Evaluation of lethal and non-lethal assessment methods of muscle fat content in European eel (*Anguilla anguilla*)

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**Abstract**

Individual fat reserves are considered a key factor for the reproductive fitness of the endangered European eel (*Anguilla anguilla*). In contrast to most established standards, microwave measurements enable the determination of fat contents without sacrificing individual fish, offering a broad range of ecological applications. In order to test the reliability of non-lethal assessment methods of the muscle fat content in eels, the performance of microwave measurements was compared to the prevailing standard of measuring fat in a distinct subsample of muscle tissue by solvent extraction. Results indicate that either method is prone to error due to physiological and morphological changes during the sexual maturation of eels. Since microwave measurements were systematically affected by life stage and body length, it was possible to calibrate the method accordingly, putting it at least on par with the prevailing standard and further facilitating its use for scientific purposes.
Introduction

The individual fat content is an important determinant for the reproductive fitness of European eels (*Anguilla anguilla*, e.g. Svedäng & Wickström 1997; Dainys et al. 2018). After spending a period of approx. 8 to 15 years in European and North African continental waters as so called yellow eels, they metamorphose into silver eels and engage in a spawning migration of approx. 5000 – 7000 km, travelling to their spawning grounds in the Sargasso Sea (Schmidt 1923; Tesch 1999). Since eels are believed to cease feeding after the onset of migration, they solely rely on internal energy reserves to cover the cost of transport and maturation (Clevestam et al. 2011), e.g. intramuscular fat deposits, built up prior to the migration. The development of reliable methods to measure the fat content of eels therefore contributes towards a better assessment of spawner quality and provides valuable insights into the species ecology. Furthermore, it has been demonstrated in other species (e.g. Atlantic salmon, *Salmo salar*, Mørkøre et al. 2001) that the muscle fat content affects processing yield and the quality of the flesh (e.g. colour and texture), which is of general interest to the fish producing and processing industry.

Most methods for measuring the fat content of fish require sacrificing individuals in order to take tissue samples for subsequent solvent extraction and gravimetric quantification of fat (e.g. Bligh & Dyer 1959; Anon. 1996; van Ginneken 2007). Hence, they do not allow for repeated measurements and the often time consuming analyses limit the number of samples that can be processed. Applications for the European eel involve different sampling and fat extraction procedures (Belpaire et al. 2009; Couillard et al. 2014; Caron et al. 2016). Most frequently, fat is quantified in a subsample of muscle tissue taken approx. 5cm behind the anterior end of the anal fin (hereafter referred to as ‘reference muscle’) (e.g. Larsson...
and Lewander 1972; Sühring et al. 2012; Marohn et al. 2013). Despite being a well-established standard method, to the authors’ knowledge, no data has been published that investigated the relationship of whole muscle fat content to this reference muscle. Since the fat content of European eels is known to vary along the body axis, with the tail being the fattest part (McCance 1944; Tesch 1999), it remains to be tested if, or to which degree, this subsample is representative for the actual muscle fat content of eels.

The development of non-lethal assessment methods of the fat content of fish offers alternative approaches, which enable tracking the fat content over time and greatly increase the potential number of specimens to be investigated. They further conform to the ethical obligation to reduce the number of sacrificed fish for scientific purposes to the required minimum. The latest technical applications of these methods include bioelectric impedance analysis (BIA, Cox and Hartmann 2005) and microwave measurements in handheld devices (fatmeters, FM, Kent 1990; Crossin and Hinch 2005). Either method provides an indirect measurement of the water content in fish (by electric resistance or microwave absorption/reflection), which can be translated into fat content, based on an inverse relationship of water and fat content in fish (Schreckenbach et al. 2001). The only study that tested the reliability of these methods for European eels so far was conducted by Klefoth et al. (2013). The authors concluded that FM measurements constantly performed better than BIA. However, they tested the performance of these methods in predicting relative dry mass, which only implies that FM measurements provide accurate estimates of the fat content if the above mentioned assumption of constant correlation of water and fat content holds for European eels; yet, respective data is missing in scientific literature. Furthermore, eels undergo considerable morphological and physiological changes during the silvering
process, which potentially affect the relation of water to fat content or the measurement of
the water content itself. Among these changes, the increase in skin thickness from yellow to
silver eel (Pankhurst 1982b) is most likely to affect FM measurements since fish skin usually
has a high water content (Kent 1990) and is located directly between the sensor and the
muscle tissue to be measured.

