

## ***anyFish*: an open-source software to generate animated fish models for behavioural studies**

Thor Veen<sup>1</sup>, Spencer J. Ingley<sup>2</sup>, Rongfeng Cui<sup>3</sup>, Jon Simpson<sup>4</sup>,  
Mohammad Rahmani Asl<sup>4</sup>, Ji Zhang<sup>5</sup>, Trisha Butkowski<sup>4</sup>, Wen Li<sup>5</sup>,  
Chelsea Hash<sup>6</sup>, Jerald B. Johnson<sup>2</sup>, Wei Yan<sup>4</sup> and Gil G. Rosenthal<sup>3</sup>

<sup>1</sup>*Biodiversity Research Centre, University of British Columbia, Vancouver, British Columbia, Canada,*

<sup>2</sup>*Evolutionary Ecology Laboratories, Department of Biology, Brigham Young University, Provo, Utah, USA,* <sup>3</sup>*Department of Biology, Texas A&M University, College Station, Texas, USA,*

<sup>4</sup>*College of Architecture, Texas A&M University, College Station, Texas, USA,*

<sup>5</sup>*Department of Computer Science, Texas A&M University, College Station, Texas, USA*

*and* <sup>6</sup>*Lively Disposition, Cohoes, New York, USA*

---

### **ABSTRACT**

**Problem:** Using an experimental approach to study behaviours based on visual signals is severely limited due to the difficulty of combining realistic models (e.g. live fish) with the manipulation of signals in isolation.

**Solution:** Computer animations allow the manipulation of a single cue while maintaining the rest of the behavioural phenotype of a realistic three-dimensional (3D) model.

**Software:** We introduce the open-source software *anyFish* for the creation of 3D-animated fish. Both the animated model and its behaviour can be modified by the end-user to suit specific needs.

**Applications:** Computer-animated fish facilitate the identification of factors influencing behaviours based on visual cues, and ultimately the way they both drive and respond to selection. For our research, we vary nuptial colour and size and shape of animated male stickleback to quantify female choice for these characters. The software has many other applications, as other fish species can be animated and characters like swimming speed and direction can be manipulated as well.

*Keywords:* communication, computer animation, *Gasterosteus*, playback, stickleback, visual signals.

### **INTRODUCTION**

Animal communication is fundamental to a host of interactions within and among species, ranging from interspecific cooperation and competition, through mate choice and coordinated hunting, to provisioning of offspring by parents (Bradbury and Vehrencamp, 2011).

---

Correspondence: T. Veen, Biodiversity Research Centre, University of British Columbia, 2212 Main Mall, Vancouver, BC V6T 1Z4, Canada. e-mail: veen@biodiversity.ubc.ca

Consult the copyright statement on the inside front cover for non-commercial copying policies.

---

Communication can be very complex, involving multiple signals in different sensory modalities influenced by both the social and ecological environment (e.g. Bro-Jørgensen, 2010; Bretman *et al.*, 2011). Understanding the mechanisms, evolution, and fitness consequences of communicative interactions requires the ability to experimentally manipulate and present signals in a controlled manner. Using the natural variation of phenotypes may give the researcher access to a wide spectrum of signals but this approach is limited, as signals are not decoupled from correlated traits potentially used in the same communication context. For decades, researchers have used audio playback to study acoustic communication. Our understanding of acoustic communication has been greatly facilitated by the ability to perform quantitative manipulations of songs and calls, and conduct playbacks to animals in field or laboratory contexts (e.g. Shaw and Lesnick, 2009; Ramage-Healey *et al.*, 2010; Akre *et al.*, 2011). Individual signal components can be removed or exaggerated, and workers can create entirely artificial signals mimicking supernormal stimuli (Drăgănoiu *et al.*, 2002) or hypothesized ancestral states (Phelps *et al.*, 2001).

While acoustic communication is restricted to a few taxa, visual communication is ubiquitous, and sexual diphenism (Andersson, 1994; Gray and McKinnon, 2007) and speciation (e.g. Schluter, 2000; Coyne and Orr, 2004; Ritchie 2007) are often accompanied by divergence in complex visual signals. Understanding how receiver behaviour shapes signal evolution, and more generally the role that visual communication plays in evolutionary processes, requires the multivariate manipulation of signals within and outside their existing range and the decoupling of correlated traits.

Many studies have accomplished this via playback of manipulated video stimuli, which combines the advantage of realistic images with the opportunity to manipulate signal components (Rosenthal and Evans, 1998; Langerhans *et al.*, 2005). This is a major step forward and has been applied successfully across a variety of questions and organisms (for reviews, see Rosenthal, 2000; Woo and Rieucou, 2011). Manipulating signals is time-consuming, however, and the experimenter has limited control over many aspects (e.g. swimming speed) of the focal individual in the animation. In recent years, synthetic computer animations of visual signals have offered a resolution to these methodological problems. The use of animations has proven useful in manipulating visual signals in multiple systems, including spiders (Harland and Jackson, 2002), birds (Watanabe and Troje, 2006), lizards (Ord *et al.*, 2002), and in particular fishes, including cichlids (Baldauf *et al.*, 2009), poeciliids (e.g. Fisher *et al.*, 2006, 2009; Wong and Rosenthal 2006; Verzijden and Rosenthal, 2011), and stickleback (McKinnon and McPhail, 1996; Künzler and Bakker, 1998; for a review, see Woo and Rieucou, 2011).

There are three major limitations to the use of computer animations in animal behaviour. The first is that many interesting visual displays are complex and currently require the use of sophisticated animation software, which demands specialized technical expertise. A prime example is the courtship behaviours of fish, which are often comprised of ritualized swimming patterns in concert with morphological traits such as fins or coloration, interacting with the distribution of incident light and contrasting with the background (Rosenthal *et al.*, 1996; Rosenthal and Lobel, 2005). Second, animations present very different visual stimuli relative to the natural stimuli they are attempting to mimic, particularly with regard to colour fidelity (Fleishman and Endler, 2000) and depth cues (Zeil, 2000). Some of these problems, including the absence of light in the ultraviolet range and the absence of three-dimensional depth cues, are constraints of currently available output systems (computer monitors), but signal fidelity can be enhanced by incorporating information about animal visual perception into the stimuli that are presented. Specifically, Fleishman *et al.* (1998) propose an

algorithm for correcting monitor white balance to account for species-specific differences in visual physiology, and Zeil (2000) suggests several ways in which appropriate depth cues can be represented in a two-dimensional image. Third, the difficulties inherent in constructing animations mean that exemplars are often based on representative behaviour of a single individual. If only one animation based on a single individual is used, the trials will not be independent and testing for effects of manipulation will lead to pseudo-replication [i.e. ‘the use of inferential statistics to test for treatment effects with data from experiments where either the treatments are not replicates (though samples may be) or replicates are not statistically independent’ (Hulbert, 1984, p. 187; McGregor, 2000)]. Using multiple individuals and behavioural sequences avoids this problem but is currently time consuming.

