



Shaping up? A direct comparison between 2D and low-cost 3D shape analysis using African cichlid mandibles

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Received: 19 October 2018 / Accepted: 24 March 2019 / Published online: 16 May 2019
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Abstract Shape is a complex trait which can be investigated through a variety of methods that have been developed over the past century. Currently, ecologists and evolutionary biologists employ the use of geometric morphometrics on 2D images as their standard approach. Recently, there has been increased interest in the use of 3D methods. However, while low-cost 3D methods of data collection are becoming available their potential benefits are often more implied rather than quantified. Using the mandibles from two species of African cichlids (*Maylandia zebra* and *Tropheops* “Red Cheek”), this study aimed to evaluate the use of a low-cost 3D method of shape capture versus a range of 2D data sets (termed ‘standard’, ‘even’, and ‘extended’). Our findings indicated that while both 2D and 3D methods could discriminate differences in species and sexes there was only a slight improvement using 3D when landmark datasets were held even. Further, the standard approaches to data collection that would be taken by most researchers clearly outperformed our 3D approach. Therefore, as 3D methods become more accessible researchers should consider a cost/benefit ratio in terms of the time required to obtain 3D data versus shape information gained.

Keywords Statistical power · 3D Morphometrics

Introduction

The study of shape has been integral to biology for well over a century (Thompson 1917). Shape has been important for informing us about the changes involved with evolutionary change and adaptation at both micro and macro evolutionary scales (Adams et al. 2013). However, biological shape remains an especially challenging trait to measure because it is multidimensional and multivariate by nature. Shape was initially described using qualitative methods (i.e. the simple drawing and description of morphological structures) and advanced to include quantitative methods involving the analysis of multiple variables, often collections of linear distances (Parsons et al. 2003). These approaches were greatly enhanced by the methods of geometric morphometrics which has matured over the past fifteen years (Adams et al. 2004; Adams et al. 2013). These methods brought the conceptions of geometric shape changes proposed by D’Arcy Thompson (1917) to empirical reality and have become standard in the field.

Geometric morphometrics (hereafter GM) resolved many of the limitations presented by the use of collections of linear distances to quantify shape (i.e. ‘traditional morphometrics’). For example, linear distances were often highly correlated with body size, a problem dealt with by a range of ‘size correction’ techniques. However, no standard technique was ever adopted, and results were prone to vary across size correction approaches (Parsons et al. 2003). GM has largely resolved this issue by providing the ability to isolate size from shape, and by adopting the standard use of a more

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holistic measure of size (i.e. geometric centroid size). Further, GM provided a more anatomically consistent measure of shape by its reliance on homologous regions across specimens (Parsons et al. 2003; Adams et al. 2004) and with variance in shape reported relative to other structures (Rohlf and Marcus 1993).

Currently, the use of two dimensional (2D) approaches are standard for GM studies but opportunities for three dimensional (3D) studies are now increasing due to the greater availability of low-cost 3D scanning equipment. Specifically, these include laser and structured light scanners, photogrammetry, and alternative methods which do not require any specialized equipment such as the procedures used by Stereomorph package for R (Olsen and Westneat 2015). However, in terms of GM it has not been made empirically clear what advantages 3D can offer (see Jamniczky et al. 2015; Navarro and Maga 2016). It has been suggested that 2D approaches may be less able to capture shape variation, indeed, there is debate over why 2D morphometrics is the standard methodology as most biological structures are three dimensional (Cardini 2014).

If 2D approaches miss biologically important variation, they may also limit interpretations that can be made. For example, in a study on Oligocottinae fish head shape, 2D and 3D data collection was compared, and although both methods captured general trends of the shape variation, the 3D method showed a relationship between mouth and prey size which was not highlighted by the 2D data (Buser et al. 2017). On the other hand, while whole morphological phenotypes (e.g. the skull) can be captured by 3D, it may not necessarily improve the ability to detect the most salient biological variation (Navarro and Maga 2016). Instead, additional information from 3D quantification may be largely redundant. This raises a key question of whether the benefits of 3D are marginal or substantial compared to 2D, and in turn whether the effort and costs needed to collect and process 3D data are worthwhile.

