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TECHNICAL NOTE

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Technical note: A freeware, equitable approach to dental topographic analysis

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Abstract

Dental topographic analysis has proved a valuable tool for quantifying dental morphology. Established workflows often use proprietary software for pre-processing dental surfaces, rendering the method expensive and inaccessible to many. This study explores the use of freeware pre-processing alternatives. We tested 4 decimation tools and 13 smoothing tools across 7 different freeware packages. Surfaces generated via proprietary software could not be replicated, but it was possible to obtain statistically similar measurements using freeware. Based on this investigation, we propose a freeware workflow for researchers to conduct dental topographic analysis, with the expectation that their results will be comparable to that obtained through proprietary methods.

KEYWORDS
dental topographic analysis, freeware, MeshLab, molaR, smoothing

1 | INTRODUCTION

Dental topographic analysis (DTA) is a popular method for quantifying dental form Berthaume et al. (2020). DTA's landmark-free approach makes it applicable to a wide range of structures (Pamfilie et al., 2022; Stamos & Weaver, 2020; Wallace et al., 2017), and teeth at various stages of wear, thus allowing for distantly related taxa of various stages of life to be directly compared (Berthaume, 2016; Ungar & Williamson, 2000). Most analyses use proprietary software limiting the application of DTA to labs/groups that can afford this software. The development of a freeware method for conducting DTA would increase accessibility and equitability.

A typical DTA workflow consists of scanning a specimen, segmenting scans (if necessary), rendering a 2.5D or 3D mesh, and quantifying the mesh (Spradley et al., 2017). Scanning is usually conducted with a laser or micro-computed tomography (micro-CT) scanner, although a variety of scanning technologies, including light and tactile scanning, can be used (Boyer, 2008; Jernvall & Selänne, 1999; Ungar & Williamson, 2000; Zuccotti et al., 1998). While access to specimens, equipment, and scan reconstruction software is a barrier, the increase in public repositories of reconstructed scan and mesh data (e.g., www.morphosource.org/, (Boyer et al., 2017), www.digimorph.org), provides researchers free access to previously collected data.

During scan segmentation and mesh generation, aspects of morphology of interest are identified, isolated, and used to create a digital mesh representation of the tooth. The mesh is then cropped, simplified/decimated to a constant resolution (Evans et al., 2007) or triangle count (Winchester et al., 2014), and smoothed (Figure 1). Generally, Avizo's or Amira's (FEI Visualization Sciences Group, Berlin, Germany) inbuilt Simplify and Smooth Surface tools are used for these steps, despite these being expensive, proprietary software and the presence of freeware alternatives. The segmentation capabilities of these freeware alternatives are effective and well-tested (Egger et al., 2013; Sokolowski et al., 2019) and could immediately be implemented into a DTA workflow. Yet, the sensitivity of dental topographic measures to factors such as mesh resolution and smoothing has prevented the adoption of freeware alternatives for simplification/decimation and smoothing into DTA (Berthaume et al., 2018, 2019b; Spradley et al., 2017).

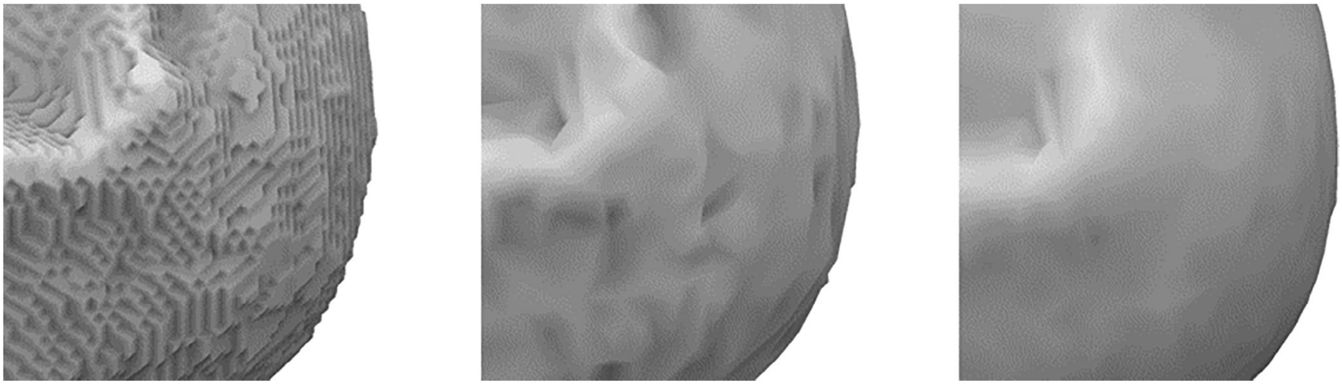

FIGURE 1 Section of a tooth during three stages of mesh preparation. (Left) Raw mesh surface downloaded from Morphosource. (Center) Simplified to a triangle count of 10 k. (Right) Smoothed with Rvcg's Taubin algorithm. Tooth displayed is AMNH 87279, M2 of Nycticebus bengalensis, originally appearing in Boyer (2008).

During simplification/decimation, mesh face sizes and shapes are changed, changing the locations of face edges and vertices. Similarly, during smoothing, the locations of the mesh vertices are altered to improve element shape and mesh quality, for example, by decreasing variation in vertex location between adjacent triangles. Differences in simplification/decimation and smoothing methodologies are unlikely to place vertices in exactly the same locations, thus creating different meshes. Even the same methodology in two different programs is unlikely to produce the same mesh due to differences in coding protocols and optimization algorithms (Table S1). Certain DTA metrics quantify shape through variation in triangle orientation between adjacent triangles, for example, Dirichlet Normal Energy (DNE) and Orientation Patch Count Rotated (OPCR), making them more sensitive to vertex location than metrics which do not rely on variation in triangle orientation, for example, Relief Index (RFI) and Portion de Ciel Visible (PCV; Berthaume et al., 2019b).