Here we introduce an open-source program, *anyFish*, which addresses many of the above concerns. The program produces custom-made animations of fishes based on biological data. *anyFish* can currently be applied to a range of model fish species; we focus here on *Gasterosteus stickleback* as an illustrative example.

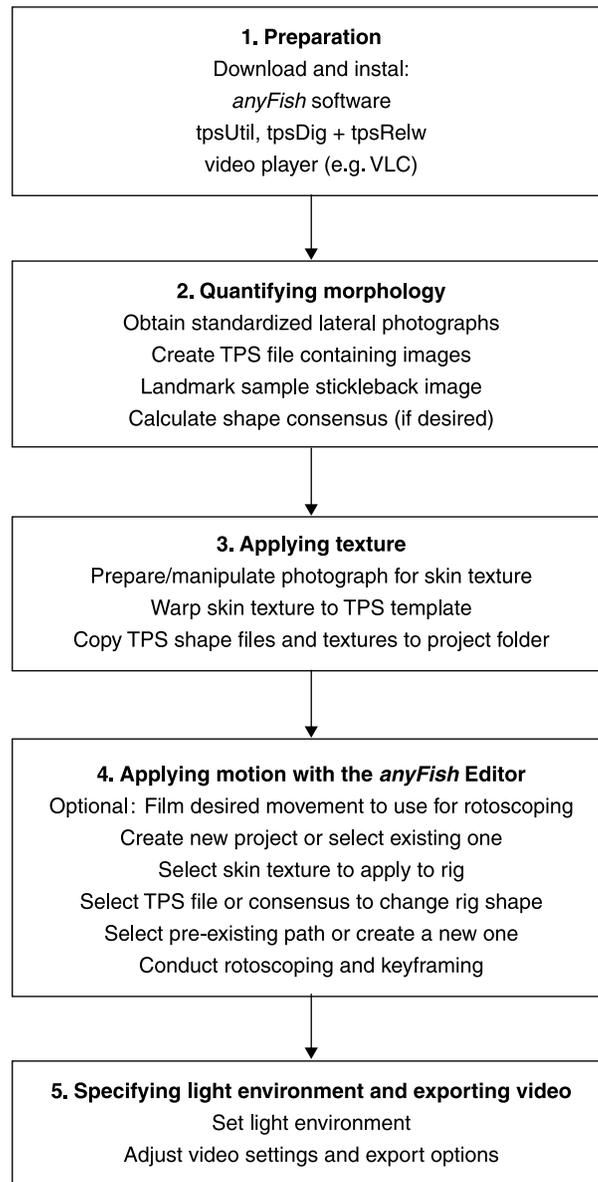
Possibly the first playback experiments in animal behaviour were Tinbergen’s (1951) use of wooden dummies to study aggressive communication in threespine stickleback. A number of studies used manipulated video playback (McKinnon, 1995; Rowland, 1995). Shortly after, McKinnon and McPhail (1996) and Theo Bakker and colleagues developed animated threespine stickleback (e.g. Künzler and Bakker, 1998). Variations of these animations were used to study female mating preferences for male courtship intensity, size (Künzler and Bakker, 2001), and asymmetries in spine length (Mazzi *et al.*, 2004), the effects of male courtship and body size on ejaculate size (Zbinden *et al.*, 2003, 2004), male aggression (McKinnon and McPhail, 1996), and kin recognition (Mehlis *et al.*, 2008). These studies showed that animations can be successfully used with stickleback and that relatively small differences in morphological traits (e.g. spine length) resulted in measurable behavioural preferences. Stickleback therefore present an excellent opportunity to provide a proof of concept for the *anyFish* animations, by which the responses to animations can be ground-truthed by replicating earlier experiments and comparing the results. After confirming the response of stickleback fish to animated stimuli, these animations can be used to investigate previously difficult (or impossible) experimental questions, such as quantifying female preferences for nuptial colour outside their natural range or for exaggerated body size and/or shape [e.g. for size in the benthic–limnetic species pair (Nagel and Schluter, 1998; Boughman *et al.*, 2005; Conte and Schluter, 2013), the marine–stream species pair (McKinnon *et al.*, 2004), and the Japanese species pair (Kitano *et al.*, 2009); for nuptial colour in the benthic–limnetic species pair (Boughman, 2001; Albert *et al.*, 2007), freshwater (Bakker, 1993; Kraak *et al.*, 1999) and marine and freshwater populations (Rowland, 1994; McKinnon, 1995)].

In the following sections, we describe the workflow of *anyFish*, paying specific attention to the methods we used to produce custom animations of a courting male stickleback representing mean body shape of benthic and limnetic males from Paxton Lake and variation in throat colour from a freshwater population in Vancouver (both locations in British Columbia, Canada). We then describe a variety of questions for which animations are a well-suited methodology, and highlight both the potential for and possible limitations of using computer-generated animations in studies of stickleback behaviour and discuss possible future extensions of *anyFish*.

## METHODS

### Generating animations

In the following sections, we outline the steps required to create a three-dimensional (3D) animated stickleback model, how to animate motion, and how the animated background used in the stimulus is constructed (Fig. 1). The *anyFish* editor is available for download on



**Fig. 1.** A flow diagram outlining the steps required to construct a stickleback animation.

**Table 1.** Recommendations for programs, other than *anyFish*, needed to perform tasks required to create an animation (see text for details)

Task	Software	URL
Image manipulation	Adobe Photoshop	www.adobe.com
	GIMP*	www.gimp.org
	TPS-transform*	http://swordtail.tamu.edu/anyfish
Morphometrics	tpsDig*	http://life.bio.sunysb.edu/morph
	tpsRelw*	http://life.bio.sunysb.edu/morph
Creating and playback video	Adobe Premiere	www.adobe.com
	VLC*	www.videolan.org

\*Free of charge (e.g. freeware, open-source).

the *anyFish* website (<http://swordtail.tamu.edu/anyfish>). To generate animated scenes, *anyFish* requires standard thin-plate spline (TPS) morphometric measurements (Rohlf, 2004, 2008, 2009) and digital images. Programs for creating and manipulating these items are commonly used among biologists and are listed in Table 1.

