# Assessment of digital image analyses for use in wildlife research

Authors: Mott, Cy L., Albert, Scott E., Steffen, Michael A., and Uzzardo, John M.

Source: Wildlife Biology, 16(1) : 93-100

Published By: Nordic Board for Wildlife Research

URL: https://doi.org/10.2981/09-010

**Wildl. Biol. 16: 93-100 (2010)**
DOI: 10.2981/09-010


**Short communication**

# Assessment of digital image analyses for use in wildlife research

**Cy L. Mott, Scott E. Albert, Michael A. Steffen & John M. Uzzardo**

Due to the decreased cost and increased availability of digital camera technology, digital image analyses are rapidly replacing traditional caliper-based approaches to wildlife morphometry. The substitution of image-based measurement methods over those utilizing calipers is based on the assumption that both methods are comparable with respect to accuracy, precision, time required and inter-observer variation, yet this hypothesis has not been explicitly tested. In this article, we evaluate these aspects of each method using three life stages of the marbled salamander *Ambystoma opacum*. We found that digital image-based measurements were significantly more accurate, faster to obtain, and resulted in reduced inter-observer measurement variation relative to caliper measurements, yet the former was associated with reduced precision among repeated measurements relative to the latter. Based on our observations, we recommend that digital image-based measurements represent a useful alternative to caliper measurements and are particularly useful for organisms that pose risks to investigators, normally maintain non-linear body orientations, or cannot easily be removed from their habitat.

*Key words: calipers, camera, image analyses, Image J, morphometry*

*Cy L. Mott, Cooperative Wildlife Research Laboratory Department of Zoology, Carbondale, Illinois, USA - e-mail: cm8755@siu.edu*
*Scott E. Albert, Michael A. Steffen, & John M. Uzzardo*, Department of Zoology 351 Life Science II, Southern Illinois University Carbondale, Illinois 62901-6501, USA - e-mail addresses: ilherps@siu.edu (Scott E. Albert); Bass2187@aol.com (Michael A. Steffen); j.uzzardo@ttu.edu (John M. Uzzardo)*

** Present address: Department of Natural Resources Management, Goddard Building 005, Box 42125, Texas Tech University Lubbock, Texas 79404, USA*

*Corresponding author: Cy L. Mott*

*Received 23 January 2009, accepted 4 September 2009*

*Associate Editor: Claude Miaud*

Traditional approaches to morphometry in wildlife research typically entail measurements obtained via hand-held digital or traditional dial calipers. However, these methods are increasingly being replaced by *in situ* digital photography of focal subjects, with subsequent digital image analysis software programs employed to obtain measurements. The potential functions of this emerging technique are diverse, as recent applications of digital image analyses include studies of body size (Hill et al. 2005) and shape (Relyea 2004), growth (Davis et al. 2008), sexual dichromatism (Todd & Davis 2007), pattern asymmetry (Wright & Zamudio 2002) and histological changes (McFadzen et al. 1994). In addition, the reduced costs of digital cameras and increased availability of digital image analysis software will likely contribute to increased use of digital technology and facilitate future novel approaches to wildlife research.

The potential advantages of digital image analysis for wildlife studies include beneficial aspects for both researchers and focal organisms. Davis & Grayson (2007) show that a wider variety of data can be collected from photographs than from caliper measurements, as digital image collections can be subsequently used for analyses of size, shape, colour and symmetry (Grill 1999, Leclair et al. 2005, Davis & Maerz 2007). Image analyses are particularly advantageous when attempting to measure organisms which typically do not assume a linear body position conducive to caliper measurements (Walston & Mullin 2005), such as reptiles and amphibians. Digital image analyses are also beneficial when focal organisms pose a particular risk to the researcher, as contact is often maximized when using calipers (Luiselli 2005). These analyses also minimize stress for focal organisms by reducing contact time between the researcher and focal animal. Furthermore, the focal organism may be photographed in its natural state (Davis et al. 2008); this may be of particular benefit to aquatic or otherwise sensitive organisms that may suffer when even briefly removed from their habitat.

While the benefits of digital image analyses are numerous, a further review of this approach is necessary before it is fully integrated into wildlife research. Of particular interest is the assumption that measurements derived from digital images are at least as accurate and precise as those obtained from calipers; this assumption of digital image analyses is critical to its acceptance as a viable alternative to caliper measurements, yet has not been directly examined. Davis et al. (2008) claim that digital image analyses are 'arguably faster' than traditional methods; however, this hypothesis has not been tested explicitly.

