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ARTICLE

Minimally Invasive Collection of Biometric Data Including Maturation Stage on European Eel Using Photography

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Abstract

In response to the severe decline of the European Eel *Anguilla anguilla* stock in recent decades, various data frameworks and research efforts toward improved management rely on the availability of site-specific biometric data. At the same time, scientists are obligated to minimize the negative effects (stress, harm, and sacrifice) of their samplings on individuals and the population without compromising data quality. In-field methods for biometric measurements must be quick, precise, and practical for the user. Essential information that is typically required in (large-scale) eel monitoring programs includes body length, mass, sex, and maturation stage. As live eels are difficult to handle, individuals are typically anesthetized or killed (and sometimes stored frozen to postpone measurements) to obtain the necessary biometrics. The primary purpose of this paper was to explore the suitability of a nonlethal method based on photography for obtaining essential biometrics and maturation stage from live European Eels *A. anguilla* in a timely manner. In addition, we evaluated the relative accuracy of measuring the parameters that are necessary for assessing maturation stages in eels after defrosting and examined the necessity of correcting for potential shrinkage of eyes and pectoral fin. Both procedures were compared against a standard reference of measurements from freshly killed eels. We found that the minimally invasive method using alive measurements of eels' body length and mass together with digital measurements of eyes and pectoral fin from photographs had the highest agreement for maturation stage outcome with the fresh reference. Our results further reveal the necessity of correcting for shrinkage of eyes and pectoral fins (in addition to length and mass) after freezing to maximize reliability in stage classification. Consequently, we provide specialized formulae to apply shrinkage corrections for eye diameter and pectoral fin length.

The European Eel *Anguilla anguilla* is an iconic species of ecological and socioeconomic value, inhabiting freshwater, estuarine, and coastal habitats across Europe and North Africa (Dekker 2003a, 2003b; Hanel et al. 2019). European Eels are semelparous and grow up in continental waters as so-called "yellow eels." Toward the end of this growth phase, individuals undergo various morphological adaptations for their upcoming marine reproductive migration, such as increase in eye and pectoral fin size and a change of body coloration, called "silvering" (Tesch 2003; Durif et al. 2005). "Silver eels" then leave their feeding and growth habitats to conduct a 5,000–10,000-km migration to reproduce and die in their spawning grounds, a large area of the Sargasso Sea in the central Atlantic Ocean (Righton et al. 2016; Miller et al. 2019; Wright et al. 2022). After spawning, their leptocephalus larvae swim and drift back to the European and North African continental shelves, where they metamorphose into glass eels and start to repopulate their growth habitats (Bonhommeau et al. 2009; Westerberg et al. 2018).

Recruitment time-series studies that have monitored numbers of arriving glass eels show a severe decline in

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recent decades to only 1–7% of the levels that were monitored between 1960 and 1979 (ICES 2021a). As a result, the European Eel is currently rated as critically endangered in the International Union for Conservation of Nature red list of endangered species (Pike et al. 2020). In addition, despite the broad range of distribution of the species, yellow and silver eel biomass has likewise shown evidence of decline in recent decades (Mateo et al. 2021; ICES 2021a). And although recruitment time series remain a crucial metric in eel management, experts strive for an improvement of demographic data for stock assessment. Landings data, yellow eel abundance, and silver eel escapement data are gaining importance, and experts in dedicated workshops (Mateo et al. 2021; ICES 2021b, 2021c) have highlighted the necessity of also considering and incorporating abundance data of later continental life history stages into stock assessment.

Starting in 2007, eels were included into the Data Collection Framework (DCF), a community framework for the collection, management, and use of data in the fisheries sector and support for scientific advice regarding the common fisheries policies of the European Union (EU) (Council Decision 2008/949/EC). In the same year, the EU adopted a regulation that targeted ensuring the escapement of 40% of silver eel biomass from continental waters as compared with pristine conditions (Council Regulation [EC] 1100/2007). For this, member states were obligated to monitor or model whether escapement targets are currently met. As a consequence of these frameworks, the need for site-specific eel data has increased and sampling efforts have been high across the distribution range in recent years. The required data for better-informed management of eels include individual biometrics such as age, length, body mass, and maturation stage, as well as stock indices in terms of yellow eel abundance, silver eel escapement, and sex ratios (Regulation [EU] No. 2017/1004). To obtain these data for monitoring and management, the differentiation between life stages and sex is necessary. Given the strong sexual size dimorphism in eels, methods for classifying sex and maturation stage typically rely on length and body mass information, complemented by external biometrics related to the silvering metamorphosis, such as eye size (Pankhurst 1982; Acou et al. 2005; Durif et al. 2005). One well-established method for determining the life history stage of eels is the "silvering index" after Durif et al. (2005, 2009). This index distinguishes between two stages of yellow eels, a premigrant stage, and three fully migrant stages (two silver female and one silver male stage). To classify the silvering index of any eel, measurements of individual body mass, length, pectoral fin length, and eye diameter are required.

As live eels are slippery and therefore difficult to handle, biometric measurements are usually performed on killed or anesthetized individuals. Eel migration and therefore monitoring can be highly seasonal, often requiring scientists to handle large sample sizes in restricted amounts of time. However, a precise in-field measurement on a fresh eel is time consuming (Acou et al. 2006), as it is important to ensure the appropriate degree of anesthesia for each individual when processing large samples. Alternatively, the samples can be frozen and biometrics obtained on thawed eels in the laboratory at a later date. Besides being sacrificial, freezing will result in shrinkage due to the loss of water content, and previous studies have offered correction factors for body mass and total length to facilitate better comparability with freshly obtained metrics (Wickström 1986; Simon 2013). In contrast, the necessity of correcting for possible effects of freezing on eye diameter or pectoral fin length has not yet been examined.