Here, we aimed to address these issues through an objective comparison of 2D acquisition methods against a low-cost approach toward the collection of 3D shape variation (i.e. structured light). Although much more sophisticated methods are available for 3D data collection, they may still be inaccessible to some researchers, therefore our aim was to test the performance of this type of 3D scanning as its cost now makes it widely available to many labs currently conducting 2D shape acquisition. We focused on shape variation in the

mandible from two species of African cichlids belonging to the exemplar adaptive radiation of Lake Malawi and for which existing 2D studies already show shape differences (Cooper et al. 2010; Parsons et al. 2011a). The mandible is also an especially important structure for determining feeding strategies and ecology in cichlids, other fishes, and wider vertebrates (Parsons and Albertson 2009; Parsons et al. 2011b). Therefore, these data and our approaches are widely relevant to those interested in evolution and adaptation (see Foster et al. 2007; Aguirre and Akinpelu 2010; Parsons et al. 2010; Sherratt et al. 2014; Dollion et al. 2015). Because of the inherent ability to account for an additional dimension, we predicted that a 3D approach would yield more statistical power to discern known groups (species, sex) within our mandible data than a comparable 2D approach. Further, we also predicted that interpretations from 3D morphometric approaches would enhance our understanding of adaptation by providing a broader picture of how variation changes among groups.

Methods and materials

The cichlid mandible as a model for comparing 2D and 3D shape

We focused on assessing shape variation in the mandible of two species namely *Tropheops* “Red Cheek” (TRC) and *Maylandia zebra* (MZ). Both species are rock-dwelling mbuna with MZ considered a generalist for its ability to both actively suction feed on zooplankton and forage on algae through biting/scraping on the surfaces of rocks (Ribbink et al. 1983). Relative to other rock-dwelling mbuna it possesses a long, thin and gracile mandible (Albertson et al. 2005). Contrasting this, TRC has a relatively short and narrow mandible and feeds by grazing on strands of algae through a ‘plucking’ motion (Parsons and Albertson 2009; Parsons et al. 2015). Therefore, the jaws of these species differ in both width, depth, and length; characteristics which allowed us to objectively assess the abilities and limitations of 2D and 3D methods using the same individuals.

We quantified shape in ways that would facilitate objective and conventional comparisons between 2D and 3D methods. The adult mandibles from a total of 18 female and 18 male MZ ($n = 36$) along with 13 female and 13 male TRC ($n = 26$) were used for all

methods; fish were a mixture of wild-caught and first-generation lab-reared. All animals were sacrificed with an overdose of benzocaine following UK Home Office guidelines. To prepare the mandibles for imaging, excess flesh was manually removed, and mandibles were then left to dry for 48 h to reduce glare (a potential problem for our structured light 3D scanning procedure).

2D imaging and shape quantification

For 2D imaging we photographed each mandible on both the left and right side using a dissecting microscope (Leica M165, Leica, Wetzlar, Germany) mounted with a digital camera (Leica DFC450 C, Leica, Wetzlar, Germany). Each mandible was placed in the field of view of the microscope with the lateral view held perpendicular to the lens using plasticine. To account for size variation, a scale bar was added to each image using the LAS application suite version 4.4 (Leica, Leica Camera AG, Wetzlar, Germany).

To assess shape variation 2D landmarks were collected across images using the TPS suite of software (available at <http://life.bio.sunysb.edu/ee/rohlf/software.html>). Using tpsUtil64, the images were linked into a single '.tps' file which was opened in tpsDig2 for landmarking. We used three approaches toward collecting 2D landmark data to allow for comparisons to take place. Hereafter we refer to these approaches as 'even', 'standard' and 'extended' data sets. First, to facilitate an objective comparison between methods, we only selected landmarks that were similarly obtainable across 2D and 3D methods; this was the 'even' 2D method and consisted of 4 homologous landmarks collected on the left side of the mandible (Fig. 1). To represent a 'standard' 2D morphometrics approach for cichlid mandibles (see Parsons et al. 2011a) we collected 8 homologous landmarks also on the left side (Fig. 1). For both approaches, landmarks were selected from functionally relevant regions of the mandible following previous studies (Albertson and Kocher 2001; Parsons et al. 2011a).

To facilitate a more comprehensive assessment of 2D shape that partially emulated 3D data, we 'extended' our data in an approach that combined landmark data from the left and right side (Fig. 1). Data analysis for this approach was achieved through the separate subset method proposed by Adams (1999) whereby shape variables for each side are combined for multivariate

