Form, function, development and evolution of intraoral food processing in salamanders

Dissertation

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Glossary

This section contains definitions and information on many technical terms used often throughout the thesis - but please always keep in mind that:

> “Words have meaning only in context. The meaning of any word depends upon the sentence or on the paragraph in which it's found.”
>
> **- Alan Wilson Watts**

Chewing	Intraoral food processing using mandibular jaws.
Feature	Part, trait, or character of an organism.
Feeding	Multistage behaviour of food acquisition, processing, transport, and swallowing. *(**also referred to as** the process of nourishment or eating)*
Feeding apparatus	Entirety of organs actively used during feeding. *(**also referred to as** feeding morphology)*
Feeding system	Relation of feeding morphology and behaviour. (***also referred to as*** form-function relation of feeding)
Heterochrony	Change in timing or rate of development relative to an ancestor.
Hyobranchial system	Remnant of the visceral skeleton (splanchnocranium); assists in feeding and/or lung ventilation of vertebrates. *(**also referred to as** hyobranchium)* ***[!] equivocal use for [!]*** *hyobranchial system in larval and hyolingual in metamorphosed salamanders, respectively*
Intraoral food processing	Mechanical food reduction or preparation in the oral cavity before swallowing.
Ontogeny	Developmental history of individual organisms within their lifetime.
Phylogeny	Evolutionary history of organic taxa or species.
Tongue	Complex, muscularly driven and motile lingual system of tetrapods (e.g. metamorphic salamanders) evolved from the ancestral hyobranchial system.

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Abstract

Every animal must consume food (i.e., feed), at least during some life stages, to survive, since the energy required for almost all processes in animal life comes from the assimilation of existing organic compounds. Intraoral food processing is one of the four stages of feeding (acquisition, processing, transport, and swallowing) and refers to any mechanical reduction or preparation of the food in the oral cavity before swallowing. Intraoral food processing involves rhythmic, cyclical, and usually coordinated movements of the skull, mandible, and tongue (i.e., the feeding apparatus). Jawed fishes (chondrichthyans, actinopterygians, and dipnoi) mostly use their mandible to process food intraorally, and thus this mechanism qualifies as chewing. The general pattern of these movements is mostly conserved across fishes. However, along with the transition from water to land during early tetrapod evolution, aquatic fish-like food processing (i.e., chewing and water-based food transport) has been replaced by terrestrial amniote-like food processing (i.e., chewing and tongue-based food transport). In fact, aquatic food processing behaviour had to adapt because of the significant difference between the physical conditions prevailing in water and on land. Although intraoral food processing occurs amongst almost all major groups of jawed vertebrates, it had been argued that recent amphibians (aside from very few potential exceptions) lacked intraoral food processing mechanisms. Previous authors argued that the demands for lung ventilation constrain intraoral food processing in amphibians. The thesis at hand aims to expand our knowledge of the ontogeny and evolution of intraoral food processing in salamanders (a group of recent amphibians). I demonstrate that intraoral food processing is common amongst salamanders and that the food processing mechanism switches from mandible-based chewing in larval and paedomorphic salamanders to a tongue-based palate rasping along with associated morphological changes during ontogeny of salamanders undergoing metamorphosis. Hence, my findings are at odds with the previously held idea that salamanders do not process their food before swallowing and, therefore, I refute the argument that lung ventilation constrains the feeding apparatus from processing food intraorally. The chewing mechanisms of salamanders with an early larval morphology (i.e., larval and paedomorphic salamanders) can be strikingly complex and even involve bending movements of the mandible (i.e., mandible wishboning) whereas, in salamanders with a later larval morphology, it can resemble simple vertical biting movements.

In contrast, tongue-based processing (i.e., tongue-palate rasping) seems to be consistent across salamanders of metamorphic morphology which exhibit remodelled tongues that allow enhanced protraction. The ontogenetic switch in intraoral food processing that occurs in metamorphosing salamanders might be argued to resemble an analogue to the phylogenetic shift that happened during the evolution of early tetrapods. This analogue suggests that direct tongue-based interactions with food have evolved under aquatic conditions – hence, suggesting that terrestrial style feeding preceded the water-land transition. However, it remains to be studied why salamanders of metamorphic morphology do not use their mandibles to chew their food in addition to the processing that occurs during tongue palate-rasping. I anticipate that this doctoral thesis provides a starting point for more sophisticated studies of the evolution of feeding across vertebrates and especially the emergence of amniote-style feeding.

Zusammenfassung

Jedes Tier muss Nahrung zu sich nehmen, um zu überleben. Die Energie, die für fast alle Prozesse im Tierleben benötigt wird, stammt aus der Assimilation vorhandener organischer Verbindungen. Die intraorale Nahrungsverarbeitung ist eine der vier Stufen der Nahrungsaufnahme und umfasst jede mechanische Reduktion oder Aufarbeitung der Nahrung in der Mundhöhle vor dem Schlucken. Die intraorale Nahrungsverarbeitung umfasst rhythmische, zyklische und koordinierte Bewegungen des Nahrungsaufnahme-Apparats, bestehend aus Schädel, Unterkiefer und der Zunge.

Fische (Chondrichthyes, Actinopterygii und Dipnoi) verwenden ihren Unterkiefer meist zur intraoralen Verarbeitung von Nahrungsmitteln. Daher stellt dieser Mechanismus eine Form des Kauens dar. Das allgemeine Muster dieser Bewegungen bleibt innerhalb der meisten fischartigen Gnatostomata (Kiefermäuler) erhalten. Während der frühen Entwicklung der Tetrapoda (Landwirbeltiere) wurde jedoch zusammen mit dem Übergang von Wasser zu Land das zur aquatischen Nahrungsverarbeitung eingesetzte Kauen in Kombination mit hydrodynamischem Nahrungstransport durch die terrestrische Nahrungsverarbeitung, welche das Kauen in Kombination mit Zungen-basierten Nahrungstransport darstellt, ersetzt. Da sich die physikalischen Bedingungen im Wasser und an Land erheblich unterschieden und somit kein hydrodynamischer Nahrungstransport möglich ist, musste die aquatische Nahrungsverarbeitung an die neuen Bedingungen angepasst werden. Obwohl fast alle Hauptgruppen der Gnathostomata ihre Nahrung intraoral verarbeiten, wurde argumentiert, dass den rezenten Amphibien, abgesehen von wenigen potenziellen Ausnahmen, intraorale Nahrungsverarbeitungsmechanismen fehlten. Frühere Autoren vermuteten, dass die Anforderungen an die Luftatmung die intraorale Nahrungsverarbeitung bei Amphibien beschränken.

Die vorliegende Arbeit zielt darauf ab, das Wissen über die Ontogenie und Evolution der intraoralen Nahrungsverarbeitung bei Salamandern, als eine Gruppe rezenter Amphibien, zu erweitern. Hierbei stellte sich heraus, dass die meisten Salamander ihre Nahrung wahrscheinlich intraoral verarbeiten und, dass sich der Nahrungsverarbeitungsmechanismus und die damit verbundene Morphologie während der Ontogenese von Salamandern verändert. Die Daten der vorliegenden Arbeit legen nahe, dass der Nahrungsverarbeitungsmechanismus von einem Unterkiefer-basierten Kauen, bei Larven und paedomorphen Salamandern, zu einer Zungen-basierten Nahrungsverarbeitung wechselt. Daher stehen die vorliegenden Ergebnisse im Widerspruch zu früheren Annahmen, dass Salamander ihre Nahrung vor dem Schlucken nicht verarbeiten. Daher widerlegen diese Ergebnisse das Argument, dass die Luftatmung die Salamander daran hindert, Nahrung intraoral zu verarbeiten. Die Kaumechanismen von Salamandern, die eine Morphologie von frühen Larven aufweisen, können auffallend komplex sein und sogar laterale Biegebewegungen des Unterkiefers beinhalten. Im Gegensatz dazu stellen die Kaumechanismen von Salamandern, welche eine Morphologie von späteren Larven aufweisen, „einfache“ vertikale Beißbewegungen dar. Die Zungen-basierte Nahrungsverarbeitung (Zungen-basiertes Gaumenraspeln) scheint in Salamandern mit metamorpher Morphologie, welche durch umgestaltete Zungen eine verbesserte Protraktion ermöglichen, konsistent zu sein.

Der ontogenetische Wechsel der intraoralen Nahrungsverarbeitung, der bei metamorphosierenden Salamandern auftritt, könnte ein Analogon zu der phylogenetischen Verschiebung, welche während der Entwicklung früher Tetrapoda auftrat, darstellen. Dieses Analogon deutet darauf hin, dass sich unter aquatischen Bedingungen direkte Zungen-basierte Wechselwirkungen mit Nahrungsmitteln entwickelt haben, was darauf hindeutet, dass die terrestrische Nahrungsverarbeitung dem Wasser-Land-Übergang vorausging. Es bleibt jedoch zu untersuchen, warum Salamander mit metamorpher Morphologie ihren Unterkiefer nicht zusätzlich zu der Verarbeitung, welche während der Zungen-basierten Nahrungsverarbeitung stattfindet, zum Kauen ihrer Nahrung verwenden. Die vorliegende Arbeit stellt einen Ausgangspunkt für differenziertere Studien zur Entwicklung des Nahrungsaufnahmeverhaltens der Vertebrata (Wirbeltiere) und insbesondere zur Entstehung der terrestrischen Nahrungsverarbeitung dar.

Chapter I

Introduction

1 Objective and methodological background

This work aims to expand our knowledge of the ontogeny and evolution of intraoral food processing in salamanders. Intraoral food processing refers to any mechanical reduction or preparation of the food in the oral cavity before it is swallowed and involves rhythmic, cyclical and usually tightly coordinated movements of the cranium, mandible and tongue (Hiiemae and Crompton, 1985; Reilly et al., 2001; Schwenk, 2000a; Schwenk and Rubega, 2005; Smith, 1984; Weijs, 1975). The present work integrates a variety of methods and schools of thought from the fields of Functional Morphology, Evolutionary Morphology, Ecological Morphology, Biomechanics, Developmental Morphology, and Constructional Morphology into a methodological framework. Depending on the size and availability of the specimens, legislative restrictions, and existing on-site equipment, the morphology of the feeding apparatus was studied using classical morphological techniques (dissection) and modern 3D approaches like micro-computed tomography (µCT). The feeding apparatus function was studied using biplanar high-speed fluoroscopy or other high-speed videography to calculate kinematics and generate 3D animations. For methodological details, see the method sections of the attached articles (Ch. II-IV and supplementary material a).

1.1 Form and function

> “The two dimensions - the form and the function - of phenotypic features […] constitute the two inseparable components of biological features and must always be considered together [...].”
>
> **(Bock and Von Wahlert, 1965)**

The interrelationship between form and function, as a matter of scientific interest likely derived from two of the four Aristotelian causes (*causa formalis* and *causa finalis* respectively) (Padian, 1995; Rieppel, 1990; Russell, 1916). Thus, the ancient conceptual relationship between form and function is at least as old as Aristotle and displays a core issue of the origin of morphology (Bock and Von Wahlert, 1965; Darwin, 1859; Padian, 1995; Russell, 1916). Form and function are different aspects of the same quality, as reverse and obverse of a coin – one implies the other (Fig. 1). The relationship between form and function described best with the term ‘form-function complex’. By introducing two new technical terms, Bock and Von Wahlert distilled the essence of the term form-function complex to the faculty of a feature (Bock and Von Wahlert, 1965). Feature and faculty, in turn, are described as any part, trait, or character of an organism and the combination of form and function of this feature respectively (Bock and Von Wahlert, 1965). In this context, form and function describe the appearance or configuration of a feature and its action or how it works (Bock and Von Wahlert, 1965). Thus, the form-function complex comprises the interaction of the appearance or configuration of a part, trait, or character of an organism, and its action or how it works. However, most features serve several functions. Thus, translated in terms of the concept of the doctoral thesis at hand, this means that the form-function complex of intraoral food processing in salamanders is the totality of the behaviours and physiological functions that are spatially and temporally interconnected with the feeding apparatus.

1.2 Development and evolution

> "Evolutionary changes must be expressed in ontogeny, and phyletic information must therefore reside in the development of individuals."
>
> **(Gould, 1977)**

Development (i) and evolution (ii) of intraoral food processing in salamanders might be translated best into the questions: (i) how does the processing behaviour evolve during the life of a salamander, and (ii) which different processing behaviours have evolved in distinct groups of salamanders? Thus, the developmental question is a question of ontogeny, and the evolutionary question is a question of phylogeny.

The respective German fundamental concepts of ontogeny and phylogeny (i.e., "Ontogenie" and "Phylogenie") were introduced by the German scientist, philosopher, and artist Ernst Heinrich Philipp August Haeckel (Haeckel, 1866) and adorn the ancient evolutionary tree on the façade of the Jena Phyletisches Museum, thus visualizing that ontogeny and phylogeny are core issues in evolution. In fact, since evolution continues to shape the predefined ontogeny of existing life and thus creates new forms of life with modified ontogenies, the processes of ontogeny and phylogeny are reciprocally linked (Fig. 1). The connection between ontogeny and phylogeny was not hidden from Charles Robert Darwin either, as he wrote: "Embryology rises greatly in interest, when we look at the embryo as a picture, more or less obscure, of the progenitor, either in its adult or larval state, of all members of the same great class." in the sixth edition of his most important and world-famous work (Darwin, 1876).

The relationships between ontogeny, phylogeny and evolution are also reviewed from different points of view in Gould's famous 1977 book 'Ontogeny and Phylogeny' (Gould, 1977). The central statement of his book is that heterochrony is a major reason for changes in the relative time of appearance and the degree of development of characteristics that were already present in ancestors and he argues that "[...] changes in developmental timing [...] produce parallels between the stages of ontogeny and phylogeny" (Gould, 1977). As an extension to this idea and based on (McNamara, 2012; Reilly, 1994; Wiens et al., 2005), the view of the present work is that changes in developmental timing (heterochrony) discombobulate or obscure the parallels between the stages of ontogeny and phylogeny. Thus, integrating the knowledge of specific heterochronic states into functional studies across ontogeny and phylogeny might facilitate the usage of these evolutionary developmental data for more detailed evolutionary interpretations.

1.3 Methodological synthesis

The English biologist, palaeontologist and morphologist Sir Richard Owen once described his idea of ‘Zoological Anatomy’ as:

> “[…] that which investigates the structure of an animal in its totality, with the view of learning how the form or state of one part or organ is necessitated by its functional connections with another, and how the co-ordination of organs is adapted to the habits and sphere of life of the species ; but does not stop here, having for its main end the comparison of these associated modifications and interdependencies of organs in all the species of animals.”
>
> (Owen, 1866a).

Apart from Owen’s biological way of thought and his idealistic view, the idea of Zoological Anatomy might be interpreted as a holistic approach to the study of animals. However, like most holistic approaches, this view of Zoological Anatomy remains theoretical due to scientific limitations, though worth striving for.

The concept of this thesis is to study the form-function complex and interconnection of the feeding apparatus and to answer questions at the interface between evolution and development (mainly concerned with Devo–Evo, Eco–Evo–Devo) (Müller, 2007). Consequently, the dissertation at hand sketches a methodological framework somewhat similar to Zoological Anatomy – however, much reduced when compared to Owen’s ambitious intentions.

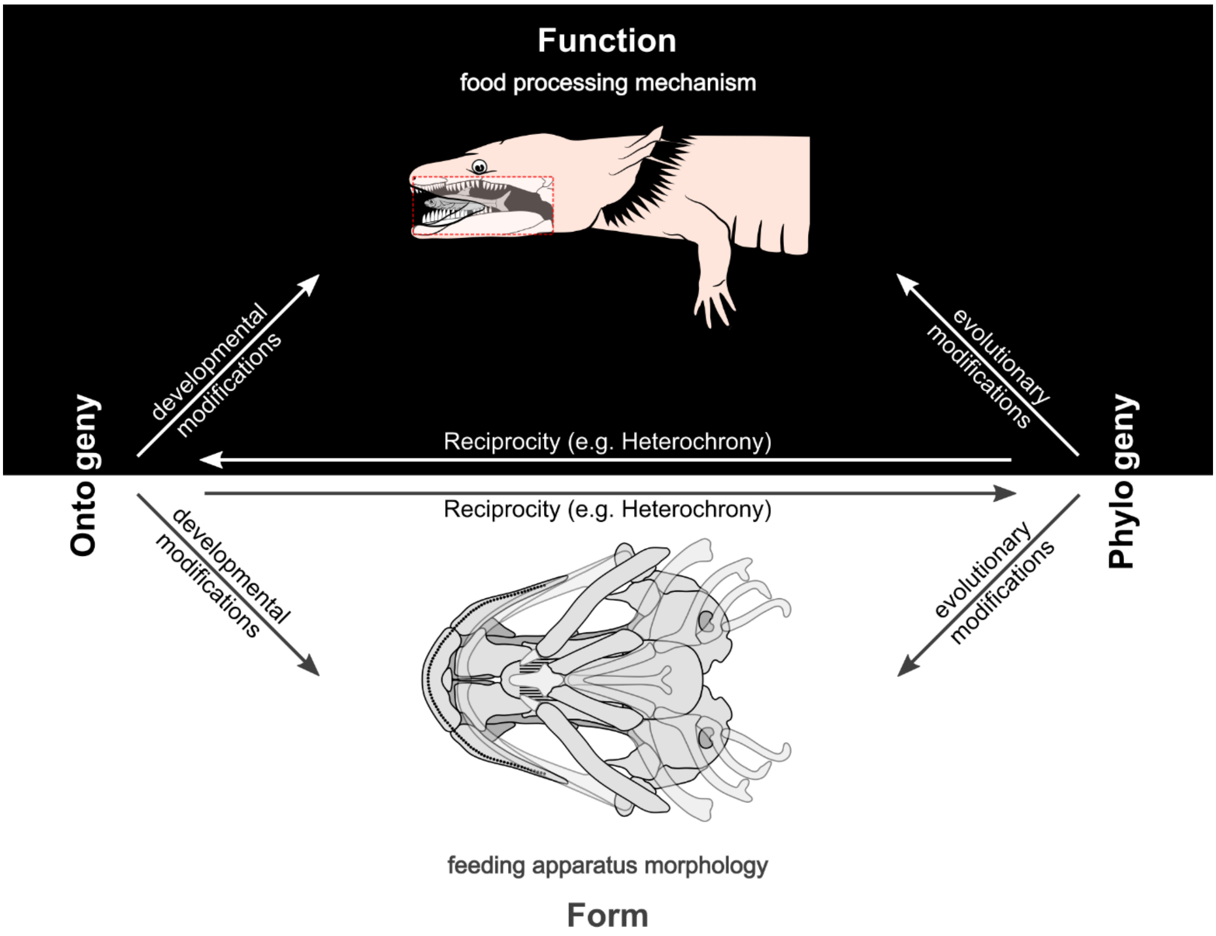

Figure 1: Connections between form, function, ontogeny, and phylogeny. Form and function are strongly linked. Ontogeny and phylogeny are reciprocally connected via developmental processes like heterochrony. Ontogeny and phylogeny potentially necessitate changes of the form-function interplay.

Since studying the behaviour and morphology of representative taxa from all families across their respective ontogenesis would go beyond the scope of a doctoral thesis, a more subtle and practical method has been applied. The specimens were selected so that they comprised examples of distinct taxa that exhibited (i) a stereotyped larval morphology, (ii) a stereotyped metamorphic morphology, and (iii) a stereotyped ontogeny during which they change from a larval to a metamorphic morphotype. This selection ultimately served the purpose of investigating the form and function of the feeding apparatus of different salamander taxa, or stages of ontogeny, to generate a model of the ontogenetic form-function relation. This ontogenetic form-function model, in turn, had been used to deduct the missing behavioural patterns from the morphology of taxa that have not been studied functionally.

Two core assumptions underlie the integrated concept of this work (Fig. 1): (i) the lines or "parallels" between ontogeny and phylogeny in salamanders can be used to conclude the respective mutual process based on knowledge about the heterochronic nature of taxa; and (ii) the simplification of the form-function complex of intraoral food processing into predefined heterochronous stages offers sufficient resolution to obtain a detailed picture of the ontogeny of a given behaviour.

2 Feeding – form, function, and evolution

All animals must consume food (feed), at least during some stages of ontogeny, to survive since the energy required for almost all processes of animal life arise from heterotrophic assimilation of organic matter. Based on this fundamental biological realization, the form, function and development of the feeding apparatus had been studied for centuries (Molyneux, 1714; Osler, 1837; Owen, 1866b). Feeding in most vertebrates involves the acquisition of food (i.e., prey), followed by its mechanical preparation to facilitate digestion and to maximize the ultimate energetic reward of a feeding event (Farrell, 1956; Lucas et al., 2002), its transport through the oral cavity, and finally swallowing to transport the food to the stomach for chemical breakdown (Bels and Whishaw, 2019; Bels et al., 1994; Schwenk, 2000a). Indeed, feeding is argued to impact on individual survival significantly and thus lifetime reproductive success (fitness consequences) (Bels and Herrel, 2019; Schwenk, 2000b). Therefore, the significance of feeding, as a fundamental part or behaviour of vertebrate life, is unquestionable.

Feeding function depends on the morphology of the feeding apparatus, which comprises all parts that are integral to the feeding behaviour of a given species (e.g. skull, jaw, and tongue). However, form and function of the vertebrate feeding apparatus have changed dramatically during evolution (Bels and Whishaw, 2019; Owen, 1866b; Parker and Bettany, 1877; Schwenk, 2000c; Starck and Wang, 2005) – ranging from aquatic filtration in the first relatively small, jawless, and fish-like vertebrates to terrestrial predation and scavenging in some mammals, with virtually all kinds of feeding functions in between. In fact, during evolution, the feeding apparatus had to adapt to various external conditions like the type of food or environmental conditions which likely imposed distinct constraints (Denny, 1993; Schwenk and Rubega, 2005). Probably the most remarkable distinguishing features are the physical differences between the fluid environments (water and air) where feeding occurs, which place drastically different demands on the form and function of the feeding apparatus (Denny, 1993; Heiss et al., 2018). In this regard, one of the most intriguing aspects of the evolution of vertebrate feeding is the rise of terrestrial feeding behaviour from an aquatic feeding behaviour in tetrapods (Reilly, 1996) – which includes the rise of amniote feeding mechanisms.

3 The rise of tetrapod feeding

Ancestral aquatic vertebrate feeding systems have been the basis from which terrestrial tetrapodean feeding systems evolved. Since the phylogenetic diversity of vertebrate feeding systems forms the basis for interpreting feeding systems of transitional forms, it seems important to understand this phylogenetic diversity to understand better how tetrapod feeding systems may have evolved.

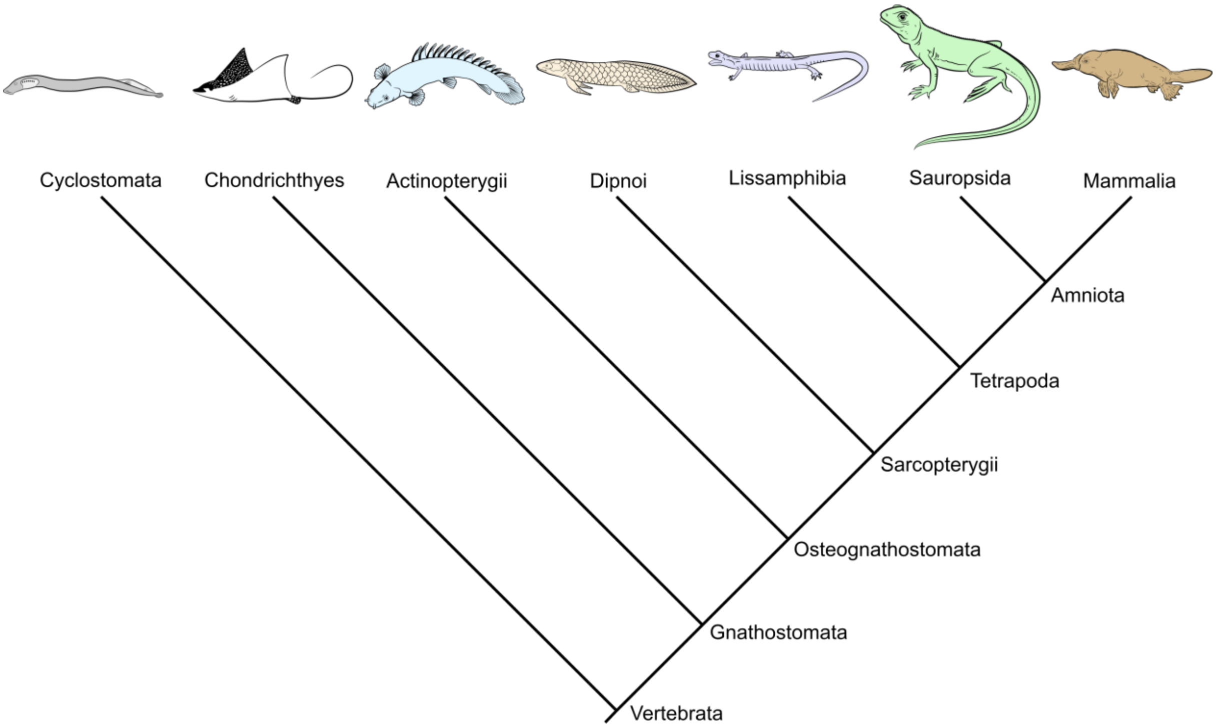

Figure 2: Generally accepted phylogenetic relationships among vertebrates. Cyclostomes (hagfishes and lampreys) represent the only extant jawless fishes; chondrichthyans are cartilaginous fishes; actinopterygians are the bony, ray-finned fishes; dipnoi or lungfish are recent lobe-finned fishes (sarcopterygians); lissamphibians are the only extant amphibians; and sauropsids consists of reptiles (including birds).

The first jawless, fish-like vertebrates evolved approximately 525 million years ago. They used their pharyngeal mucus filter (consisting partly of the hyobranchial system) to sort out detritus and suspended microorganisms (i.e., filter feeding) (Denison, 1961; Mallatt, 1981; Moy-Thomas, 1971). They likely fed by a combination of a forward movement of the body, ciliary tracts in the pharynx, and active expansion of parts of the head, particularly the floor of the mouth (Denison, 1961; Lauder, 1985).

Similar mechanisms can still be found in larval lampreys which belong to the taxa of recent jawless fish (cyclostomes) (Fig. 2) (Mallatt, 1979; Mallatt, 1981; Moore and Mallatt, 1980). However, the feeding system of adult lampreys’ changes substantially as these either bore holes into the flesh of other fish using their relatively massive laterally occluding tooth plates to eat tissue and suck blood (i.e., bulk feeding and fluid feeding) or stop feeding and live off the reserves acquired during larval life (non-feeding life) (Gill et al., 2003; Hardisty and Potter, 1971; Lafferty and Kuris, 2002). The other well-known group of cyclostomes, the hagfish, are known for their predatory or scavenging tactics for which they use their laterally occluding tooth plates to consume all or part of their prey (i.e., bulk feeding, fluid feeding and ram feeding) (Clark and Summers, 2007; Shelton, 1978; Zintzen et al., 2011). Therefore, members of both groups of cyclostomes use a feeding mechanism in which jawless, occluding tooth plates “bite”, grate, or scrape tissue from other animals, be they dead or alive. Despite the distinct feeding tactics across adult cyclostomes (i.e., mostly scavenging in hagfishes and parasitism in lampreys) the underlying form-function relations of the feeding system have been suggested to be homologues (Yalden, 1985). Consequently, it seems plausible that with the rise of mineralized tissues (i.e., teeth) in the vertebrate oral skeleton, feeding seems to have largely switched to a targeted and prey-specific mechanism. In contrast to the traditional form of filtration of numerous small particles, these prey-specific mechanisms are characterized by the consumer either hunting live animals (similar to many recent lampreys) or feeding on the cadavers of animals (similar to many recent hagfish). Interestingly, the adult cyclostome feeding mechanism appears to be an intermediate form between that of ancestral vertebrates (i.e., filter feeding) and the novel gnathostome mechanism described below (Clark and Summers, 2007). Therefore, suggesting that the ancestors of the Gnathostomes may have used a feeding mechanism similar to that of recent adult cyclostomes.

The term gnathostome, derived from the Greek *gnathos* = "jaw" and *stoma* = "mouth", and as the name suggests, the jaws, aside from the hyobranchial system, represented one of the most pivotal innovations across gnathostomes (Fig. 2). The lower jaw (i.e., mandible) brought about dramatic changes to the feeding apparatus of gnathostomes as it potentially allowed strong and fast bites to seize, incapacitate, and process food, as well as, in connection with the hyobranchial system, the rapid oropharyngeal expansion for efficient food capture and lung ventilation (Johanson et al., 2019; Schwenk, 2000a). In fact, basal gnathostomes deploy powerful suction feeding mechanisms to ingest food (Bartsch, 1996; Coates et al., 2019; Lauder, 1980a; Wilga and Motta, 1998) or biting and chewing to process food (Huber et al., 2008; Lauder, 1980a; Markey et al., 2006; Rutledge et al., 2019; Wacker et al., 2001). Thus, the evolution of the lower jaw and the hyobranchial system represents another important event in the evolution of feeding and represent a key evolutionary innovation that facilitated novel feeding mechanisms.

Actinopterygians (Fig. 2), the bony, ray-finned fishes account for approximately half of all vertebrate species and are the most numerous and most diverse group of vertebrates. One distinctive characteristic of actinopterygians is that their skeleton differs from that of chondrichthyans in that they largely replace cartilaginous elements by dermal skeletal material (bones). As a result, they have acquired a multipartite cranial skeleton made up of numerous bone plates that can move relative to one another (i.e., a kinetic cranial skeleton) (Anker, 1974; Lauder, 1980b; Liem, 1967; Stiassny, 2000). In fact, the success and power of suction feeding in ray-finned fishes, and thus the success of actinopterygians as a group, appears to be based at least in part on their highly kinetic cranial skeleton, which allows the oral cavity to expand in three dimensions (Alexander, 1967; Lauder, 1982; Westneat, 2004). The more complex cranial morphology is also argued to be the basis for the evolution of the wide range of feeding mechanisms in actinopterygians (Hulsey et al., 2005; Wainwright et al., 2004). In fact, in addition to chewing to process their food, derived taxa of ray-finned fishes have evolved two additional food processing mechanisms: (i) raking, using the tongue–bite apparatus to shred and disable prey (Camp et al., 2009; Hilton, 2001; Konow and Sanford, 2008; Konow et al., 2013; Sanford and Lauder, 1989; Sanford and Lauder, 1990) and (ii) pharyngognathy, using the pharyngeal jaw apparatus to grind food (Gidmark et al., 2014; Liem and Greenwood, 1981; Wainwright, 2002; Wainwright et al., 1989).

Dipnoi (Fig. 2) also referred to as lungfish, as lobe-finned fishes (sarcopterygians), exhibit fleshy, lobed, and paired fins, which are connected to the body by a single bone giving them their name. However, most important for their feeding behaviour is that most modern lungfish have significantly reduced and fused the bones of the skull roof and lower jaw (Clack, 2012; Kemp, 2000). It had been suggested that these changes in morphology, and with it the evolution of lungfishes, had been primarily governed by paedomorphosis, a form of heterochrony (Bemis, 1984a). In any event, these changes in the feeding apparatus morphology seemed to have constrained the feeding mechanism in dipnoi. Lungfish seem to feed only using the mechanisms seen in basal gnathostomes – they use suction feeding to ingest and chewing to process food (Bemis, 1984b; Bemis, 1986; Bemis and Lauder, 1986). Thus, the simplification of the feeding apparatus morphology (form) might be regarded as a bottleneck for its functionality.

Tetrapods (with recent representative groups including the lissamphibians, sauropsids, and mammals; Fig. 2) have evolved from their sarcopterygian ancestor approximately 390 million years ago in the middle Devonian period (Narkiewicz and Narkiewicz, 2015). Some tetrapod groups managed the transition from an ancestral, aquatic, and fish-like life to a terrestrial life. This transition had many physiological, morphological and behavioural consequences for the organisms (Carroll, 2007; Clack, 2012; Denny, 1993; Schmidt-Nielsen, 1997; Vogel, 1994). Early terrestrial vertebrates either retained the ancestral aquatic-feeding behaviour or modified it to enable terrestrial style feeding (Reilly, 1996). Indeed, some early tetrapods appear to have gone through long aquatic and juvenile development, while adults may have lived largely separately on land (Sanchez et al., 2016). It is generally agreed upon that early tetrapods likely used suction feeding to ingest food when underwater or grasped food using their jaws when feeding on land (Clack, 2012). However, an important but not yet fully understood detail about the water-land transition of early terrestrial vertebrates is what kind of mechanisms they used to process their food in new and potentially challenging conditions.

Interestingly, the transformation of the ancestral aquatic hyobranchial system into a muscle driven and flexible lingual system (i.e., mobile and free tongue that allows enhanced protraction) was an important evolutionary event in the development of tetrapods (Iwasaki, 2002; Schwenk, 2000a). Indeed, the tongue plays a vital role in and has transformed intraoral food processing of extant tetrapods (e.g. chewing and mastication) (Bels and Goosse, 1989; Hiiemae and Palmer, 1999; Schwenk, 2000a). Thus, suggesting that the appearance of such mobile and free tongues in early tetrapods might have led to changes in their food processing behaviour.

4 Modelling the evolution of tetrapod feeding

Salamanders (Caudata) seem well suited for modelling the evolution of feeding during the evolution of tetrapods, because (1) they belong to the group of Lissamphibians (Fig. 2 and Fig. S1; supplementary material c) being one of the two groups forming the extant phylogenetic bracket of tetrapods (Fig. 2); (2) salamanders are possibly the least derived lissamphibians and thus retain many of the pleisiomorphic features of the ancestral tetrapod feeding mechanism (Carroll and Holmes, 1980; Duellman and Trueb, 1994; Jarvik, 1980; Schmalhausen, 1968) including a broad and flat skull (Fortuny et al., 2011; Schoch, 2009), and a relatively robust anatomy of the hyobranchial apparatus (Witzmann, 2013); (3) many salamanders metamorphose from an aquatic larval state to a terrestrial adult during ontogeny (ontogenetic water-land transition) (Brown and Cai, 2007; Duellman and Trueb, 1994); (4) many salamanders exhibit an analogous lifestyle to that of early tetrapods and switch between aquatic and terrestrial habits (i.e., semiaquatic); (5) additionally, salamanders appear to acquire novelties in their feeding behaviour and morphology during ontogeny (Lauder and Reilly, 1994; Reilly and Lauder, 1990a). Newts (i.e., salamanders from the subfamily *Pleurodelinae*) are particularly interesting in this regard as they switch seasonally between an aquatic and a terrestrial lifestyle (i.e., they exhibit a multiphasic lifestyle) (Duellman and Trueb, 1994).

The ontogenetic and/ or seasonal habitat transition of salamanders has been argued to be a reasonable model of the changes that may have occurred during the transition to terrestrial life (Lauder and Reilly, 1994). Because we can study changes in the form and function of the feeding apparatus in individuals during ontogeny under laboratory conditions, I chose salamanders as the focus group for my thesis. Although phylogenetic relationships between the three recent amphibian groups (i.e., caudates, gymnophiones, and anurans) and fossil amphibian taxa remain controversial (Bolt, 1977; Carroll and Holmes, 1980; Duellman and Trueb, 1994; Schoch, 2014; Trueb and Cloutier, 1991), the relevance of amphibians to the evolution of vertebrate feeding and the vertebrate biology in general, is not in question. However, the evolutionary interpretations of the present work must be considered limited due to this controversy. In the following section, our current understanding, as well as unique features of salamander feeding are discussed to illustrate the background of this work.

5 Difficulties associated with studying salamander feeding

Feeding in salamanders like other vertebrates depends on distinct extrinsic (e) and intrinsic (i) influencing factors (Schwenk, 2000b). To truly understand the evolution of vertebrate feeding, one must conceive and factor in the particular characteristics of feeding - regarding its relative complex nature (i.e., multistage behaviour) (i), interconnectedness with other functions (i), dependence on the physical conditions of the environment (e), reliance on the (ontogenetically and phylogenetically) changing morphology of the feeding apparatus (i), and dependence on the properties of the food (e). These characteristics, as well as difficulties and peculiarities in interpreting them, are introduced in the following sections.

5.1 Feeding stages

Feeding is a multistage behaviour which can be divided into at least four stages or phases with distinct mechanics (Deban and Wake, 2000; Reilly et al., 2001; Schwenk, 2000a; Wake and Deban, 2000). First, ingestion (also referred to as food intake/uptake) is the initial stage of feeding when food is acquired and ingested (Fig. 3B). Second, intraoral processing (also referred to as food reduction; sometimes also referred to as manipulation; see for example (Cundall et al., 1987; Erdman and Cundall, 1984)) represents any coordinated, rhythmic, and cyclical movement of the structures of the feeding apparatus that enable the mechanical breakdown of food in the oral cavity (Fig. 3C). Third, intraoral transport (also referred to as manipulation; see for example (Larsen and Guthrie, 1975; Regal, 1966)) involves the targeted movement of food through the mouth using structures of the feeding apparatus (Fig. 3D). Fourth, swallowing (also referred to as pharyngeal emptying) is the final stage in which food particles collected in the pharynx enter the oesophagus. (Fig. 3E). The term feeding has often been used to refer to the multi-stage event on the one hand (Lauder and Shaffer, 1985; Reilly et al., 2001; Wainwright et al., 1989), but also to ingestion (stage 1) on the other (Beneski Jr et al., 1995; O’Reilly et al., 2002; Scales et al., 2016). Additionally, as indicated above, the term manipulation has been used redundantly to refer to intraoral transport (Larsen and Guthrie, 1975; Regal, 1966) and/ or intraoral processing (Cundall et al., 1987; Erdman and Cundall, 1984). These facts render many scientific works challenging to interpret, as the reader must look in the sub-text for clues as to what the authors meant.

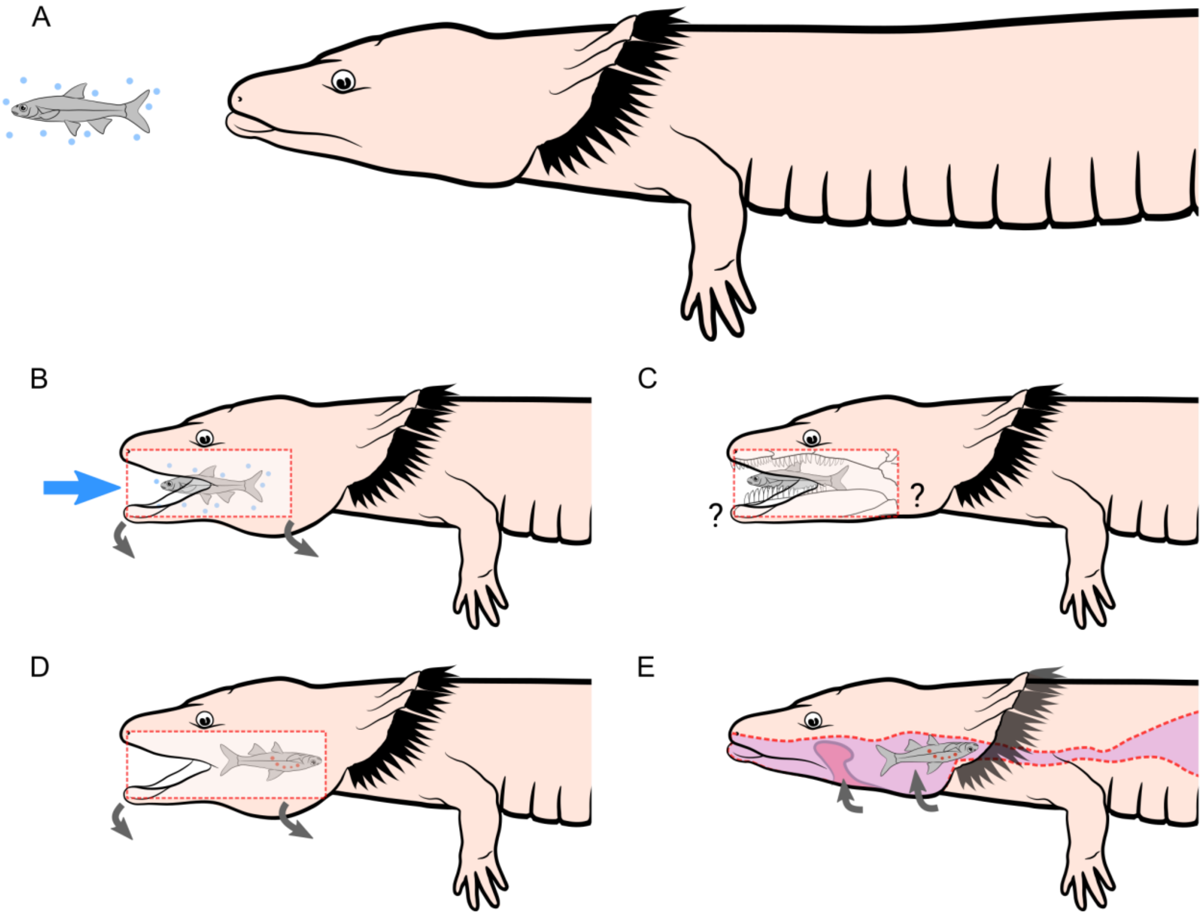

Figure 3: Schematics of the feeding stages in aquatic salamanders. (A) detection of the prey before feeding begins, (B) ingestion of the prey (stage 1), (C) intraoral food processing (stage 2), (D) intraoral food transport (stage 3), and (E) swallowing (stage 4). Note that the schematic representation points out that intraoral food transport (D) is a repetition of the ingestion movements (B). The red bulge raising towards the palate in the illustration of swallowing (E) depicts the tongue. Black arrows indicate the movement of structures or a region to which they attach. The blue arrow indicates water flow. Question marks indicate that the function or movements of structures for a particular phase are unknown. The tongue was not indicated in schematics other than (E); however, it follows the trend of the posterior arrow of the cheek region in (B) and (D).

In addition to the problematic use of terms, the sequence, as well as the concealed nature of the intraoral stages (i.e., proceeding in the oral cavity and thus concealed by the buccal skin or “cheeks”), further complicates the study of feeding. The feeding stages likely proceed in an order that allows quick and efficient feeding (Schwenk, 2000c; Schwenk and Rubega, 2005). Consequently, the prey should have little chance of escaping and will be processed sufficiently to reduce the risk of injury and to facilitate digestion. In the simplest case, the feeding sequence (order of the feeding stages) would be ingestion, processing (if any), transport, and swallowing. However, many parameters determine the feeding sequence (Hiiemae, 1984; Hiiemae, 2004; Hiiemae and Crompton, 1985). For example, if the prey initially is only partially ingested or if it is sucked too far caudal upon ingestion, then the prey must be re-positioned in the oral cavity prior to any processing. Since the order of the feeding stages cannot be predicted, and the processing mechanism of salamanders is still unknown, it is, therefore, crucial to uncover the hidden intraoral behaviours to enable their differentiation.

Intraoral food processing plays a vital role in feeding as it serves to reduce the risk of injury from prey and to facilitate swallowing and digestion (Herrel et al., 2012; Schwenk, 2000a; Schwenk and Rubega, 2005). However, (i) processing, transport, and swallowing take place in the oral cavity and therefore are difficult to observe or even to distinguish from each other and (ii) form and function of the feeding apparatus had been studied almost exclusively in terms of ingestion and transport (Larsen and Guthrie, 1975; Lombard and Wake, 1976; Peck, 1973; Regal, 1966). Not least for these reasons, it was widely believed that lissamphibians - with few potential exceptions (Cundall et al., 1987; Elwood and Cundall, 1994; Erdman and Cundall, 1984; Schwenk and Wake, 1993) - lacked intraoral food processing mechanisms and thus swallowed food whole and unreduced (De Vree and Gans, 1994; Deban and Wake, 2000; Schwenk and Rubega, 2005). In contrast, intraoral food processing is common amongst almost all other major groups of jawed vertebrates (Gintof et al., 2010; Kolmann et al., 2016; Konow et al., 2011; Lauder, 1980a; Laurence-Chasen et al., 2019; Reilly et al., 2001; Ross et al., 2007; Ross et al., 2010; Rutledge et al., 2019; Throckmorton, 1976) – suggesting that intraoral food processing might also be present amongst salamanders. Hence, my **first working hypothesis** is that: *Since intraoral food processing is an integral part of feeding behaviour that can help increase the digestive efficiency in virtually all other vertebrates, salamanders also process their food intraorally*.

5.2 Interconnectedness

In salamanders, as well as most other animals, feeding is anatomically interconnected with many other behaviours and physiological functions. Observations, as well as logical considerations, suggest that feeding, for example, is linked with aerial ventilation (i.e., air- or lung breathing) (De Vree and Gans, 1994), auditory perception (hearing) (Capshaw and Soares, 2016; Narins et al., 2006), visual perception (eyesight) (Fig. 4) (Witzmann et al., 2019), and hence locomotion (movement of the organism from one place to another). These connections can either be of a temporal or spatial character - or both. I hypothesize that a temporal relationship exists when the behaviours cannot take place simultaneously or only in a limited way and thus, in fact, resembles a temporal constraint. In contrast, a spatial connection exists when the behaviours demand the usage of structures that are also used in the respectively connected behaviour. Consequently, such behavioural interconnections could constrain a given behaviour spatially (i.e., in morphology) and/ or temporally (i.e., in the timing and duration of a behaviour). Following this logic, behaviours which are connected to feeding via both planes might impose profound constraints on the feeding behaviour.

