

SAISAQ: A novel tool for semiautomatic image based surface area quantification

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Abstract. Digital image analyses provide exciting opportunities to study morphological variation in species and to monitor ontogenetic changes. Although increasingly used analyses of images are commonly time consuming, hampering monitoring applications involving huge sample sizes. Herein we propose a novel analysis tool programmed in the open source statistics platform R.

Keywords: Cran R, larval development, monitoring, high throughput, time-efficient.

Among vertebrates, amphibians are one of the most threatened groups with more of the species as potentially endangered according to IUCN Red List criteria (i.e., near threatened, vulnerable, critically endangered; IUCN et al. 2013). Recently the Amphibian Conservation Action Plan (Gascon et al. 2007) highlighted major areas of research, wherein ex-situ and in-situ breeding programs were proposed to safeguard the future of many taxa. Basic life history data is lacking for one fourth of currently described species (category data deficient; IUCN et al. 2013). For successful ex-situ breeding, baseline data on optimal husbandry and breeding conditions are urgently required, wherein objective criteria need to be defined to quantify success rates.

Growth rates of juveniles can be used as fitness proxy in many species to assess potential effects of environmental factors and biotic interactions (Dmitriew 2011), e.g. predator response (Relyea 2004, Gonzalez et al. 2011). The development of amphibian larvae is a crucial step during ontogenesis and also commonly critical phases in conservation breeding (e.g., McGregor Reid & Zippel 2008, Gawor et al. 2012). In amphibians growth rates of larvae are frequently determined in ecological analyses, commonly either quantified by weighting the specimens (e.g., Relyea 2004) or by using the tadpoles' dorsal surface area as proxy for its mass (Davis et al. 2008). In laboratory experiments and to optimize captive breeding conditions, this frequently requires repeated measurements of single individuals, wherein a measurement procedure minimizing mortality rates is preferred.

Digital image analyses offer the possibility to quantitatively study features of a specimen with

minimal handling efforts (Davis & Grayson 2007, Mott et al. 2010). It is often used to study amphibian morphology, individual recognition, growth rate (Davis et al. 2008). Davis et al. (2008) suggested image analysis as useful tool to estimate tadpole mass based on measurements of a tadpole's surface. They detected a highly significant correlation between the log transformed dorsal area of a tadpole and the log transformed mass. Although their proposed technique allows a reduction of tadpole mortality and high efficiency in the field, image analyses in software packages such as ImageJ (<http://rsb.info.nih.gov/ij/>) or Fovea Pro (Reindeer Graphics, Inc.) is still time consuming, hampering a high throughput. Herein, we provide a novel tool programmed in the open source statistics platform R (R Developmental Core Team 2013), which allows a semiautomatic procession of standardized image files.



Figure 1. Experimental setup including light table, camera and light device.

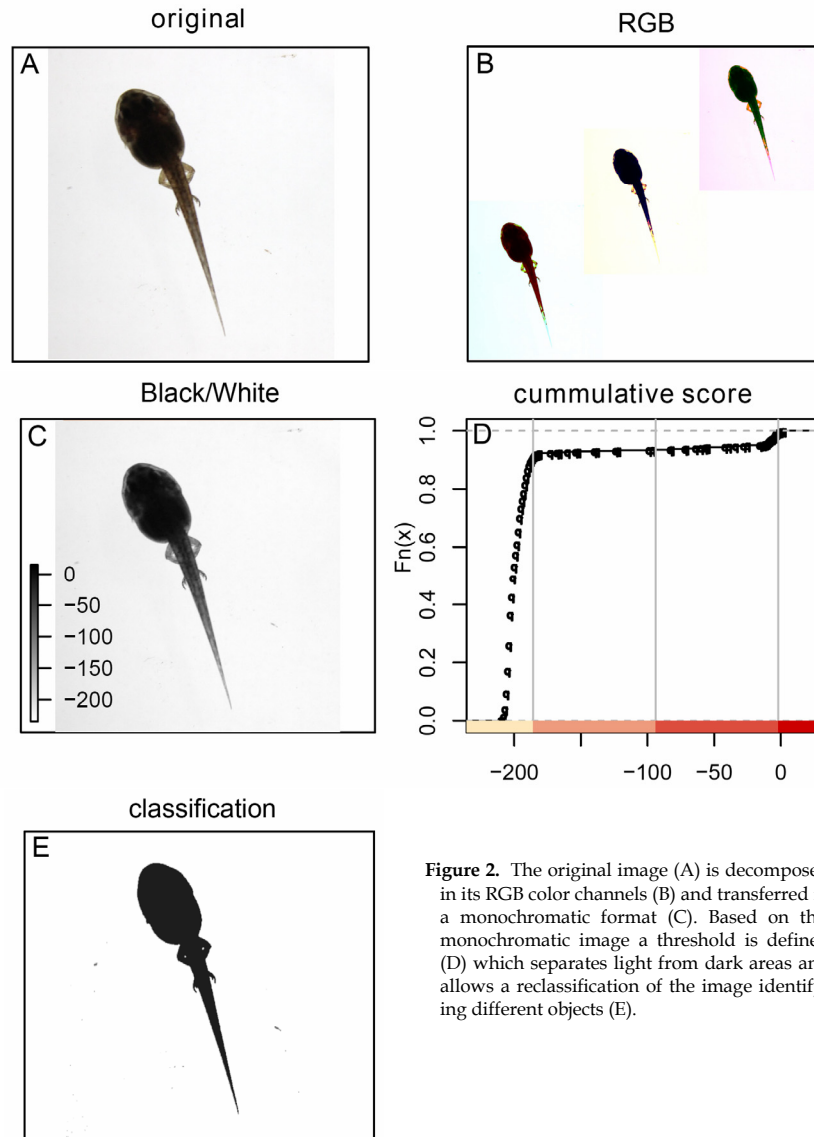


Figure 2. The original image (A) is decomposed in its RGB color channels (B) and transferred in a monochromatic format (C). Based on this monochromatic image a threshold is defined (D) which separates light from dark areas and allows a reclassification of the image identifying different objects (E).

