

RESEARCH ARTICLE

Flexibility of intraoral food processing in the salamandrid newt *Triturus carnifex*: effects of environment and prey type

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ABSTRACT

Intraoral food processing mechanisms are known for all major vertebrate groups, but the form and function of systems used to crush, grind or puncture food items can differ substantially between and within groups. Most vertebrates display flexible mechanisms of intraoral food processing with respect to different environmental conditions or food types. It has recently been shown that newts use cyclical loop-motions of the tongue to rasp prey against the palatal dentition. However, it remains unknown whether newts can adjust their food processing behavior in response to different food types or environmental conditions. Newts are interesting models for studying the functional adaptation to different conditions because of their unique and flexible lifestyle: they seasonally change between aquatic and terrestrial habitats, adapt their prey-capture mode to the respective environment, and consume diverse food types with different mechanical properties. Using X-ray high-speed recordings, anatomical investigations, behavioral analyses and mechanical property measurements, we tested the effects of the medium in which feeding occurs (water/air) and the food type (maggot, earthworm, cricket) on the processing behavior in *Triturus carnifex*. We discovered that food processing, by contrast to prey capture, differed only slightly between aquatic and terrestrial habitats. However, newts adjusted the number of processing cycles to different prey types: while maggots were processed extensively, earthworm pieces were barely processed at all. We conclude that, in addition to food mechanical properties, sensory feedback such as smell and taste appear to induce flexible processing responses, while the medium in which feeding occurs appears to have less of an effect.

KEY WORDS: Feeding, Tongue-palate rasping, Chewing, Amphibians, Kinematics, Penetration force

INTRODUCTION

Intraoral food processing is used by many jawed vertebrates to facilitate swallowing and digestion (Herrel et al., 2012; Schwenk, 2000; Schwenk and Rubega, 2005) or to immobilize prey (Dalrymple et al., 1985; Konow et al., 2013) and thus likely avoid being hurt by struggling prey. Such intraoral mechanical reduction of food is very diverse amongst gnathostomes and can involve a variety of processing systems, including oral jaws (Bemis and Lauder, 1986; Bhullar et al., 2019; Crompton and Hiimae, 1970; Gans et al., 1978;

Gintof et al., 2010; Ross et al., 2007; Smith, 1982), pharyngeal jaws (Gidmark et al., 2013, 2014; Liem and Greenwood, 1981; Wainwright et al., 2012), the tongue-bite apparatus (Camp et al., 2009; Konow et al., 2013; Sanford and Lauder, 1989) and rough palatal structures in combination with a movable tongue (Griffiths, 1978; Heiss et al., 2019; Matsumoto and Evans, 2017; Werth, 2000).

Apart from their diverse intraoral food processing systems, most gnathostomes exhibit a certain trophic niche breadth, including foods with different sizes, shapes and mechanical properties (Hanlin, 1978; Lahm, 1986; Pethybridge et al., 2011). Exploitation of a trophic niche breadth with such diverse food properties probably requires an active modulation of the food processing mechanism to allow adequate incapacitation and preparation of the prey for swallowing (Konow et al., 2013). In fact, modulation of food processing mechanisms by adapting movement patterns, muscle activation or the number of processing cycles has been reported for chondrichthyans (Gerry et al., 2008, 2010), actinopterygians (Aerts et al., 1986; Konow et al., 2013; Wainwright, 1989), lizards (Delheusy and Bels, 1999; Gorniak et al., 1982; Herrel et al., 1996, 1997a,b) and mammals (Gorniak and Gans, 1980; Thexton et al., 1980; Weijs and Dantuma, 1980). Consequently, animals that are capable of reacting flexibly to different foods might profit by increasing food processing efficiency and broadening their trophic resource spectrum. However, although modulation in prey capture is well studied in lissamphibians (Anderson, 1993; Deban, 1997; Heiss et al., 2013a, 2015; Reilly and Lauder, 1989; Valdez and Nishikawa, 1997), fewer data on the modulatory capacity of food processing behaviors are available (Rull et al., 2020).

It has generally been assumed that lissamphibians – with few potential exceptions (Cundall et al., 1987; Elwood and Cundall, 1994; Erdman and Cundall, 1984; Schwenk and Wake, 1993) – lack intraoral food processing mechanisms and thus swallow prey whole and unreduced (De Vree and Gans, 1994; Deban and Wake, 2000; Schwenk and Rubega, 2005). However, recent studies have shown that particular intraoral food processing mechanisms are present in salamanders (Heiss et al., 2019; Rull et al., 2020; Schwarz et al., 2020a,b). Here, we tested whether salamandrids also adjust their food processing mechanisms across environments or to different types of prey. Newts are ideal candidates to test hypotheses regarding processing flexibility in lissamphibians, because they generally exhibit a high degree of behavioral flexibility: post-metamorphic newts seasonally change between aquatic and terrestrial habitats (Griffiths and Teunis, 1996; Nöllert and Nöllert, 1992; Thiesmeier and Schulte, 2010; Thiesmeier et al., 2009), where they also undergo dramatic changes of their feeding behavior (Heiss et al., 2013a, 2015; Miller and Larsen, 1989, 1990). Specifically, for aquatic prey capture, newts use suction feeding, whereas for terrestrial prey capture they use quick protrusion of their sticky tongue. Considering the extremely different physical conditions of the aquatic and aerial media (water is about 850 times as dense and 50 times as viscous as air; Denny, 1993), and that newts use two fundamentally different strategies to

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capture prey in water and on land, we predict that the food processing behavior is also adjusted to the respective medium. Our approach to test the first hypothesis is to compare the food processing kinematics in water and on land by means of high-speed X-ray recordings and to compare the number of processing cycles across habitats. Additionally, newts feed on diverse prey, including insect larvae and imagoes, crustaceans, annelids, cestodes, gastropods, bivalves, tadpoles, other newts and small fishes (Avery, 1968; Cicort-Lucaci et al., 2007; Dürigen, 1897; Joly and Giacoma, 1992; Thiesmeier et al., 2009). These food types likely differ in their mechanical properties. This observation motivates our second hypothesis: that newts possess the ability to flexibly adapt their food processing behavior to the mechanical requirements of the respective type of prey. Our approach to test the second hypothesis is to quantify the number of processing cycles used across different prey types with distinct mechanical properties which we determine using prey penetration force measurements.

MATERIALS AND METHODS

Animal care

Nine adult Italian crested newts [*Triturus cristatus* (Laurenti 1768)] with mean±s.d. snout–vent lengths of 80.5±10.6 mm and masses of 10.4±2.6 g were used in this study. The animals were collected in their aquatic phase between April and June 2011 and 2012 in Lower Austria, Austria, with collection permission (RU5-BE-18/022-2011) granted by the local government of Lower Austria. Animals were group-housed in large tanks with water levels of 20 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 were covered with a removable mosquito net to prevent newts from escaping. The animals were fed twice a week with a variety of lake fly larvae (Chironomidae), earthworms (*Lumbricus* sp.), crickets (*Acheta domestica*) and maggots (*Lucilia* sp.). Preliminary experiments (data used for observation purposes only) were performed at the University of Antwerp, Belgium, and the main experimental part at the Friedrich-Schiller-University of Jena, Germany. Accordingly, husbandry and experiments were approved by the Ethical Commission for Animal Experiments of the University of Antwerp (code: 2010-36) and the Committee for Animal Research of the State of Thuringia, Germany (codes animal experiments: 02-042/14, 02-008/15, code animal husbandry: J-SHK-2684-05-04-05-07/14).

Surgical procedure

At the University of Antwerp, five newts were surgically implanted with radio-opaque metal markers on the skeletal structures of interest, following protocols modified from Herrel et al. (2000) and Manzano et al. (2008) (see also Heiss et al., 2019). The animals were anesthetized with buffered (pH 7.2) aqueous 0.05% MS222 (tricaine methanesulfonate) solution and markers were percutaneously implanted on the basibranchial ('tongue bone'), the snout tip (between the premaxillary upper jaw bones) and the lower jaw tip (in the region of the dentary symphysis) using hypodermic needles. Immediately after implantation, the marker placement was verified using X-ray images. All animals were given at least 3 days of post-surgery recovery before the start of X-ray recordings.

X-ray motion analysis

X-ray experiments were performed at the Department of Biology at the University of Antwerp and at the Institute of Zoology and Evolutionary Research at Jena University, and are described in Heiss et al. (2019). To record feeding events, newts were placed on a moistened tissue in an acrylic glass enclosure mounted on the

experimental table (terrestrial condition) and in an acrylic glass aquarium (ground area 40×40 cm with a 25×7 cm tunnel where animals were lured for the X-ray recordings; height of the aquarium: 30 cm) filled with 7 cm water (aquatic condition). For the preliminary experiments performed at the University of Antwerp, we used a Tridoros-Optimatic 880 X-ray apparatus (Siemens, Erlangen, Germany), whereas a custom-built biplanar Neurostar setup (Siemens, Erlangen, Germany) was used for the experiments at Jena University. After acclimation, newts were fed maggots (29.8±5.1 mg; mean±s.d.) and in order to visualize the maggots in X-ray recordings, we glued small tantalum markers (diameter of 0.5 mm) on their cuticle. In total, 50 terrestrial and 50 aquatic feeding events were recorded, from which 106 terrestrial and 113 aquatic food processing cycles were extracted for statistical analyses described below (11, 23, 24, 26 and 28 terrestrial cycles and 16, 17, 31, 12 and 37 aquatic cycles for individuals 1–5, respectively). Terrestrial X-ray recordings were taken from the latero-lateral and ventro-dorsal projections at 40 kV and 53 mA. Aquatic X-ray recordings were taken from the latero-lateral projections at 40 kV and 80 mA and from the ventro-dorsal projections at 50 kV and 120 mA. The sampling frequency for both terrestrial and aquatic recordings was 250 Hz. The ventro-dorsal recordings were performed to determine lateral movements of tongue and jaw systems during food processing, but because no clear lateral movements were measured, they were excluded from further analyses. Next, the resulting raw video recordings were undistorted and filtered (e.g. gamma correction, contrast, sharpness), and the horizontal (x-axis) and vertical (y-axis) coordinates of the landmarks (Fig. 1) were tracked frame by frame using SimiMotion software (SimiMotion Systems, Unterschleißheim, Germany). The 2D displacement of the landmarks was used to calculate the following movements: (1) jaw movements: angular displacement of upper and lower jaw (Fig. 1A); (2) head rotation: angular displacement between the two lines connecting (i) the points 'occipital' and 'snout tip' and (ii) the points 'first vertebra' and 'fifth vertebra' (Fig. 1A); (3) longitudinal tongue movement: horizontal displacement of the tongue relative to the point 'occipital' (i.e. parallel to a line connecting the points 'occipital' and 'snout tip'); (4) vertical tongue movement: vertical displacement of the tongue relative to a line connecting the points 'occipital' and 'snout tip'; (5) longitudinal transport of the prey: horizontal displacement of the point 'prey' relative to the point 'occipital'; and (6) vertical movement of the prey: displacement of the point 'prey' relative to a line connecting the points 'occipital' and 'snout tip' (Fig. 1B).