Locomotion and eyesight are temporally connected with feeding in salamanders (Fig. 4), as these physiological functions are integrated with multiple feeding stages (Roth, 1987a) and because during feeding, vigorous movements of the skull are used (Gillis and Lauder, 1994; Grigsby, 2009; Reilly, 1996; Reilly and Lauder, 1992; Shaffer and Lauder, 1985). Additionally, eyesight seems to be spatially connected with feeding in salamanders (Fig. 4) because the eyes may be deployed actively during swallowing (Levine et al., 2004; Witzmann et al., 2019). Thus, a focused vision and/or oriented locomotion may be temporarily suppressed once feeding has begun. Aerial ventilation and audition are temporally and spatially connected to the feeding behaviour of salamanders. The spatial connection can easily be explained by the fact that, hearing (Capshaw and Soares, 2016; Mason, 2007), aerial ventilation (Brainerd, 1994; Brainerd et al., 1993), and feeding (Gillis and Lauder, 1994; Shaffer and Lauder, 1985) share overlapping basic structures (i.e., skull, tongue, and mandible). Thus, the temporal connection of lung ventilation and audition with feeding can, in turn, be explained by the temporary occupation that the underlying structures experience during each behaviour.

Aerial ventilation is an integral part of the behaviours of many amphibians (Brainerd, 1998; Brainerd, 1999; Brainerd et al., 1993) and interconnected with feeding via both planes (temporal and spatial) (Fig. 4) might be likely to constrain feeding in amphibians. Accordingly, it had been argued that the state of lung breathing in amphibians represents a redevelopment which constrains the usage of mandible and tongue, and their bridging muscles, and that they, therefore, appear to be functionally limited in supporting intraoral food processing (De Vree and Gans, 1994).

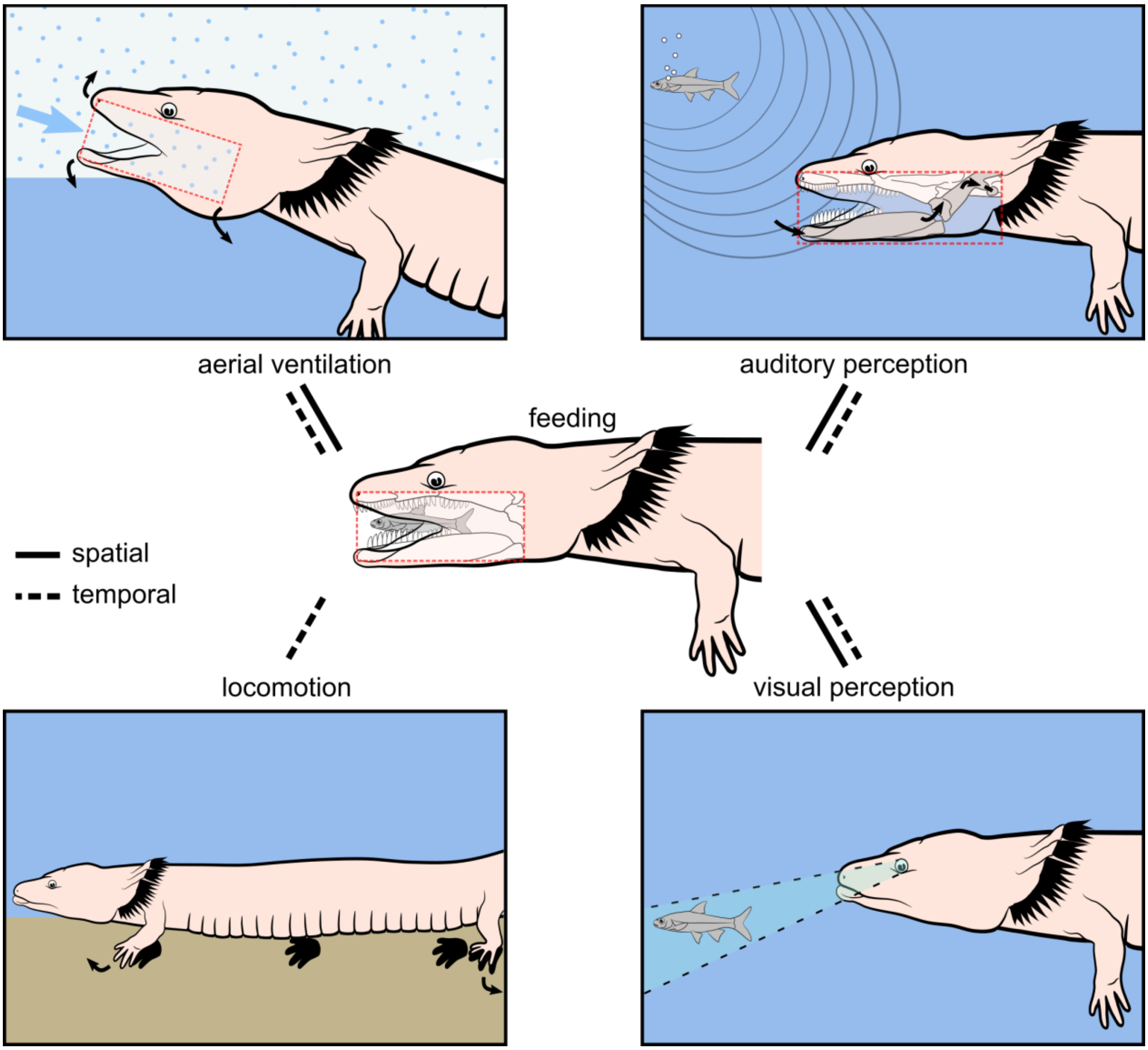

Figure 4: Exemplary behaviours interconnected with feeding in salamanders. Continuous lines connecting to feeding show spatial (also referred to as morphological) connections and dashed lines indicate temporal relationships between the behaviours. Note that aerial ventilation (lung breathing), as well as the sense of hearing, are connected to feeding via both planes – the temporal and the spatial.

This assumption contrasts with my first hypothesis that salamanders process food intraorally. However, many salamander taxa rely on bi-modal or tri-modal systems of gas exchange and thus on various respiratory surfaces (i.e., gills, lung and skin) (Guimond and Hutchison, 1972; Guimond and Hutchison, 1973; Rahn and Howell, 1976). In fact, lung respiration is rarely the primary source of oxygen uptake (Szarski, 1964). Additionally, the biggest group of salamanders (i.e., plethodontids) have lost their lungs altogether, and thus do not engage in lung ventilation (Gatz et al., 1974). Therefore, it is argued here that the behavioural interconnection with aerial ventilation is not so strong that the feeding apparatus is wholly prevented from applying any form of processing.

5.3 Morphology

Feeding has a form-function relationship with the head, jaw and hyobranchial morphology (i.e., the feeding apparatus morphology) (Herrel et al., 2012; Schwenk and Rubega, 2005) which vary substantially with ontogeny and phylogeny in lissamphibians (Rose, 2003; Wiedersheim, 1877; Ziermann, 2019). An example is the ontogenetical and phylogenetical morphologies of the hyobranchial apparatus across salamanders (Fig. 5). In fact, the feeding apparatus morphology of salamanders can vary in the structure of the hyobranchial apparatus (either as a gill-bearing or as a tongue-bearing apparatus; (Fig. 5) (Djorović and Kalezić, 2000; Heiss and Grell, 2019; Noble, 1929; Reilly, 1987; Reilly and Lauder, 1988a); the structure, position and number of the teeth (Clemen and Greven, 1994; Clemen and Greven, 2013; Greven et al., 2017; Regal, 1966); the muscular and ligamentous suspension of the hyobranchial apparatus (Findeis and Bemis, 1990; Reilly and Lauder, 1989a; Rose, 2003); the morphology of the mandible and skull (Heiss and Grell, 2019; Ivanović et al., 2014; Reilly, 1986; Schoch et al., 2019); as well as muscular and ligamentous suspension of skull and mandible (Reilly and Lauder, 1990b; Ziermann and Diogo, 2013). The morphological adaptations of the hyobranchial apparatus with respect to ontogeny and phylogeny of salamanders (i.e., differences in the form; Fig. 5) might necessitate changes in hyobranchial and fluid dynamics properties (i.e., differences in function). My **second working hypothesis** is therefore: *Since form and function are interconnected, and salamanders exhibit diverging feeding apparatus morphologies across phylogeny and ontogeny, the behaviors of intraoral food processing with regard to salamander taxa and developmental morphotypes must be different*.

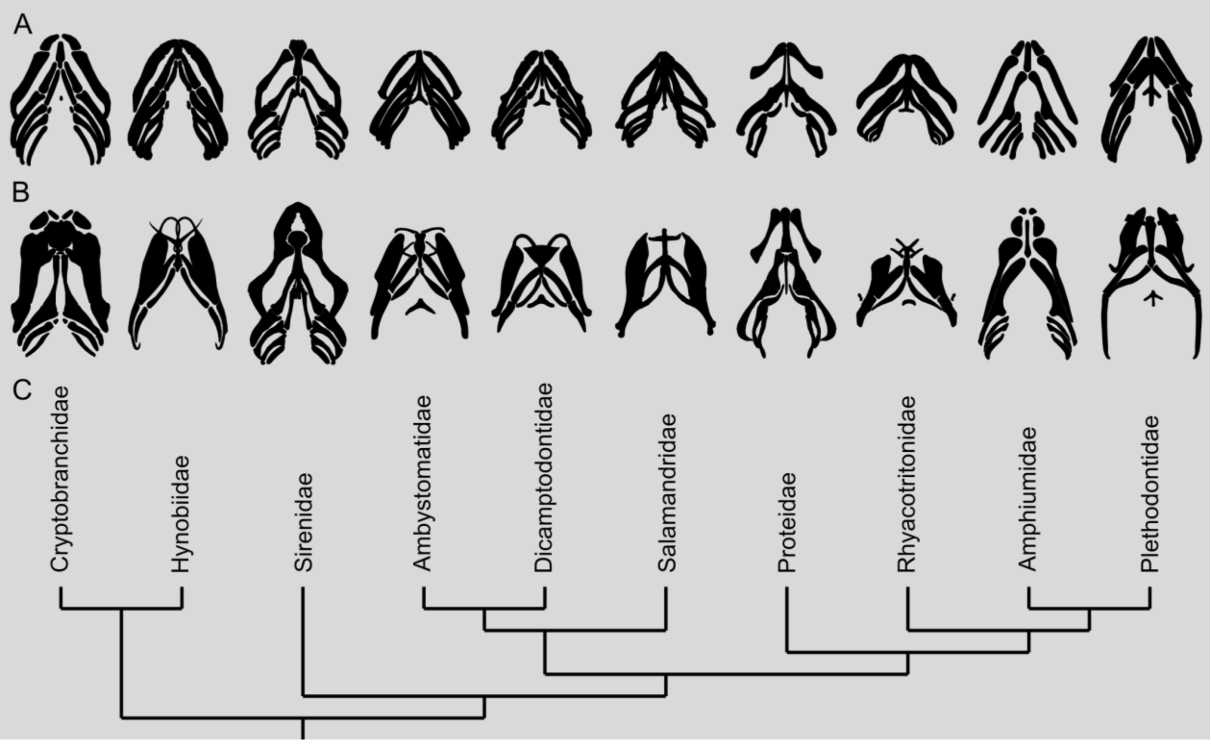

Figure 5: Hyobranchial diversity across salamander ontogeny and phylogeny. (A) Larval hyobranchial systems. (B) Adult hyobranchial systems. (C) Phylogeny of salamanders after (Pyron and Wiens, 2011). The hyobranchial morphology is redrawn from own CT scans and the following references: (cryptobranchids) (Deban, 2003; Deban and Wake, 2000), (hynobiids) (Deban, 2003; Deban and Wake, 2000; Smirnov and Vassilieva, 2002), (sirenids) (Deban and Wake, 2000; Reilly and Altig, 2006; Rose, 2003), (ambystomatids) (Lauder and Shaffer, 1988; Reilly, 1987), (dicamptodontids) (Rose, 2003; Schoch et al., 2019), (salamandrids) (Ivanović et al., 2014; Reilly, 1987), (proteids) (Marche and Durand, 1983; Parker, 1877; Wiedersheim, 1877), (rhyacotritonids) (Rose, 2003; Worthington and Wake, 1971), (amphiumids) (Deban and Wake, 2000; Erdman and Cundall, 1984; Rose, 2003), (plethodontids) (Deban, 1997; Deban, 2003; Deban and Wake, 2000).

Aside from the fact that salamander development can be complex in terms of developmental stages (Reilly, 1987; Rose, 2003), salamanders might be sorted into morphological ontogenetic stages (i.e., ontogenetic morphotypes) based on their feeding apparatus morphologies. These morphotypes appear to share commonalities associated with feeding across taxa (Lauder and Reilly, 1990; Lauder and Shaffer, 1988; Reilly, 1987; Reilly, 1990; Reilly and Lauder, 1990b; Rose, 2003). Therefore, my **third working hypothesis** is that: *As form and function are linked, and analogous morphotypes of distinct salamander taxa exhibit similar feeding apparatus morphologies, the intraoral food processing behaviour is conserved across analogous morphotypes of distinct salamander taxa*.

Form-function relationships are commonly used to investigate the ontogeny and evolution of behaviours. Yet, the ontogeny of salamanders complicates the picture of their phylogeny in that heterochrony distorts the morphological variance across taxa (Wiens et al., 2005). For example, a study of higher-level salamander relationships combined molecular and morphological data and placed three of the four paedomorphic families (Amphiumidae, Proteidae, Sirenidae) into a single group, because they contained non-transforming species with similar morphologies (Gao and Shubin, 2001). Accordingly, this suggests that when studying form-function relationships in salamanders, one cannot merely examine key taxa in order to draw conclusions about the evolution of feeding behaviour. As Gould states: "[…] changes in *developmental timing* […] produce *parallels* between the stages of ontogeny and phylogeny." (Gould, 1977) – to study the evolution of feeding behaviour in salamanders, the respective developmental strategy, and the respective morpho-development (i.e., morphogenesis) of the specimen must be considered. These results, in turn, could be plotted on a phylogenetic tree and might be used for evolutionary or phylogenetic interpretations. This line of thought motivates my idea that the phylogeny of intraoral food processing in salamanders can be resolved based on a model of the ontogenetic form-function relationship and morphogenetic data (i.e., information about the heterochronic development) of salamander taxa that have not been functionally investigated here.

However, a recent study suggested that parts of the skull associated with specializing in different feeding behaviours develop faster and more independently from others in species that undergo metamorphosis (Fabre et al., 2020). Consequently, changes in the morphology of the feeding apparatus do not have to be in accord with morphological changes in the rest of the organism. Thus, the morphology of the feeding apparatus must be used as a direct basis for the study of inferences about the developmental state of feeding morphology and feeding behaviour.

5.4 Environmental conditions

Feeding requires interaction with the surrounding medium, as the food must be extracted (ingested) from the medium as well as processed, transported, and swallowed while the medium fills the oral cavity. The interaction with the surrounding medium has been well studied in terms of ingestion and intraoral transport in aquatic salamanders. In the following part, terrestrial and aquatic ingestion and transport movements are compared; note that they have been simplified into 'two-stage' behaviours to allow a basic comparison.

Aquatic salamanders usually use suction feeding to ingest food (Cundall et al., 1987; Deban and Wake, 2000; Erdman and Cundall, 1984; Wainwright et al., 1989) and hydrodynamic transport to move the food in the oral cavity (Deban and Wake, 2000; Gillis and Lauder, 1994). Both behaviours rely on the inertial suction strategy – which means that salamanders actively generate an inertial flow in front of the 'stationary' mouth opening, which transports food towards and through the oral cavity (Heiss et al., 2018). When the food is outside of the moth, the influx of water causes the food to be drawn into the pharyngeal cavity (ingestion) (Erdman and Cundall, 1984) (Fig. 6A). As soon as the food is in the mouth, however, additional water inflows can help transport the food towards the oesophagus (caudal intraoral transport) (Gillis and Lauder, 1994) (Fig. 6C). The physical state of the aquatic medium (water) therefore, conveniently enables the same general motion pattern to solve the two problems of food ingestion and transport. Thus, aquatic ingestion and (caudal) intraoral transport is characterized by the same general motion pattern that makes up the inertial suction strategy.

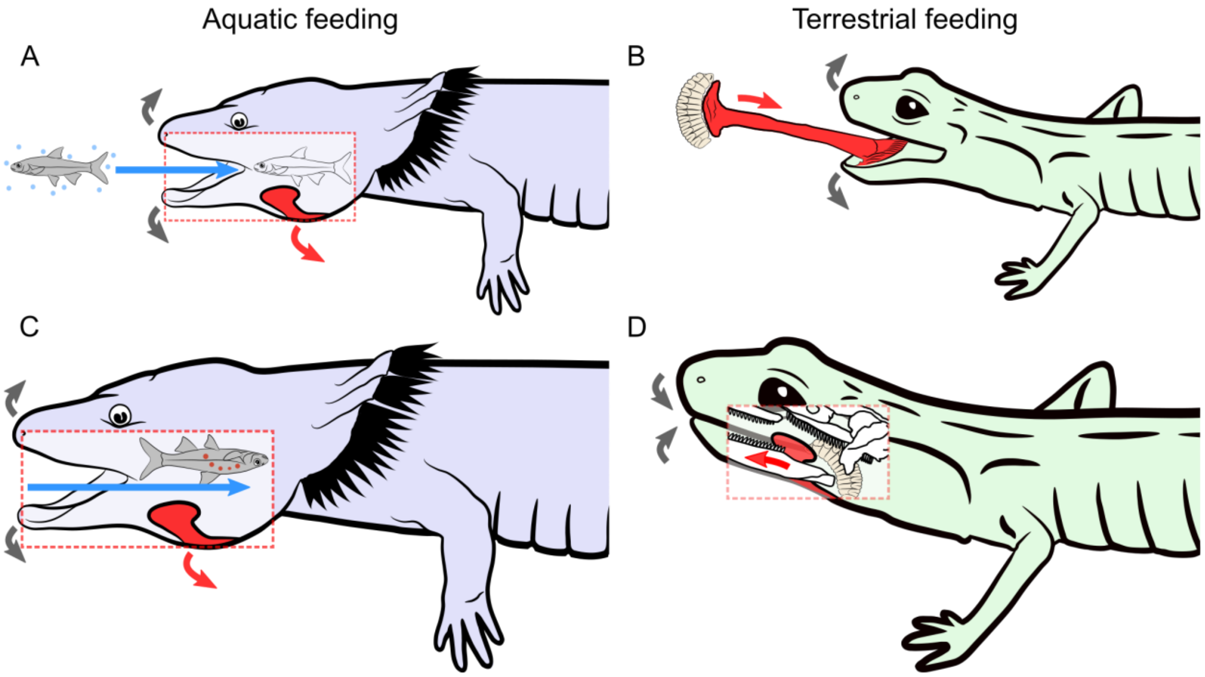

Figure 6: Connection between medium and feeding behaviour. (A) Ingestion underwater (i.e. suction feeding); (B) ingestion on land (i.e. lingual prehension); (C) intraoral transport underwater (i.e. hydrodynamic transport); and (D) intraoral transport on land (i.e. hyolingual transport). The grey arrows indicate movement of the jaws, and the red arrow indicates the movement of the tongue (i.e. hyobranchial apparatus). Note that: (i) the tongue is of fundamental importance for both feeding stages in each respective medium, (ii) the terrestrial motion sequence of the tongue stands in contrast to the aquatic during both stages, and (iii) the jaws, contrary to aquatic transport, remain or are closed during terrestrial transport.

During stage one of the inertial suction strategy the jaws open while the tongue is being depressed and retracted to expand the oropharyngeal volume – and thus to suck in water from the surrounding medium (Fig. 6A and C) (Deban and Wake, 2000; Erdman and Cundall, 1984; Heiss et al., 2015; Heiss et al., 2018). During stage two the jaws close while the tongue is slowly repositioned - thus clearing the pharyngeal cavity from dispensable water through the gill slits and/ or the remaining mouth aperture (Deban and Wake, 2000; Heiss et al., 2018; Reilly, 1995). However, like their ancestors, many salamanders exhibit biphasic life cycles and switch from an aquatic larval phase to a more terrestrial adult phase during ontogeny (Duellman and Trueb, 1994; Fabre et al., 2020; Johnson and Voss, 2013), and the physical conditions differ significantly between these respective environments. Water is about 850 times as dense and 50 times as viscous as air (Denny, 1993). Consequently, it has been suggested that these distinct physical conditions constrain aquatic and terrestrial feeding differently (Herrel and Measey, 2012). Air does not provide the physical conditions necessary for the inertial suction strategy due to its low viscosity and density (Heiss et al., 2018). Additionally, it had been suggested that muscles suitable for aquatic feeding would have to work far from their optima for strength and efficiency to compensate for the differences in the physical conditions (Hill, 1950).

As a result, salamanders are prevented from merely using the inertial suction strategy after switching from an aquatic to a terrestrial lifestyle (Heiss et al., 2018). Consequently, changes in behaviour and/ or morphology are inevitable to compensate for the different physical conditions of the two media - and thus, to enable feeding under terrestrial conditions. Hence, uptake and (caudal) intraoral transport do not show similar general movement patterns across aquatic and terrestrial salamanders (Deban and Wake, 2000; Wake and Deban, 2000).

During stage one of the terrestrial ingestion, the tongue is protracted and elevated towards the food, and the sticky tongue pad makes contact with the food as the jaws open (Fig. 6B) (Deban, 1997; Deban and Marks, 2002; Deban et al., 2007). During the second stage of terrestrial ingestion, the tongue is retracted and depressed, drawing the food into the oral cavity - while the jaws open wider, followed by fast jaw closure (Deban, 1997; Deban and Marks, 2002). However, during the first stage of the terrestrial intraoral transport, the jaws either remain closed, or they close, and the tongue is elevated and protracted (Dockx and De Vree, 1986; Reilly, 1996). Because of the limited space in the oral cavity, the tongue moves rostrally while the food is held in place by the palatal dentition (Fig. 6D) (Bramble and Wake, 1985; Larsen and Guthrie, 1975). During stage two of the caudal intraoral transport of the food, the tongue is depressed and retracted while the jaws open quickly - thus moving the food closer to the oesophagus (Dockx and De Vree, 1986; Reilly and Lauder, 1991). Hence, terrestrial ingestion and transport are similar in that the tongue is being protracted and elevated during the initial stage; however, they differ regarding their jaw movements (Fig. 6B and D). Consequently, the movement pattern must have adapted to the terrestrial condition since the terrestrial medium prevented the inertial suction strategy in early tetrapods. Therefore, the problems of (i) food intake and (ii) transport of food to the oesophagus had to be solved differently to allow feeding across the water-land transition. These findings motivate me to hypothesize that: *analogous to intraoral transport, intraoral food processing behaviour might adapt to the respective environment where feeding occurs in salamanders*.

5.5 Food properties

Feeding in salamanders also depends on various properties of the food. Ingestion in salamanders has been shown to be related to food size (Jaeger and Barnard, 1981; Johnson et al., 2003; Smith and Petranka, 1987), density (abundance) (Jaeger and Barnard, 1981), mechanical properties (Jaeger, 1990; Jaeger and Barnard, 1981), olfaction (smell) (David and Jaeger, 1981; Lindquist and Bachmann, 1982), gustatory properties (taste) (Barlow, 1998), agility (movability) (David and Jaeger, 1981; Ewert, 1972; Lindquist and Bachmann, 1982), form (Luthardt, 1981; Luthardt and Roth, 1979; Roth, 1987a), and energy content (Jaeger and Rubin, 1982).

Thus, the selection and presentation of food must enable the study of the feeding behaviour and its multiple stages. Indeed, most salamanders exhibit a considerable trophic niche breadth, including foods of varying sizes, shapes, and mechanical properties (Anderson, 1968; Hamilton, 1932; Hanlin, 1978; Scroggen and Davis, 1956). Exploitation of a trophic niche involving diverse food properties likely requires appropriate modulation of the food processing mechanism to allow adequate paralysis and preparation of the food to be swallowed (Konow et al., 2013). However, data on the modulatory capacity of food processing behaviours in salamander remain scarce (but see Rull et al., 2020).

Therefore, like most other vertebrates, as for instance chondrichthyans (Gerry et al., 2008; Gerry et al., 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; Herrel et al., 1997a; Herrel et al., 1997b) and mammals (Gorniak and Gans, 1980; Thexton et al., 1980; Weijs and Dantuma, 1980), salamanders might also modulate their food processing behaviour by adjusting movement patterns, muscle activation, or the number of processing cycles. Hence, in addition to the results regarding the impact of the environmental conditions on feeding (see ‘5.4 Environmental conditions’), the results of this section motivate my **fourth working hypothesis** that: *Since external conditions, including distinct characteristics of food and the environment, induce flexible adaptations in the food processing behaviour of different vertebrates, and the trophic niche breadth of salamanders includes foods of different sizes, shapes, and mechanical properties - salamanders can flexibly adjust their processing behaviour to external conditions*.

5.6 Difficulties and peculiarities summarized

The multistage nature of feeding behaviour is undermined by the fact that the term “feeding” is often imprecisely used to refer to suction feeding or ingestion in general. Additionally, feeding in salamanders has mostly been studied in terms of ingestion and/ or transport. Consequently, evolutionary implications remain limited to these two stages of feeding. The reduction of feeding to only include ingestion and transport to understand evolutionary processes, however, resulted in an oversimplified reading of data, and the associated scholastic inertia (ideas being published are difficult to contradict) – thus potentially prevented more detailed interpretations. In summary, feeding in salamanders is a multistage suite of behaviours with poorly studied phases (e.g. intraoral food processing) that is temporally and spatially connected to other behaviours or actions and ontogenetically and phylogenetically dependent from the changing morphology of the feeding apparatus. Hence, form and function of feeding must be studied from both a phylogenetic and ontogenetic point of view to allow detailed evolutionary interpretations. Further, feeding is extrinsically linked to the surrounding medium and food materials and their mechanical properties as it requires the interaction of the surrounding medium, the feeding complex and food. Therefore, the influence of these external factors must also be investigated in order to avoid incorrect interpretations.

6 Working hypotheses and respective chapters

The thesis is driven by the following main hypotheses, which are probed in and across the following chapters (ch. II-V). Note that the wording of the hypotheses differs slightly from that in the previous sections, as they appear here without any preceding context.

1. Since intraoral food processing is an integral part of feeding behaviour that can help increase the digestive efficiency in virtually all other vertebrates, salamanders also process their food intraorally. (ch. II-IV)

2. Since form and function are interconnected, and salamanders exhibit diverging feeding apparatus morphologies across phylogeny and ontogeny, the behaviours of intraoral food processing must differ with regard to salamander taxa and developmental morphotypes. (ch. II-III)

3. As form and function are linked, and analogous morphotypes of distinct salamander taxa exhibit similar feeding apparatus morphologies, the intraoral food processing behaviour is conserved across analogous morphotypes of distinct salamander taxa. (ch. II-III)

4. Since external conditions, including distinct characteristics of food and the environment, induce flexible adaptations in the food processing behaviour of different vertebrates, and the trophic niche breadth of salamanders includes foods of different sizes, shapes, and mechanical properties - salamanders can flexibly adjust their processing behaviour to external conditions. (ch. IV)

7 Publications overview

Chapter II A salamander that chews using complex, three-dimensional mandible movements

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Estimated own contribution: **85%**

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

Chapter III Ontogenetic plasticity in cranial morphology is associated with a change in the food processing behavior in Alpine newts

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

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Chapter II

A salamander that chews using complex, three-dimensional mandible movements

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See (CRediT taxonomy; supplementary material b) for details.

Precis:
Chapter II contains a detailed description of the form and function of the feeding apparatus in the lesser siren (Sirenoidea, see Fig. S2; supplementary material d). It is shown that the paedomorphic sirenid salamander uses complex mandible-palate rasping (i.e., mandible-palate interaction), a form of chewing in which the mandible rasps the food cyclically across and along the dentition of the palate. Form and function of the feeding apparatus of this salamander are compared with those of amniotes, with the conclusion that, contrary to previously held ideas, complex mandible movements are not exclusive to amniotes. Given the general form of the feeding apparatus of early salamander larvae, it is also suggested that mandible-palate interactions such as those of the lesser siren are common amongst salamanders with a larval morphology - which raises the question of how intraoral food processing evolves during ontogeny.

These results of this chapter support hypothesis 1
and provide further data for comparisons to support or reject hypotheses 2 and 3.

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RESEARCH ARTICLE

A salamander that chews using complex, three-dimensional mandible movements

Daniel Schwarz[1,*], Nicolai Konow[2], Yonas Tolosa Roba[1] and Egon Heiss[1]

ABSTRACT

Most non-mammal tetrapods have a hinge-like jaw operation restricted to vertical opening and closing movements. Many mammal jaw joints, by contrast, operate in more complex, three-dimensional (3D) ways, involving not only vertical but also propalinal (rostro-caudal) and transverse (lateral) movements. Data on intraoral food processing in lissamphibians and sauropsids has prompted a generally accepted view that these groups mostly swallow food unreduced, and that in those cases where lissamphibians and sauropsids chew, they mostly use simple vertical jaw movements for food processing. The exception to this generally accepted view is the occurrence of some propalinal chewing in sauropsids. We combined 3D kinematics and morphological analyses from biplanar high-speed video fluoroscopy and micro-computed tomography to determine how the paedomorphic salamander *Siren intermedia* treats captured food. We discovered not only that *S. intermedia* uses intraoral food processing but also that the elaborated morphology of its jaw joint facilitates mandibular motions in all three planes, resulting in complex 3D chewing. Thus, our data challenge the commonly held view that complex 3D chewing movements are exclusive to mammals, by suggesting that such mechanisms might have evolved early in the tetrapod evolution.

KEY WORDS: Intraoral food processing, Feeding, Amphibia, Kinematics, Functional morphology, Form and function

INTRODUCTION

Food processing refers to any mechanical reduction or preparation of food before it is swallowed (Bels and Goosse, 1989; Bramble and Wake, 1985; Schwenk and Rubega, 2005) and involves rhythmic, cyclical and usually tightly coordinated movements of the cranium, mandible and tongue (or hyobranchial apparatus in gill bearing vertebrates) (Hiiemäe and Ardran, 1968; Lauder, 1981; Schwenk and Rubega, 2005). Such behaviours are generally considered essential for immobilization and reduction of food prior to swallowing (Reilly et al., 2001; Schwenk and Schwenk, 2000; Throckmorton, 1976). The most familiar and commonly utilized processing mechanism in gnathostomes is chewing, which involves puncturing, shearing or crushing of food items by dentition set in the mandibular jaws, while cyclic motions of the hyobranchial apparatus act to move food onto the occlusal table (Davis, 1961; Hiiemäe and Ardran, 1968; Schwenk and Schwenk, 2000). Mammal chewing results in real comminution of the food (i.e. bolus formation) while non-mammals typically pierce, crush or rasp food items, resulting in little, if any, fragmentation (Schwenk and Rubega, 2005). Chewing jaw movements are diverse and involve three major elements that are directionally distinguishable: (i) vertical (arcuate or orthal, open–close) movements, (ii) propalinal (longitudinal, retraction–protraction) movements and (iii) transverse (lateral) movements of the mandible. While non-mammal tetrapods tend to use simple vertical jaw movements, most mammals combine movements in all three planes (i.e. 3D mandible movements) to varying extents (Bhullar et al., 2019; Crompton et al., 2010; Hiiemäe and Crompton, 1985; Grossnickle, 2017).

Data on chewing exist for fishes (Gintof et al., 2010; Kolmann et al., 2016; Lauder, 1980; Laurence-Chasen et al., 2019; Rutledge et al., 2019) and sauropsids (Reilly et al., 2001; Ross et al., 2010; Throckmorton, 1976) but the behaviour has mostly been studied in mammals (Bhullar et al., 2019; Crompton et al., 2010; Hiiemäe and Ardran, 1968), whereas relatively few data exist for lissamphibians (Schwenk and Wake, 1993). In fact, it has been suggested that lissamphibians generally only transport food (Dockx and De Vree, 1986; Lauder and Reilly, 1990; Schwenk and Schwenk, 2000) without processing it (De Vree and Gans, 1994; Schwenk and Rubega, 2005). The few known exceptions include the ‘head tucking’ behaviour of plethodontid salamanders following prey capture (Deban and Richardson, 2017; Schwenk and Wake, 1993) where rhythmic vertical jaw movements are used to inflict a series of strong bites to the prey. A recent study also described food processing in a salamandrid newt that rasps prey against its palatal dentition using cyclic loop movements of the tongue (Heiss et al., 2019). Together, these findings suggest that food processing could be more widespread and diverse amongst salamanders than previously thought.

Food processing depends on head, jaw and hyobranchial morphology (Herrel et al., 2012; Schwenk and Rubega, 2005), which vary substantially with ontogeny and phylogeny in lissamphibians (Heatwole and Rose, 2003; Wiedersheim, 1877; Ziermann, 2019). While at least two intraoral food processing mechanisms are known for metamorphosed salamanders, no data are currently available on the processing behavior of salamanders with larval characteristics (i.e. larval or paedomorphic morphotypes). Therefore, we analysed food processing in *Siren intermedia*, whose differentiation of somatic features is arrested in early ontogeny (Noble and Marshall, 1932; Reiss, 2002; Rose and Reiss, 1993), including retention of larval head muscles and skeletal characteristics (Clemen and Greven, 1988; Davit-Béal et al., 2007; Diogo and Abdala, 2010) that are clearly distinct from those of metamorphosed salamanders (Carroll and Holmes, 1980; Estes, 1965). For example, the prominent tooth pads on the surfaces of the mouth roof (specifically the palatine and vomerine bones) comprise the functional upper jaw, as the actual upper jaw (maxillary and

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premaxillary) bones that typically carry teeth in metamorphic tetrapods are small and toothless. Similarly, the presence of teeth on the coronoid elements of the mandible, as well as the arrested state of development of the hyobranchial elements signal an early developmental stage in larval head growth (Davit-Béal et al., 2007; Heatwole and Rose, 2003). Therefore, we propose *S. intermedia* to be a suitable model for studying food processing in salamanders with early larval traits.

The preferred prey of *S. intermedia* includes potentially damage-inflicting organisms (Hampton, 2009; Hanlin, 1978; Scroggen and Davis, 1956) so we predict that *S. intermedia* uses food processing to incapacitate and reduce food prior to swallowing. Indeed, our initial observations revealed that *S. intermedia* engages in rhythmic movements of the head, jaw and hyobranchial apparatus following prey capture. We hypothesized that these movements represent a hitherto undescribed mechanism for food processing, an idea we tested using a combination of morphological and experimental approaches.

MATERIALS AND METHODS

Specimens and animal care

Two female and two male *Siren intermedia* Barnes 1826 were chosen from the animal stock of the Institute of Zoology and Evolutionary Research at Friedrich-Schiller-University of Jena, Germany. Snout–vent length (SVL) of the animals was 29.5± 2.5 cm (mean±s.d.) and their mass was 135.9±45 g (mean±s.d.). The specimens were kept paired (SiF2/SiM2 and SiF3/SiM1). The two pairs were housed in separate glass aquariums (120×40×50 cm) with a temperature of 23±2°C, a 12 h/12 h photoperiod and fed a varied diet of maggots, small fish and mussel flesh. Husbandry and experiments were approved by the Committee for Animal Welfare of the State of Thuringia (Germany) (code for animal experiments: 02-008/15, code for animal husbandry: J-SHK-2684-05-04-05-07/14).

Marker implantation, data collection and data processing

Surgical implantation of radio-opaque tantalum markers (Bal-Tec, Los Angeles, CA, USA) on the skeletal structures of interest followed a protocol modified from prior studies (Herrel et al., 2000). The specimens were anaesthetized with buffered (pH 7.2) aqueous 0.05% MS222 (tricaine methanesulfonate) solution and the depth of the anaesthetic plane was verified by toe pinch and gently squeezing varying parts of the body with blunt tweezers. The fully anaesthetized specimen was placed on a sterile surgery tray and covered with a towel soaked in the anaesthesia solution. A hypodermic needle was used to implant spherical tantalum markers of 0.45 mm diameter to mark the tip of the upper jaw (pt), tip of the lower jaw (dt), tip of the basibranchial (indicating the hyobranchial apparatus) (bb) and back end of one hemimandible (pa) (Fig. 1A). Our initial analysis revealed that the hemimandibles of both sides move symmetrically during the transverse mandible movement. Hence, we only implanted one marker in the back end of one hemimandible and duplicated the transverse hemimandible movement of this side (Fig. 1E) to reduce the potential burden from an additional marker implant. Once recovered from anaesthesia, the animals were housed separately in recovery tanks for 1 day, after which they were returned to paired housing. The specimens were given a recovery time of at least 2 weeks to ensure complete surgical wound healing and that regular feeding had resumed.

Siren intermedia were fed maggots (*Lucilia* sp.) in water and to ensure that these food items were visible in the X-ray recordings, we glued tantalum markers of 0.45 mm diameter to their cuticle. We chose maggots as they are part of the natural prey spectrum of *S. intermedia*, though also potentially damage inflicting. It has been reported that maggots of the genus *Lucilia* can survive in the digestive tract and tissue of amphibians under certain circumstances, where they can cause severe damage (Boie, 1865; Brumpt, 1934; Zumpt, 1965).

The biplane high-speed X-ray setup consisted of two customized Neurostar TOP devices (Siemens Medical Solutions Inc.), which were mounted on two independently movable C-arms, and an acrylic aquarium. X-ray recordings were taken from the (A) dorso-ventral and (B) latero-lateral projections with a sampling frequency of 500 Hz. The following technique was used: aperture 10 mm (A), 12 mm (B); image intensifier zoom 2× (A, B); electric current 175 mA (A, B); voltage 50 kV (A, B); frame resolution 1536×1024 pixels. A total of 39 videos were recorded of which 16 recordings were selected based on the orientation of the specimen for further manual and automatic analysis.

This selection resulted in 97 cycles of post-capture movements (SiF2, 29; SiF3, 24; SiM1, 24; SiM2, 20). The videos were digitized in XMALab 1.5.0 (Knörlein et al., 2016) using a mixture of manual landmark tracking and automatic marker tracking. We calculated eight componential motions from the 3D landmark coordinates in Excel (Office 2016, Microsoft Corporation, WA, USA).

The component motions were: (1) propalinal mandible movement, measured as displacement of the tip of the lower jaw relative to the normal slope of the line connecting the points pt and oc through point oc (Fig. 1C); (2) vertical mandible movement as the distance between the tip of the lower jaw (dt) perpendicular to the line connecting the points pt and oc (Fig. 1B); (3) transverse mandible movement as the angle between the line connecting the posterior end (pa) and anterior tip (dt) of one hemimandible and the line connecting points pt and oc, times two (Fig. 1E); (4) longitudinal basibranchial movement as displacement of the anterior tip of the basibranchial (bb) relative to the normal slope of the line connecting the points pt and oc through point oc (Fig. 1C); (5) vertical basibranchial movement as normal displacement of the anterior basibranchial tip (bb) relative to the line connecting the points pt and oc (Fig. 1C); (6) longitudinal prey transport as prey (p) displacement relative to the normal slope of the line connecting the points pt and oc through point oc (Fig. 1C); (7) vertical prey transport as normal displacement of the prey (p) relative to the line connecting the points pt and oc (Fig. 1C); and (8) vertical cranial movement as the angle enclosed by the slope connecting the tip of the upper jaw (pt) and the occipital (oc) and the slope connecting the occipital (oc) and the third vertebra (v3) (Fig. 1D). The kinematic variables describing translations were normalized to the individual cranial length (% CL).

The kinematic profiles (changes in angles and distances over time) show repetitive cycles of component motions. Subdivision of the kinematic profiles into component motion cycles was achieved using a custom graph analyser tool for MATLAB 2017b (The Mathworks, Inc., Natick, MA, USA). A cycle was defined as a movement event containing three extrema: two of either low or high point and one of the other. Visual inspection of sequences suggested that propalinal movement of the mandible was the main motion component. Therefore, to generate mean kinematic profiles, the graph of the propalinal mandible movement was subdivided into componential phases (i.e. component motion cycles). All related component motion graphs were generated automatically using propalinal mandible movement as reference (Fig. 3). The kinematic variables (translations and their duration from extrema to extrema) were generated by fragmentation of the component motion cycles according to their extremes (Table S1).

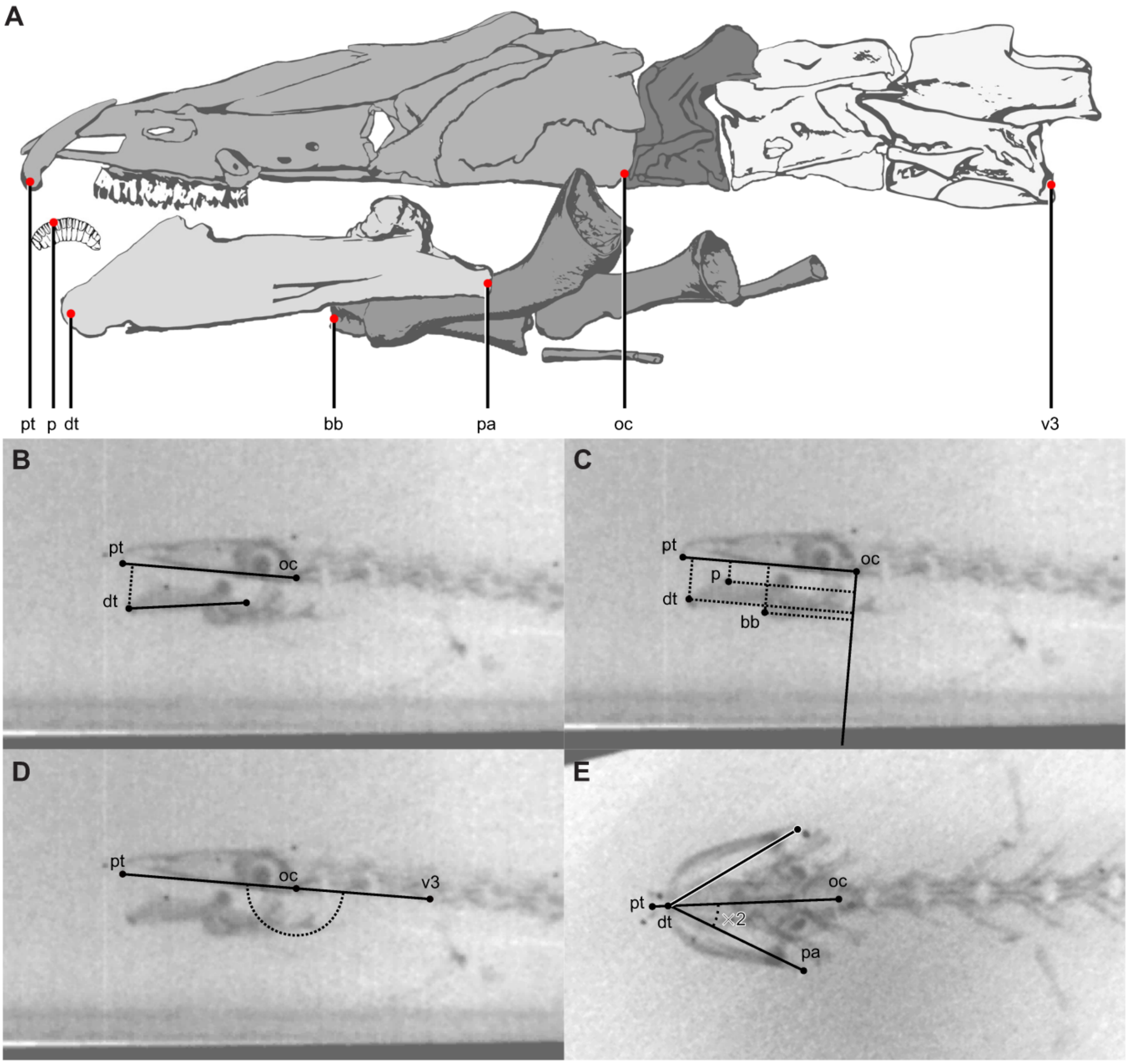

Fig. 1. Landmarks used for kinematics analyses. (A) Anatomical sketch of the *Siren intermedia* skull (lateral view) with landmarks used for kinematic analyses. (B–E) X-ray screenshots from (latero-lateral and ventro-dorsal views): (B) landmarks used for calculation of vertical mandible movements; (C) landmarks used for calculation of prey, basibranchial (indicating the hyobranchial apparatus), and mandible translations; (D) landmarks used for calculation of neck flexion–extension; and (E) landmarks used for calculation of transverse hemimandible movements. bb, basibranchial; dt, dentary tip; oc, occipital condyle; p, prey; pa, prearticular; pt, premaxilla tip; and v3, third vertebra.

Anatomical analysis

The musculoskeletal components of the feeding apparatus of two specimens (SiF2/SiM1) were reconstructed from micro-computed tomography (µCT) scans. Specimens were euthanized in a buffered 0.5% MS222 solution, fixed in 4% formaldehyde for 1 month, dehydrated in a graded series of ethanol, immersed for 2 weeks in an alcoholic iodine solution, rinsed in absolute ethanol and mounted in Falcon tubes. Two scans of the entire head region of each of the two specimens were acquired using a µCT scanner (XRadia MicroXCT-400, Carl Zeiss X-ray Microscopy, Pleasanton, CA, USA) at VetCore Facility for Research (University of Veterinary Medicine, Vienna, Austria). To apply the dual-energy µCT workflow (Handschuh et al., 2017), the first scan was performed with 40 kVp, 200 µA and the second scan with 80 kVp, 100 µA, with an isotropic voxel size of 20.3 µm for both approaches (resulting in two scans per specimen). Next, separate material fractions of mineralized and soft tissue were reconstructed according to the dual-energy µCT workflow. Volume rendering of the resulting µCT scans was performed using the Amira 6.4 software package (https://www.fei.com/software/amira). Based on tomographic image data, we threshold segmented relevant structures. The resulting 3D reconstructions were visualized in reference orientations using volume rendering from which snapshots were taken. For a more detailed anatomical analysis of the jaw joint, the skull of SiF2 and a specimen that died prior to the study were prepared for histological sections. Both specimens were decalcified using Osteomol (Merck KGaA, Darmstadt, Germany), and after complete decalcification, dehydrated in a graded ethanol series and embedded in paraffin (Histoplast-S, SERVA Electrophoresis GmbH, Heidelberg, Germany). Serial sections (8 µm) were taken on a rotary microtome (Microm HM 355 S, Thermo Fisher Scientific Inc., Waltham, MA, USA), mounted on glass slides and stained with AZAN using standard protocols (Kiernan, 1999), and documented using a light microscope (Olympus BX-51 with XC10 camera, Olympus, Tokyo, Japan).