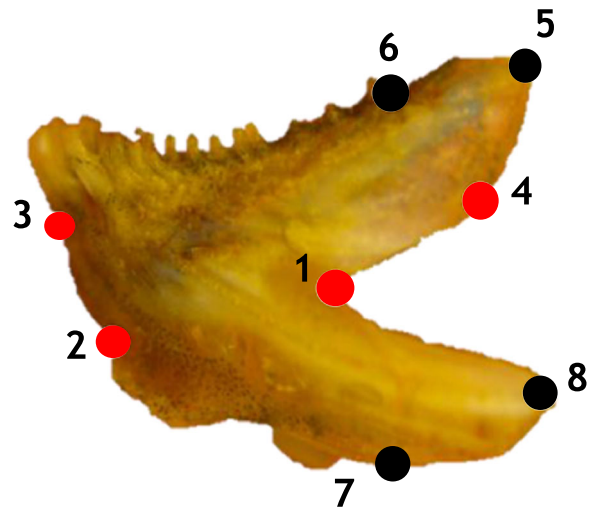


Fig. 1 The homologous landmarks placed on each of the 2D models in tpsDig2. The left side of the jaw is shown. The even 2D approach used the landmarks shown in red whereas the standard 2D used both the red and black landmarks. The landmarks correspond to the following anatomical locations (Barel et al. 1976; Albertson and Kocher 2001; Parsons et al. 2011a): (1) region where anterior of the coronoid process and articular web converge; (2) ventral midline; (3) midline at the anterior edge of the dentigerous (tooth bearing) region; (4) posterior region of the coronoid process; (5) posterior tip of the coronoid process; (6) posterior edge of the dentigerous (tooth bearing) region; (7) mandibular lateral line foramina; (8) ventral region of the obturated foramen

analysis. While this approach goes beyond what most 2D studies would conduct, this approach provided a way of adding another dimension to the 2D data (comparable to the 3D method which inherently provides data from both sides of the mandible).

3D model creation and shape quantification

To capture 3D morphology, we used a DAVID Laser Scanner system and associated software (version 4) set in structured light mode (DAVID Vision Systems GMBH, Koblenz, Germany). This low-cost system consisted of an LED projector (for projection of structured light), tripod, web camera, and a 3D calibration board along with the specialized DAVID software for image processing and model construction. Because of the small size of the mandibles (approximately 1 cm wide) we augmented the LED projector by adding a 43 mm +2 magnification lens to sharpen the focus of projected light. To standardise imaging, a darkened room was used for all specimens and calibration for size, brightness, and focal setting performed with the DAVID software. This involved imaging a calibration

board with a set of high contrast fixed pattern of circles with known pairwise distances and using the ‘Calibration’ option within the software. Once calibrated, a turntable for the specimens was placed in the field of view.

Mandibles were placed on the turntable for imaging towards the creation of a 3D model. However, to reduce glare each was sprayed with a matte white powder prior. The ‘Scan’ function in DAVID was selected to initiate the projections of sets structured lines onto the mandibles that were captured by the camera. After each image, the mandible was then manually rotated between 25 and 45° to capture the shape from a different perspective; we conducted around 8–14 scans per mandible (larger mandibles required more scans). The ‘Cleaning Tool’ within the DAVID software was then used to remove the turntable and scanning artefacts from the images. The size of our samples (1–1.5 cm across each plane) pushed the lower detection limits of our scanner making it necessary to align images manually in the DAVID software. Finally, the ‘Global Fine Registration’ mode was used to finalise the smoothing and alignment of the images to create the 3D model which was then saved as an ‘object file’ (i.e. obj format).

To collect landmark data from each 3D model, the following steps were performed. First, MeshLab (available at <http://meshlab.sourceforge.net/>) was used to convert model file formats from ‘obj’ to ‘ply’. Second, Landmark Editor (<http://graphics.idav.ucdavis.edu/research/EvoMorph>) was then used to open the ply file where six homologous landmarks were placed on each model (Fig. 2). As the finer details of the surface were not picked up by this scanner (as would be the case with more expensive μ CT scanning), only six landmarks were possible; these captured the midline and width of the mandible and were selected based on previous cichlid mandible morphometric studies (Albertson and Kocher 2001; Parsons et al. 2011a). 3D data was processed using the IMP suite of software (in the IMP suite of software (all software available at: www3.canisius.edu/~sheets/IMP%208.htm)) (Zelditch et al. 2012). Landmark data was exported as pts. (points) files and converted to a tps file using the ‘pts to tps convertor tool’ in Simple3D8. Finally, tps files were opened in Coordgen8 and converted to the x1y1z1c1c format required for our next steps.

