) values fixed at 0.8 and -0.2 for graphs A and B respectively. Dashed lines represent power functions with unconstrained iteratively derived *b*. Refer to Table 3.3 for specific parameter values.

Table 3.2. Summary of RMR MO_2 results (mean±se) for XS – XL size mulloway at 14, 20 and 26°C. ANOVA on final BW data analyzed within size class and were significant at $p<0.05$ but not $p<0.01$. Means sharing superscript letters are not significantly different ($p>0.05$) according to Tukey-Kramer test.

	XS			S			M			L			XL		
Variable	**14°C**	**20°C**	**26°C**	**14°C**	**20°C**	**26°C**	**14°C**	**20°C**	**26°C**	**14°C**	**20°C**	**26°C**	**14°C**	**20°C**	**26°C**
BW (g)	61.6±0.3	60.4±2.1	60.9±0.6	123.5±2.4	119.2±3.3	120.9±2.0	248.8±3.0^{a}	235.5±1.4^{b}	245.7±3.6^{ab}	478.6±6.4^{a}	444.4±5.0^{b}	439.2±9.5^{b}	1134±45^{a}	1011±18^{b}	976±3^{b}
MO_{2rmr-g} **(mg O_2 $fish^{-1}$ h^{-1})**	5.6±0.1^{a}	9.7±0.2^{ab}	12.4±0.2^{ab}	9.4±0.1^{ab}	16.4±0.2^{abc}	21.2±0.5^{bcd}	17.7±0.5^{abc}	28.2±2.0^{cde}	37.1±1.3^{ef}	31.4±3.0^{de}	49.7±2.3^{f}	67.1±3.4^{g}	63.9±5.2^{g}	83.3±4.8^{h}	110.1±3.0^{i}
MO_{2rmr-s} **(mg O_2 kg^{-1} h^{-1})**	90.8±1.9^{bc}	160.8±7.9^{ef}	203.2±4.5^{g}	75.8±2.0^{ab}	138.1±3.2^{de}	175.4±2.2^{f}	71.1±2.7^{ab}	119.9±9.0^{cd}	151.0±3.6^{ef}	65.6±5.7^{ab}	111.9±5.8^{c}	152.6±5.8^{ef}	56.2±2.7^{a}	82.3±3.4^{b}	112.8±2.9^{cd}

Table 3.3. Parameters of the power function $y = aM^b$ describing the relationship between body mass and $MO_{2rmr\text{-}g}$ or $MO_{2rmr\text{-}s}$ for mulloway at each experiment temperature. Data shown for iteratively derived parameters and also for coefficient values when *b* fixed at 0.8 ($MO_{2rmr\text{-}g}$) or -0.2 ($MO_{2rmr\text{-}s}$).

RMR variable	Temperature (°C)	Unconstrained *b* *a*±se	*b*±se	r^2	Constrained *b* *a*±se	r^2
$MO_{2rmr\text{-}g}$	14	0.158±0.05	0.854±0.04	0.98	0.228±0.01	**0.98**
	20	0.536±0.12	0.732±0.04	0.98	0.341±0.01	**0.98**
	26	0.622±0.13	0.754±0.03	0.99	0.461±0.01	**0.99**
$MO_{2rmr\text{-}s}$	14	174.2±22.4	-0.161±0.02	0.78	212.5±5.3	**0.74**
	20	394.4±45.7	-0.218±0.02	0.89	360.4±7.3	**0.88**
	26	**440.6±45.6**	**-0.190±0.02**	**0.90**	**465±7.4**	**0.90**

3.4.1.2 SDA

Figure 3.2 shows the SDA responses for XS, S and M size mulloway at 14, 20 and 26 °C. RFI was a significant covariate for $MO_{2sda\text{-}p}$, MO_{2scope} and MO_{2sda} (Table 3.4). No food was present in the digestive tract of any size mulloway at any temperature at the conclusion of the study.

The effect of body size on $MO_{2sda\text{-}p}$ varied significantly depending on temperature (Table 3.4). $MO_{2sda\text{-}p}$ generally increased with temperature and decreased with increasing size (Table 3.5). The relationship between peak gross MO_2 (mg O_2 $fish^{-1}$ h^{-1}) and BW was allometric (Figure 3.3) and can be described at each temperature as:

$$14^oC \text{ (mg } O_2 \text{ fish}^{-1} \text{ h}^{-1}) = 0.144BW(g)^{0.938} \quad (r^2 = 0.989) \qquad (3.3)$$

$$20^oC \text{ (mg } O_2 \text{ fish}^{-1} \text{ h}^{-1}) = 0.321BW(g)^{0.882} \quad (r^2 = 0.998) \qquad (3.4)$$

$$26^oC \text{ (mg } O_2 \text{ fish}^{-1} \text{ h}^{-1}) = 0.692BW(g)^{0.799} \quad (r^2 = 0.985) \qquad (3.5)$$

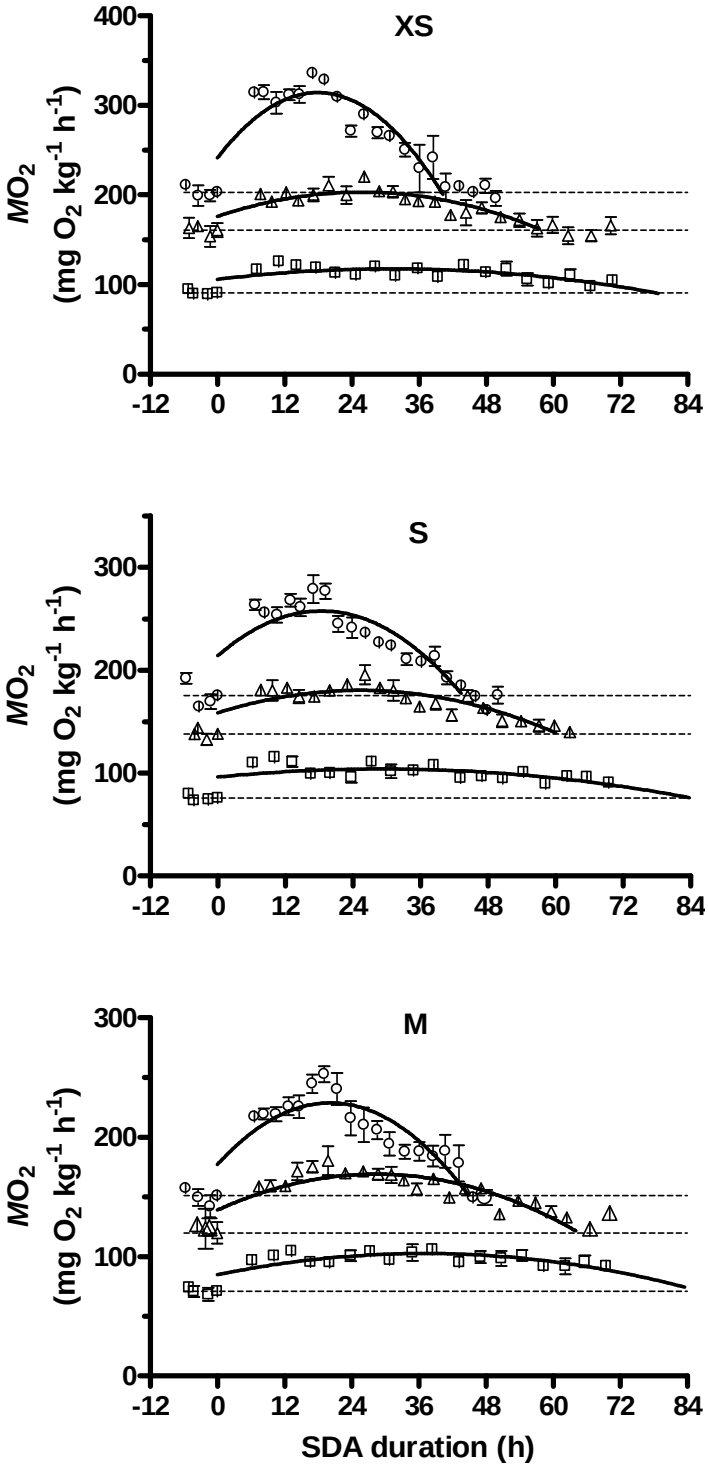

Figure 3.2. Temporal pattern of SDA measured as mean MO_2 (mg O_2 kg^{-1} h^{-1}) (±se; n = 3) of XS, S and M size mulloway at 14 (squares), 20 (triangles) and 26 ^oC (circles). Horizontal dashed lines represent $MO_{2rmr\text{-}s}$ at each temperature and size treatment. Refer to Table 2 for $MO_{2rmr\text{-}s}$ values. Quadratic functions shown fitted for $MO_{2sda\text{-}d}$.

Table 3.4. 2-way ANCOVA on MO_2 SDA variables for XS, S and M size mulloway with RFI as a significant co-variate. ns = not significant at $p<0.05$, * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$.

Source of Variation		MO_{2sda-p}			MO_{2scope}			MO_{2sda}		
	DF	**MS**	***F***	***P***	**MS**	***F***	***P***	**MS**	***F***	***P***
X(RFI)	1	384.3	12.3	**	0.035	4.8	*	884959	5.6	*
A: Temperature	2	7703.9	246.6	***	0.046	6.3	**	562757	3.5	ns
B: Size	2	4048.0	129.6	***	0.004	0.5	ns	247352	1.6	ns
AB	4	547.7	17.5	***	0.007	0.9	ns	362705	2.28	ns
Residual	17	31.2			0.007			159119		

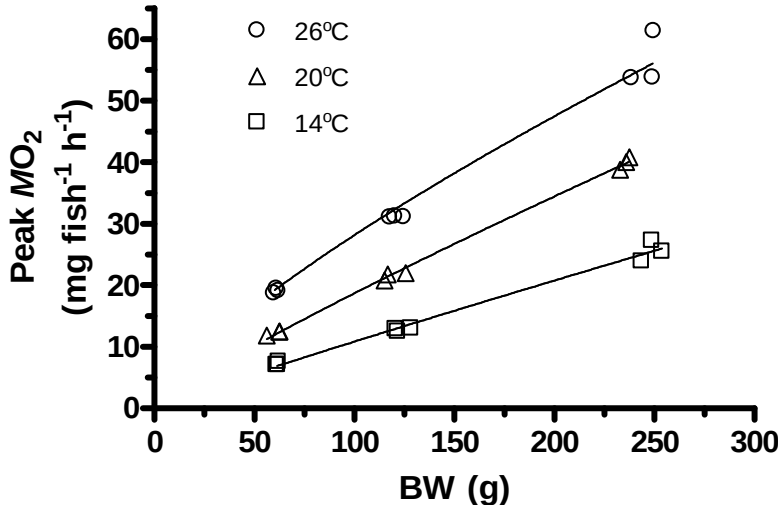

Figure 3.3. Allometric relationship between BW (g) and peak gross MO_2 (mg $fish^{-1}$ h^{-1}) (n = 9).

Exponent values for peak gross MO_2 at 14 and 20°C differed significantly ($P<0.0001$) from 0.8.

Temperature and body size significantly, but independently, influenced $MO_{2sda-pd}$ and MO_{2sda-d} (Table 3.6). MO_{2sda-d} ranged from approximately 41 – 89 h and generally decreased with increasing temperature and increased with increasing size (Table 3.5). $MO_{2sda-pd}$ ranged from 17 – 38 h. The relationship between

temperature and both $MO_{2sda\text{-}pd}$ and $MO_{2sda\text{-}d}$ was linear (Figure 3.4) with duration decreasing with increasing temperature. There was no effect of temperature between slopes (p>0.5) with regard to $MO_{2sda\text{-}d}$ however the y-intercepts differed significantly (p<0.05). Therefore, when slope = -3.467 (r^2 = 0.93), $MO_{2sda\text{-}d}$ = 128.4 (r^2 = 0.96), 132.7 (r^2 = 0.92) and 135.3 h (r^2 = 0.91) at x = 0 for XS, S and M size mulloway respectively.

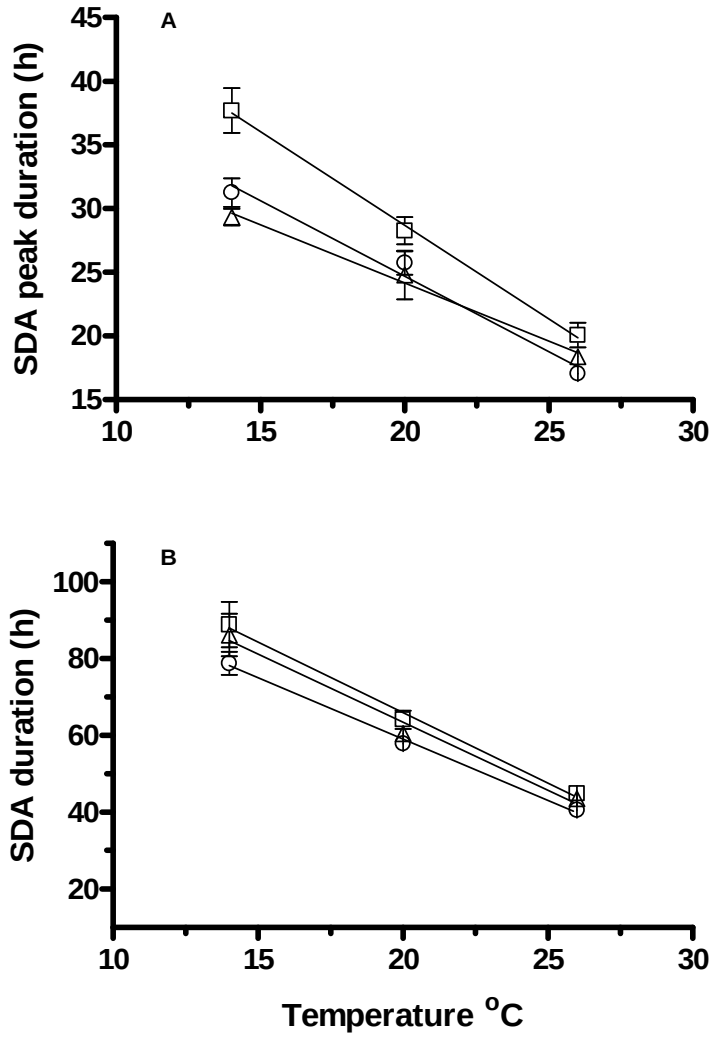

Figure 3.4. Linear relationship between temperature and A. $MO_{2sda\text{-}pd}$ and B. $MO_{2sda\text{-}d}$ (±se; n = 3) for XS (circles), S (triangles) and M (squares) size mulloway.

The relationship between temperature (T; 14-26 ^{o}C) and $M\dot{O}_{2sda\text{-}pd}$ for XS, S and M size mulloway can be described as:

$$XS = -1.185T + 48.36 \quad (r^2 = 0.94) \quad (3.6)$$

$$S = -0.914T + 42.44 \quad (r^2 = 0.87) \quad (3.7)$$

$$M = -1.470T + 58.07 \quad (r^2 = 0.94) \quad (3.8)$$

Temperature, but not body size, significantly affected $M\dot{O}_{2scope}$ (Table 3.4). $M\dot{O}_{2scope}$ demonstrated a small but statistically significant increase of, on average, 0.16 at 26 ^{o}C ($M\dot{O}_{2scope}$ = 1.51) compared to both 14 and 20 ^{o}C ($M\dot{O}_{2scope}$ = 1.37, 1.34 respectively) (Table 3.6).

There was a trend for $M\dot{O}_{2sda}$ to increase with temperature (Table 3.5); however, this was not significant (Table 3.4). $M\dot{O}_{2sda}$ was, on average, 1812, 1967 and 2799 mg at 14, 20 and 26 ^{o}C respectively (Table 3.5). There was no obvious trend indicating the relationship between $M\dot{O}_{2sda}$ and size (Table 3.5).

Table 3.5. 2-way ANOVA on SDA variables for XS, S and M size mulloway with RFI as a non-significant covariate. Results of 2-way ANOVA for RFI also shown. ns = not significant at p<0.05, * = p<0.05, ** = p<0.01, *** = p<0.001.

Source of		**$M\dot{O}_{2sda\text{-}pd}$**			**$M\dot{O}_{2sda\text{-}d}$**			**SDA coef**			**SDA_E**			**RFI**		
Variation	**DF**	**MS**	***F***	***P***	**MS**	***F***	***P***	**MS**	***F***	***P***	**MS**	***F***	***P***	**MS**	***F***	***P***
A: Temperature	2	459.6	118.5	***	3920.6	134.7	***	19.9	9.4	**	146.9	8.9	***	240.4	47.1	***
B: Size	2	55.0	14.2	***	108.8	3.7	*	20.9	9.9	**	33.0	2.0	ns	126.0	24.7	***
AB	4	9.6	2.5	Ns	8.5	0.3	ns	7.2	3.4	*	23.5	1.4	ns	18.0	3.5	*
Residual	18	3.9			29.1			2.1			16.4			5.1		

The influence of temperature on RFI and the SDA coefficient varied significantly depending on the body size of mulloway (Table 3.6). The average proportion of energy expended on SDA ranged from approximately 7 – 13 % of the total DE intake (Table 3.5).

Temperature, but not body size, significantly influenced SDA_E (Table 3.6) with the greatest SDA energy expenditure above RMR occurring at 26 ^{o}C.

When expressed independent of mass, total energy expenditure (TE) (kJ $kg^{-0.8}$) increased linearly with relative energy intake (kJ DE $kg^{-0.8}$) (Figure 3.5). Post-hoc comparisons between regressions of each temperature treatment indicated that one set of global parameters could be used to describe the data for 20 and 26 ^{o}C (p>0.1). The slopes of the regression did not differ significantly among the three temperature treatments (p>0.5), consequently a common regression coefficient can be used across all temperatures:

$$TE\ (20\text{-}26^{o}C;\ kJ\ kg^{-0.8}) = 0.068DE + 72.88 \quad (r^2=0.55;\ n=18) \qquad (3.9)$$

$$TE\ (14^{o}C;\ kJ\ kg^{-0.8}) = 0.068DE + 64.00 \quad (r^2=0.45;\ n=9) \qquad (3.10)$$

3.4.2 MO_{2SDA} Curve fitting

Quadratic equations over the $MO_{2sda\text{-}d}$ responses (Figure 3.2) are given below. Estimates for the 14 ^{o}C treatment have poor coefficient of determination (r^2) values as estimates were based on data collected up to 72 h post feeding and before the full SDA response was completed.

XS mulloway

$$MO_{2SDA}\ (14\ ^{o}C) = 105.9 + 0.75t - 0.012t^2 \quad (r^2 = 0.26) \quad (3.11)$$

$$MO_{2SDA}\ (20\ ^{o}C) = 176.1 + 2.11t - 0.041t^2 \quad (r^2 = 0.57) \quad (3.12)$$

$$MO_{2SDA}\ (26\ ^{o}C) = 241.5 + 8.12t - 0.227t^2 \quad (r^2 = 0.68) \quad (3.13)$$

S mulloway

$$MO_{2SDA}\ (14\ ^{o}C) = 96.0 + 0.54t - 0.009t^2 \quad (r^2 = 0.18) \quad (3.14)$$

$$MO_{2SDA}\ (20\ ^{o}C) = 158.5 + 1.72t - 0.0341t^2 \quad (r^2 = 0.59) \quad (3.15)$$

$$MO_{2SDA}\ (26\ ^{o}C) = 214.0 + 4.75t - 0.129t^2 \quad (r^2 = 0.70) \quad (3.16)$$

M mulloway

$$MO_{2SDA}\ (14\ ^{o}C) = 84.9 + 0.96t - 0.013t^2 \quad (r^2 = 0.29) \quad (3.17)$$

$$MO_{2SDA}\ (20\ ^{o}C) = 139.0 + 2.12t - 0.037t^2 \quad (r^2 = 0.58) \quad (3.18)$$

$$MO_{2SDA}\ (26\ ^{o}C) = 177.2 + 5.12t - 0.127t^2 \quad (r^2 = 0.61) \quad (3.19)$$

Table 3.6. Summary of SDA MO_2 results (mean±se) for XS, S and M size mulloway at 14, 20 and 26°C. All data analyzed by ANOVA except for $MO_{2sda\text{-}p}$, MO_{2scope} and MO_{2sda} which were analyzed using ANCOVA (RFI as covariate). Means sharing superscript letters are not significantly different (p>0.05) according to Tukey-Kramer test.

	XS			S			M		
Variable	**14°C**	**20°C**	**26°C**	**14°C**	**20°C**	**26°C**	**14°C**	**20°C**	**26°C**
RFI (g kg^{-1})	10.8±0.4^{a}	19.9±1.8^{bc}	24.5±0.4^{c}	11.1±0.6^{a}	16.8±1.3^{ab}	23.3±0.4^{bc}	21.3±1.2^{bc}	24.8±2.0^{c}	26.2±2.0^{c}
***MO*$_{2sda\text{-}p}$ (mg O_2 kg^{-1})**	117.7±2.6^{b}	203.2±3.8^{e}	313.5±3.0^{g}	103.9±1.7^{b}	180.5±3.3^{d}	257.6±4.0^{f}	102.7±3.5^{a}	169.2±1.6^{c}	229.0±8.8^{e}
***MO*$_{2sda\text{-}d}$ (h)**	78.8±3.0^{de}	57.9±1.3^{bc}	40.5±1.5^{a}	86.2±5.6^{e}	60.5±0.9^{c}	43.6±0.3^{ab}	88.9±5.9^{e}	64.1±2.4^{cd}	44.9±1.3^{ab}
***MO*$_{2sda\text{-}pd}$ (h)**	31.2±1.1^{d}	25.7±0.9^{cd}	17.0±0.4^{a}	29.3±0.7^{cd}	24.8±1.9^{bc}	18.4±0.1^{a}	37.7±1.8^{e}	28.3±1.1^{cd}	20.1±1.0^{ab}
***MO*$_{2scope}$ (*MO*$_{2sda\text{-}p}$ *MO*$_{2rmr\text{-}s}^{-1}$)**	1.30±0.02^{ab}	1.27±0.02^{a}	1.54±0.03^{ab}	1.37±0.03^{b}	1.31±0.03^{ab}	1.47±0.03^{ab}	1.45±0.06^{ab}	1.43±0.13^{ab}	1.52±0.04^{ab}
***MO*$_{2sda}$ (mg O_2 kg^{-1})**	1586±139^{ab}	1756±227^{a}	3371±200^{b}	1827±65^{ab}	1844±148^{ab}	2555±173^{ab}	2022±154^{a}	2299±587^{a}	2472±252^{a}
SDA_E (%)	18.1±1.0^{ab}	15.9±2.2^{a}	29.0±0.8^{b}	22.0±1.5^{ab}	18.1±1.3^{ab}	25.0±1.4^{ab}	24.5±2.8^{ab}	22.8±5.2^{ab}	26.6±1.4^{ab}
SDA co-ef (%)	11.5±0.9^{abc}	7.1±1.4^{a}	10.7±0.5^{abc}	12.8±0.6^{c}	8.6±0.8^{ab}	8.5±0.5^{ab}	7.4±0.2^{ab}	7.0±1.3^{a}	7.3±0.4^{ab}

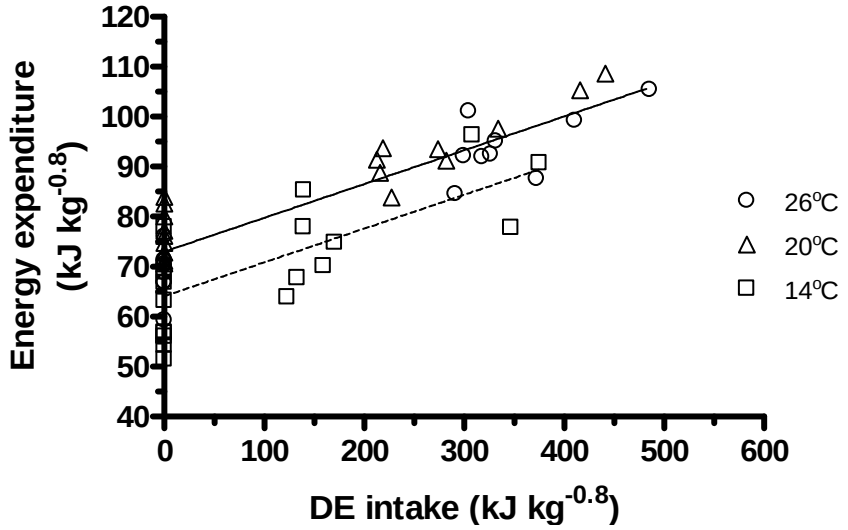

Figure 3.5. Total energy expenditure over the duration of the SDA response (kJ $kg^{-0.8}$) as a function of increasing DE intake (kJ $kg^{-0.8}$). Regression lines shown for 20 and 26 ^{o}C (global r^2=0.55; n=18; solid line) and 14 ^{o}C (r^2=0.45; n=9; dashed line). Refer to Eqs. (3.9) and (3.10) for regression parameter values. Energy expenditure at zero intake estimated from the proportion of cumulative RMR (n=27) over the SDA duration shown for comparison and not used to formulate regression lines.

3.5 Discussion

3.5.1 Influence of mass and temperature on RMR

In fish, the mass scaling exponent of resting metabolic rate has been shown to be approximately 0.8 (Winberg 1956; Clarke and Johnston 1999) although *b* is known to vary with temperature in some species (Beamish 1964; du Preez et al. 1986; Xie and Sun 1990; Hölker 2003). The influence of body size on the RMR of mulloway was also shown to vary depending on temperature which was reflected in *b* which varied from 0.73-0.85. However, as *b* did not vary significantly from 0.8, this value can therefore be considered as appropriate to describe the influence of body mass on the routine metabolism of mulloway over the temperature range used in this study. By constraining *b* important biological variability may be obscured

(Clarke and Johnston 1999), however, *a* then exclusively describes the influence of temperature, and experimental conditions, on metabolic rate and the error associated with *a* is considerably reduced (Table 3.3). Eqs. (3.1) and (3.2) provide predictions of the routine metabolic demand for O_2 by juvenile mulloway based on BW and temperature (14 to 26 ^{o}C) and give estimations which are very close to those published for this species using different size fish to those used in the current study (Fitzgibbon et al. 2007b; Chapter 2).

It is important to consider where in the metabolic scope of activity measurements are derived (i.e. from basal through to maximal metabolic rate). In mammals *b* is positively correlated with increasing levels of metabolic activity (Savage et al. 2004b; Weibel et al. 2004; White and Seymour 2005a) and similar trends were observed with mulloway in the current study; across all temperatures *b* increased with the increased metabolic activity of peak MO_2 associated with SDA. In mulloway the relationship between mass and metabolism appears to become less allometric and more isometric (i.e. as *b* approaches 1) with increasing levels of metabolic activity, particularly at lower temperatures. However, this relationship requires further validation as the size range of mulloway used in the present study may influence the value of *b*. The conditions in which data are derived and the size ranges of animals used are known to effect exponent values (White and Seymour 2005b) emphasizing the importance of standardizing experimental procedures, clearly defining the metabolic level being measured and, consequently, raising caution when attempting to make inter or intraspecific comparisons across different studies.

