METABOLIC PHYSIOLOGY OF THE SOUTHERN BLUEFIN TUNA (*THUNNUS MACCOYII*) AND MULLOWAY (*ARGYROSOMUS JAPONICUS*).

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ABSTRACT

The bluefin tuna have a variety of distinctive anatomical and physiological adaptations that enhance performance. However, our understanding of bluefin tuna physiology is limited by the logistical difficulties of studying these large pelagic fish. This thesis examines some aspects of the metabolic physiology of the southern bluefin tuna. It provides insight into the high-performance, high-energy demand physiology of bluefin. It also examines the metabolic physiology of the mulloway, another important aquaculture species for which physiological information is currently limited.

1. Routine metabolic rate (RMR) of southern bluefin tuna (SBT) (*Thunnus maccoyii*), the largest tuna specimens studied so far (body mass = 19.6 kg (± 1.9 SE)) was measured in a large (250,000 l) flexible polypropylene respirometer "mesocosm respirometer". Mean mass-specific RMR was 460 mg kg^{-1} h^{-1} (± 34.9) at a mean water temperature of 19°C. When total RMR is added to published values of other tuna species at equivalent swimming speeds, there is a strong allometeric relationship with body mass ($654 \bullet M_b^{0.95}$, $R^2 = 0.97$). This demonstrates that interspecific RMR of tuna scale with respect to body mass similar to that of other teleosts, but is approximately 5-fold higher than the standard metabolic rate (SMR) of other active teleost species.

2. This study reports on the first measurements of the metabolic cost of food digestion and assimilation (specific dynamic action, SDA) of a tuna species. Oxygen consumption ($\dot{M}O_2$) and swimming velocity of southern bluefin tuna (SBT) (*Thunnus maccoyii*) were elevated for periods between 20-45 h (longest for the largest rations) post-ingestion of sardines (*Sardinops sagax*). It is suggested that the purpose of increased swimming velocity was to increase ventilation volume as a response to the enhanced metabolic demand associated with SDA. The magnitude of SDA as a proportion of gross energy ingested (SDA coefficient) averaged 35 ± 2.2 %. This demonstrates that the absolute energetic cost of SDA in SBT is approximately double that recorded in other teleost species.

3. This study examines the effects of sardines (*Sardinops sagax*) with high- (12.9%) or low- (1.8-4.0%) lipid level on specific dynamic action (SDA) and swimming velocity of southern bluefin tuna (SBT) (*Thunnus maccoyii*). Fish swam faster during the SDA period with the increase in velocity being greatest for the fish that ingested the high-lipid sardine. Magnitude of SDA was also greater for fish that ingested the high-lipid sardines. However, the energetic cost of SDA as a proportion of ingested energy was not significantly different between fish that ingested the high- (34.3 ± 2.4%) and low-lipid sardines (31.5 ± 2.9%). These results confirm that the high energetic cost of SDA is ecologically relevant.

4. In this study the metabolic and behavioural responses of both fasted and postprandial southern bluefin tuna (*Thunnus maccoyii*, SBT) to low dissolved oxygen (DO) was examined. In moderate hypoxia (4.44 and 3.23 mg l^{-1}), swimming velocity (*U*) and routine metabolic rate (RMR) of fasted fish was mildly enhanced. At 2.49 mg l^{-1}, *U* increase to over double in the normoxic speed, possibly as an escape response. At 1.57 mg l^{-1}, both *U* and RMR were suppressed and SBT failed to survive the entire 20 h exposure period. This reveals that SBT are remarkably well adapted to low DO. Feeding did not greatly influence their hypoxia tolerance. In a subsequent experiment there were no significant differences in *U*, RMR and gastric evacuation rates of postprandial SBT in hypoxia (2.84 mg l^{-1}) compared to those in normoxia (7.55 mg l^{-1}).

5. In this study, 768 h of simultaneous recordings of metabolic rate (MR, = heat production) and visceral temperature were made in both fasted and postprandial southern bluefin tuna (SBT, *Thunnus maccoyii*) of two sizes (~10 and 20 kg) and at two water temperatures (~19 and 16°C). Duration and magnitude of specific dynamic action (SDA) were strongly related to duration and magnitude of postprandial visceral warming providing the first empirical evidence of a link between SDA and postprandial visceral warming. Visceral temperature of fasted SBT was also directly related to MR. In this case, source of heat is thought to be metabolic work performed within the red muscles which warmed the viscera through thermal conductance. Visceral excess temperatures were over 1°C warmer in larger than smaller SBT. Better heat retention ability of the larger SBT is likely attributed to improved *retia mirabilia* development and greater thermal inertia. SBT at 16°C maintained visceral excess temperatures significantly warmer than similarly sized fish at 19°C. This demonstrates some ability of SBT to physiologically regulate visceral warming.

6. In this study, the effect of progressively severe hypoxia levels on the swimming performance and metabolic scope of juvenile mulloway (*Argyrosomus japonicus*) were investigated. In normoxic conditions (6.85 mg l^{-1}), standard metabolic rate (SMR) and cost of transport were typical for sub-carangiform fish species. Mulloway had a moderate scope for aerobic metabolism (5 times the SMR). The critical dissolved oxygen level was 1.80 mg l^{-1} revealing that mulloway are well adapted to hypoxia. In all levels of hypoxia (5.23, 3.64, and 1.86 mg l^{-1}) the active metabolic rate was reduced however, the critical swimming velocity was reduced only at 3.64, and 1.86 mg l^{-1}. Mulloway metabolic scope was significantly reduced at all hypoxia levels, suggesting that even mild hypoxia may reduce growth productivity.

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Publications arising

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INTRODUCTION

Tuna share an assortment of anatomical and physiological traits that enhance performance and make them distinct from most other teleosts. They have evolved muscle, bone and tendon anatomy that supports swimming with minimal body lateral movement (thunniform swimming mode) which is specialized for both high-performance and long-distance locomotion (Altringham and Shadwick, 2001; Westneat and Wainwright, 2001). Their cardio-respiratory systems are adapted for high rates of oxygen uptake and transport, and include large gill surface areas, thin gill epithelia, large hearts, high heart rates, high cardiac outputs, high ventricular pressures, high proportions of compact myocardium, and well-developed coronary circulations (Muir and Hughes, 1969; Farrell et al., 1992; Brill and Bushnell, 2001; Blank et al., 2004). Their enhanced ability for oxygen uptake and delivery supports high metabolic rates. The standard metabolic rates of tuna have been shown to be 2 to 5 times greater than that of most other active fish (Brill, 1979; Brill, 1987; Dewar and Graham, 1994). High standard metabolism is thought to be due to energetically expensive adaptations that support a great metabolic scope (Brill and Bushnell, 1991; Korsmeyer et al., 1996). A great metabolic scope provides the power to maintain multiple metabolic functions, such as rapid sustained swimming, fast growth rates, rapid digestion, and potential for quick repayment of oxygen debt. Tuna also have counter-current vascular heat exchangers (*retia mirabilia*) that enable them to accumulate metabolically generated heat to maintain the temperature of certain tissues (red muscle, cranial tissues and the viscera) above that of water temperature (regional endothermy). It is hypothesized that regional endothermy enhances aerobic swimming performance and has allowed tuna to expand their thermal niche (Block et al., 1993; Graham and Dickson, 2000; Dickson and Graham, 2004). Due to these adaptations, tuna are commonly classed as “high performance fishes”.

Amongst tuna, bluefin (*Thunnus maccoyii*, *T. orientalis* and *T. thynnus*) could be considered to have the highest performance physiology. Pacific bluefin (*T. orientalis*) have higher heart rates, cardiac outputs, can maintain cardiac performance at lower temperatures and have higher metabolic rates at given swimming velocities than yellowfin tuna (*T. albacares*) (Blank et al., 2002; Blank et al., 2004; Blank et al., 2007). Bluefin also have the most advanced endothermy anatomy (Collette et al.,

2001), with well developed lateral *retia mirabilia* to warm the muscles (Carey and Teal, 1969b) and visceral *retia mirabilia* to accumulate heat within the peritoneal cavity (Carey et al., 1984; Fudge and Stevens, 1996). It is hypothesized that this physiology has allowed bluefin to extend their distribution further into productive cooler waters of higher latitudes (Gunn and Block, 2001; Block et al., 2005).

Although our understanding of tuna physiology is rapidly increasing, it still lags far behind that other teleosts such as salmonids. Many fundamental aspects of tuna physiology remain unknown. This is because tuna possess many attributes that make them difficult experimental specimens. They are large, strong, and difficult to maintain in culture conditions. Furthermore, they need to swim continuously in order to ventilate their gills and to maintain hydrodynamic equilibrium (Brown and Muir, 1970; Magnuson, 1973). Indeed, only recently has the first measurement of bluefin tuna metabolism been conducted (Blank et al., 2007).

For tuna, maintaining their high performance physiology comes at a great energetic cost. This energetic cost is reflected in tunas' high metabolic intensity, which in many respects parallels that of mammals and birds (Korsmeyer and Dewar, 2001). Tuna must balance the high energetic costs of these adaptations with the benefits they provide for energy acquisition (prey capture and processing). It is for this reason that tuna are referred to as "energy speculators", investing large amounts of energy on the potential for higher rates of energy returns (Korsmeyer et al., 1996). According to the optimal foraging theory, an individual's fitness increases with their ability to maximise the ratio of energy acquisition to energy expenditure. Tuna have proved to be extremely successful, inhabiting all of the world's oceans from the tropics into cool sub-polar waters. Further studies on metabolism and energetics are likely to give more insight into the role of energy speculation on the success of tuna within their expansive environment that is largely depauperate of prey.

The rapid development of intensive bluefin tuna aquaculture practices worldwide (Farwell, 2001) has resulted in a need for a greater understanding of their metabolic physiology. Metabolism is the physiological engine that powers all activities such as swimming, growth and reproduction. An understanding of its fundamental processes and the effects of environmental factors is required to optimize it for culture objectives (i.e. growth or reproduction). Precise metabolic data are required for calculation of aquaculture system oxygen requirements, fish energy

requirements, environmental impact assessments, and species-specific physiological thresholds.

Further species-specific metabolic information is required to support the expanding international aquaculture industry. Teleosts from the family Sciaenidae have many attributes that make them suitable for intensive aquculture, and commercial production of sciaenid species is rapidly increasing worldwide (Battaglene and Talbot, 1994; Thomas et al., 1996; Drawbridge, 2001; Holt, 2001). In fact, sciaenid fishes are now the major fish species for artificial propagation in the world's leading producer of aquaculture products, China (Hong and Zhang, 2003). In Australia and South Africa, mulloway (*Argyrosomus japonicus*) is a large sciaenid that has recently become an important aquaculture species (Fielder and Bardsley, 1999; Hecht and Mperdempes, 2001). There is currently no metabolic information on the mulloway and little on other Sciaenidae fishes.

This thesis examines some aspects of the metabolic physiology of Southern bluefin tuna (*T. maccoyii*) and mulloway. It outlines the development of a novel approach for measuring the metabolic rates of large, free-swimming teleost species. It uses this technique to make the first recordings of the metabolic rate of active southern bluefin tuna and the metabolic cost of specific dynamic for any tuna species. It examines the effect of low dissolved oxygen on the metabolism of both southern bluefin and mulloway. Finally, it examines the relationship between southern bluefin tuna visceral warming and metabolic rate.

1. Routine metabolic rate of southern bluefin tuna (*Thunnus maccoyii*)

Abstract

Routine metabolic rate (RMR) of the largest tuna specimens studied so far (southern bluefin tuna, *Thunnus maccoyii*, body mass (M_b) = 19.6 kg (± 1.9 SE)), was 460 mg kg^{-1} h^{-1} (± 34.9) at a mean water temperature of 19°C. When total RMR is added to published values of other tuna species at equivalent swimming speeds, there is a strong allometeric relationship with body mass ($654 \bullet M_b^{0.95}$, $R^2 = 0.97$). This suggests that interspecific RMR of tuna scale with respect to body mass similar to that of other teleosts, but is approximately 5-fold higher than the standard metabolic rate (SMR) of other active teleost species. RMR (not SMR) is most appropriate metabolic index for this species and other tuna which are ram-ventilators and unable to cease swimming. Metabolic measurements were made in a large (250,000 l) polypropylene respirometer (mesocosm respirometer) that was deployed *in-situ* within a marine-farm sea cage for 29 days. Fasted fish were maintained within the respirometer up to 42 h while dissolved oxygen dropped by 0.056 (± 0.004) mg l^{-1} h^{-1}. Fish showed no obvious signs of stress. They swam at 1.1 (± 0.1) fork lengths per second and several fed within the respirometer immediately after measurements.

Introduction

Although metabolic physiology has been extensively examined in many teleost species, our knowledge of metabolic processes in active pelagic species is limited. The advent of the Brett-type tunnel respirometer revolutionalized fish metabolism research, allowing comprehensive investigations of oxygen consumption rates ($\dot{M}O_2$) with respect to controlled swimming velocities and environmental factors (Brett, 1964). To date, metabolic studies of swimming active teleost species have principally concentrated on salmonids (Brett, 1972; Brett and Glass, 1973; Grottum and Sigholt, 1998; Leonard et al., 2000; Gallaugher et al., 2001; Farrell et al., 2003; Geist et al.,

2003; Lee et al., 2003a; Lee et al., 2003b), and small individuals of the tuna and mackerel family (Scombridae) (Gooding et al., 1981; Graham and Laurs, 1982; Graham et al., 1989; Dewar and Graham, 1994; Sepulveda and Dickson, 2000; Sepulveda et al., 2003; Blank et al., 2007). However, the logistics of handling large, active teleosts has limited tunnel respirometry to small species or juvenile individuals of species whose adult size far exceeds practical water tunnel size. Thus, respiratory work on free-swimming tuna has generally been conducted with fish of body mass (M_b) under ~10 kg (Gooding et al., 1981; Graham and Laurs, 1982; Graham et al., 1989; Dewar and Graham, 1994; Sepulveda and Dickson, 2000; Blank et al., 2007). Given the maximum recorded size of southern bluefin tuna (SBT) (*Thunnus maccoyii*, 158 kg) and Atlantic bluefin tuna (*T. thynnus*, 679 kg) (Collette and Nauen, 1983), there may be inherent difficulties in extrapolation of metabolic information from existing work on small tunas to these large adult animals. Because of this limited experimental size range, there is much work yet to be done to improve our understanding of the metabolic physiology of large tuna (Korsmeyer and Dewar, 2001).

The $\dot{M}O_2$ rate of animals does not have an isometric relationship with M_b. Instead, $\dot{M}O_2$ is best scaled relative to M_b according to the allometric equation (Schmidt-Nielsen, 1984):

$$\dot{M}O_2 = a \bullet M_b^{\,b}$$

where a is the elevation, and b is the scaling exponent or slope of the linearized form. The exponent is generally less than 1.0, characterising a reduction of the mass-specific metabolic rate in larger animals. In teleost fish, the standard metabolic rate (SMR, the metabolic state of the animal at rest) is most commonly utilized for intraspecific evaluation of the effect of M_b on the metabolism. Brill (1979, 1987) compared the metabolic rate of tuna paralysed with a neuromuscular blocking agent to calculate an intraspecific mass-scaling exponent of $b = 0.5$ for kawakawa (*Euthynnus affinis*, 0.5-2.2 kg), $b = 0.57$ for yellowfin (*Thunnus albacares*, 0.6-3.9 kg) and $b = 0.56$ for skipjack tuna (*Katsuwonus pelamis*, 0.3-4.7 kg). Dewar and Graham (1994) found a similar exponent ($b = 0.6$) for yellowfin tuna (0.5-2.2 kg) swum in a water tunnel, with the SMR determined by extrapolation of the oxygen consumption:

swimming velocity relationship to zero. These studies suggest that the mass scaling exponent in tuna is considerably lower than the most extensively studied active teleost, i.e. sockeye salmon, *Oncorhynchus nerka*, (b = 0.88; Brett and Glass, 1973) and lower than the mean teleost exponents, both intraspecific (b = 0.79; Clarke and Johnston, 1999) and interspecific (b = 0.88; White and Seymour, 2005). However, the size and difficulty of handling tuna has meant the above-mentioned tuna mass-scaling studies were all conducted with juvenile tuna (less than 5 kg) with greatest mass range for comparison of just over one order of magnitude. An interspecific compilation of published SMR data by Korsmeyer and Dewar (2001) over several orders of magnitude however did support a lower scaling exponent for tuna than for other teleosts, but this comparison did not take into account considerable differences in study temperatures (up to 10°C). Because the accuracy of the scaling exponent depends on the body size range, it is desirable to extend measurements to larger fish.

The recent worldwide expansion of the sea farming of tuna has made large individuals available to researchers. The following study takes advantage of these sea-farming practices and outlines a novel approach for measuring the metabolic rates of large free-swimming teleost species. It is the first study to report on the metabolic rate of SBT.

Methods

Mesocosm design and manufacture

SBT routine metabolic rate (RMR) was determined by the reduction in dissolved oxygen (DO) over time within a large static respirometer (mesocosm respirometer). The mesocosm was manufactured from denier polyester scrim reinforced 1.14 mm polypropylene (R-PP 45, Stevens Geomembranes, http://www.stevensgeomembranes.com), bonded by thermal welding by Fabtech SA Pty. Ltd (Adelaide, Australia). The mesocosm was a 12 m diameter, 2.5 m deep enclosed cylinder with a wave break wall that extended 1 m above water level (Fig. 1). Entry into the mesocosm was possible through a 2 m diameter, 2 m high access port made from 0.75 mm polypropylene and positioned in the roof so that it could be rolled and clamped to completely seal the system. In addition, five 2 m diameter,

clear 0.75 mm polyvinyl chloride windows were positioned in the roof to allow entry of natural light, and eleven capped tank fittings (STF 50, Hansen Products Ltd, http://www.hansenproducts.co.nz/) allowed sealed access for experimental equipment and manual sampling.

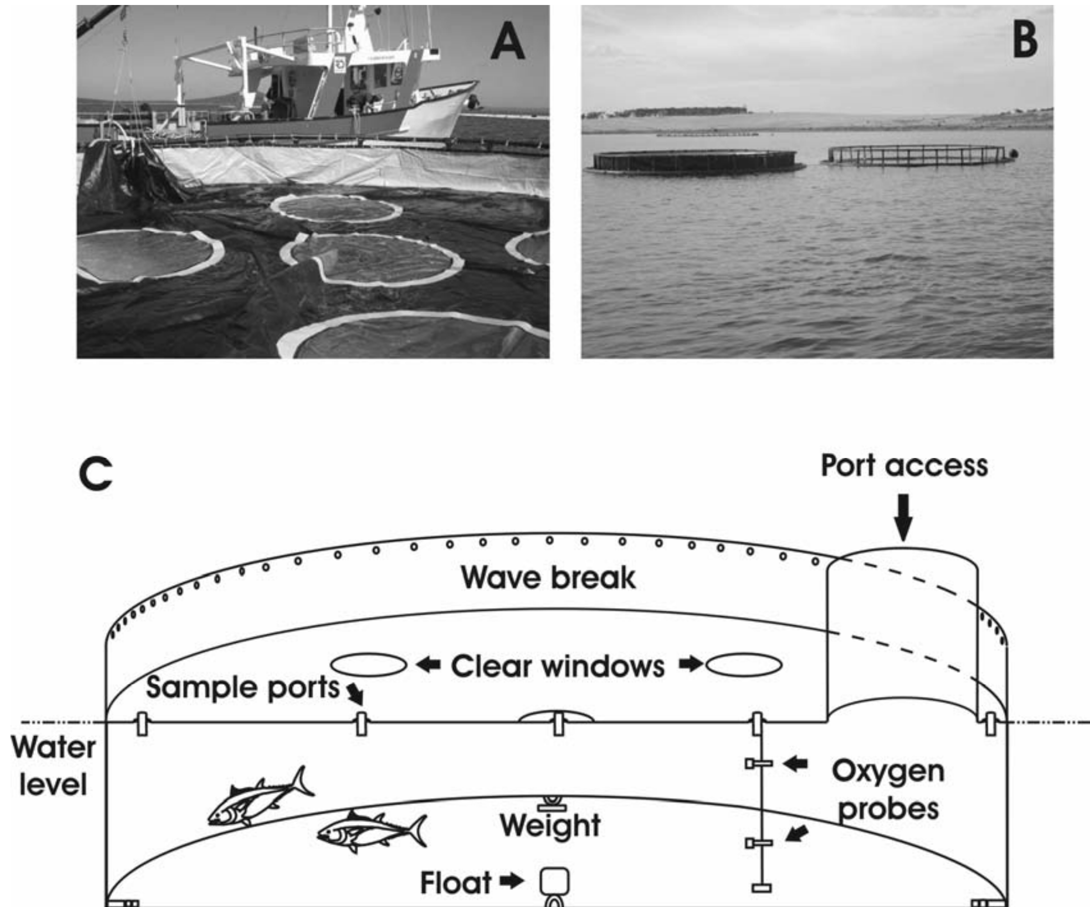

Fig. 1: **A)** The mesocosm respirometer surface when deployed showing the wave break wall, top entry port and five clear windows. **B)** The respirometer (left) and adjacent fish holding cage (right) deployed in Rotten Bay, Port Lincoln, Australia. **C)** A cross section diagram of the respirometer showing the entry port, clear windows, threaded capped fittings, wave break wall, oxygen probes, central float and stabilizing weights.

Mesocosm deployment and maintenance

The mesocosm was deployed into a 12 m diameter floating pontoon positioned in Rotten Bay (135°56'26"E, 34°43'44"S) on the southern side of Boston Island, Port Lincoln, South Australia, for 29 days. It was secured to the pontoon by the lacing of the wave break wall to the pontoon arm-rail with 5 mm diameter rope through stainless steel eyelets positioned at 0.2 m intervals around the top perimeter of the wave break wall. Once in position, the mesocosm was pump-filled with seawater. A float (10 l plastic drum filled with air) attached to the bottom and four 5 kg lead weights positioned around the internal perimeter helped retain its shape which was dynamic depending on the prevailing tide and weather conditions, but generally maintained a cylindrical form. This inherent flexibility also gave the mesocosm its resilience, allowing it to withstand winds up to 30-knots and 1 m swells on several occasions without failing.

The mesocosm floor was vacuum-cleaned with a 0.6 kW electric pump (Dynaprime X201, Davey Pty. Ltd., http://www.davey.com.au/) after every experimental trial to remove detritus. The water removed in this process was immediately replaced with new seawater with the same pump. This resulted in an approximately 5% water exchange following every trial but maintained the mesocosm's original water volume. Water ammonia levels were tested daily onsite with a portable ammonia test kit and they never exceeded 0.6 mg l^{-1}. Two temperature loggers (iButton, Dallas Semiconductor, http://www.maxium-ic.com) positioned at 1 m and 2 m depth, 1.5 m from the mesocosm edge, logged internal water temperatures (± 0.5°C) every 10 min.

Mesocosm volume was measured at the end of the experimental period by timed emptying with three calibrated pumps. Pump flow rates were calibrated at the beginning, once during and at the end of the pumping session by timing the filling rate of a 1,600 l tank. Total water volume was 248, 000 l ± 3, 570 l (mean ± SE; n = 3 pump calibrations).

Fish handling

Ten similar sized juvenile SBT (19.6 ± 1.9 kg) were randomly selected from a commercial sea-farm (DI Fishing Pty. Ltd., Port Lincoln, Australia) approximately 4 km offshore from Boston Island in early April, 2004. These fish had been purse-seine netted in the Great Australian Bight, most likely from the same school, approximately 2 months previously. In the commercial sea-farm, the fish had been weaned onto a commercial pellet diet consisting of 45% protein and 20% fat (Skretting, Tuna Growers 45:20, Hobart, Australia).

Transfer from the commercial sea-farm into a 12 m diameter holding pontoon involved hooking individual fish with a baited, barb-less hook and sliding the fish across a soft, wet slip (wet canvas over a foam mattress) into the adjacent cage. Transfer of individual fish typically took less than 15 s. The holding pontoon was towed 2 km to the Rotten Bay site and secured beside the mesocosm (Fig. 1B). They were fed to satiation twice a day and were feeding well before the beginning of any experimental trials, but were starved for at least 36 h before any individual trial. Studies of the effects of feeding on visceral warming (Gunn et al., 2001) and metabolic rate (Chapter 2) show that digestion and assimilation of previous meals are likely complete within 36 h.

On the morning of a respiratory trial a baited, barb-less hook was used to transfer two fish from the holding cage directly into the mesocosm as described above. On most occasions, the bait would fall from the hook on striking and not be ingested, eliminating the metabolic effects associated with specific dynamic action. However, bait size was kept as small as possible (less than 25 g), so that on the odd occasion that the bait was swallowed its metabolic effect would be minimal. Two fish were chosen for each trial, as it was believed that these schooling fish would be more comfortable within the mesocosm if introduced with a partner.

Experimental protocols

Five respiratory trials were completed on days 8, 17, 19, 22 and 25 of the experimental period (Table 1). Following introduction, the fish were then left undisturbed for 6 h to become accustomed to the mesocosm environment. This period

was longer than used in other tuna studies for recovery and acclimation (3-4 h) (Dewar and Graham, 1994; Sepulveda and Dickson, 2000). During this period trapped air under the roof was expelled by rolling it towards the entrance port. The port was then rolled-up and closed with a 3 m, hinged timber clamp. The swimming velocity was estimated by taking digital video recordings (Sony DCR-HC30, www.sony.com) by a researcher lying on top of the mesocosm and recording through the mesocosm roof windows at the start and end of each experimental period. Filming did not cause any disturbance as the researcher was not visible to the fish during filming. Footage was later analysed with video motion analysis software (World in Motion, http://www.logint.com.au). Care was taken to keep the camera still during filming and only footage of fish swimming directly below the camera was used for analysis. Selected video footage (at least 1 s portions) was analysed and measured tuna lengths were used to calibrate image scale, therefore correcting for variable distances between the fish and the camera. Individual fish were easily identified from the pair by comparison of overall lengths, as the two fish usually swam side by side. On termination of the experiment, the mesocosm was opened, the fish removed, body length (BL), and body mass (M_b) recorded. Immediately after each trial, the dissolved oxygen was raised to approximately 100% saturation with an oxygen diffuser (Aqua & Co Force 7, BOC gasses, NSW, Australia).

Background respiration trials were conducted at regular intervals (days 1, 9, 19 and 26) as a measurement of net respiration and photosynthesis of planktonic organisms in the water column and on the mesocosm surfaces. These trials consisted of the same protocol as the respiratory trials, however no fish were present.

To determine the oxygen integrity of the mesocosm respirometer, oxygen leakage was measured on day 27. This trial used the procedure outlined for the background trial, however 3000 g of sodium sulphite (Ace Chemical Company Pty. Ltd., Adelaide, Australia) and 50 g of the reaction catalyst cobalt chloride (Sigma-Aldrich, www.sigmaaldrich.com) were added to the mesocosm and water mixed with two, 4 hp pumps for 30 min and sealed (Ruttanagosrigit et al., 1991; Bennett and Beitinger, 1995). This experiment was designed to reduce dissolved oxygen level (DO) in the mesocosm by approximately 20% saturation, to provide a sufficient oxygen gradient across the mesocosm membrane.

Dissolved oxygen measurement

The DO of the mesocosm water was monitored by two methods. First by two oxygen electrodes (Vickie Cheshire Pty. Ltd., Adelaide, Australia) that were installed at 1 and 2 m depths, 1.5 m from the mesocosm edge, and held in place on a leaded line set on a pulley system that allowed positioning with minimal disturbance. Electrodes were calibrated in 100 and 0% oxygen saturation solutions (aerated or sodium sulphite-saturated seawater, respectively) immediately before and after to each experiment. If measurable electrode drift was recorded, data from this electrode were excluded from calculations. These electrodes were connected to a data logger (Datataker DT500, www.datataker.com) that recorded the electrode mV every 2 min. The electrodes were housed in 90 mm PVC pipe with a 12 V, propeller driven stirrer that delivered a constant stream of water over the electrode surface. The power supplies for the stirrers, oxygen electrodes and data logger were stored in sealed boxes attached to the pontoon arm rail. The second method involved taking manual readings at the beginning and end of each experimental trial with portable oxygen meter (Handy Gamma, OxyGuard International, www.oxyguard.dk). This meter was used to take 2 min readings through three screw-cap access ports in the mesocosm roof, 0.5, 3 and 6 m from the mesocosm edge and at three depths (0.25, 1.25 and 2.25 m). These recordings served three purposes, to backup logging meters, to check calibration of installed probes and to check mesocosm mixing (i.e. any oxygen gradient within the respirometer). The DO level of the environment outside the pontoon at a 1 m depth was also recorded at the beginning and end of each trial.

DO levels were converted from mV to mg l^{-1} at the water temperature of 19°C, salinity of 35 ppt and assuming standard atmospheric pressure of 101.3 kPa resulting in oxygen solubility of 7.67 mg l^{-1}. RMR was evaluated according to the equation:

$$\text{RMR (mg kg}^{-1}\text{ h}^{-1}) = (\Delta DO_t - \Delta DO_b + \Delta DO_l) \bullet V M_b^{-1}$$

Where ΔDO_t is the trial drop in respirometer dissolved oxygen (mg l^{-1} h^{-1}), ΔDO_b is the background respiration rate for the trial (mg l^{-1} h^{-1}), ΔDO_l is calculated oxygen leak across the wall (O_2 leak calculated every 2 min relevant to the pertinent oxygen

gradient) (mg l^{-1} h^{-1}), V is the respirometer volume (l) and M_b the fish mass (kg, mean of two fish used in each trial).

Results

Mesocosm performance

All fish introduced to the mesocosm survived the entire experimental period. No obvious signs of stress such as escape attempts or elevated swimming velocities were observed. The experimental period ranged from 21-22 h, except for one trial when poor weather prevented access and the fish were left for 42 h (Table 1). Water temperatures remained between 18.7 and 19.3°C. Coefficients of variation between portable meter DO recordings from the nine sample positions did not exceed 2.5% for any trial. These results suggest that the mesocosm remained well mixed at all times, most probably due to wave-induced fluctuations in mesocosm surfaces and swimming of the fish.

Table 1: Experimental day, trial length, ambient water temperatures (T_w), fish mass (M_b), body length (BL) and recorded swimming velocities (U) of the two southern bluefin tuna (*Thunnus maccoyii*) in each trial.

Trial	Day	Trial Length (h:min)	T_w (°C)	Fish	M_b (kg)	BL (cm)	U Start (BL s^{-1})	U End (BL s^{-1})
1	8	42:17	19.3 ± <0.1	1	20.8	108.5	0.77	0.56
				2	16.4	100.5	1.06	1.01
2	17	21:45	19.0 ± 0.1	1	21.6	117.0	0.63	1.29
				2	19.8	110.0	1.40	1.01
3	19	20:55	18.7 ± <0.1	1	22.8	107.7	1.29	1.21
				2	17.9	103.5	1.15	0.94
4	22	21:52	18.7 ± <0.1	1	23.2	107.5	1.17	1.39
				2	19.3	102.5	1.18	0.95
5	25	21:52	19.0 ± <0.1	1	20.4	104.6	1.05	0.82
				2	14.0	93.0	0.69	1.23

Temperature values are means ± SE, n = >144.

Experimental trials

Fish mass ranged from 14.0 to 23.2 kg and BL from 93 to 117 cm (Table 1). Fish were observed to be swimming slowly and calmly as a pair within the mesocosm. Mean velocity across all trials was 1.1 (± 0.1) BL s^{-1} (n = 10) (Table 1). Eight of the ten fish fed within the mesocosm when offered either a pellet feed or baitfish immediately following a trial; three of the fish struck a baited hook.

There was a net drop in DO in all background trials (presumably due to background respiration). Background respiration increased over the experimental period from 0.009 mg l^{-1} h^{-1} on day 1 to a maximum of 0.024 mg l^{-1} h^{-1} on day 26 (Fig. 2). It is believed that this increase in background respiration during the mesocosm deployment period was due to a proliferation of microbial colonies within the mesocosm environment. Respiratory trials were adjusted for background respiration according to the linear relationship of the increase in background relative to the experimental day (0.013-0.025 mg l^{-1} h^{-1}, Table 2). Mesocosm DO during SBT $\dot{M}O_2$ measurement fell between 0.048 and 0.068 mg l^{-1} h^{-1}.

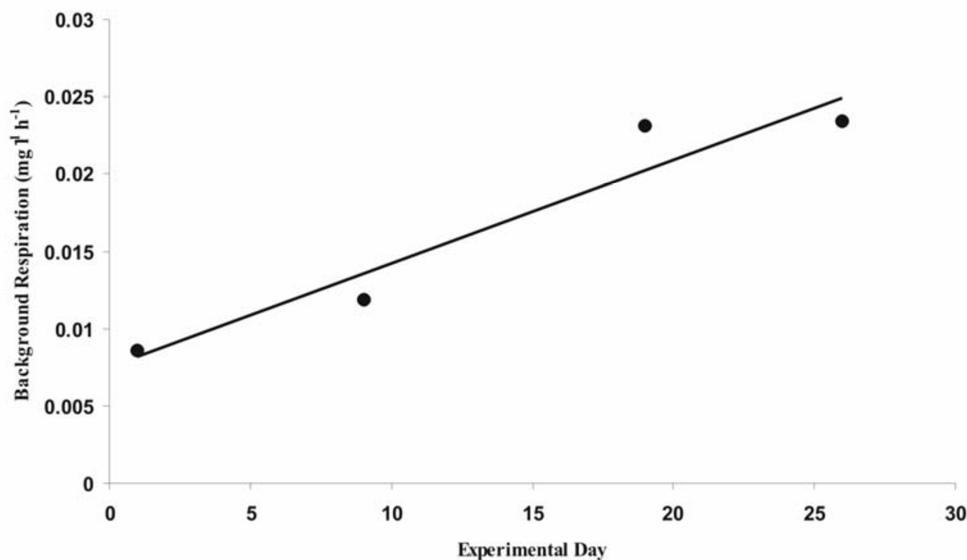

Fig. 2: Background respiration rates (mg l^{-1} h^{-1}) throughout the experimental period with fitted linear relationship (R^2 = 0.92).