Generally, the sacrifice of eels solely for the collection of external biometrics does not comply with ethical obligations and the long-standing scientific advice to keep anthropogenic mortality as low as possible (ICES 2020). Even though in some cases it may be necessary to sacrifice eels for certain methodologies (e.g., age reading, otolith microchemistry, or certain contaminant analyses), it is generally desirable to collect biometric data on live animals with limited harm by using minimally invasive methods to avoid the unnecessary sacrifice of these endangered specimens. This would complement available minimally invasive methods for the determination of muscle fat content (Pohlmann et al. 2019), salinity-habitat history (Bertolini et al. 2022), and parasite infection or damage (Beregi et al. 1998; Crean et al. 2003; De Noia et al. 2022). Harmless methods are of special importance if the data are acquired for monitoring purposes and the respective fish are supposed to be released with no harm, as is the case, for example, in electrofishing surveys or mark–recapture studies.

Although obtaining the body mass data of a live eel (for example in a simple bucket on a scale or in a net, using a hanging scale) is relatively unproblematic, the determination of length, eye diameter, and pectoral fin length on live eels is challenging. Eye diameter and pectoral fin length are usually measured with a caliper. Using these precision tools, however, requires stable and steady conditions, and measuring eye diameter and fin length of an anxious or agitated animal may not be fast and unproblematic.

A quick and potentially less stressful alternative could be facilitated by using photographs of an animal or the focal body part together with a reference scale next to it. The measurements are then subsequently conducted digitally using specialized image software, such as ImageJ (http://rsb.info.nih.gov/ij/; Schneider et al. 2012) or Image Tool 3.0 (UT Health Science Center, San Antonio, Texas, USA), that allows the determination object sizes based on known dimensions on the reference scale. The precision of this method has previously been evaluated with respect to

the body length of small-sized fish (≤50 cm) by Andrialovanirina et al. (2020), attesting that accuracy can be very high. More recently, Sundin et al. (2022) introduced the use of photography for measurements of eye size (but not pectoral fin length) of eel. Their analyses suggested that digital measurements from photographs could reduce the variability of measurements and the resulting maturation stages across different observers, compared with caliper measurements. It was therefore recommended to measure eye size from digital measurements in future monitoring efforts. Importantly, however, the precision of the photography method for obtaining body, eye, and fin metrics for eels and its suitability for determining maturation stage remains unvalidated.

In this study, we compared the accuracy and measurement repeatability of different methods that rely on photography or caliper measurements (on fresh and defrosted eels) to obtain length, body mass, and maturation stages of eel, thereby examining their suitability for data collection in the field. Special emphasis was given to the reliability and usability of methods that prevent anesthetizing or sacrificing endangered freshwater eels while obtaining the necessary data in line with various monitoring directives.

METHODS

Study fish.— A total of 144 European Eels covering a length range from 27 to 89 cm were sampled between April 6, 2021, and May 31, 2022, from local fisheries in the German rivers Ems, Elbe, and Weser (n = 107, 27, and 10 eels, respectively). Because the eels were sampled from stow net fisheries, catches were naturally dominated by silver eels, particularly stages F V (female stage V, n = 52; 36%) and M II (male stage II, n = 40; 28%). However, all maturation stages (and length-classes) were sufficiently represented in the final sample composition. From n = 117 eels, a complete set of biometrics (i.e., body length, body mass, eye diameter, and pectoral fin length) was measured at least once by each of the different methods described below. For the remaining n = 27 individuals, one of each total length measurement approaches was missing but measurements of eye diameter and pectoral fin length were available. A summary of the sampled eels is given in Table 1.

Methods of measurements.— To obtain the abovementioned biometrics, the eels were measured using three different methods and settings: fresh measurements (which served as reference values) and defrosted and live photo measurements. For the fresh measurements, body length was measured and rounded down to the nearest cm shortly after killing the eels with an overdose of 2-Phenoxyethanol. Thereafter, pectoral fin length and eye diameter (horizontal and vertical) were measured on the left side of the eel with a digital caliper to the nearest 0.1 mm (digiMax; Wiha Werkzeuge GmbH, Schonach, Germany). The defrosted measurements were conducted, respectively, after the eels were frozen in batches that were packed in plastic bags at −20°C for 1–44 d (18.6 ± 14 d [mean ± SD]). Body mass was measured once alive and once after freezing and complete thawing (without removing coagulated mucus). Mass was determined by placing the eel in a bucket on a scale (compact scale Type 9121; Soehnle Professional, Backnang, Germany) and was rounded down to the nearest gram.

To photograph the eye and fin of the live eels, it proved useful to cover the eyes of the nonsedated, live eel (with a wet towel) prior to the procedure until it was calm, as eels are known to be sensitive to light (Lowe 1952; Haddering et al. 1999; Elvidge et al. 2018). Then, photographs of the left pectoral fin and left eye were taken directly beside a reference scale (here, a piece of laminated graph paper) for subsequent digital measurements (camera model: RX 100 IV; Sony, Minato, Tokyo, Japan). Particular care was taken that the object was centered in the photograph, the camera was pointed straight at the object, and the scale was held on the same level as the object to avoid potential sources of distortion (see examples in Figure 1). For live body length measurements, a customized

TABLE 1. Sample sizes by maturation stage and length-classes underlying the method comparisons. The global data set consisted of N = 144 individuals, which were used in the analyses of eye diameter and pectoral fin length by procedure. Body length measures were incomplete for N = 27 individuals, whereby the numbers in parentheses represent the sample sizes underlying the comparison of body length by procedure and the determination of correct stage classification percentage. Maturation stage and length-classes are given as obtained from measurements on freshly killed eels.