3.5.2 SDA response

3.5.2.1 SDA duration

The SDA responses in mulloway were typical of those exhibited by other fish and ectothermic species: metabolic rate increased following ingestion of feed and then gradually declined over a number of days with the duration of the SDA and peak time response markedly affected by both temperature and body size (Jobling and Davies 1980; Boyce and Clarke 1997; McCue 2006; Secor 2009). SDA durations of approximately 40 to 90 h for mulloway fall within the ranges reported for other temperate fish species (reviewed by McCue 2006) but are much lower than some Antarctic species which are reported at 240-390 h at ~0 ^{o}C (Boyce and Clarke 1997). Gastric evacuation time is strongly correlated with SDA duration (Jobling and Davies 1980) and this was indicated in mulloway at each temperature with the absence of feed in the digestive tract of fish sub-sampled at the conclusion of MO_2 readings. Although MO_2 readings at 14 ^{o}C were ended at 72 h, and before the full SDA duration was completed, the absence of feed combined with MO_2 rates which had returned to RMR levels at day 6 post-feeding lends support to our estimation of approximately 80-90 h SDA duration at this temperature.

3.5.2.2 SDA factorial scope

While body size clearly influences the overall SDA duration, it has been shown to have little effect on the post-prandial factorial scope in fish (Jobling and Davies 1980; Johnston and Battram 1993) which was also confirmed with mulloway in this study. Temperature is also considered not to have a large influence on factorial scope (Jobling and Davies 1980; Johnston and Battram 1993) however

mulloway were shown to have a significantly greater factorial scope at 26 ^{o}C than at 14 or 20 ^{o}C. The largest difference between temperature treatments however, although statistically significant, was on average 0.17. This tends to support the conclusions of other studies that the influence of temperature on factorial scope is quite small. It should be noted however, that factorial scope is a relative unit expressed as a multiple of RMR levels and large differences can be seen in peak MO_2 between treatments when expressed in absolute terms (Peck 1998; Figure 3.2). The factorial scope of mulloway demonstrated in the current study (1.3-1.5) is at the low end compared to those for other fish species which can range from 1.4 – 4.1 (see review by McCue 2006) however; there are several contributing reasons for this. Firstly, the magnitude of the SDA response is greatly affected by meal size (Hamada and Maeda 1983; Boyce and Clarke 1997; Fu et al. 2005). In this study mulloway ate approximately 2 % of their body weight which, although typical for this species fed to satiation on the type of feed used in this study (Chapter 4), may be considered small compared to other species such as southern catfish (*Silurus meridionalis*) which have a correspondingly higher factorial scope of 4.1 when the relative meal size is 24 % (Fu et al. 2005). Secondly, the factorial scope in the current study is reported relative to RMR which will vary among species depending on their normal resting or routine level of activity. Thirdly, factorial scope is sometimes reported relative to standard or basal metabolic rates (e.g. Beamish 1974; Chen et al. 2008) which will increase values. Lastly, our values for mulloway are derived from the models fitted to the data and will therefore slightly underestimate the maximal recorded values.

3.5.2.3 SDA coefficient and energy expenditure

At approximately 7-13 %, the average SDA coefficient for mulloway, i.e. the energy devoted to the SDA response as proportion of the energetic content of a meal, was within the range reported for most temperate fish species (6-23 %) (Pandian and Vivekanandan 1985; McCue 2006). The SDA coefficient is known to be influenced by body size (Beamish 1974), meal size (Carter and Brafield 1992; Fu et al. 2006), meal type (Secor and Boehm 2006) and, in the case of mulloway, the influence of body size on the SDA coefficient varied depending on temperature which was likely due to the corresponding interaction between body size and temperature on relative feed intake (Table 3.6). The influence of these variables on the SDA coefficient therefore makes direct comparison amongst other studies very difficult (Beaupre 2005; McCue 2006). Expressing total energy expenditure as a function of DE intake independent of body weight (Figure 3.5) perhaps gives a somewhat better insight into SDA energetics as it at least avoids the potential confounding caused by the allometric relationships associated with body mass and meal size inherent when making comparisons of coefficients derived from mass-specific data (Beaupre 2005). When expressed this way total energy expenditure by mulloway was shown to increase linearly with increasing DE intake. Temperature is generally considered to have little influence on SDA expenditure (see reviews by McCue 2006; Secor 2009) although temperature effects have been noted in some fish species (Guinea and Fernandez 1997; Peck et al. 2003; Luo and Xie 2008). Although values for $MO_{2sda\text{-}p}$, $MO_{2sda\text{-}pd}$ and $MO_{2sda\text{-}d}$ all differed among temperatures irrespective of the size class of mulloway, energy expenditure (kJ $kg^{-0.8}$) relative to intake (kJ $kg^{-0.8}$) was shown to be very similar at 20 and 26 ^{o}C and approximately only 9 kJ $kg^{-0.8}$ less at 14 ^{o}C. The absolute difference among these

temperatures remained constant because the DE utilization efficiency was approximately the same at all temperatures for any given quantity of feed (see also Chapter 4). The difference in magnitude however will decrease exponentially with increasing feed intake from approximately 12 % difference at zero intake to 8 % difference at an intake of 500 kJ $kg^{-0.8}$.

Mulloway are a eurythermal species typically found in warm-temperate to sub-tropical estuaries and near-shore waters (Harrison and Whitfield 2006; Silberschneider and Gray 2008) where temperatures of 20 or 26 ^{o}C are not uncommon (Harrison 2004; Harrison and Whitfield 2006). If metabolic rates are dependent on the temperature-sensitive properties of enzymes and cellular components which in turn determine thermal optima (Hochachka and Somero 2002), the similar net response on energy expended due to SDA by mulloway at 20 and 26 ^{o}C may be indicative of the biochemical rate processes operating within a thermal range suitable for normal metabolic function. In ectotherms there is a negative correlation between peak SDA metabolism and the duration of the SDA response which is dependant on temperature (McCue 2006). When peak SDA increases there is a corresponding decrease in SDA duration; the resultant net energy expenditure being similar (Wang et al. 2003; Secor et al. 2007; Luo and Xie 2008). This response was seen with mulloway and is typical of most temperature performance curves recorded for ectotherms (Angilletta et al. 2002) and demonstrates the trade off between the “specialist” (high narrow peak) and the “generalist” (low broad peak) metabolic responses (see Huey and Hertz 1984; Gilchrist 1995).

It is important to note that RMR represents the major proportion of the total energy expended during the SDA period (Tables 3.6), the greatest proportion of

SDA above RMR occurred at 26 ^{o}C accounting for approximately 27 % of the total energy expenditure and is likely to be related to the energetic cost incurred for increased protein turnover and synthesis (Houlihan et al. 1988; Brown and Cameron 1991). This indicates a greater potential for growth at this temperature which has been confirmed in other feeding studies with mulloway (Chapter 4). Although mulloway are a relatively sedentary species, the above values demonstrate a relatively high proportion of DE intake dedicated to maintaining routine metabolism and, although comparable to values reported for some teleost species (Carter and Brafield 1992; Xie et al. 1997; Owen 2001), indicates a moderate scope for growth particularly when compared to the high-energy-demand species such as the yellowtail (*Seriola quinqueradiata*) (Watanabe et al. 2000a) and southern bluefin tuna (*Thunnus maccoyii*) (Fitzgibbon et al. 2007a).

3.5.3 Conclusion

RMR and SDA were shown to represent significant energetic costs in the overall energy budget of mulloway. Many of the SDA indices measured in this study were within the ranges of those reported for other temperate marine fish; however, we have demonstrated that these values are not fixed and are highly dependent on temperature, body size and feed intake. We have therefore presented equations as a function of these variables which will allow greater accuracy in the bioenergetic modeling of metabolic expenditure for this species.

If the greatest proportion of SDA energy is channeled towards the biochemical processes that contribute to growth (Wieser 1994; Willmer et al. 2000), it would then appear that the growth rate potential of mulloway may be limited at 14

and 20^{o}C (compared to 26 ^{o}C). The gathering body of information on the temperature responses of various metabolic, growth and preference parameters measured for mulloway thus far indicate that a temperature of approximately 26±2 ^{o}C to be optimal for growth and metabolic function (Bernatzeder and Britz 2007; Collett et al. 2008; Chapter 2, 4). It is not known what the SDA response in mulloway is at temperatures above 26 ^{o}C; however, there are indications the SDA coefficient may be reduced in some ectotherms at elevated temperatures (Cui and Wootton 1988; Toledo et al. 2003).

Chapter 4

Protein and Energy Utilization and the Requirements for Maintenance in Juvenile Mulloway[3]

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4.1 Abstract

This study described the digestible protein (DP) and digestible energy (DE) utilization in juvenile mulloway and determined the requirements for maintenance. This was achieved by feeding triplicate groups of fish weighing 40 or 129 g held at two temperatures (20 ^{o}C or 26 ^{o}C) a commercial diet (21.4 g DP MJ DE^{-1}) at four different ration levels ranging from 0.25% initial body weight to apparent satiation over 8 weeks. Weight gain and protein and energy retention increased linearly with increasing feed intake. However, energy retention efficiency (ERE) and protein retention efficiency (PRE) responses were curvilinear with optimal values, depending on fish size, approaching or occurring at satiated feeding levels. Maximum predicted PRE was affected by body size but not temperature; PRE values were 0.50 and 0.50 for small mulloway and 0.41 and 0.43 for large mulloway at 20 ^{o}C and 26 ^{o}C respectively. ERE demonstrated a similar response; with values of 0.42 and 0.43 for small and 0.32 and 0.34 for large mulloway at 20 ^{o}C and 26 ^{o}C respectively. Utilization efficiencies for growth based on linear regression for DP

(0.58) and DE (0.60) were independent of fish size and temperature. The partial utilization efficiencies of DE for protein (k_p) and lipid (k_l) deposition estimated using a factorial multiple regression approach were 0.49 and 0.75 respectively. Maintenance requirements estimated using linear regression were independent of temperature for DP (0.47g DP $kg^{-0.7}$ day^{-1}) while maintenance requirements for DE increased with increasing temperature (44.2 to 49.6 kJ DE $kg^{-0.8}$ day^{-1}). Relative feed intake was greatest for small mulloway fed to satiation at 26 ^{o}C and this corresponded to a greater increase in growth. Large mulloway fed to satiation ate significantly more at 26 ^{o}C but did not perform better than the corresponding satiated group held at 20 ^{o}C. Mulloway should be fed to satiation to maximize growth potential if diets contain 21.4 g DP MJ DE^{-1}.

4.2 Introduction

The utilization of digestible protein (DP) and digestible energy (DE) by growing animals is dependant on the composition of the diet and the efficiency with which deposition occurs (van Milgen and Noblet 2003; Schroeder and Titgemeyer 2008). In fish, patterns of protein deposition with increasing levels of DP intake vary considerably between species, diet and experimental conditions and responses have been described as linear (Lupatsch et al. 2001a; Fournier et al. 2002; Lupatsch and Kissil 2005; Peres and Oliva-Teles 2005) or curvilinear (Huisman et al. 1979; McGoogan and Gatlin 1998; Watanabe et al. 2000b; Bureau et al. 2006). These responses indicate that the utilization efficiencies are either constant or tend to plateau with increasing protein intake. Unfortunately, such variations emphasize the need to determine nutrient retention profiles and utilization efficiencies of growing

fish on a species by species basis. Understanding how nutrients are utilized is an essential step towards developing bioenergetic models that predict growth responses, feeding requirements and nutrient losses to the environment (Bureau et al. 2002).

The concept of maintenance requirements is one that may be considered as paradoxical with regard to growing animals but it is a concept that has proved useful for animal nutritionists because it allows the partitioning of production and maintenance costs based on the assumption that the two are additive (van Milgen et al. 2000; Bureau et al. 2002). Maintenance DE requirements for fish have been shown to range from 32 – 77 kJ DE $kg^{-0.8}$ day^{-1} (Watanabe et al. 2000a; Lupatsch and Kissil 2005) and vary depending on temperature, species and fish size. Published maintenance requirement values for DP are less common in the literature but values of 0.45 - 0.96 g DP $kg^{-0.7}$ day^{-1} have been recorded (Lupatsch et al. 1998; Lupatsch and Kissil 2003; Peres and Oliva-Teles 2005; Glencross 2008).

The objectives of this study were to determine i) the protein and energy utilization responses to increasing DE and DP intake, ii) the efficiencies of DP and DE utilization, and iii) the maintenance requirements of juvenile mulloway. This was achieved using two size classes of mulloway (40 or 129 g) at two temperatures (20 ^{o}C or 26 ^{o}C).

4.3 Materials and methods

4.3.1 Experiment design

The protein and energy utilization of mulloway was tested by feeding four different ration levels ranging from 0.25 % of initial body weight (ibw) using a

commercial diet (Ridley AquaFeed Pty. Ltd., Narangba, Qld. Australia) to two size treatments (small or large; ibw (mean±SD) = 40.2±5.7 g and 129.3±17.2 g) at two temperatures (20 or 26 ^{o}C). The experiment was run over 8 weeks using fish produced at the New South Wales Department of Primary Industries, Port Stephens Fisheries Institute (PSFI). Fish were stocked into 200 l white opaque tapered cylindrical tanks (Dimensions: Top diameter = 78 cm; Bottom diameter = 68 cm; Height = 55 cm) at 40 small or 12 large fish tank^{-1}. Mulloway are a gregarious species and stocking densities were chosen to optimize growth potential (Appendix 1). Each size and ration treatment were randomly assigned to triplicate tanks within each temperature treatment with each tank constituting an experimental unit.

4.3.2 Experiment system

The experiment system consisted of two separate 1700 l recirculating bio-filtration units each supplying 24 x 200 l replicate tanks (each unit total volume ≈ 6500 l). The temperature for each unit was controlled with a chiller and immersion heater in an antagonistic mode which allowed precise temperature control of ±0.5 ^{o}C of the set temperature. All fish were initially stocked at 23 ^{o}C and the temperature adjusted 1 ^{o}C day^{-1} until the experiment temperatures were reached. Flow to each tank was approximately 4 l min^{-1} and orientated to create a weak centripetal current which allowed the retention of feed pellets in the tank while removing faeces via a central upright 32 mm diameter pvc overflow pipe which was fixed approximately 1 cm off the bottom of each tank. Black plastic sheets were placed around each tank and across the top front half to minimise disturbance. All tanks were exposed to indirect natural light (photoperiod 13L:11D). Ammonium

(NH_4^+) (<0.1 mg l^{-1}), dissolved oxygen (>5.0 mg l^{-1}), pH (7.5 - 7.8) and salinity (30 - 34 ppt) were monitored regularly throughout the duration of the experiment.

4.3.3 Feed and feeding

Proximate composition of the diet was (g kg^{-1}): 961 dry matter; 90 ash; 455 crude protein; 187 fat and 22.2 MJ kg^{-1} gross energy. The apparent digestibility co-efficient for protein was 0.88 and energy was 0.84 (Booth, unpublished data 2008).

Fish were fed 6 mm extruded sinking pellets from once up to four times daily depending on ration size to improve the likelihood of all fish obtaining pellets in the lower ration treatments or to maximize voluntary daily feed intake in the higher ration treatments. Any uneaten pellets were counted then siphoned from tanks approximately 45 min after initial feeding. Total daily feed intake was adjusted accordingly (predetermined individual pellet weight mean±SD = 0.21±0.02 g; n = 202). The commercial feed used in this study had excellent water stability and it was assumed nutrient losses through leaching were insignificant.

4.3.4 Sample preparation and analyses

Fish were fasted for 48 h prior to sampling for carcass composition. Initial representative samples of 10 fish of each size class were collected before the start of the experiment and frozen (-20 ^oC). At the conclusion of the feeding trial all fish were euthanized with an overdose of benzocaine (ethyl-*p*-aminobenzoate), weighed and stored frozen for compositional analyses. Compositional changes in energy, lipid, ash and moisture were estimated by comparing the initial fish carcass samples with those

from the feeding trial. Estimates of initial whole body protein were based on the compositional value of 191.4 g kg^{-1}. This value was derived in a separate study (Chapter 6) to establish the compositional profile of mulloway where several hundred fish were sampled in groups representing size classes ranging from 2 – 2100 g (n = 3 – 100 fish per group depending on size). Using this value was necessary because of a data transcription error with the original initial values for protein composition. All other initial compositional constituents appeared to be true representations of the initial carcass composition. Assuming a fixed initial whole body protein composition is valid as the proportional relationship between body protein and body weight in fish is known to be relatively constant (Shearer 1994; Lupatsch et al. 1998; Dumas et al. 2007).

Whole carcass composition was determined by placing the weighed fish into 5 l glass beakers, covering with aluminum foil and then autoclaving for 99 min at 121 ^{o}C. After cooling to room temperature any changes in weight were accounted for and assumed to be changes in moisture content. The samples were then homogenised *in situ* with a hand blender and a sub-sample taken for dry matter determination. A portion of the remaining homogenate was then transferred to plates and oven dried at approximately 80 ^{o}C. The desiccated samples were then finely ground in a laboratory blender and analysed in accordance with AOAC (2005). Protein was calculated from total nitrogen based on N x 6.25 using the Dumas method. Dry matter was calculated gravimetrically after oven drying at 105 ^{o}C. Ash was calculated gravimetrically. Gross energy was determined by adiabatic bomb calorimetry. Fat was measured gravimetrically after chloroform-methanol extraction.

The following performance indices were calculated for each treatment group:

Daily weight gain (g $fish^{-1}$ day^{-1}) = Final body weight (fbw) – ibw / number of days

Daily protein gain (g $fish^{-1}$ day^{-1}) = Final carcass protein content – 0.1914 x initial sample bw / number of days

Daily energy gain (kJ $fish^{-1}$ day^{-1}) = Final carcass energy content – initial carcass energy content / number of days

Feeding Efficiency (FE) = Weight gain / Total feed intake

Protein Retention Efficiency (PRE) = Protein gain / Total DP intake

Energy Retention Efficiency (ERE) = Energy gain / Total DE intake

Data are also expressed as geometric mean body weights (GMBW) and scaled using the metabolic body weight exponent value of 0.7 for protein retention data and 0.8 for energy retention data (after Brett and Groves 1979; Lupatsch et al. 1998).

4.3.5 Data analyses

The effects of varying feed ration (fixed; 4 levels) at different temperatures (fixed; 2 levels) on performance indices and compositional data were tested with 2-way ANOVA for each size class (fbw was not a significant co-variant for analyses of compositional data). Formal comparisons using ANOVA were not made between

sizes as ration levels were not orthogonal. Normality of the data was checked with skewness, kurtosis and omnibus normality tests. Assumptions of homogeneity of variances were tested using modified Levenes' equal variance test. Tukey-Kramer test was used for *a posteriori* multiple comparison of means on significant terms. All results were regarded as significant at $p<0.05$. Data were normally distributed for all performance indices for large fish at both temperatures. Data were non-normally distributed for FE, PRE and ERE for small fish at 20 ^{o}C and for FE and PRE for small fish at 26 ^{o}C; these data could not be normalized. ANOVA was still performed and due regard should be given to subsequent interpretations of the results. All performance indices and carcass composition data variances were homogenous (Levene's; $p>0.05$).

Nonlinear regression was applied to PRE data where the asymptote of the quadratic function was considered as the optimal daily dietary DP intake giving the maximum predicted PRE value (Shearer 2000).

Daily maintenance requirements for dietary protein and energy at 20 and 26 ^{o}C were estimated using linear regression of daily intake and gain where the *x*-intercept describes the daily requirement for maintenance, the slope of regression describes the utilization efficiency and the reciprocal of the slope describes the nutrient cost of production.

Partial energy efficiencies for protein and lipid deposition were further investigated using the factorial method based on Kielanowski (1965) where DE intake can be partitioned as:

$$\text{DE intake (kJ kg}^{-b}\text{ day}^{-1}) = DE_m + PD/k_p + LD/k_l$$

Where DE_m = daily maintenance energy requirement (kJ DE kg^{-b} day^{-1}); PD = energy retained as protein (kJ day^{-1}); k_p = partial energy efficiency for protein deposition; LD = energy retained as lipid (kJ day^{-1}); k_l = partial energy efficiency for lipid deposition. The metabolic weight exponent (*b*) was estimated simultaneously with the above parameters which gave values of 0.817(±0.05) and 0.784(±0.07) at 20 and 26 ^oC respectively. These values were statistically indistinguishable from the common inter-specific exponent value of 0.8 applied to energy metabolism of teleost fishes (Clarke and Johnston 1999). A fixed exponent value of 0.8 was therefore used in the model to estimate parameters DE_m, k_p and k_l. Least squares regression method assumes normally distributed residuals therefore robust multiple regression was used which minimizes the influence of outliers on coefficient estimates (Montgomery and Peck 1992).

The heat of combustion values for protein and lipid required to determine PD and LD (above) were also derived using robust multiple regression however with the *y*-intercept term removed from the regression model:

RE (kJ) = *a* x PD + *c* x L*D*

Where RE = retained energy (kJ); *a* = protein heat of combustion (kJ g^{-1}); *c* = lipid heat of combustion (kJ g^{-1}); PD = protein deposition (g); LD = lipid deposition (g)

4.4 Results

Survival at the end of the experiment was 100 %. The effect of ration on weight, protein and energy gain in small mulloway varied significantly depending

on temperature (Table 4.1). Weight, protein and energy gain were significantly higher at the highest ration level at 26^{o}C for small mulloway (Table 4.2). Ration level but not temperature affected the weight, protein and energy gain of large mulloway (Table 4.1). Large mulloway fed to satiation at 26 ^{o}C also demonstrated higher gain but this was significantly different from the satiated treatment at 20 ^{o}C only for protein retention (Table 4.2). Relative feed intake (RFI) was significantly greater at the satiated level for small mulloway at 26 ^{o}C (p<0.05) with 6.1±0.2 g kg$^{-0.7}$ day^{-1} consumed compared to 5.1±0.1 g kg$^{-0.7}$ day^{-1} at 20 ^{o}C. RFI was also significantly greater for large mulloway at 26^{o}C (p<0.05) with 5.4±0.1 g kg$^{-0.7}$ day^{-1} consumed compared to 4.4±0.2 g kg$^{-0.7}$ day^{-1} at 20 ^{o}C.

Table 4.1. Two-way ANOVA on performance indices and carcass composition (as received basis) for both small and large mulloway. ns = not significant at p<0.05, * = p<0.05, ** = p<0.01

	Source of Variation					
Variable	**Small**			**Large**		
	Temp.	**Ration**	**Interaction**	**Temp.**	**Ration**	**Interaction**
Performance Indices						
Weight gain	ns	**	**	ns	**	ns
Protein gain	ns	**	**	ns	**	ns
Energy gain	ns	**	**	ns	**	ns
FE	**	**	**	*	**	ns
PRE	ns	**	ns	ns	**	**
ERE	ns	**	ns	ns	**	ns
Carcass Composition						
Protein	**	ns	ns	**	**	ns
Lipid	ns	**	**	ns	**	**
Moisture	**	**	ns	**	**	ns
Ash	**	**	**	**	**	ns
Energy	ns	**	ns	ns	**	ns

4.4.1 Utilization efficiencies and maintenance requirements

The effect of ration on FE varied significantly depending on temperature for small mulloway while both ration and temperature significantly, but independently, affected FE for large mulloway (Table 4.1). PRE was significantly influenced by ration but not temperature in small mulloway while the influence of ration on PRE in large mulloway was dependent on temperature (Table 4.1). The interaction occurred because of the relatively better PRE value at 26 ^{o}C compared to 20 ^{o}C when large mulloway were fed the lowest ration level (Table 4.2). ERE in small and large mulloway was significantly influenced by ration but not temperature (Table 4.1).

PRE in mulloway demonstrated a curvilinear response to increasing DP intake. Temperature had little effect on the maximum predicted PRE provided that DP intake was increased with increasing temperature (Figure 4.1). Body size, however, did influence PRE (Table 4.2) with the maximum predicted PRE for small mulloway 0.50 and 0.50 and large mulloway 0.41 and 0.43 at 20 ^{o}C and 26 ^{o}C respectively. The daily protein intake to achieve maximum predicted PRE was 1.7 and 2.0 g DP $kg^{-0.7}$ day^{-1} at 20 ^{o}C and 26 ^{o}C respectively (Figure 4.1). The relationship between digestible protein intake (g DP $kg^{-0.7}$ day^{-1}) and PRE can be described as:

$$\text{PRE (20 }^{o}\text{C)} = -0.542 + 1.162\text{DP} - 0.337\text{DP}^2 \qquad (r^2 = 0.89,\ n = 24) \qquad (4.1)$$

$$\text{PRE (26 }^{o}\text{C)} = -0.418 + 0.887\text{DP} + -0.222\text{DP}^2 \qquad (r^2 = 0.87,\ n = 24) \qquad (4.2)$$

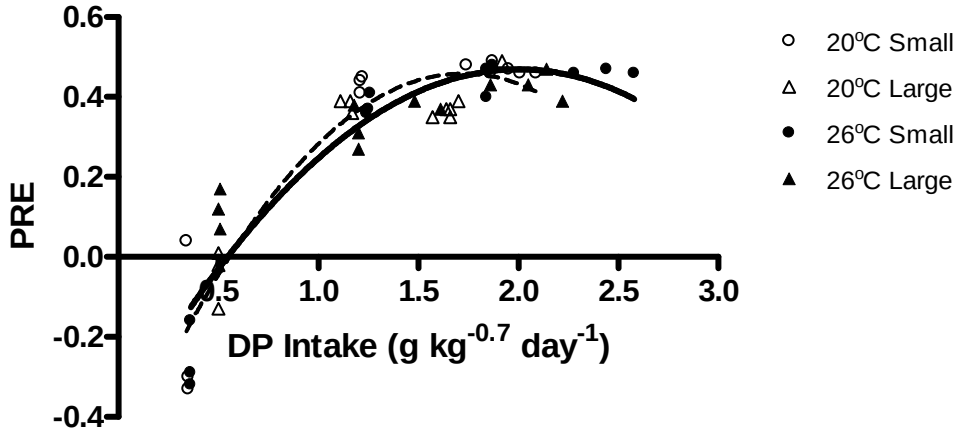

Figure 4.1. Effect of digestible protein intake (g $kg^{-0.7}$ day^{-1}) on PRE at 20 ^{o}C (dashed line) and 26^{o} C (solid line).

Table 4.2. Summary of performance indices and carcass composition of small and large mulloway held at 20°C or 26°C. Tukey-Kramer test on means between temperature treatments within each size class. Means sharing superscripts are not significantly different (p>0.05).

