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Application of bioelectrical impedance analysis as a method for estimating composition and metabolic condition of southern bluefin tuna (*Thunnus maccoyii*) during conventional tagging

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ABSTRACT

Tagging fish without gathering physiological information may be a wasted opportunity. We tested bioelectrical impedance analysis (BIA) for measurement of relative condition of southern bluefin tuna (*Thunnus maccoyii*) during conventional tagging at sea. We refined the equipment and method by measurement of 360 fish during conventional and acoustic tagging. Our results demonstrate that BIA is an accurate measure of condition for southern bluefin tuna in the same way it has been shown to be for metabolic condition and composition in other vertebrates including humans. Further, there is sufficient variation in BIA measures of the natural population to give meaningful measures of both metabolic condition and composition between groups at different times and developmental stages. Condition of tuna in this study may be related to the ocean environment just prior to measurement. BIA meets the necessary objectives for measuring fish condition during tagging as it is shown to be harmless, reliable, quick, and effective and does not disrupt conventional tagging operations. In the light of these results this type of condition measurement should be taken wherever possible in future tagging operations for this and other similar species, which will generate new insight into the ecological challenges faced by pelagic fishes. The ability to relate recent ocean environments and subsequent patterns in fish survival may lead to changes in the way tagging data is interpreted.

1. Introduction

"*An observational framework that simultaneously measures environmental and physiological variables ... will truly advance knowledge and enable us to understand these fish*" (Kirby, 2001).

Patterns of distribution and abundance, and movement and behavior, in pelagic fishes have been inferred from tagging studies (Schaefer et al., 1961; Hampton, 1986; Bayliff, 1988). Inference is limited because tagging is a binary presence or absence approach: only live fish can be tagged, and only live fish can be recaptured. Key questions related to sustainable fishing, conservation, and ecological understanding will be answered only with new approaches (Kirby, 2001; Sharp, 2001). Tuna have been the focus of many programs using both conventional (Hampton, 1986) and electronic tags (Gunn et al., 1995; Gunn and Block, 2001; Davis and Stanley, 2002). We use southern bluefin tuna (SBT, *Thunnus maccoyii*) as an example and argue that doing more than just tagging is desirable when handling the fish.

1.1. Benefits of knowing the condition of tagged fish

Conventional tagging involves capture and release of thousands of tagged individuals, for example, approximately 10,000–20,000 SBT were tagged per annum in a 5 year program (Anonymous, 2006). Each fish is handled for under a minute (Hampton, 1986) and length is commonly the only biological measurement taken. Access to so many fish in such a short time is an opportunity. We contend that additional measurements, compatible with the required speed of tagging, can yield valuable information. All fish in a size class are assumed to be equally likely to be recaptured; however, this is probably not true. For example, not all fish will be equal in body condition: fish in poor condition may not survive. A key attribute of tuna behavior and survival is body condition, and a measurement of body condition during tagging would shed insight into the following questions.

1. Are all tunas equal? Can we predict individual tuna survival based on body condition? What is the variation of condition in a school/cohort/population, and does this variation change relative to environment? Do tuna spectrum early into potential breeders and non-breeders, as has been demonstrated

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for salmon (Young, 1999; Hobday and Boehlert, 2001)? A "condition–survival relationship" could support management and conservation strategies.
2. Does environmental suitability vary for tuna? Dead fish are hard to catch, thus, fish presence in an area has been assumed to indicate existence of suitable habitat and absence as unsuitable habitat. This implicit assumption is central in many analyses of environmental relationships (Laurs et al., 1984; Perry and Smith, 1994; Block et al., 1997; Royer et al., 2004; Zagaglia et al., 2004; Zainuddin et al., 2006). Tuna have the ability to move long distances quickly, and to traverse unsuitable habitats (Polovina, 1996; Itoh et al., 2003; Block et al., 2005). Habitat suitability over the long-term can be inferred from average growth parameters from recaptured individuals, but short-term, fine scale patterns cannot be resolved. An understanding of the relationship of fish condition to local environmental conditions will improve habitat description, recruitment relationships and knowledge of migration pathways and hotspots (Kirby, 2001).
3. How does body condition change over seasonal time scales and with variable habitat use and how does this affect future life history and dispersion? Some tuna species occupy seasonal feeding grounds (Polovina, 1996; Chase, 2002; Block et al., 2005; Zainuddin et al., 2006; Golet et al., 2007). At these times, there may be a trade-off between growing fast (long and skinny), or growing fat (Golet et al., 2007). Do individuals switch from growth to fat at any time during the season? Do they generally run on empty or feast? Bimodal behaviors have been observed for some tuna—some fish migrate and some do not (Polovina, 1996). Such thresholds in behavior may be related to body condition.
4. How do feeding and body condition define interaction rates for ecosystem models? How can realistic behavior rules be incorporated into spatial models and dispersion models? Discovery of relationships between environmental conditions, body condition and subsequent behavior have the capacity to improve the descriptions for movement and ecosystem impact for both individual-based models and dynamic population models (Kirby, 2001; Sharp, 2001).

