RESEARCH ARTICLE

A peculiar mechanism of bite-force enhancement in lungless salamanders revealed by a new geometric method for modeling muscle moments

Stephen M. Deban* and Jason C. Richardson

ABSTRACT

Desmognathine salamanders possess unusual morphological features for lungless salamanders that have been proposed to aid in burrowing and biting, including well-ossified jaws and skull and a pair of robust ligaments connecting the atlas to the mandible. We evaluated the function of these and other peculiar desmognathine cranial features in biting by examining the morphology, mechanics and *in vivo* biting performance of the large *Desmognathus quadramaculatus*. We estimated theoretical biting force using a novel geometric method that we describe. Results provide quantitative evidence to bolster earlier conclusions that the unusual atlanto-mandibular ligaments couple ventral head flexion, a unique desmognathine behavior, with biting performance. Our analysis also reveals that the ligaments not only transmit, but also amplify the force of head flexion when acting together with the unusual stalked occipital condyles, enlarged atlas and massive quadratopectoralis muscles. The geometric model predicts that this mechanism contributes five times the biting force of the three jaw levator muscles combined and predicts that maximum biting force in *D. quadramaculatus* matches or exceeds forces reported for similarly sized lizards. The *in vivo* biting performance we measured was several times greater in *D. quadramaculatus* than another plethodontid salamander, *Pseudotriton ruber*, which lacks the unusual morphology and mechanism of desmognathines. The effective biting mechanism of *D. quadramaculatus* we describe is an emergent property of many of the distinguishing morphological features of desmognathine salamanders and likely plays an important role in their natural history given that desmognathines use biting in feeding, defense and even courtship.

KEY WORDS: Amphibian, Biomechanics, Feeding

INTRODUCTION

Desmognathine salamanders of the Plethodontidae possess a unique suite of morphological features that has long been used to establish the common ancestry of the group (Soler, 1950; Wake, 1966). The robust, wedge-shaped skull and mandible, enlarged anterior vertebrae and axial muscles, larger hindlimbs compared with forelimbs, and other unusual features have been interpreted as adaptations for burrowing or biting, or both (Dunn, 1926; Wake, 1966; Hinderstein, 1971; Schwenk and Wake, 1993). Desmognathines are known to burrow in soil and wedge

themselves beneath rocks; they are also atypical among salamanders in their propensity for biting strongly in defense and during feeding, displaying a characteristic behavior while biting in which the head is strongly flexed, directing the snout downward (Baldauf, 1947; Brodie, 1978; Dalrymple et al., 1985; Bakkegard, 2005). The long history of interest in the morphology and behavior of desmognathines has generated numerous propositions regarding the function and biological roles of their unique morphology. We assess one proposed function – the generation of elevated biting forces – that is an emergent property of several morphological features interacting during biting and head flexion, by examining the morphology and performance of a large desmognathine, *Desmognathus quadramaculatus* (Holbrook 1840).

A distinctive feature of desmognathine morphology, the robust atlanto-mandibular ligament on each side of the skull that originates on the atlas and inserts on the lower jaw (Figs 1 and 2), has long been a puzzle regarding its function and biological role (Dunn, 1926; Wake, 1966; Hinderstein, 1971; Dalrymple et al., 1985; reviewed by Schwenk and Wake, 1993). The ligament restricts ventral movement of the mandible relative to the trunk and thereby couples head movements with mandible movements. Its proposed functions are to prevent or limit ventral head flexion and require head lifting to open the mouth (Dunn, 1926; Wake, 1966), to hold the mandible against the quadrate (Hinderstein, 1971), to recoil elastically to close the jaws and hold them firmly closed in biting (Dalrymple et al., 1985), and to increase biting force (Larsen and Beneski, 1988; Schwenk and Wake, 1993). The atlanto-mandibular ligament may contribute to biting force only if it is placed in tension, such as when the cranium is flexed ventrally relative to the atlas or the jaws are open to a degree. Desmognathines are known to engage in head flexion during feeding, but the flexion must be forceful to contribute substantially to biting pressure.

Another distinctive desmognathine feature – large quadratopectoralis muscles – can indeed pull the cranium ventrally, and the large size of the muscles suggests they can do so forcefully. These muscles originate on the pectoral fascia and insert on the quadrate, giving them a strong mechanical advantage for head flexion about the atlas. Acting in conjunction with the atlanto-mandibular ligaments, the quadratopectoralis muscles appear to be capable of increasing biting force beyond what the jaw levator muscles that span the jaw joint can achieve (Dalrymple et al., 1985; Larsen and Beneski, 1988; Schwenk and Wake, 1993).

The ecological and evolutionary significance of the desmognathine biting mechanism is appreciated when one considers the natural history of these salamanders and the potential constraints on their morphology. Desmognathines burrow and wedge themselves beneath stones (Dunn, 1926; Wake, 1966; Worthington and Wake, 1972; Bakkegard, 2005), a behavior that limits the shape and dimensions of the head.

Department of Integrative Biology, University of South Florida, 4202 East Fowler Avenue, Science Center 110, Tampa, FL 33620, USA.

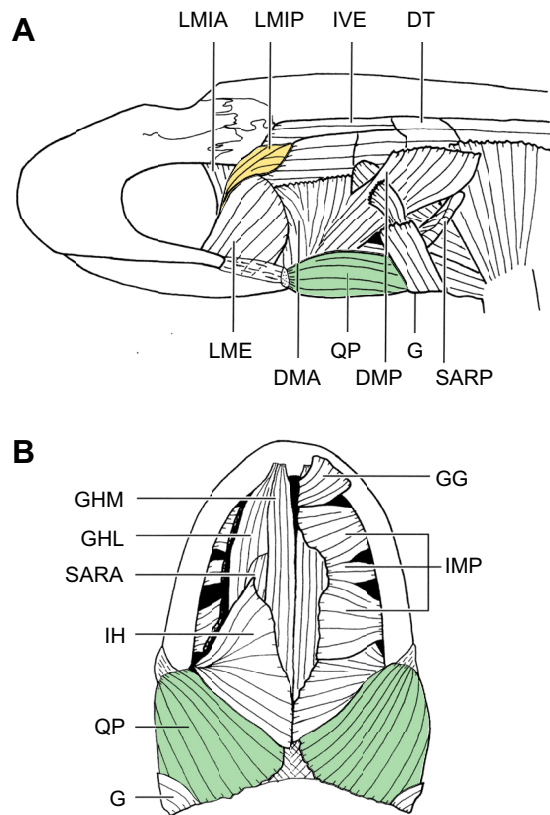*Author for correspondence (sdeban@usf.edu)

S.M.D., 0000-0002-7979-4346

Fig. 1. Musculature of *Desmognathus quadramaculatus*. (A) Dorsolateral and (B) ventral views show the enlarged quadratopectoralis muscle (QP, green) and the anterior portion of the levator mandibulae internus posterior muscle (LMIP, gold), under which lies the atlanto-mandibular ligament. The QP myofibers originate broadly on the pectoral fascia and converge to insert on the quadrate and prearticular, in a position to flex the cranium ventrocaudally. Other muscles shown include the levator mandibulae internus anterior (LMIA), intervetebral epaxial (IVE), dorsalis trunci (DT), levator mandibulae externus (LME), depressor mandibulae anterior (DMA), gularis (G), subcualis rectus posterior (SARP), genioglossus (GG), geniohyoideus medialis (GHM), geniohyoideus lateralis (GHL), subarcualis rectus anterior (SARA), intermandibularis posterior (IMP) and interhyoideus (IH).

Fig. 2. Morphology and position of the atlanto-mandibular ligaments and their associated myofibers. (A) Robust atlanto-mandibular ligaments (white arrowheads) in stained and cleared *D. quadramaculatus* originate on the atlas and insert on the coronoid processes of the prearticular of the lower jaw. Ligaments are displaced laterally, more so on the left side, to reveal trochlea in skull. Note the subocular mineralization (black arrowhead). (B–D) Levator mandibulae internus posterior muscle and ligament in dorsal (B), lateral (C) and ventral (D) views. Myofibers (B,C) originate and insert along the dorsal surface ligament in a staggered fashion, reducing muscle thickness and allowing tension placed on the myofibers to share the stress with the ligament. The proximal surface of the ligament is smooth and devoid of myofibers.

amplified force of the quadratopectoralis muscles to the mandible to be used as biting force. We explore the performance of this mechanism quantitatively using the geometric model, and compare the predicted performance with *in vivo* bite-force measurements. Additionally, we compare biting performance in *D. quadramaculatus* with that of a plethodontid of similar size, *Pseudotriton ruber*, which lacks the unusual features of desmognathines (i.e. stalked occipital condyles, reinforced quadrates, robust jaws, enlarged quadratopectoralis muscles and atlanto-mandibular ligaments).