If caliper measurements are found to be more time-consuming than digital image analyses or vice-versa, this finding would be of considerable significance to wildlife research for a variety of reasons. First, as many wildlife studies require long-term data consisting of thousands of individual measurements (Cumming & Havlicek 2002, Knouft 2003, Gibbs & Karraker 2006), even small differences in the time necessary to obtain measurements could result in reduced time required in the field in inclement conditions, reduced time working with preserved specimens, and funding allocated to either task. Also, the faster method would reduce handling stress on focal organisms if physical contact is minimized. It is also necessary to determine if reduced time required by a particular method imposes a trade-off in terms of reduced accuracy and precision of measurements. Finally, since measurements are often obtained by multiple researchers, it is necessary to determine if either measurement method results in sizeable inter-observer differences in measurements of a common subject, as this would represent a trade-off that must be considered when determining which method to employ.

The purpose of our study is to compare the utility of caliper and image-based measurement procedures with respect to time requirements, accuracy, precision and inter-observer measurement variation. We conducted these procedures using three morphologically unique life stages of a focal organism to identify characteristics of the subject that favour one measurement method over another based on life history traits. Based on our findings, we make recommendations regarding the utility of each method for wildlife research.

## Material and methods

As our focal organism, we utilized the marbled salamander *Ambystoma opacum*, a common terrestrial amphibian species that exhibits traits conducive to digital image measurements used in wildlife research. First, long-term amphibian studies, including those involving marbled salamanders, often require measuring thousands of individuals (Scott 1990, 1994, Taylor & Scott 1997), and any reduction in time required for each measurement would greatly reduce the total time devoted to this task. Second, salamanders in particular are known to exhibit non-linear body orientations when handled, and caliper measurements of linear traits such as snout-to-vent length (SVL) and total length (TL) may be skewed by the organism's attempt to coil (Wise & Buchanan 1992). Finally, many salamanders, including the larval stage of marbled salamanders, are aquatic, thus making handling and caliper measurements difficult due to viscous body surfaces and a tendency to vigorously shake the entire body when collected (C.L. Mott, pers. obs.).

Our decision to employ multiple life stages of the marbled salamander was dependent on each stage exhibiting traits that addressed each of the aforementioned potential benefits of digital image analysis. The egg stage was selected because large sample sizes are often required to identify differences in ovum morphology. By collecting large numbers of egg measurements, we determined if digital image analyses were less time consuming than caliper measurements for large sample sizes. Terrestrial adult marbled salamanders were utilized in determining if digital image analyses provided more accurate and precise measurements for organisms that typically maintain non-linear body orientations, and aquatic larval marbled salamanders were employed to represent those organisms that could not easily be collected from or measured in aquatic habitats.

### Caliper measurements

All caliper measurements were taken using model Y3110337 digital calipers (Fischer Scientific, Pittsburgh, Pennsylvania, USA); therefore, times required to collect measurements are considered minimum estimates, as traditional non-digital dial calipers do not provide readable displays and their use would require additional time to determine measurements. Calipers were recalibrated before use by each investigator, and all of the following procedures were performed by four investigators to quantify inter-observer measurement biases. Investigators were provided a 5-minute practice period to become familiar with the operation of digital calipers, and all caliper measurements were recorded on separate data sheets to best mimic the time required to collect similar data *in situ*.

#### *Eggs*

We collected 120 marbled salamander eggs from nesting females in the Shawnee National Forest, Illinois, USA, in November 2007, which were transported to the Cooperative Wildlife Research Laboratories facilities of Southern Illinois University Carbondale (SIUC). We randomly selected 10 eggs, rinsed them to remove any remaining debris, and placed them in a single line onto a moistened countertop to prevent egg drying and associated shrinkage from occurring during measurements. A digital timer began with the measurement of the diameter of the first egg and concluded when the diameter of each egg had been measured 10 times, resulting in a total of 100 measurements. For each investigator, the average time per measurement was calculated by dividing the total number of measurements by the total time required to obtain them. Repeated measurements of each egg were utilized to calculate a mean diameter ($\bar{y}$) and coefficient of variation (CV; Zar 1999), which is defined as $(\sigma/\bar{y}) \times 100$, of each egg for each investigator.

