RESEARCH ARTICLE

Masters of change: seasonal plasticity in the prey-capture behavior of the Alpine newt *Ichthyosaura alpestris* (Salamandridae)

Egon Heiss[1,2,*], Peter Aerts[1,3] and Sam Van Wassenbergh[1,4]

[1]Department of Biology, University of Antwerp, Universiteitsplein 1, B-2610 Antwerp, Belgium, [2]Department of Integrative Zoology, University of Vienna, Althanstr. 14, A-1090 Vienna, Austria, [3]Department of Movement and Sports Sciences, Ghent University, Watersportlaan 2, B-9000 Ghent, Belgium and [4]Evolutionary Morphology of Vertebrates, Ghent University, K.L. Ledeganckstraat 35, B-9000 Ghent, Belgium

*Author for correspondence (egon.heiss@ua.ac.be)

SUMMARY

Transitions between aquatic and terrestrial environments are significant steps in vertebrate evolution. These transitions require major changes in many biological functions, including food uptake and transport. The Alpine newt, *Ichthyosaura alpestris*, is known to show a 'multiphasic lifestyle' where the adult shifts from a terrestrial to an aquatic lifestyle and then back to a terrestrial lifestyle every year as a result of its breeding activity. These transitions correspond to dramatic changes in morphology, physiology and behavior, resulting in distinct aquatic and terrestrial morphotypes. We hypothesized that these shifts go along with changes in prey-capture mechanics to maintain a sufficiently high performance in both environments. We analyzed the prey-capture kinematics in the four possible modes: aquatic strikes in the aquatic phase, terrestrial strikes in the terrestrial phase, aquatic strikes in the terrestrial phase and terrestrial strikes in the aquatic phase. A multivariate comparison detected significant kinematic differences between the phase-specific feeding modes. In both the aquatic and the terrestrial phase, *I. alpestris* uses a suction-feeding mechanism for capturing prey in water. By contrast, *I. alpestris* uses a jaw-based grasping mechanism with a kinematic profile similar to the aquatic modes for terrestrial prey-capture in its aquatic phase but an elaborate lingual-based prehension mechanism to capture terrestrial prey in the terrestrial phase. These results exhibit a so-far unknown amount of behavioral plasticity in prey-capture behavior that is tuned to the seasonal demands of performance, and exemplify functional mechanisms behind aquatic–terrestrial transitions in vertebrates.

Key words: feeding, kinematics, plasticity, salamander.

Received 5 June 2013; Accepted 21 August 2013

INTRODUCTION

Prey capture is challenging when switching between aquatic and terrestrial environments. Most aquatic vertebrates use a hydrodynamic mechanism, which typically includes a fast oropharyngeal volume expansion by means of abduction of the jaws and hyobranchial elements. This generates a suction flow into the expanding mouth, which draws the prey and its surrounding water into the oral cavity (Muller and Osse, 1984; van Leeuwen and Muller, 1984; Lauder, 1985; Lauder and Shaffer, 1986; Van Damme and Aerts, 1997; Lemell et al., 2002; Marshall et al., 2008; Kane and Marshall, 2009; Heiss et al., 2013). However, as suction feeding does not work on land because of the low density and viscosity of air, most terrestrial vertebrates have evolved a highly modified capture mode where precise contact of the prey by the jaws and/or tongue plays a central role (Larsen and Guthrie, 1975; Bramble and Wake, 1985; Dockx and De Vree, 1986; Findeis and Bemis, 1990; Schwenk, 2000; Herrel et al., 2012). Consequently, this general classification of prey-capture behaviors of aquatic *versus* terrestrial vertebrates indicates that aquatic and terrestrial feeding success relies on different sets of movements of the hyobranchial apparatus and the oral jaws (Deban, 2003).

The biophysical demands on the prey-capture system as a whole are conflicting between water and air (Deban and Wake, 2000; Deban, 2003). This implies that animals feeding in the two media must either employ the same set of feeding movements in both, and thus perform suboptimally in at least one, or change their feeding behavior in response to the different biophysical demands they encounter (Bramble, 1973; Stayton, 2011). In fact, only a few species have evolved mechanisms that allow them to exploit food sources in both environments. Such amphibious feeders are mainly known within lower tetrapods, especially within salamanders and turtles (Reilly and Lauder, 1989; Miller and Larsen, 1989; Miller and Larsen, 1990; Summers et al., 1998; Deban and Wake, 2000; Deban, 2003; Heiss et al., 2008; Natchev et al., 2009; Natchev et al., 2010; Stayton, 2011). Recent studies have shown that the capability to feed in both media is mainly due to behavioral plasticity, where modulatory behavior compensates for morphological constraints (Stayton, 2011). This capacity is hypothesized to be an important pre-requisite for the invasion of new environments.

Aquatic–terrestrial transitions are integral events in the life history of most recent amphibians (Duellman and Trueb, 1994) – making them suitable models to study environmental transitions. In principle, they show a two-phasic life history where they metamorphose from an aquatic larva to a more or less terrestrial postmetamorphic stage. Within amphibians, newts (salamandrids) are of special interest to studies of aquatic–terrestrial transitions as they show a unique pattern referred to as a 'multiphasic lifestyle', where the adults change from a terrestrial to an aquatic life, and

then revert to the terrestrial stage every year because of their breeding activity (Duellman and Trueb, 1994; Griffiths, 1996; Thiesmeier and Schulte, 2010; Kopecký et al., 2012). These seasonal transitions are associated with profound changes in physiology, morphology and behavior of the whole organism (Matthes, 1934; Duellman and Trueb, 1994; Warburg and Rosenberg, 1997; Thiesmeier and Schulte, 2010). Focusing on the feeding apparatus, the disappearance and regeneration of labial lobes can be observed (Matthes, 1934), but no changes of the hyobranchial system have been documented. This is interesting because the hyobranchium plays a central role in both aquatic and terrestrial prey capture but the biophysical demands on it are conflicting between the media. Nevertheless, as newts are known to effectively capture prey in both environments, it is likely that they use behavioral strategies to compensate for morphological constraints. However, the impact of the seasonal habitat change on their prey-capture behavior is to date unknown.