MATERIALS AND METHODS
Functional morphology of *D. quadramaculatus* jaws

Four adult *D. quadramaculatus* were killed by immersion in a 2 g l^{-1} aqueous solution of MS-222 (tricaine methanesulfonate; Sigma-Aldrich, St Louis, MO, USA) buffered with sodium bicarbonate, and specimens were massed (Ohaus Corporation Scout Pro SP2001 balance, Pine Brook, NJ, USA). Head width at the jaw joints and snout–vent length (SVL) were measured using Neiko 01408A digital calipers. The head and neck of the fresh specimens were then skinned and tissue was dissected away to expose the muscles and their origins and insertions, the quadrate-prearticular joints (i.e. jaw joints) and the atlanto-occipital joints. The dorsoventral movements of the jaws and head through their range of motion were examined by manipulation before and after the specimens were skinned, paying attention to the relationship between the angle formed by the jaws and the angle formed by the head and trunk, given that these were noted to be related in previous studies (Dunn, 1926; Wake, 1966; Hinderstein, 1971; Dalrymple et al., 1985; Schwenk and Wake, 1993).

Three muscles that span the quadrate-prearticular joint and one that spans the atlanto-occipital joint are in a position to contribute to bite force: levator mandibulae externus, levator mandibulae internus anterior, levator mandibulae internus posterior and quadratopectoralis (Fig. 1). These muscles were examined with regard to their origins and insertions, trajectory, muscle fiber angles and mass.

Protruding jaw muscles would impede this behavior, yet large jaw levator muscles are commonly observed in vertebrates that engage in vigorous biting, for example in the salamanders of the genus *Aneides* (Stebbins, 1985; Staub, 1993). A system that makes use of a ventrolaterally located muscle such as the quadratopectoralis to contribute to biting forces allows the skull to be flattened, important in a salamander that wedges under rocks (Larsen and Beneski, 1988; Schwenk and Wake, 1993).

We sought to evaluate the hypothesis that the atlanto-mandibular ligaments and quadratopectoralis muscles allow desmognathines to produce elevated biting forces when they interact with other distinctive morphological features of desmognathines. We also aimed to assess the hypotheses that the atlanto-mandibular ligaments limit mouth opening or engage in elastic recoil, or both.

To achieve these aims, we examined the cranial morphology of *D. quadramaculatus* and quantified dimensions of its jaw apparatus. We derived a novel 3D geometric method for modeling moments in musculoskeletal systems such as jaws that does not require 3D coordinate data. We present a novel interpretation of the unusual desmognathine morphology and biting mechanism, revealing a mechanism in which the atlanto-mandibular ligaments transmit the
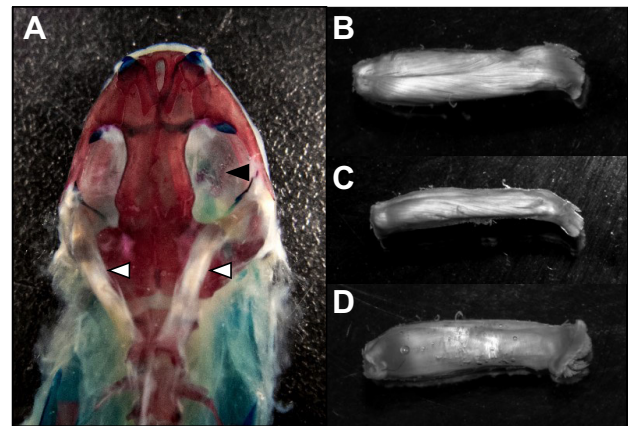
Straight-line distances between morphological landmarks were measured on specimens with the head in the resting position and the mouth closed to calculate theoretical bite force via a static musculoskeletal model, described below. A total of 27 distances were measured on each specimen, including six distances for each of the four muscles that can contribute to biting. For each of the three cranial jaw-levator muscles on the left side of each specimen (levator mandibulae externus, levator mandibulae internus anterior and levator mandibulae internus posterior), six distances were measured: muscle origin to insertion, left jaw joint to muscle origin, left jaw joint to muscle insertion, right jaw joint to muscle origin, right jaw joint to muscle insertion and left to right jaw joint center. For the left quadratopectoralis muscle, which can contribute to biting by flexing the cranium ventrally, six distances were measured: muscle origin to insertion, left atlanto-occipital joint to muscle origin, right atlanto-occipital joint to muscle origin, left atlanto-occipital joint to muscle insertion, right atlanto-occipital joint to muscle insertion and left to right atlanto-occipital joint center. Six additional distances were measured for the model: left atlanto-occipital joint center to the closest point on the left atlanto-mandibular ligament, right atlanto-occipital joint center to the closest point on the left atlanto-mandibular ligament, left jaw joint to mid-rostral tip of jaws, right jaw joint to tip of jaws, left jaw joint to the last (caudal-most) tooth on left side and right jaw joint to last tooth on the left side. Measurements of each distance were made three times using digital calipers (Neiko 01408A) and averaged to the nearest 0.01 mm.

The insertion of each of the muscles on the lower jaw and quadrate is narrow and its position for use in the model was easily located. The origin, however, is broader and its position for the model was estimated as a point in the center of the area of origin. The levator mandibulae internus anterior and the levator mandibulae externus are parallel-fibered muscles whose fiber angle relative to the muscle line of action is very shallow and was taken as zero. The myofiber angles of 10 random fibers of the quadratopectoralis and levator mandibulae internus posterior were estimated using a dissecting microscope with a camera-lucida attachment together with a protractor.

Muscles were dissected free and massed (Virtual Measurements and Control model VB-302A, Santa Rosa, CA, USA; ±0.001 g accuracy). The myofibers of the levator mandibulae internus posterior were teased apart to visualize their relationships to the atlanto-mandibular ligament on which they originate and insert (Fig. 2B–D). Dimensions of the atlanto-mandibular ligament were measured for two specimens to calculate tendon stress.

Theoretical isometric muscle force for each muscle was calculated by first determining physiological cross-sectional area (PCSA) as the cosine of average fiber angle times mass divided by density divided by fiber length. Muscle density was taken as 1.056 g cm^{-3}. Muscle force was calculated by multiplying PCSA by a value of 22 N cm^{-2} isometric stress for amphibian muscle (Edman, 1979).

Static model of theoretical bite force

A static model of bite force was developed that made use of geometric formulae to calculate muscle force vectors and lever-arm lengths from straight-line distances measured on the specimens without the use of 3D coordinates (Fig. 3). The model used these values combined with theoretical isometric muscle force to calculate the contribution of each of the three jaw muscles that insert directly on the mandible to bite force, combined with the contribution of the quadratopectoralis muscle via head flexion and
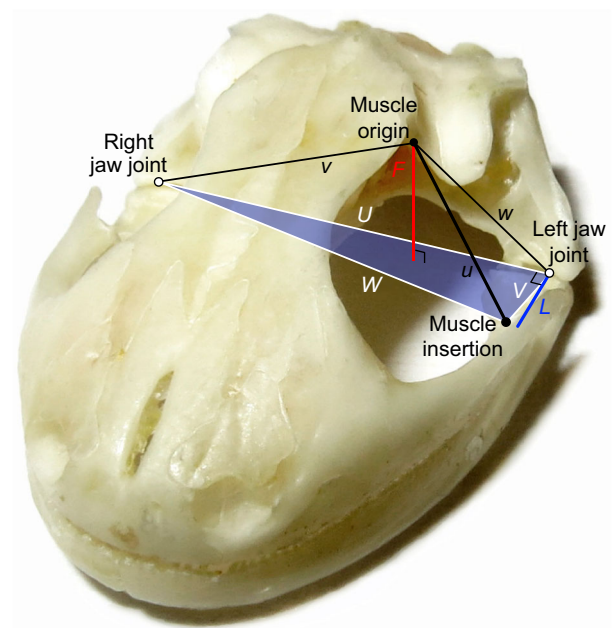

Fig. 3. Geometric model for calculating the jaw muscle moment produced by a jaw muscle that originates on the skull and inserts on the lower jaw. The base triangle (blue, with sides U, V and W) is formed by the muscle insertion and the two jaw joints, the height (L) of which defines the muscle in-lever length. Out-lever length is calculated similarly by using the bite point as the apex of the triangle. The height (F) of the tetrahedron (sides U, V, W and u, v, w) defines the component of the muscle force vector (u) that lies perpendicular to the base triangle. The moment produced by the muscle is calculated as F times L. See Materials and methods, 'Static model of theoretical bite force' for details.

the resulting tension in the atlanto-mandibular ligament that inserts on the mandible.

The muscle force vector that contributes to biting is defined for each of the three jaw muscles as the height of an irregular tetrahedron (i.e. a triangular pyramid with unequal sides) that has the muscle origin at its apex and its triangular base formed by the muscle insertion and the left and right jaw joints (Fig. 3). Heron's formula from antiquity (*Metrica*, ca. 100 BC–100 AD) was used to calculate the area of the base triangle from the lengths of its three sides, and an extension of Heron's formula was used to calculate the volume of the tetrahedron from the lengths of its six sides (Sommerville, 1939) (formulae in Workbook 1). The muscle force vector was taken as the height of the tetrahedron calculated as three times the volume of the tetrahedron divided by the area of its triangular base (applying Cavalieri's principle). The muscle in-lever length was taken as the height of the base triangle calculated as twice its area divided by the length of its base (i.e. the distance between the jaw joints). The bite out-lever length for all muscles was calculated similarly as the height of the triangle formed from the two jaw joints at the base and the bite point at the rostral tips of the jaws (or at the last tooth) at the apex. The contribution of each muscle to biting force was calculated as the muscle force vector times the in-lever length divided by the out-lever length. This quantity was doubled to account for both left and right muscles.

