The anatomy and feeding mechanism of the Japanese Giant Salamander (*Andrias japonicus*)

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ABSTRACT

The fully aquatic Japanese giant salamander (*Andrias japonicus*) is a member of the Cryptobranchidae, and is currently distributed in western Japan, with other members of this group restricted to China and North America. Their feeding behaviour is characterized by a form of suction feeding that includes asymmetric movements of the jaw and hyobranchial apparatus. Previous studies on the North American species, *Cryptobranchus alleganiensis*, have suggested that this specialised jaw movement is produced by a flexible quadrate-articular joint combined with a loosely connected lower jaw symphysis including two small fibrocartilaginous pads. However, little is known about this feeding behaviour in the Asian species, nor have the three-dimensional asymmetric jaw movements been fully investigated in any member of Cryptobranchidae. In this study, we explore the asymmetric jaw movements in *A. japonicus* using three methods: 1) dissection of musculoskeletal structures; 2) filming of feeding behaviour to understand in which situations asymmetric feeding is used; 3) analysis of 3D movement of jaws and skull. In the third component, fresh (from frozen) specimens of *A. japonicus* were manipulated to replicate asymmetric and symmetric jaw movements, with the specimens CT scanned after each step to obtain the 3D morphology of the jaws at different positions. These positions were combined and their Euler angles from resting (closed) jaw position calculated for asymmetric or symmetric jaw positions. Our filming revealed that asymmetric jaw movements are linked to the position of the prey in relation to the snout, with the jaw closest to the prey opening asymmetrically. Moreover, this action allows the salamander to simultaneously grasp prey in one side of the mouth while ejecting water on the other side, if the first suction attempt fails. The asymmetric jaw movements are performed mainly by rotation of the mandible about its long axis, with very limited lateral jaw movements. During asymmetric and symmetric jaw movements, the posterior ends of the maxilla and quadrate move slightly. The asymmetric jaw movements are permitted by a mobile quadrate-articular joint formed by wide, round cartilages, and by two small fibrocartilage pads within the jaw symphysis that act as cushions during jaw rotation. Some of these soft tissue structures leave traces on the jaws and skull, allowing feeding mode to be reconstructed in fossil taxa. Understanding cryptobranchid asymmetric jaw movement thus requires a comprehensive assessment of not only the symphysial morphology, but also that of other cranial and hyobranchial elements.

1 INTRODUCTION

Cryptobranchidae, commonly known as giant salamanders, comprises a group of fully aquatic salamanders currently restricted to eastern North America, eastern and southern China, and western Japan. Until 2018, only two extant genera and three species were recognised, *Cryptobranchus alleganiensis* (USA), *Andrias davidianus* (China), and *Andrias japonicus* (Japan), but recent genetic analysis (e.g. Yan et al., 2018; Liang et al., 2019) revealed that the Chinese Giant salamander (*A. davidianus*) could be divided into five or more different clades. However, their population structure has been modified by human activity and has resulted in genetic homogenization (Yan et al., 2018; Liang et al., 2019). More recently, one wild, genetically pure population has been recorded as *Andrias jiangxiensis* (Chai et al., 2022). The confirmed fossil record of Cryptobranchidae dates back to the Paleocene and shows a slightly wider distribution than at present, including Mongolia, Kazakhstan and also Europe (e.g. Szentesi et al., 2019).

Cryptobranchidae is characterised by a large body size and a rounded flattened skull, as well as aspects of their feeding behaviour. Cundall et al. (1987) and Elwood and Cundall (1994) reported an unusual, and apparently unique, feeding strategy in *C. alleganiensis* and *A. japonicus* whereby the jaws and hyobranchial apparatus could move asymmetrically to allow the mouth to open on one side only during suction feeding. There are no reliable records of asymmetric jaw movements in *A. davidianus*, but the potential for this movement has been deduced in previous studies (e.g. Heiss et al., 2013).

A comparative study of the kinematic patterns of aquatic prey capture in *Cryptobranchus* and other salamander families found that *Cryptobranchus* uses a bidirectional hydrodynamic motion as in *Amphiuma* (Reilly & Lauder, 1992). More recently, cryptobranchid feeding was explored using computational fluid dynamics models of Chinese giant salamanders (*Andrias davidianus*) (Heiss et al., 2013) and suggested that their suction feeding was powered largely by rapid jaw separation rather than hyoid depression as in other suction feeding salamanders. Feeding in *A. davidianus* has also been examined using 3D finite element analysis (Fortuny et al., 2015). This revealed that the position at which the prey contacts the jaw is important for feeding performance, and may linked to the absence of a bony connection between the maxilla and quadrate (Fortuny et al., 2015). Moreover, a recent developmental study of *A. japonicus* proposed that the early onset of jaw ossification, posterior expansion of the maxilla, and the posterolateral inclination of the squamosal were adaptations for a mode of suction feeding based primarily on mouth opening, but combined with hyobranchial depression (Ishikawa et al., 2022). Thus, an understanding of cryptobranchid skull morphology and feeding behaviour has gradually been developed from various perspectives. However, the mechanism of the unique asymmetric cryptobranchid feeding behaviour is known only from structural details of the jaw symphysis, the articular surface of the mandible, and the hyobranchial apparatus in *C. alleganiensis* (Cundall et al., 1987; Elwood & Cundall, 1994), and information is even more limited for *Andrias japonicus* (Cundall et al., 1987).

The unique cryptobranchid jaw movement has also been inferred for some fossil species, such as the Oligocene-Pliocene *A. scheuchzeri,* based on a ridge or angulation on the symphysial surface of the mandible that may have separated the two fibrocartilaginous pads within the symphysis (Szentesi et al., 2019). This interpretation was based on the morphological similarity of the fossil symphysis to that of a modern cryptobranchid, especially *C. alleganiensis*. Therefore, understanding the mechanism of asymmetric jaw movements in extant cryptobranchids may allow a more robust reconstruction of feeding behaviour in extinct species. Furthermore, clarification of the relationship between asymmetric jaw movements and skull and hyobranchial apparatus morphology is essential to understanding the early evolution of the group.

This study focuses on the Japanese giant salamander (*A. japonicus*), whose feeding strategy is poorly documented, to understand the circumstances under which asymmetric feeding takes place, and to investigate the three-dimensional movement of the skull and jaw during this movement. We used high-speed cine-photography to record actual feeding behaviour, examined the three-dimensional morphology and arrangement of soft tissues such as muscles and hyoid cartilage, and analysed the three-dimensional movement of the skull and jaw during asymmetric feeding. These observations are combined to yield a comprehensive discussion of the characteristic cryptobranchid feeding behaviour.

Key words

Aquatic, suction feeding, hyobranchial apparatus, Caudata, Cryptobranchidae

2 MATERIALS AND METHODS

2.1 Anatomy and dissection: Seven specimens of *Andrias japonicus* and one specimen of *Andrias davidianus* were dissected for this study (Supplementary Table 1). These specimens were obtained from Hiroshima City Asa Zoological Park, Mie Prefectural Museum, Kitakyushu Museum of Natural History & Human History, and the National Museum of Nature and Science with permission from the Ministry of the Environment. All specimens had died a natural death. Some of these specimens were scanned before dissection, using the micro-computed tomography (µCT) scanner at the National Museum of Nature and Science, Tokyo Japan. This is a TESCO, Microfocus CT TXS 320-ACTIS (slice width 0.1 mm). The software Avizo 8.0 was used to visualize 3D images of the µCT data. The myology described in our study is based on functional units. The muscle names follow those of Elwood & Cundall (1994) for jaw and hyobranchial muscles, and Francis (1934) or Erdman & Cundall (1984) for neck muscles, with innervation and function summarized from previous studies (Francis,1934; Kleinteich et al. 2014). However, possible additional functions are also proposed, based on the results of this study.

2.2 Iodine staining: In order to improve the contrast within soft tissues for µCT imaging (e.g. Jeffery et al., 2011; Gignac et al., 2016), a fresh specimen of *Andrias japonicus* (from Mie Prefectural Museum; snout-vent length [SVL] 238 mm; snout-tail length [STL] 335 mm) was stained with potassium iodide solution. An incision was made in the ventral surface of the pectoral girdle area, and the specimen was fixed in 4% paraformaldehyde for 5 days. The specimen was then immersed in a 5% iodine-potassium iodide solution (I_2Kl) for 10 days, and was µCT scanned. For the iodine-stained specimens, the x-ray source voltage of the µCT scanner was 189 kV and the current was 200µA. The scanned images were imported into the 3D visualization software, and the skull, individual muscles, and hyobranchial elements were segmented out.

2.3 Filming of behaviour: Feeding behaviour of *Andrias japonicus* (SVL 553 mm; STL 872 mm) was filmed using a high-speed camera (Ditect, HAS-L1M) with LED synchronized stroboscopic illumination at the Kitakyushu Museum of Natural History and Human History. The filming conditions were as follows: frame rate, 300/s; shutter speed, 1/2500; image, greyscale; window size, 800x600 mm; max frame number, 3080. A live weather loach (*Misgurnus anguillicaudatus*) was used as the prey during filming, as this fish is the usual food for the captive museum display specimen of *A. japonicus*. A total of 28 feeding events were recorded on the video (Supplementary Table 2). For each filming sequence, a single prey (live weather loach) was provided in the water tank. Key scenes from the film of the feeding sequence were exported as image files (TIFF) using Adobe Premier Pro 2023, and figures were created.

2.4 Asymmetric jaw movement: We used μCT to examine and quantify three-dimensional jaw and skull movement during asymmetric jaw opening. Five fresh (from frozen) *Andrias japonicus* individuals, at different ontogenetic stages (SVL 238 mm–~680 mm [STL 335–1000 mm]), were used, with each specimen being scanned in four different jaw positions: both jaws closed, left jaw only open, right jaw only open, and both jaws open (Figure 1a). The jaws of fresh carcasses were manipulated to match the jaw positions in living salamanders, in order to image them with the CT scanner. The maximum jaw gape was created without damaging the jaw joints, and a buffer material (sponge or Styrofoam block) was placed between the jaws to maintain the jaw positions. The jaw positions for asymmetric opening were manipulated by placing a buffer between the upper and mandibles on one side, while the contralateral jaws were held closed. These scanned images were imported into 3D visualization software (Avizo 8.0), and image data were segmented into three parts (skull, right and left jaws) for each specimen/jaw position. The data were exported in “stl” format, and then imported into the structural analysis software Voxelcon 2014 (Quint Co.) to combine the four different jaw positions and obtain the coordinates of the positional changes of the jaw.

2.4.1 Step 1: Data setting (Figure 1b, c)

1) For all jaw positions (closed, left open, right open, and both jaws open), X, Y, Z axes were oriented as follows:
   X axis, left–right (X=0, median plane); Y axis, dorsal-ventral; Z axis, anterior-posterior (Figure 1b)
2) For each specimen, the skulls were superimposed for the four positions. However, some skull elements, especially the maxilla and squamosal, were slightly displaced in different jaw positions. Therefore, skull position was aligned with respect to the braincase and the midline of the rostrum (e.g. nasal) (Figure 1b).
3) Based on this data, the amount of rotation of the mandible around the three axes (lateral, dorso-ventral, antero-posterior) relative to the skull (maxilla), and the amount of deformation within the skull during jaw opening and closing could be calculated.