The combination of sodium sulphite and cobalt chloride reduced the DO of the mesocosm to approximately 5.7 mg l^{-1}. Over the 21 h trial, DO fell by a further 2.2% (0.008 mg l^{-1} h^{-1}), apparently due to background respiration. This rate of DO drop is less than predicted from background trials (0.026 mg l^{-1} h^{-1}) where there was a smaller oxygen gradient between the mesocosm and the outside environment. Subsequently, oxygen leakage was calculated as 0.05 mg l^{-1} h^{-1} per mg l^{-1} oxygen gradient between the mesocosm and outside environment. This factor was used to adjust all respiratory trial 2 min samples according to the pertinent DO gradient between the mesocosm and the external environment at that time. Calculated total leakage of DO in respiratory trials were very low (-3.0×10^{-8}-2.4×10^{-8} mg l^{-1} h^{-1}) but varied considerably depending on the initial DO (Table 2). Calculated RMR of the SBT ranged between 345 and 539 mg kg^{-1} h^{-1}, and the mean was 460 ± 35 mg kg^{-1} h^{-1} (n = 5) (Table 2).

Table 2: Summary of external (External DO), start and end point dissolved oxygen (DO), calculated background respiration rate (ΔDO_b), dissolved oxygen drop during routine metabolic rate trials (ΔDO_t), calculated mean oxygen leak (ΔDO_l) and routine metabolic rate (RMR) for five respiratory trials.

Trial	**External DO (mg l^{-1})**	**Start DO (mg l^{-1})**	**End DO (mg l^{-1})**	ΔDO_b **(mg l^{-1} h^{-1})**	ΔDO_t **(mg l^{-1} h^{-1})**	ΔDO_l **(mg l^{-1} h^{-1})**	**RMR (mg kg^{-1} h^{-1})**
1	7.32	9.06 ± 0.04	7.20 ± 0.25	0.013	0.048	2.4×10^{-8}	473
2	7.44	7.20 ± 0.02*	6.02 ± 0.03*	0.019	0.053	-3.2×10^{-8}	424
3	7.47	7.88 ± 0.02*	6.92 ± 0.02*	0.020	0.049	-9.8×10^{-9}	346
4	7.32	7.31 ± 0.11	5.93 ± 0.16	0.022	0.068	-3.0×10^{-8}	539
5	7.32	8.22 ± 0.16	6.90 ± 0.15	0.025	0.063	1.1×10^{-8}	419
Mean (n=5)					**0.056 ± <0.01**		**460 ± 35**

Values are means ± SE. (n = 11), * n = 9 due to data-logger failure.

Discussion

It is difficult to measure the metabolic rate of large, swimming, active fish. Indeed, for some species it has proven to be impossible (Benetti et al., 1995), and for the remaining species, it has been restricted to small individuals (Dewar and Graham,

1994; Sepulveda and Dickson, 2000; Sepulveda et al., 2003; Blank et al., 2007). The mesocosm respirometer has several advantages that make it suitable for large teleost research, advantages that overcome some of the logistical limitations of traditional respirometry technologies (i.e. land-based water tunnel respirometer). One advantage is that the system is based at sea. Transportation of large ram-ventilating fish to shore-based facilities is difficult and risky. Infrastructure required for mesocosm respirometer research is relatively basic and, with the worldwide expansion of tuna aquaculture, is comparatively attainable. Another advantage of the mesocosm respirometer is that the stress levels within the spacious marine sea-cage based environment are likely to be low. Stress is known to elevate metabolic rates, thus respiratory studies should be designed to limit its effects (Cech Jr, 1990). As tuna require to swim continuously, a comfortable environment must have sufficient space for voluntary movement. Furthermore, previous studies recording the SMR of tuna have been conducted with fish that have been paralysed or forced to swim in a laboratory-based tunnel respirometer (Brill, 1979; Brill, 1987; Dewar and Graham, 1994; Sepulveda and Dickson, 2000). In all cases, the time allowed for transfer recovery and acclimation to these abnormal environments was limited (as low as 1 h). In the present experiment, the fish were transferred in less than 15 s and were allowed at least 6 h for recovery. Although no measurements were made to quantify stress (e.g. corticosteroid levels), the slow steady swimming speeds and voluntary feeding suggests it was low. The swimming velocities recorded in present study were within the range recorded for undisturbed Atlantic bluefin tuna within large marine sea cages (0.6-1.2 BL s^{-1}) (Wardle et al., 1989).

Some of the factors that make the mesocosm suitable for large teleost research do so at an experimental cost. The mesocosm's large volume serves to diminish the $\dot{M}O_2$ signal of the fish, extending time required for significant measurement. It is recommended that the volume of a static respirometer be thirty to fifty times that of the fish (Cech Jr, 1990). The mesocosm volume in the present study was over six thousand times the M_b of the fish. Although tuna's high metabolic rates allow a greater volume: mass ratios than could be used for other teleosts, several hours and two fish were required to make a reliable measurement. In the present experiments, the fish consumed approximately 10% of the available oxygen within 12.5 h. The use of two SBT that were not identical in mass may also introduce error associated to allometric scaling of $\dot{M}O_2$ with M_b.

A further problem with such a large water volume is the risk of insufficient mixing. This was found to be the case in a preliminary experiment were twenty-one (M_b = 3.17 ± 0.14 kg) yellowtail kingfish (*Seriola lalandi*) were introduced to the mesocosm in a single trial. After the 24 h trial, it was found that the DO recordings taken from the centre of the mesocosm were significantly less (approximately 0.7-1.0 mg l^{-1} less) than those recordings taken from the edge. In contrast to the tuna, the yellowtail kingfish formed a tight school within the centre of the mesocosm. Tuna used much greater proportion of the mesocosm, with a wider swimming arc and regular cuts through the centre. For all tuna trials, the mesocosm remained well mixed, suggesting that the swimming action of the fish was likely to have contributed to the mixing. The mesocosm respirometer may only be suitable for large teleosts that are likely to use a greater proportion of the internal respirometer environment.

Within the mesocosm respirometer, the fish is able to move freely, thus the RMR is measured. The RMR is the metabolic rate influenced by random activity in which fish movements are presumably restricted and the fish is protected from outside stimuli (Fry, 1971). For tuna however, this metabolic state is likely to be a close representation of the practical minimum metabolic rate, because locomotion is essential for life. Tuna are both negatively buoyant and obligate ram ventilators (Brown and Muir, 1970; Magnuson, 1973), thus the metabolic cost of sufficient activity to facilitate ventilation and maintenance within the water column is unavoidable. In the present experiment, the fish were able to maintain adequate activity for survival, but the physical constraints of the respirometer restricted unnecessary activity and thus would be indicative of the animals' practical minimal metabolic rate.

When RMR of SBT is represented independent of M_b (assuming a mass scaling exponent of 0.88 and a normalized study temperature of 25°C; 889 mg $kg^{-0.88}$ h^{-1}), it is higher than SMR for other tuna species; 518-252 mg $kg^{-0.88}$ h^{-1} for skipjack tuna (0.32-4.74 kg, Brill, 1979), 497-292 mg $kg^{-0.88}$ h^{-1} for kawakawa tuna (0.54-2.15 kg; Brill, 1987), 337-189 mg $kg^{-0.88}$ h^{-1} for yellowfin tuna (0.59-3.89 kg, Brill, 1987), 435-278 mg $kg^{-0.88}$ h^{-1} for yellowfin, (0.47-2.17 kg, Dewar and Graham, 1994). The higher $\dot{M}O_2$ of SBT is probably largely due to the metabolic cost of maintenance swimming.

Table 3: Summary of study temperature (T), fork length (FL), mean body mass (M_b), oxygen consumption ($\dot{M}O_2$)*, swimming velocity at 1.1 FL s^{-1}(U),* and predicted routine metabolic rate (RMR) at swimming speed of 1.1 FL s^{-1} for five species of tuna.

Species	T (°C)	FL (cm)	M_b (kg)	$\dot{M}O_2$ (mg h^{-1})	U (cm s^{-1})	RMR (mg kg^{-1} h^{-1})
Yellowfin tuna [1]	25	51 ± 1	2.17 ±0.14	$545 \bullet 10^{0.0045U}$	56.1	474
Albacore tuna [2]	13.5 - 18.0	87	8.5 - 12.0	$2574 \bullet 10^{0.003U}$	95.7	517
Skipjack tuna [3]	23 - 24	47 ± 1	1.96 ±0.31	$571 \bullet 10^{0.0038U}$	51.7	472
Kawakawa tuna [4]	24	19 ± 1	0.13 ±0.03	$90 \bullet 10^{0.0046U}$	21.7	883
Southern bluefin tuna [5]	18.7 – 19.3	105 ± 2.1	19.6 ±1.9	-	116.0	460

[1]*Thunnus albacares*, Dewar and Graham, 1994; [2]*Thunnus alalunga*, Graham *et al*, 1989; [3]*Katsuwonus pelamis*, Gooding *et al*, 1981; [4]*Euthynnus affinis*, Sepulveda and Dickson, 2000; [5]*Thunnus maccoyii*, Present study. Mass and Length data are Means ±SE. *$\dot{M}O_2 = a.10^{bU}$, were a and b are constants and U is swimming velocity (cm s^{-1}).

The major advantage of the mesocosm respirometer is that is allows the investigation of large individuals. The present study widens the mass range of tuna metabolic measurements providing further strength to investigations of mass scaling. However, for comparisons between the present and previous studies the metabolic cost of activity needs to be standardized. Table 3 outlines the swimming velocity: $\dot{M}O_2$ relationship for previously studied swimming tuna species and the predicted RMR for each species when swimming at 1.1 FL s^{-1} (swimming velocity recorded in the present study). When these are plotted dependent on mass (normalized to a temperature of 25°C, $Q_{10} = 1.65$, (White and Seymour, 2005), there is a strong interspecific allometric relationship between RMR and body mass (Fig. 3). For comparison, the relationship between the SMR and mass for the sockeye salmon is also included. As can be seen, the slope of the relationship between the tuna species and salmon are similar but the tuna is approximately 5 times greater. The elevated metabolism is associated with morphological and physiological adaptations such as large gill surface area, larger cardiac outputs, higher heart rates and elevated muscle temperatures, which contribute to greater aerobic scopes (Brill, 1979; Brill, 1987; Brill and Bushnell, 1991; Bushnell and Jones, 1994; Korsmeyer et al., 1996; Korsmeyer et al., 1997; Korsmeyer and Dewar, 2001). Enhanced aerobic scope enables tuna to achieve physiological feats, such as the ability to repay an oxygen debt whilst rapidly digesting and maintaining a swimming velocity for optimum prey

detection, that are instrumental to their success in the world's nutrient-poor oceans (Korsmeyer et al., 1996).

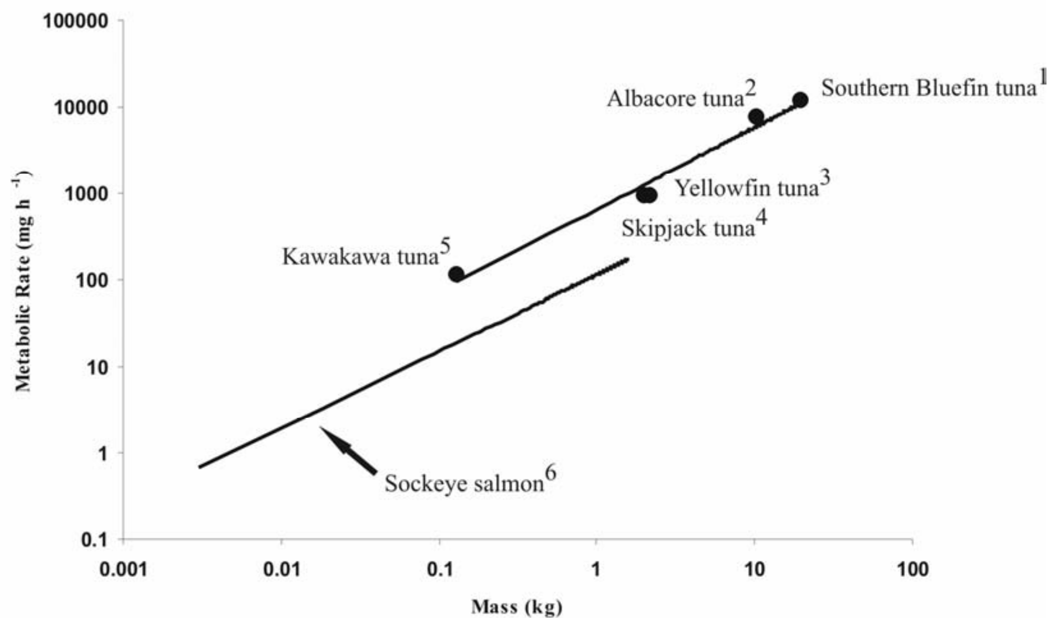

Fig. 3: The routine metabolic rate (RMR) of five species of swimming tuna when normalized for a swimming velocity of 1.1 FL s^{-1} and study temperature of 25°C (assuming Q_{10} of 1.65) with fitted allometric relationship; RMR = $654M^{0.95}$, R^2 = 0.97. Included for comparison is the standard metabolic rate (SMR) of the Sockeye salmon again normalized to a study temperature of 25°C; SMR = $115M^{0.88}$ ([1]Present study, [2]Graham *et al*, 1989, [3]Dewar and Graham, 1994, [4]Gooding et al, 1981, [5]Sepulveda and Dickson, 2000, [6]Brett and Glass, 1973).

The slope of interspecific scaling of RMR of the five tuna species is similar to slope of SMR for the sockeye salmon (Fig. 3). However, it is considerably higher than intraspecific SMR values previously reported for other tuna species (*b*=0.5-0.6) (Brill, 1979; Brill, 1987; Dewar and Graham, 1994). The reason for this discrepancy is likely due to the disparity in metabolic states in the comparison, suggesting that the mass scaling exponent for tuna RMR is higher than that for the SMR. A reason why tuna RMR may scale differently to the SMR may be related to mass-scaling of gill surface area. The surface area of the gills is considered to be related to the

requirement for oxygen, and should scale to M_b with a similar relationship to that of $\dot{M}O_2$ (Schmidt-Nielsen, 1984). As the gill surface area: mass exponent in fish is generally between 0.8 and 0.9, it supports the higher than average mass scaling exponent for fish when compared to most other vertebrates. The exponent between gill surface area and M_b in tuna appears to be no different to other teleosts (b=0.85, (Muir and Hughes, 1969). However, Hughes (1984) suggest that gill area limits maximal rates of oxygen uptake and thus should scale with the active metabolic rate , rather than the SMR. Author acknowledges that the scaling comparison made in the present study should be taken with caution. Ideally, a mass scaling exponents should be determined at a well-defined resting metabolic state over a wide range of homomorphic individuals (Schmidt-Nielsen, 1984). The logistic of working with tuna has meant that this has not been possible for the present and previous studies alike. Furthermore the present study does not take into account allometric scaling of swimming costs which may alter the relationship.

The present data for southern bluefin tuna extend the allometric analysis to the largest fish so far measured and gives greater confidence in the value of the scaling exponent. From this comparison, it appears that the slope of swimming tuna mass scaling is similar to that of other fish species, but the elevation is much higher. The mesocosm respirometer would permit measurements from even larger tuna, which would enable intraspecific analyses of M_b over one order of magnitude. Furthermore, tuna have been observed feeding within the respirometer, which suggests that the first examination of tuna specific dynamic action may be possible.

2. The energetic consequence of specific dynamic action in southern bluefin tuna (*Thunnus maccoyii*)

Abstract

The effect of feeding on the rate of oxygen consumption ($\dot{M}O_2$) of four groups of three southern bluefin tuna (SBT) (*Thunnus maccoyii*) was examined in a large static respirometer at water temperatures of 18.2-20.3°C. Six feeding events of rations between 2.1 and 8.5% body mass (%M_b) of sardines (*Sardinops sagax*) were recorded (two of the groups were fed twice). Before feeding, fish swam between 0.71 and 1.4 body lengths s^{-1} (BL s^{-1}) and the routine metabolic rate (RMR) was 366 ± 33 SE mg kg^{-1} h^{-1}. For all trials, $\dot{M}O_2$ was elevated post-feeding, presumably as a result of specific dynamic action (SDA). Swimming velocity was also elevated post-feeding for periods similar to that of $\dot{M}O_2$ (between 20-45 h, longer for the larger rations). Post-feeding swimming velocity increased to between 0.9-2.6 BL s^{-1} and was also dependent on ration consumed. It is suggested that the purpose of increased swimming velocity was to increase ventilation volume as a response to the enhanced metabolic demand associated with SDA. Peak postprandial $\dot{M}O_2$ increased linearly with ration size to a maximum of 1 290 mg kg^{-1} h^{-1}, corresponding to 2.8 times the RMR. When converted to its energy equivalent, the total magnitude of SDA was linearly correlated with ration size to a maximum of 192 kJ kg^{-1} h^{-1}. As a proportion of gross energy ingested (SDA coefficient) the magnitude of SDA averaged 34.7 ± 2.2 %. These results demonstrate that, although the factorial increase of SDA in SBT is similar to that of other fish species, the absolute energetic cost of SDA is much higher. The ration that SBT require to equal the combined metabolic costs of SDA and RMR is estimated in this study to be 3.5 %M_b of sardines per day.

Introduction

Tunas are highly specialized marine predators that are widely distributed throughout the world's tropical and temperate oceans. In this vast habitat, they have evolved

numerous unique anatomical, biochemical and physiological adaptations that have been instrumental to their success (Bushnell and Jones, 1994; Brill, 1996; Graham and Dickson, 2004). Of particular interest are the adaptations in the areas of metabolism and energetics. Tunas are known to have standard metabolic rates (SMR, the rate when theoretically at complete rest) and metabolic scopes that greatly exceed those of most other well studied fish species (Korsmeyer and Dewar, 2001). These elevated metabolic states allow tuna to achieve physiological feats, including rapid digestion and quick recovery from oxygen-debt, that enable them to take advantage of the ocean's patchy prey distribution. However, this metabolic amplification comes at an energetic cost, which has led some authors to describe tuna as "energy speculators", animals that gamble high rates of energy expenditure on potential higher rates of energy return (Brill, 1987; Korsmeyer et al., 1996).

Our understanding of tuna energetics lags far behind that of smaller species such as salmonids. The size and difficulty of handling tuna has meant only a small number of metabolic measurements have been possible. The majority of research on tuna metabolism has focused on evaluation of the SMR or quantification of swimming costs (Brill, 1979; Gooding et al., 1981; Graham and Laurs, 1982; Brill, 1987; Graham et al., 1989; Dewar and Graham, 1994; Sepulveda and Dickson, 2000; Blank et al., 2007). The metabolic cost of specific dynamic action (SDA) has yet to be examined in any tuna species.

Specific dynamic action (often referred to as apparent specific dynamic action, calorigenic effect or heat increment of feeding) refers to the total energy cost involved with ingestion, digestion, absorption and assimilation of a meal. In fish, SDA is a substantial component of total bioenergetics, representing between 5 and 20% of gross ingested energy (Muir and Niimi, 1972; Beamish, 1974; Carter and Brafield, 1992; Chakraborty et al., 1992; Peck et al., 2002; Fu et al., 2005c). However none of these studies have examined marine pelagic fish. Specific dynamic action has been hypothesized to be a significant component of tuna metabolism, because of their high rates of food consumption and digestion (Korsmeyer and Dewar, 2001). Furthermore, it is hypothesized that rapidly growing fish, such as tuna, should show the greatest postprandial increase in metabolic rate (Jobling, 1981; Brill, 1996). This theory is based on the consensus that a large proportion of the energetic cost of SDA is related to metabolic work responsible for growth, i.e. protein synthesis and turnover (Jobling, 1981; Brown and Cameron, 1991b; Brown and Cameron, 1991a; Lyndon et al., 1992).

The spacious, low-stress environment of the sea-based respirometer described in Chapter 1 supports voluntary feeding allowing this study to make the first recording of SDA of a tuna species, southern bluefin tuna (SBT) (*Thunnus maccoyii*), ingesting sardine (*Sardinops sagax*), an important food component in the diets of both wild and aquaculture SBT.

Methods

Experimental animals

SBT were purse-seine netted in the Great Australian Bight, most likely from the same wild school, in early January 2005. They were transferred to a sea-cage and slowly towed approximately 200 nautical miles back to the marine farms in the waters off Port Lincoln, South Australia. Soon after arrival, twelve juvenile SBT (10 ± 0.4 kg, mean ± SE) were sourced from the Tuna Boat Owners Association of South Australia and transferred into a 12 m diameter, 8 m deep sea-cage. Transfers involved hooking individual fish with a baited, barb-less hook and sliding the fish across a soft, wet slip (wet canvas over a foam mattress) into the adjacent cage. Transfer of individual fish typically took less than 15 s. The holding pontoon was towed 2 km to the Rotten Bay site and secured beside the mesocosm moored in a small bay at the south end of Boston Island. Fish were fed sardines to satiation twice a day and allowed 2 months to recover from the tow and transfer, and to become accustomed to the sea-cage environment.

Mesocosm respirometer

SBT oxygen consumption rates ($\dot{M}O_2$) was determined by the reduction in dissolved oxygen (DO) over time within a large static respirometer ("mesocosm respirometer"). The mesocosm was manufactured from denier polyester scrim reinforced 1.14 mm polypropylene (R-PP 45, Stevens Geomembranes, http://www.stevensgeomembranes.com), bonded by thermal welding by Fabtech SA Pty. Ltd (Adelaide, Australia). The mesocosm was a 12 m diameter, 2.5 m deep enclosed cylinder with a wave break wall that extended 1 m above water level (Fig.

1). Entry into the mesocosm was possible through a 2 m diameter, 2 m high access port made from 0.75 mm polypropylene and positioned in the roof so that it could be rolled and clamped to completely seal the system. In addition, five 1 m diameter, clear 0.75 mm polyvinyl chloride windows were positioned in the roof to allow entry of natural light, and eleven capped tank fittings (STF 50, Hansen Products Ltd, http://www.hansenproducts.co.nz/) allowed sealed access for experimental equipment and manual sampling.

The mesocosm was pump filled with sea-water to capacity (approximately 400 000 l) to reduce the likelihood of confinement stress. However, in subsequent experiments, water volume was reduced to 350 000 l as the initial volume was found to be slightly in excess of what was required to maximise available space within the respirometer.

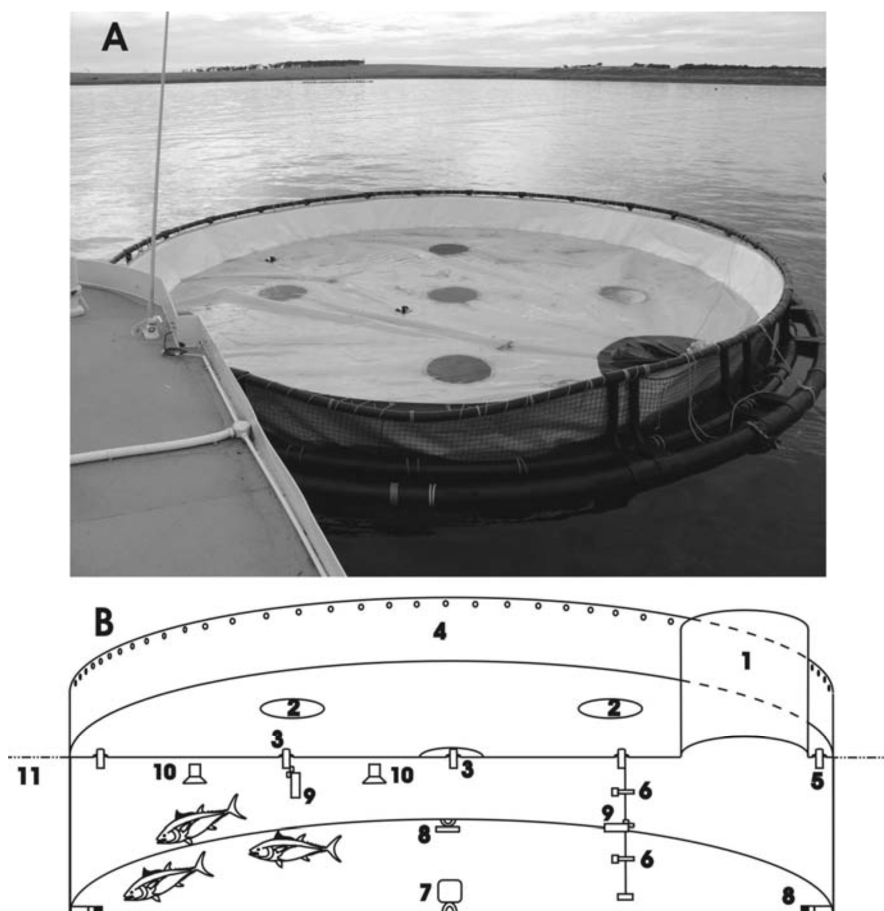

Fig. 1. (A) Mesocosm deployed in Rotten Bay, Australia. **(B)** Diagram of the mesocosm respirometer showing; [1]entrance port, [2]windows, [3] sample ports, [4]wave break wall, [5]cable entrance ports, [6]oxygen probes, [7]float, [8]lead weights, [9]video cameras, [10]lights, and [11]water level.

Fish were introduced into the mesocosm from the adjacent holding sea-cage by use of a baited, barb-less hook as described above or, in the case when the fish were hook-shy, by crowding the fish in a net and a skin-diver securing them by hand and passing the fish across the soft, wet, slipway into the respirometer. After introduction, any air bubbles that had collected on the internal surface (from scuba-diver expiration or oxygen injection) were rolled out of the access port, before the mesocosm was sealed. Oxygen consumption rates were determined by measuring the drop in mesocosm internal dissolved oxygen (DO) level over time. Mesocosm mixing was monitored by manual recordings of DO at the beginning and end of each experimental trial through screw cap access ports in the ceiling at nine positions within the respirometer (3, 6 and 9 m from the port access side and at three depths 0.25, 1.25 and 2.25m). Coefficient of variation between manual DO recordings from the nine sample positions did not exceed 2.6% for any trial, indicating that the mesocosm remained well mixed at all times presumably by the fish movement or mesocosm wall flexing.

Background respiration and photosynthesis of planktonic organisms in the water column and on the mesocosm surfaces were measured in trials immediately before and after each experiment. Low levels of background respiration were generally found, however it was dependent on time of day (net respiration rate would fall during the day due to photosynthesis). All $\dot{M}O_2$ recordings were adjusted for background respiration according to the pertinent time of day. The oxygen holding capabilities of the mesocosm was examined in a further two trials where the respirometer DO was reduced to either 1.90 or 2.26 mg l^{-1} by injecting microbubbles of pure nitrogen with a gas diffuser (Aqua & Co Force 7, BOC gases, NSW, Australia) and then removing the bubbles that collected at the water surface, before the mesocosm was sealed and oxygen monitored for more than 20 h. Subsequent oxygen leakage into the respirometer was found to be very low, representing just 0.0042 mg^{-1} h^{-1} per mg l^{-1} oxygen gradient between the respirometer and the external environment. This rate of leakage was nevertheless used to adjust all trial DO recordings according to the pertinent oxygen gradient. Dissolved ammonia concentrations were also monitored by daily analyses of water samples before, during and after each trial, and they never exceeded 0.6 mg l^{-1}.

Experimental protocol

The mesocosm was installed into the marine farm pontoon for 49 days between March and May 2005, when four trials were conducted. For each trial, three fish were transferred from the holding sea-cage into the respirometer in the late afternoon. The fish were left overnight to become accustomed to the respirometer environment. To verify whether this time was sufficient for acclimation, $\dot{M}O_2$ was recorded during the acclimation period for one trial. In this trial, $\dot{M}O_2$ was elevated immediately after introduction, but had plateaued within 7 h and remained steady for the subsequent 18 h (Fig. 2). This indicates that overnight was a sufficient period for acclimation.

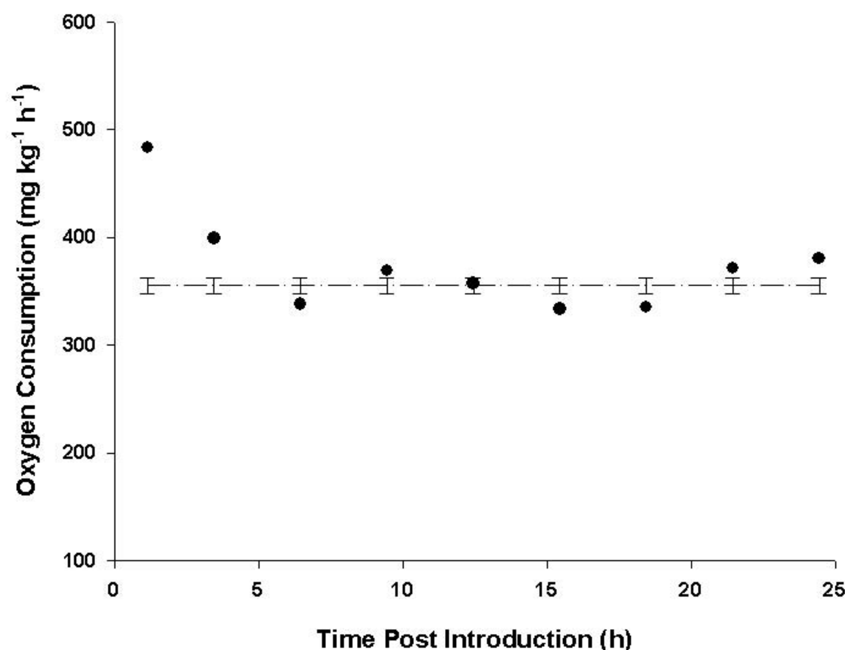

Fig. 2. Oxygen consumption rates ($\dot{M}O_2$) of three SBT after introduction into the mesocosm respirometer, showing the plateau in $\dot{M}O_2$ within 7 h from transfer and subsequently calculated mean routine metabolic rate (RMR, dotted line, ±SE of measurements (3 h periods)).

Measurement of the routine metabolic rate (RMR) was commenced the morning following introduction, when the respirometer was sealed and $\dot{M}O_2$ was monitored for 16-24 h. RMR was calculated as the mean, post-acclimation $\dot{M}O_2$ over this period (Fig. 2).

The following morning the mesocosm was re-opened and DO returned to approximately 100 % saturation by pure oxygen injected with the diffuser. The fish were then left for roughly 1 h to recover from this disturbance before they were offered sardines (Table 1) through the entrance port. Tuna consumed sardines during all attempts at feeding within the respirometer. Attempts were made to differentiate rations consumed by individual fish from both the surface and by a diver observing from within the mesocosm. However, these attempts proved largely unsuccessful as it was not always possible to differentiate individual fish from the surface and fish would often fail to ingest in the presence of a diver. After feeding, a diver collected any uneaten sardines, and this mass was subtracted from the known mass of bait offered to establish total consumption. Following feeding, the respirometer was sealed and $\dot{M}O_2$ monitored for the subsequent 40 h + (except for one trial when logging failed after 26 h).

Table 1: Nutritional content of the sardines (*Sardinops sagax*) ingested by southern bluefin tuna in specific dynamic action experiments.

Feed component	Content
Protein	20.2%
Fat	1.7%
Ash	3.2%
Dry Matter	27.4%
Moisture	72.6%
Energy	5.6 mJ kg^{-1}

Proximal analysis conducted by Weston Food Laboratories (NSW, Australia).

Feed component presented as % wet weight.

Energy value calculated on the basis of 23.6 and 39.5 kJ g^{-1} of protein and lipid (Suarez et al., 1995).

Dissolved oxygen and water temperature measurement

Two luminescence dissolved oxygen meters (Hach HQ10, Loveland, CO, USA) permanently installed at a depth of 1 and 2 m respectively, both 3 m from the wall logged mesocosm DO and water temperature. Meters were calibrated according to the manufacturer's instructions at the start of the trials and calibration checked following the completion of all trials. Meters recorded DO (0.01 mg l^{-1} resolution) that was automatically compensated for water temperature and salinity set at $35^{0}/_{00}$. Oxygen meters were set to log DO every 5 or 15 min, depending on the trial-logging

interval required (24 or 48 h). All DO recordings were broken up into 3 h blocks according to time of day (starting at midnight) and linear regressions were fitted to the change in DO (mg l^{-1}) representative for that 3 h period. Approximate drop in mesocosm DO was ~ 0.04 mg l^{-1} h^{-1} during RMR measurement and as great as 0.12 mg l^{-1} h^{-1} during SDA measurement. The high-resolution stability of these luminescent meters meant that the linear regression coefficient of determination generally remained between R^2 = 0.96-0.99 over each 3 h period. $\dot{M}O_2$ calculations were adjusted for the mean background respiration rate (mean of pre- and post-SDA trial background recording) recorded for the respective time of day (3 h block) and oxygen leakage through the respirometer walls. A similar DO meter was used to make daily recording of the DO in the external environment, which remained between 7.1 and 7.4 mg l^{-1}.

Swimming velocity analysis

Fish behaviour was monitored (both day and night) with a permanently installed video system (SciElex Pty. Ltd., Hobart, Australia) that consisted of two 0.05 lux black and white underwater video cameras and two 6 W white LED lights (housed within resin) (Fig. 1). The system was programmable through a central controller and power supply unit that were secured to the sea-cage. The cameras were programmed to record for 4 min every 90 min (first by the horizontal camera then immediately by the vertical camera) and data stored on a pocket video recorder (Archos AV400, Greenwood Village, CO, USA). The lights were programmed to turn on at dusk and remain on until dawn. The system was powered by a single 32 Ah, 12 V battery that was exchanged daily.

Video recordings were viewed in the video editing program (VirtualDub 1.5.10, Avery Lee, www.virtual.dub.org). Suitable records were defined as those containing a fish that was swimming perpendicularly to the camera and at least one frame in which body length (BL) could be measured for scaling. This sequence was imported into video motion analysis software (Video Point Version 2.5, Lenox Softworks, www.lsw.com) where swimming velocity was analysed by tracking movement across the field. Tuna BL was used to calibrate the image scale, accounting for variable distances between the fish and the camera. Any camera

movement was corrected by referring to a stable point within the respirometer on a frame-by-frame basis.