Shape analysis

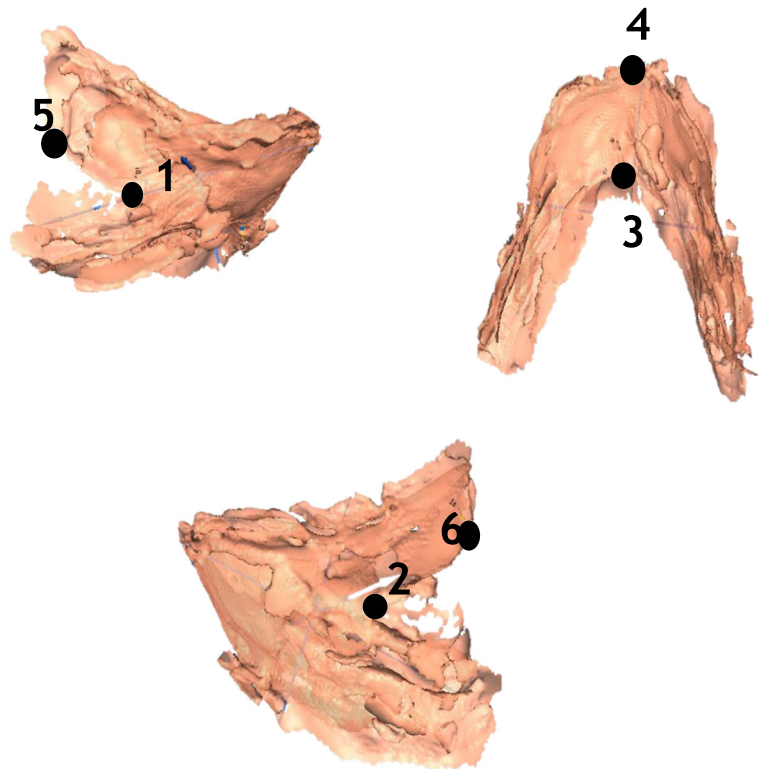
Both 2D and 3D landmark data sets provided a quantified representation of shape variation. However, data collection can create artefacts due to variation in size and orientation. Therefore, we performed a Generalised Procrustes Analysis (GPA) on all landmark datasets to translate, rotate, and scale the data for size to minimize the sum of squared distances among landmark sets (Adams et al. 2013). However, GPA does not take account of the relationship between size and shape (i.e. allometry). Therefore, we tested whether allometric effects differed amongst species. While it is common practise to minimize allometric effects in morphometric studies, there is an emerging view that this can also mask biologically important variation, and indeed a multivariate regression to remove allometry assumes allometric slopes are the parallel between groups (Klingenberg 2016). Allometric variation was tested in the R geomorph package (Adams et al. 2017) and showed that allometric slopes differed in the standard and extended 2D approaches. Allometric slopes between species did not differ in the 3D or ‘even’ 2D methods but did account for a substantial 15% of the variation. Therefore, we retained allometric variation in all of our datasets for further analyses.

Following these procedures, we applied a thin-plate spline transformation to our coordinates to derive partial warps (and their associated scores) using PCAGen (2D) and 3DPCA (3D) and to quantify the ‘bending energy’ needed for the consensus form to become the shape of a given individual. These partial warp scores provided the raw data for our statistical analysis. While it is conventional to remove the asymmetrical component of shape variation in 3D data sets when it is not the focus of the study (Klingenberg 2015) we retained this shape variation to facilitate comparison with the ‘extended 2D’ approach. Our view was that the different correction methods needed for these methods (and their dissimilar efficiencies) may artificially inflate variation between data sets.

Statistical analysis

Among multivariate methods used by biologists to summarize patterns of shape variation principal components analysis (PCA) is the most widely used. PCA can simplify complex data by combining covarying variables into new synthetic variables which are orthogonal to

Fig. 2 The six homologous landmarks placed on each of the 3D models in Landmark Editor. The landmarks correspond to the following anatomical locations (Barel et al. 1976; Albertson and Kocher 2001; Parsons et al. 2011a): (1) region where anterior of the coronoid process and articular web converge on the left lateral side (2) region where the anterior of the coronoid process and the articular web converge on the right lateral side (3) ventral midline (4) midline at the anterior edge of the dentigerous (tooth bearing) region (5) posterior region of the coronoid process on the left lateral side (6) posterior region of the coronoid process on the right lateral side



each other. Thus, while not a statistical test, PCA can identify the combination of variables that account for the largest proportions of variation in multivariate data (Fowler et al. 1998). Therefore, to follow the common practise of morphometric studies a PCA was performed using the programs PCAGen (even and standard 2D data), ThreeD PCA (3D data) and using base functions in R for the ‘extended’ 2D data (R version 3.4.1; R core team 2017). Because the extended 2D method relied on different Procrustes models for each side of the mandible, scale differences were accounted for by using a correlation-based PCA rather than the covariance approach used for the other data sets. Following PCAs, we tested for the effects of species and sex on shape variation by performing an ANOVA on each of the first two principal component axes.

We also assessed differences in explanatory power between 2D and 3D data. To do this we took advantage of the known a priori grouping variables for our specimens including sex and species. A discriminant function analysis (DFA) was used to test these groupings and examine shape difference between species and sexes. DFAs were carried out in R version 3.4.1 (R Core Team 2017) using the MASS package