	20 °C Small Feed Ration				26 °C Small Feed Ration				20 °C Large Feed Ration				26 °C Large Feed Ration			
	1	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Performance Indicies																
Initial Body Weight (g)	40.2	40.0	40.3	39.6	39.9	40.3	40.4	40.8	124.6	126.1	127.5	134.1	132.1	131.7	131.0	127.5
Final Body Weight (g)	39.2^{a}	59.1^{b}	73.3^{c}	74.7^{c}	35.7^{a}	54.4^{b}	70.5^{c}	83.7^{d}	121.8^{a}	150.7^{c}	163.6^{cd}	180.3^{de}	127.9^{ab}	147.4^{bc}	164.5^{cde}	184.3^{e}
Gain (g/fish/day)	-0.02^{a}	0.33^{c}	0.57^{d}	0.61^{d}	-0.07^{a}	0.24^{b}	0.52^{d}	0.74^{e}	-0.05^{a}	0.43^{bc}	0.63^{bd}	0.81^{de}	-0.07^{a}	0.27^{b}	0.59^{bcd}	1.00^{e}
Feed Intake (g/fish/day)	0.09	0.37	0.59	0.66	0.09	0.37	0.60	0.84	0.29	0.72	1.05	1.20	0.31	0.75	1.08	1.44
FE	-0.20^{b}	0.90^{c}	0.96^{c}	0.92^{c}	-0.79^{a}	0.66^{c}	0.87^{c}	0.88^{c}	-0.17^{a}	0.60^{bc}	0.60^{bc}	0.66^{c}	-0.24^{a}	0.36^{b}	0.54^{bc}	0.69^{c}
DP Intake (g/fish/day)	0.04	0.15	0.24	0.26	0.04	0.15	0.24	0.33	0.12	0.29	0.42	0.48	0.12	0.30	0.43	0.57
Protein Retention (g/fish/day)	-0.01^{a}	0.06^{b}	0.11^{c}	0.12^{c}	-0.01^{a}	0.06^{b}	0.11^{c}	0.15^{d}	-0.01^{a}	0.11^{bc}	0.15^{bcd}	0.20^{d}	0.01^{a}	0.10^{b}	0.17^{cd}	0.25^{e}
PRE	-0.20^{a}	0.43^{b}	0.48^{b}	0.46^{b}	-0.26^{a}	0.38^{b}	0.45^{b}	0.46^{b}	-0.05^{a}	0.38^{c}	0.36^{c}	0.41^{c}	0.12^{b}	0.32^{c}	0.40^{c}	0.43^{c}
DE Intake (kJ/fish/day)	1.71	6.80	11.08	12.28	1.69	6.84	11.09	15.55	5.37	13.36	19.54	22.35	5.70	14.01	20.09	26.81
Energy Retention (kJ/fish/day)	-1.09^{a}	2.02^{b}	4.35^{c}	4.53^{c}	-1.39^{a}	1.67^{b}	3.98^{c}	6.04^{d}	-3.20^{a}	1.58^{bc}	4.05^{c}	6.13^{cd}	-3.05^{a}	0.37^{b}	3.97^{c}	7.87^{d}
ERE	-0.64^{a}	0.30^{b}	0.39^{b}	0.37^{b}	-0.82^{a}	0.24^{b}	0.36^{b}	0.39^{b}	-0.34^{a}	0.22^{bc}	0.28^{c}	0.34^{c}	-0.28^{a}	0.13^{a}	0.27^{bc}	0.35^{c}
Carcass Composition[†]																
Moisture (%)	73.3^{c}	71.1^{b}	69.7^{ab}	69.7^{ab}	71.3^{b}	69.8^{ab}	69.6^{ab}	68.9^{a}	73.2^{e}	71.3^{cd}	70.3^{bc}	69.6^{ab}	72.0^{de}	70.7^{bcd}	68.8^{a}	68.3^{a}
Protein (%)	18.6^{a}	19.2^{ab}	19.5^{ab}	19.6^{ab}	19.6^{b}	20.1^{b}	19.7^{b}	20.0^{b}	19.3^{a}	20.2^{ab}	20.2^{ab}	20.5^{bc}	20.4^{bc}	20.8^{bc}	21.2^{c}	20.8^{bc}
Lipid (%)	2.4^{b}	5.2^{c}	6.5^{d}	6.7^{de}	1.5^{a}	5.3^{c}	6.7^{de}	7.4^{e}	2.4^{ab}	3.9c	5.0^{d}	5.6^{de}	2.0^{a}	3.3^{bc}	5.0^{d}	6.5^{e}
Ash (%)	6.4^{d}	5.1^{bc}	4.8^{ab}	4.8^{ab}	8.2^{e}	5.5^{c}	4.7^{ab}	4.3^{a}	5.9^{cd}	5.4^{ab}	5.3^{ab}	5.1^{a}	6.4^{d}	5.9^{cd}	5.6^{bc}	5.1^{ab}
Energy (MJ kg^{-1})	5.04^{a}	6.38^{b}	7.00^{cd}	6.96^{bcd}	4.97^{a}	6.57^{bc}	6.99^{cd}	7.35^{d}	5.13^{a}	6.02^{bc}	6.46^{cd}	6.76^{de}	5.33^{a}	5.93^{b}	6.54^{de}	6.92^{e}

[†] Initial carcass composition (small, large). Moisture; 72.3, 71.6. Protein (fixed value); 19.1, 19.1. Lipid; 5.5, 4.0. Ash; 3.6, 5.5. Energy; 6.48, 5.86

Energy retention efficiency demonstrated a similar curvilinear response (Figure 4.2) with increasing DE intake and body weight greatly influencing the efficiency values (Table 4.2). Temperature did not affect the maximum predicted ERE values with 0.42, 0.44 and 0.32, 0.34 for small and large mulloway at 20 ^{o}C and 26 ^{o}C respectively. The relationship between digestible energy intake (kJ DE kg$^{-0.8}$ day^{-1}) and ERE can be described as:

$$\text{ERE } (20\ ^{o}\text{C}) = -1.121 + 0.025\text{DE} - 0.0001\text{DE}^2 \qquad (r^2 = 0.94,\ n = 24) \qquad (4.3)$$

$$\text{ERE } (26\ ^{o}\text{C}) = -1.128 + 0.028\text{DE} - 0.0001\text{DE}^2 \qquad (r^2 = 0.97,\ n = 24) \qquad (4.4)$$

The daily energy intake (kJ DE kg$^{-0.8}$ day^{-1}) to achieve a maximum predicted ERE was 107 kJ DE kg$^{-0.8}$ day^{-1} at 20^{o}C and 125 kJ DE kg$^{-0.8}$ day^{-1} at 26^{o}C (Figure 4.2).

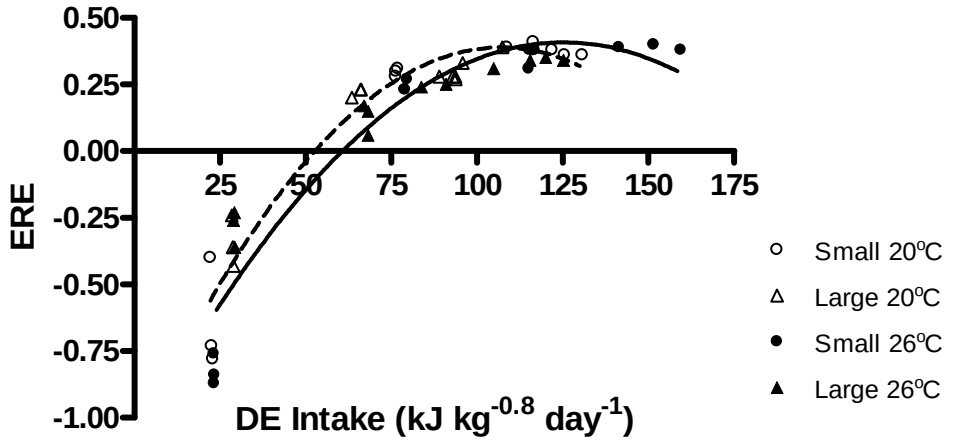

Figure 4.2. Effect of digestible energy intake (kJ kg$^{-0.8}$ day^{-1}) on ERE at 20 ^{o}C (dashed line) and 26 ^{o}C (solid line).

There was no temperature (p>0.5) or size effect (p>0.1) between the slopes of regression when considering the relationship between protein intake (g DP kg$^{-0.7}$ day^{-1}) and protein gain (g kg$^{-0.7}$ day^{-1}) (Figure 4.3). A pooled value of 0.58±0.02

describes the utilization efficiency of protein for mulloway at 20 – 26 ^{o}C. The relationship between protein gain (g kg$^{-0.7}$ day^{-1}) and DP intake (g kg$^{-0.7}$ day^{-1}) was linear and can be described as:

Protein gain = 0.581DP - 0.272 (r^2 = 0.97, n = 48) (4.5)

The corresponding cost of DP per unit of protein gain was 1.72 g g^{-1}. Temperature did not have a significant effect on maintenance requirements for protein with the greatest difference of <0.1 g fish^{-1} (Figure 4.3). Estimates of maintenance protein requirements for mulloway held at 20 – 26 ^{o}C are 0.47 g DP kg$^{-0.7}$ day^{-1}.

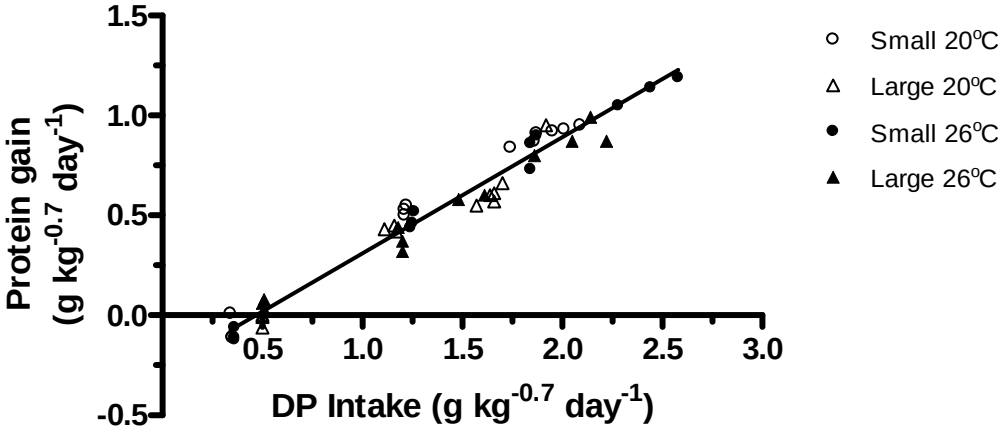

Figure 4.3. Effect of digestible protein intake (g DP kg$^{-0.7}$ day^{-1}) on protein gain (g kg$^{-0.7}$ day^{-1}).

There was no temperature (p>0.1) or size effect (p>0.5) between the slopes of regression when considering the relationship between energy intake (kJ DE kg$^{-0.8}$ day^{-1}) and energy gain (kJ DE kg$^{-0.8}$ day^{-1}) (Figure 4.4). A pooled value of 0.60±0.01 describes the utilization efficiency of energy for mulloway at 20 – 26 ^{o}C.

The corresponding cost of DE per unit of energy gain is 1.66 kJ kJ^{-1}. The relationship between energy intake (kJ $kg^{-0.8}$ day^{-1}) and energy gain (kJ $kg^{-0.8}$ day^{-1}) was linear and can be described as:

Energy gain (20 ^{o}C) = 0.604DE - 26.689 (r^2 = 0.98, n = 24) (4.6)

Energy gain (26 ^{o}C) = 0.591DE – 29.302 (r^2 = 0.98, n = 24) (4.7)

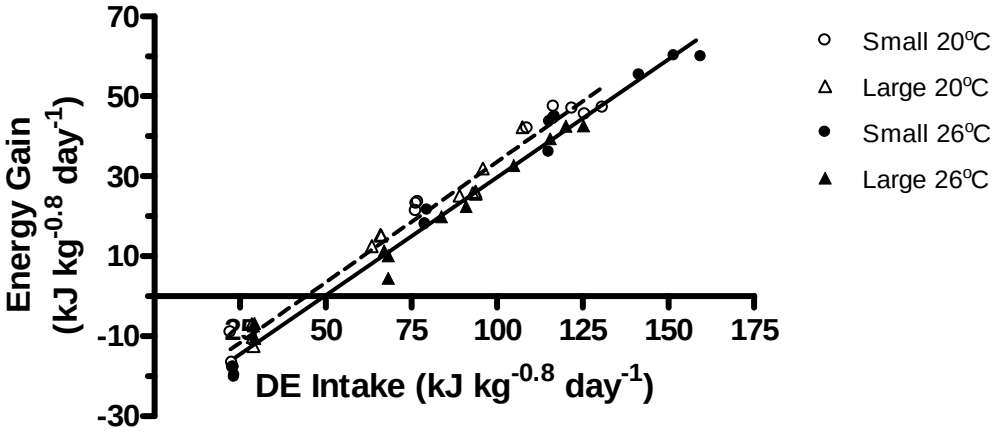

Figure 4.4. Effect of digestible energy intake (kJ $kg^{-0.8}$ day^{-1}) on energy gain (kJ $kg^{-0.8}$ day^{-1}). Dashed lines = 20 ^{o}C; Solid line = 26 ^{o}C

The maintenance requirements for energy (kJ DE $kg^{-0.8}$ day^{-1}) varied significantly depending on temperature (p<0.005) (Figure 4.4). Estimates of maintenance energy requirements were 44.2 and 49.6 kJ DE $kg^{-0.8}$ day^{-1} at 20 ^{o}C and 26 ^{o}C respectively.

Heat of combustion values derived using robust multiple regression analysis were 22.9 and 37.0 kJ g^{-1} for protein and lipid respectively. Partial energy efficiencies estimated using the factorial method were k_p = 0.49±0.09 and k_l = 0.75±0.19. Therefore, based on k_p, k_l and the heat of combustion values for protein

and lipid the energetic cost to mulloway to deposit 1 g of protein is 46.73 kJ and 1 g of lipid is 50.0 kJ.

DE_m was estimated at 43.0±3.8 kJ $kg^{-0.8}$ day^{-1} using the factorial method and compared well with estimates of 46.60±1.21 kJ DE $kg^{-0.8}$ at zero energy gain (*x*-intercept) derived using linear regression of combined temperature and size data (Figure 4.4).

Figure 4.5 shows the response of energy gain as a function of DE intake partitioned between protein energy (PE) and lipid energy (LE) where LE is calculated as the difference between total energy gain and PE gain and assumes no other contributing non-protein energy. There was no significant difference found between the slopes ($p>0.1$) when comparing PE and LE. There was no significant difference found when comparing the slopes ($p>0.1$) and *y*-intercepts ($p>0.1$) between PE deposition at 20 ^oC or 26 ^oC. A common linear regression can describe the relationship between PE deposition (kJ $kg^{-0.8}$ day^{-1}) and DE intake (kJ $kg^{-0.8}$ day^{-1}) at these temperatures:

$$\text{PE gain} = 0.288\text{DE} - 8.213 \quad (r^2 = 0.98, n = 48) \qquad (4.8)$$

There was no temperature effect between slopes ($p>0.5$) with regard to LE deposition however the *y*-intercepts differed significantly ($p<0.0001$). The relationship between LE deposition (kJ $kg^{-0.8}$ day^{-1}) and DE intake (kJ $kg^{-0.8}$ day^{-1}) can be described as:

$$\text{LE gain (20 }^oC) = 0.304\text{DE} - 17.960 \quad (r^2 = 0.97, n = 24) \qquad (4.9)$$

$$\text{LE gain (26 }^oC) = 0.310\text{DE} - 21.280 \quad (r^2 = 0.98, n = 24) \qquad (4.10)$$

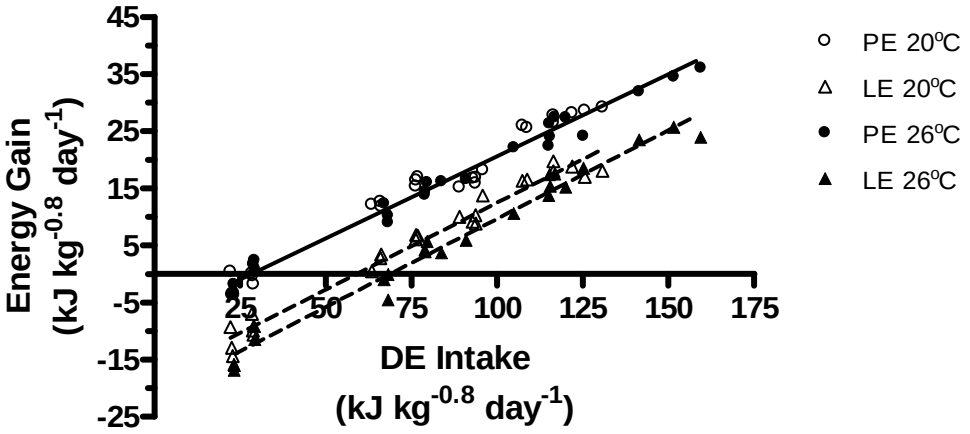

Figure 4.5. Partial retained energy as protein (PE; solid line) or lipid (LE; dashed line) as a function of increasing DE intake at 20 and 26 ^{o}C.

4.4.2 Effect of ration level and temperature on carcass composition

Protein content was significantly lower in the 20 ^{o}C treatment compared to the 26 ^{o}C treatment for both size mulloway while large mulloway protein content was also significantly affected by ration level although this occurred independent of temperature (Table 4.1). The average overall difference between temperature treatments within sizes for protein content, although statistically significant, was <10 g kg^{-1}.

Energy content was significantly affected by ration level but not temperature (Table 4.1) and there was a trend for energy content to increase with increasing ration (Table 4.2).

Lipid content generally increased with increasing ration level. The effect of feed ration on lipid composition varied significantly with temperature (Table 4.1) and the interaction occurred because lipid content at the lowest rations (Ration level 1 for small fish; Ration level 1 and 2 for large fish) was, on a relative basis, less in

the 26 ^{o}C treatment compared to the 20 ^{o}C treatment while at the higher ration levels the opposite occurred; lipid content was greater in the 26^{o}C treatment (Table 4.2).

Ash content in small mulloway demonstrated a similar, but opposite, response to lipid content (Table 4.2). Ash content in large mulloway tended to be higher at 26 ^{o}C and decrease with increasing ration level (Table 4.2).

Both temperature and ration level significantly, but independently, affected moisture content in small and large mulloway (Table 4.1).

4.5 Discussion

Understanding how growth is affected by the ration level of a particular diet is important in optimizing feeding strategies for aquaculture species. A curvilinear response dictates that feeding restricted rations will maximize feeding efficiencies while reducing waste outputs and increasing overall cost effectiveness. Conversely a linear response determines feeding to satiation to achieve optimal growth and feeding efficiencies. Growth and protein deposition in mulloway demonstrated a linear response (Figure 4.3) while optimal retention efficiencies, depending on size, approached or were at satiated intake levels (Figure 4.1). The largest difference in PRE between the predicted optimal and satiated DP intake level occurred with small mulloway at 26^{o}C with a difference of only 0.07 g DP fish^{-1} day^{-1}. The commercial diet used in this study, which is also commonly used by farmers in Australia, should therefore be fed to satiation to maximize growth potential and feeding efficiencies in mulloway.

At 0.60, the energy utilization efficiency of mulloway is within the range

reported for other fish species (0.4 - 0.7; see Bureau et al. 2006). No significant differences were found between the energy utilization efficiencies of the two size classes of mulloway or temperatures used in this study. These observations have been similarly demonstrated for European seabass (*Dicentrarchus labrax*) (Lupatsch et al. 2001a), Asian seabass (also known as barramundi, *Lates calcarifer*) (Lupatsch and Kissil 2003) and rainbow trout (*Oncorhynchus mykiss*) (Azevedo et al. 1998). However, it may be that the ranges between the sizes and temperatures studied were not sufficient to observe a shift in utilization efficiencies. Glencross (2008) demonstrated an improvement in the energy utilization efficiencies for growth with increasing size of barramundi of 0.61 for 15 g fish to 0.76 for 410 g fish although the regression model appeared heavily influenced by the data set from the satiated group of small fish in that study. This marked difference in the utilization efficiency of dietary energy with size has important implications in bioenergetic modelling and feed formulations for the species and warrants further investigation.

The protein utilization efficiency of mulloway was 0.58 and independent of temperature and size. Similar values and temperature effects have been demonstrated with European seabass (0.52; 20-26 ^{o}C) (Lupatsch et al. 2001a), barramundi (0.49-0.51; 21-30 ^{o}C) (Lupatsch and Kissil 2003; Glencross 2008) and white grouper (*Epinephelus aeneus*) (0.54; 22-27 ^{o}C) (Lupatsch and Kissil 2005). Peres and Oliva-Teles (2005) demonstrated a protein utilization efficiency of 0.64 for European seabass which is higher than that reported by Lupatsch et al. (2001a). Differences in protein utilization efficiencies can, in part, be accounted for by the amino acid composition of the diet (Sandberg et al. 2005b).

The partial energy retention efficiency of PE in mulloway (k_p = 0.49) was

similar to and falls within the SEM ranges of k_p values recorded for other carnivorous fish species such as gilthead seabream (*Sparus aurata*) (0.53), European seabass (0.53), white grouper (0.56) (Lupatsch et al. 2003b) and rainbow trout (0.53) (Azevedo et al. 2005).

k_l values for mulloway (0.75) are directly comparable to gilthead seabream (0.76) (Lupatsch et al. 2003b) but are lower than those recorded for European seabass (0.91) and white grouper (0.91) (Lupatsch et al. 2003b) although k_l for mulloway is still within the lower SEM range of these two species. Lupatsch et al. (2003b) suggested that PE was also used in lipid deposition at higher PE intake levels in gilthead seabream hence the lower k_l value for that species and supported this argument by demonstrating the non-linear response of PE deposition with increasing PE intake. However, the relationship between PE deposition and PE intake in mulloway was linear (r^2 = 0.98). k_l values of approximately 0.9 can be expected if dietary lipid is the base nutrient for body lipid synthesis (Emmans 1994; van Milgen et al. 2001; Lupatsch et al. 2003b) however, if lipids are also synthesised from dietary energy supplied by carbohydrates then a reduction in k_l may be seen. The commercial diet used in this study contained 268 g kg^{-1} nitrogen–free extract (NFE = 100 – (protein + lipid + ash) suggesting that non-lipid dietary energy was available for lipid synthesis and may, in part, explain the relatively lower k_l value.

The proportional rate of deposition of protein:lipid remained relatively constant with increasing DE intake for mulloway with LE deposited at a slightly numerically greater rate (p>0.1) than PE (Figure 4.5). This is in contrast to rainbow trout which show a clear decrease in protein:lipid deposition with increasing DE intake (Rodehutscord and Pfeffer 1999; Bureau et al. 2006). The difference between

the partial energy utilization efficiencies of protein and lipid in mulloway may not be of a sufficient magnitude to demonstrate a clear protein sparing effect if dietary lipid levels were to be increased. This was demonstrated in Chapter 5 where mulloway fed increasing levels of DP at either one of two fixed DE levels (16 or 21 MJ kg^{-1}) showed no obvious protein sparing effect.

Values for maintenance energy requirements ranged from 44.2 to 49.6 kJ DE $kg^{-0.8}$ day^{-1} depending on temperature and fall within the maintenance DE values common to other fish species (40 – 60 kJ DE kg-0.8 day-1; Bureau et al. 2002). Protein requirements for maintenance for mulloway were 0.47 g DP $kg^{-0.7}$ and were found to be independent of temperature. Similar protein maintenance requirements ~0.45 g DP $kg^{-0.7}$ day^{-1} (Lupatsch and Kissil 2003; Glencross 2008) and temperature effects (Lupatsch and Kissil 2003) have also been demonstrated in barramundi.

Feeding at maintenance energy level does not necessarily imply that a constant body weight is maintained. Pigs have been shown to maintain zero energy retention while depositing protein and gaining body weight at the expense of body lipid (Ledividich et al. 1980). In the current study the *y*-intercept for PE (Eq. 4.8) was much larger than for LE (Eqs. 4.9, 4.10) therefore a DE intake at or slightly above maintenance level will yield positive protein deposition in mulloway without lipid gain. This has been demonstrated in yellowtail (*Seriola quinqueradiata*) (Watanabe et al. 2000b), European seabass (Peres and Oliva-Teles 2005) and rainbow trout (Bureau et al. 2006) fed at or near maintenance rations. These observations support the principle that weight gain in growing animals is driven by protein deposition (van Milgen et al. 2000; Bureau et al. 2002). The separation between LE deposition (Figure 4.5) accounts for the different requirements for total energy at different temperatures (Figure 4.4) and indicates that lipids rather than

protein are mobilised as an energy source to meet the increased maintenance energy demands imposed at higher temperatures. This allows the growing animal to continue to deposit protein at a rate predetermined by its genetic potential.

The improved growth rates in small mulloway can be directly attributed to a proportional increase of feed intake at the satiated level. However, large mulloway fed to satiation ate significantly more at 26 ^{o}C than 20 ^{o}C but did not demonstrate significantly better growth. This discrepancy may be attributed to the relatively greater costs for maintenance imposed on large mulloway at the higher temperature and may also indicate a shift in the DP:DE requirements for larger fish. Brett (1971) and Kellogg and Gift (1983) suggest that the final temperature preference exhibited by fish coincides with the temperature required to optimize biochemical and physiological processes. In a temperature preference experiment Bernatzeder and Britz (2007) determined 25 ^{o}C to 26.4 ^{o}C as the preferred temperature for 20g mulloway. The improved protein and energy retention of mulloway fed to satiation at 26 ^{o}C in the current study tends to support those findings, particularly for small mulloway. However, at satiated levels the maximum retention efficiencies for protein and energy did not vary between temperatures. Below these levels there was a trend for the retention efficiencies of dietary energy to be greater at 20 ^{o}C (Figure 4.2) which is likely to be related to the reduced maintenance energy requirements at that temperature. This suggests that improved growth rates can occur at 26 ^{o}C provided that dietary intake is optimized.

The effect of ration on whole-body composition was however independent of temperature except for lipid (small and large mulloway) and ash (small mulloway) (Table 4.1). Lipid content is known to vary directly depending on intake levels (Shearer 1994) and as lipid levels changed in mulloway a corresponding change in ash and moisture was also observed. This can be attributed to a proportional shift between bone and muscle mass with increasing feed intake.

Whole-body protein content is considered to remain constant and independent of feed intake and temperature (Shearer 1994); however, protein content in mulloway was shown to vary significantly with temperature (small and large fish) and ration (large fish). The overall difference in protein content between significant treatment levels was, however, quite small at <10 g kg^{-1}on average. It can therefore be concluded that while feed intake and temperature have a statistically observable effect on the protein composition of mulloway, protein levels do indeed remain fairly well conserved. This result also supports the use of a constant value for the initial fish protein composition (191.4 g kg^{-1}) which, when compared across all combined size, temperature and ration treatment values, differed by <10 g kg^{-1}.

