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Comparison of manual measurements and computer-assisted image analysis in fish morphometry

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Abstract: Fish usually have a simple body shape and many morphological characters can be easily obtained by 2-dimensional image analysis. Measuring errors are often analyzed using data from several researchers or repeated measurements, but the testing of accuracy and repeatability based on different methods has rarely been examined. Here we report the comparison of manual measurements using Proma 150D digital calipers and computer-assisted image analysis in LUCIA software to evaluate the accuracy of both methods and the suitability of digital images for exploration of fish morphology. Eighteen morphometric characters of 20 specimens of Prussian carp were examined by 2 researchers. The mean of the relative bias within and between researchers separately for the manual and computer-assisted methods (expressed as a percentage of a character's value) varied from –4.27% to 9.48%. Comparison of interresearcher results using digital caliper, interresearcher results using LUCIA, intraresearcher results using digital caliper, and the 2 methods using manual caliper and LUCIA revealed significant differences in several characters. Only testing of intraresearcher relative bias using LUCIA showed no significant difference.

Key words: Variation, digital caliper, ichthyology, software LUCIA, Prussian carp

1. Introduction

External morphological measurement is still one of the main research approaches used in ichthyology and aquaculture, most likely due to its long tradition and simplicity. In many cases, it is a sufficient method for many the purposes for which it is required. Biometrical characters are usually used for comparison of different fish species or for describing variability within the species among different populations, breeds, or lineages. Meristic characters are defined exactly, while morphometric characters are (due to their high correlation with size) expressed generally in indexes (log, ln, or %). The most common index (%) is usually given in relation to body or head length. The raw data are mostly obtained by caliper, measuring board, graph paper, ruler, etc. (1–4). New instruments were also implemented in ichthyological and aquaculture research with the intention of making measurements easier and more accurate. Ovredal and Totland (5) described a device named "FishMeter", and digital calipers with direct computer connection are well known. Utilization of these types of instruments helps to make measurements more precise and less loaded with errors that commonly arise when raw data are rewritten to paper.

In many scientific studies, different authors compared their morphological analysis in the same species to each other, especially in cases where the species has wide geographic range of distribution and high morphological variability can be assumed (6). Accordingly, proper methodology, repeatability, and accuracy are fundamental.

The problem that still persists in conventional morphometry is the effect of the researcher who collects the data. This impact of human interaction on accuracy cannot be eliminated easily. As shown by Mazurová et al. (7), exact definition of each character is supposed to be a crucial factor for quality of measurements and repeatability. Moreover, manual measurements of fixated fish material are loaded with significant errors due to different pressures transmitted to soft tissue by calipers, causing deformation (5). The quality of measurements is also affected by the effect of fixative medium (8–11). Preservation techniques likely have the most profound effect on fishes, which may cause considerable shrinkage of the specimens (12,13).

Fish usually have a more simple body shape in contrast to some other vertebrates and many morphological characters can be easily obtained by 2-dimensional image

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analysis. Photos of fish are today commonly used in methods of geometric morphometrics. Although they are based on a completely different method of collecting data from the object than conventional morphology (14–16), they prove that the usage of an image could be a relevant source of morphological information.

Several works that deal with measurement error of researchers or with accuracy of obtaining metric characters already exist (6,17–21), but the comparison of manual measurement and computer-assisted measurement from digital images is lacking.

In this article, we question whether it is possible to make reliable conventional morphological measurements of fish using single standardized digital images and common laboratory software. We compare traditional manual measurements of fish using digital calipers with measurements done on digital images of the same specimens. Comparison of accuracy and repeatability, benefits, and disadvantages are explored and discussed.

2. Materials and methods

Twenty individuals of Prussian carp (*Carassius gibelio* Bloch, 1782) were chosen as model objects for the presented study. The species was chosen as a common wide-spread cyprinid with a typical fish body shape. All studied specimens were collected at several localities in the Czech Republic and one in Turkey. Fish were preserved for 3 months in 4% formaldehyde and stored in the fish collection of the Department of Zoology and Fisheries, Czech University of Life Sciences Prague. According to the schema of Holčík (1), 18 morphometric characters of each fish were measured as shown in the Figure: standard length (SL), head length (lCA), preorbital length (prO), postorbital length (poO), eye diameter (dO), body depth (aCO), caudal peduncle length (lPC), depth of caudal peduncle (aPC), minimum body depth (minPC), predorsal length (pD), preventral length (pV), preanal length (pA), P-V distance (P-V), P-A distance (P-A), dorsal fin base (LD), anal fin base (lA), pectoral fin length (lP), and ventral fin length (lV).