From movements 1–4, we calculated the following variables for further kinematic analyses and statistics: (1) mouth opening angle (angular displacement from start of mouth opening to maximum gape); (2) mouth closing angle (angular displacement from maximum gape to next minimum); (3) duration of mouth opening (time from start of mouth opening until maximum gape); (4) duration of mouth closing (time from maximum gape until next minimum); (5) head elevation angle (angular displacement from start of head elevation until maximum head elevation relative to the trunk); (6) head depression angle (angular displacement from maximum head elevation until maximum head depression relative to the trunk); (7) duration of head elevation (time from start until maximum head elevation); (8) duration of head depression (time from maximum head elevation until maximum head depression); (9) tongue protraction distance (distance from minimum to maximum horizontal tongue displacement, parallel to a line connecting points 'occipital' and 'snout tip' and relative to point 'occipital'); (10) tongue retraction

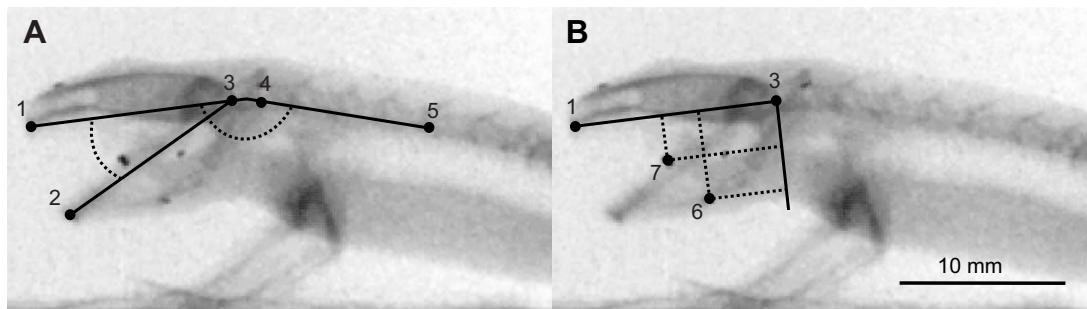


Fig. 1. X-ray frame shots with landmarks. Lateral views showing the seven landmarks used to measure movements of (A) the mouth and head and (B) the tongue and prey. Landmarks: (1) snout tip; (2) lower jaw tip; (3) ‘occipital’ (posterior part of the skull); (4) first vertebra; (5) fifth vertebra; (6) tongue (basibranchial); and (7) prey. Gape and head rotation (A) were measured as angular displacements (indicated by dashed arc), whereas tongue and prey movements were measured as vertical and horizontal translations (indicated by dashed lines in B) relative to the skull axis (line connecting points 1 and 3) and the normal line through point 3, respectively. Note that the anterior end of the esophagus is approximately at the height of point 3 (‘occipital’). Modified from Heiss et al. (2019).

distance (distance from maximum to minimum tongue displacement, parallel to a line connecting points ‘occipital’ and ‘snout tip’ and relative to point ‘occipital’); (11) duration of tongue protraction (time from minimum to maximum horizontal tongue displacement); (12) duration of tongue retraction (time from maximum to minimum tongue displacement); (13) tongue elevation distance (distance from the minimum to the maximum vertical tongue displacement relative to a line connecting the points ‘occipital’ and ‘snout tip’); (14) tongue depression distance (distance from the maximum to the minimum vertical tongue displacement relative to a line connecting the points ‘occipital’ and ‘snout tip’); (15) duration of tongue elevation (time from minimum to maximum vertical tongue displacement); and (16) duration of tongue depression (time from maximum to minimum vertical tongue displacement). To account for different head sizes between individuals, all displacement values for tongue movements were normalized to percentage of cranial length. The cranial length was measured from the latero-lateral X-ray recordings and defined as the distance between the premaxillary and occipital condyles (Fig. 1A,B). Calculations and graphic illustrations were performed using Microsoft Excel 2010 (Microsoft, USA), custom-made scripts for MATLAB (MathWorks, USA) and the open source software Inkscape.

Behavioral experiments

To test for the effects of prey type and environment, five newts (two of which were also used for the X-ray analyses) were fed different prey organisms under both aquatic and terrestrial conditions, resulting in a total of 350 feeding trials (see Table 1 for details). In order to minimize food size effects on feeding kinematics (Ferry-Graham, 1998; Gidmark et al., 2013; Montuelle et al., 2012), we carefully size-selected maggots to equal one mouth-width of the newts (12.1 ± 0.7 mm and 29.9 ± 4.9 mg; means \pm s.d.). Crickets were chosen

so that their thorax–abdomen length corresponded approximately to the length of the maggots, and earthworms with a width equal to that of the maggots were cut into ‘maggot-sized’ pieces. In addition, the crickets’ heads and thorny jumping legs were removed. Maggots soaked in earthworm blood (henceforth referred to as ‘ea-maggots’) were only used in terrestrial experiments, because blood was immediately rinsed off the maggots upon contact with water, thereby preventing aquatic trials. All feeding trials were performed in the animals’ home tanks using a high-speed camcorder (JVC GC-PX100, Japan), and the number of processing cycles used were counted from the recordings.

Measurement of food mechanical properties (prey penetration force)

As previously shown, the newt *T. carnifex* processes its prey by pressing and translating it across the palatal dentition by means of cyclic loop motions of the tongue, causing multiple perforations to the prey (Heiss et al., 2019). In order to compare the forces required to penetrate different foods, we took measurements of the penetration force of different prey types. The penetration force is not intended to represent the actual force exerted by the consumer to damage the food. Rather, it should represent an easily modellable and comparable property of food in relation to the respective consumer. Ideally, a real newt tooth would have been used to measure the prey penetration force. However, because the vomerine teeth of *T. carnifex* are approximately 200 μ m long, they are difficult to dissect out and, owing to their hollow root structure, even more difficult to mount to a force transducer. Moreover, drying of teeth or infiltration with glue would also change their mechanical properties. Therefore, we decided not to use real teeth for this measurement. As a substitute, we first analyzed the needle-like vomerine teeth of the Italian crested newt using histological sections. The histological sections were prepared as described in Heiss et al. (2016). In short, two newts were euthanized by immersion in a 0.5% aqueous solution of MS222 (tricaine methanesulfonate), buffered to pH 7.2, and decapitated, and the heads were immersed in Bouin’s fixative for 2 months (Kiernan, 1999; Mulisch and Welsch, 2015). Next, the samples were rinsed, dehydrated in a graded ethanol and acetone series, and embedded in paraffin. The paraffin blocks were mounted on a MH 360 (Zeiss, Germany) rotatory microtome and 7 μ m thick serial sections were made. The sections were mounted on glass slides and stained with Azan (standard protocols after Mulisch and Welsch, 2015) and documented using a digital camera mounted to an Axiolab microscope (Carl Zeiss Jena, Germany). From the resulting micrographs, we calculated the mean \pm s.d. tip diameter (39.2 ± 4.7 μ m) and crown angle (29.7 ± 4.6 deg) of the vomerine

Table 1. Overview showing the experimental design used to test for the effects of different prey types on the number of processing cycles

Prey type	Environment	Trials per specimen	Number of specimens
Maggot	Aquatic	10	5
	Terrestrial	10	5
Cricket	Aquatic	10	5
	Terrestrial	10	5
Earthworm pieces	Aquatic	10	5
	Terrestrial	10	5
Ea-maggot	Terrestrial	10	5

The term ‘ea-maggot’ refers to maggots soaked in earthworm blood.

teeth. Steel pins (code no. 10242930; Prym Consumer Europe GmbH, Stolberg, Germany) with very similar tip properties (45.6 μm tip diameter and 29 deg crown angle) were selected to replace real teeth for these measurements. We maintain that the strong resemblance in terms of the tip properties of real teeth of *T. carnifex* and the steel pins we selected supports the use of steel pins as ‘model teeth’ in penetration force tests, thus yielding realistic results. In addition, the original elastic modulus of fairly stiff teeth (on the order of a few dozen GPa) and the elastic modulus of steel (~ 100 GPa) would not make a significant difference in the much softer foods, whose elastic modulus ranges from hundreds of kPa to a few GPa.

The properties and the geometry of the needle tip were verified before each trial using a 3D measurement microscope (Keyence VR 3100) with associated software (Keyence One-Shot Software v. 1.4.0.0; Keyence KK, Osaka, Japan).