Our experimental setup consists of a light table and a Canon EOS 600D device mounted on a stand in a fixed distance to the light table (Fig. 1). In a first step we photograph a size standard to calibrate the image analysis software, i.e. an object with a specified rectangular surface or a scale paper. For photographing, the tadpoles are individually placed in transparent petri-dishes with a minimal amount of water just covering their dorsum (Fig. 2A). It is important that the tadpole is not moving while taking the photos to ensure sharp and contrast rich images. Subsequently the

images are transferred to a computer and analyzed with the previous mentioned tool.

First, the software tool is calibrated based on the image of the millimeter paper to establish a square centimeter-to-pixel relationship. Subsequently, each tadpole image is monochromatic, coded by summing up the RGB scores (Fig. 2B, C), the approximate location of the tadpole is determined by a rectangle and the cumulative frequency of greyscale values is plotted (Fig. 2D). Based on these plots it is possible to separate light from dark items on the picture. Using the respec-

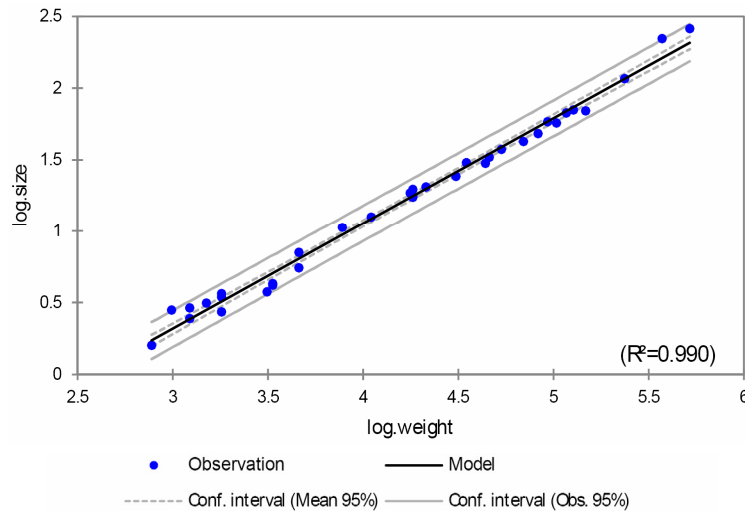


Figure 3. Simple linear regression of the log-transformed surface area measurements of the tadpoles ($n=45$) as obtained from image analyses and log-transformed mass of the specimens.

tive functions of the *raster* package (i.e., *clump*; Hijmans and van Etten 2012), coherent surfaces can be identified (Fig. 2D) and the surface size of the largest object is quantified based on the square centimeter-to-pixel relationship.

These analysis steps were implemented in a software pipeline programmed in R making use of the relevant functions in the packages *biOps* (Bordese and Alini 2012), *raster* (Hijmans & van Etten 2012), *igraph* / *igraph0* (Csardi & Nepusz 2006), *classInt* (Bivand 2012), and *adimpro* (Polzehl and Tabelow 2007). The pipeline enables the user to efficiently process large amounts of images applying either no or moderate user interaction. As output both a graphic output allowing quality assessments and a spreadsheet containing the pixels per reference area and the number of pixels per tadpole and its estimated surface area are provided.

We tested the feasibility and reliability of our approach by comparing the estimated surface area and the body mass using a standard weighting procedure. Therefore, we took each three pictures and the weights of 45 tadpoles of *Epiplatys anthonyi* with Gosner (1960) stages between 25 and 41, forcing the movement of the tadpole between each sample. Both raw mass and dorsal surface area were log-transformed and relationship was tested using a simple linear regression applying Pearson's coefficient of determination (R^2) (XLSTAT 2012; addinsoft).

In concordance with the results presented by

Davis et al. (2008), we detected a highly significant linear relationship between the dorsal surface area of the tadpoles and their body mass ($R^2 = 0.990$, $p < 0.0001$; $\log.size = -1.87 + 0.73 * \log.weight$; Fig. 3). Among our three replicates the variation introduced by tadpole movement was negligible (range $R^2 = 0.990 - 0.992$, $n=3$) wherein the absolute average deviance was only 2.2 % (95% CI: -1.36 - 5.74 %). Results may deviate after Gosner stage 41 when limbs start to develop, what might blur the dorsal surface size to mass relationship. Therefore, we recommend applying this method only until Gosner stage 41. Procession speed was reasonable, wherein approximately 130 images can be analyzed on a standard windows PC (Windows 7, 4GB RAM, Intel® Core™ i3 CPU, 2.93 GHz, 32 bit) in one hour, increasing computing power may even allow a higher efficiency.

The high reliability of our approach and its efficiency now allows the processing of large amounts of data, minimizing manual data handling and mortality rates of tadpoles. The implementation in Cran R is open source and therefore freely available. Applying the SAISAQ pipeline allows documentation, quantification and monitoring of the development of tadpoles under different environmental conditions. This enables us to document the development of poorly known species in a very time-efficient way ultimately leading to a better understanding of their husbandry requirements to improve ex-situ management.

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Supplementary material S1 (may be found in the online version of this paper):
SAISAQ R-script, example data and manual for semiautomatic image analyses for size measurements.