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A Correcting Factor for the Reduction of Body Length and Mass of European Eel After Ethanol Preservation and After Freezing

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ABSTRACT

Measurements of length and mass are used in many research fields, and such data are often collected from samples that have been preserved in ethanol or frozen prior to data collection. Since many preservation methods affect the size and shape of soft-bodied animals, species-specific correction factors are used. Here, we calculated ethanol and freezing shrinkage correction factors for the European eel, *Anguilla anguilla* Linnaeus, 1758, and investigated how preservation duration and individual size affected shrinkage. We also investigated if freezing had an impact on the size of the eyes and pectoral fins, which could affect maturation stage classification. We found that preservation in 95% ethanol and freezing decreased body length and mass, as expected. Time kept in ethanol did not affect shrinkage. Time kept in freezer had some effect on shrinkage, and the model fit suggested inclusion of days frozen for body mass shrinkage. That, however, only had negligible improvement on the model. For preservation in freezer, shrinkage was greater for lengths below 330 mm and mass below 100 g, compared to eels above these sizes. However, applying a size threshold to the analysis only generated negligible improvement of the model fit, meaning that specific shrinkage factors for different sizes are not needed. We also found that freezing induced shrinking in eye size, while the pectoral fin increased in size. The increase in pectoral fin length is however believed to be due to a measurement deviation. User-friendly formulas for all correction factors are provided. The application of these factors should be restricted to the European eel within the size range used in this paper.

1 | Introduction

Basic data, such as body length, body mass, and the length, mass, and shape of different parts of the animal, are often used as descriptive data and/or included in models as covariates or main effects depending on the study. Such data are used in virtually any discipline, from basic science such as evolutionary biology, to applied science such as aquaculture and fisheries research. The measurements are often collected on alive specimens, or on alive and sedated or recently anesthetized individuals. This is commonly done within basic (experimental) research and aquaculture, as the study animals are usually readily available, and measurements can be collected in controlled laboratory conditions. In other instances, sampling of the test animals is conducted under such circumstances that direct, accurate, measurements of length, body mass and similar cannot be collected.

TABLE 1 | Shrinkage in total body length and body mass in published papers for the European eel, *Anguilla anguilla*, and the American eel, *Anguilla rostrata*, after preservation in freezer and in ethanol (70%), detailing percent shrinkage in length and mass, body length and mass (reference 1: average length and mass, reference 2: min–max), and sample size, as presented in the papers.

Preservation	Species	Shrinkage length, mass (%)	Eel length, mass	Sample size	Ref.
Freezing	*A. anguilla*	2.4, 2.7	339 mm, 44.4 g	53	1[a]
Freezing	*A. anguilla*	1.3–4.8, 6.0–19.6	81–989 mm	2576	2
Freezing	*A. rostrata*	1.2, 1.9	Not provided	180	3
Freezing	*A. rostrata*	3.0, NA	Not provided	30	4
Ethanol	*A. rostrata*	2.8, not provided	Not provided	Not provided	5[b]

Note: References: 1: Wickström (1986), 2: Simon (2013), 3: Machut et al. (2007), 4: Morrison and Secor (2003), 5: Powles and Warlen (2002).

[a]In Swedish, not peer-reviewed, not digitalized.

[b]Mentions correction factor in discussion, referring to unpublished data.

It is then necessary to preserve the sample for later measurements and analysis. Large-bodied species (or life stages) are usually kept frozen, while small species/life stages are commonly kept in ethanol or formalin. Preservation, whether done with chemicals or freezing, however comes with the drawback that it affects size, usually leading to a reduction in both length and mass of many soft-bodied animals, such as fish, due to loss of water content (Dae-Jin 1982; Butler 1992). Since such changes could lead to inaccurate reporting of basic statistics, and/or erroneous results from statistical models, the shrinkage needs to be corrected for. The degree of shrinkage is dependent on species, time between death and preservation, time kept in preservation, and type and strength of preservative (Butler 1992). This variability means that the correction factor must be species-specific, and account for type of preservative and preservation time.