In this study we analyzed the prey-capture behavior of the Alpine newt *Ichthyosaura alpestris*, a species known for its multiphasic lifestyle by exhibiting seasonal habitat shifts where it changes between two distinct morphotypes (Matthes, 1934; Duellman and Trueb, 1994; Thiesmeier and Schulte, 2010). Alpine newts are known to feed in water when they are in the aquatic phase and on land when they are in the terrestrial phase (Denoël, 2004; Thiesmeier and Schulte, 2010). Additionally, they do occasionally go on land when they are in the aquatic phase and *vice versa*: they seek their home waters when they are in the terrestrial phase (Thiesmeier and Schulte, 2010; Kopecký et al., 2010). Accordingly, it is not unlikely that they also feed during such excursions and are capable of feeding on land when they are in the aquatic phase and *vice versa*.

Here, we compared the prey-capture kinematics in the four possible feeding modes, namely (1) aquatic feeding in the aquatic phase, (2) terrestrial feeding in the terrestrial phase, and the reciprocal modes (3) aquatic feeding in the terrestrial phase and (4) terrestrial feeding in the aquatic phase. This allowed us to test whether the prey-capture strategy changes between aquatic and terrestrial environments. Dual-phase urodels use suction as aquatic larvae and lingual prehension after metamorphosis when they gain a terrestrial lifestyle (Lauder and Reilly, 1994; Reilly, 1996; Deban and Wake, 2000; Deban, 2003). In analogy with this pattern observed in dual-phase urodeles, we predicted prey capture to be based on suction when *I. alpestris* is in the aquatic phase and feeding underwater, but to rely on lingual prehension to feed on land when this species is in the terrestrial phase. Furthermore, we hypothesized that the seasonal habitat change also impacts the reciprocal feeding modes. More specifically, if the neuromotor control of the prey-capture movements is changed with habitat, aquatic lingual prehension is expected to occur if animals are in the terrestrial phase, and terrestrial feeding using suction movements if they are in the aquatic phase. This hypothesis is based on previous findings that lingual prehension in water is in fact used by terrestrial salamanders (Deban and Marks, 2002) and, *vice versa*, movements similar to suction feeding on land are used by aquatic salamanders (Miller and Larsen, 1989; Miller and Larsen, 1990). Alternatively, if the neuromotor control of prey capture is easily reversible in the Alpine newts, they could select the appropriate, medium-specific prey-capture mode and use lingual prehension on land and suction feeding underwater regardless of the current phase that they are in.

MATERIALS AND METHODS
Study animals
The alpine newt, *I. alpestris* (Laurenti 1768), is a medium-sized salamandrid ranging on average from 80 to 120 mm total length, with females being slightly larger than males. The Alpine newt is one of the most common European salamanders and has a wide distribution area that ranges from Spain in the west to Ukraine in the east – and from Denmark in the north to Italy and Greece in the south, where it can be found from lowland areas a few meters above sea level to the mountains, up to an altitude of 2600 m (Nöllert and Nöllert, 1992; Thiesmeier and Schulte, 2010). The Alpine newt develops from an aquatic, gill-bearing larva that undergoes metamorphosis and changes to a more or less terrestrial juvenile, typical salamander-like form. Depending on the season, adult *I. alpestris* live in aquatic habitats that can range from tiny temporary pools up to lakes, or the terrestrial habitat around them. More specifically, the Alpine newt is known to exhibit a multiphasic lifestyle where it changes between aquatic and terrestrial habitats, depending on the season. In general, *I. alpestris* hibernates on land and starts its migration to its home water in the early spring (aquatic phase) – where it stays till mid-summer, roughly till breeding season is completed. Then, it leaves the water and gains a terrestrial lifestyle (terrestrial phase), hibernates on land and migrates to the home waters by the early spring again, closing the 'multiphasic cycle' (Thiesmeier and Schulte, 2010).

The natural food spectrum in adult *I. alpestris* is very broad and, because of the diverging availability of prey organisms in water and on land, its diet also differs between its aquatic and terrestrial phase (Joly, 1981; Sattmann, 1989; Denoël, 2004; Thiesmeier and Schulte, 2010). In its aquatic phase, *I. alpestris* mainly preys on aquatic insect larvae, crustaceans and tadpoles (Joly, 1981; Sattmann, 1989). During the terrestrial phase, it feeds mainly on terrestrial insects and their larvae, oligochaets, spiders and isopods (Thiesmeier and Schulte, 2010).

The animals used for the present study were collected in the aquatic phase between April and June 2011 in Lower Austria, Austria, with collection permission (RU5-BE-18/022-2011) granted by the local government of Lower Austria. The animals were kept in large tanks with water levels not exceeding 15 cm and an easily accessible land part with piles of cork bark pieces. The water was permanently filtered by an external trickle filter and the top of the tanks was covered with a removable mosquito net to prevent newts from escape. The animals were fed twice a week with a variety of red mosquito larvae (Chironomids), firebrats (*Thermobia domestica*) and maggots (*Lucilia* sp.). Animal husbandry and experiments were approved by the Ethical Commission for Animal Experiments of the University of Antwerp (code: 2010-36).

High-speed video recording
To record feeding kinematics, *I. alpestris* were habituated to feed in a small glass aquarium (base area: 12×30 cm, height: 20 cm) where they were filmed with two digital high-speed cameras (Redlake MotionScopeM3 and Redlake Motion-Pro HR1000a; Redlake Digital Imaging Systems, IDT Vision, Tallahassee, FL, USA) in lateral and ventral views at a frame rate of 500 Hz.

As light sources we used four infrared spotlights. To avoid distortive effects of different prey types on the prey-capture behavior (Maglia and Pyles, 1995; Deban, 1997), we used living maggots (*Lucilia* sp.) as standardized prey items. Maggots were also used because they are a natural prey and all newts showed a strong preference for feeding on them.

The experiments were performed when the newts were in the aquatic phase and again after they had changed to the terrestrial phase. The change of phase was determined first by the fact that animals suddenly but permanently changed from the aquatic to the terrestrial habitat in their tank (or accordingly *vice versa*) and second by external morphological features, such as the growth or
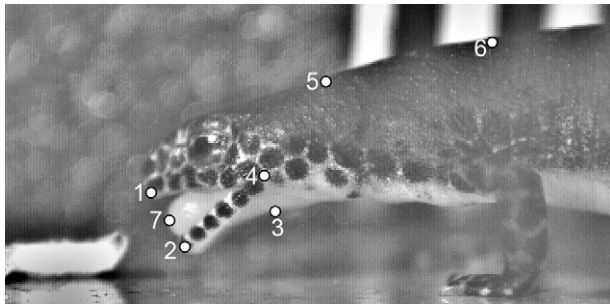
Fig. 1. Landmarks used for the kinematic analyses: 1, upper jaw tip; 2, lower jaw tip; 3, hyoid (throat); 4, jaw joint; 5, nape; 6, dorsal trunk reference; 7, tongue tip (only used if visible).

degeneration of tail fins or labial lobes. Animals were given 4 weeks after a change of phase to accommodate to the new habitat.