Prey items (animals and parts thereof) are often structurally complex and consist of a large number of materials that are interconnected in complex ways. Consequently, foods exhibit complex material properties (including strength, stiffness and toughness), and therefore conventional materials science variables have proved inadequate to quantify food properties (Evans and Sanson, 2005; Sanson et al., 2001). Therefore, we used the following technique to measure the prey penetration force, inspired by earlier experiments (Andrews and Bertram, 1997; Herrel et al., 1996, 1999). The prey penetration force measurements were conducted with an experimental set-up consisting of a motorized micromanipulator (DC3001R with controller MS314, World Precision Instruments, Sarasota, FL, USA), with an attached force transducer (FORT100, World Precision Instruments) connected to an ADC amplifier (analog to digital converter) MP100A (BIOPAC Systems, Inc., Goleta, CA, USA). The steel pin (as a model tooth) was mounted directly on the force transducer. The motorized micromanipulator was used to move the model tooth perpendicularly onto the prey samples at a constant speed of $200 \mu\text{m s}^{-1}$ to penetrate their outer integument, as indicated by a sudden slope change (eventually a drop in force) in the force–time curve. After penetration of the outer integument, the model tooth was moved back out of the specimen. A camera (iA1900-32gc, Basler AG, Ahrensburg, Germany), which was mounted on a stereo microscope (MZ 12-5; Leica Microsystems GmbH, Wetzlar, Germany), was used to monitor and record the experiments. The force–time curves were recorded on a PC using the Acq-Knowledge 3.7.0 software (BIOPAC Systems). The resulting force–time curves were used to calculate the force required to penetrate the hull of the food objects (i.e. prey penetration force) as the maximum force before the sudden drop in the force–time curve, minus the baseline force before the start of the penetration test.

The penetration force was measured in three prey types: maggots (*Lucilia* sp.), crickets (*Acheta domesticus*) and earthworms (*Lumbricus* sp.). The specimen selection was performed as described in the ‘Behavioral experiments’ section above. All prey specimens were anesthetized with CO_2 for approximately 1 min, after which they were embedded on one side in two-component dental impression silicone (AFFINIS[®] light body polyvinylsiloxane; Coltène/Whaledent AG, Altstätten, Switzerland) on a microscope glass slide. Before embedding, some specimens needed further preparation: the heads of the crickets were removed and the earthworms were cut in two pieces to fit the microscope slides. We probed the prey-penetration force for all specimens along the sagittal plane of the dorsal side. The measurements were performed with 10 maggots, five crickets and five earthworms. Maggots were only used for one measurement, earthworms were probed twice (each piece) as

their segmented anatomy resulted in stable results (i.e. owing to internal pressure of each segment), and crickets were probed once on the abdomen (median tergites) as well as on the mid thorax. The heads of the crickets were not examined, as the newts were only fed decapitated crickets. Further detail about the potential implications of this methodological simplification can be found in ‘Behavioral experiments’ as well as in the Discussion.

Statistics

The first goal was to test for differences of kinematic variables across environments (aquatic versus terrestrial) and individuals (1–5). As the variables violated the assumptions for parametric tests, non-parametric statistical tests were performed. Specifically, the variables were heteroscedastic in nature or the variables’ residuals were non-normally distributed (even after \log_{10} transformation). To test for differences across individuals, we performed a series of Kruskal–Wallis tests and corrected the *P*-value to account for multiple tests performed (to avoid type 1 error). Given that the same individuals were used in both environments, the samples could not be considered independent and a Wilcoxon signed-rank test had to be performed to test for differences across environments. To account for diverging sampling numbers across environments (e.g. for individual 1 there were 29 terrestrial cycles versus 16 aquatic cycles), we first calculated the median values for each variable and individual and then performed the Wilcoxon signed-rank tests on the medians. Given the very conservative nature of this approach (Wilcoxon signed-rank tests were performed on medians and not on direct measurements) and to avoid type 2 error, *P*-values were left uncorrected for the Wilcoxon signed-rank tests (see also Armstrong, 2014; Garamszegi, 2006; Moran, 2003 for cautious use of Bonferroni corrections).

The second goal was to test for differences in processing cycle numbers across prey type and environment. As the data violated the assumptions for parametric statistics and to account for the dependence of the samples (different prey was tested on the same animals), pairwise Wilcoxon signed-rank tests were performed. By contrast, differences between individuals were examined by using Kruskal–Wallis tests.

The third goal was to test for differences between the mechanical properties (i.e. penetration forces) of different prey types (maggot, cricket, earthworm). To determine whether the kinematic parameters differed between morphotypes, we performed a Kruskal–Wallis one-way ANOVA. Sequential pairwise multiple comparisons (i.e. Mann–Whitney *U*-tests) using ranks based on consideration of all samples, not just the two samples currently involved in a comparison (Dunn, 1964), were performed to determine which morphotypes differed. The significance values were Bonferroni adjusted to account for multiple testing.

RESULTS

Kinematics

The general mechanism used to process maggots was the same across environments; newts used tongue-palate rasping when processing maggots both on land and in water (Fig. 2, Fig. S1, Movies 1–4), resulting in generally similar kinematic profiles (Figs 2 and 3). The tongue-palate rasping processing mechanism is described in detail elsewhere (Heiss et al., 2019). In short, the prey is pressed and translated across the palatal dentition by cyclic loop motions of the tongue, causing multiple perforations to the prey. The series of Wilcoxon signed-rank tests revealed that the median ranks of nine out of 20 kinematic variables differed across environments (Table 2). Specifically, mouth opening and closing, as well as tongue protraction, differed in both magnitude and duration across environments, being

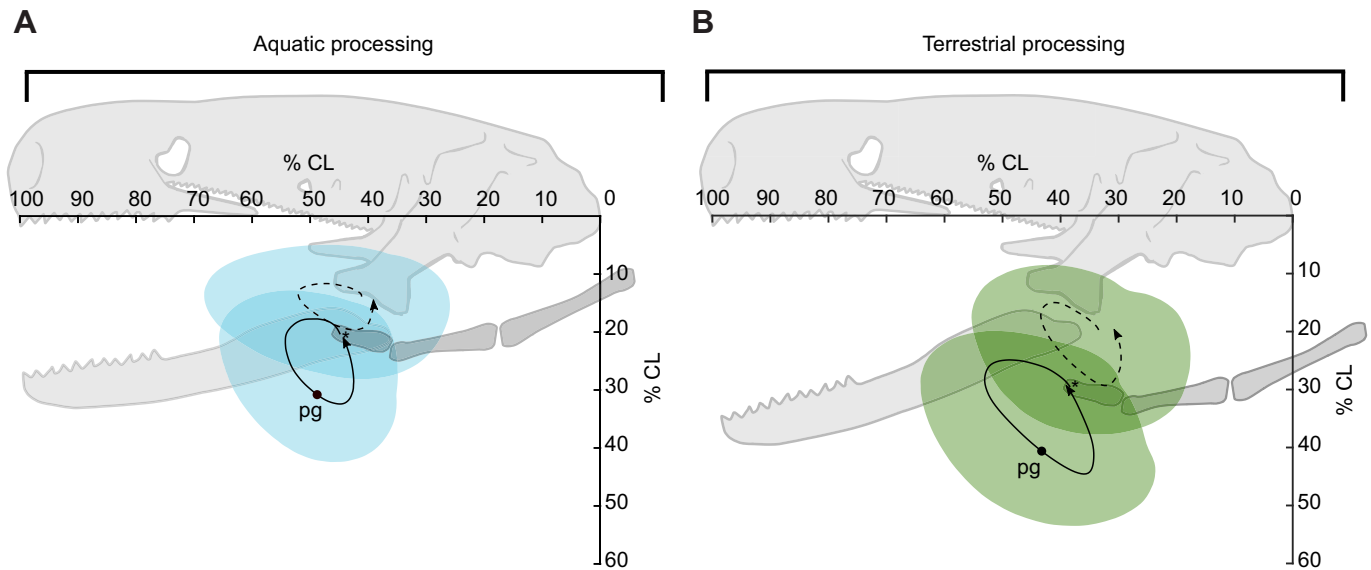


Fig. 2. Tongue and prey movements in the 2D cranial reference coordinate system in *Triturus carnifex*. The loops are running in a counter-clockwise direction and indicate motion of the tongue (continuous line) that moves prey (dashed line) along the mouth roof under aquatic (A) and terrestrial (B) conditions. To facilitate interpretation, schematic hyobranchial apparatus, skull and lower jaw have been added. The loops show the mean trajectory from 113 (A) and 106 (B) processing cycles from maggot feeding trials, with colored areas indicating 68% confidence intervals, normalized to cranial length (% CL). The start of mouth opening phases are indicated by an asterisk and the peak gape (i.e. peak mouth opening) by 'pg'. B is modified after Heiss et al. (2019).

less pronounced with shorter durations in aquatic feeding events (Fig. 3, Table 2). Similarly, head depression, tongue elevation and prey protraction durations were significantly lower during aquatic feeding. A series of Kruskal–Wallis tests revealed that significant inter-individual differences were present in 16 out of the 20 kinematic variables tested. Only the durations of mouth opening and prey protraction, along with the magnitudes of head depression and head elevation, showed no significant inter-individual differences (Table 2). In sum, differences across individuals were higher than across environments.

Behavior

Behavioral differences across environments and across different prey were quantified by comparing the number of processing cycles used. Descriptive statistics and a series of Wilcoxon signed-rank tests revealed that the number of processing cycles only differed across environments when newts processed maggots, being almost twice as high in aquatic feeding compared with terrestrial feeding events (Table 3, Fig. 4). By contrast, there were no differences across environments for crickets and earthworm pieces (Table 3, Fig. 4). On the individual level, the series of Kruskal–Wallis tests revealed significant inter-individual differences in the number of processing cycles used only related to crickets (Table 3). Feeding on maggots, earthworm pieces and ea-maggots showed no inter-individual effects (Table 3). When testing for differences in food processing cycle numbers across prey types, the series of Wilcoxon signed-rank tests showed significant differences of the median ranks across all prey types (Table 4, Fig. 4), except for one scenario: the median rank of the number of processing cycles associated with maggots rinsed in earthworm blood (ea-maggots) did not differ from regular earthworm pieces (but note that the difference between ea-maggots and regular maggots was highly significant). Descriptive statistics showed that the highest number of processing cycles was associated with maggots, followed by crickets and then earthworm pieces along with maggots rinsed in earthworm blood (see Tables 3 and 4, Fig. 4).

Mechanical properties of different prey types

A Kruskal–Wallis one-way ANOVA revealed significant differences of penetration forces between the three prey types ($H_2=19.36$, $P<0.001$). Pairwise comparisons showed that penetration forces were significantly higher for maggots (557.9 ± 147.5 mN) than both for crickets (136.7 ± 54.0 mN) and earthworms (137.9 ± 31.5 mN) (Fig. 5, Fig. S2). By contrast, the penetration forces for crickets and earthworms showed no statistically significant difference after Bonferroni correction (Fig. 5).

