Supplementary material

Technical and conceptual considerations for using animated stimuli in studies of animal behavior

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Table S1: List of studies cited in the main text, as well as additional selected examplar studies (to complement the use of study species) representing the use of computer animations and virtual reality since the early 1990s. For each study, information on the used method, the taxonomic study group, and the research topic are highlighted. For brevity, only the first author is given in the first column. Detailed references are provided at the end of the supplementary material. 2D = two-dimensional computer animation, 3D = three-dimensional computer animation, VR = virtual reality, M = mammals, B = birds, R = reptiles, A = amphibians, F = fish, I = insects, S = spiders. Different shades of grey are used to visually distinguish between 'method', 'taxonomic study group' and 'research topic'.

Reference	Method			Taxonomic study group							Research topic			
				Vertebrates					Invertebrates		Sexual signaling	Group forming	Navigation	Perception/ recognition
	2D	3D	VR	M	B	R	A	F	I	S				
Abaid, 2012	■							■				■		
Amcoff, 2013	■							■			■			
Baldauf, 2009, 2010, 2011	■							■			■			
Butkowski, 2011		■						■			■			
Campbell, 2009		■		■										■
Clark, 1999	■							■				■		
Culumber, 2013		■						■			■			
Dolins, 2014			■	■									■	
Egger, 2011	■							■			■			
Fischer, 2014	■							■						■
Fry, 2008			■						■					■
Gerlai, 2009	■							■						■
Gray, 2002			■						■					■
Harland, 2002		■								■				■
Hess, 2016	■							■						■
Hiermes, 2016		■						■			■			
Hölscher, 2005			■	■										■
Ioannou, 2012	■							■				■		
Künzler, 1998		■						■			■			
Levy, 2014	■							■			■			
Makowicz, 2010	■							■			■			

Mazzi, 2003														
McKinnon, 1996														
Mehlis, 2008														
Moffat, 1998														
Morris, 2003														
Nakayasu, 2014														
Neave, 2011														
Nelson, 2006														
Nelson, 2010														
Ord, 2002														
Parr, 2008														
Pather, 2009														
Peckmezian, 2015														
Peters, 2007														
Qin, 2014														
Reichert, 2014														
Robinson, 2010														
Rosenthal, 1998														
Rosenthal, 2004														
Rosenthal, 2005														
Roster, 1995														
Tedore, 2013														
Tedore, 2015														
Thurley, 2014														
Thünken, 2014														
Van Dyk, 2008														
Watanabe, 2006														
Wong, 2006														
Woo, 2012, 2015														
Zbinden, 2004														
Total	**21**	**24**	7	7	1	5	3	**28**	2	5	**22**	6	4	**23**

Table S2: List of selected programs (current as of 16 March 2016) for creating and presenting 2D and 3D stimuli as well as VR. Free software is given in italics.

Software	Website	Brief description
Design and animation of 2D stimuli		
Adobe Photoshop[1,2]	http://www.adobe.com/	State-of-the-art raster graphics editing program for print and web; elaborate layer processing; support for many file formats including RAW; *30-day free trial version*
Adobe After Effects[1] or Adobe Animate (formerly Adobe Flash)	http://www.adobe.com/	Animation program for 2D .psd image files prior modified with Adobe Photoshop; video compositing in After Effects; *30-day free trial version*
GIMP with *GIMP Animation Package*[2]	http://www.gimp.org/	2D raster and vector graphics editing program; *GIMP animation package* is a plugin that enables animation of images as a sequence of single frames; video editing (frame by frame); offers many of the same features as Adobe Photoshop with lower memory requirements; working with different layers possible; supports many file formats including RAW
MS PowerPoint[3]	https://www.microsoft.com/	Presentation program with limited 2D raster and vector graphics editing and

		animation capabilities
Pencil2D[4]	http://www.pencil2d.org	Realistic sketching program suited also for 2D animation; allows both creation and animation of raster and vector graphics based on keyframing timeline; alternative to Adobe Flash
Design and animation of 3D stimuli (generally also applicable for 2D animation)		
3D Studio Max	http://www.autodesk.com/	Most commonly used professional 3D modelling tool to design, visualize, and animate 3D objects and environments; animation by keyframing; more user-friendly interface than Maya; available for Windows only; *free 30-day trial version*
anyFish 2.0[5]	http://swordtail.tamu.edu/anyfish/	Generate, animate (keyframing), and share 3D fish models, for fish biologists; currently supports models for sticklebacks and poeciliid fishes
Blender[6,7]	https://www.blender.org/	Design of 3D objects and environments; free alternative to Maya; offers most tools that available elsewhere; keyframing animation; supports Python scripts; game engine included (see below); open-source
LightWave 3D[8]	https://www.lightwave3d.com/	Professional modelling and animation tool; supports Python scripts; LightWave's flocking system for simulating coordinated animal motion; needs less resources and memory compared to 3D Studio Max; *free 30-day trial version* and reduced educational version for students and faculty staff