For kinematic analyses, we selected the lateral recordings of five feeding trials for each of four similarly sized individuals (one male,

Four feeding modes in two phases were recorded: (i) terrestrial feeding in the terrestrial phase, (ii) aquatic feeding in the aquatic phase, (iii) aquatic feeding in the terrestrial phase and (iv) terrestrial feeding in the aquatic phase.

For filming aquatic feeding in the aquatic phase, prey was offered in front of the animals in the experimental aquarium with a 5 cm water level. The same setup was used to record aquatic feeding in the terrestrial phase. For filming terrestrial feeding in the aquatic phase, a Plexiglas ramp was placed into the aquarium with a 5 cm water level and the newts were then slowly lured out of the water *via* the ramp to capture the prey offered on land. For filming terrestrial feeding in the terrestrial phase, prey was offered in front of the animals in the same aquarium but without water. The ventral view recordings were performed to determine lateral expansion movements of the head during prey capture. However, as no significant lateral movements could be measured, they were excluded from further analyses and used for observations only.

Kinematics

For kinematic analyses, we selected the lateral recordings of five feeding trials for each of four similarly sized individuals (one male,
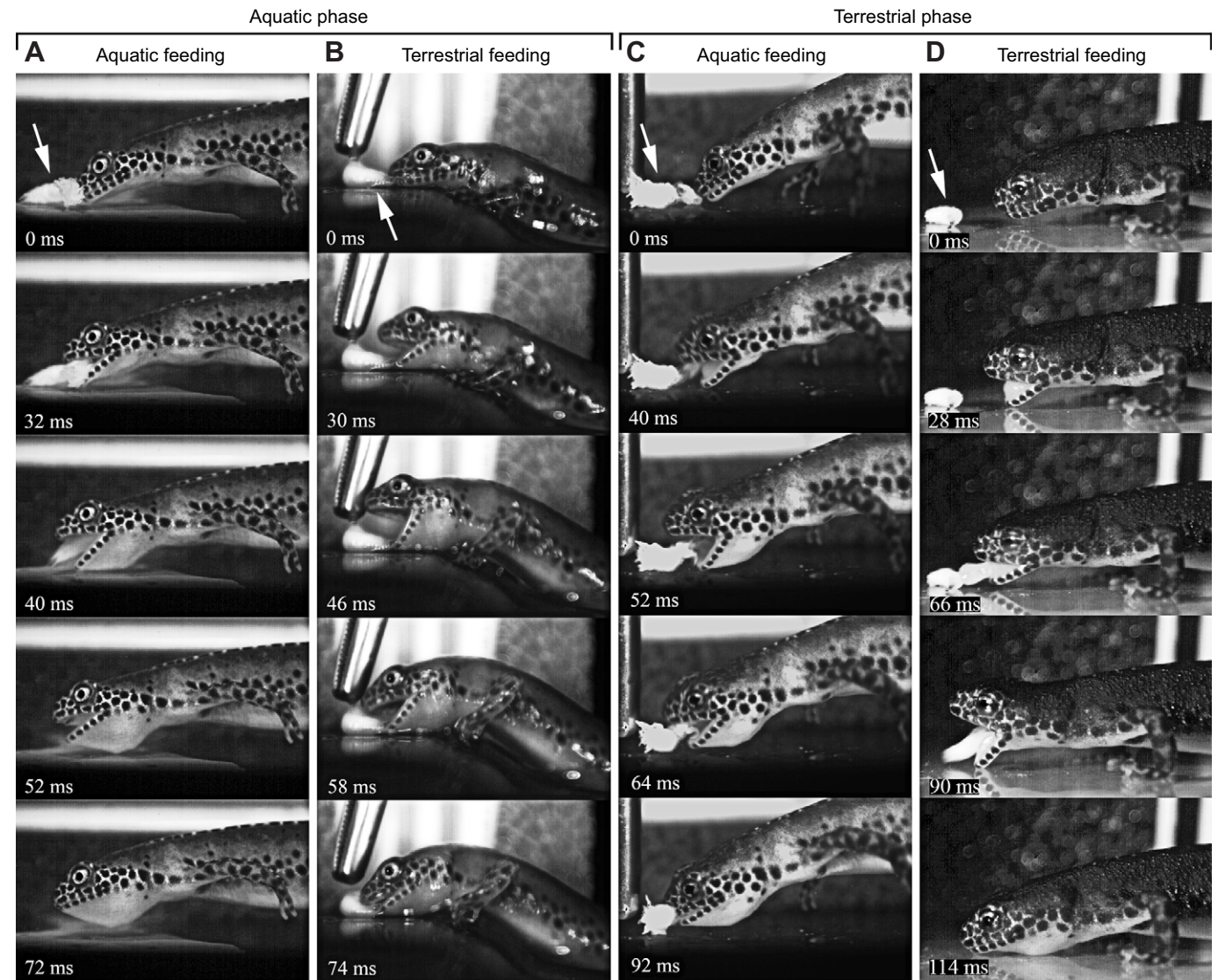

Fig. 2. Frame shots showing all four feeding modes in the same individual: (A) aquatic feeding in the aquatic phase; (B) terrestrial feeding in the aquatic phase; (C) aquatic feeding in the terrestrial phase; and (D) terrestrial feeding in the terrestrial phase. The prey (a maggot) is indicated by the arrows. Note the similar prey-capture movements in the first three modes: aquatic feeding in the aquatic phase, terrestrial feeding in the aquatic phase and aquatic feeding in the terrestrial phase; and the distinct pattern in the last mode: terrestrial feeding in the terrestrial phase, where prey is captured by the tongue.

three females, ranging in total length between 84 and 87 mm) that readily fed in all four modes and provided good quality feeding strike recordings, resulting in a total of 80 recordings.