Here, we propose a freeware-based method for conducting DTA. Such a method will produce surfaces suitable for DTA, judged by the production of realistic DTA values. We focus on two steps during the DTA workflow, decimation and smoothing, and compare our DTA results to those obtained using Avizo. Avizo is the most common program used for these steps, and a lack of a significant difference between a freeware method and Avizo would suggest results gathered using these two different methods are comparable. We hypothesize the freeware method will not produce results identical to Avizo due to differences in coding and the uniqueness of Avizo's smoothing algorithm. However, we expect results to be comparable.

1.1 | A freeware method

During DTA, teeth are scanned, segmented (if necessary), rendered, and quantified (Spradley et al., 2017). Scanning is done with lab equipment which come with equipment-specific software, meaning there is no "freeware" alternative. The design and development of increasingly affordable scanning technologies, such as scAnt (Plum & Labonte, 2021), will improve the accessibility of scanning by decreasing the price associated with scan acquisition.

Accessible freeware exists for scan segmentation, such as 3D slicer (Pieper et al., 2004) and Fiji (Schindelin et al., 2012). As this step requires high levels of user input, inter-observer error is likely to be higher than inter-program differences. As such, differences in segmenting abilities between programs will not be investigated here. Mesh analysis is often done in freeware, for example, Morphotester (Winchester, 2016), molaR (Pampush et al., 2016), doolkit (Thiery et al., 2021), and CloudCompare (Berthaume et al., 2019a). The between program comparability of some metrics has been investigated elsewhere (Pampush et al., 2016), so will not be investigated here.

Mesh simplification/decimation and smoothing require minimal user input and have a large impact on the results (Berthaume et al., 2019b; Spradley et al., 2017), implying the effect of the program and tool chosen will have a larger effect on the results than the user. The effect of program and tool choice on four dental topographic metrics (DNE, RFI, OPCR, and PCV) will be the focus of this study.

Here, we investigate freeware alternatives to the decimation and smoothing steps in DTA. To make the method accessible and equitable, we propose such a method would require software accessible to all which is relatively easy to use, not requiring expertise in programming languages other than R (as R is often used for DTA).

2 | MATERIALS AND METHODS

Freeware mesh processing programs were identified by reviewing websites, publications and manufacturer's documentation detailing and comparing software. Those with tools dedicated to simplification and smoothing of meshes were trialed, and those in which (1) simplification or smoothing could be conducted across the whole mesh uniformly, (2) the mesh could be exported, and (3) had no other drawbacks, such as limited access or requiring programming in languages other than R, were investigated further.

2.1 | Sample

Twenty-five primate M_2s, often seen as the most "average" molar (Kay, 1975), representing 25 species and 22 genera with published DTA values were selected from Berthaume et al. (2019b). This sample was originally published in Boyer (2008) and has appeared in Bunn et al. (2011); Winchester et al. (2014). To encompass variability in molar shape, five molars were selected from each dietary category (folivore, frugivore, omnivore, insectivore, and hard object feeder), as defined by Boyer (2008) and Winchester et al. (2014). Further information on dietary classifications can be found in Boyer (2008) and Winchester et al. (2014).

Meshes were downloaded from morphosource.org and rotated 180° in Meshlab (Berthaume et al., 2019b; Thiery et al., 2019), aligning the occlusal surface with the positive *z*-axis. Isolated triangles were removed using the Remove Isolated pieces (wrt Face Num) filter, and a minimum connected component size of 5000. The entire enamel cap (EEC) was used for analysis (Berthaume et al., 2018).

We also tested a hemisphere created in CAD (autodesk.com/products/inventor), (Autodesk, 2023), similar to Spradley et al. (2017) (Tables S4 and S5). Unfortunately, low variation in DTA metrics meant nearly all programs performed to similar levels. As such, we did not use the data in this analysis, but have provided it in Supplementary Material.

2.2 | Topographic calculations

Four topographic values, correlated to diet in primates, were chosen. These metrics quantify the curvature (DNE), relief (RFI), complexity (OPCR), and morphological wear resistance of the enamel crown (PCV; Berthaume et al., 2019a; Boyer, 2008; Bunn et al., 2011; Evans & Jernvall, 2009). DNE, RFI, and OPCR were calculated in molaR using R and RStudio (Pampush et al., 2016; R Core Team, 2021; RStudio Team, 2020), and PCV was calculated using the PCV/ShadeVis tool for CloudCompare (https://cloudcompare.org/) (Girardeau-Montaut, 2020). The top 1% energy × area values were excluded for DNE calculations—that is, DNE99 from Berthaume et al. (2018). To make results comparable to Berthaume et al. (2019b), boundary triangles were included in DNE calculation. Occasionally, errors were encountered, which could usually be fixed by running dental surfaces through Meshlab's Remove Zero Area Faces filter. Relief index was calculated using the following formula from Boyer (2008):

$$\mathrm{RFI} = \ln\left(\sqrt{\frac{\text{3D M2 crown area}}{\text{2D M2 crown area}}}\right)$$

with an alpha of 0.06. If an error message occurred, the alpha value was iteratively increased by increments of 0.005 until RFI was calculated. We checked 2D RFI projections with the Check2D tool when measured areas significantly deviated from expected values. Complexity (OPCR) was calculated with a minimum patch size of three triangles and eight rotations of 5.625°. Ambient occlusion was calculated over the surface of the tooth using the PCV/ShadeVis tool in CloudCompare V2.11.3 (default count of 256, Only northern hemisphere [+*Z*]), and average ambient occlusion (PCV) was calculated using the Distribution fitting: Gauss tool (Berthaume et al., 2019a).

2.3 | Decimation

Decimation reduces triangle count. Many topographic metrics are sensitive to triangle count and require triangle count to be held constant (Berthaume et al., 2019b; Melstrom & Wistort, 2021; Spradley et al., 2017). Only programs with decimation tools which simplified surfaces to a constant triangle count in a single step were considered, as iterative reductions in triangle count can create final surfaces with slight variations in final vertex number and position. The only freeware programs identified with this capability were FreeCAD (Decimation tool, www.freecadweb.org), Meshlab (Quadric Edge Collapse Decimation, www.meshlab.net) (Cignoni et al., 2008), Openflipper (Decimater, www.openflipper.org; Möbius & Kobbelt, 2010), and R, (vcgQEdecim command in Rvcg package, https://cran.r-project.org/web/packages/Rvcg/; Schlager, 2017).