Analyses of mandible and squamosal motion potential

To examine the range of motion of the mandible at the jaw joint and to test for cranial kinesis between the squamosal and skull, we dissected two specimens (SiF3/SiM2) and the skeletal elements of interest were manually manipulated following *in vivo* data collection. The specimens were euthanized in a buffered 0.5% MS222 solution, fixed in 4% formaldehyde, rinsed in tap water and dissected. After careful removal of the head muscles, propalinal and transverse movability of the mandible and the squamosal was tested by pulling and pushing on the lower jaw and squamosal using forceps.

RESULTS

Chewing motion kinematics

Following prey capture, all specimens used rhythmic jaw and hyobranchial movements to process food. Processing involved cyclic jaw opening and closing (i.e. vertical mandible movement) (Fig. 2 and Fig. 3B), mandible retraction and protraction (propalinal mandible movement) (Fig. 2B–D and Fig. 3A), and lateral abduction and adduction of the lower jaw 'arms' or hemimandibles (transverse hemimandible movement, i.e. mandible wishboning) (Fig. 2B–D and Fig. 3H). At the same time, there was rhythmic and cyclic flexion and extension of the neck (vertical cranial movement) (Fig. 2B–D and Fig. 3G), hyobranchial retraction and protraction (longitudinal hyobranchial movement) (Fig. 2B–D and Fig. 3E) as well as hyobranchial elevation and depression (vertical hyobranchial movement) (Fig. 2B–D and Fig. 3F). During these movements, prey debris was expelled from the oral cavity, indicating that the behaviour caused significant prey disintegration (see Movies 1 and 2 in figshare: https://doi.org/10.6084/m9.figshare.11881110.v1).

A processing cycle is defined from the start of lower jaw retraction to the end of its protraction. Using these propalinal jaw movements as a reference, all cycles were divided into preparatory (I) and power stroke (II) phases (see vertical dotted lines in Fig. 3A–H). In the first phase (preparatory phase), as the skull was depressed and the lower jaw retracted, the hemimandibles were spread apart along the transverse axis (i.e. abducted laterally, wishboning over the symphysis of the mandible) and the mouth closed slightly (Fig. 2C) (see Movie 3 in figshare: https://doi.org/10.6084/m9.figshare.11881110.v1). In the second phase (power stroke phase), as the skull was elevated and the lower jaw protracted, the mandibular rami were brought together along the transverse axis (adducted medially) and the mouth opened slightly (Fig. 2D). As the lower jaw was protracted, the prey was moved forward (anteriorly) and rasped against the functional upper jaw (anterior palatal dentition), whereby it was processed (Fig. 2D). During chewing, the prey was rasped forward bite by bite. When the prey was displaced too far anteriorly for further chewing (i.e. beyond the margins of the palatal dentition), water flows induced by hyobranchial depression transported the prey toward the oesophagus (posterior oropharynx) (see Movie 4 in figshare: https://doi.org/10.6084/m9.figshare.11881110.v1). The prey remained there for variable periods of time, after which it was either protracted and repeatedly processed or swallowed. Each chewing event consisted of one to several chewing bouts (or trains) and each bout consisted of between one and seven chewing cycles (or 'rasping' bites).

Head anatomy of *S. intermedia*

Detailed descriptions of the cranial anatomy of *S. intermedia* are available elsewhere (Carroll and Holmes, 1980; Clemen and Greven, 1988; Diogo and Abdala, 2010; Iordansky, 2010; Kleinteich and Haas, 2011; Reilly and Altig, 2006; Wiedersheim, 1877); this study focuses on structures relevant to food processing. The upper jaw and mouth roof consists of the very small toothless premaxilla and maxilla, along with the prominent paired vomer and palatine elements. The vomer and palatine carry single-cusped teeth that are recurved backwards and inwards (i.e. postero-medially), arranged in rows and, accordingly, form the 'functional upper jaw'. On the lower jaw, only the coronoid processes carry teeth, which show a similar morphology and orientation to the opposing dentition on the functional upper jaw. The hyobranchial apparatus (tongue) consists of massive bones, prominent muscles and cartilages filling most of the space of the floor of mouth between the hemimandibles (Fig. 4F).

The main muscles of the feeding system are shown in Fig. 4A–C and Fig. 5. The jaw closers (i.e. adductores mandibulae, am) contain an external (ame) and an internal (ami) part. The adductor mandibulae internus complex (ami) consists of a pseudotemporalis and a pterygoideus portion. The pseudotemporalis is further portioned into an anterior positioned pseudotemporalis profundus (also referred to as adductor mandibulae A3″) and a posterior pseudotemporalis superficialis (also referred to as adductor mandibulae A3′; Diogo and Abdala, 2010). The pseudotemporalis originates at the medial fission zone of both frontal and parietal bones, runs posteriorly and ventrally and inserts on the central and medial part of the hemimandible. Below the pseudotemporalis lies the pterygoideus muscle (also referred to as pterygomandibularis), which originates on the lateral wall of the skull (parasphenoid and orbitosphenoid). The pterygoideus reaches ventrally, laterally and posteriorly to the back end of the lower jaw where it wraps around the hemimandible behind the jaw joint to form a fleshy belly (Fig. 4B,C and Fig. 5) and inserts on the outer side of the mandible. Given this line of action, the pseudotemporalis is functionally a jaw opener that also protracts and adducts the mandible medially. The adductor mandibulae externus complex (ame) contains tightly interconnected muscles, originating on a tendon of the first vertebra (atlas), extending antero-ventrally and connecting to a tendon sheet (coronar aponeurosis) central on each hemimandible. The epaxial (e) neck musculature attaches on the back (occipital) of the skull and extends posteriorly along the whole body length (Fig. 4A,B and Fig. 5). The jaw opener complex (depressor mandibulae complex) lies adjacent to the adductor mandibulae complex and consist of two parts, the (anterior) depressor mandibulae (dm) and a depressor mandibulae posterior (dm′). The depressor mandibulae posterior descends from the levator hyoideus (Diogo and Abdala, 2010) and, consequently, it is often referred to as such. The smaller anterior depressor mandibulae originates at squamosal and exoccipital and inserts on the upper part of the retroarticular process (Fig. 4A,B and Fig. 5). The depressor mandibulae posterior originates on the dorsal fasciae of the neck muscles, runs along the ceratohyal cartilage and attaches on the back of the retroarticular process (Fig. 4A–C and Fig. 5). The hyobranchial skeleton, positioned within the floor of the mouth, forms the attachment site for four major muscles. The largest is the branchiohyoideus externus, extending from the upper part of the hyobranchial skeleton (cartilaginous ceratobranchial I) to the ceratohyal. The delicate geniohyoid muscle connects the foremost hyobranchial bone (basibranchial) anteriorly to the lower jaw (dentary) while the subarcualis rectus I connects the basibranchial posteriorly to the cartilaginous ceratobranchial I. The rectus cervicis

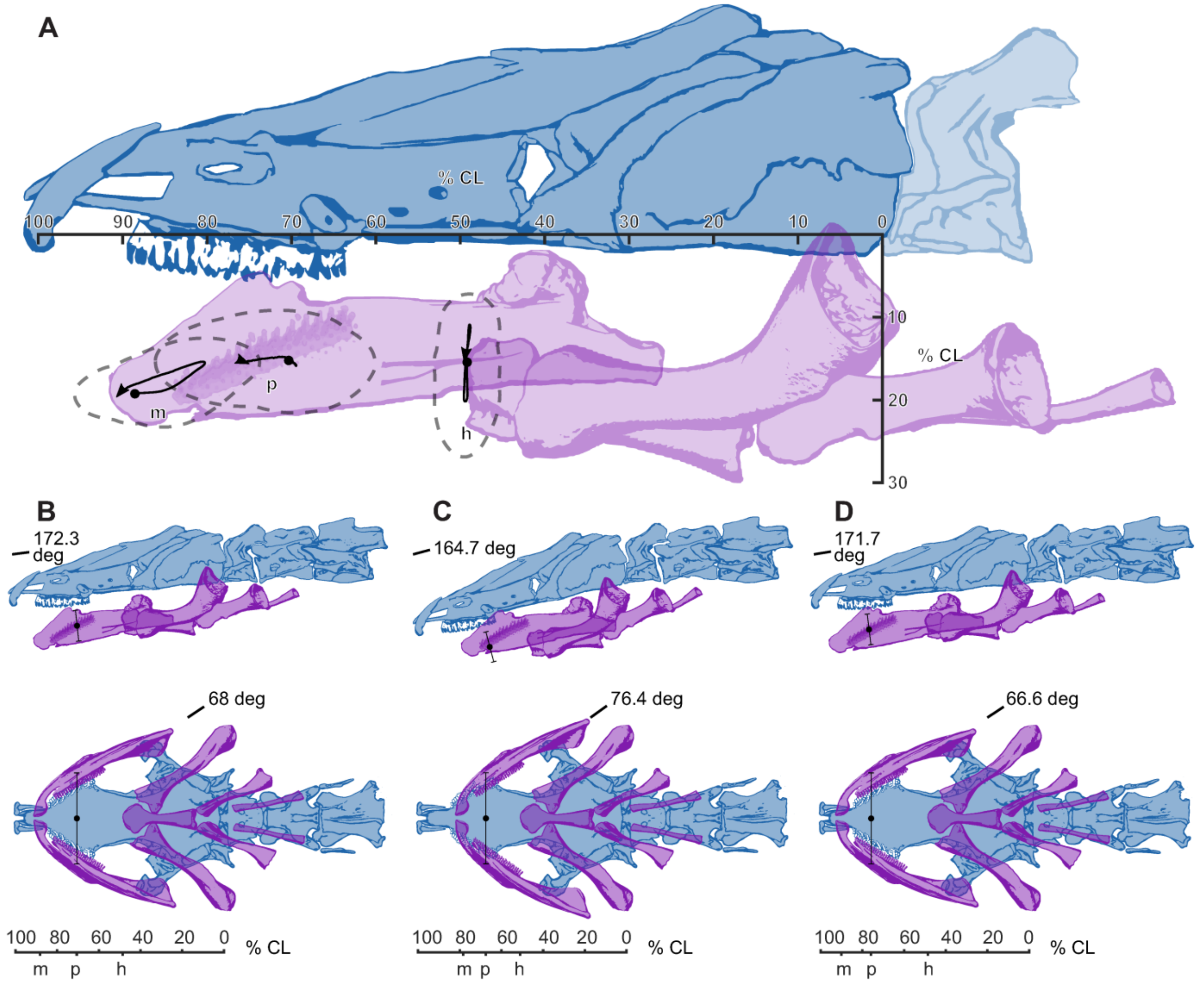

Fig. 2. Movements of cranial elements during chewing in *S. intermedia*. (A) Lateral view of the range of motion of the main chewing movements normalized to cranial length (CL). Solid lines represent means while dashed lines indicate 68% confidence interval of the 97 analysed cycles. The counter-clockwise loops indicate motion of the mandible (m) that moves prey (p) along the mouth roof while the hyobranchial apparatus (h) (tongue) moves mainly vertically. (B–D) The mean orientation of the head elements from lateral and ventral views at the different chewing stages: (B) resting, (C) fully retracted and (D) fully protracted state. Shifts in bone orientation from B to C indicate movements during stage I (preparatory phase); shifts in C to D indicate movements during stage II (power stroke phase). Mean displacements in Table S1 and raw data were used to generate the displacement panels B–D; the position of the prey (black dot) was added using the mean kinematic profiles.

(sternohyoid) muscle connects the central hyobranchial bone (hypobranchial I) posteriorly to the abdominal muscle (rectus abdominis) (Fig. 4B,C and Fig. 5).

Anatomy of the jaw joint complex

The jaw joint complex of *S. intermedia* is an elaborate structure built up by the cartilaginous mandibular suspension (quadrate), a set of cartilaginous processes and ligaments, the jaw joint with surrounding connective tissue, and parts of the mandible (Fig. 6; Fig. S1, Fig. S2). The contact area of the jaw joint consists of the cartilaginous quadrate that articulates with the cartilaginous articular of the lower jaw. The jaw joint resembles a half-saddle joint, with a saddle like or ball-and-socket like structure in its anterior region (Fig. 6B,E) that flattens up posteriorly (Fig. 6C,F). The quadrate carries two cartilaginous processes. The rear and descending (postero-ventral) branch (i.e. hyoquadrate process) links the quadrate and ceratohyal (Fig. 6D,G; Fig. S1). The upper and rising (dorso-medial) branch (i.e. ascending process) connects the quadrate to a cartilaginous lateral part of the cranium, posterior to the orbitosphenoid (Fig. 6B,E).

Analyses of the motion potential of the mandible and its squamosal suspension

To determine the potential magnitude and direction of jaw movement with respect to the skull, we dissected two *S. intermedia* specimens and used careful manipulations (Konow et al., 2008). Gentle pulling and pushing of the mandible in the longitudinal direction with respect to the long-axis of the skull resulted in clear propalinal and transverse stretching deformations at the jaw joint. The squamosal remained virtually fixed with respect to the skull. Greater forces directly applied to the squamosal only resulted in minor bending but no rotation of the

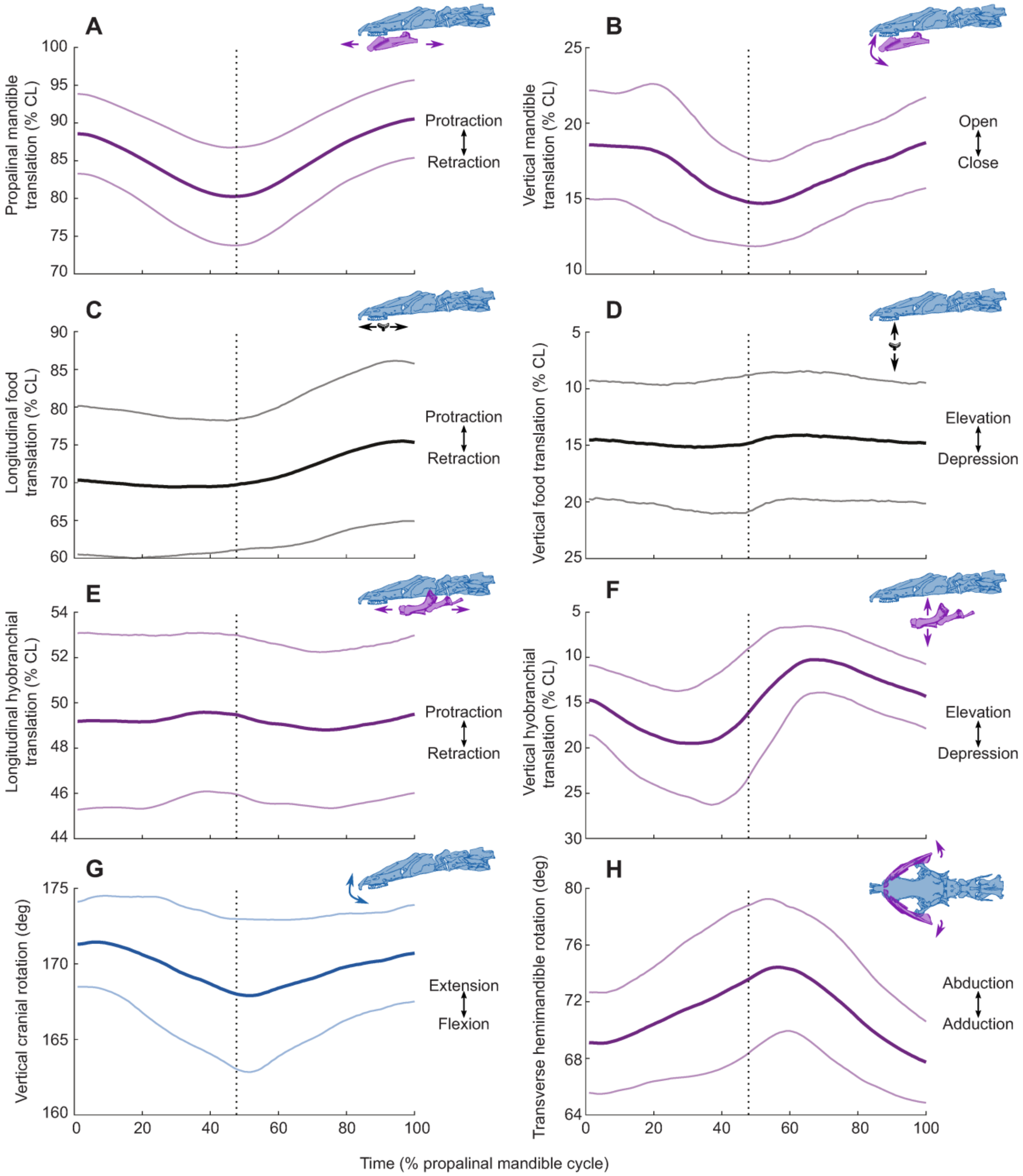

Fig. 3. Mean kinematic profiles of aquatic chewing in *S. intermedia*. Based on 97 chewing cycles (bold curves) ±s.d. (narrow curves). The profiles show motions of the feeding apparatus and prey in the cranial coordinate system (see Fig. 2A for the coordinate system reference), normalized to the timing of propalinal mandible movement. Translations are normalized to cranial length (CL), and the dotted lines separate phase I (preparatory) and phase II (power stroke). Purple, mandible and hyobranchial movements; blue, cranial movement; and black, displacement of the prey item. The comparison of each kinematic profile with the corresponding movement (Fig. 2) demonstrates the position and translation or rotation of the underlying structures. Please see Table S1 for absolute kinematics values.

squamosal relative to the cranium was observed. Our dissections revealed that dense connective tissue encapsulates and reinforces the jaw joint.

DISCUSSION

We used anatomical and biplanar high-speed video-fluoroscopy techniques to show that *S. intermedia*, after capturing its prey, uses

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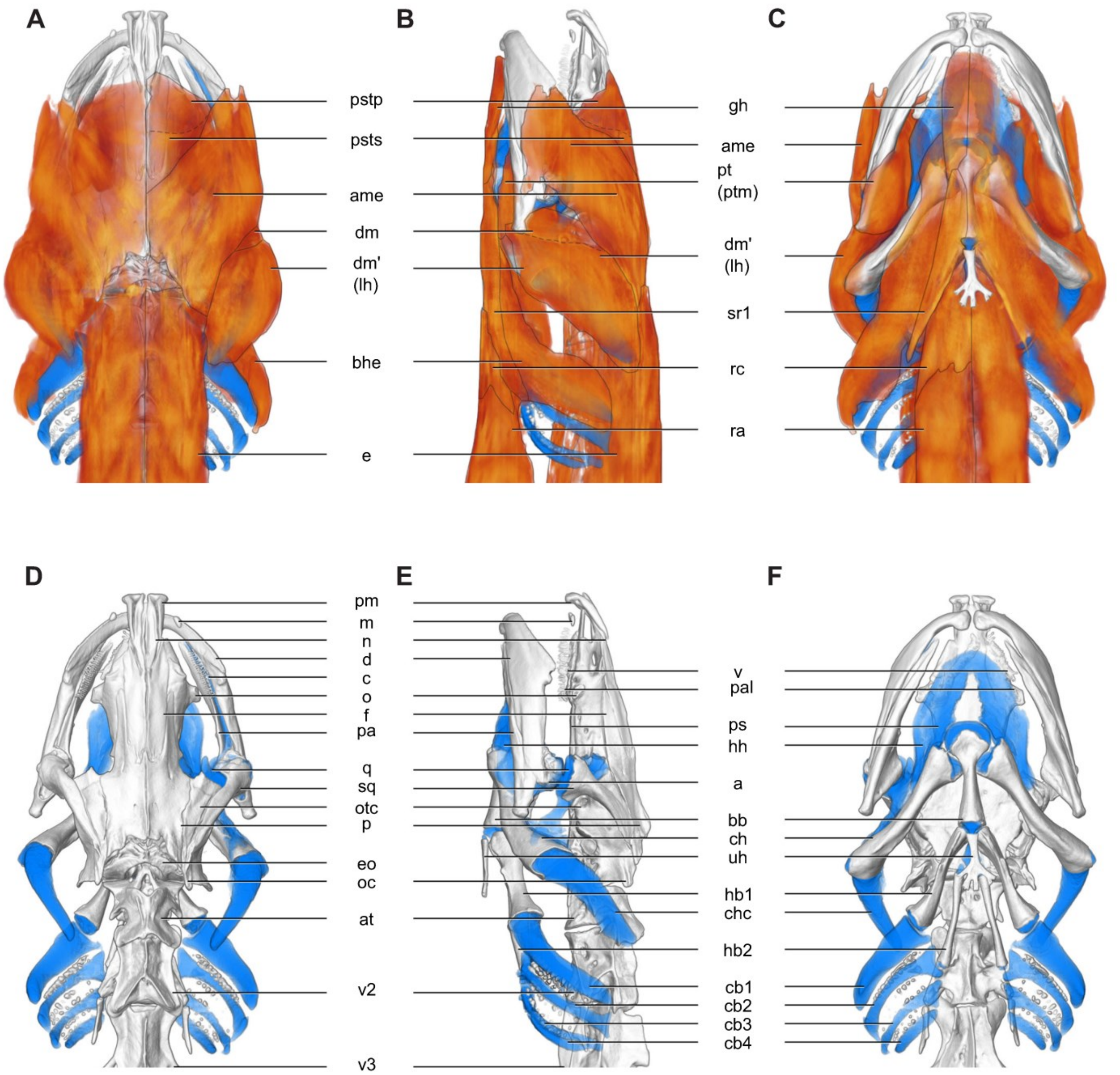

Fig. 4. Head anatomy of *S. intermedia* based on dual-energy micro-computed tomography (µCT) scans. Bone reconstruction is based on a hydroxyapatite fraction; cartilage reconstruction is based on a water fraction; and muscles were reconstructed using an iodine fraction. (A–C) Muscles (outlined to clarify their location): ame, adductor mandibulae externus (complex); bhe, branchiohyoideus externus; dm, depressor mandibulae (anterior); dm′, depressor mandibulae posterior (or levator hyoideus, lh); e, epaxialis; gh, geniohyoideus; pstp, pseudotemporalis profundus; psts, pseudotemporalis superficialis; pt, pterygoideus (also referred to as pterygomandibularis, ptm); ra, rectus abdominis; rc, rectus cervici; and sr1, subarcualis rectus 1. (D–F) Bones and cartilages: a, articular; at, atlas; bb, basibranchial; c, coronoid; cb 1–4, ceratobranchial 1–4; ch, ceratohyal; chc, ceratohyal cartilage; d, dental; eo, exoccipital; f, frontal; hb1–2, hypobranchial 1–2; hh, hypohyal; m, maxilla; n, nasal; o, orbitosphenoid; oc, occipital condyle; otc, otic capsules; p, parietal; pa, prearticular; pm, premaxilla; ps, parasphenoid; pal, palatine; q, quadrate; sq, squamosal; uh, urohyal; v, vomer; v2, second vertebra; v3, third vertebra.

cyclic and rhythmic movements of the mandible, skull and hyobranchial apparatus (tongue) to process its food. The hyobranchial apparatus drives the food dorsally (compare Fig. 3D and F) after which the mandible translates the food longitudinally (compare Fig. 3A and C) and processing occurs as prey is rasped between the teeth of the lower jaw (i.e. mandible) and the functional upper jaw (i.e. palatal dentition) (compare Fig. 2C and D) (see Movies 3 and 4 in figshare: https://doi.org/10.6084/m9.figshare.11881110.v1). Thus, food processing in *S. intermedia* qualifies as a form of chewing, which per definition, involves food processing by movements of the mandibular jaws (Reilly et al., 2001). The few processing mechanisms in other salamanders known so far differ considerably from chewing in *S. intermedia*. For example, plethodontids deploy cyclic flexion/extension at the neck, resulting in head pitching movements, and the power of this movement is amplified and transmitted to the mandible via the

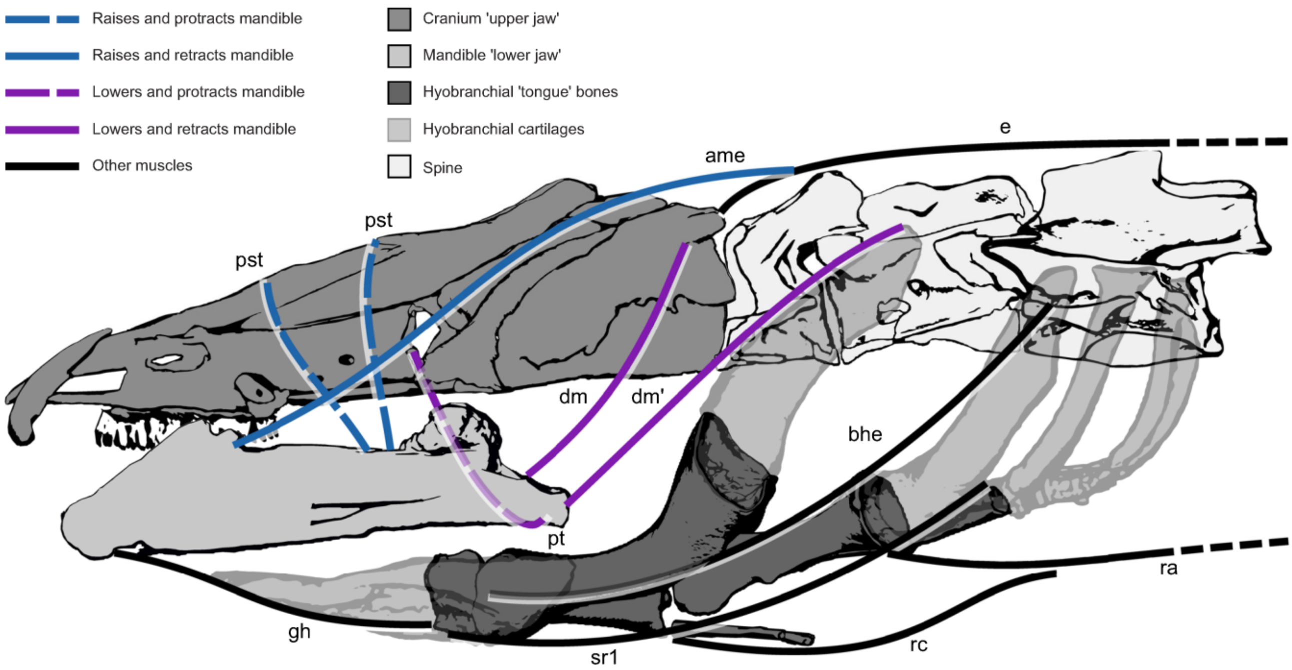

Fig. 5. Schematic diagram of the functional key components of the cranial musculoskeletal system in *S. intermedia*. Muscles: ame, adductor mandibulae externus; bhe branchiohyoideus externus; dm, depressor mandibulae; dm′, depressor mandibulae posterior (or levator hyoideus, lh); e, epaxialis; gh, geniohyoideus; pstp, pseudotemporalis profundus; psts, pseudotemporalis superficialis; pt, pterygoideus (also referred to as pterygomandibularis, ptm); rc, rectus cervicis; ra, rectus abdominis; and sr1, subarcualis rectus 1. Dashed and opaque lines indicate muscle extending further than drawn, transparent lines (or parts) indicate muscle parts hidden from skeletal elements.

atlanto-mandibular ligament in order to produce a series of strong bites onto the food (Deban and Richardson, 2017). Salamandrids use cyclic head pitching movements in combination with repetitive loop motions of the tongue to rasp the food across its palatal dentition (Heiss et al., 2019). In contrast, the chewing mechanism in *S. intermedia* is a remarkably complex 3D procedure that in addition to the rhythmic skull and hyobranchial movements involves three cyclical components of mandibular motion in the (i) median, (ii) horizontal and (iii) transverse planes. Hence, our results are at odds with the commonly accepted view that 3D mandible movements during chewing are exclusive to mammals (Reilly et al., 2001; Ungar and Sues, 2019).

Chewing is widespread across tetrapods in general (Gintof et al., 2010; Konow et al., 2011; Ross et al., 2007), but it has long been assumed that non-amniote tetrapods and many sauropsids – if they chew at all – mostly use ‘simple’ vertical mandible movements because their hinge-like jaw joints only permit dorso-ventral rotation of the lower jaw (Olson, 1961; Reilly et al., 2001; Ungar and Sues, 2019). Such vertical jaw movements are thought to be the ancestral tetrapod condition (Hotton et al., 1997; Olson, 1961) and have been assumed to be retained among lissamphibians (Olson, 1961). However, chewing movements can exhibit many degrees of freedom. For example, propalinal chewing movements have evolved independently several times in tetrapods (Reilly et al., 2001), and have been reported in mammals (Hiiemäe and Crompton, 1985; Offermans and de Vree, 1990; Weijs, 1975), turtles (Bramble, 1974; Schumacher, 1973) and some lepidosaurs (Gorniak et al., 1982; Throckmorton, 1976). Chewing in turtles is initiated with mandibular protraction and simultaneous jaw opening before jaw closure and mandible retraction during the power stroke (Bramble, 1974). Chewing in lepidosaurs differs between herbivorous and carnivorous species: the herbivorous *Uromastyx* uses a mechanism similar to that of turtles (Throckmorton, 1976), whereas the carnivorous *Sphenodon* retracts its mandible during jaw opening and protracts its mandible during jaw closure during the power stroke (Gorniak et al., 1982). Among mammals, propalinal chewing movements are especially pronounced among rodents (Byrd, 1981; Cox et al., 2012; Offermans and de Vree, 1989), whose jaw is opened as the mandible is retracted, and the power stroke results from mandibular protraction, accompanied by jaw closing (De Vree and Gans, 1994; Offermans and de Vree, 1990; Weijs, 1975).

In contrast to vertical and propalinal chewing movements, jaw displacement along the transverse axis has exclusively been reported for mammals (Bhullar et al., 2019; Grossnickle, 2017; Hiiemäe and Crompton, 1985) – but the present study suggests that at least one lissamphibian also performs transverse jaw movements during food processing. Transverse chewing movements require a flexible jaw joint and can be achieved either by the hemimandibles moving relative to one another, resulting in a change in the angle they subtend (i.e. wishboning: Hylander, 1985; Bhullar et al., 2019; Weijs, 1975), or by moving the mandible or hemimandible transversely, relative to a stationary upper jaw (transverse mandible displacement: Crompton et al., 2010). In mammals, transverse mandible displacement is characterized by a wide jaw gape followed by a lateral translation of the mandible towards and beyond the maxillary tooth row of the working side, as the jaw is closed (Crompton et al., 2010; Hiiemäe and Crompton, 1985). The main vector of the power stroke is, therefore, lateral and ends as the mandibles begin to re-separate (De Vree and Gans, 1976; Weijs and Dantuma, 1981). It has also been suggested that wishboning accompanies the propalinal mandible movements in rodents (Hiiemäe and Ardran, 1968). As shown in Figs 2 and 3, *S. intermedia* retracts and abducts its hemimandibles during jaw closure in the preparatory phase, and protracts and adducts its

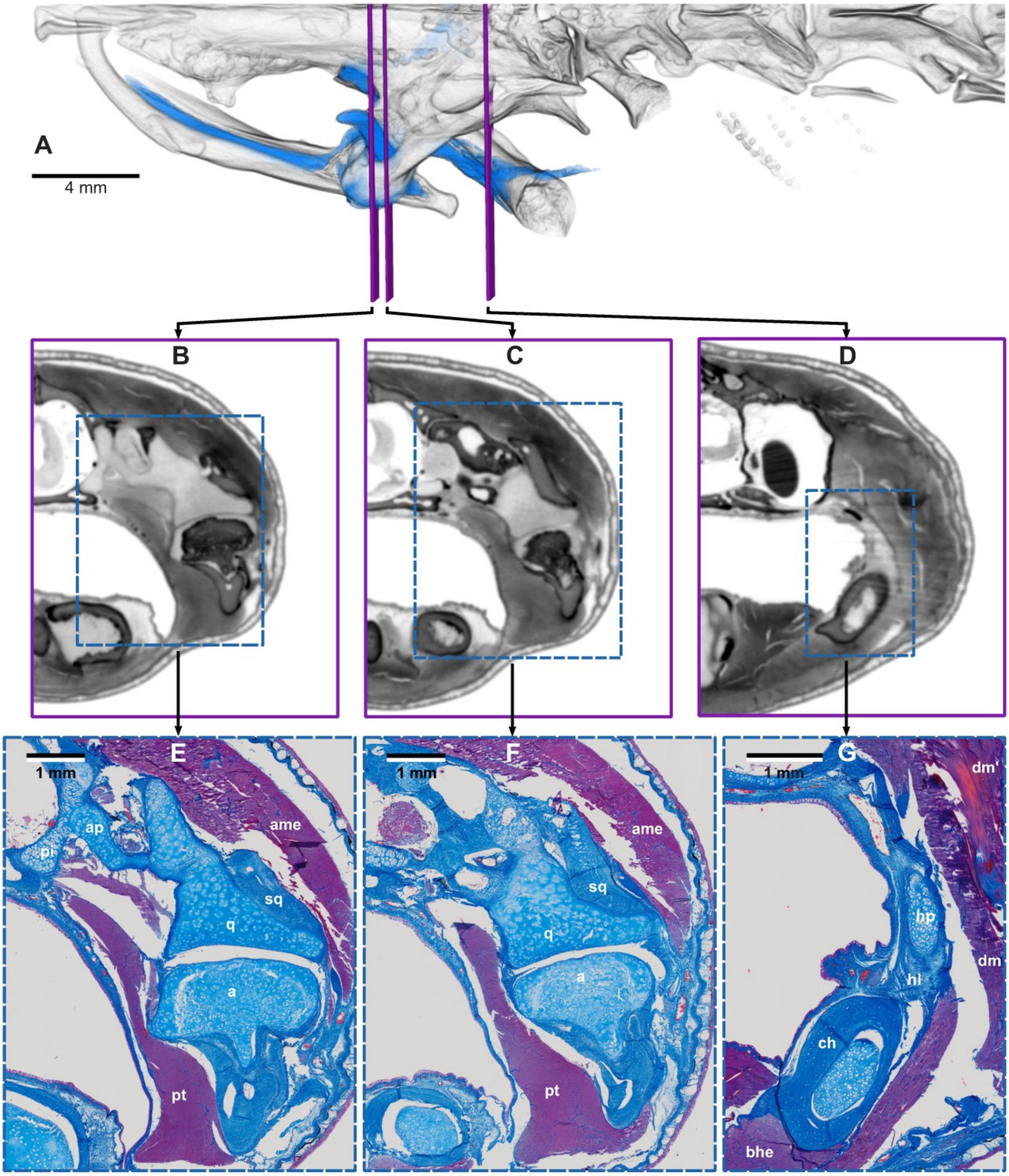

Fig. 6. Cross-sections from different areas of the jaw joint complex. (A) µCT of the skull of *S. intermedia* in grey, with the jaw joint complex in blue. (B–D) µCT cross-section planes illustrating the position of histological cross-sections (E–G). a, articular; ame, adductor mandibulae externus; ap, ascending process; bhe, branchiohyoideus externus; ch, ceratohyal; dm, depressor mandibulae (anterior); dm′, depressor mandibulae posterior; hl, hyoquadrate ligament; hp, hyoquadrate process; pt, pterygoideus; pi, pila antotica; q, quadrate; and sq, squamosal. Muscles are purple while bones and cartilages are blue in histological sections (E–G).

hemimandibles (i.e. wishboning) during jaw opening in the power stroke phase. Consequently, if we compare chewing kinematics of *S. intermedia* with that of turtles, lepidosaurs, rodents and other mammals, most similarities are seen between chewing in *S. intermedia* and rodents. Both taxa use a power stroke that results from mandible protraction and exhibits transverse mandible movements. However, transverse mandible movements in *S. intermedia* are of the wishboning type and probably result from the propalinal mandible movement in combination with the mandible morphology (i.e. loose symphysis and jaw joint) rather than being functionally necessary for food processing. By contrast, rodent chewing is likely to consist of wishboning as well as transverse mandible displacement as functionally important parts. Additionally, after maximal gape closure, *S. intermedia* opens its mouth during most of the anteriorly directed power stroke, probably attributed to the orientation of the mandibular tooth row that would collide with the teeth of the upper jaw during simple mandible protraction (Fig. 2C,D). The pterygoideus muscle that is likely to power the anteriorly directed mandible movement in *S. intermedia* also acts as a jaw opener (Fig. 5). In sum, the characteristics of the chewing mechanism in *S. intermedia* deviate strongly from those in other known tetrapod chewing systems, presumably reflecting the specialized morphology of the paedomorphic sirenid salamanders.

As noted above, mammals are generally considered to be the only tetrapod group that incorporates mandibular movements in the median, horizontal and transverse planes during chewing (Hiiemäe and Crompton, 1985). These complex chewing movements have been argued to require a specialized jaw joint anatomy, which was suggested to be exclusive to mammals (Bhullar et al., 2019; Crompton and Hylander, 1986; Grossnickle, 2017; Herring, 1993; Turnbull, 1970). However, here we show that the salamander *S. intermedia* uses 3D chewing movements during food processing, resulting in a complex chewing pattern that challenges the commonly accepted view that amphibians only use their jaws for 'scissor-like' vertical movements (Olson, 1961; Reilly et al., 2001; Ungar and Sues, 2019). Complex 3D mandible movements demand either (i) a loose jaw joint that allows vertical, propalinal and lateral mandible movements with or without jaw joint disarticulation or (ii) a movable mandibular suspension (i.e. some form of cranial kinesis). Horizontal excursions at the jaw joint have so far not been described in lissamphibians, whereas cranial kinesis (Frazzetta, 1962; Fürbringer, 1900; Iordanski, 1966) has been suggested to be present in some salamanders (Iordansky, 1989; Natchev et al., 2016). It has also been suggested that some salamanders have a cranial architecture that might permit transverse movement of the jaw suspension during suction feeding and respiration (Carroll, 2007; Lauder and Shaffer, 1985). However, as the thin bony suspension of the mandible (i.e. squamosal) in *S. intermedia* was not always visible in our X-ray recordings, we used cadaver manipulations to test for cranial kinesis. Because the mandibular suspension and skull seem tightly connected and hardly movable against one another, we conclude that cranial kinesis is unlikely to explain the complex jaw movements in *S. intermedia* on its own. Rather, it appears that the complex jaw joint in *S. intermedia* permits extensive antero-posterior and lateral sliding of the articular along the quadrate, with the joint capsules stabilizing those flexible movements.

The complex jaw movements in *S. intermedia* appear to be feasible mainly because of its peculiar jaw joint morphology that integrates an anterior ball-and-socket joint and a posterior plane joint (Fig. 6E,F). This 'ball-socket-plane joint' allows vertical (pitch), propalinal (surge) and transverse (sway and yaw) movements, for a total of four degrees of freedom. This stands in remarkable contrast to the proposed hinge-like joint movements in lissamphibians, which only permit vertical jaw movements (one degree of freedom) (Olson, 1961). The high degree of mandibular mobility also appears to result from a derived ligament arrangement. The hyomandibular ligament, which connects the hyobranchial system and mandible in most actinopterygians and metamorphic salamanders, does not connect to the mandible in *S. intermedia* and is instead rerouted to the cartilaginous quadrate (part of the mandible suspension) during early ontogeny (Reilly and Altig, 2006) and acts as a hyosuspensory ligament (Drüner, 1902; Huxley, 1874). Thus, the mandible can move relatively independently of the hyobranchial system. Propalinal jaw movements also require a specialized muscle–tendon morphology with muscles extending anteriorly and posteriorly from the mandible to the skull. Indeed, mandibular retraction in *S. intermedia* appears to result from contraction of the external mandible adductors (ame complex) while the mandible depressors (dm and dm′) may support retraction to a certain extent. Similarly, mandibular protraction appears to result from contraction of the internal mandible adductors (ami complex) consisting of pseudotemporalis profundus (pstp), pseudotemporalis superficialis (psts) and the pterygoideus muscle (pt).

There is little doubt that cranial systems allowing propalinal jaw movements evolved from cranial systems with vertical jaw movements (Olson, 1961; Reilly et al., 2001). It had been suggested that the main factor allowing systems with propalinal jaw movements to form from systems with vertical jaw movements is the appearance of a jaw joint that allows the lower jaw to slide anteriorly from the hinge socket (Reilly et al., 2001). This theory might in fact be supported by the chewing apparatus in *S. intermedia*, where a loose and highly movable jaw joint enables propalinal chewing (Figs 2, 3 and 5). Aside from propalinal jaw movements, the loose jaw joint in *S. intermedia* also allows the lower jaw to slide laterally relative to the quadrate (wishboning of the lower jaw, see Fig. 2), resulting in extraordinary complex 3D chewing movements for lissamphibians (i.e. vertical, longitudinal and transverse). Still, given that the development of *S. intermedia* gets arrested early in its ontogeny (Noble and Marshall, 1932; Reiss, 2002; Rose and Reiss, 1993), and *S. intermedia* thereby shows a cranial morphology typical for most early salamander larvae, it might well be that such complex chewing motions are a common feature for early salamander larvae and that the ability to perform complex jaw movements is lost during ontogeny. Kinematic (and especially fluoroscopic) studies on early staged salamander larvae are technically challenging because of the small size of these creatures but macro-high-speed recordings could enable testing of this hypothesis.

In conclusion, we have revealed a previously unknown and peculiar chewing mechanism in *S. intermedia* and have shown that complex mandible movements during chewing are not exclusive to amniotes. We argue that as the development of *S. intermedia* is arrested in its early ontogeny, many anatomical characteristics connected with feeding resemble those of early salamander larvae (Heatwole and Rose, 2003). Given the generalized morphology of salamander larvae (Heatwole and Rose, 2003; Reilly, 1986; Rose and Reiss, 1993), it is possible that chewing movements similar to those seen in *S. intermedia* could be a generalized feature in salamanders with an early larval morphology.

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Competing interests
The authors declare no competing or financial interests.

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Data availability

Data are available from the figshare digital repository:
https://doi.org/10.6084/m9.figshare.11881110.v1

Supplementary information

Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.220749.supplemental

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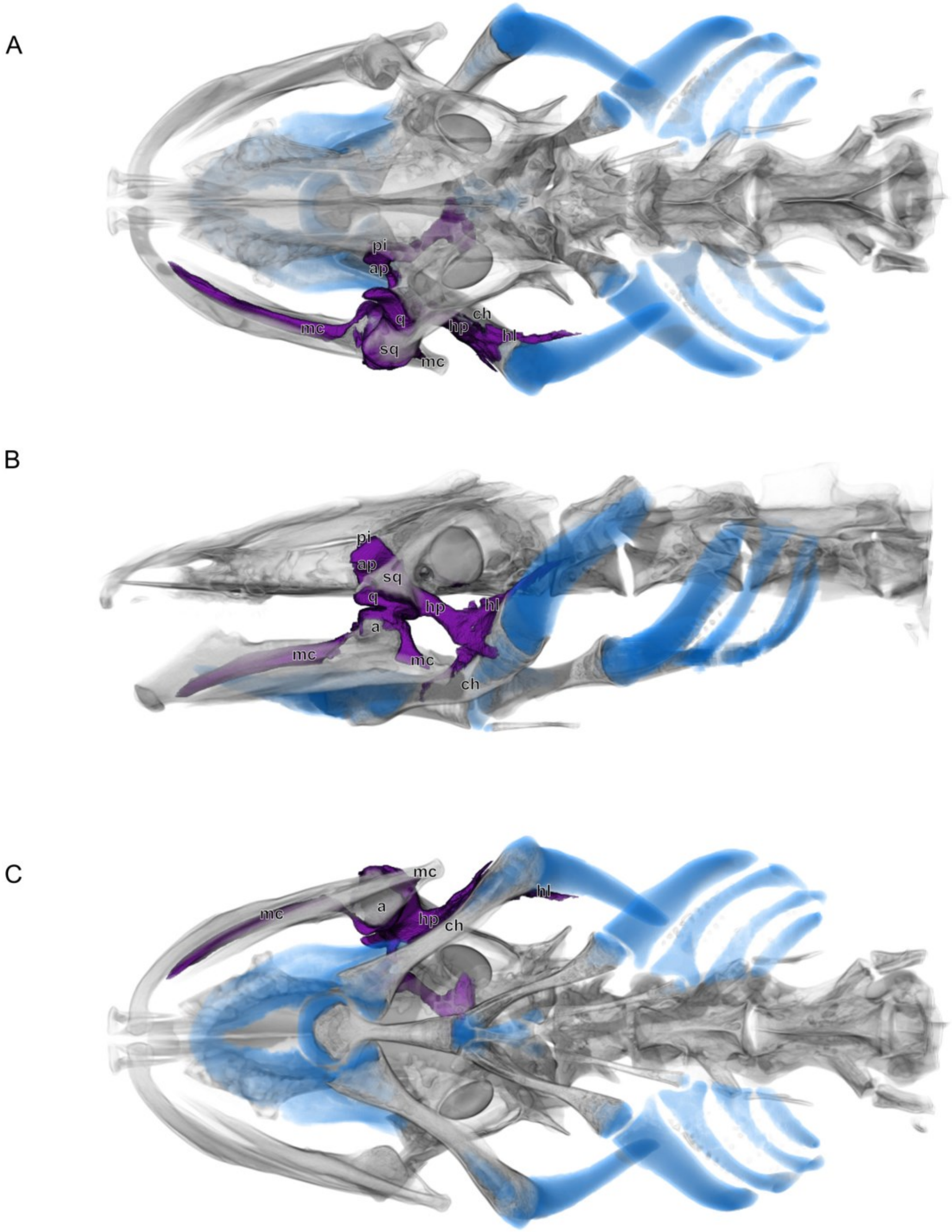

Fig. S1. Morphology of the jaw-joint-complex. A, dorsal; B, lateral and C, ventral views. Bony tissue is highlighted in grey, cartilages in blue, and the jaw-joint-complex in purple. Components of the jaw joint complex: a, articular; ap, ascending process; ch, ceratohyal; hl, hyoquadrate ligament; hp, hyoquadrate process; mc, Meckel's cartilage; pi, pila antotica; q, quadrate and sq, squamosal.