The European eel, *Anguilla anguilla* Linnaeus, 1758, is an important species within fisheries research, basic/experimental research and aquaculture. Despite being listed as Critically Endangered (Pike et al. 2020), with a decrease in recruitment of 95%–99% and a population trend in sharp decline (ICES 2024), it remains commercially important in many European countries (ICES 2024). Several management practices for the recovery of the stock are in place (Council Regulation No. 1100/2007), and it is monitored within the EU Data Collection Framework (DCF, Regulation EU 2017/1004). As part of the DCF, EU member states collect biological fisheries data, including data on length, body mass and maturation stage, to support the Common Fisheries Policy (CFP) through scientific advice. Accurate biological data is necessary in stock assessments, which is essential to ensure that management and conservation efforts work as intended. Despite the elaborate usage of this species in research, surprisingly few preservation-shrinking factors are available in the published literature, and they differ quite markedly (Wickström 1986; Simon 2013; Höhne et al. 2023) (Table 1). Some correction factors are available for the closely related American eel, *Anguilla rostrata*, however they too differ between studies (Powles and Warlen 2002; Morrison and Secor 2003; Machut et al. 2007) (Table 1). In addition, information on the size range or sample size used to calculate the correction factors is sometimes missing (Table 1), making them difficult to apply to any given dataset.

In this study, we calculated a correction factor for shrinkage in 95% ethanol using juvenile eels, since such a correction factor is seemingly not available in the literature for the European eel. We also calculated a freezing shrinkage correction factor for total body length and body mass using a large dataset with over 1900 individual measurements for length and over 1100 individual measurements for body mass. We also evaluated the effect of time kept in freezer on shrinkage, since this parameter has not been assessed in previous studies. In addition to changes in total body length and body mass, preservation can affect the size of other organs, such as eyes and fins (Höhne et al. 2023). This is particularly important for the European eel, where eyes and pectoral fins are measured in order to calculate maturation stage (i.e., Durif's silver index: Durif et al. 2005; Durif et al. 2009). It has recently been shown that freezing can affect the size of the eyes and pectoral fin, and this could impact the maturation stage classification (Höhne et al. 2023). We hence derived a correction factor formula for eyes and pectoral fins to increase the available published data.

2 | Material and Methods

2.1 | Ethical Statement

The data and samples used in this study have been collected as part of several different monitoring programmes in accordance with the animal ethical legislation valid at that time, with the latest permit being issued in 2020. The care and use of experimental animals complied with the Swedish Board of Agriculture's animal welfare laws, guidelines and policies as approved by the Swedish Board of Agriculture (permit number: Dnr 6229-2020).

2.2 | Ethanol Shrinking for Total Body Length and Mass

To calculate the ethanol shrinking correction factor, we used data from juvenile eels (n = 79, 85–187 mm, 0.556–8.709 g), imported in 2022 to Sweden from France by Scandinavian Silver Eel (a commercial importer of glass eel), and then held at the Institute of Freshwater Research, Swedish University for Agricultural Sciences, Institute of Aquatic Resources (SLU Aqua). The samples utilized here were used for other purposes within the Swedish monitoring programme for European eel, which is part of the EU DCF in the fisheries and aquaculture sector (Regulation 2017/1004). The 'fresh' measurements were collected 30 August 2022 on eels that had been killed with an overdose of analgesics

(Benzocaine E1501-100G, Sigma-Aldrich) (data collected by J.P., assisted by J.S.). The biological parameters measured were total length (± 1 mm, measured using a standard measuring board), and total body mass (± 0.001 g, using a Sartorius lab instruments, Germany). Each individual eel was then placed in an ID labelled 50 mL screw cap tube (114 × 28 mm, conical base, Sarstedt) containing 40 mL 95% ethanol. The samples were stored for 80 days and thereafter length and mass were measured again (18 November 2022, by J.P., assisted by J.S.), as described above. The samples were then returned to the respective tube with ethanol (no ethanol was refilled) and the measurement procedure was repeated after an additional 31 days (19 December 2022, total storage time: 111 days, $n = 64$), and again after an additional 339 days (2023-11-23, total storage time: 450 days, $n = 64$) (all measurements made by J.P., assisted by J.S.). Note that the measurement after 80 days was performed on all 79 eels, whereafter 15 eels were utilized for other purposes, leaving $n = 64$ eels for the remaining measurements. The correction factor for ethanol-introduced shrinkage was calculated assuming a linear relationship between fresh and ethanol treated length and body mass, respectively. Values of α and β were estimated using the 'lm' function in R (R Core Team 2024).