#### *Step 1: Preparation*

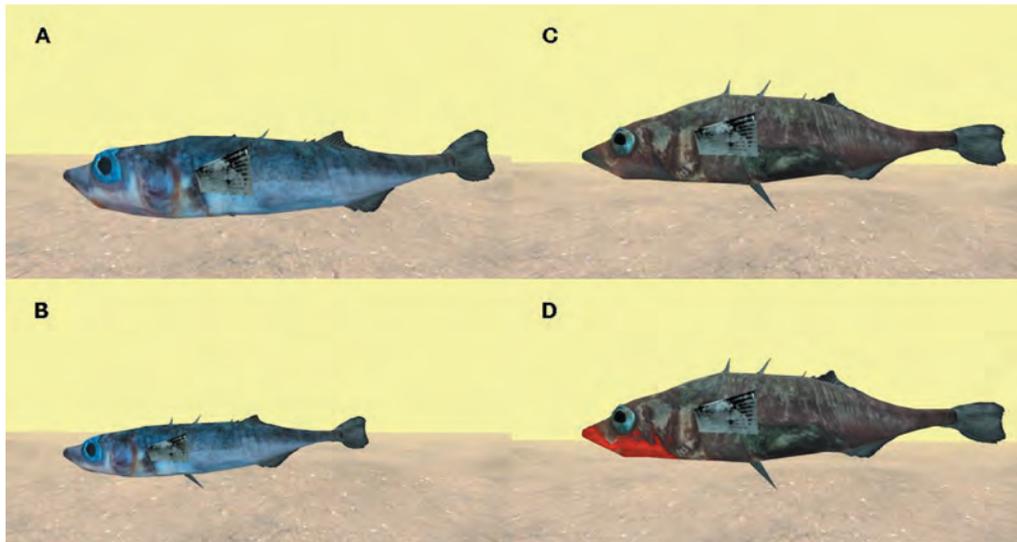
Download and instal the *anyFish* editor and additional programs, one for each task as outlined in Table 1. The *anyFish* editor loads several pre-defined fish templates, including one for stickleback fishes. Technical details on the construction of the model are available on the *anyFish* website (<http://swordtail.tamu.edu/anyfish>). The end user can manipulate the shape, texture (skin and fin colour pattern), and behaviour of this base model.

#### *Step 2: Quantifying morphology*

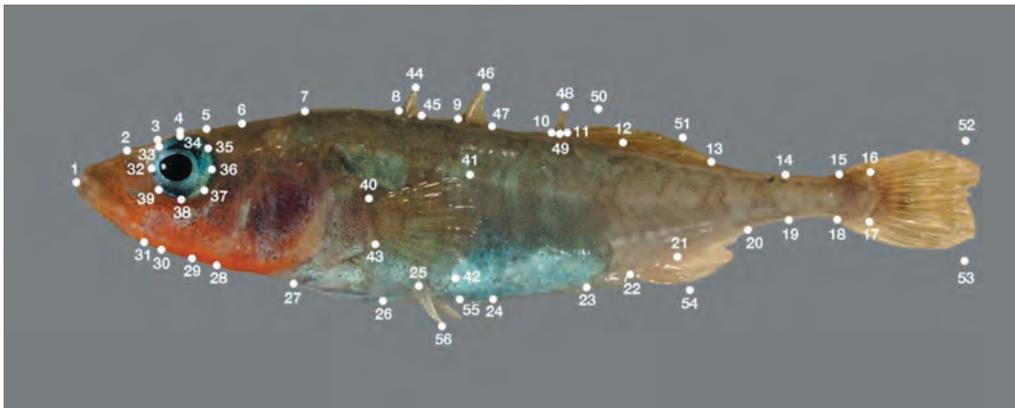
Much of the power behind *anyFish* is the ability for the end user to manipulate the appearance of the animated fish. *anyFish* comes with a generic stickleback build, which is a 'digital skeleton' – a model of the three-dimensional structure of a stickleback. This build is constructed using measurements from real fish but the shape and size can be adjusted in two general steps as outlined below. Figure 2 illustrates such variations for a typical limnetic and benthic stickleback from Paxton Lake, British Columbia, Canada.

We used standard methods from geometric morphometrics (for reviews, see Bookstein, 1996; Claude, 2008; applied to sticklebacks in, for example, Walker, 1997; Aguirre and Bell, 2012) to quantify body shape. First, we acquired standardized digital images. These photographs were used both to obtain morphological data and as skin textures to be applied to the rig (Step 3). We took digital photos of the left side of the stickleback in the same position and under standardized lighting conditions using a Nikon D300 with a Nikkor AF-S 60mm F2.8 G Micro ED lens and Nikon speedlight SB-R200 wireless flash ([www.nikon.com](http://www.nikon.com)). A size standard (e.g. a ruler) was visible in each photograph so as to apply a scale factor. We used a QPcard 201 colour standard ([www.qpcard.com](http://www.qpcard.com)). We saved images in the raw format to optimize image manipulation, but saved them as jpeg images using QPcolorsoft 501 before assigning landmarks using the TPS software.

The second step required to create a stickleback animation is to generate TPS files that contain morphological landmark data. To capture the key morphological features of the



**Fig. 2.** Animations of a typical benthic (A) and limnetic (B) stickleback from Paxton Lake. Colour-manipulated throats of an animated male stickleback from a freshwater population in Vancouver are depicted in (C) and (D). Colour manipulation was conducted in two steps. First, all the pixels of a predefined throat area of the male were assessed independently and those exceeding a threshold 'redness' value (as measured by the R value of the RGB score of an individual pixel) selected. In the second step, the R value was set to one of five predetermined values, ranging from 85 (C) to 255 (D). The G and B values were set to be equal and together with R sum to 255 by definition.



**Fig. 3.** Landmarks needed for the morphological analysis. Landmarks 52 and 53 are for the tips of a fully extended caudal fin.

stickleback, including fin size and shape, we assigned a set of 56 landmarks (i.e. morphological points on the lateral image of the fish; Fig. 3). The homologous landmarks are identified on the stickleback build. It is important to note that all landmarks in the sequence provided are required to change the generic stickleback build to make it match the TPS file coordinates of the target individual. The location of some of the landmarks is taxon-

specific. For example, stickleback vary in the number of dorsal spines, but a programmer can easily modify these. To apply this set of landmarks to each image, *tpsUtil* (Rohlf, 2004) was used to create a TPS file containing all the photos of interest. *tpsDig* (Rohlf, 2009) was then used to set the coordinates of each landmark for each image of interest. It is crucial that each landmark is correctly placed on each fish image, and that these landmarks are applied in the correct order for each image. Several landmarks reference the position of a trait, such as fin insertions. When traits are not fully visible in the photograph, for example if the tail is worn or not fully fanned, the position of the landmarks should be interpolated (e.g. landmark 52 and 53 in Fig. 3). The TPS files that are generated during this step are used to reshape the stickleback build to create the desired animated fish. It is important to note that as long as the TPS points are in consistent order, any set of coordinates can serve as TPS input (i.e. the coordinates do not need to come from landmarks of an individual fish). For example, it may be desirable to apply a shape that represents a population average or a hypothetical ancestor. Such consensus shapes can be calculated using *tpsRelw* (Rohlf, 2008). The TPS file is also used in Step 3, below, to ‘warp’ images to a standard TPS template for use as textures in the *anyFish* editor (see Step 3 for details). Multiple TPS files can be loaded into the *anyFish* editor to change the shape of the animated fish. This feature provides the flexibility that is required to create a variety of stimuli. To use either specific TPS files or a population consensus TPS file, the end user should copy these files to the ‘TPS’ folder inside the ‘Projects’ folder that comes with the *anyFish* editor download package.