The objectives of our study were thus to i) test the underlying assumption that the
correlation of water and fat content is constant and independent of life stage in European
eels, ii) compare the performance of FM measurements and fat contents in reference
muscle derived by solvent extraction for the prediction of whole muscle fat content in eels,
and iii) investigate whether potential effects of life stage on the accuracy of FM
measurements are related to skin thickness.

Material and Methods

A total of 29 yellow and 21 silver eels from four different river basin districts (Eider, Rhine,
Schlei / Trave, Weser) were analyzed in this study. All eels were wild-captured and sampled
as part of the European Data Collection Framework (DCF, EU 2008) in 2015 and 2016. Live
eels were bought from commercial fishermen and kept in flow through tanks for a
maximum of 14 days. After being anesthetized with 2-Phenoxyethanol (Karl ROTH,
Karlsruhe, Germany), eels were measured for body mass, total length, eye diameter and
pectoral fin length, and staged according to the silvering index (SI) by Durif et al. (2005), as
well as the Ocular Index (OI, Pankhurst 1982a). All individuals with SI 1 to 3 were classified
as yellow eels, whereas individuals with SI 4 and 5 were classified as silver eels. To cover a
large range of naturally occurring fat contents, all eels sampled in the DCF were measured
for muscle fat content in wet mass with a microwave fat meter (see below), aiming for the
collection of at least three yellow and silver eels per 5% increment (i.e. three eels between
5% and 10%, 10% and 15% etc.). Fat contents of collected eels ranged from 5 to 40% for
yellow and 5 to 30% for silver eels. Less than three individuals were available for fat
contents below 10% for yellow eels, all of which were included in the analysis. For silver
eels, only three individuals were available for fat contents below 15%. Since fat content is
known to be a distinctive factor for the onset of sexual maturation (Larsson et al. 1990) in
European eels, it was uncertain whether these individuals physiologically resemble yellow or
silver eels. They were therefore excluded from statistical analyses (but displayed and
marked with * in Fig. 1-4), resulting in an effective range of fat contents between 15% and
30% for silver eels.

Sampling procedure for fatmeter

Microwave fat measurements were conducted using a handheld device (FM 692, Distell Inc.,
West Lothian, Scotland), according to the manufacturer’s instructions. Briefly, anesthetized
eels were placed on a steel table and excess water was removed from the surface. The
sensor was placed along the lateral surface of the fish in eight positions from head to tail on
each side, using the ‘eel-1’ preset. Accordingly, the device displayed the average fat content
in wet mass of muscle without skin in percent, based on a species-specific calibration
provided by the manufacturer (Distell 2003). The sensor was frequently wiped with a paper
towel to remove mucus and functionality was checked with the provided calibration tool
(‘check pad’) on a daily basis. All measurements were quadruplicated and mean values were
used in subsequent analyses (i.e. 8 measurements per side × 2 sides × 4 replicates).
Quantification of muscle fat content by solvent extraction

After FM measurements, eels were killed by severing the spinal cord and the following samples were taken: liver, skin, muscle, intestines (including kidney), swim bladder, gonad, gill and carcass. For muscle, two different samples were taken (including red and white muscle tissue): i) the above mentioned ‘reference muscle’, a piece of muscle approx. 5cm long, cut off the left side of the spine behind the anterior end of the anal fin (without skin), and ii) the rest of the fillet cut off the spine from the base of the pectoral fin to the tip of the tail on both sides (without skin, hereafter referred to as ‘remaining muscle’). It should be noted that muscle tissue was removed thoroughly from the bone; yet, a small amount of tissue remained attached to the carcass. The wet mass was taken for all excised tissues using different scales (VWR-124, Sartorius, Göttingen, Germany, 0.0001g resolution for samples < 120 g and PCE-BT 2000, PCE Instruments, Alicante, Spain, 0.01g resolution for samples > 120 g). Subsequently, all samples were stored at -20°C until further use.

