Jaw and hyolingual movements during prey transport in varanid lizards: effects of prey type

Vicky Schaelaeken a,∗, Stéphane J. Montuelle b, Peter Aerts a, Anthony Herrel c

a Dept. Biology, University of Antwerp, Universiteitsplein 1, B-2610 Antwerpen, Belgium
b Ohio University, Dept. of Biomedical Sciences, 228 Irvine Hall, Athens, OH 45701, USA
c UMR 7179, Muséum National d’Histoire Naturelle, Département EGB, 57, rue Cuvier CP 55, F-75231 Paris, France

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The ability to modulate feeding kinematics in response to prey items with different functional properties is likely a prerequisite for most organisms that feed on a variety of food items. Variation in prey properties is expected to reveal variation in feeding function and the functional role of the different phases in a transport cycle. Here we describe the kinematics of prey transport of two varanid species, Varanus niloticus and Varanus ornatus. These species were selected for analysis because of their highly specialised hyolingual system and food transport mechanism (inertial food transport). In these animals, tongue and hyoid movements are expected to make no, or only a minor, contribution to prey transport. We observed statistically significant prey type effects that could be associated with prey properties such as mass, size and mobility. These data show that both species are capable of modulating the kinematics of food transport in response to different prey types. Moreover, not only the kinematics of the jaws were modulated in response to prey characteristics but also the anterior/posterior movements of the tongue and hyoid. This suggests a more important role of the tongue and hyolingual movements in these animals than previously suspected. In contrast, head movements were rather stereotyped and were not modulated in response to changes in prey type.

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1. Introduction

Feeding is important for the survival and fitness of individuals, and consequently has been studied in a wide variety of animals. Moreover, tetrapod feeding and prey capture strategies are extremely variable, ranging from, e.g., the extraordinarily protrusible tongue in ant-eating mammals used to capture prey, to the highly reduced but equally protrusible tongue specialised for chemoreception observed in snakes (for an overview see Hiiemae, 1978; Reilly and Lauder, 1990; Schwenk, 2000; Reilly et al., 2001). This extreme variation in form and function of the hyolingual system makes it a very attractive system to investigate in a comparative context.

Lizards are an ideal model to study the functional morphology of feeding because of the complexity and diversity of the squamate feeding systems that depend on the integration of the jaw, the hyolingual and the cervical systems (Bels et al., 1994; Schwenk, 2000; Herrel et al., 2001; Ross et al., 2007). Most lizards feed on a wide variety of food items that often differ in their characteristics (e.g., texture, mass, mobility; see Greene, 1982). This variability in prey properties can be expected to have an influence on the efficiency of prey capture and transport. Thus, the ability to adjust feeding behaviour in response to different prey characteristics seems essential as it may allow a more efficient food processing. A number of studies have already demonstrated that lizards modulate their intraoral food processing to some degree (e.g., Bels and Baltus, 1988; Wainwright et al., 1991; Herrel et al., 1996; Herrel and De Vree, 1999; Urbani and Bels, 1999; Schwenk, 2000; Ross et al., 2007; Schaelaeken et al., 2008).

For efficient prey transport, a strong coupling between the movements of the jaws, tongue, and hyobranchium is necessary, as in most lizards head movements that could assist prey transport play a negligible role. However, the overall form and surface morphology of the tongue are crucial in this context as the tongue makes intimate contact with the prey item during feeding and in doing so drives one of the principal phases of the intraoral transport cycle (slow open phase; see Bramble and Wake, 1985; Schwenk, 2000).