All specimens were manually measured twice by one researcher to evaluate intraresearcher differences and additionally once by a second researcher to evaluate interresearcher differences. Manual measurement of SL was done by measuring board. All other characters were measured with a Proma 150D digital caliper and recorded to the nearest 0.1 mm.

As the second method, we used semiautomated measurement of digital images of the specimens with image analysis system LUCIA for PC (Laboratory Imaging s.r.o.). Fish with straightened bodies and erected fins were photographed using a Fuji FinePix S7000 digital camera. Monotone bright plastic sheets were used as background. The digital camera was fixed on a tripod and the midsagittal body plane was perpendicular to the image plane as much as possible. Distance of the camera from

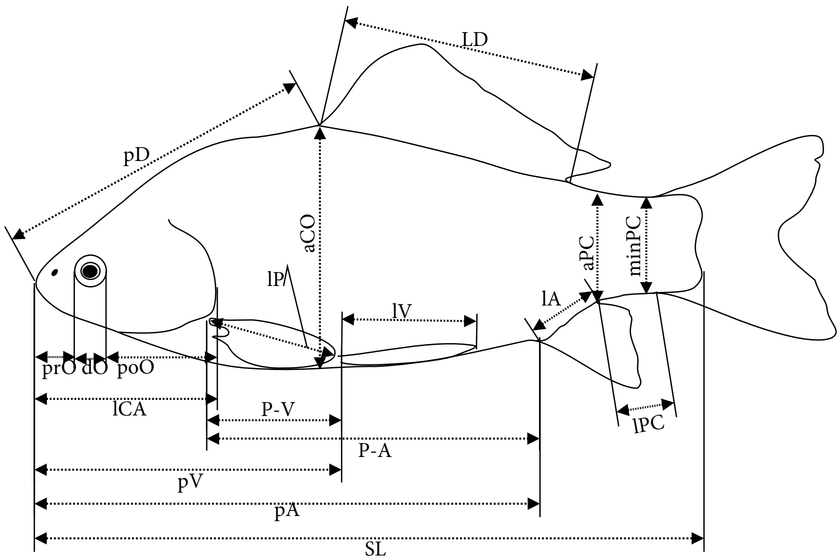

Figure. Schema of morphological characteristics measured in this study: SL = standard length, lCA = head length, prO = preorbital length, poO = postorbital length, dO = eye diameter, aCO = body depth, lPC = caudal peduncle length, aPC = depth of caudal peduncle, minPC = minimum body depth, pD = predorsal length, pV = preventral length, pA = preanal length, P-V = P-V distance, P-A = P-A distance, LD = dorsal fin base, lA = anal fin base, lP = pectoral fin length, lV = ventral fin length. Adapted from Holčík (1).

the object was the same for all specimens. The camera was set to semiautomatic aperture priority mode (f4) with sufficient depth of field. An artificial light source with uniform intensity was used for lightening the scene.

Digital images were processed using LUCIA (ver. 4.21). The program is designed for obtaining, archiving, and analyzing digital images. The program supports usage of macros, and thus we created and used a simple script allowing semiautomatization of all measurements, available at http://home.czu.cz/en/petrtyl/research/.

Since we tested the differences between 2 methods of measurement with mixed units, all measured characters were recalculated to indexes of body length (% SL). Regarding possible body asymmetry, only the left side of all specimens' bodies was examined by both methods.

There was a 1-week interval among all repeated measurements for both researchers as well as for repeated measurements of the first researcher. Within the process, the time required for measurement of each specimen was recorded and evaluated separately for both approaches.

Basic descriptive statistics (minimum, maximum, mean, and standard deviation) were calculated for all measurements. Intraresearcher and interresearcher biases (differences) in measurements were calculated and values were checked if the mean bias differed significantly from zero using the one-sample t-test. Biases were expressed as relative values in % of measured character. Differences between methods were tested by paired-samples t-test. Averaged values of the repeated measurements of the first researcher were used for testing of differences between methods. All analyses were processed using Statistica ver. 9.1 (22).

3. Results

The SL of 20 specimens ranged from 92 to 216 mm. Descriptive statistics and variation of pooled data (from both researchers and both methods) for each character (expressed as indexes % of SL) are shown in Table 1.