Maturation stage							
S I	F II	F III	F IV	F V	M II		Total
17 (9)	15 (14)	11 (9)	9 (5)	52 (49)	40 (31)		144 (117)
Length-class							
<30 cm	30–39 cm	40–49 cm	50–59 cm	60–69 cm	70–79 cm	≥80 cm	Total
4 (2)	35 (23)	26 (22)	30 (28)	24 (24)	14 (11)	11 (7)	144 (117)

shallow rectangular tray with an embedded scale next to the long side of the rectangle was used. After placing an eel, the tray was held with both hands and the fish was carefully slipped toward the scale by inclining the tray. After touching the tray wall, eels typically straighten and allow for an ad hoc measurement. Individuals differ in their vivacity, whereby sliding the eel toward the wall was repeated until it aligned to the wall, allowing the measurement of the total length reliably (Figure 2). Additionally, the total length of the eels was measured digitally from photographs by placing a reference scale in the tray and then laying the eel on top of it. Once the eel calmed and remained still, a photograph (using the same camera model mentioned before) was taken from straight above for the subsequent digital measurements (Figure 2). Lenses may cause distortions depending on their focal length (and the sensor type of the camera), which are particularly pronounced at the edges of pictures for wide-angle lenses. Although the use of appropriate optics (least distortion at approximately 50 mm focal length) was impractical due to limitations in the maximum distance between camera and eel, care was taken to center eels in the image and a camera with good distortion control (i.e., built in software postprocessing) was used. The digital measurement procedure followed the description in Sundin et al. (2022), using the open-source software ImageJ (version 1.53; Schindelin et al. 2012, 2015). The fresh, photo, and live body length measurements were taken in the field or after transporting

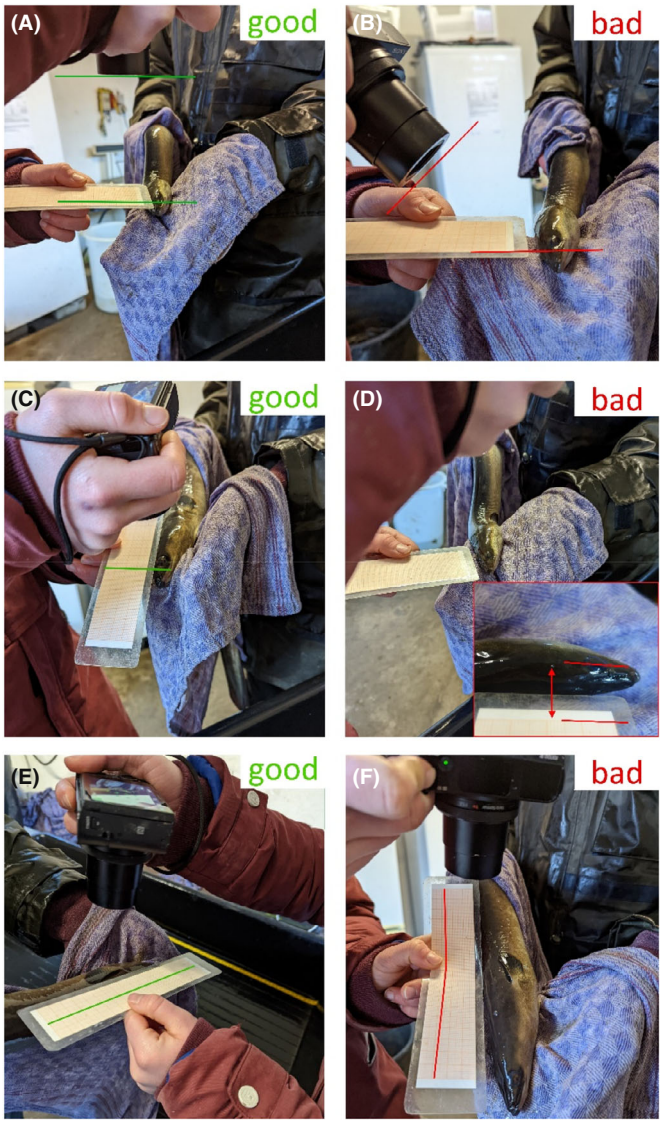

FIGURE 1. Good and bad examples of useful photographs for obtaining accurate digital measurements of eye diameter and pectoral fin length from live European Eels. **(A, B)** The focal organ must be photographed from straight above—that is, the lens must be in a parallel level to the organ. The reference scale must be held on the same level as the organ. **(C, D)** Presenting it in a higher or lower level than the organ will lead to distorted measurements. **(E, F)** If a soft reference scale is used (e.g., laminated graph paper), attention must be paid that the scale is held in plane and not bent through finger pressure, especially in the area of the focal organ.

FIGURE 2. Measuring body length of live European Eels in a customized tray (top) and an example of a good photograph with subsequent digital measurement (bottom). Photos for length measurements must be taken with a straight angle from above the eel and with the reference scale and fish both in the center of the picture. The reference scale should not be too small (e.g., DIN-A4 graph paper).

the eels to the lab; the defrosted measurements were conducted in the lab.

To estimate the consistency of repeated measurements (i.e., repeatability) within the presented methods, a subsample of $n = 82$ individuals was measured three times with respect to eye diameter and pectoral fin length (each using the fresh, frozen, and photo procedure). Because independence of repeated measures on the same individual is crucial, the caliper was always set to 0 mm prior to each (fresh or defrosted) measurement and displayed millimeters were only read after the final position was established. For the photo procedure, the same photo was measured digitally three times, reopening the image and setting the reference scale for each measurement. The eels were sampled and measured in batches of multiple individuals so that repeated measurements on the same fish were never taken in direct succession.

In our study, all of the described measurements were taken by the same observer. Eye diameter was measured along a horizontal and a vertical axis, and the two measurements were averaged. Length, body mass, averaged eye diameter, and pectoral fin length measurements were further used to calculate the silvering index following Durif et al. (2005, 2009).

