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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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) and 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 (Kn), 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...
used to index condition, because relationships change through
ontogeny and seasonally, e.g. during spawning periods (Vallestad
and Jonsson, 1986; Froese, 2006). Thus, length−weight−based
condition indices should only be used after careful examina-
tion of their underlying assumptions (Bolger and Connolly, 1989;
Cone, 1988).

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 (Cauliton
and Bursell, 1977). Indeed, dry mass has been found to constitute a use-
ful surrogate of energetic status of fish (Shearer, 1994). However,
analysis of dry mass still requires obtaining a fresh 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, pro-
tein, and ash across a range of species (brook trout Salvelinus
fontinalis Cox and Hartman, 2005; Rasmussen et al., 2012; steel-
head Oncorhynchus mykiss Hanson et al., 2010; yellow perch Perca
flavescens, walleye Sander vitreus, lake whitefish Coregonus clupe-
formis Pothoven et al., 2008; channel catfish Icterus 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 intracel-
lar 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 con-
tent 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 mus-
cle). 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 sen-
or 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 pur-
poses 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 con-
tent, 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, regres-
sions on dry mass in fish flesh should generate the most robust
results, similar to BIA. All other predictions of BIA or FM out-
puts 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 corre-
lations 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 (Cauliton 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 rela-
tive 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 (Slinger and Marchel, 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 calibra-
tion 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 Kn, 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 Euro-
pean 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 under-
lines the hypothesis that at least FM should provide robust results
in carp.

2. Materials and methods

Calibration for Kn, BIA and FM readings was conducted using
N = 80 farmed scaled carp (Nordhaus Müller, Ostercappeln,
Germany, 52 ° 19′ 53″ N, 8 ° 14′ 51″ E) and N = 40 wild−captured yellow
eel (Carl Peter Braesen 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, dif-
ferent 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 com-
mercial 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 cli-
mate chamber with a standardized temperature of 20 ° C and a light
time regime of 12:12 h. One third of the aquaria water was exchanged
weekly and all tanks were continuously filtered using external fil-
ters (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 fil-
ter. Light regime was 12:12 h. Water inflow was 11 s−1 and water
temperature ± SD was 15 ± 2 ° C. After delivery, N=20 individual
eel were directly measured for their proximate body composition.
using BIA and FM. Another N = 20 eel were starved within the circular tank for 45 days before subsequent analyses.

Prior to measurements, each fish was anaesthetized using a 1:9 clove oil/ethanol solution (0.75 ml l⁻¹ water), excess water was removed with a paper towel and fish were then measured for their total length (TL, nearest mm) and weight (nearest g). Using length–weight relationships at the time of delivery for eel and after the feeding experiment for carp as reference (carp: mean TL 207.8 ± 18.5 mm, mean weight 126.8 ± 39.2; eel: mean TL 586.1 ± 93.1 mm, mean weight 318.9 ± 177.6 g), relative condition factors as described by Le Cren (1951) were calculated for each individual to test for the reliability of $K_r$ to predict proximate body composition.

### 2.1. Sampling procedure BIA

For BIA measurements (serial resistance and reactance) a bio-electrical impedance analyzer was used (Quantum II; RJL Systems, Detroit, Michigan). The BIA system consisted of two sets of 3-gauge 10 mm long hypodermic needles. Each set included an outer transmitting and an inner detecting electrode held 1 cm apart in a plastic housing that allowed each needle to penetrate about 3 mm into the fish muscle. Electrodes were placed in the dorsal region of the fish following instructions by Cox and Hartman (2005). If necessary, 1–2 scales on the needle positions were removed in carp. Measurements took place on a non-conductive plastic board to avoid any current flow potentially biasing measurements (Cox et al., 2011). All BIA measurements were triplicated, and the distance between the two sets of needles was measured. Raw BIA measurements (resistance and reactance) and parallel-transformed raw BIA measurements (Pothoven et al., 2008) are cross-sectional measurements and should relate to relative tissue properties like relative dry mass (Rasmussen et al., 2012), whereas volumetric measurements (e.g. needle distance/parallel-transformed reactance, Cox and Hartman, 2005; Hanson et al., 2010) are three dimensional measurements and should reflect whole organism properties like total body water (Rasmussen et al., 2012). We calculated all of these parameters. Because there is no agreement whether parallel-transformed (Pothoven et al., 2008) or series-based (Rasmussen et al., 2012) measures of resistance and reactance should be used for calibration, mean values of the triplicated measures of both were used for analyses.