2.4.2 Step 2: Calculation

1) **Jaw position in Euler angles**– For the four different jaw positions, the right and left jaws were duplicated from the closed jaw (rest) position, and repositioned in the X (left-right), Y

(dorso-ventral), and Z (antero-posterior) axes to define a reference position (Figure 1c). This reference position ensured that the tooth row of the dentary was always parallel to the horizontal plane (XZ plane), and that the symphysis was on the median plane of the skull (X=0). This reference position was then voxelised. The standard vector of the mandible in this voxelised reference position corresponds to the X, Y, and Z axes in coordinate space. Subsequently, this voxelised reference position of the mandibles was imposed on the four different jaw positions (both jaws closed or open, right or left jaw open). The standard vectors (DX, DY, DZ) of the imposed jaw reference position were then obtained. On this basis, the Euler angle of the different mandible positions was calculated. The Euler angles were defined by the rotational order of the X axis (pitch: positive for jaw open), Y^a axis (yaw: positive for leftward rotation), Z^b axis (roll: positive for rightward rotation) so that the mandible follows rotations (Figure 1d).

Y^a axis of the mandible: this is a new Y axis after the mandible has been rotated around the X-axis (left-right)

Z^b axis of the mandible: this is a new Z axis, after the mandible has been rotated around the X-axis (left-right), and then around the Y^b-axis.

These Euler angles for the different jaw positions were calculated using a spreadsheet software (Excel, Microsoft), and the results of jaw positional changes during asymmetric movement were displayed as a projection on the stereographic Wulff net using the package “RFOC” in statistical software R ver. 3.2.5. (The R Project for Statistical Computing).

2) **Skull deformation**– The points at the “most posterior end of the maxilla” and the “most ventral end of the quadrate” in a closed jaw position were taken as the reference points. The degree to which the homologous points in the other three jaw positions (both jaws open, right or left jaw open) were displaced relative to these reference points in the left-right and dorsoventral axes was measured. These relative transitions of the distal end of the quadrate (Q) and caudal end of the maxilla (M) are shown as a percentage of the skull width (quadrate-quadrate width in palatal view; Supplementary Table 1).

To avoid confusion between the jaw position in Euler angle and the skull deformation, “rotation” is used to refer to the Euler angle, ”transition” to refer to the skull deformation, and “movement” to refer to general movement of jaws and skull.

3. INSTITUTIONAL ABBREVIATIONS

KPM, Kanagawa Prefectural Museum of Natural History, Japan; NSM, National Museum of Nature and Science, Japan; UCL, University College London

4. DESCRIPTION

4.1 Cranial osteology (Figure 2)

A detailed description and illustration of the *Andrias japonicus* skull has been given by previous authors (e.g. Osawa, 1902; Schumacher, 1958; Rong et al., 2021), as well as that of *Cryptobranchus alleganiensis* (e.g. Reese, 1906; Elwood & Cundall, 1994). Skull morphology is generally similar in these two genera, but the adult skull of *A. japonicus* (STL ~1500 mm) is much larger than that of *C. alleganiensis* (STL up to 750 mm) (Deban & Wake, 2000). Therefore, cartilage bones (such as the quadrate and articular) are well ossified in mature *C. alleganiensis* compared to similar sized specimens of *A. japonicus* (Figure 2: Supplementary Figure 1).

Although the skull of *A. japonicus* is closely similar to that of *C. alleganiensis*, there are some minor differences (e.g. Reese, 1906; Meszoely, 1966), and these are summarised in Table 1. Most of these differences have been reported in previous studies (e.g. Reese, 1906; Meszoely, 1966), but some characters have been added based on the current study. Few of the morphological differences between these two species are likely to affect feeding, but the size of the coronoid process of the prearticular and the pterygoid-maxilla relations may be exceptions. In *A. japonicus*, the coronoid process of the prearticular is well developed, regardless of growth stage (SVL 238 mm [STL 335 mm], RM pers. obs), but it is slightly smaller in *C. alleganiensis.* The larger coronoid process may reflect a stronger, tendinous attachment of the adductor muscles. In addition, although both genera lack a pterygoid-maxilla contact (connected by pterygomaxillary ligament), the two bones approach one another more closely in *A. japonicus* (with a shorter pterygomaxillary ligament), possibly allowing a stronger bite compared to *C. alleganiensis.* However, bite force has yet to be measured in either species.

Individual variation in skull morphology may be observed in *A. japonicus*. For example, the midline suture between the nasals is not always straight, and may be interdigitated in large individuals (RM pers. obs.). In some young adult specimens (SVL 365 mm [STL 580 mm]), the prefrontal and frontal are fused on one side but separate on the other (Figure 2a). This condition has also been reported in *C. alleganiensis* (Elwood & Cundall, 1994). The midline suture of the premaxilla may be closed in some adult *Andrias japonicus*, but patent in others. In addition, the frontal and parietal may develop a dorsal midline ridge, which presumably reflects enlargement of the deep mm. levator mandibulae anterior. In *Andrias davidianus*, the midline suture of the parietals, and the fronto-parietal sutures, are usually fused in the adult stage (Fortuny et al., 2015). Nonetheless, some sutures remain open, such as the vomer-parasphenoid and the premaxilla-maxilla-nasal-frontal sutures, which are considered to have a shock absorption function during biting (Fortuny et al. 2015). However, even in the large specimens of *A. japonicus* used in this study (SVL 564 [STL 820 mm]), the midline parietal and fronto-parietal sutures remained patent (the CT data for the largest specimen examined, STL 1000 mm, is not clear). The mid-parietal suture appears to be open, but the condition of the fronto-parietal suture is uncertain).

4.2 Symphysis (Figure 3)

The detailed structure of the symphysis has been described in *Cryptobranchus alleganiensis* (Cundall et al., 1987; Elwood & Cundall, 1994). According to Elwood & Cundall (1994), two small median cartilages are contained within the symphysis, with each cartilage surrounded by a different collagen structure: woven collagen for the dorsal cartilage and scattered collagen fibres for the ventral cartilage. In addition, these two cartilages are separated by a band of dense collagen (the median symphyseal ligament). This ligament tightly connects the symphyseal plates on both sides of the dentaries. We confirmed the presence of these cartilages in *Andrias japonicus* (Figure 3a–d). A small, thin triangular dorsal cartilage is wrapped by woven collagen which is attached to the dorsal convexity of the symphysis (Figure 3c–f). The ventral cartilage is larger and softer than the dorsal pad, and this cartilaginous pad lies in a small compartment surrounded by collagen fibres, with a slight cavity between the ventral cartilage and the fibres. A medial symphyseal ligament runs between these two pads and tightly binds the jaws. The attachment of this medial ligament corresponds to the angled line between the dorsal convexity and the ventral concavity in a dry jaw. In addition, the symphysial joint is wrapped by two further ligaments: the central and superficial symphyseal ligaments. The anterior surfaces of both dentaries are bound with a transversally aligned fibrous band, the central symphyseal ligament (Figure 3c, d). A trace of this ligament may remain on the anterior symphysial margin of the dentary as a roughened transversally-oriented structure. The ventral margins of the symphysis are connected by the superficial symphyseal ligament, of which a trace remains as a shallow depression on the ventral surface of dentary (Figure 3b, ssl). During jaw movement, the dorsal symphysial pad mainly has a role as the centre of the rotation axis, while the ventral pad is a cushion for the joint.

As noted above, osteological specimens of *A. japonicus* and *C. alleganiensis* retain traces of the symphysial structures (Figure 3e–g). The dorsal part of the symphysis is smooth and slightly convex (Figure 3e) and some dry specimens bear radially-arranged tissues that may be the remains of woven collagen (Figure 3f). In contrast, the ventral (or posterior) half of the symphysis is concave and the surface is slightly roughened (Figure 3e, f), with a distinct angulation marking the boundary between the dorsal convexity and ventral concavity, and probably also the insertion line of the median symphyseal ligament. This angulation becomes clearer in larger individuals: contrast Figure 3e (skull width 66.6 mm) vs. Figure 3f (skull width 78 mm). The absence of the cartilages in dry specimens makes it difficult to articulate the mandibular symphysis firmly, leaving a small gap and a rounded surface between the two bones.

4.3 Quadrate-articular joint (Figure 4)

The structure of the quadrate-articular joint of *Andrias japonicus* is similar to that of *Cryptobranchus alleganiensis* (Elwood & Cundall, 1994). The mandible of *A. japonicus* is composed of the dentary, prearticular, angular, and articular bones (Figure 2a, b). The jaw surface of the articular is cartilaginous and is broad anteriorly and narrower posteriorly (Figure 4a–d).

The cranial component of the jaw joint is formed by the ossified quadrate bone and a thick unossified portion of pterygoquadrate cartilage filling the space between the lateral process of the pterygoid and the squamosal (Figure 4a, d). The articular surface of the quadrate is also covered by a thin layer of cartilage (Figure 4c), which was not visible on the diceCT images. Together, quadrate and pterygoid form a smooth concave surface for articulation with the mandible (Figure 4c, d). The quadrate mainly contacts the broad anterior part of the articular surface of the mandible, while the cartilage on the pterygoid meets the narrow posterior part of the articular cartilage on the mandible (Figure 4a–d). The jaw joint is enclosed by a robust ligament (detailed description in Elwood & Cundall, 1994) that allows for flexible rotation around the longitudinal and transverse axes of this broad jaw joint during asymmetric jaw movements (Figure 4b, c).

4.4 The hyobranchial apparatus in Cryptobranchidae (Figure 5)

Within Cryptobranchidae, some morphological variation occurs in hyobranchial morphology (e.g., Cox & Tanner, 1989; Edgeworth, 1923). In *Andrias japonicus*, there is no clear separation between the first and second hypohyal, whereas these elements are separate in *Cryptobranchus alleganiensis*. The ossification of hyobranchial elements is limited to the second hyobranchial and the second ceratobranchial in *A. japonicus*, but the posterior parts of the ceratohyals and the third ceratobranchial are also ossified in *C. alleganiensis.* Moreover, the third hyobranchial, and third and fourth ceratobranchials, are absent in *A. japonicus*, but are retained in the adult stage of *C. alleganiensis*. In the Chinese species, *A. davidianus*, these hyobranchial structures are basically similar to those of the Japanese species, but they differ from all other species in the enlargement of the hypohyals and arrangement of the basihyals

(Cox & Tanner, 1989). Furthermore, in *A. davidianus,* the first ceratobranchial has developed a small flange on the posteromedial end, which may provide a strong muscle attachment for the m. subarcualis rectus I.

4.5 The hyobranchial apparatus in *Andrias japonicus* (Figure 5, Supplementary Figure 2)

The hyobranchial elements of the adult stages of *Andrias japonicus* have been described in several studies (e.g. Schmidt et al., 1862; Fischer, 1864; Hyrtl, 1865; Parker, 1882; Osawa, 1902), as have the larval hyobranchial skeleton and musculature, and growth stage modifications (e.g. Edgeworth,1923; Ishikawa et al. 2022).

The first and second hypohyal (HHI, II) form an M shape that lies along the anterior margin of the mandible (Figure 5a–c). These elements form the posterior margin of the anterior fold of buccal mucosa (Supplementary Figure 2). A tiny basihyal (BH) is attached to the HHI in the midline, but is free from the basibranchial (BB) (Figure 5a, b).

A small median cordiform basibranchial is situated posterior to the BH in the middle of the other hyobranchial elements (Figure 5a–c). It has a small prominence on its ventral surface with two distinctive, shallow concavities on each side: a lateral cavity receives the first ceratobranchial (CBI), and a posterior cavity accommodates the second hyobranchial (HBII) (Figure 5d, e). The corresponding proximal articular surfaces of the CBI and the HBII are smooth and rounded, and there is some space within the joint between CBI + HBII and the BB (Figure 5b–d). These joints primarily allow mediolateral flexion of the HBII and the CBI on the BB, but they also permit very restricted rotation and dorsoventral movement.