Data analysis

Swimming velocity data were collated within the same 3 h time periods as DO. For each 3 h period, between four and seven video footage segments were analysed for swimming velocity and the mean taken as representative for that period (a total of four hundred and forty eight footage segments were analysed). Due to poor quality footage, four suitable footage segments were not available for some 3 h periods. Video records after 24 h in trial 6 were lost due to video failure.

Mean swimming velocity recordings made during the RMR measurement are subsequently referred to as the routine swimming velocity (U_r) and velocity measurements made post-feeding as the SDA swimming velocity (U_{sda}). U_{sda} refers to the duration that post-feeding swimming velocity remained one standard error (SE) above *U*. The effect of meal size on metabolic rate was quantified by the following parameters: SDA peak (maximum post-prandial $\dot{M}O_2$), SDA factorial peak (SDA peak divided by RMR), SDA duration (duration that post prandial $\dot{M}O_2$ remained greater than one SE above the RMR), SDA magnitude (total oxygen consumed above the RMR within the SDA duration period) and SDA coefficient (SDA magnitude as a percentage of gross ingested energy). Conversion from oxygen consumed to the energy equivalent was calculated assuming 14.32 J of energy are expended per milligram oxygen consumed (Beamish and Trippel, 1990).

Results

Table 2: Southern bluefin tuna body mass (M_b), body length (BL), water temperature (T_w), routine swimming velocity (U_r), calculated routine metabolic rate (RMR), ingested ration of sardines, total ingested energy (IE), mean swimming velocity during the SDA period (U_{sda}), duration of elevated postprandial swimming velocity (U_{sda} duration) and SDA peak, factorial peak, duration, magnitude and coefficient.

Trial	1	2		3		4	Mean (n = 4 or 6)
M_b (kg)	9.4 ± 1.6	9.9 ± 0.9		10.5 ± 1.3		11.3 ± 1.4	**10.3 ± 0.4**
BL (cm)	81 ± 5	83 ± 2		88 ± 3		86 ± 2	**84 ± 2**
T_w (°C)	19.7-20.1	20.1-20.3		19.1-19.7		18.2-18.5	**19.3 ± 0.4**
U_r (BL s^{-1})	0.71	1.40		0.78		0.83	**0.92 ± 0.15**
RMR (mg kg^{-1} h^{-1})	300	456		355		354	**366 ± 33**
Feed trials	**1**	**1**	**2**	**1**	**2**	**1**	**-**
Ration (%M_b)	5.7	6.0	8.5	2.1	6.8	4.1	**5.5 ± 0.9**
IE (mJ)	8.96	10.0	14.1	3.70	12.1	7.73	**9.48 ± 1.48**
U_{sda} (BL s^{-1})	0.9	2.0	2.6	0.8	1.7	1.4	**1.6 ± 0.9**
U_{sda} Duration (h)	26	38	45+	20	45+	24+	**33 ± 5**
Peak (mg kg^{-1} h^{-1})	728	1, 240	1, 290	469	840	669	**872 ± 133**
Factorial peak	2.4	2.7	2.8	1.3	2.3	1.9	**2.2 ± 0.2**
Duration (h)	24	23	42	27	45+	25	**31 ± 4**
Magnitude (kJ kg^{-1})	99	130	192	31	148	75	**112 ± 23.**
Coefficient (%)	31.3	38.5	40.2	26.4	38.8	32.9	**34.7 ± 2.2**

Values are mean for the three fish used in each trial. ± SE.

During the RMR measurement, three of the four groups of fish swam slowly and consistently between approximately 0.7 and 0.8 BL s^{-1}, the other group swam considerably faster at around 1.4 BL s^{-1} (U_r, Table 2). The fish that swam the fastest also consumed the most oxygen with a strong linear relationship ($R^2 = 0.94$) between swimming velocity and RMR (Fig. 3A). The evaluated RMR, taking into account background respiration and diffusion, ranged between 300 and 456 mg kg^{-1} h^{-1} corresponding to a mean of 366 ± 33 mg kg^{-1} h^{-1} (n = 4). This is less than the RMR of SBT recorded in Chapter 1 (460 mg kg^{-1} h^{-1}). The discrepancy is likely due to individual variability in voluntary swimming speeds and the longer acclimation period used in the present study (16+ h versus 6 h).

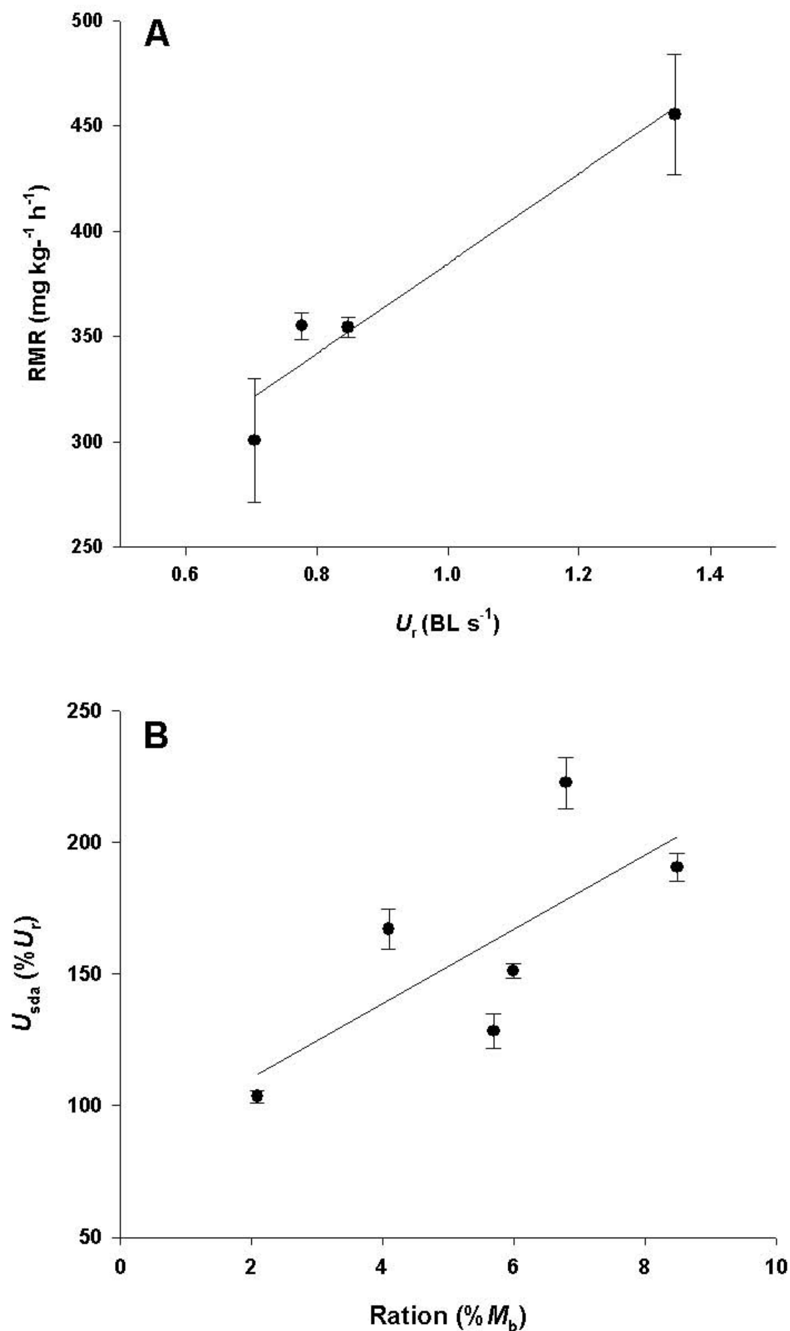

Fig. 3. (A) The relationship between the routine swimming velocity (U_r, body lengths s^{-1}) and the routine metabolic rate (RMR) of southern bluefin tuna with fitted linear regression (y = 219x – 166, R^2 = 0.94, n = 4). Values shown are means ± SE (n = 5-9). **(B)**: the relationship between ration consumed (% Body weight) and the factorial increase swimming velocity recorded during the SDA period (% U_r) with fitted linear regression (y = 14.1x + 82.5, R^2 = 0.53, n = 6). Values are means ± SE (n = 5-15).

Six feeding events of rations between 2.1 and 8.5 % body mass (%M_b) of sardines were recorded (two of the experimental groups were fed twice, the other two experimental groups fed only once) (Table 2). On all occasions, swimming velocity increased post-feeding. The duration before swimming velocity returned to pre-feeding levels (U_r) appeared to increase with ration size, from 20 h for the smallest ration to longer than the 45 h measurement period for the greatest two rations. The mean swimming velocity recorded during the SDA period (U_{sda}) was linearly related to ration size (R^2 = 0.53) (Fig. 3B).

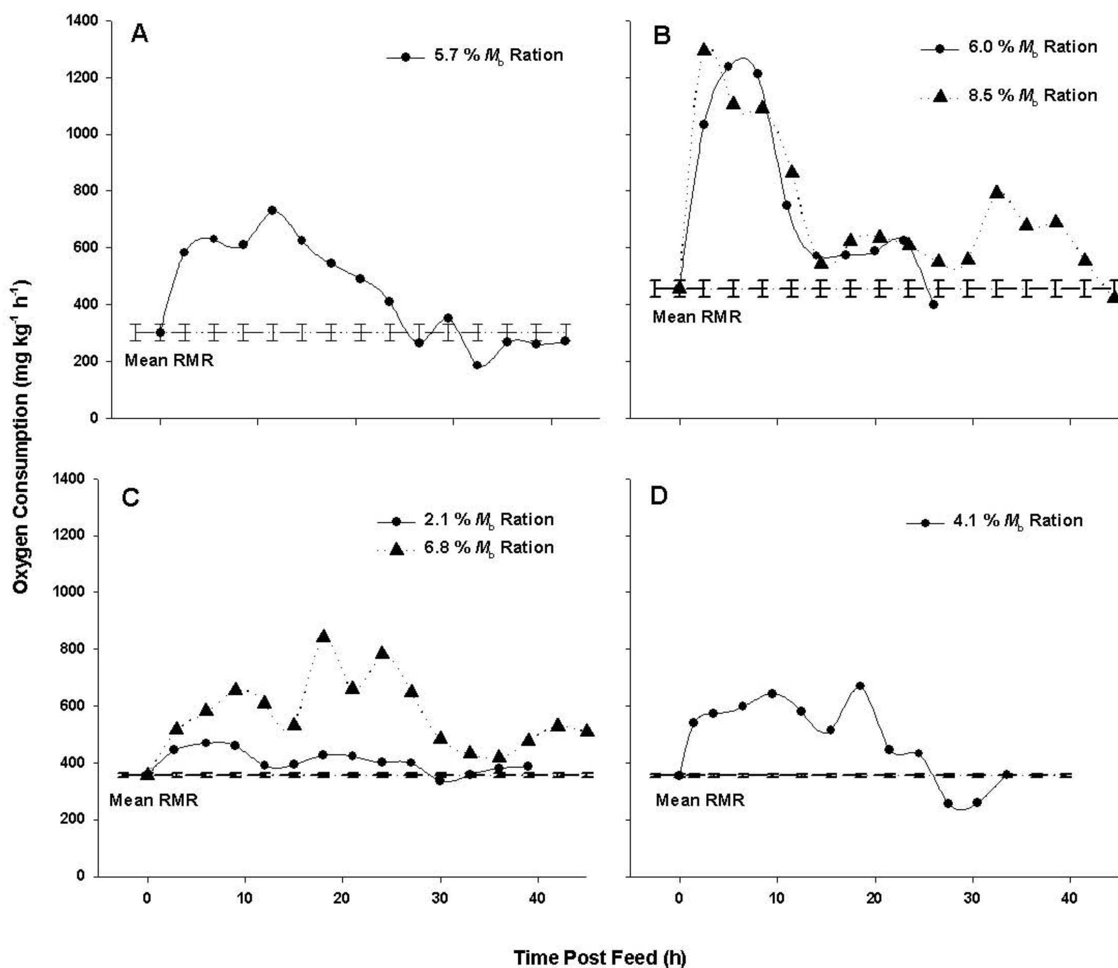

Fig. 4. Post feeding oxygen consumption rate for southern bluefin tuna for the six feeding events recorded of ration size between 2.1 and 8.5% Body weight (%M_b) in the four mesocosm respirometer experiments (**A**), (**B**), (**C**), and (**D**). Dotted line represents the calculated routine metabolic rate (RMR).

In all trials, $\dot{M}O_2$ was elevated above the RMR post-feeding, however the nature of this elevation was highly dependent on ration size and experiment (Fig. 4). Maximum postprandial $\dot{M}O_2$ (SDA peak) increased linearly with ration size (R^2 = 0.71) (Fig. 5A). At the greatest ration consumed (8.5 %M_b), SDA peak was 1 290 mg kg^{-1} h^{-1}, corresponding to 2.8 times the RMR, whilst with the smallest ration (2.2 %M_b), SDA peak was 469 mg kg^{-1} h^{-1}, or just 1.3 times the RMR (Table 2). The duration of SDA ranged between 23 and 45 h and was poorly correlated with ration size (R^2 = 0.38) (Fig. 5B). At the four lowest rations, SDA duration did not appear to be affected by ration size, however above a ration of 6 %M_b, SDA duration was greatly elevated. The magnitude of SDA ranged between 31 and 191 kJ kg^{-1} (Table 2) and was strongly linearly correlated with ration size (R^2 = 0.98) (Fig. 5C). SDA coefficient averaged 34.7 ± 2.2% (n = 6) (Table 2).

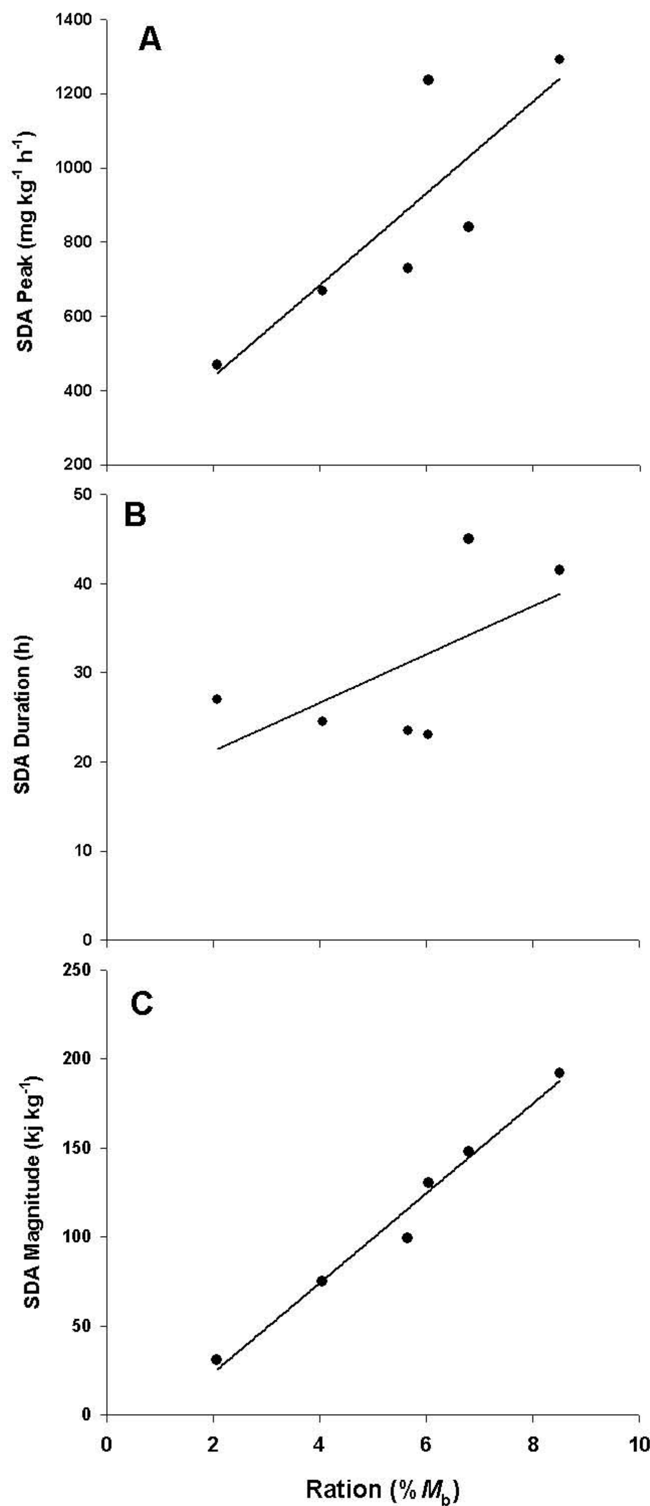

Fig. 5. Relationship between southern bluefin tuna ration size (% body weight) and, **(A)** specific dynamic action (SDA) peak with fitted regression (y = 124x + 190, R^2 = 0.71), **(B)** SDA duration (y = 2.7x + 15.8, R^2 = 0.38) and **(C)** SDA magnitude (y = 25.2x + 26.7, R^2 = 0.98), for the six feeding events recorded in the mesocosm respirometer.

Discussion

Effect of meal size on SDA

The pattern of SDA in fish is characterized by a rapid increase in $\dot{M}O_2$ after feeding before gradually falling back to the resting level (Jobling, 1981). The pattern of SDA response appears to be no different for SBT. The factorial scope and duration of SDA is comparable to many fish species examined (Jobling, 1981; Chakraborty et al., 1992; Lyndon et al., 1992; Hunt von Herbing and White, 2002; Peck et al., 2002; Fu et al., 2005b; Fu et al., 2005a). Also like most fish species, peak, duration and magnitude of SDA appear to be directly related to ration size (Muir and Niimi, 1972; Jobling and Davis, 1980; Carter and Brafield, 1992; Chakraborty et al., 1992; Fu et al., 2005c; Fu et al., 2006). However, the energetic cost of SDA is approximately double (SDA coefficient = 35 %) that recorded in other teleost species (generally between 5-20%). It appears that although the factorial pattern of SDA (SDA peak and duration) in SBT is consistent with other fish, the absolute metabolic cost of SDA is much higher.

High metabolic cost of SDA

A complete understanding of the physiological basis of SDA remains unclear, however much of its effect is attributed to the metabolic cost of growth. That is, the postprandial influx of amino acids stimulates rapid synthesis and turnover of tissue proteins resulting in much of the increase in metabolic rate known as SDA (Jobling, 1981; Brown and Cameron, 1991b; Brown and Cameron, 1991a; Lyndon et al., 1992). In fish, both protein synthesis and degradation rates have been shown to rise linearly with growth rate (Houlihan et al., 1988), and therefore are likely to contribute more to the metabolic cost of SDA in fast growing species. The growth rates of tuna, like other large pelagic fishes, are known to be high (Uchiyama and Struhsaker, 1980; Brill, 1996; Sainsbury et al., 1997; Glencross et al., 2002). Furthermore, the SBT examined in the present study although large in comparison to other specimens examined, were in fact juveniles (Collette and Nauen, 1983; Schaefer, 2001). Juvenile fish have been shown to divert a proportionately high amount of available

metabolic scope to support SDA to meet the demands of their fast growth rates (Hunt von Herbing and White, 2002). The likely discrepancy between SDA duration and food gut passage time supports the notion that SDA is mostly a post absorptive effect. The recorded duration of SDA (between 25 and 45 h) is far greater than the likely gut evacuation time, which in tuna has been shown to be greatly accelerated (~10-12 h) (Magnuson, 1969; Schaefer, 1984; Olson and Boggs, 1986). This suggests that high protein turnover and synthesis rates associated with fast growth rates of juvenile SBT are likely to elevate the metabolic cost of SDA. Unfortunately, there is no information on the cost of SDA in other fast growing pelagic fishes for comparison.

It is possible that the low energy value of the ingested sardines may have contributed to the high SDA coefficient recorded. The nutritional balance of the diet can affect SDA, in particular the protein level and its balance with non-protein energy sources. Deamination of ingested amino acids is considered to be a significant proportion of SDA in carnivorous fish (Beamish and Trippel, 1990; Cho and Kaushik, 1990). Diets with excess amounts of protein or insufficient non-protein energy sources (i.e. fat or digestible carbohydrates) can elevate the energy expenditure of SDA as a result of increased deamination of amino acids (Jobling and Davis, 1980; LeGrow and Beamish, 1986; Beamish and Trippel, 1990; Chakraborty et al., 1992; Peres and Oliva-Teles, 2001). When represented in terms of the digestible protein (P, g) and digestible energy (E, mJ), the P:E ratio of the sardines fed in the present study (34 g mJ^{-1}, assuming 90% and 85% digestible energy and protein, respectively) is considerably greater than that of diets thought to be optimal in aquaculture of salmonid species (P:E = 20 g mJ^{-1}) (Cho and Kaushik, 1990). It is likely that this excessive protein to energy ratio would have resulted in high levels of amino acid deamination contributing the high SDA coefficient recorded in the present study. However, this nutritional profile is typical for sardines caught in Southern Australian waters (Ellis and Rough, 2005) and as the sardine is an important wild-fish food source (Kemps et al., 1999), this high SDA is biologically relevant.

Elevated energy expenditure associated with postprandial visceral warming may also contribute to SDA. Tuna of the subgenus *Thunnus* can elevate their visceral temperature (Collette et al., 2001) which is characterised by a rapid increase following feeding, before slowly cooling to a basal temperature of 2°C above ambient water temperature up to 60 h post feeding (Gunn et al., 2001). The primary purpose of

visceral warming is thought to be to accelerate digestion (Stevens and McLeese, 1984). The source of heat is hypothesized to be a result of aerobic metabolism associated with SDA which is retained by counter-current exchange in *retia mirabilia* that thermally isolate the stomach, caecum, intestines and spleen (Carey et al., 1984). The liver however, is on the cold side of the *rete* and thus does not contribute to visceral warming. This is surprising, as the liver is considered to have the highest rates of protein synthesis of teleost tissues (Carter and Houlihan, 2001) and thus would be an obvious source of SDA-associated heat production. Protein synthesis rates in the livers of juvenile Atlantic cod have been shown to increase rapidly after feeding and after 6 h account for 31% of whole body protein synthesis compared to just 9% for the stomach (Lyndon et al., 1992). This suggests that only a small fraction of SDA contributes to visceral warming (related to gastrointestinal work and absorption) leaving potential for the involvement of another mechanism. Support for this was given by Gunn et al. (2001), who found that, for a given ration, the magnitude of SBT visceral warming was much greater in winter than in summer. They suggested that SBT possess a compensatory acclimation mechanism to provide increased digestive efficiency at low ambient temperatures, possibly facilitated by increased aerobic metabolism and/or the heat conservation (through changes in the activity of the visceral *retia*). Thus, it appears that visceral warming may not simply be the accumulation of heat by-product of SDA, but may be an energy-consuming mechanism to maintain optimal digestive rates.

The observed increase in swimming velocity after feeding would have contributed to the measured metabolic cost of SDA. Fish on average swam 1.7 times faster after feeding (1.6 compared to 0.92 BL s^{-1}). Based on the relationship between $\dot{M}O_2$ and swimming velocity described by Dewar and Graham (1994) for 2.2 kg yellowfin tuna (*Thunnus albacares*), an increase in swimming velocity from 0.92 to 1.6 BL s^{-1} would increase metabolic rate from 409 to 585 mg kg^{-1} h^{-1}. A potential explanation for faster swimming is to increase ventilation volume as a response to the enhanced metabolic demand associated with SDA. This theory is supported by the fact that the fish that consumed the most also swam the fastest (Fig. 3B) and for most of the feeds examined, the duration of elevated swimming velocity post feeding (U_{sda} duration) is similar to the duration of SDA. However, the pattern of post-feeding swimming velocity was more difficult to distinguish than that of SDA, as for the two greatest rations it failed to return to pre-feeding levels within the 45 h timeframe of

each experiment. At present the reason for this discrepancy between swimming velocity and SDA is unknown, however it may be due to measurement error caused by individual variation in swimming speed due to unequal ration consumption, or diurnal swimming cycles (fish appeared to swim faster at dusk and dawn). More intensive examination of the effects of feeding on individual tuna swimming velocity over a longer experimental period is required to verify the metabolic cost of faster postprandial swimming.

SDA and metabolic scope

Consistent with some fish species, SBT peak postprandial $\dot{M}O_2$ increased with ration size (Muir and Niimi, 1972; Chakraborty et al., 1992); however for other species, SDA peak reaches a maximum and plateaus with increased ingestion (Beamish, 1974; Fu et al., 2005c). In the latter, SDA peak has a species-specific maximum that limits the proportion of available metabolic scope devoted to SDA and allows other metabolic activities to occur simultaneously (Beamish, 1974). It is possible that SBT reach this plateau at a ration size greater than that examined in the present study (8.5 $\%M_b$). However, this is unlikely as anecdotal evidence from tuna-farm managers indicates that sardine rations of 8.5 $\%M_b$ are close to the maximum feed intake that SBT accept in a single meal. According to cardiorespiratory models, the factorial scope of skipjack (*Katsuwonus pelamis*) and yellowfin tuna is approximately 8-9 times the SMR (Brill and Bushnell, 1991; Korsmeyer and Dewar, 2001). If a similar scope of 7 times the RMR is assumed for SBT (less than that predicted above as the comparison is based on a low RMR not the SMR), it corresponds to 2 560 mg kg^{-1} h^{-1}, which is similar to the maximum-recorded $\dot{M}O_2$ of a tuna species, 2 500 mg kg^{-1} h^{-1} (Gooding et al., 1981). The maximum recorded postprandial metabolic rate (1 300 mg kg^{-1} h^{-1}) measured in the present study is approximately 50% of this predicted metabolic scope, suggesting that even during the peak of SDA at the greatest ration recorded, the SBT examined would still have had considerable metabolic scope available to perform other tasks (i.e. oxygen debt recovery and locomotory costs beyond the requirement for hydrostatic equilibrium and ventilation). This supports the theory that the main benefit of high aerobic scope in tunas is to simultaneously support multiple metabolic functions needed to sustain their high performance requirements (Brill and Bushnell, 1991; Korsmeyer et al., 1996).

Energetic consequence of a high metabolic cost of SDA

To estimate minimum daily energetic cost of feeding SBT, figure 6 presents total energy expenditure as a percentage of ingested energy for the six feeding trials (calculated as the total oxygen consumption the 24 h post feeding). The break-even point (feed ration at which energy expenditure was equal to ingested energy) is approximately a ration of 3.5 %M_b day^{-1}. However, if SBT are ultimately to grow and reproduce their minimum daily ration of sardines needs to be greater than this, as some ingested energy is indigestible and further energy is lost in the form of urine, gill excretion and surface losses. Furthermore, the calculated energetic cost is likely to be a minimum, as activity was restricted. With wild fish, the cost of activity is likely to be much greater due to the activity requirement of predation and competition.

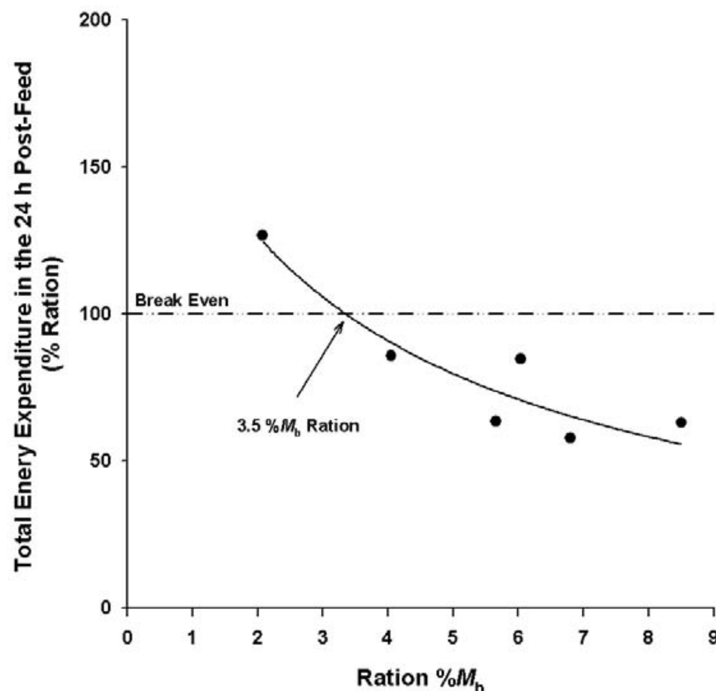

Fig. 6. Relationship between southern bluefin tuna ration size (% body mass, %M_b) and total energy consumption (calculated as the total oxygen consumption the 24 h post feeding) in the 24 h after feeding as a percentage of ingested energy for the six feeding events recorded in the mesocosm respirometer. The dotted line represents the theoretical point that the amount of energy used by the fish's metabolic processes equals the amount of ingested energy.

Several bioenergetic models for tuna species have been described (Kitchell et al., 1978; Korsmeyer et al., 1996). These show that tuna are “energy speculators”, gambling high rates of energy expenditure for potentially higher rates of energy returns. The work presented here agrees with this conclusion; however it shows one aspect of tuna energetics, the metabolic costs of SDA, may have been considerably underestimated. Previous models have estimated the cost of SDA to be only 15 % of the ingested energy (Kitchell et al., 1978; Korsmeyer et al., 1996). For SBT consuming the predominant natural food source in Australia’s southern oceans, 35 % of the ingested energy is lost in the SDA process. This finding further exemplifies the risk that tuna take in balancing performance with efficiency and highlights the importance of oceanic prey species communities to these valuable fish.

3. The effect of baitfish lipid content on the energetic cost of specific dynamic action in southern bluefin tuna (*Thunnus maccoyii*)

Abstract

This study examines the effects of feeding sardines (*Sardinops sagax*) of varying lipid levels (but similar protein content) on the rate of oxygen consumption ($\dot{M}O_2$), and swimming velocity of southern bluefin tuna (SBT) (*Thunnus maccoyii*). Five groups of two or three SBT (mean mass = 19.8 ± 0.5 SE kg, n = 14) were fed a total of thirteen times. Eight times they were fed low-lipid sardines (1.8-4.0 % lipid, 5.2-6.6 MJ kg^{-1} gross energy (GE)) and five times a high-lipid sardine (12.9 %, 9.2 MJ kg^{-1} GE). Before feeding, fish swam at 0.75 ± 0.03 body lengths per second (BL s^{-1}) (n = 5) and their routine metabolic rate was 306 ± 15 mg kg^{-1} h^{-1}. $\dot{M}O_2$ was elevated after feeding presumably as a result of specific dynamic action (SDA). However, the effect was greatest for the fish that ingested high-lipid sardines. The magnitude of SDA for fish that ingested high-lipid sardines (3.20 ± 0.23 MJ per kg sardine ingested, n = 5) was significantly greater than those that ingested low-lipid sardines (1.93 ± 0.10 MJ kg^{-1}, n = 8). Fish also swam faster during the SDA period, with the increase in velocity being greatest for the fish that ingested high-lipid sardines (1.33 ± 0.04 BL s^{-1}). When the energetic cost of SDA is represented as a proportion of ingested energy, there was no difference between fish that ingested high- (34.3 ± 2.4%) or low-lipid sardines (31.5 ± 2.9%). This demonstrates that the enhanced SDA magnitude for high-lipid sardines was proportional to the increased ingested energy, and shows that for baitfish diets with lipid contents over a range that is likely to span that of wild tuna prey, the energetic cost of SDA in SBT is approximately double that recorded in other teleosts. These results confirm that the high energetic cost of SDA is ecologically relevant. The majority of the high cost of SDA is hypothesized to be attributed to high-protein synthesis rates, therefore representative of the energetic costs of the fast growth rates of tuna.

Introduction

Specific dynamic action (SDA) is the increase in metabolic rate that occurs during a period after feeding (Jobling, 1981; McCue, 2006). The first measurements of SDA in a tuna species, southern bluefin tuna (SBT) (*Thunnus maccoyii,* Castelnau, 1872), were reported on in Chapter 2. These measurements show that the duration and factorial peak of SDA are typical for teleost fishes, but the absolute energy increment is much greater. For SBT ingesting sardines (*Sardinops sagax*), approximately 35% of gross ingested energy is used in the processes of SDA (SDA coefficient), which is approximately double the SDA coefficients (5-20%) commonly recorded in other teleosts (Chapter 2). However, in that study the lipid content (~2% wet weight) of the sardine ingested was low, corresponding to a low energy diet that was dominated by protein (20%). In carnivorous fish, diets with greater protein have been shown to elevate the energy expenditure of SDA presumably as a result of increased deamination of amino acids (Jobling and Davis, 1980; LeGrow and Beamish, 1986; Beamish and Trippel, 1990; Chakraborty et al., 1992). Furthermore, if there are insufficient non-protein energy sources (i.e. lipids), dietary protein is deaminated and oxidized to meet the energy requirement (LeGrow and Beamish, 1986; Beamish and Trippel, 1990). In this case, a decrease in the protein-to-energy ratio by increasing the lipid content, is said to "spare" protein from deamination and oxidation for energy, in turn reducing the magnitude of SDA (LeGrow and Beamish, 1986; Peres and Oliva-Teles, 2001). Therefore, it is possible that the high SDA coefficient recorded in Chapter 2 was at least partially related to the nutritional content of the sardines ingested and may not be representative of tuna ingesting higher-lipid diets.

There is a strong likelihood that wild SBT would commonly consume higher-lipid diets. The mean lipid content of sardines caught in the SBT feeding ground of the Great Australian Bight is ~4% (Ellis and Rough, 2005). Furthermore, there is evidence to suggest that SBT move to the Great Australian Bight to coincide with a period of high productivity (Austral summer-autumn) to feed on sardines when their lipid content is highest (up to 6.8%) (Ward et al., 2006). Elsewhere, SBT have been shown to feed mainly on small pelagic fishes such as jack mackerel (*Trachurus declivis*) and red bait (*Emmelichthys nitidus nitidus*) (Young et al., 1997; Kemps et al., 1998). The lipid content of these two baitfish species is considerably higher than

that of sardines: jack mackerel mean ~6% (Vlieg, 1988) and red bait mean ~9% (Ellis and Rough, 2005).

