Final Report

Aquafin CRC - SBT Aquaculture Subprogram:
Activity metabolism in live-held southern bluefin tuna
(*Thunnus maccoyii*)

R. Musgrove and Q. Fitzgibbon

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Replaced Professor Russell Baudinette
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(deceased March 2004))

OBJECTIVES:

1 To measure SBT metabolic rates in situ in a mesocosm

2 To identify and develop methodology to make reliable, realistic and repeatable measurements of metabolic rate in SBT under commercial conditions

OUTCOMES ACHIEVED

A 248.5 m^3 mesocosm respirometer was designed, built and successfully used to monitor routine metabolic rate in southern bluefin tuna (SBT, *Thunnus macoyii)* in a research pontoon environment. The mesocosm proved to be reliable and the measurements realistic and repeatable, providing the first metabolic data on commercial size tuna (16 – 22 kg) and on large teleost fish in general. The mesocosm platform is currently in use in Aquafin CRC-FRDC 2005/200, the second project of the tuna metabolism series where it will allow production of physiological data to inform different computer models, which will, in turn allow more informed husbandry decisions, improve farm efficiency and reduce environmental impacts of tuna aquaculture.

A study was also carried out to investigate the feasibility of development of a respiratory tag to be attached to SBT for monitoring oxygen consumption of large fish under commercial conditions. The tag proved not to be feasible within existing time and budget constraints, largely because of issues such as oxygen electrode stability and device power requirements. An alternative, in the form of an impedence tag designed to measure SBT heart rate, will be tested in Aquafin CRC-FRDC 2005/200.

Industry members have been kept informed of results through interactions during field-work, presentations during industry meetings and articles in Aquafin-CRC, FRDC and SBT Aquaculture Subprogram publications. The Tuna Boat Owners Association of South Australia

has been supportive of the research and its continuation through Aquafin CRC-FRDC 2005/200.

I. NON-TECHNICAL SUMMARY

This study investigated techniques for measurement of southern bluefin tuna (SBT) respiration rates *in situ*. Two avenues were investigated; measurement using a mesocosm respirometer deployed within a floating research pontoon (i.e. sea-cage) and the evaluation of the feasibility of a "respiratory tag", to be fitted onto individual SBT within commercial cages to log oxygen consumption rates.

The mesocosm was manufactured from 1.14 mm polyester mesh-reinforced polypropylene bonded by thermal welding. It takes the form of an enclosed cylinder 12 m across and 2.5 m deep, with a wave break wall that extends a further 1 m above water level. Entry into the mesocosm was through a 2 m diameter, 2 m high access port made from 0.75 mm black un-reinforced polypropylene positioned in the roof directly adjacent to the wave-break wall on one side. A smaller 1 m x 1 m drainage port is positioned at the centre of the base. Both ports can be clamped shut to completely seal the system. In addition, five 2 m diameter clear 0.75 mm polyvinyl chloride (PVC) windows are located in the roof, allowing entry of natural light; the sidewall tapers by 5^{o} to allow for marine pontoon net taper; stainless steel eyelets are set at 0.2 m intervals around the top perimeter of the wave break wall for attachment to the marine pontoon; and nine rope handles are included around the perimeter of the wave break wall at 4 m intervals for ease of handling.

Mesocosm respirometer trials were successfully carried out in March and April 2004. Five experimental respiratory trials were run using pairs of SBT. Tests were also run to investigate mesocosm membrane permeability and background respiration due to biological oxygen demand and planktonic metabolism, and the oxygen consumption data gathered from the experimental trials adjusted accordingly.

The SBT used ranged from 14.0 to 23.2 kg (mean weight 19.6 ± 1.9 kg) and 93 to 117 cm (fork length) and were apparently unstressed by the experiments as they were generally observed swimming slowly, as a pair within the mesocosm. The overall mean SBT swimming velocity was 1.1 ± 0.1 Body Lengths.sec^{-1}. Eight of the ten fish used for the experiments also fed immediately after their trial; three of the fish taking a baited hook and all surviving the mesocosm without obvious signs of stress. Coefficients of variation between portable meter dissolved oxygen (DO) recordings from the 3 depths at 3 sample positions around the mesocosm did not exceed 2.5% for any trial, suggesting the mesocosm remained well mixed at all times.

Calculated corrected oxygen consumption rates of the SBT examined ranged between 345.5 and 539.3 mg O_2 $kg^{-1}.h^{-1}$ giving a mean of 460.3 ± 34.9 mg O_2 $kg^{-1}.h^{-1}$ for the five trials. Passive diffusion and background respiration rates were in all cases less than 100^{th} of a percent of SBT

oxygen consumption rates over 22 hr. The success of these trials suggests that the mesocosm respirometer will provide a very useful platform for further work.

The second avenue of research comprised a desktop study and flume tank trials designed to evaluate the combination of an archival tag with micro-cathode oxygen electrodes (i.e. a "respiratory tag") to allow indirect determination of SBT metabolic rate by measurement of oxygen consumption. Flume tank trials were carried out to evaluate the yellowtail kingfish, (YTK, *Seriola lalandii*) as a surrogate for SBT and if suitable YTK would be used to test a prototype respiratory tag before field trials with SBT. Although the YTK flume tank trials were successful, intensive study of the technology required for the respiratory tag demonstrated that it would not be feasible at the present time.

The Aquafin CRC-FRDC has recently funded the second project in the series (FRDC 2005/200) to undertake further mesocosm trials in conjunction with archival tag studies. An alternative to the respiratory tag, in the form of an impedence tag designed to measure heart rate, will be adapted for use on SBT, in conjunction with Professor Peter Frappell, Head, Department of Zoology, La Trobe University, Melbourne. Professor Frappell and colleagues have already used this technology to measure heart rates of a number of fish species.

II. ACKNOWLEDGMENTS

This report is dedicated to the memory of the late Russell Baudinette, a wonderful teacher, colleague and scientist. Professor Baudinette was a driving force behind the development of this project and was tremendously enthusiastic about its potential for innovation in the area of large teleost metabolism research. He was Mr Fitzgibbon's primary PhD supervisor.

The authors also wish to thank Dr Jeffrey Buchanan and Dr John Carragher for assistance with project management and execution. Breakwater Bay skipper and crew Brenton Ebert, Guy Manthorp and Richard Morrison for at-sea assistance. Steven Clarke (SBT Aquaculture Subprogram Leader) for support in the area of industry communications. David Ellis (TBOASA) for in-field advice and support. Geoff Bayly and DI Fishing Pty Ltd for tuna farm industry support and BOC Gases for technical support and supply. This work formed part of a project of Aquafin CRC and received funds from the Australian Government's CRCs program and Fisheries R&D Corporation, and from other CRC participants.

III. BACKGROUND

Southern Bluefin tuna (SBT; *Thunnus maccoyii*) culture is, at present, the single most valuable sector within South Australia's aquaculture industry. In 2003/2004, tuna farms in the Port Lincoln area produced approximately 9,290 tonnes of tuna, valued at $242 million (whole weight after growout, Source: ABARE, February 2005).

Since 1991, when the Australian Tuna Boat Owners Association set up its first experimental tuna farm, considerable work has been undertaken by the industry and associated research bodies (e.g. SARDI Aquatic Sciences, Flinders University) on feed requirements and on improving understanding of the effects of pre-harvest stressors on growth rates, flesh quality, vulnerability to water quality deterioration and grow-out mortalities.

The work continues today with a large part of the research effort managed under the Aquafin CRC/FRDC SBT Aquaculture Subprogram. At its inception in 2001, there were several key issues identified, including the need for formulated feeds that yield high fish productivity and quality with low feed wastage, the need for tools so that farmers would be able to optimise SBT quality to meet market expectations and increase the value of the industry and the requirement for an understanding of the environmental impacts of sea-cage farming to ensure sustainability. The latter is of relevance to the present project, particularly in view of the perceived sensitivity of SBT to water quality fluctuations.

The key outcomes of this project (intended to be a pilot study), were to be development of the ability to carry out the first measurements of metabolism on industry-size SBT and an investigation of the use of microsensor technology for long term monitoring of SBT in a commercial setting.

In detail, the first project was to evaluate metabolic rates of a range of sizes of SBT in large, sealable plastic bags supported by pontoons (mesocosm respirometers) anchored in shallow water. SBT were to be monitored over extended periods of time, and data used as the first step in the construction of a metabolic model for SBT in culture.

The project was also to investigate the use of microsensor technology to provide a longer-term method for application to a commercial setting. The key element of the microsensor technology was to be an archival tag. Such tags have been used extensively on many large finfish, including SBT and other tunas (Gunn 2001). SBT were actually the first tuna to be archivally tagged (Gunn et al 1994) and up to 2001 such tags had produced over 15,000 days of data and provided a great deal of information on the physiology and behaviour of wild populations (Gunn, 2001).

This project was to test the utility of archival tags for studies under culture conditions. A feasibility study was to be carried out to evaluate the combination of an archival tag with micro-cathode oxygen electrodes to allow indirect determination of SBT metabolic rate by measurement of oxygen consumption. The feasibility study was to include desktop work as well as trials of a dummy system to look at attachment and placement issues. Yellowtail kingfish (*Seriola lalandii*) were to be used in this case, as a cost effective surrogate for SBT, given the latter's very high value, and for ease of handling. The data collected were to be used purely to assess the techniques that, if feasible, would be transferred to SBT in the second project of the series.

The second project, Aquafin CRC-FRDC 2005/200, will use the tools developed during the first project to measure SBT metabolism in commercial facilities and, with various key treatments applied, determine average and critical values for oxygen demands associated with feeding and tolerance thresholds for low oxygen concentrations. The mesocosms will also be used in the second project, to gather the required data to determine heart rate and oxygen demand.

The second project will link with the Aquafin CRC's SBT Production, Environment and Health Programs to develop the capacity to make metabolic measurements in relation to tuna size, water quality and feed types. It will also facilitate the development of a monitoring system to assist in improvement of feeding efficiency and further minimisation of stress, increasing survival and product value.

Data on metabolism are important to SBT culture in that they will provide fundamental information on tuna responses to handling, confinement, changes in water temperature and oxygen levels (especially hypoxia), levels of cage fouling, feeding activity, food type and stressors such as microalgae, sediment, noise and predator presence. The data derived from these two projects will be used to improve the efficiency of the SBT bioenergetic model (FRDC 97/363) and contribute to an improvement in the efficiency of SBT husbandry. For example, if SBT metabolic rates and tolerance limits to low oxygen concentration at a given temperature are known, simulations could be run to investigate whether lower stocking rate, for example, could overcome the need to stop feeding on dodge tides in summer. Also, decisions could be made on feeding frequency, given knowledge of what the food and the increased fish metabolism (activity) will do the water oxygen levels and what effect that might have on the growth rate/survival.

IV. NEED

Southern bluefin tuna are large, fast, pelagic predators and as such probably have a very different metabolism to most other species of cultured finfish. There is a need therefore, to develop a comprehensive understanding of the metabolic rates of SBT as a basis for improving our management and husbandry of this species in an aquaculture environment.

Nutrition (food conversion ratios, feed type and feeding frequency), responses to water quality (limiting oxygen concentrations), response to predators, fish health and environmental outcomes (e.g. waste production) are all linked to SBT metabolic rate. Improving the management of fish in culture including the development of new feeds, optimising production and product quality and assuring sound environmental outcomes are all dependant on developing this basic knowledge.

At the SBT Aquaculture Subprogram Steering Committee Workshop, held on the 6th November 2002 in Port Lincoln, physiology was identified, by both industry and research sectors, as a major research priority. In particular, it was recognised that a need existed to measure SBT size-specific metabolic rates under a variety of circumstances, including tow history, handling, cage acclimation effects, water temperature, changes in oxygen levels (especially hypoxia), levels of cage fouling, feeding activity and food type. Similarly, data on SBT's metabolic responses to stressors such as microalgae, sediment, noise and predator presence were also considered important.

This project also provides invaluable information about metabolic rates that will inform research being conducted on SBT in the wild and thereby enhance our understanding of the overall biology of this species. Importantly, the data could also be used to calibrate information from tagged fish currently being collected as part of CSIRO's ongoing research into the movement and ecology of SBT using archival tags.

The project was to address industry needs by producing the following outcomes:

- Measurements of oxygen consumption on commercial-sized SBT
- Incorporation of those data into metabolic model for cultured SBT
- An investigation of the usefulness of microsensor technology in monitoring SBT respiration in a commercial environment.

V. OBJECTIVES

1. To measure SBT metabolic rates in situ in a mesocosm.
2. To identify and develop methodology to make reliable, realistic and repeatable measurements of metabolic rate in SBT under commercial conditions.

The activities undertaken to address these two objectives will be described in the following three self-contained Chapters.

CHAPTER 1. THE AEROBIC METABOLISM OF SOUTHERN BLUEFIN TUNA AND YELLOWTAIL KINGFISH: A LITERATURE REVIEW

1.1 INTRODUCTION

The southern bluefin tuna (SBT; *Thunnus maccoyii*) is a highly migratory species. During the months of September and March, SBT spawn in the seas south of Java (Murphy and Majkowski, 1981). Juvenile SBT then migrate down the coast of Western Australia, before ranging widely over the oceanic waters of the southern Indian, Atlantic and south western Pacific Oceans (Murphy and Majkowski, 1981; Gunn and Block, 2001). Since the early 1950's, SBT have been subject to substantial commercial fishing pressure which has resulted in a considerable reduction in SBT numbers. The SBT biomass in 1998 was estimated to be less than 12% of the virgin stock mass before commercial fishing (reviewed by Gunn and Block, 2001).

In the early 1990's, Australia began to experiment with SBT aquaculture. Instead of passing the fish directly to market, fishermen began to on-grow wild caught juveniles to a more profitable weight and condition (Walker and Clymo, 1995). Today, nearly all SBT caught by Australian fishermen, are on-grown in large floating sea-cages. At capture they are generally 2-3 years old and weigh approximately 15-20 kg. Within a summer grow-out season, they gain up to approximately 100% of their initial body mass, although this varies with individual and with initial size (pers comm. J Buchanan). The SBT aquaculture industry in South Australia has grown to become arguably Australia's largest aquaculture industry worth over A$250 million in the 2000/2001 financial year (Knight et al., 2002).

The rapid development of the tuna aquaculture industry has highlighted the need for, and lack of, physiological information on SBT. A fishery quota currently limits the development of the SBT aquaculture industry. For the past 10 years this fishery quota has stood at 5,265 tonnes (fish whole weight). Therefore, further development of the industry must involve optimising culture conditions to obtain greater or more efficient growth from the limited supply of fish and/or ongrowing the fish for longer to achieve more growth. Gaining a further understanding of the basic physiology of the tuna is seen as an essential component in order to improve SBT aquaculture efficiency.