The horizontal (*x*-axis) and vertical (*y*-axis) coordinates of previously defined landmarks (Fig. 1) were tracked frame by frame using SIMI-MatchiX software (SIMI Reality Motion Systems, Unterschleissheim, Germany). Our landmarks were based on those used by other studies on salamander prey capture (Shaffer and Lauder, 1985; Reilly, 1995; Reilly, 1996; Deban, 1997; Deban and Marks, 2002; Deban and O'Reilly, 2005) to allow direct comparison of kinematics. According to the 2D displacement of the landmarks, we calculated the following movements: jaw movement (distance between the tips of the upper and lower jaw), head rotation (dorsoventral angle displacement of the head relative to the trunk), hyoid depression (distance between the jaw joint and throat where maximum depression occurs) and tongue movements (distance between tongue tip and jaw joint) in the terrestrial feeding mode of the terrestrial morphotype. From these kinematic profiles, 12 kinematic variables that summarize the kinematics of a whole prey-capture event were determined in analogy with previous research on prey-capture biomechanics in salamanders (Shaffer and Lauder, 1985; Reilly, 1995; Reilly, 1996; Deban, 1997). These variables were (1) time to maximum gape (i.e. time from start of mouth opening to maximum gape), (2) duration of gape closing (i.e. time from maximum gape till mouth closing), (3) maximum gape (i.e. maximum distance between upper and lower jaw tips), (4) velocity of gape opening (i.e. mean velocity of mouth opening calculated as a derivative of gape change), (5) velocity of gape closing (i.e. mean velocity of mouth closing calculated as a derivative of gape change), (6) time to start of hyoid depression (i.e. time from start of mouth opening to start of hyoid depression), (7) duration of hyoid depression (i.e. time from start of hyoid depression to maximum hyoid deflection), (8) maximum hyoid depression (i.e. maximum distance between jaw joint and ventral hyoid deflection), (9) velocity of hyoid depression (i.e. mean velocity of ventral hyoid deflection calculated as a derivative of hyoid deflection), (10) maximum head elevation (i.e. maximum angle of head relative to trunk), (11) duration of head elevation (i.e. time from start of head elevation to maximum head deflection) and (12) velocity of head elevation (i.e. mean angular velocity of head elevation calculated as a derivative of head rotation).

Statistics

After calculating descriptive statistics for each variable and individual, we checked for normal distribution of the residuals of the variables and if they were not normally distributed we $\log_{10}$ transformed the variables, after which their residuals were normally distributed. Then, we performed a multivariate ANOVA (MANOVA) where both 'individual' and 'feeding mode' were treated as fixed factors. In order to account for running multiple tests, the simultaneous Bonferroni correction was used to adjust significance levels to $P \leq 0.0042$. Additionally, we performed a principal component analysis to show the effects of (i) individual and (ii) feeding mode on the total variation of the feeding behavior. All statistical analyses were performed with Microsoft Excel 2010 and SPSS Statistics 20 software package.

RESULTS
Aquatic feeding kinematics

When feeding underwater in the aquatic phase, *I. alpestris* approached the prey slowly and then sucked it up by an anteroposteriorly directed wave of oropharyngeal expansion (Fig. 2A). The prey-capture event started with jaw opening, achieved by dorsal skull rotation and lower jaw depression. The maximum gape of 5.5±1.6 mm (mean ± s.d.) was reached after 31.4±4.5 ms, immediately after which mouth closing started. Hyoid depression started shortly after the onset of mouth opening and reached its ventral deflection peak of 4.6±1.0 mm after 30.5±6.1 ms, almost simultaneously with the peak gape. The prey was sucked in before jaw closing started (see Table 1, Fig. 2A, Fig. 3A). The angle of the skull relative to the longitudinal body axis was always smaller ('nose down') after the jaws were closed (end of gape cycle) than before jaw opening started (start of gape cycle). The whole gape cycle described a bell-shaped curve and lasted 58.5±6.4 ms (Table 1, Fig. 2A, Fig. 3A).

The kinematic profile of animals feeding underwater in the terrestrial phase showed a slightly (*ca*. 10 ms) slower gape cycle. Apart from that, it was nearly identical to the aquatic feeding profile in the aquatic phase as described above (see Table 1, Fig. 2B, Fig. 3B, and statistics below).

Terrestrial feeding kinematics

Terrestrial feeding in the terrestrial phase started with a slow approach to the prey. Then, the mouth was opened by dorsal skull

Table 1. Descriptive statistics of kinematic variables in the four different feeding modes and subsequent ANOVA testing effects of individual and feeding mode

| Variable | Aquatic phase | | Terrestrial phase | | ANOVA | | | |
| | Aquatic prey capture | Terrestrial prey capture | Aquatic prey capture | Terrestrial prey capture | Individual | | Mode | |
					F-ratio	*P*-value	*F*-ratio	*P*-value
Time to maximum gape (ms)	31.4±4.5	47±15	36±5.4	102±19	6.878	≤0.001*	175.950	≤0.001*
Duration of gape closing (ms)	27.1±4.9	32.6±7.8	32±5.4	32.8±8.5	0.521	0.669	3.375	0.024
Maximum gape (mm)	5.5±1.6	7.0±1.2	5.6±1.2	6.7±1.9	29.501	≤0.001*	13.204	≤0.001*
Velocity of gape opening (m s^{-1})	0.2±0.06	0.16±0.05	0.16±0.04	0.07±0.02	9.615	≤0.001*	42.751	≤0.001*
Velocity of gape closing (m s^{-1})	0.18±0.06	0.2±0.06	0.18±0.06	0.2±0.07	13.616	≤0.001*	1.174	0.327
Time to start hyoid depression (ms)	7.6±4.8	11.6±9.3	9.3±5.8	72.7±13.5	9.040	≤0.001*	89.692	≤0.001*
Duration of hyoid depression (ms)	30.5±6.1	39±12	31.9±6.1	58.9±14.2	2.314	0.084	31.818	≤0.001*
Maximum hyoid depression (mm)	4.6±1.0	4.0±0.9	4.2±0.9	2.7±0.7	9.196	≤0.001*	24.959	≤0.001*
Velocity of hyoid depression (m s^{-1})	0.2±0.04	0.1±0.04	0.1±0.03	0.05±0.02	1.844	0.148	47.532	≤0.001*
Maximum head elevation (deg)	15.4±5.5	17.8±5.8	15.2±4.5	21.6±7.2	4.708	0.005	6.497	0.001*
Duration of head elevation (ms)	30.2±5.8	42.7±13.4	33.3±4.7	89.1±25.3	0.834	0.480	90.465	≤0.001*
Velocity of head elevation (deg s^{-1})	518±190	450±168	458±137	249±61	3.346	0.024	15.924	≤0.001*

Prey capture data are means ± s.d.