2.3 | Freeze Shrinking for Total Body Length and Mass

To calculate the freeze shrinking correction factor, we extracted data from a database called 'Sötebasen', kept by the Institute of Freshwater Research, Swedish University for Agricultural Sciences, Institute of Aquatic Resources (SLU Aqua) (data extracted 2024-12-28, by S.S.). The database contains measurements of thousands of individual eels from several lakes and coastal areas across Sweden covering an extensive time period. For the analyses in this paper, data where both fresh and defrosted body length and body mass for an individual were available were used. Individuals with missing data for any of those variables were excluded, leaving $N = 1984$ individuals for the analysis of length (62–1089 mm), and $N = 1102$ individuals for the body mass analysis (0.29–3339.30 g), (data collected 2005–2024). The data have been collected as part of several different monitoring programmes (by several different people), with the most recent being the EU DCF in the fisheries and aquaculture sector (Regulation 2017/1004). The number of days frozen before defrosted varied between 1–1363 days for both length (mean = 217 days) and body mass (mean = 152 days) measurements. Based on previous studies and graphical examination of the data, we assumed that the relationships between fresh and defrosted measurements of body length and mass were linear, and the correction factors (intercept, α, and slope, β) were therefore estimated using the 'lm' function in R (R Core Team 2024). The individual shrinkage, in percent, was assumed to reach an asymptote for both size (mass and length) and for the number of days frozen. The relationship was therefore calculated as:

$$S_i = \rho(\rho - \nu)e^{-\omega x_i}$$

where S_i is the individual (i) shrinkage proportion for each individual fresh length, fresh body mass, or days frozen, x_i. ρ is the asymptote, ν is the intercept and ω is the relative rate of change in S_i based on the increase in x_i. Instead of dividing the potential shrinkage into predefined size-classes (length and mass) and day-classes as was done in Simon (2013), any potential size-class or number of days frozen division was evaluated by comparing the asymptote of the shrinkage function and the mean of different size- and day-classes. The parameters in the fitted asymptotic regression were estimated using the nls_multstart function from the nls.multstart package (Padfield et al. 2021).

2.4 | Freeze Shrinking for Eyes and Pectoral Fins

To test if freezing had an effect on the size of the eyes and pectoral fin, thereby potentially altering the maturation stage classification, we used data from eels ($n = 53$, 417–964 mm, 119–2049 g) sampled in 2022 within a fisheries independent survey in lake Mälaren, Sweden (59°20′04.3″N 17°52′30.6″E). The survey is part of the Swedish monitoring programme for European eel, which is part of the EU DCF in the fisheries and aquaculture sector (Regulation 2017/1004). For method details on the survey, see Myrenås et al. 2023. The 'fresh' measurements were collected on newly caught eels that had been killed with an overdose of analgesics (Benzocaine E1501-100G, Sigma-Aldrich) (data collected by J.P., 16 May to 12 July 2022). The biological parameters measured were total body length (± 1 mm, measured using a standard measuring board), total body mass (± 0.1 g, measured using a Mettler PC 4400 scale, Mettler Instruments AG, Zürich, Switzerland), vertical and horizontal diameter of the left and right eye, and left and right pectoral fin length (for both, ± 0.01 mm, measured using digital calipers, Mitutoyo Absolute Coolant proof IP67, Mitutoyo, Aurora, Illinois, USA). The eels were then labelled with a unique ID code and frozen at −18°C. Dissections were conducted on defrosted samples from 18 October to 21 December 2022 (data collected by J.P.) (freezing time: 99–180 days). For each individual eel, the same measurements were taken again (i.e., total body length, body mass [without removing coagulated mucus], left and right eye diameter, and left and right pectoral fin length), as described above. For the correction factor calculations, we did not use both the left- and right-side measurements since they do not differ (Sundin et al. 2022). We calculated eye area using the mean radius from one horizontal and one vertical measurement of the left eye. The relationship between fresh and defrosted eye area, and fresh and defrosted (left) pectoral fin, respectively, was assumed to be linear and the intercept and slope was estimated using the 'lm' function in R (R Core Team 2024).

All statistical analyses were performed by S.S. The full model for each measurement included frozen length or body mass and the number of days frozen. Each full linear model was then compared with the model including only frozen length or body mass, using the 'Anova' function in R, and non-significant full models were excluded.