Among lizards, the function and morphology of the tongue is diverse, with different degrees of specialisation being present in different clades (Cooper, 1995; Schwenk, 2000). Squamates are often divided into two main clades, iguanian and scleroglossan lizards. Generally, Iguania have a short, fleshy tongue that is used for hyolingual transport. This kind of transport involves coupled
anterior/posterior movements of the tongue and the hyobranchium underneath the prey while it is held against the roof of the mouth (Delheusy and Bels, 1992). Despite differences in tongue and hyobranchial form, intraoral transport in scleroglossans is superficially similar to that observed in iguanians. However, some clades such as varanids and teiids have evolved a more active foraging style that is dependent on the use of chemoreception (Vitt et al., 2003). The specialisation of the tongue required for chemoreception involves morphological changes including an elongation of the foretongue resulting in a decrease in total surface area, and a decoupling of tongue and hyobranchium (for a description of tongue morphology in varanids, see Smith, 1984). Consequently, these lizards rely predominantly on alternative mechanisms of food transport. Indeed, varanids and teiids use inertial food transport whereby the prey is literally thrown into the oesophagus by rapid movements of the head, neck, and entire body. During inertial transport the jaws are opened rapidly and simultaneously the head/neck system is drawn back, initiating the backward displacement of the prey after release from the jaws. At maximal gape, the entire head and body move forward and the jaws are closed (Smith, 1982; Montuette et al., 2009). Consequently, it has been hypothesised that the tongue plays no significant role in prey transport in these species (Bramble and Wake, 1985).

The present study has two main goals: (i) to quantify the kinematics of the jaws, head, tongue, and hyoid during inertial transport in varanid lizards feeding on a variety of food items and (ii) to establish whether the tongue and hyoid play an active role during inertial transport. Data on the feeding kinematics in varanids are relatively rare and most studies typically focus on species with relatively atypical morphologies such as V. exanthematicus. The species used here (Varanus niloticus and Varanus ornatus) were chosen because they are typical varanid characters by a long neck, elongate head and diverse diet.

Based on a priori reasoning and the hypotheses put forward by Metzger (2009), we make the following predictions on how transport cycles should be affected by prey properties. First, we predict the consumption of larger (more massive) prey items to be associated with increasing gape distances and transport cycle durations. These relationships are predicted as during inertial transport the jaws have to be opened extensively to allow free passage of the prey into the gular area. Consequently, the degree of gular expansion is predicted to increase with prey size as well. As the transport of larger prey is predicted to be associated with larger gape distances, the duration of a transport cycle is also predicted to increase. Metzger (2009) predicted that the consumption of prey with higher mass should be associated with an increase in the absolute duration of the slow open phase (SO). This has already been demonstrated for species using lingual intraoral transport (e.g., Thexton et al., 1980; Bramble and Wake, 1985; Herrel et al., 1996). However, as varanids use inertial prey transport, we predict that there will be no effect of prey mass or size on SO duration as the tongue is expected to make no contact with the prey item during jaw opening. Second, based on Montuette et al. (2009) we predict that an increase in prey size (mass) will lead to an increased amplitude of head movements as the head starts its movement from a lower position and ends at a higher position. Finally, following the predictions of Schaerlaeken et al. (2008), more elusive prey items are predicted to be associated with a increase in jaw opening and closing velocity and a decrease in the duration of the fast jaw open (FO) and close (FC) phase durations as these are the phases during which the teeth are not engaged with the food.

In addition to these hypotheses related to the effect of prey characteristics on the shape of the jaw cycle, predictions regarding the movements of tongue and hyoid during inertial prey transport can be made. As the tongue is predicted to make no intimate contact with the prey during transport, no or only minor anterior/posterior movements are expected (Smith, 1982, 1986; but see Elias et al., 2000). As prior studies have demonstrated a strong coordination between the movements of the tongue and hyobranchium in lizards, we also predict little or no contribution of the hyobranchium to prey transport in varanid lizards. Thus, mechanical differences between prey items should not be associated with changes in tongue or hyoid movements.

### 2. Materials and methods

#### 2.1. Species

One sub-adult specimen of the species V. niloticus and two sub-adult specimens of V. ornatus were used in the experiments (for a general description of the size of the specimens see Table 1). The specimens were obtained from commercial animal dealers and transferred to the University of Antwerp. These two species were chosen as they are very similar in overall morphology and ecology; until recently V. ornatus was considered a subspecies of V. niloticus (Böhme, 2003). The animals were housed separately in large vivaria (120 cm x 80 cm x 80 cm) on a 12 h:12 h light/dark cycle and were fed dog food, mice, raw quail eggs and small chicks ad libitum. The environmental temperature varied from 28 °C during daytime to 22 °C at night. An incandescent bulb provided the animals with a basking place at a higher temperature.