Maya	http://www.autodesk.com/	Professional software for 3D modelling, rendering and animation of objects and environments; animation by keyframing; supports Python scripts; *free 30-day trial version; 3 years free for students*
Unity 3D Personal Edition[9]	http://unity3d.com/	Design of 3D objects and environments; game engine included (see below)
Game engine		
Blender game engine[6]	https://www.blender.org/	2D/3D game engine written in the programming languages C, C++ and Python; included in *Blender* main software (see above); supports real-time rendering and external input devices
Irrlicht[6]	http://irrlicht.sourceforge.net/	2D/3D game engine developed by a small independent developer team; written in C++; using D3D, OpenGL; enables real-time rendering and external input devices
Unity 3D Engine[10]	http://unity3d.com/	Advanced 2D/3D and VR development platform written in C#, JavaScript or Boo; included in *Unity 3D Personal Edition*; supports real-time rendering and external input devices; game development for mobile devices
Design and animation of VR		
Unity 3D Engine	http://unity3d.com/	Advanced 2D/3D and development platform for VR (head mounting devices)

		included in *Unity 3D Personal Edition* (see above).
WorldViz[7]	http://www.worldviz.com/	Professional distributor for VR software and entire VR setup solutions
FreemooVR[11]	https://github.com/freemoovr	Open-source VR software for freely moving animals
Tracking software		
BIOBSERVE[12]	http://www.biobserve.com/	Company that develops software and hardware for behavioral experiments including tracking, recording and analyzing of behavior and path tracking in 2D and 3D
EthoVision XT (Noldus)[13]	http://www.noldus.com/	Software for automated video tracking (2D) and behavior analysis software specifically designed for animal behavior research; Track3D add-on available for 3D tracking; *free 30-day trial version*

[1]Tedore and Johnsen (2015); [2]Baldauf et al., (2011); [3]Fischer et al., (2014); [4]Makowicz et al., (2016); [5]Culumber and Rosenthal (2013); [6]Müller et al., (2017); [7]Thurley et al., (2014); [8]Woo (2007); [9]Ingley et al., (2015); [10]Peckmezian and Taylor (2015); [11]Stowers et al. (2014); [12]Butkowski et al., (2011); [13]Fry et al., (2008).

Table S3: List of useful online expert-written resources concerning the issue of latency with VR.

The John Carmack Blog (well-known American game developer)
• http://oculusrift-blog.com/john-carmacks-message-of-latency/682/   • https://www.twentymilliseconds.com/post/latency-mitigation-strategies/
Occulus and Valve Blogs (prominent VR and game development studios)
• http://blogs.valvesoftware.com/abrash/latency-the-sine-qua-non-of-ar-and-vr/   • https://developer.oculus.com/blog/the-latent-power-of-prediction/
Gizmondo
• http://gizmodo.com/the-neuroscience-of-why-vr-still-sucks-1691909123

Box S1. Software example for design and animation of 3D fish stimuli for biologists.

***anyFish* 2.0**

One obstacle to using animations in behavioral research is the difficulty and financial cost often associated with using advanced animation software programs (e.g. Maya). Recently, efforts have been made to create a free, open-source, user-friendly software platform for generating animations of fish for behavioral research (Veen et al., 2013; Ingley et al., 2015). '*anyFish*' is the result of this effort, and provides a model for transparency, repeatability, and collaboration in the field of animal behavior. *anyFish* provides an excellent means to create high-quality fish stimuli for behavioral research, requires only basic computational equipment to rapidly and repeatedly create animations, and is completely free and open-source, facilitating user guided changes to improve or customize the program according to their needs.

To create an animation, *anyFish* uses lateral images of fish as an input, and incorporates modern geometric morphometric methods to accurately model fish shape. Images are used to quantify body shape and provide a texture for the final 3D model. *anyFish* allows users to map fin and body textures independently, providing added flexibility in experimental design, e.g., the appearance of the fins can be manipulated independently of the appearance of the body (Culumber and Rosenthal, 2013). The shape of the model can be manipulated easily by changing the position of digitized landmarks that are applied to lateral images of the fish. By doing so, the user can manipulate body and fin shape even beyond morphological variation found in nature. The shape of the model can also be determined by generating an average body shape of a subsample of fish, and different sets of fish could be used to create multiple stimuli and avoid pseudoreplication.

The *anyFish* platform allows users to create animations of behavioral sequences using three different approaches. First, users can create an animation de novo by keyframing the animated rig in the X, Y, and Z-axes. Second, users can use a rotoscoping technique, wherein the model is matched to a video of a behavioral sequence frame by frame. Finally, motion capture data from third-party tracking systems can be used to determine the animated fish's swimming path. A major benefit of the *anyFish* workflow is that project folders can easily be shared amongst collaborators or via online data repositories (e.g., Dryad). This increases the ease of collaboration and provides added transparency and repeatability. In summary, *anyFish* provides a unique, albeit not always user-friendly, approach to creating animations for behavioral studies for fish.

Supplementary references

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