Another rapidly growing aquaculture industry in South Australia is the marine farming of yellowtail kingfish (YTK; *Seriola lalandi*). Species of the *Seriola* family are circumglobal supporting wild and recreational fisheries in many countries (Benetti et al., 2001). For many years the culture of yellowtail (*Seriola quinqueradiata*) has supported a large and profitable

aquaculture industry in Japan, where the flesh is highly regarded for raw consumption as sushi and sashimi (Poortenaar et al., 2001). Driven by a high demand and price for *Seriola sp*. in international seafood markets, countries including Australia, Ecuador, Japan and several in Europe are striving to produce these fish commercially (Benetti et al., 2001). Presently, there are at least 3 YTK marine sea-cage farms in South Australia and YTK farming is considered a growth industry for the state.

1.2 FISH METABOLISM WITH PARTICULAR REFERENCE TO TUNA

From an energy budget perspective, fish, like all organisms, use energy obtained from the ingestion of food (U) for the synthesis of tissues (P), as fuel in the metabolic process (R) with some energy being lost as waste products (W) (Calow, 1985; Jobling, 1994). The metabolic process powers the synthesis of tissues and all other physiochemical work required by the organism. As oxygen is required in the enzymatic steps involved with the metabolic process, oxygen consumption has almost universally been used to determine metabolic rate in fish (Fry, 1971; Brett, 1972). Aerobic metabolism (R) in fish can be broken into 3 main subheadings (reviewed by Calow, 1985; Priede, 1985; Jobling, 1994; Korsmeyer et al., 1996; Korsmeyer and Dewar, 2001):

1) Standard metabolic rate (Rs), the resting and fasting metabolic rate and theoretically the minimal metabolic rate of the animal.

2) Metabolism due to locomotory activity (Ra), which is typically swimming in fish, and

3) Metabolic rate attributed to the activities of food digestion and assimilation (Rf). Rf is also often referred to as “specific dynamic action”.

The above may be represented as a mathematical equation as follows:

$$U = P + R + W$$

where:

$$R = Rs + Ra + Rf$$

Due to the inherent difficulties of handling such large active fish, the study of tuna metabolism has lagged far behind the study of other species, principally salmonids and goldfish (reviewed by Brett, 1972). Initial estimations of tuna metabolism were made with *in vitro* tissue samples (Gordon, 1968) or anaesthetised and restrained whole tuna (Stevens, 1972). Later, the value of Rs in skipjack tuna (*Katsuwonus pelamis*) was estimated by measuring oxygen consumption of specimens immobilized with neuromuscular blocking agents (Brill, 1979). Gooding et al. (1981) performed the first metabolic study with freely swimming tuna by measuring oxygen

consumption of groups of skipjack tuna in a sealed fibreglass tank. Subsequent to this, tuna metabolism has been examined by a variety of other techniques including energy loss due to starvation (Boggs and Kitchell, 1991) and continuous infusion dye dilution (Bushnell and Brill, 1991). However, it took the development of large water-tunnel respirometers to allow the examination of the aerobic metabolic requirements of a variety of tuna species under controlled velocities and temperatures (Graham and Laurs, 1982; Graham et al., 1989; Dewar and Graham, 1994).

1.2.1 Standard metabolic rate (Rs)

Tuna are ram ventilators, that is, they lack ventilatory pumps, and thus must continually swim to ventilate their gills (Brown and Muir, 1970). Therefore, direct measurement of a tuna's metabolism at "resting" is physiologically impossible. Tuna researches have estimated Rs by the use of two methods: (i) Extrapolating the relationship between oxygen consumption and swimming velocities (VO_2) back to zero velocity (Gooding et al., 1981; Graham, 1989; Dewar and Graham, 1994), or (ii) Measurement of oxygen consumption of immobilized specimens (neuromuscular or spinal block) (Brill, 1979, 1987). Validation for both techniques was recorded by Brill (1987), who found that the Rs of aholehole (*Kuhlia sanduicensis*) and rainbow trout (*Salmo gairdenerii*) measured by neuromuscular blocking, were not significantly different from published data of Rs measured by reducing VO_2 back to zero velocity.

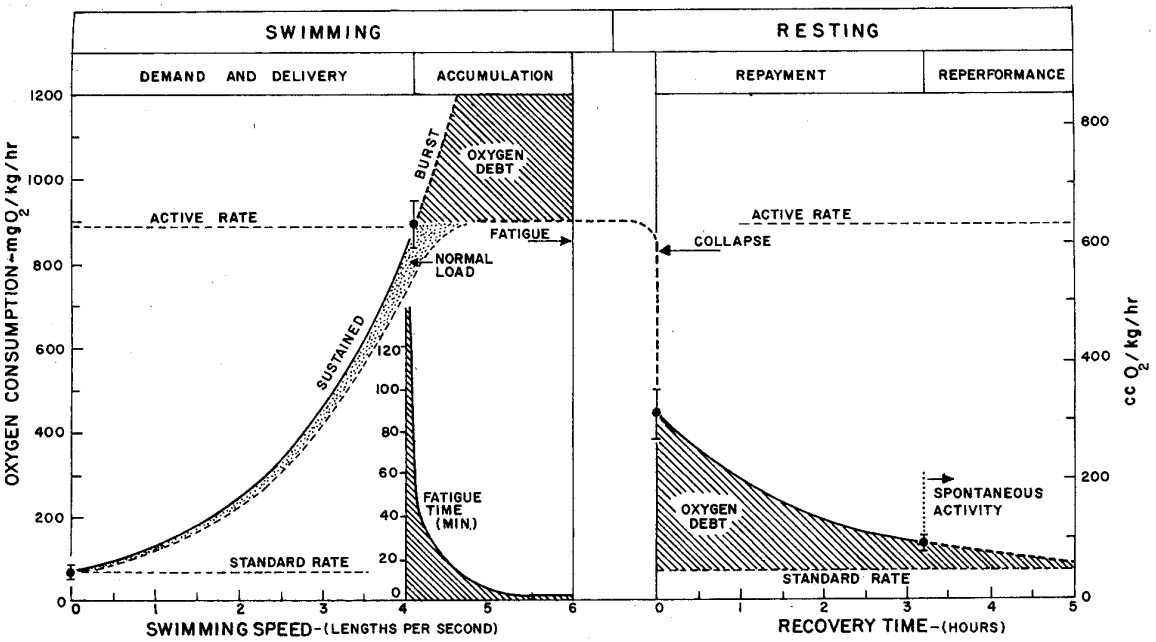
The Rs values of tunas are approximately 3 - 5 times greater than those of other active teleosts and about 10 times greater than those of sluggish bottom dwelling species (Brill, 1979, 1987). The high Rs may be related to the overall highly evolved metabolic capabilities to help them survive in a patchy nutrient-poor pelagic environment. Brill (1987) describes tuna as "energy speculators", gambling on high expenditure with high rates of energy return. Brill (1987) hypothesizes that physiological adaptations to maintain tunas' large metabolic scope (primarily large gill surface area), consequently increases the value of Rs. In fish, osmoregulation costs have been shown to account for up to 50% of Rs. Tunas large gill surface area (up to a order of magnitude larger than other fish species) is likely to greatly increase the animals' osmoregulatory cost and consequently its Rs.

1.2.2 Aerobic locomotion (Ra)

Tunas are highly active pelagic predators, specialized for continuous swimming. Thus, the metabolic cost of swimming is a permanent feature. In order to further explain the aerobic cost of locomotion, some terminology must be outlined (Brett, 1972; Korsmeyer and Dewar, 2001). Routine metabolic rate (RMR), refers to the average aerobic metabolism associated with spontaneous activity and may occur over a wide range of values. Maximum metabolic rate or active rate (MMR) refers to the maximum sustained rate (for 1 hour by definition) of aerobic

metabolism without fatigue. The difference between MMR and Rs represents the animal's aerobic capacity or metabolic scope. In fish, oxygen consumption increases exponentially with swimming speed until it reaches the "critical velocity". This critical velocity corresponds to the MMR of the animal. Higher speeds can be achieved, but they must involve anaerobic metabolism and result in an oxygen debt and fatigue. This is often referred to as burst swimming, and in fish, oxygen demand during these events can exceed oxygen supply by an order of magnitude (Brett, 1972). Figure 1.1 illustrates the relationship between aerobic metabolism and swimming speed in a teleost species.

Fig 1.1 The relationship between oxygen consumption and swimming speed, fatigue time, oxygen debt and recovery time for a 50 g salmonid (Brett, 1972).



In tuna, the metabolic rate at any swimming speed is higher than other similarly-sized active teleosts as is the cost of transport (in Joules.Newton^{-1}.m^{-1}) (Brill, 1996). This suggests that although tunas have highly streamlined hydrodynamic bodies, they are less efficient swimmers than other teleosts. In a metabolic sense (i.e. oxygen consumption per meter) this is true, however this high cost of swimming can be again attributed to elevated Rs and thus is another cost of a large metabolic scope (Brill, 1996).

1.2.3 Specific dynamic action (Rf)

Rf is defined as the increase in metabolic rate following the ingestion of food and represents the metabolic expenditures for ingestion, digestion and absorption (Jobling, 1981). In poikilothermic species, this is most commonly measured as the post-prandial increase in the rate of oxygen consumption (Tandler and Beamish, 1979; Jobling and Davies, 1980; Jobling, 1981). In fish, oxygen consumption generally peaks soon after ingestion followed by a

gradual decline to the pre-feeding or resting level (Jobling, 1981). The magnitude of this effect is measured by calculating the oxygen consumption above the resting level, commonly including quantification of peak and duration. The peak level of post-prandial oxygen consumption in fish is generally twice the resting level and, in most fish species tested, the magnitude represents 9-20% of the ingested energy (reviewed by Jobling, 1981).

No direct measurement of Rf has ever been made for tuna. However, it is hypothesized to be a substantial component of their metabolism due to a high rate of consumption and digestion (Korsmeyer and Dewar, 2001). Some species of tuna can consume up to 30% of their body mass per day and are able to clear the gut 4 to 5 times faster than other fishes of comparable size (reviewed by Brill, 1996; Korsmeyer and Dewar, 2001). These adaptations allow tuna to consume large quantities and feed frequently when food resources are abundant in the nutrient poor-oceans. The ability of tuna to elevate visceral temperatures also suggests Rf to be a substantial component of its metabolic capabilities. Gunn et al. (2001) found that the visceral temperature of SBT commonly increases more than 4^{o}C immediately following feeding and can be maintained above basal levels for up to 40 h. Gunn et al. (2001) concluded that this increase in visceral temperature results directly from the heat production due to the hydrolytic breakdown of food and from an increase in metabolic rate.

1.2.4 The effect of body mass and temperature

In general, the relationship between metabolism and weight in fish can be described by the equation (Fry, 1971; Clark and Johnson, 1999):

$$R = a.W^{b}$$

Where: “R” is the rate of metabolism, “W” is fish body weight, “a” is a constant and “b” is the scaling component. Clark and Johnson (1999) collated the published Rs - mass relationship for 69 teleost species and found that, of the 110 studies examined, 80% reported a scaling component of 0.65 – 0.95, with a total study mean of 0.79. As this coefficient is less than 1.0, fish mass-specific Rs declines proportionately, as the animal gets larger. In tuna, this scaling coefficient appears to be generally lower than the average for other teleosts. The mass scaling coefficients reported for tuna include, 0.50 – kawakawa (*Euthynnus affinis*), 0.57 and 0.60 – yellowfin (*Thunnus albacares*), and 0.57 – skipjack (Brill, 1979, 1987; Dewar and Graham, 1994). This suggests that the mass specific decline of Rs in tuna is even greater than that for other teleosts. However, it must be noted that these estimations have been made from relatively small fish. The studies mentioned above recorded the mass effect with fish from 0.3 – 4.7 kg. Considering that yellowfin tuna can grow as large as 180 kg; extreme

caution must be taken when extrapolating these relationships above the masses used in these studies (Korsmeyer and Dewar, 2001).

The relationship between environmental temperature and Rs in fish is curvilinear (Brett, 1972). Clark and Johnson (1999) collated the data from published studies examining the relationship between Rs and temperature for teleost fish and found that an Arrhenian model best described the relationship as follows:

$$Rs = A^{(-\mu / GT)}$$

Where: “A” is a constant, “μ” the Arrhenius constant, “G” the universal gas constant and “T” the absolute temperature. When they applied this model to the more commonly used Q_{10}, they found that the mean of the 69 species was 1.83, but the within-species median was 2.40 (the frequency distribution being negatively skewed). This implies that a tropical fish at 30^{o}C consumes approximately 6 times as much O_2 as does a polar fish at 0^{o}C (Clark and Johnson, 1999). Published data on tuna metabolic sensitivity to temperature suggest that they are fairly typical of teleost fish. Brill et al. (1987) found that the Q_{10} of 3 species of neuromuscular-paralysed tuna (skipjack, kawakawa and yellowfin) was 2.44, 3.16 and 2.31 respectively. However, a later study, using free-swimming yellowfin tuna, recorded a Q_{10} of 1.67 (Dewar and Graham, 1994). This discrepancy in results may be due to the ability of tuna to elevate muscle temperatures, making them less sensitive to water temperature (Dewar and Graham, 1994; Korsmeyer and Dewar, 2001).

1.3 METABOLISM AND AQUACULTURE

The basic principle of bioenergetics involves the energy budget equation stated earlier. Put simply, energy consumed is used in the metabolic process, deposited as new body tissue or lost as wastes (Calow, 1985; Jobling, 1994). Therefore bioenergetic studies are principally concerned with the physiological basis behind the relationship between feeding and growth. As feed costs represent a considerable component of aquaculture expense, and tissue growth is the ultimate aim of aquaculture farms, understanding and optimising this relationship is critical for fish farming. Defining and evaluating the energy cost associated with metabolism is an essential component of bioenergetic studies; therefore, metabolic data obtained in the present experiment, will greatly help the further examination of bioenergetics in SBT and YTK.

Metabolism also represents a considerable proportion of energy losses from the system; Ra and Rf are of particular importance to aquaculture. Rs has little direct relevance to fish farming, although it is often important to scientists as a baseline for comparison of the metabolic scopes and for other parameters, such as feeding, locomotion, physical and environmental factors. Ra represents a major component of energy expenditure of fish especially for highly active species

such as tuna. Energy expenditure of tuna has been shown to more than triple over the normal aerobic swimming velocity range (Dewar and Graham, 1994).

An increase in metabolism associated with stress is also associated with Ra (Knights, 1985). In aquaculture, stress can be caused by social, physical and environmental factors. Stress elicits a physiological response principally controlled by the endocrine tissues with the secretion of catecholamines and corticosteroids. These hormones are known to modify the fish's state of metabolism - i.e. the depletion of tissue glycogen reserves, lipolysis and increased muscle protein catabolism (Jobling, 1994). This is generally represented by an increase in metabolic rate and, when combined with other factors associated with stress (i.e. reduced feed intake), can result in a considerable reduction in growth in fish (Knights, 1985).