The anatomical origins of the levator mandibulae internus anterior and the levator mandibulae externus were used as the origins for these muscles in the model. However, for the levator mandibulae internus posterior, the rostral edge of the otic-parietal depression was used as the origin for this muscle in the model,

because the otic-parietal depression is a trochlea that redirects the force of this muscle (Fig. S1).

The contribution of the quadratopectoralis muscle to biting was calculated with analogous geometric methods, using the muscle origin on the pectoral fascia as the apex of a tetrahedron that has as its base the muscle insertion on the quadrate and the left and right atlanto-occipital joint centers about which the skull rotates dorsoventrally. The muscle force vector was calculated as the height of this tetrahedron and the muscle in-lever length as the height of the base triangle, as described above. The out-lever length was calculated as the height of the triangle formed by the two atlanto-occipital joints and the nearest point on the left atlanto-mandibular ligament, which lies just dorsal to the joint (see Fig. 4).
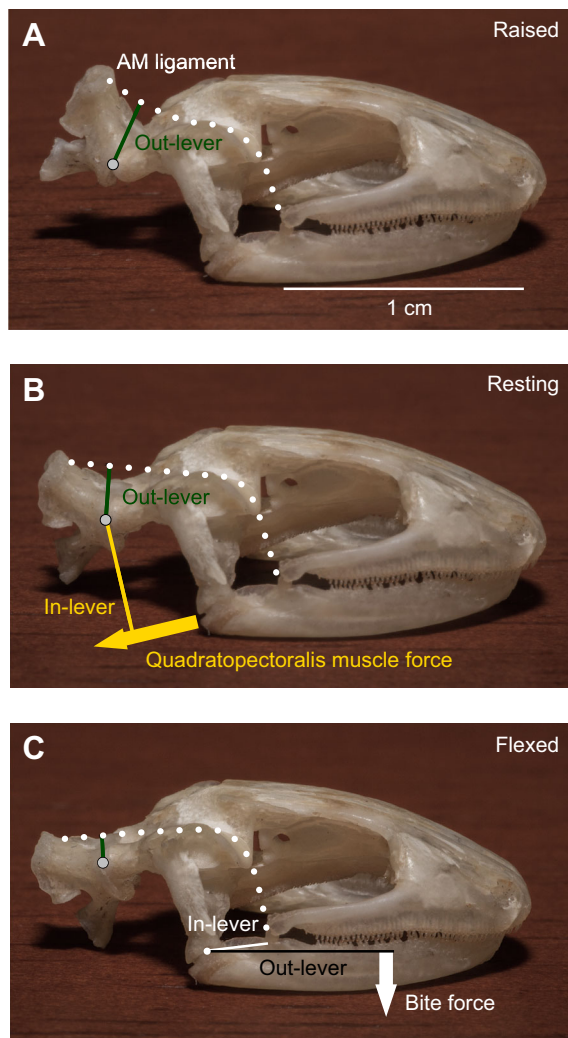

Fig. 4. Cranium of *D. quadramaculatus* in different positions relative to the atlas, showing the range of motion afforded by the stalked occipital condyles. (A) Raised; (B) resting; and (C) flexed. The atlanto-mandibular ligament (dotted line) is placed in tension by the quadratopectoralis muscle flexing the cranium at the atlanto-occipital joint (gray dot). Tension is the product of quadratopectoralis muscle force (gold arrow in B) and the ratio of in-lever length (gold line) to out-lever length (green line). The out-lever length decreases substantially when the cranium is flexed (C), increasing the mechanical advantage of the quadratopectoralis muscle and thereby increasing ligament tension. Tension is transmitted along the atlanto-mandibular ligament, over the skull, to the lower jaw. Bite force (arrow in C) is the product of atlanto-mandibular ligament tension and the ratio of jaw in-lever (white line) to out-lever (black line).

The force that the quadratopectoralis muscle produced at the ligament was calculated as the muscle force vector times the in-lever length divided by the out-lever length; this force produced an equivalent magnitude of tension in the ligament that was transmitted to the lower jaw via its path through the otic-parietal trochlea. This force was added to the force of the levator mandibulae internus posterior because the ligament acts as the tendon with which this muscle inserts on the mandible. This quantity was doubled to account for both left and right muscles.

Bite force was modeled with the jaws closed and head in the resting position. Manipulations of the model were used to examine the effect of ventral head flexion on bite force. Head flexion has three effects (see Results) that increase the contribution of the quadratopectoralis muscle to bite force: (1) placing the atlanto-mandibular ligament under tension by increasing the distance between its origin and insertion, (2) orienting the muscle so that its entire force is applied perpendicularly to the muscle in-lever and (3) positioning the atlanto-mandibular ligaments adjacent to the atlanto-occipital joints and thereby reducing the out-lever considerably. The latter two effects were incorporated into the model.

Measurement of *in vivo* bite force

Voluntary bite force was measured in four *D. quadramaculatus* (80–95 mm SVL) and three *Pseudotriton ruber* (65–75 mm SVL), all collected in North Carolina, USA. Salamanders were maintained on a diet of crickets in inclined plastic shoeboxes with a few centimeters of water at 16–18°C. All procedures were approved by the University of South Florida Institutional Animal Care and Use Committee.

Pseudotriton ruber is a large semiaquatic plethodontid with a more generalized cranial morphology than *D. quadramaculatus*; it was chosen for comparison because it is of similar size and ecology yet lacks many of the morphological features of desmognathines that are thought to enhance biting force, such as stalked occipital condyles, rigid quadrates, robust jaws, enlarged quadratopectoralis muscles and atlanto-mandibular ligaments. *Pseudotriton ruber* does, however, possess enlarged levator mandibulae externus muscles compared with desmognathines and thus is not entirely unspecialized for biting.

Voluntary bite force was measured with a custom-built bite meter (Herrel et al., 2001) with a Kistler Type 9203 force transducer connected to a Kistler Type 5995 charge amplifier. The transducer has a range of 0–50 N with a threshold force of <1 mN, making it suitable for accurately measuring the biting forces of the salamanders. The bite meter (Fig. S2) was fitted with bite plates covered in a thin layer of rubber to protect the teeth of the salamanders and elicit strong bites (Lappin and Jones, 2014). The transducer produced signals that were sampled at 1000 Hz using PowerLab 16/30 data acquisition hardware and LabChart software (ADInstruments, Bella Vista, NSW, Australia) running on a Dell Optiplex GX620 computer. Prior to biting experiments, the transducer was calibrated with weights hung from the upper bite plate.

Salamanders were induced to bite the transducer by covering the bite plates with the molted exoskeletons of crickets that were part of their captive diet or skin secretions from a prey salamander (*Desmognathus ocoee*). Salamanders were placed in a retreat (a cardboard paper-towel tube) and enticed to bite by placing the transducer a few to several millimeters in front of their snouts. After bites were obtained, salamanders were rewarded with live crickets. Bites were imaged in lateral view with a Canon Powershot S100

camera recording in HD at 60 Hz to obtain the approximate position of the jaws on the bite meter during the bite (Fig. 5; Movie 1). To visualize the moving morphology, fluoroscope images of *D. quadramaculatus* feeding were recorded at 1000 Hz in lateral and dorsal view with Visario Speedcam cameras (Weinberger GmbH, Erlangen, Germany) (Fig. 6; Movie 2). All *in vivo* experiments were conducted at an ambient temperature of 16–18°C and 61–69% relative humidity.

Bite force data of *D. quadramaculatus* and *P. ruber* were log transformed for normality and homoscedasticity and compared in R version 3.3.2 using an ANOVA (nlme package) that included species as a fixed effect and individual as a random effect. Microsoft Excel 2016 was used to calculate effect size (Cohen's *d*). Means are presented ±s.e.m.

