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*Research Article*

# A suitable ichthyometer for systemic application

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**ABSTRACT.** An excessive and inappropriate handling of live fish is currently performed to obtain its size and weight. As an ameliorative attempt, a system named “IchthyoJHOL" was devised for the measurement of live specimens of fusiform fish species. The system holds the fish ventrally between supports and is mounted upon a scale. An image is captured on camera and processed with a digital measurement system. The system generates biometric data such as length, height, and weight. This methodology creates a two-stage biometric process, the first being the capture of the images and the second their digital analysis, allowing for each stage to be carried out at distinct times and places. With this methodology, a reliability of over 96.8% is achieved, similar to that produced by the traditional system, but with a significant reduction in handling time and stress induced on the fish, allowing for large-scale biometric analysis. In addition, the library of fish images can be shared for posterior evaluation, redescription studies or a more extensive morphometric analysis.

**Keywords:** fish biometry, fish length, fish welfare, ichthyometer, digital analysis of images.

## INTRODUCTION

Determining the length of a fish is fundamental for basic biological studies such as growth, age, maturity and physiological condition of an organism; it can also be an ecological indicator in natural, experimental or production environments (Jawad *et al.*, 2009; Simeanu *et al.*, 2010; Mir *et al.*, 2013; Yuji-Sado *et al.*, 2015). Establishing size is important in fish, as it used to regulate the exploitation of some species in sports fishing and commercial fishing; it is also important in many other organisms such mollusks, crustaceans, birds, insects, and plants (Pérez-González, 2011; DEDDI, 2012; DFGWA, 2014). In cultivated fish systems, size is one of the principal indicators of the success of production processes (Rutjes *et al.*, 2009; Nunes-Rocha *et al.*, 2012), and the recording and monitoring of gain size should be a rapid and recurrent procedure which produces the lowest level of stress possible on the organisms and generating precise data.

Total length, fork length or standard length (TL, FL or SL, respectively) are the most commonly used measurements to achieve fish size and are obtained using an ichthyometer or measuring table, which consists of a flat support and a ruler. The fish is placed on its right side with its mouth closed and pointing to the left (Gulland, 1971; Simeanu *et al.*, 2010; DEDDI, 2012; Paiva *et al.*, 2015). In large fish, measurements are taken with the help of a measuring tape (Tičina *et al.*, 2011).

Determining height (H) and width of the fish is an even more complicated procedure which requires severe handling, and therefore causes significant stress on the organisms. This situation becomes even more delicate if the fish is ecologically important or has been granted some kind of protection status (González-Díaz *et al.*, 2005; Portz *et al.*, 2006; Trujillo, 2009; Simeanu *et al.*, 2010; Galván, 2011; Martins *et al.*, 2012; Yajing, 2012).

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Many biological indices in live fish are derived by correlating morphometric, meristic, biometric and genetic variables, using both *in situ* and *in vitro* techniques (Hamza, 1999; Jones *et al.*, 1999; González-Díaz *et al.*, 2005; Gaspar *et al.*, 2012; Reis-Neto *et al.*, 2012; Muto *et al.*, 2016). The modern methodology includes the use of high-definition cameras, sensors and specialized software (Harvey, 2003; Costa *et al.*, 2006).

In the case of dead fish, studies may involve systems coupled with conveyor belts that are equipped with cameras and software for digital editing and measurement, such as the identification and classification of fish (White *et al.*, 2006; Rutjes *et al.*, 2009; Shortis *et al.*, 2013). However, this new technology is complex, expensive and not accessible to the majority of fish farmers or researchers, and error management (random and systematic) associated as much with the measurement process as with the observer, could result in incorrect *a posteriori* interpretations of these estimations (White *et al.*, 2006; Goodennough *et al.*, 2010, 2012; Sidek & Halawani, 2010; Yajing, 2012).