Rf can also represent a considerable proportion of energy losses, accounting for up to 20% of the ingested energy (Jobling, 1981). Evaluation of Rf allows the further examination of the effects of ration size and different feeds on the efficiency of digestion. Furthermore, appetite in fish is considered to be directly related to Rf. Voluntary feeding in fish has been found to occur well after gut evacuation. Presumably, metabolism associated with Rf is responsible for this delay in the development of hunger (Vahl, 1979). It is also suggested that Rf can indirectly affect fish growth. It is well known that low dissolved oxygen levels detrimentally affect fish growth. For example, the appetite of the rainbow trout (*Oncorhynchus mykiss*) is suppressed when water saturation falls below approximately 60%, and feed conversion efficiency is affected when saturation falls below 70% (Jobling, 1994). This reduction in appetite is considered to be due to the decrease in oxygen availability reducing the animal's metabolic scope and lessening the available oxygen for food digestion and assimilation. Mallekh and Lagardere (2002) found that appetite of turbot (*Scophthalmus maximus*) was proportional to metabolic scope. In this study, the fish's metabolic scope was reduced by dropping dissolved oxygen concentrations or temperature, resulting in a linear reduction in the feed intake.

CHAPTER 2. MEASUREMENT OF SBT METABOLIC RATE IN A MESOCOSM

2.1 INTRODUCTION

To date, studies on respiratory physiology of tunas have been limited to fish less than 5 kg (Dewar and Graham 1994; Sepulveda and Dickson 2000; Sepulveda et al. 2003) and to flume tanks in the laboratory. The data generated are of limited utility to culturists needing to understand oxygen use in pontoon-held tuna up to two orders of magnitude larger in size. This project developed a new technology to address this question; a mesocosm respirometer, a cylindrical polypropylene bag designed to be deployed within a 12 m research pontoon and used to determine metabolic rates of commercial-sized southern bluefin tuna (SBT). In comparison with previous flume-tank studies the current work has less control over measurement interval and none over swimming velocity, but nevertheless has the potential to generate useful data on oxygen consumption of commercial-size fish that will contribute to bioenergetic models relevant to pontoon-held tuna.

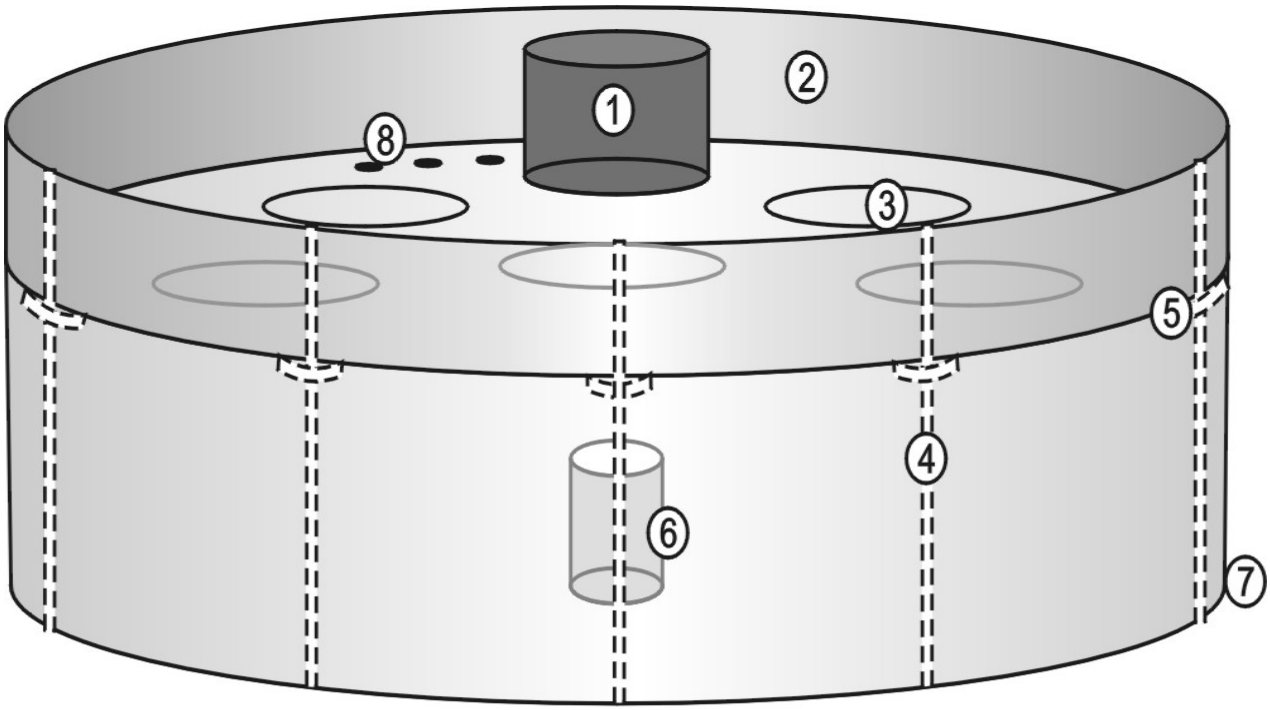
2.2 METHODS

2.2.1 Mesocosm design and manufacture

The mesocosm was manufactured largely from Stevens Geomembranes® denier polyester mesh-reinforced 1.14 mm polypropylene bonded by thermal welding by Fabtech SA Pty Ltd. The structure is a 12 m by 2.5 m (diameter by depth) enclosed cylinder with a wave break wall that extends a further 1 m above water level (Fig. 2.1). Entry into the mesocosm is through a 2 m diameter, 2 m high access port made from 0.75 mm black un-reinforced polypropylene positioned in the roof. A smaller 1 m diameter (1 m height) drainage port is positioned at the centre of the base. Both ports can be rolled and clamped shut to completely seal the system. In addition, five 2 m diameter clear 0.75 mm polyvinyl chloride (PVC) windows are positioned in the roof to allow entry of natural light; a 5^{o} taper is included in the sidewall to accommodate marine pontoon net taper; stainless steel eyelets are positioned at 0.2 m intervals around the top perimeter of the wave break wall for attachment to the marine pontoon; and nine rope handles are included around the perimeter of the wave break wall at 4 m intervals for ease of handling.

Mesocosm volume was measured at the end of the experimental period by timed pumping dry of the mesocosm with calibrated pumps. Pump flow rates were calibrated at the beginning, 3 times during and at the end of the pumping session by timing the filling rate of a 1,600 litre tank. Total mesocosm water volume was calculated to be 248,556 litres.

Fig 2.1 Mesocosm respirometer



1	Top port	5	Rope handles
2	Wave skirt	6	Bottom port
3	Windows	7	5^{o} taper
4	Handling ropes	8	Equipment ports

2.2.2 Mesocosm deployment and maintenance

a) Deployment

The mesocosm was deployed (Fig 1.2) into a 12 m diameter floating pontoon using a team of five people, although only 3 were required for day-to-day operation. The top of the wave break wall was laced to the pontoon arm-rail with 5 mm diameter rope. Once in position, the mesocosm was pump-filled with seawater (60,000 l/h) and a float (10 litre plastic drum filled with air) attached to the sealed bottom port inside the mesocosm to help retain its shape in the prevailing currents. The shape of the mesocosm was dynamic, depending on the prevailing tide and weather conditions, but generally maintained a cylindrical form.

The mesocosm was first deployed in mid-August 2003 (“winter” trials) at the SARDI Research Farm (Fig 2.2), and over the following 2 days the structure and dissolved oxygen

logging equipment were tested. During this time it was apparent that mesocosm structure could survive at least 20 knots of wind. However, on the third day winds peaked at 40 knots and the mesocosm wall was torn; necessitating on-land repairs. It was not possible to undertake any winter-time respirometry trials with SBT due to the time needed for repairs.

The following March (2004, “autumn” trials), the mesocosm was deployed in a more protected site, in Rotten Bay (135^{o}56’26”E, 34^{o}43’44”S) on the southern side of Boston Island, Port Lincoln. In this instance trials were successfully completed as described below.

Fig 2.2 Mesocosm deployment

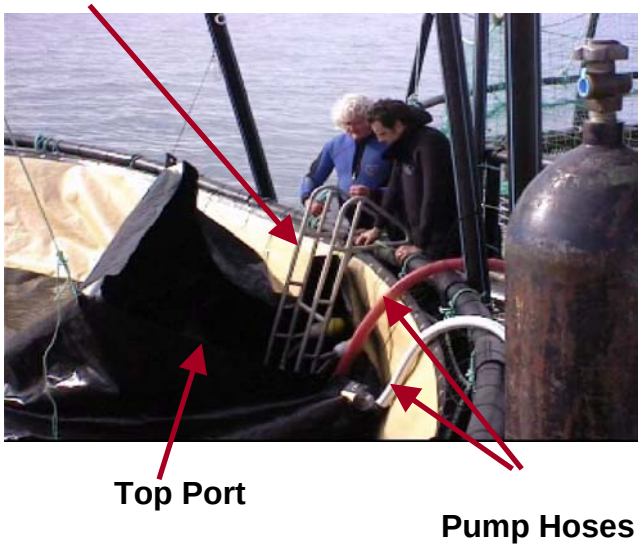
a) Unrolling

b) Lashing to pontoon

c) Deployment complete

d) Top port for entry of divers and fish and water.

Access Ladder



b) Maintenance

The mesocosm floor was vacuum-cleaned by a diver after every experimental trial to remove any detritus. The diver used the filling pump in reverse, applying the end of the intake hose in a methodical fashion to the entire floor of the mesocosm. Immediately after vacuuming, the water removed by this method was replaced with fresh seawater by use of the same pump. This resulted in an approximately 5% water exchange following every trial but maintained the mesocosm's original water volume.

c) Water Quality

Water ammonia levels were tested daily onsite with a Hagen Co. portable ammonia test kit and never exceeded 0.6 mg NH_4 l^{-1}. Two ibutton™ (Temperature Technologies Pty. Ltd.) temperature loggers ($\pm$ 0.5^{o}C) positioned at 1 m and 2 m depth, 1.5 m from the mesocosm edge, continuously logged mesocosm water temperatures at a rate of 1 reading every 10 min.

2.2.3 Fish handling and respiratory trials

a) Fish Handling

Ten similar sized juvenile SBT (mean 19.6 $\pm$ 1.9 kg) were randomly selected from a SBT commercial pontoon (DI Fishing Pty Ltd) approximately 4 km offshore from Boston Island in early April 2004. These fish had been purse-seined in the Great Australian Bight, most likely from the same school, approximately 2 months previously and had been weaned onto the Skretting Co. Tuna Growers 45:20 commercial pellet diet.

Individual fish were transferred from the commercial pontoon into a 12 m diameter holding pontoon by hooking with a baited barb-less hook then 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 then towed to the Rotten Bay site and secured alongside the mesocosm and fish left to recover for 48 h. Experimental fish were fed to satiation with pilchards (S*ardinops sagax*) 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 examining the visceral warming of SBT suggest that the fish would have most likely completed digestion and assimilation of previous meals within 36 h (Gunn et al. 2001).

b) Respiratory Trials

Basic Protocol:

Five SBT respiratory trials were completed over 29 days; commencing on days 8, 17, 19, 22 and 23 (Table 2.1). 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 and not be ingested as striking would occur the moment the fish took the bait. Two fish were chosen for each trial, as it was believed that due to the schooling nature of SBT, they would be less stressed within the mesocosm if introduced with a partner.

The fish were then left undisturbed for 6 h to acclimatize to the mesocosm environment. This acclimation period is greater than used for other tunas to recover from transport and acclimate into a respirometer environment (3-4 h; Dewar and Graham 1994; Sepulveda and Dickson 2000). During the acclimation period, trapped air under the ceiling of the mesocosm was expelled by rolling it towards the top port. The port was then rolled up and clamped shut with a 3 m hinged timber clamp, completely sealing the mesocosm. The fish were left for the duration of the experiment, then the mesocosm was opened, the fish removed and fork length (LF) and body weight recorded. Immediately preceding each trial, an Aqua & Co®, Force 7 oxygen diffuser (BOC Gases) was used to raise the dissolved oxygen levels back to approximately 100% saturation before the beginning of the next trial.

Dissolved oxygen monitoring:

The dissolved oxygen level (DO) of the mesocosm water was monitored by two methods. The first used two Clarke-type oxygen electrodes (Vickie Cheshire Pty. Ltd.) 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 sulphide-saturated seawater, respectively) immediately before and after each experiment. If electrode drift was recorded to be greater than 10%, data from this electrode was excluded from calculations. These electrodes were connected to a Datataker® DT500 logger that recorded the oxygen concentration 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 allowing remote oxygen monitoring and servicing.

The second method of DO monitoring involved taking readings at the beginning and end of each experimental trial with an OxyGuard International® Handy Gamma portable oxygen meter. This meter was used to take 2 min readings through three 40 mm 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). 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.

Swimming velocity assessment:

The swimming velocity of each SBT was estimated by taking digital video footage of fish (Sony DCR-HC30) through the mesocosm roof windows at the start and end of each experimental period and later analysed using the World in Motion video analysis software. 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 ($\geq$ 1 s portions) was analysed at a rate of 30 frames per sec and the known tuna lengths were used to calibrate image scale so as to correct for variable distance of fish from the camera. Individual fish were generally easily identified from the pair by comparison of overall lengths as the two fish usually swam side by side.

c) Background Trials

Background respiration trials were conducted at regular intervals (days 1, 9, 19 and 26) as a measurement of biochemical oxygen demand and planktonic respiration in the water column and on the mesocosm surfaces. These trials consisted of the same protocol as for the SBT respiratory trials, however no fish were introduced to the mesocosm.

d) Passive Diffusion Trial

Passive diffusion through the mesocosm material was measured on day 27 using the procedure outlined for the background trial. In this case 3 kg of sodium sulphite (Ace Chemical Co Pty Ltd) and 50 g of the catalyst cobalt chloride (Sigma-Aldrich) were added to the mesocosm and the water mixed with two 4 hp pumps for 30 min before sealing. This experiment was designed to reduce DO level in the mesocosm by approximately 20% saturation, to gain a better understanding of the oxygen diffusive qualities of the mesocosm membrane (*sensu* Ruttanagosrigit et al. 1991; Bennett and Beitinger 1995).

e) Data Analysis

Dissolved oxygen levels were corrected to a water temperature of 19^{o}C, salinity of 35 ppt and the standard atmospheric pressure of 760 torr resulting in oxygen solubility of 7.627 mg O_2 l^{-1}. SBT Oxygen consumption rates (Vo_2) were evaluated according to the equation:

$$Vo_2 = Vm \cdot \Delta o_2 m / \Delta t \cdot Mb$$

where: “Vm” is the total mesocosm water volume, “$\Delta o_2 m$” is the change in dissolved oxygen concentration of the mesocosm water, “Δt” is the corresponding experimental time period and “Mb” is the total SBT body mass. Results represented are means $\pm$ SE (n = 11) of both logged and portable data. Logged data for trials 2 and 3 were not available due to data logger failure, however portable DO meter recordings were taken successfully for these trials.