RESULTS
Functional morphology of *D. quadramaculatus* jaws
Manipulation of fresh specimens revealed an interaction between the rotation of the mandible on the cranium and the rotation of the cranium on the atlas. The jaws of *D. quadramaculatus* closed when the head was flexed ventrally, and opening the mouth required the cranium to be either in line with the trunk or rotated (extended) dorsally about the atlanto-occipital joint (Dunn, 1926; Wake, 1966; Hinderstein, 1971). Despite this constraint on mouth opening, the cranium could be flexed ventrally to nearly 90 deg to the trunk with the jaws in occlusion. Dorsal rotation of the cranium appeared to slacken the atlanto-mandibular ligaments to allow jaw depression up to a gape angle of approximately 90 deg. Flexing the cranium ventrally on the atlas to bring the line of the upper jaw below the plane of the trunk placed the ligaments under tension; the taut ligaments pulled the jaws closed or applied force to a finger placed between the jaws when the cranium was further flexed ventrally. Cranial flexion also moved the atlanto-mandibular ligaments close to the atlanto-occipital joints; when the cranium was fully flexed, the ligaments were in contact with the dorsal surfaces of the occipital condyles. In skinned specimens with the mouth closed and the ligaments slackened by raising the cranium, the ligaments could be easily displaced laterally out of the otic-parietal trochleas. When the atlanto-mandibular ligaments were severed, interaction between jaw movements and head movements was eliminated, confirming
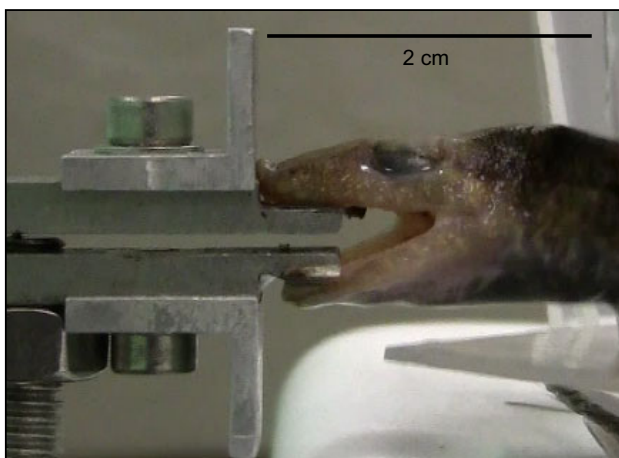
Table 1. Morphological measurements of *Desmognathus quadramaculatus* used in the biomechanical model

Variable	Mean±s.e.m.
Body mass (g)	18.4±2.2
Snout–vent length (mm)	93.5±3.6
Total length (mm)	169±6.2
Head width at jaw joints (mm)	14.6±1.0
Distance between jaw joint centers (mm)	11.6±0.4
Jaw joint to jaw tip (mm)	14.2±0.5
Jaw joint to last tooth (mm)	4.3±0.3

earlier conclusions that these ligaments are responsible for coupling head movements with jaw movements (Dalrymple et al., 1985; Schwenk and Wake, 1993).

Manipulation and dissection of specimens confirmed that the three jaw levator muscles that span the jaw joint (Fig. 1) contribute to bite force directly by elevating the mandible relative to the cranium, and that the quadratopectoralis muscle contributes indirectly by flexing the cranium ventrally. This ventral flexion of the cranium is coupled with jaw elevation or the application of bite force via the atlanto-mandibular ligaments, which are placed in tension when the cranium is ventroflexed. Movement of the atlanto-mandibular ligaments in the otic-parietal trochlea occurs with no noticeable friction.

Morphology and estimated bite performance of *D. quadramaculatus*
The *D. quadramaculatus* specimens examined for morphology had an SVL of 93.5±3.6 mm, total length of 169±6.2 mm, body mass of 18.4±2.2 g and head width at the jaw joints of 14.6±1.0 mm. Distance between the jaw joint centers was 11.6±0.4 mm, and jaw length from a line connecting the jaw joints to the rostral-most tooth was 13.0±0.5 mm (Table 1). The dorsal surface of the cranium is devoid of musculature, although the jaw levators bulge slightly laterally just rostral to the jaw joints. The enlarged quadratopectoralis muscles bulge visibly, extending from just
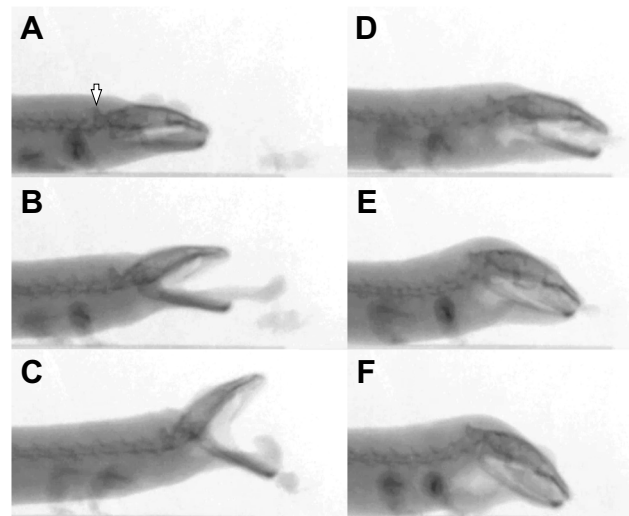

Fig. 5. Salamanders voluntarily bit the bite plates of the force transducer baited with exoskeletons of crickets or skin secretions of *Desmognathus ocoee*, a prey salamander species. Bite plates were covered with rubber strips to prevent tooth damage. Note the mouth is opened without head elevation. Details of the bite-force transducer are shown in Fig. S2.

Fig. 6. Fluoroscope images of feeding in *D. quadramaculatus*. (A) The cranium and atlas (arrow) in the resting position. The cranium is raised during tongue projection (B) and further during prey transport (C). It is lowered upon mouth closing (D) and strongly flexed relative to the atlas during biting (E,F) placing the atlanto-mandibular ligaments under tension. Note the curvature of the anterior vertebral column (E,F) that changes the angle of the atlas relative to the cranium.

caudal to the jaw joints to the insertion of the forelimb and giving the salamanders a 'neckless' appearance (Fig. S3).

The levator mandibulae externus (LME) muscle originates on the rostrolateral surfaces of the occipito-otic and squamosal and travels ventrally between the quadratomaxillary ligaments to insert on and just caudal to the coronoid process of the prearticular. Muscle fibers travel rostroventrally and laterally, nearly in parallel from origin to insertion, and are in a position to draw the mandible dorsally to close the mouth or apply biting force, as well as caudally towards the quadrate-prearticular joint. The LME had a mass of 0.023±0.003 g and was calculated to contribute a moment of 0.0023±0.0003 Nm at the jaw joint (unilaterally) and to exert a bite force of 0.18±0.023 N at the jaw tips or 0.55±0.08 N at the last tooth (Table 2). These forces and moments are unilateral, as for the muscles discussed below, unless otherwise noted.

The levator mandibulae internus anterior (LMIA) muscle originates on the lateral surface of the rostralmost edge of the parietal and the palatoquadrate cartilage just caudal to the eye. Fibers run ventrolaterally nearly in parallel and insert on the medial projection of the coronoid process of the prearticular via a narrow tendon, and can thus raise the mandible or apply biting force. The medial component of force that this muscle exerts on the mandible may be redirected dorsally by the medial quadratomaxillary ligament acting as a retinaculum. The LMIA had a mass of 0.006±0.001 g and was calculated to contribute a moment of 0.0006±0.0003 Nm at the jaw joint (unilaterally) and to exert a bite force of 0.048±0.023 N at the jaw tips or 0.15±0.082 N at the last tooth (Table 2).

The levator mandibulae internus posterior (LMIP) muscle lies lateral to the LMIA. It originates on the rostral surface of the atlas crest and runs rostrolaterally, then rostroventrally through the otic-parietal depression and thence ventrolaterally to insert between the quadratomaxillary ligaments on the coronoid process of the prearticular via a large tendon. The LMIP tendon is in fact the terminus of the robust atlanto-mandibular ligament that shares with the LMIP its origin, insertion and trajectory. Muscle fibers originate from and insert onto the atlanto-mandibular ligament in a complex pennate arrangement (Fig. 2) at an average angle of 11.2 deg, but do not extend from origin to insertion. The myofibers of the LMIP, by virtue of this arrangement, can place tension on the atlanto-mandibular ligament when they contract, but are limited in how far they can be stretched when tension is placed on the ligament during cranial flexion. The LMIP plus atlanto-mandibular ligament can raise the mandible or can prevent mandibular depression or apply biting force when the cranium is flexed ventrally by the quadratopectoralis muscles. As with the LMIA, the medial quadratomaxillary ligament may act as a retinaculum to redirect medial force dorsally. The atlanto-mandibular ligament without myofibers averaged 0.27±0.06 mm in thickness, 2.02±0.84 mm in width and 11.26±0.12 mm in length; cross-sectional area was thus 0.489±0.112 mm^2. The LMIP had a mass of 0.009±0.0005 g and was calculated to contribute a moment of 0.0013±0.00031 Nm at the jaw joint (unilaterally) and to exert a bite force of 0.105±0.024 N at the jaw tips or 0.33±0.08 N at the last tooth (Table 2).