Surfaces were decimated to 10,000 triangles. Only target triangle count could be altered in FreeCAD. Parameters in Openflipper were left to default values, as altering parameters often resulted in the wrong triangle count (Decimation Order = by distance; Constraints: Distance = 0.05 and Normal Dev. = 5). The effects of Topography Preserving (True vs. False), Scale Independence = (True vs. False), Quality Threshold (0.0001–1, 0.25 increments, and "not considered"), Normal Threshold (0 –3pi/4, pi/4 increments, and "not considered"), and Boundary Threshold (0–1, 0.25 increments and "not considered") were investigated in Rvcg along with default values, yielding 22 datasets (Table 1). When a parameter was investigated, all other parameters were assigned default values (Topography preservation = F, Quality consideration = T, Boundary preservation = F, Scale independence = T, Normal consideration = F, Quality threshold = 0.3).

If >5% of surfaces in a dataset were unusable, the process/tool was unsuitable for DTA. Resultant DTA metrics were compared to metrics of 10 k triangle count unsmoothed surfaces from Berthaume et al. (2019b), using paired student's *t*-tests to determine if results were comparable to those generated with Avizo ($\alpha = 0.05$, Bonferroni correction for multiple comparisons). Number of significant differences was counted, with fewer differences indicating more similar results. Ties were resolved by comparing mean % difference in DTA values. The "optimal" set of parameters for Rvcg were used to create a 23rd dataset.

Meshlab had many of the same parameters as Rvcg. We assumed variations in parameter values would yield similar results in both programs, and used the "optimal" parameter set from Rvcg in Meshlab (Preserve Normal selected, Quality threshold = 1). Boundary preservation settings were different. The effects of Preserve Boundary (True vs. False), Preserve Topology (True vs. False), and Boundary Preserving Weight (0.1, 1, 10) were investigated in Meshlab, using the same protocol as in Rvcg with the following values for other parameters (Preserve normal selected; Quality threshold = 1.0; Percentage

TABLE 1 Programs, tools and parameter values used to simplify surfaces in this study.

Program	Tool	Parameter	Values	Datasets created
FreeCAD	Decimation tool	N/A	N/A	1
Meshlab	Quadric Edge Collapse Decimation	Boundary Preserving	True/False	12
		Boundary Weight	0.001, 0.01, 0.1, 1, 10, 100, 1000	
		Topology Preserving	True/False	
Openflipper	Decimater	N/A	N/A	1
Rvcg	vcgQEdecim	Boundary Threshold	Not considered, 0, 0.25, 0.5, 0.75, 1, default	23
		Normal Threshold	Not considered, 0, pi/4, pi/2, 3pi/4, default	
		Quality Threshold	Not considered, 0.0001, 0.25, 0.5, 0.75, 1, default	
		Scale Independence	True/False	
		Topography Preserving	True/False	

Note: Default values in Rvcg produced the same dataset for each parameter. An extra dataset was produced for both Meshlab and Rvcg, using optimal values.

TABLE 2 Programs, tools and parameter values used to smooth surfaces in this study.

Program	Tool	Parameter	Values	Datasets created
Meshlab	Laplacian Smooth	Iterations	1, 2, 3, 4, 5	5
Openflipper	Smoother	Iterations	1, 2, 3, 4, 5	5
Blender	Smooth vertices (Laplacian)	Iterations	1, 2, 5, 10, 20, 50, 100	14
		Lambda Factor	0.03–0.3	
	Smooth vertices	Iterations	1, 2, 5, 10, 20, 50, 100	
		Smoothing	0.02–0.8	
CloudCompare	Smooth (Laplacian)	Iterations	1, 2, 5, 10, 20, 50, 100	7
		Smoothing Factor	0.02–0.8	
FreeCAD	Mesh Smoothing (Laplace)	Iterations	1, 2, 5, 10, 20, 50	6
		Lambda	0.03–0.9	
	Mesh Smoothing (Taubin)	Iterations	1, 2, 5, 10, 20, 50	18
		Lambda	0.3–0.8	
		Mu	0.02, 0.05, 0.1	
Rvcg	vcgSmooth (angWeight)	Iterations	1, 2, 5, 10, 20, 50, 100	7
		Delta	0.0009–0.04	
	vcgSmooth (fujiLaplace)	Iterations	1, 2, 5, 10, 20, 50, 100	7
		Delta	0.0001–0.005	
	vcgSmooth (surfPreserveLaplace)	Iterations	1, 2, 5, 10, 20, 50, 100	7
		Delta	0.6–4	
	vcgSmooth (Taubin)	Iterations	1, 2, 5, 10, 20, 50, 100	21
		Lambda	0.4–0.9	
		Mu	–Lambda—0.02, –Lambda—0.05, –Lambda—0.1	
Meshmixer	Smooth (Shape preserving)	Constraint rings	1, 3, 10	3
		Smoothing Scale	1–2	
	Smooth (Uniform triangles)	Constraint rings	1, 3, 10	3
		Smoothing Scale	0.9–1	
Unsmoothed		NA	NA	1

Note: Iterations had a stronger effect on smoothing in Meshlab and Openflipper, so smaller values were used. FreeCAD did not allow for 100 smoothing iterations. See Section 2 for explanation of value ranges.