2.3 RESULTS

2.3.1 Mesocosm performance

All fish survived the mesocosm without obvious signs of stress. The experimental period ranged from 21 – 22 h (Table 2.1), except for one trial when poor weather prevented access and the fish were left for 42 h. Experimental temperatures remained between 18.7 and 19.3 ^{o}C. Coefficients of variation between portable meter DO recordings from three depths at three sample positions did not exceed 2.5% for any trial (data not shown). These results suggest that the mesocosm remained well mixed at all times.

Table 2.1 Fish weight (kg) and fork length (LF, cm) of the two SBT in each trial, trial day, trial length, recorded swimming velocities and mean temperature (± SE, n = 144) for the respiratory trials.

Trial	Day	Duration (h:min)	Temperature ±SE (^{O}C)	Fish	Weight (kg)	LF (cm)	Swimming Velocity Start (LF sec^{-1})	Swimming Velocity End (LF sec^{-1})
1	8	42:17	19.3 ± 0.03	1	20.8	108.5	0.77	0.56
				2	16.4	100.5	1.06	1.01
2	17	21:45	19.0 ± 0.09	1	21.6	117.0	0.63	1.29
				2	19.8	110.0	1.40	1.01
3	19	20:55	18.7 ± 0.02	1	22.8	107.7	1.29	1.21
				2	17.9	103.5	1.15	0.94
4	22	21:52	18.7 ± 0.01	1	23.2	107.5	1.17	1.39
				2	19.3	102.5	1.18	0.95
5	25	21:52	19.0 ± 0.02	1	20.4	104.6	1.05	0.82
				2	14.0	93.0	0.69	1.23

Temperature values are means ± SE

2.3.2 Fish size and behaviour

Fish size ranged from 14.0 to 23.2 kg and from 93 to 117 cm (Table 2.1). Fish were observed swimming slowly as a pair within the mesocosm. Mean SBT swimming velocity across all trials was 1.1 ± 0.1 LF sec^{-1} (n = 20) (Table 2.1). Eight of the ten fish fed within the mesocosm when offered either a pellet or baitfish feed immediately after a trial. Further, three of the fish took a baited hook.

2.3.3 Background trials

Oxygen was consumed in all background trials. Net background respiration (i.e. respiration > photosynthesis) increased over the experimental period (22 h) from 0.009 mg O_2 $l^{-1}.h^{-1}$ on day 1 to a maximum of 0.024 mg O_2 $l^{-1}.h^{-1}$ on day 26 (Fig. 2.3). It is believed that this increase in background respiration during the experimental period was due to a proliferation of zooplankton within the mesocosm. All respiratory trials were adjusted for background respiration according to the linear relationship (R^2 = 0.92) of the increase in background relative to the experimental day. Calculated background respiration for SBT respiratory trials ranged from 0.013 mg O_2 $l^{-1}.h^{-1}$ for trial 1 to 0.025 mg O_2 $l^{-1}.h^{-1}$ for trial 5 (Table 2.2).

Fig 2.3 Mesocosm background respiration rates (R_{mb})(averaged over 21 h) throughout the experimental period.

R_{mb} = 0.0007x Day + 0.0075, R^2 = 0.9232

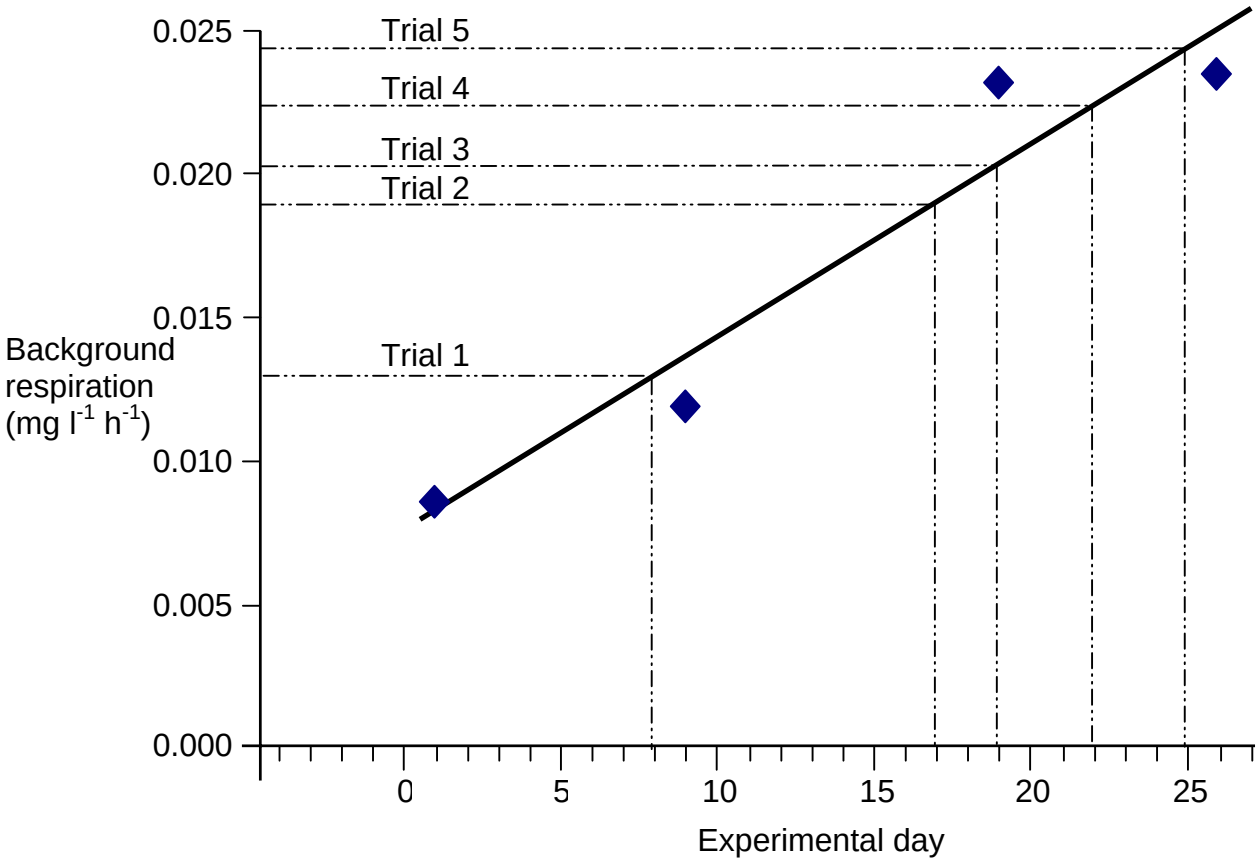


Table 2.2 Summary of external oxygen level, start and end point oxygen levels, calculated background respiration for the five SBT respiratory trials.

Values are means ± SE, n = 11/trial except for Trials 2 and 3 (*) where n = 9 due to data-logger failure and for external O_2 data where n = 2/trial.

Trial	External O_2 (mg O_2 l^{-1})	Start O_2 (mg O_2 l^{-1})	End O_2 (mg O_2 l^{-1})	Calculated Background Respiration (mg O_2 $l^{-1}.h^{-1}$)
1	7.32	9.06 ± 0.04	7.20 ± 0.25	0.013
2	7.44	7.20 ± 0.02*	6.02 ± 0.03*	0.019
3	7.47	7.88 ± 0.02*	6.92 ± 0.02*	0.020
4	7.32	7.31 ± 0.11	5.93 ± 0.16	0.022
5	7.32	8.22 ± 0.16	6.90 ± 0.15	0.025

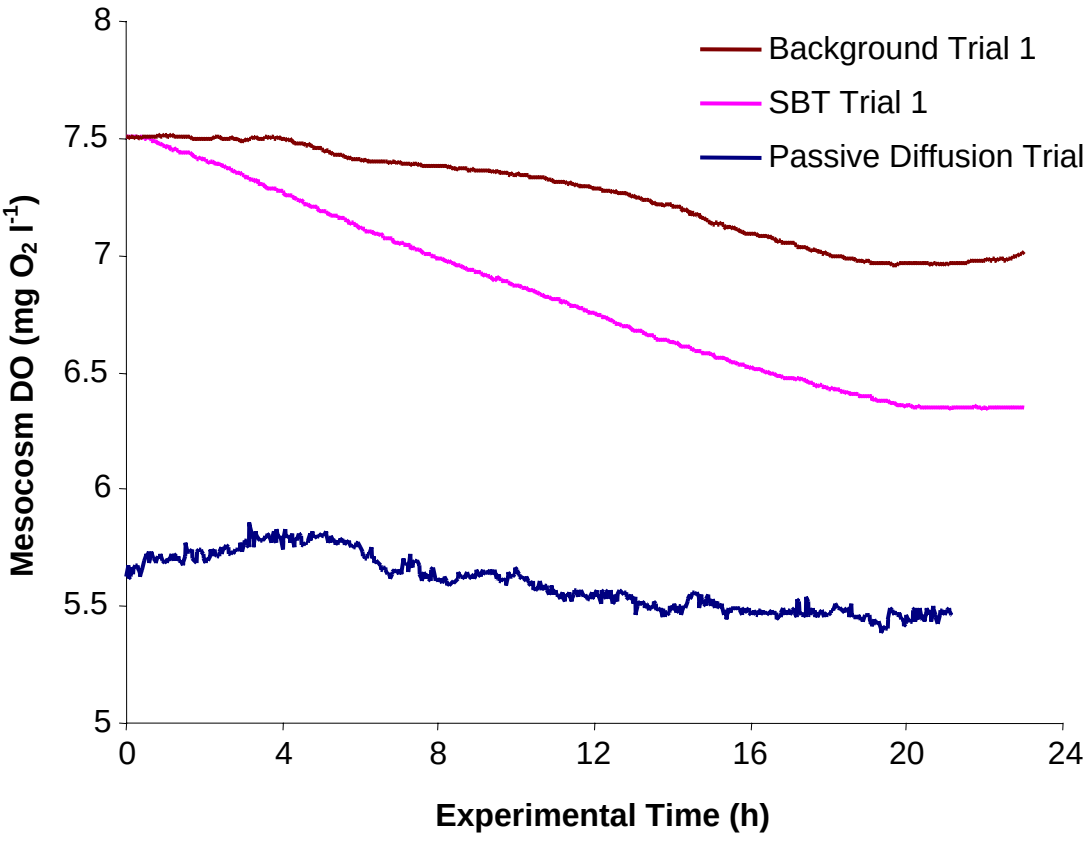
2.2.4 Passive diffusion trial

The combination of sodium sulphite and cobalt chloride dropped the DO saturation of the mesocosm to approximately 5.7 mg O_2 l^{-1}. Over the 21 h trial, the oxygen content of the mesocosm dropped by a further 2.2% (0.008 mg O_2 $l^{-1}.h^{-1}$), most likely due to background respiration (Fig. 2.4). This rate of oxygen consumption is less than predicted from the background trials (Day 27; 0.026 mg O_2 $l^{-1}.h^{-1}$) where there was no initial oxygen gradient between the mesocosm and the outside environment. At this oxygen gradient extreme the calculated passive oxygen diffusion rate through the mesocosm material was 0.0026 - 0.008 = 0.018 mg O_2 $l^{-1}.h^{-1}$. When represented as passive diffusion rate per oxygen partial pressure difference between the mesocosm and outside environment this equates to 0.0005 mg O_2 $l^{-1}.h^{-1}.torr^{-1}$. This diffusion rate factor was used to progressively adjust all respiratory trial 2 min samples according to the pertinent oxygen gradient between the mesocosm and the external environment. For data collected with the portable oxygen electrode, it was assumed that the oxygen consumption rates were recorded as 2 min samples in a linear relationship between the start and finish samples. Calculated net passive diffusion (i.e. influx - efflux) of oxygen in SBT respiratory trials was generally low (-0.172 to 0.245 mg O_2) (Table 2.3), but varied considerably depending on the initial DO.

Table 2.3 Calculated total passive diffusion over the experimental period for the five SBT respiratory trials.

Trial	Calculated Total Passive O_2 Diffusion (mg O_2)
1	0.245
2	-0.172
3	-0.051
4	-0.164
5	0.058

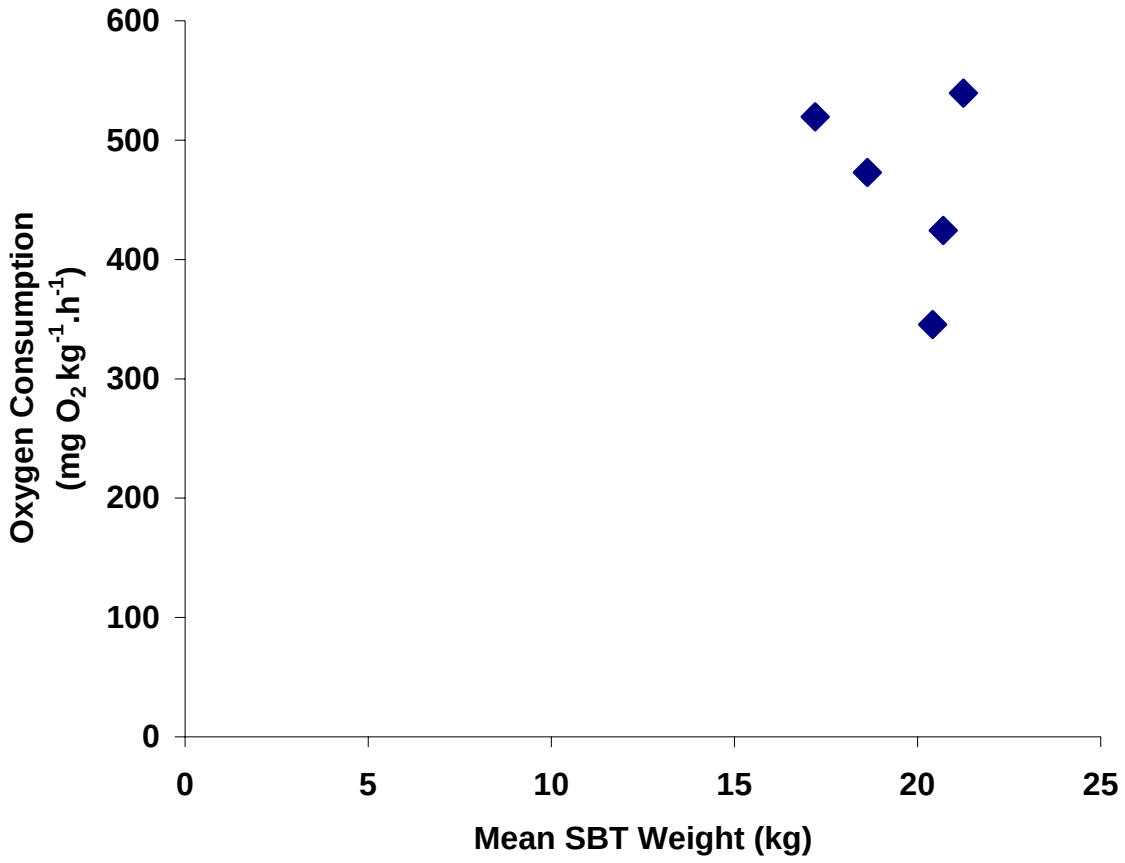
Fig 2.4 Mesocosm dissolved oxygen (mg O_2 l^{-1}) logged throughout the first SBT respiratory trial, the first background trial and the passive diffusion trial.