Correcting for shrinkage of defrosted eels.—A correction formula for the shrinkage of eye diameter and pectoral fin length was derived by linear regression of fresh measurements over frozen measurements (Figures 3 and 4). For the length and body mass of the defrosted eels, published correction factors were available to account for the shrinkage and loss in body mass associated with freezing (Wickström 1986; Simon 2013). Wickström-corrected values had slightly lower mean absolute differences from the freshly measured values, which might be partially

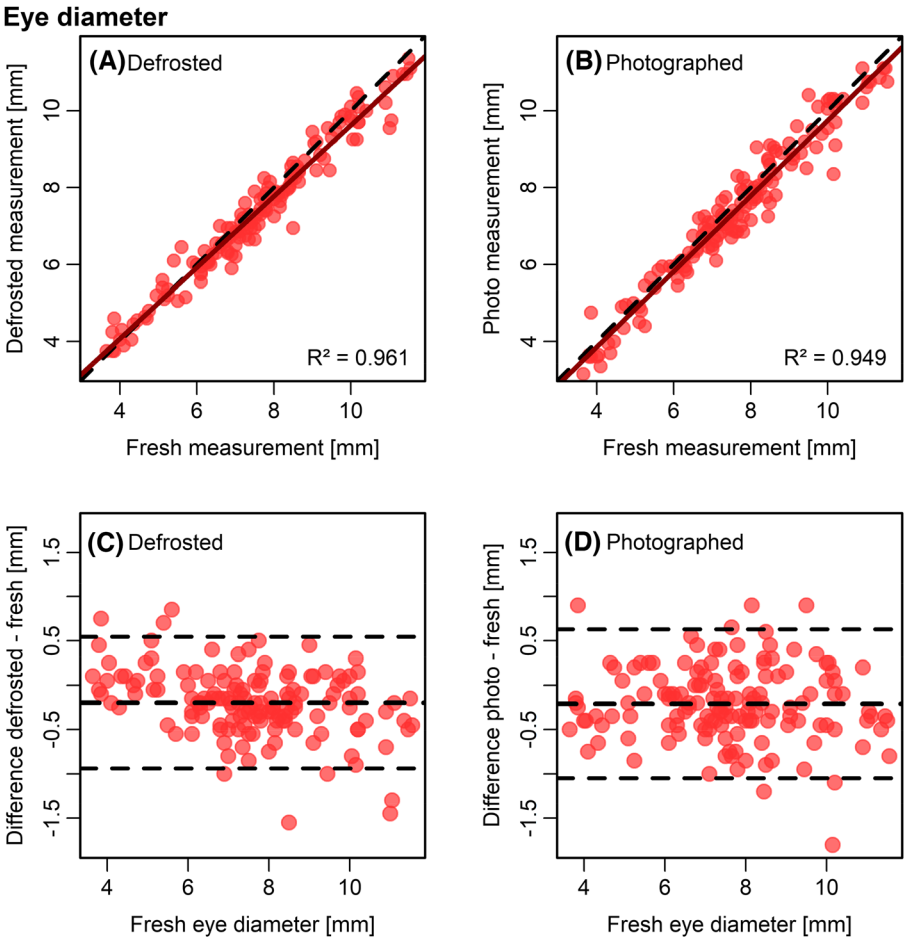

FIGURE 3. Comparison plots of eye diameter measurements on defrosted or photographed eels with the standard reference of caliper measurements on freshly killed eels. **(A, B)** Illustrate regression results, with dashed lines indicating the "line of equality"—that is, a hypothetical line on which all data points would lie at perfect agreement between the two compared methods. Solid lines represent the regression line of actual observations. **(C, D)** Show the difference between methods against the fresh measurements, with the middle line indicating the mean difference between the alternative and the standard procedures and the outer lines indicating the limits of agreement as mean ± 1.96 SD. Eye diameter means the average of two measurements taken along the horizontal and vertical axis of the left eye. $N = 144$.

because Simon (2013) removed coagulated mucus from thawed eels, which we did not do. In addition, we derived and applied our own correction formulae for length and mass as described for eye and fin. The use of both the Wickström and our own formulae led to the exact same Durif-stage outcome for all individuals. Our own formulae, presented in the results section, were thus used to correct for shrinkage and body mass loss in further analyses.

Data analysis.— Our general analytic approach was to compare each of the alternative measuring procedures (using live or defrosted eels) for body length, eye diameter, and pectoral fin length against measurements that were conducted on fresh eels as the reference methodology. From the subset of individuals whose eyes and fins were measured three times to estimate repeatability, only the first-recorded observation was taken for the analyses of measurement accuracy and correct stage classification in order to have independent samples (Bland and Altman 1986).

For visualization, the observed measurements from any alternative method were regressed over the standard reference of fresh measurements. Although the values for R^2 are given in the resulting regression plots for convention (Figures 3A,B, 4A,B, 5A–C), it is not a suitable statistic for assessing the agreement of measurements with the standard reference (see explanations in Bland and Altman 1986). Instead, the differences between alternative and fresh measurement were plotted against the reference of fresh measurements. We then calculated the limits of agreement for any alternative method with the fresh measurement as the mean difference ± 1.96 SD (Bland and Altman 1986). This measure reflects the range within which 95% of alternative measurements deviate from the fresh measurements. It was used to compare the