Due to the importance of some biometric indices in fish and the interest in reducing levels of stress habitually caused by the measurement process, a two-phase method is proposed; the first for the collection of information and the second for processing the data, which allows the tasks to be separated and developed with greater efficiency, as suggested by Chang *et al.* (2010) and Yajing (2012). In the first phase, the use of a support which allows the live fish to be secured in place for the acquisition of a photograph is proposed, which is then analyzed digitally (second phase) to determine the biometric dimensions of interest (TL, SL, H). Weight is determined directly by placing the ichthyometer on a tared scale (Fig. 1). The whole procedure is carried out in a few seconds (3 to 8), and the fish is immediately returned to the water. Collection times, information sorting and classification could be superior with the proposed method than with traditional methodology. It is estimated that using the traditional method, weighing and registering the size of an organism takes between 45 and 70 s (Portz *et al.*, 2006; Martins *et al.*, 2012; Upton & Riley, 2013).

As well as estimating standard length (SL), this method, with the help of a digital image analyzer, allows estimation of other parameters such as size, shape of eyes and fins, and distances and proportions between biometric features; all important criteria in the optimum management of aquatic resources (Jawad *et al.*, 2009; Gaspar *et al.*, 2012; Reis-Neto *et al.*, 2012; Yajing, 2012; Klima *et al.*, 2013; Paiva *et al.*, 2015; Muto *et al.*, 2016).

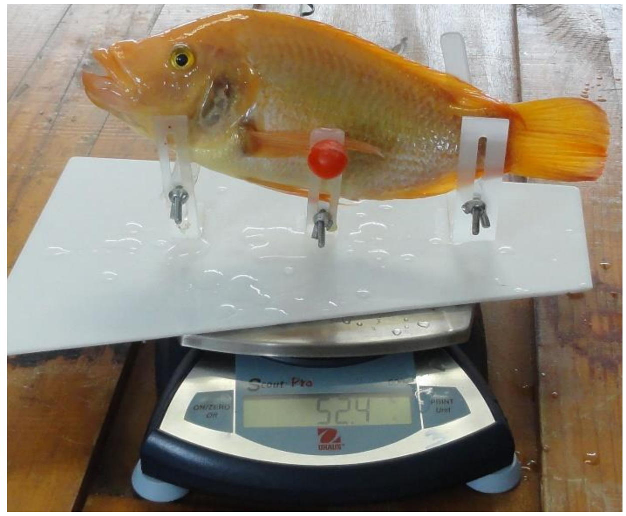


**Figure 1.** Fish mounted in the icthyometer and placed on the scale. a) The forks holding the fish, b) the calibration sphere, and c) the screen of the scale can be seen.

## MATERIALS AND METHODS

### Structural design of the ichthyometer

The IchthyoJHOL consists of a rigid, inert plate, upon which various fork-shaped supports are fixed in a straight line for the fish to be placed in. The plate can be acrylic or polyamide, having dimensions of 15×35 cm and a thickness of less than 6 mm. The acrylic Y-shaped supports are 1 cm thick and 4 cm tall from the base with a fork length of 4 cm (Fig. 1a).

### Device management

### Orthogonal plane

Accuracy in the size estimation of a fish depends upon the orthogonal plane established between the Ichthyo JHOL and the camera. A basic calibration device is used to represent a fish (polystyrene fish), which contains a conical cylinder on the left side; when the circumference of the cylinder base and its outer edge are concentric the orthogonal plane has been achieved (Fig. 2b).

### Image acquisition procedure (Phase 1)

In a well-illuminated environment and close to the fish reservoir, a semi-analytical scale (0.1 g accuracy) is placed on a level surface and the IchthyoJHOL is then placed upon the scale. A camera tripod with a digital camera attached is placed in front of the system. The fish calibration device is placed in the supports (Fig. 2) facing left. The distance between the camera and the IchthyoJHOL system depends upon the morphometric characteristics in question being within the visual field of the camera. Adjustments are made to the camera or