1.2. *The urgency for condition measures – why now?*

Tuna populations are under pressure, with similar abundance, growth and condition trends noted in many species and oceans (Polacheck et al., 2004; Pauly and Palomares, 2005; Golet et al., 2007). Additional impacts are expected from a changing ocean climate. Knowledge of the relationship between condition, survival, and the environment is required for prediction of future patterns. It is imperative that environmental conditions, suitability and ecosystem impacts such as interaction rates be incorporated into management plans (Sinclair and Valdimarsson, 2003; King and McFarlane, 2006).

1.3. *Condition information is useful to ecosystem modelers*

Data to inform increasing complex ecosystem models with movement parameters is needed (Dagorn et al., 2000; Lehodey, 2001). Indeed, one of the greatest challenges facing ecosystem modelers is the effect of spatial organisation on interaction rates (Sharp, 2001; Fulton et al., 2003). Temperate tuna in particular, are not well represented by diffusion-type parameters often incorporated in movement models (Kleiber and Hampton, 1994; Adam and Sibert, 2002). More explicit rules, related to local environmental conditions are needed, which can be derived if movement is related to body condition (Kirby, 2001). These rules are needed on a scale relevant to ecosystem models, which are generally smaller scale than the annual range of tuna (Fulton et al., 2004).

1.4. *Designing a system for condition measurement*

Tuna movement is likely motivated by feeding and spawning migrations, both of which are related to fish condition (Goldstein et al., 2007; Golet et al., 2007). Fish condition can be measured in a variety of ways, each with limitations and tradeoffs (Vogt et al., 2002). The perfect system would be cheap, quick, and non-invasive, suitable for use on a moving boat on live fish, and without labor-intensive post-processing of samples. It could be undertaken by field staff as part of regular tagging operations, and would require little additional measurement and calibration. Given the scale of tuna movements, a suitable approach for measuring body condition would also reflect the tuna's response to local conditions: a scale of days to weeks and 10s to 100s of km.

1.5. *Current options for measuring the condition of fish*

The most common method of measuring fish condition is to derive a length–weight relationship and measure condition as deviations from the expected relationship (Hampton, 1986). This method has recently been criticized as inaccurate and irrelevant to condition and likelihood of survival (Green, 2001). This method is limited for large delicate fish by handling time, potential handling damage, and the difficulty of accurate weight measurement of fish, which can be large and energetic, on a moving vessel in rough seas often encountered during tagging. Biochemical approaches are effective, but require a tissue sample (potentially damaging fish) and substantial onshore processing (Vogt et al., 2002). Processing large samples may also be prohibitive in terms of transport, long-term investment and consistency. We suggest that bioelectrical impedance analysis (BIA) is useful for tuna, particularly during tagging experiments. We first describe how this analysis works, demonstrate it is applicable to fish, and finally report some preliminary results on tuna body composition across two seasons.

1.6. *History and use of BIA*

Bioelectrical impedance analysis shows that human body composition is correlated to the electrical impedance of the whole body (Kushner, 1992). Impedance is a vector sum of reactance and resistance, each of which can be measured in body tissue by the application of a high frequency (50 kHz) current of imperceptible amplitude (800 μA) (Kushner, 1992). The technique is highly effective for measuring human body composition (fat content, lean muscle, total water) (Lukaski et al., 1985; Dittmar and Reber, 2001; Dittmar, 2003) and nutritional status (Barbosa-Silva et al., 2003; Mushnick et al., 2003; Mika et al., 2004; Wirth and Miklis, 2005) and there is abundant supporting literature from the medical studies demonstrating effectiveness of the approach. Specifically, phase angle (angle between the vector components of impedance) is sensitive to nutritional status in humans (Barbosa-Silva et al., 2003; Mika et al., 2004), and is an important predictor of survival in a number of human diseases, which are conditional on the body's capacity to take advantage of food intake, and malnutrition (Wirth and Miklis, 2005). This is because BIA is an accurate measure of the ratio of intracellular to extracellular water (Barbosa-Silva et al., 2003). There is an important distinction between composition and nutritional status, because, for instance, highly trained athletes have body compositions close to those of malnourished people but their nutritional status might be diametrically opposite. This is due to high metabolic rate induced by training, in contrast to the lower than normal metabolic rate of malnourished people, or people with diseases that impede food uptake. Intra/extracellular water ratio is correlated to metabolic turnover in humans and thus indicated by BIA phase angle (Marra et al., 2005). BIA is used in the treatment of anorexia nervosa and various cancers because of this metabolic sig-