#### 2.2. X-ray recordings

Small lead markers were inserted on the right side or along the mid-sagittal plane at the anterior tip of the upper and lower jaw, at the anterior and posterior aspect of the parietal and frontal bones, in the neck at the level of cervical vertebrae 2 and 4, in the anterior and posterior regions of the tongue, and at the joint between the basihyal and the hypobyal (Fig. 1). Before implantation of these markers the animals were anaesthetised using ketamine (100 mg/kg; ketamine hydrochloride, 50 mg/ml; Parke-Davis, Brussels, Belgium). Markers on the parietal and frontal bones were placed in small holes made with a dental drill, while the other markers were implanted using hypodermic needles. Marker placement was checked using dorso-ventral and lateral radiographs. Animals were filmed in lateral view while feeding on large grasshoppers (Locusta migratoria), pinkies (newborn mice, Mus musculus), pre-killed mice (M. musculus) and intact snails (Cornu aspersum). These prey items were selected because they are part of the natural diet of both species (Losos and Greene, 1988) and vary in their functional properties (Table 2). A Redlake MotionPro2000 digital high speed camera (Integrated Design Tools, Inc., Tallahassee, FL, USA) attached to a Philips 14-inch image intensifier (Philips,

### Table 1

<table>
<thead>
<tr>
<th>Species</th>
<th>SVL (mm)</th>
<th>HL (mm)</th>
<th>HW (mm)</th>
<th>HH (mm)</th>
<th>LJ (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V. ornatus</td>
<td>450</td>
<td>80.15</td>
<td>43.08</td>
<td>36.38</td>
<td>94.69</td>
</tr>
<tr>
<td>V. ornatus</td>
<td>435</td>
<td>81.07</td>
<td>44.34</td>
<td>36.6</td>
<td>93.08</td>
</tr>
<tr>
<td>V. niloticus</td>
<td>480</td>
<td>68.56</td>
<td>34.44</td>
<td>26.86</td>
<td>77.79</td>
</tr>
</tbody>
</table>

SVL, snout–vent length; HL, head length; HW, head width; HH, head height; LJ, lower jaw length.

### Table 2

<table>
<thead>
<tr>
<th>Food type</th>
<th>Size (mm)</th>
<th>Mass (g)</th>
<th>Hardness (N)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grasshopper</td>
<td>43.68 ± 3.8</td>
<td>0.51 ± 0.60</td>
<td>2.33 ± 2.01</td>
</tr>
<tr>
<td>Pinkie</td>
<td>38.44 ± 3.6</td>
<td>1.74 ± 0.40</td>
<td>4.17 ± 0.66</td>
</tr>
<tr>
<td>Mouse</td>
<td>87.83 ± 7.54</td>
<td>30.82 ± 1.77</td>
<td>17.62 ± 6.76</td>
</tr>
<tr>
<td>Snail</td>
<td>34.85 ± 1.79</td>
<td>8.13 ± 1.67</td>
<td>79.71 ± 15.74</td>
</tr>
</tbody>
</table>

* See Herrel et al. (1999) for a description of the measurement of food hardness.
Amsterdam, Netherlands) was used to record the feeding events at 250 Hz. X-rays were generated using a Philips Optimus M200 X-ray generator. All procedures were approved by the animal ethics committee at the University of Antwerp.

2.3. Video analysis

Cineradiographic recordings were analysed using Midas Player software version 2.1.7 (Redlake, San Diego, CA, USA). Only feeding cycles in which all phases of a transport cycle (slow opening, SO; fast opening, FO; fast closing, FC; slow closing, SC) were present and in which no prey reduction or prey swallowing was involved were used for further analysis. Prey reduction can easily be distinguished from prey transport by features such as a pronounced slow close phase during crushing. Swallowing, on the other hand, can be distinguished from prey transport by features such as smaller gape angles, more extensive tongue movements and the lack of extensive head and neck movements.