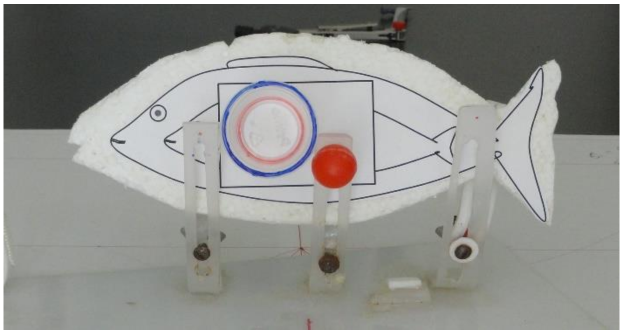

**Figure 2.** Image of fish calibrator. a) The outline of the fish, b) the conical cylinder showing concentric circumferences, and c) the reference sphere mounted on the forks.

tripod to maintain the object central and orthogonal. It must be verified that as well as the camera-IchthyoJHOL orthogonality, the screen on the scale is clearly visible so that the weight of each individual can be registered using the photograph (Fig. 1c).

Once the above process has been carried out, the fish are placed in a nearby tank. One by one the fish are placed in the supports; a photograph is taken and then each fish is returned to the tank. To avoid jolting and ensure high-quality images, clove oil or Tricaine-S may be used as an anesthesia; in which case the fish are introduced into oxygen-saturated water before returning them to their original tank in order to guarantee an adequate recuperation.

**Internal scale**

The IchthyoJHOL uses a dual calibration system, the primary being the fish calibrator, which considers two circumscribed fish (Fig. 2a), and as an alternative design, a sphere placed in the forks that are used to sustain the organisms (Figs. 1b, 2c). These elements are of known size and are used as a reference to measure biometric characteristics in the images during their processing.

The fish calibrator is used as an internal scale or software calibrator. In the first instance, the magnitude of the calibrator is used as an *a posteriori* reference to obtain the magnitude of the biometric characteristics SL, TL, or H from the image. For processing of the image, Motic Images Plus© 2.0 (Motic China Group Co. Ltd., China) software was used to estimate the size of the organisms (with permission from Motic for this application).

**Image analyses procedure** (Phase 2)

A procedure without calibration was used, following the guidelines below. The fish-image.jpg file is opened in Motic Analyzer Software. The commands measure and line are used to measure (in pixels) the dimension in question (length or height), first in the fish calibrator and then in the fish. The results are then transferred to a database for processing.

**Biometric data transformation**

Using the length of the fish calibrator as an internal reference, the biometric characteristics are obtained by multiplying the magnitude of the characteristic in the image (px) by a proportionality factor (Pf), which integrates the conversion from px to cm, as the homothetic effect (homogenous dilation) of the measurement system, from the following expression:

Pf = (calibrator length/calibrator size), where Pf is the proportionality factor; the calibrator length and size are the actual sizes and that observed in the image, in cm and px respectively, obtained from one of the fish drawn on the fish calibrator. The Pf value may oscillate between 0.010 and 0.030 for images around 2 Mpx in size.

The diameter of the sphere (Fig. 2c) is of great use as an internal reference when the measurement system has been moderately altered during the course of the biometric process, and can temporarily substitute the reinstallation of the orthogonal plane. To do this, the value of the sphere is used to obtain the Pf index.

Having digitally calibrated the software, direct biometric parameters are generated, which facilitate classification and analysis; this option is for advanced software users, and for this reason, the procedure described here is the most simple and suitable for non-expert users.

**Procedure beyond the common problems**

In the biometric process, the main stress factor is the extraction and handling of the organisms, and for this reason, a protocol should be designed to effectively manage this operation and the process should preferably be started early in the morning. Then, select the appropriate scale, taking the weight of the IchthyoJHOL and the organisms into account, with a proposed accuracy of 0.1 g and maximum measuring time of 3 s. Choose a camera with a resolution of over 10 Mpx (suggested), taking images of at least 1920×1440 px that do not exceed the size of the computer monitor, taking memory storage and extra batteries into account. Finally, the familiarization with the digital analysis program using the fish calibrator is recommended, procuring a fixed optic and carrying out the adjustments needed to maintain the orthogonal plane (Phase 1).