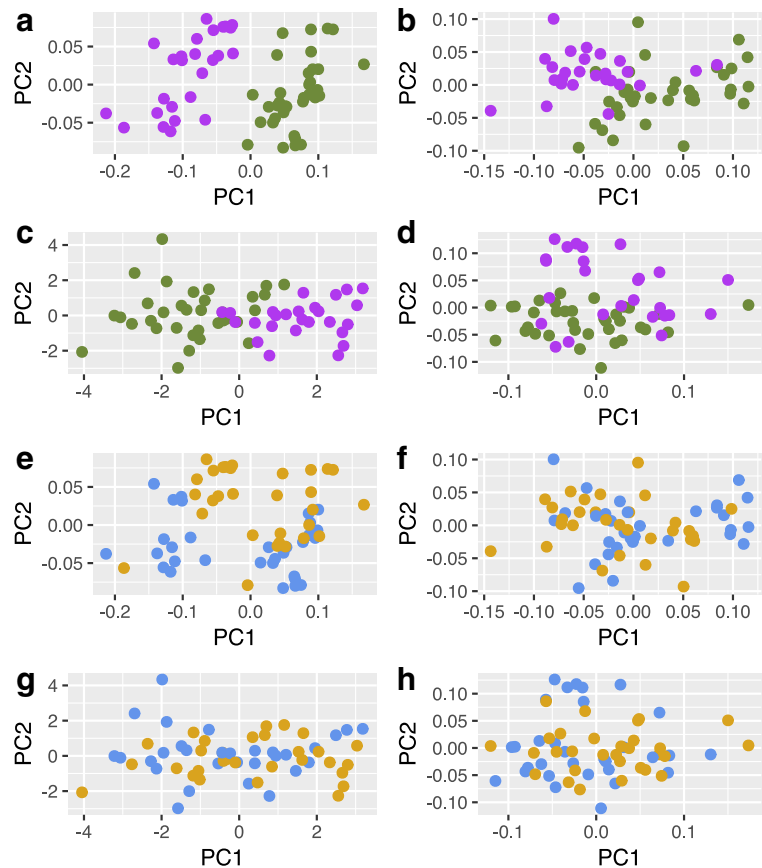
(Venables and Ripley 2002). For 2D shape (even and standard data sets), the linear discriminant (LD1) scores obtained from the DFA were then regressed on the Procrustes coordinates using geomorph and magnified by a factor of 2 to visualise shape differences across species and sex. Similarly, for 3D shape, the LD1 scores obtained from the DFA were regressed on the Procrustes coordinates to visualise differences. Following DFAs, MANOVA models were used to test the effect and significance of species and sex groupings.

Results

Data simplification: PCA of 2D and 3D methods

Qualitatively there were clear groupings visible for all of the methods for species based on the first two PCs (Fig. 3). Visually, the standard 2D approach appeared to perform best at distinguishing species. In line with this for sex, only the standard 2D method displayed a grouping on the PC plot, although with slight overlap (Fig. 3). Quantitatively, ANOVAs from the standard 2D approach indicated an effect of species, sex and an

Fig. 3 Scatter plots of the first two principal components for mandible shape using different data collection approaches. The top 4 panels represent groupings based on species (TRC represented in purple and MZ in green) while the bottom 4 panels represent sex (males represented in yellow and females in blue). The data collection methods include standard 2D (a, e), ‘even’ 2D (b, f), an ‘extended’ 2D (c, g), and 3D (d, h) approaches



interaction between the two factors on PC1 and an effect of species and sex on PC2. In comparison, the ANOVAs for the even 2D data indicated an effect of species and sex on PC1 and of species on PC2 (Table 1). While for 3D data there was an effect of species on PC1, and an effect of species and an interaction between sex and species on PC2 (Table 1). For the extended 2D method, there was an effect of species on shape along PC1 but no interaction; none of the factors were significant for the ANOVA for PC2.

Testing a priori groupings: DFA and MANOVA of 2D and 3D data

The DFAs showed that a priori groupings for species were well supported (Fig. 4). For the standard 2D approach, classification was 100% for both MZ and TRC. This was slightly less for the even 2D method which categorised 94% of MZ and 81% of TRC, while for 3D

data this classification was 91% for MZ and 85% for TRC. The extended 2D method performed comparatively better at classifying species with 97% of MZ and 92% of TRC correct. For sex, classification rates were generally lower than for species. While the standard 2D method had over 97% correct classification for both sexes, the even 2D method only correctly classified 58% of females and 60% of males. In addition, the extended 2D method classified 71% of females and only 60% of males correctly. The 3D method showed an improvement when compared with the even 2D method whereby 71% of females and 70% of males were correctly categorised. MANOVA tests confirmed the findings from the DFAs for both 2D, 3D and extended 2D data sets (Table 2). Specifically, models showed that there was a significant effect of species on shape for all methods. Additionally, for the standard 2D and 3D data there was a significant effect of sex on shape and for the extended 2D and 3D data there was an interaction between sex and species.