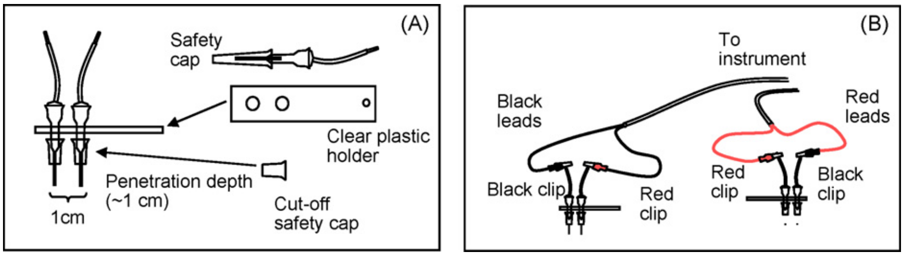

Fig. 1. Construction and connection of electrode pairs. The single electrodes were made in advance by crimping a wire within the barrel of an epoxy resin filled standard medical syringe needle. Spare safety caps can be cut off, in the field, to adjust penetration depth. The electrodes were easy to clean, cheap, and safe to store and use. Diagram used in field guide (Bradford et al., 2007).

nal (Scalfi et al., 1999; Schwenk et al., 2000; Mushnick et al., 2003; Gupta et al., 2004; Barbosa-Silva and Barros, 2005).

1.7. BIA use for animals and fish in comparison to humans

BIA is also an accurate predictor of body composition of animals (Marchello et al., 1999; Tierney et al., 2001) and fish (Bosworth and Wolters, 2001; Cox and Hartman, 2005; Duncan et al., 2007). Body composition factors such as total water, or proportion of fat tissue to lean tissue are correlated to BIA measurements through regression equations built on multiple measurements of control groups (Barbosa-Silva et al., 2005). Composition relationships to BIA data for humans are similar to those of animals and fish but with different regression constants, reflecting different body shape and composition. In the medical field there is a move toward using BIA phase angle instead of regression analysis of composition. This is because phase angle has a direct linkage to medium term (weeks) metabolic rate and nutritional status, which are more important predictors of survival and condition, and because it avoids regression errors and additional measurements introduced by regression analysis (Scalfi et al., 1999; Barbosa-Silva et al., 2003). BIA works very similarly for a wide range of vertebrates from humans to fish, and phase angle is an effective measure of condition, related to nutrition and basal metabolic rate.

1.8. Suitability of BIA

To address the questions outlined, in addition to the earlier requirements, a procedure must (1) be fast (up to five fish are tagged each minute over several hours by an effective conventional tagging team), (2) work reliably and accurately without additional measurements, and (3) differentiate body condition in wild fish which may be in states previously not observed. BIA gives multiple measures related to body condition, these are body composition (non-skeletal mass, such as protein, fat, lean weight, and water content) and metabolic condition (presumed to be correlated to basal metabolic rate and a general indication of health in humans). We refer to condition as the general state of fish health that may incorporate elements of both metabolic condition and composition.

The ubiquity of BIA for medical and farming applications means that low cost, mass produced, field tested, and reliable equipment is available. The cost of gathering BIA data is low relative to the cost of tagging programs, and we believe that more programs should collect such information. Thus, this paper is about technique and applicability of the BIA method, which in turn will allow the questions we pose above to be addressed.

2. Methods

We used a Quantum II Bioelectrical Body Composition Analyzer from RJL Systems (Clinton Township, MI, USA, http://www.rjlsystems.com). This instrument was manufactured for human subjects and reports reactance and resistance between two sets of two electrodes. We manufactured electrodes using standard syringe needles for human medical use (Terumo Corporation, Tokyo, Japan). We used 28 G (0.35 mm × 25 mm), and 20 G (0.9 mm × 25 mm) needles. We preferred to use the thinnest needles that were practical so as to keep potential tissue damage to a minimum, the 28 G were effective on all fish sizes, but on the larger fish (over 1 m length) thicker 20 G needles were more resilient over multiple measurements. These needles are finer than the applicator used to implant the conventional tags. There was very rarely a discernable reaction from the fish to insertion of either needle size, the fish usually remained passive. Pairs of needles were held 1 cm apart by a plastic jig that had holes to firmly hold the syringe needle collar (Fig. 1). An electrical connection was made by feeding several strands of uncoated electrical flex down the barrel of the syringe and crimping. The barrel of the syringe was filled with epoxy resin and a commercial industrial grade neutral cure silicone sealant (bathroom sealer) was used to fill the remaining gap in the syringe needle collar and embed the insulator from the electrical flex in the collar with a watertight seal. The electrodes were reliable and resilient and were tested with the manufacturer's test resistor regularly to check for accuracy.