3 | Results

3.1 | Ethanol Shrinking for Total Body Length and Mass

Preservation in 95% ethanol reduced both length and body mass (Figure 1, Table 2), with an average shrinkage of −6.2 mm and

TABLE 2 | The mean difference (percent) in measured metrics between fresh and 95% ethanol stored/preserved samples of European eel, *Anguilla anguilla*, and between fresh and defrosted eel, and the 95% confidence intervals. Total size range used for ethanol was 85–187 mm, 0.556–8.709 g ($n = 79$). For freezing, the size range for length was 62–1089 mm ($n = 1984$) and the size range for weight was 0.29–3339.30 ($n = 1102$).

Metric	Preservation	Mean difference (%)	95% CI
Body length	Ethanol	−4.84	−5.06 to −4.62
Body mass	Ethanol	−20.27	−22.00 to −18.54
Body length	Freezing	−3.07	−3.15 to −2.99
Body mass	Freezing	−7.77	−8.36 to −7.19
Eye area	Freezing	−2.74	−6.48 to 1.01
Pectoral fin	Freezing	3.35	2.24 to 4.47

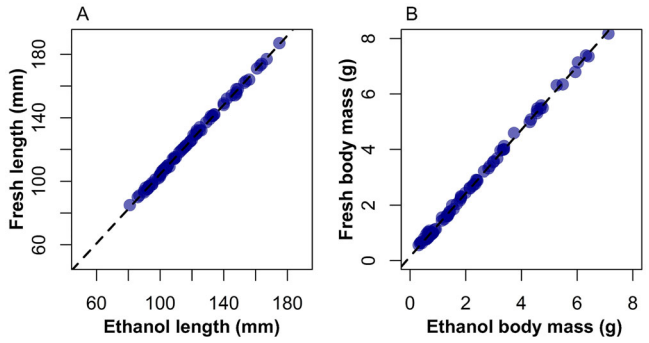

FIGURE 1 | Relationship between measurements on fresh samples of European eel, *Anguilla anguilla*, and after storage for 80 days in 95% ethanol in (A) length and (B) body mass. Blue data points show each individual measurement, dashed black lines show the fitted linear relationship (length: $n = 79$, mass: $n = 79$).

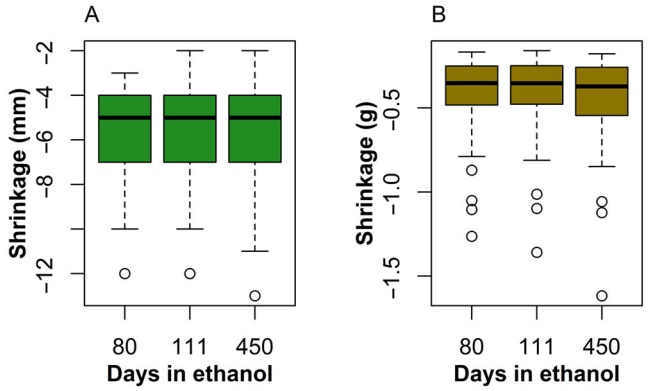

FIGURE 2 | Shrinkage in (A) length and (B) body mass of European eel, *Anguilla anguilla*, shown as comparison between fresh measurements ($N = 64$) and number of days is stored in 95% ethanol (80, 111, and 450 days). Boxes show 50% of the data, black lines in boxes show median values, whiskers show 1.5 × IQR (interquartile range) and circles show data point outliers.

−0.48 g after 80 days. Keeping the eels for longer in ethanol essentially did not induce any additional shrinkage, with an average shrinkage of −0.09 mm and −0.001 g between 80 and 111 days, and an average shrinkage of −0.05 mm and −0.029 g between 111 and 450 days (Figure 2). Therefore, only the data obtained after 80 days in ethanol were used to calculate the correction factor, since that allowed us to include more data ($n = 79$ compared to $n = 64$). The decrease in body mass was greater for the ethanol preservation compared to freezing (Table 2).

