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ARTICLE

Evaluation of Sampling Methods for Maturation Stage Determination in the European Eel *Anguilla anguilla*

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Abstract

Monitoring data is important in ecological research, but differences between and within areas or species in data collection methods could introduce bias in the analyses. Standardizing data collection is particularly important when monitoring migratory species that have a distribution that crosses several national borders. The European Eel *Anguilla anguilla* is an extreme example of such a species since it constitutes one stock across the entire distribution area. One important variable collected for the European Eel is maturation stage. This data is needed to monitor silver eel escapement to assess population trends. To determine maturation, data on length, weight, diameter of the eyes, and pectoral fin length are used to calculate Pankhurst eye index and Durif's silver index. In this study, we investigated effects of precision and interobserver variability on data collection relevant for maturation stage determination according to Pankhurst and Durif's indices. We found that eye diameter differed in size between the left and right eyes; however, the mean difference (0.19 mm) is probably an artifact of the large sample size ($n = 16{,}977$) and can be regarded as being within the measurement precision. Meanwhile, there was no significant difference in pectoral fin length. These results suggest that either side of the eel could be used without losing precision. Visually determined maturation stage classifications differed from those calculated with Pankhurst and Durif's indices but could still provide useful information; hence, it is recommended to collect this variable. Measurements performed using computer software generated greater precision than using calipers, which increased interobserver variability. Since the difference was relatively small and since computer analysis of images may not always be an option, measuring method can be decided based on the level of precision needed in each case. These suggested implementations can reduce observation bias and streamline the data collection used for stock assessments of the European Eel.

Monitoring is an important tool in ecological research, and data from monitoring programs are used in national and international policy making, applied science, and basic life science (reviewed in Lovett et al. 2007). Fish monitoring programs, however, sometimes suffer from a lack of structure, leaving the programs ineffective and unable to reach the targeted aims (reviewed in Radinger et al. 2019). In addition, methodological differences in data collection between and within areas or species, as well as changes of sampling methods over time, can be poorly quantified and/or justified, which could introduce bias when analyzing long-term data sets (reviewed in Radinger et al. 2019). Within the European Union, there is an established framework for the collection of data in the fisheries sector (Regulation 2017/1004). This regulation aims to establish rules for collection, management, and use of biological, environmental, technical, and socioeconomic data concerning the fisheries sector. Collecting data on biological variables, such as length, weight, sex, and age, is incorporated in this regulation, as such data is needed for stock assessments of harvested fish species.

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Standardized methods for data collection are however not specified, which could lead to ineffective monitoring (e.g., collecting data not used in assessments, overlooking collecting data that would be needed, collecting data with insufficient precision) and incomparable data between countries and over time.

If data collection methods are inconsistent and variable, international stock assessments may be particularly difficult when the distribution of a species crosses national borders. The European Eel *Anguilla anguilla* is an extreme example of such a species, consisting of one population and one stock across the entire distribution area. The genetic structure of the European Eel has been discussed (Wirth and Bernatchez 2001), but most analyses from that time (i.e., about two decades ago) suggest that the European Eel is a panmictic species (using microsatellite markers [Palm et al. 2009] and single nucleotide polymorphisms [Pujolar et al. 2014]). More recent data using whole-genome sequencing indeed confirm that there is a complete lack of geographical genetic differentiation (Enbody et al. 2021). This has implications for monitoring since the data collection across all monitoring countries must be comparable to reduce method-based biases that affect stock assessment. The European Eel is monitored within the European Union (EU) Data Collection Framework (Regulation EU 2017/1004), wherein the EU member states collect fisheries data to support the Common Fisheries Policy through scientific advice. There are several end users, with the joint European Inland Fisheries and Aquaculture Advisory Commission (EIFAAC)/International Council for the Exploration of the Sea (ICES)/General Fisheries Commission for the Mediterranean (GFCM) working group on eels (known as WGEEL) being one of the most important. The Data Collection Framework provides a possibility to conduct streamlined data collection between countries for a great part of the distribution range (albeit not the total range). However, the EU data collection framework does not specify standardized data collection methods. This implies that despite having a joint data collection framework across many countries, methodological differences could still exist and induce biases in the international stock assessment. This is of particular concern for the critically endangered European Eel (Pike et al. 2020), where recruitment has decreased by 95–98% and the population trend is in sharp decline (ICES 2021).

One important variable collected within the EU data collection framework for the European Eel is maturation stage. The European Eel undergoes several life stages during its life cycle, starting off with the leptocephalus larvae hatching from eggs in the Sargasso Sea (Schmidt 1912). The eel larvae drift towards Europe and North Africa and ascend rivers in search of foraging grounds, often migrating in river–lake systems more than 1,000 km over several years (Aprahamian 1988; Tesch 2003). They spend 6 to >20 years in rivers and lakes during their growth period, which is the yellow eel stage (Tesch 2003). In their final life stage, they prepare for their migration back to the Sargasso Sea, cease feeding, and transform to silver eels. Data on maturation stage is needed to monitor silver eel escapement, assess population trends, and provide data as a proxy for spawning stock biomass. The proportion of mature eels (silver eels) can also be used to calculate the production potential of a specific water body. The transition from yellow to silver eel is however not readily observable, but researchers early noted that eye size increased with maturity (Pankhurst 1982 and references therein) (Figure 1), and later it was found that female eels commencing their migration had longer pectoral fins (Durif et al. 2005). Using these observations, Pankhurst (1982) developed an index to determine maturity (silvering stages) based on eye size. Later, Durif's silver index was developed (Durif et al. 2005), where length, weight, vertical and horizontal diameter of the eyes, and pectoral fin length are used to calculate maturation (Durif et al. 2009). These are the two most commonly used eel maturation stage classification indices.