#### *Step 3: Applying texture*

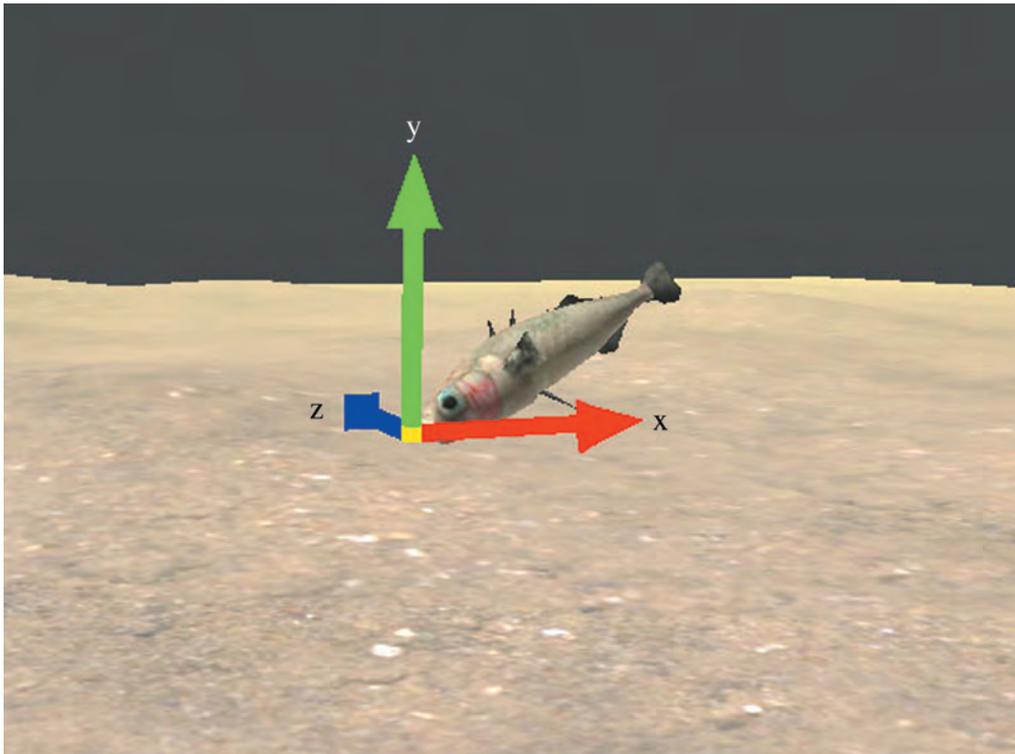
We selected photos to be used as skin texture and used image manipulation programs (e.g. Adobe Photoshop) to remove or eliminate specular highlights, and manipulate colour patterns as desired (e.g. change the intensity of the nuptial colour). Any image can be used as long as the appropriate landmarks can be placed on the image. In later steps, this will allow the end user to select among different textures for their animation. These textures can be of different individuals for proper replication and/or manipulated, e.g. the colour of the throat of males (Fig. 2). The next step requires a novel program that we have developed, *TPS-transform* (M.R. Asl and W. Yan, unpublished data), which is installed along with *anyFish* by default or freely available from the *anyFish* website. For the purposes of *anyFish*, *TPS-transform* is used to warp photos that the end user desires to use as skin textures to conform to a default stickleback TPS template. The template is a TPS file that is provided in the ‘Projects’ folder of the *anyFish* editor download package. For an image to be used as a texture, the end user must open *TPS-transform*, select the desired image, select the image’s corresponding TPS file, and finally select the stickleback TPS template from the *anyFish* editor project folder. *TPS-transform* will warp the fish images to match the stickleback TPS template, and prepare them for use as textures in the *anyFish* editor. Once this process is complete, the texture images should be copied to the ‘Texture’ folder found within the ‘Projects’ folder that comes with the *anyFish* editor download package.

#### *Step 4: Applying motion with the anyFish editor*

Once the above steps are completed, the end user is ready to create an animation using the *anyFish* editor. Upon opening *anyFish*, the end user will be able to select a pre-existing project or create a new one. A menu is provided in which the end user can specify all of the key features of the animation, including the TPS files and textures created in Steps 2 and 3 above. *anyFish* comes preloaded with a generic stickleback build, or digital skeleton.

However, the open-source nature of *anyFish* allows for creation of new builds if desired. The generic build has a series of springs situated in the central part of the fish, resembling a backbone. The springs are stiff and are activated sequentially (e.g. a movement starts at the anterior side and moves towards the posterior side). The resistance varies among springs to simulate labriform swimming. The user can adjust the spring resistance to better match the motor patterns he or she is modelling; future versions of *anyFish* will explicitly use motion capture data to determine spring parameters. The shape of the build is specified by the TPS files that the end user created during Step 2 above. These TPS files should be copied to the Project TPS folder, and can consist of either population consensus files or a specific TPS file from a given individual. The skin textures, which should be copied to the 'Textures' folder for a given project, are those stickleback images described in Step 3.