3.2 | Freeze Shrinking for Total Body Length and Mass

Graphical analysis of shrinkage as a function of size (length and body mass) suggested a separation between eels with a fresh length of 330 mm and a body mass of 100 g (Figure 3A,B). The analysis showed that for lengths over 330 mm and mass over 100 g, the average shrinkage was equal to the asymptote. This means that sizes above these two thresholds will not introduce any additional shrinkage that is dependent on a specific length or body mass. Instead, sizes above the thresholds will shrink at a relatively constant rate with increasing size. Moreover, shrinkage was greater in smaller individuals below the threshold values, and there was more variation, both in length and mass (Figure 3A,B). However, if applying the size threshold to the correction factor analysis, this only introduced negligible improvement of the model fit of the linear model (Figure 4, Table 3), meaning that a size separation is not needed when calculating freezing shrinkage.

The effect of time kept in freezer (days frozen) showed a similar pattern as the effect of size on shrinkage (Figure 3C,D), but the maximum time frozen available from the dataset was not long enough to reach the asymptote in percentage shrinkage for body mass, as predicted by the model (Figure 3D). Shrinkage and variation in shrinkage for body mass as a function of days frozen were higher compared to the shrinkage for length (Figure 3C,D). Thus, for the effects of days frozen, model fit suggested inclusion of days frozen for the body mass shrinkage (Table 3). For length, visual evaluation suggested that after 100 days frozen, the shrinkage in length would on average have reached an asymptote and would not be affected by increased time frozen, whereas the average shrinkage in body mass did not reach an asymptote after 100 days (Figure 3C,D). However, including days frozen as a covariate only had a negligible improvement on the model fit (Table 3).