To summarize, the utilization efficiencies of DE (0.60) and DP (0.58) for growth in mulloway were shown to be constant and independent of fish size, ration level or temperature used in this study. The partial utilization efficiencies of DE for protein (k_p) and lipid (k_l) deposition were 0.49 and 0.75 respectively. Maintenance requirements for protein (0.47 g DP $kg^{-0.7}$ day^{-1}) were influenced by body size but were independent of temperature while maintenance requirements for energy increased with increasing temperature (44.21 to 49.59 kJ DE $kg^{-0.8}$ day^{-1}) and were also influenced by body size. Mulloway should be fed to satiation to maximize growth potential if diets contain 21.4 g DP MJ DE^{-1}.

Chapter 5

The Interactive Effects of Dietary Protein and Energy on Feed Intake, Growth and Protein Utilization of Juvenile Mulloway[4]

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[4]The following chapter is published as:

Pirozzi, I., Booth, M.A., Allan, G.L., In press. The interactive effects of dietary protein and energy on feed intake, growth and protein utilization of juvenile mulloway (*Argyrosomus japonicus*). Aquac. Nutr. doi: 10.1111/j.1365-2095.2007.00641.x

5.1 Abstract

The objectives of this study were to describe the interactive effects of varying digestible protein (DP) and digestible energy (DE) content on the feed intake, growth, protein utilization and whole body composition of juvenile mulloway (*Argyrosomus japonicus*) and to determine the optimal DP:DE ratio for growth. This was achieved by feeding mulloway diets containing one of four different DP levels (250 - 550 g kg^{-1}) at two DE levels (16 or 21 MJ kg^{-1}). Juvenile mulloway were stocked at each of two different sizes (70 or 200 g) in triplicate groups for each dietary treatment and fed twice daily to apparent satiation over 58 days. The results indicated that feed intake was not governed solely by energy demands but was also dependant on the DP content of the diet. Protein utilization did not improve with diets containing decreasing protein and increasing lipid content indicating that mulloway have a limited capacity to spare dietary protein. Optimal DP content was found to be 444-491 g kg^{-1} depending on the DE content of the diet and the size of mulloway and is within the range reported for other sciaenid species. The use of

formulated diets with 28.6 g DP MJ DE^{-1} will achieve optimal growth and protein deposition for 70 – 275g mulloway.

5.2 Introduction

Mulloway, *Argyrosomus japonicus* (Pisces: Sciaenidae), are a euryhaline, gregarious, fast growing and highly fecund species that are easily reproduced in captivity. Mulloway have a wide distribution covering the east, western and southern seaboards of Australia (Silberschneider and Gray 2008) and can be grown successfully in different culture systems including sea cages, ponds and recirculating aquaculture systems (Quartararo 1996; Fielder et al. 1999; O'Sullivan and Ryan 2001; Doroudi et al. 2006). Aquaculture of mulloway is relatively new in Australia beginning in the mid 1990's (Gooley et al. 2000). As such the industry is in its relative infancy although there has been a steady increase in production in recent years. Production of mulloway in Australia for 2004/05 was 558.4 t (O'Sullivan et al. 2007), up from 6.8 t in 1997/98 (O'Sullivan and Roberts 2000). Development of the industry is currently restricted by a lack of knowledge of the nutritional requirements of mulloway. To date there is no published information on the requirements for digestible protein (DP) and digestible energy (DE) for mulloway and, as a consequence, no specific diet formulations are available. As a carnivorous species it is expected that mulloway will have a high requirement for DP and this is reflected in the current practice by industry of feeding mulloway commercial diets formulated for other carnivorous species such as barramundi (*Lates calcarifer*) or more generic 'marine fish' formulations.

Aquaculture feeds are formulated to maximize nutrient retention and

minimize nutrient loss. This strategy is driven by both economic and environmental considerations. Nutrient utilization efficiencies have been shown to be influenced by many different factors such as species effects (Refstie et al. 2000; Azevedo et al. 2004), fish size (Einen and Roem 1997; Azevedo et al. 2004), temperature (Bendiksen et al. 2003; Moreira et al. 2008) and the DP:DE ratio of the diet (Lupatsch et al. 2001b; Booth et al. 2007). Considerable advances have been made in improving protein retention in Atlantic salmon (*Salmo salar*) by increasing the energy content of the diet with lipid levels sometimes in excess of 30% (e.g. Einen and Roem 1997; Hemre and Sandnes 2008). The improved efficiencies are a result of the sparing of dietary protein from catabolism for energy by incorporating sufficient non-protein dietary energy from lipid or carbohydrate. There are, however, many examples of carnivorous marine fish such as grouper (*Epinephelus coioides*) (Luo et al. 2005), cobia (*Rachycentron canadum*) (Chou et al. 2001) and the large yellow croaker (*Pseudosciaena crocea*) (Duan et al. 2001) that show a much lower tolerance to elevated levels of dietary lipid thereby limiting potential protein sparing effects. Diets formulated with excess energy may also promote excessive lipid deposition (Shearer 1994) and reduce feed intake (Marais and Kissil 1979). Supplying formulated feeds with the optimal DP:DE content appropriate to a particular species, size and culture conditions is therefore crucial in maximizing nutrient retention.

The objectives of this study are to i) describe the interactive effects of varying DP and DE content on feed intake, growth, protein utilization and whole body composition of juvenile mulloway, ii) determine the optimal DP content for juvenile mulloway fed fishmeal based diets and iii) to determine the optimal DP:DE ratio for growth.

5.3 Materials and methods

5.3.1 Experiment design and system

The effects of varying the DP and DE content on growth and protein retention efficiency of mulloway was tested by feeding fish one of eight different diets formulated with a DP:DE ratio ranging from approximately 12 - 35 g DP MJ^{-1} to each of two size treatments from different cohorts stocked at 10 (large; initial body weight (ibw mean±SD) = 199.5±11.6 g) or 20 (small; ibw = 68.7±8.5 g) fish $tank^{-1}$. There was no significant difference between initial weights within size treatments (p>0.5). The experiment was run over 58 days using fish produced at the New South Wales Department of Primary Industries, Port Stephens Fisheries Institute (PSFI). The experimental system consisted of two integrated 1700 l recirculating bio-filtration units supplying 48 x 200 l replicate tanks (total volume ≈ 13000 l). The temperature was held at 26±0.5^{o}C which is known to promote good growth rates in mulloway (Chapter 4) and is within the preferred temperature range for this species (Bernatzeder and Britz 2007). Flow to each tank was approximately 4 l min^{-1} and orientated to create a weak centripetal current which allowed the retention of feed pellets in the tank while removing feces via a central upright 32mm diameter pvc overflow pipe which was fixed approximately 1cm off the bottom of each tank. Black plastic sheets were placed around each tank and across the top front half to minimise disturbance. Each size and feed treatment were randomly assigned to triplicate tanks with each tank constituting an experimental unit. All tanks were exposed to indirect natural light (photoperiod 12L:12D). Ammonium (NH_4^+) (<0.1 mg l^{-1}), dissolved oxygen (>5.0 mg l^{-1}), pH (7.5 - 7.9) and

salinity (28 - 33 ppt) were monitored regularly throughout the duration of the experiment.

5.3.2 Feeds and feeding

Feeds were formulated using the linear method in Winfeed 2.8 (Winfeed Ltd., Cambrdige, UK). Each diet was formulated with either a low DE (16 MJ kg^{-1}) (LE) or high DE (21 MJ kg^{-1}) (HE) content. Digestibility coefficients of the ingredients were identified in a previous study by Booth (unpublished data, 2008). Dry ingredients were mixed in a Hobart mixer (Troy Proprietary Ltd, Ohio, USA) to make the four base diets. Each summit/diluent pair was then blended to give four different DP levels while maintaining the respective DE content giving eight diets in total (Table 5.1). The dry ingredients were then combined with distilled water before being pelleted through a mincer (Barnco Australia Proprietary Ltd, Leichhardt, NSW, Australia) with a 10 mm diameter die and cut to 6mm lengths. Pellets were dried in convection drier at < 35 °C for about 6 h. Fish were fed to apparent satiation twice daily and any uneaten pellets were counted then siphoned from tanks approximately 45 min after initial feeding. Total daily feed intake was then adjusted to account for uneaten feed using the average weight derived from a sub-sample of feed pellets (n = 150 pellets for each diet) (Table 5.1). Fish were fasted for 48 h prior to final sampling.

5.3.3 Sample preparation and analyses

Initial representative samples of 10 fish of each size class were collected before the start of the experiment, euthanized with an overdose of benzocaine (ethyl-*p*-aminobenzoate) and frozen (-20 ^{o}C). At the conclusion of the feeding trial all fish were euthanized, weighed and stored frozen for compositional analyses.

Compositional changes were estimated by comparing the initial fish samples with those from the feeding trial. All values and subsequent reference to the nutrient and energy composition of mulloway in this study are based on whole body composition. Whole body composition was determined by placing the weighed fish into 5 l glass beakers, covering with aluminum foil and then autoclaving for 99 min at 121 ^{o}C. After cooling to room temperature any changes in weight were accounted for and assumed to be changes in moisture content. The samples were then homogenised *in situ* with a hand blender and a sample taken for dry matter determination. A portion of the remaining homogenate was then transferred to plates and oven dried at approximately 80 ^{o}C. The desiccated samples were then finely ground in a laboratory blender and analysed in accordance with AOAC (2005). Protein was calculated from total nitrogen based on N x 6.25 using the Dumas method. Dry matter was calculated gravimetrically after oven drying at 105 ^{o}C. Ash was calculated gravimetrically after incineration at 550 ^{o}C for 2 h. Gross energy was determined by adiabatic bomb calorimetry. Lipid was measured gravimetrically after chloroform-methanol extraction.

5.3.4 Performance indices

Mass-specific data are expressed as the geometric mean of initial and final body weights of fish (GMBW) and scaled using the metabolic body weight exponent values of 0.7 for protein retention data and 0.8 for energy retention data (after Brett and Groves 1979; Lupatsch et al. 1998). The following performance indices were calculated for each treatment group:

Daily weight gain (g $fish^{-1}$ day^{-1}) = Final body weight – initial body weight / number of days

Daily protein gain (g $fish^{-1}$ day^{-1}) = Final whole body protein content – initial whole body protein content / number of days

Daily energy gain (kJ $fish^{-1}$ day^{-1}) = Final whole body energy content – initial whole body energy content / number of days

Daily lipid gain (g $fish^{-1}$ day^{-1}) = Final whole body lipid content – initial whole body lipid content / number of days

Feeding Efficiency (FE) = Weight gain / Total feed intake

Protein Retention Efficiency (PRE) = Protein gain / Total protein intake

Relative Feed Intake (RFI) (g $kg^{-0.7}$ day^{-1}) = Total feed intake / $(GMBW/1000)^{0.7}$ / number of days

5.3.5 Data analyses

The effects of varying DE content (fixed; 2 levels), DP content (fixed; 4 levels) and mulloway size (fixed; 2 levels) on RFI, FE and PRE were tested using a 3-way ANOVA. Size directly influences the nutrient and energy composition of fish (Shearer, 1994); therefore data were pooled across the size term and the effects of diet on whole body composition tested as a 2-way ANCOVA with final body

weight (fbw) as the co-variate. Normality of the data were checked with skewness, kurtosis and omnibus normality tests. Assumptions of homogeneity of variances were tested using modified Levenes' equal variance test. Tukey-Kramer test was used for *a posteriori* multiple comparison of means on significant terms. All results were regarded as significant at $p<0.05$ except for the compositional analyses where α was set at 0.01 as differences between means of <0.5 % were detected when α = 0.05. All analyses were carried out using untransformed data unless otherwise stated.

Data for PRE were non-normally distributed due to a single outlier for small mulloway fed the HE1 diet (PRE = -0.49). PRE variances were homogeneous. PRE data could not be normalized; however, ANOVA results were significant regardless of the inclusion of the outlier or not. ANOVA data are given inclusive of the outlier and due consideration should therefore be given to interpretations of the subsequent multiple comparison tests.

Water flow and air failed to one tank during the experiment resulting in the loss of all fish in that tank (large fish; HE4 diet). Survival in all the remaining tanks was 100 %. Statistical analysis was completed with the mean of the remaining set substituted and degrees of freedom adjusted accordingly (Underwood 1997).

Nonlinear regression was applied to the PRE values to determine the DP requirements of mulloway for each energy level. The asymptote of the quadratic function was considered as the optimal DP content giving the maximum PRE value.

Correlations (r) were determined between performance indices (FE and PRE) and dietary nutrients (protein, lipid and starch). Protein gain (g $kg^{-0.7}$ day^{-1}) was also correlated with the DP:DE of the diets.

The optimal DP:DE ratio for protein gain (g $kg^{-0.7}$ day^{-1}) was predicted using

a biphasic linear model based on Koops and Grossman (1993) where protein gain (g $kg^{-0.7}$ day^{-1}) was described as:

$$A - BSln(1 + exp^{(C-x)/s})$$

Where A = asymptote (second phase); B = linear slope (first phase); S = transition smoothness (0.5); C = transition point.

Table 5.1. Nutrient and ingredient profile of experimental diets (as fed basis).

	Low Energy Diet				**High Energy Diet**			
	LE1	**LE2**	**LE3**	**LE4**	**HE1**	**HE2**	**HE3**	**HE4**
Diet Ingredient (g kg^{-1})								
Fish Meal	344	405	435	496	191	311	372	492
Fish Oil	167	104	73	10	304	236	201	133
Blood Meal	10	103	150	243	10	66	94	150
Bovine Meal	10	10	10	10	150	138	132	120
Pregel Starch	200	152	128	80	200	152	128	80
Vit. PreMix	5	5	5	5	5	5	5	5
Diatomaceous Earth	264	221	199	156	141	92	68	20
Pellet weight (g)	0.27	0.29	0.3	0.29	0.3	0.29	0.28	0.28
Nutrient Composition								
DM (g kg^{-1})	951	949	947	945	949	946	944	941
Protein (g kg^{-1})	266	397	462	592	263	393	458	588
Fat (g kg^{-1})	196	142	115	61	331	27	248	193
Ash (g kg^{-1})	307	274	258	225	172	140	125	93
GE (MJ/Kg)	17.12	17.30	17.44	17.65	22.50	22.54	22.58	22.66
DP (g kg^{-1})	257	376	436	555	251	373	434	555
DE (MJ/Kg)	15.92	15.82	15.77	15.67	20.95	20.96	20.96	20.97
DP:DE (g/MJ)	16.1	23.8	27.6	35.4	12.0	17.8	20.7	26.5

5.4 Results

5.4.1 Diet and fish size interactions on feed intake, FE and PRE

The effects of varying DE and DP content on RFI (g $kg^{-0.7}$) were not dependant on the size of mulloway (i.e. there was no second-order interaction)

(Table 5.2) however; the effect of DE on RFI (g $kg^{-0.7}$) varied significantly depending on the DP content of the diet. The influence of DE as well as DP on RFI also varied significantly depending on the size of mulloway (Table 5.2; Figure 5.1).

Table 5.2. 3-way ANOVA on relative feed intake (RFI), feeding efficiency (FE) and protein retention efficiency (PRE). Size = small and large mulloway (ibw ~70g and 200g); Dietary DE level = 16 and 21 MJ kg^{-1}; Dietary DP level 1 – 4 = 255, 375, 435 or 555 g DP kg^{-1}. ns = not significant at $p<0.05$, * = $p<0.05$, ** = $p<0.01$.

		RFI			FE			PRE		
Source of Variation	**DF**	**MS**	***F***	***P***	**MS**	***F***	***P***	**MS**	***F***	***P***
A: Size	1	34.33	816.30	**	0.02	6.25	*	0.01	7.79	**
B: DE Level	1	26.77	636.56	**	0.13	42.14	**	0.16	98.14	**
AB	1	0.39	9.22	**	0.08	27.81	**	0.06	36.84	**
C: DP Level	3	0.53	12.72	**	1.09	363.89	**	0.23	141.15	**
AC	3	0.16	3.73	**	0.07	24.83	**	0.04	21.38	**
BC	3	2.43	57.87	**	0.18	59.92	**	0.10	59.72	**
ABC	3	0.05	1.28	ns	0.02	6.03	**	0.02	12.64	**
Residual	31	0.04			0.00			0.00		

Relative feed intake (g $kg^{-0.7}$) was higher for the LE diets than the HE diets for both fish sizes and the DE x DP interaction occurred because significantly more of the LE1 diet was consumed. The DP x size interaction occurred because, in relative terms, small mulloway consumed less of the HE1 and HE2 diets. Large mulloway consumed a proportionally greater amount of the LE diet over the HE diet hence the DE x size interaction.

The effect of dietary DE on total FE and PRE varied significantly with DP content and these interactions were different for each size class (Table 5.2; Figure 5.1). The highest FE values (mean±se) were 0.93±0.05 and 0.75±0.02 for small and large mulloway respectively fed the HE4 diets (Table 5.3; Figure 5.1). FE generally improved with increasing DP content with the HE diet but began to plateau from the LE2 and LE3 diets for small and large mulloway respectively.

The highest PRE values (mean ±se) were 0.32±0.01 and 0.35±0.02 for small and large mulloway fed diets LE2 and LE3 respectively. PRE began to plateau from DP level 2 regardless of the DE content (Table 5.3; Figure 5.1). The second-order interaction for both FE and PRE occurred because of a relative improvement in efficiencies for the HE2 diet by large mulloway (Figure 5.1).

Correlation responses to dietary protein (r = 0.86 and 0.87; $p < 0.001$) and dietary starch (r = -0.84 and -0.84; $p<0.001$) with respect to FE were found to be virtually identical between small and large mulloway respectively. Dietary protein (r = 0.66 and 0.65; $p<0.001$) and dietary starch (r = -0.62 and -0.62; $p<0.001$) were also correlated with PRE and virtually identical between the small and large mulloway (Figure 5.2). Dietary lipid (r = -0.73; $p<0.001$ and -0.60; $p<0.01$) also demonstrated a negative correlation with FE for both small and large mulloway as did PRE (r = -0.62; $p<0.001$ and -0.60; $p<0.01$). The HE1 diet was found to have a large influence on the correlation coefficients with respect to dietary lipid which could be expected at a crude lipid inclusion level of 33 %. With this dietary treatment level removed clear differences were found between the size classes. FE and PRE were negatively correlated with dietary lipid for small mulloway (r = -0.48, $p<0.05$ and -0.62; $p<0.001$); however, there was no significant correlation with large mulloway (r = -0.17, $p>0.1$; -0.04, $p>0.5$).

Protein gain (g $kg^{-0.7}$ day^{-1}) was highly correlated with the DP:DE ratio of the diet (r = 0.84; $p<0.001$).

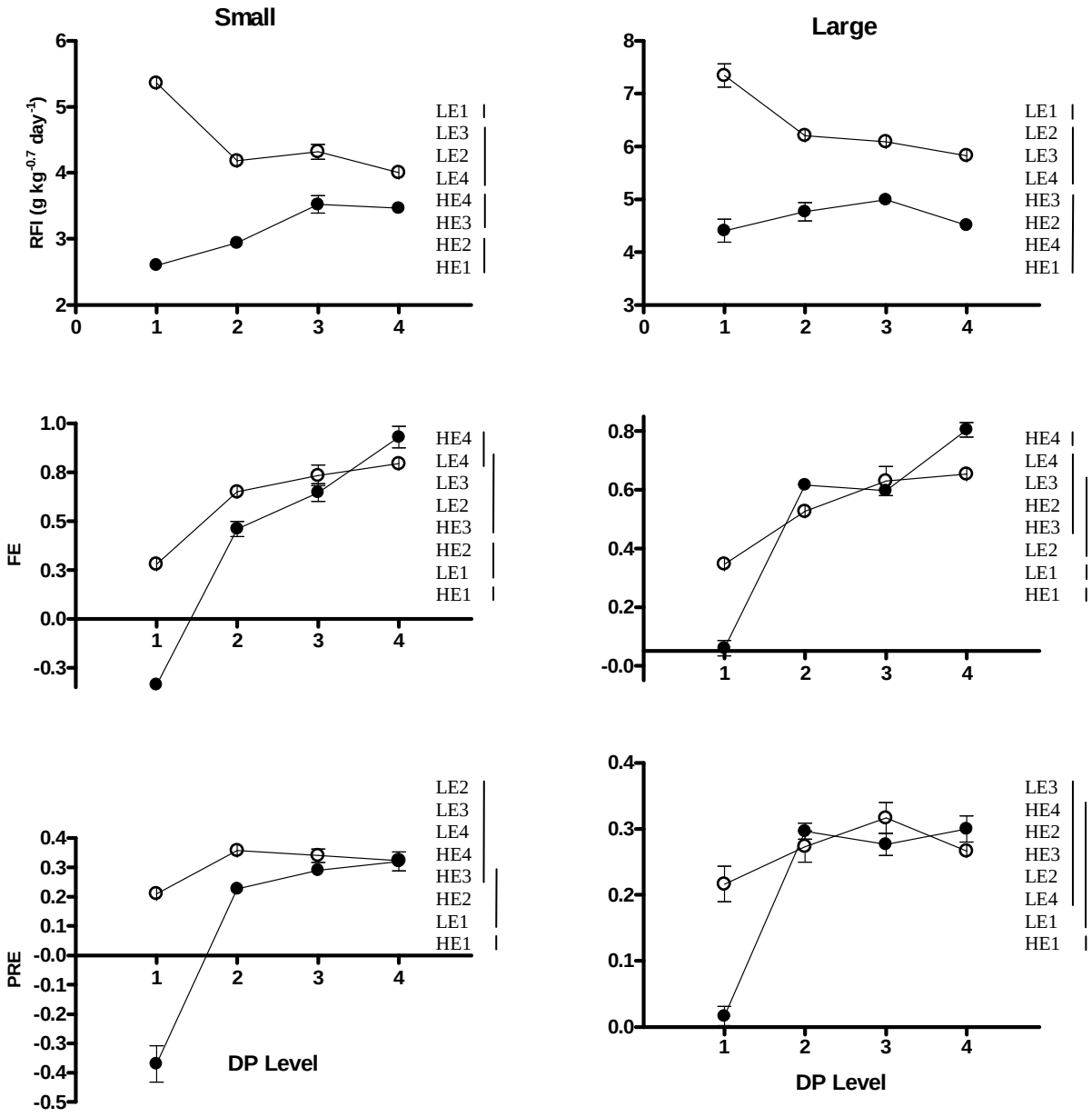

Figure 5.1. First-order interaction (DE x DP and DP x size) on relative feed intake (RFI). Second-order interaction (DE x DP x size) on feeding efficiency (FE) and protein retention efficiency (PRE). Data points are means ± se (n = 3; n = 2 for large HE4). Solid circles = high energy diets (HE; 21 MJ kg^{-1}); Open circles = low energy diets (LE; 16 MJ kg^{-1}). Dietary DP level 1 – 4 = 255, 375, 435 or 555 g DP kg^{-1}. Small ~70g; large ~200g ibw. Tukey-Kramer test on means within the DE x DP interaction term for each size class. Diets shown ranked by mean values from highest (top) to lowest (bottom). Means sharing lines are not significantly different (p>0.05).

Table 5.3. Summary of performance indices and carcass composition of mulloway. Fish size: Small ~70g ibw; Large ~200g ibw.

	Diet	Performance Indices									Carcass Composition[5]				
Fish Size	**Treatment[1]**	**Harvest Weight**	**Weight[2] Gain**	**Protein[2] Gain**	**Energy[3] Gain**	**Lipid[2] Gain**	**Feed[2] Intake**	**RFI[4]**	**FE**	**PRE**	**Moisture**	**Protein**	**Lipid**	**Ash**	**Energy**
Small	Initial	-	-	-	-	-	-	-	-	-	692	184	79	48	7.25
	LE1	82.42	0.25	0.05	1.97	0.02	0.87	5.36	0.28	0.21	683	185	82	51	7.40
	LE2	96.56	0.47	0.10	2.71	0.02	0.72	4.18	0.65	0.35	699	190	66	49	6.82
	LE3	98.83	0.55	0.11	2.57	0.00	0.75	4.32	0.73	0.34	709	190	54	50	6.43
	LE4	100.73	0.56	0.13	1.90	-0.02	0.70	4.01	0.79	0.32	719	198	40	47	6.03
	HE1	65.30	-0.13	-0.04	-1.84	-0.02	0.40	2.60	-0.33	-0.37	706	173	67	57	6.48
	HE2	82.68	0.22	0.04	1.69	0.03	0.48	2.94	0.46	0.23	684	184	84	52	7.32
	HE3	88.50	0.38	0.07	2.60	0.03	0.58	3.52	0.65	0.29	692	186	76	49	7.16
	HE4	100.19	0.56	0.11	2.93	0.01	0.60	3.46	0.93	0.32	706	186	60	51	6.57
Large	Initial	-	-	-	-	-	-	-	-	-	720	190	34	61	5.59
	LE1	245.05	0.76	0.14	11.06	0.19	2.56	7.34	0.30	0.22	688	190	73	50	7.20
	LE2	263.67	1.06	0.23	9.92	0.11	2.23	6.21	0.47	0.27	702	197	51	52	6.48
	LE3	272.30	1.28	0.31	11.32	0.11	2.19	6.09	0.58	0.32	701	204	48	51	6.48
	LE4	274.99	1.28	0.32	8.30	0.02	2.11	5.83	0.61	0.27	714	205	29	53	5.83
	HE1	199.70	0.01	0.01	1.87	0.04	1.43	4.41	0.01	0.02	713	192	46	55	6.13
	HE2	251.32	0.94	0.18	12.25	0.20	1.66	4.76	0.57	0.30	687	192	72	52	7.20
	HE3	256.02	0.97	0.21	11.64	0.16	1.76	4.99	0.55	0.28	691	196	64	51	7.00
	HE4	267.47	1.21	0.27	10.94	0.11	1.61	4.51	0.75	0.30	701	198	49	54	6.50

[1] Refer to Table 1 for diet composition
[2] (g $fish^{-1}$ day^{-1})
[4] Relative feed intake (g $kg^{-0.7}$ day^{-1})
[3] (kJ $fish^{-1}$ day^{-1})
[5] as received g kg^{-1} or MJ kg^{-1} (energy)

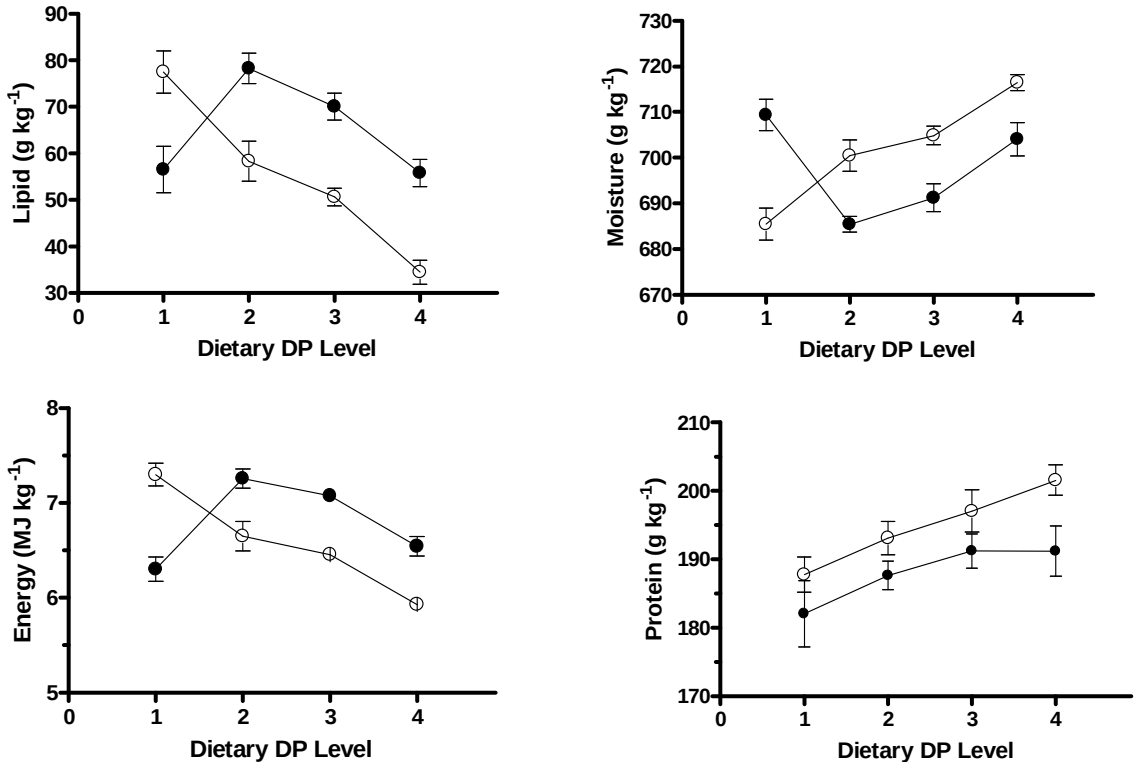

Figure 5.2. Effect of dietary DE on lipid, moisture, energy and protein composition of mulloway dependant on dietary DP content. Data are pooled means across size terms (± se; n = 6; n = 5 for HE4). Solid circles = high energy diets (HE; 21 MJ kg^{-1}); Open circles = low energy diets (LE; 16 MJ kg^{-1}). Dietary DP level 1 – 4 = 255, 375, 435 or 555 g DP kg^{-1}.