**Statistical analysis**

A descriptive and diagnostic assay was applied to the recorded data to support the parametric analysis

(Yamane, 1999; Zar, 2010). A significance level of $\alpha$ = 0.05 was applied for one-way ANOVA. In test 1; the effect of deviation (in degrees) was the fixed factor and test 2; the level of experience classified as the fixed effect and the measurement of fish size (in cm, TL) was in both tests, the dependent factor. The analysis of variance was carried out using SPSS software, version 20 (IBM Corp. USA).

**Reliability assay**

As a measure of reliability of the proposed method, the intraclass correlation coefficient (ICC) was used, generated from the variance components; this value is defined as the coefficient between the variance of the measured object and the total variance (McGraw & Wong, 1996; Doros & Lew, 2010; Zar, 2010; Yajing, 2012). The ICC ranges from $-1/(n-1) \leq ICC \leq 1$ (practically from 0 to 1); values close to zero indicate a poor level of reliability (when estimated effect of the random factor is zero) while factors close to 1.0 suggest high level of reliability (when estimated effect of error is zero). The ICC was carried out using the program SPSS, with the option of absolute agreement, and applying a significance level of $\alpha$ = 0.05.

**Experimental trials**

**Image acquisition system**

The following characteristics were used in the image acquisition process: 28 cm long IchthyoJHOL with three supports, non-professional Sony Cyber-shot DSCH55, 14.1 Mpx digital camera mounted on a tripod at a height of 90-110 cm at a physical distance of 75-85 cm from the IchthyoJHOL, with optical zoom between 2.2 and 2.5x, on the automatic setting in Easy shooting, no flash, high-resolution format at 72 dpi, with a 4.0 mm focal length, set to an f-stop of f/3.5, generating jpg-files between 1 and 2 Mb in size.

**Organisms used in the biometric analysis**

In order to test the proposed method, different image sets were chosen from data recorded between March 11$^{th}$ 2013 and November 21$^{st}$ 2014, from three intensive cultures of *Oreochromis mossambicus* (Peters, 1852, Perciformes: Cichlidae), carried out in the "A-Mena" Aquaculture Laboratory of the Faculty of Marine Science at the University of Colima, Mexico. The images were digitally analysed in a random order; prior to statistical analysis, the information generated was reordered categorically, according to the numerical identifier of the original file.

**Effect of the orthogonal plane**

To evaluate the effect produced by drift on the orthogonal plane between the measuring system and the camera, which may occur in field conditions, the image of the largest fish drawn on the polystyrene model (LT = 21.74 cm) was measured in triplicate, varying the angle by ±15° from the 90° position in increments of 2-3°.

The effect of deviation from the orthogonal plane (in degrees, fixed factor) on the measurement of fish size (fish length, dependent factor) was estimated using a one-way ANOVA with $\alpha$ = 0.05, by a general linear model (GLM) conducted with SPSS v20 software.

**Influence of the observer training level**

The effect of the observer training level in the proposed method was evaluated with an experiment using experts and beginners; the experts with a wide knowledge of ichthyology and the beginners with no experience, except one informative session about the objective of the work and how to use the digital analysis program. All of the observers showed interest in the scope of the project and they measured 15 images of fish in triplicate, with a mean size of TL = 20.83 ± 0.32 cm.

In this test, a one-way ANOVA was applied with the level of experience classified as the fixed effect and fish length the random factor, with a level of significance $\alpha$ = 0.05. The values obtained were also tested using the ICC to estimate the level of variability with respect to the level of observer training.

**Verification of analysed images**

To calculate the accuracy of the fish measurements, 10% of the digital collection was analysed at random, observing the lines traced and noting any observed discrepancies.

