Optimising Growth Performance in Juvenile Hapuku (*Polyprion oxygeneios*)

An Insight into the Physiology of a Novel Finfish Aquaculture Species

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A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Marine Science.

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Abstract

Maximising production performance (growth rate and feed conversion ratio, FCR) is essential to the long term viability of finfish aquaculture. Production efficiency can also determine the viability of any new aquaculture species being considered for commercial culture. The current study aimed to understand how certain environmental and commercial conditions affect the production performance of a novel aquaculture species in New Zealand, the hapuku *Polyprion oxygeneios*. Additionally, the current study aimed to construct a simple energetic model for this species by collating the metabolic costs of feeding, growth and swimming within the physiological framework set by aerobic metabolic scope (AMS). AMS represents the physiological capacity for non-maintenance activities by setting the limit for aerobic ATP production. This work was done under the hypothesis that production performance is linked the capacity to perform physiological work and that maximal growth can only occur when AMS is non-limiting.

Juvenile hapuku were employed to assess whether respirometric tests and thermal preference methods could be used to resolve the optimum temperature for growth and feed conversion efficiency in novel culture species (Chapter 2). On the basis that the energetic costs of rapid growth are substantial and need to be accommodated physiologically, it was hypothesised that maximal growth and optimal feed conversion would coincide with temperatures where aerobic metabolic scope was maximised. It was further hypothesised that hapuku would behaviourally self-select temperatures that lead to the greatest level of AMS, growth and FCR performance. Acclimating hapuku juveniles to 12, 15, 18, 21 and 24 °C for 4 weeks resulted in a peak in specific growth rate (SGR) in the range of 18 °C – 21 °C with slower growth at lower and higher temperatures. AMS was also maximal between 18 °C – 21 °C and was tightly linked with SGR. The behavioural thermal preference (T_{pref}) range of hapuku also fell within the optimum range for growth. FCR, however, was inversely related to temperature with the most and least efficient rates of conversion occurring at 12 °C and 24 °C respectively. Though AMS and T_{pref} had no utility in predicting the optimal range for FCR, standard metabolic rate (SMR) showed a positive linear relationship to FCR. The conclusions of this initial study are three-fold: i) hapuku select temperatures that optimise both AMS and growth, ii) AMS and T_{pref} appear

tightly linked with SGR and could be used to predict the optimum temperature for growth in novel species and iii) AMS and T_{pref} have no utility in predicting the optimum temperature for FCR.

Within Chapter 3, specific dynamic action (SDA) was measured in juvenile hapuku at two temperatures and two different ration sizes: 15 ºC (0.75% body weight [BW] and 1.5% BW) and 21 ºC (1.5% and 3% BW). To resolve whether SDA is functionally related to the SGR and FCR, SDA was then compared against the SGR and FCR of hapuku raised for 6 weeks at the same temperatures and ration levels. A marked effect of temperature on SDA was found and all SDA parameters (i.e. MO_2 peak, duration, time to MO_2 peak, limitation of AMS, SDA energy and SDA coefficient) were significantly higher for the shared ration size (1.5% BW) at 21 ºC than 15 ºC. However, only a few SDA parameters increased in magnitude with ration size (i.e. MO_2 peak and SDA energy). Although SDA is thought to reflect the cost of growth in fish, larger SDA parameters were not linked with SGR which was significantly lower at 21 ºC than 15 ºC for the shared ration size of 1.5% BW. Further observations confirmed that there is a complex and interactive effect of temperature and ration size which is driving the SDA - growth response of juvenile hapuku. Specifically, elevated growth potential through larger SDA responses were present at the higher temperature of 21 ºC, but the higher maintenance cost (i.e. SMR) of fish at this temperature constrains growth if a restricted 1.5% BW ration is delivered. FCR was largely independent of ration size and was significantly less efficient at 21 ºC, despite fish having a larger SDA response. No single SDA parameter was found to predict the SGR and FCR performance of juvenile hapuku but future research might focus on the SDA response of fish to multiple (vs. single) feeds per day as this conveyed a significant growth benefit to hapuku for reasons not yet explained by metabolism.

Chapter 4 examined the applicability of exercise training for juvenile hapuku as induced-swimming can improve the growth and FCR of finfish aquaculture species, such as salmonids and *Seriola* sp. However, the response to exercise is not universal and some species (such as Atlantic cod *Gadus morhua*) show no or a negative productivity response to exercise. As a possible explanation for these species-specific differences, a recent hypothesis proposed that the applicability of exercise training, as well as the exercise regime for optimal growth gain ($ER_{opt\ growth}$), was dependent upon

the size of available AMS. This study aimed to test this hypothesis by measuring the growth and swimming metabolism of hapuku to different exercise regimes and collating the metabolic costs of swimming and growth (i.e. specific dynamic action, SDA) against AMS. Two 8-week growth trials were conducted with ERs of 0.0, 0.25, 0.5, 0.75, 1 and 1.5 body lengths per second (BL s^{-1}). Fish on a relatively high growth trajectory showed a small but positive growth response to exercise but only in the range of 0.5 BL s^{-1} to 0.75 BL s^{-1} compared to static water controls. Slightly larger fish on a slower growth trajectory, however, showed no evidence of exercise-induced growth. Long-term exposure to water flow at 0.75 BL s^{-1} and 1.5 BL s^{-1} also yielded no difference in the swimming metabolism of fish relative to static water controls. Combining the SDA of hapuku with the metabolic costs of swimming showed that hapuku have sufficient physiological capacity to support growth and swimming at all ERs. The current study therefore suggests that exercise-induced growth was not limited by AMS and possibly varies as a function of species, life stage and/or inherent growth trajectories.

As a follow on from the exercise trials, Chapter 5 examined the role of exercise in altering the flesh quality of juvenile hapuku. Consumers base the quality of fish on factors such as taste, texture, appearance, freshness and odour and it has been suggested that exercise could modify some of these factors in a way that affects consumer preferences. The effect of exercise training on the flesh firmness of juvenile hapuku was therefore investigated to determine whether exposing them to increased water currents can produce a firmer, and presumably more desirable product. Flesh samples were taken from fish that had been exercised for 8 weeks at either 0.0, 0.75 or 1.5 BL s^{-1} and used to determine firmness (textual analyses), muscle fibre density (MFD) and the distribution of muscles fibre sizes from prepared histological slides. MFD and flesh firmness were negatively related to ER and decreased significantly between 0.0 BL s^{-1} and 0.75 BL s^{-1} suggesting a stimulation of muscle fibre hypertrophy with continuous swimming. Fibre size distributions also showed an increase in median muscle fibre size with the increasing exercise regimes. The full effects of exercise training on flesh quality, particularly the effect on organoleptic properties, need to be determined before a definitive decision can be made on the applicability of exercise training to hapuku.

Within Chapter 6, the costs of SDA and swimming were collated under the metabolic framework of AMS and presented as a model of the energetics of juvenile *P. oxygeneios* in culture, with particular reference to 17 °C where the costs of swimming were measured. Additionally, the kJ requirements of hapuku under different conditions were calculated and compared to ingested digestible energy (DE) from feed. The assumptions of the energetic equations, the apparent discrepancy between DE intake and energy usage and factors that would modify energy usage are all discussed in detail and compared to other species of interest. To conclude, recommendations for farming practice and future work are made based on the empirical data of the model.

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Chap 2. Optimum temperatures for growth and feed conversion in cultured tropical (Polynesian) oysters) – Is there a link to aerobic metabolic scope and optimal temperature. Aquaculture 430, 107-115.

Nature of contribution by PhD candidate	All investigative procedures, statistical analyses and primary author of manuscript.
Extent of contribution by PhD candidate (%)	90.5

CO-AUTHORS

Name	Nature of Contribution
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M. Bruce	Manuscript editing
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Certification by Co-Authors

The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and
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Chap. 5 The effect of ration size on the SDA response and production performance of juvenile kingfish (Seriola rivoliana) Aquaculture

Nature of contribution by PhD candidate	All investigative procedures, statistical analyses and primary author of manuscript
Extent of contribution by PhD candidate (%)	90.%

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Ch 4. Accomodating the growth of swimming in fish - the applicability of exercise induced growth to juvenile barramundi Polydprion argyreiosus

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Chap 5 Sustained exercise reduces the muscle fibre density and flesh firmness of a juvenile hapuku (Polyprion oxygeneios)

Nature of contribution by PhD candidate	growth and husbandry, histological and statistical analyses and primary author of manuscript
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Chapter 1 - General Introduction

1.1 High-value finfish aquaculture

Capture fisheries are failing to meet the increasing demand of consumption. In the period 1990 – 1997, fish consumption increased by 31% while the supply from marine capture fisheries increased by only 9% (Tidwell and Allan, 2001). In fact, the global wild capture fisheries effectively reached a plateau at approximately 93 million tons in the mid-1990s (FAO, 2010) which continues to be the case today. Commercially and recreationally targeted finfish stocks are being exploited at rates well above their predicted maximum sustainable yield (MSY) and fishing effort has to be increased to maintain the current level of harvest (Maunder, 2002). There is also ample evidence to suggest that the higher the value of the fish species, the greater the degree of exploitation (Larkin, 1977; Maunder, 2002; Fromentin and Powers, 2005). Even since the establishment of New Zealand's iconic quota management system (QMS) in 1986, many local high value species have also fallen victim to over-harvesting. Species such as snapper *Pagrus auratus*, blue cod *Parapercis colias*, ling *Genypterus blacodes*, hoki *Macruronus novaezelandiae* and orange roughy *Hoplostethus atlanticus* have all experienced varying degrees of serial stock depletion under the QMS system and show declining long-term trends (Punt and Hilborn, 1997; Batstone and Sharp, 1999; Clark, 2001; Hauser *et al.*, 2002; Beentjes and Carbines, 2005).

Aquaculture has boomed in the last 50 years and now produces as much as 50 million tons per year (Read and Fernandes, 2003; FAO, 2010). It also widely considered to be one of the fastest growing industries in the world. Farming high-value finfish has the potential to reduce fishing pressure on over-exploited fish stocks by bringing an alternative and essentially equivalent product to the market. However, as with all intensive farming endeavours, there are various cost and benefits that must be considered before full-scale production can commence.

1.1.1 Benefits of finfish aquaculture

-Environmental

Increases in aquaculture efforts may reduce the need to further increase and over-exploit already devastated wild fish stocks (Tidwell and Allan, 2001). Furthermore, a reduction in fishing effort for certain species can reduce the associated effect of environment destruction. Widely applied commercial fishing methods such as trawling, bottom trawling, long-lining and gill netting. These can devastate environments and produce large numbers of low-value or unusable bycatch which are discarded overboard, illegally in some cases (Punt and Hilborn, 1997; Maunder, 2002). Intensive fish farming in localised areas can reduce the broader-scale environmental footprint of fish production into a considerably smaller and more manageable area (Tidwell and Allan, 2001).

-Economic

Fish protein is the primary source of animal protein for a large proportion of the world and, as fisheries decline, the problem of feeding an ever increasing population is looming. The development of low-cost and low-tech fish farming operations (as well as shellfish farming) in many third world nations has reduced the demand on wild fisheries and provided a reliable source of locally consumed protein (Tidwell and Allan, 2001). This has also helped to create employment in many areas where little was previously available (Murshed-E-Jahan and Pemsl, 2011). Even in developed areas such as British Columbia, the farming of Atlantic salmon *Salmo salar* has been a significant economic entity with aquaculture directly or indirectly providing in excess of 3000 jobs in smaller coastal communities with previously high unemployment rates (Gerwing and McDaniels, 2006). Indeed, as wild fisheries around the world are stabilising or declining, job creation in the industry is very limited and the sustainability of fishing communities is at risk (Muallil *et al.*, 2011; Natale *et al.*, 2013).

1.1.2 Costs of finfish aquaculture

-Environmental

Eutrophication of the local environment has always been at the forefront of the aquaculture debate. Some examples, such as the Greenshell® mussel in New Zealand, have extremely localised effects which dissipates within 50 metres of the farm site regardless of hydrological conditions (Hartstein and Stevens, 2005). Unfortunately, the environmental effects of farming finfish in seacages are more prevalent. Intensive salmonid farming in places such as Canada, Chile, Scandinavia and New Zealand have produced hypoxic/anoxic areas (usually as a result of feed and fish waste in the sediment), disease events and varying degrees of genetic disturbance [i.e. the mixture of highly bred commercial stocks with wild populations (Buschmann et al., 1996; Naylor et al., 1998; Muslow et al., 2006)]. Many of these effects can, however, be reduced significantly by appropriate site selection, periodic abandonment (cycling cages), improved technologies and optimised feeding strategies (Carroll et al., 2003).

Perhaps the most controversial aspect of farming carnivorous finfish is the feed itself. High-value finfish species that are typically carnivores at a high trophic level and therefore have high protein and oil requirements for optimum growth, health and flesh quality (Cowey, 1975; Einen and Roem, 1997). To meet these requirements, current commercial feeds usually have a proportion of fishmeal which is made up of various wild-harvested pelagic baitfishes. Previously, the rate of conversion of fishmeal into the mass of cultured fish (feed conversion ratio, FCR) meant that more fish mass was being taken out of the ocean than was being produced with the resulting feed products (Naylor et al., 1998). More recently, the increasing replacement of fishmeal with alternatives such as meat-processing waste (Sealey *et al.*, 2011; Wang *et al.*, 2013) means that the global-scale impacts of aquaculture are decreasing.

-Economic

Sheltered coastal sites deemed suitable for larger-scale aquaculture operations are often valuable to local groups, recreational fisherman and beach goers. Therefore some of the economic and sociological implications of opening up an area for marine farms of any description result from the opportunity cost of the area itself (Falconer *et al.*, 2013), which is often where the contention between marine farmers and local groups originate from. In China and south-east Asia the culture of shrimp and some fishes has resulted in the destruction of valuable mangrove forests and coastal wetlands which help supply water and soils for terrestrial crops such as rice (de Graaf and Xuan,

1998). New aquaculture ventures are also notoriously risky and it has been known to take several full-scale attempts to solve major production hurdles, particularly in the hatchery stage. This cost often has to be absorbed by the industry itself and few parties are willing to invest the necessary capital until reliable technology and procedures are in place. Even when the technology is in place, if the potential gains of a new venture/species does not outweigh the associated risk and the opportunity cost of the capital (which could be applied to a more well-known species) there is little incentive for farmers to take on the risk. This has been an issue for New Zealand, particularly as farmers receive a high price for Chinook salmon *Oncorhynchus tshawytscha* in foreign markets and there is little incentive to take on alternative species such as the yellowtail kingfish *Seriola lalandi*. The recovery of overseas farming operations such as those in Chile are likely to lower Chinook salmon prices worldwide and this may create an incentive for farmers in New Zealand to take on alternative high value species. Additionally, the New Zealand government has set out to expand the aquaculture industry up to a $1 billion NZD value by 2025, which can only be achieved with a degree of diversification (MPI, 2013).

Hapuku *Polyprion oxygeneios* are an alternative species option but, to make them an attractive venture to farmers around New Zealand, there is the need to demonstrate that this species is profitable. By understanding the physiology of this species and how simple environmental and commercial variables can be manipulated to optimise growth and feed conversion efficiency, it may be possible to reveal the potential of this species as a high-value alternative to salmon.

1.2 Hapuku

The wreckfish *P. oxygeneios* is a large reef predator commonly known in New Zealand as hapuku, hapuka or groper (Fig. 1.1). It is distributed throughout the temperate and subtropical waters of the southern Indian and Pacific oceans (Beentjes and Francis, 1999) and is highly sought after wherever it is found. Despite this, catches remain relatively low worldwide with New Zealand peaking at 2700 tonnes in 1983 - 1984 (Beentjes and Francis, 1999; Francis *et al.*, 1999). After the introduction of the QMS, annual catches have been consistently lower than 1500 tons, with yearly catches

showing a similar decline to many other fisheries for similar species around the world (Annala and Sullivan, 1997). They are the target of a significant set-netting and long-lining industry, particularly in the Cook Strait and the Canterbury Bight (Beentjes and Francis, 1999). Reduced catches in coastal areas has also led to further exploitation of a related deep water species *P. americanus* (known as the South-western Atlantic wreckfish in some parts of the world) (Anderson *et al.*, 2012) which is considered together with hapuku as a single management unit under the QMS (Annala and Sullivan, 1997; Francis *et al.*, 1999). Both species are also the target of recreational and charter fishing out of many areas and are considered one of the most desirable species to target by anglers who have the means to do so.

Figure 1.1. An approximately nine-month old hapuku (680 g, 350 mm FL) raised from broodstock (F1) at the National Institute of Water and Atmospheric Research (NIWA) Bream Bay Aquaculture Park, Ruakaka, New Zealand. Photo by Javed Khan.

Spawning in hapuku occurs from late July to October and the eggs and larvae are known to exist in pelagic surface waters (Hardy, 1978; Anderson *et al.*, 2012). The juveniles themselves are often found actively swimming with flotsam (aggregations of floating debris in the open ocean) around New Zealand. Surveys, trawling and long-lining data suggests that they settle into a more demersal (50-600 metres) habitat at approximately 50 cm in length and three to four years in age (Francis *et al.*, 1999). This assumption is based on the fact that animals smaller than this are very rarely caught commercially and recreationally in areas where large adults are targeted (Beentjes and Francis, 1999; Francis *et al.*, 1999).

This species as well as *P. americanus* are considered to be long-lived (up to 60 years) and relatively slow-growing. However, during their first years both species show reasonable growth rates with hapuku reaching approximately 60 cm within the first five years (Francis *et al.*, 1999) (Fig. 1.2) and *P. americanus* reaching approximately 50 cm within their first five years (Papandroulakis *et al.*, 2004; Peres and Haimovici, 2004). In both cases females showed slightly higher growth rates than males. The growth rate of juveniles was considered sufficiently high and *P. americanus* was classified as the seventh most suitable species for culture out of twenty-seven candidates in a European review (Quéméner *et al.*, 2002).

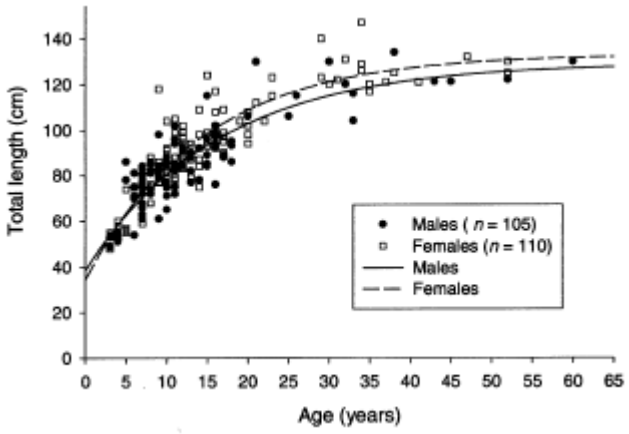

Figure 1.2. Hapuku *Polyprion oxygeneios* length-at-age data with fitted von Bertalanffy growth curves. Modified from Francis *et al*. (1999).

1.2.1 Value as an aquaculture species

The growth rate of the juveniles and the perceived high quality of the flesh suggest that hapuku would be an excellent species for culture. Additionally, New Zealand is an exporter of many unique or high-value primary products and the aquaculture industry has followed that trend i.e. Greenshell® mussels and Chinook salmon. Therefore, if it is to become a billion dollar industry by 2025, research will have to be centred on diversifying the industry into other high-value species (MPI, 2013). Research carried out at NIWA's Bream Bay Aquaculture Park (Ruakaka, New Zealand) has already shown that this species is amenable to aquaculture conditions and able to be bred successfully and reliably in captivity (Anderson *et al.*, 2012).This being the case, and with commercial catches remaining low, there is ample evidence to

suggest that there is a market for this species both locally and internationally which has space for a high quality aquaculture product.

1.3 Energy budgets and their applications

Traditional energetic models ascertain the energy from food intake and partition it into various experimentally measureable areas of energy consumption associated with an organisms life stage or ecosystem functioning (Nisbet *et al.*, 2012). The basic concept is very simple: energy from food is ultimately lost to the environment (nitrogenous waste and faeces), consumed in metabolic processes or deposited as *de novo* tissues (growth) (Jobling, 1993; Jobling, 1994; Bevelhimer, 2002; Sun and Chen, 2014). Taking an individual fish as an example, bioenergetic models often encompass aspects such as the energetic cost of growth, reproduction, activity, ingestion and excretion in relation to variables such as temperature, oxygen availability, ammonia concentration and salinity (Warren and Davis, 1967; Cuenco *et al.*, 1985; Jobling, 1993). These models often have a strong empirical basis which integrate several measures of performance and provide a useful tool when examining energy dynamics in different kinds of biological systems. In a general sense, they can be used to determine how different species balance the competing physiological costs of energetically expensive process, growth and activity, for example, against available aerobic metabolic capacity and the energy available from ingested feed (Cuenco *et al.*, 1985; Myrick, 1998; Owen, 2001).

The simplest way to represent these processes is with a commonly used equation which uses the NRC nomenclature:

$$RE = IE – FE – UE – ZE – HiE – HjE - HeE$$

Where RE is the retained energy (growth and storage), IE is the ingested energy, FE and UE are the energy lost to the environment through excretion, ZE is the branchial excretion, HiE is the heat increment, HjE is the energy associated with voluntary activity and HeE is basal cost of metabolism (Halver and Hardy, 2002). The specific nomenclature of the different elements can change with different authors but the key purpose remains the same: track the fate of ingested energy through the physiological

processes of the animal (Fig. 1.3). Obtaining all these elements for a single species is a very lengthy process and often requires the work of several authors or research groups over a long period of time. Some of the broader elements of the equation, such as maintenance metabolism, swimming activity and the heat increment can be obtained over a shorter period and can provide the basis for decision making in culture and future research directions.

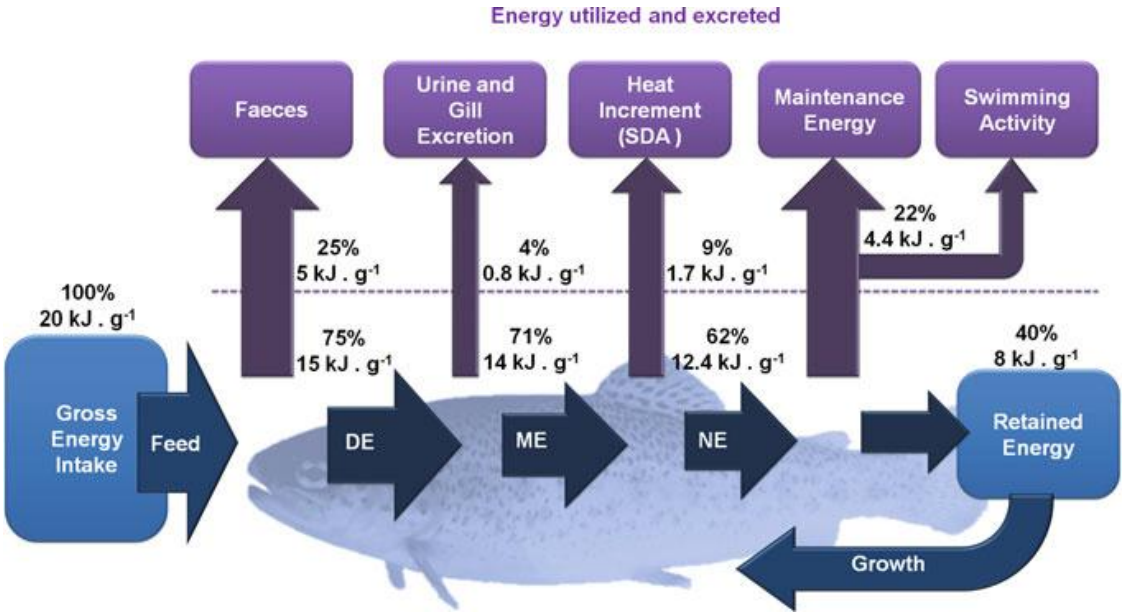

Figure 1.3. An example of energy use/allocation and excretion in a 1 kg rainbow trout *Oncorhynchus mykiss*. Here the nomenclature is different compared to that of Halver and Hardy (2002). DE = digestible energy, ME = metabolisable energy, NE = net energy. The image is taken from Magnoni *et al.* (2013a) using the data of Cho and Bureau (1995a).

One of the distinct advantages of aquaculture systems is that many of the interacting environmental variables are not only measured but directly controlled. By developing a model of energy use from feed and using this data to ascertain the proportion of feed that is converted to actual fish mass (i.e. growth) it may be possible to reduce costs or increase output from current levels of expenditure. Energy budgets have been developed for several species that are of interest to the aquaculture industry, including: white sturgeon *Acipenser transmontanus* (Bevelhimer, 2002; Mayfield and Cech, 2004), Nile tilapia *Oreochromis niloticus* (Liu and Chang, 1992; Xie *et al.*, 1997; Yang, 1999), European eel *Anguilla anguilla* (Owen, 2001) and salmonids

(Beauchamp *et al.*, 1989; Cho and Bureau, 1995a; Myrick, 1998) which integrate many measures of physiological performance and often include data from several sources. In the case of a novel aquaculture species such as hapuku, it is essential to demonstrate two things. Firstly, that the growth rate of this species is competitive (in terms of time required to reach a marketable size) compared to other available species. Secondly, that the amount of feed and conditions required are realistic and aren't going to increase costs to a point where hapuku have a lower potential to produce profits compared to other species. Using principles and components from energy budget models will help develop an understanding of how different environmental conditions affect growth and FCR which can be provided to the industry.

1.3.1 Energy and oxygen consumption

There are two major catabolic, biochemical pathways by which animal cells release energy from foodstuffs to produce energy in the form of ATP (adenosine triphosphate) which can be used to perform physiological and mechanical work. These are aerobic metabolism, which requires oxygen, and anaerobic metabolism which can occur in the absence of oxygen.

Aerobic catabolic pathways completely oxidise glucose to CO_2 and H_2O and capture the released energy in ATP bonds through four major sets of reactions: i) glycolysis, ii) the Krebs cycle (citric acid cycle), iii) the electron-transport chain and iv) oxidative phosphorylation (Hill *et al.*, 2008). When oxygen is available as a final electron acceptor and able to be reduced, there can be a net yield of 34 mol of ATP per mol of glucose. For fish, metabolic fuel is not limited to glucose. Fatty acids are known to be a preferred fuel source during sustained swimming (Weber and Haman, 1996; Magnoni and Weber, 2007; Magnoni *et al.*, 2013a) and can provide as much 130 mol of ATP per mol of substrate (in reference to palmitic acid). Similarly protein, though it is often conserved where possible, can provide as much 24 kJ g^{-1} (Magnoni *et al.*, 2013a) in times of high energy demand and low feed intake (migration, for example).

For tissues to produce ATP without the presence of O_2 (i.e. in the case of anaerobic metabolism), they have to be able to maintain redox balance without O_2 as a final electron acceptor. This is achieved by $NADH_2$ donating its protons to pyruvic

acid to create lactic acid ($C_3 H_5 O_3$) which regenerates NAD and allows pyruvic acid to act as the final electron acceptor (West, 2008). This produces a net yield of only two mol of ATP molecules per mol of glucose. Unlike CO_2 and H_2O which are fully oxidised, the products of anaerobic metabolism are organic compounds and can be further oxidised to produce more energy. Once O_2 becomes available again, lactic acid can be converted back into pyruvic acid (by re-donating its electrons to NAD) and can re-enter the citric acid cycle and proceed through the aerobic process to form ATP, or it can be converted back to glucose or glycogen (gluconeogenesis) at the cost of six molecules of ATP (West, 2008). This process is often called excess post-exercise oxygen consumption (EPOC) and the level of anaerobic debt can be estimated through the amount of oxygen required (above maintenance) in the resting periods following exhaustive exercise (Wood, 1991).

Shifts in the rate of production of ATP, and consequently the required rate of oxygen uptake are considered as changes in metabolic rate. For this reason the rate of oxygen consumption has been used as a proxy for metabolic rate and metabolism in general (Fry, 1947; Jobling, 1994) but is only reliable during aerobic metabolism where the rate of oxygen consumption and ATP are tightly linked. The rate of oxygen uptake during the aerobic recovery from anaerobic metabolism (the re-payment of "oxygen debt" or EPOC) is often applied to measure maximal rates of aerobic activity (Reidy *et al.*, 1995; West, 2008; Khan and Herbert, 2012).

1.3.2 Aerobic metabolic scope (AMS)

Fry (1947) historically suggested that processes such as growth, digestion and assimilation, reproduction and activity are the result of the integrated metabolism of an organism. Since many of these activities are carried out simultaneously, no one process fully exhausts total metabolic potential. Therefore, the capacity for an animal's metabolism to provide the energy for physiological and mechanical work is governed: i) by the difference between the minimum metabolic rate required to maintain physiological function and ii) the maximum possible metabolic rate under the immediate environmental conditions.

This "scope" for physiological and mechanical work is now much more widely understood and applied (Jobling, 1994; Clark *et al.*, 2013). It represents the difference

between two empirically measureable values: i) the standard metabolic rate (SMR), defined as the minimum metabolic rate/rate of ATP production of an animal that is starved and at rest and, ii) the maximum metabolic rate ($\dot{M}O_{2max}$), defined as the highest obtainable rate of metabolism/oxygen uptake (Farrell, 2009) (Fig. 1.4). It also represents a more encompassing measure of physiological performance than those traditionally applied such as heart rate, stroke volume, activity levels *etc*. and gives insight into the broader scale physiological processes driving these measures (Claireaux *et al.*, 2000). SMR ideally represents the energy cost [or rate of oxygen uptake as it is usually measured (Steffensen, 1989)] of only those physiological and motor processes that are immediately vital to life. These would include (using fish as an example) the oxygen required to maintain the heart, gill and ventilatory tissues, among other essential physiological and neural functions (Jobling, 1994). SMR is essentially the best possible estimate of basal metabolic rate (BMR) under laboratory conditions (Steffensen, 1989; Khan and Herbert, 2012).

$\dot{M}O_{2max}$ simply represents the highest rate of metabolic turnover and is usually measured by exercising the animal (again, using fish as an example) in a swim flume or through chasing to exhaustion. Different ecotypes are more suited to different methods of inducing $\dot{M}O_{2max}$ but, in the Atlantic cod *Gadus morhua*, it was found that chasing in a tank consistently produced higher estimates of $\dot{M}O_{2max}$ than a swim flume (Reidy *et al.*, 1995). Both methods contain an element of stress and anaerobic metabolism but in many species, particularly the more sedentary examples, measuring the EPOC is considered the most valid method of measuring $\dot{M}O_{2max}$. This is because oxygen is required to metabolise the products of anaerobic metabolism and repay "oxygen debt" from anaerobic activities (West, 2008; Khan and Herbert, 2012).

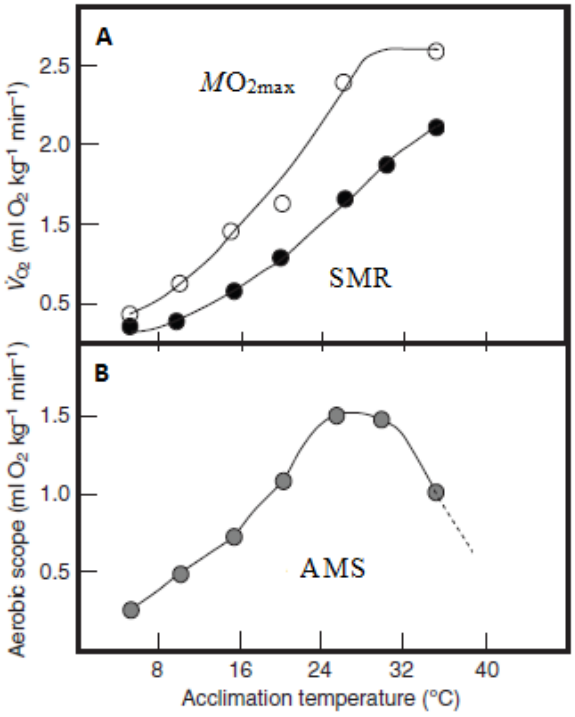

Figure 1.4. (A) Measurements of the SMR and MO_{2max} of goldfish *Carassius auratus* in relation to temperature (top) and (B) the difference between these values, termed aerobic metabolic scope (AMS). Values are shown as V_{O2} which is alternative notation based on measuring the volume of oxygen rather than the amount in mg (MO_2). These notations are interchangeable and depend on the equipment used during measurement. Modified from Farrell (2009), original data from Fry (1947).

The difference between SMR and MO_{2max} is considered to be the aerobic metabolic scope (AMS) which is the capacity to perform aerobic physiological work (Blier *et al.*, 1997; Khan and Herbert, 2012; Clark *et al.*, 2013). At any given point in time and at any given life stage or under specified environmental conditions, AMS represents the aerobic capacity to produce ATP for non-maintenance processes. One of the most energetically demanding of those processes is growth, or more specifically the protein synthesis associated with growth (Jobling, 1994; McCarthy *et al.*, 1999). There is a strong link in the literature between the maximisation of AMS and growth rates. Examples such as the sockeye salmon *Oncorhynchus nerka* (Brett, 1976), brown trout *salmo trutta* (Elliot, 1976), plaice *Pleuronectes platessa* and flounder *Platichthys flesus* (Fonds *et al.*, 1992) show a strong positive relationship between the magnitude

of AMS and growth. AMS is increased and decreased by environmental variables that increase or decrease the capacity to deliver oxygen to working tissues or govern the rate at which chemical reactions can occur (Clark *et al.*, 2013). For example, low-environmental oxygen levels and sub-optimal temperatures reduce the rate at which oxygen is delivered to tissues, reducing AMS (Cook *et al.*, 2011; Domenici *et al.*, 2013; Dupont-prinet *et al.*, 2013) while optimal temperatures and oxygen saturated environments allow for the maximum potential rate of oxygen delivery, increasing AMS and physiological performance (Claireaux and Lagardère, 1999; Claireaux and Lefrançois, 2007; Fitzgibbon *et al.*, 2007; Khan *et al.*, 2014a). This relationship is commonly referred to as the oxygen- and capacity-limited thermal tolerance (OCLTT) paradigm, which has been recently reviewed by Clark *et al.* (2013).

Because AMS represents the energetic potential for non-maintenance processes, it is appropriate to consider the AMS of hapuku as a metabolic framework, within which, processes such as growth and activity must be reconciled (Jobling, 1994; Owen, 2001). It is possible to measure how different environmental variables not only affect the difference between SMR and MO_{2max} and thus limits or increases physiological potential, but also how aquaculture related processes such as swimming/exercise and feeding/protein synthesis fit into that metabolic framework.

1.3.3 Measuring oxygen consumption as an indicator of metabolism

To ascertain the metabolic rate of aquatic breathers like fish, a common technique known as respirometry is applied. There are many applications and forms of this technique but, in the field of fish physiology, automated intermittent flow respirometry is now considered to be the most accurate (Steffensen, 1989; Clark *et al.*, 2013) and is certainly the most widely applied in modern investigations (Jordan and Steffensen, 2007; Clark *et al.*, 2013; Frisk *et al.*, 2013). This technique measures the oxygen uptake of fish by measuring the decline in oxygen saturation inside a sealed chamber containing the specimen. The chamber is periodically flushed with a pump and returned to the desired saturation before being sealed and allowed to decrease again (see Fig. 1.5).

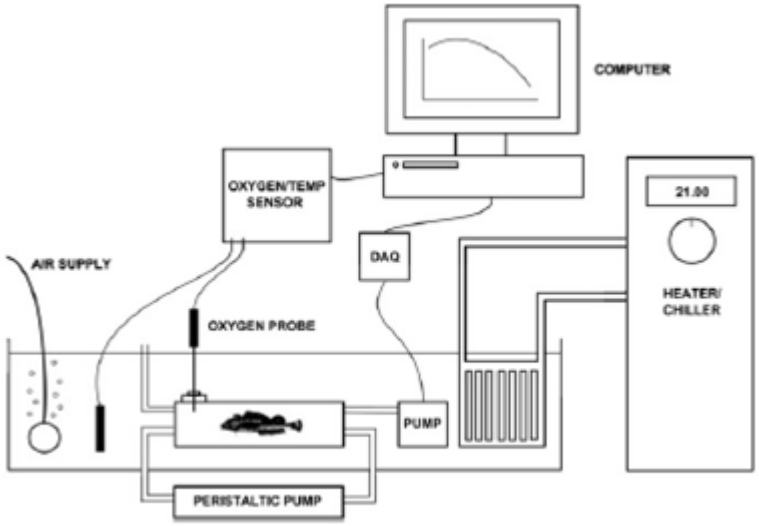

Figure 1.5. A representation of a basic automated, intermittent respirometry system. The fish is contained in a sealed chamber which is flushed with aerated water periodically by a pump which is in turn controlled electronically (in this case by a custom DAQ system). When the pump isn't running the chamber is sealed and the oxygen sensing system records the rate of decline in oxygen saturation inside the chamber caused by respiratory oxygen uptake by the fish. From Khan and Herbert (2012).

These systems can be either static (as shown in Fig. 1.5) where the fish remains stationary or in a swim flume arrangement where a flow is created that fish have to swim against (as seen in Brown *et al*. 2011). The periodic flushing of chambers allows for measurements to continue over long periods of time and thus more accurate estimates of SMR can be obtained through repeated and prolonged sampling (Steffensen, 1989; Jordan and Steffensen, 2007; Khan and Herbert, 2012; Khan *et al.*, 2014a; Khan *et al.*, 2015). Recently published methods have applied an "open-top" approach to respirometery where the top of the chamber containing the fish is exposed to air (Pirozzi and Booth, 2009; Gamble *et al.*, 2014) which could aid in several aspects of measuring metabolism (such as feeding metabolism, for example). This method, however, has not been validated in many species and data may not be considered comparable to other examples in the literature which applied a more standard methodology.

Oxygen uptake data often contains period of spontaneous activity or reaction to external stimuli (where oxygen uptake would increase) and periods of hypercapnia

where oxygen consumption can be conspicuously low. To account for this variation, it is necessary to have a standard and widely applied method of calculating SMR from the respirometry data. Considering the current state of the field, the 15^{th} quantile (q = 15) method of Chabot and Claireaux (2008b) best demonstrates this variation and effectively prevents periods of spontaneous activity and hypercapnia from affecting SMR estimations. This method has been applies to several species since its introduction (Cook *et al.*, 2011; Dupont-prinet *et al.*, 2013; Khan *et al.*, 2014a) and SMR estimates often coincide with the modal value of the distribution of MO_2 for any individual fish.