### 2.2. Sampling procedure fat meter

After BIA measurements, the microwave fat meter (MMF 992; Distell Inc., West Lothian, Scotland) was applied on the same region of the dorsal muscle. The FM sensor (frequency 2 GHz, power 2 mW) was placed along the dorsal surface of the fish at four positions for eel, and at one position for carp on both sides of the fish’s body. Additional measurements on carp were not possible, because the FM microstrip sensor already covered most of the dorsal region. For measurements, the “carp–1” calibration and the “eel–1” calibration provided by the manufacturer were used. The manufacturer had previously conducted species–specific calibrations by chemical analyses of relative fat content of dorsal muscle tissue, which were then regressed against the FM readings (Distell, 2003). The device displays the result of this calibration as % lipids. Thus, species–specific settings provided by the manufacturer are most likely to provide the best results for dorsal muscle tissue, rather than for the whole body’s nutrient composition (Crossin and Hinch, 2005).

We applied the FM readings to predict individual’s relative dry mass similar to the BIA approach. All measurements were triplicated at all positions. Mean values were then used in subsequent analyses.

### 2.3. Assessment of temperature-dependency

To test for the effects of temperature on the performance of BIA and FM in predicting relative dry mass, individuals were first measured at temperatures of the holding tanks and subsequently cooled down using iced water. Eel were initially measured at $15^\circ$C and cooled down to $10^\circ$C. Carp were initially measured at $20^\circ$C and cooled down to $10^\circ$C. While cooling, temperature of the fish within the body cavity was scanned using a digital slate thermometer (GTH 175/Pt, Greisinger electronic GmbH, Regenstauf, Germany), and BIA and FM readings were repeated once the target temperature was reached. During repeated BIA measurements, needle distances were held constant.

### 2.4. Laboratory analyses of proximate body composition

After BIA and FM measurements, anaesthetized fish were killed with an overdose of the anaesthetic (5 ml l⁻¹ water) and whole bodies of $N = 40$ eel and $N = 40$ carp were homogenized using an electrical meat grinder (Krefft R-70, Krefft, Solingen, Germany) followed by grinding using a stirring staff (ESGE Zauberstab M100, ESGE AG, Mettlen, Switzerland). The remains carp ($N = 40$) were filleted and the white dorsal muscle was separated from bones and skin before homogenization to also calibrate BIA, FM and $K_r$ for the dorsal muscle of carp. This was done to test if calibration of BIA and FM would in principle be possible using dorsal muscle tissue only. If so, non-lethal FM and BIA measurements could be taken in future applications in the field. Afterwards in the same fish, non-lethal muscle biopsies could be taken for calibration purposes, instead of killing the fish for subsequent laboratory analyses. Using this approach, few fish would die during the calibration process on purpose, which could be necessary if studies were to be conducted on protected species or rare specimens. Triplicated subsamples of homogenized tissue ($4.3 ± 0.8$ g) were used for laboratory analyses of dry mass, individual nutrients (lipids) and energy density (see for procedures below). Replicated homogenates were separately packed into plastic screw cap containers (40 ml) and stored at $−80^\circ$C until analyses.