## RESULTS

**Outcomes from consistency trials**

**Orthogonal plane**

The effect of deviation from the orthogonal position in the measuring system revealed significant differences ($P < 0.05$) with respect to the total length (TL) in images of the fish calibrator. It can be seen in Figure 3 that the accuracy of the total length of the fish calibrator varies in function to the magnitude of the separation angle from the perpendicular. The average magnitude of error for a variation of ±5° with respect to the orthogonal plane was 0.5%, with a maximum of 1.1%. For a deviation between -10 and +10° an average error of 1.1% is produced, with a maximum of 3.1%, and if the ichthyometer oscillates between ±15° the average error is 1.7% with maximum error value over 4%.

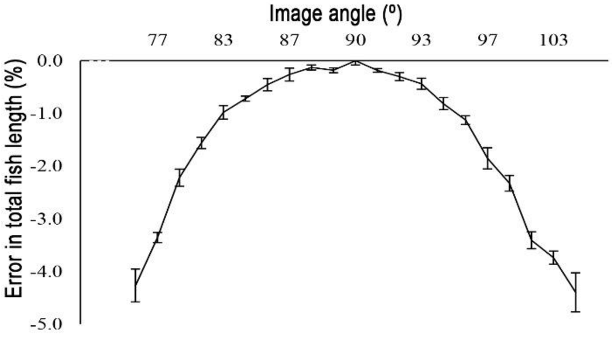


**Figure 3.** Mean error in total length (% and ± SD; line and bars respectively) measured on fish calibrator as an effect of the angle of deviation from the orthogonal measuring system (n = 3).

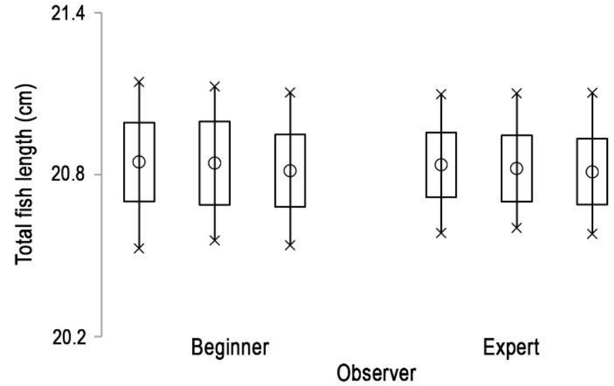


**Figure 4.** Total length (mean, SD and upper & lower limit; circle, box and cross respectively) measured on images of fish by both beginner and experienced observers (n = 15).

### Observer experience

The results of the one-way ANOVA to test the efficiency in expert and beginner observers (3 per group) revealed a similitude of variance ($P > 0.05$), registering an average total length (TL) of 20.82 cm and a standard deviation of 0.12 cm for the expert group, while the beginners calculated an average of 20.83 cm for TL and a standard deviation of 0.15 cm (Fig. 4).

The general intraclass correlation coefficient (ICC) was 0.937 with a confidence interval of 95% ($IC_{95\%}$) from 0.884 and 0.974, and with respect to the level of training, although there is no evidence of significant differences, the beginners group present an ICC = 0.925, less than that of the experts (ICC = 0.971).

Of the six observers, evaluator number 3 of the beginner's group registered the lowest ICC of 0.913 and an $IC_{95\%}$ of 0.810 and 0.967, whereas observer 6 (experts) registered the highest index with an ICC = 0.985 and $IC_{95\%}$ of 0.962 and 0.995.

### *Post-hoc* evaluation of processed images

Of the evaluation of the 228 images of fish used in the measurement of biometric characteristics, 21.1% of the lines traced were under or overestimated by between 1.2 and 3.7 px with respect to the actual edges of the measured characteristic, which represents a discrepancy of between 0.6 and 2 mm.

## DISCUSSION

### Orthogonality on bidimensional plane

Achieving an orthogonal plane (90°), between the measuring system and the fish calibrator results in a minimum level (%) of error, as it can be seen in Figure 3. Therefore, the characteristic being measured tends towards its greatest dimension (actual dimension); in contrast, a diagonal plane from the measuring system (higher or lower than 90°) results in a perspective error, which reduces the size of the object being measured, thus the error of 0.49% indicates a bias of -0.49%. This error increases in proportion to the drift from the orthogonal plane, which coincides with Sidek & Halawani (2010) and Rahim *et al.* (2012), who registered the lowest error in the orthogonal position.