The quadratopectoralis (QP) muscle originates broadly on the fascia of the pectoral-gular region and its fibers travel rostrodorsally at an average angle of 14.8 deg to converge and insert on the lateral surface of the ventral edge of the quadrate beneath the lateral quadratomaxillary ligament (Fig. 1). The QP is the largest of the muscles that contribute to bite force, which it accomplishes by flexing the cranium ventrally and thereby placing tension on the atlanto-mandibular ligament. The QP muscle had a mass of

Table 2. Morphological and biomechanical quantities from four muscles that contribute to biting in _Desmognathus quadramaculatus_

	Levator mandibulae externus	Levator mandibulae internus anterior	Levator mandibulae internus posterior	Quadratopectoralis	
				Cranium at rest	Cranium ventroflexed
Origin	Occipito-otic and squamosal	Parietal and palatoquadrate	Atlas crest	Pectoral fascia	Pectoral fascia
Insertion	Coronoid process of prearticular	Coronoid process of prearticular	Coronoid process of prearticular	Quadrate	Quadrate
Joint spanned	Quadrate-articular (i.e. jaw joint)	Quadrate-articular	Quadrate-articular and atlanto-occipital	Atlanto-occipital	Atlanto-occipital
Mass (g)	0.023±0.003	0.006±0.001	0.009±0.0005	0.087±0.013	0.087±0.013
PCSA (cm^2)	0.055±0.008	0.02±0.008	0.019±0.004	0.081±0.01	0.081±0.01
Raw force (N)	1.22±0.17	0.43±0.17	0.41±0.079	1.79±0.23	1.79±0.23
Fraction of force transmitted	0.73±0.052	0.48±0.1	0.81±0.041	0.84±0.016	1.0
Contributing force (N)*	0.88±0.108	0.23±0.13	0.34±0.07	1.5±0.19	1.79±0.23
In-lever (mm)	2.77±0.6	2.94±0.51	3.93±0.23	3.35±0.24	3.35±0.24
Out-lever (mm)	12.98±0.48	12.98±0.48	12.98±0.48	1.57±0.059	0.81±0.043
Muscle moment about jaw joint (Nm)	0.0023±0.00033	0.0006±0.0003	0.0013±0.00031	0.011±0.0022	0.024±0.004
Bite force contribution at jaw tips (N)	0.18±0.023	0.048±0.023	0.105±0.024	0.81±0.153	1.81±0.29
Bite force contribution at last tooth (N)	0.55±0.08	0.15±0.082	0.33±0.08	2.52±0.54	5.65±1.07

Values are means±s.e.m. Bite force and torque contributions are unilateral. Contribution from the quadratopectoralis is calculated as applied through the atlanto-mandibular ligament of the levator mandibulae internus posterior muscle. PCSA, physiological cross-sectional area.
*Contributing force is the component of raw muscle force that produces a moment about the jaw joint.

0.087±0.013 g and was calculated to contribute a moment of 0.005±0.001 Nm at the atlanto-occipital joint (unilaterally) and a tension in the atlanto-mandibular ligament of 3.35±0.63 N with the head in the resting position (Table 2). The QP muscle is calculated to exert, via the tension in the atlanto-mandibular ligament, a moment of 0.011±0.0022 Nm at the jaw joint (unilaterally), and exert a bite force of 0.808±0.153 N at the jaw tips or 2.52±0.54 N at the last tooth. With the head flexed ventrally, the QP contributed a moment of 0.006±0.001 Nm and a tension in the ligament of 7.44±1.06 N. The QP muscle is calculated to exert, via the tension in the atlanto-mandibular ligament, a moment of 0.024±0.004 Nm at the jaw joint (unilaterally), and exert a bite force of 1.81±0.29 N at the jaw tips or 5.65±1.07 N at the last tooth.

All muscles together contributed an estimated total moment of 0.015±0.003 Nm at the joint unilaterally with the head in the resting position and 0.028±0.005 Nm with the head flexed (Table 3). The QP muscle contributes 73% of this moment with the head at rest and 85% when the head is flexed, while the LME muscle contributes 15% and 8%, the LMIA contributes 4% and 2%, and the LMIP contributes 9% and 5%, respectively. With the head in the resting position, the total moment resulted in a unilateral calculated bite force of 1.14±0.20 N at the jaw tips and 3.55±0.72 N at the last tooth. With the head flexed, the unilateral bite force is estimated at 2.14±0.34 N at the jaw tips and 6.68±1.27 N at the last tooth. Doubling these values to account for all bilaterally paired muscles and head flexion yields a maximum theoretical applied bite force of 4.29±0.69 N at the jaw tips and 13.37±2.55 N at the last tooth. Maximum stress in the atlanto-mandibular ligament is calculated at 5.5±1.6 MPa with the head in the resting position and 12.9±1.6 MPa with the head flexed.

Given that the QP muscle contributes up to 85% of bite force and acts indirectly via the atlanto-mandibular ligament that is absent or poorly developed in other salamanders, we calculated the required muscle force and PCSA of the second greatest contributor, the LME, to produce the estimated maximum moment and bite force with the head flexed in the absence of the contribution of the QP. Adding the unilateral moment of the QP of 0.024 Nm to the moment of the LME of 0.0023, the combined moment is 0.0263 Nm. Producing this moment through the measured average in-lever of 2.76 mm and an applied muscle force fraction of 62.4% (owing to the angle of the line of action of the LME) would require an LME muscle force of 15.27 N. At a specific tension of 22 N cm^{-2}, the LME would need to have a PCSA of 0.69 cm^2 or 12.5 times the average calculated PCSA of 0.055 cm^2 based on morphological measurements.

In vivo bite performance

Both salamander species readily bit the force transducers, after a forward lunge of the entire body towards the transducer and tongue projection in many trials. The salamanders typically pressed their snouts against the stop on the transducer, positioning the bite point near the mid-point of the jaws (Fig. 5). The bite point therefore fell between the bite points that we included in the biomechanical model: the jaw tips and the last tooth. The rigidity and immobility of the transducer prevented the salamanders from depressing their heads fully during biting. They presumably could flex or extend their anterior vertebral column dorsally to change the angle of the atlanto-occipital joint; however, we could not determine whether or not they did so. Thus, the measured bite forces for *D. quadramaculatus* are likely to fall between the extreme conditions of our biomechanical model, with respect to both head angle and bite-point position.

Desmognathus quadramaculatus achieved a peak bite force of 8.2±1.3 N and a bite impulse of 0.75±0.12 Ns, with a bite duration of 0.20±0.01 s (*N*=21). The highest single bite from *D. quadramaculatus* was 14.3 N (Fig. 7). *Pseudotriton ruber* achieved a peak bite force of 0.64±0.20 N, a bite impulse of 0.057±0.015 Ns and a bite duration of 0.19±0.02 s (*N*=9). The highest single bite from *P. ruber* was 1.1 N. *Desmognathus quadramaculatus* had significantly higher peak bite force (*P*=0.0011, Cohen's *d*=3.8) and bite impulse (*P*=0.0009, *d*=4.2) than *P. ruber*, but similar bite duration (*P*=0.3942, *d*=0.39; Fig. S4).

Fluoroscope images of *D. quadramaculatus* capturing a cricket (Fig. 6) reveal the range of motion of the atlanto-occipital joints, as well as the head-tucking behavior after the prey has been brought between the jaws by the tongue. These images reveal that the anterior vertebral column is extended dorsally while the cranium is flexed ventrally. These movements combine to increase the angle between the atlas and the cranium, and thereby increase the tension on the atlanto-mandibular ligament.

DISCUSSION

Functional morphology of *D. quadramaculatus* jaws

The musculoskeletal cranial features of the *D. quadramaculatus* examined here are largely consistent with descriptions of these features in this species and other desmognathine species by previous researchers (Dunn, 1926; Wake, 1966; Hinderstein, 1971; Dalrymple et al., 1985; Schwenk and Wake, 1993). Our observation of the solid cranium and jaws with reinforced quadrate, robust atlanto-mandibular ligaments, stalked occipital condyles, and enlarged atlas and anterior axial muscles are
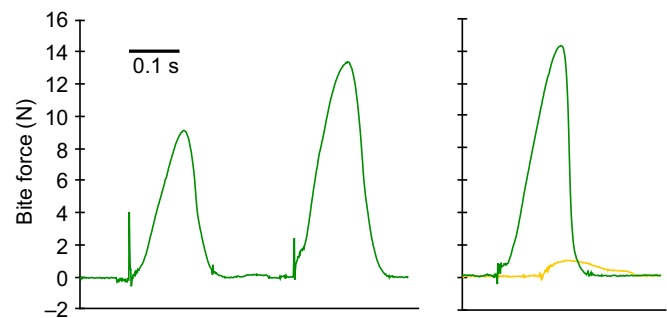
Table 3. Total forces and moments from all muscles that contribute to biting in *Desmognathus quadramaculatus*

	Unilateral	Bilateral
Cranium in resting position		
Total moment at jaw joint (Nm)	0.015±0.003	0.03±0.006
Total bite force at jaw tips (N)	1.14±0.202	2.28±0.404
Total bite force at last tooth (N)	3.55±0.722	7.1±1.445
Tension in A-M ligament (N)	3.35±0.632	6.69±1.263
Stress in A-M ligament (MPa)	5.54±1.605	5.54±1.605
Cranium flexed ventrally		
Total moment at jaw joint (Nm)	0.028±0.005	0.056±0.01
Total bite force at jaw tips (N)	2.14±0.34	4.29±0.69
Total bite force at last tooth (N)	6.68±1.27	13.37±2.55
Tension in A-M ligament (N)	7.44±1.06	14.89±2.13
Stress in A-M ligament (MPa)	12.89±1.55	12.89±1.55

A-M, atlanto-mandibular. Values are means±s.e.m.