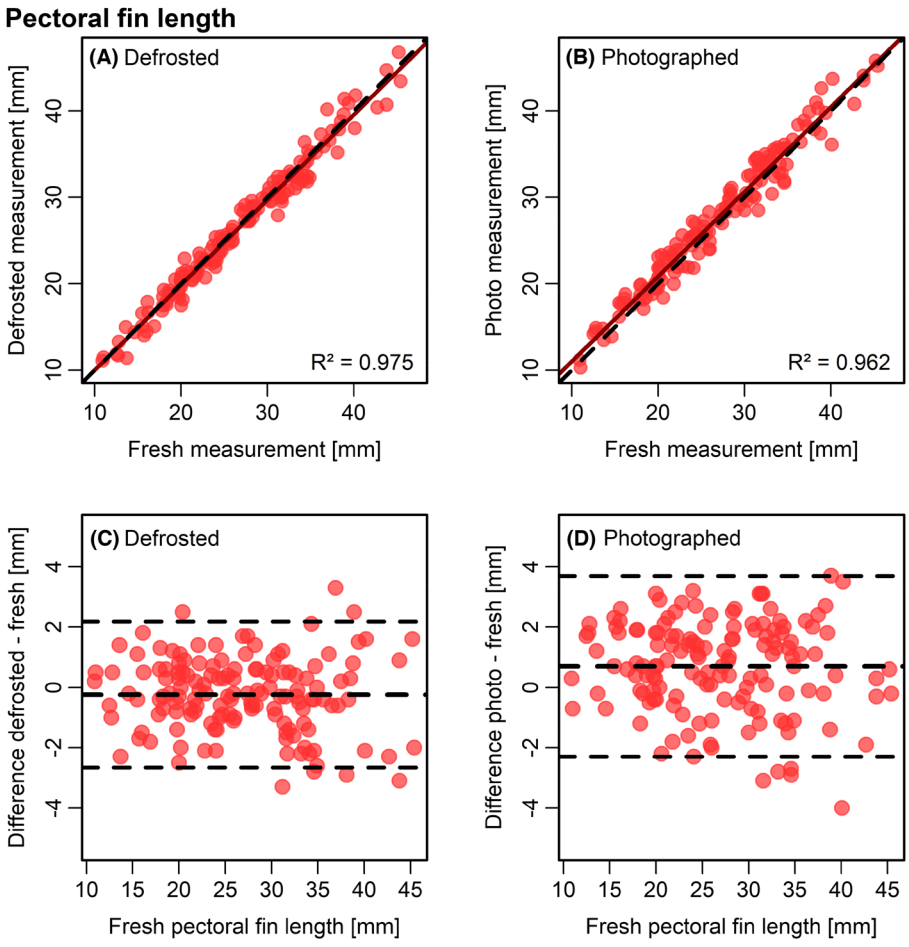

FIGURE 4. Comparison plots of pectoral fin length measurements on defrosted or photographed eels with the current standard reference of caliper measurements on freshly killed eels. **(A, B)** Illustrate regression results, with dashed lines indicating the "line of equality"—that is, a hypothetical line on which all data points would lie at perfect agreement between the two compared methods. The solid lines represent the regression line of actual observations. **(C, D)** Show the difference between methods against the fresh measurements, with the middle line indicating the mean difference between the alternative and standard procedures and the outer lines indicating the limits of agreement as mean ± 1.96 SD. $N = 144$.

agreement with fresh measurements across different candidate methods, with narrower limits of agreement indicating higher accuracy of the method. Frequency distributions of eye diameters, pectoral fin lengths, and body lengths obtained by any of the procedures were visually checked for normality to meet the assumptions of determining the limits of agreement (Bland and Altman 1986).

To assess the reliability of the silvering index classifications by any alternative procedure, we calculated the percentage of individuals whose silvering index was correctly classified—that is, consistent with the index that was obtained from the fresh measurements. Again, only the first observation was used from individuals with repeated measurements for independency of observations. All individuals for which body length, eye diameter, and pectoral fin length measurements were measured by any of the presented methods were used for this analysis ($N = 117$ individuals). The following methods were evaluated (1) using defrosted eels without any correction of measurements; (2) using defrosted eels with values of body length and mass corrected for shrinkage; (3) using defrosted eels with corrected length, body mass, and eye diameter, and pectoral fin length, corrected using the formulae presented in this study (see Correction Formulae for Silvering Metrics on Defrosted Eels); (4) using photography-obtained measurements for body length, eye diameter, and pectoral fin length with fresh mass; and (5) using photography-measured eye diameter and pectoral fin length, fresh mass, and body length measured in the customized tray.

Finally, two statistics were used to assess the degree of variation within the three repeated measurements that were conducted for each procedure on a subset of 82 eels. First, a repeatability coefficient (RC) was calculated according to Bland and Altman (1996). This measure is based on the within-subject SD across the three repeated measurements. An RC value of x implies that the difference between two repeated measurements on the same object using the same method will be less than x in 95% of the cases (Bland and Altman 1996). Smaller RC values therefore indicate a higher consistency (i.e., lower variation) across repeated measurements for a given method. Second, an intraclass correlation coefficient (ICC) was calculated as a measure of the homogeneity within repeated measurements in relation to the total variation that was observed for the parameter and method of interested. The ICC scores get higher and approach 1 the lower the variability across repeated measurements with a specific method is. Precisely, the two-way mixed-effects formulation for absolute agreement was used following Koo and Li (2016) and McGraw and Wong (1996; "ICC [A, 1]" formulation).

All statistical analyses and visualizations were performed in R version 4.2.0 (R Core Team 2022). The package "irr" was used to calculate the ICC values (Gamer et al. 2019).

Ethical statement.— All of the eels that were used in this study were sampled as part of the obligatory data collection in line with the DCF German national programme, and no additional animals were killed. The sampling and treatment of eels used in this study followed current German legislation.

RESULTS

Silvering Metrics (Eye Diameter and Pectoral Fin Length) Obtained from Alternative Methods

The measurements of silvering metrics on defrosted eels were on average smaller than the reference from the fresh caliper measurements by −0.20 mm for eye diameter and −0.24 mm for pectoral fin length. The limits of agreement (i.e., the range within which 95% of measures deviated from the reference) were −0.94 to +0.55 mm for eye diameter and −2.66 to +2.18 mm for pectoral fin length measured on defrosted eels (Figures 3A,C and 4A,C).

The measurements of eye diameter from the photographed eels were on average smaller than fresh measurements (mean difference Δ: −0.21 mm), whereas the pectoral fin measurements exceeded the reference method on average (mean Δ: +0.70 mm). The limits of agreement were −1.05 to +0.63 mm for eye diameter and −2.30 to +3.69 mm for pectoral fin length measured on photographed eels (Figures 3B,D and 4B,D).