#### *Adults*

We collected 10 adult marbled salamanders from pond basins in the Shawnee National Forest, Illinois, USA, in May 2008, which were transported to the Cooperative Wildlife Research Laboratories facilities of SIUC. We obtained measurements of SVL using the 'salamander stick' technique (Walston & Mullin 2005), which has previously been shown to provide more accurate and precise measurements than either freehand techniques or those where salamanders are restrained in tubes. In this approach, individual marbled salamanders were placed in transparent plastic Zip-loc® bags, which were then drawn through a 2-mm opening between two 38 mm-diameter polyvinylchloride (PVC) tubes joined in a parallel fashion with duct tape. When the plastic bag was pulled with even mild tension, the cranio-caudal axis of the salamander was linearly aligned with the trough formed by the PVC tubes, facilitating caliper measurements of SVL, which is defined as the distance from the anterior end of the head to the anterior edge of the vent. Adult marbled salamanders were individually housed in numbered $15 \times 15 \times 5$ mm plastic containers, and a digital timer was initiated when the first salamander was removed from its container. Snout-to-vent length of each salamander was measured 10 times, resulting in a total of 100 measurements. After all measurements were collected, the timer was stopped, and a time per measurement and CV value of each salamander for each investigator was calculated as described for egg measurements.

#### *Larvae*

We collected 10 larval marbled salamanders from a vernal pond at the Touch of Nature Environmental Education Center, Carbondale, Illinois, USA, in May 2008, which were transported to the Cooperative Wildlife Research Laboratories facilities of SIUC. In place of SVL, we utilized total length (TL), which is defined as the distance from the anterior end of the head to the posterior end of the tail, as the focal measurement because the vent is often difficult to discern in larval salamanders (C.L. Mott, pers. obs.) and previous studies have utilized TL as a metric of larval body size (Gamradt & Kats 1996, Ziemba et al. 2000). Larvae were individually housed in numbered $15 \times 12 \times 5$ cm plastic containers filled to a depth of 3 cm with reconstituted soft water (Horne & Dunson 1995); the depth of the containers allowed caliper tips to be placed directly in the water when measuring larvae. A digital timer began with the measurement of the first larva, and each larva was measured 10 times as described for eggs and adults. In the case where a larva maintained a non-linear position when attempting to measure TL, it was touched gently with a blunt probe to cause an escape movement and associated straightening of its cranio-caudal axis. Most larvae stimulated in such a manner required only one touch before measuring. Once all larvae were measured 10 times, the digital timer was stopped, and a time per measurement and CV value of each larva for each investigator was calculated as described previously.

## Digital measurements

All marbled salamander life stages were photographed using a Nikon D70 digital single lens reflex (DSLR) camera. Separate photographs of each life stage were taken by each of four investigators, and repeated measurements of a single individual in each life stage were obtained from 10 separate photographs per life stage rather than repeated measurements of a single photograph, as the latter approach best mimics measurements obtained *in situ*. Photographs were titled according to the life stage, investigator and repetition number, and all photographs were stored on a laptop computer using ~ 550 MB of hard drive storage space (pixel dimensions: 3008 × 2000). All image analyses were performed with Image J, a free software program available through the National Institutes of Health, Bethesda, Maryland, USA (available at: http://rsb.info.nih.gov/ij/). All investigators were initially provided with a 10-minute orientation period to learn the functions of Image J required in obtaining digital measurements. Using Image J, a stored image must first be calibrated using the 'set to scale' function. To accomplish this, a 15-cm ruler was included in each photograph along the same plane as the focal organism, and a 10-cm segment of the ruler was highlighted using the 'straight line' function. The 'set to scale' function then allowed us to calibrate the picture according to the highlighted segment. After calibrating the photograph, a 1-cm test measurement was performed to ensure the accuracy of measurements within 0.05 mm. For all digital image measurements, the time required to photograph subjects, create and store digital images, and calibrate photographs were incorporated into the total time required to employ this technique. The same subjects utilized for caliper measurements were photographed for digital image analyses, and photographs were taken immediately following caliper measurements to prevent temporal changes in length due to growth. As with caliper measurements, the following procedures were performed by four investigators to quantify inter-observer measurement variation.