The anterior portion of the basibranchial overlaps the third hypohyal (Figure 5b). The hypohyals are small and rounded, attached to the ceratohyals laterally, and provide a strong attachment for the tendon of the m. subarcualis rectus I. Dorsal to the first ceratobranchial, a plate-like ceratohyal covers most of the floor of the mouth (Figure 5a–c). This element gradually curves posterolaterally, with a thickening along the medial and posterior margins (Figure 5c). The posterior margin is covered by a hyoquadrate ligament, which extends towards the posterior margin of the squamosal where the quadrate cartilage is located (Supplementary Figure 2). Within this ligament, a small thin triangular cartilage (‘epi-hyal’ in Parker, 1882) lies over the posterior margin of the squamosal and also overlaps the posterior end of the ceratohyal, forming a loose joint (Figure 4b). This joint contributes to stabilizing the ceratohyal during its posterolateral swing as the buccal cavity expands. The hyobranchial apparatus has a flattened shape in its resting position, as seen in lateral view. In this resting position, the epi-hyal is also horizontal at its attachment to the posterior end of the ceratohyal. However, during buccal expansion, the epi-hyal is pushed slightly dorsally as the ceratohyal moves posterolaterally to form the lateral wall of the oral cavity, broadening the pharynx. In addition, as noted above, the first ceratobranchial and the second branchial arch (HBII + CBII) can move at their joint on the basibranchial, and these also contribute to the lateral wall of the oral cavity when the mouth is expanded. The CH and CBI are connected by the m. subarcualis rectus I, and the posterior ends of CBI and the second epibranchial (EBII) are connected by short ligaments. Therefore, the movement of the HBII+CBII+EBII can be linked to those of the CH+CBI during expansion or compression of the buccal cavity (Figure 5a–c).

When asymmetric hyobranchial movements occur, they are mainly produced by independent movement of the anterior hyobranchial elements (hypohyal, basibranchial, branchial arches I and II), due to their loose attachments (Cundall et al. 1987). This flexibility of these hyobranchial articulations was confirmed during the dissection of *Andrias japonicus* on unfixed specimens.

4.6 Myology

Our dissections of the adductor and hyobranchial apparatus of adult *Andrias japonicus* largely confirmed previous studies of *A. japonicus* and *Cryptobranchus alleganiensis* (e.g. Schmidt et al., 1862; Hyrtl, 1865; Osawa, 1905; Elwood & Cundall, 1994) and the more specific muscle attachments shown in Figure 6, but there are some minor differences among Cryptobranchidae. The cranial muscles of *A. japonicus* are mainly divided into four functional units: mandibular elevators; mandibular depressors; hyobranchial depressors; and hyobranchial elevators.

4.6.1 Mandibular muscles (Figure 6, 7)

4.6.1.1 M. levator mandibulae externus (LME): This large rounded muscle mass occupies the space behind the tooth row, between the posterior edge of the maxilla and the anterior portion of the squamosal (Figure 6a, b; 7a). The fleshy LME arises from the antero-lateral portion of the squamosal, and these muscle fibres insert into the posterolateral surface of the dentary, just behind the level of the tooth row (Figure 7a). The origin of the LME is marked on the dorsal surface of the squamosal by a transverse keel, and a short ridge along the suture for the parietal separates the origins of the LME and the anterior part of the m. depressor mandibulae (ADM). The insertion of the LME is marked by slightly roughened surface and a shallow groove on the lateral surface of the dentary. A strong ligament at the posterior end of tooth row firmly connects the maxilla to the dentary, and anterior superficial fibres of the LME may attach to this ligament.

Innervation: Mandibular ramus of the trigeminal nerve (V_3).

Function: The primary action of this muscle is jaw closure, but the mediolateral orientation of the muscle fibers suggests that this muscle might also function in the internal (as in roll) and medial (as in yaw) rotations of the mandibles.

4.6.1.2 Deep m. levator mandibulae anterior (DLMA): The deep m. levator mandibulae anterior is a large, fan-shaped muscle occupying the space behind the orbit (Figure 6a; 7a). Although this muscle is divided into three parts in *Cryptobranchus alleganiensis* (Elwood and Cundall, 1994)*,* only two bundles are evident in *Andrias japonicus*, as noted in Osawa (1902). The deepest sheet (DLMA II) arises from three bones: the posterior two thirds of the prefrontal, the dorsal surface of the frontal; and roughly the anterior half of the parietal (Figure 6a–d; 7a). The superficial sheet (DLMA I) arises near the midline of the frontal and the parietal (Figure 7a). A trace of these muscle attachments remains on the prefrontal, frontal and parietal as a shallow longitudinal depression. In large specimens (e.g. SVL 564 mm [STL 820 mm], KPM-NFA 50), there is a well-developed midline keel on the frontal and parietal, indicating strong muscle attachments for the DLMA. The muscle layers of DLMA I and II are attached ventrally to tendinous sheets (aponeuroses) and these sheets are attached to the aponeurosis of the superficial m. levator mandibulae anterior (SLMA) (Figure 6a–c). Together, these three aponeuroses converge to form a tendon that is attached to the medial margin of the coronoid process of the prearticular (Figure 7a). This tendon attachment is marked by a distinctive ridge on the prearticular, even in relatively small individuals (e.g. skull width of 37.3 mm; SVL 238 mm [STL 335 mm]).

Innervation: Mandibular ramus of the trigeminal nerve (V_3).

Function: The primary function of this muscle is jaw closure, but the mediolateral orientation of the muscle fibers suggests that it might also function in the internal (as in roll) and medial (as in yaw) rotations of the mandibles.

4.6.1.3 Superficial m. levator mandibulae anterior (SLMA): This long muscle is roughly L-shaped in appearance (Figure 6a–c). It arises from aponeuroses attached from the first to third vertebrae in *Andrias japonicus*, but from the first and second vertebrae in *Cryptobranchus alleganiensis* (Elwood & Cundall, 1994). The muscle runs along the midline to the posterior part of the skull extending above the epaxial musculature. Anterior muscle fibres overlap the deep m. levator mandibulae anterior (DLMA I) on the posterior part of the parietal (Figure 7c). At the insertion, all the sheets of muscles converge into a strong band-like tendon that is attached to the dorsally expanded coronoid flange of the prearticular, as noted under DLAM (Figure 6a, 7c).

Innervation: A separate branch of the mandibular ramus of the trigeminal nerve V_3.

Function: The primary function of this muscle is jaw closure, but as for the DLMA, the orientation of the muscle fibres suggests it may also have a role in the internal (as in roll) and medial (as in yaw) rotations of the mandibles.

4.6.1.4 M. levator mandibulae posterior (LMP): This muscle is located deep to the m. levator mandibulae externus (LME) and fills a small space in the anterior margin of the squamosal (Figure 6b). Fleshy fibres of LMP originate on the anterior margin of the squamosal, ventral to the origin of the LME (Figure 7a). These two sheets of muscle are hardly distinguishable near the origin, but they are separated by the mandibular branch of the trigeminal nerve as noted in *Cryptobranchus alleganiensis* (Elwood & Cundall, 1994). Superficial fibres of the LMP insert on the dorsal margin of the dentary, medial to the LME. The deeper fibres insert on the dorsal surface of Meckel’s cartilage and the lateral margin of the coronoid process of the prearticular (Figure 7a).

Innervation: Mandibular ramus of the trigeminal nerve (V_3).

Function: The mediolaterally orientated muscle fibers suggest that it may function in the internal (as in roll) and medial (as in yaw) rotation of the mandibles, as well as jaw closure.

4.6.1.5 Anterior m. depressor mandibulae (ADM): This is a large, rounded muscle that is superficially similar to the anteriorly located LME in its morphology and volume (Figure 6a, b). This muscle wraps the posterior margin of the squamosal, the pterygoquadrate cartilage, and the posterior end of the ceratohyal. The anterior m. depressor mandibulae divides into two bundles (Figure 6a–c). The anterior bundle (ADM I) originates from the posteromedial margin of the squamosal (immediately lateral to the parietal suture) via a tendon and fleshy fibres (Figure 6a, b). Ventrally, the fibres insert into the posterodorsal edge of the articular by a thick tendon. The anterior bundle (ADM I) is wrapped by a slightly smaller posterior bundle (ADM II) (Figure 6a–c). The latter originates on the posteromedial edge of squamosal, medial to the ADM I, and inserts on the posterior tip of the articular, posteromedial to the insertion of the ADM I (Figure 7a, b).

Innervation: R. jugularis of the facial nerve (VII).

Function: Jaw opening.

4.6.1.6 Posterior m. depressor mandibulae (PDM): This fan-shaped muscle lies posterior to the ADM (Figure 6a–d). The muscle originates from the dorsal fascia, and gradually increases in thickness towards its insertion on the articular, posteromedial to the insertion of the ADM II (Figure 7b).
Innervation: R. jugularis of the facial nerve (VII).
Function: Jaw opening.

4.6.2 HYOBRANCHIAL MUSCLES (Figures 8, 9)

Four thin sheets of muscle, the m. intermandibularis anterior (IMA), m. intermandibularis posterior (IMP), m. interhyoideus (IH) and m. interhyoideus posterior (IHP), extend transversally and form the floor of the mouth (Figure 8a).

4.6.2.1 M. intermandibularis anterior (IMA): This small, semicircular muscle extends transversally to connect the two dentaries on their ventromedial surfaces (Figure 8a, 9a). A trace of the muscle origin remains on the dentary as a flat surface or shallow concavity, medial to the ridge for the m. geniohyoideus attachment (Figure 10).
Innervation: Mandibular ramus of the trigeminal nerve (V_3).
Function: Ventroflexion of the symphysis, which corresponds to the external rotation of the mandible (as in roll).

4.6.2.2 M. intermandibularis posterior (IMP): This muscle is located posterior to the m. intermandibularis anterior (Figure 8a). It inserts into the dorsomedial margin of the subdental ridge, formed by the dentary and prearticular, via a tendinous sheet that thickens posteriorly (Figure 10). Like the IMA, the muscle fibres extend transversely, and insert into a midline tendinous raphe (Figure 8a). Schumacher (1958) described the muscle as an elevator of the posterior septum of the floor of the mouth, contributing to the floor of the pharynx.
Innervation: Mandibular ramus of the trigeminal (V_3).
Function: The primary function of this muscle is elevation of the buccal floor, but the transversally orientated muscle fibers may function in external rotation (as in roll) and medial rotation (as in yaw) of the mandible.

4.6.2.3 M. interhyoideus (IH): This thin sheet of muscle is overlapped anteriorly by the m. intermandibularis posterior (IMP), and posteriorly by the m. interhyoideus posterior (IHP) in ventral view (Figure 8a). The IH attaches to the posterolateral margin of the ceratohyal and the posteromedial margin of the quadrate and pterygoid articular cartilages, mostly on the pterygoid side (Figure 10). In *Cryptobranchus alleganiensis* and other salamanders, this muscle originates from the quadrate (Elwood & Cundall, 1994; Francis, 1934). This difference may be due to the ossification stage of the *Andrias japonicus* specimens examined. Although large specimens were dissected in this study (SVL 483 mm [STL 710 mm]), the quadrate was not completely ossified unlike that of *C. alleganiensis* (Supplementary Figure 1). The m. interhyoideus muscle fibres fan out across the floor of mouth, and insert on the midventral raphe, posterior to the IMP (Figure 8a). Although no clear traces of muscle attachment were left on the bone, the ventromedial rim of the quadrate is considered to be a muscle attachment surface (Figure 7a).

Innervation: R. jugularis of facial nerve (VII).

Function: This muscle assists breathing and deglutition by constriction of the hyobranchial elements and the posterior part of the mouth. However, like the IMP, the transverse orientation of these muscle fibres suggest it may also have a role in external rotation (as in roll) and medial rotation (as in yaw) of the mandibles.