Body Length Measurements Obtained from Alternative Methods

The body length measurements of the defrosted eels were on average −1.06 cm smaller than the fresh length (limits of agreement: −2.22 to +0.10 cm; Figure 5A,D). The body length measurements of the photographed eels were on average +0.77 cm larger than the fresh length, with limits of agreement ranging from −0.95 to +2.48 mm. A linear regression revealed that both the raw Δ of photo to fresh length and absolute differences from fresh lengths (i.e., magnitude of measurement error of the photo method) were positively related to the size of the individual ($\beta_{\Delta} = 0.0227$, $P_{\Delta} < 0.001$; $\beta_{\Delta abs} = 0.0216$, $P_{\Delta abs} < 0.001$; Figure 5B,E). The value for mean change of body length measured in a customized tray versus fresh length was +0.06 cm (limits of agreement: −1.28 to +1.40 mm; Figure 5C,F).

Correction Formulae for Silvering Metrics on Defrosted Eels

The formulae for correcting for the shrinkage in eye diameter (ED; in mm) after freezing derived from our

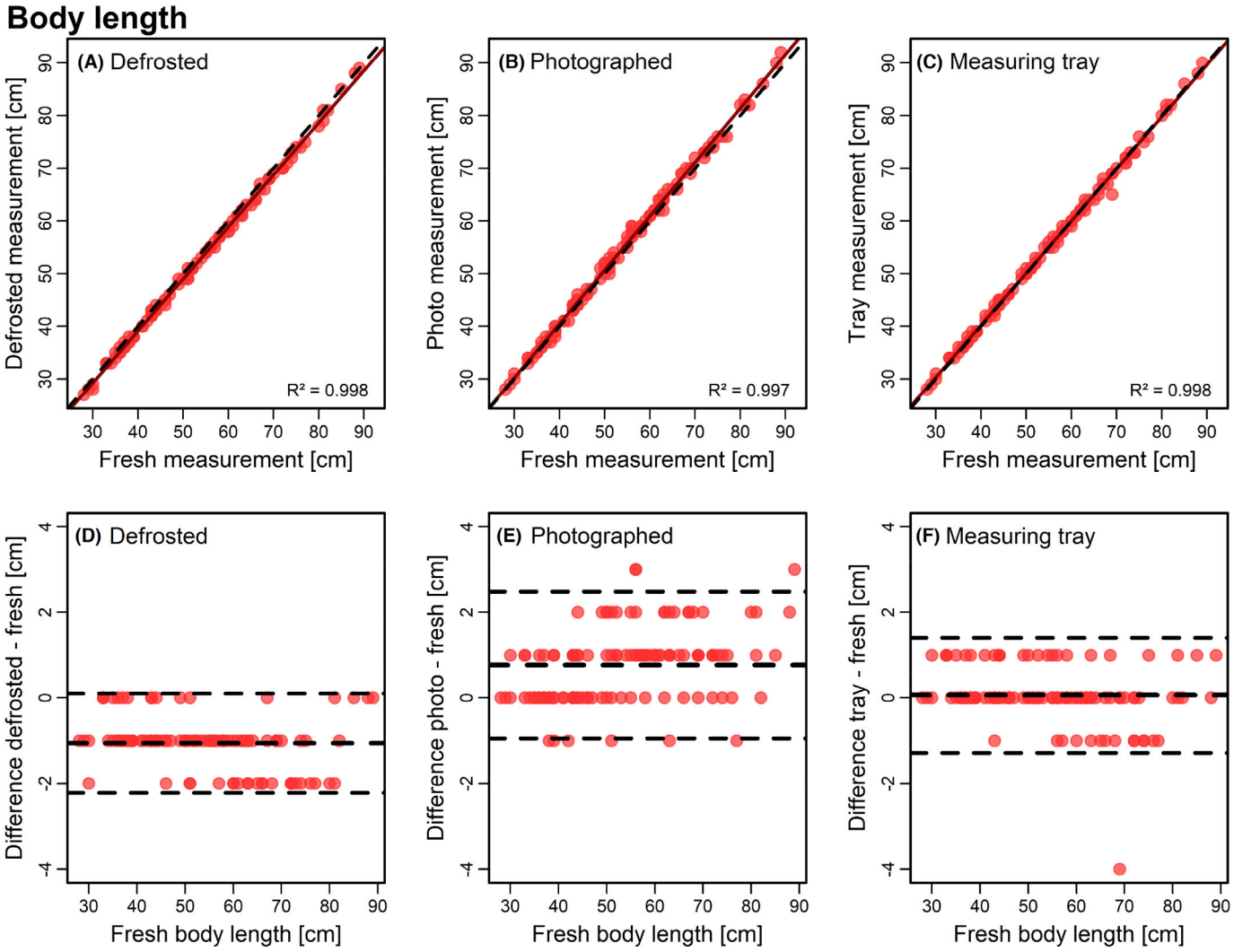

FIGURE 5. Comparison plots of body length obtained from alternative procedures (measured after defrosting, from photography, or in a specific measuring tray), with the standard reference of measuring freshly killed eels on a ruler. **(A–C)** Illustrate regression results, with dashed lines indicating the line of equality. The solid lines represent the regression line of the actual observations. **(D–F)** Show the difference between methods against the fresh measurements, with the middle line indicating the mean difference between the alternative and standard procedures and the outer lines indicating the limits of agreement as mean ± 1.96 SD. $N = 117$.

data set was $ED_{fresh} = 1.039307 \times ED_{defrosted} - 0.0933568$. The conversion formula for the pectoral fin length (PFL; in mm) of thawed eels was $PFL_{fresh} = 0.9898703 \times PFL_{defrosted} + 0.5106442$. The formula correcting for body length (TL; in mm) shrinkage was $TL_{fresh} = 1.010542 \times TL_{defrosted} + 5.024445$, and for mass ($W$, in grams) loss, $W_{fresh} = 1.035511 \times W_{defrosted} + 3.603632$.