The aim of this study is to determine whether lipid content of baitfish diets significantly affects the SDA coefficients in SBT and to establish if the previously recorded high SDA coefficients were greatly influenced by the diet ingested. The results have significant bioenergetic implications for SBT, as it will ascertain if the high energetic cost of SDA is ecologically relevant and likely associated with their unique high performance physiology thus, common for all tuna.

Methods

Experimental animals

In January 2006, fourteen SBT were sourced from a marine aquaculture farm in the waters off Port Lincoln, South Australia, through the Tuna Boat Owners Association of South Australia. These SBT had been purse-seine netted in the Great Australian Bight one month earlier. From the commercial farm, individual SBT were hooked with a baited, barb-less hook and transferred into a 12 m diameter, 8 m deep holding sea-cage that was towed and moored in a protected bay at the southern end of Boston Island, where all trials were conducted. SBT were then left to recover from transfer for 2 months and were fed both high- and low-lipid sardines to satiation twice per day. They were starved for a minimum of 48 h before any experimental trial. SBT were transferred into the respirometer by crowding them within a purse-seine net and skin diver securing the fish by hand and passed it across a soft, wet slipway into the adjacent respirometer.

Mesocosm respirometer

SBT oxygen consumption rates ($\dot{M}O_2$) was determined by the drop in dissolved oxygen (DO) over time within a large static respirometer (“mesocosm respirometer”). The mesocosm was manufactured from denier polyester scrim reinforced 1.14 mm polypropylene (R-PP 45, Stevens Geomembranes, http://www.stevensgeomembranes.com), bonded by thermal welding by Fabtech SA

Pty. Ltd (Adelaide, Australia). The mesocosm was a 12 m diameter, 2.5 m deep enclosed cylinder with a wave break wall that extended 1 m above water level (Chapter 2). Entry into the mesocosm was possible through a 2 m diameter, 2 m high access port made from 0.75 mm polypropylene and positioned in the roof so that it could be rolled and clamped, to completely seal the system. In addition, five 1 m diameter, clear 0.75 mm polyvinyl chloride windows were positioned in the roof to allow entry of natural light, and eleven capped tank fittings (STF 50, Hansen Products Ltd, http://www.hansenproducts.co.nz/) allowed sealed access for experimental equipment and manual sampling.

The mesocosm was pump filled with sea-water to a total water volume of between 400 000-402 000 l (variation between experiments due to water exchanges).

Experimental protocol

Five experimental trials were conducted between March and May 2006. As SBT were obviously distressed when confined within the respirometer alone, either two or three fish were used for each trial. SBT were introduced in the late afternoon, respirometer sealed, and DO measured for the subsequent 36 h. SBT $\dot{M}O_2$ was commonly elevated after introduction, presumably as a result of transfer stress, but would plateau within 6-20 h of transfer. Routine metabolic rate (RMR) was determined as the mean $\dot{M}O_2$ after this period.

The following morning, the mesocosm access port was opened and DO returned to ~100-110 % saturation by oxygen injection with an oxygen diffuser (Force 7, Aqua & Co, BOC Gases, NSW, Australia). After 1 h to allow for recovery from this disturbance, SBT were fed weighed sardines to satiation through the access port and any uneaten baitfish removed by a diver to obtain the net consumption. Two different sardines were fed in separate trials, a low-lipid (1.8-4.0%) and a high-lipid (12.9% lipid) sardine (Table 1). Any collected bubbles were then removed from the respirometer before it was sealed and DO monitored until the morning approximately 48 h later. The experiment was then either terminated, SBT removed and body length (BL) and mass (M_b) recorded, or the DO was returned to ~100% saturation and SBT fed again with the same procedure.

Table 1. Nutritional content of sardines (*Sardinops sagax*) ingested by southern bluefin tuna in the specific dynamic action experiments.

Feed Component	Low-Lipid	High-Lipid
Lipid	1.8-4.0	12.9
Protein	17.2-20.5	17.1
Ash	3.3-3.5	3.3
Water	70.9-76.0	64.4
Gross Energy (MJ kg^{-1})	5.2-6.6	9.2

Analysis conducted by the South Australian Research and Development Institute, Pig & Poultry Production Institute, (Adelaide, Australia).
Feed components presented as % wet weight.
Gross energy derived by bomb calorimetry.
Low-lipid sardines were sourced from local commercial fish fleets from the Port Lincoln, Australia. High-lipid sardines were imported from commercial fishing fleets in northern California, USA.
Variation in low lipid sardine nutritional content due to use of different sardine batches. Only one batch of high lipid sardines was used.

Background respiration and photosynthesis of planktonic organisms in the water column and on the mesocosm surfaces were measured in trials immediately before and after each experiment. Low levels of background respiration were generally found, however it was dependent on time of day (net respiration rate would fall during the day due to photosynthesis). All $\dot{M}O_2$ recordings were adjusted for background respiration according to the pertinent time of day. The oxygen holding capabilities of the mesocosm was examined in a further trial where the respirometer DO was reduced to 2.2 mg l^{-1} by injecting micro-bubbles of pure nitrogen with a gas diffuser (Aqua & Co Force 7, BOC gases, NSW, Australia) and then removing the bubbles that collected at the water surface, before the mesocosm was sealed and oxygen monitored for more than 20 h. Subsequent oxygen leakage into the respirometer was found to be negligible. This was improved from Chapter 2 where low levels of oxygen leakage into the respirometer were recorded. Improved oxygen holding capacity was attributed to minor modification made between experiments, including double sealing of material bonds and improved access port sealing procedure. Dissolved ammonia concentrations were also monitored by daily analyses of water samples before, during and after each trial, and they never exceeded 0.6 mg l^{-1}.

Dissolved oxygen, water temperature, and swimming velocity measurement.

Two luminescence dissolved oxygen meters (Hach HQ10, Loveland, CO, USA) permanently installed at a depth of 1 and 2 m respectively, both 3 m from the wall, logged mesocosm DO and water temperature. Meters were calibrated according to the manufacturer's instructions at the start of the trials and calibration checked following the completion of all trials. Meters recorded DO (0.01 mg l^{-1} resolution) that was automatically compensated for water temperature and salinity set at $35^{0}/_{00}$. Oxygen meters were set to log DO every 5 min.

Fish behaviour was monitored (both day and night) with a permanently installed video system (SciElex Pty. Ltd., Hobart, Australia) that consisted of two 0.05 lux black and white underwater video cameras and two 6 W white LED lights (housed within resin). The system was programmable through a central controller and power supply unit that were secured to the sea-cage. The cameras were programmed to record for 4 min every 90 min (first by the horizontal camera then immediately by the vertical camera) and data stored on a pocket video recorder (Archos AV400, Greenwood Village, CO, USA). The lights were programmed to turn on at dusk and remain on until dawn. The system was powered by a single 32 Ah, 12 V battery that was exchanged daily.

Video recordings were viewed in the video editing program (VirtualDub 1.5.10, Avery Lee, www.virtual.dub.org). Suitable records were defined as those containing a fish that was swimming perpendicularly to the camera and at least one frame in which body length (BL) could be measured for scaling. This sequence was imported into video motion analysis software (Video Point Version 2.5, Lenox Softworks, www.lsw.com) where swimming velocity was analysed by tracking movement across the field. Tuna BL was used to calibrate the image scale, accounting for variable distances between the fish and the camera. Any camera movement was corrected by referring to a stable point within the respirometer on a frame-by-frame basis.

Data analysis

Dissolved oxygen recordings were broken up into 3 h blocks according to time of day (starting at midnight) and linear regression fitted to the change in DO to establish SBT

$\dot{M}O_2$ (mg kg^{-1} h^{-1}). All $\dot{M}O_2$ calculations were adjusted for background respiration/photosynthesis effects (mean of pre- and post-trial background recordings) representative for the time of day. Swimming velocity data were also collated within the same 3 h time periods, where between four and seven footage segments were analysed and the mean taken as representative of swimming velocity for that period. A total of 692 segments were analysed for swimming velocity with a minimum of 35 for any individual trial. Due to video logger failure, swimming velocity measurement is not available for one feeding event (Trial 1; Feeding 2).

The effect of meal size on $\dot{M}O_2$ was quantified by the following parameters: SDA peak (maximum postprandial); SDA duration (duration that postprandial $\dot{M}O_2$ remained greater than one standard error (SE) above the RMR); SDA magnitude (total energy consumed above the RMR within the SDA duration period); ingestion-specific magnitude (magnitude per kg ration ingested) and SDA coefficient (magnitude as a percentage of gross ingested energy). Conversion from oxygen consumed to the energy equivalent was calculated assuming 14.32 J of energy were expended per mg oxygen consumed (Beamish and Trippel, 1990). Significant differences of means were established by the use of two-tailed Students T-tests, and regression slopes and intercepts by ANOVA within the StatistiXL program (version 1.5, www.statistixl.com).

Results

As would be expected with larger SBT, mean mass-specific RMR (306 ± 15 mg kg^{-1} h^{-1}, 19.8 kg, Table 2) was less than recorded in Chapter 2 (366 mg kg^{-1} h^{-1}, 10.3 kg). However, this difference did not prove to be statistically significant ($P = 0.11$) most probably due to poor of statistical strength of comparison ($df = 7$) and variation between SBT cohorts studied in the different years. Mean U (0.75 ± 0.03 BL s^{-1}) were also less than recorded in Chapter 2 (0.93 BL s^{-1}) but, again not statistically significantly ($P = 0.33$).

Table 2. Experimental parameters for the groups of two or three southern bluefin tuna examined in each of the five trials before feeding.

Trial	1	2	3	4	5	Mean ± SE
M_b (kg)	17.9 ± 2.0 (3)	20.2 ± 0.1 (3)	19.8 ± 0.3 (3)	20.9 ± 1.8 (2)	20.4 ± 1.3 (3)	**19.8 ± 0.5**
BL (cm)	102 ± 1	106 ± 1	104 ± 1	108 ± 4	106 ± 2	**105 ± 1**
T_w (°C)	20.1-19.6	18.7-18.2	17.1-16.6	16.9-16.4	16.1-15.7	**17.5 ± 0.7**
U (BL s^{-1})	0.83	0.68	0.79	0.71	0.74	**0.75 ± 0.03**
RMR (mg kg^{-1} h^{-1})	272	292	286	357	320	**306 ± 15**

M_b, body mass; BL, body length; T_w, experimental water temperature; U, routine swimming velocity; RMR, routine metabolic rate Values are means ±SE, () = number of fish

Thirteen feeding trials were recorded, eight with low-lipid, and five with high-lipid sardines (Table 3). Rations of high-lipid sardines (1.72 ± 0.19 kg) were significantly greater (P <0.01) than that of low-lipid sardines (1.08 ± 0.08 kg). As SBT were fed to satiation, this suggests a dietary preference for the high-lipid sardines. The reason for this is not known however, the high-lipid sardines were larger (~80 g fish^{-1}) and in better condition (i.e. less freezing and storage damage) than the low-lipid sardines (~40 g fish^{-1}), which may have contributed to their preference. U of all SBT increased post-feeding, however was significantly greater (P <0.01) for SBT that ingested the high-lipid (1.33 ± 0.04 BL s^{-1}) than the low-lipid baitfish (0.94 ± 0.06 BL s^{-1}).

Table 3. Specific dynamic action parameters.

Trial\ feedings	Lipid level	Ration (kg)	U (BL s^{-1})	IE (MJ)	Peak (mg kg h^{-1})	Dur. (h)	Mag. (MJ)	IS Mag. (MJ kg^{-1})	SDA Coef. (%)
1\1	**Low**	1.59	1.21	10.49	668	26	3.24	2.04	30.9
1\2	**Low**	1.04	-	5.40	595	12	2.26	2.17	41.8
2\1	**Low**	0.98	0.75	6.47	430	18	1.85	1.89	28.6
2\2	**Low**	1.00	0.93	5.20	596	13	2.23	2.22	42.9
3\1	**Low**	0.84	0.82	5.54	413	16	1.32	1.57	23.7
3\2	**Low**	0.98	0.99	6.47	476	20	2.01	2.05	31.0
4\1	**Low**	1.16	0.89	7.65	549	23	2.07	1.79	27.1
5\1	**Low**	1.08	0.97	7.13	535	22	1.82	1.69	25.6
Mean		**1.08**	**0.94**	**6.80**	**531**	**18**	**2.10**	**1.93**	**31.5**
± SE		**± 0.08**	**± 0.06**	**± 0.08**	**± 36**	**± 2**	**± 0.19**	**± 0.10**	**± 2.9**
3\3	**High**	1.28	1.32	11.77	647	26	3.20	2.40	27.1
3\4	**High**	1.60	1.29	14.72	735	29	5.23	3.27	35.5
4\2	**High**	1.68	1.20	15.45	727	44	4.50	2.68	29.1
4\3	**High**	1.60	1.42	14.72	869	31	5.58	3.48	37.9
5\2	**High**	2.42	1.42	22.26	1015	36	9.26	3.83	41.6
Mean		**1.72**	**1.33**	**15.79**	**798**	**33**	**5.56**	**3.20**	**34.3**
± SE		**± 0.19****	**± 0.04****	**± 1.74****	**± 59****	**± 7****	**± 1.01***	**± 0.23***	**± 2.4**

Ration, total mass of sardines ingested; *U*, swimming velocity during the specific dynamic period; IE, ingested energy; Peak, maximum postprandial $\dot{M}O_2$; Dur., duration of specific dynamic action; Mag., magnitude energy consumed above the routine metabolic rate within the SDA duration period; IS Mag., magnitude of SDA per kg ration consumed; and SDA Coef., energy consumed by SDA as a proportion of ingested energy.

*mean significantly different to low-lipid mean (P<0.05), **(P<0.01).

$\dot{M}O_2$ increased post-feeding in all trials presumably as a result of SDA (Fig. 1). Similar to that recorded in Chapter 2, magnitude of SDA was directly related to ration size (R^2 = 0.94 and 0.83, for the high- and low-lipid sardines, respectively) (Table 4). Comparisons by ANOVA found no significant difference between regressions recorded with low-lipid sardines in the present study with that recorded in Chapter 2 (P = 0.46 and 0.26 for regression slope and intercept, respectively) (Fig. 2).

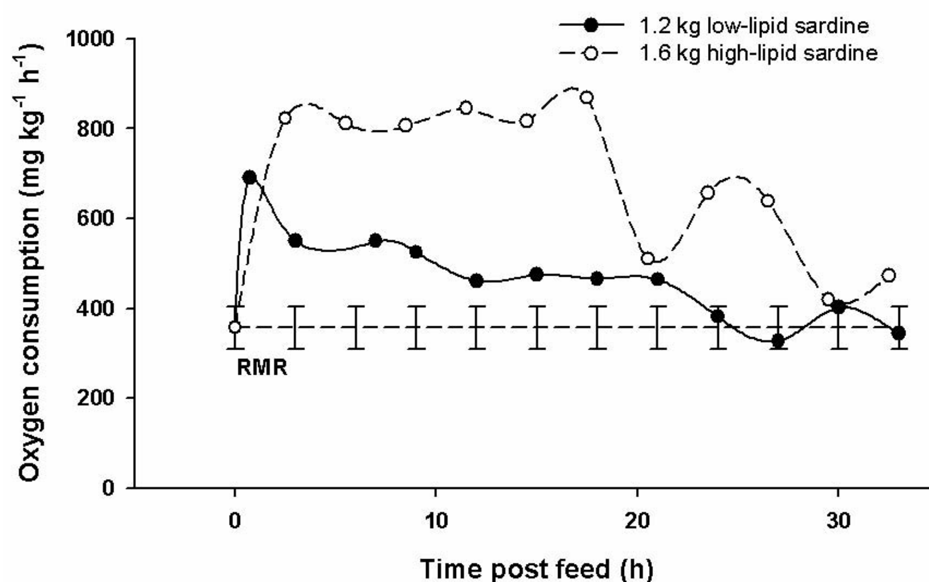

Fig. 1. Example of increase in oxygen consumption rate for southern bluefin tuna after ingesting similar sized rations of low-lipid (1.2 kg, trial\feed = 4\1, filled circles) and of high-lipid sardines in the same trial (1.6 kg, trial\feed = 4\3, open circles). Flat, dashed line represents the pre-feeding routine metabolic rate (±SE).

Table 4. Parameters of least squares regression (y = bx - a) relating specific dynamic action magnitude (MJ) and ration size (kg) in southern bluefin tuna ingesting high- and low-lipid sardines.

Lipid level	Ration range (kg)	n	a	95% C.I.	b	95% C.I.	R^2	ANOVA P (slope)
Low	0.84-1.59	8	0.32	0.18-0.97	2.23	2.14-2.70	0.83	<0.01
High	1.28-2.42	5	3.37	0.87-7.60	5.20	2.79-7.61	0.94	

n = number of measurements.

ANOVA P (slope), ANOVA probability that slopes of regressions are not significantly different.

Comparisons between the high- and low-lipid sardines demonstrate that SDA was greater for the SBT that ingested the high-lipid sardines. SDA peak, SDA duration, SDA magnitude, ingestion-specific magnitude and regression slope were all significantly greater for SBT that ingested the high-lipid than those the low-lipid sardines (Tables 3 and 4, Fig. 2). The greater intake-specific magnitude and regression slope confirms that the larger magnitude was not due to the larger rations

of high-lipid sardines ingested. However, when the energetic cost of SDA was represented as a proportion of ingested energy, there was no significant (P = 0.95) difference between the high- (34.3 ± 2.4%) and low-lipid sardines (31.5 ± 2.9%) (Table 3).

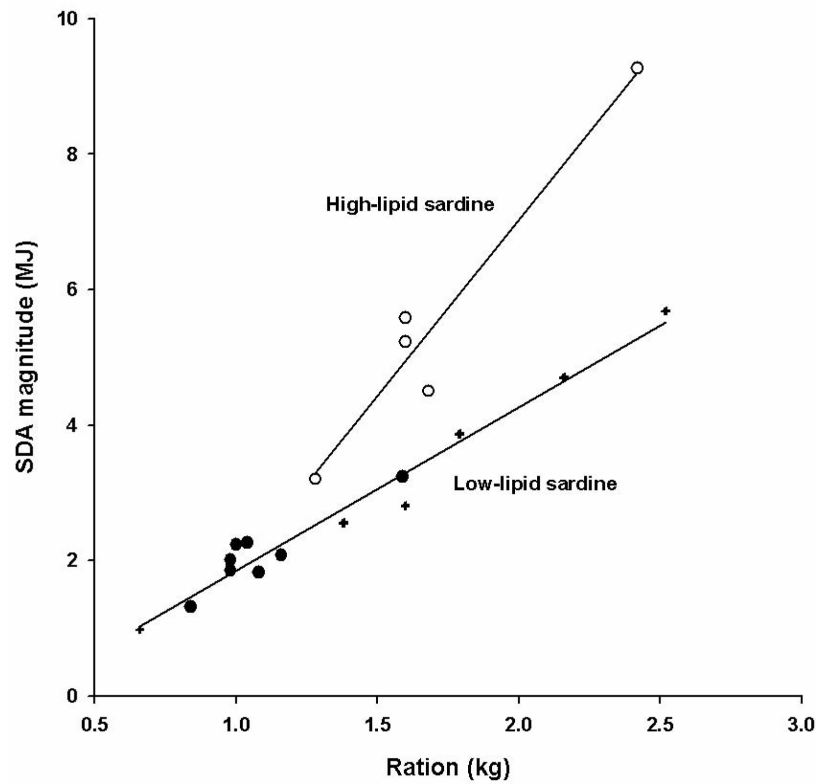

Fig. 2. Magnitude of Specific Dynamic Action (SDA) for southern bluefin tuna ingesting low-lipid (closed circle) and high-lipid (open circles) sardines in comparison to results recorded in Chapter 2 for southern bluefin tuna also consuming low-lipid sardines (crosses). Solid lines are linear regressions for high-lipid sardines (y = 5.2x - 3.7, R^2 = 0.94, n = 5) and for low-lipid sardines (present study data and Chapter 2 combined, y = 2.4x - 0.6, R^2 = 0.96, n = 14).

Discussion

Ingestion of high-lipid sardines induced a greater SDA response than that of low-lipid sardines (Fig. 2). The increase in response was proportionate to the increase in energy ingested, as evident by the similar SDA coefficients (~35%, Table 3). As protein content was similar between the sardines (17.1-20.5%), it can be assumed that this effect was a result of the increased lipid level and subsequent enhanced energy content. A similar result was recorded with rainbow trout (*Oncorhynchus mykiss*) where increasing lipid content of low-lipid compound diets increased SDA magnitude but had little effect on the SDA coefficient (LeGrow and Beamish, 1986). However, with this species increasing lipid content of a low protein diet resulted in significant reduction in SDA magnitude and coefficient. In this case, it was assumed that increasing lipid level served to spare protein utilization for energy catabolism resulting in a reduction in magnitude of SDA (LeGrow and Beamish, 1986; Beamish and Trippel, 1990). This protein sparing effect was largely absent when lipid levels were increased with higher-protein, higher-energy diets. This led LeGrow and Beamish (1986) to propose a dietary energy intake "threshold", below which energy is limiting and high levels of deamination and oxidation of dietary protein for energy results in the elevation of SDA. The apparent lack of protein sparing effect in the present study suggests that the span of dietary energy content examined did not cross this energy threshold for SBT. At present, it is not known if this energy threshold is below the energy level of the low-lipid or above the energy level of the high-lipid sardine diet examined. However, as the baitfish lipid content range (1.8-12.9%), is likely to span that of wild tuna prey (~4-9%)(Ellis and Rough, 2005; Ward et al., 2006), this confirms that the high energetic cost of SDA is ecologically relevant.

The results of this study support those found in Chapter 2, that the energetic cost of SDA in SBT is higher (approximately double) than recorded in other teleost species. In that study it was proposed that the mechanisms of SDA in tuna are no different to that of other teleosts, but some metabolic aspects are enhanced in association with other adaptations specific to the high-performance fishes and Bluefin tuna (*T. maccoyii, T. thynnus and T. orientalis*).

It is likely that the major contributing factor to the high-recorded metabolic cost of SDA the high protein synthesis and degradation rates associated with the fast

growth rates of juvenile SBT. Protein synthesis is known to be a highly energy consuming process (Carter and Houlihan, 2001) and much of SDA represents the costs of protein anabolism and hence growth (Brown and Cameron, 1991b; Brown and Cameron, 1991a; Lyndon et al., 1992). Unfortunately, there is no information on the cost of SDA in other fast-growing, high-performance fish for comparison, however high protein synthesis costs have been hypothesized to greatly contribute to the postprandial $\dot{M}O_2$ of these fish (Brill, 1996). Comparisons with other fast-growing species would yield insight into the proportion of SDA that is specifically associated with fast growth rates and not related to tuna specific adaptations.

As previously recorded, swimming speed was elevated during the SDA period, however in this case, the increase in velocity was dependent on the baitfish ingested, being greatest for the high-lipid sardine. As the high-lipid sardines also induced the greatest postprandial metabolic demand, this supports the theory that the purpose of faster post-feeding swimming is to increase ventilation volume in order to supply the enhanced oxygen demand (Chapter 2). To increase ventilation, obligate ram-ventilating fish must increase swimming speed and/or mouth gape. Ram ventilating sharks have been shown to enhance ventilation by increasing both speed and mouth gape in response to hypoxia (Parsons and Carlson, 1998; Carlson and Parsons, 2001). Skipjack (*Katsuwonus pelamis*) and yellowfin tuna (*Thunnus albacares*) also increase velocity in response to hypoxia, however in these cases, there is debate whether the purpose was to increase ventilation or a escape response (Dizon, 1977; Gooding et al., 1981; Bushnell and Brill, 1991). As a postprandial escape response seems implausible, it is likely that SBT in the present study increased swimming speed in order to enhance oxygen extraction and was probably coupled with an increase in mouth gape. This increase in speed would have contributed to the metabolic cost of SDA. An estimation of its energetic cost can be made by using the relationship between $\dot{M}O_2$ and velocity of fasted bluefin tuna (Blank et al., 2007) (Table 5). From this estimation, the metabolic cost of faster postprandial *U* is a significant component of total magnitude of SDA (up to ~30%), however is unlikely to account for the high SDA coefficient recorded. Furthermore, increased post-feeding ventilatory costs are not exclusive to ram-ventilating fish and are also likely to be a significant component of SDA magnitude in buccal-pumping species. Interestingly, the increase in post-feeding swimming speed of fish that ingested the high-lipid sardines was

proportionately greater than the increase in ingested energy, as evident by the greater proportion of SDA used (~30% compared to ~15% for low-lipid sardines). It is possible that this is related to the counter-productive effect of increasing speed to facilitate increased ventilation. Increasing swimming velocity incurs its own metabolic demand and must be supported by another increase, and so on. The greater the metabolic load of SDA, the greater the increase in *U*, and thus the greater compounding effect.

Table 5. Estimation of the metabolic cost of faster postprandial swimming as a proportion of southern bluefin tuna (SBT) specific dynamic action (SDA).

	U (BL s^{-1})	$\dot{M}O_2$ (mg $kg^{0.88}$ h^{-1})[ab]	SDA Duration (h)	$\dot{M}O_2$ Magnitude (mg $kg^{0.88}$)[a] b	SBT SDA Magnitude (mg $kg^{0.88}$)[b]	% SDA Magnitude
Pre-feeding	0.75	233	-	-	-	-
Low lipid	0.94	272	18	541	3808	14
High lipid	1.33	364	33	3343	10662	31

U, mean swimming velocity of southern bluefin tuna (SBT) before and after ingestion of low- and high-lipid sardines; $\dot{M}O_2$, calculated oxygen consumption rate of fasted Pacific bluefin tuna (*Thunnus orientalis*); SDA Duration, mean duration of SBT specific dynamic action (SDA); $\dot{M}O_2$ Magnitude, post-feeding $\dot{M}O_2$ subtracted from the pre-feeding level and multiplied by SDA duration; % SDA Magnitude, $\dot{M}O_2$ magnitude presented as a proportion of total magnitude of SBT SDA magnitude measured in the present study.

[a]Based on the relationship between swimming velocity and $\dot{M}O_2$ of a 8.3 kg Pacific bluefin tuna (*Thunnus orientalis*) (Blank *et al.*, 2007).

[b]Mass independent assuming a scaling coefficient of 0.88 ((White and Seymour, 2005; Fitzgibbon et al., 2006) adjusted to the present study temperature of 17.5°C assuming a Q_{10} = 1.65 (White and Seymour, 2005).

As the exact mechanism of postprandial visceral warming is still poorly understood, it is difficult to estimate its metabolic cost. However, it is unlikely to greatly contribute to the high metabolic cost of SDA recorded with SBT. The source of visceral heat is thought to be an accumulation of heat by-product of SDA (Carey et al., 1984). However, the major tissue sites for SDA associated protein synthesis (thus heat production), the liver and white muscle (Houlihan et al., 1988; Carter and Houlihan, 2001), are thermally isolated from the viscera (Carey et al., 1984). Therefore, only a fraction of SDA associated heat production (produced in the stomach, caecum, intestines and spleen) contributes to visceral warming. However, it has been estimated that the heat released by metabolizing less than 5% of gross ingested energy is sufficient to account for visceral warming in large Atlantic bluefin tuna (*Thunnus thynnus*) (Carey et al., 1984). This is less than 15% of the SDA

magnitude observed in SBT. The proportion of SDA associated with physiological processes that take place within the tissues of the viscera, are likely to be much greater than this. These include pre-absorptive and absorptive processes such as gut peristalsis, enzyme and acid secretion, protein catabolism, and intestinal absorption (McCue, 2006). The cost of gut peristalsis alone has been shown to contribute as much as 30% of the SDA response in fish (Tandler and Beamish, 1979). This combined with the effective heat retention by highly efficient *retia mirabilia* (Carey and Gibson, 1983) and efficient insulation by an overlying gas bladder and a thick belly wall (Carey et al., 1984), suggests that although only a small proportion of the heat by-product of SDA contributes to visceral warming, it is likely to be largely sufficient to account for visceral warming. Furthermore, the energetic cost of visceral warming appears unlikely to account for the observed differences in SDA magnitude between the baitfish diets. Gunn et al., (2001) found that the magnitude of visceral warming to be twice as great for SBT ingesting low-lipid (3.2%) than high-lipid (7.0%) baitfish. This suggests that the metabolic cost of visceral warming is greatest for low-lipid baitfish. However, this result was confounded by a substantial difference in study ambient water temperature (6-7°C). Therefore it is unknown if the result was related to the lipid content of the diet or a compensatory mechanism to increase digestion rate at low temperatures. Clearly, more research is required to decipher the effects of diet energy levels on bluefin tuna visceral warming.

The results of this study confirm that the metabolic cost of SDA for SBT is approximately double that recorded in other teleosts (~35% of gross injected energy) and it is most likely related to their high-performance physiology (principally their fast growth rates) and thus common for all tuna. This verifies another aspect of tuna metabolic physiology that is greatly enhanced when compared to other teleosts species (including standard metabolic rates and metabolic scopes), which emphasizes their great aerobic requirements. However, this does not necessarily mean that tuna SDA is less energy efficient than that of other teleosts. As it appears that the majority of the high cost of SDA is attributed to rapid protein anabolism, it mainly represents the “cost of growth” and thus reducing SDA is unlikely to improve dietary energy utilization efficiency (Lyndon et al., 1992).

4. Effects of hypoxia on oxygen consumption, swimming velocity and gut evacuation in southern bluefin tuna (*Thunnus maccoyii*)

Abstract

Of the few measurements of the behavioural and physiological responses of tuna to hypoxia, most are restricted to shallow diving tropical species. Furthermore, when wild tuna experience low dissolved oxygen (DO), they are likely to have an increased oxygen demand associated to the metabolic cost of food digestion and assimilation (specific dynamic action, SDA). However the response of postprandial tuna to hypoxia has never been examined. This study focuses on the metabolic and behavioural responses of both fasted and postprandial southern bluefin tuna (*Thunnus maccoyii*, SBT) to low DO. Separate groups of three fasted SBT (10.0 kg ± 0.9 SE, water temperature (T_w) = 19.1-20.3°C) were exposed to mean DO levels of 4.44, 3.23, 2.49, and 1.57 mg l^{-1} for 20-21 h. In moderate hypoxia (4.44 and 3.23 mg l^{-1}), swimming velocity (U) was enhanced (1.5 and 1.3 times normoxic speed, respectively) presumably to increase ventilation volume. Routine metabolic rate (RMR) was similarly elevated (1.3 and 1.2 times normoxic RMR, respectively), most likely due to increased metabolic demand of faster swimming. At 2.49 mg l^{-1}, U increased to over double the normoxic speed, possibly as an escape response. At 1.57 mg l^{-1}, both U and RMR were suppressed (0.8 and 0.9 times normoxic level, respectively), and SBT failed to survive the entire 20 h exposure period. This reveals that the critical oxygen level of SBT is between 1.57 and 2.49 mg l^{-1}, demonstrating that SBT are remarkably well adapted to low DO. Feeding did not greatly influence their hypoxia tolerance, as a further group of three fish (11.4 ± 1.4 kg, T_w = 18.2-18.5°C) survived exposure to DO levels between 2.96 and 1.81 mg l^{-1} for 21 h, after ingestion of a 6.7 % body weight ration of sardines (*Sardinops sagax*). In a subsequent experiment to determine the effects of hypoxia on digestion rate, three groups of three SBT (19.4 ± 0.7 kg, T_w = 15.7-20.1°C) were fed to satiation at low DO (4.57 mg l^{-1}) immediately before the DO was further reduced (to 2.84 mg l^{-1}) for an exposure period of 6.5-8 h, and compared to a single control group of two SBT (20.9

± 1.8 kg, T_w = 16.4-16.9°C) in normoxia. There was no significant difference in *U*, RMR and gastric evacuation rates of SBT in hypoxia compared to those in normoxia. This demonstrates that in moderate to severe hypoxia, SBT are still capable of aerobically supporting maintenance metabolism, routine swimming and SDA. It is hypothesized that adaptations which support the large metabolic scope of tuna are also likely to be beneficial for oxygen extraction and delivery in conditions of hypoxia.

Introduction

Tuna achieve oxygen consumption rates ($\dot{M}O_2$) that exceed those of most other teleosts. Their standard metabolic rate is 2 to 5 times greater than that of most other active fish (Korsmeyer and Dewar, 2001; Graham and Dickson, 2004). This elevated basal metabolism has been attributed to energetically expensive adaptations that support a greater metabolic scope (Brill and Bushnell, 1991; Brill, 1996; Korsmeyer et al., 1996; Korsmeyer and Dewar, 2001). Because of high oxygen demands it seems reasonable to assume that tuna would be very sensitive to hypoxia (Bushnell et al., 1990). However, the oceans are not homogeneous, and significant reductions in dissolved oxygen (DO) occur at depths frequented by tuna (Sund et al., 1981). Bigeye tuna (*Thunnus obesus*) spend substantial amounts of the day time at great depths (>500m), in waters that can be severely hypoxic (as low as 1.5 mg l^{-1}) (Hanamoto, 1987; Dagorn et al., 2000; Schaefer and Fuller, 2002; Musyl et al., 2003), and Atlantic (*Thunnus thynnus*) and southern bluefin tuna (SBT, *Thunnus maccoyii*) dive to greater than 400 m (Block et al., 2001; Gunn and Block, 2001). Although high oxygen requirements might be expected to limit tolerance to hypoxia, at least some tuna species are well adapted to low DO.