1.4 Thermal physiology and temperature preference

Temperature is the most pervasive environmental variable that any ectothermic organism will encounter and it has a significant effect on their performance. Within the context of finfish aquaculture, the thermal tolerances of a species is one of the largest considerations as to whether a species is a viable choice for a commercial venture. It governs site selection for seacages as well as influencing growth rate (Jobling, 1981; Jobling *et al.*, 1993b) and FCR (Jørgensen and Jobling, 1993; Nordgarden *et al.*, 2003b; Van Ham *et al.*, 2003) which both affect running costs, turnover and profitability. In hatcheries and inshore farms it also effects site selection in addition to the cost associated with heating and cooling water. It may also have an effect on maturity and energy investment in some species which can divert energy away from growth and into gonads and gametogenesis (Adams and Thorpe, 1989; Cox and Hinch, 1997). Of particular interest to this investigation, temperature has a measureable effect on the AMS of fish (Jobling, 1994) and thus their capacity to produce ATP aerobically (Fig. 1.4). To understand energy investment at different temperatures it is essential to have an idea of the how temperature affects AMS across a range that a species will likely be exposed to.

1.4.1 Temperature and metabolic rate

The metabolic rate of ectotherms is highly dependent on environmental temperature and will generally increase as environmental temperatures increase (as

seen in Fig 1.4A). This increase in metabolic rate with temperature is primarily attributable to the increases in the speed and efficiency of enzymatically driven biochemical pathways, changes in the expression and structure of proteins and changes in mitochondrial density and functionality of important organ systems such as the heart, liver and gills (Bowler and Tirri, 1990; Claireaux *et al.*, 2000; Hilton *et al.*, 2010). Enzymatic reactions that increase passively with temperature also increase the demand for ATP which will lead to an increase in oxygen demand if the fish is to remain aerobic (Jobling, 1994; West, 2008).

- *Standard metabolic rate (SMR)*

SMR will continue to increase with increasing environmental temperature (Fig. 1.4A). This will occur up to a critical point at which the animal can no longer produce adequate supplies of energy, adequate oxygen cannot be obtained from the external environment/delivered to the tissues or enzymatic systems begin to break down due to protein denaturation (Claireaux *et al.*, 2000; Pörtner, 2001; Lefrancois and Claireaux, 2003). Similarly, the slowing of enzymatic processes at extremely cold temperatures can prevent ectotherms from being able to produce adequate amounts of energy to sustain function (Fry, 1947; Hill *et al.*, 2008). These thresholds represent the critical temperatures at which the long term survival of the organism is highly compromised (Pörtner, 2001). The rate of the increase in the SMR of ectothermic animals is often expressed as a Q_{10} value which represents the change in the metabolic rate over a 10 °C temperature range as a ratio which can be compared across species and environmental variables, often falling between 2 and 3 (Jobling, 1994).

- *Maximum metabolic rate (MO_{2max})*

Like SMR, maximum metabolic rate (MO_{2max}) is highly dependent on the environmental temperature. In most ectothermic organisms, MO_{2max} will show a species-specific parabolic or asymptotic relationship with temperature (Fig. 1.4A) increasing up to a certain point where it is maximised and then often decreasing rapidly as temperatures exceed the physiological thresholds of the animal (Fry, 1971; Pörtner, 2001; Frisk *et al.*, 2012). In teleost fishes the temperature dependence of MO_{2max} is thought to be primarily attributable to the oxygen binding properties of the various forms of fish haemoglobins, their physiological capacity to perform and sustain exercise and their nutritional or reproductive state (Clarke and Johnston, 1999).

Recent analyses from authors such as Farrell (2002) and Hilton *et al.* (2010) have also determined the importance of highly aerobic cardiac tissue and the link between temperature and the efficiency of cardiac mitochondria in determining maximum energetic output at elevated temperatures.

- *Aerobic metabolic scope (AMS)*

Variations in AMS with temperature are the direct result of the differential between SMR and MO_{2max} for any given species (Fig. 1.4B). Reductions in AMS results in trade-offs between maintenance and non-maintenance processes, resulting in the limitation of non-maintenance processes such as growth, activity or reproduction (Jobling, 1993; Jobling, 1994; Owen, 2001). Increasing AMS increases the capacity to simultaneously accommodate these processes (assuming there is an adequate nutrient intake) (Fry, 1971; Claireaux *et al.*, 2000). What is often seen in ectotherms is the characterisation of their physiologies around an ecologically relevant temperature where physiological performance (AMS) and organismic performance (e.g. growth or fecundity *etc*.) are maximised (Johnston and Dunn, 1987). Outside of this range the performance is reduced by their inability to maintain oxygen and reagent supplies to all tissues (the OCLTT paradigm), inducing varying degrees of anaerobic metabolism (Pörtner and Knust, 2007; Pörtner and Farrell, 2008; Pörtner, 2010; Clark *et al.*, 2013). Generally, the further from the optimal temperature range the more physiological performance decreases and eventually reaches a point either side of the optimum where investment in non-vital processes such as growth is not possible.. These are known as “pejus” temperatures (see Pörtner, 2001 for a review) and in terms of aquaculture, represent the absolute upper and lower limits of culture temperature. They also represent the upper and lower thermal limits of the long-term survival of an organism.

1.4.2 Behavioural thermoregulation and temperature preference

In contrast to many terrestrial mammals and birds, mobile ectothermic animals will not inhabit their widest possible thermal range (Pörtner, 2001). When presented with a choice of environmental temperatures, usually in the form of a thermal gradient, mobile organisms will typically stay within a very narrow temperature range (Reynolds and Casterlin, 1979). This temperature range is considered to be their

preferred temperature [T_{pref} (Johnston and Dunn, 1987)] and the active selection of that range is termed behavioural thermoregulation (Reynolds and Casterlin, 1979).

Acute thermoregulatory behaviour, usually within the first few hours of exposure to a gradient, is highly influenced by the temperature at which the organism has been acclimated or previously exposed to. Therefore they are considered to be exhibiting an acute preference (Reynolds and Casterlin, 1979). However, after lengthy exposure to a gradient, usually more than 48 hours, organisms tend to gravitate towards a range of preferred temperatures that is species-specific and independent of previous exposure (Neill *et al.*, 1972; Ward *et al.*, 2010). This temperature has been termed the "final preferendum" and defined as: "a temperature around which all individuals [of a given species] will ultimately congregate, regardless of their thermal experience before being placed in the gradient" as well as "that temperature at which the preferred temperature is equal to the acclimation temperature" (Fry, 1947). Therefore the final preferendum paradigm is a useful tool in determining the thermal characteristics of a species, particularly when matched to other physiological characteristics.

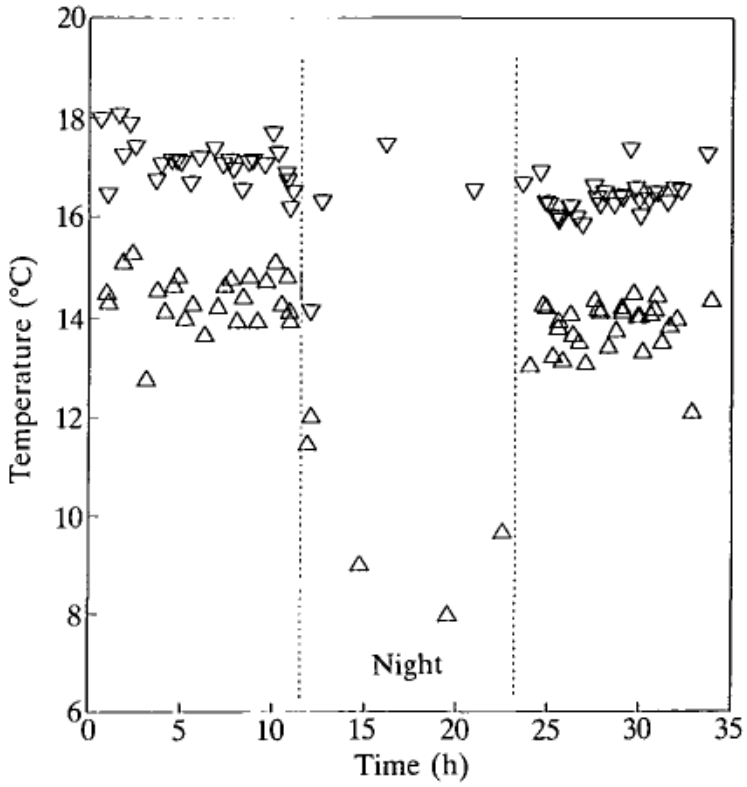

Figure 1.6. An example of measured thermoregulatory behaviour in a rainbow trout *Oncorhynchus mykiss*. Downward pointing triangles show the temperature that the fish left a hot chamber and the upward pointing triangles show the temperature that the fish left a cold chamber. T_{pref} is considered to be between these two temperatures. Note the decrease in thermoregulatory efficiency at night. From Schurmann *et al.* (1991).

This tool has been used extensively in determining the thermal characteristics of many species of interest. Schurmann *et al.* (1991) and Schurmann and Steffensen (1992) determined the preferred temperature of the brown trout and Atlantic cod respectively in both normoxic (Fig. 1.6) and hypoxic conditions. Both species preferred cooler water during hypoxia, likely as a result of a reduction in SMR that reduced oxygen demand. The final thermal preference of the bluegill *Lepomis macrochirus* has shown to be resistant to changes in light patterns, acute thermal shocks and even the introduction of conspecifics into an experimental system (Beitinger, 1974, 1975; Medvick *et al.*, 1981) which agrees with the final preferendum paradigm of Reynolds and Casterlin (1979). Several species, such as the green sunfish *Lepomis cyanellus*, show a wide range between their upper and lower avoided temperatures [up to 5.1 °C between upper and lower avoidance thresholds (Beitinger, 1975)] while others such as the rainbow trout *Oncorhynchus mykiss* show a

comparatively narrow range between their upper and lower avoidance temperatures (sometimes less than 1 °C at normoxia, Schurmann *et al*. 1991**,** Fig. 1.6). These differences between species likely represent their specific thermal tolerances and the temperature range which they show their highest organismic performance i.e. stenothermic *vs* eurythermic species (Jobling, 1981).

Perhaps the most exciting development to come out the work on thermal preference is the discovery that the final temperature preference of a species may represent its optimum temperature for growth. Work by authors such as Beitinger and Magnuson (1979) and a review by Jobling (1981) revealed that, in almost every species investigated, the optimum temperature for growth coincided precisely with their final temperature preferendum. This has several implications. Firstly, as AMS is positively related to growth performance (Brett, 1976), T_{pref} can indicate the range in which the optimal temperature for AMS resides. Secondly, if validated, this method could provide a practical alternative to direct measurements of growth and physiological performance (AMS) for determining the optimum temperature range for growth. According to the final preferendum paradigm (Reynolds and Casterlin, 1979), it can be applied to species acclimated to any temperature or conditions and should ultimately result in an estimation of the optimum temperature for growth (Jobling, 1981). In an aquaculture research setting, this method could be applied to any new species to rapidly determine the optimal temperature for growth without having to set up a large, replicated growth trial that encompasses a wide variety of temperatures.

1.4.3 Measuring temperature preference

Historic approaches to measuring temperature preference in fishes were to place them in a tank or tube with a temperature gradient and quantify the temperature range where specimens spent the most time (Fry, 1947). These systems were somewhat limited, however, in the range of temperatures that could be maintained effectively. More modern approaches arose from authors such as Neill *et al.* (1972), who designed an experimental system that used the movement of fish to activate cooling and heating triggers and Myrick *et al.* (2004) who developed an annular chamber.

Since its inception the original "shuttlebox" design of Neill *et al.* (1972) has been modified continuously to suit different purposes. An updated system developed by Schurmann *et al.* (1991) and Schurmann and Steffensen (1992) used a photo-cell system to track when the fish moved between the hot and cold side of the chambers which was fully automated. Khan and Herbert (2012) further developed this so that it employs open-source tracking software to determine the position of the fish at all times (Fig. 1.7). In almost all versions, the chambers themselves remain 2 °C apart at all times with the hot chamber always being 2 °C warmer than the cold chamber. This means that when the fish does switch sides it can feel an immediate temperature difference. When the fish moves from the cold side to the hot side the entire system begins to heat up at a rate of 2 °C per hour (with the warm side always remaining 2 °C warmer than the cold side). If the fish moves from the hot chamber to the cold chamber then the entire system starts to cool down at 2 °C per hour while still maintaining the 2 °C separation (see Khan and Herbert, 2012 or Schurmann *et al*. 1991 for a more detailed explanation). From this apparatus it is possible to measure the maximum and minimum temperatures behaviourally tolerated as well as the final T_{pref}. Further modifications to this system have the potential to work with much larger animals or changing the variables in question (hypoxia, salinity *etc.*). As this method has been the standard procedure for measuring thermal preference through the bulk of the published literature, it is probably the most appropriate to apply to a new species such as the hapuku.

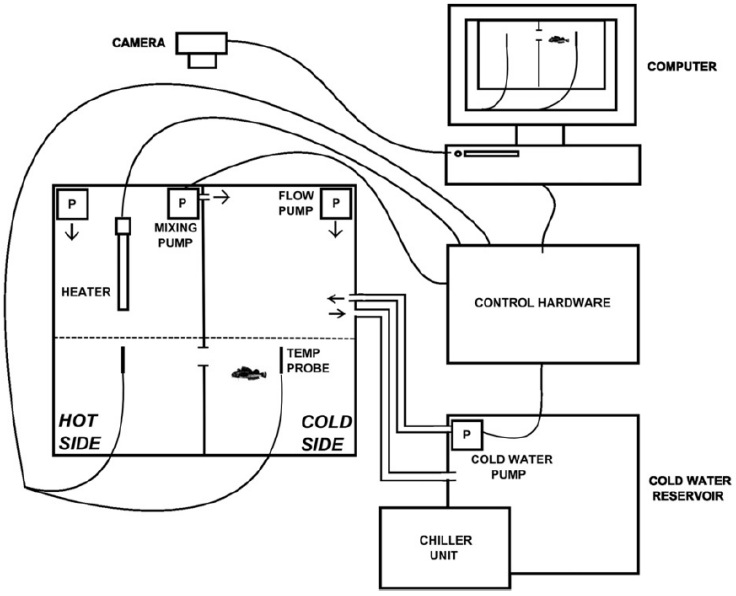

Figure 1.7. A representation of a behavioural thermoregulation apparatus that was based on the original design of Neill *et al.* (1972) with further refinements by Schurmann *et al.* (1991). See text for details. From Khan and Herbert (2012).

The annular system of Myrick *et al.* (2004) employs a circular channel where water of different temperatures overflow into the swimming channel from external channels. The advantage of this system is that the channel is continuous and there are no obstacles to overcome to experience a range of temperatures (such as the passage that divides the two sides of a shuttlebox). This may suit species that are less exploratory or particularly sedentary. Active swimmers, however, are likely to mix and break down temperature gradients as they swim along. A particularly large/wide channel can reduce the mixing caused by swimming fish, though there is a tradeoff as to how much energy is required to heat/cool larger systems and how big it has to be to work with a particular species. As juvenile hapuku are known to be very active swimmers (S. Walker, *pers. comm.* Jan, 2011), the shuttlebox apparatus is the more appropriate technique for determining temperature preference.

1.5 Feeding metabolism and specific dynamic action

Only a portion of the energy in food ingested by fish is available for processes such as maintenance, growth and activity (Tandler and Beamish, 1981; Seth *et al.*, 2010) (Fig. 1.3). Valuable food energy is lost as heat, faeces, metabolic excretion or in the energy needed to drive components of specific dynamic action (SDA) (Jobling, 1983, 1994). SDA was originally defined by Rubner (1902) and has been investigated for decades in terrestrial endotherms (generally farm animals, see Kleiber, 1975 for a review) in an attempt to better understand the conversion of pasture to protein and milk. SDA can now be defined as the increase in metabolic rate associated with feeding (see Fig. 1.8), the digestion/assimilation of nutrients into the tissues (Elliot, 1976; Brown and Cameron, 1991; Jobling, 1994) and the extensive post-absorptive costs associated with protein synthesis, turnover and growth (Jobling, 1983; Grigoriou and Richardson, 2008; Millidine *et al.*, 2009; Seth *et al.*, 2010). This is a highly encompassing description and covers aspects such as: i) excited locomotor and incidental activities, ii) the mastication, digestion and absorption of food in the gut and the iii) biochemical processes that follow, with protein synthesis being the most investigated (Tandler and Beamish, 1979; Soofiani and Hawkins, 1982; Boyce and Clarke, 1997).

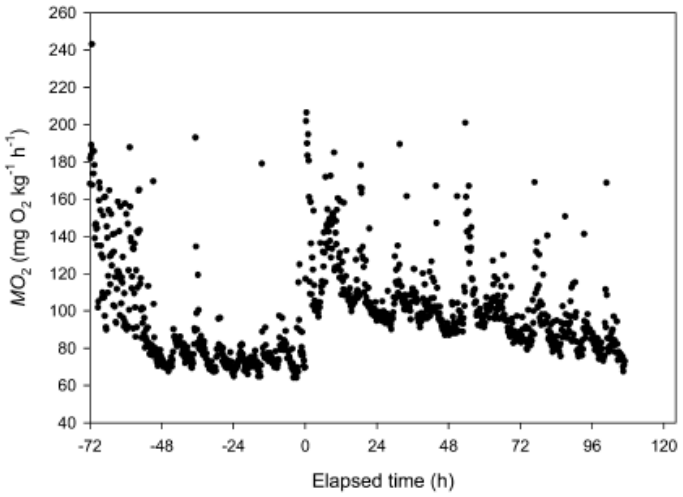

Figure 1.8. An example of an individual Atlantic cod's *Gadus morhua* (174 g) SDA response to a 5% wet weight ration of herring fillets at time 0. Note the sharp increase in oxygen consumption (left hand axis) after feeding. From Jordan and Steffensen (2007).

Because SDA describes such a wide variety of interacting mechanical and physiological factors, many investigators have attempted to break it into individual and empirically measureable components. Tandler and Beamish (1979) attempted to separate SDA out into its mechanical and biochemical components by feeding largemouth bass *Micropterus salmoides* non-digestible cellulose pellets. This allowed them to separate out the mechanical aspects, such as the mastication and gut motility. These values can then be subtracted from the full SDA response when they were fed a proper meal. The difference was considered the biochemical component of SDA (digestion, assimilation and protein synthesis) which rose exponentially with meal size, reflecting the increasing cost of protein catabolism. Brown and Cameron (1991) came to a similar conclusion by infusing essential amino acids directly into the bloodstream of the channel catfish *Ictalurus punctatus* which induced the anabolic response of protein synthesis. The greater the amount of amino acids the greater the SDA response, confirming that a large part of the prolonged SDA response measured in fish is a result of post-absorptive protein synthesis (Jobling, 1983; Secor, 2009). By understanding the various components of SDA it may be possible to further optimise feeds and feeding protocols to optimise growth and FCR. Indeed, the link between the magnitude of SDA parameters [post-feeding MO_2 peak, time to the MO_2 peak, total duration of the response and the energy consumed during the response (SDA energy), for example] and growth has been established for the grass carp *Ctenopharyngodon idella* where larger SDA response was associated with higher protein retention (Carter and Brafield, 1992). In novel species such as hapuku, it is important to first identify the extent of the response itself and how it is involved in the retention of energy and protein from feed. The term "apparent SDA" appears in the literature as a less precise term that encompasses all possible aspects of feeding that may cause an increase in metabolism during and after feeding events (Averett, 1969; Pierce and Wissing, 1974; Tandler and Beamish, 1979), and hereafter (for simplicity's sake), when the term SDA is used, it is referring to apparent SDA.

1.5.1 Measurement of SDA

In the context of energetics and aquaculture, the cost of the entire feeding process, from search and ingestion to the deposition of new protein is of interest and needs to be measured. SDA was traditionally measured in homeotherms by increases

in heat increments post-feeding (Saunders, 1963; Kleiber, 1975; Tandler and Beamish, 1979, 1981). In fish, as with almost all measurements of energy expenditure or consumption, it primarily involves measuring the increase in oxygen consumption after a meal (Fig. 1.8). Most methodologies involve feeding in a static, automated respirometer (Soofiani and Hawkins, 1982; Jordan and Steffensen, 2007) or directly injecting food into the gut or infusing purified nutrients into the bloodstream (Goolish and Adelman, 1987; Brown and Cameron, 1991; Fu *et al.*, 2007). The latter method is often applied to separate out some of the components that make up the SDA response while the first method is often applied to simply measure the size and length of the full SDA response. In measuring SDA, there are three primary factors of interest: i) the highest peak in oxygen consumption of the response, ii) the magnitude of the response (i.e. the area under of the curve above SMR) and iii) the duration of the response (Jobling and Davies, 1980; Jobling, 1994).

1.5.2 Specific dynamic action within the energy budget of fish

The size and the duration the SDA response can have a significant effect on the investment of energy on growth (Carter and Brafield, 1992; Secor, 2009). In a review of energy budgets compiled by Brett and Groves (1979) it was determined that the energy used for respiration after a meal accounted for 37% of the energy in the meal in herbivorous fish and 44% in carnivorous fish as they incur higher metabolic costs associated with high protein diets (Cowey, 1975; Houlihan *et al.*, 1995; Smith and Houlihan, 1995). Elliot (1976) found that respiration accounted for up to 88% of the energy after a feed in the brown trout while Ciu and Liu (1990) found that respiration accounted for between 50% and 69% of energy after feeding in six different teleost species. In a later review by Cho and Kaushik (1985), it was determined that more often than not, less energy is retained from meals than is expended in respiration from the resulting SDA. This large proportion of energy that is being invested in the digestion, assimilation and handling of nutrients can reduce the retained energy (Fig. 1.3).

The magnitude of the SDA response is affected by a number of variables. In the common cuttlefish *Sepia officinalis* the magnitude of the SDA response was correlated with temperature as the duration was two hours shorter for any given ration size at 20 °C than 15 °C (Grigoriou and Richardson, 2008). A similar result was found by

Jobling and Davies (1980) in plaice where the duration of the response was half as long at 20 °C than at 10 °C (averaging 26 h and 51.4 h respectively). Body weight and ration size had little effect on the factorial scope of the feeding response of Antarctic plunderfish *Harpagifer antarcticus*, (Boyce and Clarke, 1997) and the body size of common cuttlefish showed no effect on the size or duration of SDA (Grigoriou and Richardson, 2008). Jobling and Davies (1980) also noted that the proportion of digestible protein in the diet had a significant effect on SDA. SDA was significantly higher in diets with a higher proportion of protein than those with a lower proportion of protein but higher digestible energy (DE) values. This result is consistent with the literature (Carter and Brafield, 1992; Jobling, 1994; Koskela *et al.*, 1997; Secor, 2009) and further emphasises the high cost of protein handling and synthesis (Smith and Houlihan, 1995).

Table 1.1. An example of SDA in the Atlantic cod *Gadus morhua* in relation to ration size (2.5% or 5% body mass). Limitation of aerobic scope is the proportion of AMS taken up by the post-feeding $\dot{M}O_{2peak}$, SDA energy is the area under the entire SDA response curve (peak and duration above SMR) and SDA coefficient is SDA energy (kJ) expressed as a proportion of the digestible energy in the meal fed to induce the SDA response. Modified from Jordan and Steffensen (2007).

	Normoxia (19.8 kPa)	
SDA Parameters	2.5% BM	5% BM
Mass (g)	188.4 ± 12.2	147.1 ± 5.6
Prefeeding metabolic rate	59.7 ± 9.1	71.3 ± 2.0
MMR (mg O_2 kg^{-1} h^{-1})	235.6	235.6
$\dot{M}O_{2peak}$ (mg O_2 kg^{-1} h^{-1})	128.2 ± 13.3	160.6 ± 12.6
Limitation of aerobic scope for activity (%)	39.8 ± 8.0	54.5 ± 7.0
t_{peak} (h)	6 (–40)	10 (1–34)
Duration (h)	48.6 ± 13.1	95.1 ± 5.6
SDA energy (kJ)	3.9 ± 1.3	7.1 ± .9
SDA coefficient (%)	8.1 ± 2.8	9.7 ± 1.3
N	5	8

Ration size (meal size) is the most common factor investigated in the field of fish SDA and Table 1.1 shows the large extent of the affect this can have on the energetics of feeding and post-prandial metabolism. In this example from Jordan and Steffensen (2007) the limitation of AMS was 15% higher when the ration was doubled and the peak in oxygen consumption was significantly higher in the Atlantic cod. Soofiani and Hawkins (1982) came to the same conclusion with much smaller (1 – 8 g) Atlantic cod when they were fed progressive meals in a respirometry system; the peak and duration of the SDA response increasing linearly as food intake increased. They also found that the peak of the post-feeding $\dot{M}O_2$ response in resting satiated fish was close to the $\dot{M}O_{2max}$ for starved fish, suggesting that large meals can saturate aerobic capacity in this species, which leaves little capacity for factors such as swimming. When AMS is limited, there may have to be a trade-off between energetic demands. A good example of this is the European eel *Anguilla anguilla* where swimming performance was decreased after feeding as a form of metabolic prioritisation of growth over activity (Owen, 2001). In the Nile tilapia increasing the ration size also had a significant effect on the duration, the peak and the magnitude of the apparent SDA response (Ross *et al.*, 1992) (Fig 1.9).

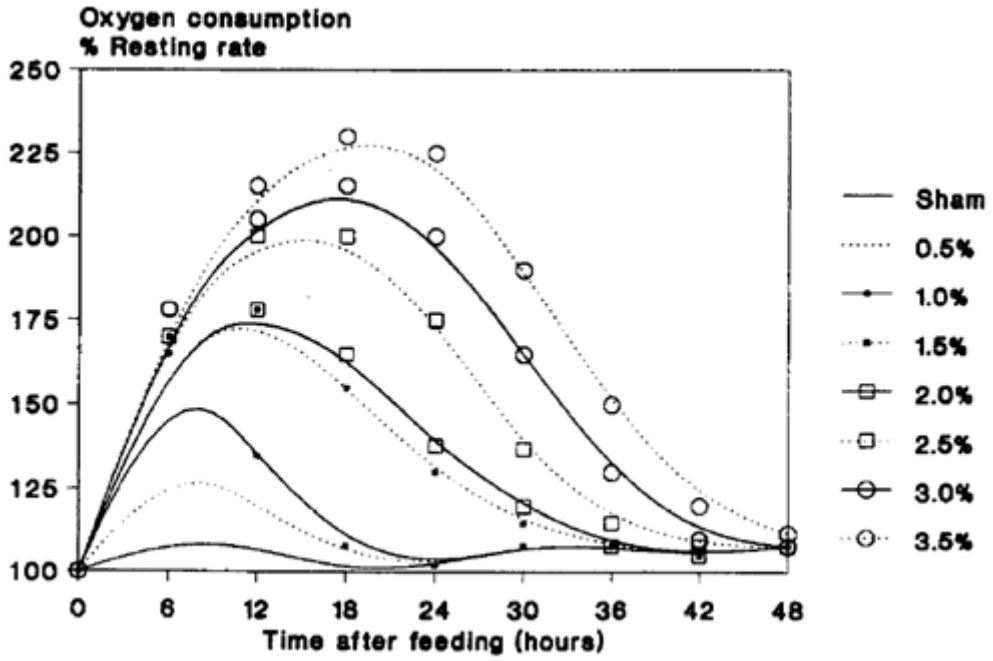

Figure 1.9. Post-feeding change in the oxygen consumption rate in Nile tilapia *Oreochromis niloticus* fed different ration levels (expressed as a proportion of body weight) at 28 °C. From Ross *et al.* (1992).

From data such as these it is clear that there are significant energetic considerations of different feeding regimes and that these need to be measured empirically in new species. Higher magnitude SDA responses should be associated with higher levels of protein synthesis and growth (Carter and Brafield, 1992) but larger meals may cause inefficiencies associated with mechanical costs and evacuation rates (Jobling, 1983). Ideal feeding regimes need to be determined for novel species and understanding the physiological response to feeding is one effective way of ensuring feed is delivered optimally.

1.5.3 Feeding high-value finfish in culture

Efficient FCRs could be the difference between the success and failure of any aquaculture venture. It is generally expressed as the amount of feed in kilograms required to produce one kilogram of fish mass and can range widely from below 1 in dry feeds that absorb water, to above 3 or 4 in wet feeds or unfavourable conditions (Jobling, 1994). By improving FCR it is possible to reduce the feeding costs associated with each kilogram of fish output, though this does not always go hand in hand with

improved growth rates in general (Kleiber, 1975). In effect, there may be a trade-off between the rearing cost and the time to get a fish to the desired weight.

FCR refers broadly and most crudely to the ratio between feed weight input and fish weight gain. Optimisation of FCR generally comes from two sources. The first of these is the feed itself. As a result of the SDA responses described in section 1.5.2, increasing protein levels can increase post-feeding levels of protein synthesis (Jobling and Davies, 1980; Carter and Brafield, 1992) but come at an economic and environmental cost (see section 1.1.2). Reducing protein levels below a certain point can also have an adverse effect of FCR as protein becomes too limited to sustain protein accretion (Jobling, 1994; Lupatsch *et al.*, 2001). Attempts to substitute fishmeal as a protein source with alternatives such as soybean protein have also produced poor FCR's, reduced quality of the final product and even led to morphological issues (Rumsey *et al.*, 1994; Kaushik *et al.*, 1995; Francis *et al.*, 2001; Ostaszewska *et al.*, 2005). Ideal feed compositions for FCR will likely involve species-specific ratios of protein to energy, as well as a carefully considered protein sources (Lupatsch *et al.*, 2001; Lupatsch *et al.*, 2003; Tacon and Metian, 2008). The second method is to identify under what environmental and commercial conditions FCR is optimised in a particular species. FCR has been shown to be increasingly inefficient with increasing temperature in juvenile turbot *Scophthalmus maximus* (Van Ham et al., 2003) and juvenile barramundi (Bermudes *et al.*, 2010). Juvenile Atlantic cod however, show an optimality curve over a functional temperature range (Björnsson *et al.*, 2001), suggesting that temperature effects could be species specific. Induced swimming been shown to significantly improve FCR in some more athletic species such as certain salmonids (Christiansen and Jobling, 1990; Jobling *et al.*, 1993a) and up to 8% in *Seriola spp*. (Brown *et al.*, 2011) (see following section). The conditions that produce the most efficient FCRs are usually unknown in novel aquaculture species though a combination of physiological and production performance measures will offer insight into this uncertainty.

1.6 Swimming and exercise

Fish move through a very dense and viscous environment (Videler, 1993) and the energetic costs of locomotion have to be balanced against other costs such as growth and feeding. Swimming has been shown to account for up to 40% of total energy expenditure in more active species (Jobling, 1994; Ohlberger *et al.*, 2005) and therefore, it is very important to consider and directly measure the cost of swimming when constructing an energy budget for a species. In terms of aquaculture it is also important to understand how different levels of sustained swimming (i.e. exercise training) can affect energy usage, growth, FCR and the quality of the final product (Davison, 1989, 1997; Palstra and Planas, 2011) as these can have implications for site selection and desired tank conditions.

1.6.1 Swimming musculature in fish

A significant proportion of fish mass is made up of the trunk musculature [~85% (Rasmussen *et al.*, 2013)], which is primarily used for swimming. Trunk musculature also happens to be the most desirable part of the fish for human consumption. In most fishes there are two distinct colourations of the trunk flesh which are superficially named red muscle and white muscle for their appearance. These muscles have different tastes and textures as a result of their physiological and functional differences.

Red muscle (RM, or oxidative muscle) is commonly seen just under the skin in a thin strip along the trunk of the fish. It is primarily used for slow-twitch aerobic swimming (cruising) and, because it is often applied for long periods of time or for long-distance swimming, it uses aerobic respiration to produce ATP (McGlinchey *et al.*, 2001; West, 2008). This requires a higher concentration of myoglobin (an-iron based oxygen binder in the muscle), mitochondria and extensive vascularisation, resulting in the red-brown appearance of the flesh and its stronger flavour. The result of this is muscle that can be in action for long periods of time (induced exercise training, for example) without the build-up of excess metabolites. It can, if necessary, be recruited in short anaerobic bursts where the high degree of vascularisation helps remove detrimental metabolites faster than its counterpart, white muscle (WM, or

glycolytic muscle) (Jayne and Launder, 1994) but it fatigues rapidly and is not ideal for this purpose.

WM makes up the bulk of muscle mass in most fish and is by far the most desirable part of the fish to eat. It is used primarily for fast-start and unsteady short anaerobic bursts and has far lower concentrations of myoglobin, mitochondria and significantly lower vascularisation (Domenici and Blake, 1997). It is considered high energy turnover, fast-twitch muscle that almost entirely relies on anaerobic pathways for ATP production. Prolonged use of this muscle results in a high concentrations of lactate and muscle fatigue (Videler, 1993) though the capacity for anaerobic activity is still far greater than that of RM (Johnston, 1999; Sänger and Stoiber, 2001).

1.6.2 Measurement of swimming costs

As with the measurement of other expenditures of energy, the rate of oxygen consumption is the most applied method to measure the cost of swimming. Intermittent respirometry systems are able to be modified to incorporate a mechanism to control flow speed in a loop or raceway[see Ohlberger *et al.* (2005) for an example]. These systems are capable of determining: i) $\dot{M}O_2$ at a range of swim speeds in terms of both relative (body lengths per second, BL s^{-1}) and absolute (metres per second, m s^{-1}), ii) SMR through the extrapolation of $\dot{M}O_2$ at different swim speeds down to 0 BL s^{-1} and iii) U_{crit} (critical swimming speed), the swimming speed that corresponds to the highest level of oxygen consumption (Itai, 2001; Nelson *et al.*, 2002; Peake and Farrell, 2004; Brown *et al.*, 2011).

To standardise measures of swimming performance between species and investigations, parameters such as the gross cost of transport (GCOT) are usually calculated (Davison, 1989; Jobling, 1994; Brown *et al.*, 2011). GCOT essentially describes the energy cost of moving the animal through the water at any given speed (usually expressed in mgO_2 kg^{-1} m^{-1} or mgO_2 kg^{-1} body $length^{-1}$) and is often shown with the net cost of transport (NCOT, GCOT less SMR estimated by extrapolating down to 0 BL s^{-1}). The relationship between GCOT/NCOT and swim speed is usually parabolic and the lowest value is referred to as the optimal swimming speed (U_{opt}) and represents the lowest cost of swimming per unit distance travelled (Behrens *et al.*, 2006; Claireaux *et al.*, 2006; Fitzgibbon *et al.*, 2007). U_{opt} is a particularly well-

reported measure in swimming physiology as it has very important implication in athletic and migratory species such as eels (Methling *et al.*, 2011; Burgerhout *et al.*, 2013; Righton *et al.*, 2013) and salmonids (Hasler *et al.*, 2012; Eliason *et al.*, 2013).

Swimming has the potential to consume a significant proportion of the energy budget and aerobic scope in active species so understanding the factors that modify the rate of energy consumption will have direct applications to aquaculture. Temperature is the most pervasive of environmental variables and has been shown to have a significant effect on not only the amount of energy consumed at any given swimming speed but also in other measures of physiological significance such as U_{crit} (Fitzgibbon *et al.*, 2007; Hanna *et al.*, 2008; Pang *et al.*, 2011). This effect has been clearly demonstrated in species such as the common carp *Cyprinus carpio*, Pacific cod *Gadus macrocephalus*, sea bass *Diecentrarchus labrax* and the largemouth bass *Micropterus salmoides* with all these species showing optimal and sub-optimal swimming performance over their ecological temperature range (Beamish, 1970; Heap and Goldspink, 1986; Koumoundouros *et al.*, 2002; Hanna *et al.*, 2008; Brown *et al.*, 2011). As with growth and other measures of physiological performance, other environmental stressors have been implicated in the reduction of swimming performance. There are numerous examples but factors such as reduced salinity in marine species (Nelson *et al.*, 1996) and oxygen (Vagner *et al.*, 2008; Domenici *et al.*, 2013) are common in the literature. Body size too is known to have a significant effect on the swimming energetics of many species. Ohlberger *et al.* (2005) found that $\dot{M}O_2$ at any swim speed was positively related to body size in the roach *Rutilus rutilus* and the common carp, with a more pronounced effect in the carp. In a review by Brett and Glass (1973) it was found that the costs of swimming also increased with body size in the sockeye salmon in relation to all other variables investigated, particularly temperature. These results are generally species-specific (Jobling, 1994; Palstra and Planas, 2011) and investigations need to made into the costs of swimming in new species to determine how different environmental and aquaculture variables affect swimming energetics.

1.6.3 Potential benefits of exercise

The considerable cost of swimming has been described in the previous section and it would be easy to come to the simple conclusion that if swimming costs were

reduced (e.g. by slowing down the flow the fish is swimming against) there would be a larger amount of energy retained for growth. This, however, is not always the case. There is ample evidence in the literature to show that sustained exercise in farmed fish can improve productivity, welfare and the quality of the product itself (Davison and Herbert, 2013; Huntingford and Kadri, 2013; Rasmussen *et al.*, 2013). Exercise-induced growth is, in its very nature, paradoxical in that expending more energy swimming could lead to improved growth, but there are more than enough examples to show this phenomenon in action. In stating that, there are species where sustained exercise has shown nil or negative effects, with the Atlantic cod being a prime example (Karlsen *et al.*, 2006). The potential benefits of exercise warrant investigation of this phenomena in any new finfish aquaculture species. It is important to note that when considering exercise training it is referring to a sustained and essentially constant level of exercise (swimming). Intermittent swim training has shown positive effects, such as improved immune function in Atlantic salmon *Salmo salar* (Castro *et al.*, 2011) while examples of periodic sprint training in the literature have resulted in a high stress response and nil or negative aspects regarding fish growth and welfare (Hernández *et al.*, 2002; Palstra and Planas, 2011). Therefore, these kinds of training regimes will not be applied in the scope of this investigation.

- *Growth and FCR*

Exposing fish to higher velocities (generally 1 BL s^{-1} – 2 BL s^{-1}) has led to positive growth responses up to 40% faster than static controls (0 BL s^{-1}) in salmonids (Davison and Goldspink, 1977; Houlihan and Laurent, 1987; Jørgensen and Jobling, 1993). Houlihan and Laurent (1987) determined that rainbow trout grew twice as fast when exposed to a continuous 1 BL s^{-1} current compared to tank-rested control fish and investigations into brown trout (Davison and Goldspink, 1977), Arctic charr *Salvelinus alpinus* (Grunbaum *et al.*, 2008) and Atlantic salmon (Jørgensen and Jobling, 1993) have consistently showed improved growth and FCRs at speeds from 0.75 BL s^{-1} – 2 BL s^{-1}. Interestingly, the Chinook salmon consistently show nil or negative responses to the exact same regimes, though the mechanism for this is not well understood (Palstra and Planas, 2011). Beyond the salmonids, there have been significant results found in species such as the striped bass *Morone saxatilis* which showed significantly improved growth and FCRs at speeds ranging from 1.2 BL s^{-1} to 2.4 BL s^{-1} (Young and Cech Jr, 1993). Kingfish *Seriola lalandi* showed a significant

increase in growth rate at 0.75 BL s^{-1} which coincided with an 8% improvement in FCR (Brown *et al.*, 2011) while the study of Yogata and Oku (2000) on *S. quinqueradiata* found an approximately 22% increased weight gain between trained and untrained fingerlings. The difference in the exercise regimes that produce the best growth response ($ER_{opt\ growth}$) in different species has recently been reviewed by Davison and Herbert (2013) who suggested that available AMS may be a significant driving force in the ability to grow faster whilst swimming as a larger AMS can better accommodate the costs of swimming and growth (SDA) simultaneously (see Fig. 1.11). If this is the case, it may be possible to use AMS to estimate the $ER_{opt\ growth}$ for new aquaculture species. It should be noted, however, that this relationship does not ignore the general increase in appetite associated with increased swimming activity.