The field procedure was formalized for rapid understanding and consistency in use by multiple technicians in the field as part of conventional tagging programs for southern bluefin tuna (*Thunnus maccoyii*) and is included in the field procedure guide (Bradford et al., 2007). We used morphological characters for consistent placement of electrodes (Fig. 2). The dorsal positions were used as the measurement was made in one of the longest single muscles (myosepta) (Westneat, 2000), thus avoiding fascia boundaries between muscle groups that may lead to inconsistency between fish. Additionally, dorsal placement was shown to result in maximum discrimination in fat content of the closely related albacore tuna (*Thunnus alalunga*) (Dotson, 1978). Dorsal placement also avoids damage to the lateral line, or other sensitive areas, important if the fish is to be released alive. Major blood vessels are near the surface at the mid line in SBT.

The depth of electrode penetration was adjustable in the field by cutting pairs of syringe safety caps down to expose the needle

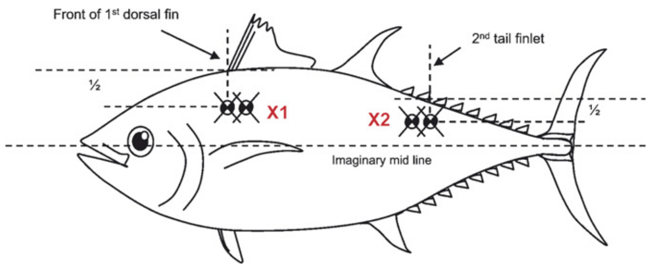

Fig. 2. Positions for placement of the electrodes on a tuna. X1 and X2 positions were used for this study.

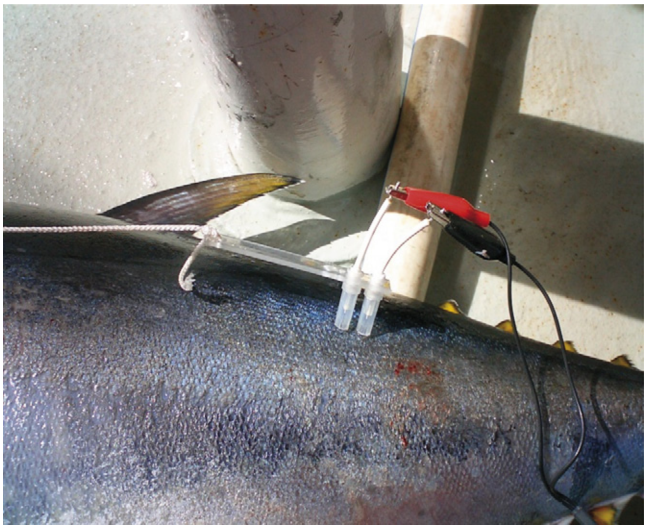

Fig. 3. Electrode placement on southern bluefin tuna (*Thunnus maccoyii*) (control specimen). Posterior pair in line with second dorsal finlet, note use of cut off safety caps to limit penetration.

the required length (Fig. 1). A depth of approximately 1 cm was best for penetration of the skin (3–4 mm) and for stable readings during use (Fig. 3).

2.1. *Measurement and treatment of observations*

The volume of a tuna is well approximated by a uniform cylinder which is consistent with the theory for the efficacy of BIA (Kushner, 1992). The BIA device measures resistance (ohms) and reactance (ohms). We took one pair of measurements from each fish tagged, as well as a number of control fish. The metabolic condition index, phase angle (degrees), is determined from resistance and reactance (Barbosa-Silva et al., 2003):

$$\text{phase angle}\,(^\circ) = \left(\arctan\left(\frac{\text{reactance}}{\text{resistance}}\right)\right) \times \frac{180^\circ}{\pi} \qquad (1)$$

Unlike composition measurements using BIA, phase angle avoids calibration based on regression equations built from previous measurement of a representative sub-population (e.g. Cox and Hartman, 2005). The equation does not require any chemical analysis and is independent of separation between electrodes (approximated by LCF for fish, or height for humans). Thus, phase angle avoids errors introduced by these methods and limitations of the ranges of calibration studies.