DISCUSSION

Italian crested newts show a moderate level of modulation of their intraoral food processing behavior across fluid environments, but respond flexibly to different prey and modulate their processing behavior in accordance to the different prey types. Maggots are processed extensively, crickets moderately and earthworm pieces barely, if at all. Below, we discuss how these differences might be explained.

Links between food mechanical properties and food processing flexibility

Mechanical tests of different prey types showed that maggots require significantly higher forces to be perforated by the vomerine tooth model than crickets and earthworms. Accordingly, the greater number of intraoral food processing cycles that the newts use while feeding on maggots could be explained, at least in part, by the significantly greater prey-penetration force. Furthermore, maggots might also be dangerous prey. Specifically, it has been reported that maggots – if not processed adequately – can survive for some time after being swallowed and cause severe damage to the predator (Brumpt, 1934; Zumpt, 1965). The tough cuticle of maggots seems to protect them from the digestive enzymes, so piercing the cuticle before swallowing might be essential not only to kill a potentially dangerous prey, but also to allow enzymes to enter the maggot and facilitate digestion. Compared with maggots, crickets and earthworms require significantly less force to be perforated.

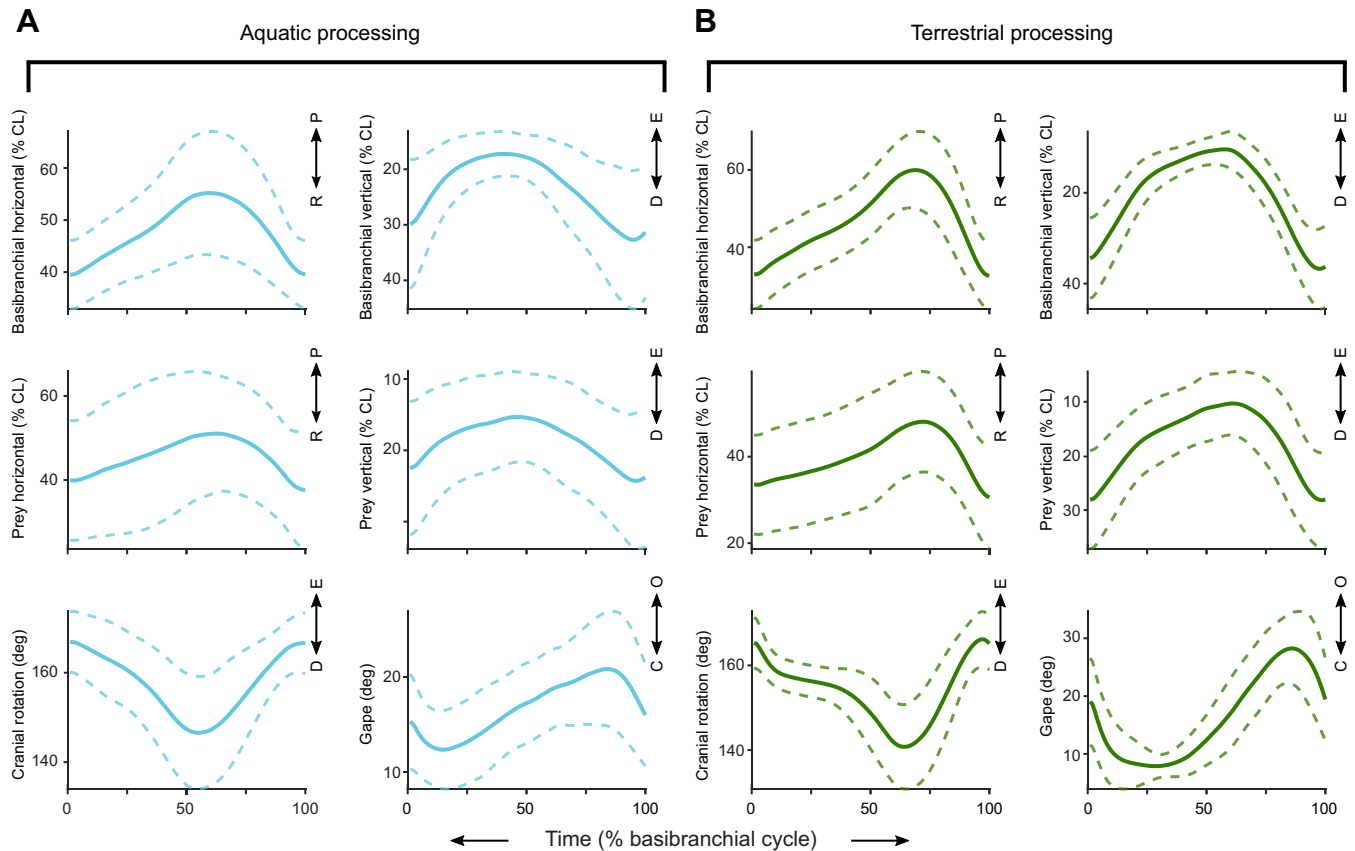


Fig. 3. Kinematic profiles of aquatic and terrestrial food processing in *T. carnifex*. Solid lines indicate mean values and dashed lines indicate \pm s.d. from 113 (A) and 106 (B) processing cycles from maggot feeding trials. The time scale on the x-axes is normalized as percentage of basibranchial cycle. Vertical and horizontal translations of tongue (basibranchial, bb) and prey on the respective y-axes are normalized as percentage of individual cranial length (% CL). C and O, closure and opening; D and E, depression and elevation; and R and P, retraction and protraction.

Interestingly, although crickets do not require significantly higher penetration forces than earthworms, they are processed more intensively. At first sight, the case that two prey types that demand similar penetration forces are processed with different intensities might sound contradictory. However, as the newts were only fed decapitated crickets, we only measured the penetration forces of the thorax and abdomen, but the head capsule is usually one of the strongest sections of insects (Hillerton et al., 1982). If newts had to effectively incapacitate a cricket under natural conditions, it would behave them to pierce the head capsule. Consequently, the greater number of processing cycles for crickets in our study might be partially explained by the mechanical properties of the cricket's head capsule – although it had been removed in the experiments. The question of how the mechanical properties of removed cricket heads can affect the processing behavior of newts could be explained by the fact that the newts used in this study were caught in the wild where they probably already had contact with a wide range of prey organisms – including armored and potentially dangerous prey, such as crickets. Furthermore, the newts were fed with living house crickets during husbandry, so that the newts may have learned that crickets possess hard head capsules and require more intensive processing. In fact, prey-related learning effects are well known for salamanders (Crane et al., 2018). Aside from the hard head capsule of crickets, the newts might also have learned that crickets, unlike earthworms, possess forceful mandibles (Hack, 1997) that can be

used to inflict damage to the predator. Indeed, just like maggots, crickets are potentially dangerous prey, and processing such prey reduces the probability of injuries for the predator. In contrast to maggots and crickets, earthworms are hardly processed at all. In fact, earthworms seem to be harmless prey as they have no mandibles and, apart from mucus and very short bristles, no mechanical system that could somehow be used against a predator (Edwards and Bohlen, 1996). Accordingly, earthworms seem not to require post-capture processing and are safely swallowed unreduced. Future studies are encouraged to analyze in more detail the kinematic differences between prey types. Although we have shown that different prey are processed with different numbers of processing cycles, the question of how the kinematics changes between prey types remains open. Because important kinematics of intraoral food processing in salamandrid newts are visible from the outside [mouth opening and closure (i.e. gape), vertical cranial rotation, vertical hyobranchial movement], simple kinematic studies from high-speed videography could potentially answer this question in the future.

Sensory control of the food processing behavior

Our data suggest that salamanders may be generally able to adjust their food processing behavior to different prey. However, the question remains as to how they can distinguish between different types of prey. Studies on amniote chewing have shown that sensory feedback is important for fine-tuning food processing behavior to the respective

Table 2. Descriptive statistics of kinematic variables, along with differences across individuals (individuals 1–5) and environment (aquatic versus terrestrial)

Variable	Aquatic processing	Terrestrial processing	Individual		Environment	
			Kruskal–Wallis H_4	P	Wilcoxon Z	P
Mouth opening angle (deg)	15.1±3.9	25.1±3.9	48.40	<0.001*	15	0.043*
Mouth closing angle (deg)	15.1±4.2	25.1±4.0	49.83	<0.001*	15	0.043*
Duration mouth opening (ms)	239±65	313±89	12.94	0.012	15	0.043*
Duration mouth closing (ms)	143±65	207±90	28.18	<0.001*	15	0.042*
Head depression angle (deg)	27.9±9.6	29.7±10.7	6.21	0.184	6	0.686
Head elevation angle (deg)	27.5±8.1	29.6±9.6	7.99	0.092	5	0.500
Duration head depression (ms)	211±70	340±109	19.27	0.001*	15	0.043*
Duration head elevation (ms)	178±73	168±63	41.66	<0.001*	9	0.686
Tongue protraction distance (% CL)	17.6±8.9	28.7±7.8	16.46	0.002*	15	0.043*
Tongue retraction distance (% CL)	17.7±8.9	29.1±8.1	19.15	0.001*	14	0.080
Duration tongue protraction (ms)	213±86	346±118	35.90	<0.001*	15	0.043*
Duration tongue retraction (ms)	171±61	161±40	18.49	0.001*	5	0.500
Tongue elevation distance (% CL)	19.0±9.9	27.6±9.4	23.12	<0.001*	11	0.345
Tongue depression distance (% CL)	20.9±10.0	29.4±9.2	28.14	<0.001*	12	0.225
Duration tongue elevation (ms)	196±67	300±92	19.66	0.001*	15	0.042*
Duration tongue depression (ms)	189±62	209±76	22.26	<0.001*	12	0.225
Prey protraction distance (% CL)	18.2±12.1	17.8±10.8	34.09	<0.001*	7	0.893
Prey retraction distance (% CL)	20.5±11.7	20.7±9.0	41.71	<0.001*	9	0.686
Duration prey protraction (ms)	204±85	307±121	10.35	0.035	15	0.042*
Duration prey retraction (ms)	181±75	200±97	18.35	0.001*	9	0.686