### *Eggs*

A timed measurement session of 10 marbled salamander eggs began when an investigator opened the first image on the laptop computer. Following image calibration, the diameter of each egg was measured once, after which the second photograph was opened and the process repeated, until diameter measurements were obtained from 10 photographs of the 10 eggs, resulting in 100 total measurements. The measurement session concluded after all measurements were transferred to a separate data file. The total time required for each investigator, as calculated previously, was divided by the number of measurements to calculate a time per measurement value for each investigator, and a CV value of each egg for each investigator was derived as described for caliper measurements.

### *Adults*

The 10 adult marbled salamanders utilized for caliper measurements were initially arranged in a compartment box that isolated all salamanders from one another. Upon removal of the box, 10 photographs of the group of 10 marbled salamanders were taken by each investigator. As all marbled salamanders assumed a non-linear orientation following removal of the dividers, SVL was measured using the 'bent line' function of Image J, which facilitates length measurements for curved lines (Fig. 1). SVL for digital measurements was defined as the length from the anterior end of the head to the posterior edge of the rear legs, as the latter region is synonymous with the anterior edge of the vent. The measurement session concluded after all measurements were transferred to a separate data file. Time per measurement values, as well as CV values of each adult salamander, for each investigator were derived as described for caliper measurements.

### *Larvae*

The 10 marbled salamander larvae utilized for caliper measurements were initially arranged in a plastic compartment box filled to a depth of 3 cm with reconstituted soft water, each compartment housing a single individual. Measurements of TL, calculation of time per measurement values, and CV values of each larva for each investigator were then

Figure 1. Measurement of adult marbled salamander snout-vent length (SVL) using the 'bent line' function of Image J image analysis software. Plastic rulers are included in each photo for the purposes of calibration, and Image J provides data in tabular form after each measurement (see upper right corner), which can easily be transferred to other formats.

conducted as described for adult marbled salamanders.

All statistical analyses were performed using SAS Version 9.1 (SAS Institute, Cary, North Carolina, USA), and only $P < 0.05$ were deemed significant. We determined if caliper or digital image methods resulted in significant differences in recorded measurements using separate paired t-tests for each life stage, with measurement method as the independent variable and the 10 repeated measurements by each investigator for each of 10 individuals (egg, adult or larva) using both methods as the paired dependent variables. We also determined if either measurement method resulted in significant inter-observer measurement variation using a nested analysis of variance (ANOVA) for each life stage and measurement method; these analyses utilized investigator and the nested factor of subject (#1-10 for each life stage) as independent variables and the 10 repeated measurement values of each subject as the dependent variables. For adult and larval marbled salamander life stages, we assumed *a priori* the method resulting in larger measurement values to be more accurate, as the non-linear body positioning exhibited by these stages tends to artificially reduce body lengths (Wise & Buchanan 1992). We also assumed larger values to be more accurate for egg stage salamanders, as measurement of any plane of the egg aside from that crossing the true diameter would result in artificially low values, given the true diameter is the widest possible plane. To determine if there were significant differences in time required to obtain an equivalent number of measurements with traditional caliper methods or digital image analyses, we conducted separate paired t-tests for each life stage, with measurement method as the independent variable and time per measurement values of each investigator as the dependent variable. Finally, we determined if measurement

Table 1. Results of paired t-tests of caliper (CP) and digital image (DI) measurements for the three life stages of the marbled salamander. Mean measurement values (in mm ± 1 STD) for egg, larval and adult stages consisted of the diameter, total length (TL) and snout-vent length (SVL), respectively.

| Life stage | $\bar{y}$ CP | $\bar{y}$ DI | df | t | P |
|---|---|---|---|---|---|
| Egg | 6.23 ± 0.69 | 6.62 ± 0.67 | 399 | -24.40 | < 0.0001 |
| Larva | 38.96 ± 4.78 | 37.91 ± 4.59 | 399 | 6.99 | < 0.0001 |
| Adult | 55.08 ± 4.87 | 56.55 ± 5.10 | 399 | -7.09 | < 0.0001 |

Table 2. Results of the nested analysis of variance for inter-observer bias among measurements recorded with caliper (CI) or digital image analysis (DI) methods for the three life stages of the marbled salamander.

| Stage | Method | Numerator df | Denominator df | F | P |
|---|---|---|---|---|---|
| Egg | CP | 3 | 36 | 0.86 | 0.4839 |
| Egg | DI | 3 | 36 | 1.44 | 0.2478 |
| Larva | CP | 3 | 36 | 21.39 | < 0.0001 |
| Larva | DI | 3 | 36 | 4.89 | 0.0059 |
| Adult | CP | 3 | 36 | 14.91 | < 0.0001 |
| Adult | DI | 3 | 36 | 0.46 | 0.7096 |

methods differed in their degree of precision using paired t-tests for each life stage, with measurement method and pooled CVs for all investigators as independent and dependent variables, respectively.