There have been few measurements of response of tuna to hypoxia, and of these most have been with tropical species. Laboratory studies with either paralysed or swimming skipjack (*Katsuwonus pelamis*), yellowfin (*Thunnus albacares*), and bigeye tuna, have shown that all respond to relatively mild reductions in DO (~5-5.5 mg l^{-1}) with significant cardio-respiratory adjustments including decreased heart rate, and increased mouth gape and ventilation volume (Bushnell et al., 1990; Bushnell and Brill, 1991; Bushnell and Brill, 1992). The $\dot{M}O_2$ of swimming albacore tuna (*Thunnus alalunga*) was reduced in DO levels between 2.6 and 5.1 mg l^{-1} (Graham et al., 1989).

Paralysed yellowfin and skipjack tuna were able to maintain $\dot{M}O_2$ at normoxic levels at 3.85, but not at 2.14 mg l^{-1} (Bushnell and Brill, 1992). However, in that study skipjack drew from venous oxygen reserves at 3.85 mg l^{-1} whereas yellowfin did not, suggesting that yellowfin are more hypoxia tolerant. The greater hypoxia tolerance of yellowfin is supported by tank studies that found they could survive periods in excess of 3 h at DO levels of just 1.4-1.6 mg l^{-1} (Dizon, 1977), whereas skipjack survived only about 60 min 3 mg l^{-1} (Dizon, 1977; Gooding et al., 1981). However, this finding has been largely ignored, as the depth distribution of yellowfin tuna is believed to be limited to waters with DO above 6 mg l^{-1} (Cayré and Marsac, 1993). Indeed, intraspecific variation in hypoxia tolerance is thought to correlate with the species' vertical movement patterns, with more tolerant tuna diving deeper and for longer than other species (Hanamoto, 1987; Cayré and Marsac, 1993; Brill, 1994). Consequently it is hypothesized that deeper diving tuna such as bluefin (*T. thynnus*, *T. maccoyii*, and *T. orientalis*) and bigeye tuna may be better adapted to low DO. However, the tolerance of these species to severe hypoxia has yet to be examined, which emphases our limited understanding of the physiological constraints of low DO on tuna species.

The response of wild tuna to hypoxia is likely to be complicated by the circumstances in which they experience it. Unlike many marine fish species that avoid hypoxia (Claireaux et al., 1995; Schurmann et al., 1998; Claireaux et al., 2000), tuna voluntarily enter areas of low DO in the pursuit of food. There is a strong body of evidence showing that some tuna forage on deep prey, especially in the deep scattering layer (Dagorn et al., 2000; Gunn and Block, 2001; Marcinek et al., 2001; Schaefer and Fuller, 2002; Musyl et al., 2003). Feeding increases oxygen demand because of prey capture and the metabolic cost of digestion and assimilation (often referred to as specific dynamic action, SDA). In Chapter 2 it was shown that the $\dot{M}O_2$ of postprandial SBT can be as much as three times the basal routine metabolic rate. The effect of hypoxia on teleost SDA is largely unknown. Only recently have the first measurements of SDA of a fish species in reduced DO been made (Jordan and Steffensen, 2007). In that study, Atlantic cod (*Gadus morhua*) SDA continued at DO level of 2.7 mg l^{-1}, however maximum postprandial $\dot{M}O_2$ was depressed and duration of SDA prolonged compared with that in normoxia (Jordan and Steffensen, 2007). However, the Atlantic cod is a relatively inactive species in comparison to tuna. How hypoxia affects tuna SDA is unknown. It is likely that the elevated metabolic load

associated with feeding and SDA greatly amplifies the challenge that tuna face balancing oxygen demand with oxygen availability in deep hypoxic waters. Therefore, the effect of feeding must be assessed in order to understand the response of wild tuna to hypoxia.

This study examines the metabolic and behavioural responses of both fasted and postprandial SBT to low DO. SBT can spend much time at great depths (to 400 m) feeding on omastrophic squid associated with the deep scattering layer, a similar behavioural pattern to bigeye tuna (Gunn and Block, 2001). Physiological data on environmental thresholds are important for understanding how tuna interact with their environment, for population assessments and fisheries management (Brill, 1994), and critical for captive fish management in the expanding bluefin tuna aquaculture industry (Farwell, 2001).

Methods

Experimental animals

Two separate trials were conducted in early 2005 and 2006. In January of both years, SBT were sourced from a marine aquaculture farm in the waters off Port Lincoln, South Australia, through the Tuna Boat Owners Association of South Australia. These SBT had been purse-seine netted in the Great Australian Bight one month earlier. From the commercial farm, individual SBT were hooked with a baited, barb-less hook and transferred into a 12 m diameter, 8 m deep holding sea-cage that was towed and moored in a protected bay at the southern end of Boston Island, where all trials were conducted. SBT were then left to recover from transfer for 2 months and were fed sardines (*Sardinops sagax*) to satiation twice per day. They were starved for a minimum of 48 h before any experimental trial. SBT were transferred into the respirometer by crowding them within a purse-seine net and skin diver securing the fish by hand and passed it across a soft, wet slipway into the adjacent respirometer.

Mesocosm respirometer

SBT oxygen consumption rates ($\dot{M}O_2$) was determined by the reduction in dissolved oxygen (DO) over time within a large static respirometer ("mesocosm respirometer"). The mesocosm was manufactured from denier polyester scrim reinforced 1.14 mm polypropylene (R-PP 45, Stevens Geomembranes, http://www.stevensgeomembranes.com), bonded by thermal welding by Fabtech SA Pty. Ltd (Adelaide, Australia). The mesocosm was a 12 m diameter, 2.5 m deep enclosed cylinder with a wave break wall that extended 1 m above water level. Entry into the mesocosm was possible through a 2 m diameter, 2 m high access port made from 0.75 mm polypropylene and positioned in the roof so that it could be rolled and clamped to completely seal the system. In addition, five 1 m diameter, clear 0.75 mm polyvinyl chloride windows were positioned in the roof to allow entry of natural light, and eleven capped tank fittings (STF 50, Hansen Products Ltd, http://www.hansenproducts.co.nz/) allowed sealed access for experimental equipment and manual sampling.

The mesocosm was pump filled with sea-water to a total water volume of between 350 000-402 000 l (varied between experiments due to water exchanges).

Dissolved oxygen was manipulated by high pressure injection of micro-bubbles of either pure oxygen or nitrogen with a gas diffuser (Force 7, Aqua & Co, BOC Gases, NSW, Australia). Injection of oxygen would rapidly raise DO (~6 mg l^{-1} h^{-1}) with nearly all bubbles being completely absorbed before reaching the surface. However, nitrogen injection was slower to reduce DO (~1.5 mg l^{-1} h^{-1}) because oxygen had to be carried away in the nitrogen bubbles. Bubbles that collected within the respirometer were removed by rolling them out of the access port.

General experimental procedures

All experiments involved two or three fish. They were introduced in the late afternoon, and routine metabolic rate (RMR) was determined over the subsequent 36 h. $\dot{M}O_2$ was commonly elevated after introduction (presumably as a result of transfer stress) but would plateau within 6-20 h. RMR was determined as the mean $\dot{M}O_2$ after this period. Fish were then used in SDA experiments as described in Chapters 2 and 3

therefore, before the commencement of hypoxia trials, fish had spent a minimum of 3 days within the respirometer, during which time they had ingested at least one substantial meal of sardines. Hypoxia trials did not begin until 48-72 h after feeding to allow the effects of SDA to subside. Fish were fed weighed sardines through the access port and any uneaten baitfish removed by a diver to obtain the net consumption.

Background respiration and photosynthesis of planktonic organisms in the water column and on the mesocosm surfaces were measured in trials immediately before and after each experiment. Low levels of background respiration were generally found, however it was dependent on time of day (net respiration rate would fall during the day due to photosynthesis). All $\dot{M}O_2$ recordings were adjusted for background respiration according to the pertinent time of day. The oxygen holding capabilities of the mesocosm was examined in further trials where the respirometer DO was reduced to between 1.9 and 2.3 mg l^{-1} before the mesocosm was sealed and oxygen monitored for more than 20 h. Very low levels of leakage (0.0042 mg^{-1} h^{-1} per mg l^{-1} DO gradient between the respirometer and external environment) were found in 2005 and negligible levels in 2006. These rates of leakage were nevertheless used to adjust all trial DO recordings according to the pertinent oxygen gradient. Dissolved ammonia concentrations were also monitored by daily analyses of water samples before, during and after each trial, and they never exceeded 0.6 mg l^{-1}.

Two luminescence DO meters (Hach HQ10, Loveland, CO, USA) permanently installed at a depth of 1 and 2 m respectively, both 3 m from the wall logged mesocosm DO and water temperature. Meters were calibrated according to the manufacturer's instructions at the start of the trials and calibration checked following the completion of all trials. Meters recorded DO (0.01 mg l^{-1} resolution) that was automatically compensated for water temperature and salinity set at $35^0/_{00}$. Oxygen meters were set to log DO every 5 min.

Fish behaviour was monitored (both day and night) with a permanently installed video system (SciElex Pty. Ltd., Hobart, Australia) that consisted of two 0.05 lux black and white underwater video cameras and two 6 W white LED lights (housed within resin). The system was programmable through a central controller and power supply unit that were secured to the sea-cage. The cameras were programmed to record for 4 min every 90 min (first by the horizontal camera then immediately by the vertical camera) and data stored on a pocket video recorder (Archos AV400,

Greenwood Village, CO, USA). The lights were programmed to turn on at dusk and remain on until dawn. The system was powered by a single 32 Ah, 12 V battery that was exchanged daily.

Video recordings were viewed in the video editing program (VirtualDub 1.5.10, Avery Lee, www.virtual.dub.org). Suitable records were defined as those containing a fish that was swimming perpendicularly to the camera and at least one frame in which body length (BL) could be measured. This sequence was imported into video motion analysis software (Video Point Version 2.5, Lenox Softworks, www.lsw.com) where swimming velocity was analysed by tracking movement across the field. Tuna BL was used to calibrate the image scale, accounting for variable distances between the fish and the camera. Any camera movement was corrected by referring to a stable point within the respirometer on a frame-by-frame basis.

Experiment 1

Trials were conducted in 2005 following SDA experiments as described in Chapter 2 with SBT with a mean mass (M_b) of 10.3 ± 0.6 kg (n = 12) (Table 1). For each trial, respirometer DO was reduced before SBT $\dot{M}O_2$ and behaviour were monitored for the subsequent 20-21 h. Initial DO levels were 4.66, 3.42, 2.79 or 1.65 mg l^{-1} (60, 40, 30 and 20% trials, respectively). A further trial (30% feed trial) was conducted to determine if feeding affected hypoxia tolerance. At DO level of 4.75 mg l^{-1}, SBT were fed to satiation before respirometer DO was further reduced to 2.96 mg l^{-1} and $\dot{M}O_2$ and behaviour monitored for the subsequent 21 h. All trials were conducted with different groups of three SBT except the 20% trial, which was performed with the same fish as the 40% experiment after a 24 h recovery period in normoxia.

Experiment 2

Trials were conducted in 2006 following SDA experiments as described in Chapter 3 with SBT with a mean M_b of 19.7 ± 0.8 kg (n = 11) (Table 2). At DO between 3.25-5.17 mg l^{-1}, three groups of three SBT were fed sardines to satiation before the DO was further reduced to between 2.90 and 3.13 mg l^{-1} and $\dot{M}O_2$ and behaviour monitored for 6.5-8.0 h from the time of feeding. SBT were then removed by divers, killed according to normal commercial harvesting procedures and contents of the

stomach removed, blotted dry, and weighed. At this stage, divers also inspected the respirometer for any regurgitated sardines. This was compared to a control group of two SBT run at 7.81 mg l^{-1} using the same procedures.

Data analysis

DO recordings were broken up into 3 h blocks according to time of day (starting at midnight) and linear regression fitted to the change in DO to establish fish $\dot{M}O_2$ (mg kg^{-1} h^{-1}). All $\dot{M}O_2$ calculations were adjusted for background respiration/photosynthesis effects (mean of pre- and post-trial background recordings) representative for the time of day. Swimming velocity data were also collated within the same 3 h time periods, where between four and seven video footage segments were analysed and their mean taken as representative of swimming velocity for that period. Significant differences were established using Students T-test (StatistiXL program version 1.5, www.statistixl.com). Values are means ± standard errors of means.

Results

Experiment 1

In the 60% trial, mean DO level was 4.44 mg l^{-1} and all fish survived the 20 h experimental period (Table 1). *U* and RMR were greater than that recorded in normoxia, being 1.5 and 1.3 times greater, respectively (Fig. 1). A similar result was recorded at a mean DO of 3.23 mg l^{-1} (40% trial), with *U* being 1.3 times, and RMR 1.2 times greater, then in normoxia. However, at 2.49 mg l^{-1} (30% trial), increase in *U* was 2.2 times greater than in normoxia. RMR was also elevated (1.4 times normoxic level) but not to the extent of *U*. The first lethal effects of hypoxia were observed at 1.57 mg l^{-1} (20% trial). Video footage showed that two of the three SBT died after 5 h, the other after 7 h. Before mortality, both *U* and RMR were suppressed to 0.8 and 0.9 times the level in normoxia, respectively.

Table 1: Parameters for experiment 1 of groups of three fasted southern bluefin tuna (SBT) when exposed to different levels of hypoxia (60, 40, 30, and 20% trials), and one group of SBT when exposed to hypoxia after being fed sardines to satiation (30% feed trial).

Trial	60%	40%	30%	20%	30% feed
M_b (kg)	9.4 ± 1.6	9.9 ± 0.9	10.6 ± 1.3	9.9 ± 0.9	11.4 ± 1.4
BL (cm)	81 ± 5	83 ± 2	88 ± 3	83 ± 2	86 ± 2
T_w (°C)	19.7-20.1	20.1-20.3	19.1-19.7	20.1-20.3	18.2-18.5
U_r (BL s^{-1})	0.71	1.35	0.78	1.35	0.83
RMR (mg kg^{-1} h^{-1})	300	456	355	456	354
Ration (%BW)	0	0	0	0	6.7
DO_{range} (mg l^{-1})	4.66-4.21	3.42-3.03	2.79-2.18	1.65-1.49	2.96-1.81
DO_{mean} (mg l^{-1})	4.44	3.23	2.49	1.57	2.39
Duration (h)	20.0	21.0	20.0	20.0	21.0
U_h (BL s^{-1})	1.05	1.84	1.68	1.09	1.53
RMR_h (mg kg^{-1} h^{-1})	400	525	497	405	715

Values are means for groups of three SBT ± SE (n = 3).

M_b, body mass; BL, body length; T_w, experimental water temperature; U_r, routine swimming velocity (normoxia); RMR, routine metabolic rate (normoxia); DO_{range}, range in experimental dissolved oxygen level; DO_{mean}, mean experimental dissolved oxygen level; Duration, duration of experimental trial; U_h, swimming velocity in hypoxia; RMR_h, routine metabolic rate in hypoxia.

Similar to fasted fish at a comparable hypoxia level, the U of SBT at 2.39 mg l^{-1} after ingestion of a 6.7 %M_b ration of sardines (30% feed trial) was twice that before feeding in normoxia (Fig 1). RMR however, was substantially greater than in fasted fish at this hypoxia level, being twice that before feeding in normoxia. By the end of this feeding experiment, DO had fallen to 1.81 mg l^{-1}. Although all fish survived the entire 21 h experimental period, two of the three died when stressed by divers during capture. This suggests that 1.81 mg l^{-1} was close to their lethal limit and similar to that of fasted fish (20% trial).

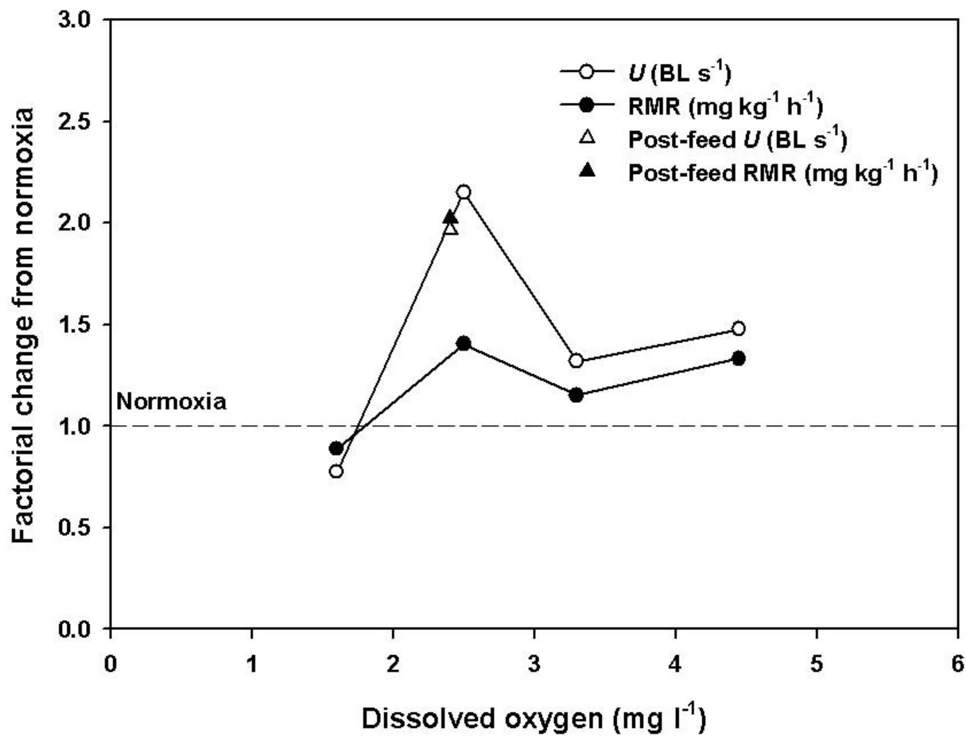

Fig. 1. Factorial difference from normoxia of groups of three fasted southern bluefin tuna (SBT) swimming velocity (*U*, open circles) and routine metabolic rate (RMR, closed circles) when exposed to dissolved oxygen (DO) levels of 4.66, 3.42, 2.79 or 1.65 mg l^{-1} (60, 40, 30 and 20% trials, respectively). Also included is the *U* (open triangle) and RMR (closed triangle) of SBT in reduced DO, after ingestion of a 6.7 % body mass ration of sardines (30% feed trial). Values are means of three SBT.

Experiment 2

There were no significant differences in SBT M_b, BL, *U* or RMR between hypoxia trials and the normoxia control (Table 2.). Despite the low DO levels at feeding in hypoxia trials (4.57 ± 0.66 mg l^{-1}), SBT still voluntarily ingested substantial rations of sardines (2.7 ± 0.3 %M_b). However, this was significantly less than ingested in the normoxic control (4.0 %M_b, P = 0.04). In hypoxia (2.84 ± 0.07 mg l^{-1}), mean *U* was 1.8 ± 0.1 times greater, and RMR 1.6 ± 0.1 times greater, than before feeding in normoxia (Fig. 2). *U* and RMR were similarly elevated post-feeding in the normoxic control (1.9 and 1.8 times pre-feeding level, respectively). On no occasion were regurgitated sardines found within the respirometer at the end of experiments. A

mean of 17.3 ± 4.3 % of ingested ration mass remained within the stomach of fish in hypoxia, which was not significantly different from those in the normoxia (16.7 %). When corrected for variable experimental duration, there was no difference in gastric evacuation rate of fish in hypoxia (11.1 ± 0.7 % h^{-1}) compared to those in normoxia (12.8 % h^{-1}).

Table 2. Parameters for experiment 2 of southern bluefin tuna (SBT) before and after ingestion of a ration of sardines and exposured to a period of hypoxia or normoxia.

	Hypoxia 1	Hypoxia 2	Hypoxia 3	Hypoxia mean	Normoxia
M_b (kg)	17.9 ± 2.	19.8 ± 0.3	20.4 ± 1.3	**19.4 ± 0.7**	20.9 ± 1.8
BL (cm)	102 ± 1	104 ± 1	106 ± 2	**104 ± 1**	108 ± 4
Tw (°C)	20.1-19.6	17.1-16.6	16.1-15.7	**17.8-17.3**	16.9-16.4
U (BL s^{-1})	0.83	0.79	0.74	**0.79 ± 0.03**	0.71
RMR (mg kg^{-1} h^{-1})	272	286	320	**293 ± 14**	357
Ration (%M_b)	2.2	3.1	2.9	**2.7 ± 0.3***	4.0
DO_{feed} (mg l^{-1})	5.17	5.30	3.25	**4.57 ± 0.66***	7.81
N_2inject time (h)	2.50	2.25	0.00	**1.58 ± 0.79**	0.00
DO_{range} (mg l^{-1})	3.13-2.84	2.90-2.67	3.06-2.43	**3.03-2.65**	7.81-7.29
DO_{mean} (mg l^{-1})	2.99	2.79	2.75	**2.84 ± 0.07****	7.55
Duration (h)	8.0	6.5	8.0	**7.5 ± 0.5**	6.5
U_h (BL s^{-1})	1.46	1.42	1.39	**1.42 ± 0.02**	1.34
RMR_h (mg kg^{-1} h^{-1})	391	499	557	**482 ± 49**	655
Gut contents (%ration)	8.6	21.9	21.5	**17.3 ± 4.3**	16.7
Gut evacuation (% h^{-1})	11.4	12.0	9.8	**11.1 ± 0.7**	12.8

*mean significantly different to normoxia (P<0.05), **(P<0.01). Values are means of three SBT ± SE (n = 3), except for normoxia trial were two SBT were used. M_b, body mass; BL, body length; T_w, water temperature; U, swimming velocity (U); RMR, routine metabolic rate; ration of sardines ingested; DO_{feed}, dissolve oxygen (DO) at time of feeding; N_2inject time, time require to lower dissolved oxygen to experimental level; DO_{range}, range in experimental DO; DO_{mean}, mean experimental DO level; Duration, time from feeding until end of experiment; U_h, U in hypoxia; RMR_h, RMR in hypoxia; gut contents, mass of contents of gut at end of experiment; Gut evacuation, % of intake ration evacuated from the gut per hour from feeding.

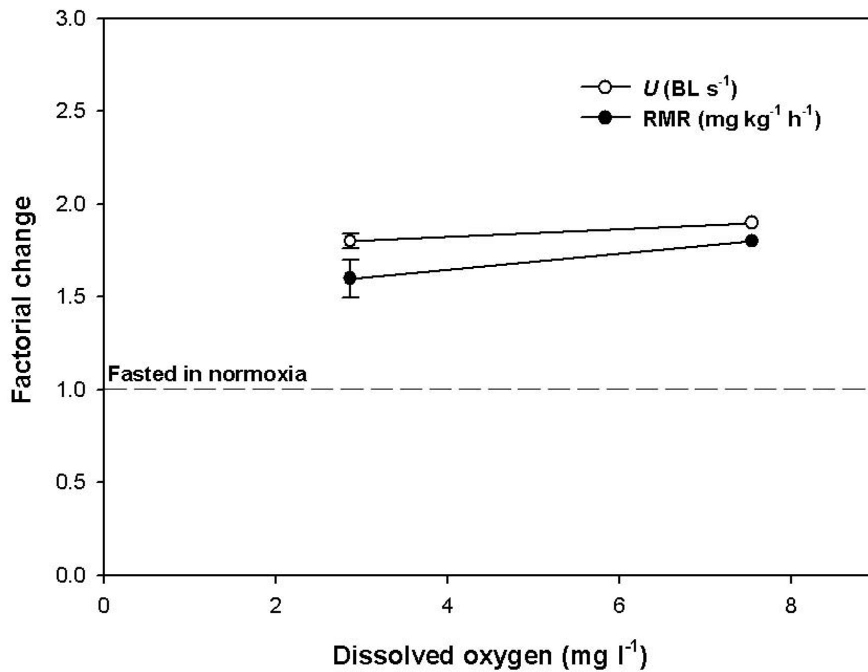

Fig. 2: Factorial change of swimming velocity (*U*, open circles) and routine metabolic rate (RMR, closed circles) of three groups of three SBT after ingestion of 2.7 % body weight (%M_b) ration of sardines in hypoxia (mean 2.84 mg l^{-1}) (values are means ± SE) compared to that of a control group of two fish that ingested a 4.0%M_b ration in normoxia (7.55 mg l^{-1}).

Discussion

Fasted fish

SBT swim faster in moderate hypoxia (4.44 and 3.23 mg l^{-1}) than in normoxia (1.3 and 1.5 times normoxic speed, respectively, Fig. 1). This is similar to that recorded with skipjack (Dizon, 1977; Gooding et al., 1981; Bushnell and Brill, 1991), and yellowfin tuna (Bushnell and Brill, 1991). It is likely that faster swimming is required to increase ventilation volume in response to decreased oxygen availability. Obligate ram-ventilating fish such as tuna must increase speed and/or mouth gape in order to increase ventilation. Video footage taken by divers at the end of hypoxia trials, confirm a noticeable increase in SBT mouth gape with decreasing DO (Q. P.

Fitzgibbon, personal observation). Ram-ventilating sharks increase ventilation volume by increasing both speed and mouth gape in response to hypoxia (Parsons and Carlson, 1998; Carlson and Parsons, 2001). However, increasing speed also increases oxygen demand. Indeed, RMR and swimming speed of SBT are similarly enhanced in moderate hypoxia (1.2-1.5 times), compared to normoxia. As the $\dot{M}O_2$ of immobilized tuna remains constant from normoxia through to moderate hypoxia (Bushnell and Brill, 1992), this indicates that the increase in RMR is due to the faster swimming. This seemingly paradoxical effect has led to debate over whether the metabolic costs of faster swimming outweigh its benefits to oxygen delivery (Bushnell and Brill, 1991). Oxygen demand and delivery models for yellowfin and skipjack indicate that increasing swimming velocity above routine levels is an ineffective response to hypoxia, and that adequate oxygen delivery is best achieved by doubling mouth gape without a increase in velocity (Bushnell and Brill, 1991). For these species, it is suggested that the increase in speed is a response to escape from hypoxic waters. It should be noted however, that the speed of SBT in normoxia (study mean = 0.75 BL s^{-1}) was substantially less than that recorded for yellowfin (~1.2 BL s^{-1}) and skipjack (~1.6 BL s^{-1}) (Dizon, 1977; Gooding et al., 1981; Bushnell and Brill, 1991). Instead, the velocity of SBT in moderate hypoxia (1.05-1.84 BL s^{-1}) was similar to that found to be optimal for oxygen delivery for these other tuna species (1.2-1.4 BL s^{-1}). Therefore the observed increase in swimming velocity combined with an increase in mouth gape was likely to have been effective for improving oxygen delivery. In severe hypoxia (2.49 mg l^{-1}) however, swimming speed increased to 2.2 times the speed in normoxia. The metabolic cost of this great increase in swimming velocity is likely to outweigh its benefits to oxygen delivery, suggesting that it was an escape response.

All fish are able to extract enough oxygen to maintain minimal metabolic function throughout some range of hypoxia (Fry, 1971; Hughes, 1973). Cardio-respiratory studies show that tuna do so mainly by increasing ventilation volume (Bushnell et al., 1990; Bushnell and Brill, 1991; Bushnell and Brill, 1992). However, below the critical oxygen level (S_{crit}), they need to resort to anaerobic metabolism, which is not sustainable. SBT are able to maintain minimal aerobic uptake, down to and including the DO level of 2.49 mg l^{-1}. When DO is reduced to 1.57 mg l^{-1}, they are no longer able to support their minimal metabolic requirements, and die within 5-7

h. Therefore the S_{crit} of SBT is between 1.57 and 2.49 mg l^{-1}. As the S_{crit} of fish has been widely examined, it proves a useful tool for comparing hypoxia tolerance between species. However when comparing the S_{crit} of SBT, it must be noted that the S_{crit} is most commonly evaluated with resting fish as the DO level in which the fish is no longer able to maintain its standard metabolic rate (SMR). The present study evaluates the S_{crit} with unrestrained swimming tuna. As the active metabolism of fish is more strongly influenced by DO than SMR (Fry, 1971), the S_{crit} of a swimming fish will be at a higher DO than that for a resting fish. Even so, the S_{crit} of SBT is comparable to many other teleosts, especially those of other marine species (Table 3), which reveals that SBT are well adapted to low DO. This tolerance of hypoxia seems counterintuitive considering the well documented elevated oxygen demands of tuna (Korsmeyer and Dewar, 2001; Graham and Dickson, 2004). However, much of the high oxygen demand of tuna is associated with adaptations that enhance oxygen uptake and delivery, including large gill surface area and thin gill epithelia (Muir and Hughes, 1969; Hughes, 1984), and high heart rates and cardiac output (Brill and Bushnell, 2001; Blank et al., 2004). These energetically expensive adaptations also improve the oxygen extraction and utilization capabilities of tuna when exposed to low DO.

Table 3: Interspecific comparison of the critical oxygen level (S_{crit}) of some teleost species.

Common name	Species	Habitat	M_b (kg)	T_w (°C)	U (BL s^{-1})	S_{crit} (mg l^{-1})
Southern bluefin tuna[1]	*Thunnus maccoyii*	O	10	18.2-20.3	0.71-1.84	1.57-2.49
Mulloway[2]	*Argyrosomus japonicus*	C,E	0.34	22.0	0.9	1.5
Atlantic cod[3]	*Gadus morhua*	C	0.30	15	0.0	2.58
Common dentex[4]	*Dentex dentex*	C	0.39	20.8	0.0	2.5
Atlantic salmon[5]	*Salmo salar*	C, FW_s, FW_m	0.04	12.5	0.0	4.0
Brook trout[6]	*Salvelinus fontinalis*	FW_s,FW_m	0.10-0.28	15	0.0	3.8
Rainbow[7] trout	*Oncorhynchus mykiss*	FW_s,FW_m	0.54	20	0.0	1.6
Carp[7]	*Cyprinus carpio*	FW_s	1.6	25	0.0	1.1
Cichlid[8]	*Oreochromis niloticus*	FW_s,FW_m	0.22	20	0.0	1.1
Darter[9]	*Etheostoma rufilineatum*	FW_m	-	20	0.0	6.1
Darter[9]	*Etheostoma flabellare.*	FW_s,FW_m	-	20	0.0	2.1
Darter[9]	*Etheostoma duryi*	FW_s,FW_m	-	20	0.0	2.0
Darter[9]	*Etheostoma squamiceps*	FW_s,FW_m	-	20	0.0	2.3
Darter[9]	*Etheostoma boschungi*	FW_s,FW_m	-	20	0.0	1.7
Darter[9]	*Etheostoma fusiforme*	FW_s	-	20	0.0	2.9
European eel[10]	*Anguilla anguilla*	FW_s	0.12	25	0.0	1.3
Trahia[11]	*Hoplias malabaricus*	FW_s,FW_m	0.04	25	0.0	0.94-1.67
Trairao[12]	*Hoplias lacerdae*	FW_s,FW_m	0.3-0.5	25	0.0	1.83
Gulf killifish[13]	*Fundulas grandis*	E, FW_s	0.01	25	0.0	1.73

[1]Present study; [2]Chapter 6; [3]Schurmann and Steffensen (1997); [4]Valverde *et al*., (2006); [5]Stevens *et al*., (1998); [6]Beamish, (1964); [7]Ott *et al*., (1980); [8]Fernandes and Rantin, (1989); [9]Ultsch *et al*., (1978); [10]Cruz-Neto and Steffensen, (1997); [11]Kalinin *et al*., (1993); [12]Rantin *et al*., (1992), [13]Virani and Rees, (2000).

M_b, body mass; T_w, temperature; U, swimming velocity.

O, oceanic; C, coastal; E, estuarine; FW_s, still fresh water; FW_m, moving freshwater

Tolerance of SBT to hypoxia is similar to that of yellowfin tuna (Dizon, 1977), suggesting that the ability to tolerate severe hypoxia may be common to the *Thunnus* genera. This implies that the depth distribution limitation of yellowfin tuna to well oxygenated waters (Cayré and Marsac, 1993) is a behavioural response and not a physiological threshold. Similar behavioural avoidance of moderate hypoxia have also been recorded in other marine teleosts such as Atlantic cod and sea bass (*Dicentrarchus labrax*) (Claireaux et al., 1995; Schurmann et al., 1998; Claireaux et al., 2000). Bigeye tuna are hypothesized to be more tolerant of hypoxia than other tuna due to lower blood oxygen affinity (Jones et al., 1986; Bushnell et al., 1990; Lowe et al., 2000; Brill and Bushnell, 2006), which is supported by an frequent inhabitation of hypoxic waters (Dagorn et al., 2000; Schaefer and Fuller, 2002; Musyl et al., 2003). However, their behavioural patterns are not necessarily evidence of greater hypoxia adaptation. Although bigeye tuna may briefly frequent waters with

DO levels as low as ~1.5 mg l^{-1} (Hanamoto, 1987; Musyl et al., 2003), they are largely limited to DO levels above ~3 mg l^{-1} with regular (1-2.5 hourly) ascents into the mixed surface waters (Dagorn et al., 2000; Musyl et al., 2003). This type of hypoxia exposure is probably well within the physiological capabilities of SBT. The prolonged deep diving behaviour of bigeye tuna may be largely a behavioural/predatory preference or a greater tolerance of low water temperatures that are often closely associated with deep hypoxic waters.

