DIPLOMARBEIT

Geometric morphometrics of body shape of *Salamandra salamandra* larvae from diverse water bodies

verfasst von

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**Introduction**

Phenotypic plasticity is the ability of a single genotype to produce more than one alternative form of morphology, physiological state, and/or behavior in response to environmental conditions [1]. Larvae of a variety of different amphibian species show phenotypic adaptations in behavior and morphology to different environmental conditions, such as presence of predators or high intraspecific competition. For example, predators can induce large brightly coloured tails and short bodies [2, 3]. Walls et al. [4] detected a diet-induced variation in head shape in larvae of *Ambystoma macrodactylum columbianum*. Increased predation risk induced lower activity, deeper tails, and shorter bodies, which collectively caused slower growth. Increased competition also caused slower growth inducing higher activity, shallower tails, and longer bodies [5]. When exposed to caged dragonfly larvae, newts of two species (*Triturus alpestris* and *Triturus helveticus*) spent more time hiding in the leaf litter, had darker pigmentation in the tail fin, and developed larger heads and tails relative to their body size, in comparison with newts in predator-free ponds [6]. Moreover Van Buskirk [7] showed that several amphibian species had relatively large tails in ponds that were shaded or thickly vegetated.

Steinfartz et al. [8] reported that fire salamander larvae *Salamandra salamandra* (Linnaeus, 1758) from North Rhine–Westphalia near Cologne (Germany) inhabited both small streams as well as shallow ponds, which are ecologically very different from streams. Besides showing differences in their metamorphic behavior, mitochondrial and microsatellite analyses of the larvae showed signs of genetic differentiation. Steinfartz et al. [8] discussed these signs in the context of sympatric speciation as well as ecological separation.
Schulte [9] examined new born fire salamander larvae from streams and ponds. His study was carried out in Germany, in the Kottenforst, where Steinfartz et al. [8] has been working as well. The larvae from standing water bodies showed significantly higher tail fin depth than those from streams. He stated that the higher tail fins might be of advantage when it comes to escaping predators. For the stream larvae, he presumed, the lower tail fin depth prevents them from being swept away by the current. A similar situation can be found in the Vienna Woods (Austria), where fire salamander larvae live in streams as well as in ponds and transitory habitats.

The aim of this study is to compare larval body shape across different habitats. Despite the close geographic proximity of the sampling locations, we expect that larval body shape differs between the habitats because of environmentally induced phenotypic plasticity. We further assess to which degree body shape differences are an allometric effect of differences in body size.

In the last few years geometric morphometrics has become a useful tool in zoology for studying shape variation not only of bones or single body parts but also of the whole individual [6, 10-14]. Geometric morphometrics has been used for shape analysis in larvae of different amphibian species before [6, 7, 13, 15, 16] but not on fire salamander larvae yet.
Material and Methods

Study locations

This study was carried out in the Vienna Woods (Austria) between May and July 2010, at four locations, Lainzer Tiergarten (N48° 9’ 53.651” E16° 14’ 46.622”), Neuwaldegg (N48° 15’ 5.789” E16° 15’ 44.888”), Maurer Wald (N48° 8’ 56.926” E16° 14’ 32.662”) and Moosgraben (N48° 13’ 27.455” E16° 17’ 17.974”), comprising 10 different water bodies. Spatial information was recorded on a pocket computer (MobileMapper 6, Ashtech, Westminster, CO, USA) using the mobile GIS software ArcPad 8.0 (ESRI, Redlands, CA, USA).

The water bodies were categorized as ponds, spring pools, streams, and intermittent streams. Intermittent streams carry water during spring and after heavy rainfalls, but can dry out during the summer. There was one pond in an erosion gully; in our analysis this pond was treated as an intermittent stream. Spring pools and intermittent streams together are regarded as transitory habitats.

The sampling sites in Lainzer Tiergarten were one intermittent stream (LTB), one spring pool (LT1) and one pond (LT2). One clear stream (N) in Neuwaldegg was sampled three times (ascending numeration: N1, N2, N3). The sampling was done stream upwards to avoid measuring the same larvae more than once. In Maurer Wald, three ponds were sampled, one of them cloudy (MS), one clear (MD) and one clear but of dark coloration (MW), as well as one spring pool (Q) and a pond in an erosion gully (P2), which dried out during summer. The
sampling site of Moosgraben (H) was a clear stream. Date of collection, water quality, sample size, type of water body and canopy cover are shown in Table 1.