TABLE 3 *p* Values of paired Student's *t*-tests between datasets generated by each tested decimation tool and the Avizo dataset.

		p Value between Avizo and program dataset			
Program	**Tool**	**RFI**	**DNE**	**OPCR**	**PCV**
FreeCAD	Decimation tool	9.55E-08	7.84E-06	0.0271	0.0002
Meshlab	Quadric Edge Collapse Decimation	0.0944	0.0413	0.6554	0.0097
Openflipper	Decimater	2.40E-09	6.84E-11	4.73E-08	0.0033
Rvcg	vcgQEdecim	0.0081	1.55E-10	5.17E-10	2.2E-16

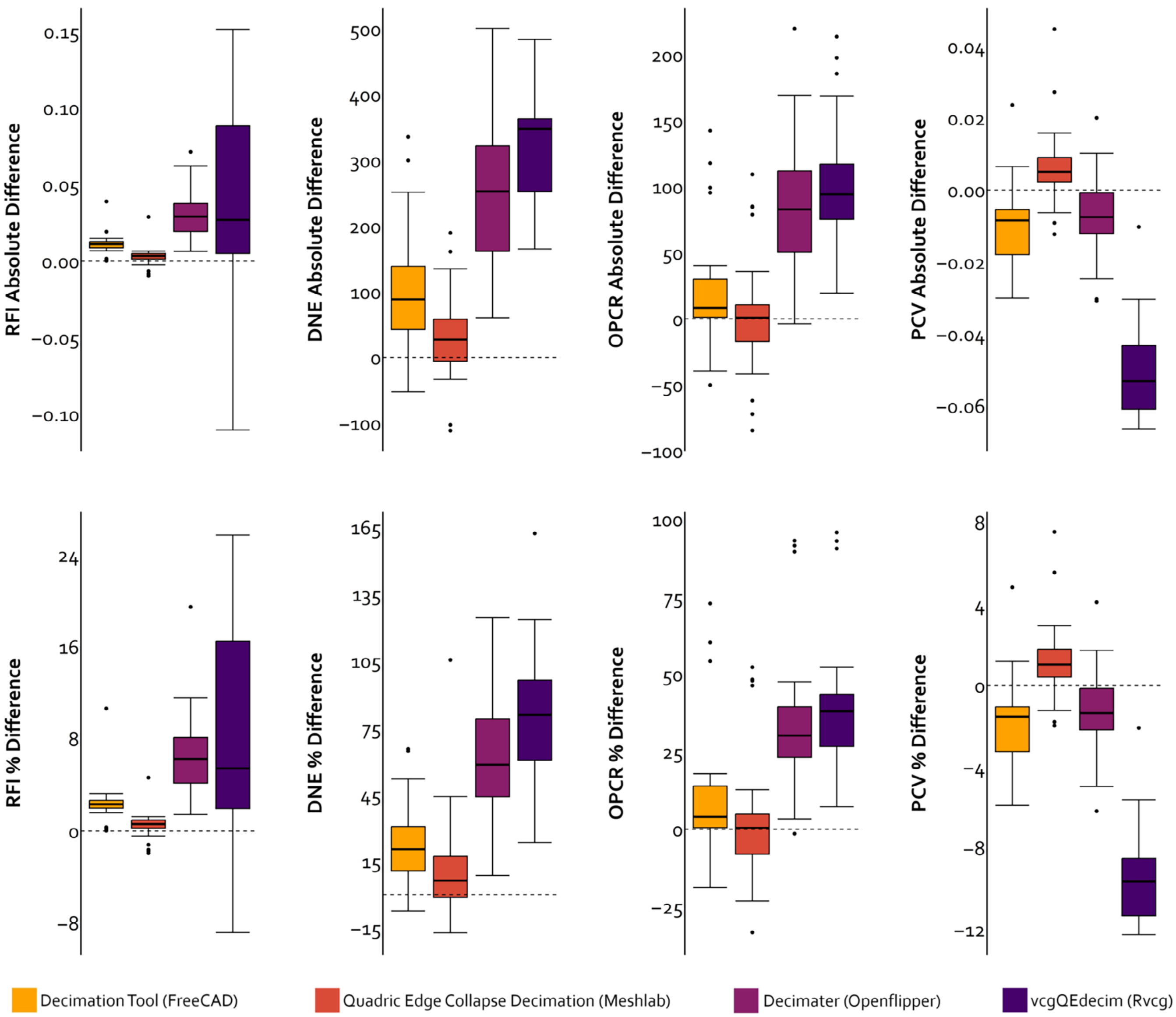

FIGURE 2 Absolute (above) and % (below) differences in RFI, DNE, OPCR, and PCV between decimated surfaces and Avizo (Lautenschlager, 2021).

reduction = 0; Preserve boundary of mesh, Preserve topology, Planar simplification, Weighted simplification, and Simplify only selected faces all unselected; Optimal position of simplified vertices and Post-simplification cleaning selected).

Thirty-seven decimation datasets were created. The "optimal" decimation tool was used to investigate smoothing.

2.4 | Smoothing

Smoothing tools in 44 mesh editing freeware programs were investigated (Table S2). Seventeen programs contained 37 tools for uniformly smoothing the mesh in a single step and were considered further (Table S3). Twenty-four were excluded as they (1) produced

TABLE 4 *p* Values of Signed Rank Wilcoxon tests between datasets generated by each tested smoothing tool and the Avizo dataset.

		p Value between Avizo and program dataset			
Program	**Tool**	**RFI**	**DNE**	**OPCR**	**PCV**
Blender	Smooth vertices (Laplacian)	0.0002	0.5720	0.7879	1.31E-05
Blender	Smooth vertices	2.51E-05	0.0207	0.9571	1.31E-05
Cloudcompare	Smooth (Laplacian)	2.21E-05	0.0207	0.9464	1.31E-05
FreeCAD	Mesh Smoothing (Laplace)	0.0010	0.0040	0.9143	1.31E-05
FreeCAD	Mesh Smoothing (Taubin)	3.04E-05	1.31E-05	0.0383	1.31E-05
Meshlab	Laplacian Smooth	0.2584	1.31E-05	1.31E-05	0.0003
Meshmixer	Smooth (Shape Preserving)	0.0851	0.1186	0.0495	1.48E-05
Meshmixer	Smooth (Uniform Triangles)	0.1264	0.0436	0.0071	1.48E-05
Openflipper	Smoother	0.0097	0.5012	0.0758	1.48E-05
Rvcg	vcgSmooth (angWeight)	0.1573	1.31E-05	1.31E-05	0.0091
Rvcg	vcgSmooth (fujiLaplace)	2.13E-05	0.0001	0.0192	0.0003
Rvcg	vcgSmooth (surfPreserveLaplace)	1.67E-05	0.0335	0.4675	1.31E-05
Rvcg	vcgSmooth (Taubin)	0.2713	0.0040	0.7879	2.70E-05
Unsmoothed		3.84E-05	1.31E-05	1.31E-05	0.0124

identical results to another smoothing tool, (2) significantly altered the triangle count, or (3) required knowledge of programming in a language other than R. As DTA generally requires R, we assume some literacy in R, but not other programming languages.