To specify movement, the end user can either select and modify a pre-existing path or create a new one. Three standard movement paths are currently included as defaults: the 'zigzag' courtship swim of a male towards the female, the subsequent 'heads-down' position towards the nest, and random swimming. The end user can either select and modify these behaviours or model novel swimming patterns as desired. To create a new path, the end user can manually adjust the position of the model in the x, y, and z-axes, as well as rotating the model in all three axes (Fig. 4). The movement pattern can be done *de novo*, or if desired, the end user can record video of the movement of interest and import the frames from the video



**Fig. 4.** The stickleback model with three levers to adjust its position in a three-dimensional space (y = green, x = red, and z = blue). The position of the stickleback model will change by dragging the levers.

to manually match the position of the stickleback model with that of the stickleback in the video footage (i.e. 'rotoscoping'). More detailed information regarding this option is available on the *anyFish* website. In short, the video should have the individual visible both from the side and top of the tank, which can be achieved by positioning a mirror at a 90° angle above the tank. The background should be uniform and ideally blue to improve contrast. In the *anyFish* editor, the end user can load the frames from the video sequence and manually match the position of the stickleback model with that of the stickleback in the video footage (i.e. 'rotoscoping'). This process does not require that the end user specify the position of the model for each frame. By 'keyframing', the end user can assign the fish position at intervals and the physics system built into the model will interpolate the model's position. Rotoscoping allows the end user to make as many replicas of movement from different video segments as needed, or create animation segments entirely *de novo*. Alternatively, existing paths can be modified to add user-controlled variation or vary a key component of behaviour, e.g. the number of zigzags performed in each sequence. An image sequence can be produced for the rotoscoped sequence of movements that can be used to create video sequences using *anyFish-VM*.

#### *Step 5: Specifying light environment and exporting video*

Visual communication depends critically on the spatial, temporal, and wavelength distribution of downwelling light and of background elements (Rosenthal, 2007). *anyFish* allows the end user to manipulate both of these parameters. In the standard *anyFish* scene, most of the downwelling light comes from two directional lights at opposite angles. The intensity of the directional light source providing upwelling light is lowered to simulate global illumination. The colour and brightness of each of these sources are simulated from irradiance measurements provided by the user. To simulate turbidity, the user can provide overall colour and brightness of a distance-based fog. The background terrain is constructed by using a height map to simulate variation in elevation. The nest is based on a partly transparent image of a real nest.

In the last step, the animation can be exported to video using *anyFish-VM*, which provides options to the user to choose the appropriate frame rate and specify whether the exported video contains one single or two side-by-side animation sequences. The video sequences can subsequently be edited in video editing software (Table 1) to make longer sequences or link different movements into one sequence (e.g. zig-zag, heads-down, and swim around for a male courtship sequence).

Colour reproduction on computer and video monitors involves combinations of red, green, and blue (RGB) light mixed in different combinations to create the perceptual illusion of colour. By default, the mapping of RGB to perceived colour is based on standard CIE colour-space viewer, which assumes human spectral sensitivity. The manipulation of colour in playback experiments thus poses special challenges (D'Eath, 1998). One option is to use monochrome monitors and manipulate the spectral distribution by overlaying colour filters (Kingston *et al.*, 2003). Alternatively, monitor white balance – the relative intensity of red, green, and blue emissions – can be manipulated to take into account receiver visual sensitivity, as detailed by Fleishman and Endler (2000). Forthcoming versions of *anyFish* will provide the option to globally adjust white balance given data on spectral sensitivity and on monitor output characteristics.

## DISCUSSION

The *anyFish* program makes the construction and sophisticated manipulation of computer animations accessible to researchers working on stickleback. We see several research areas for which stickleback animations can provide a powerful methodology.

Mating behaviour is intensively studied in stickleback (for a review, see Huntingford and Ruiz-Gomez, 2009) and animations provide new possibilities to extend the pioneering work by Bakker and colleagues and incorporate other sexual traits such as red pelvic spines in threespine (Yong *et al.*, 2013) and even brook stickleback (Hodgson *et al.*, 2013). Animations can focus on the role of a single signal, but also the interaction of multiple signals if needed. Although multiple sexual signals are widespread in nature (Candolin, 2003), they are hard to study experimentally due to the difficulty of manipulating the traits of interest in isolation. A good example is the role of red pelvic spines and how they covary with other traits such as the red throat (Yong *et al.*, 2013). Size and shape have been suggested to be important factors in causing reproductive isolation between stickleback populations (Nagel and Schluter, 1998; McKinnon *et al.*, 2004; Boughman *et al.*, 2005; Vines and Schluter, 2006; Kitano *et al.*, 2009). Size can be manipulated by regulating the density of juvenile fish during rearing (McKinnon *et al.*, 2004; Conte and Schluter, 2013) or through cross-breeding individuals from different populations. These methods are time-consuming, costly, and may have undesirable side-effects, which may be avoided by using animations.

The visual system underlying the processing of visual signals may influence signal-receiver dynamics and their evolutionary trajectory. Males may exploit pre-existing sensitivities of the visual system to increase their mating probability (Ryan and Rand, 1990; Hodgson *et al.*, 2013). Such biases can be quantified through systematic manipulation of the reflectance properties of the animation (even outside their natural range) and measuring the response of the females. The scope for sensory exploitation can be investigated from a more mechanistic angle by modelling the spectral sensitivity through linking opsin expression with their specific spectral absorbance (Govardovski *et al.*, 2000; Rowe *et al.*, 2004), which can be compared with the female's response to animated males differing in their reflectance. This approach can be relevant for species divergence as hypothesized by Rowe *et al.* (2004). Differential expression of sets of cones can create a situation where different signal functions [e.g. intraspecific mate choice and species recognition (Pfennig, 1998)] can be combined, which would increase reproductive isolation. We aim to incorporate the information on spectral absorbance and make it easily adjustable in a future version of *anyFish*. The light environment affects the way in which signals are perceived, and thus only focusing on the reflectance of the body of the stickleback may be biased (Rosenthal, 2007). Animations provide a way to experimentally test how strong the effect of, for example, background matching (Clarke and Schluter, 2011) is by changing both the environmental light conditions and the reflectance properties of the stickleback model in the animation.

The use of animations can be extended to include behaviours other than mate choice, such as male–male aggression (Candolin, 1999), as pioneered by McKinnon and McPhail (1996). Intra-sexual interactions can be studied in a similar way as female choice. Shoaling or sociability assays (Wark *et al.*, 2011) may benefit from using animations, as the stimulus of the environment can be standardized while retaining a biologically relevant context (e.g. a group of conspecifics). Spatial learning is important in stickleback and depends on foraging ecology (Park, 2013) and landmarks in their environment (Odling-Smee and Braithwaite, 2003). Furthermore, social cues such as the presence of conspecific stickleback may influence the

decision-making process (Nomakuchi *et al.*, 2009) and animations may provide a novel way to standardize and manipulate conspecific behaviour.