To quantify water content and its inverse, dry mass, tissues were dried in a vacuum dryer (Zirbus technology GmbH, Bad Grunz, Germany) for 24 h at $−20^\circ$C and relative dry mass per individual wet mass was calculated. Due to malfunction of the vacuum dryer $N = 18$ samples of whole body carp and $N = 2$ samples of white dorsal muscle of carp were corrupted, resulting in a final sample size of $N = 22$ for whole body carp and $N = 38$ for white dorsal muscle of carp. Dry matter tissue lipids were extracted following the procedure outlined in Folch et al. (1957), and lipid content was calculated as percentage of dry mass. In addition, energy content of dry mass was estimated by bomb calorimetry (Parr 6400 Calorimeter, Parr, Franklin M., Germany) and energy density ($kJ g^{-1}$) of wet mass was then re-calculated considering the water content initially estimated per sample tissue.

### 2.5. Statistics

Initially, we conducted correlations between relative lipid content, energy density and relative dry mass in whole body samples of eel and carp and dorsal white muscle of carp using linear regressions. Subsequently, to derive relationships between our non-lethal measurements and observed relative dry mass of the sampled fish at $10^\circ$C, 12 different multiple regression models were used. As independent variables the models contained (1) BIA derived serial resistance, serial reactance and needle distance, (2) BIA derived parallel-transformed resistance, parallel-transformed reactance and needle distance, (3) BIA derived volumetric reactance based on serial values, (4) BIA derived volumetric reactance...
based on parallel-transformed values, (5) FM readings, and (6) relative condition factor only. Models 7–12 were similar to models 1–6, but additionally included relative condition and total length (TL) as a covariate. To select the most parsimonious model, i.e. the best methodology to non-lethally assess dry mass of fish, second order Akaike information criteria for small sample sizes (AICc) were calculated (Burnham and Anderson, 1998). Afterwards the lowest AICc value was subtracted from the other AICc of alternative models to create a rank index referred to as $\Delta_i = \text{AICc}(min)$, where the best model has an index value of $\Delta_i = \text{AICc}(\text{min}) = 0$. To test for the dependency of needle distances on size of the fish in BIA measurements, measured needle distances were correlated with TL of the fish using Pearson’s correlations.

To test for the effects of temperature on BIA and FM measurements in whole body carp we first compared BIA and FM outputs at $10{^\circ}\text{C}$ and $20{^\circ}\text{C}$ using paired T-tests. In addition, measurements taken at $20{^\circ}\text{C}$ were used to predict relative dry mass of the fish based on the calibrated regression model results at $10{^\circ}\text{C}$. Thereby, we simulated researchers using calibrations from low temperature to predict dry mass of fish from samples taken at a higher temperature ($20{^\circ}\text{C}$). These predicted values for relative dry mass at $20{^\circ}\text{C}$ were then compared with laboratory-derived values using paired T-tests. Further, the same values for relative dry mass were tested for their rank order consistency using Spearman correlations. Statistics were conducted using SPSS 15.0 with an error probability of alpha = 0.05.

3. Results

3.1. Correlations between dry mass, lipid, and energy density in eel and carp

We found relative dry mass in whole bodies of eel and carp and in dorsal white muscle of carp to be significantly correlated with both relative fat content and energy density (all $P<0.05$). Linear regression models explained 96.7% (whole body eel), 76.7% (whole body carp) and 88.9% (dorsal white muscle of carp) of the variance in relationships between relative dry mass and relative fat content, and 99.1% (whole body eel), 96.4% (whole body carp) and 97.2% (dorsal white muscle of carp) in relationships between relative dry mass and energy density (kJ g$^{-1}$). Thus, relative dry mass is a very useful proxy of the whole energetic status of eel and carp.