Fig. 7. Representative bites of *D. quadramaculatus* (green) measured at approximately mid-mandible. Force far exceeds that produced by the jaw muscles. Bites of *Pseudotriton ruber* (gold) are much weaker. Vertical spikes were caused by the salamander's snout striking the stop on the bite transducer.

consistent with previous descriptions. The coupled movements of the cranium and mandible we observed are likewise in accord with previous descriptions. However, we found that the head can be flexed to nearly 90 deg relative to the trunk, indicating the atlanto-mandibular ligaments do not prevent head depression. Another difference with earlier work lies in the description of the atlanto-mandibular ligament by Hinderstein (1971), who describes it as being encompassed by muscle fibers in the species he examined, including *D. quadramaculatus*. We found that the muscle fibers of the LMIP are the only myofibers attaching to the ligament and these are restricted to the dorsal surface of the ligament, and that the ventral surface is smooth and devoid of myofibers. This permits the atlanto-mandibular ligament to slide without noticeable friction in the otic-parietal trochlea, an important condition for the transmission of the amplified force of the QP muscle to the bite that is central to the unique desmognathine biting mechanism.

The atlanto-mandibular ligament on each side of the skull is such an unusual and conspicuous feature of desmognathine salamander cranial morphology that its function has long been pondered (Dunn, 1926; Wake, 1966; Hinderstein, 1971; Dalrymple et al., 1985; reviewed by Schwenk and Wake, 1993). Our observations agree with the findings of previous researchers that the linkage between the atlas and mandible provided by this ligament limits the extent of lower jaw depression relative to the trunk (e.g. Wake, 1966) and thereby couples cranial movements with jaw movements. However, we find no evidence to support the proposition that desmognathines must raise their heads to initially open their mouths (Hinderstein, 1971), finding instead that the ligament limits but does not prevent mouth opening when the head is in its resting posture in line with the trunk (Fig. 5). Further, head lifting is ubiquitous during mouth opening in salamander feeding (Deban and Wake, 2000; Wake and Deban, 2000), and most species lack this mechanical linkage. The atlanto-mandibular ligament has also been proposed to recoil elastically to accelerate mouth closing (Hinderstein, 1971; Dalrymple et al., 1985); however, we found this conclusion to be inconsistent with our mechanical analysis and our observation that the ligament is far too robust to be appreciably stretched by the small jaw depressor muscles with their narrow tendons and poorer mechanical advantage. The atlanto-mandibular ligament couples mouth closing and head depression, and can cause the jaws to 'snap' shut rapidly in manipulated specimens when the snout is pressed downward, but this mechanism does not require elastic recoil.

Based on our manipulations of specimens, we found that the robust atlanto-mandibular ligament functions as an inextensible link, like a cable, between the atlas and mandible, in agreement with a previous account (Schwenk and Wake, 1993). We also observed that the position of the cranium over which the ligament passes alters the length of its path and thus the degree of mouth opening permitted. This coupling allows the cranial depressor muscle, the QP, to contribute to biting force when the head is flexed, increasing bite performance beyond the force provided by the jaw levator muscles (LME, LMIA and LMIP).

Novel biting mechanism
Our mechanical analysis reveals a novel mechanism of force amplification in which the force contribution of the atlanto-mandibular ligament to biting is greater than the force produced by the QP muscle in flexing the cranium – a biting mechanism that may be unique to desmognathine salamanders. The high tension produced in the atlanto-mandibular ligament is generated by the high mechanical advantage of the QP muscle resulting from the long in-lever of the QP muscle, defined as the distance of the

quadrate to the atlanto-occipital joint, and the very short out-lever, defined as the distance from the atlanto-occipital joint to the atlanto-mandibular ligament (Fig. 4). With the head fully flexed, the QP force is better aligned to produce a moment about the atlanto-occipital joint, using the full length of the in-lever from the QP insertion to the atlanto-occipital joint, a length that is increased by the presence of stalked occipital condyles. Perhaps more importantly, the distance from the atlanto-mandibular ligament to the atlanto-occipital joint drops by half when the head is fully flexed, by virtue of the stalked occipital condyles. As a result of this increase in mechanical advantage, the QP muscle contributes an estimated 7.4 N to the tension of the atlanto-mandibular ligament when the cranium is fully flexed, despite exerting an estimated force of only 1.8 N at its insertion on the quadrate (Tables 2 and 3, Fig. 4) – a force amplification of over four times.

The novel biting mechanism of desmognathines may provide benefits in jaw mechanics beyond simply increasing peak biting force. A dual mechanism of mouth closing may expand the range of head and jaw angles over which high bite force can be applied. The intrinsic jaw levator muscles (LME, LMIA and LMIP) may be used to close the jaws with relatively low force and high velocity at a range of head angles, such as when prey are first engulfed, while the QP may be incapable of producing rapid mouth closing because of its high mechanical advantage but instead may be recruited subsequently to apply high biting forces. Such an expanded functional range of force application might be demonstrated with additional biomechanical modeling and *in vivo* biting experiments.

The biting mechanism described here is an emergent property of many of the distinguishing morphological features of desmognathine salamanders. The robust skull and jaws are able to withstand elevated biting forces compared with other salamanders. The enlarged QP muscles that give desmognathines their distinctive 'neckless' appearance account for 70% of biting-muscle mass but exert 85% of biting force by virtue of the robust atlanto-mandibular ligament that originates on the enlarged atlas. The stalked occipital condyles are critical; they enable a wide range of head movements, including the characteristic 'head tuck' that desmognathines exhibit when biting (Dalrymple et al., 1985). During the head tuck, the condyles improve mechanical advantage of the QP muscles and amplify its force contribution by simultaneously increasing the in-lever length and decreasing the out-lever length.

Static model of theoretical bite force
We derived a novel geometric method to compute muscle force components (i.e. vectors) and lever-arm lengths in *D. quadramaculatus* that makes use of straight-line distances measured using calipers on dissected specimens without the need for 3D coordinates of morphological features. The vectors and lever arms produced were combined with estimates of muscle force (e.g. via PCSA) to calculate muscle moments. The same geometric methods were then used to convert muscle moments to force output at the end of a lever, such as a jaw tip (see Workbook 1).

Our geometric method enabled us to quantify the contribution of each of the jaw muscles and the unusual jaw mechanism described above to bite force in *D. quadramaculatus*. We conclude from these analyses that the unusual mechanism of force amplification and transmission via the atlanto-mandibular ligament significantly increases biting performance in desmognathine salamanders beyond what is possible with intrinsic jaw levator muscles. In addition, flexion of the head is important for fully engaging this mechanism and achieving the highest bite forces.

In vivo bite performance

Bite force calculated from the model, a maximum of 13.4 N at the last tooth with the head flexed, is comparable to the maximum measured *in vivo*, 14.3 N, indicating that the model captures the essential elements of the biting mechanism. We do not compare predicted with *in vivo* bite force statistically because we cannot be certain of the exact bite position along the jaw in each bite (Lappin and Jones, 2014) or the degree of flexion of the cranium relative to the atlas, in the absence of fluoroscope images for each *in vivo* bite.

Our measured biting performance of *D. quadramaculatus* is impressive for a salamander and significantly exceeds what the comparably sized *P. ruber* exhibited (14.3 versus 1.1 N maximum single bite; Fig. 7, Fig. S4). However, we conclude that the measured biting force of *D. quadramaculatus* falls below what they are capable of for three reasons. First, the bite transducer is rigid and therefore prevented full head flexion, which our model indicates is important for generating maximum biting force with the jaws in occlusion. Second, we measured the force of rapid feeding bites (Movie 1) rather than static-pressure bites used in defense, in combat or to crush prey (Brodie, 1978; Schwenk and Wake, 1993). Third, the value of isometric muscle stress that we used in the model (22 N cm^{-2}) is conservative because it is derived from frog-limb myofibers operating at 2.9°C, myofibers whose temperature coefficient of isometric force was calculated at 1.24 (Edman, 1979). We estimate that maximum *in vivo* bite forces may be 1.36 times greater at 18°C (the room temperature of our biting experiments), at which temperature the isometric muscle force is extrapolated to be 30 N cm^{-2}. Estimated peak bite force for *D. quadramaculatus* at 18°C is therefore approximately 18 N at the last tooth.

Significance and comparison with other systems

Desmognathine salamanders of all species possess the same unusual morphological features of the jaw mechanism described here and elsewhere (Dunn, 1926; Wake, 1966; Schwenk and Wake, 1993) and are therefore expected to display similarly elevated biting performance to what we observed in *D. quadramaculatus*. Why might desmognathines produce high biting forces? Desmognathines, like most salamanders, are opportunistic feeders that will eat most prey that can fit in the mouth (Deban and Wake, 2000; Wake and Deban, 2000), including arthropod prey such as insects and, in the case of *D. quadramaculatus*, crayfish that must be subdued by a powerful bite. The ability to inflict crushing bites on prey likely expands the dietary range of desmognathines beyond that of other comparably sized salamanders. That some prey may inflict injury may explain the presence in *D. quadramaculatus* of unusual subocular mineralizations (Fig. 2A) that are perfectly positioned to protect the eyes from damage by prey that are inside the buccal cavity. Desmognathines are unusual among salamanders in biting readily when cornered or handled (Baldauf, 1947; Dalrymple et al., 1985; Bakkegard, 2005); notably, *D. quadramaculatus* was found to use biting to repel the attacks of shrews (Brodie, 1978). We have observed *D. ocoee* defend against attacks by a larger plethodontid salamander species, *Gyrinophilus porphyriticus*, by tenacious biting, and have observed scars on *G. porphyriticus* of the appropriate size and shape to be the result of *D. ocoee* or small *D. monticola* bites. Finally, the diminutive *D. aeneus* and *D. wrighti* are unusual among plethodontids in using prolonged biting during courtship (Promislow, 1987), biting that is forceful enough that the male breaks the skin of the female and is able to lift her from the substrate.