Values are means±s.d. from 113 (aquatic) and 106 (terrestrial) processing cycles from maggot feeding trials. The P -values for Kruskal–Wallis tests were corrected after Bonferroni ($P\leq 0.003$), while P -values for Wilcoxon tests were not corrected (see Materials and Methods). Significant P -values are indicated by asterisks.

demands (Gans and De Vree, 1986; Herrel et al., 2008; Hiiemae and Crompton, 1985; Thexton et al., 1980). However, data are lacking on the impact of sensory feedback upon the food processing behavior in lissamphibians. Previous studies on prey capture in lissamphibians found that vision, mechanoreception, electroreception and olfaction are the main sensory systems used for food detection and discrimination (Anderson and Nishikawa, 1993; Deban, 1997; Roth, 1987; Valdez and Nishikawa, 1997). In salamanders and frogs, vision and (to a lesser degree) mechanoreception were suggested to be the most important senses involved in the guidance of feeding (Anderson and Nishikawa, 1993; Roth, 1987). However, vision and mechanoreception alone are unlikely to trigger the different food processing behavior observed in *T. carnifex*. Specifically, in our experiment, where newts were fed maggots rinsed in earthworm blood (ea-maggots), the newts processed the ea-maggots just like regular earthworm pieces, although visual and tactile cues were still most similar to those of regular maggots. The response of newts to ea-maggots suggests that in this case, chemical cues are more important for triggering prey-specific processing behavior than visual or mechanosensory information. In fact, salamanders possess a high number of taste buds in their oropharyngeal cavity (Northcutt et al., 2000; Zuwala and Jakubowski, 2007), but their importance in feeding behavior has not yet been specifically

addressed. It was hypothesized that salamanders might reject distasteful or toxic prey items (Avery, 1968), but with the present study we showed that chemical cues are also likely to trigger more subtle prey-specific intraoral prey handling.

Alternatively, one could argue that the sense of taste is only used to determine whether prey have been processed sufficiently. In fact, earthworm pieces and crickets were at least partially coated with blood or hemolymph before intraoral processing began. In this way, they may have spread aromas that indicated that adequate intraoral processing was carried out earlier than with maggots. Accordingly, it could be argued that the sense of taste does not serve to distinguish between prey types, but only between damaged and undamaged prey. However, some preliminary tests using whole crickets gave the same results as prepared crickets (i.e. decapitated and jumping legs removed), although this test procedure turned out to be unsuitable, because the newts temporarily refused further intake after feeding on whole crickets. We therefore encourage further investigation of this scenario – using prey or foods of uniform size that can be fed undamaged or with no aromatic signs of damage (i.e. without loss of blood or hemolymph), and that can also be supplied with the flavors of other prey or foods. See for example these interesting findings and approaches: Chases (2008); David and Jaeger (1981); Lindquist and Bachmann (1982).

Table 3. Comparison of the number of processing cycles used for different prey in aquatic and terrestrial feeding events in five individuals

Prey type	Aquatic feeding events	Terrestrial feeding events	Individual effect		Environmental effect	
			Kruskal–Wallis H_4	P	Wilcoxon Z	P
Maggot	18.7±7.2	8.4±3.2	5.426	0.249	–5.992	<0.001*
Cricket	2.8±3.6	2.6±4.0	54.117	<0.001*	–0.543	0.587
Earthworm	0.8±1.2	0.4±0.9	3.451	0.485	–2.229	0.026
Ea-maggot	N/A	0.7±1.2	5.391	0.249	N/A	N/A

Values are means±s.d. P -values were corrected after Bonferroni ($P\leq 0.013$). Significant P -values are indicated by asterisks. Kruskal–Wallis and Wilcoxon signed-rank tests indicate differences between individuals and environments.

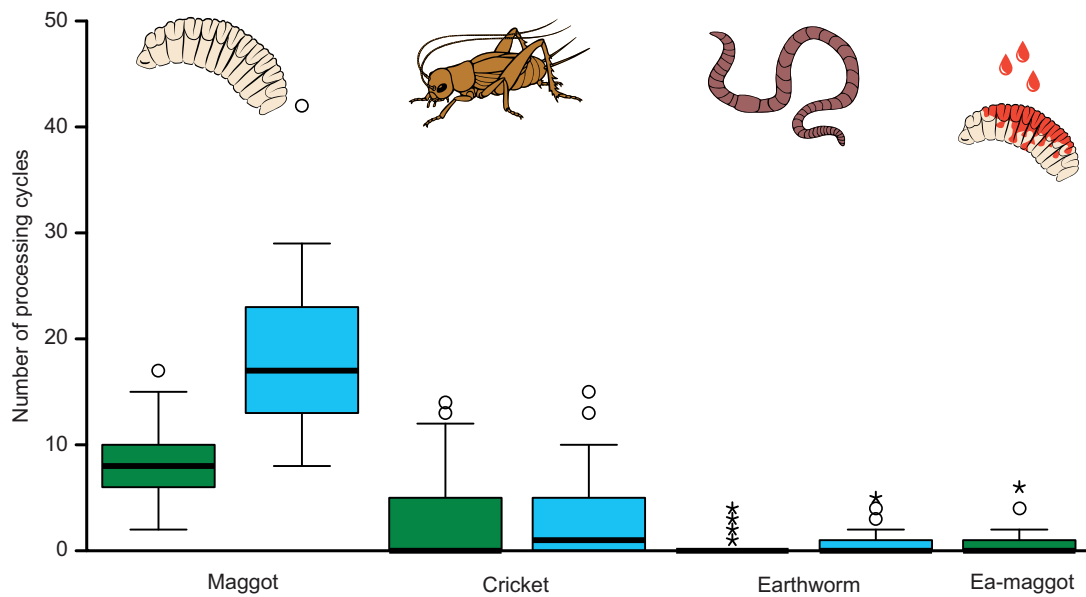


Fig. 4. Boxplots showing the different number of processing cycles used by *T. carnifex* for different prey and across aquatic and terrestrial feeding events. Green plots (left) indicate terrestrial feeding; blue plots (right) indicate aquatic feeding. Note that untreated maggots inflict most processing cycles but almost twice as many cycles are used under aquatic conditions. By contrast, such an environmental-based difference is not present in crickets and earthworms. Whereas untreated maggots generated the highest number of processing cycles, ea-maggots (maggots rinsed in earthworm blood) were hardly processed at all. For detailed statistics, see Table 3. Circles indicate values that differ significantly from the remaining values; asterisks indicate significant outliers.

Impacts of the environment on food processing

Aside from the flexible processing response to different prey, some modulation was also detected across environments. However, the general food processing mechanism only showed minor differences between aquatic and terrestrial events – with differences between individuals being greater than between environments. Newts used tongue-palate rasping to process prey regardless of the medium in which feeding occurred. The low level of behavioral modulation across environments was unexpected, given that a shift in motion-coordination between hyobranchial structures and mandible across aquatic and terrestrial chewing had been hypothesized (Konow et al., 2011) and prey capture differs fundamentally between aquatic and terrestrial habitats in salamanders (Heiss et al., 2018; Miller and Larsen, 1989, 1990; Reilly, 1996; Stinson and Deban, 2017a). To capture prey under water, salamanders employ suction feeding, where a fast oropharyngeal volume expansion induces a drop in intraoral pressure that drives prey and surrounding water to flow into the mouth (Deban and Wake, 2000; Heiss et al., 2013b; Lauder and Shaffer, 1985). On land, the low viscosity and density of air compared with water makes suction feeding inefficient (Bramble and Wake, 1985;

Heiss et al., 2018), and most salamanders use their sticky tongue that is accelerated out of the mouth to capture prey (Deban, 2003; Findeis and Bemis, 1990; Stinson and Deban, 2017b). Why is prey capture heavily affected by the medium while intraoral food processing is not? In prey capture, there is more influence from the surrounding medium (water versus air) as prey has to be removed from the surrounding environment and moved into the mouth. Once the prey is in the mouth, the conditions are likely more homogeneous, and

Table 4. Differences in the number of processing cycles used across different prey

	Wilcoxon Z	P
Maggot versus cricket	−8.316	<0.001*
Maggot versus earthworm	−8.686	<0.001*
Cricket versus earthworm	−4.647	<0.001*
Maggot versus ea-maggot	−6.103	<0.001*
Cricket versus ea-maggot	−3.150	0.002*
Earthworm versus ea-maggot	1.419	0.156

P-values were corrected after Bonferroni ($P \leq 0.008$). Significant *P*-values are indicated by asterisks. All comparisons involve data from both aquatic and terrestrial feeding events, except for comparisons with ea-maggots, where only terrestrial feeding events were included. The term 'ea-maggot' refers to maggots soaked in earthworm blood.

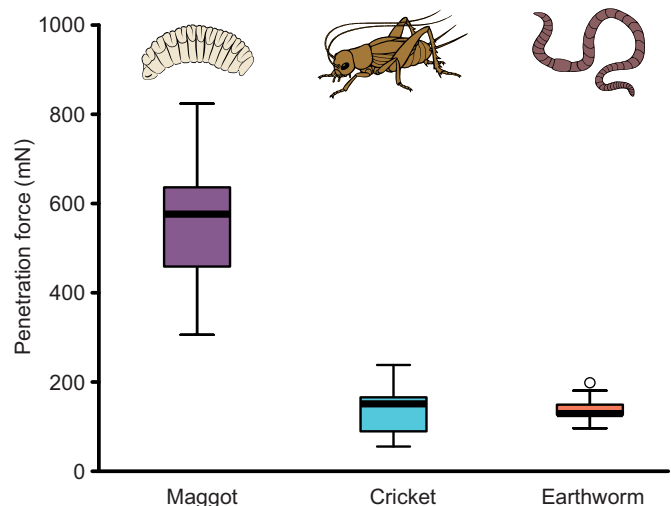


Fig. 5. Boxplots showing different penetration forces (mean \pm s.d.) measured for three different prey types: maggots, crickets and earthworms. Circles symbolize values that differ significantly from the remaining values. Pairwise Mann–Whitney *U*-tests comparing the penetration forces across the three different prey types revealed significant differences between maggots and crickets (Mann–Whitney $U_1=15.0$, $P<0.001$) and between maggots and earthworms (Mann–Whitney $U_1=15.0$, $P<0.001$), but not between crickets and earthworms (Mann–Whitney $U_1=0.0$, $P>1.000$). *P*-values were corrected after Bonferroni ($P \leq 0.017$).

therefore the environment has less influence on intraoral food processing, which leads to similar kinematics under both conditions.