Prior to the extraction of fat, the water content of reference muscle and remaining muscle was determined gravimetrically by freeze-drying (Lyovac GT 2, GEA Pharma Systems, Wommelgem, Belgium). Dry samples were then re-suspended in distilled water and homogenized using different grinders (Bosch MCM4100, Kenwood HDP40, IKA A11 basic), depending on the size of the sample. Subsequently, samples were freeze-dried again and shortly ground in order to achieve a good degree of homogenization and avoid the separation of fat from the rest of the sample. The fat content of each sample was determined following the protocol of Smedes (1999), modified by Schlechtriem et al. (2003). Accordingly, fat was extracted from approx. 100mg sample using a mixture of cyclohexane (2.50mL), propan-2-ol (2.00mL) and water (2.75mL), following a second extraction with
cyclohexane (2.175mL) and propan-2-ol (0.325mL), collecting the organic phase after each extraction. After evaporating the solvent, the remaining fat was determined gravimetrically. All measurements were conducted as quadruplicates and the mean values were used in subsequent analyses. Parallel to the extraction of fat, analytical dry mass was determined in 250mg of the same sample, dried at 105°C for 4 h to remove the residual moisture. Measurements were conducted as duplicates and the fat contents were corrected for the average water content of both measurements to derive the fat content per dry mass, which was converted to fat content per wet mass using the previously determined water content.

Given the small amount of tissue used for the analyses, homogeneity of the sample was considered a key factor for the reliability of the results. Therefore, the following criteria for quality control were defined: For fat, single measurements must not deviate more than 1 percentage point from the mean or 2.5 percentage points if the relative deviation was smaller than 5%. For analytical dry mass, single measurements must not deviate more than 1 percentage point from the mean. In case any of the criteria were not met, the procedure was repeated, starting with the homogenization of the sample. In a single case, only three values for the fat content were available, which were well within the limits of the quality control and were therefore used as is in subsequent analyses.

While the measurements provided direct estimates of the reference muscle water and fat content, the remaining muscle did not depict an accurate estimate of the whole muscle water and fat contents since the reference muscle was removed. Thus, the whole muscle fat content was calculated as the weighted mean of reference muscle and remaining muscle (i.e. sum of absolute fat/water content of both samples in g divided by the sum of absolute wet mass of both samples in g), which is considered the best estimate of the actual whole
muscle water/fat content and will therefore be referred to as observed muscle water/fat content in the following. Furthermore, this estimate allows for a direct comparison with FM derived estimates since the measurement refers to the same part of the body (i.e. two trimmed fillets without skin).

Calculation of skin thickness

To calculate skin thickness, we assumed that the eel body has density of 1g·cm\(^{-3}\) and resembles a cylindrically shaped object. Accordingly, body weight could be directly converted to volume, allowing the calculation of skin surface based on body length and the geometrical properties of a cylinder. Given the wet mass of the skin and assuming that the density of eel skin is constant, an indirect estimate of skin thickness was derived as the average skin mass per skin surface area (g·cm\(^{-2}\)) for each individual.