5.4.2 Effect of dietary DE and DP on whole body composition

The effect of DE content on the moisture, lipid and energy composition of mulloway varied significantly with DP content (Table 5.4; Figure 5.2). The interaction occurred because of the relative low lipid and energy content and high moisture composition for mulloway fed the HE1 diet (Figure 5.2). The effect of DE on protein composition was independent of the DP content. However, both DP and DE were found to effect the protein composition of mulloway (Table 5.4). There was a trend for protein composition to be greater for mulloway fed LE diets and to increase with increasing DP although protein composition tended to plateau from the

HE3 diets (Figure 5.2). The overall difference between the effect of diets on the protein composition of mulloway, although statistically significant, were quite small at less than 1 % for across DE or DP content.

Table 5.4. 2-way ANCOVA on protein, lipid and energy composition with final body weight (FBW) as the co-variate. Lipid data arcsine transformed. 2-way ANOVA on moisture and ash composition. ns = not significant at $p<0.05$, * = $p<0.05$, ** = $p<0.01$, ***= $p<0.001$.

		Protein			**Lipid**			**Energy**				**Moisture**			**Ash**		
Source of Variation	**DF**	**MS**	***F***	***P***	**MS**	***F***	***P***	**MS**	***F***	***P***	**DF**	**MS**	***F***	***P***	**MS**	***F***	***P***
X (FBW)	1	11.54	46.37	**	26.12	53.11	**	0.33	5.62	*							
A: DE Level	1	3.71	14.92	**	14.48	29.44	**	0.42	7.13	*	1	2.16	4.37	*	0.48	5.08	*
B: DP Level	3	2.03	8.17	**	18.47	37.55	**	1.07	18.07	**	3	6.20	12.51	***	0.20	2.11	ns
AB	3	0.16	0.62	ns	19.65	39.95	**	1.96	33.26	**	3	10.59	21.39	***	0.20	2.14	ns
Residual	38	0.25			0.49			0.06			39	0.50			0.10		

FBW was a significant co-variate for protein and lipid composition although, on average, the differences were small; large mulloway had only 1 % greater protein and 1.2 % less lipid composition than small mulloway. There was no significant effect of DE or protein on ash composition at $\alpha=0.01$ (Table 5.4).

5.4.3 Dietary DP requirements

Estimates of optimal DP content based on maximum predicted PRE derived from the asymptotic values of 2nd order polynomial regressions were LE diet: 452 and 444 g DP kg^{-1} and HE diet: 491 and 478 g DP kg^{-1} for small and large mulloway respectively (Figure 5.3).

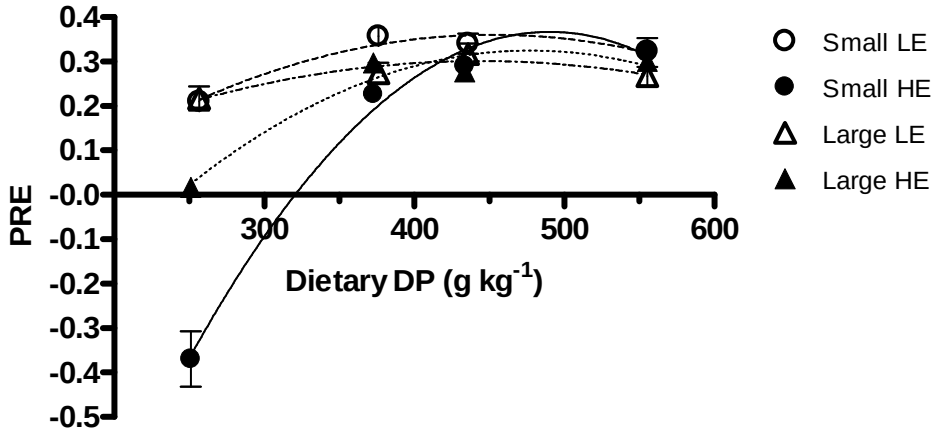

Figure 5.3. Effect of dietary DP and DE on PRE. Asymptote of quadratic functions represent required dietary DP for optimal PRE. (± se; n = 3; n = 2 for Large HE at 555 g DP kg^{-1}). HE = 21 MJ kg^{-1}; LE = 16 MJ kg^{-1}.

Figure 5.4 depicts the effect of DP content on protein gain (g $kg^{-0.7}$ day^{-1}) for both LE and HE diets. This relationship can be described by the quadratic functions:

LE diet:

protein gain (g $kg^{-0.7}$ day^{-1}) = -0.697 + 0.053DP - 0.0005DP^2 (r^2 = 0.76)

HE diet:

protein gain (g $kg^{-0.7}$ day^{-1}) = -1.836 + 0.088DP - 0.0008DP^2 (r^2 = 0.85)

Estimates of optimal DP:DE for maximum protein gain (g $fish^{-1}$ day^{-1}) derived from the bi-phasic growth model were 29.8 (r^2 = 0.86) and 27.3 (r^2 = 0.88) g DP MJ DE^{-1} for small and large fish respectively. The protein gain response to the HE1 diet (negative gain) heavily influenced the model estimates for small mulloway therefore the model was fitted excluding the HE1 data for that size. Comparison with

estimates of optimal DP:DE derived from the asymptotes of quadratic functions fitted to the PRE response (HE1 data removed for small mulloway) were 29.4 (r^2 = 0.74) and 27.2 (r^2 = 0.78) for small and large mulloway respectively; these results are very similar to the estimates derived using the above bi-phasic growth model with protein gain (g fish^{-1} da y^{-1}) as the dependent variable.

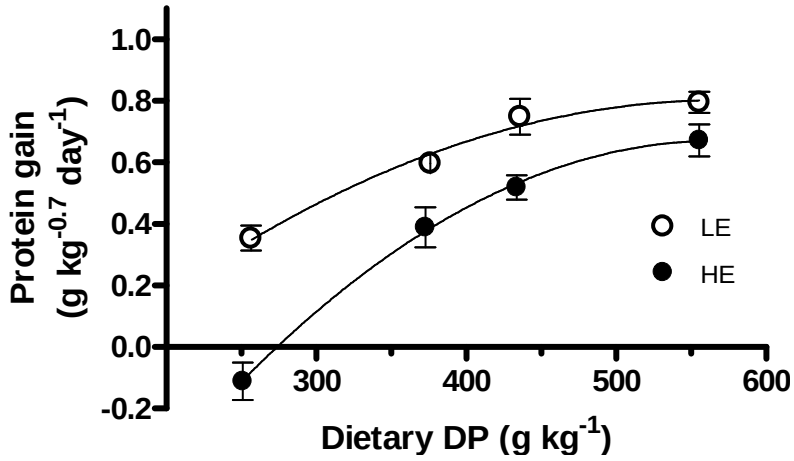

Figure 5.4. Effect of dietary DP and DE on protein gain (g kg$^{-0.7}$ day^{-1}). (± se; n = 6; n = 5 for HE at 55.5 g DP 100g^{-1}). HE = 21 MJ kg^{-1}; LE = 16 MJ kg^{-1}.

Ranges for DP:DE based on 95 % CI of the fitted bi-phasic growth model overlapped for the different size classes (small, 26.6 - 33.1; large, 23.8 - 30.8 g DP MJ DE^{-1}) suggesting that a common DP:DE ratio of 28.6 g DP MJ DE^{-1} would be suitable for formulating practical diets for mulloway of at least 70 g to approximately 275 g BW (Figure 5.5).

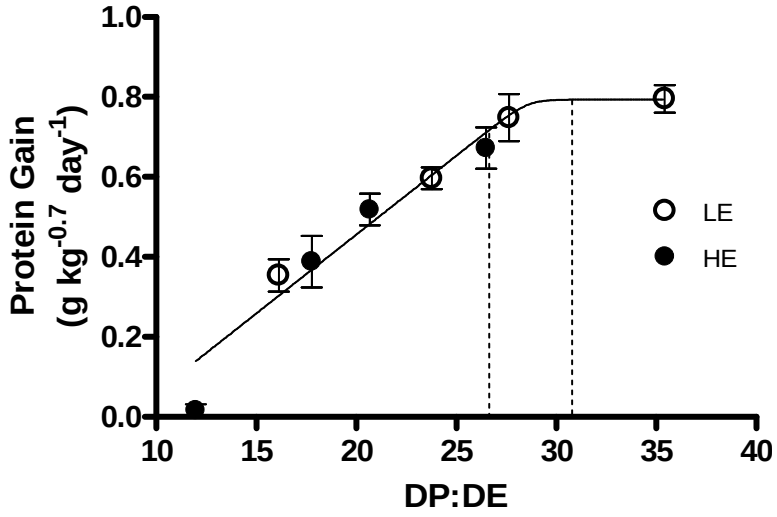

Figure 5.5. Effect of dietary DP:DE ratio (g DP MJ DE^{-1}) on protein gain (g $kg^{-0.7}$ day^{-1}). (± se; n = 6; n = 5 for 26.5 g DP MJ DE^{-1}; HE1 data for small mulloway removed from analyses). Maximum protein deposition at 28.6 g DP MJ DE^{-1}. Dashed vertical lines represent 95 % confidence limits at 26.6 - 30.8 g DP MJ DE^{-1}. HE = 21 MJ kg^{-1}; LE = 16 MJ kg^{-1}.

5.5 Discussion

It has been widely hypothesized that fish regulate feed intake to satisfy their energy requirements (Cho and Kaushik 1990; Kaushik and Medale 1994). The findings from the current study also support this theory however; the magnitude of the effect of DE on the relative feed intake of mulloway was shown to vary depending on the DP content of the diet. This indicates that energy requirements alone do not drive feed intake but the requirements for nutrients, in this case protein, also play a very important role.

Both the effects of DP and DE content on relative feed intake were shown to vary significantly depending on the size of mulloway. On a relative basis the demand for protein for somatic growth is known to be greater for smaller than larger fish and, conversely, the demand for energy is greater for larger than smaller fish (Garcia-Alcazar et al. 1994; Lupatsch et al. 2001b). This fact is reflected in formulated diets

which provide greater DP:DE content for smaller, rapidly growing fish. Large mulloway in this study consumed relatively more LE diet (compared to HE diet) than small fish presumably to meet a greater demand for metabolic energy. However, the greater overall relative feed intake demonstrated by large mulloway was likely compensatory as indicated by the initial body composition (Xie et al. 2001)

If fish also consume feed to satisfy their nutrient requirements then it would be expected that, on a relative basis, smaller fish would consume more of a low protein diet than larger fish to meet those metabolic demands for protein. However, the DP x size interaction occurred because, on a relative basis, small mulloway ate significantly less of the HE low protein diets (HE1 and HE2) compared to the higher protein diets (HE3 and HE4) than large mulloway. Indeed, small fish consuming the HE1 diet were the only group to lose weight while the corresponding feed intake for large fish was approximately equivalent to maintenance level. This response may have occurred because high energy diets can sometimes suppress appetite (Marais and Kissil 1979) and may indicate a shift in taste preference between size classes (Kasumyan and Doving 2003); although this remains to be tested in mulloway. In real terms however, the difference between the amounts of HE1 and HE2 diets compared to HE3 and HE4 diets consumed by small mulloway was, on average, only 0.73 g $kg^{-0.7}$ and would not have been significant at α.= 0.01. The different responses between small and large mulloway to diets with high DE and low DP may indicate a subtle shift in the protein:energy demands between the two sizes and highlights the importance of the correct DP:DE ratio in formulated feeds.

The effect of DE on the moisture, lipid and energy composition of mulloway was shown to vary depending on the DP content of the diet. The general trend was for lipid composition to decrease with increasing dietary protein. The exception

occurred with the HE1 diet and this is concomitant with reduced feed intake at this level. Similar results have been demonstrated with silver perch (*Bidyanus bidyanus*) which show a significant linear decrease in whole body lipid composition when fed 17 MJ DE kg^{-1} diets with increasing DP content of 173-424 g kg^{-1}; however, at 129 g DP kg^{-1} silver perch also show a significant relative reduction in whole body lipid composition (Allan and Booth 2004). Protein composition was shown to be affected by DE and DP content with a greater protein composition with increasing DP and decreasing DE content. A similar trend for the effect of DE on the protein composition has been reported for the cuneate drum, *Nibea miichthioides* (Wang et al. 2006b); however, the influence of DP in their study, while significant, was unclear. This is possibly due to the limited range of DP (360 – 400 g kg^{-1}) used in their diets.

Mulloway in this study were found to have DP requirement of 444 – 491 g kg^{-1} depending on the size of the fish and the dietary energy level. These values are similar to those reported for other sciaenid species such as the giant and Atlantic croakers (450 g CP kg^{-1}; Davis and Arnold 1997; Lee et al. 2001), the large yellow croaker (470 g CP kg^{-1}; Duan et al. 2001) the cuneate drum (≥400 g DP kg^{-1}; Wang et al. 2006b) and red drum (350 - 440 g DP kg^{-1}; Daniels and Robinson 1986; McGoogan and Gatlin 1998).

The use of dietary protein can potentially be maximized for growth if adequate amounts of non-protein energy can be supplied to satisfy metabolic demands for energy. This protein sparing effect has been demonstrated in some fish species (e.g. Erfanullah and Jafri 1995; Company et al. 1999; Lee et al. 2002; Azevedo et al. 2004) but not others (e.g. Peres and Oliva-Teles 1999; De Pedro et al. 2001; Regost et al. 2001; Azevedo et al. 2004). PRE values did not significantly

improve with diets containing decreasing protein and increasing lipid content; this occurred irrespective of the energy content of the diet and indicates that mulloway have a limited capacity to spare dietary protein. This effect has similarly been demonstrated in other sciaenid species (Duan et al. 2001).

Carnivorous fish are known to efficiently catabolize dietary proteins as an energy source (Gatlin III 1995); however, whether catabolism of dietary protein by mulloway for energy is performed preferentially over non-protein energy sources (i.e. lipids) is unclear although the lack of a positive correlation between dietary lipid for both FE and PRE indicates this may be likely. Rasmussen et al. (2000) and Azevedo et al. (2004) did not observe a significant correlation between PRE and lipid intake in rainbow trout while at the same time demonstrating a significant correlation between protein intake and PRE indicating an absence of a protein sparing effect of non-protein energy nutrients. Further research is required to establish the energy utilization potential of non-protein energy sources as they pertain to mulloway.

The correct proportion of dietary DP to DE is not only important to maximize growth but imbalances have economic and environmental consequences. Diets containing excessive protein will generally be less cost effective and produce excessive nitrogenous waste (Kaushik 1998). Diets with excessive lipid content will increase lipid deposition to the visceral cavity, liver and some muscle tissue of fish (e.g. Nanton et al. 2007) although this may be desirable in some aquaculture species such as those consumed as sashimi (Chou et al. 2001). Protein deposition in mulloway was found to be highly correlated with the DP:DE ratio of the diet with 28.6 g DP MJ DE^{-1} considered appropriate for 70 – 275 g mulloway.

While the experimental design used in this study was sensitive enough to discriminate subtle differences between the DP and DE requirements of 70 and 200 g (ibw) fish; it is important to keep in perspective that mulloway can grow up to 75 kg and 1.8 m in length (Griffiths and Heemstra 1995). Both the sizes used in this study therefore represent rapidly growing juveniles with a high demand for dietary protein; 444 - 491 g kg^{-1} DP depending on the fish size and energy content of the diet. Protein utilization did not improve with diets containing decreasing protein and increasing lipid content indicating that protein sparing in mulloway may be limited and underscores the importance of supplying adequate amounts of dietary protein. Protein deposition in juvenile mulloway from 70 – 275 g can be optimized with diets containing 28.6 g DP MJ DE^{-1}.

Chapter 6

A Factorial Approach to Diet Formulation and Feeding Regimes for Mulloway Based on the Requirements for Protein and Energy

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6.1 Abstract

This study builds upon the information established in the previous chapters to develop a factorial model to predict protein and energy requirements for mulloway throughout the production range. Assessments of the growth potential of mulloway and the allometric relationships between body size and protein and energy metabolism and protein and energy whole body composition were combined with previously established data on the utilization efficiencies and maintenance requirements for DP and DE. Factorial modelling of the data allowed estimations of the decreasing requirement of the ratio of DP:DE for mulloway with increasing body size up to 2 kg. From this information diet formulations and feeding regimes were then iteratively derived applicable for the different dietary needs dependant on body size.

6.2 Introduction

The factorial modelling method for defining nutrient requirements in fish has seen significant advances made in recent years with the work on non-salmonid marine fish by Lupatsch et al. (1998), Lupatsch et al. (2001a) and Lupatsch and Kissil (2005) and work on salmonids by Cho and Bureau (1998) and Azevedo et al.

(2005). The premise behind the factorial method being that the requirements for protein and energy can be partitioned into production and maintenance costs based on the assumption that the two are additive. This can expressed as:

$$\text{Total nutrient requirement} = a \times BW(kg)^{b} + c \times \text{Growth} \quad (6.1)$$

where a = maintenance requirement; b = weight exponent; c = utilisation coefficient

The advantage of this method over the more traditional empirical based dose response methods such as that used in Chapter 5 is that it can be used to describe DP and DE requirements for growing fish throughout the production cycle and estimations are not necessarily restricted to within the size range of the test species. Key to achieving this however are establishing the utilization efficiencies and maintenance requirements for DP and DE (achieved in Chapter 4), an assessment of the protein and energy composition as a function of fish size, establishing the growth potential under a given set of culture conditions and describing the allometric relationship between body size and protein and energy metabolism.

The main objectives of this study were twofold; firstly, to use the factorial method to describe the requirements for DP and DE for mulloway up to 2 kg and then, secondly, to iteratively derive diet formulations and feeding regimes based on the requirements for protein and energy. Several assumptions relating to Eqn. (6.1) with respect to temperature and fish size effects on the utilization efficiencies (c) and maintenance requirements (a) of DP and DE in mulloway were tested in Chapter 4. In this study the influence of methodology on deriving the value of the metabolic weight exponent (b) is also tested.

6.3 Materials & Methods

6.3.1 Growth data

A data set was compiled from growth records of mulloway held at PSFI and a commercial mulloway farm. Farm data were based on cohorts held in sea cages or ponds where fish were fed to apparent satiation with commercial diets. Data from mulloway at PSFI were obtained from mulloway grown in 10000 l recirculating aquaculture systems or 1 m^3 cages in an outdoor pond. Water temperatures ranged from approximately 18 – 30 ^oC and averaged approximately 23 ^oC. All growth data were expressed as mean body weight (BW g) values of sub-sampled cohorts where total n > 3000 individual fish. Outliers or cohorts where feed intake was considered spurious were excluded from the analyses. The growth model component in this study is based on body weight however workers on commercial farms often measure growth based as body length as it is a much more convenient measurement to obtain particularly if sampling from sea cages. Therefore the relationship between standard body length (SL mm) and BW was established to allow conversion from length based data to estimate BW. SL allows accurate body length measurements as it is not influenced by the condition of the caudal fin which can sometimes be damaged; however, total length (TL) is still often used. Using a range of fish from approximately 25 – 1860 g the relationship between SL and TL was also established to allow conversions based on TL. This relationship was linear and can be described as:

$$SL = 0.9428(TL) - 13.3832 \quad (r^2 = 0.997; n = 1072) \qquad (6.2)$$

The relationship between SL and BW was allometric (Figure 6.1) and can be described as:

$$BW = 6.163x10^{-5}(SL)^{2.758} \quad (r^2 = 0.99; n = 3531) \qquad (6.3)$$

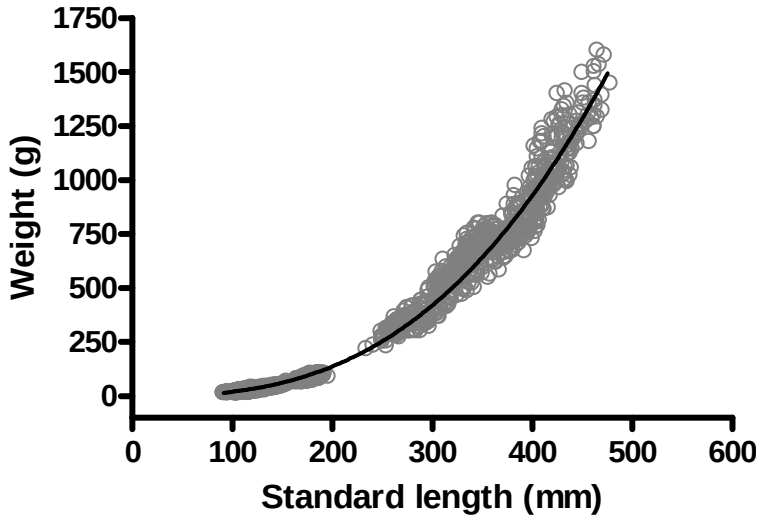

Figure 6.1. Relationship between standard length (mm) and body weight (g) of mulloway. Weight measurements range from 12 – 1600 g. (r^2 = 0.99; n = 3531).

6.3.2 Body composition

The proportional content of energy, lipid and moisture to the BW of fish are not constant throughout the growing phase and composition also varies between species (Shearer 1994; Lupatsch et al. 2003b). The relationship between the proximate composition and body weight of mulloway was determined using groups of equal size fish ranging from 2 – 2100 g (n = 3 to 100 fish depending on size). Samples were prepared for proximate analysis as per Chapter 4.

6.3.3 Dietary protein and energy utilization

The dietary protein and energy utilization efficiencies for mulloway used to populate the factorial model in this study were established in Chapter 4. Based on the slopes of regression, utilization efficiencies for DP and DE were 0.58 and 0.60 respectively. The respective corresponding cost per unit of protein or energy deposition is 1.72 g DP g^{-1} and 1.67 kJ DE kJ^{-1}.

6.3.4 Metabolic body weight

The effect of body weight on metabolic rate (*M*) can be described by the general allometric equation:

$$M = aBW^b \quad (6.4)$$

Where *a* is the normalizing constant, BW is the body mass in g and *b* is the scaling exponent describing the influence of mass on metabolism. The metabolic weight exponent for routine metabolism (RMR) of mulloway based on oxygen consumption was established in Chapter 3 and found to be 0.8. An alternative method is the comparative carcass analysis of energy loss on starvation. This method also has the advantage of allowing the metabolic weight loss of any body tissue such as protein to be established if required. To test if fasting duration and temperature effected the allometric relationship between daily protein and energy loss on starvation 3 size classes of mulloway (S, M or L; initial BW approximately 25, 90 and 645 g respectively) were fasted at two durations (2 or 4 weeks) at two temperatures (14 or 20^oC). Treatments are referred to as 14:2, 14:4, 20:2 and 20:4 denoting each

respective temperature and fasting duration group. Fish were stocked into replicate 200 l tanks (n = 4) at 45, 25 or 6 fish $tank^{-1}$ for S, M, or L fish respectively for each temperature and fasting duration treatment. A description of the system and temperature acclimation protocols can be found in Chapter 2. Stocking densities were chosen to approximate those established as appropriate for mulloway in Appendix 1. A representative initial sample of each size class (n = 10 for S and M fish or n = 5 for L fish) were euthanized at the beginning of the study and stored frozen until the completion of the fasting trial. At the conclusion of each fasting treatment, fish were euthanized, weighed and also stored frozen until preparation for carcass analyses. All samples for proximate whole carcass analyses were prepared as per Chapter 4. Compositional changes in protein and energy were estimated by comparing the initial fish carcass samples with those from the fasting trial.

6.3.5 Maintenance requirements

The daily maintenance requirements for energy and protein were established in Chapter 4. Maintenance requirements for energy varied depending on temperature and were 44.2 and 49.60 kJ DE $kg^{-0.8}$ day^{-1} at 20 and 26 ^{o}C respectively. Routine metabolic rate (RMR) (Chapters 2 & 3) and peak MO_2 after feeding (Chapter 3) were both shown to increase linearly with temperature; therefore, a linear relationship with maintenance energy requirement (kJ DE $kg^{-0.8}$ day^{-1}) and temperature was also assumed which can be expressed as $26.28+0.897T$ (when T = 20 to 26^{o}C).

The daily maintenance requirement for protein was found to be independent of the temperatures used in Chapter 4 and was estimated at 0.47 g DP $kg^{-0.7}$ day^{-1}

6.3.6 Data analyses

Allometric relationships were iteratively derived using the non-linear least squares method in Graphpad Prism V4 (GraphPad Software, San Diego, CA, USA). All data are based on the mean of tanks or experimental units. Comparisons of individual model parameters were made using the extra sum-of-squares *F*-test. Statistical significance was regarded at $P < 0.05$.

Piecewise linear analysis was used to determine breakpoints describing key changes in the relationship between BW and the requirement for the ratio of DP:DE using NCSS (2004, Kaysville, Utah).