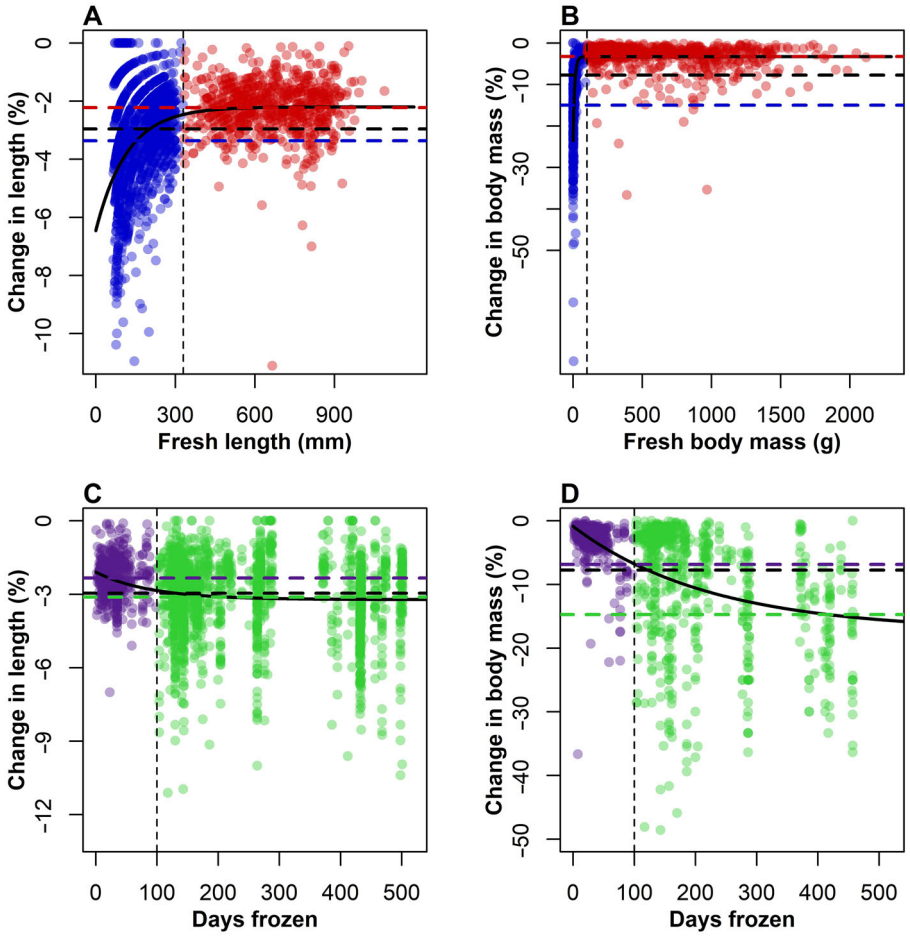

FIGURE 3 | Freezing shrinkage (change) in percent as a function of (A) fresh length, (B) fresh body mass, and as a function of days frozen for (C) length and (D) body mass in European eel, *Anguilla anguilla*. Solid black lines show applied asymptotic relationship between shrinkage, and black dashed lines indicate the average shrinkage, using the full dataset (i.e., using all data points per panel, A and C: $N = 1984$, B and D: $N = 1102$). The colour separation of data points and the coloured dashed lines in panel A and B show the average shrinkage based on sizes below and above the threshold values of 330 mm for length and 100 g for body mass (blue data points and lower dashed line, and red data points and upper dashed line) (below threshold: A: $n = 1274$, B: $n = 420$, above threshold: A: $n = 710$, B: $n = 682$). In panel C and D, the colour separation of data points and the coloured dashed lines show the average shrinkage after less and more than 100 days frozen (purple data points and upper dashed line, and green data points and lower dashed line) (< 100 days: C: $n = 401$, D: $n = 401$, > 100 days: C: $n = 1583$, D: $n = 701$). Note the different *y*-axis scales between panels.

3.3 | Freeze Shrinking for Eyes and Pectoral Fins

There was no difference in shrinkage between the left and right measurements of the eye area ($t = -0.49$, df = 99.95, $p = 0.62$), or the pectoral fin length ($t = -1.42$, df = 104.78, $p = 0.16$), wherefore results based on the left side measurements are included in the study. The eyes of the eels were shrinking after freezing whereas pectoral fin length increased after freezing (Figure 5, Tables 2 and 3).

4 | Discussion

In this paper, we provide user-friendly correction factor formulas that can be applied to correct for shrinkage of total length and mass of samples preserved in 95% ethanol or by freezing, as well as correction factors for eye and pectoral fin length after samples have been frozen. The application of these factors should be restricted to the European eel within the size range included in this paper.

4.1 | Ethanol Shrinking for Total Body Length and Mass

Preservation in ethanol caused shrinkage, as expected, and it was most pronounced for body mass.

Time kept in ethanol did not affect shrinkage, since the additional shrinkage, after the initial 80 days, essentially was within the measurement error (± 1 mm and ± 0.001 g). The correction factor provided here for ethanol shrinkage is particularly important since we were unable to find any such correction factors for the European eel in the scientific literature. A study using the American eel (Jessop 2010) reported that they used a correction factor of 2.8% for preservation in 70% ethanol, citing Powles and Warlen (2002). Jessop (2010) further states that they used a conversion equation from Jessop et al. (2004) to enable usage of that shrinking factor for samples preserved in 95% ethanol. It however proved difficult to backtrack these references. It is difficult to understand which conversion equation in Jessop et al. (2004) that should be used. In addition, the Powles and Warlen

TABLE 3 | Correction factors for linear relationships between fresh and preserved (frozen or stored in 95% ethanol) measurements in European eel, *Anguilla anguilla*, for all significant model specifications. α is the intercept and β is the slope of the linear function. R^2 is the goodness of fit for each linear model. Total size range used for ethanol was 85–187 mm, 0.556–8.709 g (n = 79). For freezing, the size range for length was 62–1089 mm (n = 1984) and the size range for weight was 0.29–3339.30 (n = 1102).

Linear model	α	β	β_2	R^2
Fresh length = $\alpha + \beta \times$ frozen length	2.1594	1.0194	NA	0.9997
Fresh length < 330 mm = $\alpha + \beta \times$ frozen length < 330 mm	2.3598	1.0182	NA	0.9987
Fresh length > 330 mm = $\alpha + \beta \times$ frozen length > 330 mm	1.7164	1.0200	NA	0.9982
Fresh body mass = $\alpha + \beta \times$ frozen body mass	0.4926	1.0360	NA	0.9979
Fresh body mass < 100 g = $\alpha + \beta \times$ frozen body mass < 100 g	0.5537	1.0283	NA	0.9983
Fresh body mass > 100 g = $\alpha + \beta \times$ frozen body mass > 100 g	0.5447	1.0359	NA	0.9963
Fresh body mass > 100 g = $\alpha + \beta \times$ frozen body mass + $\beta 2 \times$ days frozen	−2.4553	1.0368	0.0173	0.9979
Fresh length = $\alpha + \beta \times$ ethanol length	−4.7572	1.0926	NA	0.9987
Fresh body mass = $\alpha + \beta \times$ ethanol body mass	0.1481	1.1412	NA	0.9988
Eye area fresh = $\alpha + \beta \times$ frozen eye area	−3.0408	1.1578	NA	0.9554
Fresh pectoral fin length = $\alpha + \beta \times$ frozen pectoral fin length	−0.6854	0.9942	NA	0.9852