4.6.2.4 M. interhyoideus posterior (IHP): This has also been called the superficial m. levator mandibulae anterior (Elwood & Cundall, 1994), but the terminology of Francis (1934) is used here as it is more consistent with the ventral muscle position. It is the most posteriorly located of the superficial muscles, wrapping around the m. depressor mandibular. The IHP is very thin, especially on the dorsal side, and is tightly attached to the dorsal fascia that covers the lateral surface of the head behind the mandible. This tight fascial attachment makes it difficult to detach the muscle from the skin without damaging the muscle laterally. The muscle fibres run medially and terminate on the midline raphe which also receives the m. intermandibularis posterior and m. interhyoideus (Figure 8a).

Innervation: R. jugularis of facial nerve (VII).

Function: Elevates the buccal floor

4.6.2.5 M. geniohyoideus (GH): The m. geniohyoideus lies deep to the m. intermandibularis posterior (IMP), m. interhyoideus (IH) and m. interhyoideus posterior (IHP) (Figure 8, 9a, d). It arises from the ventral margin of the dentary via a connective tissue sheet that forms the anterior fold of the buccal lining (Figure 8b). This connective tissue is also attached medially to the posterior margin of the m. intermandibularis anterior (IMA). The ventral surface of the dentary, near the symphysis, has clear traces of this muscle attachment in the form of sharp ridges (Figure 10). The anterodorsal fibres of m. geniohyoideus are tightly attached to the anterior fold of the buccal lining. The movement of this anterior fold may therefore be directly controlled by the GH. Posteriorly, the muscle fibres divide into superficial and deep parts (Figure 9d). The superficial part tapers posteriorly and inserts on the ventral epimysium of the m. rectus cervicis, a muscle that extends to the level of the middle part of the pectoral girdle. The deeper part terminates at posterolateral margin of the second hypobranchial.

Innervation: Hypoglossal nerve.

Function: Depression of the mandible or whole head, or it may pull the *os triangulare* forward.

4.6.2.6 M. genioglossus (GG): This is a very tiny strap-like muscle, running dorsal to the m. geniohyoideus (Figure 8b). The muscle originates from the lingual surface of the dentary, just above the origin of the m. geniohyoideus, and inserts on the first and second hypohyals (Figure 8b). It is tightly attached to the anterior fold of the buccal lining. The muscle is too small to be identified on the Dice CT images and is therefore not figured.

Innervation: Terminal twigs of the hypoglossal nerve

Function: Mainly extrusion of tongue

4.6.2.7 M. subarcualis rectus I (SRI): This is a relatively thick and elongated muscle that links the ceratohyals (CH) and first ceratobranchials (CBI) (Figure 8a, 9d). It arises from a thick tendon that attaches to the third hypohyal and lies along the anterior margin of the ceratohyal. Deep fleshy fibres also attach to the ventral surface of the ceratohyal, over approximately the anterior third of the area. The muscle fibres run parallel to this cartilage and insert on the first ceratobranchial, wrapping around its posterior end. In *Cryptobranchus alleganiensis*, a small muscle slip arising from the medial edge of the ceratohyal inserts on the lateral surface of the joint between the second hyobranchial and ceratobranchial (Elwood & Cundall, 1994). However, this muscle slip has not been identified in the seven specimens of *Andrias japonicus* dissected in this study, nor was it mentioned in Osawa (1902). In the larger Chinese species, *Andrias davidianus* (SVL 740 mm [STL 1180 mm]), a medial muscle bundle attaches to the joint between the second hyobranchial and ceratobranchia, but most of its fibres attach to the buccal lining between the ceratohyal and the second hyobranchial. This could be an interspecific or size related difference.

The mm. branchiohyoideus externus (Edgeworth, 1935), or the ceratohyoideus externus (Drüner, 1901) also connects CBI and CH, like the m. subarcualis rectus I, but they differ in innervation (cranial nerve VII for the m. branchiohyoideus externus) (Kleinteich & Haas, 2011). The mm. branchiohyoideus externus and subarcualis rectus I are both found in the larval stage of *A. japonicus* and *C. alleganiensis* (Kleinteich et al. 2014). However, the muscle has not been recognized in adult stages (e.g. Elwood & Cundall, 1994). In the description of *A.*

japonicus by Osawa (1902), the name “cerato-hyoideus externus” is used for the muscle attached to the CBI and CH, but this is the same muscle as here termed “the m. subarcualis rectus I”, and is different from a muscle with the same name in Drüner (1901) (Francis, 1934). Although the m. branchiohyoideus externus has not been identified, it is possible that this muscle is reduced or lost in the adult stage. A small muscle bundle associated with the joint between the second hyobranchial and ceratobranchial in *C. alleganiensis* and *A. davidianus* may be a remnant of the m. branchiohyoideus externus.

Innervation: Glossopharyngeal nerve (IX) and vagus to visceral arches (X).

Function: The muscle contributes to protraction of the branchial arches and their associated copula.

4.6.2.8 M. rectus cervicis (RC): This large muscle bundle runs along the midline (Figure 9a–f). The superficial fibres converge on a tendon that attaches to the point at which three elements (basibranchial, first ceratobranchial, and second hyobranchial) meet at the midline. The tendon attaches to a small area on the ventral prominence of the basibranchial, the anterodorsal tip of the first ceratobranchial, and the anterior margin of the second hyobranchial. The muscle fibres have a fleshy attachment to the buccal lining between the first ceratobranchial and the second hyobranchial, and wrap around the anterior part of the second hyobranchial. Deep anterior fibres run to the ventral side of the second hyobranchial and posteriorly the muscle merges into the m. rectus abdominis as a broad muscle sheet.

Innervation: The first three spinal nerves.

Function: Supports the m. geniohyoideus, while retracting the tongue.

4.6.3 Neck muscles (Figure 11, 12)

4.6.3.1 Epaxial muscles: Three major components of epaxial musculature were identified in *Andrias japonicus,* as in other salamanders (e.g. Elwood & Cundall, 1994; Deban & Wake, 2000): m. intermyoseptalis (Erdman & Cundall, 1984) (or m. dorsalis trunci; Francis, 1934), m. myoseptal-vertebralis (or m. intertransversarium capitis superior), and the m. intervertebralis. The m. intermyoseptalis (IME) is a large muscle that mainly covers the dorsal part of the body (Figure 11a). This muscle divides anteriorly into two layers, superficial and deep, both of which insert into the occipital region (Figure 6b). The superficial layer (IME I) is a small triangular muscle that inserts into the posterior crest of the parietal, along the suture with the squamosal (posterior to the ADM attachment) (Figure 6b). The deeper layer of the m. intermyoseptalis (IME II) inserts just below the superficial layer, and its insertion is marked by a shallow concavity on the posterior part of the parietal (Figure 7b). The epaxial m. myoseptal-vertebralis (MVE) originates from the dorsolateral margin of the neural spine of the atlas (Figure 7b, 11c). This small muscle slip inserts on the posterior part of the parietal, medial to the insertion of IME II (Figure 11a, b). The deepest epaxial muscle, m. intervertebralis (IVE), originates lateral to the neural spine of the atlas, and inserts on the dorsal surface of the exoccipital, along line of the parietal suture (Figure 7b, 11c). The muscle attachment on the exoccipital is marked by a flat surface (Figure 7b).

4.6.3.2 Hypaxial muscles: The hypaxial muscles are arranged in two bundles, the m. subvertebralis medialis (MSV) and the m. subvertebralis lateralis (LSV), both of which attach to the braincase (Figure 11d). The lateral branch is separated from the medial branch (MSV) around the second vertebra and it is attached to the posteroventral surface of the exoccipital and parasphenoid by an aponeurosis (Figure 11d). The medial branch (MSV) inserts on the same aponeurosis and attaches to the posteroventral margin of the parasphenoid (Figure 11d). These muscle attachments are marked by a shallow groove on the posterior margin of the parasphenoid.

4.7 Feeding behaviour in *Andrias japonicus* (Figure 12, Supplementary Figures 3, 4)

Vertebrate feeding behaviour is generally divided into three phases: 1, prey capture; 2, manipulation; 3, swallowing. In fully aquatic salamanders, these three strategies generally involve water flow. Suction feeding (or ‘gape and suck feeding’; Deban & Wake, 2000) is accomplished by rapid expansion of the buccal cavity combined with depression of the mandible and hyobranchial apparatus. During mouth opening, prey is drawing into the buccal cavity with water. At this time the gill slits (if retained) are closed, and the mouth closes as the prey is manipulated into the buccal cavity. The gill slits then open and water is expelled through them or through the mouth via a narrow slit-like opening, concomitant with elevation of the hyobranchial apparatus which returns to its original position.

In *Cryptobranchus alleganiensis,* prey capture occurs either by inertial suction or a strike combined with suction (Elwood & Cundall, 1994). In inertial suction negative pressure is created in the oral cavity by buccal expansion combined with the depression of the hyobranchial apparatus. On the other hand, a strike is distinguished from inertial suction by the initial movements of the head. During jaw opening, elevation of the braincase is combined with lateral and anterior movement of the head. Jaw closing during a strike is similar to that in inertial suction, with greater displacement of the branchial arches, but a shorter period for the recovery phase (Elwood & Cundall, 1994).

Like *Cryptobranchus alleganiensis*, feeding in *Andrias japonicus* is generally either by inertial suction or a strike with suction. *A. japonicus* is occasionally observed to approach and attack the prey by moving its head and body, but it usually waits until the prey is close enough to establish a suction distance. When the prey is located above the head or at the bottom of the aquarium, *A. japonicus* sometimes elevates the head to open the mouth (Supplementary Table 2). However, in most cases, the mouth is opened by the depression of the mandible and hyobranchial apparatus, followed by elevation of the skull on the neck. During suction, the buccal cavity is enlarged by the lateral and posterior expansion of the hyobranchial apparatus. As the buccal cavity is expanded like a balloon, the prey is swallowed with water. Once the prey is caught, the jaws are immediately tightly closed, but subsequently a narrow gap is created between the upper jaws and mandibles to expel water from the mouth. Due to the reaction of the suction force, the salamander’s body is often seen floating in the water like a large balloon. In the recovery phase, the hyobranchial apparatus returns to its original rest position by gradual compression of the buccal cavity, and the salamander returns to the floor of the aquarium at the end of this phase. This recovery phase is the longest of the feeding sequence, taking approximately 2–3 minutes to complete, probably due to the use of small prey in relation to body size, as reported by Elwood & Cundall (1994).

In this study, asymmetric jaw movements were observed in *A. japonicus* in the following situations (Supplementary Figure 4): 1) when the prey approached the mouth on one side, or 2) when *A. japonicus* failed to capture the prey in the first attack (Figure 12). When the prey approached the mouth on one side, the jaw closer to the prey was preferentially opened and prey was captured by a combination of lateral strikes and suction. Eventually both mandibles were opened (Supplementary Table 2). In some cases, asymmetric hyobranchial depression was also seen after the asymmetric mandibular depression. When the prey approached the front of the snout, near the symphysis, both of the mandibles were opened at the same time.

Asymmetric jaw movement was also observed when *A. japonicus* failed to swallow the prey in the first attack (Figure 12a–l). The prey was grasped by the maxilla and dentary teeth on one side (Figure 12c–e), with the contralateral jaw slightly open to drain water from the mouth as a preparation for a second suction movement (Figure 12f, g). After draining half its mouthful of water, the mouth was opened widely by depression of both jaws and elevation of the skull (Figure 12h). Once the prey was released by the teeth, it was then sucked back into the buccal cavity (Figure 12i). Subsequently, the prey was transported from the mouth into the esophagus by water flow (Figure 12i). The mouth closed again and the recovery phase began (Figure 12j, k). Asymmetric jaw movement was also observed at this stage, with the mandible closed on the side where the prey was caught and the contralateral jaw slightly open to drain water (Figure 12k).