Statistical analyses

Simple linear correlations were tested by Pearson’s product-moment correlation test if the assumptions for normality and linearity were met. Further data exploration was conducted following the protocol described in Zuur et al. (2010). Due to the high collinearity we initially performed separate linear regressions to investigate the relationship between the following parameters (all fat contents in wet mass): 1) observed muscle fat content (OM) vs. muscle water content (WA) and life stage (ST), 2) observed muscle fat content vs. FM measurement of muscle fat content (FM) and life stage, 3) observed muscle fat content vs. fat content of the reference muscle (RM) and life stage and 4) observed muscle fat content vs. FM measurement of muscle fat content and skin thickness (SK). Life stage (i.e. yellow or silver eel) was included as a categorical predictor. Since life stage is not independent of length (i.e.
length is used to calculate the SI, Durif et al. 2005), but potentially affects FM
measurements and fat content in the reference muscle (Fig. 2b & 3b, see discussion), we
additionally ran separate models for either life stage: 5) observed muscle fat content vs. FM
measurement of muscle fat content and length (L) for yellow eels, 6) observed muscle fat
content vs. FM measurement of muscle fat content and length for silver eels, 7) observed
muscle fat content vs. fat content of the reference muscle and length for yellow eels and 8)
observed muscle fat content vs. fat content of the reference muscle and length for silver
eels. All models included the interactions. If an interaction was found non-significant, the
interaction term was removed from the model. If we found heterogeneity in the residuals,
we applied generalized least squares and incorporated a variance structure into the model
(Pinheiro and Bates, 2000; Zuur et al., 2009). The level of significance for all tests was α =
0.05. All statistical analyses were conducted with the R statistical software version 3.4.1 (R
Development Core Team, 2017) using the car (Fox and Weisenberg, 2011) and the nlme
(Pinheiro et al., 2017) packages.

Results

Correlation between fat content vs water content and life stage

The observed muscle fat content was highly and inversely correlated with muscle water
content (p < 0.001). The effect of life stage was also significant (p < 0.01), but no significant
interaction was found (Fig. 1, Tab. 1). Thus, the relation of water to fat content was
constant, but not independent of life stage, with yellow eels having approx. 2% less fat than
silver eels for a given water content.
Performance of FM vs reference muscle to assess muscle fat content

We found that the observed muscle fat content was significantly correlated with both, FM measurements (p < 0.001) and life stage (p < 0.001), but no significant interaction was found (Fig. 2a, Tab. 1). FM measurements were on average 5.09 ± 3.26% higher for yellow and 4.94 ± 2.20% lower for silver eels, as compared to the observed muscle fat content, with min/max deviations of the FM being -2.08%/13.13% and -10.54%/6.1% for yellow and silver eels, respectively. It should be noted, that repeated measurements of the same individual, showed considerably less variation with an average standard deviation of 0.5 ± 0.27% (0.09% - 1.21%) averaged over all eels investigated in this study. Separate models for yellow and silver eels further revealed a significant effect of length in the prediction of the observed muscle fat content by FM measurements for yellow and silver eels (p < 0.05, Fig 2b, Tab. 1: Models 5 and 6).

The observed fat content was significantly correlated with reference muscle fat content (p < 0.01) and life stage (p < 0.001). In contrast to the FM, the interaction of independent variables was also significant (p < 0.05, Fig. 3a, Tab. 1). The accuracy of reference muscle derived fat contents was higher than for the FM, as reflected by the lower average deviation from the observed muscle fat content, with yellow eels being 3.12 ± 3.75%, and silver eels being 0.22 ± 3.22% higher than the observed muscle fat content. Min/max deviations were -8.04%/9.85% and -10.82%/4.68% for yellow and silver eels, respectively. The variation of errors was, however, more pronounced than for FM measurements for both life stages, as indicated by the higher standard deviation. While length had no significant effect on the prediction of the observed muscle fat content by reference muscle fat content for yellow
eels (Fig. 3b, Tab. 1: Model 7), both the effect of length and the interaction term were significant for silver eels (p < 0.05, Fig. 3b, Tab. 1: Model 8).

Effect of skin thickness on FM measurement

The linear regression showed that skin thickness and the difference between observed muscle fat content and FM measurement (ΔFM) were highly and significantly correlated (Fig. 4, R² = 0.83, p < 0.001). Multiple linear regression (model 4) further revealed a significant interaction between skin thickness and FM measurements (p < 0.01) and explained for > 94% of the observed variance (Tab. 1).