2.2.5 Respiratory trials

Figure 2.4 demonstrates the drop in mesocosm DO level as recorded for SBT respiratory trial 1 relative to background trial 1 and the passive diffusion trial. The relationship between these three factors was used to calculate the SBT oxygen consumption rates for each trial (as discussed above). The calculated oxygen consumption rates of the SBT examined when corrected for background respiration and passive diffusion ranged between 345.5 and 539.3 mg O_2 $kg^{-1}.h^{-1}$ (Figure 2.5) corresponding to a mean of 460.3 $\pm$ 34.9 mg O_2 $kg^{-1}.h^{-1}$ (n = 5).

Fig 2.5 Oxygen consumption rate for the five trials completed when adjusted for background respiration and passive diffusion.

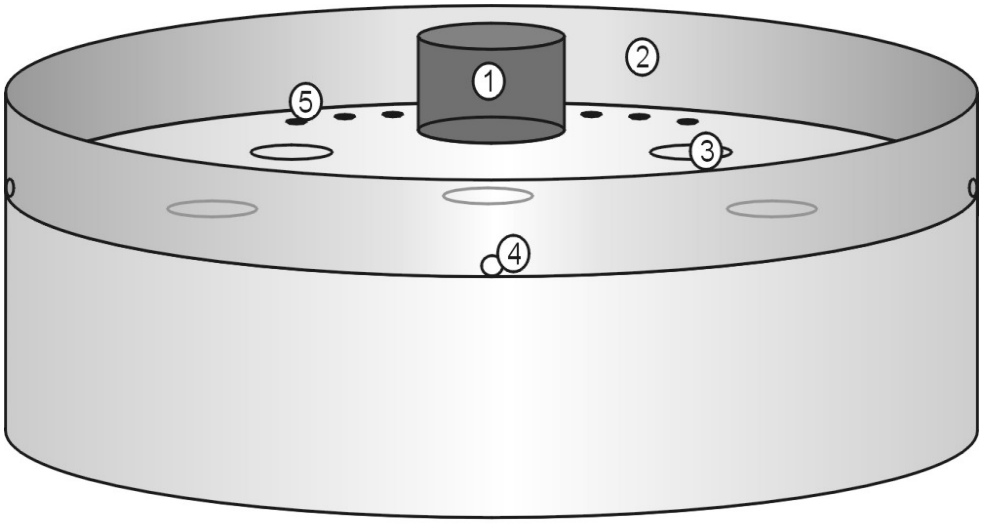


2.3 DISCUSSION

The Mesocosm

The mesocosm performed very well given the conditions to which it was exposed. Its inherent flexibility allowed it to withstand 30 knot winds and 1 m swells on several occasions without any obvious signs of stress. Neither the effects of background respiration nor passive diffusion were sufficient to obscure SBT respiration data, allowing the collection of valuable information which has led to the continuation of this work through a second project recently funded by the Aquafin CRC. A second mesocosm has been constructed (Fig 2.6) with the following modifications to reduce costs and improve handling efficiency and general operation.

Fig 2.6 Mesocosm respirometer MkII



1 Top port
2 Wave skirt
3 Windows
4 Non return valves
5 Equipment ports

The surface window diameter has been reduced from 2 m to 1 m diameter. This will reduce incident light, and therefore associated phytoplanktonic growth and zooplanktonic growth, both of which contribute to background respiration. The 5^{o} taper in the sides of the mesocosm, the bottom port and the sidewall rope handles have been omitted as unnecessary. Five strong attachment points have also been added to the inside of the floor of the mesocosm to allow use of a central buoy to improve stability, and nets, when necessary, to handle SBT. The external rim of the top port has been reinforced and stainless steel eyelets installed at 250 mm intervals around its circumference. Extra equipment ports have been added to the mesocosm roof to allow access for camera equipment and oxygen probes and an extra 50 mm port has been

added to the wall below the access port near the junction with mesocosm bottom to facilitate drainage of residual water from the mesocosm upon emptying. Finally, four non-return valves (valve opening to outside) have been inserted in the wave break wall, close to the mesocosm roof, to allow for exit of water collecting on the top surface via wave action and rain.

These modifications will improve the operational efficiency and handling of the original mesocosm and through project Aquafin CRC-FRDC 2005/200 enable a significant contribution to be made to the bioenergetics of SBT under commercial culture conditions.

SBT in the mesocosm

The SBT oxygen consumption rates estimated in the mesocosm respirometer represent the fish's SMR plus the metabolic cost associated with the maintenance of a mean swimming velocity of 1.1 LF sec^{-1}. Table 2.4 outlines the relationship between oxygen consumption (VO_2) and swimming speed (U) for previously-studied free-swimming tuna species and sockeye salmon, and the predicted RMR for each species when swimming at 1.1 LB sec^{-1}.

Table 2.4 Summary of study temperature (T), mean body mass, with corresponding Vo_2 relationship and predicted routine metabolic rate (RMR) and for each species at swimming speed (cm sec^{-1}, U) of 1.1 LB sec^{-1} for three species of tuna and the sockeye salmon.

Species	T (^oC)	Length (cm)	Mass (kg)	Vo_2 (mg O_2 h^{-1})	U (cm sec^{-1})	RMR (mg O_2 $kg^{-1}.h^{-1}$)
Yellowfin tuna (*Thunnus albacares*)[1]	25	51 ± 0.866	2.17 ± 0.14	$545.10^{0.0045U}$	61.2	473.6
Albacore tuna (*Thunnus alalunga*)[2]	13 - 18	87	8.5 - 12.0	$2574.10^{0.003U}$	104.4	516.7
Skipjack tuna (*Katsuwonus pelamis*)[3]	23 - 24	47 ± 1.20	1.96 ± 0.31	$571.10^{0.0038U}$	56.4	472.4
Kawakawa Tuna (*Euthynnus affinis*)[4]	24	19 ± 1.35	0.13 ± 0.03	$90.10^{0.0046U}$	23.7	882.6
Sockeye salmon (*Oncorhynchus nerka*)[5]	15	53.9	1.43	$60.10^{0.0086U}$	64.7	134.9

[1]Dewar and Graham, 1994, [2]Graham et al, 1989, [3]Gooding et al, 1981, [4]Sepulveda and Dickson, 2000, [5]Brett, 1965. Mass and Length data are Means ± SE.

The mean oxygen consumption rate of 460.3 mg O_2 $kg^{-1}.h^{-1}$ for SBT recorded in the present study is far greater than that of sockeye salmon but similar to the four tuna species shown, suggesting that the measured metabolic profile of SBT is in line with the other members of the *Thunnini* tribe. From the data available from studies with both free swimming and immobilised individuals, it is evident that the aerobic metabolic capacities of *Thunnini* tunas are higher than those of most co-familial and more distantly-related species studied (Brill 1979; Brill 1987;

Dewar and Graham 1994). The SMR's of tuna are generally at least twice those of other active species, probably a consequence of physiological adaptations such as large gill surface area, enhanced cardiac output, and elevated muscle temperature that allow tuna to achieve great aerobic scopes (Brill and Bushnell 1991; Bushnell and Jones 1994). Only the dolphin fish (*Coryphaena hippurus*), an extremely fast-growing active pelagic species with a gill surface area equivalently to tuna, has been recorded to have a similar SMR (Benetti et al. 1995)

It is difficult to swim active ram-ventilating fish within controlled environments for metabolism studies, indeed, for some species it has proven to be impossible (eg Benetti et al. 1995), and for the remaining species tested, it has been restricted to small individuals (Dewar and Graham 1994; Sepulveda and Dickson 2000; Sepulveda et al. 2003). The mesocosm respirometer offers an alternative that can accommodate much larger individuals, but with some loss of control, in particular that of swimming velocity and metabolic measurement-interval acuity. The mesocosm allows the measurement of routine metabolic rate (RMR) where RMR is influenced by random activity under experimental conditions in which fish movements and behaviours are presumably somewhat restricted and fish are protected from outside stimuli (*sensu* Fry 1971). Routine metabolic rate data are the most commonly derived metabolic information from fish (Fry 1971) but the SMR (i.e. RMR scaled back to a theoretical point of complete rest) has become more accepted as it allows better standardization of metabolic data and comparison between studies and species (Schmidt-Nielsen 1984; Korsmeyer and Dewar 2001). However, complete rest for tuna is physiologically impossible. Tuna are both negatively buoyant and obligate ram ventilators and must always swim to maintain position in the water column and ventilate their gills (Brown and Muir 1970; Magnuson 1973). Thus, the use of the SMR in tuna physiology research complicates the required experimental design and limits the physiological relevance of the studies. Published studies recording SMR of other tuna species have used fish paralysed with neuromuscular blocking agents (Brill 1979; Brill 1987; Dewar and Graham 1994) or have extrapolated the VO_2 relationship to a swimming velocity of zero (Dewar and Graham 1994). Validation for both techniques was recorded by Brill (1987), who found that SMR of aholehole (*Kuhlia sanduicensis*) and rainbow trout (*Oncorhynchus mykiss*), measured by neuromuscular blocking, are not significantly different from published data on standard metabolic rates estimated by extrapolating VO_2 back to zero velocity. However, it is questionable whether a comparison of two relatively small, neutrally buoyant, pump-ventilating, exothermic fish species can be used to validate a technique with negatively buoyant, ram-ventilating and endothermic species such as tuna.

Minimisation of stress is critical when working with large, highly responsive, sensitive animals such as tuna. Steady swimming rates and willingness to feed within the mesocosm respirometer suggested that experimental SBT were not overly stressed. The swimming

velocities recorded in the present study were within the range recorded for undisturbed northern bluefin tuna within large marine pontoons (0.6-1.2 LB sec^{-1}; Wardle et al. 1989). Stress levels within the spacious, marine pontoon-based environment of the mesocosm are likely to be much lower than those measured using more traditional teleost metabolic physiology technologies such as land-based water tunnel respirometers. Thus, the non-stressed and post-absorptive routine metabolic rate measured in the present study is probably a good way of standardizing metabolic data with tuna. The metabolic states of the tuna examined in the present study are likely to be fairly physiologically representative of unfed SBT swimming at typical swimming speeds within aquaculture cages.

CHAPTER 3 RESPIRATORY TAG DEVELOPMENT

3.1 INTRODUCTION

The second objective of this project was to identify and develop methodology to make reliable, realistic and repeatable measurements of metabolic rate in SBT under commercial conditions. This involved an assessment of whether a small device, to be called a respiratory tag, could be developed for attachment to a SBT in a commercial cage to indirectly provide information on metabolic rate by measurement of oxygen consumption. The device was to be a modified archival tag carrying two small oxygen electrodes, fixed to the fish with a clip on or near the operculum (Fig. 3.1). The optimum position for the archival tag on the fish was to be determined during the study. One electrode would project into the gill cavity and monitor downstream oxygen levels; the other would monitor the water in which the fish was swimming and the data would be stored on an archival tag for later retrieval and analysis. An additional probe would be fitted to the archival tag to monitor water temperature.

A feasibility study was carried out to evaluate the above combination using micro-cathode oxygen electrodes. The study was to include sourcing/modification of required technology as well as flume tank trials of a prototype tag to look at attachment and placement issues. Yellowtail kingfish (YTK; *Seriola lalandi*) were to be evaluated for the flume tank work as easier to handle and cost effective surrogates for SBT. They are also similar to tuna in pelagic habit and ventilatory style (YTK are facultative ram ventilators). Once evaluation had been carried out, the equipment and techniques would, if proven, be transferred to SBT in the second project of the series.

It was necessary to establish metabolic parameters for YTK before testing and calibration of respiratory metabolic tag components. This work was done in conjunction with the requirements of FRDC project 2003/222 “Innovative solutions for aquaculture planning and management – Project 2a, Spatial impacts and carrying capacity: Further developing, refining and validating existing models of environmental effects of finfish farming”. FRDC 2003/222 details the relationship between metabolic rate and swimming speed and temperature and investigates the metabolic cost of digestion in YTK. The present project required these data but only as a baseline from which to carry out work on the respiratory tag.