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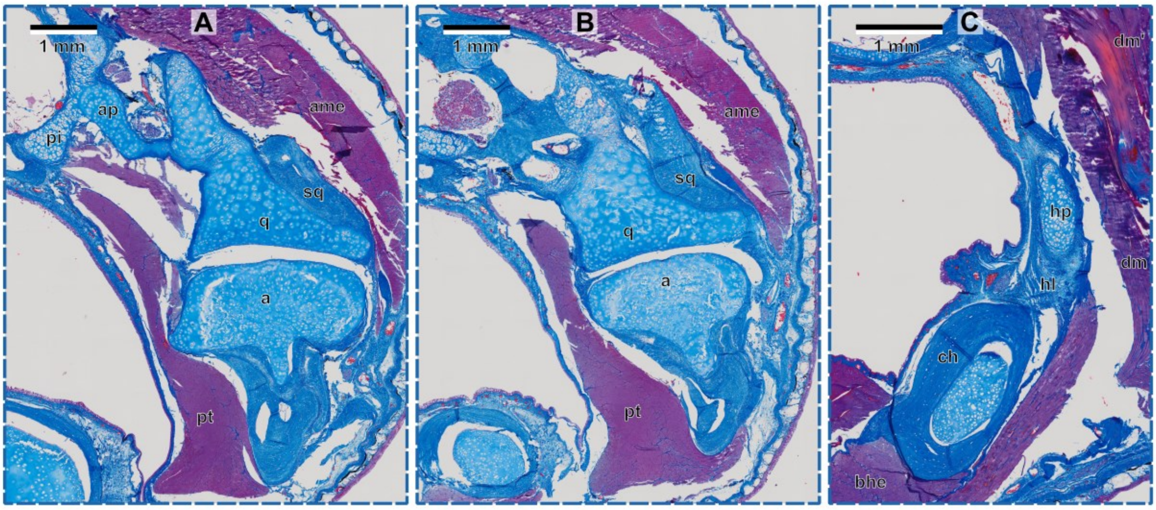

Fig. S2. Cross sections from different areas of the jaw joint-complex. Abbreviations: a, articular; ame, adductor mandibulae externus; ap, ascending process; bhe, branchiohyoideus externus; ch, ceratohyal; dm, depressor mandibulae (anterior); dm', depressor mandibulae posterior; hl, hyoquadrate ligament; hp, hyoquadrate process; pt, pterygoideus; pi, pila antotica; q, quadrate and sq, squamosal. Muscles are purple while bones and cartilages are blue in histological sections.

Table S1. Kinematic variables of chewing in *S. intermedia* with descriptive statistics

Component motion	Variable	Description	Value
Propalinal mandible movement	Mandible retraction	Distance from start to maximal retracted position of the mandible	9.77 ± 3.53 % CL
	Mandible protraction	Distance from maximal retracted to maximal protracted position of the mandible	11.72 ± 3.49 % CL
	Duration mandible retraction	Time from start retraction until maximal retraction of the mandible	146 ± 59 ms
	Duration mandible protraction	Time from start protraction until maximal protraction of the mandible	178 ± 41 ms
Vertical mandible movement	Gape closing	Distance from start of closure to maximal gape closure	7.74 ± 3.59 % CL
	Gape opening	Distance from closed gape to maximal gape opening	6.82 ± 2.88 % CL
	Duration gape closing	Time from start gape closure until maximal closure	99 ± 49 ms
	Duration gape opening	Time from gape closure until maximal gape opening	141 ± 57 ms
Longitudinal hyobranchial movement	Hyobranchial protraction	Distance from start to maximal protracted position of the hyobranchium	2.89 ± 1.57 % CL
	Hyobranchial retraction	Distance from maximal protracted position to maximal retracted position of the hyobranchium	3.12 ± 2.05 % CL
	Duration hyobranchial protraction	Time from start protraction until maximal protraction of the hyobranchium	95 ± 55 ms
	Duration hyobranchial retraction	Time from maximal protraction until maximal retraction of the hyobranchium	112 ± 57 ms
Vertical hyobranchial movement	Hyobranchial depression	Distance from start to maximal depressed position of the hyobranchium	11.79 ± 5.71 % CL
	Hyobranchial elevation	Distance from maximal depressed position to maximal elevation position of the hyobranchium	15.38 ± 5.36 % CL
	Duration hyobranchial depression	Time from start depression until maximal depression of the hyobranchium	129 ± 62 ms
	Duration hyobranchial elevation	Time from maximal depression until maximal elevation of the hyobranchium	114 ± 103 ms
Vertical cranial movement	Neck flexion (cranial 'ventroflexion')	Angular displacement from start to maximum neck flexion relative to the trunk	7.59 ± 3.16 °
	Neck extension (cranial 'dorsoflexion')	Angular displacement from maximum neck flexion to maximum neck extension relative to the trunk	7.10 ± 3.37 °
	Duration neck flexion	Time from start flexion until maximal flexion of the neck	99 ± 42 ms
	Duration neck extension	Time from maximal flexion until maximal extension of the neck	128 ± 67 ms
Transverse mandible movement	Medio-lateral mandible abduction	Angular displacement from start to maximum medio-lateral abduction of the mandible	4.22 ± 1.70 °
	Medio-lateral mandible adduction	Angular displacement from maximum medio-lat. abduction to maximum medio-lat. adduction of the mandible	4.95 ± 2.05 °
	Duration medio-lateral mandible abduction	Time from start medio-lateral abduction until maximal medio-lateral abduction of the neck	156 ± 60 ms
	Duration medio-lateral mandible adduction	Time from maximal medio-lateral abduction until maximal medio-lateral adduction of the neck	146 ± 53 ms

Data are means ± SD. Distances normalized by cranial length (% CL).

Chapter III

Ontogenetic plasticity in cranial morphology is associated with a change in the food processing behavior in Alpine newts

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Estimated own contribution: **85%**.

Conceptualization: **D.S.**, E.H.; Methodology: **D.S.**, E.H.; Formal analysis: **D.S.**; Investigation: **D.S.**, E.H.; Resources: E.H.; Data curation: **D.S.**; Writing - original draft: **D.S.**, E.H., L.B.P., N.K.; Writing - review & editing: **D.S.**, E.H., L.B.P., N.K.; Visualization: **D.S.**; Supervision: **D.S.**, E.H.; Project administration: **D.S.**; Funding acquisition: E.H.

See (CRediT taxonomy; supplementary material b) for details.

Precis:

Chapter III contains detailed descriptions of the form and function of the feeding apparatus of distinct morphotypes in the Alpine newt (Salamandroidea, see Fig. S2; supplementary material d). It is shown that paedomorphic salamandrids use mandible-palate clenching (i.e., mandible-palate interaction), a form of chewing in which the mandible bites the food repeatedly against the palate with the teeth of the lower jaw impinging between the two rows of teeth of the upper jaw. Further, it is shown that the food processing mechanism switches to tongue-based processing as seen in *Triturus carnifex* (tongue-palate rasping) along with the morphological changes that occur during development. Form and function of the feeding apparatus of the ontogenetic morphotypes are compared with those of other salamanders, suggesting that the switch from mandible-based to tongue-based intraoral food processing is typical for salamanders undergoing metamorphosis. Given the differences of the chewing mechanisms as well as the form of the feeding apparatus of the lesser siren and the Alpine newt, it is also suggested that complex 3D mandible-palate interactions as seen in the lesser siren might be common amongst salamanders with a very early larval morphology – while later larval morphotypes likely change to vertical chewing.

These results of this chapter support hypotheses 1, 2 and 3.

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Ontogenetic plasticity in cranial morphology is associated with a change in the food processing behavior in Alpine newts

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Abstract

Background: The feeding apparatus of salamanders consists mainly of the cranium, mandible, teeth, hyobranchial apparatus and the muscles of the cranial region. The morphology of the feeding apparatus in turn determines the boundary conditions for possible food processing (i.e., intraoral mechanical reduction) mechanisms. However, the morphology of the feeding apparatus changes substantially during metamorphosis, prompting the hypothesis that larvae might use a different food processing mechanism than post-metamorphic adults. Salamandrid newts with facultative metamorphosis are suitable for testing this hypothesis as adults with divergent feeding apparatus morphologies often coexist in the same population, share similar body sizes, and feed on overlapping prey spectra.

Methods: We use high-speed videography to quantify the in vivo movements of key anatomical elements during food processing in paedomorphic and metamorphic Alpine newts (*Ichthyosaura alpestris*). Additionally, we use micro-computed tomography (μCT) to analyze morphological differences in the feeding apparatus of paedomorphic and metamorphic Alpine newts and sort them into late-larval, mid-metamorphic and post-metamorphic morphotypes.

Results: Late-larval, mid-metamorphic and post-metamorphic individuals exhibited clear morphological differences in their feeding apparatus. Regardless of the paedomorphic state being externally evident, paedomorphic specimens can conceal different morphotypes (i.e., late-larval and mid-metamorphic morphotypes). Though feeding on the same prey under the same (aquatic) condition, food processing kinematics differed between late-larval, mid-metamorphic and post-metamorphic morphotypes.

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Conclusions: The food processing mechanism in the Alpine newt changes along with morphology of the feeding apparatus during ontogeny, from a mandible-based to a tongue-based processing mechanism as the changing morphology of the mandible prevents chewing and the tongue allows enhanced protraction. These results could indicate that early tetrapods, in analogy to salamanders, may have developed new feeding mechanisms in their aquatic environment and that these functional innovations may have later paved the way for terrestrial feeding mechanisms.

Keywords: Salamander, Kinematics, Micro-CT, Functional morphology, Feeding apparatus, Ontogeny, Chewing, Intraoral food processing, Feeding, Flexibility

Background

Most salamanders switch from a feeding larval- to a post-metamorphic stage during ontogeny via metamorphosis [1–3]. A recent study suggests that in species undergoing metamorphosis, parts of the skull associated with feeding develop faster and more independently from the rest [4]. This in turn suggests that the form and function of skeletal elements associated with feeding may be more flexible. From an ontogenetic perspective, this flexibility is particularly useful for metamorphic species that change their food spectrum over the course of their lives due to the transition from aquatic to terrestrial environments. However, some salamanders do not undergo metamorphosis but instead attain sexual maturity while retaining larval traits [5, 6]. This somatic developmental arrest is referred to as paedomorphosis, and is particularly common among salamanders [7–10]. In some salamander species, individuals can either undergo or skip metamorphosis (i.e., facultative paedomorphosis) [8, 11], resulting in paedomorphic and metamorphosed adults co-populating similar niches of a habitat whilst differing in morphology (i.e., heterochronic morphotypes). Prior studies have hypothesized that, due to their different morphologies, heterochronic morphotypes differ in their feeding performance (capture success-rate) and feeding behavior [12–14]. Behavioral studies have shown that paedomorphs tend to have greater aquatic prey capture performance [12, 14], but surprisingly, despite diverging prey capture performance and major differences in head morphology, there are only minor differences in prey capture kinematics between heterochronic morphotypes [13–16].

Prey capture is followed by intraoral behaviors, which can include distinct processing and transport cycles. Similar to prey capture kinematics, transport kinematics do not seem to differ significantly between larval and post-metamorphic morphotypes [17–20]. However, it is unclear whether intraoral processing kinematics follow the same pattern as capture and transport. First, although recent evidence suggests that intraoral food processing is more common in salamanders than previously thought [21–23], processing remains little studied in salamanders compared to other taxa [24, 25]. Second, processing might be affected more from differing feeding apparatus morphologies than capture and transport. This latter point becomes more evident if we consider changes in the structure, position and number of the teeth [26–29]; structural changes of the hyobranchial apparatus (i.e., developing from a gill-bearing to a tongue-bearing apparatus) [14, 15, 30–32]; changes in the muscular and ligamentous suspension of the hyobranchial apparatus [33–35]; morphological changes of mandible and skull [15, 36–38]; as well as dramatic muscular reorganization [39, 40] during metamorphosis in salamanders. All of the aforementioned characteristics impact intraoral food processing kinematics in salamanders [21, 23].

Food processing in salamanders involves a mix of structural and functional traits seen in fishes and amniotes [21]. Salamanders, being lissamphibians, are especially interesting from an evolutionary point of view because of their phylogenetic position near the base of the tetrapod radiation, with lissamphibians being considered the extant sister-group of amniotes. As a result, salamanders are critical to our understanding of the functional evolution of tetrapods, because they might retain many basal features in the musculoskeletal system [41, 42], including a broad and flat skull [43, 44], and a similarly robust anatomy of the hyobranchial apparatus [45]. The lissamphibian metamorphosis enables the experimental investigation of developmental water-land transitions in recent tetrapods [41] – as an analogy to the evolutionary water-land transitions of early tetrapods.

Accordingly, our objectives in the present study are: a) to compare the intraoral food processing kinematics and feeding apparatus morphologies of the heterochronic morphotypes of the Alpine newt (*Ichthyosaura alpestris*) and b) to propose a possible evolutionary scenario of the prey-processing behavior in early tetrapods. We quantify how changes in form of the feeding apparatus can induce shifts in feeding kinematics. We hypothesize that while prey capture and transport kinematics are similar between paedomorphic and post-metamorphic Alpine newts, intraoral processing kinematics will differ between heterochronic morphotypes.

Results

Functional morphology of the feeding apparatus

Detailed descriptions of the cranial anatomy of *Ichthyosaura alpestris* and other salamandrids can be found elsewhere [26, 32, 46–52] and we focus on structures relevant for processing and on specific differences between morphotypes.

Cranial osteology

The feeding apparatus of the Alpine newt consists of an osseous skull and mandible, and a complex, partially cartilaginous hyobranchial system (i.e., hyobranchial in larval or hyolingual in metamorphosed salamanders, respectively) (see Fig. 1) and prominent muscles (Fig. 2). We group the paedomorphic (p) and metamorphic (m)

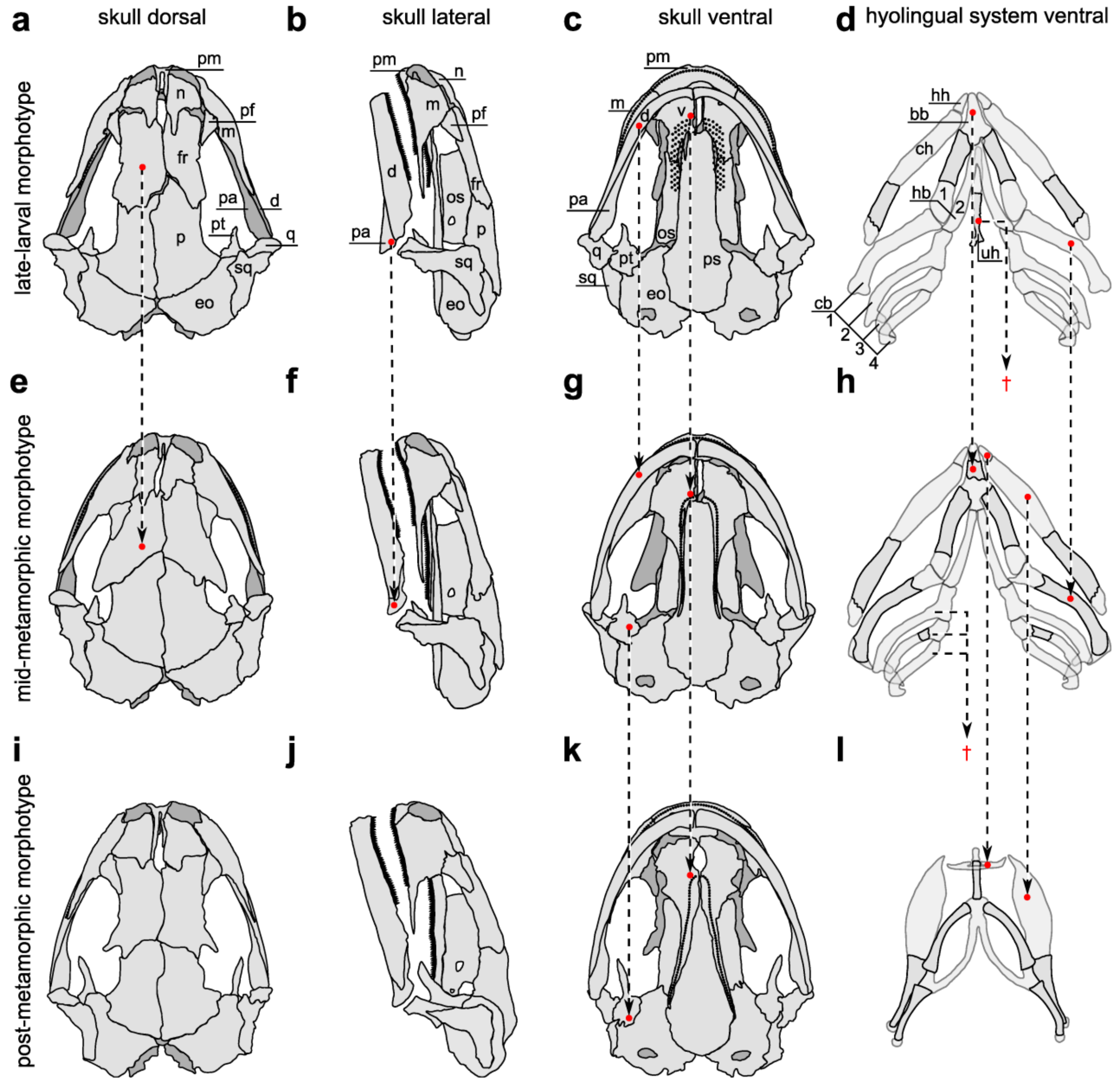

Fig. 1 Skeletal morphology of the feeding apparatus of different morphotypes in *I. alpestris*. **a**-**d** (row 1) late-larval morphotype (LLM), **e**-**h** (row 2) mid-metamorphic morphotype (MMM), **i**-**l** (row 3) post-metamorphic morphotype (PMM). Abbreviations: (bb) basibranchial, (cb 1–4) ceratobranchial 1–4, (ch) ceratohyal, (d) dentary, (eo) exoccipital, (fr) frontal, (hh) hypohyal (also referred to as radial), (hb 1–2) hypobranchial 1–2, (m) maxilla, (n) nasal, (os) orbitosphenoid, (p) parietal, (pa) prearticular, (pf) prefrontal, (pm) premaxilla, (ps) parasphenoid, (pt) pterygoid, (q) quadrate, (sq) squamosal, (uh) urohyal, (v) vomer. Arrows connecting different morphotypes (rows) highlight significant structural differences. Arrows ending in the space between morphotypes marked with † indicate the reduction of the structure

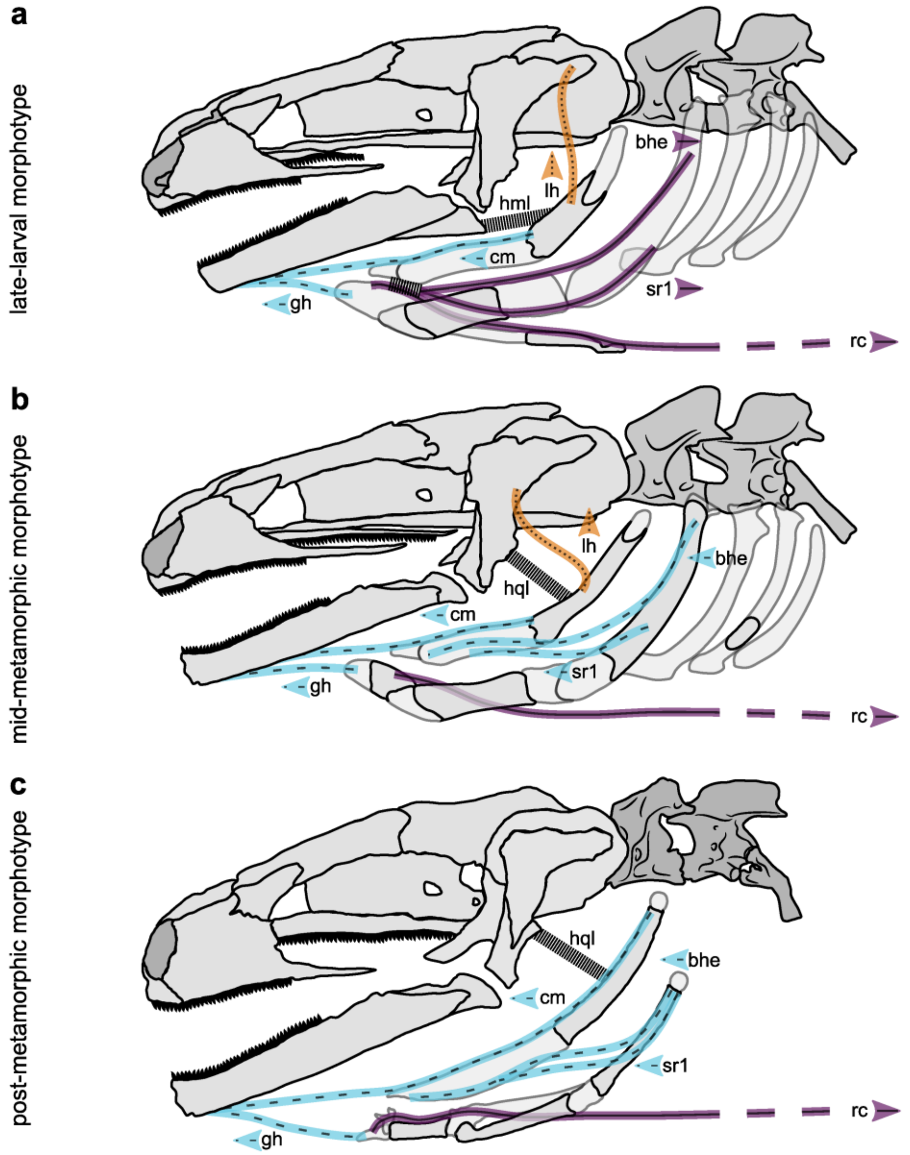

Fig. 2 Muscular morphology of the feeding apparatus of three different morphotypes of *I. alpestris*: (**a**) the late-larval morphotype (LLM), (**b**) the mid-metamorphic morphotype (MMM), (**c**) the post-metamorphic morphotype (PMM). Muscles: (bhe) branchiohyoideus externus, (cm) ceratomandibularis, (gh) geniohyoid, (lh) levator hyoideus, (rc) rectus cervicis and (sr1) subarcualis rectus 1. Ligaments: (hml) hyomandibular ligament and (hql) hyoquadrate ligament. The directional effect of each muscle on the movement of the tip of the hyobranchial apparatus (i.e., the basibranchial) is encoded by the arrows, protractors (blue and dashed), retractors (purple and solid), and elevators (orange and dotted). Note that there is no direct hyobranchial elevator in the PMM. Depression of the hyobranchial system is achieved by a combination of rectus cervicis activity and the ligamentous and muscular suspension of the hyobranchial skeleton to the skull. Please note that the course of the ligaments was obtained from other morphological descriptions [33, 35, 48] and could not be verified in this study

specimens into three distinct morphotypes: (i) late-larval (p), (ii) mid-metamorphic (p), and (iii) post-metamorphic (m), based on their developmental state. The anterior skull plates of the late-larval morphotype (LLM) are largely unfused while in the mid-metamorphic morphotype (MMM) and the post-metamorphic morphotype (PMM) the enlarged frontal bones fill those gaps. The pterygoids of LLM and MMM are relatively small compared to those of the PMM. All morphotypes carry two functional upper jaw systems: the first consists of the tooth bearing maxilla and premaxilla (i.e., "primary" upper jaw), and the second of the tooth bearing vomerine and palatine bones of the mouth roof (i.e., "secondary" upper jaw or palatal jaw).

The palatal dentition pattern of the LLM is U-shaped and the teeth organized in rows, the mandible is slightly

V-shaped in ventral view and the functional occlusal surface for the lower jaw dentition is the palate between "primary" and "secondary" upper jaws. The mandibles of MMM and PMM are U-shaped in ventral view and the occlusal surface for the lower jaw are the maxillary teeth of the "primary" upper jaw. The palatal dentition of the MMM and the PMM are distinct as the MMM has a U-shaped single row of denticles and the PMM exhibits a V-shaped single row of denticles.

Hyobranchial musculoskeletal anatomy

The hyobranchial apparatus shows the most striking differences between morphotypes. In the LLM, the hyobranchial apparatus is a complex and mainly cartilaginous system with small ossification centers in ceratohyal, hypobranchial and urohyal. The hyobranchial apparatus of MMM shows enlargement of these ossified centers, additional ossification centers in basibranchial, ceratobranchial 1 and 3, as well as the reduction of the urohyal. The hyolingual apparatus of the PMM exhibits a typical morphology for metamorphosed salamandrids. Thus, in the PMM the ceratobranchial 2–4 are reduced and the hypohyals merge to form a buckle around basibranchial (often referred to as the radial).

Our functional descriptions of the hyobranchial apparatus focus on muscles responsible for the main movements of the anterior tip of the hyobranchial system (i.e., the basibranchial). The 3D muscle morphology is considered but the main function of each muscle is assessed from a lateral perspective (i.e., simplified to a 2D movement). More complex inter-hyobranchial movements are likely to occur due to the 3D orientation of the hyobranchial apparatus and its muscles (see for example [53]). The hyobranchial system of all morphotypes forms the attachment site for several major muscles (i.e., six in the LLM and MMM, and five in the PMM). The muscles can be differentiated according to their initial attachment to hyoid arch or branchial arch during ontogeny [54]. The hyoid arch (paired ceratohyals) is connected with the ceratomandibularis (CM) and branchiohyoideus externus (BHE) mucles in all morphotypes, and also with the levator hyoideus (LH) in the LLM and MMM. The CM extends between the ossified area of the ceratohyal and the dentary in all morphotypes, acting as a protractor of the hyobranchial system. The fleshy BHE extends from the lateral side of the postero-dorsal ceratobranchial I to a tendoninous sheet connecting the anterior regions of the hyoid- and branchial arch in the LLM. Because of the ligamentous connection of the ceratobranchial to the mandible (HML), the BHE serves as a retractor of the anterior part of the branchial system while it adduces the anterior tip of the hyobranchial apparatus and the posterior part of ceratobranchial I. In contrast, in the MMM and the PMM, the insertion of the BHE shifted completely to the antero-ventral part of ceratohyal and therefore acts as a protractor of the branchial arch. In the LLM, the LH originates on the dorsal squamosal process and attaches to the upper osseous part of the ceratohyal, while in the MMM the LH originates from the mid-squamosal and attaches to the upper osseous part of ceratohyal. Accordingly, the LH serves as a hyobranchial elevator in LLM and MMM. The LH is missing in the PMM because the LH detaches from the hyobranchial system during development in order to attach to the lower jaw and thus form the depressor mandibulae posterior. Apart from the development of the depressor mandibulae posterior, the cranial muscles for opening and closing the jaw showed no significant differences between the morphotypes.

The branchial arch is connected with geniohyoid (GH), subarcualis rectus 1 (SR1), and rectus cervicis (RC) muscles. The thin GH muscle extends from the basibranchial to the dentary in all morphotypes, thus enabling protraction of the hyobranchial system. A peculiarity in metamorphs is that some fibers of the GH extend from the pericardium to the dentary [47]. In the LLM, the SR1 extends from the antero-ventral side of the cartilaginous ceratobranchial I anteriorly to a tendoninous sheet connecting the anterior regions of the hyoid- and branchial arch. Thus, the SR1 acts similarly as BHE in the LLM, retracting the tip of the hyobranchial system, while in the MMM and the PMM the SR1 extends from the medial part of the ceratobranchial I to the medio-lateral part of the ceratohyal to act as a protractor of the branchial arch. The most prominent muscle of the hyobranchial system in all morphotypes is the RC that originates from the ventral abdominal trunk muscles and inserts onto the basibranchial. Due to its course and the ligament and muscle suspension of the hyobranchial apparatus on the skull (hyomandibular or hyoquadrate ligament and levator hyoideus), the RC facilitates retraction and depression of the hyobranchial apparatus.

Intraoral food processing

After initial ingestion via suction feeding, one or two transport movements were used by all morphs to position prey prior to a consecutive set of processing cycles. The mean total processing cycles were 5.7 ± 3.2 (mean ± S.D.) for the late-larval, 5.6 ± 2.4 for the mid-metamorphic, and 5.9 ± 2.5 for the post-metamorphic morphotypes. A processing cycle was defined from start of gape opening until the next start of gape opening. Processing involved the cyclical opening and closing of the jaw (i.e., arcuate mandible movement), elevation and depression of the hyobranchial apparatus (i.e., the tongue) and, in the post-metamorphic morphotype only, additional rhythmic flexion and extension of the neck (vertical cranial

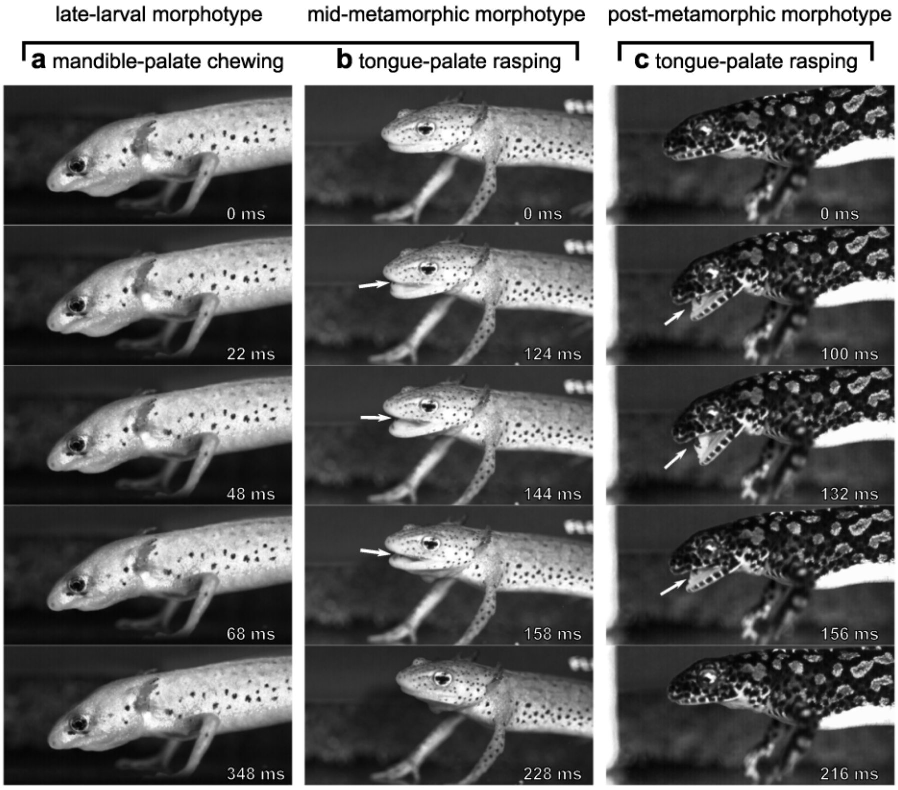

Fig. 3 Exemplary snapshots of food processing in three different morphotypes of *I. alpestris*: **a** mandible-palate chewing in late-larval morphotype (LLM), **b** tongue-palate rasping in mid-metamorphic morphotype (MMM), and **c** tongue-palate rasping in the post-metamorphic morphotype (PMM). Note the similar prey processing patterns of mid-metamorphic morphotype and the post-metamorphic morphotype versus the distinct pattern of late-larval morphotype. The arrows point to the position of the prey item when it is visible from the outside

movement) (Fig. 3c). During these movements, prey debris and haemolymph were occasionally expelled from the oral cavity, indicating that the behavior caused significant prey disintegration. After a processing bout (i.e., a series of processing cycles), water flows induced by hyobranchial movement transported the food backwards, after which it was either repeatedly processed or swallowed.

Kinematics of intraoral food processing

Intraoral food processing cycles were clearly distinguishable from food transport in that hyobranchial elevation accompanied gape opening during processing, whereas during transport hyobranchial depression accompanied gape opening. During processing, at the onset of gape opening, the LLM initiated hyobranchial elevation, which continued past peak gape opening and reached its peak coincident with complete gape closure. Then, in a returning motion, the hyobranchial apparatus was depressed while the mouth remained shut (i.e., stationary phase). The MMM started elevating the hyobranchial apparatus at the onset of gape opening. Both movements peaked approximately at the same time, after which simultaneous gape closing and hyobranchial depression (i.e., resetting movements) occurred (Fig. 4b and h). Neither the LLM nor the MMM had stereotypic cranial movements, as indicated by their relative featureless cranial kinematic profiles (Fig. 4d, e). In the PMM gape and vertical cranial flexion peaks were approximately coincident; thus, gape opening and cranial ventroflexion (or head depression) as well as gape closing and cranial dorsoflexion were aligned (Fig. 4c and f). The vertical hyobranchial movement had a ~ 10% phase shift (i.e., delay) from the gape cycle as hyobranchial elevation started at ~ 90% of the preceding gape cycle (compare Fig. 4c and i).

Table 1 shows the kinematic parameters of food processing in the three morphotypes. The stationary gape phase in the LLM clearly differed from the other two morphotypes (compare Fig. 4a with b and c) as did the cranial flexion of the PMM (compare Fig. 4 f with d and e).

Table 2 shows the statistical analysis of the kinematic parameters of food processing in the three morphotypes. Some significant changes concern the duplication of the vertical hyobranchial magnitude of the PMM compared to the MMM (compare with Fig. 4h and i), the duplication in gape magnitude from the MMM to PMM (compare

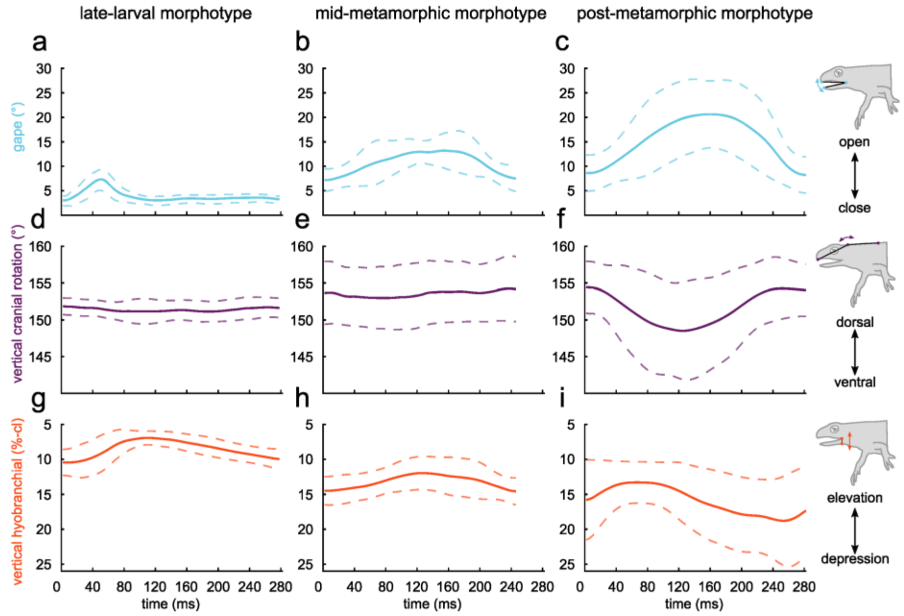

Fig. 4 Profiles of the kinematic variables during food processing in three different morphotypes of *I. alpestris*. Kinematic means (dark and bold profiles) ± SD (slender, dashed and pale curves) of synchronous motions plotted in normalized coordinate systems with group-normalized timescales (x-axes) for comparison. Gape (light blue) (**a**, **b**, **c**) and cranial flexion (purple) (**d**, **e**, **f**) in degree, vertical hyobranchial movement (orange) (**g**, **h**, **i**) normalized to cranial length (i.e., %-cl)

Fig. 4b and c), and the significantly higher mean mandible acceleration from peak gape opening to reaching maximal gape-closing speed in the LLM compared to the MMM. The durations of the gape and vertical hyobranchial movement cycle are the same across all morphotypes.

Ordination analysis of processing kinematics

A principal component analysis (PCA) was performed to analyze how the processing kinematics of the three morphotypes relate to each other and to visualize differences. Distribution of the chewing cycles among the processing modes and morphotypes on the first two principal components axes are shown in Fig. 5, and the loadings of the kinematic parameters on principal component 1 and 2 (i.e., PC1 and PC2) are given in Table 3. Hyobranchial kinematics load more strongly on PC1 while mandible kinematics loaded more strongly on PC2. Processing in PMM and LLM are separated in kinematic space with no overlap, but MMM processing overlaps with PMM while coming close to (i.e., being similar to) LLM processing.

The coefficient of variation (C_V) was calculated for each kinematic parameter (Table 1) in order to quantify the stereotypy of the processing behavior of each morphotype [55]. The stationary gape phase (i.e., parameter 6) was only part of the processing mechanism in the LLM and parameters concerning vertical cranial flexion (8–12) could only be analyzed for the PMM. Consequently, these parameters were excluded for comparison.

Stomach content analysis

Post-metamorphic newts used in stomach content analysis applied suction feeding to ingest lake fly larvae (*Chironomidae*). After ingestion, the newts used cyclic processing movements involving ventral cranial flexion and mouth opening accompanied by hyolingual elevation. Microscopic examinations of the processed lake fly larvae extracted from the stomachs of freshly euthanized newt specimens revealed clear lesions and other structural damage. Lesions were recognized by intensified methylene blue staining, which gradually attenuated along the unharmed part of the prey (Fig. 6b - d). By contrast, unprocessed lake fly larvae (control) only showed blue coloration in the posterior most region (Fig. 6a) and no structural damage. From a total of 100 processed lake fly larvae, 61 exhibited minor to major structural damage (Fig. 6b - c), 18 were ruptured (Fig. 1d) and 21 did not show evidence of damage (Fig. 6a).

Discussion

We found distinct intraoral food processing kinematics (Fig. 5) and feeding apparatus morphologies (Figs. 1 and 2) in the three heterochronic morphotypes of the Alpine newt. Thus, this study shows that externally similar animals can have different internal anatomies, which in turn may result in different behaviors.

It was recently shown that metamorphosed salamandrid newts use loop-like movements of their hyolingual apparatus (i.e., tongue) to translate food across the

Table 1 Kinematic parameters of intraoral food processing of three morphotypes of *I. alpestris*

Kinematic variable	Kinematic parameter	Late-larval morphotype (LLM)		Mid-metamorphic morphotype (MMM)		Post-metamorphic morphotype (PMM)	
		Mean ± S.D.	C_V	Mean ± S.D.	C_V	Mean ± S.D.	C_V
Gape	1 Opening (°)	5.35 ± 1.54	0.29	9.82 ± 4.28	0.44	19.30 ± 5.90	0.24
	2 Closure (°)	5.51 ± 1.21	0.22	9.48 ± 4.68	0.49	19.66 ± 5.86	0.23
	3 Opening duration (s)	0.04 ± 0.02	0.45	0.13 ± 0.06	0.48	0.16 ± 0.06	0.32
	4 Closure duration (s)	0.06 ± 0.02	0.36	0.11 ± 0.04	0.38	0.12 ± 0.06	0.44
	5 Closure acceleration (10^{-3} deg/s^2)	21.40 ± 6.15	0.29	12.25 ± 6.99	0.57	18.75 ± 10.47	0.45
	6 Open-close duration (s)	0.10 ± 0.03	0.25	0.24 ± 0.07	0.27	0.28 ± 0.07	0.21
	7 stationary duration (s)	0.17 ± 0.08	0.45	n/a	n/a	n/a	n/a
	8 Cycle duration (s)	0.28 ± 0.09	0.33	0.24 ± 0.07	0.27	0.28 ± 0.07	0.21
Vertical cranial flexion	9 Ventral (°)	n/a	n/a	n/a	n/a	12.61 ± 6.51	0.39
	10 Dorsal (°)	n/a	n/a	n/a	n/a	12.30 ± 6.46	0.40
	11 Ventral duration (s)	n/a	n/a	n/a	n/a	0.10 ± 0.05	0.41
	12 Dorsal duration (s)	n/a	n/a	n/a	n/a	0.17 ± 0.07	0.33
	13 Cycle duration (s)	n/a	n/a	n/a	n/a	0.28 ± 0.07	0.21
Vertical hyobranchial movement	14 Elevation (%-cl)	4.29 ± 1.99	0.46	4.93 ± 1.47	0.30	10.57 ± 6.18	0.53
	15 Depression (%-cl)	3.87 ± 1.34	0.35	5.43 ± 1.50	0.28	12.46 ± 5.64	0.36
	16 Elevation duration (s)	0.08 ± 0.02	0.24	0.14 ± 0.06	0.45	0.13 ± 0.05	0.34
	17 Depression duration (s)	0.20 ± 0.09	0.44	0.10 ± 0.03	0.35	0.14 ± 0.05	0.31
	18 Cycle duration (s)	0.28 ± 0.10	0.35	0.23 ± 0.07	0.28	0.27 ± 0.07	0.23

Abbreviations: *S.D.* Standard deviation, C_V Coefficient of variation and n/a not applicable. Note that parameters 6 and 8 are identical for both MMM and the PMM. This is because both MMM and PMM lack a stationary phase during processing (parameter 6), so that opening and closing the mouth corresponds to the gape cycle. Note the stereotypy of the magnitude of gape movements (parameter 1 and 2) in the LLM, the flexibility of the gape movements (parameter 1–4) in the MMM, the stereotypy of the hyobranchial movements (parameter 13,14, and 17) in the MMM, the stereotypy of the gape movements (parameter 1,2,3, and 5) in the PMM, and the flexibility of hyobranchial movements (parameter 13–15) in the PMM

Table 2 Statistical analysis of intraoral food processing kinematics in *I. alpestris*

Kinematic variable	Kinematic parameter	All Morphotypes		LLM vs. MMM		LLM vs. PMM		MMM vs. PMM	
		Kruskal-Wallis H	*p*-value	Mann-Whitney U	*p*-value	Mann-Whitney U	*p*-value	Mann-Whitney U	*p*-value
Gape	Opening	78.08	0.00*	−21.42	0.32	−80.09	0.00*	−58.67	0.00*
	Closure	79.09	0.00*	−17.58	0.55	−78.61	0.00*	−61.03	0.00*
	Opening duration	44.82	0.00*	−57.24	0.00*	−73.85	0.00*	−16.61	0.23
	Closure duration	30.18	0.00*	60.29	0.00*	59.85	0.00*	−0.43	1.00
	Closure acceleration	17.15	0.00*	51.24	0.00*	22.12	0.12	−29.12	0.00*
	Open-close duration	48.69	0.00*	−61.43	0.00*	−77.14	0.00*	−15.72	0.28
	Cycle duration	3.56	1.00	n/a	n/a	n/a	n/a	n/a	n/a
Vertical hyobranchial movement	Elevation	32.10	0.00*	−10.04	1.00	−49.48	0.00*	−39.45	0.00*
	Depression	64.27	0.00*	−18.06	0.52	−71.98	0.00*	−53.92	0.00*
	Elevation duration	18.37	0.00*	−49.55	0.00*	−45.79	0.00*	3.76	1.00
	Depression duration	33.78	0.00*	70.21	0.00*	23.89	0.09	−46.32	0.00*
	Cycle duration	5.22	0.88	n/a	n/a	n/a	n/a	n/a	n/a

Statistical analysis was calculated using Kruskal-Wallis 1-way ANOVA and only performed on parameters present in all morphotypes. *P*-values were Bonferroni adjusted to account for multiple testing; significant *p*-values are indicated by asterisks

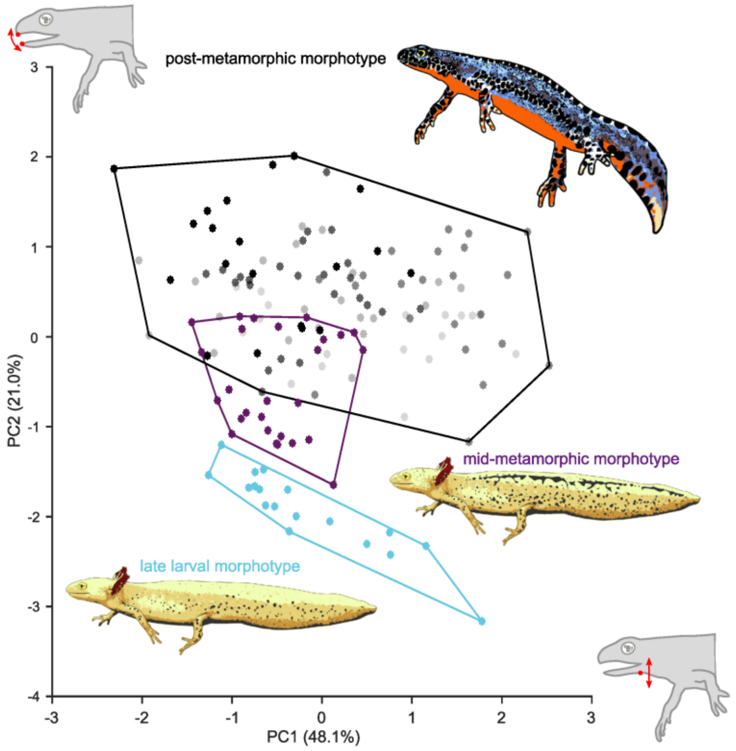

Fig. 5 Scatterplots of the first two principal components. The principal components (PC1 and PC2) derived from 5 out of 12 kinematic parameters and illustrate differences between the three processing modes in kinematic space. Light blue, processing in the late-larval morphotype (LLM); purple, processing in the mid-metamorphic morphotype (MMM); and black, processing in post-metamorphic morphotypes (PMM) (5 shades of grey code the post-metamorphic individuals). The convex hulls display the largest possible area which contains all observations of the respective feeding mode. PC1 explains 48.1% of the total variance and is mostly defined by hyobranchial parameters, while PC2 explains 21.0% of the total variance and is most strongly defined by gape parameters (Table 3). Note that the MMM and PMM show overlap, while LLM and MMM as well as LLM and PMM show no overlap in kinematic space

palatal dentition (i.e., tongue-palate rasping) [21]. It has also been suggested that salamandrids with a larval morphology cannot employ the same processing mechanism as metamorphic animals because of morphological constraints, including the lack of a flexible tongue with sufficient internal movement-potential and diverging dentition patterns in larval morphotypes [15, 16]. To address the question of how processing differs between larval and metamorphosed salamandrid morphotypes of the same species, we examined heterochronic morphotypes of adult Alpine newts.