The remaining 13 tools had 1+ parameter which could be altered. Parameter values are listed in Table 2. When more than one parameter could be altered, a single tooth (*Nycticebus bengalensis*, AMNH-87279) was used to determine optimal parameter values for each value of iterations/Constraint rings (Meshmixer). This secondary parameter was initially altered from 0.1 to 1.0 at increments of 0.1. DNE, RFI, and OPCR were calculated and compared to the Avizo smoothed, 10,000 triangle results from Berthaume et al. (2019b). PCV was omitted due to time associated with data collection. The magnitude of % difference in results was calculated and the parameter value with the lowest % difference was chosen. If a boundary value (0.1 or 1.0) was chosen, additional values with one significant figure were investigated until a non-boundary value was chosen.

Two tools had three parameters that could be altered. In Mesh Smoothing (Taubin), FreeCAD, mu values were set to 0.02, 0.05, and 0.1 and the optimal lambda value was determined for every combination of iteration and mu. In vcgSmooth (Taubin), Rvcg, lambda (λ) and mu (μ) were functions of each other ($\mu = -\lambda - a$). The difference between negative lambda and mu (a) was held constant at 0.02, 0.05, and 0.1, and the optimal lambda value for each combination of iterations and *a* was determined.

One hundred and three smoothed datasets plus the one unsmoothed dataset were generated using the 25 teeth. Shapiro–Wilk tests revealed DTA data were largely non-normally distributed ($p < 0.05$); non-parametric statistical tests were then used. Three Wilcoxon signed-rank tests per dataset were used to compare the DTA metrics from the datasets to the DTA values obtained from Avizo (above, Bonferroni corrected $\alpha = 0.0167$, ESM). The parameter values for each tool which produced results most similar to Avizo were chosen as "optimal." If there was a tie, the parameters that yielded the lowest mean % difference from Avizo were chosen. PCV was calculated for the 13 optimal datasets, and the unsmoothed dataset. A fourth Wilcoxon signed-rank test was calculated, comparing PCV values to those obtained using Avizo (Bonferroni-corrected $\alpha = 0.0125$).

3 | RESULTS

3.1 | Decimation

Decimation data were always normally distributed ($p > 0.05$, ESM). Student's *t*-tests showed that FreeCAD, Meshlab and Openflipper were suitable for DTA, but the "optimal" settings in Rvcg were unsuitable (8/25 surfaces failed DTA). Meshlab produced results most comparable to Avizo (Table 3, Figure 2), producing similar results for RFI, DNE, and OPCR ($p = 0.0944$, $p = 0.0413$, $p = 0.6554$, respectively, Bonferroni corrected $\alpha = 0.0125$) but not PCV ($p = 0.0097$). All other programs produced significant differences in all metrics ($p < 0.0125$) excepting OPCR in FreeCAD ($p = 0.0271$).

3.2 | Smoothing

Out of 104 datasets 77 were suitable for DTA. Meshmixer's Smooth (Shape Preserving) tool yielded results most comparable to Avizo (Table 4, Figures 3 and 4) with Rvcg's vcgSmooth (Taubin) the next most comparable.