Using animations is not without its limitations (reviewed in depth by Woo and Rieucou, 2011). Animations are restricted to the visual modality, although they can be paired with acoustic, olfactory, and possibly even tactile signals for presentation. Care must also be taken to ensure that the visual properties of computer animations do not produce spurious results. In particular, the visual sensitivity of stickleback is different from that of humans. Stickleback have four different types of cone photoreceptors versus three in humans with the major difference the presence of a cone sensitive to the ultraviolet (UV) spectrum (Rowe *et al.*, 2004; Boulcott and Braithwaite, 2005; Rennison *et al.*, 2012). Within the human-visible range, colour output can be corrected to account for differences in cone sensitivities as discussed above, but the absence of UV output from monitor hardware (Fleishman *et al.*, 1998; Baldauf *et al.*, 2008) presents a problem. Animations using standard output equipment should not be used to address questions about receiver response to ultraviolet signals. It also causes a more fundamental problem, namely that we cannot control for interactions between UV and other signals which may change the appearance of the animated fish. However, previous experiments using animations, which differed in morphological characteristics other than colour [e.g. spine asymmetries (Mazzi *et al.*, 2004)] showed a positive response indicating a successful manipulation. Furthermore, animations consist of two-dimensional projections of a three-dimensional world. The cues used to gauge depth vary across species, and care must be taken to ensure that receivers are gauging size and apparent distance appropriately (D'Eath, 1998; Zeil, 2000). In our animation, depth cues are provided by the fish's shadow on the bottom as well as by occlusion cues. Also, only the initial stages of the courtship ritual are captured by the animations. Subsequent steps involve physical contact, such as the male biting the female (e.g. ter Pelwijk and Tinbergen, 1937). These steps may be crucial for the female to retain interest in the male, and it is therefore important to establish over what duration to test the female's preference using animations. This may vary among populations and species. Interactions between the synthetic animation and the test subject can be made more realistic by integrating real-time rendering of the animation with automated tracking of the test subject (Butkowski *et al.*, 2011).

During behavioural experiments with stickleback we noticed significant differences in behaviour, such as response to disturbance, among populations in British Columbia (Canada). This may have consequences for how individual fish respond to a new environment and animation playback. Specifically, receivers may fail to respond simply because they have not habituated to a laboratory environment. We strongly recommend validating the use of animations before starting the experiment of interest, as well as always including positive controls in the experimental design. D'Eath (1998) provides a review on and validation of video playback experiments and Künzler and Bakker (1998) and Baldauf *et al.* (2009 – for cichlids) provide useful guidance for animations.

Computer-generated animations of stickleback allow for a great level of control of morphological and behavioural characteristics. Combined with the ability to adjust the environmental light conditions and the reflectance of the stickleback model makes animation a great tool to study behaviour in general, and sensory ecology in particular. Stickleback are particularly well suited to cast this in an evolutionary context. The recent and repeated invasion of freshwater by marine stickleback provides a unique opportunity to elucidate the evolution of ecological adaptation and speciation (McKinnon and Rundle, 2002). The presence of the ancestral state, availability of high-quality genomic tools, combined

with the power of manipulating traits in isolation provided by animations make the stickleback a great candidate to study the genetic underpinning of behaviour and its evolutionary trajectory by comparing the ancestral and derived states.

### ACKNOWLEDGEMENTS

We would like to thank the Schluter lab for continuous support during practical stickleback work and providing valuable feedback, and the contributors to this special issue for discussing the use of animations in their research. We thank Arianne Albert for providing the photograph used in Fig. 3 and Andrew Hendry, Katie Peichel, and an anonymous reviewer for much appreciated comments. The enthusiasm and support of members of the stickleback research community for the *anyFish* project is much appreciated. Funding for *anyFish* software was provided by the US National Science Foundation (IOS-1045226). T.V. was supported by the NSERC-CREATE Training Program in Biodiversity Research.

### REFERENCES

- Aguirre, W.E. and Bell, M.A. 2012. Twenty years of body shape evolution in a threespine stickleback population adapting to a lake environment. *Biol. J. Linn. Soc.*, **105**: 817–831.
- Akre, K.L., Farris, H.E., Lea, A.M., Page, R.A. and Ryan, M.J. 2011. Signal perception in frogs and bats and the evolution of mating signals. *Science*, **333**: 751–752.
- Albert, A.Y.K., Millar, N.P. and Schluter, D. 2007. Character displacement of male nuptial colour in threespine sticklebacks (*Gasterosteus aculeatus*). *Biol. J. Linn. Soc.*, **91**: 37–48.
- Andersson, M. 1994. *Sexual Selection*. Princeton, NJ: Princeton University Press.
- Bakker, T.C.M. 1993. Positive genetic correlation between female preference and preferred male ornament in sticklebacks. *Nature*, **363**: 255–257.
- Baldauf, S.A., Kullmann, H. and Bakker, T.C.M. 2008. Technical restrictions of computer-manipulated visual stimuli and display units for studying animal behaviour. *Ethology*, **114**: 737–751.
- Baldauf, S.A., Kullmann, H., Thünken, T., Winter, S. and Bakker, T.C.M. 2009. Computer animation as a tool to study preferences in the cichlid *Pelvicachromis taeniatus*. *J. Fish Biol.*, **75**: 738–746.
- Bookstein, F.L. 1996. Biometrics, biomathematics and the morphometric synthesis. *Bull. Math. Biol.*, **2**: 313–365.
- Boughman, J.W. 2001. Divergent sexual selection enhances reproductive isolation in sticklebacks. *Nature*, **411**: 944–948.
- Boughman, J.W., Rundle, H.D. and Schluter, D. 2005. Parallel evolution of sexual isolation in sticklebacks. *Evolution*, **59**: 361–373.
- Boulcott, P. and Braithwaite, V. 2005. Ultraviolet light and visual behaviour in the three-spined stickleback, *Gasterosteus aculeatus*. *Physiol. Biochem. Zool.*, **78**: 736–743.
- Bradbury, J.W. and Vehrencamp, S.L. 2011. *Principles of Animal Communication*. Sunderland, MA: Sinauer Associates.
- Bretman, A., Westmancoat, J.D., Gage, M.J.G. and Chapman, T. 2011. Males use multiple, redundant cues to detect mating rivals. *Curr. Biol.*, **21**: 617–622.
- Bro-Jørgensen, J. 2010. Dynamics of multiple signaling systems: animal communication in a world of flux. *Trends Ecol. Evol.*, **25**: 292–300.
- Butkowski, T., Yan, W., Gray, A.M., Cui, R., Verzijden, M.N. and Rosenthal, G.G. 2011. Automated interactive video playback for studies of animal communication. *J. Vis. Exp.*, **48**: e2374.
- Candolin, U. 1999. Male–male competition facilitates female choice in sticklebacks. *Proc. R. Soc. Lond. B*, **266**: 785–789.
- Candolin, U. 2003. The use of multiple cues in mate choice. *Biol. Rev.*, **78**: 575–595.