Discussion

The comparison of FM readings to the observed muscle fat content showed a considerable lack in accuracy, with yellow eels being systematically over- and silver eels being underestimated. In contrast, sampling of reference muscle provided comparably accurate estimates of the fat content for silver eels, whereas estimates for yellow eels were on average higher than the observed muscle fat content. For either method, the effect of life stage was significant (Tab. 1) and can be calibrated accordingly; yet, the overall smaller variation of errors in FM-derived estimates indicates that this method is better suited to predict the observed fat content, after correction. In addition, we found that FM readings were affected by body length (Tab. 1, Fig. 2b), resulting in lower values for larger eels, whereas for the reference muscle an effect of length was only significant for silver eels (Tab. 1, Fig. 3b). Accordingly, FM-derived values can be corrected for length and life stage, however, with separate models for yellow and silver eels (Tab. 1, Model 5 and 6), since life
stage is not independent of length (see above). In summary, FM does not provide reliable estimates of an individual’s fat content as is, but given the here provided calibrations (Models 5 & 6) it outperforms the prevailing standard of subsampling reference muscle and is therefore favorable, especially taking into account that this method does not require sacrificing fish.

In contrast to other species, the observed deviation of FM readings for the European eel was comparably high. For example, in studies on albacore tuna (*Thunnus alalunga*, Goñi & Arrizabalaga 2010), sockeye salmon (*Oncorhynchus nerka*, Crossin & Hinch 2005) and Atlantic herring (*Clupea harengus*, e.g. Davidson & Marshall 2010) the authors concluded that FM readings produced accurate readings of individual fat contents. Nielsen et al. (2004), however, highlighted that lipid and water content in herring differed according to their gonadal development status and therefore influenced FM readings. Accordingly, the lowest correlation of FM readings and fat contents derived from solvent extraction was found during periods of maturation, ripeness and spawning. Furthermore, they found significant differences between FM readings on the skin and muscle side of trimmed fillets, indicating that the two sides of the fillet respond differently to the microwaves. Even though FM readings were more accurate on the skin side, these results indicate that any change in skin properties (e.g. thickness, water or fat content) will affect the accuracy of FM readings. These findings are in line with the original studies on microwave measurements performed by Kent (1990) who pointed out that variation in water and fat content, as well as the effect of skin thickness are amongst the major sources of error in microwave measurements.

Due to the physiological and morphological changes from yellow- to silver eel, similar effects were to be expected for the European eel. Our results clearly show a change in the relation
of water and lipid levels between different life stages (Tab. 1, Fig. 1), indicating that FM
readings can only be as accurate as approx. 2% without accounting for the life stage, which
is in line with the manufacturer’s specifications (Distell 2003). The actual deviation was,
however, notably higher. Pankhurst (1982b) described an increase in skin thickness in
European eels with body length and sexual maturation. Given the high moisture content of
eel skin (Brinkmann et al. 2015) and its close proximity to the FM sensor, this could lead to
an overestimation of the water content in large, mature eels (i.e. silver eels), resulting in an
underestimation of the fat content, which is in line with our findings. Though the here
presented data do not necessarily establish a causal link between skin thickness and the
variation in FM readings, their high predictive power for the observed fat content (model 4)
and the notable correlation of the measurement error with skin thickness (Fig. 4) strongly
suggests a relevant influence and at least renders skin thickness a useful indicator to
calibrate FM readings. Therefore, the implementation of non-invasive methods for
measuring skin thickness (e.g. ultrasound, Moore et al. 2003) could further improve
microwave measurements of the fat content in fish. Since the application of such methods
is, however, not trivial, the here suggested calibration by stage and length provides a more
practicable approach for the European eel.