Agreement Rate of Maturation Stage Classification

Using raw, uncorrected measurements of defrosted eels led to correct maturation stage classification for 90.6% of the individuals, with the "correct" stage reference being based on our defined standard, the fresh measurements (Table 2). Using shrinkage-corrected length and body mass measurements together with raw measurements on eye diameter and pectoral fin length of defrosted eels did not alter the agreement rate (90.6% correct classification). When the values for eye diameter and pectoral fin length of the defrosted eels were corrected for shrinkage using the formulae given in Correction Formulae for Silvering Metrics on Defrosted Eels, together with the length- and body-mass-corrected values, the agreement rate improved to 92.3%. The Durif stages of 93.2% of eels were classified correctly when body length, eye diameter, and pectoral fin length were digitally measured from photographs and using the fresh mass. The highest agreement rate for maturation stage (94%) was obtained when digital measurements of eye diameter and pectoral fin length were used

TABLE 2. Agreement rates of maturation stages (Durif index) obtained from alternative procedures with the reference (from caliper measurements on freshly killed eels). Defrosted L-W-corrected = thawed eels with corrected length and body mass measurements, but raw eye diameter and pectoral fin length. Defrosted all corrected = thawed eels with a correction applied to length, body mass, eye diameter, and pectoral fin length ($N = 117$ eels).

Procedure	Defrosted raw	Defrosted L-W-corrected	Defrosted all corrected	All photo	Photo + tray length
% correct classification	90.6	90.6	92.3	93.2	94.0

together with body length as measured in a customized tray and the fresh body mass (Table 2).

Repeatability of Measurements

The ICCs of the repeated measurements of eye diameter and pectoral fin length by any of the presented methods (fresh, defrosted, or photographed) were all higher than 0.99 and overall very similar (Table 3). For eye diameter, the measurements on defrosted eels were among the highest ICC (0.996, $P < 0.001$) and lowest RC values (0.297) across all methods. The lowest ICC (0.994, $P < 0.001$) and highest RC value (0.377) for eye diameter measurements were found for measurements on fresh eels. In contrast, the repeatability indices for pectoral fin length were better for fresh measurements (ICC = 0.995, $P < 0.001$; RC = 1.56) than for defrosted (ICC = 0.994, $P < 0.001$; RC = 1.707) and digital measurements from photographs (ICC = 0.994, $P < 0.001$; RC = 1.732).

DISCUSSION

Maturation stages that were determined from the minimally invasive methods based on photography and live length measurement showed the highest agreement with freshly determined stages (93.2–94%) across the compared methods. We estimated that digitally assessing eels' silvering stages with the aid of photographs is possible, exhibiting a deviation range of ca. −1.1 to +0.6 mm from fresh measurements for eye diameter and −2.3 to +3.7 mm for pectoral fin length (95% limits of agreement). The eye diameter measurements from photographs were on average smaller than fresh eye diameter, whereas the digital fin measurements were on average larger than the fresh reference. The opposing directions of bias suggest that deviations from fresh measurements might be explained by an observer effect rather than a consistent distortion that is caused by the procedure (e.g., through lens distortion). This is corroborated by (Sundin et al. 2022), who stated that distortions by camera lenses are thought to be minimal if the focal organ is in the center of the picture.

In combination with digital measurements of eye and fin, measuring body length of a live eel in a customized tray as described earlier was superior in correct stage classification over photographed length measurements. This is likely due to the stronger deviation of photographed body length measurements from the fresh length, with an average overestimation of length (mean $\Delta = +0.8$ cm) compared with the tray measurements, which were in close agreement with fresh length (mean $\Delta < 0.1$ cm). Importantly, measuring body length from photographs became more imprecise and overestimation became more severe as body length increased. One potential explanation for the bias in photographed body length measurements is that the flat reference scale is located below the animal whereas only the dorsal side of the eel can be measured on screen, causing a difference in the image plane between the scale and measured object (Figure 2). Moreover, the anguillid shape of eels requires a segmented measuring line to follow their body curvature, possibly introducing more error than in fusiform fish, which can be measured with a straight line (Andrialovanirina et al. 2020). Thus, digital body length measurements of eels might be more prone to error than those of fusiform fish species. We therefore suspect that the photo method will lose accuracy when eel samples are dominated by large individuals. Our result is an extension to Andrialovanirina et al. (2020), who reported high precision for digital body length measurements of fish ≤50 cm in length, but our study cautions for an increased potential measurement error above this length threshold.

A general benefit of using photography to measure any silvering metric of eels is that photos can be digitally stored and measurements could be checked and

TABLE 3. Measures of repeatability in eye diameter and pectoral fin length measurements across $N = 82$ eels that were measured three times with each method. ICC = intraclass correlation coefficient; RC = repeatability coefficient (***$P < 0.001$).

	Fresh measurement		Defrosted measurement		Photo measurement	
	RC (mm)	ICC	RC (mm)	ICC	RC (mm)	ICC
Eye diameter	0.377	0.994***	0.297	0.996***	0.323	0.996***
Pectoral fin length	1.56	0.995***	1.707	0.994***	1.732	0.994***

remeasured anytime, if needed. For that purpose, researchers need a reliable concept for matching photographs with the individual's information or ID. Such linkage can be achieved by placing a physical tag with identification information in the photograph or by immediate protocoling of the digital file names displayed on camera beside an individual's biometric information. Average handling times (for experienced raters) were 16.8 s to measure body length in a tray and 25 s to obtain both photographs of eyes and pectoral fins for digital measurements (N = 177 individuals). In addition, Andrialovanirina et al. (2020) estimated the average time for an on-screen digital fish length measurement to be 12.8 s and outlined how automatic procedures will further minimize these times in the future. These aspects highlight the potential of the nonlethal methods of minimizing the handling times of eels while being time-efficient to the user in the field. However, in contrast to conducting measurements on anesthetized or dead fish, the photograph procedure requires two persons for picture taking in the field. Collectively, we conclude that the use of digital measurements from photographed eyes and pectoral fins together with live measurements of mass and body length (e.g., in a specialized tray as outlined here) enable nonsacrificial and nonanesthetic collection of essential biometrics and maturation stages from eels at high reliability. Evaluating whether this method also reduces stress levels below anesthetic procedures would involve measurements of blood parameters (e.g., cortisol, glucose, and lactate) during handling. This was beyond the scope of this study but can be seen as a future research need toward identifying the most fish-friendly techniques for biometric data collection of eels.