3.2. Suitability of non-lethal assessment methods for dry mass

The regression models used to calibrate BIA and FM for carp and eel without inclusion of TL and $K_o$ revealed FM readings performing better in all cases and for both species compared to either BIA or the relative condition factor $K_o$ (Table 1). Differences between models using series-based and parallel-transformed BIA measurements as independent variables were negligible and therefore, only results based on parallel-transformed values are presented. Significant linear relationships with high predictive power (67.6–81.4%)
<table>
<thead>
<tr>
<th>Species</th>
<th>Model</th>
<th>Standardized coefficients</th>
<th>R²</th>
<th>P</th>
<th>ΔAICc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyprinus carpio</td>
<td>DM = 10.65 + 0.70(FM)</td>
<td>0.91(FM)</td>
<td>0.814</td>
<td>&lt;0.001</td>
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<td></td>
<td>+ 9.24 + 15.18(Xp)</td>
<td>0.75(Kn)</td>
<td>0.544</td>
<td>&lt;0.001</td>
<td>24.9</td>
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<td></td>
<td>+ 20.81 + 1.37(DN / XP)</td>
<td>0.63(DN / XP)</td>
<td>0.161</td>
<td>0.008</td>
<td>51.1</td>
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<td></td>
<td>+ 20.13 + 0.08(DN) - 0.08(Kn) · 0.35(DN / XP) + 0.43(DN)</td>
<td>-0.19(DN / XP) - 0.01(Kn)</td>
<td>0.124</td>
<td>0.001</td>
<td>72.0</td>
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<td></td>
<td>+ 12.24 + 0.18(DN) + 3.05(XP) + 0.01(TL)</td>
<td>0.76(DN) 0.16(XP) 0.07(TL)</td>
<td>0.820</td>
<td>&lt;0.001</td>
<td>3.2</td>
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<td></td>
<td>+ 0.95 - 0.01(DN) + 0.00(XP) - 0.11(DN) + 14.37(Kn) + 0.08(TL)</td>
<td>0.15(Kn) 0.21(XP) - 0.33(DN) 0.72(Kn) 0.58(TL)</td>
<td>0.576</td>
<td>&lt;0.001</td>
<td>39.5</td>
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<td>+ 0.92 + 13.17(Kn) + 0.08(TL)</td>
<td>0.68(Kn) 0.22(TL)</td>
<td>0.575</td>
<td>&lt;0.001</td>
<td>28.9</td>
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<td>+ 0.83 - 0.16(DN / XP) + 0.80(Kn) + 0.03(TL)</td>
<td>-0.05(DN / XP) 0.68(Kn) 0.26(TL)</td>
<td>0.562</td>
<td>&lt;0.001</td>
<td>26.9</td>
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<td>Anguilla anguilla</td>
<td>DM = 23.10 + 0.11(FM)</td>
<td>0.85(FM)</td>
<td>0.707</td>
<td>&lt;0.001</td>
<td>5.1</td>
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<tr>
<td>whole body</td>
<td>DM = 0.01 + 0.00(XP) + 0.00(DN) + 1.45(DN / XP)</td>
<td>0.04(DN) 0.59(XP) 0.08(DN)</td>
<td>0.285</td>
<td>0.002</td>
<td>56.6</td>
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<td></td>
<td>+ 23.65 + 16.65(Kn)</td>
<td>0.41(Kn)</td>
<td>0.143</td>
<td>0.009</td>
<td>33.9</td>
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<td></td>
<td>+ 37.87 + 71.67(DN / XP)</td>
<td>0.25(DN / XP)</td>
<td>0.028</td>
<td>0.152</td>
<td>33.5</td>
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<td>+ 9.75 + 0.55(FM) + 7.98(Kn) + 0.01(TL)</td>
<td>0.77(FM) 0.19(Kn) 0.10(TL)</td>
<td>0.763</td>
<td>&lt;0.001</td>
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<td>+ 45.57 + 0.01(DN) + 0.00(XP) + 2.64(DN / XP) + 23.42(Kn) + 0.14(TL)</td>
<td>0.50(Kn) 0.51(XP) - 1.24(DN) 0.57(Kn) 2.17(TL)</td>
<td>0.665</td>
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<td>+ 23.51 - 309.6(DN / XP) + 24.61(Kn) + 0.09(TL)</td>
<td>-1.19(DN / XP) + 0.68(Kn) 1.46(TL)</td>
<td>0.142</td>
<td>&lt;0.001</td>
<td>17.5</td>
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<td>+ 12.76 + 16.49(Kn) + 0.02(TL)</td>
<td>0.40(Kn) 0.10(TL)</td>
<td>0.212</td>
<td>0.005</td>
<td>41.4</td>
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<td>Cyprinus carpio</td>
<td>DM = 21.04 + 1.07(FM)</td>
<td>0.83(FM)</td>
<td>0.676</td>
<td>&lt;0.001</td>
<td>3.1</td>
</tr>
<tr>
<td>whole body</td>
<td>DM = 13.81 + 14.69(Kn) - 0.01(XP) + 0.01(DN)</td>
<td>0.73(Kn) - 0.35(XP) 0.01(DN)</td>
<td>0.532</td>
<td>&lt;0.001</td>
<td>24.0</td>
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<td></td>
<td>+ 24.86 + 3.38(DN / XP)</td>
<td>0.54(DN / XP)</td>
<td>0.261</td>
<td>0.007</td>
<td>14.5</td>
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<tr>
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<td>+ 6.06 + 27.59(Kn)</td>
<td>0.52(Kn)</td>
<td>0.234</td>
<td>0.011</td>
<td>11.7</td>
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<tr>
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<td>+ 21.07 + 1.12(FM) - 16.49(Kn) + 0.01(TL)</td>
<td>0.87(FM) - 0.31(Kn) 0.30(TL)</td>
<td>0.707</td>
<td>&lt;0.001</td>
<td>0.0</td>
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<tr>
<td></td>
<td>+ 58.72 + 0.17(DN) - 0.02(XP) - 0.69(DN / XP) + 10.05(Kn) + 0.32(TL)</td>
<td>0.74(Kn) -0.05(XP) -1.00(DN) 0.15(Kn) 1.36(TL)</td>
<td>0.661</td>
<td>&lt;0.001</td>
<td>15.0</td>
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<td></td>
<td>+ 21.56 + 374(DN / XP) + 14.26(Kn) + 0.23(TL)</td>
<td>-0.08(DN / XP) + 0.27(Kn) 0.09(TL)</td>
<td>0.130</td>
<td>0.014</td>
<td>7.9</td>
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<td></td>
<td>+ 0.56 + 9.01(Kn) + 0.11(TL)</td>
<td>0.18(Kn) 0.40(TL)</td>
<td>0.130</td>
<td>0.007</td>
<td>14.8</td>
</tr>
</tbody>
</table>