In addition to enhancing biting force, the distinctive skull and jaw morphology and mechanism of desmognathines have been proposed to improve the ability of these salamanders to burrow and to wedge themselves beneath stones, behaviors that they exhibit (Dunn, 1926; Wake, 1966; Worthington and Wake, 1972; Bakkegard, 2005). The tapered skull and head that lacks dorsally protruding jaw levator muscles would require less force to penetrate a substrate or crevice than a skull with greater diameter, while its heavy ossification can withstand the forces involved. The atlanto-mandibular ligaments would indeed hold the mouth closed to prevent the entry of debris when the head is flexed.

The desmognathine system represents a means of generating high bite forces while eliminating dorsally protruding jaw levator muscles that would increase head diameter and the consequent cost of burrowing (Larsen and Beneski, 1988; Schwenk and Wake, 1993). Other salamanders that achieve high biting performance do so with enlarged jaw levator muscles, in the case of *Aneides* (Stebbins, 1985; Staub, 1993), or by a combination of large levator muscles and large body size, as in *Amphiuma* and *Cryptobranchus* (Elwood and Cundall, 1994; Erdman and Cundall, 1984). Our biomechanical analysis reveals that for *D. quadramaculatus* to produce the predicted maximum biting force in the absence of the QP muscle and its force-amplification mechanism that we describe, the LME would need to be 12 times larger in cross-sectional area, which would greatly increase the diameter of the head. Another group of burrowing amphibians, the limbless caecilians, have evolved an unusual bite-force enhancing mechanism in which a post-cranial muscle, the interhyoideus posterior, pulls ventrocaudally on the retroarticular process of the mandible. The caecilian jaw mechanism is thought to have evolved in response to a similar selective pressure to reduce head cross-sectional area and thereby reduce the cost of constructing burrows (Bemis et al., 1983; Kleinteich et al., 2008; Nussbaum, 1983; Summers and Wake, 2005). Interestingly, the interhyoideus posterior muscle of caecilians is a homologue of the QP of desmognathines (Bauer, 1992). An alternative strategy of increasing bite force in the face of space constraints is seen in crevice-dwelling chuckwalla lizards and involves widening rather than deepening the head (both dimensions are increased in many lizards); males have four times the bite force of females and wider but not deeper heads (Lappin et al., 2006).

The highest *in vivo* biting forces of *D. quadramaculatus* measured here (14.3 N maximum) fall within the range of those measured in lizards of similar size. *Anolis* were measured to produce ~11 N at 10 cm SVL (Herrel and O'Reilly, 2006), which *D. quadramaculatus* can exceed. Xenosaurid lizards produced up to 20.4 N at 11 cm SVL (Herrel et al., 2001), which exceeds our measured biting force in *D. quadramaculatus* but is close to the force predicted by the model (18 N) operating with full head flexion at a comparable temperature. If *D. quadramaculatus* could withstand the higher temperatures that many lizards prefer, they might well outperform lizards. That the bite force of *D. quadramaculatus* is comparable to that of lizards, especially when compared with the maximum performance we measured in *P. ruber* of 1.1 N, is a testament to the effectiveness of the force amplification mechanism. The importance of biting in the natural history of desmognathine salamanders – in feeding, defense and courtship – suggests that selection for elevated biting performance played a role in the evolution of their unique morphological features and highly effective jaw mechanism.

Systematic Zoology and Evolutionary Biology, Friedrich-Schiller-University in Jena, Germany. Charlotte Stinson, Jeffrey Olberding, Mary Kate O'Donnell and Christian Brown provided helpful comments on the manuscript.

References

Bakkegard, K. A. (2005). Antipredator behaviors of the red hills salamander, *Phaeognathus hubrichti*. *Southeast. Nat.* **4**, 23-32.

Baldauf, R. J. (1947). *Desmognathus f. fuscus* eating eggs of its own species. *Copeia* **1947**, 66.

Bauer, W. J. (1992). A contribution to the morphology of the m. interhyoideus posterior (VII) of urodele Amphibia. *Zool. Jahrb. Anat* **122**, 129-139.

Bemis, W. E., Schwenk, K. and Wake, M. H. (1983). Morphology and function of the feeding apparatus in *Dermophis mexicanus* (Amphibia: Gymnophiona). *Zool. J. Linn. Soc.* **77**, 75-96.

Brodie, E. D., Jr (1978). Biting and vocalization as antipredator mechanisms in terrestrial salamanders. *Copeia* **1978**, 127-129.

Dalrymple, G. H., Juterbock, J. E. and La Valley, A. L. (1985). Function of the atlanto-mandibular ligaments of desmognathine salamanders. *Copeia* **1985**, 254-257.

Deban, S. M. and Wake, D. B. (2000). Aquatic feeding in salamanders. In *Feeding: form, Function and Evolution in Tetrapod Vertebrates* (ed. K. Schwenk), pp. 95-116. San Diego, CA: Academic Press.

Dunn, E. R. (1926). *The Salamanders of the Family Plethodontidae*. Northampton, MA: Smith College.

Edman, K. A. (1979). The velocity of unloaded shortening and its relation to sarcomere length and isometric force in vertebrate muscle fibres. *J. Physiol.* **291**, 143-159.

Elwood, J. R. L. and Cundall, D. (1994). Morphology and behavior of the feeding apparatus in *Cryptobranchus alleganiensis* (Amphibia: Caudata). *J. Morphol.* **220**, 47-70.

Erdman, S. and Cundall, D. (1984). The feeding apparatus of the salamander *Amphiuma tridactylum*: morphology and behavior. *J. Morphol.* **181**, 175-204.

Herrel, A. and O'Reilly, J. C. (2006). Ontogenetic scaling of bite force in lizards and turtles. *Physiol. Biochem. Zool.* **79**, 31-42.

Herrel, A., De Grauw, E. and Lemos Espinal, J. A. (2001). Head shape and bite performance in xenosaurid lizards. *J. Exp. Zool.* **290**, 101-107.

Hinderstein, B. (1971). The desmognathine jaw mechanism (Amphibia: Caudata: Plethodontidae). *Herpetologica* **27**, 467-476.

Kleinteich, T., Haas, A. and Summers, A. P. (2008). Caecilian jaw-closing mechanics: integrating two muscle systems. *J. R. Soc. Interface* **5**, 1491-1504.

Lappin, A. K. and Jones, M. E. H. (2014). Reliable quantification of bite-force performance requires use of appropriate biting substrate and standardization of bite out-lever. *J. Exp. Biol.* **217**, 4303-4312.

Lappin, A. K., Hamilton, P. S. and Sullivan, B. K. (2006). Bite-force performance and head shape in a sexually dimorphic crevice-dwelling lizard, the common chuckwalla [*Sauromalus ater* (=*obesus*)]. *Biol. J. Linn. Soc.* **88**, 215-222.

Larsen, J. H., Jr and Beneski, J. T., Jr (1988). Quantitative-analysis of feeding kinematics in dusky salamanders (*Desmognathus*). *Can. J. Zool.* **66**, 1309-1317.

Nussbaum, R. A. (1983). The evolution of a unique dual jaw-closing mechanism in caecilians (Amphibia: Gymnophiona) and its bearing on caecilian ancestry. *J. Zool.* **199**, 545-554.

Promislow, D. E. L. (1987). Courtship behavior of a plethodontid salamander, *Desmognathus aeneus*. *J. Herpetol.* **21**, 298-306.

Schwenk, K. and Wake, D. B. (1993). Prey processing in *Leurognathus marmoratus* and the evolution of form and function in desmognathine salamanders (Plethodontidae). *Biol. J. Linn. Soc.* **49**, 141-162.

Soler, E. I. (1950). On the status of the family Desmognathidae (Amphibia, Caudata). *Univ. Kansas Sci. Bull.* **33**, 459-480.

Sommerville, D. M. L. Y. (1939). *Analytical Geometry of Three Dimensions*. Cambridge: Cambridge University Press.

Staub, N. L. (1993). Intraspecific agonistic behavior of the salamander *Aneides flavipunctatus* (Amphibia: Plethodontidae) with comparisons to other plethodontid species. *Herpetologica* 271-282.

Stebbins, R. C. (1985). *A Field Guide to Western Reptiles and Amphibians*. Boston, MA: Houghton Mifflin.

Summers, A. P. and Wake, M. H. (2005). The retro-articular process, streptostyly and the caecilian jaw closing system. *Zoology* **108**, 307.