Still, depending on the prey type, there can be a functional effect of the environment as the number of processing cycles can differ across aquatic and terrestrial feeding events. Specifically, the number of processing cycles differed for maggots but not for crickets or earthworm pieces: under aquatic conditions, maggots were processed with almost twice as many cycles compared with under terrestrial conditions. Why do newts use almost twice as many processing cycles in water than on land when feeding on maggots? One might first think that more drag and less friction under water would first decrease the grip between tongue pad and prey, and second decrease the grinding effect as the tongue rasps prey against the palatal dentition. Our results only partly support this hypothesis: tongue protraction distance varied significantly between environments, being lower under aquatic conditions, while anterior translation of prey did not differ. In other words, although the tongue is moved anteriorly over a shorter distance, the prey is still translated over a similar distance across the palatal dentition in aquatic feeding events. So, under aquatic conditions, the protraction of the prey seems likely to be accomplished by the tongue, but this effect might additionally be enforced by the anteriorly directed water current induced by the protracting and elevating the lingual apparatus. Additionally, decreased friction between the prey and the palatal dentition in water would enhance prey protraction but at the same time reduce the processing efficiency. Decreased processing efficiency in turn would explain why almost twice as many processing cycles were used in aquatic compared with terrestrial feeding events. Thus, it might be argued that twice as many processing cycles are necessary for the same processing effect. When feeding on crickets and earthworms, the newts showed no significantly different processing cycle numbers across environments. However, crickets and earthworms were generally processed less extensively, implying that intraoral food processing is less relevant when feeding on such prey compared with maggots.

Food processing flexibility from an evolutionary perspective

Considering the results of this study from an evolutionary perspective might allow us to extrapolate traits of food processing to the Devonian fish–tetrapod (water–land) transition of early tetrapods. Food processing in salamanders shows traits akin to both fish and amniote food processing (Heiss et al., 2019). Therefore, salamanders might provide a suitable model for revealing changes in feeding behavior during this seminal transition (Lauder and Reilly, 1994). It has been suggested that early tetrapods may have developed new feeding mechanisms in their aquatic environment and that these innovations could have later paved the way for terrestrial feeding mechanisms (Ahlberg et al., 2005; Clack, 2012; Markey and Marshall, 2007; Porro et al., 2015; Schwarz et al., 2020b). From this point of view, the differences and similarities in food processing behavior between the two fluid media could reflect an analogous adaptation or preservation of the processing mechanism of earlier tetrapods during the water–land transition. In particular, the present study suggests that, despite the extremely different physical conditions between water and air (Denny, 1993), only minor kinematic changes are needed to allow tongue–palate rasping on land (i.e. in air). Consequently, because of the projected relatively consistent conditions in the oral cavity, the new aquatically developed processing mechanism may have been retained during the transition from fish-like to more terrestrial tetrapods – as ultimately only minor behavioral changes are required to maintain intraoral food processing abilities during water–land transitions.

Conclusions

We show that *Triturus carnifex* adapts its processing behavior to the type and/or processing status of the prey, whereas the medium (i.e. the fluid environment) in which feeding occurs (water versus land) has less of an effect. *Triturus carnifex* actively discriminates between prey types, and aside from the mechanical properties of prey, gustation appears to play an important role in the guidance of feeding behavior. The present study might allow parallels to be drawn to the evolution of terrestrial feeding in early tetrapods. Analogous to *T. carnifex*, owing to relatively constant conditions in the oral cavity, early tetrapods may have shown only a slight change in their food processing behavior between aquatic and terrestrial environments.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: D.S., E.H.; Methodology: D.S., E.H., S.N.G., A.K.; Software: D.S.; Validation: D.S., E.H.; Formal analysis: D.S., E.H.; Investigation: D.S., E.H., S.N.G., A.K.; Resources: E.H., D.S., S.N.G., A.K.; Data curation: D.S., E.H.; Writing - original draft: D.S., E.H., N.K., S.N.G., A.K.; Writing - review & editing: D.S., E.H., N.K., S.N.G., A.K.; Visualization: D.S., E.H.; Supervision: D.S., E.H.; Project administration: D.S., E.H.; Funding acquisition: E.H., N.K.

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Supplementary information

Supplementary information available online at <https://jeb.biologists.org/lookup/doi/10.1242/jeb.232868.supplemental>

References

- Aerts, P., De Vree, F. and Vandewalle, P. (1986). Pharyngeal jaw movements in *Oreochromis niloticus* (Teleostei, Cichlidae) - preliminary-results of a cineradiographic analysis. *Ann. Soc. R. Zool. Belg.* **116**, 75-81.
- Ahlberg, P. E., Clack, J. A. and Blom, H. (2005). The axial skeleton of the Devonian tetrapod *Ichthyostega*. *Nature* **437**, 137-140. doi:10.1038/nature03893
- Anderson, C. W. (1993). The modulation of feeding behavior in response to prey type in the frog *Rana pipiens*. *J. Exp. Biol.* **179**, 1-12.
- Anderson, C. W. and Nishikawa, K. C. (1993). A prey-type dependent hypoglossal feedback system in the frog *Rana pipiens*. *Brain. Behav. Evol.* **42**, 189-196. doi:10.1159/000114152
- Andrews, C. and Bertram, J. E. A. (1997). Mechanical work as a determinant of prey-handling behavior in the Tokay gecko (*Gekko gekko*). *Physiol. Zool.* **70**, 193-201. doi:10.1086/639576
- Armstrong, R. A. (2014). When to use the Bonferroni correction. *Ophthalmic Physiol. Opt.* **34**, 502-508. doi:10.1111/oppo.12131
- Avery, R. A. (1968). Food and feeding relations of three species of *Triturus* (Amphibia, Urodela) during the aquatic phases. *Oikos* **19**, 408-412. doi:10.2307/3565028
- Bemis, W. E. and Lauder, G. V. (1986). Morphology and function of the feeding apparatus of the lungfish, *Lepidosiren paradoxa* (Dipnoi). *J. Morphol.* **108**, 81-108. doi:10.1002/jmor.1051870108
- Bhullar, B.-A. S., Manafzadeh, A. R., Miyamae, J. A., Hoffman, E. A., Brainerd, E. L., Musinsky, C. and Crompton, A. W. (2019). Rolling of the jaw is essential for mammalian chewing and tribosphenic molar function. *Nature* **566**, 528-532. doi:10.1038/s41586-019-0940-x
- Bramble, D. M. and Wake, D. B. (1985). Feeding Mechanisms of lower Tetrapods. In *Functional Vertebrate Morphology* (ed. M. Hildebrand, D. Bramble, K. F. Liem and D. B. Wake), pp. 230-261. Belknap Press.
- Brumpt, E. (1934). Recherches expérimentales sur la biologie de la *Lucilia bufonivora*. *Ann. Parasitol. Hum. Comparée* **12**, 81-97. doi:10.1051/parasitol/1934122081
- Camp, A. L., Konow, N. and Sanford, C. P. J. (2009). Functional morphology and biomechanics of the tongue-bite apparatus in salmonid and osteoglossomorph fishes. *J. Anat.* **214**, 717-728. doi:10.1111/j.1469-7580.2009.01056.x