Many trials had to be discarded because of out-of-plane movements of the head and were thus not included in our analyses. For those sequences retained, seven landmarks were digitised on each frame using Didge (Image Digitising Software version 2.2.0; by Alistair Cullum, Creighton University, Omaha, NE, USA). These landmarks included the anterior tip of the lower jaw, the anterior tip of the upper jaw, the neck at the level of cervical vertebrae 2 and 4, the marker on the parietal bone, the posterior tongue marker and the hyoid marker placed at the connection between the basihyal and hypohyal (Fig. 1).

Based on the x–y coordinates of these markers, the following variables were calculated (Fig. 1): the distance between the upper and lower jaw (gape distance), the distance between the hyoid and neck (gular extension), the distance between lower jaw and tongue (tongue distance) and the distance between lower jaw and hyoid (hyoid distance). Based on the plots of the movements of the jaws, tongue and hyoid during prey transport, the maximal gape distance, the maximal tongue displacement relative to the lower jaw, the maximal displacement of the hyoid relative to the lower jaw and the maximal distance between neck and hyoid were determined.

To quantify the movements of the head during inertial feeding, changes in head angle, head velocity and head acceleration were calculated. The head angle was defined as the angle between a line interconnecting the markers on the parietal bone and the tip of the upper jaw, and a line interconnecting the two neck markers (Fig. 1).

The raw displacement profiles of the jaws and the changes in head angle were smoothed using a zero phase shift, fourth-order low-pass Butterworth filter at 5 Hz. Velocities and acceleration profiles were then calculated based on the filtered data by taking the first and second derivatives. From these data the maximal instantaneous jaw opening and jaw closing velocity, the maximal and minimal instantaneous angular velocity of the head, and the maximal instantaneous angular peak acceleration and deceleration of the head were extracted for each transport cycle. The different phases within a transport cycle were determined based on the acceleration profiles calculated from the changes in gape velocity over time as outlined in Schraerlaeken et al. (2007, 2008).

Based on the transport cycles, 16 variables in total were calculated and used in the statistical analyses: gape distance, the durations of the different transport phases (dSO, dFO, dFC, dSC), the total duration of a transport cycle, the maximal jaw opening and closing velocity, the maximal tongue displacement relative to the lower jaw, the maximal displacement of the hyoid relative to the lower jaw, the maximal distance between the neck and hyoid, the total variation in head angle, the maximal and minimal head angle velocity, and the maximal and minimal head acceleration. A total of 57 transport cycles were analysed for V. niloticus, and a total of 31 transport cycles for V. ornatus.

2.4. External video recordings

To quantify the number of transport cycles needed to transport different prey, a data set was gathered using a regular video camera (Sony DCR HC24; 30 Hz; Sony Corp., Tokyo, Japan) and filming the prey transport of the same 2 individuals of V. ornatus while eating grasshoppers, pinkies and mice (dead and alive) (graphically depicted in Fig. 2).