Wake, D. B. (1966). Comparative osteology and evolution of the lungless salamanders, family Plethodontidae. *Mem. South. Calif. Acad. Sci.* **4**, 1-111.

Wake, D. B. and Deban, S. M. (2000). Terrestrial feeding in salamanders. In *Feeding: form, Function and Evolution in Tetrapod Vertebrates* (ed. K. Schwenk), pp. 95-116. San Diego, CA: Academic Press.

Worthington, R. D. and Wake, D. B. (1972). Patterns of regional variation in the vertebral column of terrestrial salamanders. *J. Morphol.* **137**, 257-277.
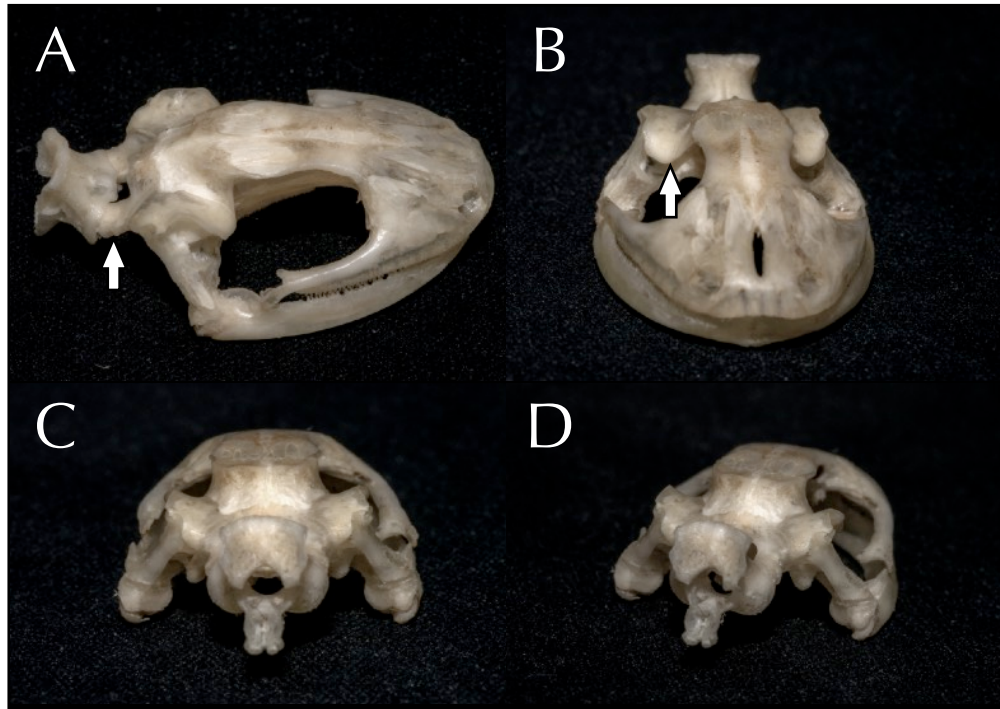
Fig. S1. Cranium and atlas of *D. quadramaculatus* showing the stalked occipital condyles (arrow in A), and the trochlea formed by the parietal and occipito-otic bones (arrow in B) through which the atlanto-mandibular ligament runs. The ligament slides freely in the trochlea, which is also visible in caudal views (C, D).
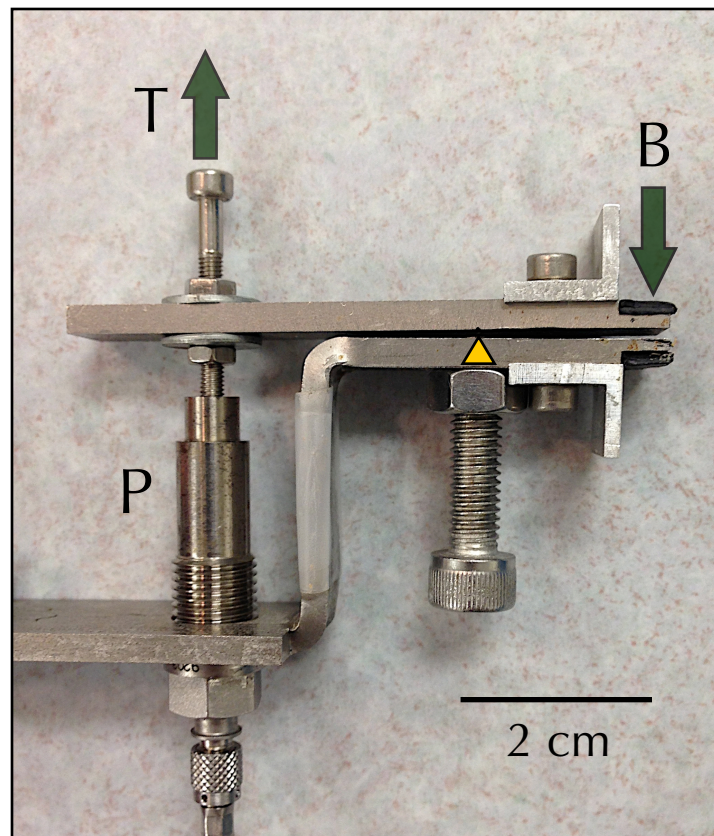
Fig. S2. Bite force transducer indicating the tension (T) applied to the piezo- electric transducer (P) via the fulcrum (triangle) when bite force (B) is applied to the bite plate.

Fig. S3. Adult *Desmognathus quadramaculatus* specimen in life. Note the relatively smooth dorsal surface of the cranium and the "neckless" appearance due to the enlarged quadratopectoralis muscles. These features and the enlarged hind limbs are characteristic of desmognathine salamanders. Head width is approximately 14 mm.
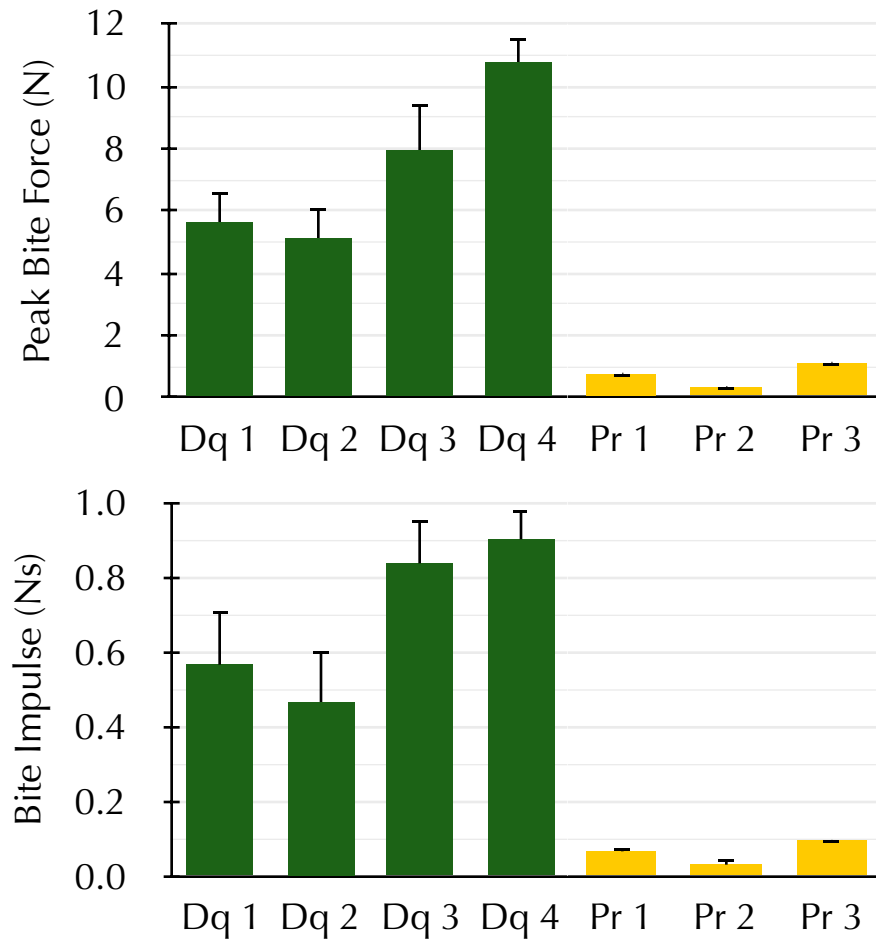
Fig. S4. Mean and SEM of peak bite force and bite impulse from *D. quadramaculatus* (green; n=6, 2, 4, 9) and *P. ruber* (gold; n=5, 3, 1).

Movie 1. *Desmognathus quadramaculatus* rapidly biting the bite transducer baited with a cricket exoskeleton.

Movie 2. Fluoroscope images of *Desmognathus quadramaculatus* capturing a cricket with tongue projection, followed by biting and prey transport as well as the characteristic desmognathine head-flexion behavior. Note the mobility of the atlanto-occipital joint between the enlarged atlas and the cranium during head flexion (upper panel) and the stalked occipital condyles (lower panel).

Workbook 1. Microsoft Excel Workbook with formulae and description of the geometric methods used to calculate muscle moments.

Click here to Download Workbook 1