All SBT were measured for length to caudal fork (LCF), to nearest cm, as part of the conventional tagging process. We calculated an impedance index for measurement of body composition using a constant conversion of LCF to separation between electrodes of 0.4, the physical similarity between fish made this an accurate constant, to at least an accuracy of the LCF measurement (within 1 cm). The composition indices were of the form:

$$\text{composition index} = \frac{\text{separation}^2}{\text{impedance component}} \qquad (2)$$

where the impedance component could be serial resistance, parallel resistance, serial reactance, or parallel reactance. All components are recorded directly by the instrument (serial cases) or can be calculated from the two readings with simple equations (Liedtke, 1998). In studies of humans and other vertebrates all these impedance components used in the composition index have a similar relationship to total weight, lean mass, fat mass, and other body composition factors. In previous studies, minor differences in the regression constants have lead to one component being selected over another (Kushner, 1992; Cox and Hartman, 2005). We used serial reactance as the impedance component for calculation of composition index (Eq. (2)) because this component most accurately correlated to the weight of the control group, but all other components gave similar results.

For a group of 29 SBT in the Great Australia Bight we took BIA observations on both sides of the fish, measured separation between electrodes and measured the weight of the fish in January 2005. In the same season we fitted a further 29 SBT in the GAB with acoustic tags and measured BIA at time of release, the experimental procedure is described in a separate paper (Willis and Hobday, 2007). SBT fitted with acoustic tags are expected to be monitored for several days or weeks after tagging and we intended to use this to detect any adverse effects of BIA procedure relative to other acoustic tagging studies of SBT (Hobday and Kawabe, 2005). As part of CCSBT southern bluefin tuna conventional tagging programs (Anonymous, 2006) we measured the BIA of 162 juvenile SBT from southern western Australia in 2005/2006 and 2006/2007. After each fish had two conventional tags applied, the BIA measurements were taken, which took 10–20 s per fish. The condition of these fish was related to the environmental conditions in each year as an example of how these data might shed light on subsequent survival patterns.

Our statistical approach to the analysis of the results was based on this work being a pilot study for future designed experiments and a thorough test of the equipment under field conditions. Thus, we used simple *t*-tests and *z*-tests wherever possible and linear least squares regression using in-built functions of the Matlab$^{\text{TM}}$ computing environment (*t*-test2.m, *z*-test.m, and regress.m http://www.mathworks.com). Broad correlations to environmental variables and clear differentiation between defined groups were the goals and thus simple tests were appropriate (Murtaugh, 2007).

3. Results

3.1. *Survival of fish tagged and measured with BIA*

During the season of 2005/2006 in the Great Australian Bight we fitted 29 fish with surgically implanted acoustic tags in a separate experiment (Willis and Hobday, 2007). During this tag insertion we also took BIA measurements of the fish. Fish with the acoustic tags were detected acoustically for up to 60 days after tagging. All fish which were tagged within 5 km of the acoustic receivers (which have a range of 500 m) were detected and the majority of fish (6 of 10) that were tagged 40 km away were also detected, there was no statistical difference between the metabolic conditional or composition measures between the fish detected and those not. We also detected fish which had been acoustically tagged the year before and had traveled over 1000 km which suggests fish which have been acoustically tagged can survive similarly to untagged fish. For the purposes of this BIA study we offer this as evidence that the BIA had no effect on the normal behavior of tagged fish even when they underwent a surgical procedure in combination with attachment of conventional tags. The BIA readings for these fish are included in the data set used in this study.

3.2. *Control group*

During the 2005/2006 season 29 southern bluefin tuna, troll-caught as part of the conventional tagging program in the Great Australia Bight were used as a control group. The impedance index (Eq. (2)) shows a significant positive correlation with weight for the control group (Fig. 4). Measurement error was estimated using the relative error of the control group as a pooled sample with-

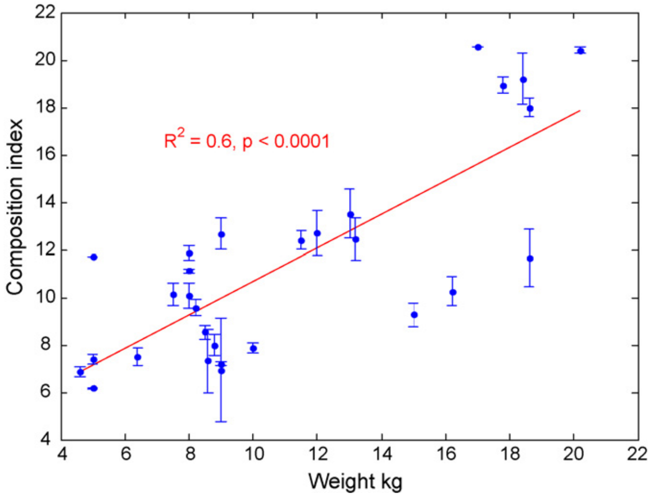

Fig. 4. Correlation of weight to composition index from measurements taken from either side of 29 southern bluefin tuna (*Thunnus maccoyii*) in the Great Australian Bight in 2005. Bars indicate the two measurements and the dot is the mean.