Table 1 The results from ANOVAs testing for species and sex effects on the first two PC axes for the 2D (four and eight landmarks, ‘even’ and ‘standard’ approaches respectively), 3D and extended

2D data sets describing mandible shape for two species of African cichlids (*Maylandia zebra* and *Tropheops* “Red Cheek”)

View	Response	Factor	DF	F value	p value
2D (‘even’)	PC1 (55% of the variance)	Species	1	35.719	<0.001**
		Sex	1	4.922	0.03*
		Species:Sex	1	1.683	0.199
	PC2 (24% of the variance)	Species	1	9.731	<0.001**
		Sex	1	0	0.985
		Species:Sex	1	0.603	0.44
2D (standard)	PC1 (48% of the variance)	Species	1	275.264	<0.001**
		Sex	1	9.168	0.004*
		Species:Sex	1	7.616	0.008*
	PC2 (16% of the variance)	Species	1	8.488	0.005*
		Sex	1	29.520	<0.001**
		Species:Sex	1	0.651	0.4230
Extended 2D	PC1 (40% of the variance)	Species	1	88.46	<0.001**
		Sex	1	3.38	0.071
		Species:Sex	1	0	0.99
	PC2 (19% of the variance)	Species	1	1.019	0.317
		Sex	1	0.719	0.400
		Species:Sex	1	0.659	0.420
3D	PC1 (35% of the variance)	Species	1	4.073	0.049*
		Sex	1	3.400	0.07
		Species:Sex	1	0.057	0.811
	PC2 (26% of the variance)	Species	1	26.075	<0.001**
		Sex	1	0.599	0.442
		Species:Sex	1	4.441	0.039*

*p value < 0.05; **p value < 0.001

Interpretable shape differences for species and sex were present in the mandible for both 2D and 3D shape and were similar. Species differed with TRC mandibles being wider along the midline with a shorter coronoid process relative to MZ (Fig. 4). For sex, the male shape (Fig. 4) is slightly broader along the midline and the coronoid processes are longer than in females (Fig. 4).

Discussion

The aim of this study was to directly compare morphometric methodologies involving 2D and 3D data collection and our results suggest there is not a strong difference in power between methodologies when using a comparable number of landmarks. However, the standard 2D approach using eight landmarks performs the best amongst all of the

approaches applied. For all methods, shape variation between species was more discernible than variation between sexes. This suggests that all methods were detecting somewhat similar aspects of shape variation, specifically species differences between MZ and TRC. It is possible that the small number of landmarks, which enabled direct comparisons between 3D and 2D methods, were unable to accurately reflect the differences between the two sexes. The standard 2D approach was the only method with >95% correct classification for sex. Nonetheless, the results and trend of shape variation are similar for both the 2D and 3D methods and match previous results about the mandible of these African cichlids suggesting that our data was biologically meaningful (Albertson and Kocher 2001; Parsons et al. 2015).

Given that groups were slightly more discernible in 3D from a discriminant function analysis than in ‘even’ 2D demonstrates some potential for additional insights