Maturation stage determination from measurements on defrosted eels showed somewhat lower agreement with the fresh eel's reference than the alive procedures (90.6–92.3%). We assessed that the eye diameter of defrosted eels can be measured within ca. −0.9 to +0.6 mm and pectoral fin length within −2.7 to +2.2 mm of deviation from the fresh reference (both procedures using calipers). Freezing and thawing induced a mean shrinkage of both eye diameter and pectoral fin length by ca. 0.2 mm compared with fresh measurements. This implies that relative to the size of the organ, eyes shrunk more than did pectoral fins. Likewise, the body length of the eels was smaller after freezing by ca. 1 cm on average. Shrinkage after freezing has been reported before with respect to body length (and mass), and correction factors are available (Wickström 1986; Simon 2013). This is not the case for pectoral fin and, importantly, eye measurements, which are primary metrics for determining eels' maturation stage by all established indices such as the ocular index (Pankhurst 1982), the three criteria method from Acou et al. (2005), and the widely used Durif index (Durif et al. 2005). We herein derived specific equations to correct for shrinkage in eye diameter and pectoral fin length of defrosted eels, which are provided in the results section.

The maturation stages that were calculated from raw and uncorrected silvering metrics of the defrosted eels agreed in 90.6% of cases with the fresh stage. Using correction-factor-adjusted values for length and body mass alone did not improve the agreement rate with the fresh reference. When eye diameter and pectoral fin length were adjusted for shrinkage in addition to length and body mass correction (using our presented conversion formulae), agreement with the fresh stages increased to 92.3%. This result likely reflects the strong relative influence of pectoral fin length and eye diameter as the major contributor on the Durif silvering index outcome, outweighing the influence of body mass and length (Durif et al. 2009). We therefore suggest that correcting only length and body mass may be insufficient to maximize the accuracy of stage classifications of defrosted eels. Instead, eye diameters and pectoral fin lengths of defrosted eels should be adjusted for shrinkage as well, and our presented correction formula can be used for that purpose. There was no significant effect of freezing duration on any of the focal biometrics within our storage period range of 1–44 d (Figure S1 available in the Supplementary Material separately online). However, the accuracy of the herein presented correction formulae cannot be warranted for freezing periods or storage methods that deviate from the protocol of this study.

All of the examined methods for measuring the eye diameter and pectoral fin length of eels showed "excellent repeatability" according to the definition by Portney and Watkins (2009; i.e., all ICC values >0.9). Across the different methods for obtaining eye diameter, consistency across repeated measurements was highest for defrosted caliper measurements and lowest for fresh eels. A likely explanation for this finding is that the delimitation between eye and the surrounding skin may sometimes be difficult to judge on fresh eels, depending on the coloration of the individual (Sundin et al. 2022). The authors further reported that defrosted samples may be easier to measure, as the delimitation is often clearer after thawing. This matches our observations of the eye color becoming whitish after thawing, whereby determination of the edges is less subjectively. The repeatability of pectoral fin length measurements was very comparable across the methods. Measurement consistency of fins was slightly higher for the methods using caliper measurements, particularly on fresh eels. This could reflect the circumstance that pectoral fin length measurements on the actual eel can make use of tactile cues in addition to visual cues to determine measurement limits, such as feeling the base of the fin by touch with the caliper. We emphasize that the reported percentages of "correct" stage classification by any of the

presented methods cannot necessarily be generalized across contexts. Our results primarily apply to samples with a similar (i.e., silver-dominated) composition of maturation stages as in our case. In addition, our reference of the presumed "true" stage, derived from fresh caliper measurements, cannot be assessed without certain measurement error itself (as discussed above, measuring the same fish twice with the same method could result in different stages). This element of stochasticity could have dampened the agreement rates of the alternative methods with the references. Independent of the potential sources of noise, it appears safe to conclude that the examined methods, especially the nonsacrificial approaches, demonstrate high reliability.

Biologists and natural resource managers bear the responsibility of maximizing research benefits to humans and wildlife stocks while minimizing harm, stress, and sacrifice of individuals (Sloman et al. 2019). This demand is not only ethically self-evident, but also anchored in the animal welfare legislation of many countries (Sneddon et al. 2017). Correspondingly, the strong need for minimally invasive methods for scientific data collection on rare and endangered species has been recognized by the fish and fisheries science community (Pohlmann et al. 2019; Sloman et al. 2019). In this study, we demonstrated the suitability of a nonlethal and nonanesthetic method for obtaining accurate biometrics and reliable maturation stage classifications of European Eels. We recommend measuring the body length of live eels in a specialized tray (or a similar tool, if accuracy is certain) and to digitally measure eye diameter and pectoral fin length from photographs with a reference scale (or object). This approach was time efficient and therefore constitutes a way to cope with a large number of individuals in restricted time windows, a typical scenario of the highly seasonal in-field eel data collection. Our presented method helps minimizing adverse repercussions of scientific data collection on individual eels and the stock in future monitoring efforts, while being practical to the applying researcher.

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SUPPORTING INFORMATION

Additional supplemental material may be found online in the Supporting Information section at the end of the article.