Table 1: Detailed description of the different sample sites. Canopy cover (1-present, 0-absent).

<table>
<thead>
<tr>
<th>Location</th>
<th>Date</th>
<th>Water Quality</th>
<th>Sample Size (n)</th>
<th>Canopy Cover</th>
<th>Type of Water Body</th>
</tr>
</thead>
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<tr>
<td>Neuwaldegg</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>N1</td>
<td>16.05.2010</td>
<td>clear</td>
<td>20</td>
<td>1</td>
<td>stream</td>
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<tr>
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<td>20</td>
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</tr>
<tr>
<td>N3</td>
<td>23.06.2010</td>
<td>clear</td>
<td>18</td>
<td>1</td>
<td>stream</td>
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<tr>
<td>Mauer Wald</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MD</td>
<td>09.06.2010</td>
<td>clear</td>
<td>10</td>
<td>1</td>
<td>pond</td>
</tr>
<tr>
<td>MS</td>
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<td>19</td>
<td>0</td>
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<tr>
<td>MW</td>
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<td>22</td>
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<tr>
<td>P2</td>
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<td>15</td>
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<tr>
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<td>25</td>
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<tr>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>29.07.2010</td>
<td>clear</td>
<td>26</td>
<td>1</td>
<td>stream</td>
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<tr>
<td>Lainzer Tiergarten</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LTB</td>
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<td>clear</td>
<td>11</td>
<td>1</td>
<td>intermittent stream</td>
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<tr>
<td>LT1</td>
<td>03.07.2010</td>
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<td>14</td>
<td>1</td>
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</tr>
<tr>
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<td>03.07.2010</td>
<td>turbid</td>
<td>16</td>
<td>1</td>
<td>pond</td>
</tr>
</tbody>
</table>

Data collection

A total of 216 fire salamander larvae were collected during this study. They were caught using a dip net aiming for a sample size of 20 individuals per sampling site. Immediately after capture they were put into a photo cuvette (for lateral images) and a specifically adapted cell culture flask (for dorsal images) filled with tap or pond water depending on the air temperature to avoid misting up of the vessels. The vessels were placed on a small carton
ensuring a level surface. Subsequently, dorsal and lateral high-resolution images were taken (Nikon D90, Sigma 105 mm macro lens) from a constant distance: 85 mm for the dorsal images and 90, 115 or 185 mm for the lateral images, depending on the light conditions. For scaling a plotting paper was attached to the outside of the photo cuvette and cell culture flask. Immediately after the photographs were taken, the larvae were released into the water bodies.

Data evaluation

Geometric Morphometrics

Seven landmarks and 47 semilandmarks were digitized on the two-dimensional lateral images and additionally 12 landmarks and 44 semilandmarks on the two-dimensional dorsal images using the tps software package [17]. For the lateral images, the 7 landmarks represent the tip of the nose, the tip of the tail, the centre of the eye, the insertion of the limbs and the beginning of the tail fin (dorsal and ventral). The 12 landmarks on the dorsal images represent the tip of the nose, the centre of each eye, the anterior edge of the gills, the insertions of the limbs and the tip of the tail (Figure 1).
Landmarks are anatomically homologous point locations. Semilandmarks lie on homologous curves, yet their exact position along these curves is unclear and hence statistically estimated. They are allowed to slide along their curves in order to minimize the thin plane spline's bending energy from the actual landmark configuration to the sample average configuration [18, 19].

The landmark configurations were superimposed using GPA (Generalized Procrustes Analysis), involving three steps [20, 21]. First, all the landmark configurations were translated so that they share the same centroid. Subsequently, they were scaled so they all had the same centroid size (square root of the summed square deviations of the coordinates from their centroid), and then rotated until the sum of the squared distances between corresponding landmarks was minimized. The dorsal landmark configurations were symmetrized by
averaging each configuration with its relabelled reflection [22, 23]. The resulting shape coordinates were analysed by a between-group principal component analysis which is recommended for Procrustes data as an alternative to descriptive discriminant analysis (bgPCA; [24]).