Basic statistics for relative biases (expressed as a percentage of the character's value) within and between researchers are provided in Table 2, separately for the manual (caliper) and computer-assisted (LUCIA) methods. The mean of the relative bias for interresearcher comparisons varied between –3.53% and 4.84% and –4.27% and 9.48% for caliper and LUCIA methods, respectively. The mean of the relative bias for intraresearcher comparisons varied between –1.28% and 3.21% and –2.97% and 0.86% for caliper and LUCIA measurements, respectively. Intraresearcher biases were generally smaller, although some extreme values (–74%, 31%) appeared for dO measurements.

The one-sample t-test indicated in interresearcher comparisons 7 and 6 characters with significant relative biases as measured by caliper and LUCIA, respectively. Intraresearcher comparisons contained 6 and 0 characters with significant relative biases as measured by caliper and LUCIA, respectively. For details, see the last columns of Table 2.

Table 1. Basic statistics of measurements of morphological characteristics of 20 specimens (*Carassius gibelio*) used in this study. All characteristics are expressed as indexes (% of SL).

Character	Min	Max	Mean	SD
lCA	23.67	35.56	28.28	2.16
aCO	33.12	43.76	38.80	2.70
pD	46.86	55.67	51.36	1.79
pA	72.79	83.03	76.69	1.89
pV	38.80	53.59	47.54	2.14
P-V	18.05	26.02	20.99	1.56
P-A	43.18	56.40	50.36	2.36
minPC	13.38	16.82	14.77	0.67
aPC	14.60	19.79	17.24	1.02
LD	31.59	42.27	37.18	1.85
lA	7.44	13.13	10.02	1.00
lV	16.99	24.48	21.16	1.52
lP	17.77	22.86	19.77	1.01
prO	3.96	9.40	6.97	1.52
poO	14.36	19.46	15.86	0.93
dO	3.78	8.06	5.92	0.81

Testing of intermethod variability by the same researcher, including manual measurement by digital caliper and measurement by LUCIA, revealed that measurements of 10 compared characters differed significantly. Basic descriptive statistics and significance for each character are given in Table 3.

Characters for which we generally found the lowest number of statistically significant differences were pD, pA, pV, P-V, P-A, minPC, prO, and poO for both comparison of measurements made by 1 researcher repeatedly and comparison of measurements of 2 researchers independently for the chosen method

On the other hand, characters with the highest amount of differences within our tests were lCA, aCO, LD, lV, and lP.

Average time of measurements for a specimen manually using digital caliper was 240 s, whereas when using LUCIA it was 105 s. We only considered the time of measuring itself; we did not include time necessary for preparing and manipulating fish or taking photos.

4. Discussion

Although measured morphological characters are precisely determined (1), delimitating characters on fish during

Table 2. Summary statistics for relative biases for intraresearcher and interresearcher results of measuring morphological characteristics. Biases are expressed as percent of trait's value. Positive bias indicates larger measurement value by the first researcher vs. the second researcher or in the first measurement vs. the repeated measurement by the same researcher. P is the probability of the null-hypothesis that the mean bias does not differ from zero. Characteristics with significant values (**P < 0.05**) are in bold.

Character	Interresearcher caliper					Interresearcher LUCIA				
	Min	Max	Mean	SD	P	Min	Max	Mean	SD	P
lCA%	**–1.15**	**7.36**	**1.62**	**1.91**	**0.00**	–8.49	13.38	–0.14	4.87	0.90
aCO%	**–1.24**	**2.12**	**0.48**	**0.92**	**0.03**	–11.12	9.13	–0.37	5.11	0.75
pD%	–4.57	2.22	–0.22	1.74	0.58	**–3.13**	**1.70**	**–0.71**	**1.42**	**0.04**
pA%	–6.15	1.81	–0.20	1.69	0.61	–3.11	2.32	–0.60	1.56	0.10
pV%	–5.37	6.88	0.38	2.76	0.54	**–8.99**	**2.17**	**–2.09**	**3.21**	**0.01**
P-V%	–12.60	4.46	–0.34	4.07	0.71	**–21.50**	**3.54**	**–4.27**	**5.98**	**0.00**
P-A%	–7.90	9.16	0.41	2.88	0.54	–7.27	5.57	–0.57	3.27	0.44
minPC%	–5.06	4.67	0.35	2.32	0.51	–3.46	5.72	0.57	2.67	0.35
aPC%	**–0.94**	**18.54**	**4.84**	**5.29**	**0.00**	–7.67	11.18	1.44	5.29	0.24
LD%	**–1.92**	**4.72**	**1.45**	**1.57**	**0.00**	–11.32	7.67	–1.83	5.68	0.17
lA%	**–13.52**	**5.30**	**–3.53**	**4.63**	**0.00**	**–17.66**	**21.70**	**5.74**	**10.62**	**0.03**
lV%	**–1.48**	**3.43**	**0.91**	**1.32**	**0.01**	**–4.18**	**20.15**	**9.48**	**5.98**	**0.00**
lP%	**–0.91**	**6.53**	**1.63**	**2.14**	**0.00**	**–6.83**	**11.10**	**4.28**	**5.19**	**0.00**
prO%	–6.27	10.59	1.38	3.27	0.07	–20.91	15.03	–1.49	8.39	0.44
poO%	–3.81	2.65	0.12	1.75	0.76	–10.34	12.48	0.39	4.78	0.72
dO%	–7.86	8.76	1.36	3.87	0.13	–26.67	25.73	–0.50	13.41	0.87