Bold P values indicate significant regression models. * Significant coefficients (P < 0.05).
were found between FM readings and laboratory-derived values for relative dry mass in all cases (Table 1, Figs. 1–3). Significant relationships were also found between $K_{a}$ and relative dry mass of the fish, but the degree of variances explained was comparatively low (14.3–54.4%; Table 1, Figs. 1–3). Compared to FM, all BIA outputs were found to perform similarly to $K_{a}$-based models when predicting dry mass of eel and carp (Table 1, Figs. 1–3). Comparisons of volumetric BIA measurements and cross-sectional BIA measurements revealed inconsistent results (Table 1). However, in most cases, parallel-transformed cross-sectional BIA measurements were not significant (Table 1), indicating a low contribution of these variables to the degree of explained variances. By contrast, needle distance and volumetric measurements including needle distances were found to be significant in most cases (Table 1), and needle distances were highly correlated with the size of the fish (Pearson correlation, $r = 0.964$, $P < 0.001$). This suggests that length of the fish rather than BIA measurements itself might have been responsible for the somewhat small predictive power of the BIA device. This assumption was supported by the models including TL and $K_{a}$. Explained variances in BIA models increased substantially after inclusion of these variables (57.1–66.5%; Table 1), whereas FM and $K_{a}$ models only slightly changed (Table 1). In addition, AIC, values of BIA and $K_{a}$ models differed substantially from those of the FM models, indicating generally higher reliability of FM measurements compared to both $K_{a}$ and BIA measurements.