Table 3 Loadings of processing parameters on the first two principal components (PC1 and PC2)

Parameter	PC1	PC2
(18) Duration hyobranchial movement cycle	0.836[a]	−0.123
(15) Magnitude hyobranchial depression	0.779[a]	0.353
(14) Magnitude hyobranchial elevation	0.663[a]	0.480
(1) Magnitude gape opening	−0.036	0.872[a]
(3) Duration gape opening	0.300	0.699[a]
Total variance explained (%)	48.1	21.0

Parameters marked with [a]load strongly (> 0.5) on each respective principal component. Note that parameters connected to hyobranchial movement load more strongly in PC1 while parameters connected to gape movements load more strongly on PC2

Our data support the hypothesis that larval salamandrids process their food differently than metamorphic salamandrids, as we observed many differences in prey processing behavior across heterochronic morphotypes (Table 1 and Fig. 5). For example, the late-larval morphotype (LLM) was the only morphotype to exhibit a stationary phase after the gape cycle and the post-metamorphic morphotype (PMM) was the only morphotype to show cranial flexion during processing (Fig. 4 and Table 1). Even if only kinematic parameters are compared that apply to all morphotypes, the mid-metamorphic morphotype (MMM) differed in 6 out of 12 kinematic parameters from the LLM, the PMM in 8 out of 12 kinematic parameters from the LLM and the PMM differed in 6 out of 12 kinematic parameters from MMM – suggesting that each morphotype could potentially use a different food processing mechanism.

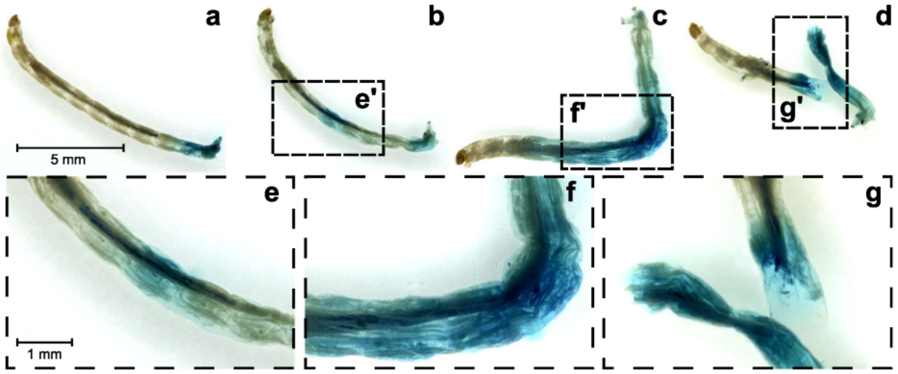

Fig. 6 Lake fly larvae (*Chironomidae*) after intraoral processing. **a** Control specimen, and (**b**-**c**) processed larvae. The processed specimen exhibited (**b**) minor injuries, (**c**) major injuries, and (**d**) ruptures. Methylene blue staining highlight structural damages in the cuticle. Images **e**-**g** show details of the image sections **e**'-**g**'. Note that all samples (including the control) have a blue colored posterior area, probably due to the anal opening

The PMM of the Alpine newts used its tongue to cyclically and rhythmically drive the food against the vomerine dentition on the palate (Video 1 and Fig. 3c), very similar to movement patterns reported for the crested newt [21], that uses tongue-palate rasping to process prey. In fact, our stomach content analysis revealed that processing in *I. alpestris* caused substantial mechanical damage to the food objects (Fig. 6). Tongue-palate rasping in the PMM was characterized by relatively flexible tongue movements (C_V between 0.31–0.53, except for the relatively stereotypical hyobranchial cycle duration with 0.23), which may indicate that the tongue movements must be flexibly fine-tuned during processing. Similar to the PMM, the MMM showed evidence of a tongue-palate rasping mechanism being used as during processing the tongue was used to move the prey across the palate (Video 2) and haemolymph occasionally left the mouth (Video 3). The tongue-palate rasping mechanism of the MMM deviated from that of the PMM in that it showed a modified tongue motion pattern (compare Fig. 4b and h to c and i) and small and sporadic cranial movements (Fig. 4e). Tongue-palate rasping in the MMM was characterized by relatively stereotypical movements of the tongue (C_V between 0.28–0.35, excluding the relative flexible duration of hyobranchial elevation with 0.45). With regard to the switch from chewing (LLM) to tongue-palate rasping (MMM), this could suggest that a relatively stereotypical motion sequence is used first when mastering a new behavior pattern, while this motion sequence can become more flexible during ontogenesis (as seen in the PMM).

The LLM used a processing mechanism with a limited mouth opening, which initially prevented us from determining how food was processed. However, further observations showed that the LLM chewed its food because the prey occasionally protruded from the mouth and therefore made clear how the jaws act upon the prey (Video 4). We could distinguish the post-ingestion behavior (i.e., jaw and hyobranchial movements) into prey transport (characterized by hyobranchial depression during gape opening [17–19]) and rhythmic food processing (characterized by hyobranchial elevation throughout or during some of the gape opening cycle [21, 23]). Food-processing kinematics in the LLM involved the highest mean gape-closure acceleration (Fig. 4a – c and Table 1). As the mandibles of all morphotypes are of approximately the same size and therefore likely have approximately the same mass, the finding that the LLM showed the highest mean gape-closure acceleration might suggest that they exhibit the highest bite force. This, in turn, supports the idea that the dentition of the mandible is directly involved in intraoral food processing (i.e., chewing). We use the term bite force to describe the result of the action of the mandible elevator muscles modified by the craniomandibular biomechanics [56] and thus the force that the mandible can transmit onto an object in the oral cavity (therefore not merely equivalent to adductor muscle force). Additionally, one of the most striking characteristics of the LMM cranio-mandibular anatomy is its overbite, causing dentition on the mandible to occlude between the two functional upper jaw systems, creating an effective shearing bite against the palate (Fig. 1b and c). Consequently, the morphology of the LLM supports the idea that it chews its food using the tooth-bearing mandible (Fig. 1) to pierce the prey against the palate (i.e., 'mandible-palate clenching') while the tongue and dentition on both functional upper jaws hold the prey in place. The kinematic profiles support this assumption as initial gape opening is followed by hyobranchial elevation, which potentially act to position and hold the prey in the area of the occlusal surface on the palate, before the mandible accelerates towards the palate (Fig. 4a and g) to bite the prey.

Externally, the processing behavior of LLM showed striking similarities with the chewing behavior of another paedomorphic salamander, *Siren intermedia*. It was shown using high-speed x-ray analyses that *S. intermedia* use its mandible to rasp the prey across the dentition of the palate [23]. Larval Alpine newts, however, chew their food using 'simple' arcuate movements of the mandible (i.e., opening-closing), and switch from chewing to tongue-palate rasping during ontogeny. Tongue-palate rasping appears to become the main food processing mechanism before the tongue is completely remodeled during metamorphosis (Fig. 2b, Fig 3b, and Video 2). The behavioral shift from mandible-palate interactions (i.e., chewing) to tongue-palate rasping corresponds with the key morphological changes between morphotypes. Whereas in the LLM the teeth of the V-shaped mandible impinge on the palate between the dentition of both functional upper jaw systems (allowing shearing action), the U-shaped mandible of the MMM and the PMM would occlude with more latero-caudal elements of the "primary" upper jaw (i.e., maxilla) (Fig. 1 f-g and j-k) upon jaw closing. The change in mandible shape might prevent mandible-based processing in a progressed mid-metamorphic morphotype as (i) there is only a limited occlusal surface between the mandible and the latero-caudal part of the "primary" upper jaw for chewing and (ii) food loading might be insufficient, given that there is no bone bridge between the rear end of the primary maxilla and the anterior quadrato-squamosal region [57]. Food processing is often argued as being important for the immobilization and break-down of food items before swallowing [58–61] so salamanders might need alternative food processing mechanisms once their mandible outgrows its chewing function. Interestingly, this flexible switch from one processing mechanism to another took place in a single stage of development as both morphotypes (LLM and MMM) were paedomorphic. This appears to reflect the complex morphological life cycle of many salamanders, in which there may be different morphological expressions of paedomorphism, with the morphology of some paedomorphic animals being very similar to that of metamorphs [30]. Not least for this reason, we suspect that the sequence of behaviors we observed could be typical for the development of many salamanders.

It has been previously hypothesized that salamanders show a phylogenetic trend of evolving tongues with greater protrusion potential, increased freedom of the branchial arch in relation to the hyoid arch, and that tongue prehension might have evolved from a intraoral "manipulation" function [28]. Intraoral "manipulation", which was originally understood as pure transport behavior [19, 28], has recently been interpreted as a continuum of processing and transport behavior in terrestrial salamanders [21]. In line with Regal's idea, we found a concurrent ontogenetic process of remodeling in the tongue apparatus. As during the newt ontogeny the tongue develops from a bulky relatively inert system (i.e., hyobranchial system) with small protrusion ability in the LLM (Fig. 1d and Fig. 2a) to a delicate and relatively mobile system (i.e., hyolingual system) with greater protrusion ability in the PMM (Fig. 1l and Fig. 2c). The LLM hyobranchial system has a muscular anatomy that creates motion-potential in all directions of the median plane. However, tongue protraction is limited to geniohyoid and ceratomandibularis muscles which act as the primary tongue protractor complex in larval salamanders (Fig. 2a). During the metamorphosis in the MMM, the branchiohyoideus externus and subarcualis rectus 1 muscles are rearranged to functionally suspend the branchial arch on the paired ceratohyal (i.e., hyoid arch) (Fig. 2b). This muscle rearrangement enables a more effective protraction of the branchial arch, since it can now be moved by the suspension on the hyoid arch and thus pulled further anteriorly (Fig. 2b). This secondary tongue protractor complex allows the tip of the tongue to be ejected out of the mouth which has been described for post-metamorphic salamandrids [33] and in turn is the functional basis for tongue-palate rasping [21]. Our data suggest that aquatic salamandrid larvae begin to use their tongue for intraoral food processing (Fig. 3b and Video 2) as soon as the mandibular reorganization prevents them from chewing but their tongue morphology enables improved protraction during development. Thus, we hypothesize that salamanders that are able to protract their tongue effectively and have a metamorphic palatal dentition (Fig. 1c and g) are potentially able to combine these elements to achieve tongue-palate rasping. Consequently, it is likely that tongue-palate rasping is the general processing mechanisms in salamanders with a metamorphic feeding apparatus morphology. Additionally, our data support Regal's hypothesis that tongue prehension likely evolved from a "manipulation" function of the tongue [28] as our animals mastered tongue-palate rasping before they were apt to leave the water and thus before they used their protractible tongue to catch prey.

Mid-metamorphic Alpine newts develop the ability to rasp a food item against the palatal dentition and engage in tongue-palate rasping due to rearrangements of the branchiohyoideus externus and subarcualis rectus 1 muscles during metamorphosis. At the same time mid-metamorphic morphotypes also retain the ability to forcefully elevate their tongue using the levator hyoideus muscle (Fig. 2b). The tongue of the post-metamorphic morphotype loses this muscular connection to the skull (i.e., levator hyoideus) and its motion is limited to elevation based on muscles spanning the mouth floor and the hyobranchial system laterally [35]. As a result, the tongue is likely to lose the ability to forcefully press a food against the palatal dentition, possibly reducing the effectiveness of tongue-palate rasping. It had been hypothesized that the coordination between hyolingual motion and depression of the skull may aid food processing efficacy in post-metamorphic salamandrids [21]. Coordinated head and hyolingual movement patterns also appear in post-metamorphic Alpine newts (Fig. 4f, i), but not in the mid-metamorphic stage, suggesting that coordinated depression of the skull and hyolingual movements might be a compensatory behavior for the loss of the levator hyoideus (Fig. 2c).

From an evolutionary perspective the findings presented here might shed light on the fish-tetrapod transition (water-land transition) of early tetrapods. While tongue and jaw kinematics are similar across amniotes [24, 25, 62], food processing in salamanders shares traits with both fish and amniotes [21]. Accordingly, salamanders may be a good analog model to reveal functional changes in feeding behavior across the fish-tetrapod transition [41]. From this point of view, the morphological and behavioral differences between the two aquatic larval stages (LLM and MMM) could reflect analogous changes in the early tetrapods. In particular, the present study shows that the MMM – a stage with both larval and post-metamorphic traits and without a freely movable tongue - utilizes a new feeding mechanism (tongue-palate rasping) before the presumed morphological adjustments for this function have fully developed and while the animal remains fully aquatic. It is possible that behavioral changes may have preceded obvious morphological evolution of the feeding system across the fish-tetrapod transition, resulting in new feeding mechanisms. Thus, understanding the timing of changes in feeding mechanism across the transition may require precise quantification and characterization of morphology as well as rigorous biomechanical testing, which can reveal biomechanical differences in similarly-shaped structures [63]. Furthermore, our results support findings from previous studies that morphological and behavioral changes facilitated the evolution of “terrestrial style feeding” in early tetrapod taxa that were still primarily aquatic [64–67].

Conclusions

We found differences in the skeleton, soft tissue and food processing kinematics between the late-larval morphotype (LLM) and the mid-metamorphic morphotype (MMM), suggesting previously unappreciated diversity between superficially similar paedomorphic stages. Further, our data show that prey processing kinematics differ between all three morphotypes (late-larval, mid-metamorphic and post-metamorphic morphotype) in the Alpine newt, contrary to the previously established pattern of stereotypy of prey capture or intraoral transport kinematics for this species. Our data indicate a degree of plasticity not previously demonstrated in the ontogeny of intraoral food processing behaviors. Based on a similar development in the feeding apparatus morphologies of most larval salamanders, our data also suggest that salamanders may undergo similar food processing ontogenies in general. Additionally, we found, that salamanders that are able to protract their tongue effectively and have proper palatal dentition, are potentially equipped to use tongue-palate rasping. Consequently, it is likely that tongue-palate rasping is a generalized pattern in salamanders with a metamorphic feeding apparatus morphology (i.e., MMM and PMM). Finally, the present study might allow some parallels to be drawn about the evolution of terrestrial feeding in early tetrapods. In analogy to salamanders, early tetrapods might have evolved new feeding mechanisms in their aquatic environments and these functional innovations later might have paved the way for terrestrial feeding mechanisms.

Methods

Specimens and animal care

The paedomorphic and metamorphic specimens used in this study were collected in September of 2012 from an artificial irrigation reservoir in the Province of Bolzano (South Tyrol, Italy) under collection permit No. 63.01.05/120963, granted by the local government of the Province of Bolzano. For further information on the pond and the paedomorphic character of the specimens, see [68]. The natural prey spectrum of metamorphic and paedomorphic *Ichthyosaura alpestris* is very broad. In the aquatic habitat, the Alpine newt feeds on insect larvae (e.g. *Chironomidae*), small crustaceans and amphibian eggs or larvae [69, 70].

Kinematic analyses were conducted using five post-metamorphic individuals (five PMM) and two paedomorphic individuals (one LLM, one MMM). The kinematic analyses of the LLM and the MMM were therefore limited to the repetitions of one specimen each (for details to the number of repetitions see “High-speed recording and kinematic analysis”). The SVL of paedomorphic specimens (43 and 45 mm) did not differ significantly from the SVL of post-metamorphic specimens (44.6 ± 3.4 mm). The animals were group-housed with both paedomorphic newts in one aquarium (60 × 30 × 40 cm; (length × width × depth)) and the post-metamorphic newts in a larger aquarium (120 × 60 × 40 cm). The animals were kept at 20 ± 2 °C temperature, 12/12 h photoperiod and were exclusively fed with lake fly larvae (*Chironomidae*) a week before the recordings.

High-speed recording and kinematic analysis

The newts were placed in a glass aquarium (30 × 12 × 20 cm) with a water level of approximately 10 cm.

Paedomorphic and post-metamorphic individuals were fed with lake fly larvae (*Chironomidae*). A chessboard pattern was placed in the background of the aquarium to allow calibration of the videos. The test setup was illuminated with reduced heat emission spotlights (VD-7000 LP; Vision Devices GmbH, Metzingen, Germany). A high-speed camera (Photron FASTCAM model 100KC; Photron, Tokyo, Japan) was used to record the feeding events at 500 fps with a 1024 × 512 pixel frame format. Recordings of paedomorphic feeding trials was conducted using a 60 mm macro lens while a 50 mm standard lens was used for post-metamorphic trials. A total of 49 recordings from paedomorphic and 50 recordings from post-metamorphic newt feeding were acquired.

Recordings for kinematic analyses were selected according to overall sharpness (focus on the specimen) as well as specimen orientation. Landmark tracking was carried out using Simi Motion 8.0.0.315 software (Simi Reality Motion Systems GmbH, Unterschleißheim, Germany). Three component motions - gape cycle, vertical cranial flexion, and dorso-ventral hyobranchial movement - were analyzed. To do so, we tracked six landmarks: (1) tip of the upper jaw, (2) back of the head, (3) reference point on the back approximately over the shoulder girdle (4) tip of the mandible, (5) corner of the mouth, and (6) point ventral to the corner of the mouth which lowers as the hyobranchial apparatus is depressed (Fig. 7a-b). Every fifth frame was used for manual landmark tracking, the missing intermediate time steps were spline interpolated, and areas of the resulting motion graphs that showed very small movements were locally smoothed using a moving average filter of the tracking software. Using the smoothing and interpolation functions of the tracking software allowed confirmation of the markers' positions on their specific landmarks. In total, 105 processing cycles of post-metamorphic newts (PMM) and 45 processing cycles of paedomorphic newts (27 MMM and 18 LLM) were analyzed. We used trigonometry on the 2-D landmark coordinates to calculate the kinematic parameters gape (Fig. 7c), vertical cranial flexion (Fig. 7d), and vertical hyobranchial movement (Fig. 7e) in Excel (Microsoft Corporation, WA, USA). Subdivision of the kinematic profiles into component motion cycles was achieved using a custom graph analyzer tool for MATLAB R2019b (The Mathworks, Inc., Natick, MA, USA). A cycle was defined as a movement event that contained three extremes: two low or high points and the opposite point. The custom-written script additionally computed the 18 kinematic parameters out of the kinematic variables by using the high and low point information.

Statistical analysis and ordination approach

The aim of the statistical analysis was to test the kinematic parameters for differences between the morphotypes (LLM, MMM and PMM). Since the parameters violated the assumptions for parametric tests, nonparametric statistics were carried out. To determine if the kinematic parameters differed between morphotypes, we performed a Kruskal-Wallis 1-way ANOVA. The sequential pairwise multiple comparisons (i.e., Mann-Whitney U tests), using ranks based on considering all samples, not just the two samples that are currently involved in a comparison [71], were performed to determine where the differences are among the morphotypes. All significance values were Bonferroni adjusted to account for multiple testing.

The aim of the ordination approach was to visualize how the processing kinematics of the three morphotypes are related and thus by implication to highlight their differences. First, processing parameters that do not apply to all morphotypes were excluded (7, 9–13 of Table 1). Subsequently, seven dimension reductions were performed prior to the principal component analysis (PCA) to remove processing parameters (2,4–8, 16–17 of Table 1) which do not load strongly (< 0.5) on any of the components. The PCA was performed on the correlation matrix and the resulting Anderson-Rubin factor scores were saved in order to show the effects of (i) individual, (ii) heterochronic state, and (iii) processing mode on the total variance. The factor scores of the PCA were plotted with the related convex hulls, displaying the biggest possible area, which contains all observations of the respective feeding mode. The principal component analysis was performed using SPSS 25 (IBM Corporation, Armonk, NY, USA).

Stomach content analysis

Once the feeding trials and the kinematic analysis were performed, we fed the post-metamorphic animals with lake fly larvae ad libitum. They were presented with a multitude of lake fly larvae, which they ingested and processed one to several at a time. After feeding, the animals were anaesthetized and subsequently euthanized by immersion in an aqueous solution of 0.5% MS222, buffered to pH 7.0. The stomachs were removed and voided post-mortem and the stomach contents were fixed in 70% ethanol for 1 week. The processed lake fly larvae were stained using methylene blue (1 min) and then washed with 70% ethanol. The processing injuries were recorded using a digital microscope (Keyence, VHX-2000; Keyence Corporation, Osaka, Japan). The paedomorphic samples had to be euthanized after a fungal infection before data collection was completed; thus no paedomorphic stomach contents could be analyzed.

Anatomical analysis (μCT)

The musculoskeletal components of the feeding apparatus of paedomorphic and post-metamorphic specimens were reconstructed from μCT scans [15]. Euthanized specimens

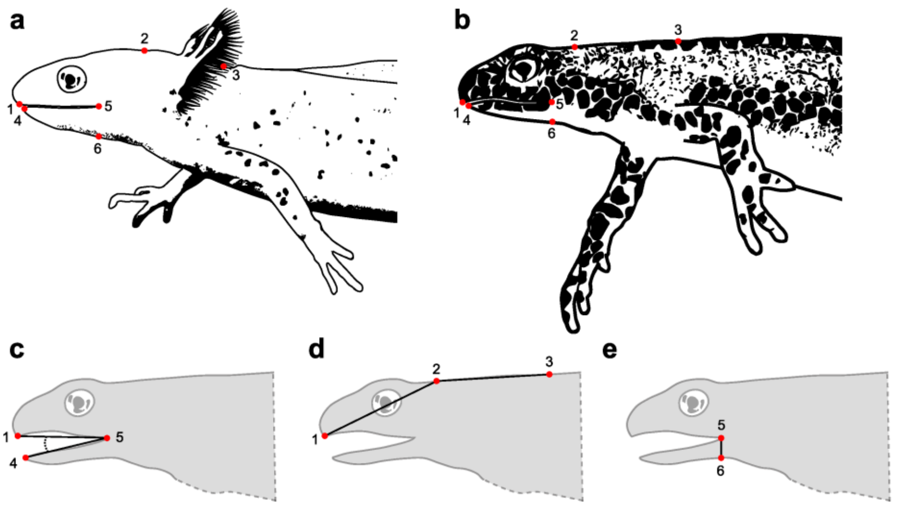

Fig. 7 Landmark overview for kinematic analyses. **a** Paedomorphic and (**b**) post-metamorphic *I. alpestris* with landmarks used for kinematic analyses. Lower row depicts the calculation of (**c**) gape, (**d**) vertical cranial flexion and (**e**) vertical hyobranchial displacement. Abbreviations: (1) tip of the upper jaw, (2) back of the head, (3) reference point on the back approximately over the shoulder girdle (4) tip of the mandible, (5) corner of the mouth, and (6) point ventral to the corner of the mouth which lowers as the hyobranchium is depressed

were fixed in 4% formaldehyde for 1 month, dehydrated in a graded series of ethanol, immersed for 2 weeks in an alcoholic iodine solution, rinsed in absolute ethanol and securely mounted in Falcon tubes to avoid motion artifacts in the scans. Scans of the entire paedomorphic specimens were acquired using a μCT scanner (SkyScan 2211; Bruker, Billerica, MA, USA) with a source voltage of 100 kV, an electric current of 180 μA, a 0.5 mm Titan filter, and an isometric voxel resolution of 8.00 μm. X-ray projections were then reconstructed in NRecon Reconstruction Software 1.7.3.1 (Micro Photonics, Allentown, PA) with an automatic beam hardening correction factor of 45%. For the post-metamorphic specimen only scans of the head region were acquired using a μCT scanner (SkyScan 1174) with a source voltage of 50 kV, an electric current of114 μA, a 0.5 mm aluminum filter, and an isometric voxel resolution of 7.39 μm. X-ray projections were also reconstructed in NRecon Reconstruction Software. Volume rendering of the μCT scans was performed using the Amira 6.4 software package (https://www.fei.com/software/amira). Based on tomographic image data, we threshold-segmented bones and used manual segmentation for muscles, cartilage and teeth. Both paedomorphic specimens are kept in the State Museum of Natural History Stuttgart (SMNS 16344 and SMNS 16345).

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12983-020-00373-x.

Additional file 1: Video 1. High-speed movie from a lateral perspective, showing a post-metamorphic *I. alpestris* processing a lake fly larva (Chironomidae) under water. Three consecutive processing cycles can be observed. Note how the tongue cyclically rasps the prey along the palate. The movie was recorded at 500 Hz and is played back at 50 Hz, which corresponds to 10% of the actual speed.

Additional file 2 Video 2. High-speed movie from an anterio-lateral perspective, showing a mid-metamorphic *I. alpestris* processing a lake fly larva (Chironomidae) under water. Five full processing cycles can be observed, cycle 1, 4, and 5 are mixed, or immediately followed, by transport movements. Note how the tongue cyclically rasps the prey along the palate. The movie was recorded at 500 Hz and is played back at 50 Hz, which corresponds to 10% of the actual speed.

Additional file 3 Video 3. High-speed movie from a lateral perspective, showing a mid-metamorphic *I. alpestris* processing a lake fly larva (Chironomidae) under water. Five full processing cycles can be observed, cycle 3 is immediately followed by a transport movement. Note how in every except the third cycle the haemolymph of the prey leaves the mouth during processing (i.e., an indication of structural damage). The movie was recorded at 500 Hz and is played back at 100 Hz, which corresponds to 20% of the actual speed.

Additional file 4 Video 4. High-speed movie from a lateral perspective, showing a late-larval *I. alpestris* processing a lake fly larva (Chironomidae) under water. Five full processing cycles can be observed, cycle 5 is mixed with a transport movement. Note how the lower jaw is used to bite the prey. The movie was recorded at 500 Hz and is played back at 100 Hz, which corresponds to 20% of the actual speed.

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Authors' contributions

DS and EH designed the study. EH, LBP, and NK oversaw the experimental design. DS collected data (high-speed experiments), designed and performed the data analysis, created the three-dimensional reconstruction, conducted the morphological description, prepared the figures and interpreted the results. DS wrote the first manuscript with help, guidance and review from EH, LBP, and NK. The authors read and approved the final manuscript.

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Availability of data and materials

The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Experiments were performed at the Friedrich-Schiller-University of Jena, Germany. The Committee for Animal Research of the State of Thuringia, Germany, approved husbandry and experiments (codes animal experiments: 02–042/14, 02–008/15; code animal husbandry: J-SHK-2684-05-04-05-07/14).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Publisher's Note

Chapter IV

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

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Daniel Schwarz, Stanislav N. Gorb, Alexander Kovalev, Nicolai Konow, and Egon Heiss

Estimated own contribution: **75%**.

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

See (CRediT taxonomy; supplementary material b) for details.

Precis:
Chapter IV concerns the flexible adjustment of intraoral food processing in the Italian crested newt (Sirenoidea, see Fig. S2; supplementary material d). The results suggest that the intraoral food processing mechanics adapt to the type of prey eaten while the medium in which processing occurs seems to be of less effect. Given the switch in the food processing mechanisms that seems to occur during the early, aquatic development of salamanders (ch. III), the here presented also suggest that terrestrial style feeding (i.e., feeding where the tongue directly moves the food) evolved before the transition to land.

These results of this chapter support hypothesis 4

and provide further data for comparisons to support or reject hypotheses 1, 2 and 3.

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RESEARCH ARTICLE

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

Daniel Schwarz[1,*], Stanislav N. Gorb[2], Alexander Kovalev[2], Nicolai Konow[3] and Egon Heiss[1,4]

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 Hiiemae, 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-Lucaciu 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 carnifex* (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 domesticus*) 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

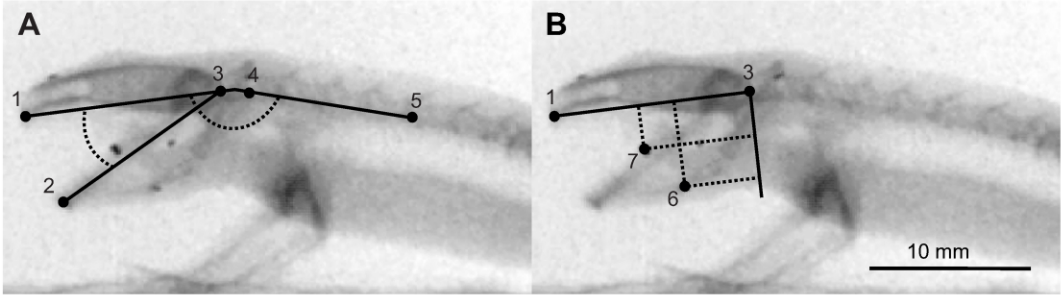

Fig. 1. X-ray frame shots with landmarks. Lateral views showing the seven landmarks used to measure movements of (A) the mouth and head and (B) the tongue and prey. Landmarks: (1) snout tip; (2) lower jaw tip; (3) 'occipital' (posterior part of the skull); (4) first vertebra; (5) fifth vertebra; (6) 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±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.

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.

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 µm 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 µm 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±s.d. tip diameter (39.2±4.7 µm) and crown angle (29.7±4.6 deg) of the vomerine

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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 macroscope (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 µm 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

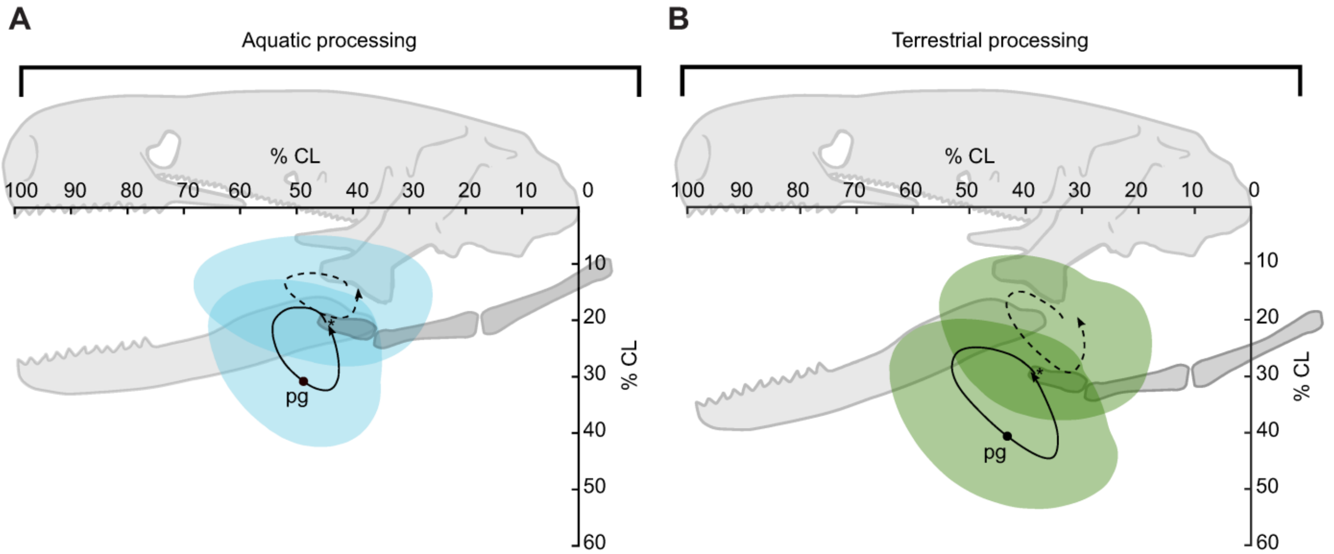

Fig. 2. Tongue and prey movements in the 2D cranial reference coordinate system in *Triturus carnifex*. The loops are running in a counter-clockwise direction and indicate motion of the tongue (continuous line) that moves prey (dashed line) along the mouth roof under aquatic (A) and terrestrial (B) conditions. 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.

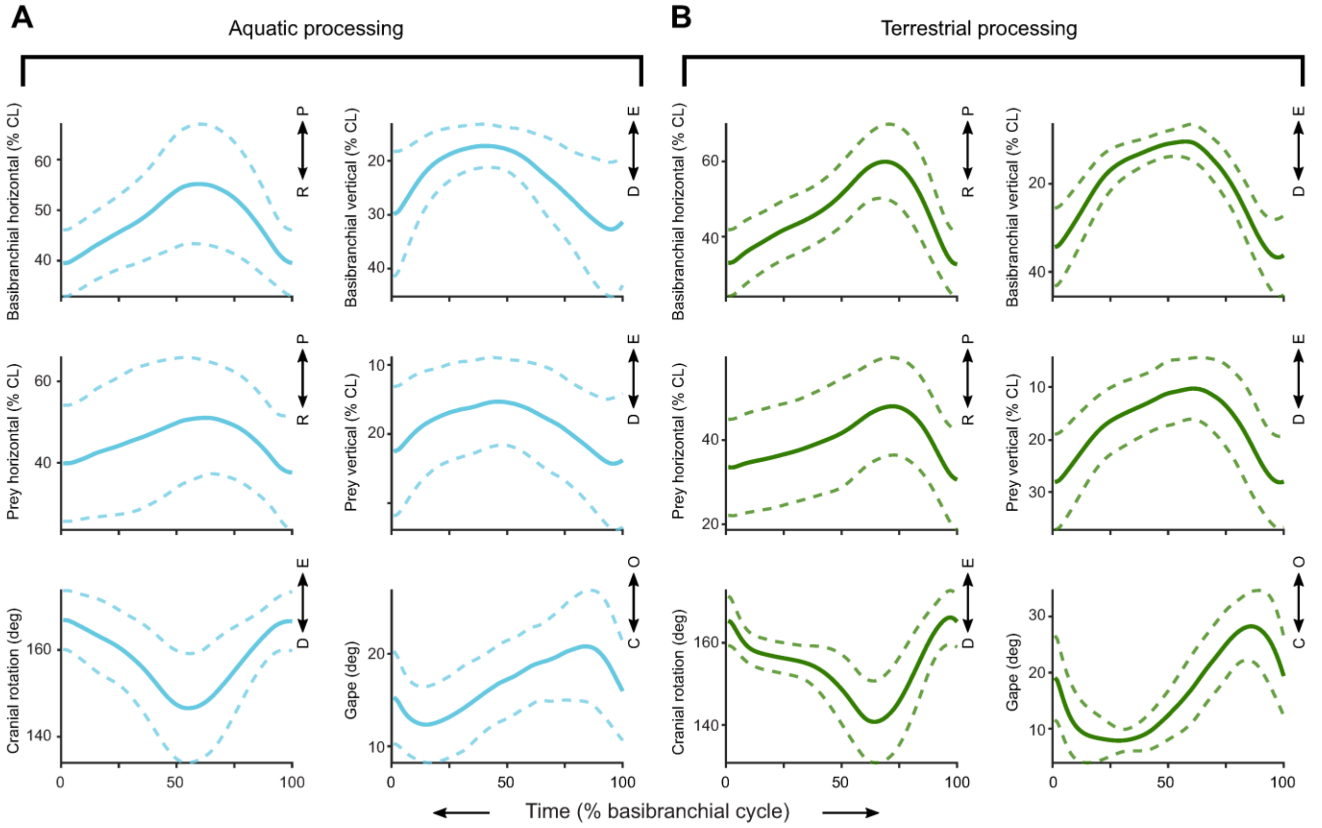

Fig. 3. Kinematic profiles of aquatic and terrestrial food processing in *T. carnifex*. Solid lines indicate mean values and dashed lines indicate ±s.d. from 113 (A) and 106 (B) processing cycles from maggot feeding trials. The time scale on the *x*-axes is normalized as percentage of basibranchial cycle. 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 behove 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)

			Individual		Environment	
Variable	Aquatic processing	Terrestrial processing	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±90	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 ($P\leq0.003$), 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

			Individual effect		Environmental effect	
Prey type	Aquatic feeding events	Terrestrial feeding events	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 ($P\leq0.013$). Significant P-values are indicated by asterisks. Kruskal–Wallis and Wilcoxon signed-rank tests indicate differences between individuals and environments.

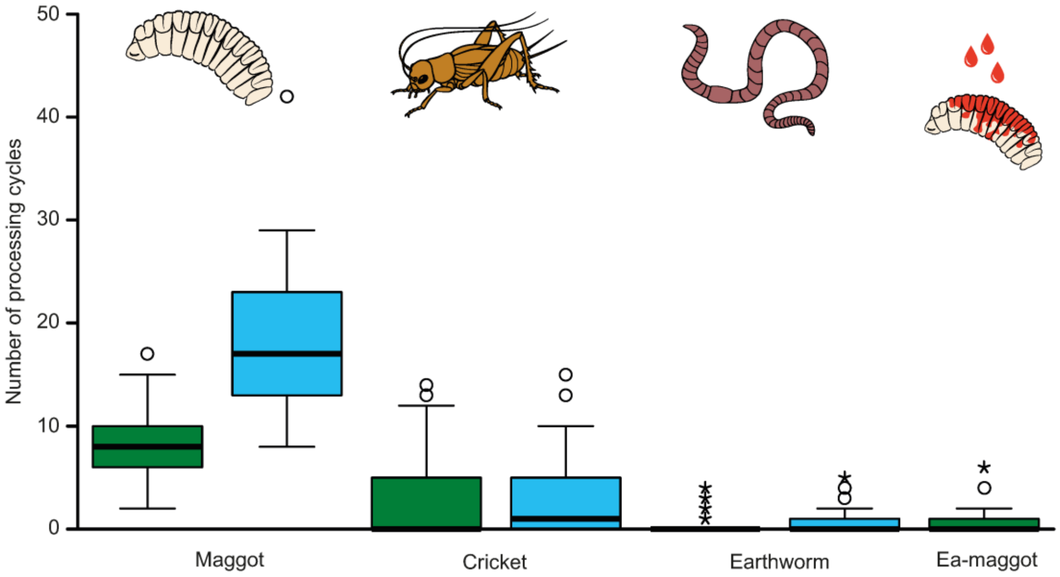

Fig. 4. Boxplots showing the different number of processing cycles used by *T. carnifex* for different prey and across aquatic and terrestrial feeding events. Green plots (left) indicate terrestrial feeding; blue plots (right) indicate aquatic feeding. Note that untreated maggots inflict most processing 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 ($P\leq0.008$). 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.

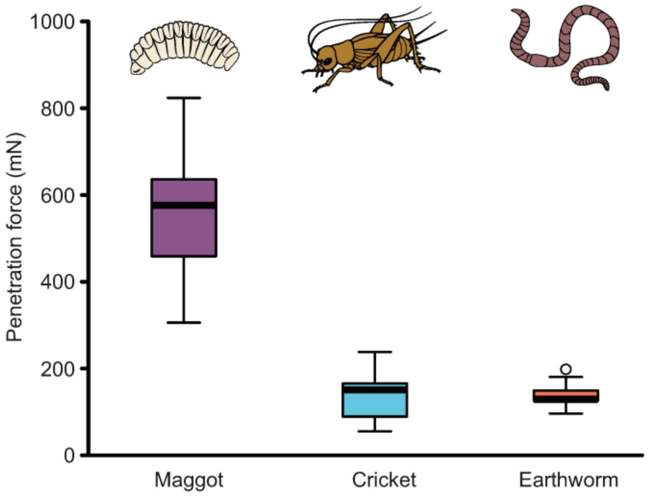

Fig. 5. Boxplots showing different penetration forces (mean±s.d.) measured for three different prey types: maggots, crickets and earthworms. Circles symbolize values that differ significantly from the remaining values. Pairwise Mann–Whitney *U*-tests comparing the penetration forces across the three 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 ($P\leq0.017$).

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

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https://jeb.biologists.org/lookup/doi/10.1242/jeb.232868.supplemental

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Supplementary information

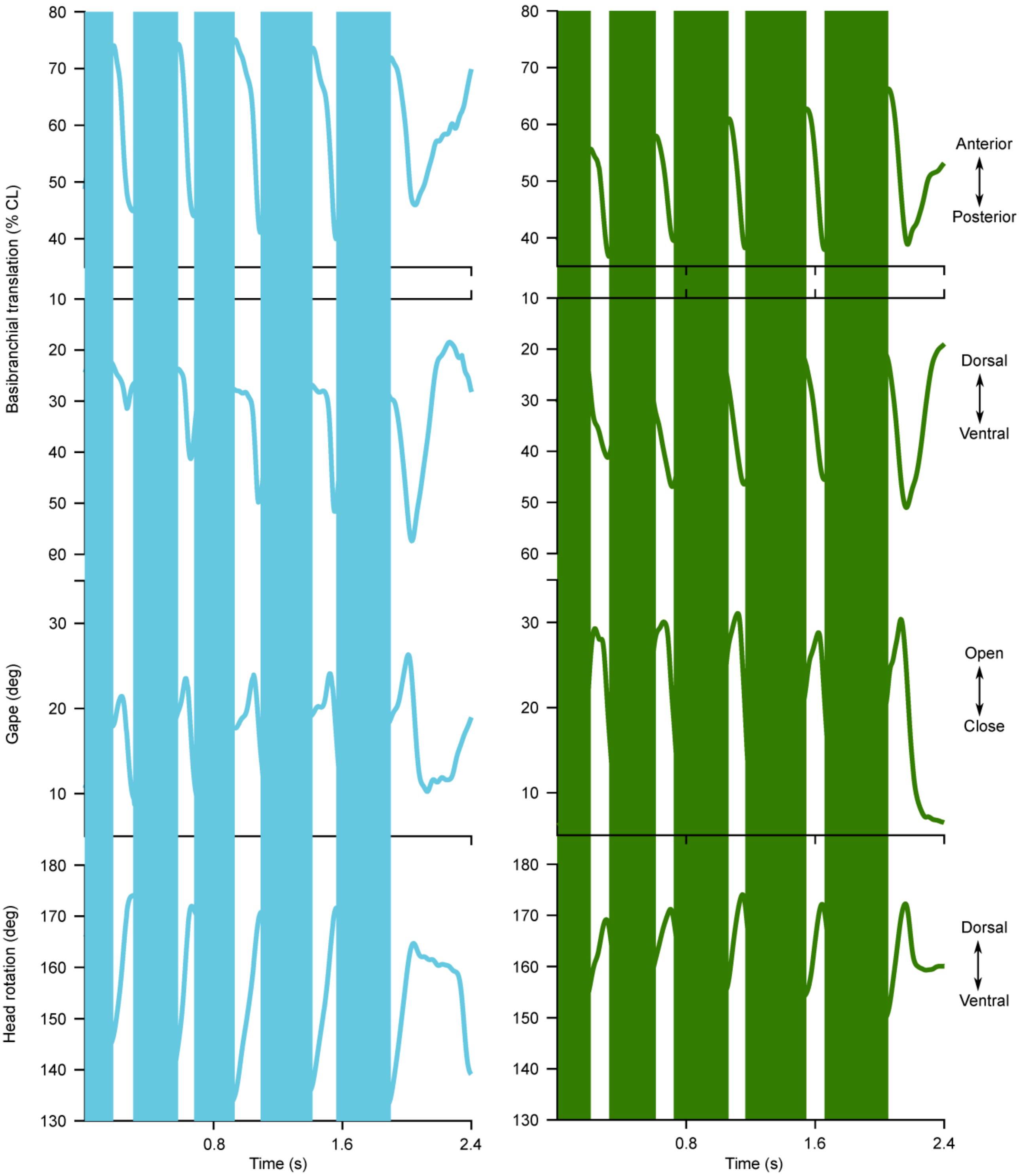


Figure S1. Representative kinematic profiles during aquatic (blue) and terrestrial feeding (green) from the same animal. Basibranchial (tongue), gape and skull displacements are shown during a typical intraoral processing event in *Triturus carnifex*. As a reference, tongue protraction phases are indicated by grey bars. CL, cranial length. The terrestrial part of the figure is modified after Heiss et al. (2019).