The vertical and horizontal eye diameters are central measurements in both maturation stage classification indices for European Eel and are traditionally measured with calipers. However, the same data can be obtained using digital image analysis software. Those two methods each have their advantages and disadvantages, such as the potential for higher precision if using software compared with direct measurements with calipers, while the former method might decrease time efficiency. In addition, measurements of the eye and pectoral fin are sometimes taken on both the left and right side of each individual eel, but this might not be needed to quantify maturation stage. Obtaining measurements from only one side would increase time efficiency and, moreover, reduce handling time when measurements are taken on live animals (thereby minimizing handling induced stress; Barton et al. 1980). In addition to those measuring methods, maturation stage is sometimes subjectively defined by visually observed traits, including the presence of neuromasts and the color of the eel. Even though this visual classification is usually not intended to function as an exact maturation stage classification in itself, data collected on subjective terms could introduce bias due to differences between observers. Hence, it is important to be aware of bias and the potential errors it could lead to.

Using a large data set from the Swedish EU data collection program consisting of more than 16,000 measured European Eel individuals from the years 2003 to 2020, this study explores how data collection could be more streamlined and how precision in key measurements could be improved. This was achieved by comparing (1) left- and right-side measurements of the eye diameter and

FIGURE 1. Photographs illustrating how eye size of the European Eel (female) can differ between individuals at different silvering stages.

pectoral fin and the subsequent maturation classification (Pankhurst and Durif's indices), (2) the potential difference in eel maturation classification (Pankhurst and Durif's indices) between regular measuring methods (calipers) and visual determination, and (3) the precision among observers in eye diameter measurements and subsequent maturation classification (Pankhurst and Durif's indices) using both calipers and digital (ImageJ) measurements. The predictions based on the research questions were the following: (1) the diameter of the eye and pectoral fin were predicted to be similar between the left and right sides of the eel, and hence, there should be no difference in maturation stage classification if using left- or right-side data; (2) maturation classifications based on visual appearance were predicted to differ from those calculated with Pankhurst and Durif's indices, given the subjective nature of visual classification; and (3) precision was predicted to increase when measuring eye diameter using digital image analysis since that should reduce observer bias compared with measuring with calipers.

METHODS

Pankhurst eye index and Durif's silver index.— Using Pankhurst eye index, the maturation or silvering stage can be calculated using body length (bl) and the mean diameter (md) of the eye calculated from the horizontal and vertical diameter (Pankhurst 1982):

$$100\frac{\pi\left(\frac{\mathrm{md}}{2}\right)^2}{\mathrm{bl}}. \quad (1)$$

An index below 6.5 indicates an immature yellow eel, and an index above 6.5 indicates a mature silver eel (Pankhurst 1982). Durif's silver index can be calculated using body length, body mass, eye diameter, and pectoral fin length (Durif et al. 2009). This index produces six maturation stages: I, MII, and FII–FV, with I denoting resident undifferentiated males and females, MII migrant males, FII resident females, FIII premigrant females, and FIV–FV indicating migrant females (Durif et al. 2005, 2009). Durif's silver index was calculated following the methods described in Durif et al. (2009); the index parameters and script are available on figshare (see Data Availability Statement).

Comparison between left- and right-side measurements.— Left- and right-side measurements of eye diameter and pectoral fin length were compared using data from a database containing data on European Eels from several lakes across Sweden collected during many years (database called "Sötebasen"). The database is kept by the Institute of Freshwater Research, Swedish University for Agricultural Sciences. For the analyses, data on body length (±1 mm, measured using a standard measuring board), body mass (±0.1 g), diameter of left and right eyes (±0.01 mm, measured using calipers), and length of the left and right pectoral fins (±0.01 mm, measured using calipers) for each individual eel was used. Individuals with missing data for any of those six variables were excluded, leaving $N = 16{,}977$ individuals for the analysis (Table 1; data collected 2003–2020, measured in the field [alive, sedated] or in the laboratory [alive sedated, dead fresh, or frozen and thawed], extracted from the database on March 30, 2021). For the eels that were measured as frozen and thawed, we corrected weight and length using the Simon (2013) freeze shrinking correction factor before further analyses. Given the large time span, the data has been collected as part of several different monitoring programs, with the most recent being the EU data collection framework in the fisheries and aquaculture sector (Regulation 2017/1004).

Comparison between visual and measurement-based maturation classification.— To investigate whether maturation stage determined by an observer based on visual information (the color of the eel, contrast in color between the ventral and dorsal area, visually estimated size of eyes and

TABLE 1. Sample size, data collection year, and length range (mm) for each of the three analyses: comparison between left- and right-side measurements, comparison between visual and measurement-based maturation classification, and comparison of precision between measuring methods.

Analysis	*N*	Data collected	Length range
Left- and right-side measurements	16,977	2003–2020	162–1,230
Visual versus measurement maturation classification	11,498	2003–2020	331–1,218
Precision comparison, measuring methods	50	2016	602–856

TABLE 2. Difference and coherence in silver eel index classification based on measurements of the left and right side of each individual (eye diameter and pectoral fin length). Green cells indicate coherence in silver eel index calculation based on the left or right side of each individual. Red cells indicate a difference in silver eel index calculation based on left- or right-side measurements of each individual. The total number of European Eels was $N = 16,977$ (for sample size details, see Table 1).

Durif's silver index, left eye	Durif's silver index, right eye					
	MII	I	FII	FIII	FIV	FV
MII	68	10	13	9	0	2
I	0	276	4	1	0	0
FII	1	48	789	58	3	1
FIII	1	0	324	5,796	316	189
FIV	0	1	3	609	6,495	46
FV	2	0	6	554	32	1,320

pectoral fin, and the presence of neuromasts) generated the same maturation stage classification as when using eye diameter and pectoral fin length to calculate Pankhurst and Durif's maturation stage indices, we also used data from the database "Sötebasen" (described above). For these analyses, data on body length (±1 mm, measured using a standard measuring board), body mass (±0.1 g), diameter of left and right eyes (±0.01 mm, measured using calipers), length of the left and right pectoral fins (±0.01 mm, measured using calipers), and a visually determined maturation stage (yellow eel, transitional, and silver eel) for each individual European Eel was used. Individuals in the database with missing data for any of those seven variables were excluded. Since males are rarely caught within the data collection programs in Sweden and since they can be difficult to disentangle from juveniles, both visually (unless dissected) but also when using the indices, all individuals classified as I (resident undifferentiated males and females) and MII (migrant males) according to Durif's silver index ($n = 354$; Table 2) were excluded from the analysis. These two exclusions left $N = 11,498$ individual females for the analysis (Table 1; data collected 2003–2020, measured in the field [alive, sedated] or in the laboratory [alive sedated, dead fresh, or frozen and thawed], extracted from the database on March 30, 2021).