3.3. Effects of temperature on non-lethal dry mass assessments

We found BIA measurements to be significantly temperature-dependent using whole body carp (mean parallel-transformed resistance at 10 and 20 °C ± SD 305.3 ± 23.0 and 257.9 ± 36.7 and mean parallel-transformed reactance at 10 and 20 °C ± SD 965.0 ± 124.7 and 809.4 ± 137.1, respectively, paired T-tests, $N = 22$, $T = 7.4$, $P < 0.001$ and $N = 22$, $T = 14.1$, $P < 0.001$), whereas FM readings did not differ between 10 and 20 °C (mean FM readings at 10 and 20 °C ± SD 9.9 ± 3.1 and 10.0 ± 3.4 respectively, paired T-test, $T = 0.3$, $P = 0.875$). Predicted values of mean relative dry mass were found to be significantly lower than those achieved under laboratory conditions when applying BIA measurements at 20 °C for dry mass content estimation using the model calibrated to 10 °C (mean relative dry mass measured under laboratory conditions and mean calculated relative dry mass for measurements at 20 °C ± SD 31.7% ± 4.3% and 29.7% ± 3.1% respectively, paired T-test, $T = 2.7$, $P = 0.013$; Fig. 4). Therefore, one would underestimate dry matter content at warm water if a model calibrated to cooler water is used. This systematic bias did not change the rank order of dry matter content across individuals (Spearman correlation, Spearman’s rho = 0.498, $P = 0.016$), but biased the absolute estimated value downwards. No such contrast was conducted for whole body eel and dorsal white muscle of carp because the initial calibration results for BIA measurements were not significant, suggesting no predictive power.

4. Discussion

Our analyses showed that FM consistently performed better than BIA and $K_{a}$ to predict relative dry mass as an index of energetic density in whole body carp and white muscle of carp. These results confirmed that highly reliable non-lethal dry mass estimations are possible using a FM in fish species that are comparably rich in lipids such as eel and carp. Due to the correlation of dry mass and lipid content, and particularly energy density, the FM approach can thus be considered useful for predicting the energetic status of eel and carp. Our results, however, raise a cautionary note as to the usefulness of relative condition metrics and BIA assessments to infer insights about the energetic status in these species. We are not claiming that the use of BIA and $K_{a}$ is superfluous in fish in general, as indeed some weak correlation between measures and relative dry mass were found in our study species. Instead, we contend that in carp and eel, FM-based models will outperform BIA models, and thus, BIA is inferior to FM in these species.

![Fig. 3. Observed and predicted relative dry mass values for whole body carp derived from fat meter, cross-sectional parallel-transformed bioelectric impedance analyses (BIA) and relative condition factor.](image)

![Fig. 4. Temperature dependency of cross-sectional parallel-transformed BIA measurements for whole body carp. Black dots indicate calibration results at 10 °C and white dots indicate calculated values of relative dry mass at 20 °C based on calibration results at 10 °C.](image)
Our study was confined to predict relative dry mass, based on the fact that both BIA and FM directly measure water content as the inverse of dry mass (Schoeller, 2000). Dry mass is a suitable index of the energetic status of fish if it is strongly correlated with energy density or with stored energy, which is closely related to lipid content in most species that are rich in fat (Chellappa et al., 1995; Schrenckenbach et al., 2001). As expected, we found relative fat content and energy density to be highly correlated with relative dry mass. Thus, relative dry mass can be used as a proxy for relative fat content and energy density in fish. We propose that the simplest index value of energetic status that may be estimated even from small tissue samples (such as those stemming from non-lethal muscle biopsies) is tissue dry matter. In this context, FM is useful as a non-lethal assessment tool to infer dry mass of fish. Therefore, future calibration studies could be confined to relative dry mass only without loss of information (see also Jonas et al., 1996). It is straightforward to conduct FM on a live fish and then a rapid muscle biopsy that is later dried in the laboratory to assess dry mass. This way, potentially spurious and more variable co-variables relative to specific body constituents such as protein and ash and BIA and FM readings are avoided and more importantly, time and cost intensive laboratory analyses can be cut down and the fish remains alive.