The results showed that a deviation of ±5° from the orthogonal plane (angle between 85 and 95°) generates an average error of -0.49% and maximum error of -1.13%; with a larger divergence of ±10 and ±15° the maximum error increases to -3.41 and -4.73% respectively. Using the drift error for ±5°, the TL of a 20.84 cm fish is reduced on average to 20.74 cm and to a maximum of 20.60 cm. Perhaps these values do not generate significant differences; however, this level of error is not trivial, since according to Goodenough *et al.* (2010) no matter how small the incidence of errors, it is magnified during calculation of biometric indices, and therefore it is important that this error, if present, is minimal (Hamza, 1999; Jawad *et al.*, 2009; Rutjes *et al.*, 2009; Masson *et al.*, 2011; Gaspar *et al.*, 2012; Reis-Neto *et al.*, 2012).

To reduce deviation from the orthogonal plane as a source of error, a cylindrical cone was integrated into the fish calibrator (Fig. 2b); this ensures the position of the orthogonal plane in the measuring system by adjusting the X-Y plane between the camera and the IchthyoJHOL until the circumference of the cylinder become concentric. This simple application achieves a reduction in deviation to less than 3°, which consequently significantly reduces error to an average value of -0.29% and a maximum of -0.41%.

High levels of deviation from the orthogonal plane produce a higher level of error (Fig. 3), which causes bias in the biometric information; therefore, this deviation was a critical variable in the validation of the IchthyoJHOL, a factor controlled by the cylindrical cone. To reduce distortion of the focal plane when measuring fish *in vivo*, Harvey (2003), Costa *et al.* (2006) and Shortis *et al.* (2013) use a stereo-video system, while Chang *et al.* (2010) and Hsieh *et al.* (2011) minimize projection error in photographic images using geometric transformations.

**Variability in observer's measurements**

The similitude obtained between expert and beginner observers with respect to total length of fish (Fig. 4; ANOVA, $P > 0.05$) indicated that the proposed methodology is independent of observer experience, however, the variability registered with beginner observers is higher than that of experts, with an ICC of 0.925 and 0.971 respectively.

This slight variability suggests a statistical similitude in fish length between expert and beginner observers (Fig. 4), which can be attributed both to the reliability of the methodology and the length and linear nature of this variable (fish with TL > 10 cm), as suggested by Goodenough *et al.* (2012) by indicating that linear biometric data generated in digital processes rarely produces a significant variability, and that linear parameter is more precise than curved or angular parameters. The similarity between values obtained by experts and beginners also coincides with Unis *et al.* (2010) by not encountering differences in bone measurements (tibial plateau analysis) between observers with differing levels of experience. In contrast, when the biometric variable is very small or non-linear, it is very common to find significant differences which lead to a certain level of imprecision (Goodenough *et al.*, 2012; Yajing, 2012).

The bias calculated in this experiment, although small, should not be disregarded, and it is necessary to identify the source of error to control its magnitude (Arnqvest & Mårtensson, 1998; Chang *et al*., 2010; Kerekes-Máthé & M. Székely, 2014). In this case, the intraclass correlation and its interval registered for the beginner observers indicate the possibility of reducing the imprecision of the measurements by increasing knowledge of the system through training and experience sharing sessions with other observers, which, in accordance with O'Neal *et al.* (2002) and Chang *et al.* (2010), can increase the level of precision as long as the observer demonstrates a talent for the measurement process. Meanwhile, Yajing (2012) indicates that a clear definition of the biometric characteristic, which is to be measured, reduces the bias among observers, by allowing them to better recognize the edges of the object of interest.

Aptitude and attitude are both important in a dependable observer, the second strongly influences the first and, according to O'Neal *et al.* (2002), it can transcend the methodology, as they demonstrated by measuring foliage characteristics using manual digital equipment with higher precision than using an automated system.