FIGURE 4 | Relationship between fresh and defrosted measurements of (A) length and (B) body mass in European eel, *Anguilla anguilla*. Black dashed lines show linear relationships using all data (length: N = 1647, mass: N = 785). Blue data points show individuals below the threshold values of 330 mm for length (left panel) and 100 g for body mass (right panel) (length: n = 1274, mass: n = 420). Red data points show individuals above the threshold values (length: n = 710, mass: n = 682). Blue and red dashed lines show linear relationships based on blue (below threshold) values and red (above threshold values), respectively.

FIGURE 5 | Relationship between fresh and defrosted measurements in European eel, *Anguilla anguilla*, of (A) the mean eye area (n = 59) and (B) the pectoral fin length (n = 59). Black dashed lines show fitted linear regression for the fresh and defrosted measurements.

(2002) study, that is, the original paper cited for the correction factor in Jessop (2010), only mentions the shrinking factor in the discussion, referring to unpublished data. Hence, it is not possible to find information about the size range or sample size used to calculate that correction factor, meaning that it cannot be applied to any given dataset. The correction factor presented here hence constitutes an important contribution and offers researchers the possibility to correct for ethanol shrinkage in their analyses.

4.2 | Freeze Shrinking for Total Body Length and Mass

Preservation by freezing also led to shrinkage in total length and mass, as expected. Using visual evaluation of the data, we found that small individuals (< 330 mm, < 100 g) might be more affected by freezing than larger individuals. However, the goodness of fit in the correction factors shows that such a division will have minor effects in the prediction of the fresh measurements, wherefore one correction factor can be used regardless of size. For precaution, we however suggest that this is only applied

for samples within the size range used in this paper. It has previously been suggested that different correction factors should be used for different size-classes based on 100 mm intervals (Simon 2013), but no statistical or biological rationale was given for that specific interval division. The range in shrinkage reported in other papers for European and American eel is relatively large, varying between 1.2% and 4.8% (Table 1). Wickström (1986) reported that length and body mass on average decreased with 2.4% and 2.7%, respectively. That study used relatively small eels and is based on a rather small sample size (mean 339 mm, 44 g, $n = 53$), and in addition, the publication is a report written in Swedish that is not peer-reviewed nor digitally available, meaning that it might be inaccessible to many researchers. Another study reported average shrinkage of 1.3%–4.8% in length and 6.0%–19.6% in mass (Simon 2013). The study is based on 2576 eels between 81 and 989 mm, and is hence sufficient for a large part of the size range of European eel (Simon 2013). Freezing correction factors are also available from studies using the American eel. Although not the same species, they are similar in many aspects. Machut et al. (2007) reported that freezing reduced length and body mass by 1.2% and 1.9%, respectively, but the length range that this reduction was calculated on is not given (size range of the total sample is given, but not of the subsample used to calculate the freezing correction factor) (Machut et al. 2007). Another study using the American eel reported that freezing caused approximately 3% decrease in total length, however again the size range of the subsample used to calculate the freezing factor is not given, but the size range of the total sample in the study indicates that relatively small eels were used (Morrison and Secor 2003). The reason for the variation in reported shrinkage between papers could hence be due to differences in sample size and/or size range used, but it could also be due to time kept in freezer, which is not always reported or used as a factor in the analyses. Here, we found that including days frozen as a covariate only had a negligible improvement on the model fit, following the results in Simon (2013). The maximum time frozen available in our dataset for body mass was however not long enough to reach the asymptote in percentage shrinkage, while the data for length indicated that after 100 days, the shrinkage would on average have reached an asymptote and would not be affected by increased time frozen. Other factors that could explain the differences in shrinkage between studies are the specific methods used, for example, freezing the eels in plastic bags with air or under vacuum, defrosting the samples in air or water, and whether the samples are measured with or without mucus (Ersoy et al. 2008; Simon 2013). In this study, we measured mass of the eels without removing the mucus (similar to Höhne et al. 2023). Simon (2013) specifically investigated the potential effects of keeping or removing the mucus (also termed desliming) and found that the average amount of coagulated mucus was 3.3% ± 3.4% (SD) of body mass, based on a sample size of 273 eels. A similar loss in mass after removal of mucus was reported in another study on the New Zealand eel, *Anguilla dieffenbachii*, and the short-finned eel, *Anguilla australis* (Beentjes and Chisnall 1998). Hence, whether or not coagulated mucus was removed can potentially induce a 3% difference in decrease in mass after storage in freezer, indicating the importance of accurate method reporting, and of knowing whether mucus was removed or not before applying a published correction factor.