Our findings contrast with previous work in several fish species using BIA who reported strong relationships between BIA outputs and proximate body composition (e.g., Cox and Hartman, 2005; Duncan et al., 2007; Hanson et al., 2010). In contrast to our work, these studies involved the regression of BIA measurements on absolute values of proximate body composition rather than relative values. Pothoven et al. (2008) compared calibration results for BIA measurements between total and relative body composition values. Similar to our work, they found weak relationships between absolute values of body composition, and strong ones for absolute values. All above mentioned studies used volumetric impedance measures in their regression analyses. Because this three-dimensional approach reflects the mass of water within the electric field induced by BIA (Liedtke, 1997), strong correlations between total mass of body constituents and volumetric BIA measurements are to be expected (Rasmussen et al., 2012). However, the volumetric approach is confined by the TL of the fish or needle distances (Cox and Hartman, 2005; Hanson et al., 2010). We found both variables to be highly correlated, if needles are placed along morphological landmarks as outlined in Cox and Hartman (2005). Therefore, needle distances are just a proxy of fish size and strong correlations between BIA and proximate body composition may just be caused by the co-variance of absolute body composition with fish length. Similar to the volumetric approach, analyses using cross-sectional BIA measurements commonly include measures of size (Pothoven et al., 2008) or weight (Bosworth and Wolters, 2001) of the fish as co-variates. Due to the strong relationship between size and absolute values of nutrients carried by an individual (Caulton and Bursell, 1977; Weatherley and Gill, 1983), strong relationships can be expected in joint models involving size or weight and cross-sectional BIA outputs. Non-lethal assessments of proximate body composition can be considered most valuable if measurements directly correlate with relative dry mass of the fish. Although the inclusion of TL and Kc in regression models strongly increased the explanatory power of BIA in our study, high variance explanation seems to be unrelated to the BIA measurements per se. Consequently, BIA might be of less use than claimed before, and it was indeed found to be of low utility in carp and eel in our study.

From an ecological perspective it is most important to discern among-individual differences in relative body constitution, because this reflects the potential for fitness differences or past foraging success. In this context, in our study BIA measurements did not work to a degree that renders the device of use in the field. This finding contrasts with the results presented by Hartman et al. (2011) and Rasmussen et al. (2012). Hartman et al. (2011) combined dorsal and ventral BIA measurements in their analyses, and they used several electrical equations to calculate a broad range of candidate predictor variables for explaining relative dry mass of the fish. This approach led to up to ten variables within the same model, most of them relying on the same two measurements of resistance and reactance. Further, variables included in the regression models of Hartman et al. (2011) strongly differed between different size classes of the fish and with different temperatures at measurement. Thus, calibration results created by Hartman et al. (2011) cannot easily be compared with other studies without additional calibration work for the size classes of interest at a specific temperature. By contrast, measurement techniques between our study and Rasmussen et al. (2012) did not differ. Using parallel-transformed reactance as independent variable, Rasmussen et al. (2012) also found similarly weak relationships between BIA values and relative body water content as we did in carp and eel. Rasmussen et al. (2012) only observed strong relationships between relative body composition values and BIA measurements when applying serial-based (i.e. raw) BIA measurements. This finding was not supported in our study where usage of serial-based and parallel-transformed values created negligible differences in the results of regression analyses. However, Rasmussen et al. (2012) used brook trout as a model species and calibration of BIA for relative body constituents might be species-specific. Although our study did not support the usefulness of simple BIA assessments to predict dry mass in eel or carp, it cannot be ruled out that combined dorsal and ventral BIA measurements, as conducted by Hartman et al. (2011), and/or application to different species can be a way to substantially improve the performance of BIA applications.