2.5. Statistical analyses

To meet the assumption of homoscedasticity and normality for regression analyses (Sokal and Rohlf, 1981; Kachigan, 1991), all kinematic data were log_{10} transformed prior to analyses. Univariate F-tests coupled to Bonferroni post hoc tests were performed on the kinematic variables to test for differences between prey types. Individual was entered as a random factor in the ANOVAs and significance levels were corrected for multiple testing (Rice, 1989). All analyses were performed using SPSS 15.0.
Table 3
Results of the univariate F-tests on the kinematic variables testing differences between different prey types and individuals.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Prey F</th>
<th>Prey p</th>
<th>Individual F</th>
<th>Individual p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gape distance (mm)</td>
<td>21.470</td>
<td>&lt;0.001</td>
<td>7.671</td>
<td>0.001</td>
</tr>
<tr>
<td>Duration of the slow open phase (s)</td>
<td>4.691</td>
<td>0.004</td>
<td>0.517</td>
<td>0.598</td>
</tr>
<tr>
<td>Duration of the fast open phase (s)</td>
<td>5.685</td>
<td>&lt;0.001</td>
<td>1.299</td>
<td>0.278</td>
</tr>
<tr>
<td>Duration of the fast close phase (s)</td>
<td>3.628</td>
<td>0.016</td>
<td>2.327</td>
<td>0.103</td>
</tr>
<tr>
<td>Duration of the slow close phase (s)</td>
<td>0.683</td>
<td>0.565</td>
<td>1.136</td>
<td>0.326</td>
</tr>
<tr>
<td>Total duration transport cycle (s)</td>
<td>3.844</td>
<td>0.12</td>
<td>1.359</td>
<td>0.262</td>
</tr>
<tr>
<td>Maximal jaw opening velocity (mm/s)</td>
<td>30.549</td>
<td>&lt;0.001</td>
<td>9.594</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Minimal jaw opening velocity (mm/s)</td>
<td>44.968</td>
<td>&lt;0.001</td>
<td>4.053</td>
<td>0.020</td>
</tr>
<tr>
<td>Total tongue displacement (mm)</td>
<td>8.374</td>
<td>&lt;0.001</td>
<td>7.443</td>
<td>0.001</td>
</tr>
<tr>
<td>Total hyoid displacement (mm)</td>
<td>17.197</td>
<td>&lt;0.001</td>
<td>1.761</td>
<td>0.177</td>
</tr>
<tr>
<td>Distance neck–hyoid (mm)</td>
<td>3.282</td>
<td>0.025</td>
<td>8.197</td>
<td>0.001</td>
</tr>
<tr>
<td>Head angle (°)</td>
<td>3.797</td>
<td>0.013</td>
<td>10.686</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Minimal head velocity (mm/s)</td>
<td>1.485</td>
<td>0.224</td>
<td>2.740</td>
<td>0.070</td>
</tr>
<tr>
<td>Maximal head velocity (mm/s)</td>
<td>3.418</td>
<td>0.021</td>
<td>3.456</td>
<td>0.036</td>
</tr>
<tr>
<td>Minimal head acceleration (mm/s²)</td>
<td>4.488</td>
<td>0.006</td>
<td>9.565</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Maximal head acceleration (mm/s²)</td>
<td>3.147</td>
<td>0.029</td>
<td>4.794</td>
<td>0.011</td>
</tr>
</tbody>
</table>

Bold values: significant at alpha = 0.05 after sequential Bonferroni correction.
* Significant after correction for multiple testing.

3. Results

Feeding in varanid lizards can be divided into 4 different stages starting with prey capture, followed by prey repositioning and prey transport and ending with swallowing or pharyngeal packing (Smith, 1982). For our quantitative analyses only the prey transport cycles, crush bites not included, were used.

3.1. Modulation of prey transport – prey type effects

Univariate F-tests were performed on the kinematic variables and revealed significant prey type and individual effects for gape distance (prey type: $F_{3,94} = 21.470; P < 0.001$; individual: $F_{2,94} = 7.671; P = 0.001$), maximal jaw opening velocity (prey type: $F_{3,94} = 30.549; P < 0.001$; individual: $F_{2,94} = 9.594; P < 0.001$), minimal jaw opening velocity (prey type: $F_{3,94} = 44.968; P < 0.001$; individual: $F_{2,94} = 4.053; P = 0.020$) and total tongue displacement (prey type: $F_{3,94} = 8.374; P < 0.001$; individual: $F_{2,94} = 7.443; P = 0.001$). Univariate F-tests performed on the duration of the slow open phase ($F_{3,94} = 4.691; P = 0.004$), the duration of the fast open phase ($F_{3,94} = 5.685; P = 0.001$) and the total hyoid displacement ($F_{3,94} = 17.197; P < 0.001$) revealed only a significant prey type effect. Finally, univariate F-tests performed on the distance between neck and hyoid ($F_{2,86} = 8.197; P = 0.001$), head angle ($F_{2,86} = 10.686; P < 0.001$) and the minimal head acceleration ($F_{2,86} = 4.794; P = 0.011$) revealed only a significant individual effect (see Table 3).

Table 4 summarises the results of the post hoc tests performed on those variables for which prey type effects and/or individual effects were significant. Most remarkably, the differences between snails and the other prey types (grasshopper, mouse and pinkie) were significant in all individuals. Gape distance, total hyoid displacement, duration of the slow open phase, maximal jaw opening and closing velocity and total tongue and hyoid displacement were smaller when transporting snails compared to grasshoppers, mice and pinkies (Fig. 3). The total tongue displacement was also smaller during the transport of pinkies compared to mice. Only the duration of the fast open phase was greater during the transport of snails compared to mice, grasshoppers and pinkies (see Table 5).