Character	Intraresearcher caliper					Intraresearcher LUCIA				
	Min	Max	Mean	SD	P	Min	Max	Mean	SD	P
lCA%	**–1.71**	**15.00**	**1.59**	**3.39**	**0.05**	–25.16	25.62	–0.52	10.32	0.83
aCO%	**–0.70**	**4.07**	**1.01**	**1.31**	**0.00**	–20.29	15.14	–1.52	9.40	0.48
pD%	–7.54	2.02	–0.30	2.12	0.53	–10.67	3.92	–1.19	3.71	0.17
pA%	–2.87	4.70	0.21	1.56	0.55	–7.54	3.00	–0.90	2.63	0.14
pV%	–1.28	18.15	1.59	4.41	0.12	–9.42	9.34	–0.79	5.16	0.50
P-V%	–3.08	5.32	0.99	2.20	0.06	–19.86	10.28	–1.36	7.34	0.42
P-A%	–7.45	3.28	0.39	2.15	0.42	–19.91	6.66	–0.88	5.87	0.51
minPC%	–5.12	13.58	0.74	4.01	0.42	–8.79	12.15	–0.19	4.90	0.86
aPC%	–19.87	3.85	–1.28	5.79	0.34	–19.56	13.48	–2.97	8.30	0.13
LD%	**–4.46**	**6.88**	**1.59**	**2.36**	**0.01**	–14.45	9.88	–0.54	6.11	0.70
lA%	–6.41	7.89	0.68	4.24	0.48	–24.31	26.06	0.86	16.17	0.82
lV%	**–1.42**	**3.98**	**1.29**	**1.54**	**0.00**	–13.76	13.94	–1.94	7.57	0.27
lP%	**–3.71**	**5.74**	**1.09**	**2.13**	**0.03**	–18.90	10.24	–2.58	7.49	0.14
prO%	–7.85	9.91	–1.69	4.06	0.08	–42.48	26.04	–1.88	17.13	0.63
poO%	–5.25	5.03	0.58	2.56	0.32	–26.57	18.17	–1.35	8.39	0.48
dO%	**–5.22**	**9.77**	**3.21**	**4.19**	**0.00**	–74.45	31.10	–2.53	26.79	0.68

Table 3. Summary statistics for values of morphological characteristics separately for 2 measurement devices (caliper vs. LUCIA). P in the last column represents the probability of significant difference between devices based on t-test. Characteristics with significant values (**P < 0.05**) are in bold.

Character	Digital caliper				LUCIA				
	Min	Max	Mean	SD	Min	Max	Mean	SD	P
lCA	**27.35**	**34.00**	**29.47**	**1.65**	**23.99**	**30.46**	**27.23**	**1.64**	**<0.01**
aCO	33.77	43.31	38.97	2.83	34.89	41.78	38.64	1.98	0.4
pD	**48.05**	**52.49**	**50.32**	**1.46**	**49.63**	**54.15**	**52.25**	**1.23**	**<0.01**
pA	**73.23**	**77.99**	**75.68**	**1.42**	**75.49**	**80.11**	**77.50**	**1.20**	**<0.01**
pV	**43.10**	**49.61**	**46.82**	**1.73**	**45.13**	**50.33**	**47.99**	**1.39**	**0.02**
P-V	**18.13**	**21.85**	**20.14**	**1.20**	**19.84**	**23.18**	**21.52**	**0.86**	**<0.01**
P-A	**44.15**	**52.52**	**49.44**	**2.6**	**47.71**	**53.95**	**51.24**	**1.43**	**<0.01**
minPC	**14.28**	**16.33**	**15.7**	**0.61**	**13.70**	**15.40**	**14.52**	**0.46**	**<0.01**
aPC	15.80	19.14	17.34	0.94	16.44	18.69	17.52	0.63	0.32
lD	32.39	39.12	36.97	1.69	34.96	40.13	37.36	1.24	0.19
lA	8.95	11.33	9.89	0.68	9.13	11.68	10.23	0.66	0.08
lV	**19.46**	**24.39**	**21.89**	**1.9**	**19.42**	**22.79**	**21.17**	**0.89**	**0.02**
lP	18.1	22.27	19.85	0.93	19.5	21.20	20.10	0.65	0.3
prO	**7.80**	**9.21**	**8.42**	**0.36**	**4.22**	**6.47**	**5.54**	**0.54**	**<0.01**
poO	**14.39**	**18.34**	**15.65**	**0.85**	**14.96**	**17.41**	**16.10**	**0.64**	**0.03**
dO	4.92	7.74	6.06	0.75	4.75	6.87	5.80	0.55	0.1