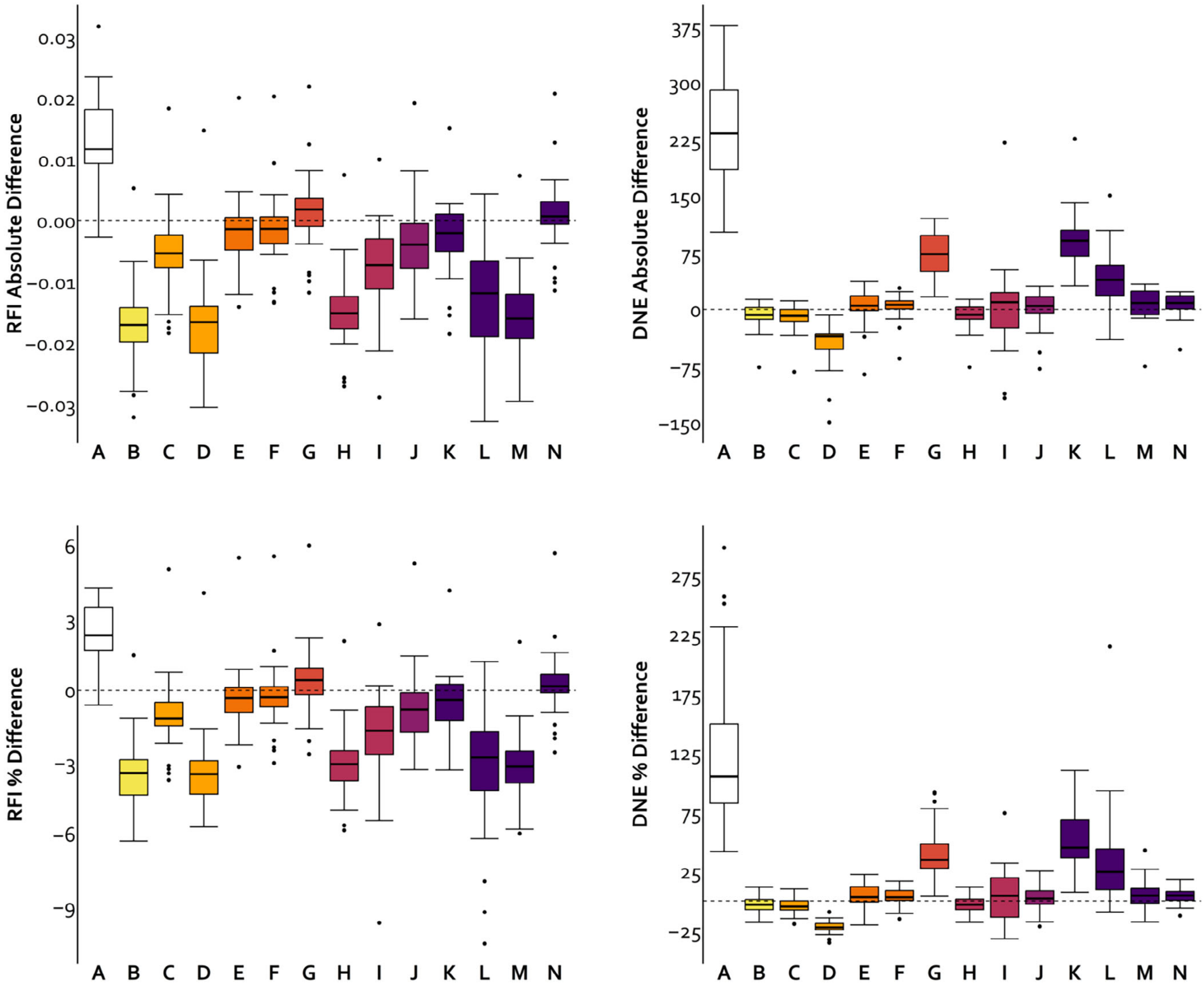

FIGURE 3 Absolute and % differences in RFI (left) and DNE (right) between smoothed surfaces and Avizo. (a) Unsmoothed surfaces. (b) Smooth (Laplacian) (Cloudcompare). (c) Mesh Smoothing (Laplace) (FreeCAD). (d) Mesh Smoothing (Taubin) (FreeCAD). (e) Smooth (Shape Preserving) (Meshmixer). (f) Smooth (Uniform Triangles) (Meshmixer). (g) Laplacian Smooth (Meshlab). (h) Smooth Vertices (Blender). (i) Smooth Vertices (Laplacian) (Blender). (j) Smoother (Openflipper). (k) vcgSmooth (angWeight) (Rvcg). (l) vcgSmooth (fujiLaplace) (Rvcg). (m) vcgSmooth (surfPreserveLaplace) (Rvcg). (n) vcgSmooth (Taubin) (Rvcg).

Of the 14 smoothing options tested (13 smoothing tools and the unsmoothed surfaces) Meshmixer's Smooth (Shape Preserving) tool produced results most comparable to Avizo ($p = 0.0851$, $p = 0.1186$, $p = 0.0495$ for RFI, DNE, and OPCR, respectively, Bonferroni-corrected $\alpha = 0.0125$) although it did produce significant difference in PCV values ($p = 1.48\text{E-}5$). Rvcg's vcgSmooth (Taubin) produced insignificant differences in RFI and OPCR ($p = 0.2713$, $p = 0.7879$, respectively) but significant differences in DNE and PCV ($p = 0.0040$, $p = 2.70\text{E-}5$).

Blender's Smooth Vertices and Smooth Vertices (Laplacian), Cloudcompare's Smooth (Laplacian), Openflipper's Smoother, and Rvcg's vcgSmooth (SurfPreserveLaplace) all produced insignificant differences in DNE and OPCR values ($0.0207 < p < 0.9571$) and significant differences in RFI and PCV ($1.31\text{E-}5 < p < 0.0097$). Meshmixer's Smooth (Uniform Triangles) produced insignificant differences in RFI and DNE, and significant differences in OPCR and PCV ($p = 0.1264$, $p = 0.0436$, $p = 0.0071$, $p = 1.48\text{E-}5$, respectively). Of the tools producing insignificant difference in two or more metrics, Rvcg's vcgSmooth (Taubin) produced the lowest mean percentage difference across all metrics (0.2578% diff in RFI, 4.7661% diff in DNE, 1.4842% diff in OPCR, 1.4319% diff in PCV).

FreeCAD's Mesh Smoothing (Laplace) and Mesh Smoothing (Taubin), and Rvcg's vcgSmooth (fujiLaplace) gave insignificant differences in OPCR ($0.0192 < p < 0.9143$), but significant differences in all other metrics ($1.31\text{E-}5 < p < 0.0091$). Meshlab's Laplacian Smooth and Rvcg's vcgSmooth (angWeight) gave insignificant differences in RFI ($p = 0.2584$, $p = 0.1573$) but significant differences in all other metrics ($1.31\text{E-}5 < p < 0.0091$). Finally, the unsmoothed surfaces gave significant differences in all metrics ($1.31\text{E-}5 < p < 0.0124$).