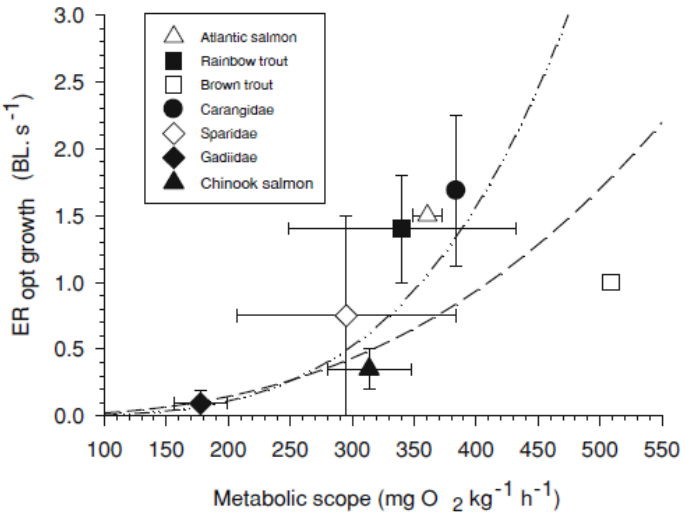

Figure 1.10. The relationship between the exercise regime (ER) for optimal growth and available AMS in a variety of well-studied fish species. The broken line is the relationship if the anomalously high AMS value for the brown trout (open squares) is included. The broken and dotted line shows the relationship when it is excluded. Metabolic scope is equivalent to AMS. From Davison and Herbert (2013).

Some increases in growth were attributed to an increase in appetite with sustained exercise which is a common phenomenon (Davison, 1989, 1997; Kieffer, 2010; Davison and Herbert, 2013). However, further investigation has since found that exercised fish fed on the same rations as non-exercised fish may still show improved

growth rates (Jobling *et al.*, 1993a) and hence improved FCR, indicating a direct role of exercise in growth acceleration. Davison (1997) and Kieffer (1998) describe a switch to lipid as a primary fuel source as this produces a larger yield of ATP per unit of substrate. In this way the proportion of protein in the feed is less likely to be used as a consumable fuel, leaving more protein for somatic growth [protein sparing (Kaushik and Médale, 1994)], which may be one of the mechanisms driving improvements in the FCR of many species. Transcriptomic processes as a result of sustained swimming have been also been recently measured in rainbow trout and reported in Planas *et al.* (2013) and Magnoni *et al.* (2013b). In total, there was an upregulation of 354 and 206 genes for RM and WM respectively, as well as a downregulation of similar number in each muscle type. These upregulated genes are involved in processes such as muscle contraction (actin, malate dehydrodgenase, creatine kinase *etc*.), glycolysis (GAPDH) and protein synthesis (40S, 60S and translation elongation and initiation factors) which explains the increased swimming and growth performance of exercised rainbow trout (Houlihan and Laurent, 1987; Alsop and Wood, 1997; Hernández *et al.*, 2002).

- *Stress and welfare*

Another potential mechanism involved in the increased growth of exercised fish could be an increase in general welfare. Dominance hierarchies are well understood in salmonid culture (Adams *et al.*, 1998; Cubitt *et al.*, 2008) and result in decreased feed intake and increased aggression towards subordinate fish (Adams *et al.*, 1995). Aggressive activity from dominant fish also lead to a great deal of spontaneous activity in both dominant and subordinate fish (turning, chasing and being chased) and results a large amount of energy consumption (Jönsson *et al.*, 1998; Castro *et al.*, 2006; Huntingford and Kadri, 2013). There are plenty of examples of exercise training leading to a significant reduction in aggressive behaviour (and increased schooling behaviour) which decreases spontaneous activity costs as well as increasing the feed consumption of what would otherwise be subordinate fish (Christiansen *et al.*, 1992; Adams *et al.*, 1995; Brännäs, 2009). Data from Jørgensen and Jobling (1993) and Christiansen and Jobling (1990) have supported this by showing a significant reduction in the proportion of fish with bite marks and/or fin damage in Arctic charr and Atlantic salmon exposed to exercise.

- *Muscle Structure, Product Quality and Texture*

The quality of the flesh in farmed fish is a combination of the characteristics of skeletal muscle, which include its chemical composition [fat content, fatty acid profile and glycogen stores (Haard, 1992; Hagen *et al.*, 2007)] and its structural components [muscle fibre density (MFD) and fibre size distribution (Johnston *et al.*, 2000; Sänger and Stoiber, 2001; Rasmussen *et al.*, 2013)]. One of the major contributors to flesh quality and value is texture which is primarily determined by MFD and connective tissue characteristics (Hatae *et al.*, 1990; Johnston, 1999; Rasmussen *et al.*, 2011). The relationship between MFD and the firmness of the fillets have been demonstrated for Atlantic salmon (Johnston *et al.*, 2006; Johnsen *et al.*, 2011), sea bass (Periago *et al.*, 2005) and Atlantic halibut *Hippoglossus hippoglossus* (Hagen *et al.*, 2007), though these relationships are not universal and the interactive effects of factors such as genetics, temperature, slaughter method, post-slaughter treatment and measurement method can cause variable results (Johnston *et al.*, 2000; Ashton *et al.*, 2010; Rasmussen *et al.*, 2013). Exercise is known to have variable effects on the structural and textual characteristics of the flesh of farmed fish species, but generally stimulates muscle fibre hypertrophy (increase in the average size of muscle fibres, reducing MFD) over hyperplasia (the recruitment of new muscle fibres, increasing MFD) (Davison and Goldspink, 1977; Johnston, 1999; Sänger and Stoiber, 2001; Rasmussen *et al.*, 2013). Muscle fibre hypertrophy generally occurs as a result of the proliferation of myonuclei in active muscle fibres (Haard, 1992; Martin and Johnston, 2006; Johnston and Børresen, 2008; Rasmussen *et al.*, 2013) and can reduce fillet firmness compared to static controls (Bjørnevik *et al.*, 2003; Rasmussen *et al.*, 2011). Species such as the Atlantic cod and Chinook salmon which have traditionally responded poorly to induced exercise, also show nil or marked decreases in MFD/fillet texture with prolonged exposure to currents (Davison, 1989, 1997; Bjørnevik *et al.*, 2003). As far as nutritional content is concerned, few studies have come to a consensus and the overall change as a result of exercise may be very species-specific. Examples of increased protein content in the fillets of salmonids have been found in moderately exercised examples (Davison and Goldspink, 1977; Houlihan and Laurent, 1987) but exposure to excessive speeds may reduce the protein levels below that of the controls (Davison and Goldspink, 1977). Lipid content is often found to be higher in moderately exercised fish which is accompanied by an increase in moisture content, making a tastier and moister fillet (Davison and Goldspink, 1977; Jobling *et al.*, 1993a). Exercised fish may have higher concentrations of buffering agents in their

flesh which can result in a slower onset and less intense rigor with a reduced drop in pH (Haard, 1992), leading to improved overall product quality (Totland *et al.*, 1987). If exercise has the potential to improve the growth rates and FCR of novel aquaculture species such as the hapuku, the effects on swimming musculature and fillet quality must also be considered.

1.7 Experimental aims

The encompassing aim of this investigation is to determine under what conditions the growth rates and FCR of juvenile hapuku are optimised. This is the first time that the physiology and metabolism of this species has been explored and the information gathered here can be used to make informed decisions about the future aquaculture of this species. Using primarily respirometric techniques, a rudimentary metabolic framework will be formulated for juvenile hapuku with respect to i) temperature, ii) SDA and iii) swimming and exercise training. By measuring the physiological costs and consequences of these three factors and budgeting them into a metabolic framework (AMS), it will be possible to understand how different (and potentially competitive) physiological processes are modified by environmental and culture conditions. In an attempt to approach this in as logical a way as possible, this thesis will be structured as follows:

Chapter 2 – There have been no prior attempts to measure the AMS of juvenile hapuku. As AMS represents the metabolic framework of this species, it is necessary to determine how AMS is affected by environmental variables such as temperature. Additionally, AMS is considered to be tightly linked to growth performance (Jobling, 1994) and this needs to be confirmed in hapuku. Under the hypothesis that growth performance and FCR improve with increasing AMS, this chapter aims to determine:

- The AMS of juvenile hapuku over the range of 12 °C – 24 °C.
- Production performance (growth and FCR) over the range of 12 °C – 24 °C.
- The behaviourally selected T_{pref} of juvenile hapuku.

- The utility of AMS and T_{pref} in predicting the optimal temperature range for growth and FCR in novel aquaculture species.

Chapter 3 – SDA is a representation of the energetic investment in growth (Jobling, 1983) and, while being affected by aspects such a meal size, composition and temperature (Fu *et al.*, 2005b; Frisk *et al.*, 2013), it is confined to the metabolic framework of AMS. Under the hypothesis that the magnitude of the SDA response is positively related to growth performance in juvenile hapuku, this chapter aims to determine:

- The effect of both temperature (15 °C and 21 °C) and ration size on the magnitude and duration of the SDA response of juvenile hapuku.
- The functional link between the magnitude of SDA parameters and growth and FCR.

Chapter 4 – Sustained exercise has been shown to have many potential benefits to intensively cultured fish species (Davison and Herbert, 2013), as well as having nil or detrimental effects in others (Kiessling *et al.*, 1994; Karlsen *et al.*, 2006). Davison and Herbert (2013) also hypothesised that the optimal exercise regime for growth is positively related to AMS. By examining the growth performance of juvenile hapuku to a range of exercise regimes, this hypothesis can be tested directly. Therefore, this chapter aims to determine:

- The effect of different levels of sustained exercise on the growth and FCR of juvenile hapuku.
- The metabolic cost of swimming at different speeds, as well as the effect of different levels of sustained exercise on swimming efficiency.
- The applicability of the model of Davison and Herbert (2013) in predicting the optimal exercise regime for optimal growth (based on available AMS) in a novel finfish aquaculture species.

Chapter 5 – Sustained exercise in finfish aquaculture species has also been shown to effect the product quality of farmed fish [fillet constituents, texture and appearance, for example (Rasmussen *et al.*, 2013)]. As cultured hapuku are ultimately intended for consumption, it is of use to know how different levels of swimming are affecting the end product. Under the hypothesis that exercise training will induce muscle hypertrophy and reduce fillet texture, this chapter aims to determine:

- The effect of different levels of sustained exercise on the gross muscle structure of juvenile hapuku.
- The effect of different levels of sustained exercise on the firmness of raw hapuku fillets.

Chapter 6 – This final chapter aims to integrate the findings and construct a rudimentary energetic model for juvenile hapuku. The assumptions of the model, factors that may influence energetics, limitations and future research directions will also be presented and discussed.

Chapter 2 – The Effect of Temperature on the Physiology and Growth of Juvenile Hapuku

Published as:

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2.1 Introduction

The expansion of fish farming has driven diversification into new species. The farming of novel species presents exciting new opportunities, but an adequate understanding of how environmental variables affect the production efficiency of different species is often lacking. With diversification in mind, there may be utility in using simple physiological and behavioural tests to rapidly determine the optimal growing conditions of novel finfish aquaculture species.

Temperature has a direct effect on the rate at which ATP energy is produced and utilized by ectotherms (Fry, 1971; Jobling, 1994; Pörtner and Farrell, 2008). Temperature, therefore, has a major influence on the performance of fish in aquaculture and can have a strong bearing on the viability of any new venture, especially since it impacts growth (Jobling, 1981; Jobling *et al.*, 1993b) and feed conversion ratio (FCR) (Jørgensen and Jobling, 1993; Nordgarden *et al.*, 2003a; Van Ham *et al.*, 2003). Because standard metabolic rate (SMR) and maximum metabolic rate (MO_{2max}) both increase with temperature within a non-critical range, but not necessarily linearly or in proportion with each other, the difference between these two

measures (termed aerobic metabolic scope, AMS) is not constant across a range of temperatures (Jobling, 1994; Claireaux *et al.*, 2000; Mallekh and Lagardère, 2002). AMS is an estimate of the capacity for aerobic ATP production and non-maintenance metabolic work such as growth (Clark *et al.*, 2013). High rates of growth and other non-maintenance activities are only permissible when AMS is high and non-limiting (Healy and Schulte, 2012) and studies in species such as gadoids (Soofiani and Priede, 1985b; Claireaux *et al.*, 2000), salmonids (Cutts *et al*., 2002) and flatfish (Mallekh and Lagardère, 2002; Lefrancois and Claireaux, 2003) support this paradigm [termed the oxygen- and capacity-limited thermal tolerance (OCLTT) hypothesis, recently reviewed by Clark *et al.* (2013)].

Growth is a focal performance measure, but as feed represents a significant proportion of costs, the feed conversion ratio (FCR) must also be taken into account. The processes driving the relationship between temperature and FCR are poorly understood and only preliminary investigations have been made.

Behavioural measures could also be used to predict the optimum temperature for growth/FCR. The accumulated evidence suggests that the behaviourally selected final thermal preference of fish (hereafter referred to as T_{pref}) approximates the optimum temperature for growth in many commercial and model fish species [see Reynolds and Casterlin (1979) and Jobling (1981) for a review]. The application of this technique as an investigative tool in aquaculture research, however, has perhaps been underestimated. The T_{pref} protocol involves presenting fish with a choice of temperatures and allowing them to self-select their preferred temperature over a period of time, which should, according to available information, should reflect the temperature where growth rates are maximised (Brett, 1952; Cherry *et al.*, 1977; Jobling, 1981; Jorgensen *et al.*, 2012). Therefore, if validated for a greater range of species, T_{pref} and AMS could readily predict the temperature at which finfish productivity is optimal. Measurement of AMS and T_{pref} is relatively straightforward, so once optimal conditions are resolved for one size class, a range of body sizes or strains could then be examined further, potentially with the interactive effects of environmental challenge (e.g. low oxygen levels, increased swimming speeds etc.) also taken into consideration as they have been in relation to climate change and conservation physiology (Pörtner and Farrell, 2008; Jorgensen *et al.*, 2012; Khan and Herbert, 2012). In this scenario, simple respirometric and/or behavioural experiments

could provide a favourable alternative to extended growth trials that are lengthy, laborious and expensive to run.

These techniques were applied to a novel finfish aquaculture species in New Zealand, the hapuku *Polyprion oxygeneios*. Hapuku are a wreckfish species found around the coasts of New Zealand and in the temperate and subtropical waters of the southern Indian and Pacific oceans (Beentjes and Francis, 1999). It is both commercially and recreationally targeted wherever it is found and is highly prized for its firm white flesh, but commercial catch rates are low in New Zealand (Beentjes and Francis, 1999; Francis *et al*., 1999). Hapuku therefore represents a valuable aquaculture opportunity. Hatchery technologies have been developed in New Zealand and the further research into the applicability of this species to intensive culture is underway (e.g. Anderson *et al*. 2012).

The aims of the current investigation were to establish whether a physiological measure, AMS, and behavioural measure, T_{pref}, are linked with growth and FCR optimisation in juvenile hapuku across a range of temperatures. In addition, the utility of these measures to predict the optimal conditions for novel finfish species was also discussed.

2.2 Materials and methods

2.2.1 Experimental animals

A total of 300 juvenile hapuku, approximately four months post hatch (46.2 ± 0.8 g), were obtained from the NIWA Bream Bay Aquaculture Facility in Ruakaka, Northland, New Zealand. These fish were implanted intraperitoneally with a passive integrated transponder (PIT) tag under anaesthesia (Aqui-S® 0.01 mL L^{-1} followed by 2-phenoxy-ethanol 0.3 mL L^{-1}) approximately 3 weeks prior to the start of the trial. Fish were then treated with Chloramine-T (0.005 g L^{-1}) to prevent infection post-tagging. Any individuals that showed signs of infection were either treated further with formalin (0.15 mL L^{-1}) or euthanized.

2.2.2 Acclimation, feeding and growth trials

Experimental animals were reared in cylindrical 170 L bottom draining tanks in a temperature controlled room at the NIWA Bream Bay Aquaculture facility (approximately 20 animals per tank with similar total biomasses, 3 tanks per temperature). The tanks were supplied with flow through filtered (1 µm) UV sterilised (ALX2/8, 150,000 µW sec cm^{-2}, Davey Water Products, Australia) seawater. Dissolved oxygen levels were maintained at between 9 and 12 mg L^{-1} through low flow pure oxygen diffusers. The water temperature in all tanks was initially maintained at 18 °C. Tank water temperatures were then adjusted 1 °C per day towards their experimental temperatures of 12, 15, 18, 21 and 24 ± 0.25 °C (standard facility practice).

Once target tank temperatures were stable, fish were anaesthetised (Aqui-S® 0.01 mL L^{-1} followed by 2-phenoxy-ethanol 0.3 mL L^{-1}) and their initial weight measured. After one full day of recovery, each tank was hand fed to satiation twice daily (1000 h and 1600 h) with Skretting Nova FF 5 mm pellets (Skretting, Australia, 50.0% protein, 17.0% lipid, digestible energy 18.6 MJ kg^{-1}). Uneaten feed was recovered 15 mins after feeding behaviours had ceased and recorded. Reclaimed feed was corrected for water absorption by a standard saturation factor (determined by soaking known weights of feed pellets and then re-weighing). The amount of feed consumed by each tank was recorded at the end of each day. After 28 days the fish were food deprived for two days, anaesthetised (as above) and the weight for each individual fish was re-measured (see Table 2.1).

Mass specific growth rate (SGR, % body weight day^{-1}) was calculated according to:

$$SGR = \ln m_2 - \ln m_1 / t_2 - t_1 \times 100$$

where, m_1 is the initial weight at the start of the growth period t_1 and m_2 is the final weight at the end of the growth period t_2. Post growth trial (PGT) SGR was also measured for the fish used in the respirometry trials with m_1 being the weight of the fish at the end of the growth trial and m_2 was the weight at the time of respirometry. Feed conversion ratio (FCR), measured as the weight of dry feed intake per unit weight gain for the period, and was calculated for each tank using the following formula:

FCR = weight of dry feed consumed in tank / wet weight gained in tank

Feed consumption per individual was measured as the total weight of dry feed (in kg) consumed in each tank, corrected for any uneaten feed, divided by the number of fish in the tank.

Table 2.1. The mean weight (g) of the juvenile hapuku at the start and end of the 4-week growth trial as well as the mean weight of the specimens used in the respirometry and behavioural thermoregulation experiments.

Acclimation Temperature (°C)	Mean Growth Trial Start Mass (g)	Mean Growth Trial End Mass (g)	Mean Respirometry Mass (g)	Mean Behav Thermoregulation Mass (g)
12	49.29 ± 0.16	79.76 ± 0.27	118.95 ± 4.57	
15	47.44 ± 0.86	118.44 ± 1.80	135.62 ± 8.02	169.90 ± 25.20
18	47.03 ± 0.85	144.88 ± 2.28	167.18 ± 6.28	180.91 ± 32.56
21	47.25 ± 0.97	150.83 ± 2.92	160.53 ± 6.48	
24	42.96 ± 0.68	109.27 ± 2.29	110.58 ± 5.48	

All values are shown with their associated standard error (s.e.)

2.2.3 Respirometry

Fish were food deprived for 48 hours prior to respirometric measurements to remove the confounding effects of specific dynamic action (SDA) on metabolic rate (Ross *et al.*, 1992; Thuy *et al.*, 2010a). Metabolic rate was determined in 40 individuals (8 individuals from each temperature, two to three individuals per tank) as the mass-specific rate of oxygen consumption (MO_2) in mg of oxygen consumed per kg of wet weight per hour ($mgO_2\ kg^{-1}\ h^{-1}$). Measurements were carried out using two identical automated intermittent flow respirometry systems over approximately 100 days, with similarities to the methodology detailed in Steffensen (1989), Jordan and Steffensen (2007) and Khan and Herbert (2012). Over the 100 day measurement period, feeding levels were maintained at satiation and acclimation temperatures were cycled through in sequence in an attempt to standardise the time between the end of the

growth trial and respirometry between temperatures. Two custom-built 1.85 L acrylic cylinders, with a Sicce® Mimouse submersible "flush" pump at one end and an outlet tube extending above the water level at the other, were placed in fully aerated 60 L reservoirs filled with filtered (1 µm) UV sterilised (Pondmaster UV-C, Danner Manufacturing, New York) seawater. Water circulation within the chambers was provided by an external loop of tubing containing a modified Sicce® Mimouse submersible pump and an oxygen probe cuvette. The water supplied to the reservoir was continuously UV sterilised and then aerated via a separate 40 L air diffused cooling/gassing tower. Aluminium coils of warm or cold water within the tower were connected to an external reservoir and water was supplied via a relay on/off system to maintain the desired temperature (i.e. 12, 15, 18, 21 or 24 ± 0.1 °C).

The level of oxygen saturation in the respirometer was measured using a WTW Cellox® 325 galvanic probe inserted into the external loop of the chamber and connected to a WTW® 3310 meter (WTW, Germany) operating at a frequency of 1 Hz. Oxygen saturation was measured constantly across three cycled phases (flushing, wait and measure) that were repeated every 12 min (see Khan and Herbert, 2012). The Cellox® probe was calibrated at the beginning of every experiment to account for changes in atmospheric pressure and treatment temperature. $M\mathrm{O}_2$ was calculated using the same formulae as Jordan and Steffensen (2007) and Khan and Herbert (2012).

Approximately 280 $M\mathrm{O}_2$ values were obtained from fish in a rested state over ~ 60 h and these were used to estimate standard metabolic rate (SMR) using the quantile method (q = 0.15) of Chabot and Claireaux (2008a), Franklin *et al.* (2013) and Dupont-prinet et al. (2013). The 0.15 quantile typically coincides with the modal value of $M\mathrm{O}_2$ and this was the case for juvenile hapuku.

After the SMR estimate was complete, fish were removed from the chambers and chased to exhaustion with a net in a 50 L circular tank of the appropriate temperature. This was done until the fish failed to respond to light physical stimulation (approximately 10 mins). The fish were then returned to the chambers and underwent four to eight more 12 min cycles, after which, $M\mathrm{O}_2$ was clearly decreasing towards normal levels. $M\mathrm{O}_{2\mathrm{max}}$ was then estimated using a q = 0.99 method (Dupont-prinet *et al.*, 2013). For this reason, $M\mathrm{O}_{2\mathrm{max}}$ usually corresponded to either the $M\mathrm{O}_2$ values recorded immediately after the specimen was initially placed in the chamber or after

the exhaustive exercise period. This method of measuring oxygen debt repayment as a proxy for MO_{2max} is often used in species that are not necessarily suited to Brett-type swim flume respirometers (Khan and Herbert, 2012; Clark *et al.*, 2013; Dupont-prinet *et al.*, 2013).

Aerobic metabolic scope (AMS) was defined as the difference between MO_{2max} and SMR for each individual. After each set of measurements was complete, background oxygen consumption readings (rate of oxygen decline in the respirometer after the fish had been removed) were taken to ensure that bacterial respiration was negligible. All equipment was cleaned thoroughly before a new fish was introduced using a high pressure water blaster and a mild hypochlorite solution (0.015 mL L^{-1}). The entire system was shrouded to minimise human disturbance and buffered against vibrations with foam under the reservoirs.

2.2.4 Behavioural thermoregulation

Juvenile hapuku acclimated to either 15 °C or 18 °C for a minimum of four weeks were allowed to thermoregulate in an electronic shuttlebox modified from Neill et al. (1972), Schurmann et al. (1991) and Khan and Herbert (2012) (see Fig. 2.1). 15 °C and 18 °C acclimated fish were selected *post-hoc* as they represented a sub-optimal and a near-optimal/optimal temperature for growth. The shuttlebox was constructed from transparent acrylic panels to form an 1100 mm × 900 mm × 300 mm chamber. This was split with a non-transparent acrylic panel to create two identical chambers hereafter referred to as the "hot" and "cold" chambers. The behavioural arena (two chambers each approximately 850 mm × 450 mm × 300 mm) was separated from a working area where pumps and other equipment were submerged by a perforated panel that was perpendicular to the separating panel, 250 mm from one end of the chambers. Two Eheim® 600 submersible "flow" pumps ensured that water was well mixed between the working area and the behavioural arena. A drain in the working area on the cold side of the apparatus allowed excess water to drain and maintained a water depth of 250 mm. The hot and cold sides of the apparatus were connected by a 120 mm diameter hole in the separating plate allowing the fish to move freely between the chambers. Temperatures in the hot and cold chambers were measured by fixed temperature probes. The temperature on the hot side was maintained at 2 °C above the cold side. Once the heating and cooling cycles were initiated the movement of the fish

between the chambers was tracked with a Monacor® IR camera connected to Swistrack® freeware and custom components. Depending on the location of the fish and the temperature in each chamber, the custom Swistrack® components activated pumps via a custom hardware and DAQ control system (designed by John Atkins, Leigh Marine Laboratory). When the fish was on the cold side both chambers cooled down at a rate of 2 °C h^{-1} maintaining the 2 °C difference between the chambers. The cooling was achieved by an on/off relay attached to an Eheim® 600 submersible pump in a 125 L cold sump that pumped water at a rate of 300 L h^{-1} into the working area of the cold side. The cold sump was chilled to 10 °C via a Hailea® HC-1000A aquarium chiller. When the fish was on the hot side of the chamber, both chambers were heated at a rate of 2 °C h^{-1} but the 2 °C difference between the cold and the hot side was maintained. Heating was achieved with an on/off relay connected to an Eheim® 600 submersible pump in a 125 L hot sump that pumped water at a rate of 300 L h^{-1} into the working area of the hot side. The hot sump was heated to 35 °C with a custom 2000 W bar heater. If the temperature separation between the hot and cold side became larger than 2 °C, an Eheim® 300 "mixing" pump (see Fig. 2.1) would pump water from the hot side to the cold side to reduce the difference. If the temperature separation between the hot and cold side became less than 2 °C, water from both sumps would be pumped into the chambers simultaneously to maintain the 2 °C separation. Water levels in both sumps were maintained via a ballcock valve. The sumps were supplied with oxygenated, filtered (1 µm) and UV sterilised (ALX2/8, 150,000 µW sec cm^{-2}, Davey Water Products, Australia) seawater. Fresh seawater flowing into the sumps and older water draining out of the behavioural shuttlebox resulted in the water in the system being replaced at least three times daily. The shuttle box was insulated where possible and shrouded in black plastic to minimise external disturbance. To allow effective behavioural tracking of fish, the shuttlebox was placed on a white background and backlit by a strip of white LED lights around the edge of the 1200 mm × 1200 mm Perspex™ base plate.

To prevent any potentially confounding effects of SDA on T_{pref}, all specimens were food deprived for 24 h prior to being placed in the shuttlebox and were then left for a further 48 h in the apparatus before the experiment began (total of 72 h of starvation). T_{pref} was estimated in seven fish acclimated to 15 °C and seven fish acclimated to 18 °C. The initial temperatures in the shuttlebox were set with the hot

chamber being 1 °C above, and the cold chamber 1 °C below the specimens acclimation temperature (e.g. for a fish acclimated to 15 °C, the hot chamber was initially 16 °C and the cold chamber 14 °C, maintaining the differential of 2 °C). A single fish was then introduced randomly to one side of the chamber and allowed to explore the chambers and temperature gradient for 48 h before temperature changes were initiated and continued for a further 72 h. During this time, the position of the fish, the temperature of each chamber, the temperature difference between the chambers and the pump states (on/off) were recorded every second by the DAQ system.

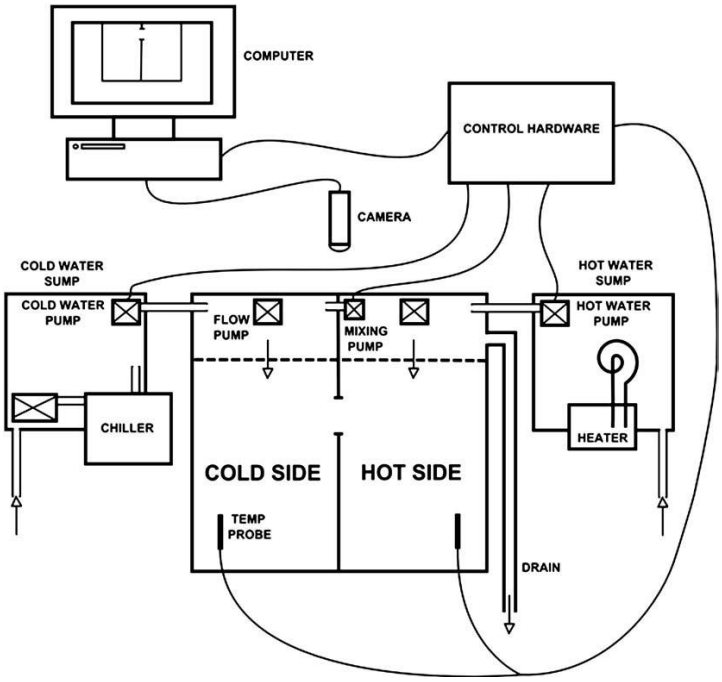

Figure 2.1. Illustration of the electronic shuttlebox apparatus used to determine temperature preference (T_{pref}) in juvenile hapuku *P. oxygeneios*. See section 2.2.4 for details.

T_{pref} was defined as the median temperature experienced by any individual fish in accordance with the findings of Schurmann et al. (1991) and Khan and Herbert (2012). Upper avoidance temperature (UAT) and lower avoidance temperature (LAT) were defined as the median value of the temperatures at which a fish left the hot or cold chamber respectively i.e. the temperature they elected to leave in favour of the temperature on the other side.

2.2.5 Statistical analyses

The differences in AMS, SGR, FCR and the feed per individual across the five experimental temperatures were examined using a parametric one-way analysis of variance (ANOVA), after ensuring that data were both equally variant and normally distributed. Where a temperature effect was detected, a Holm-Sidak method was applied for specific *post-hoc* comparisons. The relationship between production parameters (i.e. SGR, PGT SGR and FCR) and measures of metabolism (i.e. AMS and SMR) were examined using either a simple linear or, a second-order (non-linear) polynomial regression. SMR was not scaled allometrically as there was no significant effect of scaling over the size range investigated (linear regression, $R^2 = 0.02$, $P = 0.42$). Subsequently, MO_{2max} and AMS are also shown in their raw form. The difference in T_{pref}, UAT and LAT for fish acclimated to 15 °C and 18 °C were analysed using student's t-tests. The effect of body size on T_{pref} was analysed using an analysis of co-variance (ANCOVA). All analyses were conducted using SigmaPlot® 11.0 and JMP 5.0.

2.3 Results

There was a parabolic relationship between temperature and the following three performance measures: AMS, SGR and feed per individual (Fig. 2.2A, B and C). These parameters all increased with temperature and then declined as temperatures increased to 24 ºC. ANOVA tests confirmed that these measures were all significantly affected by acclimation temperature (one-way ANOVA F = 63.04, 67.13, 110.49 and 201.07 respectively, $P < 0.001$ in all cases). Specific *post-hoc* comparisons revealed that AMS and SGR were both optimised in the range 18 °C to 21 °C and that there were no significant differences for these parameters at these two temperatures. In contrast, feed per individual was highest at 21 °C. Interestingly, FCR did not follow the same parabolic relationship with temperature (Fig. 2.2D); rather, it increased with temperature between 12 °C and 24 °C with a particularly large increase between 21 °C and 24 °C (Fig. 2.2D).

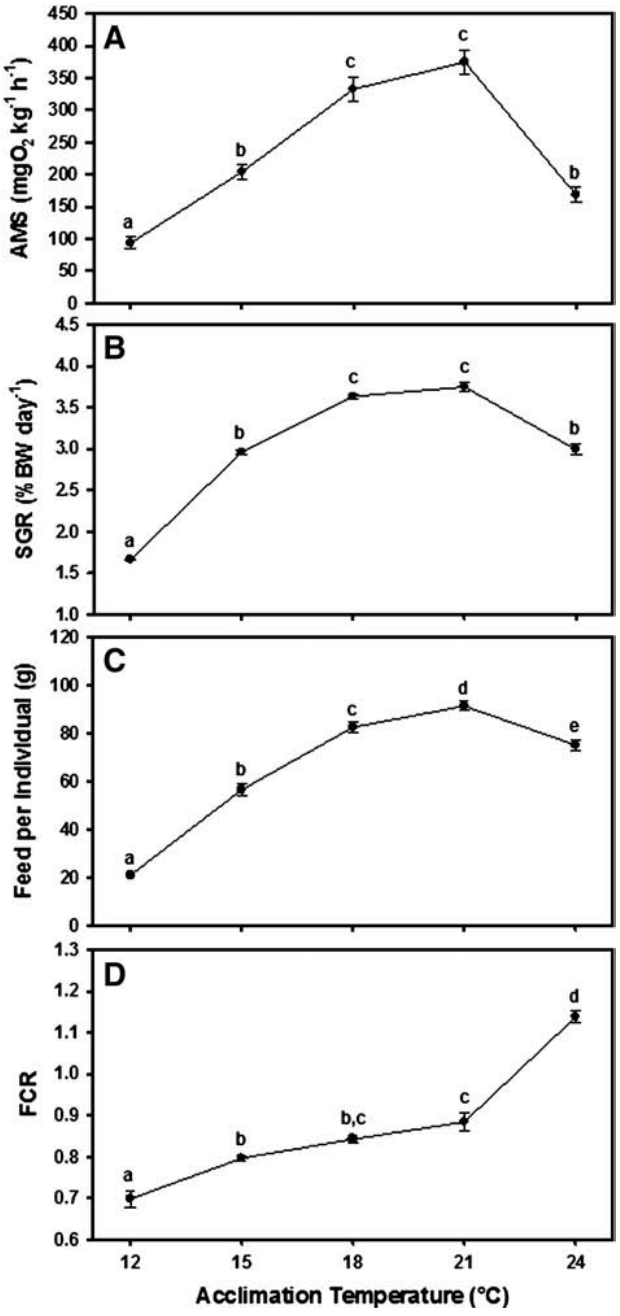

Figure 2.2. (A) Aerobic metabolic scope (AMS, measured as the difference between SMR and MO_{2max} estimates, N = 8 per temperature), (B) specific growth rate (SGR, N = approximately 60 per temperature), (C) the average feed consumption per individual (calculated by dividing total feed consumption in each tank by the number of fish, N = 3 per temperature) and (D) feed conversion ratio (FCR, measured as the amount of dry feed intake per unit weight gain, N = 3 per temperature) for each of the 5 temperatures that juvenile hapuku *P. oxygeneios* were acclimated to for a minimum of 4 weeks (12, 15, 18, 21 and 24 °C). Data are means ± SE. Superscripts between acclimation temperatures show a significant difference at $P < 0.05$.

There was a positive but non-linear relationship between AMS and SGR (Fig. 2.3A, $R^2 = 0.92$, $P = 0.03$) and AMS and PGT SGR (Fig. 2.3B, $R^2 = 0.99$, $P < 0.001$). FCR, however, did not show any relationship to AMS (Fig. 2.3B, $R^2 = 0.11$, $P = 0.87$), though it did show a significant positive and linear relationship to SMR (Fig. 2.3C, $R^2 = 0.94$, $P = 0.003$).

The T_{pref} of 15 °C and 18 °C acclimated hapuku (19.58 and 19.13 °C respectively, Table 2.2) were not significantly different from one another ($F = 0.12$, $P = 0.74$) and fell within the 18 to 21 °C range where the optimal values for SGR and AMS were observed (Fig. 2.2A, 2.3A and 2.3B). The UAT and LAT for both the 15 and 18 °C acclimated fishes also bordered the optimal range for SGR and AMS, as did the 0.25 and 0.75 quartiles (Table 2.2). There was no significant interaction effect of body size and acclimation temperature on T_{pref} ($F = 0.68$, $P = 0.53$).

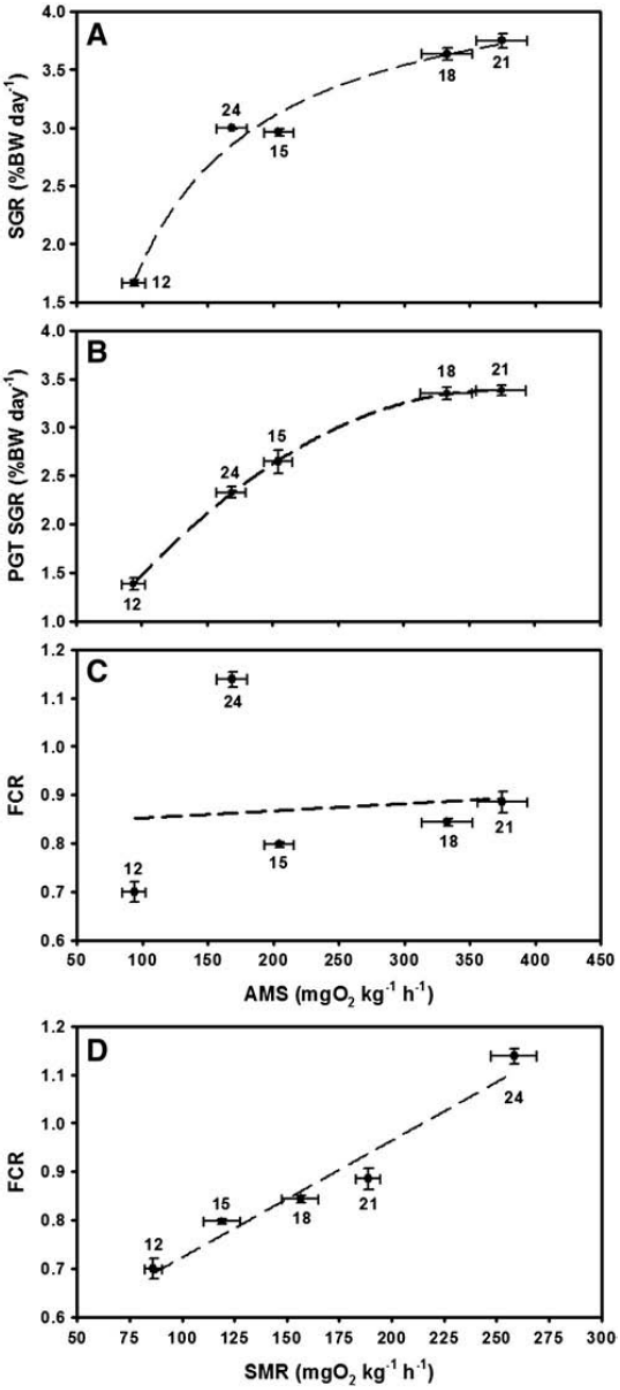

Figure 2.3. Relationships in juvenile hapuku (*P. oxygeneios*) between: (A) Specific growth rate (SGR, N = approximately 60 per temperature) and aerobic metabolic scope (AMS, measured as the difference between SMR and MO_{2max} estimates, N = 8 per temperature). The broken line shows a second order polynomial regression (y = -3E-05x^2 + 0.0222x - 0.059, R^2 = 0.92, P = 0.03) between these parameters; (B) Post growth trial (PGT) SGR and AMS (N = 8 per temperature for both parameters). The broken line shows a second order polynomial regression (y = -3E-05x^2 + 0.0193x - 0.2019, R^2 = 0.99, P < 0.001) (C) Feed conversion ratio (FCR, measured as the amount of dry feed intake per unit weight gain, N =3) compared to

AMS (N = 8 per temperature). The broken line shows a linear regression (y = 0.0001*x* + 0.84, R^2 = 0.11, *P* = 0.87) and (D) Feed conversion ratio (FCR) and standard metabolic rate (SMR). The broken line shows a linear regression (y = 0.0024*x* + 0.48, R^2 = 0.94, *P* = 0.002). All values are shown as the mean values ± SE for both SGR/FCR and AMS/SMR. The acclimation temperatures that relate to each data point are shown above or below it. R^2 values are for the presented data means.