Since the visual maturation stage determination divides adult European Eels in three maturation stage groups (yellow eel, transitional, silver eel) and the Pankhurst eye index only consists of two groups (yellow and silver eels), two of the visually determined groups had to be pooled for the analyses. We pooled the transitional and silver eel groups since the eels visually classified as transitional are closer to being silver eels than yellow eels (J. Persson, personal observation). The fact that the transition from yellow to silver eel happens fast once initiated, relative to the many years spent as a yellow eel, further supports pooling the transitional and silver eel stages. For the comparison to Durif's silver index, the groups were matched according to the following: individuals visually classified as yellow eels matched with FII (resident females), individuals visually classified as transitional eels matched with FIII (premigrant females), and individuals visually classified as silver eels matched with FIV and FV (migrant females).

Comparison of precision between measuring methods.—To investigate potential differences in precision between measuring methods, caliper and digital (ImageJ) measurements were compared. European Eels ($n = 50$ females; Table 1) collected as part of the EU data collection framework (Regulation 2017/1004) were used; they were fished in Lake Vombsjön (latitude: 55.684069, longitude: 13.584766) and Lake Mälaren (latitude: 59.446755, longitude: 16.240534) in 2016 by commercial fishers. Data collection was conducted in three steps. First, total body length (±1 mm, measured using a standard measuring board), body mass (±0.1 g, measured using a Mettler PC 4400 scale [Mettler Instruments AG, Zürich, Switzerland]), and pectoral fin length (±0.01 mm, measured using digital calipers [Mitutoyo Absolute Coolant proof IP67; Mitutoyo, Aurora, Illinois]) were measured on eels that had been frozen and then thawed (freeze shrinking was corrected for before analyses using the Simon [2013] freeze shrinking correction factor). All measurement data were collected at the Institute of Freshwater Research, Swedish University for Agricultural Sciences, Drottningholm, Sweden, in 2016. Since the aim was to explore how the precision in eye diameter measurements was affected by measurement method and observer, the same observer measured body length, body mass, and pectoral fin length (Persson). Secondly, three observers (Persson, N. Sjöberg, and O. Renman) with extensive knowledge in eel biology and monitoring procedures measured eye diameter (vertical and horizontal diameter) on the 50 eels in a

consecutive order using digital calipers (±0.01 mm; Mitutoyo Absolute Coolant proof IP67 [Mitutoyo, Aurora, Illinois] or C.E. Johansson Jocal–digital caliper [Eskilstuna, Sweden]). Thirdly, a plastic ring (inner diameter of 12.66 mm) was placed over the right eye and each individual eel was photographed using a digital camera (Olympus Tough F2.0 camera [4× wide optical zoom 4.5–18 mm 1; 2.0–4.9]; Olympus, Tokyo, Japan) (Figure 2) mounted on a

