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Reliability of non-lethal assessment methods of body composition and energetic status exemplified by applications to eel (*Anguilla anguilla*) and carp (*Cyprinus carpio*)

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ABSTRACT

Non-lethal assessments of proximate body composition of fish can help unravelling the physiological and condition-dependent mechanisms of individual responses to ecological challenges. Common non-lethal methods designed to index nutrient composition in fish include the relative condition factor (K_n), bioelectric impedance-based assessments of body composition (BIA), and microwave-based “fat” meters (FM). Previous studies have revealed mixed findings as to the reliability of each of these. We compared the performance of K_n, BIA and FM at different temperatures to predict energetic status of the whole bodies of live eel (*Anguilla anguilla*) and carp (*Cyprinus carpio*) and the dorsal white muscle of carp. Homogenized fish flesh was used for calibration. Relative dry mass was strongly correlated with relative fat content (R^2 up to 96.7%) and energy density (R^2 up to 99.1%). Thus, calibrations were only conducted for relative dry mass as an index of energetic status of a fish. FM readings were found to predict relative dry mass of whole body in eel ($R^2 = 0.707$) and carp ($R^2 = 0.676$), and dorsal white muscle of carp ($R^2 = 0.814$) well. By contrast, BIA measurements and K_n were much less suited to identify variation in relative dry mass. BIA-based models were also temperature-dependent. As a result, a regression model calibrated at 10 °C and applied to BIA measurements at 20 °C was found to underestimate energetic status of a fish. By contrast, no effects of temperature on FM calibration results were found. Based on our study, the FM approach is the most suitable method to non-lethally estimate energetic status in both, carp and eel, whereas BIA is of limited use for energetic measurements in the same species, in contrast to other reports in the literature.

1. Introduction

Proximate body composition of fish, usually measured as the relative amount of moisture, lipid, protein, and ash within fish flesh, is influenced by a range of exogenous and endogenous factors (Shearer, 1994). Macronutrient composition in fish flesh constitutes an integrative endpoint of complex ecological processes that involve catabolism and anabolism and is also a key determinant of behaviour, maturation and survival, e.g., over winter (Ursin, 1967; Gardiner and Geddes, 1980; Post and Parkinson, 2001; Biro et al., 2005). Nutrient content and the composition of nutrients in fish flesh thus provides important insights into the physiological and energetic status of fish, which in turn can help predicting an individual’s condition for wintering (Schreckenbach et al., 2001) or its propensity to engage in migration (Larsson et al., 1990) or spawning (Ludsin and DeVries, 1997). The proximate body composition of fish is usually measured in tissue samples taken from sacrificed fish (e.g., Hendry et al., 1999; Mathes et al., 2010). Such studies can only offer a snap-shot picture at the population-level, because individual fish cannot be tracked over time. Non-lethal assessment techniques of body composition in fish were developed to offer an alternative that allows for repeated measures on individual fish to study fitness in the wild or in aquaculture conditions.

A range of non-lethal methods have been developed. The earliest were length–weight-regression-based condition indices such as Fulton’s condition factor (Ricker, 1975) or the relative condition factor (K_n), which relates an individual’s actual weight to a standard average weight in the studied fish population (Le Cren, 1951). However, length–weight relationships are not without problems when

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used to index condition, because relationships change through ontogeny and seasonally, e.g. during spawning periods (Vøllestad and Jonsson, 1986; Froese, 2006). Thus, length–weight-based condition indices should only be used after careful examination of their underlying assumptions (Bolger and Connolly, 1989; Cone, 1989).

A further opportunity is to analyze the ratio of dry mass to wet mass of an individual (Hartman and Brandt, 1995). Due to strongly inverse relations of water and lipid contents in fish flesh (Schreckenbach et al., 2001) a higher dry mass should correlate positively with energetic density and hence condition (Caulton and Bursell, 1977). Indeed, dry mass has been found to constitute a useful surrogate of energetic status of fish (Shearer, 1994). However, analysis of dry mass still requires obtaining a flesh sample of the fish, either by sacrificing the fish or by muscle biopsy as a non-lethal alternative approach (Hendry et al., 2001).

The latest technical developments for estimating proximate body composition and/or energetic status of fish were based on the inverse correlation of lipid content and water content. In these applications water content in fish flesh is measured using electric currents [bioelectric impedance analysis, BIA, Cox and Hartman (2005)] or microwaves in handheld devices [fat meters, FM, Crossin and Hinch (2005)]. Calibration studies using BIA devices regressed various nutrients in fish flesh against BIA readings, in particular total body water, body fat, body fat-free mass, protein, and ash across a range of species (brook trout *Salvelinus fontinalis* Cox and Hartman, 2005; Rasmussen et al., 2012; steelhead *Oncorhynchus* mykiss Hanson et al., 2010; yellow perch *Perca flavescens*, walleye *Sander vitreus*, lake whitefish *Coregonus clupeaformis* Pothoven et al., 2008; channel catfish *Ictalurus punctatus* Bosworth and Wolters, 2001). It is worth noting that BIA cannot measure any of these variables directly (Schoeller, 2000). In fact, BIA measures the resistance and its inverse, reactance, of an animal's body to an electrical current where resistance is dependent on the quantity (and not the properties) of intracellular and extracellular water. Thus, the quantity of water within fish flesh mainly influences the degree of resistance (Schoeller, 2000). Because body water is inversely related to body fat content as mentioned before (Craig, 1977; Schreckenbach et al., 2001) and hence water relates inversely to energy density (Chellappa et al., 1995), BIA measures have been found to correlate well with a range of body composition metrics in fish (e.g., Bosworth and Wolters, 2001; Pothoven et al., 2008; Hanson et al., 2010; Rasmussen et al., 2012).