- Clarke, J.M. and Schluter, D. 2011. Colour plasticity and background matching in a threespine stickleback species pair. *Biol. J. Linn. Soc.*, **102**: 902–914.
- Claude, J. 2008. *Morphometrics with R*. New York: Springer.
- Conte, G.L. and Schluter, D. 2013. Experimental confirmation that body size determines mate preference via phenotype matching in a stickleback species pair. *Evolution*, **67**: 1477–1484.
- Coyne, J.A. and Orr, H.A. 2004. *Speciation*. Sunderland, MA: Sinauer Associates.
- D’Eath, R. 1998. Can video images imitate real stimuli in animal behaviour experiments? *Biol. Rev.*, **73**: 267–292.
- Drăgănoiu, T.I., Nagle, L. and Kreutzer, M. 2002. Directional female preference for an exaggerated male trait in canary (*Serinus canaria*) song. *Proc. R. Soc. Lond. B*, **269**: 2525–2531.
- Fisher, H.S., Wong, B.B.M. and Rosenthal, G.G. 2006. Alteration of the chemical environment disrupts communication in a freshwater fish. *Proc. R. Soc. Lond. B*, **273**: 1187–1193.
- Fisher, H.S., Mascuch, S. and Rosenthal, G.G. 2009. Multivariate male traits misalign with multivariate female preferences in the swordtail fish, *Xiphophorus birchmanni*. *Anim. Behav.*, **78**: 265–269.
- Fleishman, L.J. and Endler, J.A. 2000. Some comments on visual perception and the use of video playback in animal behavior studies. *Acta Ethol.*, **3**: 15–27.
- Fleishman, L.J., McClintock, W.J., D’Eath, R.B., Brainard, D.H. and Endler, J.A. 1998. Colour perception and the use of video playback experiments in animal behaviour. *Anim. Behav.*, **56**: 1035–1040.
- Govardovski, V.I., Fyhrquist, N., Reuter, T., Kuznub, D.G. and Donner, K. 2000. In search of the visual pigment template. *Visual Neurosci.*, **17**: 509–528.
- Gray, S.M. and McKinnon, J.S. 2007. Linking colour polymorphism maintenance and speciation. *Trends Ecol. Evol.*, **22**: 71–79.
- Harland, D.P. and Jackson, R.R. 2002. Influence of cues from the anterior medial eyes of virtual prey on *Portia fimbriata*, an araneophagic jumping spider. *J. Exp. Biol.*, **205**: 1861–1868.
- Hodgson, A., Black, A.R. and Hull, R. 2013. Sensory exploitation and indicator models may explain red pelvic spines in the brook stickleback, *Culaea inconstans*. *Evol. Ecol. Res.*, **15**: 199–211.
- Hulbert, S.H. 1984. Pseudoreplication and the design of ecological field experiments. *Ecol. Monogr.*, **54**: 187–211.
- Huntingford, F.A. and Ruiz-Gomez, M.L. 2009. Three-spined sticklebacks *Gasterosteus aculeatus* as a model for exploring behavioural biology. *J. Fish Biol.*, **75**: 1943–1976.
- Kingston, J.J., Rosenthal, G.G. and Ryan, M.J. 2003. The role of sexual selection in maintaining a colour polymorphism in the pygmy swordtail, *Xiphophorus pygmaeus*. *Anim. Behav.*, **65**: 735–743.
- Kitano, J., Ross, J.A., Mori, S., Kume, M., Jones, F.F., Chan, Y.F. *et al.* 2009. A role for a neo-sex chromosome in stickleback speciation. *Nature*, **461**: 1079–1083.
- Kraak, S.B.M., Bakker, T.C.M. and Mundwiler, B. 1999. Sexual selection in sticklebacks in the field: correlates of reproductive, mating, and paternal success. *Behav. Ecol.*, **10**: 696–706.
- Künzler, R. and Bakker, T.C.M. 1998. Computer animations as a tool in the study of mating preferences. *Behaviour*, **135**: 1137–1159.
- Künzler, R. and Bakker, T.C.M. 2001. Female preference for single and combined traits in computer animated stickleback males. *Behav. Ecol.*, **12**: 681–685.
- Langerhans, R.B., Layman, C.A. and DeWitt, T.J. 2005. Male genital size reflects a tradeoff between attracting mates and avoiding predators in two live-bearing fish species. *Proc. Natl. Acad. Sci.*, **102**: 7618–7623.
- Mazzi, D., Künzler, R., Largiadèr, C.R. and Bakker, T.C.M. 2004. Inbreeding affects female preferences for symmetry in computer-animated sticklebacks. *Behav. Genet.*, **34**: 417–424.
- McGregor, P.K. 2000. Playback experiments: design and analysis. *Acta Ethol.*, **3**: 3–8.
- McKinnon, J.S. 1995. Video mate preferences of female three-spined sticklebacks from populations with divergent male coloration. *Anim. Behav.*, **50**: 1645–1655.