We found FM readings to be highly correlated with relative dry mass in all our investigated cases in both species, whereas the predictive power of relative condition-factor-only models for predicting relative dry mass was comparably low. Although linear regressions with Kc as independent variable were found to be highly significant, the degree of variance in dry mass explained by the condition-factor-only models were found to be substantially lower than those using FM as explanatory variable. Also, AICc values were much higher compared to regression models based on FM indicating less supported models. Both, high reliability of FM readings for predicting energetic status of fish, and high uncertainty of relative condition factors for doing the same job are in agreement with previous studies (Kent, 1992; Vogt et al., 2002; Crossin and Hinch, 2005; Davidson and Marshall, 2010; Hanson et al., 2010). However, other studies found strong relationships between relative body composition values and relative condition factors (e.g. Perca fluviatilis Craig, 1977; Morone saxatilis Brown and Murphy, 1991), which contrasts with our results. Reasons for the lack of reliability of relative condition factors to predict energetic status in carp and eel in our study might have been caused by imprecision in calculating relative condition factors. Commonly, relative condition factors relate the actual weight to a calculated average weight using reference fish other than the subsample used for experimentation (Le Cren, 1951; Coné, 1989), whereas we used the same fish. Thus, our study does not generally discount the use of relative condition factors.

BIA measurements were significantly influenced by temperature, and measurements at 20 °C resulted in significantly different predictions for relative dry mass when calculated based on the calibration at 10 °C. No such dependency of FM measurements on temperature was observed, confirming previous studies that used energy meters on fresh and iced fish without any differences in FM readings (Vogt et al., 2002). Susceptibility of BIA measurements to fluctuating temperatures is known (Slanger and Marchello, 1994; Cox et al., 2011; Hartman et al., 2011), and reasons for this are likely based on differences in blood flow and velocity at different temperatures.
environments and modes of use. These measurements are typically not feasible in large scale experiments, where the relative proportion of each type of ship is limited. Results are typically obtained from small sample sizes, which may not be representative of larger populations. Moreover, the use of dynamometers is limited to specialized applications, such as the measurement of wind force in meteorological studies. Further, because lipids stored in white muscle can be correlated with whole body lipid content in fish (Viola et al., 1988; Regost et al., 2001), future calibration of FM readings might sometimes also be done using muscle biopsy without killing the fish (Hendry and Beall, 2004). This can be beneficial if the study objects belong to a protected species or killing of individuals is impossible. However, in cases where body constituents are stored along a head–tail gradient, as it is known for some migrating salmon (Herbinger and Friars, 1991), multiple biopsy samples might be necessary.

A range of authors have discussed advantages and disadvantages of BIA and FM to non-lethally infer indices of condition and energetic status, or even proximate body composition in fish (Table 2). Based on the existing literature (Table 2) and the results of our study, we conclude that FM provides the most robust and repeatable results, unless lipid contents are too low (~2.5% body fat content (Crossin and Hinch, 2005); Table 2). Also, compared to BIA, the FM device is less prone to misapplication based on inexperience (Cox et al., 2011). Nevertheless, applicants are recommended to test the data generation in the field, and pay attention to the angle by which the FM device is placed on the tissue of interest. Correct angles can be trained using a calibration tool that is provided with the device. Also, replicated readings are encouraged to minimize measurement error. However, in cases where the reading head of the FM is bigger than the dorsal region of the fish, the FM technique cannot be used anymore (Table 2). For such cases we propose relative condition factors as an alternative (Table 2).

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Table 2

<table>
<thead>
<tr>
<th>Challenge/functionality</th>
<th>BIA</th>
<th>FM</th>
<th>K_{r}</th>
<th>References</th>
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<td>Assessment of body moisture (water content) rather than direct assessment of proximate body constituents</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>Caution and Bursell (1977), Kent (1992), and Schoeller (2000)</td>
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<td>Reliability of measurements</td>
<td>−</td>
<td>−</td>
<td>+</td>
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<td>Reliability at very high water levels</td>
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<td>Reliability against temperature fluctuations</td>
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<td>−</td>
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<td>User experience needed</td>
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<td>NA</td>
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