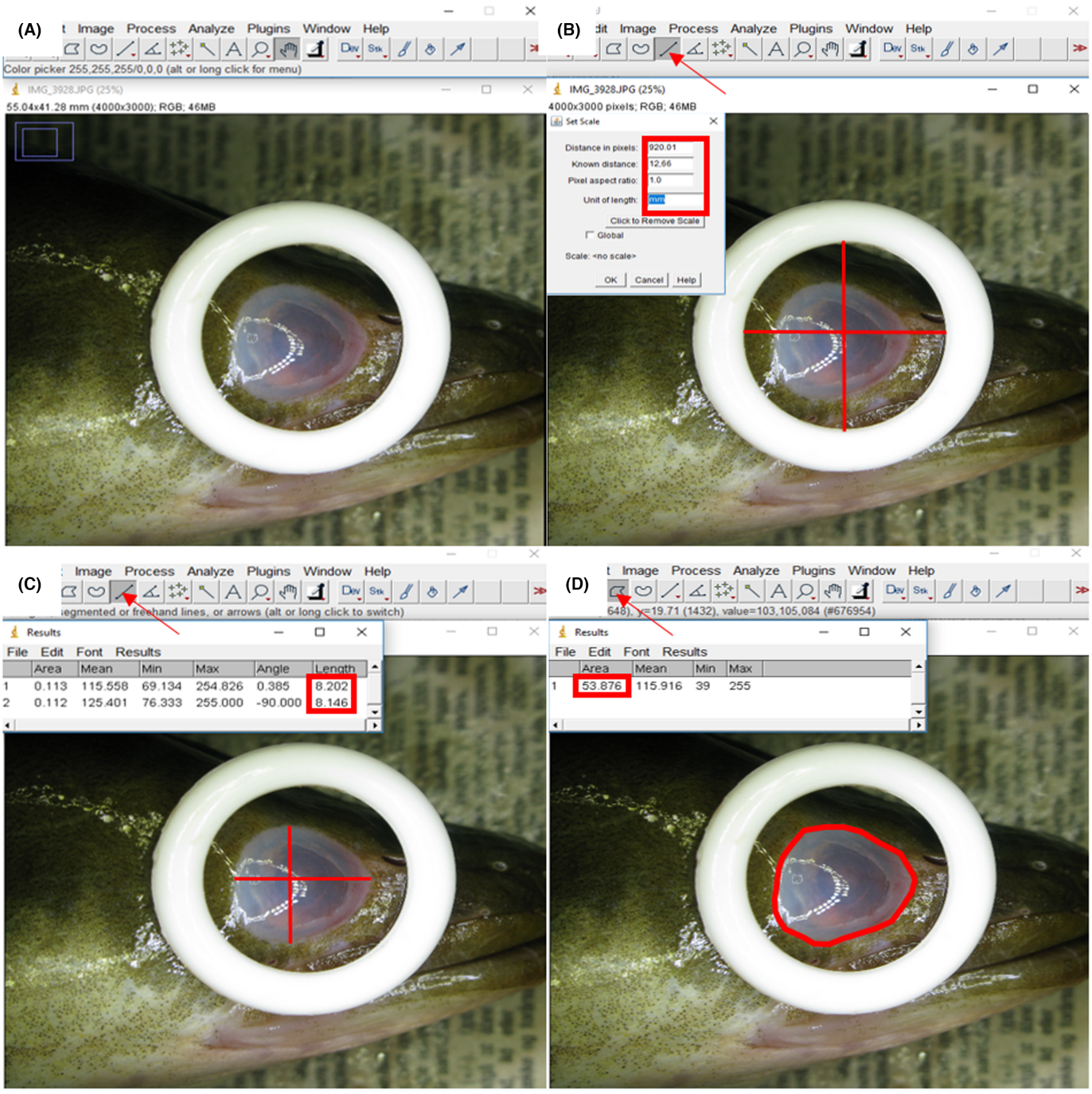

FIGURE 2. Processing images of European Eels in ImageJ: **(A)** the original image, when opened the timing started (to quantify duration to measure each image); **(B)** using the "Straight" function the scale was set using "Analyze–Set scale" based on the mean diameter of the horizontal and vertical measurement of the object with known diameter, in this case the white plastic ring (ø = 12.66 mm); **(C)** horizontal and vertical measurement of the eye, using the "Straight" function and pressing ctrl+m; and **(D)** measuring the circumference using the "Polygon" function. When all measurements had been inserted in the protocol and the picture was closed, timing was stopped.

stand. The Olympus Tough camera has in-camera distortion correction, leaving a small measured geometric barrel distortion in JPEG files (at full wide angle) of ~0.1% along the top edge and ~0.3% along the bottom edge, but since the eye was always in the center of the image, distortion was considered to be minimal. The plastic ring served as a reference object and was later used to set the scale for the digital measurements of the eye. The images were then used to digitally measure the horizontal and vertical eye diameters and the area of the eye using the free software ImageJ (version 1.53g) (Schindelin et al. 2012, 2015). The images of the 50 eels were digitally measured independently by all three observers (Persson, Sjöberg, and Renman) in February 2021. In ImageJ, the scale was set by measuring the object with known size, in this case the plastic ring with known inner diameter (Figure 2A). The tool "Straight" was used, and the inner diameter of the ring was measured horizontally and vertically by pressing ctrl+m. The average of the measured inner diameter was used to set the scale, under "Analyze–Set Scale" (Figure 2B). When the scale had been set, the eye was measured using the "Straight" tool to measure the diameters horizontally and vertically (Figure 2C), and the "Polygon" tool was used to measure the circumference (Figure 2D). The duration to measure each digital image was also quantified, from opening the image in ImageJ until closing the image (i.e., including all three eye measurements and adding each data point into an electronic spreadsheet).

Statistical analyses.— All statistical analyses were performed in R version 4.1.1 (R Core Team 2021) and visualized using the package ggplot2 (Wickham 2016). Both Pankhurst eye index (Pankhurst 1982) and Durif's silver index (Durif et al. 2005, 2009) use the average eye diameter. Therefore, both the vertical and horizontal measurements of the eye diameter were used in all models. To account for the fact that some European Eels were measured fresh and some were measured after freezing and thawing, a correction factor accounting for the shrinkage was applied for all eels measured after freezing and thawing (Simon 2013).

To analyze if there were differences between the eye diameter and pectoral fin length on the left and right sides of the individual European Eel, a Welch two-sample *t*-test was used ($N = 16{,}977$; Table 1). To analyze whether the left or right measurements would result in individual eels being differently classified according to Pankhurst eye index and Durif's silver index, Pearson's chi-square tests were used.

To test the potential difference in maturation stage classification between visual and measurement-based (Pankhurst eye index and Durif's silver index) methods, chi-square tests were used ($N = 11{,}498$; Table 1). Note that this was tested both for the measurements made on the left and right sides of the eel, while there is only one visual stage determination per individual eel.

The difference in precision (precision in terms of standard deviation) between the measuring methods of the mean diameter measurements for each individual European Eel ($n = 50$ eels, measured by three observers) was compared using an ANOVA. Standard deviation values per eel were the response variable, while measuring method with three levels was the fixed effect (*Caliper Diameter*: mean diameter measurements of the eye using digital calipers, *ImageJ Diameter*: mean diameter measurements of the eye using digital image measurements, and *ImageJ Diameter from Area*: mean diameter calculated from digital image measurements of eye area). Tukey's honestly significant difference post hoc tests (Tukey HSD) were used to further determine significant effects where appropriate. To analyze whether the different measuring methods would result in individual eels being differently classified between the three observers, according to Pankhurst eye index and Durif's silver index, chi-square tests were used.

Time spent on image-based measurements (including both diameter and circumference measurements) was analyzed with a nonlinear function: measuring time = $\alpha e^{(-\beta\ \text{eel individual})}$, where α is the intercept and β the slope. A negative exponential function was chosen since the measuring time was expected to decrease with the accumulated number of eels measured as an effect of increased observer experience.

RESULTS

Comparison between Left- and Right-Side Measurements

There was a statistical difference in diameter between the right and left eyes, where the left eye was larger than the right eye on average ($t_{33,885} = -12.54$, $P < 0.001$, mean difference = 0.19 mm ± 0.022 mm [95% CI]) (Figure 3A). The most pronounced difference was found for smaller European Eels (small eye diameter), where left eye measurements were larger than right eye measurements for the eye diameter as indicated by the 1:1 ratio (Figure 3A). There was no significant difference in the measurements of the right and left pectoral fins ($t_{33,950} = 0.62$, $P = 0.53$), with a mean difference of 0.045 mm ± 0.025 mm (95% CI) (Figure 3B).