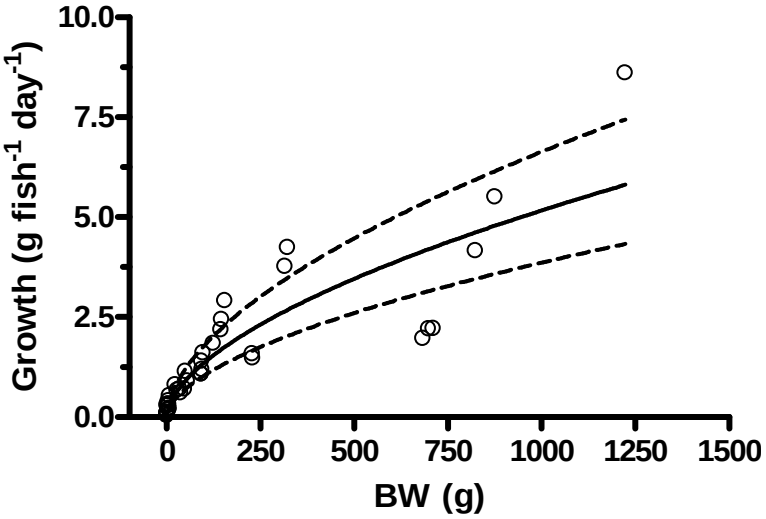

Figure 6.2. Relationship between BW (g) and growth rate (g $fish^{-1}$ day^{-1}) of mulloway held at an average temperature of approximately 23 ^{o}C (solid line). Data points represent mean values of groups of fish (n = 44). Dashed lines represent estimations of growth rates at the lower and upper ranges of temperatures occurring during growth trials (18 – 30 ^{o}C) based on Eqn. (6.5).

6.4 Results

6.4.1 Growth model

Figure 6.2 shows the allometric relationship between growth rate and BW of mulloway held at an average temperature of 23.6 ^{o}C (SD±2.5 ^{o}C). This can be expressed as a function of temperature (T) within the range sampled (~18 to 30 ^{o}C):

$$\text{Gain (g fish}^{-1}\text{ day}^{-1}) = 0.03344 \times \text{BW(g)}^{0.5699} \times \exp(0.0451 \times T)$$

$$(r^2 = 0.77;\ n = 44) \qquad (6.5)$$

Eqn. (6.5) can be expressed in terms of predicted BW based on initial weight (BW_0) after time (t) in days as:

$$BW = (BW_0^{0.4301} + 0.0144 \times \exp^{0.0451xT} \times t)^{2.3248} \qquad (6.6)$$

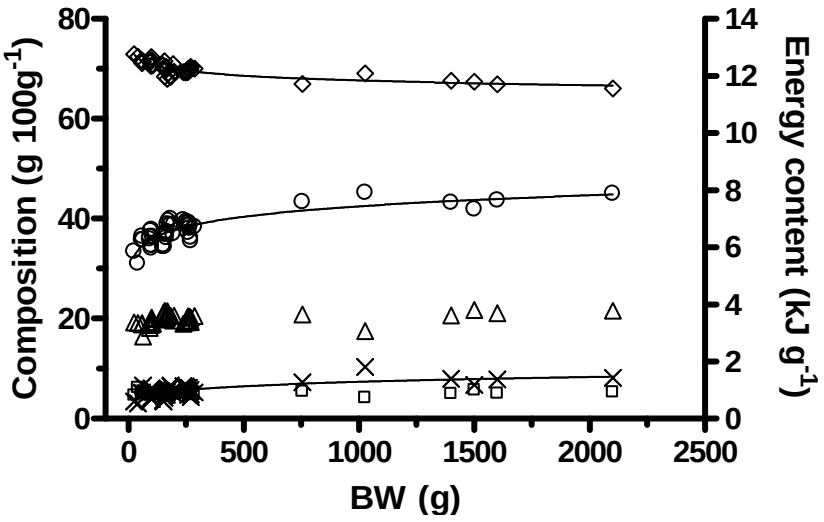

Figure 6.3. Relationship between proximate body composition and live weight (g) (n = 45 groups). Diamonds = moisture; Circles = energy; Triangles = protein; Crosses = lipid; Squares = ash.

6.4.2 Body composition

The whole body composition of mulloway (n = 45 groups) can be seen in Figure 6.3 Average whole body protein (19.13 g 100 g^{-1}) and ash (5.2 g 100 g^{-1}) content remained relatively constant independent of fish BW while energy, lipid and moisture demonstrated an allometric response:

$$\text{Energy (kJ g}^{-1}) = 4.492 \times \text{BW(g)}^{0.0729} \quad (r^2 = 0.75) \quad (6.7)$$

$$\text{Lipid (g 100 g}^{-1}) = 2.063 \times \text{BW(g)}^{0.1838} \quad (r^2 = 0.53) \quad (6.8)$$

$$\text{Moisture (g 100 g}^{-1}) = 77.80 \times \text{BW(g)}^{-0.02} \quad (r^2 = 0.73) \quad (6.9)$$

6.4.3 Metabolic body weight

The coefficient and metabolic weight exponent values for protein and energy loss on starvation can be seen in Table 6.1. The relationship between daily protein loss and geometric mean body weight at different temperatures and fasting durations can be seen in Figures 6.4 and 6.5.

Table 6.1. Parameters of the power function $y = ax^b$ describing the relationship between body mass and protein (g $fish^{-1}$ day^{-1}) or energy loss (kJ $fish^{-1}$ day^{-1}) on starvation.

Temperature & Fasting Duration (^oC:weeks)	**Protein loss**			**Energy loss**		
	***a*(±se)**	***b*(±se)**	r^2	***a*(±se)**	***b*(±se)**	r^2
14:2	0.0004(±0.0003)	0.992(±0.13)	0.90	0.0565(±0.018)	0.836(±0.05)	**0.99**
14:4	0.0017(±0.002)	0.782(±0.19)	0.84	0.1066(±0.089)	0.778(±0.13)	**0.92**
20:2	0.0026(±0.003)	0.805(±0.21)	0.84	0.1661(±0.050)	0.768(±0.05)	**0.99**
20:4	**0.0050(±0.002)**	**0.683(±0.05)**	**0.98**	**0.2415(±0.099)**	**0.687±(0.06)**	**0.97**

6.4.3.1 Protein loss

Exponent values for protein loss ranged from 0.683 for the 20:4 group to 0.992 for the 14:2 group. However, when comparing *b* values amongst treatments a global value of 0.764 could be used to describe *b* for all data sets ($F = 0.30$; $P > 0.5$). When comparing against H_0: $b = 0.7$ only the 14:2 group differed significantly ($F = 7.88$; $P < 0.05$). No statistical difference was found when comparing *b* of all fasted treatments against H_0: $b = 0.8$.

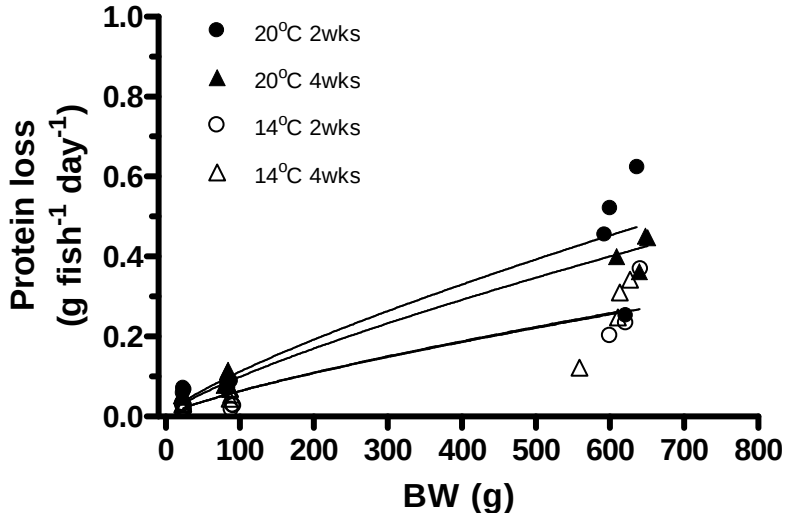

Figure 6.4. Protein loss (g fish day^{-1}) of mulloway after 2 or 4 weeks fasting at 14°C or 20°C (n = 12).

6.4.3.2 Energy loss

Exponent values for energy loss ranged from 0.687 for the 20:4 group to 0.836 for the 14:2 group. A global value of 0.748 could be used to describe *b* for data sets ($F = 0.50$; $P > 0.5$). No statistical difference was found when comparing *b* of all fasted treatments against H_0: $b = 0.8$

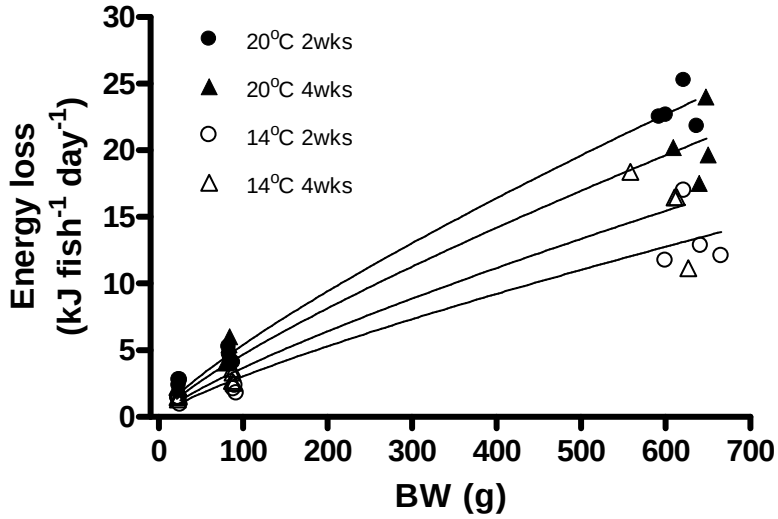

Figure 6.5. Energy loss (g fish day^{-1}) of mulloway after 2 or 4 weeks fasting at 14^{o}C or 20^{o}C (n = 12).

6.4.4 Protein and energy requirements

A summary of the parameters used to populate the factorial model are presented in Table 6.2. From Eqn. (6.1) the total requirement can be described for dietary protein as:

$$\text{DP requirement (g fish}^{-1}\text{ day}^{-1}) = 0.47\times\text{BW(kg)}^{0.7} + 1.72\times\text{protein gain} \qquad (6.10)$$

and for dietary energy as:

$$\text{DE requirement (kJ fish}^{-1}\text{ day}^{-1})$$
$$= (26.28+0.897T)\times\text{BW(kg)}^{0.8} + 1.67\times\text{energy gain} \qquad (6.11)$$

From Eqns. (6.10) and (6.11) the total daily protein and energy requirements can then be calculated for the production range of mulloway (Table 6.3).

Table 6.2. Summary of parameter values used to populate the factorial model.

Term	Equation / value
Growth rate (g fish^{-1} day^{-1})	$0.03344BW^{0.5699} \times \exp(0.0451 \times T)$
Whole body composition (energy) (kJ/g)	$4.492BW^{0.07288}$
Whole body composition (protein) (g/kg)	191.27
Utilization efficiency (energy)	0.60
Utilization efficiency (protein)	0.58
Maintenance requirement DE (kJ kg$^{-0.8}$ day^{-1})	$26.28+0.897T$
Maintenance requirement DP (g kg$^{-0.7}$ day^{-1})	0.47

6.4.5 Feed formulations and practical diet assignment

Based on the protein and energy requirements calculated in Table 6.3 the theoretical feed intake and feed conversion ratio's (FCR'S) can then be predicted for feeds with a pre specified energy content for any size mulloway up to 2 kg (Figure 6.6). Figure 6.6 is based on the "ideal" DP:DE requirement at each body weight which in practice would require many different diets with a DP:DE content to reflect this shifting requirement. Piecewise analyses identified significant changes in DP:DE requirement at 111, 582 and 1120 g (Figure 6.7). Practical feed formulations based on 3 growth stages, each with a fixed DP:DE content, can be seen in Table 6.4.

Table 6.3. Energy and protein requirements for mulloway at 20 and 26°C.

	Live weight g	10	50	100	200	500	800	1100	2000
	MBW ($kg^{-0.8}$)[a]	0.03	0.09	0.16	0.28	0.57	0.84	1.08	1.74
Temperature	**MBW ($kg^{-0.7}$)[a]**	0.04	0.12	0.20	0.32	0.62	0.86	1.07	1.62
20 °C	**Growth (g $fish^{-1}$ day^{-1})[b]**	0.31	0.77	1.14	1.69	2.85	3.72	4.46	6.27
	DE Maintenance (kJ $fish^{-1}$ day^{-1})[c]	1.11	4.02	7.00	12.19	25.38	36.96	47.69	76.93
	Energy gain (kJ $fish^{-1}$ day^{-1})[d]	1.63	4.58	7.15	11.16	20.11	27.20	33.38	49.02
	DE growth (kJ $fish^{-1}$ day^{-1})[e]	2.71	7.63	11.91	18.60	33.51	45.33	55.63	81.70
	DE total (kJ $fish^{-1}$ day^{-1})[f]	3.82	11.65	18.91	30.79	58.89	82.30	103.32	158.63
	%DE for maintenance	29.04	34.52	37.02	39.60	43.09	44.91	46.16	48.50
	DP Maintenance (g $fish^{-1}$ day^{-1})[g]	0.02	0.06	0.09	0.15	0.29	0.40	0.50	0.76
	Protein gain (g $fish^{-1}$ day^{-1})[h]	0.06	0.15	0.22	0.32	0.54	0.71	0.85	1.20
	DP growth (g $fish^{-1}$ day^{-1})[i]	0.10	0.25	0.38	0.56	0.94	1.23	1.47	2.07
	DP total (g $fish^{-1}$ day^{-1})[j]	0.12	0.31	0.47	0.71	1.23	1.63	1.97	2.83
	%DP for maintenance	15.58	18.53	19.93	21.41	23.49	24.60	25.38	26.88
	DP:DE (g DP MJ DE^{-1})[k]	**31.30**	**26.62**	**24.77**	**23.01**	**20.83**	**19.77**	**19.08**	**17.83**
26 °C	**Growth (g $fish^{-1}$ day^{-1})[b]**	0.40	1.00	1.49	2.21	3.73	4.88	5.85	8.22
	DE Maintenance (kJ $fish^{-1}$ day^{-1})[c]	1.25	4.51	7.86	13.68	28.48	41.47	53.51	86.32
	Energy gain (kJ $fish^{-1}$ day^{-1})[d]	2.13	6.00	9.37	14.63	26.36	35.66	43.76	64.26
	DE growth (kJ $fish^{-1}$ day^{-1})[e]	3.55	10.00	15.62	24.38	43.93	59.43	72.93	107.10
	DE total (kJ $fish^{-1}$ day^{-1})[f]	4.80	14.51	23.47	38.06	72.41	100.90	126.44	193.42
	%DE for maintenance	25.95	31.09	33.48	35.95	39.33	41.10	42.32	44.63
	DP Maintenance (g $fish^{-1}$ day^{-1})[g]	0.02	0.06	0.09	0.15	0.29	0.40	0.50	0.76
	Protein gain (g $fish^{-1}$ day^{-1})[h]	0.08	0.19	0.29	0.42	0.71	0.93	1.12	1.57
	DP growth (g $fish^{-1}$ day^{-1})[i]	0.13	0.33	0.49	0.73	1.23	1.61	1.93	2.71
	DP total (g $fish^{-1}$ day^{-1})[j]	0.15	0.39	0.59	0.88	1.52	2.01	2.43	3.47
	%DP for maintenance	12.34	14.79	15.96	17.21	18.97	19.93	20.60	21.90
	DP:DE (g DP MJ DE^{-1})[k]	**31.46**	**26.78**	**24.93**	**23.16**	**20.97**	**19.91**	**19.21**	**17.95**

[a]Live weight scaled using exponents of 0.8 or 0.7 for energy and protein respectively
[b]Daily weight gain derived from growth model Eqn. (6.5)
[c]DE maintenance = 26.28+0.897*T* x MBW
[d]Energy gain = Growth x $4.492BW^{0.07288}$
[e]DE growth = Energy gain / 0.60
[f]DE total = DE Maintenance + DE Growth
[g]DP maintenance = 0.47 x MBW
[h]Protein gain = Growth x 0.191
[i]DP growth = Protein gain / 0.58
[j]DP total = DP Maintenance + DP Growth
[k]DP:DE = (DP total / DE total) x 1000

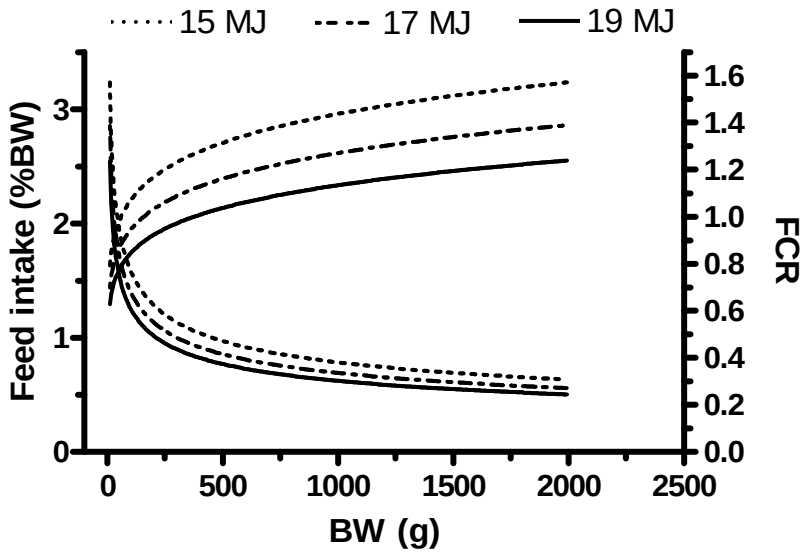

Figure 6.6. Relationship between theoretical FCR and feed intake values (%BW) and BW for mulloway fed diets with three different DE contents (15, 17 or 19 MJ kg^{-1}). Predicted FCR's increase with increasing BW, feed intake as a proportion of BW decreases with increasing BW. Values based on theoretical feed intake at 26 ^{o}C with diets optimized for decreasing DP:DE demands with increasing BW.

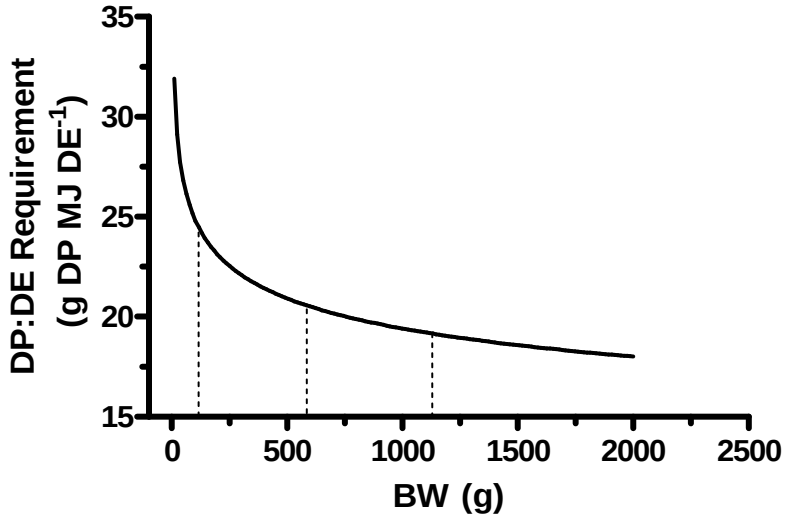

Figure 6.7. Theoretical requirement for DP:DE ratio at 20 to 26^{o}C. Breakpoints (dashed vertical lines) derived from piecewise analysis occur at 111, 582 and 1120 g.

Table 6.4. Iteratively derived feed formulations and feeding regimes at 20 and 26 ^{o}C. Estimates derived from fixed DP:DE ratios over 4 growth stages; 10-100 g = 31.3 g DP MJ DE^{-1} , 100-500 g = 24.8 g DP MJ DE^{-1}, 500-1100 g = 20.8 g DP MJ DE^{-1}, 1100-2000 g = 19.1 g DP MJ DE^{-1}. Suggested appropriate diet specifications and feeding regimes shaded in boxes.

	Live weight (g)							
	10	**50**	**100**	**200**	**500**	**800**	**1100**	**2000**
DE content 15.0 MJ kg^{-1}								
DP content (g kg^{-1})[a]	469.5	469.5	371.5	371.5	312.4	312.4	286.2	286.2
20 ^{o}C								
Intake (g fish^{-1} day^{-1})[b]	0.25	0.78	1.26	2.05	3.93	5.49	6.89	10.58
Intake (%BW day^{-1})	2.55	1.55	1.26	1.03	0.79	0.69	0.63	0.53
Expected FCR[c]	0.83	1.01	1.11	1.22	1.38	1.47	1.54	1.69
26 ^{o}C								
Intake (g fish^{-1} day^{-1})[b]	0.32	0.97	1.56	2.54	4.83	6.73	8.43	12.89
Intake (%BW day^{-1})	3.20	1.94	1.56	1.27	0.97	0.84	0.77	0.64
Expected FCR[c]	0.80	0.96	1.05	1.15	1.29	1.38	1.44	1.57
DE content 17.0 MJ kg^{-1}								
DP content (g kg^{-1})[a]	532.1	532.1	421.0	421.0	354.1	354.1	324.3	324.3
20 ^{o}C								
Intake (g fish^{-1} day^{-1})[b]	0.22	0.69	1.11	1.81	3.46	4.84	6.08	9.33
Intake (%BW day^{-1})	2.25	1.37	1.11	0.91	0.69	0.61	0.55	0.47
Expected FCR[c]	0.73	0.89	0.98	1.07	1.22	1.30	1.36	1.49
26 ^{o}C								
Intake (g fish^{-1} day^{-1})[b]	0.28	0.85	1.38	2.24	4.26	5.94	7.44	11.38
Intake (%BW day^{-1})	2.82	1.71	1.38	1.12	0.85	0.74	0.68	0.57
Expected FCR[c]	0.70	0.85	0.93	1.01	1.14	1.22	1.27	1.38
DE content 19.0 MJ kg^{-1}								
DP content (g kg^{-1})[a]	594.7	594.7	470.6	470.6	395.7	395.7	362.5	362.5
20 ^{o}C								
Intake (g fish^{-1} day^{-1})[b]	0.20	0.61	1.00	1.62	3.10	4.33	5.44	8.35
Intake (%BW day^{-1})	2.01	1.23	1.00	0.81	0.62	0.54	0.49	0.42
Expected FCR[c]	0.66	0.80	0.88	0.96	1.09	1.16	1.22	1.33
26 ^{o}C								
Intake (g fish^{-1} day^{-1})[b]	0.25	0.76	1.24	2.00	3.81	5.31	6.65	10.18
Intake (%BW day^{-1})	2.53	1.53	1.24	1.00	0.76	0.66	0.60	0.51
Expected FCR[c]	0.63	0.76	0.83	0.91	1.02	1.09	1.14	1.24

[a]DP content = Fixed DP:DE (values noted in Table 6.4 caption) x DE content
[b]Intake = DE total (from Table 6.3) / DE content
[c]Expected FCR = Intake / Growth (from Table 6.3)

6.5 Discussion

6.5.1 Feed formulation & feed requirements

This study applied a factorial approach to quantifying protein and energy requirements using those parameters established in the previous chapters relating to protein and energy utilization efficiencies and protein and energy requirements for maintenance combined with whole body compositional and growth data to formulate practical diets and feeding regimes for mulloway. Estimates of 26.7 and 24.4 g DP MJ DE^{-1} for a 70 and 200 g fish respectively at 26 ^{o}C using the current factorial modelling method fall close to those ranges established using the empirical dose response method in Chapter 5. Comparison of DP:DE values between these two independent studies, which used different methodologies to arrive at similar values, appear to mutually validate the estimations of protein and energy requirements in mulloway.

The current practice of feeding mulloway feeds formulated for barramundi or more generic “marine fish” formulations may not be ideal particularly for fish <500 g if growth rates are to be maximized. This is because some commercial feeds can typically contain 21.4 g DP MJ DE^{-1} (e.g. Chapter 4) which, when considering Table 6.3, may not provide an adequate proportion of DP:DE for rapidly growing smaller fish particularly if fish were fed restrictively (see Chapters 4 and 5).

The assignment of different diets with appropriate DP:DE content at key growth stages throughout the production cycle will assist in maximizing growth potential in mulloway. Piecewise polynomial analysis (Figure 6.7) specified key growth stages although, for practical purposes, we can consider 100, 500 and 1100 g to represent appropriate BW indicators at which point to change diets for mulloway. At each successive designated growth stage the DP:DE content will decrease as indicated in

Table 6.3. Although demand for DE increases with increasing BW there may, however, be little scope to supplement diets with non-protein energy sources as mulloway have been shown to have a limited capacity to spare dietary protein (Chapter 5). The potential for mulloway to utilize non-fishmeal based protein sources and non-protein based energy sources requires further investigation.

As the requirement for DP:DE decreases with increasing fish size so to does the maximum capacity for voluntary relative feed intake. Diets in Table 6.4 are presented at three different energetic contents to accommodate feeding smaller fish a low energy 15 MJ diet and larger fish with higher energy 19 MJ diets. This is necessary firstly because, on a relative basis, smaller fish consume more feed than larger fish and issues of inadequate nutrient intake may occur in larger fish unable to ingest adequate feed volumes to meet their nutrient requirements. Secondly, to maintain an appropriate DP:DE content high energy diets require a proportionately high protein content and this may be impractical to make particularly with, for example, 19 MJ diets containing 595 g DP kg^{-1} as indicated in Table 6.4.

6.5.2 Protein and energy composition

The DP:DE requirements derived using the factorial method (Table 6.3) show mulloway to have a relatively high requirement for dietary protein not dissimilar to that established for white grouper (*Epinephelus aeneus*) (Lupatsch and Kissil 2005) and barramundi (Glencross 2008) although greater than that required by gilthead seabream (Lupatsch et al. 2003c) and European sea bass (Lupatsch et al. 2001a). While protein composition tends to remain fairly constant between fish species energy composition can vary considerably and can also vary with body weight. The reason for the above

differences seen in DP:DE requirements between species is largely due to the different requirements for energy. It would therefore appear prudent to calibrate compositional estimations of mulloway with more fish samples >500 g as these may be underrepresented in this study (Figure 6.3). This will assist in refining the energy model presented in Eqn. (6.7).