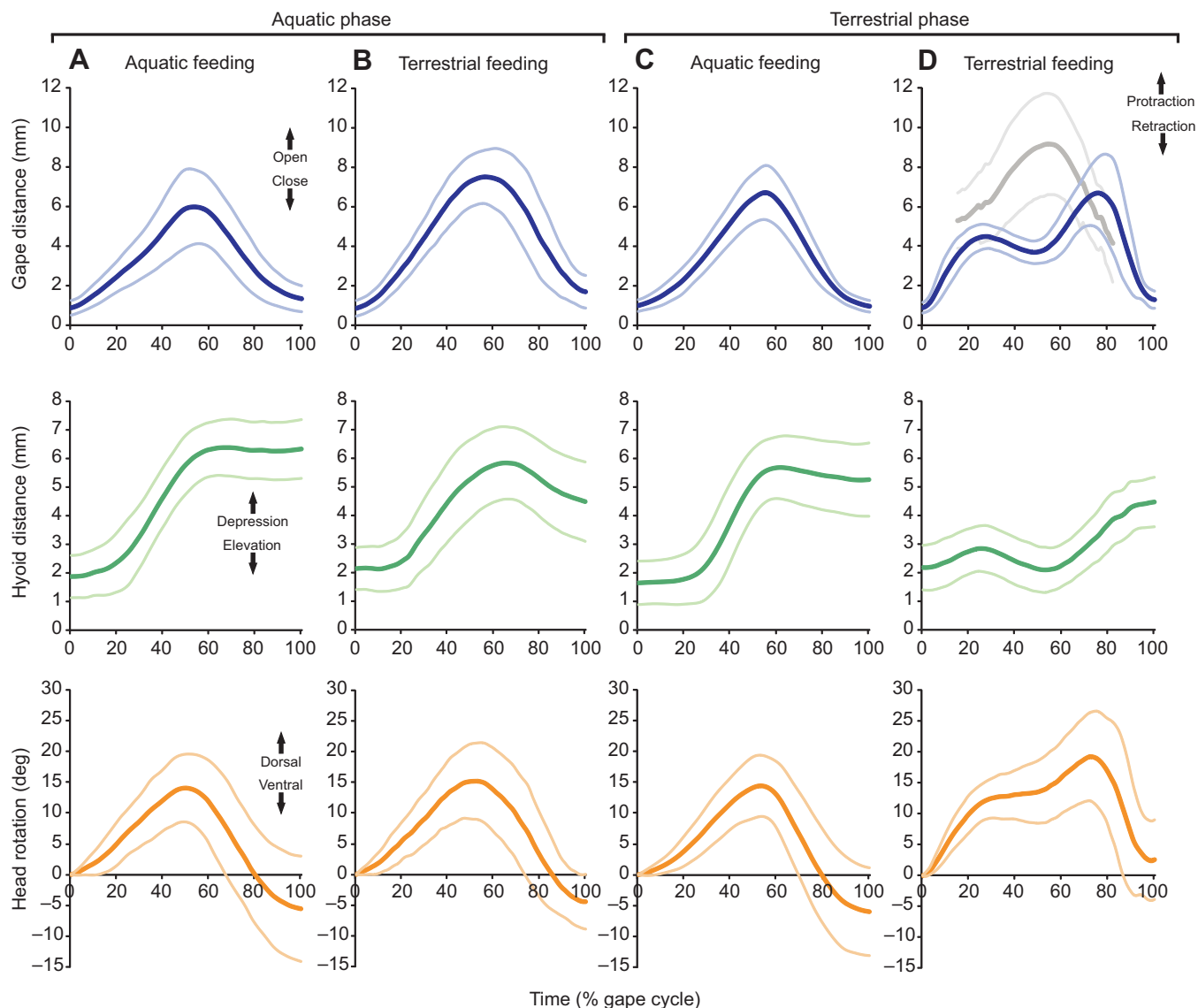*Significant difference at $P \leq 0.0042$ (simultaneous Bonferroni-corrected significance level).

Fig. 3. Kinematic means (bold curves) ±s.d. (light curves) of gape (blue), hyoid (green), head rotation (orange) and tongue movement (gray, only shown in D). The time scale on the *x*-axes is normalized as a percentage of total gape cycle; see Table 1 for absolute values.

rotation and lower jaw depression, and the gape reached a first peak (Fig. 2D, Fig. 3D). During this first jaw-opening phase, the tongue was slowly protracted to the margins of the lower jaw. Next, the mouth was slightly closed again and the tongue was projected out of the mouth to contact the prey. When the tongue with the adhering prey was retracted, the gape increased again and reached its second peak of 6.7±1.9 mm after 102±19 ms, just at the instant when the prey was engulfed (Fig. 2D, Fig. 3D). Then, the mouth was quickly closed. The kinematics of the gape described a two-peaked profile where the first peak was always lower than the second and the whole gape cycle lasted 135±22 ms (Table 1, Fig. 3D). When the mouth started opening, the hyoid was slightly depressed and then became elevated after the gape reached its first peak. The peak of the hyoid elevation was correlated with the local gape minimum (r_{20}=0.87, P<0.001) which appeared between the two gape peaks, and then the hyoid was depressed again until the mouth was closed.

For recording terrestrial feeding in the aquatic phase, the newts had to be lured out of the water onto a shallow ramp (Fig. 2C). The capture of prey involved jaw opening by dorsal skull rotation and lower jaw depression. The maximum gape of 7.0±1.2 mm was reached after 47±15 ms to produce a gape profile with a bell-shaped curve. Mouth opening was followed by hyoid depression, which reached its maximum ventral deflection of 4.0±0.9 mm after 39±12 ms, and the hyoid was elevated thereafter (Table 1, Fig. 2C). The prey was grasped by the closing jaws and immediately brought back to the water for further intraoral transport and swallowing. The angle of the skull relative to the longitudinal body axis was usually smaller after the jaws were closed than before jaw opening started, just as observed in the aquatic feeding patterns described above (compare Fig. 3A–C). The whole gape cycle lasted 79±18 ms.