- Cicort-Lucaciu, A.-Ş., David, A., Covaci, R., Toader, S. and Diaconu, I. (2007). Feeding of some *Triturus cristatus* population in Turţ area (Oas Mountains, Romania). *Herpetol. Rom.* **1**, 30-37.
- Clack, J. A. (2012). *Gaining Ground: The Origin and Evolution of Tetrapods*. Indiana University Press.
- Crane, A. L., Helton, E. J., Ferrari, M. C. O. and Mathis, A. (2018). Learning to find food: evidence for embryonic sensitization and juvenile social learning in a salamander. *Anim. Behav.* **142**, 199-206. doi:10.1016/j.anbehav.2018.06.021
- Crompton, A. W. and Hiemae, K. (1970). Molar occlusion and mandibular movements during occlusion in the American opossum, *Didelphis marsupialis* L. *Zool. J. Linn. Soc.* **49**, 21-47. doi:10.1111/j.1096-3642.1970.tb00728.x
- Chases, L. G. (2008). The Behavioral Response of Larval Coastal Giant Salamanders, *Dicamptodon Tenebrosus*, to Chemical Stimuli. Humboldt State University. <http://humboldt-dspace.calstate.edu/handle/2148/377>
- Cundall, D., Lorenz-Elwood, J. and Groves, J. D. (1987). Asymmetric suction feeding in primitive salamanders. *Experientia* **43**, 1229-1231. doi:10.1007/BF01945537
- Dalrymple, G. H., Juterbock, J. E. and La Valley, A. L. (1985). Function of the atlanto-mandibular ligaments of Desmognathine salamanders. *Copeia* **1985**, 254-257. doi:10.2307/1444826
- David, R. S. and Jaeger, R. G. (1981). Prey Location through Chemical Cues by a Terrestrial Salamander. *Copeia* **2**, 435-440. <https://www.jstor.org/stable/1444234>.
- De Vree, F. and Gans, C. (1994). Feeding in tetrapods. *Adv. Comp. Environ. Physiol.* **18**, 93-118. doi:10.1007/978-3-642-57906-6_5
- Deban, S. M. (1997). Modulation of prey-capture behavior in the plethodontid salamander *Ensatina eschscholtzii*. *J. Exp. Biol.* **200**, 1951-1964.
- Deban, S. M. (2003). Constraint and convergence in the evolution of salamander feeding. In *Vertebrate Biomechanics and Evolution* (ed. V. L. Bels, J.-P. C. and A. C.), pp. 161-178. Oxford: BIOS Scientific Publishers.
- Deban, S. M. and Wake, D. B. (2000). Aquatic Feeding in Salamanders. In *Feeding: Form, Function, and Evolution in Tetrapod Vertebrates* (ed. K. Schwenk), pp. 65-94. Academic Press.
- Delheuty, V. and Bels, V. L. (1999). Feeding kinematics of *Phelsuma madagascariensis* (Reptilia: Gekkonidae): testing differences between Iguania and Scleroglossa. *J. Exp. Biol.* **202**, 3715-3730.
- Denny, M. W. (1993). *Air and Water: the Biology and Physics of Life's Media*. Princeton University Press.
- Dunn, O. J. (1964). Multiple comparisons using rank sums. *Technometrics* **6**, 241-252. doi:10.1080/00401706.1964.10490181
- Dürigen, B. (1897). *Deutschlands Amphibien und Reptilien: Eine Beschreibung und Schilderung sämtlicher in Deutschland und den angrenzenden Gebieten vorkommenden Lurche und Kriechthiere*. Magdeburg: Creutz'sche Verlagsbuchhandlung.
- Edwards, C. A. and Bohlen, P. J. (1996). *Biology and Ecology of Earthworms*. Springer Science & Business Media.
- Elwood, J. R. L. and Cundall, D. (1994). Morphology and behavior of the feeding apparatus in *Cryptobranchus alleganiensis* (Amphibia: Caudata). *J. Morphol.* **220**, 47-70. doi:10.1002/jmor.1052200106
- Erdman, S. and Cundall, D. (1984). The feeding apparatus of the salamander *Amphiuma tridactylum* - morphology and behavior. *J. Morphol.* **181**, 175-204. doi:10.1002/jmor.1051810206
- Evans, A. R. and Sanson, G. D. (2005). Biomechanical properties of insects in relation to insectivory: cuticle thickness as an indicator of insect 'hardness' and 'intractability'. *Aust. J. Zool.* **53**, 9-19. doi:10.1071/ZO04018
- Ferry-Graham, L. A. (1998). Effects of prey size and mobility on prey-capture kinematics in leopard sharks *Triakis semifasciata*. *J. Exp. Biol.* **201**, 2433-2444.
- Findeis, E. K. and Bemis, W. E. (1990). Functional morphology of tongue projection in *Taricha torosa* (Urodela: Salamandridae). *Zool. J. Linn. Soc.* **99**, 129-157. doi:10.1111/j.1096-3642.1990.tb00565.x
- Gans, C. and De Vree, F. (1986). Shingle-back lizards crush snail shells using temporal summation (tetanus) to increase the force of the adductor muscles. *Experientia* **42**, 387-389. doi:10.1007/BF02118620
- Gans, C., De Vree, F. and Gorniak, G. C. (1978). Analysis of mammalian masticatory mechanisms: progress and problems. *Anat. Histol. Embryol.* **7**, 226-244. doi:10.1111/j.1439-0264.1978.tb00798.x
- Garamszegi, L. Z. (2006). Comparing effect sizes across variables: generalization without the need for Bonferroni correction. *Behav. Ecol.* **17**, 682-687. doi:10.1093/beheco/ark005
- Gerry, S. P., Ramsay, J. B., Dean, M. N. and Wilga, C. D. (2008). Evolution of asynchronous motor activity in paired muscles: Effects of ecology, morphology, and phylogeny. *Integr. Comp. Biol.* **48**, 272-282. doi:10.1093/icb/ict055
- Gerry, S. P., Summers, A. P., Wilga, C. D. and Dean, M. N. (2010). Pairwise modulation of jaw muscle activity in two species of elasmobranchs. *J. Zool.* **281**, 282-292. doi:10.1111/j.1469-7998.2010.00703.x
- Gidmark, N. J., Konow, N., LoPresti, E. and Brainerd, E. L. (2013). Bite force is limited by the force-length relationship of skeletal muscle in black carp, *Mylopharyngodon piceus*. *Biol. Lett.* **9**, 20121181. doi:10.1098/rsbl.2012.1181
- Gidmark, N. J., Tarrant, J. C. and Brainerd, E. L. (2014). Convergence in morphology and masticatory function between the pharyngeal jaws of grass carp, *Ctenopharyngodon idella*, and oral jaws of amniote herbivores. *J. Exp. Biol.* **217**, 1925-1932. doi:10.1242/jeb.096248
- Gintof, C., Konow, N., Ross, C. F. and Sanford, C. P. J. (2010). Rhythmic chewing with oral jaws in teleost fishes: a comparison with amniotes. *J. Exp. Biol.* **213**, 1868-1875. doi:10.1242/jeb.041012
- Gorniak, G. C. and Gans, C. (1980). Quantitative assay of electromyograms during mastication in domestic cats (*Felis catus*). *J. Morphol.* **163**, 253-281. doi:10.1002/jmor.1051630304
- Gorniak, G. C., Rosenberg, H. I. and Gans, C. (1982). Mastication in the tuatara, *Sphenodon punctatus* (reptilia: Rhynchocephalia): Structure and activity of the motor system. *J. Morphol.* **171**, 321-353. doi:10.1002/jmor.1051710307
- Griffiths, R. (1978). *The Biology of the Monotremes*. Academic Press New York.
- Griffiths, M. A. and Teunis, B. (1996). *Newts and Salamanders of Europe*. T & AD Poyser London.
- Hack, M. A. (1997). The energetic costs of fighting in the house cricket, *Acheta domestica* L. *Behav. Ecol.* **8**, 28-36. doi:10.1093/beheco/8.1.28
- Hanlin, H. G. (1978). Food habits of the greater siren, *Siren lacertina*, in an Alabama coastal plain pond. *Am. Soc. Ichthyol. Herpetol.* **1978**, 358-360. doi:10.2307/1443585
- Heiss, E., Aerts, P. and Van Wassenbergh, S. (2013a). Masters of change: seasonal plasticity in the prey-capture behavior of the Alpine newt *Ichthyosaura alpestris* (Salamandridae). *J. Exp. Biol.* **216**, 4426-4434. doi:10.1242/jeb.091991
- Heiss, E., Natчев, N., Gumpenberger, M., Weissenbacher, A. and Van Wassenbergh, S. (2013b). Biomechanics and hydrodynamics of prey capture in the Chinese giant salamander reveal a high-performance jaw-powered suction feeding mechanism. *J. R. Soc. Interface* **10**, 20121028. doi:10.1098/rsif.2012.1028
- Heiss, E., Aerts, P. and Van Wassenbergh, S. (2015). Flexibility is everything: prey capture throughout the seasonal habitat switches in the smooth newt *Lissotriton vulgaris*. *Org. Divers. Evol.* **15**, 127-142. doi:10.1007/s13127-014-0187-1
- Heiss, E., Handschuh, S., Aerts, P. and Van Wassenbergh, S. (2016). Musculoskeletal architecture of the prey capture apparatus in salamandrid newts with multiphasic lifestyle: does anatomy change during the seasonal habitat switches? *J. Anat.* **228**, 757-770. doi:10.1111/joa.12445
- Heiss, E., Aerts, P. and Van Wassenbergh, S. (2018). Aquatic-terrestrial transitions of feeding systems in vertebrates: a mechanical perspective. *J. Exp. Biol.* **221**, jeb154427. doi:10.1242/jeb.154427
- Heiss, E., Schwarz, D. and Konow, N. (2019). Chewing or not? Intraoral food processing in a salamandrid newt. *J. Exp. Biol.* **222**, 12. doi:10.1242/jeb.189886
- Herrel, A., Cleuren, J. and De Vree, F. (1996). Kinematics of feeding in the lizard *Agama stellio*. *J. Exp. Biol.* **199**, 1727-1742.
- Herrel, A., Cleuren, J. and de Vree, F. (1997a). Quantitative analysis of jaw and hyolingual muscle activity during feeding in the lizard *Agama stellio*. *J. Exp. Biol.* **200**, 101-115.
- Herrel, A., Wauters, I., Aerts, P. and de Vree, F. (1997b). The mechanics of ovophagy in the beaded lizard (*Heloderma horridum*). *J. Herpetol.* **31**, 383-393. doi:10.2307/1565667
- Herrel, A., Verstappen, M. and De Vree, F. (1999). Modulatory complexity of the feeding repertoire in scincid lizards. *J. Comp. Physiol. A* **184**, 501-518. doi:10.1007/s003590050350
- Herrel, A., Meyers, J. J., Aerts, P. and Nishikawa, K. C. (2000). The mechanics of prey prehension in chameleons. *J. Exp. Biol.* **203**, 3255-3263.
- Herrel, A., Schaeerlaeken, V., Ross, C., Meyers, J., Nishikawa, K., Abdala, V., Manzano, A. and Aerts, P. (2008). Electromyography and the evolution of motor control: limitations and insights. *Integr. Comp. Biol.* **48**, 261-271. doi:10.1093/icb/ict025
- Herrel, A., Van Wassenbergh, S. and Aerts, P. (2012). *Biomechanical Studies of Food and Diet Selection*. eL.S. John Wiley Sons.
- Hiemae, K. M. and Crompton, A. W. (1985). Mastication, food transport and swallowing. In *Functional vertebrate morphology* (ed. M. Hildebrand, D. M. Bramble, K. F. Liem and D. B. Wake), pp. 262-290. Belknap Press.
- Hillerton, J. E., Reynolds, S. E. and Vincent, J. F. V. (1982). On the indentation hardness of insect cuticle. *J. Exp. Biol.* **96**, 45-52.
- Joly, P. and Giacoma, C. (1992). Limitation of similarity and feeding habits in three syntopic species of newts (*Triturus*, Amphibia). *Ecography* **15**, 401-411. doi:10.1111/j.1600-0587.1992.tb00050.x
- Kiernan, J. A. (1999). Histological and histochemical methods: theory and practice. *Shock* **12**, 479. doi:10.1097/00024382-199912000-00012
- Konow, N., Herrel, A., Ross, C. F., Williams, S. H., German, R. Z., Sanford, C. P. J. and Gintof, C. (2011). Evolution of muscle activity patterns driving motions of the jaw and hyoid during chewing in gnathostomes. *Integr. Comp. Biol.* **51**, 235-246. doi:10.1093/icb/ict040
- Konow, N., Krijestorac, B., Sanford, C. P. J., Boistel, R. and Herrel, A. (2013). Prey processing in the Siamese fighting fish (*Betta splendens*). *J. Comp. Physiol. A Neuroethol. Sensory, Neural, Behav. Physiol.* **199**, 641-651. doi:10.1007/s00359-013-0819-5
- Lahm, S. A. (1986). Diet and habitat preference of *Mandrillus sphinx* in Gabon: implications of foraging strategy. *Am. J. Primatol.* **11**, 9-26. doi:10.1002/ajp.1350110103