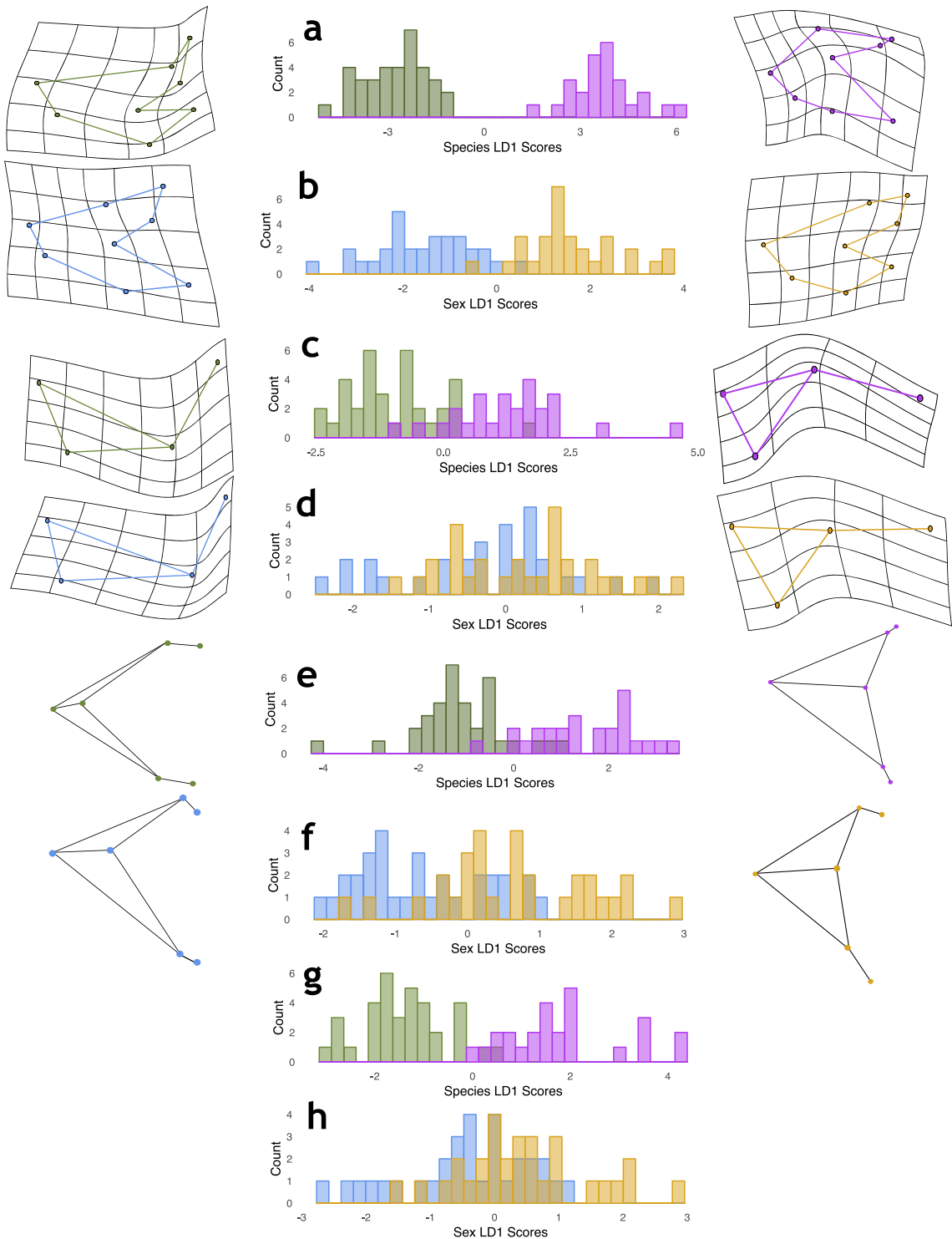


Fig. 4 Deformation grids and wireframes representing the regression of the LD1 values for each specimen from the discriminant function analyses of species and sex on the mean shape, alongside the frequency histograms for each DFA conducted for the standard

2D (a, b), ‘even’ 2D (c, d), 3D (e, f), and extended 2D (g, h). Males represented in yellow and females in blue; TRC are represented in purple and MZ in green

Table 2 The results from MANOVAs reliant on shape data from cichlid (*Tropheops* “Red Cheek” and *Maylandia zebra*) mandibles generated from different methodologies including 2D (four and

eight landmarks, even and standard approaches respectively), 3D, and extended 2D data

View	Factor	DF	Approx. F value	Pillai’s trace	p value
2D (‘even’)	Species	1	19.444	0.590	<0.001**
	Sex	1	2.008	0.129	0.107
	Species:Sex	1	1.17	0.0804	0.33
2D (standard)	Species	1	52.814	0.932	<0.001**
	Sex	1	12.051	0.759	<0.001**
	Species:Sex	1	1.896	0.331	0.06
Extended 2D	Species	1	18.383	0.746	<0.001**
	Sex	1	1.683	0.212	0.126
	Species:Sex	1	2.798	0.309	0.01*
3D	Species	1	8.067	0.654	<0.001**
	Sex	1	2.278	0.348	0.025*
	Species:Sex	1	2.598	0.378	0.01*

*p value < 0.05; **p value < 0.001

through 3D morphometric data when using a low-cost method. Intuitively, an additional dimension may provide more opportunity to capture shape information although the benefit is apparently lost when compared with the standard 2D approach. However, benefits of 3D are likely reliant on how strongly a third dimension correlates with two. In other words, it could be that our results indicate that mandible shape in 2D serves as a reasonably good proxy for shape in 3D because data from an extra dimension are redundant. But, for other cases we can envision structures where this is not the case. For example, in a comparison of methods for measuring egg shape by Attard et al. (2018), the 3D approach captured finer differences in shape when compared with traditional 2D methods. Also, a comparative study by Jamniczky et al. (2015) on three spine sticklebacks used much higher resolution 3D models from micro-CT scans and compared results with a 2D approach. The 3D approach allowed for a much higher resolution and substantially more landmarks than we could currently provide and reported that a 3D approach, while finding many of the same results, was more powerful than 2D for understanding shape variation. Indeed, it is obvious that a 3D approach can often capture more variation of a 3D structure than a 2D approach (Cardini 2014), but our data indicate this is not a universal situation.

Whilst no methods outperformed the standard 2D approach, all were able to discriminate species successfully. For discriminating sex, the 3D method performed better

when compared with the ‘even’ and extended 2D method. However, the 3D method showed slight differences in results when compared with the standard 2D approach with a significant interaction between species and sex in the MANOVA and for PC2 in the ANOVA. This could be due to the increased degrees of freedom in this approach, or there may be additional biologically relevant variation captured by measuring and including shape data from both sides of the mandible. Indeed, jaw asymmetry, which this approach may have been capturing, is a well-described feature of cichlids (Stewart and Albertson 2010).