Kinematic differences between the four feeding modes
The MANOVA showed significant differences between the four feeding modes (Wilks' lambda *F*=10.481, *P*<0.001) and between individuals (Wilks' lambda *F*=4.031, *P*<0.001), and a significant interaction effect between individual and mode (Wilks' lambda *F*=1.862, *P*<0.001). The subsequent series of ANOVA showed that the differences between modes comprised 10 out of the 12 variables

tested (Table 1). Only the duration of gape closing and the velocity of gape closing showed no significant difference between the modes. The *post hoc* test (Tukey HSD) revealed that the differences between the modes were mainly based on significant differences between terrestrial feeding in the terrestrial phase and all other modes with respect to eight out of the 10 variables, and on significant differences between aquatic feeding in the aquatic phase and terrestrial feeding in the aquatic phase for seven out of the 10 variables. Aquatic feeding in the aquatic phase and aquatic feeding in the terrestrial phase only showed significant differences in the velocity of hyoid depression; it was faster when animals were in the aquatic phase.

The differences between individuals were less distinct and the series of ANOVA showed significant differences in all but one gape variable (closing duration) and two out of four hyoid variables (Table 1).

A significant interaction effect of individual on feeding mode was detected in three out of the 12 variables tested: velocity of gape opening (F=3.993, P<0.001), maximum hyoid depression (F=3.603, P=0.001) and duration of head elevation (F=5.108, P<0.001), and might have less biological importance.

Bivariate dispersion of kinematics among the four feeding modes and the four individuals on the first two principal component axes are illustrated in Fig. 4, and the loadings of the variables along the first four principal component axes are given in Table 2. The aquatic feeding modes (namely aquatic feeding in the aquatic phase and aquatic feeding in the terrestrial phase) were very similar to each other with a large overlapping area (Fig. 4A). Terrestrial feeding showed a certain amount of overlap with aquatic feeding when animals where in the aquatic phase (mode 3). By contrast, terrestrial feeding in the terrestrial phase (mode 4) was clearly separated in kinematic space from the aquatic feedings, but showed a small overlap with terrestrial feeding in the aquatic phase.

Fewer differences were detected in the kinematic space regarding the four individuals, which are quite similar with broadly overlapping distributions (Fig. 4B).

DISCUSSION

The aquatic–terrestrial transition is among the most challenging of transitions in vertebrates and certainly marks one of the most significant steps in tetrapod evolution (Stayton, 2011). These transitions require major morphological and functional changes of almost every organ system, including amongst other things the feeding biology. The aquatic–terrestrial transition of the feeding system, however, is most challenging because the biophysical demands on the feeding apparatus are conflicting between water and air (Deban, 2003). Consequently, only a few tetrapods are able to manage the biophysical constraints and have found a way to effectively exploit food sources from both environments. As a prerequisite to feeding in both environments, the capacity for behavioral plasticity seems to be used to compensate for morphological constrains.

Newts are of special interest in studying aquatic–terrestrial transitions, including the underlying role of behavioral plasticity, as they show a multiphasic lifestyle whereby they seasonally change from aquatic to terrestrial habitats as adults (Duellman and Trueb, 1994). Our study shows that the Alpine newt exhibits an extraordinary behavioral plasticity in its prey-capture behavior. When changing phase (morphotype), *I. alpestris* also changes its prey-capture kinematics. In fact, it is one of very few tetrapods, if not the only known one, that as adults use effective suction feeding in water and lingual prehension on land, which are known to be
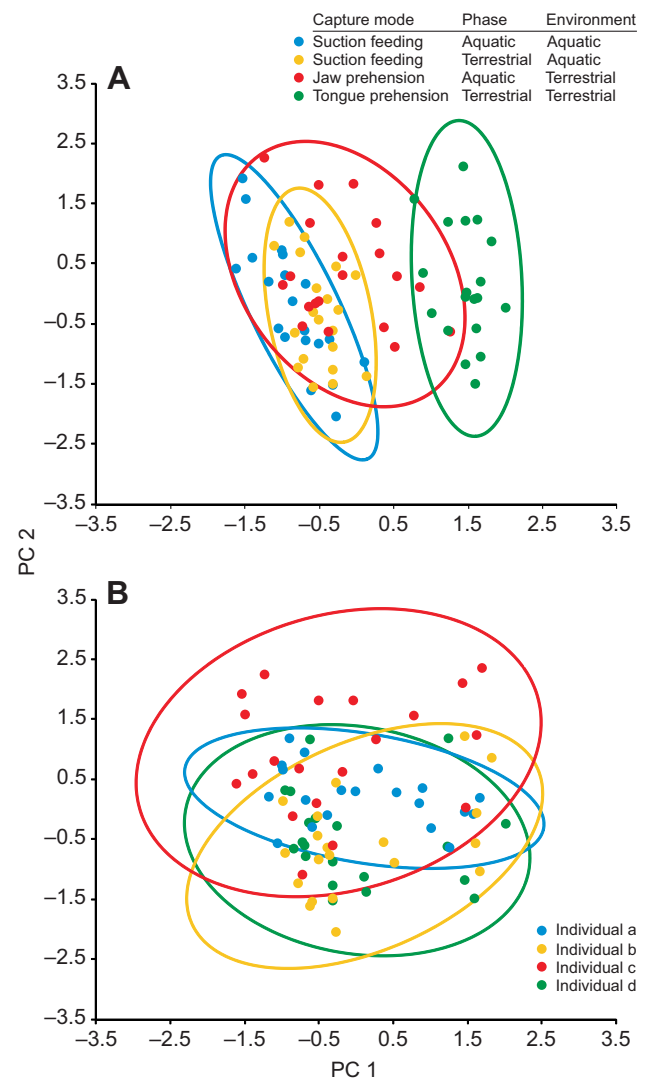

Fig. 4. Scatterplots of the first two principal components (PC1 and PC2) derived from the 12 kinematic variables to illustrate the multivariate relationship among kinematic patterns for the four feeding modes (A) and the four individuals (B). Each point represents one feeding event and groups are coded by color with corresponding 95% confidence interval (indicated by ellipses). PC1 explains 49% and PC2 17.6% of the total variance.

among the dominant modes in the respective media (Deban and Wake, 2000; Wake and Deban, 2000).