Journal of Experimental Biology: doi:10.1242/jeb.232868: Supplementary information

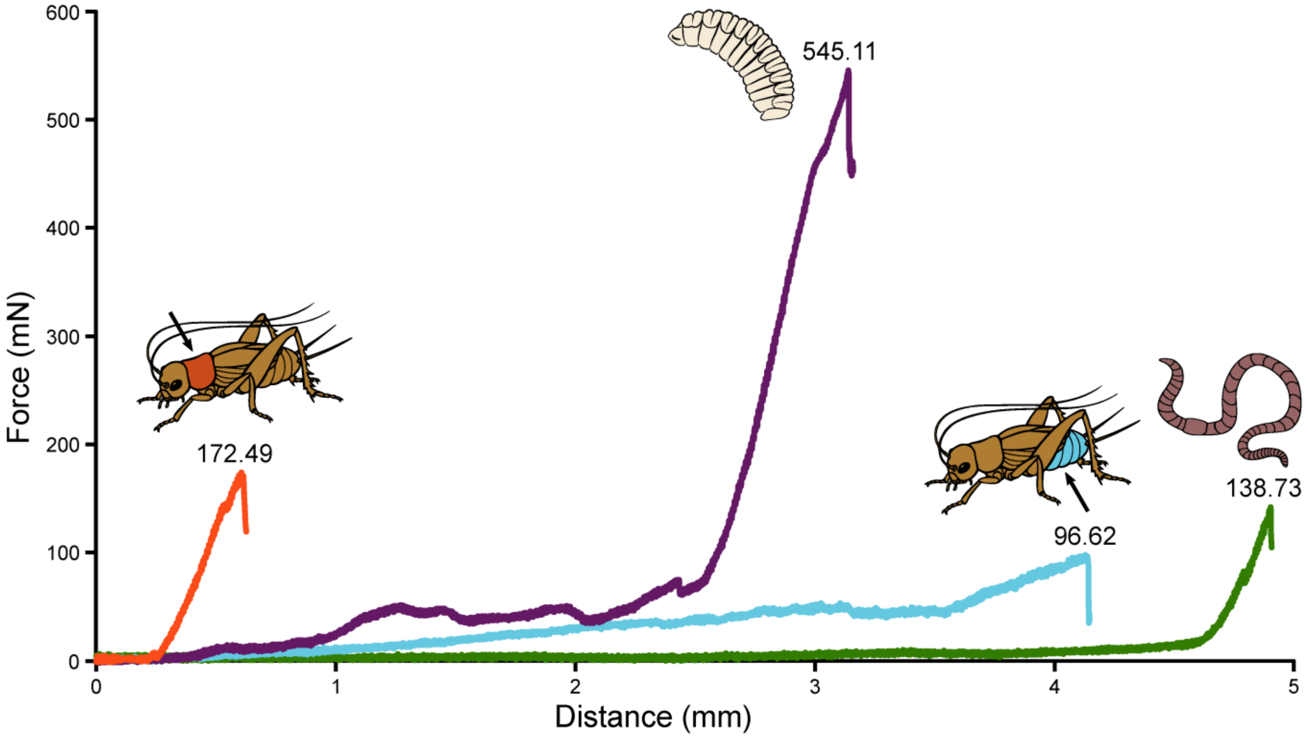
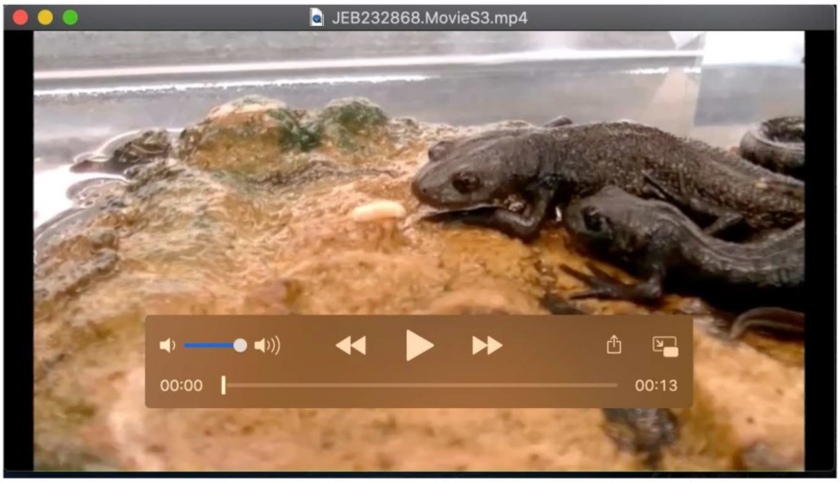


Figure S2. Representative force-distance curves of a cricket thorax (orange), maggot (purple), cricket abdomen (blue), and earthworm (green) measurement. Every trend begins with the contact between the specimen and the needle. The trends are cut behind the drop in the force measurement.

Journal of Experimental Biology: doi:10.1242/jeb.232868: Supplementary information

Movie 1. Movie from a lateral perspective, showing T. carnifex feeding on a maggot (Lucilia sp.) under water. T. carnifex first ingests the prey by suction feeding, after a short stationary phase the newt orients the maggot in the oral cavity in order to prepare it for the subsequent processing. The newt then begins to process the maggot immediately, followed by a caudal transport of the prey to prepare for swallowing. In terms of intraoral movements; one initial transport cycle (i.e. orientation of the food in the oral cavity), seventeen consecutive processing cycles (tongue-palate rasping), and two (caudal) transport movements (preparation for swallowing) can be observed. The movie was recorded at 50 Hz and is played back at 25 Hz, which corresponds to half the actual speed.

Movie 2. X-ray movie from a latero-lateral perspective, showing T. carnifex processing a maggot (Lucilia sp.) under water. Eight consecutive processing cycles (i.e. tongue-palate rasping) can be observed. The large radiopaque marker in the high-speed x-ray recording indicates the position of the prey (maggot). The movie was recorded at 250 Hz and is played back at 50 Hz, which corresponds to 1/5 of the actual speed.



Movie 3. Movie from a lateral perspective, showing T. carnifex feeding on a maggot (Lucilia sp.) on land. The newt first ingests the prey (maggot) by a mixture of tongue and jaw prehension, after a very short stationary phase and two transport cycles the animal begins to process the maggot. Six consecutive processing cycles (i.e. tongue-palate rasping) can be observed. The movie was recorded at 50 Hz and is played back at 25 Hz, which corresponds to half the actual speed.

Movie 4. X-ray movie from a latero-lateral perspective, showing T. carnifex processing a maggot (Lucilia sp.) on land. The movie begins with the maggot being already ingested. After a short stationary phase, the newt orients the maggot in the oral cavity in order to prepare it for the subsequent intraoral processing. In terms of intraoral movements; one initial transport cycle (i.e. orientation of the food in the oral cavity) and six processing cycles (tongue-palate rasping) can be observed. Intraoral processing cycle 4 is mixed with transport movements. The large radiopaque marker in the high-speed x-ray recording indicates the position of the prey (maggot). The movie was recorded at 250 Hz and is played back at 50 Hz, which corresponds to 1/5 of the actual speed.

Chapter V

Discussion

The results presented here extend and substantiate our knowledge about salamander food processing mechanisms and contrast the great support from literature for the idea that salamanders do not process their food intraorally (De Vree and Gans, 1994; Deban and Wake, 2000; Schwenk and Rubega, 2005). Thus, these findings suggest that salamanders seem to engage in intraoral food processing in general. The data also suggest that different salamander taxa use distinct processing mechanisms and that these mechanisms may change during the ontogeny of individual salamanders. Despite the disparate physical conditions of water and air, evidence from one group of salamanders, the multiphasic newts suggest that the medium in which feeding takes place appears to have less influence on the processing mechanism of salamanders than the food consumed.

8. Intraoral food processing in salamanders

In line with the **first hypothesis**, that *Since intraoral food processing is an integral part of feeding behaviour that can help increase the digestive efficiency in virtually all other vertebrates, salamanders also process their food intraorally* - the data presented here suggest that intraoral food processing might be common amongst salamanders. Thus, these results stand in contrast to the prevailing belief that salamanders, like other lissamphibians, do not process their food intraorally but rather swallow it whole and unreduced (Bemis et al., 1983; De Vree and Gans, 1994; Deban and Wake, 2000; Lauder and Gillis, 1997; Reilly and Lauder, 1990a; Schwenk and Rubega, 2005). The fact that processing appears to be shared amongst salamanders reflects the pattern seen in all other vertebrates; that intraoral food processing is a generalized trait of vertebrate feeding (Bemis and Lauder, 1986; Dean et al., 2005; Gans and De Vree, 1986; Gans et al., 1978; Gintof et al., 2010; Herrel et al., 1999; Sanford and Lauder, 1989; Schwenk and Rubega, 2005; Schwenk and Wake, 1993; Wainwright et al., 1989).

The first mechanism of intraoral food processing described in this work (ch. II) is that of the Italian crested newt (*Triturus carnifex*; Laurenti, 1768). Food processing in the metamorphic Italian crested newt, a salamandrid (Fig. 7A) involves cyclic pitching movements of the head (i.e., vertical cranial rotation) in combination with repetitive loop motions of the tongue, which rasp the food across the palatal dentition (i.e., tongue-palate rasping) as the jaws cyclically open and close (Heiss et al., 2019). The first stage of the processing cycle is defined by protraction and elevation of the food-bearing tongue to rasp the food against and across the palatal dentition. At the same time, the cranium is depressed (i.e., rotated ventrally). Later during the first stage, the jaws initially close from the previous cycle, then peak and start opening again (Fig. 7A). The second stage is defined by retraction and depression of the tongue to reposition the food while the cranium is elevated (i.e., rotated dorsally). During the second stage, the jaws first open further, reach their maximum opening (i.e., peak) and start rapid jaw closure, which in turn continues into the subsequent processing cycles.

The results of this work also support the **second hypothesis** that: *Since form and function are interconnected, and salamanders exhibit diverging feeding apparatus morphologies across phylogeny and ontogeny, the behaviours of intraoral food processing must differ with regard to salamander taxa and developmental morphotypes.* Since another mechanism of intraoral food processing was found in the lesser siren (*Siren intermedia*; Barnes, 1826) (ch. **III;** Fig. 7B), a member of the sirenids, the relatively basal sister taxon of the salamandrids (Pyron and Wiens, 2011) (Fig. S2; supplementary material d).

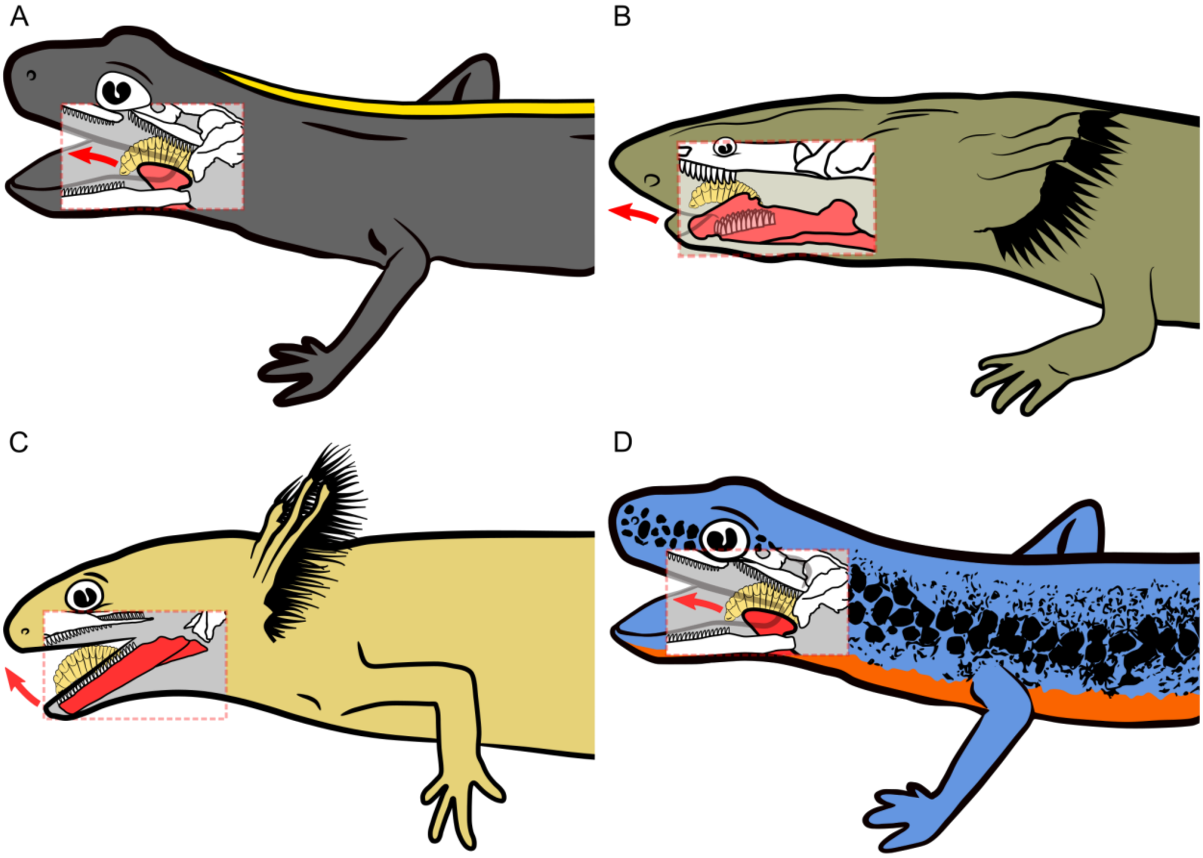


Figure 7: Intraoral food processing mechanisms in salamanders. (A) tongue-palate rasping in the metamorphic Italian crested newt (*Triturus carnifex*), (B) mandible-palate rasping in the paedomorphic lesser siren (*Siren intermedia*), (C) mandible-palate clenching in the paedomorphic Alpine newt (*Ichthyosaura alpestris*), and (D) tongue-palate rasping in the metamorphic Alpine newt. The red arrows indicate the working direction of the processing organ (tongue or mandible), which in turn is highlighted in red. The prey item (maggot) is highlighted in yellow.

The lesser siren uses water flow induced by its hyobranchial apparatus to drive the food antero-dorsally, after which the mandible (i.e., lower jaw) translates the food longitudinally. Processing occurs as the prey is rasped antero-dorsally between the teeth of the mandible and the palatal dentition (i.e., a mandible-palate rasping) (Fig. 7B). Since the lesser siren processes its food using its jaws, this mechanism reflects a form of chewing in a practical sense, which by definition represents any mandible-based form of intraoral food processing (Reilly et al., 2001). During chewing in the lesser siren, the hemimandibles (also referred to as mandibular arms) abduct and adduct laterally (i.e., mandibular wishboning) as the mandible is cyclically retracted and protracted, and the jaws cyclically close and reopen. This behaviour represents a peculiarly complex chewing motion that has been claimed to require a unique mandibular joint anatomy and symphysis that was believed to be exclusive to mammals (Bhullar et al., 2019; Grossnickle, 2017; Herring, 1993; Hylander and Crompton, 1986; Turnbull, 1970). Thus, the results of this work demonstrate that complex 3D movements of the lower jaw during chewing are not exclusive to amniotes, specifically mammals.

In addition to the extraordinarily complex chewing of the lesser siren and the tongue-palate rasping of the Italian crested newt, another food processing mechanism was revealed in the Alpine newt (*Ichthyosaura alpestris*; Laurenti, 1768) (ch. IV). Paedomorphic Alpine newts use their mandible to bite the food against the palate (i.e., mandible-palate clenching) (Fig. 7C), between the two functional upper jaw systems (i.e., the “primary” upper jaw and the palatal jaw). The first stage of the processing cycle is defined by jaw opening and coincident hyobranchial elevation (i.e., bite preparation and food transport), followed by a greatly accelerated jaw closure (Fig. 7C) during which the hyobranchial apparatus keeps elevating (i.e., bite and food transport). Both movements, the jaw closure and hyobranchial elevation, peak simultaneously. During the second stage, the jaws remain shut while the hyobranchial apparatus is depressed (i.e., repositioning). Interestingly, in contrast to the paedomorphic Alpine newts, metamorphic Alpine newts used a processing mechanism homologous to that of metamorphic Italian crested newt (i.e., tongue-palate rasping) (Fig. 7D). Since the development of salamandrids is stereotypical and different species exhibit very similar morphotypes during their ontogeny, it seems plausible to assume that paedomorphic Italian crested newts also apply a processing mechanism analogous to that of the Alpine newts.

In summary, the most intriguing implications come from comparing the remarkable complex food processing mechanisms in the relatively conformal group of salamanders. The data obtained suggest that the lesser siren and the larval Alpine newt use their mandibles, while the Italian crested newt and the metamorphic Alpine newt use their tongues to process food (Fig. 7). Besides the apparent superficial homology; that these different mechanisms resemble forms of palatal processing, it is intriguing that such diverse processing mechanisms (i.e., chewing vs tongue-palate rasping) are used within a group, and even across an individual's ontogeny. The presence of such distinctive processing mechanisms raises the question: What causes these disparate behaviours, and why do some taxa apply similar forms of food processing?

8.1. Form and function

Since form and function are firmly related, and represent different aspects of the same quality (i.e., of the form-function complex), each side of the quality provides information about the other (see ‘1.1 Form and function’ and Bock and Von Wahlert, 1965). The most pronounced changes in the form of the cranial region that occur during early larval development and metamorphosis of salamanders involve structures that are directly involved in feeding (Fabre et al., 2020; Rose, 2003). Thus, as one side of the form-function complex, the feeding apparatus morphology, changes during ontogeny, it seems to necessitate the observed changes of the other side, the food processing mechanism. Hence, the answer to the question of what causes the different processing mechanisms and why some taxa employ similar mechanisms is likely to be contained in their form (i.e., morphology). The change in the form of an individual, the morphological development or morphogenesis, however, takes time and can therefore be viewed as a function of ontogenesis (Bonett et al., 2014; Hanken, 1999; McNamara, 2012). Thus, form and function, as well as the ontogeny and the underlying developmental patterns of salamanders, are considered in the following part.

Salamanders can exhibit distinct strategies of morphological development (i.e., heterochronic strategies), which involve different speeds as well as outcomes (i.e., ontogenetic morphotypes) (Fig. 8) (Zug et al., 2001). The ancestral and widespread mechanism among salamanders is metamorphosis (i.e., isomorphosis), in which individuals develop from a larval to a metamorphic stage in a short period during ontogenesis (Hanken, 1999; Just et al., 1981; Lofts, 1976; Zug et al., 2001). During metamorphosis, an discrete and accelerated developmental period causes dramatic changes in the morphology of salamanders (Greven et al., 2017; Reilly, 1986; Reilly, 1994; Rose and Reiss, 1993; Schoch et al., 2019) (Fig. 8). Another widespread morphogenetic strategy, however, is paedomorphy (a form of heterochrony), in which salamanders do not undergo metamorphosis but rather reach sexual maturity while maintaining larval characteristics (Denoël and Poncin, 2001; Gould, 1977; Hayes, 1997; Pierce and Smith, 1979; Semlitsch, 1987; Wiens et al., 2005; Zug et al., 2001) (Fig. 8).

Paedomorphosis comes in different variations, and some paedomorphic salamanders can still experience delayed metamorphosis (Clemen and Greven, 2018; Denoel and Joly, 2000; Greven et al., 2015; Jömann et al., 2005). However, the most common form of development in salamanders is direct development, in which as a result of peramorphosis (another form of heterochrony), salamanders develop through a single “metamorphosis” directly inside the egg (Bonett et al., 2014; McNamara, 2012; Wake and Hanken, 2004). These distinct morphogenetic strategies and their outcomes (i.e., ontogenetic morphotypes) must be factored in when working comparatively with the morphology of different groups of salamanders (Wiens et al., 2005).

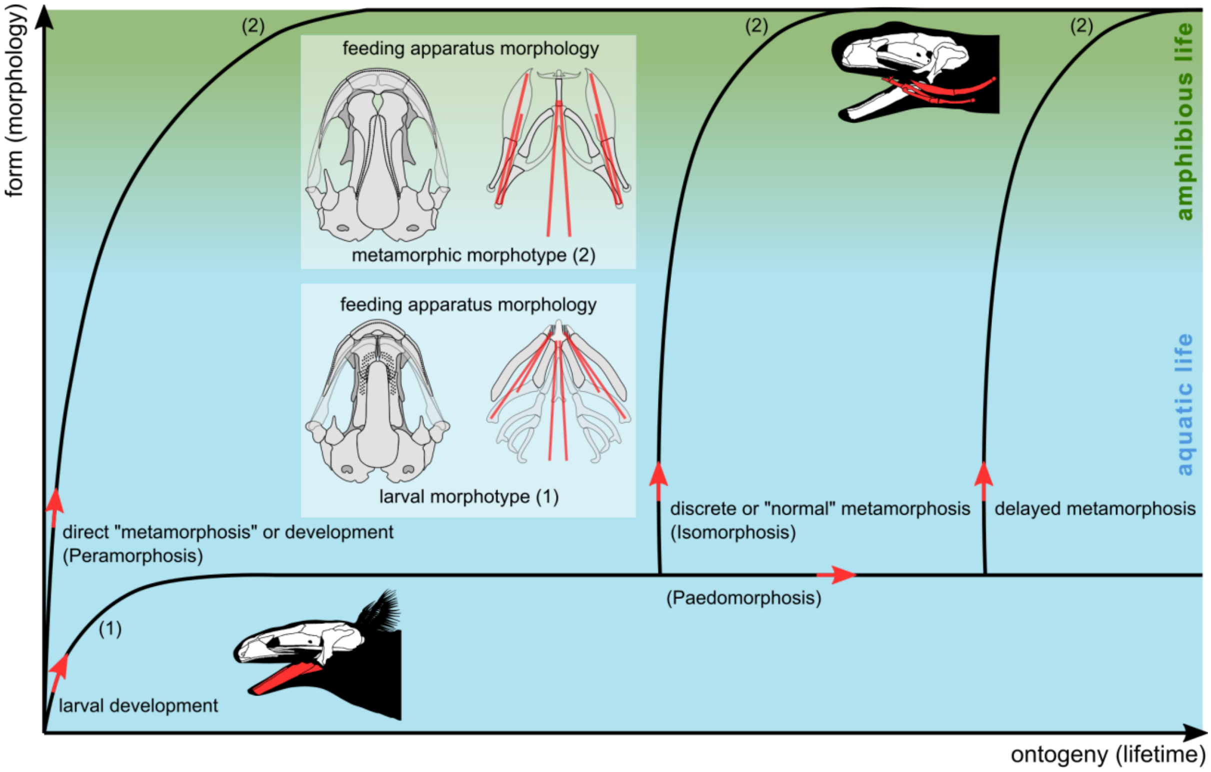


Figure 8: Major morphogenetic strategies and intraoral food processing in salamanders. The labelled arrows on the sketched trajectories indicate different strategies or stages thereof. Cranial morphology of the larval (1) and metamorphic morphotype (2) are displayed (for more details see Fig. 9), and their emergence is positioned on the sketched life-history trajectories. However, since the larval morphotype represents a realm of morphotypes rather than a state of morphological development, its positioning can only be approximated. The position of a mid-metamorphic morphotype remains unclear and is thus neglected. The colour in the background of the coordinate system indicates the lifestyle (aquatic vs amphibious) of a salamander at a given height of the trajectory. The idea of this morphogenetic scheme is derived from previous attempts to display metamorphosis and heterochrony, see for example (Reilly, 1994; Schoch, 2009; Schoch, 2010; Zug et al., 2001). The black sketches represent the processing mode applied by given ontogenetic morphotypes (with a given morphology); larval morphotypes use mandible-based processing (i.e., chewing) as indicated by a red mandible while metamorphic morphotypes apply tongue-palate rasping as indicated by a red hyobranchial system.

To include the ontogenetic morphotypes of salamanders in the comparative analysis of the form-function complex of food processing, I identified (i) larval (Bonebrake and Brandon, 1971; Jömann et al., 2005; Lauder and Shaffer, 1988; Reilly, 1986; Reilly, 1987; Rose, 2014; Worthington and Wake, 1971) as well as (ii) metamorphic feeding apparatus morphotypes (Edgeworth, 1923; Erdman and Cundall, 1984; Greven and Clemen, 2009; Hyrtl, 1865; Kleinteich et al., 2014; Mivart, 1869; Reilly and Altig, 2006; Rose, 2003) based on their presence in states of either (i) larvae and paedomorphic forms or (ii) adult transforming and direct-developing forms, respectively. It has been argued that the accurate determination of a trait as larval or paedomorphic depends on the nature and ontogeny of its phylogenetically close relatives (i.e., local phylogenetic interpretation) for phylogenetic analysis (Fink, 1982). However, as 'only' the evolution of intraoral food processing across the currently accepted phylogeny of salamanders (Pyron and Wiens, 2011) shall be interpreted in the following, a global phylogenetic interpretation was used. In other words, the ontogenetic morphotypes of the feeding apparatus are compared across all salamander families to identify typical larval (or paedomorphic) and metamorphic characteristics.

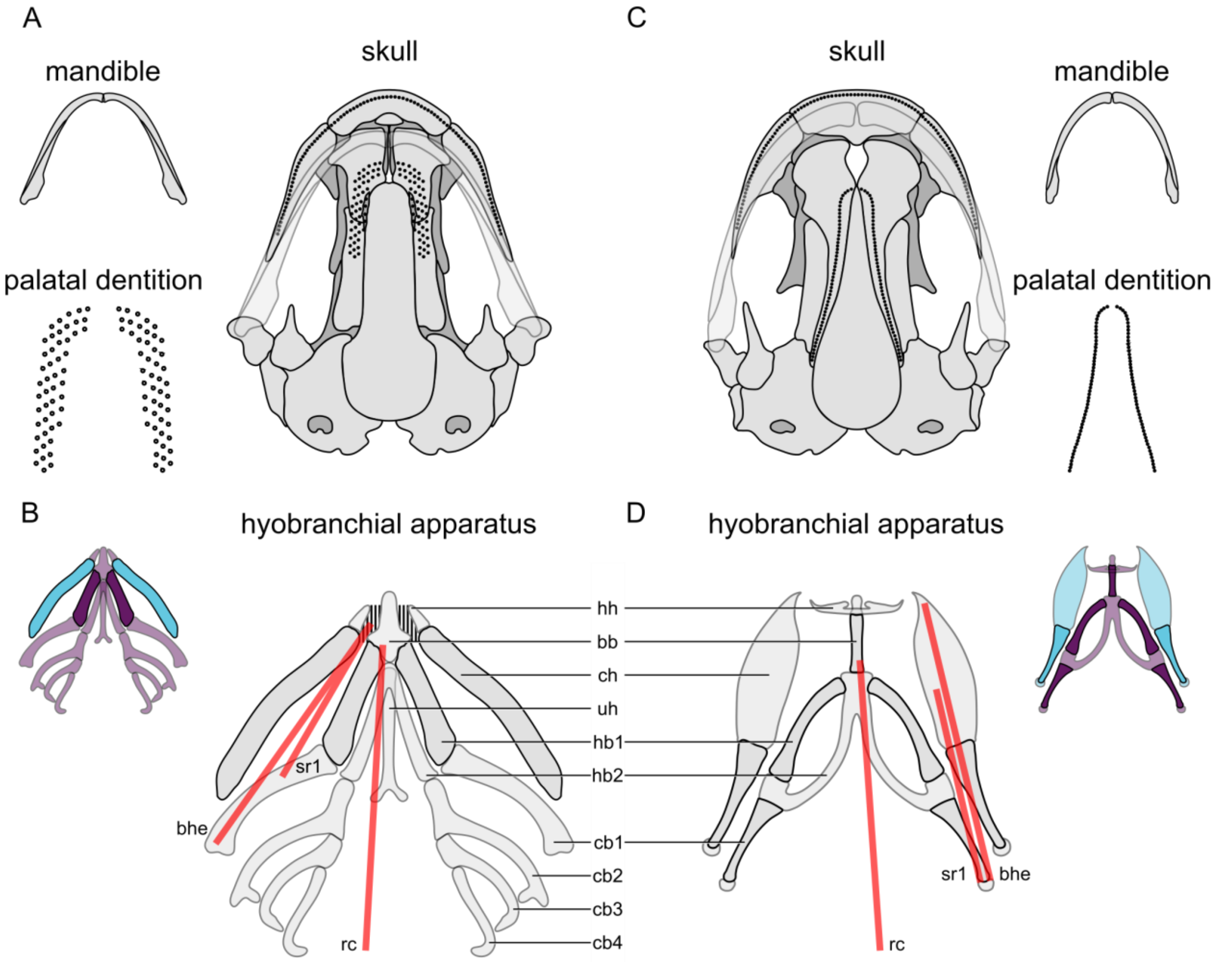


Figure 9: Morphological development of the feeding apparatus. (A) skull and (B) hyobranchial apparatus of a generalized salamander larva and (C) skull and (D) hyobranchial apparatus of a generalized metamorphosed salamander. All schematics are from a ventral perspective**.** During metamorphosis, salamanders reduce the posterior branchial arches (cb2-4) as well as urohyal and mechanically decouple the hyoid arch (blue) from the branchial arch (purple); a rearrangement specific for metamorphic salamanders that allows protraction of the tongue pad-bearing tip of the branchial arch (i.e., basibranchial) by contraction of the subarcualis rectus and the branchiohyoideus externus muscles. Retraction is powered by the rectus cervicis muscle. Note that, for the sake of clarity, only the respective sides of the muscles that point away from the marking in the middle of the two hyobranchial systems (B/D) are shown. Abbreviations: (bb) basibranchial, (bhe) branchiohyoideus externus, (cb1–4) ceratobranchial 1–4, (ch) ceratohyal, (hb1–2) hypobranchial 1–2, (hh) hypohyal, (uh) urohyal, (rc) rectus cervicis, and (sr1) subarcualis rectus.

The structures of the ontogenetic morphotypes that are associated with feeding exhibit remarkable similarities between salamander taxa. For example, larval morphotypes possess tongues with relatively little internal movement potential (Erdman and Cundall, 1984; Heiss and Grell, 2019), V-shaped mandibles (Lauder and Shaffer, 1985; Schoch et al., 2019), and palatal dentition patterns, in which the teeth are located mostly in the anterior region (Clemen and Greven, 2013; Greven et al., 2017; Rose, 2003). In contrast, metamorphic morphotypes possess tongues with relatively great internal movement potential (Findeis and Bemis, 1990; Heiss and Grell, 2019; Reilly and Lauder, 1990b), U-shaped mandibles (Schoch et al., 2019), and palatal dentition patterns, in which the teeth often reach into more posterior regions (Accordi and Mazzarani, 1992; Reilly, 1986; Rose, 2003) (Fig. 9).

Given the details of these morphotypes, the data on the change in the food processing behaviour in Alpine newts presented here (ch. III) suggest that this switch is directly linked to the change from a larval to a metamorphic morphotype. The form-function complex of food processing of the Alpine newt switched from mandible-based chewing in the larval morphotype (Fig. 8A) to tongue-based processing in the metamorphic morphotype (Fig. 8B) during ontogeny. This represents an intriguing ontogeny of form and function of the feeding apparatus in an exemplary salamander species. As suggested in the methodological background of this work (1.3 Methodological synthesis), the comparative study of form and function of food processing across salamander taxa could help to understand the development and evolution of intraoral food processing in salamanders.

Regarding the distinct processing mechanisms of the Italian crested newt and the lesser siren, these data initially are not intuitive since both salamanders are sexually mature (adults) and thus did not show different ontogenetic stages *per se*. However, when comparing the underlying morphology of both salamanders and their respective mechanisms of food processing with those of the ontogenetic morphotypes of the Alpine newt, it becomes apparent that there are similarities to be found between those species. On the one hand, the Italian crested newt and the metamorphic morphotype of the Alpine newt had a metamorphic morphology and used tongue-palate rasping to process their food (Fig. 7A and D); while on the other hand, the lesser siren and the larval morphotype of the Alpine newt exhibit a larval morphology and a processing mechanism that qualifies as chewing (i.e., mandible-based intraoral food processing) (Fig. 7B and C).

In fact, the lesser siren is known to exhibit paedomorphy, which is characterized by an arrest in the differentiation of somatic features during early ontogeny (Noble and Marshall, 1932; Reilly and Altig, 2006; Reiss, 2002; Rose and Reiss, 1993). Thus, the lesser siren features larval musculoskeletal characteristics (Clemen and Greven, 1988; Davit-Béal et al., 2007; Diogo and Abdala, 2010; Reilly and Altig, 2006) distinct from those of metamorphosed salamanders (Carroll and Holmes, 1980; Estes, 1965). Hence, since the form of the feeding apparatus represents that of larval salamanders (i.e., a larval morphotype), so does the food processing mechanism (i.e., chewing). Consequently, these results suggest a model of the intraoral food processing ontogeny in salamanders, which involves a change from chewing to tongue-palate rasping (function) and from a larval- to a metamorphic morphotype (form) via metamorphosis (as suggested in Fig. 8). Salamanders with heterochronous morphogenetic strategies (i.e., peramorphosis and paedomorphosis), however, only exhibit the food processing mechanism typical of their respective morphotype. This model is supported by the fact that metamorphic newts (i.e., the Alpine newt and the Italian crested newt) apply consistent food processing mechanisms (tongue-palate rasping), paedomorphic salamanders (i.e., the lesser siren and the Alpine newt) deploy consistent food processing mechanisms (chewing), and larval and paedomorphic as well as isomorphic and peramorphic salamanders (i.e., larval morphotypes and metamorphic morphotypes respectively) exhibit overall similar morphologies (Rose, 2003), which in turn likely facilitate similar behaviours. Regarding the particularly early arrest of the differentiation of somatic features in the lesser siren resulting in an early larval morphotype (Carroll and Holmes, 1980; Reilly and Altig, 2006; Rose, 2003) as well as its mandible-based rasping mechanism (ch. III) and the fact that salamanders eat virtually during their entire larval development (Kuzmin, 1997), the model might be extended by the idea that early larval morphotypes generally apply complex 3D mandible-based chewing as seen in the lesser siren, while later larval morphotypes apply more simple vertical chewing as seen in paedomorphic Alpine newts. This idea is corroborated by my own unpublished data from fire salamander early-stage larvae (*Salamandra salamandra*; Linnaeus, 1758). The shift from 3D chewing to simple vertical chewing might be explained by the larval development of the jaw joint, ligaments, and muscles attached to the lower jaw, which in combination prevent mobile lower jaws and thus constrain 3D chewing. However, since detailed data on the early larval development of cranial structures are scarce for salamanders, this idea had not been added to the model.

The model for the food processing ontogeny in salamanders contrasts with the ontogeny of aquatic food intake, as salamanders have been shown to change their food processing during ontogeny, while suction feeding (i.e., a form of the inertial suction strategy) is used to ingest food across all ontogenetic stages (Heiss and Grell, 2019). The fact that aquatic, intraoral food processing changes during ontogeny, while aquatic ingestion remains similar, can be explained by changes in the underlying morphology. Despite ontogenetically changing morphology and the resulting shift in suction feeding performance, the inertial suction strategy is not prevented (Heiss et al., 2013a; Heiss et al., 2015; Lauder and Reilly, 1990; Reilly, 1995; Reilly, 1996; Reilly and Lauder, 1988a) while the aquatic food processing might either be functionally constrained (i.e., due to changes in morphology) or the changing morphology enables a more efficient mechanism which replaces the ancestral mechanism. The idea that larval chewing is replaced by a more efficient mechanism seems to be corroborated by the fact that longitudinal processing movements (grinding and rasping) are better suited to facilitate the breakdown of food as compared to the relatively simple, vertical mechanism (Takanobu et al., 1998). At first glance, the lesser sirens' longitudinal chewing seems to contradict this assumption, but as discussed earlier, its feeding apparatus likely resembles the form and function of very early larval salamanders. Since the lower jaw becomes firmly attached to the skull during normal larval development in salamanders (Rose, 2003), it can be assumed that larval salamanders can only exert simple vertical chewing movements from a particular stage onwards. However, the question of what causes the switch from mandible-based to tongue-based food processing in salamanders cannot be answered here, since the Alpine newt's mandible has lost its chewing function in the same morphotype (i.e. mid-metamorphic morphotype) in which its tongue became more mobile and free (ch. IV). However, apart from the unresolved reason of the ontogenetic change, the model of these behavioural changes is consistent with the **third hypothesis** that: *As form and function are linked, and analogous morphotypes of distinct salamander taxa exhibit similar feeding apparatus morphologies, the intraoral food processing behaviour is conserved across analogous morphotypes of distinct salamander taxa.* Since the processing mechanics appear to be conserved across comparable morphotypes of salamander taxa. These results raise the question of how these processing mechanisms are distributed across the phylogeny of salamanders.

8.2. Ontogeny and phylogeny

As shown in the previous section, the developmental state of the feeding apparatus, and thus its morphology, appears to define the food processing mechanism. Therefore, to answer the question of how these processing mechanisms are distributed across the phylogeny of salamanders, the model of the ontogeny of food processing in salamanders was combined with morphological data of larval and metamorphic stages of basal representatives of each salamander family (from literature (Bonett and Blair, 2017; Bonett et al., 2014; Ehmcke and Clemen, 2000; Erdman and Cundall, 1984; Fabre et al., 2020; Macaluso et al., 2020; Marks, 2000; Parker, 1882; Reilly and Altig, 2006; Rose, 2003; Schoch et al., 2019; Vassilieva et al., 2013; Worthington and Wake, 1971) and my own investigations) (Fig. 10A) and plotted on the currently accepted phylogenetic tree of salamanders (Pyron and Wiens, 2011) (Fig. 10B).

Since form and function are two aspects of the same quality (i.e., the form-function relationship), the presence of a larval or metamorphic morphotype during ontogeny is represented by chewing or tongue-palate rasping, respectively. Hence, the ubiquitous presence of chewing indicates paedomorphosis (e.g., neoteny), the exclusive presence of tongue-palate rasping indicates peramorphosis (e.g., direct development), and the presence of both processing mechanisms indicates isomorphosis (i.e., metamorphic development) to be common in the respective family.

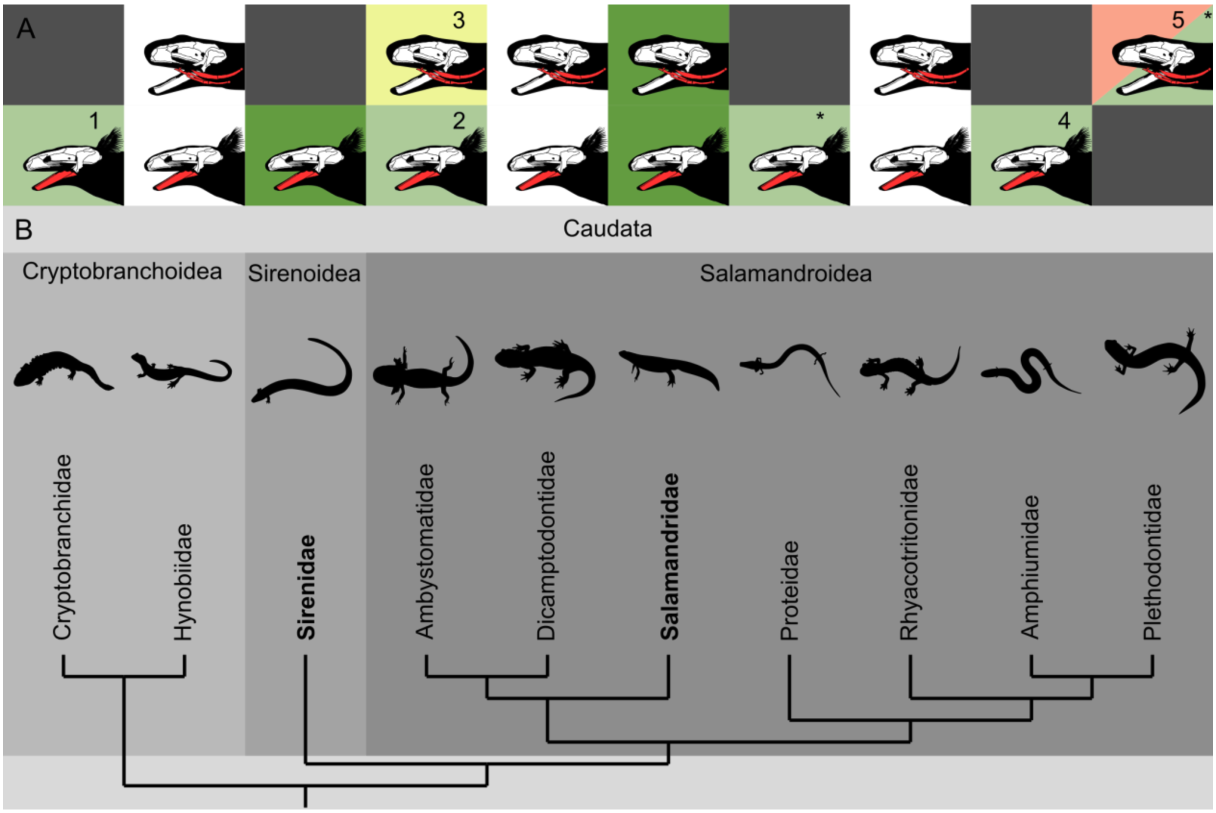


Figure 10: Phylogeny of the food processing ontogeny in salamanders. (A) Model of the ontogeny of intraoral food processing across salamanders based on the basal-most morphogenesis of each family (Bonett and Blair, 2017; Bonett et al., 2014; Ehmcke and Clemen, 2000; Erdman and Cundall, 1984; Fabre et al., 2020; Macaluso et al., 2020; Marks, 2000; Parker, 1882; Reilly and Altig, 2006; Rose, 2003; Schoch et al., 2019; Vassilieva et al., 2013; Worthington and Wake, 1971). The ontogeny of food processing is divided into two phases: mandible-based chewing in larval morphotypes (lower row, i.e., larval and paedomorphic salamanders) and tongue-palate rasping in metamorphic morphotypes (upper row, i.e., peramorphic and metamorphic salamanders). Highlighting: (dark green) primary support, (light green) secondary support, (yellow) potential support, (orange) counter-evidence. Split highlighting indicates conflicting data; no highlighting indicates a lack of data or references. The absence of a "feeding stage" during ontogeny is indicated by dark grey blocks. Own unpublished data are marked with (*) and with references (reference numbers 1-5). References: 1, (Cundall et al., 1987); 2, (Rull et al., 2020); 3, (Dockx and De Vree, 1986); 4, (Erdman and Cundall, 1984); 5, (Schwenk and Wake, 1993). (B) exhibits the relationships among the ten salamander families after (Pyron and Wiens, 2011). Groups (suborders) are framed with different shades of grey.

The data that was used to generate the model (ch. II-V) are classified as primary support (Fig. 10A; dark green). Additionally, my own unpublished findings from the common mudpuppy (*Necturus maculosus*; Rafinesque, 1818) as well as that of the northern slimy salamander (*Plethodon glutinosus*; Green, 1818) were added to test the model (Fig. 10A, marked with *). The paedomorphic common mudpuppy (Proteidae), on the one hand, exhibits a typical larval morphotype and uses mandible-based intraoral food processing, while the peramorphic northern slimy salamander (Plethodontidae), on the other hand, shows a metamorphic morphotype and applies tongue-palate rasping. Hence, the unpublished data support the model of the food processing ontogeny of salamanders. However, as this data has not yet been published and reviewed, they have been classified as secondary support (Fig. 10A; light green).

Further, available data from literature has been added (Fig. 10A, marked with numbers). However, the literature on food processing in salamanders is scarce, and is mainly concerned with other stages of feeding, with the processing function being of minor importance (Dockx and De Vree, 1986; Erdman and Cundall, 1984). The few articles directly focusing on food processing in salamanders leave out the general kinematic pattern and/ or the result of the processing mechanism (Rull et al., 2020; Schwenk and Wake, 1993). Thus, as neither of these articles reports a combination of a detailed description of the food processing mechanism (e.g., kinematics) and nor its result (i.e., processing marks), they have been classified as secondary support (Fig. 10A; light green). However, the few pieces of information gathered from these articles mostly seem to support the model of the food processing ontogeny of salamanders. Since the paedomorphic cryptobranchids and amphiumids, as well as a paedomorphic member of the ambystomatids, exhibit a larval morphotype and chew their food (Cundall et al., 1987; Erdman and Cundall, 1984; Rull et al., 2020) (Fig. 10A).

It is unsurprising, however, that all articles reporting on intraoral food processing in amphibians include chewing, a mechanism that is known because we use it ourselves and seems unmistakable due to typical jaw movement. However, early approaches to the application of X-ray videography for kinematics showed that metamorphic ambystomatids use an intraoral transport mechanism that appears homogeneous to tongue-palate rasping (Dockx and De Vree, 1986). As shown in chapter II, from the point of view that during tongue-palate rasping, the prey is processed but also transported back and forth across the oral cavity, it may not be possible to classify it as either intra-oral processing or transport clearly. In fact, it is argued that tongue-palate rasping, because of its ambiguous nature, resembles a mixture of processing and transportation. This could indicate that the movement of the food during tongue-palate rasping, which is evident from the X-ray videos, resulted in the behaviour being interpreted as mere food transport and that, therefore, food processing went unnoticed. The kinematics described are homogeneous to tongue-palate rasping; however, as no information is available about potential damage to the food, the source is classified as potential support (Fig. 10A; yellow).

In contrast to the previous data that support the model of the food processing ontogeny of salamanders, the plethodontid *Desmognathus* uses a mandible-based processing mechanism (referred to as head-tucking) yet possesses a metamorphic morphology (Schwenk and Wake, 1993). In fact, the head-tucking mechanism and its result (mechanical preparation of the food) are well documented, and this mandible-based processing mechanism is known to commonly snap foods in half (Dalrymple et al., 1985). As a result, this plethodontid head-tucking behaviour appeared to contradict the model of the ontogeny of intraoral food processing in salamanders and was therefore classified as counterevidence (Fig. 10A; orange). In contrast to the food processing behaviour reported here, however, plethodontid food processing has been studied using relatively large foods (earthworms and waxworms) (Dalrymple et al., 1985; Schwenk and Wake, 1993), although (i) such foods are not a large part of the prey spectrum of members of this family (Keen, 1979; Sites Jr, 1978) and (ii) parts of the food regularly protruded from the mouth (Dalrymple et al., 1985; Schwenk and Wake, 1993). Since it had been argued that comparisons between taxa used to study the evolution of feeding behaviour required the use of natural and comparable prey (Maglia and Pyles, 1995), one could argue that the head-tucking of the Plethodontid should not be included in the comparison. In fact, the head-tucking behaviour of plethodontids is significantly associated with feeding upon such relatively large and uncommon foods (Dalrymple et al., 1985). Thus, it might be argued that as the food commonly protruded the jaws of plethodontids, the head-tucking behaviour may not resemble intraoral processing *per se*. According to this logic, processing in plethodontids appears to resemble a red herring for modelling the ontogeny of intraoral food processing in salamanders and should therefore be excluded. This idea is corroborated by my own unpublished findings that show that the plethodontid northern slimy salamander, besides using tongue-palate rasping to process maggots, relied on head-tucking movements when feeding on whole earthworms. Consequently, since all comparable behaviours from the literature seem to support the model of the intraoral food processing ontogeny of salamanders, the model appears to be correct.