Using the left- or right-side measurements resulted in 11% of the individual European Eels being differently classified according to Pankhurst eye index ($n = 1{,}930$ eels out of the total $N = 16{,}977$ eels; $\chi^2_1 = 101.86$, $P < 0.001$; Figure 4). For Durif's silver index, 13% ($n = 2{,}233$) of the individual eels were differently classified ($\chi^2_5 = 92.98$, $P < 0.001$; Table 2). Most of those differences were due to the left eye being larger than the right eye (Figures 3 and 4) at small sizes.

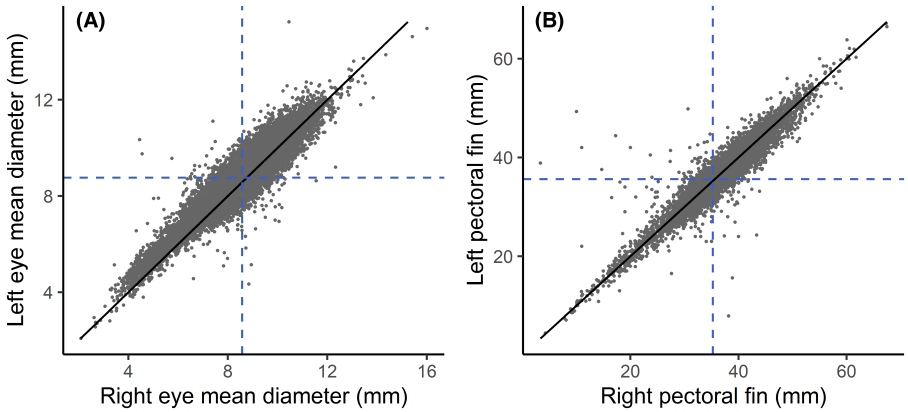

FIGURE 3. Comparison of **(A)** mean eye diameter (mean of one vertical and one horizontal measurement per eye) and **(B)** pectoral fin length for the left and right sides of European Eels. Each data point represents the mean from one individual's measurements ($N = 16{,}977$ females, males, and juveniles; for sample size details, see Table 1). The solid black line represents the 1:1 ratio (no difference) between the left and right sides. The blue dashed vertical and horizontal lines represent (A) the grand mean measurement of the diameter for the right and left eyes, respectively and (B) the grand mean length of the left and the right pectoral fin measurements, respectively.

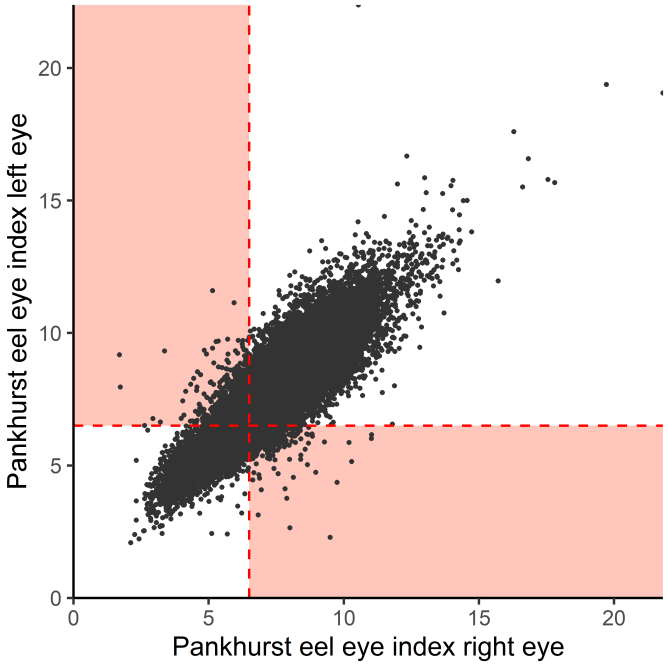

FIGURE 4. Difference in maturation stage classification when using left or right eye measurements for Pankhurst eye index for European Eels. An index below 6.5 indicates an immature yellow eel, and an index above 6.5 indicates a mature silver eel (red dashed lines) according to Pankhurst. Each data point represents the left- and right-side measurements of one individual. Points inside red shaded areas represents individuals classified differently depending on the side of measurement (total number of eels was $N = 16{,}977$ females, males, and juveniles; for sample size details, see Table 1).

Comparison between Visual and Measurement-Based Maturation Classification

There was a significant difference in the number of European Eels classified into the different maturation stages when comparing eels classified based on visual information to classifications calculated from measured variables (Table 3; Figure 5). These results were consistent for all comparisons and for both Pankhurst and Durif's indices, regardless of whether data from the left or right side was used (Table 3; Figure 5). The visual classifications estimated a greater number of the eels as silver eels, and the left eye–left side data classified more eels as silver eels

TABLE 3. Number of European Eels being classified into the different maturation stages according to Pankhurst eye index (yellow and silver eels) and Durif's silver index (FII = resident females, FIII = premigrant females, FIV and FV = migrant females) for the left and right side when using measured variables and when stage was determined based on visual information by an observer (visual classification). Chi-square comparisons between the number of eels in each maturation stage is based on visual information and Pankhurst eye index and Durif's silver index (using left- and right-side measurements). The total number of eels was $N = 11{,}498$ (for sample size details, see Table 1).

Index and comparison	Left eye	Right eye	Visual determination	χ^2	df	P
Pankhurst						
Yellow	2,981	2,089	1,393			
Silver	8,517	9,409	10,105			
Durif's						
FII	900	1,186	1,393			
FIII	4,357	4,808	2,642			
FIV and FV	6,241	5,504	7,463			
Chi-square comparisons						
Pankhurst left eye versus visual				163.47	1	<0.001
Pankhurst right eye versus visual				711.05	1	<0.001
Durif's left side versus visual				635.20	2	<0.001
Durif's right side versus visual				942.31	2	<0.001

compared with the right eye–right side data for both Pankhurst and Durif's indices (Figure 5).