We quantified the behavioral changes during the aquatic-terrestrial transition in detail and showed behavioral modulations that accompany the habitat change and guarantee a prey-capture system that is fine-tuned to the corresponding medium. The kinematic profiles of gape, hyoid movement and skull rotation were similar in the following three feeding modes: aquatic feeding in the aquatic phase, aquatic feeding in the terrestrial phase and terrestrial feeding in the aquatic phase. The kinematic pattern observed for these three modes will from here on be summarized as 'the aquatic feeding pattern'. In fact, the aquatic feeding pattern was very different to the fourth mode: terrestrial feeding in the terrestrial phase. In general, mouth movements (gape) are performed by both skull rotation and lower jaw depression in all four feeding modes. The gape profile in the aquatic feeding pattern is typically bell-shaped and hyoid depression starts shortly after the onset of mouth

Table 2. Loadings of variables to the first four principal components

Variable	PC1	PC2	PC3	PC4
Time to maximum gape	0.939	0.230	0.116	–0.051
Duration gape closing	0.267	–0.219	0.472	0.797
Maximum gape	0.095	0.878	0.297	0.280
Velocity of gape closing	–0.072	–0.922	–0.052	–0.292
Velocity of gape opening	–0.866	0.331	0.061	0.233
Time to start hyoid depression	0.868	0.096	–0.017	0.051
Duration of hyoid depression	0.788	0.205	0.120	–0.166
Maximum hyoid depression	–0.732	0.312	0.346	–0.057
Velocity of hyoid depression	–0.903	0.077	0.153	0.092
Maximum head elevation	0.406	0.309	–0.707	0.412
Duration head elevation	0.930	0.124	–0.027	0.119
Velocity head elevation	–0.637	0.119	–0.644	0.252
Total variance explained (%)	49	17.6	11.7	9.6

opening as prey is sucked up, resulting in a profile generally found in suction feeders (Lauder and Shaffer, 1985; Reilly, 1995; Reilly and Lauder, 1992; Deban and Wake, 2000; Deban and Marks, 2002). Gape, skull and hyoid profiles in the aquatic feeding pattern are different to those in terrestrial feeding in the terrestrial phase where jaw, skull and hyoid movements seem to be coupled to tongue movements. The tongue is not used in the aquatic feeding pattern.

The hyobranchial apparatus is the central component of both aquatic and terrestrial feeding modes in salamanders (Lauder and Reilly, 1994). However, it was shown that the hyobranchial apparatus and its musculature cannot be used to perform both suction feeding and tongue protraction simultaneously in the largest group amongst salamanders, the plethodontids (Deban, 2003). Deban showed that adult plethodontid salamanders are either suction feeders (aquatic species) or lingual feeders (terrestrial or secondarily aquatic species) and cannot switch between those modes (Deban, 2003). Other groups, however, might be more flexible in this regard (Miller and Larsen, 1989; Miller and Larsen, 1990; Reilly and Lauder, 1989; Findeis and Bemis, 1990) but because of the conflicting biophysical demands between aquatic and terrestrial feeding, it was thought that they perform sub-optimally in at least one medium (Miller and Larsen, 1989; Deban and Wake, 2000). Suction feeding is used by all larval salamanders to capture prey and is likely the most ancestral aquatic prey-capture mode for salamanders (Lauder and Reilly, 1994; Reilly, 1996). In adult metamorphosed salamanders that retain an aquatic lifestyle and rely on suction feeding, tongues are small or completely absent to increase oropharyngeal volume that can be filled with water and reduce the turbulence of inflowing water, which is advantageous for hydrodynamics in suction feeding (Özeti and Wake, 1969; Miller and Larsen, 1989; Elwood and Cundall, 1994; Deban and Wake, 2000; Deban, 2003). In contrast, the tongue systems in terrestrial salamanders are well developed and large, as the tongue plays a central role in terrestrial feeding. Terrestrial feeding is secondarily derived and salamanders in principle use two modes: jaw prehension, where the prey is grasped by the jaws, and lingual prehension, where the prey is caught and brought into the mouth by the quickly protruded and retracted sticky tongue (Deban and Wake, 2000; Wake and Deban, 2000).

Despite the functional conflict, the Alpine newt uses all three feeding modes known for salamanders – to our present knowledge without undergoing changes of the feeding apparatus on a gross morphological level. We showed that the Alpine newt uses a stereotypical suction-feeding mode underwater regardless of its current phase. By contrast, it uses jaw prehension for terrestrial prey capture if it is in the aquatic phase, but tongue prehension when it

is in the terrestrial phase. A terrestrial prey-capture behavior, basically relying on suction-feeding movements, was previously observed in the aquatic salamandrid *Pachytriton brevipes*. In contrast to *I. alpestris*, *P. brevipes* is not capable of lingual prehension (Miller and Larsen, 1989; Miller and Larsen, 1990), and shows a similar behavior to the aquatic morphotype of *I. alpestris* when feeding on land. However, it has been shown that originally terrestrial desmognathine salamanders with a secondarily derived aquatic lifestyle are not capable of suction feeding but do use their terrestrial feeding modes, namely jaw prehension and lingual prehension, when capturing prey in water (Deban and Marks, 2002). The Alpine newt is an accomplished suction feeder and uses highly stereotyped suction-feeding movements regardless of its current lifestyle. However, the Alpine newt requires a morpho-functional change to its terrestrial phase to be capable of switching to lingual prehension for terrestrial prey capture. Contrary to aquatic feeding, terrestrial feeding does differ between aquatic and terrestrial phases and tongue-based prey capture must be gained through stepwise modified behavior. The terrestrial prey-capture mechanism employed in the aquatic phase is in fact more similar to the suction-feeding mechanism used for aquatic prey capture in both phases than the lingual terrestrial prey capture in the terrestrial phase. Despite these overall similarities, *I. alpestris* does slightly change its behavior as a response to the feeding environment as shown by the significant differences between aquatic and terrestrial feeding behavior in the aquatic phase. These differences in feeding kinematics cannot be explained as a passive result caused by the differences in the physical properties between water and air. More specifically, it is unlikely that the animals use the identical pre-programmed motor pattern when feeding in the two media and that the different physical properties of air interact with the neuro-motor inputs to result in a different behavior (Stayton, 2011). For example, the lower density and viscosity of air would cause faster movements, but in fact movements were observed to be slower on land. This corroborates the hypothesis that the terrestrial feeding mechanism in the aquatic phase is in fact a modulated aquatic feeding behavior.