Effects of feeding

SBT voluntarily ingest substantial rations of sardines at DO levels as low as 3.25 mg l^{-1}, demonstrating their willingness to feed in hypoxic conditions. Indeed, rations ingested in hypoxia (6.7 and 2.7 ± 0.25 %M_b for experiments 1 and 2, respectively) were greater than recorded with the same fish in normoxia (5.5 ± 1.2 %M_b, n = 6, Chapter 2 and 2.0 ± 0.2 %M_b, n = 8, Chapter 3, respectively). This suggests that moderate hypoxia has little effect on the appetite of SBT. The opposite is true for Atlantic cod, turbot (*Scophthalmus maximus*) and European sea bass where feed intake is reduced in hypoxia (Chabot and Dutil, 1999; Thetmeyer et al., 1999; Pichavant et al., 2001). Furthermore, SBT retain ingested food when the DO is further reduced to as low as 2.5 mg l^{-1}. Regurgitation in severe hypoxia has been demonstrated in Atlantic cod (Claireaux et al., 2000). Hypoxia-induced reduction in both feed intake and retention is suggested to be as a result of the limiting effect of hypoxia on the metabolic scope (Claireaux et al., 2000; Mallekh and Lagardere, 2002). It is thought that fish adjust the energy necessary for SDA according to their capacity to provide energy to support other simultaneous metabolic requirements. Hypoxia would almost certainly reduce the metabolic scope of tuna as it does in other teleosts (Fry, 1971; Claireaux et al., 2000). However, tuna are known to have metabolic scopes that greatly exceed that of most other fish (Korsmeyer et al., 1996; Korsmeyer and Dewar, 2001; Graham and Dickson, 2004). Therefore, even with a substantial reduction in scope, they are still likely to retain a large aerobic capacity to support SDA and other essential metabolic requirements. Indeed, the present study shows that at a DO level as low as 2.84 mg l^{-1} (~40%), SBT do support digestion at a similar rate as in normoxia. Postprandial RMR is similarly elevated in hypoxia (1.6

times pre-feeding level in normoxia) as it was in normoxia (1.8 times), indicating the metabolic cost of SDA is similar. Gastric evacuation rates during hypoxia are as rapid (11.1 ± 0.7% wet mass h^{-1}) as in normoxia (12.8% wet mass h^{-1}) and similar to that documented for other tuna species tuna (Schaefer, 1984; Olson and Boggs, 1986). This is in contrast to Atlantic cod where at a similar level of hypoxia (2.7 mg l^{-1}), maximum postprandial $\dot{M}O_2$ was reduced by 25% and to compensate, the duration of SDA more than doubled (Jordan and Steffensen, 2007). With this species, the peak in postprandial $\dot{M}O_2$ in hypoxia occupied ~70% of the metabolic scope leaving little energy for other activities.

This study has shown that both fasted and postprandial SBT are highly tolerant of low DO. This conclusion is based on the examination of relatively small SBT (~10 kg). Larger tuna may be better adapted to low DO as the hypoxia tolerance of fish improves with increasing body mass (Kalinin et al., 1993). Oxygen uptake and delivery adaptations that support the great metabolic scope of tuna are also likely to be beneficial in hypoxic waters. Furthermore, it appears that the large metabolic scope of tuna maintains appetite and permits digestion at low DO levels (less then 40% saturation). It is widely considered that the great metabolic scope of tuna has evolved to allow the simultaneous support of multiple metabolic functions associated with their high performance physiology and behaviour, principally that of rapid sustained swimming, fast growth rates, rapid digestion, regional endothermy and potential for quick repayment of oxygen debt (Brill and Bushnell, 1991; Brill, 1996; Korsmeyer et al., 1996; Korsmeyer and Dewar, 2001; Graham and Dickson, 2004). Here it is suggested, that a large aerobic capacity is also integral in supporting their ability to cope with low DO, allowing some tuna species to exploit prey resources in deep, hypoxic waters.

5. Relationship between metabolic rate and visceral warming in southern bluefin tuna (*Thunnus maccoyii*)

Abstract

The ability of tuna to conserve metabolic heat to maintain tissues warmer than ambient water temperatures (T_w) is thought to have allowed them to expand their thermal niche, and contributed to their evolutionary success. Only tuna of the subgenus *Thunnus*, which includes bluefin, have the ability to warm the viscera. Species capable of visceral warming occur in cooler waters, possibly due to improved thermoregulatory capabilities. However, the mechanisms of visceral endothermy are poorly understood. The source of visceral heat is hypothesised to be a by-product of the metabolic cost of food digestion and assimilation (specific dynamic action, SDA) and it is not known if tuna are capable of regulating the efficiency of visceral heat retention. In this study, 768 h of simultaneous recordings of metabolic rate (MR = to heat production) and visceral temperature (T_{vis}) were made in both fasted and postprandial southern bluefin tuna (SBT, *Thunnus maccoyii*) of two sizes (~10 and 20 kg) and at two water temperatures (~19 and 16°C). Similar to previously recorded, basal T_{vis} were 2-3°C above T_w but would increase quickly after feeding taking hours to days to fall back to basal levels. In all trials, excess temperatures (the difference between visceral and water temperature) were directly related to postprandial MR. Duration and magnitude of SDA were strongly related to duration and magnitude of postprandial visceral warming. This provides the first empirical evidence of a link between SDA and postprandial visceral warming. Visceral temperature of fasted SBT was also directly related to MR. In this case, the source of heat is thought to be metabolic work performed within the red muscles which appears to have warmed the viscera through thermal conductance. At any level of heat production (postprandial MR), visceral excess temperatures were significantly warmer (over 1°C warmer) in larger (~20 kg) than smaller SBT (~10 kg) at equivalent water temperature. Better heat retention ability of the larger SBT is likely attributed to improved *retia mirabilia* development and greater thermal inertia. Also, at any level of heat production, SBT at 16°C maintained visceral excess temperatures significantly warmer (0.5^{o}C warmer)

than similarly sized fish at 19°C. This demonstrates some ability of SBT to physiologically regulate visceral warming.

Introduction

Unlike the majority of teleost fishes that have body temperatures close to ambient water temperatures (T_w) (ectotherms), tuna are capable of warming certain tissues substantially above T_w (regional endothermy). Regional endothermy has evolved independently in several fish lineages including lamnid sharks, billfishes and tuna, all of which are large, high-energy demand fishes (Dickson and Graham, 2004). Development of specializations for endothermy varies considerably within the Thunnini tribe and correlates strongly with tuna phylogeny (Block et al., 1993; Collette et al., 2001). Tissues capable of being warmed include locomotor muscles, cranial tissues (eyes and brain), and the viscera (Graham and Dickson, 2000; Graham and Dickson, 2001; Dickson and Graham, 2004). Physiological benefits believed to result from elevated body temperatures include enhanced rates of muscle contraction and power output (Carey and Teal, 1969a; Brill, 1978; Johnson and Brill, 1984; Wardle et al., 1989; Altringham and Block, 1997), cellular respiration (Stevens and Carey, 1981), visual acuity and neural processing (Block and Carey, 1985) and digestion (Carey et al., 1984; Stevens and McLeese, 1984). It is thought that the major evolutionary advantages of endothermy are that it allows tuna to expand their thermal niche (both horizontally and vertically) and enhances their aerobic swimming performance (Block et al., 1993; Graham and Dickson, 2000; Dickson and Graham, 2004).

Amongst tuna species examined, most can elevate temperatures of the muscles and cranial tissues. However, only species within the subgenus *Thunnus* are capable of visceral endothermy, including southern bluefin (*Thunnus maccoyii*), Atlantic bluefin (*T. thynnus*), Pacific bluefin (*T. orientalis*), albacore (*T. alalunga*) and bigeye tuna (*T. obesus*) (Collette et al., 2001). Large Atlantic bluefin (>200 kg) achieve excess temperatures (temperature above T_w) in the viscera of up to 21°C and can maintain excesses of 8-15°C for months (Carey, 1973; Carey et al., 1984; Stevens et al., 2000; Block et al., 2001; Gunn and Block, 2001). Smaller southern and Pacific bluefin (<50 kg) maintain visceral excess temperatures between 3-9°C (Kitagawa et

al., 2000; Gunn et al., 2001; Marcinek et al., 2001; Itoh et al., 2003; Kitagawa et al., 2006).

Endothermy requires a source of heat and a mechanism to retain it. The source of heat is commonly thought to be a by-product of normal metabolic processes. This heat is retained within the tissue by arterial and venous blood vessels arranged as counter current heat exchanges (*retia mirabilia*) that reduce convective heat loss from the tissue and loss to the surrounding water across the gills (Graham, 1975; Fudge and Stevens, 1996). The source of visceral heat is thought to be the by-product of metabolic work associated with food digestion and assimilation (commonly referred to as specific dynamic action, SDA), which is retained within the viscera by *retia* which supply blood to the stomach, spleen, caecum and intestines (Carey et al., 1984; Fudge and Stevens, 1996). Postprandial visceral warming and SDA display a similar abrupt rise following feeding followed by a steady decline (Carey et al., 1984; Gunn and Block, 2001; Gunn et al., 2001) and the magnitude (total increase above basal level) of SDA and postprandial visceral warming are proportional to the amount of food ingested (Gunn et al., 2001).

Thermoregulatory options for tuna can be behavioural, passive or physiological (Dizon and Brill, 1979b). Behavioural thermoregulation can be achieved by the spatial selection of favourable environmental conditions or by the control of heat production (i.e., regulation of swimming velocity or possibly feed intake). Passive thermoregulation refers to physical processes that are not physiologically controlled such as thermal inertia. Physiological thermoregulation refers to the physiological ability to alter the effectiveness of the heat-conserving mechanism. The ability of tuna to physiologically thermoregulate has historically provoked much debate due to the inherent difficulties in substantiating it (Neill and Stevens, 1974; Dizon and Brill, 1979b). In order to demonstrate physiological thermoregulation, the study must observe altered heat retention that is independent of T_w and heat production. This has been achieved in laboratory studies where it has been demonstrated that tuna can regulate red muscle heat conservation independently of T_w and heat production (evaluated as swimming velocity) that has been either defined or controlled (Dizon and Brill, 1979b; Dizon and Brill, 1979a; Graham and Dickson, 1981; Dewar et al., 1994). However, the ability of tuna to regulate visceral warming has not been examined. Examination of visceral thermoregulation is complicated by the difficulties in evaluating or controlling heat production (presumed

to be SDA). Indeed, only recently the first observations of tuna SDA have been made (Chapter 2).

By simultaneously recording postprandial metabolic rate and T_{vis}, this study aims to investigate the relationship between SDA and visceral warming. It examines two size classes of SBT (~10 kg and 20 kg) to determine the effects of juvenile development and increased thermal inertia on visceral warming. Also, the study aims to determine whether the SBT is capable of visceral physiological thermoregulation by examining the relationship between metabolic heat production and visceral warming at two different T_w (~19°C and 16°C). The thermal range of bluefin extends from the warm tropics where they spawn, to cool temperate and sub-polar waters as low as 2-3°C (Block et al., 2001; Block et al., 2005). Their derived endothermic anatomy and capabilities, including that of visceral endothermy, are likely to be involved in this thermal range expansion.

Methods

Experimental animals

Two separate trials were conducted in early 2005 and 2006. In January of both years SBT were sourced from a marine aquaculture farm in the waters off Port Lincoln, South Australia, through the Tuna Boat Owners Association of South Australia. These SBT had been purse-seine netted in the Great Australian Bight one month earlier. From the commercial farm, individual SBT were hooked with a baited, barb-less hook and transferred into a 12 m diameter, 8 m deep holding sea-cage that was towed and moored in a protected bay at the southern end of Boston Island, where all trials were conducted. Fish were left to recover from transfer for 1 month during which time they were fed sardines (*Sardinops sagax*) twice per day to satiation.

Archival temperature loggers (TX, Vemco, www.vemco.com) were then surgically implanted into the body cavity to record visceral temperature (T_{vis}). Individual fish were removed from the holding cage by hook (as described above) or by hand whereby the fish were crowded within a small purse-seine net, captured by a skin diver and passed to waiting deck crew. The fish was then inverted, placed into a padded cradle and a wet cloth put over its eyes to aid with settling. A 5-8 cm incision

into the belly wall along the central line approximately 10-12 cm anterior to the vent was made with a sharp knife and the peritoneum perforated by the surgeons' finger. The logger, which was coated in antiseptic (Betadine, Purdue Pharma, www.pharma.com) was then inserted through the hole so that it lay along the belly wall ventral to the stomach and caecum with the thermistor pointing towards the head. A 2.5 ml dose of broad spectrum antibiotic (Amoxil, GlaxoSmithKline, www.gsk.com) was then injected into the opening and wound sealed with two stitches (Vicryl suture, Ethicon, www.ethicon.com). The animal was then released, the entire process taking ~1-1.5 min. The loggers were programmed to record T_{vis} every 4 min.

Following logger implantation the fish were left to recover for at least one month. Again during this period they were fed sardines twice per day but were starved for a minimum of 48 h before introduction to the respirometer to allow the effects of SDA to subside (Chapter 2). Fish were transferred into the respirometer by hand as described above.

Mesocosm respirometer

SBT oxygen consumption rates ($\dot{M}O_2$) was determined by the reduction in dissolved oxygen (DO) over time within a large static respirometer ("mesocosm respirometer"). The mesocosm was manufactured from denier polyester scrim reinforced 1.14 mm polypropylene (R-PP 45, Stevens Geomembranes, http://www.stevensgeomembranes.com), bonded by thermal welding by Fabtech SA Pty. Ltd (Adelaide, Australia). The mesocosm was a 12 m diameter, 2.5 m deep enclosed cylinder with a wave break wall that extended 1 m above water level. Entry into the mesocosm was possible through a 2 m diameter, 2 m high access port made from 0.75 mm polypropylene and positioned in the roof so that it could be rolled and clamped to completely seal the system. In addition, five 1 m diameter, clear 0.75 mm polyvinyl chloride windows were positioned in the roof to allow entry of natural light, and eleven capped tank fittings (STF 50, Hansen Products Ltd, http://www.hansenproducts.co.nz/) allowed sealed access for experimental equipment and manual sampling.

The mesocosm was pump filled with sea-water to a total water volume of between 350 000-402 000 l (varied between experiments due to water exchanges).

General experimental procedures

Fish were obviously distressed when confined within the respirometer alone, but settled down when accompanied by others. Therefore all experiments involved three fish. They were introduced in the afternoon, the respirometer was sealed and metabolic rate (MR) was recorded until the morning approximately 36-42 h later. Data collected during this period were subsequently used as representative of fasted fish. Respirometer dissolved oxygen (DO) was then returned to between 100-115% saturation by oxygen injection with a gas diffuser (Force 7, Aqua & Co, BOC Gases, NSW, Australia). After 1 h recovery from this disturbance, fish were then fed weighed sardines through the access port and any uneaten baitfish removed by a diver to obtain the net consumption. The respirometer was again sealed and MR recorded for 48 h. Data collected during this period were considered representative of postprandial fish. Feeding was subsequently repeated between two and four times in each trial with the same procedures.

Background respiration and photosynthesis of planktonic organisms in the water column and on the mesocosm surfaces were measured in trials immediately before and after each experiment. Low levels of background respiration were generally found, however it was dependent on time of day (net respiration rate would fall during the day due to photosynthesis). All $\dot{M}O_2$ recordings were adjusted for background respiration according to the pertinent time of day. The oxygen holding capabilities of the mesocosm was examined in further trials where the respirometer DO was reduced to between 1.9 and 2.3 mg l^{-1} by injecting micro-bubbles of pure nitrogen with a gas diffuser (Aqua & Co Force 7, BOC gases, NSW, Australia) and then removing the bubbles that collected at the water surface, before the mesocosm was sealed and oxygen monitored for more than 20 h. Very low levels of leakage (0.0042 mg^{-1} h^{-1} per mg l^{-1} DO gradient between the respirometer and external environment) were found in experiment 2005 and negligible levels in 2006. These rates of leakage were nevertheless used to adjust all trial DO recordings according to the pertinent oxygen gradient. Dissolved ammonia concentrations were also monitored by daily analyses of water samples before, during and after each trial, and they never exceeded 0.6 mg l^{-1}.

Two luminescence dissolved oxygen meters (Hach HQ10, Loveland, CO, USA) permanently installed at a depth of 1 and 2 m respectively, both 3 m from the

wall, logged mesocosm DO and water temperature. Meters were calibrated according to the manufacturer's instructions at the start of the trials and calibration checked following the completion of all trials. Meters recorded DO (0.01 mg l^{-1} resolution) that was automatically compensated for water temperature and salinity set at $35^{0}/_{00}$. Oxygen meters were set to log DO every 5 min.

Fish behaviour was monitored (both day and night) with a permanently installed video system (SciElex Pty. Ltd., Hobart, Australia) that consisted of two 0.05 lux black and white underwater video cameras and two 6 W white LED lights (housed within resin). The system was programmable through a central controller and power supply unit that were secured to the sea-cage. The cameras were programmed to record for 4 min every 90 min (first by the horizontal camera then immediately by the vertical camera) and data stored on a pocket video recorder (Archos AV400, Greenwood Village, CO, USA). The lights were programmed to turn on at dusk and remain on until dawn. The system was powered by a single 32 Ah, 12 V battery that was exchanged daily.

Video recordings were viewed in the video editing program (VirtualDub 1.5.10, Avery Lee, www.virtual.dub.org). Suitable records were defined as those containing a fish that was swimming perpendicularly to the camera and at least one frame in which body length (BL) could be measured. This sequence was imported into video motion analysis software (Video Point Version 2.5, Lenox Softworks, www.lsw.com) where swimming velocity was analysed by tracking movement across the field. Tuna BL was used to calibrate the image scale, accounting for variable distances between the fish and the camera. Any camera movement was corrected by referring to a stable point within the respirometer on a frame-by-frame basis.

Experimental trials

Three experiments were conducted to examine the effects of body mass (M_b) and water temperature (T_w), each consisting of two trials with three fish.

10 kg/ 19°C experiment: These trials were conducted in 2005 with mean body mass (M_b) of 10.2 ± 1.0 kg at a mean T_w of 19.3 ± 0.1°C (Table 1). MR and T_{vis} were recorded for a total of 195 h equating to 65 (17 fasted and 48 postprandial)

simultaneous measurements. Fish were fed twice in each of the two trials. Mean ration voluntarily ingested was 5.8 ± 1.3 % body mass (%M_b).

20 kg/ 19°C experiment: To examine the effects of increased M_b, this experiment was conducted at an equivalent T_w (19.0 ± 0.1°C), however with fish approximately double the M_b (19.1 ± 0.7 kg) as in the first experiment. It was carried out in 2006 and consisted of 84 simultaneous measurements. Again, fish were fed twice in each of the two trials, however rations voluntarily ingested (2.1 ± 0.3 %M_b) were less than the experiment with 10 kg fish.

20 kg/ 16°C experiment: This experiment aimed to examine the effects of reduced T_w and was performed with the same cohort of fish (M_b = 20.1 ± 0.6 kg) as the second experiment, but later in the Austral autumn season when mean T_w was ~3°C cooler (16.2 ± 0.1°C). One hundred and seven simultaneous measurements of MR and T_{vis} were recorded. Fish were fed twice in the first trial and four times in the second. Rations ingested were similar (2.3 ±0.5 %M_b) to the 19°C trial.

Attempts were made to conduct experiments during the Austral winter when ambient water temperatures were further reduced. However these proved unsuccessful, as rough weather restricted access and caused substantial equipment failure. Furthermore, fish failed to ingest baitfish voluntarily, presumably due to a reduced appetite associated with the low T_w.

Table 1. Parameters for southern bluefin tuna trials.

Trials	No. of fish	M_b (kg)	T_w range (°C)	T_w mean (°C)	n (Fasted)	n (postprandial)	No. of feedings	Ration (%M_b)
10 kg/ 19°C	6	10.2 ±1.0	18.2-20.7	19.3 ±0.1	17	48	4	5.8 ±1.3
20 kg/ 19°C	6	19.1 ±0.7	18.4-20.2	19.0 ±0.1	31	53	4	2.1 ±0.3
20 kg/ 16°C	6	20.1 ±0.6	15.6-16.8	16.2 ±0.1	28	79	6	2.3 ±0.5

M_b, body mass; n, number of measurements; T_w, water temperature.

Data analysis

DO recordings were divided into 3 h blocks according to the time of day (starting at midnight) and linear regression fitted to the change in DO to establish fish MR (mg kg^{-1} h^{-1}). T_{vis} (mean of the three fish) and water temperature (T_w) data were also collated within the same 3 h time periods, where the mean was taken. Immediately following feeding, T_{vis} would commonly drop due to the cooling effect of sardines

ingested (which were at or below T_w) (Fig. 1). For this reason, data collected within 3 h of feeding was excluded from comparison. The difference in T_{vis} from T_w is referred to as T_{vis} excess.

Specific dynamic action (SDA) was quantified with the following parameters: SDA duration (duration that postprandial MR remained greater than the pre-feeding level), and SDA magnitude (total oxygen consumed above the pre-feeding MR within the SDA duration period). Postprandial visceral warming was quantified with the following parameters: T_{vis} duration (duration that postprandial T_{vis} remained greater than the pre-feeding level) and T_{vis} magnitude (total temperature (in ^{o}h) above the pre-feeding T_{vis} within the T_{vis} duration period). Significant differences of means were established by the use of two-tailed students T-tests, and regression slopes and intercepts by ANOVA within the StatistiXL program (version 1.5, www.statistixl.com).

Results

Fig. 1 demonstrates data recorded in one trial of the 20 kg/ 19°C experiment. Measurements began immediately after the transfer of fish into the respirometer and continued for ~6 days. Similar to that previously recorded with equivalent sized SBT (15-37 kg) (Gunn and Block, 2001; Gunn et al., 2001), basal T_{vis} temperatures were always 2 to 3°C above T_w, which was common for all trials. After transfer, MR and T_{vis} were initially elevated, but gradually decreased over ~20 h to a plateau. It is presumed that this was due to physical exertion and stress associated with capture and transfer. For transfer, fish were crowded within a net and captured by a skin diver. Fish would struggle when accidentally caught in the net and when grasped by the diver. During this trial, fish ingested two small rations of sardines (1.7 % body weight) and data taken in the subsequent 48 h of each feed was used as representative of postprandial fish. Again similar to previously recorded with SBT (Gunn and Block, 2001; Gunn et al., 2001), feeding events were characterized by an initial drop in T_{vis} (due to the lower temperature of sardines ingested) followed by a rapid increase of T_{vis} and MR, both of which appear to take ~20-30 h to fall to the pre-feeding level. T_w fluctuated between 18.4 and 18.8 ^{o}C in what appeared to be a diurnal cycle.

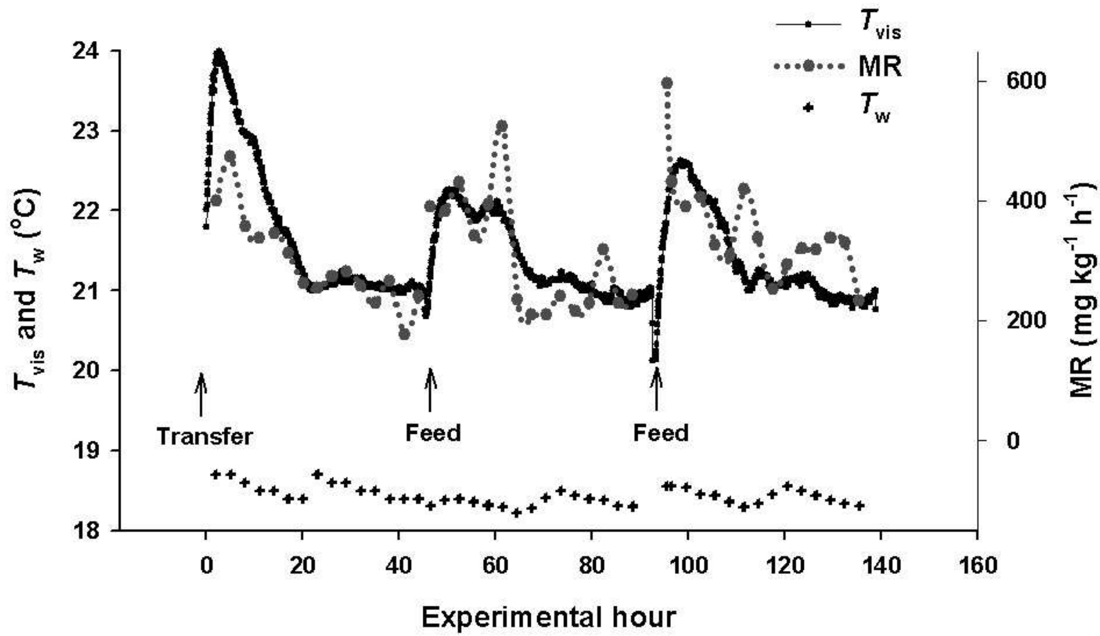

Fig. 1: Simultaneous measurement of southern bluefin tuna visceral temperature (T_{vis}) and metabolic rate (MR) in one trial of the 20 kg/ 19°C experiment. Measurements began immediately after transfer into the respirometer and the fish were fed sardines twice (both 1.7 % body mass rations) during the trial. T_w are ambient water temperatures. Values are means of three fish.

Fasted fish

T_{vis} of fasted fish ranged from 18.7 to 23.7 °C (Fig. 2A) and was related to MR in all groups (P <0.01, <0.01 and 0.01 for 10 kg/ 19°C, 20 kg/ 19°C, 20 kg/ 16°C experiments, respectively) (Table 2). Regressions slopes were not significantly different between groups however, regression intercepts were, with the 20 kg SBT at 19°C having the warmest T_{vis} and the fish at 16°C the coolest (Table 2).

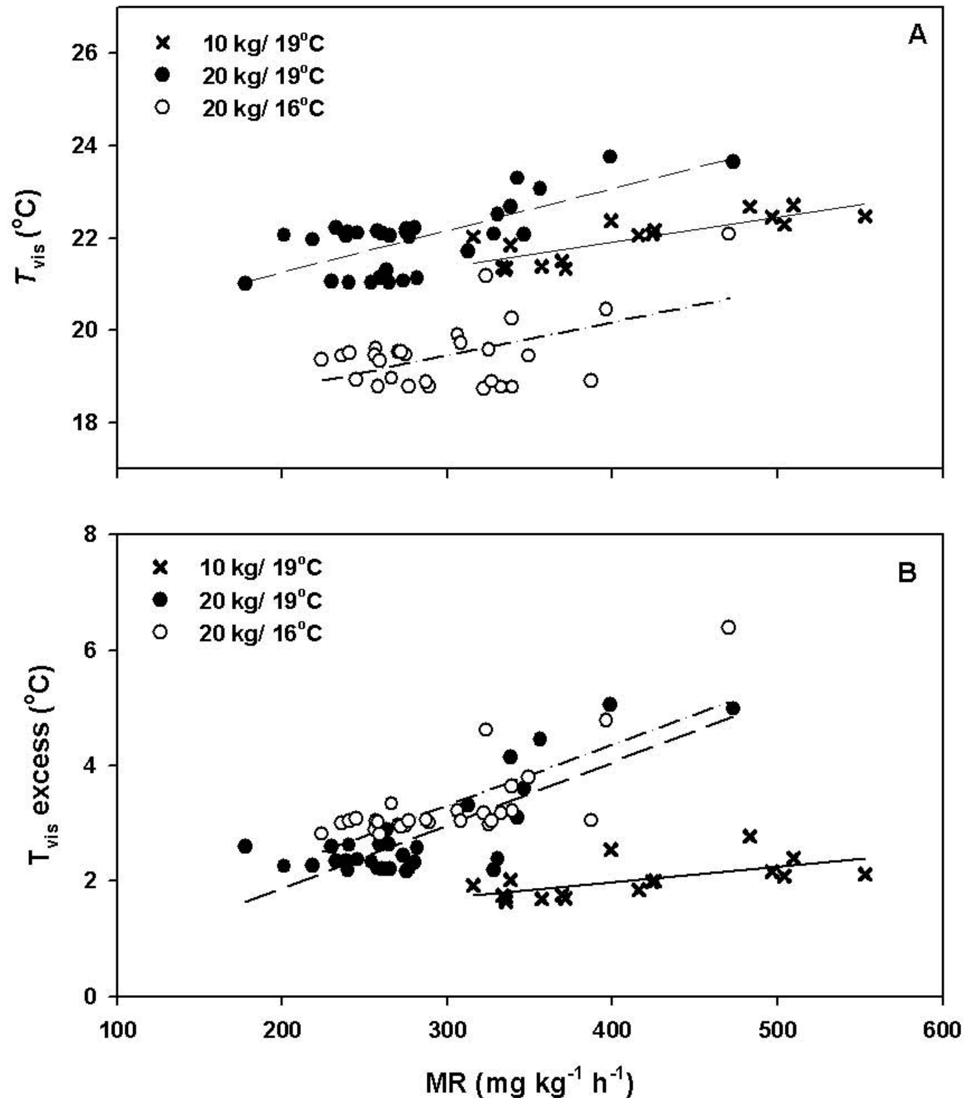

Fig. 2. Relationship between fasted southern bluefin tuna metabolic rate (MR) and **A)** visceral temperature (T_{vis}), and **B)** visceral temperature difference from ambient water temperature (T_{vis} excess), for fish with body mass (M_b) of ~10 kg at a ambient water temperatures of 19°C (crosses), M_b ~20 kg at ~19°C (filled circles), and M_b ~20 kg at ~16°C (open circles). Full details of trials are given in Table 1. Lines represent linear regressions, solid line for 10 kg/ 19°C, long dashes for 20 kg/ 19°C and dashes and dots for 20 kg/ 16°C. Details for regressions are given in Table 2. Each data points are the means of three fish over a 3 h period.

T_{vis} excess of fasted fish ranged from 1.7 to 6.4°C (Fig. 2B). T_{vis} excess was directly related to MR for all groups (P = 0.01, <0.01 and <0.01 for 10 kg/ 19°C, 20 kg/ 19°C, and 20 kg/ 16°C experiments, respectively) (Table 2). The slope of regression for the 10 kg/ 19°C trial was significantly less than the other two groups (Table 2). Comparison of regression intercept between the 20 kg/ 19°C and 20 kg/ 16°C groups revealed that fish at 16°C maintained T_{vis} excess significantly warmer (~0.5°C) than the fish at 19°C relative to MR.

Table 2. Parameters for fasted and postprandial southern bluefin tuna. Regressions relating metabolic rate (MR, mg kg^{-1} h^{-1}), with visceral temperature (T_{vis}, °C) and difference in visceral temperature from water temperature (T_{vis} excess, °C) (T_{vis} or T_{vis} excess = b • MR +a) as recorded in trials described in Table 1.

			n	**b**	**95% C.I.**	**a**	**95% C.I.**	**R^2**	**P (slope)**	**Diff. (slope)**	**Diff. (int.)**
Fasted	***T_{vis}***	**10 kg/ 19°C**	17	0.0055	0.0033-0.0076	19.73	18.85-20.63	0.67	<0.01	x	x
		20 kg/ 19°C	31	0.0091	0.0057-0.0120	19.45	18.49-20.40	0.52	<0.01	x	y
		20 kg/ 16°C	28	0.0072	0.0024-0.0120	17.30	15.82-18.77	0.27	0.01	x	z
	***T_{vis}* excess**	**10 kg/ 19°C**	17	0.0027	0.0008-0.0045	0.92	0.14-1.68	0.39	0.01	x	-
		20 kg/ 19°C	31	0.0109	0.0078-0.0141	-0.31	-1.22-0.60	0.63	<0.01	y	x
		20 kg/ 16°C	28	0.0105	0.0069-0.0141	0.15	-0.94-1.25	0.58	<0.01	y	y
Post-prandial	***T_{vis}***	**10 kg/ 19°C**	48	0.0051	0.0039-0.0064	18.97	18.18-19.76	0.60	<0.01	x	x
		20 kg/ 19°C	53	0.0058	0.0040-0.0076	19.97	19.37-20.57	0.45	<0.01	x	y
		20 kg/ 16°C	79	0.0032	0.0019-0.0045	19.21	18.59-19.83	0.25	<0.01	y	-
	***T_{vis}* excess**	**10 kg/ 19°C**	48	0.0035	0.0027-0.0044	0.71	0.20-1.23	0.62	<0.01	x	x
		20 kg/ 19°C	53	0.0041	0.0028-0.0053	1.81	1.40-2.21	0.47	<0.01	x	y
		20 kg/ 16°C	79	0.0043	0.0032-0.0054	2.27	1.73-2.81	0.43	<0.01	x	z

n, number of measurements; 95% C.I., 95% Confidence intervals; R^2, regression coefficient; P, probability that regression slope is significantly different from zero; Diff. (slope) and Diff. (int.), significant differences between experiments (P<0.05) of linear regression slope and intercept (significant differences of intercepts only analysed when there was no significantly different in regression slopes) by ANOVA with Post Hoc analysis. Different letters (x,y and z) denote significant differences between regressions.

Postprandial fish

T_{vis} of postprandial fish ranged from 18.9 to 26.1°C (Fig. 3A). T_{vis} was significantly related to MR in all groups ($P < 0.01$) (Table 2). The regression slope of the 20 kg SBT at 16°C was significantly different to the other two groups. Comparison of regression intercepts between the 10 and 20kg SBT at 19°C revealed that the 20 kg fish maintained T_{vis} approximately 1°C warmer relative to MR.

T_{vis} excess of postprandial fish ranged from 1.8 to 7.1°C (Fig. 3B). T_{vis} excess was again significantly related to MR in all groups ($P < 0.01$) (Table 2). Slopes of regressions were not significantly different between groups. Intercepts of regression were however significantly different with T_{vis} excess of 10 kg SBT approximately 1°C cooler than the 20 kg fish, and T_{vis} excess of 20 kg SBT at 16°C approximately 0.5°C warmer than similar sized fish at ~19°C relative to MR.