Fig 3.1a Respiratory tag concept diagram

(blue arrow = water stream through mouth and over gills)

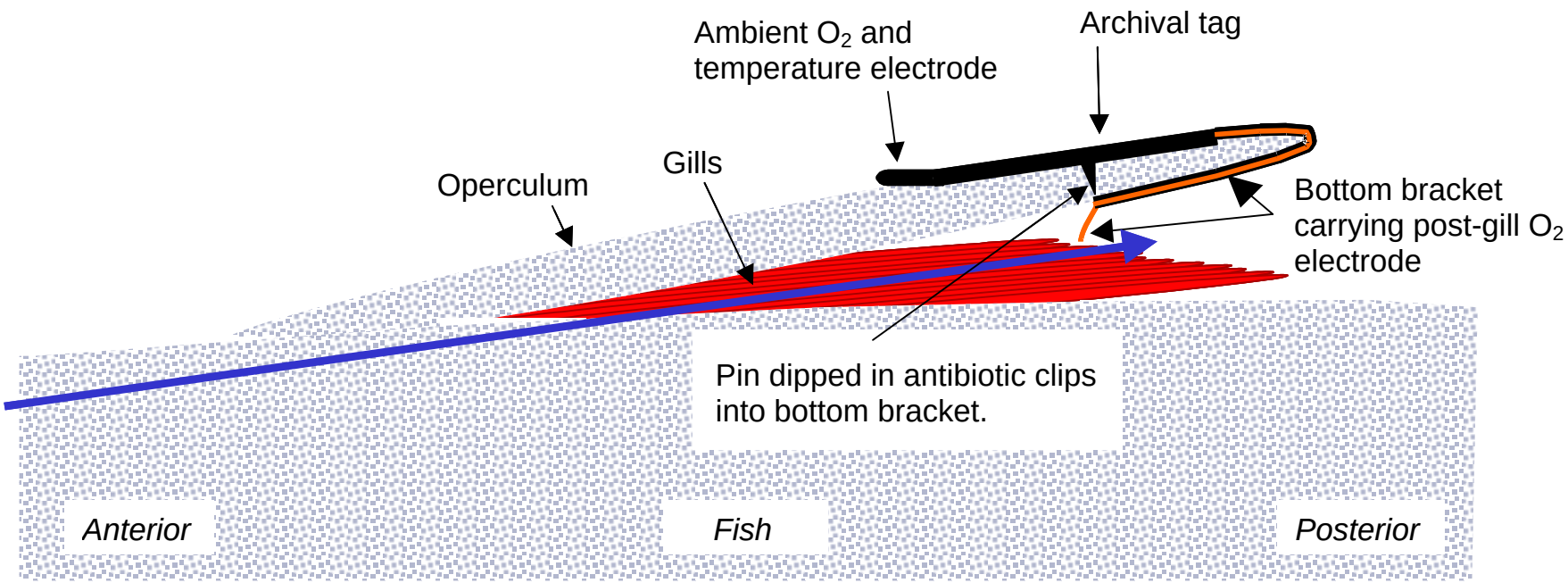
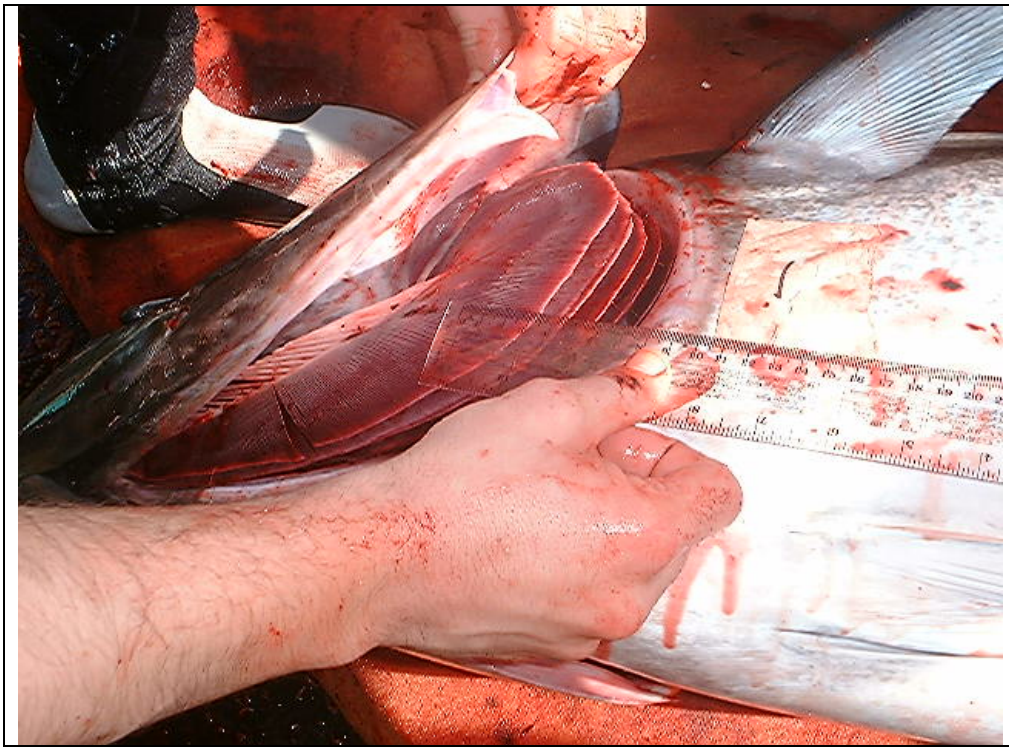


Fig 3.1b: The gill cavity of a 42 kg SBT



3.2 METHODS

The feasibility study was to involve the following 5-stage process. The first two were carried out as follows:

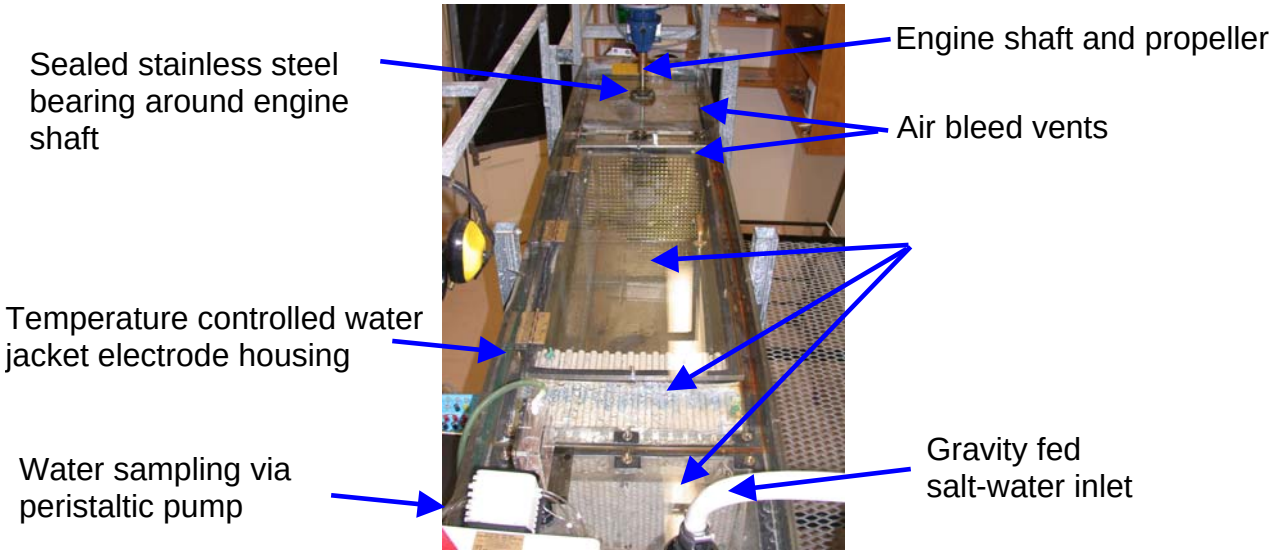
Appropriate literature was reviewed to determine the requirements for such a tag.

Technological feasibility was determined in two stages as follows:

i) Flume trials

Flume trials were carried out to test YTK's suitability as a surrogate for SBT. The flume trials were carried out in a flume tank situated in the respiratory physiology laboratory at the University of Adelaide. (Figs. 3.2a and b).

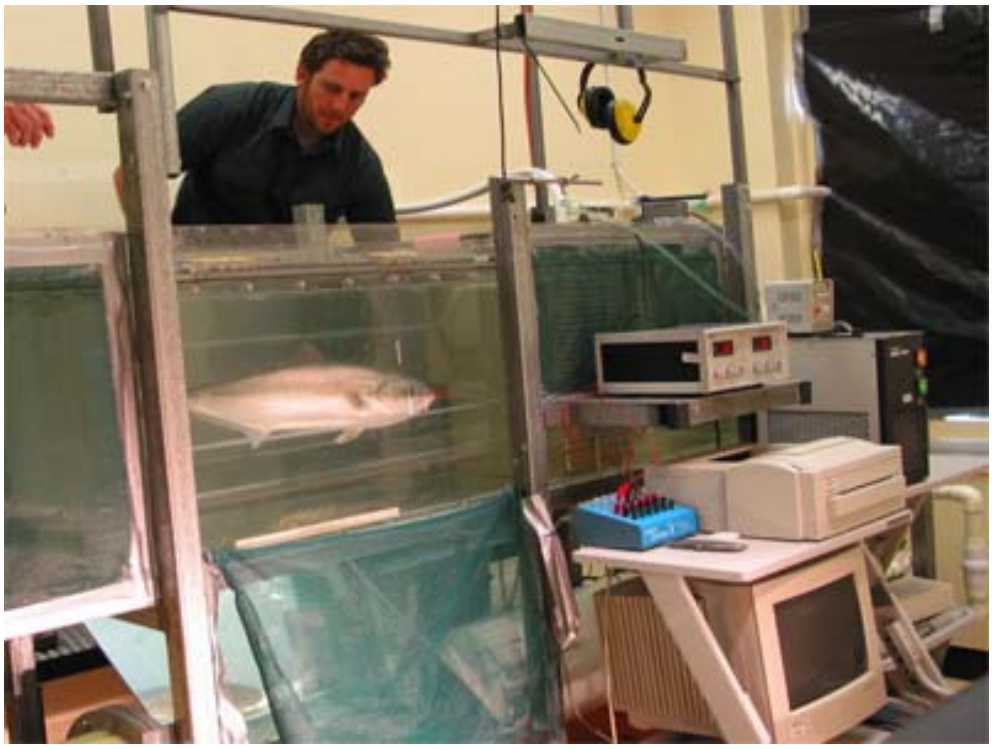
Fig 3.2a Flume tank used for YTK trials.



In December 2003 the respirometer was calibrated with respect to flow hydrodynamics and oxygen-holding capabilities. Methods required to measure metabolic rates of YTK were developed in January 2004. The respirometer was used to record the metabolic rates of YTK between 950 and 4350 g with respect to swimming velocity and size. These data allowed calculation of the mass-scaling coefficient for YTK at summer sea water temperatures (22^{o}C).

Fig 3.2b The flume with a YTK in the swim chamber.

The flume was covered in black plastic and the room lights turned off during trials.



ii) Sourcing and assembly of tag components.

It was intended that the prototype tag's components be sourced and assembled with the assistance of the Adelaide-based engineering company Measurement Engineering Pty Ltd. The study was curtailed at this point for the reasons discussed below. The following three stages were not carried out as proposed.

Biological feasibility testing. The hard-wired components of the respiratory tag were to be tested on YTK as a surrogate species for SBT.

A prototype respirometry tag was to be produced.

The respiratory tag would then be scaled-up for use with SBT and tested and calibrated in the mesocosm.

3.3 RESULTS AND DISCUSSION

3.3.1 Literature Review

Archival tags as their name suggests, store data. They are basically miniature computers incorporating 8 or 16 bit microprocessors, memory and 3 - 4 environmental sensors (Gunn and Block, 2001). They have low power budgets allowing data to be collected every few minutes for periods of up-to 5 years. To date environmental parameters that archival tags are able to measure include powerful light, depth and temperature sensors. From these 3 environmental factors, researches have used archival tags to estimate migratory, behavioral and physiological patterns of tuna over extended periods (reviewed by Gunn and Block, 2001).

The archival tag proposed by the present study was to slip over the operculum of the fish like a paper clip (Fig 3.1). It was to incorporate two oxygen sensors; one recording the oxygen concentration of the water passing the outside of the operculum (Ci – oxygen concentration of inhalant water), and the second recording the oxygen concentration of the water leaving the gills within the opercula (Ce – oxygen concentration of exhalent water). The difference between Ce and Ci (U) would be regarded as the fraction of oxygen removed by the gills. By estimating the quantity of water perfusing the gills (Vg - ventilation volume), total oxygen consumption (O_2) could be determined by using the “Fick principle” (Rahn, 1966):

$$O_2 = Vg.(Ci - Ce)$$

Researchers have previously used the Fick principle to determine oxygen consumption of tuna using a variety of experimental designs (Stevens, 1972; Jones et al., 1990, Bushnell and Brill, 1991). In most studies, evaluation of Ci is relatively easy, however determination of Ce proves to be less simple. Historically, researchers have used two methods of determining Ce. The first involves the use of a plastic membrane tightly around the fish’s snout behind the mouth to separate incoming water from the water that passes throw the gills (Stevens, 1972). The second method uses catheters inserted through the skin at the posterior margin of the opercula and directed into the opercular cavity to sample exhalant water before it leaves the gills (Jones et al., 1990, Bushnell and Brill, 1991). The drawbacks of these techniques are that both are likely cause considerable stress to the fish, and to restrict its movement. The use of the plastic membrane requires the fish to be restrained (lightly anesthetized) and force ventilated. If catheters are inserted, the fish must tow these around the tank while a researcher follows to take the samples.

Estimation of tuna gill ventilation volume has proven equally difficult. Tunas are ram ventilators, that is, the mouth is held open while swimming (Brown and Muir, 1970) allowing water to flow over the gills. Medo and Pauly (1988) proposed that ventilation volume of bonito (*Sarda*

chiliensis) could be simply estimated by multiplying the mouth gape cross-sectional area by the fish's swimming speed. The limitation of this method is that it assumes the buccal cavity and gills offer no resistance to the incoming water. However, studies on restrained tuna show that gills do resist ventilation flow and furthermore, this resistance is not consistent and can be regulated by the fish (Stevens, 1972). Stevens (1972) prevented skipjack and kawakawa tuna regulating ventilation rate by changing mouth gape, and concluded that the observed resistance was the sum of the resistance offered by the gills, and that the variation in resistance to flow was due to changes in the opercular slit (Stevens, 1972).

Jones et al. (1990) performed the first direct measurement of ventilation volume in swimming tuna by using a continuous infusion dye-dilution technique. This technique involves the continuous and consistent dispersal of a fluorescent dye into the buccal cavity of a swimming tuna. Catheters inserted into the posterior region of the opercular cavity then sample the dye and ventilation volume is determined by the dilution of the sampled dye. In this study it was found that ventilation volume of tuna studied was not related to swimming speed, thus mouth gape or opercular resistance determined ventilation volume.

Jones et al. (1990) also found that the flow result could vary depending on the position of sampling catheters, dorsal, medial or ventral on the left or right opercular margin. At the extreme, oxygen utilization could vary by up-to 20% depending on catheter position. Jones et al. (1990) attributed this variation to differences in either water flow over the gills or blood flow through various regions of the gills. A similar effect of catheter position on oxygen extraction efficiency of the gills has also been observed in other species of fish (Davis and Watters, 1970). However, the significance of the effect of sampling catheter position is debatable. On closer examination of the results produced by Jones et al. (1990), it can be seen that near 60% of flow results for individual trials were within 5% of each other irrespective of catheter position. Also, the variation recorded by Jones et al. (1990) is based on a single sample/region. Oxygen utilization recorded from a singular cannular in a rainbow trout has been shown to vary with time (Davis and Watters, 1970). Therefore, replication of samples potentially may buffer flow variation between sampling positions. This argument is strengthened by Bushnell and Brill (1991), who by the use of two sampling catheters (left and right side) and 3 sets of samples, obtained VO_2 results for yellowfin tuna comparable to results obtained in a traditional tunnel respirometer (e.g. Dewar and Graham, 1994).

A limitation of using the Fick principle as a method to measure oxygen consumption in fish is cutaneous oxygen uptake. The Fick principle assumes that all oxygen uptake in fish occurs through the gills, however oxygen uptake through the skin (or cutaneous uptake) has been shown to be a substantial component of the overall oxygen uptake of fish. During normoxia, the cutaneous oxygen uptake of the marine fish species tested, have been shown to vary between

5 and 27% of the fish's total oxygen uptake (Nonnotte and Kirsch, 1978; Steffensen et al., 1981). The cutaneous oxygen uptake component for pelagic fish (including tuna) has never been tested. However, when using the Fick principal, a correction can be made for cutaneous uptake as it has been found to be constant and steady within normal oxygen ranges (Moigne et al., 1986; Kalinin et al., 1999). In order to define cutaneous oxygen uptake for a fish species, simultaneous comparison must be made between total VO_2 (recorded in a respirometer) and gill O_2 extraction recorded using the Fick principle (Kalinin et al., 1999).