The differences between the aquatic feeding pattern on the one hand and the terrestrial strike in the terrestrial phase on the other hand are highly significant in *I. alpestris*. These feeding patterns are comparable with the diverse behaviors of aquatic prey capture in larval salamanders and terrestrial prey capture in metamorphosed salamanders (Lauder and Shaffer, 1988; Reilly, 1996). However, with closer scrutiny the terrestrial gape cycle can be subdivided into two distinct 'phases'. The first phase comprises movements from the start of mouth opening until the instant of the local gape minimum, and the second phase encompasses movements from the
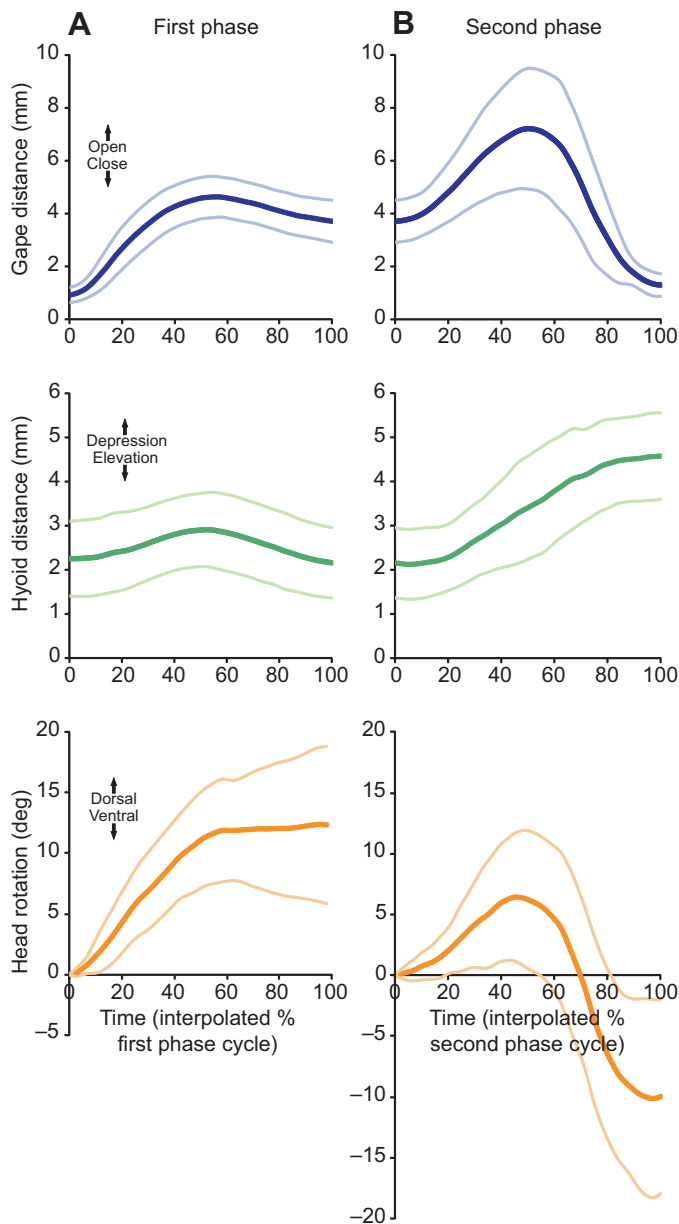
Fig. 5. First (A) and second (B) phase of the terrestrial feeding pattern shown in Fig. 3D. The time axes are normalized to percentages of corresponding phase duration. Both phases can therefore be directly compared with the kinematic profiles shown in Fig. 3. Note the striking similarities of movement patterns of the second phase (B) and the aquatic feeding patterns shown in Fig. 3A–C.

known from aquatic intraoral prey transport both in fish and salamanders (Reilly and Lauder, 1989; Lauder and Reilly, 1994; Konow and Sanford, 2008; Konow et al., 2008). Accordingly, even the first phase in terrestrial prey capture may not be a new achievement but an altered motor pattern already present in aquatic prey transport (Regal, 1966; Reilly and Lauder, 1989; Lauder and Reilly, 1994). In terms of neuromotor control, this would mean that terrestrial feeding in *I. alpestris* is not a fundamental change of the aquatic motor pattern but the result of recombination of existing motor patterns. In turn, this might indicate that the feeding biology of *I. alpestris* is primarily adapted to aquatic life and that the terrestrial feeding mode is secondarily derived through recombination and modulation of components of the aquatic feeding behavior.

Modulation of feeding behavior by recombination of modular neuromotor components may have the advantage of allowing an animal to adjust its behavior in response to changing circumstances (Deban et al., 2001; Konow et al., 2011), without the need for a fundamental change in motor program. Accordingly, analogs to the feeding behavior of the Alpine newt can be found in other vertebrate groups that undergo aquatic–terrestrial transitions. Semi-terrestrial fish species, for example, use slightly modified suction-feeding mechanisms when capturing prey on land (Sponder and Lauder, 1981; Van Wassenbergh et al., 2006). Similarly, aquatic turtles rely on modified aquatic feeding mechanics when grasping food on land (Summers et al., 1998; Natchev et al., 2009; Stayton, 2011). This shows that during invasion of land, vertebrates in general use slightly modified aquatic prey-capture mechanisms, corroborating the importance of behavioral plasticity in aquatic–terrestrial transitions. However, the example of the Alpine newt shows that a single species can go a step further within one life cycle and repeatedly modify the ancestral aquatic behavior by alternative recruitment of existing motor patterns, which has finally resulted in a prey-capture mechanism that periodically and seamlessly can be fine-tuned to the demands of the terrestrial environment.

AUTHOR CONTRIBUTIONS

E.H., P.A. and S.V.W. designed the study; E.H. conducted the experiments and the analyses; E.H., P.A. and S.V.W. discussed and interpreted the final results and drafted and revised the manuscript.

COMPETING INTERESTS

No competing interests declared.

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local minimum until the mouth is closed. The first phase is distinct from what is observed in aquatic feeding. The second phase, however, shows striking similarities to the kinematics of the aquatic feeding pattern if plotted against normalized time (Figs 3, 5). These similarities in kinematics become more interesting if we consider that the second phase shares an intriguing pattern with suction feeding: retraction of the hyobranchial system. Accordingly, the movement patterns of terrestrial prey capture in the terrestrial phase might be the result of a functional combination of the ancestral aquatic feeding motor pattern (still present as the second phase) with the addition of a new motor pattern, the first phase. One may equally well argue that this first phase with tongue protraction is not new but instead is similar to the pattern of the preparatory phase

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