Within-group allometry was estimated by a pooled regression of shape on centroid size (CS). Group differences and allometry were visualized by thin-plate spline deformation grids [25] and tested for significance by permutation tests (5000 random permutations) using Procrustes distance and explained variance as test statistics. In order to remove allometric shape variation from the data, the individuals were projected in the subspace perpendicular to the allometry vector (vector of regression coefficients; [26]).

The total length (TL) of the fire salamander larvae (dorsal images) was calculated by measuring the distance between the tip of the nose and the tip of the tail, using PAST version 2.12 [27]. Statistical analysis of total TL was done using SPSS versions 19 and 20 (IBM Co., Armonk, USA). The data was tested for normality using a Shapiro-Wilk test and indicated normal distribution, allowing the usage of parametric tests. Total length were statistically analysed with one-way analysis of variance (ANOVA) and post-hoc Least Significant Difference (LSD) tests. Probability levels of \( p < 0.05 \) (95% confidence interval) were considered statistically significant.

All geometric morphometric computations and statistical analyses were performed using the Mathematica 8.0 software package (Wolfram Research Inc., Champaign, IL, USA) [28].
Results

Between-group principal component analysis clearly separated pond populations (MS, MW, and LT2) from stream populations (H, N1, N2, and N3). Populations from transitory habitats (LT1, LTB, P, and Q) had an intermediate position (Figure 1). The first two between-group principal components accounted for 81% of the variation in body shape for the dorsal images and for 70% for the lateral images.

Figure 2 Between-group principal component analysis (bgPCA) of the 12 population mean shapes (A: dorsal images; B: lateral images). A: bgPC1 – variation in head shape, bgPC1- shape variation of the abdomen; B: bgPC1 – shape variation of tail fin, bgPC2 – variation of head shape. The arrows indicate the average within-population allometry. The length of the arrows corresponds to a shape change induced by a change in size of four standard deviations.
Allometry contributed to group separation in the dorsal images, but only minimally in the lateral images (Figure 2). Pond populations, on average, showed a higher tail fin and a narrower head. Stream populations had relatively shallow tail fins and a broader head than pond populations. Permutation tests suggest that the average shape of all pond populations differs significantly from the average shape of all stream populations ($P<0.001$). These differences are still apparent independently of allometry (Figure 3).

Larvae in standing water bodies were generally longer than larvae in streams and transitory habitats. Stream larvae, on average were $3.99\pm0.79$ cm ($n=84$), pond larvae $5.15\pm0.88$ cm ($n=67$) and larvae from transitory habitats $5.04\pm0.88$ cm ($n=65$) long (Figure 4). Statistically significant differences were found between the larvae from pond and stream populations and between the larvae from pond and transitory populations ($F_{2, 157}=44.457$, $p<0.0005$), whilst there were no statistical differences present between the pond and transitory populations ($p=0.133$).
Figure 3 (A) Deformation grids visualizing the average shape differences between populations in ponds and streams, extrapolated by a factor of three (B). Average shape differences between pond and stream populations independent of allometry (in the subspace perpendicular to allometry). (C) Visualization of pooled within-group allometry.
**Figure 4** Distribution of the total length (cm) between all samples (for n-values see Figure 1). Streams: N1, N2 (light grey), ponds (open), transitory habitats (dark grey). Boxes show the 50th percentiles. The boundary of the box closest to zero indicates the 25th percentile and the boundary of the box farthest from zero indicates the 75th percentile. The band inside the box shows the median. Whiskers indicate the 10th and 90th percentile, and dots outside of the boxes are outliers. An outlier is any data point that is more than 1.5 times the interquartile length from either end of the box.
Discussion