The distance between neck and hyoid, the head angle and the minimal head acceleration were different for different indi-
4. Discussion

Overall, intraoral transport cycles are rather similar in Iguania and Scleroglossa, despite the differences in tongue and hyobranchial form (Cooper, 1995; Schwenk, 2000). However, a few clades of Scleroglossa (varanids and teiids) are characterised by the use of inertial prey transport whereby a rapid movement of the cranio-cervical system is used to accelerate and transport the prey posteriorly (Gans, 1969; Smith, 1982; Schwenk, 2000; Montuelle et al., 2009). In this study, the food items presented to the lizards allowed us to test whether varanids are capable of modulating their prey transport kinematics and whether the tongue and hyoid play a role in inertial transport.

In accordance with the predictions of Metzger (2009), the gape distance during the transport of snails, being small prey (Table 2), is smaller than during the transport of grasshoppers, pinkies and mice. Moreover, the total number of transport cycles needed to transport pinkies is lower than that for transporting grasshoppers and mice. Thus, the transport of smaller prey is associated with smaller gape distances and fewer transport cycles.

In contrast to the prediction that no effect of prey mass on SO duration should be observed in lizards with highly specialised tongues like varanids, SO duration was greater during the transport of mice compared to the other prey items. This suggests that the tongue might indeed play some role in prey transport. This is similar to what has been observed for inertial feeding in crocodilians where the highly reduced tongue and hyobranchium retain an important function during prey transport (Cleuren and De Vree, 1992, 2000). The details of tongue and hyobranchial movements are, however, different between crocodilians and varanids, with anterodorsal movements being associated with the slow opening phase in varanids, but the fast opening phase in crocodilians (Cleuren and De Vree, 1992, 2000).

According to Montuelle et al. (2009), the transport of heavier prey items should be associated with more extensive head movements and head accelerations. However, only individual differences in head angle and minimal head acceleration could be detected, with prey type effects being non-significant. This suggests that head movements associated with inertial transport are very stereotyped. We also suggested that with more elusive prey the jaw opening and closing velocity would increase and the fast jaw open (FO) and close (FC) phase durations would decrease. Following this prediction, we expected that snails, which are slow and unlikely to escape, would be associated with longer fast opening/closing phase durations and a decrease of jaw velocity during transport. In fact, in all individuals the jaw opening and closing velocity was lower during the transport of snails compared to other prey items. Moreover, the duration of the fast open phase was also increased during the transport of snails.

Finally, we predicted that as the tongue should make no intimate contact with the prey item during inertial prey transport, no or only minor anterior/posterior tongue and hyoid movements are to be expected. This has also been suggested by Smith (1982, 1986), but Elias et al. (2000) found an extensive use of the tongue and hyoid in monitor lizards during prey transport. Here, we observed significant differences in total tongue and hyoid displacement associated with the transport of different prey types. The total tongue displacement was, for example, greater during the transport of mice. The total hyoid displacement, on the other hand, was smallest during the transport of snails. These results illustrate that the tongue and hyoid show significant anterior/posterior movements and that these movements are modulated in response to food type, suggesting an active contribution of the tongue and hyoid apparatus to prey transport.

In summary, our data suggest that varanid lizards are capable of modulating their transport kinematics in response to different prey individuals (individual effect). The distance between neck and hyoid when transporting snails was smaller in one individual than for transporting grasshoppers, mice and pinkies (see Table 5).

Based on the external video recordings, a significant prey type effect ($F_{1,32} = 10.066; P < 0.001$) was detected for the total number of transport cycles. The total number of transport cycles needed to transport pinkies was smaller than that needed to transport grasshoppers and mice (dead or alive) in 2 individuals of V. ornatus (Fig. 2).
types. Although these lizards use inertial prey transport, they also modulate tongue and hyoid movements in response to different prey types. Further experiments controlling single food properties are needed to better understand the role of tongue and hyoid movements during feeding.

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