- Lauder, G. V. and Reilly, S. M. (1994). Amphibian Feeding Behavior: Comparative Biomechanics and Evolution. In *Advances in Comparative and Environmental Physiology 18 Biomechanics of Feeding in Vertebrates* (ed. V. L. Bels, M. Chardon and P. Vandewalle), pp. 163–195. Berlin: Springer-Verlag Berlin Heidelberg GmbH.
- Lauder, G. V. and Shaffer, H. B. (1985). Functional morphology of the feeding mechanism in aquatic ambystomatid salamanders. *J. Morphol.* **185**, 297–326. doi:10.1002/jmor.1051850304
- Liem, K. F. and Greenwood, P. H. (1981). A functional approach to the phylogeny of the pharyngognath teleosts. *Am. Zool.* **21**, 83–101. doi:10.1093/icb/21.1.83
- Lindquist, S. B. and Bachmann, M. D. (1982). The Role of Visual and Olfactory Cues in the Prey Catching Behavior of the Tiger Salamander, *Ambystoma Tigrinum*. *Copeia* **1**, 81–90. https://www.jstor.org/stable/1444271
- Manzano, A. S., Abdala, V. and Herrel, A. (2008). Morphology and function of the forelimb in arboreal frogs: specializations for grasping ability? *J. Anat.* **213**, 296–307. doi:10.1111/j.1469-7580.2008.00929.x
- Markey, M. J. and Marshall, C. R. (2007). Terrestrial-style feeding in a very early aquatic tetrapod is supported by evidence from experimental analysis of suture morphology. *Proc. Natl. Acad. Sci. USA* **104**, 7134–7138. doi:10.1073/pnas.0701706104
- Matsumoto, R. and Evans, S. E. (2017). The palatal dentition of tetrapods and its functional significance. *J. Anat.* **230**, 47–65. doi:10.1111/joa.12534
- Miller, B. T. and Larsen, J. H., Jr. (1989). Feeding performance in aquatic postmetamorphic newts (Urodela: Salamandridae): are bidirectional flow systems necessarily inefficient? *Can. J. Zool.* **67**, 2414–2421. doi:10.1139/z89-342
- Miller, B. T. and Larsen, J. H., Jr. (1990). Comparative kinematics of terrestrial prey capture in salamanders and newts (Amphibia: Urodela: Salamandridae). *J. Exp. Zool.* **256**, 135–153. doi:10.1002/jez.1402560204
- Montuelle, S. J., Herrel, A., Libourel, P.-A., Daillie, S. and Bels, V. L. (2012). Flexibility in locomotor–feeding integration during prey capture in varanid lizards: effects of prey size and velocity. *J. Exp. Biol.* **215**, 3823–3835. doi:10.1242/jeb.072074
- Moran, M. D. (2003). Arguments for rejecting the sequential Bonferroni in ecological studies. *Oikos* **100**, 403–405. doi:10.1034/j.1600-0706.2003.12010.x
- Mulisch, M. and Welsch, U. (2015). *Romeis - Mikroskopische Technik*. Springer-Verlag.
- Nöllert, A. and Nöllert, C. (1992). *Die Amphibien Europas: Bestimmung - Gefährdung - Schutz*. Stuttgart: Franckh-Kosmos Verlags.
- Northcutt, R. G., Barlow, L. A., Braun, C. B. and Catania, K. C. (2000). Distribution and innervation of taste buds in the axolotl. *Brain. Behav. Evol.* **56**, 123–145. doi:10.1159/000047200
- Pethybridge, H., Daley, R. K. and Nichols, P. D. (2011). Diet of demersal sharks and chimaeras inferred by fatty acid profiles and stomach content analysis. *J. Exp. Mar. Biol. Ecol.* **409**, 290–299. doi:10.1016/j.jembe.2011.09.009
- Porro, L. B., Rayfield, E. J. and Clack, J. A. (2015). Descriptive anatomy and three-dimensional reconstruction of the skull of the early tetrapod *Acanthostega gunnari* Jarvik, 1952. *PLoS ONE* **10**, e0124731. doi:10.1371/journal.pone.0118882
- Reilly, S. M. (1996). The metamorphosis of feeding kinematics in *Salamandra salamandra* and the evolution of terrestrial feeding behavior. *J. Exp. Biol.* **199**, 1219–1227.
- Reilly, S. M. and Lauder, G. V. (1989). Physiological bases of feeding behaviour in salamanders: do motor patterns vary with prey type? *J. Exp. Biol.* **141**, 343–358.
- Ross, C. F., Eckhardt, A., Herrel, A., Hylander, W. L., Metzger, K. A., Schaeferlaeken, V., Washington, R. L. and Williams, S. H. (2007). Modulation of intra-oral processing in mammals and lepidosaurs. *Integr. Comp. Biol.* **47**, 118–136. doi:10.1093/icb/icm044
- Roth, G. (1987). *Visual Behavior in Salamanders*. Springer Science & Business Media.
- Rull, M., Solomon, J. and Konow, N. (2020). Elastic recoil action amplifies jaw closing speed in an aquatic feeding salamander. *Proc. R. Soc. B* **287**, 20200428. doi:10.1098/rspb.2020.0428
- Sanford, C. P. and Lauder, G. V. (1989). Functional morphology of the 'tongue-bite' in the osteoglossomorph fish *Notopterus*. *J. Morphol.* **202**, 379–408. doi:10.1002/jmor.1052020307
- Sanson, G., Read, J., Aranwela, N., Clissold, F. and Peeters, P. (2001). Measurement of leaf biomechanical properties in studies of herbivory: opportunities, problems and procedures. *Austral. Ecol.* **26**, 535–546. doi:10.1046/j.1442-9993.2001.01154.x
- Schwarz, D., Konow, N., Roba, Y. T. and Heiss, E. (2020a). A salamander that chews using complex, three-dimensional mandible movements. *J. Exp. Biol.* **223**, 12. doi:10.1242/jeb.220749
- Schwarz, D., Konow, N., Porro, L. B. and Heiss, E. (2020b). Ontogenetic plasticity in cranial morphology is associated with a change in the food processing behavior in Alpine newts. *Frontiers in Zoology*.
- Schwenk, K. (2000). An Introduction to Tetrapod Feeding. In *Feeding: Form, Function, and Evolution in Tetrapod Vertebrates* (ed. K. Schwenk), pp. 21–61. San Diego: Academic Press.
- Schwenk, K. and Rubega, M. (2005). *Diversity of Vertebrate Feeding Systems*. Science Publishers.
- Schwenk, K. and Wake, D. B. (1993). Prey processing in *Leurognathus marmoratus* and the evolution of form and function in desmognathine salamanders (Plethodontidae). *Biol. J. Linn. Soc.* **49**, 141–162. doi:10.1006/bijl.1993.1027
- Smith, K. K. (1982). An electromyographic study of the function of the jaw adducting muscles in *Varanus exanthematicus* (Varanidae). *J. Morphol.* **173**, 137–158. doi:10.1002/jmor.1051730203
- Stinson, C. M. and Deban, S. M. (2017a). Functional trade-offs in the aquatic feeding performance of salamanders. *Zoology* **125**, 69–78. doi:10.1016/j.zool.2017.08.004
- Stinson, C. M. and Deban, S. M. (2017b). Functional morphology of terrestrial prey capture in salamandrid salamanders. *J. Exp. Biol.* **220**, 3896–3907. doi:10.1242/jeb.164285
- Thexton, A. J., Hiemae, K. M. and Crompton, A. W. (1980). Food consistency and bite size as regulators of jaw movement during feeding in the cat. *J. Neurophysiol.* **44**, 456–474. doi:10.1152/jn.1980.44.3.456
- Thiesmeier, B. and Schulte, U. (2010). *Der Bergmolch: im Flachland wie im Hochgebirge zu Hause*. Laurenti.
- Thiesmeier, B., Kupfer, A. and Jehle, R. (2009). *Der Kammmolch: ein "Wasserdrache" in Gefahr*. Laurenti.
- Valdez, C. M. and Nishikawa, K. C. (1997). Sensory modulation and behavioral choice during feeding in the Australian frog, *Cyclorana novaehollandiae*. *J. Comp. Physiol. A* **180**, 187–202. doi:10.1007/s003590050040
- Wainwright, P. C. (1989). Prey processing in haemulid fishes: patterns of variation in pharyngeal jaw muscle activity. *J. Exp. Biol.* **141**, 359–375.
- Wainwright, P. C., Smith, W. L., Price, S. A., Tang, K. L., Sparks, J. S., Ferry, L. A., Kuhn, K. L., Eytan, R. I. and Near, T. J. (2012). The evolution of pharyngognath: a phylogenetic and functional appraisal of the pharyngeal jaw key innovation in labroid fishes and beyond. *Syst. Biol.* **61**, 1001–1027. doi:10.1093/sysbio/sys060
- Weijs, W. A. and Dantuma, R. (1980). Functional anatomy of the masticatory apparatus in the rabbit (*Oryctolagus cuniculus* L.). *Netherlands J. Zool.* **31**, 99–147. doi:10.1163/002829680X00212
- Werth, A. J. (2000). Feeding in marine mammals. In *Feeding: form, function and evolution in tetrapod vertebrates* (ed. K. Schwenk), pp. 475–514. Academic Press.
- Zumt, F. (1965). *Myiasis in Man and Animals in the Old World. A Textbook for Physicians, Veterinarians and Zoologists*. Butterworth & Co.
- Zuwala, K. and Jakubowski, M. (2007). Structural diversification of the gustatory organs during metamorphosis in the alpine newt *Triturus alpestris*. *J. Anat.* **211**, 371–375. doi:10.1111/j.1469-7580.2007.00758.x