**Improving use of the IchthyoJHOL**

The discrepancy of 0.6 to 2 mm between the magnitude of the actual biometric characteristics and the measurements obtained from 228 images suggests a bias attributable to different levels of understanding of the definition of object length by each observer; they may click on the edges of the object before or after the exact point to be measured (Chang *et al.*, 2010; Yajing, 2012).

To reduce this imprecision, it is proposed that the measurements of the biometric traits of the fish (phase 2) are taken using the following: 1) trained, motivated observers with a clear concept of the biometric traits of interest, 2) a digital analysis program appropriate to observer's ability, but analyzing the generated images through a high-resolution video-projector (1600×1200 px) and if possible with two observers, and 3) a digital analysis software with edge recognition.

**Advantages in the application of the proposed ichthyometer**

Taking into account only the collection of biometric data (phase 2), the proposed methodology for the external biometry of fish becomes a laborious process, as much or even more so than the traditional methodology; however, this time-consuming process is clearly justified in experiments with live fish because of the significant reduction in the time the fish is out of its environment and the corresponding reduction in the effect of stress on the fish, physiological tension which was evaluated through biochemical and behavioral indicators (Portz *et al.*, 2006; Martins *et al.*, 2012; Klima *et al.*, 2013; Upton & Riley, 2013) and which contrast significantly from that seen in organisms subject to the traditional biometric methodology, information which forms part of another publication.

**IchthyoJHOL, a reliable device**

This technological system induces an average integrated error of 3.2% (1-0.9681) when the recommendations are followed. Despite this level of bias, which could be reduced, a similarity can be seen between observers which suggests a substantive precision from the digital measurement process, coinciding with Costa *et al.* (2006), whose error with live fishes, ranges around 2 to 5%. Misimi (2007), using a computerized analysis system to identify and classify salmon on the processing line, with an accuracy of over 87%. Mir *et al.* (2013) also found a high morphometric efficiency in the classification of snow trout (90.6%) by discriminant analysis. But contrasts with White *et al.* (2006) upon measuring and identifying different species of flounder (TL ± 400 mm) and obtaining a high level of precision (SD = 1.2 mm). Also, Abdulah *et al.* (2009) achieved an accuracy of 99.8% in fish length measurements. This demonstrates that the biometric measurements obtained using digital methods are as or more precise than those registered manually.

## CONCLUSIONS

The use of the IchthyoJHOL represents an efficient alternative for measuring fusiform fish species; it reduces damage due to handling, it reduces exposure time of the fish (time out of its environment) and it maintains the fish in a posture similar to that when swimming, as well as reducing the level of stress.

The use of the proposed methodology generates a fish measuring system with accessible equipment, a simple calibration system and an accuracy of 96.8%, equal or superior to that of the traditional system.

With the proposed model, the most time-consuming aspect is the processing of data, which does not affect the fish, and the time invested is similar to that of microscopic analysis of bacteria or plankton.

The error caused by using beginner observers can be effectively reduced by training, motivation, and auto-assessment between observers; also by using a video-projector or digital analysis software with edge recognition.

With the proposed method, instrument associated error can be reduced by developing the geometric correction process and the analysis of measurements in an automated form.

The images of the fish, both new and processed, can be stored for posterior re-evaluation, redescription or a more extensive morphometric analysis.

The proposed method facilitates the analysis of the images in a different place and time to where the images were taken, allowing them to be taken in laboratory A and processed in laboratory B.

This instrument with appropriate variants could be used for the capture of morphometric traits in other live organisms, such as birds and reptiles.

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## ETHIC DECLARATION

We should mention that all specimens were obtained by ourselves in the aquaculture lab “A-Mena” at Colima University. We provided food at least twice per day. No diseases were observed. All the fishes were measured after anaesthetization and the experiments were conducted according to “Directive 2010/63/EU” and the “Guidelines published by the Ichthyological Society of Japan”, on the welfare of animals used for scientific purposes.

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