Comparison of Precision between Measuring Methods

There was a significant difference in precision (standard deviation) between the three different methods ($F_{2,\ 147} = 52.48$, $P < 0.001$; Figure 6A). Measuring the eye diameter using ImageJ generated greater precision compared with using calipers, regardless of whether diameter was measured directly in ImageJ or derived from area in ImageJ (Tukey HSD; ImageJ Diameter versus Caliper Diameter: $P < 0.001$, ImageJ Diameter from Area versus Caliper Diameter: $P < 0.001$; Figure 6A). In addition, diameter derived from area in ImageJ generated higher precision compared with diameter measured directly in ImageJ; however, this difference was only marginal (Tukey HSD; ImageJ Diameter from Area versus ImageJ Diameter: $P = 0.039$; Figure 6A).

The lower precision from measurements taken with calipers resulted in significantly more European Eels (22%) being differently classified between the three observers for the Pankhurst eye index (Table 4; Figure 6B; Table S1 [available in the Supplementary Material]). Measurements using ImageJ resulted in fewer eels being differently classified between the observers for the Pankhurst eye index (12% for ImageJ Diameter and 10% for ImageJ Diameter from Area; Table 4; Figure 6B). For Durif's silver index, 12% of the eels were differently classified between the observers when measurements were made with calipers, which was nonsignificant (Table 4; Figure 6B). The percentage of eels being differently classified for Durif's silver index when using ImageJ were low and nonsignificant (Table 4; Figure 6B; Table S1).

Time spent measuring eye diameter of European Eels from digital images using ImageJ (vertical and horizontal, data from all three observers) was decreasing for each measured individual, with the first 7–10 images taking a longer time to measure than the last 40 individuals (Figure 7). The mean time spent measuring each individual eel was 130 s (95% CI = 9 s).

DISCUSSION

Comparison between Left- and Right-Side Measurements

There was a difference in diameter between the left and right eyes in European Eels, where the left eye was larger than the right eye on average, while there was no size difference in length between the right and left pectoral fins. The statistically significant difference in eye diameter is probably explained by the large amount of data ($n = 16{,}977$ individual eels) rather than a biologically relevant difference given that the average difference in actual size was very small (mean difference = 0.19 mm). Even though caliper measurements of the eyes are recorded at a 0.01-mm level, the mean difference of 0.19 mm may still be regarded as being within the range of observation error. The delimitation between the eye and the surrounding skin may sometimes be difficult to assess, depending on factors such as the color of the eel. Samples that have been frozen and thawed can be easier to measure since the delimitation may then be clearer (C. Durif, Institute of Marine Research in Norway, personal communication). Freezing and thawing could on the other hand have potential effects on the shape of the eyes, but both indices are

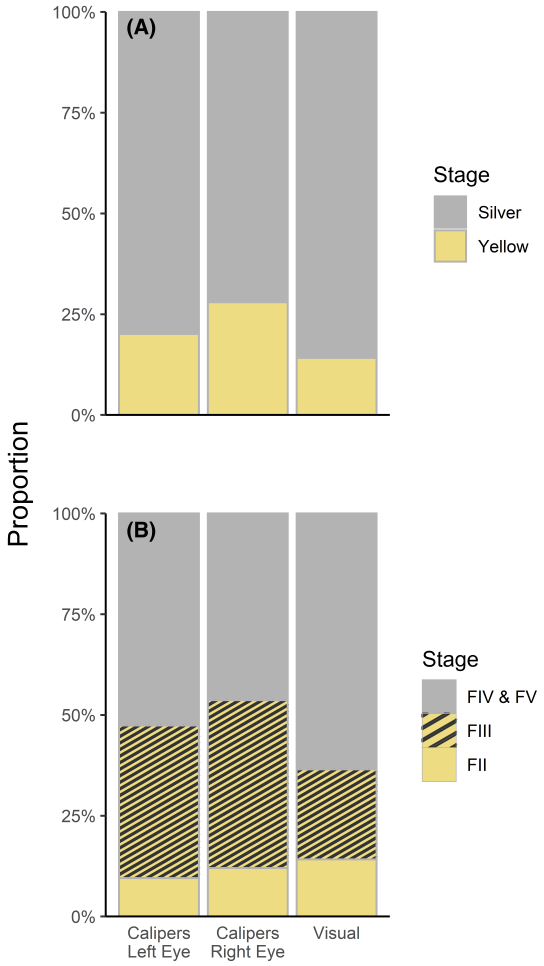

FIGURE 5. Percentage of European Eels **(A)** classified as yellow eel and silver eel based on computation of Pankhurst eye index and classified visually as yellow eel or transitional and silver eel by an observer and **(B)** classified as FII (resident females), FIII (premigrant females), or FIV and FV (migrant females) computed with Durif's silver index and classified visually as yellow eel (corresponding to FII), transitional (corresponding to FIII), or silver eel (corresponding to FIV and FV). The total number of eels was $N = 11{,}498$ females (for sample size details, see Table 1).

TABLE 4. Percent of European Eels being differently classified between the three observers for Pankhurst eye index and Durif's silver index based on measurements made using calipers and ImageJ (ImageJ Diameter and ImageJ Diameter from Area), and chi-square comparisons between observers for the number of eels being differently classified for each index and method. The total number of eels was $N = 50$ (for sample size details, see Table 1).

Measurement method	%	χ^2	df	*P*
Pankhurst eye index				
Calipers	22	7	2	0.03
ImageJ diameter	12	1.38	2	0.5
ImageJ area	10	0.19	2	0.91
Durif's silver index				
Calipers	12	1.86	4	0.76
ImageJ diameter	4	1.06	6	0.98
ImageJ area	2	0.06	6	1

frequently applied on data from frozen and thawed samples and this is not thought to have an impact on the classification (Durif, personal communication).