## Results and discussion

There were significant differences in recorded measurements of focal subjects between caliper and digital image methods for each life stage of marbled salamanders (Table 1). Egg diameters and adult SVLs obtained from digital images were significantly larger than those from calipers, while the converse was true for larval TLs. In addition to strict differences in measurement values, we also observed significant inter-observer measurement variation using both methods (Table 2). For both larval and adult stage of marbled salamanders, measurements of the same focal subjects differed significantly among investigators when utilizing caliper methods, but only larval stage measurements differed significantly when utilizing digital image analyses. There was no significant inter-observer measurement variation detected for egg stage measurements obtained with either caliper or digital image methods. With respect to time per measurement values, digital image analyses were significantly faster than equivalent numbers of caliper measurements for each life stage, with digital image analyses requiring 38-46% less time than caliper methods (Table 3). Finally, the paired t-tests of CV values identified a significant effect of measurement method on precision of repeated measurements, with digital image analyses associated with significantly higher variation than caliper methods among egg and adult stages of marbled salamanders (Table 4).

Table 3. Results of paired t-tests of mean time per measurement (in seconds/measurement ± 1 STD) for caliper (CP) and digital image (DI) measurement methods for the three life stages of the marbled salamander.

| Life stage | $\bar{y}$ CP | $\bar{y}$ DI | df | t | P |
|---|---|---|---|---|---|
| Egg | 14.45 ± 2.80 | 7.82 ± 0.28 | 3 | -4.95 | 0.0158 |
| Larva | 64.76 ± 14.55 | 36.38 ± 9.14 | 3 | -5.99 | 0.0093 |
| Adult | 57.53 ± 12.42 | 35.68 ± 11.76 | 3 | -7.16 | 0.0056 |

Table 4. Results of paired t-tests of mean coefficient of variation (CV ± 1 SE) for caliper (CP) and digital image (DI) measurement methods for the three life stages of the marbled salamander.

| Life stage | $\bar{y}$ CP | $\bar{y}$ DI | df | t | P |
|---|---|---|---|---|---|
| Egg | 2.35 ± 0.24 | 4.11 ± 1.24 | 39 | 4.96 | < 0.0001 |
| Larva | 2.49 ± 0.75 | 2.29 ± 0.17 | 39 | -1.04 | 0.3044 |
| Adult | 3.57 ± 0.94 | 5.09 ± 0.32 | 39 | 5.78 | < 0.0001 |

The results of our study indicate that there are significant differences in accuracy, precision, inter-observer variation and time requirements between measurements obtained with calipers and digital image analyses. Overall, digital image analyses were considerably faster than caliper methods when measuring all three life stages of marbled salamanders, but the latter exhibited greater precision among repeated measurements. With respect to the accuracy of measurements, our *a priori* assumptions were supported for egg and adult stage salamanders, as diameters, SVLs and TLs were significantly larger when utilizing digital image analyses. In contrast, our assumption was not supported for larval salamanders as caliper methods resulted in significantly larger TLs. Finally, measurements of the same focal subjects among investigators resulted in significantly different values when utilizing either method among larval salamanders or calipers among adult salamanders.

During the measurement process, the behaviour of focal subjects contributed to the observed discrepancies in measurement accuracy between methods. When utilizing the 'salamander stick' for caliper measurements, adult salamanders consistently maintained non-linear body orientations even when restrained within plastic bags, laterally contorting the head, body and tail. Consequently, our best efforts to linearly orient focal subjects resulted in SVLs that were smaller than the known values. In contrast, the use of digital images allowed adult salamanders to move freely, as this technique does not require linear body orientations, resulting in more accurate SVL measurements. Based on these observations, we hypothesized the freedom of movement afforded to focal subjects by digital image analyses, combined with reduced handling times, results in minimized handling stress relative to caliper measurements as well as increased measurement accuracy.