While we aimed to make 2D and 3D datasets that were comparable, we recognize limitations in our data collection. For example, the small size of the mandibles pushed the extreme limits of the structured light scanning system. While accurate in reflecting width and overall shape of the mandibles, our 3D models lacked the detail that can be obtained from larger structures in low-cost methodologies such as our DAVID scanning system, or from other higher cost methods such as μ -CT (e.g. Jamniczky et al. 2015). It is therefore acceptable to hypothesise that specimens of a larger size with more landmarks would result in this 3D scanner outperforming the traditional 2D data collection. A recent study by Marcy et al. (2018) compared the use of μ -CT scanning with a 3D surface scanner. Although the surface scanner produced low quality models, they did still contain relevant shape information showing that in some cases a surface scanner is sufficient. However, because of the low resolution of our scans due to the

small size of the mandibles, this limited our choice of homologous landmarks across methods. Indeed, low resolution surface scans can introduce measurement errors due to difficulty in landmark identification and placement (Arnqvist and Martensson 1998; Fruciano 2016; Marcy et al. 2018). The simplicity of our 2D and 3D data was necessary for direct comparisons across methods and showed that our 3D method was accurate in discerning a priori species groupings but did not perform as well as the standard 2D approach for capturing details of shape variation relating to sex.

Three-dimensional methods for shape quantification were not widely available to biologists until recently. Therefore, the vast majority of the studies have relied on 2D data obtained from digital photos. However, GM has the potential to quantify shape variation in much greater detail when coupled with 3D. Given that we found slightly increased power in favour of the 3D methods under our restrictive ‘even’ data collection we can assume that greater gains are possible. For example, in a study exploring the genetic basis of mandible shape variation Navarro and Maga (2016) detected additional QTL with high-resolution 3D shape data relative to a comparable 2D approach. However, most of the shape variation was located in the genomic region where previous 2D shape QTL had been discovered. Therefore, this also shows 2D data collection is still a useful technique but perhaps lacks the ability to uncover the depth of understanding that can be gained from 3D (Navarro and Maga 2016). This gain of insight from 3D could be especially important in some contexts, such as in studies of natural selection where slight changes in phenotype can sometimes cause major shifts in fitness (Smith 1993; Parsons et al. 2011b).

Cost and time considerations of 2D vs 3D

We show that usable 3D data for morphometrics can be obtained using a low-cost and simple system to discern biologically-meaningful group differences. However, standard 2D data collection, beyond the superior performance here, may still be preferable as it is cheaper, faster, and more straightforward than that typically available for 3D (including the DAVID system used here). Currently, most studies using 3D shape assessments use relatively inaccessible and costly CT scanning. Whilst such 3D scanning can produce high quality models, the purchase of a scanner is prohibitive to most labs making it necessary to outsource scanning which

can also be exorbitant (£10–120 per scan) (Abel et al. 2012). Another limiting factor for such 3D morphometrics stems from the training and computing power needed to prepare a 3D model using costly software which lack adequate documentation (e.g. ScanIP, VG Studio Max, Amira) (Abel et al. 2012). Unfortunately, given the intricacies and small size of some anatomical features, u-CT scanning may be the only feasible option for study in some cases; the small size of the cichlid mandible used in this study provided a suitable test of the limits of a low-cost 3D data collection system.

Fortunately, alternatives for gaining 3D shape data are arising that can likely be applied to a wide range of situations. For example, our structured light scanner can be created by users from off the shelf accessories (e.g. projector, tripod) but relies on specialized but affordable user-friendly software (complete scanning of our mandibles took less than 2 h, comparable to traditional 2D photographing). Additionally, low cost stereo camera set ups can now be used with the StereoMorph package for the R statistical language to create 3D models and collect landmarks (Olsen and Westneat 2015). These are just two examples of low-cost 3D systems and given current advances in imaging we are hopeful that more accessible methods will become widely available in the future.

Conclusions

As morphometrics incorporates new techniques for 3D data collection we predict that it will drive the need for more analytical approaches. For example, the GeoMorph package has been released for the R statistical language and is continually updated (Adams and Otárola-Castillo 2013) whilst more established software such as IMP and MorphoJ have also grown to incorporate 3D data (Klingenberg 2011). New packages are continually being added to the R statistical language such as Shape Rotator which enables the user to process landmarks from articulated structures such as different parts of the skeleton which previously was not possible to do in three dimensions (Vidal-García et al. 2018). Therefore, the number of options available to researchers for 3D shape analysis is increasing, but we conclude that the decision to invest in these techniques are best assessed by the researcher and situation. We hope that our data makes clear that in some cases the conventional 2D approach is adequate, if not a superior option for the measurement of shape.

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