Observations of *A. japonicus* in this study suggest that asymmetric jaw movement may be correlated directly with prey position relative to the head, supporting the findings of *C. alleganiensis* by Cundall et al. (1987). In addition, we also observed that asymmetric jaw movement can be used to hold the prey during the recovery phase (Figure 12). Our study also recorded, for the first time, that when *A. japonicus was* swallowing prey held by the jaw on one side, the prey was briefly released followed by rapid suction, instead of moving the jaws and the floor of mouth to reposition the prey for intraoral transport. However, asymmetric hyobranchial movements were more difficult to observe consistently and correlation with prey position relative to the jaw or buccal cavity as reported in *C. alleganiensis* by Cundall et al. (1987) could not be confirmed. There could be some delay in the depression of the hyobranchial apparatus on one side during asymmetric jaw movements.

4.8 Three-dimensional analysis of jaw and skull movements in *Andrias japonicus* (Figure 13; Supplementary Figure 5)

Three-dimensional jaw and skull movements during asymmetric jaw opening were examined in five different sized individuals of *Andrias japonicus*, but data from one of these (SVL 315 mm [STL 470 mm]) were set aside as the specimen was found to be pathological, with jaws of different length. These unfixed fresh specimens were manipulated for each jaw movement (closing, synchronous opening, asymmetric opening) and then µCT scanned (as explained above, section 2.4). Using the resulting 3D images, Euler angles for the four different jaw positions were calculated using the 3D measurement and structural analysis software Voxelcon 2014 (Quint Co.) and the spreadsheet software (Excel, Microsoft). The braincase was fixed for all four jaw positions and the reference position of the mandible was set as that in which the tooth row of the dentary was parallel to the horizontal plane. Based on this reference mandible position, Euler angles of entire jaw movements, pitch (dorsoventrall), yaw (medio-lateral), and roll (rotation), were examined. The results are tabulated in Supplementary Table 3, and these jaw movements were also projected on a stereographic Wulff net as shown in Supplementary Figure 5.

Asymmetric and synchronous jaw movements in various sized individuals of *Andrias japonicus* can be summarised as follows (Supplementary Figure 5):

Pitch (jaw closing-opening: dorsoventral rotation)**:** *A. japonicus* was able to flex the mandibles at the symphysis by a maximum of ~30º.

Yaw (mediolateral rotation): this movement was limited (< 10º)

Roll: Both internal and external rotation around the long axis of the jaw of ~30º was allowed. Thus, no significant differences were found in asymmetric jaw opening angles in individuals of different size (from SVL 238 mm – ~680 mm [STL 300 mm –1000 mm]). In a specimen of SVL ~680 mm (STL 1000 mm), jaw opening was very limited, possibly due to dry, stiff jaw muscles.

4.8.1 Synchronous jaw opening and closing

In *Andrias japonicus*, minor asymmetric jaw movements occur during synchronous jaw opening and closing. Therefore, standard, synchronous, jaw movements were defined by pitch angle.

Pitch: Standard, synchronous, jaw movements are defined as having only a small difference (< 10º) between the left and right pitch angles.

Yaw: This does not change angle (<5º) with different jaw positions, which suggests the jaws normally move with a simple dorsoventral action. However, there is tendency for the right and left mandibles to rotate in slightly different directions (medialward or lateralward) separately, when the jaws open (pitch) more than 20º.

Roll: This movement is limited (< 10º) both internally and externally. However, when the jaws open at a similar pitch angle, both left and right sides are either internally rotated (SVL 483 mm [STL 710 mm]; SVL ~680 mm [STL1000 mm]) or externally rotated (SVL 238 mm [STL 335 mm]; SVL 365 mm [STL 580 mm]).

4.8.2 Asymmetric jaw movement

Pitch: Asymmetric jaw movement is defined as a difference of more than 10º between the pitch angles of the left and right jaws.

Yaw: There is a slightly greater angle of yaw rotation than in standard (synchronous) jaw opening and closing, but usually less than 10 degrees. A tendency for a slight medial (leftward or rightward) rotation was observed when one jaw was opened more than 20º in relation to the other, but this is negligible.

Roll: When the degree of pitch of the open mandible is greater than that of the closed jaw, the open jaw rolls externally and the closed jaw rolls internally. The greater the angle of jaw opening (pitch), the greater the degree of rotation (roll angle).

4.8.3 Skull deformation

In both standard (synchronous) and asymmetric jaw movements, the ventral end of the quadrate and the posterior end of the maxilla translated laterally, medially, dorsally and ventrally within 5% (transition distance shown as a percentage of the skull width, quadrate-quadrate in palatal view) from their rest positions. There was no consistency in the direction of this movement. In some cases, elevation of the maxilla may have been the result of pressure from buffers used to maintain jaw opening during CT scanning. Regardless of whether asymmetric or standard jaw movements were manipulated, the lateral skull bones moved when the jaws opened or closed.

The results suggest that asymmetric jaw movement is produced mainly by the rotation (roll) of the mandible about its long axis. Slight rolling movements occur during standard jaw opening and closing (synchronous jaw movements), which suggest flexibility of the quadrate-articular joints, as well as the symphysis. This is supported by the observation of skull deformation during standard jaw opening and closing, but this deformation is usually less than 5% and is not linked to the asymmetric jaw movements. Limited cranial kinesis has been reported in *Cryptobranchus alleganiensis* (e.g. Iordansky, 1990; Iordansky, 2000), and our observations suggest that there is a small amount of kinesis in *A. japonicus*.

4.8.4 The role of individual muscles in three-dimensional jaw movements (Figure 13)

Three-dimensional jaw movements (pitch, yaw, and roll) and the main muscles producing these movements are summarized in Figure 13. According to the positional relationship between the joint axes and the line of muscle action, each muscle may have multiple roles, for pitch, yaw, and roll rotations of the mandible. The jaw closing muscles (DLMA, SLMA, LMP, LME) also produce medial and internal rotation (Figure 13). On the other hand, the jaw opening muscles (ADM, PDM) may also function in the lateral rotation of the lower jaw. The muscles IH and IMP can be regarded as jaw opening muscles when the buccal floor is positioned lower than the jaw joint, but these muscles simultaneously rotate the lower jaw medially and externally (Figure 13).

5 DISCUSSION

5.1 Comparison of *Andrias* and *Cryptobranchus*

Comparisons of jaw and hyobranchial musculature, osteology, and feeding behaviour between *Andrias japonicus* and *Cryptobranchus alleganiensis* indicate that both species engage in asymmetric suction feeding. Observation of feeding behaviour in *A. japonicus* also confirmed that asymmetric jaw movement is correlated with the prey position. Asymmetric jaw movement may be advantageous, especially for aquatic ambush predators, as it allows the animal to open the jaw quickly with limited movement of the head and body, and therefore little water disturbance. Moreover, Cryptobranchidae are also specialized for suction feeding and lack a fleshy mobile tongue to reposition prey captured in their jaws before swallowing.

In *Andrias japonicus*, the skull morphology and muscle arrangements are essentially similar to those of *C. alleganiensis*, but there are also some minor differences, such as the components of the skull bordering the external narial opening, and the division of deep m. levator mandibulae muscle (see Supplementary Table 1). More notable differences, possibly relating to feeding behaviour, are found in the degree of metamorphosis of the hyobranchial apparatus. Adults of *C. alleganiensis* retain larval hyobranchial elements (e.g. HBIII, CBIII and CBIV), but unlike the larval condition, there is ossification in the posterior part of the ceratohyal and CBIII (Cox & Tanner, 1989). Possibly in relation to its larval hyobranchial condition, *C. alleganiensis* has small strap-like muscles (e.g. m. subarcualis rectus II, III) connecting the posterior hyobranchial elements (Elwood & Cundall, 1994). These muscles have not been found in *Andrias japonicus*. However, given the adult size difference between *A. japonicus* and *C. alleganiensis*, the specimens of *A. japonicus* used in this study (SVL 238–690 mm [STL 335–960 mm]) were still skeletally immature despite being of almost equal size to adult *C. alleganiensis*. This may explain the differences observed in the ossification status of the articular and quadrate in the two species.

5.2 Comparison with other suction feeders

Fully aquatic salamanders generally ingest and manipulate prey by suction feeding. However, the kinematic patterns of aquatic feeding vary between taxa due to phylogenetically related differences in skull morphology (Reilly & Lauder, 1992). Suction feeding mainly requires mobility of the hyobranchial apparatus (depression and elevation) and mandibles (Deban & Wake, 2000), and the hyobranchial muscle arrangements are generally simplified compared to terrestrial feeding salamanders. During suction feeding, expansion of the buccal cavity results from hyobranchial depression due to the posteroventral swing of the hyobranchial apparatus. The major muscles responsible for this movement are the m. rectus cervicis (profundus and superficialis), and either the m. branchiohyoideus externus (BHE) or m. subarcualis rectus I (SRI) (Deban & Wake, 2000). The m. rectus cervicis (RC) is a large muscle, continuous posteriorly with the rectus abdominus and it generally inserts on the first ceratobranchial. Both the BHE and SRI extend between ceratobranchial I and the ceratohyal, but they differ in their innervation (cranial nerve VII for BHE; IX for SRI) (Kleinteich & Haas, 2011). Thus, these two muscles are not homologous, but the position and function of the SRI are replaced by BHE in some species. The BHE and SRI are found in the larval stage of most salamanders including *Cryptobranchus alleganiensis* and *Andrias japonicus* (BHE absent in *Amphiuma means*; Kleinteich & Haas, 2011), and these muscles were likely present in larvae of the common ancestor of the Caudata (Kleinteich et al., 2014). However, in partially metamorphosing taxa, such as cryptobranchids and *Amphiuma*, the SRI is considered to be the major muscle with the BHE present in the larval stage (absent after the larval stage in *Amphiuma*), but reduced or absent in adults (e.g. Erdman & Cundall, 1984; Elwood & Cundall, 1994). The situation is reversed in other aquatic species such as *Siren* (Schwarz et al. 2020) and *Ambystoma dumerilii* (Shaffer & Lauder, 1985). The SRI (or BHE) also functions to expand the buccal cavity; pulling the epibranchials/ceratobranchial I ventrally and rotating the ceratohyal (Deban & Wake, 2000). These are important movements for suction feeding.

Toward the end of suction feeding, the buccal cavity is compressed, water is expelled from mouth, and the jaws and hyobranchial apparatus return to their original rest position. This compressive movement is caused by hyobranchial elevation resulting from an anterodorsal swung of the hyobranchial apparatus (Deban & Wake, 2000). The principal muscles responsible for the elevation of the hyobranchial apparatus are generally the mm. geniohyoideus (GH), intermandibularis posterior (IMP), interhyoideus (IH) and interhyoideus posterior (IHP) (Deban and Wake, 2000). Of these muscles, GH is the only muscle that is directed anteroposteriorly and connects the mandible to the trunk region. The posterior attachments of this muscle vary among salamanders: the epimysium of the m. rectus cervicis in Cryptobranchidae and *Amphiuma*; the urohyal in some taxa (e.g. *Ambystoma*), and the basibranchial in *Siren*. The other three muscles, IMP, IH and IHP (in anterior to posterior order), are thin sheets of transversally arranged fibres that form the floor of the buccal cavity. The IMP is the primary muscle used to compress the buccal cavity because the muscle connects the mandible to the medial aponeurosis in the floor of the mouth. However, IH is also considered to be linked to the hyobranchial apparatus and to constriction of the posterior part of the mouth as the muscle connects the skull (quadrate) and the floor of mouth (midventral raphe) (e.g. Francis, 1934; Elwood & Cundall, 1994; Deban & Wake, 2000). The posteriorly located IHP is generally attached, through the ventro-lateral surface of the quadrate and squamosal, and in conjunction with IMP and IH, to the posterior part of the continuous midline raphe. Activation of IHP may also be linked to jaw adductor muscle contraction.