FM devices are an alternative to BIA, and they are based on a sensor for microwave moisture measurements (Kent, 1992). The sensor is directly placed on the tissue of interest (e.g., fish muscle). Because materials with polar constituents like water can be described by complex dielectric permittivity where the material is able to store energy (Kent, 1992), the loss of energy in the sensor can be used to predict the water content of the material (Kent, 1992). Thus, similar to BIA, FM is supposed to measure the water content of the tissue of interest. FM readings have been regressed on species-specific nutrient composition values for calibration purposes of the device (Pacific salmon *Oncorhynchus* spp. Crossin and Hinch, 2005; North Sea herring *Clupea harengus* Davidson and Marshall, 2010). Using such calibration results, the commercially available FM device displays the result of the regression using a species-specific regression of FM readings and relative lipid content, and not what the device actually measured (i.e., energy loss). FM has been applied to study lipid levels in live fish (Crossin et al., 2008), dead fish (Quillet et al., 2005), and fillets (van Sang et al., 2009). Because FM assesses the water content of this tissue, regressions on dry mass in fish flesh should generate the most robust results, similar to BIA. All other predictions of BIA or FM outputs with body constituents such as protein, fat or ash, are likely to be more spurious and variable across species and ecological contexts.

Previous calibration studies that developed BIA reported correlations of device outputs (e.g., impedance measurements in BIA) with total (e.g., absolute g per individual) rather than relative nutrient levels [e.g., g per g fish flesh; Cox and Hartman (2005)]. However, the total mass of a proximate component should be strongly related to the size of the fish (Caulton and Bursell, 1977; Weatherley and Gill, 1983) and is therefore less suitable to discern inter-individual differences in relative body composition levels of fish that are of similar size. Ecologically it is often the relative differences among individuals that are of interest to the researcher (Beamish and Mahnken, 2001) and thus, it is important to calibrate BIA and FM devices also to relative measures of body composition.

So far, the effect of temperature on calibration quality of BIA in fish has only been considered in a single study (Hartman et al., 2011). However, the temperature dependency of impedance (BIA) is well known from studies on mammals (Slanger and Marchello, 1994; Gudivaka et al., 1996). Assuming that the benefits of non-lethal body composition estimates are related to the possibility of repeated measurements on individual fish over time at fluctuating temperatures, there is a need for temperature-dependent calibration of the assessment methods. The reliability of calibration results derived at a given temperature should ideally be high when applied to a different temperature in the field (Hartman et al., 2011).

The objectives of our study were to (i) compare the performance of K_n, BIA, and FM, to predict dry mass content as an indicator of energetic status using carp (*Cyprinus carpio*) and European eel (*Anguilla anguilla*) as model species, and (ii) to test for the effects of temperature on the functionality of BIA and FM. We choose carp as a recreationally and commercially important species in European fisheries and aquaculture (Arlinghaus and Mehner, 2003) and eel due to its currently declining status, which demands non-lethal assessments of energetic status to help understanding migration propensity or failure (Larsson et al., 1990). Both of these species have not undergone rigorous testing as to the suitability of BIA and FM. The only study published so far in carp has used FM readings and has reported positive correlations (Oberle, 2008), which underlines the hypothesis that at least FM should provide robust results in carp.

2. Materials and methods

Calibration for K_n, BIA and FM readings was conducted using $N = 80$ farmed scaled carp (Nordhauser Mühle, Ostercappeln, Germany, 52°19′53″ N, 8°14′51″ E) and $N = 40$ wild-captured yellow eel (Carl Peter Brasen eel export, Hemmet, Denmark). To increase the among-individual contrast in body composition of carp and eel and thus to increase the power of the calibration procedure, different feeding regimes were applied to the fish. Carp were kept under four different feeding regimes in aquaria ($N = 20$ carp in each treatment) for 117 days before measurements [Ø 5 mm commercial carp pellets, Trouw Nutrition carp pellets C-5, Trouw Nutrition, Burgheim, Germany; 0.2%, 1%, 2% and 4% of total body weight per day]. The aquaria (110 cm × 60 cm × 80 cm) were placed in a climate chamber with a standardized temperature of 20 °C and a light regime of 12:12 h. One third of the aquaria water was exchanged weekly and all tanks were continuously filtered using external filters (Eheim professional 3 type 2080, Eheim, Deizisau, Germany). Eel were kept in a circular laboratory tank (diameter 2 m). The tank was connected to a circulating water system and a biological filter. Light regime was 12:12 h. Water inflow was 1 l s^{-1} and water temperature ± SD was 15 ± 2 °C. After delivery, $N = 20$ individual eel were directly measured for their proximate body composition