Table 2.2. The temperature preference (T_{pref}), upper avoidance temperature (UAT) and lower avoidance temperature (LAT) of juvenile hapuku (*P. oxygeneios*) acclimated to either 15 °C or 18 °C. The 0.25 and 0.75 quartile temperatures are also shown to give an indication of the data spread and how time in the shuttlebox was spent. The values for T_{pref}, UAT and LAT are shown as the mean of the median temperatures for each individual fish (see section 2.2.4 for more details).

		Mean of Median Temperatures (°C)				
Acclimation Temperature (°C)	N	LAT	UAT	Temperature Preference	0.25 Quartile	0.75 Quartile
15	7	18.11 ± 1.26	21.38 ± 1.21	19.58 ± 1.06	18.02 ± 1.08	21.25 ± 0.99
18	7	17.65 ± 1.14	21.47 ± 1.11	19.13 ± 0.82	17.72 ± 0.72	20.99 ± 0.83

All values are shown with their associated standard error (s.e.)

2.4 Discussion

T_{pref} and the temperature range where AMS was maximised were both consistent with the optimum temperature for growth performance in juvenile hapuku (Fig. 2.2). An expansion of AMS can be defined as an increased capacity to produce ATP aerobically and when given adequate energy supplies, the enlarged AMS may allow for greater and simultaneous investment in non-maintenance processes such as growth (Jobling, 1994; Blier *et al.*, 1997; Clark *et al.*, 2013). The link between high AMS and improved growth performance is not novel and has been demonstrated for economically important finfish species such as the Atlantic cod *Gadus morhua* (Claireaux et al., 2000) and sockeye salmon *Oncorhynchus nerka* (Brett, 1976). Juvenile hapuku also show a positive relationship between AMS and SGR (Fig. 2.3A)

as well as PGT SGR (Fig. 2.3B) with all measures being maximised in the range of 18 °C to 21 °C. These data support the oxygen- and capacity-limited thermal tolerance (OCLTT) hypothesis which has been recently reviewed by Clark *et al.* (2013). The hypothesis suggests that performance measures in fish (growth, for example) will be optimised where AMS is at its highest as they are ultimately governed by the oxygen transport/delivery capacity of the gills, blood and heart under the specified conditions. When the capacity to deliver oxygen is maximised, the capacity to produce ATP aerobically is maximised and there is a greater pool of ATP able to be invested into non-maintenance physiological processes.

Contrary to the link between AMS and SGR, FCR in juvenile hapuku was not optimised in the range of 18 °C to 21 °C (Fig. 2.2D), nor did it show any relationship to AMS (Fig. 2.3C). A positive relationship between FCR and temperature has been also been observed in juvenile turbot *Scophthalmus maximus* (Van Ham et al., 2003) and juvenile barramundi (Bermudes *et al.*, 2010), though juvenile Atlantic cod show an optimality curve over a functional temperature range (Björnsson *et al.*, 2001). One potential component of this observation is the apparent link between FCR and SMR in juvenile hapuku (Fig. 2.3D). Any temperature related increase in SMR requires a larger energetic investment to maintain basic physiological function (Jobling, 1994), so a smaller proportion of the energy from feed would be retained for growth. The reverse is true at lower temperatures where a smaller proportion of the energy in feed is required to maintain a lower SMR. Modifications to meal size and feeding rates can help cover the increased energy demands of a higher SMR but considerations must be made as to the effects of increased feeding on SDA, which can also affect feed conversion (Soofiani and Hawkins, 1982; Jobling, 1994; Jordan and Steffensen, 2007; Secor, 2009).

The separation of the optimum temperature for growth and the most efficient FCR observed in this study may result in a conflict between reducing the time to market size and lowering overall feed costs. Because feed is such a large component of finfish farming costs (Naylor and Burke, 2005), the optimal temperatures for the culture of this species will involve a trade-off between the best conditions for feed conversion and those for growth. In the case of juvenile hapuku, the optimum observed temperature for FCR (12 °C, Fig. 2.2D) reduces growth performance to approximately a third of that at 21 °C. On the other hand, the temperature at which growth is

optimised (21 °C, Fig. 2.2B) requires 30% more feed per kg of biomass than at 12 °C (Fig. 2.2C and D). Growth performance is also depressed above 21 °C (Fig. 2.2B) and although 24 °C does not appear to represent the thermal or physiological limit to growth in juveniles, the sharp decrease in growth performance and increase in FCR likely represents the upper thermal limit of potential seacage sites. The ideal temperature regime for juvenile hapuku would encompass the greatest area under the SGR curve as possible and also the region where FCR is low, thus minimising rearing cost. Based on the above, temperatures should probably not exceed 21 °C for any extended period of time. Thermal regimes within the range of 15 °C to 21 °C may represent the best balance of low cost growth, with a rearing temperature of 18 °C being ideal.

Because AMS and T_{pref} are known to be linked with growth in a variety of commercial fish species (Brett, 1976; Beitinger and Magnuson, 1979; Jobling, 1981; Soofiani and Priede, 1985b; Jobling, 1994), the link between these variables in juvenile hapuku is to be expected. However, the relatively simple techniques of AMS and T_{pref} are being under-utilised in terms of screening fish for growth performance. The strong link between AMS and growth (and the persistence of this relationship after the growth trials, Fig. 2.3B) allows exploration into the effect of different environmental and culture variables (Claireaux and Lagardère, 1999; Mallekh and Lagardère, 2002; Lefrancois and Claireaux, 2003) as well as inter-family performance (Pakkasmaa *et al.*, 2006; Gore and Burggren, 2012). The only major caveats to the use of AMS are the effect of body size on metabolic measures (Boyce and Clarke, 1997; Wuenschel *et al.*, 2004) and acclimation state. This may cause data from one size class, or fish that have been exposed to temperature regimes for differing periods of time, to be unrepresentative of growth performance in another (Johnston and Dunn, 1987). The time to produce the current AMS data may also seem excessive (100 days) but this is primarily a result of the available equipment (i.e. two, single channel oxygen meters). More modern and effective multi-channel oxygen sensors are becoming cheaper and more accessible (Pyroscience Firesting®, for example) meaning that larger scale experiments can be set up in commercial environments and the cost per unit datum decreased significantly. In this way, the rate of data collection is effectively limited by the level of investment.

Like AMS, T_{pref} is an accurate gauge of the optimum temperature for growth in juvenile hapuku and this result is consistent with the findings of other studies examining the link between T_{pref} and growth (Beitinger and Magnuson, 1979; Jobling, 1981). T_{pref} is independent of acclimation state in many species (Reynolds and Casterlin, 1979) meaning that it can potentially be used for determining the optimum temperature for growth in novel species, or in species under variable thermal regimes. In the case of hapuku, it is interesting that the UAT and LAT values border the 18 °C to 21 °C optimal range for growth performance and AMS (Table 2.2 and Fig 2.2A and B). In this way, the thermoregulatory behaviour of juvenile hapuku has not only revealed the optimum temperature for growth performance, but it has also indicated the upper and lower limits for maximal growth performance. Variability between individuals has been reported in the literature (Beitinger, 1974; Jobling, 1981; Reyes et al., 2011) and this appears to be a result of individual fish thermoregulating more or less precisely than others, or variation in thermal tolerances and final temperature preference between individuals or families (Ward *et al.*, 2010). If the variation between individuals is high then more replicates will be required to be confident in the observed T_{pref} range and, depending on the species and temperature ranges being investigated, this could add a lot of time to the investigation. Investment into simplification of the experimental design itself, so that multiple low-cost systems could be run simultaneously, would probably be the best solution to individual variation. Additionally, tracking software could be modified to follow several fish in larger systems with more animals, in this way data could be gathered from multiple fish simultaneously or even entire tanks at a time, reducing the cost and time per fish used.

2.5 Conclusions

Both AMS and T_{pref} are effective tools in determining the optimum temperature for growth in novel finfish aquaculture species. However, neither measure provides any indication as to the optimum temperature for FCR. FCR, instead, appears to be related to SMR, and thus, the fish's maintenance costs at a particular temperature. Full commercial application of these tools would require investigation into cost-reduction (application of the newest multi-channel oxygen sensors, for example) and output maximisation (running as many fish in each trial as possible) but they may ultimately reduce the need for extended growth trials. Experiments, such as those performed

herein, are the first step in validating the use of these techniques in an intensive culture environment, but further investigations would be needed to answer the following questions: i) How effectively can these techniques be applied to other farmed fish species? and ii) how robust are these measures to changes in body size and associated thermal preferences?

Chapter 3 – The Effects of Temperature and Ration Size on Growth and Specific Dynamic Action

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3.1 Introduction

Specific dynamic action (SDA) is the sum of all of the mechanical and physiological processes that follow a meal and is usually measured in terms of energy expenditure through the rate of oxygen consumption (MO_2) (Tandler and Beamish, 1979; Jobling, 1983; Carter and Brafield, 1992; Jobling, 1994; Fu *et al.*, 2005b). Jobling (1983) proposed that the three primary components of SDA in fish are: i) intestinal work (cost of gut motility), ii) amino acid oxidation/urea synthesis (digestion and assimilation) and iii) post-absorptive protein synthesis and deposition. There is a contribution of i) and ii) above to the elevation of metabolic rate after a meal but it is generally accepted that up to 80% of the magnitude of the SDA response is associated with post-absorptive costs, such as protein turnover and growth (Coulson and Hernandez, 1979; Brown and Cameron, 1991; Whiteley *et al.*, 2001; Seth *et al.*, 2010; Li *et al.*, 2013b). As a result of this, SDA could represent the metabolic cost of growth in ectothermic organisms and larger SDA responses could be associated with faster growth (Coulson *et al.*, 1978; Grigoriou and Richardson, 2008; Seth *et al.*, 2010). This has been demonstrated in species such as fast-growing transgenic Coho salmon *Oncorhynchus kisutch* which show higher postprandial MO_2 values than their slower-growing wild equivalents (Leggatt *et al.*, 2003), the grass carp *Ctenopharyngodon*

idella (Carter and Brafield, 1992) and in the rock carp *Procypris rabaudi* where decreased growth rates were associated with lower postprandial MO_2 values (Li *et al.*, 2013a). However, SDA has also shown to be unrelated to changes in growth performance in species such as exercise-trained qingbo *Spinibarbus sinensis* where growth performance was increased but the energy expended on SDA was unchanged (Li *et al.*, 2013b). There does not appear to be a consistent relationship between SDA and growth performance and the results may depend on the species and the SDA-altering factors that are being investigated [see Secor (2009) for a review]. This study therefore aims to determine the relationship between SDA and growth in a novel species, the hapuku *Polyprion oxygeneios,* under the basic hypothesis that the magnitude of the SDA response is positively related to growth performance.

Factors that affect the different components of SDA will ultimately affect the rate at which energy and nutrients from food are converted to tissues (Jobling, 1994). Temperature, for example, can restrict the capacity to produce ATP aerobically through the limitation of physiological capacity (Pörtner, 2001; Clark *et al.*, 2013), as well as appetite (Grove *et al.*, 1978) and the maximal rates of physiological processes such as protein synthesis through both enzyme dynamics and the limitation of oxygen carrying capacity (Smith and Houlihan, 1995; McCarthy and Houlihan, 1997; McCarthy *et al.*, 1999). Similarly, ration size and the periodicity of feeding also affect the magnitude and duration of SDA (Boyce and Clarke, 1997; Guinea and Fernandez, 1997) and these SDA components are thought to have a functional association with growth (Elliot, 1976; Grigoriou and Richardson, 2008; Millidine *et al.*, 2009). Ultimately, different combinations of feeding and environmental regimes appear to affect the size and duration of SDA (Jobling, 1994), as well as what proportion of energy in the meal is designated to the mechanical and physiological components of SDA (Jordan and Steffensen, 2007; Seth *et al.*, 2010).

The potential role of SDA in the production performance of fish is especially important in an aquaculture setting as fast growth and efficient feed conversion ratios (FCR, the rate at which feed is converted to fish mass) are vital to the long term viability of any farming venture. If SDA is understood and found to be linked with growth and FCR under a variety of farming conditions, there is potential to optimise SDA for a deliberate and physiologically-informed maximisation of production. Assuming that rearing temperature and meal size can be controlled in most intensive

culture scenarios, it is first logical to test how these parameters affect SDA and whether there exists a functional relationship between SDA measures (e.g. peak and duration) and growth and FCR. This research becomes even more crucial when attempting to adapt feeding models from established aquaculture species such as salmonids, to entirely novel ones such as the hapuku (a novel aquaculture species in New Zealand), which may have entirely different requirements for feed.

This study therefore aims to understand: i) how temperature and ration size affect the SDA response of juvenile hapuku and ii) whether the magnitude of the SDA response is positively related to growth performance. More specifically, the current set of experiments will resolve how SDA responds to single feed rations of differing size at 15 °C (0.75% and 1.5% body weight) and 21 °C (1.5% and 3% body weight) which will be compared to the growth and FCR performance of fish raised for 6 weeks at the same temperature and ration treatments as above.

3.2 Materials and methods

3.2.1 Specimens and tagging

360 juvenile hapuku (*P. oxygeneios*, approximately seven months post hatch, 112.2 g ± 1.8 g) were obtained from the NIWA Bream Bay Aquaculture Facility in Ruakaka, Northland, New Zealand. These fish were previously tagged intraperitoneally with passive integrated transponders (PIT) under anaesthesia (0.01 mL L^{-1} Aqui-S® followed by 0.3 mL L^{-1} 2-phenoxy-ethanol). Specimens were treated with Chloramine-T (0.005 mL L^{-1}) to prevent infection post-tagging. Any individuals that showed signs of infection were treated further with formalin (0.15 mL L^{-1}) or euthanized.

3.2.2 Growth trials

All fish were divided evenly into eighteen 900 L bottom draining tanks (nine per temperature, three per feeding treatment) in a temperature controlled room at the NIWA Bream Bay Aquaculture Facility in Ruakaka, Northland, New Zealand. There were 20 animals per tank and densities never exceeded 10 kg m^{-3}. Each tank was

connected to one of two 3 m^3 header tanks via a filtered (1 µm) and UV sterilised (ALX2/8, 150,000 µW sec cm^{-2}, Davey Water Products, Australia) seawater supply. All tanks were maintained initially at 18 °C with a commercial heat pump attached to each of the header tanks. The temperature in the tanks was then manipulated by heating one header tank and cooling the other at a rate of 1 °C d^{-1}, ultimately producing nine tanks each at 15 °C and 21 °C. Each tank and the headers were supplied with a low flow of oxygen through a diffuser plate and maintained at 100 to 105% O_2 saturation.

Once 15 °C and 21 °C were achieved, initial morphometrics (fork length and weight) were taken for every individual in every tank under anaesthesia (0.01 mL L^{-1} Aqui-S® followed by 0.3 mL L^{-1} 2-phenoxy-ethanol). After a day of recovery, the nine tanks at each temperature were divided into three groups of three and assigned a specific feeding regime. The nine 15 °C tanks were divided into three groups receiving feed at a rate of either: i) 0.75% of total tank biomass once d^{-1} (a.m.), ii) 0.75% twice d^{-1} (a.m. and p.m.) and iii) 1.5% once d^{-1} (a.m.). The nine 21 °C tanks were also divided into three groups receiving feed at a rate of either: i) 1.5% once d^{-1} (a.m.), ii) 1.5% twice d^{-1} (a.m. and p.m.) and iii) 3% once d^{-1} (a.m.). Feeding regimes were based on the temperature-dependent appetites of juvenile hapuku from Khan *et al.* (2014a) which equated to a maximum daily feed intake of approximately 1.5% and 3% (dry weight) of tank biomass at 15 ºC and 21 ºC respectively.

These regimes were maintained for 12 days on Skretting Nova FF 5 mm pellets (Skretting, Australia, 50.0% protein, 17.0% lipid, digestible energy 18.6 MJ kg^{-1}). Any uneaten feed was recovered 15 minutes after feeding behaviours had ceased. The weight of recovered feed was corrected for water absorption using a standard saturation factor (determined by soaking a known weight of feed pellets and then re-weighing, equating to 1.6 x dry weight). After the 12 days, all specimens were food deprived for 48 hours and morphometrics were measured again under anaesthesia (0.01 mL L^{-1} Aqui-S® followed by 0.3 mL L^{-1} 2-phenoxy-ethanol). This was followed by another 12 days on the same feeding regimes but with the feed amount adjusted for the increased tank biomass. This cycle was repeated once more to give a total of three 12-day feeding periods interspersed with weight assessments and adjustments to the feed amount. All tanks were treated with Chloramine-T (0.005 mL L^{-1}) once per day for three days following any handling event and approximately 3 hours before feeding.

There was no difference in feeding behaviour between days with Chloramine-T treatments and those without. The morphometrics of each individual fish was recorded at the end of the trial under anaesthesia (0.01 mL L^{-1} Aqui-S® followed by 0.3 mL L^{-1} 2-phenoxy-ethanol).

Mass specific growth rate (SGR, % body weight day^{-1}) was calculated for each individual using the formula:

$$SGR = \ln m_2 - \ln m_1 / t_2 - t_1 \times 100$$

where, m_1 is the initial weight at the start of the growth period t_1 and m_2 is the final weight at the end of the growth period t_2.

Feed conversion ratio (FCR), measured as the weight of dry feed intake (corrected for uneaten feed) per unit weight gain for the period, and was calculated for each tank using the following formula:

FCR = weight of dry feed consumed in tank / wet weight gained in tank

3.2.3 Respirometry

All specimens were food deprived for 48 h prior to experimentation to remove any confounding effects on metabolic rate (based on observation) and were maintained at either 15 ºC or 21 ºC during the entire respirometry period. Metabolic rate was measured in 24 individuals (n = 12 at each of 15 °C and 21 °C) as the mass-specific rate of oxygen consumption (MO_2) in mgO_2 kg^{-1} h^{-1}. Measurements were carried out using two automated intermittent flow respirometers similar to the methodologies outlined by Steffensen (1989), Jordan and Steffensen (2007) and Khan *et al.* (2014a). Two custom-built 7.41 L acrylic box-type respirometers (with an Eheim® 600 submersible "flush" pump at one end and an outlet tube extending above the water level through the top) were placed in a fully aerated 130 L reservoir filled with 1 µm filtered, UV sterilised (ALX2/8, 150,000 µW sec cm^{-2}, Davey Water Products, Australia) seawater. Built into each end of the chamber lids were two hinged ports that could be opened to insert feed into the chamber. The feeding ports were completely sealed when closed. Water circulation within the chambers was provided by an external loop of tubing containing a modified Sicce® Mimouse submersible pump and a cuvette for the oxygen sensing probe. Temperature was maintained at the correct

temperature (either 15 °C or 21 °C ± 0.1 °C) using a custom bar heater and Hailea® HC-1000A chiller unit working in unison.

O_2 saturation in the respirometer was measured constantly by a Firesting® 2-channel oxygen meter (Pyroscience, Germany) connected to a Firesting® dipping probe in the external loop of the chamber. O_2 saturation was measured constantly across three cycled phases (flushing, wait and measure) that were repeated every 12 min. The oxygen dipping probes were calibrated at the beginning of every experiment to account for changes in atmospheric pressure and treatment temperature.

Fish were weighed to determine the appropriate ration size prior to respirometry. A short exposure to Aqui-S® (0.01 mL L^{-1}) was used and they were allowed to recover for approximately 2 h before being placed in the respirometry chamber. MO_2 values obtained from resting fish over the next 36 h to 48 h (spontaneous activity levels in this species decrease significantly after 24 h in a static respirometer) and were used to estimate $MO_{2standard}$ using the 15% quantile method of Chabot and Claireaux (2008b), Cook *et al.* (2011) and Khan *et al.* (2014a). After a maximum of 48 hours, the appropriate ration (either 0.75% or 1.5% body weight at 15 °C and 1.5% or 3% body weight at 21 °C) of Skretting Nova FF 5 mm or 7 mm pellets (Skretting, Australia, 50.0% protein, 17.0% lipid, digestible energy 18.6 MJ kg^{-1}) was fed slowly into the chambers through one of the ports in the lids. The fish were then left for another period of approximately 72 h. If the fish did not feed, or did not take their full ration ± 10% (minimum unit of feed delivery was 0.2 g), the data from that specific fish was not used. To accommodate feeding behaviour and allow for periodic visual inspections, the system was not shrouded and the fish were exposed to a normal light regime for the entire duration of the experiment (this requirement was determined during pilot studies as hapuku would not feed in the respirometry chambers in darkened conditions). Fish were not disturbed as foot traffic in the experimental area was limited to careful observations by the primary investigator during feeding. To prevent the build-up of waste products, all water in the system was replaced every 24 h without the need for direct disturbance or any change in temperature. After each experiment was complete, background oxygen consumption readings were taken to ensure that it was negligible. All equipment was cleaned thoroughly between specimens using a high pressure water blaster and a mild hypochlorite solution.

3.2.4 Data handling and analysis

SDA variables were calculated by subtracting the SMR estimate from all post-feeding MO_2 values for each fish and then converting them to 1 h averages (Jordan and Steffensen, 2007). Six SDA variables were extracted from the post-feeding MO_2 data for each fish as follows: (1) SDA_{peak}, the maximum 1 h averaged value of post-feeding MO_2; (2) t_{peak}, the time taken to reach SDA_{peak} (h) post-feeding; (3) Duration, the time between feeding and the return of MO_2 values to ± 5% of SMR (in h); (4) SDA energy, expressed as the total energy used (kJ) between feeding and when MO_2 values return to SMR; (5) SDA coefficient, expressed as the SDA energy (kJ) as a proportion of the digestible energy content of the ration fed to induce SDA. MO_2 values were converted to energy consumption using an oxycalorific coefficient of 14.06 kJ g O_2^{-1} (Gnaiger, 1983; Jordan and Steffensen, 2007). One-way ANOVAs (shown with their associated F values) were used to compare each SDA variable for each of the different ration sizes within temperatures, and between the 15 °C and 21 °C fish that were fed the standard 1.5% ration. Where normality conditions were not met, a non-parametric Kruskal-Wallis ANOVA on ranks was applied (shown with their associated H values). The differences in SGR and FCR between the two temperatures and three ration sizes at each temperature were examined using a one-way ANOVA, after ensuring that data were both equally variant and normally distributed. FCR data from each temperature was also pooled and compared using a student's t-test. Where an effect was detected, a Holm-Sidak method was applied for specific *post-hoc* comparisons. There were no detectable tank effects on either SGR or FCR. All analyses were carried out on SigmaPlot® 11.0 and significance was accepted at $P \leq 0.05$.

3.3 Results

3.3.1 Prefeeding SMR and SDA

SMR was not significantly different for different ration size treatments within each temperature but SMR was affected by temperature and was significantly higher at 21 °C than 15 °C (see Table 3.1).

SDA variables were determined from the post-feeding MO_2 values of 24 juvenile hapuku (combined mean weight 205.9 ± 12.7 g, See Fig. 3.1 for examples). Temperature effects were detected for all SDA variables whereas ration size effects were less certain for a number of variables (see Table 3.1). For example, SDA_{peak} was significantly higher for the higher ration size within each temperature (15 °C, F = 24.03, $P < 0.01$ and 21 °C, H = 4.08, $P < 0.05$) and was also higher at 21 °C than 15 °C for the standard 1.5% ration (F = 229.35, $P < 0.01$). t_{peak} was not significantly different between ration sizes at 15 °C (F = 0.79, $P > 0.05$) but was significantly higher for the larger ration at 21 °C (F = 7.96, $P < 0.05$) and between the 15 °C and 21 °C fish fed the standard 1.5% ration (H = 4.69, $P < 0.05$). Duration increased significantly with ration size at 15 °C (F = 13.05, $P < 0.05$) but not at 21 °C (F = 0.99, $P > 0.05$) and was significantly higher at 21 °C for the shared 1.5% ration (F = 76.82, $P < 0.01$).

SDA energy increased significantly with ration size at both temperatures (15 °C, F = 8.81, $P < 0.05$ and 21 °C, F = 5.44, $P < 0.05$) and it was also higher at 21 °C than 15 °C following the standard 1.5% ration (F = 51.86, $P < 0.01$). SDA coefficient was not significantly different between ration sizes at 15 °C (F = 0.21, $P > 0.05$) but was significantly higher for the larger 3% ration treatment at 21 °C (F = 15.31, $P < 0.01$). The SDA coefficient was also temperature sensitive, being significantly higher at 21 °C than 15 °C for the standard 1.5% ration (F = 235.01, $P < 0.01$).

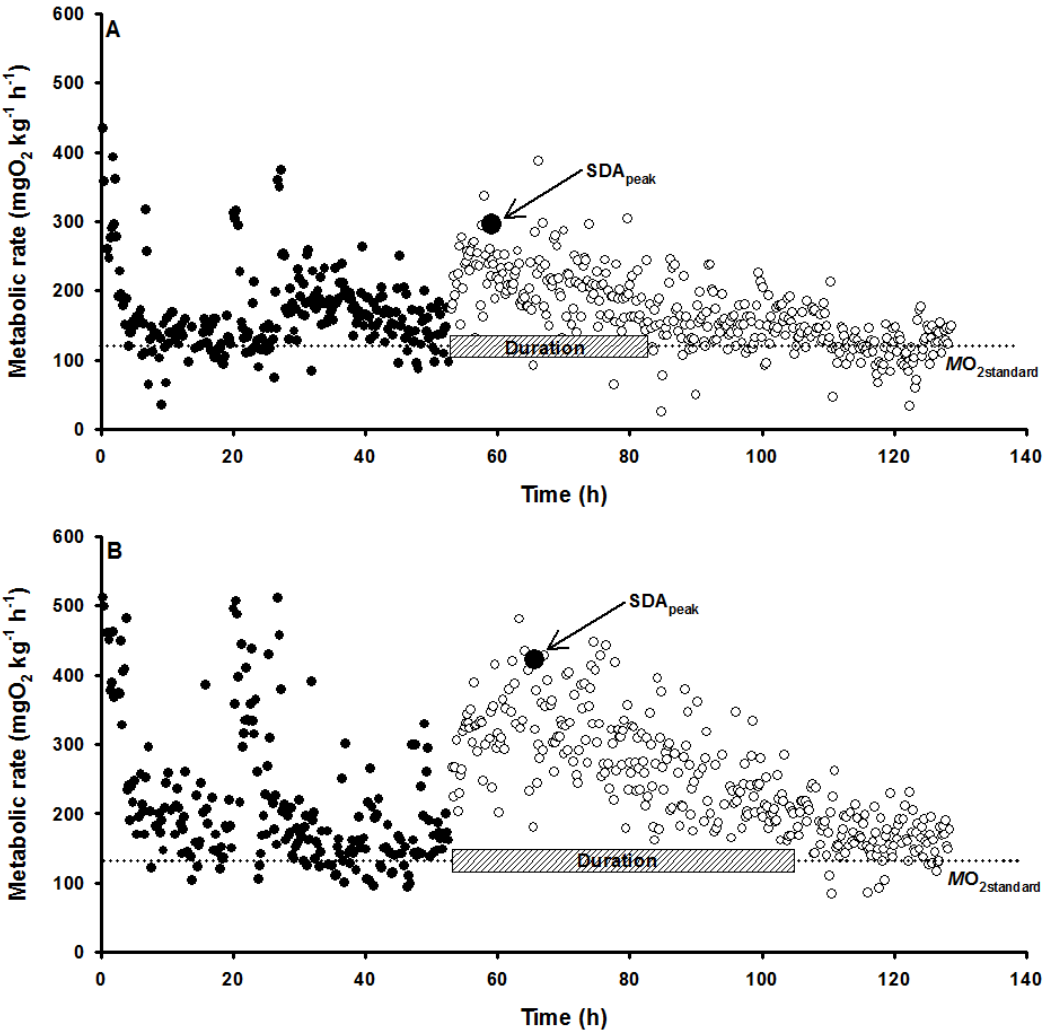

Figure 3.1. An example of the raw (not 1-h averaged) pre-feeding (filled circles) and post-feeding (open circles) MO_2 data for two individual juvenile hapuku at 21 °C fed (A) a 1.5% body weight and (B) a 3% body weight ration of dry pellets (fish were 185 g and 175 g respectively). Feeding occurred at the transition between the filled circles and the open circles. The dotted lines show the SMR estimate for each fish, shown here as $MO_{2standard}$. The duration and SDA_{peak} of the SDA response are shown by the shaded box and the large data point circle respectively.

Table 3.1. Standard metabolic rate (SMR) estimates and specific dynamic action (SDA) parameter estimates for juvenile hapuku at 15 °C, fed either a 0.75% or a 1.5% body weight ration and at 21 °C, fed either a 1.5% or a 3% body weight ration of dry pellets. Comparisons are shown wither their associated *P* values.

	15 °C		21 °C		ANOVA	
SDA Parameters	0.75% BM	1.5% BM	1.5% BM	3% BM	Within Temp *P* (15 °C)/(21 °C)	Between 15 °C and 21 °C 1.5% BM *P*
Prefeeding SMR ($mgO_2\ kg^{-1}\ h^{-1}$)	75.6 ± 2.3	69.9 ± 2.2	128.3 ± 6.9	131.8 ± 3.7	(0.11)/(0.67)	< 0.01
N	6	6	6	6		
SDA_{peak} ($mgO_2\ kg^{-1}\ h^{-1}$)	117.5 ± 3.9	138.8 ± 1.8	291.5 ± 9.9	409.4 ± 15.4	(< 0.01)/(< 0.01)	< 0.01
t_{peak} (h)	5.2 ± 1.4	3.8 ± 0.6	9.5 ± 1.8	17.7 ± 2.4	(0.39)/(< 0.05)	< 0.05
Duration (h)	15.5 ± 2.4	27.4 ± 2.2	58.2 ± 2.7	61.6 ± 2.1	(< 0.05)/(0.35)	< 0.01
SDA Energy (kJ)	3.4 ± 0.5	6.7 ± 1.0	19.5 ± 1.5	26.0 ± 2.4	(< 0.05)/(< 0.05)	< 0.01
SDA Coefficient (%)	10.5 ± 2.0	9.5 ± 1.0	32.8 ± 1.4	42.0 ± 1.9	(0.66)/(< 0.01)	< 0.01

3.3.2 SGR and FCR

SGR increased with ration size at both 15 °C and 21 °C ($F = 142.10$, $P < 0.01$ and $F = 303.70$, $P < 0.01$ respectively, Fig. 3.2A). A positive effect of temperature on SGR was not readily distinguishable from the data because the fish at 21 °C had a significantly lower SGR than the 15 °C fish when fed the standard 1.5% ration d^{-1}. SGR was significantly higher at 21 °C with the higher 3% rations d^{-1} than all other values. SGR decreased significantly at all temperatures and ration sizes during the course of the 6-week growth trial with increasing fish size. The SGRs of the 15 °C fish at 14 days were 0.78, 1.34 and 1.32 for the 0.75%, 0.75%/0.75% and 1.5% ration size treatments respectively while the SGRs of the 21 °C fish were 0.97, 2.09 and 1.87 for the 1.5%, 1.5%/1.5% and 3% ration treatments respectively. The statistical relationships between these values were the same as those at the end of the 6-week growth trial (as shown in Fig. 3.2A).

The SGR of fish at 15 °C was not significantly different when they were fed twice d^{-1} but, at 21 °C, the SGR of the 1.5% twice d^{-1} feed treatment was significantly higher than the 3% once d^{-1} treatment ($t = 5.29$, $P < 0.01$).In contrast to SGR, FCR was not affected by ration size nor the number of rations d^{-1} at either 15 °C or 21 °C ($F = 0.27$, $P > 0.05$ and $F = 3.4$, $P > 0.05$ respectively, Fig. 3.2B) but FCR was significantly higher (i.e. less efficient) for all ration treatments at 21 °C than at 15 °C when all of the FCR data from each temperature was pooled ($t = -7.67$, $P < 0.01$).

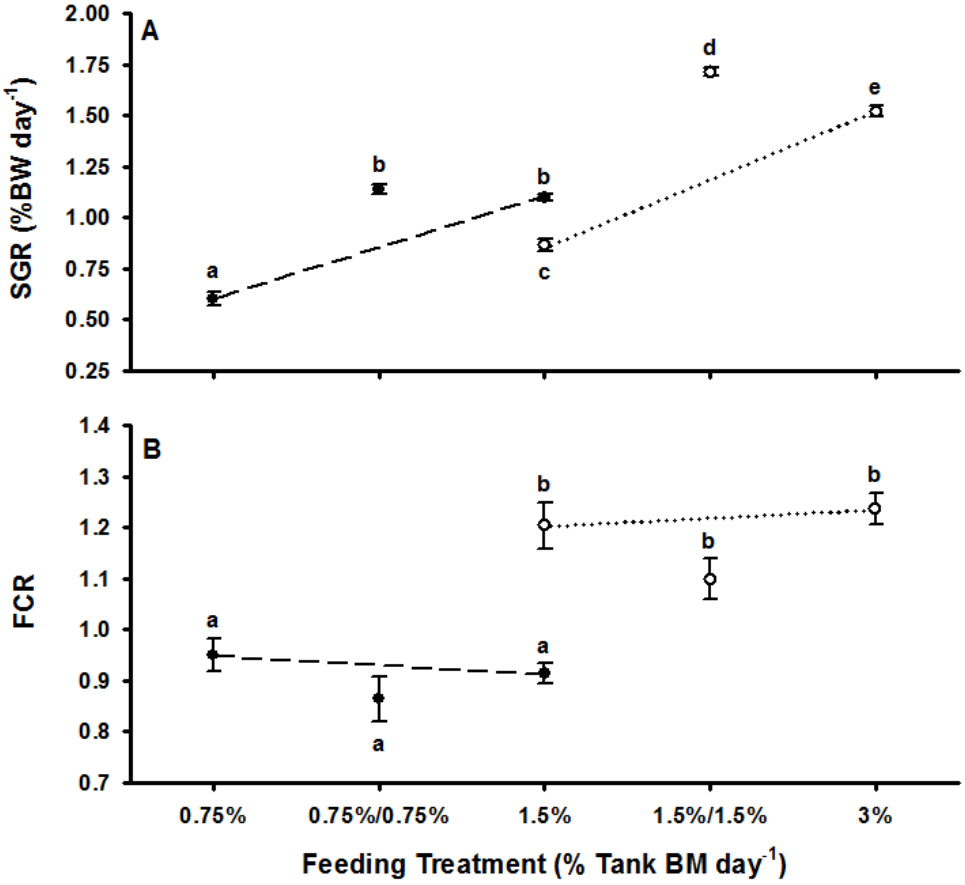

Figure 3.2. (A) The specific growth rate (SGR, % body weight gained per day) and (B) the feed conversion ratio (FCR, weight of feed required per unit weight gain) of fish at 15 °C (filled circles, black lines) and 21 °C (open circles, dotted lines). Fish at 15 °C were fed either a single 0.75%, two 0.75% or a single 1.5% of total tank biomass in dry pellet rations per day (% tank BM d^{-1}). Fish at 21 °C were fed either a single 1.5%, two 1.5% or a single 3% ration of total tank BM d^{-1}. Broken lines connect the SGR and FCR response of feeding treatments that match the rations fed to fish during the respirometric determination of SDA (i.e. the SGR and FCR of the two half ration d^{-1} treatment represents a subsidiary dataset). All data is shown ± standard error and letters denote significant differences between treatments.

The SGR of fish at 15 °C, was not significantly affected by twice daily feeding (i.e. the SGR of fish delivered 0.75% twice d^{-1} and 1.5% once d^{-1} was the same) but, at 21 °C, the SGR of the 1.5% twice d^{-1} feed treatment was significantly higher than the 3% once d^{-1} treatment ($t = 5.29$, $P < 0.01$). To summarise this simply, the SGR of fish fed the same daily ration twice (vs. once) grew significantly faster at 21 °C, but not at 15 °C (Fig. 3.2A). Unfortunately, SDA data was not available for these twice daily

feed treatments due to the reluctance of fish to feed twice within the constraints of this experiment (despite multiple attempts during pilot studies with various ration sizes).

In contrast to SGR, FCR was not affected by ration size or the number of rations d^{-1} at either 15 °C or 21 °C ($F = 0.27$, $P > 0.05$ and $F = 3.4$, $P > 0.05$ respectively, Fig. 3.2B) but FCR was significantly higher (i.e. less efficient) for all ration treatments at 21 °C than at 15 °C when all of the FCR data from each temperature was pooled ($t = -7.67$, $P < 0.01$).

3.3.3 The relationship between SDA and SGR

There was not a consistent positive relationship between SDA_{peak}, duration, SDA energy or SDA coefficient on SGR (Fig. 3.3). SDA_{peak}, duration and SDA energy increased significantly with a significant increase in SGR (Fig. 3.3A, C and E and Table 1) at both 15 °C and 21 °C. However, SDA coefficient did not change significantly despite an increase in SGR (Fig. 3G and Table 1) at both 15 °C and 21 °C. After delivering the shared ration size of 1.5% body weight, SGR was significantly lower at 21 °C despite a significant increase in each of SDA_{peak}, duration, SDA energy and SDA coefficient (Fig. 3A, C, E and G) between 15 °C and 21 °C.