Interestingly, fat contents derived from solvent extraction in the reference muscle were also
affected by life stage, resulting in an overestimation of yellow eels. In some fish species,
muscle fat is unevenly distributed along the body axis (e.g. Atlantic salmon, Salmo salar,
Herbinger & Friars 1991), which is also the case for the European eel (McCance 1944; Tesch
1999). Accordingly, fat contents increase towards the posterior part of the body, where the
sample was taken, causing the observed overestimation in yellow eels. The more accurate
values for silver eels indicate more evenly distributed body fat for this life stage, which is possibly related to the remobilization of energy stores during sexual maturation (Lewander et al. 1974). This is also in line with our observations during FM measurements, where a single value is displayed for every point of measurement. Yellow eels displayed the above described increase of muscle fat content towards the tail, while it was less pronounced, yet noticeable, in silver eels. Furthermore, sampling a muscle piece of constant length could have added to this effect, since the proportion of the fatty posterior part is higher in samples from smaller fish (i.e. yellow eels). However, one would consequently expect to find a negative correlation of the measurement error with body length, which should be less pronounced for silver eels, where the range of sampled body lengths is smaller and fat is more evenly distributed. In fact, no such effect was found in yellow eels, while it was significant for silver eels (Tab 1, Fig. 3b). Under the assumption that smaller eels utilize energy for growth rather than building up fat deposits, and the deposition of fat starts in the posterior part of the body, the above described increase of fat content towards the tail would be less pronounced in smaller individuals, therefore masking the expected increase of the measurement error in yellow eels. Given the available dataset, this aspect is speculative, however, and parts of the results remain inconclusive. Accordingly, the provided models have limitations when it comes to the calibration of fat contents derived from subsamples of eel muscle tissue. Nonetheless, the presented results highlight considerable uncertainties of this method, putting a prevailing standard for the estimation of muscle fat contents in eels in question, particularly if applied to yellow eels.

Four notable sources of error should be considered in the presented study: the small sample size used for solvent extraction of fat, handling of the FM device, the classification of yellow
and silver eels by the silvering index (Durif et al. 2005) and the relatively small number of measured individuals. The small sample size is prone to cause large, random measurement errors, if samples are not evenly homogenous, which was sufficiently controlled by conducting quadruple measurements and is therefore considered negligible. On the contrary, handling of the FM device almost certainly introduces an unknown error, since it is practically impossible to keep the conditions constant for each measurement (e.g. residual surface moisture). These errors are, however, most likely not systematic and did not contribute towards the here observed patterns. Furthermore, previous studies stated that FM devices are less prone for misapplication, as compared to other non-invasive methods (e.g. BIA, Cox et al. 2011; Klefoth et al. 2013) and are therefore considered of little concern.

The staging of eels by the silvering index causes more complex problems: Since the classification is categorical and based on a number of morphometric parameters (e.g. eye diameter, pectoral fin length) small measurement errors of these parameters could result in a false classification, especially for fish that are in transition from one stage to another, which was presumably the case for the three silver eels that were treated as outliers. Therefore, we strongly advise to use the calibrations only for the here presented ranges of fat contents of yellow and silver eels, respectively. Also, silvering is not an immediate change, but rather a continuous process and therefore a continuous index (e.g. OI, Pankhurst 1982a) might be better suited. However, a comparison with models where stage was replaced with the OI, which is not categorical, did not result in an improvement of the model fits (results not shown). Though the number of sampled eels is relatively small, it covers a large range of fat contents, body length and life stages. While it is certainly desirable to generate larger datasets, particularly to quantify the uncertainty in the
calibrations, the here presented data provides the best means to evaluate the applicability
of the investigated methods, up to date.

In summary, our analyses showed that fat determination via solvent extraction in a distinct
subsample of muscle tissue was the more accurate method to predict the whole muscle fat
content of eels, as compared to FM derived values. In both cases the life stage of eels, and
in case of the FM also body length, systematically affected the accuracy of measurements.
The findings of this study can be used to calibrate either method, and – given the remaining
uncertainties in the determination of fat contents in subsamples of muscle tissue –
ultimately render non-lethal assessment of muscle fat content by microwave measurements
an improvement over the prevailing standard, without the necessity of sacrificing fish. It
should be noted, that individual fat contents derived by either method showed considerable
variation and need to be interpreted with caution, though the estimation of fat contents in
batches is less prone to error.