In addition to the hyobranchial apparatus, movements of the mandible are also essential for suction feeding. Generally, the m. depressor mandibulae (DM) acts to open the jaws and m. levator mandibulae (SLMA, LMP, DLMA, LME) acts to close the jaws (e.g. Francis, 1934; Deban & Wake, 2000). The m. geniohyoideus (GH) might also contribute to jaw opening as well as hyobranchial depression. Its mechanical advantage (for jaw opening) is increased when the hyobranchial apparatus is depressed (Deban & Wake, 2000). In addition, the anterior part of the GH is tightly attached to the anterior fold of the buccal lining in Cryptobranchidae. As a result, the buccal lining may follow the movement of GH, either in elevation or depression of the mandible.

Although the general sequence of suction-feeding in adult aquatic salamanders is for rapid jaw opening followed by the depression of the hyobranchial apparatus (e.g. Stinson & Deban, 2017), a delay in the depression of the hyobranchial apparatus has been reported in adults of paedomorphic forms such as *Amphiuma* and Cryptobranchidae (Reilly & Lauder, 1992). Based on computational fluid dynamics models, suction feeding in *Andrias davidianus* is considered to be powered largely by rapid jaw separation, which generates a drop in intra-oral pressure and it is then followed by depression of the hyobranchial apparatus (Heiss et al. 2013). However, the depression of the hyobranchial apparatus occurs late in comparison to the rapid jaw opening, and is thought to help maintain the water flow into the pharyngeal cavity (Heiss et al., 2013). For these powerful movements of the jaws and hyobranchial apparatus, it might be expected that the hyobranchial elements in *Andrias* and *Cryptobranchus* would be well ossified. However, ossification is limited to the second hyobranchial and the second ceratobranchial (to some degree in *Cryptobranchus*: Cox & Tanner, 1989). In contrast, some other aquatic genera (e.g. *Amphiuma, Proteus*, and *Siren*) have well ossified hyobranchial elements, retaining all larval elements (e.g. Marche & Durand, 1983; Erdman & Cundall, 1984; Deban & Wake, 2000; Schwarz et al., 2020). A study comparing the semi-aquatic genus (*Paramesotriton*) and the fully aquatic genera (*Pleurodeles*, *Notophthalmus*, *Triturus* and *Cynops*) found greater ossification of the hyobranchial apparatus in fully aquatic species, with a narrower basibranchial and wider ceratobranchial I and II (Stinson & Deban, 2017).   This hyobranchial morphology in fully aquatic species accelerates hyobranchial depression (Stinson & Deban, 2017). Ossification of the hyobranchial apparatus is therefore important to enhance suction feeding, but Cryptobranchidae, especially the large *Andrias*, show the reverse trend. The limited ossification of the hyoid and first branchial arches may be a structural response to a requirement for flexibility of the jaws and hyobranchial apparatus in asymmetric movement (Elwood & Cundall, 1994). Thus, cryptobranchids use jaw-based suction feeding, and their large body size and broad skull morphology may compensate for the less robust cartilaginous hyobranchial apparatus. Instead of strengthening the hyoid region, there may have been selection for a greater flexibility of the hyobranchial apparatus that allowed for maximum expansion of the pharyngeal cavity and asymmetric movement of the hyobranchial apparatus and mandible. The greater expansion of the pharyngeal cavity would be advantageous in capturing a variety of prey, such as other amphibians, turtles, snakes, juvenile birds, and small mammals (bats, moles, rats), as well as fish and arthropods, that are available in their habitat (e.g. Tochimoto, 2002, 2005; Naito, 2018; Hamanaka & Nishikawa, 2020).

Elwood & Cundall (1994) proposed that the unique asymmetric jaw movement of cryptobranchids was produced by a combination of the loose contact between the mandibles at the symphysis, through cartilage and ligaments, and the wide quadrate-articular joint with extensive articular cartilages. The strongly curved mandibles may also facilitate this movement (Deban & Wake, 2000). The work presented herein revealed that the primary jaw movements are external and internal rotation (roll) about the long axis, and that lateral rotation of the jaw (yaw) is very limited during asymmetric jaw movements. Furthermore, although the open jaw mainly rolls externally (outward), the contralateral closed jaw rolls internally (inward). The multiple functions hypothesised for the jaw muscles explain the three-dimensional positions of the lower jaws in asymmetric movement, in that internal rotation (roll) of the closed jaw is mainly controlled by jaw closing muscles (DLMA, SLMA, LMP, LME), whereas external rotation (roll) of the open jaw is controlled by jaw opening muscles (IH, IMP) (Figures 1a, 12i, and 13). These movements are also permitted by the flexibility of the symphysis (Figure 3) and the width of the quadrate-articular joint (Figure 4), as proposed by Elwood & Cundall (1994).

According to Cundall et al. (1987), the angle of flexion between the jaws at the symphysis during prey capture and manipulation in *Cryptobranchus alleganiensis* could reach as high as 40º, but in *Andrias japonicus* that angle was limited to 20º. In our study, where we reproduced asymmetric jaw movements of fresh (unfixed) specimens while CT scanning, we found that *A. japonicus* was able to flex one mandible in relation to the other by about 30º. However, high speed video recording of asymmetric feeding behaviour in living *A. japonicus* showed flexion angles of only 20º. Thus, although the anatomy of the jaw joint and symphysis in *A. japonicus* permits flexion at the symphysis up to 30º, the actual movement *in vivo* is less than this. The lower angle of symphysial flexion in *A. japonicus* compared to *C. alleganiensis* might reflect larger body size (as well as skull size) and therefore greater overall suction power with less need to open the mouth as wide as that of *C. alleganiensis*.

As well as mandible movements, it has been suggested that the structures in the anterior part of braincase might allow minor torsion or flexion in order to absorb forces resulting from asymmetric jaw movements during feeding (Elwood & Cundall ,1994). Among Urodela, cranial kinesis is not considered to be well developed in *C. alleganiensis*, but dorsoventral mobility has been reported in the posterolateral parts of the skull (e.g. quadrate, pterygoid, squamosal), as well as limited mobility in the rostrum (maxilla, prefrontal, vomer) (e.g. Iordansky, 1990; Iordansky, 2000). Our analysis suggests that in both symmetric and asymmetric feeding, some skull deformation occurs in *A. japonicus*, especially in the posterolateral skull regions and the maxilla. In other aquatic salamanders, reports of cranial kinesis are variable. Kinesis is said to be well developed in the anterior part of the skull in *Amphiuma*, limited in *Siren*, and absent in *Paramesotriton* (e.g. Iordansky, 1990, 2000, 2001). However, based on the firm symphysial connection and narrow cranio-mandibular articular surfaces in these taxa, it is unlikely that any of them (except *Siren*) have asymmetric jaw movements. In addition, the strongly arched mandible in Cryptobranchidae may increase the leverage for the internal and external rotations of the lower jaw that are essential for asymmetric jaw movements, but the lever action is less effective in *Siren* and *Amphiuma* which have relatively straight jaws. *Siren* is unique in having a very flexible symphysis that, together with a ball-and-socket jaw joint, permits transverse jaw movements (Schwarz et al. 2020), instead of asymmetric jaw movement. Salamandrids also have arched mandibles, but internal and external rotation may be restricted by the tight connection between the mandibles at the symphysis and the fit of the quadrate-articular joint (RM pers. obs.).

Asymmetric hyobranchial movements are permitted by the mobile cartilaginous joints of the hyobranchial apparatus and contribute mainly to movement of the broad and flexible buccopharyngeal floor. Movement mainly occurs in the anterior part of the hyobranchial apparatus: the hyoid arch, basibranchial, and branchial arches 1 and 2. However, flexibility of the connections to the basibranchial in particular may be a key component of this movement. The hypohyal and ceratohyal are in loose contact with one other, and the dorsal surface of the basibranchial is attached to the flexible buccopharyngeal floor. When one side of the hyobranchial apparatus is pulled posterolaterally, the basibranchial is moved along with the attached buccopharyngeal floor. The repositioning of the basibranchial is followed by relocation of the associated branchial arches 1 and 2. The well-developed articular surfaces on the basibranchial for branchial arches 1 and 2 permit this mobility. In other aquatic salamanders the joint between the basibranchial and associated elements (e.g. hyobranchial 1) is not as well developed, with narrower joint cavities (e.g. *Siren*). In *Amphiuma*, the branchial arm (composed of fused ceratobranchial I and epibranchial I: Erdman & Cundall, 1984) can be rotated by up to 90º around the basibranchial in any plane in fresh specimens (Erdman & Cundall, 1984; Elwood & Cundall, 1994). However, the joint between the basibranchial and the branchial arm is very tight and asymmetric flexion is unlikely. Thus, asymmetric movement of the hyobranchial apparatus is unique to Cryptobranchidae. The ability to produce asymmetric or asynchronous movements of the jaw and hyobranchial apparatus may be advantageous in sucking the prey from the water volume from a position at the side of the mouth as well as in front of it, using limited gape and skull movement (Elwood & Cundall, 1994), as observed in their feeding behaviour. This attack strategy, which minimizes head movement, and therefore water disturbance, would contribute to successful hunting for sit-and-wait predators, such as Cryptobranchidae.

5.3 Terrestrial vs aquatic feeding (Figure 14)

In Urodela, it is difficult to find clear morphological differences between terrestrial and aquatic feeders. Although, all terrestrial salamanders use tongue prehension for prey capture, this method is also used by metamorphosing plethodontids, even the fully aquatic desmognathine, *Desmognatus marmoratus* (Schwenk & Wake, 1988). In addition, some newts switch their feeding grounds between water and land depending on the season (Wassenberg & Heiss, 2016). However, suction feeding species tend to reduce their tongue size and have simpler tongue muscles, whereas aquatic species that utilise tongue prehension under water are known to have relatively large and complex tongues similar to those found in terrestrial species (Deban & Wake, 2000). There are some differences in the morphology of the hyobranchial apparatus and its musculature in terrestrial taxa using tongue prehension compared with fully aquatic suction feeders.

Terrestrial feeders tend to possess small transversally oriented hyobranchial elements such as the radial and otoglossal cartilages, which connect the basibranchial and ceratohyal and support a relatively large tongue pad (e.g. Ambystomatidae, Hynobiidae, Plethodontidae; Deban & Wake, 2000) (Figure 15c). These small cartilages are not found in many suction feeders including: Cryptobranchidae, *Amphiuma*, *Proteus*, *Necturus*, and *Siren*. The ossification pattern of the hyobranchial apparatus in terrestrial feeders varies, but is generally very reduced and limited to the central components: basibranchial, urohyal and the distal end of some branchial arches (e.g. branchial arch II in hynobiids).

In taxa using tongue prehension, the movement of the hyobranchial apparatus is generally produced by the following muscles: mm. subarcualis I (SRI) and subhyoideus (SH) for tongue protraction; and mm. rectus cervicis superficialis (RCS) and rectus cervicis profundus (RCP) for tongue retraction (Deban & Wake, 2000). In addition, the medial and lateral divisions of m. genioglossus are also involved in protracting the tongue, instead of m. subhyoideus (Larsen & Guthrie, 1975). A comparison of the major muscles functioning in tongue prehension vs suction feeding is presented in Supplementary Table 4. As shown in the list, the RC has a similar function in both feeding groups in terms of drawing the hyobranchial apparatus backwards, but the action of SRI is less clearcut. According to Reilly & Lauder (1989), stimulation of only m. subarcualis rectus I does not produce tongue projection and the muscle works with other three muscles (mm. geniohyoideus, intermandibularis posterior, interhyoideus). The SRI contributes to depression of the hyobranchial apparatus in suction feeding, whereas it drives the apparatus forward in terrestrial feeding. Furthermore, the other two muscles used for tongue protraction in terrestrial salamanders, mm. genioglossus and subhyoideus, are very reduced in suction feeders, such as Cryptobranchidae. In suction feeders, the m. geniohyoideus is important in compressing the buccal cavity, but this muscle acts to stabilize the mandible, which is supported by RCS, during skull elevation in *Ambystoma* (Larsen & Guthrie, 1975).