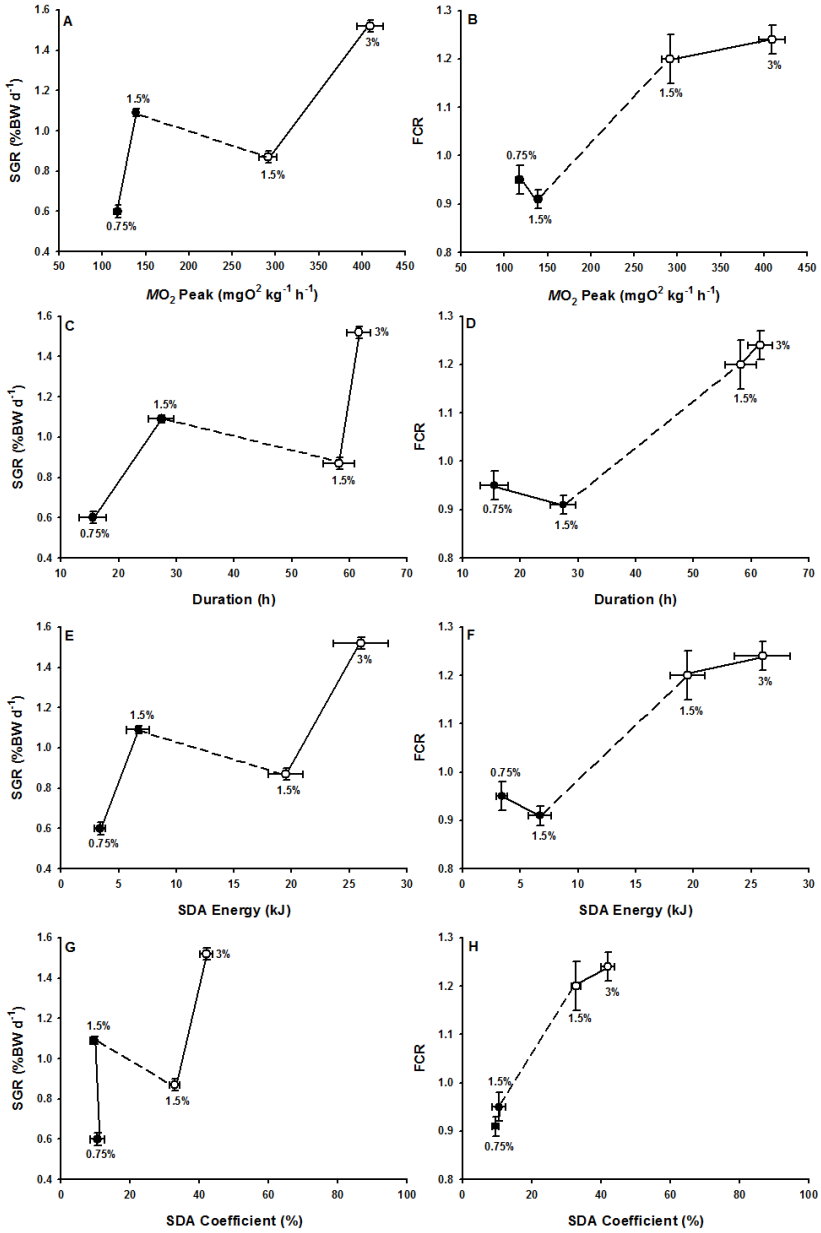

Figure 3.3. The relationship between SGR, FCR and i) MO_2 peak (mgO_2 kg^{-1} h^{-1}) (A and B respectively), ii) SDA Duration (h) (C and D respectively), iii) SDA energy (kJ) (E and F respectively) and iv) SDA coefficient (%) (G and H respectively). The 15 ºC data is shown as the filled circles and the 21 ºC is shown as the open circles. Ration sizes are shown above their corresponding data point. The solid lines show the connection between ration sizes within temperatures and the broken lines show the relationship between temperatures for the standard 1.5% ration. In the absence of statistical analysis, all 'lines of best fit' are supplied as a visual aid to gauge whether the temperature and/or ration size effects for each SDA variable might be positively or negatively related to SGR and/or FCR. See the discussion for more detail. All data is shown ± standard error.

3.4 Discussion

All measured SDA parameters were significantly higher at 21 ºC than at 15 ºC for the standard 1.5% ration and this is consistent with the temperature sensitivity of SDA shown by other researchers for a number of other fish species. For example, there is evidence of a temperature-related increase in the peak and duration of SDA in some species (Jobling and Davies, 1980; Tandler and Beamish, 1981; Guinea and Fernandez, 1997) and decreases in duration with increasing temperature in certain freshwater species (Luo and Xie, 2008; Pang *et al.*, 2010) suggesting that the response may be species/ecotype specific. Increasing ration size had a less consistent effect over SDA than temperature, with only SDA_{peak} and SDA energy increasing significantly with higher ration at both 15 °C and 21 °C. Whilst the ration size effect was not universal, our results do concur with the general conclusions of Secor (2009) and the specific findings of Jordan and Steffensen (2007) who observed a significant increase in the SDA_{peak} and duration of the SDA response in Atlantic cod *Gadus morhua* following a doubling of ration size (2.5% to 5% body weight) at 10 °C. The review of Secor (2009) also concludes that the SDA coefficient generally increases with increasing meal energy and this is not only consistent with our own data at 21 °C, but also with species at temperatures as low as 1 °C (Boyce and Clarke, 1997).

A positive relationship between the magnitude of the SDA response and growth has been established for fish species such as the grass carp *Ctenopharyngodon idella* (Carter and Brafield, 1992), transgenic Coho salmon (Leggatt *et al.*, 2003) as well as other non-fish groups such as isopods (Carefoot, 1990). This relationship is not universal, however, as several fish species have shown changes in either SDA magnitude or growth performance without a corresponding change in the other (Li *et al.*, 2013b) or have shown that these processes are competitive within the available aerobic capacity (Cui and Liu, 1990). The data from juvenile hapuku do not suggest that SDA and growth are competitive but that the relationship between SDA and growth is not clear-cut, probably because it appears heavily influenced by the interaction of temperature and ration size together. For example, the increased SDA_{peak} and duration of fish fed the standard 1.5% ration fish at 21 ºC were not associated with improved rates of growth as fish fed the standard 1.5% ration at 15 ºC had a significantly higher SGR than their 21 ºC counterparts. This particular phenomenon,

however, is also likely due to a 1.5% body weight ration being restrictive at 21 °C. The significantly increased SMR at 21 °C requires a larger energetic investment to maintain basic physiological function and thus, for the same amount of feed ingested, a lower proportion of the energy in that feed is available for growth (Cuenco *et al.*, 1985; Guinea and Fernandez, 1997; Dietz *et al.*, 2012). As another example of the apparent interaction of temperature and ration size on SDA, larger rations improved SGR at both temperatures but were only associated with an increase in SDA coefficient or t_{peak} at 21 °C, with no significant change in these parameters with increasing ration size at 15 °C. Generally, the relationship between temperature, ration size, SDA parameters and SGR is a matrix of positive and negative interactions, as seen in Figs. 3.3A, C, E and G.

Like SGR, FCR does not improve consistently with temperature and ration size and there are both positive and negative relations to increasing SDA parameters (Fig. 3.3). Indeed, where changes in ration size had a significant effect on SGR, ration size had no significant effect on FCR at either temperature. The lack of a significant effect of ration size on FCR at either temperature, and all ration sizes having significantly better feed conversion at 15 ºC than 21 ºC, suggests that temperature, and not SDA, is the primary driving force behind FCR. This was first suggested for this species by Khan *et al.* (2014a) and appears to be the case even when this species is fed the same ration at different temperatures. Data from the current experiment therefore provides further evidence for the hypothesis of Khan *et al.* (2014a) that FCR is related to SMR and is thus a function of maintenance costs in juvenile hapuku.

The effect of multiple meals in a single day on the SDA response was not measured in the current study but it does warrant further investigation on the basis that growth performance was significantly higher for fish at 21 ºC fed a 1.5% ration twice (*vs.* once) d^{-1}. If enlarged SDA parameters are indeed associated with higher rates of protein synthesis and growth as previous workers have suggested (Hidalgo and Alliot, 1988; Carter and Brafield, 1992; McCarthy *et al.*, 1999), future research may possibly discover that the total SDA energy and SDA coefficient of hapuku at 21 ºC are significantly higher for fish fed multiple smaller rations d^{-1}. The basis of this stems from the rationale that any change in SDA energy is unlikely to come from a higher individual peak in MO_2 but rather an increase in the number of peaks (one for each meal during the day) and an increase in SDA duration as feeding metabolism would

effectively restart with every meal (Fu *et al.*, 2005a). However, considering that there has not been a consistent relationship between SDA magnitude and growth in this species, it is difficult to speculate the exact physiological consequences of multiple meals. On the basis of the current set of results, future investigations may therefore strive to: i) measure the SDA response of juvenile hapuku fed multiple meals d^{-1} to resolve whether shifts in feeding metabolism do indeed have a functional link to growth performance and ii) separate the costs of SDA into mechanical (handling) and physiological (protein synthesis, digestion and assimilation) components as per the studies of Tandler and Beamish (1979), Tandler and Beamish (1980), Carefoot (1990) and Brown and Cameron (1991) in order to determine what aspects of SDA are being modified by different dietary and environmental regimes in aquaculture.

3.5 Conclusions

The magnitude of the SDA response has been positively linked to growth performance in some fish species and found to be unrelated in others, suggesting that the link may be species- or situation-specific. The current study therefore investigated the simple hypothesis that the magnitude of the SDA response is positively related to growth performance in a novel aquaculture species. The current data, however, does not show a consistent relationship between the magnitude of the SDA response and growth performance, meaning that the hypothesis has to be rejected. Increasing ration size at either 15 °C or 21 °C increased SGR in juvenile hapuku and SGR did increase with some SDA parameters under some scenarios but the SDA-growth relationship was not a consistent pairing across a range of temperature and ration size. In fact, temperature and ration size appear to be highly interactive in their effect on SDA and growth, resulting in changes in growth performance that rely on the physiological costs of maintenance and the changing cost of feeding, digestion and assimilation. More work is therefore required to further understand the complexity of the SDA response if temperature-specific diets and/or feeding regimes are to be developed as a way of encouraging the best possible rates of growth and feed conversion under a wide variety of different conditions.

Chapter 4 – The Effects of Sustained Exercise on Growth and the Cost of Swimming

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4.1 Introduction

There is ample evidence in the literature showing that induced swimming, or exercise training, can improve the growth and feed conversion efficiency of many species of farmed fish (Davison, 1989; Palstra and Planas, 2011; Davison and Herbert, 2013). Most of this evidence has been accumulated in the salmonid groups *Oncorhynchus* (Houlihan and Laurent, 1987; Alsop and Wood, 1997; Hernández *et al.*, 2002), *Salmo* (Davison and Goldspink, 1977; Totland *et al.*, 1987; Boesgaard *et al.*, 1993) and *Salvelinus* (Leon, 1986; Christiansen *et al.*, 1989; Christiansen and Jobling, 1990) but there are examples of exercise-induced growth from other groups, with species such as the striped bass *Morone saxatilis* (Young and Cech Jr, 1993) and the yellowtail kingfish *Seriola lalandi* (Brown *et al.*, 2011). The global aquaculture industry is expanding rapidly and the potential for continuous exercise to accelerate the growth of fish has direct application due to the potential for fast biomass gain, improved flesh quality and the flexibility of production it can provide. However, exercise-induced growth is often perceived as a paradoxical concept as it seems illogical that fish can expend considerable energy on exercise whilst also committing

to the extra expense of accelerated growth. This view is reinforced by a number of studies showing that exercise has either nil, or only negative effects on the growth of fish such as the Atlantic cod *Gadus morhua* (Bjørnevik *et al.*, 2003) and Chinook salmon *Oncorhynchus tshawytscha* (Kiessling et al., 1994). Therefore, to stand any chance of exploiting the economic gains of exercise-induced growth in aquaculture, an in-depth understanding of how fish balance the metabolic costs of growth and exercise needs to be ascertained, particularly in the case of information-poor species that are new to farming.

There has been a rekindled interest in the mechanisms and applicability of exercise-induced growth in recent years (Palstra and Planas, 2011) and new efforts have been made to predict the levels of exercise required for the best rate of growth in novel species using readily accessible measures of behaviour and physiology (Davison and Herbert, 2013; Herbert, 2013). In particular, the aerobic metabolic scope (AMS) of fish and the speed where the energetic cost of transport (COT) is at its lowest, termed the optimal swimming speed (U_{opt}), appears to explain a significant proportion of the variation between different fish that show exercise-induced growth (Davison and Herbert, 2013). Aerobic metabolic scope (AMS) is the difference between maintenance and maximal metabolic rates, and thus represents a physiological framework, within which non-maintenance physiological work operates (Jobling, 1994; Clark *et al.*, 2013). In light of this belief, it has been suggested that a larger AMS better accommodates the energetic costs of swimming in addition to other processes, such as protein synthesis associated with growth and feeding (von Herbing and White, 2002; Davison and Herbert, 2013). There are few experimental studies on this topic but the work of Owen (2001) on the European eel *Anguilla anguilla* appears to support this assertion. Indeed, where AMS was insufficient, or the costs of feeding (specific dynamic action, SDA) were excessive, the swimming speed of eels were reduced to accommodate SDA as a form of energetic prioritisation (Owen, 2001). On the basis of these observations, Davison and Herbert (2013) examined how the required exercise regime for optimal growth acceleration (termed $ER_{opt\ growth}$, in units of body lengths s^{-1}, BL s^{-1}) co-varied with AMS in a variety of well-studied species. As a positive but non-linear correlation was found between $ER_{opt\ growth}$ and AMS, Herbert (2013) proposed that AMS might have value in predicting the $ER_{opt\ growth}$ of novel fish species in which the effects of exercise have yet to be investigated. Of

particular relevance to those species that show both a positive growth response to exercise and a direct relationship between U_{opt} and ER $_{opt\ growth}$ is the suggestion that that U_{opt} speeds are preferentially selected by some migratory fish across extended periods (Hinch and Rand, 2000; Tudorache *et al.*, 2011). This would imply that, when swimming is required, a minimisation of swimming costs per unit distance may allow for a greater proportion of available AMS to be allocated to somatic growth. Therefore, to summarise this collective background, fish with a sufficiently high AMS are expected to have the capacity to swim and grow fast at the same time (Davison and Herbert, 2013; Herbert, 2013) and, in this scenario, U_{opt} is also believed to predict the best swimming speed for growth (Davison and Herbert, 2013).

In an attempt to test and validate the proposed models of Davison and Herbert (2013) and Herbert (2013), the exercise-induced growth performance of juvenile hapuku, *Polyprion oxygeneios*, a novel farmed finfish species from New Zealand, was quantified and compared against experimentally derived measures of AMS and U_{opt}. Specifically, if the AMS – ER $_{opt\ growth}$ model of Davison and Herbert (2013) is applicable to a wider range of species, then the predicted AMS value of hapuku at 17 °C [300 mg O_2 kg^{-1} h^{-1} at 17 °C, based on the data of Khan *et al.* (2014a)] is hypothesised to provide the metabolic capacity for optimal exercise-induced growth in the vicinity of ~0.4 – 0.5 BL s^{-1} (Herbert, 2013). If exercise-induced growth is indeed observed in hapuku, U_{opt} and ER $_{opt\ growth}$ should also be relatively well matched (Davison and Herbert, 2013). As a further step in this validation and testing process, the metabolic costs of swimming at different speeds and the recently measured cost of specific dynamic action (SDA) (Khan *et al.*, 2015), which is largely comprised of post-absorptive protein synthesis and growth (Secor, 2009; Seth *et al.*, 2010) was also reconciled against the available AMS. This allowed for the experimental resolution of whether hapuku have metabolic capacity to accommodate swimming and the physiological costs associated with growth.

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4.2 Materials and methods

4.2.1 Specimens, tagging and growth trials

Two full- and half-sibling groups of ~120 juvenile hapuku (*P. oxygeneios*, approximately eight months post hatch) were used for growth trials at the NIWA Bream Bay Aquaculture Facility in Ruakaka, Northland, New Zealand. "Trial 1" fish (128.8 g ± 3.1 g) were hatched 12 weeks prior to "trial 2" fish (172.4 ± 4.5 g) and were also smaller at the start of the growth trials as they were 4 weeks younger at the point when they entered the experimental tanks. Both groups of fish were held at 17 °C in larger 4 m^3 tanks prior to the start of both trials. To track the growth and performance attributes of individuals, all fish were tagged intraperitoneally with a 5 mm passive integrated transponder (PIT) under anaesthesia (0.01 mL L^{-1} Aqui-S® followed by 0.3 mL L^{-1} 2-phenoxy-ethanol, standard facility practice). Specimens were treated with chloramine-T (0.005 mL L^{-1}) to prevent infection post-tagging (added to flowing tank water, standard facility practice). Any individuals that showed signs of infection were treated further with formalin (0.15 mL L^{-1}) or euthanized with an excessive dose of Aqui-S® (0.1 mL L^{-1}). Thereafter, two sequential and identical growth trials were conducted incorporating six different exercise regimes (ER, corresponding to six in-tank flow speeds of 0.0, 0.25, 0.5, 0.75, 1 and 1.5 body lengths per second, BL s^{-1}). Each of the two trials were conducted in six identical 1.6 m^3 circular tanks (560 mm water depth, 1900 mm diameter). All tanks were housed in a purpose-built building under ambient light conditions (11L: 13D) and supplied with fresh 1 µm filtered and UV-sterilised (ALX2/8, 150 mW sec cm^{-2}, Davey Water Products, Australia) seawater at 17 ± 0.3 ºC. A continuous non-directional inflow of water (30 L min^{-1}) was present at the side of each tank and all tanks were central draining. Water flow around the tank was negligible in the 0.0 BL s^{-1} (control) tank but the remaining water flow ER treatments (i.e. 0.25, 0.5, 0.75, 1 and 1.5 BL s^{-1}) were maintained through the use of external water pumps (Leader® Ecopool 15, Leader Pumps, Italy) plumbed over the side of each tank via a 25 mm PVC intake and outlet. Pump outlets were connected to a spray bar at a water depth level of 100 mm from the surface and the spray bar extended 500 mm into the tank at a perpendicular angle. Water flow through the spray bars (and thus flow speed in the tanks) was controlled through a ball valve plumbed between the pumps and the spray bars. Flow speeds were set in the tanks by measuring

water velocity (in m s^{-1}) 200 mm from the tank wall at 100 mm depth on the side directly opposite the spray bar with a Höntzsch® HFA anemometer (V 1.5, Höntzsch technologies, Waiblingen, Germany) and making the necessary correction to the flow of water according to the average body length of the fish at regular fortnightly intervals. Each tank had a single projection of PVC pipe (200 mm high, 100 mm diameter) off the floor, approximately half way between the wall and the centre. They were entirely submerged, impossible to remove and created a small low-flow area in their wake. Water chemistry was checked regularly and remained at normal levels at all times throughout both trials.

The fish intended for trial 1 were anaesthetised (as described above) in their pre-trial holding tank at 17 ± 0.3 ºC and their initial weight and length were measured. They were then divided randomly and evenly (approximately 20 per tank) into one of the six experimental tanks and allowed to recover for 4 h with no directional flow. Once swimming behaviour appeared normal, flow speeds in the tanks were increased slowly towards one of the six exercise training speeds in BL s^{-1} according to the average BL of all fish in each tank. All tanks were fed to satiation twice a day (at approximately 0800 and 1600 h) for the following 12 days on Skretting Nova FF 5/7 mm pellets (Skretting, Australia, 50.0% protein, 17.0% lipid, digestible energy 18.6 MJ kg^{-1}). Any uneaten feed was recovered 15 mins after feeding behaviour had ceased (low tank densities allowed feeding behaviour to be observed accurately by an observer). The weight of recovered feed was corrected for water absorption by a standard saturation factor (determined by soaking a known weight of feed pellets and then re-weighing, equating to 1.6 x dry weight at saturation). After 12 days all specimens were starved for 48 hours and their weight and length recorded under anaesthesia (as described above). Water speeds in each tank (other than the control) were then increased to match the increased length of the fish, in order to maintain treatments of 0.25, 0.5, 0.75, 1 and 1.5 BL s^{-1}. This was followed by another 12-day period on the same feeding regime. This cycle was repeated twice more to give a total of four 12-day feeding periods interspersed with length assessments and adjustments to water speeds. All tanks were treated with Chloramine-T (0.005 mL L^{-1}) once per day for three days after any handling event and at least 3 hours before feeding. There was no measureable difference in feeding behaviour between days with Chloramine-T treatments and those without. To ensure ER regimes were maintained at a target level,

water flow was checked daily at a position on the opposite side to the spray bar, and at regular spacing intervals around the tank weekly. The weight and length of each individual fish were recorded at the end of the trial under anaesthesia (anaesthetised as described above).

One week after the end of trial 1, trial 2 commenced and fish were treated in exactly the same way as trial 1 but with ER treatments (0.0, 0.25, 0.5, 0.75, 1 and 1.5 BL s^{-1}) randomly reassigned to each of the six tanks. The only other exception was a reduction in the number of fish per tank in the second trial (exactly 17 fish per tank in trial 2 *vs.* approximately 20 per tank in trial 1) and therefore a non-significant difference in biomass density between trials (trial 1 = 1.99 ± 0.12 kg m^{-3}; trial 2 = 2.03 ± 0.22 kg m^{-3}).

Mass specific growth rate (SGR, % body weight day^{-1}) was calculated for each individual using the formula:

$$SGR = (\ln m_2 - \ln m_1) / (t_2 - t_1) \times 100$$

where, m_1 is the initial weight at the start of the growth period t_1 and m_2 is the final weight at the end of the growth period t_2.

Feed conversion ratio (FCR), measured as the weight of dry feed intake (corrected for uneaten feed) per unit weight gain for the period, and was calculated for each tank using the following formula:

FCR = weight of dry feed consumed in tank / wet weight gained in tank

The initial and final condition factor (CF) of fish was also calculated using the formula:

$$CF = mass / length^3 \times 100$$

The relative change in CF (ΔCF) over the course of the growth trials was then calculated as the difference between final and initial CF.

4.2.2 Respirometry

Swim flume respirometry was performed on fish from three ER treatments (0.0, 0.75 and 1.5 BL s^{-1}) to resolve the effect of exercise training on metabolic cost

functions. Aside from understanding the potential metabolic effects of long-term exercise, this information was important for section 4.3.3 where an attempt was made to reconcile the energetic costs of 300 - 500 g hapuku. All specimens were starved for 48 hours prior to respirometry to remove any confounding effects of feeding on metabolic rate (Ross *et al.,* 1992; Thuy *et al.,* 2010b). The mass specific rate of oxygen consumption (MO_2, $mgO_2\ kg^{-1}\ h^{-1}$) was then determined from 24 fish from trial 2 (i.e. 8 fish from the 0.0, 0.75 and 1.5 BL s^{-1} ER groups) over a period of 30 days in the 38.4 L Brett-type swim flume respirometer described by Brown *et al*. (2011). The change in oxygen saturation in the respirometer was measured continuously using a Firesting® 2-channel oxygen meter (Pyroscience, Germany) connected to an oxy-dipping probe (Pyroscience, Germany) which was sealed into the respirometer in a position anterior to the swimming section. The respirometer was operated through a custom software interface which controlled water flow speed in the swimming section and the cycling of the flush, wait and measure periods (5, 1 and 4 mins respectively, 10 mins total). MO_2 and its components were calculated using the same formulae as Brown *et al*. (2011).

After measuring the weight, length, depth and width of fish [to compensate for the solid-blocking effect (Steffensen, 1989)], specimens were placed in the sealed swimming section of the respirometer (530 x 130 x 155 mm). This occurred at approximately 1600 h and provided fish an overnight period of acclimation to the conditions of the respirometer with a low flow of water (0.25 BL s^{-1}) and with the system cycling automatically through a repeated series of flush, wait and measure. From 0800 the following day, a critical swimming speed (U_{crit}) test commenced where the flow speed inside the swimming section was increased by 0.25 BL s^{-1} every 30 mins (i.e. after three 10 min flush-wait-measure cycles). This continued until ⅓ of the body was pressed up against the rear of the swimming section or erratic and non-directional burst activity was observed. Fish swimming behaviour was monitored at all times with a CCD camera (KT & C 19mm, Seoul, Korea) attached to an external monitor. After each experiment was complete, background oxygen consumption levels were measured without a fish and confirmed that bacterial respiration was essentially nil and negligible in all runs. All equipment was cleaned thoroughly between experiments with freshwater and a mild hypochlorite solution (0.005 g L^{-1}).

For each individual fish, critical swimming speed (U_{crit}) was calculated using the same formula as Brett (1964), Brown *et al.* (2011) and Yanase *et al.* (2012). The 15% quantile method of Chabot and Claireaux (2008a) and Franklin *et al.* (2013) was used to obtain a near-resting value of MO_2 from overnight measures at 0.25 BL s^{-1} in order to remove erroneously-low vales associated with hypercapnia or weak oxygen probe signals. Thereafter, three MO_2 values obtained from each of the three flush-wait-measure cycles at each speed were averaged to resolve the relationship between swimming speed and MO_2 (Korsmeyer *et al.*, 2002; Brown *et al.*, 2011) at speeds that were considered to be exclusively aerobic i.e. up to 2.5 BL s^{-1} (Roche *et al.*, 2013). In order to yield an estimate of standard metabolic rate (SMR) for every individual fish, average MO_2 at all speeds was extrapolated back to 0.0 BL s^{-1} using an exponential regression function as used previously by other authors (Pettersson and Hedenström, 2000; Yanase *et al.*, 2012) as power functions underestimated SMR values compared to other investigations on the same species (Khan *et al*., 2014, 2015). Using all MO_2 values from the point that fish first entered the respirometer, MO_{2max} was calculated using the 99% quantile method of Khan *et al.* (2014a) as this yielded higher, and produced less inter-individual variation, than MO_2 values at U_{crit}. Aerobic metabolic scope (AMS) was calculated by subtracting SMR from MO_{2max} and the gross cost of transport (GCOT, mgO_2 kg^{-1} BL^{-1}) was calculated by dividing MO_2 by their corresponding swimming velocity (BL s^{-1}).

4.2.3 Statistical analysis

The data relating the effect of ER treatments on SGR, ΔCF, FCR and feed per individual (g) from trial 1 and 2 were each initially described with a second-order (non-linear) polynomial regression of the form: $y = ax^2 + bx + c$. Non-linear polynomial regressions were also used to effect of ER on GCOT, as well as being used to calculate U_{opt} (Pettersson and Hedenström, 2000). Exponential regressions were used to analyse the effect of ER on the relationship between MO_2 and swimming speed. Due to the presence of non-normal data, the effect of ER on SGR and ΔCF in both trials was tested with a non-parametric Kruskal-Wallis one-way analysis of variance (ANOVA) test. When this test identified a significant effect of ER, a Dunn's comparison test was then used to locate a specific *post hoc* difference in SGR or ΔCF from the control 0 BL s^{-1} ER treatment. After ensuring that data was compliant for

normality and homoscedasticity, a repeated measures (RM) two-way ANOVA was used to test the effect of swim speed on MO_2 (factor 1) as well as the effect of long-term ER on MO_2 (factor 2). The same 2-way RM ANOVA was also used to test the effect of the same two factors on GCOT. The optimal (i.e. least cost) swimming speed (U_{opt}) of individual fish was calculated from the non-linear speed-GCOT regression and taken as the speed that yielded a minimum level of GCOT. The effect of long-term ER on U_{opt}, SMR, MO_{2max}, AMS and U_{crit} was then tested with individual one-way ANOVA tests, followed by a Tukey *post-hoc* test for specific pairwise comparisons where appropriate. Significance was accepted at $P \leq 0.05$ and all data are displayed ± standard error. All statistical analyses were performed using SigmaPlot® version 11.0.

4.3 Results

4.3.1 Effects of exercise training on growth

Non-linear regressions did not provide convincing evidence that ER was positively linked with weight-specific growth (SGR) for either trial 1 ($F = 1.91$, $R^2 = 0.56$, $P > 0.05$) or trial 2 ($F = 0.56$, $R^2 = 0.27$, $P > 0.05$) (Fig. 4.1A). Kruskal-Wallis tests confirmed that ER did not have any effect on the SGR of fish in Trial 2 ($H = 5.23$, $P > 0.05$) where starting weights were higher (Table 4.1) but a strong positive effect of ER on the SGR of fish in trial 1, where starting weights were lower, was identified ($H = 18.93$, $P < 0.01$) (Fig. 4.1A and Table 4.1). Specific *post-hoc* comparisons against the control 0.0 BL s^{-1} treatment revealed that fish were subject to a significant 3.5% increase in SGR at 0.5 BL s^{-1} ($P < 0.05$) and a 4.8% increase in SGR at 0.75 BL s^{-1} ($P < 0.05$) (Fig. 4.1A). No other ER treatment was subject to a change in SGR. The regressions detailing the link between ER and ΔCF were non-significant within the scale of responses observed in trial 1 ($F = 1.33$, $R^2 = 0.47$, $P > 0.05$) and trial 2 ($F = 0.65$, $R^2 = 0.3$, $P > 0.05$) (Fig. 4.1B). ANOVA tests revealed that ΔCF was positively affected by increasing ER in both trial 1 ($H = 12.29$, $P < 0.05$) and trial 2 ($H = 13.76$, $P < 0.05$). However, specific *post-hoc* comparisons against the 0.0 BL s^{-1} control only revealed a significantly higher ΔCF following long-term swimming at 0.5 BL s^{-1} in trial 1 (Fig. 1B). Therefore, in addition to the positive effect on SGR, fish at 0.5 BL s^{-1} had a relatively deeper body shape.

FCR varied little as a function of ER across trial 1 ($F = 4.98$, $R^2 = 0.77$, $P > 0.05$) and trial 2 ($F = 1.67$, $R^2 = 0.53$, $P > 0.05$) (Fig. 4.1C). Feed intake per individual (g) was positively related to ER in trial 1 fish ($F = 115.48$, $R^2 = 0.98$, $P < 0.05$) but showed no relationship with ER in trial 2 ($F = 0.78$, $R^2 = 0.34$, $P > 0.05$, Fig. 4.1D).

Table 4.1. The starting weight (g), final weight (g), total feed consumed (g) and number of fish in each of Trial 1 and Trial 2.

	Trial 1				Trial 2			
Tank Speed (BL s^{-1})	Start Weight (g)	End Weight (g)	N	Total Feed Intake (g)	Start Weight (g)	End Weight (g)	N	Total Feed Intake (g)
0.0	119.9 ± 3.1	341.4 ± 10.1	20	4331.4	170.2 ± 6.1	382.6 ± 12.1	17	3635.2
0.25	131.4 ± 4.7	366.7 ± 9.9	19	4360.1	170.2 ± 8.0	372.8 ± 5.6	17	3359.3
0.5	128.6 ± 4.6	380.6 ± 12.6	20	4702.2	170.9 ± 5.7	397.2 ± 14.1	17	3841.9
0.75	122.7 ± 4.6	365.2 ± 11.8	19	4576.2	187.5 ± 10.6	423.8 ± 19.7	17	4205.7
1.0	133.0 ± 4.3	375.8 ± 7.1	16	4015.2	171.8 ± 10.1	388.4 ± 19.0	17	3678.9
1.5	138.8 ± 3.1	381.9 ± 7.6	17	4384.3	167.4 ± 6.2	372.3 ± 6.2	17	3732.4

All values shown ± standard error

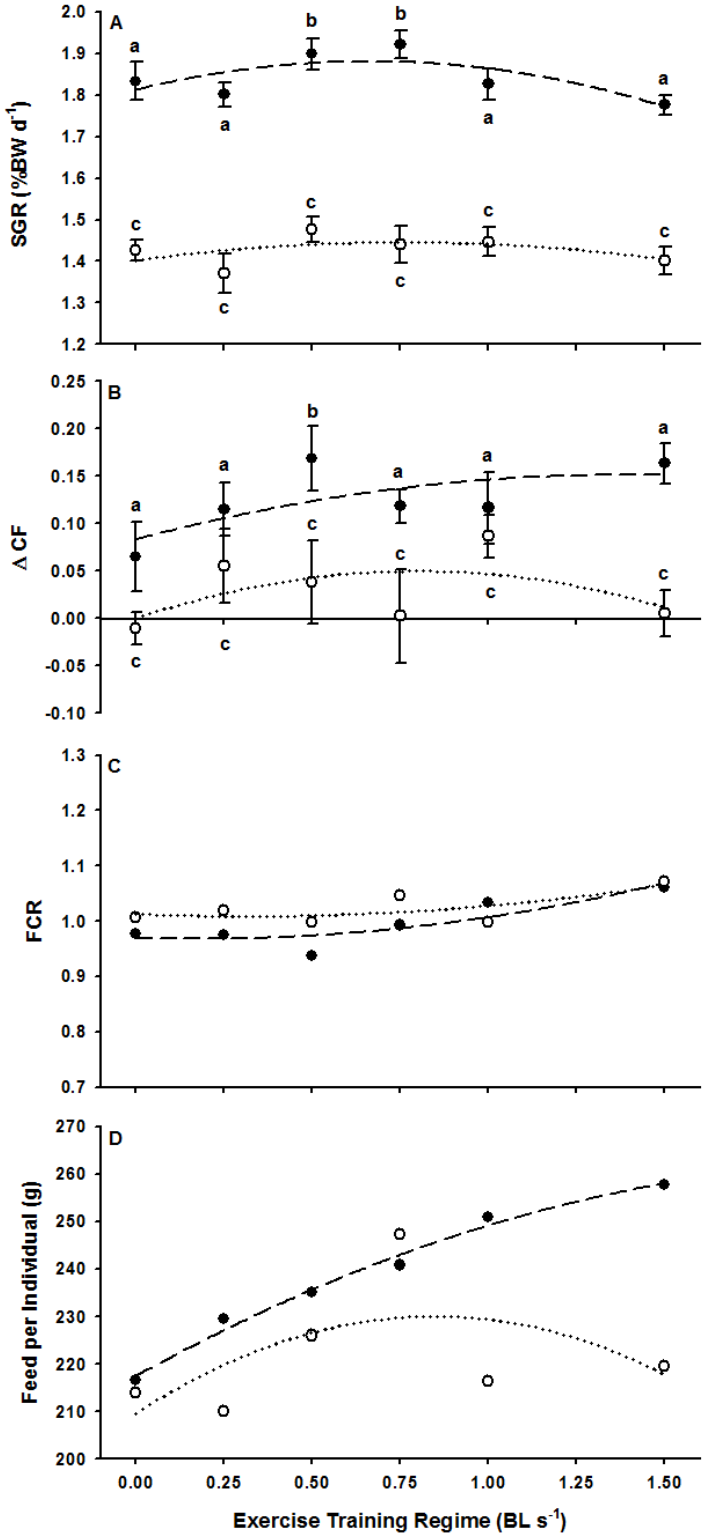

Figure 4.1. The effect of exercise regimes (ER, in BL s^{-1}) on various production parameters of *P. oxygeneios* in trial 1 (closed circles, broken line) and trial 2 (open circles, dotted line). (A) Average SGR. Regressions are second order non-linear polynomials described as: $y = -0.155x^2 + 0.205x + 1.813$ and $y = -0.075x^2 + 0.114x + 1.402$ for the trial 1 and 2 respectively. (B)

Average ΔCF. Regressions are: $y = -0.035x^2 + 0.098x + 0.083$ and $y = -0.078x^2 + 0.125x$ for trial 1 and 2 respectively. (C) Feed conversion ratio, FCR. Regressions are: $y = 0.058x^2 - 0.02x + 0.97$ and $y = 0.0401x^2 - 0.025x + 1.013$ for trial and 2 fish respectively. (D) Feed intake per individual (g). Regressions are $y = -9.390x^2 + 41.138x + 217.51$ and $y = -28.539x^2 + 48.304x + 209.61$ for the trial 1 and 2 fish respectively. Dissimilar letters in each of the plots represent a significant difference between swim speed treatments ($P < 0.05$).

4.3.2 Effects of exercise training on swimming performance

MO_2 increased linearly with swimming speed for each of the 0.0, 0.75 and 1.5 BL s^{-1} ER groups (linear regressions with $R^2 = 0.78$, $R^2 = 0.78$, $R^2 = 0.76$ and $P < 0.05$ for the 0.0, 0.75 and 1.5 BL s^{-1} ER groups respectively, Fig. 4.2A) and a highly significant effect of swimming speed on MO_2 was confirmed from the two-way RM ANOVA tests ($F = 136.11$, $P < 0.01$). There was, however, no significant difference in MO_2 between the three ER treatments ($F = 1.41$, $P > 0.05$) and there was no significant interaction between swimming speed and ER on MO_2 ($F = 0.61$, $P > 0.05$).

GCOT showed a significant parabolic relationship with swimming speed for each of the 0.0, 0.75 and 1.5 BL s^{-1} ER groups ($R^2 = 0.74$, $R^2 = 0.73$, $R^2 = 0.73$ and $P < 0.05$ for the 0.0, 0.75 and 1.5 BL s^{-1} ER groups respectively, Fig. 4.2B) and a highly significant effect of swimming speed on GCOT was once again confirmed with the two-way RM tests ($F= 138.47$, $P < 0.01$). However, there was no significant difference in GCOT between the three ER groups ($F = 0.51$, $P > 0.05$) and there was no interactive effect of swimming speed and ER on GCOT ($F = 0.46$, $P > 0.05$). U_{opt} estimations were also not significantly different between the three ER treatments ($F = 1.26$, $P > 0.05$) and were essentially identical to the pooled U_{opt} estimation of 1.86 BL s^{-1} with a GCOT minima of 0.03 mgO_2 kg^{-1} BL^{-1}.