With regard to the accuracy of larval measurements, we initially hypothesized that larger measurement values would be more accurate estimates of TL for the same reasons as indicated for adults. However, when attempting to obtain caliper measurements, we observed that contact between the calipers and either the anterior end of the snout or the posterior end of the tail resulted in larvae fleeing and/or rapidly undulating the body. Given this response, in most cases we were unable to ascertain the most accurate estimate of total length, which would require simultaneous contact of the calipers with both the snout and tail while larvae maintained a linear body position. Consequently, we believe caliper measurements overestimated larval lengths and digital image analyses, although providing relatively smaller measures of larval size, actually provided more accurate measurements. The frequent escape/stress behaviours observed during caliper measurements also suggest that digital image analyses minimize handling stress for aquatic organisms, as the latter method does not require physical contact between investigator and focal organism.

Although our results indicate overall increases in measurement accuracy with digital images relative to calipers, we also identified a significant trade-off between measurement accuracy and precision. For both egg and adult stage salamanders, repeated measurements of a single focal subject were, on average, 43-75% more variable with digital image analyses than with calipers, but there were no differences in measurement variability between methods for larvae. To determine if our estimate of measurement variation could be considered within the acceptable levels for research, we derived CV values for 38 populations of *Ambystoma* spp. (15 adult SVL CVs, 13 larvae TL or SVL CVs, and 10 egg diameter CVs) from previous studies (Kaplan 1980, Semlitsch 1985, Walls & Altig 1986, Semlitsch et al. 1988, Scott 1994). Adult SVL, larval TL/SVL and egg diameter CVs from these populations ranged within 1.2-21.1, 4.2-15.6, and 3.0-28.2, respectively, with mean CVs (± 1 SD) of 6.7 ± 5.4, 6.6 ± 3.1, 9.0 ±8.0 for adults, larvae and eggs. Although these CVs are not derived from repeated measurements of a single focal subject as in our study, they provide a relative indication of variation inherent in studies of these species. Therefore, despite the reduced precision of digital image measurements relative to those from calipers, measurement variation by the former is still well within the established range for these species, and these findings indicate that the increases in accuracy afforded by digital image analyses are not completely offset by reduced precision.

With regard to inter-observer variation, both measurement methods resulted in significantly different values when multiple investigators measured a common focal subject. However, digital image analyses slightly outperformed caliper methods, as significant differences in measurement values were seen in two marbled salamander stages using the former, whereas only one stage exhibited significant variation with digital image analyses. As many field studies employ multiple technicians and/or investigators in obtaining measurements, reduced inter-observer measurement variation represents a considerable advantage of digital image analyses.

In conclusion, increased accuracy and reduced time, inter-observer variation and limited handling of focal subjects afforded by digital image analyses make this technique an attractive alternative to traditional caliper measurement techniques utilized in wildlife research. Although our study was limited to a single species, we believe digital image analyses may be suitable for obtaining linear and non-linear length measurements from any focal subject or focal measurement characterized by a planar surface. Such subjects may include, but are not limited to, snakes, feathers, bones, fish and leaves, as well as linear segments of non-linear objects such as egg, tree and bone diameters. In addition, the recent reductions in the cost of digital cameras and image analysis software packages, minimal storage space required for digital images and ease of learning digital image analysis procedures provide even further support for their utility in future research.

*Acknowledgements* - all procedures associated with this research were conducted in accordance with SIUC Institutional Animal Care and Use Committee (IACUC) protocol #05-037 and Illinois Department of Natural Resources collection permit #A08.4048. We would like to thank L.K. Berkman, E.K. Croteau and L.J. Kjaer for their comments on a previous version of the manuscript. We also thank the United States Forest Service and the Touch of Nature Environmental Center for permission to collect eggs, larvae and adult salamanders. This research was funded by a Delyte and Dorothy Morris Fellowship awarded to C.L. Mott.

## References

Cumming, G.S. & Havlicek, T.D. 2002: Evolution, ecology, and multimodal distributions of body size. - Ecosystems 5: 705-711.

Davis, A.K. & Grayson, K.L. 2007: Improving natural history research with image analysis: the relationship between skin color, sex, age, and stage in adult red-spotted newts (*Notophthalmus viridescens viridescens*). - Herpetological Conservation and Biology 2: 65-70.