8.3. Flexibility

Behavioural flexibility is the ability of animals to reversibly change or adapt their actions in response to new challenges or conditions and thus results in adaptations and diversity of behaviours (Fagen, 1982; Wainwright et al., 2008). Since (i) intraoral food processing facilitates the digestion, and the energy consumption from a food source (Bels and Whishaw, 2019; Bels et al., 1994; Schwenk, 2000a; Schwenk and Rubega, 2005) and (ii) animals are exposed to a variety of different external conditions that might affect processing, it seems plausible to assume that flexibility plays an essential role in intraoral food processing. These external conditions (e.g., environment, the activity of food, and mechanical properties of food) potentially affect food processing. In fact, flexible adjustment of food processing to such external conditions has been suggested to be present in chondrichthyans (Dean et al., 2005; Gerry et al., 2008; Gerry et al., 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), and mammals (Gorniak and Gans, 1980; Thexton et al., 1980; Weijs and Dantuma, 1980) – and thus, across most vertebrates.

Since it was argued that intraoral food processing is absent in salamanders, data on the modulation capacity of food processing behaviour was also lacking. However, it has been shown that salamanders flexibly adapt their food ingestion behaviours to different external conditions (Deban, 1997; Heiss and De Vylder, 2016; Heiss et al., 2013a; Heiss et al., 2015; Maglia and Pyles, 1995) - hence, suggesting that flexibility could also be necessary for food processing. Indeed, the results of the present work indicate that metamorphic salamanders adapt their processing behaviour to the type of prey, while the medium in which feeding occurs does not seem to have a pronounced effect (Fig. 11), since metamorphic salamanders use tongue-palate rasping regardless of the medium (ch. V). Thus, the results of this work support the fourth hypothesis that salamanders can flexibly adjust their processing behaviour to external conditions.

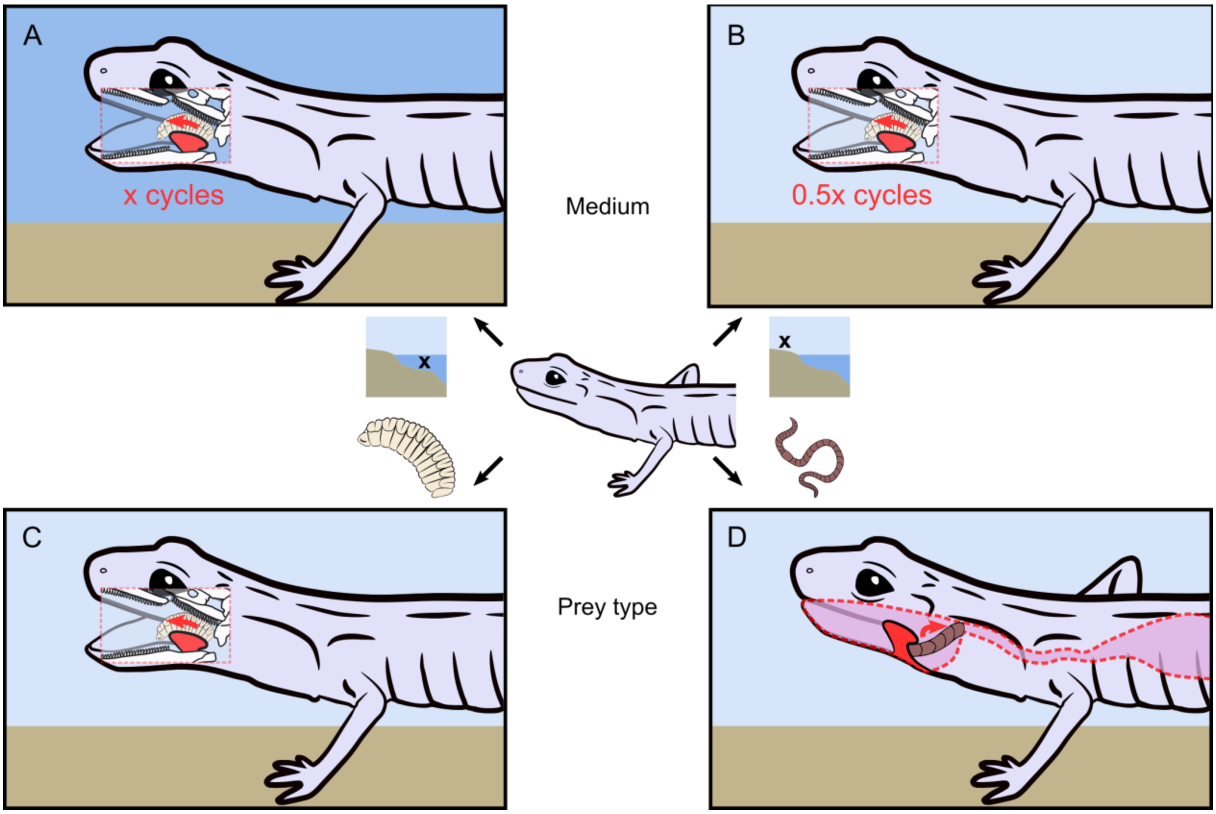


Figure 11: Flexibility of intraoral food processing in salamanders. Each arrow with a schematic in the middle indicates the test condition (A and B medium, C, and D prey type) shown in the subsection of the figure. Feeding (A) underwater, (B) on land, (C) on a maggot, (D) on an earthworm piece. Note that feeding on land required approximately half of the processing cycles (0.5x cycles) that were used underwater (x cycles) and that when feeding earthworm pieces, these were usually swallowed immediately after ingestion while maggots were processed more intensely.

In addition to the results presented here, a recent study has shown that salamanders of a larval morphotype can adapt their processing behaviour to different types of prey (Rull et al., 2020). The adaptation of the processing behaviour to the prey type of salamanders exhibiting larval (Rull et al., 2020) and metamorphic (ch. V) morphotypes was not surprising since such flexible adaptations are common amongst vertebrates (Gerry et al., 2010; Gorniak and Gans, 1980; Throckmorton, 1980; Wainwright, 1989). In contrast, the fact that the medium appears to have little impact on food processing (mainly changes in the number of processing cycles and minor kinematic changes, see Fig. 11A-B and ch. V) was quite surprising as water and air have very distinct physical conditions (Denny, 1993) and in fact, ingestion has been shown to change with the switch from aquatic to terrestrial conditions (Heiss and De Vylder, 2016; Heiss et al., 2013a; Heiss et al., 2015). This raises the question of why intraoral food processing, in contrast to ingestion, does not adapt to the respective medium in metamorphic salamanders. It could be argued that the more or less self-contained system of feeding apparatus and food is relatively liberated from the physical conditions of the environment, not least because of the saliva production of salamanders (Francis, 1961). Regardless of the reason, the independence of the processing behaviour from the surrounding medium is a remarkable feature for the evolution of the feeding behaviour. Because, while other stages of the feeding behaviour (ingestion and food transport) had to adapt to the new medium to enable feeding under the divergent conditions, the processing mechanism remained unaffected and stable in the Alpine newt. The results on form, function, development, and flexibility of how intraoral food processing in salamanders raise the question of how this behaviour might have evolved in tetrapods.

8.4. The Evolution of tetrapod feeding

Several key innovations in the vertebrate feeding apparatus seem to have occurred during the evolution of the intraoral food processing mechanism of salamanders (see ‘3 The rise of tetrapod feeding’). The hyobranchial system in fishes (chondrichthyans, actinopterygians, and dipnoi) and the hyolingual system (tongues) in terrestrial tetrapods are considered to be homologous structures (Reilly and Lauder, 1988b). Therefore, both hyobranchial and hyolingual systems will, from now on, simply be referred to as "tongue" to be able to describe the evolutionary processes as simple as possible.

The rise of tetrapods had been coupled with further remodelling and reduction of elements of the tongue, which thus became more free and moveable (Witzmann, 2013). Further, with the reduction of internal gills during the evolution of tetrapods, the feeding apparatus had been decoupled from aquatic ventilation. Thus, the muscular activities and mechanical processes of the feeding apparatus were able to evolve independently (Schoch, 2014). However, the feeding apparatus of tetrapods were still temporally and structurally related to at least three behaviours (i.e., aerial ventilation, food ingestion, and food transport). Basal tetrapods, like recent amphibians, likely relied on bi-modal or tri-modal systems of gas exchange (Schoch, 2014), and just like in recent amphibians, they likely did not rely critically on aerial respiration. Thus, it might be assumed that their feeding apparatus, like in salamanders, had been relatively freed from functional constraints imposed by respiration altogether. Therefore, the liberation of the feeding apparatus from respiration constraints in tetrapods might be regarded as a license for the evolution of relatively time-intensive, cyclical, tongue-based feeding mechanisms in tetrapods. However, tongue modification that supports enhanced protraction (i.e., terrestrial tongues) similar as seen in metamorphic salamanders is scarce in early tetrapods. Thus, since early tetrapods seemed to have lacked such more mobile and free tongues (Witzmann, 2013), they likely also lacked tongue-palate interactions, as seen in metamorphic salamanders, and relied on aquatic chewing (i.e., fish-like) to process food intraorally. Terrestrial tongues have been argued to have evolved independently within temnospondyls (e.g., lissamphibians) and early amniotes, and thus convergently across tetrapods (Witzmann, 2013). Thus, the tongue-palate rasping of salamanders might have evolved independently of similar behaviours.

In contrast to the evolution of tongue-palate rasping in salamanders (from the amphibian lineage of tetrapods), reptiliomorph tetrapods seem to have evolved terrestrial (or amniote) chewing alongside or next to their transition from water to land. Terrestrial chewing differs from aquatic chewing in that the muscularly and highly mobile sticky tongue is used to transport food mostly directly (i.e., without hydrodynamic drag) across the oral cavity (Hiiemae, 1984; Hiiemae and Crompton, 1985; Iwasaki, 2002). Since water-land transitions are associated with a significant change in the physical conditions of the medium (Denny, 1993), early terrestrial tetrapods were prevented from using hydrodynamic food transport while chewing (Heiss et al., 2018). They, therefore, had to rely on a different intraoral food transport mechanism to coordinate the food during processing and to move it towards the oesophagus in preparation for swallowing. In fact, tetrapods both the amphibian lineage (e.g., salamanders) and the reptiliomorphs developed and improved adhesive and highly moveable terrestrial tongues (Bramble and Wake, 1985; Erdoğan and Iwasaki, 2014; Iwasaki, 2002; Kleinteich and Gorb, 2015), which could be used to manipulate food. Therefore, the terrestrial tongues in reptiliomorphs enabled tongue-based transport, which, despite the new physical conditions, continued to allow the same general chewing mechanism (vertically occluding jaws).

Interestingly, the terrestrial chewing and intraoral food processing of metamorphic salamanders exhibit very similar mechanics (Bels and Goosse, 1989; Bramble and Wake, 1985; Hiiemae, 1984; Hiiemae and Crompton, 1985; Hiiemae and Palmer, 1999; Hiiemae et al., 1981). When broken down into two stages, both terrestrial chewing and tongue-palate rasping basically follow the same trend (compare ch. II-V and (Bramble and Wake, 1985)). During stage one, the mouth opens, and the tongue elevates (or still elevates in consecutive cycles), and more or less shortly before the mouth reaches the maximal opening, the tongue starts depressing (i.e., preceding gape closure). Stage two begins with the closure of the mouth while the tongue still depresses, reaching maximal depression before the mouth reaches maximal closure and then beginning to elevate once more, which in turn marks the start of a potential successive processing cycle. The similar mechanics of amniote-like chewing and tongue-palate rasping might be explained by the fact that both mechanisms rely on the tongue to move the food against and across the palate and/ or dentition. Hence, tongue-palate interactions are at the core of amniote chewing and tongue-palate rasping in salamanders. This raises the question of why salamanders only process food with their tongue but not with their jaws (i.e., chewing).

Considering that a relatively pronounced dentition on the palate was widespread across reptiliomorphs and early amniotes and was only gradually reduced during their evolution (Matsumoto and Evans, 2017), this might suggest that terrestrial chewing initially started as tongue-palate rasping and that the jaws were only secondary incorporated into the processing mechanism. The idea that the jaws had been incorporated later to facilitate enhanced processing might be explained by the fact that intrinsic tongue muscles that allow fine-tuned coordination of the food evolved later within amniotes (Iwasaki, 2002; Matsumoto and Evans, 2017). This idea is further supported by the fact that adductors became more powerful (permitting increased bite-forces) across amniotes once their tongues became more fine-tuned and precise (Matsumoto and Evans, 2017).

However, both mechanisms exhibit temporal shifts, so that as a result, the peaks of tongue and jaw movements can be delayed across distinct taxa (potentially resembling another mechanism) and different foods (potentially resembling behavioural flexibility) (compare ch. II-V and (Hiiemae et al., 1981)). These delays need to be studied in a comparative context and deserve future attention. Despite the unknown nature of these delays, tongue-palate interaction, either (i) mainly as a processing mechanism as seen in salamanders or (ii) mostly as a transport mechanism as seen in recent amniotes (Bels and Goosse, 1989), is likely to resemble the origin of cyclic loop-like food transport motions of fleshy amniote tongues during terrestrial chewing. Terrestrial chewing has been suggested to have a common origin related to the water-land transition (Reilly et al., 2001); however, regarding the data acquired here, it seems plausible to assume that it developed under aquatic conditions – preceding the transition to land, supporting the idea that tetrapods may have developed new feeding mechanisms in their aquatic environment and that these later paved the way for terrestrial feeding mechanisms (Ahlberg et al., 2005; Clack, 2012; Markey and Marshall, 2007; Porro et al., 2015). In any case, mobile and sticky tongues like seen in salamanders likely enabled the usage of new feeding behaviours across tetrapods, thus representing another key innovation in the vertebrate feeding system and the origin of tongue-palate interactions. However, since tongue-palate interactions during feeding (in food transport or processing) appear to be broadly distributed across tetrapods (Witzmann, 2013) and the phylogeny and morphogenesis of early tetrapods still remain controversial (Bolt, 1977; Carroll and Holmes, 1980; Duellman and Trueb, 1994; Marjanović and Laurin, 2019; Schoch, 2014; Trueb and Cloutier, 1991) the question of whether these interactions evolved convergent or divergent remains unanswered.

Conclusion

Amphibians represent the last class of vertebrates whose processing mechanisms remained relatively unknown. Contrary to previously accepted ideas, the present work suggests that salamanders generally engage in intraoral food processing after ingestion, thus expanding the known picture of vertebrate food processing mechanisms. Further, it has been shown that salamanders undergo considerable changes in the form and function of the feeding apparatus during ontogeny. In fact, the development from a larval to a metamorphic morphotype (i.e., metamorphosis) is connected to a switch from mandible-based chewing to tongue-based rasping. This model of the development of the form and function of intraoral food processing has been combined with pre-existing data of morphogenetic peculiarities (i.e., heterochrony) as well as a phylogenetic tree of the currently accepted relationships among the salamanders to generate a phylogeny of the food processing ontogeny in salamanders (Fig. 10).

The model has been used to extend the evolutionary history of intraoral food processing in vertebrates by reconstructing a scenario of how tetrapod feeding might have evolved. The ontogenetic switch from chewing to tongue-palate interactions in salamanders seems to reflect the phylogenetic shift from chewing in fishes (chondrichthyans, actinopterygians, and dipnoi) to tongue-palate interaction in recent tetrapods (lissamphibians and amniotes). Unsurprisingly, the underlying morphological changes in salamanders (i.e., switch from an aquatic- to a terrestrial tongue) also reflect the morphological changes which occurred across the evolution from fishes to tetrapods. Since terrestrial tongues seem to have evolved independently across tetrapods, cyclic loop-like food transport motions of the fleshy amniote tongues during terrestrial chewing (i.e., tongue-palate interaction) likely emerged convergently to tongue-palate rasping in salamanders and thus resemble analogous behaviours. Based on my salamander food processing model, it can be argued that tongue-palate interactions and terrestrial tongues both evolved under aquatic conditions – hence, suggesting that terrestrial style feeding, which might be regarded as a license for a permanent life on land, preceded the water-land transition. However, the most significant difference between the tongue movements of amniotes and that of salamanders is that salamanders rely on tongue-palate interaction to process food while amniotes mostly seem to use these to transport and reorient food intraorally while the jaws perform processing. In fact, kinematically, this resembles a minor difference because the jaws actually move in a way that would allow mandible-based processing (chewing) – the food, however, is not transported to come into contact with the jaws or their dentition. Kinematically, this can be explained by the temporal shift in tongue movement; functionally, however, it remains to be studied why metamorphic salamanders do not chew their food in addition to tongue-palate rasping.

Finally, it should also be mentioned that in the same way that heterochrony complicates the interpretation of the morphological phylogeny of salamanders, it also complicates the interpretation of the tetrapod evolution. Since early tetrapods, like recent amphibians, presumably lived amphibiously, distinct morphotypes of different species could have inhabited a single aquatic habitat during a given time. These morphotypes of different species may have exhibited similar morphologies, complicating a form-based classification. The problem of similar forms during ontogeny, aside from other limitations of palaeontology (e.g., taphonomy), illustrates why the evolution of early tetrapods is an unresolved and heavily debated problem. As a result, the interpretations of this work, which has been based strongly on the current paleontological view of the evolution of tetrapods, are limited in this regard.

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Supplementary Material

a. First article on intraoral food processing in salamanders

Chewing or not? Intraoral food processing in a salamandrid newt

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Egon Heiss, **Daniel Schwarz** and Nicolai Konow

Estimated own contribution: **40%**.

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

See (CRediT taxonomy; supplementary material b) for details.

***Precis*:**
This supplementary material contains the first detailed description of the form and function of the feeding apparatus in a salamandrid newt (Salamandroidea, see supplementary material c), which also demonstrates that mechanical reduction and preparation of food occurs during intraoral food processing. It is shown that metamorphic newts use tongue-palate rasping, a form of tongue-palate interaction in which the tongue that carries the food performs cyclical loop motions to rasp the food across and along the dentition of the palate. Form and function of the feeding apparatus in this salamander are compared to those of fishes and amniotes, with the conclusion that tongue-palate interactions are common amongst gnathostomes – hence prompting the question of whether these evolved convergent or share a common origin.

The results of this chapter support hypothesis 1

and provide initial data for future comparisons to support or reject hypotheses 2 and 3.

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RESEARCH ARTICLE

Chewing or not? Intraoral food processing in a salamandrid newt

Egon Heiss[1,*], Daniel Schwarz[1] and Nicolai Konow[2]

ABSTRACT

Food processing refers to any form of mechanical breakdown of food prior to swallowing. Variations of this behaviour are found within all major gnathostome groups. Chewing is by far the most commonly used intraoral processing mechanism and involves rhythmic mandibular jaw and hyobranchial (tongue) movements. Chewing occurs in chondrichthyans (sharks and rays), actinopterygians (ray-finned fishes), dipnoi (lungfishes) as well as amniotes and involves similarities in the patterns of muscle activity and movement of the feeding apparatus. It has been suggested that amniote chewing, which involves the interaction of movements of the mandibular jaw and the muscular tongue, has evolved as part of the tetrapod land invasion. However, little is known about food-processing mechanisms in lissamphibians, which might have retained many ancestral tetrapod features. Here, we identified a processing mechanism in the salamandrid newt, *Triturus carnifex*, which after prey capture displays cyclic head bobbing in concert with rhythmic jaw and tongue movements. We used high-speed fluoroscopy, anatomical reconstructions and analyses of stomach contents to show that newts, although not using their mandibular jaws, deploy a derived processing mechanism where prey items are rasped rhythmically against the dentition on the mouth roof, driven by a loop motion of the tongue. We then compared patterns and coordination of jaw and tongue movements across gnathostomes to conclude that food processing in this newt species shares traits with processing mechanisms in fish as well as amniotes. This discovery casts salamanders as promising models for reconstructing the evolution of intraoral processing mechanisms at the fish–tetrapod split.

KEY WORDS: Foraging, Manipulation, Mastication, Tongue, Palatal dentition, Amphibia

INTRODUCTION

Food processing involves any type of mechanical manipulation of food before swallowing and includes crushing, puncturing, shearing and grinding (Schwenk and Rubega, 2005). Mechanical processing of food facilitates chemical dissociation and nutrient resorption by the digestive tract and, thus, increases the efficiency of energy exploitation from a given food source (Bramble and Wake, 1985; Schwenk, 2000a,b). Processing mechanisms differ substantially across vertebrate groups but coordinated rhythmic and cyclic movements of the jaw, skull and hyobranchial (tongue) system are common in cartilaginous and ray-finned fishes, lungfishes and amniotes (Bemis and Lauder, 1986; Dean et al., 2005; Gans et al., 1978; Gans and Vree, 1986; Gintof et al., 2010; Herrel et al., 1999; Sanford and Lauder, 1989; Schwenk and Rubega, 2005; Schwenk and Wake, 1993; Wainwright et al., 1989). Whereas some cartilaginous fishes, including sharks and rays, use rhythmic chewing to process food within their mandibular jaw systems (Dean et al., 2005; Kolmann et al., 2016), ray-finned fishes exhibit three 'jaw systems' for food processing: (i) raking, using the tongue–bite apparatus (Camp et al., 2009; Hilton, 2001; Konow et al., 2013; Konow and Sanford, 2008; Sanford and Lauder, 1989, 1990), (ii) grinding, using the pharyngeal jaw apparatus (referred to as 'pharyngognathy') (Gidmark et al., 2014; Liem and Greenwood, 1981; Wainwright, 2002; Wainwright et al., 1989) and (iii) chewing, using the mandibular jaw apparatus (Fernandez and Motta, 1997; Gintof et al., 2010; Konow and Sanford, 2008; Lauder, 1981). While raking and pharyngognathy are derived mechanisms that only occur in some ray-finned fish groups, chewing occurs in both fishes and amniotes (Gans et al., 1978; Gintof et al., 2010; Herring et al., 2001; Hiiemae and Crompton, 1985; Schwenk, 2000a; Schwenk and Rubega, 2005). By contrast to fishes, amniotes additionally rely on a derived anatomical feature for intraoral processing: their muscular and highly movable tongue (Iwasaki, 2002). However, the coordination of jaw and tongue movements across amniotes is strikingly similar and it has been suggested that cyclic intraoral processing shares a common origin associated with the tetrapod terrestrialization process (Reilly et al., 2001). If so, and considering that behaviours are genetically determined, with more closely related species generally showing more similarities than distantly related ones (Katz, 2011), we hypothesize that there are similar mechanisms for food processing among members of the extant sister group to amniotes: the lissamphibians.

The question whether lissamphibians process their food, however, remains virtually unaddressed as it has become widely accepted that lissamphibians nearly universally omit food processing and, with only a few exceptions, swallow their food whole and unreduced (Bemis et al., 1983; Lauder and Gillis, 1997; Reilly and Lauder, 1990; Schwenk and Rubega, 2005). Aside from examples of rudimentary processing such as powerful bites, prey shaking or spinning (Bemis et al., 1983; Deban and Wake, 2000; Fortuny et al., 2015; Lukanov et al., 2016; Measey and Herrel, 2006; O'Reilly, 2000; Summers and Wake, 2005; Tanner, 1971; Wake and Deban, 2000), it had been suggested that some salamanders might use palatal dentition and tongue movements to manipulate prey (Deban and Wake, 2000; Regal, 1966; Reilly, 1996). Still, the only elaborate processing mechanism involving complex and rhythmic movements demonstrated so far occurs in plethodontid salamanders from the genus *Desmognathus*. These salamanders employ cyclic head bobbing movements once prey is held between the mandibular jaws, which deliver a series of strong bites (Dalrymple et al., 1985; Deban and Richardson, 2017; Larsen and Beneski, 1988; Schwenk and Wake, 1993). We have observed a similarly elaborate behaviour that follows food capture in the salamandrid

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newt *Triturus carnifex*. The behaviour involves of the order of 9 sequential cycles of 'head bobbing', in concert with rhythmic movements of the jaw and tongue apparatus (Movie 1).

Here, we studied the kinematics of the rhythmic post-capture behaviour in *T. carnifex*. The rhythmicity of this intraoral behaviour, as well as the apparent similarities with intraoral processing behaviours seen in other gnathostomes, led us to hypothesize that *T. carnifex* might use a hitherto undescribed food-processing mechanism. We combined data from high-speed fluoroscopy, three-dimensional anatomical reconstructions by means of micro-computed tomography (µCT), and analyses of stomach content to describe the mechanism underlying the processing behaviour. Our comparisons of the mechanism seen in *T. carnifex* and processing mechanisms used by other gnathostomes seek to develop a better understanding of the diversity and evolution of food-processing and intraoral cyclic behaviours across gnathostome vertebrates.

MATERIALS AND METHODS

Animal care

Seven adult alpine crested newts, *Triturus carnifex* (Laurenti 1768), with snout–vent lengths of 80.5±10.6 mm and a mass of 10.4±2.6 g (mean±s.d.), 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 a water level of 20 cm and an easily accessible land area 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 escaping. The animals were fed twice a week with a variety of red mosquito larvae (Chironomids), firebrats (*Thermobia domestica*), earthworms (Lumbricids) and maggots (*Lucilia* sp.). For the experiments, we fed maggots as standardized prey items and because dipteran larvae are part of the natural food source of *T. carnifex* (Romano et al., 2012). Like other newts, *T. carnifex* seasonally changes between aquatic and terrestrial lifestyles (Griffiths, 1997) but for the experiments described herein, all newts were in their terrestrial phase for at least 3 weeks prior to data collection. 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 (animal experiment codes: 02-042/14, 02-008/15, animal husbandry code: 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 modified protocols of Herrel et al. (2000) and Manzano et al. (2008)]. The animals were anaesthetized with buffered (pH 7.2) aqueous 0.05% tricaine methanesulfonate (MS222) solution and markers were percutaneously implanted by using hypodermic needles on the basibranchial ('tongue bone') and in two animals on the snout tip (between the premaxillary upper jaw bones) and the lower jaw tip (in the region of the dentary symphysis). Immediately after implantation, 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

The newts were placed on a moistened tissue in a Plexiglas enclosure mounted on the experimental table of the X-ray setup. For the preliminary experiments performed at the University of Antwerp, we used a Tridoros-Optimatic 880 X-ray apparatus (Siemens, Erlangen, Germany); for the experiments at the University of Jena, a custom-build biplanar Neurostar setup (Siemens, Erlangen, Germany) was used. 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) to their cuticle. In total, 50 feeding events were recorded from which 106 processing cycles were extracted for statistical analyses described below (10, 21, 22, 24, 29 cycles for individuals 1–5, respectively). X-ray recordings were taken from the laterolateral and dorsoventral projections at 40 kV and 53 mA with a sampling frequency of 250 Hz. The dorsoventral recordings were performed to determine lateral movements of tongue and jaw systems during processing, but as no clear lateral movements were measured, they were excluded from further analyses. However, the dorsoventral image plane was used for the X-ROMM analyses (see below). Next, the resulting raw video recordings were filtered (e.g. gamma correction, contrast, sharpness) and the horizontal (x-axis) and vertical (y-axis) coordinates of previously defined 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 the upper and lower jaw at the point 'occipital' (jaw joint was not always visible in the X-ray movies so jaw displacement was measured at the point 'occipital') (Fig. 1A); (2) head rotation: angular displacement between the two linear slopes connecting (i) the points 'occipital' and 'snout tip' and (ii) the points 'first vertebra' and 'fifth vertebra' (Fig. 1B); (3) longitudinal tongue movement: horizontal (i.e. parallel to the linear slope connecting the points 'occipital' and 'snout tip') displacement of the tongue relative to the point 'occipital'; (4) vertical tongue movement: vertical displacement of the tongue relative to the linear slope connecting the points 'occipital' and 'snout tip'; (5) longitudinal transport of the prey: horizontal (i.e. parallel to the linear slope connecting the points 'occipital' and 'snout tip') displacement of the prey relative to the point 'occipital'; (6) vertical movement of the prey: displacement of the point 'prey' relative to the linear slope connecting the points 'occipital' and 'snout tip' (Fig. 1C).

From movements 1–4, we calculated the kinematic variables summarized in Table 1. To account for different head sizes between individuals, all displacement values for tongue movements were normalized as percentage of the respective cranial length. The cranial length was measured from the laterolateral X-ray recordings and defined as the distance between premaxillary and the occipital condyles (Fig. 1B,C). Calculations and graphic illustrations were performed using Microsoft Excel 2010, custom-written scripts for Matlab (MathWorks, Natick, MA, USA) and the open source software Inkscape.

Statistics

From the descriptive kinematics (examples shown in Figs 2 and 3), we determined relationships between tongue, head and jaw movements and used bivariate correlations to compare coordination between movements (Wainwright et al., 2008). Specifically, we hypothesized tongue, head and gape cycles to be temporally linked. Furthermore, tongue protraction, tongue elevation and head depression on the one

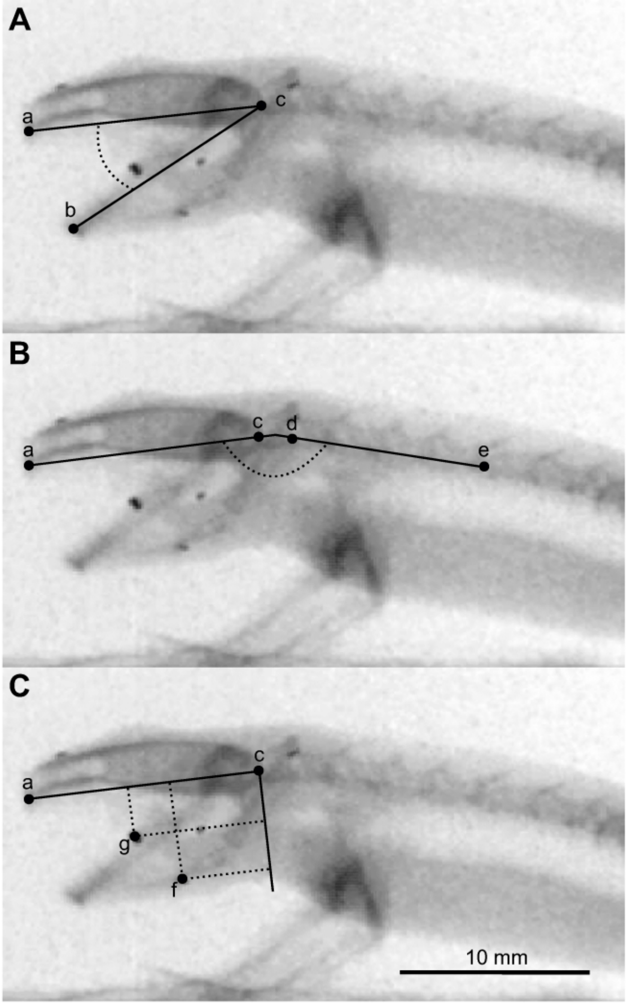

Fig. 1. X-ray frame samples with landmarks. Lateral views showing the seven landmarks that were used to measure movements of (A) gape, (B) head and (C) tongue and prey. Landmarks: a, snout tip; b, lower jaw tip; c, 'occipital'; d, first vertebra; e, fifth vertebra; f, tongue (basibranchial); g, prey. Gape (A) and head rotation (B) 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 C) relative to the skull axis (line connecting points a and c) and the normal line through point c, respectively. Note that the anterior end of the oesophagus is approximately at the height of point c ('occipital').

hand and tongue retraction, tongue depression and head elevation on the other hand were expected to show a high degree of temporal overlap. Based on these hypothesized links and temporal overlaps, we expected functional coordination that was quantitatively tested by performing bivariate correlations between the respective kinematic variables. We tested for (i) correlations between durations of total gape, head and tongue cycles (tongue: both horizontal and vertical movements) and (ii) correlations between tongue protraction, tongue elevation and head depression as well as between tongue retraction, tongue depression and head elevation. In the first approach (i), we only tested temporal variables while in the latter (ii), we tested temporal and magnitude variables (the latter measured as translations or rotations). To account for the multiple tests performed (18), the *P*-value was corrected after Bonferroni to $P \leq 0.0028$. All statistical analyses were performed using Microsoft Excel 2010 and SPSS Statistics 20 software package (IBM).

Specimen fixation and analysis of stomach contents

In order to (i) analyse the condition of processed and swallowed maggots and (ii) study the morphology of the skull with special emphasis on the dentition pattern, two metamorphosed adult newts (both males with snout–vent lengths of 62 and 70 mm) were used (they had not been used in the X-ray experiments). The newts were first fed maggots *ad libitum*: in total 19 (8 and 11, respectively) maggots were consumed by the two newts. The animals were then anaesthetized and subsequently euthanized by immersion in an aqueous solution of 0.5% MS222 buffered to pH 7.2 (Leary et al., 2013). The heads were removed post-mortem and fixed in 4% buffered formaldehyde solution. Next, the stomachs were removed and the maggots contained within were transferred into 70% ethanol solution for preservation. After 2 days, all maggots were analysed using a stereo-microscope and photographed to document punctures and lacerations caused by intraoral processing. As a control, we used (i) 10 unprocessed intact maggots and (ii) 10 unprocessed maggots that were pierced with a needle to visualize a puncture in the cuticle (to prevent misinterpretation of natural structures such as tracheal openings as punctures). Both controls were immersed in 70% ethanol for 2 days.

µCT

For µCT scanning, two newts (both males) were fixed in 4% formaldehyde for 1 month. Then, specimens were dehydrated in a graded series of ethanol and mounted in Falcon tubes. A scan of the whole head was acquired using a SkyScan 1174 (Bruker, Belgium) µCT scanner with a source voltage of 50 kV and an isovolumetric voxel resolution of 7.39 µm. After image acquisition, image stacks were imported into the 3D software package AMIRA 4 (FEI Visualization Sciences Group, Merignac Cedex, France). Based on tomographic image data, relevant structures were segmented by threshold segmentation and visualized using surface renderings.

XROMM

The goal of our XROMM analyses (see Movie 2) was to animate and reconstruct 3D skeletal movements. We followed the standard protocol for Scientific Rotoscoping (Brainerd et al., 2010; Gatesy et al., 2010). In short, polygonal mesh models of the skull, lower jaw and bony hyobranchial elements (derived from µCT scans) were built and a digital avatar of the skeletal elements was constructed. Next, the avatar was aligned to the calibrated biplanar X-ray projections using the XROMM toolbox in Maya (Alias Systems Corporation, Toronto, ON, Canada) and animations were created (Brainerd et al., 2010).

RESULTS

Kinematics

Prey capture was always by the tongue and tongue retraction resulted in placement of the maggot prey (i) between the jaws (only a few cases) or (ii) directly behind the margins of the jaws as the gape was closed. After prey had been transported into the oral cavity, 8.8±3.4 (mean±s.d.) rhythmic cycles involving movements of the skull, jaw and tongue skeletal elements started. The head was rhythmically elevated and depressed, the jaw opened and closed (Movies 1–3) and the tongue moved in an elliptic loop in the lateral view (Figs 2 and 3). With the nose of the subjects pointing left, the tongue motion loop progressed in the counter-clockwise direction. The movement of the prey inside the oral cavity also progressed in a counter-clockwise loop; the prey was first moved dorsally and slightly anteriorly and then ventrally and posteriorly. Accordingly,

Table 1. Kinematic variables of prey processing in *Triturus carnifex* and descriptive statistics

Variable	Description	Value
Mouth opening	Angular displacement from start of mouth opening to maximum gape	25.1±3.9 deg
Mouth closing	Angular displacement from maximum gape to next minimum	25.1±4 deg
Duration of mouth opening	Time from start of mouth opening until maximum gape	313±90 ms
Duration of mouth closing	Time from maximum gape until next minimum	207±91 ms
Head elevation	Angular displacement from start of head elevation until maximum head elevation relative to the trunk	29.6±9.7 deg
Head depression	Angular displacement from maximum head elevation until maximum head depression relative to the trunk	29.7±10.8 deg
Duration of head elevation	Time from start until maximum head elevation	168±64 ms
Duration of head depression	Time from maximum head elevation until maximum head depression	340±109 ms
Tongue protraction	Distance from minimum to maximum horizontal tongue displacement, parallel to linear slope connecting point 'occipital' and point 'snout tip' and relative to point 'occipital' (see Fig. 1)	28.7±7.9% CL
Tongue retraction	Distance from maximum to minimum tongue displacement, parallel to linear slope connecting point 'occipital' and point 'snout tip' and relative to point 'occipital' (see Fig. 1)	29.1±8.1% CL
Duration of tongue protraction	Time from minimum to maximum horizontal tongue displacement	346±119 ms
Duration of tongue retraction	Time from maximum to minimum horizontal tongue displacement	161±41 ms
Tongue elevation	Distance from the minimum to the maximum vertical tongue displacement relative to the linear slope connecting the points 'occipital' and 'snout tip' (see Fig. 1)	28.8±9.4% CL
Tongue depression	Distance from the maximum to the minimum vertical tongue displacement relative to the linear slope connecting the points 'occipital' and 'snout tip' (see Fig. 1)	29.4±9.3% CL
Duration of tongue elevation	Time from minimum to maximum vertical tongue displacement	300±94 ms
Duration of tongue depression	Time from maximum to minimum vertical tongue displacement	208±76 ms

Data are means±1 s.d. % CL, data normalized by cranial length.

the prey was first pressed against the roof of mouth during tongue protraction and then moved away from the mouth roof during tongue retraction. One processing cycle was defined as being from the start of tongue protraction until the completion of tongue retraction. Horizontal tongue movements were chosen as the reference, because in contrast to gape movements they could be clearly assigned to dorsal and ventral head rotation as well as tongue elevation and depression phases. A representative kinematic profile is shown in Fig. 2 and descriptive statistics (Table 1) reveal that during a given processing cycle, the tongue was protracted within 346±119 ms (mean±s.d.) to a peak of 28.7±7.9% cranial length and elevated within 300±94 ms to a maximum of 28.8±9.4% cranial length. Meanwhile, the head was depressed within 340±109 ms over an angle of 29.7±10.8 deg. After the tongue was maximally protracted, it was retracted within 161±41 ms to 29.1±8.1% cranial length and depressed within 208±76 ms to 29.4±9.3% cranial length. At about the same time as tongue retraction, the head was elevated within 168±64 ms over an angle of 29.6±9.7 deg. Gape opening (25.1±3.9 deg within 313±90 ms) and gape closing (25.1±4 deg within 207±91 ms) could not be categorically assigned to any phase of horizontal tongue movement as the tongue is partly protracted and retracted during both gape opening and closing phases. Gape phases only seemed to roughly correspond with vertical tongue movements.

Statistics

Spearman's Rho correlation revealed 18 significant correlations (see Figs 4–6). We tested for correlations between (i) total cycle duration and (ii) sub-movements. Regarding total cycle duration, all variables correlated with each other. Specifically, the duration of the horizontal tongue cycle correlated significantly with the duration of the vertical tongue (r_s=0.72; P<0.001; Fig. 4A), head (r_s=0.96; P<0.001; Fig. 4B) and gape cycles (r_s=0.83; P<0.001; Fig. 4C). The duration of the vertical tongue cycle correlated with the duration of the head (r_s=0.76; P<0.001; Fig. 4D), gape (r_s=0.82; P<0.001; Fig. 4E) and horizontal tongue cycles (see above). The duration of the head cycle correlated with gape cycle duration (r_s=0.86; P<0.001; Fig. 4F), as well as horizontal and vertical tongue cycle duration (see above). When testing the single movement phases, we found significant correlations between the following variables: duration of tongue protraction correlated significantly with duration of tongue elevation (r_s=0.85; P<0.001; Fig. 5A) and head depression (r_s=0.90; P<0.001; Fig. 5B); duration of tongue elevation correlated significantly with duration of tongue protraction (see above) and head depression (r_s=0.84; P<0.001; Fig. 5C); duration of tongue retraction correlated significantly with duration of tongue depression (r_s=0.52; P<0.001; Fig. 5D) and head elevation (r_s=0.56; P<0.001; Fig. 5E); duration of tongue depression correlated significantly with duration of tongue retraction (see above) and head elevation (r_s=0.82; P<0.001; Fig. 5F). The magnitude of tongue protraction correlated significantly with the magnitude of tongue elevation (r_s=0.68; P<0.001; Fig. 6A) and head depression (r_s=0.68; P<0.001; Fig. 6B). The magnitude of tongue elevation correlated significantly with the magnitude of tongue protraction (see above) and head depression (r_s=0.56; P<0.001; Fig. 6C). Similarly, the magnitude of tongue retraction correlated significantly with the magnitude of tongue depression (r_s=0.74; P<0.001; Fig. 6D) and head elevation (r_s=0.74; P<0.001; Fig. 6E). The magnitude of tongue depression correlated significantly with the magnitude of tongue retraction (see above) and head elevation (r_s=0.69; P<0.001; Fig. 6F).

Morphology

Salamandrid skull morphology is described in detail elsewhere (e.g. Francis, 1934; Ivanović and Arntzen, 2017; Trueb, 1993); we focus here on observations relevant to food processing. Teeth are found on both the upper (premaxilla and maxilla) and lower (dentary) jaw bones, as well as on the roof of the mouth, specifically the vomerine bones (Fig. 7A). The vomers are flattened bony plates positioned anteriorly in the oral roof between the premaxillae and maxillae. From these flattened plates, a tooth bearing posterior vomerine process extends caudally up to the level of the squamosal base (Fig. 7A) where the rod-like vomerine process overlies the large parasphenoid. The vomerine teeth are arranged in an arc-like fashion at the interface between the flattened vomer and posterior vomerine process. The dentition extends posteriorly along the vomerine process to form two parallel rows of teeth. The vomerine teeth are

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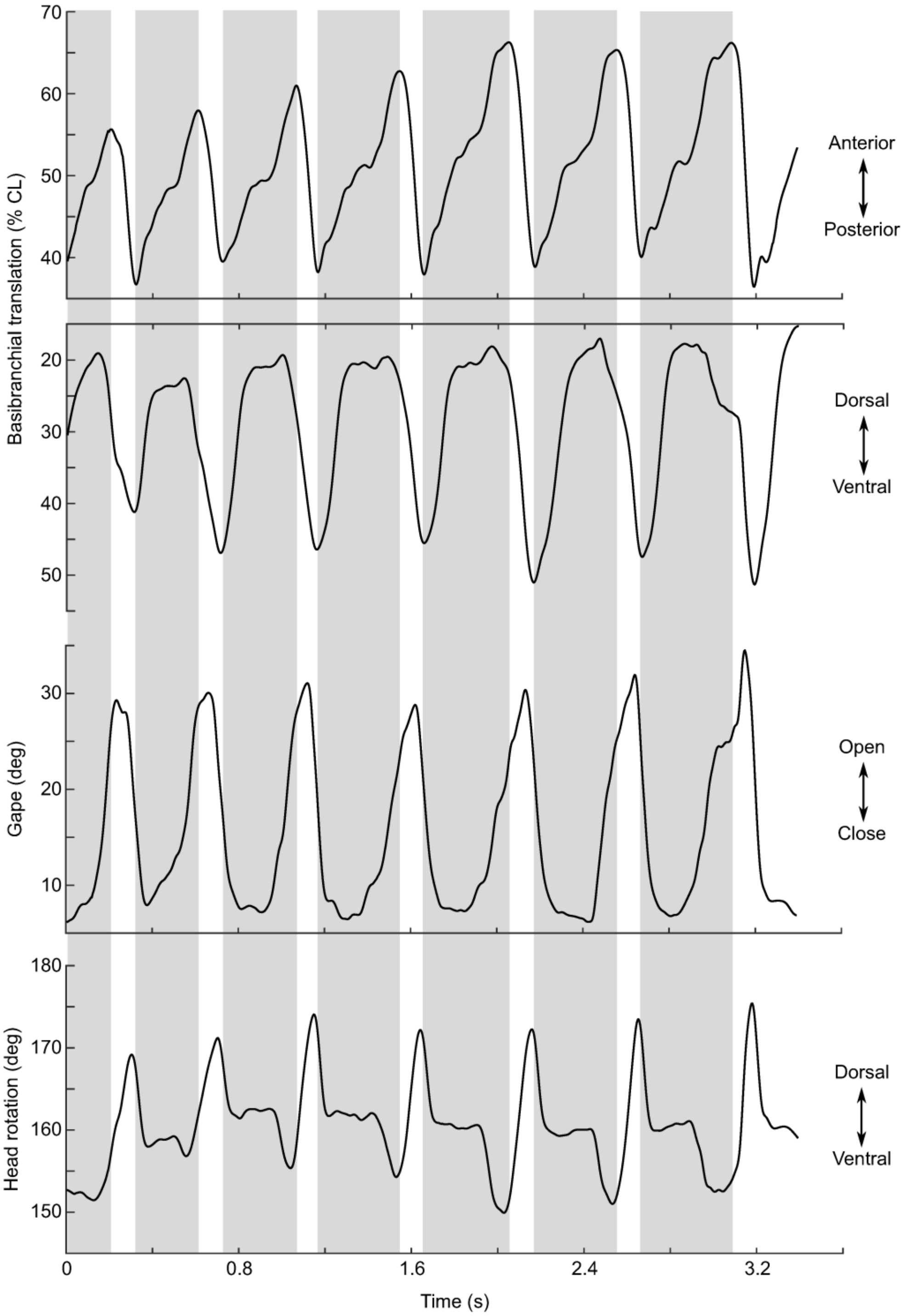

Fig. 2. Representative kinematic profile. Basibranchial (tongue), gape and skull displacements are shown during a typical intraoral processing event in *Triturus carnifex* following prey capture. As a reference, tongue protraction phases are indicated by grey bars. CL, cranial length.

about half the size of the teeth found on the jaws, but densely arranged, sharply pointed and slightly posteriorly recurved (Fig. 7A). Medially to the vomerine tooth rows lie two additional rows of small denticles (Fig. 7A).