Using the left-side measurements of the eye compared with the right-side measurements resulted in 11% of the individual European Eels being differently classified according to the Pankhurst eye index; for Durif's silver index, 13% of the individual eels were differently classified. Most of those differences were due to the left eye being larger than the right eye. Although the effects were statistically significant, the number of eels being differently classified might be an acceptable amount depending on the precision needed. Again, given the large data set, the statistically significant effects must be interpreted with care, both regarding the difference in eye diameter and the subsequent effect on maturation stage classification. Since statistical significance depends on both sample size and effect

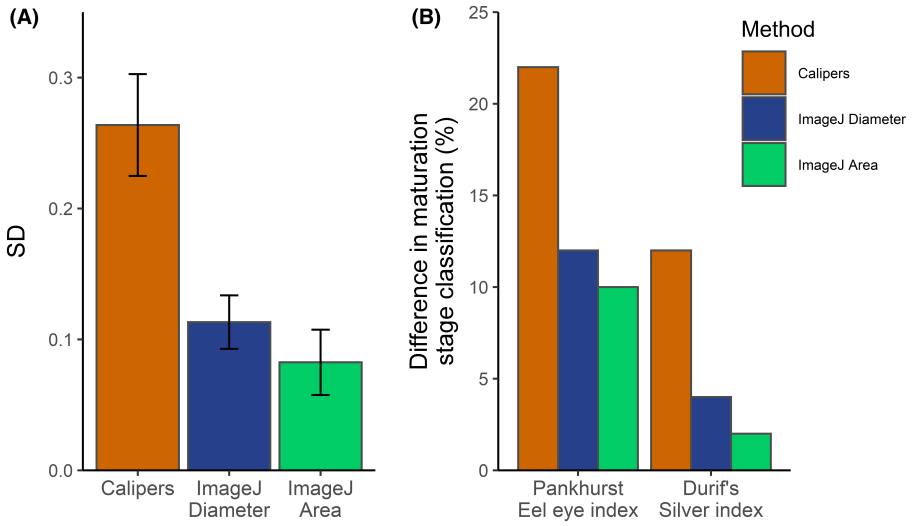

FIGURE 6. Panel **(A)** shows the difference in precision (in terms of standard deviation [SD]) when measuring eye diameter using calipers (orange bar) or ImageJ diameter (blue bar) and when diameter was derived from area in ImageJ (green bar). Each bar represents the mean standard deviation for $N = 50$ female European Eels measured by three different observers (for sample size details, see Table 1). Error bars indicate 95% confidence intervals. Panel **(B)** shows the percent of eels being differently classified between the three observers for Pankhurst eye index and Durif's silver index when the eels were measured using calipers (orange bars) or ImageJ diameter (blue bars) and when diameter was derived from area in ImageJ (green bars).

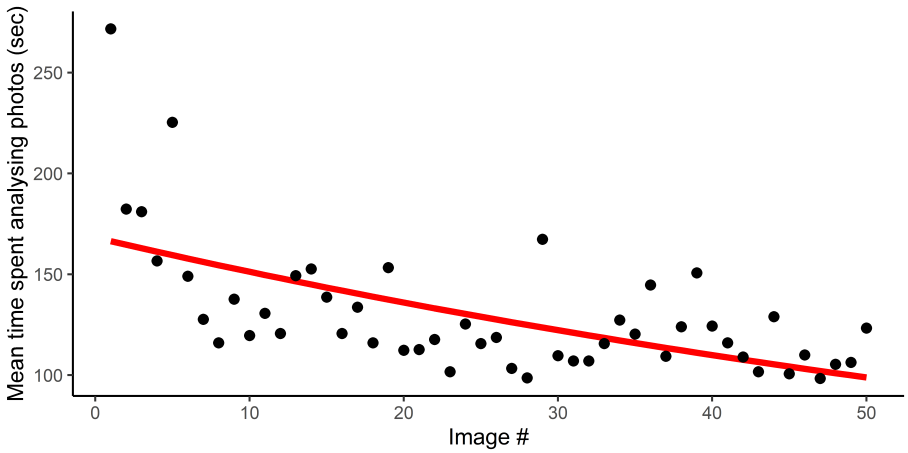

FIGURE 7. Mean time spent measuring individual European Eel eye diameter (vertical and horizontal) from digital images using ImageJ ($N = 50$; for sample size details, see Table 1) based on the measuring time from three observers. The red line represents the fitted nonlinear function for the data points.

size, *P*-values are confounded because of their dependence on sample size, meaning that a statistically significant result could be a consequence of using a very large sample size (Sullivan and Feinn 2012). Hence, the differences in actual numbers must be taken into account, and here those differences were indeed very small. Another factor to consider is that Durif 's silver index uses continuous data to calculate categorical indices, where it can be expected that small differences in measurements of the eel would result in the majority of the misclassifications being within the range of adjacent classes (e.g., FII being classified as FI or FIII), which our results also suggest. However, our results also indicate that for classification FIII, there was a considerable number of eels being classified as FV (two classes apart), which could have an impact on classification of migratory individuals since FIII is determined as premigratory and FV classified as migratory. This is something that might need further analysis in the

future; however, this misclassification constituted less than 10% in our study, which might fall into reasonable variation for Durif's silver index.

While data are collected on both sides of the eel within the Swedish EU data monitoring program, and potentially in other countries as well, it is standard to collect data on one side only in some countries (e.g., France, Spain, Portugal). In the protocols for the monitoring program within those countries, it is specified that the eye and pectoral fin on the left side should be measured, unless malformed (protocols from the SUDOANG project: https://sudoang.eu/en/). Given the small difference in actual size of the eye and in the actual number of eels being differently classified, it is suggested that measuring only one side is sufficient to enable accurate maturation stage classification, even though a statistical difference between the left and right sides was detected. Reducing data collection to one side will reduce data collection time (and handling time when data is collected on live animals) while still generating data comparable to other countries, thereby increasing cost effectiveness of the monitoring program.