Among terrestrial salamanders, the arrangement of these muscles is variable (see detailed illustration in Özeti & Wake, 1969: e.g. Figure 12, 16), but a comparison of muscle arrangements in terrestrial (*Ambystoma tigrinum*) and aquatic (*Andrias japonicus*) taxa is shown in Figure 14 as an example. Ambystomatidae were taken as an example because their tongue protraction is relatively modest and the gape cycle is simpler (Deban & Wake, 2000). Most differences are in the anterior part of the tongue. The m. genioglossus is well developed in *Ambystoma* and is divided into two bundles, lateralis and medialis, that attach on the dorsal surface of the tongue pad and the hyobranchial elements (Figure 14c, d). This dorsal attachment of muscles is not found in *Andrias* and other fully aquatic species such as *Amphiuma* and *Siren*. Furthermore, in *Ambystoma*, the hyobranchial apparatus is more robust, with ossification in the centre of the basibranchial, the distal end of the first epibranchial, and in the urohyal (Larsen and Guthrie, 1975). These elements are essential for the attachment of muscles (SRL, RC, GH) that retract and protract the tongue (Figure 14c, d).

5.4 Asymmetric jaw movement in extinct Cryptobranchidae

Comparisons among aquatic salamanders, and between suction feeders and those using tongue prehension, highlight the unique asymmetric feeding behaviour of Cryptobranchidae. In order to understand the evolution of cranial morphology and feeding behaviour in Cryptobranchidae, it is important to explore the fossil evidence for the acquisition of asymmetric jaw mobility. Hynobiidae is the sister group of Cryptobranchidae, within the Cryptobranchoidea and includes both aquatic and semiaquatic species. Semiaquatic species (e.g. *Salamandrella keyserlingii*, *Batrachuperus persicus*) tend to use jaw prehension under water, whereas fully aquatic taxa (e.g. *Pachyhynobius*) have labial lobes and a pleated buccal lining, which suggest suction feeding (Deban & Wake, 2000). However, asymmetric jaw movements have not been reported in hynobiids, suggesting that this unique jaw movement may be derived within cryptobranchoids.

The oldest fossil records of *Andrias* and *Cryptobranchus* are from the Paleocene; *C.*

saskatchewenesis from the Paleocene to Miocene of North America (Naylor, 1981); *Andrias matthewi* from the Miocene of North America (Naylor, 1981); and *Andrias scheuchzeri* from the Oligocene-Pliocene of Eurasia (e.g., Szentesi et al., 2019). Of these, asymmetric movement of mandible was proposed for the extinct Eurasian species, *Andrias scheuchzeri,* and was listed as a diagnostic character of this species based on a convex symphysial contact that was taken to imply the presence of symphysial cartilages like those found in extant taxa (Vasilyan & Böhme, 2012). The oldest representative of crown Cryptobranchidae, the relatively large (up to 2 m STL) *Aviturus exsecratus,* is recorded from the late Paleocene of Mongolia. Bilateral asymmetric jaw movement was again proposed for this species because it has a convex symphysial surface (Vasilyan & Böhme, 2012; Vasilyan et al., 2013). However, the vomerine tooth row in this species has a posteriorly shifted "zigzag form" (Vasilyan & Böhme, 2012), whereas *Andrias* retains the transversally oriented vomerine dentition of the larval condition. A zigzag-form vomerine dentition is characteristic of "pond-type" salamanders (Zhang et al., 2006), which feed on small terrestrial prey using tongue movements (Vasilyan & Böhme, 2012). Terrestrial habits were suggested for *Aviturus* based on the elongation of the femur and the development of the olfactory region, whereas increased bite force implied by jaw muscle attachment scars could be adaptations to either underwater or terrestrial feeding, or both (Vasilyan & Böhme, 2012). However, the Miocene species, *Ukrainurus hypsognathus*, which is placed as the sister taxon of crown Cryptobranchidae, has less space for cartilage pads at the symphysis, suggesting less potential for asymmetric movement (Vasilyan et al., 2013).

The perennibranchiate Mesozoic *Chunerpeton* from China was originally described as a stem-cryptobranchid (Gao & Shubin, 2003). However, the phylogenetic position of this genus has recently been revised and the genus has been placed either as a stem group caudate, unrelated to Cryptobranchidae (Rong et al. 2021; Jones et al. 2022) or as a stem cryptobranchoid (Jia et al. 2021). Most *Chunerpeton* specimens are two-dimensionally preserved, which makes examination of jaw and symphysial joints difficult. The specimen described by Rong et al. (2021) had a small space between the jaws at the symphysis, which could indicate the presence of a soft tissue pad, but there is no evidence for asymmetric jaw movement. In addition, the ossification pattern and morphology of the hyobranchial elements (hypobranchial I, II, and medial basibranchial II) differs from the pre- and post-metamorphic states of the modern species (Edgeworth, 1923; Ishikawa et al. 2022). These morphological variations suggest differences in feeding behaviour between this fossil and living genera.

The fossil record thus provides limited evidence that the ability to perform asymmetric jaw movements may have evolved in the stem group of Cryptobranchidae. The origin of this unique feeding behaviour could extend back to the Paleocene (e.g. Vasilyan &

Böhme, 2012). However, the evidence for this movement is limited to the morphology of the symphysis, and the presence of a ridge/angulation on the symphyseal surface in *Andrias scheuchzeri*, which was interpreted as separating two cartilaginous pads as in extant species (Szentesi et al. 2019). However, the symphysis of *A. scheuchzeri* appears somewhat damaged and the ridge is not clear from the published images (Szentesi et al. 2019; Figure 3), although a shallow concavity is visible in medial view. In *Andrias japonicus* and *Cryptobranchus alleganiensis*, there is a dorsal convexity and a ventral concavity on the symphysial surface, and this concavo-convex structure creates an angulation (rather than a distinct ridge) that separates the two cartilaginous pads, as well as marking the attachment of the medial symphysial ligament. As shown herein, asymmetric jaw movement not only involves the jaw symphysis. Furthermore, as in the case of *Siren*, a loose jaw symphysis does not necessary mean that asymmetric jaw movements can occur. Other structures, such as the quadrate-articular joint, hyobranchial apparatus, intracranial movements, and muscle attachments on the ventral surface of the mandible (e.g. m. intermandibularis, m. geniohyoideus, contributing to jaw rotation) should be discussed comprehensively in reconstructing feeding behaviour. The hyobranchial apparatus plays an important role in the feeding strategy of Cryptobranchidae, but most of its components are unossified, and cartilage is rarely preserved in fossils. However, as in extant species, ossified hyobranchial elements are likely to retain traces of the attachment of important hyobranchial muscles. Thus, examination of any ossified hyobranchial elements may be useful in reconstructing feeding behaviour in extinct species.

6 CONCLUSIONS

In *Andrias japonicus*, asymmetric jaw movements are linked to the position of the prey in relation to the snout, with the jaw closest to the prey opening. This action allows the salamander to simultaneously grab prey in their jaws (closing one side of the mouth) while ejecting water (opening the mouth on the other side), if the first suction attempt fails. This asymmetric movement is performed by rotation (rolling) of the mandible at the symphysis and at the quadrate and articular joint, with limited lateral rotation (yaw). The open jaw rotates externally (as in roll) about its long axis and the closed jaw rotates in the opposite direction. This is permitted by the flexibility of the jaw symphysis and the wide cartilaginous articular surfaces of the quadrate and articular at the jaw joint. In addition, asymmetric hyobranchial movement is made possible by flexibility of the wide buccal lining, and by the mobile joints between the basibranchial and the first ceratobranchial and second hyobranchial. There is limited skull kinesis in *Andrias japonicus*. During jaw opening and closing, the posterolateral parts of the skull (quadrate and maxilla) move slightly ventrally. Thus, asymmetric jaw movements involve not only the jaw symphysis, but also other cranial structures including the quadrate-articular joint and hyobranchial apparatus, so the morphology of these elements needs to be interpreted comprehensively.

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AUTHOR CONTRIBUTIONS

Matsumoto R. and Evans S.E. dissected jaw, hyoid and neck muscles of the specimens. Matsumoto R. performed iodine staining, CT scanning, digital segmentation, filming feeding behaviour, made the figures, and wrote the first draft of manuscript. Fujiwara S. set up CT data by using structural analysis software, and calculated jaw position in Euler angles, and made the figures. All authors reviewed and edited the final manuscript.

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SUPPLEMENTARY TABLE CAPTIONS

Supplementary Table 1. List of specimens of Cryptobranchidae specimens used in this study.

Supplementary Table 2. Summary of filming suction feeding movements in *Andrias japonicus.*

Supplementary Table 3. Jaw positions of studied specimens. The table lists: specimen number (specimen), SVL in mm, CT-scanned jaw position (position), jaw side (Side: right, left), Euler angles for the jaw position (Pitch, Yaw, Roll), and the relative transition of the distal end of the quadrate (Q) and caudal end of the maxilla (M) as a percentage of the width of the skull. The Euler angles are positive for jaw open (pitch), left-ward yaw (yaw), and right-ward rotation (roll). The deformations of the quadrate and maxilla are listed for medio-lateral transition which is positive for lateral transition (Q_med and M_med, respectively), and for dorso-ventral, which is positive for dorsal-ward transition (Q_dors, and M_dors, respectively). Specimen “STL 470 mm” is a pathological specimen, with mandibles of different lengths.

Supplementary Table 4. The function of the hyobranchial apparatus muscles in aquatic and terrestrial salamanders.

SUPPLEMENTARY FIGURE CAPTIONS

Supplementary Figure 1. 3D reconstructions of skulls and mandibles of *Andrias japonicus* (SVL 365 mm [STL 580 mm]) and *Cryptobranchus alleganiensis* (UMZC R 305) for comparison: skull of *A. japonicus* in dorsal (a) and ventral (b) views; mandible of *A. japonicus* in dorsal (c) and ventral (d) views; skull of *C. alleganiensis* in dorsal (e) and in ventral (f) views; mandible of *C. alleganiensis* in dorsal (g) and ventral (h) views. Abbreviations: ar, articular; co. p, coronoid process; d, dentary; exo, exoccipital; fr, frontal; mx, maxilla; na, nasal; os, orbitosphenoid; pa, parietal; pmx, premaxilla; pra, prearticular; prf, prefrontal; pro, prootic; psh, parasphenoid; pt pterygoid; q, quadrate; rid, ridge; sq, squamosal; stp, stapes; v, vomer.

Supplementary Figure 2 3D reconstruction and dissection of the hyobranchial apparatus in jaw closing and opening for *Andrias japonicus*: (a), Resting position of the skull and hyobranchial apparatus in dorsal view of *A. japonicus* (SVL 483 mm [STL 710 mm]); (b), in lateral view with jaw closed; (c), hyobranchial apparatus in lateral view with jaws open; (d), dissected hyobranchial apparatus of *A. japonicus* (SVL 365 mm [STL 580 mm]) in the open jaw position. Abbreviations: af, anterior fold of buccal mucosa; BB, basibranchial; CBI–II, ceratobranchial I–II; CH, ceratohyal; d, dentary; ep-hy, epi-hyal; HHI–II, hypohyal I–II; lig, ligament; mx, maxilla; RC, m. rectus cervicis; ptq. c, pterygoquadrate cartilage; v1–3, vertebrae 1–3.

Supplementary Figure 3. Suction feeding sequence in *Andrias japonicus* when the prey is positioned in front of the mouth (a–h).

Supplementary Figure 4. Suction feeding sequence in *Andrias japonicus* when the prey is positioned on the left side of the mouth in (a–h).