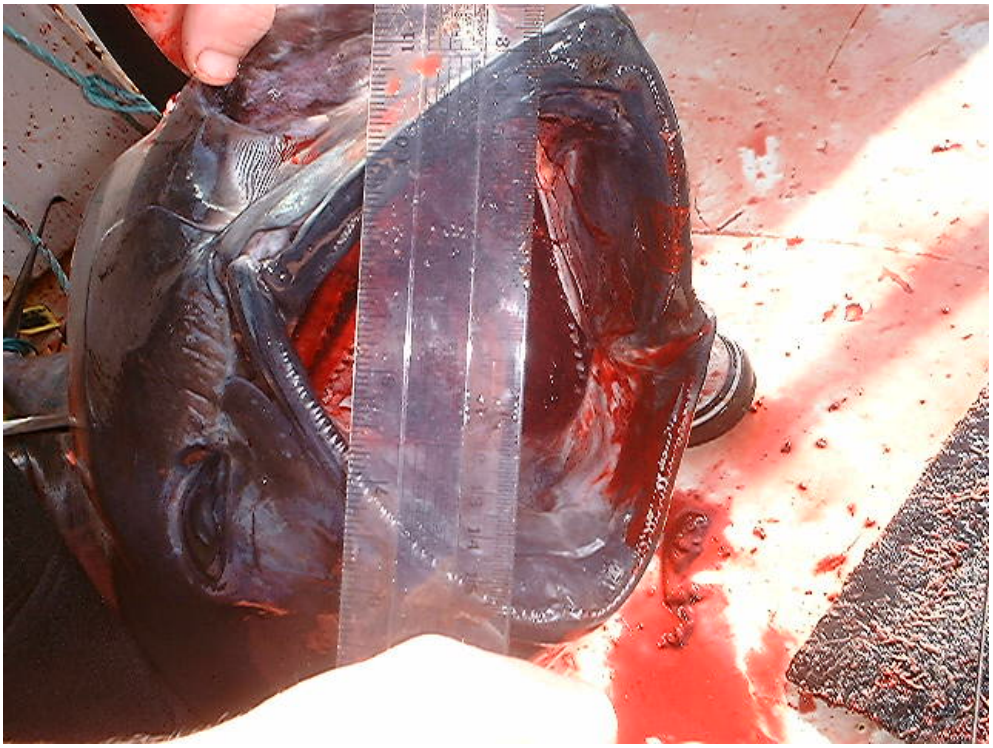
Thus, to measure VO_2, an archival tag must, reliably and non-restrictively, measure ventilation volume, sample frequently to reduce irregularity in gill oxygen removal and take into account cutaneous oxygen uptake. Very few studies have directly measured ventilation volume in free-swimming fish. The first study to directly measure ventilation volume in fish used electromagnetic blood-flow transducers that were passed through cannulars into the buccal and opercular cavities of carp (*Cyprinus carpio*) (Holeton and Jones, 1975). Since then, thermal anemometry probes have been used to measure ventilatory flow in two pump-ventilatory species (bass; *Micropterus salmoides* and blackfish; *Orthodon microlepidotus*) and one ram-ventilating species (paddle fish; *Polyodon spathula*) (Lauder, 1984; Sanderson et al., 1991; Sanderson et al., 1994). In all of the above experiments, buccal flow probes were passed through cannulars implanted into the neurocranium (on the fish's nose) or through the hyomandibular bone (cheek before the gill rakers). Probes had to penetrate the buccal cavity by >1.5 mm to overcome flow-frictional effects from the sides of the mouth and there was no significant difference if the probes were placed through the neurocranium or hyomandibular (Sanderson et al., 1994).

The use of flow probes allows the measurement of water flow velocity (V) at the probe position but not ventilation rate. Total water flow through the gills (Vg) is equal to V multiplied by the cross sectional area (CSA) of the cavity at the probe (Vogue, 1994). Therefore, in order the measure ventilation rate, the CSA of the cavity at the site of the probe must be known. With pump-ventilatory species, using the above relationship to measure ventilation volume is difficult because of the extensive variation in magnitude of peak velocities within the respiratory tract, and the variation in velocity with time (Lauder, 1984). However, this variation in velocity with time and position will not be present with ram ventilating species such as YTK and SBT. Unfortunately, the only study to measure respiratory flow velocity in a ram-ventilating species did not estimate total ventilation volume (Sanderson et al., 1994). To allow the examination of the relationship between localized flow velocity and total ventilation flow, the probe must be positioned where the CSA cannot change. The buccal cavity (Fig 3.3) is the most likely position, as the fish cannot regulate the CSA (unlike the opercular cavity). Furthermore, the CSA of the buccal cavity is likely to be related to body length and thus, the buccal CSA for an individual can be predicted if the relationship for the species is defined. Evaluating the relationship between

buccal CSA and body length can be done by the physical examination and measurement of the buccal cavity in a wide range of fish sizes.

Fig 3.3 The buccal cavity of a 42 kg SBT

The distance between the corner of the mouth and the gill rakers is approximately 10 cm. It was envisioned that buccal flow velocity would be sampled from this position.



Thus, the brancial oxygen consumption of the fish (BVO_2) can be determined by use of the Fick principle by the following equation:

$$BVO_2 = V \,.\, CSA\ (Ci - Ce)$$

However, this calculation does not take into account cutaneous oxygen uptake (CVO_2). By simultaneously measuring oxygen consumption of the fish in the respirometer (VO_2) and using the tag to measure BVO_2 by the Fick principle, the cutaneous oxygen uptake component for the fish can be determined by the following relationship:

$$CVO_2 = VO_2 - BVO_2$$

This was to allow the examination of the cutaneous component of oxygen uptake in YTK (tunnel respirometer) and SBT (mesocosm experiment). The relationship between fish body length and cutaneous oxygen uptake can then be examined with experimental replication over a wide range of fish sizes. If cutaneous uptake is found to be constant and related to body length,

cutaneous oxygen uptake could be simultaneously accounted for by using the reverse Fick principle to calculate a cutaneous oxygen uptake adjusted CSA (CSAa):

$$CSAa = (VO_2 / V) / (C - Ce)$$

The relationship between CSAa and body length could again be determined, with replication, allowing automatic adjustment for cutaneous oxygen uptake in field trials of the tag.

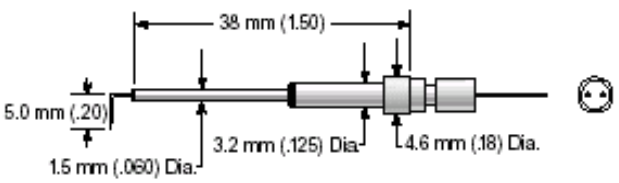
The Sensors

The sensors used with the tag must (i) be compatible with the archival tag, (ii) be small and light weight, producing minimal drag, (iii) be robust enough to withstand salt-water immersion and tuna movement, (iv) have the capacity to measure the desired parameters within the specified range, and, finally, (v) have small power and voltage requirements.

Thermal anemometry probes, similar to that in previous studies, may be suitable to measure ventilation flow. Thermal anemometer probes measure fluid velocity by sensing changes in heat transfer from a small electrically heated sensor. This heated sensor is thermostatically controlled to maintain a particular temperature. Thus, the cooling effect of fluid flowing past the sensor is compensated by an increase in current flow to the sensor. The magnitude of the current required to keep the temperature consistent is directly related to heat transfer and thus flow velocity. Thermal probes come in a variety of sizes and shapes that may make them physically suitable for this application. A diagram with specifications of a possible probe is shown in Fig 3.4. This type of probe comes in a wide range of sizes and its right angle tip would make it suitable to pass through the operculum into the buccal cavity. The body of the probe could then be secured to the side of the fish's head. Unfortunately, thermal anemometry probes have a large power requirement, creating a potential problem, as they must be continuously supplied with energy to ensure that the thermistor bridge is in a steady state (Voegeli and Pincock, 1980).

Fig 3.4 TSI Incorporated 1262A miniature thermal anemometer sensor

(TSI, Internet).



Clark-type oxygen electrodes are the most widely used oxygen sensors. These sensors are based on the reduction of oxygen at a working electrode and are dependant on the diffusion

of oxygen across a permeable membrane. In recent years, Clark-type sensor have been miniaturized and come in a variety of sizes. An example of a potential oxygen electrode that may be useful for this application is shown in Fig 3.5.

Fig 3.5 The SDR Clinical Technology durable oxygen electrode

The electrode is designed to be robust and to measure oxygen concentration in small volumes of liquids (SDR, Internet).

In salt-water, Clark oxygen electrode membranes may be subject to server modification that results in signal drift. Some suggest that this makes Clark type electrodes unsuitable for prolonged marine application without recalibration (Gouin et al., 1997). However, a new fiber-optic oxygen sensor technology has recently been praised as extremely useful for aquatic applications (Klimant et al., 1995; Gouin et al., 1997). Fiber-optic sensors are based on the dynamic quenching of a fluorescent indicator by oxygen. The advantages of fiber-optic oxygen micro sensors are that (i) they are small and light weight, (ii) they show excellent long term stability, (iii) they are not reliant on stirring, (iv) other chemicals (hydrogen sulfide or carbon dioxide) do not interfere with the measurements, (v) they are highly resistant to biofouling and (vi) they do not consume oxygen (Klimant et al., 1995). Fiber-optic probes are produced by several companies and to a variety of specifications. However, it is unknown whether fiber-optic sensors are compatibile with archival tags. Examples of potential fiber-optic oxygen sensors are shown in Figs 3.6 and 3.7. The micro-flow type probe could be passed through the opercula and directed into the exhalent water flow. A small flat, flared catchment cone could also be attached to the T inlet, thus directing water flow over the probe from a wider cross section of the gill. This could potentially reduce variation in oxygen measurements due to inconsistencies in oxygen removal from different areas of the gill.

Fig 3.6 The OxyMicro Systems micro tip fiber-optic oxygen sensor

The oxygen sensor tip consists of a 140 μm fiber tapered to a 50 μm tip housed in a 22 mm stainless steel needle (WPI, Internet).

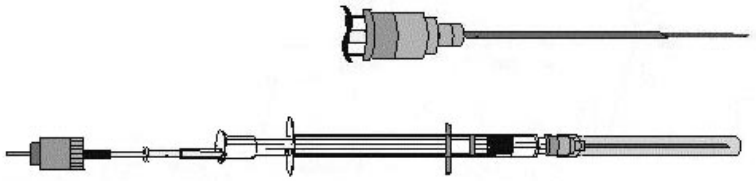
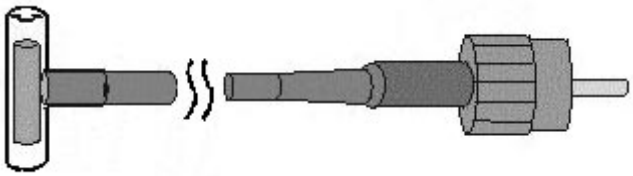


Fig 3.7 The OxyMicro Systems micro flow fiber-optic oxygen sensor

The 50 μm tip is integrated in a T-shaped flow cell (WPI, Internet).



Using available literature it was concluded that the respiratory tag using the Fick principle would be feasible. The tag would have to be small, non invasive and capable of carrying two oxygen sensors. In addition, the tag must estimate gill flow rate directly (e.g. using a flow probe) and facilitate exhalent water mixing before sampling.

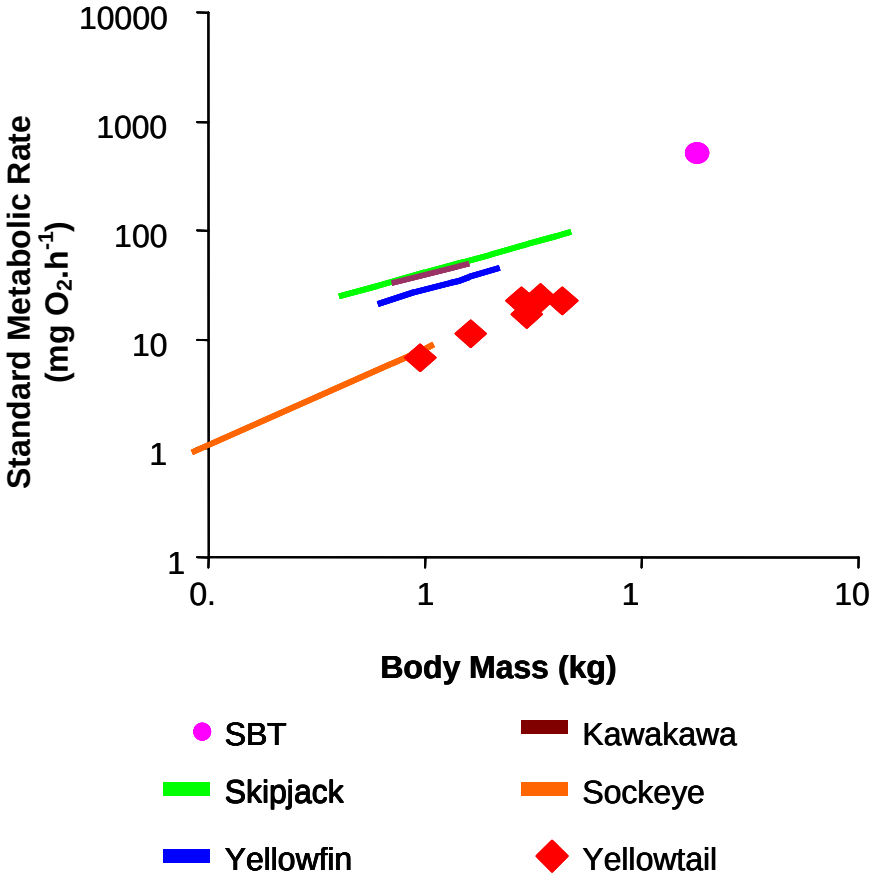
3.3.2 Technological Feasibility

3.3.2.1 Surrogate Testing

Analysis of flume tank data suggests that YTK have standard metabolic rates similar to sockeye salmon (Fig 3.8) but somewhat lower than the tunas tested to date. The latter notwithstanding they would be useful surrogates for SBT because of their relative ease of handling, their willingness to swim in the flume tank and their facultative ram ventilatory habit. The project now has the technology, methodology and experience required for development of the respiratory tag or other such technology using YTK as a surrogate to further SBT metabolism research. Detailed YTK metabolism results and discussion are presented in the final report for FRDC 2003/222.

Fig 3.8 Standard metabolic rates of active fish species including new data from YTK (FRDC 2003/222) and present study (incl SBT).