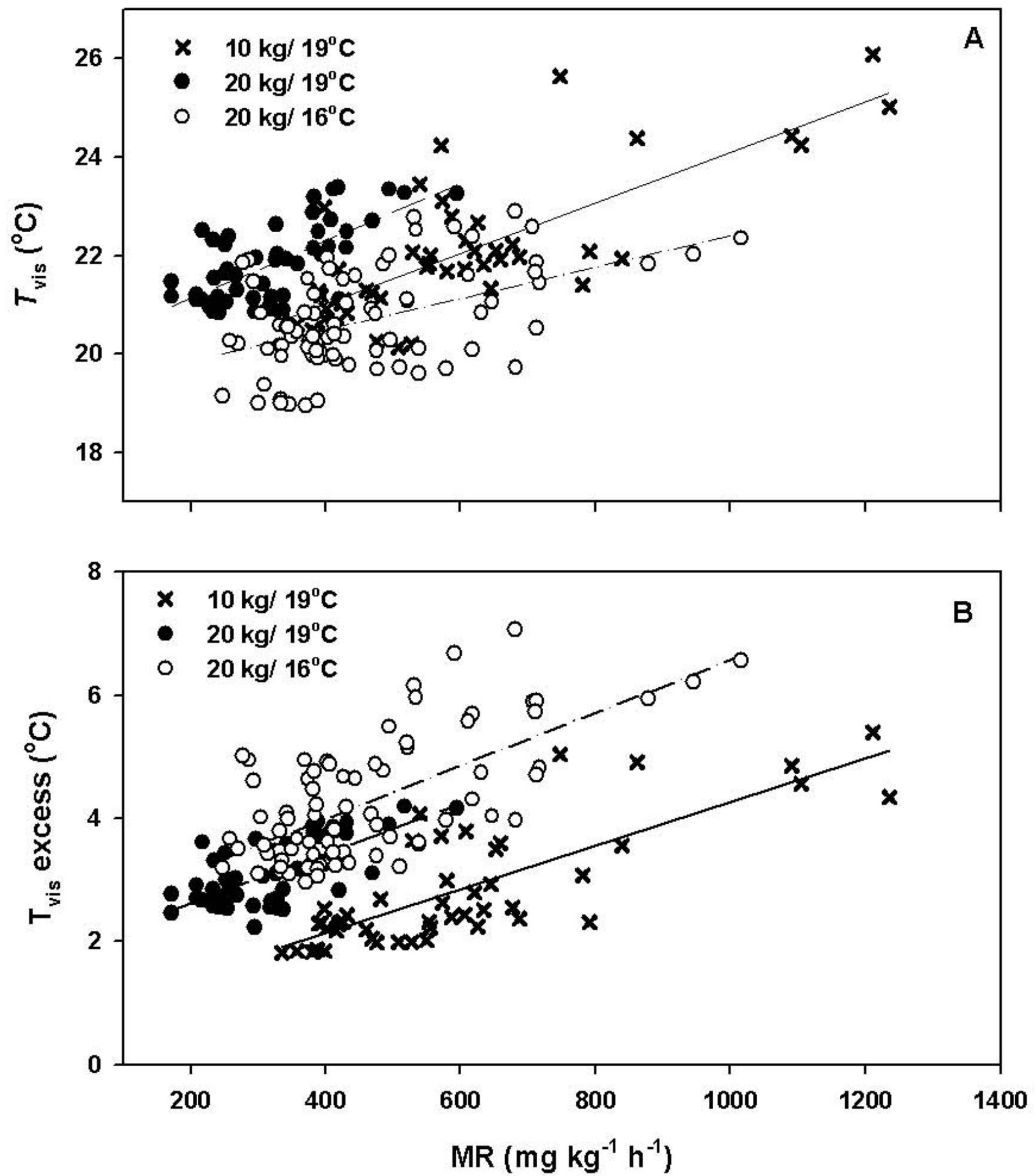

Fig. 3. Relationship between postprandial southern bluefin tuna metabolic rate (MR) and **A)** visceral temperature (T_{vis}), and **B)** visceral temperature difference from ambient water temperature (T_{vis} excess), for fish with body mass (M_b) of ~10 kg at an ambient water temperatures of 19°C (crosses), M_b ~20 kg at ~19°C (filled circles), and M_b ~20 kg at ~16°C (open circles). Full details of trials are given in Table 1. Lines represent linear regressions, solid line for 10 kg/ 19°C, long dashes for 20 kg/ 19°C and dashes and dots for 20 kg/ 16°C. Details for regressions are given in Table 2. Each data point is the means of three fish over a 3 h period.

Specific dynamic action parameters

Duration of SDA was significantly related to the duration of postprandial visceral warming for the 20 kg SBT at 19°C and 16°C (P = 0.01 and 0.04, respectively) but not the 10 kg SBT at 19°C (P = 0.19). Regression slopes and intercepts were not significant different between groups (Table 3). When the data from all experiments are combined, duration of SDA and visceral warming was significantly related (P <0.01) (Fig. 4A).

The magnitude of SDA was significantly related to the magnitude of postprandial visceral warming for the 10 kg SBT at 19°C and the 20 kg SBT at 16°C (P = 0.02 and 0.01, respectively) but not the 20 kg fish at 19°C (P = 0.06). Slopes of regressions were not significantly different between groups. Regression intercept of the 10 kg SBT at 19°C was significantly less than in the 20 kg SBT at 16°C. When the data for the 20kg fish were combined, SDA and T_{vis} magnitude was significantly related (P <0.01) (Fig. 4B).

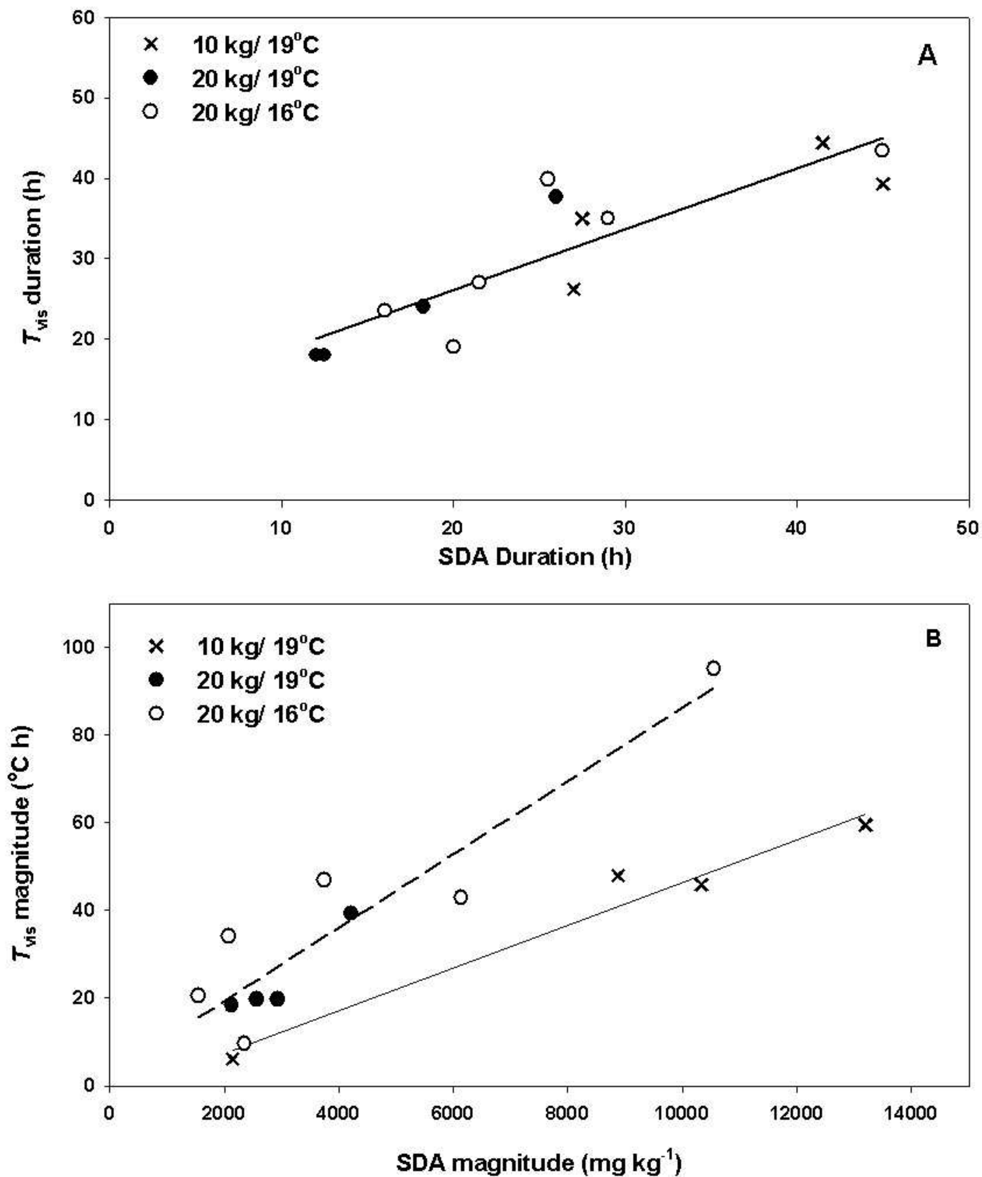

Fig. 4. Relationship between **A)** duration of southern bluefin tuna specific dynamic action (SDA duration) and duration of postprandial visceral warming (T_{vis} duration) and **B)** magnitude of specific dynamic action (SDA magnitude) and magnitude postprandial visceral warming (T_{vis} magnitude). Crosses are for fish with body mass (M_b) of ~10 kg at a ambient water temperatures of 19°C, filled circles for those with M_b ~20 kg at ~19°C, and open circles for those with M_b ~20 kg at ~16°C. Full details of trials are given in Table 1. Lines represent linear regression for all data points in Fig. 4A, and for 10 kg (solid line) and 20 kg (dashes) fish in Fig. 4B. Details for regressions are given in Table 3.

Table 3. Details of linear regressions (T_{vis} duration or magnitude = b • SDA duration or magnitude +a) for correlations between parameters of southern bluefin tuna specific dynamic action (mg kg^{-1}) and postprandial visceral warming (^{o}C h) as recorded in trials described in Table 1.

		n	b	95% C.I.	a	95% C.I.	R^2	P (slope)	Diff. (slope)	Diff. (int.)
Duration	**10 kg/ 19°C**	4	0.6607	-0.8399-2.1613	12.93	-41.34-67.20	0.64	0.19	x	x
	20 kg/ 19°C	4	1.4097	0.7931-2.0264	0.20	-10.96-11.35	0.97	0.01	x	x
	20 kg/ 16°C	6	0.7750	0.4180-1.5082	11.00	-9.37-31.38	0.68	0.04	x	x
	All data	14	0.7568	0.4895-1.0241	10.91	3.36-18.46	0.76	<0.01	-	-
Magnitude	**10 kg/ 19°C**	4	0.0049	0.0019-0.0079	-2.20	-30.85-26.46	0.96	0.02	x	x
	20 kg/ 19°C	4	0.0106	-0.0001-0.0213	-7.23	-40.02-25.56	0.90	0.05	x	xy
	20 kg/ 16°C	6	0.0080	-0.0033-0.0125	6.43	-18.58-31.43	0.85	0.01	x	y
	20 kg combined	10	0.0084	0.0056-0.0111	2.60	-10.23-15.42	0.86	<0.01	-	-

Duration, relationship between duration of SDA and postprandial visceral warming; Magnitude, relationship between SDA and visceral warming magnitude.

n, number of measurements; 95% C.I., 95% Confidence intervals; R^2, regression coefficient; P, probability that regression slope is significantly different from zero; Diff. (slope) and Diff. (int.), significant differences between experiments ($P<0.05$) of linear regression slope and intercept (significant differences of intercepts only analysed when there was no significantly different in regression slopes) by ANOVA with Post Hoc analysis. Different letters (x,y and z) denote significant differences between regressions.

Discussion

The finding of a relationship between the duration and magnitude of postprandial visceral warming and SDA (Fig. 4) is the first empirical documentation of a link between the two parameters. However, it is unlikely that all of the heat by-product of SDA contributes to visceral warming because not all of the physiological processes involved in SDA occur in the organs supplied with blood through visceral *retia mirabilia* (stomach, spleen, caecum and intestines). Processes of SDA that do occur within these tissues include enzyme production and secretion, gut peristalsis, acid secretion, mechanical digestion, and intestinal absorption (McCue, 2006). These pre-absorptive and absorptive processes are likely completed well before that of visceral

warming. Gut evacuation rates of tuna are extremely fast (~10-12 h) (Magnuson, 1969; Schaefer, 1984; Olson and Boggs, 1986), however the visceral temperatures of bluefin can remain elevated for longer than 48 h after feeding. SDA in fish is considered mostly a post-absorptive effect attributed to catabolic events such as amino acid deamination (Beamish and Trippel, 1990; Cho and Kaushik, 1990) or anabolic processes like protein synthesis (Brown and Cameron, 1991b; Brown and Cameron, 1991a; Lyndon et al., 1992). The liver is major tissue site of postprandial catabolism with the majority of tissue anabolism occurring within the white muscle (Carter and Houlihan, 2001; McCue, 2006). Blood supply to the liver or white muscle does not pass through *retia mirabilia* thus they should not accumulate heat (Carey et al., 1984; Fudge and Stevens, 1996). However, the ventral surfaces of the liver of visceral warming tuna species have distinctive patterns of radiating vessels, which may act as crude counter-current heat exchangers (Carey et al., 1984; Fudge and Stevens, 1996). Visceral warming in bluefin tuna may be best explained as a spike of heat production due to heat released during hydrolysis of food and the pre- and absorptive processes of SDA which is maintained within the viscera by *retia mirabilia* and insulated by a partially warm liver which forms a barrier between the viscera and the cool heart, kidney and gills. The finding that peak postprandial T_{vis} corresponds to the gut evacuation time in northern bluefin (Carey et al., 1984) supports this pattern of visceral warming. As the magnitude of pre- and absorptive stage of SDA is likely to be proportional to absolute SDA, it thus explains the relationship between visceral warming and SDA recorded in the present study.

Elevated visceral temperatures (as great as 6.4^{o}C above water temperature) of fasted SBT were observed immediately after introduction to the respirometer (Fig. 1). It is likely that the source of this metabolic heat was metabolic work performed within the red muscles, resulting from physical exertion and stress associated with capture and transfer, which warmed the viscera through thermal conductance. Muscle temperatures of Pacific bluefin, blue marlin (*Makaira nigricans*) and swordfish (*Xiphias gladius*) have been shown to be greatly elevated after capture (Carey and Robinson, 1981; Block et al., 1992; Marcinek et al., 2001). The slope of regression between MR (presumed to equal heat production) and T_{vis} excess of fasted fish was significantly greater than that for postprandial fish (20 kg SBT, $P < 0.01$) which is probably due to the fish being in a non-steady state of heat transfer, with much of the

heat generation occurring before commencement of experiments (during capture and transfer). High T_{vis} excess of fasted fish (maximum 6.4°C), which were only 0.7°C less than maximum T_{vis} excess of postprandial fish (7.1°C), does however suggest a great potential for conductive heat transfer between the muscles and the viscera. SBT swim faster after feeding (Chapter 2) which suggests that conductance of heat from the muscles may contribute to postprandial visceral warming.

At any level of heat production (postprandial MR), 20 kg SBT maintained a T_{vis} excess over 1°C warmer than 10kg fish (Fig. 3B) demonstrating that the larger SBT have greater heat retention ability. This is also true when MR is represented independent of mass, assuming a mass scaling coefficient of 0.88, White and Seymour, 2005). In the same vein, 70 cm Pacific bluefin maintain appreciably greater visceral excess temperatures than 50 cm fish (Kitagawa et al., 2001). Red muscle *retia mirabilia* length and number of vessel rows in juvenile tuna increases with growth which may improve efficiency of heat conservation (Funakoshi et al., 1985; Dickson et al., 2000). Larger tuna also have a lower surface area to volume ratio, thicker belly walls, more subcutaneous fat that acts as an insulation, and greater thermal inertia (Graham and Dickson, 2001). Heat budget models suggest that reduced whole body thermal conductance coefficients results in larger bluefin maintaining greater visceral excess temperatures (Kitagawa et al., 2006). A reduction in thermal inertia may explain why visceral excess temperatures of smaller SBT (10 kg) were only mildly affected by fasted MR (presumed to equal red muscle heat production) (Fig. 2B). Larger tuna warming more extensive regions of their body as a result of greater thermal inertia (Carey et al., 1971; Linthicum and Carey, 1972; Carey et al., 1984), therefore the potential for heat transfer between the muscles and viscera is likely to increase with mass.

Tuna at 16°C maintained visceral excess temperatures significantly warmer (0.5°C warmer) at any level of heat production (postprandial MR) than similar sized fish at 19°C (Fig. 3B). This suggests SBT have an ability to control thermal conductance and thus to physiologically regulate T_{vis}. The ability of tuna to physiologically control heat balance of muscles has been previously recorded. In tank studies, yellowfin (*Thunnus albacares*) and skipjack tuna (*Katsuwonus pelamis*) muscle excess temperatures were not related to swimming speed (assumed to equal red muscle heat production, (Dizon et al., 1978; Dizon and Brill, 1979b; Dizon and Brill, 1979a). Restrained albacore tuna can alter red muscle thermal conductance and

reduce their rate of cooling (Graham and Dickson, 1981). Yellowfin tuna swimming at constant velocities within a large water tunnel alter their muscle thermal rate coefficient, dependant on both T_w and the magnitude and direction of T_w change (Dewar et al., 1994). It is hypothesized that this enables yellowfin to suppress heat loss during descents into cool waters and increase heat gain when back in warmer surface waters (Dewar et al., 1994), a pattern observed in telemetry studies with bigeye tuna (Holland et al., 1992). Although the mechanisms involved are not completely understood, it is likely achieved by modulating blood flow through *retia*, thereby adjusting heat-exchange efficiency (Graham and Dickson, 2001). *Rete* arterial and venule walls have layers of smooth muscle which may provide a mechanism to regulate blood flow (Graham and Dickson, 2001). The observed greater heat retention of SBT at 16°C was probably to maintain preferably warm T_{vis}. Long term physiological reductions in thermal conductance coefficients are likely to be instrumental for the ability of bluefin to maintain prolonged visceral excess temperatures, which have been recorded as high as 15°C for several months (Block et al., 2005).

One of the primary functions of visceral warming may be as an added source of heat to maintain elevated core and muscle temperatures to improve thermoregulatory capability. As heat generated within the muscle can warm the viscera through thermal conductance, it is reasonable to assume that, given the opposite thermal gradient, heat generated within the viscera would warm the muscles. The ability to accumulate some of the heat by-product of SDA within the viscera likely provides a substantial source of heat that is not available to non-visceral warming species. The magnitude of SDA in SBT is twice that of other fish species examined, attributing to 35% of ingested energy (Chapter 2). Postprandial visceral warming during foraging dives below the thermocline would provide heat when the thermoregulatory challenge is most severe and may be critical in allowing tuna to dive deeper and forage longer. Unfortunately there have been few measurements of the muscle temperature of free swimming tuna and they have been insufficient in duration and corresponding data to decipher the effects of feeding-related heat production (Carey and Lawson, 1973; Holland et al., 1992; Marcinek et al., 2001). In one study the muscle temperature of impounded giant Atlantic bluefin (>400 kg) were compared to the T_{vis} of another tuna within the same pound (Stevens et al., 2000). Apart from immediately following ingestion of partially frozen baitfish, T_{vis} exceeded muscle

temperature by 4 to 5°C. Assuming thermal conductance between the tissues, accumulated heat within the viscera should have warmed the muscles. However, fluctuations in T_{vis} were not reflected in the muscles which led these authors to propose that giant bluefin actively regulate muscle temperature (Stevens et al., 2000). The stability of muscle temperature could also be attributed to the great thermal inertia of these very large animals. Clearly more data, covering a range of fish sizes, is required to evaluate the relationship between heat accumulated within the viscera and muscles.

6. Metabolic scope, swimming performance and the effects of hypoxia in the mulloway, *Argyrosomus japonicus* (Pisces: Sciaenidae)

Abstract

The culture of the mulloway (*Argyrosomus japonicus)*, like many other Sciaenidae fishes, is rapidly expanding. However there is no information on their metabolic physiology. In this study, the effects of various hypoxia levels on the swimming performance and metabolic scope of juvenile mulloway (0.34 ± 0.01 kg, mean ± SE, n = 30) was investigated (water temperature = 22°C). In normoxic conditions (dissolved oxygen = 6.85 mg l^{-1}), mulloway oxygen consumption rate ($\dot{M}O_2$) increased exponentially with swimming speed to a maximum velocity (U_{crit}) of 1.7 ± <0.1 body lengths s^{-1} (BL s^{-1}) (n = 6). Mulloway standard metabolic rate (SMR) was typical for non-tuna fishes (73 ± 8 mg kg^{-1} h^{-1}) and they had a moderate scope for aerobic metabolism (5 times the SMR). Mulloway minimum gross cost of transport ($GCOT_{min}$, 0.14 ± 0.01 mg kg^{-1} m^{-1}) and optimum swimming velocity (U_{opt}, 1.3 ± 0.2 BL s^{-1}) were comparable to many other sub-carangiform fish species. Energy expenditure was minimised when swimming between 0.3 and 0.5 BL s^{-1}. The critical dissolved oxygen level was 1.80 mg l^{-1} for mulloway swimming at 0.9 BL s^{-1}. This reveals that mulloway are well adapted to hypoxia, which is probably adaptive from their natural early life history within estuaries. In all levels of hypoxia (75% saturation = 5.23, 50% = 3.64, and 25% = 1.86 mg l^{-1}), $\dot{M}O_2$ increased linearly with swimming speed and active metabolic rate (AMR) was reduced (218 ± 17, 202 ± 14 and 175 ± 10 mg kg^{-1} h^{-1} for 75%, 50% and 25% saturation respectively). However, U_{crit} was only reduced at 50% and 25% saturation (1.4 ± <0.1 and 1.4 ± <0.1 BL s^{-1} respectively). This demonstrates that although mild hypoxia (75% saturation) reduced their metabolic capacity, mulloway are able to compensate to maintain swimming performance. $GCOT_{min}$ (0.09 ± 0.01 mg kg^{-1} m^{-1}) and U_{opt} (0.8 ± 0.1 BL s^{-1}) were significantly reduced at 25% dissolved oxygen saturation. As mulloway

metabolic scope was significantly reduced at all hypoxia levels, it suggests that even mild hypoxia may reduce growth productivity.

Introduction

The mulloway (*Argyrosomus japonicus,* formerly *A. hololepidotus)* is a large sciaenid (maximum size 75 kg) that has recently become an aquaculture species (Battaglene and Talbot, 1994; Fielder and Bardsley, 1999; Hecht and Mperdempes, 2001). The mulloway is naturally distributed in the coastal waters of the Indian and western Pacific oceans and has long been an important fishery species in Australia and South Africa (Griffiths and Heemstra, 1995; Griffiths, 1997b). Juvenile mulloway reside in estuaries, whilst adults move close offshore and to surrounding surf zones (Gray and McDonal, 1993; Griffiths, 1996; Griffiths, 1997b; Griffiths, 1997a). Attributes that make mulloway suitable for aquaculture include their high price, marketability, high fecundity, fast growth, non-territorial non-cannibalistic nature, and wide salinity tolerance. These make mulloway appropriate for both marine cage and on-land saline pond culture.

There is no metabolic information on the mulloway and little on other Sciaenidae fishes. This is despite sciaenid species becoming increasingly important to aquaculture world wide (Thomas et al., 1996; Drawbridge, 2001; Holt, 2001). In fact, sciaenid fishes are now the major fish species for artificial propagation in the world's leading producer of aquaculture products, China (Hong and Zhang, 2003). Consequently, there is a lack of precise metabolic data for calculations of aquaculture system oxygen requirements, fish energy requirements, environmental impact assessments and species-specific physiological thresholds.

Metabolism is the physiological engine that powers all activities such as swimming, growth and reproduction (Neill et al., 1994). The potential power that this engine can generate is determined by the aerobic metabolic scope, the difference between metabolic rate at the maximum sustained swimming speed (active metabolic rate; AMR) and the fish's minimum metabolic rate (standard metabolic rate; SMR). The greater the aerobic scope, the greater the potential for growth (Fry, 1971). Maintaining culture conditions for optimum metabolic scope will result in optimum potential productivity. For this reason, metabolic scope is considered to be an integral

correlate of environmental quality for aquaculture (Neill and Bryan, 1991). The environment influences the activity of an organism through its metabolism. Dissolved oxygen is considered to be a limiting factor, setting the upper limit of aerobic metabolism thus defining the metabolic scope (Fry, 1971). With fish, a decrease of dissolved oxygen results in the reduction of metabolic scope (Fry, 1971; Claireaux et al., 2000; Jordan and Steffensen, 2007). Although the effects of reduced dissolved oxygen may not be acutely apparent, prolonged exposure can result in reduced growth performance (Priede, 1985).

The following study aims to define some of the metabolic parameters of the mulloway and considers how these interact with intensive aquaculture conditions. In particular, it examines the relationship between swimming velocity and metabolic rate ($\dot{M}O_2$) and determine the cost of transport (COT), SMR, AMR, metabolic scope, and critical swimming velocity (U_{crit}). Furthermore, it examines the effects of hypoxia on these parameters with the purpose of determining not just the minimum oxygen requirements, but also the potential production-limiting effects of hypoxia through modulation of the metabolic scope.

Methods

Experimental animals

Thirty juvenile mulloway (0.34 ± 0.08 kg, Table 1) were randomly selected from a 10,000 l, flow-through (3 mm gravel filtered seawater) stock tanks at the South Australian Research and Development Institute, Aquatic Sciences, Adelaide facility. These fish had been raised on-site from fertilized eggs supplied by Clean Seas Pty. Ltd., commercial marine finfish hatchery (Spencer Gulf, South Australia). Fish were maintained at ambient light and water temperature. All experimental trials were conducted between December 2004 and March 2005 when ambient water temperatures remained between 21 and 23°C. Fish were fed commercial marine diet (Nova, Skretting, Hobart, Australia) to satiation once a day but were starved for a minimum of 36 h before the beginning of all experimental trials.

For each trial, individual fish were scoop-netted from the stock tank and immediately transferred into a 2,000 l fish transport container filled with seawater and

carried 13 km to the University of Adelaide campus where all respiratory trials were conducted. Seawater from the transport container was used to gravity fill the respirometer, and the fish were introduced. Each fish was introduced into the respirometer late in the afternoon and then left overnight to acclimate for a minimum of 16 h. During the acclimation period, water quality was maintained by continuous water exchange (~10% h^{-1}), gently bubbled air maintained dissolved oxygen above 7 mg l^{-1}, and water flow velocity set at 7.5 cm s^{-1} (~0.25 body lengths s^{-1}, BL s^{-1}) facilitated steady swimming and respirometer mixing. Following experimental trials, fish were anaesthetized in 0.05 mg l^{-1} clove oil, and body length (BL) and body mass (M_b) recorded.

Experimental apparatus

All respiratory trials were conducted in a large (850 l) water tunnel respirometer. The respirometer was predominantly constructed from acrylic plastic and large diameter unplasticised polyvinyl chloride storm-water pipe. Water flow was driven by a single 20 cm diameter propeller powered by a 1.5 kW electric motor (CMG, www.cmggroup.com) with variable speed controller (Nord AC, www.nord.com). Maximum water flow velocity was 80 cm s^{-1}. Circulating water passed through parallel tubes (1 cm internal diameter × 25 cm long) to induce laminar flow before entry into the working section. Laminar flow was verified by video observation of neutrally buoyant particles (wet cotton-wool) drifting through the working section with a high-speed video system (MotionScope PCI, Redlake, www.redlake.com). The working section was 100 × 40 × 40 cm (length × width × height) however, fish were restricted from within 8 cm of the walls by a rigid metal wire cage (3 cm stay and line wire spacing), to reduce wall effects. Water velocity calibrations were made with a acoustic doppler velocimeter (Sontek ADV, www.sontek.com) (mean 25hz for 10 s), from 10 positions (five horizontal × two vertical intervals) in the active working section. The coefficient of variation between mean flow velocity and sample position was found to be low (<6.1%, n = 10).

Fish were introduced into the working section through a hinged hatch, which was sealed immediately, and the up-stream half covered to limit visual disturbance. Fish generally maintained position within the forward shaded half of the working

section, only falling back to the visible rear section when unable to maintain position against the water current. Dissolved oxygen was manipulated by bubbling air, nitrogen, or oxygen through a large air stone when $\dot{M}O_2$ measurements were not being made. Trapped gas was bled from the system through twelve bleeder valves positioned in the respirometer roof. Water quality was maintained by a partial water exchange (~5%) between experimental measurements. An external water circuit powered with a 2.4 kW pump circulated respirometer water through a 4 kW chiller and 500 W heater element that maintained respirometer water temperature via a digital controller unit (Carel IR series, www.carel.com). During all experiments, respirometer water temperatures were maintained between 21.8 and 22.2°C.

Dissolved oxygen measurement

Respirometer water was continually sampled and replaced by a peristaltic pump (Ismatic SA Vario, Cole-Parmer, www.coleparmer.com) and temperature-adjusted dissolved oxygen (mg l^{-1}) logged every 10 s by an oxygen optode (Microx TX3, PreSens GmbH, www.presens.de). The optode was calibrated at the start of each trial according to the manufacturer's instructions, and calibration checked at the end of the trial. Meter drift was never found to be significant. Recorded dissolved oxygen was later adjusted for salinity, assuming an oxygen solubility of 7.20 mg l^{-1} in oceanic seawater (salinity of 35 ‰) at 22°C. A minimum of 15 min at any velocity was required to obtain a reliable oxygen consumption measurement.

Experimental protocols

For all treatments, $\dot{M}O_2$ was recorded over a 1 h period before either the swimming velocity or dissolved oxygen was changed and the next swim trial begun. This would continue until the critical swimming velocity (U_{crit}) was reached and the experiment terminated. The U_{crit} was determined when the fish resorted to burst and glide swimming and brushed its tail up against the back screen more than three times in 30 s, and it was calculated according to the equation (Brett, 1964):

$$U_{crit} = U_c + (T_f \; T_i^{-1}) \bullet U_i \qquad (1)$$

where U_c is the last speed at which the fish swam the entire 1 h period, T_f is the time the fish swam at the final speed, T_i is the time interval at each speed (1 h), and U_i is the velocity increment (7.5 cm s^{-1}).

At the end of the experiment the fish was removed from the respirometer and background respiration measured. Background trials were conducted at the same dissolved oxygen level as the pertinent respiratory trial to account for any changes in diffusion. Although background respiration was found to be small, all fish $\dot{M}O_2$ recordings were corrected accordingly.

Hypoxia levels (100%, 75%, 50%, and 25% dissolved oxygen saturation): Beginning at a swimming velocity of 7.5 cm s^{-1}, $\dot{M}O_2$ was recorded for a period of 1 h, before the swimming velocity was increased in a stepwise fashion by 7.5 cm s^{-1} for periods of 1 h until U_{crit} was reached. This was performed at ~100% saturation (mean ± SE trial start dissolved oxygen level = 6.85 ± 0.02 mg l^{-1}), ~75% (5.23 ± 0.02 mg l^{-1}), ~50% (3.64 ± 0.03 mg l^{-1}), and ~25% (1.86 ± 0.02 mg l^{-1}) (n = 47, 44, 36 and 37, respectively) (Table 1). Swim trials at each dissolved oxygen level were replicated with six fish.

Routine critical dissolved oxygen level (R_{crit}): At a constant water flow velocity of 30.0 cm s^{-1} (0.9 BL s^{-1}), $\dot{M}O_2$ was recorded over periods of one hour whilst dissolved oxygen was reduced at the end of each hour. Trials began at ~50% (3.60 mg l^{-1}) and progressively stepped down to ~40% (2.92 mg l^{-1}), ~30% (2.19 mg l^{-1}), ~25% (1.82 mg l^{-1}), ~20% (1.46 mg l^{-1}), ~15% (1.11 mg l^{-1}), ~12.5% (0.91 mg l^{-1}) and finally lowered to approximately ~10% saturation (0.73 mg l^{-1}) (Table 1). This trial was replicated with six fish.

Table 1. Fish mass (M_b) and body length (BL), trial start point dissolved oxygen (DO), swim velocity range (U) achieved by mulloway swimming at 22°C at progressively greater velocities and in the routine critical dissolved oxygen level experiment (R_{crit})

	M_b(kg)	BL (cm)	DO (mg l^{-1})	U (cm s^{-1})
100%	0.39 ± 0.02	31.5 ± 0.5	6.85 ± 0.02	7.5-60.0
75%	0.34 ± 0.01	32.0 ± 0.3	5.23 ± 0.02	7.5-52.5
50%	0.36 ± 0.03	32.4 ± 0.9	3.64 ± 0.02	7.5–52.5
25%	0.33 ± 0.01	31.6 ± 0.4	1.86 ± 0.02	7.5-45.0
R_{crit}	0.36 ± 0.01	32.1 ± 0.4	3.60-0.73	30.0

Values are means ± SE, n = 6 for M_b and BL, n = 47-36 for DO

Data analysis

Linear regressions were applied to dissolved oxygen recordings and fish $\dot{M}O_2$ determined by the following equation:

$$\dot{M}O_2 \text{ (mg kg}^{-1} \text{ h}^{-1}) = (\Delta DO_t - \Delta DO_b) \bullet V M_b^{-1} \quad (2)$$

Where ΔDO_t is the trial drop in respirometer dissolved oxygen (mg l^{-1} h^{-1}), ΔDO_b is the background respiration rate for the trial (mg l^{-1} h^{-1}), V is the respirometer volume (l) and M_b the fish mass (kg). The relationship between swimming velocity and $\dot{M}O_2$ was described as either a linear or exponential function by the following equations:

$$\dot{M}O_2 \text{ (linear)} = b \bullet U + a \quad (3)$$

and:

$$\dot{M}O_2 \text{ (exponential)} = a \bullet e^{bU} \quad (4)$$

Where a and b are constants and U the swimming velocity (cm s^{-1}).

Standard metabolic rate (SMR) was determined by extrapolation of the $\dot{M}O_2$ exponential relationship back to a swimming velocity of 0 cm s^{-1}, corresponding to the constant a in equation 4.

Net cost of transport (NCOT) was determined by first subtracting the SMR from each $\dot{M}O_2$, dividing this by the U at which it was obtained, and plotting the results as a function of U (BL s^{-1}).

Gross cost of transport (GCOT) was determined by dividing each $\dot{M}O_2$ by U (BL s^{-1}) at which it was obtained, and fitting a nonlinear regression according to the equation:

$$GCOT\ (mg\ kg^{-1}\ m^{-1}) = a \bullet e^{bU} U^{-1} \quad (5)$$

The optimum swimming speed (U_{opt}) was determined as where the first derivative of the GCOT equation equaled zero according the equation;

$$U_{opt} = 1\ b^{-1} \quad (6)$$

Minimum gross and net cost of transport ($GCOT_{min}$ and $NCOT_{min}$, respectively) were determined by inserting U_{opt} into their regression equations.