Analysis of stomach contents

Both the newts that were fed maggots for further stomach contents analyses showed the characteristic – presumably processing – behaviour (described in the kinematics section) after prey capture. Maggots were captured by the tongue and transported directly beyond the jaws, so puncturing of the maggots by the closing jaws can be excluded in these experiments. Microscopic examinations revealed clear lesions characterized by a distinct outline all over the surface of the processed maggots (Fig. 7B). In contrast, the control maggots only showed the puncture that was manually caused with a needle (Fig. 7C). Apart from that manually induced puncture, no further lesions were evident. The lesions caused by processing were characteristically small roundish perforations with a diameter of 30–50 µm or elongated incisions of up to 500 µm length. On average, the 19 processed maggots showed 21.6±11.6 (mean±s.d.) lesions.

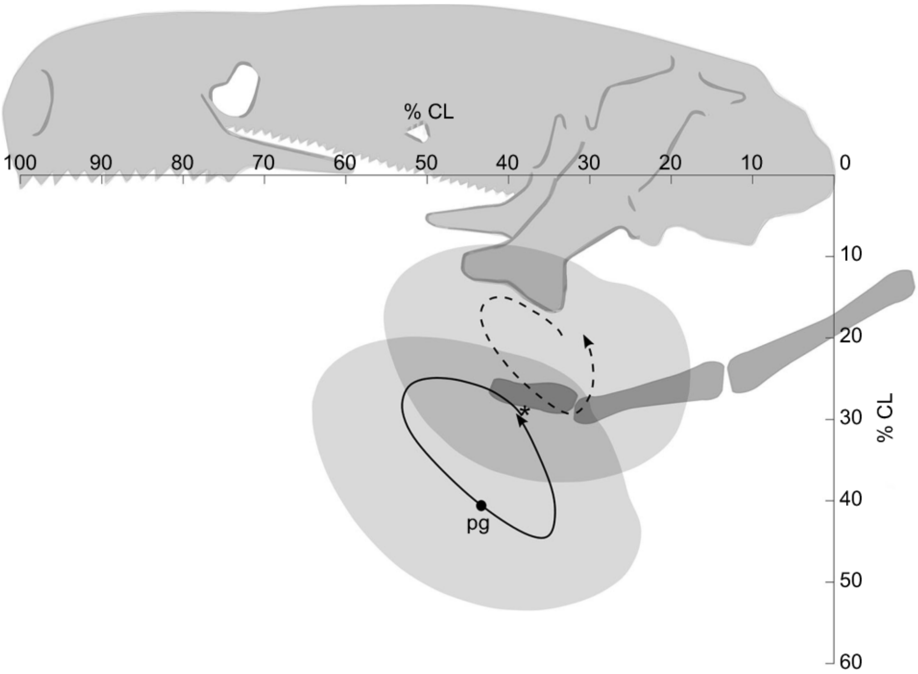

Fig. 3. Diagram showing tongue (basibranchial) and prey movements with the 2D cranial reference coordinate system in *T. carnifex*. The counter-clockwise loops indicate motion of the tongue (continuous line) that moves prey (dashed line) along the mouth roof. The loops prescribe the mean trajectory, with grey areas indicating 68% confidence interval of 106 cycles from five individuals, normalized to cranial length (see also Fig. 1C). The start of gape opening (asterisk) and peak gape (pg) are indicated on the tongue loop.

DISCUSSION

Our experiments on *T. carnifex* reveal a previously undescribed processing mechanism for lissamphibians that involves rhythmic and cyclic movements of the skull, jaw and tongue (hyolingual) elements. Below, we discuss how food processing is achieved using these element movements, and how this behaviour compares with food-processing behaviours in other vertebrate groups.

How and where does food processing take place inside the mouth of *Triturus*? Like most other post-metamorphic salamandrids, *T. carnifex* has two parallel-running lateral rows of vomerine teeth on the roof of the mouth (Trueb, 1993). Our kinematics data reveal that as the tongue moves anteriorly and dorsally, the skull is depressed, and when the tongue moves posteriorly and ventrally, the skull is elevated. In a cyclic context, the effect of this motion pattern is an anteriorly and dorsally directed movement of the tongue to translate food across the palate, which is adorned with dentition (Figs 2, 3 and 7A). Accordingly, prey is cyclically pressed against and translated across the needle-like vomerine teeth, causing the prey to be pierced. The coordination of anterior tongue movement with respect to head depression (Figs 2 and 5) may increase the mechanical resistance between the protracting tongue and the palate, which in turn is likely to increase rasping efficiency. Our observations of processed maggots from newt stomachs revealed multiple cuticle perforations that are lacking in control maggots that have not undergone food processing (Fig. 7B,C).

The rhythmic and cyclic oral behaviour observed in *T. carnifex* results in mechanical processing of food. This is a distinct behaviour from the intraoral transport of food described for ambystomatid salamanders (Reilly and Lauder, 1990, 1991). Ambystomatid intraoral behaviours are rhythmic, but supposedly only serve to move food away from the mouth aperture and towards the oesophagus and so do not process food. The transport cycles described for ambystomatid salamanders are different from the processing cycles in *T. carnifex*. For instance, the ambystomatid tongue is retracted and depressed during gape opening and the first part of gape closing, stays relatively stationary during the second part of gape closing and only slowly starts protracting and elevating after gape closure. In *T. carnifex*, during the gape-opening phase, the tongue is first protracted and elevated and then retracted and depressed. During the gape-closing phase, the tongue first continues retracting and depressing after which it starts protracting and elevating (Fig. 2). Therefore, there are obvious differences in the coordination of tongue and jaw movements between ambystomatid transport and salamandrid processing. However, it remains unclear how the mechanics of food processing in *T. carnifex* compare with processing mechanisms in other lissamphibians.

There have only been a few descriptions of mechanisms for food processing in lissamphibians and these mechanisms appeared to differ from that of *T. carnifex*. Food processing in *T. carnifex* is different because of the involvement of rhythmic head and jaw movements in concert with cyclic tongue movements to reduce food intraorally. However, there are some superficial similarities with the processing mechanism of desmognathine salamanders (Schwenk and Wake, 1993). Both taxa use rhythmic 'head bobbing' in concert with gape cycles but the mechanisms also involve obvious differences: in *Desmognathus*, the head is elevated during gape opening, followed by rapid depression of the skull and gape closure. Skull depression places the massive, pulley-like atlantomandibular ligaments (connections between the cervical vertebra and lower jaw) under tension to transmit force from head flexion to assist the jaw adductor muscles with gape closure and amplify bite force (Dalrymple et al., 1985; Deban and Richardson, 2017; Schwenk and Wake, 1993). Our anatomical observations reveal that *T. carnifex* lacks the atlantomandibular ligament. Head bobbing in *Desmognathus* applies strong bites to food contained between the mandibular arch elements (Deban and Richardson, 2017). By contrast, our data suggest that *T. carnifex* processes food by rasping it against the palatal dentition with its tongue and not between mandibular arch elements, resulting in a fundamentally different mechanism for food processing.

How does the mechanism of food processing in *T. carnifex* compare with processing mechanisms across gnathostomes? To address this question, we focus on two aspects: (i) coordination between tongue

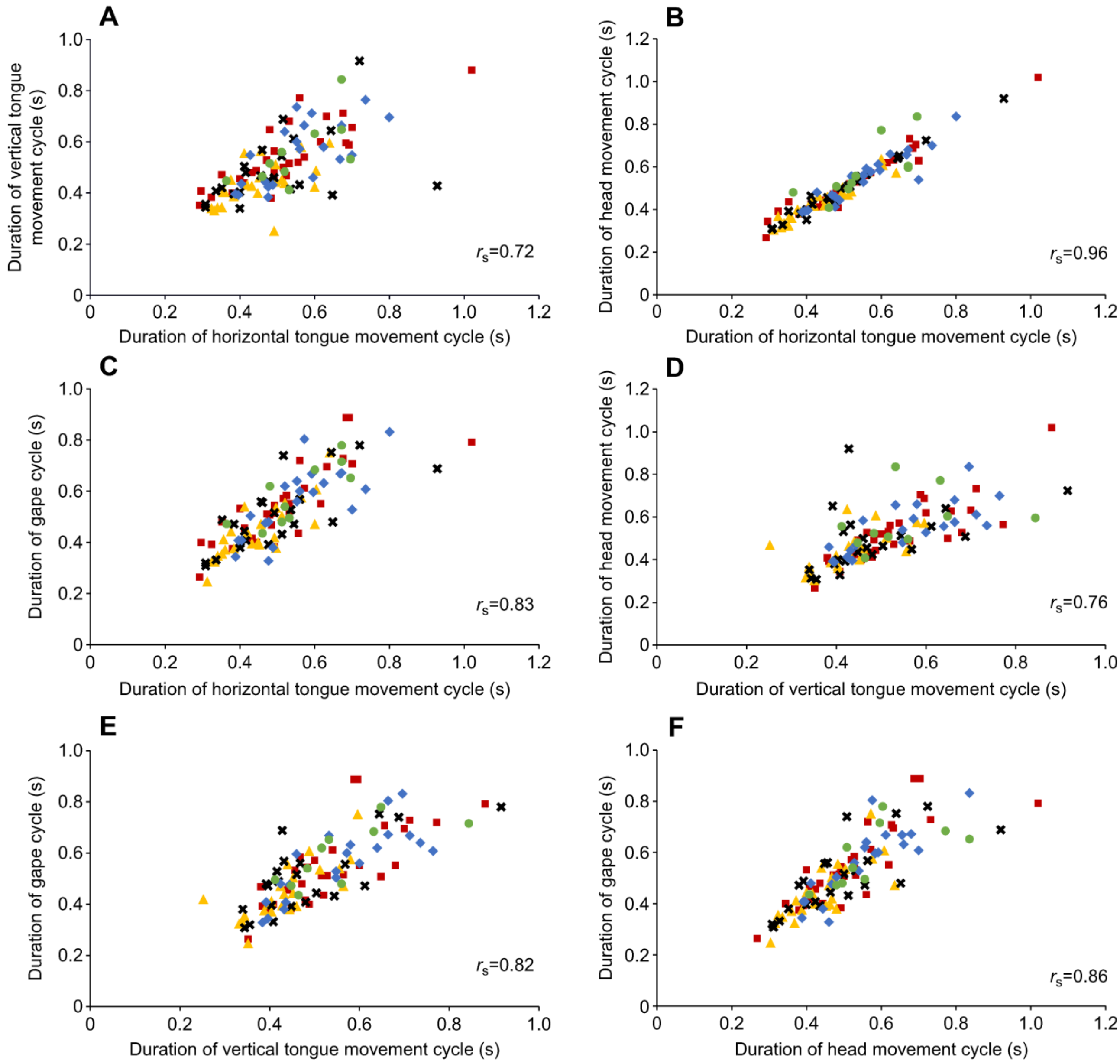

Fig. 4. Significant correlation plots of the duration of total gape, head and tongue cycles. Duration of vertical tongue cycle (A), head cycle (B) and gape cycle (C) against horizontal tongue cycle; head cycle (D) and gape cycle (E) against vertical tongue cycle; and gape cycle against head cycle (F). The five individuals are coded by different symbols and colour. *P*≤0.0028, Bonferroni correction.

and gape cycles, because tongue movements are traditionally associated with – and interpreted relative to – jaw movements in gnathostomes and (ii) use of similar mechanical systems where the tongue rasps against rough palatal surfaces. The hyobranchial system in fishes and the hyolingual system in tetrapods (tongue) are here considered homologous structures (Reilly and Lauder, 1988) and to simplify the interpretations, both hyobranchial and hyolingual systems are henceforth simply referred to as ‘tongue’.

The most commonly occurring mechanism for food processing across gnathostomes is grinding or puncturing of food between the occlusal surfaces of mandibular arch dentition via a repetitive series of bites, also known as chewing. These rhythmic bites serve to crush, grind or puncture food whereas a carefully coordinated and cyclic movement loop of the tongue system serves to reposition food items in between chew cycles. This pattern is recognizable for most gnathostomes but the mechanisms for repositioning food differ between aquatic and terrestrial forms. Aquatic gnathostomes such as sharks, rays, bony fishes and lungfishes use the action of their tongue system to generate water flow to move and reposition food (Bemis and Lauder, 1986; Dean et al., 2005; Gintof et al., 2010; Lauder, 1985). Among fishes that chew, the tongue system moves in the caudal and ventral direction during gape opening so food is transported inwards and repositioned as the gape is closed (Konow and Sanford, 2008; Reilly and Lauder, 1990). Furthermore, in lungfishes (Bemis and Lauder, 1986) and probably also in some ray-finned fishes (Lauder, 1981; Van Wassenbergh et al., 2016) the tongue system can alternatively be elevated and protracted during gape opening to induce a posterior–anteriorly directed flow if food items have to be

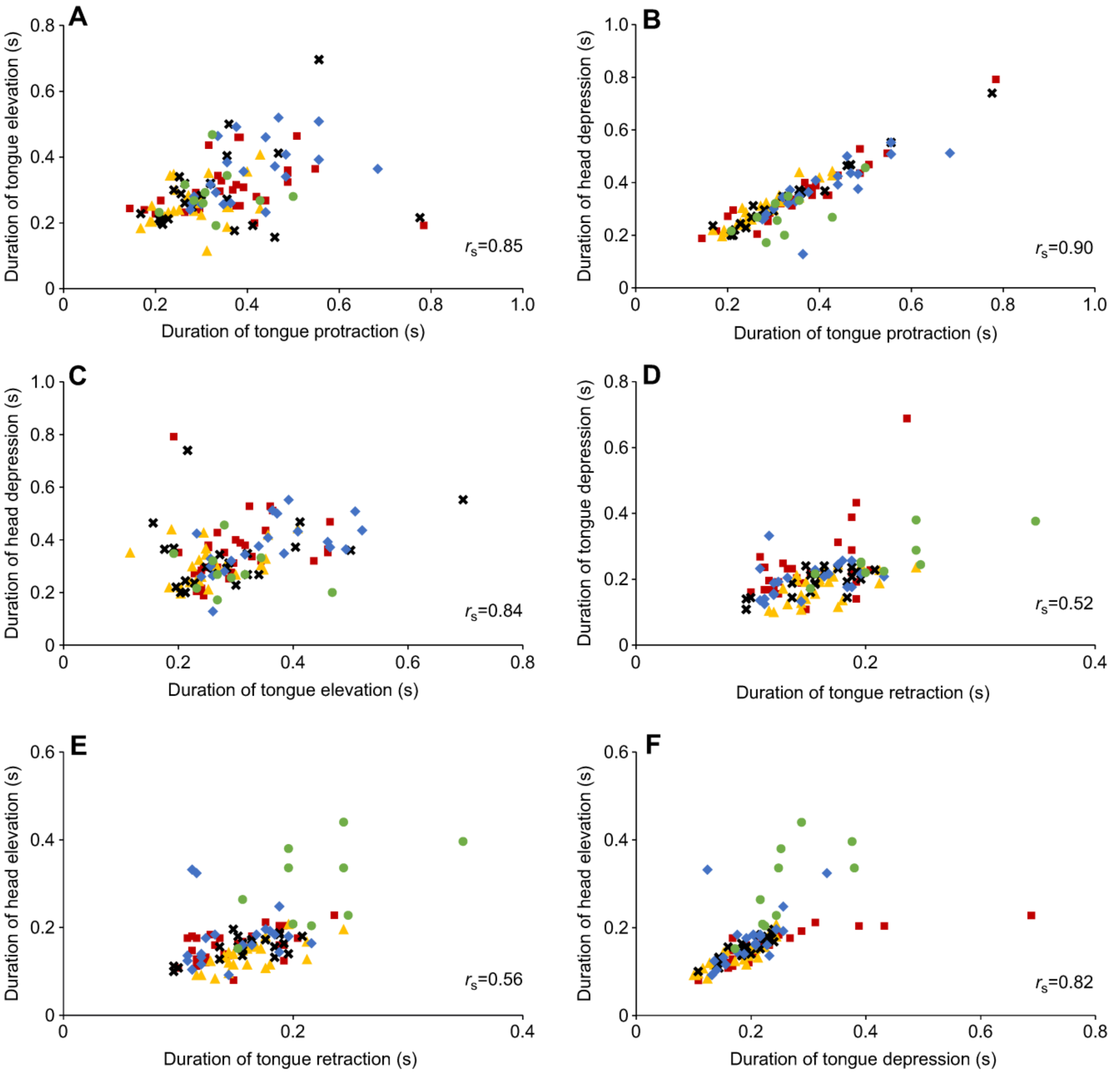

Fig. 5. Significant correlation plots of kinematic variables showing coordination between tongue and head movements in time. Duration of tongue elevation (A) and head depression (B) against tongue protraction; head depression against tongue elevation (C); tongue depression (D) and head elevation (E) against tongue retraction; and head elevation against tongue depression (F). The five individuals are coded by different symbols and colour. $P \leq 0.0028$, Bonferroni correction.

transported from back to front. Hence, coordination of jaw and tongue-system cycles appears to depend on functional requirements. In *T. carnifex*, the coordination of tongue and gape cycles shows some overlap with that of bony fishes and lungfishes but also some differences. The newt tongue initially remains stationary and is then depressed during gape opening and is elevated during most of gape closure. Furthermore, the tongue is partly protracted and retracted during both gape opening and gape closing. In other words, the relationship between vertical tongue movements and gape movements is overall similar to that of fishes in the first phase of the gape cycle, whereas horizontal tongue movements show a phase shift in relation to the gape cycle, when compared with fishes (see also Figs 2 and 3).

In some ray-finned fishes, a further processing behaviour that superficially resembles the tongue–palate rasping in newts is raking. In raking, food is stabilized between the mandibular jaws while the skull is elevated and the tongue apparatus forcefully retracted, causing food shredding by dentition on the mouth roof and tongue tip (basihyal) (Camp et al., 2009; Konow and Sanford, 2008; Sanford and Lauder, 1989, 1990). Raking in ray-finned fishes and tongue–palate rasping in newts appear similar but the coordination of tongue, jaw and skull movements differs. In raking, the tongue loops in the reverse, clockwise direction (rostrum facing left) and although the tongue shreds the food against palatal dentition in both systems, the power stroke in raking involves tongue retraction versus tongue protraction in the newt system. However, some groups of spiny-rayed

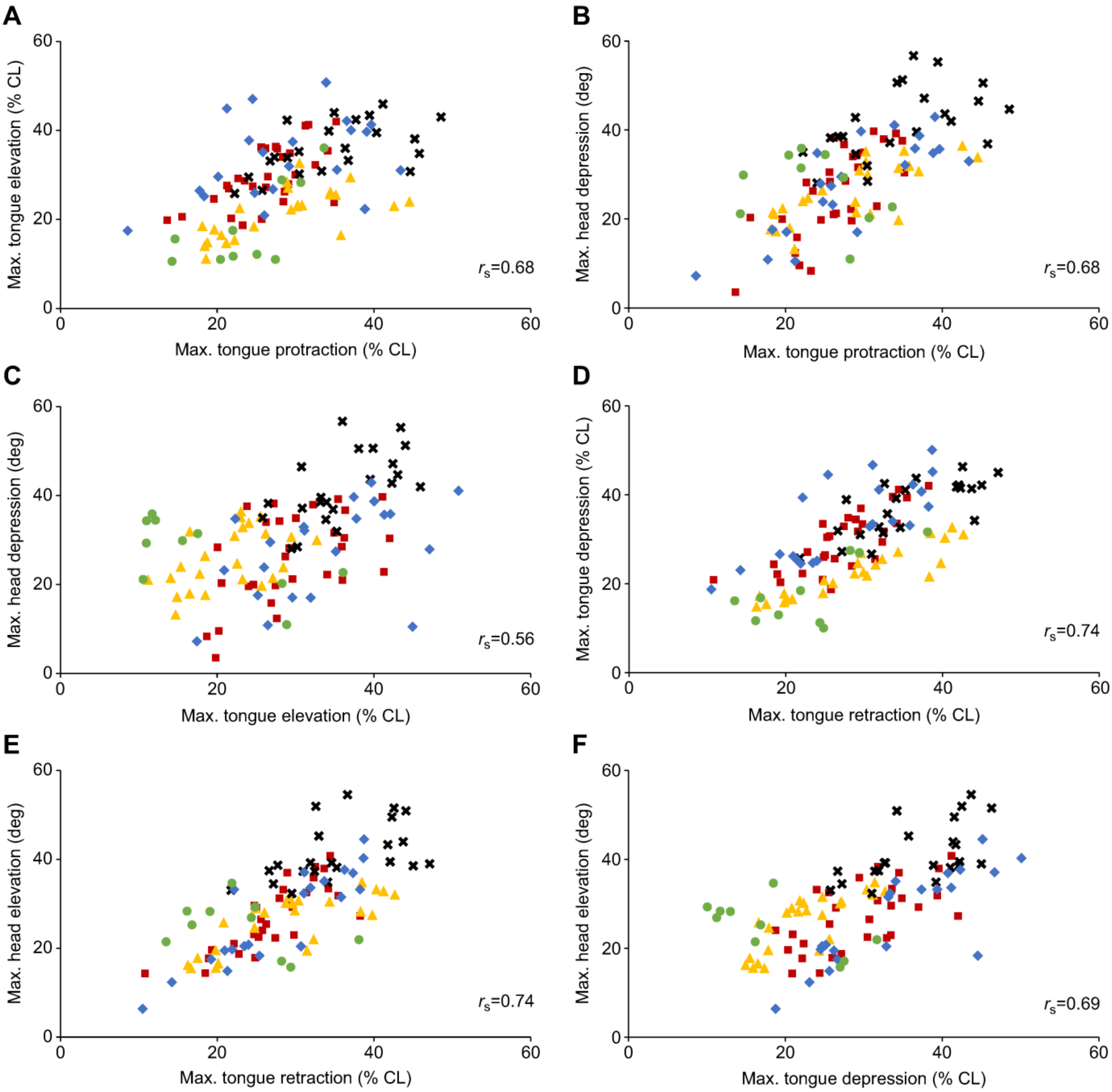

Fig. 6. Significant correlation plots of kinematic variables showing coordination between tongue and head movements in magnitude (translation or rotation). Maximum tongue elevation (A) and head depression (B) against maximum tongue protraction; maximum head depression against maximum tongue elevation (C); maximum tongue depression (D) and maximum head elevation (E) against maximum tongue retraction; and maximum head elevation against maximum tongue depression (F). The five individuals are coded by different symbols and colour. $P \leq 0.0028$, Bonferroni correction.

(acanthomorph) fishes, including anabantoids (Konow et al., 2013; Liem and Greenwood, 1981), may have evolved a raking analogue with the same tongue motion pattern as seen in the newt.

In amniotes, cyclic movements of the tongue system during a bout of chewing first help position the food between the teeth and later help gradually transport food towards the oesophagus in preparation for swallowing (Bramble and Wake, 1985; Herrel et al., 2008; Herrel and De Vree, 1999; Hiiemae and Crompton, 1985; Smith, 1984). Although feeding mechanics differ substantially between amniote groups, a general pattern has been described using a simplified model (Hiiemae and Crompton, 1985; Bramble and Wake, 1985; Reilly and Lauder, 1990): during most of the mouth-opening phase, the tongue is first protracted and elevated and then partly retracted and depressed. During mouth closure, the tongue is mostly retracted and depressed. With the snout oriented to the left, the tongue describes a counter-clockwise loop. In newts, the tongue also loops in a counter-clockwise direction, yet the coordination of this loop with the gape cycle differs from the amniote pattern. During mouth opening, the newt tongue is first stationary and is then depressed (versus elevated and then partly depressed in the amniote model), while during mouth closing, the newt tongue is mostly elevated (versus depressed in the

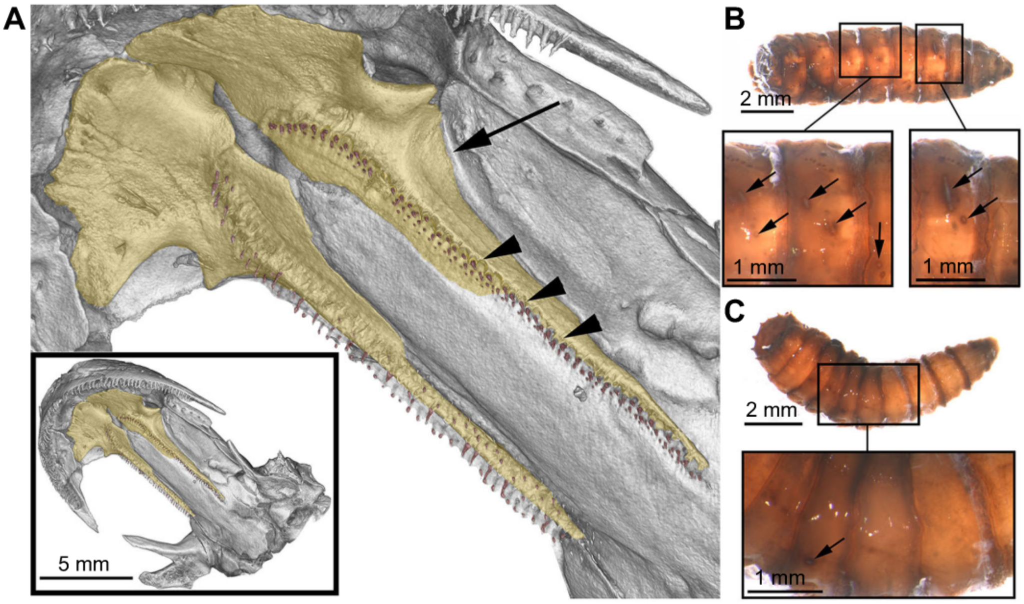

Fig. 7. Morphology of the skull of *T. carnifex* and processed and unprocessed maggots. (A) Overview (inset bottom left) and detail showing a graphical reconstruction of the skull of *T. carnifex* from ventrolateral view, based on a μCT scan. The vomerine bones on the roof of the mouth are highlighted in gold (arrow) and the small vomerine teeth forming two longitudinally running rows are in purple (arrowheads). (B) Micrographs showing a maggot extracted from the stomach of a newt after being processed and (C) a control maggot that was manually pierced with a needle and fixed the same way as those extracted from newt stomachs. Note the multiple perforations and incisions indicated by arrows in the processed maggot while the control only shows the manually induced perforation (arrow).

amniote model). As the gape is opened, the newt first protracts and then retracts its tongue (similar pattern to that in amniotes) and during gape closure, the tongue first continues to retract and then starts protracting (versus mostly retraction in amniotes). Compared with amniotes, the newt therefore shows a phase shift of vertical and partly horizontal tongue movements relative to the gape cycle, yet the direction of the loop is the same. The phase shifts might be due to different mechanisms underlying food processing: amniotes use their tongue to move food into jaw occlusion, which leads to a requirement for tightly coordinated tongue and jaw movements (Alfaro and Herrel, 2001; Hiiemae and Crompton, 1985; Lund, 1991). In contrast, the tongue–palate rasping mechanism in newts appears to require tight coordination of tongue and head movements, more so than tongue and jaw movements. Still, the ubiquitous counter-clockwise loop motion of the tongue suggests that amniotes also use their tongue to move food over the palate (Hiiemae, 2000; Palmer et al., 1997; Reilly et al., 2001; Schwenk and Rubega, 2005). Might this tongue–palate interaction play a role in food reduction in amniotes too? In many groups, mechanical reduction of food might be a minor element of the tongue sliding forward during the chewing cycle but in certain groups like sea cows, the rough keratinized palate is significantly involved in food processing (Werth, 2000). In the echidna and platypus, which are basal, edentulous mammals, tongue–palate interaction replaces tooth function and the tongue is adorned with keratinous spines that interact with similar palatal structures to grind food into a viscous slurry (Doran and Baggett, 1972; Griffiths, 1978; Schwenk and Rubega, 2005). Tongue–palate rasping mechanisms in amniotes remain poorly understood and the kinematics are relatively unstudied but we predict that the movement patterns are similar to those involved in food processing in the newt.

Despite the apparent differences outlined above, intraoral food-processing systems where the tongue rasps food against rough palatal structures are found in different gnathostome groups, opening up the question of whether the underlying motor patterns might have evolved convergently or have a common neuromechanics ancestry. Salamanders may be promising models to begin testing associated motor control hypotheses on the evolution of processing mechanisms in tetrapods because (i) lissamphibians are the only extant anamniote tetrapods that might have retained many ancestral tetrapod features and (ii) contrary to earlier assumptions (for reviews, see Deban and Wake, 2000; Schwenk and Rubega, 2005), salamanders do use rhythmic processing involving tongue, head and jaw systems.

The detection of tongue–palate rasping systems is complicated by movements of the tongue system being only partially visible to the eye, because they occur deep within the oral cavity. This makes X-ray, or alternatively invasive measurement techniques like sonomicrometry, necessary for analysis. A clear distinction between intraoral transport (sensu Gillis and Lauder, 1994; Reilly, 1996; Reilly and Lauder, 1990, 1991) and tongue–palate rasping might not always be possible because the transition from one to the other function is often continuous. For example, cyclic intraoral behaviours in lissamphibians have been suggested to only involve food transport. However, only a few lissamphibian taxa have been studied so far and we think it is highly likely that the electromyographic and light videography approaches used by earlier authors (Gillis and Lauder, 1994; Reilly, 1996; Reilly and Lauder, 1990, 1991) might have caused food-processing behaviours to go unnoticed. Data from lissamphibians are critical for unravelling and reconstructing the evolution of food-processing systems because, aside from being the only extant anamniote tetrapod clade that might have retained many ancestral tetrapod features, salamanders also permit studies of food processing across aquatic and terrestrial environments given the many semi-terrestrial species. Lissamphibians also allow observations of processing changes across the transformation of a hyobranchial to a hyolingual system as they metamorphose from a gill-bearing larva to a tongue-bearing post-metamorphic animal. Taken together, lissamphibians have many traits that make them suitable analogues of early tetrapods that similarly had to undergo structural and functional changes of their oropharyngeal system during terrestrial evolution.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

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

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Data availability

Raw data are available from the corresponding author upon request.

Supplementary information

Supplementary information available online at
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Supplemental material

Movie 1. High-speed light recording and high-speed x-ray recording at 250fps (frames per second) showing intraoral processing in the newt *T. carnifex* played at 30fps. The large radiopaque marker in the high-speed x-ray recording indicates the position of the prey (maggot).

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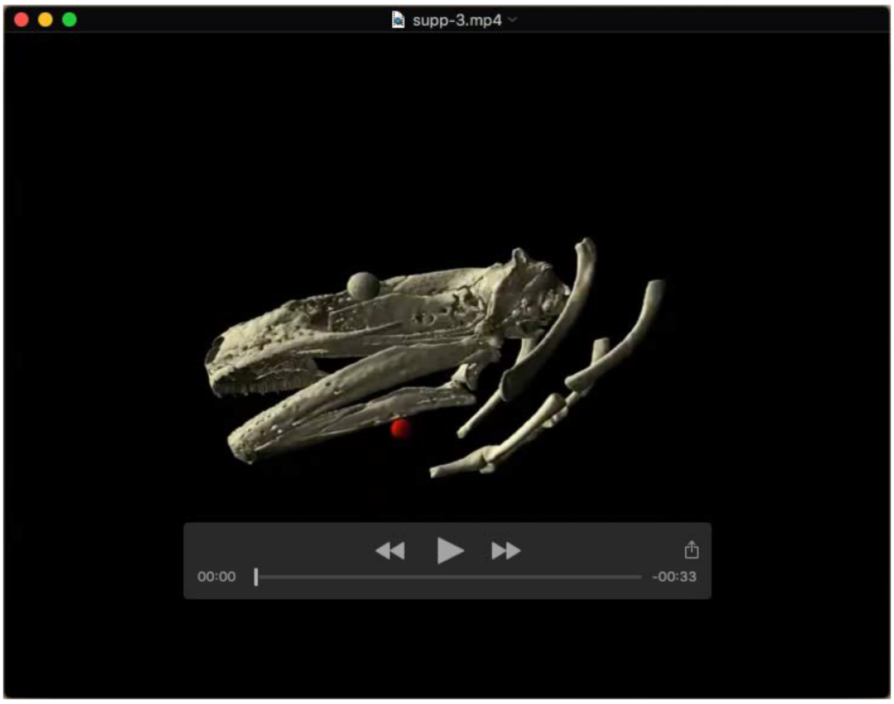

Movie 2. XROMM-animation of processing in the newt *T. carnifex*. The red sphere indicates the position of the prey.

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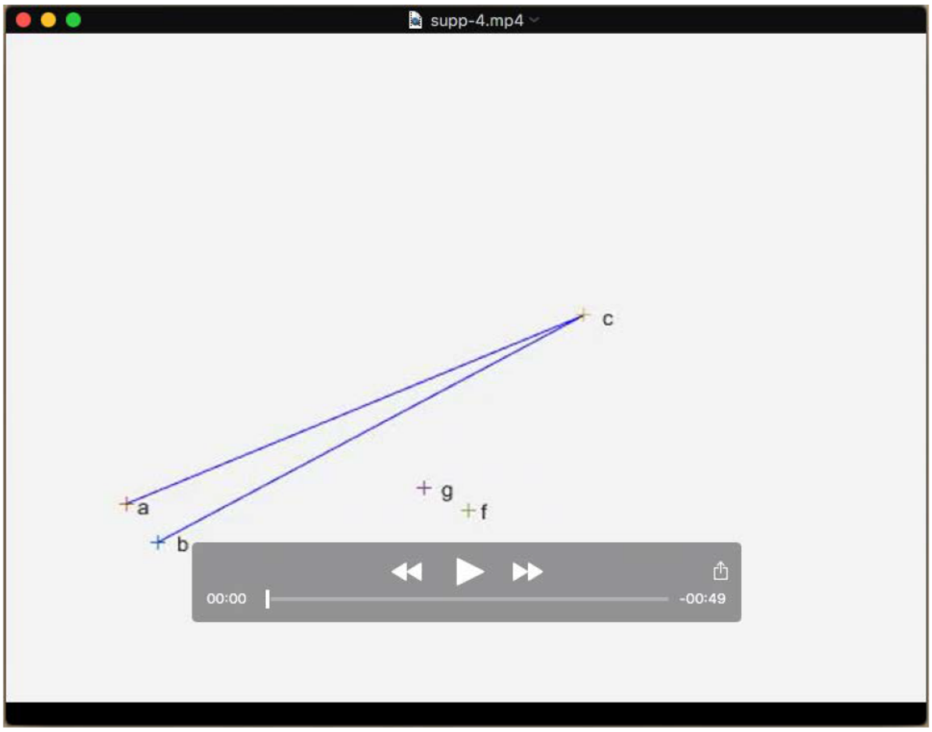

Movie 3. Kinematic animation of processing in *T. carnifex* (same feeding event as shown in Fig. 2). Abbreviations as indicated in Fig. 1: a, snout tip; b, lower jaw tip; c, occipital; f, tongue (basibranchial); g, prey.

b. Author contributions

CRediT taxonomy

Contributor role	**Role definition**
Conceptualization	Ideas; formulation or evolution of overarching research goals and aims.
Methodology	Development or design of methodology; creation of models
Software	Programming, software development; designing computer programs; implementation of the computer code and supporting algorithms; testing of existing code components.
Validation	Verification, whether as a part of the activity or separate, of the overall replication/reproducibility of results/experiments and other research outputs.
Formal analysis	Application of statistical, mathematical, computational, or other formal techniques to analyze or synthesize study data.
Investigation	Conducting a research and investigation process, specifically performing the experiments, or data/evidence collection.
Resources	Provision of study materials, reagents, materials, patients, laboratory samples, animals, instrumentation, computing resources, or other analysis tools.
Data curation	Management activities to annotate (produce metadata), scrub data and maintain research data (including software code, where it is necessary for interpreting the data itself) for initial use and later reuse.
Writing – original draft preparation	Creation and/or presentation of the published work, specifically writing the initial draft (including substantive translation).
Writing – review and editing	Preparation, creation and/or presentation of the published work by those from the original research group, specifically critical review, commentary or revision – including pre- or post-publication stages.
Visualization	Preparation, creation and/or presentation of the published work, specifically visualization/data presentation.
Supervision	Oversight and leadership responsibility for the research activity planning and execution, including mentorship external to the core team.
Project administration	Management and coordination responsibility for the research activity planning and execution.
Funding acquisition	Acquisition of the financial support for the project leading to this publication.

The breakdown of the authors' contributions was adopted from the Journal of Experimental Biology (2020.10.15), *Author Contributions: CRediT Taxonomy*, retrieved from: https://jeb.biologists.org/content/author-contributions

c. Phylogeny of lissamphibians

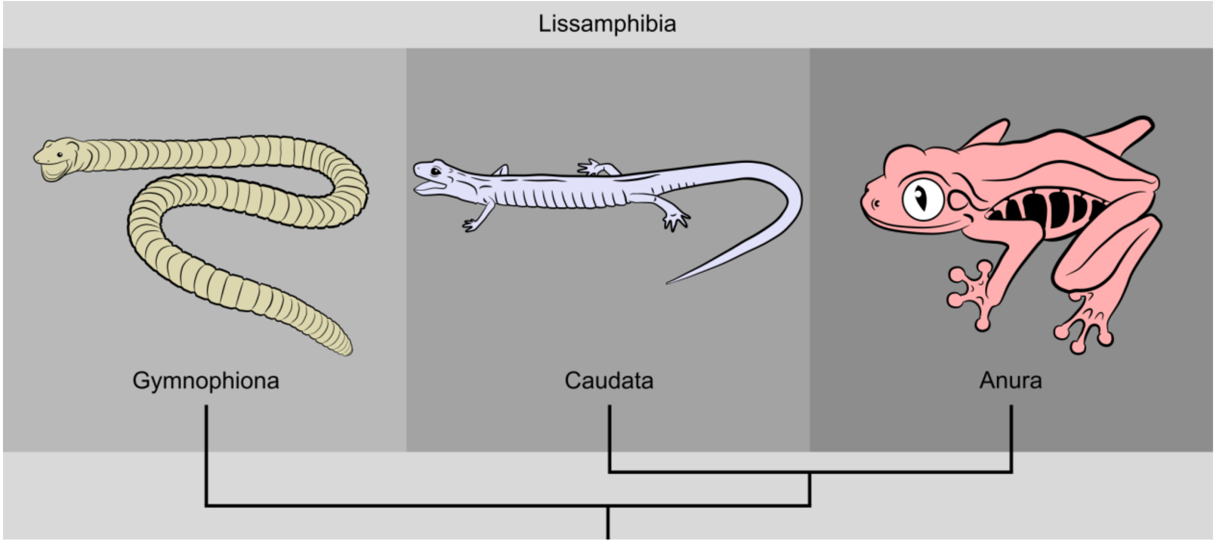

Fig. S1: Phylogeny of lissamphibians. Relationships between recent amphibians, which are framed by different shades of grey, after (Pyron and Wiens, 2011).

d. Phylogeny of salamanders

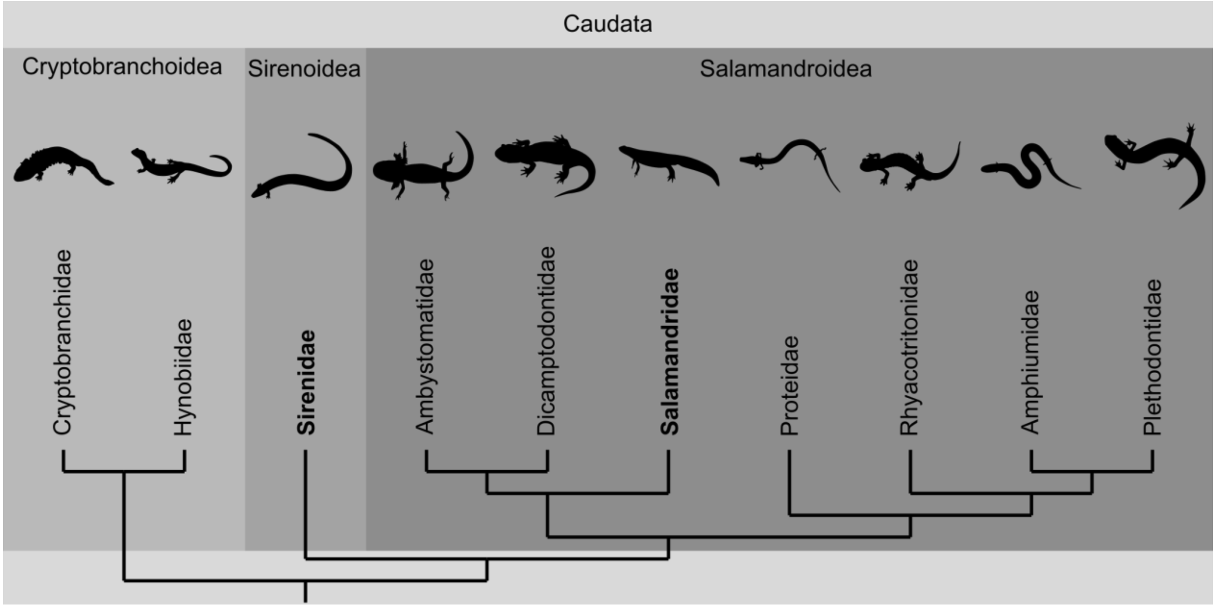

Fig. S2: Phylogeny of salamanders. Relationships among the ten salamander families after (Pyron and Wiens, 2011). Groups (suborders) are framed with different shades of grey.

e. Correction of supplementary figure S1 from page 68

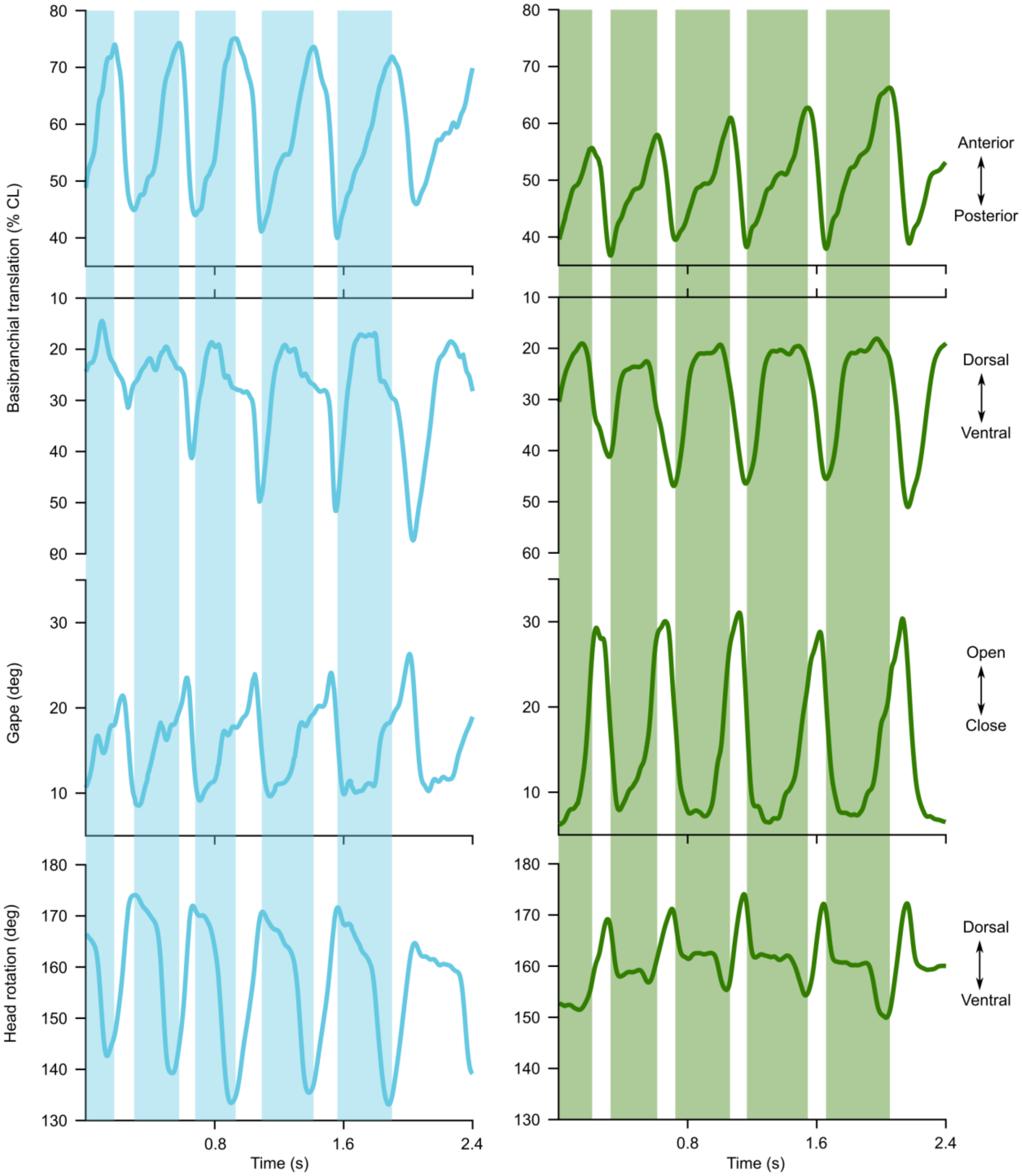

Fig. S3: Representative kinematic profiles during aquatic (blue) and terrestrial feeding (green) from the same animal. Basibranchial (tongue), gape and skull displacements are shown during a typical intraoral processing event in Triturus carnifex. As a reference, tongue protraction phases are indicated by grey bars. CL, cranial length. The terrestrial part of the figure is modified after Heiss et al. (2019).

Ehrenwörtliche Erklärung (Declaration of Honor)

Hiermit erkläre ich, Daniel Schwarz, dass:

- ich mit der Promotionsordnung der Fakultät für Biowissenschaften der Friedrich-Schiller-Universität Jena vertraut bin;
- die vorliegende Dissertation von mir verfasst wurde und dass weder Textabschnitte von Dritten noch eigener Prüfungsarbeiten ohne Kennzeichnung übernommen wurden;
- alle von mir verwendeten Hilfsmittel, persönliche Mitteilungen und Quellen als solche kenntlich gemacht wurden;
- alle Personen, welche bei der Erstellung der Publikationen unterstützend tätig waren, als Co-Autoren aufgeführt sind;
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Ort, Datum