4.3 | Freeze Shrinking for Eyes and Pectoral Fins

The eyes of the eels were shrinking after freezing, which is in agreement with previously published data (Höhne et al. 2023). The pectoral fin however increased in length after freezing. This is unexpected and it does not follow previously published work (Höhne et al. 2023), however Höhne et al. (2023) did report that pectoral fin length was measured with a −2.7 to +2.2 mm deviation from the fresh measurement. The mean difference between fresh and defrosted pectoral fins in our study was 3.35%, which equals to an actual difference of 0.85 mm. This unexpected increase might point to difficulties in measuring the pectoral fin, rather than an actual increase. Höhne et al. (2023) reported that measurement consistency of fins was particularly high on fresh eels and discuss that this could be due to the use of tactile cues to determine measurement limits, such as feeling the base of the fin. While this was mainly discussed in comparison to measuring the pectoral fin digitally from a photo, it is possible that locating the base of the fin differs in a fresh sample compared to a defrosted sample, which might explain the unexpected result found here. It is also a possibility that the base of the fin, consisting of muscle tissue, shrank more from the freezing preservation than the fin (in particular due to the fin rays), potentially implying that the base of the fin is measured further towards the body of the eel in a thawed sample, while the fin itself is still approximately equally long as the fresh sample, in that case generating a longer fin measurement. Obtaining accurate measurements of eye and pectoral fin is important given that these measurements are used to calculate maturation stage (i.e., Durif's silver index: Durif et al. 2005; Durif et al. 2009). Furthermore, Durif's silver index is frequently applied on data from both fresh and defrosted samples (Höhne et al. 2023; Sundin et al. 2022), and it has previously been reported that a correction factor is indeed needed (Höhne et al. 2023).

5 | Conclusion

We conclude that preservation in 95% ethanol and freezing decreased body length and mass, as expected, and that the decrease was most pronounced for body mass after preservation in ethanol. Applying correction factors is thus essential for accurate data reporting and statistical analyses. Some minor effects of time kept in preservation were observed, however controlling for preservation time only led to negligible improvement of the models. Similarly, some effects of size were observed, but applying a size threshold to the analysis generated a marginal improvement to the model fit. Correction factors should also be applied on eye and pectoral fin size, which is particularly important for the European eel to calculate accurate maturation stages. While our results showed a decrease in eye size after shrinkage, which was expected, we found that the pectoral fin size increased in size. This result must be interpreted with care and could potentially be due to differences in locating the base of the fin between the fresh and defrosted samples. In general, our results, together with previously published data, show that factors such as sample size, size range, storage time, specific methods used (for example whether the samples are measured with or without mucus), can have implications for the difference between

data collected on a fresh sample compared to after preservation. Thus, we suggest that readers use published correction factors for samples within the same size range and that used the same methods.

Author Contributions

Josefin Sundin: conceptualization, methodology, validation, investigation, resources, writing – original draft, writing - review and editing. **John Persson**: methodology, validation, investigation, writing – review and editing. **Stefan Skoglund**: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing – review and editing, visualization. In this manuscript, methods are reported using the author's initials to clarify contributor roles for reproducibility and replicability using the Method Reporting with Initials for Transparency (MeRIT) guidelines (Nakagawa et al. 2023). Additional author contribution roles are listed here according to the Contributor Role Taxonomy (CRediT) guidelines (https://credit.niso.org).

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data and script for this study are archived in the figshare repository (https://figshare.com/s/f045e9a9482f780e54f5, https://doi.org/10.6084/m9.figshare.28309352) following best practices (Roche et al. 2015) and were available to editors and reviewers upon initial submission.

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