Routine critical dissolved oxygen level (R_{crit}) was determined as the intercept of two linear regressions applied to the $\dot{M}O_2$ data. One regression was applied to $\dot{M}O_2$ data within one standard deviation of the mean $\dot{M}O_2$ recorded at U = 30 cm s^{-1} in the normoxia and hypoxia level experiments. A second linear regression was applied to data $\dot{M}O_2$ that fell below one standard deviation.

Statistical differences were determined from Analysis of Variance with Tukey Post-Hoc analysis (P = <0.05) performed using StatistiXL.com software. Values are mean ± standard error of the means (SE).

Results

Hypoxia levels experiment

In normoxia (100% saturation), an exponential regression best described the relationship between swimming velocity and $\dot{M}O_2$ (R^2 = 0.86) (Table 2, Fig. 1a). However in all levels of hypoxia, linear regressions best fit the relationship (R^2 = 0.82, 0.65, 0.85 for 75%, 50% and 25% dissolved oxygen saturation, respectively).

Table 2. Regression details describing the relationship between swimming velocity (U), and oxygen consumption ($\dot{M}O_2$, represented as both an exponential (Exp.) and linear relationship), net cost of transport (NCOT, also represented as both an exponential (Exp.) and linear relationship), and gross cost of transport (GCOT).

	$\dot{M}O_2$ (Exp.)	R^2	$\dot{M}O_2$ (linear)	R^2	NCOT ($mg\ kg^{-1}\ m^{-1}$) (Exp.)	R^2	NCOT ($mg\ kg^{-1}\ m^{-1}$) (linear)	R^2	GCOT ($mg\ kg^{-1}\ m^{-1}$)	R^2
100%	$70.9e^{0.027U}$	0.86	5.19U+27.0	0.84	$0.05e^{0.48U}$	0.31	0.04U+0.06	0.28	$0.07e^{0.79U}U^{-1}$	0.94
75%	$54.1e^{0.026U}$	0.75	3.19U+36.4	0.82	$0.04e^{0.47U}$	0.33	0.03U+0.04	0.34	$0.05e^{0.90U}U^{-1}$	0.94
50%	$38.9e^{0.038U}$	0.58	3.76U+26.6	0.65	$0.02e^{1.21U}$	0.29	0.05U+0.04	0.30	$0.04e^{1.15U}U^{-1}$	0.82
25%	$25.8e^{0.045U}$	0.75	3.67U+3.8	0.85	$0.03e^{0.88U}$	0.40	0.05U+0.03	0.49	$0.03e^{1.23U}U^{-1}$	0.91

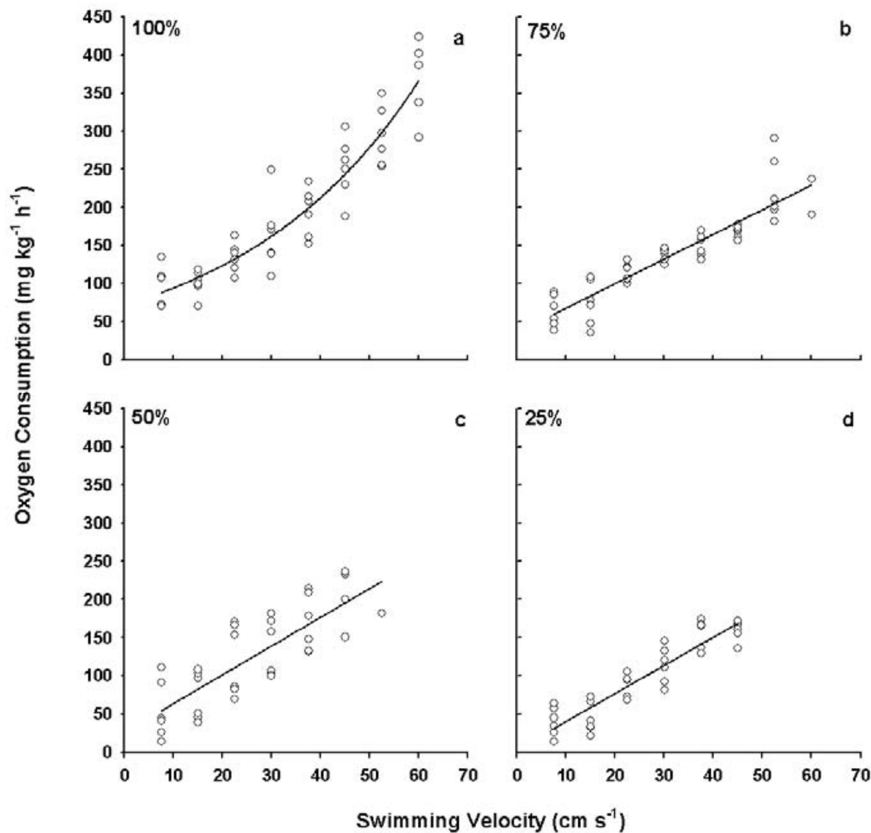

Fig. 1. The relationship between oxygen consumption rate and swimming velocity of mulloway (n = 6) at 22°C when swum at progressively increasing velocities (interval of 7.5 cm s^{-1}) for 1 h periods at dissolved oxygen levels of **a)** 6.85 mg l^{-1} (100%), **b)** 5.23 mg l^{-1} (75%), **c)** 3.64 mg l^{-1} (50%) and **d)** 1.86 mg l^{-1} (25%). Solid lines are either an exponential or linear regressions. Details of regression are given in Table 2.

Standard metabolic rate (determined by extrapolating the $\dot{M}O_2$ exponential relationship back to a swimming velocity of 0.0 cm s^{-1}) at 25% saturation (29 ± 6 mg kg^{-1} h^{-1}) was significantly less than in normoxia (73 ± 8 mg kg^{-1} h^{-1}) (Table 3). Metabolic rate at the maximum swimming velocity (AMR) in normoxia was 365 ± 20 mg kg^{-1} h^{-1}, which equates to a five times the SMR. AMR in all levels of hypoxia was significantly less than in normoxia. Metabolic scope was also significantly reduced in all levels of hypoxia. Maximum sustainable swimming velocity (U_{crit}) at 75% saturation was the same as in normoxia, however was significantly reduced at 50 and 25% saturation

Table 3. Standard metabolic rate (SMR), active metabolic rate (ARM), metabolic scope (Scope), critical swimming velocity (U_{crit}), optimum swimming speed (U_{opt}), minimum cost of transport ($GCOT_{min}$) and net cost of transport (NCOT) at U_{opt} of mulloway swimming at 100, 75, 50 and 25% dissolved oxygen saturation at 22°C.

	100%	**75%**	**50%**	**25%**
SMR (mg kg^{-1} h^{-1})	73 ± 8[a]	55 ± 7[ab]	45 ± 13[ab]	29 ± 6[b]
AMR (mg kg^{-1} h^{-1})	365 ± 20[a]	218 ± 17[b]	202 ± 14[b]	175 ± 10[b]
Scope (mg kg^{-1} h^{-1})	292 ± 18[a]	209 ± 16[b]	153 ± 9[c]	150 ± 11[c]
U_{crit} (BL s^{-1})	1.7 ± <0.1[a]	1.7 ± 0.1[a]	1.4 ± <0.1[b]	1.4 ± <0.1[b]
U_{opt} (BL s^{-1})	1.3 ± 0.2[a]	1.1 ± 0.1[a]	0.9 ± 0.1[a]	0.8 ± 0.1[a]
$GCOT_{min}$ (mg kg^{-1} m^{-1})	0.14 ± 0.01[a]	0.11 ± 0.01[ab]	0.11 ± 0.01[ab]	0.09 ± 0.01[b]
NCOT at U_{opt}	0.09 ± 0.01[a]	0.07 ± 0.01[ab]	0.07 ± 0.01[ab]	0.06 ± 0.01[b]

Values are means ±SE (n=6). Differences in letter superscripts indicate significant differences ($P<0.05$)

Similar to $\dot{M}O_2$, NCOT was best described by an exponential relationship in normoxia but by linear relationships at all hypoxic levels (Fig. 2a-d, Table 2). In normoxia and all levels of hypoxia, GCOT displayed a shallow U-shaped function with swimming speed (Fig. 2e-f). In normoxia, the minimum GCOT ($GCOT_{min}$) was 0.14 mg kg^{-1} m^{-1} corresponding to an optimal swimming velocity (U_{opt}) of 1.3 BL s^{-1} (Table 2). Optimal swimming velocity appeared to reduce with hypoxia level, but this effect did not prove to be significant. Minimum GCOT at 25% saturation was however, significantly less than in normoxia. This effect was not due to the reduction in SMR, as the NCOT at the U_{opt} was also significantly reduced at 25% saturation.

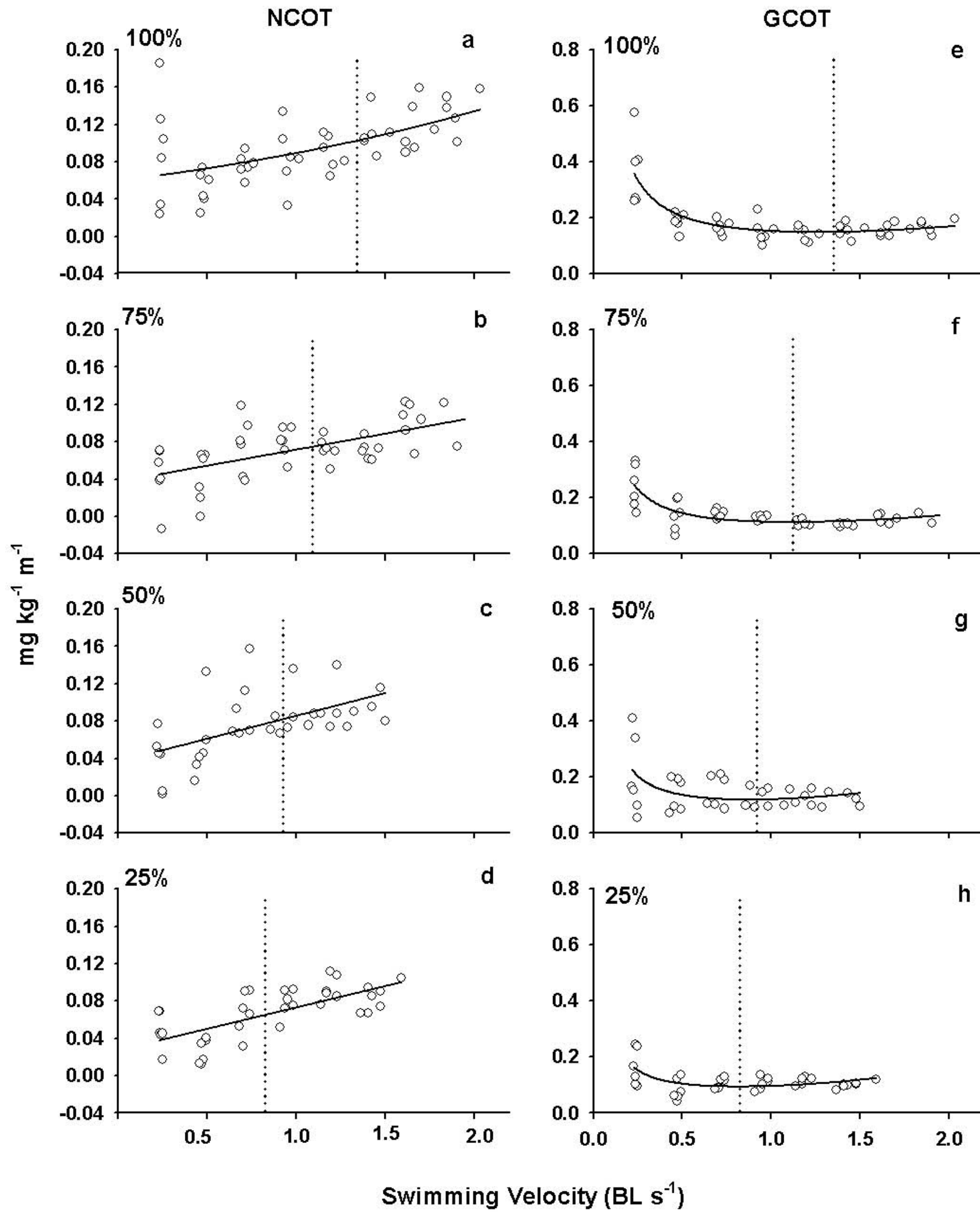

Fig. 2. Net cost of transport (NCOT) and gross cost of transport (GCOT) plotted versus swimming velocity (U) for mulloway (n = 6) swum at **a** and **e)** 100%, **b** and **f)** 75%, **c** and **g)** 50%, and **d** and **h)** 25% dissolved oxygen saturation, respectively (details are as for figure 1). Solid lines are regression. Details of regression are given in Table 2. Vertical dotted line indicates the swimming velocity that GCOT is minimum ($GCOT_{min}$), where this line crosses the GCOT curve equates to the optimum swimming velocity (U_{opt}).

Routine critical dissolved oxygen level (R_{crit}) experiment

$\dot{M}O_2$ of mulloway swimming at 0.9 BL s^{-1} was not significantly influenced by reductions in dissolved oxygen until the R_{crit} was reached at ~1.8 mg l^{-1} or ~25% saturation (Fig. 3). Above this dissolved oxygen level, $\dot{M}O_2$ remained within a standard deviation of that recorded with mulloway swimming at 0.9 BL s^{-1} in the normoxia and other hypoxia experiments. However, below 1.8 mg l^{-1}, $\dot{M}O_2$ fell sharply. One fish lost the ability to sustain swimming at 0.91 mg l^{-1} (~12.5% saturation), the remaining five fish at 0.73 mg l^{-1}.

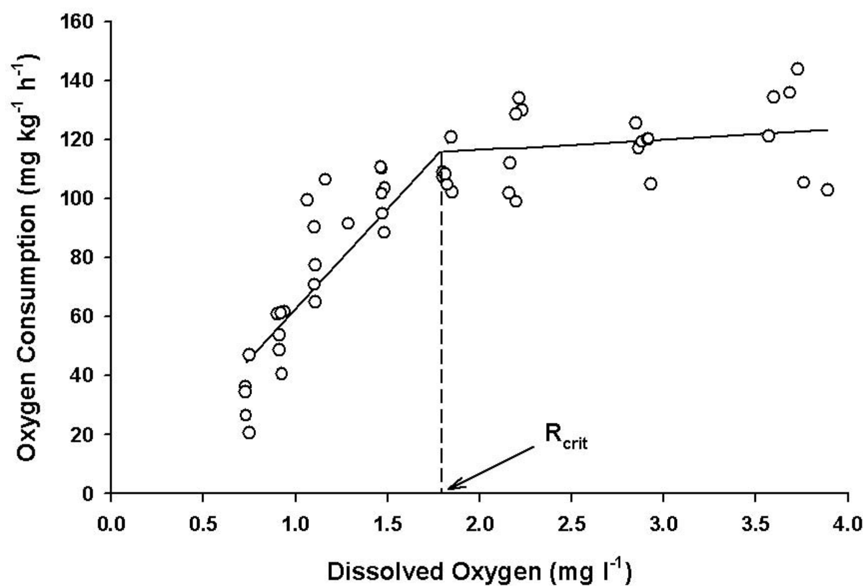

Fig. 3. Oxygen consumption rate of mulloway (n = 6) swimming at 30 cm s^{-1} for 1 h periods at progressively lower dissolved oxygen levels between 3.60 and 0.73 mg l^{-1}. The dotted line indicates the determined critical oxygen level (R_{crit}).

Discussion

Normoxic interspecific comparison

The SMR of most teleost fishes appears to be similarly related to body size with a allometric mass scaling exponent between 0.8 and 0.88 (Brett and Glass, 1973; Clarke and Johnston, 1999; White and Seymour, 2005). The exceptions to this are the high

performance fishes such as tunas and dolphin fish (*Coryphaena hippurus*), whose SMRs are considerably elevated when compared to most other fish species (Brill, 1979; Brill, 1987; Dewar and Graham, 1994; Benetti et al., 1995). The elevated SMRs of these fish are associated with energetically expensive adaptations that support their great aerobic scope potential (Bushnell and Jones, 1994). The SMR of mulloway appears to be typical for non-tuna fishes (Fig. 4), suggesting they lack the adaptations associated with the high performance fishes.

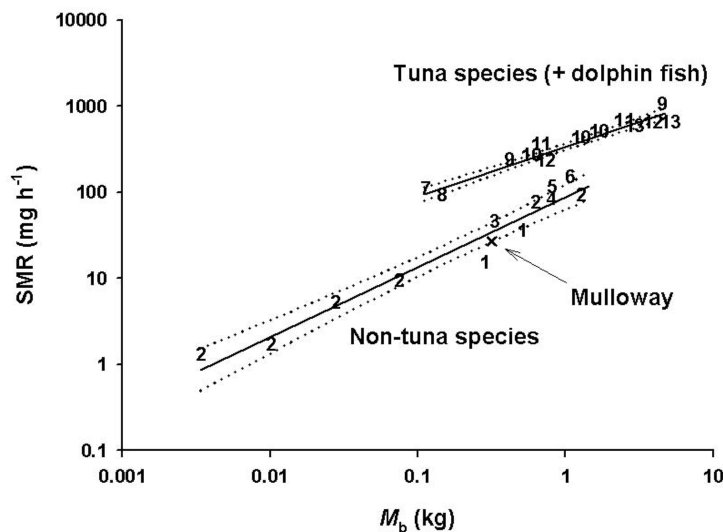

Fig. 4. Intraspecific comparison of the relationship between standard metabolic rate (SMR) and body mass (M_b) of non-tuna species ([1]rainbow trout, *Oncorhynchus mykiss* (Brill, 1987), [2]sockeye salmon, *Oncorhynchus nerka* (Brett, 1965), [3]Atlantic cod, *Gadus morhua* (Schurmann and Steffensen, 1997), [4]yellowtail kingfish, *Seriola lalandi* (Fitzgibbon, unpublished data), [5]Yellowtail, *Seriola quinqueradiata* (Yamamoto and Itazawa, 1981), [6]Pacific bonito, *Sarda chiliensis* (Sepulveda et al., 2003)); ($SMR = 85 \bullet M_b^{0.81}$) and, tuna and dolphin fish ([7]Yellowfin tuna, *Thunnus albacares* (Sepulveda and Dickson, 2000), [8]Kawakawa, *Euthynnus affinis* (Sepulveda and Dickson, 2000), [9]skipjack tuna, *Katsuwonus pelamis* (Brill, 1979), [10]Yellowfin tuna (Dewar and Graham, 1994), [11]Kawakawa (Brill, 1979), [12]yellowfin tuna (Brill, 1979), [13]Dolphin fish, *Coryphaena hippurus* (Benetti et al., 1995)); $SMR = 342 \bullet M_b^{0.57}$. With mulloway SMR (×) included for comparison. All SMR have been normalized to a study temperature of 25°C with a $Q_{10} = 1.65$ (White and Seymour, 2005). Solid line is least square linear regression with corresponding 95% confidence intervals (dotted line).

The maximum sustained swimming speed (U_{crit}) and corresponding AMR ranges widely in fish and is representative of a diverse locomotory and power (metabolic scope) potential. Although the U_{crit} has been widely measured in fishes, interspecific comparisons are problematic because it can vary depending on testing protocol (principally velocity increment and the amount of time spent at each speed) (Bushnell et al., 1984). Furthermore, body mass/length, study temperature, population and life history can affect the maximum sustainable swimming velocity of individuals within a species (McDonald et al., 1998; Gallaugher et al., 2001; Lee et al., 2003b; Rodnich et al., 2004). Considering the mulloway examined here were cultured fish and the duration of swimming interval was long (both of which are likely

to reduce U_{crit}), they appear to have moderate swimming potential, similar to that of rainbow trout and Atlantic cod (Table 4). Anatomical adaptations that support swimming performance include large, wedge-shaped caudal fins and a deep caudal peduncle (Griffiths, 1997a). Similarly, the metabolic scope of mulloway is significantly less than that of tuna and other highly active teleosts (i.e., sockeye salmon (*Oncorhynchus nerka*) and yellowtail kingfish (*Seriola lalandi*)) but comparable to that of the rainbow trout (*Oncorhynchus mykiss*) and Atlantic cod (*Gadus morhua*) (Table 4).

Table 4. Critical swimming velocity (U_{crit}), and metabolic scope (active metabolic rate minus the standard metabolic rate) of the mulloway compared to other teleosts.

Common Name	M_b (kg)	T (°C)	RC	Duration (h)	U_{crit} (BL s^{-1})	Scope*(mg $kg^{-0.88}$ h^{-1})
Mulloway[1]	0.34	22	cultured	1	1.7	290
Yellowfin tuna[2]	2.1	25	wild	-	-	2451
Kawakawa[3]	0.024-0.255	24	wild	0.5	3.4-5.1	-
Chub mackerel[4]	0.26-0.144	24	wild	0.5	3.8-5.8	-
Sockeye salmon[5]	0.746	15	cultured	1.0	3.0	1050
Yellowtail kingfish[6]	2.1	20	cultured	1.0	2.3	790
Saithe[7]	0.485	10	wild	0.5	2.5	480
Rainbow trout[8]	0.300	15	cultured	1.0	1.9	337
Atlantic cod[9]	0.298	15	wild	0.5	1.9	249
European sea bass[10]	0.147	22	cultured	0.5	-	134
Whiting[11]	0.297	10	wild	0.5	2.1	337

M_b = body mass, T = study temperature, RC = rearing condition, duration = time fish swam at each velocity.

[1]*Argyrosomus japonicus*, (Present study), [2]*Thunnus albacaresi*, (Korsmeyer et al., 1996), [3]*Euthynnus affinis* (Sepulveda and Dickson, 2000), [4]Scomber japonicus (Sepulveda and Dickson, 2000), [5]*Oncorhynchus nerka (Brett, 1965),* [6]*Seriola lalandi* (Clark and Seymour, 2006), [7]*Pollachius virens* (Steinhausen et al., 2005), [8]*Oncorhynchus mykiss* (Bushnell et al., 1984), [9]*Gadus morhua* (Schurmann and Steffensen, 1997), [10]*Dicentrarchus labrax* (Claireaux et al., 2006), , [11]Merlangius merlangus (Steinhausen et al., 2005)

*mass independent assuming a mass scaling exponent of 0.88 (Brett and Glass, 1973; Clarke and Johnston, 1999) and normalized study temperature of 25°C with a Q_{10} = 1.65 (White and Seymour, 2005)

GCOT is the energy required to travel a unit distance. When plotted versus swimming speed (U), it typically results in a U-shaped function. GCOT is high at low swimming speeds due to the SMR contributing relatively more to total $\dot{M}O_2$, and increases after the U_{opt} due to the increase in hydrodynamic resistance with U. The mulloway GCOT displays a shallow U-shaped relationship with U (Fig. 2a-d), that is GCOT increases only slightly after the U_{opt}. The lack of a significant increase in

GCOT following U_{opt} may indicate that mulloway have partially resorted to anaerobic metabolism to support swimming performance at higher velocities. Indeed, Lee et al., (2003a) suggest that anaerobic swimming in sockeye and coho salmon (*Oncorhynchus kisutch*) begins at approximately 60% of U_{crit} and energetic cost of anaerobic swimming greatly contributes to the GCOT above this velocity. The U_{opt} and $GCOT_{min}$ serve as valuable indices to compare swimming efficiency between species. Videler (1993) collated the GCOT (J N^{-1} m^{-1}) and U_{opt} for fourteen sub-carangiform teleosts and found both to be allometrically related with body mass. When the mulloway U_{opt} (1.3 Bl s^{-1}) and $GCOT_{min}$ (when converted into common units, 0.21 J N^{-1} m^{-1}) are included in this comparison, they fall neatly within the 95% confidence limits suggesting their swimming efficiency is typical of sub-carangiform swimming teleosts (Fig. 5).

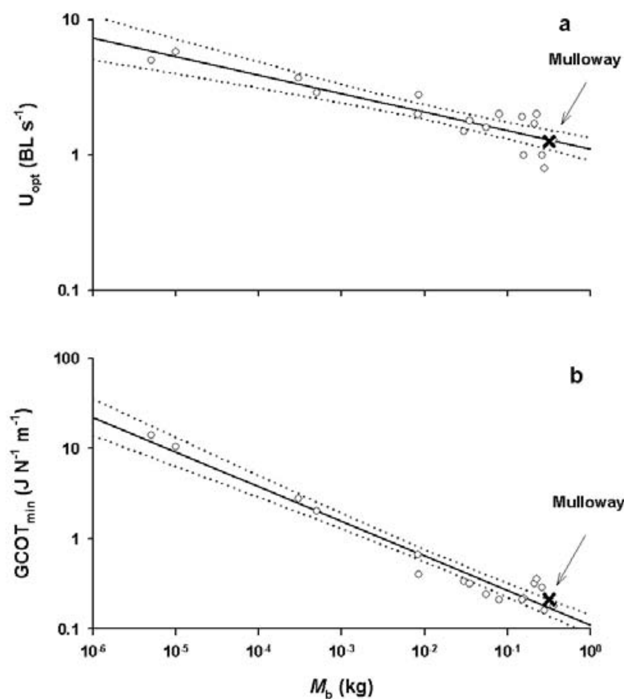

Fig. 5. The allometric relationship between body mass (M_b) and **a)** optimum swimming speed ($U_{opt} = 1.1 \bullet M_b^{-0.14}$), and **b)** Minimum gross cost of transport ($GCOT_{min} = 0.11 \bullet M_b^{-0.38}$) for fourteen fish species collated by Videler (1993) with mulloway (×) included for comparison. Solid line is least square linear regression with corresponding 95% confidence intervals (dotted line).

For some species U_{opt} proves to be a good indication of routine swimming velocity (Videler, 1993; Dewar and Graham, 1994; Tanaka et al., 2001; Lowe, 2002) however, this is likely only true with species where covering long distances is required for optimal prey search or during migration. For more sedentary species, routine swimming speed is often considerably less than the U_{opt} (Steinhausen et al.,

2005). Juvenile mulloway (<100 cm) in their natal estuaries rarely move great distances, however adults they make coastal migrations (Gray and McDonal, 1993; Griffiths, 1996). In South Africa, a large proportion of the adult population participates in a long distance (~1000 km) migration to spawn (Griffiths, 1996) and swimming close to the U_{opt} would minimize the energetic cost, thereby conserving energy for gonad development and spawning behaviour. In culture conditions however, GCOT has arguably little relevance as fish have no requirement to travel for feeding or migration. Instead, it is desirable to minimize the absolute energetic cost of swimming. As with most fish species, an exponential increase best describes the relationship between swimming velocity and $\dot{M}O_2$ of mulloway. However, at the two slowest velocities (0.24 and 0.48 BL.s^{-1}) there is little difference in $\dot{M}O_2$ (Fig. 1a), with $\dot{M}O_2$ actually being slightly greater at the slower velocity. This is most probably due to an energetic cost associated with stability control at the low velocity. Indeed, Webb (1998) suggested a J-shaped curve best describes $\dot{M}O_2$ of negatively buoyant fish, and that the elevated $\dot{M}O_2$ of fishes at low velocities should not be dismissed. This could have considerable influence in aquaculture system design, where the fish are required to swim against a defined current. For the mulloway it appears that a swimming speed between 0.3 and 0.5 BL s^{-1} results in minimal energy expenditure (Fig. 1).

Effect of hypoxia

All fish have some ability to cope with fluctuations in dissolved oxygen. During mild hypoxia, fish are able to maintain constant metabolic function mainly by increasing respiratory volume (Randall, 1982) and when doing so are referred to as oxygen regulators (Hughes, 1973). However, below a critical dissolved oxygen level (S_{crit}), the fish is unable to maintain its SMR, and $\dot{M}O_2$ decreases linearly with dissolved oxygen level. At this point the fish is now termed an oxygen conformer and if the situation persists, it will potentially suffocate. In the present study S_{crit} was determined at a routine metabolic state (R_{crit}); at a swimming velocity of 0.9 BL s^{-1} corresponding to a metabolic load of 45% of the AMR. It is believed that the R_{crit} is more relevant to cultured fish as they are likely to have a metabolic load associated with specific dynamic action when hypoxic conditions are experienced. However

when compared to the S_{crit}, the R_{crit} will be at a higher dissolved oxygen level as it has been shown to increase with swimming speed (Kutty and Saunders, 1973). Taking this into account, it appears that the mulloway is well adapted to hypoxia as the R_{crit} (1.8 mg l^{-1}, Fig. 3) is considerably less than that of the S_{crit} of Atlantic cod (2.58 mg l^{-1}, 15°C) (Schurmann and Steffensen, 1997), similar to the S_{crit} of rainbow trout (1.6 mg l^{-1}, 20°C) (Ott et al., 1980) and only slightly higher than the S_{crit} of the hypoxia tolerant carp (*Cyprinus carpio*, 1.1 mg l^{-1}, 25°C) (Ott et al., 1980) or nile tilapia (*Oreochromis niloticus*, 1.1 mg l^{-1}, 20°C) (Fernandes and Rantin, 1989). It is most likely that this hypoxia tolerance is an adaptation to support the early life history within estuaries, where water quality could be variable, and fluctuations in dissolved oxygen likely. This hypoxia tolerance gives further evidence to support the suitability of mulloway for intensive aquaculture where hypoxia may be apparent.

At the maximum sustained aerobic metabolic rate (AMR), it is assumed that the respiratory and circulatory systems are working at their maximum capacity. The AMR of mulloway was significantly reduced at all levels of hypoxia (Table 3). This demonstrates that the metabolic capacity on mulloway was impaired by even mild hypoxia (75% saturation). Although the aerobic metabolic capacity of fish is thought to be primarily limited by the ability of the gills to extract oxygen (Priede, 1985), a multitude of factors are involved in the respiratory transport chain, any one of which may have been limiting. The suppressive effect of hypoxia was not only limited to the maximum metabolic rates but also apparent at intermediate swimming velocities. At all levels of hypoxia, $\dot{M}O_2$ increased linearly with U (not exponentially as in normoxia). This resulted in a progressive reduction of GCOT with hypoxia level, with the GCOT and NCOT at the U_{opt} (COT_{min}) being significantly reduced at 25% saturation. A similar result is found with brook trout (*Salvelinus fontinalis*) and carp, where $\dot{M}O_2$ is lower in hypoxia, particularly at higher activity levels (Beamish, 1964). With these species, it was hypothesized that in hypoxia some of the metabolic requirement was derived anaerobically. With mulloway, the flattening of the swimming velocity curves in hypoxia could be achieved by a greater recruitment of anaerobic metabolism at high velocities. An increased anaerobic swimming may also explain how mulloway were able to maintain their swimming performance (U_{crit}) even though their metabolic capacity (AMR and scope) was suppressed in mild hypoxia (75% saturation). However, reduced $\dot{M}O_2$ whilst swimming in hypoxic conditions could also be achieved by decreasing the aerobic requirements of tissues not directly

involved in the muscular work (i.e. liver, gut and kidneys). In response to increased locomotory oxygen demand, chinook salmon (*Oncorhynchus tshawytscha*) reduce blood flow to the intestines, reducing the rate of intestinal oxidative metabolism and thus overall maintenance requirements (Thorarensen et al., 1993). As mulloway SMR was significantly reduced at 25% saturation it suggests a similar regional vasoconstrictive mechanism in response to hypoxia. However, as circulatory parameters were not measured in the present study, we cannot verify the exact mechanism.

The negative effect of hypoxia on fish growth rate is well documented. For the most part, the reduction in growth is attributed to a reduced in feed intake and is not a consequence of decreased feed conversion ratios (Chabot and Dutil, 1999; Thetmeyer et al., 1999; Pichavant et al., 2001; Mallekh and Lagardere, 2002). Energy budget conflicts caused by the limiting effect of hypoxia on the metabolic scope are thought to reduce appetite. Mallekh and Lagardere (2002) found a linear relationship between feed intake and metabolic scope in turbot (*Scophthalmus maximus*), and suggested that their appetite is regulated by the capacity to provide the energy necessary for digestion. This is supported by Claireaux et al., (2000) who found a direct relationship between hypoxia-related reduced growth rate and metabolic scope in Atlantic cod. In the present study, the metabolic scope of mulloway was significantly suppressed at a dissolved oxygen level of just 75% saturation. This is the same as recorded for the common sole (*Solea solea*, 4-24°C) (Lefrancois and Claireaux, 2003) and similar to that recorded with turbot (6-22°C), where below 78 and 90% air saturation the AMR was suppressed (Mallekh and Lagardere, 2002). For these species, even mild hypoxic conditions may cause energy budgeting conflicts that reduce production performance. Such an effect was recorded with Atlantic cod (6-10°C) where below 73% air saturation growth was suppressed (Chabot and Dutil, 1999). This highlights the importance of maintaining dissolved oxygen levels close to air saturation in mulloway culture

APPENDIX A. LIST OF ABBREVIATIONS

SBT	Southern bluefin tuna
$\dot{M}O_2$	Rate of oxygen consumption
SDA	Specific dynamic action
M_b	Body mass
% M_b	Percent body mass
BL	Body length
SMR	Standard metabolic rate
RMR	Routine metabolic rate
MR	Metabolic rate
RMR_h	Routine metabolic rate in hypoxia
AMR	Active metabolic rate
U	Swimming velocity
U_r	Swimming velocity during routine metabolic rate measurement
U_{sda}	Swimming velocity during the specific dynamic action period
U_h	Swimming velocity in hypoxia
DO	Dissolved oxygen
T_w	Water temperature
T_m	Muscle temperature
T_{vis}	Visceral temperature
T_{vis} excess	Temperature of viscera above water temperature.
U_{crit}	Critical swimming velocity
S_{crit}	Critical dissolved oxygen level of a resting fish
R_{crit}	Critical dissolved oxygen level of a fish at a defined routine activity level
NCOT	Net cost of transport
GCOT	Gross cost of transport
U_{opt}	Optimum swimming speed
$NCOT_{min}$	NCOT at the optimum swimming speed
$GCOT_{min}$	GCOT at the optimum swimming speed

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