Tuna other than SBT were immobilised with a neuromuscular block. Note logarithmic scale on both axes.



3.3.2.2 Sourcing and assembly of tag components

After a thorough investigation of all equipment sources it was concluded that we could not progress to (3) Biological feasibility testing, because we could not assemble the components of the tag within existing time and budgetary constraints. The technical constraints on advancement were as follows. The available electrodes were not stable enough to be used for the intended respiratory tag. The power requirements of the proposed device, given the necessity to measure oxygen tension and water flow over the gills, were too great to be practical for a tag to be attached to the gill operculum of an SBT. The oxygen extraction variability of the gills is likely to be too great to allow production of useful data taken from one point in the exhalant water stream. Miniaturisation of the equipment proved to be too complex and costly.

It is therefore unlikely that such a tag could be constructed. An alternative, in the form of an impedence tag designed to measure SBT heart rate, will be tested in the recently-funded Aquafin CRC-FRDC 2005/200.

VI. BENEFITS AND ADOPTION

The development of the mesocosm respirometer technology and the subsequent collection of data on metabolic oxygen demand from free-swimming SBT have opened the way for further physiological research to address several of the priorities identified in the SBT Strategic R&D Plan (2001-2006), namely:

- Nutritional studies
- Maintaining a healthy environment
- Improved farm husbandry and management practices resulting in increased production and product quality at reduced cost
- SBT health.

Although the respiratory tag was not developed as proposed in the original application the results of the project have been met with considerable enthusiasm by Tuna Boat Owners Association of South Australia and by other scientists involved in the Aquafin CRC, the latter particularly including those also addressing the abovementioned priorities.

VII. FURTHER DEVELOPMENT

The success of this pilot study led to the development and funding of a two year project (Aquafin CRC-SBT Aquaculture Subprogram: activity metabolism in live-held southern bluefin tuna (*Thunnus maccoyii*) Phase 2. FRDC 2005/200).

VIII. PLANNED OUTCOMES

The planned outcomes were as follows:

1. Efficient measurement of SBT metabolic rates in mesocosms.
2. Assessment of the feasibility of microsensors.
3. Increased depth of knowledge of SBT physiology allowing more informed husbandry decisions and improving farm efficiency.

Outcome 1 was achieved in full. We now have the experience and technology to efficiently measure the metabolic rates of large SBT in mesocosms. Outcome 2 was also achieved, however the combination of the archival tag and microsensor was found to be not feasible at

the present time. The development of an alternative (an impedence tag measuring heart rate) will be explored in the upcoming project (Aquafin CRC – FRDC 2005/200).

This project has added significantly to the body of knowledge on SBT physiology, as described in Outcome 3, but, more importantly it has opened the way for more in-depth studies that will provide detailed physiological information to a bioenergetic model, which will, in turn allow more informed husbandry decisions and improved farm efficiency for SBT aquaculture.

IX. CONCLUSION

The project objectives were as follows:

To measure SBT metabolic rates in situ in a mesocosm.

To identify and develop methodology to make reliable, realistic and repeatable measurements of metabolic rate in SBT under commercial conditions.

Objective 1 has been completed. A sealable mesocosm respirometer was developed for southern bluefin tuna (SBT; *Thunnus maccoyii*) metabolism studies and 5 trials run over 29 days in March/April 2004.

All fish survived the mesocosm trials without obvious signs of stress. SBT also fed while in the mesocosm and mean swimming velocity across all trials was 1.1 ± 0.1 LF sec^{-1}. Spot dissolved oxygen (DO) readings indicated the mesocosm was well mixed at all times. Passive diffusion and background respiration rates were in all cases less than 100^{th} of a percent of SBT oxygen consumption rates.

Calculated oxygen consumption rates of SBT, when corrected for background respiration and passive diffusion, ranged between 345.5 and 539.3 mg O_2 $kg^{-1}.h^{-1}$ corresponding to a mean of 460.3 ± 34.9 mg O_2 $kg^{-1}.h^{-1}$ (n = 5 trials).

These results demonstrate that the mesocosm respirometer can be a useful platform for respiration studies on commercial-sized SBT. The mesocosm is robust and flexible and aside from the deployment and retrieval, can be operated with a team of no more then three people, including boat skipper.

Objective 2 has been partially completed. Flume tank trials were successful, showing yellowtail kingfish to be useful potential surrogates for SBT in the development and testing of new technology, in this case the respiratory tag. Although progress on the latter was thwarted by the limitations in available technology, the equipment, methodology and experience accumulated during the flume trials can now be turned to the development of the impedence tag (heart rate monitor) as proposed in Aquafin CRC – FRDC 2005/200.

REFERENCES

Bennett W., A., Beitinger T., L. (1995) Overview of techniques for removing oxygen from water and a description of a new oxygen depletion system. The Progressive Fish-Culturist 57: 84-87

Benetti D. D., Brill R. W., Kraul Jr. S. A. (1995) The standard metabolic rate of dolphin fish. Journal of Fish Biology 46: 987-996

Benetti D. D., Nakada M., Minemoto Y., Hutchinson W., Shotton S., Tindale A. (2001) Aquaculture of yellowtail amberjacks *Carangidea*: Current status, progress and constraints. Aquaculture 2001: Book of Abstracts: 56

Bennett W. A., Beitinger T. L. (1995) Overview of techniques for removing oxygen from water and a description of a new oxygen depletion system. The Progessive Fish-Culturist 57: 84-87

Boggs C. H., Kitchell J. F. (1991) Tuna metabolic rates estimated from energy losses during starvation. Physiological Zoology 64: 502-524

Brett J. R. (1972) The metabolic demand for oxygen in fish, particularly salmonids, and a comparison with other vertebrates. Respiration Physiology 14: 151-170

Brill R. W. (1979) The effect of body size on the standard metabolic rate of skipjack tuna, *Katsuwonus pelamis*. Fishery Bulletin 77: 494-498

Brill R. W. (1987) On the standard metabolic rates of tropical tunas, including the effect of body size and acute temperature change. Fishery Bulletin 85: 25-35

Brill R. W. (1996) Selective advantages conferred by the high performance physiology of tunas, billfish, and dolphin fish. Comparative Biochemistry and Physiology 113A: 3-15

Brill R. W., Bushnell P. G. (1991) Metabolic and cardiac scope of high energy demand teleost, the tunas. Canadian Journal of Zoology 69: 2002-2009

Brown C. E., Muir B. S. (1970) Analysis of ram ventilation of fish gills with application to skipjack tuna (*Katsuwonus pelamis*). Journal of the Fisheries Research Board of Canada 27: 1637-1652

Buchanan, J (2006) Personal communication 3/02/06

Bushnell P. G., Brill R. W. (1991) Responses of swimming skipjack (*Katsuwonus pelamis)* and yellowfin (*Thunnus albacares*) tuna to acute hypoxia, and a model of their cardiorespiratory function. Physiological Zoology 64: 787-811

Bushnell P. G., Jones D. R. (1994) Cardiovascular and respiratory physiology of tuna: adaptations for support of exceptionally high metabolic rates. Environmental Biology of Fishes 40: 303-318

Calow P. (1985) Adaptive aspects of energy allocation. In: Tytler P., Calow P. (eds) Fish Energetics New Perspectives. Croom Helm, London

Clarke A., Johnston N. M. (1999) Scaling of metabolic rate with body mass and temperature in teleost fish. Journal of Animal Ecology 68: 893-905

Davis J., C., Watters K. 1970 Evaluation of opercular catheterization as a method for sampling water expired by fish. Journal of the Fisheries Research Board of Canada 27: 1627-1635

Dewar H., Graham J. B. (1994) Studies of tropical tuna swimming performance in a large water tunnel I. Energetics. The Journal of Experimental Biology 192: 13-31

Fry F. E. J. (1971) The effect of environmental factors on the physiology of fish. In: Hoar W. S., Randall D. J. (eds) Fish Physiology. Academic Press, San Diego

Gooding R. M., Neill W. H., Dizon A. E. (1981) Respiration rates and low-oxygen tolerance limits in skipjack tuna, *Katsuwonus pelamis*. Fishery Bulletin 79: 31-48

Gordon M. S. (1968) Oxygen consumption of red and white muscle from tuna fishes. Science 159: 87-90

Gouin J. F., Baros F., Birot D., Andre J. C. (1997) A fiber-optic oxygen sensor for oceanography. Sensors and Actuators B38-39: 401-406

Graham J. B., Laurs R. M. (1982) Metabolic rate of the albacore tuna *Thunnus alalunga*. Marine Biology 72: 1-6

Graham J. B., Lowell W. R., Lai N. C., Laurs R. M. (1989) O_2 tension, swimming-velocity, and thermal effects on the metabolic rate of the Pacific albocore *Thunnus alalunga*. Experimental Biology 48: 89-94

Gunn J., Block B. (2001) Advances in acoustic, archival, and statellite tagging of tunas. In: Block B. A., Stevens D. E. (eds) Fish Physiology. Academic Press, San Diego

Gunn J., Hartog J., Rough K. (2001) The relationship between food intake and visceral warming in southern bluefin tuna (*Thunnus maccoyii*). In: Sibert J., R., Nielsen J., L. (eds) Electronic Tagging and Tracking in Marine Fisheries. Kluwer Academic Publishers

Holeton G., F., Jones D., R. (1975) Water flow dynamics in the respiratory tract of the carp (*Cyprinus carpio* L.). The Journal of Experimental Biology 63: 537-549

Jobling M. (1981) The influences of feeding on the metabolic rate of fishes: a short review. Journal of Fish Biology 18: 385-400

Jobling M. (1994) Fish bioenergetics. Chapman and Hall, London

Jobling M., Davis J. C. (1980) Effect of feeding on the metabolic rate, and the specific dynamic action in plaice, *Pleuronectes platessa* L. Journal of Fish Biology 16: 629-638

Jones D., R., Bill R., W., Butler P., J., Bushnell P., G., Heieis M., R., A. (1990) Measurement of ventilation volume in swimming tunas. The Journal of Experimental Biology 149: 491-498

Kalinin A., L., Glass M., L., Rantin F., T. (1999) A comparison of direct measured and estimated gill ventilation in the Nile tilapia, *Oreochromis niloticus.* Comparative Biochemistry and Physiology A122: 207-211

Klimant I., Volker M., Kuhl M. (1995) Fiber-optic oxygen microsensors, a new tool in aquatic biology. Limnology and Oceanography 40: 1159-1165

Knights B. (1985) Energetics and fish farming. In: Tytler P., Calow P. (eds) Fish Energetics New Perspectives. Croom Helm, London

Korsmeyer K. E., Dewar H. (2001) Tuna metabolism and energetics. In: Block B, A., Stevens E. D. (eds) Fish Physiology. Academic Press, San Diego

Korsmeyer K. E., Dewar H., Lai N. C., Graham J. B. (1996) The aerobic capacity of tunas: Adaptation for multiple metabolic demands. Comparative Biochemistry and Physiology 113A: 17-24

Lauder G., V. (1984) Pressure and water flow patterns in the respiratory tract of the bass (*Micropterus salmoides*). The Journal of Experimental Biology 113: 151-164

Magnuson J. J. (1973) Comparative study of adaptations for continuous swimming and hydrostatic equilibrium of scombroid and xiphoid fishes. Fishery Bulletin 71: 337-356

Mallekh R., Lagardere J. P. (2002) Effect of temperature and dissolved oxygen concentration on the metabolic rate of the turbot and the relationship between metabolic scope and feeding demand. Journal of Fish Biology 60: 1105-1115

Mendo J., Pauly D. (1988) Indirect estimation of oxygen and food consumption in bonito, *Sarda chiliensis* (*Scombridea*). Journal of Fish Biology 33: 815-817

Moigne J., Le., Soulier P., Peyraud-Waitzensgger M., Peyraud C .(1986) Cutaneous and gill O_2 uptake in the european eel (*Anguilla anguilla* L.) in relation to ambient PO_2, 10-400 torr. Respiration Physiology 66: 341-354

Murphy G. I., Majkowski J. (1981) State of the southern bluefin tuna population: fully exploited. Australian Fisheries: 21-19

Nonnotte G., Kirsch R. (1978) Cutaneous respiration in seven sea-water teleosts. Respiration Physiology 35: 111-118

Poortenaar C. W., Hooker S. H., Sharp N. (2001) Assessment of yellowtail kingfish (*Seriola lalandi lalandi*) reproductive physiology, as a basis for aquaculture development. Aquaculture 201: 271-286

Priede I. G. (1985) Metabolic scope in fishes. In: Tytler P., Calow P., (eds) Fish Energetics New Perspectives. Croom Helm, London

Rahn H. (1966) Aquatic gas exchange: theory. Respiration Physiology 1: 1-12

Ruttanagosrigit W., Musig Y., Boyd C. E. (1991) Effect of salinity on the oxygen transfer by propeller-aspirator-pump and paddle wheel aerators used in shrimp farming. Aquacultural Engineering 10: 121-131

Sanderson S., L., Cech J., J., Patterson M., R. (1991) Fluid dynamics in suspension-feeding blackfish. Science 251: 1346-1348

Sanderson S., L., Cech J., J., Cheer A., Y. (1994) Paddlefish buccal flow velocity during ram suspension feeding and ram ventilation. The Journal of Experimental Biology 186: 145-156

Schmidt-Nielsen K. (1984) Scaling: Why is animal size so important. Cambridge University Press, Cambridge

Sepulveda C., Dickson K. A. (2000) Maximum sustainable speeds and cost of swimming in juvenile kawakawa tuna (*Euthynnus affinis*) and chub mackerel (*Scomber japonicus*). The Journal of Experimental Biology 203: 3089-3101

Sepulveda C., Dickson K. A., Graham J. B. (2003) Swimming performance studies on the eastern Pacific bonito *Sarda chiliensis*, a close relative of the tunas (family *Scombridae*). The Journal of Experimental Biology 206: 2739-2748

Steffensen J., F. (1989) Some errors in respirometry of aquatic breathers: how to avoid and correct for them. Fish Physiology and Biochemistry 6: 49-45

Stevens E. D. (1972) Some aspects of gas exchange in tuna. The Journal of Experimental Biology 56: 809-823

Tandler A., Beamish F. W., H. (1979) Mechanical and biochemical components of apparent specific dynamic action in largmouth bass, *Micropterus salmoides Lacepede*. Journal of Fish Biology 14

Vahl O. (1979) An hypothesis on the control of food intake in fish. Aquaculture 17: 221-229

Voegeli F., A., Pincock D., G. (1980) Determination of fish swimming speed by ultrasonic telemetry. Biotelemetry Patient Monitoring 7: 215-220

Vogue S. (1994) Life in moving fluids, the physical biology of flow. Princeton University Press, New Jersey

APPENDIX 1: INTELLECTUAL PROPERTY

The results of this study will be published and widely disseminated. Aquafin CRC does not intend to register any intellectual property.

APPENDIX 2: STAFF

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