Supplementary Figure 5. Results of analysis of asymmetric jaw movement in *Andrias japonicus* with specimens of various size (a–e) projected on a stereographic Wulff net. The images show orientations of the occlusal margins of right and left mandibles from a rostral view of the skull. The planes of occlusal margins are displayed as ‘great circle’ on the upper hemisphere of the Wulff net. The plot on each ‘great circle’ indicates the rostral orientation of the mandibular element. Specimen “STL 470 mm” in (b) is a pathological specimen, with mandibles of different lengths, which may explain the unusual results.

TABLE CAPTION

Table 1. Summary of skull differences between *Andrias japonicus* and *Cryptobranchus alleganiensis*.

FIGURE CAPTIONS

Figure 1. Data setup process for asymmetric jaw movement analysis and images to demonstrate calculation of Euler angles. (a), Fresh (from frozen) *Andrias japonicus* scanned in four different mandible positions (positions were manipulated); (b), scanned jaw positions were repositioned with respect to the braincase and midline, with definitions of X, Y, and Z axes as left-right, dorso-ventral, and anterior-posterior of the skull, respectively; (c) a reference jaw position with tooth row aligned on XZ plane, was created; (d), Euler angles (pitch, yaw, and roll in order) of the mandible with respect to the reference position were calculated in

each jaw position. Abbreviations: ant-post, anterior-posterior; asym, asymmetric; dors-vent, dorsoventral; L-R, left-right; sym, symmetric.

Figure 2. 3D reconstructions of the skull and mandible in *Andrias japonicus* (SVL 365 mm [STL 580 mm]): the skull in dorsal (a), and ventral (b) views; the mandible in dorsal (c), and ventral (d) views. Abbreviations: ar, articular; co. p, coronoid process; d, dentary; exo, exoccipital; fr, frontal; mx, maxilla; na, nasal; os, orbitosphenoid; pa, parietal; pmx, premaxilla; pra, prearticular; prf, prefrontal; pro, prootic; psh, parasphenoid; pt pterygoid; q, quadrate; sq, squamosal; stp, stapes; v, vomer.

Figure 3. Symphyseal structure in *Andrias japonicus* and *Cryptobranchus alleganiensis*; (a), dissection of symphysis in *A. japonicus* (SVL 483 mm [STL 710 mm]) in ventral view; (b), 3D reconstruction of the mandible showing ligament attachments of *A. japonicus* (SVL 483 mm [710 mm]) in ventral view; (c), dissected symphysial surface (left and right) of *A. japonicus* (SVL 483 mm [STL 710 mm]) in medial view; (d), 3D reconstruction of symphysial surface showing soft tissue attachments of *A. japonicus* (SVL 483 mm [STL 710 mm]) in medial view; (e), right symphysis of *A. japonicus* (SVL 365 mm [STL 580 mm]) in medial view; (f), right symphysis of *A. japonicus* (NSM uncatalogued specimen) in medial view; (g), right symphysis of *C. alleganiensis* (UCL uncatalogued specimen) in medial view. Abbreviations: ca, concavity; csl, central symphyseal ligament; cv, convexity; dsc, dorsal symphyseal cartilage; msl, medial symphyseal ligament; nb, nub; ru, rugosity; ssl, superficial symphyseal ligament; vsc, ventral symphyseal cartilage; w. cl, woven collagen.

Figure 4. Quadrate-articular joint of *Andrias japonicus,* showing soft-tissues in 3D reconstruction based on diceCT, and dissection images. (a) a skull of *A. japonicus* (SVL 238 mm [STL 335 mm]) in lateral view with ossified quadrate and squamosal, and cartilaginous pterygoquadrate and articular coloured; (b) dissected left jaw joint of *A. japonicus* in lateral view; (c), articular surface of the left jaw joint, and dotted line showing the posterior margin of the ossified quadrate; (d), dorsal (on articular) and ventral (on quadrate) view of the jaw joint cartilages. Abbreviations: ar. c, articular cartilage; CH, ceratohyal; ep-hy, epi-hyal; lig, ligament; pa, parietal; psh, parasphenoid; pt, pterygoid; ptq. c, pterygoquadrate cartilage; q, quadrate; sq, squamosal.

Figure 5. Hyobranchial apparatus of *Andrias japonicus* in a resting position. (a) Dissected hyobranchial apparatus of *A. japonicus* (SVL 483 mm [STL 710 mm]) in ventral view; (b) 3D reconstruction of hyobranchial apparatus based on diceCT of *A. japonicus* (SVL 238 mm [STL

335 mm]) in ventral view; (c) posterodorsal view of the hyobranchial apparatus; (d) anterodorsal view of the CBI and HBII with transparent image of BB; (e) posteroventral view of the BB. Abbreviations: af, anterior fold of buccal mucosa; ar. c articular cartilage; BB, basibranchial; Bh, basihyal; CBI–II, ceratobranchial I–II; CH, ceratohyal; HH I–III, hypohyal I–III; d, dentary; EBII, epibranchial II; f. CBI, facet for ceratobranchial I; f. HBII, facet for hyobranchial.

Figure 6. 3D reconstructions of the musculoskeletal architecture of the jaw system in *Andrias japonicus* (SVL 238 mm [STL 335 mm]); (a), full jaw muscle arrangements in right anterolateral view; (b), LME rendered transparent to show the underlying LMP; (c), LME and LMP removed, and SLMA and ADM I rendered transparent to show underlying structures; (d), SLMA and DLMA I removed and ADMII rendered transparent. Abbreviations: ADM I II, anterior m. depressor mandibulae I–II; DLMA I–II, deep m. levator mandibulae anterior I–II; IME I–II, m. intermyoseptalis; LME, m. levator mandibulae externus; LMP, m. levator mandibulae posterior; MVE, epaxial m. myoseptal-vertebralis; PDM, posterior m. depressor mandibulae; SLMA, superficial m. levator mandibulae anterior; tend, tendon; v2, vertebra 2.

Figure 7. Mandibular muscle attachment positions marked on a 3D reconstruction of the skull of *Andrias japonicus* (SVL 365 mm [STL 580 mm]); (a) in right dorsolateral view; (b) posterodorsal view with atlas. Abbreviations: ADM I–II, anterior m. depressor mandibulae I–II; at, atlas; DLMA I–II, deep m. levator mandibulae anterior I–II; exo, exoccipital; fr, frontal; IME, m. intermyoseptalis; IVE, epaxial m. intervertebralis; SLMA, superficial m. levator mandibulae anterior; LME, m. levator mandibulae externus; LMP, m. levator mandibulae posterior; MVE, epaxial m. myoseptal-vertebralis; na, nasal; pa, parietal; PDMI, posterior m. depressor mandibulae I; prf, prefrontal; pt, pterygoid; q, quadrate; sq, squamosal.

Figure 8. The superficial hyobranchial muscles of *Andrias japonicus* (SVL 365 mm [STL 580 mm]) in ventral (a) and anteroventrolateral views (b). On the right side of (a), IMP, IH and IHP cut along the midline, to show the dorsal surface of these muscles; (b) shows the position of the very small m. genioglossus (dotted line) which underlies m. geniohyoideus. Abbreviations: af, anterior fold of buccal mucosa; CBI–II, ceratobranchial I–II; d, dentary; GG, m. genioglossus; GH, m. geniohyoideus; tend rap, tendinous raphe; HHI & II, hypohyal I & II; IH, m. interhyoideus; IHP, m. interhyoideus posterior; IMA, m. intermandibularis anterior; IMP, m. intermandibularis posterior; RC, m. rectus cervicis; SRI, m. subarcualis rectus I.

Figure 9. 3D reconstructions of the musculoskeletal architecture of the hyobranchial apparatus of *Andrias japonicus* (a–c: SVL 238 mm [STL 335 mm]) and corresponding dissection images

(d–f: SVL 483 mm [STL 710 mm]) of *A. japonicus* in ventral view. Ossified HBII and CBII are coloured yellow. Abbreviations: d. br. GH, deep branch of m. geniohyoideus; s. br. GH, superficial branch of m. geniohyoideus; other abbreviations are the same as in Figure 5 and 8.

Figure 10. Hyobranchial and hypaxial muscle attachments of *Andrias japonicus* shown on a 3D reconstruction of the skull (left) and on an osteological specimen (right) of *A. japonicus* in ventral view. Abbreviations; ar, articular; apo, aponeurosis; d, dentary; exo, exoccipital; LME, m. levator mandibulae externus; LSV, .m. subvertebralis lateralis; MSV, m. subvertebralis medialis; PDM, posterior m. depressor mandibulae; psh, parasphenoid; pt, pterygoid; ptq. c, pterygoquadrate cartilage; q, quadrate. Other abbreviations are the same as in Figure 8.

Figure 11. 3D reconstructions of the epaxial and hypaxial muscles of *Andrias japonicus* (SVL 238 mm [STL 335 mm]) based on diceCT; (a), epaxial muscle arrangement in dorsal view with IME I rendered transparent on right side; (b), IME I–II removed and MVE rendered transparent on right side; (c) MVE removed and IVE rendered transparent on right side; (d), hypaxial muscles in ventral view. Abbreviations: ADM, anterior m. depressor mandibulae; apo, aponeurosis; at, atlas; IME I–II, m. intermyoseptalis I–II; IVE, epaxial m. intervertebralis; LME, m. levator mandibulae externus; LSV, m. subvertebralis lateralis; MSV, m. subvertebralis medialis; MVE, epaxial m. myoseptal-vertebralis; pa, parietal; PDM, posterior m. depressor mandibulae; v2, vertebra 2.

Figure 12. Image sequence of suction feeding in *Andrias japonicus* (a–l). The times (ms) are from the start of lateral head movement. White arrows indicate the position of the prey (pond loach), and grey arrows indicate asymmetric jaw opening for draining water.

Figure 13. Schematic diagram of the main muscles involved for jaw rotations (a, pitch; b, yaw; c, roll) of *Andrias japonicus* in a posture with both lower jaws open and the subdental ridge directed ventrally in each jaw. Line of muscle actions for each rotation are shown as arrows with a colour that indicates the potential function of that muscle. (a) Pitch rotation around X axis (orange, jaw opening; pink, jaw closure). (b) Yaw rotation around Y^a axis (light green, medial rotation; green, lateral rotation). (c) Roll rotation around the Z^b axis (light blue, internal rotation; blue green, external rotation). See main text for the definitions of the axes and the muscle abbreviations. Note that the Y and Z axes in (b) and (c) are not parallel to the plane of the page.

Figure 14. Comparative diagram of the hyobranchial apparatus and its muscle arrangements in aquatic (*Andrias japonicus*) and terrestrial (*Ambystoma tigrinum*) feeders; (a), *A. japonicus* in

ventral view, and (b) in lateral view; (c), *Am. tigrinum* in ventral view, and (d) in lateral view. In (b) and (d), the hyobranchial apparatus has been moved ventrally from its original position to show the lateral structures. The muscle arrangement of *Am. tigrinum* is based on Reilly & Lauder (1989) and Larsen & Guthrie (1975). The function of these muscle is outlined in Supplementary Table 2. Muscles for retraction of the tongue/ buccal cavity are shown by orange lines; muscles for protraction are shown in pink lines. Grey circles indicate muscles attached to the dorsal surface of the elements. Abbreviations: BB, basibranchial; CBI–II, ceratobranchial I–II; CH, ceratohyal; EP, epaxial muscles; GG, m. genioglossus; GH, m. geniohyoideus; GL, m. genioglossus, lateral division; GM, m. genioglossus medial division; SRI, m. subarcualis rectus I; HG, m. hypoglossus; IMA, m. intermandibularis anterior; OG, otoglossal cartilage; R1, 2, radial 1, 2; RC, m. rectus cervicis; RCP, m. rectus cervicis profundus; RCS, m. rectus cervicis superficialis; UH, urohyal.