Comparison between Visual and Measurement-Based Maturation Classification

There were differences in the number of European Eels classified into the different maturation stages when comparing eels classified based on visual information to classifications calculated from measured variables, with the visual classifications estimating a greater number of the eels as silver eels. The notion that the common visual classification of maturity overestimates the proportion of silver eels compared with the two index-based methods has previously been reported when analyzing a data set consisting of 86 European Eels from the Swedish west coast (Andersson et al. 2019). This difference might not be due to observer bias since previous studies have shown that migrating eels from many locations in Sweden seem to be in an earlier stage of maturation than those from elsewhere in Europe, and the eyes may also grow during the migration in the Baltic Sea (Sjöberg et al. 2009). In that study, European Eels with a Pankhurst eye index below 6.5 still had a silvery appearance (i.e., black back, silver–white belly, and conspicuous lateral line) and were caught in the same gears and at the same time and at the same fishing sites as more mature eels (Sjöberg et al. 2009). In addition, the visual classification is not intended to function as an exact maturation stage classification in itself; rather, these data are collected as a complement to the variables needed to calculate the indices. The silver eel characters (neuromasts, color, contrast in color between the ventral and dorsal area, size of eyes and pectoral fin) may not all appear at the same time, and they might not appear in the same order for all individuals (Persson, personal observation), meaning that the visual classification can be useful in cases where individuals are on the margin for any of the index categories. Since eels with relatively small eyes but with the exterior look of silver eels do migrate towards the Baltic outlet, the indices can only be used as proximate indicators of migrant behavior (Sjöberg et al. 2017).

It is still important, however, to limit the subjectivity between observers. The criteria for visual maturation stage determination in European Eels should be clearly defined, and such standards have been published by Acou et al. (2005). They consist of precise descriptions of the appearance of neuromasts and the color of the eel. If the neuromasts are small (<1 mm in diameter) and grouped into white points, that indicates the yellow eel stage (Acou et al. 2005). During silvering, the white points are gradually surrounded by a black circle and increase in diameter and lastly become black with a diameter of 1–2 mm (Acou et al. 2005). Fully mature silver eels have a clearly differentiated lateral line (Zacchei and Tavolaro 1988). Histological and photometric investigations have shown that changes in skin color during maturation are subtle and varied and, as such, do not form a good basis for determining developmental stages (Pankhurst and Lythgoe 1983). The relative contrast between the ventral and dorsal areas is generally apparent in mature silver eels and can therefore serve as a more objective color criterion (Acou et al. 2005).

In addition to the difference between the visual classification and the indices for European Eels, data from the left and right sides of the eel also generated different classifications for both Pankhurst and Durif's indices. The left eye–left side data classified more eels as silver eels compared with the right eye–right side data. This result is expected given that the results also showed that the left eye was larger than the right eye on average. Similar to the analyses between the left and right sides, however, these differences were small in actual numbers and the statistical significance can be considered an artifact of analyzing a very large data set (Sullivan and Feinn 2012).

Comparison of Precision between Measuring Methods

This study shows that precision increased when measurements of European Eels were made using digital image analysis software compared with measurements using calipers. The lower precision obtained when measuring with calipers resulted in significantly more eels being differently classified between the three observers according to the Pankhurst eye index. For Durif's silver index, measuring with calipers or from digital images did not result in a greater amount of the eels being differently classified between the observers. Issues related to interobserver variability when collecting data to determine European Eel maturation stage have been noted previously in a study where 13 European Eels were measured by three observers

(Acou et al. 2006). The study concluded that measurement error (bias and imprecision) can be present even under optimal measurement conditions (Acou et al. 2006). The fact that humans are prone to errors and that this may influence fisheries data monitoring has also been described for data of fish length (Bunch et al. 2013). It could therefore be suggested that eye measurements should be made using digital image analysis software, rather than with calipers, to increase precision. This method might, however, increase data collection time since all steps combined (i.e., photographing the eels, image handling [labelling, storing], and analysis in ImageJ) are probably more time consuming than making direct measurements with calipers, particularly if the data is collected under field conditions compared with in the laboratory. This was, however, not quantified in this study. The gain in precision should nonetheless be put in relation to a potential increase in the time needed to collect the data. If applying this method within a monitoring program, it might be valuable to automate the measurements in ImageJ and restrain the eye measurement to only include the area measurement since it gave the highest interobserver precision, which also would decrease analysis time. The accuracy for automatic measurements (when setting measurements using a measuring board as the reference value) in ImageJ and other programs can be very high (Andrialovanirina et al. 2020). Though they measured total length of rather small fishes only ($n = 180$ from 19 families, no eels), their example seems promising.

CONCLUSIONS

Based on the results from this study, it is suggested that eye measurements in European Eels should be made using digital image analysis software rather than calipers to increase precision. Eye diameter and pectoral fin length can be measured on the left or right side only without losing precision when determining maturation stage. Lastly, classification made by observers may not be as reliable as calculated indices but could still provide useful information. These implementations will generate a more precise collection of biological data necessary to accurately determine stock abundance and trends of the European Eel. For most of the analyses and comparisons, it is important to keep in mind that the true maturation stage is unknown. The maturation stages in the Pankhurst eye index and Durif's silver index were, however, originally defined based on sexual maturity determined using histological preparations (Pankhurst 1982; Durif et al. 2005). Hence, the maturation stage calculated here using the indices should be accurate.

The importance of measurement precision in fisheries has been discussed elsewhere, and previous findings show that measurement errors can influence results, such as error in spawner biomass estimations leading to flawed stock–recruitment relationships (Walters and Ludwig 1981) and errors in otolith age reading causing aging and growth estimate errors (Campana 2001; Kullmann et al. 2018). Ultimately, the statistical methods required to analyze the data will decide the level of precision needed and it is important to be aware of error factors, in particular when the results have implications for management (Bunch et al. 2013).

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DATA AVAILABILITY STATEMENT

The data and script for this study are archived in the repository figshare following best practices (Roche et al. 2015). It was made available to editors and reviewers upon initial submission as Supplementary Material. Figshare link: https://figshare.com/projects/Evaluation_of_sampling_methods_for_maturation_stage_determination_in_the_European_eel/139771.

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SUPPORTING INFORMATION

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