Davis, A.K. & Maerz, J.C. 2007: Spot symmetry predicts body conditions in spotted salamanders, *Ambystoma maculatum*. - Applied Herpetology 4: 195-205.

Davis, A.K., Connell, L.L., Grosse, A. & Maerz, J.C. 2008: A fast, non-invasive method of measuring growth in tadpoles using image analysis. - Herpetological Review 39: 56-58.

Gamradt, S.C. & Kats, L.B. 1996: Effect of introduced crawfish and mosquitofish on California newts. - Conservation Biology 10: 1155-1162.

Gibbs, J.P. & Karraker, N.E. 2006: Effects of warming conditions in eastern North American forests on red-backed salamander morphology. - Conservation Biology 20: 913-917.

Grill, C.P. 1999: Development of colour in an aposematic ladybird beetle: the role of environmental conditions. - Evolutionary Ecology Research 1: 651-662.

Hill, M.G., Mauchline, N.A., Cate, L.R. & Connolly, P.G. 2005: A technique for measuring growth rate and survival of armoured scale insects. - New Zealand Plant Protection 58: 288-293.

Horne, M.T. & Dunson, W.A. 1995: Effects of low pH, metals, and water hardness on larval amphibians. - Archives of Environmental Contamination and Toxicology 29: 500-505.

Kaplan, R.H. 1980: The implications of ovum size variability for offspring fitness and clutch size within several populations of salamanders (*Ambystoma*). - Evolution 34: 51-64.

Knouft, J.H. 2003: Convergence, divergence, and the effect of congeners on body size ratios in stream fishes. - Evolution 57: 2374-2382.

Leclair, R., Leclair, M.H. & Levasseur, M. 2005: Size and age of migrating eastern red efts (*Notophthalmus viridescens*) from the Laurentian Shield, Quebec. - Journal of Herpetology 39: 51-57.

Luiselli, L. 2005: Snakes don't shrink, but "shrinkage" is an almost inevitable outcome of measurement error by the experimenters. - Oikos 110: 199-202.

McFadzen, I.R.B., Lowe, D.M. & Coombs, S.H. 1994. Histological changes in starved turbot larvae (*Scophthalmus maximus*) quantified by digital image analysis. - Journal of Fish Biology 44: 255-262.

Relyea, R.R. 2004: Fine-tuned phenotypes: tadpole plasticity under 16 combinations of predators and competitors. - Ecology 85: 172-179.

Scott, D.E. 1990: Effects of larval density in *Ambystoma opacum*: an experiment in large-scale field enclosures. - Ecology 71: 296-306.

Scott, D.E. 1994: The effect of larval density on adult demographic traits in *Ambystoma* opacum. - Ecology 75: 1383-1396.

Semlitsch, R.D. 1985: Reproductive strategy of a facultatively paedomorphic salamander *Ambystoma talpoideum*. - Oecologia 65: 305-313.

Semlitsch, R.D., Scott, D.E. & Pechmann, J.H.K. 1988: Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. - Ecology 69: 184-192.

Taylor, B.E. & Scott, D.E. 1997: Effects of larval density dependence on population dynamics of *Ambystoma opacum*. - Herpetologica 1997: 132-145.

Todd, B.D. & Davis, A.K. 2007: Sexual dichromatism in the marbled salamander, *Ambystoma opacum*. - Canadian Journal of Zoology 85: 1008-1013.

Walls, S.C. & Altig, R. 1986: Female reproductive biology and larval life history of *Ambystoma* salamanders: a comparison of egg size, hatchling size, and larval growth. - Herpetologica 42: 334-345.

Walston, L.J. & Mullin, S.J. 2005: Evaluation of a new method for measuring salamanders. - Herpetological Review 36: 290-292.

Wise, S.E. & Buchanan, B.W. 1992: An efficient method for measuring salamanders. - Herpetological Review 23: 56-57.

Wright, A.N. & Zamudio, K.R. 2002: Color pattern asymmetry as a correlate of habitat disturbance in spotted salamander (*Ambystoma maculatum*). - Journal of Herpetology 36: 129-133.

Zar, J. H. 1999: Biostatistical analyses. 4th edition. - Prentice Hall, Upper Saddle River, 931 pp.

Ziemba, R.E., Myers, M.T. & Collins, J.P. 2000: Foraging under the risk of cannibalism leads to divergence in body size among tiger salamander larvae. - Oecologia 124: 225-231.