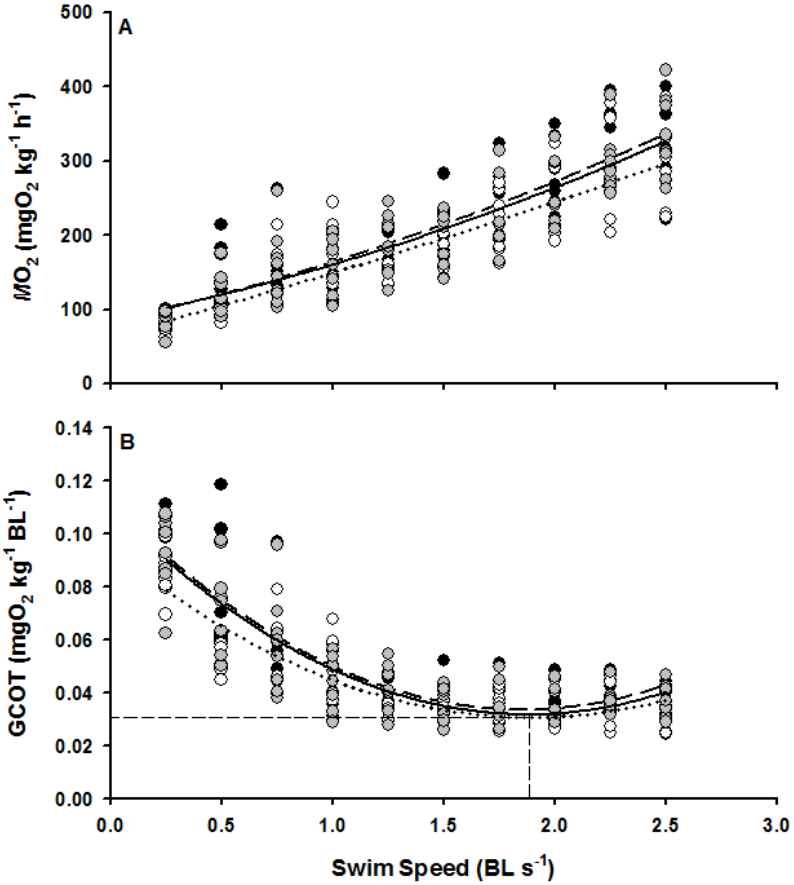

Figure 4.2. The effect of swim flume speed (BL s^{-1}) on the MO_2 (A) and GCOT (B) of juvenile hapuku raised for 6 weeks at either 0.0 BL s^{-1} (black circles, broken line), 0.75 BL s^{-1} (open circles, dotted line) or 1.5 BL s^{-1} (gray circles, solid line). MO_2 regressions are $y = 13.856x^2 + 66.59x + 83.8$, $y = 5.355x^2 + 79.871x + 64.135$ and $y = 14.963x^2 + 58.821x + 86.701$ respectively. GCOT regressions are $y = 0.023x^2 - 0.084x + 0.111$, $y = 0.018x^2 - 0.067x + 0.094$ and $y = 0.021x^2 - 0.081x + 0.109$ respectively. As no significant differences were detected between ER treatments (see results), the horizontal dashed lines refers to the calculated pooled $GCOT_{min}$ (0.03 mgO_2 kg^{-1} BL^{-1}) and the vertical dashed line refers to pooled U_{opt} (1.86 BL s^{-1}).

Long-term exposure to the three ER treatments had no significant effect on SMR ($F = 1.17$, $P > 0.05$), MO_{2max} ($F = 1.15$, $P > 0.05$), AMS ($F = 0.75$, $P > 0.05$) or U_{crit} ($F = 2.63$, $P > 0.05$) (Table 4.1).

Table 4.2. The average weight (g), standard metabolic rate (SMR), maximum metabolic rate (MO_{2max}) and aerobic metabolic scope (AMS) of juvenile hapuku (measured as $mgO_2\ kg^{-1}\ h^{-1}$) as well the critical swimming speed (U_{crit}, $BL\ s^{-1}$) of juvenile hapuku raised for 6 weeks at either 0.0, 0.75 or 1.5 $BL\ s^{-1}$ and measured in a swim-flume respirometer. No significant effect of swimming speed treatment was detected in any of the listed variables ($P > 0.05$).

	Swim Speed Treatment		
	0.0 BL s^{-1}	0.75 BL s^{-1}	1.5 BL s^{-1}
Average weight (g)	469.12 ± 5.96	496.38 ± 8.14	481.44 ± 6.77
Standard metabolic rate (SMR, $mgO_2\ kg^{-1}\ h^{-1}$)	91.33 ± 5.37	80.58 ± 5.46	90.67 ± 5.80
Maximum metabolic rate (MO_{2max}, $mgO_2\ kg^{-1}\ h^{-1}$)	324.72 ± 7.44	294.05 ± 5.99	337.90 ± 8.59
Aerobic metabolic scope (AMS, $mgO_2\ kg^{-1}\ h^{-1}$)	233.39 ± 9.04	213.47 ± 5.49	247.23 ± 8.23
Critical swimming speed (U_{crit}, $BL\ s^{-1}$)	2.72 ± 0.13	2.55 ± 0.08	2.94 ± 0.14
	All values shown ± standard error		

4.3.3 Reconciling the cost of swimming

A summary of hapuku metabolic costs across a temperature range of 15 °C – 24 °C was amalgamated and graphically represented (Fig. 4.3) for the purpose of reconciling metabolic components against available aerobic metabolic scope at 17 °C. SMR values for 15 °C and 21 °C were measured in a different study in similarly sized fish using a static respirometry system (Khan *et al.*, 2015) and the line between these two values intersected 17 °C at 91.31 $mgO_2\ kg^{-1}\ h^{-1}$ which is very similar to the SMR estimate from the current study (87.53 ± 5.21 $mgO_2\ kg^{-1}\ h^{-1}$) at 17 °C (Table 4.1). SDA estimates were also measured in Khan *et al.* (2015) for fish fed a 1.5% BW d^{-1} ration at both 15 °C and 21 °C. An estimate of SDA at 17 °C was then estimated from the straight line function between these two SDA values (Q_{10} = 3.44, $MO_2 = 25.45temp - 242.95$). It was therefore assumed that peak SDA follows a linear relationship between these two temperatures when fed the same-sized ration. (NB. ration size varied 1.3% - 1.8% BW d^{-1} in the current study so was close to the standard 1.5% BW ration in Khan *et al.* (2015). ER had no significant effect on swimming costs at 17 °C (see above) so the MO_2 values from each ER were pooled to calculate an average cost of swimming at 0.25, 0.5, 0.75, 1.0 and 1.5 $BL\ s^{-1}$. MO_{2max} values are shown as the highest and lowest

estimates from the current study (i.e. 1.5 BL s^{-1} = 337.90 ± 8.59 mgO_2 kg^{-1} h^{-1} and 0.75 BL s^{-1} = 294.05 ± 5.99 mgO_2 kg^{-1} h^{-1} respectively, Table 4.2).

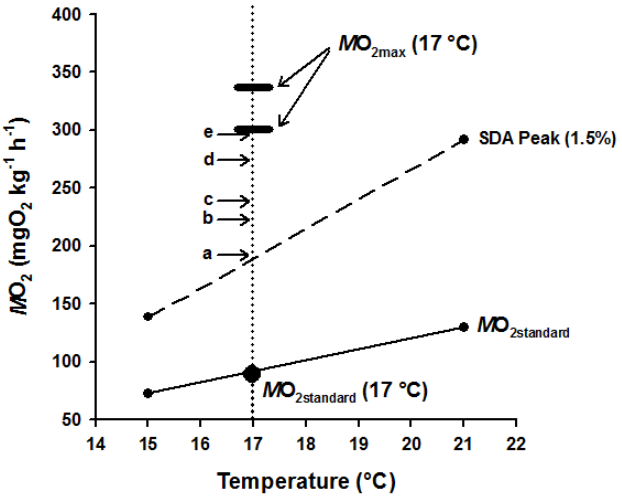

Figure 4.3. Graphical summary of hapuku metabolic components as a function of temperature (15 °C – 21 °C) with particular detail at 17 °C, allowing the costs of specific dynamic action (SDA) and swimming to be balanced within the boundaries of aerobic metabolic scope (AMS = MO_{2max} – SMR, shown as $MO_{2standard}$). $MO_{2standard}$ at and between 15 °C and 21 °C (small filled circles adjoined by the lowest solid line) was taken from Khan *et al*. (2015) using a static respirometry system whilst $MO_{2standard}$ at 17 °C (large filled circle) was measured directly within the current study using the swim-flume respirometer. The two small filled circles adjoined by the broken line show the peak in the SDA response of 300 – 500 g hapuku fed a 1.5% BW d^{-1} ration at and between 15 °C and 21 ºC (Khan *et al*., 2015). It is likely that the SDA costs measured at 15 °C and 21 ºC can be used to accurately interpolate the feeding costs of fish in the current study at 17 °C because the fixed 1.5% BW d^{-1} ration of Khan *et al*. (2015) closely approximates the 1.3% – 1.8% BW d^{-1} *ab libitum* ration level of fish from the different ER treatments. The thickened horizontal dash indicates the MO_{2max} of 300 – 500 g hapuku measured in the swim flume at 17 °C during the current study. Overlaid above $MO_{2standard}$ (solid line) and peak SDA (broken line) are horizontal arrows with letters (a-e) showing the additional measured costs of swimming from the current study at 17 °C as follows: (a) 0.25 BL s^{-1}, (b) 0.5 BL s^{-1}, (c) 0.75 BL s^{-1}, (d) 1.0 BL s^{-1} and (e) 1.5 BL s^{-1}. The vertical dotted line therefore represents the transect through the accumulated costs of maintenance ($MO_{2standard}$), feeding/growth (SDA) and exercise of *P. oxygeneios* at 17 °C and shows that the costs of SDA and exercise fall comfortably within the limits of available aerobic metabolic scope at this temperature (see results and discussion for more detail).

4.4 Discussion

In order to validate the model of Davison and Herbert (2013), exercise-induced growth in juvenile hapuku would be expected in the range of ~0.4 – 0.5 BL s^{-1}. However, the current study does not provide compelling evidence of exercise-induced growth at 17 °C (Fig. 4.1). This is a stark contrast to salmonids that show up to a 40% increase in growth from sustained exercise in the region of 0.75 – 1.5 BL s^{-1} (Davison and Goldspink, 1977; Houlihan and Laurent, 1987; Jørgensen and Jobling, 1993). Indeed, hapuku with an average starting weight of 130 g in trial 1 only showed a maximum of a 4.8% increase in growth at 0.5 and 0.75 BL s^{-1} respectively (Fig. 4.1A) whereas the larger 170 g (starting weight) hapuku showed no sign of exercise-induced growth in trial 2 (Table 4.1 and Fig. 4.1A). Earlier studies on salmonids considered that the exercise regimes at which optimal growth is ascertained (i.e. $ER_{opt\ growth}$) was attributed to their active ecotype, as well as the physiological and behavioural requirements of schooling, migration and river spawning [e.g. position holding in strong water flows (Jobling *et al.*, 1993a)]. Hapuku would not be described as highly active so the data is consistent with the view of Jobling *et al*. (1993). However, the recent review on exercise-induced growth in fish by Davison and Herbert (2013) went further to propose that $ER_{opt\ growth}$ is a function of aerobic metabolic scope (AMS). Most salmonids, with their active ecotype and high AMS (~350 – 500 $mgO_2\ kg^{-1}\ h^{-1}$), show exercise-induced growth at relatively fast swimming speeds (Walker and Emerson, 1978; Houlihan and Laurent, 1987; Jørgensen and Jobling, 1993; Bugeon *et al.*, 2003) and therefore provide data to support the upper end of the Davison and Herbert (2013) model. In contrast, the lower end of the model is based on species such as gadoids that have a small AMS in the region of ~150 – 200 $mgO_2\ kg^{-1}\ h^{-1}$ (Hammer, 1994; Karlsen *et al.*, 2006) and show little to no growth response to exercise training (Bjørnevik *et al.*, 2003; Karlsen *et al.*, 2006). On the basis of these observations, the current study aimed to assess the AMS – $ER_{opt\ growth}$ model of Davison and Herbert (2013) by testing whether the ~300 $mgO_2\ kg^{-1}\ h^{-1}$ AMS level of Khan *et al.* (2014a) does indeed lead to an $ER_{opt\ growth}$ of 0.4 – 0.5 BL s^{-1}. At least for trial 1, the Davison and Herbert (2013) model prediction does appear to provide a reasonable fit. However, the lack of exercise-induced growth in trial 2 is not consistent with the Davison and Herbert (2013) model and the very modest levels of growth acceleration do not validate the model for this novel species.

The AMS values measured from 480 g hapuku at the end of the current study ranged from 213 – 247 $mgO_2\ kg^{-1}\ h^{-1}$ (Table 4.2) and are therefore lower than the 300 $mgO_2\ kg^{-1}\ h^{-1}$ AMS value ascertained for 180 g hapuku at 17 °C in the study of Khan *et al.* (2014a). Whilst these larger AMS values were used initially to formulate our hypothesis, the recently established values of AMS are considered more valid because they originate from a size class of fish that corresponds to the current $ER_{opt\ growth}$ data. However, applying these lowered AMS values to the model of Davison and Herbert (2013) predicts an $ER_{opt\ growth}$ of between 0.15 and 0.3 BL s^{-1} which does not correspond to the observed $ER_{opt\ growth}$ range of fish in trial 1 or the total lack of exercise-induced growth in trial 2 (Fig. 4.1A). These data further suggest that the relationship between AMS and $ER_{opt\ growth}$ is not validated in this species.

In relation to the second model of Davison and Herbert (2013), the $ER_{opt\ growth}$ range observed in trial 1 (0.5 – 0.75 BL s^{-1}, Fig. 1) does not even vaguely correspond to the measures of U_{opt} in the current study (1.86 BL s^{-1}, Fig. 4.2B). The U_{opt} estimation for juvenile hapuku was unaffected by ER and is considerably higher than one might expect for a species that is less active than Atlantic salmon *Salmo salar*, brown trout *Salmo trutta* and brook charr *Salvelinus fontinalis* which all have U_{opt} values in the range of 0.9 to 1.1 BL s^{-1} (Beaumont *et al.*, 2000; Deitch *et al.*, 2006; Tudorache *et al.*, 2011). Atlantic cod and gilthead seabream *Sparus aurata* also have unusually high U_{opt} estimations [ranging from 1.2 – 1.6 BL s^{-1} in the cod and up to 2.3 BL s^{-1} in the gilthead seabream (Schurmann and Steffensen, 1997; Steinhausen *et al.*, 2010)]. Alternative methods of calculating the minimum cost of transport [i.e. those suggested by Pettersson and Hedenström (2000)] produce a similarly high U_{opt} estimate of 1.84 BL s^{-1} for the pooled GCOT data (Fig. 4.2B). It may be that these less active ecotypes do not have an ecologically functional or relevant U_{opt} as would be the case for migratory or highly active species (Hinch and Rand, 2000; Tudorache *et al.*, 2011) though this is speculation and would require further investigation.

The hypothesis that AMS places a capacity limitation on exercise-induced growth (Davison and Herbert, 2013) is not supported by the current data for juvenile hapuku. For 480 g hapuku at 17 °C, the costs of exercise and specific dynamic action [SDA, which can be comprised of up to 80% protein synthesis (Coulson and Hernandez, 1979; Brown and Cameron, 1991; Seth *et al.*, 2010; Li *et al.*, 2013b)] are easily accommodated within available AMS, even at the highest swimming speed used

in the growth trials (1.5 BL s^{-1}, Fig. 4.3). It is generally accepted that the energetic costs associated with SDA are largely comprised of post-absorptive protein synthesis and is thought to represent the cost of growth (Whiteley *et al.*, 2001; Grigoriou and Richardson, 2008; Secor, 2009) and, in less active species with low AMS, SDA often consumes a large proportion of AMS potential (Jobling, 1983; Soofiani and Priede, 1985a; Jordan and Steffensen, 2007). This has led researchers to propose that an inability to reconcile the metabolic costs of growth and exercise simultaneously would either lead to a reduction in the rate of protein synthesis (as a prioritisation of exercise over growth, Davison and Herbert, 2013) or, as shown in the European eel *Anguilla anguilla* in the study of Owen (2001), a reduction in swimming activity as a prioritisation of growth over exercise. Therefore, with an ability to accommodate the costs of exercise and growth simultaneously and with metabolic costs of swimming (Fig. 4.2) and SDA not vastly different to other ecotypes (Fu *et al.*, 2005b; Jordan and Steffensen, 2007; Ohlberger *et al.*, 2007; Yanase *et al.*, 2012; Frisk *et al.*, 2013), it is proposed that the weak exercise growth response of hapuku is a species-specific effect and not due to capacity limitation of aerobic metabolism.

The data in Fig. 4.3 provides evidence that AMS does not limit the ability of juvenile hapuku to swim and grow simultaneously but, on a cautionary note, it does not take into the account the extra metabolic costs of spontaneous activity (Boisclair and Tang, 1993; Tang *et al.*, 2000) nor does it necessarily prove that hapuku have the metabolic capacity to grow *faster* whilst swimming. With respect to the latter point, the SDA costs of supplementary fast growth from exercise were not measured within static respirometry chambers (Khan *et al.*, 2015) and is therefore still not yet resolved. Interestingly, there is other recent data suggesting that the costs of SDA and exercise can act additively in the darkbarbel catfish *Peltebargus vachelli* (Li *et al.*, 2010) and the sea bass *Dicentrarchus labrax* (Altimiras *et al.*, 2008) to the point where total costs exceed measured MO_{2max}. This is relevant to the current discussion as it suggests a potential disconnect between AMS and the combined costs of exercise and growth. More importantly, this data opposes the AMS – $ER_{opt\ growth}$ hypothesis of Davison and Herbert (2013) as exercise and growth could potentially occur simultaneously in catfish and sea bass without their costs being limited by AMS. The presence of additive SDA has not yet been addressed in hapuku and, whilst this species appears suited to U_{crit} swimming tests in a swim-flume respirometer, feeding attempts have not

yet been successful. To investigate this issue further, it may be necessary to implement a gavage protocol or directly infuse food or amino acids into the gut or bloodstream (Brown and Cameron, 1991; Li *et al.*, 2010).

4.5 Conclusions

The data from the current study is not consistent with the hypothesis of Davison and Herbert (2013) that AMS sets a limit to, and therefore determines, the likelihood of seeing exercise-induced growth in finfish aquaculture species such as hapuku. This is essentially based on the fact that; i) juvenile hapuku showed a modest and inconsistent exercise-induced growth response in a narrow band of swimming speeds (0.5 – 0.75 BL s^{-1}, and ii) the AMS of these fish appears sufficient to accommodate the physiological costs SDA and swimming simultaneously. It may be that this species is generally not responsive to exercise training but, before that conclusion is reached, future research should possibly strive to examine the response of different-sized hapuku across a greater range of (optimal) temperatures as a means of disentangling the potential role of these factors in exercise-induced growth (e.g. Brown *et al*., 2011).

Chapter 5 – The Effects of Sustained Exercise on Muscle Fibre Density and Fillet Texture

In review as:

Khan, J.R., Pether, S., Bruce, M., Walker, S.P., Herbert, N.A. Sustained exercise reduces the muscle fibre density and flesh firmness of juvenile hapuku (*Polyprion oxygeneios*). *New Zealand Journal of Marine and Freshwater Research.*

5.1 Introduction

Aquaculture has a distinct advantage over fisheries because product quality and appearance can be manipulated more easily to suit consumer preference and demand (Johnston *et al.*, 2000). For example, farmed fish are generally considered to be of lower quality than wild counterparts due to softer flesh (Haard, 1992; Periago *et al.*, 2005) but sustained exercise is now under the spotlight as a viable technique to increase product quality and consumer preference in a variety of farmed finfish species [e.g. Totland *et al.* (1987), Davison (1997) and Palstra and Planas (2011)]. Of particular interest is the fact that exercise has the potential to improve flesh quality by manipulating the structure and constituents of fish swimming muscle (Rasmussen *et al.*, 2013).

The textural properties of a fillet, and firmness in particular, are often related to the pattern and distribution of muscle fibres. For example, where muscle has a more tightly packed arrangement of smaller white muscle fibres (i.e. muscle fibre density, MFD, is high) it tends to yield firmer flesh which is considered more desirable (Hatae *et al.*, 1990). On the other hand low MFD tends be associated with softer, less desirable flesh (Johnston *et al.*, 2000). This relationship between MFD, firmness and

desirability has been demonstrated in the Atlantic salmon *Salmo salar* (Johnston *et al.*, 2006; Johnsen *et al.*, 2011), sea bass *Dicentrarchus labrax* (Periago *et al.*, 2005) and Atlantic halibut *Hippoglossus hipposglossus* (Hagen *et al.*, 2007), though these relationships are not universal and the interactive effects of factors such as genetics, temperature, slaughter method, post-slaughter treatment and measurement method can lead to variable results (Johnston *et al.*, 2000; Ashton *et al.*, 2010; Rasmussen *et al.*, 2013). In terms of the manner in which exercise affects the flesh quality of fish it is perhaps surprising that changes in fillet texture and white muscle cellularity are induced at swimming speeds where the bulk of the white muscle may not even be activated [i.e. steady straight line (aerobic) swimming at ~ 0.8 – 1.0 body lengths per second, BL s^{-1} (Johnston and Moon, 1980; Martin and Johnston, 2005b; Rasmussen *et al.*, 2013)]. A further consideration is that sustained exercise generally promotes hypertrophy (larger muscle fibre size) rather than hyperplasia (new fibre growth) in fish muscle (Davison and Goldspink, 1977; Davison, 1997; Ibarz *et al.*, 2011) which should yield softer flesh as a result of lower MFD (Johnston and Børresen, 2008; Rasmussen *et al.*, 2013). However, on the basis that both softer (Bugeon *et al.*, 2003) and firmer flesh (Rasmussen *et al.*, 2011; Rasmussen *et al.*, 2013) has been found in exercised salmonids showing muscle hypertrophy, the role of exercise in improving flesh quality and altering muscle fibre characteristics clearly requires more investigation across a wider range of cultured fish species.

Based on the assumption that firmer fillets are more desirable to consumers and are easier and more efficient to process mechanically (Haard, 1992; Johnston *et al.*, 2006), the level of sustained swimming required to produce changes in fillet firmness in a novel aquaculture species, the hapuku *Polyprion oxygeneios*, was investigated. This was done in fish exposed to water currents equivalent to 0.0, 0.75 or 1.5 BL s^{-1} across an 8 week period. The secondary aim of this study was to investigate the link between flesh texture, white-muscle MFD and hypertrophy/hyperplasia in this novel species as a means of resolving whether exercise-induced hypertrophy is indeed associated with softer flesh and lower MFD. Exercise has been shown to have a marginal growth benefit for *P. oxygeneios* (Khan *et al.*, 2014b) so the current study provides a more focussed view into the quality of farmed hapuku and the role of exercise in their production.

5.2 Materials and methods

5.2.1 Specimens, tagging and growth trials

The 56 juvenile hapuku used in the current study (n = 20, 19 and 17 for the 0.0, 0.75 and 1.5 BL s^{-1} exercise regimes respectively, 371.3 ± 4.2 g pooled average final weight) are those from "trial 1" of Khan *et al.* (2014b) which exhibited a small but significant growth response to a 0.75 BL s^{-1} exercise regime. The 8-week exercise period (growth trial) was conducted incorporating six different exercise regimes corresponding to flow speeds of 0.0, 0.25, 0.5, 0.75, 1 and 1.5 BL s^{-1}, though only fish from the 0.0, 0.75 and 1.5 BL s^{-1} regimes were used for histological and textural analysis. These regimes were chosen as they represented a control, a mid-point where growth was maximised (Khan *et al.*, 2014b) and an extreme value. The trial was conducted in six identical 1.6 m^3 circular tanks (560 mm water depth, 1900 mm diameter). All tanks were housed in a purpose-built building under ambient light conditions (11L: 13D) and supplied with fresh 1 µm filtered and UV-sterilised (ALX2/8, 150 mW sec cm^{-2}, Davey Water Products, Australia) seawater at 17 ± 0.3 ºC. A continuous non-directional inflow of water (30 L min^{-1}) was present at the side of each tank and all tanks were central draining. Water flow around the tank was negligible in the 0.0 BL s^{-1} (control) tank but the remaining exercise treatments were maintained through the use of external water pumps (Leader® Ecopool 15, Leader Pumps, Italy) plumbed over the side of each tank via a 25 mm PVC intake and outlet. Flow speeds were set in the tanks by measuring water velocity (in m s^{-1}) 200 mm from the tank wall at 100 mm depth on the side directly opposite the spray bar with a Höntzsch® HFA anemometer (V 1.5, Höntzsch technologies, Waiblingen, Germany) and making the necessary correction to tanks speeds according to the average body length of the fish which was measured at regular fortnightly intervals under anaeasthesia (0.01 mL L^{-1} Aqui-S® followed by 0.3 mL L^{-1} 2-phenoxy-ethanol, standard facility practice). Water chemistry was checked regularly and remained at normal levels at all times throughout both trials. All husbandry practices as well as the details of the 8-week growth trial are reported in greater detail by Khan *et al.* (2014b) and in the previous chapter.

5.2.2 Sampling

Immediately upon completion of the 8-week trial all fish from the 0.0, 0.75 and 1.5 BL s^{-1} exercise regimes were euthanized with an overdose of anaesthetic (0.04 mL L^{-1} Aqui-S®) and placed on ice. Fillets were taken off both sides of each fish over the following 3 h and were skinned immediately. From each fillet a 20 mm (anterior to posterior plane) wide steak was removed from immediately behind the gut cavity which traversed the entire depth of the fillet (See Fig. 5.1). The steaks were allowed to air dry for one minute on a piece of cardboard and then fully submerged in a labelled bottle of 10% formalin for fixation.

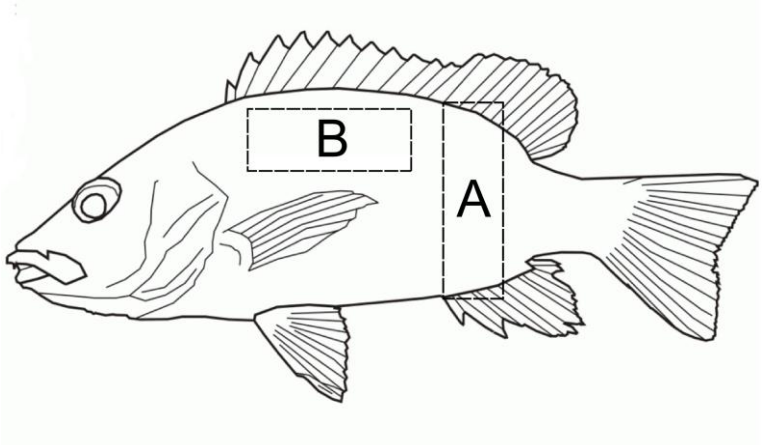

Figure 5.1. The positions of samples taken for (A) the muscle fibre density and fibre size investigation and (B) the texture analysis. Samples were taken from both sides of each fish.

From the dorsal portion of each fillet (above the spine and gut cavity) a 30 × 20 × 10 mm section was removed. These were placed individually in labelled zip-lock bags and stored immediately in a 4 ºC chiller (See Fig. 5.1). There were therefore two steaks (fixed in formalin) and two dorsal 30 × 20 × 10 mm sections (fresh and chilled) taken from each fish.

5.2.3 Staining and Image analysis

Steaks were fixed for a minimum 1 week in 10% formalin before they were prepared for imaging by Gribbles Veterinary Pathology, Mt Wellington, New Zealand. Two 7 mm^2 samples were taken from as close to the midpoint of each steak as possible, producing two subsamples per steak and four per fish. Each subsample was

stained with haematoxylin and eosin and then embedded in paraffin wax. Photomicrographs were taken using an Olympus XC50 camera on an Olympus BX41 microscope at 100× to 400× magnification (10× - 40× objective, 10× eyepiece), and processed via AnalySIS GetIT software (v 5.1, Build 1693). Multiple areas of every subsample were imaged so that images without any tearing (resulting from the cutting process) could be used to determine fibre size frequency and MFD. The incidence of tearing in samples was generally low.

The images associated with 10 random fish from each of the 0.0, 0.75 and 1.5 BL s^{-1} exercise regimes were used to determine fibre size distribution and MFD. All image analyses were performed on ImageJ freeware (v 1.47). One random image per subsample was analysed, meaning that two images were analysed per steak and four images in total for each fish. For each image, the outlines of 100 random muscle fibres were traced and the area of each fibre calculated in μm^2 (hereafter referred to as fibre size). Fibre size data were pooled for each fish (400 values per fish) and the frequency distribution of fibre size determined using 1000 μm^2 bins. MFD (fibres per mm^2) was calculated as the sum of the area of all of the muscle fibres sampled for a single fish (mm^2) / the number of fibres sampled.

5.2.4 Textural analysis

All of the 30 × 20 × 10 mm dorsal sections were maintained at 4 ºC for the 24 h between removal from the fillet and the start of the textural analyses. Analyses were performed using a TA.XT Plus texture analyser (Stable Microsystems, Surrey, England) equipped with a 25 kg load cell and a Warner-Bratzner blade (Sigurgisladottir *et al.*, 1999). Peak force (N) and total work done (N mm, not including the force required for retraction) were measured for every dorsal section using a test and retraction speed of 2 mm s^{-1}. All samples were taken out of the 4 ºC chiller immediately before analysis and were placed on the apparatus skin side down. Measurements were taken to a 7 mm depth from the initiation of a 5 g force trigger. The sections contained no bones and the blade and platform was cleaned and dried between each sample.

5.2.5 Statistical analyses

A parametric two-way analysis of variance (ANOVA) was used to analyse the peak force and total work data using the exercise regime (0.0, 0.75 or 1.5 BL s^{-1}) and the side of the fish (left or right) as factors, after ensuring that data were both equally variant and normally distributed. MFD and median fibre size were compared using a non-parametric Kruskal-Wallis one-way ANOVA. Where significant effects were detected, a *post-hoc* Holm-Sidak pairwise comparison was used to determine specific treatment differences. The relationship between MFD and peak force/total work was evaluated using linear regression analysis. A Kolmogorov-Smirnov test was used to test for normality in the distributions of all raw fibre size data and a related samples Friedman's two-way analysis of variance by ranks was used to compare raw fibre size distributions between the different exercise regimes. Pairwise differences in fibre distributions were analysed using a Friedman's two-way analysis of variance by ranks on raw fibre size data. Analyses were carried out on SigmaPlot 11.0 and IBM SPSS 22. Significance was accepted at $P \leq 0.05$.

5.3 Results

5.3.1 Fillet texture

Peak force and total work were significantly affected by exercise regime (F = 3.967 and F = 3.149 for peak force and total work respectively, $P < 0.05$ for both) but were not significantly affected by the side of the fish (F = 2.21 and F = 0.32 for peak force and total work respectively, $P > 0.05$ for both). No significant interaction was detected between force variables and the side of the fish (F = 0.20 and F = 1.01 for peak force and total work respectively, $P > 0.05$ for both). In terms of specific pairwise comparisons against the control 0.0 BL s^{-1} regime, both peak force and total work were significantly lower in the 0.75 BL s^{-1} and 1.5 BL s^{-1} treatment groups ($t = 2.34$ and $t = 2.61$ respectively, $P < 0.05$ for both. Fig. 5.2A and B). Values of peak force and total work were not significantly different between the 0.75 BL s^{-1} and 1.5 BL s^{-1} treatments ($t = 1.88$ and $t = 2.12$ respectively, $P > 0.05$ for both. Fig. 5.2A and B).

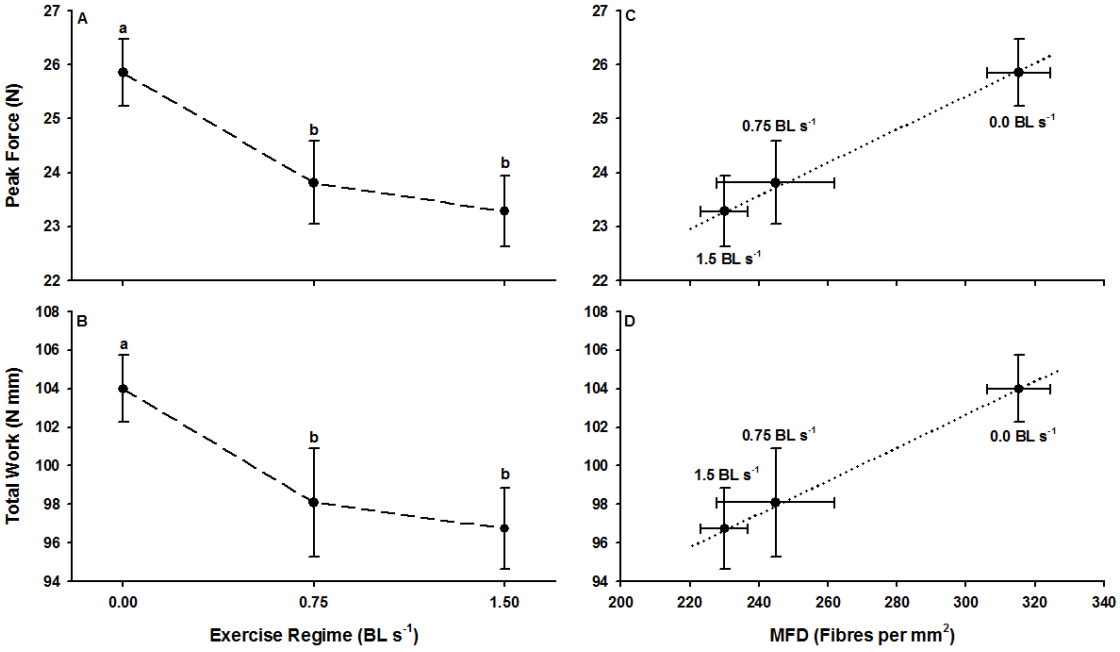

Figure 5.2. The (A) peak force (N) and (B) total force required (N mm) to crush a 30 × 20 × 10 mm piece of dorsal flesh taken from juvenile hapuku that were raised for 8 weeks at either 0.0, 0.75 or 1.5 BL s^{-1}. Texture analyses were performed with a Warner-Bratzner blade. (C) and (D) show the relationship between the same texture measurements and muscle fibre density (MFD, fibres per mm^2), regressions are $y = 0.03x + 16.46$ and $y = 0.08x + 77.29$ respectively. Values are shown with their associated standard errors and letters denote significant differences between treatments.

5.3.2 Muscle fibre size and density and the link to fillet firmness

None of the fibre size distributions from 0.0, 0.75 and 1.5 BL s^{-1} exercise regimes were normally distributed (K = 0.58, 0.458 and 0.055 respectively, $P < 0.01$) but the distributions of the raw fibre size data were significantly different from one another when an appropriate test was applied (Kolmogorov-Smirnov test, F = 247.73, $P < 0.01$, Fig. 5.3). Fibre size distributions appeared to flatten and widen with progressively higher levels of exercise and pairwise comparisons confirmed that each of the fibre size distributions were significantly different between the three exercise treatments (F = 62.90, F = 230.41 and F = 43.88 for the 0.0 – 0.75, 0.0 – 1.5 and the 0.75 – 1.5 BL s^{-1} comparisons respectively, $P < 0.01$ for each, Fig. 5.3). More specifically, there was a significant effect of exercise regime on both MFD (H = 17.52,

$P < 0.01$) and median fibre size (H = 344.47, $P < 0.01$). In terms of a specific pairwise comparison against the 0.0 BL s^{-1} control treatment, MFD decreased significantly with exercise in the 0.75 BL s^{-1} and 1.5 BL s^{-1} treatments ($q = 4.23$, $P < 0.05$). But, similar to the patterns observed for firmness (above and Figs. 5.2A and B), there was no observable difference in MFD between the 0.75 BL s^{-1} and 1.5 BL s^{-1} groups ($q = 1.51$, $P > 0.05$). As such, a strong and positive relationship was observed between MFD and i) peak force and ii) total work showing that fillet firmness increases with increasing MFD (F = 3.97 and F = 4.58 for peak force and total work respectively, $R^2 = 0.99$ and $P < 0.01$ for both, Fig. 5.2C and D).

There was a strong and positive relationship observed between MFD and i) peak force and ii) total work (F = 3.97 and F = 4.58 for peak force and total work respectively, $R^2 = 0.99$ and $P < 0.01$ for both, Fig. 5.2C and D), with fillet firmness increasing as MFD increased.

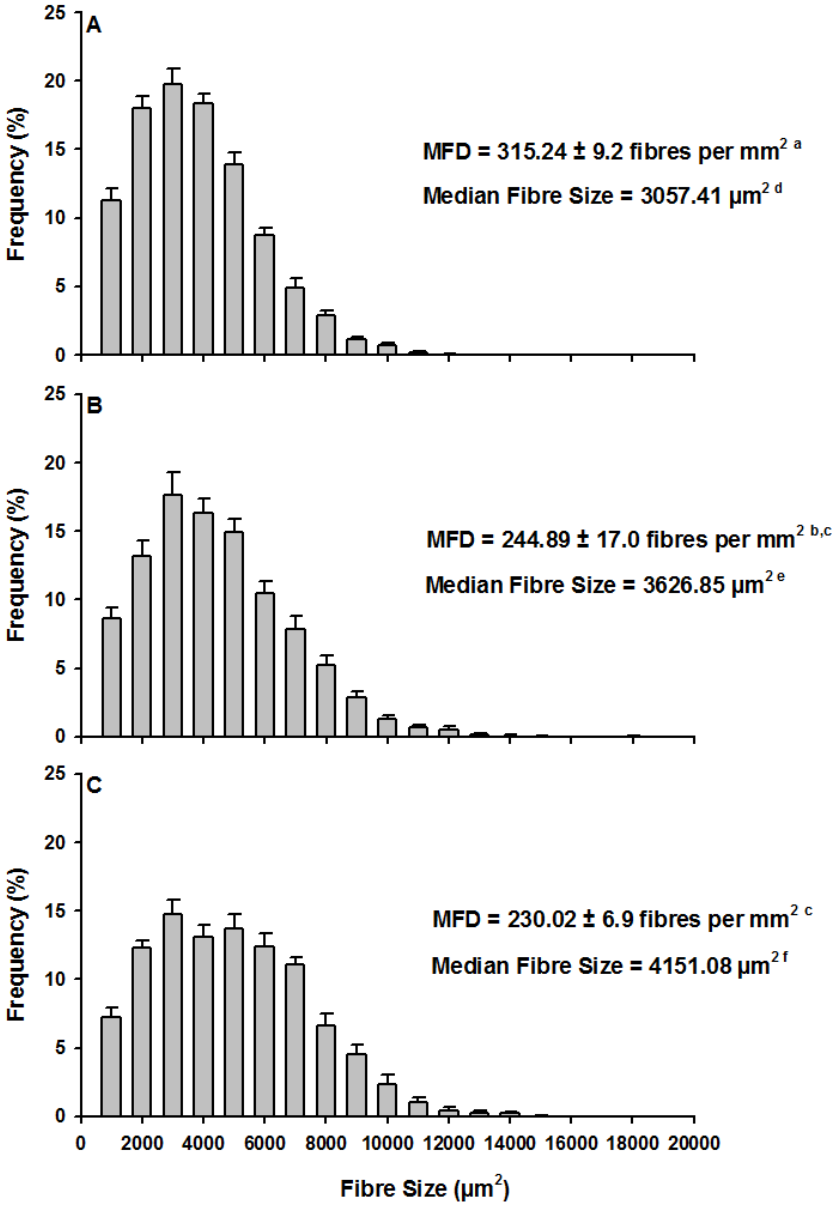

Figure 5.2. Fibre size (measured as fibre area in μm^2) distributions for juvenile hapuku raised for 8 weeks at either (A) 0.0, (B) 0.75 or (C) 1.5 BL s^{-1} exercise regimes (ER). Distributions are displayed as a proportion of measured fibres (400 per fish, 200 per side) with their associated muscle fibre density (MFD) and the median fibre size. Superscripts denote a significant difference between measures. MFD values and histogram bars are shown with their associated standard errors.