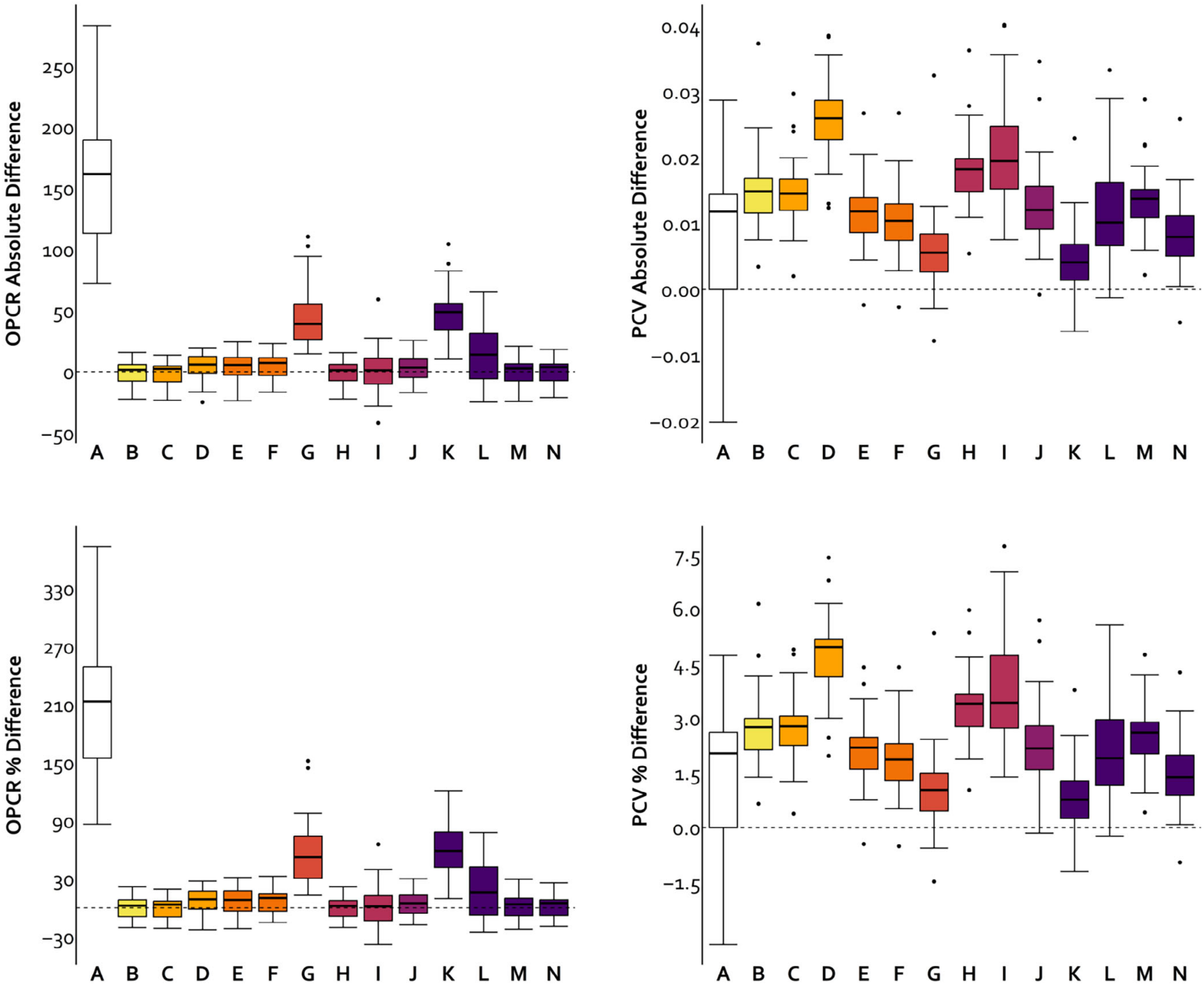

FIGURE 4 Absolute and % differences in OPCR (left) and PCV (right) between smoothed surfaces and Avizo. (a) Unsmoothed surfaces. (b) Smooth (Laplacian) (Cloudcompare). (c) Mesh Smoothing (Laplace) (FreeCAD). (d) Mesh Smoothing (Taubin) (FreeCAD). (e) Smooth (Shape Preserving) (Meshmixer). (f) Smooth (Uniform Triangles) (Meshmixer). (g) Laplacian Smooth (Meshlab). (h) Smooth Vertices (Blender). (i) Smooth Vertices (Laplacian) (Blender). (j) Smoother (Openflipper). (k) vcgSmooth (angWeight) (Rvcg). (l) vcgSmooth (fujiLaplace) (Rvcg). (m) vcgSmooth (surfPreserveLaplace) (Rvcg). (n) vcgSmooth (Taubin) (Rvcg).

4 | DISCUSSION

Here, we investigated the effect of decimation and smoothing tools in different freeware programs on DTA results. Differences in decimation/smoothing and coding methodologies mean no workflow produced results identical to those produced in Avizo, but similar results could be obtained with the following freeware workflow (Figure 5).

Pampush et al. (2016) suggests Meshlab (Cignoni et al., 2008) can be used as a freeware alternative to Avizo or Amira for the purposes of mesh preparation for analysis in molaR. Indeed, López-Torres et al. (2018) and Prufrock et al. (2016) use a single iteration of Meshlab's Laplacian Smooth as the primary smoothing operation in mesh preparation. López-Aguirre et al. (2022); Melstrom et al. (2021); Melstrom and Wistort (2021); and Rannikko et al. (2020) also use this tool for smoothing, as well as Meshlab's Quadric Edge Collapse Decimation tool for mesh simplification. The application within Melstrom et al. (2021) of freeware tools to saurian teeth demonstrates potential for expansion of the freeware method proposed within this article for taxa outside *Primates*, and indeed, even outside *Mammalia*.

The establishment of Meshlab's mesh preparation tools as a freeware alternative to proprietary tools matches our results here. As demonstrated, the decimation step is best performed in Meshlab. Although the precise algorithm used for decimation in Avizo is unknown due to the proprietary nature of this program, it has been stated to be an edge collapse algorithm (ThermoFisher Scientific, 2019), likely functioning similarly to Meshlab's quadric edge collapse tool, and as demonstrated here, producing similar results in DTA metrics of the resultant mesh.