- McKinnon, J.S. and McPhail, J.D. 1996. Male aggression and colour in divergent populations of the threespine stickleback: experiments with animations. *Can. J. Zool.*, **74**: 1727–1733.
- McKinnon, J.S. and Rundle, H.D. 2002. Speciation in nature: the threespine stickleback model system. *Trends Ecol. Evol.*, **17**: 480–488.
- McKinnon, J.S., Mori, S., Blackman, B.K., David, L., Kingsley, D.M., Jamieson, L. *et al.* 2004. Evidence for ecology's role in speciation. *Nature*, **429**: 294–298.
- Mehlis, M., Bakker, T.C.M. and Frommen, J.G. 2008. Smells like sib spirit: kin recognition in three-spined sticklebacks (*Gasterosteus aculeatus*) is mediated by olfactory cues. *Anim. Cogn.*, **11**: 643–650.
- Nagel, L. and Schluter, D. 1998. Body size, natural selection, and speciation in sticklebacks. *Evolution*, **52**: 209–218.
- Nomakuchi, S., Park, P.J. and Bell, M.A. 2009. Correlation between exploration activity and use of social information in threespine sticklebacks. *Behav. Ecol.*, **20**: 340–345.
- Odling-Smee, L.C. and Braithwaite, V.A. 2003. The influence of habitat stability on landmark use during spatial learning in the three-spined stickleback. *Anim. Behav.*, **65**: 701–707.
- Ord, T., Peters, R., Evans, C. and Taylor, A. 2002. Digital video playback and visual communication in lizards. *Anim. Behav.*, **63**: 879–890.
- Park, P.J. 2013. Spatial learning ability of the threespine stickleback (*Gasterosteus aculeatus*) in relation to inferred ecology and ancestry. *Evol. Ecol. Res.*, **15**: 213–239.
- Pfennig, K.S. 1998. The evolution of mate choice and the potential for conflict between species and mate-quality recognition. *Proc. R. Soc. Lond. B*, **265**: 1743–1748.
- Phelps, S.M., Ryan, M.J. and Rand, A.S. 2001. Vestigial preference functions in neural networks and túngara frogs. *Proc. Natl. Acad. Sci. USA*, **98**: 13161–13166.
- Remage-Healey, L., Coleman, M.J., Oyama, R.K. and Schlinger, B.A. 2010. Brain estrogens rapidly strengthen auditory encoding and guide song preference in a songbird. *Proc. Natl. Acad. Sci. USA*, **107**: 3852–3857.
- Rennison, D.J., Owens, G.L. and Taylor, J.S. 2012. Opsin gene duplication and divergence in ray-finned fish. *Mol. Phylogenet. Evol.*, **62**: 986–1008.
- Ritchie, M.G. 2007. Sexual selection and speciation. *Annu. Rev. Ecol. Evol.*, **38**: 79–102.
- Rohlf, F. 2004. *tpsUtil*, file utility program. Version 1.26. Stony Brook, NY: Department of Ecology and Evolution, State University of New York at Stony Brook. Available at: <<http://life.bio.sunysb.edu/morph/>>.
- Rohlf, F.J. 2008. *tpsRelw*, *Relative Warps Analysis*. Version 1.46. Stony Brook, NY: Department of Ecology and Evolution, State University of New York at Stony Brook. Available at: <<http://life.bio.sunysb.edu/morph/>>.
- Rohlf, F.J. 2009. *tpsDig*. Version 2.14. Stony Brook, NY: Department of Ecology and Evolution, State University of New York. Available at: <<http://life.bio.sunysb.edu/morph/>>.
- Rosenthal, G.G. 2000. Design considerations and techniques for constructing video stimuli. *Acta Ethol.*, **3**: 49–54.
- Rosenthal, G.G. 2007. Spatiotemporal dimensions of visual signals in animal communication. *Annu. Rev. Ecol. Evol. Syst.*, **38**: 155–178.
- Rosenthal, G.G. and Evans, C.S. 1998. Female preference for swords in *Xiphophorus helleri* reflects a bias for large apparent size. *Proc. Natl. Acad. Sci. USA*, **95**: 4431–4436.
- Rosenthal, G.G. and Lobel, P. 2005. Communication. In *Behaviour and Physiology of Fish*, Vol. 24 (K. Sloman, S. Balshine and R. Wilson, eds.), pp. 39–78. San Diego, CA: Elsevier Academic Press.
- Rosenthal, G.G., Evans, C.S. and Miller, W.L. 1996. Female preference for a dynamic trait in the green swordtail, *Xiphophorus helleri*. *Anim. Behav.*, **51**: 811–820.
- Rowe, M.P., Baube, C.L., Loew, E.R. and Phillips, J.B. 2004. Optimal mechanisms for finding and selecting mates: how threespine stickleback (*Gasterosteus aculeatus*) should encode mate throat colors. *J. Comp. Physiol. A*, **190**: 241–256.

- Rowland, W.J. 1994. Proximate determinants of stickleback behaviour: an evolutionary perspective. In *The Evolutionary Biology of the Threespine Stickleback* (M.A. Bell and S.A. Foster, eds.), pp. 297–344. Oxford: Oxford University Press.
- Rowland, W.J. 1995. Do female stickleback care about male courtship vigour? Manipulation display tempo using video playback. *Behaviour*, **132**: 951–961.
- Ryan, M.J. and Rand, A.S. 1990. The sensory basis of sexual selection for complex calls in the tungara frog, *Physalaemus pustulosus* (sexual selection for sensory exploitation). *Evolution*, **44**: 305–314.
- Schluter, D. 2000. *The Ecology of Adaptive Radiation*. Oxford: Oxford University Press.
- Shaw, K.L. and Lesnick, S.C. 2009. Genomic linkage of male song and female acoustic preference QTL underlying a rapid species radiation. *Proc. Natl. Acad. Sci. USA*, **106**: 9737–9742.
- ter Pelwijk, J.J. and Tinbergen, T. 1937. Eine reizbiologische Analyse einiger Verhaltensweisen von *Gasterosteus aculeatus* L. *Z. Tierpsychol.*, **1**: 193–200.
- Tinbergen, N. 1951. *The Study of Instinct*. Oxford: Clarendon Press.
- Verzijden, M.N. and Rosenthal, G.G. 2011. Effects of sensory modality on learned mate preferences in female swordtails. *Anim. Behav.*, **82**: 557–562.
- Vines, T.H. and Schluter, D. 2006. Strong assortative mating between allopatric sticklebacks as a by-product of adaptation to different environments. *Proc. R. Soc. Lond. B*, **273**: 911–916.
- Walker, J.A. 1997. Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus* L (Gasterosteidae) body shape. *Biol. J. Linn. Soc.*, **61**: 3–60.
- Wark, A.R., Wark, B.J., Lageson, T.J. and Peichel, C.L. 2011. Novel methods for discriminating behavioral differences between stickleback individuals and populations in a laboratory shoaling assay. *Behav. Ecol. Sociobiol.*, **65**: 1147–1157.
- Watanabe, S. and Troje, N.F. 2006. Towards a ‘virtual pigeon’: a new technique for investigating avian social perception. *Anim. Cogn.*, **9**: 271–279.
- Wong, B.B.M. and Rosenthal, G.G. 2006. Female disdain for swords in a swordtail fish. *Am. Nat.*, **167**: 136–140.
- Woo, K.L. and Rieucau, G. 2011. From dummies to animations: a review of computer-animated stimuli used in animal behavior studies. *Behav. Ecol. Sociobiol.*, **65**: 1671–1685.
- Yong, L., Guo, R., Wright, D.S., Mears, S.A., Pierotti, M. and McKinnon, J.S. 2013. Correlates of red throat coloration in female sticklebacks and their potential evolutionary significance. *Evol. Ecol. Res.*, **15**: 453–472.
- Zbinden, M., Mazzi, D., Künzler, R., Largiadèr, C.R. and Bakker, T.C.M. 2003. Courting virtual rivals increases ejaculate size in sticklebacks (*Gasterosteus aculeatus*). *Behav. Ecol. Sociobiol.*, **54**: 205–209.
- Zbinden, M., Largiadèr, C.R. and Bakker, T.C.M. 2004. Body size of virtual rivals affects ejaculate size in sticklebacks. *Behav. Ecol.*, **15**: 137–140.
- Zeil, J. 2000. Depth cues, behavioural context, and natural illumination: some potential limitations of video playback techniques. *Acta Ethol.*, **3**: 39–48.