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References

content, category B, 1996 (ISO/DIS 6492), Geneva, Switzerland. International Organisation
for Standardisation


https://mc06.manuscriptcentral.com/cjfas-pubs


Goñi, N., Arrizabalaga, H. 2010. Seasonal and interannual variability of fat content of juvenile albacore (Thunnus alalunga) and bluefin (Thunnus thynnus) tunas during their feeding migration to the Bay of Biscay. Prog. Oceanogr. 86: 115-123


Klefoth, T., Skov, C., Aarestrup, K., Arlinghaus, R. 2013. Reliability of non-lethal assessment methods of body composition and energetic status exemplified by applications to eel (Anguilla anguilla) and carp (Cyprinus carpio). Fish. Res. 146: 18-26


McCance, R.A. 1944. The chemistry of growth and the food value of the common eel (Anguilla anguilla (L.)). Biochem. J. 38:474-480


Lipid content in herring (Clupea harengus L.) – influence of biological factors and comparison of different methods for analyses: solvent extraction, Fatmeter, NIR and NMR. LWT Food Sci. Technol. 38: 537-548


Tab. 1 Summary of models for the prediction of relative fat content in muscle wet mass of European eels (all proportions in decimal numbers).

<table>
<thead>
<tr>
<th>Model</th>
<th>Coefficients</th>
<th>β</th>
<th>SE</th>
<th>p</th>
<th>lower 95% CI</th>
<th>upper 95% CI</th>
<th>R²</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) OM = 0.8477 - 1.0196 x WA - 0.0213 x ST</td>
<td>Intercept 0.8477 0.0250</td>
<td>&lt; 0.001</td>
<td>0.7973</td>
<td>0.8982</td>
<td>GLS</td>
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<tr>
<td></td>
<td>WA -1.0196 0.0451</td>
<td>&lt; 0.001</td>
<td>-1.1106</td>
<td>-0.9286</td>
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<td></td>
<td>ST -0.0213 0.0065</td>
<td>&lt; 0.01</td>
<td>-0.0345</td>
<td>-0.0081</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(2) OM = 0.0634 + 0.9407 x FM - 0.1003 x ST</td>
<td>Intercept 0.0634 0.0175</td>
<td>&lt; 0.001</td>
<td>0.0281</td>
<td>0.0987</td>
<td>GLS</td>
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<tr>
<td></td>
<td>FM 0.9407 0.0708</td>
<td>&lt; 0.001</td>
<td>0.7980</td>
<td>1.0833</td>
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<td></td>
<td>ST -0.1003 0.0080</td>
<td>&lt; 0.001</td>
<td>-0.1165</td>
<td>-0.0841</td>
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<tr>
<td>(3) OM = 0.171 + 0.397 x RM - 0.1599 x ST + 0.4077 x RM x ST</td>
<td>Intercept 0.1710 0.0402</td>
<td>&lt; 0.001</td>
<td>0.0898</td>
<td>0.2521</td>
<td>GLS</td>
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<tr>
<td></td>
<td>RM:ST 0.3970 0.1389</td>
<td>&lt; 0.01</td>
<td>0.1169</td>
<td>0.6770</td>
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<tr>
<td></td>
<td>ST -0.1599 0.0428</td>
<td>&lt; 0.001</td>
<td>-0.2463</td>
<td>-0.0735</td>
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<td></td>
<td>RM:ST 0.4077 0.1521</td>
<td>&lt; 0.05</td>
<td>0.1009</td>
<td>0.7145</td>
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<tr>
<td>(4) OM = -0.0258 + 0.315 x FM - 0.1251 x SK + 7.9866 x FM x SK</td>
<td>Intercept -0.0258 0.0488</td>
<td>0.5998</td>
<td>-0.1242</td>
<td>0.0726</td>
<td>OLS</td>
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<td>FM 0.3150 0.2117</td>
<td>0.1442</td>
<td>-0.1120</td>
<td>0.7420</td>
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<td></td>
<td>SK -0.1251 0.5653</td>
<td>0.8259</td>
<td>-1.2652</td>
<td>1.0150</td>
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<tr>
<td></td>
<td>FM:SK 7.9866 2.4683</td>
<td>&lt; 0.01</td>
<td>3.0089</td>
<td>12.9643</td>
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<tr>
<td>(5*) OM = -0.1375 + 0.9483 x FM + 0.0017 x L</td>
<td>Intercept -0.1375 0.0390</td>
<td>&lt; 0.01</td>
<td>-0.2176</td>
<td>-0.0573</td>
<td>GLS</td>
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<tr>
<td></td>
<td>FM 0.9483 0.0645</td>
<td>&lt; 0.001</td>
<td>0.8157</td>
<td>1.0809</td>
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<tr>
<td></td>
<td>L 0.0017 0.0008</td>
<td>&lt; 0.05</td>
<td>0.0001</td>
<td>0.0033</td>
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<tr>
<td>(6**) OM = 0.1125 + 0.524 x FM + 0.0006 x L</td>
<td>Intercept 0.1125 0.0241</td>
<td>&lt; 0.001</td>
<td>0.0612</td>
<td>0.1638</td>
<td>GLS</td>
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<tr>
<td></td>
<td>FM 0.5240 0.0719</td>
<td>&lt; 0.001</td>
<td>0.3707</td>
<td>0.6773</td>
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<tr>
<td></td>
<td>L 0.0006 0.0003</td>
<td>&lt; 0.05</td>
<td>0.0000</td>
<td>0.0012</td>
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<tr>
<td>(7*) OM = -0.0119 + 0.8233 x RM + 0.0003 x L</td>
<td>Intercept -0.0119 0.0348</td>
<td>0.7342</td>
<td>-0.0835</td>
<td>0.0596</td>
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<tr>
<td></td>
<td>RM 0.8233 0.0489</td>
<td>&lt; 0.001</td>
<td>0.7229</td>
<td>0.9238</td>
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<td>L 0.0003 0.0007</td>
<td>0.6585</td>
<td>-0.0012</td>
<td>0.0018</td>
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<tr>
<td>(8**) OM = 0.9181 - 2.3476 x RM - 0.0095 x L + 0.0349 x RM x L</td>
<td>Intercept 0.9181 0.3022</td>
<td>&lt; 0.01</td>
<td>0.2701</td>
<td>1.5662</td>
<td>GLS</td>
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<td></td>
<td>RM -2.3476 1.0137</td>
<td>&lt; 0.05</td>
<td>-4.5217</td>
<td>-0.1734</td>
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<td>L -0.0095 0.0040</td>
<td>&lt; 0.05</td>
<td>-0.0181</td>
<td>-0.0009</td>
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<tr>
<td></td>
<td>RM:L 0.0349 0.0133</td>
<td>&lt; 0.05</td>
<td>0.0063</td>
<td>0.0635</td>
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</table>