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5.4 Discussion

This study set out to resolve whether sustained exercise training would provide beneficial adjustments to the fillet texture of juvenile hapuku and it is now apparent that exercise yields a statistically significant reduction in textural firmness in this species (Fig. 5.2A and B). This observation reflects negatively on the role of exercise in aquaculture because reduced firmness would generally be considered a negative trait by consumers who often perceive firmer flesh as being of higher quality (Rasmussen *et al.*, 2013). However, while sustained exercise has generally shown positive effects in exogenous characteristics such as reduced fin damage and healthier looking skin (Davison, 1989; Jørgensen and Jobling, 1993; Davison, 1997), the primary result of this study should not be viewed as anomalous or unusual as the effects of exercise on fillet firmness do appear to vary with species, training regimes and the method of measurement (Rasmussen *et al.*, 2011; Rasmussen *et al.*, 2013). For example, no change in the fillet texture of Atlantic cod *Gadus morhua* was detected above the general level of variability after being exercise trained for 9 months at either 0.5 and 1.0 BL s^{-1} (Bjørnevik *et al.*, 2003). Brown trout *Salmo trutta* from the study of Bugeon *et al.* (2003) did however show a significant increase in fillet firmness after 8 months of sustained exercise at 2 BL s^{-1}, which also coincided with a significant increase in weight gain compared to unexercised controls. Specific examples of sustained exercise decreasing fillet firmness, such as that observed in the current study, are not as common in the literature but examples of exercise inducing muscle hypertrophy are common (Davie *et al.*, 1986; Martin and Johnston, 2005b, a, 2006; Ibarz *et al.*, 2011) and this change in muscle structure is generally associated with a decrease in firmness though firmness is not always measured directly (Sato *et al.*, 1986; Hatae *et al.*, 1990; Coppes *et al.*, 2002; Periago *et al.*, 2005; Johnsen *et al.*, 2011).

The second aim of this study was to investigate the link between exercise, MFD and flesh quality in a novel finfish aquaculture species. Sustained exercise increases the median fibre size and reduces white muscle MFD in the fillets of juvenile hapuku compared to static controls (Fig. 5.3) which is also observed in salmonids (Davison and Goldspink, 1977; Bugeon *et al.*, 2003) and other non-salmonid species (Love, 1970; Davison and Goldspink, 1978; Broughton *et al.*, 1980; Young and Cech Jr, 1993) as a result of muscle fibre hypertrophy (Rasmussen *et al.*, 2013). Muscle

fibre hypertrophy was also induced in the common carp *Cyprinus carpio* as a result of exercise (2.5 BL s^{-1}, 16 h per day) though primarily in the slow-twitch red muscle (Martin and Johnston, 2006). Examples of sustained exercise inducing hyperplasia are not as prevalent, though it has been found in the white muscle of zebrafish *Danio rerio* exercised from hatching (van der Meulen *et al.*, 2006). The proportion of aerobic red muscle is also known to increase in several non-salmonid species in response to sustained exercise (Sänger, 1992; Meyer-Rochow and Ingram, 1993; Young and Cech Jr, 1993; Sänger and Stoiber, 2001) as a result of both hypertrophy and hyperplasia. However, aerobic red muscle is generally considered to be a less desirable part of the fillet due its dark colour and strong taste (McGlinchey *et al.*, 2001; Videler, 2011; Rasmussen *et al.*, 2013). Therefore, practices that increase the proportion of red muscle may not be looked upon favourably by the aquaculture industry, particularly if the benefits of exercise training are otherwise marginal. This has not yet been investigated in hapuku but considering that there is a limited and variable effect of exercise on growth in this species (Khan *et al.*, 2014b), it may warrant investigation if exercise training is to be fully considered.

Fillet firmness is known to be positively linked to the amount of connective tissue in white muscle (collagen, elastin, glycoproteins *etc.*), particularly in raw fish products (Hatae *et al.*, 1990; Rasmussen *et al.*, 2013). It is also known that the amount of connective tissue is positively related to MFD as a large proportion of the connective tissue exists between muscle fibre bundles (Sato *et al.*, 1986; Rasmussen *et al.*, 2013). Therefore, as MFD increases then logically fillet firmness should also increase due to there being more connective tissues holding the fibres together (Kanoh *et al.*, 1988; Hatae *et al.*, 1990). In juvenile hapuku, fibre sizes are considerably smaller, and MFD is considerably higher than most salmonids (Johnston *et al.*, 2000; Bugeon *et al.*, 2003; Johnston *et al.*, 2006; Rasmussen *et al.*, 2011) and several non-salmonid species (Davison and Goldspink, 1978; Periago *et al.*, 2005) but is comparable to that of farmed Atlantic halibut (Hagen *et al.*, 2007). Because of this, it is not surprising that hapuku flesh is firmer than the above mentioned species and that decreasing MFD in hapuku is tightly correlated with decreasing fillet firmness (Fig. 2C and D). It is important to note that though higher MFDs are generally positively related to fillet firmness, the two variables were found to be unrelated in the Atlantic cod (Bjørnevik *et al.*, 2003) suggesting that this relationship may not be universal.

Firmness is an important component of the perceived product quality of fish and has the potential to significantly affect retail values of farmed fish, though it cannot always be determined visually. Unlike the exogenous and visually comparable characteristics of fish that sustained exercise can effect [reduced fin damage with reduced aggression, improved condition factors, skin colour and homogeneity *etc.* (Sveinsdottir *et al.*, 2003; Rødbotten *et al.*, 2009; Rasmussen *et al.*, 2013)], fillet firmness is more likely to affect repurchasing rates and perhaps the likelihood of farmed hapuku finding its way on to the menus of high-end eateries. It would also be of interest to compare the general quality (organoleptic properties, for example) of exercised and non-exercised hapuku to their wild equivalents. The firmness of farmed fish is usually found to be less than that of wild-caught fish [sea bass (Periago *et al.*, 2005) and Atlantic salmon (Johnston *et al.*, 2006) for example] but, as of yet, there are no published data on the flesh firmness of wild hapuku or related wreckfish species (*Polyprion americanus*, for example). Similarly, potential changes in the gross fillet constituents of the exercised and non-exercised hapuku have not been measured so it is not yet known how they compare to wild fish. Comparisons between the two will show whether the farmed product can match, or even exceed, the quality of the wild product and where it fits into local and export markets.

5.5 Conclusions

Sustained exercise induces white-muscle hypertrophy and reduces the MFD of juvenile hapuku which, due to its presumed link with the amount of connective tissue in the flesh, also reduces fillet firmness. Considering that softer flesh is generally considered to be less desirable, it appears that exercise training may not increase the quality of farmed hapuku. However, the effects of exercise on fillet constituents and organoleptic properties still requires investigation and there may yet be improvements to factors such as protein, moisture and lipid proportions which could also be altered in some way by exercise for a positive effect on consumer preference. Additionally, a general comparison needs to be made between wild and farmed hapuku in terms of factors such as MFD, texture and taste to determine how the farmed product compares.

Chapter 6 – General Discussion

6.1 Introduction

Understanding aspects of production performance such as growth and the feed conversion ratio (FCR) are the ultimate goals for many investigations into finfish aquaculture. These investigations also give the empirical basis for management decisions based around maximising production performance and economic output (Ernst *et al.*, 2000). For species that are classically associated with aquaculture, or are of considerable commercial interest, there is an ever-expanding pool of data available which can be integrated into production, husbandry, engineering and management decisions [salmonids, for example (Brett, 1976; Maxime *et al.*, 1989; Jobling *et al.*, 1993b; Thorarensen and Farrell, 2006; Aunsmo *et al.*, 2014)]. Novel finfish species have little empirical data to base decisions on, and as this information is often commercially sensitive during the development of hatchery and on-growing practices, most of it is kept in-house. Commercialisation of any new species will require a base of empirical data to determine financial viability, the appropriateness of proposed farming locations, and the opportunity cost of one species compared to any other (Kam *et al.*, 2003; Duncan *et al.*, 2013).

To allow for growth, the physiological costs of protein synthesis have to be balanced against all other immediate physiological costs (Jobling, 1994; Owen, 2001). As a result, growth rates can only be maximised when: i) the capacity for physiological work is maximised and several costs can be accommodated simultaneously, and/or ii) all other physiological costs are reduced and growth is able to take up a large proportion of metabolic capacity (aerobic metabolic scope, AMS) (Brett and Groves, 1979; Jobling, 1994). This balancing of metabolic resources with changing demand is the basis of ectothermic bioenergetics (Jobling, 1994; Owen, 2001). In the preceding chapters, the metabolic consequences of changes in temperature, feeding and swimming speed have been measured and compared to production performance (growth and FCR) in juvenile hapuku *Polyprion oxygeneios*. This concluding chapter aims to produce a simple but integrated model of their bioenergetic budget which can be used to make physiologically informed decisions regarding the culture of this

species. Additionally, this chapter aims to explore some of the factors that would affect the model and propose future testable hypotheses of interest to the successful commercialisation of hapuku.

6.2 Modelling the energetic budget of juvenile hapuku

6.2.1 The model

There are several different approaches to modelling the energetics of fish metabolism which generally depend on the breadth of data available. Factorial approaches, such as those used by Pirozzi *et al.* (2010) are becoming more common but require information regarding the energetic content of fish carcasses which was not in the scope of the current study. Considering the data available on hapuku, a more simplistic approach, such as that of Owen (2001) is more appropriate. Within the integrated energetic model of Owen (2001) for the European eel *Anguilla anguilla*, respiratory metabolism (R) was divided into three primary components and expressed as:

$$R = R_s + R_f + R_a$$

where R_s is the resting metabolic rate (SMR), R_f is SDA and R_a is the metabolic cost of locomotion (Priede, 1985). For the sake of simplicity and continuity, the formula applied here will read:

$$R\ (17\ °C) = \text{SMR}\ (17\ °C) + \text{SDA}\ (17\ °C) + MO_{2swim}\ (17\ °C)$$

As this thesis only contains data for the physiological costs of swimming at 17 °C, most of the summaries will be based around this temperature. The SDA values are given as the post-feeding MO_2 peak taken at the point where the line between the 1.5%

ration SDA values at 15 °C and 21 °C intersect 17 °C, less the SMR estimates at 17 °C [see Khan *et al.* (2014b) and Chapter 4]. The 1.5% ration size which was representative of the feeding levels in the exercise trials (Chapter 4) and is therefore the most appropriate for these analyses. MO_{2swim} is the metabolic cost of swimming at the stated swim speed which were calculated as the pooled average of each of the 0.0, 0.75 and 1.5 BL s^{-1} ER trained groups, less SMR, at 17 °C.

A more extensive model based on energy intake was proposed by Secor (2009) and expressed as:

$$EI = FE + UE + SMR + SDA + AMR + growth + reproduction + fat$$

where the EI is the ingested energy, FE and UE are the amount of energy excreted as faeces and urine, AMR is the active metabolic rate (equivalent to a standard level of MO_{2swim}) and growth/reproduction and fat represent the amount of energy incorporated into the body during these processes (Elliot, 1976). Though this model is more comprehensive, it contains several elements that were not measured in the current set of investigations and cannot be applied reliably. This immediately raises an issue as only energetic requirements under a limited number of conditions can be estimated. It is not yet possible to balance all energy inputs and losses from feed as has been done recently in other species such as the cobia *Rachycentron canadum* and the yellow grouper *Epinephelus awoara* (Sun *et al.*, 2006; Sun *et al.*, 2007). In this scenario, it has to be assumed that excess energy from feed (not used in the measured processes of SMR, SDA and swimming) is lost to the environment as faeces (FE) and urine (UE) and branchial excretion (Evans *et al.*, 2005). The limitation of this approach will be discussed in the next section.

6.2.2 Balance of oxygen and energy allocation

Assuming that the SMR of a 500 g hapuku at 17 °C is constant for an entire 24 h period (Chapter 4), the oxygen requirements of a juvenile hapuku fed two rations of ~1.5% body weight (BW) of dry feed, swimming at ~**0.0 BL s^{-1}** (stationary), can be modelled as:

$$R = 87.52\ mgO_2\ kg^{-1}\ h^{-1}\ (SMR,\ 17\ °C) + 102.28\ mgO_2\ kg^{-1}\ h^{-1}\ (SDA,\ 1.5\%) + 0.0\ mgO_2\ kg^{-1}\ h^{-1}\ (MO_{2swim},\ 0.0\ BL\ s^{-1}) = 189.8\ mgO_2\ kg^{-1}\ h^{-1}$$

which equates to a daily oxygen requirement of 4555.2 $mgO_2\ kg^{-1}$ or an energetic requirement of 64.05 kJ kg^{-1} [using the oxycalorific coefficient of 14.06 kJ g O_2^{-1} (Gnaiger, 1983; Jordan and Steffensen, 2007)]. If this was standardised to a 500 g fish, they would require approximately 32.02 kJ d^{-1} yet, from two 1.5% BW rations of Skretting Nova FF 5 mm pellets (Skretting, Australia, 50.0% protein, 17.0% lipid, digestible energy 18.6 MJ kg^{-1}), they would receive 279 kJ of digestible energy.

The energetics of a fish under the same conditions but swimming at **~0.25 BL s^{-1}** (considered to be "milling about" in a tank with low directional flow) could be modelled as:

$$R = 87.52\ mgO_2\ kg^{-1}\ h^{-1}\ (SMR,\ 17\ °C) + 102.28\ mgO_2\ kg^{-1}\ h^{-1}\ (SDA,\ 1.5\%) + 2.1\ mgO_2\ kg^{-1}\ h^{-1}\ (MO_{2swim},\ 0.25\ BL\ s^{-1}) = 191.9\ mgO_2\ kg^{-1}\ h^{-1}$$

which equates to a daily oxygen requirement of 4605.6 $mgO_2\ kg^{-1}$ or an energetic requirement of 64.75 kJ kg^{-1}. In this scenario, a 500 g fish would require ~32.4 kJ d^{-1} and would receive 279 kJ of digestible energy from two 1.5% BW rations.

A fish swimming at **~1.5 BL s^{-1}** (the highest speed applied in the growth trials of Chapter 4) could be modelled as:

$$R = 87.52\ mgO_2\ kg^{-1}\ h^{-1}\ (SMR,\ 17\ °C) + 102.28\ mgO_2\ kg^{-1}\ h^{-1}\ (SDA,\ 1.5\%) + 107.52\ mgO_2\ kg^{-1}\ h^{-1}\ (MO_{2swim},\ 1.5\ BL\ s^{-1}) = 297.32\ mgO_2\ kg^{-1}\ h^{-1}$$

which equates to a daily oxygen requirement of 7135.68 mgO_2 kg^{-1} or an energetic requirement of 100.33 kJ kg^{-1}. A 500 g fish would require ~50.16 kJ d^{-1} and would receive 279 kJ of digestible energy from two 1.5% BW rations.

A basic model of the energy consumption of juvenile hapuku exposed to different swim speeds are shown in Table 6.1.

Table 6.1. Oxygen and energy requirements for juvenile hapuku *P. oxygeneios* swimming at different speeds at 17 °C. SMR, SDA and MO_{2swim} values are taken from Chapters 3 and 4 respectively. kJ requirements are calculated using the oxycalorific value of 14.06 kJ g O_2^{-1} from Gnaiger (1983). Note that a standard 500 g fish receiving two 1.5% body weight rations of Skretting Nova FF pellets (Skretting, Australia, 50.0% protein, 17.0% lipid, digestible energy = 18.6 MJ kg^{-1}) per day would receive a total of 279 kJ of digestible energy.

Swim speed (BL s^{-1})	SMR + SDA (mgO_2 kg^{-1} h^{-1})	MO_{2swim} (mgO_2 kg^{-1} h^{-1})	Daily O_2 requirements (mgO_2 kg^{-1})	Daily kJ requirements (kJ kg^{-1})	Daily kJ required for a standard 500 g fish
0.0	189.8	0.0	4555.2	64.05	32.02
0.25	189.8	2.1	4605.6	64.75	32.4
0.5	189.8	35.98	5418.72	76.19	38.1
0.75	189.8	66.35	6147.6	86.44	43.22
1.0	189.8	73.04	6308.16	88.69	44.35
1.5	189.8	107.52	7135.68	100.33	50.16
2.0	189.8	174.55	8744.4	122.95	61.47
2.5	189.8	230.97	10098.48	141.98	70.99

6.2.3 Assumptions of the model

The energetics shown in Table 6.1 assume that the physiological costs of SDA are constant with changing swim speeds. This assumption appears to hold true in the rainbow trout *Oncorhynchus mykiss* up to speeds of up to 3 BL s^{-1} at which point the combined costs of swimming and SDA in fish fed a 3% ration increase faster than those fed a 1% ration (Alsop and Wood, 1997). Similarly, in the qingbo *Spinibarbus sinensis* swimming up to 4 BL s^{-1}, there is a very limited change in the combined costs of swimming and SDA over the observed range of speeds (Pang *et al.*, 2011; Li *et al.*, 2013b). A constant difference between the swimming costs of fed and starved goldfish *Carassius auratus* and common carp *Cyprinus caprio* suggests that the costs of SDA

are unchanged at different swimming speeds (Pang *et al.*, 2011). Therefore it seems safe to assume that the costs of SDA for a juvenile hapuku at 17 °C fed a 1.5% ration do not change significantly between 0 BL s^{-1} and 1.5 BL s^{-1}. At speeds greater than 1.5 BL s^{-1}, Fig. 6.1 would suggest that there may have to be trade-off between the costs of swimming and SDA, though this does not apply to the growth trials in Khan *et al.* (2014b)/Chapter 4 where the maximum treatment was 1.5 BL s^{-1}.

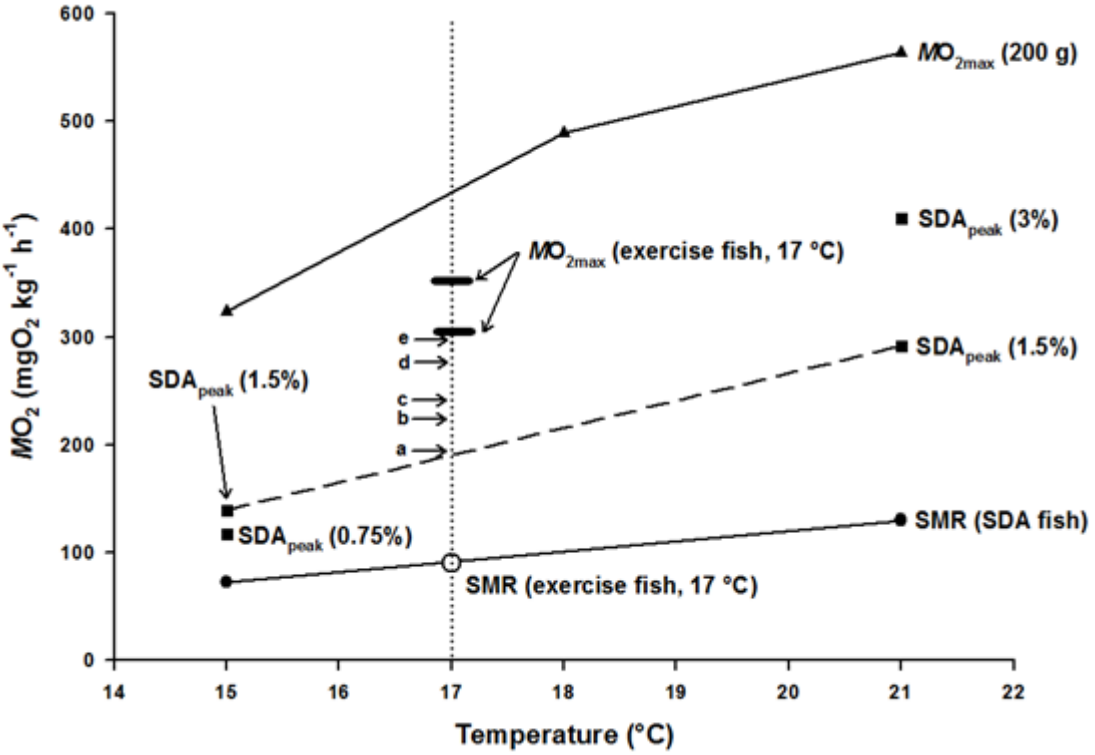

Figure 6.1. A model of the energy budget of juvenile hapuku *P. oxygeneios* in the range of 200 g – 500 g. SMR values for 15 °C and 21 °C (filled circles) are taken from Chapter 3 whilst SMR estimates for similarly-sized fish (~500 g) at 17 °C were measured directly (Chapter 4) (large open circle). SDA_{peak} ration values (post-feeding MO_2 peak, filled squares and broken lines, shown with their corresponding ration sizes) are taken from Chapter 3. Only the 1.5% ration size was fed to fish acclimated to both 15 °C and 21 °C and hence they are the only connected points. It is likely that the SDA costs shown can accurately interpolate the feeding costs of fish in the current study at 17 °C because the fixed 1.5% BW d^{-1} ration of Chapter 3 closely approximates the 1.3% – 1.8% BW d^{-1} *ab libitum* ration level of fish from the different ER treatments (Chapter 4). The thickened horizontal marks at 17 °C show the highest and lowest average MO_{2max} values of fish as measured in a Brett-type swim flume respirometer at 17 °C while the small arrows show the cost of swimming (in addition to SDA) at; (a) 0.25, (b) 0.5, (c), 0.75, (d) 1.0 and (e) 1.5 body lengths per second (BL s^{-1}) (taken from Chapter 4). MO_{2max} values (filled triangles and solid lines) are taken from Khan *et al.* (2014a) and measured in smaller, 200 g fish.

The calculation of daily energy requirements in Table 6.1 also assumes that the applied swimming speed treatment is not variable and is maintained all day by each individual. This assumption is not necessarily valid as: i) flows vary spatially in a circular tank so the desired treatment speed was not maintained in all areas at all times, ii) fish can actively choose to position hold or swim faster or slower than the flow speed they are experiencing, iii) the small projection PVC pipe in each tank in our experiments created a low flow wake (described in Chapter 4, though was probably only relevant at speeds $\geq$ 0.5 BL s^{-1}) and could act as a refuge from currents and iv) spontaneous activity rates have not yet been measured. This assumption is applied for simplicity and consistency across different swimming treatments. Swimming behaviour was recorded for the last 2 weeks of trial 1 and the entire 8-week duration of trial 2 and will ultimately provide valuable insight into daily swimming behaviour patterns as well as the effectiveness of the different swimming treatments. Unfortunately it was not able to be analysed in the scope or time-frame of this PhD but is being analysed by an MSc student with the intention of publishing it as a complement to Khan *et al.* (2014b).

It is also assumed that oxycalorific value 14.06 kJ g O_2^{-1} (Gnaiger, 1983) that is used to convert rates of oxygen to energy usage is relevant to juvenile hapuku. The oxycalorific conversion values given by Gnaiger (1983) have been used in more than 300 studies to calculate bioenergetics for a range of different marine organisms such as sea urchins (Stumpp *et al.*, 2011), shellfish (Beyer *et al.*, 2013; Hawkins *et al.*, 2013) and fishes (Finn and Fyhn, 2010; Behrens *et al.*, 2012). Though this value has not been confirmed to be directly relevant to juvenile hapuku, it is based on the stoichiometry of proton release during respiration in reactions under standard conditions [pH = 7, for example (Gnaiger, 1983)], which gives it a high degree of applicability to a wide range of animals. To confirm it directly for this species would require a comparison between metabolic rates measured through the rate of oxygen consumption (respirometry) and direct calorimetry (Regan *et al.*, 2013) but, considering the prevalence of these assumptions, to use a different oxycalorific value or to not use it all would reduce the strength of comparisons to other investigations.

As the energetic losses from faeces and urine have not been measured in the current investigations, it is not possible to fully determine the final fate of the energy in feed as only the energy use through oxygen consumption has been measured. Taking

the energy requirements of a standard 500 g fish swimming at 1.0 BL s^{-1} as an example (Table 6.1), there is an excess of 234.65 kJ in the diet compared to the requirements of maintenance (SMR), feeding and growth (SDA) and swimming (MO_{2swim}) which equates to an excess of 84.1% of ingested energy. Where the energy content of faecal matter has been measured in other species, values are consistent and in the range of approximately 12.9% – 14.2% of ingested energy for six species of teleost fish (Ciu and Liu, 1990) and 9.1% – 14.9% of ingested energy at various temperatures and rations sizes in the barramundi *Lates calcarifer* (Bermudes *et al.*, 2010). Where the energy content of both faeces and urine have been measured, the higher values are in the range of approximately 25% of ingested energy (Du Preez and Cockroft, 1988a; Du Preez and Cockroft, 1988b). This creates a considerable discrepancy between the estimates for a juvenile hapuku swimming at 1.0 BL s^{-1} or any other speed (Table 6.1). There are several potential sources of error associated with these calculations which need to be considered. One possible major source of error is that the measurements of energy requirements in juvenile hapuku may have been underestimated. To determine if this is the case, the energy requirements and usage of juvenile hapuku will be discussed in the next section, along with factors that would modify energy usage and have consequences for the proposed model of the energetic budget for this species (Fig. 6.1).

6.3 Energy requirements, usage, modifiers and the implications for growth performance

6.3.1 The discrepancy between feed input (DE) and usage

A 500 g juvenile hapuku requires 12.27 kJ d^{-1} at 15 °C and 21.94 kJ d^{-1} at 21 °C to maintain SMR (SMR data from Chapter 3) and 14.77 kJ d^{-1} at 17 °C (Chapter 4 and Table 6.1) making their requirements comparable to other fish species using several different methods [see Cho and Bureau (1995b) for an explanation of different methods]. For an equivalent 500 g fish, maintenance digestible energy requirements (DE, to maintain body weight) are 27.51, 26.06 and 19.56 kJ between 19 °C and 27 °C for the gilthead seabream *Sparus aurata*, European seabass *Dicentrarchus labrax* and

white grouper *Epinephelus aeneus* respectively calculated using weight loss during starvation (Lupatsch *et al.*, 2003). Using the same method, red tilapia *Oreochromis niloticus* require somewhere between 14.36 kJ d^{-1} at 26 °C (Meyer-Burgdorff *et al.*, 1989) and 22.97 kJ d^{-1} in the temperature range of 20.9 °C – 24.3 °C (Hepher *et al.*, 1983). From measuring metabolic rate, the rainbow trout *Oncorhynchus mykiss* required 26.62 kJ d^{-1} at 20 °C (Cho and Kaushik, 1990). These values are not dissimilar from the values estimated for juvenile hapuku in the current study and suggest that maintenance costs are not where the discrepancy between DE input and usage is occurring.

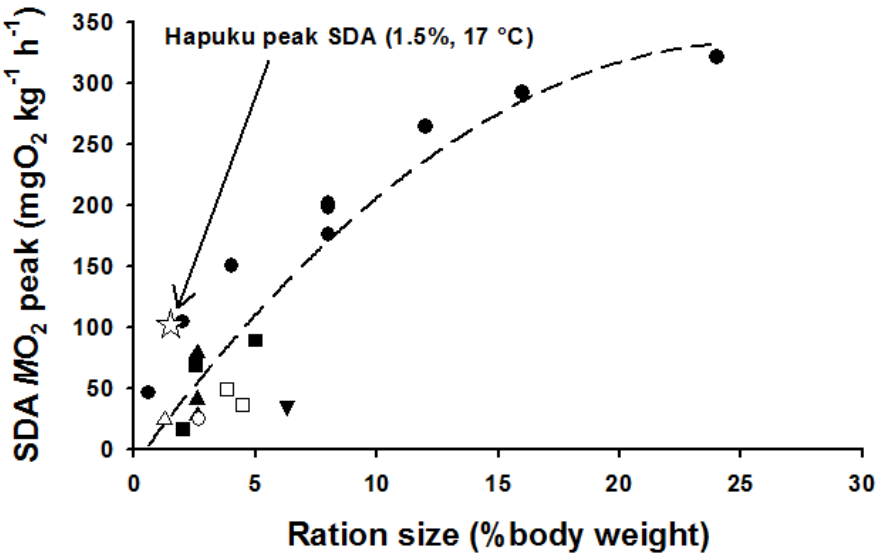

Figure 6.2. An analysis of the peak of the SDA response in mgO_2 kg^{-1} h^{-1} in a range of fish species in relation to the size of the ration ingested. Filled circles are *Silurus meridionalis* at 27.5 °C (Fu *et al.*, 2005a; Fu *et al.*, 2005b), filled squares are *Gadus morhua* at 10 °C (Jordan and Steffensen, 2007; Behrens *et al.*, 2012), filled upright triangles are *Mugil saliens* at 20 °C (Guinea and Ferna´ndez, 1991), filled upside down triangles are *Paranotothenia magellanica* at 10 °C, open circles are *Paranotothenia sima* at 10 °C, open squares are *Harpagifer bispinis* at 10 °C and the open triangles are *Austrolycus depressiceps* at 10 °C (Vanella *et al.*, 2010). The relationship between the peak of the SDA response and ration size is described with a second-order polynomial regression (broken line) and is significant ($F = 5.69$, $R^2 = 0.83$, $P < 0.05$). The larger open star shows the estimate of the peak of the SDA response for a juvenile hapuku at 17 °C fed a 1.5% ration which was not used in the regression analysis.

As with the costs of maintenance, it does not appear that the energetic costs associated with SDA and growth have been underestimated in juvenile hapuku compare to other fish species (Fig. 6.2). SDA varies with each of species (Secor, 2009), fish size (Secor and Faulker, 2002; Fu *et al.*, 2005b), temperature (Guinea and Fernandez, 1997; Pang *et al.*, 2011; Frisk *et al.*, 2013) and ration size (Guinea and Fernandez, 1997; Secor and Faulker, 2002; Jordan and Steffensen, 2007) making generalisations about energetic costs difficult. However, the estimated peak in MO_2 incurred in juvenile hapuku after eating a 1.5% ration at 17 °C fits into the upper end of the range of responses incurred by a range of species with different ecotypes, fed different sized rations and at different temperatures (Fig. 6.2). If the values for the southern catfish *Silurus meridionalis*, which cover the largest range of both MO_2 peaks and ration sizes [0.6% – 24% of body weight, 46.1 – 321.4 $mgO_2\ kg^{-1}\ h^{-1}$ (Fu *et al.*, 2005a; Fu *et al.*, 2005b)] were removed the estimate for juvenile hapuku would be considerably higher than the estimates from species fed similar ration sizes such as the 23.9 $mgO_2\ kg^{-1}\ h^{-1}$ peak for a 1.24% ration in *Austrolycus depressiceps* at 10 °C (Vanella *et al.*, 2010) and the 78.4 $mgO_2\ kg^{-1}\ h^{-1}$ peak for a 2.6% ration in *Mugil saliens* at 20 °C (Guinea and Ferna´ndez, 1991). These comparisons suggest that the peak SDA estimates for juvenile hapuku fed a 1.5% ration at 17 °C are not lower than would be expected and that these values are probably not the source of the discrepancy between DE input and usage.

The sustained swimming costs of a standard 500 g juvenile hapuku are also in the range of other fish species and, again, this suggests that these estimates are not the source of discrepancy between energy input and usage. In Table 6.1, when the 17.26 kJ d^{-1} associated with SDA are removed, it shows that a 500 g hapuku at 17 °C requires 27.09 kJ d^{-1} and 44.21 kJ d^{-1} to maintain 1 BL s^{-1} and 2 BL s^{-1} respectively. These estimates are slightly lower than those of the kingfish *Seriola lalandi* calculated from the MO_2 values of Brown *et al.* (2011) and also standardised to 500 g which were 40.49 kJ d^{-1} and 60.74 kJ d^{-1} at 1 BL s^{-1} and 2 BL s^{-1} respectively. The estimates for juvenile hapuku were equivalent, however, to the energy requirements for kingfish from Yanase *et al.* (2012) which were 26.32 kJ d^{-1} and 39.48 kJ d^{-1} for 500 g kingfish at 1 BL s^{-1} and 2 BL s^{-1} respectively. Coho salmon *Oncorhynchus kisutch* exhibit higher sustained swimming costs of approximately 43.12 kJ d^{-1} for transgenic fish and 65.19 kJ d^{-1} for ocean ranched fish at 1 BL s^{-1} (500 g) (Lee *et al.*, 2003a) while

juveniles of the pelagic scombrid, the kawakawa *Euthynnus affinis*, have considerably lower daily energy costs of 16.65 kJ d^{-1} and 21.1 kJ d^{-1} for maintaining 1 BL s^{-1} and 2 BL s^{-1} respectively as calculated from the average relationship between MO_2 and swimming speed for 10 individuals (Sepulveda and Dickson, 2000). These examples span a range of swimming modes, from subcarangiform (hapuku and coho salmon) to carangiform (kingfish) to thunniform (kawakawa) [see Blake (2004) for a review] and these different swimming modes are reflected in their daily swimming costs. The fact that the daily swimming costs of juvenile hapuku are consistent with those of other species suggests that the swimming costs shown in Table 6.1 are representative of the cost of sustained swimming. They do not, however, take into account the considerable costs of spontaneous activity/swimming behaviour (Boisclair and Tang, 1993; Tang *et al.*, 2000) which will be discussed in a later section.

If the discrepancy between DE input and energy usage cannot be attributed to the underestimation of the costs of maintenance (SMR), feeding and growth (SDA) or sustained swimming then the other potential sources of error are mainly limited to the DE of the feed itself and the energy lost to the environment. The DE values of the feed used in the current investigations (18.6 MJ kg^{-1}) were provided directly by Skretting, Australia and are not dissimilar from the DE content of pelleted feeds used in a variety of experiments based on the production performance of finfish species [21 – 23 MJ kg^{-1} (Raven *et al.*, 2006), 14.2 – 20.1 MJ kg^{-1} (Lupatsch *et al.*, 1997), 10.2 – 21.57 MJ kg^{-1} (Lupatsch *et al.*, 2001), for example]. Therefore, it seems unlikely that these values have been overestimated. The next logical step in addressing this issue is to measure the energy content of the faecal and nitrogenous output of juvenile hapuku under standardised conditions. It is notoriously difficult to provide accurate information regarding the energy lost through the excretion of nitrogenous waste as this will vary with both the amount and the composition of excretory products measured (Cho and Kaushik, 1985; Jobling, 1993). However, Cho and Kaushik (1985) have suggested that a conversion factor of 25 kJ g^{-1} of N excreted be applied to standardise comparisons between species. Based on this factor, total nitrogen excretion should account for between 4% and 15% of the energy in ingested feed. This conversion factor, as well as studies in other species would suggest that these values would not be large enough to balance the considerable excess of energy that is seemingly available to hapuku under the investigated conditions (Du Preez and Cockroft, 1988b; Ciu and Liu, 1990;

Bermudes *et al.*, 2010). As an aside, rates on nitrogen excretion are extremely useful in determining the potential environmental effects of the full-scale commercial culture of novel species (Read and Fernandes, 2003; Islam, 2005; Vezzulli *et al.*, 2008) and thus warrant further investigation regardless.

6.3.2 Meal size, meal energy, growth and SDA

The DE input for a 500 g fish fed two 1.5% rations per day is in excess to the energy required to maintain SMR, feeding, growth and swimming. Even the DE of a single 1.5% ration (139.5 kJ) is adequate for maintaining for these costs for an entire 24 h period (Table 6.1). This then raises the question as to why juvenile hapuku show variable growth rates between the single 1.5% ration per day, two 1.5% rations per day and a single 3% ration per day feeding treatments at 21 °C [Khan *et al.* (2015), Chapter 3] when there is an excess of DE for all treatments. It is known that the amount of DE in the diet is positively related to growth performance in several species including the gilthead sea bream *Sparus aurata* (Lupatsch *et al.*, 1997; Lupatsch *et al.*, 2001) and the turbot *Psetta maxima* (Dietz *et al.*, 2012). If DE is positively related to growth performance in juvenile hapuku, then significantly faster growing fish at 21 °C that were fed either two 1.5% rations or a single 3% ration per day would be expected to grow faster than those fed only a single 1.5% ration. The positive relationship between DE and growth does not hold, however, for the comparison between fish that were fed two small 1.5% rations per day and the group that were fed one large 3% ration per day at 21 °C where DE content is equivalent but growth performance is significantly lower for fish fed the single large ration (Fig. 3.2, Chapter 3). Furthermore DE does not appear to be limiting in the current investigations so it is not possible to come to the same simple conclusion as Lupatsch *et al.* (2001) that DE is positively related to growth.

As the significantly higher growth of fish at 21 °C fed two 1.5% rations per day compared to those fed a single 3% ration per day cannot be attributed to changes in DE, it could be that the observed difference in growth is driven by changes in feeding/digestive efficiency with increasing meal size and the associated SDA response (Jobling, 1983; Secor and Faulker, 2002; Fu *et al.*, 2005b; Secor, 2009). Increasing meal size increases the post-feeding peak in $\dot{M}O_2$, the duration of SDA and the total energy consumed during the response (SDA energy), primarily due to the

increasing effort needed to digest and assimilate a larger meal (Secor and Diamond, 1995; Jordan and Steffensen, 2007). When looking at the difference in the SDA parameters for the single 1.5% and 3% meals at 21 °C, peak MO_2, SDA energy and SDA coefficient all increase significantly as ration size increases, along with growth performance [Khan *et al.* (2015), Chapter 3]. It is therefore hypothesised that the second meal (two discrete 1.5% rations *vs.* a single 3% ration) during the day would maintain increased levels of post-absorptive protein synthesis, observed as an increase in oxygen consumption, for longer periods. This may be the result of the SDA response being restarted after the second meal, as seen in the southern catfish *Silurus meridionalis* where the repeat feeding of a 2% ration every 12 h maintained MO_2 at levels considerably higher than SMR at all times (Fu *et al.*, 2005a). In effect, a second SDA response in a day would increase the total SDA energy and also potentially the SDA coefficient, which would explain the significantly improved growth performance observed for fish at 21 °C fed two 1.5% rations (*vs*. a single 3% ration) per day.

Hapuku swimming at high speeds can push MO_2 towards MO_{2max} estimates when the estimated SDA value for a 1.5% ration at 17 °C is included (Fig. 6.1). If ration sizes were to increase above 1.5% of body weight per feed, then presumably the SDA peak would also increase (Secor and Faulker, 2002; Jordan and Steffensen, 2007; Secor, 2009) and the ability to swim at high speeds might be constrained by aerobic capacity (AMS) (Owen, 2001). Doubling of ration size increases the peak of the SDA response by 25.3% (Jordan and Steffensen, 2007) and 31.2% (Soofiani and Hawkins, 1982) at 10 °C in the Atlantic cod *Gadus morhua*, 17.1% at 30 °C in the marine toad (Secor and Faulker, 2002) and 20.93% at 27.5 °C in the southern catfish (Fu *et al.*, 2005b). Juvenile hapuku showed an 18.1% increase in their peak post-feeding MO_2 between 0.75% and 1.5% rations at 15 °C and a 40.4% increase between 1.5% and 3% rations at 21 °C, suggesting that they are generally within the range of ration-related increases observed in other species. If hapuku at 17 °C were able to take a 3% ration, it is likely that there could also be an approximately 40% increase in the post-feeding MO_2 peak. This results in an increase from 102.28 $mgO_2\ kg^{-1}\ h^{-1}$ to 143.19 $mgO_2\ kg^{-1}\ h^{-1}$ which pushes the combined costs of SDA and swimming at 1.0 BL s^{-1} and 1.5 BL s^{-1} above the lowest estimate for MO_{2max} in equivalently sized fish. Previously, where SDA and activity costs have had to be balanced within available AMS, it is activity, or the capacity for activity, that is first reduced to accommodate the increasing costs of

digestion and protein synthesis. This has been demonstrated in the European eel where peaks in protein synthesis and activity did not occur at the same time and spontaneous swim speeds were lower post-feeding (Owen, 2001). Similarly, the rainbow trout and southern catfish both experienced a significant reduction in U_{crit}, the maximum sustainable swimming speed, in fed fish (Alsop and Wood, 1997; Pang *et al.*, 2010). It is therefore hypothesised that larger meals would also reduce the swimming performance of juvenile hapuku as activity levels, or at least the capacity for aerobic activity, will be reduced to accommodate the rise in energetic costs of protein synthesis and growth (Alsop and Wood, 1997; Pang *et al.*, 2010).