measuring is always in the hand of the researcher. Even if experienced researchers follow standard methodology, subjective influence on the measurement due to slightly different personal habits cannot be excluded. This implies that repeated measurements by 1 researcher on identical material should grant more aligned data in comparison to measurements gained from identical material by 2 or more researchers (6,7,18).

In contrast with other studies, we chose nonprofessional morphologists as researchers since we wanted to test both methods (manual measurement and the LUCIA system) without impact of personal experience. This probably led to bigger biases and differences obtained among several manual measurements as compared to, for example, the study of Roitberg et al. (6). The mean of relative biases ranged from –4.27% to 9.48% (Table 2). However, as seen above, we obtained very similar general findings as authors cited within our study in conventional manual measurement analyses.

The results also clearly indicate that measurements are influenced not only by different researchers but also by chosen instruments and methods. According to the collected data, we conclude that conventional manual measurement with calipers is performed with a higher probability of significant errors and biases due to different approaches to measurement processes among different researchers, although each method (i.e. use of calipers or digital images) is connected with a specific error compared to true values. High variance of data obtained by manual measuring allows us to argue that this approach has lower accuracy and repeatability and requires more experience, which is difficult to evaluate. When we compare repeated measurements made by the same researcher with calipers, statistically significant differences were observed in some characters. Measurements made with LUCIA showed no significant differences (Table 2).

All measurements by LUCIA were done repeatedly on one identical set of photographs. It can be assumed that there may be more differences found between measurements when digital photos of the same specimen are obtained by various photographing approaches. To eliminate this possible error, standard uniform protocol for photographing the material must be employed.

Semiautomated workflow in LUCIA by using macros accelerates the process of measurements by automatic recalculation of real measurements in pixel count to percentage. When there is a need to measure in real values, recalculation is done by software using the calibration scale that is present on each picture. The skipping of the handwriting phase of obtained data due to simultaneous automatic logging by computer eliminates possible errors and leads to improvement of time efficiency. Advantages of computer-assisted measurements were also reported by several other authors (2,3,23,24).

The obvious disadvantage of morphological measurements made from digital pictures is 2-dimensionality. Not all morphological characters can be evaluated from one image (e.g., interorbital distance, body widths). To overcome this disadvantage, a second photograph with perpendicular to longitudinal body-axis orientation is necessary. However, there is usually no need to obtain all morphological characters for sufficient morphological comparison (25–29).

Another aspect of converting an object from its natural 3D state to a 2D digital photo is that some characters are distorted due to reduction of a dimension based on that fact that some characters (e.g., preorbital distance) are shorter in photos than on the real specimen. The more complex the shape of a studied specimen is, the more biased the data are (with reference to the real object) when observed from a 2D image.

Our results show that in some characters there is one-way value shift. When using LUCIA, the lCA, minPC, lV, prO, and dO values are always smaller than in the case of caliper measurements. However, the referred influence of transformation from 2D to 3D is always the same when using standardized photographing and does not affect the comparison of obtained values.

On the other hand, there are characters that always give higher values when measured by LUCIA: pD, pA, pV, P-V, P-A, LD, lA, lP, and poO. This can be explained by distortion related to different pressure of tips of the caliper to scales or soft tissue around bones, mainly of the head. When the specimen is measured from a digital picture, there is no such distortion and the gained values are larger and less variable.

Although we did not evaluate the time needed for photographing or manipulating fish, since those activities can vary considerably, net time collection of values is clearly in favor of computer-assisted image analysis

In conclusion, the most important benefit of using digital photos in fish morphology is the availability. Collections of digital photos can be posted online (secured if needed) and serve as easily accessed and sharable archives for all interested scientists independent of their locations. The protection of the original valuable material from possible unwanted damage or even loss is also of significant value. Finally, digital photos offer lower stress for live fish as compared to overall manual measurement.

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