The ability of amphibian larvae to alter their phenotype in the presence of predators or due to competitors has been studied intensively [5, 7, 12-14, 16, 29-34]. We assessed if larval body shape is influenced by the water body in which they develop. Schulte [9] showed that fire salamander larvae from pond populations had higher tail fins than larvae from stream populations what is in line with our findings. The larvae from our pond populations had higher tail fins and a narrower head, whereas the ones from streams had a broader head and relatively shallow tail fins. The higher tail fins could be used for luring predators away from the fragile body to the tail area in order to survive an attack [35, 36]. Additionally a higher tail fin could be important as respiratory surfaces, influence swimming performance and relating to the swimming, enhance hunting success. Although fire salamander larvae living in streams prefer microhabitats exposed to only minor hydraulic stress [37], having a shallower tail fin could be advantageous for avoiding being swept away. The broader head shape of the pond populations could be an adaptation to the presence of predators or an adaptation to prey size or volume of consumed prey [12]. It has also been shown that a higher competitor density may induce a broader head in order to compensate for resource limitation by increasing food consumption [4, 7, 12].

On average the larvae from standing water bodies were larger than those from streams or transitory habitats (Figure 4). Csillery & Lengyel [38] found that fire salamander larvae increase their body length in response to high density in a field experiment. Furthermore, density can affect both age and size at metamorphosis [39]. Although this could be one reason for the size differences between our study-populations, food availability may also play an
important role. Zahn [40] ascertained that with higher food availability growth rate increases as well. A higher birth weight might be of advantage, for instance Weitere [41] calculated in his study a time saving of about a week of feeding before metamorphosis. In Csillery & Lengyel’s experiment the fire salamander larvae that were kept in shallower water were heavier and they assume that the larvae accelerate their metamorphosis to avoid desiccation [38, 42]. Sadeh did an experiment in which he exposed fire salamander larvae (S. *infraimmaculata*) to two water level regimes in combination with chemical cues and tested for a developmental response. The final larval size at metamorphosis differed significantly between water level regimes, independently of the presence of the chemical cues. Sadeh supposed that morphological changes occur throughout larval ontogeny before the final stage of metamorphosis, indicating that differentiation is occurring during the whole larval period along with growth in body size [43]. We showed in our study that fire salamander larvae show allometric differences in body shape allometry, however allometry had no effect on the shape differences we found between our populations. Our findings indicate that the different body shapes are environmentally induced.

Steinfartz et al. [8] found early stages of genetic differentiation in the *S. salamandra* population and discussed them in the context of adaptive differentiation. Zutz [44] investigated the situation from two populations from the Vienna Woods, one from Maurer Wald and one from Neuwaldegg. Her study showed that the genetic diversity was not significant between the two sites. The phenotype of an animal is a product of both the genotype and the environment [1]. As our populations are only minimally genetically differentiated- if at all -, the phenotypic differences between the populations are unlikely to be genetically determined.
In conclusion, it could be shown that fire salamander larvae display different phenotypes, depending on their habitats. Larvae in ponds generally had higher tail fins and narrower heads, whilst larvae in streams had relatively shallow tail fins and broad heads. The geometric morphometrics approach allows to document shape alteration and presents a powerful analytical method for investigations of differences in larval body shapes.
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Abstract

Shifts in life histories, from lotic to lentic larval habitats or to direct development, have been important events in the evolutionary diversification of salamanders. Recently, a habitat change from streams to ponds in fire salamanders in Western Germany was discussed in the context of sympatric speciation (Steinfartz et al. 2007). We investigate a similar situation in Vienna (Austria) where fire salamander larvae not only inhabit streams, but also ponds and transitory habitats. To probe the generality of patterns of adaptive change, we analyzed variation in larval body shape from a gradient of habitats, ranging from fast running streams over intermittent streams to stagnant pools, with geometric morphometric methods. We found that larvae differ in their body shape, depending on the water body they live in. Larvae from ponds and transitory habitats were generally longer, showed a higher tail fin and a narrower head whereas larvae from streams were shorter, had a shallower tail fin and a broader head depending on the habitat they live in. A study on the genetic diversity of the fire salamander populations in Vienna showed that there were no significant differences between these populations (Zutz 2012) and a process of speciation could not be shown. In conclusion it could be shown that fire salamander larvae display different phenotypes and they are likely to be environmentally induced.
Zusammenfassung

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