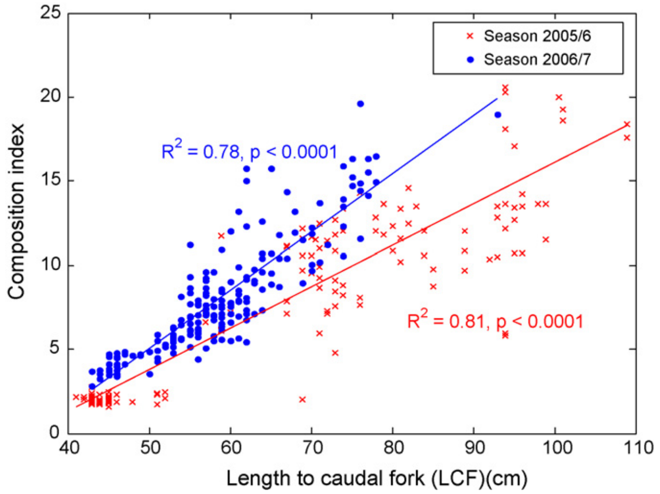

Fig. 5. Composition index from bioelectrical impedance analysis of 360 juvenile southern bluefin tuna (*Thunnus maccoyii*) of length to caudal fork between 41 and 109 cm during the seasons of 2005/2006 (×) and 2006/2007 (●) ($n=32$) and 2006/2007, in southern Australian coastal waters between latitude 115° and 135° east.

out implication of correlation to weight which was measured with unknown bias. The errors were normally distributed (determined by t-test, $t_{28}=-1.54$, $p=0.14$) with standard deviation of 8% of measured value.

3.3. BIA indices

A total of 360 fish over 2 years were measured when conventional tags were applied in western and southern Australia, and this slowed tagging to between 10 and 20 s per fish compared with application of conventional tags only. Composition index (Eq. (2)) against LCF for all subjects, shows the expected correlation overall to weight/LCF but also highlights clear differentiation for smallest size class between years. The relationships between the composition index and the length were significantly different between the seasons (regression parameters: Season 2005/2006, intercept $=-8.51\pm1.26$, gradient $=0.246\pm0.018$, $n=162$. Season 2006/2007, intercept $=-12.27\pm1.54$, gradient $=0.346\pm0.026$, $n=198$. Confidence alpha $=0.05$, gradients $t_{358}=15{,}401$, intercepts $t_{358}=243$, $p<0.0001$ for both) (Fig. 5).

The composition index shows a clear differentiation at the smallest size class (<50 cm) (Fig. 5). Fish below 50 cm LCF, tagged in the 2005/2006 season have on average a leaner relative body composition than those of similar length class tagged in 2006/2007. This differentiation is also evident from the BIA metabolic condition index (phase angle) (Fig. 6). *T*-tests indicate rejection of the null hypothesis that the metabolic condition and composition indices means are equal at the 0.05 level (metabolic condition index: $t_{91}=9.89$, $p<0.0001$, composition index: $t_{91}=25.33$, $p<0.0001$). Sea surface temperature from composite satellite images for the period of 15 days immediately prior to the majority of the BIA measurements (Fig. 7) show a major difference in the environment for the two groups, although they were tagged at approximately the same calendar date each year (Fig. 6) and at approximately the same geographic location (Fig. 7). In the 2005/2006 season the mean (±1 S.D.) was 17.5 ± 1.16 °C, while in the 2006/2007 season the mean was 19.4 ± 0.98 °C in the same geographic region (Fig. 7).