6.5.3 Growth model

The growth model presented in Eqn. (6.5) is based on the growth assessment of several cohorts of fish representing the growth potential for mulloway over a range of temperatures. Care was taken to exclude cohorts performing poorly where feed intake was dubious and any outliers were also removed from the data set to ensure that the model represented the growth potential of mulloway under the given culture conditions. However, as indicated above, the diets fed to mulloway, also currently used by industry, may not provide an optimal DP:DE content particularly for smaller fish <300 g. Growth assessments using diets formulated according to Table 6.4 will allow further refinement of the growth model. Although estimations in Table 6.3 fall close to the 95 % confidence interval range of DP:DE requirements estimated in Chapter 5, increasing the value of the coefficient in Eqn. (6.5) will in turn increase estimations in the relative demand for dietary protein (Eqn. (6.10)) pushing estimates even closer to those values established in Chapter 5. It should also be noted that the growth model presented is relevant for temperatures ranging from ~18 – 30 ^{o}C and care should be taken when extrapolating outside these ranges.

The growth model also provides a useful management tool to ascertain if general husbandry and feeding practices are of an adequate standard by comparing

actual vs. predicted growth rates. Growth rates found to be well below those predicted in Eqn. (6.5) could indicate problems associated with feed intake such as the quality and/or quantity of feed offered, poor water quality, inappropriate stocking densities (see Appendix 1) or any number of other issues which can potentially retard growth.

6.5.4 Metabolic body weight

Body size and temperature are two of the fundamental determinants of metabolic rate in living organisms (Clarke and Johnston 1999; Willmer et al. 2000; Gillooly et al. 2001). The magnitude with which body mass (BW) influences metabolism (*M*) is reflected in the value of the mass scaling exponent (*b*) in the general form shown in Eqn. (6.4). In animals, *b* is generally <1 therefore the proportionality between body mass and metabolic rate of different size animals is not constant and the mass-specific metabolic rate tends to decrease with increasing body mass. To accurately model protein and energy requirements throughout the production cycle metabolic rate must be expressed as a proportion of the metabolic body weight; however, this study demonstrated that the value of *b* will also vary depending on methodology. While this study determined protein and energy loss on starvation in mulloway using a replicated fully orthogonal design, the error associated with each treatment resulted in acceptance of the null hypothesis. Methodology, data transformation, sample size and animal size range are all known to influence the value of the allometric exponent (White and Seymour 2005b; Hui and Jackson 2007; Packard and Boardman 2009) making estimations of the "true" exponent value difficult. Hui and Jackson (2007) demonstrated that large sample sizes are necessary (up to 61 % of population size) to obtain reliable estimates of the allometric scaling exponent and reduce the likelihood of Type II error.

When sample sizes are small, they argue as is the case with most studies, erroneous acceptance of a false null hypothesis can occur. A value of 0.8 describing the metabolic weight loss of energy determined in the current study supports those for energy metabolism established in Chapters 3 and 4 and the common inter-specific exponent value applied to the energy metabolism of teleost fishes (Clarke and Johnston 1999). A value of 0.7 describing the metabolic weight loss of protein was used which also allows comparison with published data on other fish species (Lupatsch et al. 2001a; Lupatsch et al. 2003c; Lupatsch and Kissil 2005; Glencross 2008) and is close to the 2/3 rather than 3/4 power scaling of basal metabolic rate seen across different phyla (White and Seymour 2003; Kozlowski and Konarzewski 2004; McKechnie and Wolf 2004). The allometric relationship seen between BW and SL (solving for SL in Eqn. (6.3); b = 0.341) is also very close to 1-b = 0.333 power scaling used to describe the thermal-unit growth co-efficient based on BW or length derived data (Iwama and Tautz 1981; Cho 1992).

6.5.5 Conclusion

The data presented in this chapter will assist in the formulation of appropriate diets and feeding regimes to optimize the growth performance of mulloway in intensive aquaculture throughout the production cycle to 2 kg BW. While estimations for DP and DE in this study were found to be close to those established in Chapter 5 further growth studies are required to validate those diets and feeding regimes prescribed in Table 6.4.

Chapter 7

General Discussion

Prior to the commencement of this research there were no published data on the requirements for digestible protein (DP) and digestible energy (DE) for mulloway and, as a consequence, no specific diet formulations or feeding standards were available. To address this, a comprehensive series of studies quantifying the requirements for DP and DE for maintenance and growth and the description of mulloway metabolism relating to aspects of fasting and feeding physiology were undertaken. The results from these studies were consolidated in Chapter 6 using a factorial modelling approach to produce theoretical diet formulations and feeding tables applicable for mulloway throughout the grow-out stage up to 2 kg body weight. This research has contributed towards addressing several problems currently faced by marine fish farmers and aquafeed manufacturers in Australia with regard to appropriate diet specifications and feed management practices for mulloway.

The following discussion firstly addresses some of the influences of methodology on establishing the requirements of DP and DE for maintenance and growth obtained in the previous chapters. Particularly insightful is a sensitivity analysis of the parameters used to populate the factorial model. The application of bioenergetic principles to the varying planes of nutrition as they pertain to mulloway is then discussed and an energy flow schematic is presented. The implications for industry are

then addressed in terms of the current feeding and culture strategies being used by industry in light of the findings of this thesis. Future research directions are considered including the further development of the feed evaluation system for mulloway. The general discussion then concludes with a brief overview of the major findings of this thesis.

7.1 Methodological considerations

7.1.1 Factors influencing estimations of protein and energy requirements based on the factorial method: Individual parameter sensitivity

Individual parameter sensitivity analyses is a simple method to test the response of the factorial model (Chapter 6) to small perturbations of individual parameter values to identify which of the sub-model parameters have the greatest influence on the predicted output, i.e. the predicted ratio of DP:DE. The change in model output relative to the models response for a nominal set of parameter values can be calculated as:

$$S = \frac{(R_a - R_n)/R_n}{(P_a - P_n)/P_n}$$

Where S is the single parameter sensitivity, R_a and R_n are the models response to altered and nominal parameter values respectively, and P_a and P_n are the altered and nominal parameters respectively (Haefner 2005). Altered parameter values were calculated as ±10% of nominal values from Table 6.2. This method tests the influence of individual parameters and does not consider the potential multiplicative effect of the simultaneous change in two or more parameter values. The following discussion relates to parameter

sensitivity at 20 ^{o}C although stochastic variables such as temperature can, depending on the output criteria, influence parameter sensitivity (e.g. Zhou et al. 2005). This temperature was chosen as it was the common temperature used throughout this thesis and is close to the average annual temperature experienced at mulloway sea cage operations in Botany Bay, NSW (see Fig. 7.5).

The sensitivity analyses results presented in Table 7.1 are insightful as they demonstrate, on several levels, the dynamic effect that small adjustments in individual parameter values have on the overall estimates for DP:DE. Several generalisations can be made. Firstly, the factorial model is fairly robust as there is very little compounding of output values with adjustments of individual model parameter values, i.e. with only minor exception, the magnitude of change in the output value was always less than the magnitude of change of the input value over the size range tested. Secondly, because the output is a ratio, an increase or decrease in any individual parameter value will directly change the output value to reflect the influence of that parameter relative to the requirement for DP. For example, an increase in protein utilisation efficiency will decrease the requirement for DP while an increase in energy utilisation efficiency will increase the requirement for DP. Thirdly, the magnitude of change of the absolute output value will generally differ depending on the direction of parameter change. The exception to this is the whole body protein constant where the magnitude of change in absolute terms is equal regardless of the direction of parameter change. Lastly, the relationship of any individual parameter influence on the magnitude of change for a given body weight on the output value is allometric.

From Table 7.1 it can be seen that the individual parameters which have the greatest influence on the predicted requirement for DP:DE for mulloway up to 2 kg are the protein and energy utilisation coefficients and the whole body composition

coefficients for protein and energy while the growth model exponent value becomes increasingly influential for fish >200 g. The accuracy of the utilization coefficients can be assumed with some confidence as these were determined from controlled experiments (Chapter 4) and were also found to be consistent with published values for other fish species (Azevedo et al. 1998; Lupatsch et al. 2003b). The whole body composition for protein is known to remain fairly constant in fish (Shearer 1994) and was shown to be consistent across all the relevant studies in this thesis (Chapters 4, 5 & 6). However, unlike protein composition, relative whole body energy composition will vary with body size (Figure 6.3) necessitating a comparatively large sample size to accurately determine whole body energy composition over the desired size range. Feeding history also strongly influences whole body energy composition making previous DE intake an important consideration when attempting to establish energy compositional profiles representative of a “normally” feeding population. This also has implications for compositional analyses when comparing initial and treatment samples (Chapters 4, 5). The influence of intake on whole body protein and energy composition is clearly demonstrated in Figure 7.1.

Table 7.1. Parameter sensitivity analysis. Values represent % change in the predicted DP:DE values at 20 ^{o}C (Table 6.3) after altering individual model parameter values ±10%. Refer to Table 6.2 for original individual model parameter values. Parameters shown ranked in order of greatest to least influence on predicted DP:DE requirement based on the average (absolute) value over the fish weight range shown.

		Live fish body weight (g)								
Altered value	**Parameter**	**10**	**50**	**100**	**200**	**500**	**800**	**1100**	**2000**	**Average**
+10%	**Whole body composition constant (protein)**	8.4	8.1	8.0	7.9	7.7	7.5	7.5	7.3	**7.8**
	Utilisation efficiency coefficient (protein)	-7.9	-7.6	-7.5	-7.4	-7.2	-7.1	-7.0	-6.9	**-7.3**
	Growth weight exponent	1.7	3.4	4.3	5.3	6.7	7.5	8.1	9.2	**5.8**
	Utilisation efficiency coefficient (energy)	6.9	6.3	6.1	5.8	5.5	5.3	5.1	4.9	**5.7**
	Whole body composition coefficient (energy)	-6.6	-6.1	-5.9	-5.7	-5.4	-5.2	-5.1	-4.9	**-5.6**
	Metabolic weight exponent (energy)	9.8	7.9	6.6	5.0	2.4	0.8	-0.4	-2.7	**4.5**
	Maintenance constant (energy)	-2.8	-3.3	-3.6	-3.8	-4.1	-4.3	-4.4	-4.6	**-3.9**
	Maintenance constant (protein)	1.7	2.1	2.2	2.4	2.6	2.7	2.8	3.0	**2.4**
	Whole body composition exponent (energy)	-1.2	-1.9	-2.1	-2.3	-2.6	-2.7	-2.7	-2.8	**-2.3**
	Metabolic weight exponent (protein)	-4.3	-3.5	-3.0	-2.3	-1.1	-0.4	0.2	1.3	**-2.0**
	Growth coefficient	1.3	1.5	1.6	1.7	1.9	1.9	2.0	2.1	**1.7**
	Growth temperature exponent	1.2	1.4	1.5	1.6	1.8	1.8	1.9	1.9	**1.6**
-10%	**Utilisation efficiency coefficient (protein)**	9.7	9.4	9.2	9.1	8.8	8.7	8.6	8.4	**9.0**
	Whole body composition constant (protein)	-8.4	-8.1	-8.0	-7.9	-7.7	-7.5	-7.5	-7.3	**-7.8**
	Whole body composition coefficient (energy)	7.6	7.0	6.7	6.4	6.0	5.8	5.7	5.4	**6.3**
	Utilisation efficiency coefficient (energy)	-7.3	-6.8	-6.5	-6.3	-5.9	-5.8	-5.6	-5.4	**-6.2**
	Growth weight exponent	-1.8	-3.7	-4.6	-5.6	-7.0	-7.8	-8.3	-9.3	**-6.0**
	Metabolic weight exponent (energy)	-11.5	-8.5	-7.0	-5.2	-2.4	-0.8	0.4	2.7	**-4.8**
	Maintenance constant (energy)	3.0	3.6	3.8	4.1	4.5	4.7	4.8	5.1	**4.2**
	Metabolic weight exponent (protein)	5.9	4.3	3.5	2.6	1.2	0.4	-0.2	-1.3	**2.4**
	Whole body composition exponent (energy)	1.2	1.9	2.1	2.3	2.6	2.7	2.8	2.9	**2.3**
	Maintenance constant (protein)	-1.6	-1.9	-2.0	-2.2	-2.4	-2.5	-2.6	-2.8	**-2.3**
	Growth coefficient	-1.4	-1.7	-1.8	-1.9	-2.1	-2.1	-2.2	-2.3	**-2.0**
	Growth temperature exponent	-1.2	-1.5	-1.6	-1.7	-1.8	-1.8	-1.9	-2.0	**-1.7**

Note: Ranked average values are for illustrative purposes and will obviously change depending on nominated body weight and range.

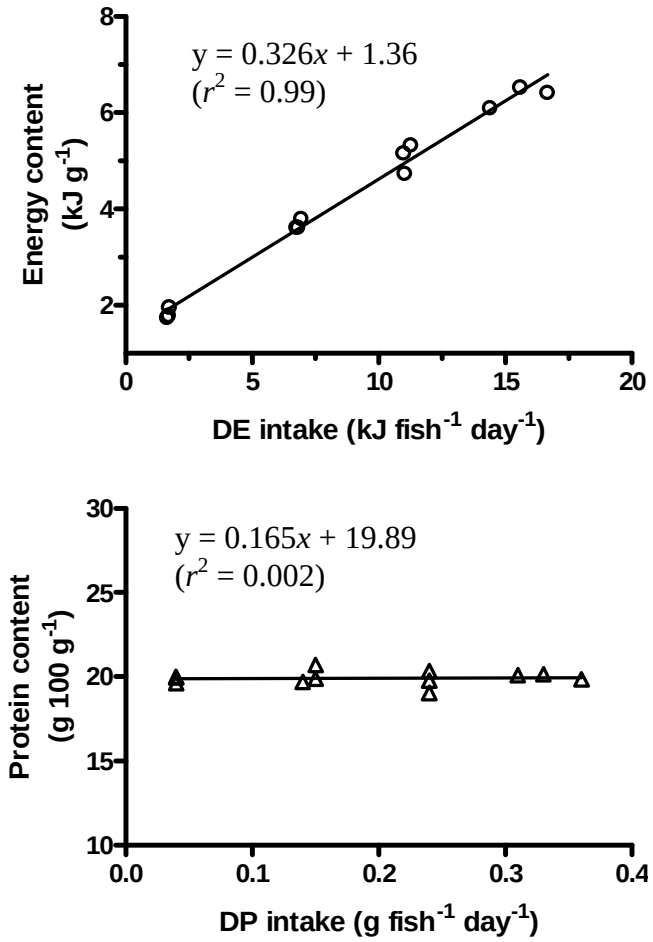

Figure 7.1. Energy or protein whole body content as a function of DE or DP intake for 40 g mulloway (IBW) held at 26 ^{o}C for 57 days (n = 12). Data adapted from Chapter 4.

As indicated above, model parameters which respond allometrically with body weight require a substantial data set over which reliable predictions can be made. This is particularly the case when considering sub-models which have more than one independent variable such as the growth model presented in Chapter 6. In this case, integrating a temperature function requires an increase in the quantity of growth data by an order of magnitude representing the different levels of growth as a function of both body weight and temperature. The growth model for mulloway can therefore be further calibrated with the collation of more data over the desired production size and temperature ranges. While there are few published data on the growth rates of mulloway to compare with, particularly for fish exceeding 200 g, the model in its current form indicates a similar growth trajectory to that of barramundi and white grouper when reared at the same temperature (Figure 7.2).

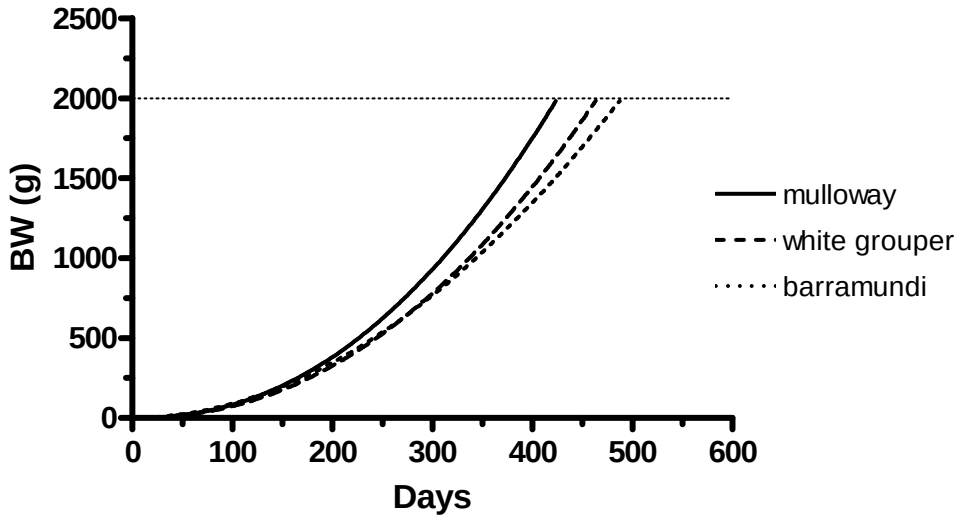

Figure 7.2. Comparison of predicted body weight over time between mulloway, white grouper (Lupatsch and Kissil 2005) and barramundi (Lupatsch and Kissil 2003) growing from 1 g to 2 kg at 26 ^{o}C.

7.1.2 Estimating maintenance energy requirements

Values derived from RMR (Chapters 2 & 3), Eqns. (4.6) and (4.7) (Chapter 4) and energy loss on starvation for 4 weeks (Chapter 6) all gave similar estimates for the maintenance energy requirements for unfed mulloway (Figure 7.3). Figure 7.3 also shows that estimates derived from mulloway fasted for 2 weeks will, on average, be approximately 20% higher compared to those estimated using the other three methods at 20 ^{o}C. A cascade of physiological and behavioural responses occurs from the onset of starvation in animals which are dependant upon several factors including the duration of food deprivation (Wang et al. 2006a). The onset of food deprivation may initially promote the animal to search more actively for food inturn increasing stress levels leading to an increase in energy expenditure in the short term phase of starvation. This initial phase is then generally followed by the animal becoming more quiescent and down regulating metabolism leading to a relative reduction of energy expenditure over the longer term (Wang et al. 2006a). The starvation method can be used to accurately determine maintenance energy requirements in mulloway; however, 2 weeks starvation at 20 ^{o}C may not be a sufficient length of time and 4 weeks duration appears to be more appropriate at this temperature.

SDA and the utilisation efficiency of a diet for production are often discussed in reciprocal terms (Bureau et al. 2002) and can be regarded as two sides of the same coin. From Eqns. (4.6) and (4.7) SDA represents approximately 40% of the total maintenance requirements for mulloway. Using this data as a proportion of the requirements for maintenance estimated in Table 6.2 the theoretical SDA coefficient can be calculated as approximately 12 to 16% of DE intake depending on temperature (20 – 26 ^{o}C) and fish size (60 – 240 g) which is within the range

reported for most temperate fish species (6-23%) (Pandian and Vivekanandan 1985; McCue 2006) and not too dissimilar to the SDA coefficients (7 – 11%) derived empirically in Chapter 3 based on the oxyenergetic equivalent of MO_2 rates at similar temperatures and fish sizes.

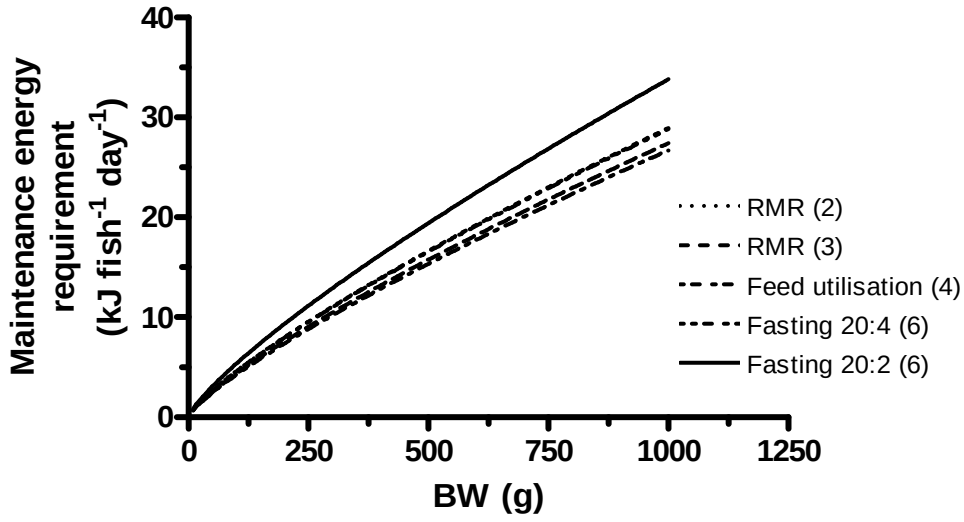

Figure 7.3. Comparison of maintenance energy requirements of unfed mulloway at 20 ^{o}C using the different methodologies presented in this thesis. Legend codes are reference to the specific studies and chapter numbers: RMR (2) = Eqn. (2.13) (Chapter 2); RMR (3) = derived from Eqn. (3.1) using the oxyenergetic coefficient of 13.59 kJ mg^{-1} O_2 (Chapter 3); Feed utilisation = y-intercept of Eqn. (4.6) (Chapter 4); Fasting 20:2 and 20:4 power functions adapted from Table 6.1 when b = 0.8 (Chapter 6).

7.2 Bioenergetic application

The studies in the preceding chapters partitioned and quantified the energetic contributions to growth and maintenance in mulloway. Across all studies a key finding was the highly variable nature of many of the parameter values dependent on the influence of individual and/or the interactive effects of fish size, temperature, diet and other factors. This has important implications on the accuracy of any system attempting to model rates of nutrient deposition and subsequent feed requirements in animals; predictions can only be valid within the physiological and environmental contexts from which the parameter values were derived. Clearly the inappropriate use of fixed parameter values in bioenergetic models will lead to spurious predictions of energy requirements (Bureau et al. 2002).

An example of the variable nature of energy flow within mulloway can be seen in Figure 7.4. The schematic is based on the experimental conditions in Chapter 3 where different size mulloway were fed a commercial diet to apparent satiation at 14, 20 or 26 ^{o}C. Non-faecal energy was not measured in that study but is assumed to range from approximately 3-9% (Chakraborty et al. 1995; Kaushik 1998). The energy budget of growing, fed fish can be expressed as:

$$IE = M + UE + FE + P \quad (7.1)$$

where ingested energy (IE) can be partitioned into energy used for metabolism (M=RMR+SDA), nitrogenous waste (UE), and faecal waste (FE). From this the proportion of recovered energy (P; production) in mulloway can be predicted to be between 10-54% of total IE (Figure 7.4). In Chapter 4, the proportion of IE retained

as P in mulloway which were fed to apparent satiation with the same commercial diet at 20 and 26 ^{o}C fell within this range (P = ~30-40%; measured directly).

Energy budgets of mulloway fed below maintenance levels (Chapters 4 and 5) must take into account the catabolism of body tissue (i.e. loss of weight) as an energy source and can be derived using a modification of Eq. (7.1):

$$IE - P = M + UE + FE \quad (7.2)$$

Similarly, unfed fish will also catabolize body tissue although the parameter M in this case will exclude any SDA effect:

$$-P = M + UE + FE \quad (7.3)$$

Some authors exclude FE from Eq. (7.3) (e.g. Carter and Brafield 1991); however, mulloway were observed to excrete faecal-like matter throughout the starvation periods noted in Chapter 6. This may partly explain differences seen in predicted vs. measured values for P estimates in some species resulting in energy budgets of unfed fish being somewhat less accurate than those of fed fish (e.g. Carter and Brafield 1991; Chakraborty et al. 1995). As P, M and UE can be measured directly in Eq. (7.3); FE in unfed fish can then be estimated by difference with reasonable accuracy. However, this remains to be validated for mulloway.

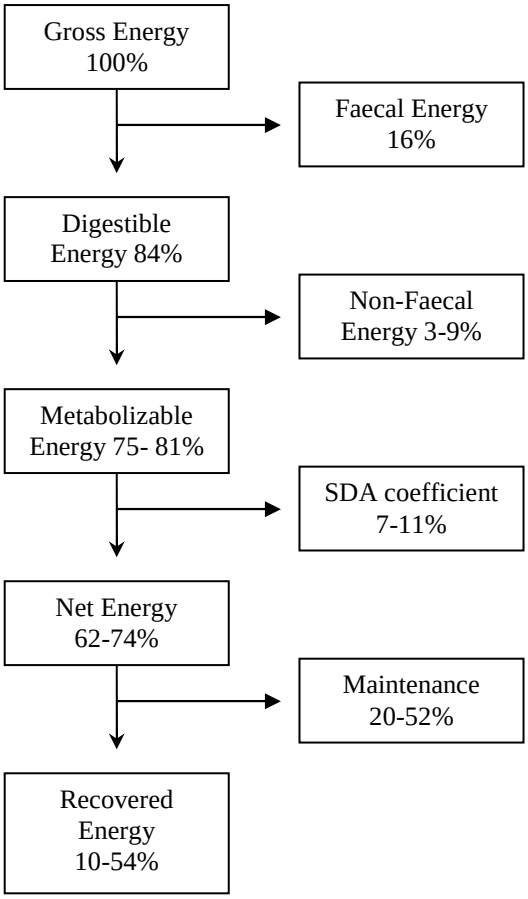

Figure 7.4. Dietary energy partitioning schematic for fed mulloway based on the culture conditions presented in Chapter 3. Digestibility coefficient from Booth (unpublished data, 2008). SDA and maintenance (RMR) energetic values from Chapter 3. Non-faecal energy assumed to be ~3-9% (Chakraborty et al. 1995; Kaushik 1998). Recovered energy estimated by difference.

7.3 Industry implications

The influence of nutritional and environmental factors and their interactions on the growth of mulloway reported in this thesis have direct implications for the mulloway aquaculture industry. Chapter 2 also provided information describing the thermosensitivities of yellowtail kingfish and mulloway which have implications with regard to the appropriate thermal range with which to culture these species and also highlighted the critical importance of maintaining high DO levels for yellowtail kingfish.

While mulloway are a eurythermal species, a temperature of around 26 ^{o}C is likely to be the most suitable to optimise growth. Currently many commercial sites in Australia are located where mean annual water temperatures are below 20^{o}C. At these established sites growth rates may be improved by the use of more efficient feeds and improved feeding regimes, as indicated above and in the preceding chapters. However, if optimised diets and feeding regimes are used in combination with grow out at sites or facilities at or near optimal temperatures then the time to market will be significantly reduced. The impact of temperature on growth rates and subsequent time to market is clearly illustrated in Figure 7.5. From Eqn. (6.6), the difference between the time taken for mulloway (BW_0 = 1 g) to reach 2 kg when exposed to different temperature profiles at Port Lincoln, SA compared to Kurnell, NSW will be approximately 100 days. It should be noted that, apart from temperature, Figure 7.5 assumes the same set of rearing conditions, water quality, diet and feeding regimes.