6.3.3 Protein synthesis as a limiting factor for growth

It is clear that there is ample DE in the diet to grow and that the costs of growth and swimming are easily encompassed within available AMS. This raises the question as to what is limiting growth rates below 17 °C and driving improved growth rates at temperatures up to 21 °C. AMS ultimately limits the maximum capacity for protein synthesis associated with growth (Houlihan *et al.*, 1995; Smith and Houlihan, 1995) as growth is very unlikely to occur anaerobically. However, protein synthesis itself is also known to be limited by temperature by controlling the maximal rate of enzymatic reactions (Houlihan *et al.*, 1995; Katersky and Carter, 2007). In fish, protein synthesis rates are generally positively and linearly related to temperature (Haschemeyer *et al.*, 1979; McCarthy *et al.*, 1999; Doherty *et al.*, 2012), as seen in the rainbow trout (Mathers *et al.*, 1993) and the Atlantic wolffish *Anarhichas lupus* (McCarthy *et al.*, 1999). Rates of protein synthesis were also positively related to temperature in juvenile barramundi *Lates calcarifer*, though they did plateau within an optimal temperature range of 27 °C – 33 °C (Katersky and Carter, 2007). In oxygen and feed saturated environments, such as those used in the current investigations, it may simply be that the major limiting factor for the growth of juvenile hapuku is the rate at which they can synthesise protein. Interestingly, though the Atlantic wolffish demonstrated a linear relationship between protein synthesis rates across the suspected temperature range of this species (Moksness and Pavlov, 1996), it also showed an overall parabolic relationship between the efficiency of protein retention (synthesis rates – degradation rates) and temperature. This is the same relationship seen in juvenile hapuku between temperature, AMS and SGR (Khan *et al.*, 2014a). Specifically measuring rates of

protein synthesis and degradation in juvenile hapuku in the future may reveal a major mechanism in the relationship between growth and temperature in this species and, based on the results obtained for other species (Houlihan *et al.*, 1995; Katersky and Carter, 2007; Doherty *et al.*, 2012), it is hypothesised that the rate of protein retention would show the same parabolic relationship with temperature as AMS and SGR (Khan *et al.*, 2014a).

6.3.4 The costs of swimming

At 17 °C, the combined costs of SDA and swimming at speeds up to 1.5 BL s^{-1} do not appear to reach the ceiling of aerobic capacity in juvenile hapuku (Fig. 6.1). However, while the costs associated with SDA have been measured at both an optimal and suboptimal temperature (15 °C and 21 °C), the cost of swimming has only been measured at 17 °C and this gives little insight into the potentially limiting effects of AMS on the capacity to balance the costs of SDA and swimming at different temperatures. At the species level, the response to increasing temperature is an increase in the cost of swimming at any speed (Sepulveda and Dickson, 2000; Dickson *et al.*, 2002; MacNutt *et al.*, 2006; Ohlberger *et al.*, 2007), though the interaction between the cost of swimming and temperature appears to be species/ecotype dependant. As an example of the variation; between 18 °C and 24 °C, swimming costs increase 56.6% at 1 BL s^{-1} and 14.4% at 2 BL s^{-1} in the chub mackerel *Scomber japonicus* (Sepulveda and Dickson, 2000; Dickson *et al.*, 2002) whereas, between 4 °C and 8 °C, net swimming costs increase by 150% at 1 BL s^{-1} in the vendace *Coregonus albula*, with a further 60% increase between 8 °C and 15 °C (Ohlberger *et al.*, 2007). Salmonids in particular appear to show a great deal of variation between studies. The cost of swimming at 1 BL s^{-1} in the sockeye salmon *Oncorhynchus nerka*, for example, varies from ~510 mgO_2 kg^{-1} h^{-1} at 13 °C (Lee *et al.*, 2003b) to ~300 mgO_2 kg^{-1} h^{-1} at 15.8 °C (MacNutt *et al.*, 2006) and ~450 mgO_2 kg^{-1} h^{-1} at 17 °C (Eliason *et al.*, 2013). As an additional complication, the limiting effect of AMS with increasing swimming costs also depends on how aerobic capacity scales with temperature (Pörtner, 2010; White and Seymour, 2011; Clark *et al.*, 2013). AMS in juvenile hapuku increases from 204.44 mgO_2 kg^{-1} h^{-1} at 15 °C to 374.86 mgO_2 kg^{-1} h^{-1} at 21 °C (Q_{10} = 2.75, 83.4% increase) while the costs associated with SDA increase from 66.03 mgO_2 kg^{-1} h^{-1} to 161.48 mgO_2 kg^{-1} h^{-1} (Q_{10} = 4.44, 144.6% increase) over the same temperature range.

This difference in the rate of increase suggests that there is a decreasing capacity to physiologically accommodate the (presumably) increasing costs of swimming at any speed and, therefore, it is hypothesised that at 21 °C there would be a necessary trade-off between swimming performance and SDA in fed fish. This could manifest as a significant decrease in U_{crit} or voluntary swimming behaviour between fed and starved fish at any temperature as has been seen in other species (Alsop and Wood, 1997; Owen, 2001; Pang *et al.*, 2010).

As mentioned in section 6.2.3, the energetic model presented in Table 6.1 assumes that swimming rates are constant, and at the treatment speed, for an entire 24 h period. This is unlikely to be the case as flows vary spatially in tanks and fish are free to swim at whatever speed they choose at any time. Fish swimming costs are known to increase with swimming complexity (Boisclair and Tang, 1993) and the costs of spontaneous swimming (turning, social interactions, *etc*.) may provide some insight as to the discrepancy between DE input and energy usage in this species.

Table 6.2. An adjusted model of oxygen and energy requirements for juvenile hapuku *P. oxygeneios* swimming at different speeds at 17 °C. SMR, SDA and MO_{2swim} values are taken from Chapters 3 and 4 respectively. MO_{2swim} values were then increased 8-fold as per the average increase in swimming costs associated with spontaneous activity as suggested by Tang *et al.* (2000). kJ requirements are calculated using the oxycalorific value of 14.06 kJ g O_2^{-1} from Gnaiger (1983). The 25% lost energy value is the highest estimate for the combined energy of faeces and urine as a proportion of digestible energy (DE) input from Du Preez and Cockroft (1988a) and Du Preez and Cockroft (1988b). Note that a standard 500 g fish

Swim speed (BL s^{-1})	SMR + SDA (mgO_2 kg^{-1} h^{-1})	Spontaneous MO_{2swim} (mgO_2 kg^{-1} h^{-1})	Daily kJ requirements for a 500 g fish	Daily kJ req. as a prop. of DE intake	Prop. of DE intake less 25% lost energy
0.0	189.8	0.0	32.02	11.48	15.3
0.25	189.8	16.8	34.86	12.49	16.66
0.5	189.8	287.84	80.59	28.89	38.51
0.75	189.8	530.8	121.58	43.58	58.1
1.0	189.8	584.32	130.61	48.81	62.42
1.5	189.8	860.16	177.15	63.49	84.66
2.0	189.8	1396.4	267.62	95.92	127.89
2.5	189.8	1847.6	343.75	123.21	164.28

receiving two 1.5% body weight rations of Skretting Nova FF pellets (Skretting, Australia, 50.0% protein, 17.0% lipid, DE = 18.6 MJ kg^{-1}) per day would receive a total of 279 kJ of DE.

Spontaneous swimming costs for the brook trout *Salvelinus fontinalis* at 4 °C, 8 °C and 12 °C were, on average, 8-fold higher than those of fish swimming at a set speed [ranged from 3-fold to 22-fold higher than forced swimming costs (Tang et al., 2000)]. If the swimming costs of juvenile hapuku are increased 8-fold, total energy usage increases considerably and there is a smaller discrepancy between DE input and the energy used in SMR, SDA and swimming (Table 6.2). Additionally, if the ~25% energy loss from faeces and urine (Du Preez and Cockroft, 1988a; Du Preez and Cockroft, 1988b) are also factored in, the discrepancy decreases further still. The model is speculative as the costs of spontaneous swimming behaviours and the energy lost as faeces and urine have yet to be quantified for juvenile hapuku. Swimming behaviours were, however, recorded during the growth trials of Chapter 4 and are being analysed currently. These analyses, in combination with the known costs of swimming at speeds of up to 2.5 BL s^{-1} at 17 °C, could allow for the exact calculation of spontaneous swimming behaviours in juvenile hapuku which will refine the energetic models shown in Tables 6.1 and 6.2.

6.3.5 Swimming and product quality

Despite the fact that the combined costs of SDA and swimming at speeds ≤ 1.5 BL s^{-1} at 17 °C are encompassed within available AMS (Fig. 6.1) there is not a consistent and significant exercise-induced growth response in hapuku [Khan *et al.* (2014b) Chapter 4]. There is, however, a significant reduction in fillet firmness and muscle fibre density (MFD) for juvenile hapuku trained at speeds ≥ 0.75 BL s^{-1} (Chapter 5). This reduction in firmness is likely driven by the observed decrease in muscle fibre density (MFD) which has also been linked in other species such as the goldfish (Davison and Goldspink, 1978), the roach *Rutilus rutilus* (Broughton *et al.*, 1980) and the European sea bass (Periago *et al.*, 2005). A decrease in MFD as a result of sustained exercise in the rainbow trout, however, did not result in a reduction in fillet firmness (Rasmussen *et al.*, 2011). The bodies of hapuku, like most fish, are primarily comprised of white muscle and alterations to the quality or desirability of white muscle can have significant effects for the viability of a venture (Sänger and Stoiber, 2001; Rasmussen *et al.*, 2013). Indeed, farmed fish are generally found to be

softer than their wild equivalents (Haard, 1992; Periago *et al.*, 2005) though this can be countered with marketing based around perceived positive aspects of farmed fish such as sustainability and "quality mark" labelling (Jaffry *et al.*, 2004). Changes in fillet texture, however, only give some insight into the overall effects of sustained exercise on the final product quality as organoleptic differences (taste, smell, mouthfeel *etc*.) are driven by more than just texture (Rasmussen *et al.*, 2013). Modification to fillet constituents, such as fat and connective tissue (soluble and insoluble collagen) concentrations, have been reported for many fish species as a result of sustained exercise (Periago *et al.*, 2005; Johnsen *et al.*, 2011; Rasmussen *et al.*, 2011) and play an important role in the perceived quality of raw and cooked fish products (Guillou *et al.*, 1995; de Francesco *et al.*, 2004; Izquierdo *et al.*, 2005). Therefore, the full spectrum of exercise effects on the product quality of hapuku needs to be determined before an informed decision on the applicability of exercise training to this species can be made.

6.3.6 FCR drivers

Feed comprises the biggest expense in finfish aquaculture and the rate at which feed is converted to fish mass is an important consideration in determining the viability of novel species (Alvarez-Lajonchère and Ibarra-Castro, 2013). From the current investigations it appears that temperature is the primary driver of FCR in hapuku (Khan *et al*. (2014), Chapter 3) and that ration size, meal periodicity and swimming speed have negligible effects over the ranges investigated (Chapters 3 and 4). It is interesting to note, however, that when hapuku were fed half the ration of other fish at the same temperature (a single 0.75% ration vs. double at 15 °C or a single 1.5% ration vs. double at 21 °C) that their FCR was equivalent to fish that had received twice as much feed and that had grown significantly faster (Fig. 3.2, Chapter 3). Protein gain in the gilthead seabream was asymptotically related to the amount digestible crude protein (DCP) in the diet (Lupatsch *et al*., 2001), as was growth in the brown-marbled grouper *Epinephelus fuscoguttatus* (Shapawi *et al*., 2014), suggesting that they are more efficient at converting protein to fish weight when it is limiting in the diet (Lupatsch *et al*., 2001; Lupatsch *et al*., 2003). If juvenile hapuku also use dietary protein more efficiently when it is limiting in the diet it could possibly explain why

fish fed half rations are able to convert feed as efficiently as those fed full rations, regardless of only receiving half the DCP.

The hypothesised relationship between DCP and FCR could also explain some of the observed changes in FCR with temperature. FCR became increasingly inefficient with increasing temperature in juvenile hapuku and this was contributed to increases in maintenance costs associated with temperature as SMR and FCR were positively and significantly related (Khan *et al.*, 2014a). Feed intake, and hence DCP intake, also increased with temperature up to 21 °C, then decreased at 24 °C (Khan *et al.*, 2014a). Taking the feed per individual and FCR data from Khan *et al.* (2014a), there is a positive and significant linear relationship between DCP and FCR when the values for 24 °C are not included, though it is not significant when the values at 24 °C are included (Fig 6.3). This may be evidence for more efficient protein conversion when dietary protein is limited by appetite, but it does not hold up as strongly as the SMR – FCR relationship which was significant across the whole range of 12 °C – 24 °C (Khan *et al.*, 2014a). If increasing DCP decreases FCR as it does in the gilthead seabream (Lupatsch *et al.*, 2001), it may be possible to rework diets for hapuku based on optimal (and potentially lower) levels of DCP, in combination with high levels of DE for growth from (primarily) lipids (Lupatsch *et al.*, 1997; Lupatsch *et al.*, 2001; Dietz *et al.*, 2012). DE from both carbohydrates and lipids have been shown to be used effectively for growth, and even create a protein-sparing effect, in species such as redbelly tilapia *Tilapia zilli* (El-Sayed and Garling Jr, 1988), coho salmon (Leggatt *et al.*, 2009) and kingfish (Booth *et al.*, 2013), though the overall effects of decreased DCP and increased DE from other sources on the flesh quality of hapuku is not yet known. The reason for the comparatively large decrease in conversion efficiency at 24 °C (Fig. 6.3) is also not known but may be a result of decreased phosphorylation efficiency in mitochondria creating inefficiencies at excessive temperatures (Hilton *et al.*, 2010; Hickey and Iftikar, 2012) or an increase in protein degradation rates (Houlihan *et al.*, 1995; Smith and Houlihan, 1995; McCarthy and Houlihan, 1997).

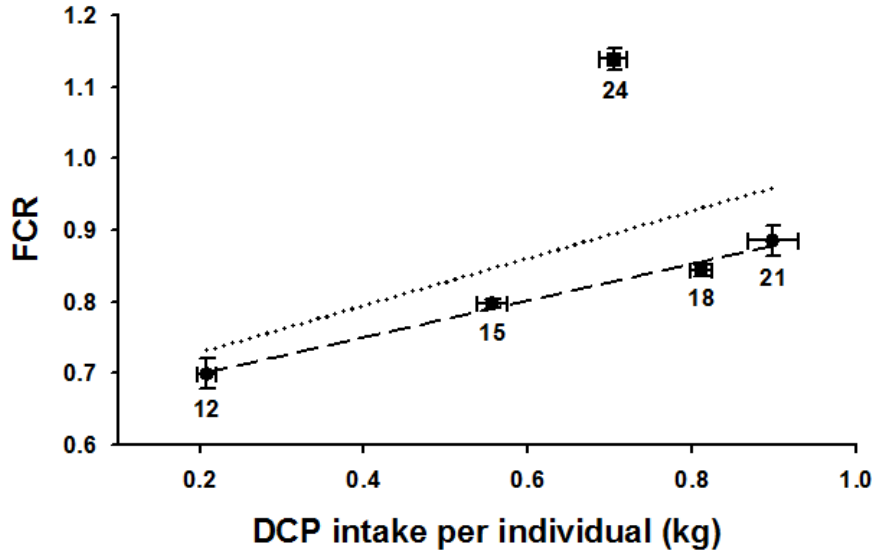

Figure 6.3. The relationship between digestible crude protein (DCP) received per fish and FCR in the 4-week growth trial of Khan *et al.* (2014a). DCP ± SE was calculated as 50% of the feed intake per individual as per the protein proportion of Skretting Nova FF pellets (Skretting, Australia, 50.0 % protein, 17.0 % lipid, digestible energy 18.6 MJ kg^{-1}). FCR values ± SE were taken directly from Khan *et al.* (2014a). Data points are given with their associated temperatures (°C). The data is described with linear regressions including the values at 24 °C (filled circles and filled square, dotted line, $F = 1.26$, $R^2 = 0.29$, $P > 0.05$) and excluding the values at 24 °C (filled circles, broken line, $F = 149.25$, $R^2 = 0.99$, $P < 0.01$).

6.4 Recommendations for aquaculture practice

As stated earlier in this chapter, ideal aquaculture practices should aim to either increase the capacity for physiological work or, to decrease supplementary costs in a fashion that increases the proportion of AMS available for growth and protein synthesis. This section aims to explore a few simple recommendations that, based on the preceding investigations and explanations, should lead to improved growth performance in a commercial scenario. It is important to note that all of the current analyses were carried out in tanks and are likely to apply better to tank-based culture than sea cages. However, *in lieu* of other investigations regarding the production performance of this species, speculative recommendations will be made regarding the culture of this species in sea-cages.

6.4.1 Temperature

In terms of production and physiological performance, temperature had the most measurable effect. The smaller 40 g – 180 g animals used in Khan *et al.* (2014a) displayed optimal growth and AMS in the range of 18 °C – 21 °C while the larger 300 g – 500 g fish displayed significantly higher growth, with adequate feed at 21 °C than 15 °C [Khan *et al*. (2015), Chapter 3]. Additionally, at all swim speeds at 17 °C (Khan *et al.*, 2014b), growth was higher than all feeding regimes at 15 °C for similarly-sized fish. This data confirms that SGR and AMS are optimised in the range of 18 °C – 21 °C in fish up to 500 g. In contrast, FCR becomes less efficient with increasing temperature in both the smaller size class (Khan *et al.*, 2014a) and larger size class (Khan *et al.*, 2015) suggesting that there is a trade-off between the optimal temperatures for growth and feed conversion. As mentioned in Khan *et al.* (2014a), controlled or ambient temperature regimes should ideally not exceed 21 °C for any extended period of time and probably not drop below 15 °C. These temperatures encompass the fastest growth rates of Khan *et al.* (2014a), which is the only investigation that exceeds the 15 °C – 21 °C temperature range, and avoids the lowest SGRs and the least efficient FCR (Khan *et al.*, 2014a). The economic costs associated with maintaining these temperatures will depend on the location and whether the system is based on tanks or sea-cages. Ultimately, site location and the associated ambient temperature regimes will all have to be balanced against any potential gains or losses in SGR or FCR, as well as the logistical, infrastructural, environmental and social considerations of any particular farm site (Halide *et al.*, 2009; Alvarez-Lajonchère and Ibarra-Castro, 2013; Falconer *et al.*, 2013).

Due to the potential difference in thermal optima between smaller and larger individuals, it may be necessary to have separate locations or infrastructure for the different size classes. Warm-tolerant smaller fish may be better suited to more northern New Zealand locations, sheltered harbours or even land-based, partially recirculating systems attached to hatcheries where higher temperatures can be maintained at lower cost (Losordo and Westerman, 1994; Blancheton, 2000). Larger individuals (> 500 g or more) could then be moved to colder sea-cage locations (more southern, or outside of sheltered water bodies) when their thermal optima begins to decrease, though this particular size has yet to be deciphered. Another possible solution is to take advantage

of thermal stratification and have sea-cage locations in water that is adequately deep to separate size classes within the cage itself. Larger fish with colder thermal preferences can be kept in the bottom section where temperatures could be as much as 2 °C – 3 °C colder than the surface water where the smaller individuals would be kept. Feeding the lower half of the cage could be achieved through a central chute which could deliver a larger, sinking pellet with limited access to the smaller fish above.

6.4.2 Feeding

In simple terms, breaking up the total daily feed ration into multiple smaller meals can have a significant effect on SGR when growth trajectories are high enough to separate out the increase in growth efficiency of multiple small meals (i.e. at 21 °C, Chapter 3). Unfortunately, the exact mechanism for the improved SGR with more frequent feeding, in terms of measured SDA parameters and the limitation of AMS, are still uncertain and warrant further investigation. There was, however, no measureable effect of ration size/feeding periodicity on FCR within the temperature range of the study which has two consequences: i) there are no significant gains in FCR to be made by modifying feeding regimes, but ii) there are no significant losses in FCR to be made which means that SGR can be prioritised and management plans can be designed accordingly. Though only a maximum of two meals per day were fed to juvenile hapuku in the current study, it may be possible to get an even larger increase in SGR if the number of meals was increased further. Constant feeding approaches, such as belt feeders, would likely maintain constant levels of protein synthesis throughout the day and for a post-feeding period that would be dependent on species, ration size and temperature (Haschemeyer *et al.*, 1979; Smith and Houlihan, 1995; McCarthy *et al.*, 1999; Whiteley *et al.*, 2001; Katersky and Carter, 2007). SGR/feeding periodicity data from the current investigation (Khan *et al.*, 2015) do not oppose the general trend seen in other species, suggesting that constant feeding may be an effective approach to maximising growth in this species, but not necessarily FCR which was not significantly affected by ration size.

6.4.3 Swimming

The variable results between trial 1 and trial 2 in Chapter 4 make definitive recommendations difficult. The 4.8% gain in SGR in the range of 0.5 BL s^{-1} to 0.75

BL s^{-1} over static controls may only be achievable when growth trajectories are high. However, larger gains may be possible in scenarios where growth trajectories are particularly high, such as small size classes in an optimal thermal regime of 18 °C – 21 °C, though this still requires investigation. In general, 0.5 BL s^{-1} to 0.75 BL s^{-1} may represent an "optimal zone of exercise" where the energetic cost of swimming is low, production performance is potentially increased (and not decreased) and small gains could be also made in terms of parameters such as condition factor (CF, Fig. 4.1, Chapter 4). These speeds are also, in absolute terms, low for small fish and are easily generated with a directional inflow in tank systems and without the need for specialised equipment. Fish in sea-cages, however, often experience variable and uncontrollable flows dependant on cage location and intermittent periods of higher flows associated with tidal movements (Oppedal *et al.*, 2011; Pinkiewicz *et al.*, 2011). Intermittent flows have caused both improved growth and disease resistance in Atlantic salmon *Salmo salar* (Castro *et al.*, 2011) and improved metabolic fuel efficiency in rainbow trout (Pearson *et al.*, 1990) where speeds remained within available aerobic capacity. However, decreased growth performance and FCR have been observed in rainbow trout where intermittent flow speeds became excessive (i.e. sprint training) (Gamperl *et al.*, 1988). As the effects of intermittent and sprint training on juvenile hapuku performance are not known, considerations may need to be made when introducing small fish to tidal sites. The combination of SDA and swim speeds ≥ 1.0 BL s^{-1} at 17 °C are encroaching upon maximal aerobic capacity (Fig. 4.3, Chapter 4) and should any other physiological cost increase (SDA, for example), fish may have to balance the cost of swimming and protein synthesis/growth with some form prioritisation of one over the other (Owen, 2001). Investigations into the swimming performance of fed fish would be the first step in determining how this species will prioritise its physiological capacity into either activity or growth/protein synthesis.

6.5 Central paradigms and limitations

The applicability of the current investigations is based on certain paradigms in ectothermic physiology that require a brief discussion. Additionally, previously mentioned caveats will be briefly summarised.

6.5.1 Thermal acclimation

Thermal acclimation refers to a phenotypic response to environmental temperature that alters performance and improves fitness, though the latter aspect is rarely measured directly (Lagerspetz, 2006; Angilletta, 2009). The current investigations apply measurements after a set period of exposure and are ultimately measuring the resulting phenotypes of those exposures. No direct connections between physiological/production performance and fitness have been made (Wilson and Franklin, 1999; Hazel, 2002; Angilletta, 2009), as these are generally not required in aquaculture production physiology. There is, however, an underlying assumption that improvements in these factors relate to improved organismic performance, and therefore fitness. For examples of where thermal acclimation/tolerance has been applied to fitness, see Pörtner and Knust (2007), Pörtner and Farrell (2008) and Clark *et al.* (2013).

6.5.2 The OCLTT paradigm

The oxygen- and capacity-limited thermal tolerance (OCLTT) hypothesis assumes that the biochemical and physiological capacities of aquatic ectotherms are maximised within a specific temperature range, and are reduced outside of this range. This is based on the perceived inability of ectotherms to maintain oxygen and substrate supplies to all tissues, inducing varying degrees of anaerobic metabolism (Pörtner and Knust, 2007; Pörtner and Farrell, 2008; Pörtner, 2010; Clark *et al.*, 2013). This assumption is the basis of a large number of investigations into the ecophysiology of ectotherms where the temperature-performance-fitness relationship is examined (Pörtner, 2010, 2012; Clark *et al.*, 2013). The recent review by Clark *et al.* (2013) states that the OCLTT hypothesis needs to be tested rather than just assumed in ecophysiological investigations. This was done in the first experimental series where it was hypothesised that maximal growth and optimal feed conversion (FCR) would be maximised at temperatures where aerobic metabolic scope (AMS) was also maximised (Khan *et al.*, 2014a). Indeed, growth and AMS were maximised in the range of 18 °C – 21 °C and were significantly lower at each of 12 °C, 15 °C and 24 °C, though FCR was not related to AMS. These results, in combination with the relationship between temperature and growth performance in Chapters 3 and 4, confirm that the OCLTT hypothesis is applicable to these investigations and has been thoroughly tested.

6.5.3 Additive SDA

It has been shown in the European sea bass *Dicentrarchus labrax* (Altimiras *et al.*, 2008) and the darkbarbel catfish *Peltebagrus vachelli* (Li *et al.*, 2010) that the MO_2 of fed fish in a swim flume can exceed MO_{2max} estimates at U_{crit} in fasting fish. This suggests that SDA is being effectively added on to MO_{2max} and that AMS does not always represent the full aerobic capacity. The potential presence of "additive SDA" has two consequences for the current set of analyses: i) SDA_{peak} does not necessarily represent a proportion of available AMS being used and, ii) the energetic model presented in Fig. 4.3 in Chapter 4 and Fig. 6.1 could have underestimated aerobic capacity as the combined costs of swimming and SDA could exceed MO_{2max}. The first of these points is relatively inconsequential as SDA was not saturating AMS under any of the scenarios investigated (Table 3.1, Chapter 3). The second of these points would only be relevant if Fig. 6.1 showed that the ability of juvenile hapuku to simultaneously accommodate the physiological costs of SDA and swimming was limited by AMS. However, it does not, so additive SDA is not considered a major issue. Additionally, in the primary literature examples, MO_{2max} (or rather, MO_2 at U_{crit}) estimates were measured in fish swimming in a swim flume which might not actually capture the full extent of aerobic MO_{2max}. This has already been shown in the Atlantic cod where MO_2 at U_{crit} was significantly lower than MO_2 induced by manual chasing or burst sprinting (Reidy *et al.*, 1995). This is also supported by the use of the 99% quantile method of Khan *et al.* (2014a) where the highest and most consistent MO_2 values for juvenile hapuku in a swim-flume often occurred immediately after fish were handled, air exposed and placed in the respirometer, rather than the MO_2 at U_{crit}. As there are few data measuring the MO_{2max} of the European sea bass and dark barbel catfish after chase or air-exposure protocols it is difficult to confirm, but in all examples concerning juvenile hapuku, post-chase and air exposure MO_{2max} estimates have been higher than MO_2 at U_{crit} [see Khan *et al*. (2014) and Khan *et al.* (2014b)]. Therefore, it seems plausible that the presence of additive SDA may actually be a function of the method used to measure MO_{2max} and that physiological work still has to be balanced within AMS that is derived from more realistic measures of MO_{2max}.

6.5.4 Size and thermal optima of hapuku

As with all new species of interest, there are more questions than answers. This largely manifests in the subject of hapuku growth performance as "does the thermal tolerance/preference of hapuku shift with size/age?" This has not been addressed as it requires working with a large size range of animals, though it does warrant discussion. Hapuku juveniles in the wild are associated with flotsam in warmer surface-waters up to about 5 years of age and 50 cm FL (Francis *et al.*, 1999) which fits the current data as smaller fish are warm-tolerant and show significantly improved growth and a larger AMS in the range of 18 °C – 21 °C (Khan *et al.*, 2014a). Adults in the wild recruit to deep reefs (Francis *et al.*, 1999) where temperatures are considerably colder than surface waters. From these conclusions it is possible to hypothesise that this transition to deeper water is associated with a shift in thermal optima/tolerance. Additionally, egg/larval development is most successful at 13 °C – 14 °C (Anderson *et al.*, 2012) which is a sub-optimal temperature range for the growth of 40 g – 180 g hapuku (Khan *et al.*, 2014a). In terms of full-scale commercial culture, this transition probably represents a total shift in the required thermal regime or a change in the appropriateness of a sea-cage site. In particular it's important to determine whether this transition occurs during on-growing (before market size is reached) and whether this process is sudden, or whether thermal optima decreases slowly with size/age. Experimental approaches to answering these questions will be discussed in the next section.

6.5.5 Applicability of the data to aquaculture

It is reasonably safe to assume that the initial idea was to on-grow this species in sea cages at appropriate sites around New Zealand. However, the current investigations have all been performed in tank systems and in individual respirometric or behavioural systems. Unfortunately, during the early stages of research and development around the culture of this species, investigations regarding the performance of this species in sea-cages were not possible. *In lieu* of actual cages, tank-based experiments do provide the best means of disentangling the effects of different environmental conditions such as temperature, feeding and swimming. Additionally, they provide very accurate and replicated responses to tightly controlled environmental which is extremely advantageous when investigating the effects of

broad environmental variables on a new species. As a specific example, investigating the effects of different temperatures on growth and FCR in sea cages is complicated by the fact that: i) there are unlikely to be multiple cages in different thermal regimes, ii) temperature changes are seasonal and growth responses to temperature are complicated by differing size classes at each temperature and iii) fish could be exposed to any number of sporadic environmental events which may disrupt growth at different rates depending on time of year, size *etc*. The data contained within this thesis can therefore provide a basis for more applied investigations into how this species will perform in sea cages or can be used to establish the conditions for a land-based early on growing facility.

6.6 Future research directions

These investigations are some of the first to consider the effects of environmental and aquaculture variables on the performance and physiology of juvenile hapuku. These preliminary investigations will always raise more questions than answers and some of these questions are raised here along with some new hypotheses and potential experimental approaches to investigate them.

6.6.1 Thermal optima

As mentioned above, the next step forward in terms of temperature is to determine at which point hapuku transition between "warm tolerant" and "warm intolerant" physiologies (Khan *et al.*, 2014a). Unfortunately, the growth trials and measurements of physiological performance (AMS) in Khan *et al.* (2014a) become increasingly difficult to apply and replicate on larger fish, primarily due to the size of the tanks and apparatus required (Clark *et al.*, 2013). To further complicate the issue, it is particularly important to determine the optimal conditions for large and valuable fish, such as broodstock in aquaculture. Early determinations of optimal temperatures in large broodstock fish (particularly those that are wild-caught) usually come from measures of reproductive output or appetites *etc*. and this can come at the risk of variable reproductive performance seasons as a result of unreliable "trial and error" data. The link between behaviourally-derived preferred temperature (T_{pref}) and the optimal temperature for growth (Jobling, 1981; Khan *et al.*, 2014a) does give a

potential method for working with larger fish. The shuttlebox apparatus described in Khan *et al.* (2014a) was sufficient in size and water turnover for fish up 1 kg and it is not a difficult design to scale up to systems as large as 1000 L – 5000 L by connecting two normal tanks via a tunnel that allows fish to move between them freely (some large choice chambers are available from Loligo Systems®, but at considerable cost). Larger systems, however, become increasingly difficult to heat and cool meaning that the 2 °C h^{-1} rate of change will probably have to be decreased and experiments drawn out longer to provide reliable data. Claireaux *et al.* (1995) resolved the thermal preference of large 1.2 kg – 1.5 kg Atlantic cod in a staggering 125,000 L, three-storey tower which could be thermally stratified. With some development, this method could be applied to determining the preferred temperature range of larger broodstock fish in their own tanks (70,000 L) by pumping in water of different temperatures at different heights to create thermal stratification. Ideally, the thermal preference of juvenile hapuku needs to be resolved between 500 g, the upper end of the size range of the current investigations, market size (~2 kg – 10 kg) and broodstock (~10 kg – ~60 kg). The hypothesised T_{pref} for mature broodstock hapuku, based on the temperature selection for egg culture presented in Anderson *et al.* (2012) and unpublished data regarding appetite and reproductive performance, would be in the range 13 °C – 16 °C.

6.6.2 SDA and feeding

As mentioned in the conclusions of Chapter 3, there are two logical progressions from the investigations presented. The first of these is to determine the effect of multiple meals in a day on the SDA response of juvenile hapuku. Having their daily ration split into two smaller feeds produced a significantly higher SGR in fish at 21 °C (Khan *et al.*, 2015) and, as fish refused to take feed a second time in the confinement of respirometry chambers, there is yet to be any measurement on how the second meal affects postprandial MO_2. Multiple daily meals maintained MO_2, and presumably the rate of protein synthesis, at elevated levels at all times in the southern catfish (Fu *et al.*, 2005a). From these data and the significantly higher growth performance of the hapuku that were fed two 1.5% rations per day at 21 °C, it is hypothesised that the SDA coefficient [expressed as the SDA energy (kJ) as a proportion of the digestible energy content of the ration fed to induce SDA] would increase significantly as a result of the elevated energetic cost of protein synthesis and

growth. To measure this, it may be necessary to apply the forced gavage methodology of Li *et al.* (2010), but this was not deemed appropriate for the current study due the added stress of handling. Fish could receive a direct infusion of feed/amino acids into the blood or gut (Brown and Cameron, 1991) which is plausibly the most appropriate for hapuku although it has yet to be attempted in this species. Whole tank MO_2 estimations are also possible in feeding fish, assuming that the passive rate of oxygen across the surface of the tank is accounted for (Pirozzi and Booth, 2009; Gamble *et al.*, 2014) and that background respiration rates are able to be measured reliably. Initial investigations should aim to determine if there are differences in SMR and the SDA parameters between the current study and those determined with the use of direct infusion or whole-tank methods, and then follow with a periodic infusion or feeding at controlled intervals or constantly.

An additional proposal for future work would be to separate the costs of SDA into mechanical (handling) and physiological (digestion, assimilation and protein synthesis) components as per the studies of Tandler and Beamish (1979), Tandler and Beamish (1980), Carefoot (1990) and Brown and Cameron (1991). The application of these techniques should determine the apparent inefficiency of large meals which could be associated with increasing mechanical costs. Based on the fact that hapuku fed a single 3% ration at 21 °C grew significantly slower than those that were fed two 1.5% rations per day (Fig. 3.2, Chapter 3), it is hypothesised that larger meals incur larger mechanical costs (i.e. not associated with protein synthesis) in juvenile hapuku which leads to inefficiency. Increasing mechanical costs with larger meals have been demonstrated largemouth bass *Micropterus salmoides* where the mechanical costs of SDA increased asymptotically with meal volume (Tandler and Beamish, 1979). The direct infusion of amino acids into the blood can be used to separate the digestion, assimilation and protein synthesis components of SDA from the mechanical components (Brown and Cameron, 1991). Similarly, indigestible cellulose pellets can be used to determine the mechanical components of the SDA response, including feed handling and gut peristalsis (Tandler and Beamish, 1979).

6.6.3 Swimming and exercise

Juvenile hapuku, under the conditions of the current study, did not show a significant enough improvement in growth or FCR where the application of exercise

would provide significant economic gains (Fig. 4.1, Chapter 4). Additionally, the induction of swimming speeds ≥ 0.75 BL s^{-1} significantly reduced MFD and fillet firmness (Fig. 5.4, Chapter 5), though the full effect of exercise on the organoleptic properties of the flesh are unknown. Considering the difference in the growth response to exercise between the smaller trial 1 fish and the larger trial 2 fish (Khan *et al.*, 2014b), it is first necessary to repeat the growth experiments in a way that disentangles the effect of temperature and body size on exercise-induced growth. In the current investigations, variation in growth trajectories were seen between the smaller fish in trial and the larger fish in trial 2 (Khan *et al.*, 2014b) and fish were held just outside their optimal temperature range for growth of 18 °C – 21 °C (Khan *et al.*, 2014a). Therefore, the maximal 4.8% improvement in growth rate seen in trial 1 at 0.5 BL s^{-1} – 0.75 BL s^{-1} is unlikely to be the largest potential gain from sustained exercise and small fish in ideal conditions could show consistent and higher growth gains at 0.5 BL s^{-1} – 0.75 BL s^{-1}. Along the same lines, it may be prudent to determine the effects of different swimming speeds on the growth of fish closer to market size and at various temperatures to be fully aware the effects at both ends of the on-growing process.

In terms of product quality, investigations need to be made in to the organoleptic and textural differences between wild-caught and farmed hapuku. This would ideally cover not only MFD and texture, both of which are potentially higher in wild hapuku (Johnston, 1999; Periago *et al.*, 2005; Rasmussen *et al.*, 2013), but also the differences in flesh constituents. Similarly the differences in flesh constituents and organoleptic properties (Johnston *et al.*, 2006; Grigorakis, 2007; Rasmussen *et al.*, 2013) between exercised and non-exercised hapuku should be investigated to fully understand the role of swimming in the quality of the final product.

6.7 Concluding remarks

As aquaculture grows and diversifies in species and technologies, it becomes increasingly important to identify which species are the best suited to intensive culture and what the associated economic implications and opportunities are. The preceding investigations are some of the first to empirically measure the effects of environmental and culture variables on hapuku, a novel finfish aquaculture species, and have

provided valuable insight into some of the optimal conditions for production performance. The information provided here can be used by farmers and other invested parties to make informed decisions on many important aspects of the commercial culture of hapuku including farm location, thermal regimes, feeding regimes, swimming conditions and the viability of this species compared to others. The energetic models presented here can also be used to infer as to how some of these factors interact, as well as providing a basis for continued exploration into the bioenergetics of this species. In general, juvenile hapuku have proven to be an ideal species for investigating the physiological basis of production performance in fish and are highly amenable to the methodologies applied in this thesis. They should be considered as ideal candidates for future investigations into the physiology of fish.

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