 Abbreviations: observed relative fat content in whole muscle wet mass derived by solvent extraction (OM), relative muscle water content (WA), relative fat content in muscle wet mass derived by fatmeter (FM), relative fat content of reference muscle in wet mass derived by solvent extraction (RM), life stage (ST, categorical: Y = 1 / S = 0), skin thickness (SK) and total length in cm (L). *: yellow eels only **: silver eels only.
Fig. 1 Observed muscle fat content and muscle water content of yellow (○, open circles, dashed line) and silver European eels (●, filled circles, solid line). Individuals marked by * were not included in statistical analyses.
Fig. 2 (a) Observed relative muscle fat content and FM derived muscle fat content of European eels and (b) difference between FM derived muscle fat content and observed muscle fat content (Δ FM) in relation to body length, with linear regressions for yellow (○, open circles, dashed line) and silver eels (●, filled circles, solid line). Individuals marked by * were not included in statistical analyses.
Fig. 3 (a) Observed relative muscle fat content and fat content of the reference muscle of European eels and (b) difference between the reference fat content measured in a subsample and observed muscle fat content ($\Delta$ reference muscle) in relation to body length, with linear regressions for yellow (○, open circles, dashed line) and silver European eels (●, filled circles, solid line). Individuals marked by * were not included in statistical analyses.
**Fig. 4** Difference between observed muscle fat content and FM measurement (Δ FM) in relation to skin thickness of yellow (○, open circles) and silver (●, filled circles) European eels.