4. Discussion

4.1. Applicability of BIA technique

In this study we showed that BIA is a technique that can be applied during normal tagging operations, at high speed and on the wet deck of a moving vessel. The benefits of obtaining an immediate measurement without damage to the fish and without the complexity of taking, storing and managing tissue samples are obvious. The equipment and the additional electrodes we manufactured from inexpensive and widely available components worked well, were resilient, and were simple and safe to handle, use and store. The electrodes caused little or no reaction from the fish or apparent damage to the skin of the fish. The acoustically tagged fish demonstrated a range of movement patterns after tagging traveling

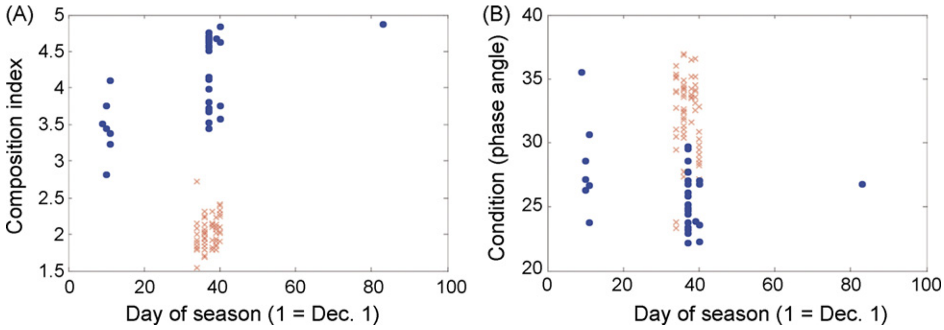

Fig. 6. Composition index (A) and condition index (B) from bioelectrical impedance analysis of 93 juvenile southern bluefin tuna (*Thunnus maccoyii*) of length to caudal fork between 41 and 50 cm during the seasons of 2005/2006 (×) ($n=32$) and 2006/2007 (●) ($n=61$), in southern Australian coastal waters between latitude 115° and 122° east.

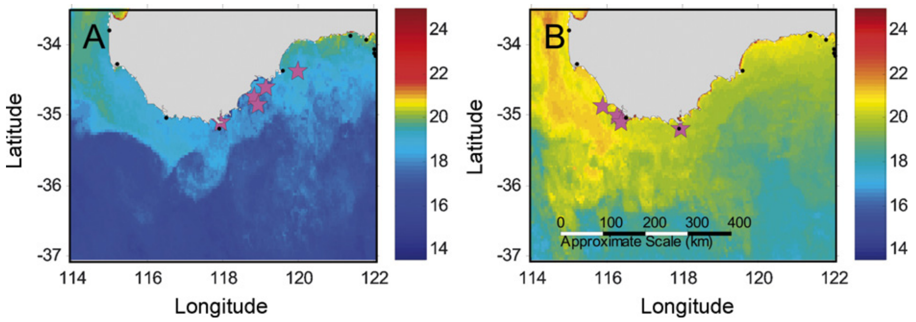

Fig. 7. Tagging position (star) of 93 juvenile southern bluefin tuna (*Thunnus maccoyii*) of length to caudal fork between 41 and 50 cm during the seasons of 2005/2006 (A) and 2006/2007 (B), in southern Australian coastal waters between latitude 115° and 122° east, shown against sea surface temperature (NOAA AVHRR) averaged from 15 days preceding January 5th.

upwards of 40 km over 60 days (Willis and Hobday, 2007), and thus we are confident that BIA adds no detectable additional stress to a fish over and above that caused by tagging. Therefore, BIA satisfies all of our criteria for an effective method of condition measurement during normal tagging operations.

4.2. BIA performs for tuna in a similar way to other vertebrates

The results confirm that BIA can be used to measure broad composition of tuna as it can for many other vertebrates including humans. This is demonstrated by the good correlation of BIA composition measures to size and weight of tuna across both the control group and the whole data set. This indicates that BIA is measuring compositional changes between fish of similar length and also that BIA phase angle is a good indicator of basal metabolic rate and related indices in tuna as for other vertebrates. This is the key criteria for the continued use of BIA to measure metabolic condition and composition of fish during tagging, because the overall aim is to attempt to match the relative measures of composition and metabolic condition with the future life history of tuna. The eventual tag returns from a statistically meaningful number of fish that had also been measured for metabolic condition and composition may give insight into how these relative measures relate to the future movement and health of the fish in question. The use of this technique therefore is bound into the analysis of the results of the whole tagging study, anything else that we can infer from the initial measurements is a bonus when viewed in reference to this primary objective.

4.3. Variation of fish condition between years

The results show that there is differentiation between groups of fish in the natural population. The relationship of length to composition is different between the 2 years. This may be due to environment during the season or life history reflecting different habitats used by the two groups in the past, or indeed might represent some other unknown difference between the two groups such as a phenotypic or genetic difference. Immediate environmental conditions are perhaps the most likely factor and for one length class this differentiation is likely related to the environment immediately preceding measurement. Our results show that for the smallest group of fish tested (<50 cm LCF) there was the clearest differentiation between the 2 year groups and this was evident in both the compositional measures and the metabolic conditional measures. Although the fish were in approximately the same place and measured at the same point in the season, the sea surface temperature data over the preceding 2 weeks suggests that the environmental conditions were quite different. This difference in terms of health or survival cannot be evaluated until a larger number of fish are measured and recaptured. As mentioned above, subsequent tag returns may shed some new light on these points. This is a pilot study and as such the differentiation is the key result supporting the use of BIA measurements during tagging. We can, however, make some reasonable assumptions based on comparative BIA studies and current knowledge of tuna life history.