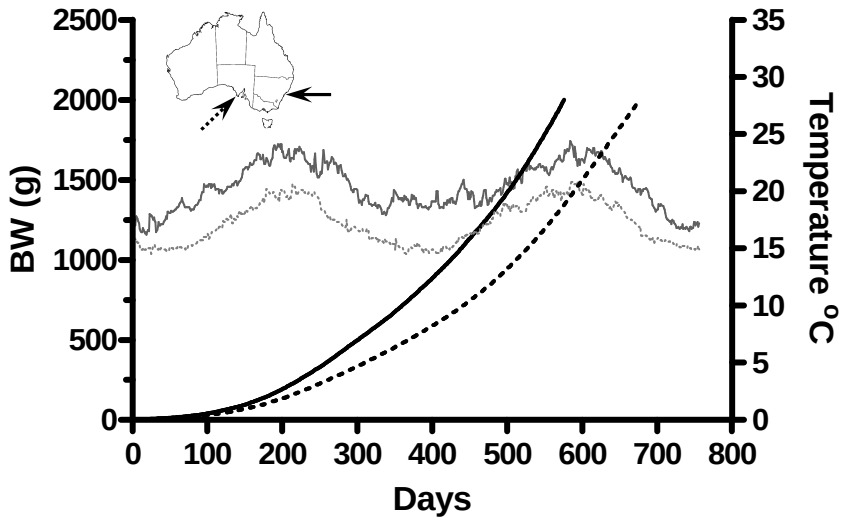

Figure 7.5. Predicted growth rates of 1g mulloway to 2 kg at two different site locations in Australia. Growth rates calculated from Eqn. (6.6) based on 2005 – 07 daily sea surface temperatures (secondary *y*-axis) at Port Lincoln, SA (dotted lines) and Kurnell, NSW (solid lines). SST source: Bureau of Meteorology, Australia.

As indicated in Chapters 5 and 6, some commercial feeds currently being used for mulloway may not provide an adequate proportion of DP:DE particularly for rapidly growing fish <300 g. Aquafeeds currently marketed for marine fish in Australia and generally containing approximately 20 g DP MJ DE^{-1} may be suitable for mulloway > 500 g however, as clearly illustrated in Figure 5.5, mulloway <200 g fed these diets will show a reduced rate of protein accretion (g $kgBW^{-0.7}$ day^{-1}) of approximately 40% when compared to those fed diets with 28.6 g DP MJ DE^{-1} (Chapter 5).

Dietary protein, particularly in the form of fishmeal, is usually the main driver of aquafeed ingredient costs. Therefore diets formulated as suggested in Table 6.3 will be more expensive than some of the less nutrient dense diets currently available. However, a diet which is optimised to match the nutritional requirements of a species will promote faster growth and improved feed conversion ratios. The cost of feeds also represents the major expense associated with running

aquaculture farms; therefore the economic returns on a reduced time to market and improved FCR's need to be carefully considered when making decisions about the most appropriate feeds and feeding regimes to use. A low cost feed does not necessarily mean that it will be cost effective.

More efficient feeds, i.e. feeds that are better utilised, combined with better feeding practices will also help mitigate environmental impacts in sea cage operations by reducing excess excretion and feed wastage. This is particularly important in oligotrophic environments where excessive nutrient loading from intensive aquaculture may, for example, cause a shift in the diversity and abundance of algal assemblages in near-shore natural systems inturn impacting on local faunal communities (Mannino and Sara 2008).

7.4 Mulloway feed evaluation system - future direction

The factorial model applied to the development of feed formulations and feeding strategies for mulloway in Chapter 6 was derived from a series of laboratory based empirical studies and growth data obtained under both farm and laboratory culture conditions. Although estimates for DP and DE requirements using the factorial method in Chapter 6 were similar to those derived independently in Chapter 5, engendering confidence in the results of both studies, the suggested feed formulations and strategies presented in Chapter 6 are theoretical and remain to be tested under commercial culture conditions. Successful validation through a series of feeding trials performed under commercial culture conditions will assist in the decision by the mulloway aquaculture industry in Australia to adopt the suggested feed formulations and feeding strategies.

The factorial method used to define the requirements for DP and DE of mulloway in this thesis is based predominantly on the work originally described by Lupatsch et al. (1998). The theoretical framework of this method is highly adaptive and could be applied to describe the requirements for other nutrients such as lipids or individual amino acids. Optimization of feed formulations and feeding strategies could also be achieved using alternative feed ingredients to the fishmeal based diets used in the current study (see section 7.4.2 below). The growth model, which underpins the factorial approach, can be integrated with other stochastic functions such as salinity or dissolved oxygen etc. to better predict growth targets under specific culture conditions. This is particularly pertinent to estuarine sea-cage operations which experience large tidal and/or seasonal fluctuations in the physicochemical profile of water quality parameters.

The use of more efficient feeds and feeding practices will invariably reduce the amount of nutrient output on fish farms; however, accurate estimations of nutrient loading are a necessary requirement in the legislation of modern commercial aquaculture farms in Australia and other parts of the world. This is also the case with regard to complying with environment protection policies for new farm proposals. Application of the factorial method to estimate waste output has been achieved for some species (Lupatsch and Kissil 1998; Lupatsch et al. 2003a; Hua et al. 2008) and could readily be applied to mulloway.

7.5 Conclusions

The studies presented in this thesis have contributed directly to our understanding of the nutritional energetics and the fundamental requirements for

digestible protein and digestible energy in mulloway. These studies provide the knowledge foundation from which future nutritional studies and diet development research can be built upon for this species. The fasting metabolism of yellowtail kingfish was also described with implications relating to the optimal temperature for routine metabolic function for this species.

Overview of the major conclusions in this thesis:

- The majority of parameter values for establishing bioenergetic budgets for mulloway are not fixed and are highly variable dependent on body size, temperature, diet and nutritional plane
- Mulloway have a broader temperature range for optimal routine metabolic function compared to yellowtail kingfish. RMR for mulloway and yellowtail kingfish are least thermally dependant at 28.5^{o}C and at 22.8^{o}C respectively
- The magnitude of the effect of temperature on the mass-specific RMR of mulloway will vary depending on body size
- Both SDA duration and time to peak SDA are influenced by temperature and body weight of mulloway. SDA duration will occur 41-89 h and peak SDA will occur within 17 – 38 h of feeding at temperatures 14 – 26 ^{o}C
- A temperature of approximately 26±2 ^{o}C appears to be optimal for the growth and metabolic function of mulloway
- Utilization efficiencies for DP and DE in mulloway are independent of fish size, ration level or temperature

- Maintenance requirements for DP are influenced by body size of mulloway but not temperature while maintenance requirements for DE increase with increasing temperature and are also influenced by body size
- Mulloway were shown to have a limited capacity to spare dietary protein
- Adoption of diet formulations and feeding strategies described in this thesis will facilitate an improvement in the nutritional management of mulloway

Appendix 1

Pilot Study: The Effect of Stocking Density and Repeated Handling on the Growth of Juvenile Mulloway[5]

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[5]The following Appendix is published as:

Pirozzi, I., Booth, M.A., Pankhurst, P.M., 2009. The effect of stocking density and repeated handling on the growth of juvenile mulloway, *Argyrosomus japonicus* (Temminck & Schlegel 1843). Aquac. Int. 17, 199-205.

A1.1 Abstract

The effect of stocking density on the growth of mulloway, was tested with 17 g fish stocked at 4.08, 8.16 or 16.32 kg m^{-3} in 50 L aquaria. Weight checks were carried out every two weeks to track performance. Each density treatment was also compared to a non-handled control group to establish if handling during weight checks influenced the growth of mulloway. Mulloway performed poorly at the lowest density and, under the current experimental conditions, growth did not appear to be negatively affected by regular handling.

A1.2 Introduction

Stocking density is one of the most important biotic factors influencing growth and feed intake of fish in culture (Kestemont and Baras 2001) directly modifying feeding behaviour (Boujard et al. 2002), social interactions (Barcellos et

al. 1999), water quality (Ellis et al. 2002), and has also been shown to influence sexual dimorphism (Davis et al. 2002). Stocked densities of 15 kg m^{-3} at harvest have been achieved for mulloway (Quartararo 1996) however the relationship between stocking density and growth of mulloway is currently unknown.

The primary objective of this study was to identify the effects of stocking density on the growth of juvenile mulloway as evidenced by survival, body weight and length, condition factor, size heterogeneity and feeding efficiency. This information will be of use in determining appropriate stocking densities of mulloway for both future growth studies and aquaculture of the species.

During growth studies on fish it is common practice to track performance (growth) over time by sampling periodically and measuring some physical parameter, e.g. weight, length, etc. Anesthetics are commonly used to minimize the stress response when handling fish; however, anesthesia can itself produce a stress response (Ortuno et al. 2002a; Ortuno et al. 2002b) and can also have a negative effect on growth (Hoskonen and Pirhonen 2006). Each stocking density treatment was therefore also compared to a non-handled control group to identify if the growth of mulloway is compromised from routine handling during regular weight checks.

A1.3 Materials and Methods

The effect of density on the growth of mulloway was tested over 37 days using 17 g fish (±3.5 g), 4 month old, F2 juveniles of broodstock held at the New South Wales Department of Primary Industries, Port Stephens Fisheries Centre (PSFC). Fish were sedated with using 20 mg L^{-1} benzocaine (ethyl *p*-

aminobenzoate) and stocked into 50 L aquaria at one of three stocking densities: 4.08, 8.16 or 16.32 kg m^{-3} (12, 24 or 48 fish $aquaria^{-1}$), nominally low (LD), medium (MD) and high (HD) densities respectively. There were four replicate aquaria for each density treatment. The control (non-handled) group consisted of an additional four replicate aquaria for each of the three stocking densities. Once stocked, the control fish were not handled until the completion of the experiment. In this experiment the combined effects of anesthesia and handling cannot be separated and therefore the terms 'handling' or 'handled' are used to denote both.

The experiment system consisted of 24 x 50 L replicate acrylic aquaria integrated via a semi-recirculating bio-filtration unit. A moderate flow-through rate allowed twice daily renewal of water to the system. Flow to each aquarium was approximately 2 L min^{-1} ensuring similar water quality between all treatment aquaria. Ranges and means (±SD) for water quality parameters were: temperature (^{o} C) 19.6 – 22.5, 20.8 (0.9); NH_4^+ (mg/L) 0.1 – 0.8, 0.4 (0.1); DO (mg/L) 5.3 – 7.2, 6.1 (0.3); pH 7.8 – 8.3, 8.0 (0.1); salinity (ppt) 26.0 – 32.3, 29.4 (1.4). Black plastic sheets were placed between each aquarium and across the front to minimize disturbance. All aquaria were exposed to 12L:12D photoperiod using fluorescent lighting (<1 µE m^{-2} s^{-1} at aquaria surface).

Analysis of variance of initial weights ($F_{2,21}$ = 3.35; P >0.05) and initial CV ($F_{2,21}$ = 0.76; P >0.1) demonstrated no significant difference between treatments. An additional 100 individuals were also measured for weight and total length (L_T) for initial condition factor (K) comparison. Refer to Table 1 for summary of initial data.

Weight checks were carried out every two weeks on the handled treatment group. To ensure that handling protocols during weight checks remained consistent between all density treatments, fish in the highest density were sampled first and the

exposure time to handling and benzocaine per aquarium noted. This time (approx. 15 min.) was then applied to the remaining densities and also to subsequent weight checks.

Fish were fed by hand twice daily (08:30 and 15:00) to apparent satiation with a commercial barramundi (*Lates calcarifer*) diet (Ridley AquaFeed Pty. Ltd., Narangba, Qld. Australia; reported nutrient composition: 50% crude protein, 12% crude fat, 2.5% fibre, 18 MJ kg^{-1} gross energy) which was reground and repelleted (3mm) to sink.

Table A1.1. Summary of initial and final data. Initial data are means ±SD. Final data are pooled mean values (±se; n = 8) for each density tested. Tukey-Kramer test on means between densities shown as superscripts. Means sharing superscripts are not significantly different (P >0.05).

Treatment	Survival (%)	Weight (g)	Length (mm)	Condition (*K*)	CV (%)	FE
Initial	-	16.5 (1.9)	117 (2.9)	1.04 (0.06)	11.2 (1.4)	-
Final						
LD	92.8 (3.3)	23.8 (0.7)^{a}	131 (0.7)^{a}	1.03 (0.02)	24.5 (2.3)	0.45 (0.04)^{a}
MD	88.0 (2.5)	28.7 (0.8)^{b}	139 (0.9)^{b}	1.05 (0.01)	28.5 (1.9)	0.84 (0.03)^{b}
HD	85.7 (1.9)	27.5 (0.7)^{b}	138 (0.9)^{b}	1.03 (0.01)	26.9 (1.2)	0.90 (0.04)^{b}

Aquaria were inspected daily and any mortalities were replaced with similar size fish in order to maintain treatment densities. Replacement fish were fin clipped (left pectoral) for ease of identification and were not used in the final analyses; all data were derived from the tank means of the remaining original fish. Faeces and feed debris were siphoned from tanks daily. Shoaling and feeding behaviour and responses to routine aquaria maintenance were observed daily; however, these were not quantified.

Co-efficient of variation (CV) of weight (%), Condition factor (K) and feeding efficiency (FE) were calculated as:

$CV = s.\bar{x}^{-1} \times 100$

$K = [W / L_T^{3}] \times 100$

Where: W = wet weight (g) and L_T = total length (cm)

FE = wet weight gain (g) / total feed intake (g)

A 2-way ANOVA was used to determine density and handling effects on the dependent variables: survival (%), final weight (W_f), final length (L_f), FE, CV and *K*. Cochran's *C* test was used to test homogeneity of variances. Tukey-Kramer test was used for *a posteriori* multiple comparison of means on significant terms. Probability of Type I error was set at $\alpha = 0.05$ for all analyses.

A1.4 Results

There was no significant interaction or handling main effect between densities for all variables (survival (%), W_f, L_f, *K*, CV, FE) (Table A1.2). The handling term was therefore removed and all subsequent analyses performed as a single factor ANOVA on pooled data.

Mean individual weights were significantly different between density treatments from the first weight check two weeks after initial stocking ($F_{2,9} = 6.35$; $P < 0.02$) (Figure A1.1). At week two MD fish were larger than LD fish but not

significantly different from the HD fish. By week four both the MD and HD fish were larger than the LD fish ($F_{2,9}$ = 8.05; P <0.01) (Figure A1.1). The effect of stocking density was also significant on final weight ($F_{2,21}$ = 12.35; P <0.001) and final length ($F_{2,21}$ = 20.48; P <0.001) with MD and HD fish larger than LD fish (Table A1.1). Stocking densities (±SD; n = 8) at the conclusion of the experiment were 5.7(0.5), 13.8(1.1) and 26.4(1.9) kg m^{-3} for LD, MD and HD respectively.

Total overall survival was 88 %. There was a trend for greater survival with decreasing density; however, this was not statistically significant ($F_{2,21}$ = 1.87; P >0.1) (Table A1.1).

Final FE was significantly poorer for the LD treatment than MD and HD treatments (Table A1.1). CV increased from initial stocking (Table A1.1); however there was no significant difference between final density treatments ($F_{2,21}$ = 1.07; P >0.2; Table A1.1).

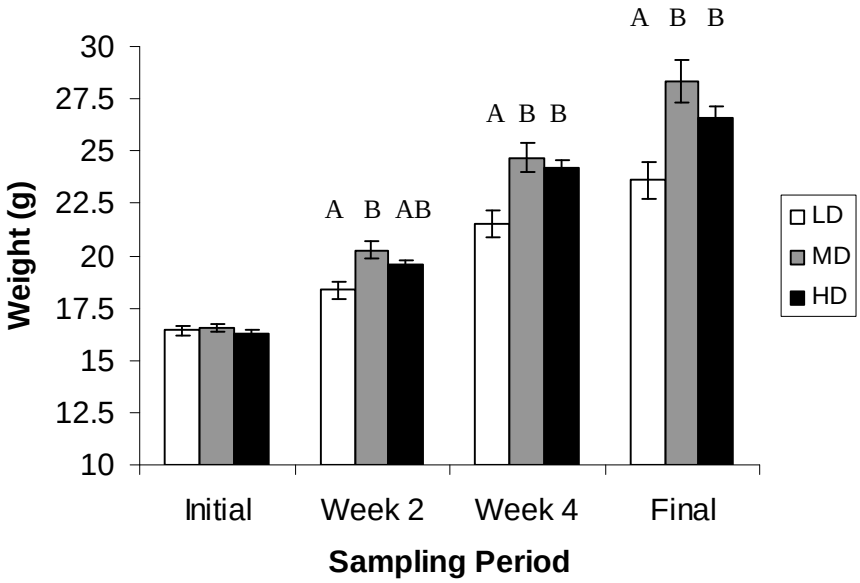

Figure A1.1. Initial mean stocking weight (g) and mean weight of handled group over time (+/- se; n = 4). LD = 12, MD = 24 and HD = 48 fish $aquaria^{-1}$. Tukey-Kramer test on means between densities shown for each sampling period. Means sharing letters are not significantly different (P >0.05).

Initial and final condition co-efficients were similar (Table A1.1). Stocking density did not have a significant effect on final *K* ($F_{2,21}$ = 0.72; *P* >0.5). Heterogeneity of variances could not be removed from final *K* data; however, ANOVA was still performed. The result is valid as heterogeneous data increases the chance of Type I error (Underwood 1997) and, in this case, there were no significant differences.

There was no agonistic behaviour observed during feedings or at other times in any of the aquaria. LD fish appeared to be quite timid for the first two weeks; often staying in the back corner of aquaria huddled together and taking longer to approach food. In contrast, MD and HD fish were evenly dispersed throughout aquaria. Fish did not appear to be disturbed by daily siphoning of aquaria. Lights switching on and off startled the fish causing them to swim rapidly for several seconds and collide with the aquaria surfaces; however, normal behaviour appeared to resume quite quickly after each event.

Table A1.2. Two-factor analysis of variance for survival, final weight, final length, condition (K), CV and FE. ns indicates not significant at P < 0.05, * significant at p < 0.05, ** significant at p < 0.01.

		Survival(%)			**$Weight_f$**			**$Length_f$**			**Condition (*K*)**			**CV**			**FE**		
Term	**DF**	**MS**	***F***	***P***	**MS**	***F***	***P***	**MS**	***F***	***P***	**MS**	***F***	***P***	**MS**	***F***	***P***	**MS**	***F***	***P***
A. Handled v Control	1	3.99	0.07	ns	1.39	0.31	ns	12.14	2.07	ns	0.00	0.05	ns	1.43	0.05	ns	0.01	0.60	ns
B. Density	2	105.06	1.79	ns	51.99	11.55	**	118.39	20.17	**	0.001	0.67	ns	30.13	1.08	ns	0.47	40.42	**
AB	2	61.65	1.05	ns	2.99	0.66	ns	1.79	0.30	ns	0.001	0.71	ns	43.39	1.55	ns	0.01	0.85	ns
Residual	18	58.56			4.50			5.87			0.001			27.99					

A1.5 Discussion

The results indicate an appropriate initial (~17g fish) lower stocking threshold for mulloway of above 4.08 kg m^{-3} while growth between MD and HD were similar suggesting an initial stocking density in excess of 16.32 kg m^{-3} may be achievable. While the direct extrapolation of MD or HD stocking densities used in this experiment to commercial scale culture or different size classes of mulloway may not be appropriate it is important to note that this study demonstrated the significant negative effect of low stocking density on the growth of mulloway after only two weeks.

Under the current experiment conditions mulloway were not negatively affected by regular handling. Negative growth responses to anesthesia may be anesthetic specific (e.g. Hoskonen and Pirhonen 2006) and in this case mulloway appear to be able to tolerate regular weight checks using benzocaine. It should be noted however that exposure to a repeated stressor can potentially reduce the ability of fish to respond to an additional acute stressor (Barton 2002). It is unclear to what extent, if any, that the daily switching on and off of lights (repeated stressor) masked the additional effect of handling (acute stressor) on the growth of mulloway in this experiment. Growth of MD mulloway in this experiment were however comparable to those of juvenile mulloway in intensive culture using 10000 l tanks (~0.35g day^{-1}) (Booth, Allan and Losordo, unpub. data, 2002).

LD fish fed erratically; reluctant to feed when food was introduced into the aquaria then darting over to pellets often stirring them up. MD and HD fish in contrast fed well from the experiment outset. The FE value of the LD treatment should be regarded with some caution as the erratic feeding behaviour of the LD fish made accurate quantification of feed intake difficult. However; the low FE

value for this group does provide an indication of the overall inefficient feeding behaviour of mulloway at low densities.

Qualitative observations during the present study did not identify any obvious agonistic behaviour among any of the density treatments while the similarity of growth heterogeneity between the density treatments reinforced this observation. This implies a moderate social hierarchy independent of the stocking densities used in this experiment (Brett 1979). This also occurred despite the introduction of replacement fish to maintain density compliments.

One of the primary functions of shoaling behaviour in fish is predator avoidance (Pitcher 1986) and the size of the shoal has been shown to directly influence the behaviour of individuals (Magurran and Pitcher 1983). Magurran (1986) proposed that as a fish shoal increases in size, “corporate vigilance” for predators decreases. This relationship is not unique to fish and has been documented extensively in many animal behavioral studies such as birds (Pulliam 1973), wild boar (Quenette and Gerard 1992) and rabbits (Roberts 1988). The results and observations from this study indicate that a lower threshold of stocking density may also apply to mulloway; we hypothesize that, at a certain density, there forms a social cohesiveness which encourages a reduction in corporate vigilance and a change to normal feeding and behaviour. Below this threshold mulloway may become increasingly skittish and vigilant for (perceived) predators, increasing general activity and inefficient feeding behaviour. Growth and feeding studies combined with quantifiable behavioral data would test this hypothesis.

Appendix 2

Theoretical estimates for O_2 re-aeration in seawater (~30 ppt) used to establish atmosphere-seawater oxygen transfer parameters. Data applicable to experiment system used in Chapters 2 and 3.

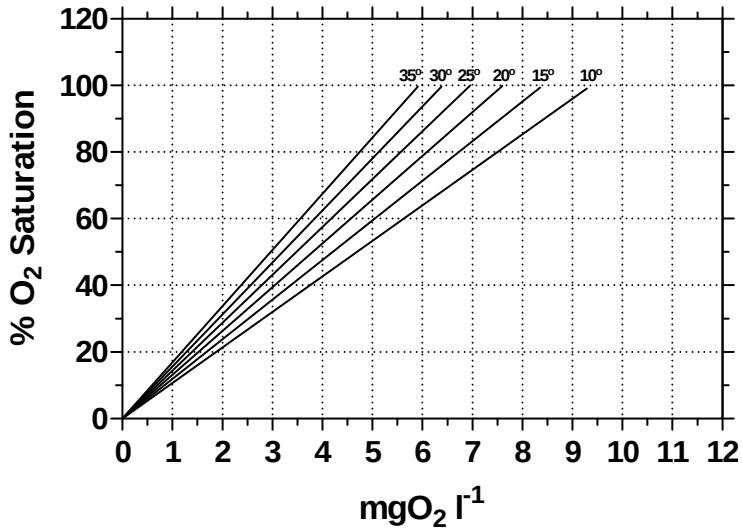

Figure A2.1. Theoretical relationship between O_2 concentration (mg/l) and % saturation at 10 – 35 ^{o}C.

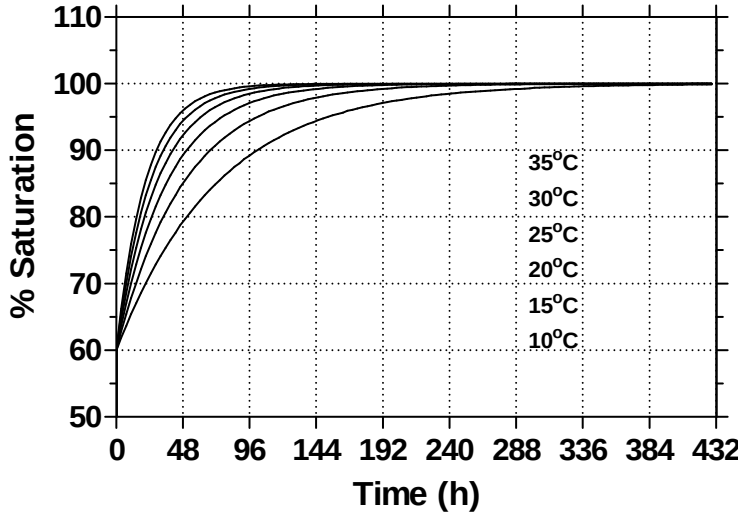

Figure A2.2. Re-saturation rate

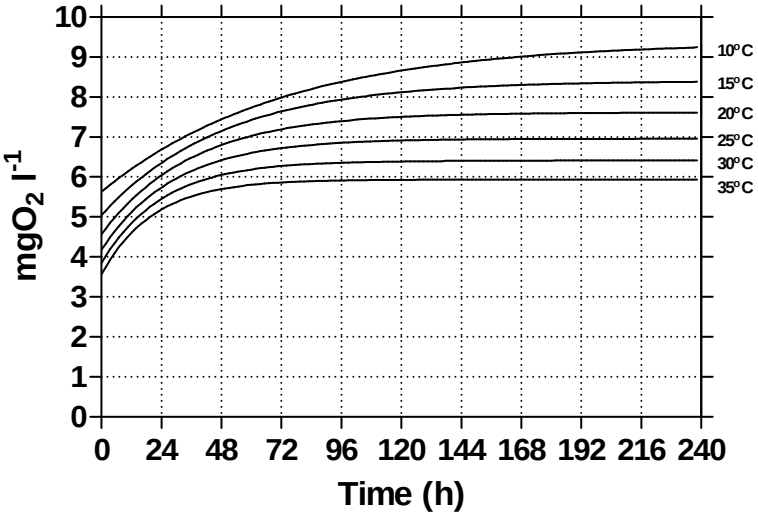

Figure A2.3. Re-aeration rate

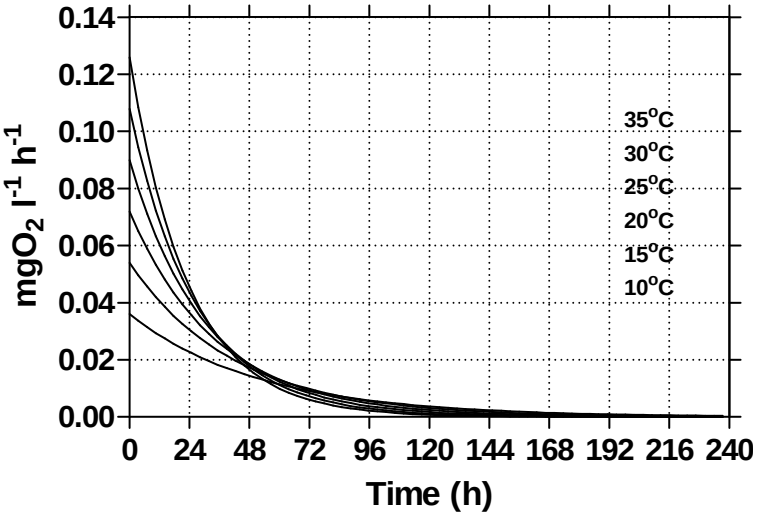

Figure A2.4. Re-aeration rate change

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