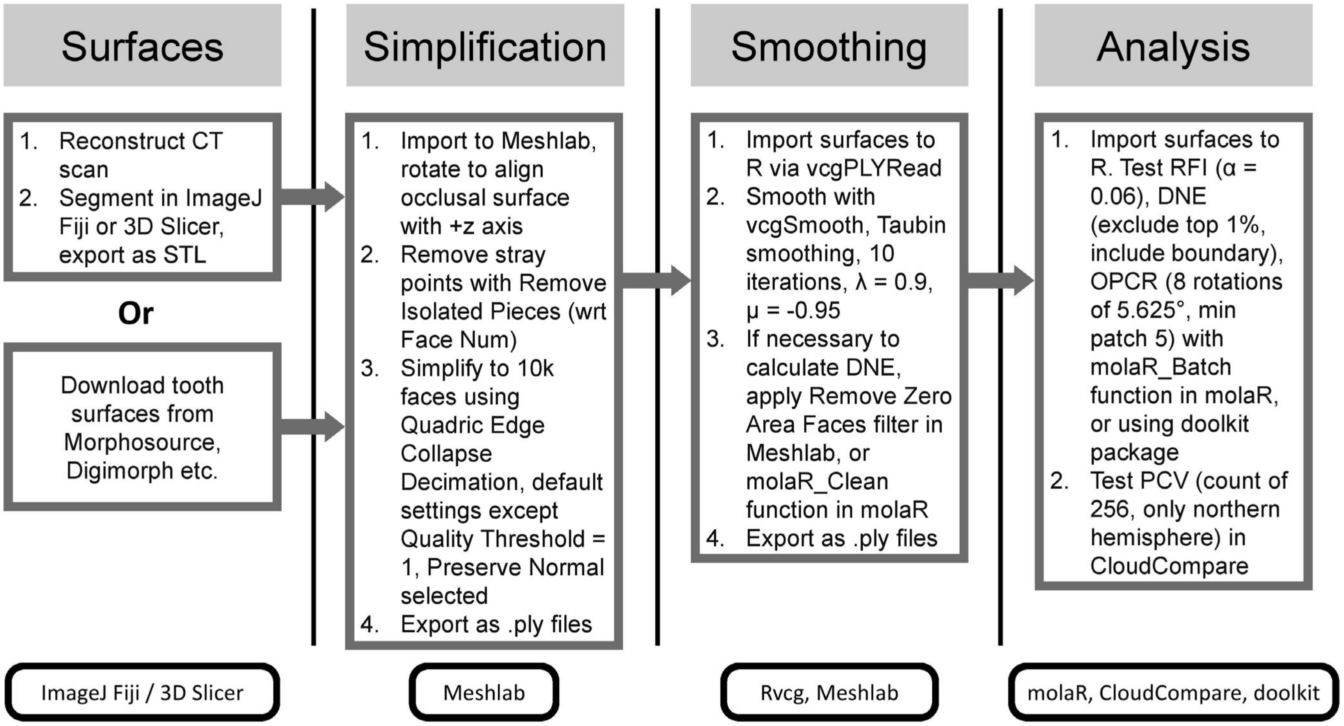

FIGURE 5 The proposed freeware workflow for conducting DTA. An alternative package for conducting analysis, Morphotester (Winchester, 2016) is not included, as it has not been recently recompiled, limiting accessibility.

Although some studies have promoted Meshlab's Laplacian Smooth tool as a freeware smoothing alternative, our results showed that using 2+ iterations of this tool result in 5% + meshes being unsuitable for DTA. When only one iteration was applied, other smoothing tools produced results much closer to values produced by Avizo. Foremost among these was Meshmixer's Smooth (Shape Preserving), giving DTA results most comparable to Avizo values. However, the authors would recommend using the vcgSmooth (Taubin) tool within Rvcg to complete the smoothing step for DTA. This smoothing tool produces the second most comparable results to Avizo, as well as having a few other benefits, including automated batch processing which can be used in combination with molaR for the automated processing of a large number of teeth. This is reinforced by Veneziano et al. (2018), who found that smoothing tools based on the Taubin algorithm are best-suited for similar applications.

When selecting a mesh preparation methodology, an important factor to consider is uniformity in triangle size. It is likely that triangle size uniformity, or lack thereof, has a significant impact on resultant metrics: in uniform meshes, all portions of the tooth contribute equally to the topographic calculation, but in non-uniform meshes, some portions of the tooth (i.e., those with smaller triangles) contribute to topographic calculation more than other portions of the tooth (i.e., those with larger triangles). Although not quantified in this study, we noticed that the smoothing tools resulting in more uniform meshes tended to produce results more similar to Avizo. This suggests that Avizo's smoothing algorithm, based on the principle of lambda-connectedness (Chen et al., 2000), also promotes triangle uniformity. While no conclusions about the relative importance of triangle uniformity can be drawn from this study, it may be a valuable avenue for future research.

Interestingly, a disproportionate number of studies using proprietary mesh preparation methods are still focused on primate teeth, while those using freeware methods of mesh preparation focus on non-primate teeth (López-Aguirre et al., 2022; Melstrom et al., 2021; Melstrom & Wistort, 2021; Rannikko et al., 2020; Renaud et al., 2018). The freeware workflow proposed in this study may therefore further increase the accessibility of DTA for non-primate taxa.

Although no single, standardized DTA workflow exists, greater similarity in workflows has allowed for easier interpretation of results and inter-study comparisons. It is the authors' hope that the present study will provide a methodological baseline to encourage more reliable inter-study comparisons between different freeware studies, and between freeware and proprietary studies.

As with any study, ours has limitations. We sampled from a single order of mammals, and the shape variation in our sample is relatively small compared to intertaxonomic variability. We also used a relatively small number of unworn teeth and did not investigate the effect of sex on our results. Time constraints meant we did not investigate all possible programs or topographic parameters.

We used the Bonferroni correction to reduce probability of Type I error. When comparing populations, the Bonferroni correction reduces the likelihood of finding differences between populations. As we are performing several comparisons using the same dataset, a post-hoc correction factor is needed. However, here, we are not interested in differences, but rather similarities—that is, which method produces the most statistically insignificant results. The use of the Bonferroni correction may therefore be problematic, as it will make more programs seem similar to Avizo than is actually true.

5 | CONCLUSION

Our results demonstrate that freeware methods of mesh preparation can produce results comparable to those obtained using proprietary software, enabling DTA to be accessible to a wider range of researchers. When reviewing the literature, we noted a wide range of methodologies employed by different studies for mesh preparation. Our results suggest establishment of one or more standardized workflow(s) for DTA is important, both for inter-study comparability and study longevity. Having a standardized, freeware workflow, such as the one proposed in the current study, promotes inclusive, inter-study comparability.

AUTHOR CONTRIBUTIONS

Matthew John Morley: Conceptualization (equal); data curation (lead); formal analysis (lead); investigation (lead); methodology (lead); project administration (lead); writing – original draft (lead); writing – review and editing (supporting). **Michael Berthaume:** Conceptualization (equal); funding acquisition (lead); methodology (supporting); project administration (supporting); supervision (lead); writing – review and editing (lead).

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in LSBU Open Research at https://doi.org/10.18744/lsbu.93322 (Morley & Berthaume, 2023).

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SUPPORTING INFORMATION

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