4.4. Environmental explanation for variation between years

For the group of fish less than 50 cm LCF measured in approximately the same place and time during season there is differentiation in both composition and metabolic condition measures. These seem to be giving contrary indications. In humans BIA phase angle (the metabolic condition measurement in this study) is used as a general indicator of health (Barbosa-Silva et al., 2005), for instance, of people's likely recovery from surgery (Mushnick et al., 2003), and it is also used as indicator for basal metabolic rate which shows, for instance, when people with anorexia nervosa are metabolizing food and becoming healthier (Marra et al., 2005). For this group of SBT, BIA phase angle was higher in the 2005/2006 season indicating perhaps that they were in a better state of health. In contrast, the BIA compositional measure (Eq. (2)) indicated that this group had less fat, protein and other non-skeletal mass in 2005/2006 as is confirmed by other studies of fish composition (Cox and Hartman, 2005). Tuna aggregate in this area of the continental shelf of southern Australia and form surface schools to feed on lipid rich pilchard and anchovy that feed and spawn in the same area (Gaughan et al., 2004; Ward et al., 2006). Experienced fishers and taggers suggest SBT form visible surface schools in shelf waters after the temperatures exceed 18 to 20 °C (personal communication Clive Stanley). Thus, the sea surface temperature records show it is likely that the fish tagged in 2006/2007 had been feeding on lipid rich pilchards to a greater extent than those in 2005/2006 which would account for their higher compositional measurements. The apparent higher basal metabolic rate of the less well fed fish (indicated by the higher BIA phase angle [metabolic condition]) measurement may be explained by reference to experiments on healthy humans under starvation conditions, because this apparent reversal of metabolic condition indicator in the face of recovery form starvation has been noted in human studies. "... adaptive thermogenesis at the onset of semistarvation caused a rapid drop in resting metabolic rate, which then decreased slowly as lean tissue was catabolized and protein turnover decreased. During refeeding, the levels of adaptive thermogenesis and the energy costs of de novo lipogenesis and protein turnover were increased, resulting in a higher resting metabolic rate at the same lean mass during refeeding vs. semistarvation" (Hall, 2006). Given that BIA phase angle has been shown to be a sensitive indicator of basal metabolic rate, it is likely that the differences in metabolic condition index between the two groups of fish relate to short-term metabolic differences related to their physical position in the cycle of feeding and growth, and might be an indicator of nutritional stress if it is at elevated levels. Thus, our results suggest composition is a more definitive indicator of medium term nutritional health, but metabolic condition might also have an important role in indicating the current state of the fish. As stated above the true test of these results will be several years away after a significant number of tags have been returned and we are able to analyse the results in the light of the initial fish composition and metabolic condition measurements.

4.5. The need for BIA in more tagging studies

The main benefits of the use of BIA in tagging studies are likely to come after a statistically meaningful number of tags have been returned. However, like many other large pelagic predators, southern bluefin tuna are a species under pressure from fishing and potentially from climate change (Pauly and Palomares, 2005). It is estimated that less than 10% of the 1960's biomass remain (Anonymous, 2006). We suspect there are long-term changes to school composition (Dell and Hobday, 2008) and growth rates (Polacheck et al., 2004). We currently have no direct way of detecting seasonal condition variation in the tuna we tag and rely solely on inference. The school composition and suspected growth changes may indicate similarities to long-term deterioration in fish quality reported for the closely related northern bluefin tuna (*Thunnus thynnus*) (Golet et al., 2007). The northern bluefin tuna is under similar fishing pressure (Golet et al., 2007). Deterioration in condition might have a direct bearing on the future long-term viability of fisheries dependent on these species, as is already the case for the northern species (Golet et al., 2007). This study shows that BIA has the potential to differentiate seasonal variation in condition purely from initial measurements before tag return. Therefore, it would be immediately valuable to build a longer and more comprehensive time series.

This study shows that BIA is a practical solution to the problem of measuring metabolic condition and composition without disruption to conventional tagging operations. BIA is unique in our experience in combining cost effectiveness, practicality and accuracy in a single simple operation without overheads in lab time or management of samples. The more BIA condition measurements of this and other species, the more likely we are able to make breakthroughs regarding habitat use by pelagic fishes, and discover 'rules' for fish migration and movement.

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